CHALLENGING SAMPLES

IMPROVING RELIABILITY AND ROBUSTNESS

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RATIONAL

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
IMPROVING RELIABILITY AND ROBUSTNESS

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
IMPROVING RELIABILITY

SAMPLE PREPARATION - IMPROVING HETEROGENEITY

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

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)?

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

Images courtesy of Vojta Pražák
SAMPLE PREPARATION – IMPROVING HETEROGENEITY

Can we change the arrangement of cells for different samples?

Advantages:

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

Virus

Receptor

Merge

LM slide

EM grid

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BETTER SAMPLE PREPARATION – HOW?

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

Vojta Pražák
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BETTER SAMPLE PREPARATION - HOW?

- **Micropatterning**
  - A. Microcontact printing
  - B. Photo-patterning
  - C. Laser-patterning

- **Micro/Nanofabrication**
  - In vitro - gel
  - micropatterned lines/track
  - inside microchannels


For the project

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

"improving reliability"

data acquisition - getting the most from each sample
IMPROVING ROBUSTNESS

DATA ANALYSIS – GETTING THE MOST FROM EACH SAMPLE

› For the project
› For the lab
› For the community

META DATA COLLECTION & TRACKING

Sample preparation

Microscopy parameters

› Improved reproducibility
› Better understanding of cellular behaviours
IMPROVING RELIABILITY AND ROBUSTNESS

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