Photo-Micropatterning - Creating functionalized cryoEM grids for studying cell architecture EMBLEM Ref. 2019-027

Challenge

- Cryo-Electron Microscopy is one of the major imaging technologies for structural biology
- Sample preparation of intact cells for cryo-ET by cryo-FIB relies on the proper positioning of cells on the cryo-EM grids
- Spatial distribution of cells on the grids with traditional methods is subject to coincidence, resulting in many samples not suitable for cryo-FIB

Commercial Opportunity

 EMBL offers a Technology Evaluation Programme, as well as licensing and collaboration opportunities

Technology

- Allows to study the intracellular architecture in their native, functional cellular environments, by enabling to control cell shape and differentiation in a predictable manner
- Photo-micropatterning of cryo-EM grids allows for spatially controlled adhesion of cells
- The technology can be employed on a variety of grid materials
- The functionalization of micropatterns generate predictable cellular organization and therefore allow for the direct correlation between cell architecture and in-cell threedimensional structural characterization
- The technology can be employed on a variety of grid materials
- compared, to traditional sample preparation this allows for high-throughput generation of specimen with cryo-FIB

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Intellectual Property

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The method comprises several steps:

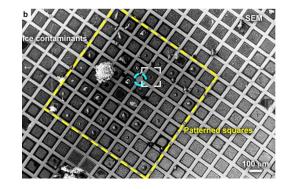
- Passivation: Grid passivation with antifouling agent PLL-g-PEG generates an organized repulsive PEG brush at the surface
- 2. Photo-Micropatterning using a laser
- 3. Spatially constrained ablation of the PLL-g-PEG passivation layer
- 4. Grid functionalization with extracellular matrix (ECM)-related proteins
- 5. Cell seeding at the functionalized micropatterned areas
- 6. Storage up to 30 days under hydrated conditions at 4°C

Grid materials suitable for Photo-Micropatterning:

- Gold or titanium mesh
- SiO₂ films
- full gold grids
- continuous amorphous carbon or graphene film

References

Toro-Nahuelpan, M., Zagoriy, I., Senger, F. et al. Tailoring cryo-electron microscopy grids by photo-micropatterning for in-cell structural studies. Nat Methods **17**, 50–54 (2020). https://doi.org/10.1038/s41592-019-0630-5



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Internal Reference

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