

# *Single Cell Imaging*

*What can be learned about the single whole cell using EM?*

*Where are we now?*

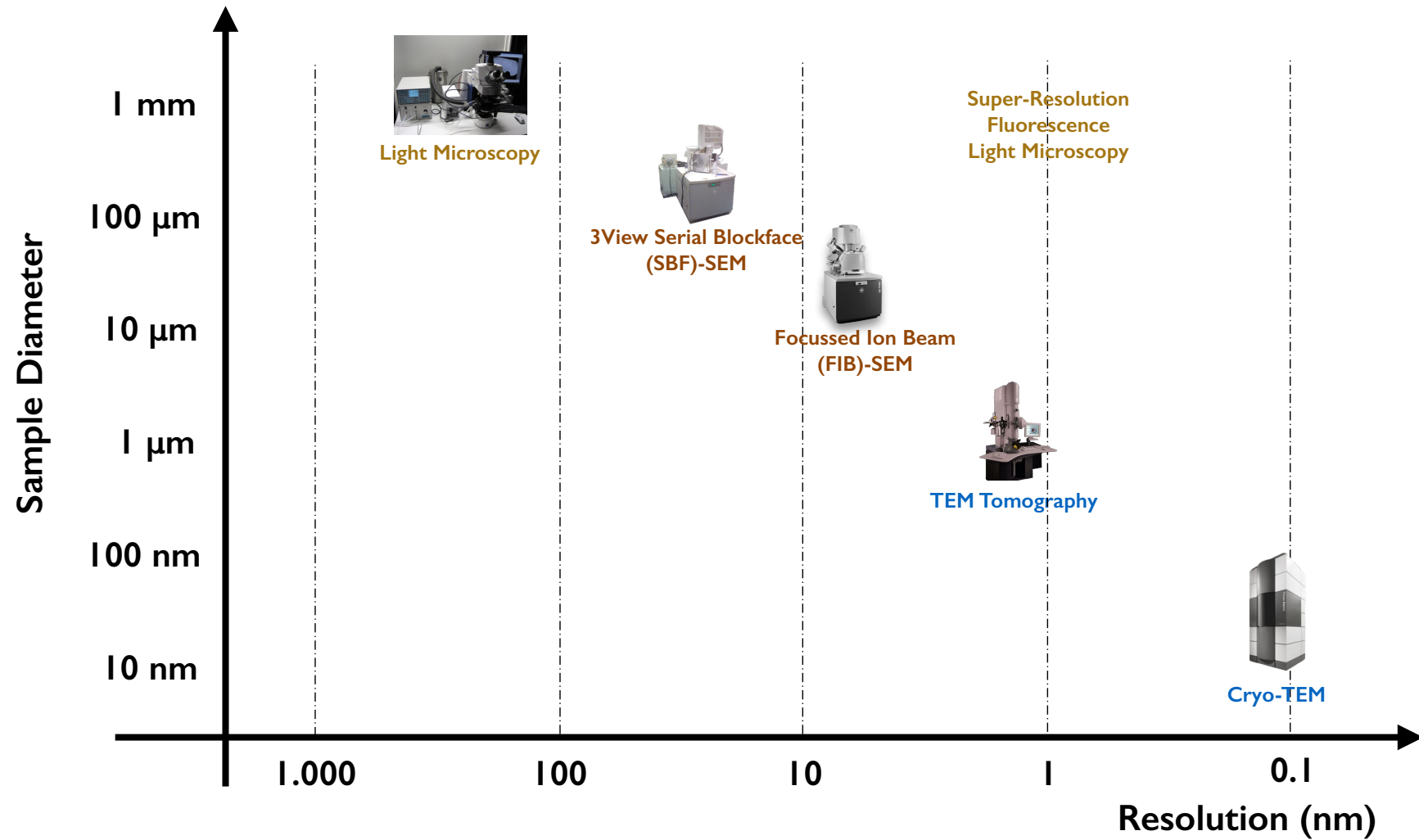
*What are the challenges going forward?*

Henning Stahlberg

*Center for Cellular Imaging and NanoAnalytics (C-CINA)*

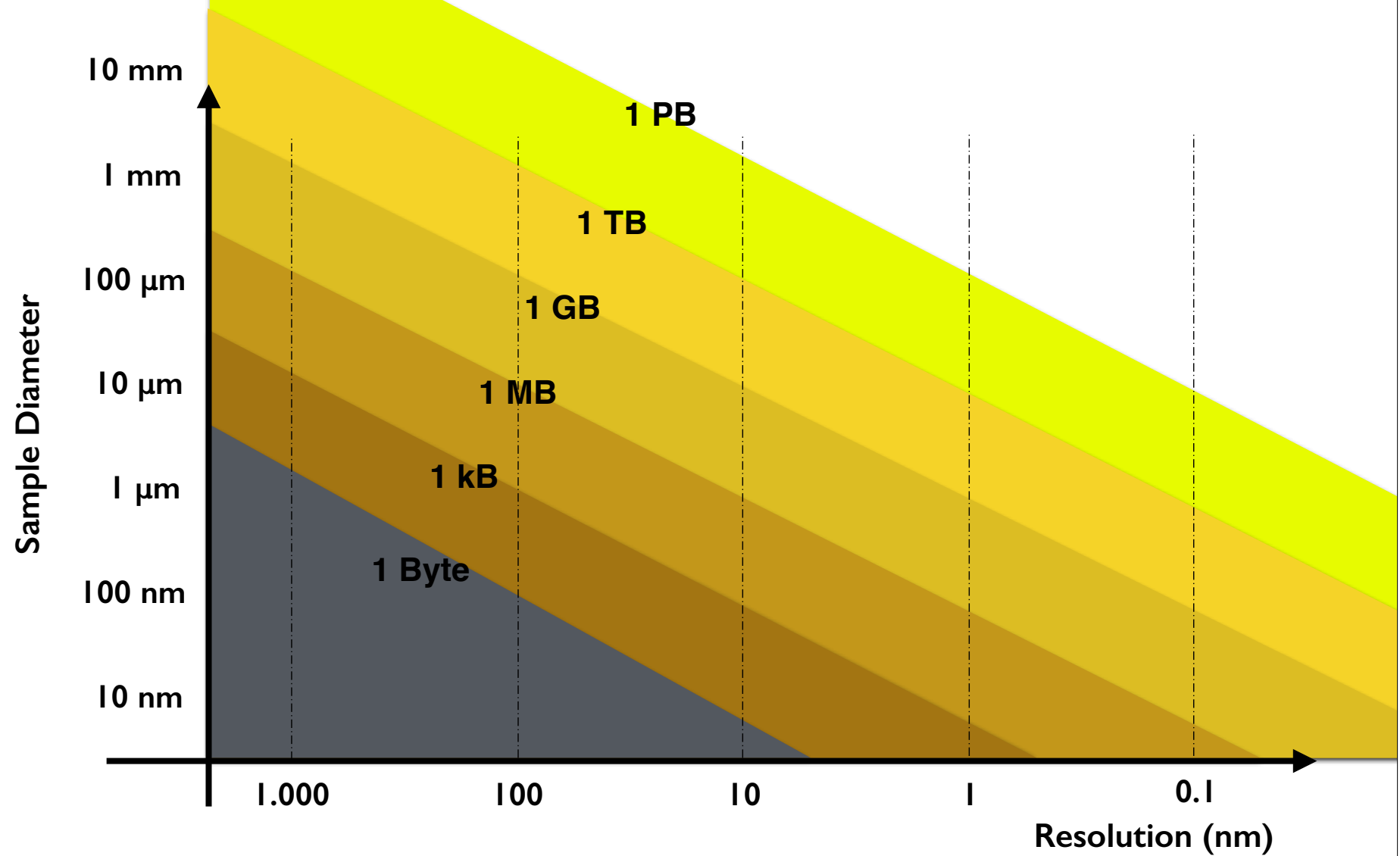
*Biozentrum, University of Basel, Switzerland*

# Multi-Resolution 3D Microscopy



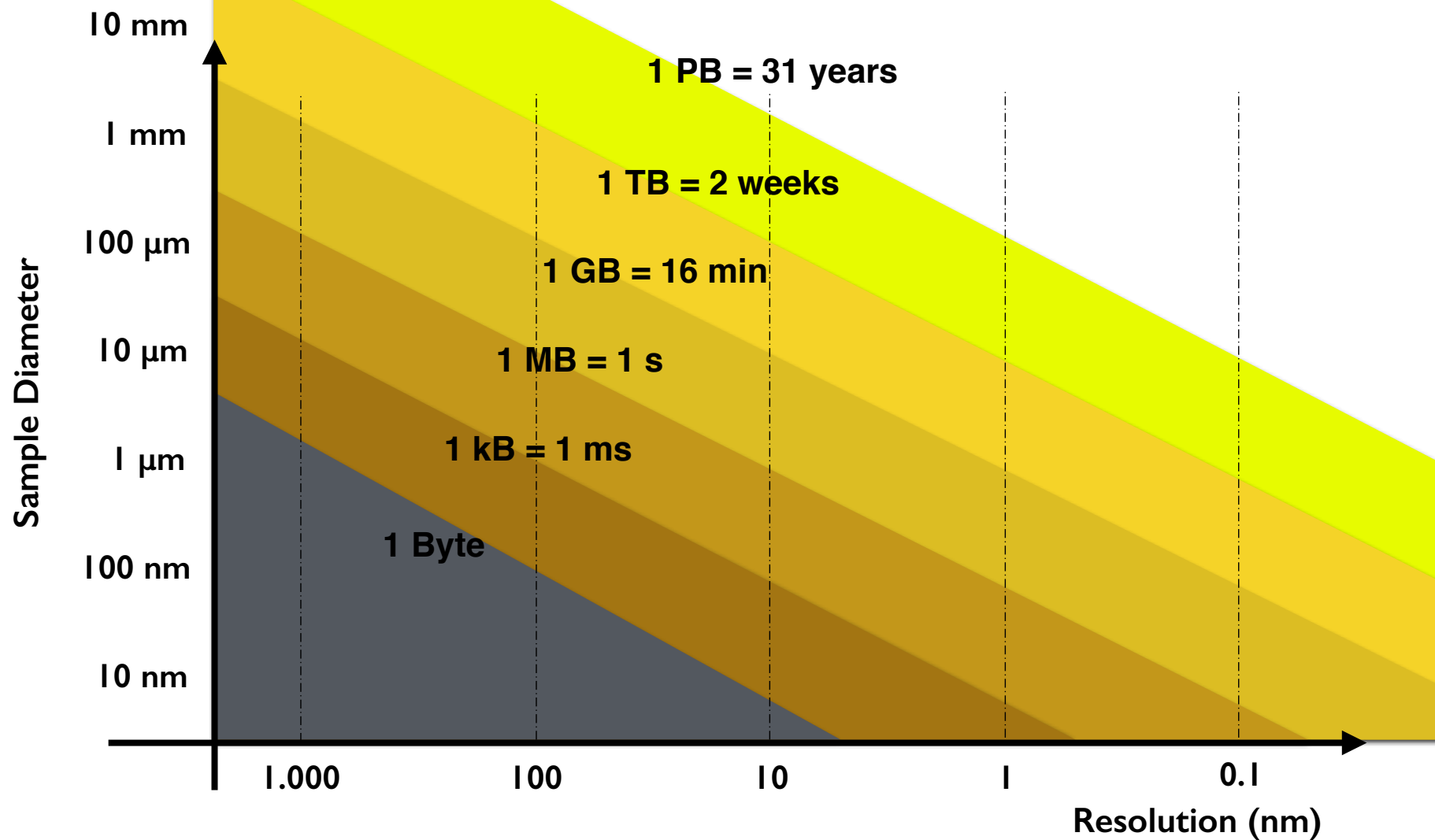
No single instrument can cover all scales.

# File size of a volume at a certain resolution



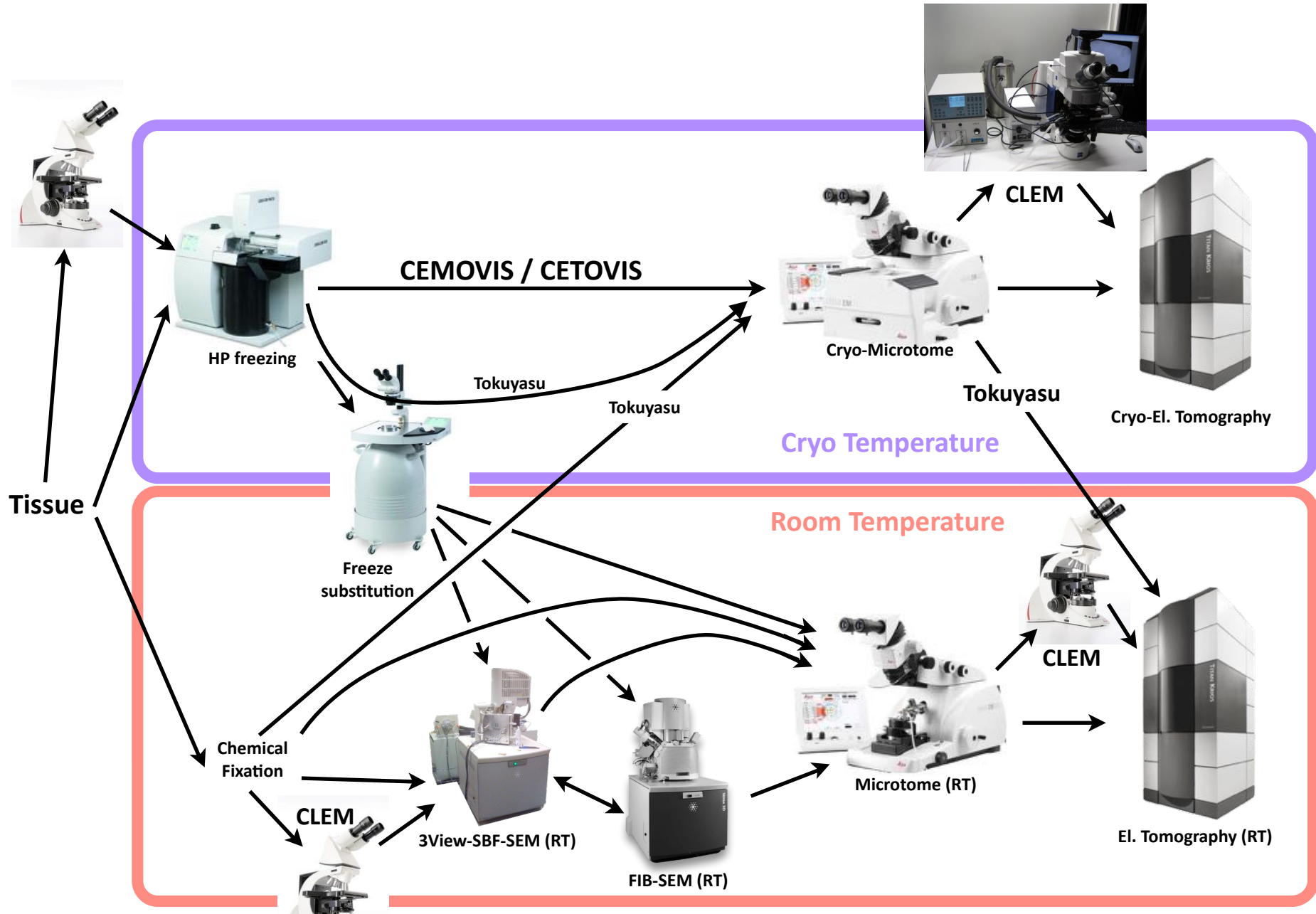
No single instrument can cover all scales.

# Data recording time, if recording at $1 \text{ px}/\mu\text{s}$



No single instrument can cover all scales.

# Specimen Workflows





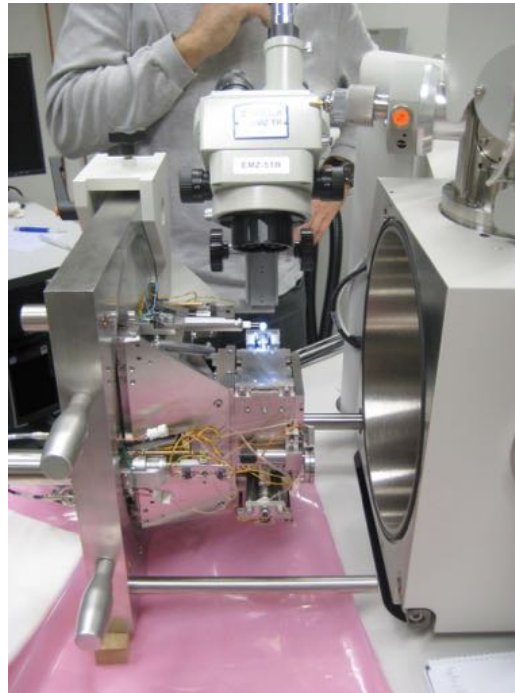
Christel Genoud (FMI)

# Quanta-200/3View: Serial Block Face SEM

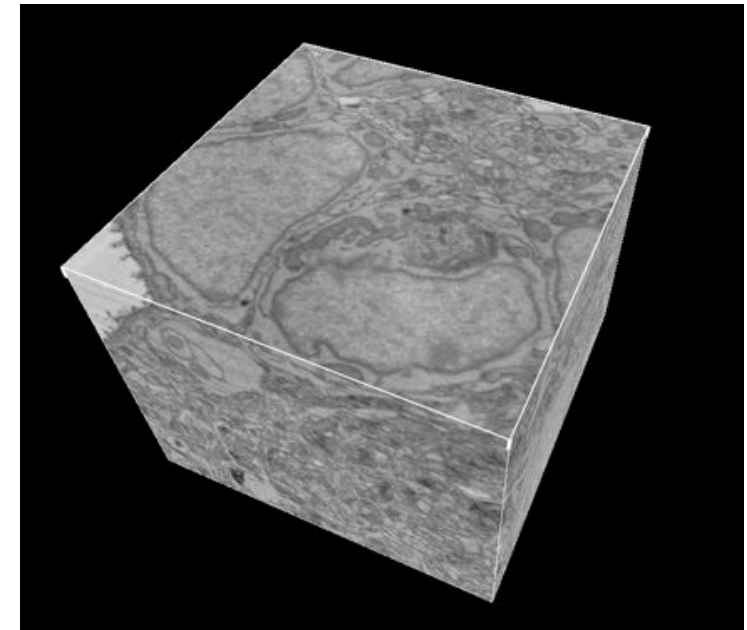
Olfactory bulb of zebrafish embryo:  
15x15x15  $\mu\text{m}$ : 500 cuts@30nm. 6nm per pixel  
Saved with isotropic voxel size:  
volume can be observed in all dimensions



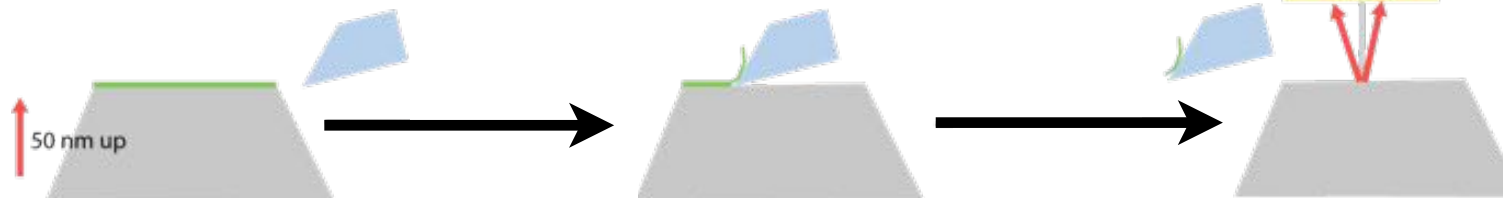
Room-Temperature  
Block in SEM



Shaved with microtome  
inside of the SEM



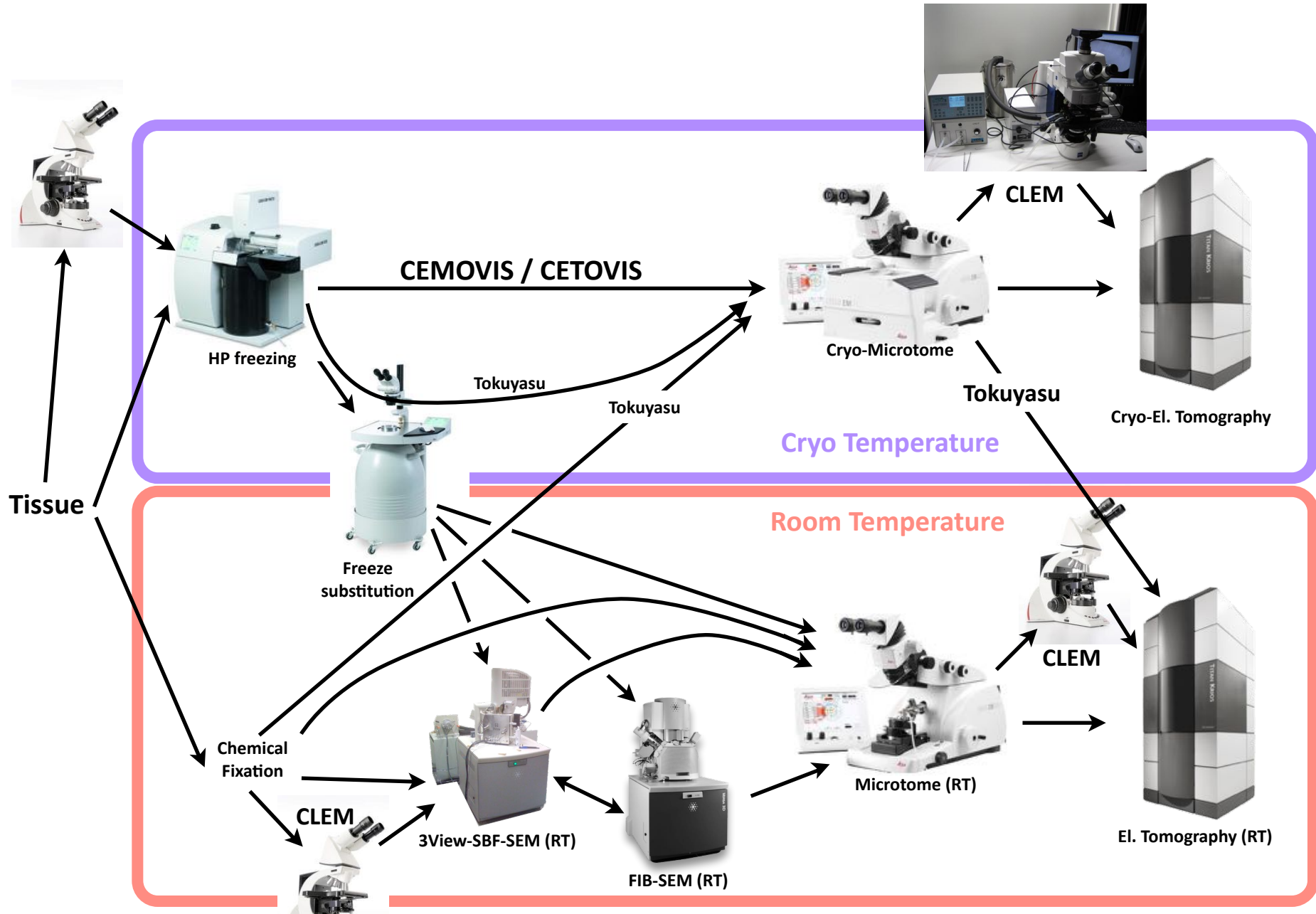
Remaining Block is  
imaged with SEM



Collaboration with Susan Gasser & Rainer Friedrich, FMI

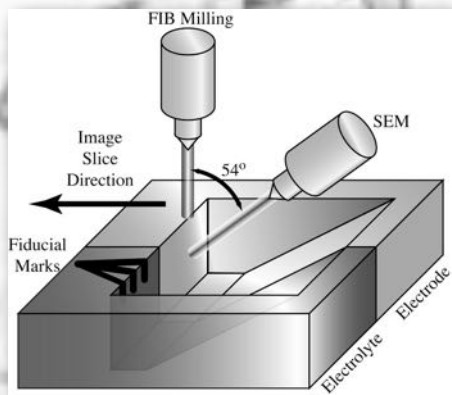
Denk W, Horstmann H (2004) PLoS Biology 11:e329

# Specimen Workflows



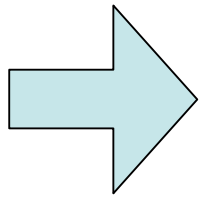
# Focussed Ion Beam SEM: FIB SEM, also called: Dual-beam

HeLa cell, 72hrs post infection with *Brucella abortus* bacteria  
(with Christoph Dehio, InfectX)



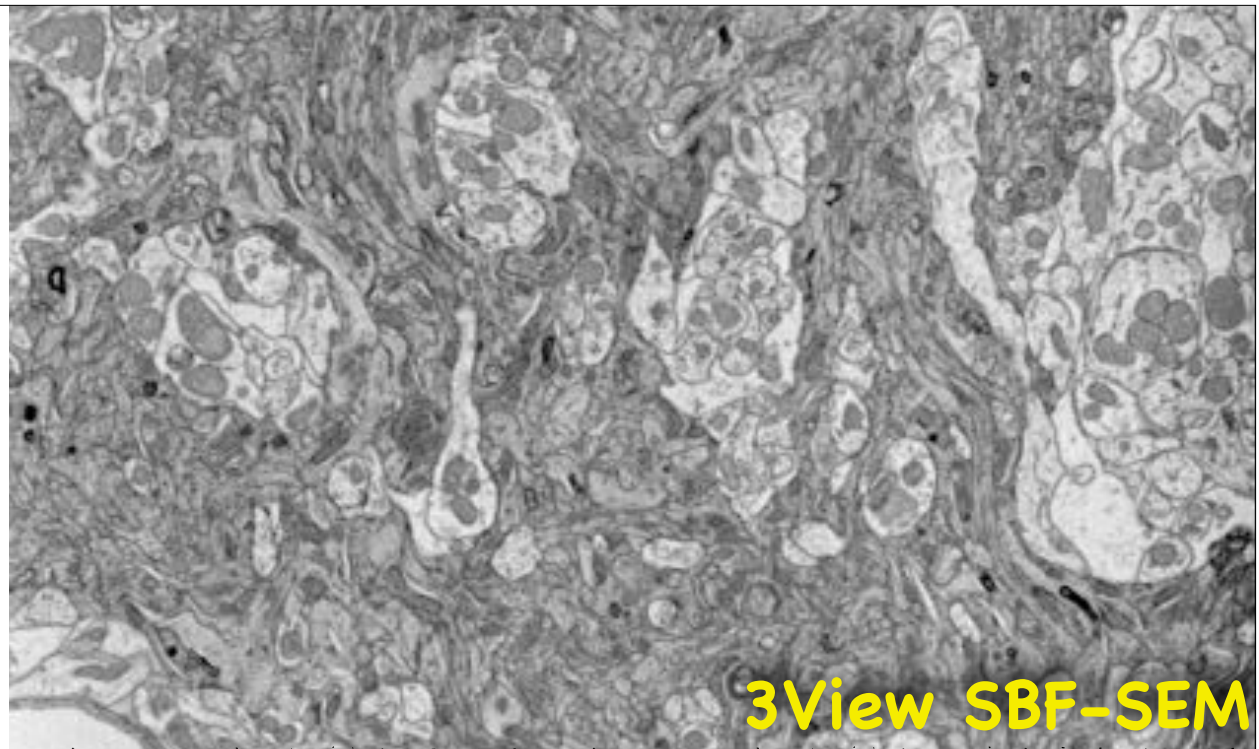
(c) Jarek Sedzicki, C-CINA



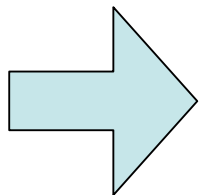


(3View) Serial Block Face  
Scanning Electron Microscopy  
(Christel Genoud, FMI/C-CINA)

*up to 700 $\mu$ m diameter  
at 10 nm resolution*

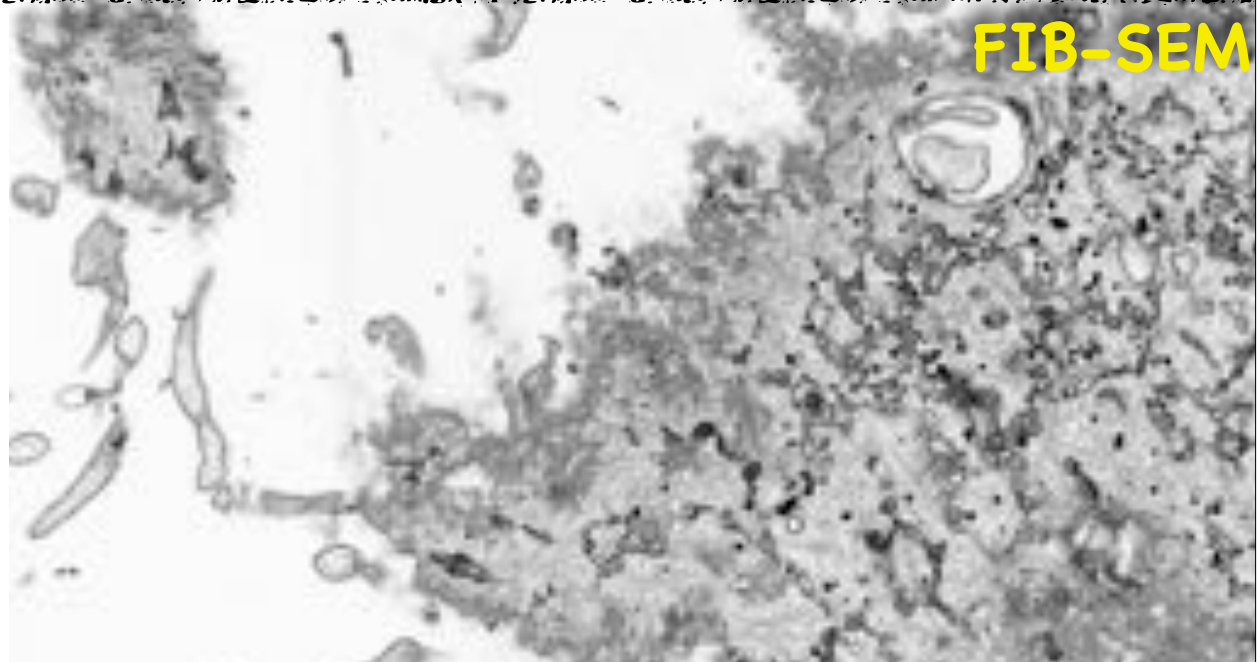


**3View SBF-SEM**

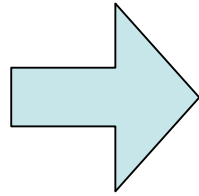


Focussed Ion Beam  
Scanning Electron Microscopy  
(Jarek Sędzicki, C-CINA)

*up to 100 $\mu$ m diameter  
at 3 nm resolution*



**FIB-SEM**



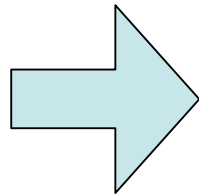
(3View) Serial Block Face  
Scanning Electron Microscopy  
(Christel Genoud, FMI/C-CINA)

*up to 700 $\mu$ m diameter  
at 10 nm resolution*

- (3View) SBF-SEM is ideal to study the morphology of biological tissue.
- Currently only on room-temperature, fixed and stained samples.
- Can easily be combined with thin-sectioning TEM.
- Might be extended to CLEM, and combined with EDAX, cathodoluminescence imaging, ion mass spectrometry, ...

**3View SBF-SEM**

**FIB-SEM**

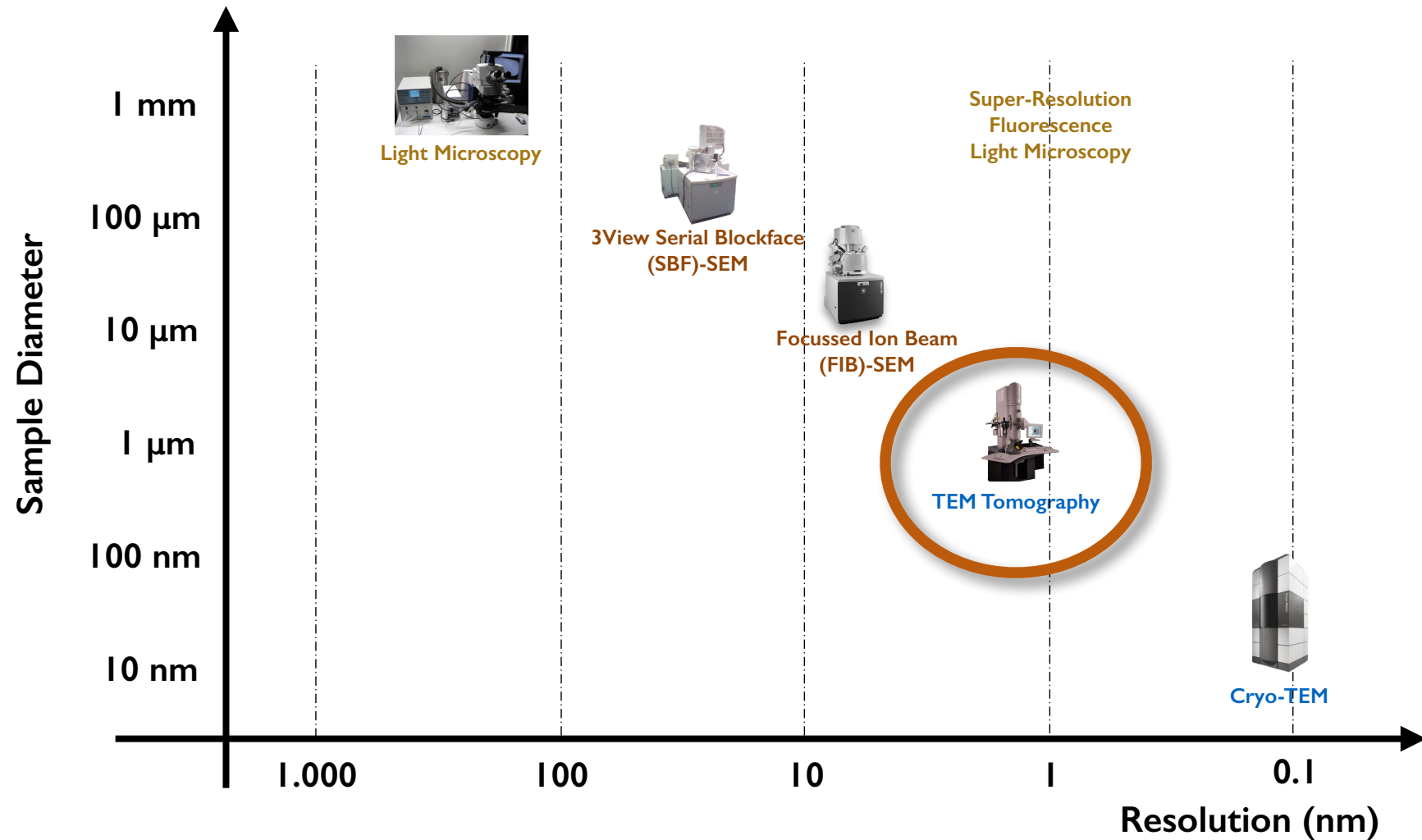


Focussed Ion Beam  
Scanning Electron Microscopy  
(Jarek Sędzicki, C-CINA)

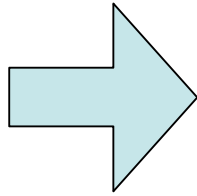
*up to 100 $\mu$ m diameter  
at 3 nm resolution*

- FIB-SEM is ideal to study the morphology of one cell.
- Mostly at room-temperature, difficult in cryo.
- Can be combined with 3View or thin-sectioning TEM.
- Might be extended to CLEM, and combined with EDAX, cathodoluminescence imaging, ion mass spectrometry, ...

# Multi-Resolution 3D Microscopy



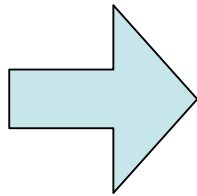
No single instrument can cover all scales.



### TEM Tomography

*up to 1  $\mu\text{m}$  in diameter  
at 2 nm resolution*

- Electron tomography is the best possible method to study thin cells (<1  $\mu\text{m}$ ) by EM (thinner is better).
- Can be applied to sections of cells (CETOVIS), can be done in cryo.
- Can easily be combined with LM (CLEM).
- Can be applied to serial sections (e.g., Brad Marsh's work)
- Can be extended by sub-volume averaging.

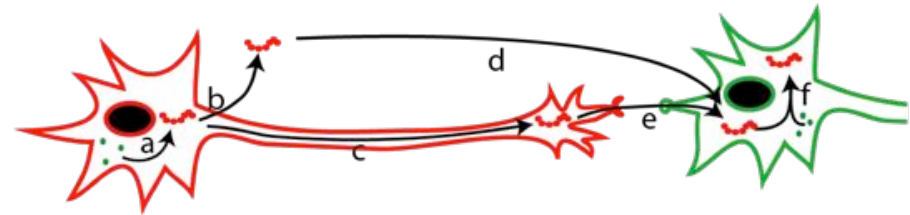
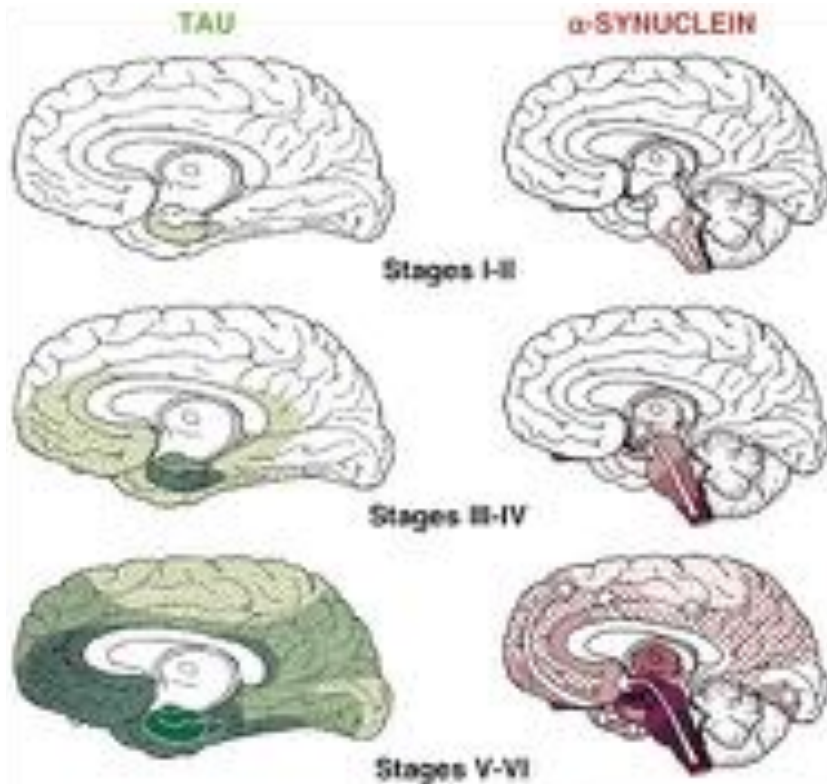


### STEM Tomography

*up to 2  $\mu\text{m}$  diameter  
at 3 nm resolution*

- STEM Tomography allows to image slightly thicker samples than TEM tomography.
- Only amplitude contrast can be recorded.
- The resolution is better in the upper half of the sample.
- Technically challenging.

# Prionoid fibril strains and Neurodegeneration



Investigations at the level of

- Tissue,
- Cellular,
- Membrane,
- and Fibrils.

Goedert *et al.*, Trends in Neurosciences 33(7), 317-325 (2010)

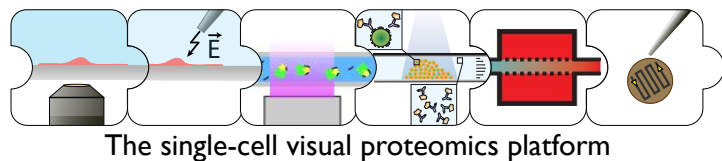
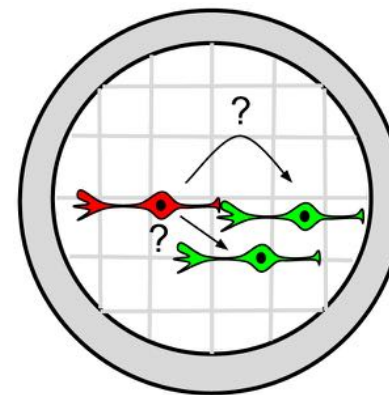
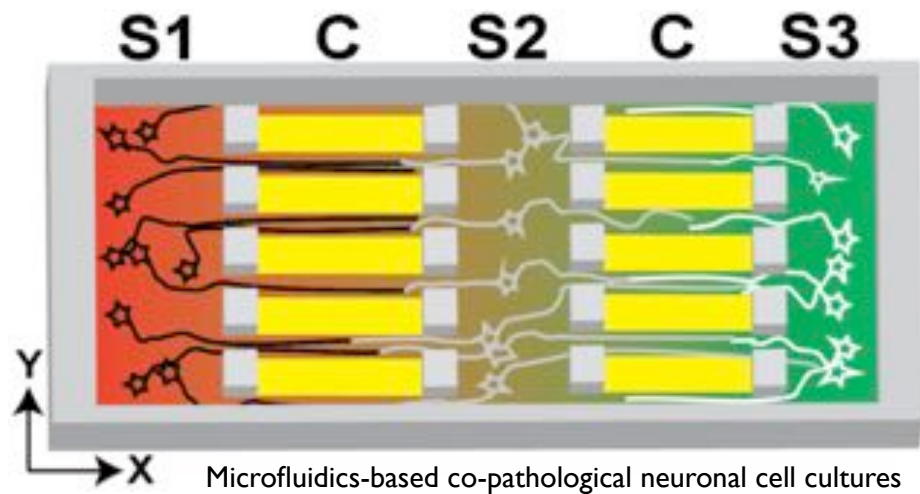
## Diseases related to tau neurofibrillar tangles

- Alzheimer's Disease (AD)
- Agyrophilic Grain Disease
- Corticobasal Degeneration
- Frontotemporal Dementia (Pick's Disease)
- Progressive Supranuclear Palsy
- Tangle-only Dementia
- White matter tauopathy with globular glial inclusions

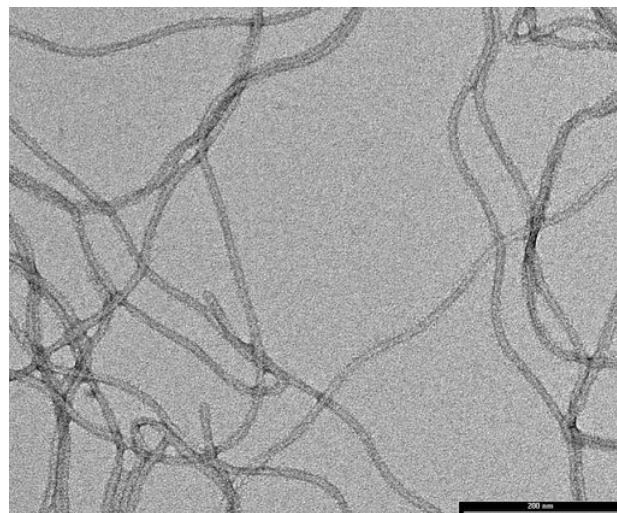
## Diseases related to $\alpha$ -Synuclein fibrils

- Parkinson's Disease (PD)
- Dementia with Lewy Bodies
- Lewy Body variant of AD
- Multiple System Atrophy
- Neurodegeneration with Brain Iron Accumulation (NBIA) Type I
- Parkinson's Disease with Dementia
- Pure Autonomic Failure (PAF) Disease

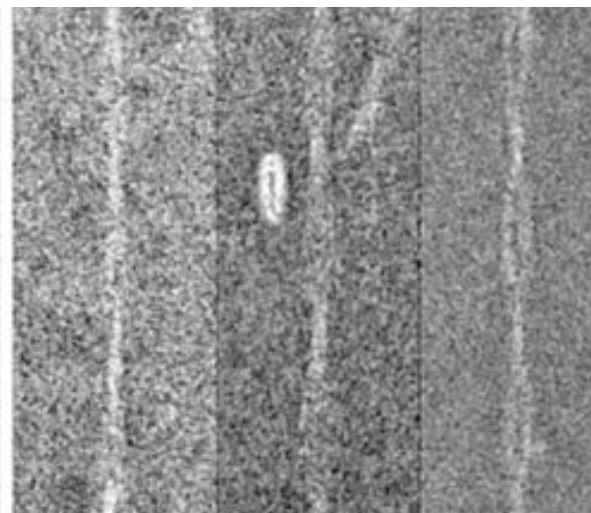
# Following the Spreading Process at the Single Cell Level



LUHMES cells (Marcel Leist, Konstanz):  
human mesencephalic cells that can be differentiated  
into neuron-like dopaminergic cells

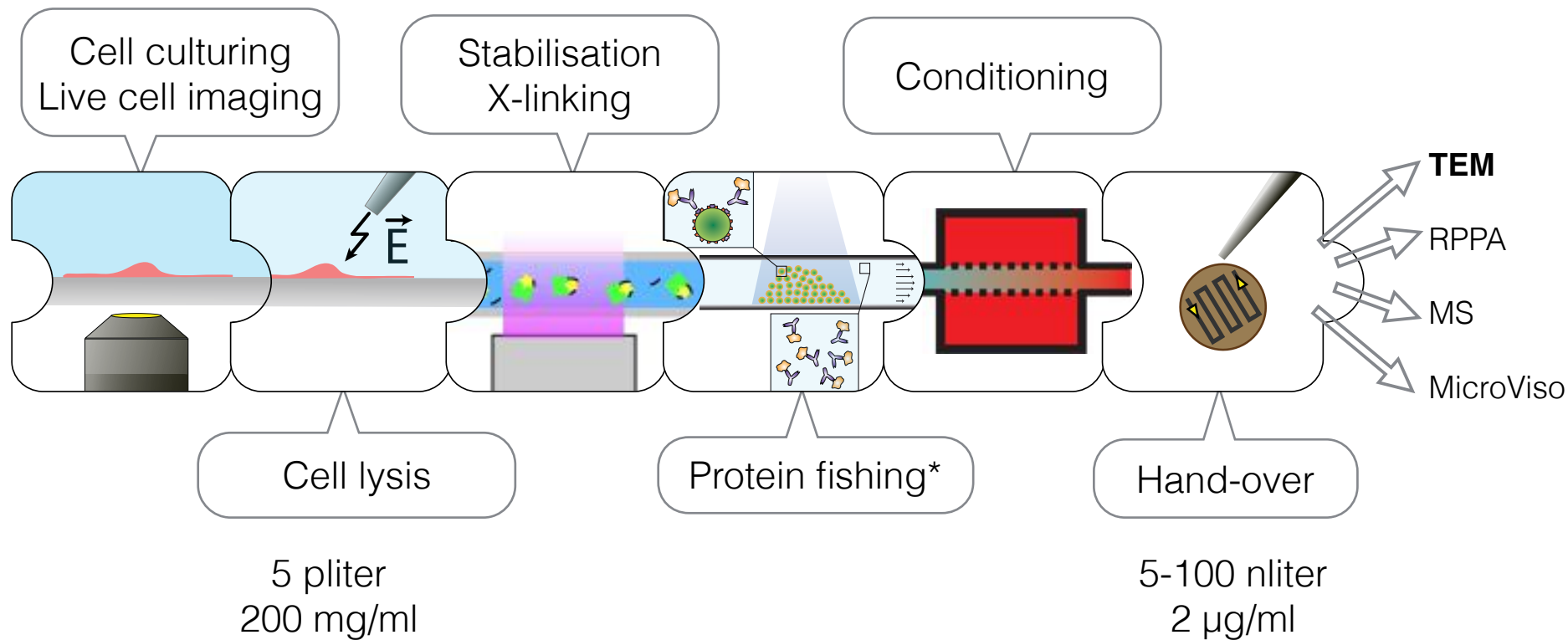
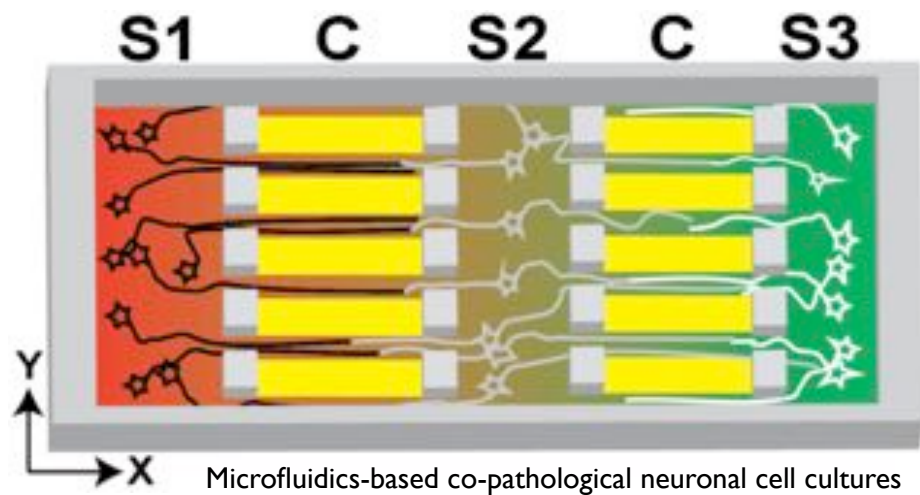


tau  
(Stahlberg lab)



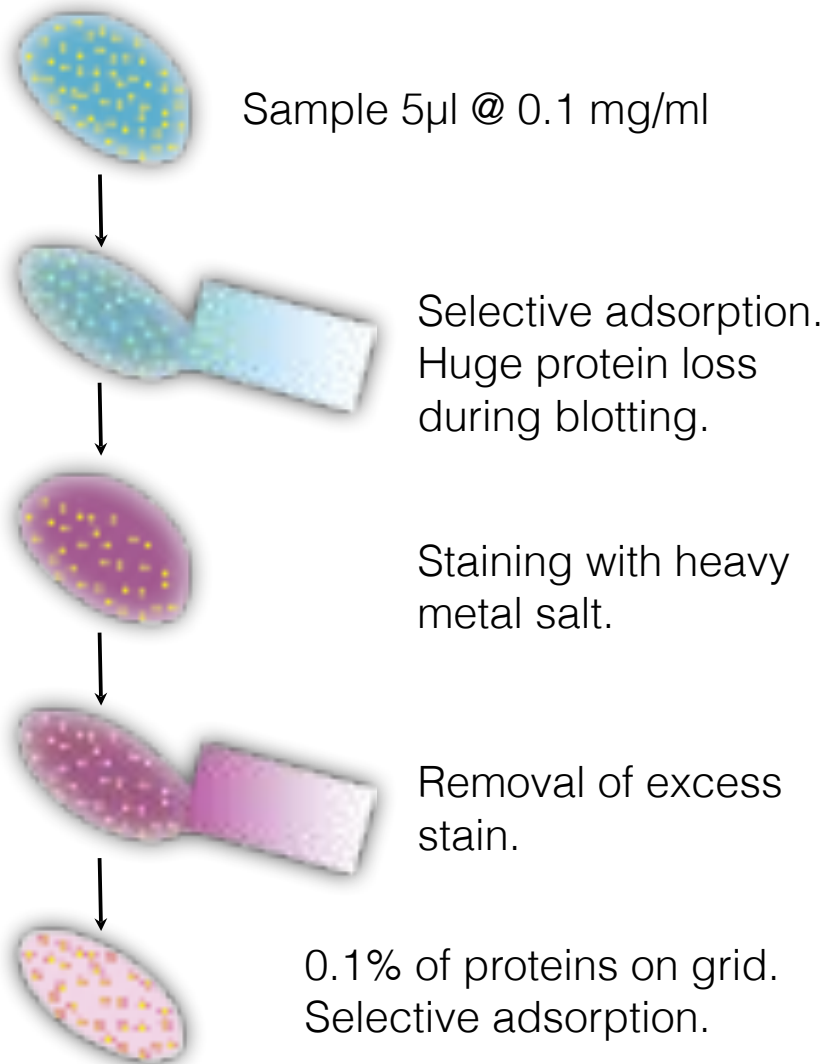
$\alpha$ -synuclein  
(Villars, Stahlberg, *et al.*, PNAS 2008)

# The single-cell visual proteomics pipeline



# Negative stain EM grid preparations

## “Classical”



## Total proteomics

**Single cell 5 pL @ 200 mg/ml**  
Cell lysis, mixing with heavy metal salt

Sample preparation in  $\mu$ -fluidics.



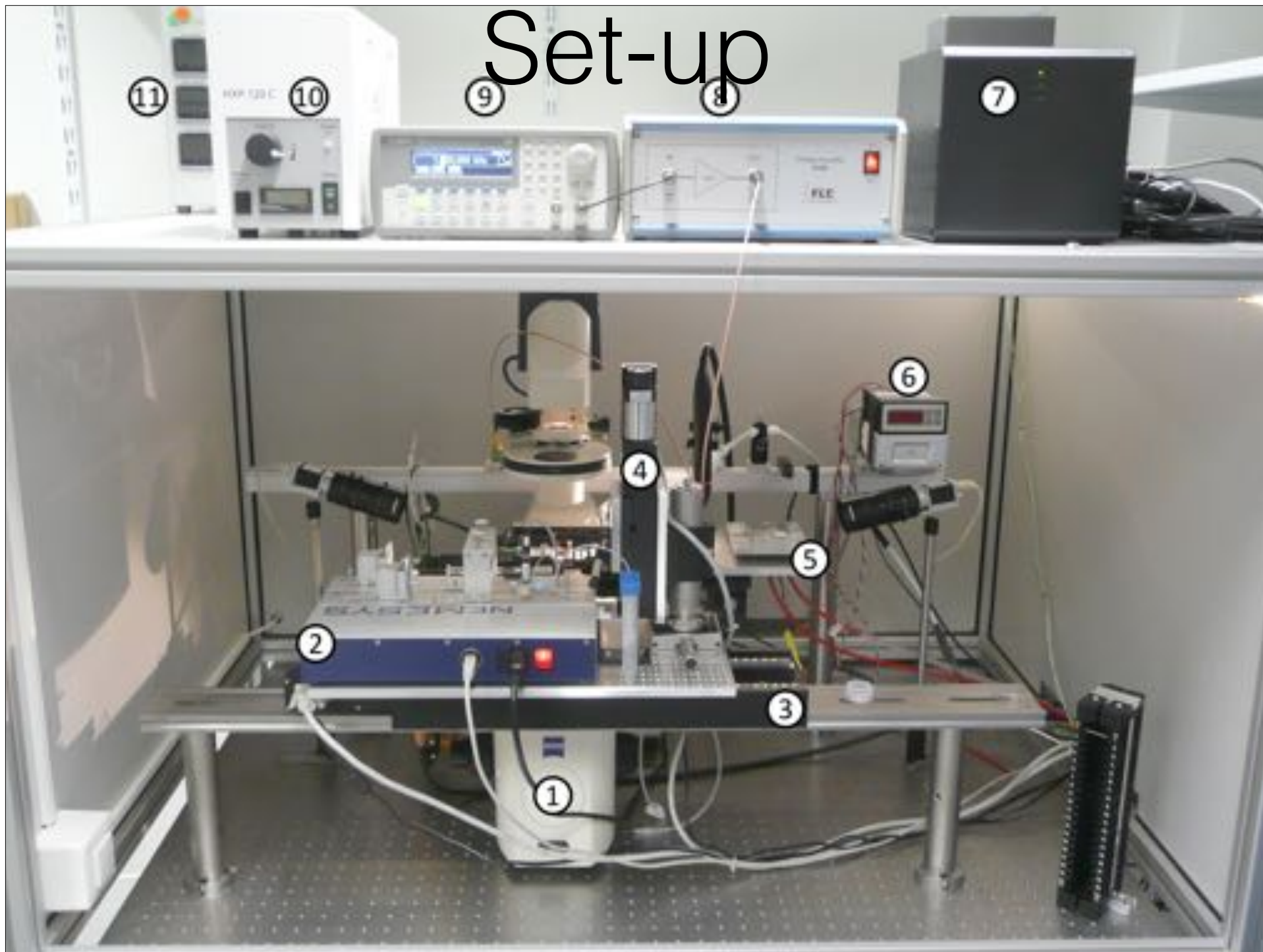
Drying of complete droplet.  
Up-concentration on grid.

100% of proteins on grid.  
No selective adsorption.

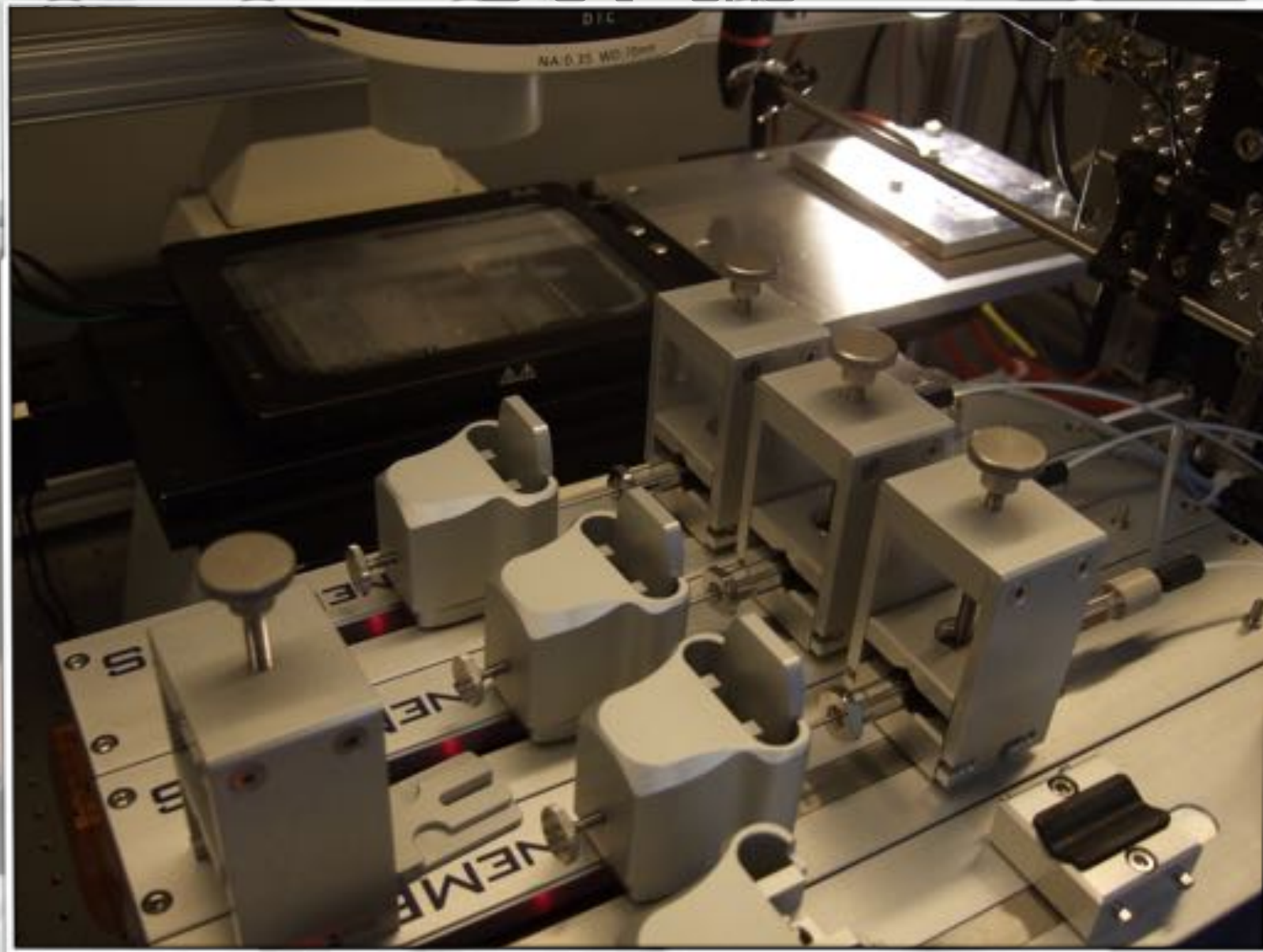
Kemmerling et al., 2012



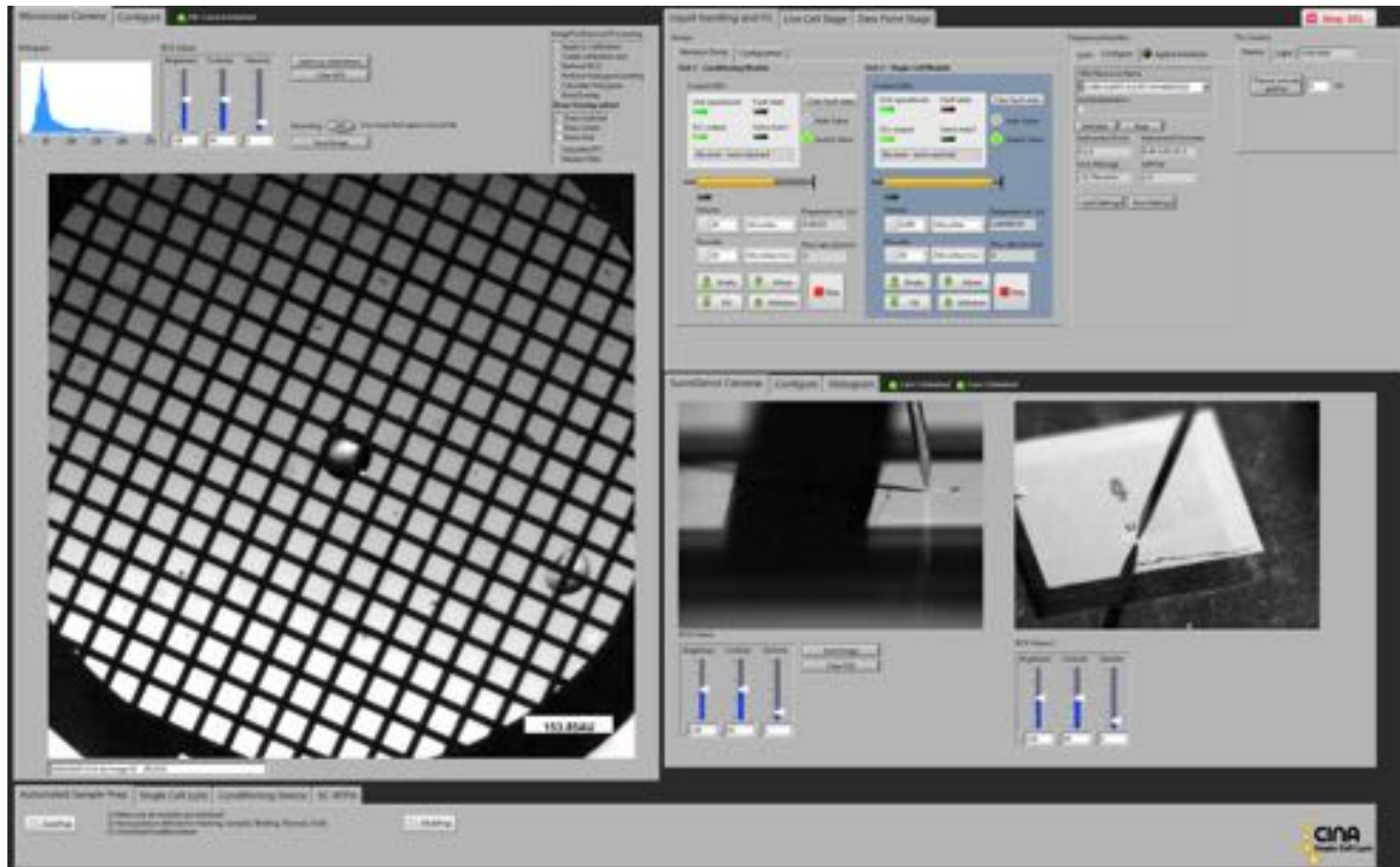
# Set-up



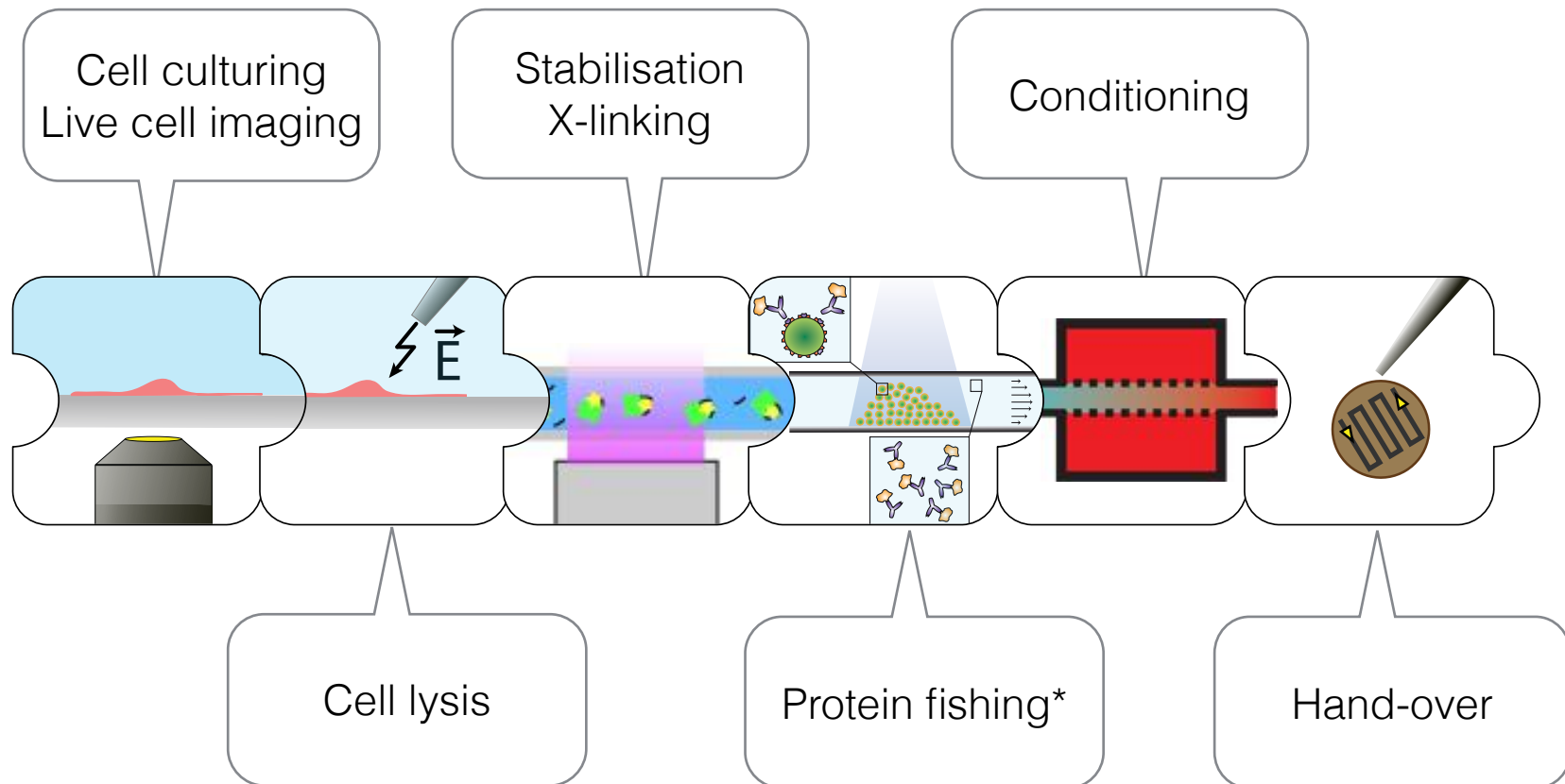
# Set-up



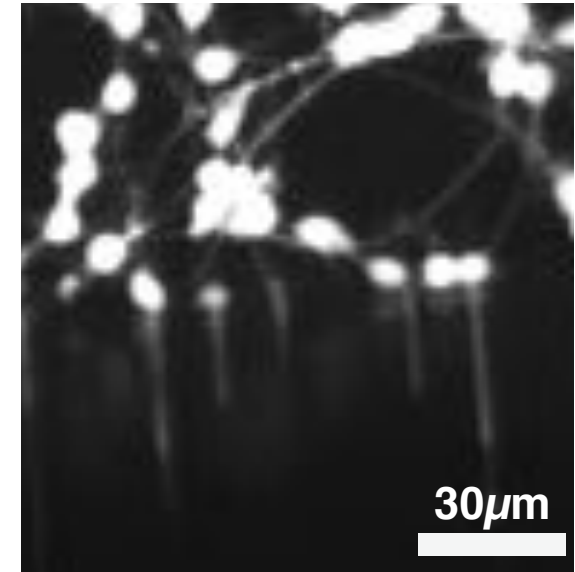
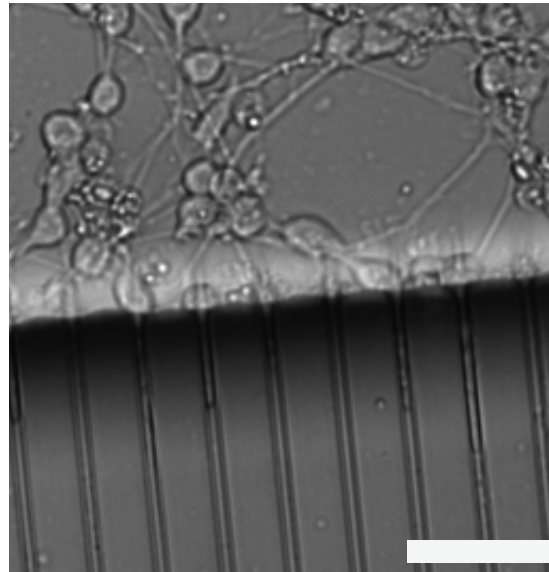
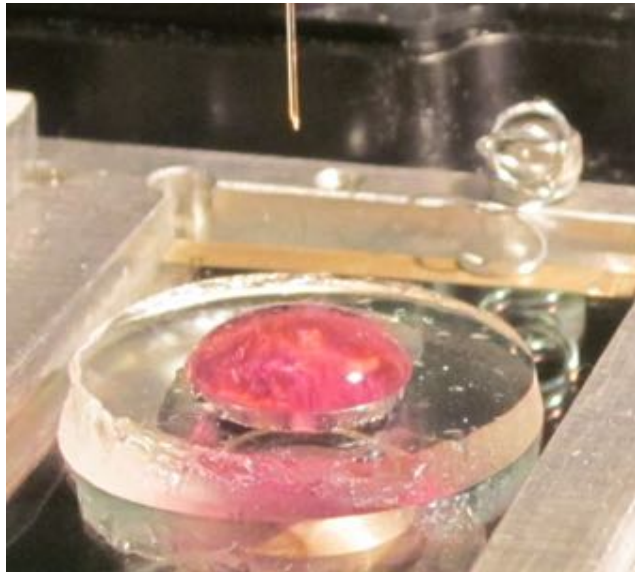
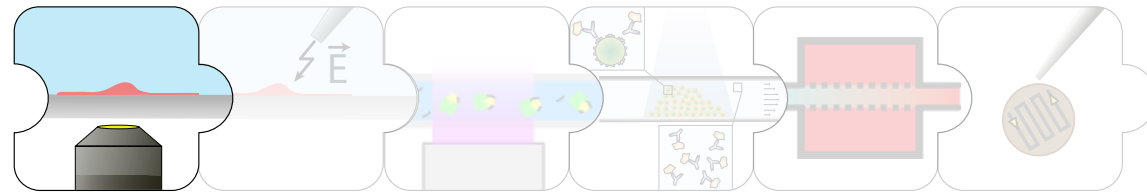
# OpenBEB – LabView-based instrument control with database integration



# Pipeline

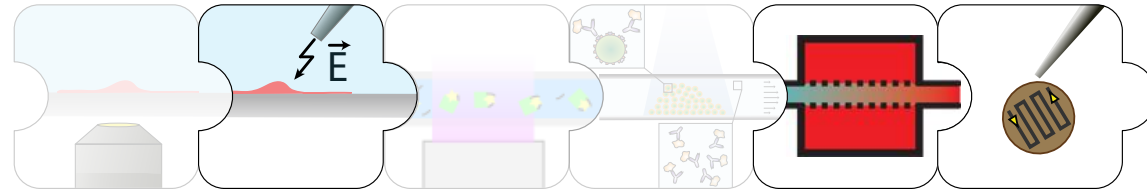


# Cell cultures



LUHMES (Lund Human Mesencephalic) cells, can be differentiated into dopaminergic, neuron-like cells. Cells express  $\alpha$ -synuclein and tau, and cytosolic GFP.

# Single cell lysis



Electro-lysis &  
aspiration of cytosol

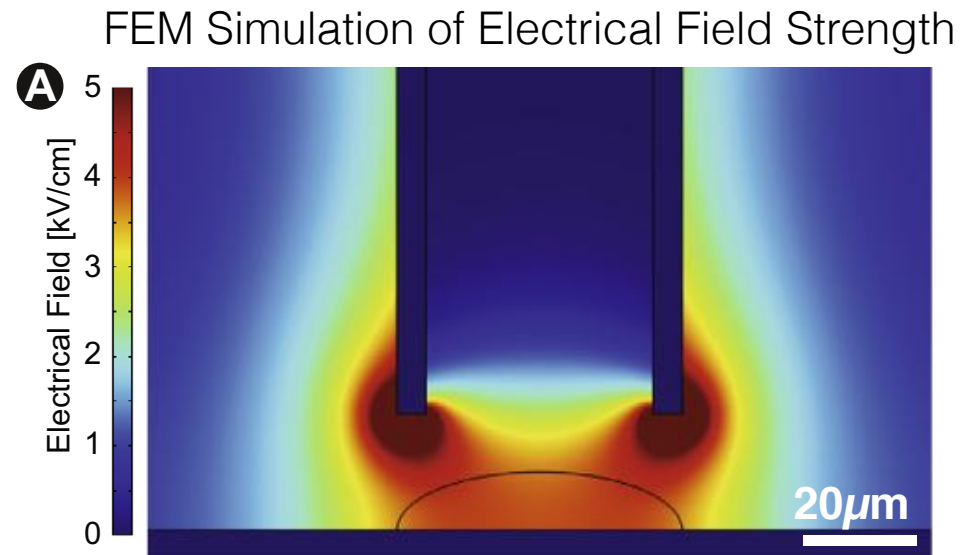
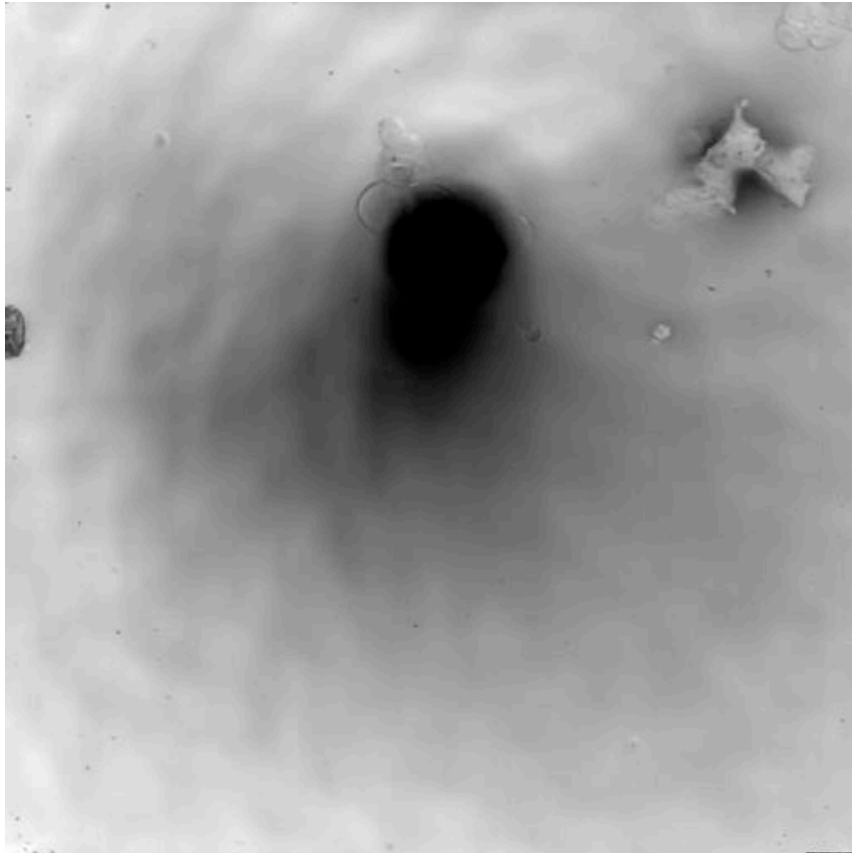


“Writing” onto  
EM grid or substrate



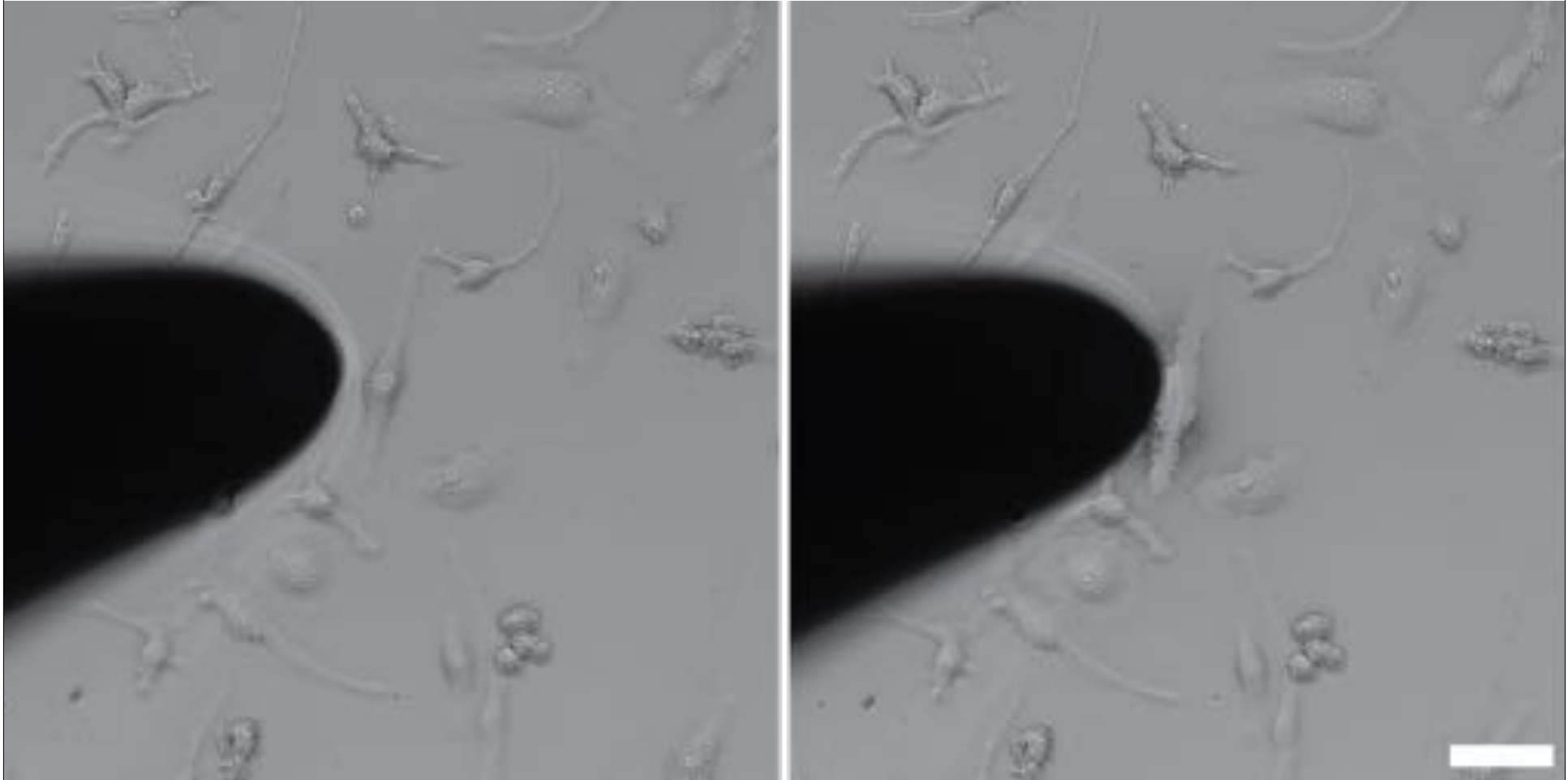
Volume handled: 5-100nl

# Single cell lysate



Arnold SA, *et al.* Single-cell lysis for visual analysis by electron microscopy. *J. Struct. Biol.* 2013; 183(3):467–73.

# Single cell lysate



Microganglion cells, collaboration with Petr Broz, Biozentrum Basel

Arnold SA, *et al.* Single-cell lysis for visual analysis by electron microscopy. *J. Struct. Biol.* 2013; 183(3):467–73.



# Single cell lysate

Stain: 0.1% Ammonium Molybdate

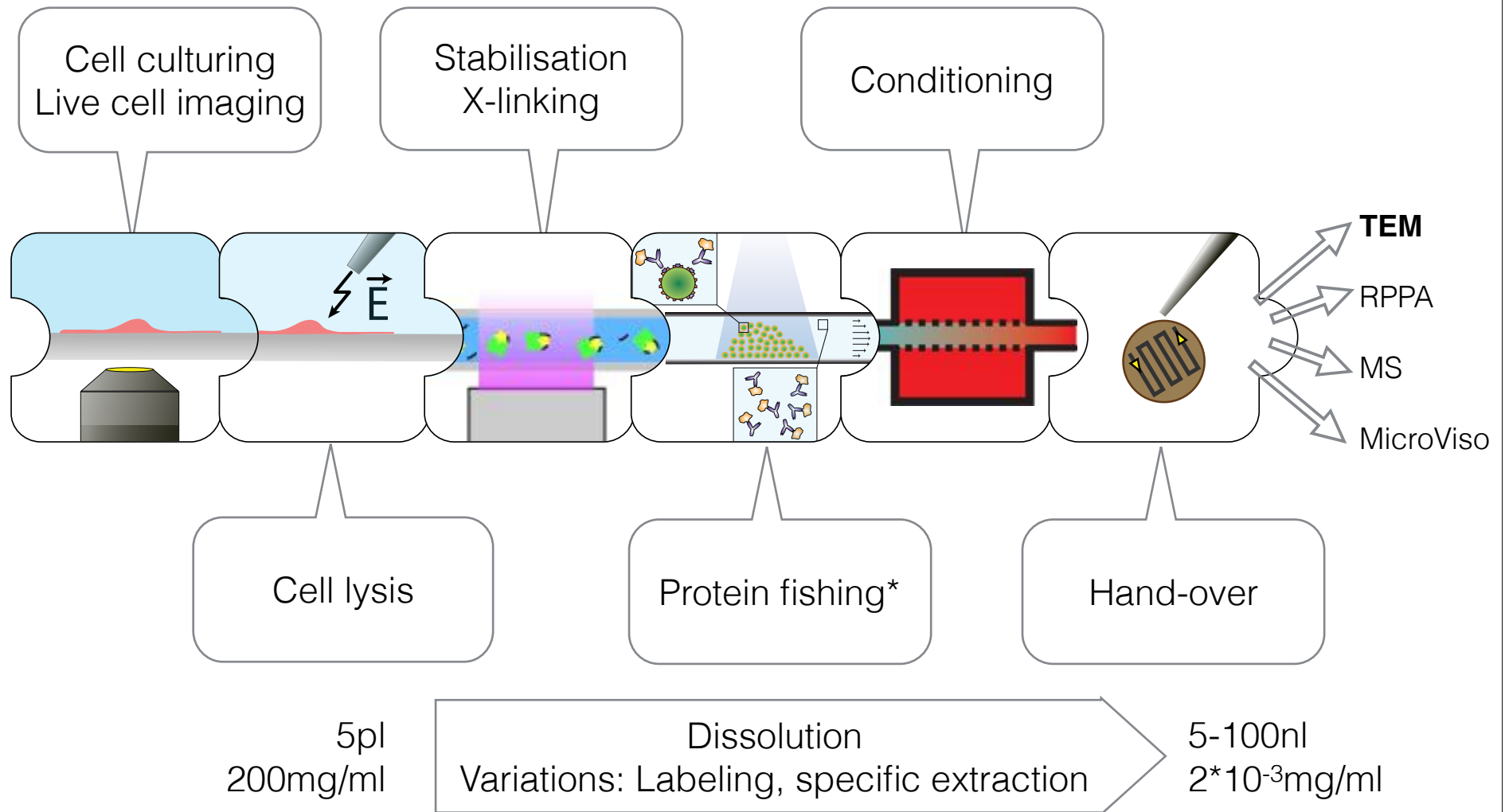
200nm

**Vault organelles**

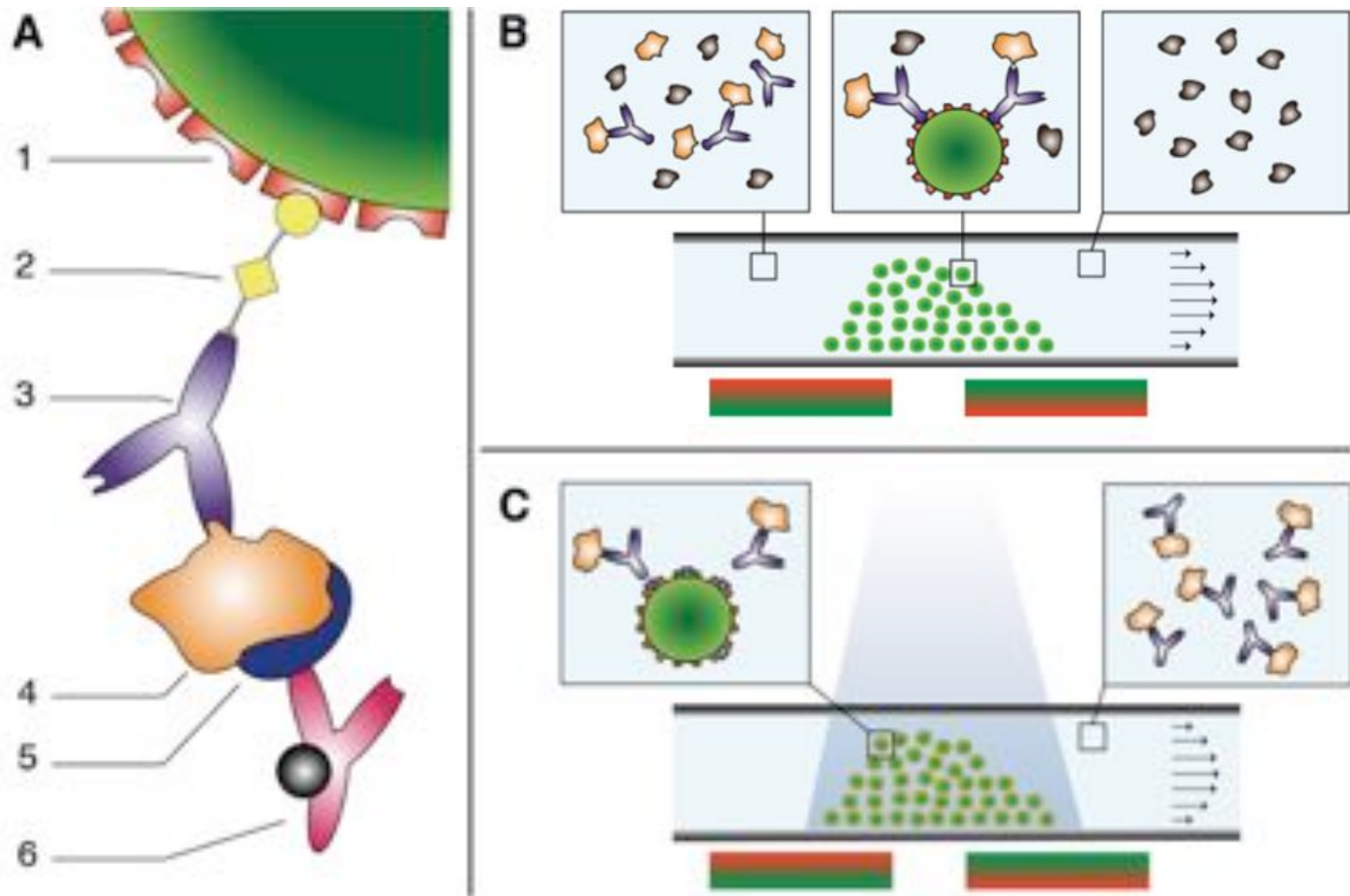
**Actin filaments**

50nm

# Single Cell Proteomics

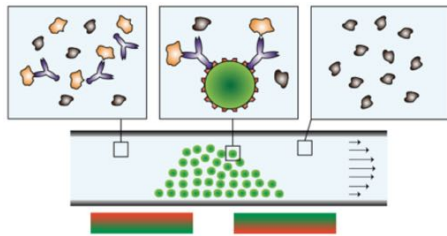


# Microfluidic Protein Purification



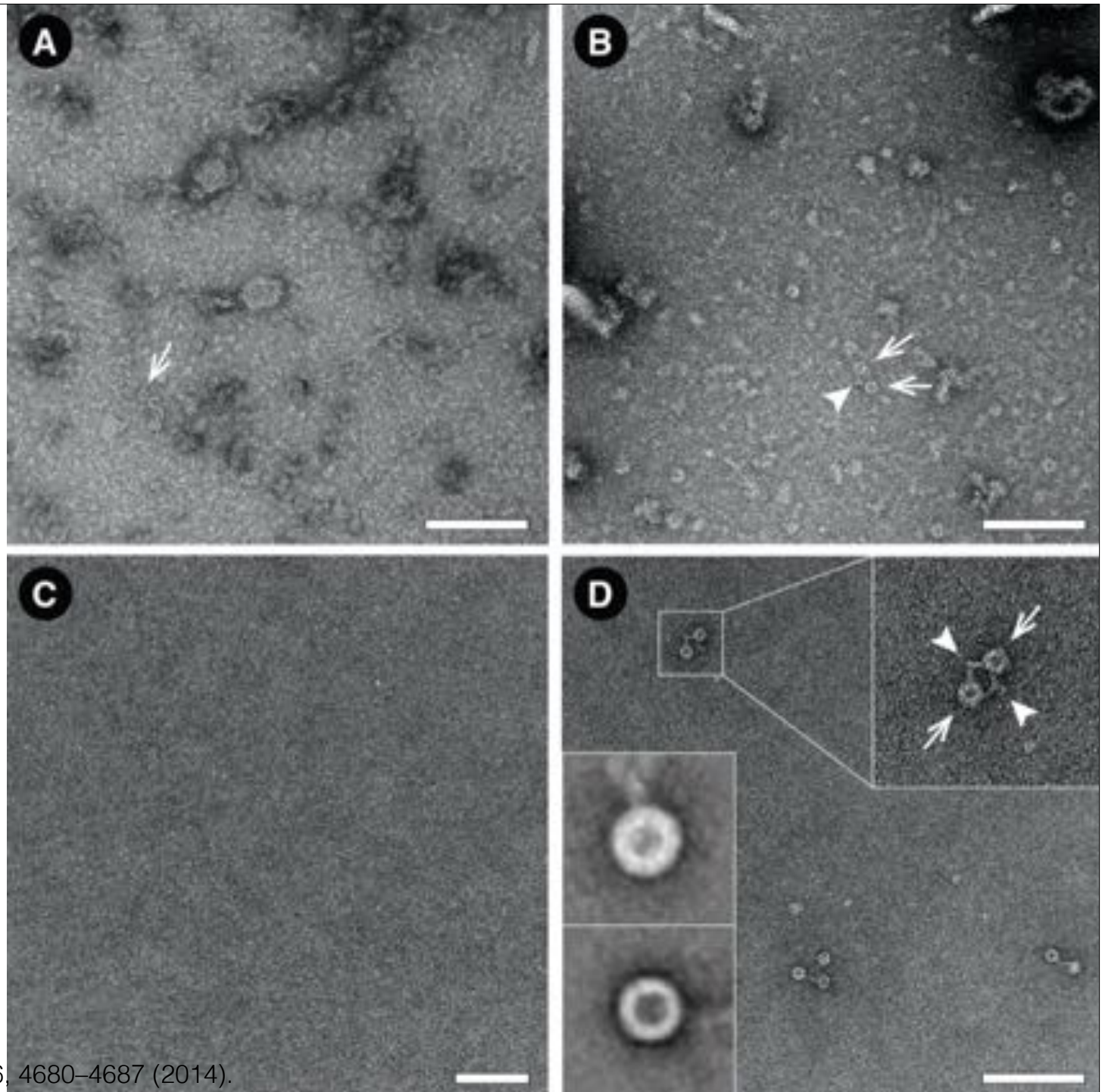
Giss, D., et al., Anal. Chem. 86, 4680–4687 (2014).

# Microfluidic Protein Purification



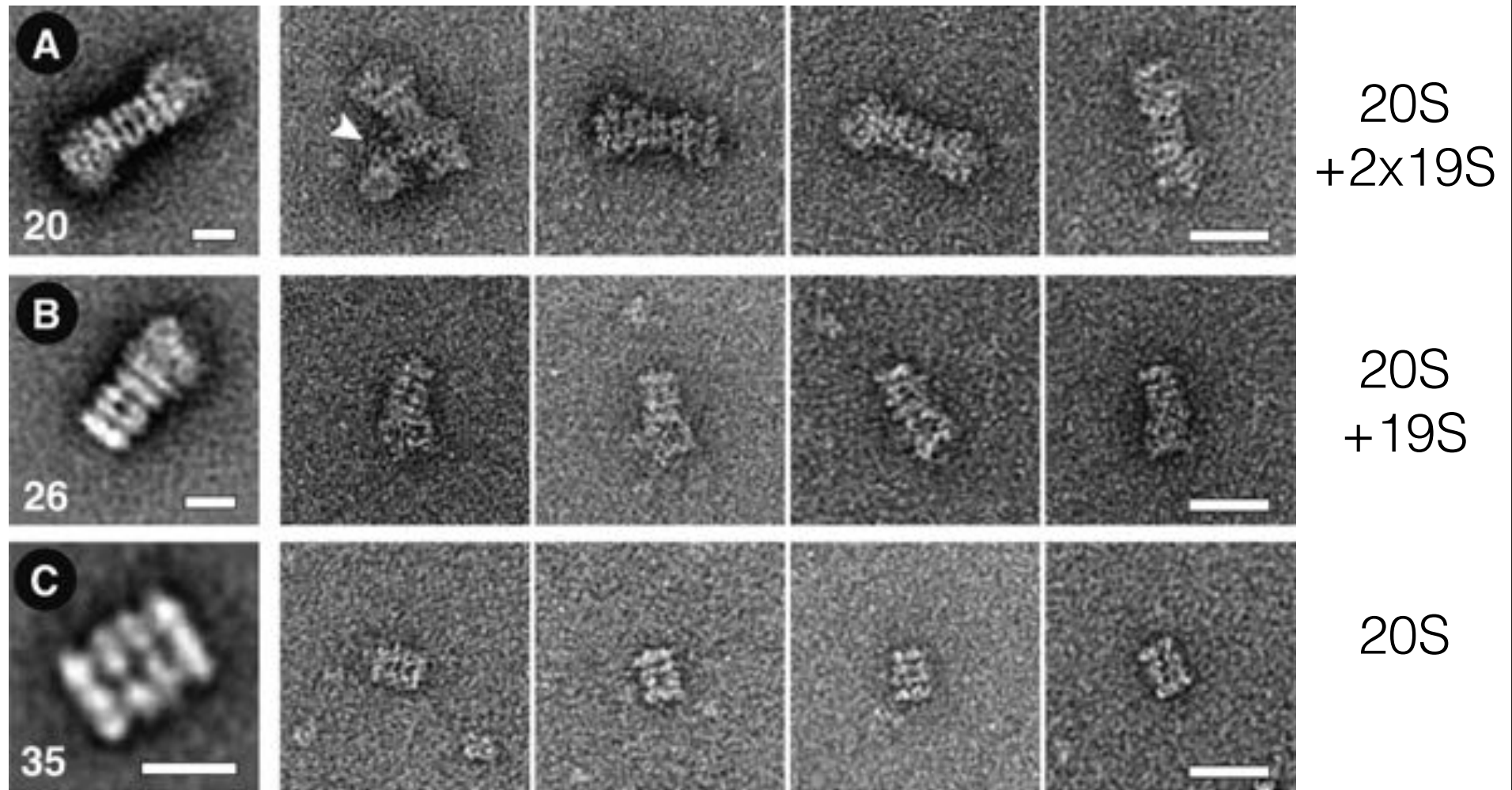
BHK cells,  
expressing  
apoferritin

A: Cytosol  
B: 1st Wash  
C: Last Wash  
D: Photo-Elute



Giss, D., et al., *Anal. Chem.* 86, 4680–4687 (2014).

# 20S proteasome fishing

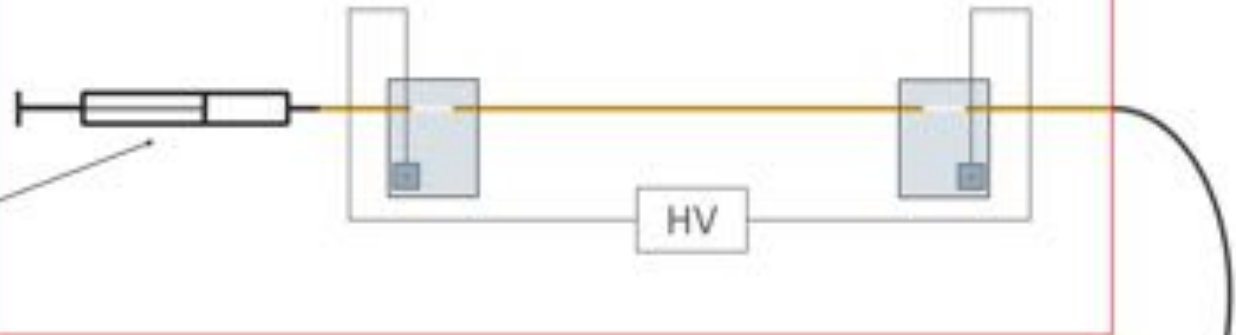


Giss, D., et al., Anal. Chem. 86, 4680–4687 (2014).

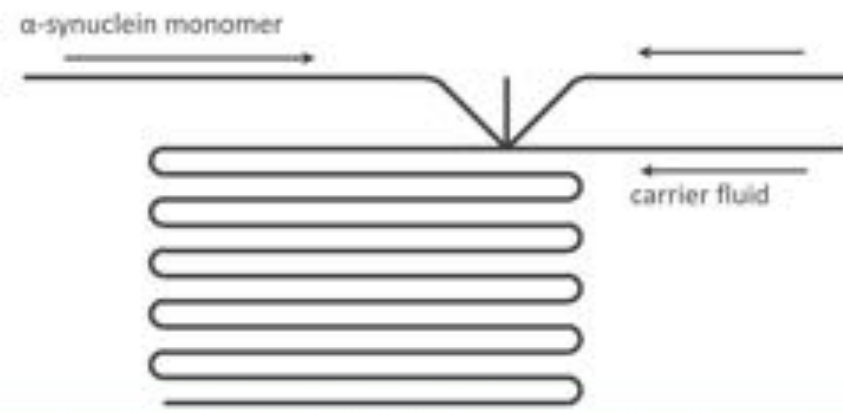
# What is the inoculator for fibril formation?



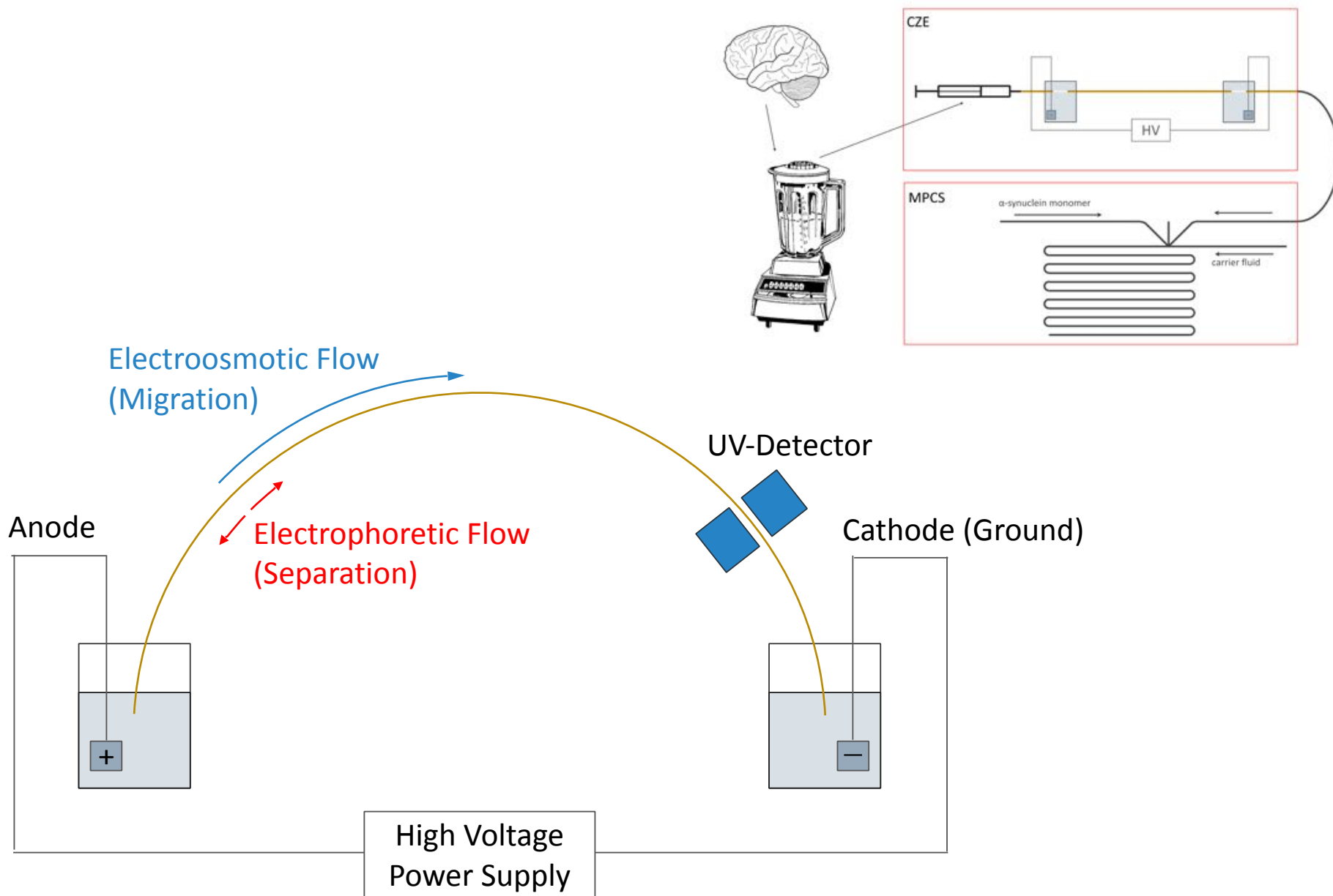
## Capillary Zone Electrophoresis



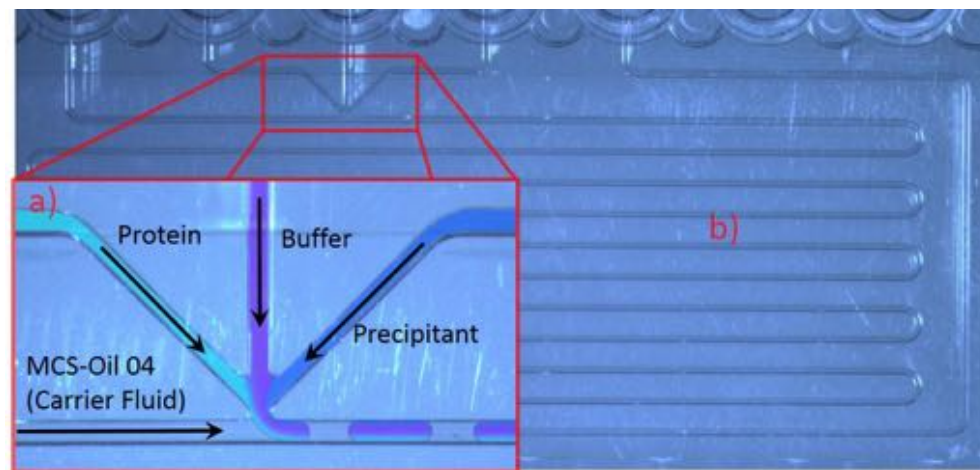
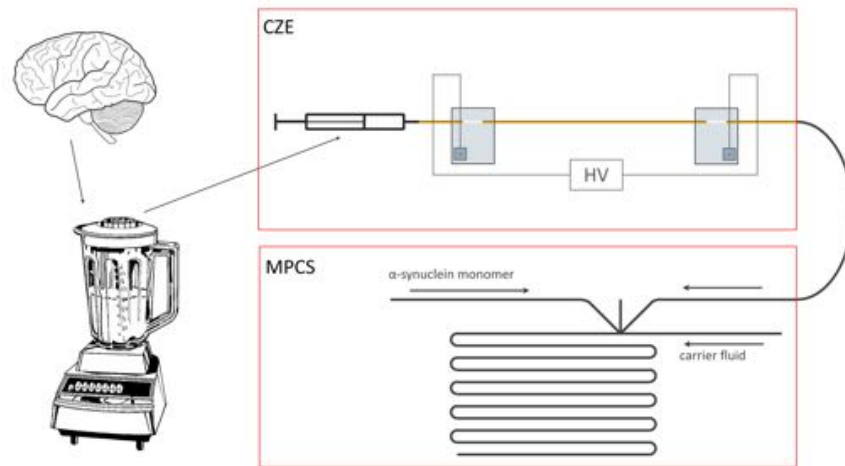
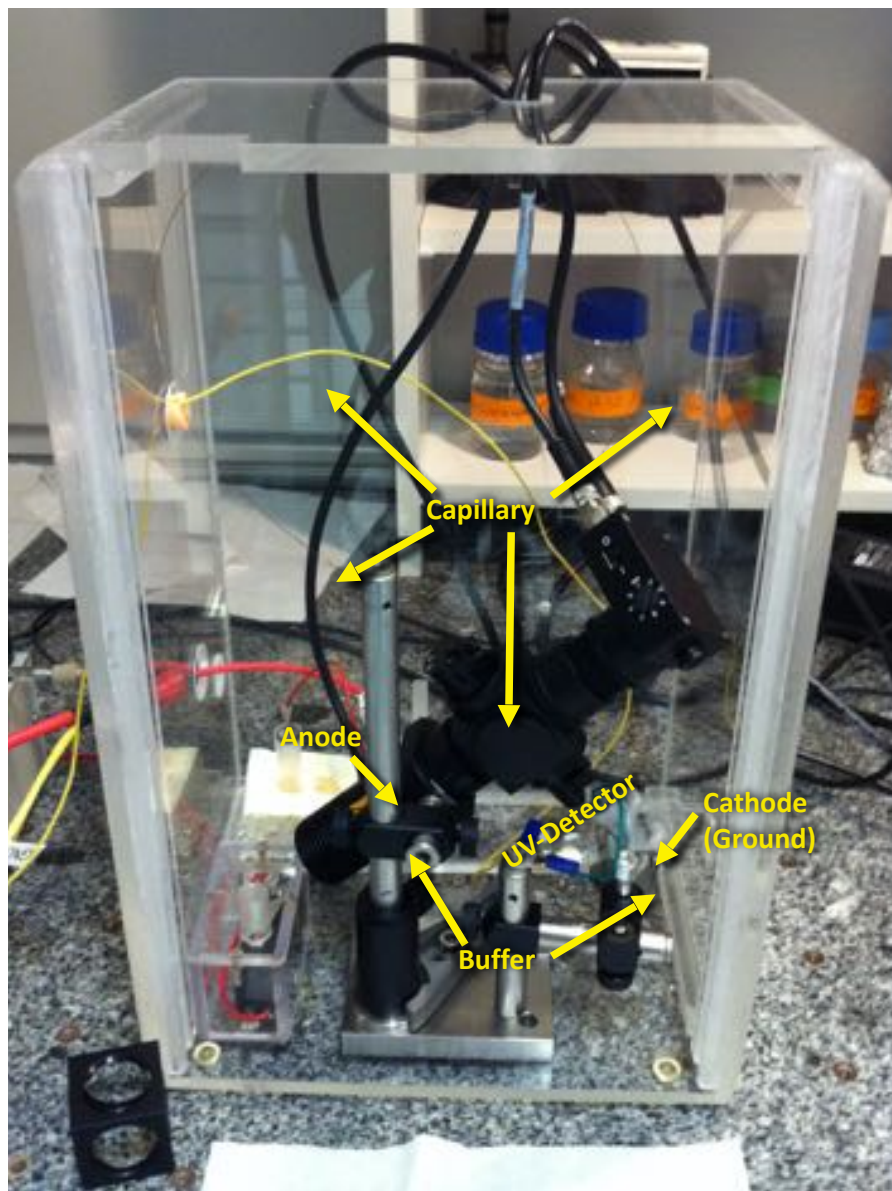
## Microcapillary Protein Crystallization System



# What is the inoculator for fibril formation?

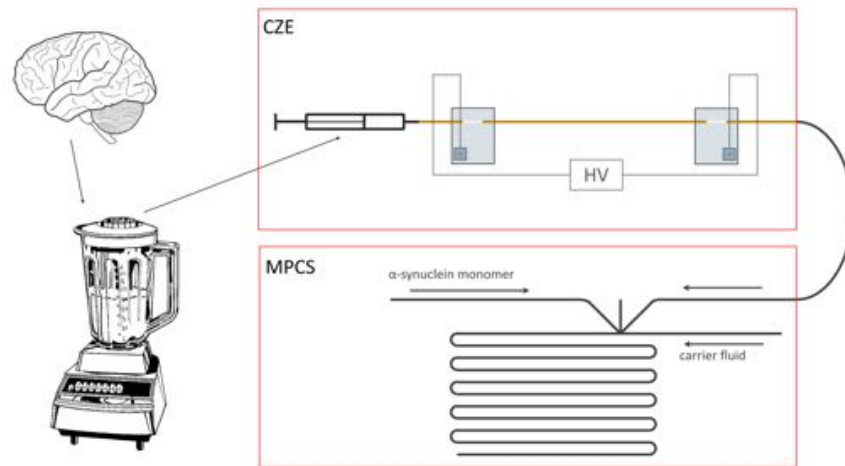
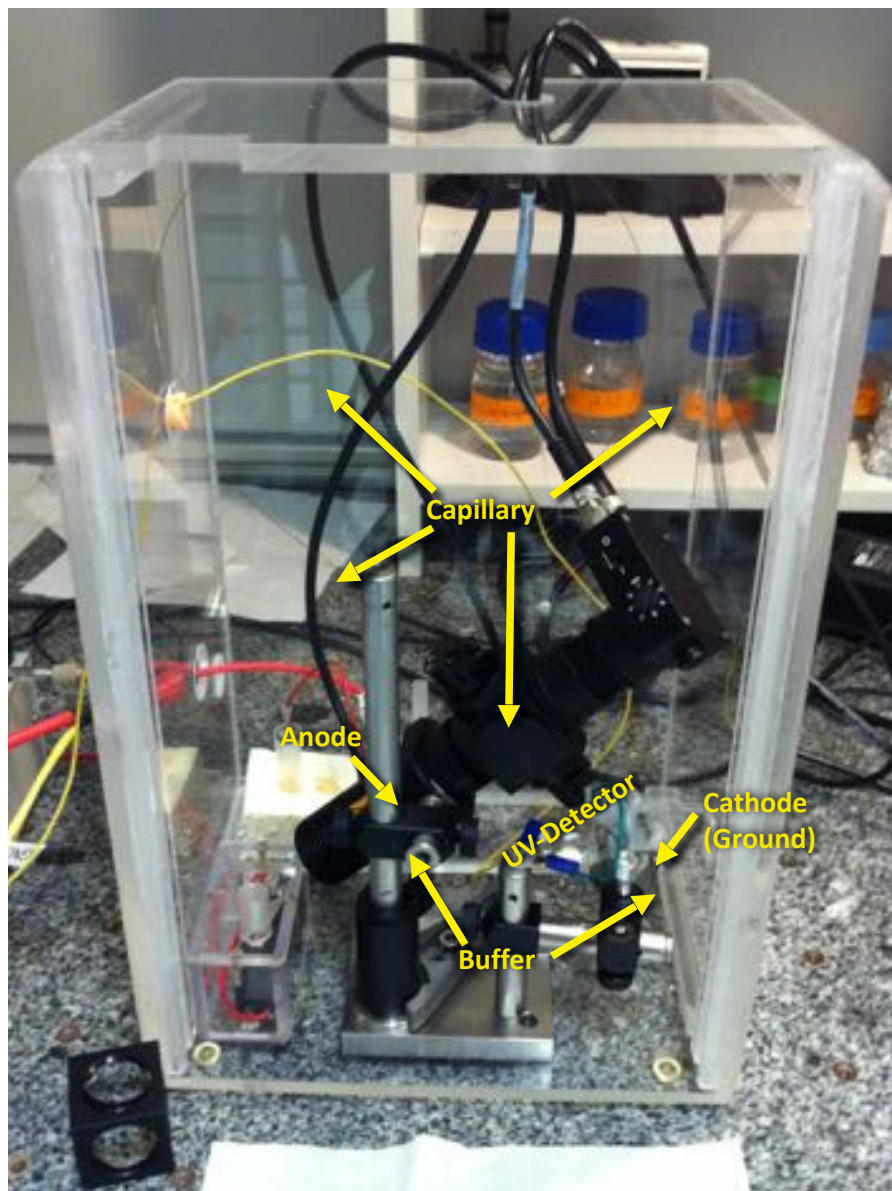


# What is the inoculator for fibril formation?

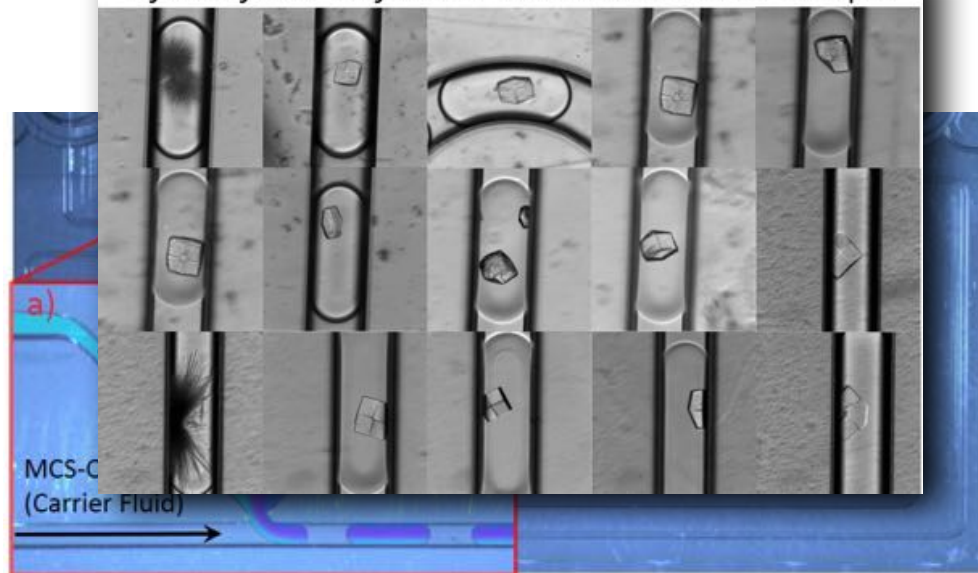




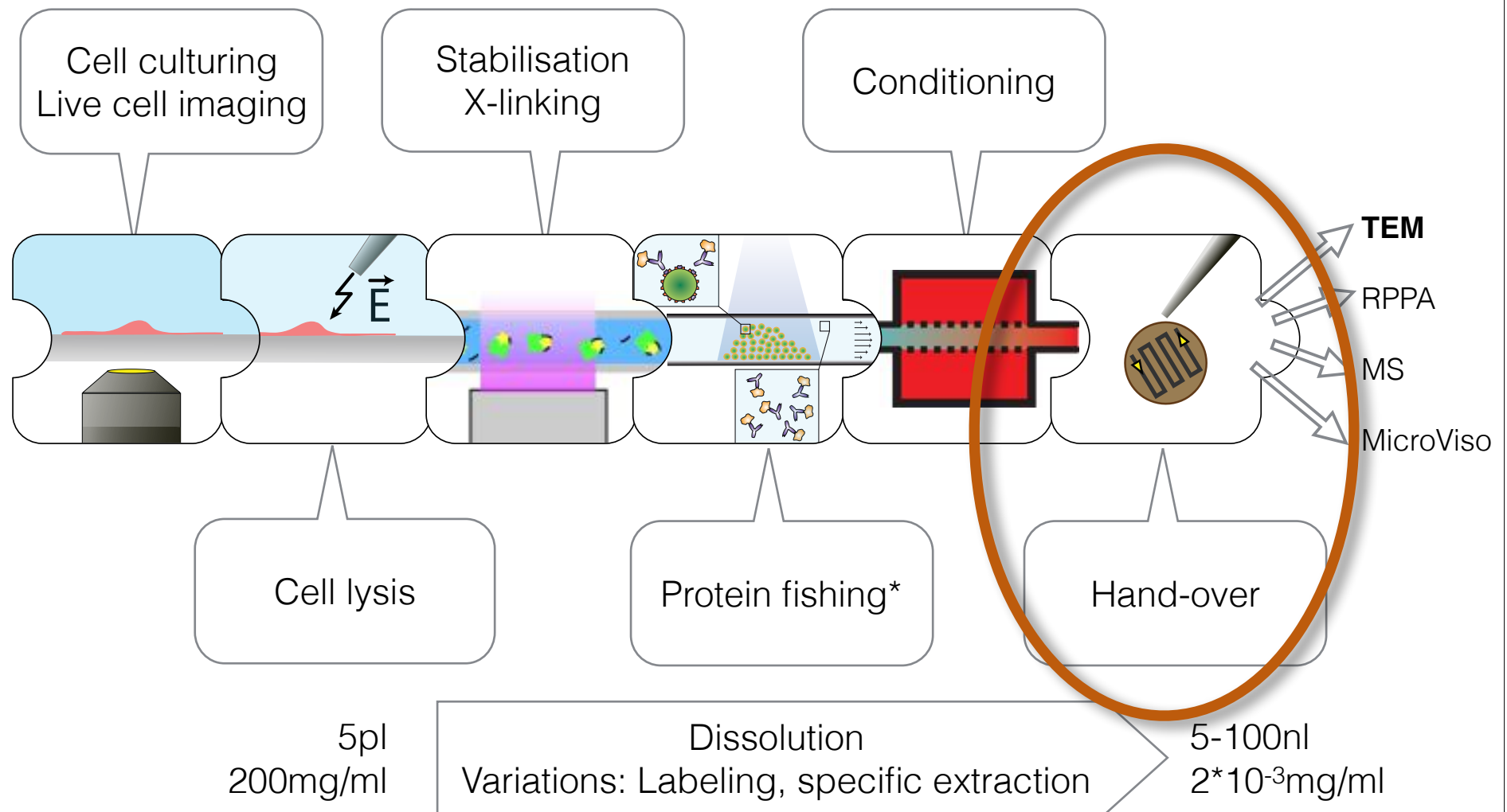
# What is the inoculator for fibril formation?



Lysozyme crystals in microfluidic drops



# Single Cell Proteomics



# Condition & Handover

Kemmerling *et al.*, J Struct Biol. 177(1):128–134 (2012)

Grid activation by a Helium plasma beam (without vacuum)

relyon<sup>®</sup> plasma

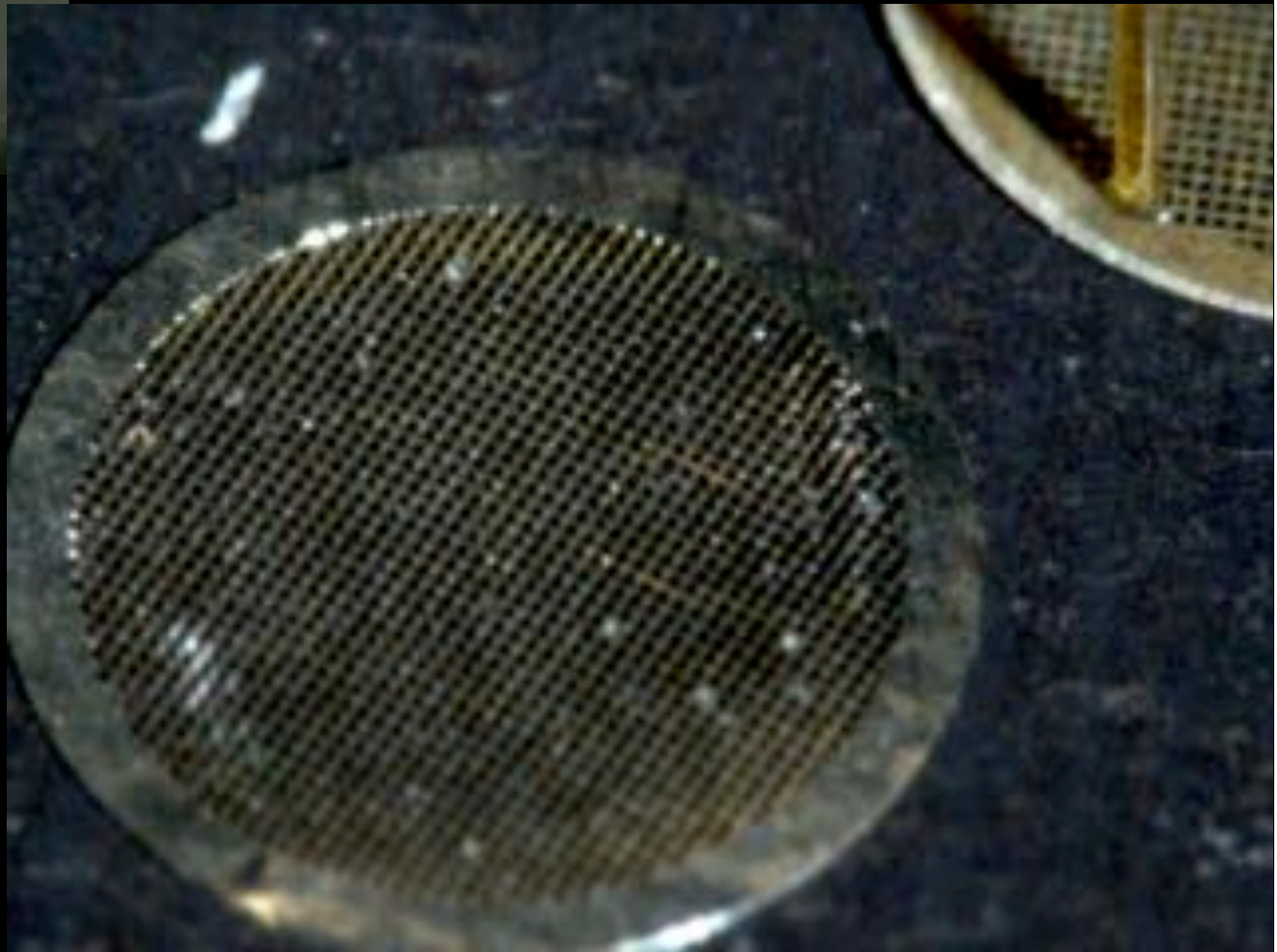


# Condition & Handover

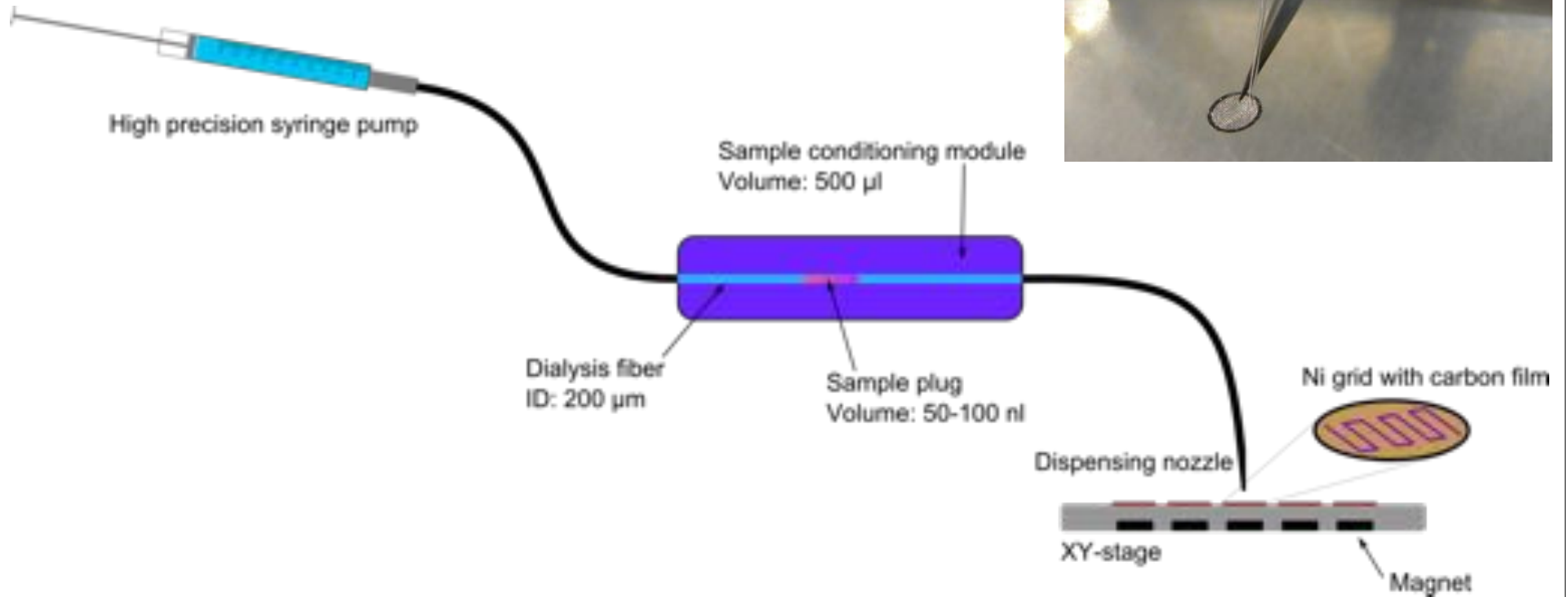
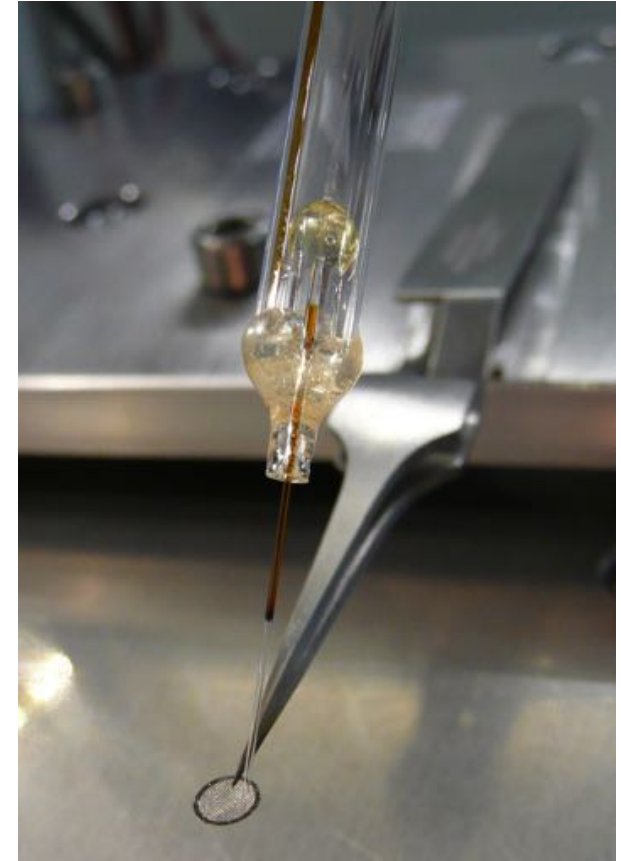
Kemmerling *et al.*, J Struct Biol. 177(1):128–134 (2012)

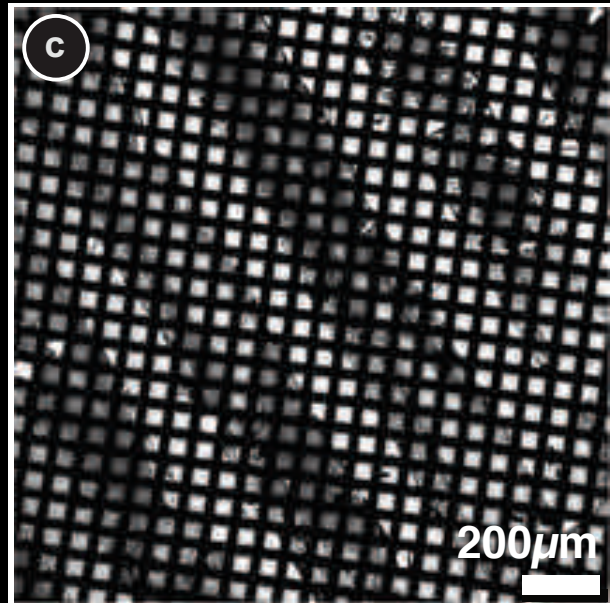
Grid activation by a  
Helium plasma beam  
(without vacuum)

Writing on an EM grid  
with a microfluidic capillary

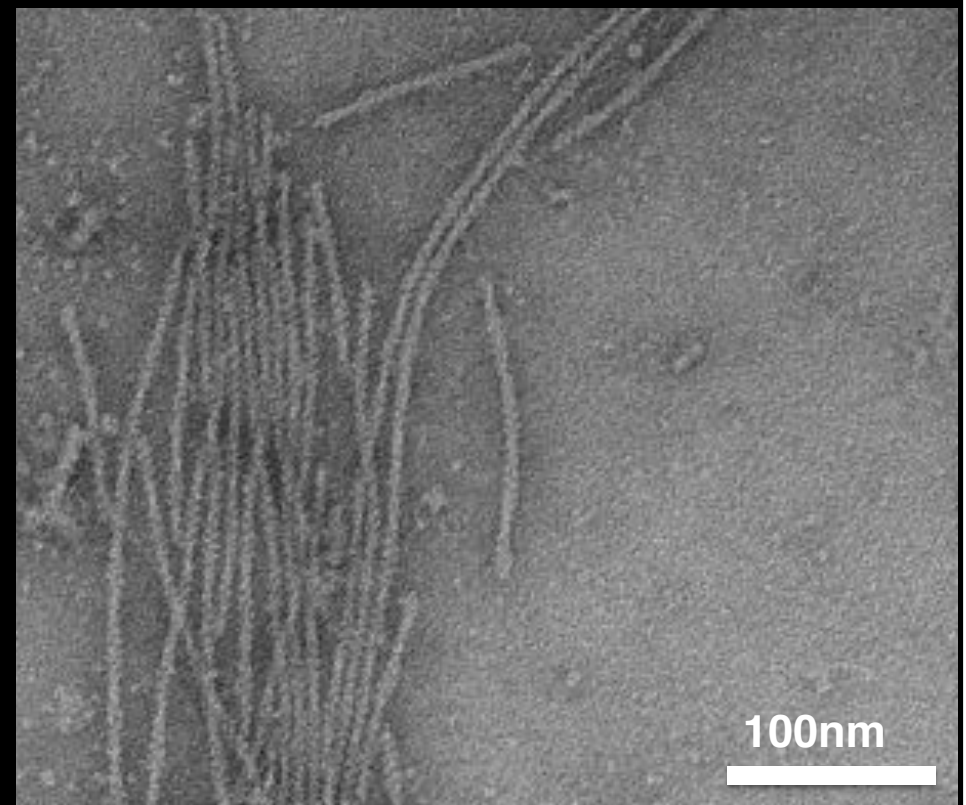
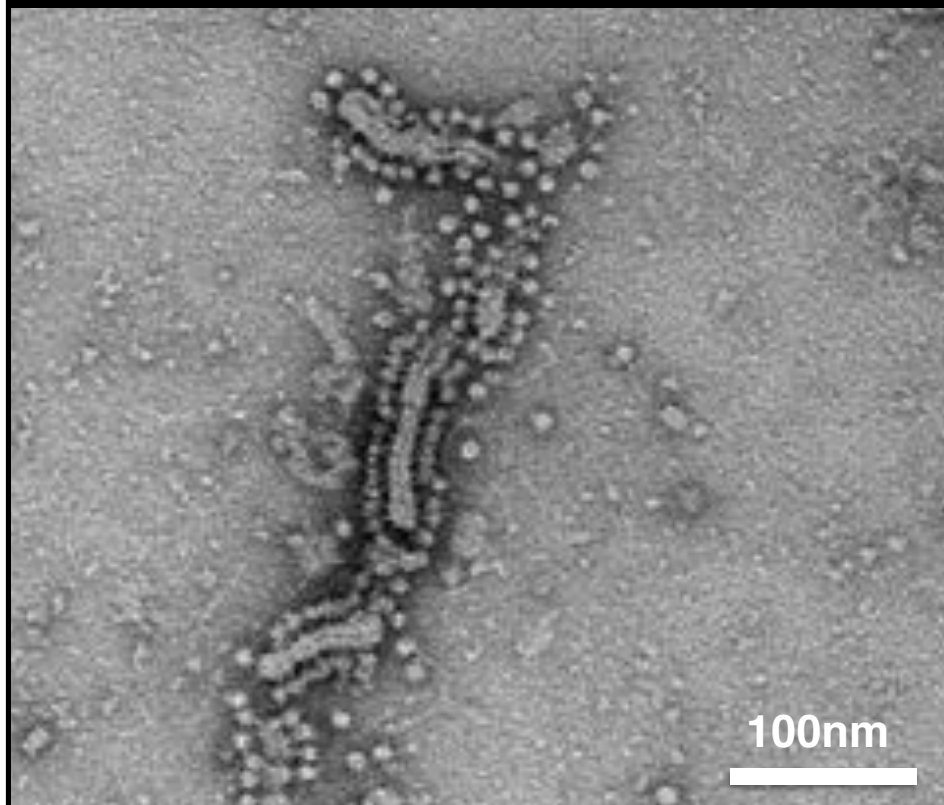


# Aspiration and Writing in the same capillary

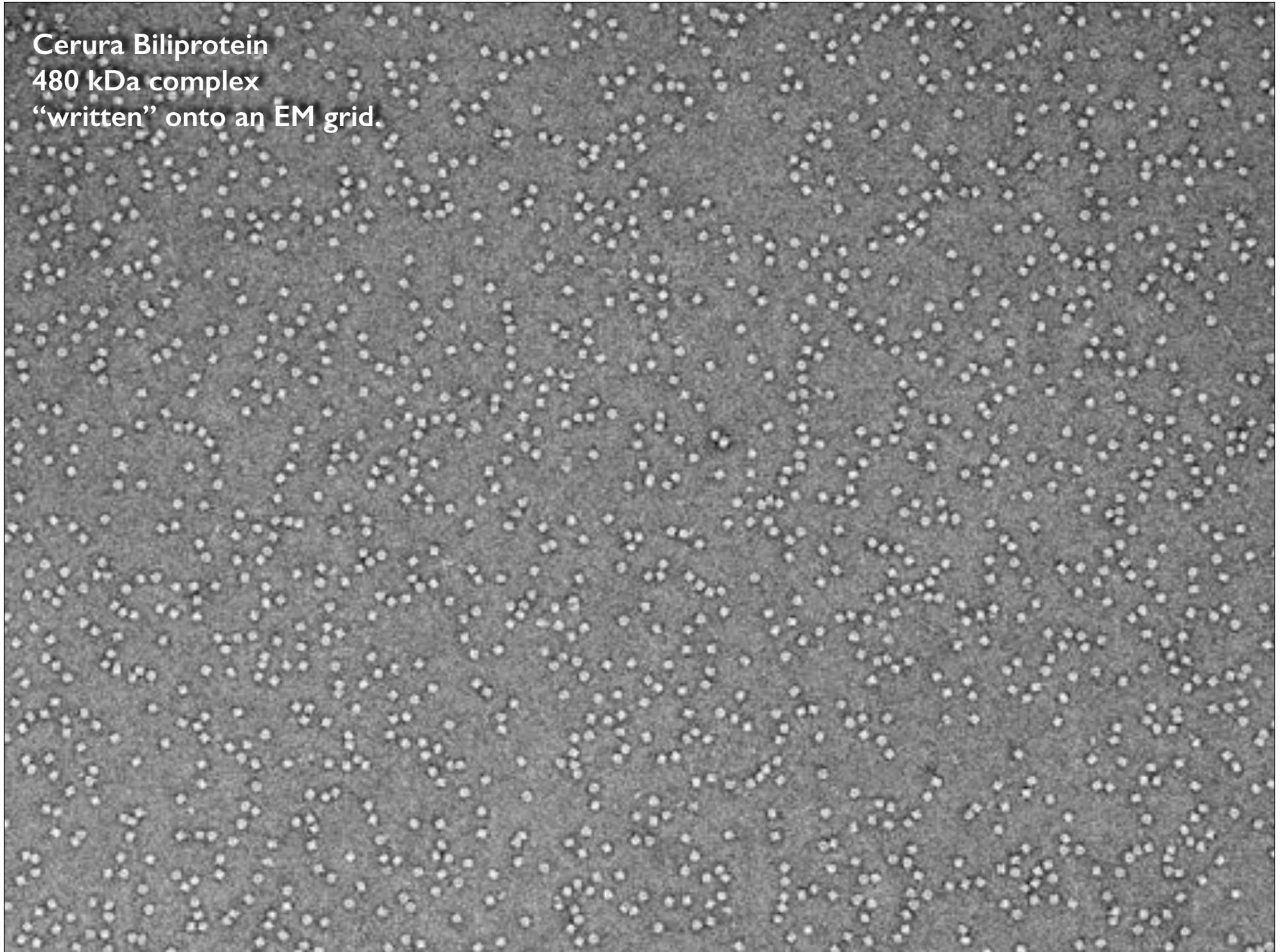




# Single cell cytosol in the EM



Cerura Biliprotein  
480 kDa complex  
“written” onto an EM grid.

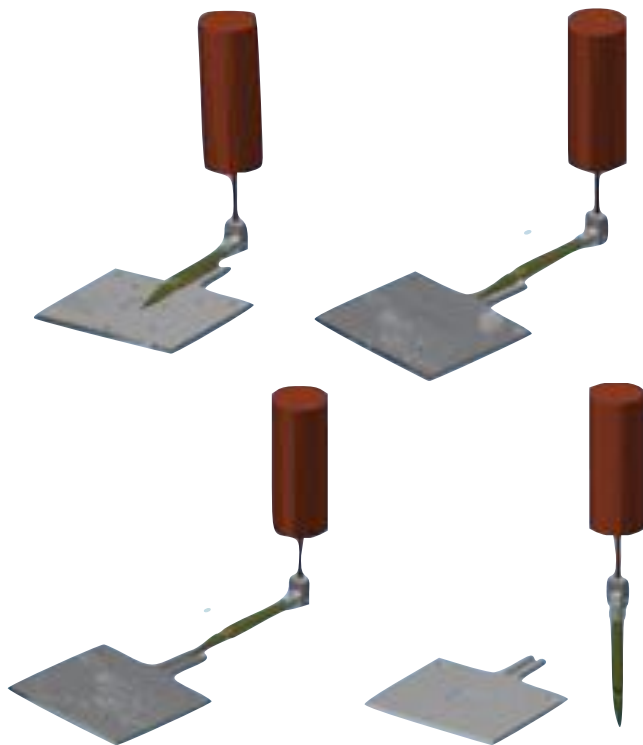


Cerura Biliprotein  
480 kDa complex  
“written” onto an EM grid.

“Lyse and Spread” *Single Cell Visual Proteomics*

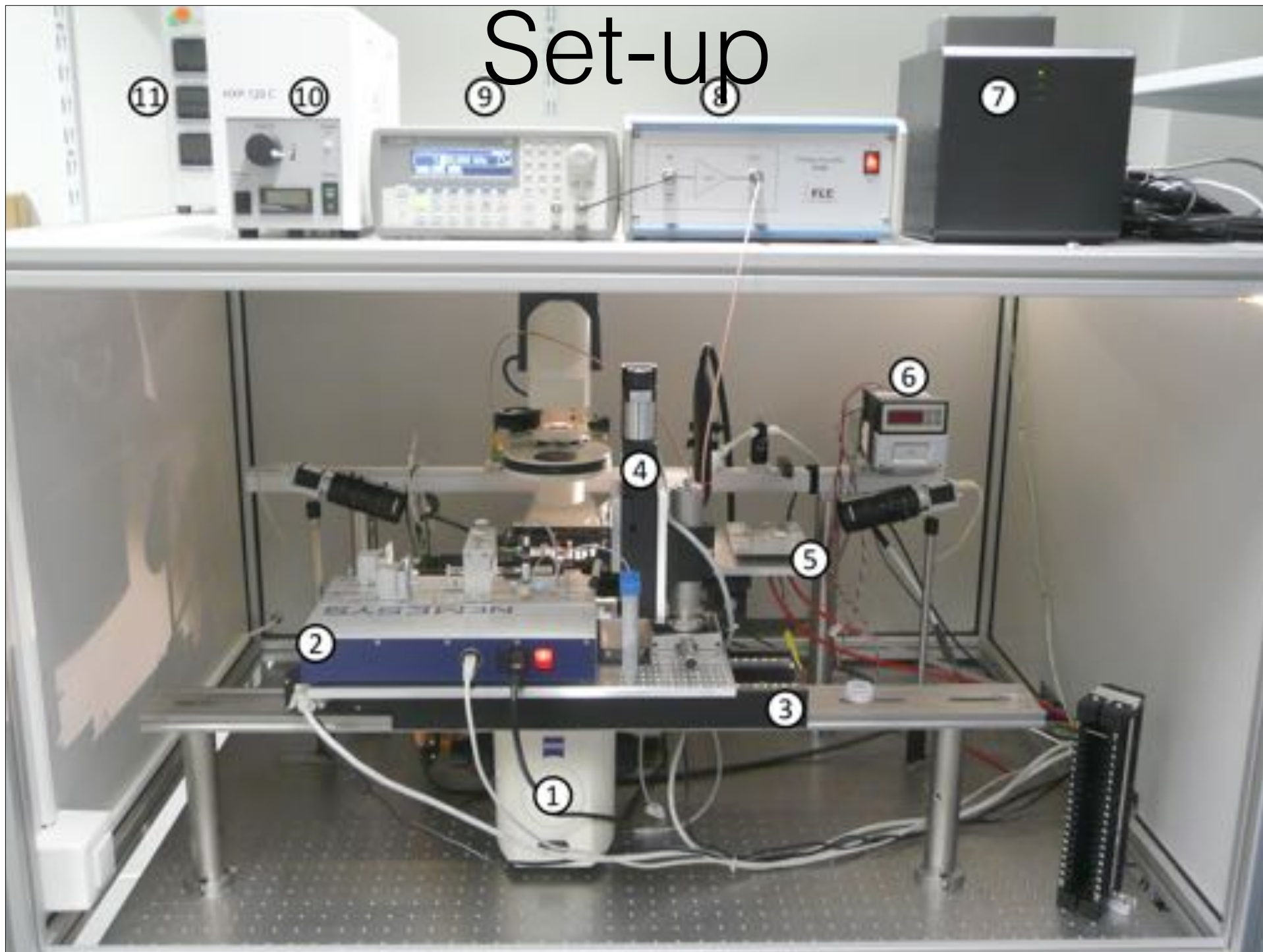


# Single Cell Cytosol Cryo-EM Grid Preparation



at dew point

# Set-up



## ***What can be learned about the single whole cell using EM?***

- 3D overview by 3View-SBF-SEM & FIB-SEM
- 3D structure by *Electron Tomography* (of slices)
- Proteome structural inventory by *Single Cell Visual Proteomics*

### ***Applications:***

- *Intracellular morphological changes due to Growth, Development, Cancer, Aging, Neurodegeneration, ...*
- *Impact of external factors (e.g., nanoparticles, bacteria, toxins, ... ) onto the cellular health*
- *\*omics studies at the single cell: Proteomics, Metabolomics,*
- *Patient-specific studies of diseases mechanism, or of the efficacy of drugs on diversified patient-derived stem cells.*

## ***Where are we now?***

- **3View-SBF-SEM & FIB-SEM:** Fly brain, mouse brain, intracellular bacterial infections, nanoparticle incorporation.
- **3D structure by *Electron Tomography* (of slices):** Bacteria ultrastructure, NPCs, intracellular location of larger complexes (e.g. ribosomes, proteasomes)
- **Proteome structural inventory by *Single Cell Visual Proteomics*:** Enables study of spreading of fibrils in neurodegeneration.

## ***What are the challenges going forward?***

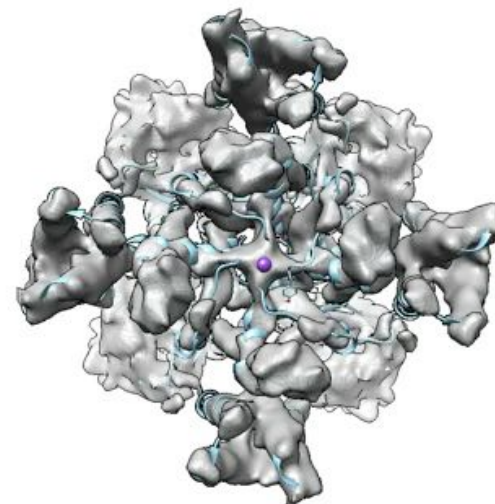
- **3D:** Data collection speed, resolution, data storage, data transfer, segmentation, analysis, visualization.
- **Single Cell Visual Proteomics:**
  - Minimize preparation speed
  - Minimize non-specific loss due to adsorption to walls
  - (Re-) Implement inline purification
  - Extension towards cryo

### Mechanisms of Neurodegeneration in Parkinson's Disease



image: wikipedia.org

### Membrane Protein Structure & Function



MloK1 Potassium Channel

# Acknowledgements

**C-CINA.org**

## ETH Basel

Andreas Hierlemann

Carlos Escobeda

Bernd Rinn

Ramakrishnan Chandrasekhar

## EPF Lausanne

Horst Vogel

Sophie Roizard

## Universität Konstanz

Marcel Leist

## ROCHE

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