A structural and mechanical assessment of thrombus analogs formed under physiological pressure and flow

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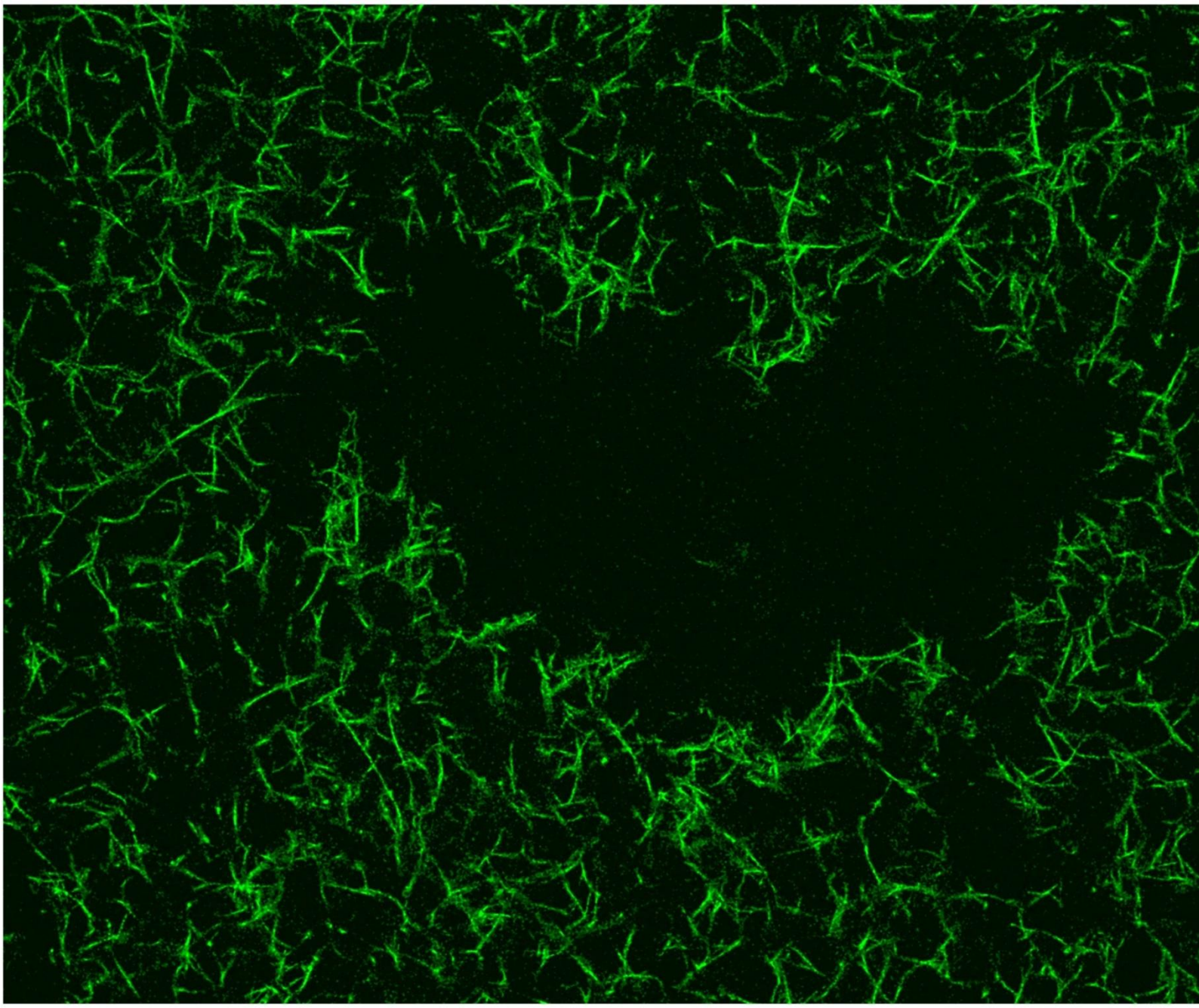

TU Delft Delft University of Technology

Erasmus MC Universitair Medisch Centrum Rotterdam

A structural and mechanical assessment of thrombus analogs formed under physiological pressure and flow

by
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to obtain the degree of
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Contents

1 Preface

This thesis is the product of the last proud member of the "Thrombus crew." The subject combines a structural and mechanical assessment of clot analogs. This thesis was written in the format of a scientific paper. In addition, appendices were added with more details about this thesis.

During this thesis, I learned practical lab skills like how to pipette chemicals, fixate tissue with formaldehyde, coat microscopic slides with tissue factor, and make buffers by mixing all sorts of chemicals (refreshing my ancient chemistry skills). In addition, I learned a lot about handling machinery like a confocal microscope, a micro-indentation machine, an unconfined compression tester, and a tensile tester. Also, a lot of software knowledge was needed, like Matlab, ImageJ, and SPSS. I finally really mastered Matlab, which was one of my main goals.

I am glad and thankful that I got the opportunity to learn from and work on this project. I hope you enjoy reading it.

2 Acknowledgements

I want to thank my girlfriend for giving me the advice to rest in between writings and for always supporting me. I want to thank my mother for keeping me sain during my thesis. Furthermore, I want to thank the rest of my family and friends for always supporting me during this thesis.

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3 Abstract

BACKGROUND - The success of the mechanical thrombectomy treatment in acute ischemic stroke is highly dependent on mechanical clot behavior and characteristics like stiffness. This mechanical treatment is tested in clot analogs. Realistic clot analogs could potentially be made in a realistic formation environment. The effects of blood pressure and flow were mimicked in this study. Factors affecting the mechanical behavior of thrombus analogs should be investigated to understand treatment possibilities better.
METHODS - First, the effect of blood pressure was mimicked in a static pressure experiment by putting a weight on blood which generates a constant force. The clot analogs were mechanically assessed by unconfined compression testing. The effect of whole blood clot formation under pressure, the effect of different heights, and the effect of different hematocrit levels (a volumetric hematocrit of 1%H, 40%H, and 99%H) were investigated. Secondly, fibrin clots are formed by clotting platelet poor plasma on tissue factor under flow, with four different shear rates (shear=0/s, 50/s, 150/s, and 300/s). These clots were assessed by micro-indentation and confocal imaging.
RESULTS - A total of 50 clot analogs under static conditions were successfully analyzed. Hyperelastic strain stiffening and visco-elastic behavior was seen in all clots, higher heights resulted in a higher stiffness in strain >60%, and 1%H clots were stiffer than 99%H clots. Furthermore, a total of 43 fibrin clots formed under flow were successfully investigated. It was seen that flow reduced the clot heights, fibers seemed to align with the flow direction, flow reduced the density in the top of the clots, and clots formed under shear had a higher stiffness than similar statically formed clots.
CONCLUSION - A range of clot analogs under static conditions and flow were made and tested. The significant differences in mechanical properties and microstructure found can have new implications in thrombectomy research.

4 Introduction

4.1 Background of stroke, current treatments, and problems

Stroke causes 10% of deaths.[1] In 87% of the cases, stroke is ischemic as opposed to hemorrhagic.[2] Ischemic stroke can be defined as an episode of neurologic dysfunction caused by focal cerebral, retinal, or spinal infarction.[3] Ischemic stroke is mainly caused by a thromboembolus, in which a blood clot formed elsewhere travels via the bloodstream to intracranial arteries, acutely blocks the blood flow, and leads to infarction over the supplied brain territory.[4] Treatment of ischemic stroke in the acute phase involves re-perfusion by intravenous alteplase (t-PA) or endovascular treatment (EVT). These treatments can be broadly divided into the chemical dissolution of thrombi with locally delivered thrombolytic agents and thrombus retrieval or thrombectomy with mechanical devices such as stent retrievers or aspiration catheters. Specifically, thrombectomy was beneficial in large vessel (arterial) occlusion. An absolute difference of 13.5% points (95% CI, 5.9 to 21.2) in the rating scale of functional independence (modified Rankin score, 0 to 2) in favor of the EVT (thrombectomy and alteplase) over only t-PA (32.6% vs. 19.1%) was shown, which is a breakthrough in stroke research.[5] Though limitations arise with these new treatments. Examples are the need for trained specialists who can perform a thrombectomy, technical difficulties of the device, vessel access, maneuvering the wire through weak vessels, dissection of vessels, the occurrence of distal thromboemboli when manipulating the thrombus, vasospasms, and issues in removability of thrombi.[6] One of the main challenges seems to be the different characteristics of the thrombi, which can affect removability,[7,8] as thrombi have different strength, viscoelasticity, permeability, and resilience to fibrinolysis.[9–11] One of the main important mechanical characteristics of thrombi is stiffness. The stiffness of the fibrin scaffold of occlusive thrombi is a major determinant for the effectiveness of their removability to restore the impaired blood flow.[12] The main components of thrombi include fibrin, red blood cells (RBCs), platelets, leukocytes, and neutrophil extracellular traps.[13] Hematocrit is the volume of red blood cells (RBCs) relative to WB.[14] An almost full range of hematocrit levels seems to exist in thromboemboli.[15,16] It seems that a high hematocrit percentage, gives lower levels of stiffness.[15,17] Furthermore, different clot heights are found in stroke patients.[18,19] It is shown that a large thrombus

height is associated with a decrease in recanalization success and increased complication rates in thrombectomy.[20] It is unknown if these dimensions also affect clot stiffness. It is also seen that thrombus parameters are highly dependent on features specifically based on the fibrin fibers, an association with fiber diameter, branching, and cross-linking is seen.[9–11] More research is being done to address the limitations of these relatively new therapies in ischemic stroke.

4.2 Thrombus analogs and mimicking conditions in the human body

These mechanical devices are tested in thrombus analogs or clots. As real thrombi have variating characteristics, realistic clots are needed. A realistic clot fits the pathological conditions in which it is formed in the body. So ideally, the formation environment in which clot analogs are generated mimics the body accurately. Blood flow is one of the most important physical factors that affect the formation of the fibrin network, its structure, and its properties.[21,22] The effects of blood pressure and flow were mimicked in this study. The effect of blood pressure was mimicked in a static pressure experiment by putting a weight on blood which generates a constant force. The effect of blood flow was mimicked in a dynamic flow experiment done with three different shears. In the experiments, clotting was influenced in different ways. Clotting all begins with the clotting cascade, which ultimately results in the polymerization of fibrin and the activation of platelets, leading to a thrombus. Therefore, it can be concluded that clotting mainly depends on fibrin and platelets.[23] With the clot formation in static conditions, the clot formation process finishes when all soluble fibrinogen is converted to insoluble fibrin. The fibrin fibers are predominantly randomly oriented in a static environment without clot contraction.[24] After solidification of the blood, platelets are the drivers for clot contraction. Clot contraction is when tightening the fibrin network takes place by gathering fibrin strands to adhered platelets.[25] Clot contraction caused by platelets significantly affects clot volume and microstructure, and in turn, clot stiffness.[26] Tightening of the fibrin network might be further enhanced by mechanical compaction of the clot. Mechanical compression of the clot can be caused by high blood pressure or mimicked in a static pressure experiment, such that fluid (serum) is expelled from a clot.[27] Alternatively, clot formation under flow, results in the adding of more fibrinogen to the forming clot. Adding more fibrinogen to the clot will result in a higher protein level and denser, thicker, and more bundled fibers.[28,29] Under flow conditions, fibrin fibers can get orientated along the direction of flow.[22,30] Orientation of the fibers can change mechanical properties.[22,31]

4.3 The purpose of this study

Firstly, homogeneous clots were made under static pressure, mimicking physiological blood pressure. The effect of blood pressure, clot dimensions, and specific components in WB on clot stiffness were assessed. Then, unconfined compression was performed in all of these clots to find the stiffness. This study provides a detailed characterization of these clot analogs showing cyclic loading-unloading hysteresis. It aims to provide mechanical information on a range of clot analogs formed under static pressure. It could help understand the effect of physiologic static pressure, clot dimensions, and hematocrit level on clot stiffness and, in turn, on the effectiveness of removability of clots with certain stiffnesses. Also, information found in this study about clot dimensions could help interpret the variating clot dimensions used in the unconfined compression testing of clot analogs.[32,33] Secondly, clots with an aligned fibrin network formed under flow with three different shear rates were investigated. By using platelet poor plasma (PPP), there was specifically looked at the effect of controlled flow on fibrin networks. Fibrin is the predominant loading bearing component of a thrombus,[34] making it the most physiologically relevant element to asses in thrombus mechanics. Fibrin networks facilitate the load-bearing in clots. The effect of controlled flow on the structure of fibrin networks and how this would affect the mechanical properties was investigated. Micro-indentation and confocal imaging were done in the fibrin clots formed under flow to obtain mechanical and structural information from the clots. This study links the mechanical and microscopic qualities of the generated fibrin clots to different flow rates. It aims to help understand fibrin network structures, their effects on mechanical features, and the pathophysiology of flow rates in the bloodstream.

5 Method and materials

5.1 Whole blood: retrieval and separation of constituents

All experiments were performed in the Erasmus Medical Center labs. For the clots formed under static conditions, fresh human blood was obtained from a cubital vein in four volunteers with no known hemostatic conditions and no anti-coagulation use. The volunteers were males and females with the age range of 22 to 53 years. This venous whole blood, was anticoagulated in 0.105 M buffered trisodium citrate solution (BD Vacutainer®, United States), which is specified in protocol 13.2.1 "Retrieving blood."[35] WB is used to assess the 6.1.1 "Clot formation under pressure," and the 6.1.2 "Different heights in clots." To separate the WB constituents (protocol 13.2.2 "Making clot analogs: ratios"), centrifuging was done. Platelet-rich plasma (PRP) was separated from the hematocrit by centrifuging at 120 g for 20 minutes (brakes/stops: 4), at room temperature. PRP was then pipetted from the fluid. The remainder was centrifuged at 2000 g for 10 minutes (brakes/stops: 7) at room temperature, after which the PPP was separated from the RBCs. The hematocrit and PRP were used in 6.1.3 "Different hematocrit levels." The PPP can be stored at -80 °C. Pooled PPP was used in 6.2 "Fibrin clot formation under flow", from different volunteers (± ten of unknown age).

5.2 Thrombus analogs formed under static conditions

5.2.1 Testing workflow

The general steps for the clots formed under static pressure are shown in figure 1, it consists of testing the effect of the formation of clots under compaction or pressure, testing the effect of different heights, and testing the effect of varying hematocrit levels, all by unconfined compression testing.

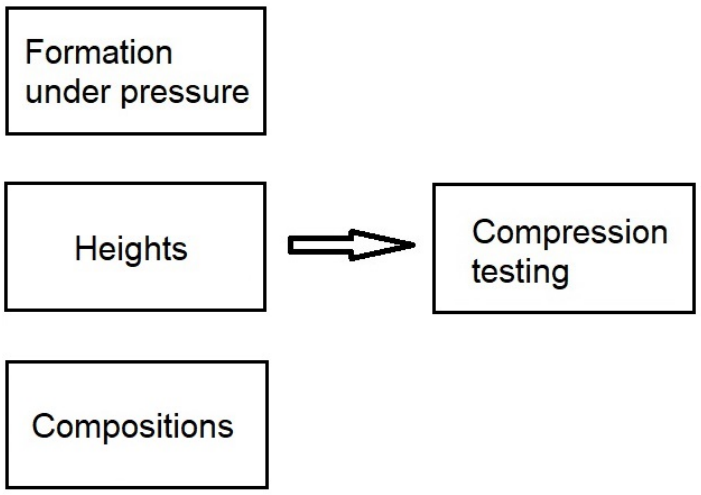

Figure 1. The general steps for making clots under static pressure.
Clots were first formed under different conditions and then tested by unconfined compression testing.

Firstly, we used the WB of one donor (29-year-old female) to test the effect of the formation of samples with or without applying pressure. Five samples were made with pressure and four samples without pressure. Secondly, the effect of different heights in samples was tested. The WB of one person (female 29 years old) was used to test clots with a height of 1 mm, 2 mm, and 5 mm, with five samples per height. Thirdly, the differences in hematocrit levels were tested, blood from two donors was used (a 53-year-old male and a 27-year-old female), and three different hematocrit compositions were made. Hematocrit is expressed as a % of the mixture and is written as H%. Five samples were made per donor with 1%H and 99%H. As for the 40%H samples, three samples were made per donor. The specific samples per topic are summarized in table 1.

Table 1. Number of different clots formed under static conditions.

	Blood donor			Clot type 1				vs	Clot type 2				vs	Clot type 3			
Topics	Sex	Age	Draw	H%	n	h [mm]	Weight		H%	n	h [mm]	Weight		H%	n	h [mm]	Weight
(Not) pressured	Female	29	A	WB	4	1	No		WB	5	1	Yes		x	x	x	x
Heights	Female	29	B	WB	5	1	Yes		WB	5	2	Yes		WB	5	5	Yes
Compositions	Male	53	C	1%	5	1	Yes		40%	3	1	Yes		99%	5	1	Yes
	Female	27	D	1%	5	3	Yes		40%	3	3	Yes		99%	5	3	Yes

The different clot types that are compared and the corresponding samples are depicted above. The topics can be seen in the left column. Blood draws were done in one batch, performed at date A, B, C, or D. This blood was then used in all the different samples seen in that row. The sample heights were created by cutting the main clot in different heights. All samples above were matured at 37 °C overnight in 5mL syringes. H%, hematocrit%, n: amount of samples, h: height, Weight: if a weight was used to mimick physiologic pressure.

5.2.2 Clot formation under pressure

Mechanical compression or compaction can be described as a volume reduction, which changes thromboemboli.[36] It can be done by using a physical element or force to promote the contraction of clots. This force can be translated as the presence of blood pressure during clotting. When blood pressure was mimicked in the fabrication of clots, blood pressure was mimicked in a static way for this experiment. Blood pressure was simplified as a static force, neglecting characteristics like flow and pulsation, which can be added to experiments in the future. The pulsatile character falls out of the scope of this study. The effects of flow were treated separately in subsection 6.2 "Fibrin clot formation under flow". Overall force or pressure which is caused by blood pressure is the mean arterial pressure (MAP), which can be described with formula 1,[37] with diastolic blood pressure (DBP) systolic blood pressure (SBP) in mmHg:

$$MAP = \frac{2DBP + SBP}{3} \tag{1}$$

MmHg relates to newtons per square meter, as 1 mmHg is approximately 133 N/m^2. A blood pressure of 140/90 mmHg, is normal in adults in the upper arm (no hypertension).[38] The normal mean arterial pressure was calculated from this normal blood pressure, which resulted in 107 mmHg, or 1.4.10^4 N/m^2. A syringe was filled with blood and was pressured to mimic blood pressure. The syringe exists of the piston which pushes against the fluid or blood. The diameter of the cylinder piston is called the bore, and the piston goes into the baron or suction tube. A constant force was exerted by a cylindrical shell weight on the baron. The constant force exerted by the cylindrical shell weight on the baron causes an opposite force on the piston. Therefore, pressure on the clots is exerted. The fluid (liquids and gases) pressure is in a closed condition in this situation, and is called a "closed conduit". The closed body of the syringe with this fluid is "static". Pascal's principle states that pressure applied to a static fluid in a closed container is transmitted equally and undiminished throughout the fluid.[39] So when the blood pressure mimicking force was applied at one side of the fluid (a.k.a. the piston), it was naturally transmitted in the column of blood. In this study, we took the example of a 5 mL volume syringe filled with 5 mL of blood in a 37 °C waterbath. A syringe of Terumo 5 mL (613-0917, Terumo),[40] with an inner diameter or bore of 13 mm was used.[41] The syringe needed a Luer lock cap (135290rot, Teqler) to assure an enclosed environment.[42] So the surface area of the circle-shaped piston touching the liquid was 1.34^{-4} m^2. A normal MAP is 107 mmHg, which gives a MAP with the Pascal principle of 1.9 N in this example. Besides the MAP, there needs to be corrected for other forces which are present and can be calculated with formula 2. In this formula, the apparent force exerted by the cylindrical weight (F$_{apparent}$), and the friction force between the piston and the baron (F$_{friction}$) are shown as:

$$F_{apparent} = F_{MAP} + F_{friction} \tag{2}$$

First F$_{friction}$ was calculated by using a tensile tester. The syringe was cut at the backside of the suction tube to make it fit the range of motion of the tensile tester. The piston was fixated at one clamp, and a needle that could be screwed tight was fixated at the other side. The friction force in the syringe was then measured by measuring the resistance when tension was applied. The set-up can be seen in figure 2 (a). It resulted in a force of around 1.0 N of compression resistance, which can be seen in figure 2 (b) as the peak of the graph (before constant force occurs).

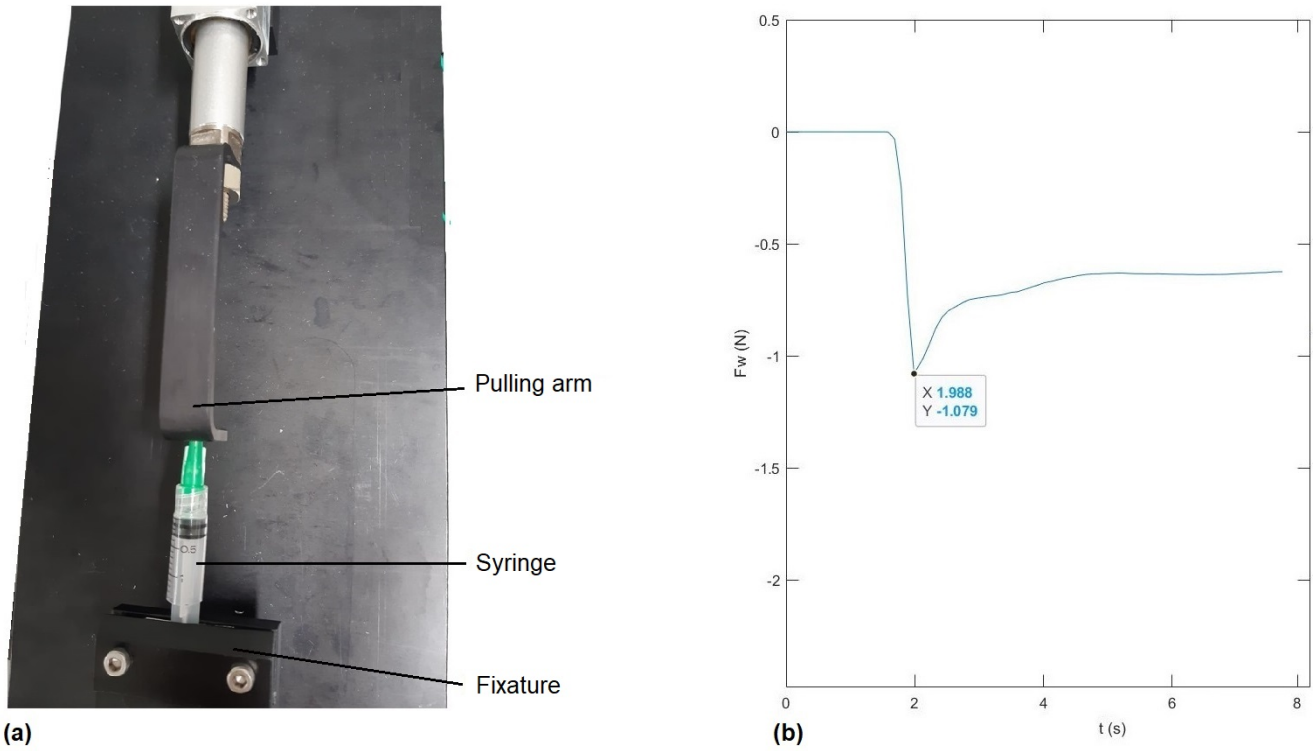

Figure 2. Syringe friction force was measured with a tensile tester.
(a) The set-up with a syringe in the tensile tester and **(b)** the resulting illustrative graph of friction force measured in the syringe, which was around 1.0 N.

So this friction force resulted in outcome:

$$F_{apparent} = F_{MAP} + F_{friction} \tag{3}$$
$$= 1.9 + 1.0 \tag{4}$$
$$= 2.9 \tag{5}$$

When the cylindrical shell weight was put in the water, its weight was diminished by buoyancy. The apparent immersed weight is the weight of the object minus the weight of the displaced fluid, which is expressed in formula 6 with forces in [N], the force exerted by the weight of the cylindrical shell in air ($F_{cylinder}$), the force exerted by the weight of displaced water ($F_{displaced}$). Furthermore it is extended in formula 7 with gravitational force (g) in [m/s^2], the volume of the cylindrical shell weight ($V_{cylinder}$), water displaced by the object in it ($V_{displaced}$), with both volumes in [m^3], and the brass density (ρ_{brass}) and density water (ρ_{water}) in [kg/m^3]:

$$F_{apparent} = F_{cylinder} - F_{displaced} \tag{6}$$
$$= \rho_{brass} g V_{cylinder} - \rho_{water} g V_{displaced} \tag{7}$$

When the cylinder is fully submerged, $V_{cylinder} = V_{displaced}$,[43] so $\rho_{corrected}$ is marked as ρ_{brass} impact corrected for ρ_{water}, which gives formula 9:

$$F_{apparent} = (\rho_{brass} - \rho_{water}) g V_{cylinder} \tag{8}$$
$$= (\rho_{corrected}) g V_{cylinder} \tag{9}$$

The volume of the cylindrical shell was rewritten in formula 10 with cylindrical shell outer radius ($R_{cylinder}$), cylindrical shell inner radius ($r_{cylinder}$), and height of cylindrical shell (h) all in [m]:

$$F_{apparent} = (\rho_{brass} - \rho_{water}) g (R^2_{cylinder} - r^2_{cylinder}) \tag{10}$$

The outer radius of the cylindrical shell is required, so the formula was rewritten:

$$R_{cylinder} = \sqrt{\frac{F_{apparent} + \rho_{corrected} g^2_{cylinder}}{\rho_{corrected} g}} \tag{11}$$

The water density in the case of 37 °C is 9.93[2] kg/m^3,[44] which was mimicked because this is the average body temperature. The material which was used for the weight is brass, and this has a density of 8.51[3] kg/m^3.[45] The outer diameter of the syringe was 14 mm, so the inner diameter of the cylindrical shell weight should be around 15 mm to fit over the syringe, the shell can be seen in figure 3. When formula 11 was used, an outer shell radius of 17.4 mm was calculated, and a mass of 335 grams. When the weight was fabricated, it resulted in weights of 331 grams. The slight difference is negligible as this is a 1% difference. The calculations were summarized in a Matlab code which can be seen in the codes: 11.1 "Cylinder weight". In conclusion, almost all the clots were formed under static pressure (protocol 13.3.2 "Making thrombus analogs under pressure"). The blood mixtures were put in the syringes, compacted by the cylindrical shell weight, and matured overnight in a waterbath at 37 °C.

Figure 3. Cylindrical shell weights which mimicked blood pressure in a static way.
The constant force exerted by the cylindrical shell weight on the baron caused an opposite force on the piston.

5.2.3 Different heights in clots

Different heights in samples were made by cutting the clots with a scalpel in a trimming fixture shown in figure 4. There was chosen to investigate the differences between certain heights in an experiment, as seen in other studies performing compression testing. These heights were chosen to make results comparable. The investigated heights also seen in other studies for this experiment were 1 mm,[15] 2 mm,[32,33] and 5 mm.[17,46] Additionally, a clot height of 3 mm is tested in the different clot compositions experiment. This clot height was tested to assess how practical this specific clot height was in unconfined compression testing.

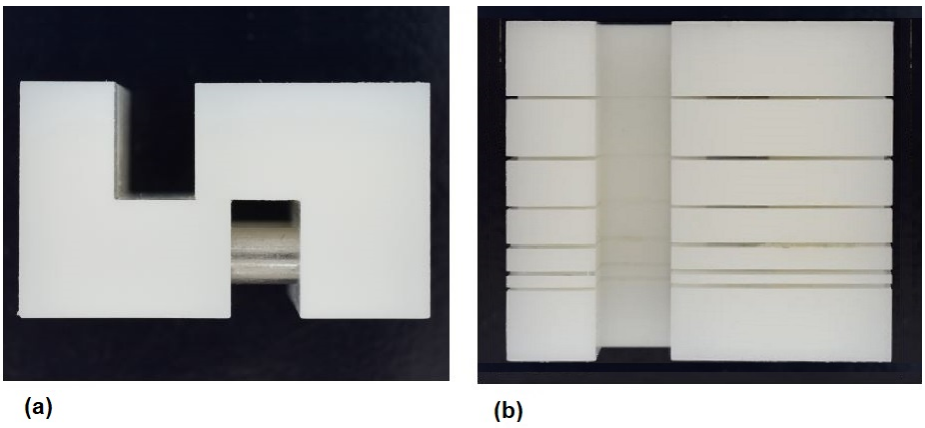

Figure 4. The trimming fixture for making different clot heights.
A trimming fixature was used to cut the sample to the desired height. Available dimensions were 1 mm, 2 mm, 3 mm, 4 mm, and 5 mm. **(a)** Sideview of the trimming fixture. **(b)** Topview of the trimming fixture.

5.2.4 Different hematocrit levels

The blood mixture was recalcified with $CaCl_2$ (C5670, Sigma Aldrich) to initiate clotting (protocol 13.2.3 "$CaCl_2$ and thrombin"), then clots were made (protocol 13.3.1 "Workflow thrombus analogs formed under static conditions"). Different hematocrit percentages were used, which resulted in various types of clots. Separated hematocrit and PPP were mixed in a certain ratio. The different clots made have a hematocrit % of 1%, 40%, or 99%, mixed with PRP. Another type of clot made were WB clots in which no separation of blood took place.

5.2.5 Mechanical data acquisition

The unconfined compression set-up of Boodt et al. (2021) was used.[15] The compression rate used in our study was comparable to the scheme used in Boodt et al. (2021) and Johnson et al. (2020 July), which allows comparison to these studies.[15,17] One unconfined compression cycle is performed. The clots were compressed until 80% of the total height, at a strain rate of 10% per second. Then, retraction of the compression head took place for 80%, also with a strain rate of 10% per second. In the unconfined compression data, MEXE02 and Labview software were used. These softwares are described in protocol 13.3.3 "General: software compression/tensile test". The step-by-step process of the unconfined compression, preparing the clots by cutting them into different heights, and testing them in the machine, is described in protocol 13.3.4 "Unconfined compression." Preliminary experiments showed that 40%H clots with a height of 3 mm start floating during the compression experiment. In the final experiments with these specific samples, an additional thin layer of double-sided (waterproof) tape with fine grid sandpaper or superglue was added to prevent the floating of these samples. The tensile test protocol for clots is also added to the protocols in this study, but it is used in an adapted way to measure the $F_{friction}$ in syringes, (protocol 13.3.5 "Tensile test"). The data is recorded in Labview and converted to a text file as the force used over time (function 11.4.3 "Matlab text output compression text"). A text output is preferred over an excel output because less resolution is lost in this format.

5.2.6 Mechanical data analysis

The data was analyzed with the help of Matlab codes 11.1 "Matlab excel output compression test" and 11.2 "Matlab text output compression test". The nominal axial stress was calculated by formula 12[17] with the compressive axial stress (σ), the force exerted by the load cell (F), and the surface of the undeformed sample A_0:

$$\sigma = F/A_0 \tag{12}$$

The undeformed samples were imaged before the compression experiment. The A_0 was calculated by processing these images with the help of Fiji (protocol 13.5.1.2 "Image processing"). As for the hysteresis loops, one needs the axial compressive strain or ε [%] which can be calculated by using formula 13. The height before application of load (h_0) was known because the clots were cut in a certain height. The deformed height during the application of load (h) was known because the compression % was set as a certain height in the compression testing machine:

$$\varepsilon = (h_0 - h)/h_0 \tag{13}$$

17

The force-time output from Labview was converted to stress-strain data, stress-strain graphs were made. Force peaks occurred at maximum strain (80%), and minimal force occured at minimum strain (0%). Timepoints where minimal and maximal force occurred were identified as these specific strains, and in between strains with corresponding timepoints were identified as well. The known strain was plotted as an independent variable on the horizontal axis, replacing the variable time. The corresponding stress was then plotted as the dependant variable on the vertical axis, replacing force. The stress plotted as a function of strain (Matlab function 11.4.1 "Hysteresis"), was sometimes smoothed with the help of 11.4.2 "Baseline fit." The tangent modulus was also calculated, which is defined as the slope of a line tangent to the stress-strain curve at a point of interest.[47] In this study, the tangent stiffness is calculated at ε= 10 to 80% with steps of 10%, which makes it comparable to other studies taking these intervals.[15,17] The ε= 80% data was not used in tangent stiffness calculations, as preliminary experiments show unusable data at that instant.

5.3 Fibrin clot formation under flow

5.3.1 Testing workflow

The general steps for the fibrin clots formed under flow are shown in figure 5, it consists of three main steps: the formation of the fibrin clots under flow, mechanical testing the clots by indenting them, and doing confocal imaging.

Figure 5. The general steps for the clots formed under flow.
The main steps were the formation of the clots under flow, testing the clots by indenting them, and doing confocal imaging.

A total of four pooled plasma groups was used. These groups were marked as experiments one till four. Table 2 shows the number of samples made per experiment.

Table 2. Number of fibrin clots formed under different shears.

	Shear= 0 /s	Shear=50 /s	Shear= 150 /s	Shear= 300 /s
Experiment	n	n	n	n
1	0	3	3	3
2	3	1	3	3
3	3	3	3	3
4	3	3	3	3

Topics: 1. heights of fibrin clots 2. fiber angles, 3. fiber densities, 4. indentation.

Platelet poor plasma from different donors is pooled per experiment, and is labeled as experiment one till four. All fibrin clots were used in all topics. The experiments per shear had a corresponding sample size as depicted above.
n: amount of samples.

5.3.2 Flow chamber design

A flow set-up was made. The different components and features are explained. A stiff flow system and displacement pumps where displacement rates can be entered were needed to reach the desired flow rates. The right displacement rate in the system is reached when the system itself is stiff enough, and the material which has to be displaced is incompressible. If parts of the system change in volume during flow because of low stiffness, it is not possible anymore that the displacement indicated in the pump is reached. The material inside the flow system used in this experiment was a fluid, which can be assumed to be incompressible. All gasses (air bubbles) should be removed from the fluid to ensure it is incompressible. Furthermore, it should be assured that the system is not leaking, as air can be introduced this way. When calculating shears, remember that these calculations are only valid in the central area of the width of the flow channel depicted in figure 6. All shear stress calculations are valid in the large (in the example orange) area of width only. Side effects near the wall were ignored. Observations should be done at least in a distance comparable to the channel height.[48]

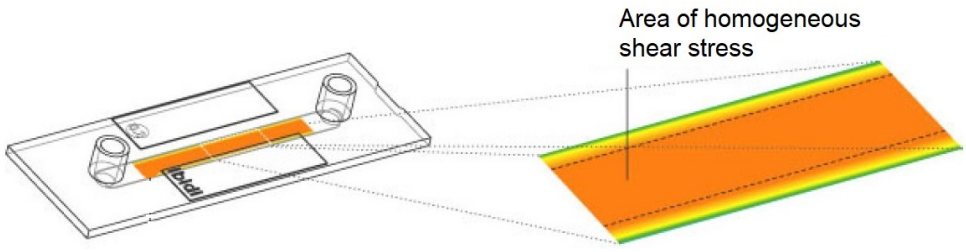

Figure 6. Area valid for shear calculations in flow experiment.
All shear stress calculations are valid in the large area of width only (in orange). Side effects near the wall were ignored. Observations should be done at least in a distance comparable to the channel height (from the dotted line). A homogeneous flow profile is expected in the center region of the channel. No calculations should be done near the wall (green and yellow area), because of the lack of homogeneous flow.[48]

The desired fluid displacement per time unity can be easily entered in the syringe pump machines. Syringe pumps were used to gain precise flow rates, which are precise to the level of 1 μL per 1 minute.[49] A standard infuse pump (Harvard apparatus, America) was used. It is set at the desired flow rate, pumping a total volume of 500 μL per microscopic slide. Fluid was pumped through PVC tubings (B.Braun, Netherlands) which can be used with blood products.[50] A stiff tube is put around this PVC tubing, which can be seen in figure 7. These tubings were equipped with three-way tabs used in infusion lines. The effect of stiffer tubings was compared to soft tubings in a separate two pump experiment with mixing and can be seen in appendix 12.5.1 "Mixing part."

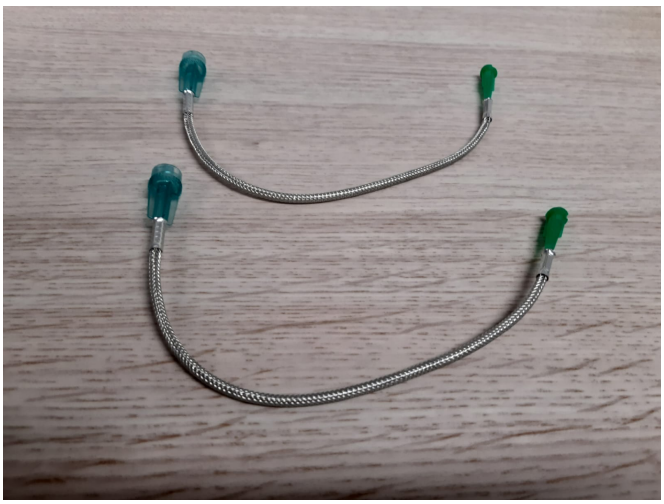

Figure 7. Tubings used in flow experiment.
The used tubing is made of PVC, which can be used with blood products blood. This tubing is surrounded by a stiff material.

A flow chamber where the clotting occurs was designed to make fibrin clots under controlled flow. This flow chamber can be seen in figure 8. It was made from PVC. A microscope glass (631-0108, Slaughter) was fitted inside,[51] a silicon ring surrounds the flow channel to prevent leakage. The flow channel was the part where the actual blood flow takes place inside the flow chamber. The flow chamber design was a redesign of the flow chamber of van Kruchten et al. (2012).[52] It has the same dimensions for the actual flow channel part, with a height of 0.05 mm, width of 5 mm, and length of 30 mm. The actual flow channel part is indicated as the red rectangular part in figure 9. The flow chamber also has 20° angles for the inlet and outlet connection to the tubing. To mimic more physiological flow patterns, less sharp angles were introduced: the outer apart of the inlet and outlet are also in a 20° angle, as opposed to the flow chamber of van Kruchten et al. (2012), whereas this is tangent to the sidewall of the chamber, creating a sharp angle.

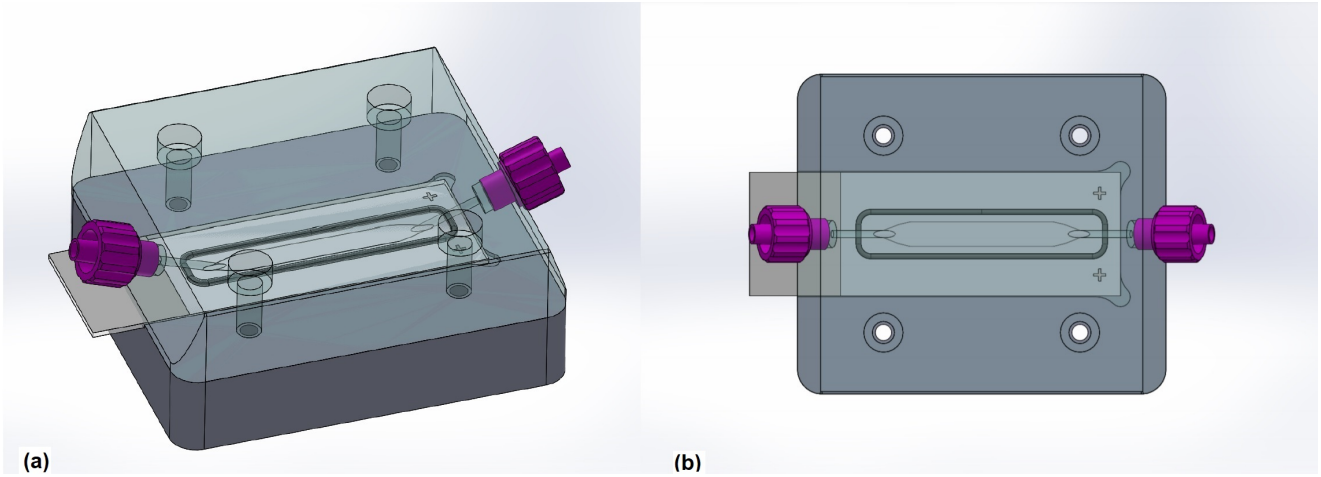

Figure 8. Flow chamber design in AutoCAD.
(a) Angled view, and **(b)** topview.

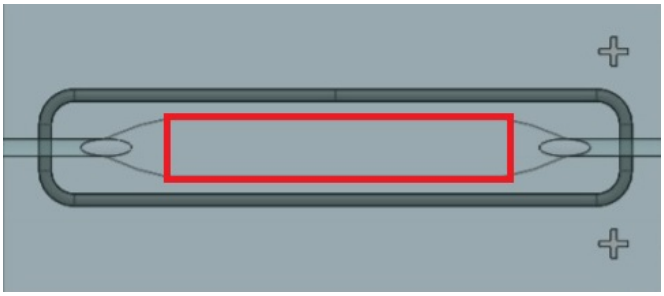

Figure 9. Flow channel part where the flow takes place marked in red.
The dimensions of this red square are: height 0.05 mm, width 5 mm, length 30 mm.

The flow channel and three dots named A, B, and C were marked on the microscope glasses. The marked dots were the regions of interest during testing. The flow channel was also marked by a thin yellow tape functioning as a mold for the liquid Tissue factor (TF). The TF crystallizes on the microscopic glass and is used as a coating during the flow experiments. An example of the microscopic glass is shown in figure 10.

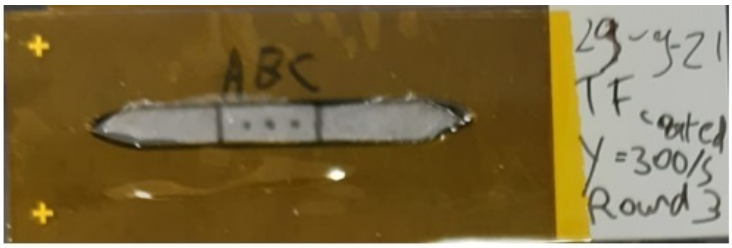

Figure 10. Coated TF.
The flow channel part where the flow takes place is coated with crystallized TF. The yellow tape functioned as a mold to keep the fluid in place. Point A, B, and C are marked as regions of interest.

The TF pathway or extrinsic clotting cascade,[23] causes both arterial and venous thrombosis.[53] The plasma portion of blood contains soluble proteins (enzymes) that act together, culminating in the formation of fibrin. TF powder (Dade® Innovin®, Germany)[54] was mixed with buffer mixture and was frozen at -80 °C. TF was applied at the microscopic glass just before the experiment, on the part where the flow channel was placed (figure 9). When the fluids evaporated, a solid coating was left on the glass, which can be seen in figure 10. An extensive description of this procedure in protocol 13.4.2 "Preparing materials needed for fibrin clot formation under flow". Pooled fresh-frozen PPP was used, fibrinogen with Alexa-Fluor 488 (0.025 mg/ml) was added in a ratio of 59.28:1 to visualize fibrin strands with the confocal microscope. Citrate from the blood collection tubes binds calcium and prevents clotting. Clotting is initiated by adding back an excess of calcium ions at a later moment. Calcium was added back to the PPP solution right before starting the experiments, with PPP+fibrinogen being 97% and calcium 3% of the solution. HEPES buffer was used to pre-flush the flow system, wash excess fibrin away, and could be used in a parallel flow experiment with thrombin.

5.3.3 Shear experiments

The desired fluid displacement per time unity was inserted in the pump. The pump then produced the different flow rates, which will give different shear rates. The shear rates tested are: 0 /s (control), 50 /s (venous rate), 150 /s (venous rate), and 300 /s (arterial rate). These different shear rates with corresponding wall shear stresses can be seen in table 3. Mind that fluid viscosity is independent of the shear rate.

Table 3. Venous and arterial mean wall shear rates, and wall shear stress.[55]

Vessel type	Shear rate [/s]	Shear stress [N/m]
Veins	15-200	0.1-0.9
Large arteries	300-800	1.4-3.6
Arterioles	450-1600	2.0-7.2
Stenotic vessels	800-10000	3.6-45
The shear rate in veins and large arteries were mimicked in our study. Tested shear rates are: 0 /s, 50 /s, 150 /s, and 300 /s.		

The required shear rate was achieved with certain flow rates related to the dimension of the flow chamber. Assuming a parabolic flow pattern, in a parallel-plate chamber the flow rate (Q) in [ml/min] is described by the formula 14,[56] with shear rate (γ) in [/s], height of the flow chamber (h), and width of the flow chamber (w) in [mm]:

$$Q = \frac{(\gamma * h^2)}{100 * w} \tag{14}$$

The TF coated slide was placed in the flow chamber. The top of the chamber was closed tight on the bottom, with the microscopic glass in between. The pump and flow chamber was attached to the tubings with three-way tabs. The attached syringes were filled with recalcified PPP labeled with AF488-fibrinogen and HEPES buffer. The HEPES buffer was used to remove air in the total set-up. At the start, the PPP mixture was pushed until the flow chamber, which can be seen as a yellow coloring (excess PPP mixture in the flow chamber was then flushed away with HEPES buffer). Then the pump was started and pushed 500 μL thorough the flow chamber with the desired flow rate. When the pump was done, the microscopic glass was removed and directly put in a petri-dish filled with HEPES buffer. After gently washing away the excess fibrin by immersing the slide in HEPES buffer, it was put in a clean petri-dish also filled with HEPES buffer (preventing dehydration). At this point, the sample was ready for micro-indentation. The washing step was done in clots that were formed under shear because it was shown in preliminary experiments that loosely attached fibrin fibers disturbed micro-indentation. The HEPES buffer was also used to wash excess fibrin away from the flow chamber after removing the newly formed fibrin clot. The system should be cleaned with buffer before starting a new round of fibrin clot formation. The total set-up is shown in figure 11, and a step-by-step protocol can be found in 13.4.3 "Final flow experiment protocol (1 pump)". Furthermore, a static control sample was made. The static sample was made by applying PPP mixture on a TF coated slide with the yellow tape functioning as a mould. The sample was then placed on a heating plate for 40 minutes at 37 °C. Afterwards, the sample was also immersed in HEPES buffer. It was shown that shear= 0 /s control samples didn't need an extra washing step for removing excess fibrin strands.

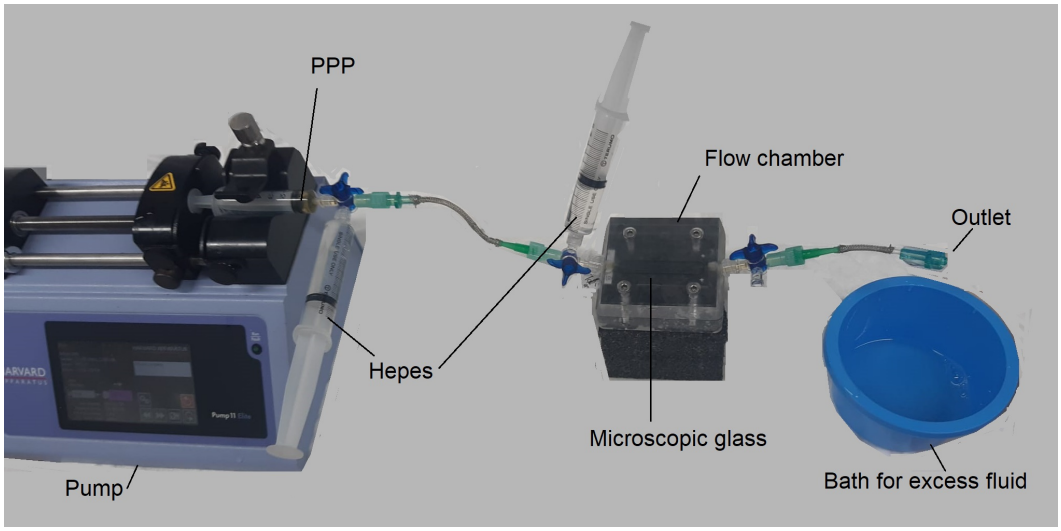

Figure 11. The flow experiment set-up.
The syringe with the PPP mixture was placed in the displacement pump. The syringe was connected to three-way tabs, tubes, syringes with HEPES, and the flow chamber. The microscopic glass was placed inside the flow chamber. A bath for the excess fluid was placed at the outlet tube.

5.3.4 *Micro indentation*

A commercial nano-indenter (Piuma, Optics 11 live, Amsterdam) was used to perform the micro-indentation experiments. Piuma tips were used with a cantilever stiffness of 0.42-0.48 N/m with a tip radius of 100-105 μm. A step-by-step protocol of the use of the Piuma is described in 13.4.4 "Piuma." A standardized method of performing micro indentations in clots does not exist. There is seen variation in micro indentation methods in the literature.[21,57] The Piuma set-up is depicted in figure 12.

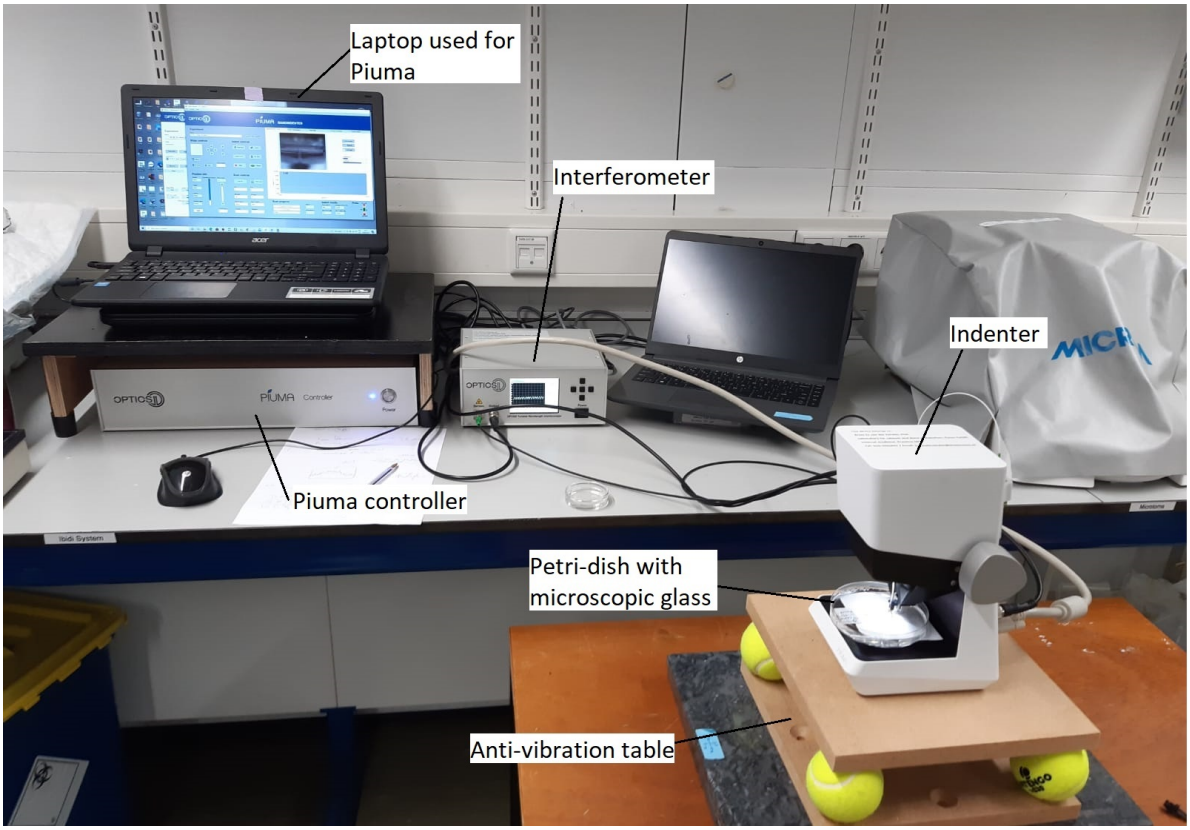

Figure 12. The total Piuma set-up.
It consists of a laptop with Optics 11 software, the Piuma controller, the interferometer, the Piuma indenter, wires connecting all the parts, an anti-vibration table, and a petri-dish with a microscopic glass.

A single or double ferrule tube fitting is possible. For our Piuma device, a single ferrule tube fitting is more suitable because of the camera's range of motion. For this study, a much bigger tip than the average pore size was chosen to get the local bulk properties of the fibrin clots.[58] A cantilever stiffness is chosen, which can sense a sample stiffness between $\pm$ 200 Pa to 300 kPa, based on PPP TA stiffness found in the literature.[59–62] A final indent depth of at least 1 μm should be achieved in the fibrin clots, based on the heights of clots found in preliminary experiments (table 17 in the appendix). After testing, bigger indentation depths than allowed were corrected in the Piuma data viewer software. There is a 5-10% maximal indent depth (in respect to the clot height) for the clots, but sometimes a bigger indent depth is used during the measurements to prevent missing information (as exact clot heights are unknown during the Piuma indentations). Furthermore, the maximum indentation depth shouldn't be more than 16% of the tip radius,[63] with the tip radius of 100-105 μm this is an easy condition to achieve. A final indentation depth of 6 μm is chosen with a speed 1 μm/s, and a holding period of 20 s in between, which can be seen in the protocol (13.4.4) "Piuma." It was decided to do three indents per region of interest (A, B, C), giving nine indents per sample. In preliminary experiments, it was shown that the effect of viscoelasticity was limited in the fibrin clots (appendix 12.3.1 "Piuma outcomes viscoelasticity"). Therefore, data could be collected by indenting the same spot within the fibrin clot multiple times, which was done in some clots. When there would be a big viscoelastic effect, this should be taken into account when indenting at the same spots. In that case, the maximum indention depth (h_{max}) plays a big role. The h_{max} can also be described by formula 15 with the final contact depth (h_f), and elastic recovery depth (h_e):

$$h_{max} = h_r + h_e \tag{15}$$

A big enough step size is needed in the case of a viscoelastic effect. Then the radius of the contact area (a) of the indent should be known, which can be calculated with formula 16, with the h_{max}, and the radius of the indentor/probe head (R_i):

$$a = sqrt(h_{max} R_i) \tag{16}$$

The step size (formula 17) should be at least two times the contact radius a, to avoid oversampling:

$$stepsize \geq 2 * a \tag{17}$$

Other used terms are the h_c, which is the contact depth between indentor and material at the beginning of the unloading, and the maximum load (P_{max}). It is all illustrated in figure 13, together with the loading-unloading curve. For example, a h_{max} of 2 μm in calculations, with a radius of 100 μm probe, leads to a contact area of 14.14 μm. So the stepsize should be a=28.28 μm.

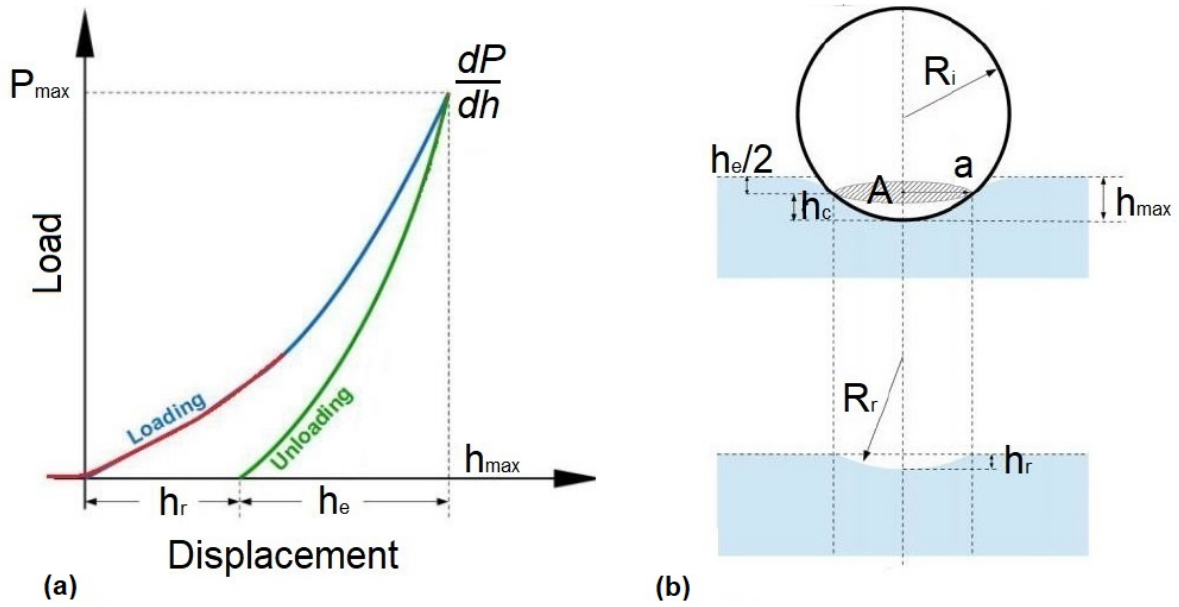

Figure 13. The theory behind the indentation.
(a) The loading (blue line), unloading (green line) curve is depicted, a fitting is done (red line) to calculate the effective Young's modulus. **(b)** The indentation head is depicted as a sphere with a radius R_i, the head indents an elastic half-space to depth h_{max} and thus creates a contact area of radius a=$\sqrt{R_i h_{max}}$ (right). The elastic recovery depth (h_e), the contact depth between indentor and material at the beginning of the unloading (h_c), and final contact depth (h_f) are also depicted.[64]

5.3.5 Mechanical data analysis

Piuma data viewer version 2.4 was used. The Piuma software enables to directly calculate the 'Bulk Young's Modulus' (E). The Hertzian contact model is used to calculate the effective Young's modulus. Hertz model assumes material to be purely linear elastic. The fit of the loading curve is calculated with the equation 18, with force (F), effective Young's modulus (E_{eff}), the radius of the spherical indenter tip (R), and the indentation-depth (h):

$$F = \frac{4}{3} E_{eff} R^{\frac{1}{2}} h^{\frac{3}{2}} \tag{18}$$

The Poisson ratio was not considered in this fitting.[65] The square of the correlation (R^2) value and Root Mean Square Error (RMSE) were calculated with the software. R^2 tells us how well the predictor variables can explain the variation in the response variable, expressed as a value between 0 to 1. The higher the R^2 value, the better a model fits a dataset. RMSE tells us the typical distance between the predicted value made by the regression model and the actual value. The lower the RMSE, the better a model fits a dataset.[66] A rule of thumb is to use data with an $R^2 > 0.6$, and RMSE < 10% of the range

of target property value.[67] All results which fail to meet this rule of thumb were not used in data analysis. Therefore, three indents from shear=0/s fibrin clots from experiment three, were not considered in the data analysis. So 97.68% of all data was used for analysis, having an average R^2 of 0.96, and an average RMSE of 3.90%.

5.3.6 Image acquisition

A confocal microscope (Leica SP5) was used, the set-up can be seen in figure 14. Confocal imaging is used to visualize the fibrin structures, a brief protocol is given in appendix 13.4.6 "Confocal - Leica 604 - Quick guide".

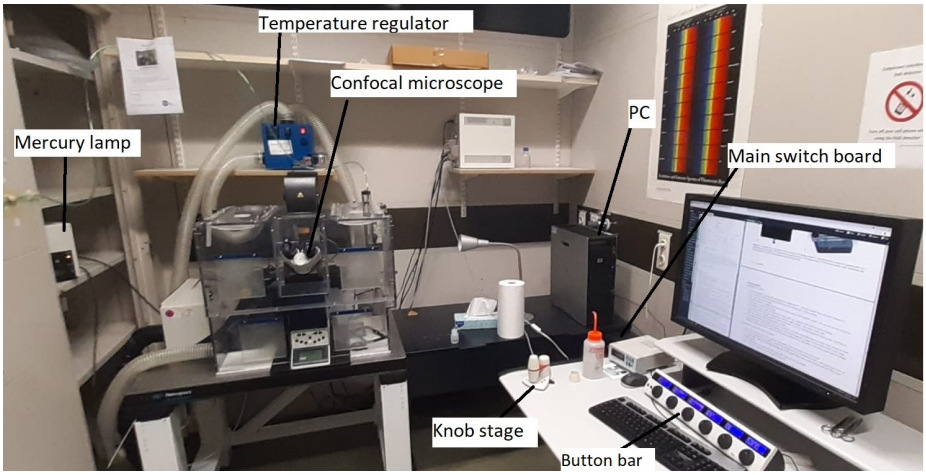

Figure 14. The confocal microscope set-up.
It consisted of a PC, knob stage, button bar, switch board (not really visible in this figure), mercury lamp, and temperature regulator.

The following settings are used to achieve good results in the Leica SP5 confocal microscope (table 4):

Table 4. Confocal settings for imaging fibrin clots.

Tab	Component	Settings
Acquire	Lens	40x oil immersed
	Bits	8
	Format	2048x2048
	Zoom in	1.9x
	Gain in shear clot	1250 V
	Gain in static clot	721 V
	Pinhole	65.35 μm (=1 airy unit)
	Z-step size	0.15-0.5 (used 0.17) μm
	Line averaging	2
	x and y	both 203.95 μm
	Interval imaging	200 Hz
Acquisition	Fluorescent laser	488 nm
	488 laser in shear clot	20%
	488 laser in static clot	8%

The setting used in this study for the confocal microscope are depicted. The lasers can be activated in the configuration tab of the software, then the settings in the acquire and acquistion tab were done.

These settings give a pixel size of x and y <100 μm (99.63x99.63 μm), and a certain z-size which can be bigger because it is not used for analysis (167.85 μm). The total xy scanning area will be 204 μm, the approximate diameter of a dot (A, B, or C) or region of interest on the microscopic glass is 200 μm, so this will be within these dimensions. The diameter of the area of contact with indentation is approximately 30 μm, so area can be imaged easily with the scanning area used. The resultant confocal settings are summarized in table 5.

Table 5. Confocal image size.

Specification	Size
X-y scanning area	204x204 μm
X-y pixel size	99.63x99.63 μm
Z pixel size	167.85 μm

5.3.7 Image analysis

A z-stack of images of the total clot height was made in most clots. Fiji image analysis software was used to analyze these z-stacks. The z-stack was projected to a single image using Fiji's standard deviation method.[68] Preliminary experiments showed that the standard deviation method gave the clearest projections. Projections of the total clot height and top 2 μ were made. The height was defined as the highest height seen in the clots. This clot height should not be underestimated in the case of indentation, as there is a maximum indentation depth. Two methods were used to estimate the clot height. Firstly, the total number of frames in a z-stack was multiplied by the z-step. This was done in almost all cases. Secondly, when an incomplete z-stack was made, the z-height was measured in a xy-side image. This image was made by changing the confocal acquisition mode from "xyz" to "xy". This latter method was used to estimate the z-height in some clots, as these z-stacks were limited. Although these clots were partially imaged, their projections were marked as the "total" clot height during data analysis. This is the case for the shear= 0/s clots from experiment two, in which a z-stack of $\pm$ half the height is made to reduce imaging time. It was hypothesized that only a part of the z-stack was needed in these shear= 0/s clots to assess the fibrin because fiber alignment would be homogeneously random thorough the stack, as these clots were formed without flow. Furthermore, point C in shear=300/s clots from experiments two and four were not imaged totally because of lack of time, although these z-stacks are only slightly incomplete. Next, the fiber orientation analysis (FOA) tool was used. This tool can detect fibers by analyzing the local tissue structure on multiple scales based on the local Hessian matrix.[69] The orientation in the FOA tool is counterclockwise and starts at 0 at the east, which is illustrated in figure 15. It computes a histogram or probability density function (PDF), indicating the number of structures in a given direction, indicated as yellow vectors. An example of a result is shown in figure 16. The anisotropic fraction, dominant direction, and SD can be calculated from these results (figure 16 **(b)**. The anisotropic fraction is defined as:

$$Anisotropic\ fraction = 1 - Isotropic\ fraction \tag{19}$$

The (an)isotropy of the fiber directions is determined first by the baseline and anisotropic fraction, and second by the width (or SD) of the peak, independent of the baseline.[70] A high anisotropic fraction is associated with anisotropy, aswell as a low SD. Images with completely isotropic content are expected to give a flat histogram, whereas images in which there is a preferred orientation are expected to give a histogram with a peak at that orientation. Isotropy versus anisotropy is illustrated in figure 17. Preliminary experiments were done to find the best method for measuring fibrin fibers density. Self-developed Macro's in Fiji were suitable for finding the density. These Macro's are a combination of smoothing, thresholding, despeckling, making the figure binary and selecting the fibers to measure the area covered by fibrin.

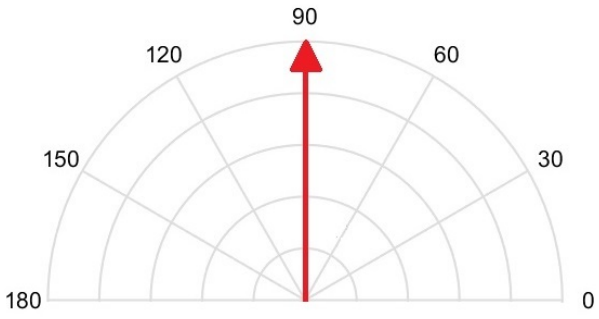

Figure 15. FOA orientation.
The orientation in the FOA tool, the red arrow points towards the direction of flow.

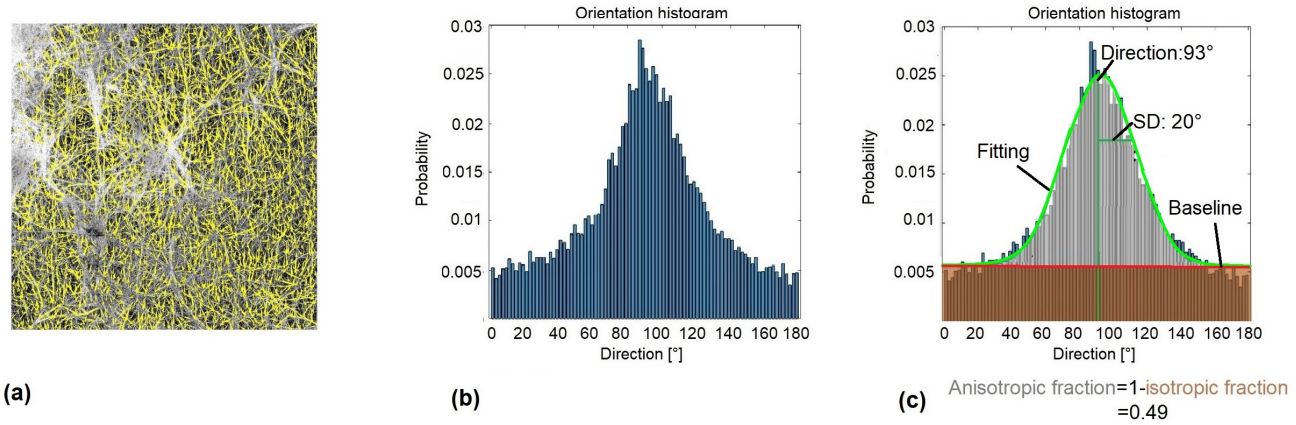

Figure 16. Histogram fiber orientation.
An example of fiber orientations is shown, with **(a)** an overlay picture with yellow vectors indicating the fiber directions. **(b)** The probability that a certain fiber direction occurs as a normalized histogram. **(c)** The calculated histogram characteristics. The green line in this latter figure indicates the fitting curve. It is also used for determining the anisotropic fraction. The red line indicates the baseline used for determining the isotropic fraction.

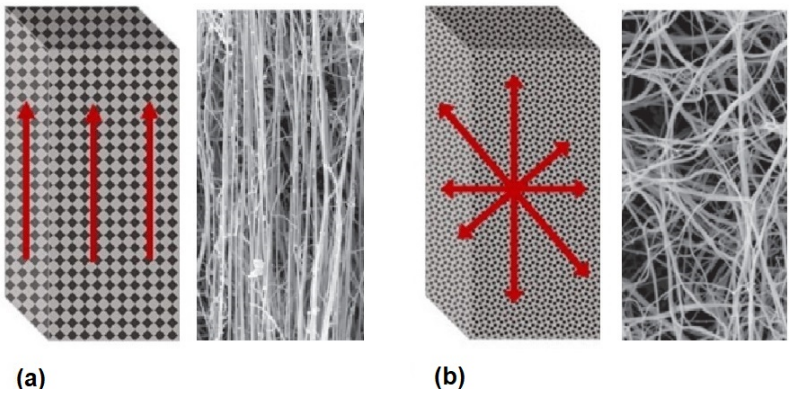

Figure 17. Anisotropy versus isotropy.
The red arrows indicate the fiber directions. In **(a)** an anisotropic morphology is shown, and in **(b)** an isotropic morphology.[71,72]

5.4 Statistical analysis

The Shapiro-Wilk test is performed in all continuous variables to test if the data follows a normal distribution. The p-values shown following a Kruskal-Walis test, are Bonferroni-corrected p-values.

5.4.1 Thrombus analogs formed under static conditions

An independent samples test is used to analyze if there are differences between the tangent moduli from WB clots matured with and without applying pressure. A Friedman's two-way analysis was done per strain rate, to analyze differences in tangent moduli between the different strain rates. The effect of different heights in WB clots on tangent moduli were assessed with an independent-samples Kruskal-Wallis test in strain=10%, 50% and 60% samples, and a one-way ANOVA in the other strains with a Bonferroni posthoc test. A related-samples Friedman's two-way analysis was performed in the samples with different heights, to analyze differences in tangent moduli between the different strain rates. The effect of different hematocrit levels in tangent moduli are assessed by a Kruskal-Wallis test. A Kruskal-Wallis test was also used to compare the samples in different donors with the same H%. A related-samples Friedman's two-way analysis was performed in the samples with different hematocrit levels, to analyze differences in tangent moduli between the different strain rates.

5.4.2 Fibrin clot formation under flow

A one-way ANOVA test, Gabriel posthoc test, and Hochberg's GT2 posthoc test were used to compare fibrin clot heights. In order to compare the fiber directions in the fibrin clots, there is performed a One-way ANOVA in the top 2 μm z-projections and a Kruskal-Wallis test in the total z-projections. A

Kruskal-Wallis test is performed to compare fiber densities from all the individual fibrin clots formed at different shears. To compare fiber densities from fibrin clots originating from different experiments, a one-way ANOVA test is performed in all clots, except for the total z-projections of shear=50/s clots whereas a Kruskal-Wallis test is done. In shear=0/s, and shear=150/s total clot projections, a Bonferroni post-hoc test was done. In shear=300/s total clot projections, a Games-Howel post-hoc test was done. 0.17

6 Results

6.1 Thrombus analogs formed under static conditions

6.1.1 Clot formation under pressure

The effect of applying pressure during clot maturation was investigated. Five WB clots were matured while applying pressure, and four were matured without applying pressure. Blood donated by one 29-year-old female donor was used to make all of the nine samples. Unconfined compression testing was performed in all the samples. Each sample was compressed for one time, which resulted in one stress-strain loading-unloading curve for each sample. In figure 18 **(a)** stress-strain loading-unloading cycles for each sample are shown, so each loading-unloading cycle represents one sample. In **(b)** the resulting average tangent moduli from all of the samples at the different strain rates are shown (mean $\pm$ SD). No significant difference between clots for the different strain rates was found for the tangent moduli, which is depicted in table 6. Furthermore, it was seen that samples at strain=10-20% had a significantly ($p<0.04$) lower tangent modulus than at strain=60-70%. No histology was done, so possible significant histological changes are not detected. A big variation in stiffness found with unconfined compression was seen within sample groups.

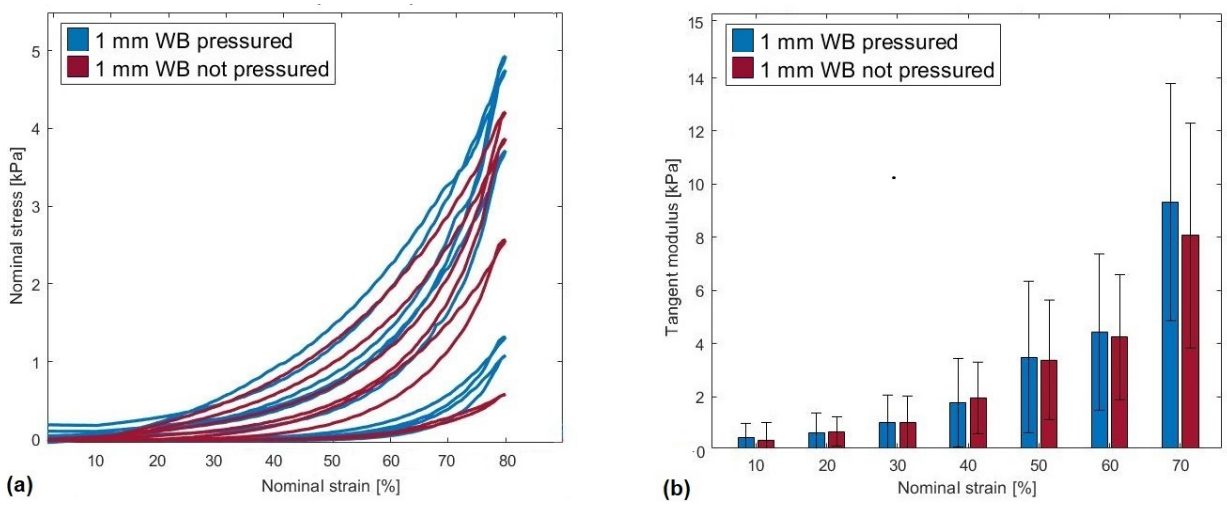

Figure 18. Comparison of WB clots matured with applying pressure versus WB clots without using pressure. Unconfined compression results are shown, with **(a)** each loading-unloading curve representing one sample, and **(b)** tangent moduli (mean $\pm$ SD), with errors bars representing the SDs.

Table 6. Tangent moduli (mean $\pm$ SD) of WB clots matured with applying pressure versus without using pressure.

	Clot pressured	**Clot not pressured**	**Clots pressured vs not pressured**	
ε **[%]**	Tangent stiffness [kPa]		One sided p-value	Two sided p-value
10	0.44 ± 0.68	0.45 ± 0.56	0.49	0.97
20	0.69 ± 0.56	0.62 ± 0.76	0.45	0.90
30	1.0 ± 0.99	1.0 ± 1.1	0.49	0.99
40	2.0 ± 1.4	1.8 ± 1.7	0.44	0.87
50	3.4 ± 2.3	3.5 ± 2.9	0.48	0.95
60	4.2 ± 2.4	4.4 ± 2.9	0.46	0.92
70	8.1 ± 4.2	9.3 ± 4.5	0.34	0.68

A statistical comparison was performed on clot types with similar pre-coagulated composition and/or similar percentage plasma/hematocrit in the coagulated sample. A p-value 0.05 was considered statistically significant (marked with *). ε: strain.

6.1.2 *Different heights in clots*

The results from WB clots made with different heights (1 mm versus 2 mm versus 5 mm) from clots made from the blood of one 29-year-old female donor are shown. In figure 19 **(a)** the stress-strain curve from one loading-unloading cycle and **(b)** the corresponding tangent stiffness are shown. Results are also shown in table 7. Significant differences could be seen when applying relatively high strains (>60% strain). It is shown that higher heights from clots at high strains resulted in more stiffness. This was seen in all clots at strain=70%. At strain=60%, this was shown for 1 mm clots versus the other clots. In the zoom-in box in **(b)** at strain=10% and 20%, it seems like clots with smaller heights had a relatively high tangent modulus, but these were not significantly higher than in other clots at this strain. Furthermore, it was seen that samples at strain=10-20% had a significantly ($p<0.04$) lower tangent modulus than at strain=60-70%.

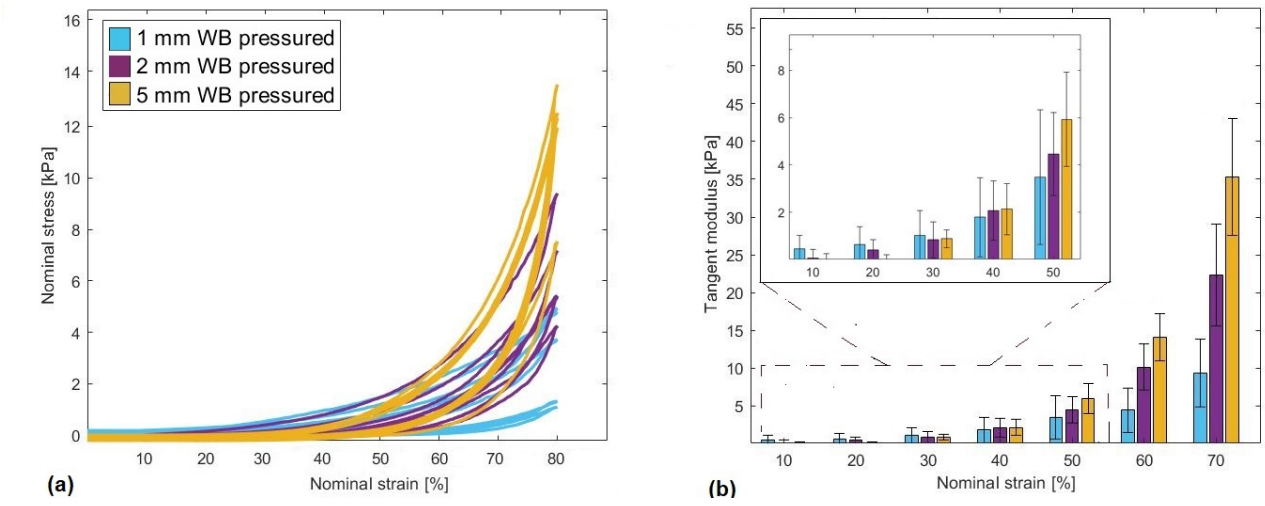

Figure 19. Comparison of WB clots with a height of 1 mm, 2 mm, or 5 mm.
Unconfined compression results are shown, with **(a)** each loading-unloading curve representing one sample, and **(b)** tangent moduli (mean ± SD), with errors bars representing the SDs.

Table 7. Comparison tangent moduli (mean ± SD) from WB clots with a height of 1mm, 2mm, or 5mm.

	Clot 1 mm	Clot 2 mm	Clot 5 mm	Clots 1 mm vs 2 mm	Clots 1 mm vs 5 mm	Clots 2 mm vs 5 mm
ε [%]	Tangent stiffness [kPa]			P-value		
10	0.45 ± 0.56	0.06 ± 0.36	0.03 ± 0.21	0.23	0.23	0.23
20	0.62 ± 0.76	0.40 ± 0.45	0.02 ± 0.18	0.21	0.21	0.21
30	1.0 ± 1.0	0.83 ± 0.77	0.87 ± 0.38	0.93	0.93	0.93
40	1.8 ± 1.7	2.1 ± 1.3	2.1 ± 1.1	0.91	0.91	0.91
50	3.5 ± 2.9	4.5 ± 1.8	5.9 ± 2.0	0.26	0.26	0.26
60	4.4±2.9	10±3.1	14±3.1	0.04*	<0.001*	0.19
70	9.3±4.5	22 ± 6.8	35±7.8	0.02*	<0.001*	0.02*

A statistical comparison was performed on clot types with similar pre-coagulated composition and/or similar percentage plasma/hematocrit in the coagulated sample. A p-value 0.05 was considered statistically significant (marked with *). ε: strain.

6.1.3 *Different hematocrit levels*

The tangent moduli from the clots with different hematocrit levels from two donors were investigated. Individual results from these two donors are shown in figure 20, table 8 and table 9. It can be seen that clots with 1%H have significantly higher stiffness than 99%H clots, which was shown in both donors. When the stiffness in different H% were analyzed, a decreasing stiffness is seen with 1%H, 40%H, and 99%H. No significant difference was seen between the tangent of 1%H versus 40%H clots or 40%H versus 99%H clots except for two exceptions which can be seen in the tables. It is seen that the 40%H clots from the 27 year old female showed a more profound hysteresis loop, which is very wide (figure 20 **(b)** and **(d)**). Although these 40%H clots were (not significantly) higher in stiffness than 1%H clots at strain=20 till 40%. When comparing the tangent stiffness in samples with the same H% between the different donors, no significant difference was seen between these samples ($p=1$). A significantly ($p<0.05$) higher tangent modulus was seen in all samples when comparing the same sample at strain=10% versus strain=70%.

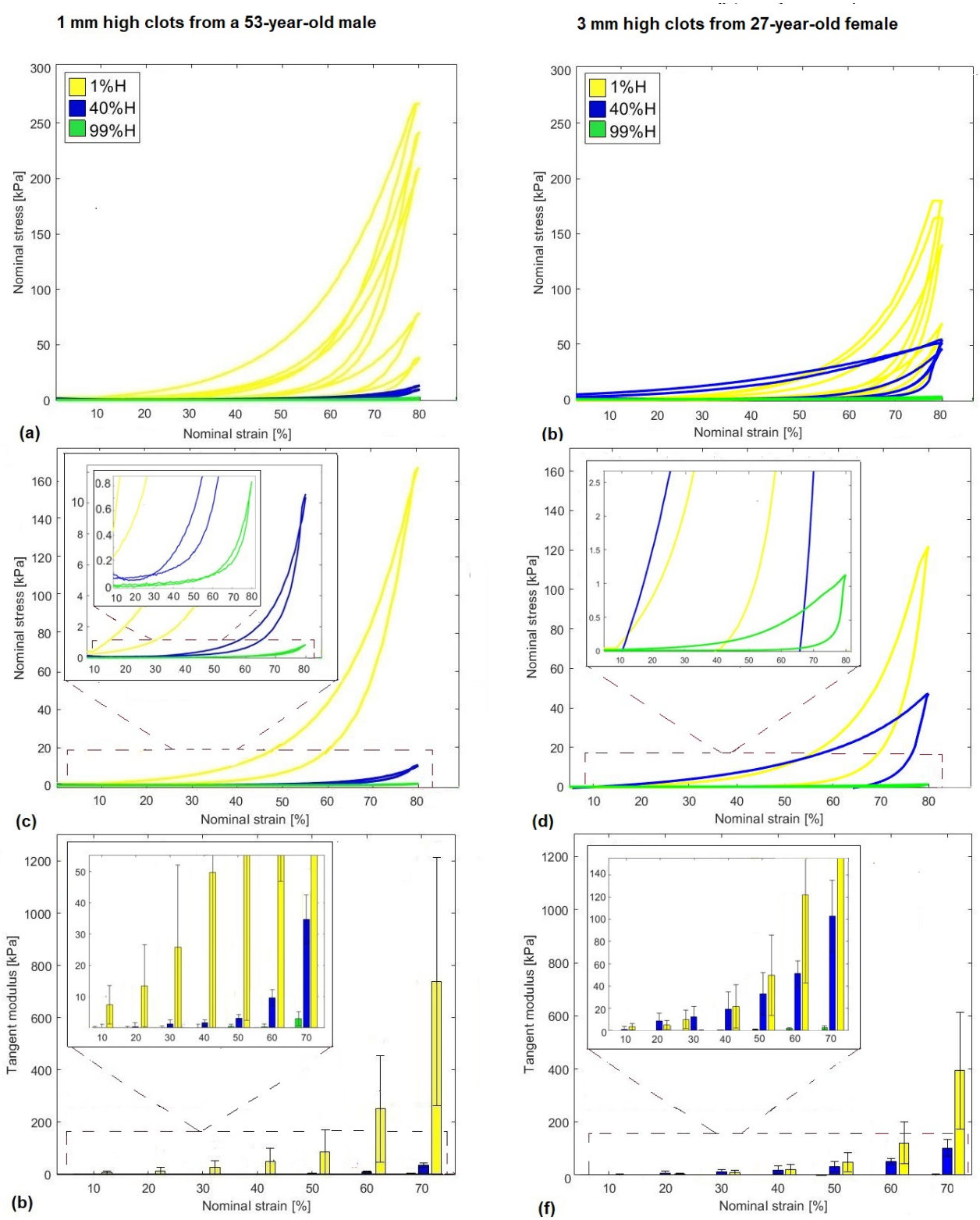

Figure 20. Comparison of clots with 1%H, 40%H, and 99%H.
Unconfined compression results are shown, with each loading-unloading curve representing one sample in **(a)** 1 mm high clots from a 53-year-old male, and **(b)** 3 mm high clots from a 27-year-old female. These stress-strain curves from the different samples were averaged as one curve per H%, for **(c)** 1 mm high clots from a 53-year-old male, and in **(d)** 3 mm high clots from a 27-year-old female. Furthermore, tangent moduli (mean $\pm$ SD) are shown in which errors bars represent the SDs, in in **(e)** 1 mm high clots from a 53-year-old male, and **(f)** 3 mm high clots from a 27-year-old female.

Table 8. Comparison tangent moduli (median [25th-75th percentile]) with different clot H% from clots made with the blood from one 27-year-old female donor.

	Clot 1%H	Clot 40%H	Clot 99%H	Clot 1%H vs 40%H	Clot 1 vs 99%H	Clot 40%H vs 99%H
ε [%]	Tangent stiffness [kPa]			P-value		
10	3.0 [0.40-6.0]	1.8 [-0.17-2.3]	0.14 [0.07-0.17]	0.17	0.17	0.17
20	4.9 [1.1-8.6]	12 [6.3-12]	0.10 [0.10-0.13]	1	0.05*	0.03*
30	8.9 [2.6-17]	16 [8.3-17]	0.09 [0.09-0.22]	1	0.02*	0.08
40	19 [3.9-37]	20 [12-27]	0.37 [0.14-0.39]	1	0.02*	0.08
50	49 [19-81]	41 [26-44]	0.56 [0.30-0.87]	1	0.01*	0.14
60	126 [56-181]	45 [45-55]	1.5 [0.69-1.6]	1	0.01*	0.18
70	434 [214-540]	105 [87-120]	1.5 [1.4-3.5]	0.67	0.005*	0.38

A statistical comparison was performed on clot types with different pre-coagulated composition or different percentages plasma/hematocrit. A p-value 0.05 was considered statistically significant (marked with *). H%: hematocrit%, ε: strain.

Table 9. Comparison tangent moduli (median [25th-75th percentile]) with different clot H% from clots made with the blood from one 53-year-old male donor.

	Clot 1%H	Clot 40%H	Clot 99%H	Clot 1%H vs 40%H	Clot 1 vs 99%H	Clot 40%H vs99%H
ε [%]	Tangent stiffness [kPa]			P-value		
10	6.7 [2.6-9.3]	-0.40 [-2-0.35]	0.05 [-0.29-0.11]	0.04*	0.04*	1
20	14 [2.2-15]	0.31 [-0.25-0.97]	-0.08 [-0.42-0.29]	0.11	0.02*	1
30	24 [5.0-29]	0.31 [-0.25-0.97]	0.34 [-0.46-0.47]	0.24	0.008*	1
40	48 [11-59]	1.5 [0.65-2.0]	-0.68 [-2.0-0.22]	0.30	0.006*	0.91
50	81 [26-102]	2.6 [2.5-3.5]	0.56 [0.22-0.76]	0.67	0.005*	0.38
60	269 [91-345]	8.2 [8.1-10]	0.73 [0.06-0.80]	0.5	0.003*	0.5
70	959 [355-1087]	31 [30-37]	3.1 [1.3-3.6]	0.5	0.003*	0.5

A statistical comparison was performed on clot types with different pre-coagulated composition or different percentages plasma/hematocrit. A p-value 0.05 was considered statistically significant (marked with *). H%: hematocrit%, ε: strain.

6.2 Fibrin clot formation under flow

6.2.1 Heights of fibrin clots formed under flow

Fibrin clot formation under flow resulted in different fibrin clots with different heights. The heights of the clots are depicted in figure 21 and all heights are shown in appendix 12.4.2. Interestingly, it was seen that clots show a variation in height over the surface area. An illustrative example of the variation in clot height can be seen in figure 22. Compared to the clots under shear, there only was a significant difference (P<0.001) in height compared to clots formed at shear= 0/s. It was seen that the clots formed at shear=0/s had a bigger height (26$\pm$6.2) than the other clots. The clots formed under shear had a height of 7.4$\pm$4.6 (shear=50/s), 13$\pm$7.6 (shear=150/s), and 13$\pm$5.4 (shear=300/s). The heights of clots formed under shear were limited by a flow chamber height of 0.05 mm. No significant difference in height was found between clots formed with shear. Furthermore, no significant difference (p=0.38) was found when intragroup differences were investigated.

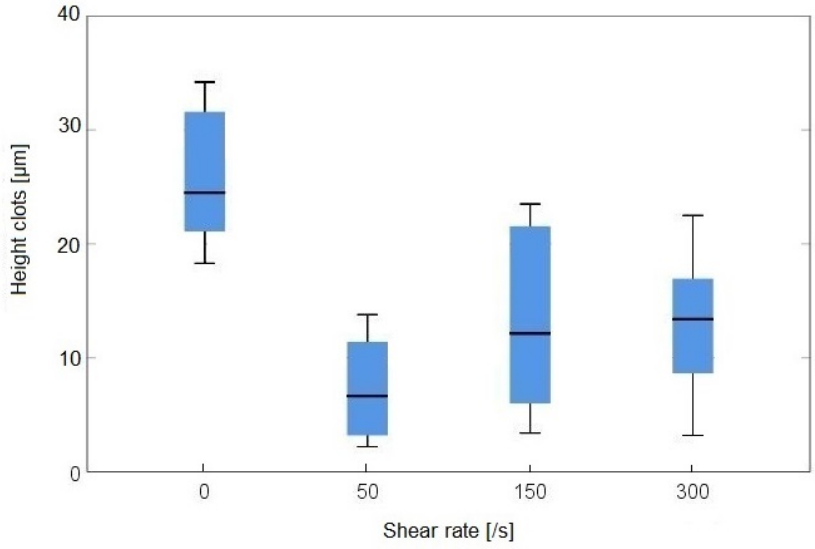

Figure 21. Heights from fibrin clot formed under flow.
The variance is pictured as error bars.

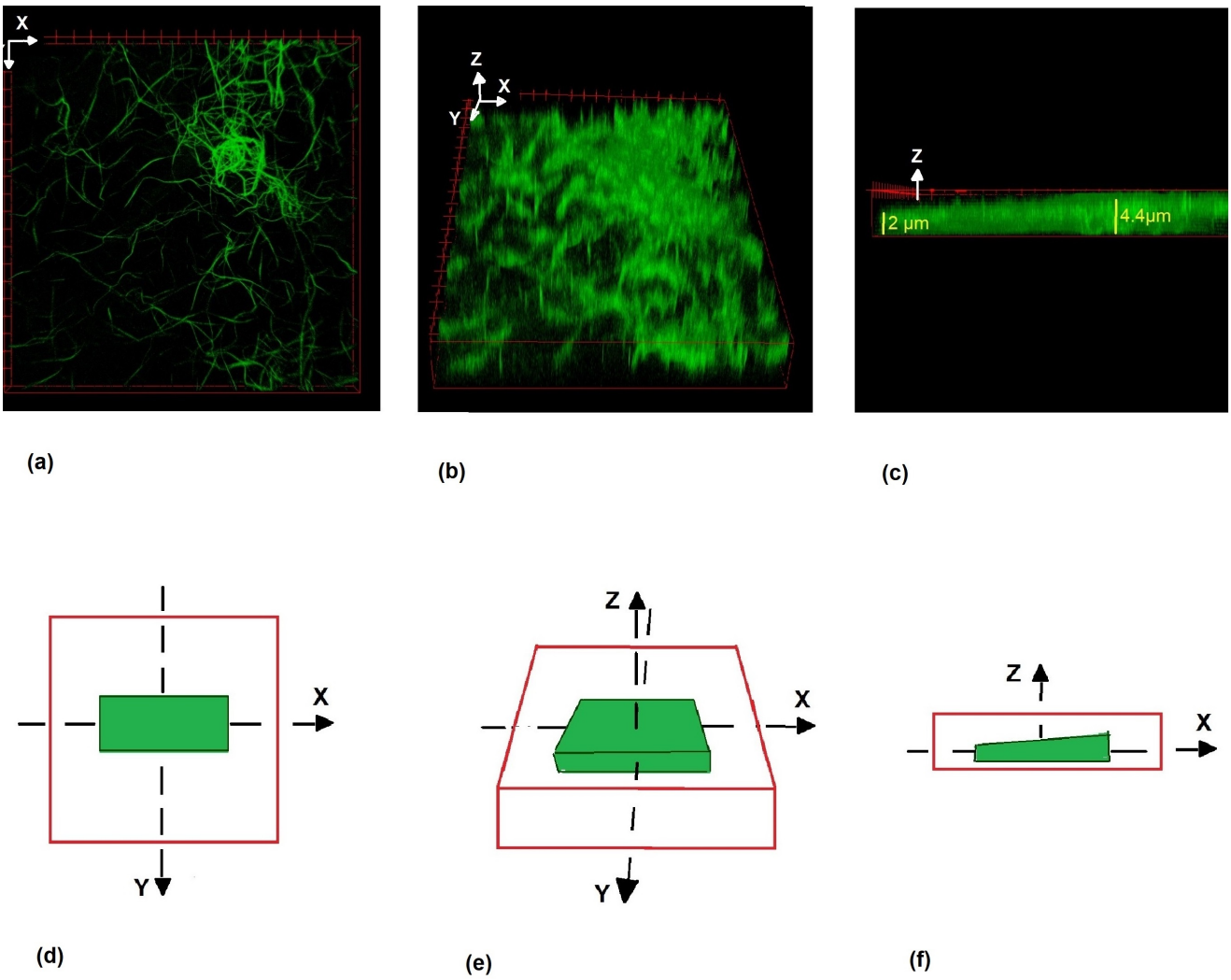

Figure 22. An illustrative example of the variance in clot height.
This is an image of point A from experiment two, formed at shear=150 /s. In **(a)** the front view is shown, **(b)** an angled view, and **(c)** side view (in which it is seen that the resolution is a lot lower). This is simplified in figures below these projections ((**d**), **(e)** and **(f)**. The variation in clot height in this latter view was pretty high. On one side, a height of $\pm$ 2μm was seen. On the other side, a height at least twice as large was seen.

6.2.2 The effect on fibrin network alignment

6.2.2.1 Illustrative example

We take the illustrative example in figure 23, starting with the total clot projection in **(a)** and **(b)**. We move from top to bottom as can be seen in figure 23 **(c)** till **(j)**, an increasingly clear peak (with a lower baseline, so lower isotropic fraction) in the fiber directions could be seen. This is shown as an increasingly high anisotropic fraction or proportion of angles which goes into the peak.[70] The PDFs in these figures showed that the main direction of fibers at the base of the clot is $\pm$ 90°, which is equal to the flow direction. The reported SD and anisotropic fraction of these PDFs vary at the different heights. There seems to be a relatively high amount of anisotropy in the total clot projection, compared to the clot projections seen in **c** till **h**. In the total clot projection an anisotropic fraction of 0.49 (the second highest value seen) and a relatively low SD of 20°was seen. When moving from the top of the clot to the bottom, an increasing anisotropic fraction is seen, but the SDs variate. In **(d)**, **(f)**, and **(h)** one can see that the baseline is at a probability of $\pm$ 0.005. In **(d)** one can see a small low peak in the middle, which gives the small SD. In **(f)** a slightly clearer Bell Shape is seen in comparison to **(d)**, so the curve wrapped around this peak which is higher and wider, gives a higher SD. In **(h)** an even clearer peak can be seen indicated by the increase in anisotropic fraction, the peak of this PDF is wide which gives a high SD. In the bottom of the clot the clearest anisotropy is suggested **(i)**. Its PDF **(j)** with 0.62 has the highest anisotropic fraction, and almost has the lowest SD with 13°.

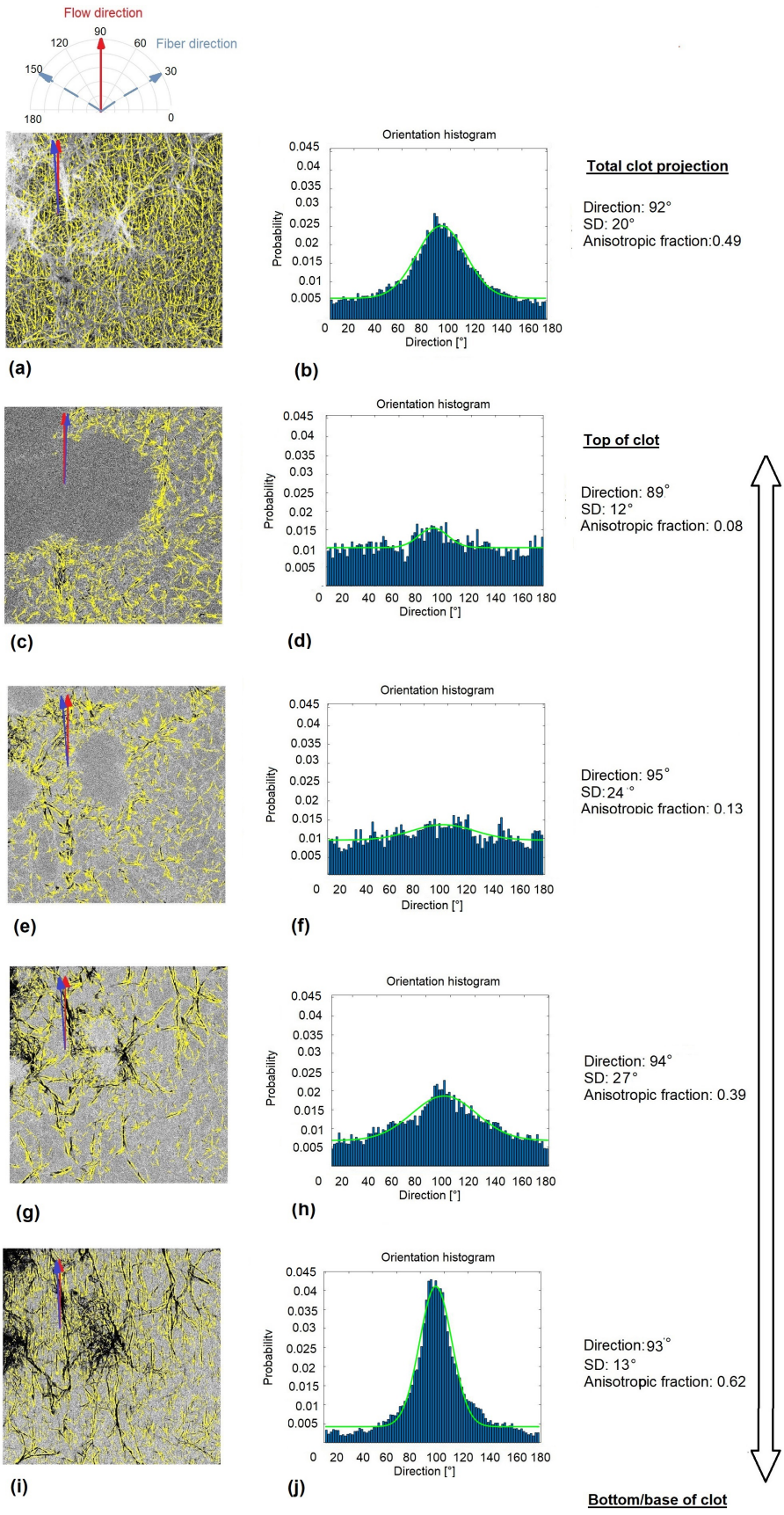

Figure 23. Example of fiber directions in different clot heights.
A total clot projection **(a)** and its histogram of the fiber orientation with a green line illustrating the fitting curve **(b)**, are shown. Furthermore, z-projections of 2 μm thickness of this clot were made at different heights. The different z-projections are depicted from top to bottom **(c, e, g, i)** in the same order as to how they were located in the clot, with the fiber directions depicted in histogram respectively **(d, f, h, j)**.

6.2.2.2 Analyzing the different fiber angles

Fiber angles from the top 2 μm layer and the total clot were analyzed. All fiber directions can be seen in appendix 12.4.3. The fiber angles were illustrated in figure 24, with the red horizontal line indicating the 90°flow direction. In table 10 with the mean fiber directions, it can be seen that shear=150/s total clot projections had a fiber direction of 94°, which was closest to the flow direction. Shear=300/s total clot projections had a combination of the highest anisotropic fraction (0.35) and a relatively low SD, which could suggest that these clots are the most anisotropic. The highest SDs were seen in the static clots (4-15°higher than other SDs). Though these static clots had relatively high anisotropic fractions, the combination with a high SD might suggest isotropy. It is seen that shear=0/s clots have a dominant direction of 69.36°for the top 2μm, this angle deviates the most from the flow direction. Though in the shear=0/s total clot projections, the fiber angle approaches the flow angle. Next, the fiber orientation occurrence probability per angle (0-180°) for the different pooled plasma groups/experiments (one to four) was illustrated in figure 25. These figures show the results as the average from the different sample points (A, B, and C), while distinguishing between the shear rates. The previous findings were visually represented in these figures. In these figures, one can see that static clots (**(a)** and **(b)** have relatively wide peaks. Relatively thinner and higher peaks can be seen in the clots formed under shear (**(c)** till **(h)**). Finally, no significant differences in fiber directions were found for all the different clots (top 2 μm p=0.154, and total z-projections p=0.252), but a trend for shear dependant angles in fibrin fibers was seen.

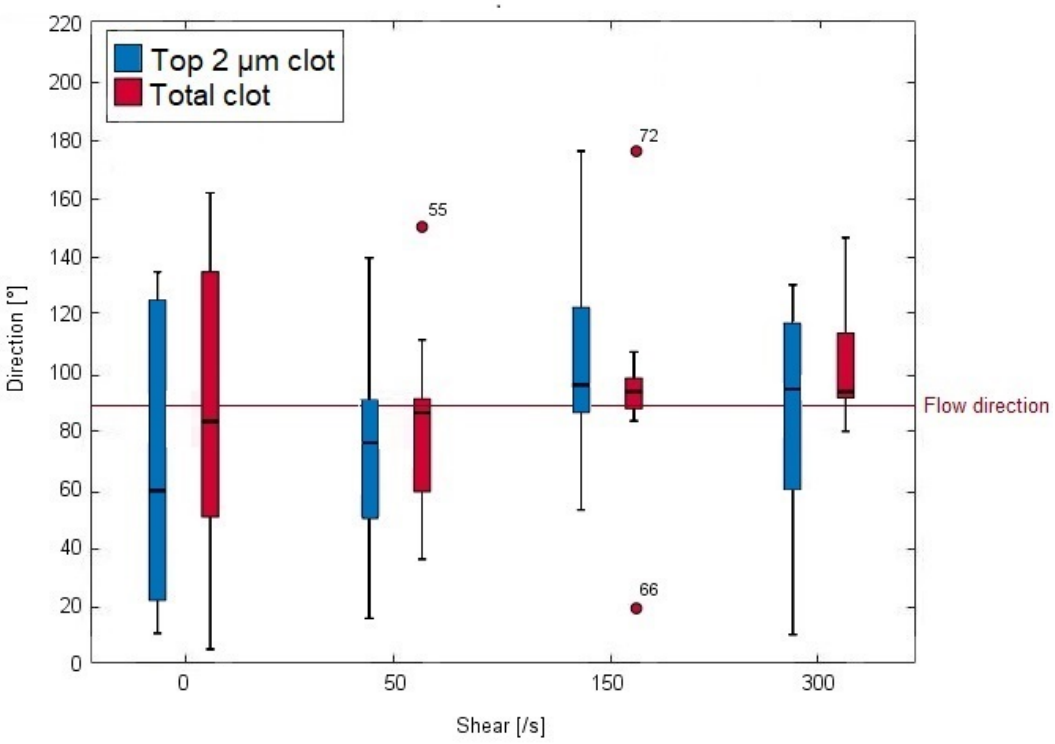

Figure 24. The fiber angles.
The fiber angles in the z-projection from the top 2 μm and the fiber angles from the total z-projection are shown.

Table 10. Mean fiber angles in top 2 μm and total z-projection.

	Means top 2 μm clot				**Means total clot**			
Shear [/s]	Angle [°]	Deviation [°]	SD [°]	Anisotropic fraction	Angle [°]	Deviation [°]	SD [°]	Anisotropic fraction
0	69	21	41	0.33	85	5	30	0.32
50	73	17	23	0.23	84	6	17	0.20
150	106	16	26	0.25	94	4	22	0.21
300	85	5	17	0.25	103	13	21	0.35

The mean top 2 μm and total clot fiber angles, deviation from 90 (the flow direction), standard deviation and anisotropic fraction are shown.

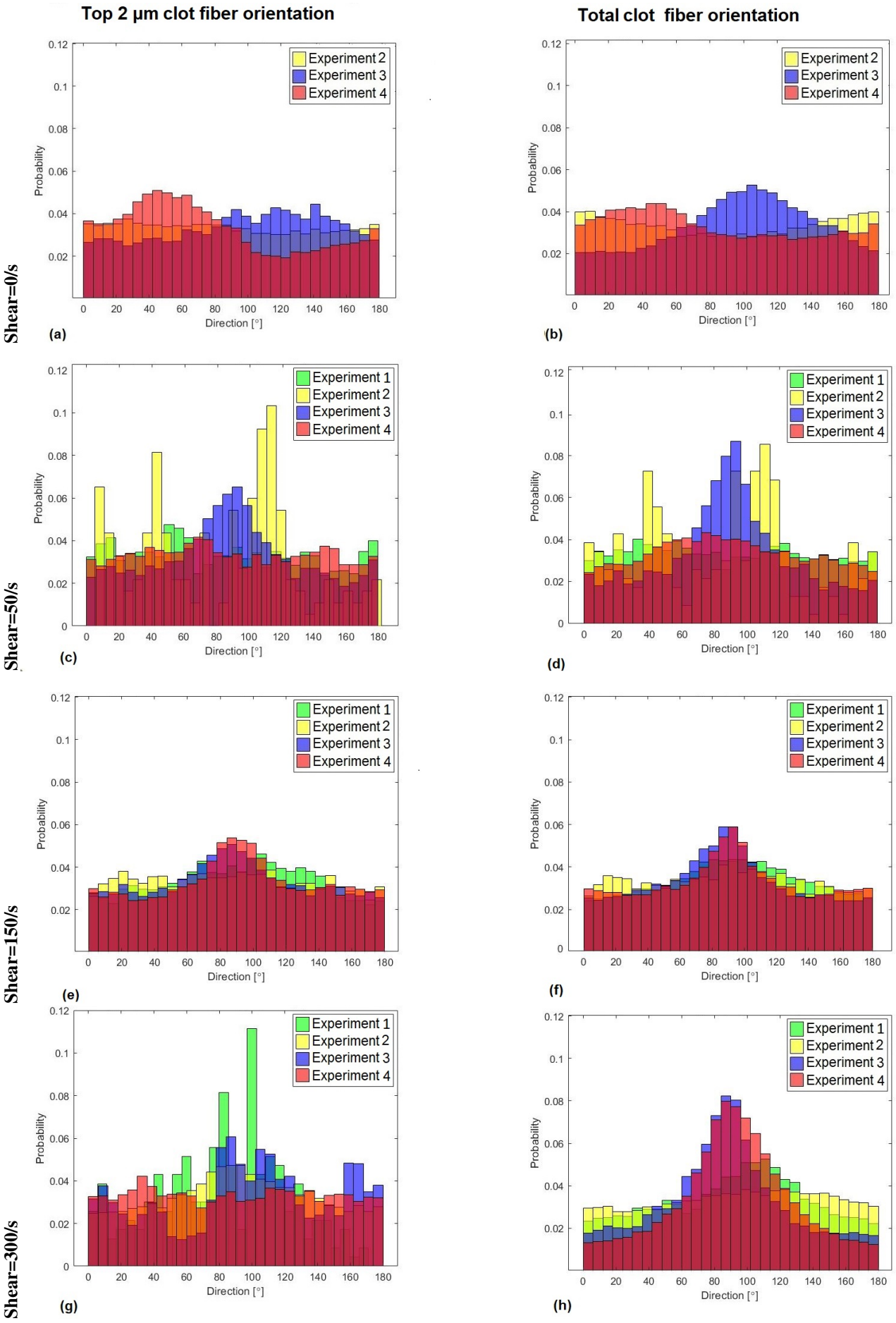

Figure 25. Fiber orientation.
The results are shown per shear rate: in shear=0/s **(a)** top 2 μm and **(b)** total clot, in shear=50/s **(c)** top 2 μm and **(d)** total clot, shear=150/s **(e)** top 2 μm and **(f)** total clot, shear=300/s **g)** top 2 μm and **(h)** total clot.

6.2.3 Densities of fibrin clots formed under flow

All the fiber densities from the clots were analyzed, results can be seen in figure 26 and median densities can be seen in table 11. All results and the used method for calculating the fiber density per sample can be seen in appendix 12.4.4. The top 2 μm projections from shear=0/s clots have the highest median density, followed by projections at shear=150/s, shear=50/s, and shear= 300/s. Significant differences were found between the top 2 μm projections at shear=0/s versus shear=50/s and shear=0/s versus shear=300/s. In the total clot projections, shear= 300/s clots have the highest median density, followed by shear=50/s, shear=150/s, and shear=0/s clots. Significant differences in density were found between the total clot projections of shear=50/s clots compared to all the other clots.

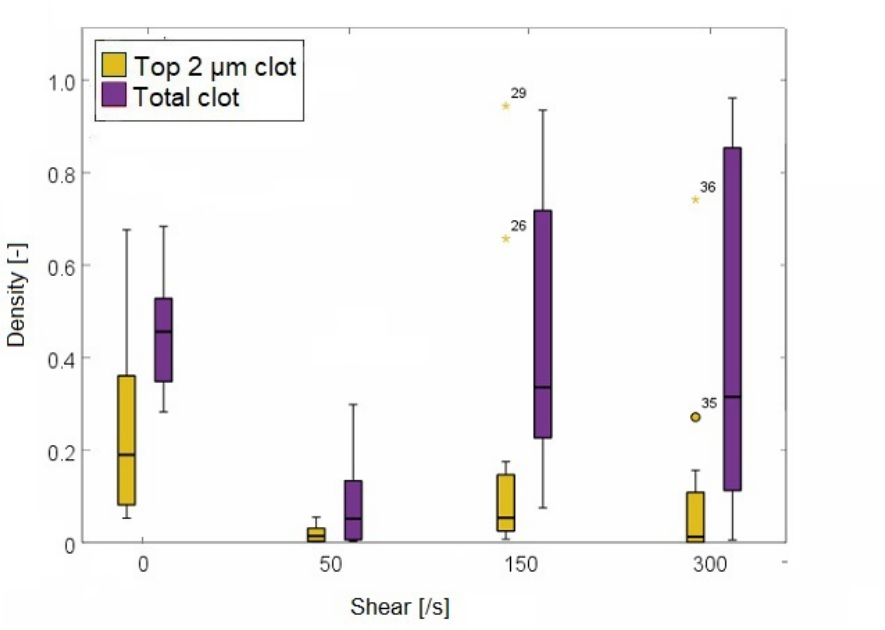

Figure 26. The fiber density per shear.
The density per shear for the top 2 μm and total clot height are shown.

Table 11. Fibrin clot densities (median [25th-75th percentile]) from all clots formed under different shears.

	Top 2 μm	Total clot		Top 2 μm	Total clot
Shear [/s]	Density [-]		**Shear [/s]**	P-value	
0	0.19[0.80-0.43]	0.09[0.05-0.52]	0 vs 50	0.005*	0.003*
50	0.01[0.003-0.03]	0.30[0.06-0.67]	0 vs 150	0.97	1.0
150	0.05[0.20-0.16]	0.25[0.60-0.39]	0 vs 300	0.02*	1.0
300	0.10[0.006-0.13]	0.56[0.07-0.90]	50 vs 150	0.19	0.01*
			50 vs 300	1.0	0.02
			150 vs 300	0.48	1.0

A statistical comparison was performed between clots formed under different shears.
A p-value of 0.05 was considered significant (marked with *).

6.2.4 Results from the Piuma

The median effective Young's moduli per shear measured at the top of clots, are shown in figure 27 and table 12. All results can be seen in appendix 12.4.1. Shear=50/s clots have the highest effective Young's moduli, followed by shear=300/s, shear=150/s, and shear=0/s clots. The shear=50/s are significantly stiffer than all the other clots ($p \leq 0.01$), and the shear=0/s clots are significantly less stiff than all other clots ($p<0.001$). Other results are not significant.

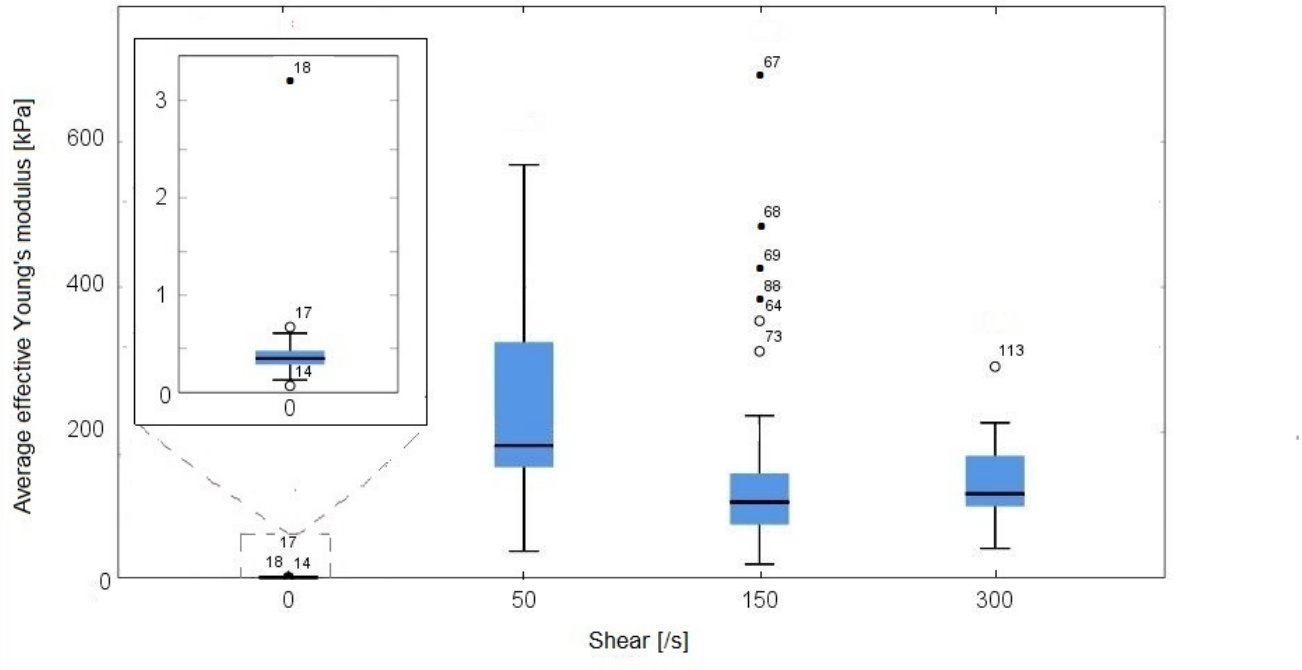

Figure 27. The median effective Young's modulus is shown per shear rate.

Table 12. The median effective Young's modulus (median [25th-75th percentile]) is shown per shear rate.

	Clots made at shear [/s]		**Clots compared with shear [/s]**
Shear [/s]	Effective Young's modulus [kPa]	**Shear [/s]**	P-value
0	0.35[0.28-0.43]	0 vs all	0.001*
50	182[152-335]	50 vs 150	0.003*
150	104[71-147]	50 vs 300	0.01*
300	115[98-168]	150 vs 300	1.0

A statistical comparison between clots formed under different shears. A p-value of 0.05 was considered significant (marked with *).

6.2.5 Linking the effective Young's modulus and morphological features

Lastly, a possible link between the effective Young's modulus and morphological features was investigated. In this comparison, we should only look at the top 2 μm morphological characteristics, as only the top of the clot is indented. An exception is the total clot height, which is mentioned as a separate feature. The top 2 μm morphological findings and the stiffness per shear are summarized in table 13. It was seen that the effective Young's modulus in shear=0/s clots was the lowest compared to all other clots ($p<0.001$). The shear=0/s clots had a larger height than all other clots ($p<0.001$), and their density was higher than in shear=50/s and shear=300/s clots ($p\leq0.02$). Furthermore, shear=50/s clots were significantly stiffer than all clots ($p\leq0.01$) and were significantly lower in height and density than shear=0/s clots. The shear=150/s and shear=300/s clots weren't significantly different in stiffness, but both were significantly stiffer than shear=0/s clots. Shear=150/s clots were significantly lower in height than shear=0/s clots. Lastly, shear=300/s clots were significantly lower in height and density than shear=0/s clots.

Table 13. Linking the effective Young's modulus and morphological features (mean±SD or median[25th-75th percentile]).

	Mechanical feature	**Morphologic features**		
	Effective Young's modulus [kPa]	Height [μm]	Angle fibers [°]	Density [-]
Shear [/s]	Top 2 μm	Total clot	Top 2 μm	Top 2 μm
0	0.35[0.28-0.43]*	26±6.2*	69±41	0.19[0.80-0.43]**
50	182[152-335]***	7.4±4.6	73±23	0.01[0.003-0.03]
150	104[71-147]	13±7.6	106±26	0.05[0.20-0.16]
300	115[98-168]	13±5.4	85±17	0.10[0.006-0.13]

A statistical comparison between clots formed under different shears. Significant findings are marked as: *p<0.001 compared to all other clots, **p≤0.02 compared to shear=50/s and 300/s clots, ***p≤0.01 compared to the shear=150/s and 300/s clots.

7 Discussion

7.1 Thrombus analogs formed under static conditions

This is the first study in which the stiffness of a clot matured with physiologic pressure versus a clot matured without applying pressure was investigated. It is also the first study investigating the effect of different heights on a clot's stiffness. The results found in the thrombus analogs formed under static conditions can be listed as four findings. The first finding is that WB, 1%H, 40%H, and 99%H clots exhibit non-linear hyperelastic strain stiffening and visco-elastic behavior. Secondly, by investigating WB clots formed with or without applying physiological pressure during maturation, it was seen that no significant differences were found in the stiffness of these clots. Thirdly, when using relatively high strains (>60%), it was seen that WB clots with a bigger height were significantly stiffer than clots with a smaller height. Fourthly, it was shown that 1%H clots were significantly stiffer than 99%H clots.
The first finding was found by investigating all clots made with static pressure using unconfined compression testing. Similarly to other studies, there was seen non-linear hyperelastic strain stiffening[26,32,33] and visco-elastic behaviour[32,33,73] in our clot analogs. This behavior was observed in all samples when comparing the tangent stiffness at strain=10% versus strain=70%. As mechanical thrombectomy causes large strain in clots,[26] it is thought that large strains are most interesting when comparing analogues to thromboemboli.
The second finding was seen in the tangent moduli from WB clots formed with or without physiological pressure during maturation. In order to make realistic clots, it is essential to know if the effect of blood pressure should be mimicked in developing clot analogs. As statically mimicking blood pressure by putting a weight on the developing clot is relatively simple, it could be easily applied in future research. Almost all studies don't apply pressure in the developing clot analog, but only after maturing through unconfined compression testing.[17,26,32,33] One other study was found in which pressure was applied during maturation, but no unpressured control sample was made to compare differences in stiffness.[74] It could be seen that no significant difference was found between the tangent moduli from WB clots formed with or without applying physiological pressure during maturation. Therefore, it can be concluded that although it is simple to apply static physiologic pressure in clot analog development, it is not needed to make clot analogs with a realistic stiffness. Furthermore, a large SD over 100% (bigger than the mean tangent modulus itself) was seen in the stiffness of the WB clots. Platelet contractile forces might have played a role in this increased SD. In contracted clots, it is shown by Cines et al. (2014) that fibrin and platelets are primarily seen on the outside of these clots and erythrocytes on the inside.[27] Our samples were cut as cylindrical-shaped slices from the total column of WB clot. Samples cut from the bottom or top may contain more fibrin and platelets than samples from the middle of the clot, as the latter might include a more exterior part of the clot. The middle part might contain more erythrocytes. Though no histology was done in our clots to confirm this histologic difference. In addition, the possible differences in histology might lead to a different stiffness. As it is seen in other studies individual elastic moduli of erythrocytes (a several hundred Pa to a few kPa)[75] are a lot lower than in platelets (32-224 kPa)[76] and fibrin (uncrosslinked and crosslinked 2-15 MPa).[77] Furthermore, high SDs of about 80% were also seen in ovine WB clot analogs from the study of Johnson et al. (2020), also suggesting that WB samples variate in stiffness. The WB clots in this study were stiffer than our clots, ranging from 0.55 to 25 kPa when increasing strains from 0 to 50%. As compared to 0.44 to 3.5 kPa with similar strains in our study. This difference in stiffness with Johnson et al. (2020) was expected, as these clots were made from ovine blood, which leads to a higher stiffness.[78]
The third finding was seen in WB clots with variating heights, when applying a high level (>60%) of strain. In our study it was seen that at high strains, higher heights in WB clots resulted in more stiffness. This was seen when comparing clots with heights of 1 mm, 2 mm, and 5 mm. Although stiffness is usually seen as a static material property[79] assumed to be independent of sample volume, significant differences were seen. The strain and strain rates were the same for the different clots when clots were tested, so this cannot explain these differences. It might be possible that larger clots (for example with a height of 5 mm) contain fibers with a longer length, as cutting the clots in smaller samples (height 1 mm) might result in shorter fiber lengths. A bigger stiffness was seen in fibrin fibers

with a bigger length in the Collet et al. (2005) study, which could explain the higher stiffnesses seen in our clots. Though it is not known if this higher stiffness depends more on fiber diameter associated with these bigger lengths.[77] In the study of Johnson et al. (2020) a similar testing scheme was used in ovine clot analogs with a similar height. It was seen that 5 mm high WB clots from Johnson et al. (2020) were stiffer than 5 mm clots from our study. Respectively at strain=50% 11±1 versus 5.9±2.0 kPa. A big difference was seen at higher shears, with 200±100 at strain=80% kPa versus 35±7.8 at strain=70%, which is not totally comparable because of the difference in strain. Again a higher stiffness was expected in these clots made from ovine blood.[78] The findings in our study implie that clot heights should be carefully picked and be uniform thorough studies, in order to make studies comparable. It also implies that this higher stiffness found in clot analogs with bigger heights, might also be found in real thrombi with larger heights. A role for a higher stiffness in recanalization rates might be possible, as these higher thrombi are associated with a decrease in recanalization succes.[20] The fourth finding was the higher stiffness seen in 1%H clots compared to 99%H clots, this was seen in samples from both donors. A higher RBC content leads to a lower stiffness is in accordance with other studies.[15,17,80] It is thought that denser fibrin networks can form in clots with less RBCs, due to the contracting platelets pulling on the fibrin fibres causing them to become compacted around the few RBCs present.[26,27,81] Although it should be noted that volumetric RBC ratio used in the formation of our clot analogs, might differ from the RBC content which could be found in these clots.[82] No histology is done in our study, so the exact RBC contents remain unknown. In the study by Johnson et al., compression tests were performed in ovine clot analogs with similar volumetric RBC ratios of 0%H and 40%H. In the clots with these same %H a mean stiffness range from 0.1 – 1.1 kPa at strain=10% and from 100-400 kPa at strain=80% is seen. When comparing the tangent moduli values to the Johnson et al. (2020) study, a higher mean tangent range was seen in our samples, ranging from 0-16.2 kPa at strain=10% and 170-1200 kPa at strain=70%. This was surprising as ovine blood and a higher strain were expected to give a higher stiffness. This variation could a consequence of the fact that they used a different centrifugation scheme with higher speeds.[83] The speeds and times used in the Johnson et al. (2020) study versus our study were respectively, 2200 g (10 minutes) versus 2000 g (10 minutes) and 180 g (10 minutes) versus 120 g (20 minutes). This theory is further confirmed by the fact that the WB clots in this same study are a lot stiffer than similar WB clots from our study shown before. Furthermore, inter-donor variation may have played a role as our WB clots were made from the blood of a different donor than the clots with varying hematocrit. In the study of Johnson et al. (2019)

7.2 Fibrin clot formation under flow

In this study, a new flow set-up was designed with a flow chamber which was partly based on the Kruchten et al. (2012) design.[52] In this new set-up, it was possible to formate fibrin clots on TF-coated slides. Shears ranging from 0 to 300/s were applied. The results of the fibrin clots which were formed under flow can be listed as four findings. The first finding is that flow reduced the clot height, as compared to clots formed without shear. The second finding is that fibers seemed to align with the flow direction, although no significant difference in fiber direction was seen compared to the statically formed clots. The third finding is that flow significantly reduced the density in the top 2 mμ at some shears. The fourth finding was that clots formed under shear have a significantly higher stiffness than clots formed without shear.

The first finding was seen in the different fibrin clot heights. In our study, shear=0/s clots were significantly larger in height than the clots formed under shear at the TF-coated slides. Other perfusion studies showed that flowing blood with an increasing wall shear rate leads to decreased fibrin deposit.[84–86] This might be the case in the samples created under flow versus the samples formed without flow. This decreased fibrin deposit might be caused by the shear-dependant effect on coagulation due to the dilution of the thrombin and fibrin monomers by the flow field,[87] which was seen in the study of Shen et al (2008). Furthermore, it should be noted that the shear=0/s samples were formed differently compared to the samples created under flow. Respectively, by applying PPP for 40 minutes on a slide at 37°C versus a flow experiment. Also, an extra washing step in clots formed under flow was used, which might have caused fibrin to be washed away. No significant difference was seen

between the heights in clots formed under flow. These clot heights ranged from 7.3±4.6 to 13.15±5.4 μm. Onasogo-Jarvis et al. (2014) did a flow study with PPP flow over TF-coated silica beads, finding a similar clot height range (6.9±1.8 to 15.3±2.4 μm) when looking at shears from 50/s till 250/s, and found a significant decrease in clot height with increasing shears.[88] This difference in shear dependency might be explained by the fact that in our study TF was coated on a relatively large area in terms of millimeters, as opposed to the nanometer scaled beads in the Onasoga et al. study. They showed that a minimum TF spot size was needed to result in a thrombin burst.[88] Our increased TF level might have led to higher thrombin concentrations, which were not diluted in an amount that led to a shear-dependent decrease in clot height. Although thrombin could still have trouble reaching the luminal side of the clot, as the fibrin could block access of zymogens to the TF-rich surface.[89]
The second finding was seen when the fibrin network alignment was investigated. In our study, it seemed that fibrin fibers align to the flow direction. It is thought that applying a tensile drag force or shear stresses on fibrin fibers affects the entire fibrin clot, optimally resisting these forces by orientating fibers along flow lines.[22] This orientation is not expected in clots without applying flow, whereas it is thought that fibers are randomly orientated.[90] Though no significant difference in fiber direction was seen when comparing the fibers formed under flow versus the statically formed fibrin clots. In our study, peak angles of <10° relative to the line of flow were seen, which seems to confirm alignment to flow. This was seen in z-projections of the top 2 mμ in clots at shear=300/s and total z-projections in clots at shear=0/s, 50/s, and 150/s. Remarkably, the shear=0/s total z-projection had an angle close to the flow direction. In contrast, the highest difference relative to the line of flow (when looking at all clots) was seen in the top 2 mμ z-projection of shear=0/s clots. The shear=0/s total z-projection peak angle seemed a considered coincidence, as no shear was applied in this clot. Interestingly, the different z-projections of clots at a certain shear showed different degrees of alignment. In the illustrative example described in this study, there seemed to be more alignment of fibers closer to the bottom of the clot. This relationship of alignment and location in the clot is still hypothetical but might explain the difference in alignment between total and top 2 μm z-projections. In the Campbell et al. (2010) study, fibers significantly aligned parallel to flow vectors (peak angles <10° relative to the line of flow).[22] In this study by Campbell et al. (2010), the flow of PPP was done in a set-up with TF-bearing cells (immortalized dermal fibroblasts) on coverslips, and static clots were made as well. They used a flow rate of 0.1 ml/min (± the flow used in our shear=150/s clots) but didn't specify the shear rate. In the Campbell et al. (2010) study, networks formed under stasis exhibited an isotropic fiber distribution instead of anisotropy, which was seen in fibrin clots formed under flow. This finding was different from what we saw in our study. Our static clots had a relatively high anisotropic fraction. Though the highest SDs were also seen in these static clots, which in turn implies isotropy. Differences with the Campbell et al. study might be caused by unspecified shears, so it is not known if the applied shears are comparable to our study. Furthermore, in the previously mentioned study of Onasoga et al. (flow of PPP on TF patches), the angle distribution of fibrin fibers was determined. In this study, the fibrin fibers aligned in the flow direction in a shear rate-dependent manner at higher shears (250/s to 1000/s) but were isotropically orientated in a starburst pattern at shears of 50/s and 100/s.[88] This might explain why a certain degree of isotropy was seen in the clots formed in our study at a shear lower than 250/s.
The third finding was seen when investigating the density of the fibrin clots. Different density patterns were seen when comparing the same shear in the top 2μm versus the total clot projections. The shear=0/s clots had the highest density in the top 2 μm z-projections, without being significantly denser than shear=150/s clots. These findings might imply that shear generally causes a lower density in the top 2μm of clots. The shear=0/s having the highest density in the top 2 μm is in accordance with the study of Onasogo-Jarvis et al. (2014), in which a higher density of fibrin fibers at lower shear rates was seen.[88] In the total clot projections of our study, a different density was seen. The top 2 μm projections are thought to be more trustworthy than the total clot projections. These top 2 μm projections are homogeneous in height, and the results are more comparable to the literature. Total z-projections of clots with bigger heights could potentially cause a denser network, as there is a bigger chance of projection fibers over each other. An underestimation in density of total clot z-projections of shear=0/s

clots, might be explained by the partial imaging of some of these clots. Also, different Fiji Macro's were used to calculate densities in the various z-projections, which might explain some differences. The fourth finding was investigated in the effective Young's modulus of the fibrin clots. It was seen that the effective Young's modulus was significantly different between some clots formed with varying rates of shear. The shear=50/s clots were significantly stiffer than all other clots, and the shear=0/s are significantly the least stiff. In the study of Campbell et al. (2010) it was seen that thicker fibers aligned under flow,[22] these thicker fibers are associated with a higher stiffness.[91] Since fibers exhibit a higher stiffness under tensile stretching than in bending,[77] networks in which fibers are predominantly aligned along flow vectors would be more resistant to deformation than isotropic networks formed under stasis. Furthermore, the effective Young's modulus was linked to the morphological findings found in our study. The morphologic features of the top 2 μm were used in this comparison, as this the indented part. It was seen that the shear=0/s clots significantly had the lowest stiffness and largest height compared to all other clots. This suggests that a low stiffness seems to be linked to a large clot height. Furthermore, a higher density might be associated with lower stiffness. This was seen in shear=0/s clots versus shear=50/s and 300/s clots.

8 Limitations

8.1 Thrombus analogs formed under static conditions

No histology was done in this study. This results in the fact that exact RBC concentrations in the clot analogs remain unknown. Furthermore, there is a possibility that glue introduced an artifact in 40%H clots from the 27 year-old-female donor, as these samples show an unexpected higher value than 1%H clots in strains ranging from 20 to 40%. Though differences between the clots were non-significant. It was seen that small clots ($\pm$ 1 mm height) tend to fold or lie on their side, which raised the question if a constant surface area was pictured for the stress calculations. Though it is possible that the samples have a preferred positioning configuration which is repeated when the samples are placed under the compression tester and are pictured by the camera. .

8.2 Fibrin clot formation under static conditions

Exact flow rates cannot be continuously maintained in the flow experiments because of the narrowing of the flow channel caused by clot formation. Shear rates will increase because of this smaller channel height. It was calculated that the shear would theoretically increase by 2-3 % during this process (with the heights seen in our clots), which was considered negligible. Furthermore, when the top part of the flow chamber was removed after the experiment, a potential artifact was introduced into the clot. This could happen by forces from the top pulling on the fibrin fibers when these are attached to the top. Though this attachment is less likely as the clotting process is initiated at the bottom of the flow chamber where TF is located, and the chamber is relatively high (compared to the clot heights). Furthermore, by immersing the clots in buffer and formaldehyde, forces are also exerted on the fibrin fibers, which can again introduce artifacts.

9 Future research

9.1 Thrombus analogs formed under static conditions

Firstly, it would be interesting to mimic hypertension in a static pressure experiment in the future. As applying physiological pressure didn't lead to differences in stiffness, it would be interesting to know if stiffening of the clot analogs occurs under a pathologic high pressure. This hypertension experiment could give more insight into the pathology of hypertension in patients. Secondly, more research on the effect of different heights in clot stiffness could be done in clot analogs formed under static conditions. It would be interesting to know if this height relationship is also seen with tensile testing, as these tensile forces mimic the removal of thrombi by thrombectomy devices more accurately. Thirdly, it would be interesting to investigate the exact effect on clot stiffness by applying different centrifugation

speeds and times, as these vary between studies. For example, an experiment can be done in which centrifugated WB versus non-centrifugated WB is used for making static clot analogs, which are tested by unconfined compression testing. Centrifugated WB would be made by remixing the constituents after centrifugation to make WB again.

9.2 Fibrin clot formation under flow

Firstly, it would be interesting to investigate if there a relationship between fiber alignment and localization within a clot. Indications of this relationship were seen in this study, but more data is needed to confirm this. Secondly, it would be interesting to add the pulsatile character of blood flow to the flow set-up to see if this affects clot characteristics. As the effects of blood flow in terms of pressure and flow were investigated in this study, it would be interesting to add its pulsatile character. Thirdly, as our flow experiments were done at room temperature, it would be interesting to know if there would be an effect on these clots by mimicking body temperature. This could be done by heating the flow chamber where the clotting takes place or heating up the whole system. Fourthly, it would be interesting to repeat the flow experiments with this same set-up but with different reagents, like PRP or whole blood. Adding these blood constituents would be interesting to understand the effect of flow on various blood components.

10 Bibliography

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11 Codes

11.1 Cylinder weight

```
clear all; clc
%%
syms DBP
syms SBP
syms h
syms F_MAP
syms F_friction
syms rho_brass
syms rho_water
syms bore
syms d_cylinder
syms g
%% Variables:
% control + r this block for seeing formulas, or control + t to enter variables
DBP=90; % diastolic blood pressure, mmHg
SBP=140; % systolic blood pressure, mmHg
bore=13.04E-3; % bore or diameter piston, m

h=50E-3; % approximate height of syringe, m
F_friction= 1; % friction force of piston with syringe, N

rho_brass= 8.5*10^3; % density of brass, kg/m^3
rho_water= 9.933316*10^2; % density of water, kg/m^3

g=9.81;
d_cylinder= 14.5E-3; % inner diameter cylindrical shell, m
mmHg_to_Pa=133.322387415; % 1 mmHg is 133.322387415, Pa
%% Formulas:
MAP=(2*DBP+SBP)/3; % mean arterial pressure, mmHg
P_MAP= MAP*mmHg_to_Pa; % pressure, Pa or N/m^2

r_syringe= bore/2; % radius syringe, m
A_syringe=pi*r_syringe^2; % surface area piston, m^2

F_MAP= P_MAP*A_syringe; % force exerted by mean arterial pressure, N
F_apparent=F_MAP + F_friction; % apparent force, N
rho_corrected=rho_brass-rho_water; %density brass minus density water, kg/m^3
r_cylinder= d_cylinder/2;% inner radius of weight, m

R_cylinder= sqrt((F_apparent+(rho_corrected*g*pi*h*r_cylinder^2))/(rho_corrected*g
    *pi*h)) % outer radius cylindrical shell, m
mass_cylinder=pi*((R_cylinder^2)-(r_cylinder^2))*h*rho_brass %mass of needed
    cylinderical shell to mimick BP, kg
```

11.2 Matlab text output compression test

```
%%Written by Manouk Mondeel
clearvars -except H99_19_02 H_WB19_03_1mm_compr_S5
clc
%doc for information
%%
clear all
close all
clc
%% analysis tensile test data
%T = readtable('40%H_compr_80%_S1_1mm.txt'); %load excel table / or from text
```

```
for i=1:5 %1:number of samples
    %19-02-2021:
    T1=strcat('19-02-2021/1%H_compr_80%_S',num2str(i),'_1mm.txt'); %strcat: save
       all tables as cells
    data1_19_02{i}={readtable(T1)};
    T1_19_02_60=strcat('19-02-2021/1%H_compr_60%_S',num2str(i),'_1mm_relax.txt');
    data1_19_02_60{i}={readtable(T1_19_02_60)};
    T40=strcat('19-02-2021/40%H_compr_80%_S',num2str(i),'_1mm.txt'); %save all
       tables as cells
    data40_19_02{i}={readtable(T40)};
    T40_19_02_60=strcat('19-02-2021/40%H_compr_60%_S',num2str(i),'_1mm_relax.txt')
       ;
    data40_19_02_60{i}={readtable(T40_19_02_60)};
    T99=strcat('19-02-2021/99%H_compr_80%_S',num2str(i),'_1mm.txt');
    data99_19_02{i}={readtable(T99)};

    %02-03-2021:
    T1_2_3=strcat('2-3-2021/1%H_compr_80%_S',num2str(i),'_3mm.txt');
    data1_2_3{i}={readtable(T1_2_3)};
    T40_2_3=strcat('2-3-2021/40%H_compr_80%_S',num2str(i),'_3mm.txt');
    data40_2_3{i}={readtable(T40_2_3)};
    T99_2_3=strcat('2-3-2021/99%H_compr_80%_S',num2str(i),'_3mm.txt');
    data99_2_3{i}={readtable(T99_2_3)};
    T99_2_3_60=strcat('2-3-2021/99%H_compr_60%_S',num2str(i),'_1mm.txt');
    data99_2_3_60{i}={readtable(T99_2_3_60)};

    %19-03-2021:
    TWB19_03_uncompr=strcat('19-03-2021/1mm_uncompr/WB%H_compr_80%_S',num2str(i),'
       _1mm_uncompr.txt');
    dataWB19_03_1mm_uncompr{i}={readtable(TWB19_03_uncompr)};
    TWB19_03_1mmcompr=strcat('19-03-2021/1mm_compr/WB%H_compr_80%_S',num2str(i),'
       _1mm.txt');
    dataWB19_03_1mm_compr{i}={readtable(TWB19_03_1mmcompr)};
    TWB19_03_2mm=strcat('19-03-2021/2mm/WB%H_compr_80%_S',num2str(i),'_2mm.txt');
    dataWB19_03_2mm{i}={readtable(TWB19_03_2mm)};
    TWB19_03_5mm=strcat('19-03-2021/5mm/WB%H_compr_80%_S',num2str(i),'_5mm.txt');
    dataWB19_03_5mm{i}={readtable(TWB19_03_5mm)};
end
clear T1 T40 T99 T1_2_3 T99_2_3_60 i T40_2_3 T99_2_3 T1_19_02_60 T40_19_02_60
   TWB19_03_uncompr TWB19_03_1mmcompr TWB19_03_2mm TWB19_03_2mm TWB19_03_5mm

%% Extract time and force column :

% Extract as matrix:
% data1{1,1}{1,1}(:,1) % to extract as a matrix
% data1{1,1}{1,1}(:,'Var1')
%doc hms

for i=1:5  %use {} for a double format!
dt=data1_19_02{1,i}{1,1}{:,1} ;% extract time
timei=timefunc(dt);
timeT1_19_02{i}={timei};
Fi=data1_19_02{1,i}{1,1}{:,2}; %force column
F1_19_02_80{i}={Fi};

dt=data1_19_02_60{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeT1_19_02_60{i}={timei};
Fi=data1_19_02_60{1,i}{1,1}{:,2};
F1_19_02_60{i}={Fi};
```

```
dt=data40_19_02{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeT40_19_02_80{i}={timei};
Fi=data40_19_02{1,i}{1,1}{:,2};
F40_19_02_80{i}={Fi};

dt=data99_19_02{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeT99_19_02{i}={timei};
Fi=data99_19_02{1,i}{1,1}{:,2};
F99_19_02{i}={Fi};

dt=data1_2_3{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeT1_2_3{i}={timei};
Fi=data1_2_3{1,i}{1,1}{:,2};
F1_2_3{i}={Fi};

dt=data40_2_3{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeT40_2_3{i}={timei};
Fi=data40_2_3{1,i}{1,1}{:,2};
F40_2_3{i}={Fi};

dt=data99_2_3{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeT99_2_3{i}={timei};
Fi=data99_2_3{1,i}{1,1}{:,2};
F99_2_3{i}={Fi};

dt=data99_2_3_60{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeT99_2_3_60{i}={timei};
Fi=data99_2_3_60{1,i}{1,1}{:,2};
F99_2_3_60{i}={Fi};

dt=dataWB19_03_5mm{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeWB19_03_5mm{i}={timei};
Fi=dataWB19_03_5mm{1,i}{1,1}{:,2};
FdataWB19_03_5mm{i}={Fi};

dt=dataWB19_03_1mm_compr{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeWB19_03_1mm_compr{i}={timei};
Fi=dataWB19_03_1mm_compr{1,i}{1,1}{:,2};
FdataWB19_03_1mm_compr{i}={Fi};

dt=dataWB19_03_1mm_uncompr{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeWB19_03_1mm_uncompr{i}={timei};
Fi=dataWB19_03_1mm_uncompr{1,i}{1,1}{:,2};
FdataWB19_03_1mm_uncompr{i}={Fi};

dt=dataWB19_03_2mm{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeWB19_03_2mm{i}={timei};
Fi=dataWB19_03_2mm{1,i}{1,1}{:,2};
FdataWB19_03_2mm{i}={Fi};
end
```

```
for i=1:4 % a test round missing
dt=data40_19_02_60{1,i}{1,1}{:,1} ;
timei=timefunc(dt);
timeT40_19_02_60{i}={timei};
Fi=data40_19_02_60{1,i}{1,1}{:,2};
F40_19_02_60{i}={Fi};
end
clear dt hi i mi si time time0i timei Fi
clear data40_2_3 data1_19_02 data1_19_02_60 data1_2_3 data40_19_02 data40_19_02_60
    data 20_2_3 data99_19_02 data99_2_3 data99_2_3_60



%% Calculate stress:

% Stress [N/m^2]= force[N]/area [m^2] --> 1 Pa, so 1 kPa is /1000
% Area is measured with ImageJ

area = readtable('surface_area_1.csv'); %in mm^2
area=area(:,6); %selecting area column
Area1_19_02_80=area(23:27,1);
Area1_19_02_60=area(29:33,1);
Area40_19_02_80=area(36:40,1);
Area40_19_02_60=area(42:46,1);
Area99_19_02=area(48:52,1);
Area1_2_3=area(60:64,1);
Area1_2_3_after=area(66:70,1);
Area40_2_3=area(72:76,1);
Area99_2_3=area(78:82,1);
Area99_2_3_60=area(84:88,1);
AreaWB19_03_1mm_compr=area(90:94,1);
AreaWB19_03_1mm_uncompr=area(96:100,1);
AreaWB19_03_2mm=area(102:106,1);
AreaWB19_03_5mm=area(108:112,1);

%%
%in kPa=
for i= 1:5
    Stress=(F1_19_02_80{1,i}{1,1}/(Area1_19_02_80{i,1}))*(1000);
    Stress1_19_02_80{i}={Stress};

    Stress=(F1_19_02_60{1,i}{1,1}/(Area1_19_02_60{i,1}))*(1000);
    Stress1_19_02_60{i}={Stress};

    Stress=(F40_19_02_80{1,i}{1,1}/(Area40_19_02_80{i,1}))*(1000);
    Stress40_19_02_80{i}={Stress};

    Stress=(F99_19_02{1,i}{1,1}/(Area99_19_02{i,1}))*(1000);
    Stress99_19_02{i}={Stress};

    Stress=(F1_2_3{1,i}{1,1}/(Area1_2_3{i,1}))*(1000);
    Stress1_2_3{i}={Stress};

    Stress=(F40_2_3{1,i}{1,1}/(Area40_2_3{i,1}))*(1000);
    Stress40_2_3{i}={Stress};

    Stress=(F99_2_3{1,i}{1,1}/(Area99_2_3{i,1}))*(1000);
    Stress99_2_3{i}={Stress};

    Stress=(F99_2_3_60{1,i}{1,1}/(Area99_2_3_60{i,1}))*(1000);
    Stress99_2_3_60{i}={Stress};
```

```
    Stress=(FdataWB19_03_1mm_compr{1,i}{1,1}/(AreaWB19_03_1mm_compr{i,1}))*(1000);
    StressWB19_03_1mm_compr{i}={Stress};

    Stress=(FdataWB19_03_1mm_uncompr{1,i}{1,1}/(AreaWB19_03_1mm_uncompr{i,1}))
        *(1000);
    StressWB19_03_1mm_uncompr{i}={Stress};

    Stress=(FdataWB19_03_2mm{1,i}{1,1}/(AreaWB19_03_2mm{i,1}))*(1000);
    StressWB19_03_2mm{i}={Stress};

    Stress=(FdataWB19_03_5mm{1,i}{1,1}/(AreaWB19_03_5mm{i,1}))*(1000);
    StressWB19_03_5mm{i}={Stress};
end

for i=1:4
    Stress=(F40_19_02_60{1,i}{1,1}/(Area40_19_02_60{i,1}))*(1000);
    Stress40_19_02_60{i}={Stress};
end

%% Post-test samples:

h1=3; %first height, mm
h2=0.6; %lowest height during compression, mm

Area_difference_1_2_3=  (Area1_2_3_after{:,:}-Area1_2_3{:,:});
increased_area_percentage_1_2_3=(Area_difference_1_2_3*100)./Area1_2_3{:,:};
Volumetric_1_2_3_theoretically= Area1_2_3{:,:}*h1; % 3mm height. So area * height.

%So squeezed 80% compressed for 20 cycles. Untill 0.6 mm height. Permanent
%deformation?

%theoretical area at lowest height:
Area1_2_3_lowest=Volumetric_1_2_3_theoretically/h2;

%%
clear F1_19_02 F1_19_02_60 F1_2_3 F40_19_02_80 F40_2_3 F99_19_02 F99_2_3
    F99_2_3_60 i Stress area
clear Area1_19_02_60 Area1_19_02_80 Area40_19_02_60 Area40_19_02_80 Area40_2_3
    Area99_19_02
%% 19-02 80% compression
%% 19-02:
% plotting the right timeframes(check if force is right? out of range load cell)

t_1_19_02_S1=timeT1_19_02{1,1}{1,1}(1:9126); %9126 is endpoint 20 cycle loop
S_1_19_02_S1=Stress1_19_02_80{1,1}{1,1}(1:9126)+0.33;
t_1_19_02_S2=timeT1_19_02{1,2}{1,1}(1:9126);
S_1_19_02_S2=Stress1_19_02_80{1,2}{1,1}(1:9126)+2.1; %9126 is endpoint 20 cycle
    loop
t_1_19_02_S3=timeT1_19_02{1,3}{1,1}(1:9126);
S_1_19_02_S3=Stress1_19_02_80{1,3}{1,1}(1:9126)+3.3;
t_1_19_02_S4=timeT1_19_02{1,4}{1,1}(1:9126);
S_1_19_02_S4=Stress1_19_02_80{1,4}{1,1}(1:9126)+3.8;
t_1_19_02_S5=timeT1_19_02{1,5}{1,1}(78:9203)-timeT1_19_02{1,5}{1,1}(78);
S_1_19_02_S5=Stress1_19_02_80{1,5}{1,1}(78:9203)+3.21; % the +3 is tear to zero.
    The minus is to bring the x-axis to zero, and the selected time frame to get
    the right window of 20 cycles


% average:
```

```
H1_19_02_avg_S=(S_1_19_02_S1+S_1_19_02_S2+S_1_19_02_S3+S_1_19_02_S4+S_1_19_02_S5)
    /5;
H1_19_02_avg_t=(t_1_19_02_S1+t_1_19_02_S2+t_1_19_02_S3+t_1_19_02_S4+t_1_19_02_S5)
    /5;

H1_19_02_avg={H1_19_02_avg_t,H1_19_02_avg_S};

H1_19_02={t_1_19_02_S1,S_1_19_02_S1;........
        t_1_19_02_S2,S_1_19_02_S2;
        t_1_19_02_S3,S_1_19_02_S3;
       t_1_19_02_S4,S_1_19_02_S4;
       t_1_19_02_S5,S_1_19_02_S5;};

figure(1)
plot(H1_19_02_avg_t,H1_19_02_avg_S);
title('1 mm height 1%H average 19-02-2021: 80% compression.')
lgd=legend('1%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

% hysteresis loop preparation H1_19_02:
data=H1_19_02
stepsize_valley=455;
cycles=20;%normally 20 % amount of cycles on the sample
samples=5; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=30; %should give output: to be equal to the amount of cycles
peakdistance=40; %should give output: to be equal to the amount of cycles
new_time_vector=(0:(strain_level/(desired_vector_lengths-1)):strain_level)

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end

% plotting H1_19_02:
[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
    first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
    First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
    first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
    Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
    std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
    new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
    cycles,samples,strain_level,desired_vector_lengths)

%first_cycle_is={newy_is{1,1}{1,1} newy_is{1,2}{1,1} newy_is{1,3}{1,1}  newy_is
    {1,4}{1,1} newy_is{1,5}{1,1}}


%new_time_vector200=0:(80/199):80;
peakH1_19_02=Absolute_1_line(end);
for i=1:samples
One_line_meanH1_19_02(1,i)=One_line_mean{1,i}(end)
Young_per_sampleH1_19_02(1,i)=Young_per_sample{1,i}(end)
end

figure(2)
for j=1:samples
for i=1:cycles
hold all
```

```
plot(new_time_vector/100,newy_ds{1,j}{1,i},'y');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'y');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'y'); % plotting the first cycle as
    well {1,j}{1,1}
end
end
title('1 mm height 1%H all samples + all cycles, 19-02-2021 80% compression.')
lgd=legend('1%H')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(3)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
title('1 mm height 1%H average 19-02-2021 hysteresis as 1 line the 5, 80%
    compression.')
lgd=legend('1%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(4) %graph std's
plot(new_time_vector/100,Absolute_1_line,'y')
title('1 mm height 1%H average 19-02-2021 hysteresis as 1 line: 80% compression.')
lgd=legend('1%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')




figure(5)
plot(new_time_vector/100,dy)
title('1 mm height 1%H samples 19-02-2021: tangent modulus average hysteresis in 1
     line.')
lgd=legend('1%H')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')


figure(6)%average
bar(perc, Young_avg_per_percentage,'y')
title('19-02-2021 samples, height of 1 mm,  1%H.')
lgd=legend('1%H')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')
```

```
Young_avg_per_percentage_1H_19_02=Young_avg_per_percentage; %safe

%save the data:
samples1_19_2=samples;
cycles1_19_2=cycles;
newy_ds1_19_2=newy_ds;
newy_is1_19_2=newy_is;
Absolute_1_line1_19_2=Absolute_1_line;
One_line_mean1_19_2=One_line_mean;
points_mean1_19_2=points_mean;
dy1_19_2=dy;
perc1_19_2=perc;
Young_avg_per_percentage1_19_2=Young_avg_per_percentage;
std_10_20_etc1_19_2=std_10_20_etc;

%save first cycle data
One_line_is_and_ds1_19_2=One_line_is_and_ds;
First_cycle_One_line_mean1_19_2=First_cycle_One_line_mean;
first_cycle_points_mean1_19_2=first_cycle_points_mean;
first_cycle_std_10_20_etc1_19_2=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_1_19_2=first_cycle_Young_avg_per_percentage;

%% 19-02;
t_40_19_02_S1=timeT40_19_02_80{1,1}{1,1}(1:8500); %9126 is endpoint 20 cycle loop
S_40_19_02_S1=Stress40_19_02_80{1,1}{1,1}(1:8500)+0.1;
t_40_19_02_S2=timeT40_19_02_80{1,2}{1,1}(1:8500)+11.64;
S_40_19_02_S2=Stress40_19_02_80{1,2}{1,1}(1:8500)+0.1; %9126 is endpoint 20 cycle
    loop
t_40_19_02_S3=timeT40_19_02_80{1,3}{1,1}(1:8500);
S_40_19_02_S3=Stress40_19_02_80{1,3}{1,1}(1:8500)+0.14;
t_40_19_02_S4=timeT40_19_02_80{1,4}{1,1}(1:877);
S_40_19_02_S4=Stress40_19_02_80{1,4}{1,1}(1:877)+0.11;
t_40_19_02_S5=timeT40_19_02_80{1,5}{1,1}(1:200);
S_40_19_02_S5=Stress40_19_02_80{1,5}{1,1}(1:200); % the +3 is tear to zero. The
    minus is to bring the x-axis to zero, and the selected time frame to get the
    right window of 20 cycles

H40_19_02_avg_t=(t_40_19_02_S1+t_40_19_02_S3)/2
H40_19_02_avg_S_part2=(S_40_19_02_S1(485:8500)+S_40_19_02_S2(485:8500)+
    S_40_19_02_S3(485:8500))/3
H40_19_02_avg_S_part1=(S_40_19_02_S1(1:484)+S_40_19_02_S3(1:484))/2
H40_19_02_avg_S=[H40_19_02_avg_S_part1;H40_19_02_avg_S_part2];

H40_19_02={t_40_19_02_S1,S_40_19_02_S1;.....
          t_40_19_02_S2,S_40_19_02_S2;
         t_40_19_02_S3,S_40_19_02_S3;}
        %  t_40_19_02_S4,S_40_19_02_S4;
        % t_40_19_02_S5,S_40_19_02_S5;}


%clear t_40_19_02_S1 t_40_19_02_S2 t_40_19_02_S3 t_40_19_02_S4 t_40_19_02_S5
    S_40_19_02_S1 S_40_19_02_S2 S_40_19_02_S3 S_40_19_02_S4 S_40_19_02_S5

% hysteresis loop preparation H40_19_02:
data=H40_19_02
stepsize_valley=455;
cycles=4; % amount of cycles on the sample
samples=3; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=1; %should give output: to be equal to the amount of cycles
```

```
peakdistance=4; %should give output: to be equal to the amount of cycles

% for i=1:3
%     [peakH40_19_02{i,2},peakH40_19_02{i,1}]=findpeaks(H40_19_02{i,2},'
    MinPeakProminence', peakheight, 'MinPeakDistance', peakdistance) %gives 5
% end

[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
    first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
    First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
    first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
    Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
    std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
    new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
    cycles,samples,strain_level,desired_vector_lengths)
%new_time_vector200=0:(80/199):80;
peakH40_19_02=Absolute_1_line(end);
for i=1:samples
One_line_meanH40_19_02(1,i)=One_line_mean{1,i}(end)
Young_per_sampleH40_19_02(1,i)=Young_per_sample{1,i}(end)
end

%plot(t_40_19_02_S4,S_40_19_02_S4) % dont use, fell of platform, low peaks because
    of it
%plot(t_40_19_02_S5,S_40_19_02_S5) % dont use, fell of platform

figure(7)
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'b');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'b');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'b');
end
end
title('1 mm height 40%H all samples + all cycles, 19-02-2021 80% compression.')
lgd=legend('40%H')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(8)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
title('1 mm height 40%H average 19-02-2021 hysteresis as 1 line the 5, 80%
    compression.')
lgd=legend('1%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
```

```
figure(4) %graph std's
plot(new_time_vector/100,Absolute_1_line)
title('1 mm height 40%H average 19-02-2021 hysteresis as 1 line: 80% compression.'
    )
lgd=legend('1%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

figure(10)
plot(new_time_vector/100,dy)
title('1 mm height 40%H samples 19-02-2021: tangent modulus average hysteresis in
    1 line.')
lgd=legend('40%H')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(11)
bar(perc, Young_avg_per_percentage,'b')
title('Youngh modulus 1 mm height 1%H samples 19-02-2021: tangent modulus average
    hysteresis in 1 line.')
lgd=legend('40%H')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')

Young_avg_per_percentage_40H_19_02=Young_avg_per_percentage;

%safe the data:
samples40_19_2=samples;
cycles40_19_2=cycles;
newy_ds40_19_2=newy_ds;
newy_is40_19_2=newy_is;
Absolute_1_line40_19_2=Absolute_1_line;
One_line_mean40_19_2=One_line_mean;
points_mean40_19_2=points_mean;
dy40_19_2=dy;
perc40_19_2=perc;
Young_avg_per_percentage40_19_2=Young_avg_per_percentage;
std_10_20_etc40_19_2=std_10_20_etc;

%save for the first cycle
One_line_is_and_ds40_19_2=One_line_is_and_ds;
First_cycle_One_line_mean40_19_2=First_cycle_One_line_mean;
first_cycle_points_mean40_19_2=first_cycle_points_mean;
first_cycle_std_10_20_etc40_19_2=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_40_19_2=first_cycle_Young_avg_per_percentage;


%% 19-02 fitting needed!
t_99_19_02_S1=timeT99_19_02{1,1}{1,1}(1:9125); %9126 is endpoint 20 cycle loop
S_99_19_02_S1=Stress99_19_02{1,1}{1,1}(1:9104);
t_99_19_02_S2=timeT99_19_02{1,2}{1,1}(1:7922);
S_99_19_02_S2=Stress99_19_02{1,2}{1,1}(1:7922); %9126 is endpoint 20 cycle loop
t_99_19_02_S3=timeT99_19_02{1,3}{1,1}(1:9125);
S_99_19_02_S3=Stress99_19_02{1,3}{1,1}(1:9125);
t_99_19_02_S4=timeT99_19_02{1,4}{1,1}(1:9125);
S_99_19_02_S4=Stress99_19_02{1,4}{1,1}(1:9125);
t_99_19_02_S5=timeT99_19_02{1,5}{1,1}(1:9125);
```

```
S_99_19_02_S5=Stress99_19_02{1,5}{1,1}(1:9125); % the +3 is tear to zero. The
    minus is to bring the x-axis to zero, and the selected time frame to get the
    right window of 20 cycles

[y,yfit1] = bf(S_99_19_02_S1,'confirm','linear')
S_99_19_02_S1=S_99_19_02_S1-yfit1 % fitting needed
[y,yfit2] = bf(S_99_19_02_S2,'confirm','linear')
S_99_19_02_S2=S_99_19_02_S2-yfit2 % fitting needed
[y,yfit3] = bf(S_99_19_02_S3,'confirm','linear')
S_99_19_02_S3=S_99_19_02_S3-yfit3 % fitting needed
[y,yfit4] = bf(S_99_19_02_S4,'confirm','linear')
S_99_19_02_S4=S_99_19_02_S4-yfit4 % fitting needed
[y,yfit5] = bf(S_99_19_02_S5,'confirm','linear')
S_99_19_02_S5=S_99_19_02_S5-yfit5 % fitting needed

H99_19_02={t_99_19_02_S1,S_99_19_02_S1;........
          t_99_19_02_S2,S_99_19_02_S2;
         t_99_19_02_S3,S_99_19_02_S3;
         t_99_19_02_S4,S_99_19_02_S4;
         t_99_19_02_S5,S_99_19_02_S5;}

% align peaks!
%
% for i=1:4
%     [peakH99_19_02{i,2},peakH99_19_02{i,1}]=findpeaks(H99_19_02{i,2},'
    MinPeakProminence', 0.1, 'MinPeakDistance', 4) %gives 5
% end
% %Bring the only 17 peak sample forward

%nummering s2: 1205 = 1.
%H99_19_02_avg_t=(H99_19_02{1,1}+H99_19_02{3,1}(1:9125)+H99_19_02{4,1}(1:9125)+
    H99_19_02{5,1})/4;


H99_19_02_avg_S_part1=(S_99_19_02_S1(1:1204)+S_99_19_02_S3(1:1204)+S_99_19_02_S4
    (1:1204)+S_99_19_02_S5(1:1204))/4;
%H99_19_02_avg_S_part2=(S_99_19_02_S1(1205:9125)+S_99_19_02_S2(1:7921)+
    S_99_19_02_S3(1205:9125)+S_99_19_02_S4(1205:9125)+S_99_19_02_S5(1205:9125))/5;
%H99_19_02_avg_S=[H99_19_02_avg_S_part1; H99_19_02_avg_S_part2];
%%
% hysteresis loop preparation H99_19_02:
data=H99_19_02
stepsize_valley=455;
cycles=16;%normally 20 % amount of cycles on the sample
samples=5; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=0.2; %should give output: to be equal to the amount of cycles
peakdistance=4; %should give output: to be equal to the amount of cycles

% for i=1:4
%     [peakH99_19_02{i,2},peakH99_19_02{i,1}]=findpeaks(data{i,2},'
    MinPeakProminence', peakheight, 'MinPeakDistance', peakdistance) %gives 5
% end
% peakH99_19_02=Absolute_1_line(end);
```

```
[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
    first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
    First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
    first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
    Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
    std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
    new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
    cycles,samples,strain_level,desired_vector_lengths)

for i=1:samples
One_line_meanH99_19_02(1,i)=One_line_mean{1,i}(end)
Young_per_sampleH99_19_02(1,i)=Young_per_sample{1,i}(end)
end
figure(7)
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'g');
end
end
title('1 mm height 99%H all samples + all cycles, 19-02-2021 80% compression.')
lgd=legend('99%H')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(8)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
title('1 mm height 99%H average 19-02-2021 hysteresis as 1 line the 5, 80%
   compression.')
lgd=legend('99%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(9)
plot(new_time_vector/100,Absolute_1_line)
title('1 mm height 99%H average 19-02-2021 hysteresis as 1 line: 80% compression.'
   )
lgd=legend('99%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

figure(10)
plot(new_time_vector/100,dy)
```

```
title('1 mm height 99%H samples 19-02-2021: tangent modulus average hysteresis in
    1 line.')
lgd=legend('99%H')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(11)
bar(perc, Young_avg_per_percentage,'b')
title('Youngh modulus 1 mm height 99%H samples 19-02-2021: tangent modulus average
     hysteresis in 1 line.')
lgd=legend('99%H')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')

Young_avg_per_percentage_99H_19_02=Young_avg_per_percentage

%safe the data:
samples99_19_2=samples;
cycles99_19_2=cycles;
newy_ds99_19_2=newy_ds;
newy_is99_19_2=newy_is;
Absolute_1_line99_19_2=Absolute_1_line;
One_line_mean99_19_2=One_line_mean;
points_mean99_19_2=points_mean;
dy99_19_2=dy;
perc99_19_2=perc;
Young_avg_per_percentage99_19_2=Young_avg_per_percentage;

One_line_is_and_ds99_19_2=One_line_is_and_ds;
std_10_20_etc99_19_2=std_10_20_etc;
First_cycle_One_line_mean99_19_2=First_cycle_One_line_mean;
first_cycle_points_mean99_19_2=first_cycle_points_mean;
first_cycle_std_10_20_etc99_19_2=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_99_19_2=first_cycle_Young_avg_per_percentage;

%clear t_99_19_02_S1 t_99_19_02_S2 t_99_19_02_S3 t_99_19_02_S4 t_99_19_02_S5
    S_99_19_02_S1 S_99_19_02_S2 S_99_19_02_S3 S_99_19_02_S4 S_99_19_02_S5
%% FINAL GRAPHS 80% compresion 19_2
%%
% 19-02-2021

y=First_cycle_One_line_mean1_19_2
t=new_time_vector/100
dy=gradient(y)./gradient(t)
k=indices(1,7)
tang=(t-t(k))*dy(k)+y(k)


figure(1001)
a=plot(new_time_vector/100,First_cycle_One_line_mean1_19_2,'y');
hold on
b=plot(new_time_vector/100,First_cycle_One_line_mean40_19_2,'b');
hold on
c=plot(new_time_vector/100,First_cycle_One_line_mean99_19_2,'g');
title('TAs: 1 mm high, first cycle of compression.')
xlabel('Nominal strain [-]')
ylabel('Nominal stress [kPa]')
lgd=legend([a b c],'1%H','40%H','99%H')
hold on
plot(t,tang)
```

```
errorbar(new_time_vector(indices)/100,first_cycle_points_mean1_19_2,
    first_cycle_std_10_20_etc1_19_2,'.k')
errorbar(new_time_vector(indices)/100,first_cycle_points_mean40_19_2,
    first_cycle_std_10_20_etc40_19_2,'.k')
errorbar(new_time_vector(indices)/100,first_cycle_points_mean99_19_2,
    first_cycle_std_10_20_etc99_19_2,'.k')




figure(1002)
hold all
a=bar(perc, first_cycle_Young_avg_per_percentage_1_19_2,'y')
b=bar(perc, first_cycle_Young_avg_per_percentage_40_19_2,'b')
c=bar(perc, first_cycle_Young_avg_per_percentage_99_19_2,'g')
title('TAs: 1 mm high, first cycle of compression.')
lgd=legend([a b c],'1%H','40%H','99%H')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')

figure(1011)
perc10=[first_cycle_Young_avg_per_percentage_1_19_2(1,1)
    first_cycle_Young_avg_per_percentage_40_19_2(1,1)
    first_cycle_Young_avg_per_percentage_99_19_2(1,1)];
perc20=[first_cycle_Young_avg_per_percentage_1_19_2(1,2)
    first_cycle_Young_avg_per_percentage_40_19_2(1,2)
    first_cycle_Young_avg_per_percentage_99_19_2(1,2)];
perc30=[first_cycle_Young_avg_per_percentage_1_19_2(1,3)
    first_cycle_Young_avg_per_percentage_40_19_2(1,3)
    first_cycle_Young_avg_per_percentage_99_19_2(1,3)];
perc40=[first_cycle_Young_avg_per_percentage_1_19_2(1,4)
    first_cycle_Young_avg_per_percentage_40_19_2(1,4)
    first_cycle_Young_avg_per_percentage_99_19_2(1,4)];
perc50=[first_cycle_Young_avg_per_percentage_1_19_2(1,5)
    first_cycle_Young_avg_per_percentage_40_19_2(1,5)
    first_cycle_Young_avg_per_percentage_99_19_2(1,5)];
perc60=[first_cycle_Young_avg_per_percentage_1_19_2(1,6)
    first_cycle_Young_avg_per_percentage_40_19_2(1,6)
    first_cycle_Young_avg_per_percentage_99_19_2(1,6)];
perc70=[first_cycle_Young_avg_per_percentage_1_19_2(1,7)
    first_cycle_Young_avg_per_percentage_40_19_2(1,7)
    first_cycle_Young_avg_per_percentage_99_19_2(1,7)];
perc80=[first_cycle_Young_avg_per_percentage_1_19_2(1,8)
    first_cycle_Young_avg_per_percentage_40_19_2(1,8)
    first_cycle_Young_avg_per_percentage_99_19_2(1,8)];
all=[perc10'
    perc20'
    perc30'
    perc40'
    perc50'
    perc60'
    perc70'
    perc80'];
figure(1)
b=bar(perc,all)
b(1).FaceColor = 'y'
b(2).FaceColor = 'b'
b(3).FaceColor = 'g'
title('TAs: 1 mm high, first cycle of compression.')
xlabel('Nominal strain [%]')
```

```
ylabel('Tangent modulus [kPa]')


%% ONE WAY ANOVA

% The ANOVA test makes the following assumptions about the data in X:
%
% All sample populations are normally distributed.
% All sample populations have equal variance.
% All observations are mutually independent.

for i = 1:numel(One_line_is_and_ds1_19_2)
  One_line_is_and_ds1_19_2{1,i} = transpose(One_line_is_and_ds1_19_2{1,i});
end
G1_19_2=cell2mat(One_line_is_and_ds1_19_2);

for i = 1:numel(One_line_is_and_ds40_19_2)
  One_line_is_and_ds40_19_2{1,i} = transpose(One_line_is_and_ds40_19_2{1,i});
end
G40_19_2=cell2mat(One_line_is_and_ds40_19_2);

for i = 1:numel(One_line_is_and_ds99_19_2)
  One_line_is_and_ds99_19_2{1,i} = transpose(One_line_is_and_ds99_19_2{1,i});
end
G99_19_2=cell2mat(One_line_is_and_ds99_19_2);

First_cycle_all_19_2=[G1_19_2 G40_19_2 G99_19_2];

BF_test_values=First_cycle_all_19_2(:)
BF_test_label=zeros((200*(numel(One_line_is_and_ds1_19_2)+numel(
    One_line_is_and_ds40_19_2)+numel(One_line_is_and_ds99_19_2))),1)
R1=1;
R2=2;
R3=3;
BF_test_label(1:(200*(numel(One_line_is_and_ds1_19_2))),:)=R1
BF_test_label((200*(numel(One_line_is_and_ds1_19_2)))+1:(200*(numel(
    One_line_is_and_ds1_19_2)+numel(One_line_is_and_ds40_19_2))),:)=R2
BF_test_label(1+(200*(numel(One_line_is_and_ds1_19_2)+numel(
    One_line_is_and_ds40_19_2))):(200*(numel(One_line_is_and_ds1_19_2)+numel(
    One_line_is_and_ds40_19_2)+numel(One_line_is_and_ds99_19_2))),:)=R3

BF_test_19_2=[BF_test_values BF_test_label]; % put this in spps

%per percentage


%% 19-02 60% compression
%%
t_40_19_02_60_S2=timeT40_19_02_60{1,2}{1,1}(1:1659);
S_40_19_02_60_S2=Stress40_19_02_60{1,2}{1,1}(1:1659)+0.326427;
t_40_19_02_60_S3=timeT40_19_02_60{1,3}{1,1}(1:1659);
S_40_19_02_60_S3=Stress40_19_02_60{1,3}{1,1}(1:1659)-0.225;
t_40_19_02_60_S4=timeT40_19_02_60{1,4}{1,1}(1:1659);
S_40_19_02_60_S4=Stress40_19_02_60{1,4}{1,1}(1:1659)-0.06465;

H40_19_02_60_avg_S=(S_40_19_02_60_S2+S_40_19_02_60_S3+S_40_19_02_60_S4)/3;
H40_19_02_60_avg_t=(t_40_19_02_60_S2+t_40_19_02_60_S3+t_40_19_02_60_S4)/3;

H40_19_02_60={t_40_19_02_60_S2,S_40_19_02_60_S2;.......
         t_40_19_02_60_S3,S_40_19_02_60_S3;
        t_40_19_02_60_S4,S_40_19_02_60_S4;};
```

```
figure(77)
plot(H40_19_02_60_avg_t,H40_19_02_60_avg_S)

clear t_40_19_02_60_S2 S_40_19_02_60_S2 t_40_19_02_60_S3 S_40_19_02_60_S3
    t_40_19_02_60_S4 S_40_19_02_60_S4
%%
t_1_19_02_60_S1=timeT1_19_02_60{1,1}{1,1}(1:1659);
S_1_19_02_60_S1=Stress1_19_02_60{1,1}{1,1}(1:1659);
t_1_19_02_60_S2=timeT1_19_02_60{1,2}{1,1}(1:1659);
S_1_19_02_60_S2=Stress1_19_02_60{1,2}{1,1}(1:1659);
t_1_19_02_60_S3=timeT1_19_02_60{1,3}{1,1}(1:1659);
S_1_19_02_60_S3=Stress1_19_02_60{1,3}{1,1}(1:1659);
t_1_19_02_60_S4=timeT1_19_02_60{1,4}{1,1}(1:1391);
S_1_19_02_60_S4=Stress1_19_02_60{1,4}{1,1}(1:1391);
t_1_19_02_60_S5=timeT1_19_02_60{1,5}{1,1}(1:1659);
S_1_19_02_60_S5=Stress1_19_02_60{1,5}{1,1}(1:1659);


H1_19_02_60_avg_S_part1=(S_1_19_02_60_S1(1:1391)+S_1_19_02_60_S2(1:1391)+
    S_1_19_02_60_S3(1:1391)+S_1_19_02_60_S4(1:1391)+S_1_19_02_60_S5(1:1391))/5;
H1_19_02_60_avg_S_part2=(S_1_19_02_60_S1(1392:1659)+S_1_19_02_60_S2(1392:1659)+
    S_1_19_02_60_S3(1392:1659)+S_1_19_02_60_S5(1392:1659))/4;

H1_19_02_60_avg_t_part1=(t_1_19_02_60_S1(1:1391)+t_1_19_02_60_S2(1:1391)+
    t_1_19_02_60_S3(1:1391)+t_1_19_02_60_S4(1:1391)+t_1_19_02_60_S5(1:1391))/5;
H1_19_02_60_avg_t_part2=(t_1_19_02_60_S1(1392:1659)+t_1_19_02_60_S2(1392:1659)+
    t_1_19_02_60_S3(1392:1659)+t_1_19_02_60_S5(1392:1659))/4;


H1_19_02_60_avg_S=[H1_19_02_60_avg_S_part1;H1_19_02_60_avg_S_part2];
H1_19_02_60_avg_t=[H1_19_02_60_avg_t_part1;H1_19_02_60_avg_t_part2];

H1_19_02_60={t_1_19_02_60_S1,S_1_19_02_60_S1;
        t_1_19_02_60_S2,S_1_19_02_60_S2;........
         t_1_19_02_60_S3,S_1_19_02_60_S3;
        t_1_19_02_60_S4,S_1_19_02_60_S4;
        t_1_19_02_60_S5,S_1_19_02_60_S5;};

clear t_1_19_02_60_S1 t_1_19_02_60_S2 t_1_19_02_60_S3 t_1_19_02_60_S4
    t_1_19_02_60_S5 S_1_19_02_60_S1 S_1_19_02_60_S2 S_1_19_02_60_S3 S_1_19_02_60_S4
     S_1_19_02_60_S5
%% 02-03
%%
t_99_2_3_60_S1=timeT99_2_3_60{1,1}{1,1}(1:1405);
S_99_2_3_60_S1=Stress99_2_3_60{1,1}{1,1}(1:1405)-0.06494;
t_99_2_3_60_S2=timeT99_2_3_60{1,2}{1,1}(1:1405);
S_99_2_3_60_S2=Stress99_2_3_60{1,2}{1,1}(1:1405)-0.05376;
t_99_2_3_60_S3=timeT99_2_3_60{1,3}{1,1}(1:1260);
S_99_2_3_60_S3=Stress99_2_3_60{1,3}{1,1}(1:1260)+0.07383;
t_99_2_3_60_S4=timeT99_2_3_60{1,4}{1,1}(1:1049);
S_99_2_3_60_S4=Stress99_2_3_60{1,4}{1,1}(1:1049);
t_99_2_3_60_S5=timeT99_2_3_60{1,5}{1,1}(1:1405);
S_99_2_3_60_S5=Stress99_2_3_60{1,5}{1,1}(1:1405)-0.1526;

H99_2_3_60_avg_S_part1=(S_99_2_3_60_S1(1:1049)+S_99_2_3_60_S2(1:1049)+
    S_99_2_3_60_S3(1:1049)+S_99_2_3_60_S4(1:1049)+S_99_2_3_60_S5(1:1049))/5;
H99_2_3_60_avg_S_part2=(S_99_2_3_60_S1(1050:1260)+S_99_2_3_60_S2(1050:1260)+
    S_99_2_3_60_S3(1050:1260)+S_99_2_3_60_S5(1050:1260))/4;
H99_2_3_60_avg_S_part3=(S_99_2_3_60_S1(1261:1405)+S_99_2_3_60_S2(1261:1405)+
    S_99_2_3_60_S5(1261:1405))/3;
```

```
H99_2_3_60_avg_t_part1=(t_99_2_3_60_S1(1:1049)+t_99_2_3_60_S2(1:1049)+
    t_99_2_3_60_S3(1:1049)+t_99_2_3_60_S4(1:1049)+t_99_2_3_60_S5(1:1049))/5;
H99_2_3_60_avg_t_part2=(t_99_2_3_60_S1(1050:1260)+t_99_2_3_60_S2(1050:1260)+
    t_99_2_3_60_S3(1050:1260)+t_99_2_3_60_S5(1050:1260))/4;
H99_2_3_60_avg_t_part3=(t_99_2_3_60_S1(1261:1405)+t_99_2_3_60_S2(1261:1405)+
    t_99_2_3_60_S5(1261:1405))/3;

H99_2_3_60_avg_S=[H99_2_3_60_avg_S_part1;H99_2_3_60_avg_S_part2;
    H99_2_3_60_avg_S_part3];
H99_2_3_60_avg_t=[H99_2_3_60_avg_t_part1;H99_2_3_60_avg_t_part2;
    H99_2_3_60_avg_t_part3];

H99_2_3_60={t_99_2_3_60_S1,S_99_2_3_60_S1;
        t_99_2_3_60_S2,S_99_2_3_60_S2;........
         t_99_2_3_60_S3,S_99_2_3_60_S3;
        t_99_2_3_60_S4,S_99_2_3_60_S4;
        t_99_2_3_60_S5,S_99_2_3_60_S5;};

figure(15)
plot(H99_2_3_60_avg_t,H99_2_3_60_avg_S)

%% 2-3: 3 mm plots

t_1_2_3_S1=timeT1_2_3{1,1}{1,1}(1:8380); %9126 is endpoint 20 cycle loop
S_1_2_3_S1=Stress1_2_3{1,1}{1,1}(1:8380)%+0.1062;
t_1_2_3_S2=timeT1_2_3{1,2}{1,1}(1:8380);
S_1_2_3_S2=Stress1_2_3{1,2}{1,1}(1:8380)%+0.04249; %9126 is endpoint 20 cycle loop
t_1_2_3_S3=timeT1_2_3{1,3}{1,1}(1:8380);
S_1_2_3_S3=Stress1_2_3{1,3}{1,1}(1:8380)%+0.7419;
t_1_2_3_S4=timeT1_2_3{1,4}{1,1}(1:6441);
S_1_2_3_S4=Stress1_2_3{1,4}{1,1}(1:6441);
t_1_2_3_S5=timeT1_2_3{1,5}{1,1}(1:8380);
S_1_2_3_S5=Stress1_2_3{1,5}{1,1}(1:8380)%-0.04051; % the +3 is tear to zero. The
    minus is to bring the x-axis to zero, and the selected time frame to get the
    right window of 20 cycles

H1_2_3_avg_t=(t_1_2_3_S1+t_1_2_3_S2+t_1_2_3_S3+t_1_2_3_S5)/4;
H1_2_3_avg_S=(S_1_2_3_S1+S_1_2_3_S2+S_1_2_3_S3+S_1_2_3_S5)/4;

H1_2_3={t_1_2_3_S1,S_1_2_3_S1;........
         t_1_2_3_S2,S_1_2_3_S2;
        t_1_2_3_S3,S_1_2_3_S3;
        t_1_2_3_S4,S_1_2_3_S4;
        t_1_2_3_S5,S_1_2_3_S5;};

figure(16)
plot(H1_2_3_avg_t,H1_2_3_avg_S)


%clear t_1_2_3_S1 t_1_2_3_S2 t_1_2_3_S3 t_1_2_3_S4 t_1_2_3_S5 S_1_2_3_S1
    S_1_2_3_S2 S_1_2_3_S3 S_1_2_3_S4 S_1_2_3_S5


clear peakS peakt newy_ds olds_d newy_d old_d Young_per_sample
    Young_avg_per_percentage dy points_mean indices Absolute_1_line std_per_strain
    std_10_20_etc One_line_mean mean_per_sample_decr mean_per_sample_incr newy_is
    new_time_vector perc
% hysteresis loop preparation H1_2_3:
data=H1_2_3;
stepsize_valley=455;
```

```
cycles=3; % why so low??????
samples=5; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=30; %should give output: to be equal to the amount of cycles
peakdistance=40; %should give output: to be equal to the amount of cycles

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end



[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
   first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
   First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
   first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
   Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
   std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
   new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
   cycles,samples,strain_level,desired_vector_lengths)
% 1x200 newy_ds,olds_d,newy_d,old_d,

%[Young_per_sample,Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line
   ,std_per_strain,std_10_20_etc,One_line_mean,sum_per_sample_decr,
   sum_per_sample_incr,newy_is,newy_ds,new_time_vector]=hysteresis2(peakheight,
   peakdistance,data,stepsize_valley,cycles,samples,strain_level,
   desired_vector_lengths)
%new_time_vector200=0:(80/199):80;

figure(120)
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'y');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'y');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'y');
end
end
title('1 mm height 1%H 3mm height all samples + all cycles, 19-02-2021 80%
   compression.')
lgd=legend('1%H')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(13)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
```

```
title('1 mm height 1%H 3mm height average 19-02-2021 hysteresis as 1 line the 5,
    80% compression.')
lgd=legend('1%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(14)
plot(new_time_vector/100,Absolute_1_line)
title('1%H 3mm height average 02-03-2021 hysteresis as 1 line: 80% compression.')
lgd=legend('1%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

new_time_vector_1H_2_3=new_time_vector;
std_10_20_etc_1H_2_3=std_10_20_etc;
Absolute_1_line_1H_2_3=Absolute_1_line;
indices_1H_2_3=indices;
points_mean_1H_2_3=points_mean;

One_line_is_and_ds1_2_3=One_line_is_and_ds;
std_10_20_etc1_2_3=std_10_20_etc;
First_cycle_One_line_mean1_2_3=First_cycle_One_line_mean;
first_cycle_points_mean1_2_3=first_cycle_points_mean;
first_cycle_std_10_20_etc1_2_3=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_1_2_3=first_cycle_Young_avg_per_percentage;

figure(15)
plot(new_time_vector/100,dy)
title('1 mm height 1%H 3mm height samples 19-02-2021: tangent modulus average
    hysteresis in 1 line.')
lgd=legend('1%H')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(16)
bar(perc, Young_avg_per_percentage,'y')
title('Youngh modulus 1%H 3mm height samples 19-02-2021: tangent modulus average
    hysteresis in 1 line.')
lgd=legend('1%H')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')

Young_avg_per_percentage_1H_2_3=Young_avg_per_percentage









%% 2-3: 3 mm: avg grafiek is niet goed nog
b=8279;
a=110;
t_40_2_3_S1=timeT40_2_3{1,1}{1,1}(1:8280);
S_40_2_3_S1=Stress40_2_3{1,1}{1,1}(1:8280)-0.011;
t_40_2_3_S2=timeT40_2_3{1,2}{1,1}(a:a+b);
```

```
S_40_2_3_S2=Stress40_2_3{1,2}{1,1}(a:a+b);
t_40_2_3_S3=timeT40_2_3{1,3}{1,1}(a:a+b);
S_40_2_3_S3=Stress40_2_3{1,3}{1,1}(a:a+b)+0.59;
t_40_2_3_S4=timeT40_2_3{1,4}{1,1}(a:a+b);
S_40_2_3_S4=Stress40_2_3{1,4}{1,1}(a:a+b)+0.03577;
t_40_2_3_S5=timeT40_2_3{1,5}{1,1}(a:a+b);
S_40_2_3_S5=Stress40_2_3{1,5}{1,1}(a:a+b)+0.2;


H40_2_3={t_40_2_3_S1,S_40_2_3_S1;........
         t_40_2_3_S2,S_40_2_3_S2;
        t_40_2_3_S3,S_40_2_3_S3;
        t_40_2_3_S4,S_40_2_3_S4;
        t_40_2_3_S5,S_40_2_3_S5;};

H40_2_3_avg_S=(S_40_2_3_S1+S_40_2_3_S2+S_40_2_3_S3+S_40_2_3_S4+S_40_2_3_S5)/5  ;
H40_2_3_avg_t=(t_40_2_3_S2+t_40_2_3_S3+t_40_2_3_S4+t_40_2_3_S5)/4   ;

%eerste piek is 9.399 van S1
% eerste piek rest is

figure(71)
plot(t_40_2_3_S1,S_40_2_3_S1)
hold on
plot(t_40_2_3_S2,S_40_2_3_S2)


figure(9)
plot(H40_2_3_avg_t,H40_2_3_avg_S)


clear peakS peakt timestamp_sample_d_rev newy_ds olds_d newy_d old_d
    Young_per_sample Young_avg_per_percentage dy points_mean indices
    Absolute_1_line std_per_strain std_10_20_etc One_line_mean mean_per_sample_decr
     mean_per_sample_incr newy_is new_time_vector perc peakheight peakdistance data
     stepsize_valley cycles samples strain_level desired_vector_lengths

% hysteresis loop preparation H1_2_3:
data=H40_2_3;
stepsize_valley=455;
cycles=4; % waarom zo laag????
samples=5; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=10; %should give output: to be equal to the amount of cycles
peakdistance=40; %should give output: to be equal to the amount of cycles

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end

[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
    first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
    First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
    first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
    Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
    std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
    new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
    cycles,samples,strain_level,desired_vector_lengths)
```

```
figure(17)
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'g');
end
end
title('3 mm height 40%H 3mm height all samples + all cycles, 19-02-2021 80%
    compression.')
lgd=legend('40%H')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(18)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
title('3 mm height 40%H 3mm height average 19-02-2021 hysteresis as 1 line the 5,
    80% compression.')
lgd=legend('40%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(19)
plot(new_time_vector/100,Absolute_1_line)
title('3 mm height 40%H 3mm height average 19-02-2021 hysteresis as 1 line: 80%
    compression.')
lgd=legend('40%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

new_time_vector_40H_2_3=new_time_vector;
Absolute_1_line_40H_2_3=Absolute_1_line;
new_time_vector_40H_2_3=new_time_vector;
indices_40H_2_3=indices
points_mean_40H_2_3=points_mean;
std_10_20_etc_40H_2_3=std_10_20_etc;
Young_avg_per_percentage_40H_2_3=Young_avg_per_percentage;

One_line_is_and_ds40_2_3=One_line_is_and_ds;
std_10_20_etc40_2_3=std_10_20_etc;
First_cycle_One_line_mean40_2_3=First_cycle_One_line_mean;
first_cycle_points_mean40_2_3=first_cycle_points_mean;
first_cycle_std_10_20_etc40_2_3=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_40_2_3=first_cycle_Young_avg_per_percentage;
```

```
figure(20)
plot(new_time_vector/100,dy)
title('3 mm height 40%H 3mm height samples 19-02-2021: tangent modulus average
    hysteresis in 1 line.')
lgd=legend('40%H')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(21)
bar(perc, Young_avg_per_percentage,'y')
title('Youngh modulus 40%H 3mm height samples 19-02-2021: tangent modulus average
    hysteresis in 1 line.')
lgd=legend('40%H')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')



%clear t_40_2_3_S1 t_40_2_3_S2 t_40_2_3_S3 t_40_2_3_S4 t_40_2_3_S5 S_40_2_3_S1
    S_40_2_3_S2 S_40_2_3_S3 S_40_2_3_S4 S_40_2_3_S5
%% 2-3: 3 mm
t_99_2_3_S1=timeT99_2_3{1,1}{1,1}(1:8280);
S_99_2_3_S1=Stress99_2_3{1,1}{1,1}(1:8280)-0.00669; % last 10 cycli
t_99_2_3_S2=timeT99_2_3{1,2}{1,1}(1:3250);
S_99_2_3_S2=Stress99_2_3{1,2}{1,1}(1:3250)+0.006538; % only 3 real tops
t_99_2_3_S3=timeT99_2_3{1,3}{1,1}(1:8380);
S_99_2_3_S3=Stress99_2_3{1,3}{1,1}(1:8380);
t_99_2_3_S4=timeT99_2_3{1,4}{1,1}(1:8380);
S_99_2_3_S4=Stress99_2_3{1,4}{1,1}(1:8380);
t_99_2_3_S5=timeT99_2_3{1,5}{1,1}(1:8380);
S_99_2_3_S5=Stress99_2_3{1,5}{1,1}(1:8380);

H99_2_3_avg_part1_S=(S_99_2_3_S1(1:3250)+S_99_2_3_S2(1:3250)+S_99_2_3_S3(1:3250)+
    S_99_2_3_S4(1:3250)+S_99_2_3_S5(1:3250))/5;
H99_2_3_avg_part2_S=(S_99_2_3_S1(3251:8280)+S_99_2_3_S3(3251:8280)+S_99_2_3_S4
    (3251:8280)+S_99_2_3_S5(3251:8280))/4;
H99_2_3_avg_part3_S=(S_99_2_3_S3(8280:8380)+S_99_2_3_S4(8280:8380)+S_99_2_3_S5
    (8280:8380))/3;
H99_2_3_avg_S=[H99_2_3_avg_part1_S;H99_2_3_avg_part2_S;H99_2_3_avg_part3_S];

H99_2_3_avg_part1_t=(t_99_2_3_S1(1:3250)+t_99_2_3_S2(1:3250)+t_99_2_3_S3(1:3250)+
    t_99_2_3_S4(1:3250)+t_99_2_3_S5(1:3250))/5;
H99_2_3_avg_part2_t=(t_99_2_3_S1(3251:8280)+t_99_2_3_S3(3251:8280)+t_99_2_3_S4
    (3251:8280)+t_99_2_3_S5(3251:8280))/4;
H99_2_3_avg_part3_t=(t_99_2_3_S3(8280:8380)+t_99_2_3_S4(8280:8380)+t_99_2_3_S5
    (8280:8380))/3;
H99_2_3_avg_t=[H99_2_3_avg_part1_t;H99_2_3_avg_part2_t;H99_2_3_avg_part3_t];

H99_2_3={t_99_2_3_S1,S_99_2_3_S1;.......
        t_99_2_3_S2,S_99_2_3_S2;
        t_99_2_3_S3,S_99_2_3_S3;
        t_99_2_3_S4,S_99_2_3_S4;
        t_99_2_3_S5,S_99_2_3_S5;};

    figure(89)
    plot(H99_2_3_avg_t,H99_2_3_avg_S)
clear t_99_2_3_S1 t_99_2_3_S2 t_99_2_3_S3 t_99_2_3_S4 t_99_2_3_S5 S_99_2_3_S1
    S_99_2_3_S2 S_99_2_3_S3 S_99_2_3_S4 S_99_2_3_S5

clear peakS peakt
% hysteresis loop preparation H1_2_3:
```

```
data=H99_2_3;
stepsize_valley=455;
cycles=3; % amount of cycles on the sample
samples=5; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=0.018; %should give output: to be equal to the amount of cycles
peakdistance=15; %should give output: to be equal to the amount of cycles

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end
peakS
peakt
[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
   first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
   First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
   first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
   Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
   std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
   new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
   cycles,samples,strain_level,desired_vector_lengths)

figure(22)
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'g');
end
end
title('3 mm height 40%H 3mm height all samples + all cycles, 19-02-2021 80%
   compression.')
lgd=legend('40%H')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(23)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
title('3 mm height 40%H 3mm height average 19-02-2021 hysteresis as 1 line the 5,
   80% compression.')
lgd=legend('40%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(24)
```

```
plot(new_time_vector/100,Absolute_1_line)
title('3 mm height 40%H 3mm height average 19-02-2021 hysteresis as 1 line: 80%
    compression.')
lgd=legend('40%H')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

new_time_vector_99H_2_3=new_time_vector;
Absolute_1_line_99H_2_3=Absolute_1_line;
new_time_vector_99H_2_3=new_time_vector;
indices_99H_2_3=indices;
points_mean_99H_2_3=points_mean;
std_10_20_etc_99H_2_3=std_10_20_etc;
One_line_is_and_ds99_2_3=One_line_is_and_ds;
std_10_20_etc99_2_3=std_10_20_etc;

First_cycle_One_line_mean99_2_3=First_cycle_One_line_mean;
first_cycle_points_mean99_2_3=first_cycle_points_mean;
first_cycle_std_10_20_etc99_2_3=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_99_2_3=first_cycle_Young_avg_per_percentage;


figure(25)
plot(new_time_vector/100,dy)
title('3 mm height 40%H 3mm height samples 19-02-2021: tangent modulus average
    hysteresis in 1 line.')
lgd=legend('40%H')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(26)
bar(perc, Young_avg_per_percentage,'y')
title('Youngh modulus 40%H 3mm height samples 19-02-2021: tangent modulus average
    hysteresis in 1 line.')
lgd=legend('40%H')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')


Young_avg_per_percentage_99H_2_3=Young_avg_per_percentage
%%
figure(1012)
perc10=[first_cycle_Young_avg_per_percentage_1_2_3(1,1)
    first_cycle_Young_avg_per_percentage_40_2_3(1,1)
    first_cycle_Young_avg_per_percentage_99_2_3(1,1)];
perc20=[first_cycle_Young_avg_per_percentage_1_2_3(1,2)
    first_cycle_Young_avg_per_percentage_40_2_3(1,2)
    first_cycle_Young_avg_per_percentage_99_2_3(1,2)];
perc30=[first_cycle_Young_avg_per_percentage_1_2_3(1,3)
    first_cycle_Young_avg_per_percentage_40_2_3(1,3)
    first_cycle_Young_avg_per_percentage_99_2_3(1,3)];
perc40=[first_cycle_Young_avg_per_percentage_1_2_3(1,4)
    first_cycle_Young_avg_per_percentage_40_2_3(1,4)
    first_cycle_Young_avg_per_percentage_99_2_3(1,4)];
perc50=[first_cycle_Young_avg_per_percentage_1_2_3(1,5)
    first_cycle_Young_avg_per_percentage_40_2_3(1,5)
    first_cycle_Young_avg_per_percentage_99_2_3(1,5)];
perc60=[first_cycle_Young_avg_per_percentage_1_2_3(1,6)
    first_cycle_Young_avg_per_percentage_40_2_3(1,6)
```

```
    first_cycle_Young_avg_per_percentage_99_2_3(1,6)];
perc70=[first_cycle_Young_avg_per_percentage_1_2_3(1,7)
    first_cycle_Young_avg_per_percentage_40_2_3(1,7)
    first_cycle_Young_avg_per_percentage_99_2_3(1,7)];
perc80=[first_cycle_Young_avg_per_percentage_1_2_3(1,8)
    first_cycle_Young_avg_per_percentage_40_2_3(1,8)
    first_cycle_Young_avg_per_percentage_99_2_3(1,8)];
all=[perc10'
    perc20'
    perc30'
    perc40'
    perc50'
    perc60'
    perc70'
    perc80'];
figure(1)
b=bar(perc,all)
b(1).FaceColor = 'y'
b(2).FaceColor = 'b'
b(3).FaceColor = 'g'
title('TAs: 3 mm high, first cycle of compression.')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')

%% FINAL GRAPHS 80% compresion 2_3
%%
%
figure(1003)
a=plot(new_time_vector/100,First_cycle_One_line_mean1_2_3,'y');
hold on
b=plot(new_time_vector/100,First_cycle_One_line_mean40_2_3-3,'b'); % -3 gedaan!!!!
hold on
c=plot(new_time_vector/100,First_cycle_One_line_mean99_2_3,'g');
title('TAs: 3 mm high, first cycle of compression.')
xlabel('Nominal strain [-]')
ylabel('Nominal stress [kPa]')
lgd=legend([a b c],'1%H','40%H','99%H')
hold on
errorbar(new_time_vector(indices)/100,first_cycle_points_mean1_2_3,
    first_cycle_std_10_20_etc1_2_3,'.k')
errorbar(new_time_vector(indices)/100,first_cycle_points_mean40_2_3-3,
    first_cycle_std_10_20_etc40_2_3,'.k')
errorbar(new_time_vector(indices)/100,first_cycle_points_mean99_2_3,
    first_cycle_std_10_20_etc99_2_3,'.k')

figure(1004)
hold all
a=bar(perc, first_cycle_Young_avg_per_percentage_1_2_3,'y')
b=bar(perc, first_cycle_Young_avg_per_percentage_40_2_3,'b')
c=bar(perc, first_cycle_Young_avg_per_percentage_99_2_3,'g')
title('TAs: 3 mm high, first cycle of compression.')
lgd=legend([a b c],'1%H','40%H','99%H')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')

for i = 1:numel(One_line_is_and_ds1_2_3)
  One_line_is_and_ds1_2_3{1,i} = transpose(One_line_is_and_ds1_2_3{1,i});
end
G1_2_3=cell2mat(One_line_is_and_ds1_2_3);

for i = 1:numel(One_line_is_and_ds40_2_3)
```

```
  One_line_is_and_ds40_2_3{1,i} = transpose(One_line_is_and_ds40_2_3{1,i});
end
G40_2_3=cell2mat(One_line_is_and_ds40_2_3);

for i = 1:numel(One_line_is_and_ds99_2_3)
  One_line_is_and_ds99_2_3{1,i} = transpose(One_line_is_and_ds99_2_3{1,i});
end
G99_2_3=cell2mat(One_line_is_and_ds99_2_3);

First_cycle_all_2_3=[G1_2_3 G40_2_3 G99_2_3];

BF_test_values=First_cycle_all_2_3(:)
BF_test_label=zeros((200*(numel(One_line_is_and_ds1_2_3)+numel(
    One_line_is_and_ds40_2_3)+numel(One_line_is_and_ds99_2_3))),1)
R1=1;
R2=2;
R3=3;
BF_test_label(1:(200*(numel(One_line_is_and_ds1_2_3))),:)=R1
BF_test_label((200*(numel(One_line_is_and_ds1_2_3)))+1:(200*(numel(
    One_line_is_and_ds1_2_3)+numel(One_line_is_and_ds40_2_3))),:)=R2
BF_test_label(1+(200*(numel(One_line_is_and_ds1_2_3)+numel(
    One_line_is_and_ds40_2_3))):(200*(numel(One_line_is_and_ds1_2_3)+numel(
    One_line_is_and_ds40_2_3)+numel(One_line_is_and_ds99_2_3))),:)=R3

BF_test_2_3=[BF_test_values BF_test_label];

%% final graphs 19-2 and 2-3 together
%%

%ANOVA per stress

First_cycle_perc_10p_stress_19_2=[1 1 1 1 1 2 2 2 3 3 3 3 3]'
for i=1:size(First_cycle_all_19_2,2)
First_cycle_perc_10p_stress_19_2(i,2)=[First_cycle_all_19_2(indices(1,1),i)]
First_cycle_perc_20p_stress_19_2(i,2)=[First_cycle_all_19_2(indices(1,2),i)]
First_cycle_perc_30p_stress_19_2(i,2)=[First_cycle_all_19_2(indices(1,3),i)]
First_cycle_perc_40p_stress_19_2(i,2)=[First_cycle_all_19_2(indices(1,4),i)]
First_cycle_perc_50p_stress_19_2(i,2)=[First_cycle_all_19_2(indices(1,5),i)]
First_cycle_perc_60p_stress_19_2(i,2)=[First_cycle_all_19_2(indices(1,6),i)]
First_cycle_perc_70p_stress_19_2(i,2)=[First_cycle_all_19_2(indices(1,7),i)]
First_cycle_perc_80p_stress_19_2(i,2)=[First_cycle_all_19_2(indices(1,8),i)]
end

First_cycle_perc_10p_stress_2_3=[1 1 1 1 1 2 2 2 2 2 3 3 3 3 3]'
for i=1:size(First_cycle_all_2_3,2)
First_cycle_perc_10p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,1),i)]
First_cycle_perc_10p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,1),i)]
First_cycle_perc_20p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,2),i)]
First_cycle_perc_30p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,3),i)]
First_cycle_perc_40p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,4),i)]
First_cycle_perc_50p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,5),i)]
First_cycle_perc_60p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,6),i)]
First_cycle_perc_70p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,7),i)]
First_cycle_perc_80p_stress_2_3(i,2)=[First_cycle_all_2_3(indices(1,8),i)]
end

%% Anova per youngs

% first_cycle_df=diff(First_cycle_One_line_mean);
% first_cycle_dy=gradient(First_cycle_One_line_mean(:))./gradient(new_time_vector
    (:)/100)
```

```
% first_cycle_Young_avg_per_percentage=[first_cycle_dy(index10) first_cycle_dy(
    index20) first_cycle_dy(index30) first_cycle_dy(index40) first_cycle_dy(index50
    ) first_cycle_dy(index60) first_cycle_dy(index70) first_cycle_dy(index80)]

grad_big=[(new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100)];
first_cycle_dy_19_2_all=gradient(First_cycle_all_19_2(:))./gradient(grad_big(:))

grad_big2=[(new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100)];
first_cycle_dy_2_3_all=gradient(First_cycle_all_2_3(:))./gradient(grad_big2(:))

perc10_1H_19_2_all=zeros(13,1)
for i=0:1:12
perc10_19_2_all(i+1,1)=[first_cycle_dy_19_2_all((indices(1,1)+i*200),1)]
perc20_19_2_all(i+1,1)=[first_cycle_dy_19_2_all((indices(1,2)+i*200),1)]
perc30_19_2_all(i+1,1)=[first_cycle_dy_19_2_all((indices(1,3)+i*200),1)]
perc40_19_2_all(i+1,1)=[first_cycle_dy_19_2_all((indices(1,4)+i*200),1)]
perc50_19_2_all(i+1,1)=[first_cycle_dy_19_2_all((indices(1,5)+i*200),1)]
perc60_19_2_all(i+1,1)=[first_cycle_dy_19_2_all((indices(1,6)+i*200),1)]
perc70_19_2_all(i+1,1)=[first_cycle_dy_19_2_all((indices(1,7)+i*200),1)]
perc80_19_2_all(i+1,1)=[first_cycle_dy_19_2_all((indices(1,8)+i*200),1)]
end

first_cycle_dy_19_2_all((indices(1,7)+12*200),1)
first_cycle_dy_19_2_all((indices(1,8)+12*200),1)

for i=0:1:14
perc10_2_3_all(i+1,1)=[first_cycle_dy_2_3_all((indices(1,1)+i*200),1)]
perc20_2_3_all(i+1,1)=[first_cycle_dy_2_3_all((indices(1,2)+i*200),1)]
perc30_2_3_all(i+1,1)=[first_cycle_dy_2_3_all((indices(1,3)+i*200),1)]
perc40_2_3_all(i+1,1)=[first_cycle_dy_2_3_all((indices(1,4)+i*200),1)]
perc50_2_3_all(i+1,1)=[first_cycle_dy_2_3_all((indices(1,5)+i*200),1)]
perc60_2_3_all(i+1,1)=[first_cycle_dy_2_3_all((indices(1,6)+i*200),1)]
perc70_2_3_all(i+1,1)=[first_cycle_dy_2_3_all((indices(1,7)+i*200),1)]
perc80_2_3_all(i+1,1)=[first_cycle_dy_2_3_all((indices(1,8)+i*200),1)]
end
%% 19-03:

%% 1 mm compr
S_WB19_03_1mm_compr_S1=StressWB19_03_1mm_compr{1,1}{1,1}(1:9100);
t_WB19_03_1mm_compr_S1=timeWB19_03_1mm_compr{1,1}{1,1}(1:9100);
S_WB19_03_1mm_compr_S2=StressWB19_03_1mm_compr{1,2}{1,1}(1:9100);
t_WB19_03_1mm_compr_S2=timeWB19_03_1mm_compr{1,2}{1,1}(1:9100);
S_WB19_03_1mm_compr_S3=StressWB19_03_1mm_compr{1,3}{1,1}(1:9100);
t_WB19_03_1mm_compr_S3=timeWB19_03_1mm_compr{1,3}{1,1}(1:9100);
S_WB19_03_1mm_compr_S4=StressWB19_03_1mm_compr{1,4}{1,1}(1:9100);
t_WB19_03_1mm_compr_S4=timeWB19_03_1mm_compr{1,4}{1,1}(1:9100);
S_WB19_03_1mm_compr_S5=StressWB19_03_1mm_compr{1,5}{1,1}(1:9100)+0.25;
t_WB19_03_1mm_compr_S5=timeWB19_03_1mm_compr{1,5}{1,1}(1:9100);

[y,yfit2] = bf(S_WB19_03_1mm_compr_S2,'confirm','linear')
S_WB19_03_1mm_compr_S2=S_WB19_03_1mm_compr_S2-yfit2 ;% fitting needed
[y,yfit3] = bf(S_WB19_03_1mm_compr_S3,'confirm','linear')
```

```
S_WB19_03_1mm_compr_S3=S_WB19_03_1mm_compr_S3-yfit3 ;% fitting needed
[y,yfit4] = bf(S_WB19_03_1mm_compr_S4,'confirm','linear')
S_WB19_03_1mm_compr_S4=S_WB19_03_1mm_compr_S4-yfit4 ;% fitting needed

H_WB19_03_1mm_avg_S=(S_WB19_03_1mm_compr_S1+S_WB19_03_1mm_compr_S2+
    S_WB19_03_1mm_compr_S3+S_WB19_03_1mm_compr_S4+S_WB19_03_1mm_compr_S5)/5;
H_WB19_03_1mm_avg_t=(t_WB19_03_1mm_compr_S1+t_WB19_03_1mm_compr_S2+
    t_WB19_03_1mm_compr_S3+t_WB19_03_1mm_compr_S4+t_WB19_03_1mm_compr_S5)/5;

H_WB19_03_1mm_compr={t_WB19_03_1mm_compr_S1,S_WB19_03_1mm_compr_S1;........
t_WB19_03_1mm_compr_S2,S_WB19_03_1mm_compr_S2;
t_WB19_03_1mm_compr_S3,S_WB19_03_1mm_compr_S3;
t_WB19_03_1mm_compr_S4,S_WB19_03_1mm_compr_S4;
t_WB19_03_1mm_compr_S5,S_WB19_03_1mm_compr_S5;};

figure(78)
plot(H_WB19_03_1mm_avg_t,H_WB19_03_1mm_avg_S)
%%
clear peakS peakt
% hysteresis loop preparation H1_2_3:
data=H_WB19_03_1mm_compr;
stepsize_valley=455;
cycles=6%20; % amount of cycles on the sample
samples=5; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=0.05; %should give output: to be equal to the amount of cycles
peakdistance=0.5; %should give output: to be equal to the amount of cycles

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end

[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
    first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
    First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
    first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
    Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
    std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
    new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
    cycles,samples,strain_level,desired_vector_lengths)

figure(27)
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'g');
end
```

```
end
title('1 mm height WB compressed all samples + all cycles, 19-03-2021 80%
    compression.')
lgd=legend('WB')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(28)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
title('1 mm height  WB compressed average 19-03-2021 hysteresis as 1 line the 5,
    80% compression.')
lgd=legend('WB')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(29)
plot(new_time_vector/100,Absolute_1_line)
title('1 mm height  WB compressed average 19-03-2021 hysteresis as 1 line: 80%
    compression.')
lgd=legend('WB')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

new_time_vector_1WB=new_time_vector;
Absolute_1_line_1WB=Absolute_1_line;
indices_1WB=indices;
points_mean_1WB=points_mean;
std_10_20_etc_1WB=std_10_20_etc;

One_line_is_and_ds19_03_1=One_line_is_and_ds;
std_10_20_etc19_03_1=std_10_20_etc;
First_cycle_One_line_mean19_03_1=First_cycle_One_line_mean;
first_cycle_points_mean19_03_1=first_cycle_points_mean;
first_cycle_std_10_20_etc19_03_1=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_19_03_1=first_cycle_Young_avg_per_percentage;


figure(30)
plot(new_time_vector/100,dy)
title('1 mm height  WB compressed 19-03-2021: tangent modulus average hysteresis
    in 1 line.')
lgd=legend('WB')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(31)
bar(perc, Young_avg_per_percentage,'g')
title('Youngh modulus 1 mm height  WB compressed 19-02-2021: tangent modulus
    average hysteresis in 1 line.')
lgd=legend('WB')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')

Young_avg_per_percentage_1WB=Young_avg_per_percentage;

%% 1 mm uncompr: 5de sample ruined
```

```
S_WB19_03_1mm_uncompr_S1=StressWB19_03_1mm_uncompr{1,1}{1,1}(1:9100);
t_WB19_03_1mm_uncompr_S1=timeWB19_03_1mm_uncompr{1,1}{1,1}(1:9100);
S_WB19_03_1mm_uncompr_S2=StressWB19_03_1mm_uncompr{1,2}{1,1}(1:9100);
t_WB19_03_1mm_uncompr_S2=timeWB19_03_1mm_uncompr{1,2}{1,1}(1:9100);
S_WB19_03_1mm_uncompr_S3=StressWB19_03_1mm_uncompr{1,3}{1,1}(1:9100);
t_WB19_03_1mm_uncompr_S3=timeWB19_03_1mm_uncompr{1,3}{1,1}(1:9100);
S_WB19_03_1mm_uncompr_S4=StressWB19_03_1mm_uncompr{1,4}{1,1}(1:9100);
t_WB19_03_1mm_uncompr_S4=timeWB19_03_1mm_uncompr{1,4}{1,1}(1:9100);
% S_WB19_03_1mm_uncompr_S5=StressWB19_03_1mm_uncompr{1,5}{1,1}(1:5087);
% t_WB19_03_1mm_uncompr_S5=timeWB19_03_1mm_uncompr{1,5}{1,1}(1:5087);

[y,yfit1] = bf(S_WB19_03_1mm_uncompr_S1,'confirm','linear')
S_WB19_03_1mm_uncompr_S1=S_WB19_03_1mm_uncompr_S1-yfit1 ;% fitting needed
[y,yfit2] = bf(S_WB19_03_1mm_uncompr_S2,'confirm','linear')
S_WB19_03_1mm_uncompr_S2=S_WB19_03_1mm_uncompr_S2-yfit2 ;% fitting needed
[y,yfit3] = bf(S_WB19_03_1mm_uncompr_S3,'confirm','linear')
S_WB19_03_1mm_uncompr_S3=S_WB19_03_1mm_uncompr_S3-yfit3 ;% fitting needed
[y,yfit4] = bf(S_WB19_03_1mm_uncompr_S4,'confirm','linear')
S_WB19_03_1mm_uncompr_S4=S_WB19_03_1mm_uncompr_S4-yfit4 ;% fitting needed
% [y,yfit5] = bf(S_WB19_03_1mm_uncompr_S5,'confirm','linear')
% S_WB19_03_1mm_uncompr_S5=S_WB19_03_1mm_uncompr_S5-yfit5 ;% fitting needed

H_WB19_03_1mm_uncompr_avg_S=(S_WB19_03_1mm_uncompr_S1+S_WB19_03_1mm_uncompr_S2+
    S_WB19_03_1mm_uncompr_S3+S_WB19_03_1mm_uncompr_S4)/4;
H_WB19_03_1mm_uncompr_avg_t=(t_WB19_03_1mm_uncompr_S1+t_WB19_03_1mm_uncompr_S2+
    t_WB19_03_1mm_uncompr_S3+t_WB19_03_1mm_uncompr_S4)/4;

H_WB19_03_1mm_uncompr={t_WB19_03_1mm_uncompr_S1,S_WB19_03_1mm_uncompr_S1;.....
t_WB19_03_1mm_uncompr_S2,S_WB19_03_1mm_uncompr_S2;
t_WB19_03_1mm_uncompr_S3,S_WB19_03_1mm_uncompr_S3;
t_WB19_03_1mm_uncompr_S4,S_WB19_03_1mm_uncompr_S4;};

figure(79)
plot(H_WB19_03_1mm_uncompr_avg_t,H_WB19_03_1mm_uncompr_avg_S)
%%
clear peakS peakt
data=H_WB19_03_1mm_uncompr;
stepsize_valley=455;
cycles=20; % amount of cycles on the sample
samples=4; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=0.05; %should give output: to be equal to the amount of cycles
peakdistance=0.5; %should give output: to be equal to the amount of cycles

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end

[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
    first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
    First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
    first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
    Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
    std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
    new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
    cycles,samples,strain_level,desired_vector_lengths)


figure(32)
```

```
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'g');
end
end
title('1 mm height WB uncompressed all samples + all cycles, 19-03-2021 80%
    compression.')
lgd=legend('WB')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(33)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
title('1 mm height  WB uncompressed average 19-03-2021 hysteresis as 1 line the 5,
     80% compression.')
lgd=legend('WB')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(34)
plot(new_time_vector/100,Absolute_1_line)
title('1 mm height  WB uncompressed average 19-03-2021 hysteresis as 1 line: 80%
    compression.')
lgd=legend('WB')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

new_time_vector_1WB_uncompr=new_time_vector;
Absolute_1_line_1WB_uncompr=Absolute_1_line;
indices_1WB_uncompr=indices;
points_mean_1WB_uncompr=points_mean;
std_10_20_etc_1WB_uncompr=std_10_20_etc;

One_line_is_and_ds19_03_1_uncompr=One_line_is_and_ds;
std_10_20_etc19_03_1_uncompr=std_10_20_etc;
First_cycle_One_line_mean19_03_1_uncompr=First_cycle_One_line_mean;
first_cycle_points_mean19_03_1_uncompr=first_cycle_points_mean;
first_cycle_std_10_20_etc19_03_1_uncompr=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_19_03_1_uncompr=
    first_cycle_Young_avg_per_percentage;


figure(35)
```

```
plot(new_time_vector/100,dy)
title('1 mm height  WB uncompressed 19-03-2021: tangent modulus average hysteresis
    in 1 line.')
lgd=legend('WB')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(36)
bar(perc, Young_avg_per_percentage,'g')
title('Youngh modulus 1 mm height  WB uncompressed 19-02-2021: tangent modulus
   average hysteresis in 1 line.')
lgd=legend('WB')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')

Young_avg_per_percentage_1WB_uncompr=Young_avg_per_percentage;

%% 2 mm

S_WB19_03_2mm_S1=StressWB19_03_2mm{1,1}{1,1}(1:8569);
t_WB19_03_2mm_S1=timeWB19_03_2mm{1,1}{1,1}(1:8569);
S_WB19_03_2mm_S2=StressWB19_03_2mm{1,2}{1,1}(1:8569);
t_WB19_03_2mm_S2=timeWB19_03_2mm{1,2}{1,1}(1:8569);
S_WB19_03_2mm_S3=StressWB19_03_2mm{1,3}{1,1}(1:8569);
t_WB19_03_2mm_S3=timeWB19_03_2mm{1,3}{1,1}(1:8569);
S_WB19_03_2mm_S4=StressWB19_03_2mm{1,4}{1,1}(1:8569);
t_WB19_03_2mm_S4=timeWB19_03_2mm{1,4}{1,1}(1:8569);
S_WB19_03_2mm_S5=StressWB19_03_2mm{1,5}{1,1}(1:8569);
t_WB19_03_2mm_S5=timeWB19_03_2mm{1,5}{1,1}(1:8569);

H_WB19_03_2mm_avg_S=(S_WB19_03_2mm_S1+S_WB19_03_2mm_S2+S_WB19_03_2mm_S3+
   S_WB19_03_2mm_S4+S_WB19_03_2mm_S5)/5;
H_WB19_03_2mm_avg_t=(t_WB19_03_2mm_S1+t_WB19_03_2mm_S2+t_WB19_03_2mm_S3+
   t_WB19_03_2mm_S4+t_WB19_03_2mm_S5)/5;

H_WB19_03_2mm={t_WB19_03_2mm_S1,S_WB19_03_2mm_S1;........
t_WB19_03_2mm_S2,S_WB19_03_2mm_S2;
t_WB19_03_2mm_S3,S_WB19_03_2mm_S3;
t_WB19_03_2mm_S4,S_WB19_03_2mm_S4;
t_WB19_03_2mm_S5,S_WB19_03_2mm_S5;};

figure(1111)
plot(H_WB19_03_2mm_avg_t,H_WB19_03_2mm_avg_S)
clear peakS peakt
data=H_WB19_03_2mm;
stepsize_valley=455;
cycles= 6%9; % amount of cycles on the sample
samples=5; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=2.7; %should give output: to be equal to the amount of cycles
peakdistance=13; %should give output: to be equal to the amount of cycles

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end
```

```
[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
    first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
    First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
    first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
    Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
    std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
    new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
    cycles,samples,strain_level,desired_vector_lengths)


figure(37)
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'g');
end
end
title('2 mm height WB uncompressed all samples + all cycles, 19-03-2021 80%
    compression.')
lgd=legend('WB')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(38)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
title('2 mm height  WB uncompressed average 19-03-2021 hysteresis as 1 line the 5,
     80% compression.')
lgd=legend('WB')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(39)
plot(new_time_vector/100,Absolute_1_line)
title('2 mm height  WB uncompressed average 19-03-2021 hysteresis as 1 line: 80%
    compression.')
lgd=legend('WB')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

new_time_vector_2WB=new_time_vector;
Absolute_1_line_2WB=Absolute_1_line;
indices_2WB=indices;
points_mean_2WB=points_mean;
std_10_20_etc_2WB=std_10_20_etc;
```

```
One_line_is_and_ds19_03_2mm=One_line_is_and_ds;
std_10_20_etc19_03_2mm=std_10_20_etc;
First_cycle_One_line_mean19_03_2mm=First_cycle_One_line_mean;
first_cycle_points_mean19_03_2mm=first_cycle_points_mean;
first_cycle_std_10_20_etc19_03_2mm=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_19_03_2mm=
    first_cycle_Young_avg_per_percentage;



figure(40)
plot(new_time_vector/100,dy)
title('2 mm height  WB uncompressed 19-03-2021: tangent modulus average hysteresis
     in 1 line.')
lgd=legend('WB')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(41)
bar(perc, Young_avg_per_percentage,'g')
title('Youngh modulus 2 mm height  WB uncompressed 19-02-2021: tangent modulus
    average hysteresis in 1 line.')
lgd=legend('WB')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')

Young_avg_per_percentage_2WB=Young_avg_per_percentage;

%% 5 mm: niet geheel kloppende avg

S_WB19_03_5mm_S1=StressWB19_03_5mm{1,1}{1,1}(1:8248);
t_WB19_03_5mm_S1=timeWB19_03_5mm{1,1}{1,1}(1:8248);
S_WB19_03_5mm_S2=StressWB19_03_5mm{1,2}{1,1}(1:8248);
t_WB19_03_5mm_S2=timeWB19_03_5mm{1,2}{1,1}(1:8248);
S_WB19_03_5mm_S3=StressWB19_03_5mm{1,3}{1,1}(1:8248);
t_WB19_03_5mm_S3=timeWB19_03_5mm{1,3}{1,1}(1:8248);
S_WB19_03_5mm_S4=StressWB19_03_5mm{1,4}{1,1}(1:8248);
t_WB19_03_5mm_S4=timeWB19_03_5mm{1,4}{1,1}(1:8248);
S_WB19_03_5mm_S5=StressWB19_03_5mm{1,5}{1,1}(1:8248);
t_WB19_03_5mm_S5=timeWB19_03_5mm{1,5}{1,1}(1:8248);


x=6773; %x=271
H_WB19_03_5mm_avg_S_part1=(S_WB19_03_5mm_S1(1:x)+S_WB19_03_5mm_S2(1:x)+
    S_WB19_03_5mm_S3(1:x)+S_WB19_03_5mm_S4(1:x)+S_WB19_03_5mm_S5(1:x))/5
H_WB19_03_5mm_avg_t_part1=(t_WB19_03_5mm_S1(1:x)+t_WB19_03_5mm_S2(1:x)+
    t_WB19_03_5mm_S3(1:x)+t_WB19_03_5mm_S4(1:x)+t_WB19_03_5mm_S5(1:x))/5

y=8148;
H_WB19_03_5mm_avg_S_part2=(S_WB19_03_5mm_S1(1+x:y)+S_WB19_03_5mm_S2(1+x:y)+
    S_WB19_03_5mm_S3(30+x:y+29)+S_WB19_03_5mm_S4(1+x:y)+S_WB19_03_5mm_S5(1+x:y))/5
H_WB19_03_5mm_avg_t_part2=(t_WB19_03_5mm_S1(1+x:y)+t_WB19_03_5mm_S2(1+x:y)+
    t_WB19_03_5mm_S3(1+x:y)+t_WB19_03_5mm_S4(1+x:y)+t_WB19_03_5mm_S5(1+x:y))/5

H_WB19_03_5mm_avg_S=[H_WB19_03_5mm_avg_S_part1;H_WB19_03_5mm_avg_S_part2];
H_WB19_03_5mm_avg_t=[H_WB19_03_5mm_avg_t_part1;H_WB19_03_5mm_avg_t_part2];

H_WB19_03_5mm={t_WB19_03_5mm_S1,S_WB19_03_5mm_S1;.......
t_WB19_03_5mm_S2,S_WB19_03_5mm_S2;
t_WB19_03_5mm_S3,S_WB19_03_5mm_S3;
```

```
t_WB19_03_5mm_S4,S_WB19_03_5mm_S4;
t_WB19_03_5mm_S5,S_WB19_03_5mm_S5;};


figure(2)
plot(H_WB19_03_5mm_avg_t,H_WB19_03_5mm_avg_S)
clear peakS peakt
data=H_WB19_03_5mm;
stepsize_valley=455;
cycles=6; % amount of cycles on the sample
samples=5; %amount of clot analog samples evaluated
strain_level=80; % for 80% compression
desired_vector_lengths=200; %just a random number minus 1
peakheight=1.9; %should give output: to be equal to the amount of cycles
peakdistance=0.5; %should give output: to be equal to the amount of cycles

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end

[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
   first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
   First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
   first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
   Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
   std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
   new_time_vector,perc]=hysteresis(peakheight,peakdistance,data,stepsize_valley,
   cycles,samples,strain_level,desired_vector_lengths)

figure(42)
for j=1:samples
for i=1:cycles
hold all
plot(new_time_vector/100,newy_ds{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,i},'g');
end
end
for j=1:samples
for i=2:cycles
hold all
plot(new_time_vector/100,newy_is{1,j}{1,1},'g');
end
end
title('5 mm height WB uncompressed all samples + all cycles, 19-03-2021 80%
   compression.')
lgd=legend('WB')
xlabel('Nominal strain')
ylabel('Nominal stress [kPa]')

figure(43)
for j=1:samples
    hold all
plot(new_time_vector/100,One_line_mean{1,j})
end
```

```
title('5 mm height  WB uncompressed average 19-03-2021 hysteresis as 1 line the 5,
    80% compression.')
lgd=legend('WB')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')

figure(44)
plot(new_time_vector/100,Absolute_1_line)
title('5 mm height  WB uncompressed average 19-03-2021 hysteresis as 1 line: 80%
   compression.')
lgd=legend('WB')
xlabel('Time [s]')
ylabel('Nominal stress [kPa]')
hold on
errorbar(new_time_vector(indices)/100,points_mean,std_10_20_etc,'.')

new_time_vector_5WB=new_time_vector;
Absolute_1_line_5WB=Absolute_1_line;
indices_5WB=indices;
points_mean_5WB=points_mean;
std_10_20_etc_5WB=std_10_20_etc;

One_line_is_and_ds19_03_5mm=One_line_is_and_ds;
std_10_20_etc19_03_5mm=std_10_20_etc;
First_cycle_One_line_mean19_03_5mm=First_cycle_One_line_mean;
first_cycle_points_mean19_03_5mm=first_cycle_points_mean;
first_cycle_std_10_20_etc19_03_5mm=first_cycle_std_10_20_etc;
first_cycle_Young_avg_per_percentage_19_03_5mm=
   first_cycle_Young_avg_per_percentage;

figure(45)
plot(new_time_vector/100,dy)
title('5 mm height  WB uncompressed 19-03-2021: tangent modulus average hysteresis
    in 1 line.')
lgd=legend('WB')
xlabel('Nominal strain [-]')
ylabel('Tangent/young modulus [kPa]')

figure(46)
bar(perc, Young_avg_per_percentage,'g')
title('Youngh modulus 5 mm height  WB uncompressed 19-02-2021: tangent modulus
   average hysteresis in 1 line.')
lgd=legend('WB')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')

Young_avg_per_percentage_5WB=Young_avg_per_percentage;
%%
% %% 19-02 80% plots
%
% figure(1)
% for i=1:5;
% plot(H1_19_02{i,1},H1_19_02{i,2}) %matured all with weight
% hold on
% end
% title('Male 53 years, test 80% compression: 1%H Samples 1 mm height.
   19-02-2021')
% xlabel('Time [s]')
% ylabel('Stress [kPa]')
% legend('S1','S1','S3','S4','S5')
%
```

```
% figure(2)
% plot(H40_19_02{1,1},H40_19_02{1,2}) %matured all with weight
% hold on
% plot(H40_19_02{2,1},H40_19_02{2,2}) %matured all with weight
% title('Male 53 years, 80% compression: 40%H Samples 1 mm height. 19-02-2021')
% xlabel('Time [s]')
% ylabel('Stress [kPa]')
% legend('S1','S1')
% %REMARK: S2: missed first cycle!! S4 S5 ruined
%
% figure(3)
% for i=1:5;
%     plot(H99_19_02{i,1},H99_19_02{i,2}) %matured all with weight
% hold on
% end
% title('Male 53 years, 80% compression: 99%H Samples 1 mm height. 19-02-2021')
% xlabel('Time [s]')
% ylabel('Stress [kPa]')
% legend('S1','S1','S3','S4','S5')
% %% 19-02 60% plots
% figure(4)
% plot(H40_19_02_60{1,1},H40_19_02_60{1,2}) %matured all with weight
% hold on
% plot(H40_19_02_60{2,1},H40_19_02_60{2,2}) %matured all with weight
% hold on
% plot(H40_19_02_60{3,1},H40_19_02_60{3,2}) %matured all with weight
% title('Male 53 years, 60% compression: 40%H Samples 1 mm height. 19-02-2021')
% xlabel('Time [s]')
% ylabel('Stress [kPa]')
% legend('S1','S1','S3')
%
% figure(5)
% for i=1:5;
% plot(H1_19_02_60{i,1},H1_19_02_60{i,2}) %matured all with weight
% hold on
% end
% title('Male 53 years, 60% compression: 1%H Samples 1 mm height. 19-02-2021')
% xlabel('Time [s]')
% ylabel('Stress [kPa]')
% legend('S1','S1','S3','S4','S5')
% %% 2-3 plots
% figure(6)
% for i=1:5;
% plot(H99_2_3_60{i,1},H99_2_3_60{i,2}) %matured all with weight
% hold on
% end
% title('Female 28 years, 60% compression: 99%H Samples 1 mm height. 2-3-2021')
% xlabel('Time [s]')
% ylabel('Stress [kPa]')
% legend('S1','S1','S3','S4','S5')
%
% figure(7)
% for i=1:5;
% plot(H40_2_3{i,1},H40_2_3{i,2}) %matured all with weight
% hold on
% end
% title('Female 28 years, 80% compression: 40%H Samples 3 mm height. 2-3-2021')
% xlabel('Time [s]')
% ylabel('Stress [kPa]')
% legend('S1','S1','S3','S4','S5')
```

```
%% Final plots:
%%
%% 1 mm height samples, 80% compression
figure(8)
for i=1:5;
a=plot(H1_19_02{i,1},H1_19_02{i,2},'y') %matured all with weight
hold on
end
b=plot(H40_19_02{1,1},H40_19_02{1,2},'b') %matured all with weight
hold on
plot(H40_19_02{2,1},H40_19_02{2,2},'b') %matured all with weight
hold on
for i=1:5;
  c=  plot(H99_19_02{i,1},H99_19_02{i,2},'g') %matured all with weight
hold on
end
title('1 mm height samples, 80% compression.')
xlabel('Time [s]')
ylabel('Stress [kPa]')
lgd=legend([a b c],'1%H','40%H','99%H')
lgd.FontSize=14;
clear a b c lgd


%% 1 mm height samples, 60% compression
figure(9) %40%H (3 samples) and 1%H (5 samples, 3 samples weak) with from male 53
    years, 99%H (5, 1 weak) from female 28 years.

for i=1:5;
d=plot(H1_19_02_60{i,1},H1_19_02_60{i,2},'y'); %matured all with weight
hold on
end
e=plot(H40_19_02_60{1,1},H40_19_02_60{1,2},'b'); %matured all with weight
hold on
plot(H40_19_02_60{2,1},H40_19_02_60{2,2},'b'); %matured all with weight
hold on
plot(H40_19_02_60{3,1},H40_19_02_60{3,2},'b'); %matured all with weight
hold on
for i=1:5;
f= plot(H99_2_3_60{i,1},H99_2_3_60{i,2},'g'); %matured all with weight
hold on
end
title('1 mm height samples, 60% compression.')
xlabel('Time [s]')
ylabel('Stress [kPa]')
lgd=legend([d e f],'1%H','40%H','99%H')
lgd.FontSize=14;
clear a b c d e f lgd
%% 3 mm height samples, 80% compression
figure(10)
for i=1:4;
a=plot(H1_2_3{i,1},H1_2_3{i,2},'y'); %matured all with weight
hold on
end
for i=1:5;
b=plot(H40_2_3{i,1},H40_2_3{i,2},'b'); %matured all with weight
hold on
end
for i=1:5;
c=plot(H99_2_3{i,1},H99_2_3{i,2},'g'); %matured all with weight
hold on
```

```
end
title('3 mm height samples, 80% compression.')
xlabel('Time [s]')
ylabel('Stress [kPa]')
lgd=legend([a b c],'1%H','40%H','99%H')
lgd.FontSize=14;
clear a b c lgd

%% WB graphs pressure versus no pressure:
%%
figure(1008)
a=plot(new_time_vector/100,First_cycle_One_line_mean19_03_1,'Color','#0072BD');
hold on
b=plot(new_time_vector/100,First_cycle_One_line_mean19_03_1_uncompr,'Color','#77
    AC30');
xlabel('Nominal strain [-]')
ylabel('Nominal stress [kPa]')
title('TAs: WB (un)pressured formation, first cycle of compression testing.')
lgd=legend([a b],'1mm WB compressed','1mm WB uncompressed')
hold on
errorbar(new_time_vector(indices)/100,first_cycle_points_mean19_03_1,
    first_cycle_std_10_20_etc19_03_1,'.k')
e=errorbar(new_time_vector(indices)/100,first_cycle_points_mean19_03_1_uncompr,
    first_cycle_std_10_20_etc19_03_1_uncompr,'.')
e.Color='#A2142F'

figure(1009)
hold all
a=bar(perc, first_cycle_Young_avg_per_percentage_19_03_1)
b=bar(perc, first_cycle_Young_avg_per_percentage_19_03_1_uncompr)
title('TAs: WB (un)pressured formation, first cycle of compression testing.')
lgd=legend([a b],'1mm WB compressed','1mm WB uncompressed')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')



perc10=[first_cycle_Young_avg_per_percentage_19_03_1(1,1)
    first_cycle_Young_avg_per_percentage_19_03_1_uncompr(1,1)];
perc20=[first_cycle_Young_avg_per_percentage_19_03_1(1,2)
    first_cycle_Young_avg_per_percentage_19_03_1_uncompr(1,2)];
perc30=[first_cycle_Young_avg_per_percentage_19_03_1(1,3)
    first_cycle_Young_avg_per_percentage_19_03_1_uncompr(1,3)];
perc40=[first_cycle_Young_avg_per_percentage_19_03_1(1,4)
     first_cycle_Young_avg_per_percentage_19_03_1_uncompr(1,4)];
perc50=[first_cycle_Young_avg_per_percentage_19_03_1(1,5)
        first_cycle_Young_avg_per_percentage_19_03_1_uncompr(1,5)];
perc60=[first_cycle_Young_avg_per_percentage_19_03_1(1,6)
       first_cycle_Young_avg_per_percentage_19_03_1_uncompr(1,6)];
perc70=[first_cycle_Young_avg_per_percentage_19_03_1(1,7)
       first_cycle_Young_avg_per_percentage_19_03_1_uncompr(1,7)];
perc80=[first_cycle_Young_avg_per_percentage_19_03_1(1,8)
       first_cycle_Young_avg_per_percentage_19_03_1_uncompr(1,8)];

all=[perc10'
    perc20'
    perc30'
    perc40'
    perc50'
    perc60'
    perc70'
```

```
    perc80'];
figure(1012)
bar(perc,all)
title('TAs: WB (un)compressed formation, first cycle of compression testing.')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')
legend




for i = 1:numel(One_line_is_and_ds19_03_1)
  One_line_is_and_ds19_03_1{1,i} = transpose(One_line_is_and_ds19_03_1{1,i});
end
G19_03_1=cell2mat(One_line_is_and_ds19_03_1);

for i = 1:numel(One_line_is_and_ds19_03_1_uncompr)
  One_line_is_and_ds19_03_1_uncompr{1,i} = transpose(
     One_line_is_and_ds19_03_1_uncompr{1,i});
end
G19_03_1_uncompr=cell2mat(One_line_is_and_ds19_03_1_uncompr);

First_cycle_all_19_03_1=[G19_03_1 G19_03_1_uncompr];



BF_test_values_19_03_1=First_cycle_all_19_03_1(:);
BF_test_label_19_03_1=zeros(1800,1);
R1=1;
R2=2;
BF_test_label_19_03_1(1:(200*(numel(One_line_is_and_ds19_03_1))),:)=R1;
BF_test_label_19_03_1(1000+1:1800,:)=R2;
BF_test_19_03_1=[BF_test_label_19_03_1 BF_test_values_19_03_1];

First_cycle_perc_10p_stress_19_03_1=[1 1 1 1 1 2 2 2 2 2]'
for i=1:size(First_cycle_all_19_03_1,2)
First_cycle_perc_10p_stress_19_03_1(i,2)=[First_cycle_all_19_03_1(indices(1,1),i)]
First_cycle_perc_20p_stress_19_03_1(i,2)=[First_cycle_all_19_03_1(indices(1,2),i)]
First_cycle_perc_30p_stress_19_03_1(i,2)=[First_cycle_all_19_03_1(indices(1,3),i)]
First_cycle_perc_40p_stress_19_03_1(i,2)=[First_cycle_all_19_03_1(indices(1,4),i)]
First_cycle_perc_50p_stress_19_03_1(i,2)=[First_cycle_all_19_03_1(indices(1,5),i)]
First_cycle_perc_60p_stress_19_03_1(i,2)=[First_cycle_all_19_03_1(indices(1,6),i)]
First_cycle_perc_70p_stress_19_03_1(i,2)=[First_cycle_all_19_03_1(indices(1,7),i)]
First_cycle_perc_80p_stress_19_03_1(i,2)=[First_cycle_all_19_03_1(indices(1,8),i)]
end

% Anova per youngs

% first_cycle_df=diff(First_cycle_One_line_mean);
% first_cycle_dy=gradient(First_cycle_One_line_mean(:))./gradient(new_time_vector
    (:)/100)
% first_cycle_Young_avg_per_percentage=[first_cycle_dy(index10) first_cycle_dy(
    index20) first_cycle_dy(index30) first_cycle_dy(index40) first_cycle_dy(index50
    ) first_cycle_dy(index60) first_cycle_dy(index70) first_cycle_dy(index80)]


grad_big_19_3=[(new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector
    (:)/100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100) (new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)
    /100)];
```

```
first_cycle_dy_19_3_all=gradient(First_cycle_all_19_03_1(:))./gradient(
    grad_big_19_3(:))

for i=0:1:8
perc10_19_3_all(i+1,1)=[first_cycle_dy_19_3_all((indices(1,1)+i*200),1)]
perc20_19_3_all(i+1,1)=[first_cycle_dy_19_3_all((indices(1,2)+i*200),1)]
perc30_19_3_all(i+1,1)=[first_cycle_dy_19_3_all((indices(1,3)+i*200),1)]
perc40_19_3_all(i+1,1)=[first_cycle_dy_19_3_all((indices(1,4)+i*200),1)]
perc50_19_3_all(i+1,1)=[first_cycle_dy_19_3_all((indices(1,5)+i*200),1)]
perc60_19_3_all(i+1,1)=[first_cycle_dy_19_3_all((indices(1,6)+i*200),1)]
perc70_19_3_all(i+1,1)=[first_cycle_dy_19_3_all((indices(1,7)+i*200),1)]
perc80_19_3_all(i+1,1)=[first_cycle_dy_19_3_all((indices(1,8)+i*200),1)]
end

%% WB height info for stats from WB 2mm and WB 5mm.




%% WB height graphs:

figure(1010)
hold all
a=plot(new_time_vector/100,First_cycle_One_line_mean19_03_1,'Color','#0072BD');
b=plot(new_time_vector/100,First_cycle_One_line_mean19_03_2mm,'g');
c=plot(new_time_vector/100,First_cycle_One_line_mean19_03_5mm, 'Color', '#EDB120')
    ;
xlabel('Nominal strain [-]')
ylabel('Nominal stress [kPa]')
title('TAs: WB, formation under pressure, first cycle of compression testing.')
lgd=legend([a b c], '1mm WB pressured','2mm WB pressured','5mm WB pressured')
errorbar(new_time_vector(indices)/100,first_cycle_points_mean19_03_1,
    first_cycle_std_10_20_etc19_03_1,'.k')
errorbar(new_time_vector(indices)/100,first_cycle_points_mean19_03_2mm,
    first_cycle_std_10_20_etc19_03_2mm,'.k')
errorbar(new_time_vector(indices)/100,first_cycle_points_mean19_03_5mm,
    first_cycle_std_10_20_etc19_03_5mm,'.k')
hold off


figure(1011)
hold all
a=bar(perc, first_cycle_Young_avg_per_percentage_19_03_1)
b=bar(perc, first_cycle_Young_avg_per_percentage_19_03_2mm)
c=bar(perc, first_cycle_Young_avg_per_percentage_19_03_5mm)
title('TAs: WB compressed formation, first cycle of compression testing.')
lgd=legend([a b c],'1mm WB compressed','2mm WB compressed','5mm WB compressed')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')


perc10=[first_cycle_Young_avg_per_percentage_19_03_1(1,1)
    first_cycle_Young_avg_per_percentage_19_03_2mm(1,1)
    first_cycle_Young_avg_per_percentage_19_03_5mm(1,1)];
perc20=[first_cycle_Young_avg_per_percentage_19_03_1(1,2)
    first_cycle_Young_avg_per_percentage_19_03_2mm(1,2)
    first_cycle_Young_avg_per_percentage_19_03_5mm(1,2)];
perc30=[first_cycle_Young_avg_per_percentage_19_03_1(1,3)
    first_cycle_Young_avg_per_percentage_19_03_2mm(1,3)
    first_cycle_Young_avg_per_percentage_19_03_5mm(1,3)];
perc40=[first_cycle_Young_avg_per_percentage_19_03_1(1,4)
```

```
     first_cycle_Young_avg_per_percentage_19_03_2mm(1,4)
    first_cycle_Young_avg_per_percentage_19_03_5mm(1,4)];
perc50=[first_cycle_Young_avg_per_percentage_19_03_1(1,5)
        first_cycle_Young_avg_per_percentage_19_03_2mm(1,5)
    first_cycle_Young_avg_per_percentage_19_03_5mm(1,5)];
perc60=[first_cycle_Young_avg_per_percentage_19_03_1(1,6)
       first_cycle_Young_avg_per_percentage_19_03_2mm(1,6)
    first_cycle_Young_avg_per_percentage_19_03_5mm(1,6)];
perc70=[first_cycle_Young_avg_per_percentage_19_03_1(1,7)
       first_cycle_Young_avg_per_percentage_19_03_2mm(1,7)
    first_cycle_Young_avg_per_percentage_19_03_5mm(1,7)];
perc80=[first_cycle_Young_avg_per_percentage_19_03_1(1,8)
       first_cycle_Young_avg_per_percentage_19_03_2mm(1,8)
    first_cycle_Young_avg_per_percentage_19_03_5mm(1,8)];

all=[perc10'
    perc20'
    perc30'
    perc40'
    perc50'
    perc60'
    perc70'
    perc80'];
figure(1013)
b=bar(perc,all)
b(2).FaceColor = 'g'
title('TAs: WB compressed formation, first cycle of compression testing.')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')
legend

%% WB2 and 5


for i = 1:numel(One_line_is_and_ds19_03_2mm)
  One_line_is_and_ds19_03_2mm{1,i} = transpose(One_line_is_and_ds19_03_2mm{1,i});
end
G19_03_2mm=cell2mat(One_line_is_and_ds19_03_2mm);

First_cycle_all_19_03_2mm=[G19_03_2mm];

BF_test_values_19_03_2mm=First_cycle_all_19_03_2mm(:);
BF_test_label_19_03_2mm=zeros(1000,1);
R1=3;
BF_test_label_19_03_2mm(1:(200*(numel(One_line_is_and_ds19_03_2mm))),:)=R1;

BF_test_19_03_2mm=[BF_test_label_19_03_2mm];
First_cycle_perc_10p_stress_19_03_2mm=[3 3 3 3 3]'
for i=1:size(First_cycle_all_19_03_2mm,2)
First_cycle_perc_10p_stress_19_03_2mm(i,2)=[First_cycle_all_19_03_2mm(indices(1,1)
    ,i)]
First_cycle_perc_20p_stress_19_03_2mm(i,2)=[First_cycle_all_19_03_2mm(indices(1,2)
    ,i)]
First_cycle_perc_30p_stress_19_03_2mm(i,2)=[First_cycle_all_19_03_2mm(indices(1,3)
    ,i)]
First_cycle_perc_40p_stress_19_03_2mm(i,2)=[First_cycle_all_19_03_2mm(indices(1,4)
    ,i)]
First_cycle_perc_50p_stress_19_03_2mm(i,2)=[First_cycle_all_19_03_2mm(indices(1,5)
    ,i)]
First_cycle_perc_60p_stress_19_03_2mm(i,2)=[First_cycle_all_19_03_2mm(indices(1,6)
    ,i)]
```

```
First_cycle_perc_70p_stress_19_03_2mm(i,2)=[First_cycle_all_19_03_2mm(indices(1,7)
    ,i)]
First_cycle_perc_80p_stress_19_03_2mm(i,2)=[First_cycle_all_19_03_2mm(indices(1,8)
    ,i)]
end

grad_big_19_3_2mm=[(new_time_vector(:)/100) (new_time_vector(:)/100) (
    new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)/100)];
first_cycle_dy_19_3_all_2mm=gradient(First_cycle_all_19_03_2mm(:))./gradient(
    grad_big_19_3_2mm(:))

%per youngs modulus:
for i=0:1:4
perc10_19_3_all_2mm(i+1,1)=[first_cycle_dy_19_3_all_2mm((indices(1,1)+i*200),1)]
perc20_19_3_all_2mm(i+1,1)=[first_cycle_dy_19_3_all_2mm((indices(1,2)+i*200),1)]
perc30_19_3_all_2mm(i+1,1)=[first_cycle_dy_19_3_all_2mm((indices(1,3)+i*200),1)]
perc40_19_3_all_2mm(i+1,1)=[first_cycle_dy_19_3_all_2mm((indices(1,4)+i*200),1)]
perc50_19_3_all_2mm(i+1,1)=[first_cycle_dy_19_3_all_2mm((indices(1,5)+i*200),1)]
perc60_19_3_all_2mm(i+1,1)=[first_cycle_dy_19_3_all_2mm((indices(1,6)+i*200),1)]
perc70_19_3_all_2mm(i+1,1)=[first_cycle_dy_19_3_all_2mm((indices(1,7)+i*200),1)]
perc80_19_3_all_2mm(i+1,1)=[first_cycle_dy_19_3_all_2mm((indices(1,8)+i*200),1)]
end


%% 5mm
for i = 1:numel(One_line_is_and_ds19_03_5mm)
  One_line_is_and_ds19_03_5mm{1,i} = transpose(One_line_is_and_ds19_03_5mm{1,i});
end
G19_03_5mm=cell2mat(One_line_is_and_ds19_03_5mm);

First_cycle_all_19_03_5mm=[G19_03_5mm];

BF_test_values_19_03_5mm=First_cycle_all_19_03_5mm(:);
BF_test_label_19_03_5mm=zeros(1000,1);
R1=3;
BF_test_label_19_03_5mm(1:(200*(numel(One_line_is_and_ds19_03_5mm))),:)=R1;

BF_test_19_03_5mm=[BF_test_label_19_03_5mm];
First_cycle_perc_10p_stress_19_03_5mm=[4 4 4 4 4]'
for i=1:size(First_cycle_all_19_03_5mm,2)
First_cycle_perc_10p_stress_19_03_5mm(i,2)=[First_cycle_all_19_03_5mm(indices(1,1)
    ,i)]
First_cycle_perc_20p_stress_19_03_5mm(i,2)=[First_cycle_all_19_03_5mm(indices(1,2)
    ,i)]
First_cycle_perc_30p_stress_19_03_5mm(i,2)=[First_cycle_all_19_03_5mm(indices(1,3)
    ,i)]
First_cycle_perc_40p_stress_19_03_5mm(i,2)=[First_cycle_all_19_03_5mm(indices(1,4)
    ,i)]
First_cycle_perc_50p_stress_19_03_5mm(i,2)=[First_cycle_all_19_03_5mm(indices(1,5)
    ,i)]
First_cycle_perc_60p_stress_19_03_5mm(i,2)=[First_cycle_all_19_03_5mm(indices(1,6)
    ,i)]
First_cycle_perc_70p_stress_19_03_5mm(i,2)=[First_cycle_all_19_03_5mm(indices(1,7)
    ,i)]
First_cycle_perc_80p_stress_19_03_5mm(i,2)=[First_cycle_all_19_03_5mm(indices(1,8)
    ,i)]
end

grad_big_19_3_5mm=[(new_time_vector(:)/100) (new_time_vector(:)/100) (
    new_time_vector(:)/100) (new_time_vector(:)/100) (new_time_vector(:)/100)];
```

```
first_cycle_dy_19_3_all_5mm=gradient(First_cycle_all_19_03_5mm(:))./gradient(
    grad_big_19_3_5mm(:))

%per youngs modulus:
for i=0:1:4
perc10_19_3_all_5mm(i+1,1)=[first_cycle_dy_19_3_all_5mm((indices(1,1)+i*200),1)]
perc20_19_3_all_5mm(i+1,1)=[first_cycle_dy_19_3_all_5mm((indices(1,2)+i*200),1)]
perc30_19_3_all_5mm(i+1,1)=[first_cycle_dy_19_3_all_5mm((indices(1,3)+i*200),1)]
perc40_19_3_all_5mm(i+1,1)=[first_cycle_dy_19_3_all_5mm((indices(1,4)+i*200),1)]
perc50_19_3_all_5mm(i+1,1)=[first_cycle_dy_19_3_all_5mm((indices(1,5)+i*200),1)]
perc60_19_3_all_5mm(i+1,1)=[first_cycle_dy_19_3_all_5mm((indices(1,6)+i*200),1)]
perc70_19_3_all_5mm(i+1,1)=[first_cycle_dy_19_3_all_5mm((indices(1,7)+i*200),1)]
perc80_19_3_all_5mm(i+1,1)=[first_cycle_dy_19_3_all_5mm((indices(1,8)+i*200),1)]
end

%% averages
%%
H1_19_02_avg={H1_19_02_avg_t;H1_19_02_avg_S};
H40_19_02_avg={H40_19_02_avg_t;H40_19_02_avg_S};
H99_19_02_avg={H99_19_02_avg_t; H99_19_02_avg_S};
H40_19_02_60_avg={H40_19_02_60_avg_t;H40_19_02_60_avg_S}
H1_19_02_60_avg={H1_19_02_60_avg_t; H1_19_02_60_avg_S};
H99_2_3_60_avg={H99_2_3_60_avg_t; H99_2_3_60_avg_S};
H_40_2_3_avg={H40_2_3_avg_t;H40_2_3_avg_S};
H99_2_3_avg={H99_2_3_avg_t;H99_2_3_avg_S};
H_WB19_03_1mm_uncompr_avg={H_WB19_03_1mm_uncompr_avg_S;H_WB19_03_1mm_uncompr_avg_t
    };
H_WB19_03_1mm_avg={H_WB19_03_1mm_avg_t;H_WB19_03_1mm_avg_S};
H_WB19_03_2mm_avg={H_WB19_03_2mm_avg_t;H_WB19_03_2mm_avg_S};
H_WB19_03_5mm_avg={H_WB19_03_5mm_avg_t;H_WB19_03_5mm_avg_S};

avg80=[H1_19_02_avg H40_19_02_avg H99_19_02_avg H_40_2_3_avg H99_2_3_avg
    H_WB19_03_1mm_uncompr_avg H_WB19_03_1mm_avg H_WB19_03_2mm_avg H_WB19_03_5mm_avg
    ];
avg60=[H40_19_02_60_avg H1_19_02_60_avg H99_2_3_60_avg];

%%
%% statistics:

% Question 1: is there a statistical difference between the peak stresses (in
    different sample groups) and tangent stiffnesses??

%  normal distribution?
normal_distribution= kstest(Absolute_1_line) %The result h is 1 if the test
    rejects the null hypothesis at the 5% significance level, or 0 otherwise.

%The Bonferroni:
  %model (alpha = 0.05) was used to compare the
  % peak stress and tangent stiffnesses of each of the clot analogues.

  %peak stress:
  for j=1:samples;
  peakS{j,2}
  end

  %tangent stiffnesses
Young_per_sample{j}

%%
%%GRAPHS 19-02:
```

```
figure(101)
hold all
for j=1:samples1_19_2
for i=1:cycles1_19_2
hold all
a=plot(new_time_vector/100,newy_ds1_19_2{1,j}{1,i},'y');
end
end
for j=1:samples1_19_2
for i=2:cycles1_19_2
hold all
a=plot(new_time_vector/100,newy_is1_19_2{1,j}{1,i},'y');
end
end
for j=1:samples1_19_2
for i=2:cycles1_19_2
hold all
a=plot(new_time_vector/100,newy_is1_19_2{1,j}{1,1},'y');
end
end

for j=1:samples40_19_2
for i=1:cycles40_19_2
hold all
b=plot(new_time_vector/100,newy_ds40_19_2{1,j}{1,i},'b');
end
end
for j=1:samples40_19_2
for i=2:cycles40_19_2
hold all
b=plot(new_time_vector/100,newy_is40_19_2{1,j}{1,i},'b');
end
end
for j=1:samples40_19_2
for i=2:cycles40_19_2
hold all
b=plot(new_time_vector/100,newy_is40_19_2{1,j}{1,1},'b');
end
end

for j=1:samples99_19_2
for i=1:cycles99_19_2
hold all
c=plot(new_time_vector/100,newy_ds99_19_2{1,j}{1,i},'g');
end
end
for j=1:samples99_19_2
for i=2:cycles99_19_2
hold all
c=plot(new_time_vector/100,newy_is99_19_2{1,j}{1,i},'g');
end
end
for j=1:samples99_19_2
for i=2:cycles99_19_2
hold all
c=plot(new_time_vector/100,newy_is99_19_2{1,j}{1,1},'g');
end
end
title('19-02-2021: 1mm high TAs, hysteresis lines')
lgd=legend('99%H')
xlabel('Nominal strain [-]')
```

```
ylabel('Nominal stress [kPa]')
lgd=legend([a b c],'1%H','40%H','99%H')


%% 19-02


figure(102)
hold all
a=plot(new_time_vector/100,Absolute_1_line1_19_2,'y')
b=plot(new_time_vector/100,Absolute_1_line40_19_2, 'b')
c=plot(new_time_vector/100,Absolute_1_line99_19_2, 'g')
title('19-02-2021: 1mm high TAs, hysteresis line (average and standard deviation)'
    )
xlabel('Nominal strain [-]')
ylabel('Nominal stress [kPa]')
lgd=legend([a b c],'1%H','40%H','99%H')
% errorbar(new_time_vector(indices)/100,points_mean1_19_2,std_10_20_etc1_19_2,'.y
    ')
% errorbar(new_time_vector(indices)/100,points_mean40_19_2,std_10_20_etc40_19_2,'.
    b')
% errorbar(new_time_vector(indices)/100,points_mean99_19_2,std_10_20_etc99_19_2,'.
    g')


figure(1022)%average
a=bar(perc, Young_avg_per_percentage_1H_19_02,'y')
hold on
b=bar(perc, Young_avg_per_percentage_40H_19_02,'b')
hold on
c=bar(perc, Young_avg_per_percentage_99H_19_02,'g')
title('19-02-2021: 1mm high TAs, Tangent modulus.')
lgd=legend([a b c],'1%H','40%H','99%H')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')



%Compare
%%
figure(104)
hold all


x=[20 50 80]
y=[Young_avg_per_percentage1_19_2(1,2) Young_avg_per_percentage40_19_2(1,2)
    Young_avg_per_percentage99_19_2(1,2); Young_avg_per_percentage1_19_2(1,5)
    Young_avg_per_percentage40_19_2(1,5) Young_avg_per_percentage99_19_2(1,5);
    Young_avg_per_percentage1_19_2(1,8) Young_avg_per_percentage40_19_2(1,8)
    Young_avg_per_percentage99_19_2(1,8)];
ybar=bar(x, y)
title('Youngh modulus 1 mm height 99%H samples 19-02-2021: tangent modulus average
     hysteresis in 1 line.')
lgd=legend('99%H')
xlabel('Nominal strain [%]')
ylabel('Tangent/young modulus [kPa]')
%% STATISTICS:
%The Bonferroni model (alpha = 0.05) was used
%to compare the peak stress and tangent stiffnesses of each of the clot analogues.

% you have 1 peak stress per sample, matching with 80% compr:
```

```
%%
%elke keer van cycle 1 en van 4de cylce
% One_line_meanH1_19_02
% One_line_meanH40_19_02
% One_line_meanH99_19_02
cycle1_stress1_19_2;
cycle1_stress40_19_2;
cycle1_stress99_19_2;

data_cycle1=[cycle1_stress1_19_2 cycle1_stress40_19_2 cycle1_stress99_19_2];
group={'G1','G1','G1','G1','G1','G2','G2','G2','G3','G3','G3','G3'};
[p1]=anova1(data_cycle1,group)

%Question:
%are the means of cycle 1 different in the 3 different clot analogs?
%Awnser: yes
% Because p1 <0.05


%post hoc: bonferonni

%%
%Question:
% Are the cycles of 1 and 4 are same in the same clots?
% Awnser: yes
% Because p2 > 0.05
cycles1_19_2=[cycle1_stress1_19_2,cycle4_stress1_19_2];
group={'G1','G1','G1','G1','G1','G2','G2','G2','G2','G2'};
[p2]=anova1(cycles1_19_2,group)



%% GLM (like cerenovus)
% the relationship between a response variable and independent variables using
    coefficients that can vary with respect to one or more grouping variables
mdl = fitglm(Young_per_sampleH1_19_02','Young modulus at 10%');

Young_avg_per_percentage1_19_2
Young_avg_per_percentage_40_2_3
%%
cycle4_stress1_19_2'
cycle4_stress40_19_2'
cycle1_stress99_19_2'
cycle4_stress99_19_2'
Young_per_sampleH1_19_02
Young_per_sampleH40_19_02
Young_per_sampleH99_19_02

% fitglme()
stats=anova(glme)

%one way anova Laerd spss
%%
% p = anova1(X)
% multcompare(stats)
[p,t,stats] = anovan(One_line_meanH1_19_02,{Young_per_sampleH1_19_02})% %[c,m,h,
    nms] = multcompare(ANOVA1)

%Similarly, the initial stiffness, calculated at 10% strain
```

```
%% 2-3
%3 mm high clots

figure(1023)%average
a=bar(perc, Young_avg_per_percentage_1H_2_3,'y')
hold on
b=bar(perc,Young_avg_per_percentage_40H_2_3,'b')
hold on
c=bar(perc, Young_avg_per_percentage_99H_2_3,'g')
title('02-03-2021: 3mm high TAs, Tangent modulus')
lgd=legend([a b c],'1%H','40%H','99%H')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')


figure(1024)
a=plot(new_time_vector_1H_2_3/100,Absolute_1_line_1H_2_3,'y')
hold on
b=plot(new_time_vector_40H_2_3/100,Absolute_1_line_40H_2_3,'b')
hold on
c=plot(new_time_vector_99H_2_3/100,Absolute_1_line_99H_2_3,'g')
title('02-03-2021: 3mm high TAs, hysteresis line (average and standard deviation)'
    )
lgd=legend([a b c],'1%H','40%H','99%H')
xlabel('Nominal strain [-]')
ylabel('Nominal stress [kPa]')
hold on
% errorbar(new_time_vector_1H_2_3(indices_1H_2_3)/100,points_mean_1H_2_3,
    std_10_20_etc_1H_2_3,'.y')
% errorbar(new_time_vector_40H_2_3(indices_40H_2_3)/100,points_mean_40H_2_3,
    std_10_20_etc_40H_2_3,'.b')
% errorbar(new_time_vector_99H_2_3(indices_99H_2_3)/100,points_mean_99H_2_3,
    std_10_20_etc_99H_2_3,'.g')
%
%


for i = 1:numel(One_line_is_and_ds1_19_2)
  One_line_is_and_ds1_19_2{1,i} = transpose(One_line_is_and_ds1_19_2{1,i});
end
G1_19_2=cell2mat(One_line_is_and_ds1_19_2);

for i = 1:numel(One_line_is_and_ds40_19_2)
  One_line_is_and_ds40_19_2{1,i} = transpose(One_line_is_and_ds40_19_2{1,i});
end
G40_19_2=cell2mat(One_line_is_and_ds40_19_2);

for i = 1:numel(One_line_is_and_ds99_19_2)
  One_line_is_and_ds99_19_2{1,i} = transpose(One_line_is_and_ds99_19_2{1,i});
end
G99_19_2=cell2mat(One_line_is_and_ds99_19_2);

First_cycle_all_19_2=[G1_19_2 G40_19_2 G99_19_2];

BF_test_values=First_cycle_all_19_2(:)
BF_test_label=zeros((200*(numel(One_line_is_and_ds1_19_2)+numel(
    One_line_is_and_ds40_19_2)+numel(One_line_is_and_ds99_19_2))),1)
R1=1;
R2=2;
R3=3;
```

```
BF_test_label(1:(200*(numel(One_line_is_and_ds1_19_2))),:)=R1
BF_test_label((200*(numel(One_line_is_and_ds1_19_2)))+1:(200*(numel(
    One_line_is_and_ds1_19_2)+numel(One_line_is_and_ds40_19_2))),:)=R2
BF_test_label(1+(200*(numel(One_line_is_and_ds1_19_2)+numel(
    One_line_is_and_ds40_19_2))):(200*(numel(One_line_is_and_ds1_19_2)+numel(
    One_line_is_and_ds40_19_2)+numel(One_line_is_and_ds99_19_2))),:)=R3

BF_test_19_2=[BF_test_values BF_test_label];
BFtest(BF_test_19_2,0.05)
group={'G1_19_2','G1_19_2','G1_19_2','G1_19_2','G1_19_2','G40_19_2','G40_19_2','
    G40_19_2','G99_19_2','G99_19_2','G99_19_2','G99_19_2','G99_19_2'};

[p,tbl,stats]=anova1(First_cycle_all_19_2,group)


%% WB

x=[1980 200]

perc10=[Young_avg_per_percentage_1WB(1,1)
    Young_avg_per_percentage_1WB_uncompr(1,1)
    Young_avg_per_percentage_2WB(1,1)
    Young_avg_per_percentage_5WB(1,1)];
perc20=[Young_avg_per_percentage_1WB(1,2)
    Young_avg_per_percentage_1WB_uncompr(1,2)
    Young_avg_per_percentage_2WB(1,2)
    Young_avg_per_percentage_5WB(1,2)];
perc30=[Young_avg_per_percentage_1WB(1,3)
    Young_avg_per_percentage_1WB_uncompr(1,3)
    Young_avg_per_percentage_2WB(1,3)
    Young_avg_per_percentage_5WB(1,3)];
perc40=[Young_avg_per_percentage_1WB(1,4)
    Young_avg_per_percentage_1WB_uncompr(1,4)
    Young_avg_per_percentage_2WB(1,4)
    Young_avg_per_percentage_5WB(1,4)];
perc50=[Young_avg_per_percentage_1WB(1,5)
    Young_avg_per_percentage_1WB_uncompr(1,5)
    Young_avg_per_percentage_2WB(1,5)
    Young_avg_per_percentage_5WB(1,5)];
perc60=[Young_avg_per_percentage_1WB(1,6)
    Young_avg_per_percentage_1WB_uncompr(1,6)
    Young_avg_per_percentage_2WB(1,6)
    Young_avg_per_percentage_5WB(1,6)];
perc70=[Young_avg_per_percentage_1WB(1,7)
    Young_avg_per_percentage_1WB_uncompr(1,7)
    Young_avg_per_percentage_2WB(1,7)
    Young_avg_per_percentage_5WB(1,7)];
perc80=[Young_avg_per_percentage_1WB(1,8)
    Young_avg_per_percentage_1WB_uncompr(1,8)
    Young_avg_per_percentage_2WB(1,8)
    Young_avg_per_percentage_5WB(1,8)];


all=[perc10'
    perc20'
    perc30'
    perc40'
    perc50'
    perc60'
    perc70'
    perc80'];
```

```
figure(1)
bar(perc,all)
title('19-03-2021: WB TAs, hysteresis line (average and standard deviation)')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')
legend


%%
figure(1025)%average
hold all
a=bar(perc, Young_avg_per_percentage_1WB,'b')
hold on
b=bar(perc,Young_avg_per_percentage_1WB_uncompr,'c')
hold on
c=bar(perc, Young_avg_per_percentage_2WB,'r')
hold on
d=bar(perc, Young_avg_per_percentage_5WB,'y')
bar(a,b,c,d)
title('19-03-2021: 3mm high TAs, Tangent modulus')
lgd=legend([a b c d],'1mm WB','1mm WB uncompressed','2mm WB','5mm WB')
xlabel('Nominal strain [%]')
ylabel('Tangent modulus [kPa]')
%%
figure(1026)
a=plot(new_time_vector_1WB/100,Absolute_1_line_1WB,'Color','#0072BD')
hold on
b=plot(new_time_vector_1WB_uncompr/100,Absolute_1_line_1WB_uncompr,'Color','#
    D95319')
hold on
c=plot(new_time_vector_2WB/100,Absolute_1_line_2WB,'Color','#EDB120')
hold on
d=plot(new_time_vector_5WB/100,Absolute_1_line_5WB,'Color','#7E2F8E')
title('19-03-2021: WB TAs, hysteresis line (average and standard deviation)')
lgd=legend([a b c d],'1mm WB','1mm WB uncompressed','2mm WB','5mm WB')
xlabel('Nominal strain [-]')
ylabel('Nominal stress [kPa]')
hold on
% errorbar(new_time_vector_1WB(indices_1WB)/100,points_mean_1WB,std_10_20_etc_1WB
    ,'.k')
% errorbar(new_time_vector_1WB_uncompr(indices_1WB_uncompr)/100,
    points_mean_1WB_uncompr,std_10_20_etc_1WB_uncompr,'.k')
% errorbar(new_time_vector_2WB(indices_2WB)/100,points_mean_2WB,std_10_20_etc_2WB
    ,'.k')
% errorbar(new_time_vector_5WB(indices_5WB)/100,points_mean_5WB,std_10_20_etc_5WB
    ,'.k')
```

11.3 Matlab excel output tensile test syringe

```
%Written by Manouk Mondeel
clear all; clc
%doc for information
%% analysis tensile test data
T = readtable('syringe5ml_in1keer.xlsx'); %load excell table
%% make column 1 of elapsed time with excell input format dd-mm-yyyy  hh:mm:ss.ms
dt=T{:,1}; %use {} for a double format!
[h,m,s]=hms(dt); %extract hours, minutes, seconds (and miliseconds)
h=h*60*60; %hours in seconds
m=m*60; %minutes in seconds
time0= h(1)+ m(1)+ s(1); %substract starting time for duration
```

```
time=[(h+m+s)- time0]; % in seconds

%% Make a plot of Force values
force=T{:,2}; % select 1 column
%%
plot(time, force)
```

11.4 Functions used

11.4.1 Hysteresis

```
function[first_cycle_Young_avg_per_percentage,first_cycle_points_mean,
    first_cycle_std_10_20_etc,first_cycle_std_per_strain,One_line_is_and_ds,
    First_cycle_One_line_mean,first_cycle_is_mean,first_cycle_ds_mean,
    first_cycle_is,first_cycle_ds,newy_ds,olds_d,newy_d,old_d,Young_per_sample,
    Young_avg_per_percentage,dy,points_mean,indices,Absolute_1_line,std_per_strain,
    std_10_20_etc,One_line_mean,mean_per_sample_decr,mean_per_sample_incr,newy_is,
    new_time_vector,perc]=hysteresis4(peakheight,peakdistance,data,stepsize_valley,
    cycles,samples,strain_level,desired_vector_lengths)
for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance) %should give n=cycles peaks
end


%% hysteresis loop
new_time_vector200=0:(80/199):80;
new_time_vector=(0:(strain_level/(desired_vector_lengths-1)):strain_level);
%2. valleys:
for cycles=1:cycles
valley(cycles)=stepsize_valley*cycles; %stepsize is an index number
end
valley=valley.';

for i=1:samples
    [peakS{i,2},peakt{i,1}]=findpeaks(data{i,2},'MinPeakProminence', peakheight, '
       MinPeakDistance', peakdistance); %gives 5
end

% Step 3: making increasing and decreasing timestamps:

desired=0:(strain_level/desired_vector_lengths):strain_level; %desired vector
    length
%0:(strain_level/length_d{1,i}(1,j)):strain_level


%% decreaseing steps:
%bij spline ook decr gebruiken

timestamp_samples_decr_rev=cell(1,samples);
lengths_d=cell(1,samples);
olds_d=cell(1,samples);
newy_ds=cell(1,samples);
for j=1:samples;
for i=1:cycles;
timestamp_sample_d{1,i}=peakt{j,1}(i,1):valley(i);
timestamp_sample_d_rev{1,i}=timestamp_sample_d{1,i}(end:-1:1);
length_d{1,i}=length(timestamp_sample_d{1,i});
old_d{1,i}=0:strain_level/(length_d{1,i}-1):strain_level;
newy_d{1,i}=spline(old_d{1,i},data{j,2}(timestamp_sample_d_rev{1,i}),
    new_time_vector);
```

```
end
timestamp_samples_decr_rev{1,j}=timestamp_sample_d_rev;
lengths_d{1,j}=length_d;
olds_d{1,j}=old_d;
newy_ds{1,j}=newy_d;
mean_per_sample_decr{1,j}=mean(cell2mat(newy_d'),1)
end
%% increasing steps:
s=0
timestamp_samples_incr=cell(1,samples);
lengths_i=cell(1,samples);
olds_i=cell(1,samples);
for j=1:samples;
for i=2:cycles;
timestamp_sample_i{1,i}=valley(i-1):peakt{j,1}(i,1);
timestamp_sample_i{1,1}=1:peakt{j,1}(1,1);
length_i{1,i}=length(timestamp_sample_i{1,i});
length_i{1,1}=length(timestamp_sample_i{1,1});
old_i{1,i}=0:strain_level/(length_i{1,i}-1):strain_level;
old_i{1,1}=0:strain_level/(length_i{1,1}-1):strain_level;
newy_i{1,i}=spline(old_i{1,i},data{j,2}(timestamp_sample_i{1,i}),new_time_vector);
newy_i{1,1}=spline(old_i{1,1},data{j,2}(timestamp_sample_i{1,1}),new_time_vector);
end
timestamp_samples_incr{1,j}=timestamp_sample_i;
timestamp_samples_incr{1,j}{1,1}=1:peakt{j,1}(1,1);
lengths_i{1,j}=length_i;
newy_is{1,j}=newy_i;
mean_per_sample_incr{1,j}=mean(cell2mat(newy_i'),1)
end

%% sum
for j=1:samples
One_line_mean{1,j}=(mean_per_sample_incr{1,j}+mean_per_sample_decr{1,j})/2
Absolute_1_line=mean(cell2mat(One_line_mean'),1)
std_per_strain=std(cell2mat(One_line_mean'),0,1)
end

[n,index10]=(min(abs(new_time_vector-10)));
[n,index20]=(min(abs(new_time_vector-20)));
[n,index30]=(min(abs(new_time_vector-30)));
[n,index40]=(min(abs(new_time_vector-40)));
[n,index50]=(min(abs(new_time_vector-50)));
[n,index60]=(min(abs(new_time_vector-60)));
[n,index70]=(min(abs(new_time_vector-70)));
[n,index80]=(min(abs(new_time_vector-80)));

indices=[index10 index20 index30 index40 index50 index60 index70 index80];

%select std s at 10% strain 20% ... etc
std_10_20_etc=std_per_strain(indices)
points_mean=[Absolute_1_line(index10) Absolute_1_line(index20) Absolute_1_line(
    index30) Absolute_1_line(index40) Absolute_1_line(index50) Absolute_1_line(
    index60) Absolute_1_line(index70) Absolute_1_line(index80)]


%Tangent modulus is defined as the slope of a line tangent to the stress-strain
    curve at a point of interest.
df=diff(Absolute_1_line);
dy=gradient(Absolute_1_line(:))./gradient(new_time_vector(:)/100)
Young_avg_per_percentage=[dy(index10) dy(index20) dy(index30) dy(index40) dy(
    index50) dy(index60) dy(index70) dy(index80)]
```

```
perc=10:10:80
perc=[perc]


diff_per_sample=cell(1,samples)
for j=1:samples;
diff_per_sample{j}=diff(One_line_mean{1,j})
Young_per_sample{j}=gradient(One_line_mean{1,j}(:))./gradient(new_time_vector(:)
    /100)
end



%% Only taking the first cycle per sample into account:
first_cycle_is=cell(1,samples)
first_cycle_ds=cell(1,samples)
One_line_is_and_ds=cell(1,samples)
for i=1:samples
first_cycle_is(1,i)={newy_is{1,i}{1,1}}
first_cycle_ds(1,i)={newy_ds{1,i}{1,1}}
One_line_is_and_ds(1,i)={(newy_is{1,i}{1,1}+newy_ds{1,i}{1,1})/2}
%first_cycle_ds={newy_ds{1,1}{1,1} newy_ds{1,2}{1,1} newy_ds{1,3}{1,1} newy_ds
    {1,4}{1,1} newy_ds{1,5}{1,1}}
end

first_cycle_std_per_strain=std(cell2mat(One_line_is_and_ds'),0,1)

first_cycle_is_mean=mean(cell2mat(first_cycle_is'),1)
first_cycle_ds_mean=mean(cell2mat(first_cycle_ds'),1)

First_cycle_One_line_mean=(first_cycle_is_mean+first_cycle_ds_mean)/2

first_cycle_std_10_20_etc=first_cycle_std_per_strain(indices)
first_cycle_points_mean=[First_cycle_One_line_mean(index10)
    First_cycle_One_line_mean(index20) First_cycle_One_line_mean(index30)
    First_cycle_One_line_mean(index40) First_cycle_One_line_mean(index50)
    First_cycle_One_line_mean(index60) First_cycle_One_line_mean(index70)
    First_cycle_One_line_mean(index80)]

first_cycle_df=diff(First_cycle_One_line_mean);
first_cycle_dy=gradient(First_cycle_One_line_mean(:))./gradient(new_time_vector(:)
    /100)
first_cycle_Young_avg_per_percentage=[first_cycle_dy(index10) first_cycle_dy(
    index20) first_cycle_dy(index30) first_cycle_dy(index40) first_cycle_dy(index50
    ) first_cycle_dy(index60) first_cycle_dy(index70) first_cycle_dy(index80)]

%% example plotting:
% new_time_vector200=0:(80/199):80;
% figure(112)
% for j=1:samples
% for i=1:cycles
% hold all
% plot(new_time_vector200,newy_ds{1,j}{1,i})
% end
% end
% for j=1:samples
% for i=2:cycles
% hold all
% plot(new_time_vector200,newy_is{1,j}{1,i})
% end
% end
```

```
% for j=1:samples
% for i=2:cycles
% hold all
% plot(new_time_vector200,newy_is{1,j}{1,1})
% end
% end
```

11.4.2 Baseline fit

```
function [ycorr,yfit] = bf(y,varargin)
%     https://www.mathworks.com/matlabcentral/fileexchange/24916-baseline-fit
%Mirko Hrovat (2021). Baseline Fit (https://www.mathworks.com/matlabcentral/
   fileexchange/24916-baseline-fit), MATLAB Central File Exchange. Retrieved
   February 8, 2021.

% Baseline Fit each column in "x".
% Syntax: [ycorr,yfit] = bf(y,pts,avgpts,method,confirm);
%   ycorr = bf(y);  ycorr = bf(y,method); ycorr = bf(y,avgpts);
%   ycorr = bf(y,pts);  ycorr = bf(y,pts,avgpts);
% A baseline fit is interpolated from selected points and then applied to the data
   .
%   "y" is a vector or array.
%       If an array, the baseline fit is performed for each column of data (dim 1)
   .
%   Arguments following "y" may be in any order.
%   "pts" is vector specifying the indices of the points for a baseline fit.
%       If not specified then a plot is displayed and the user is instructed to
%       interactively select points for the baseline fit.
%       End points are always automatically included for interactive "pts"
   selection,
%       and do not need to explicitly selected. It is recommended that the end
   points
%       be included in any list of "pts".
%       It is not necessary to order or sort "pts" before use.
%   "avgpts" determines the width in points for the calculation of the mean y(x)
%       value, where x is a selected point in "pts". (Default = 3).
%       This can be helpful for noisy data.
%   "method" controls the algorithm applied for the baseline fit. The routine uses
%       Matlab's interp1 command. "method" must be one of the methods supported by
%       interp1. (Default is 'spline').
%   "confirm", if specified as the string 'confirm', will allow the user to see
   the
%       result and to confirm it is acceptable. If not the user can reslect "pts".
%   "ycorr" is the baseline corrected data in the same format as "y".
%   "yfit" is a vector or array with the interpolated baseline fit.
%
% Examples:
%   [y,yfit] = bf(y,'confirm','linear');
%       "y" will be plotted and the user is instructed to select points for the
   fit.
%       A baseline will be linearly interpolated from the selected points and will
    be
%       plotted together with "y". The user is prompted as to whether to redo the
%       baseline selection. Upon completion, the corrected data "y" and the fitted
%       baseline "yfit" are output.
%   ycorr = bf(y,5);
%      "y" is plotted and the user is instructed to select points for the fit.
%       The baseline fit is based on the mean value of "y" over 5 points centered
   on
%       the selected points. Cubic spline interpolation is used for the baseline
   fit.
%       The corrected data "ycorr" is output.
```

```
%   ycorr = bf(y,[5,10,15,30,35,40],'pchip');
%       Points with the specified indices are used to calculate a baseline fit
    using
%       the piecewise cubic Hermite interpolation method. No data is plotted.
%       The baseline fit is based on the mean value of "y" over 3 points centered
    on
%       the selected points. The corrected data "ycorr" is output.
%
% See Also:   interp1, spline, ginput
% Copyright 2009 Mirtech, Inc.
% Created by    Mirko Hrovat    08/01/2009  contact:mhrovat@email.com
def_method  = 'spline';
def_avgpts  = 3;
method = [];
avgpts = [];
pts    = [];
confirm = false;
for n = 2:nargin,
    f = varargin{n-1};
    if ischar(f),
        if strcmpi(f,'confirm'),
            confirm = true;
        else
            method = f;
        end
    elseif isnumeric(f) && numel(f) == 1,
        avgpts = f;
    elseif isnumeric(f) && numel(f) > 1,
        pts = f;
    elseif isempty(f),
        continue
    else
        error ('  Invalid input argument!')
    end
end
if isempty(method),     method = def_method;        end
if isempty(avgpts),     avgpts = def_avgpts;        end
dimy = size(y);
lst = dimy(1);
newdimy = [dimy(1),prod(dimy(2:end))];
y = reshape(y,newdimy);
x = 1:lst;
if isempty(pts),
    interactive = true;
else
    interactive = false;
end
if interactive || confirm,
    bffig = figure;
else
    bffig = 0;
end
ok = false;
while ~ok,
    if interactive,
        plot(x,real(y(:,1)))
        set(bffig,'Name','Baseline Fit - Select points')
        fprintf(['\n Now select baseline points to fit by positioning cursor,',...
            '\n   and selecting points with mouse button or key press.',...
            '\n Press Enter key when done.\n'])
        [a,b] = ginput;                                 %#ok
```

```
        pts = round(a.');
    end
    pts = sort(pts);
    pts(diff(pts)==0) = [];          % delete duplicate points
    if pts(1)~=1,        pts = [1,pts];          end     %#ok
    if pts(end)~=lst,    pts = [pts,lst];        end     %#ok
    npts = numel(pts);
    pss = zeros(npts,2);
    pss(:,1) = pts - floor(avgpts/2);
    pss(:,2) = pss(:,1) + avgpts;
    pss(pss < 1) = 1;
    pss(pss > lst) = lst;
    yavg = zeros([npts,newdimy(2)]);
    for n = 1:npts,
        yavg(n,:) = mean(y(pss(n,1):pss(n,2),:),1);
    end
    yfit = interp1(pts,yavg,x,method);
    if size(yfit,1) ==1,
        yfit = shiftdim(yfit,1);    % make yfit a column if it is a row vector
    end
    if confirm,
        interactive = true;
        figure(bffig)
        plot(x,real(y(:,1)),'b',x,real(yfit(:,1)),'r',pts,real(yavg(:,1)),'ob')
        set(bffig,'Name','Baseline Fit - Verify baseline')
        answer = input('  Do you to redo fit and reselect baseline points?[N] ','s
            ');
        if isempty(answer),     answer = 'n';   end
        if strcmpi(answer,'y'),
            ok = false;
        else
            ok = true;
        end
    else
        ok = true;
    end
end
if any(findobj('Type','figure')==bffig),
    close(bffig),                   % close figure if it exists
end
ycorr = y - yfit;
ycorr = reshape(ycorr,dimy);
yfit = reshape(yfit,dimy);
```

11.4.3 Timefunc

```
%written by Manouk Mondeel
function [timei]=timefunc(dt)
[hi,mi,si]=hms(dt); %extract hours, minutes, seconds (and miliseconds)
hi=hi*60*60; %hours in seconds
mi=mi*60; %minutes in seconds
time0i= hi(1)+ mi(1)+ si(1); %substract starting time for duration
timei=[(hi+mi+si)- time0i]; % in seconds
end
```

11.4.4 Mwe

```
clear all, close all
%%
nbin = 180; % number of bins for estimation / histogram / plotting

%%% single peak, estimation
```

```
% load FOA output
angles= double(degrees);

% convert from row to column
angles = angles';

% convert from degrees to radians
angles = deg2rad(angles);

% have FibLab do its thing (single Gaussian peak)
param = fib_estdist('phi', angles, 'nbin', nbin) % [peak centre, peak std ,
    anisotropic fraction]


%%% single peak, feedback

figure
% get histogram counts & bin centres (180 bins)
[n, bp] = fib_hist(angles, nbin);
% plot (nomalised) angle histogram (convert to degrees)
h = bar(rad2deg(bp), n/sum(n));
% make `pretty'
set(h, 'facecolor', [.8 .8 .8], 'linestyle', 'none')


% get a line of the estimated distribution / (Guassian) peak (180 bins)
edist = fib_gendist(param, nbin);

% add (normalised) envelope of estimated distribution to figure
hold on
plot(linspace(0, 180, nbin), edist, 'b', 'linewidth', 2)


% convert estimated param(1) and param(2) (back) to degrees?
param(1:2) = rad2deg(param(1:2))




%% two peaks, estimation

%clear all, close all
nbin = 180;

% load FOA output
%angles = load('degrees.mat');
angles= double(degrees);

% convert from row to column
angles = angles';

% convert from degrees to radians
angles = deg2rad(angles);

% have FibLab do its thing (two Gaussian peaks)
param = fib_estdist('phi', angles, 'nbin', nbin, 'npi', 2) % [peak centre, peak
    std , anisotropic fraction]


%%% two peaks, feedback
```

```
figure
% get histogram counts & bin centres (180 bins)
[n, bp] = fib_hist(angles, nbin);
% plot (nomalised) angle histogram (convert to degrees)
h = bar(rad2deg(bp), n/sum(n));
% make `pretty'
set(h, 'facecolor', [.8 .8 .8], 'linestyle', 'none')


% get a line of the estimated distribution / (Guassian) peak (180 bins)
edist = fib_gendist(param, nbin);

% add (normalised) envelope of estimated distribution to figure
hold on
plot(linspace(0, 180, nbin), edist, 'b', 'linewidth', 2)


% convert estimated param(1) and param(2) (back) to degrees?
param(:, 1:2) = rad2deg(param(:, 1:2))
```

11.4.5 Ibidi sticky slide I 0.6 Luer example

```
clear all
clc

%% Written by Manouk Mondeel with help of Sheila Serra
% calculating Shear stress and shear rate in Ibidi channel slides, based on:
   Application Note 11    ibidi GmbH, Version 3.5, Roman Zantl, July 25th, 2012
% http://www.thundersci.com/d/file/PRODUCT1/ibidi/instrument1/2016-01-22/
   c1a9df838bb5e36b0bcafb651e326050.pdf page 15-16.

%% quantity: [SI unit]:
syms tau gamma eta dpdz  b l inf n phi% making variables
% phi: total flow rate through channel [ml/ min]
% tau: shear stress [dyn/cm ]
% gamma: shear rate [1/s]
% eta: dynamical viscosity [dyn s/cm ]
% h: half of the height of the channel [cm]
% b: half of the width of the channel [cm]
% A: cross sectional area of a rectangular channel [cm^2]
% l: length of the channel [cm]

%% Fill in the values for height and width, by dividing by 2:

%specific slide geometry! Sticky slide I Luer --> type 0.6 (this is the height)
b=(0.5/2); %[cm]
x=0; %when one wants to know the tau in the center of the channel, then x=0;

l=5; %[cm]
h=(0.065/2); %[cm]
y=-h; %when one wants to know the tau in the bottom of the channel, then y=-h;

% % Dynamical viscocity/or absolute viscocity blood:
eta=0.01; % [dyn s/cm ] %between 3 - 4 for blood %[dyn s/cm ] (=100 mPa s = 1
   cP).
%%
% % Dimensions:
A=2*h*2*b %the xy plane
volume=A*l
%%
syms n m k r
```

```
%% q
sum2=symsum((1/((2*n+1)^5))*tanh(((2*n+1)*pi*h)/(2*b)),n,0,Inf) ;% domain 0 to inf
sum2_fin=vpa(sum2);

q2=((4/3)*h*(b^3))-(((8*b^4)*((2/pi)^5)*sum2_fin));

% dpdz=-eta*(phi/q)
% phi=-(1/eta)*dpdz*q % total flow
%% first way to get tau

sum3=symsum((((b*pi)*((-1)^m))/(2*m+1)^2)*((2/pi)^3))*((sinh((2*m+1)*((pi*y)/(2*b
    )))/cosh((2*m+1)*((pi*h)/(2*b))))*cos(((2*m+1)*pi*x)/(2*b))),m,0,Inf);
sum3_fin=vpa(sum3)/60; %/60 because of units
tau1= -1*eta*(phi/q2)*sum3_fin %*-1 because the sign should be positive

%% second way to get tau

sum4=symsum(((b*pi*(-1)^k)/(2*k+1)^2)*((2/pi)^3)*tanh((2*k+1)*((pi*h)/(2*b))),k,0,
    Inf);
sum4_fin=vpa(sum4)/60;

tau2=eta*(phi/q2)*(sum4_fin)
```

11.4.6 Ratios in single pump experiment

```
%%Written by Manouk Mondeel
clear all
clc
%% FILL THIS BLOCK IN

PPP_for_1_session=500; %volume that you want to pass the flow chamber[microL]
amount_of_samples=5;
mistakes=500; % it is best to give some room for spilling fluids [microL]

%labeled PPP_desired_end_volume? Than use:
AF= 0.025; % AF488-labeled fibrinogen [microg/microl] in this case
AF_stock= 1.5; %stock solution [mg/ml] or [microg/microliter]

h=0.13;%0.05;% height channel [mm] % is 50 micrometer
w=5; % width channel [mm]
L=30; %length channel [mm]
% The same dimensions as Judith her flow chamber!!

gamma=300; % desired shear rate[/s]!

HEPES_buffer_total= 1000000; %amount of HEPES buffer that you want to make [
    microliter]
%% Needed volume PPP estimation:

PPP_desired_end_volume=(amount_of_samples*PPP_for_1_session) + (3*mistakes)
CaCl2=(PPP_desired_end_volume/97)*3 % PPP_desired_end_volume is 97% and CaCl2 is
    3% of total solution
%% AF488-labeled fibrinogen needed and PPP needed to be added:

AF=AF*PPP_desired_end_volume; % needed [microL]
fibrinogen= AF/AF_stock;%
PPP_needed_to_be_added= PPP_desired_end_volume-fibrinogen;
%% HEPES buffer making

HEPES_mol=238.3;% g/mol or mg/mmol or microliter/micromol
NaCl_mol=58.44; % g/mol
KCl_mol= 74.56; % g/mol
```

```
BSA_perc=1; %

temp1=(137*HEPES_buffer_total)/1000;% NaCl=137 mM
NaCl=NaCl_mol*temp1 ;%amount of mg needed!

temp2=(3.5*HEPES_buffer_total)/1000; % KCl=3.5 mM
KCl=KCl_mol*temp2; %amount of mg needed!

temp3=(25*HEPES_buffer_total)/1000; %HEPES=25 mM
HEPES=HEPES_mol*temp3 ;%amount of mg needed!

MiliQ=HEPES_buffer_total; % in microliter

BSA=(MiliQ*10) ;% microgram needed of  1% (pH 7.4)
%% 9:1 ratio for plasma : activator mix

phi_plasma=((gamma*(h^2)*w)/100)*60; %flow rate [ml/h]
%gamma=(100*phi)/(w*h^2) %shear rate
%% volumes chamber:
volume=h*w*L; % mm^3=microliter
%% time needed for 1 session thorough chamber

phi_plasma_min=(phi_plasma*1000)/60; %ml/h to microliter/min
time_flow=PPP_for_1_session/phi_plasma_min; % minutes needed of flow in flow
    chamber


%% In a table
streep=0;
specimen={'Amount of samples';'PPP desired end volume';'PPP needed to be added to
    solution';'AF488-labeled fibrinogen';'CaCl2 addition';'.............';'height'
    ;'width';'length';'volume chamber';'.............';'shear rate';'phi plasma';'
    PPP for 1 session thorough chamber';'time flow thorough chamber';'
    .............';'HEPES buffer making';'NaCl';'KCl';'HEPES';'MiliQ';'BSA'};
value=[amount_of_samples;PPP_desired_end_volume;PPP_needed_to_be_added;fibrinogen;
    CaCl2;streep;h;w;L;volume;streep;gamma;phi_plasma;PPP_for_1_session;time_flow;
    streep;HEPES_buffer_total;NaCl;KCl;HEPES;MiliQ;BSA];
unities={'integer number';'microL';'microL';'microL';'microL';'.......';'mm';'mm';
    'mm';'mm^3 = microliter';'.......';'/s';'ml/h';'microL';'minutes';'.......';'
    microL';'microg';'microg';'microg';'microL';'microg'};
dimensions=table(specimen,value,unities)
```

11.4.7 Ratios in two pump experiment

```
%%Written by Manouk Mondeel
clear all
clc
%% FILL THIS BLOCK IN

PPP_for_1_session=500; %volume that you want to pass the flow chamber[microL]
amount_of_samples=12;
mistakes=500; % it is best to give some room for spilling fluids [microL]

%labeled PPP_desired_end_volume? Than use:
AF= 0.025; % AF488-labeled fibrinogen [microg/microl] in this case
AF_stock= 1.5; %stock solution [mg/ml] or [microg/microliter]

h=0.13;%0.05;% height channel [mm] % is 50 micrometer
w=5; % width channel [mm]
L=30; %length channel [mm]
% The same dimensions as Judith her flow chamber!!
```

```
gamma=50; % desired shear rate[/s]!

HEPES_buffer_total= 1000000; %amount of HEPES buffer that you want to make [
    microliter]
%% Needed volume PPP estimation:

PPP_desired_end_volume=(amount_of_samples*PPP_for_1_session) + (3*mistakes)
%% AF488-labeled fibrinogen needed and PPP needed to be added:

AF=AF*PPP_desired_end_volume; % needed [microL]
fibrinogen= AF/AF_stock;%
PPP_needed_to_be_added= PPP_desired_end_volume-fibrinogen;
%%  Ratios for Thrombin+Calcium and PPP_desired_end_volume in flow experiment:
% The concentrations of CaCl2 and thrombin in this solution are 10 times as
% high as you want them to be in the clot. This is needed for the shear
% ratios.

activator_mix=PPP_desired_end_volume/9; % fill in desired value in microliters!
HEPES_buffer= 0.7*activator_mix; %microl
CaCl2=0.2*activator_mix ;%microl (my stock is 850 mM)
Thrombin=0.1*activator_mix;  %microl, (stock is 100 U/ml)
end_volume_PPP_desired_end_volume=HEPES_buffer+CaCl2+Thrombin;
%% HEPES buffer making

HEPES_mol=238.3;% g/mol or mg/mmol or microliter/micromol
NaCl_mol=58.44; % g/mol
KCl_mol= 74.56; % g/mol
BSA_perc=1; %

temp1=(137*HEPES_buffer_total)/1000;% NaCl=137 mM
NaCl=NaCl_mol*temp1 ;%amount of mg needed!

temp2=(3.5*HEPES_buffer_total)/1000; % KCl=3.5 mM
KCl=KCl_mol*temp2; %amount of mg needed!

temp3=(25*HEPES_buffer_total)/1000; %HEPES=25 mM
HEPES=HEPES_mol*temp3 ;%amount of mg needed!

MiliQ=HEPES_buffer_total; % in microliter

BSA=(MiliQ*10) ;% microgram needed of  1% (pH 7.4)
%% 9:1 ratio for plasma : activator mix

phi_plasma=((gamma*(h^2)*w)/100)*60; %flow rate [ml/h]
%gamma=(100*phi)/(w*h^2) %shear rate
%% volumes chamber:
volume=h*w*L; % mm^3=microliter
%% time needed for 1 session thorough chamber

phi_plasma_min=(phi_plasma*1000)/60; %ml/h to microliter/min
time_flow=PPP_for_1_session/phi_plasma_min; % minutes needed of flow in flow
    chamber


%% In a table
streep=0;
```

```
specimen={'Amount of samples';'PPP desired end volume';'PPP needed to be added to
    solution';'AF488-labeled fibrinogen';'.............';'height';'width';'length'
    ;'volume chamber';'.............';'shear rate';'phi plasma';'PPP for 1 session
     thorough chamber';'time flow thorough chamber';'.............';'activator mix
     making';'HEPES buffer in activator mix';'CaCl2';'Thrombin';'.............';'
    HEPES buffer making';'NaCl';'KCl';'HEPES';'MiliQ';'BSA'};
value=[amount_of_samples;PPP_desired_end_volume;PPP_needed_to_be_added;fibrinogen;
    streep;h;w;L;volume;streep;gamma;phi_plasma;PPP_for_1_session;time_flow;streep;
    activator_mix;HEPES_buffer;CaCl2;Thrombin;streep;HEPES_buffer_total;NaCl;KCl;
    HEPES;MiliQ;BSA];
unities={'integer number';'microL';'microL';'microL';'.......';'mm';'mm';'mm';'mm
    ^3 = microliter';'.......';'/s';'ml/h';'microL';'minutes';'.......';'microL';'
    microL';'microL';'microL';'.......';'microL';'microg';'microg';'microg';'microL
    ';'microg'};
dimensions=table(specimen,value,unities)
```

11.5 Macro's: Fiji

1. **Threshold_94**
   run("8-bit"); run("Invert"); run("Smooth"); setAutoThreshold("Default dark"); //run("Threshold..."); setThreshold(0, 94); //setThreshold(0, 94); run("Convert to Mask"); run("Despeckle"); run("Make Binary"); run("Create Selection"); run("Measure");
2. **Inverse_threshold_94**
   run("8-bit"); run("Invert"); run("Smooth"); setAutoThreshold("Default dark"); //run("Threshold..."); setThreshold(0, 94); //setThreshold(0, 94); run("Convert to Mask"); run("Despeckle"); run("Make Binary"); run("Create Selection"); run("Measure");
3. **Threshold_5**
   run("8-bit"); run("Smooth"); setAutoThreshold("Default dark"); //run("Threshold..."); setThreshold(0, 55); //setThreshold(0, 55); run("Convert to Mask"); run("Despeckle"); run("Make Binary"); run("Create Selection"); run("Measure");
4. **Threshold_155_more_noise**
   run("8-bit"); run("Smooth"); setAutoThreshold("Default dark"); //run("Threshold..."); setThreshold(0, 115); //setThreshold(0, 115); run("Convert to Mask"); run("Despeckle"); run("Make Binary"); run("Create Selection"); run("Measure");

12 Appendices

12.1 Thrombus analogs formed under pressure: results

12.1.1 Surface areas

The resultant surface areas of the clots formed under pressure can be seen in table 14 and 15.

Table 14. Surface area TAs formed under pressure.

Blood donor	Date testing	Sample: %H, height	Test	Sample number	Surface [mm^2]
Male, 22 years	04-2-2021	1%H, 1 mm	80% compression	1	5.79
				2	8.18
				3	3.78
				4	5.78
				5	4.54
		40%H, 1 mm	80% compression	1	7.06
				2	22.29
				3	7.13
				4	20.73
				5	13.20
		99%H, 1 mm	80% compression	1	8.79
				2	11.94
				3	7.74
				4	8.62
				5	7.95
Male, 53 years	19-02-2021	1%H, 1 mm	80% compression	1	11.23
				2	9.51
				3	7.13
				4	6.77
				5	8.83
		1%H, 1 mm	60% compression	1	5
				2	4.65
				3	6.61
				4	7.27
				5	15.26
		40%H, 1 mm	80% compression	1	7.77
				2	9.74
				3	7.74
				4	5.27
				5	14.68
		40%H, 1 mm	60% compression	1	12.25
				2	6.13
				3	4.39
				4	11.23
				5	8.01
		99%H, 1 mm	80% compression	1	4.35
				2	5.15
				3	7.17
				4	6.27
				5	4.94
		99%H, 5 mm	80% compression	1	68.22
				2	80.21
				3	101.76
				4	110.79
				5	66.30

Table 15. Surface area TAs formed under pressure.

Blood donor	Date testing	Sample: %H, height	Test	Sample number	Surface [mm^2]
Female, 27 years	02-03-2021	1%H, 3 mm	80% compression	1	14.67
				2	27.70
				3	15.37
				4	14.05
				5	14.86
		1%H, 3 mm (post-test)	80% compression	1	16.08
				2	28.67
				3	16.83
				4	16.19
				5	16.35
		40%H, 3 mm	80% compression	1	52.07
				2	41.72
				3	50.06
				4	46.91
				5	49.53
		99%H, 3 mm	80% compression	1	105.16
				2	50.48
				3	53.47
				4	94.73
				5	111.97
		99%H, 1 mm	60% compression	1	5.47
				2	11.25
				3	10.78
				4	6.59
				5	3.45
Female, 29 years	19-03-2021	WB, 1 mm	80% compression	1	66.46
				2	53.79
				3	60.26
				4	60.82
				5	59.40
		WB, 1 mm (no weight)*	80% compression	1	31.54
				2	32.13
				3	21.27
				4	69.41
				5	29.84
		WB, 2 mm	80% compression	1	69.89
				2	63.42
				3	59.00
				4	69.17
				5	72.02
		WB, 5 mm	80% compression	1	62.49
				2	60.31
				3	60.11
				4	61.75
				5	58.69

12.2 Fibrin clot formation under flow: methods

12.2.1 Piuma tip selection graph

In order to choose the right tip size, there should be a balance between the spatial resolution of the mapping which depends on the heterogeneity of the sample, and the scale of the measurement in terms of depth. For surface properties, a smaller indentation depth is desirable, for bulk/averaged properties larger depths with a tip with a larger radius should be considered. However, when using larger tips, step size needs to be higher and, thus, you lose spatial resolution during indentation mapping.[64] In figure 28 the probes which were bought are shown, the green is the probe used in this thesis, and the orange line is a probe which can be used in future for softer TAs.

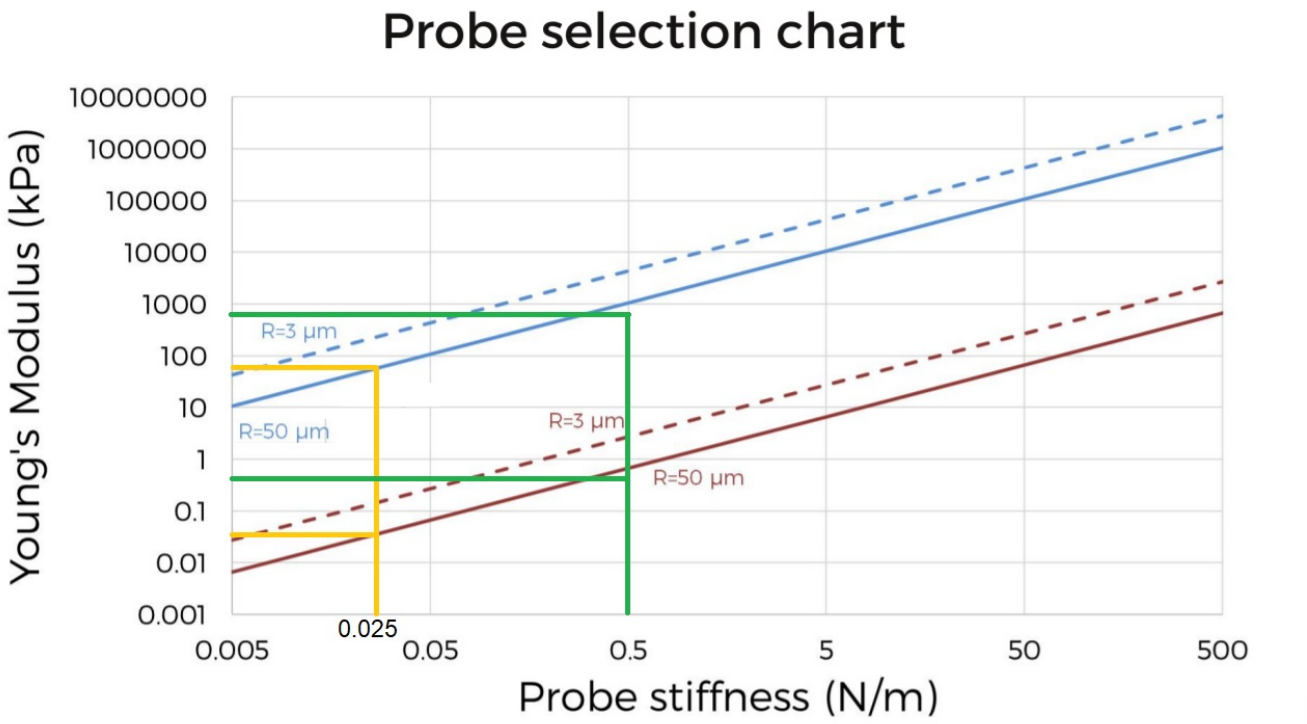

Figure 28. In this figure of Optics11, the orange and green line are added. The orange line stands for a probe with radius= 50 μm, and a stiffness= 0.025 N/m. The green line stands for a probe with radius= 100 μm, and a stiffness= 0.5 N/m.[piumaprobes]

12.2.2 Piuma mechanical data analysis

A repeatable way of fitting the data is found. In all TAs, the single fit method is used. Using the single fit method for Hertzian contact will disable Pmax % for contact point and use one fit to calculate both the contact point and the Young's modulus.[65] The Pmax is chosen to be 100 % in the case of that the maximum indentation depth (> 10 % of the TA height) is not exceeded during indentation, and is set at less than the maximum indentation depth (till a minimum of less than 20 % of the maximum indentation depth). Some curves are fitted by hand. For round 3 shear is 0, for point B and C there fitted one file by hand for these 2 points, because the load-indentation curve did not start at 0 depth. For round 3: the 300 shear one curve from point B is fitted by hand, and two curves from 150 point A, because it exceeded the max indent depth with the single fit method.

12.2.3 Confocal imaging settings

A fluorescent laser of 488 nm is used because this will excitate the used AF488-labeled fibrinogen, which makes it possible to visualize the fibrin. The detection pinhole removes all emissions not originating from the focal plane. It is therefore also referred to as "spatial filter", filtering the depth of focus and blocking extrafocal signal. For general confocal imaging, the recommendation is to just transmit the inner part of the diffraction limited feature by the pinhole. This inner part is called the "Airy disc", so there is chosen for 1 airy unit in the experiments. In addition, a variable pinhole offers the freedom to the user to increase the optical sectioning sharpness by lessening the diameter or to reduce optical sectioning performance by increasing the diameter.[92] The actual size of the scan field (the x-y dimensions of your image) itself is determined by the objective magnification and the zoom factor used. We have to distinguish between this optical zoom and digital zoom. With objective magnification, the image is magnified to a greater extent, and resolution is improved, but this improvement of resolution is limited by the optical resolution of the objective. Oversampling will not add any more details to the image (see Nyquist theorem below). Digital zoom works by mathematically increasing the image size using the original number of pixels and the image will become more blurry (less resolution), which can be done by certain software.[93] In this case a 40x zoom oil immersed lens gives an adequate zoom in of fibrin fibers (clear image), this was seen in practice during this thesis and in other papers.[21,94,95] The scan format determines how many lines are scanned and how many samples are taken along each scan line. The scan format is expressed as the number of pixels per line **x** Lines

per frame. A scan format of 2048**x**2048 therefore means that the sample area is scanned at a resolution of 2049**x**2048 pixels.
Lateral resolution (r_{xy}) can be calculated by formula 20, with wavelength (λ), and the numerical aperture (N.A.):

$$r_{xy} = 0.51 * \lambda / N.A. \tag{20}$$

So in the case of the settings in table 4, the lateral resolution will be 0.51*488/1.25=199 nm. The Nyquist sampling theorem states that the sampling frequency must be greater than twice the bandwidth of the input signal to reconstruct the original input from the sampled data. The objective has a theoretical optical resolution of 199 nm. To make full use of this resolution the pixel size should be approximately:

$$Pixelsize = r_{xy}/2 \tag{21}$$

A sampling frequency of 2 or a lower frequency of 1.5 can be used. A slightly lower frequency of 1.5 can be used because it is said that confocal can use a sampling rate lower than 2 because of its low background. This gives either a 99.5 nm or 133 nm pixel size, so a pixel size of about 100-133 can be used. At a scan format of 2048x2048 and zoom 1.9 the pixel size is 99.63 μm, which is a very safe choice in guaranteeing the quality of the images.[93,96] The x-y) dimensions of the recording should match a square of 204 μm sided square, as the diameter of dot A, B, and C is approximately 200 μm. A circular image cannot be made of the area of interest, so there is chosen to make a x=204 μm and y=204 μm sized square. There will be used a z-stack to visualize the fibrin at different heights, this height should at least visualize the top z=2 μm of the TA because this is the part in which indentation can potentially take place. In most TAs, a total z-projection is made, to also assess the TA at more depths than the indentation depth. The interval of imaging is set at 200 Hz, as a relatively big scanning field requires a higher scanning interval.[96] The z-step should be in accordance with Nyquist and can be calculated with an online calculator.[97] There is chosen for a line averaging of two to reduce noise, each line is scanned two times and the average is taken.

12.2.4 Confocal image analyzing theoretical background

The images made with the standard deviation method are directly analyzed. Preliminary experiments are done to find a good tool to assess directions of fibers. The FOA tool is found to be the best tool, as it visually confirms its findings by orientation overlays. as opposed to OrientationJ and DiameterJ.

12.2.4.1 OrientationJ

In the OrientationJ tool the following settings are used for the vector field: local θ is 10 pixels, gradient "cubic spline", grid size 75, length vector is maximum, and scale vector 100%. For the dominant direction, settings are standard. With the OrientationJ tool, the orientation is evaluated for every pixel of the image based on the structure tensor. These values are derived from the structure tensor defined (formula 22) for each pixel as the 2×2 symmetric positive matrix J, with the partial spatial derivative of the image f (x, y) along the principal direction x (f_x), along the principal direction y (f_y), and the Gaussian weighting function that specifies the area of interest w(x, y):

$$J = \begin{bmatrix} < f_x, f_x >_w < f_x, f_y >_w \\ < f_x, f_y >_w < f_y, f_y >_w \end{bmatrix} \tag{22}$$

The weighted inner product between two arbitrary images g and h is defined as:

$$< g, h >_w = \iint_{R^2} w(x,y) g(x,y) h(x,y) \mathrm{d}x \, \mathrm{d}y \tag{23}$$

[98]

Once the structure tensor is known, local orientation, energy and coherency for each pixel could be easily calculated.[99] For the dominant direction, the tool computes the average of the elements of tensor matrix, elements-by-elements. These elements live in linear scale. Then, the plugin has just to compute one orientation. This is a robust way to get the dominant orientation.[100] Orientation is the angle of the eigenvector (largest eigenvalue) of the gradient structure tensor matrix[100], the local predominant

orientation θ in the considered region corresponds to the direction of the largest eigenvector of the tensor and it is thus given by,

$$\theta = \frac{1}{2}\arctan\left(2\frac{< f_x, f_y >_w}{< f_y, f_y >_w - < f_x, f_x >_w}\right) \tag{24}$$

The energy parameter E is the trace of the tensor matrix:

$$E = \mathrm{Trace}(J) =< f_x, f_x >_w + < f_y, f_y >_w \tag{25}$$

Pixels with higher energy values correspond to less isotropic and more clearly oriented structures. The coherency parameter C is defined as the ratio between the difference and the sum of the tensor eigenvalues, with the largest eigenvalue (λ_{max}), and the smallest eigenvalue (λ_{min}):

$$\begin{aligned} C &= \frac{\lambda_{\max} - \lambda_{\min}}{\lambda_{\max} + \lambda_{\min}} \\ &= \frac{\sqrt{(< f_y, f_y >_w - < f_x, f_x >_w)^2 + 4 < f_x, f_y >_w}}{< f_x, f_x >_w + < f_y, f_y >_w} \end{aligned} \tag{26}$$

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The min-coherency is expressed as a percentage because the coherency factor is an index between 0 and 1:

$$0 <= c <= 1 \tag{27}$$

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12.2.4.2 Directionality

The following settings can be specified in directionality:

- Method: this plugin chops the image into square pieces, and computes their Fourier power spectra.
- Nbins: the number of bins (default: 90), that will partition the 180°.
- Histogram start: the starting angle (default:-90°) for analysis. Angles will run from this value to 180°plus the starting value. This option was added to avoid having peaks at +90°split at the borders of the histogram.
- Histogram end: 90°, it is chosen to let the histogram end at 90°.
- Build orientation map: the orientation map flag will cause the orientation map to be generated.
- Display color wheel: the image is colored according to its local directionality, or location orientation.
- Display table: the result table flag will generate a table containing all result value to be displayed. This table can be exported as a CSV file thereafter.
- The debug flag, if set, will cause angular filters and power spectrum to be displayed.

As for the results a toy extra analysis step fits the histogram with a Gaussian to measure the main orientation and its spread.[101] On top of the histogram, the plugin tries to generate statistics on the highest peak found. The highest peak is fitted by a Gaussian function, taking into account the periodic nature of the histogram. This will result in the following outcomes:

- The "Direction [°]" column reports the center of the gaussian.
- The "Dispersion [°]" column reports the standard deviation of the gaussian.
- The "Amount" column is the sum of the histogram from center-std to center+std, divided by the total sum of the histogram.
- The real histogram values are used for the summation, not the gaussian fit.
- The "Goodness" column reports the goodness of the fit; 1 is good, 0 is bad.

An example can be seen in figure 29. The highest peak in the histogram, gives the preferred direction.

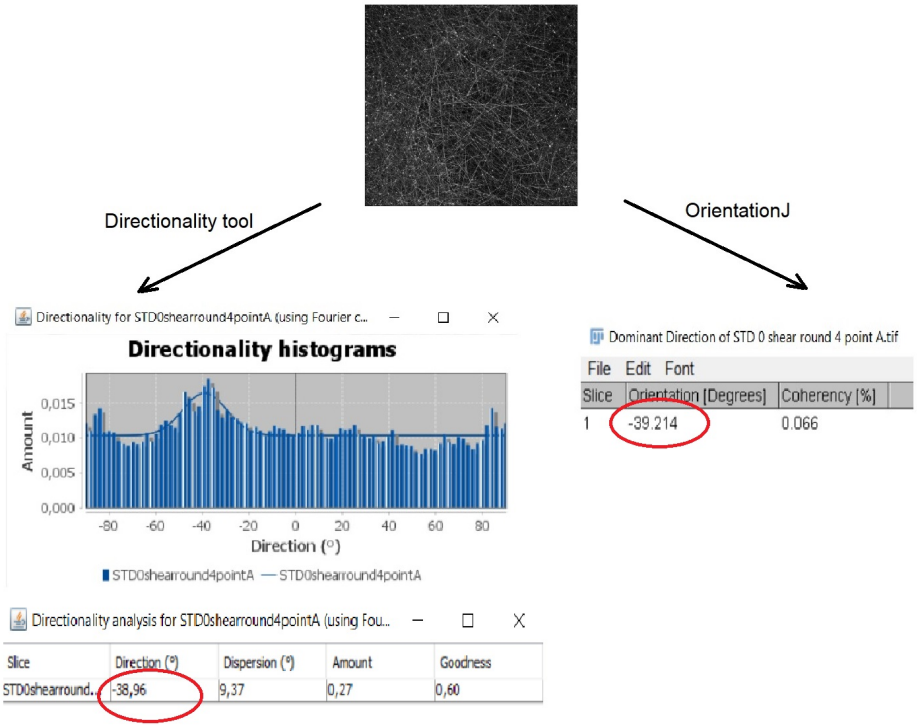


Figure 29. The top picture is analyzed, the bottom left picture gives example picture of the outcome of the Directionality tool, and bottom right by OrientationJ.[101]

12.3 Fibrin clot formation under flow: preliminary results

12.3.1 Viscoelasticity analysis

12.3.1.1 Materials and method

Some fibrin clots formed under flow are indented multiple times at exactly the same point. To assess viscoelasticity this is done at one point within point C, five indents were performed.

12.3.1.2 Results

The indents are shown in a successive order in table 16, so for example indent one is performed before indent two at the same point. No significant effect is found on the Effective Young's modulus, when indenting the samples multiple times. A one-sample Kolmogorov-Smirnov test is performed and showed p=0.20 in the shear=150/s TA sample and p=0.20 in the shear=300/s sample.

Table 16. Indents are shown in a successive order for two samples.

Specifications sample				**Outcomes of indent**				**Piuma tip used**		**Method fit**			
Round	Shear (/s)	Point	Indent	E (kPa)	E_{eff} (kPa)	R^2	RSME	radius (μm)	k	h_{max} (nm)	What fit	p_{max} (%)	Range used (nm)
2	150	C	1	115.5	154.01	0.99	0.027	103	0.42	704.95	Single fit	41	0-597
			2	58.62	78.16	0.99	0.014	103	0.42		Single fit	33	0-693
			3	232.73	310.3	0.98	0.049	103	0.42		Single fit	74	0-479
			4	99.02	132.03	0.98	0.023	103	0.42		Single fit	70	0-705
			5	61.82	82.43	0.99	0.016	103	0.42		Single fit	64	0-660
	300	C	1	105.8	141.07	0.98	0.072	103	0.42	1342.77	Single fit	83	0-1040
			2	75.26	100.35	0.98	0.074	103	0.42		Single fit	89	0-1293
			3	62.55	83.4	0.99	0.04	103	0.42		Single fit	92	0-1250
			4	78.03	104.04	0.99	0.032	103	0.42		Single fit	88	0-1040
			5	58.86	78.49	0.99	0.039	103	0.42		Single fit	91	0-1200

12.3.2 Young's modulus versus effective Young's modulus

12.3.2.1 Materials and method

During the fitting procedure, the effective Young's modulus obtained while Young's modulus E is:

$$E = E_{eff}(1 - v^2) \tag{28}$$

, where Poisson's ratio ν is set at 0.5, as the TA is seen as an incompressible materials. The results of all (effective) Young's moduli are shown below, also results with low R^2 and high RSME values are used.

12.3.2.2 Results

TAs formed under different shears

The shear=50/s TAs have the highest mean Young's modulus and effective Young's modulus, with a mean of 185.26 kPa (STD 108.55), and 247.02 (STD 144.73) kPa respectively, followed by the shear=150/s TAs with a Young's modulus of 113.63 kPa (STD 110.82) and an effective Young's modulus of 151.49 kPa (STD 147.77), followed by shear=300/s with a Young's modulus of 96.84 kPa (STD 39.95) and an effective Young's modulus of 129.12 kPa (STD 53.27), and the lowest Young's modulus and effective Young's modulus are found in the shear=0/s TAs, with 0.47 kPa (STD 0.44) and 0.63 kPa (STD 0.59) respectively. The significance levels are the same for the Young's modulus and effective Young's modulus. The differences stated before are significant when comparing the shear=50/s TAs to all the other TAs, with $p<0.001$ when comparing to shear=0/s TAs, $p=0.049$ compared to the shear=150/s TAs, and $p=0.001$ when comparing it to the shear=300/s TAs. The shear=150/s TAs have a significantly different (effective) Young's modulus from the shear=0 ($p<0.001$), but not compared to the shear=300/s TAs ($p=0.828$). The shear=300/s TAs have a significantly different (effective) Young's modulus than the shear=0/s TAs. The results of these TAs formed under different shears, is shown in figure 30.

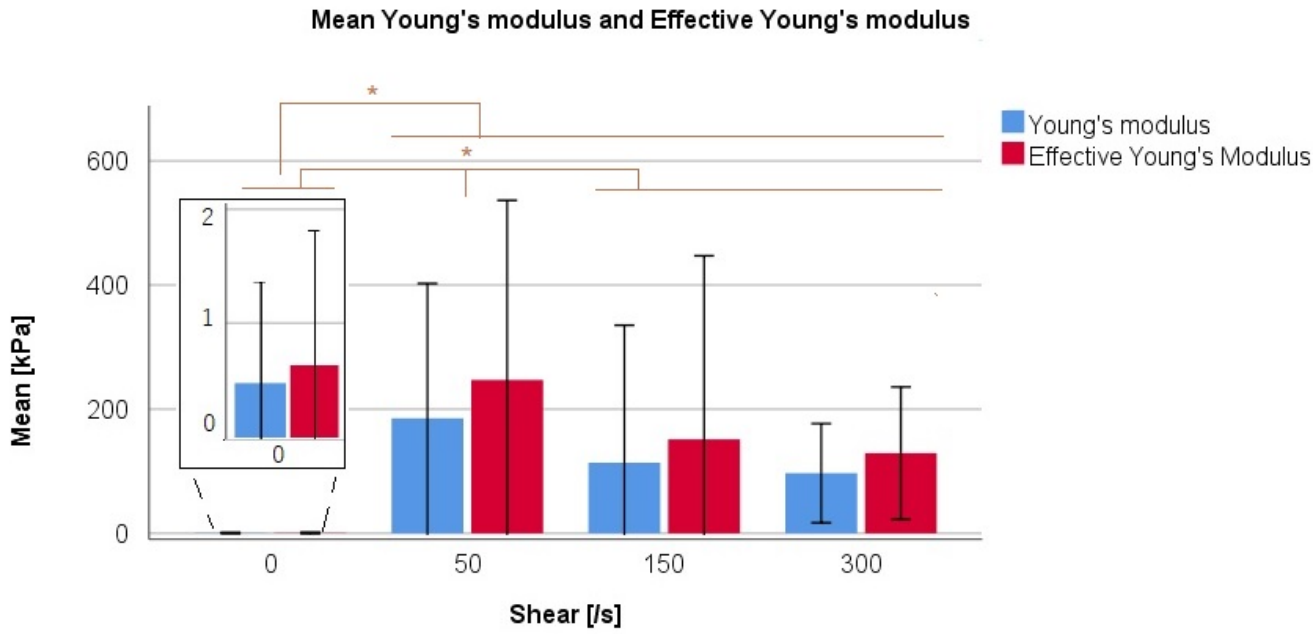


Figure 30. The mean Young's modulus and effective Young's modulus are shown per shear rate. The * shows if the differences in (effective) Young's moduli are significantly different. The error bars depict the standard deviations.

Different pooled plasma test groups, all shears taken together

The different pooled plasma groups are compared in figure 31, the overall mean Young's modulus or effective Young's modulus are shown. No significant differences for the Young's modulus or effective Young's modulus are found when there is looked at in between group differences with the pooled data ($p=0.277$). When comparing the data from different groups with the same shear rate, there are no significant differences found for shear=0, 150, or 300/s TAs. For shear=50/s TAs the Young's modulus in group three was 181.28 kPa higher than in group 1 (SE 37.57, $p=0.004$, CI 65.28 till 297.28) and also 153.45 kPa higher than in group 2 (SE 36.77, $p=0.011$, CI 37.93 till 268.97).

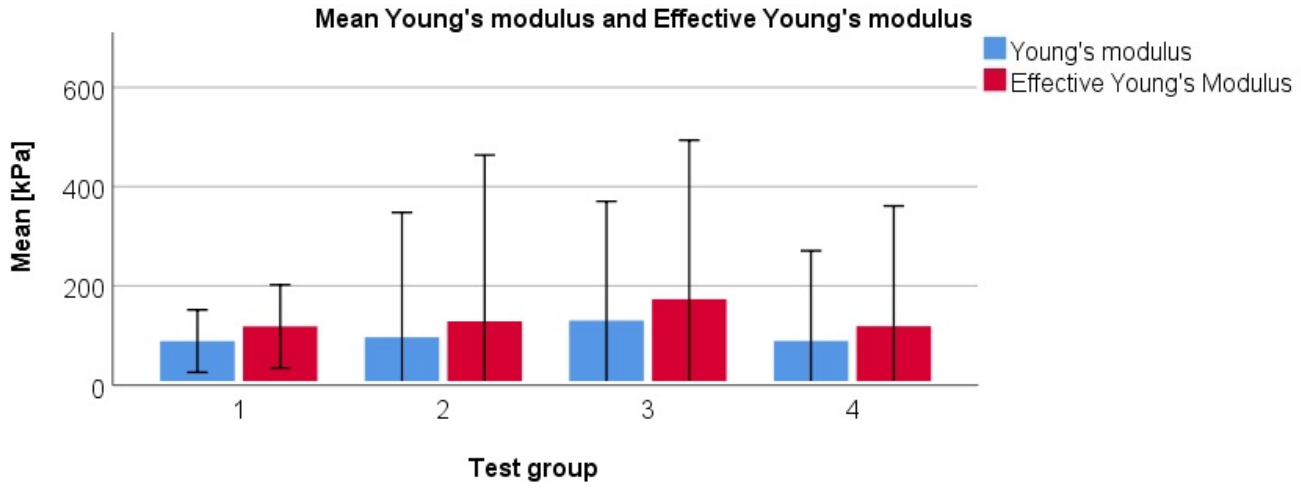


Figure 31. The different pooled plasma groups with the overal mean Young's modulus and effective Young's modulus for all shears. The error bars depict the standard deviations.

Different measurement locations, all shears and pooled plasma groups taken together

When comparing the TAs formed under a shear (>0/s) for the pooled data of all shears for different measurement locations (figure 32), it is seen that there was a significantly higher Young's modulus at point B compared to point A (67.16 kPa higher, STD 24.80, p=0.025, CI 7.09 till 127.24) and effective Young's modulus (89.56 kPa higher, STD 33.06, p=0.025, CI 9.46 till 169.67), point B and C are not significantly different from each other(p=0.176), and point A and C are not significantly different as well (p=0.535).

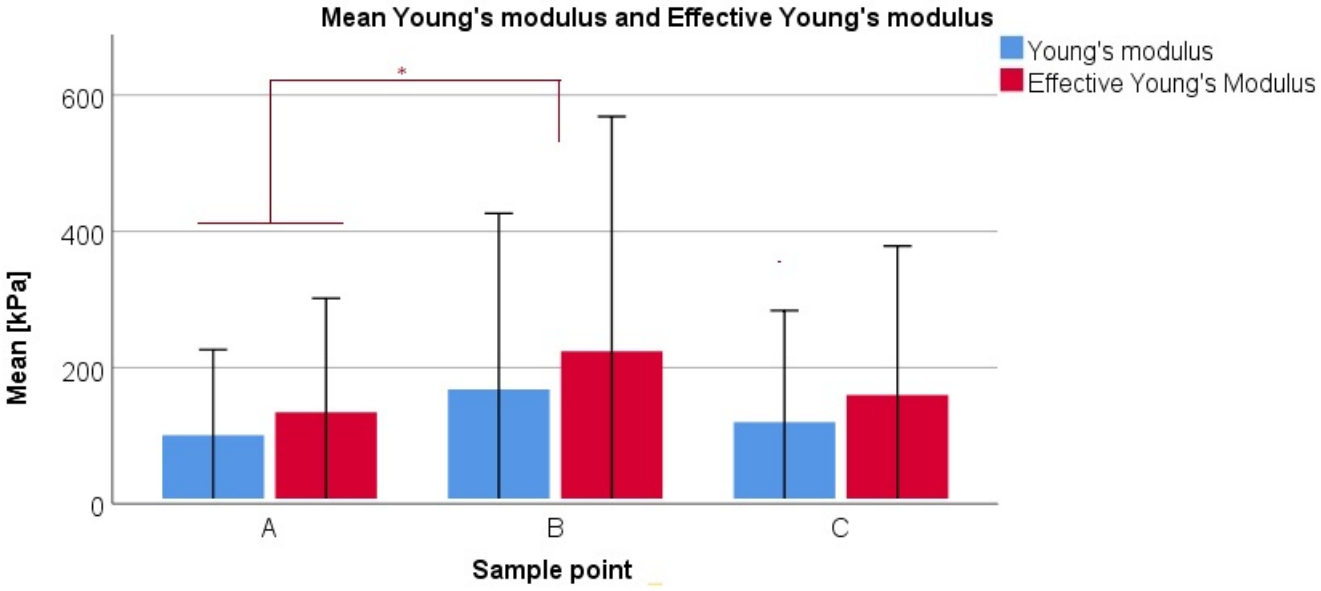


Figure 32. TAs formed under a shear (>0/s) for the pooled data of all shears for different measurement locations: A, B, and C. The * shows if the differences in (effective) Young's moduli are significantly different. The error bars depict the standard deviations.

In table 19 and 20 the outcomes of all Piuma testing is shown. In most samples it was possible to use the "single fit" method, in 11 cases a "set range" method was needed.

12.3.2.3 Discussion

It can be seen that the effective Young's modulus and Young's modulus have a clear relation, in which the effective Young's modulus is higher.

12.3.3 Preliminary outcomes confocal segmenting

It is tested by eye which method works best to make correct binary pictures of TAs. The auto-thresholding method in Fiji is tested, which gives sixteen different versions of original figure 33:

default, Huang, Huang2, intermodes, isodata, Li, Maxentropy, mean, minimumerror(I), minimum, moments, Otsu, percentile, RhenyiEntropy, Shanbhag, triangle, and Yen. It is seen that default, Huang, Huang2, isodata, Li, mean. moments, Otsu, percentile and Shanbhag, are potentially good thresholds which are analyzed in more detail. When testing other TAs different filters were preferable each time, so a new method was tested to make a more general approach in using filters. The final filters can be seen as macros in 11.5 "Macro's: Fiji".

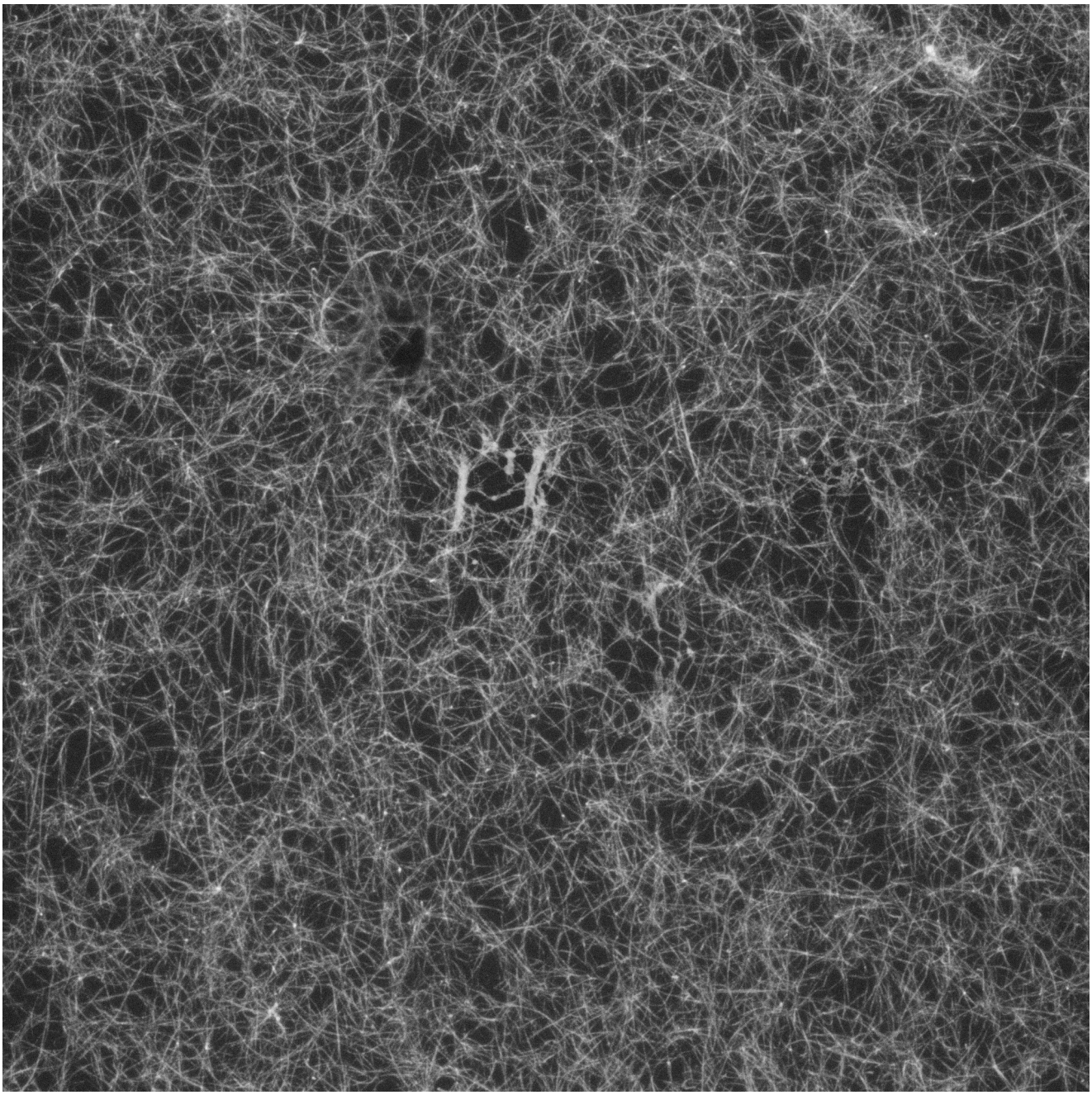


Figure 33. The analyzed picture for this experiment.

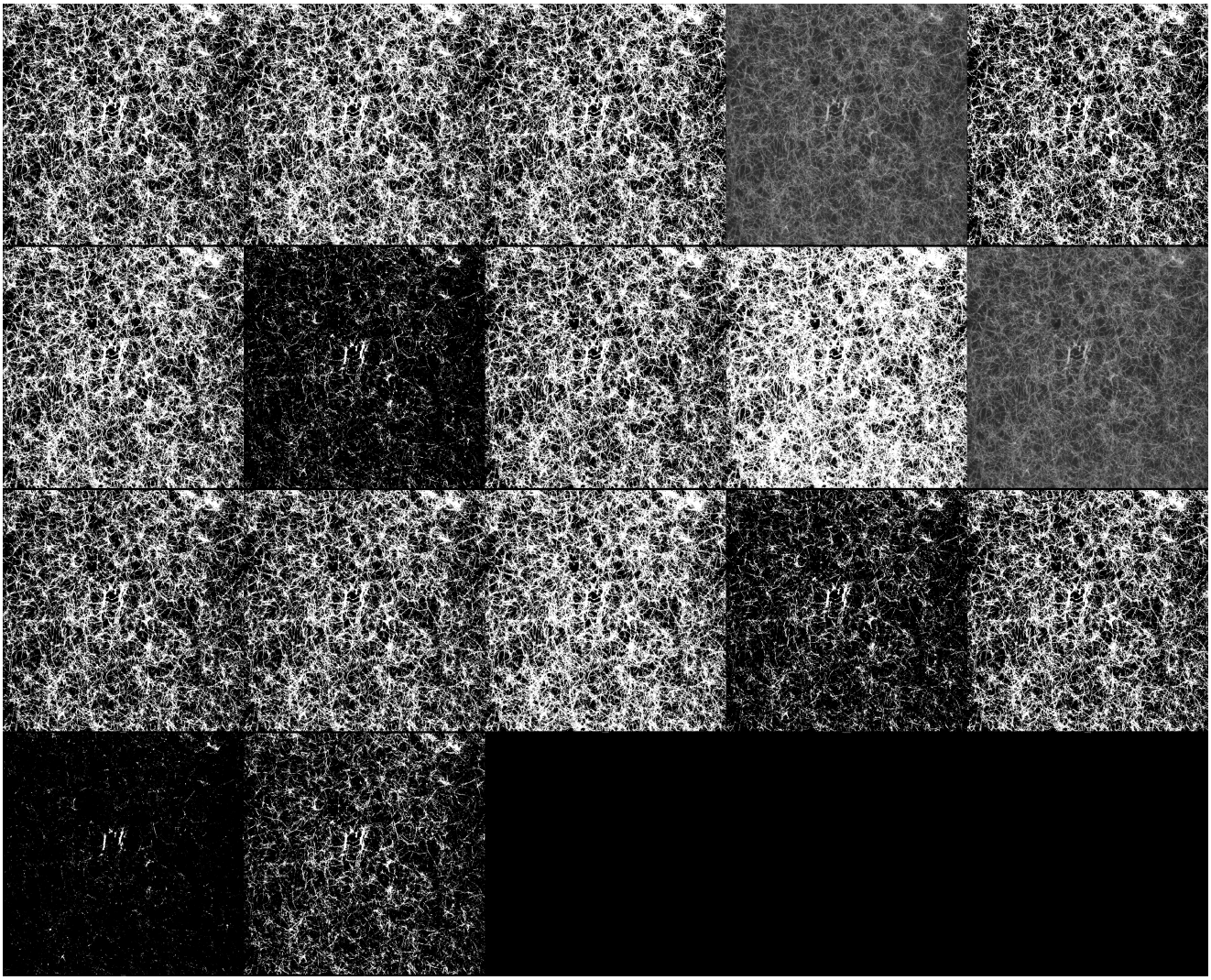


Figure 34. The result of the auto-threshold function in ImageJ of figure 33

12.3.4 Preliminary outcomes confocal for Piuma testing depth

In table 17, results are shown on which the Piuma testing depth is roughly based.

Table 17. Heights found in a performed experiment on 30-07-2021. * The 0 shear TA height is unknown.

Shear rate [/s]	**Height TA [μm]**	**Indentation depth 5-10 % [nm]**	**Total displacement [nm]**	**Time [s]**
0	-*	-*	-*	-*
50	10.1	505-1010	10505-10100	10.505-10.1
150	14.3	715-1430	10700-11430	10.7-11.43
300	5.7	285-570	10285-10570	10.285-10.57

12.3.5 Direction analysis with OrientationJ and Directionality

12.3.5.1 Method A One-way ANOVA test was used for comparing clot heights, with a Gabriel and Hochberg's GT2 post-hoc test. A Kruskal-Wallis test is done to see if there are significant differences in fiber direction for the different shear rates, for the top 2 μm projections and total projections for the directions retrieved with the Directionality tool and OrientationJ. The directions are then categorized as frequencies in which certain directions occur per 10°for the directions retrieved with OrientationJ, and a Kruskal-Wallis test was performed to see if there is a difference between the different shear rates. The Directionality tool and OrientationJ tool results are compared using a paired-samples t-test.

12.3.5.2 Results The results from OrientationJ and Directionality are shown from 0 till 90°starting at the east (going counterclockwise), and 0 till -90°(going clockwise), in table 18. It can be seen that OrientationJ tool and the directionality tool gave different results, but no significant differences were found for the top 2 μm projections (p=0.162). A significant difference (p=0.035) for the total projections retrieved with OrientationJ 0.37[-3.72-12.31]°, and directionality -17.10[-76.20-45.00]°, is found (figure 35).

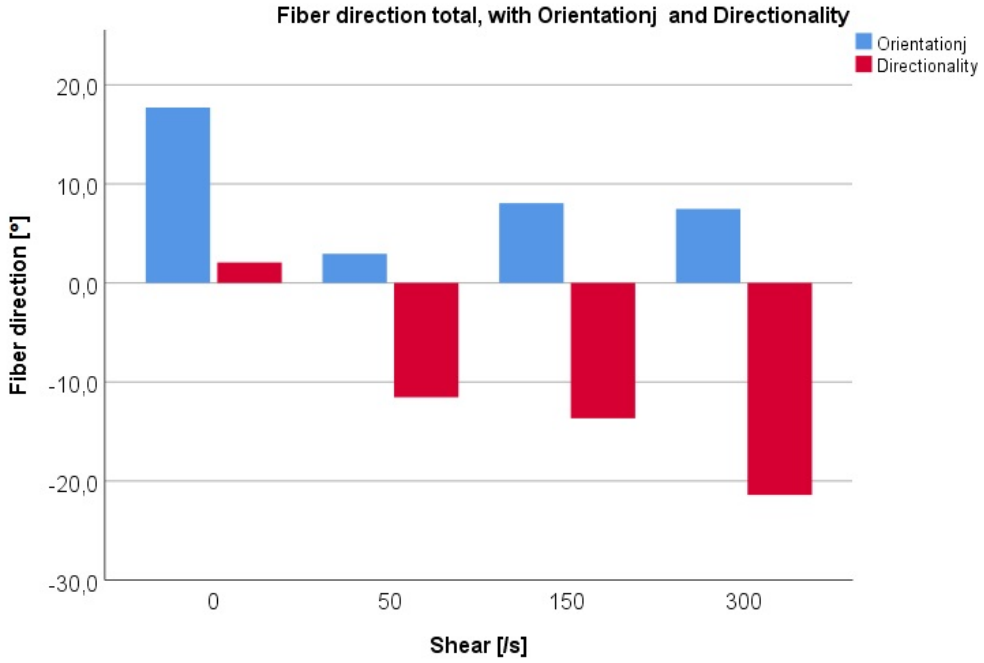


Figure 35. Orientationj versus directionality for the total z-projection.

Table 18. The direction analysis is shown for the top 2 μm of TAs and for the total TA projection. Both kind of z-projections are analyzed with the help of OrientationJ and Directionality tool.

Specifications sample			Stack of top 2 micron						Stack of total clot					
Round	Shear (/s)	Point	OrientationJ		Directionality (Fourier)				OrientationJ		Directionality (Fourier)			
			Direction (°)	Coherency (%)	Direction (°)	Dispersion (°)	Amount	Goodness	Direction (°)	Coherency (%)	Direction (°)	Dispersion (°)	Amount	Goodness
1	0	A	x	x	x	x	x	x	x	x	x	x	x	x
		B	x	x	x	x	x	x	x	x	x	x	x	x
		C	x	x	x	x	x	x	x	x	x	x	x	x
	50	A	10.95	0.00491	2.49	20.7	0.46	0.51	8.42	0.00475	17.3	34.1	0.75	0.12
		B	8.00	0.00458	40.7	45.6	0.78	0.21	27.0	0.00500	59.8	10.6	0.26	0.44
		C	-3.86	0.00853	-3.03	25.6	0.58	0.78	4.63	0.00832	-6.25	11.7	0.27	0.36
	150	A	-7.21	0.00831	0.300	27.8	0.68	0.84	-51.7	0.00593	-81.1	6.89	0.14	0.18
		B	6.31	0.00372	-73.3	3.30	0.07	0.12	90.0	0.0230	82.6	12.9	0.21	0.36
		C	-0.513	0.00722	2.17	16.2	0.36	0.62	-86.4	0.0210	-81.1	13.9	0.24	0.44
	300	A	-0.346	0.00551	1.83	35.4	0.78	0.71	-17.8	0.00339	-18.4	34.9	0.77	0.52
		B	-3.72	0.00547	1.26	31.9	0.71	0.81	-79.5	0.00500	-74.7	95.4	1.0	0.15
		C	-4.14	0.00652	-0.150	27.9	0.63	0.73	-67.4	0.00641	2.89	10.8	0.24	0.34
2	0	A	66.3	0.0388	67.3	27.0	0.49	0.77	47.9	0.0370	55.3	38.9	0.67	0.81
		B	2.22	0.0131	0.400	30.4	0.69	0.69	-18.8	0.0590	-17.3	25.4	0.62	0.95
		C	18.1	0.0178	13.6	33.3	0.76	0.93	7.49	0.0814	4.61	28.7	0.69	0.91
	50	A	-8.79	0.00420	2.35	27.2	0.61	0.53	-10.0	0.00345	0	33.9	0.76	0.39
		B	x	x	x	x	x	x	x	x	x	x	x	x
		C	x	x	x	x	x	x	x	x	x	x	x	x
	150	A	6.09	0.00630	12.0	38.6	0.85	0.57	8.47	0.00437	19.4	7.72	0.18	0.60
		B	0.366	0.00469	-3.55	13.0	0.29	0.40	-61.9	0.00100	75	23.5	0.20	0.50
		C	-4.17	0.00557	-4.10	25.1	0.57	0.81	-11.0	0.00141	-84.7	5.48	0.11	0.42
	300	A	79.3	0.00949	-91.5	10.0	0.13	0.40	84.6	0.0364	-86.2	8.49	0.14	0.32
		B	-46.8	0.00189	-14.5	14.2	0.32	0.13	-67.8	0.00300	-13.6	14.0	0.32	0.15
		C	81.9	0.00315	-40.5	15.0	0.33	0.31	-33.6	0.0245	-34.0	16.2	0.40	0.69
3	0	A	-40.7	0.0535	-39.8	6.63	0.19	0.38	-39.2	0.0659	-39.0	9.37	0.27	0.60
		B	50.0	0.0885	67.6	21.4	0.40	0.25	79.6	0.146	89.3	13.9	0.20	0.16
		C	-50.9	0.133	-48.4	26.8	0.66	0.88	-66.0	0.171	-64.0	25.6	0.59	0.89
	50	A	4.46	0.00489	1.77	22.3	0.50	0.70	7.81	0.00417	1.40	20.0	0.45	0.58
		B	12.3	0.00487	6.53	36.5	0.81	0.66	11.5	0.00489	3.06	29.9	0.66	0.68
		C	-2.71	0.00674	2.02	24.3	0.55	0.74	-6.43	0.00488	4.23	24.7	0.55	0.67
	150	A	60.3	0.00835	83.4	8.00	0.06	0.17	86.6	0.0186	80	12.2	0.09	0.18
		B	-41.2	0.00151	3.79	18.5	0.40	0.13	79.3	0.0569	-83.8	5.78	0.13	0.21
		C	3.54	0.00897	-2.41	26.0	0.58	0.77	83.2	0.0439	-89.3	4.29	0.10	0.43
	300	A	-2.78	0.00552	-0.910	34.1	0.75	0.55	87.7	0.0653	79.2	9.46	0.25	0.43
		B	-2.34	0.00548	-1.09	15.1	0.34	0.24	88.6	0.113	-89.9	8.17	0.21	0.53
		C	-0.829	0.00669	-0.210	20.7	0.47	0.81	79.2	0.107	-76.2	26.5	0.32	0.57
4	0	A	51.9	0.181	57.7	20.6	0.61	0.88	57.3	0.0724	54.2	17.0	0.47	0.86
		B	49.9	0.0361	-81.1	5.42	0.13	0.27	-83.8	0.1436	-82.4	17.4	0.34	0.40
		C	12.6	0.107	8.92	34.3	0.80	0.87	17.6	0.2029	17.8	34.1	0.85	0.97
	50	A	1.16	0.00438	-14.3	56.6	1.0	0.15	88.7	0.0118	-19.3	39.2	0.80	0.50
		B	6.91	0.00505	1.40	33.4	0.74	0.68	11.3	0.0044	-7.33	21.9	0.49	0.62
		C	12.0	0.00359	1.51	19.2	0.43	0.41	84.8	0.0280	-18	46.0	0	0.19
	150	A	-1.97	0.0298	-16.8	32.2	0.74	0.85	-2.05	0.0325	-17.1	32.8	0.75	0.83
		B	76.9	0.00683	-43	20.5	0.0	0.33	75.7	0.0244	-46	40.7	0	0.25
		C	-1.80	0.00710	-5.23	14.4	0.33	0.43	-86.7	0.0447	62.0	32.5	0.25	0.29
	300	A	-15.6	0.00123	39.9	12.0	0.26	0.06	-85.3	0.0555	75	14.8	0.14	0.38
		B	0.0765	0.00656	4.42	20.4	0.45	0.65	-89.9	0.0620	45	23.1	0.11	0.42
		C	4.82	0.00977	8.95	17.3	0.40	0.47	-88.0	0.0743	-66	19.8	0.14	0.36

12.4 Fibrin clot formation under flow: results

12.4.1 Table outcomes piuma

Table 19. Piuma outcomes of fibrin clots under flow.

Specifications sample				**Outcomes of indent**						**Piuma tip used**		**Method fit**			
Round	Shear [/s]	Point	Indent	E [kPa]	E_{eff} [kPa]	R^2	RSME [muN]	F_{max} [μN]	Ratio [%]	r [μm]	k [N/m]	h_{max} [nm]	What fit	p_{max} [%]	Range [nm]
1	0	A		x	x	x	x	x	x	x	x	x	x	x	x
		B		x	x	x	x	x	x	x	x	x	x	x	x
		C		x	x	x	x	x	x	x	x	x	x	x	x
	50	A	1	27.24	36.32	0.98	0.010	0.33	3.03	103	0.42	856.01	Single fit	21	0-768
			2	89.67	119.56	0.99	0.034	1.19	2.86	103	0.42		Single fit	55	0-815
			3	139.42	185.89	0.98	0.063	1.83	3.44	103	0.42		Single fit	70	0-810
		B	1	113.58	151.44	1.00	0.040	1.99	2.01	103	0.42	1023.86	Single fit	80	0-981
			2	130.01	173.35	0.99	0.057	2.20	2.59	103	0.42		Single fit	75	0-955
			3	113.98	151.98	0.98	0.069	2.01	3.43	103	0.42		Single fit	83	0-985
		C	1	94.84	126.46	0.99	0.050	1.75	2.86	103	0.42	1141.35	Single fit	73	0-1015
			2	126.88	169.18	0.97	0.092	1.93	4.77	103	0.42		Single fit	79	0-890
			3	111.28	148.38	0.98	0.053	1.61	3.29	103	0.42		Single fit	81	0-864
	150	A	1	47.09	62.37	1.00	0.022	1.58	1.39	103	0.42	2349.84	Single fit	90	0-1514
			2	49.17	65.56	1.00	0.033	1.64	2.01	103	0.42		Single fit	94	0-1525
			3	76.14	101.52	0.99	0.058	2.13	2.72	103	0.42		Single fit	90	0-1340
		B	1	50.66	67.55	0.99	0.051	1.79	2.85	103	0.42	2098.08	Single fit	95	0-1565
			2	58.06	77.41	0.99	0.057	2.02	2.82	103	0.42		Single fit	88	0-1550
			3	75.88	101.18	0.98	0.092	2.03	4.53	103	0.42		Single fit	85	0-1300
		C	1	84.95	113.26	0.99	0.067	2.36	2.84	103	0.42	2215.57	Single fit	85	0-1325
			2	83.79	111.73	0.98	0.057	1.67	3.41	103	0.42		Single fit	75	0-1060
			3	57.88	77.18	0.99	0.049	1.71	2.87	103	0.42		Single fit	78	0-1480
	300	A	1	159.99	213.32	0.99	0.057	1.96	2.91	103	0.42	939.93	Single fit	75	0-775
			2	76.92	102.56	0.95	0.059	1.13	5.22	103	0.42		Single fit	64	0-871
			3	71.05	94.73	1.00	0.018	1.13	1.59	103	0.42		Single fit	64	0-925
		B	1	126.97	169.29	0.99	0.045	1.92	2.34	103	0.42	1577.75	Single fit	80	0-906
			2	83.47	111.29	0.99	0.041	1.62	2.53	103	0.42		Single fit	84	0-1045
			3	71.73	95.65	1.00	0.020	1.3	1.54	103	0.42		Single fit	90	0-1002
		C	1	83.94	111.91	1.00	0.046	2.11	2.18	103	0.42	1510.61	Single fit	94	0-1250
			2	99.51	132.68	0.99	0.045	1.91	2.36	103	0.42		Single fit	80	0-1040
			3	101.75	135.66	0.99	0.046	1.91	2.41	103	0.42		Single fit	82	0-1127
2	0	A	1	0.26	0.35	0.96	0.002	0.03	7.69	105	0.48	3200.00	Single fit	20	0-3170
			2	0.31	0.42	0.95	0.002	0.03	6.25	105	0.48		Single fit	25	0-3170
			3	0.37	0.49	0.95	0.002	0.04	5.26	105	0.48		Single fit	28	0-3160
		B	1	0.26	0.35	0.96	0.002	0.03	7.69	105	0.48	3160.00	Single fit	22	0-3150
			2	0.26	0.34	0.96	0.002	0.03	7.69	105	0.48		Single fit	21	0-3150
			3	0.19	0.25	0.95	0.002	0.03	7.69	105	0.48		Set range	10	4500-7000
		C	1	0.16	0.21	0.94	0.001	0.01	7.14	105	0.48	3160.00	Single fit	12	0-2925
			2	0.21	0.28	0.92	0.003	0.04	7.50	105	0.48		Set range	50	3800-6960
			3	0.18	0.24	0.93	0.002	0.03	7.69	105	0.48		Set range	50	1500-4660
	50	A	1	128.51	171.34	0.86	0.009	0.17	5.29	103	0.42	234.98	Single fit	9	0-177
			2	122.04	162.72	0.94	0.006	0.18	3.33	103	0.42		Single fit	10	0-187
			3	148.56	198.08	0.95	0.008	0.23	3.48	103	0.42		Single fit	14	0-200
		B	x	x	x	x	x	x	x	x	x	x	x	x	x
		C	x	x	x	x	x	x	x	x	x	x	x	x	x
	150	A	1	265.15	353.53	0.96	0.033	0.84	3.93	103	0.42	335.69	Single fit	25	0-313
			2	49.63	66.18	0.89	0.029	0.40	7.25	103	0.42		Set range	100	500-835
			3	76.47	101.96	0.92	0.048	0.80	6.00	103	0.42		Set range	100	800-1135
		B	1	518.93	691.9	0.94	0.099	2.2	4.50	103	0.42	436.40	Single fit	48	0-380
			2	363.45	484.59	0.96	0.048	1.44	3.33	103	0.42		Single fit	35	0-365
			3	318.45	424.60	0.97	0.052	1.44	3.61	103	0.42		Single fit	35	0-413
		C	1	167.33	223.11	0.99	0.054	1.4	3.86	103	0.42	704.95	Single fit	49	0-701
			2	13.96	18.61	0.98	0.004	0.14	2.86	103	0.42		Single fit	8	0-675
			3	51.54	68.72	0.99	0.013	0.53	2.45	103	0.42		Single fit	30	0-688
	300	A	1	44.03	58.71	0.99	0.013	0.55	2.36	103	0.42	788.88	Single fit	29	0-784
			2	30.41	40.54	0.98	0.009	0.25	3.60	103	0.42		Single fit	25	0-764
			3	72.95	97.27	0.99	0.023	0.81	2.84	103	0.42		Single fit	50	0-755
		B	1	143.76	191.68	0.97	0.017	0.47	3.62	103	0.42	318.91	Single fit	22	0-320.5
			2	64.22	85.63	0.82	0.017	0.17	10.00	103	0.42		Single fit	15	100-300
			3	156.45	208.6	0.96	0.018	0.47	3.83	103	0.42		Single fit	23	0-304
		C	1	85.43	113.91	0.99	0.059	2.02	2.92	103	0.42	1342.77	Single fit	85	0-1200
			2	74.10	98.80	0.99	0.037	1.71	2.16	103	0.42		Single fit	90	0-1175
			3	92.86	123.81	0.99	0.052	2.03	2.56	103	0.42		Single fit	90	0-1140

Table 20. Piuma outcomes of fibrin clots under flow.

Specifications sample				**Outcomes of indent**						**Piuma tip used**		**Method fit**			
Round	Shear [/s]	Point	Indent	E [kPa]	E_{eff} [kPa]	R^2	RSME [μm]	F_{max}	Ratio [%]	r [μm)]]	k [N/m]	h_{max} [nm]	What fit	p_{max} [%]	Range [nm]
3	0	A	1	0.29	0.39	0.95	0.003	0.04	7.79	105	0.48	2114.86	Set range	100	6000-8115
			2	0.23	0.30	0.86	0.004	0.04	10.00	105	0.48		Set range	100	7300-9415
			3	0.28	0.37	0.55	0.005	0.03	17.86	105	0.48		Set range	100	2600-4600
		B	1	0.53	0.71	0.63	0.020	0.07	29.85	105	0.48	2148.43	Set range	100	3000-5148
			2	316.80	0.42	0.88	0.004	0.05	8.70	105	0.48		Set range	88	5000-7148
			3	0.30	0.40	0.84	0.003	0.03	9.38	105	0.48		Set range	30	2000-4148
		C	1	0.22	0.29	0.76	0.003	0.02	18.29	105	0.48	1829.52	Set range	100	3000-4800
			2	0.05	0.07	0.96	0.000	0.02	0.00	105	0.48		Set range	90	5000-6800
			3	0.24	0.32	0.72	0.002	0.02	10.00	105	0.48		Set range	85	1800-3800
	50	A	1	240.70	320.93	0.98	0.018	0.70	2.57	105	0.48	318.91	Single fit	21	0-294
			2	169.67	226.23	0.98	0.011	0.45	2.44	105	0.48		Single fit	18	0-283
			3	133.54	178.06	0.98	0.010	0.39	2.56	105	0.48		Single fit	15	0-294
		B	1	352.70	470.27	0.93	0.020	0.50	4.00	105	0.48	218.2	Single fit	11	0-184
			2	315.54	420.72	0.91	0.009	0.20	4.50	105	0.48		Single fit	7	0-105
			3	426.35	568.46	0.93	0.019	0.61	3.11	105	0.48		Single fit	14	0-180
		C	1	346.73	462.30	0.98	0.037	1.50	2.47	105	0.48	386.05	Single fit	43	0-384
			2	408.37	544.50	0.93	0.057	0.95	6.00	105	0.48		Single fit	27	0-256
			3	184.8	246.4	0.97	0.022	0.68	3.24	105	0.48		Single fit	23	0-348
	150	A	1	233.79	311.72	0.99	0.053	2.20	2.41	105	0.48	822.45	Single fit	75	0-655
			2	94.83	126.44	1.00	0.023	1.20	1.92	105	0.48		Single fit	51	0-805
			3	81.34	108.45	1.00	0.019	1.15	1.65	105	0.48		Single fit	46	0-8015
		B	1	96.85	129.13	0.99	0.043	1.96	2.19	105	0.48	1242.06	Single fit	91	0-1075
			2	58.37	77.82	1.00	0.018	1.41	1.28	105	0.48		Single fit	97	0-1205
			3	102.14	136.18	1.00	0.020	1.38	1.45	105	0.48		Single fit	86	0-820
		C	1	87.29	116.38	0.99	0.052	2.03	2.56	105	0.48	1191.71	Single fit	83	0-1177
			2	79.53	106.05	1.00	0.021	1.26	1.67	105	0.48		Single fit	87	0-910
			3	113.21	150.94	1.00	0.020	1.29	1.55	105	0.48		Single fit	85	0-730
	300	A	1	110.77	147.69	0.99	0.051	2.30	2.22	105	0.48	1275.62	Single fit	83	0-1090
			2	126.64	168.85	1.00	0.034	2.20	1.55	105	0.48		Single fit	86	0-970
			3	144.01	192.01	0.96	0.163	2.61	6.25	105	0.48		Single fit	88	0-1000
		B	1	158.95	211.93	0.99	0.037	1.83	2.02	105	0.48	772.09	Single fit	63	0-736
			2	217.94	290.59	0.99	0.063	2.46	2.56	105	0.48		Single fit	74	0-730
			3	125.3	167.06	0.99	0.034	1.47	2.31	105	0.48		Single fit	48	0-745
		C	1	104.64	139.52	0.99	0.094	2.64	3.56	105	0.48	1342.77	Single fit	84	0-1245
			2	77.02	102.7	1.00	0.030	2.17	1.38	105	0.48		Single fit	87	0-1337
			3	78.29	104.39	0.99	0.047	2.10	2.24	105	0.48		Single fit	89	0-1295
4	0	A	1	0.34	0.43	0.92	0.008	0.09	8.89	100	0.48	3424.06	Single fit	96	7000-10424
			2	0.50	0.67	0.84	0.005	0.05	10.00	100	0.48		Single fit	20	3000-5000
			3	2.39	3.19	0.96	0.01	0.15	6.67	100	0.48		Single fit	60	0-2300
		B	1	0.46	0.61	0.73	0.002	0.02	10.00	100	0.48	2450.55	Single fit	20	4200-4950
			2	0.32	0.43	0.96	0.003	0.06	5.45	100	0.48		Single fit	10	5000-7450
			3	0.45	0.60	0.72	0.004	0.04	10.04	100	0.48		Single fit	27	4000-5450
		C	1	0.26	0.35	0.93	0.002	0.02	10.00	100	0.48	2030.94	Set range	65	1000-3030
			2	0.25	0.33	0.95	0.002	0.02	9.52	100	0.48		Set range	72	2000-4030
			3	0.10	0.13	0.85	0.001	0.01	8.33	100	0.48		Set range	80	4000-6030
	50	A	1	64.14	85.52	0.98	0.01	0.32	3.11	100	0.48	1342.77	Single fit	95	0-430
			2	129.73	172.98	1.00	0.036	2.07	1.74	100	0.48		Single fit	89	0-940
			3	132.28	176.51	0.99	0.052	1.88	2.77	100	0.48		Single fit	86	0-860
		B	1	274.53	366.04	0.99	0.038	1.53	2.48	100	0.48	469.97	Single fit	50	0-450
			2	386.71	515.61	0.97	0.08	1.98	4.04	100	0.48		Single fit	60	0-435
			3	243.29	324.39	0.98	0.031	1.10	2.82	100	0.48		Single fit	45	0-402
		C	1	104.84	139.79	1.00	0.034	2.28	1.49	100	0.48	1376.34	Single fit	89	0-1150
			2	153.56	204.74	0.99	0.067	2.31	2.90	100	0.48		Single fit	83	0-900
			3	144.27	192.36	0.99	0.079	2.88	2.74	100	0.48		Single fit	86	0-1075
	150	A	1	28.00	37.34	1.00	0.026	1.42	1.83	100	0.48	2215.57	Single fit	99	0-2010
			2	34.59	46.12	1.00	0.02	1.43	1.40	100	0.48		Single fit	94	0-1750
			3	45.66	60.87	0.99	0.035	1.37	2.55	100	0.48		Single fit	95	0-1425
		B	1	94.37	125.83	0.99	0.028	1.45	1.93	100	0.48	1376.34	Single fit	88	0-900
			2	64.46	85.95	0.99	0.038	1.71	2.22	100	0.48		Single fit	96	0-1305
			3	69.00	92.00	0.99	0.03	1.20	2.50	100	0.48		Single fit	93	0-1000
		C	1	287.01	382.68	0.98	0.064	1.70	3.76	100	0.48	486.75	Single fit	50	0-480
			2	148.79	198.39	0.98	0.025	0.89	2.81	100	0.48		Single fit	31	0-485
			3	63.61	84.81	0.97	0.013	0.37	3.51	100	0.48		Single fit	19	0-475
	300	A	1	135.15	180.20	1.00	0.038	2.01	1.89	100	0.48	1846.31	Single fit	86	0-890
			2	66.34	88.45	0.94	0.054	0.88	6.14	100	0.48		Single fit	97	0-820
			3	87.59	116.79	0.99	0.025	1.31	1.91	100	0.48		Single fit	84	0-890
		B	1	30.14	40.18	0.99	0.031	1.05	2.95	100	0.48	2249.14	Single fit	95	0-1550
			2	45.96	61.28	0.99	0.036	1.46	2.47	100	0.48		Single fit	91	0-1480
			3	94.04	125.39	1.00	0.031	1.84	1.68	100	0.48		Single fit	84	0-1075
		C	1	99.17	132.22	0.99	0.037	1.91	1.94	100	0.48	1812.74	Single fit	85	0-1050
			2	76.60	102.13	1.00	0.025	1.37	1.82	100	0.48		Single fit	93	0-1005
			3	83.90	111.86	1.00	0.028	1.42	1.97	100	0.48		Single fit	96	0-1200

12.4.2 Table outcomes heights of the fibrin clots

Table 21. The clot height of all the fibrin clots formed under flow.

	Round	**1**	**2**	**3**	**4**
Shear [/s]	**Point**	**Height [μm]**			
0	A	x	32	21.1	34.2
	B	x	31.6	21.5	24.5
	C	x	31.6	18.3	20.3
50	A	8.6	2.4	3.2	13.4
	B	10.2	x	2.2	4.7
	C	11.4	x	3.9	13.8
150	A	23.5	3.4	8.2	22.2
	B	21	4.4	12.4	13.8
	C	22.1	7.1	11.9	4.9
300	A	9.4	7.9	12.8	19.3
	B	15.8	3.2	7.7	22.5
	C	15.1	13.4	13.4	18.1

12.4.3 Table outcomes fibrin network alignment

Table 22. Effect on fibrin network alignment analyzed with FibLab.

Specifications sample			**Stack of top 2 μm**			**Stack of total clot**		
Round	Shear [/s]	Point	Dominant direction [°]	SD [°]	Anisotropic fraction	Dominant direction [°]	SD [°]	Anisotropic fraction
1	0	A	x	x	x	x	x	x
		B	x	x	x	x	x	x
		C	x	x	x	x	x	x
	50	A	30.90*	30.10*	0.22*	35.55*	5.10*	0.01*
		B	54.67*	35.73*	0.39*	58.78	32.36	0.35
		C	15.20	5.22	0.02	149.70	20.20	0.01
	150	A	135.83	7.44	0.06	106.71	32.22	0.16
		B	92.17	37.76	0.44	94.00	30.02	0.28
		C	108.17	13.42	0.12	93.27	34.63	0.34
	300	A	9.62	6.94	0.26	123.46	7.61	0.11
		B	99.38	1.24	0.20	107.69	20.77	0.20
		C	81.83	2.72	0.22	103.24	16.62	0.23
2	0	A	71.19	34.81	0.23	50.12	38.85	0.18
		B	134.23	93.82	0.01	161.37	28.09	0.23
		C	14.96	29.38	0.29	4.58	28.91	0.22
	50	A	110.47	6.42	0.23	110.90	4.81	0.15
		B	x	x	x	x	x	x
		C	x	x	x	x	x	x
	150	A	52.44	45.31	0.15	19.72	5.80	0.03
		B	98.63	41.61	0.46	96.22	17.75	0.18
		C	103.53	46.22	0.25	99.14	39.16	0.11
	300	A	85.70	21.14	0.29	93.20	35.82	0.25
		B	112.92	35.21	0.14	118.71	38.71	0.15
		C	92.98	26.86	0.30	146.00	27.50	0.20
3	0	A	132.13	43.11	0.30	134.21	34.77	0.35
		B	59.12	27.60	0.28	82.83	28.08	0.50
		C	124.40	31.26	0.52	111.75	23.97	0.54
	50	A	90.13	14.39	0.36	90.55	13.60	0.45
		B	89.75*	14.01*	0.30*	84.70	20.32	0.23
		C	84.89*	13.45*	0.20*	86.89	16.58	0.35
	150	A	84.18	17.67	0.14	87.05	22.58	0.14
		B	88.07	16.75	0.23	83.08	22.10	0.30
		C	161.71	17.00	0.20	89.94	10.02	0.22
	300	A	37.40	0.70	0.22	90.86	22.96	0.32
		B	122.16	47.99	0.49	91.07	11.77	0.52
		C	94.90	13.40	0.20	79.46	19.23	0.46
4	0	A	56.63	26.27	0.52	55.21	23.29	0.19
		B	10.07	33.91	0.47	138.33	33.77	0.23
		C	21.47	44.42	0.36	24.78	32.12	0.45
	50	A	139.03	60.34	0.32	70.64	22.78	0.13
		B	49.55	21.55	0.13	58.75	2.57	0.05
		C	66.20	23.61	0.16	86.95	29.78	0.24
	150	A	175.71	29.99	0.42	176.01	28.03	0.39
		B	91.10	17.13	0.29	87.60	11.66	0.08
		C	83.06	15.77	0.28	92.69	14.62	0.28
	300	A	129.73	6.85	0.52	90.44	13.64	0.64
		B	29.59*	16.82*	0.07*	92.52	20.23	0.49
		C	124.72	25.86	0.06	92.83	22.09	0.59

12.4.4 Outcomes density

12.4.4.1 Method and outcome table densities

Table 23. Outcomes of density analysis of fibrin clot formation under flow.

Specifications sample			**Stack of top 2 micron**		**Stack of total clot**	
Round	Shear (/s)	Point	Density	Fitting	Density	Fitting
1	0	A	x	x	x	x
		B	x	x	x	x
		C	x	x	x	x
	50	A	0.007617	Macro threshold=94	0.083567382	Macro threshold=94
		B	0.040279	Macro threshold=94	0.083169461	Macro threshold=94
		C	0.054848	Macro threshold=94	0.133375884	Macro threshold=94
	150	A	0.022893	Macro threshold=94	0.308574919	Macro threshold=94
		B	0.075651	Macro threshold=94	0.352706675	Macro threshold=94
		C	0.031442	Macro threshold=94	0.393737797	Macro threshold=94
	300	A	0.000237	Macro threshold=94	0.005571604	Macro threshold=94
		B	0.000431	Macro threshold=94	0.024408102	Macro threshold=94
		C	0.000443	Macro threshold=94	0.019170999	Macro threshold=94
2	0	A	0.676677	First inverse, macro threshold=55	0.456182961	Contrast=1%, macro threshold=55
		B	0.36058	Macro threshold=94	0.683830984	Contrast=1%, macro threshold=55
		C	0.509015	Contrast=0.3%, macro threshold=55	0.54991246	Macro threshold=94, inverse selection
	50	A	0.001893	Macro threshold=94	0.00271015	Macro threshold=94
		B	x	x	x	x
		C	x	x	x	x
	150	A	0.118299	Macro threshold=115	0.143968584	Macro threshold=115
		B	0.018271	Macro threshold=115	0.07503581	Macro threshold=115
		C	0.031326	Macro threshold=115	0.124586583	Macro threshold=115
	300	A	0.271225	Macro threshold=94	0.304269555	First inverse, macro threshold=94
		B	0.741731	Macro threshold=94, inverse selection	0.79394413	Macro threshold=94, inverse selection
		C	0.156513	Macro threshold=94	0.913140785	Macro threshold=94, inverse selection
3	0	A	0.107489	Macro threshold=94	0.331602577	Macro threshold=55
		B	0.189869	Macro threshold=55	0.527914052	Macro threshold=55, inverse selection
		C	0.073882	Macro threshold=94	0.348290686	Macro threshold=55
	50	A	0.019052	Contrast=0.3%, despeckle, macro threshold 94	0.02099514	Contrast=0.1%, macro threshold=94
		B	0.009246	Contrast=0.3%, despeckle, macro threshold 94	0.005803346	First enhance contrast=0.1%, macro threshold=94
		C	0.002751	Contrast=0.3%, despeckle, macro threshold 94	0.013423205	First enhance contrast=0.1%, macro threshold=94
	150	A	0.657188	Macro threshold=94, inverse selection	0.934275162	Macro threshold=94
		B	0.096407	Macro threshold=94	0.669335611	Macro threshold=94, inverse selection
		C	0.00706	Macro threshold=94	0.310892348	Macro threshold=94
	300	A	0.001097	Macro threshold=94	0.200729612	Macro threshold=94
		B	0.020208	Macro threshold=94	0.212201835	Macro threshold=94
		C	0.005374	Macro threshold=94	0.325435167	Macro threshold=94
4	0	A	0.219281	Macro threshold=94	0.480572944	Macro threshold=94
		B	0.081529	Macro threshold=55	0.282708886	Contrast=3%, macro threshold=94
		C	0.05293	Macro threshold=94	0.4009769	Contrast=3%, macro threshold=94
	50	A	0.025805	Macro threshold=94	0.166552307	Macro threshold=94
		B	0.002313	Macro threshold=94	0.005827667	Macro threshold=94
		C	0.030793	Macro threshold=94	0.298710349	Macro threshold=94
	150	A	0.944486	Macro threshold=94	0.935218822	Macro threshold=94
		B	0.174831	Macro threshold=94	0.767152319	Macro threshold=94
		C	0.02654	Macro threshold=94	0.317926887	Macro threshold=94
	300	A	0.003561	First inverse, macro threshold=94	0.710776813	Macro threshold=150
		B	0.027581	First inverse, macro threshold=94	0.961461078	Macro threshold=94, inverse selection
		C	0.060762	First inverse, macro threshold=94	0.942984353	Macro threshold=150

12.4.4.2 Analyzing the differences in clot densities between pooled plasma groups per shear

The top 2 μm and total z-projections were analyzed for the densities distinguishing between the experiments, which can be seen in figure 36 and 37. Starting with the shear=0/s clots, for the top 2 μm, it was seen that the density in experiment two, 0.51 ± 0.16 was significantly higher (p=0.01) than in experiment three, 0.12 ± 0.06, and experiment two density was also higher (p=0.01) than experiment four density 0.12 ± 0.09. Secondly, if we look at shear=300/s clots from the top 2 μm z-projections, it is seen that in experiment two, a higher density of 0.27[0.16] (p=0.01) was seen than in experiment one $4.10^{-4}[2.10^{-4}]$. No other significant differences were found.

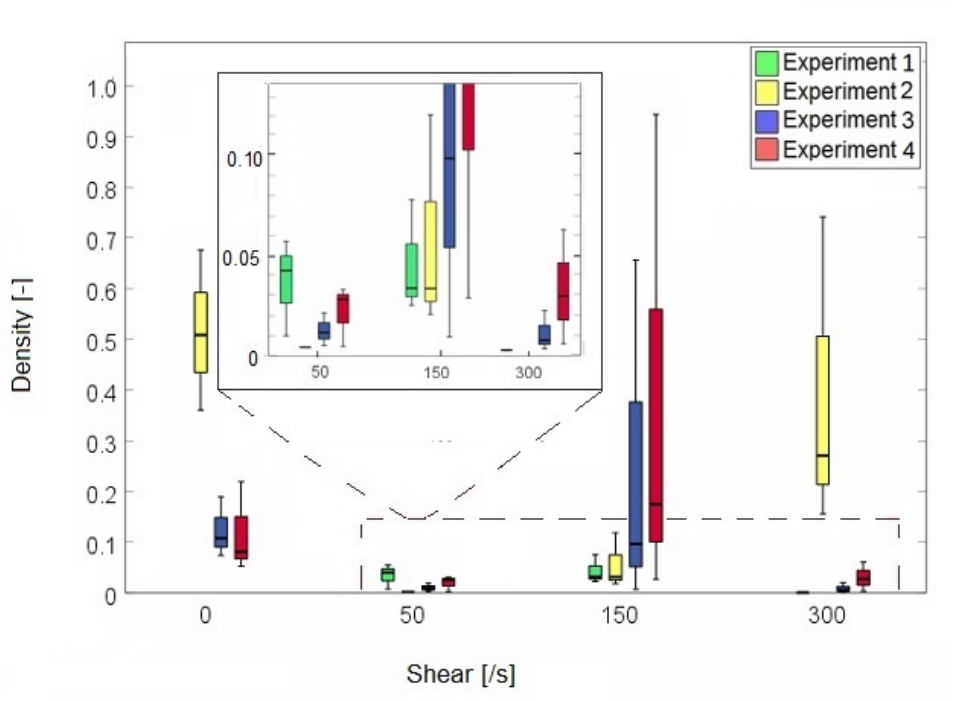


Figure 36. All the densities per shear for the top 2 μm.

For the total z-projections with clots formed at shear=300/s, clots in experiment four 0.96[0.86] had a higher density (p=0.02) than experiment one 0.02[0.01].

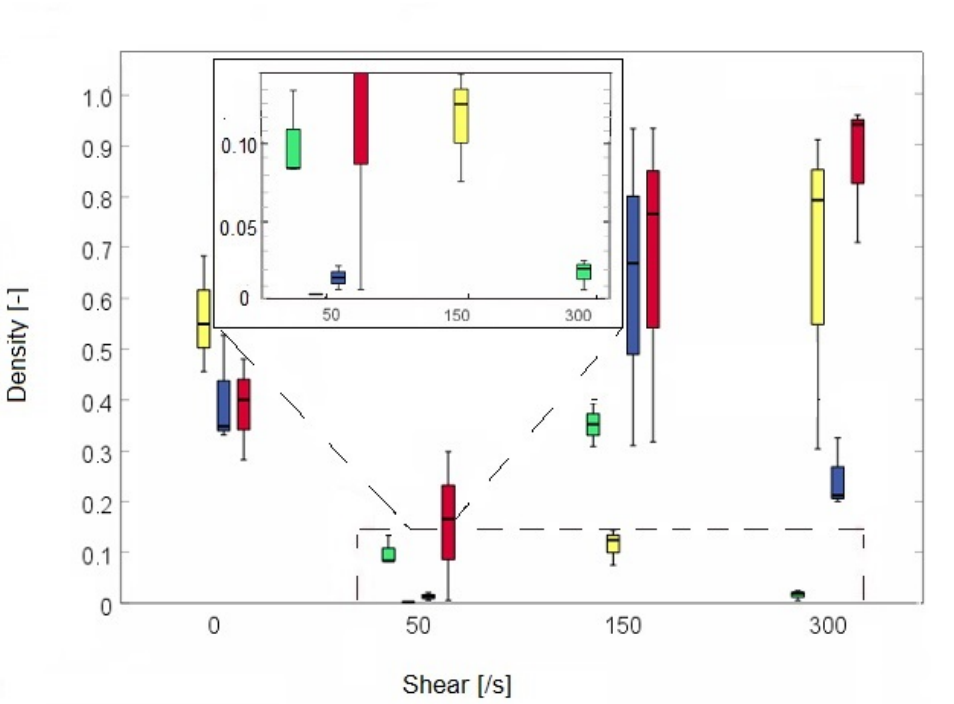


Figure 37. All the densities per shear for the total z-projection.

Table 24. Fibrin clot densities in the top 2μm z-projections (mean$\pm$SD) from all clots formed under different shears per experiment.

	Experiment				Experiments compared					
	1	2	3	4	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4
Shear [/s]	Density [-]				P-value					
0	x	0.52±0.16	0.12±0.06	0.12±0.09	x	x	x	0.01*	0.01*	1
50	0.03±0.02	0.002	0.01±0.008	0.02±0.02	0.34	0.34	0.34	0.34	0.34	0.34
150	0.04±0.03	0.06±0.05	0.25±0.35	0.38±0.49	0.5	0.5	0.5	0.5	0.5	0.5
300	3.10^{-4}±1.10^{-5}	0.39±0.31	0.009±0.01	0.03±0.03	0.19	0.19	0.19	0.19	0.19	0.19

A statistical comparison was performed between clots formed under different shears distinguishing between the different experiments. A p-value of 0.05 was considered significant (marked with *).

Table 25. Fibrin clot densities in the total clot z-projections (mean$\pm$SD) from all clots formed under different shears per experiment.

	Experiment				Experiments compared					
	1	2	3	4	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4
Shear [/s]	Density [-]				P-value					
0	x	0.56±0.07	0.40±0.11	0.39±0.10	x	x	x	0.17	0.17	0.17
50	0.1±0.03	0.003	0.01±0.008	0.16±0.15	0.52	1	1	1	0.42	0.83
150	0.35±0.04	0.11±0.04	0.64±0.31	0.67±0.32	0.17	0.17	0.17	0.17	0.17	0.17
300	0.02±0.01	0.67±0.32	0.25±0.07	0.87±0.14	0.17	0.07*	0.02*	0.34	0.77	0.02*

A statistical comparison was performed between clots formed under different shears distinguishing between the different experiments. A p-value of 0.05 was considered significant (marked with *).

12.4.5 Fibrinogen level

12.4.5.1 Materials and method

PPP aliquots (500 μL can be analysed for fibrinogen level by the department of hematology. For round three and four of the fibrin clots under flow, the fibrinogen level is quantified.

12.4.5.2 Results

These were the same: 2.3 g/L.

12.4.5.3 Discussion

The found fibrinogen levels are in the normal range (2.0 to 4.5g/L).[102]

12.5 Extra experiments under flow

12.5.1 Mixing part

12.5.1.1 Materials and method

When using thrombin a parallel flow is needed. During the experiments it was noticed (experiment 10-06-2021) that the mixing of the fluids did not take place enough which can be seen in figure 38, using normal flexible silicon tubes, and with food colouring's (blue and yellow).

Figure 38. Left 3 tubes as a result of parallel flow, right tube with food colouring's mixed in the 9:1 ratio.

A mixing part (figure 39) was designed to attach to the flow chamber. This was combined with the stiff tubings, removal of air in the fluids, and 9:1 flow rates (experiment 06-08-2021).

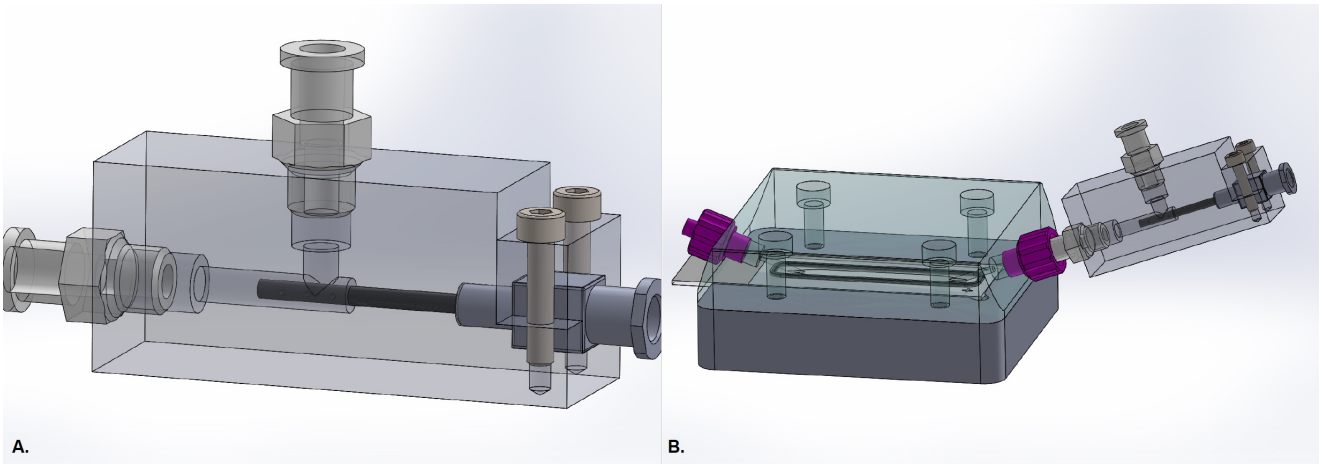


Figure 39. A. Mixing part, B. mixing part attached to flow chamber.

The stream with plasma will be 9:1 faster than the activator mix. This ratio is retrieved from van Kruchten et al (2012). The mixing chamber is designed in such a way, that the plasma stream will take the activator mix with it when they meet, and speeds up the activator mix.

12.5.1.2 Results

With food colouring's (blue and yellow) it was proven that mixing takes place in the right ratios, as a result fluid of the mixing chamber was compared to a tube with food colouring's mixed in the 9:1 ratio. The result can be seen below in figure 40 and 41.

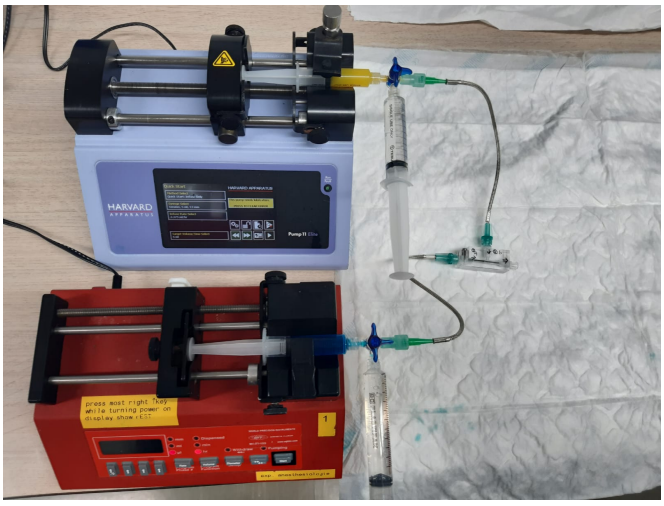


Figure 40. Set-up with food colouring's and mixing part.

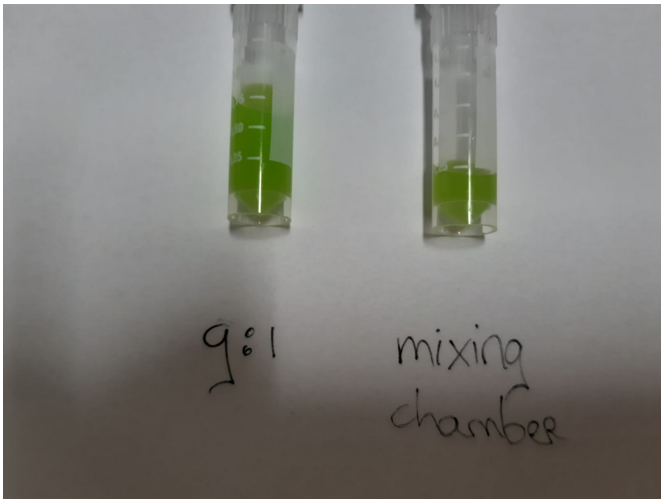


Figure 41. Left a tube with food colouring's mixed in the 9:1 ratio, on the right the result of the mixing experiment.

12.5.1.3 Discussion and conclusion

It can be concluded that a mixing chamber is a good way to mix fluids added in different speeds.

12.5.1.4 Future

When the mixing part is used in future, fabricating a smaller variant would be better, as it is quite big and thrombin is expensive.

12.5.2 Chandler loop

12.5.2.1 Materials and Method

On 15-03-2021 the Chandler loop method for making EAs dynamically was performed. Whole blood (method of protocol 13.2.3), was put into a Chandler loop (2845 μL WB in total) at 17 rpm, with the device in an angle of about 25 degrees.[103] The blood is spun around for about 2 hours, after that the whole tube is put in a waterbath (37 degrees) overnight. The blood should have enough space in the tubing to move around, without obstruction of the tubing by fast clotting, this will result in 1 big homogenous clot as can be seen in figure 44.

Figure 42. Left the chandler loop, right the Chandler loop in action with the tubes with blood around it.

12.5.2.2 Results

A macroscopicly heterogenous clot was the result (from the bigger tube seen in the picture). In the picture 43, one can see a bigger part on the left and a small part on the right which was ruptured in the

tubing. The resulting clot cut in 3 mm high pieces. An obvious color difference can be seen in some parts of the clot when it is cut into pieces.

Figure 43. The resulting clot, one can see a bigger part on the left and a small part on the right which was ruptured in the tubing. The resulting clot cut in 3 mm high pieces, the top row is the big clot, the bottom row is the small part.

Figure 44. The blood should have enough space in the tubing to move around, without obstruction of the tubing by fast clotting, this will result in 1 big homogenous clot.

12.5.2.3 Discussion and conclusion

It is suggested that the Chandler loop can easily be used as a method for generating heterogeneous clots. The color difference in the big part of the clot and the small part, could suggest some differences in mechanical properties. The rupture at a part without a colored core, could suggest that this is an indication of a weak part in the clot.

12.5.2.4 Future

The Chandler loop can easily be used as a method for generating heterogeneous clots. Interesting would be to test mechanical features of EAs. As could be seen in the experiment, the clot was fractured at some point, quantifying mechanical features of the clot at the point of fracture, would be interesting.

12.5.3 Flush excess fibrin away?

12.5.3.1 Method

On 12-08-2021 a Thrombus analogs formed under flow experiment was performed, following the method of Protocol 13.4.3. A shear rate of 50/s was applied in two experiments, using the 130 μm high flow chamber. In one of the experiments the step to remove the excess of fibrin "dip into HEPES buffer", was replaced by an equivalent flushing with HEPES buffer (again shear of 50/s, 500 μ). No formaldehyde step was applied, only direct appliance of the DAPI with a glass applied on the clot.

12.5.3.2 Results

The (normal) dipping into HEPES buffer resulted in a fibrin clot of about 10 μm height 45. The flushed 50 shear clot was washed away totally.

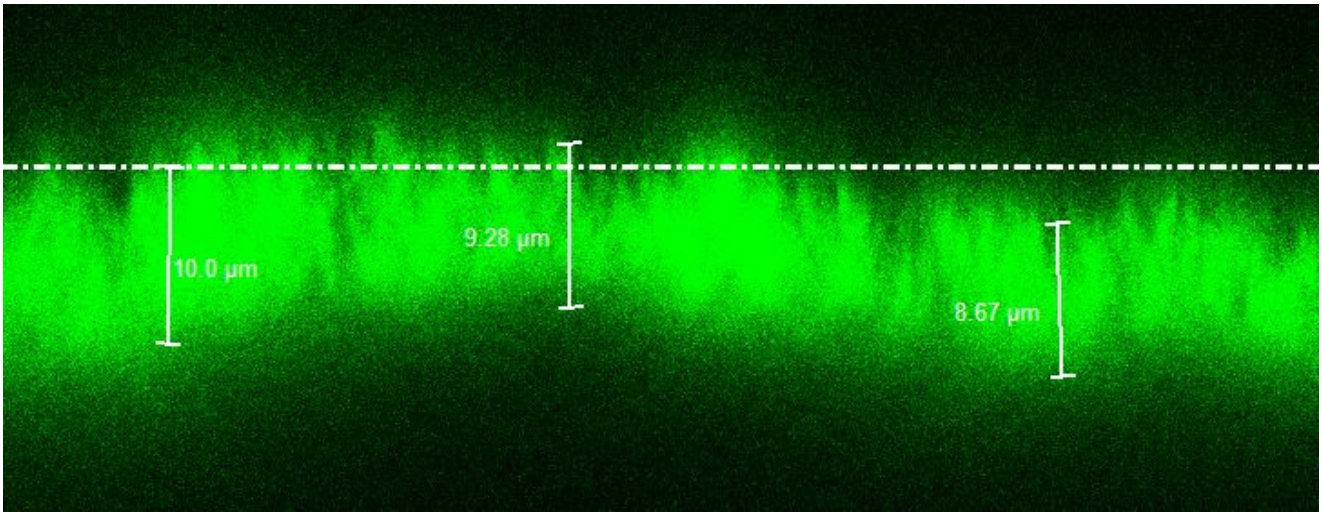


Figure 45. Xzy view or the height of the clot.

12.5.3.3 Discussion and conclusion

Removing excess fibrin by dipping the sample in HEPES is a better method than flushing with the same shear rates in which the clot was formed, as the clot is washed away totally in the latter method.

12.5.3.4 Future

Other ways of removing the excess fibrin without potentially damaging or adjusting the fibrin network, should be explored.

12.5.4 Thrombin

12.5.4.1 Method

On 15-07-2021 the method of protocol 13.6.1 was applied, after that the same protocol was followed with buffer without thrombin and with the addition of TF coated slides, with around 150 μl TF.

12.5.4.2 Results

On this point exact TF quantities were unknown because of leackage of TF fluid from the slide, it was estimated on around 150 μl of diluted TF (10 mM). The height of the different clots was estimated visually by using the oculars instead of measuring this with the software. In the 50/s sample of withouth TF, the TF on the slide was forgotten, and no clot were formed.

Shear rate (/s)	Clotting initation by	Estimated height clot (μm)
50	Thrombin	12
150	Thrombin	10
300	Thrombin	8
50	TF	0*
150	TF	40
300	TF	17

12.5.4.3 Discussion and conclusion

It seems that TF results in higher clots than thrombin.

12.5.4.4 Future

The set-up with 2 pumps could used more in future.

12.5.5 Fibrin clot formation under flow with thrombin and TF

12.5.5.1 Method

On 08-07-2021 the method of protocol 13.6.1 was applied, but than with a shear of 100/s and with the addition of TF to a slide.

12.5.5.2 Results

A fibrin clot of around 14 μm height was formed.

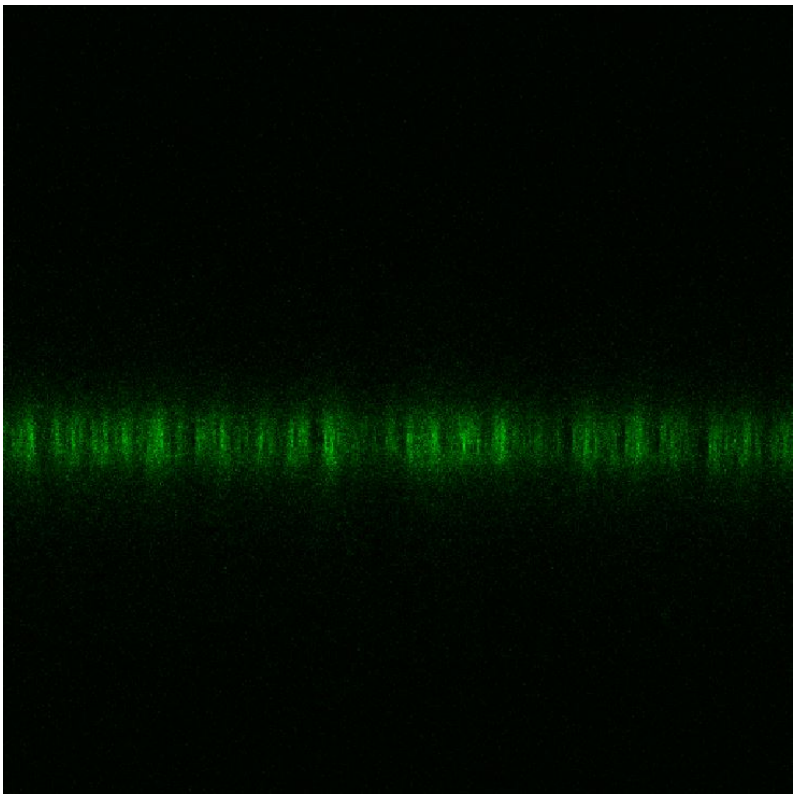


Figure 46. A height of around 14 μm.

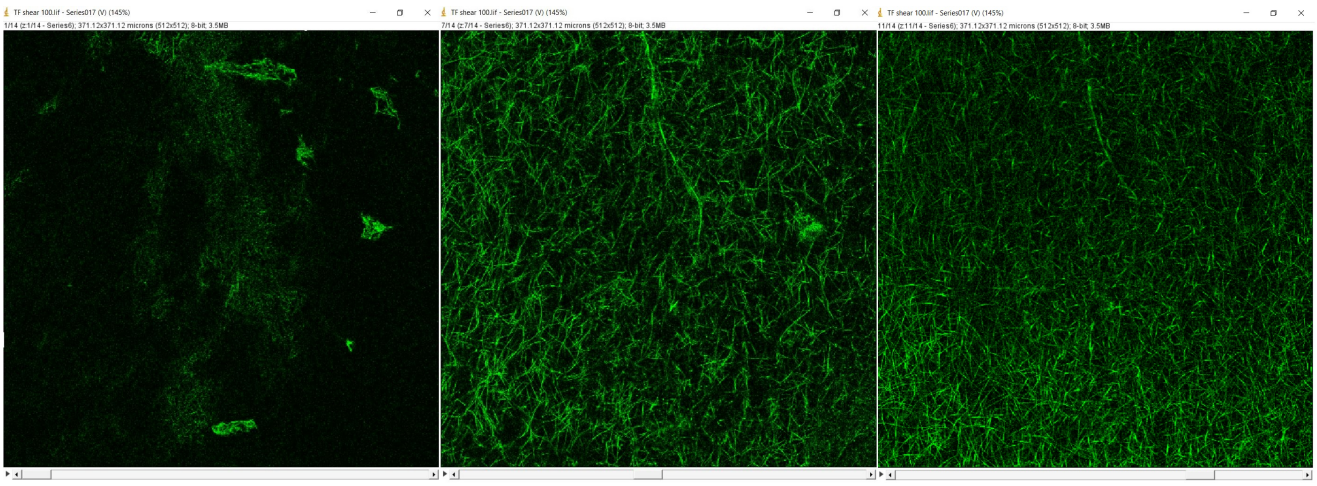


Figure 47. Left (closest to the microscope glass), and the 2 pictures higher in the clot.

12.5.5.3 Discussion and conclusion

As can be seen in figure 48, in this experiment, the clotting cascade is activated at 2 levels. A somewhat unnatural situation emerges.

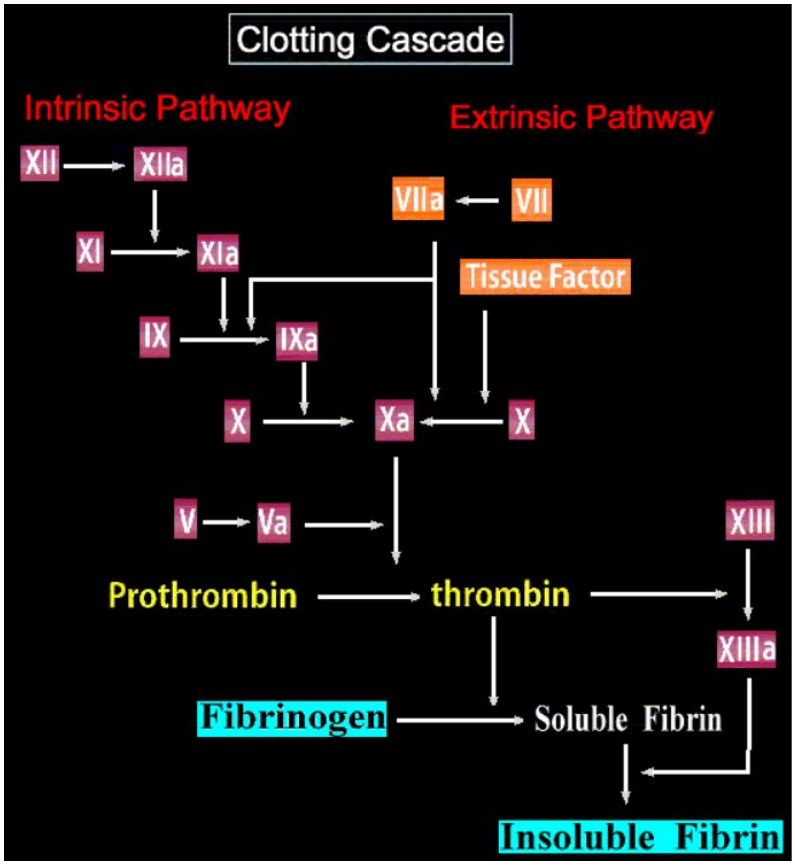


Figure 48. ..

12.5.5.4 Future

It should be investigated if there is an added value of stimulating the clotting pathway in this way, and if this is just an unnatural situation with no added value for research purposes.

12.5.6 A static control sample with TF

12.5.6.1 Method

On 27-09-2021 static control samples were made in various ways, as in previous try-outs the static samples failed. A total of 200 μL recalcified AF488-labeled PPP was used per TF coated slide 13.4.2

"Preparing materials needed for dynamic clot making". These TF coated slides are then carefully immersed in HEPES buffer to remove excess fibrin and prevent drying out. After that, the samples are prepared as can be seen in protocol 13.4.5 "Workflow clots with Piuma and confocal imaging".

Table 26. Static PPP clots.

Slide	Tape	Temperature (°C)	Time of immersion HEPES (minutes)
1	Thin	Room temperature	0
2	Thick	Room temperature	0
3	Thin	Room temperature	60
4	Thick	Room temperature	60
5	Thin	37	30
6	Thick	37	30

Figure 49. Microscopic glass slide temperature generator, with TF coated slides.

12.5.6.2 Results

12.5.6.3 Coating

The drying of TF on microscopic slides showed to be unaffective when put on 37 °C, as it is still very fluid after 45 minutes. To speed up the process, the device was put on 50 °C, after 15 more minutes the thin tape microscopic glass was coated succesfully, and after 25 more minutes the thick tape microscopic glass samples were dried as well.

12.5.6.4 Discussion and conclusion

Some useful conclusions are:

- Coating TF on microscopic slides (with thin tape) takes approximately ± 15 minutes when heated at 50 °C.

12.5.6.5 Future

13 Protocols

13.1 Location

- The experiments are conducted in the human-material lab: Ee-2307.
- The centrifuge used, is in the lab: Ee-2386.
- The miliQ and distilled water: Ee-2372.
- Some materials (BSA-solution, syringes) are retrieved in the mouse lab: Ee-2320.
- Retrieve new blue waste boxes in the back. For full bin see closet next to this room, for instructions.
- The scanning electron imaging is performed with the Leica SP5-604 microscope (allows CO2 and temperature regulation): Ee-604.

13.2 Needed for all thrombus analog making

13.2.1 Retrieving blood

13.2.1.1 Materials

1. 4.5 mL citrated tube
2. 21 gauge needle
3. Vacutainer
4. Tourniquet
5. Gazes

13.2.1.2 Methods

1. Retrieve the venous blood in a citrated tube. Blood band 15 cm above puncture place.
2. Sipon it around 4 times.

13.2.2 Making thrombus analogs: ratios

13.2.2.1 Materials

1. Whole blood
2. Syringe (function as mould), 5 ml with luer lock: link
3. Luer lock cap: link
4. Waterbath + isolation material + heating element
5. Gilson pipets: 100-1000 μL (blue marking), 50-200 μL (red), 20-100 μL (green), 2-20 μL (yellow)
6. Pipet tips: fitting sizes for pipets above. (NOTE: the blue 200-1000 μL pipet doesn't fit the same pipet tips as the 100-1000 μL pipet.)
7. Centrifuge

13.2.2.2 Methods

1. Centrifuge whole blood at 120g (120RCF) for 20 minutes (acceleration 5, brake 3. Set the total: brakes/stops: 4.) at room temperature.
2. Take off platelet-rich plasma (around 2/3 of the plasma).
3. Centrifuge remaining blood at 2000g for 10 minutes (acceleration 7, brake 7) at room temperature.
4. Take of platelet-poor plasma.
5. Make the different clots by mixing different ratios:

	Whole blood (μL)	Hematocrit (μL)	Plasma (μL)	Thrombin (μL)	$CaCl_2$ (μL)	End volume (μL)
Whole blood	291 (97%)	0	0	0	9 (3%)	300 (100%)
H 1% no thrombin	0	0	291 (97%)	0	9 (3%)	300 (100%)
H 40 % no thrombin	0	116.4 (38.8%)	174.6 (58.9%)	0	6.9 (2.3%)	300 (100%)
H 99 % no thrombin	0	291 (97%)	0	0	9 (3%)	300 (100%)
H 1% thrombin	291 (97%)	0	0	3 (1%)	6 (2%)	300 (100%)
H 40% thrombin	0	116.4 (38.8%)	174.6 (58.8%)	3 (1%)	6 (2%)	300 (100%)
H 99% thrombin	0	291 (97%)	0	3 (1%)	6 (2%)	300 (100 %)

6. Mix the blood constituetes in the right ratios, use the biggest pipet to mix all the constitutes with $CaCl_2$ and (if used for static clots) thrombin by pipetting in and out a few times in the fluid.

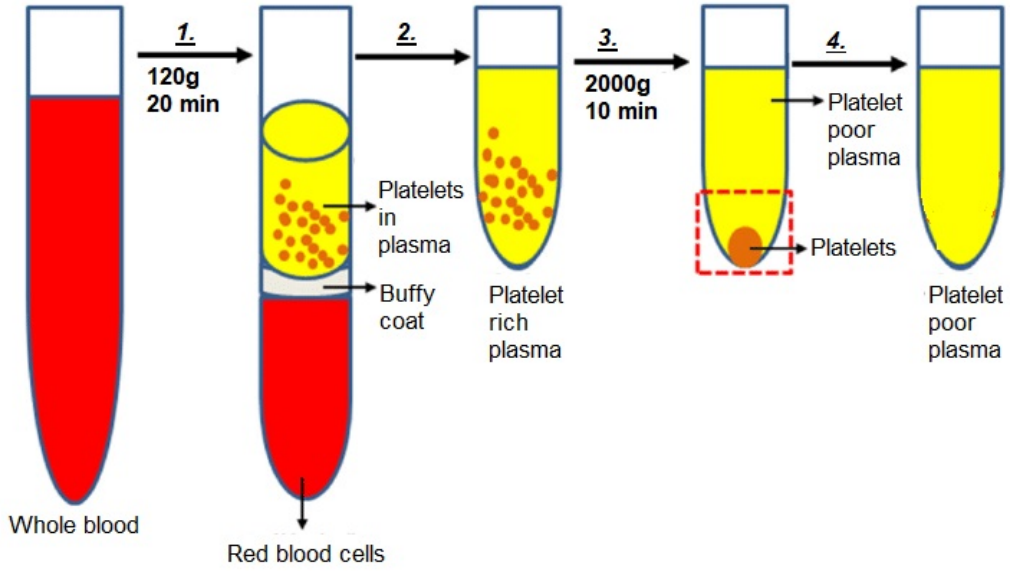


Figure 50. Step 1 till 4 from **??** analog sample making, are visualized in this figure.[104]

13.2.3 $CaCl_2$ and thrombin

13.2.3.1 Materials

Thrombin solution

1. Thrombin powder: link
2. MiliQ
3. 0.1% BSA solution (located in mouse lab)
4. Syringe
5. Pipets + pipet tips
6. Eppendorf tubes

$CaCl_2$ solution

1. $CaCl_2$ powder
2. Distilled water
3. Syringe
4. Pipets + pipet tips
5. Eppendorf tubes

Figure 51. Ee2372 lab: (left) MiliQ, (right) click for MiliQ, or use the little white tab for distilled water.

13.2.3.2 Method

Thrombin solution

1. Dissolve: 0.94 g $CaCl_2$ powder in 10 ml destiled water (this is 850 mM). This gives the "stock solution", which can conserved kept at room temperature.

$CaCl_2$ solution

1. Preferably use the powder all at once to prevent freezing and unfreezing more than needed, worse quality of the powder.
2. Solution rates depend on type of thrombin. Example 100 units (100UN): for stock solution 100 units/ml, all the powder in 1 ml of MiliQ, with 0.1% BSA in the miliQ.
3. Advice: store the total solution in a lot of small
4. Store the thrombin at -20 degrees! When using it, as cold as possible.
5. (When using thrombin:) a box with ice is needed to store the thrombin on.

13.3 Thrombus analogs formed under static conditions

13.3.1 Workflow thrombus analogs formed under static conditions

Steps in making and analyzing thrombus analogs formed under static conditions:

1. **Prepare $CaCl_2$ and (if needed) thrombin solution (protocol 13.2.3)**
2. **Prepare the clot analog constituents (protocol 13.2.1), and add these in your solution in the right ratios to prepare clots (protocol 13.2.2).**
3. **Get familiar with the used software used in the compression (and tensile) tester, and program this in the right way if needed (protocol 13.3.3).**
4. **Perform an unconfined compression test (protocol 13.3.4), or tensile test (protocol 13.3.5).**

13.3.2 Making thrombus analogs under pressure

13.3.2.1 Materials

1. Syringe (function as mould), 5 ml with luer lock: link
2. Luer lock cap: link
3. Waterbath + isolation material + heating element
4. Gilson pipets: 100-1000 μL (blue marking), 50-200 μL (red), 20-100 μL (green), 2-20 μL (yellow)

5. Pipet tips: fitting sizes for pipets above. (NOTE: the blue 200-1000 μL pipet doesn't fit the same pipet tips as the 100-1000 μL pipet.)
6. Centrifuge
7. Weights mimicking blood pressure

13.3.2.2 Method

1. Pipet the blood mixture used in a 5 mL syringe and place the luer lock cap.
2. Heat the waterbath to 38 degrees and place the weights mimicking blood pressure on the syringes. Place the syringes under water vertically, with the luer lock facing up. Waterbath:
   2.1. Yellow light: if it burns it is heating.
   2.2. Press 'P': use arrows for specific temps check temperature with device with metal pin (not built-in).
3. Let the samples mature overnight.

13.3.3 General: software compression/tensile test

13.3.3.1 Materials

1. MEXE02
2. LABVIEW: labview license needed (possible via TU Delft)

13.3.3.2 Methods

USBspeedlink

1. Make sure that the load cell is calibrated (It should be already configurated), for calibration
2. Connect USB cable (with pre-programming label) to laptop Laptop > Desktop > click tensile test > *USBSpeedLink*
3. Send the configuration saved for the Load cell. Saved as LoadCell19nov in the laptop.

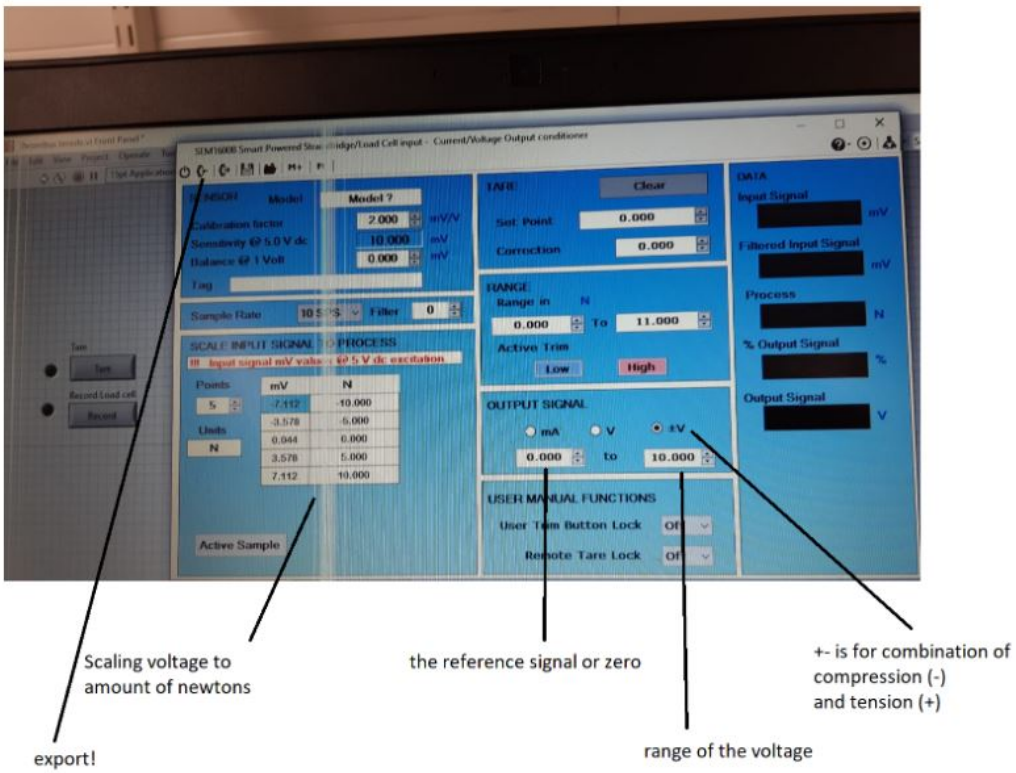


Figure 52. Interface: USBSpeedLink

MEXE02

1. Connect *pre-program* usb (labeled) to laptop
2. Pre-program the right values absolute position: is total height (so compressor above platform) Set the amount of compression, the speed, the number of loops etc.
3. Click data writing to transfer the settings to the compression tester.

Labview

1. Labview license needed (via TU Delft) connect *perform test* labeled usb cable to laptop.
2. Preferably use text as output for your data: make a .txt file and save in this file, one can select the specific file in the labview interface.
3. When using excell (excell loses resolution during testing) one cannot select this in the labview interface, use: > show block diagram block: write to measurement file > right click > save location > properties > make a new file.
4. Run labview: click on all the blocks from top to bottom.

13.3.4 Unconfined compression

13.3.4.1 Materials

Sample processing

1. Box with utensils (3x small tweezers, 2x large tweezers, micro-scissors, ruler)
2. Trimming fixture or punch blade
3. Set of blades
4. DMEM solution (stored in fridge)
5. A small and flat box (to cut the samples in) or petridishes

Measurement equipment

1. Ruler
2. Compression tester
3. Tester laptop + charger
4. Power box (for heating)
5. Digital camera + platform of 6.5 cm high

Software

1. MEXE02
2. Labview
3. ImageJ software

13.3.4.2 Methods

Sample processing

1. Remove the analog carefully from the syringe, by removing the backside.
2. Put the sample in a shallow open box with DMEM as fast as possible (prevent drying out).
3. Cut the sample in this box to the right shapes.

Measurement equipment

1. Make pictures
   1.1. Use the lighting box for an enclosed lighting setup, and a protocol for standardized camera settings.
   1.2. Place non-absorbent paper with label per sample below the DMEM box, on bottom and a ruler on top on the photography platform
   1.3. Take the overview photo.
2. Prepare the compression tester
   2.1. Fill the bath of the compression tester with DMEM solution
   2.2. Put on the heating system to plus minus 38 degrees as there exists a gradient in temperature.
3. Sample to machine
   3.1. Quickly transfer the samples in the DMEM bath of the compression tester.
   3.2. Let it slide from a thin blade to mimimize damage
4. Use MEXE02
5. Use Labview
6. Disposal:
   6.1. Biomaterial: all biomaterial (gloves, tissues): in blue+yellow biomaterial container.
   6.2. Cleaning of utensils used to handle thrombus: in sink, soap > dry > alcohol.

13.3.5 Tensile test

13.3.5.1 Materials

Measurement equipment

1. Tensile tester
2. Tester laptop + charger
3. Digital camera

Software

1. MEXE02
2. Labview
3. Axiovision

13.3.5.2 Methods

1. Prepare camera
   1.1. Open desktop computer
   1.2. Click *tensile test*
   1.3. Click *Axiovision*
   1.4. Click *F2Repeat*
   1.5. An *H* icon should appear on the lower right
2. Use MEXE02
3. Labview
   3.1. Connect *perform test* labeled usb cable to laptop
   3.2. Open a labview file
   3.3. Press *Initial position* to make sure that it starts from initial desired gauge length
   3.4. Check that the load cell gives a signal
   3.5. Choose the folder where the load cell signal is stored
4. When using axiovision

4.1. Turn both computers on (desktop computer and laptop)
4.2. Synchronize or check that both the desktop computer and the laptop have the same exact time.
4.3. Open AxioVision.
4.4. Click on Live to observe the region of interest.
4.5. Place the camera manually until the plane of interest is focused (the image).
4.6. Press on Exposure if the image color is not well processed.
4.7. Check that the camera is focusing on the middle of the clamps, and that the lower edge of the image is the border of the static clamp (parallel to the edge).
4.8. The field of View: 26 mm horizontal and 19 mm vertical
4.9. Set the storage folder (Tools>Options>Storage) and the name of the images (Tools>Options>Naming). Activate the AutoSaving mode.
4.10. Turn the light source on and check that light tubes are not in the field of vision you can dim the human lab TL-light when needed
4.11. Optional; Making a snap pic: F2
4.12. Option of high strain analysis > updating reference whole the time

5. Prepare the specimen

5.1. Fix the sample inside the metal box on the opposite side of the hole
5.2. Place the larger cardboard cylinder on top of the metal box hole.
5.3. Spray paint the top hole of the cardboard cylinder 5-10 seconds, in the direction of the sample.
5.4. Check the pattern. If it is not the desired result, try again.
5.5. If there are any problems with the spray paint, talk to Robert.

6. Back to the machine: placing clot analogs

6.1. Place double-sided foam tape (waterproof) on the bottom and top clamp, with an extra piece (4 mm width) at the outer/distal side of the clamp.
6.2. Place P400 sandpaper on the double sided tape.
6.3. Place the sample with the Velcro in the sample overlaying the tape bumps.
6.4. Place the specimen to on the clamps, use the torque wrench to tighten the screws to the same torque, (I used 10 cNm for the tensile clot analogs).
6.5. Make sure both screws are tightened to a more or less similar extent. This by not tightening them 1 by 1, because it will cause the clamp to be more tight at 1 side.
6.6. Go AxioVision and the Live mode to check the vision:
    i. Adjust the camera to focus on the sample, press on Exposure if the image color is not well processed.
    ii. Adjust the light tubes to have a proper vision of the sample and try not to have any reflections at the surface of the sample, this is critical for the DIC.
6.7. Initiate (just by clicking) the Autohotkey script: *F2Repeat.exe* that is on the desktop of the computer.
6.8. In LabVIEW, the front panel:
    i. Set the loop frequency in *interval ms*. This controls the load cell data recording frequency. (I use 50 ms)
    ii. Press *Tare* to tare the load cell
    iii. Press the *Record* button to start the measurement recordings Press the *Pre-stretching* button
    iv. Press A in the desktop computer for starting the camera acquisition right before pressing “Test Procedure” in LabVIEW.
6.9. To finish, close the Autohotkey script for stopping the image acquisition and press the “Record” button in LabVIEW

13.4 Fibrin clot formation under flow

13.4.1 Workflow

1. **Prepare $CaCl_2$ and (if needed) thrombin solution (protocol 13.2.3).**
2. **Prepare the HEPES buffer, TF coating, and (if needed) the activator mix (protocol 13.4.2).**
3. **Retrieve PPP for the flow experiment (protocol 13.2.2).**
4. **Perform the flow experiment (protocol 13.4.3).**
5. **Indent the (fresh) clot analogs as soon as possible after forming them (protocol 13.4.4).**
6. **Fixate the clot analogs (protocol 13.4.5).**
7. **Image the clot analogs in the confocal (protocol 13.4.6).**

13.4.2 Preparing materials needed for making fibrin clot formation under flow

13.4.2.1 Materials

PPP mixture

1. AF488-labeled fibrinogen (0.025 mg/ml)
2. $CaCl_2$ (stock is 850 mM)
3. PPP

TF coating

1. MilliQ
2. 25 mM HEPES
3. Stock solution of TF
4. Watertight pencil
5. Watertight tape
6. Stencil maker
7. Point marking maker
8. Bottom side flow chamber
9. Microscopic glasses: 25x75x1.0 mm (link)
10. Sharp knife

HEPES buffer

1. MilliQ
2. 25 mM HEPES
3. 137 mM NaCl
4. 3.5 mM KCl
5. 1% BSA (pH 7.4)

Activator mix (=not used in final experiment)

1. CaCl2
2. Thrombin
3. HEPES buffer

13.4.2.2 *Method* PPP mixture

Before starting the experiment, one should mix: the PPP+fibrinogen is 97% of the end-volume, the other 3% is the $CaCl_2$. The PPP:fibrinogen are in ratio of which 59.2773:1.

TF coating

1. Stock: TF powder (Dade Innovin, 10 mL)[105] can be dissolved in 10 ml MilliQ water, this is your stock solution. To prepare 10-times diluted TF, you take 1 ml and add 9 ml buffer, this needs to be stored at -80 °C.
2. Let it thaw in the normal fridge (or at RT) just before coating your slides.
3. To make the TF fluid ready to coat the microscopic glass, one can dilute the TF stock 1000x in an assay to activate coagulation. Dilute 10 μL of the x10 dilution and 990 μL of HEPES buffer. This results in a 10 mM TF buffer.
4. Put waterresistant tape upon the microscopic glass. Label the microscopic glass.
5. Draw the shape of the flow channel (watertight/alcohol resistant) on the other.
6. Mark point A, B, and C (the successive middle openings) of the point marking maker on the glass. Point A is the side closest to the white side (for labeling the slide) of the microscopic glass, and should be the inlet side of the stream, whereas B is in the middle, and C is at the outlet side.
7. Put the microscopic glass with the tape in the stencil maker (screw it tight), and cut out the shape of the flow channel (take this middle part of the tape off), figure 54.
8. Take the microscopic glass out and coat the slides with 200 μL of the TF coating solution, this should look like figure 53.
9. To speed up the process of drying the coating on the glass, one can heat up the microscopic glass slides (denaturation of TF takes place at around 55 °C,[106] so one should apply a lower temperature).
10. The TF coated microscopic glasses are then stored in a normal fridge (2 till 8 °C) or used directly.

Figure 53. The microscopic glass, marked, and with the cut out shape of the flow channel, with solid buffer on it.

Figure 54. The stencil maker (left) is screwed on the bottom part of the flow chamber.

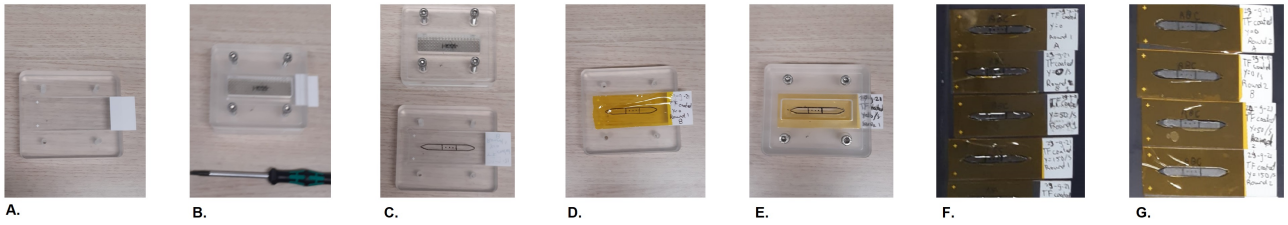


Figure 55. .

HEPES Buffer

Use the matlab code to calculate the needed amounts of materials for the HEPES buffer (code: 11.4.7). Or calculate it by hand. An example of quantities is given below. When using the table below, one should use a volume of 1 liter of MiliQ, add 0.01 BSA (1%), and the add the NaCl, KCl, and HEPES (powder), in the quantities given in the table, to create a HEPES buffer with 25 mM. One should test if the pH is 7.4, if not there can be added more BSA.

Product	Molarity (g/mol or mg/mmol)	Mass (g)
NaCl	58.44	8.01
KCl	74.56	0.261
HEPES (powder)	238.3	5.957

13.4.2.3 Activator mix (=not used in final experiment)

Use the matlab code to calculate the needed amounts of materials for the Activator mix (code: 11.4.7). Or calculate it by hand.

13.4.3 Final flow experiment protocol (1 pump)

13.4.3.1 Materials

1. 1 displacement pump
2. 2 syringes
3. Flow chamber
4. Tubes (silicon with stiff material around it)
5. Mixing chamber
6. 2 three-way tabs
7. 25 mM HEPES buffer
8. Microscopic glasses coated with TF
9. PPP
10. $CaCl_2$

13.4.3.2 Methods

1. Place the syringe on the perfusion pump (pushing mode), and select the right syringe or give in the right diameter of the syringe.
2. Place the microscopic glass in the flow chamber and screw it thight.
3. Flush the tubings and the flow chamber with HEPES buffer to remove all air. Look for leaks, and solve these by fasten every part tightly.
4. Flush PPP until mixing chamber to remove bubbles from that tube.
5. Insert the right flow rates (in the table below the flow rates for the 130 μm high flow chamber are shown, the ejected volume per test is set at 500 μL.
6. After the PPP flow is done, the microscopic glass is dipped carefully into a petri-dish filled with HEPES buffer (until it is totally covered) to remove excess fibrin.

Shear rate (/s)	Flow rate (ml/h)
50	2.535
150	7.605
300	15.21

13.4.4 Piuma

13.4.4.1 Materials

1. Piuma controller, Piuma indenter, Piuma interferrometer
2. Laptop with Piuma software
3. Probes
4. Paper
5. IPA (isopropanol or Trypsin)
6. Demiwater
7. DMEM

13.4.4.2 Methods

Prepare before:

1. Select desired probe.
2. Let the used fluid go to room temperature.
3. Clean your dishes from dust.

Piuma start machine:

1. Click all the power buttons on and start the laptop.
2. Start the Piuma software
3. Rotate the grey knobs back to move the head up as high as possible
4. Click *Home stage*.

Mount a (new) probe:

1. Carefully pick the probe from the box with a "OK-sign" of your hands.
2. Insert the probe properties in the software.
3. Pre-wet (with the selected fluid) the probe by using a plastic dropper in a way it has a drop on the inferior part.
4. Take a hard surface (petri-dish) with or without fluid, and without a sample.
5. Put down the probe without crashing it down to the glass, first manually until the camera focusses on the probe, and then with z-down. Change travel distance to about 100 micrometer per step. Make sure it does not touch the glass yet.
6. Interferrometer: Go to "Wavelength scan", click center button down.

Find surface (micrometer):

1. Click find surface (jumps down 10 micrometer), it autimatically jumps up around 10 again then.
2. Click: *Reset surface* (erase the 10), which allows you to go down again.
3. Then you go down (z-direction) 1 micrometer per step until you see the signal making a jump.
4. Go to *time data* tab in the laptop
5. Click *Set surface*
6. Click *Calibration*
7. If it is right: You will see blue and green line start at the same point. Also: Calibration factor should be same on box (not more than 0.3 difference with box).
8. If not: click *Calibration* again.
9. If it is right: click *Use new*.
10. Click *z-up* or just manually with knobs.

In the tab: indentation profile, one can insert an indentation profile.

1. First tab: displacement=0 (nm), time=0.5 (s). Always keep it the same values.
2. Second tab: displacement=... (nm), time=... (s). Changeable, this is how much the probe goes down.
3. Third tab: displacement=... (nm), time=... (s). Changeable, this is the holding phase.
4. Fourth tab: displacement=0 (nm), time=... (s). Changeable, this retraction phase.
5. Fifth tab: displacement=0 (nm), time=0.5 (s). Always keep it the same values.

Configure indentation (in nanometer!!):

Displacement (nm)	Time (s)
0	0.5
16000	16
16000	20
0	16
0	0.5

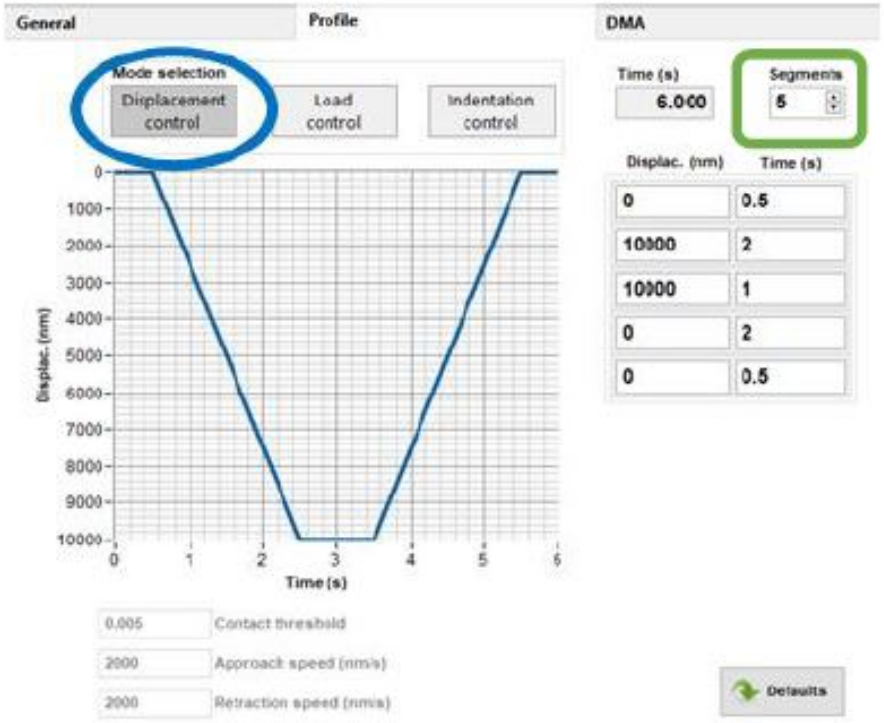


Figure 42: Mode selection: Displacement control

Figure 56. 'Profile' tab of the step and choose 'Displacement control' in the 'Mode Selection' section (blue). In the window, a graph represents the displacement profile, of which the displacement (nm) and time (s) variables can be customized for each segment of the profile. A maximum segment number of 10 can be defined; by default, the segment number is set to 5 (green).[64]

Actual measurement (micrometer):

1. Move it at the desired x/y direction.
2. Optional: preprogram *matrix scan*. Positive value is to the left.
3. Go down again until a safe distance.
4. Click *Find surface*. After finding the surface the tip should now be in focus, together with the sample surface. It should be about **10 micron** above the surface, if not make sure it is.
5. Click *Indent*
6. Time-data: When the green line starts to go down after the blue line, it indicates the indentation starts out of contact, which is preferred. Also, after the point of contact, the slope of the blue line

should be distributed over the slope of the green and red lines as the initial displacement can only result in an approach of the sample, sample deformation and cantilever bending.
7. Curve to the right from 0 (x-axis) is how deep the indent was. This old Piuma can't use *Load control* which can adjust the depth. WARNING: if you want to indent the same spot again this should be after a certain recovery time of the sample.
8. Make sure "Time Data" plot is correct.
9. Right bottom: red/green bar arrow shows if the probe is right.
10. Next indent: first move up again and then in desired direction.
11. Saves automatically to folder.
12. Click *Find surface*.
13. Click *Indent*
14. For the probe with stiffness= 0.5 N/m, and tip radius=100 micron, and indent depth of 1 micron, one should take steps of at least 20 micron in x or y directions. Perform 3 indents per dot (A, B, C), so this gives 9 indents per sample.

Clean probe:

1. Move probe up again.
2. Dropper: half fill with IPA and let it flow over probe slowly.
3. Dropper: do the same (3 times a filled dropper) with demiwater.
4. Use a tissue tip to dry very carefully.
5. Make sure cantilever is loose from probe again.

Did the indentation go alright?

1. A nice visco-elastic curve:

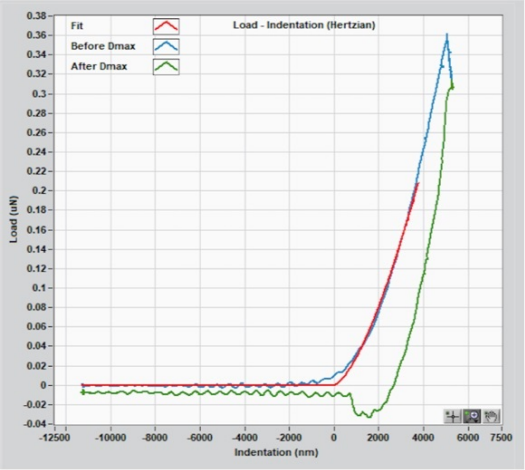


Figure 57. A typical load-displacement curve yielded from an indentation test. The blue line represent the loading/indentation phase, the green line the unloading/retraction phase and the red line the fitted Hertz model.[phillip]

2. Your tip is touching the sample before the indent: *L*oad indentation tab: blue line doesn't start at load is zero (y-axis) before the indent (x-axis < 0). This is seen a lot with soft samples after "finding the surface" automated. You should go farther up with the tip.
3. Load indentation tab: sample is floating:
4. Load indentation tab: When indent goes below 0 (y-axis) it indicates sticking of sample at the probe.

13.4.5 Fixation in formaldehyde of fibrin clots formated under flow

13.4.5.1 Materials

1. 4% formaldehyde
2. PBS
3. VectaShield
4. Coverslip

13.4.5.2 Method

After Piuma testing TAs, and before confocal imaging, TAs should be fixated:

1. Try to cover the samples as much as possible (for example with tinfoil or a closed box), light will cause fading of fluorescence.
2. Submerge the slides in 4% formaldehyde for no more than 24 hours, and at least 12 hours to fix the structure of the clot. This can be done in the histology lab under a fume hood, with special big pipets. Warning: don't tilt the miroscopic glasses until the clot is fully fixed!
3. Rinse in PBS, by emptying the petri glass first (the formaldehyde), fill it up with PBS, and than emptying it again. The fluids should be thrown away in a special waste can.
4. Apply VectaShield and add a coverslip.
5. Secure the sides of the coverslip extra on the glass by applying nail polish.
6. Store in the fridge until time of confocal imaging. Can be stored for more days afterwards in the fridge.

13.4.6 Confocal - Leica 604 - Quick guide

13.4.6.1 Materials

1. Box with ice
2. Slidebox
3. Confocal device
4. Oil for lenses

13.4.6.2 Methods

Handling the samples and placing it in the confocal

1. Try to cover the samples for light as much as possible (for example with tinfoil or a closed box), light will cause fading of fluorescence. Keep the samples cold, bring all the samples in a box with ice to the confocal.
2. When placing the samples on the confocal device, the coverslip should be down, and the microscopic glass up.
3. Choose your objective lens (40x oil) > in the configure tab > beam path settings. You can see if you are dealing with an oil lens by the furry circle around the lens.
4. Place a drop of oil on the lens, and then place the sample on the device.

Preparation

1. If needed: turn on the system by switching the green buttons (from left to right) and turning the laser key.
2. Turn on the mercury lamp (that box on the left of the confocal, turn the switch) if you want to look at fluorescence through the eyepieces.
3. Logon to Windows as TCS User (no password) > Double Click on LAS AF icon on the desktop > click: ok
4. When asked to initializing the table, make sure the microscope condenser arm is pushed back and the objective is at the lowest position.
5. Open "experiment 1ste keer alleen oefenen.lif" > Apply
6. So by opening a previous "lif" file and applying the settings a lot of settings are set. For analysis you have to do settings again every time.

Activate the lasers

1. Click on the Configuration tab > click on Laser > activate the 488 laser (you need by checking the box) and activate The Argon laser, do NOT forget to put the digital power slider at 20%.

2. Acquire tab > Click on UV and Visible to activate the laser > select the laser and their intensity by moving the sliders up or down. The 488 laser should be at 20% (in the acquire tab) in samples formated under shear, for the 0 shear samples the laserpower should be put at 6% in the same tab.
3. Desktop device > smart gain until 1250 volt in samples formed under shear (and to 721 volt in samples with shear is 0).
4. Optional: open pinhole temporary to get a better view (and go back to 1 when you found something) > standard setting is at 1 (should be 1 when making a stack).
5. Click on the Live button to check a live image of your sample.

Settings confocal

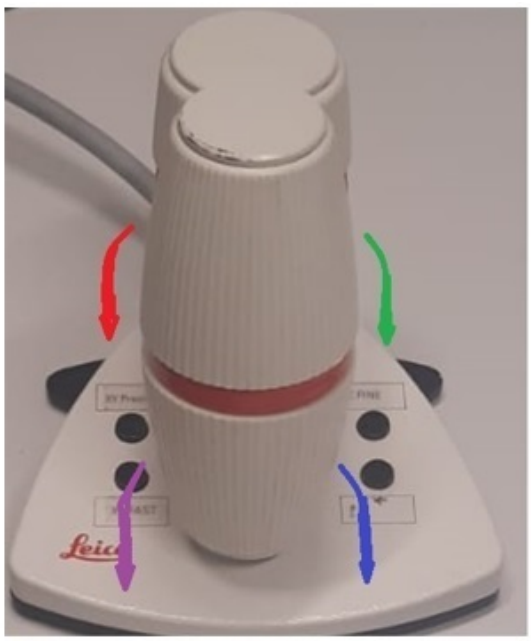
1. First use the best settings for searching the right y/x, and z-values of the fibrin clot for a z-stack, are: format 1028x1028, interval imaging 400 Hz, line averaging 1. These allow for fast recognition of fibrin.
2. When the right spot is found, one should apply the settings stated below in table 27.

The settings of table 27 can be used. The pixel size of x and y <100 micron is needed for precise analysis (99.63x99.63 micron), and a certain z-size which can be bigger because it is not used for analysis (167.85 micron).

Table 27. Options which should be used in the Leica microscope when making a z-stack.

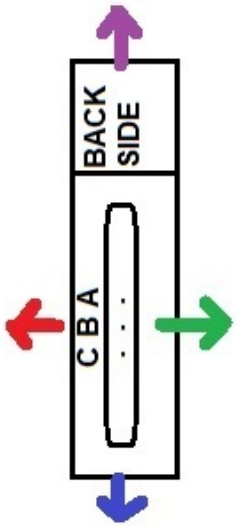
Confocal settings	**Specification**
Lens	40x oil immersed
Bits	8
Format	2048x2048
Zoom in	1.9
Fluorescent laser	488 nm
Pinhole	65.35 μm (=1 airy unit)
Interval imaging	200 Hz (standard), 0.15-0.5 (used 0.17) μm z-steps
Line averaging	2
x and y	203.95 μm
Gain shear clot	1250 V
Gain static clot	721 V
Acquisition > 488 laser shear clot	20 %
Acquisition $_{4}88 laser shear clot$	8 %

Orientation confocal

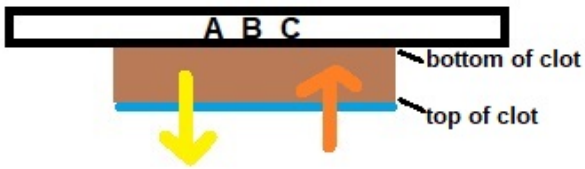


A.

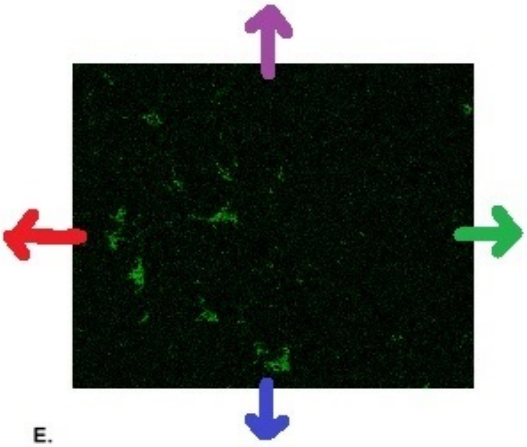
B.



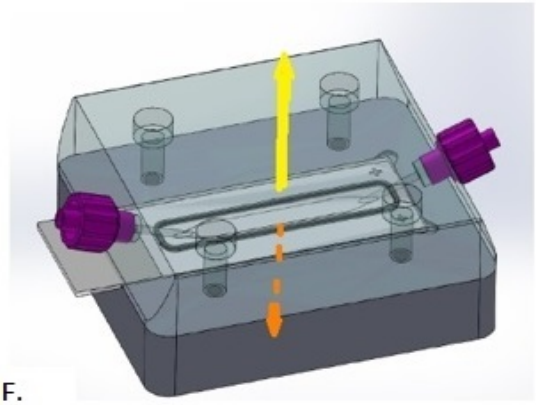
C.



D.



E.



F.

Figure 58. Orientation of: A. the joystick of the confocal, B. the desktop device, C. the slide with the sample on it (upside down as it is postitioned in the confocal), D. focusing of the sample (brown), E. the image screen, F. in the flow chamber itself. Remember that the flow direction is A to B, or the purple arrow to the blue arrow.

Doing an analysis

Finding the clot surface:

1. For activating the Argon laser> use button left middle/lowest > and click middle button (frontal): Schutter on. Use either the middle black button on the front of the microscope, or the I_3 button. First turn on the normal light.
2. Manually turn the (z-height) knob at the microscope, make contact with the sample. One can see that the oil hits the microscope, by a short flash of light.
3. Find a border in the sample (the black line which marks the chamber wall) with the normal lighting, and get a sharp image of this border.
4. Then use the argon laser and turning knob to find the right z height, where fibrin can be seen.
5. Then use the normal lighting again to find the black dot (A, B, or C) with the bright field microscope. First find the right x/y coordinates before changing the z-height.
6. Note that the whole screen lights up greenish just before and after seeing fibrin strands sharply when turning the knob at the microscope. So if you see this lighting up, one should change the z-height very slowely to find a clear image.
7. NOTE: It is better to save the z-stacks and tile-scans in different files, this makes it easier to evaluate them in ImageJ.

Figure 59. Overview of how the DiameterJ algorithm analyzes fiber diameter and other scaffold properties.

Z-stack gives in depth information of sample:

1. Get a clear (or green) view at the eyepieces by manually turning the knob at the microscope.
2. Then use the desktop device: z galvao, and turn it counterclockwise to find the first border, click on the right arrow of the z-stack image. Turn the knob until the image goes black again (lower end z-stack) Click on the left black arrow near the square.
3. Next turn the other way around and click on the other black arrow to set border.
4. Click: z step size, it will make the z-step size the same for every sample, which is needed.

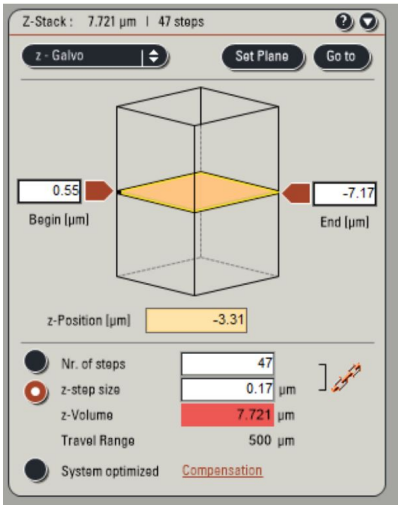


Figure 60. Always use the same method for orientation. There is chosen to make the left border setting the more positive number, and the right arrow a more negative number. If only a part of the clot is imaged, the right border should be defined first (still on the right side).

Overview picture with tile scan:

1. Search for a corner in your sample, click on tile scan icon.
2. search for 2 other corners and do the same.
3. Start scan.

Closing the system:

1. Look in the schedule if someone needs other lasers after you and put these on.
2. Put out the check-boxes of the used lasers (the 488) which are not needed anymore. Wait for 5 minutes (hear if the ventilator stops).
3. If you are the last person, push all butons and turn the key so that the system is of. WARNING: you should first unthick the box and wait for at least 5 minutes before you close down the rest, else the laser is not off and cannot cool down!

13.5 Processing software

13.5.1 Image processing

13.5.1.1 Materials

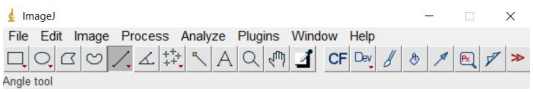
1. Use fiji (extended version ImageJ).[107]. General plugin list: https://Fiji.net/list-of-extensions. Bioformats package: https://www.openmicroscopy.org/bio-formats/downloads/
2. FOA tool and FibLab: https://gitlab.tue.nl/stem/FibLab/-/tree/master, Requires Matlab R2015b or later, Image Processing Toolbox, and Curve Fitting Toolbox.

13.5.1.2 Methods

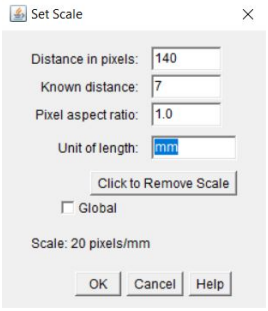
Calculate surface area in statically formed clots

1. Download: > double click the Fiji icon:

2. File > Open > Your image
3. Straight line icon > Select known distance in the picture: gives amount of pixels

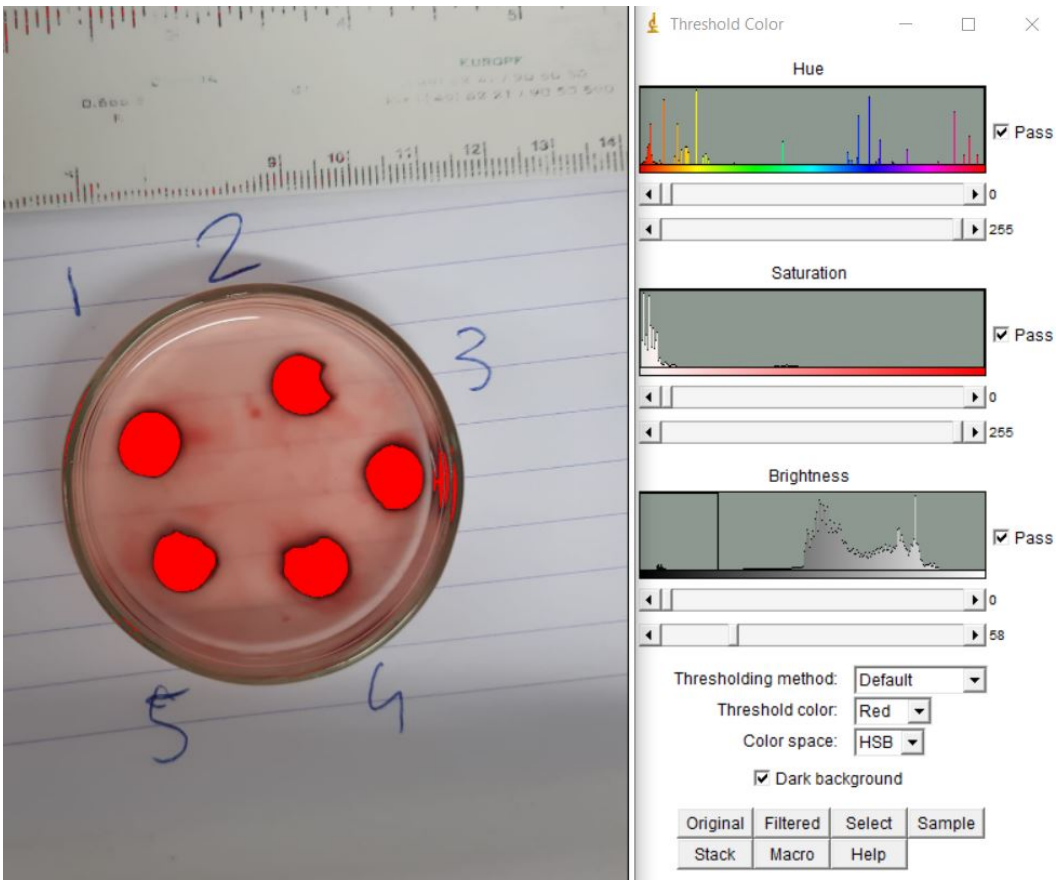


4. Analyze > set scale. For example:

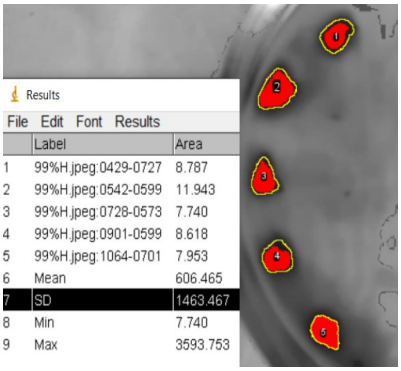


"Distance in pixels" amount of pixels measured in step 4, to "known distance" for example amount of measured mm's, it automatically gives a scale > OK

5. Optional (especially useful for less clear pictures): Edit > Invert.
6. For clear pictures: Image > Type > 8-bit and then Image > Adjust > Threshold: play around with until desired surfaces are coloured as you want.
7. For less clear contrast: Image > Adjust > Color threshold and/or Progress > Enhance contrast.



8. Analyze > Tools > ROI manager > Wand > Add [t] > click boxes Show all and Labels > you can go back and forth to step 7 to select the right area sizes
9. ROI manager > results > Area: gives the number in the chosen measure entity (for example mm's)



General

Installation tools Fiji:

1. Plugin: Place the .jar files in the *plugin* folder from *Fiji*, when you restart Fiji the plugins should appear in the plugin menu.
2. Macro: place the macro in a map in Fiji.app. The macro will appear in next to the "help" icon in your Fiji app. The macro name should contain an underscore.

Saving stacks with Fiji:

1. You can save the different stacks as a ".tif" to analyze them as seperate .lif files: save as > .tif.

Opening files with bio-formats in Fiji:

1. Drag the ".lif" that you want to open onto the Fiji bar.
2. With the settings of figure 61, you can simply open all files in the right way.

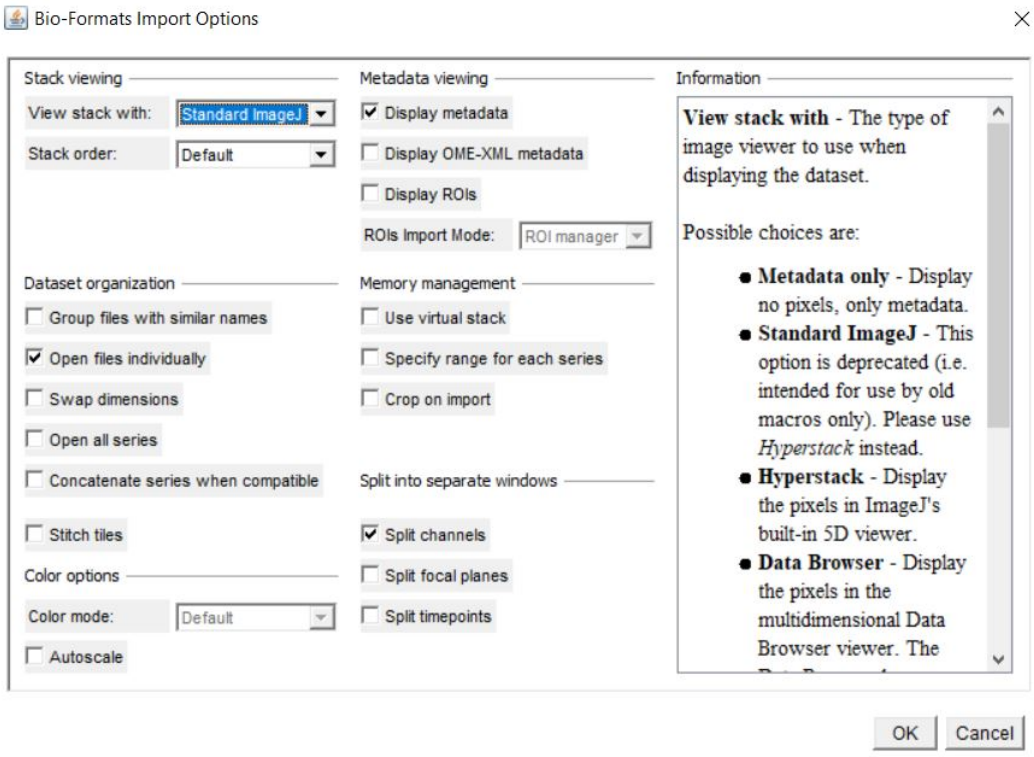


Figure 61. With these settings you can open and the images in the right way, and do all the analysis.

Making a z-projection in Fiji:

1. Make a z-projection of the top 2 micron (figure 62), one can see what is the top in figure **??**: summarizing the whole stack in 1 image: Image > stacks > z-project > standard deviation (or whichever setting which gives you the clearest image).[108]

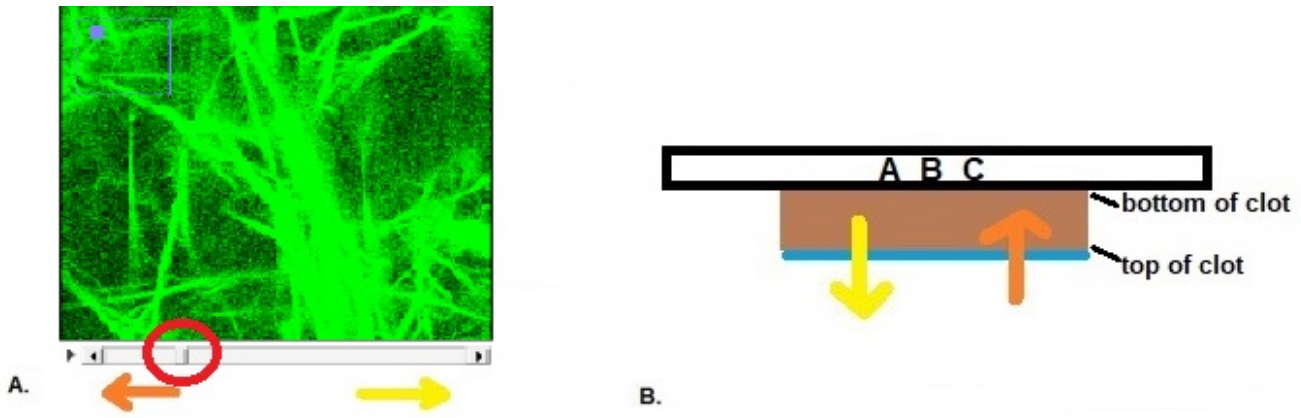


Figure 62. Scrolling to the left in Fiji stands (with button circled in red) for going to the bottom of the clot, and scrolling to the right for going up in the clot.

Fiber orientation

Fiber orientation with the FibLab:

1. Run “FOAtool.m” in same directory as images (GUI pops up, which can be seen in figure 63).
2. Load a fluorescence image, resized jpg-images are used (so TIF images are first saved as jpg images with the help of Fiji). The image won’t always show in the left window, but the tool will still work.
3. Choose R G B to filter (if needed, if it is a grey image this doesn’t matter).
4. Scales(std): [3.5 4.5 5.5].
5. Vesselness threshold: [0.9999], or when this resulted in a anisotropic fraction of 1, the vesselness threshold: [0.9995] was used in stead.
6. Press the "Calculate" button
7. Big images (2048x2048 pixels) can take 2-3 minutes to get processed, so be patient.
8. Press the "Export to workspace" button.

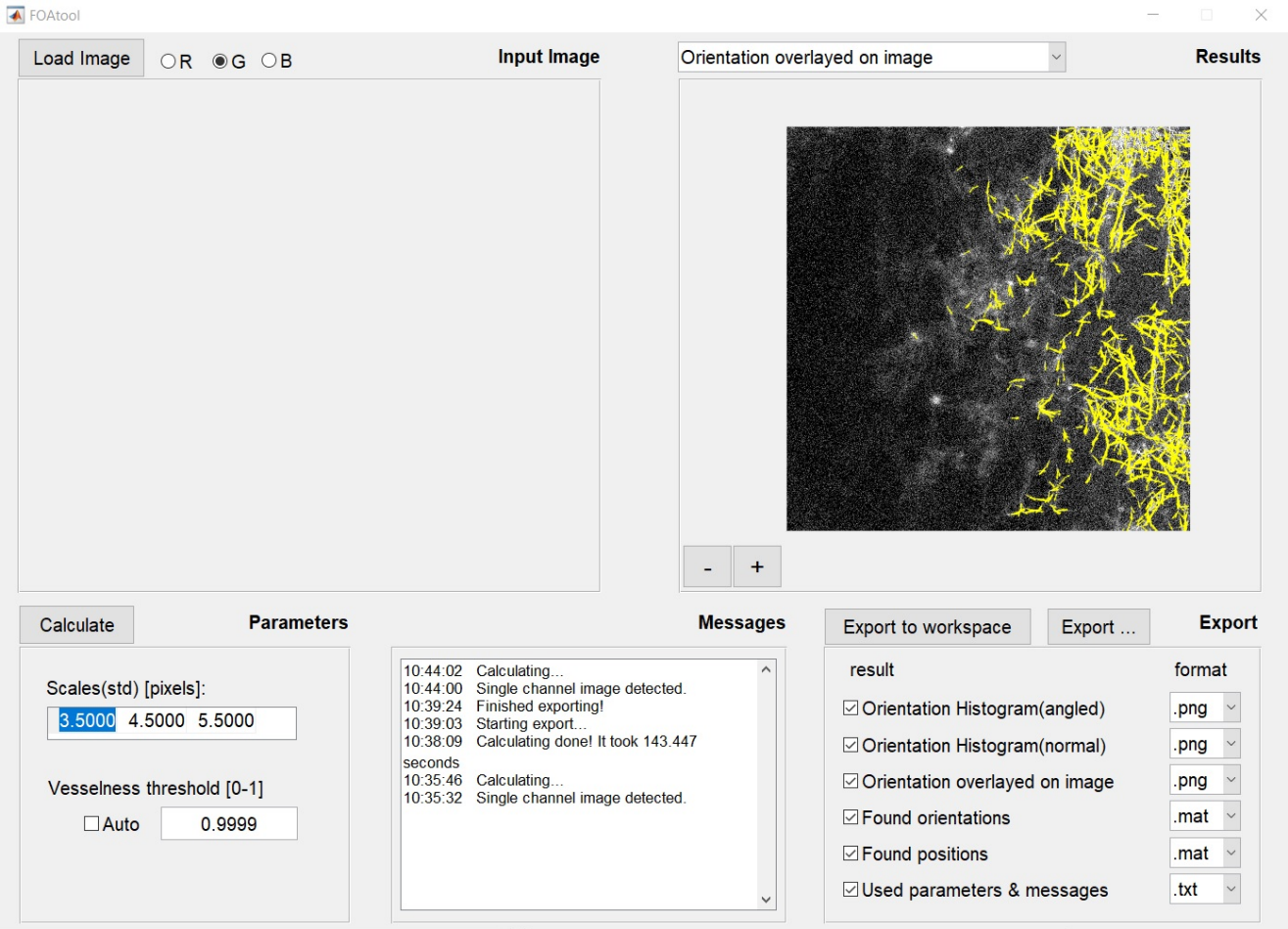


Figure 63. The FOA tool GUI interface.

Fiber orientation analysis with mwe file:

1. Open the mwe.m file (code 11.4.4).
2. First clear previous results.
3. Try the first part: "single peak", if the results contain more peaks, use the "two peaks" part,
4. Returns "param", which are [peak centre, peak std, anisotropic fraction]

Fiber density with Fiji:

1. Four macro's are used, which can be seen in: "11.5 Macro's: Fiji". Sometimes an enhance contrast step, or inverse of colors step is added, when fibers don't get selected with simply applying the different macro's.
2. The "Threshold_94" macro should be tried at first. In more noisy pictures from TAs formed under flow, the "Threshold_115_more_noise" should be tried next. The "Inv_threshold_94" macro is usefull for TAs formed under flow with a lot if fibrin. In TAs formed without flow, "Threshold_55" can be tried, especially when there are a few very bright dots in the picture.
3. These macro's will give the area covered with fibrin. The density is then calculated by dividing the selected area by the total area of the image.

13.5.2 Piuma in viewer

13.5.2.1 Materials

1. Piuma dataviewer V2.4: https://www.optics11life.com/resources/

13.5.2.2 Methods

1. Don't change the second folder name ("Indentations")!

2. Open the dataviewer
3. Options:

    3.1. Model settings > Hertzian contact.
    3.2. Pmax % for contact point: decrease the value in Pmax % for contact point until a good fit of the initial load-indentation curve is achieved with the yellow line (see Figure 64).[65] The position of 0, also called contact point, of the Indentation (nm) axis, is determined based on this fit and is needed for the correct calculation of storage and loss modulus.
    3.3. In case the Hertzian model is selected, the '% of Pmax' field controls the part of the curve to be fitted, expressed in a percentage of the maximum load in the loading part of the curve. One can also choose to not apply a model by clicking whichever model is highlighted a second time, which will grey out both sets of model parameters. The data can be reanalyzed and fitted with more models in DataViewer.

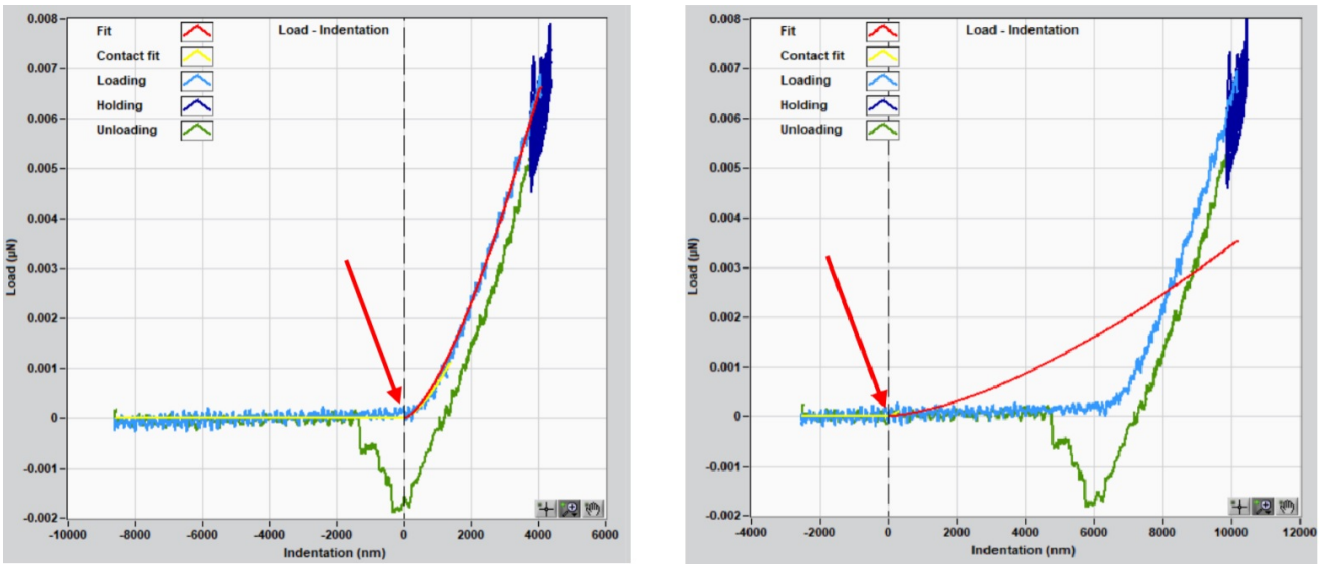


Figure 64. Left picture: good Hertz fit, a good location of the contact point. Right picture: a bad Hertz fit, a wrong location of the contact point.

4. check the box: use single fit method (this will give the results as you saw them during the experiments).
5. A Poisson's ratio (ν) can be set in the software if this is known, else use the effective Young's modulus (E_{eff}) as an outcome.
6. When the maximum 5-10% indent depth of the clot height is exceeded, one can use a different curve fitting to correct this. This can be done by adjusting the P-max%. The red line indicated which part of the indent curve is used, so with a smaller % a smaller part (beginning of the curve) is used.

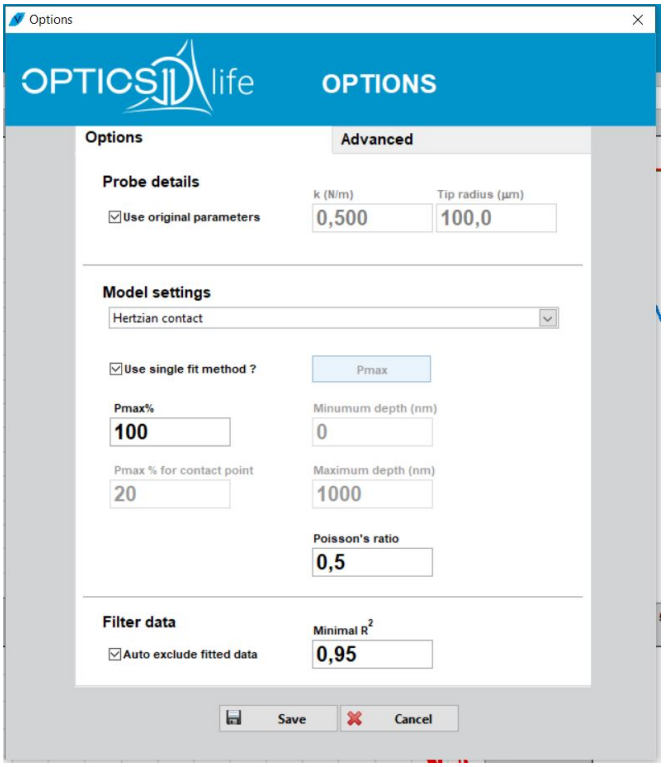


Figure 65. Options menu where one can adjust the features mentioned above.

13.6 Extra

13.6.1 Final flow experiment protocol (2 pumps, not in the final experiment)

13.6.1.1 Materials

1. 2 displacement pumps
2. 5 syringes
3. Flow chamber
4. Tubes (silicon with stiff material around it)
5. Mixing chamber
6. 5 three-way tabs
7. 25 mM HEPES buffer

13.6.1.2 Methods

Air removal:

1. Place syringes on perfusion pumps (pushing mode), and select the right syringe or give in the right diameter of the syringe.
2. Secure 2 other syringes for pushing air out of the system on the two 3 way taps.
3. Flush both tubes and the flow chamber with HEPES buffer to remove all air. Look for leaks, and solve these by fasten every part tightly.
4. Flush with activator mix until the solution reaches the mixing chamber (0.55 mL or 550 μL). For example the 3 cc syringe of Terumo, gives 2.374 ml/min (so this takes 14 seconds) in the red pump.
5. Flush PPP until mixing chamber to remove bubbles from that tube.
6. Flush at the distal end of the PPP tube with HEPES to prevent early clotting.
7. Push PPP until inlet mixing chamber (...)mL.

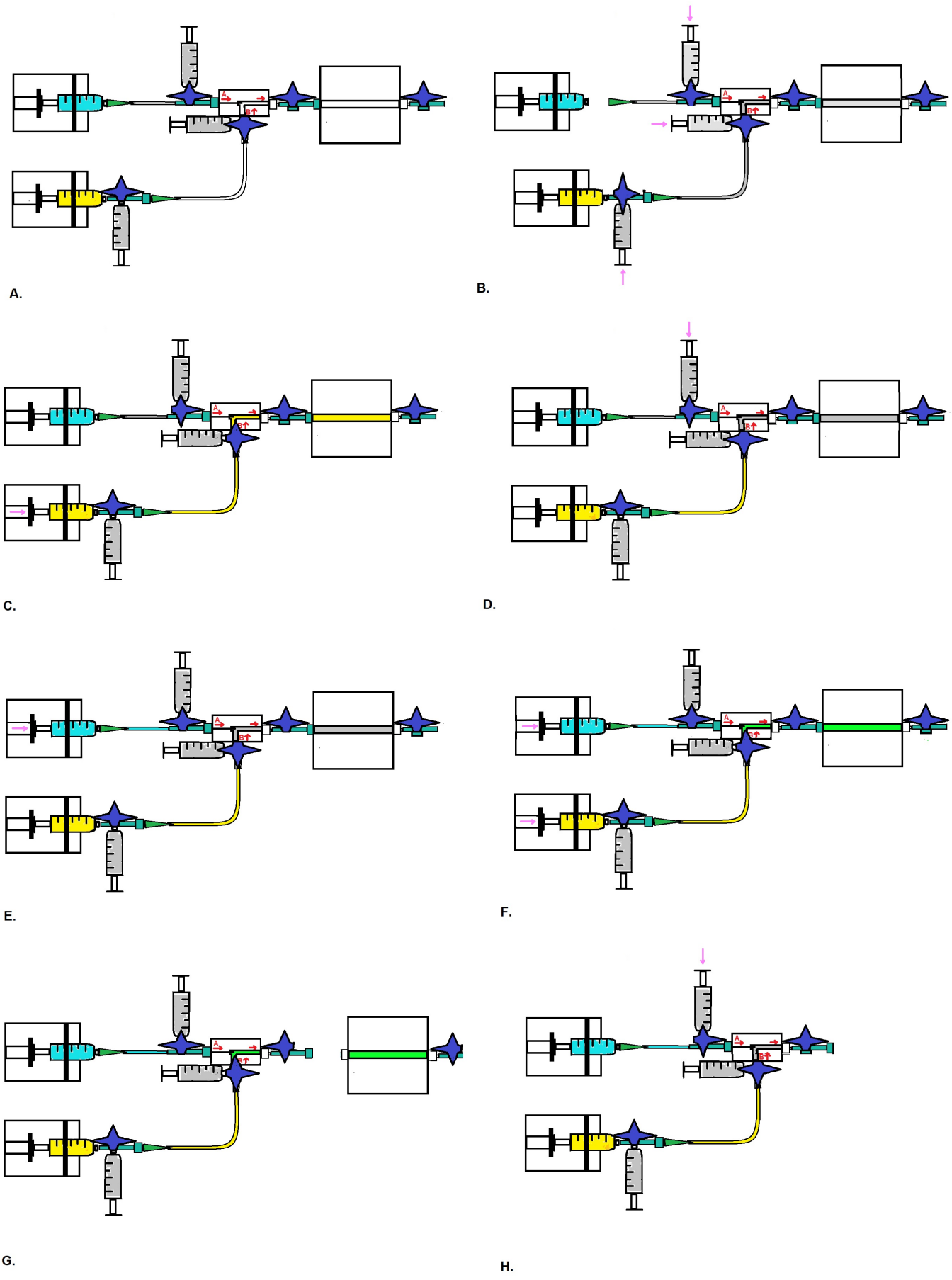


Figure 66. Schematic drawing of flow experiment (top view), the pink arrows indicate pushing of the syringes. For a full flow experiment do step A till H, for doing more samples attach the chamber again (also fill with buffer) and go to step F. **Note: do step G fast to prevent clocking of the parts. Always keep the microscopic glass with its top to the ceiling, don't tilt it to prevent shear on the fibrin (the structure is safe from shears when treated with formaldehyde).**

Actual experiment:

1. 500 μL of PPP endvolume, at the wanted shear rate.
2. 55.6 μL activator mix at the wanted shear rate.

13.6.2 Guide matlab codes

As a code written by someone else can be very difficult to use, some extra tips are written here.

13.6.2.1 Basics opening files:

1. Make sure that the folder in matlab is opened, were your ".m" file is located which you want to use. An example is shown in figure 68.
2. When you use a function, make sure this ".m" file is in the same folder as the ".m" main file were you use the function.
3. Remember to keep the file name of a function the same as how you call the function in the code.

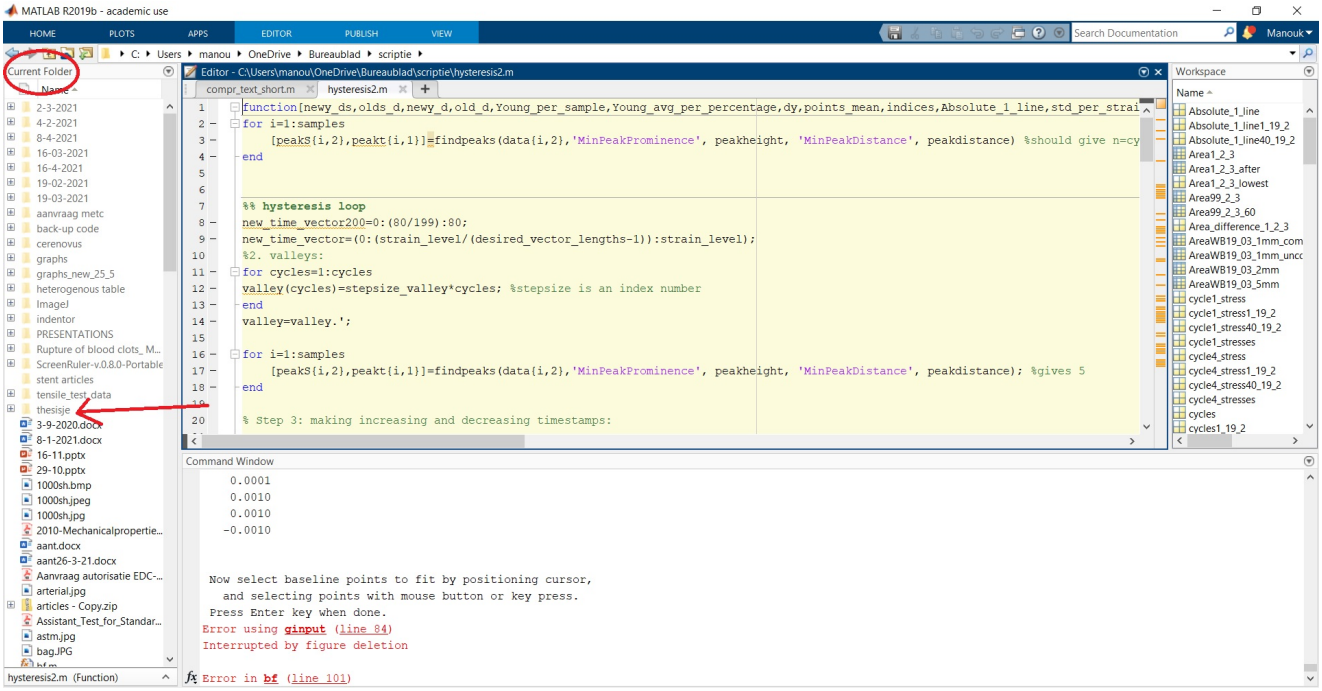


Figure 67. The red arrow indicates which folder is opened in matlab. For example the "hysteris2.m" in this example should be in this folder, else you cannot use it.

13.6.2.2 Basics using a function

The function "timefunc" is a nice example of a simple function. The input dt is generated in the main matlab file, and you want to change this data, and call the changed data timei.

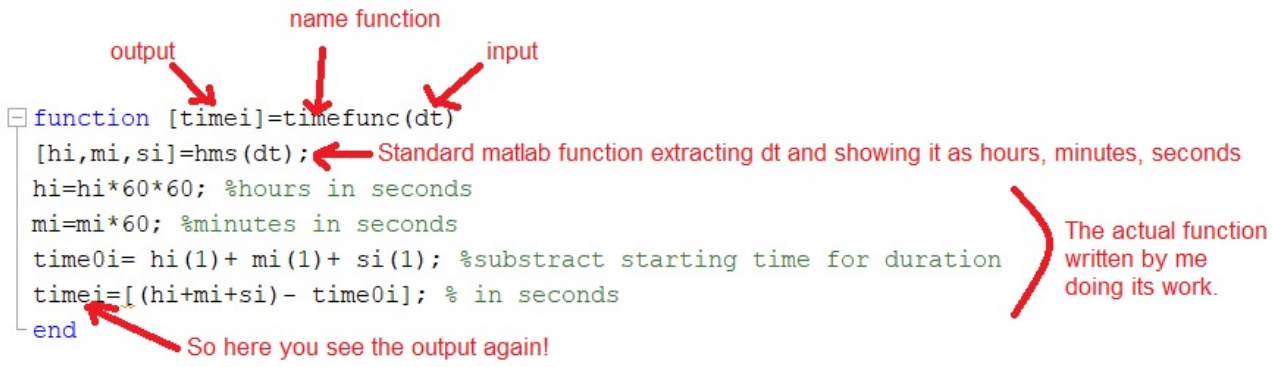


Figure 68. Some function qualities explained.[109]

13.6.2.3 Cylinder weight

This is a pretty straightforward code, simply doing a calculation for us when pressing "F5".

13.6.2.4 Baseline fit

This function corrects for baseline fluctuations.

1. Select some baseline points (where you see a deviation of the curvature), the crossing of these 2 black lines is the point of selection, really click on the blue line. If you are done press with selecting all points, press "enter".

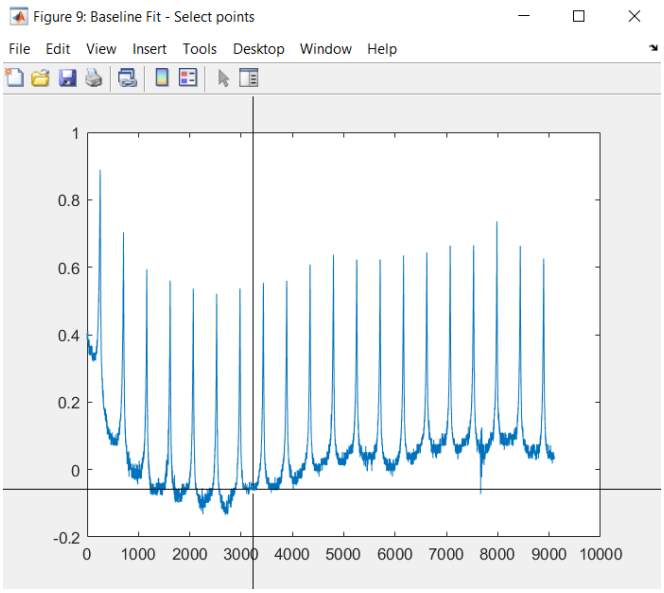


Figure 69. Click the baseline points.

2. Look if it has gone all right. The red line is the result, is this a good baseline? If yes press "Enter". Do you to redo fit and reselect baseline points? Press: "N"

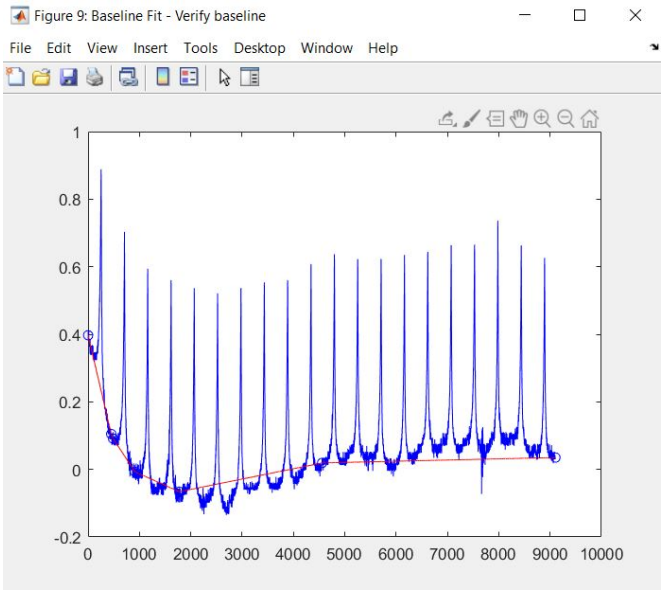


Figure 70. The red line is the result, is this a good baseline? If yes press "enter", if not press another key.

13.6.2.5 Timefunc

A function which can be used in other codes, when one wants to convert input of date with time to an output of only seconds, which can be used in a graph for example. The function is explained before in 13.6.2.2.

13.6.2.6 Ibidi sticky slide I 0.6 Luer example

Some tests were performed with the Ibidi system, shear rates can be calculated with this matlab code when using slide I 0.6 Luer. The formulas originate from the Ibidi website.[48]

13.6.2.7 Bloc shear II

Similar to the sticky slide I 0.6 Luer code, but then with the dimensions of the Bloc shear II.[48]

13.6.2.8 Activator mix and PPP amounts, and shear rates mixtures

This function explains some quantities of fluids which are needed for performing the experiments. It also gives the shear rates needed with certain dimensions of the flow chamber.[48]

13.6.3 Statistics with SPSS

13.6.3.1 Materials

1. IBM SPSS Statistics 28.0.0.0

13.6.3.2 Methods

General

Some general remarks about the data:

1. Dependant variables are the height, the mean average Young's moduli, Eff. Young's moduli.
2. Independant variables are things like used shear stress, sample point, and test group.
3. There are various variable specifications:

	Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure	Role
1	Young_mod	Numeric	6	5	Young's modul...	None	999.00000	8	Right	Scale	Input
2	Eff_Young	Numeric	6	5	Effective Young'...	None	999.00000	8	Right	Scale	Input
3	Test_group	Numeric	1	0	Test group	None	None	8	Right	Nominal	Input
4	Point	Numeric	8	0	Sample point	{1, A}...	None	8	Right	Nominal	Input
5	Shear	Numeric	3	0	Shear rate [/s]	None	None	8	Right	Scale	Input

Figure 71. Example of how variables are specified in the "Variable view tab".

Recode into categories

- Transform > recode into Different Variables
- Move desired variable in box
- Asign new name to new variable > click change
- Click old and new values
- Range, LOWEST through > enter upper bound lowest categorie > click value: "1" > add.
- Click range > specify bounds > value "2". Etc.
- Continue > ok > new variable is made
- Label the new variable
- Enter the new Value labels in the variable
- Change measure to ordinal

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How to get a subset of data into a separate data set

1. Data > select cases > if condition is satisfied
2. Double click the variable which can differentiate > "=.."
3. Click> Select: If condition is satisfied > Output: Copy selected cases to a new dataset
4. Safe as > new dataset

111

Normal distribution test

1. Analyze > descriptive statistics > explore > dependant variable in dependant box > plots: histogram and normality plots with tests.
2. Look at the Kolmogorov-Smirnov and Shapiro-Wilk test: p > 0.05 then normal distributed, p < 0.05 then not normal distributed. The Shapiro-Wilk is more valid.

[112]

One-way Anova

Applicable on quantitative data in 3 or more samples.

1. Analyze > Compare means > one-way ANOVA
2. Select the boxes in the option menu of figure 73. The Welch or Brown-Forsythe test is needed for unequal group sizes.
3. If the "Test of Homogeneity of variances" p> 0.05 than ANOVA can be used (there is homogeinity of variances), and you can go to step 5.
4. If "Test of Homogeneity of variances" is less than 0.05 (there is heterogeinity of variances), continu to the "Brown-Forsythe" or "Welch" test: this should be less than 0.05. If yes, you can proceed to step 5. If no, then ANOVA is not significant (although it can be less than 0.05 rejecting the 0 hypothesis in spss, this would give a type 1 error), there is no statistical difference in means.
5. If the ANOVA, "Brown-Forsythe" and/or "Welch" are significant, and you can say that there is a statistical difference, but you need to do a post-hoc test to see where this is the case. If there is no statistical difference, there is no point in doing a post-hoc test.
6. Post hoc: when there are equal variances, with similar sample sizes with a small amount of comparisons, use the "Bonferonni" test, with a large amount use the "Tukey" test.[113] The best for the specific analysis is the one with the smallest confidence interval.[114] If there is an unequal amount of samples use (slightly) use "Gabriel", if there is a big difference in sample amounts use "Hochberg's GT2".[113]

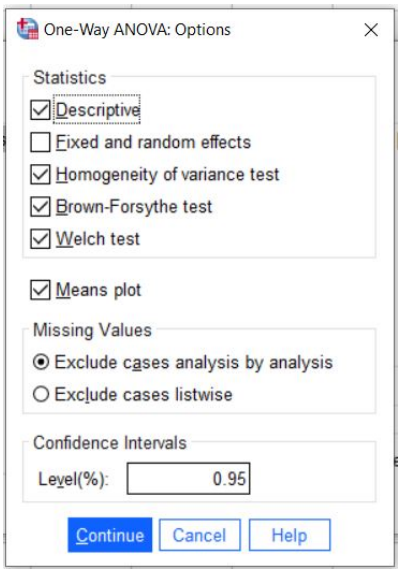

Figure 72. Select these boxes in the option menu.

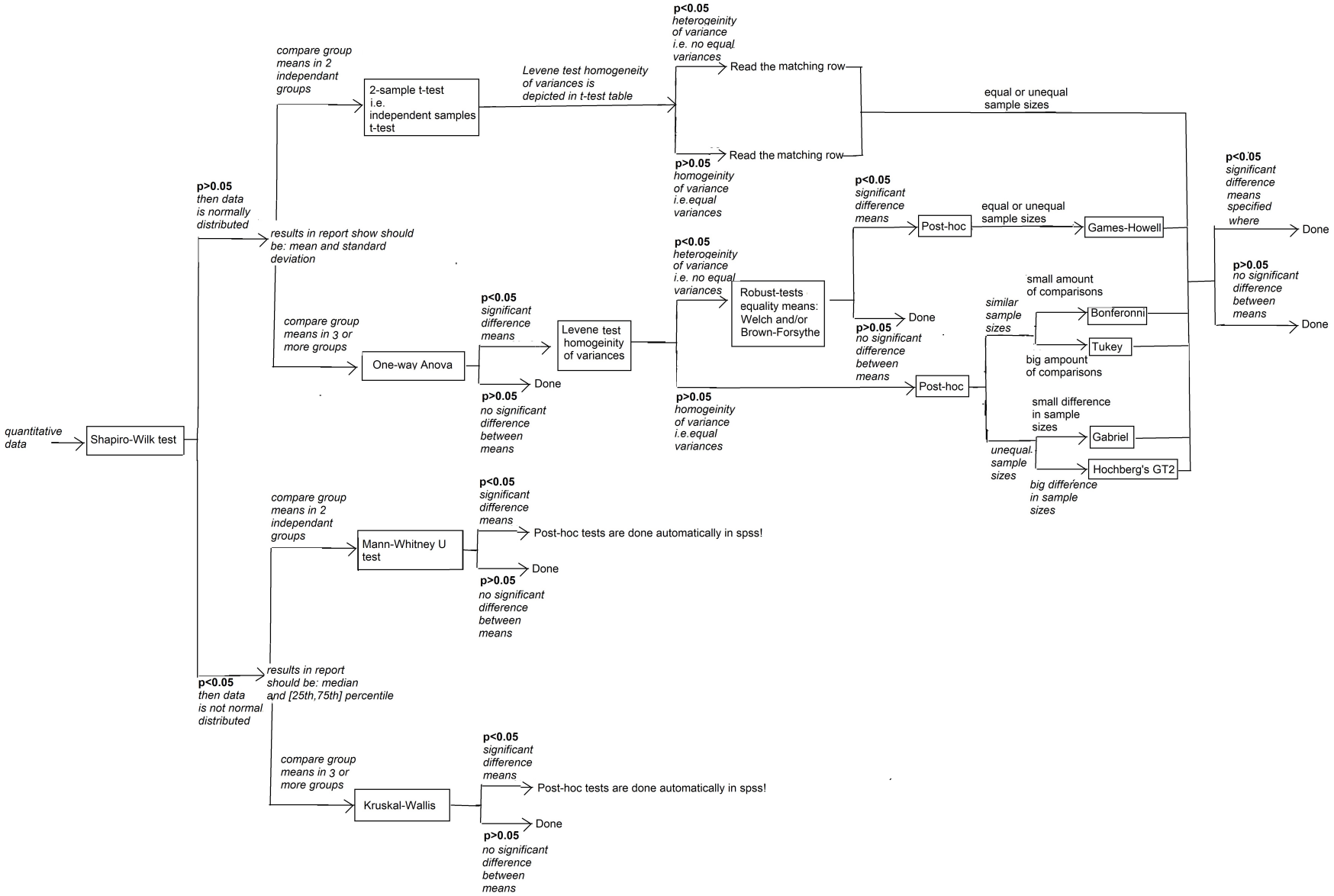

Figure 73. Summarizing the used tests in spss.

Power analysis:

1. Analyze > General linear model > Univariate.
2. Insert the dependent variable, and the "fixed factors" (a.k.a. the independant variable).
3. Options > check the boxes: Descriptive statistics, estimate of effect size, and observed power.
4. Click "ok".

Fiber orientation graphs

1. Graphs > chart builder >
2. Choose: mean (drag it in)
3. Choose: x-axis > fiber orientation (let the y-axis empty to just get a count at the y-axis)
4. On the right > click box: show error bars > choose mean > click on sd > then unclick the box show error bars
5. Then change mean (is default) > to percentage > thick the box show error bars again
6. Click on ok