Monitoring the Evaporation Process of Liquid Samples in Sub-Nanoliter Vials

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Abstract

One would expect that the evaporation process of liquid droplets was described many years ago. On the contrary, just recently some underlying principles of the dynamic process of evaporation have been published. It seems that during the evaporation of a liquid droplet, the liquid at the edge of the droplet is "pinned" to the underlying surface. This pinning prevents the droplet from shrinking. This implies that the footprint of the droplet remains constant. The evaporated liquid at the edge is replenished by liquid from the bulk of the droplet. This means that there is a flow of liquid moving towards the edge of the droplet. If the liquid droplet contains particles, like in a droplet of coffee, these particles will be transported outwards by the flow. Finally, when all the liquid has evaporated, the (coffee) particles will form a ring of stain.

The phenomena described above have also been monitored in the sub-nanoliter vials of a microarray. The behavior of the liquid inside the vials during evaporation differs a lot from the behavior of an evaporating bulk solution. We have monitored this small scale evaporation process with a wide-field epi-illumination microscope system and with a confocal scanning laser microscope system. With a conventional wide-field microscope it is only possible to monitor the projection of the liquid in the vial during evaporation, *i.e.* it is an *indirect* measurement of the shape of the meniscus, which is the interface between the air and the solution. On the other hand, with a confocal microscope system it is possible to follow the evaporation as a function of time: this is a *direct* measurement of the liquid behavior. The goal of these experiments was to monitor the shape of the meniscus during evaporation. With these measurements it is possible to validate numerical simulations of the evaporation process. When experimental results and simulation results agree, it is interesting to investigate which geometries of the vial extends the evaporation process as long as possible.

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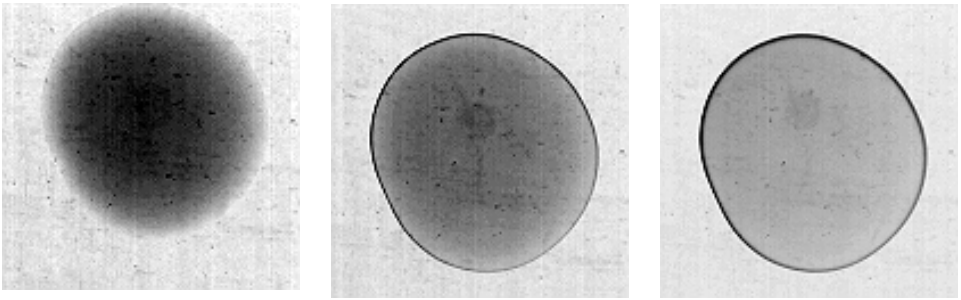
1 Introduction

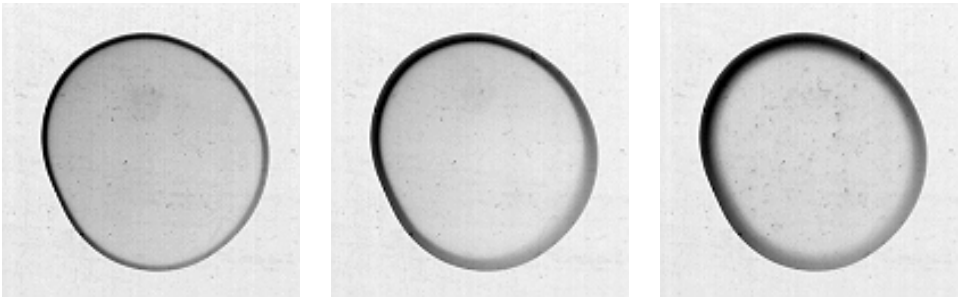
A droplet of coffee, that dries on an ordinary surface leaves a ring of stain along the perimeter of the droplet [1]. The drying of a coffee droplet is shown in Figure 1. Initially, the coffee particles were homogeneously distributed in the droplet, but during evaporation they become concentrated at the edge. From qualitative observations, it follows that rings of stain are formed for a variety of dispensed materials (solutes) and carrier liquids (solvents) as long as

- there is a non-zero contact angle between the solvent and the surface,
- the contact line between the solvent and the surface is "pinned" to its initial position, and
- the solvent evaporates.

In a *static* situation, without evaporation, the contact angle between the solvent and the surface on which the droplet lies, is determined by the balance between the surface tension of the solvent, which tries to pull the droplet into a sphere, and the adhesive forces, which try to stick the liquid to the surface. If the droplet lies on a perfectly flat and smooth surface, the edges of an *evaporating* droplet have to move inwards to maintain the required contact angle. But real surfaces are *not* that smooth. If the surface is just a little bit rough, when the edge loses some liquid, it only has



(a) t=0 min (b) t=10 min (c) t=20 min



(d) t=30 min (e) t=40 min (f) t=50 min

Figure 1: This figure shows six images of a drying coffee droplet. It can be seen the the droplet is pinned to its position, and that it leaves a ring-like stain after drying.

to move a little bit inward to find a position, where the roughness of the surface gives it the right contact angle again. This way, it moves so slowly inwards that it is effectively pinned in place. As the solvent is evaporating at the edge, the outward flow of the solvent with particles will deposite the particles at the edge, which make the surface even rougher. As a result the edge is even more firmly pinned.

Similar phenomena have been observed in the sub-nanoliter vials of a microarray. These observations will be described in Section 3.1. These microarrays have been fabricated within the frame of an inter-faculty research program *Intelligent Molecular Diagnostic Systems*. The goal of this program is to develop analytical systems that can analyze liquid samples that contain a variety of biochemical compounds, such as those associated with fermentation processes. The microarrays are fabricated at DIMES (Delft Institute for Microelectronics and Submicron Technology, Delft University of Technology, NL-2628 CD Delft, the Netherlands). These microarrays contain small vials with typical dimensions of $200 \times 200 \times 20\ \mu\mathrm{m}^3$. This translates of a volume of $\sim 0.8\,\mathrm{nl}$. The vials are fabricated in a wet etching process, resulting in the shape of a truncated pyramid. The fabrication process is described in detail in [2]. The shape of this vial is shown in Figure 2. The angle between the bottom and the sidewalls of the vial is 125.3 degrees.

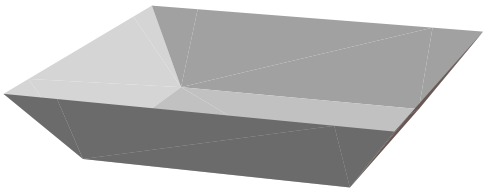


Figure 2: A $200 \times 200 \times 20\ \mu\mathrm{m}^3$ wet etched square vial. The shape of the vial is a truncated pyramid.

To deliver sub-nanoliter volumes into the vials, a special but commercially available device, the Eppendorf Transjector 5246 (Eppendorf Netheler-Hinz GmbH, 22331 Hamburg, GE) is used. This apparatus is primarily used for *in-vitro fertilization* (IVF). With the IVF technique sperm cells are injected through the cell membrane into an oval cell.

Several difficulties arise when trying to fill the vials:

- **Viscosity of the liquid.** If one would try to pipette $1\ \mathrm{nl}$ of water into such a small vial, this droplet of water would evaporate within a few

seconds. In order to extend the process of evaporation, solvents with a higher viscosity are used. The viscosity is directly related to the boiling point of the solvent: the higher the viscosity, the higher the boiling point, the lower the evaporation rate. With glycerol-water (1:1, v/v) as solvent, the evaporation process is extended to more than one hour. This mixture has a viscosity of $8.3\,\eta_0$, where η_0 is the viscosity of water. The higher viscosity lowers the evaporation rate, but it has the disadvantage that the filling of the vials gets more difficult: the higher viscosity results in a higher surface tension. This means that the meniscus of the liquid is not flat, but has a more or less spherical shape. This can easily be seen under a microscope. In order to flatten the meniscus a detergent is added to the solvent. This lowers the surface tension. The shape of the meniscus will be shown in Section 3.2.

- **Touching the bottom of the vials.** In order to release the subnanoliter droplet from the tip of the device, the tip must touch the bottom of the vial. If the tip does not touch the bottom of the vial, the drop will stick to the tip, because of the high surface tension.

- **Filling the vial completely.** It turns out from experiments that the *only* reproducible filling method is to fill the vial completely. Trying to fill a vial partially to a certain height, like in a big test tube, is *not* possible: the liquid does not spread out in the vial. On the contrary, the droplet ends up in one of the corners, for example. When filling the vial completely, it seems that the top border of the vial acts as a *pinning edge* for the liquid [1]: during the filling of the vial the liquid touches the sidewalls of the vial, but as soon as it reaches the borders of the sidewalls, the liquid pins at the edge until the vial is completely filled and the liquid is pinned to the entire border of the vial. This situation seems to be a kind of equilibrium state. In this state, there is a certain contact angle between the meniscus of the liquid and the sidewall of the vial. Trying to inject more liquid into the vial, which means that the vial overflows, requires that the contact angle has to make an abrupt change (54.7 degrees), because this angle is then between the *toplayer* of the microarray and the mensicus of the liquid. This equilibrium state makes it possible to reproducibly *(and manually)* refill the vial with a coefficient-of-variation of $5.9\ \%$ [2]. This filling error is measured with solutions that contain fluorescent molecules. The fluorescent signal is directly proportional to the amount of liquid in the vial, under the assumption that the solution is homogeneous.

In our experiments we have investigated different solvents: a mixture of glycerol and water (1:1, v/v) with a viscosity of $8.3\,\eta_0$, and mixtures of ethyleneglycol and water in different ratios. These mixtures have a viscosity of about $5\,\eta_0$. Due to the lower viscosity the evaporation process is faster in the latter cases: a $1\,\mathrm{nl}$ volume of one of these mixtures evaporates within half an hour. This evaporation process is monitored with two different microscope systems: a wide-field epi-illumination microscope system and a confocal scanning laser microscope system. With a conventional wide-field microscope system the vial is projected onto the CCD camera. With this approach the monitored process is an *indirect* measurement of the behavior of the liquid during evaporation. The 3D liquid volume is projected onto a 2D sensor. This means that there is no height information anymore. As a result, it is not possible to predict (in a simple way) the shape of the meniscus of the remaining liquid in the vial. On the other hand, it is possible to monitor this evaporation process in a direct manner. With a confocal microscope system it is possible to scan a volume (by scanning a stack of z-slices) and to get the 3D information in this way. This 3D volume can be monitored in time. One only has to distinguish between voxels with or without the fluorescent signal to know where the interface between the air and the liquid is at a certain moment. The method to distinguish between liquid and background will be discussed in Section 3.2.

2 Materials

The evaporation process of the solutions was first monitored with a conventional microscope system. This system is built around a Zeiss Axioskop. A scientific CCD camera, a KAF 1400 Photometrics Series 200 CCD camera, is mounted on this microscope via a $1.0\times$ camera mount. The CCD element contains 1317×1035 pixels with a pixel size of $6.8 \times 6.8\,\mu\mathrm{m}^2$. In our project we aim at wide Field-of-View imaging, *i.e.* we want to image the entire microarray onto the CCD array. Therefore we use low magnification optics: a $2.5\times\ /\ 0.075$ Zeiss Plan-NEOFLUAR objective. With this objective it is possible to image an area of $3.5 \times 2.7\,\mathrm{mm}^2$ onto the CCD array. Given the dimensions of the vials and a center-to-center distance of $300\,\mu\mathrm{m}$, it is possible to read out 100 vials per image. Wide field-of-view imaging with low magnification optics, however, is at the expense of absolute sensitivity. The reason is that the light-gathering power of an objective lens is proportional to the square of the NA: the lower the NA, the less efficient photons can be collected [3]. In order to detect a sufficiently strong signal, we used Rhodamine solutions with a concentration of $10.0\,\mu\mathrm{Molar}$.

As said before, a conventional wide-field micro-

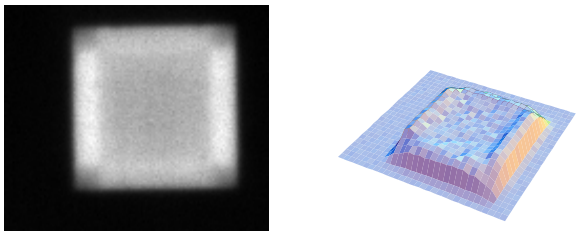
scope system does not give depth information about the shape of the meniscus during evaporation. This information can be obtained with a confocal microscope system. Only the light, that is emitted from a small confocal volume in the in-focus plane, reaches the detector. Out-of-fous light is blocked by a pinhole in front of the detector. For these experiments we have used a confocal microscope system that was built around a Zeiss Axiovert inverted microscope. Attached to this microscope was a confocal scanning unit, a Bio-Rad MRC 1000.

3 Results

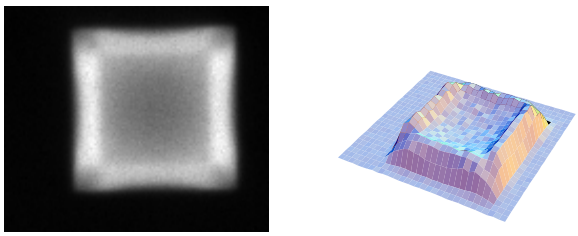
In this section we will show the results of a number of experiments. In Section 3.1, the results of the experiments on a conventional wide-field microscope system will be shown, and in Section 3.2 the results of the experiments performed on the confocal microscope system.

3.1 Conventional Microscope System

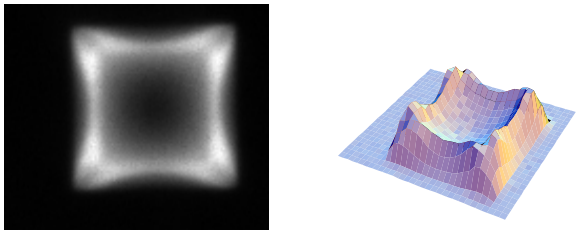
To monitor the evaporation process we have used mixtures of ethyleneglycol and water in different ratios. These mixtures have a viscosity of about $5\eta_0$. Once a vial is filled with this solvent, it evaporates in approximately 20 minutes. We monitored this process by acquiring an image every 30 seconds. Five images of this process are shown in Figure 3. At $\mathrm{t} = 0$, just after injection of the liquid, the signal from the vial is uniform, besides some geometrical effects along the sidewalls and in the corners of the vial. This implies that the meniscus of the liquid is virtually flat and the vial is completely filled. After filling the vial, the liquid is "pinned" to the edge of the vial. During evaporation, the pinning of the liquid ensures that the evaporation from the edge is replenished by liquid from the center part of the liquid in the vial [1]. This can be seen qualitatively in Figure 3: the fluorescent signal gets weaker in the center, until the bottom of the vial is reached. The signal disappears from that area some time between 10 and 15 minutes. While the evaporation continues, the liquid is stuck in the four corners of the vial. It looks that the liquid is no longer pinned at the edges of the sidewalls, but in the next section we will show that it is still the case. The concentration of fluorescing particles is increasing at the corners of the vial. Finally, when the solvent is completely evaporated, the particles loose their fluorescent behavior and the signal disappears. In Figure 4 the average fluorescence signal from the vial and the signals from the center, from one of the sidewalls and from one of the corners of the vial is shown as a function of time. It can be seen in this graph that the average fluorescence signal from the vial remains almost constant during evaporation. The slight increase of the signal in the beginning is caused by geometrical effects. These effects are less than 10% of the signal. Furthermore, it can be seen that the signal in the corners of the vial increases steadily, because of the accumulation of the fluorescent particles at these spots.



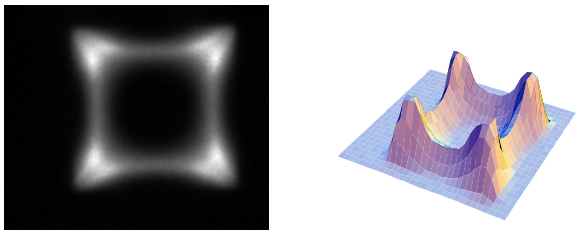
(a) $\mathrm{t} = 0$ min.



(b) $\mathrm{t} = 5$ min.



(c) $\mathrm{t} = 10$ min.



(d) $\mathrm{t} = 15$ min.

Figure 3: A series of images showing the evaporation process of ethyleneglycol-water $(90\%/10\%, v/v)$ in a vial at different times. Note that the images show the fluorescent intensity profile, *not* the shape of the meniscus.

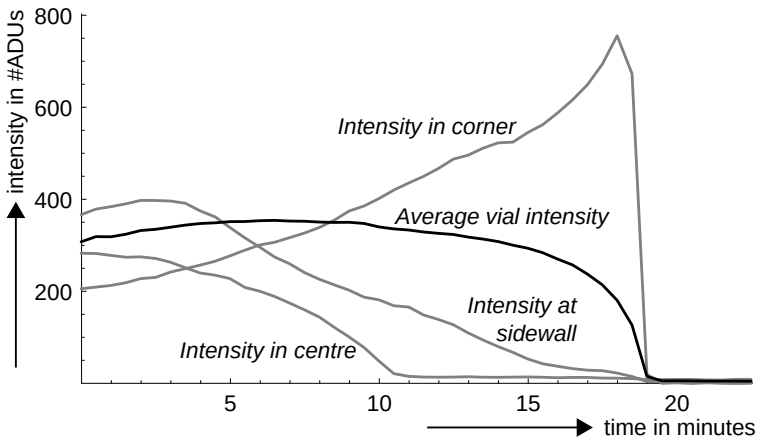


Figure 4: The average fluorescence signal from the vial, the signals from the center of the vial, the sidewalls of the vial and the corner of the vial as a function of time. It can be seen that, besides some geometrical effects, the average signal of the vial does not decrease during the evaporation process.

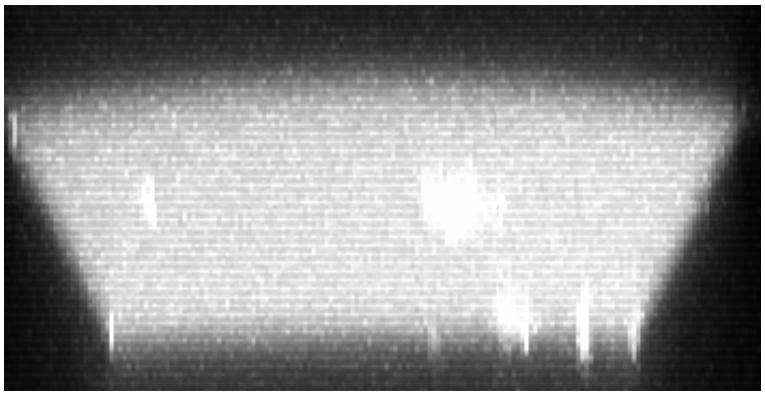


Figure 5: A vertical cross-section through the vial filled with a solution of rhodamine.

3.2 Confocal Microscope System

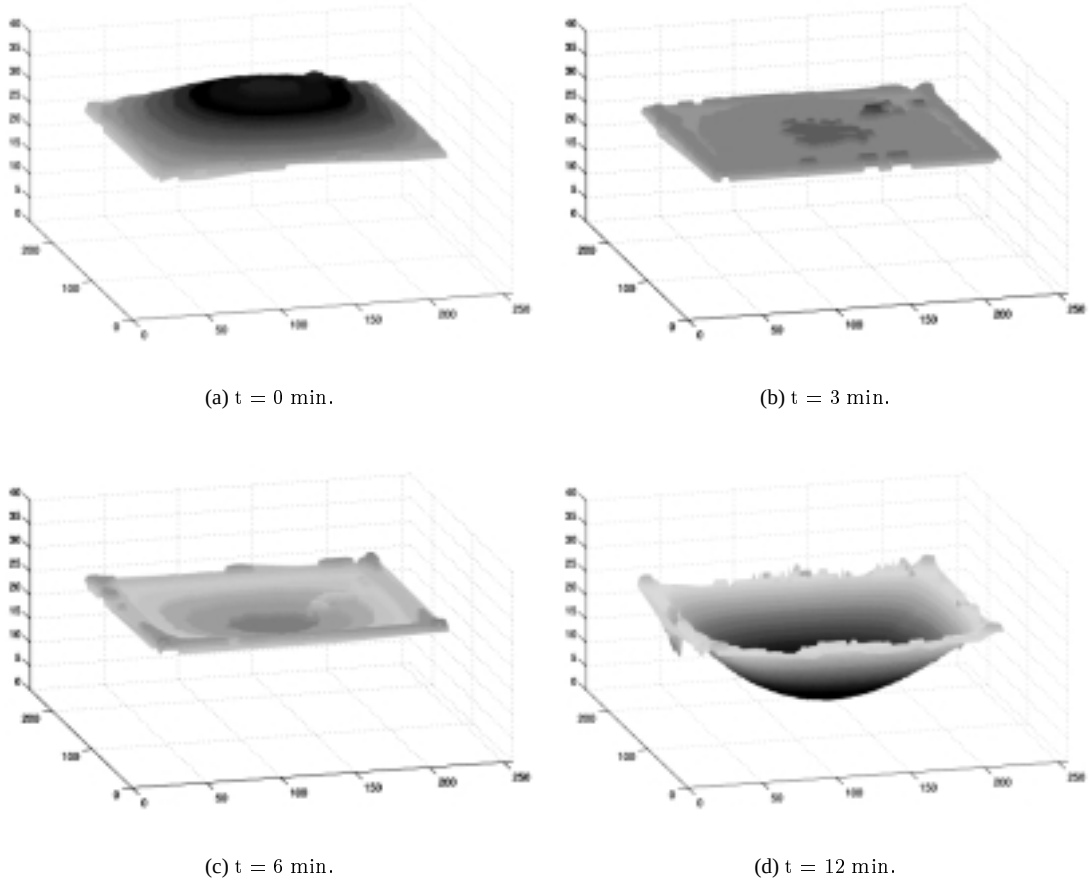
The major drawback of the experiments with a conventional microscope system is the fact that the 3D information is projected onto a 2D detector. This drawback can be overcome by performing the same experiments on a confocal microscope system. The goal of the first experiment on a confocal microscope system was to measure the shape of the meniscus right after injection of the liquid. For this experiment we used a solution with glycerol-water as solvent (Rhodamine concentration 10^{-7} M). A cross-section through the liquid in the vial is shown in Figure 5. It can be seen in this figure that the meniscus in not flat, but has a more or less spherical shape. Furthermore, it is clearly visible that the sidewalls are at an angle with respect to the bottom. This can also be seen in Figure 2. The liquid is pinned to the edge of the vial.

In the second experiment we monitored the behavior of the meniscus during evaporation of the liquid. A number of 3D scans with an imagesize of $256 \times 256 \times 50$ were acquired. At this moment we are only interested in visualizing the data with the meniscus. To extract this data, the following steps were performed:

- First, the gradient magnitude is computed by Gaussian derivatives of size $\sigma = 2.5$ Note that during evaporation, the concentration of the fluorescent particles increases. This results in a higher signal-to-noise ratio.

- The second step is to segment the gradient image by simple thesholding. This results in a hollow object. The object itself consists of the bottom and the sidewalls of the vial and the meniscus of the liquid.

- From this thick object the Euclidean skeleton is computed. This results in a one voxel thick object. From this object, the meniscus is selected.



(a) $t = 0$ min. (b) $t = 3$ min.

(c) $t = 6$ min. (d) $t = 12$ min.

Figure 6: These figures show the shape of the meniscus of the liquid during evaporation. The edge of the meniscus connects to the border of the vial. The dimensions along the axis are voxel coordinates.

Note that the xy-coordinates do not fill a rectangular grid.

- In order to visualize the datapoints of the meniscus, the Delaunay triangulation is computed, and, given this triangulation, it is possible to visualize the meniscus.

In Figure 6 four visualizations of the meniscus are shown. Figure 6(a) is acquired right after injection of the liquid sample in the vial. It can be seen in this figure that the shape of the meniscus is convex. This can also be seen in Figure 5. During evaporation, the meniscus first becomes flat and then concave as can be seen in Figures 6(b) to 6(d). Again, it can be seen that the liquid is pinned to the edge of the vial, and that the liquid first evaporates from the centepart of the vial.

From the measurements of the confocal arrangement it is straightforward to compute the liquid volume in the vial as a function of time by simple thresholding. The result of this experiment is shown in Figure 7. The evaporation speed is the decrease in liquid volume per unit of time. The evaporation speed is equal to the slope of the line in Figure 7. It can be seen in this graph that the evaporation speed is almost constant, but decreases during the evaporation process.

4 Conclusions and Discussion

In this paper we have presented the results of some experiments to monitor the evaporation process of liquid samples in sub-nanoliter vials. It seems that one of the underlying principles of evaporation is the pinning of the liquid to the surface, as can be easily demonstrated with a droplet of coffee. The experiments were performed on a conventional microscope system. The disadvantage of this setup is the fact that the 3D volume of the vial is projected onto a CCD camera. From these experiments we could only give a qualitative description of the evaporation process. The same experiments have also been performed on a confocal microscope system, from which we can derive quantitative results. From these results we could see that the shape of the meniscus after injection is convex and that the

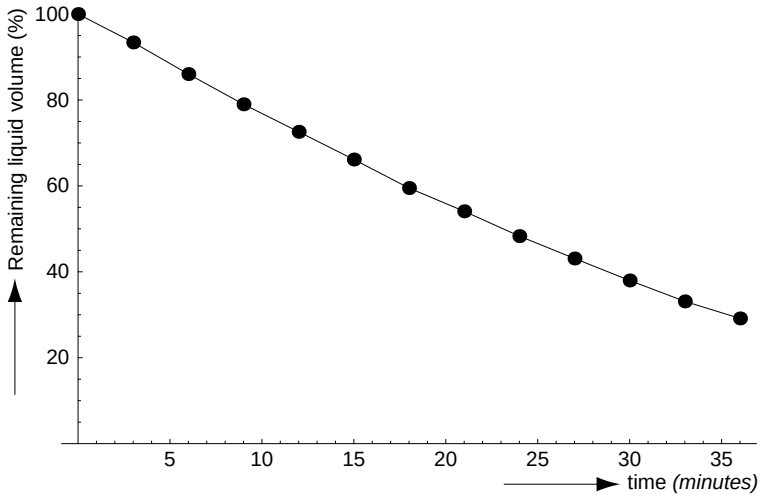


Figure 7: This figure shows the remaining liquid volume in the vial as a function of time. The evaporation speed is the slope of the line, which is almost constant, but decreases during evaporation.

liquid is pinned to the edge during evaporation. Because the menicus is pinned and the liquid volume decreases during evaporation, the shape of the mensiscus has to adjust from a convex shape via a flat shape to a concave shape.

Our future work with evaporation will focus on a number of things:

- Combining theory, experiments and simulations to get more insight in the phenomenon itself. This will be a joint research project with the Microsystems Simulations Group of the University of Freiburg.

- In the results of the experiments with the conventional micropsce system, we mentioned that the signal from the vial is uniform besides some geometrical effects. The question is now: is it possible to model the image acquisition, *i.e.* photon counting, and explain the geometrical effects, with a conventional microscope system, given the mensicus and the geometry of the vial? This would give insight in the question what would be the ideal shape of the vial for image acquisition.

- One of the observations is that the fluorescent particles end up in the corners of the vial. However, when all the solvent has evaporated, the particles loose their fluorescent behavior. If the particles would still be fluorescent, then the signal-to-noise ratio would increase, because the concentration increases and we could also apply dimension reduction: instead of measuring over a ”big” volume, it would only be necessary to measure at certain spots.

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