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

Nele Revyn[1,*], Michel Hu[2], J.-P. Frimat[2], A.M.J.M. van den Maagdenberg[2], P.M. Sarro[1], M. Mastrangeli[1]

[1]*Electronic Components, Technology and Materials, Delft University of Technology, the Netherlands*

[2]*Human Genetics and Neurology, Leiden University Medical Centre, the Netherlands*

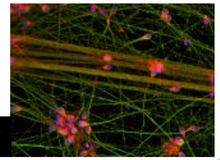
*E-mail: n.revyn@tudelft.nl

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 cortial neurons

green : beta tubulin, red : synaptophysin, blue : cell nucleus

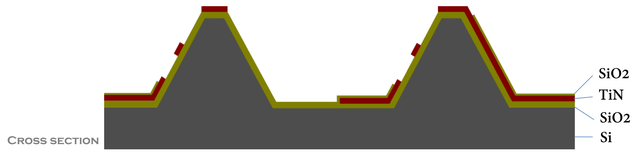
Fabrication

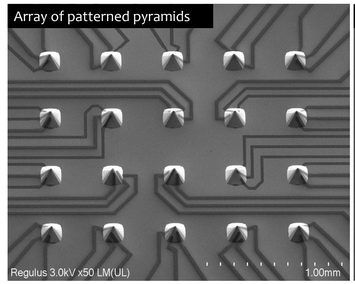
We fabricated 5-by-4 arrays of truncated Si micropyramids:

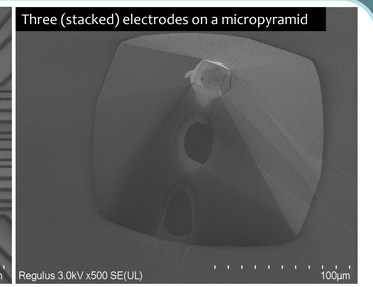
- ~90 µm-high
- ~20 µm-wide plateau
- ~46° to ~53° lateral facet slope
- 3 independent TiN electrodes on each micropyramid

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

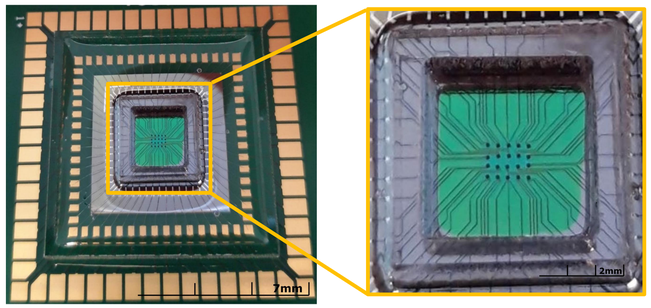
2. Passivation with SiO_2, and patterning with a 10nm/150nm-thick **Ti/TiN layer** defines the microelectrodes. A second SiO_2 layer finalizes **vertically stacked and electrically independent sampling points** on each pyramid.







3. Wire-bonding 18 by 18 mm^2 diced chips to square PCBs with 60 peripheral contact pads, makes them compatible with the commercially available MEA2100 readout from Multichannel Systems [3].

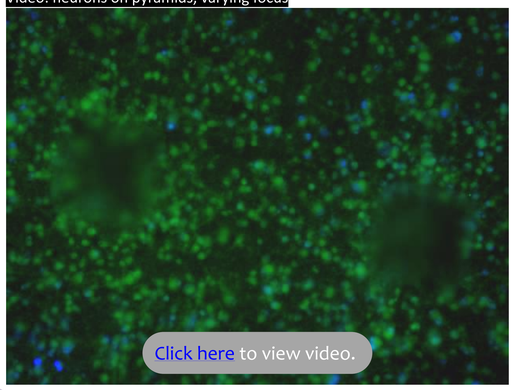


Preliminary results

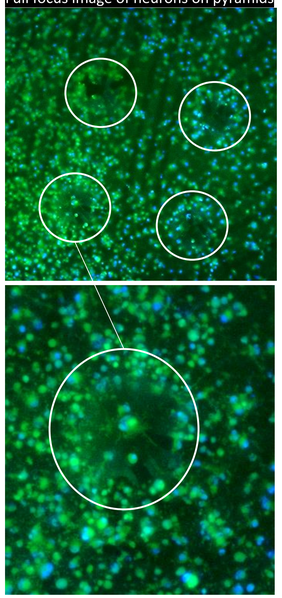
Neuron growth on pyramids (video):

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

Video: neurons on pyramids, varying focus

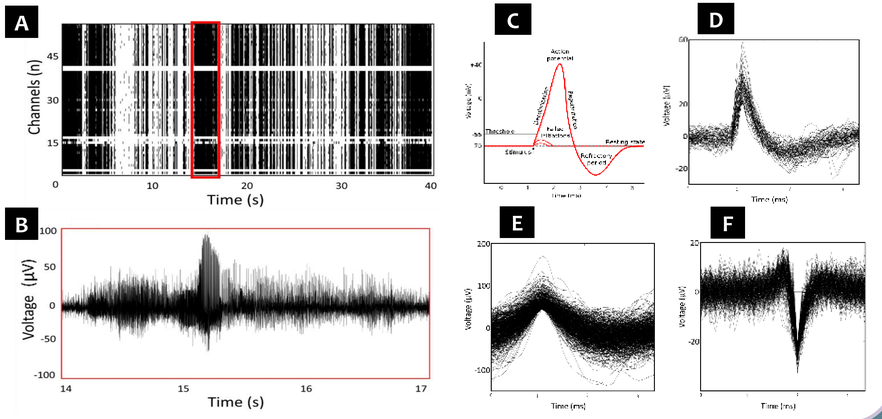


Full focus image of neurons on pyramids



Recording:

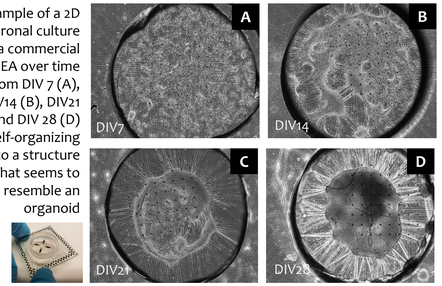
- Clear network bursting activity (A, B)
- Zoom on single spike (D):
  - Looks like intracellular waveform (C), but not exactly, as there is noise (D)
  - Comparison with spike waveform on commercial MEA with 60 electrodes (E)
  - Comparison with spike waveform on commercial 24 multiwell MEA, reported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



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 micropyramids 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.

EUROSOCS CONFERENCE 2021

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TU Delft Delft University of Technology

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