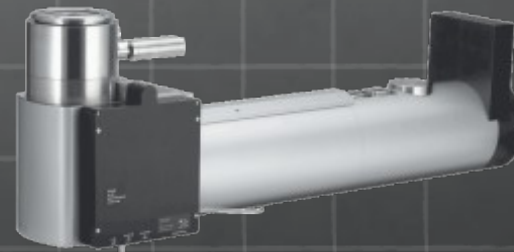
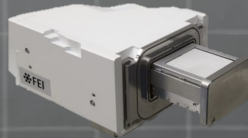
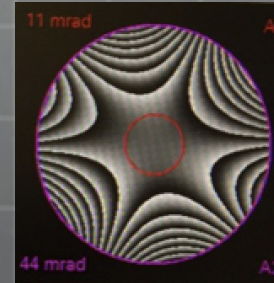


How to choose the optimal microscope/camera combinations

The Practical Matters



Anchi Cheng



National Resource for Automated
Molecular Microscopy



Common Mistakes



I have money; I will get everything, regardless.



I don't have that much money; I will buy the best scope and put it in an old lab space without renovation.

Microscope

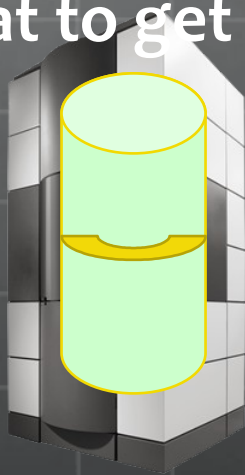
- 🌐 Stable Optics
- 🌐 Coherence and stable electron source
- 🌐 Cryo grid protection from ice buildup
- 🌐 High tension
 - 🌐 200 kV: higher contrast
 - 🌐 300 kV: smaller effect on image from lens aberration
- 🌐 Stage reproducibility and efficiency

Camera

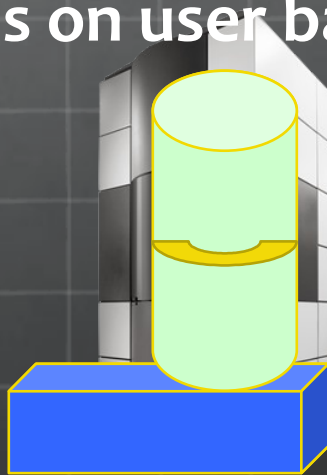
- 📺 DQE
- 📺 Frame speed
- 📺 More worse Images or Fewer better image

Add-ons to cryo-TEM

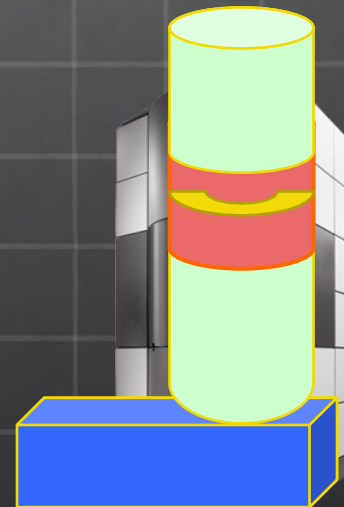
- Phase plate
 - Increase phase object contrast at low defocus
- Energy filter
 - Filter out inelastic scattering
- Image Cs corrector
 - Increase resolution limit defined by Scherzer focus
- What to get depends on user base.



SPA
Non-cellular
tomography



SPA
Tomography



SPA
Tomography
Explore new potential



I have money, I will get everything,
regardless.
**Everything you ask for,
something will be taken away**



Electromagnetic lens are not aberration free.



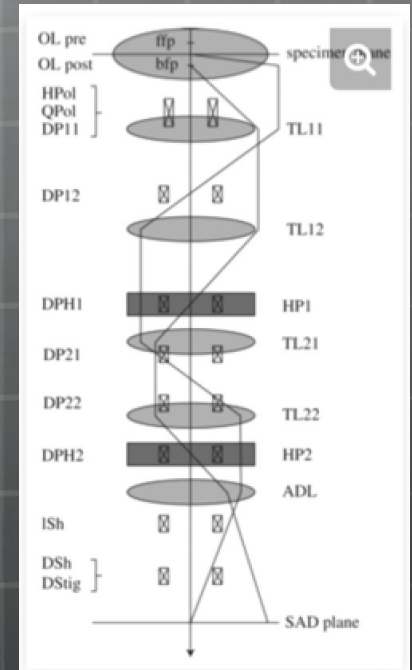
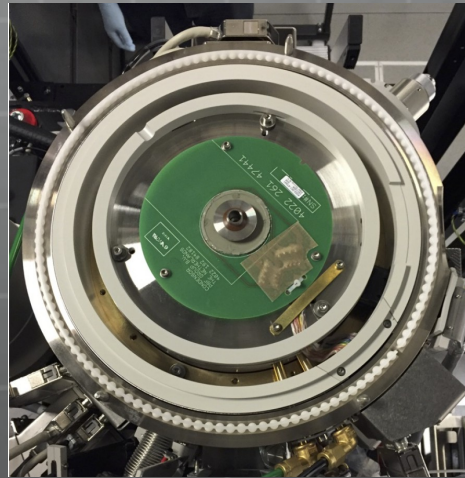
Energy filter reduces field of view that is free of distortion.



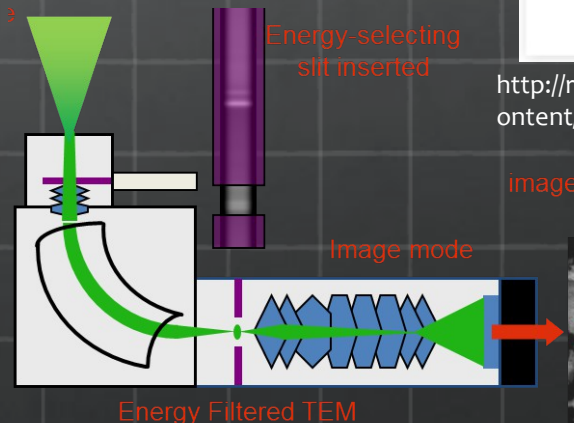
Cs corrector only offers low aberration if it is trained to compensate the deviation from the aligned condition.



Both are more sensitive to the environment change and stay tuned for shorter time.



<http://rsta.royalsocietypublishing.org/content/367/1903/3665>





How about phase plate ?

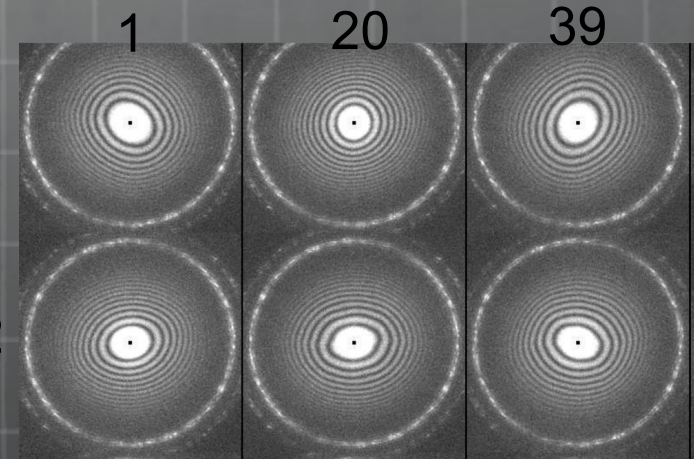


Automation challenges mostly resolved.

Phase Plate Slot 6

1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
2	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

2



Best value

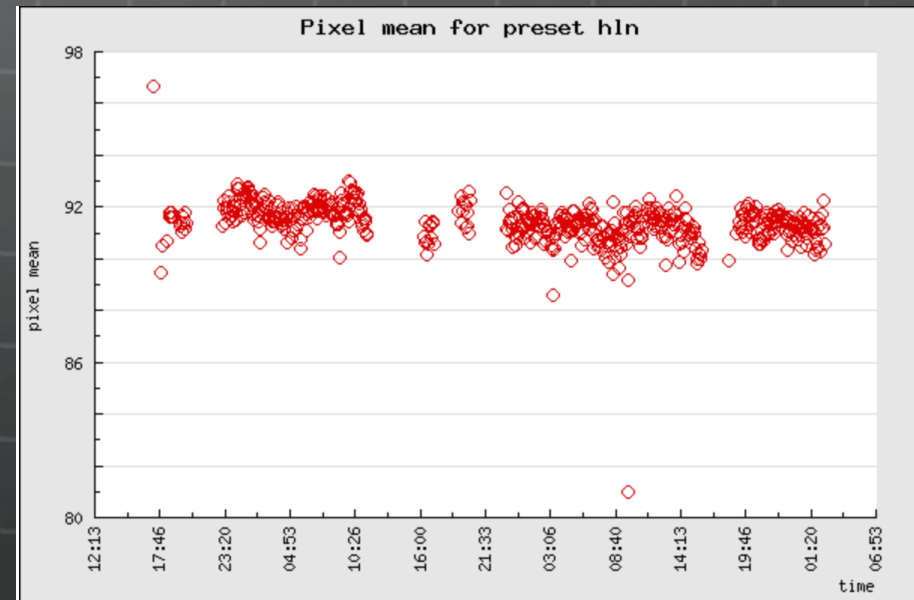


TEM is only as stable as the environment it is in

I don't have that much money, I will buy the best scope and put it in an old lab space without renovation.

- Follow the requirement from the manufacturers
- Top equipment failure resulting scope down time
 - HVAC (0.8°C p-p in 24 hr, 0.3°C p-p in 1 hr)
 - Water chiller
 - Grid autoloader
- Energy filter ZLP fail to align
- Cs corrector misalign
- Beam shifts away

3 %
↕



← 24 hr →

Validate Instrument Performance

Part of Acceptance Test



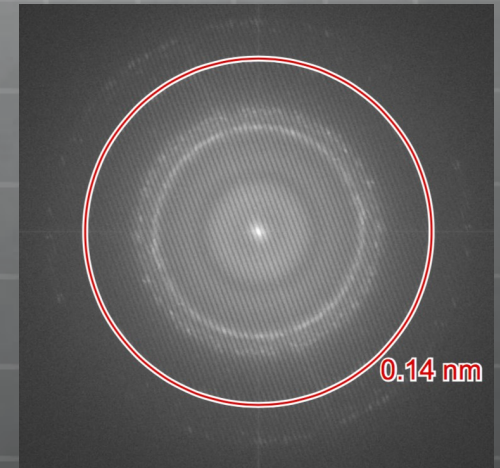
Information Limit (highest frequency that is transferred through the optical system)



Grating Replica Grid



Young's fringes in the power spectrum



Tests to show new users



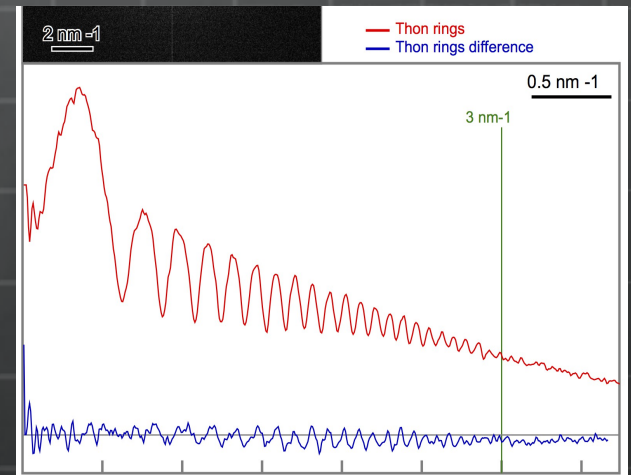
Visible Thon rings



Pt/Ir or right thickness of carbon



Grating replica if carbon is thin



Isotropic information transfer



Gold diffraction ring from

Workflow Performance Test

 Use an ideal specimen grid to test the workflow ($< 3 \text{ \AA}$)

 Proteasome

 Campbell et. al. *elife* (2015) 4:e06380

 Apo-ferritin

 Russo & Passmore *Science* (2014) 346:1377-1380

 Aldolase

 Herzik et. al.
<https://www.biorxiv.org/content/early/2017/05/25/141994>

 β -galactosidase

 Bartesaghi et. al. *Science*(2016) 348:1147-1151

 Glutamate Dehydrogenase

 Merk et. al. *Mol Pharmacol.* (2016) 89:645-51

Standard Test Result Form used @ SEMC



SEMC Operations
Krios1 testing

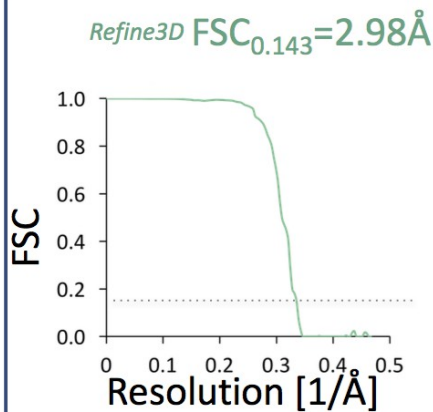


FEI
Titan Krios



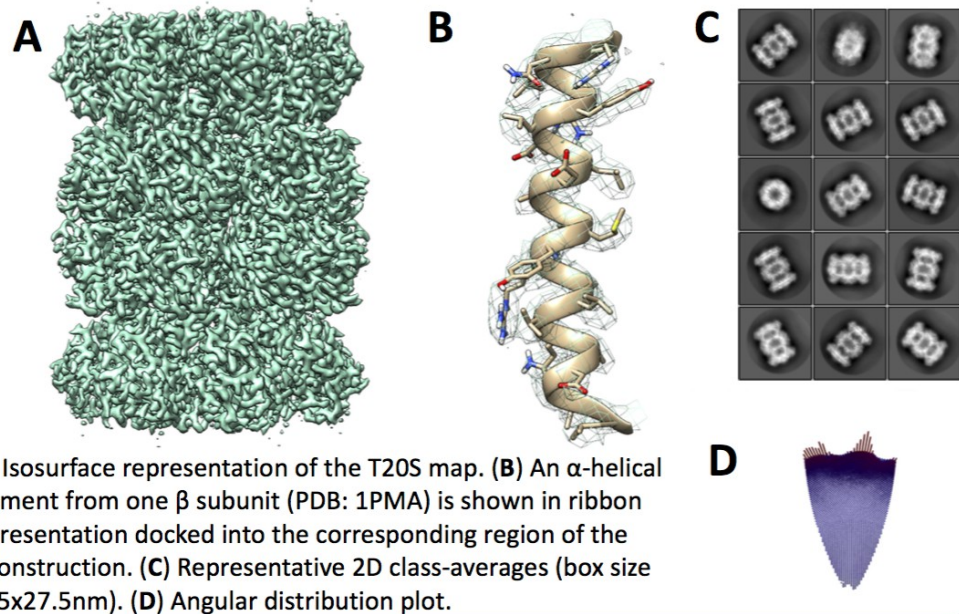
Gatan
K2 Summit
Direct Detector

Fourier shell correlation curve



Gold-standard FSC curve for the T2OS reconstruction.

CryoEM reconstruction of the T2OS
at 3.0 Å resolution



Imaging parameters

Grid loaded: 17jun09
Session: 17jun09g
Magnification: 22,500X
Pixel size: 1.07235Å/px
Camera: K2 counting, 300 ms
– 44 frames
Camera settings: 13,200 ms exp
Dose: 49.90 e⁻/Å²

Imaging statistics:

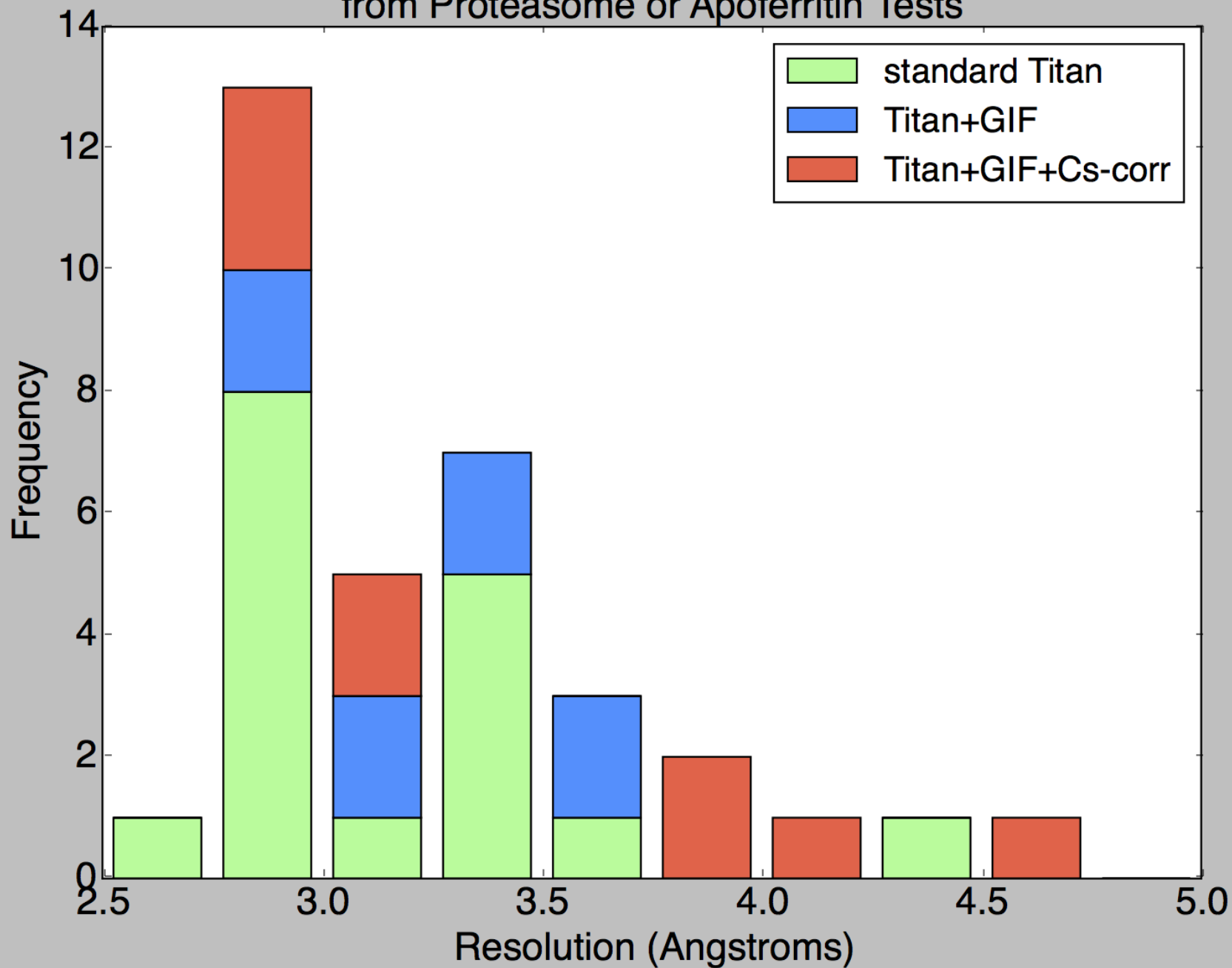
17jun09g experiment : 371 enn-a-DW images collected
130,290 Appion picks : 371 enn-a-DW images
111,721 particles : after 2D classification (3.08Å)
98,207 particles : after 3D classification (3 of 5 classes - 2.98Å)
30,585 particles (1 of 5 classes - 3.08Å); 70,640 particles (2 of 5 classes - 3.02Å);
105,373 particles (4 of 5 classes - 3.02Å)
30,585 part split into 3 and rerun: 10,150 part – 3.16Å | 20,321 part – 3.12Å | 30,585 part – 3.08Å

Sample: T2OS proteasome [gift from Yifan Cheng (UCSF)]

Reconstruction: Relion 2 3D refine



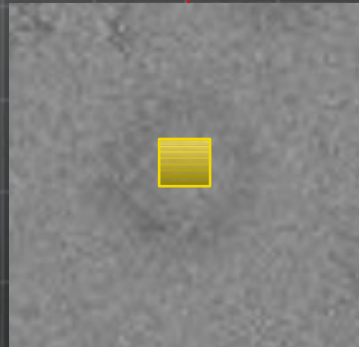
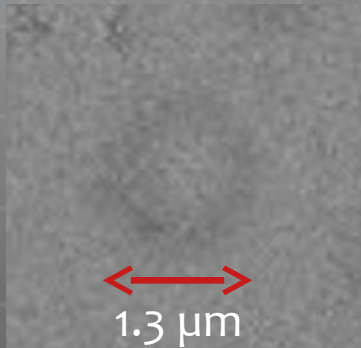
Histogram of Resolution
from Proteasome or Apoferritin Tests



All automated image collection software use some image/beam shift



Best stage: reproduce a recalled xy position within $\pm 0.2 \mu\text{m}$, typical Krios stage within ± 0.7 to $1 \mu\text{m}$.







More than 1000 images
per day ?



Beam/image shift used

EPU: Hole Centering
Leginon: Image Shift targeting
SerialEM: Beam/Image Shift targeting

Conclusions

-  Environment stability is very important for scope performance.
-  Practically, more add-ons to microscopes adds both benefit but also setup time and weak point to the microscope system.
-  Perform workflow validation test with test protein is very useful.
-  It is unclear when beam/image shift targeting will fail, yet.

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Zhening Zhang
Res. Scientist



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Res. Programmer



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Gatan
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Director



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