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Publication date

2021

Document Version

Final published version

Citation (APA)

Rebyn, N., Hu, M., Frimat, J.-P., van den Maagdenberg, A. M. J. M., Sarro, P. M., & Mastrangeli, M. (2021). *Recording 3D neuronal activity on chip with segmented 3D microelectrode arrays*. Poster session presented at European Organ-on-Chip Society Conference 2021.

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Recording 3D neuronal activity on chip with segmented 3D microelectrode arrays

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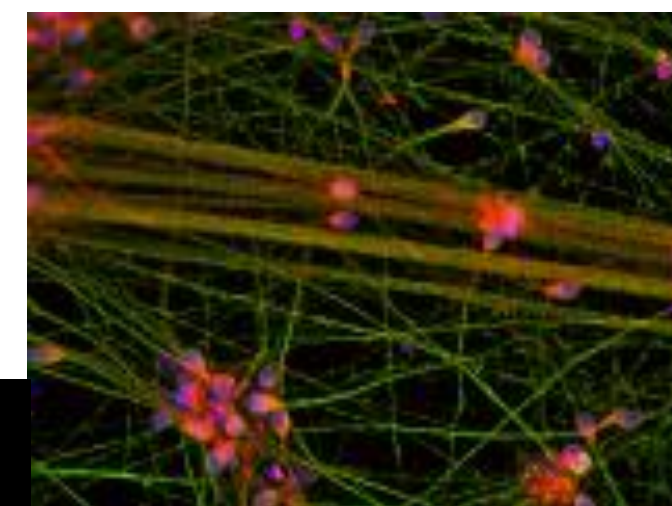
Motivation and goal

- On-chip spatial recording of distributed neuronal activity is required for in-vitro investigation of pathologies such as migraine [1,2].
- We present a 3D microelectrode array chip for neuronal activity recording along all spatial directions. This chip is fabricated by wafer-level Si-based processing, and can be seamlessly integrated with commercially available readout platforms [3].
- We validated the 3D MEA functionality with preliminary recording of neuronal activity from human-induced pluripotent stem cells (hiPSCs).

Culturing

Cortical neurons derived from hiPSCs were differentiated and matured on the 3DMEA following the provider protocol (Stemcell Tech-nologies). We measured the cultures up until 25 days in vitro (DIV) and used a custom software toolbox to analyze the data to assess the condition of the 3D neuronal cultures.

hiPSC derived cortical neurons
green : beta tubulin, red : synaptophysin, blue : cell nucleus

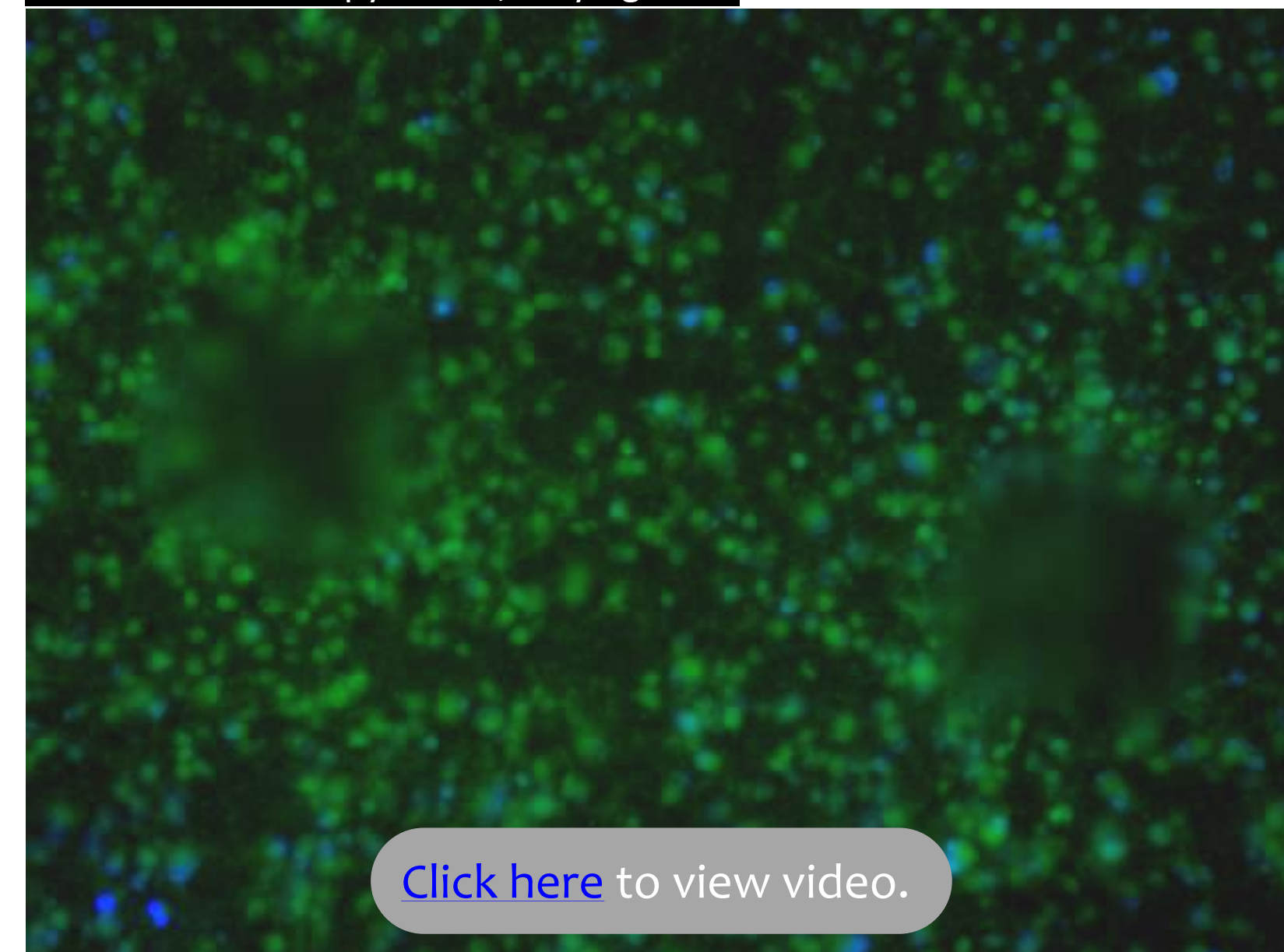


Preliminary results

Neuron growth on pyramids (video):

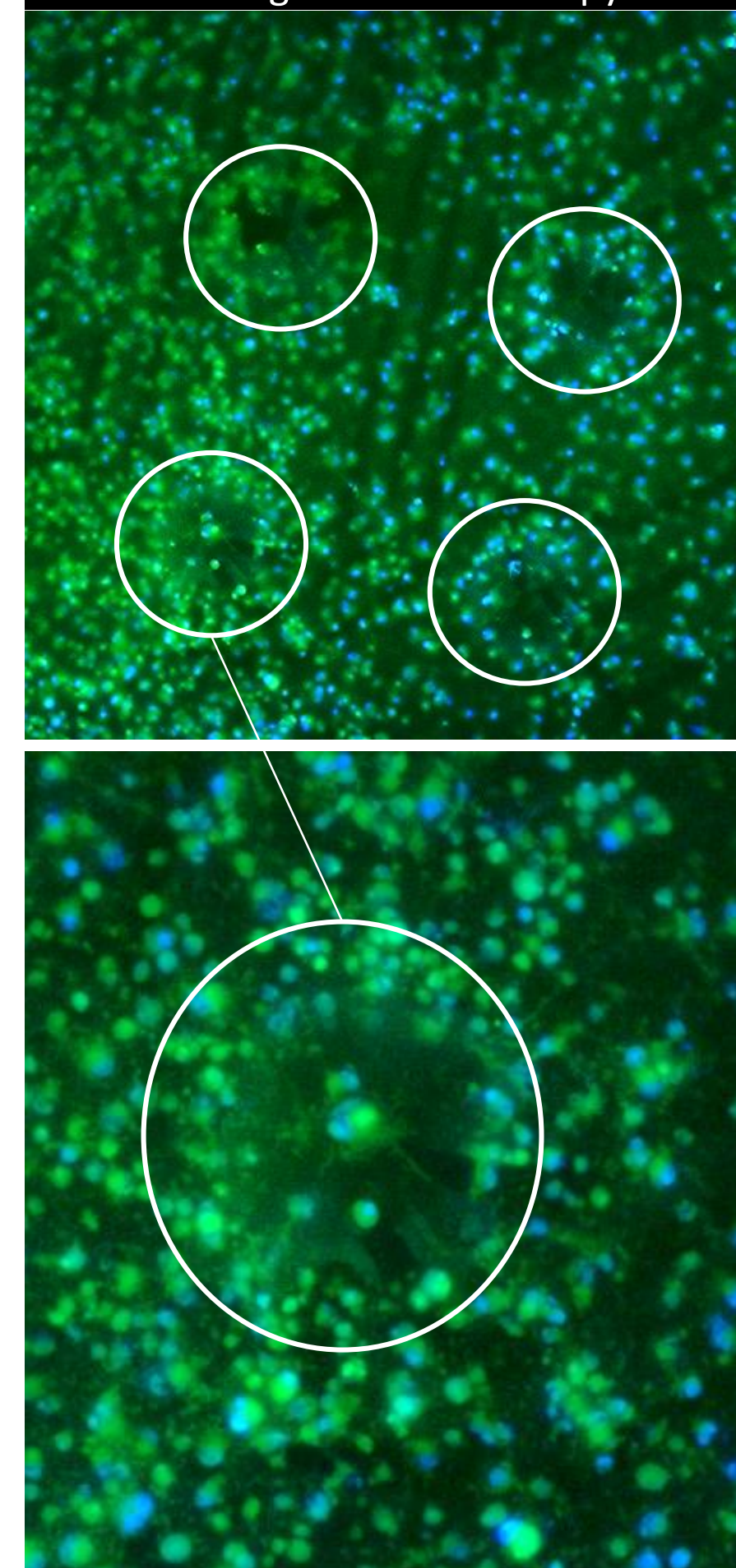
- Most neurons stay on the flat surface
- Some growth on the slopes
- One neuron sits on top

Video: neurons on pyramids, varying focus



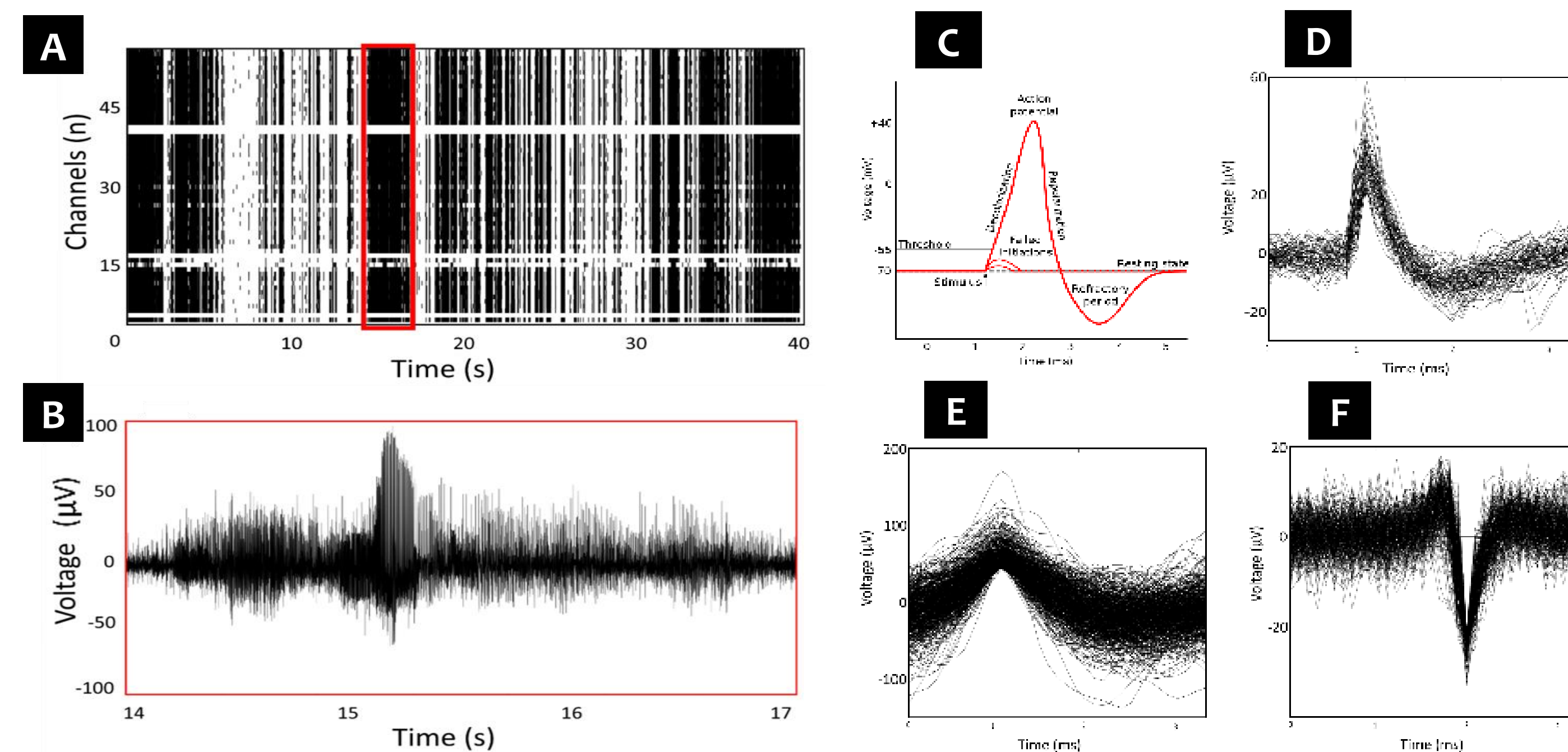
[Click here](#) to view video.

Full focus image of neurons on pyramids



Recording:

- Clear network bursting activity (A, B)
- Zoom on single spike (D):
 - Looks like intracellular waveform (C), but not exactly, as there is noise (D)
 - Comparison with spike waveform on commercial MEA with 60 electrodes (E)
 - Comparison with spike waveform on commercial 24 multiwell MEA, reported as single-unit activity for extracellular measurements in animals (F)



Fabrication

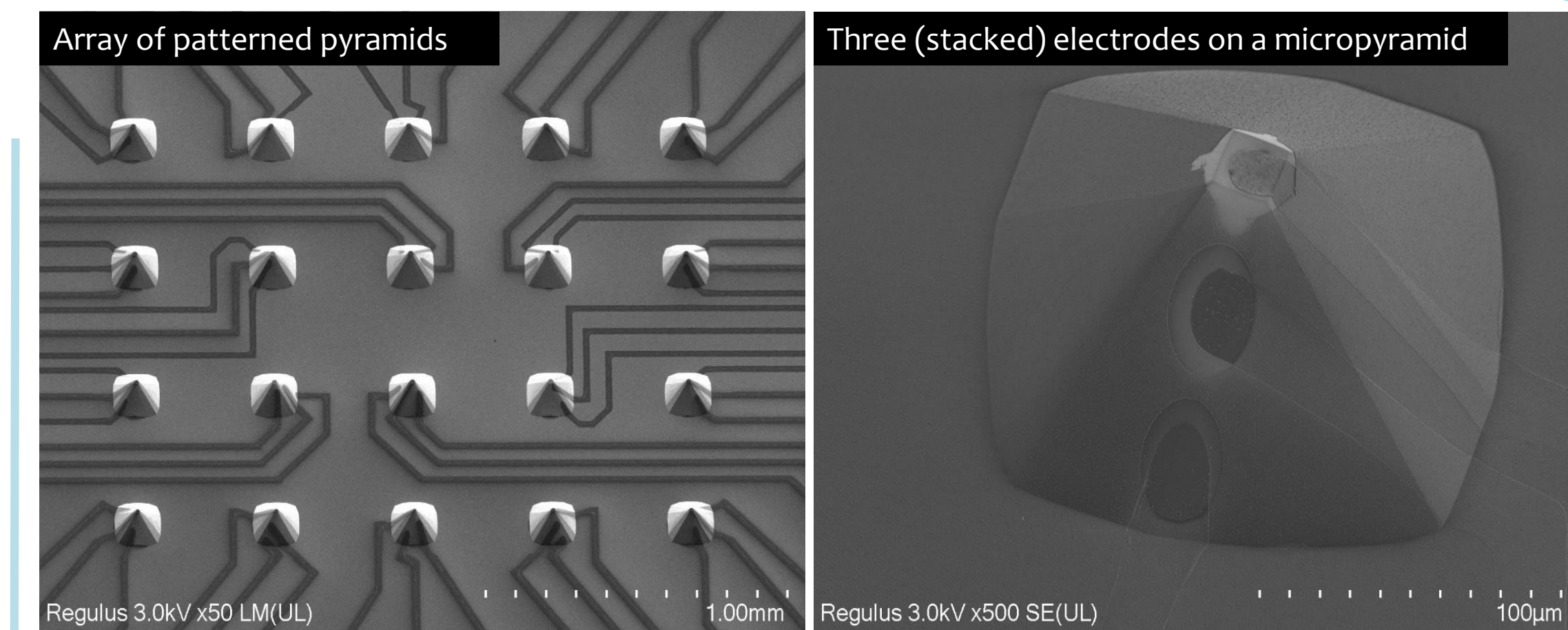
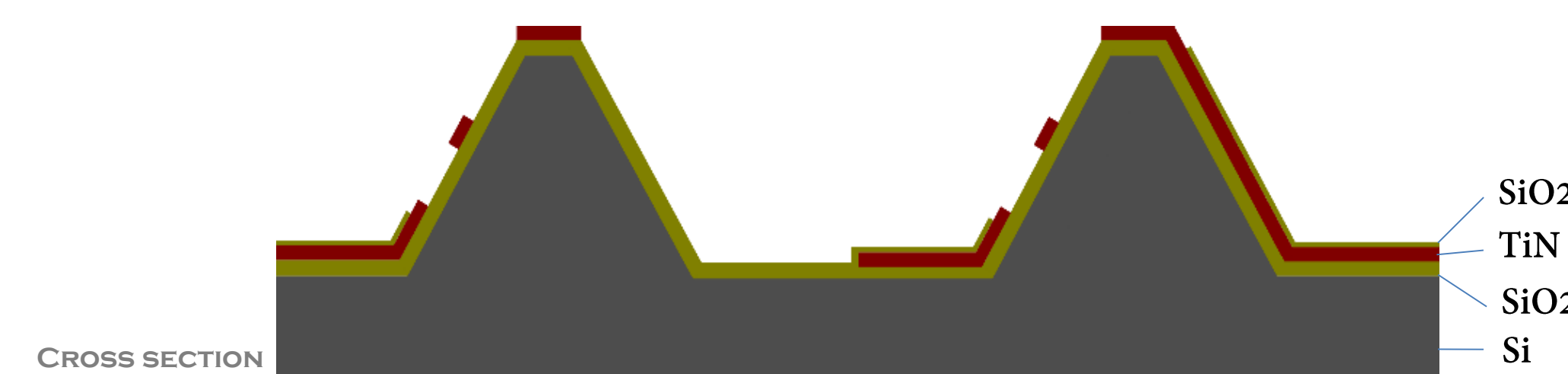
We fabricated 5-by-4 arrays of truncated Si micropylamids:

- ~90 µm-high
- ~20 µm-wide plateau
- ~46° to ~53° lateral facet slope
- 3 independent TiN electrodes on each micropylamid

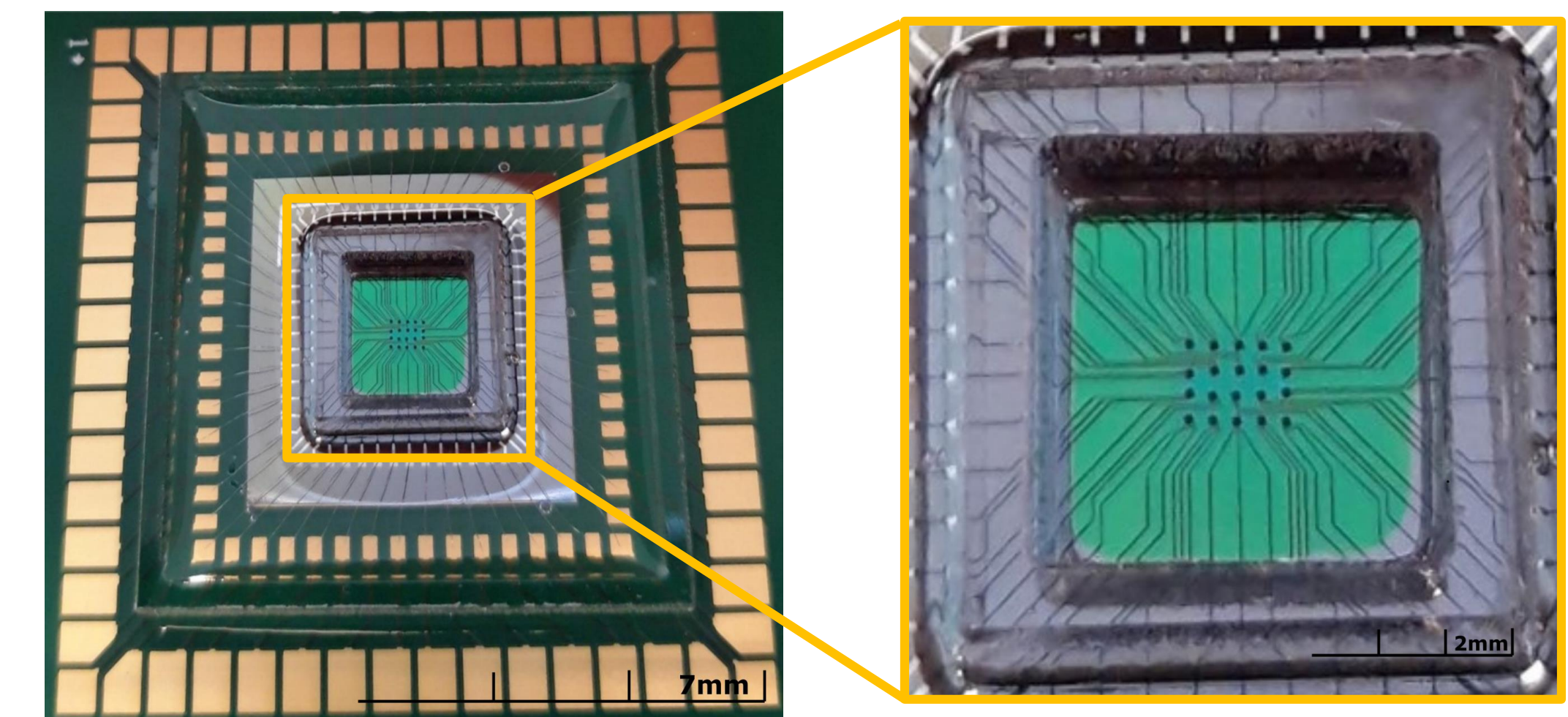
1. Timed anisotropic wet etching (25% TMAH + Triton) on 4" Si wafer through hard mask



2. Passivation with SiO₂, and patterning with a 10nm/150nm-thick Ti/TiN layer defines the microelectrodes. A second SiO₂ layer finalizes vertically stacked and electrically independent sampling points on each pyramid.



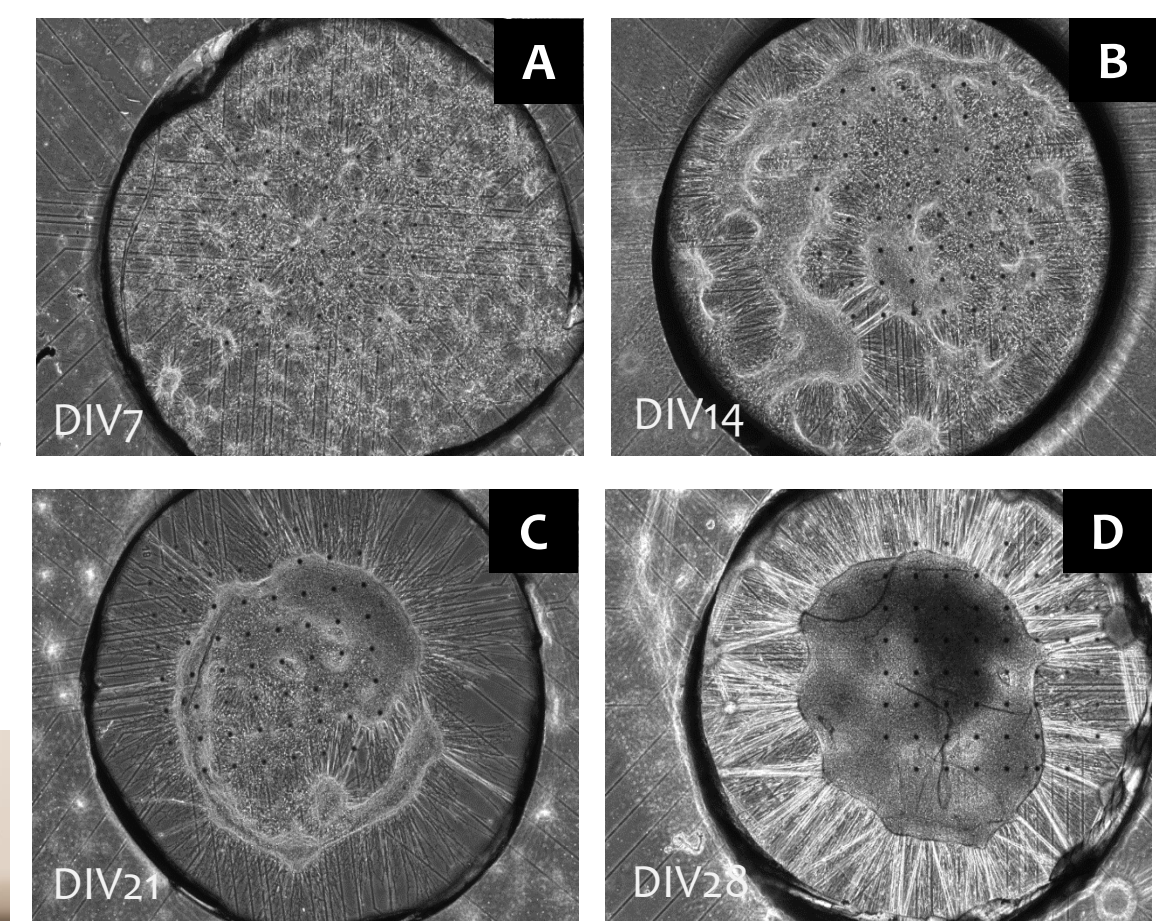
3. Wire-bonding 18 by 18 mm² diced chips to square PCBs with 60 peripheral contact pads, makes them compatible with the commercially available MEA2100 readout from Multichannel Systems [3].



Perspectives

- Transparent substrate for optical inspection.
- Optimization of electrode placement.
- Tests with thicker tissues or organoids grown on MEAs (different protocols to create organoids in process).

Example of a 2D neuronal culture on a commercial MEA over time from DIV 7 (A), DIV14 (B), DIV21 (C) and DIV 28 (D) self-organizing into a structure that seems to resemble an organoid



Conclusion

We recorded and analyzed 3D neuronal activity on chip using arrays of truncated Si micropylamids patterned with electrically distinct and vertically-stacked TiN electrodes. Encouraging preliminary results prompt further analyses and future experiments, envisaged with brain organoids or 3D cell constructs, to record full 3D electrical neuronal network activity in 3D.

[1] J. L. Bourke et al., J. Tissue Eng. Regen. Med. 12, 490-493 (2018).

[2] C. M. Didier et al., J. Micromech. Microeng. 30, 103001 (2020).

[3] K. Musick et al., Lab Chip 9, 2036 (2009).