Supplementary Material

Histograms for the different pitches

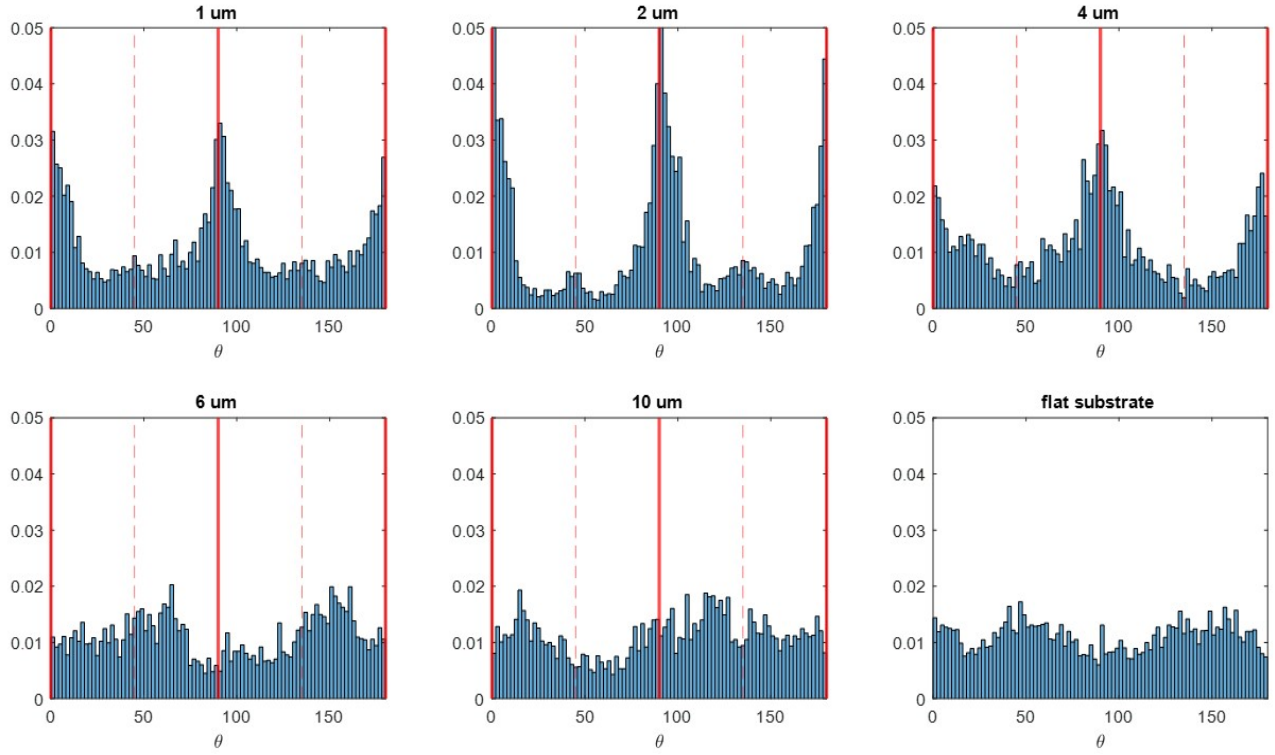

Figure S1. Histograms reporting the axon direction with respect to one of the main axis of the grid, for all the pitches present in our sample design. The detail of the analysis can be found in the main text. Red lines mark the two perpendicular directions along nearest neighbors of the array. Red dashed lines correspond to 45 and 135. Each histogram is obtained analysing a number of SEM images between 10 and 15.

Preliminary results on neuronal culture on diamond substrate

We report here the results from the first test we did about culturing neurons on diamond. As reported in literature, a thin functionalization layer is typically necessary to promote adhesion between the cells and the diamonds. In particular we tried:

- poly-l-lysine (Bio-Techne, 3438-100)
- laminin (SIGMA-ALDRICH, L2020)
- poly-l-lysine (Bio-Techne, 3438-100) and laminin (SIGMA-ALDRICH, L2020)

Only the combination of the two was successful, while using just one of the coating resulted in poor quality of the culture (visible already from optical microscope inspection).

For the final experiment we plated at a density of 150'000 cells/ml and we imaged the neurons after 10 days, with the results reported in the main text. Preliminary attempts considered higher density (2x) and longer incubation time, up to 15 days. In this cases, we observed that it was not possible to see the diamond nanostructures, since they were completely embedded in the culture. An example of this situation is reported in Fig. S2.

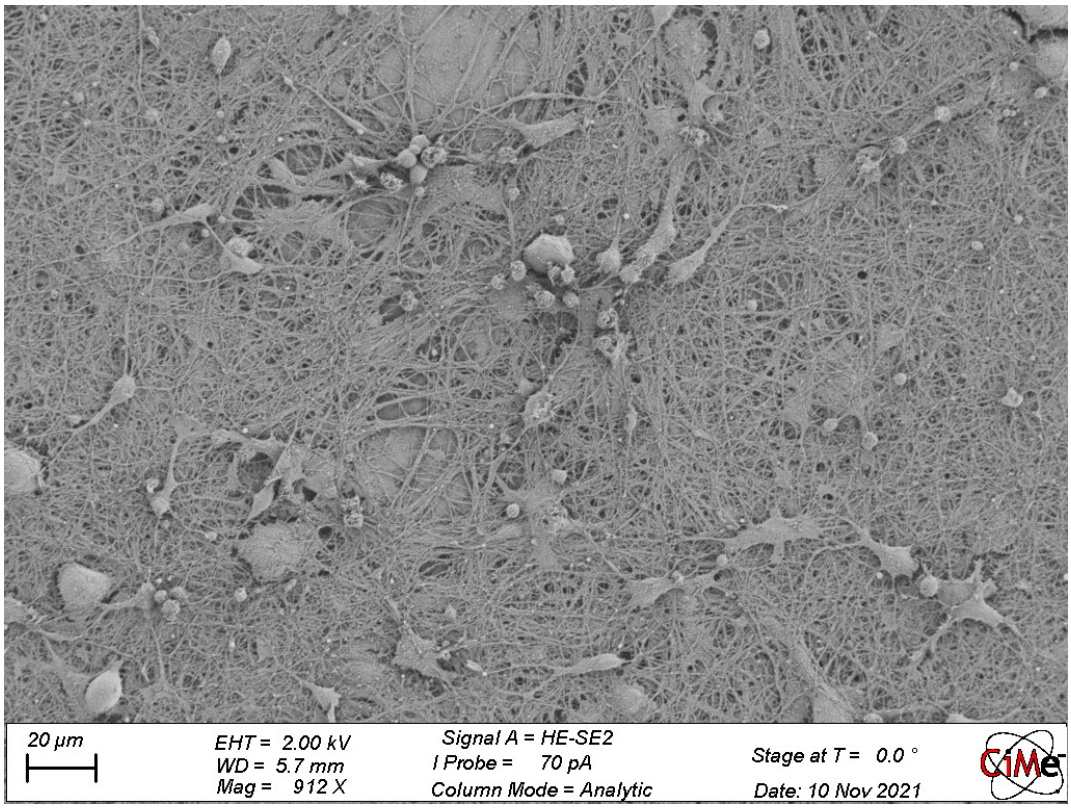

Figure S2. SEM image of primary hippocampal neuron plated on nanostructured diamond, after 14 days of incubation and with higher cell concentration respect to what was chosen for the final experiment.

Resting membrane potential

In the following table we report the average cell membrane resting potentials measured in the different tested arrays.

Resting membrane potential (mV)	$p(\mu\text{m}) \times d(\text{nm})$
-55	4×300
-60	1×500
-61	1×400
-49	10×100
-49	6×200
-63	2×200
-60	flat

On the amplitude of EPSPs recordings

As mentioned in the text the amplitude of the EPSPs signal we recorded (10 mV) is higher compared to typical values reported in literature. We here discuss better this point, suggesting some hypothesis explaining the phenomenon.

First of all, we need to take into account that the neurons are not in physiological conditions, and their neurites are not receiving the same signals for connecting than in physiology. It is possible that anomalous (in this case more than usual) connectivity happens between 2 neurons. It is possible that, due to the conditions of the culture (smaller neurite growth and higher interconnectivity), the amplitude of EPSP is observed larger than the value typically recorder for neurons in the physiology of brain tissue. Also, for the cell corresponding to the measurement reported in the figure, we were injecting -40pA to keep the recording at -65mV. Injection of negative current might have and amplifying effect on the amplitude of EPSPs.