Enhancing Cryo-EM Sample Preparation: The cryoWriter System for Uniform Thin Liquid Film Formation

Maryam Mohammadi

Thomas Braun

Biozentrum, University of Basel

University of Basel

BIOZENTRUM

Universität Basel
The Center for
Molecular Life Sciences

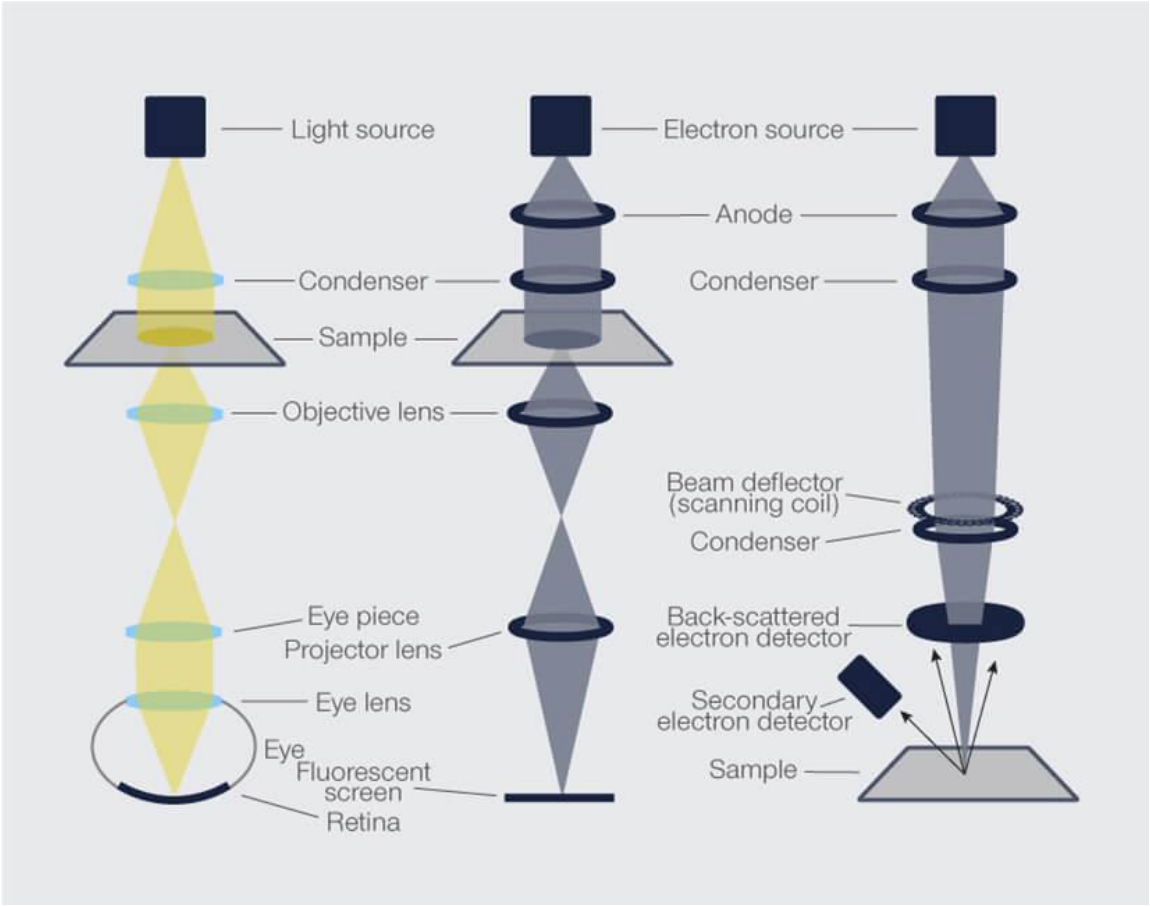
Motivation

- Biological experiments
- Method development

Single Cell Analysis Methods

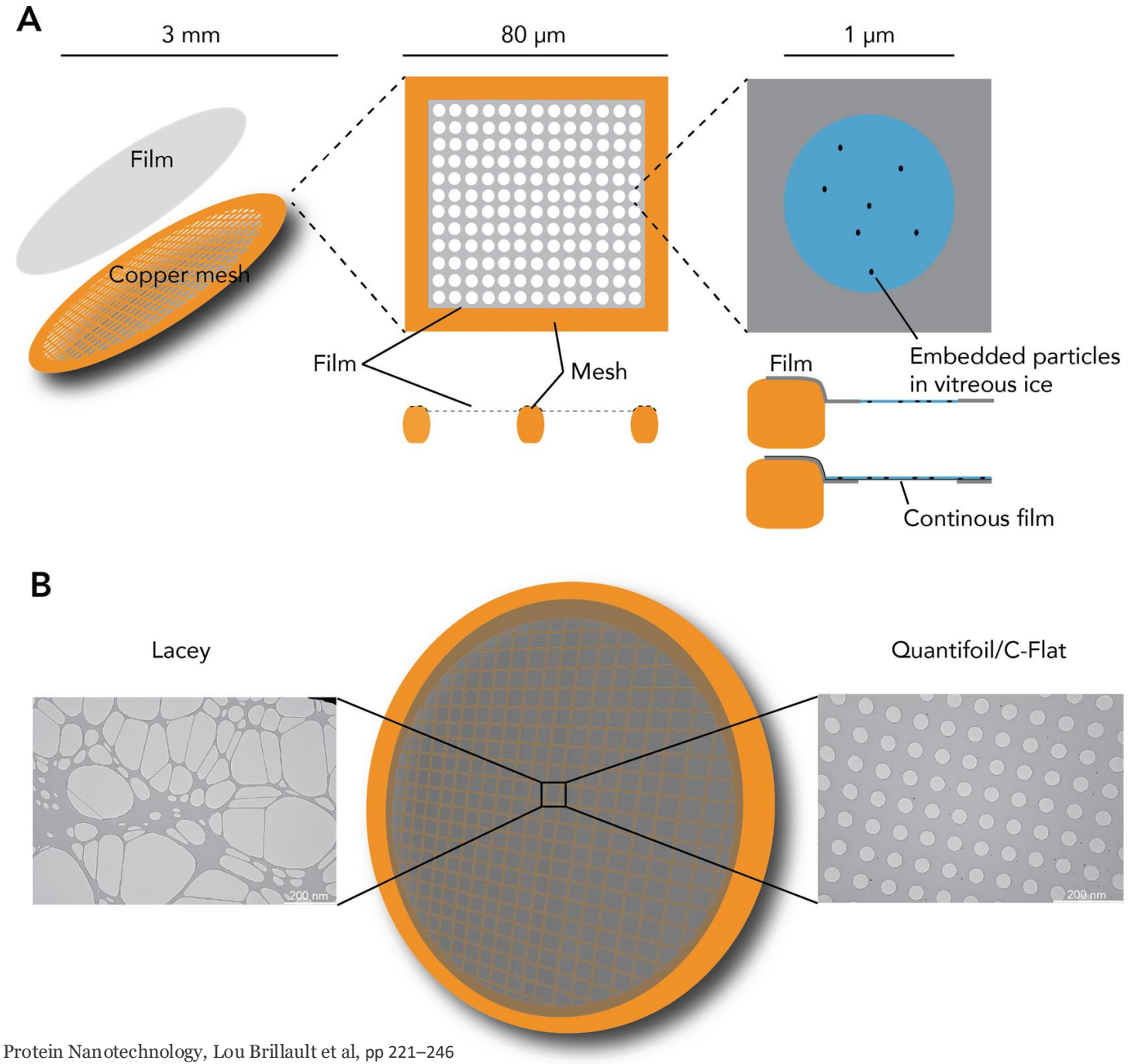
Why Electron Microscopy (EM)?

- Single molecules detection limit → visual proteomics • Enumeration and
- Structure of proteome →
- Needs new sample preparation strategies
  - Lossless
  - Conservation of native structure



Stefan A. Arnold et al., 2017

EM Grids for Thin Liquid Film Formation



Protein Nanotechnology, Lou Brillault et al, pp 221–246

Thin Liquid Film Formation:

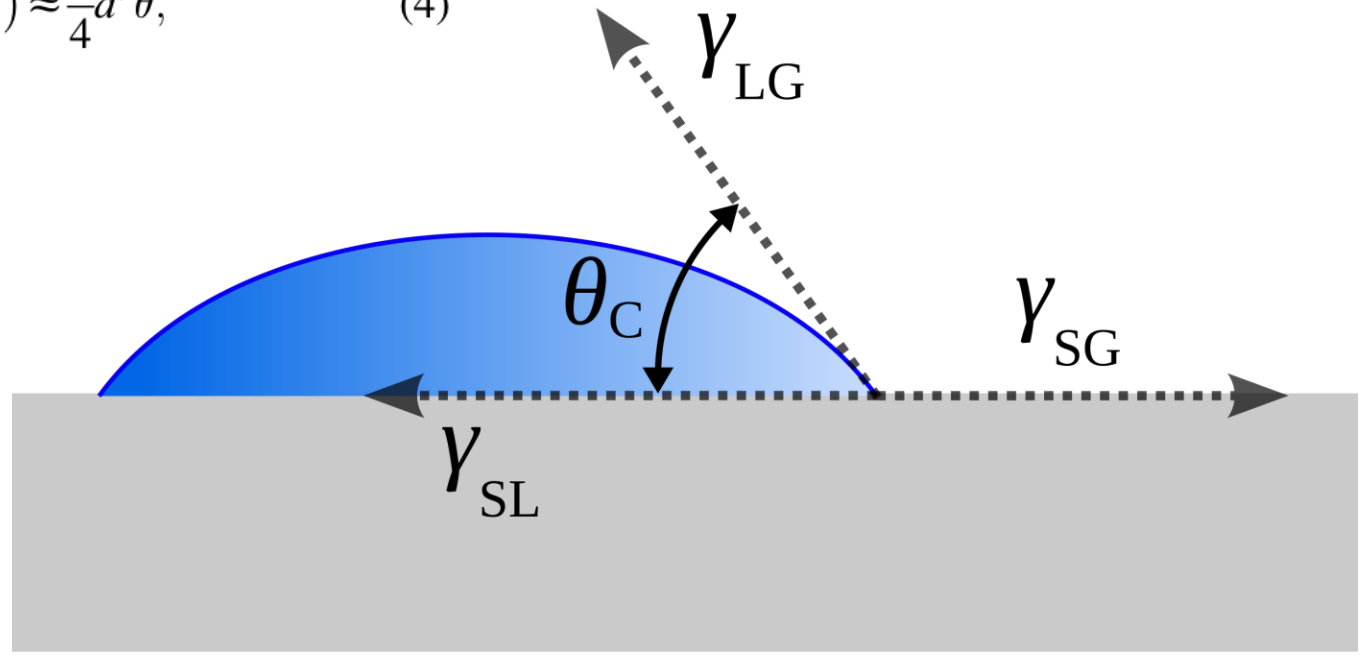
- Puddle
- Surface tension
- Contact angle

$$\cos\theta = \frac{\gamma_{SG} - \gamma_{SL}}{\gamma_{LG}}, \qquad (1)$$

$$\lambda_C = \sqrt{\frac{\gamma_{LG}}{\rho g}}, \qquad (2)$$

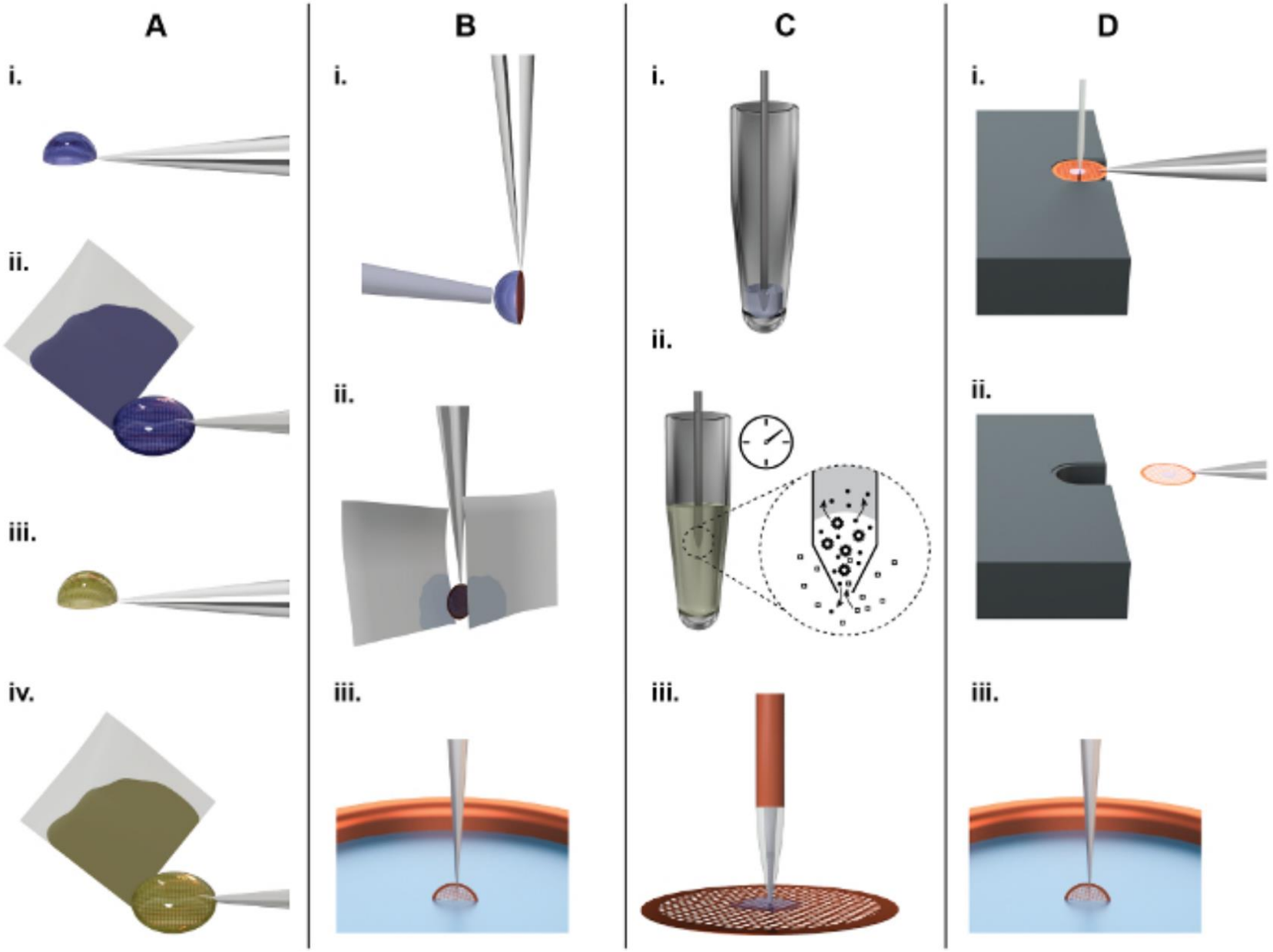
$$h = \frac{a}{\sin\theta}(1 - \cos\theta) \approx \frac{a\theta}{2}, \qquad (3)$$

$$V = \frac{\pi h}{6}\left(3a^2 + h^2\right) \approx \frac{\pi}{4}a^3\theta, \qquad (4)$$



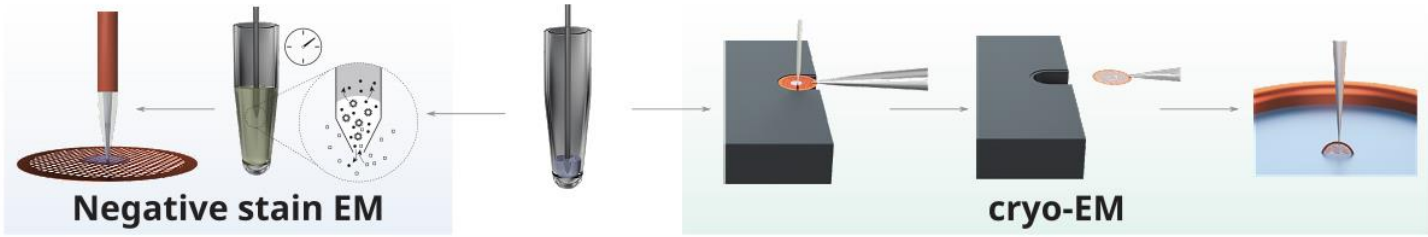
Joris Gillis~commonswiki

Visual Proteomics Classical and Microfluidic Approach

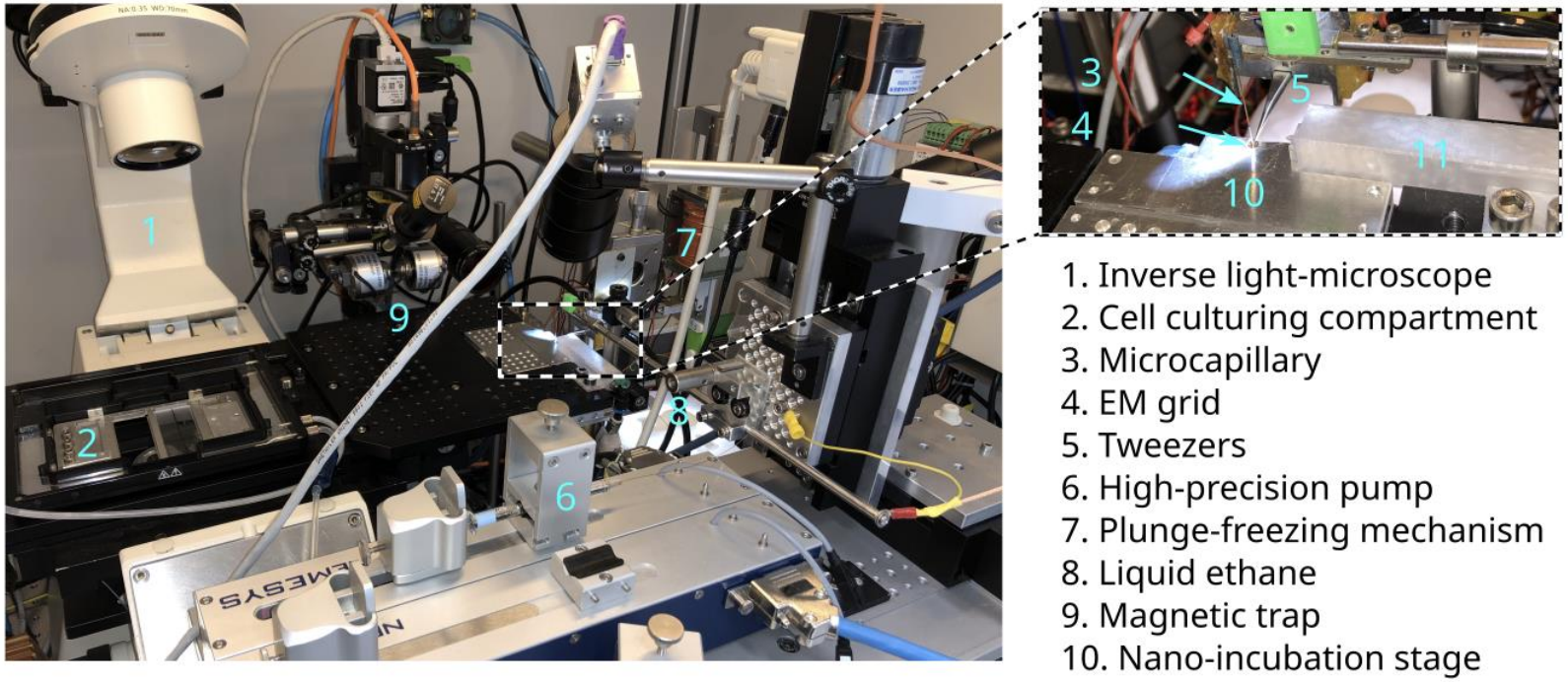


Claudio Schmidli, et al, 2018

Cryo-Writer



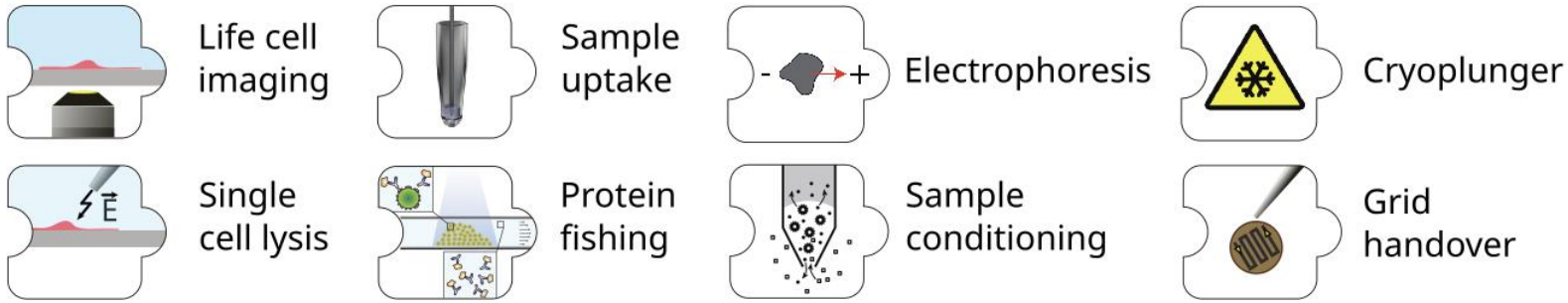
The cryoWriter system



1. Inverse light-microscope
2. Cell culturing compartment
3. Microcapillary
4. EM grid
5. Tweezers
6. High-precision pump
7. Plunge-freezing mechanism
8. Liquid ethane
9. Magnetic trap
10. Nano-incubation stage
11. ClimateJet

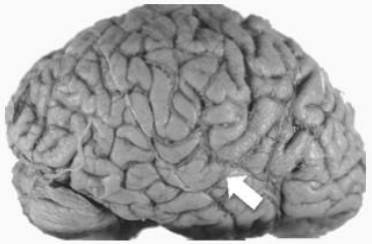
Modules

The different modules can be combined as needed for the experiment.

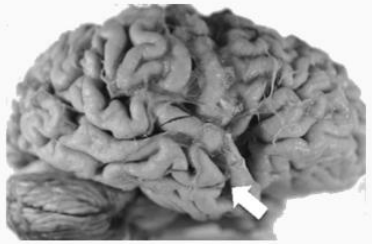


Neurodegenerative disease
Prion-like spreading
Amyloids

Normal brain



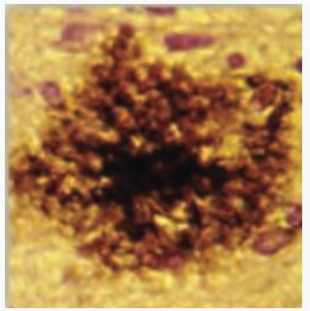
Alzheimer's Disease



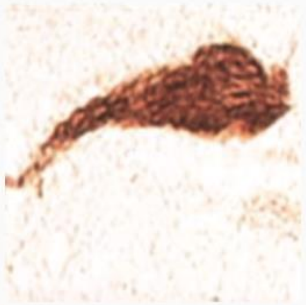
(Soto-Rojas et al., 2015)

- Progressive loss of neuronal function
- Many similarities on sub-cellular level
- Protein deposits:

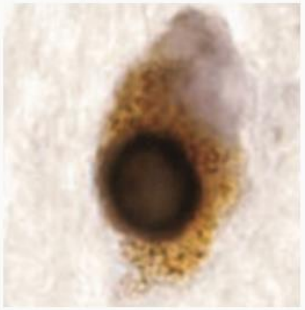
Aβ lesions



tau lesions



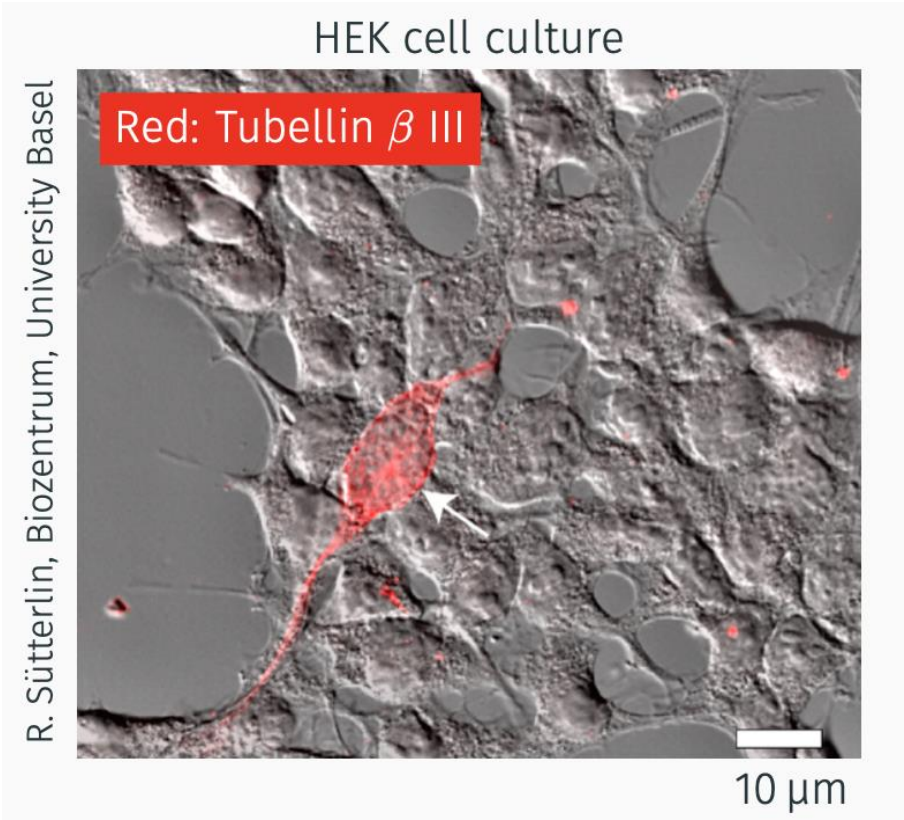
α-syn lesions



(Prusiner, 2012)

Why Single Cell Analysis?

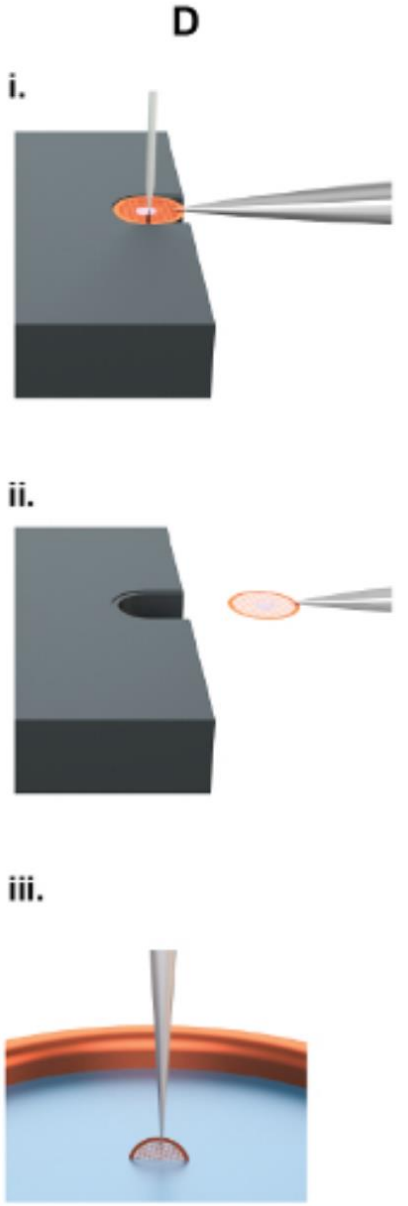
- **Biological systems are stochastic**
- **No synchronization possible**
- **→ Biological noise**



Thin Liquid Film Challenges

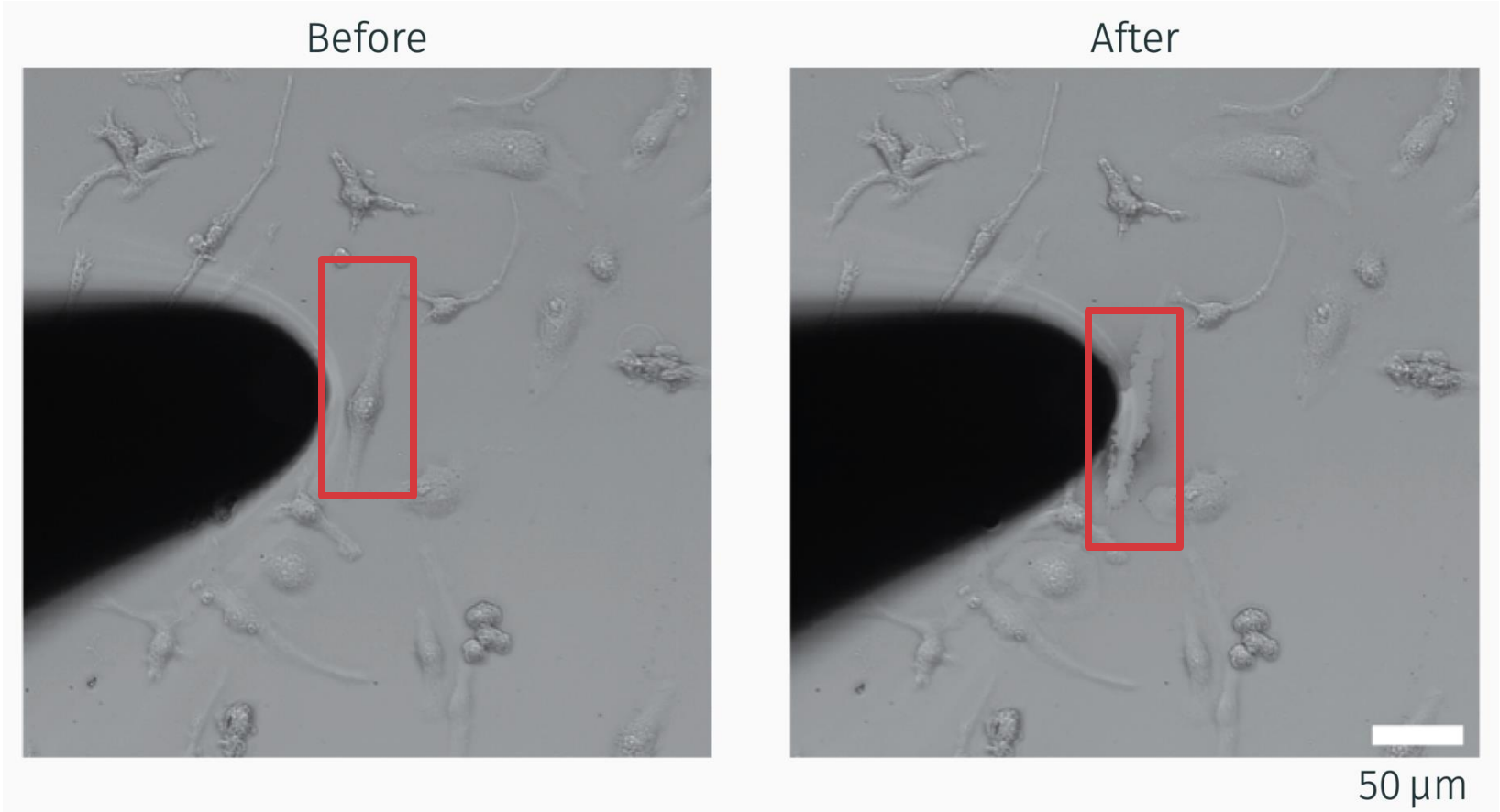
Key Properties and Dynamics of Thin Liquid Films:
Hydrodynamic and Capillarity
Stability and Rupture
Retraction
Intermolecular forces
Viscosity
Elasticity

Environmental Parameters:
Humidity,
Temperature
Pressure
Air Water Interface

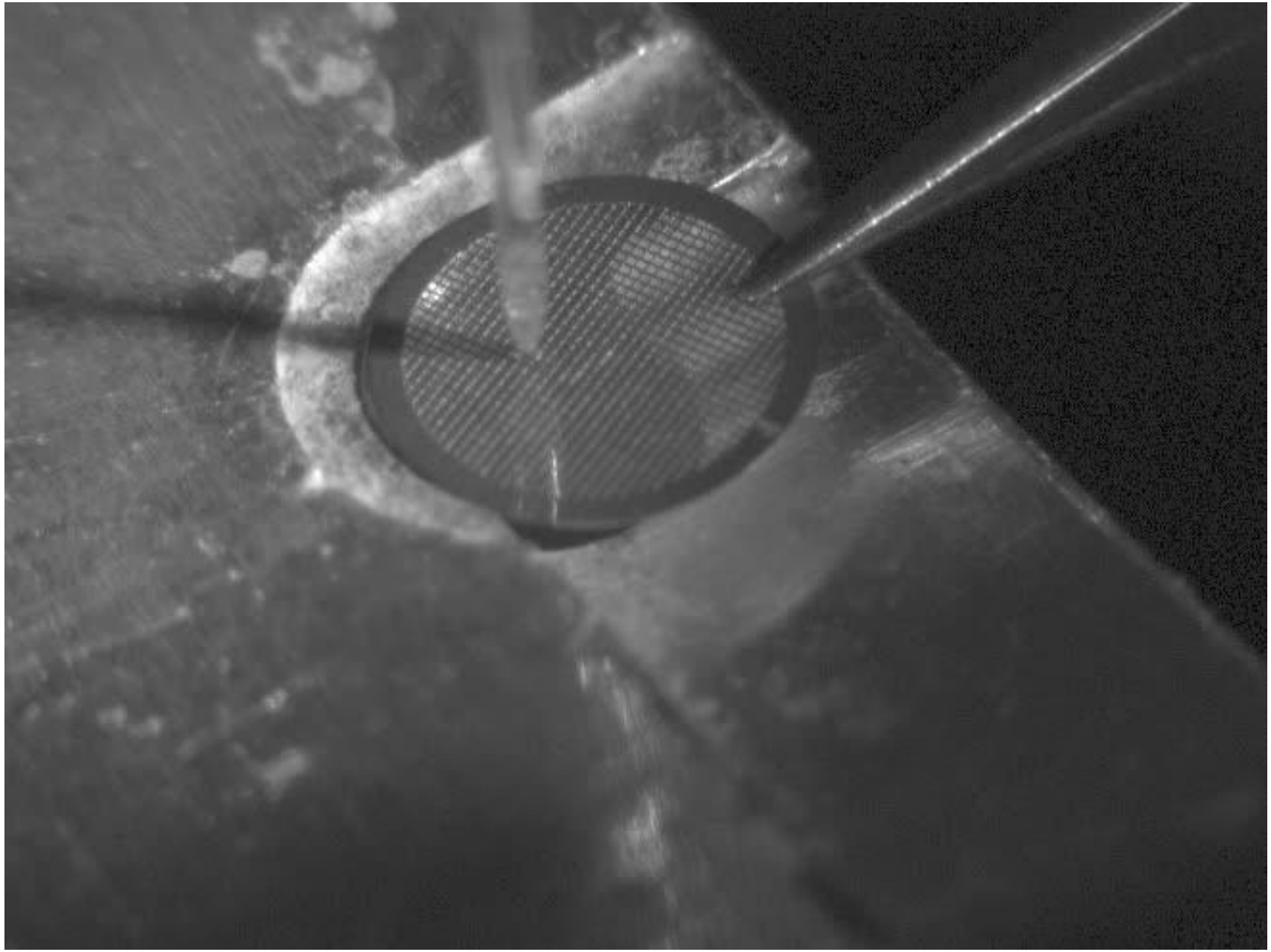


Claudio Schmidli, et al, 2018

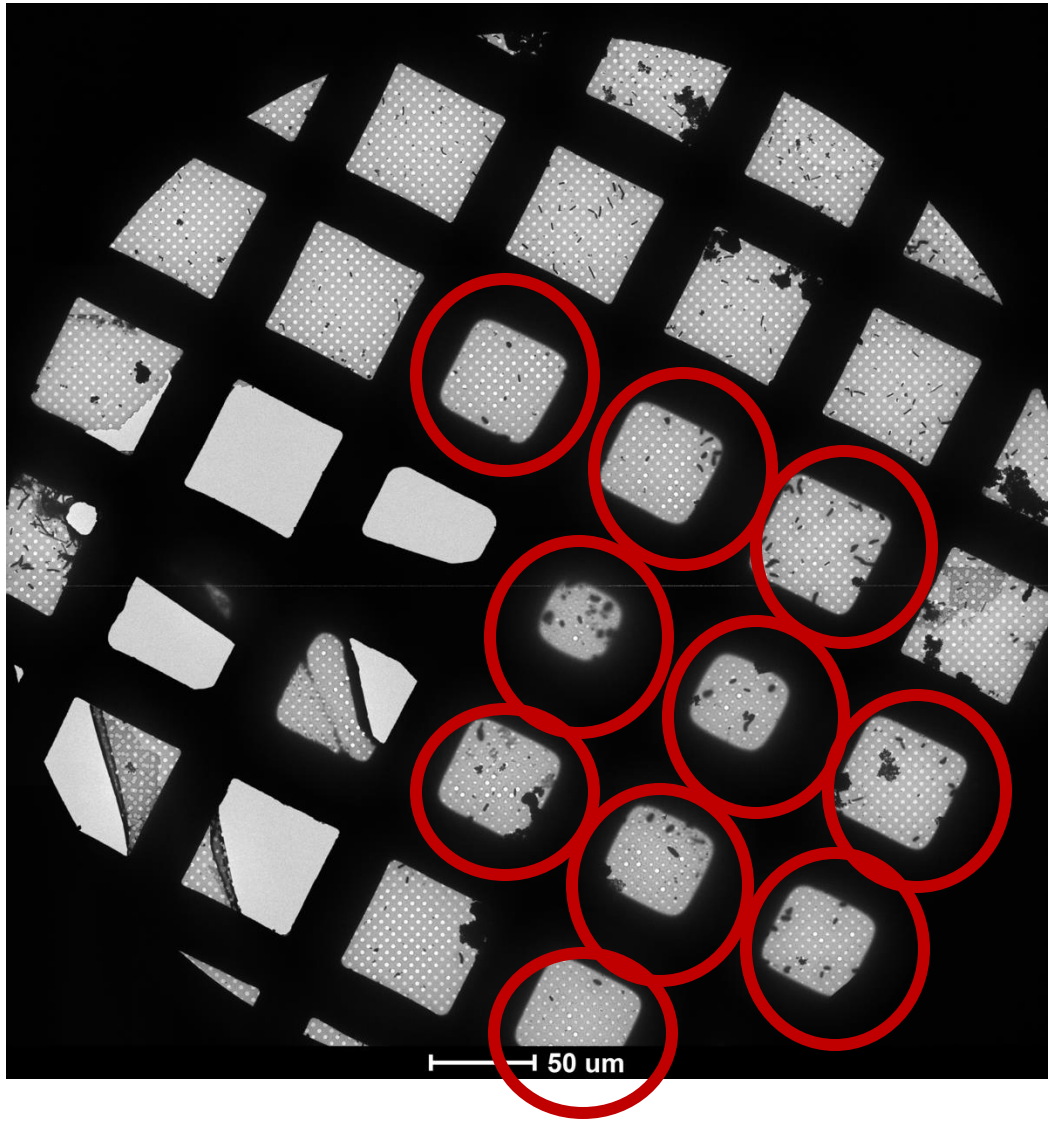
Electroporation Cell lysis



Sample stabilization and thinning by controlled water evaporation



Protocol 1: Deposition with recovery of excess sample



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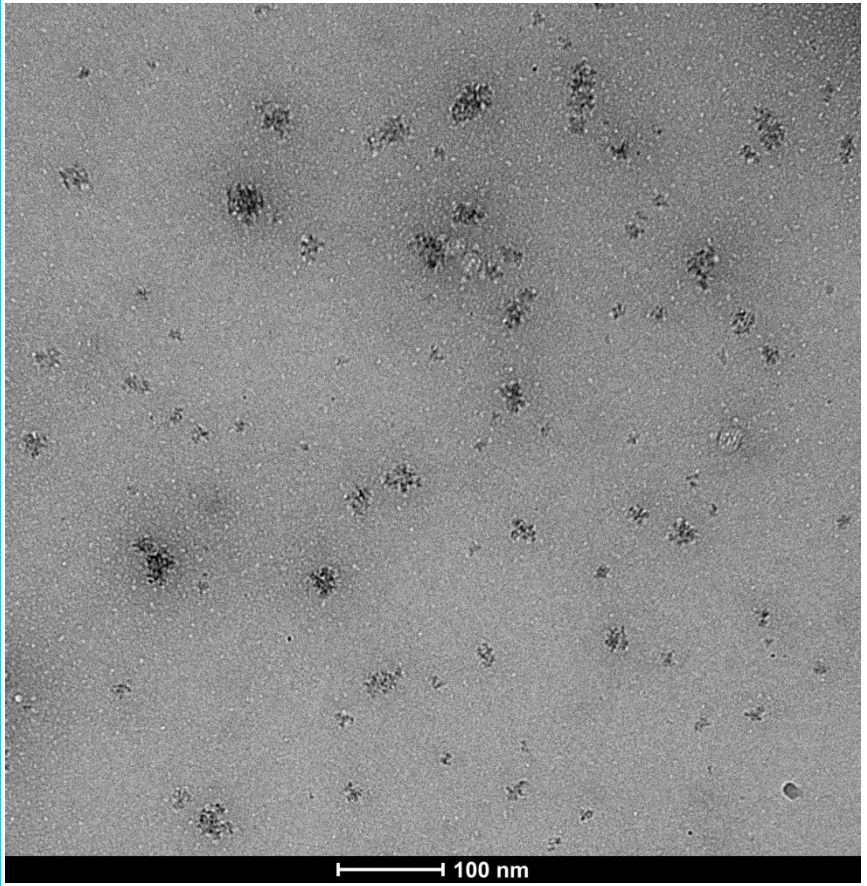
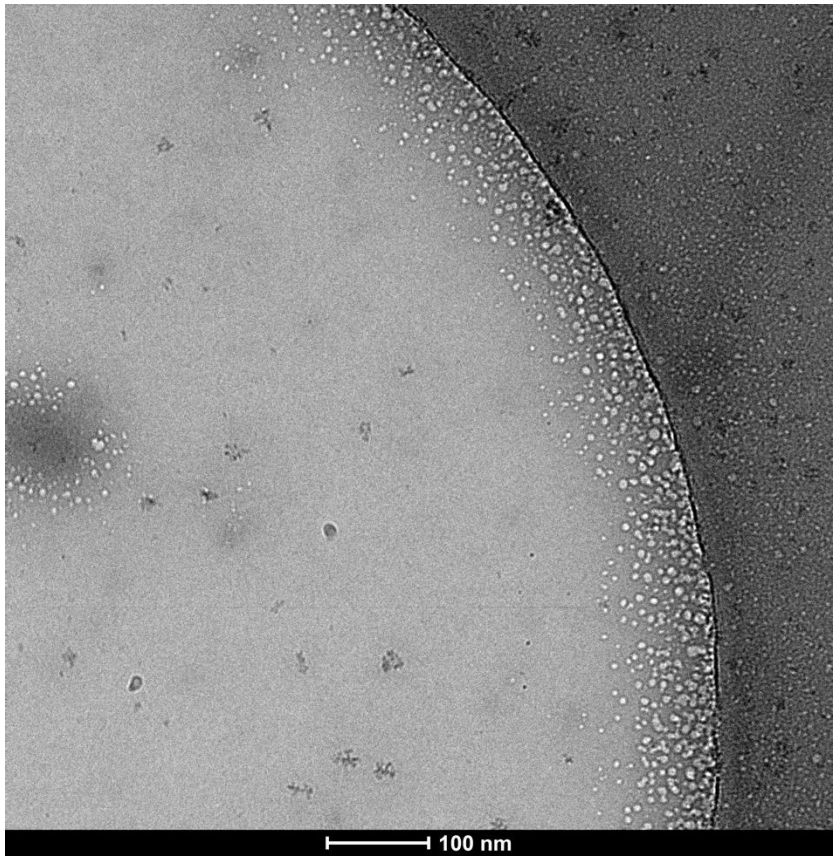
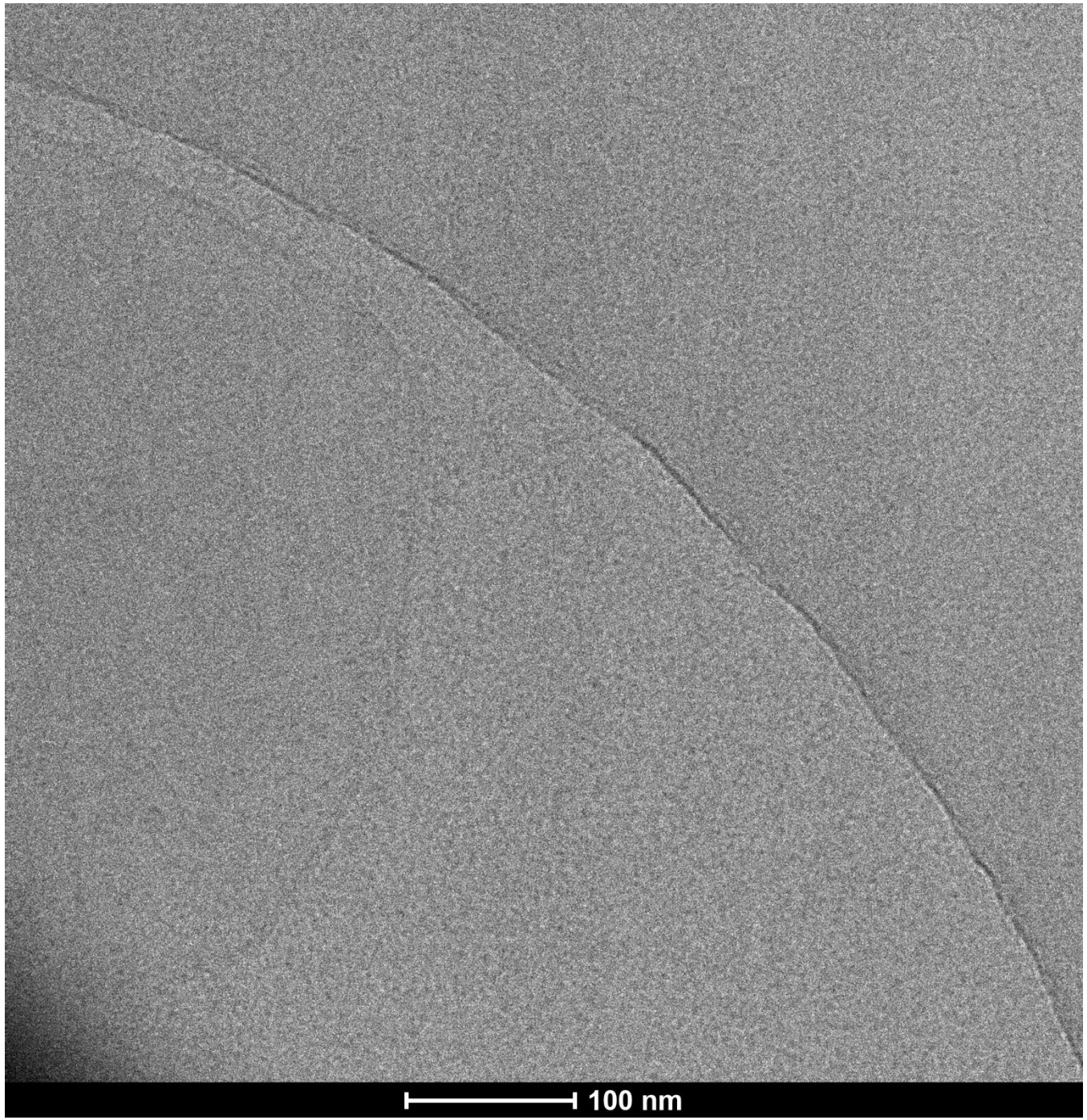
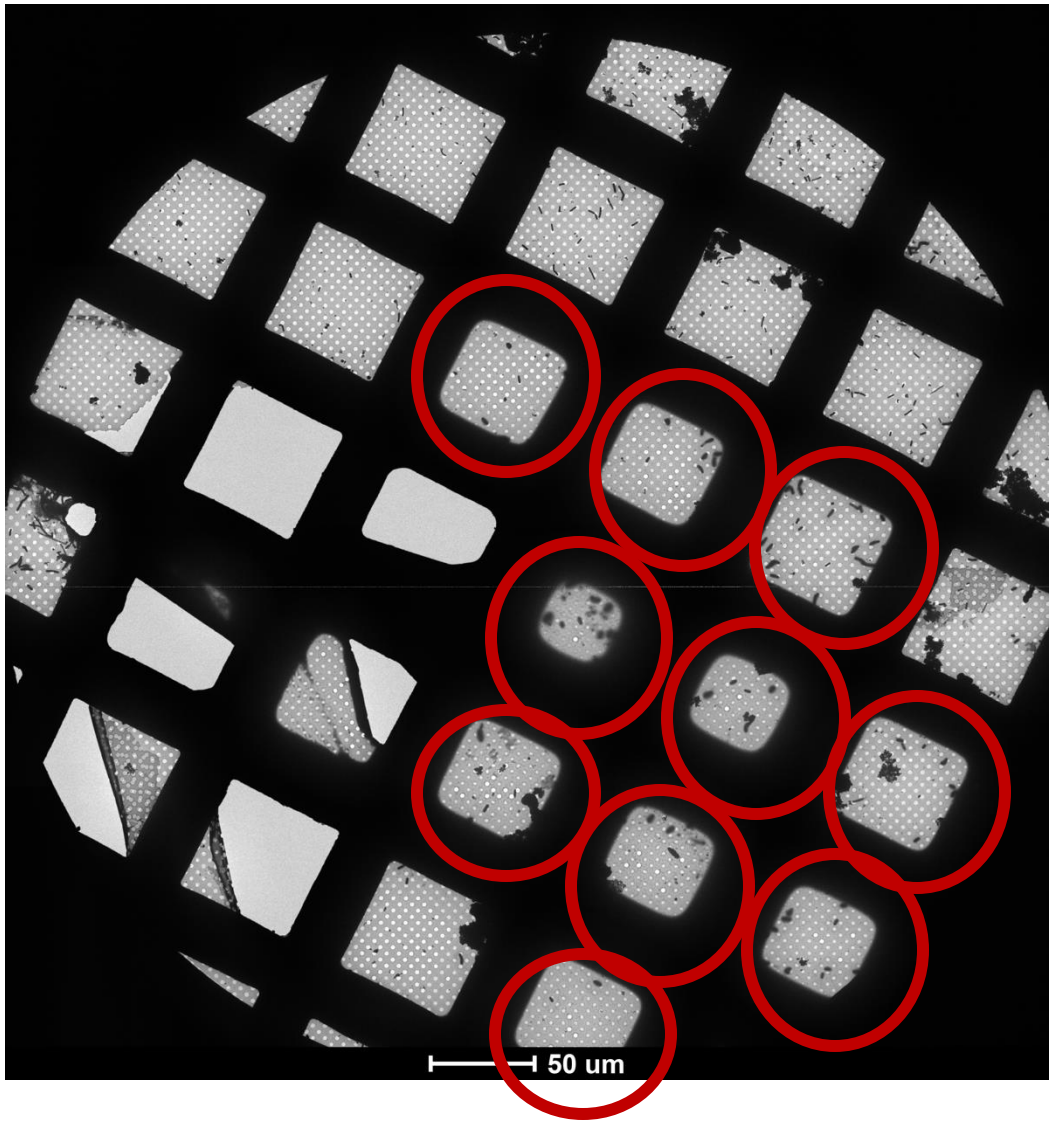





100 nm


50 um

Thanks