

Recording Optimal, Near-Atomic-Resolution Images of Cryogenic Specimens on a “Sub-Optimal” Transmission Electron Microscope

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Electron Microscopy Core Laboratory

cores.utah.edu/electron-microscopy

Nanofab and Surface Analysis Laboratory

www.nanofab.utah.edu

Acknowledgements

University of Utah
Peter Shen

FEI
Scott Richardson
Randy Meade
Chris Cunningham

Direct Electron Detectors



Direct Electron
DE-12, DE-16, DE-20, DE-64

www.directelectron.com



Gatan
K2 Base, K2 Summit, K2XP, K2 IS

www.gatan.com

FEI, Falcon I, II, III(?)

www.fei.com

State-of-the-Art Biological TEMs



FEI Titan Krios

- 300 kV
- Automated sample loader
- Isolated stage
- Instrument enclosure provides thermal and acoustic shielding
- ConstantPower™ lenses

State-of-the-Art Biological TEMs

FEI Titan Krios

FEI Polara

JEOL JEM3200FSC



What if I Don't Have a State-of-the-Art TEM?

- 200 kV
- Side-entry holders

Field-Emission Gun

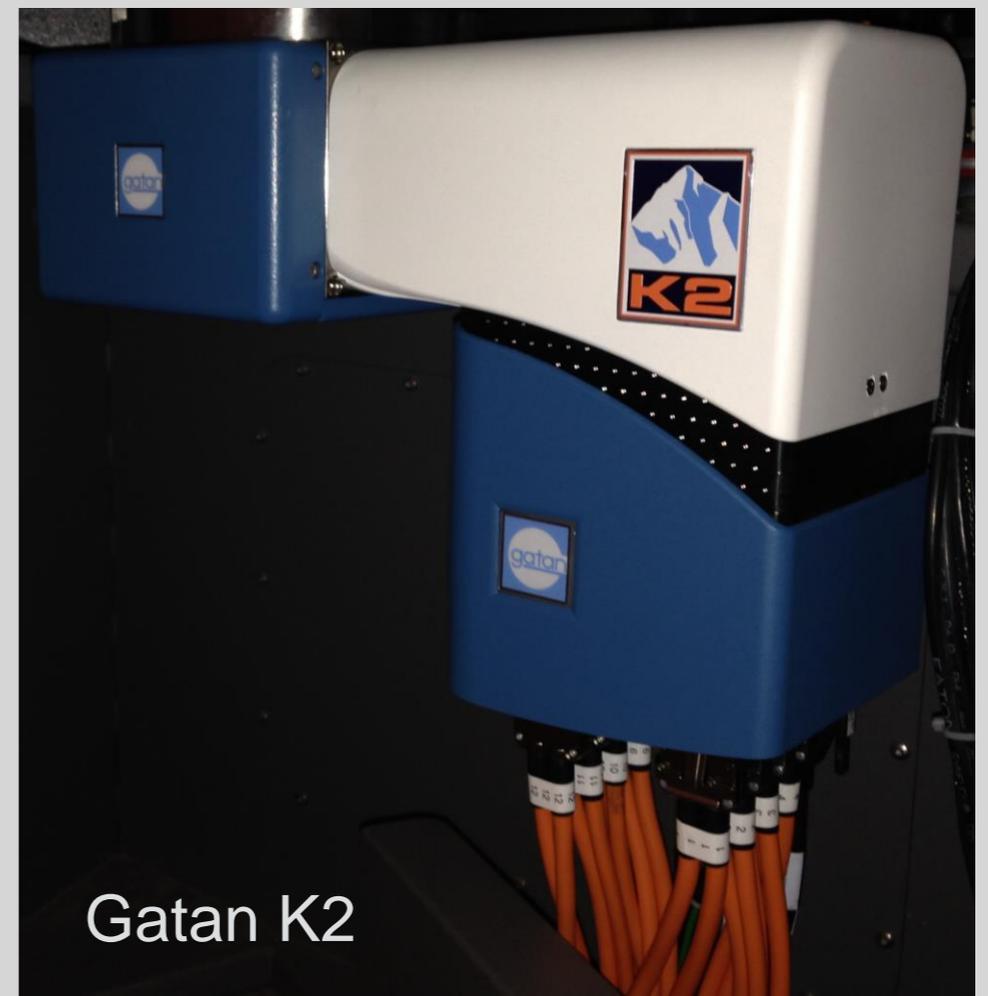
FEI Tecnai F20

**Can I Successfully Put a
Direct Electron Detector
on a Microscope Like
This?**

**Or is it a waste of
money and time?**



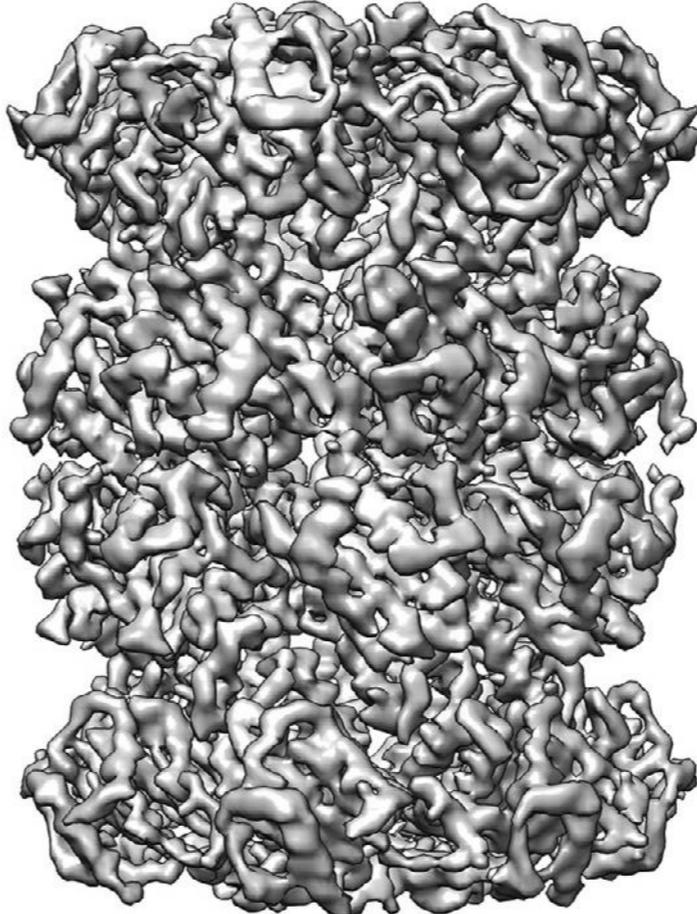
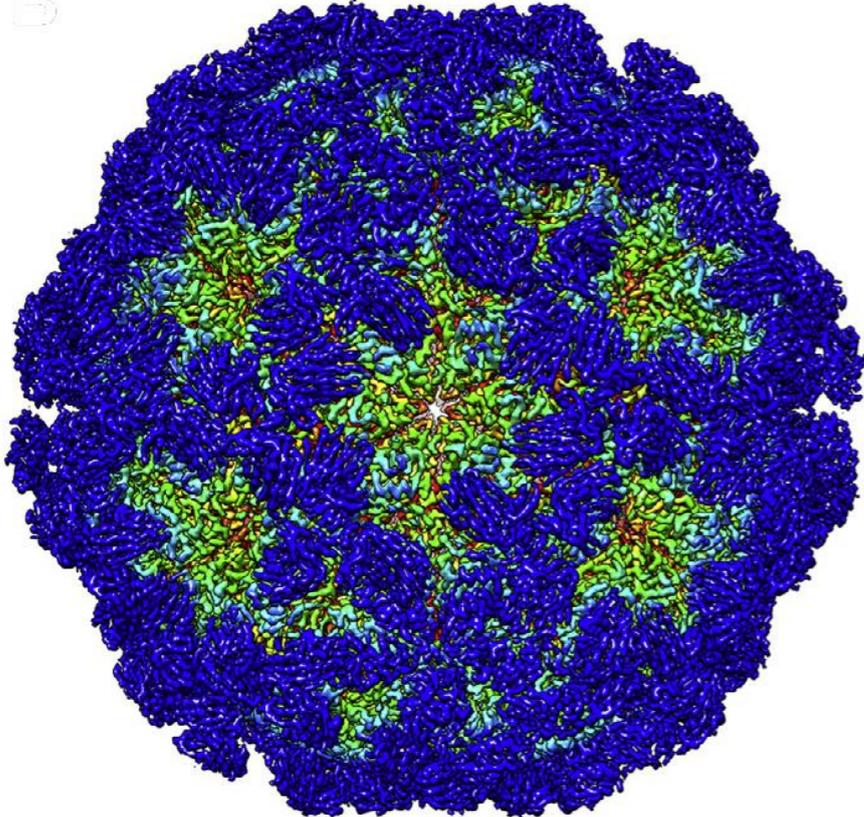
FEI Tecnai F20



Gatan K2

“Near-atomic resolution reconstructions using a mid-range electron microscope operated at 200 kV”

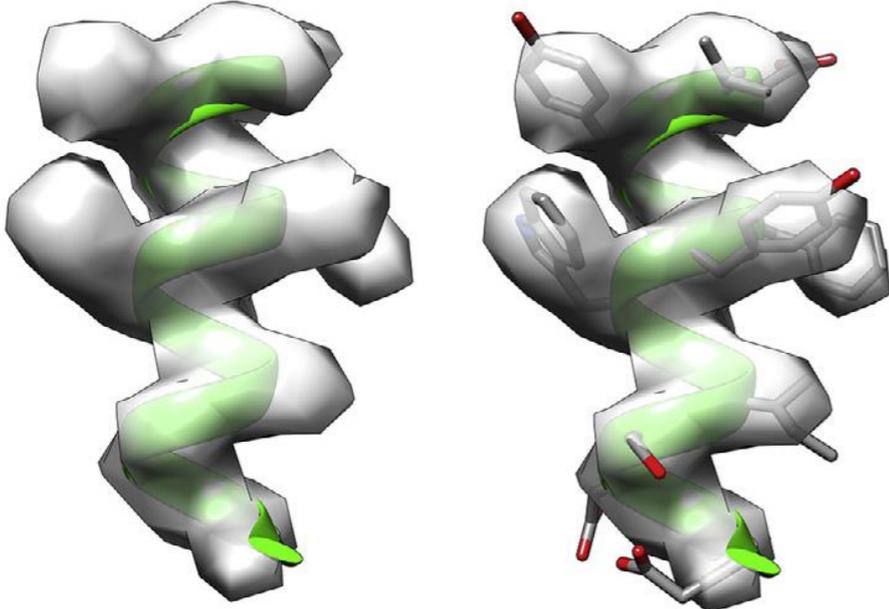
B



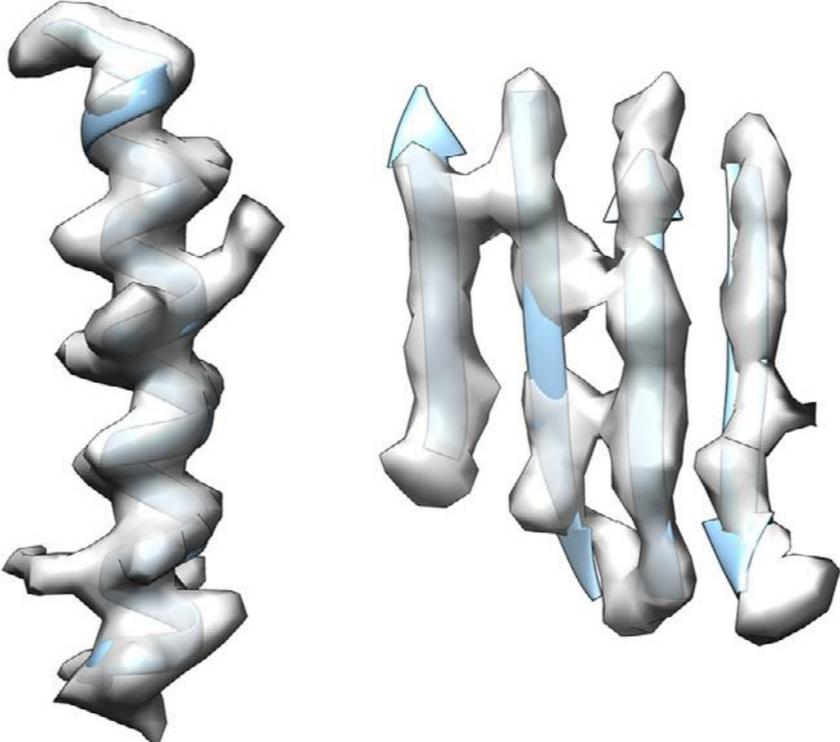
**FEI Tecnai
F20**

Proteasome
4.2 Å

D



NωV, 3.7 Å



“Near-atomic resolution reconstructions using a mid-range electron microscope operated at 200 kV”

FEI Tecnai F20

Careful Alignments, including coma-free alignment

Extraction voltage = 4150 V

Gun lens 3

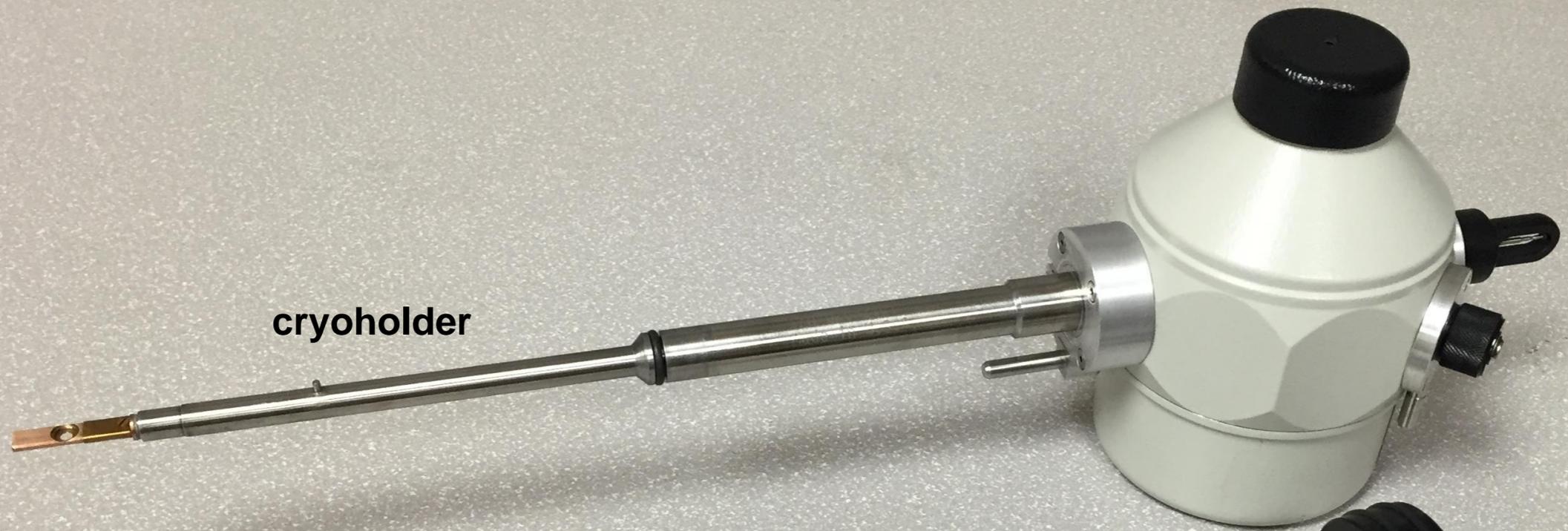
Spotsize 6 (scale 1-11)

Thon rings to ~ 3 Å over amorphous carbon

C2 aperture, 50 microns

Beam width ~ 1.2 microns (at specimen)

Tecnai F20 Holders

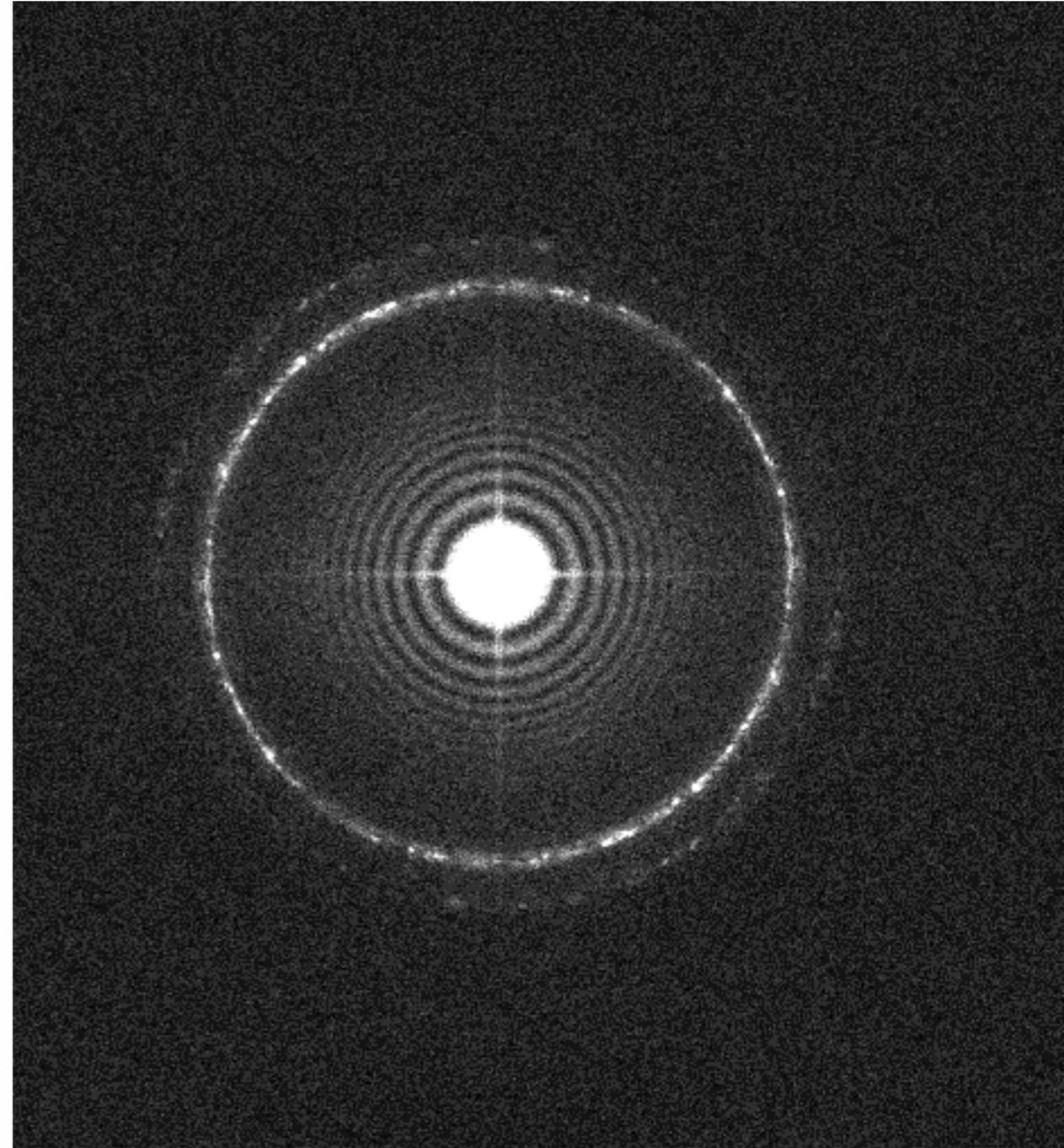


cryoholder



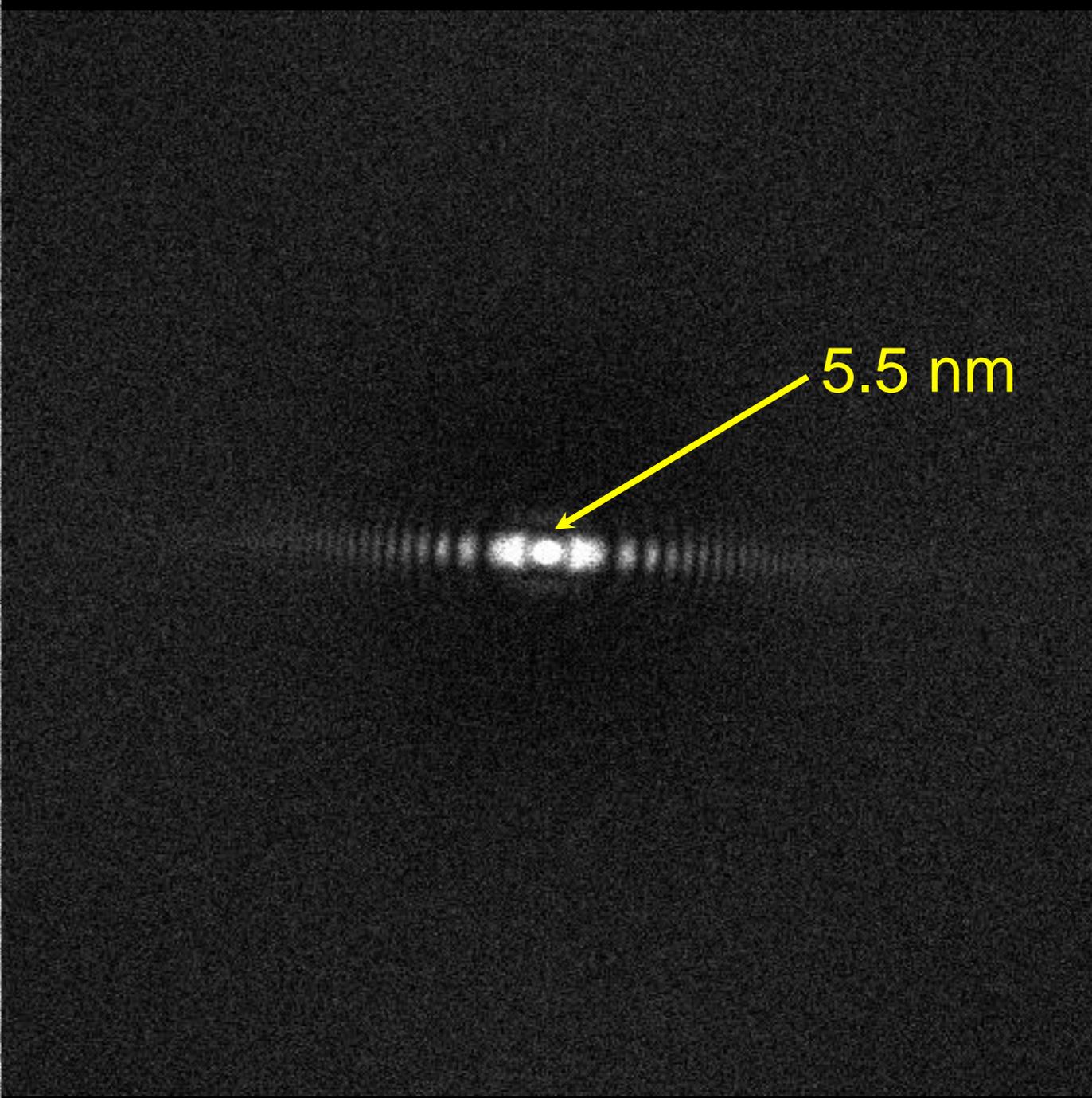
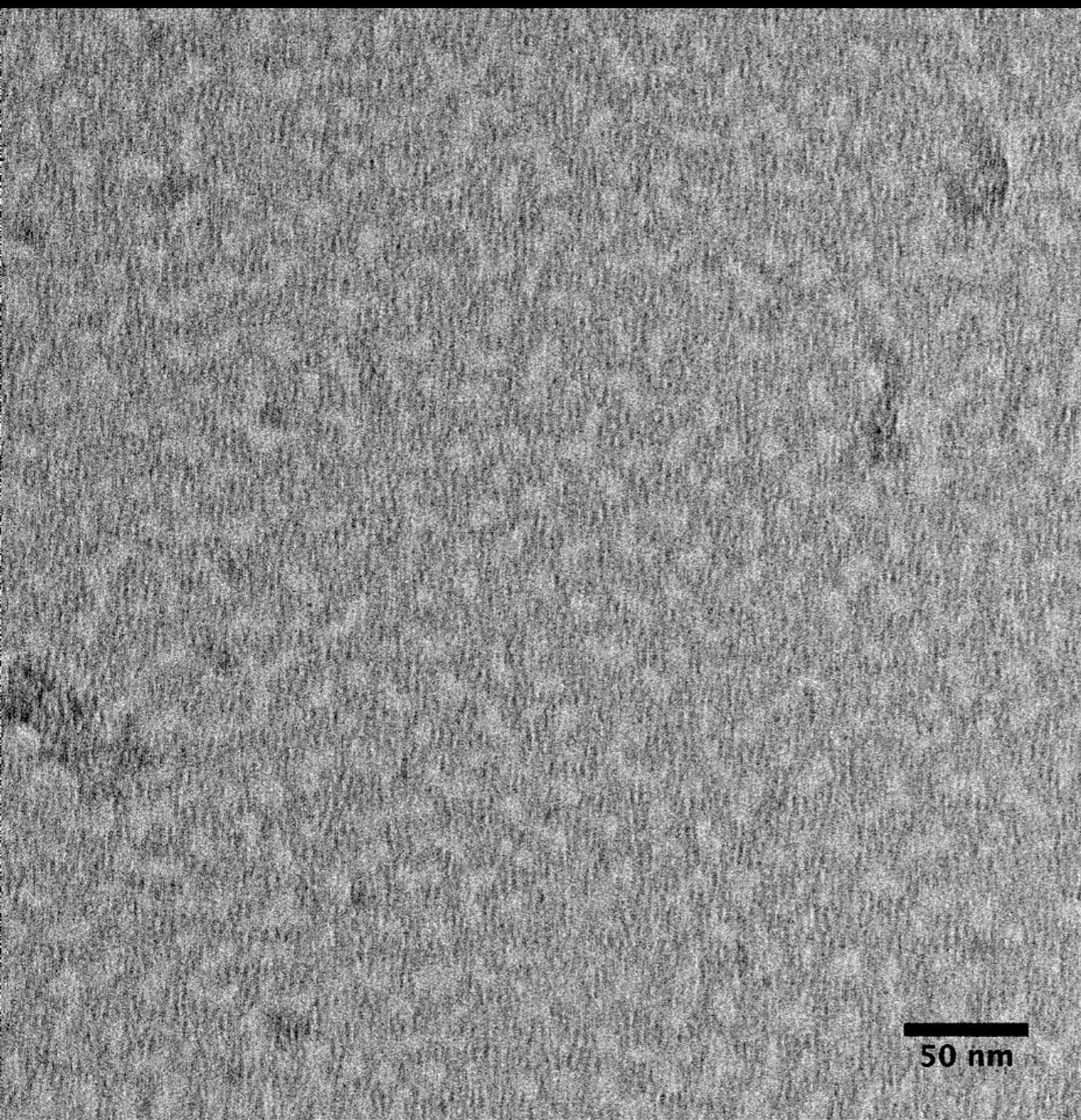
standard holder

Au on Carbon



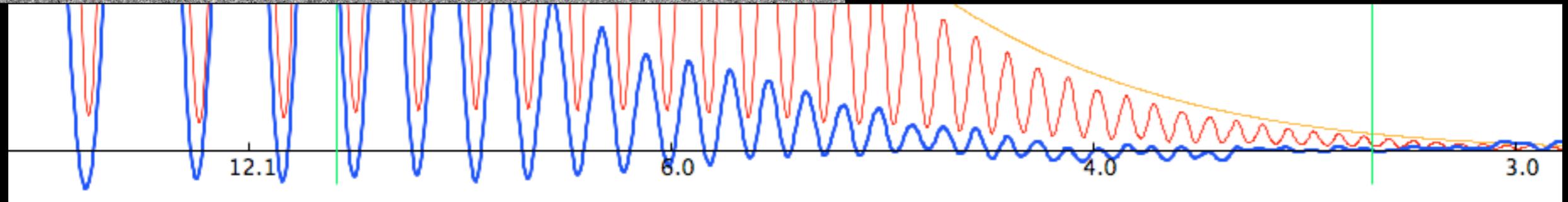
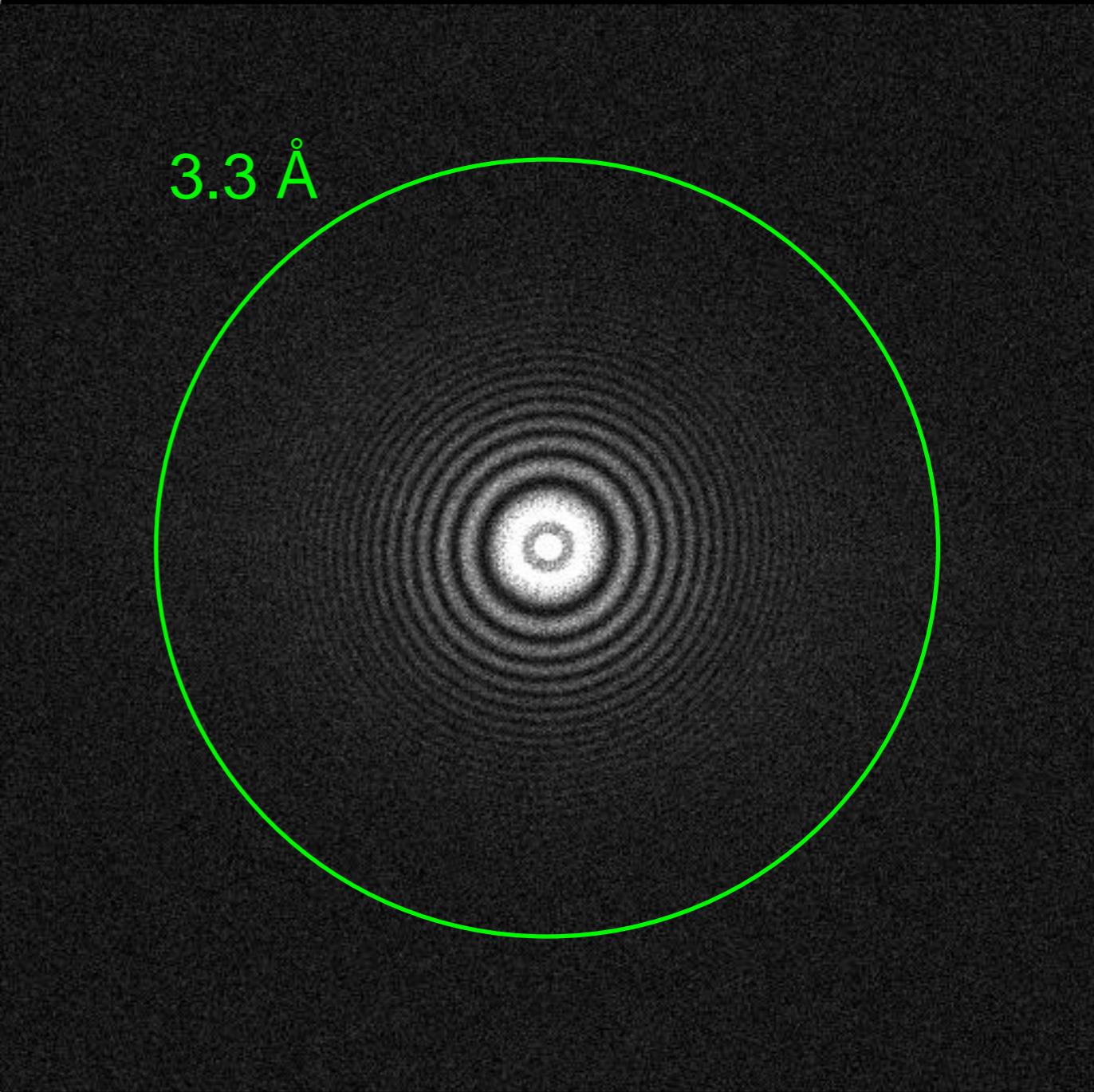
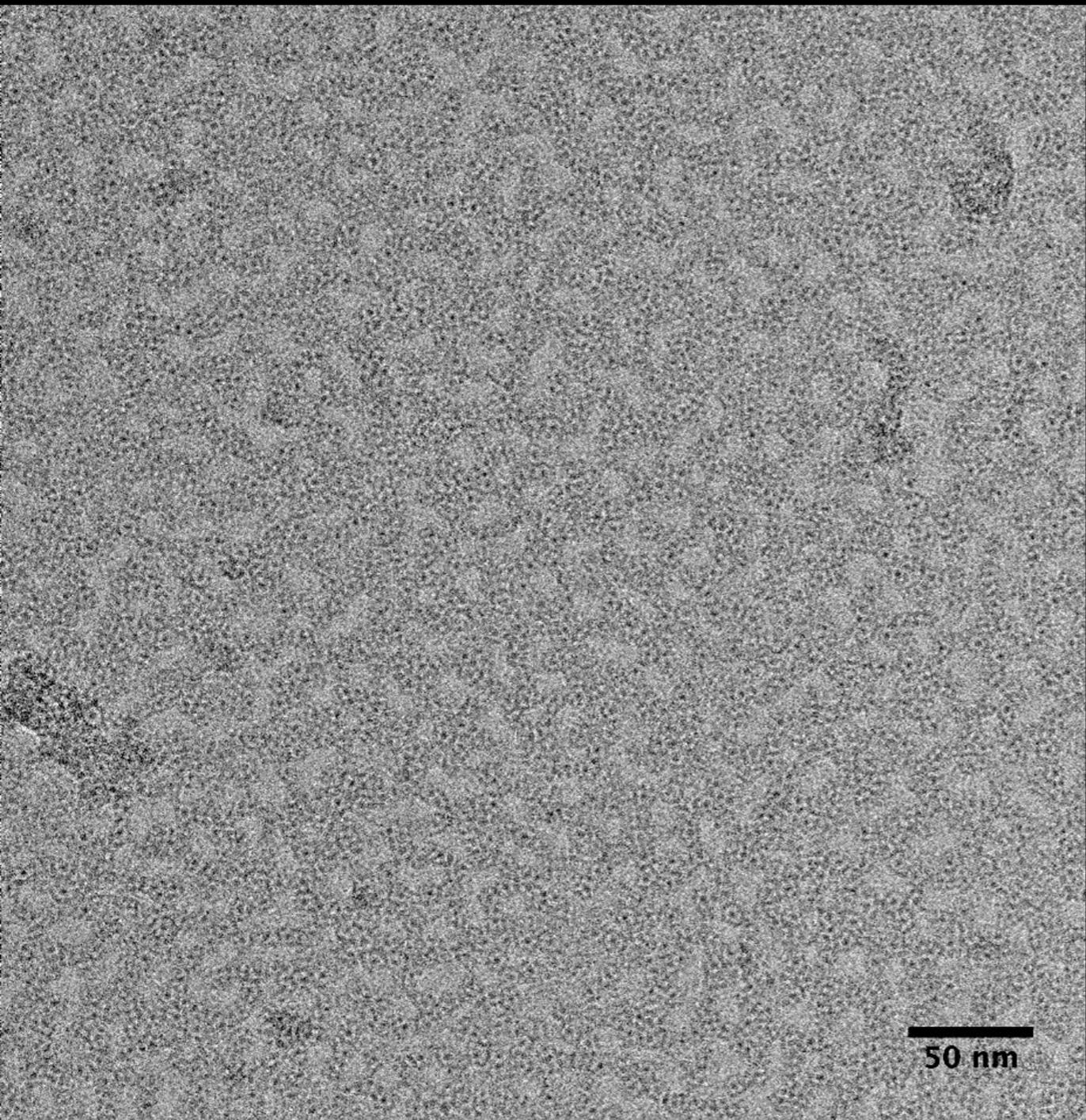
standard holder

Uncorrected Drift/Motion

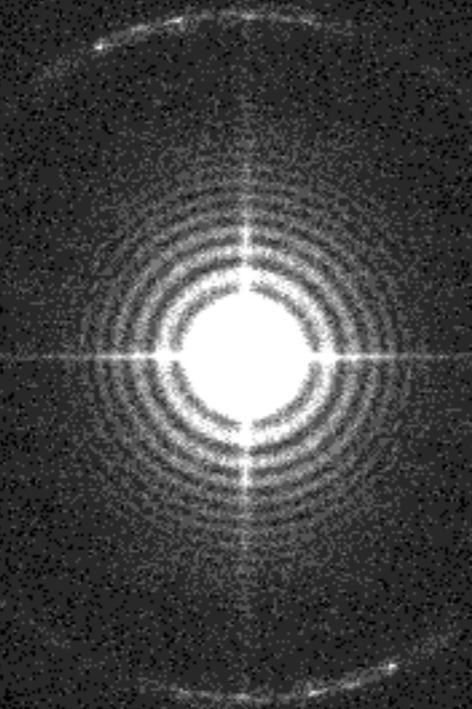


pentamers ~8 nm

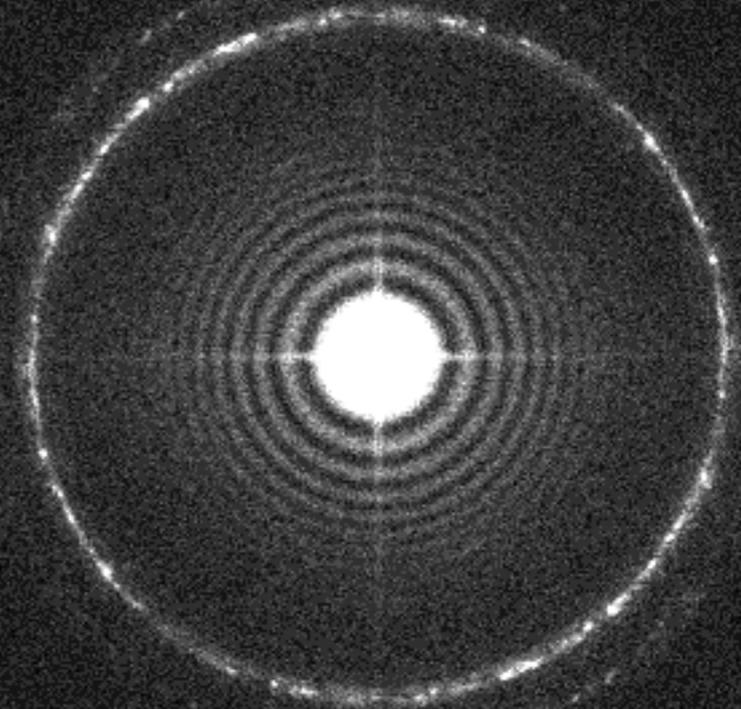
Motion Corrected



Au on Carbon
Motion Corrected

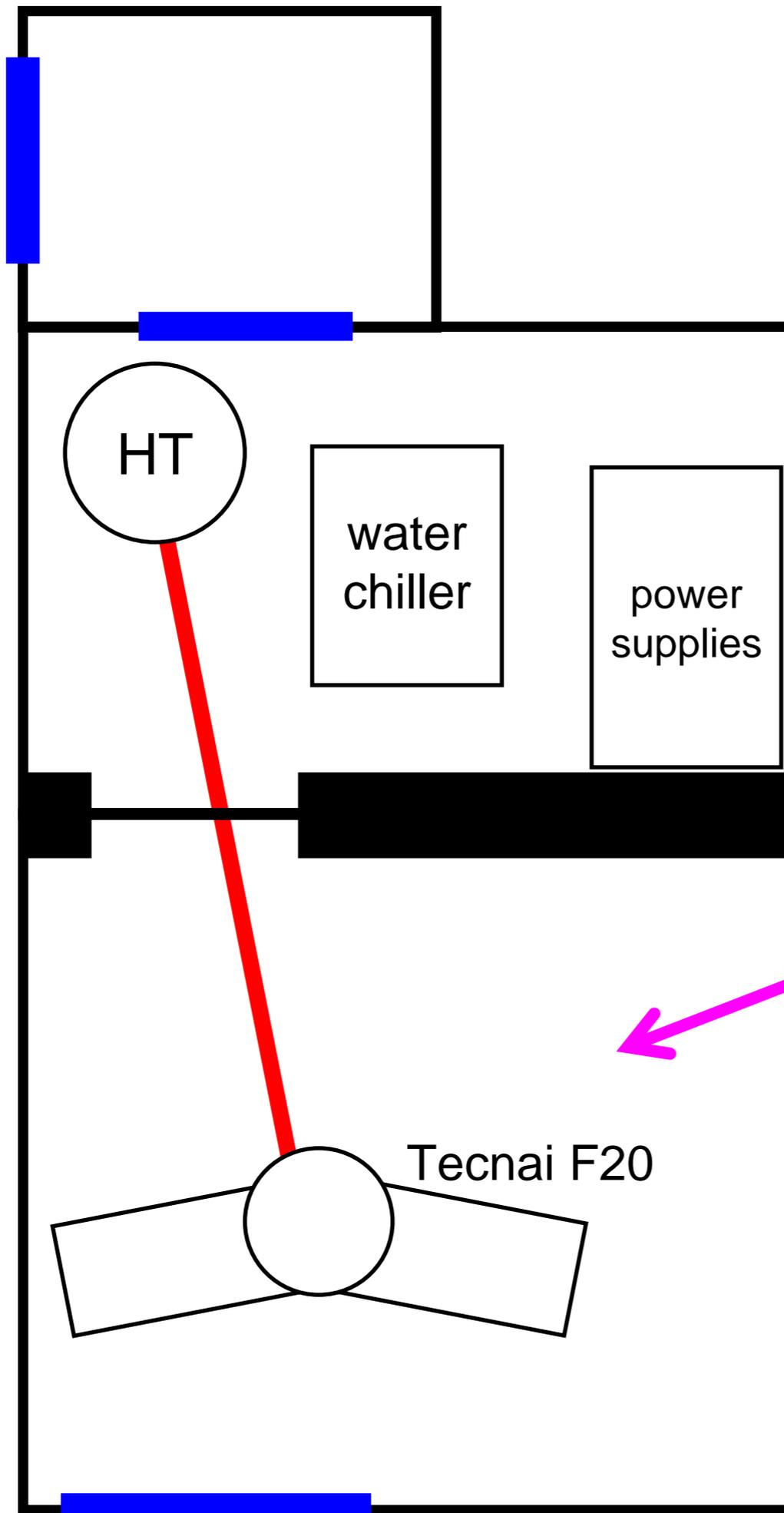


cryoholder



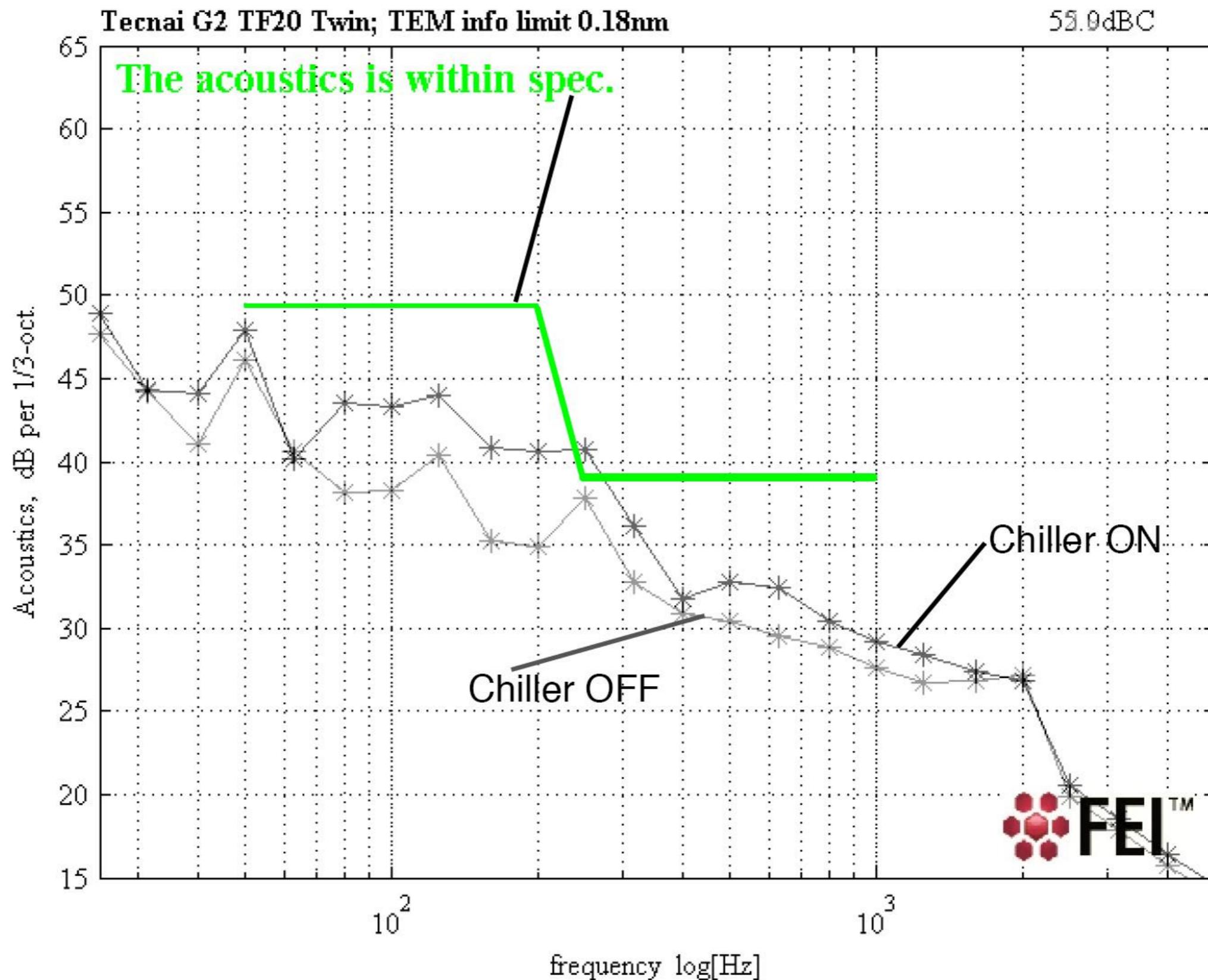
standard holder

Our Set-Up



Spicer electro-magnetic field cancellation system

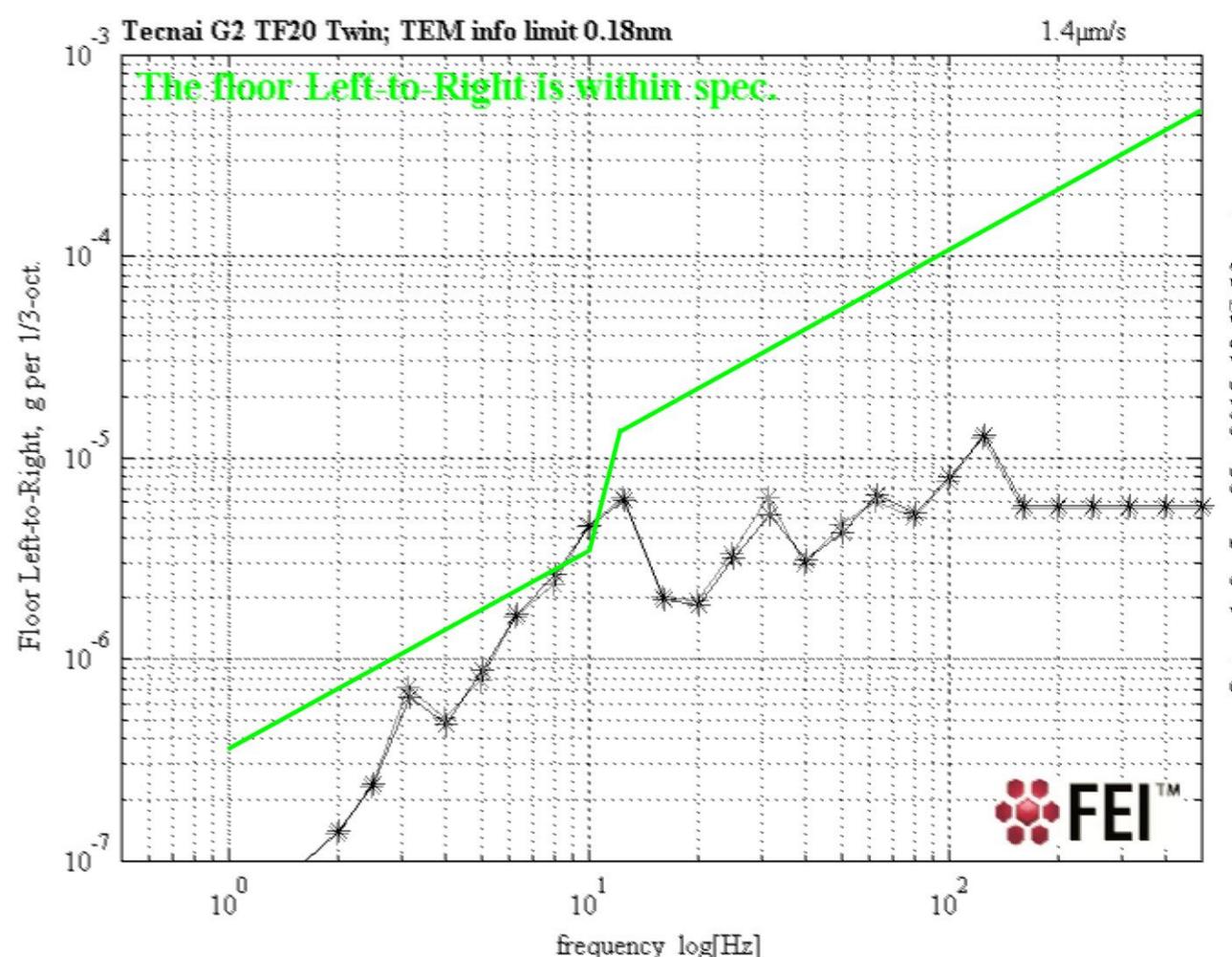
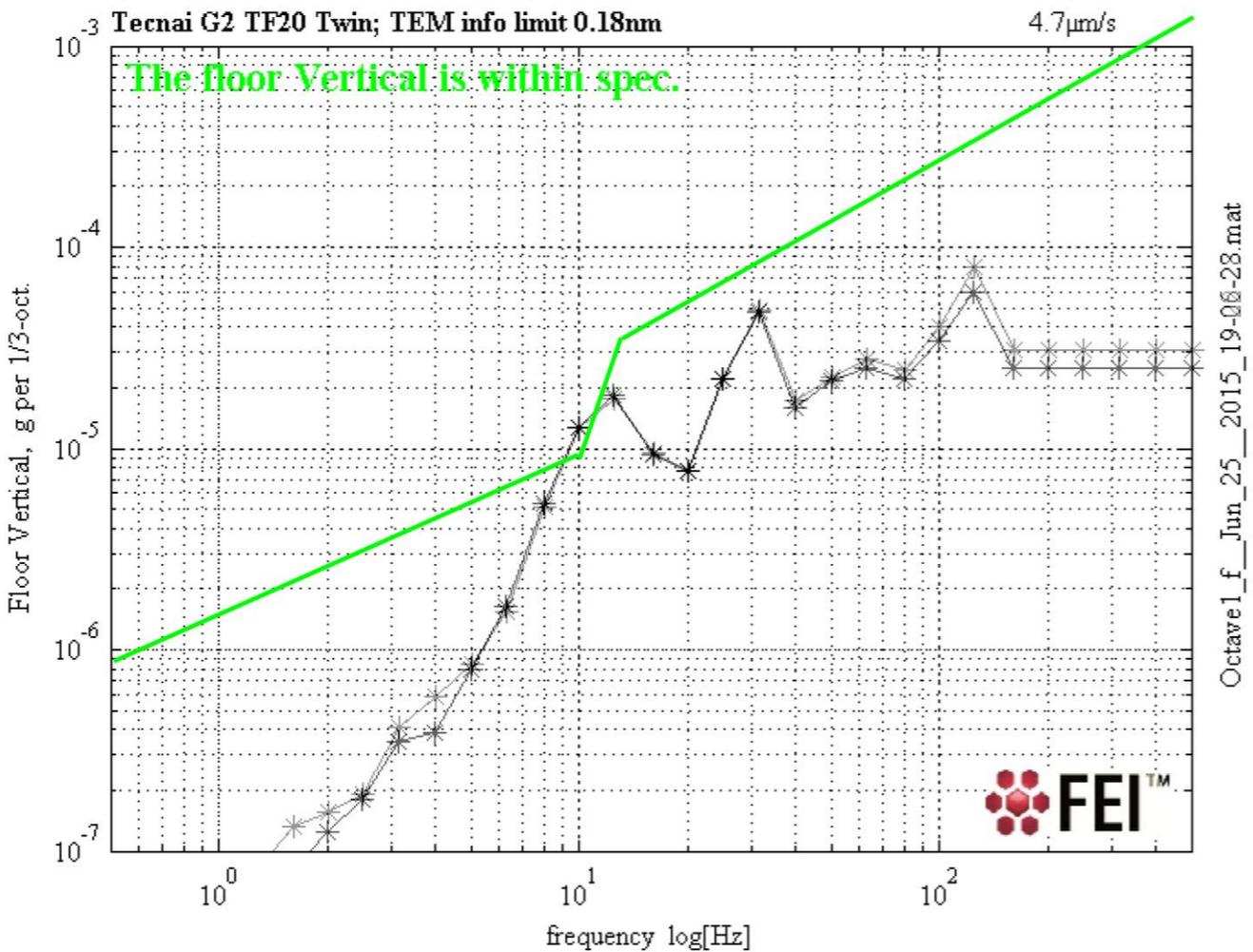
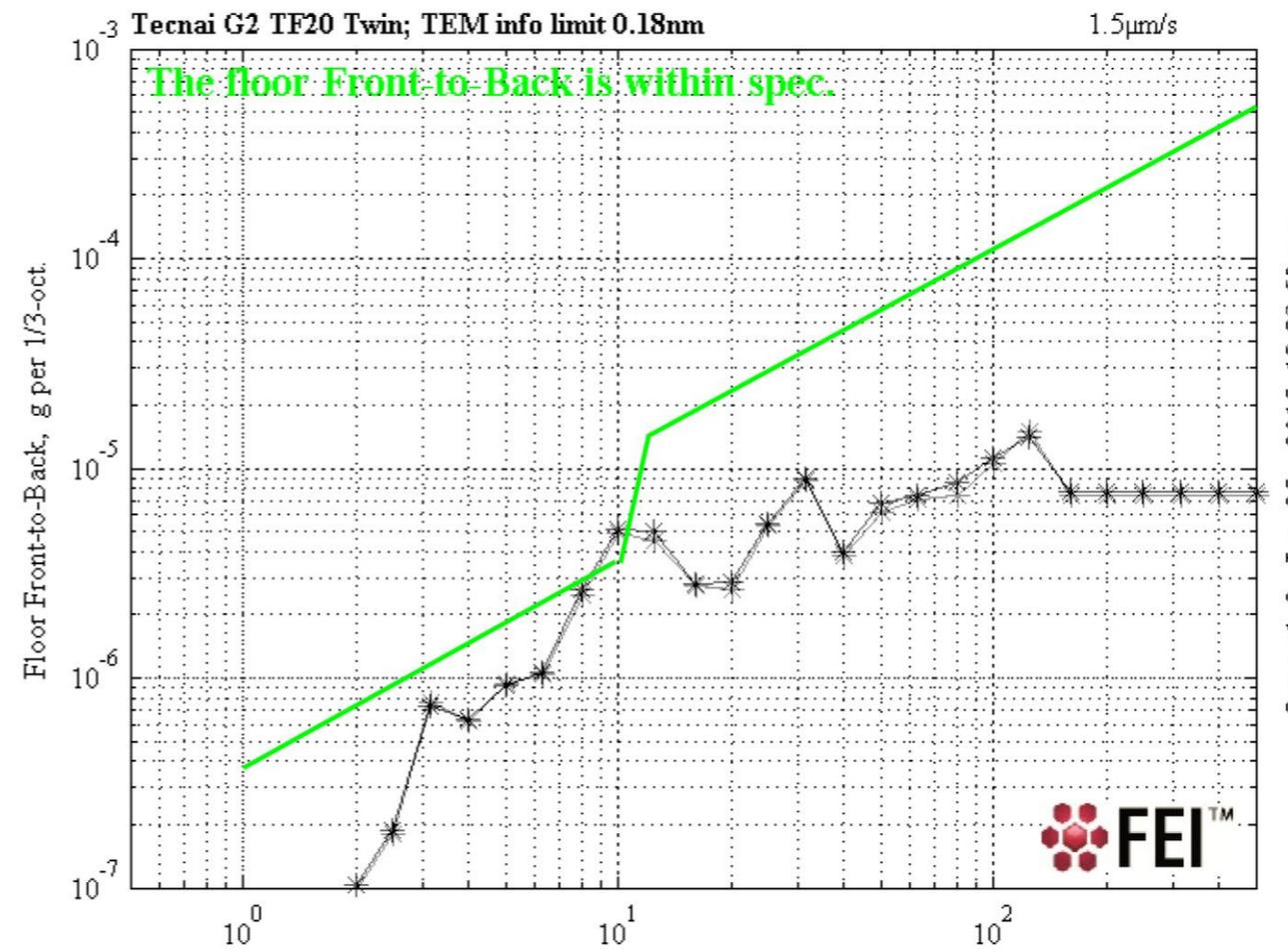
Acoustics



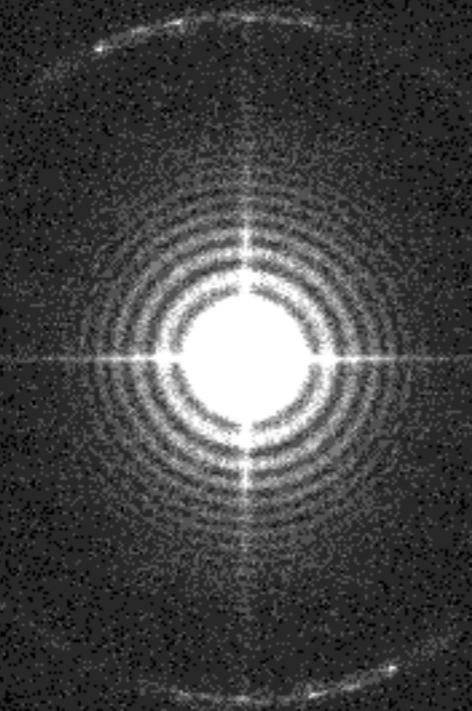
Octave1_f_Jun_25_2015_20-19-56.mat



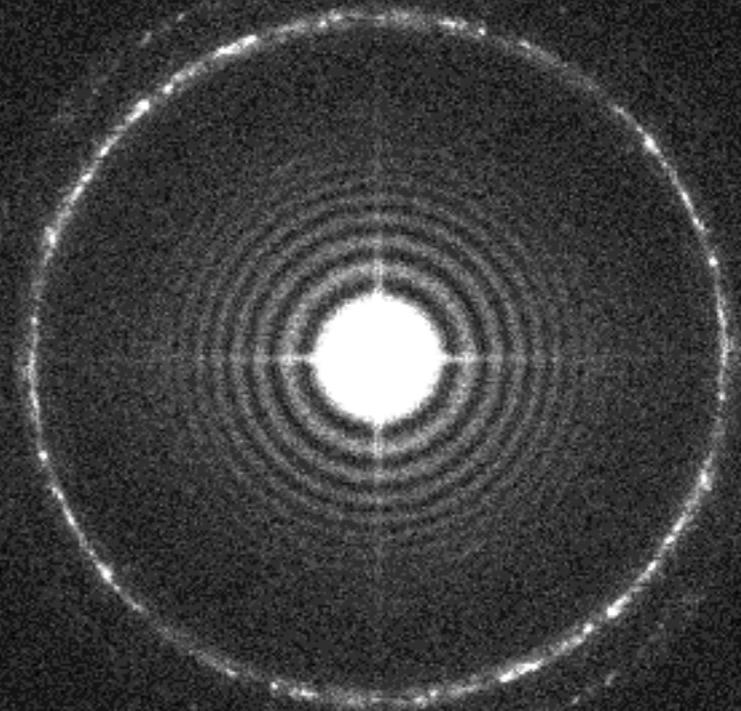
Mechanical Vibrations



Au on Carbon

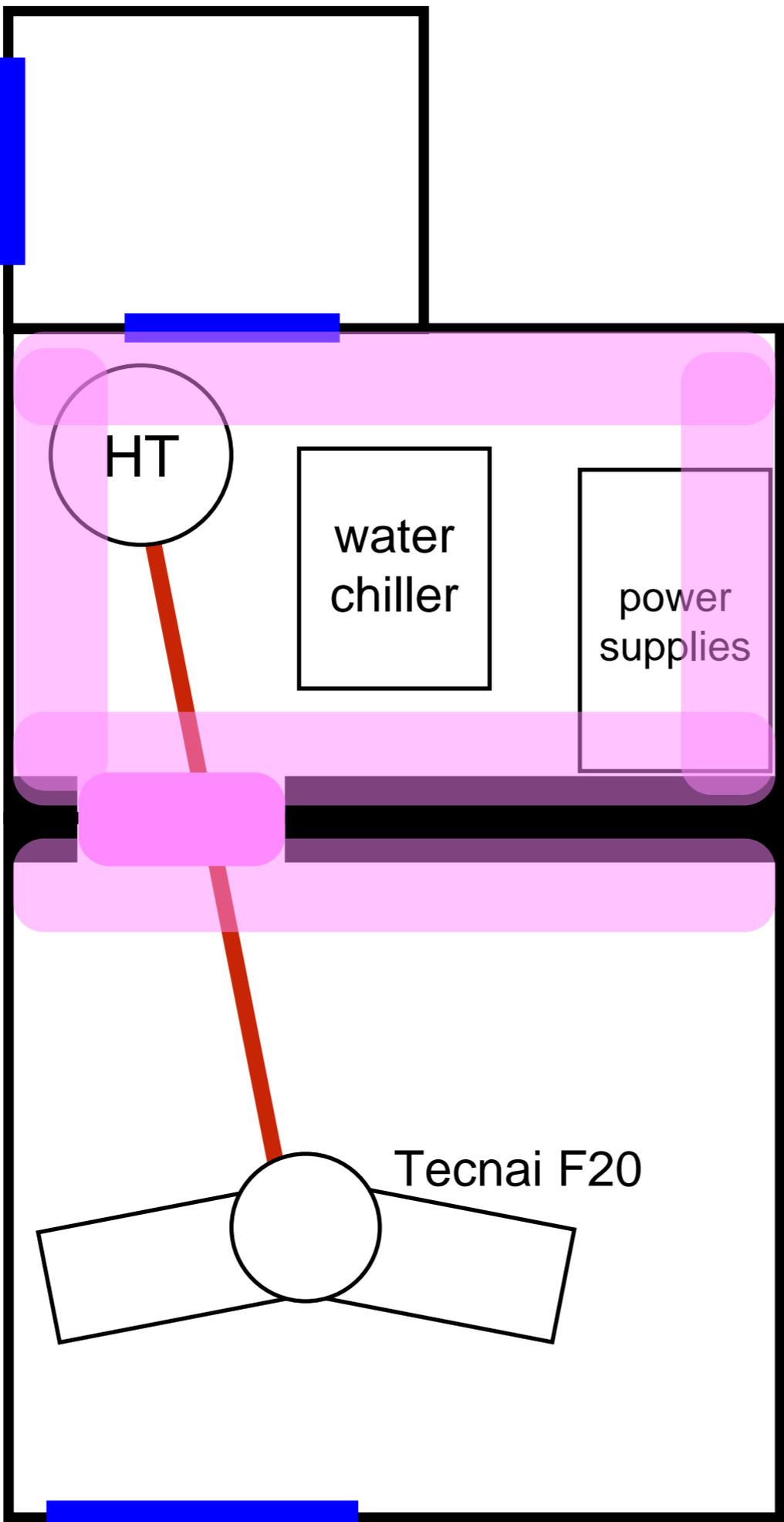


cryoholder



standard holder

Our Set-Up



Temperature Stability

Air Temperature in Room, Stable?

Temperature of Water Cooling Objective Lens, Stable?

Make Variation 0.5 °C

~22 °C

Two Big Helps

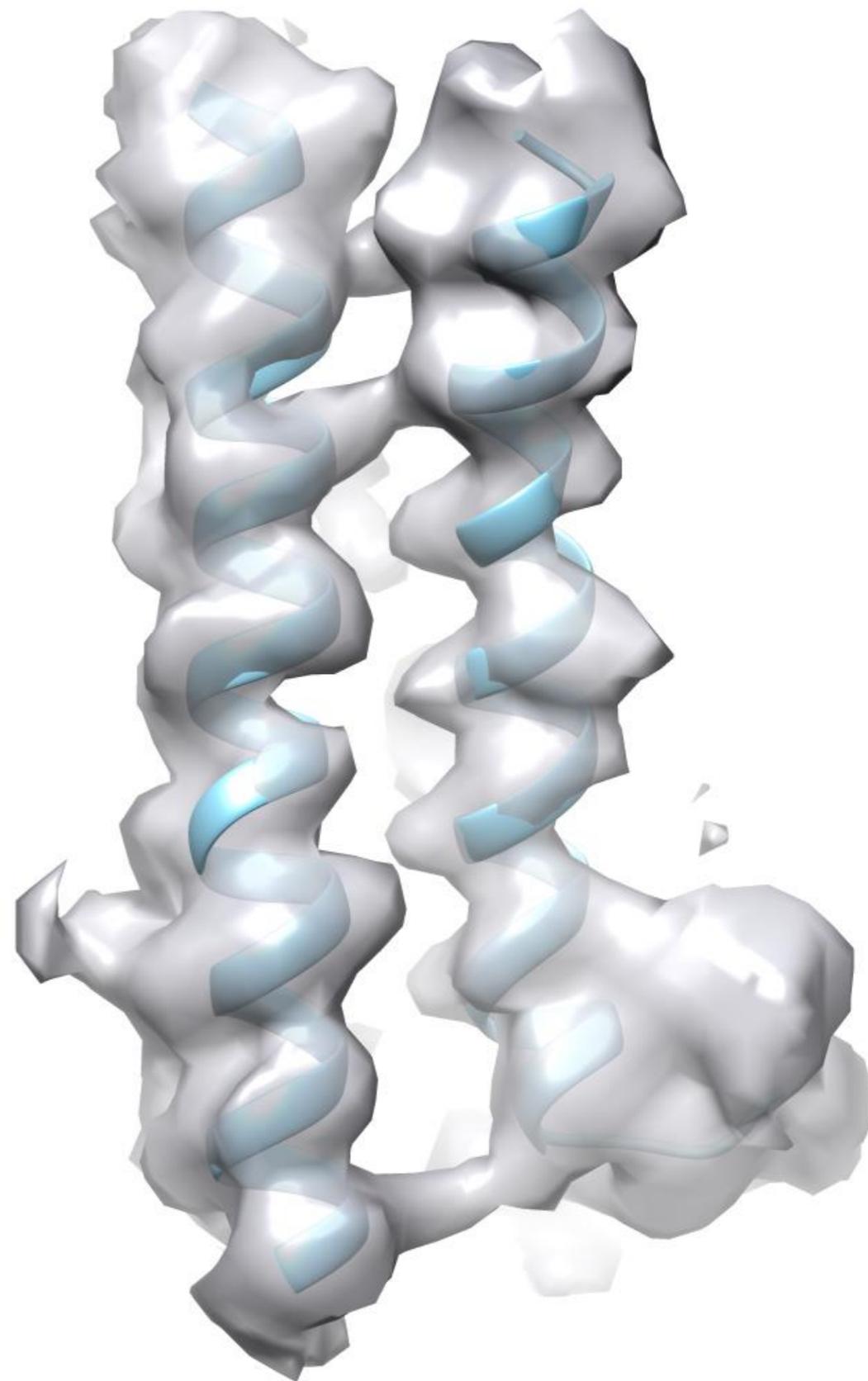
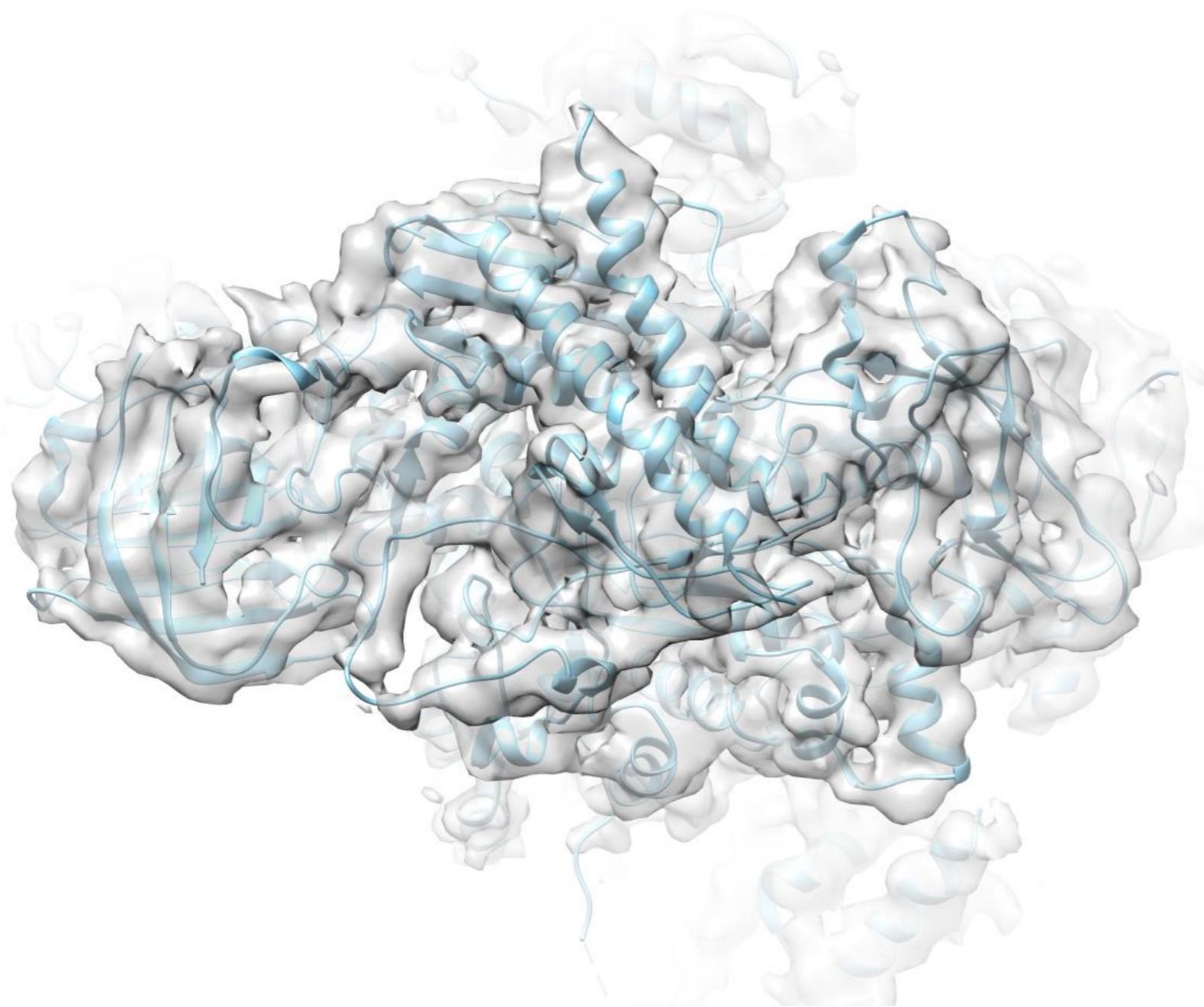
Adjustment of V-groove in Stage

Significantly Lowered the Flow Rate of Water into the Objective Lens

**20 liters/hour
OBJ1 and OBJ2**

Success!

4.2 Å



RNA Polymerase II

Detective Quantum Efficiency (DQE)

$$DQE = \frac{SNR_{output}^2}{SNR_{input}^2}$$

How efficient is a detector at converting incident energy into image information?

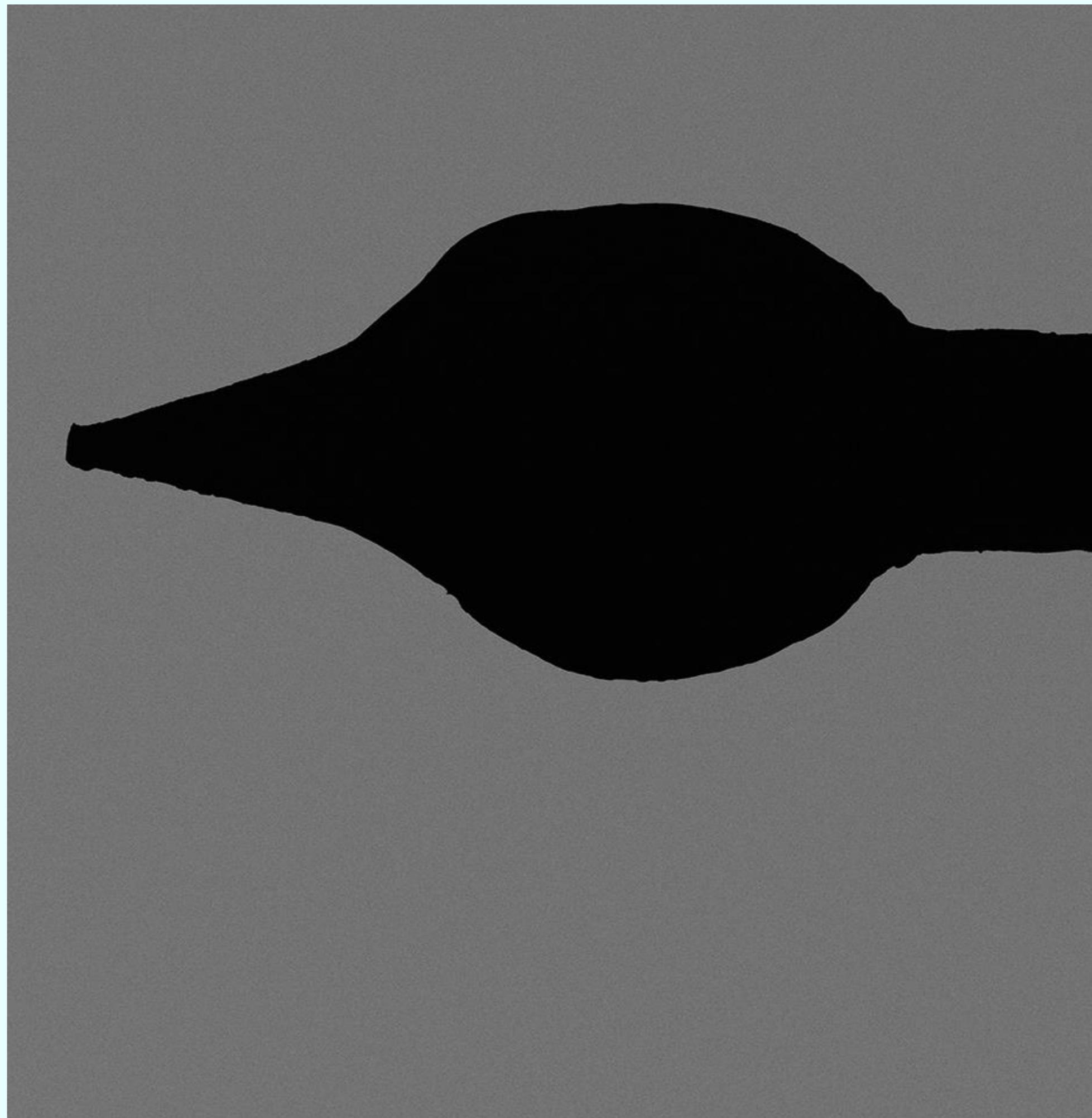
How much noise does a detector add to a recorded image?

Do It Yourself DQE!

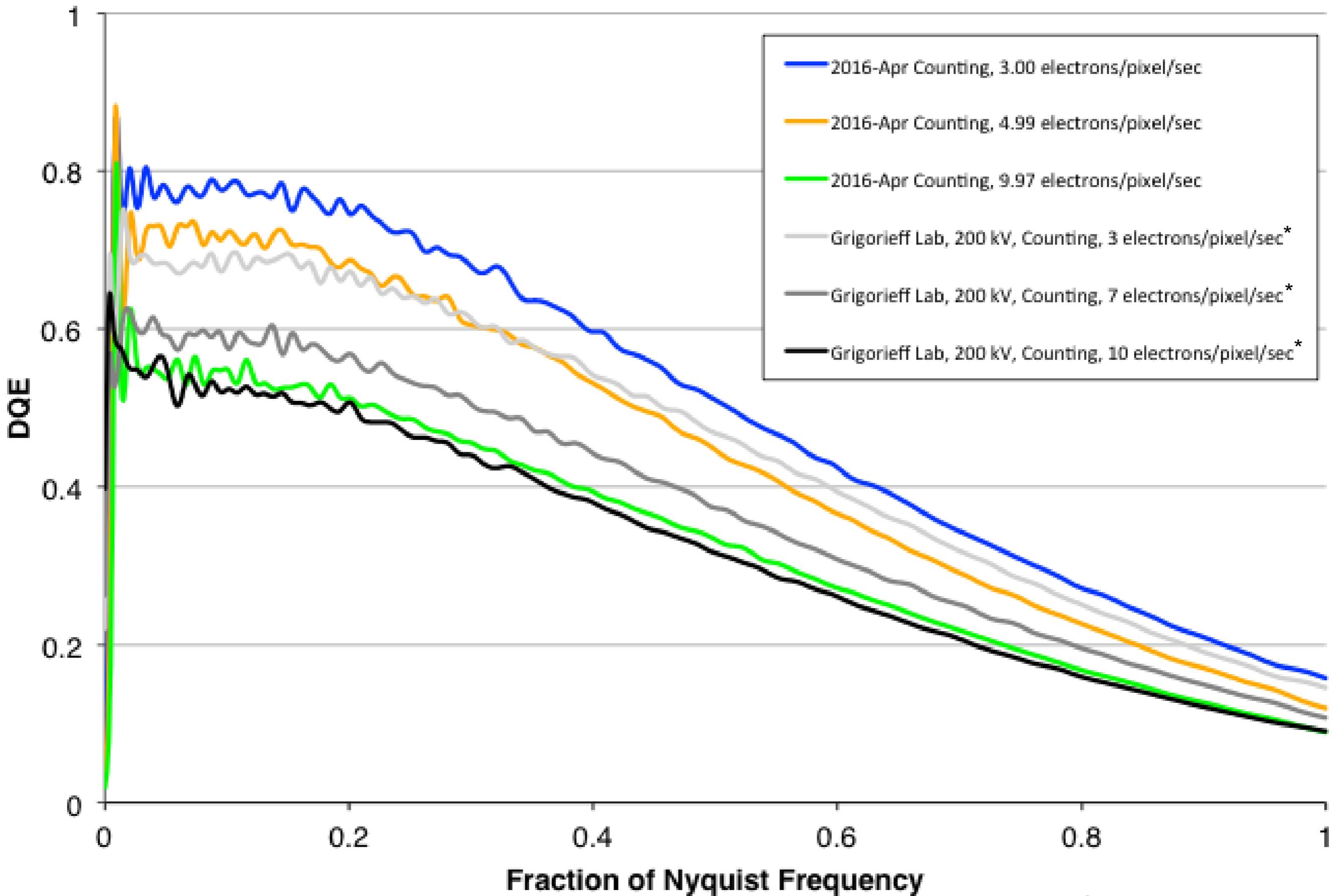
Nikolaus Grigorieff Lab 2013

J. Struct. Biol. 184:385

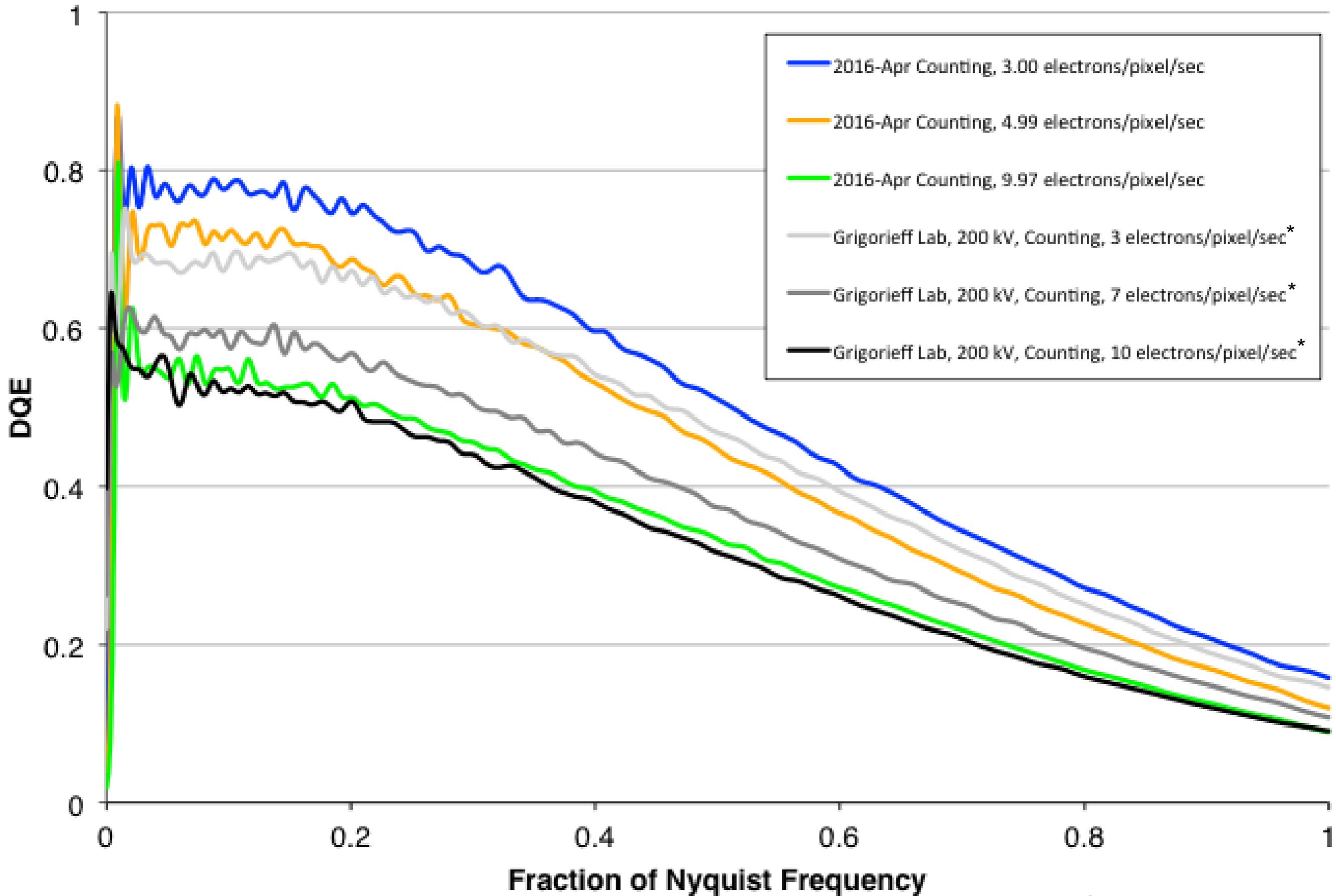
- 1. Set dose
without
pointer**
- 2. Insert
pointer**
- 3. Record
image, 50 e/p**
- 4. FindDQE**



Do It Yourself DQE!



Lower Dose = Higher DQE



Elliptical Distortion of Magnification, Magnification Anisotropy

Grigorieff Lab, Timothy Grant

J. Struct. Biol. 192:204 (2015)

between 14,000x and 37,000x saw 2.5 to 1.6% distortion

Wen Jiang, Purdue

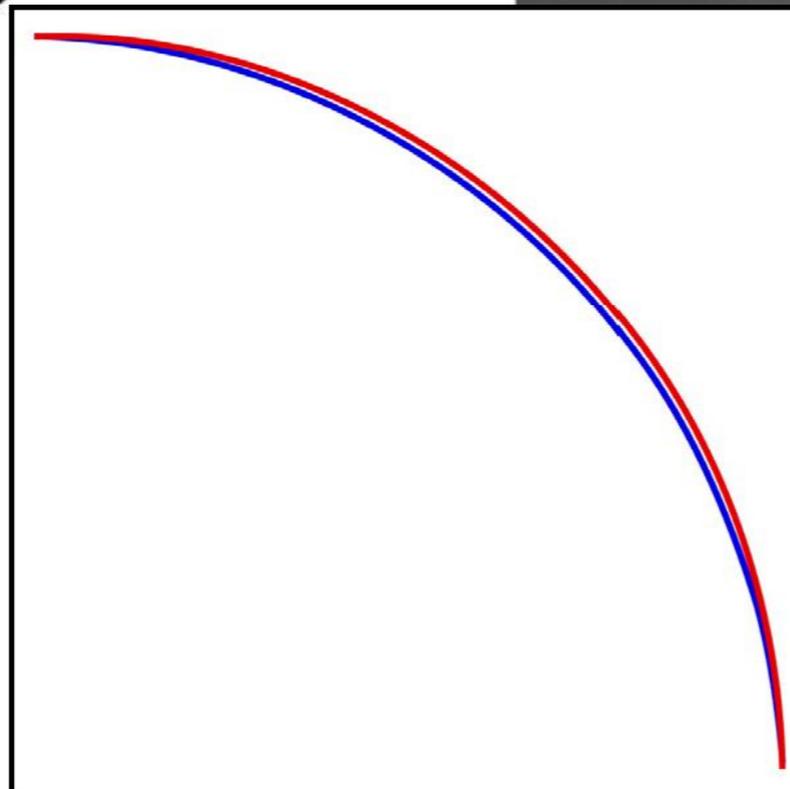
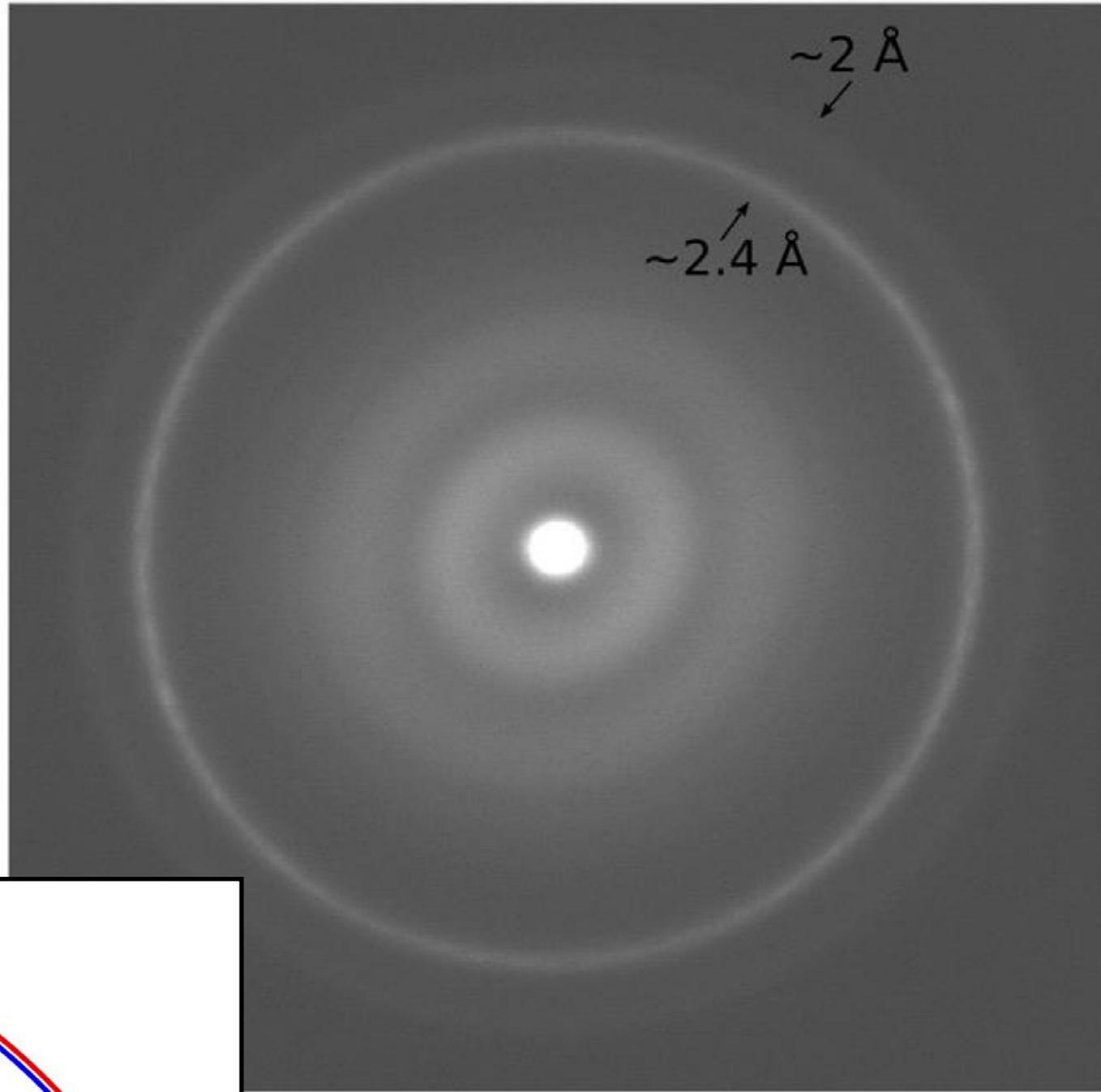
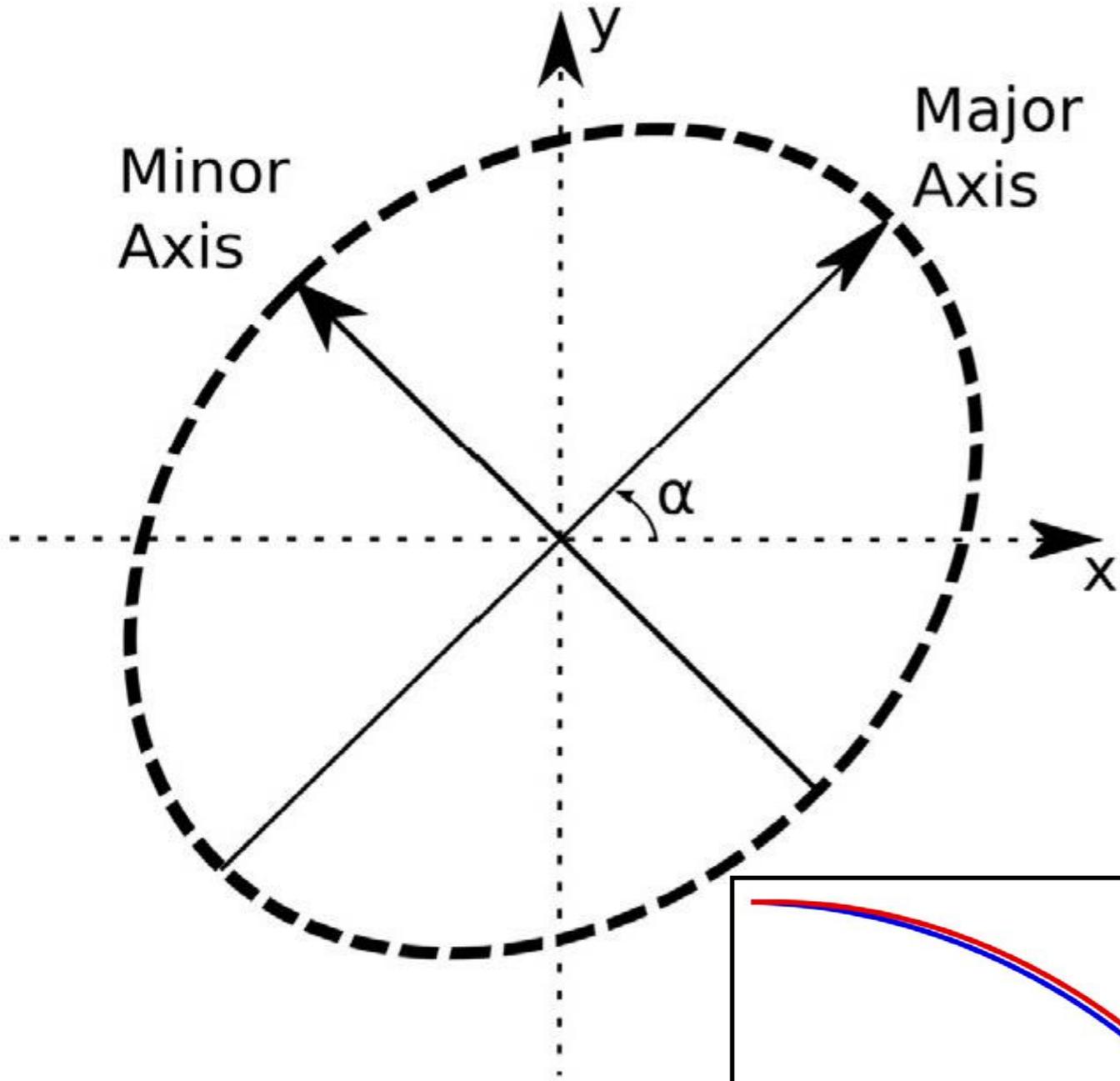
2-3% at low magnifications

significant for viruses, perhaps not for other particles

problem minimal at 59,000x and higher

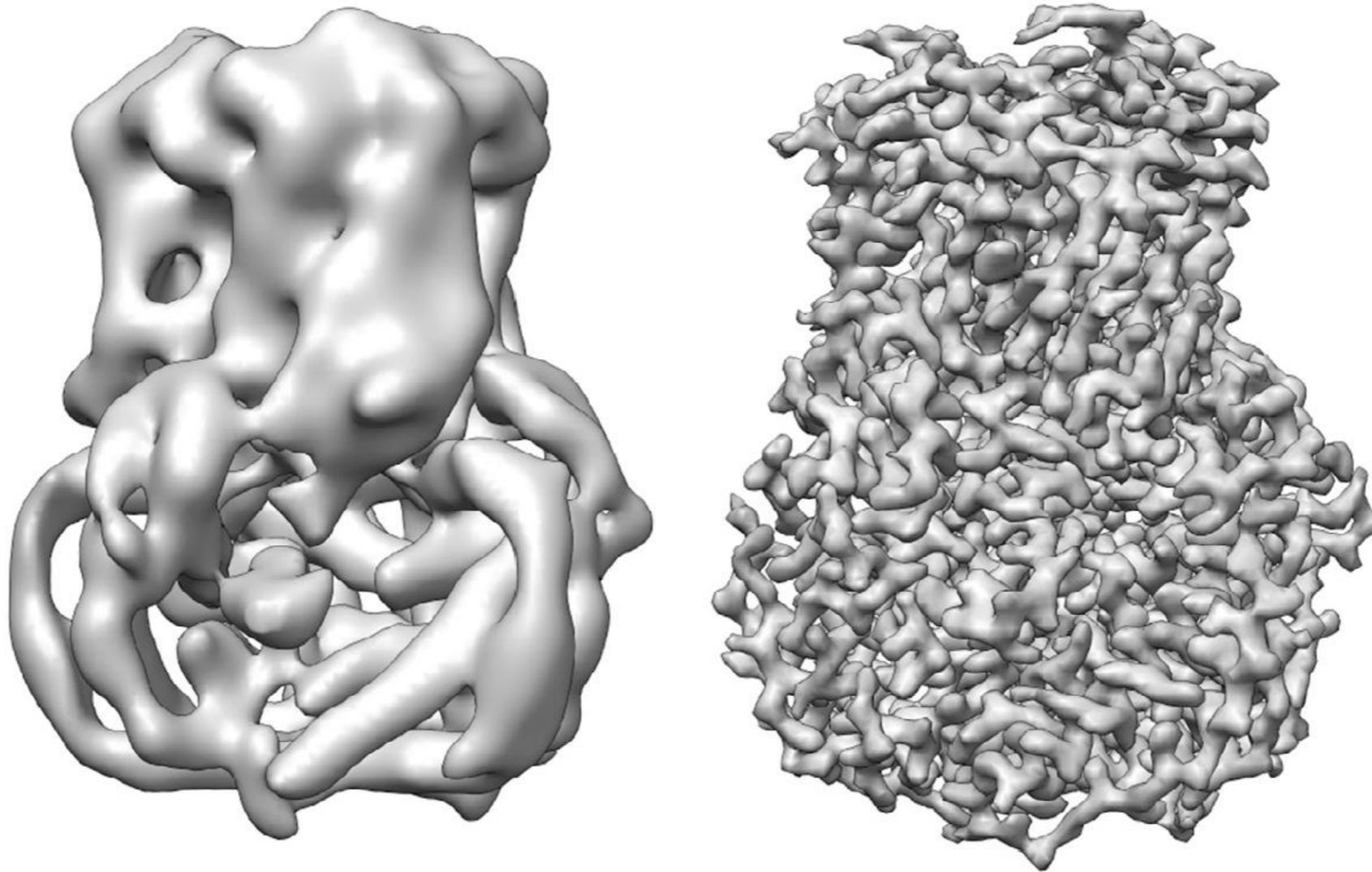
thinks wasn't noticed in film images because we used higher mags.

Elliptical Distortion of Magnification

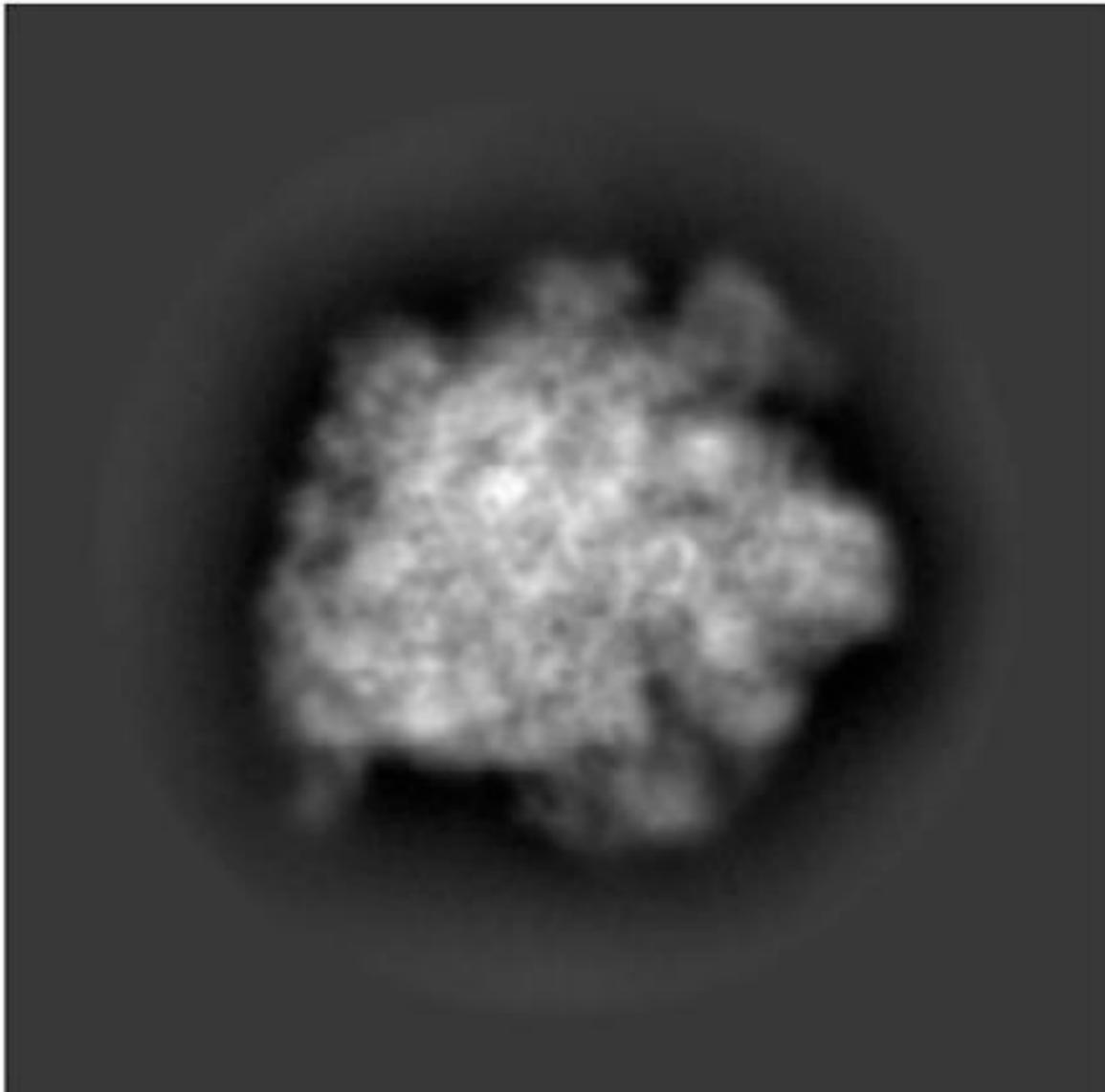


Elliptical Distortion of Magnification

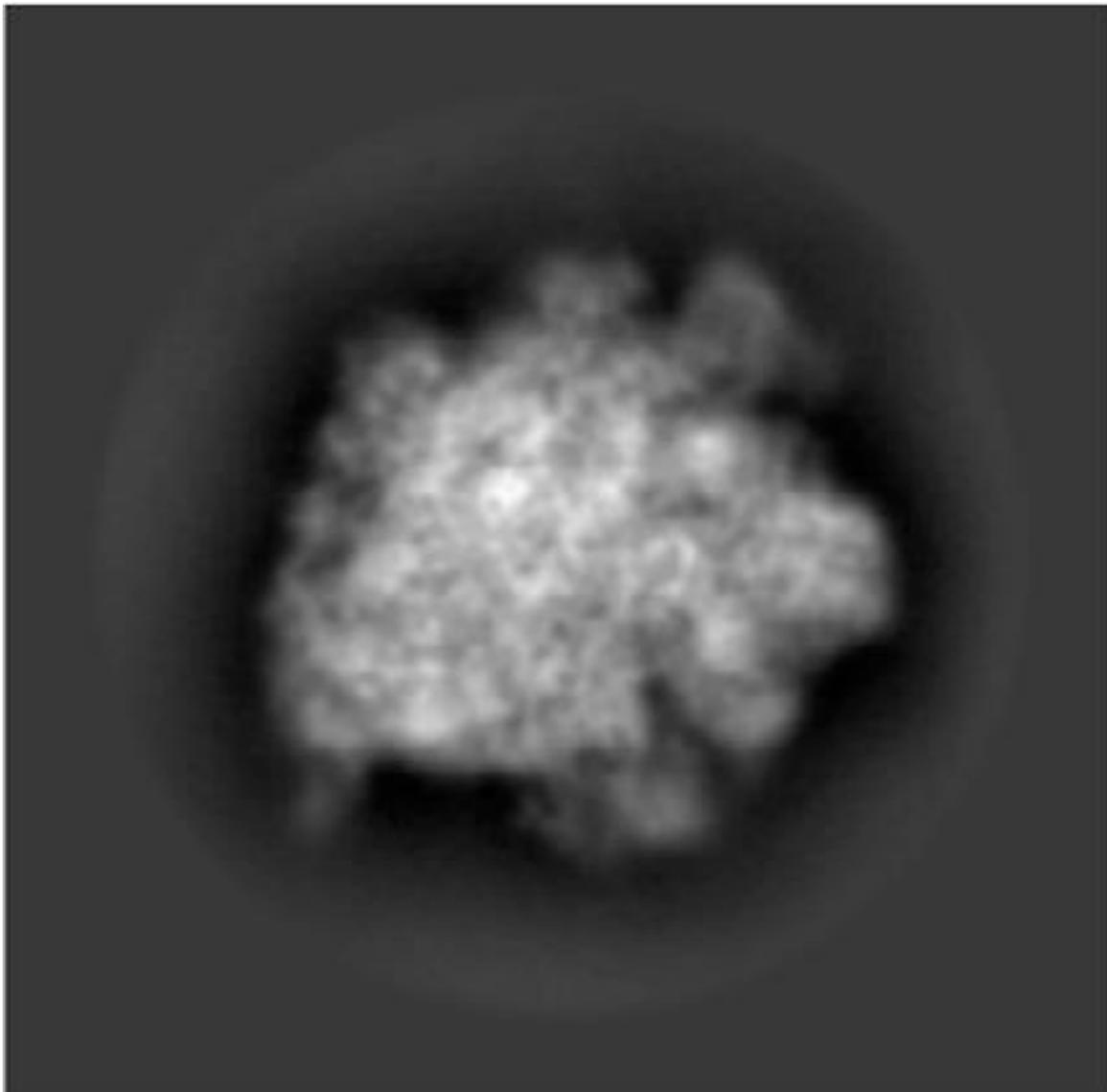
7 Å to 3 Å improvement in resolution, for rotavirus



Elliptical Distortion of Magnification



Elliptical Distortion of Magnification



Modus Operandi

Record low-dose images of cryogenic specimen

**Check a few images by doing motion correction,
compute contrast transfer function**

**Be sure CTF oscillations can be detected to a high
resolution**

Check resolution with Au specimen

Our Settings

FEI Tecnai F20

Careful Alignments, including coma-free alignment

Extraction voltage = 4500 V

Gun lens 3

Spotsize 8-9 (scale 1-11)

Check Thon rings to $\sim 3-5 \text{ \AA}$ (in output data)

C2 aperture, 50 microns

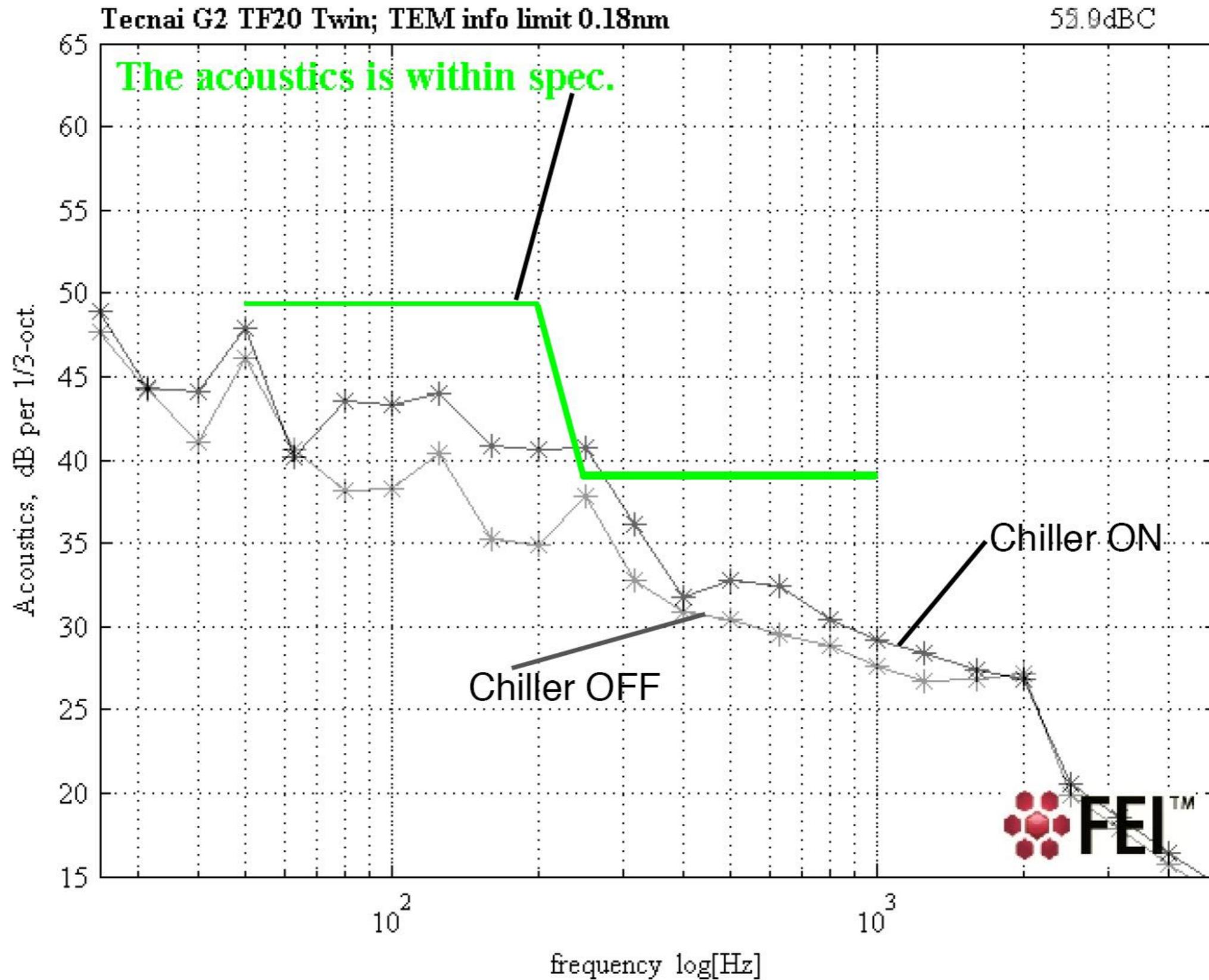
Objective aperture, 100 microns*

Wish List

Make Microscope Room Quiet (Anechoic Chamber)

Move water chiller or cover with sound-proof shell

Acoustics



Octave1_f_Jun_25_2015_20-19-56.mat



Wish List

Make Microscope Room Quiet (Anechoic Chamber)

Move water chiller or cover with sound-proof shell

Find optimal dose

Test magnification anisotropy correction

Find optimal settling time for stage

***Optimal Spot Size?**

***Wider beam and smaller C2 aperture (30 microns)?**