2818
doi:10.1017/S1431927621009855
Microsc. Microanal. 27 (Suppl 1), 2021

Using Maskless Photopatterning for Cryo-ET of Primary Drosophila Melanogaster Neurons

Joseph Kim[1], Bryan Sibert[2], Jae Yang[2], Sihui Yang[3], Josephine Mitchell[2], Jill Wildonger[4] and Elizabeth Wright[2]

[1]Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin, United States, [2]Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin, United States, [3]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California, United States, [4]Department of Biological Sciences, University of California, San Diego, La Jolla, California, United States

Cryo-electron tomography (cryo-ET) is well suited for high-resolution studies of large or pleomorphic samples that have been natively preserved by plunge-freezing. This is especially true for cellular cryo-electron tomography, where organelles and the macromolecules within cells can be studied *in situ* [1]. However, one current challenge in sample preparation for cellular cryo-ET is properly growing adherent cells directly on electron microscopy (EM) grids that are amenable for cryo-ET data acquisition. Primary neuronal cultures are a particular example of this issue. Primary neurons and their mechanistics of organelle transport, synaptic function, and neurodegenerative diseases have been studied by cryo-ET [2-4]. In particular, a unique challenge in working with primary neurons is the long neurites that emanate from the cell body, which grow extensively across the grid and grid bars and hinder cryo-ET data collection [5]. Combined with the difficulties in obtaining and culturing primary neurons, it is important to maximize the number of grid squares with neurons for data collection.

Recently, maskless photopatterning has been introduced as a way to define placement of adherent cells onto EM grids. A passivation layer of polyethylene glycol is applied on top of EM grids while ablating specific areas of the grid square away by a UV laser with a photoactivator for site-specific deposition of proteins to promote cellular adhesion. Such photopatterning technique have been used for cellular cryo-ET for a variety of cell types, including HeLa and MDCKII cells [6, 7]. Here, we report the use of maskless photopatterning for improved spatial positioning and growth of primary neurons derived from *Drosophila melanogaster* larvae on EM grids [8]. By using transgenic *Drosophila* larvae that expresses pan-neuronal GFP-tagged neurons with Alexa Fluor 350-conjugated concanavalin A as the extracellular matrix protein, we were able to monitor and track neuronal growth over several days on photopatterned EM grids by fluorescence microscopy. The neurons were well positioned on the patterns with neurites that grew out and extended within the patterns over several days. Plunge-freezing these grids and examination by cryo-EM showcased clear defined cellular density that correlated with what was observed by fluorescence microscopy. Compared with unpatterned grids, on patterned grids it was found to be easier to locate neurites and neuronal periphery that were amenable for tilt series data collection.

Reconstruction of these tomograms revealed macromolecules and organelles that have been similarly observed in tomograms of mouse neurites, including spatially-restricted mitochondria, flared microtubules, pleomorphic linkers, and dense core vesicles [4, 9-10]. Interestingly, free-floating ribosomes were also observed within these neurites, which presents opportunities for the *Drosophila* larval neuron to be used as a potential model for structural studies for ribosomes *in situ*. Combined with the genetic toolbox that the *Drosophila melanogaster* has available, many opportunities for high-resolution *in situ* studies using *Drosophila* neurons are possible [11]. Future work would extend the technological capabilities of maskless photopatterning for different neuronal cryo-ET studies, with the patterning making a direct contribution. For example, patterns can be designed with specific ECMs on the EM grid so that the neurons can form synaptic connections within the patterns, similar to past studies that were performed on glass [12]. We also hope to extend the use of

photopatterning to primary mouse neurons, where specific cellular regions can be confined on the grid squares for cryo-ET data collection.

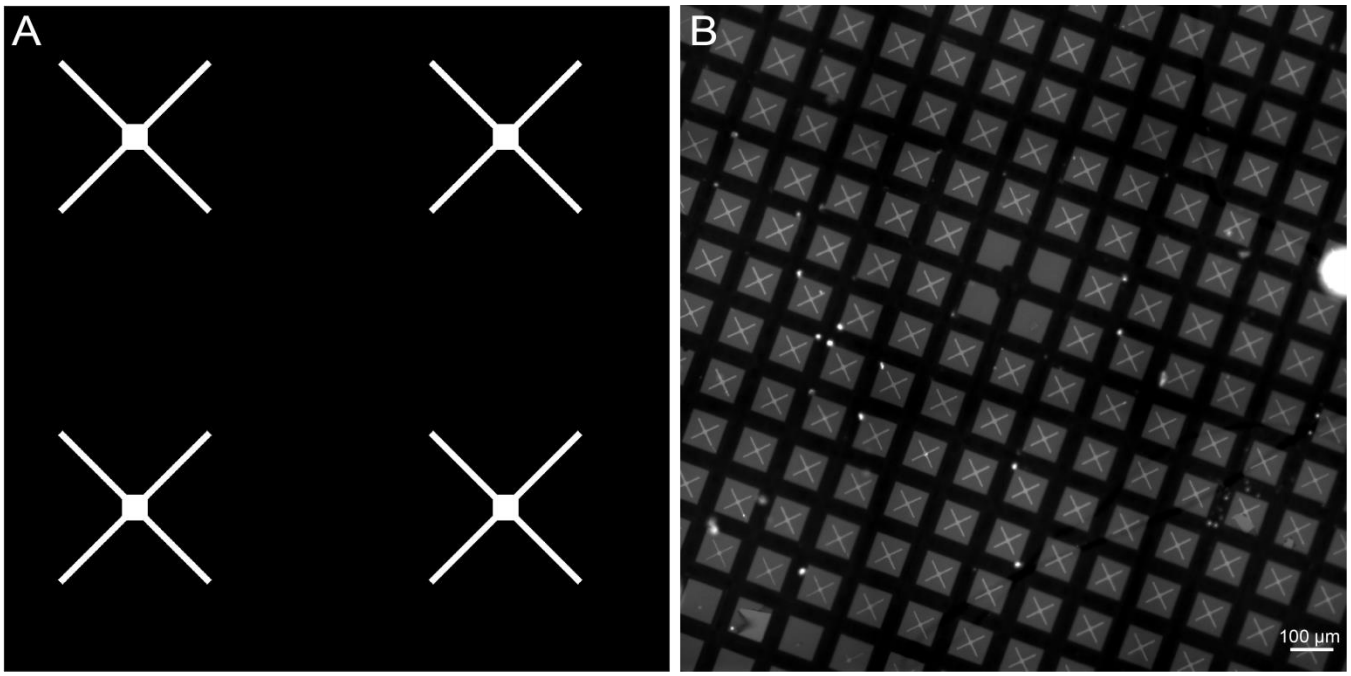

Figure 1. Figure 1. (A) Pattern that was designed for maskless photopatterning to culture drosophila melanogaster neurons. The pattern was designed to have an 'X' shape while having a small square in the middle. (B) Light microscopy image of gold Quantifoil 200 mesh R 2/1 grids that was patterned with the design from (A). The ECM that was deposited after patterning was concanavalin A that was conjugated with Alexa Fluor™ 350, which emits blue fluorescence.

https://doi.org/10.1017/S1431927621009855 Published online by Cambridge University Press

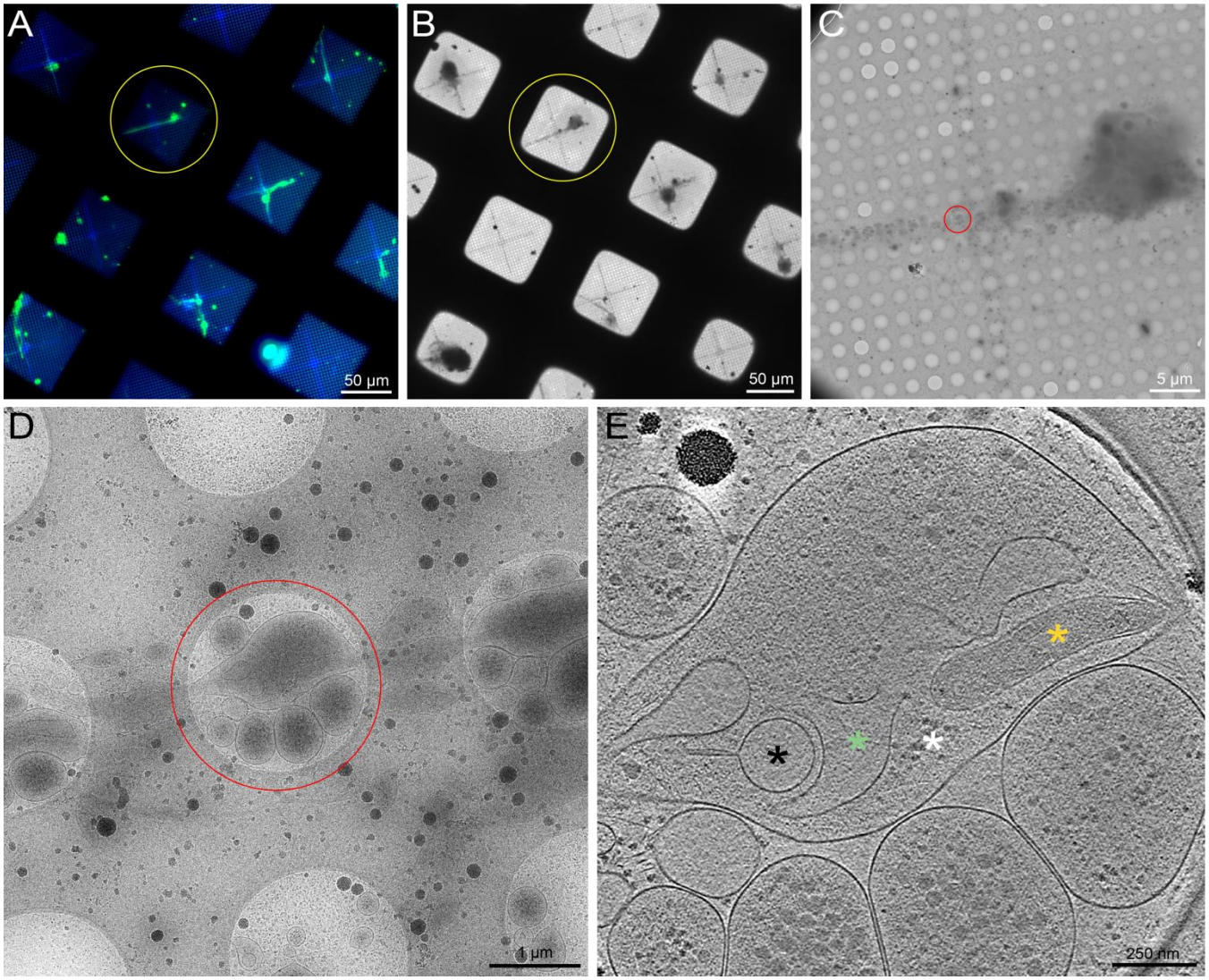

Figure 2. Figure 2. (A) Light microscopy image of GFP-tagged primary neurons from Drosophila melanogaster larvae, grown for three days on the patterned grids. Green: Neurons, Blue: Photopattern. (B) Cryo-EM grid montage of the same grid in (A) after plunge-freezing. Yellow circle shows the same grid square. (C) Magnified cryo-EM square montage of the yellow circle in (A) and (B) maps. (D) Magnified view of the red circle in (C), where a tilt series was collected on the center neurite marked by the same circle. (E) 25 nm thick slice of a low-pass filtered tomogram reconstructed from the tilt series that was acquired from the red circle in (C) and (D). Various organelles and macromolecules can be observed, including vesicles (black asterisk), the endoplasmic reticulum (green asterisk), ribosomes (white asterisk), and the mitochondria (orange asterisk).

References

1. Wagner, J. et al. Cryo-electron tomography-the cell biology that came in from the cold. FEBS Lett. 591, 2520-2533 (2017).

2. Tao, C.-L. et al. Differentiation and Characterization of Excitatory and Inhibitory Synapses by Cryo-electron Tomography and Correlative Microscopy. J. Neurosci. 38, 1493–1510 (2018).

https://doi.org/10.1017/S1431927621009855 Published online by Cambridge University Press

3. Guo, Q. et al. In Situ Structure of Neuronal C9orf72 Poly-GA Aggregates Reveals Proteasome Recruitment. Cell. 172, 696-705 (2018).
4. Fischer, T. D. et al. Morphology of mitochondria in spatially restricted axons revealed by cryo-electron tomography. PLOS Biology 16 (2018).
5. Lucic, V. et al. Multiscale imaging of neurons grown in culture: From light microscopy to cryo-electron tomography. J. Struct Biol. 160, 146–156 (2007).
6. Toro-Nahuelpan, M. et al. Tailoring cryo-electron microscopy grids by photo-micropatterning for in-cell structural studies. Nat Methods. 17, 50–54 (2020).
7. Engel, L. et al. Extracellular matrix micropatterning technology for whole cell cryogenic electron microscopy studies. J. MicromechMicroeng. 29 (2019).
8. Kim, J. et al. A New *In Situ* Neuronal Model for Cryo-ET. Microsc. Microanal. 26, 130-132 (2020).
9. Schrod, N. et al. Pleomorphic linkers as ubiquitous structural organizers of vesicles in axons. PLOS ONE 13 (2018).
10. Atherton, J. et al. Microtubule architecture in vitro and in cells revealed by cryo-electron tomography. Acta Crystallogr D Struct Biol. 74, 572-584 (2018).
11. Duffy, J. B. GAL4 system in Drosophila: a fly geneticist's Swiss army knife. Genesis (2002).
12. Czöndör, K. et al. Micropatterned substrates coated with neuronal adhesion molecules for high-content study of synapse formation. Nat Comm. 4 (2013).
13. This research was supported by funds from the University of Wisconsin-Madison, Morgridge Institute for Research, and National Institutes of Health (R01GM104540, R01GM104540-03S1, and U24 GM139168) to E.R.W. All EM data was collected at the Cryo-EM Research Center, Department of Biochemistry, University of Wisconsin-Madison.