CHALLENGING SAMPLES

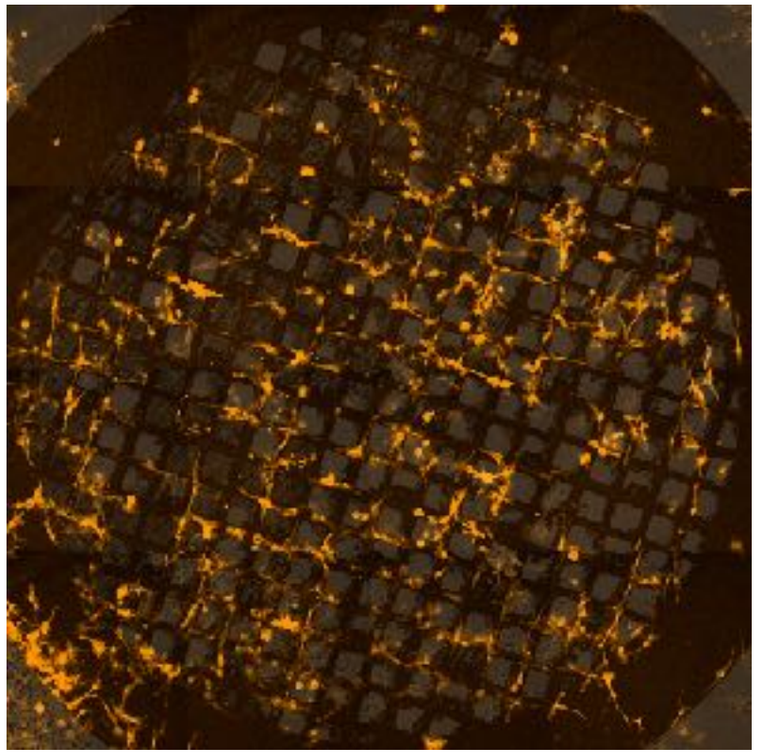
IMPROVING RELIABILITY AND ROBUSTNESS

Lindsay Baker
Kay Grünewald Lab, University of Oxford

RATIONALE

SAMPLES REMAIN A SIGNIFICANT BOTTLENECK FOR CRYO-ET PROJECTS

- Samples need to be suitable for the experiment AND exhibit biologically relevant behaviour
- Significant time, effort and resources are invested in producing appropriate samples

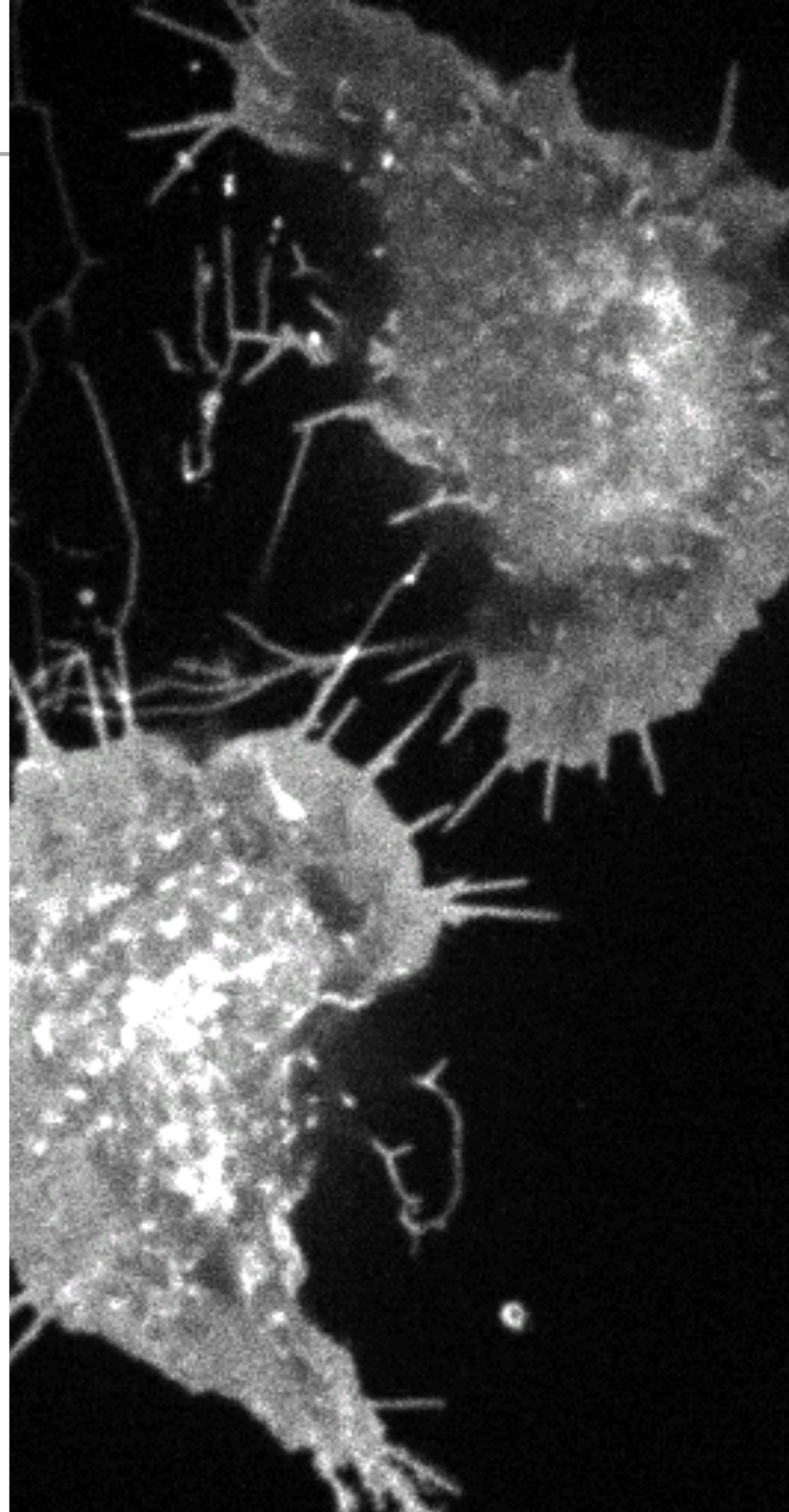


TO SPEED UP CRYO-ET…

- Samples that are ***reliably*** suitable for the experiment
- Samples that ***robustly*** exhibit biologically relevant behaviour
- Get the most out of ***every*** sample

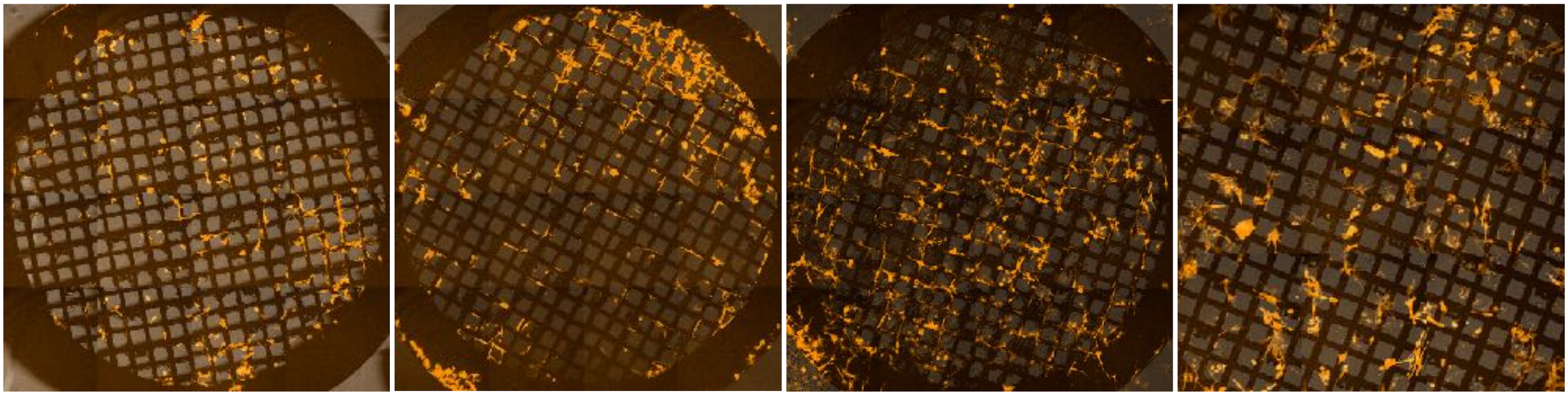
CHALLENGES

- Sample preparation
  - Reducing heterogeneity (reliability)
  - Reproducing biology (robustness)
- Data acquisition and analysis
  - Maximising data acquisition sessions (reliability)
  - Identifying patterns in complex systems (robustness)
- Community wide potential



SAMPLE PREPARATION – IMPROVING HETEROGENEITY

Distribution of cells on the support is critical for cryo-ET



Unusable | Usable if desperate | Slightly better | "Good"

"Good" = multiple grid squares have some part of a cell on them

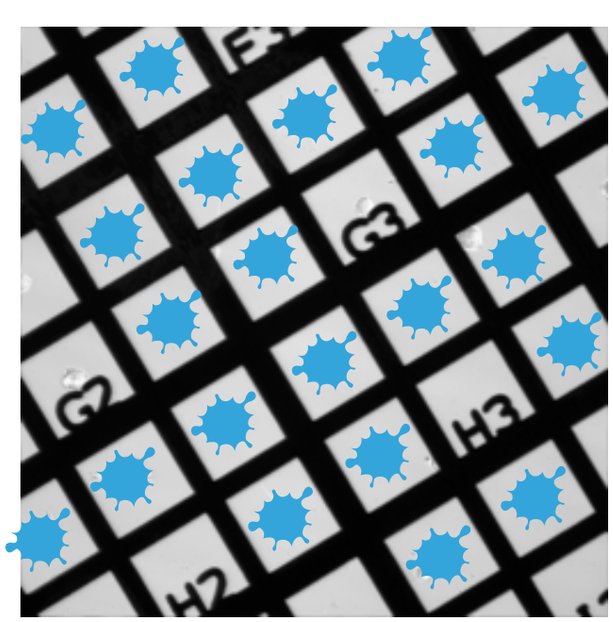
But what if…

- you're trying to target a certain region of the cell?
- you're looking for rare events?
- you want to FIB mill?
- you have unusual cell morphologies (like neurons)?

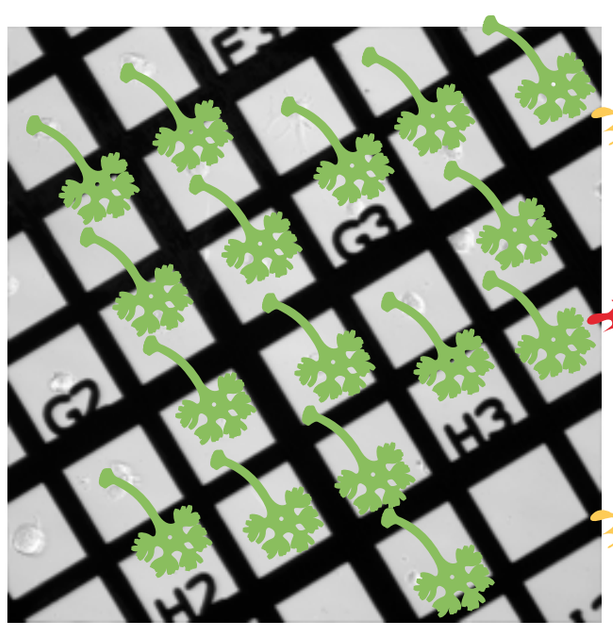
Images courtesy of Vojta Pražák

SAMPLE PREPARATION – IMPROVING HETEROGENEITY

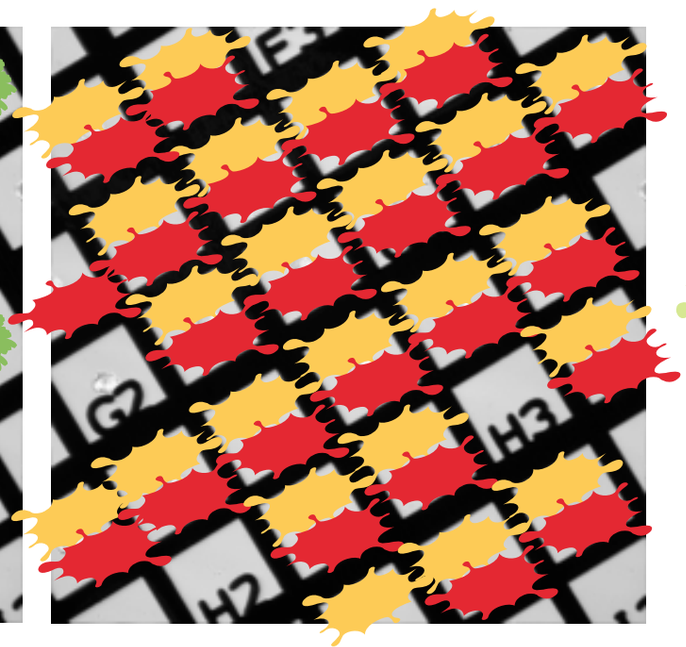
Can we change the arrangement of cells for different samples?



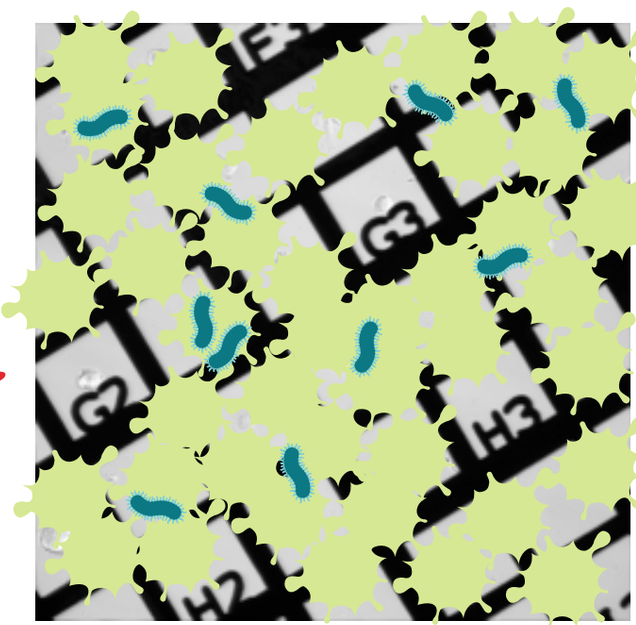
For FIB milling



For polar cells



For cell-cell interactions

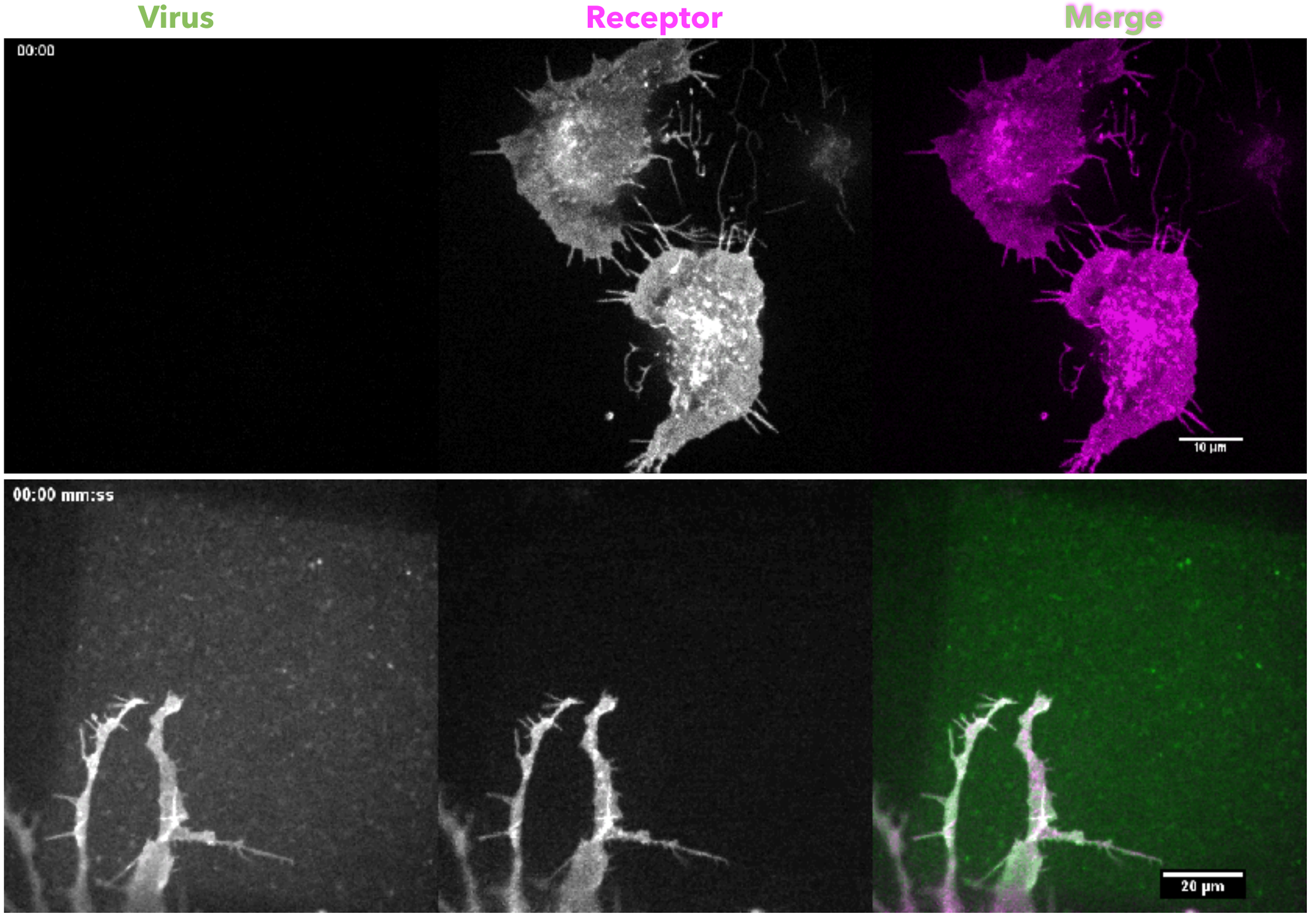


For infection/co-culture

Advantages:

- Potential for automation
- Keep Finder grid squares empty for correlation
- Increasing efficiency for all steps (LM, FIB, TEM)

SAMPLE PREPARATION – RECAPITULATING BIOLOGY



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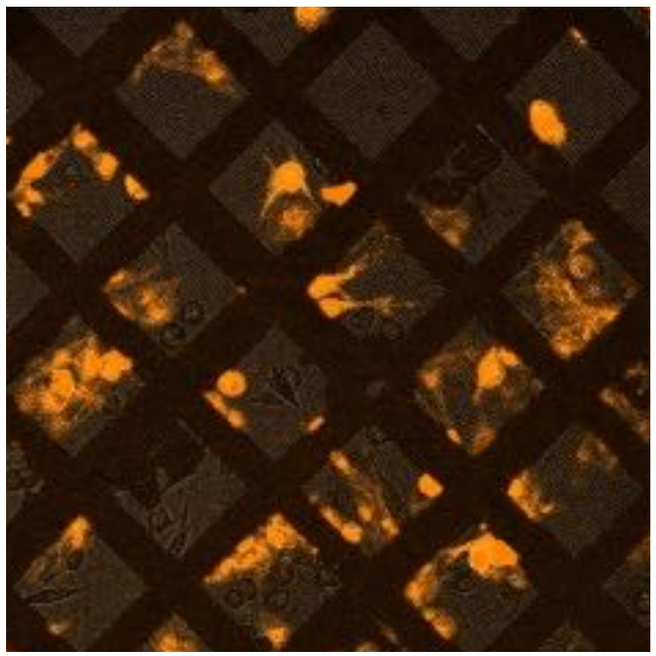
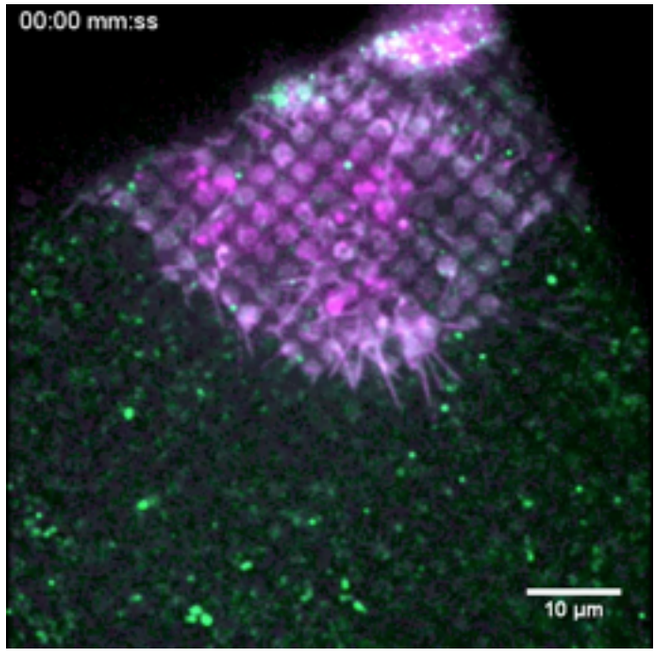
BETTER SAMPLE PREPARATION – HOW?

- Changing surface treatments
  - Carboxylic acids (colour change)
  - Different protein coatings
  - Graphene with modifications 🍔





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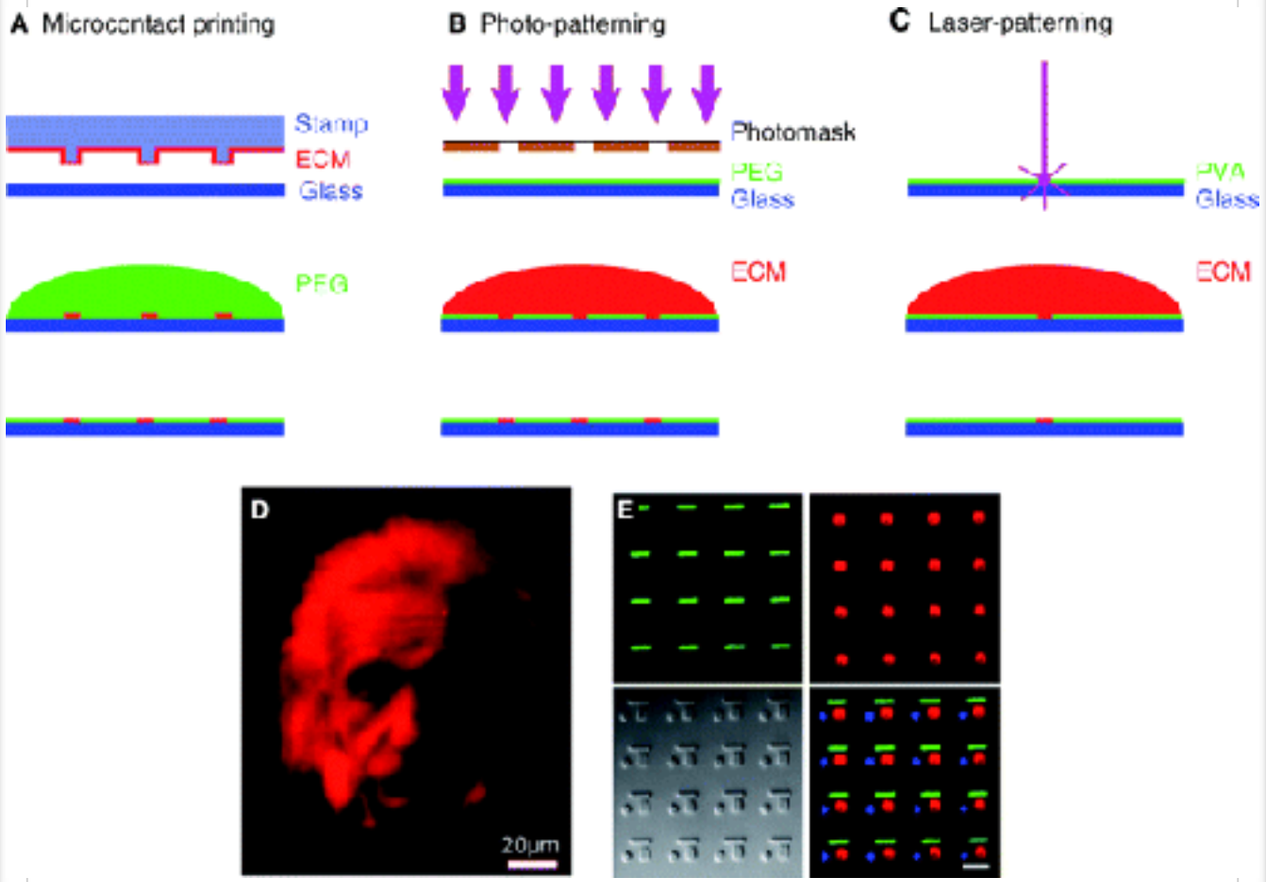
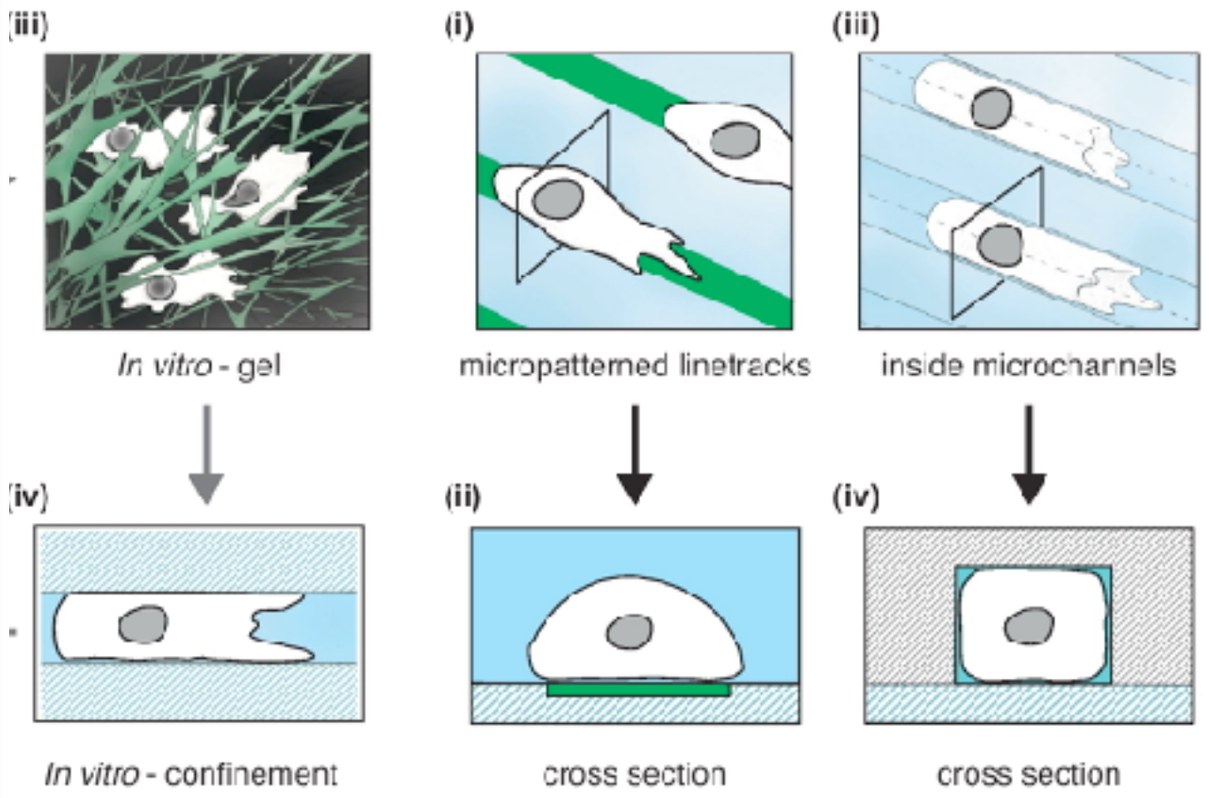
BETTER SAMPLE PREPARATION – HOW?

Micropatterning



Théry (2010) J. Cell Sci.

Micro/Nanofabrication



Lautenschläger and Piel (2013) Curr Opin Cell Biol

DATA ACQUISITION – GETTING THE MOST FROM EACH SAMPLE

- For the project
- For the lab
- For the community

REAL TIME DATA PROCESSING

Evaluate tilt series parameters and sample behaviour/quality

Best data collection efficiency/ quality

META DATA COLLECTION & TRACKING

Follow equipment behaviour to anticipate issues

Minimal sample loss and best data quality

Minimal downtime

UCSF tomography: An integrated software suite for real-time electron microscopic tomographic data collection, alignment, and reconstruction

Shawn Q. Zheng [a,b,1], Bettina Keszthelyi [b,1], Eric Branlund [a,b,1], John M. Lyle [b], Michael B. Braunfeld [a,b], John W. Sedat [b], David A. Agard [a,b,*]

Journal of Structural Biology 157 (2007) 138–147

Web portal to an image database for high-resolution three-dimensional reconstruction

Wei Dai,[a,b] Yuyao Liang,[b] and Z. Hong Zhou[a,b,*]

Journal of Structural Biology 144 (2003) 238–245

The Caltech Tomography Database and Automatic Processing Pipeline

H. Jane Ding [a], Catherine M. Oikonomou [a], Grant J. Jensen [a,b,*]

Journal of Structural Biology 192 (2015) 279–286

A relational database for cryoEM: experience at one year and 50 000 images

Denis Fellmann, James Pulokas, Ronald A. Milligan, Bridget Carragher,* and Clinton S. Potter

Journal of Structural Biology 137 (2002) 273–282

Technical Note

MRC2014: Extensions to the MRC format header for electron cryo-microscopy and tomography

Anchi Cheng [a,*], Richard Henderson [b], David Mastronarde [c], Steven J. Ludtke [d], Remco H.M. Schoenmakers [e], Judith Short [b], Roberto Marabini [f], Sargis Dallakyan [a], David Agard [g], Martyn Winn [h,*]

Journal of Structural Biology 192 (2015) 146–150

DATA ANALYSIS – GETTING THE MOST FROM EACH SAMPLE

- For the project
- For the lab
- For the community

META DATA COLLECTION & TRACKING
Sample preparation

META DATA COLLECTION & TRACKING
Microscopy parameters

- Improved reproducibility
- Better understanding of cellular behaviours

MOVING FORWARD

- Need new approaches to sample preparation to
  - improve throughput (reliability)
  - better reproduce biology (robustness)
- Need to use available but also new resources to
  - maximise data acquisition sessions (reliability)
  - include sample preparation in identifying patterns in complex systems (robustness)
- Community wide potential
  - Encourage software developers to make meta-data more accessible
  - Work with electronic lab notebooks