Leibniz ipht
LEIBNIZ INSTITUTE of PHOTONIC TECHNOLOGY

A journey through structured illumination microscopy

SIM update in our group

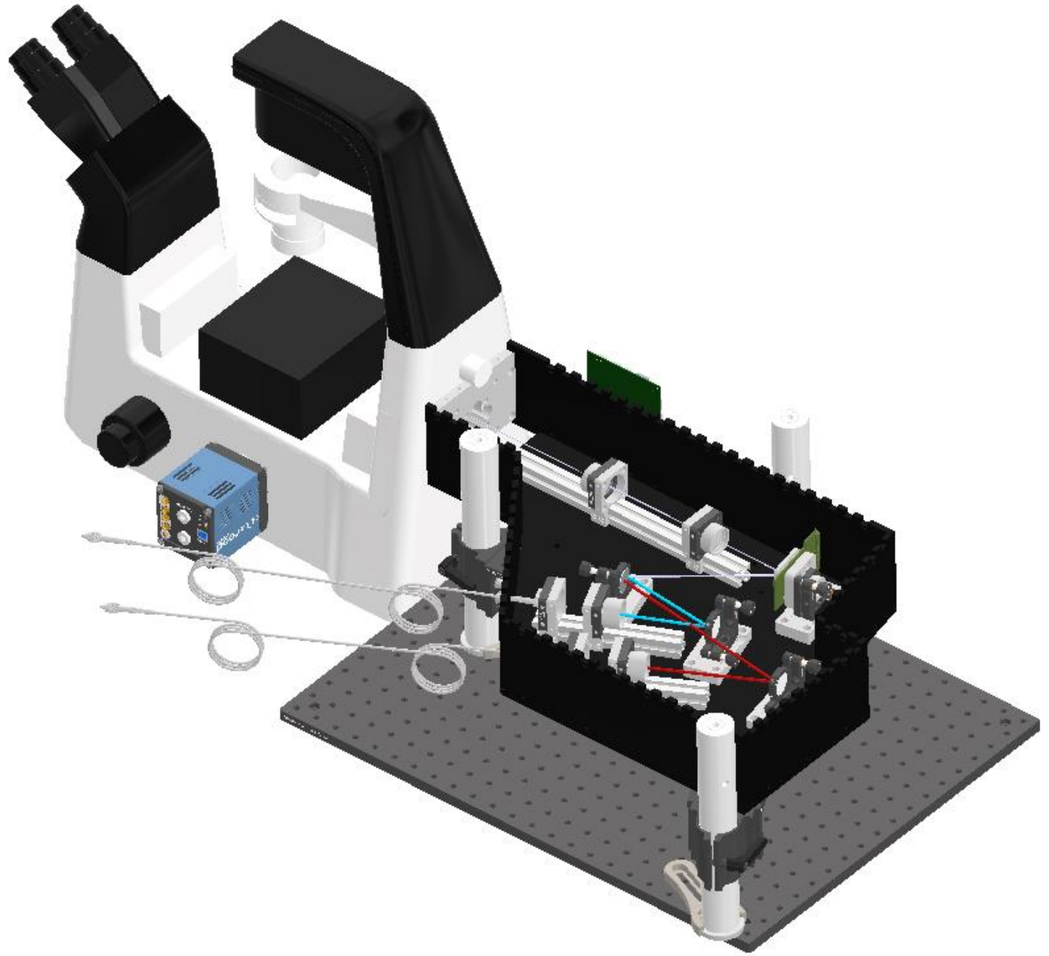
Group meeting
31.08.2023
Haoran Wang

Member of the Leibniz Association

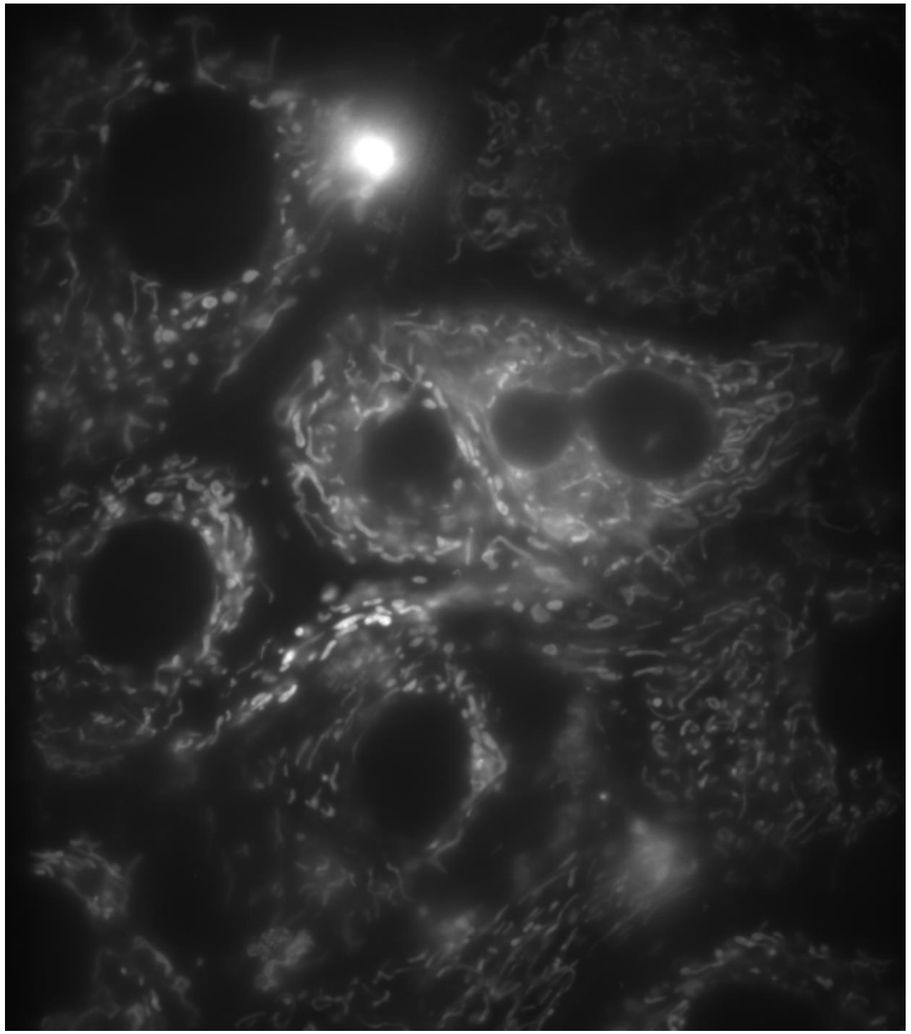
Topic

- SIM
- NL-SIM

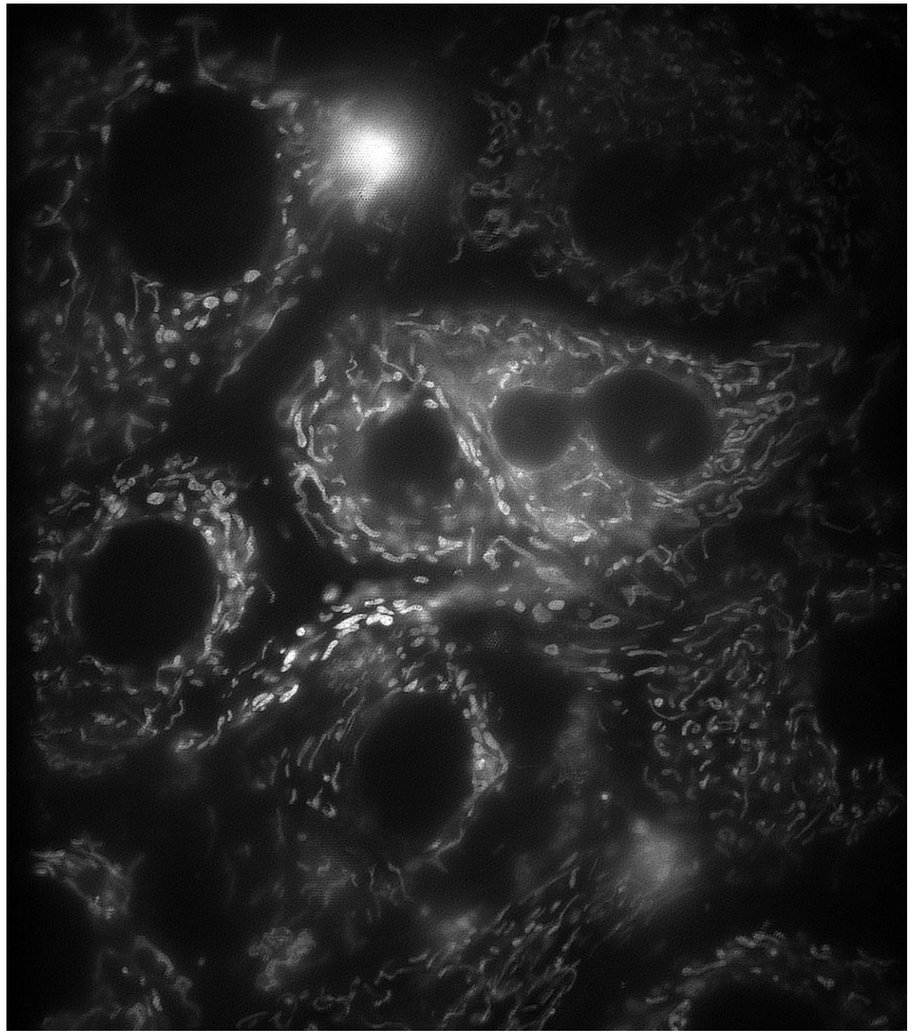
Setup



Time sequence data



Widefield



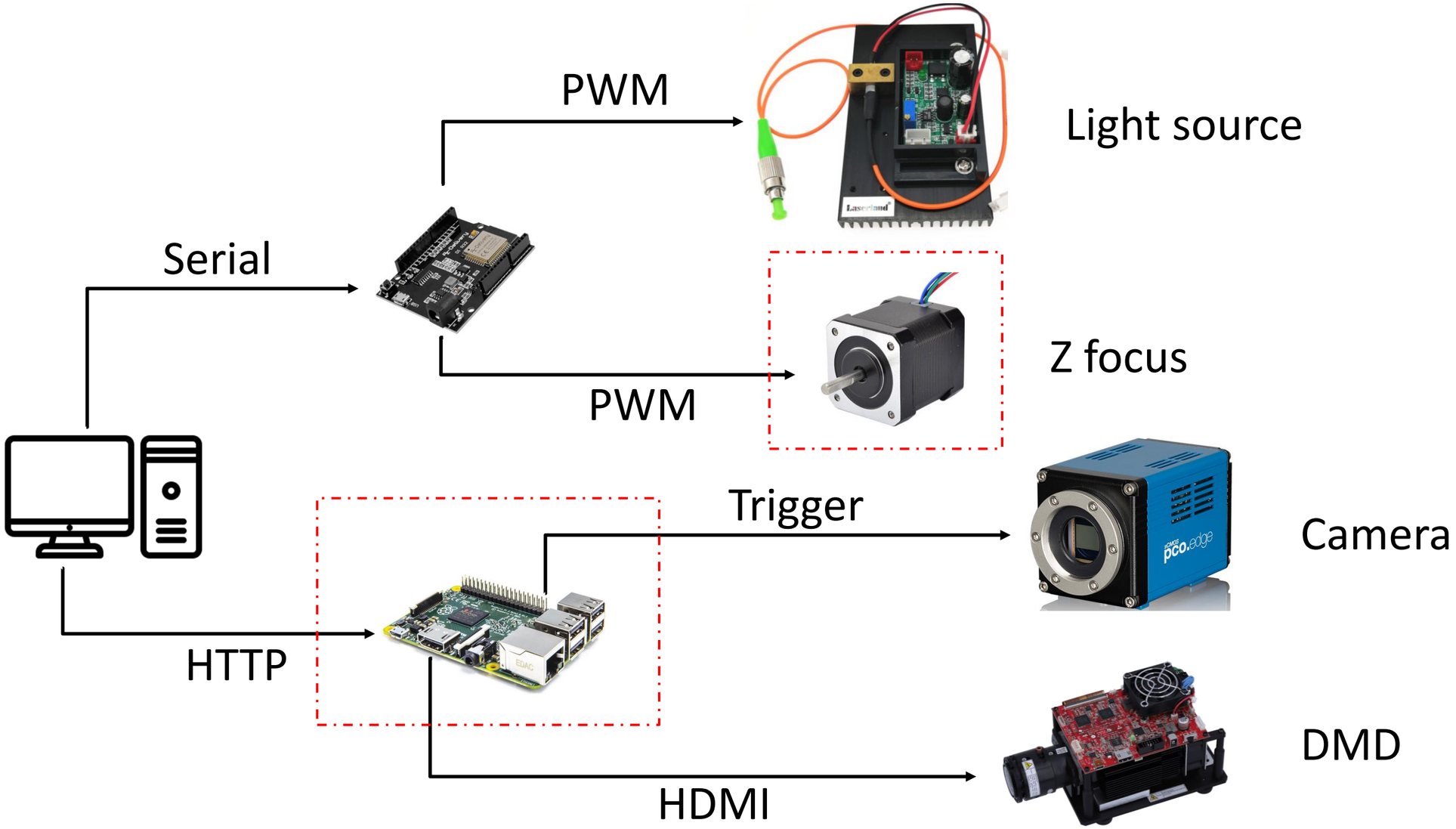
Reconstruction

HeLa
MitoTracker Green
Time interval 2min
Image slices **15**

Problem:

- Z Drift
- -> Out of focus
- Too long exposure time
- -> Fast photobleaching
- No proper synchronization
- -> Pattern image mismatch

Solution



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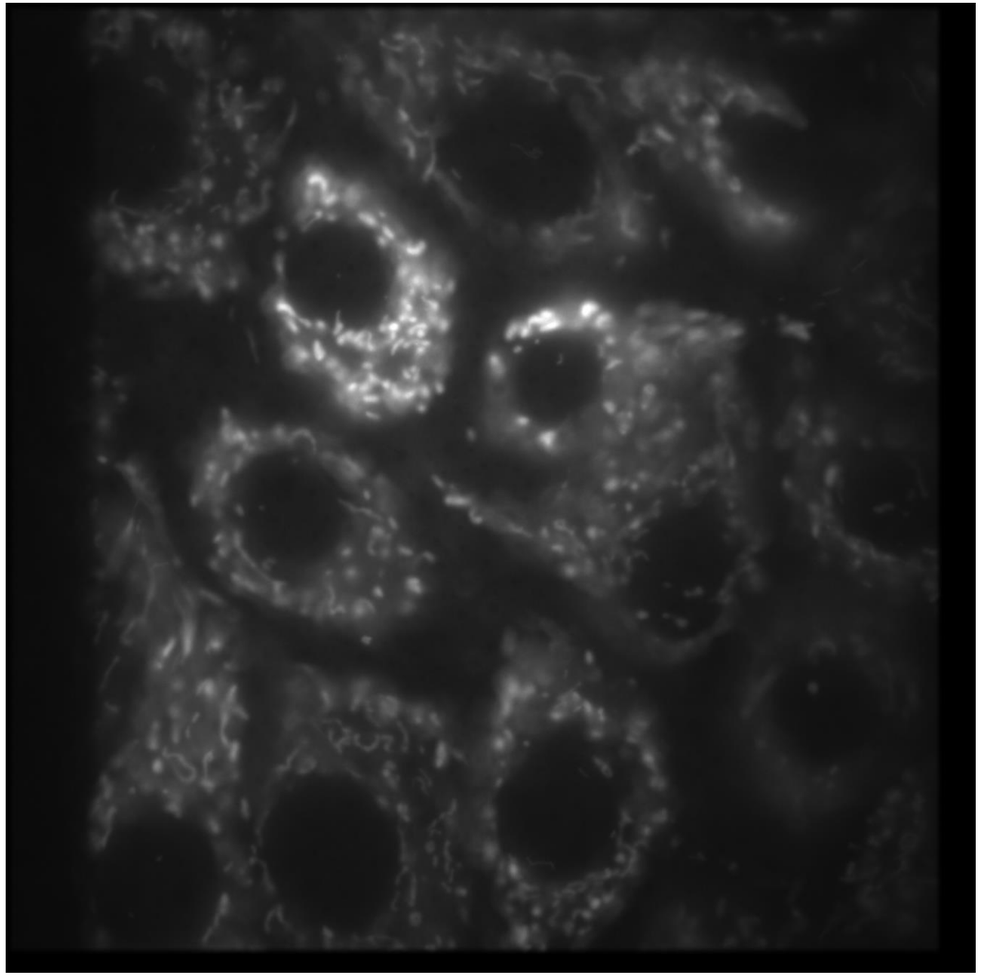
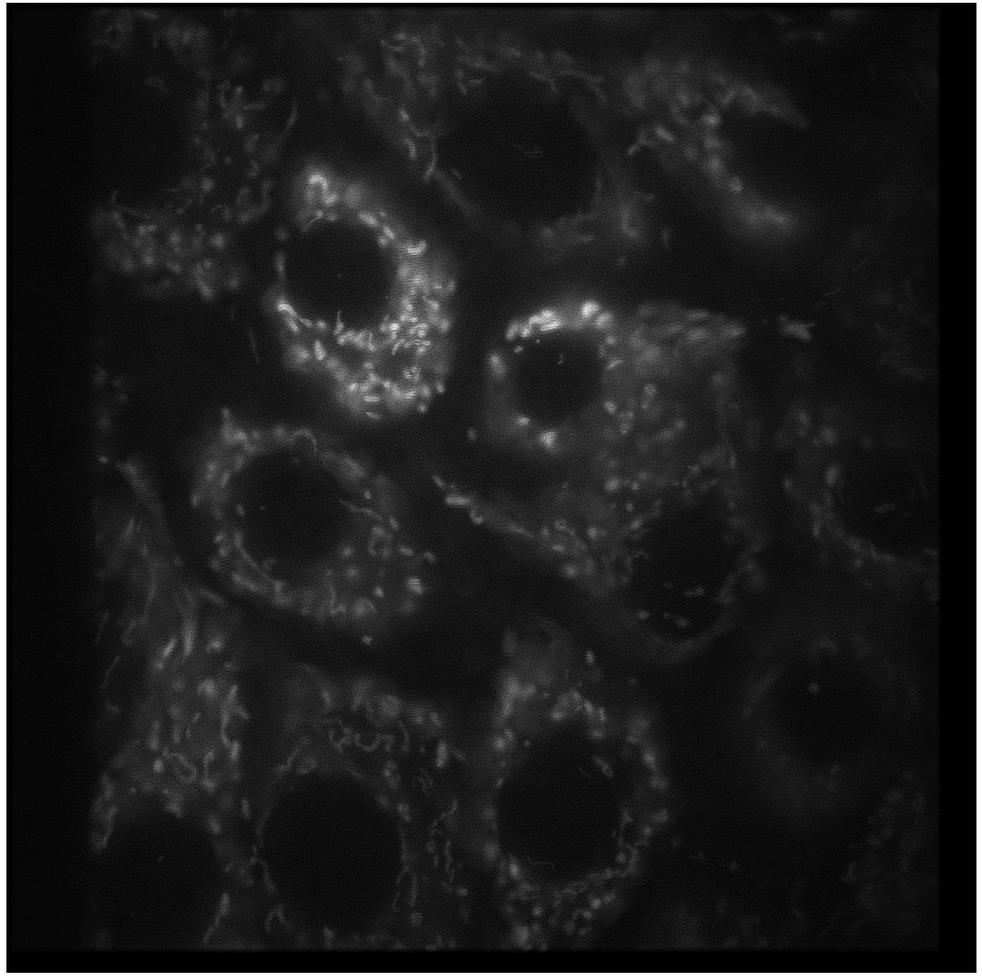
Z-axis sectioning

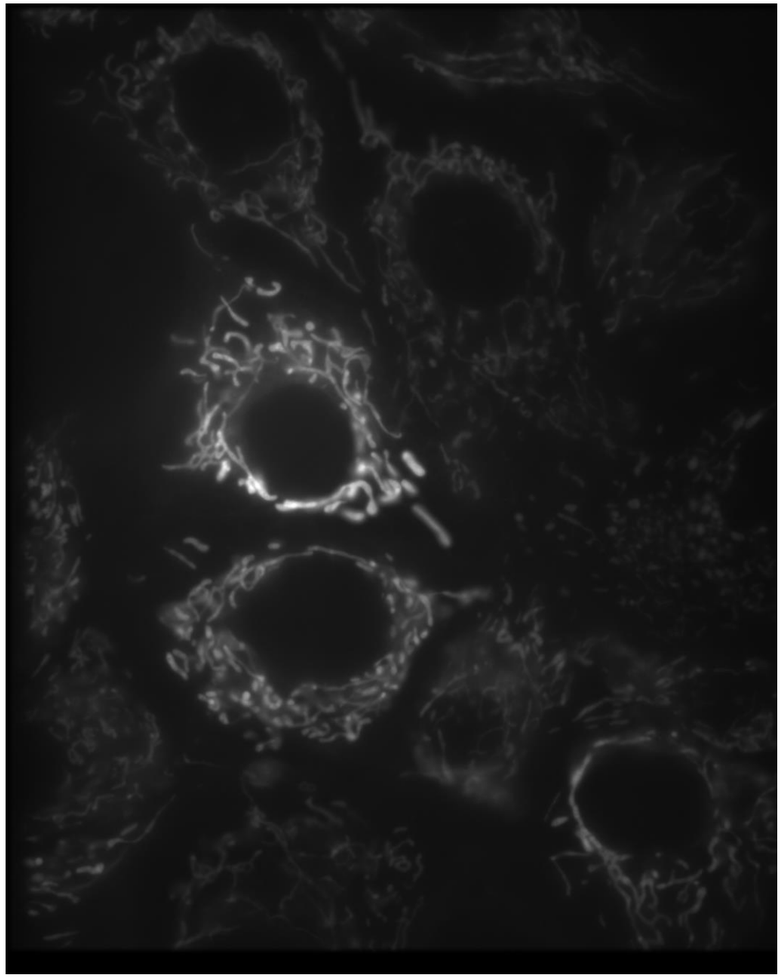


Widefield

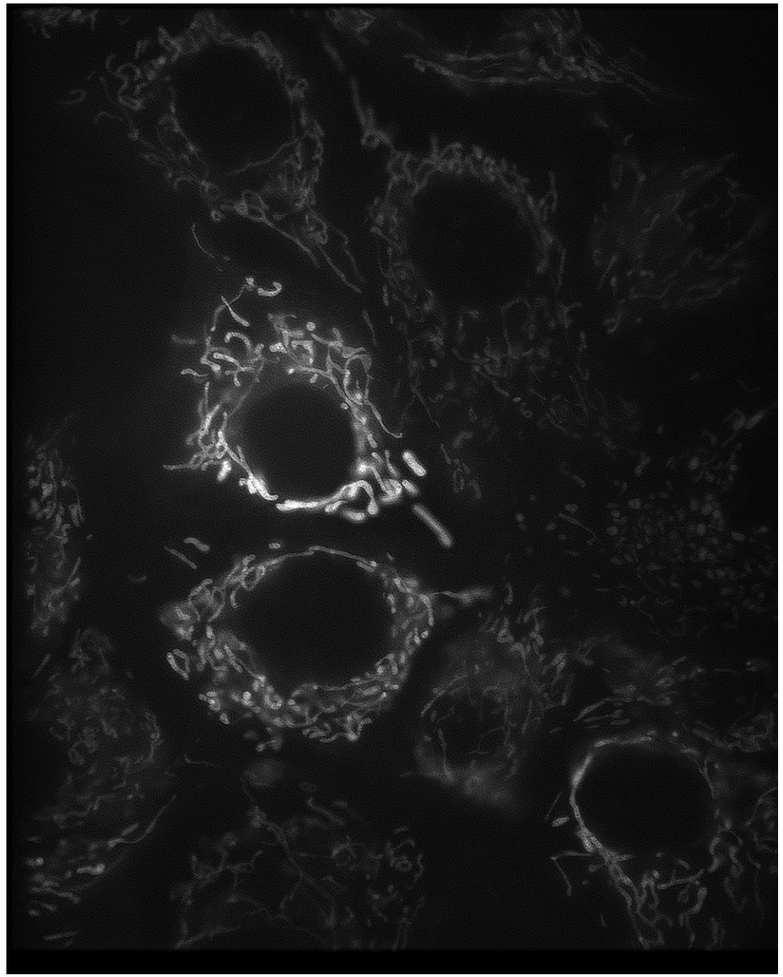


Reconstruction

Time sequence data



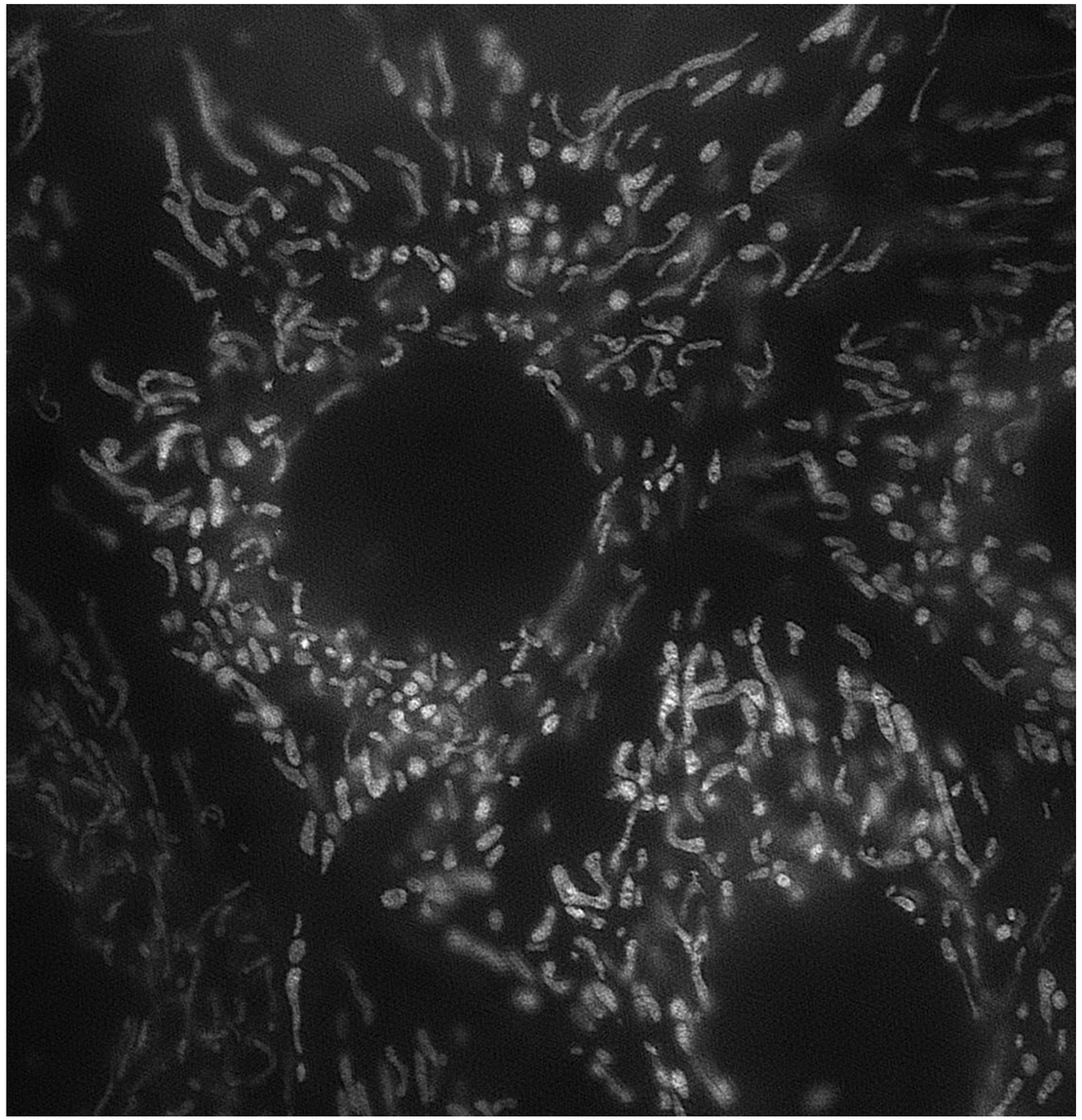
Widefield



Reconstruction

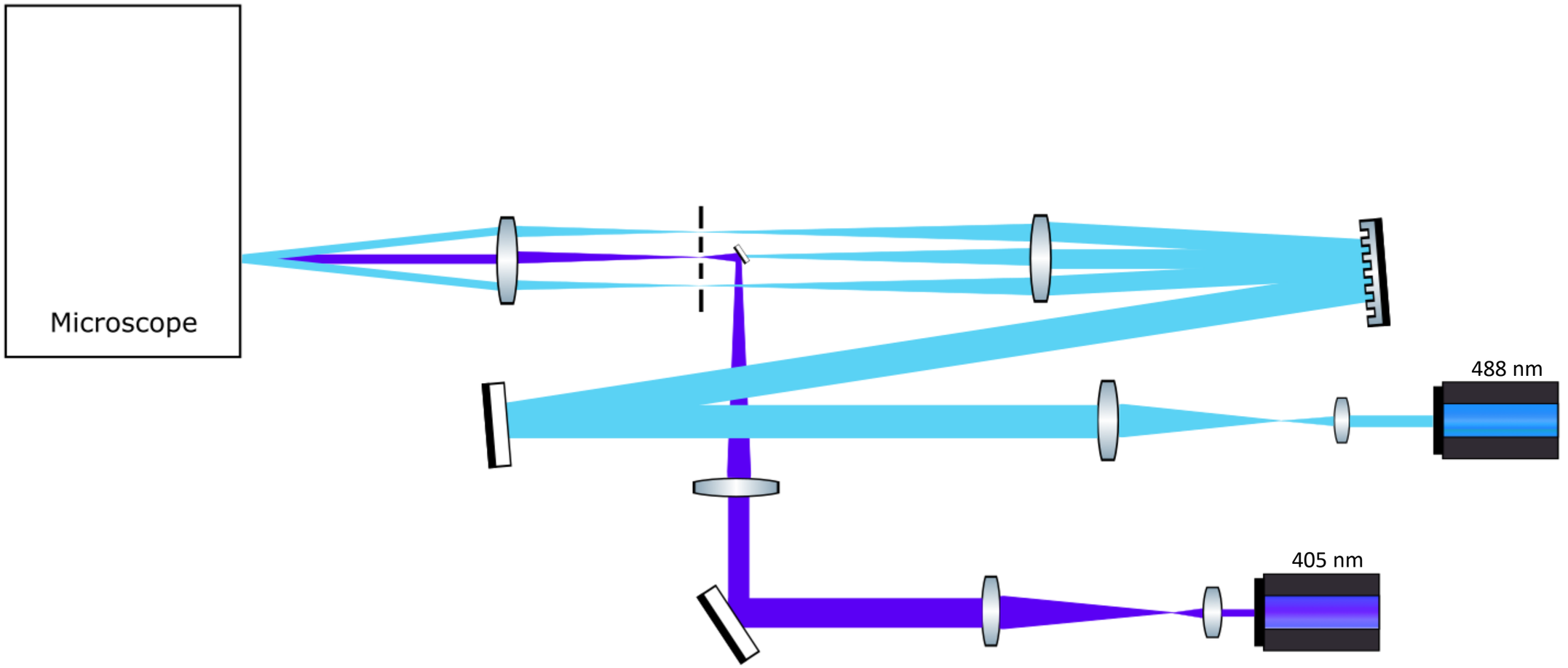
Time interval 2 min
Image slices **70**

Time sequence data



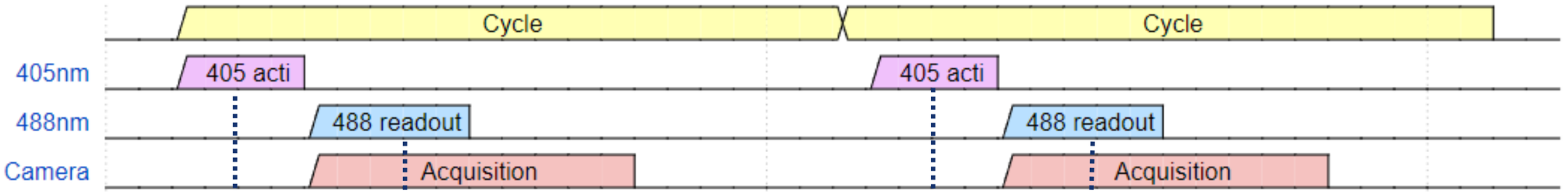
Time interval 2 min
Image slices 50

Setup design NL-SIM



Trigger Mechanism

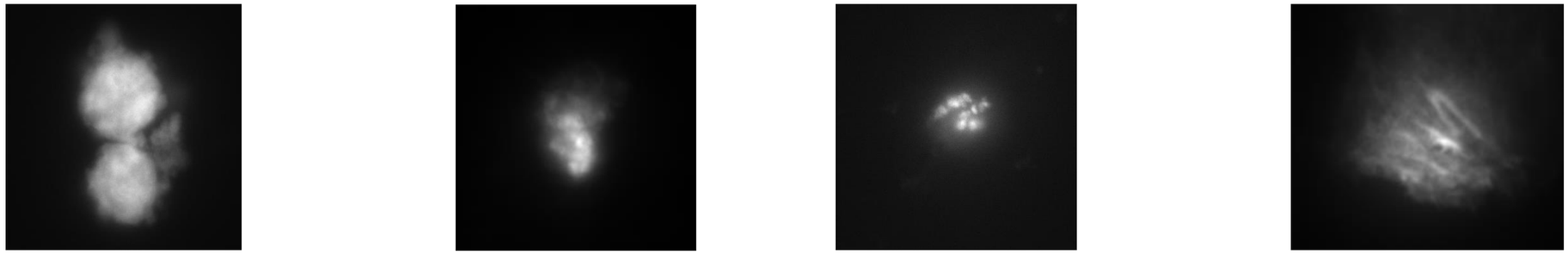
Widefield Mode



Get a plot of gaussian source

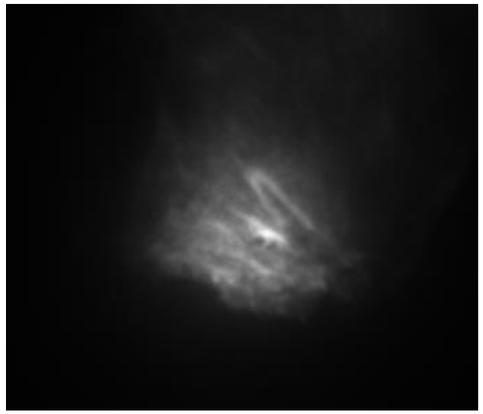
Pattern on SLM

Image

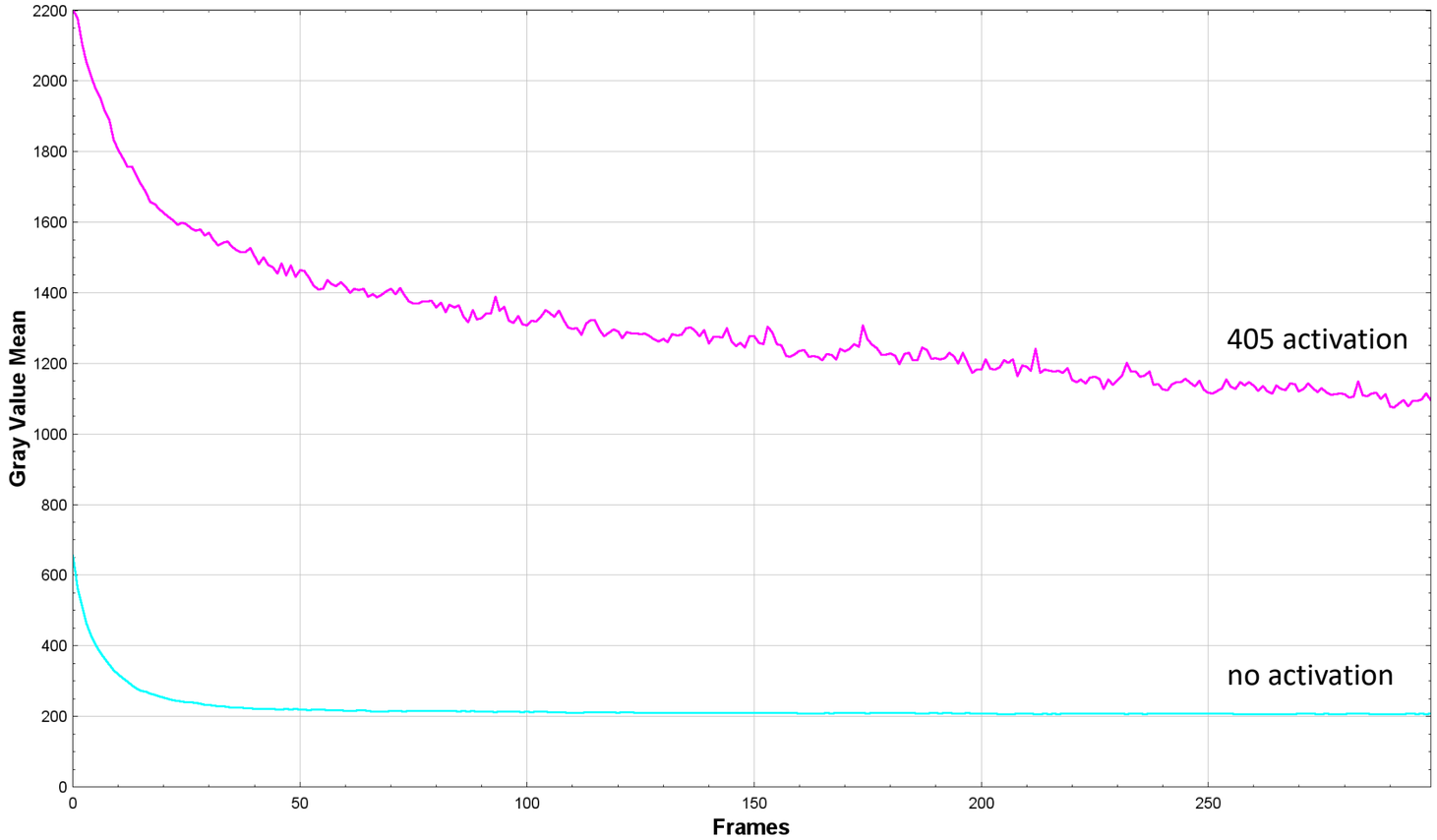


Kohinoor | Skylan | Skylan-Actin | Gamillus-Actin

Photoswitchable protein

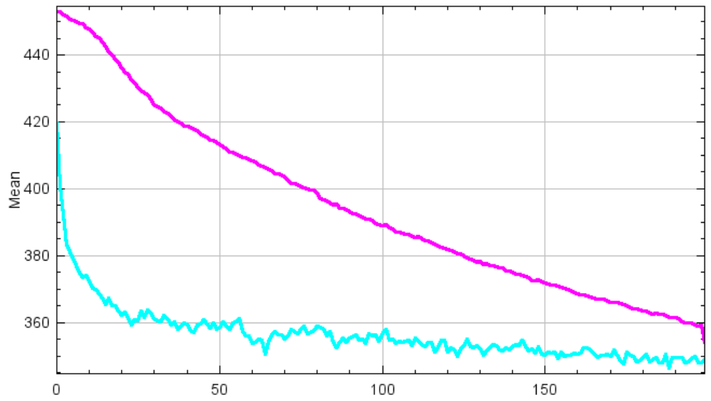


488 on

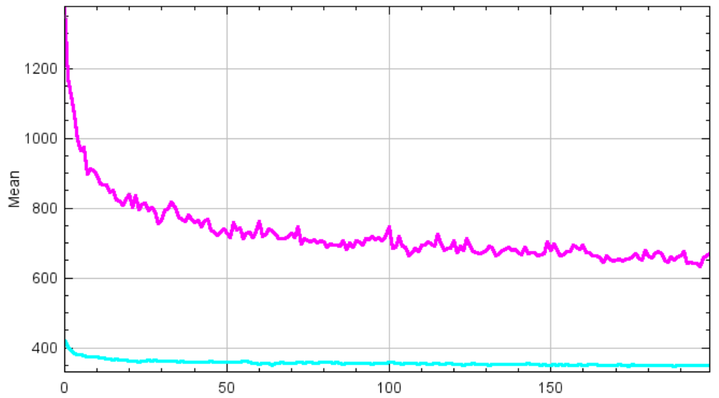


Protein Comparison

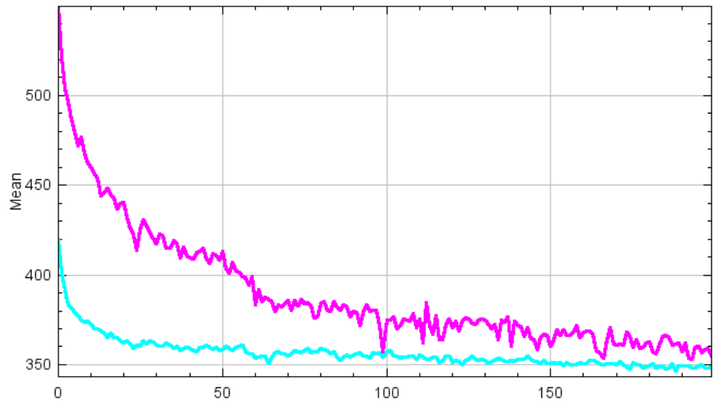
	Kohinoor	Skylan	Skylan-Actin	Gamillus-Actin
On/Off Contrast	1.14	1.84	1.07	6



Kohinoor

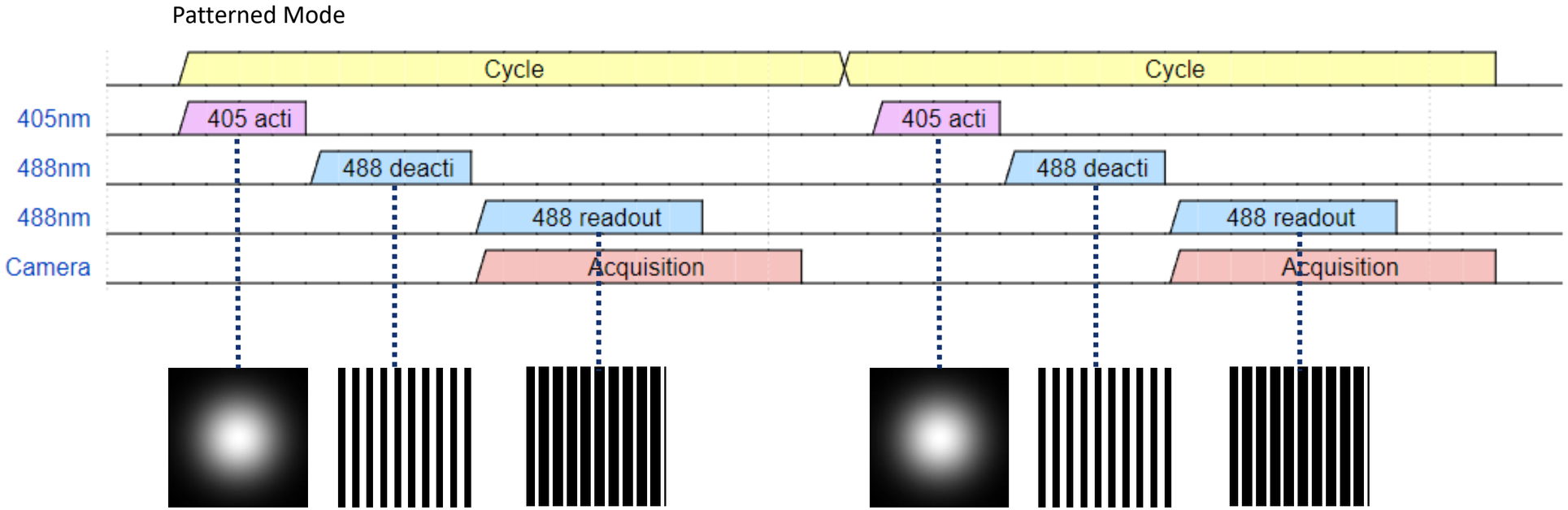


Skylan



Skylan-Actin

Next step


Patterned Mode
Cycle
Cycle
405nm
405 acti
405 acti
488nm
488 deacti
488 deacti
488nm
488 readout
488 readout
Camera
Acquisition
Acquisition

Outlook

- Transfection protocol
- Characterize other plasmid
- 488nm expension and fluctuation
- Sparse pattern illumination

Thank you

How does Z focus look like