Electron Microscopes and Cameras

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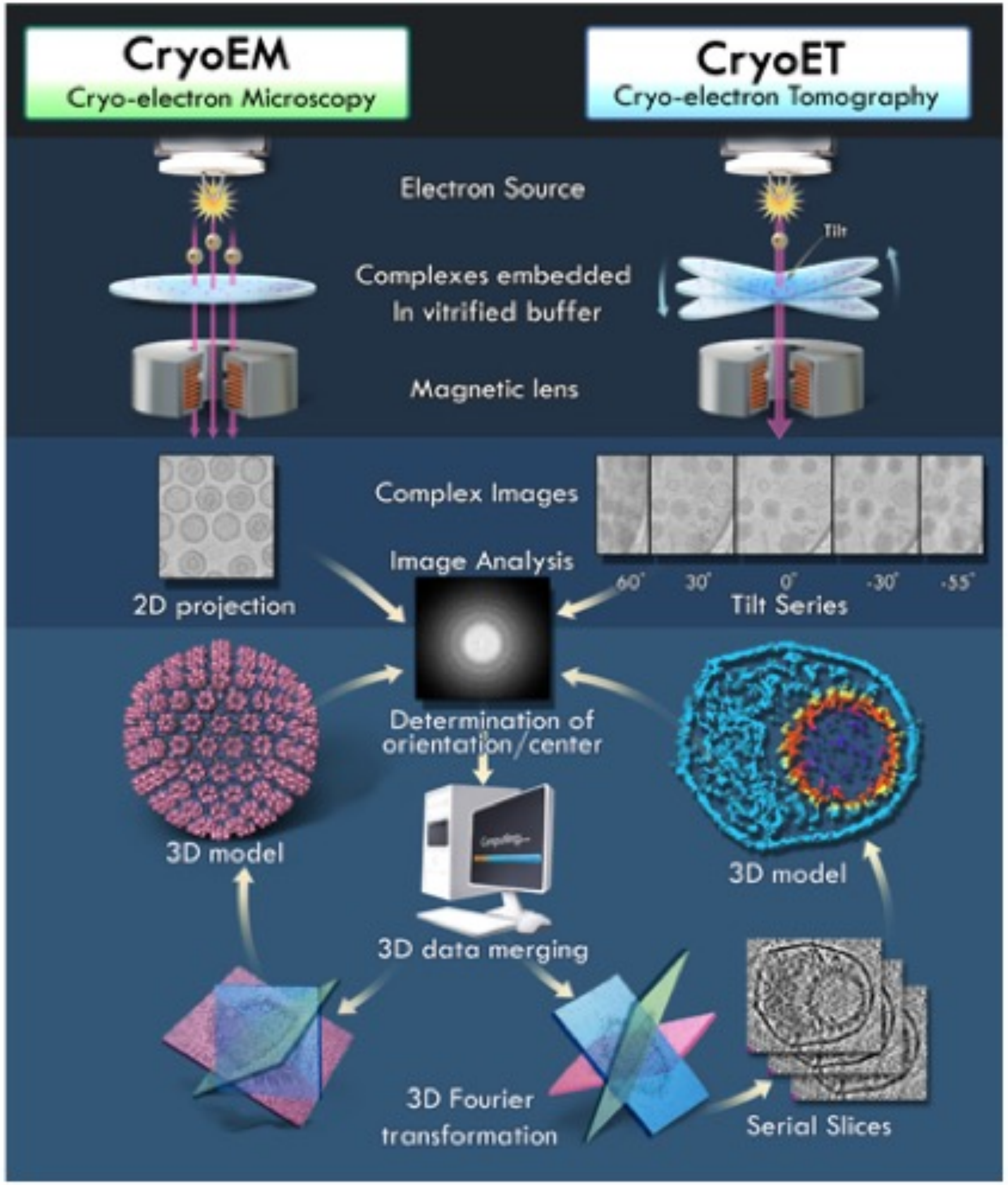
UMass Chan Medical School

LBMS cryo-EM course June 14-17, 2022

Transmission Cryo-Electron Microscopy

A tool used by structural biologists to study molecular nanomachines

Gabriel Lander, Thesis Defense 2009



(http://www.eicn.ucla.edu/xiaorui)

Outline

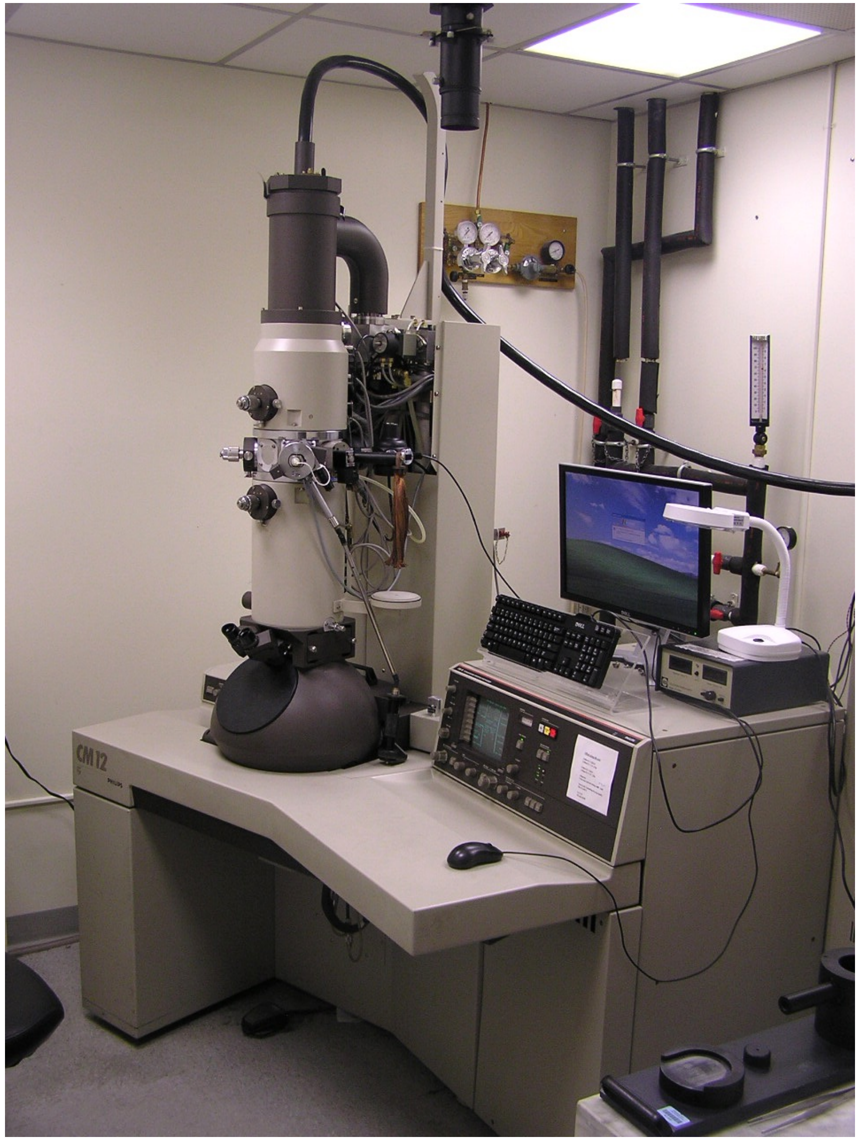
I. Electron Microscope & Optics

II. Cameras – from CCD to DED

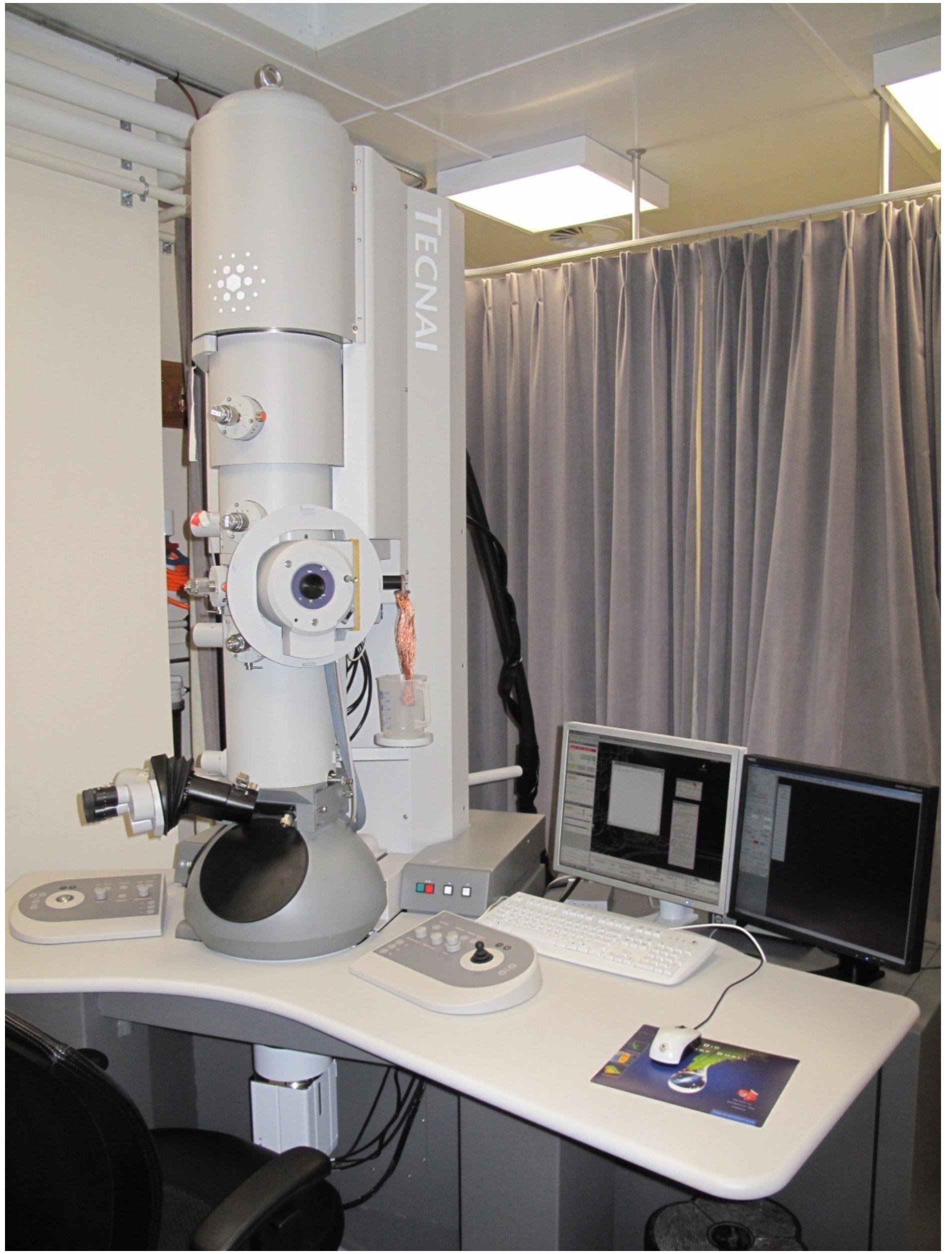
III. Advanced Software Control

I. Electron Microscope & Optics

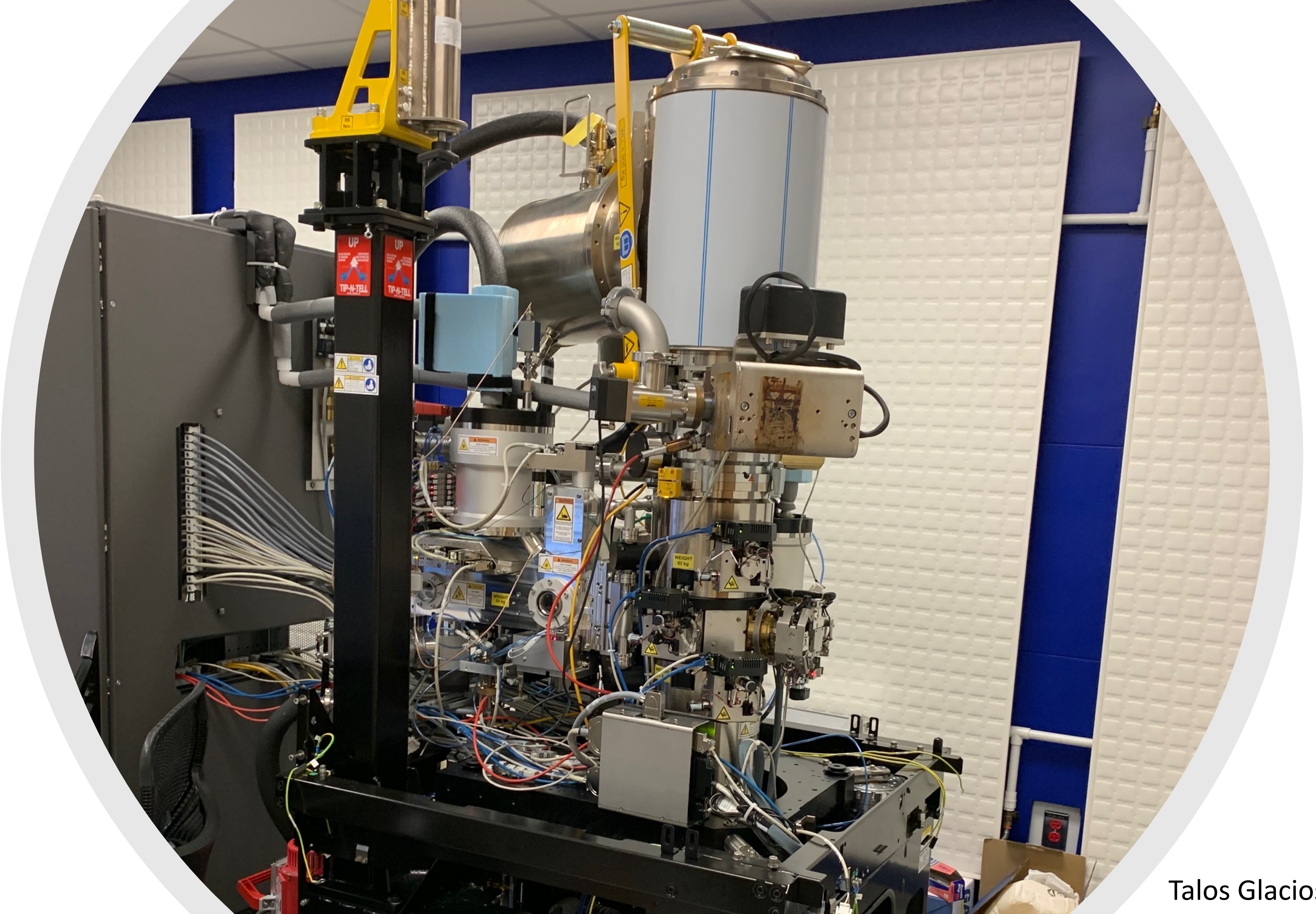
- First EM was built by Ernst Ruska and Max Knoll in 1931


CM 12

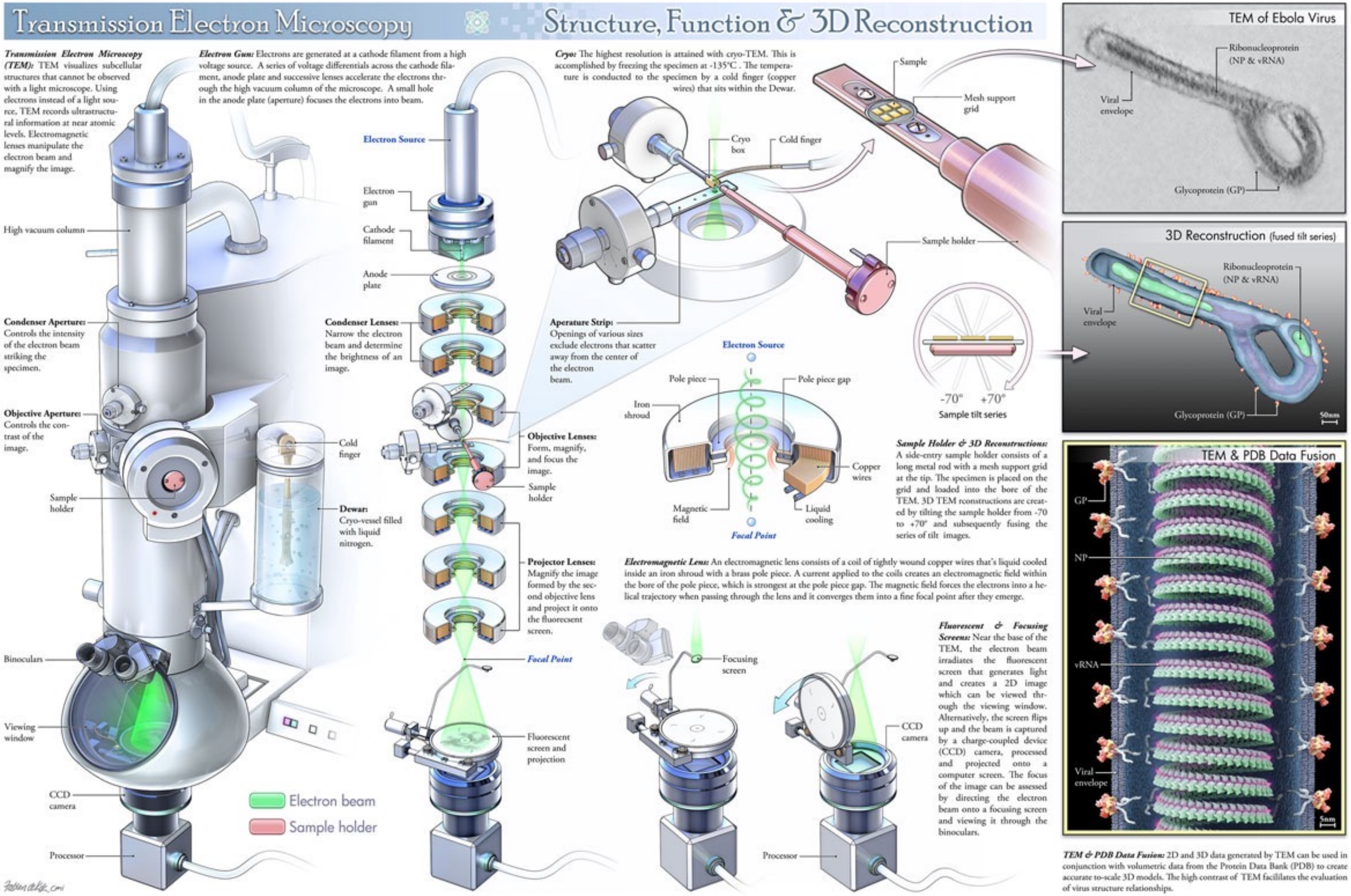

CM 12 transmission electron microscope
DATA DIM
PANEL DIM
SPOT SIZE
TEM BRIGHT FIELD-LOW DOSE
SA 40000x
HT 120kV
spot 4 300nm
focusstep 4
defocus 110nm
plate man 2.00
meter 29.0 s
exp no 0000
stock 0
IGP 11
exp delay
INCR
0.0 s
DECR
state
EXPOSURE
FOCUS
S1 S2
SEARCH
BEAM BLANK
MODES
PARAMETERS
AUTOCON-1
RSET DEFOC
MEASURING
VACUUM
TEM CAMERA
EMISSION
MICROSCOPE
ON
STAND BY
HIGH TENSION
VACUUM SYSTEM
FILAMENT
MAGNIFICATION
SHIFT
X
Y
FOCUS
STEP SIZE
MULTIFUNCTION
X | ROTATION
Y | ANGLE


TECNAI


TITAN
FEI COMPANY

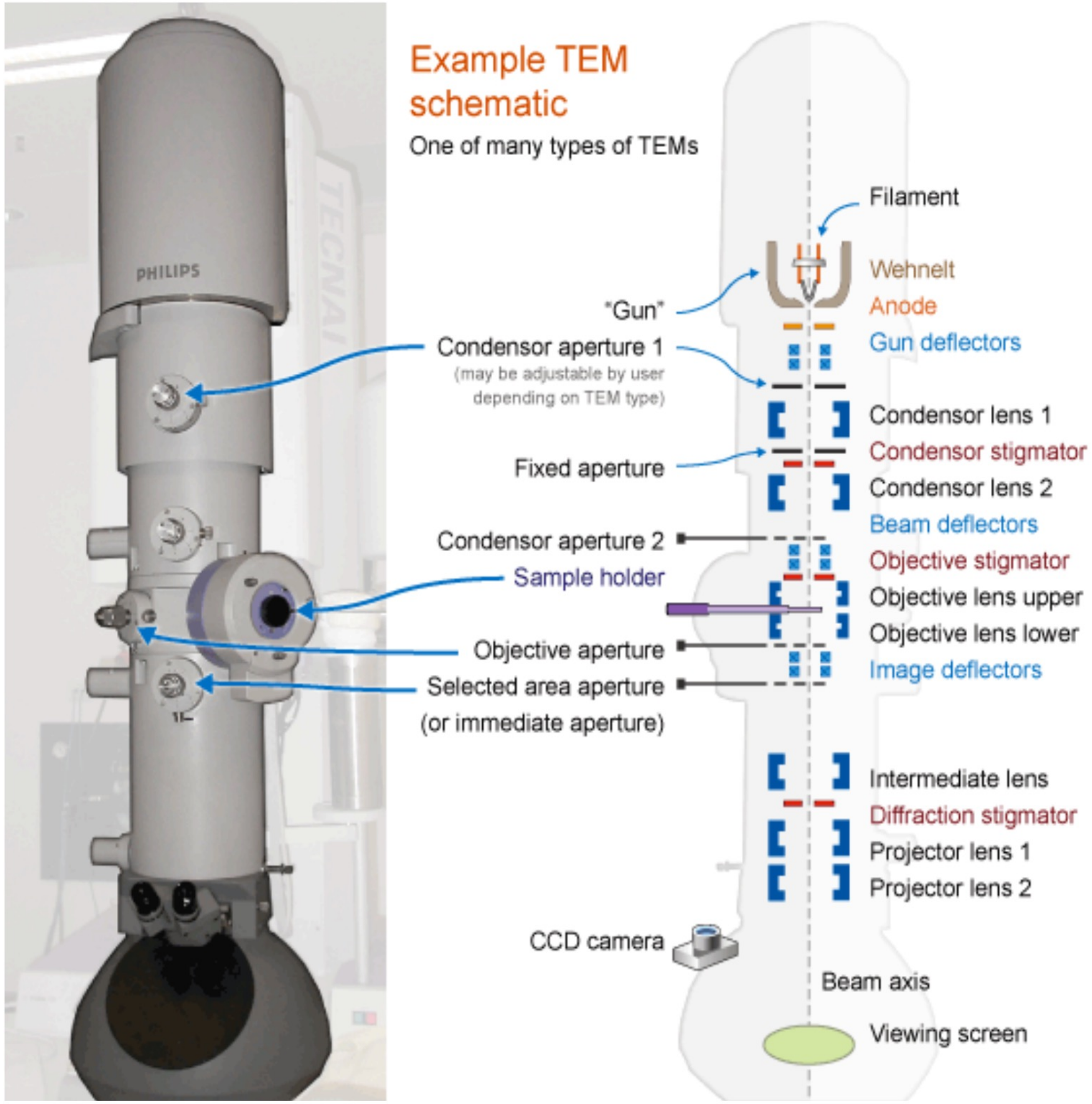


Talos Glacios @ UMass



Fabian de Kok-Mercado, Victoria Wahl-Jensen, and Laura Bollinger NIAID IRF Science 335, (2012) 6068

Anatomy of a TEM

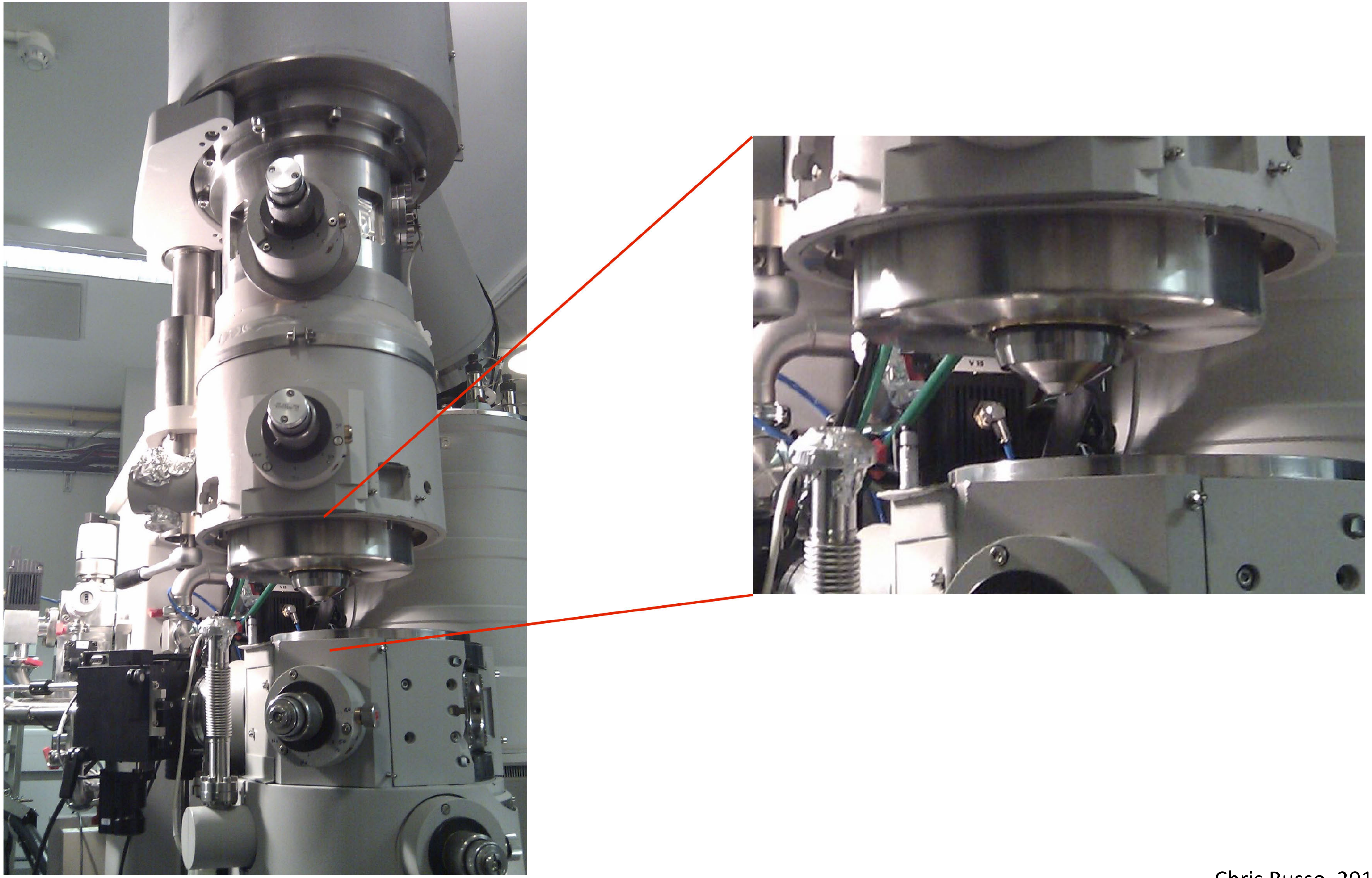


Chris Russo, 2017 MRC Course

1. Electron Gun – beam source
2. Illumination System – Condenser lenses
3. Imaging System – Objective lenses
4. Projection system – Projector lens
5. Recording system - detectors

Hardware Components

1. Electromagnetic lenses (condenser, objective, projectors)
2. Alignment coils, stigmator coils, blanker & shutter coils
3. Apertures
4. Cameras



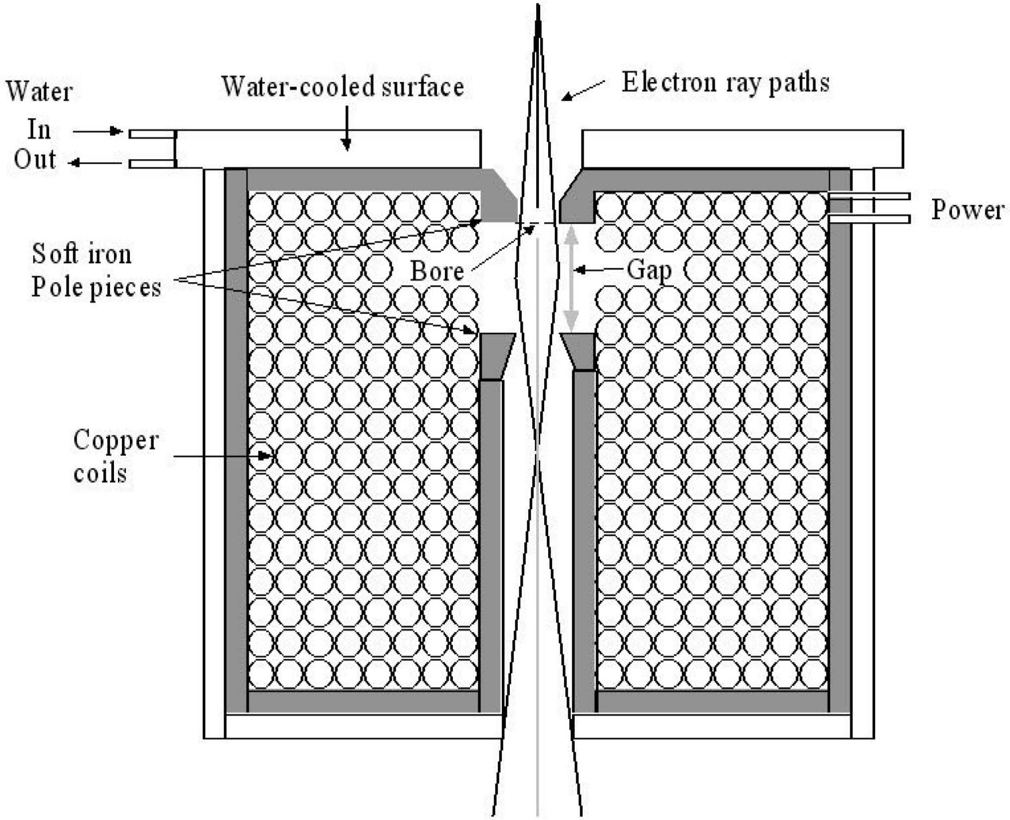
Chris Russo, 2017 MRC Course

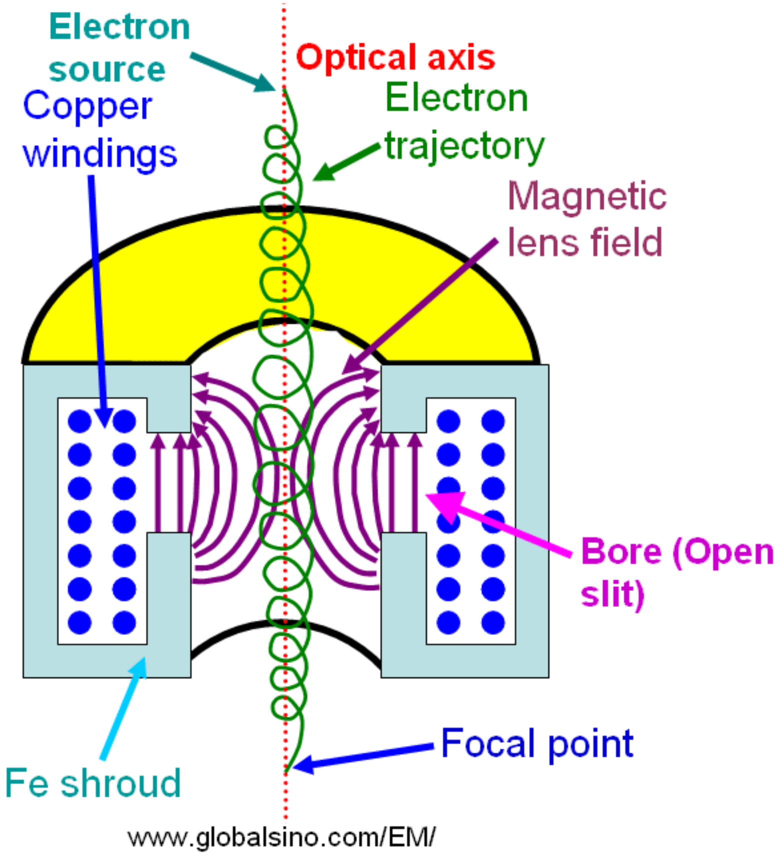
Magnetic Lens

Concentrate flux

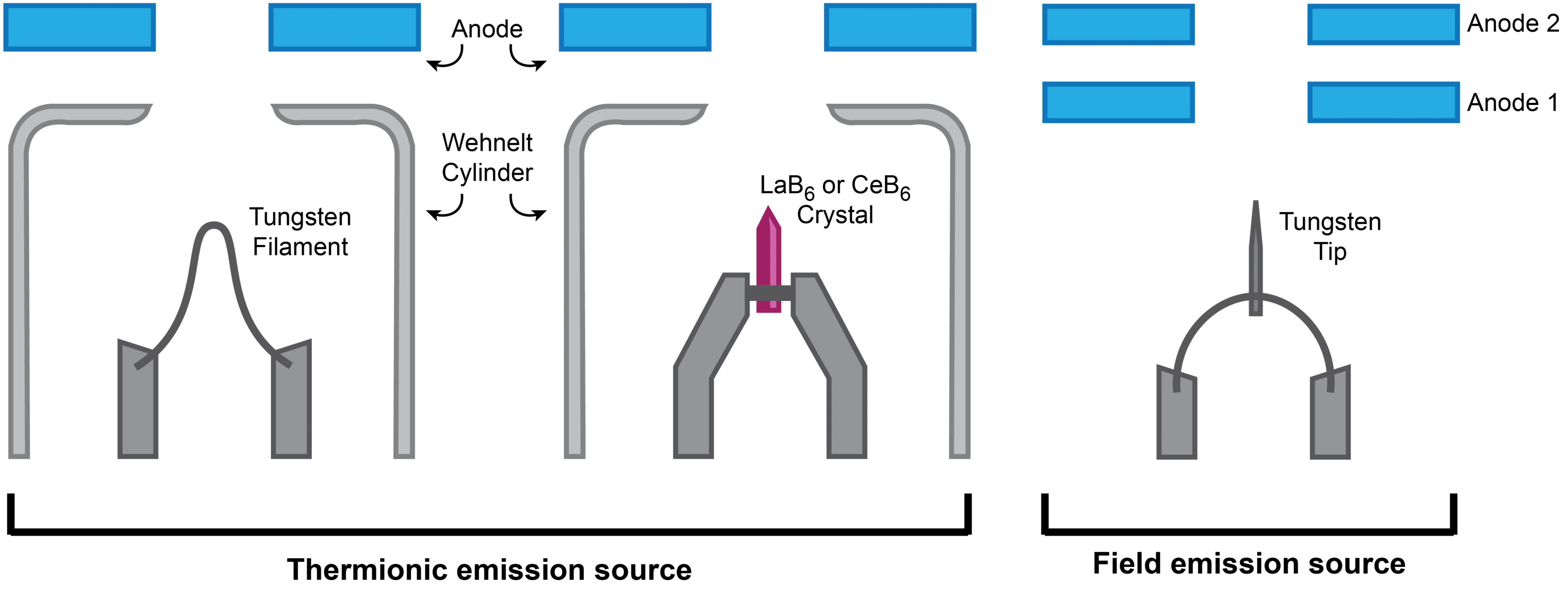
Field varies

Zero force on axial electrons



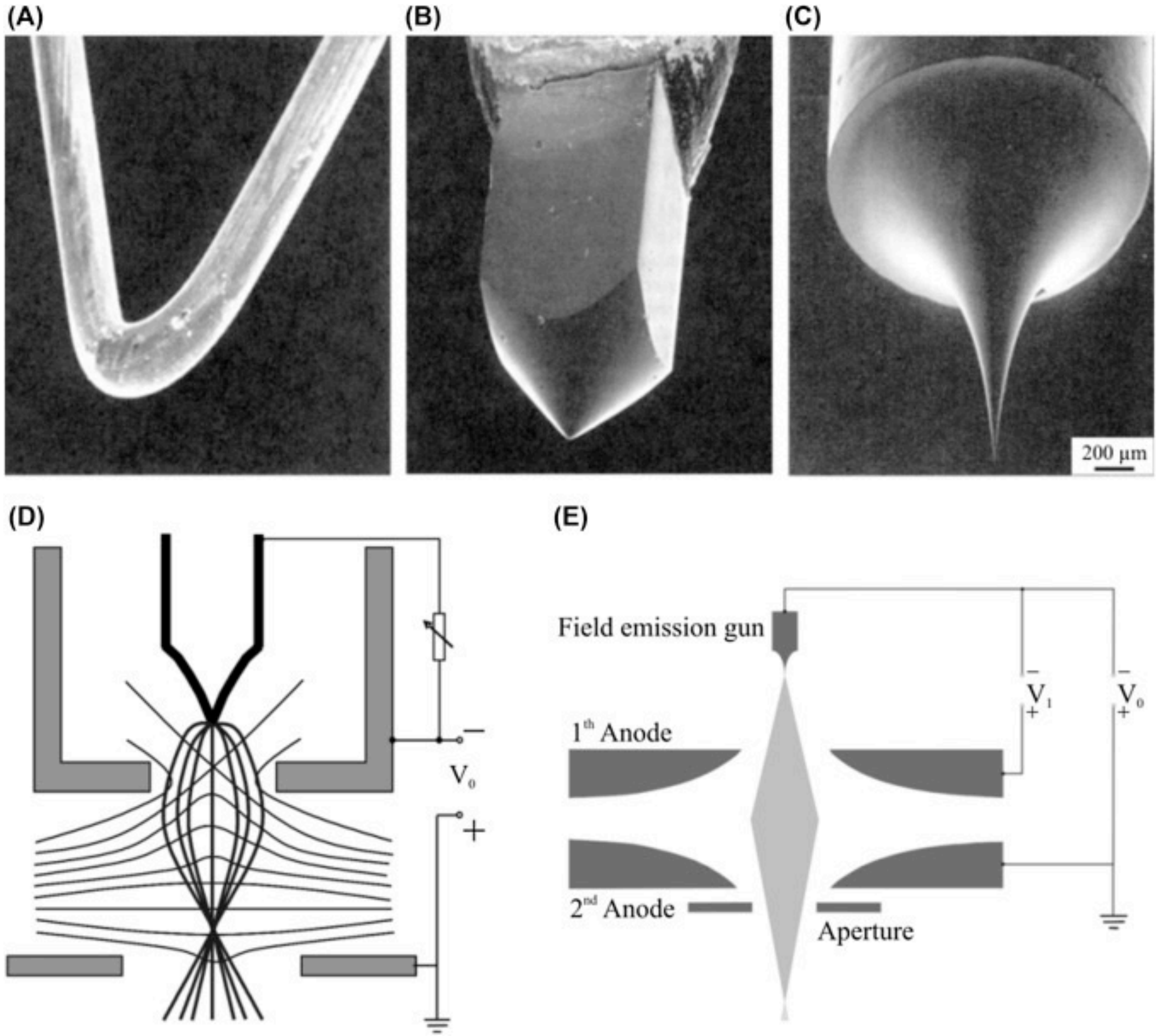


Beam Source



https://www.thermofisher.com/blog/microscopy/electron-source-fundamentals/

Beam Source



S .Henning et al, **“Microscopy Methods in Nanomaterials Characterization”, 2017**

FEG Beam Source Types

Schottky-type

1. High temperature ~ 1800 K
2. Energy spread ~ 0.7eV
3. Stable beam

Cold-FEG

1. Room temperature
2. Energy spread ~ 0.3eV
3. Decrease with time

Coherence of a Beam Source

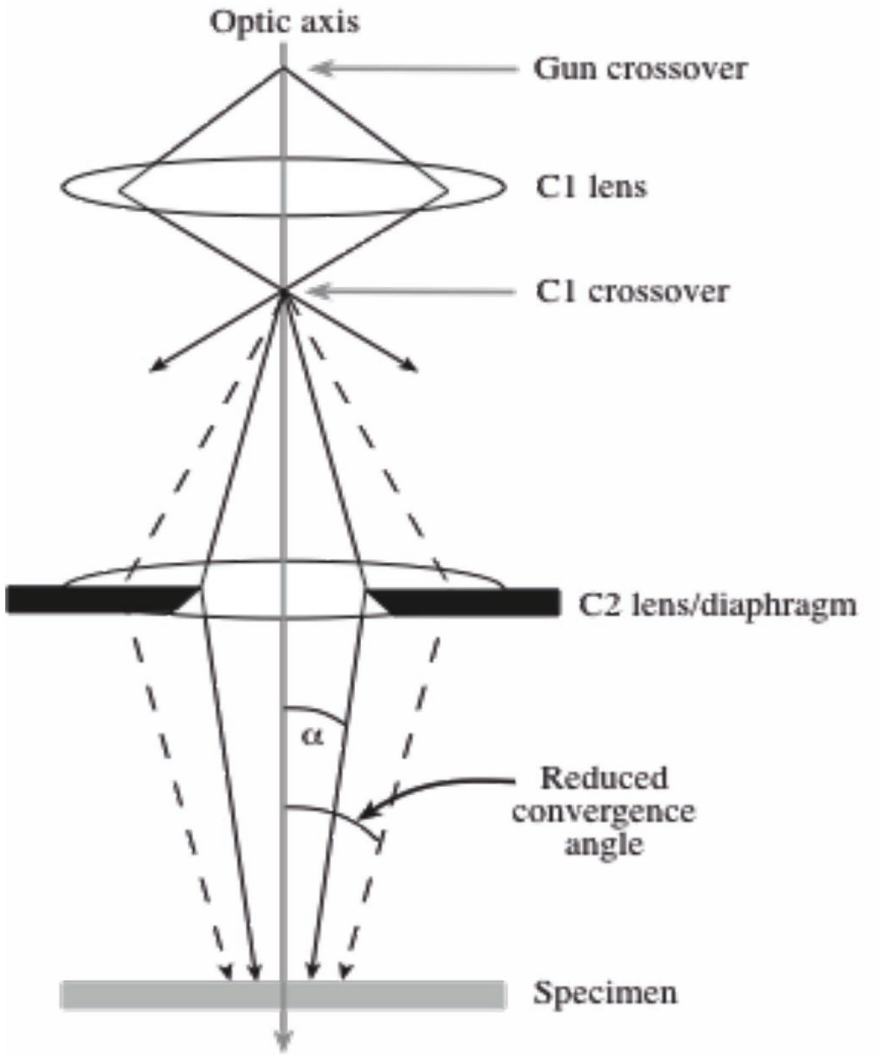
Spatial Coherence -- electrons emitting from small area

Temporal Coherence -- narrow wavelength range

The Basic Electron Condenser System

Most TEMs 2 lenses + 1 aperture

Krios: 3 lenses + 1 aperture



Aberrations due to Imperfect Lens

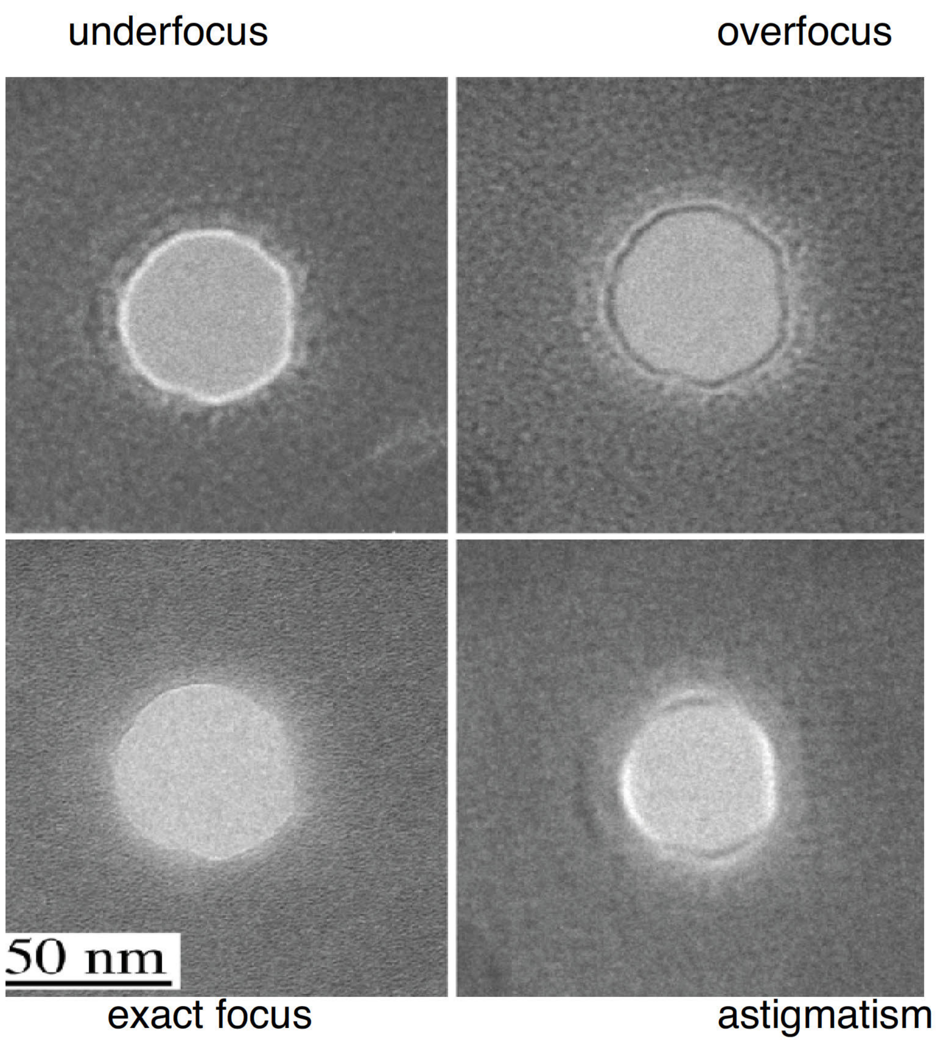
- Defocus
- Astigmatism
- Coma
- Spherical aberration
- CTF
- ……

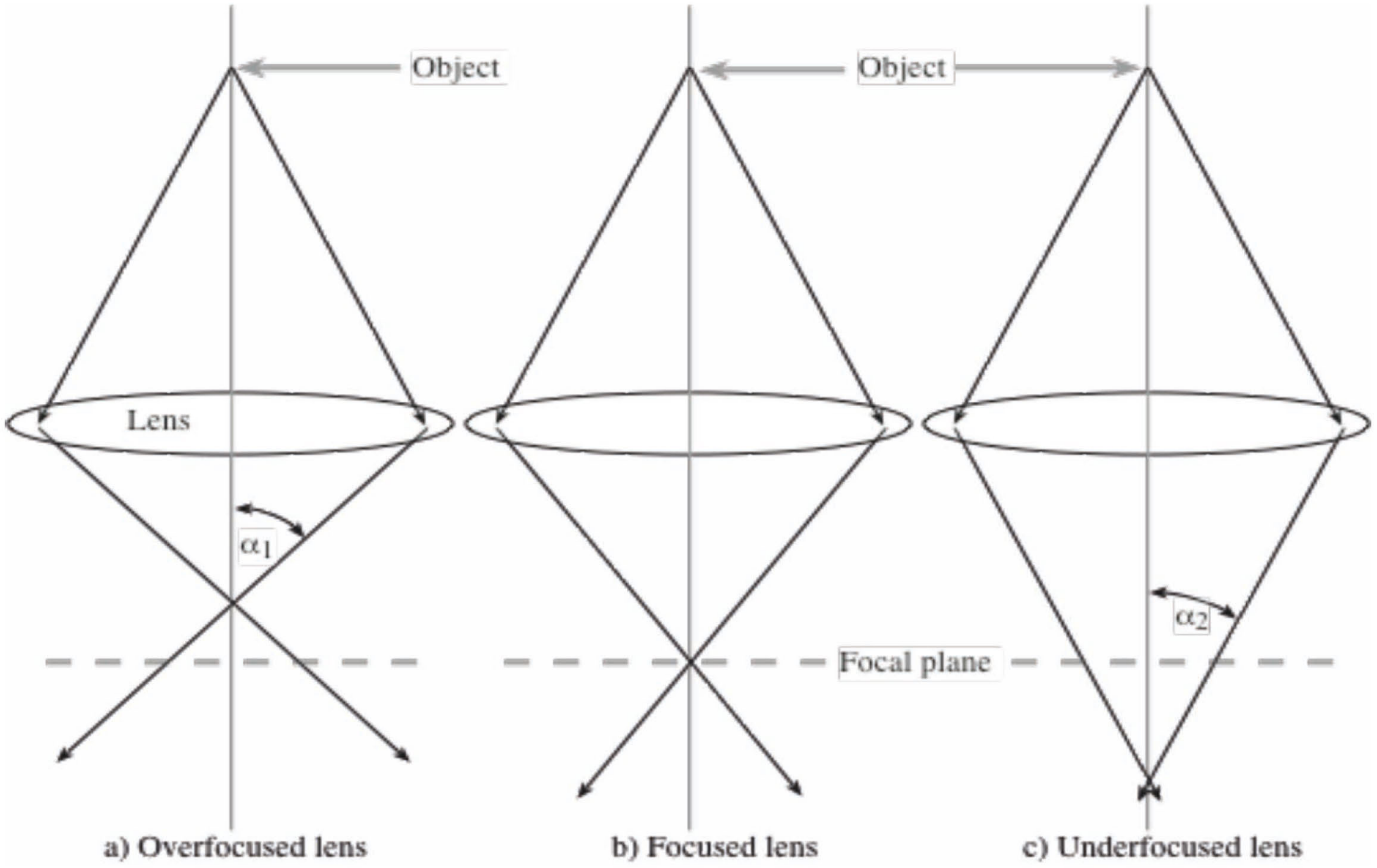
They are corrected with **additional lenses** In the microscope

OR

In **software** after the image is collected (”CTF correction”)

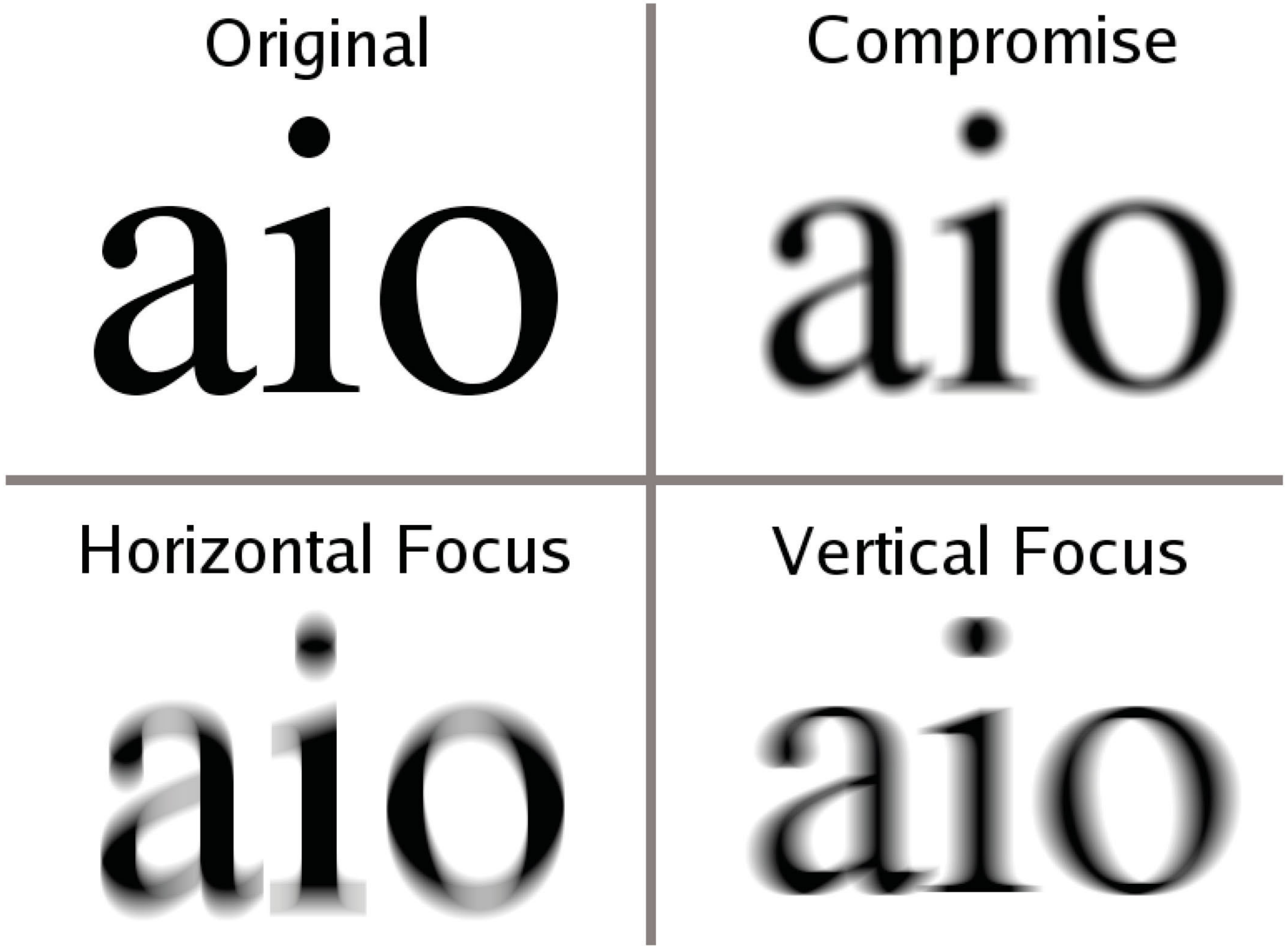
Focus terminology





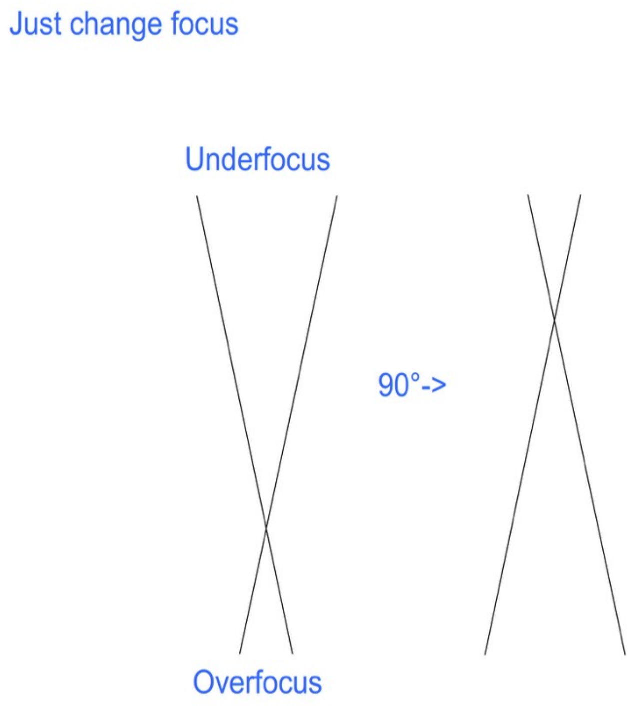
Too strong Just right Too weak

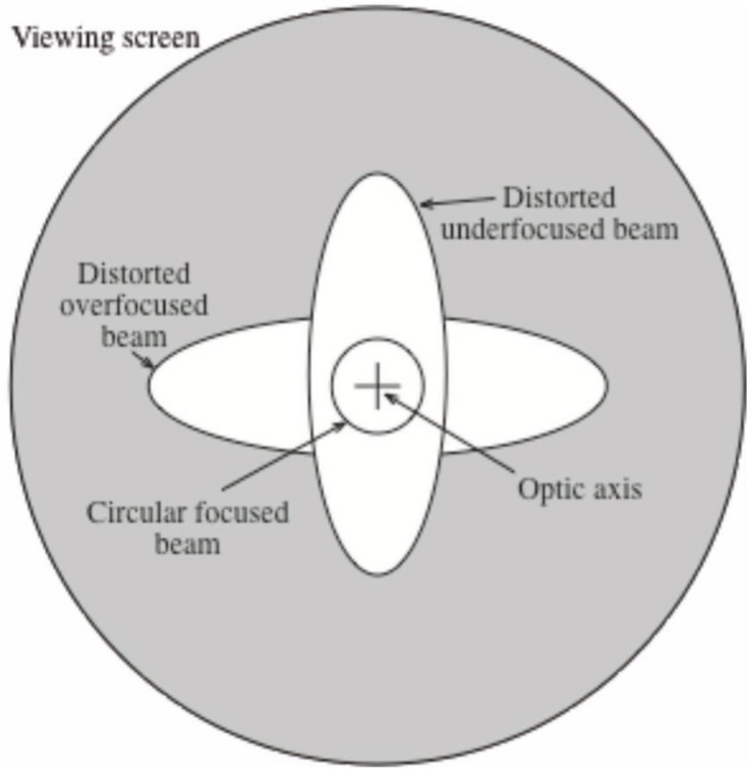
Astigmatism (example)



Chris Russo, 2017 MRC Course

Condenser Beam Astigmatism Correction





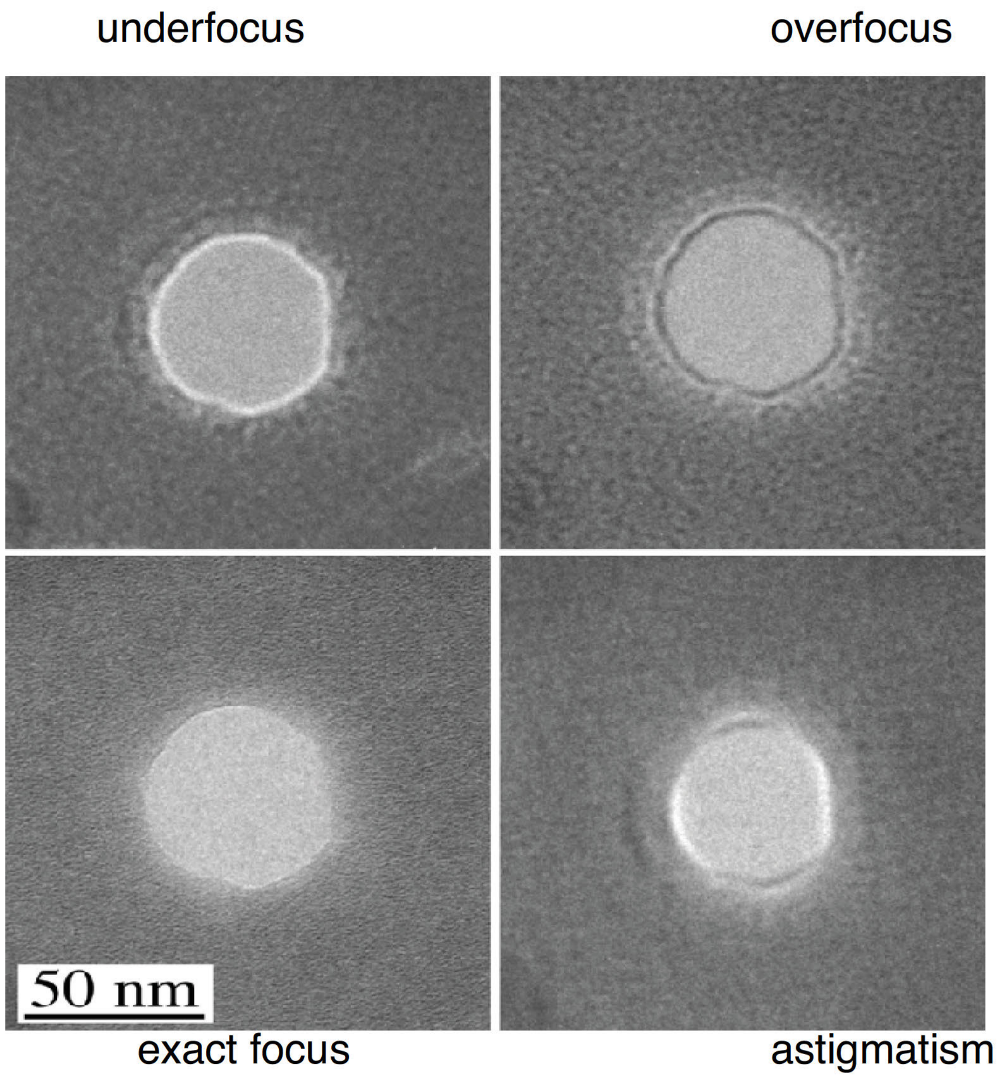
Astigmatism Correction

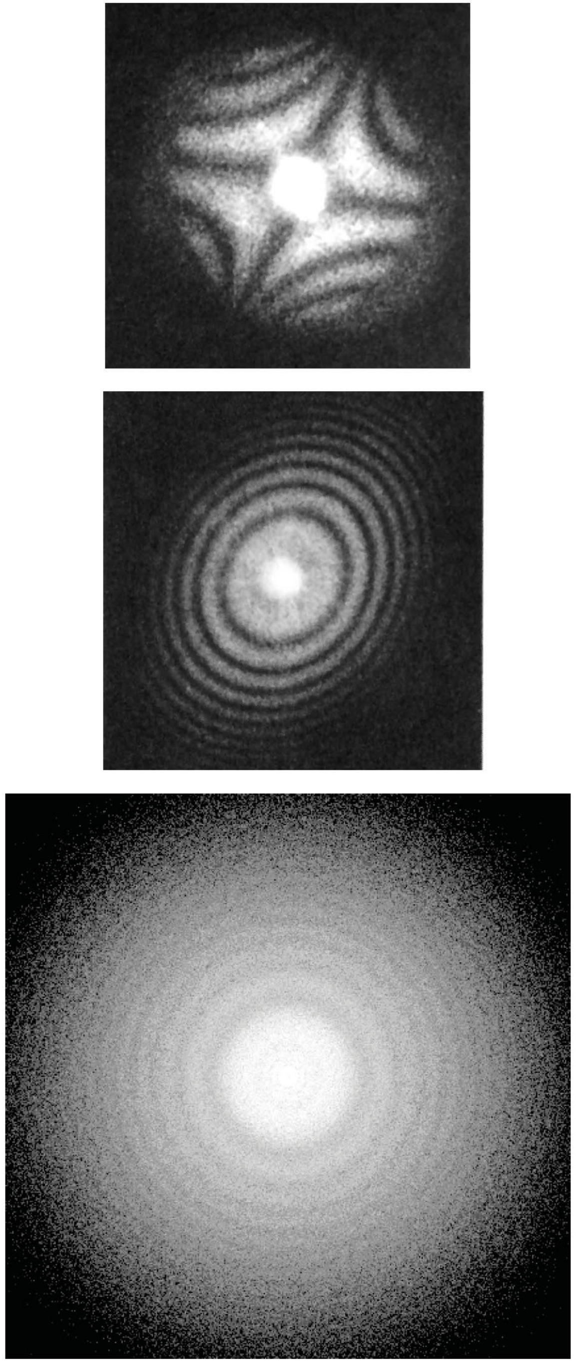
Correcting the
astigmatism
on the objective lens

Routine alignment using
Fresnel fringe

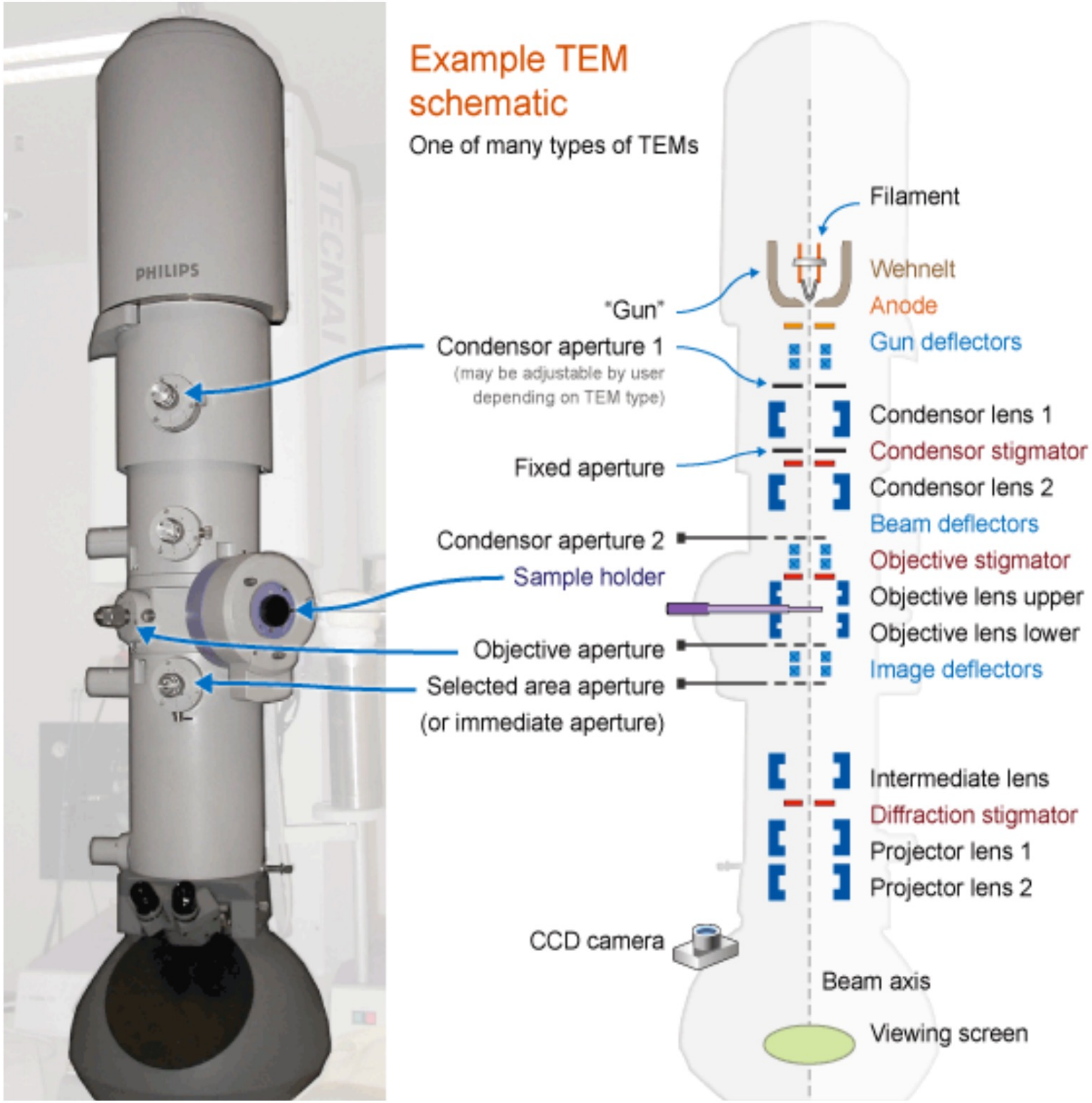
More accurate with FFT

Remember to
correct the
condenser lens too



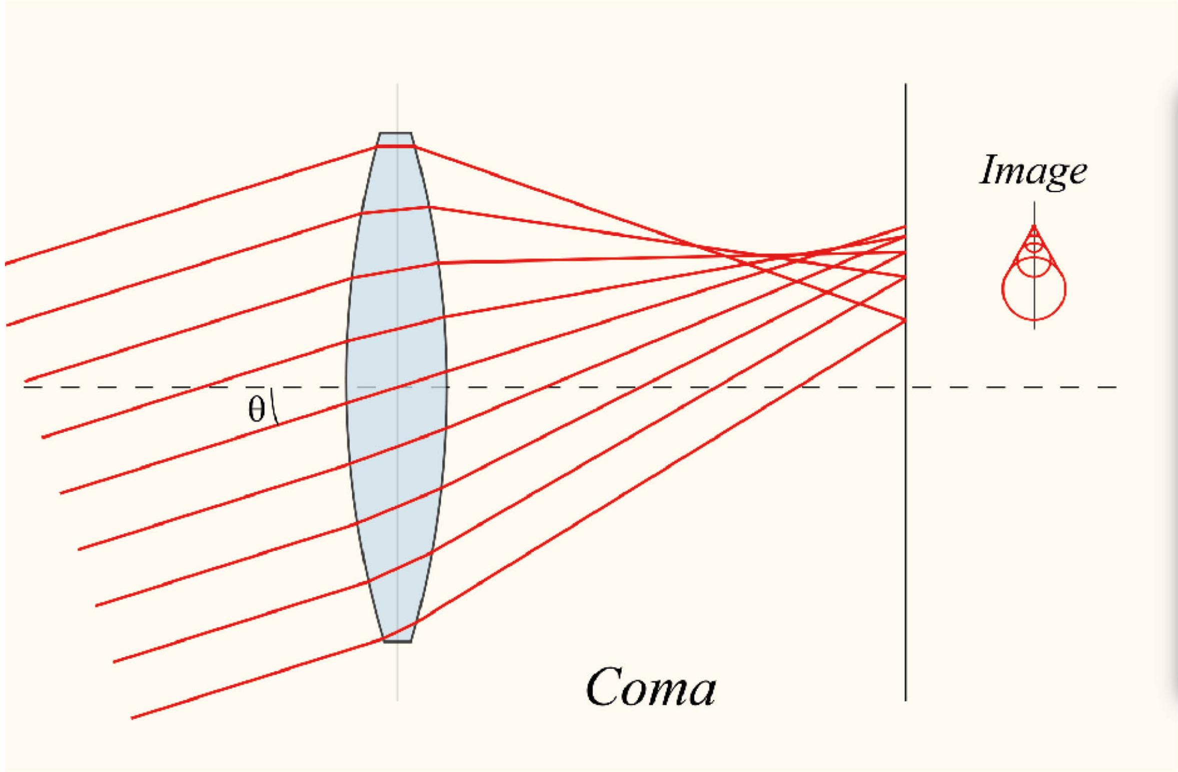


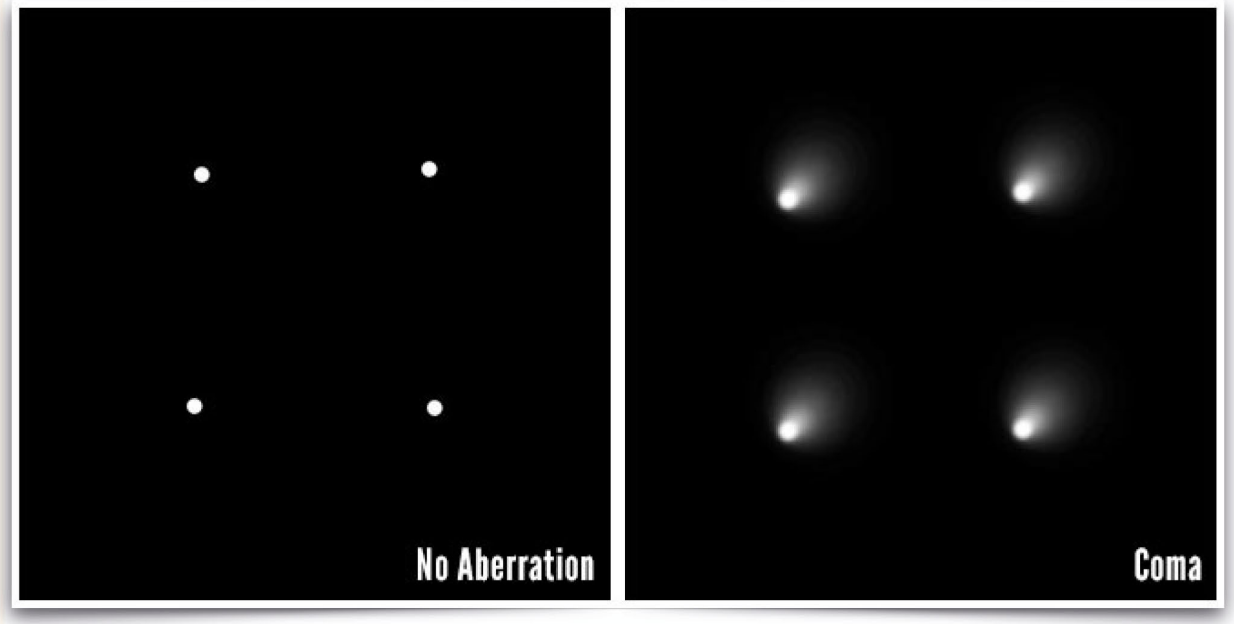
Chris Russo, 2017 MRC Course



PHILIPS
TECNAI
Example TEM schematic
One of many types of TEMs
Filament
Wehnelt
Anode
Gun deflectors
"Gun"
Condensor aperture 1
(may be adjustable by user depending on TEM type)
Condensor lens 1
Condensor stigmator
Fixed aperture
Condensor lens 2
Beam deflectors
Condensor aperture 2
Objective stigmator
Sample holder
Objective lens upper
Objective lens lower
Objective aperture
Image deflectors
Selected area aperture
(or immediate aperture)
Intermediate lens
Diffraction stigmator
Projector lens 1
Projector lens 2
CCD camera
Beam axis
Viewing screen

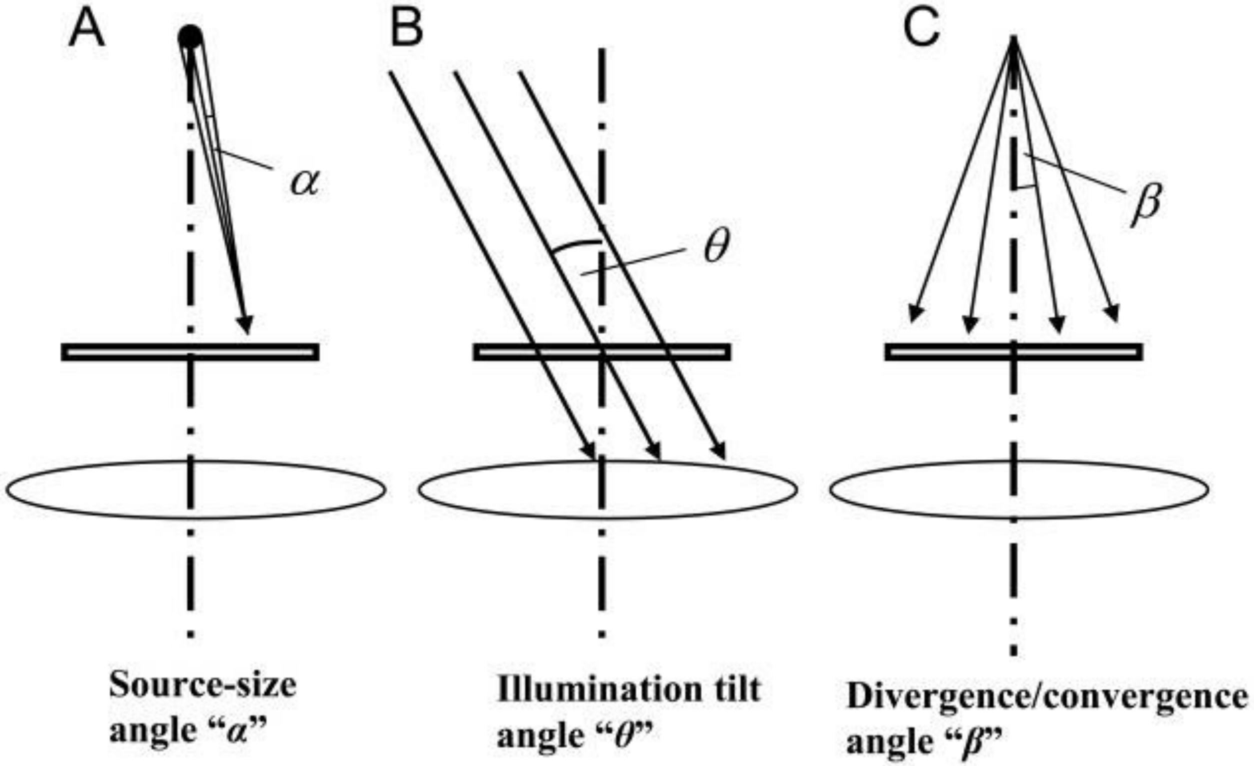
Coma





Example image from I. Norman

Illumination Conditions



Overfocused beam

Axial Coma

Off-axis Coma

R Glaeser et al, Journal of Structural Biology 174(1):1-10

Beam Tilt Introduces:

$$\Delta z_{\text{effective}} - \Delta z_{\text{no beam tilt}} = -2C_s\theta^2$$

Excess of increment of defocus

$$A_{\text{beam tilt}} = C_s\theta^2$$

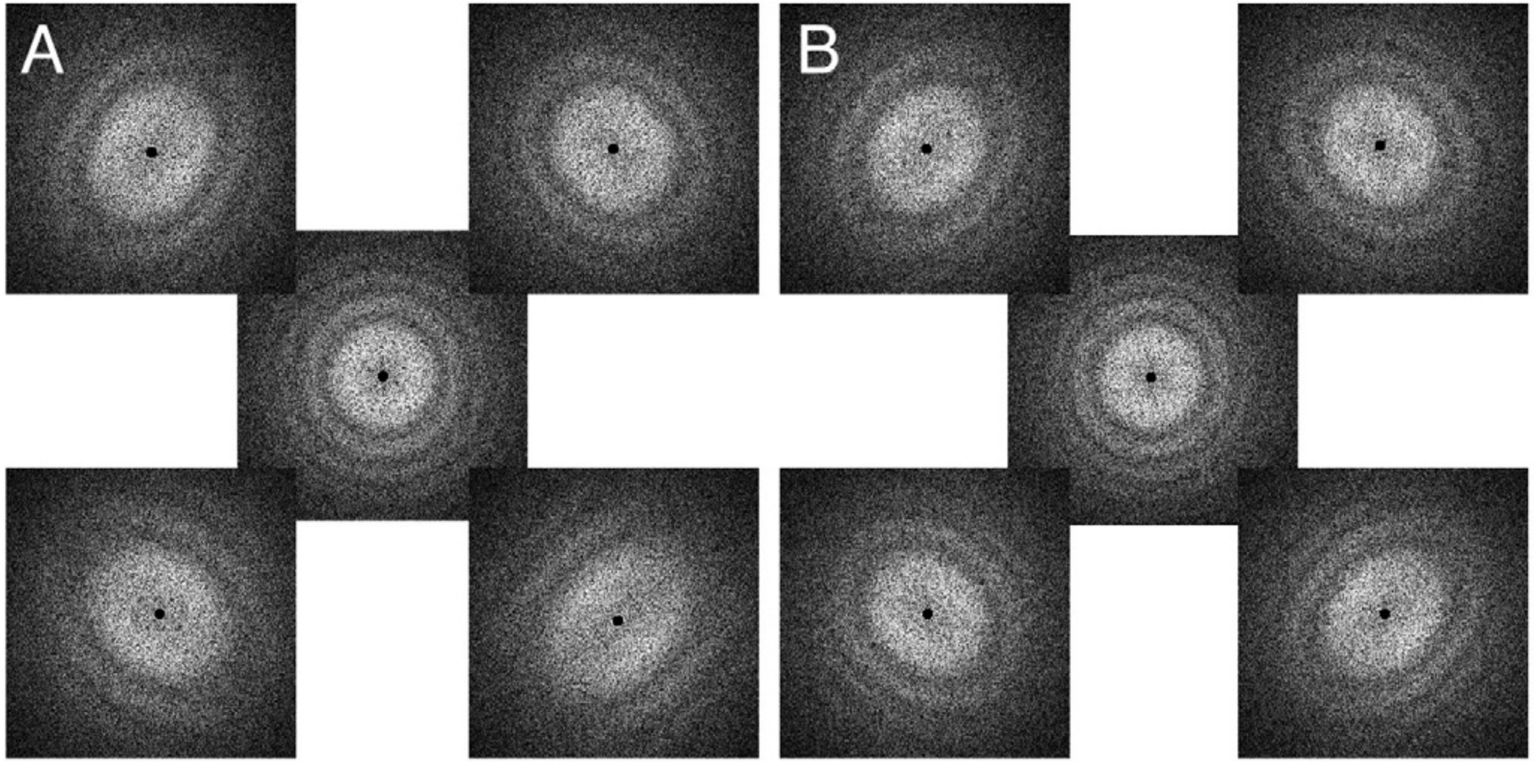
Excess astigmatism

$$\text{Phase error} = 2\pi\theta C_s\lambda^2 s^3(\hat{\boldsymbol{\theta}} \cdot \hat{\boldsymbol{s}})$$

Coma -> Phase Error

R Glaeser et al, Journal of Structural Biology 174(1):1-10

Coma Free – by minimizing beam tilt



1. **Voltage Centering**
2. **Current Centering**
3. **Zemlin Tableaux**

R Glaeser et al, Journal of Structural Biology 174(1):1-10

Parallel Beam – a verifying procedure

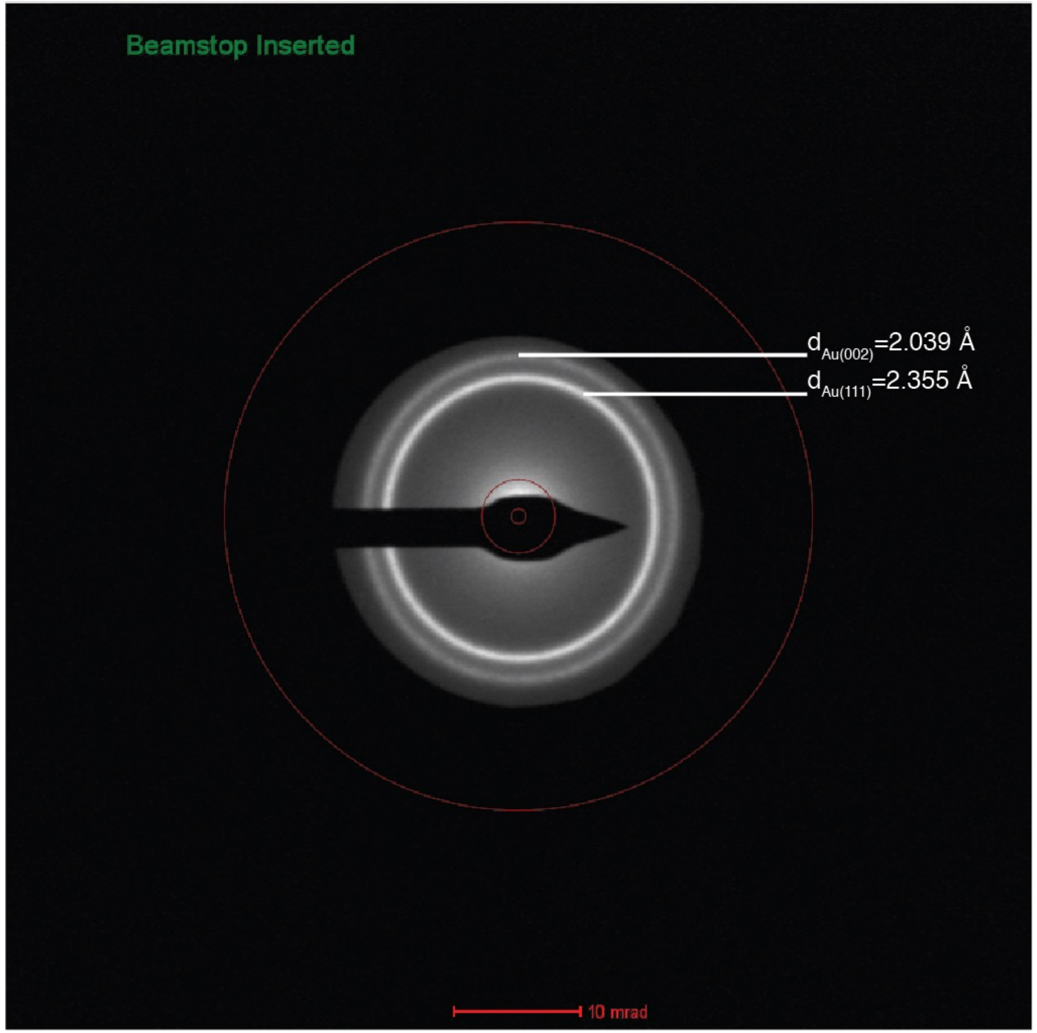
With a large beam tilt ~ 10 mrad

$$\Delta z_{\text{effective}} - \Delta z_{\text{no beam tilt}} = -2C_s\theta^2;$$

$$A_{\text{beam tilt}} = C_s\theta^2;$$

- Take an image
- Perform FFT of sub-areas
- Compare defocus and astigmatism of them
- Adjust Intensity (C2) & repeat above

Parallel Beam – a practical procedure



Parallel illumination on a Talos Arctica

- Eucentric focus
- Insert Obj Aperture in Diffr. mode
- Adjust Focus Knob until aperture edge is sharp
- Adjust Intensity (C2) until diff. rings are sharp

M Herzik et al, Nature Methods **14**, pages 1075–1078 (2017)

Parallel Beam – An Alternative Procedure

Questions / Concerns:

1. Obj Aperture might not sit at Back Focal Plane
2. Hard to find precise intermediate lens value for sharp aperture edge

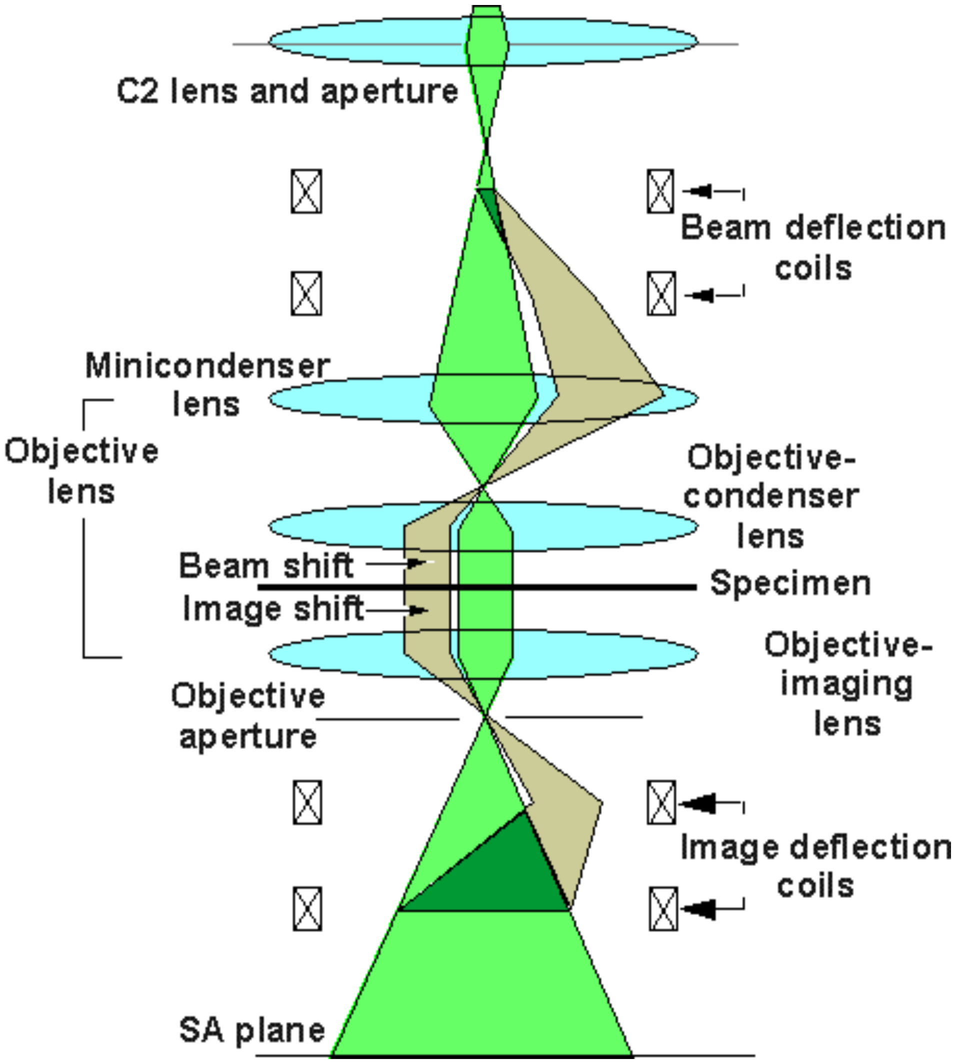
An Alternative Method

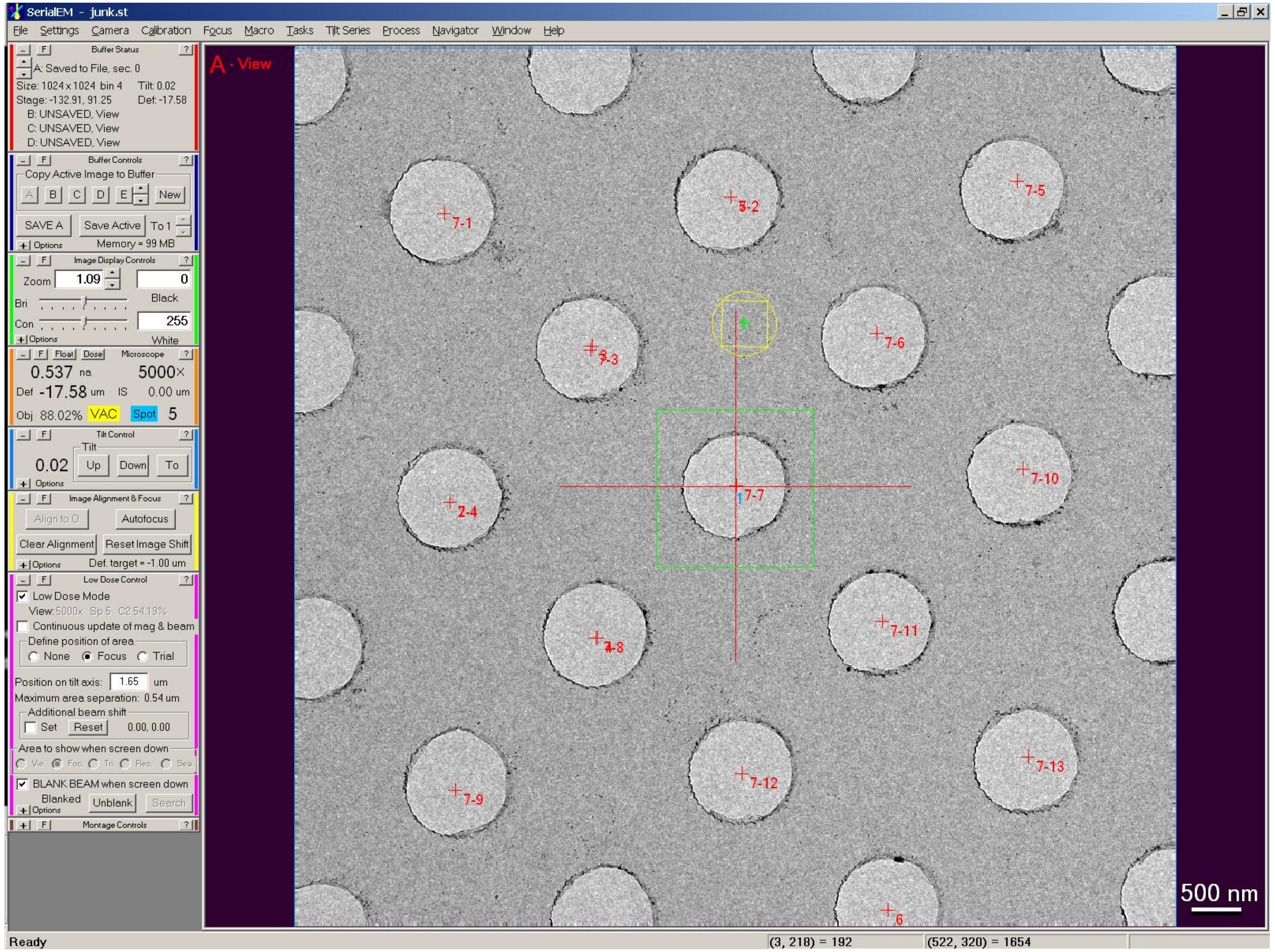
1. Z at Eucentricity Height
2. Measure pixelsizes with different C2 values
3. Z + 100 um
4. Measure pixelsize with different C2 values
5. Find interception of two lines plotted.

* On Krios with C3, the term is **ImageDistanceOffset**

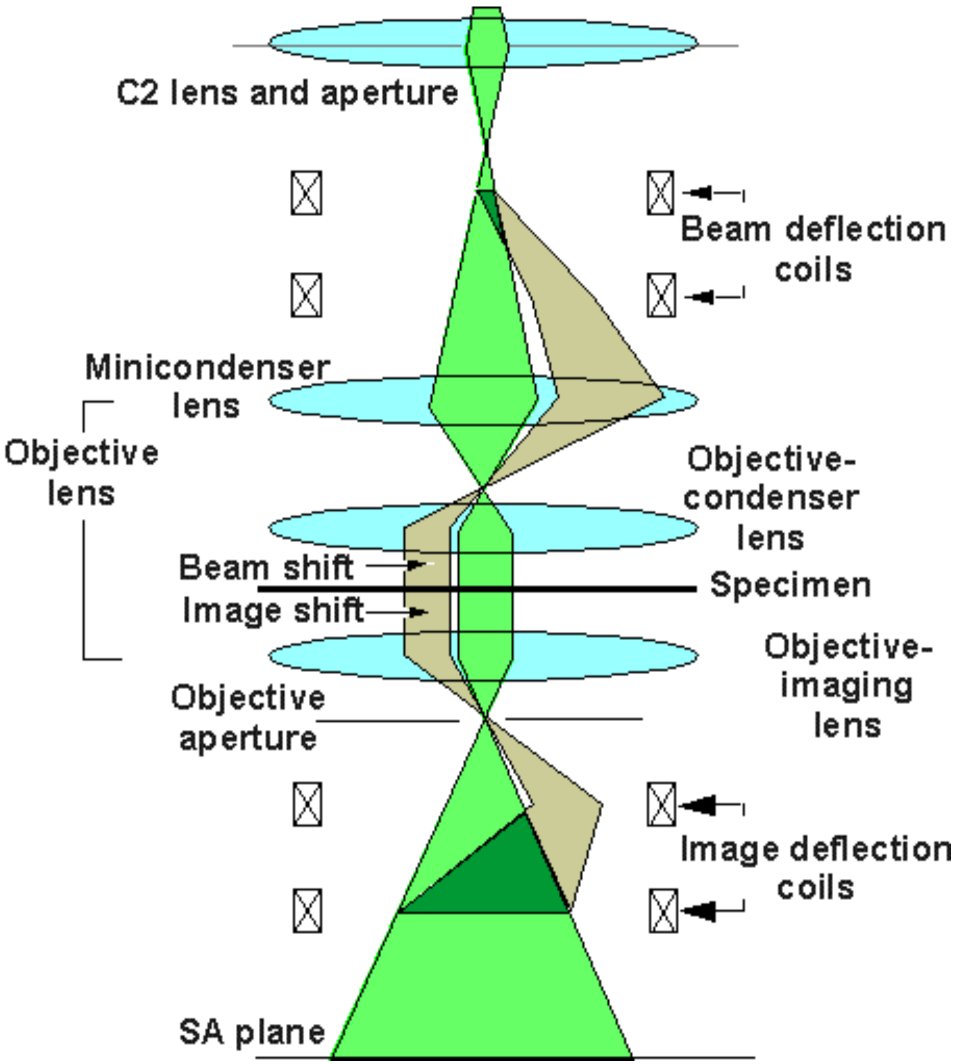
Wim Hagen, private communication

Beam-Image Shift





Beam-Image Shift

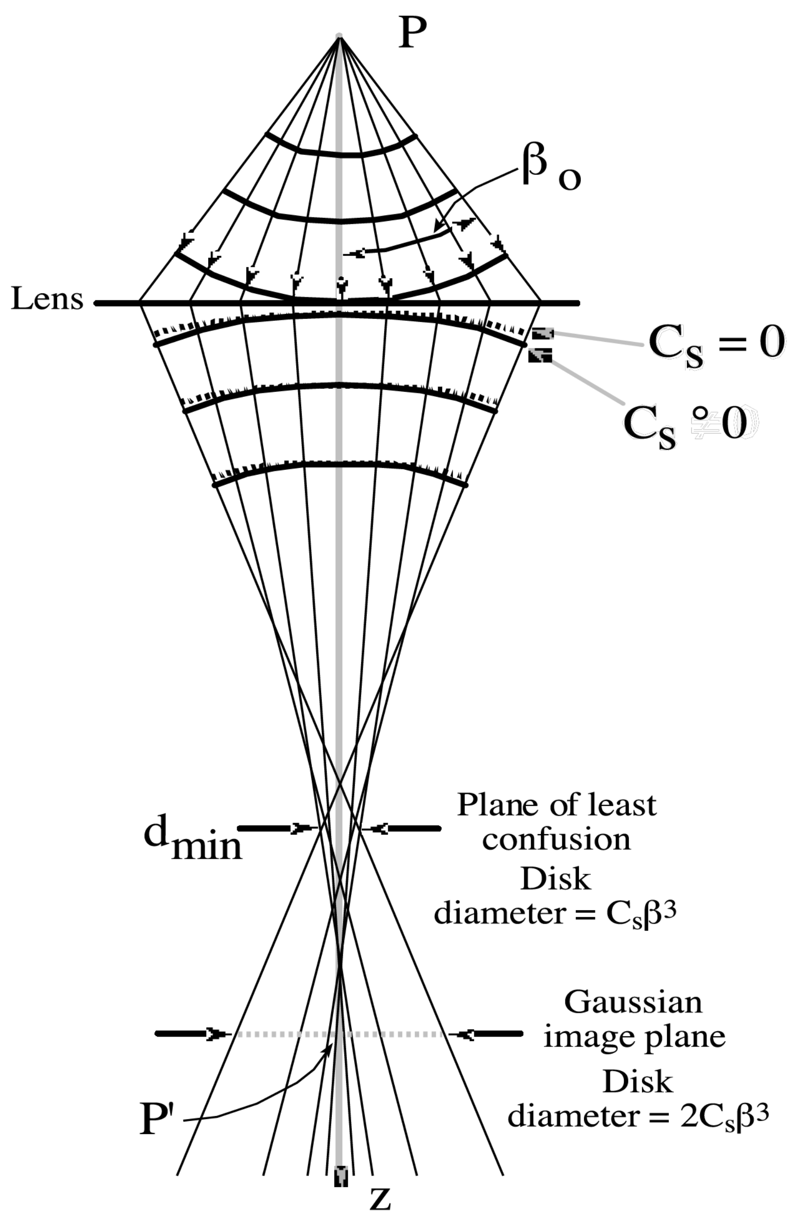


1. Is a single action of microscope
2. Used in Low Dose Conditions
3. Used in high-throughput exposure
4. Induces aberrations due to
   - Axial coma
   - Astigmatism

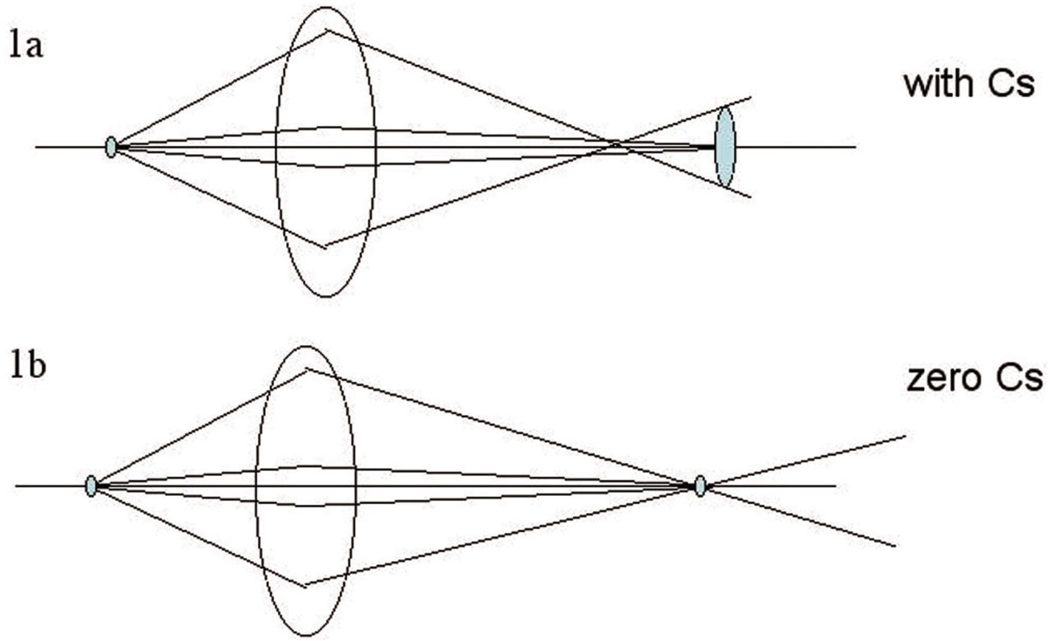
Spherical Aberration

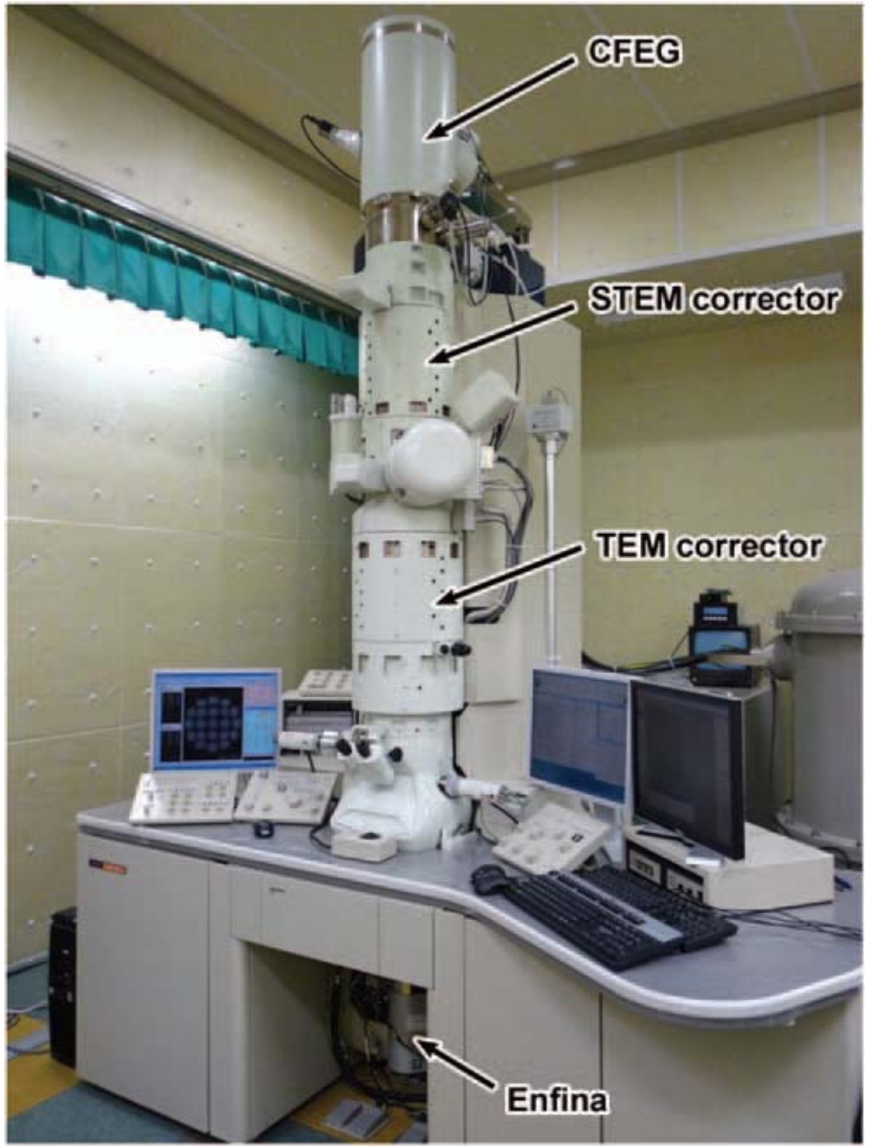
Lens is stronger off axis

Plane of least confusion



Spherical Aberration & Cs Corrector





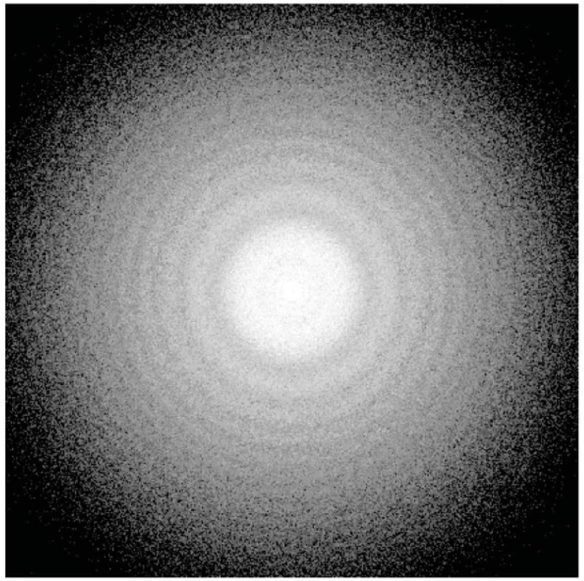
CTF

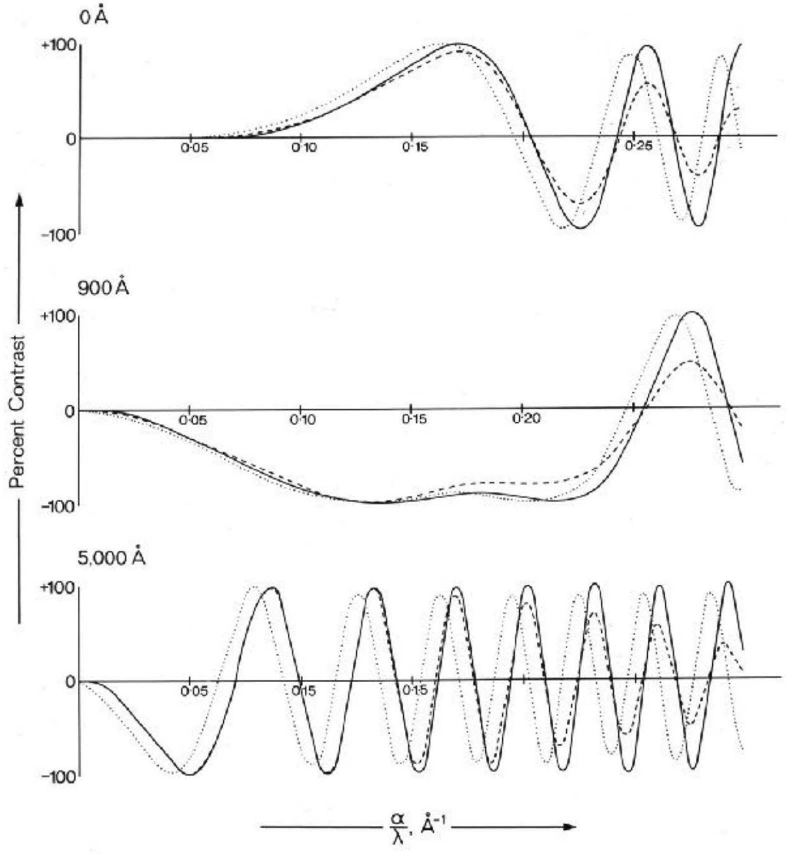
Phil. Trans. Roy. Soc. Lond. B. **261**, 105–118 (1971) [105]
Printed in Great Britain

Measurement and compensation of defocusing and aberrations by Fourier processing of electron micrographs

By H. P. Erickson and A. Klug, F.R.S.
Medical Research Council Laboratory of Molecular Biology, Cambridge

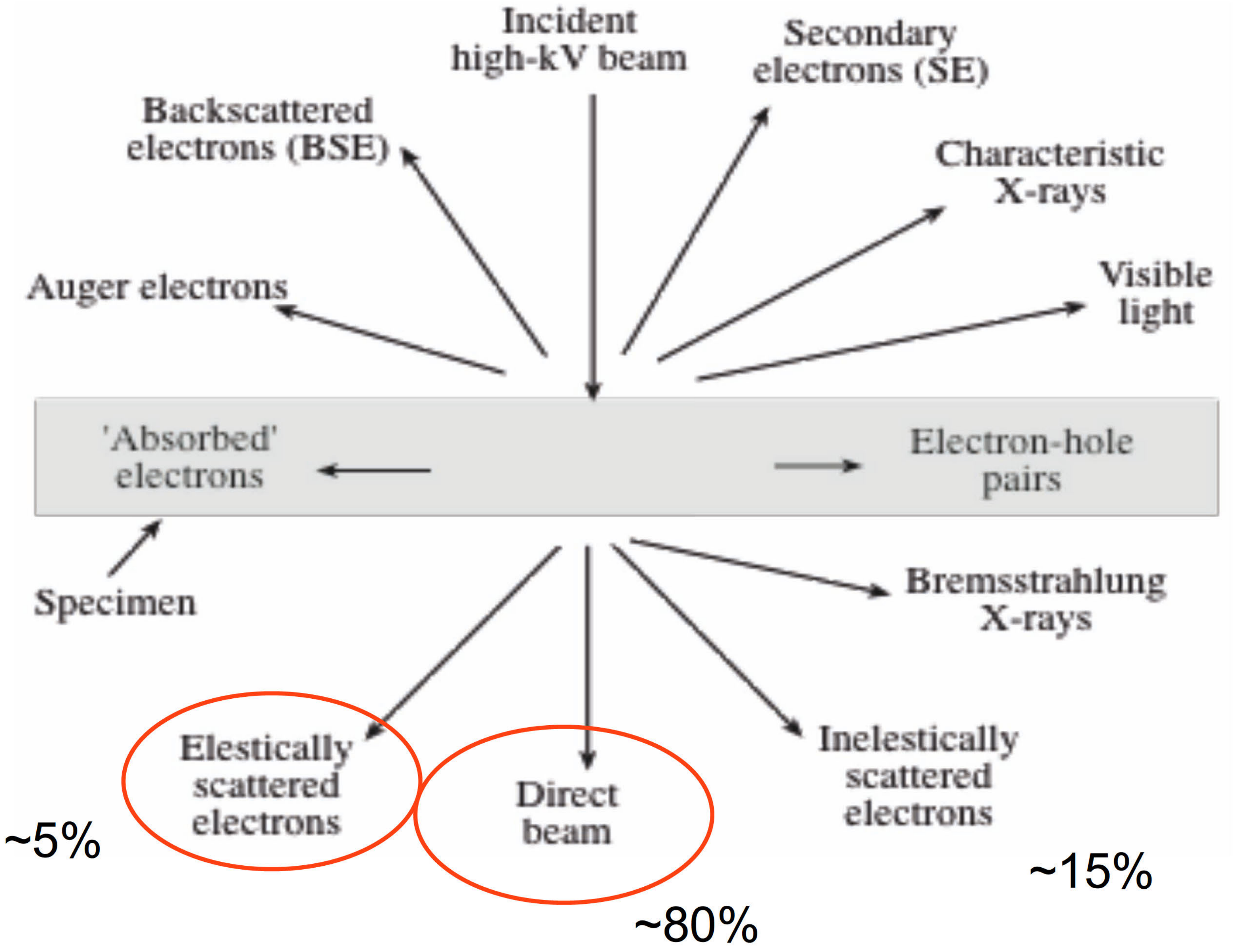
Contrast
Transfer
Function



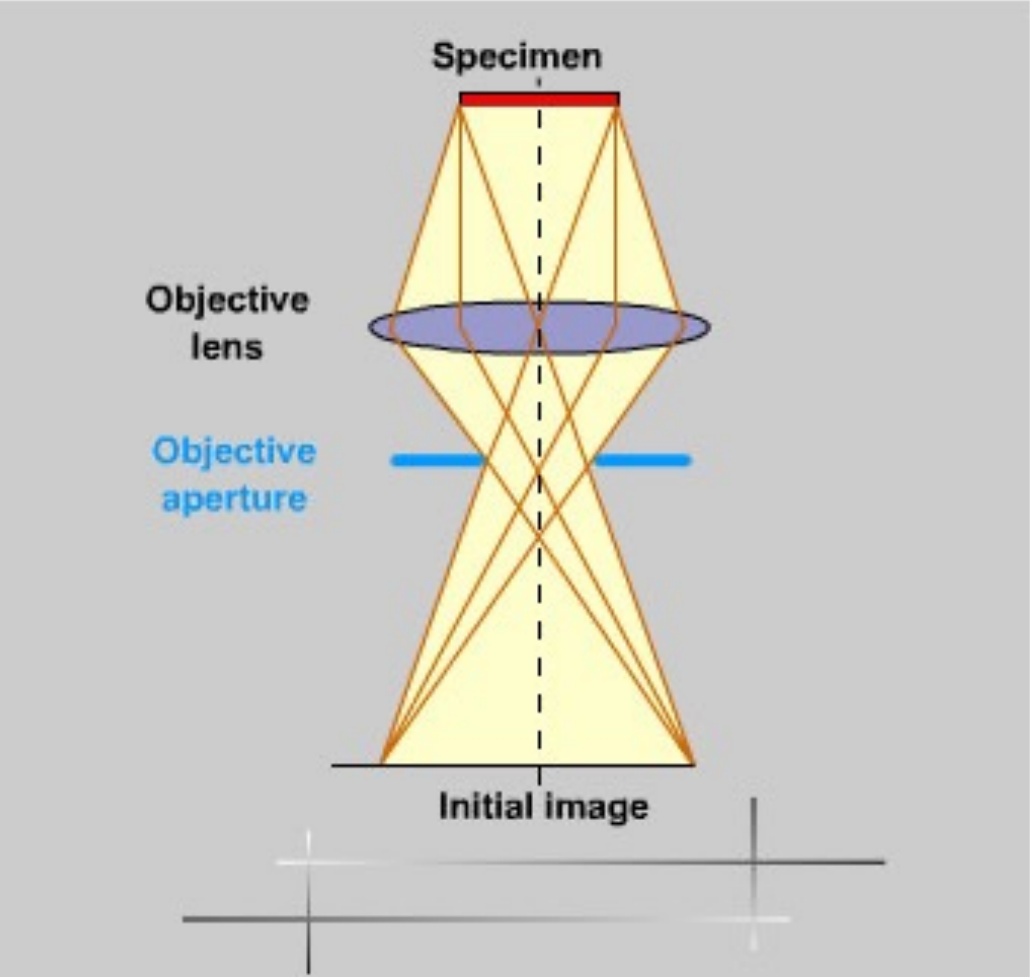


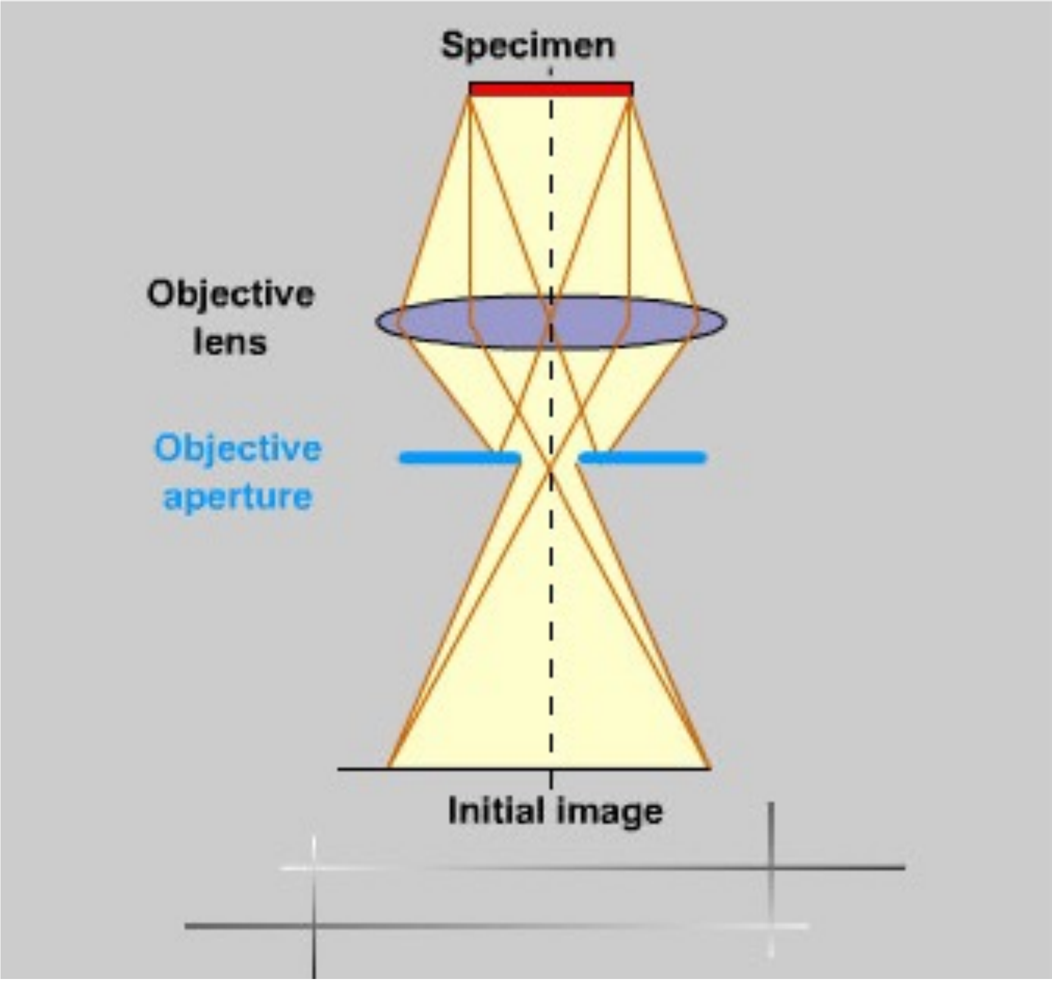
Can be corrected by software as post-process

Large Number of Signals

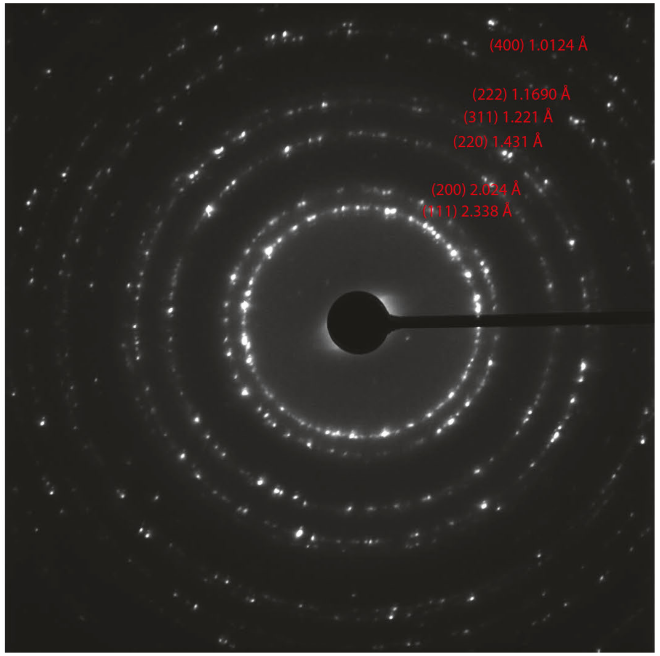


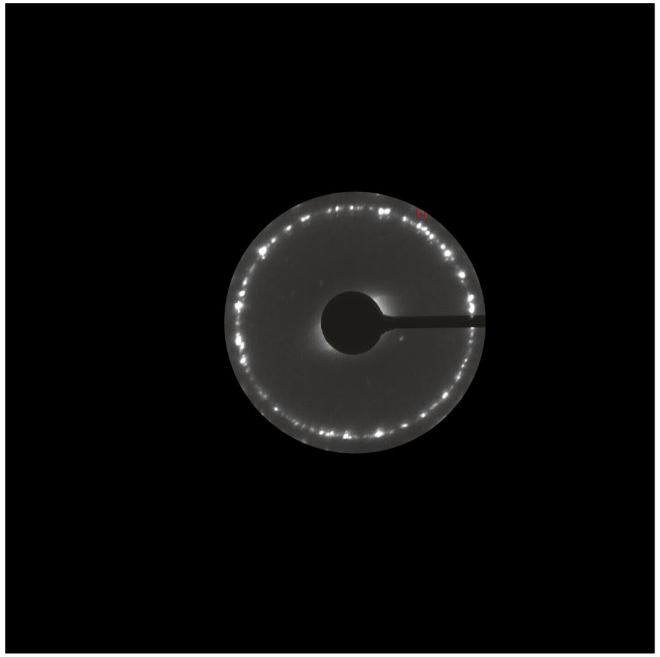
Objective Aperture



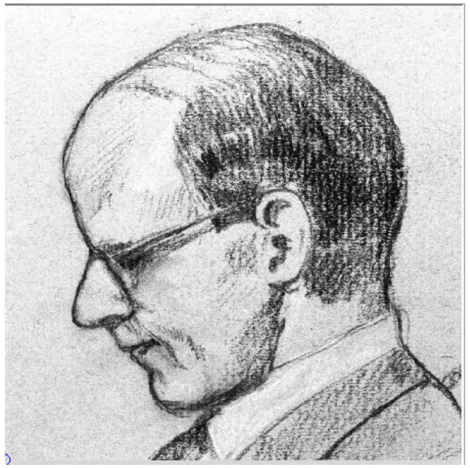


Objective aperture

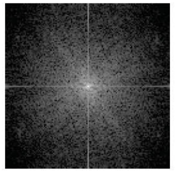




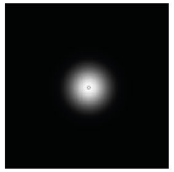
Beware...



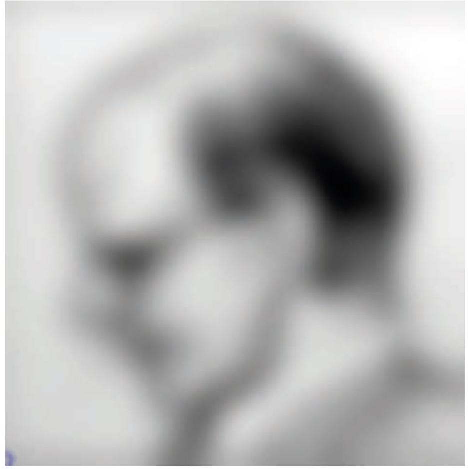
FT



x



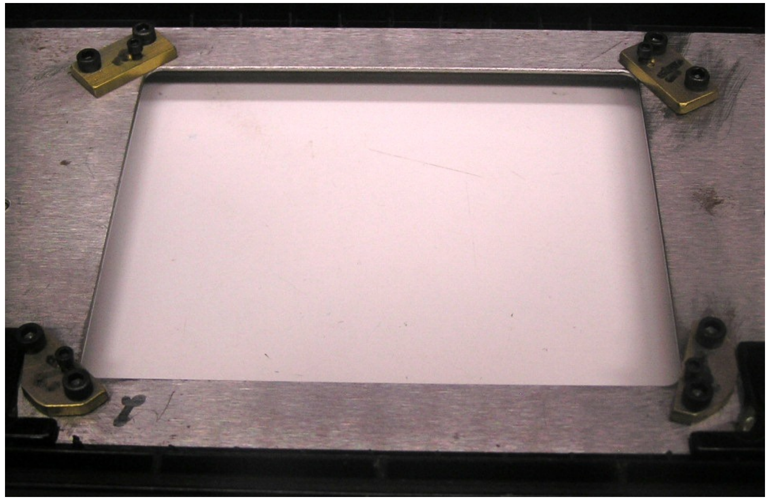
Low-pass filter

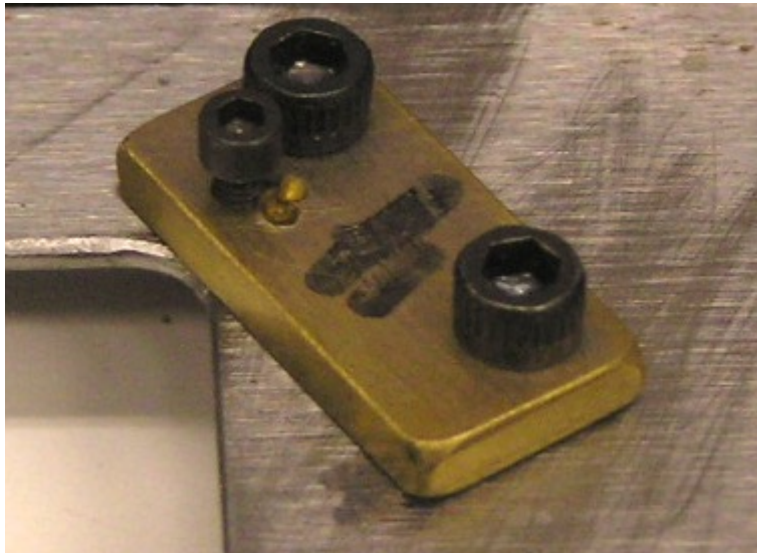


II. Cameras – from CCD to DED

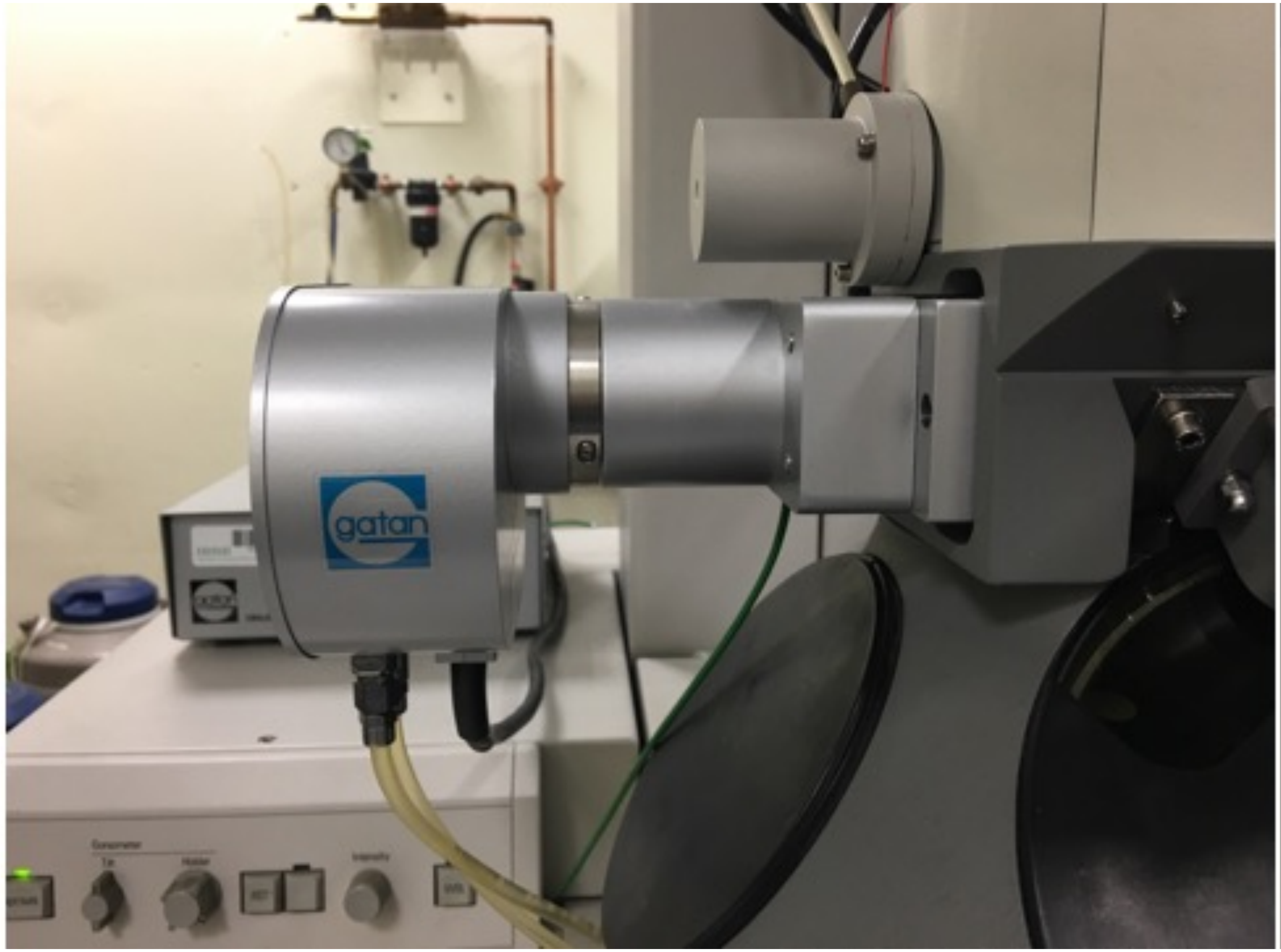

NEW
Kodak
ELECTRON
IMAGE FILM
MULTI – PACK
Kodak
ELECTRON
MICROSCOPE FILM
MULTI – PACK


SCAI



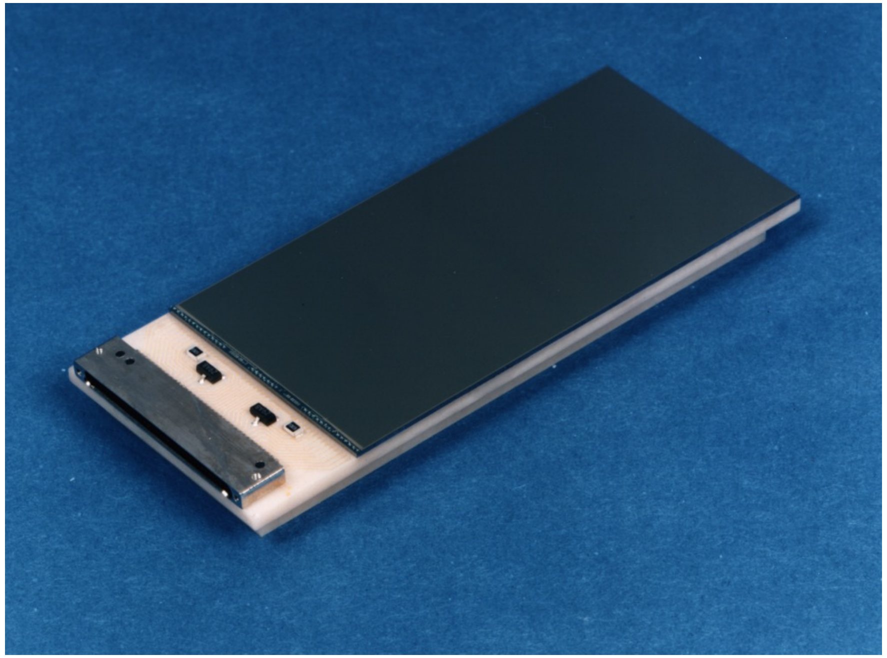
Gatan Orius CCD & TVIPS TemCam 224HD CCD cameras on CM120

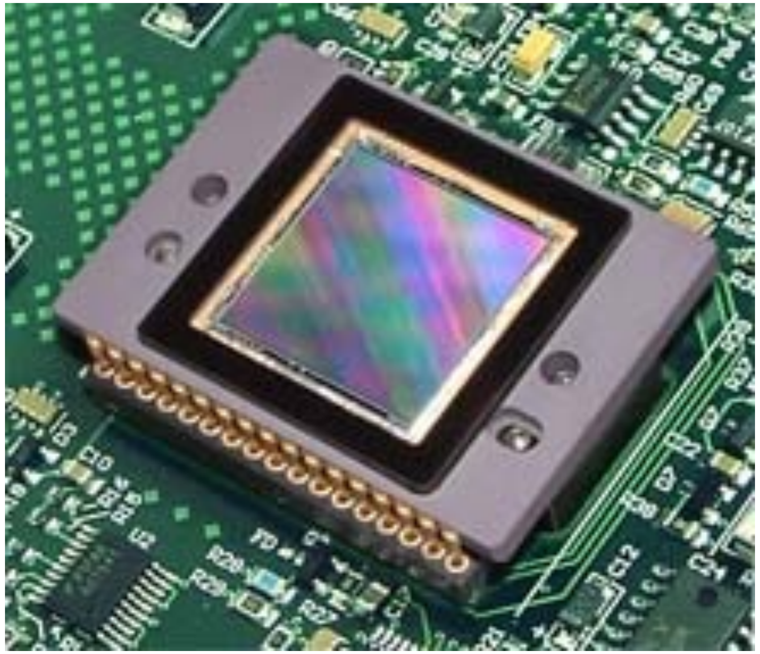


What is a CCD ?

Charge Coupled Devices (CCDs) were invented in the 1970s and originally found application as memory devices. Their light sensitive properties were quickly exploited for imaging applications and they produced a major revolution in Astronomy. They improved the light gathering power of telescopes by almost two orders of magnitude. Nowadays an amateur astronomer with a CCD camera and a 15 cm telescope can collect as much light as an astronomer of the 1960s equipped with a photographic plate and a 1m telescope.

CCDs work by converting light into a pattern of electronic charge in a silicon chip. This pattern of charge is converted into a video waveform, digitized and stored as an image file on a computer.





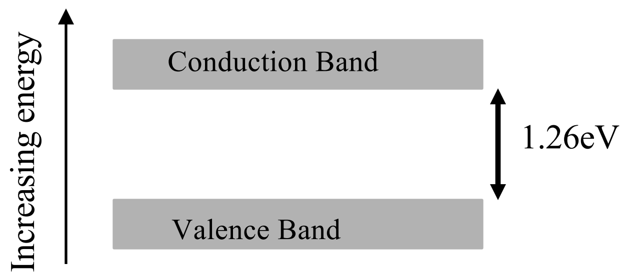
Willard S. Boyle and George E. Smith
Bell Laboratories, Murray Hill, NJ, USA

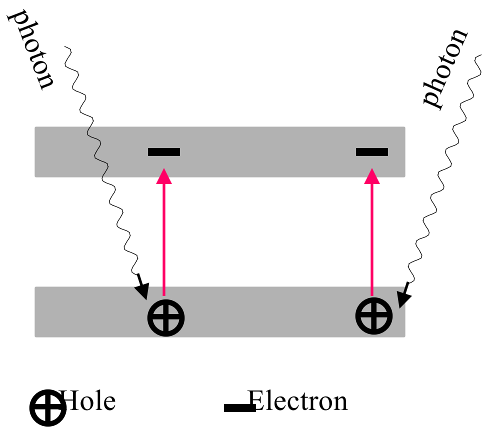
2009 Nobel Prize in Physics

"for the invention of an imaging semiconductor circuit – the CCD sensor"

Photoelectric Effect

The effect is fundamental to the operation of a CCD. Atoms in a silicon crystal have electrons arranged in discrete energy bands. The lower energy band is called the Valence Band, the upper band is the Conduction Band. Most of the electrons occupy the Valence band but can be excited into the conduction band by heating or by the absorption of a photon. The energy required for this transition is 1.26 electron volts. Once in this conduction band the electron is free to move about in the lattice of the silicon crystal. It leaves behind a 'hole' in the valence band which acts like a positively charged carrier. In the absence of an external electric field the hole and electron will quickly re-combine and be lost. In a CCD an electric field is introduced to sweep these charge carriers apart and prevent recombination.





Thermally generated electrons are indistinguishable from photo-generated electrons . They constitute a noise source known as 'Dark Current' and it is important that CCDs are kept cold to reduce their number.

1.26eV corresponds to the energy of light with a wavelength of 1μm. Beyond this wavelength silicon becomes transparent and CCDs constructed from silicon become insensitive.

CCD signal readout

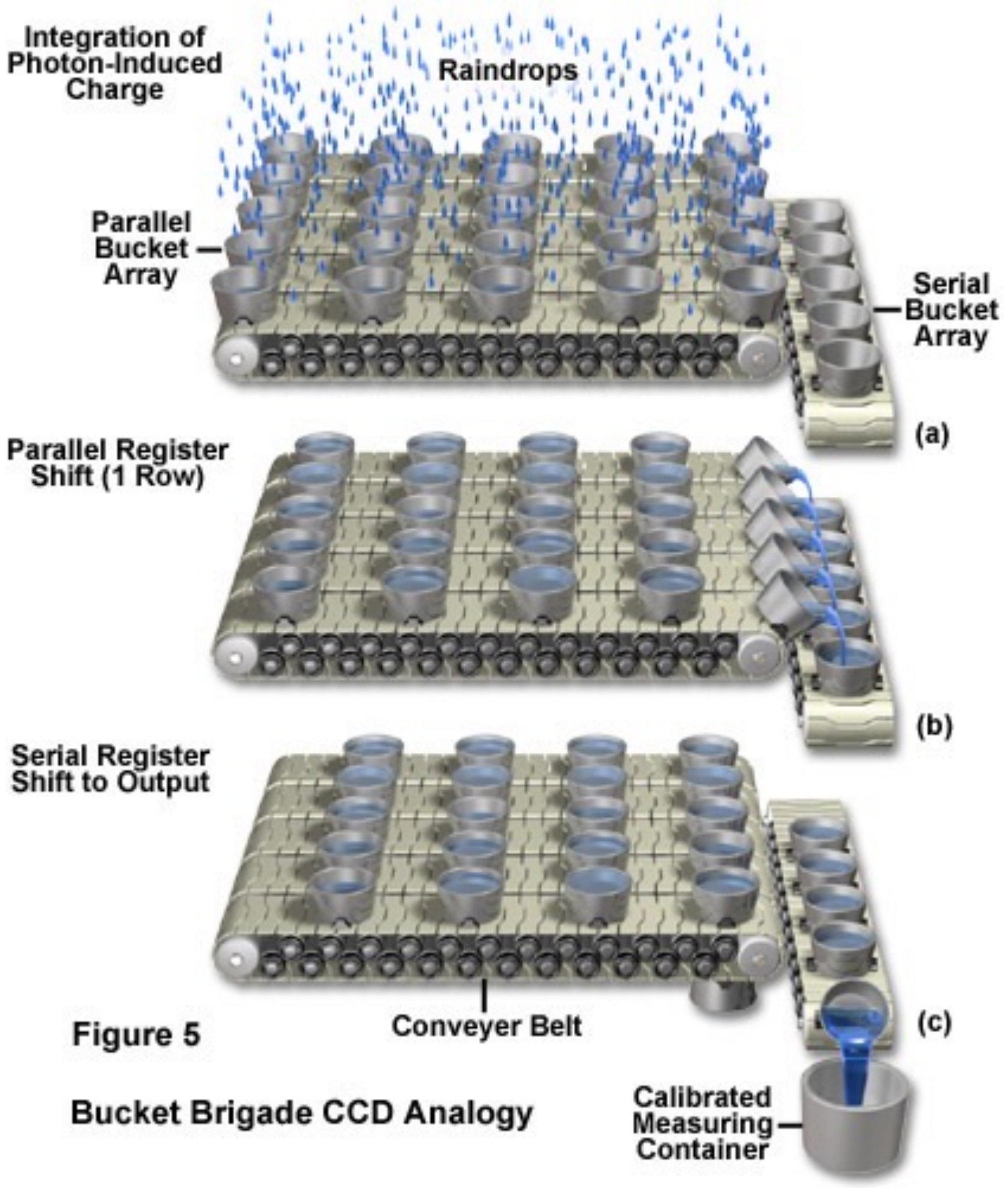
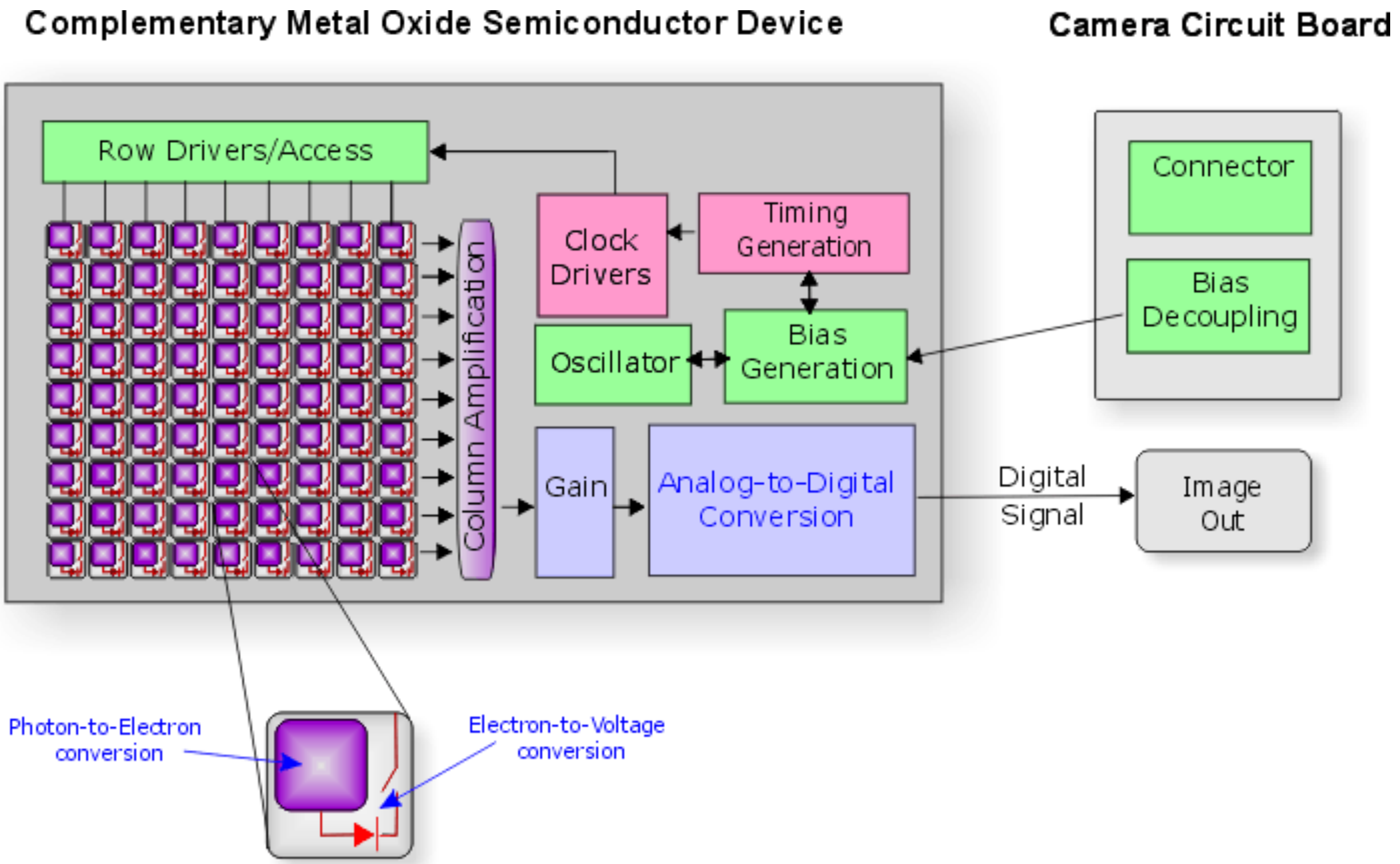


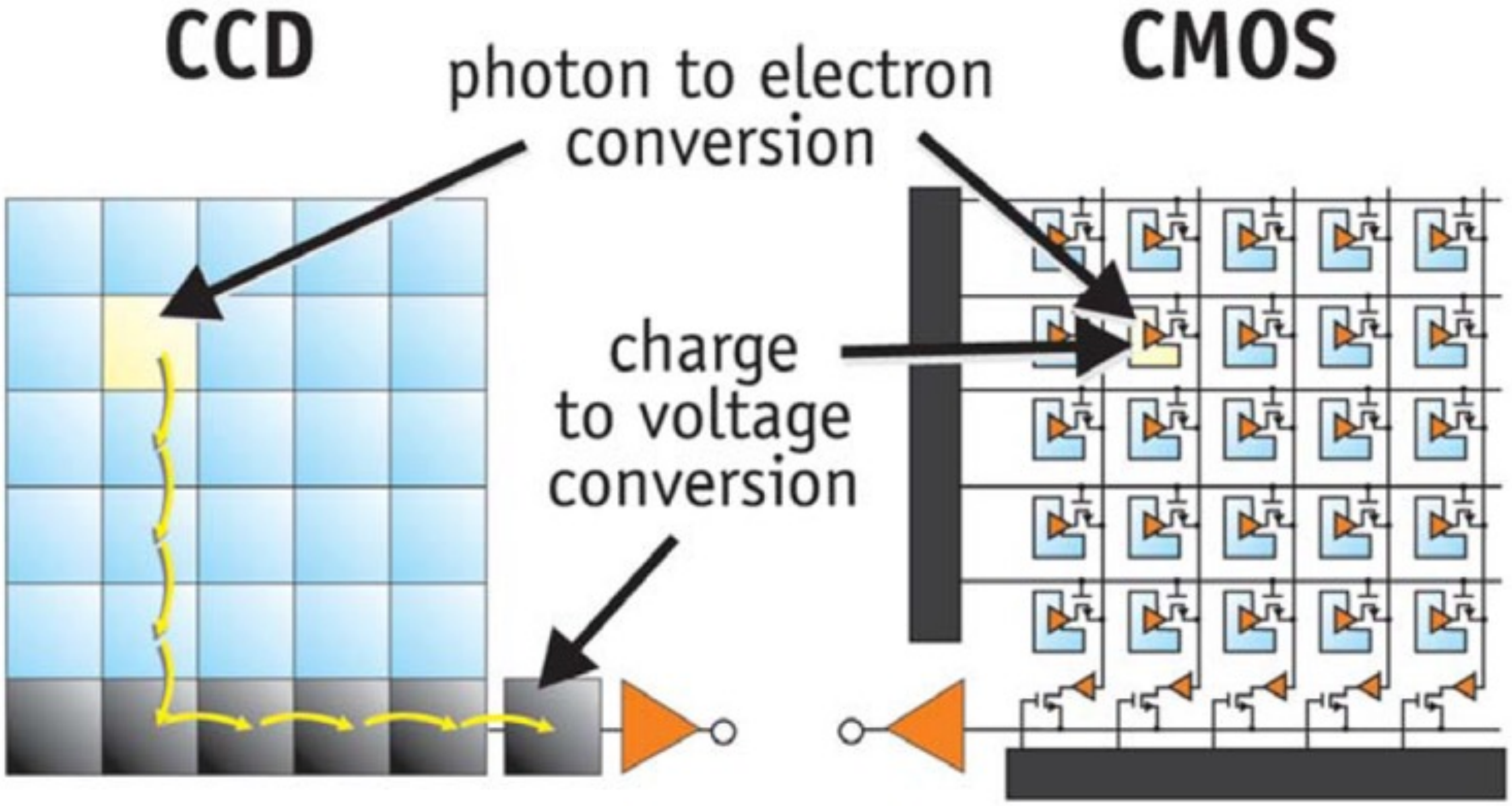
Figure 5

Bucket Brigade CCD Analogy

CMOS



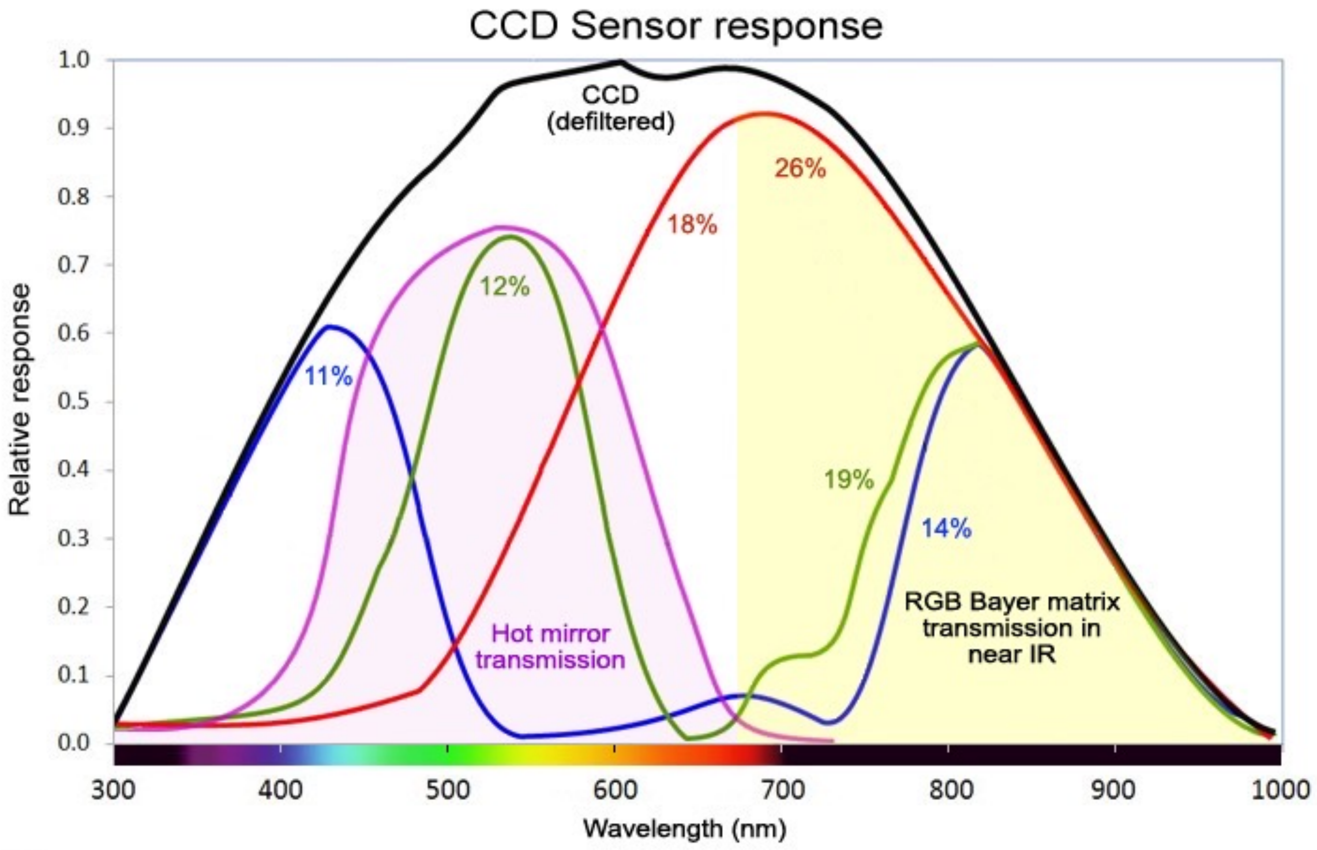
Complementary Metal Oxide Semiconductor Device
Camera Circuit Board
Row Drivers/Access
Column Amplification
Clock Drivers
Timing Generation
Oscillator
Bias Generation
Gain
Analog-to-Digital Conversion
Connector
Bias Decoupling
Digital Signal
Image Out
Photon-to-Electron conversion
Electron-to-Voltage conversion

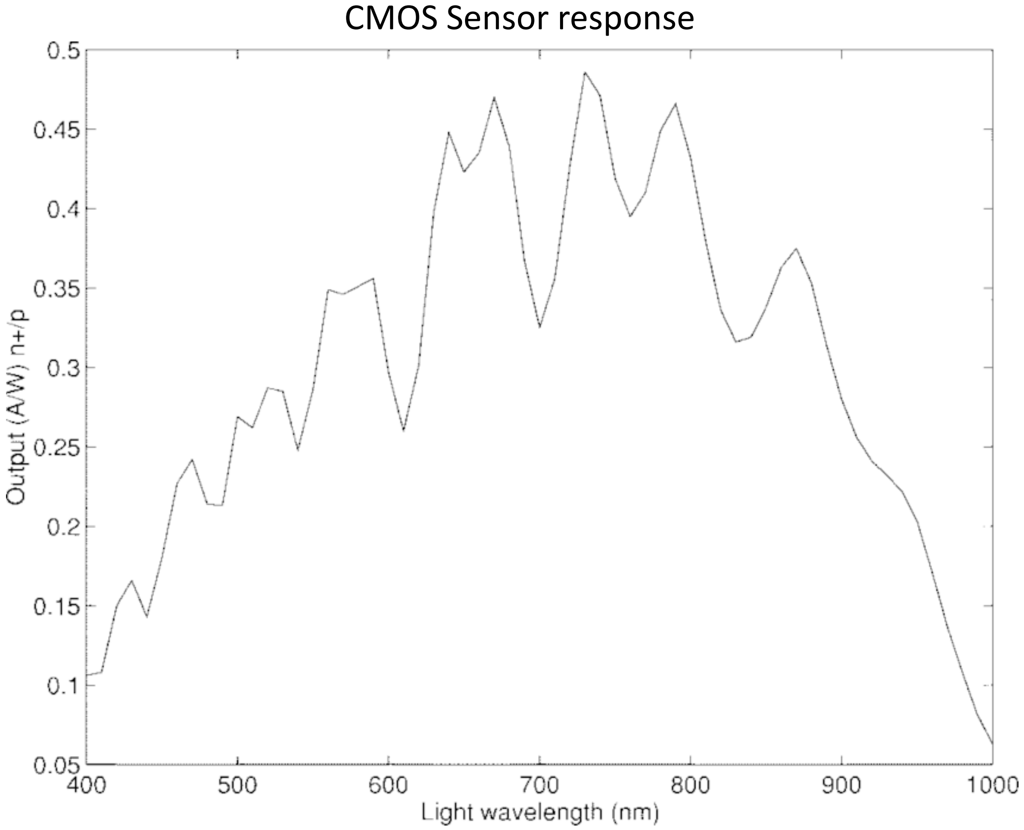


CCDs move photogenerated charge from pixel to pixel and convert it to voltage at an output node. CMOS imagers convert charge to voltage inside each pixel.

https://meroli.web.cern.ch/lecture_cmos_vs_ccd_pixel_sensor.html

Spectral Reponses for CCD and CMOS Sensors





http://www.astrosurf.com/luxorion/Physique/spectral-response-ccd.jpg

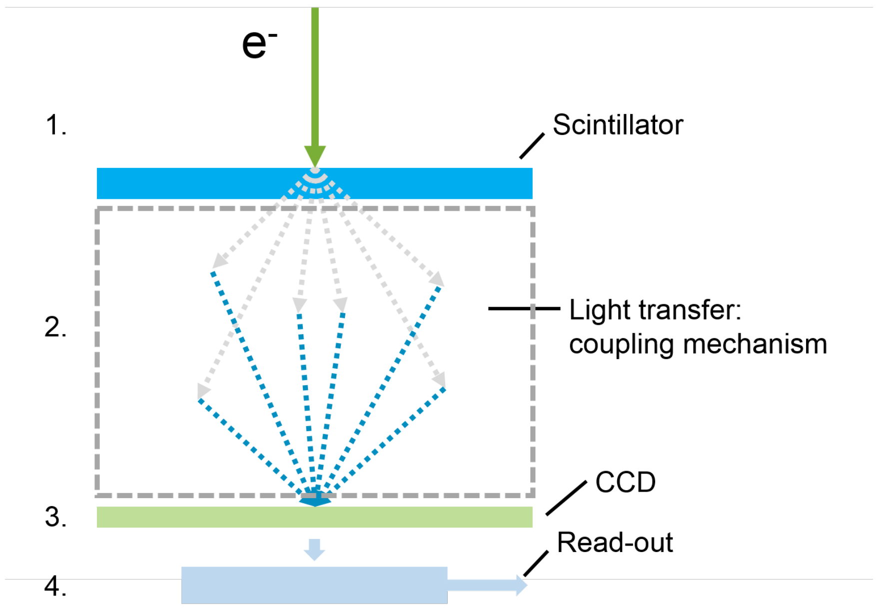
F. Pardo, IEEE Journal of Solid-State Circuits 33(6):842 - 849

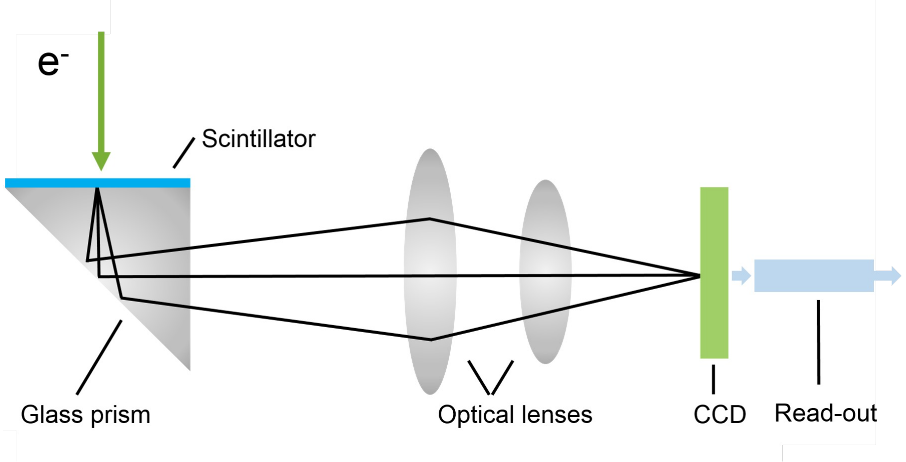
For TEM, the electrons need to be converted to photon signal so that the developed technology of CCD can be used.

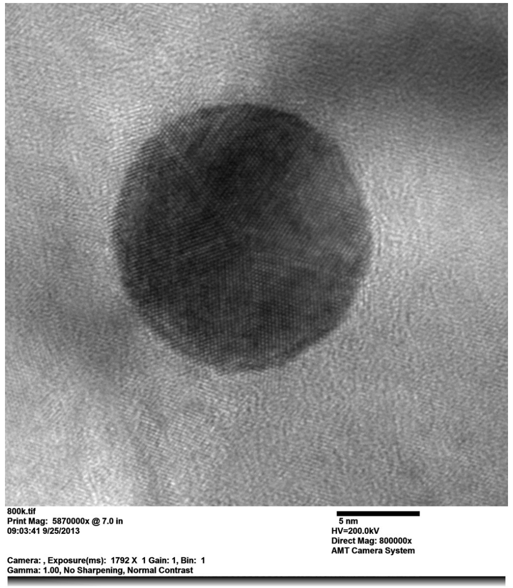
Two Key Questions for a Camera

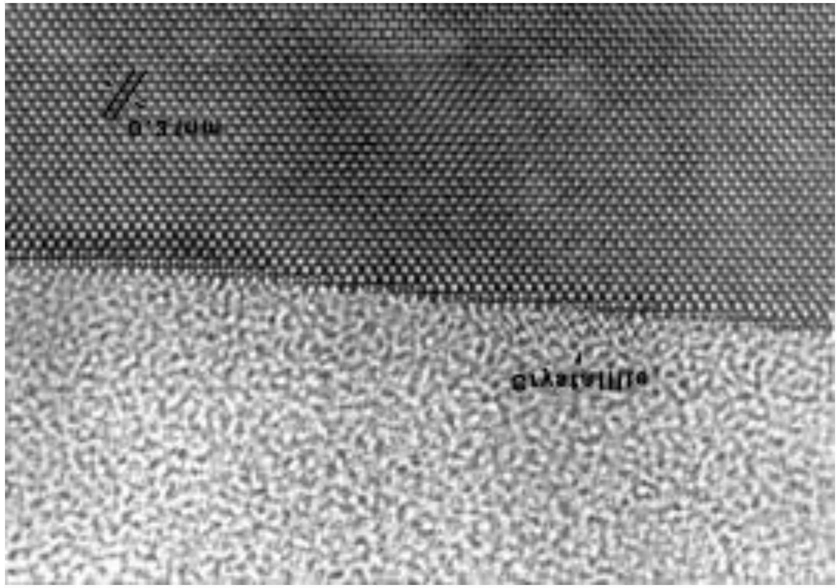
1. How is the voltage signal generated, from electron or photon?

2. How is the voltage signal read out?

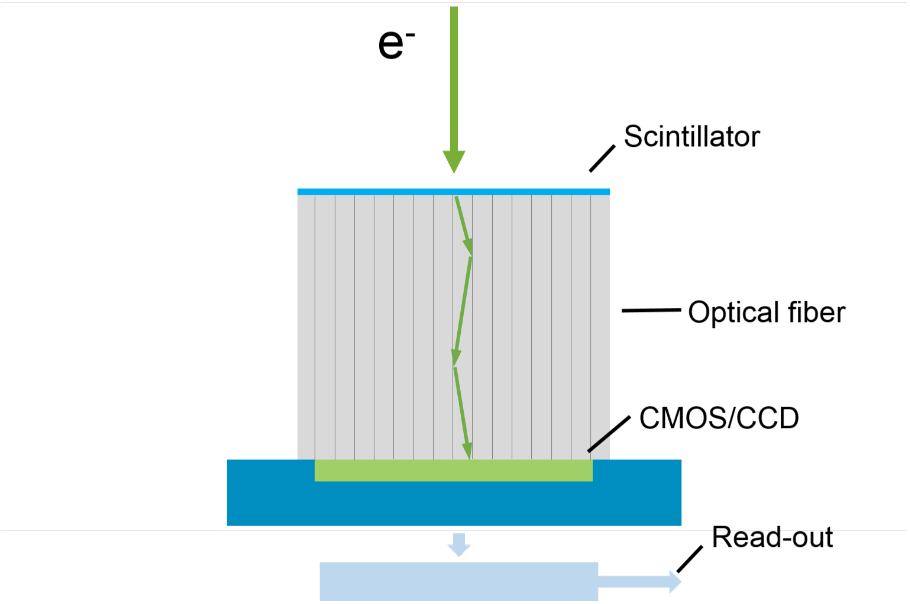

e-
1.
Scintillator
2.
Light transfer:
coupling mechanism
3.
CCD
Read-out
4.


e-
Scintillator
Glass prism
Optical lenses
CCD
Read-out

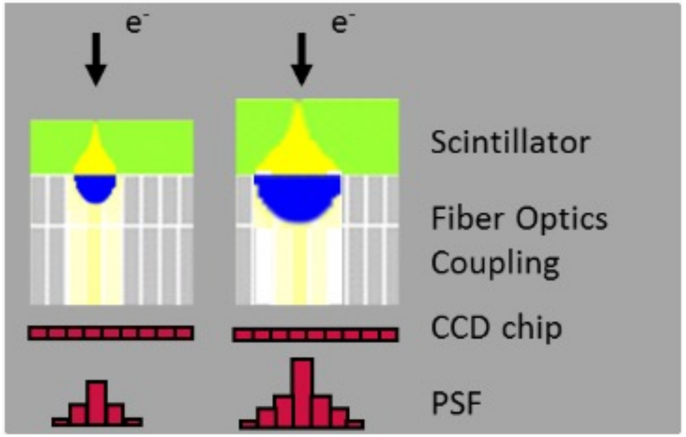

800k.tif
Print Mag: 5870000x @ 7.0 in
09:03:41 9/25/2013
5 nm
HV=200.0kV
Direct Mag: 800000x
AMT Camera System
Camera: , Exposure(ms): 1792 X 1 Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

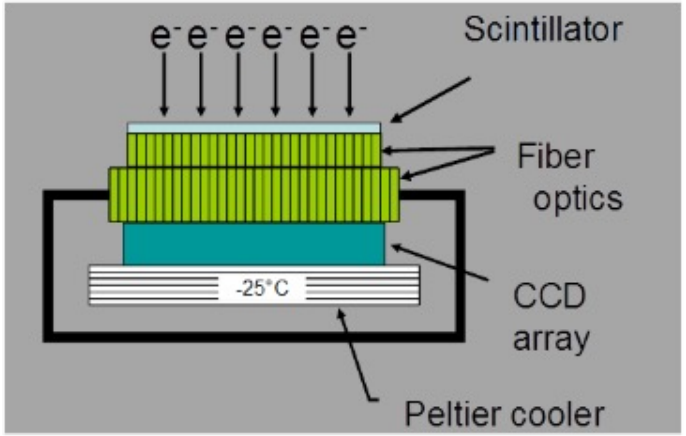


Electron to Photon Conversion in Scintillator layer Results in Information Loss



CCD: multi stage conversion of electron energy via fiber or lens optics





For cryoEM sample, with conventional camera with scintillator, it is difficult to get resolution better than 1nm!

Direct Electron Detector (DED)

DED with CMOS "Monolithic Active Pixel Sensors"

- Primary electron generates 100-300 electrons in P^- Epilayer
- Electrons collect in closest well and have to be read out frequently
- Backscatter still a big problem unless substrate is severely thinned (from 700 µm to 30-50 µm)

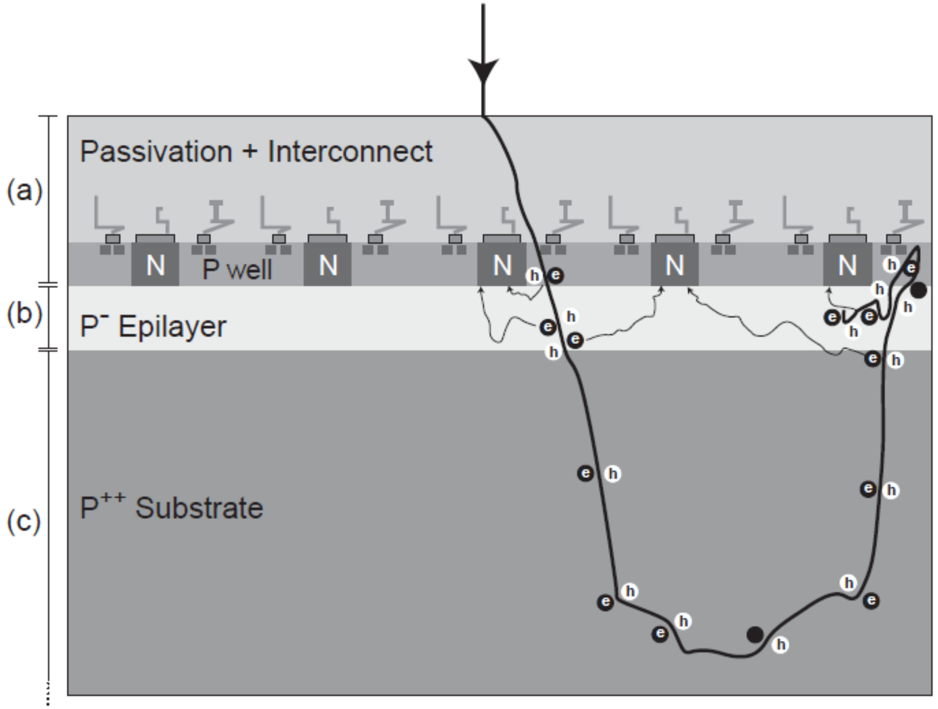
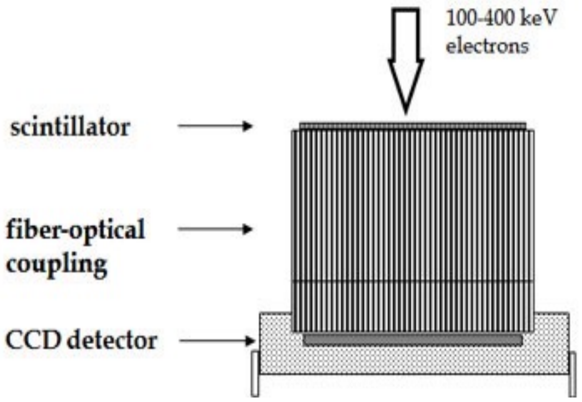


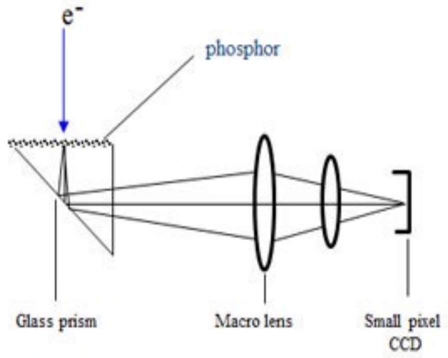
Fig. 1. Schematic of MAPS detector shown in cross-section. The detector has three main regions: (a) about 5-µm-thick passivation layer plus interconnections for readout electronics in the P well, (b) a few microns of lightly doped epilayer where the useful signal is generated, and drifts on to N wells prior to being read out, and (c) the main bulk of the detector, the substrate, which is heavily doped and which does not play a significant role in the detection process. A possible path for a single incident high-energy electron is shown to illustrate the problem with backscatter from the substrate.

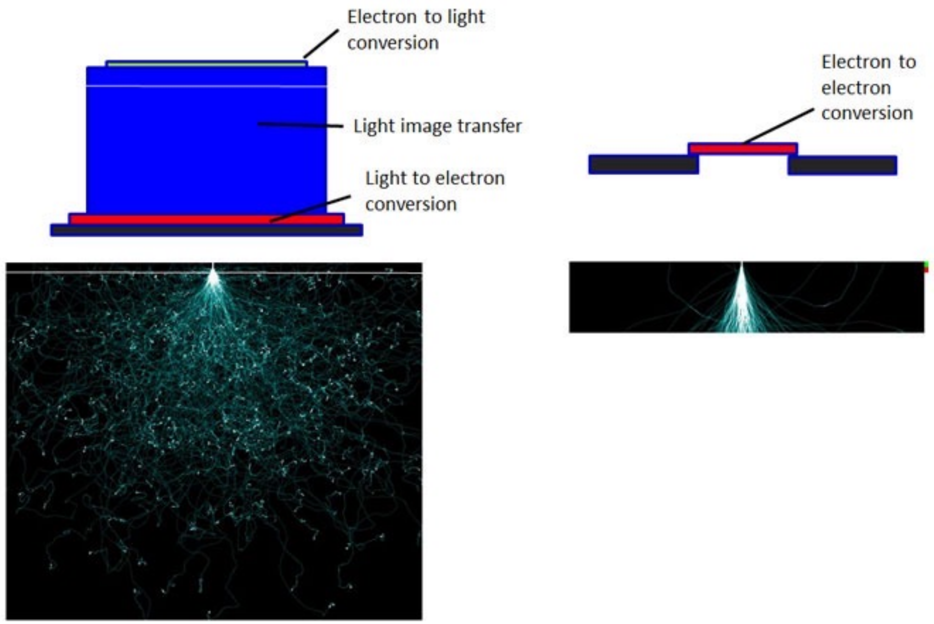
CCD vs. DED

Fiber Optical Coupling

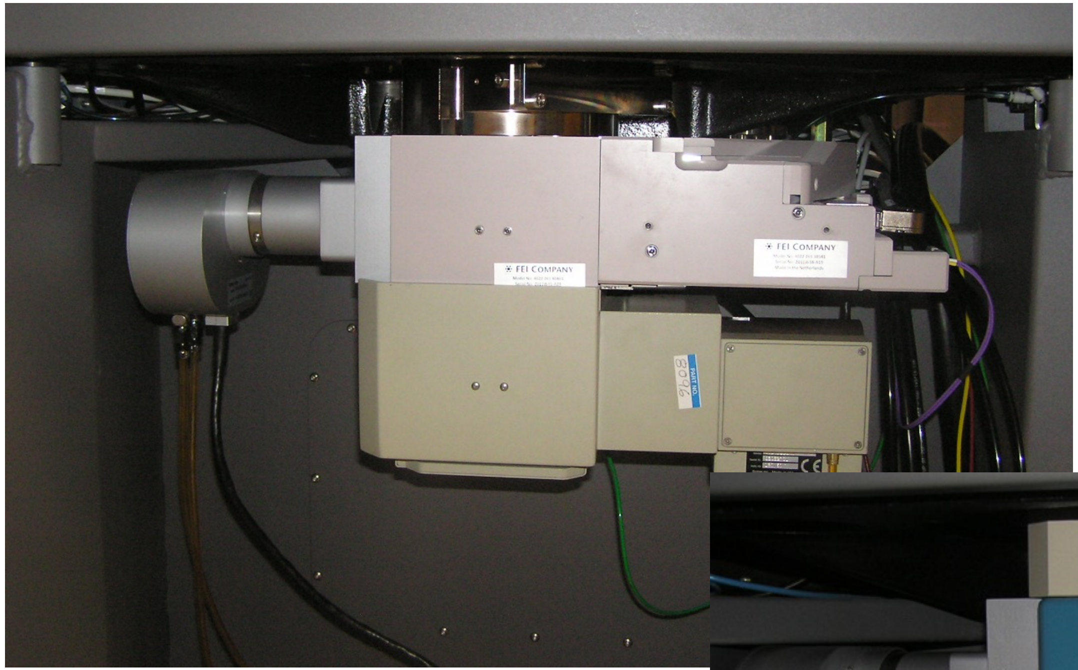


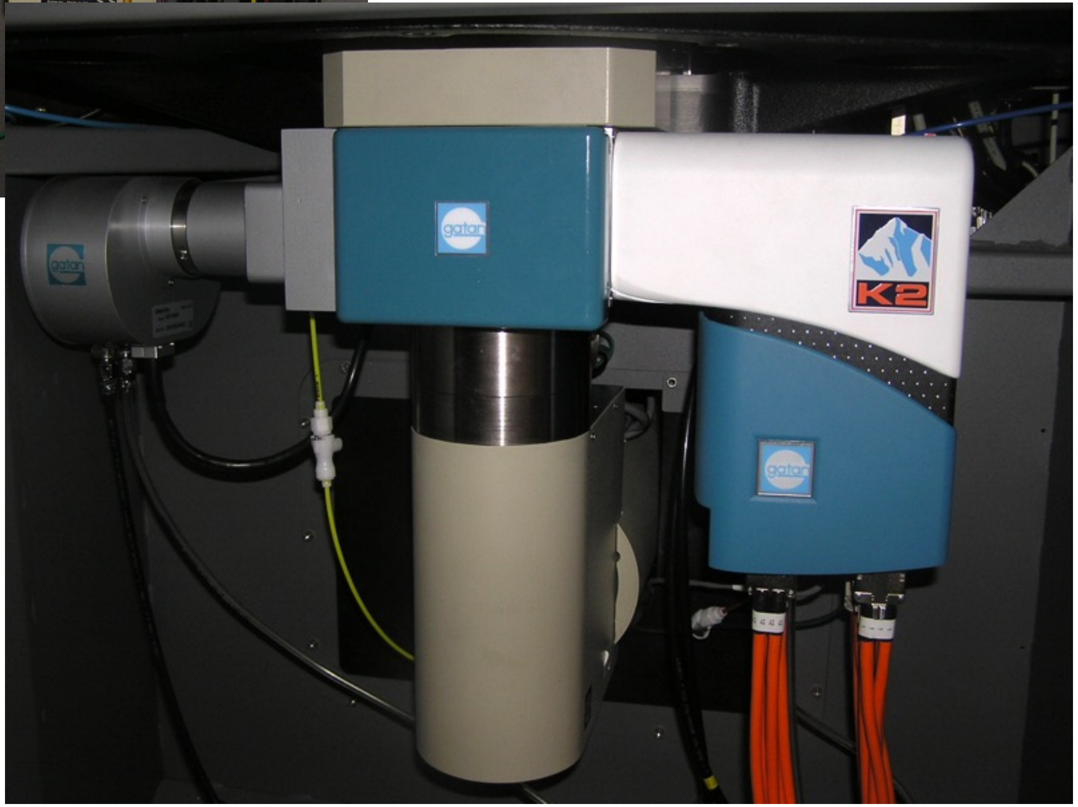
Lens Coupling





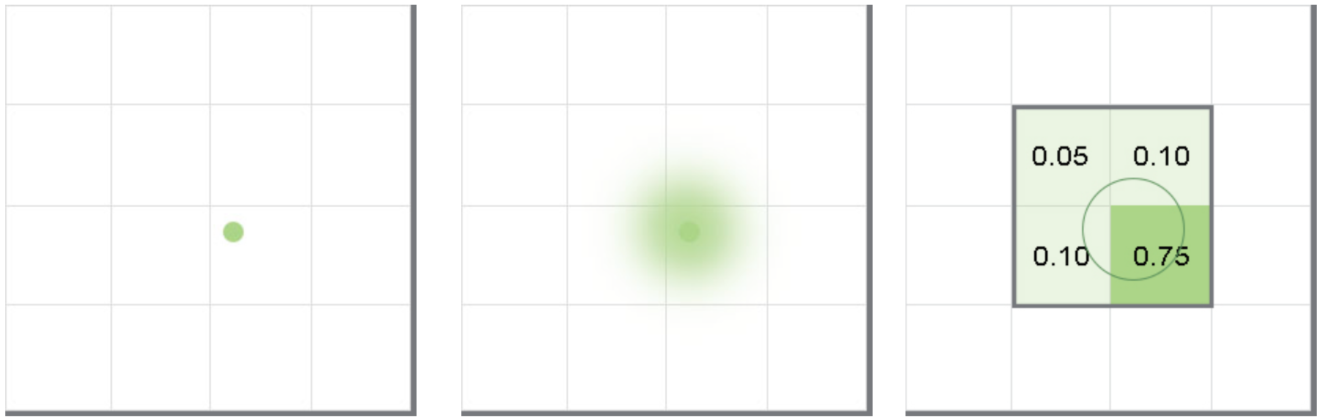
http://www.gatan.com/K2/direct/direct-detection.php


FEI COMPANY
FEI COMPANY


gatan
gatan
gatan
K2

Counting Mode

Integration vs Counting



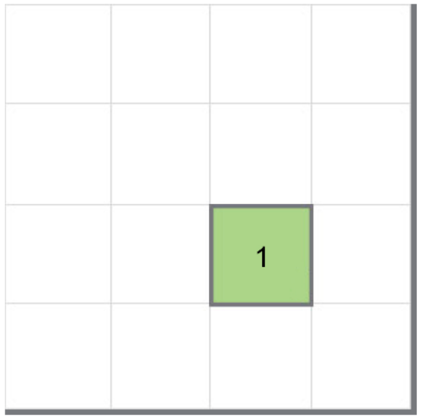
Electron enters detector.

Electron signal is scattered.

Charge collects in each pixel.

Integration

- Short exposures
- High Dose-rates applications
- Lower DQE



Events reduced to highest charge pixels.

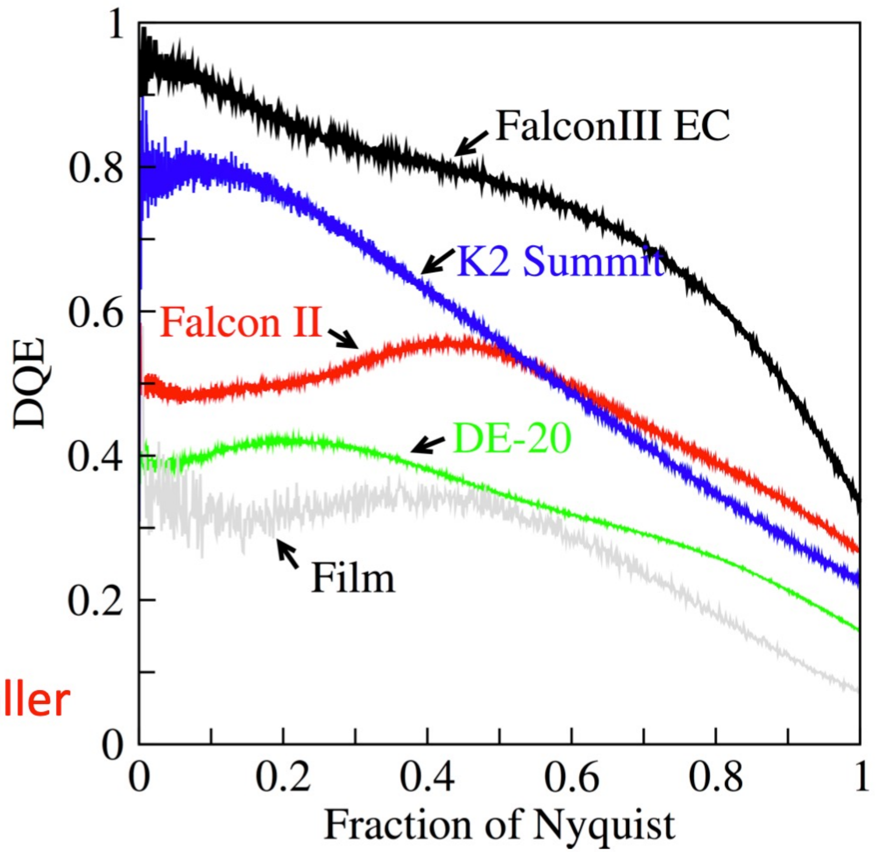
Counting

- Very low dose rate (0.5-5 e-/pixel/sec)
- Fast frame rates
- Long exposures
- Higher DQE

www.gatan.com

Detection Quantum Efficiency (DQE)

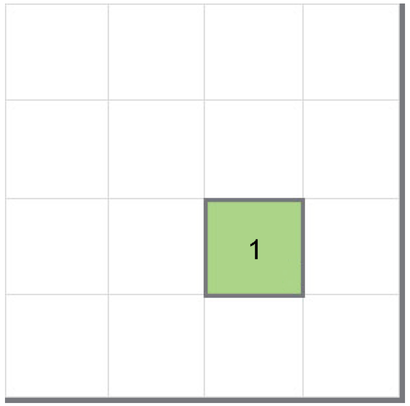
- Detective Quantum Efficiency
- DQE $=SNR^2o/SNR^2i$
- A measure of the signal to noise ratio degradation
- Perfect detector has DQE of 1



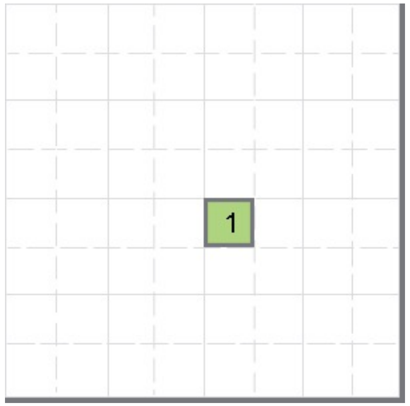
Counting detector advantageous for smaller complexes > boost low frequencies

Greg McMullan

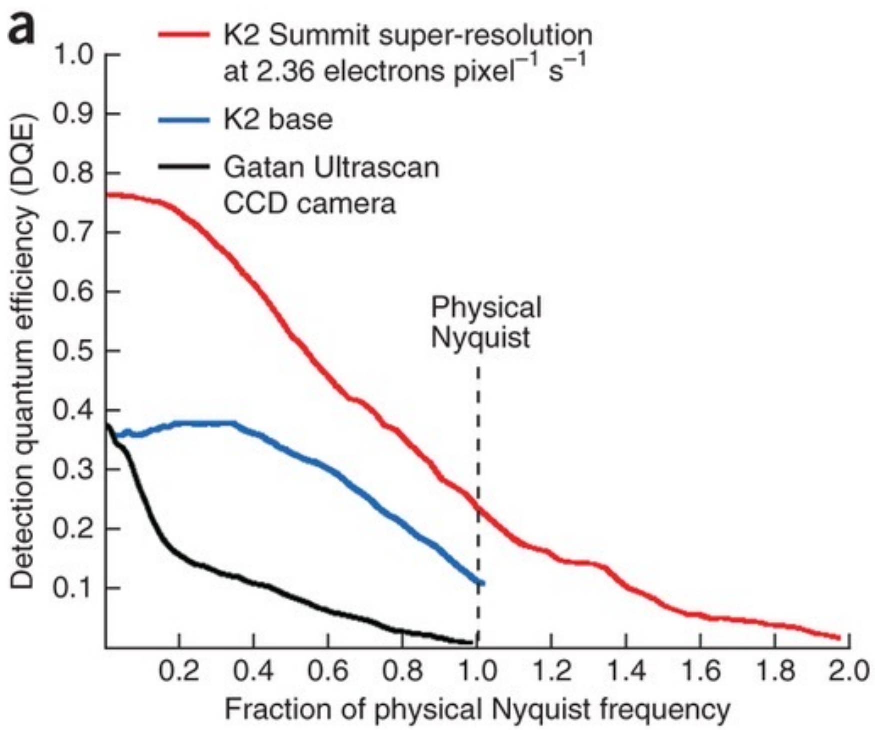
Super-resolution



Counting



Counting with Super-Res



On the Market

- **Direct Electron**
  DE-12 (4k x 3k)
  DE-20 (5k x 4k)
  Apollo (4k x 4k)
- **TFS**
  Falcon 2, 3, 4 (4i)
- **Gatan**

  K2/3 Summit
- Others…….?

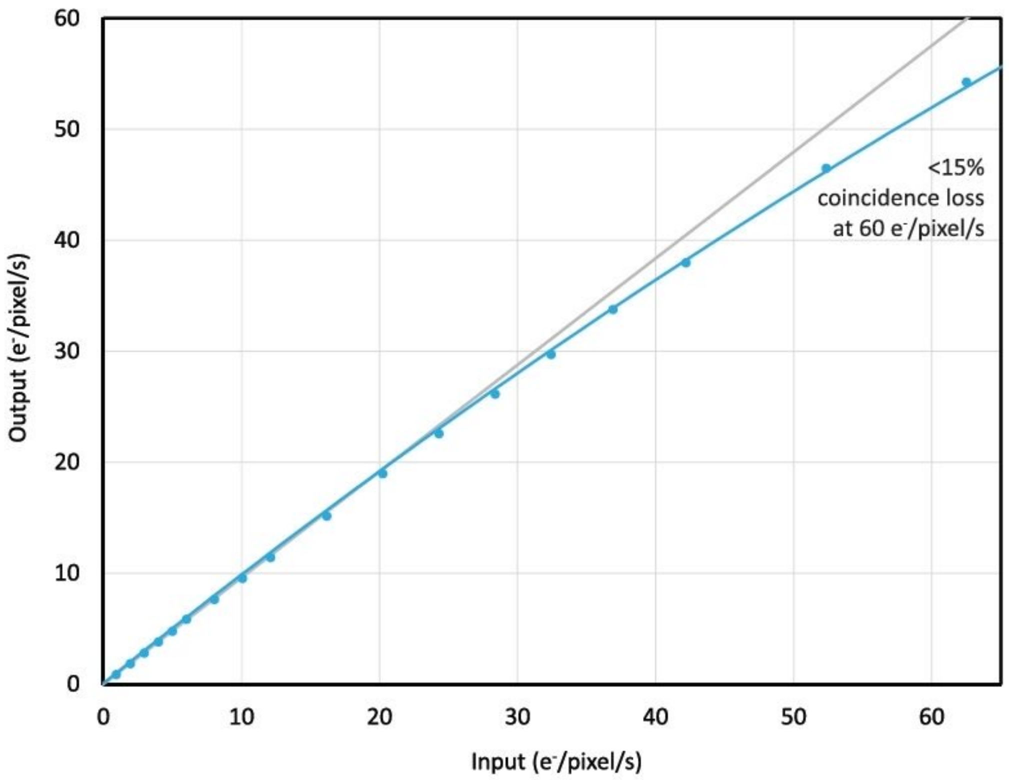
Apollo

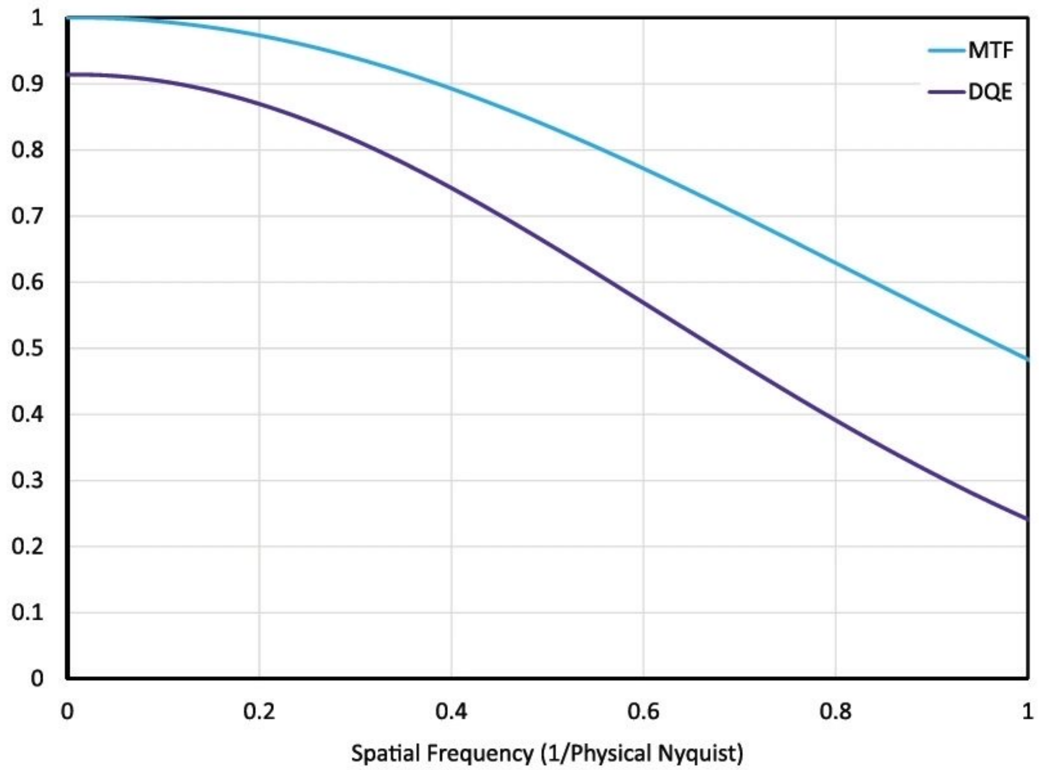
PROPELLING DISCOVERY

World's First Event-Based Active Pixel Direct Detector

- Our novel direct detection device (DDD®) delivers **ultra-low noise** and **extraordinary resolution** for almost **any beam brightness**.
- **Electron counting in hardware** through a combination of sensor technology & FPGA edge computing.
- Speed equivalent to a large-pixel direct detector **counting at 2,400 fps**.
- **On-chip CDS** minimizes noise and maximizes detection efficiency.
- **4k × 4k** (16.8 million) physical pixels with larger **8 µm pixel size** to maximize resolution (MTF).
- **Super-resolution** 8k × 8k (67.1 MP) readout to the computer at **60 fps** for motion correction, dose filtering, etc.
- Elegant, powerful, and **easy-to-use** for cryo-EM.
- Integrated with SerialEM for **automated** data acquisition.

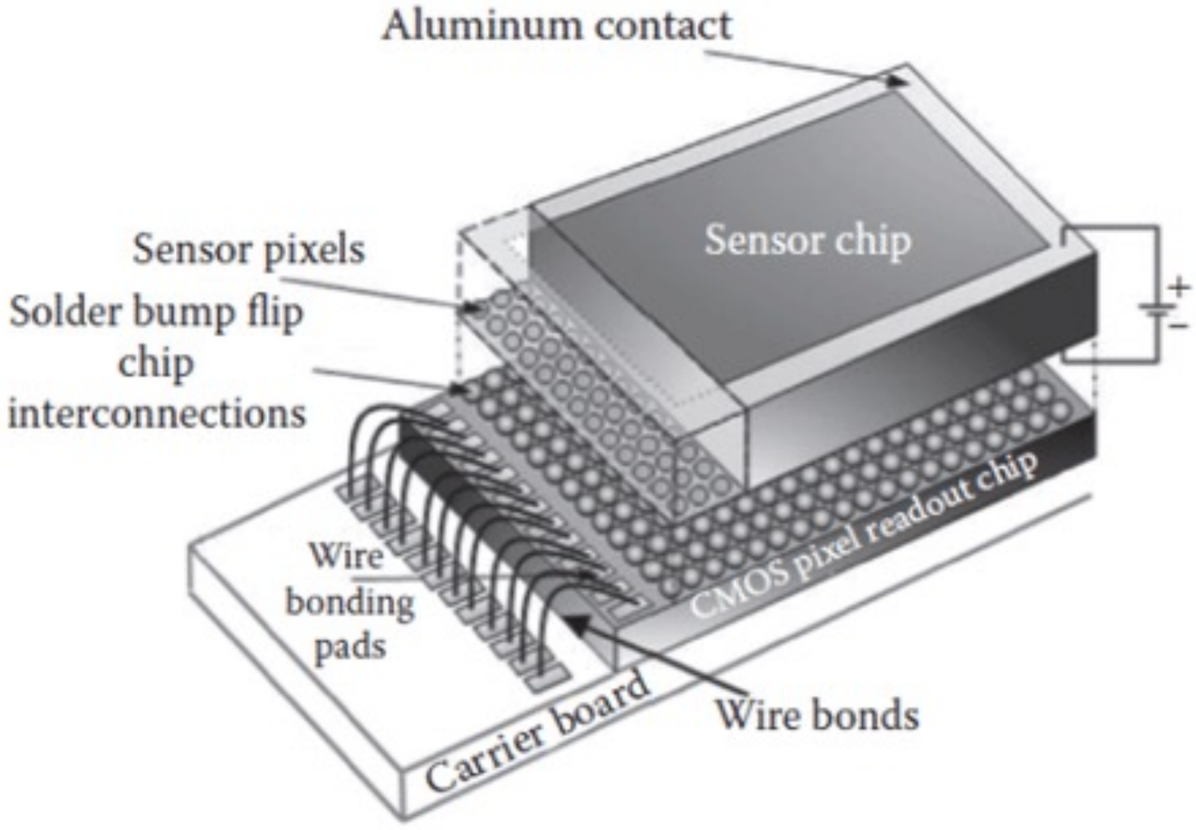
Apollo

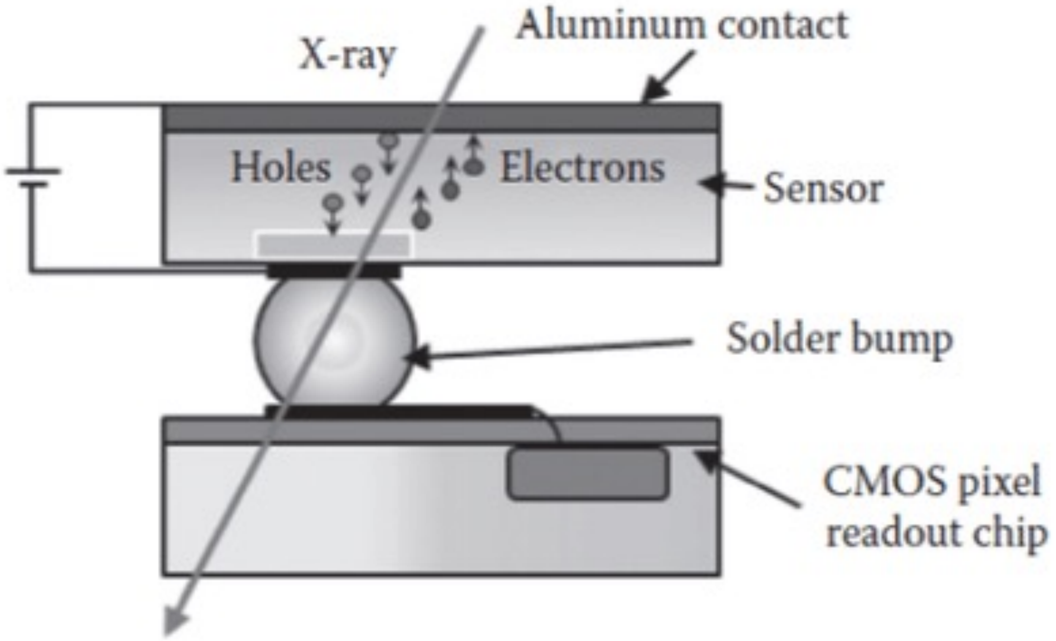




1. Insensitive to dose rate – comfortable to use
2. Low cost!

Hybrid-Pixel Camera – a different type of DED



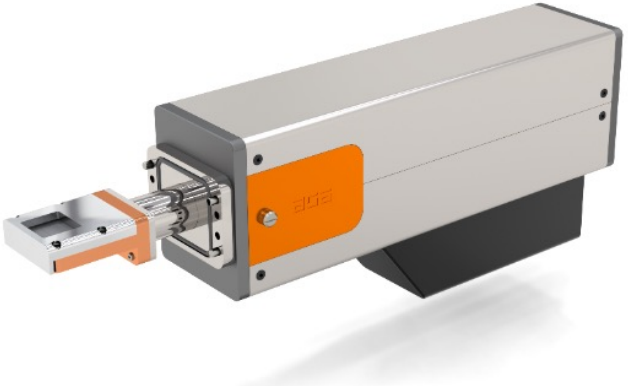


- **Ionizing radiation type of detector (semiconductor sensor + ASIC readout chip)**
- **Solder bump to couple them together (hybrid)**

Hybrid-Pixel Camera – a different type of DED

- Direct (electron) detector with good DQE
- Noiseless
- **Very high readout frame rate (4500Hz, 8bit)**
- 30-200 keV
- ~ 512 x 512 – 1000 x 1000 pixels
- Pixel size ***75*** um
- Commonly used in X-ray and synchrotron beam line
- Very good for diffraction data

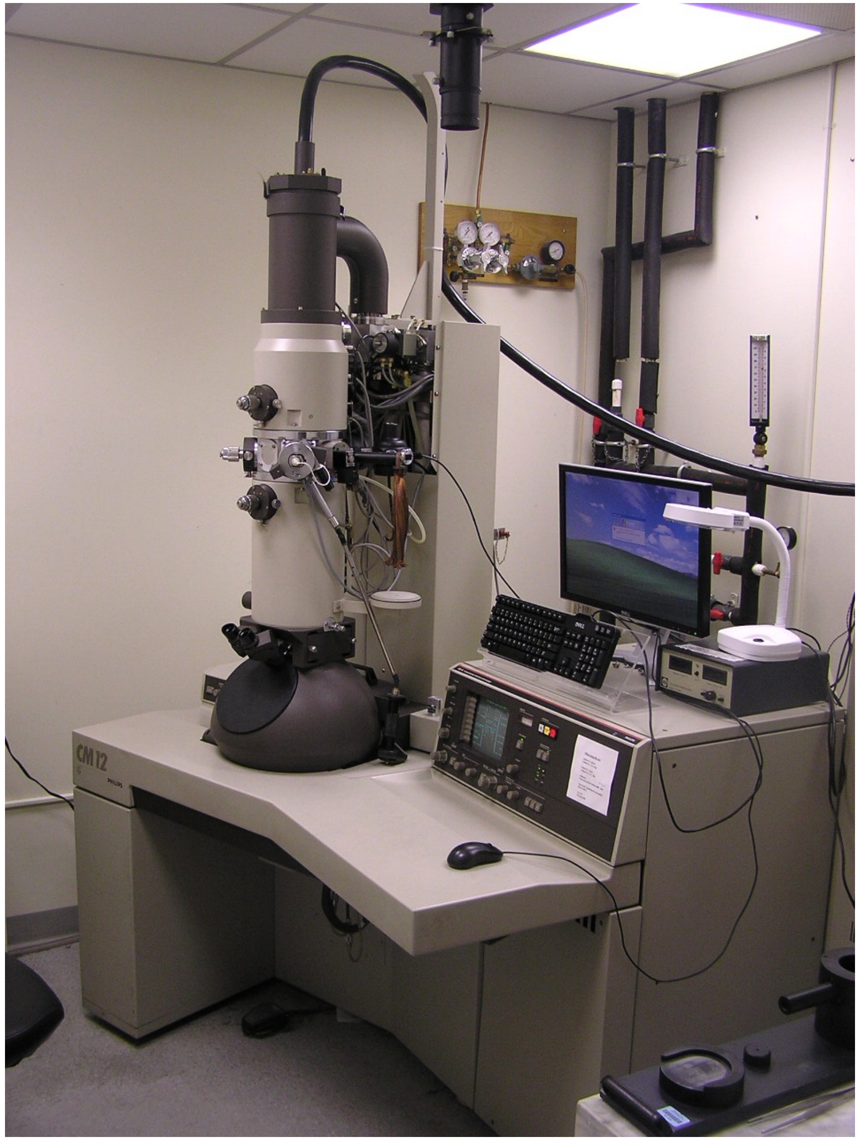
Singla, Quadro, ELA



CheeTa - Diffraction

Stela – 4D STEM

III. Advanced Software Control

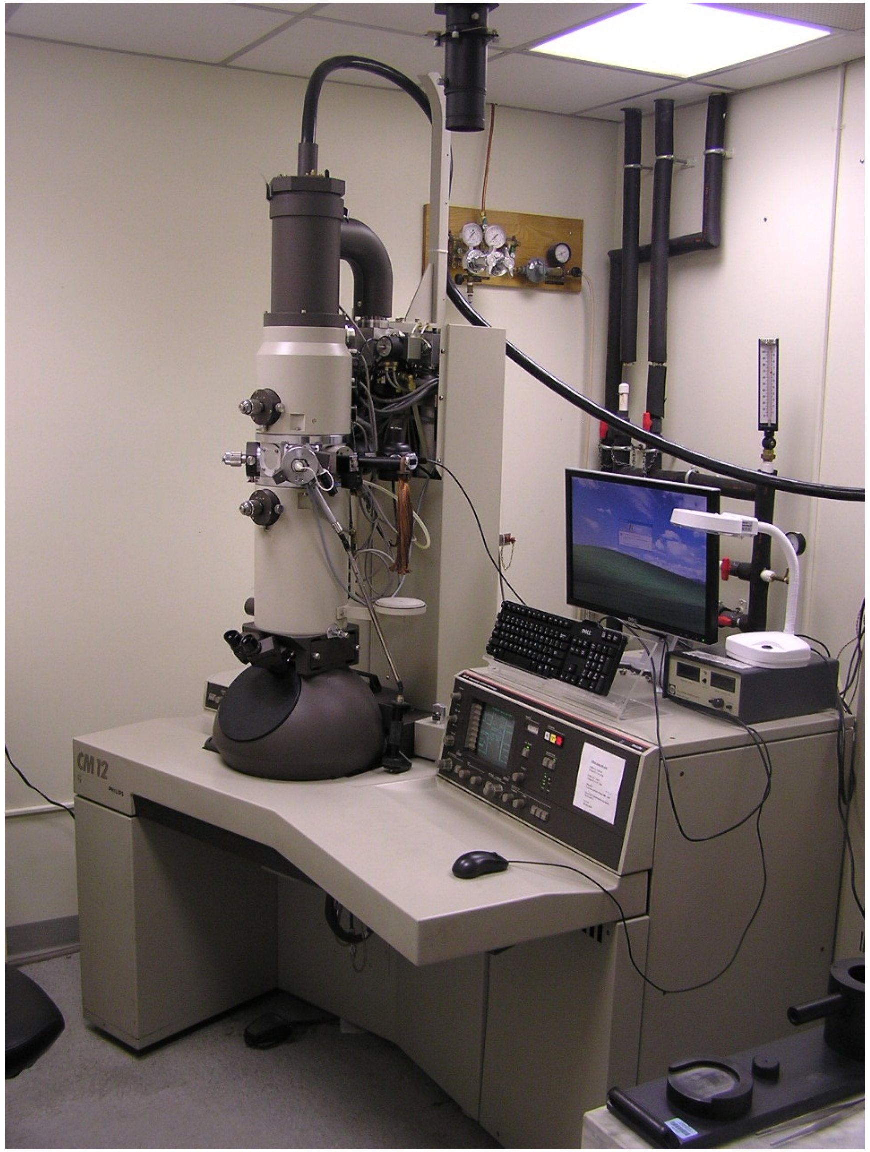

CM 12


CM 12 transmission electron microscope
DATA DIM
PANEL DIM
TEM BRIGHT FIELD-LOW DOSE
SA 40000x
MODES
PARAMETERS
AUTOCON-1
RSET DEFOC
MEASURING
EMISSION
MICROSCOPE
HIGH TENSION
VACUUM SYSTEM

Operator ⟷ TEM Hardware

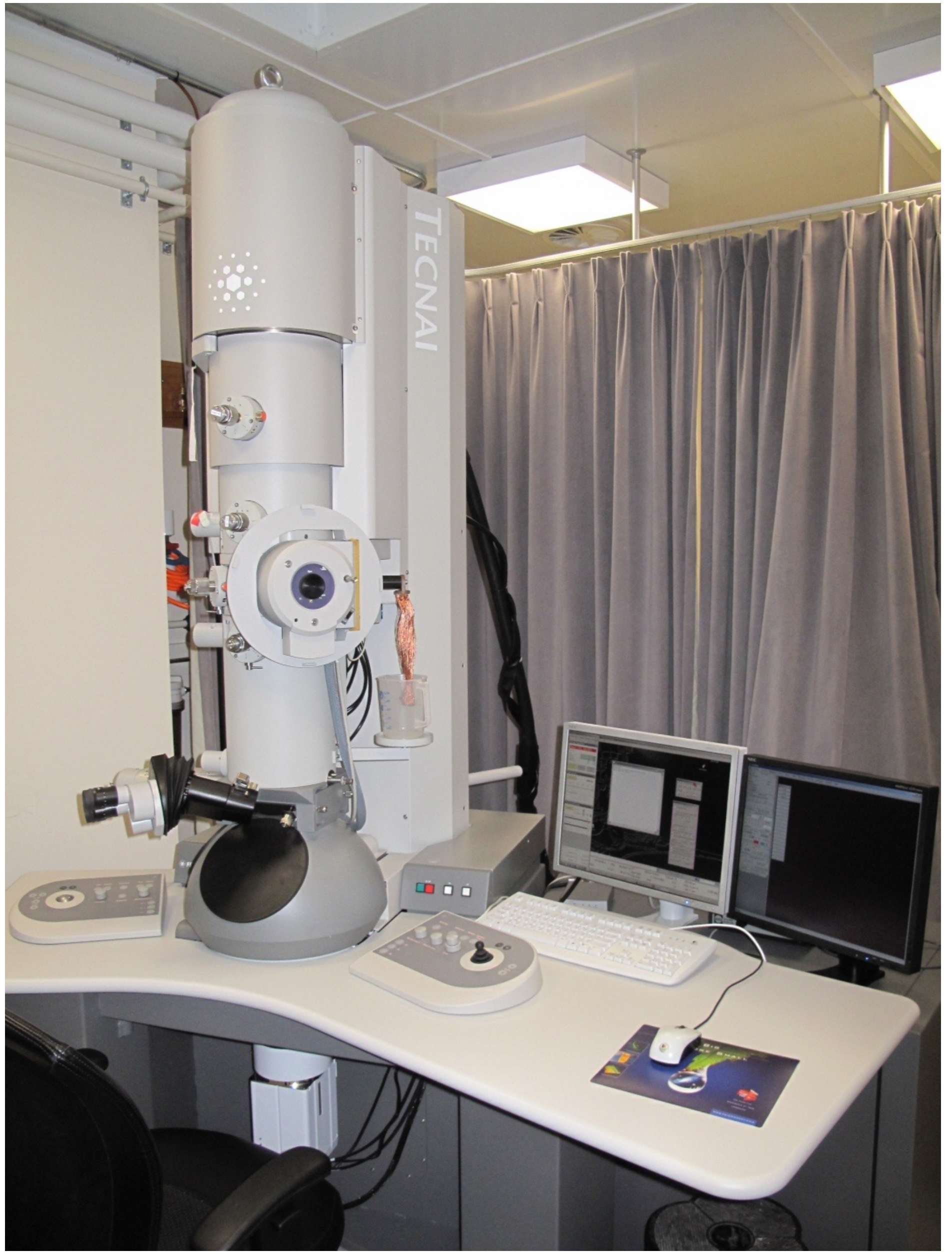
What has happened?

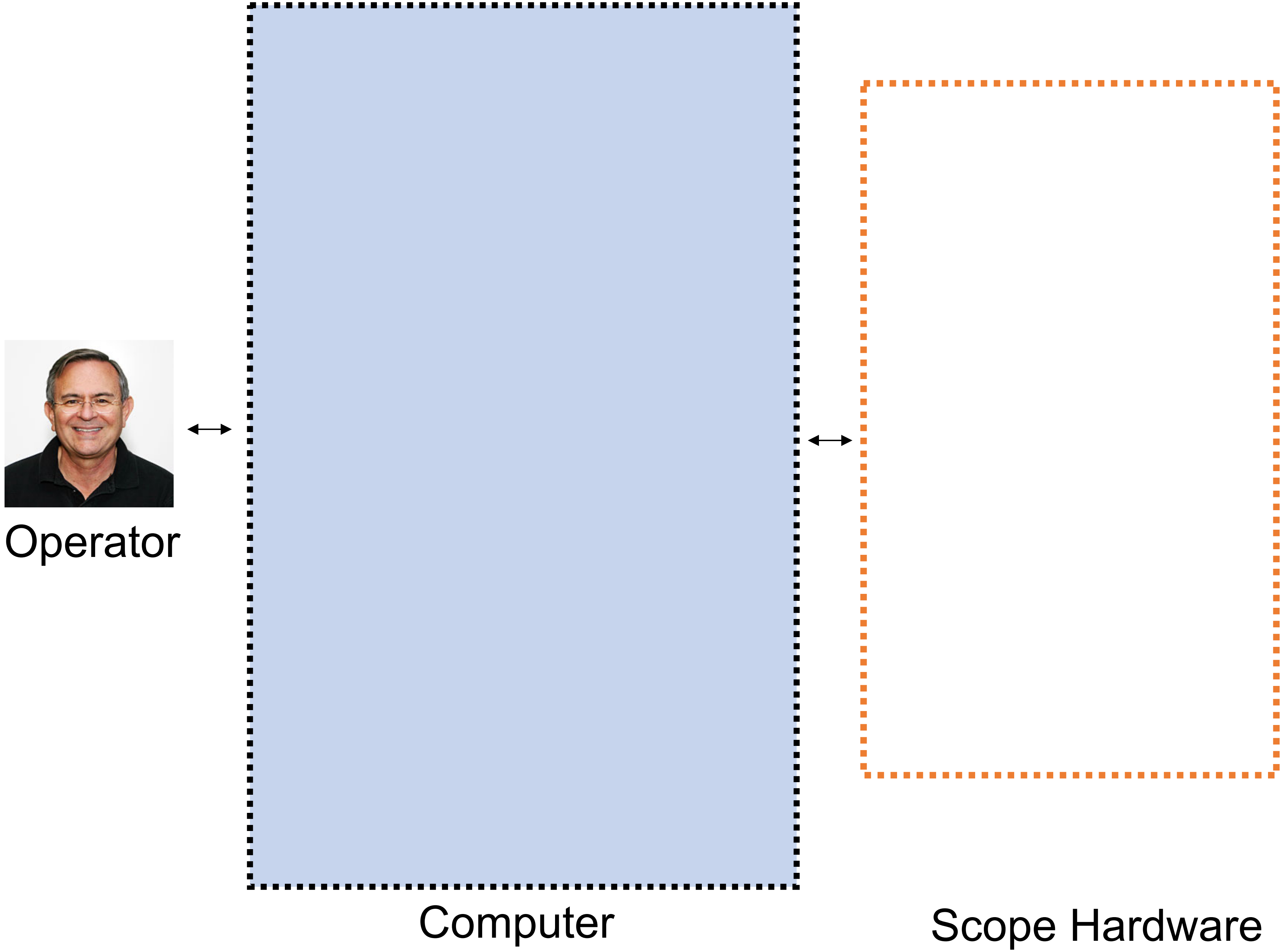
- (large-scale) Integrated Circuit
- Personal Computer
- Internet

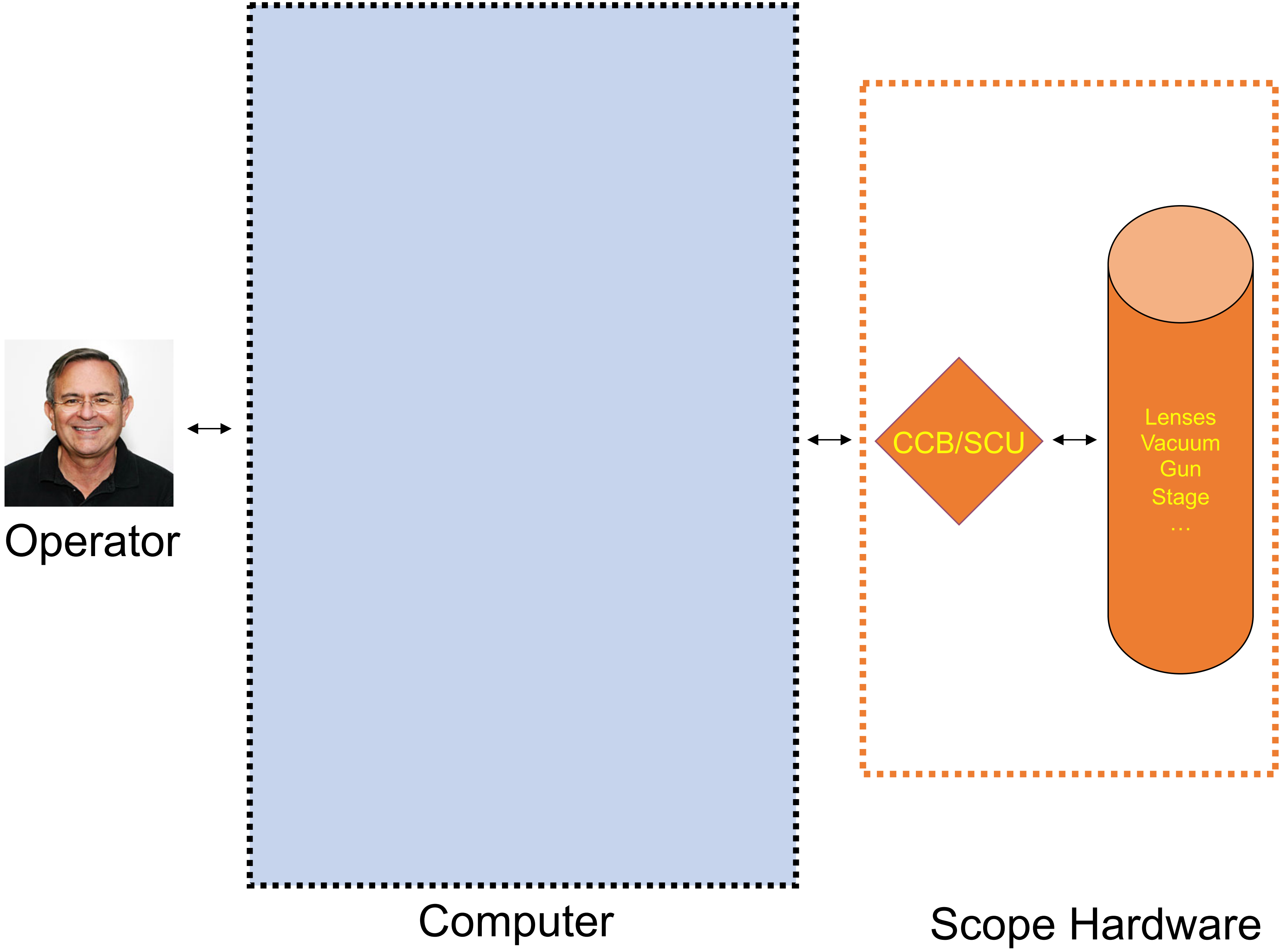


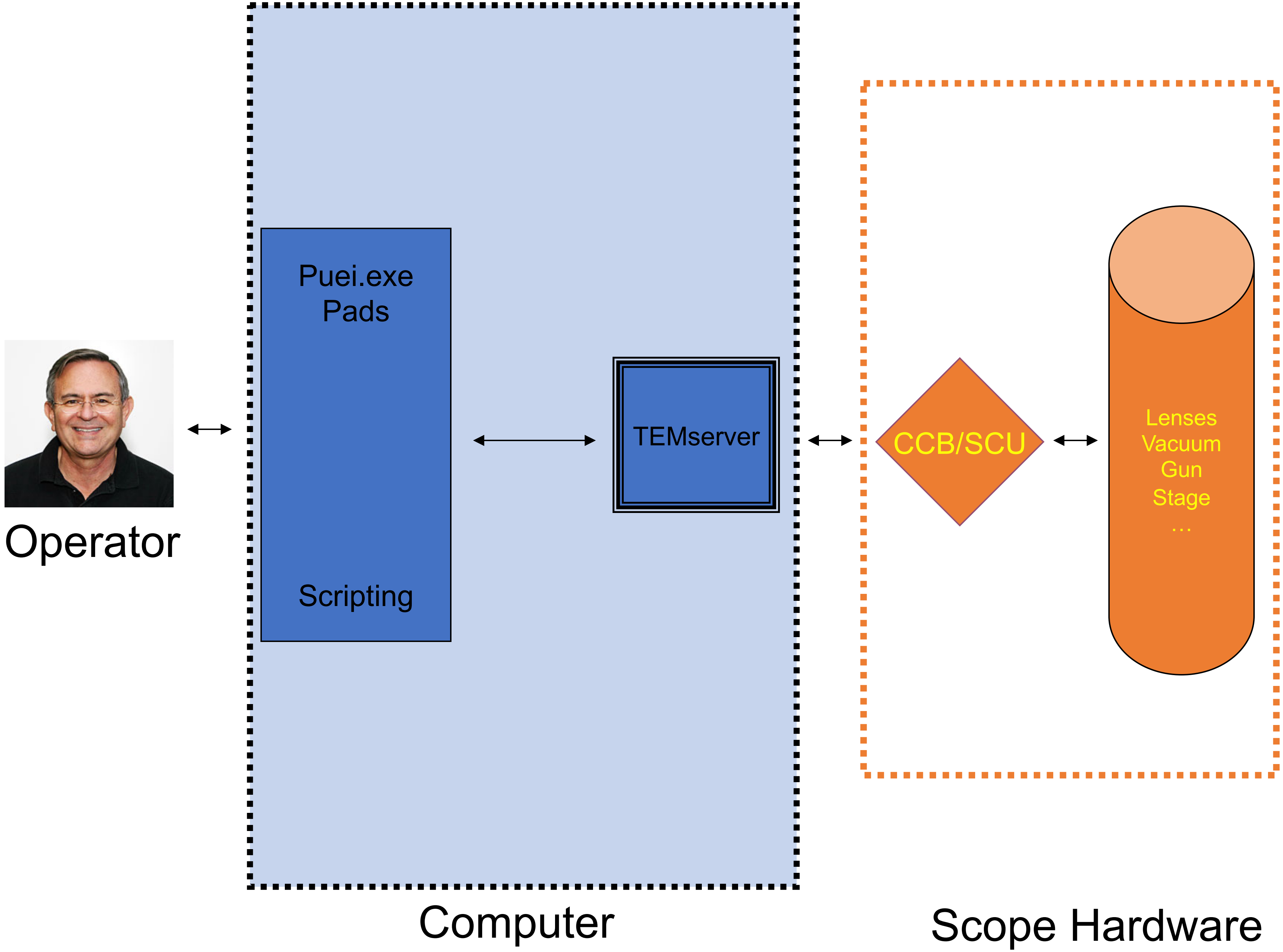
Operator ⟷ Computer ⟷ TEM Hardware

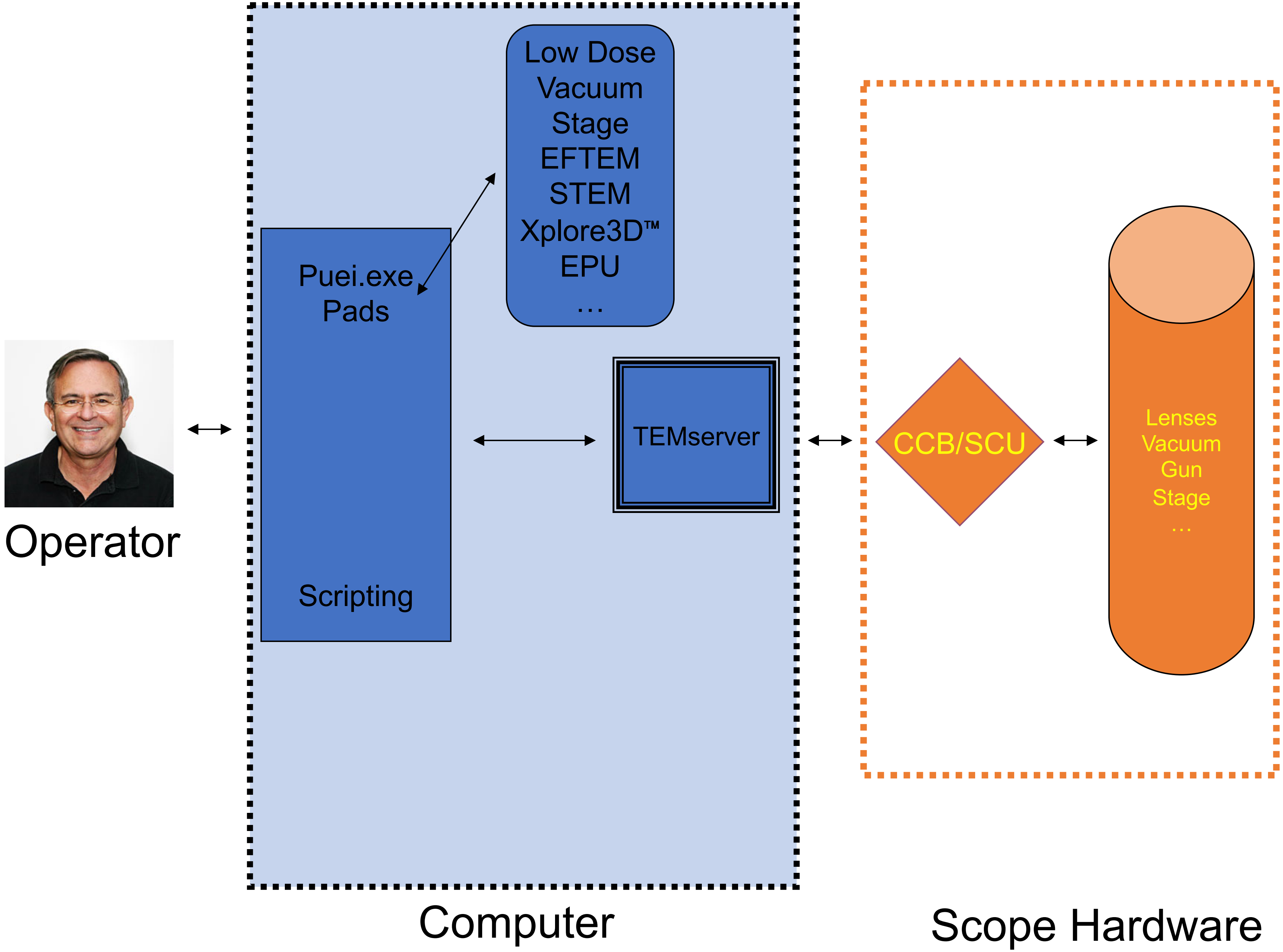
(Specimen Stage & holder, Apertures)

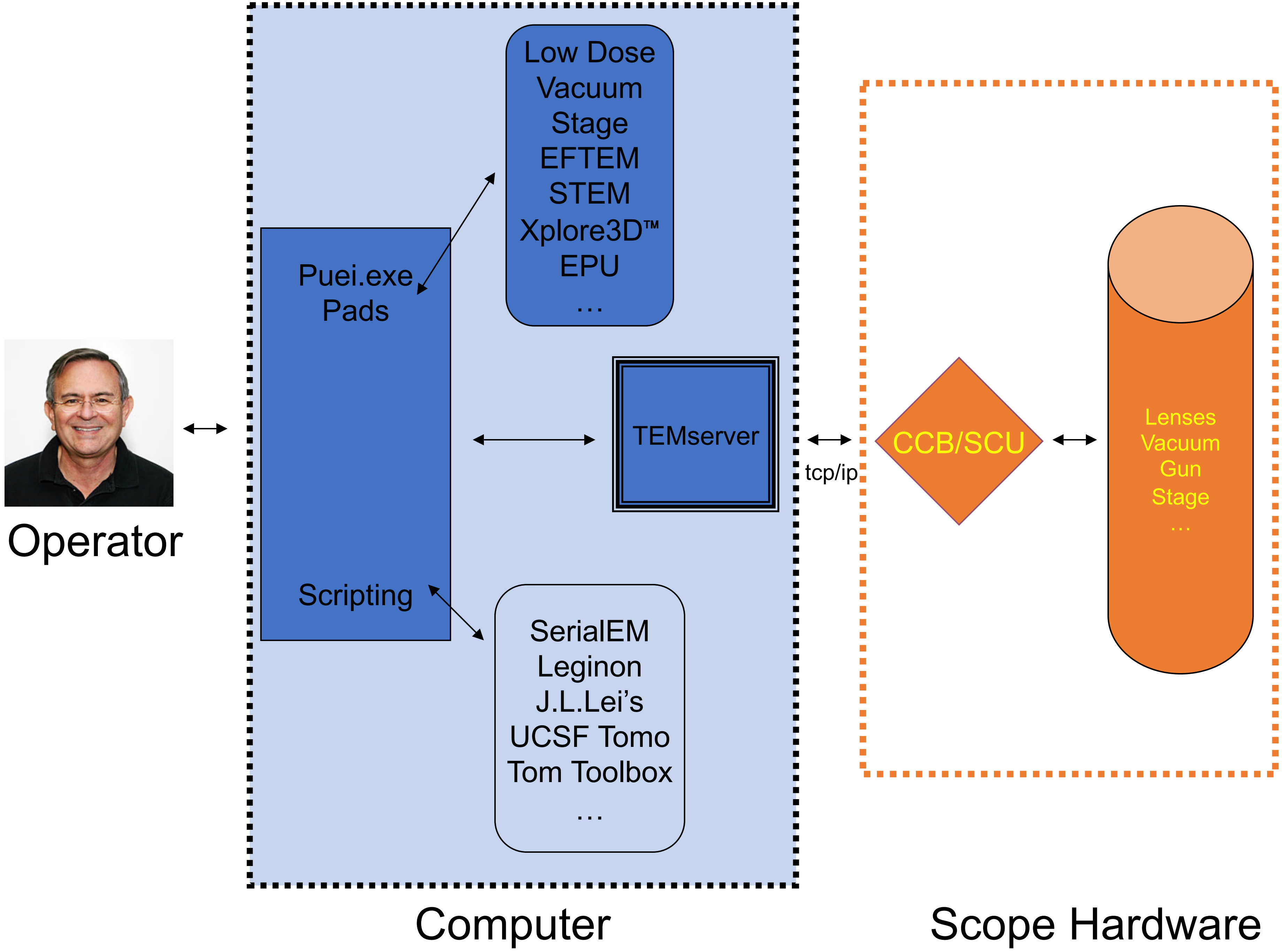

TECNAI


Operator
Computer
Scope Hardware


Operator
CCB/SCU
Lenses
Vacuum
Gun
Stage
…
Computer
Scope Hardware


Operator
Puei.exe
Pads
Scripting
TEMserver
Computer
CCB/SCU
Lenses
Vacuum
Gun
Stage
…
Scope Hardware


Low Dose
Vacuum
Stage
EFTEM
STEM
Xplore3D™
EPU
…
Puei.exe
Pads
Scripting
TEMserver
CCB/SCU
Lenses
Vacuum
Gun
Stage
…
Operator
Computer
Scope Hardware


Operator
Puei.exe
Pads
Scripting
Low Dose
Vacuum
Stage
EFTEM
STEM
Xplore3D™
EPU
…
TEMserver
SerialEM
Leginon
J.L.Lei's
UCSF Tomo
Tom Toolbox
…
tcp/ip
CCB/SCU
Lenses
Vacuum
Gun
Stage
…
Computer
Scope Hardware

Advanced Control

SerialEM
Leginon
J.L.Lei's
UCSF Tomo
Tom Toolbox
…

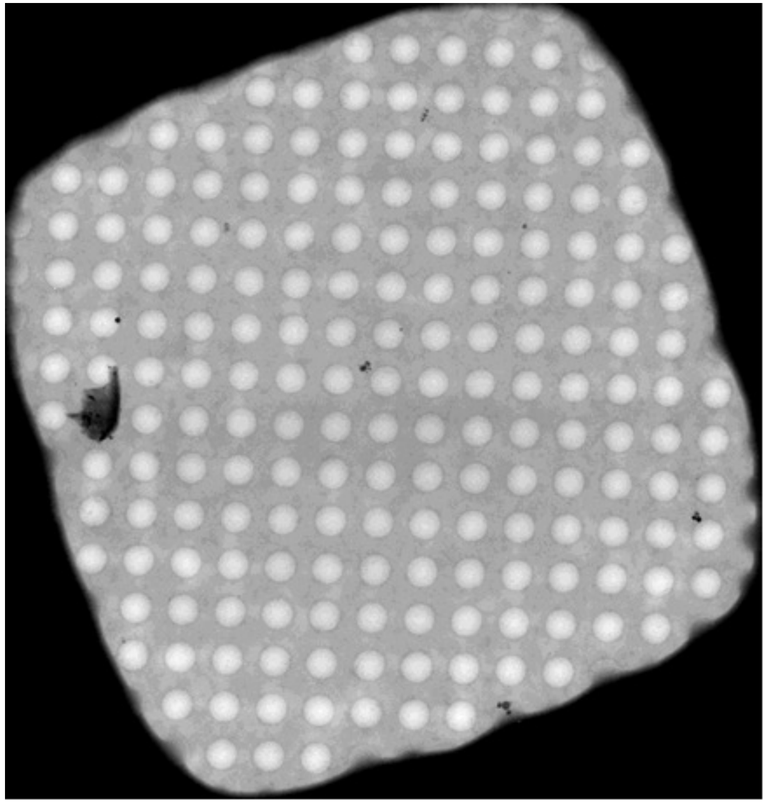
→

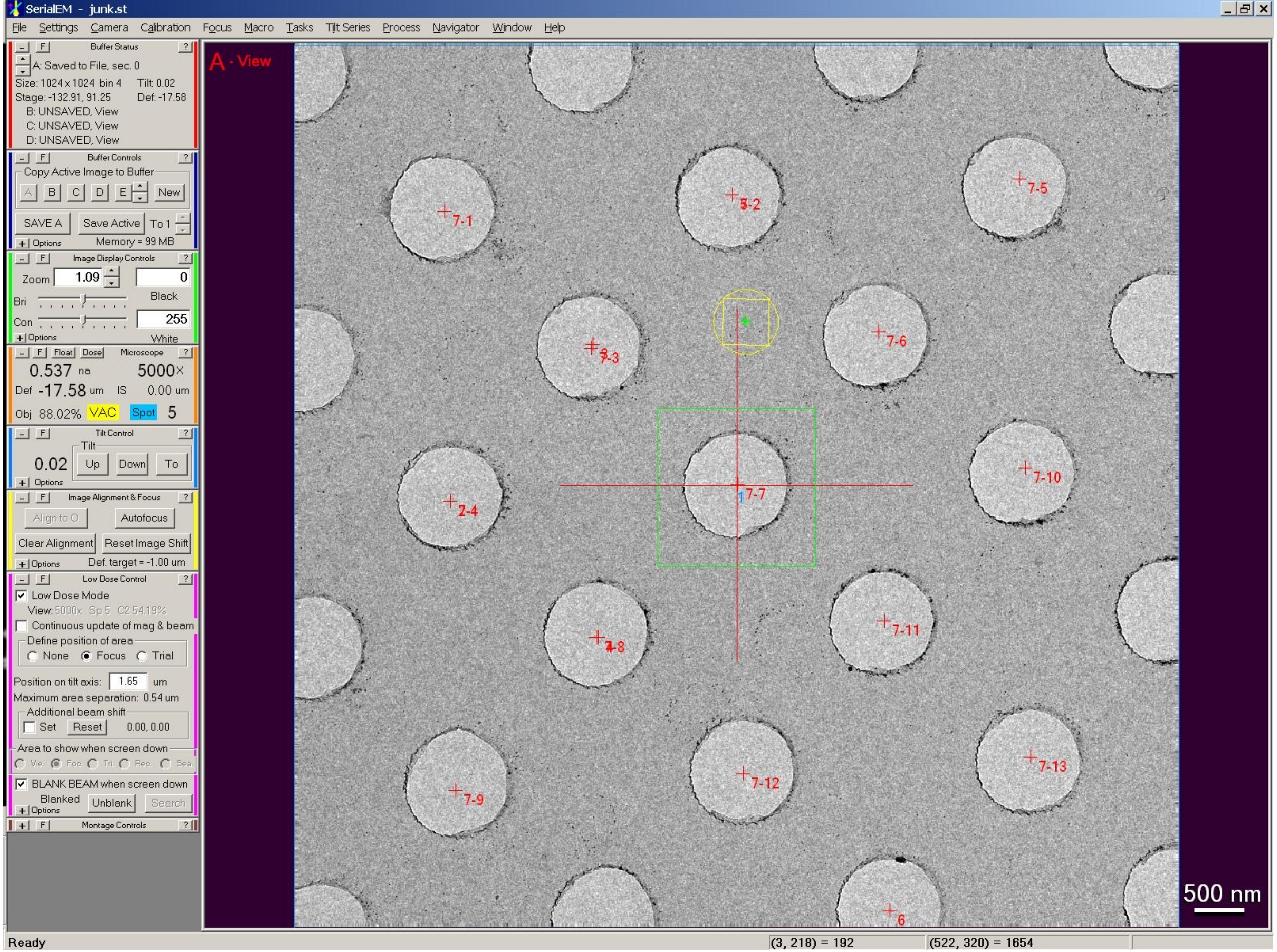
- Flexible control
- Automation
- Remote control
- ……

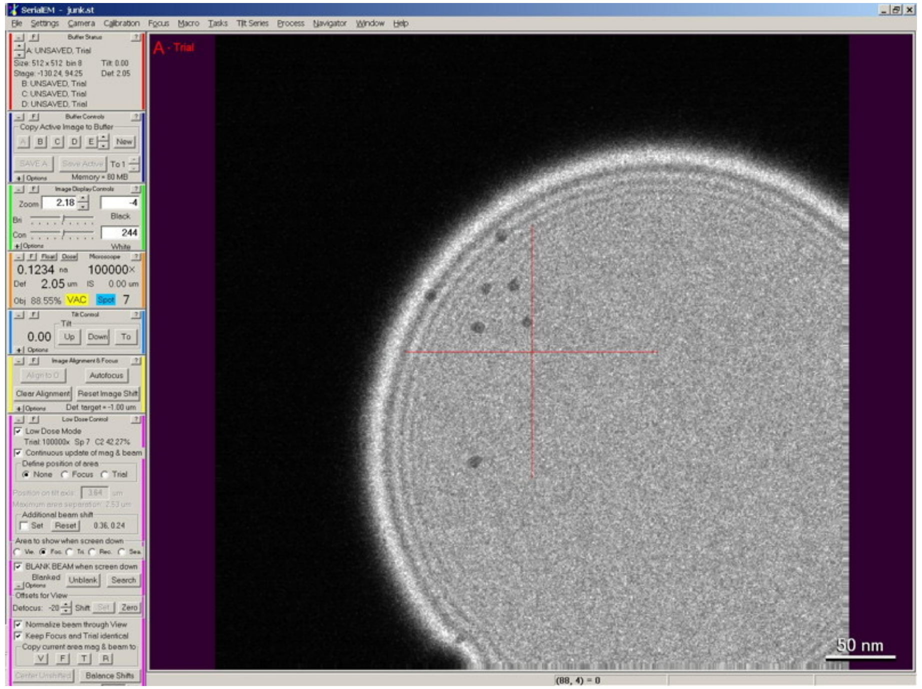
The Task of a TEM Operator

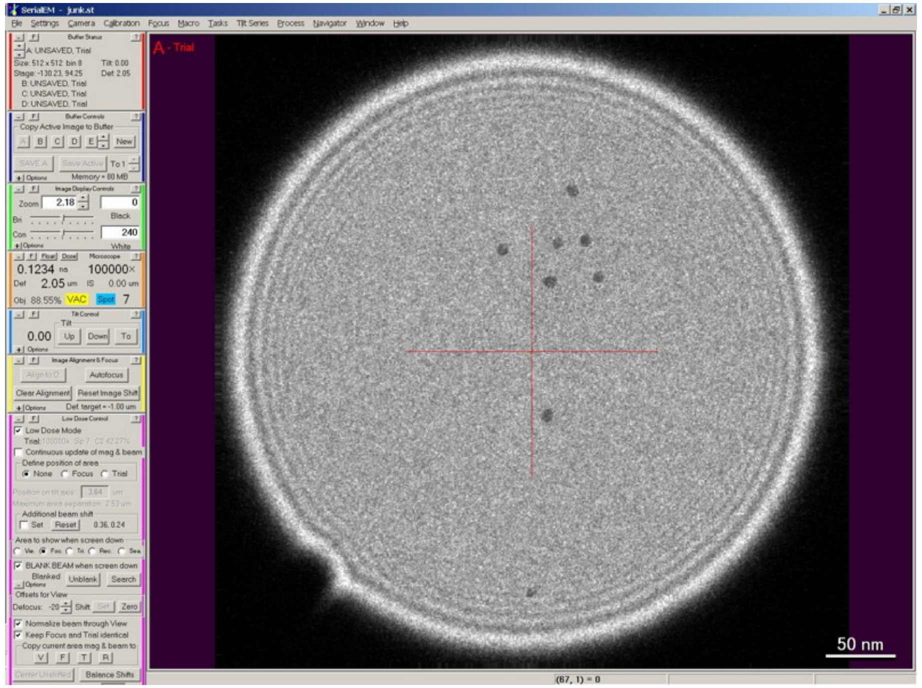
- *With the best beam conditions, then*

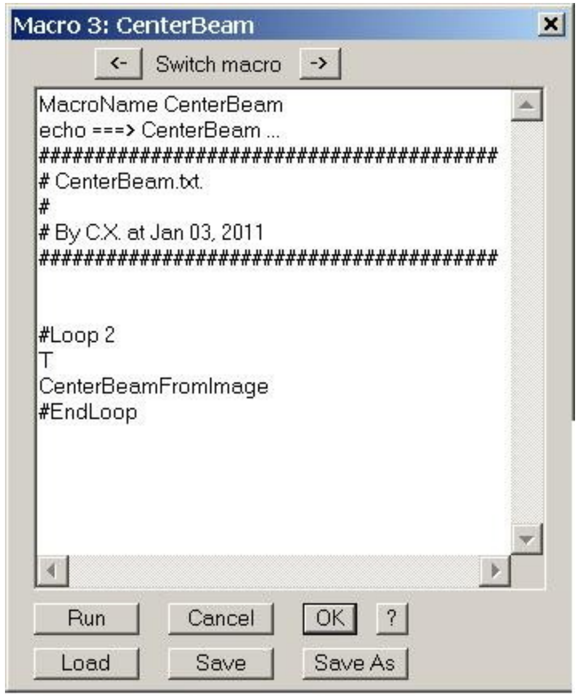
- Go to a spot - x,y,z,α,(β)
- Focus
- Record
- (repeat)




SerialEM - junk.st
File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help
Buffer Status
A: Saved to File, sec. 0
Size: 1024 x 1024 bin 4 Tilt: 0.02
Stage: -132.91, 91.25 Def: -17.58
B: UNSAVED, View
C: UNSAVED, View
D: UNSAVED, View
Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 99 MB
Image Display Controls
Zoom 1.09 0
Black
Bri
Con 255
Options White
Float Dose Microscope
0.537 na 5000x
Def -17.58 um IS 0.00 um
Obj 88.02% VAC Spot 5
Tilt Control
Tilt
0.02 Up Down To
Options
Image Alignment & Focus
Align to 0 Autofocus
Clear Alignment Reset Image Shift
Options Def. target = -1.00 um
Low Dose Control
Low Dose Mode
View: 5000x Sp 5 C2 54.19%
Continuous update of mag & beam
Define position of area
None Focus Trial
Position on tilt axis: 1.65 um
Maximum area separation: 0.54 um
Additional beam shift
Set Reset 0.00, 0.00
Area to show when screen down
Vie. Foc. Tri. Rec. Sea.
BLANK BEAM when screen down
Blanked Unblank Search
Options
Montage Controls
A - View
7-1
5-2
7-5
3-3
7-6
7-7
7-10
2-4
7-11
4-8
7-9
7-12
7-13
6
500 nm
Ready
(3, 218) = 192
(522, 320) = 1654


SerialEM - junk.st
A - Trial
50 nm

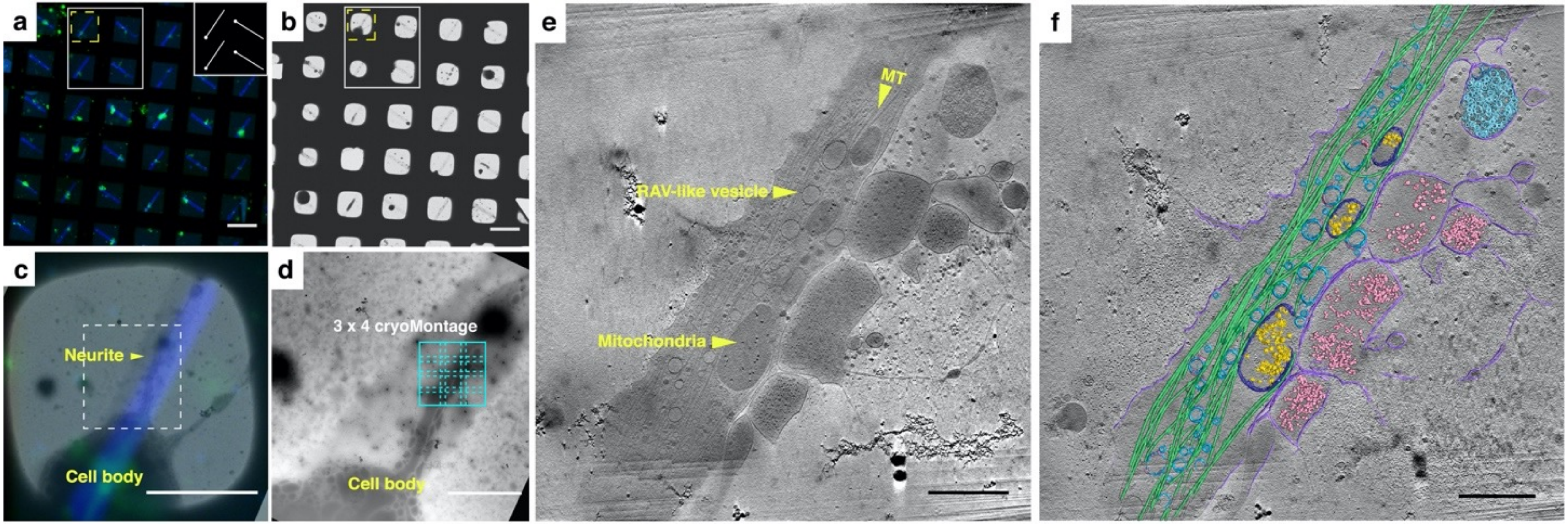

SerialEM - junk.st
A - Trial
50 nm


Macro 3: CenterBeam
<- Switch macro ->
MacroName CenterBeam
echo ===> CenterBeam ...
#######################################
CenterBeam.txt.
#
By C.X. at Jan 03, 2011
#######################################
#Loop 2
T
CenterBeamFromImage
#EndLoop
Run
Cancel
OK
?
Load
Save
Save As

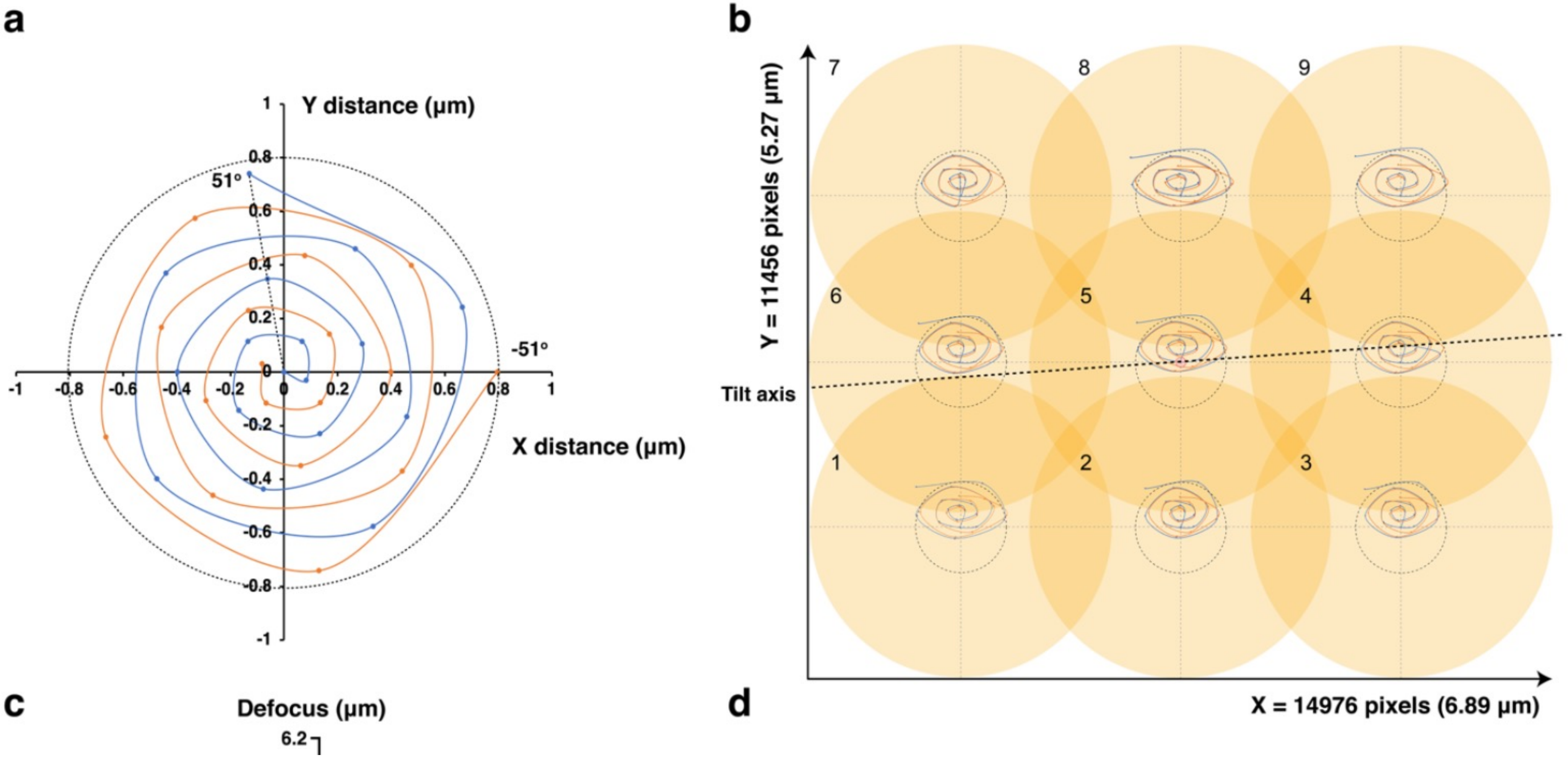
```
MacroName RT
# at 0
G
G
R
S

WalkUpTo 45

#at 45
G
G
R
S

TiltTo 0
```

```
MacroName RT
################################
# RT.txt
#
# by Chen Xu @ Dec. 23, 2010
################################
#
# A main macro for Rondom Contical Tilt (RT)
# data collection for a single point picked
#

#===========
# set angles
#===========
ang_1 = 0
ang_2 = 45

ResetClock

#========================================
# start with 0, in case last one exits at non-0 angle
#========================================
TiltTo 0

#========================
# Center Beam using T beam
#========================
Call CenterBeam

#===============
# collect tilting pairs
#===============
Loop 2 no
if $no == 1
   ang = $ang_1
else
   ang = $ang_2
endif
WalkUpTo $ang
ResetImageShift
Call Z_byG
Call Focus
Call Drift_new
Call Shot
EndLoop

#============
# back to 0
#============
TiltTo 0

ReportClock
#=== end ===
echo --------------------------------------------------
```

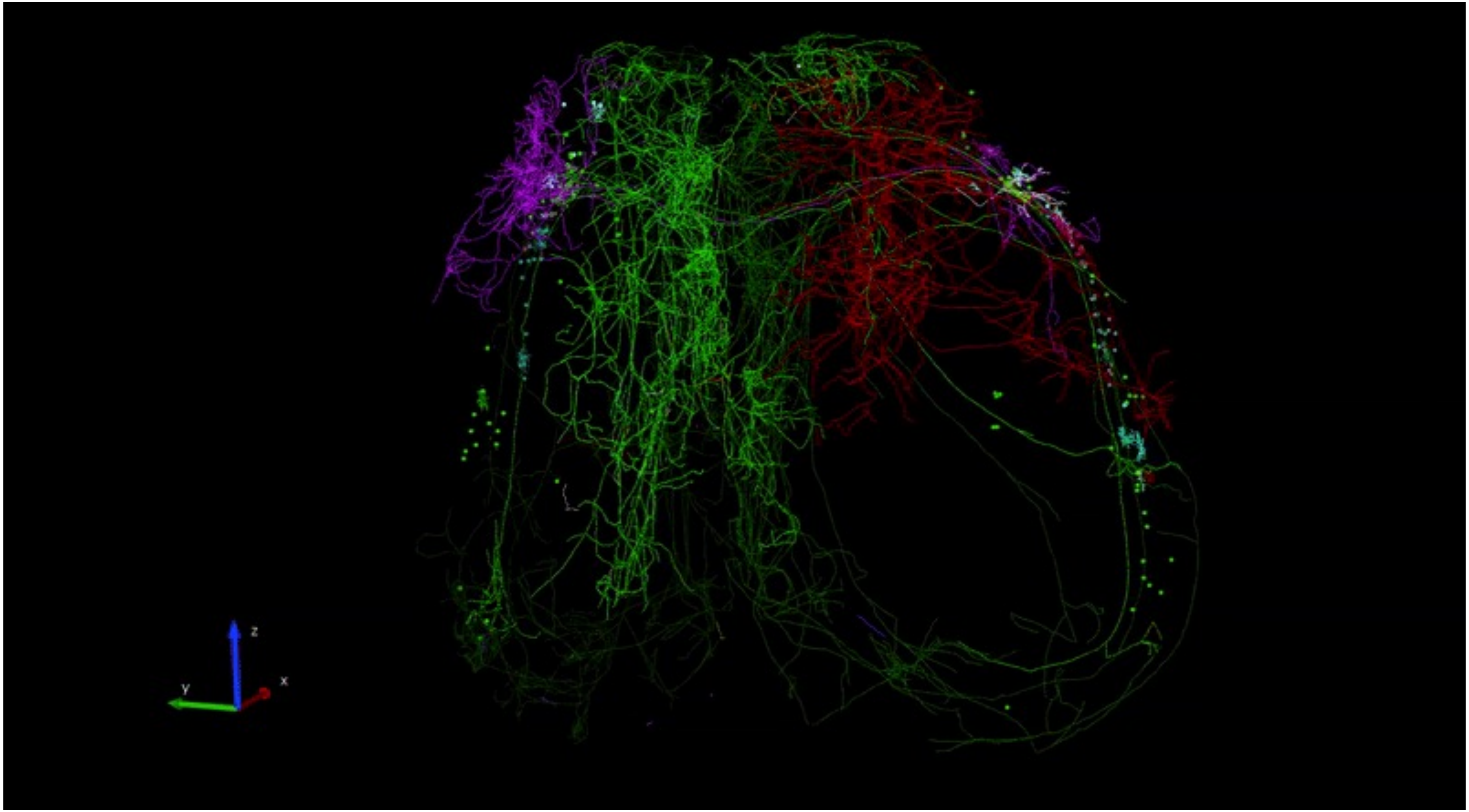
Advanced Control – an example



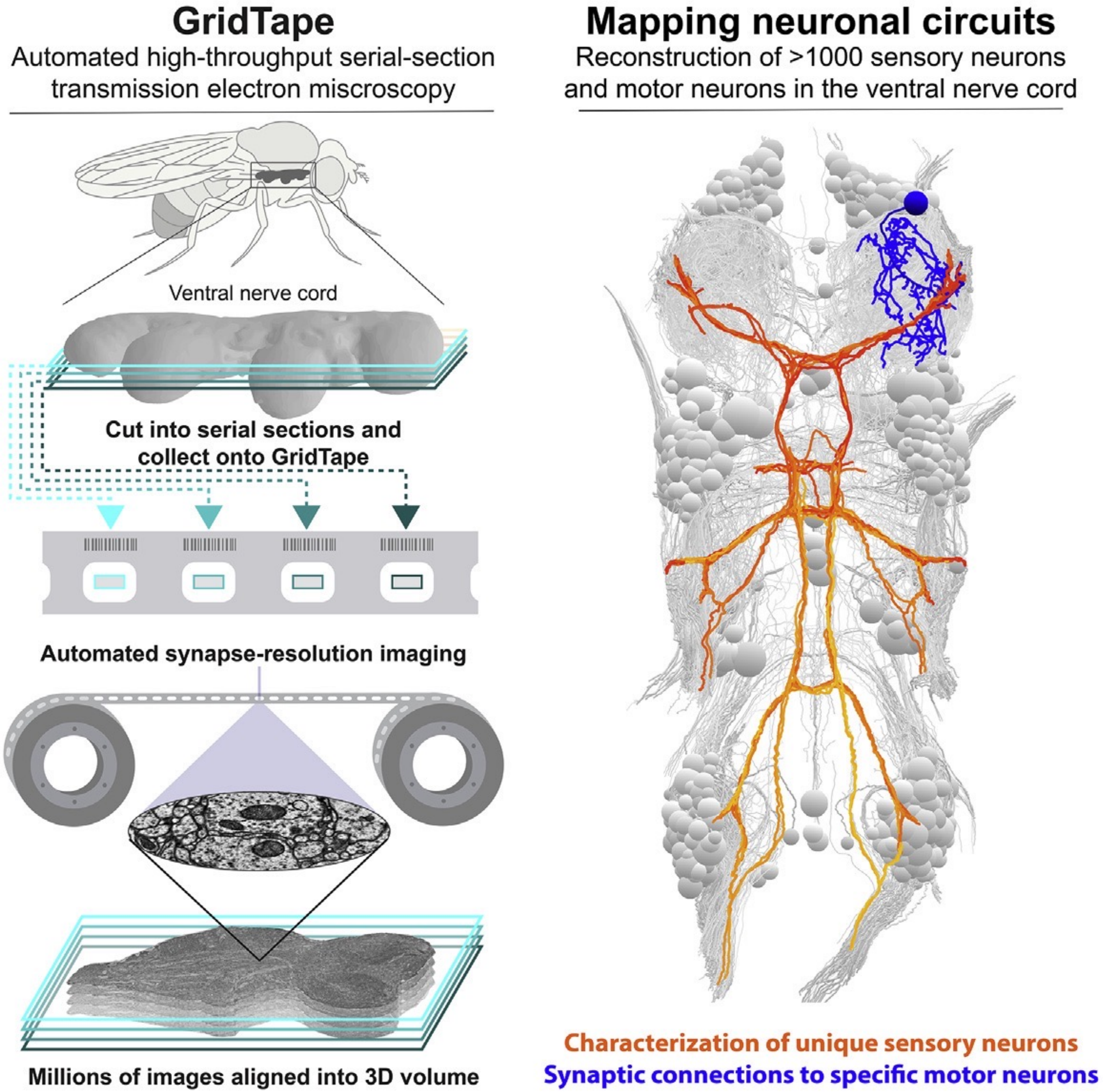
Jie E. Yang et al, "**Correlative cryogenic montage electron tomography for comprehensive in-situ whole-cell structural studies** ", (2022) bioRxiv preprint doi: https://doi.org/10.1101/2021.12.31.474669


a
Y distance (µm)
X distance (µm)
51°
-51°
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
b
Y = 11456 pixels (5.27 µm)
X = 14976 pixels (6.89 µm)
Tilt axis
7
8
9
6
5
4
1
2
3
c
Defocus (µm)
6.2
d

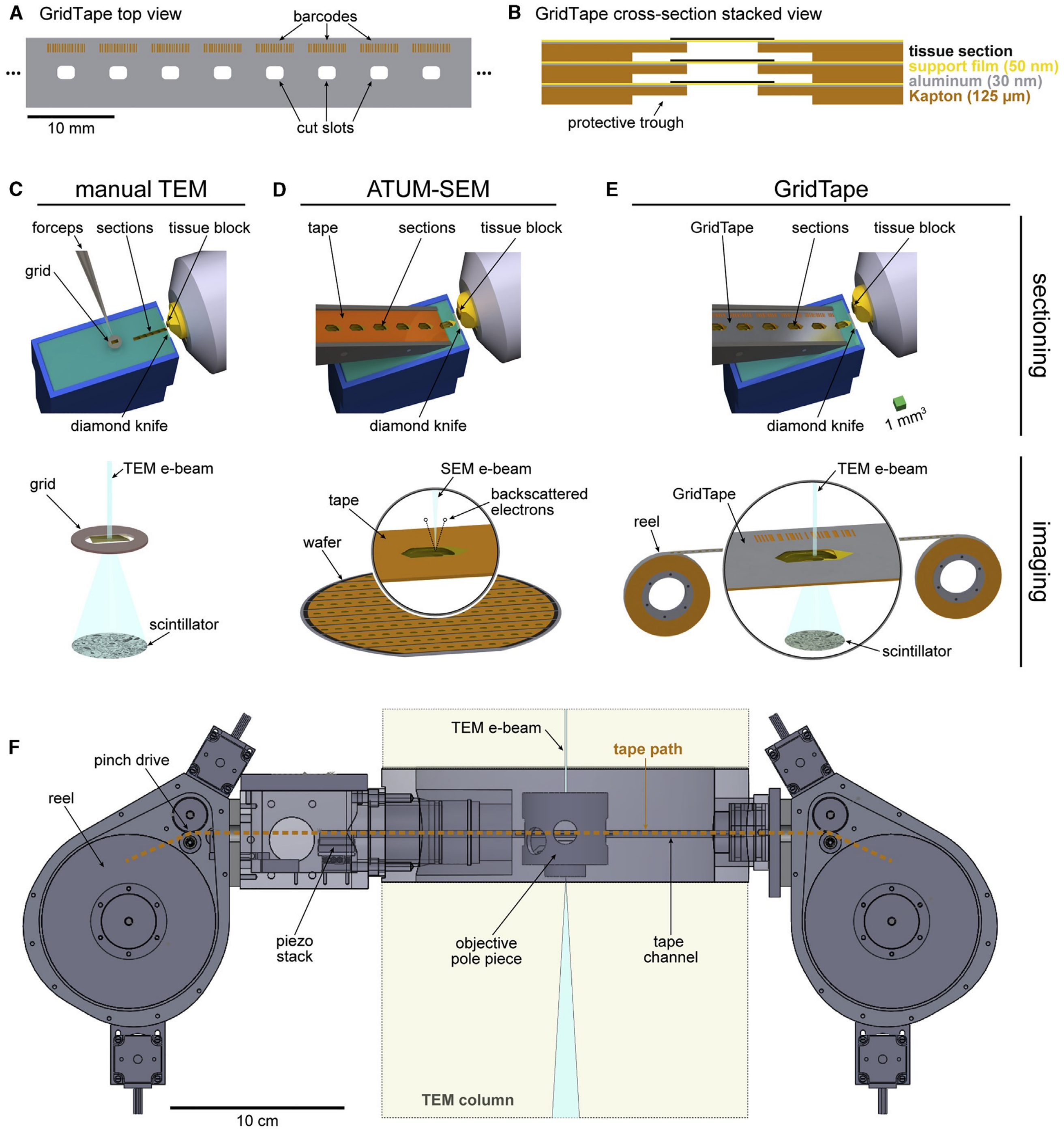
High-throughput, Large-scale Volume EM



https://alleninstitute.org/what-we-do/brain-science/news-press/articles/now-stunning-whole-brain-resolution-neurons

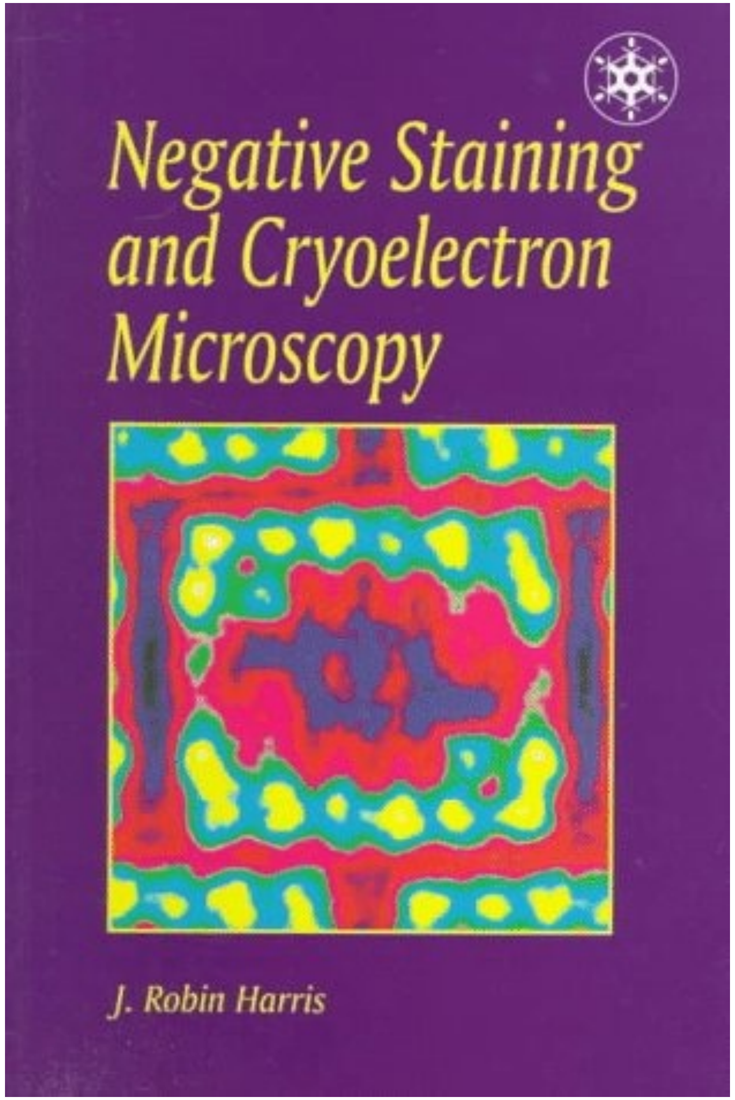


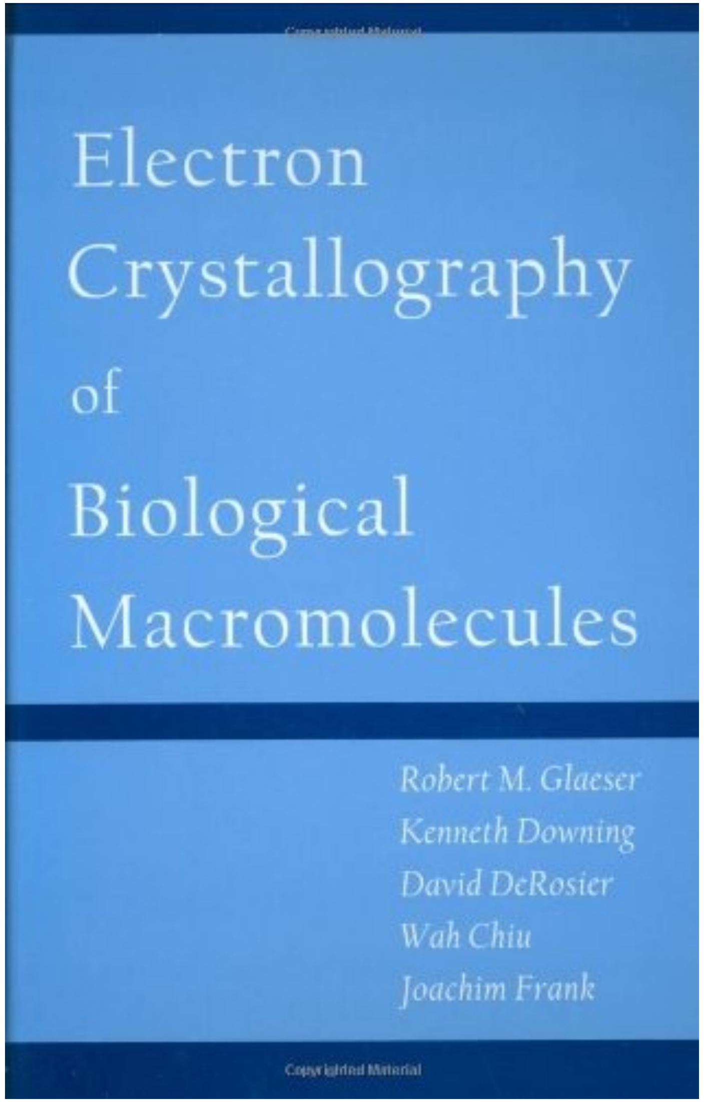
Jasper S. Phelps et al, Cell *184*, 759, 2021



Jasper S. Phelps et al, Cell *184*, 759, 2021

Two Books that I like





THANK YOU