



CINA
Cellular Imaging
and Nano Analytics

SpotScan TEM vs. STEM

Henning Stahlberg

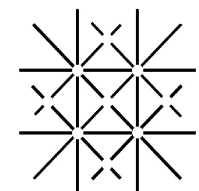
Center for Cellular Imaging and NanoAnalytics (C-CINA)

Biozentrum, University Basel

Switzerland

BIOZENTRUM

Universität Basel
The Center for Molecular Life Sciences



Uni Basel

3:15 – 5:00 Additional High-End Instrumentation Forum

Bob Glaeser, Discussion Leader

3:15 – 3:30 Holger Stark

3:35 – 3:50 Henning Stahlberg

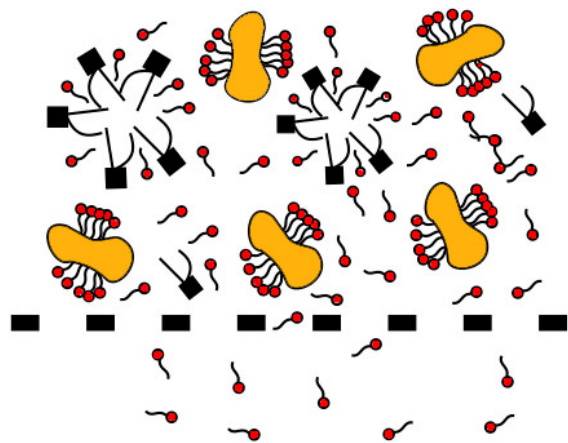
3:55 – 4:10 Bob Glaeser

4:15 – 4:30 Q&A on topics of interest that were not covered by the speakers

The session will start with three talks on selected topics about not-yet-conventional, “frontier instrumentation” that currently is in an early stage of development and/or characterization. Topics may be selected from: 80kV vs 300kV scopes, STEM vs CTEM, brighter guns (e.g. X-FEG), wet-specimen holders, dynamic TEM, correctors, condenser zoom (parallel illumination), phase plates, FIB, cryo-sectioning, sample-spotter, what else? These additional features are expensive! Speakers are charged to give a critical review of:

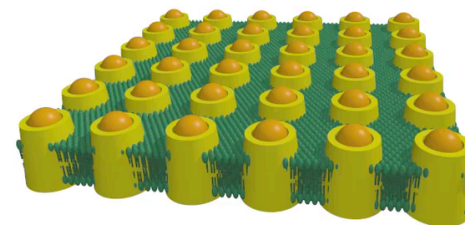
Are they worth it? What do they get us? What does the future hold?

Membrane Protein 2D Crystallization by Dialysis

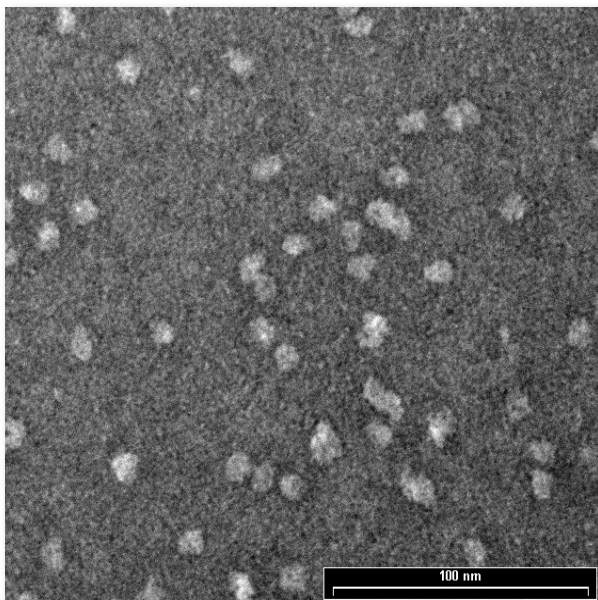


Detergent removal

2D crystal formation



2D crystal



Fourier Transform of a 2D crystal image

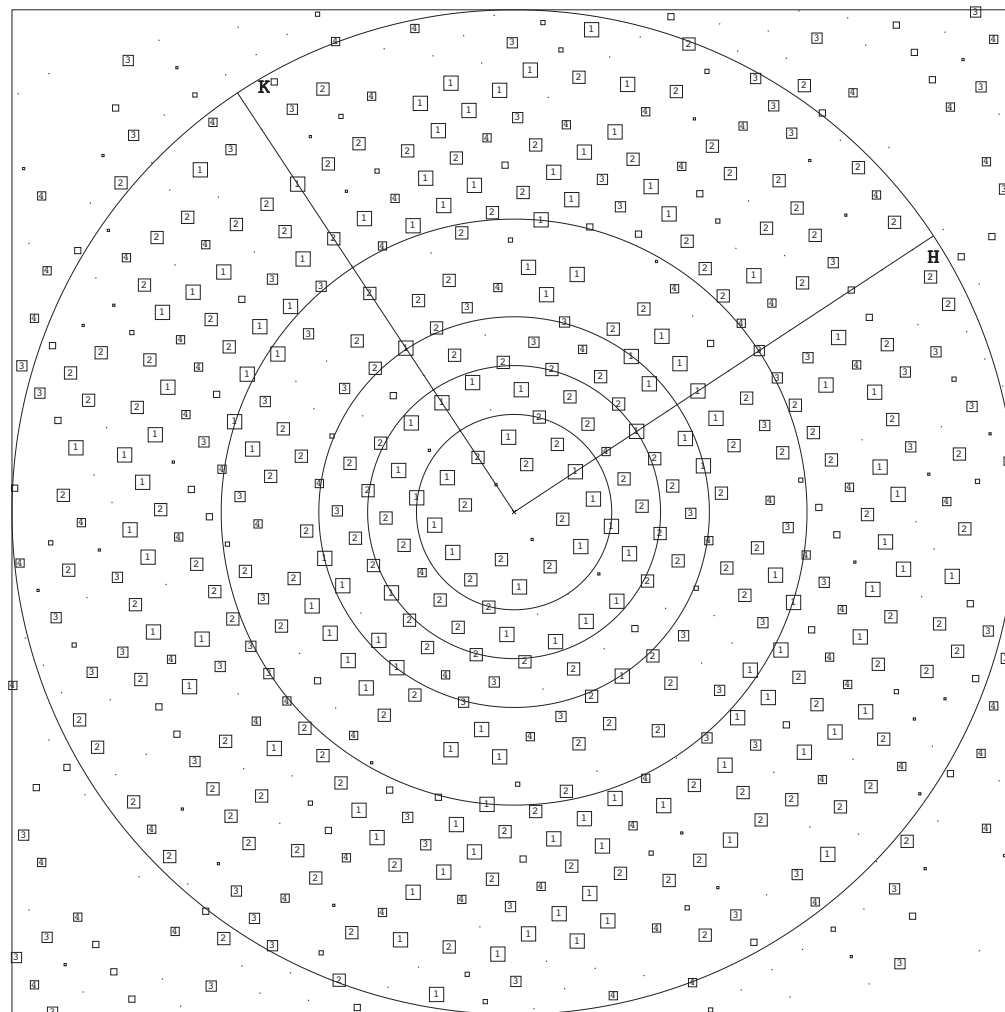
Tilt Angle: 0°



IQ plot (\sim FFT) of a non-tilted 2D crystal

Resolution Ring at 36.00 A
Resolution Ring at 24.00 A
Resolution Ring at 18.00 A
Resolution Ring at 12.00 A
Resolution Ring at 7.00 A

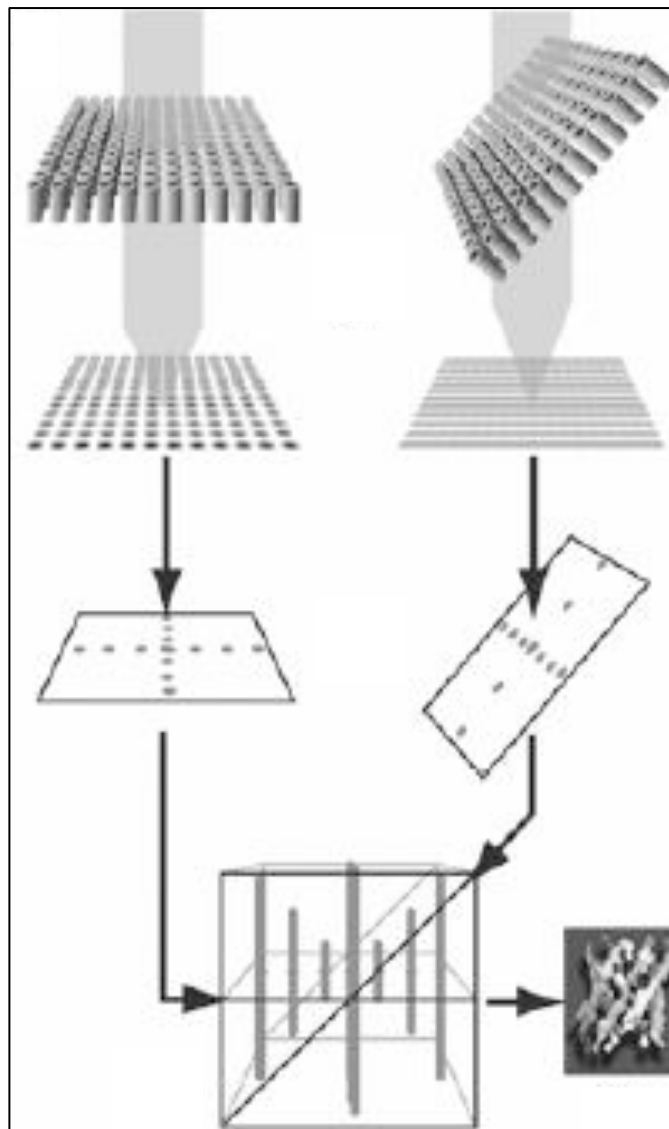
Right-handed lattice



Plot is based on measured reciprocal lattice: $U = 99.758, 65.688$ $V = -58.685, 88.929$
Plot shows the measured data.

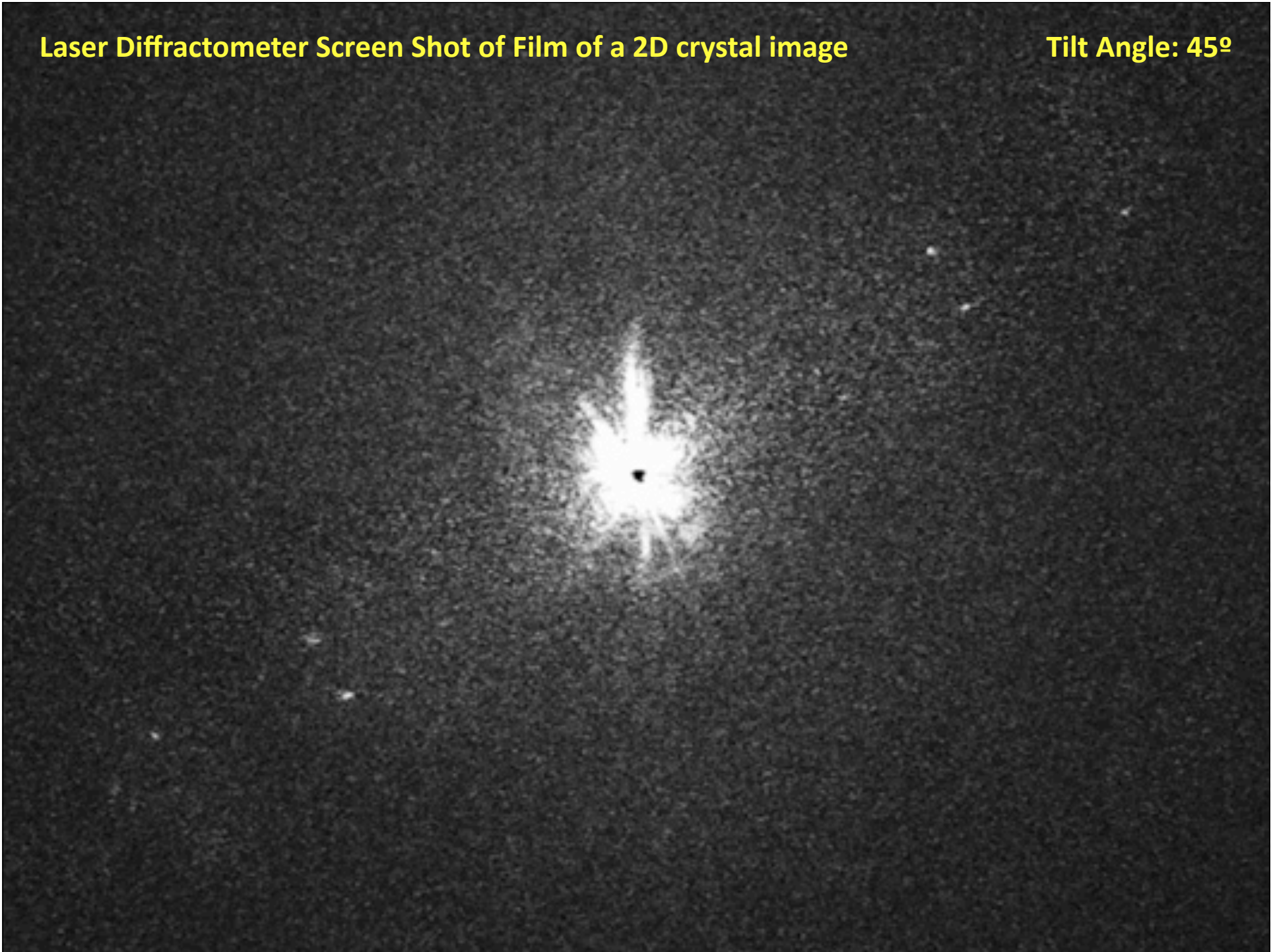
Electron crystallography: **The third dimension**

Tilting: Filling the Fourier space



Laser Diffractometer Screen Shot of Film of a 2D crystal image

Tilt Angle: 45°



Laser Diffractometer Screen Shot of Film of a 2D crystal image

Tilt Angle: 45°

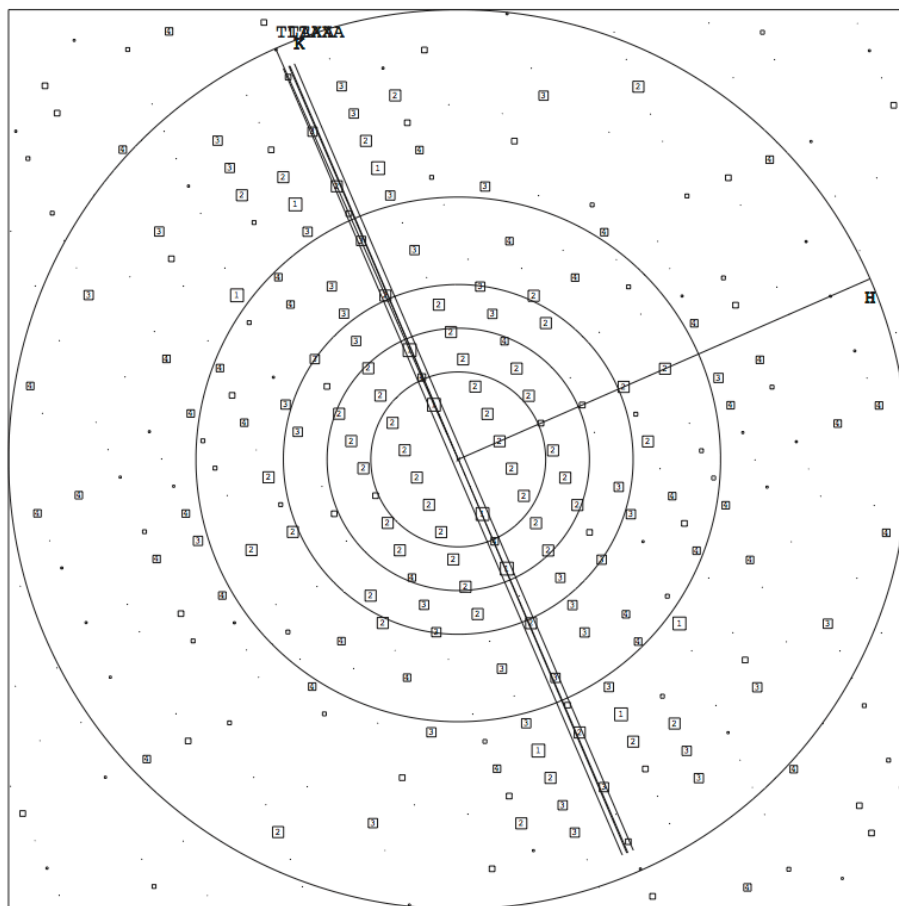


IQ plots (\sim FFT) of tilted 2D crystals

(Beam-induced resolution loss in the direction perpendicular to the tilt axis is visible)

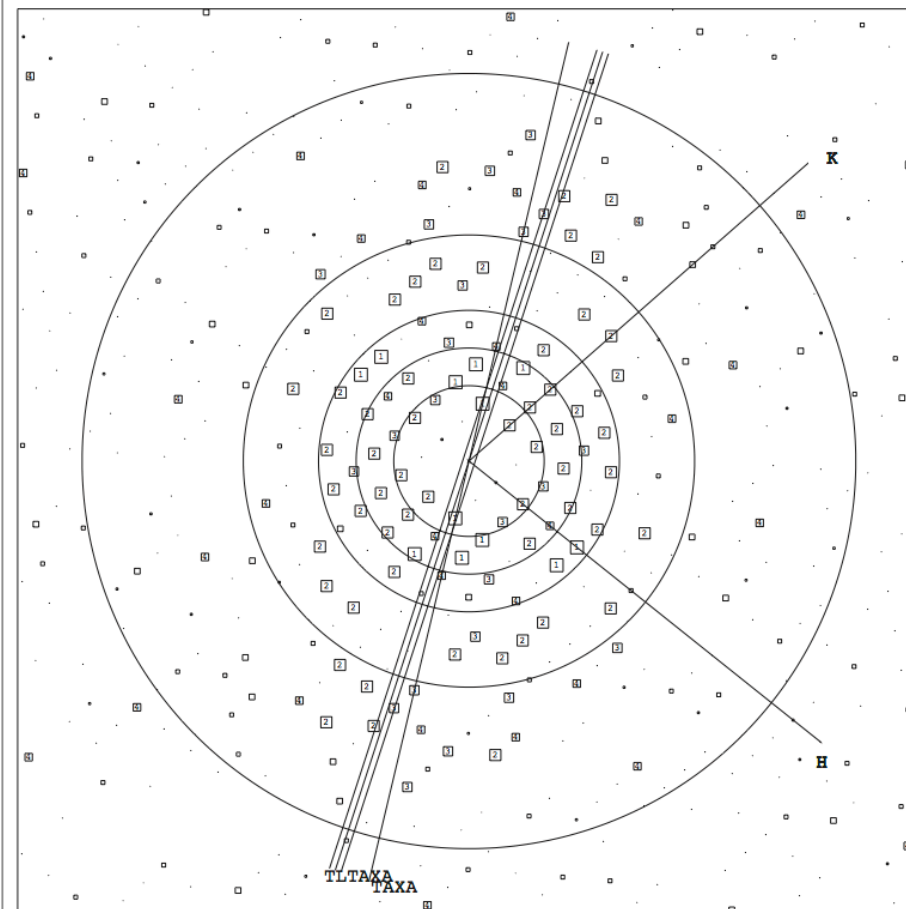
45 degree tilt

Resolution Ring at	36.00 A	TAXA = -89.520
Resolution Ring at	24.00 A	TLTAXA= -89.649
Resolution Ring at	18.00 A	TANGL = 43.043
Resolution Ring at	12.00 A	
Resolution Ring at	7.00 A	Right-handed lattice



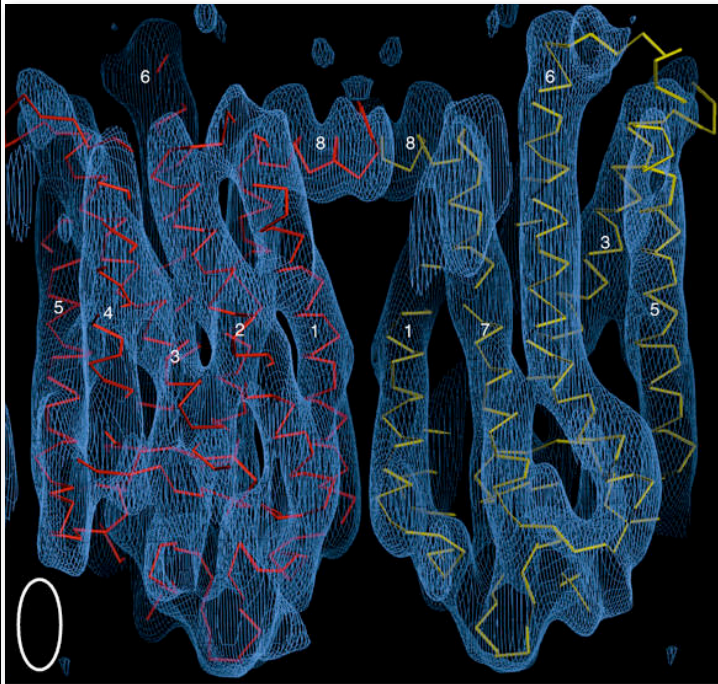
40 degree tilt

Resolution Ring at	36.00 A	TAXA = 64.834
Resolution Ring at	24.00 A	TLTAXA= 69.553
Resolution Ring at	18.00 A	TANGL = 37.483
Resolution Ring at	12.00 A	
Resolution Ring at	7.00 A	Right-handed lattice



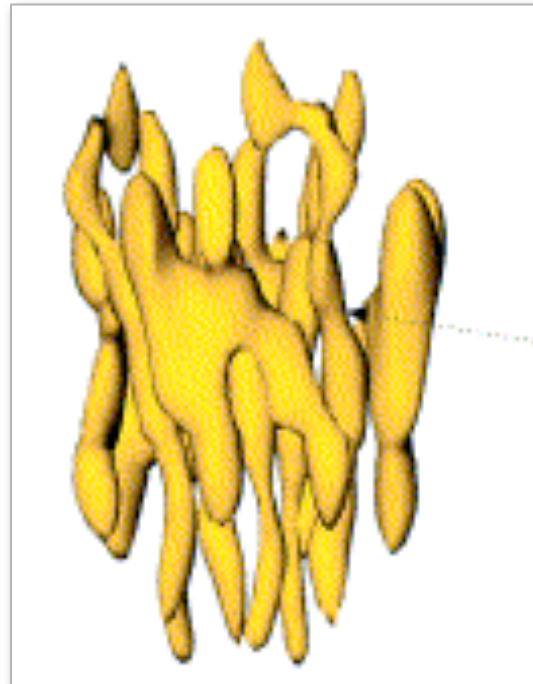
The third dimension: $x,y / z$ Anisotropy

Metarhodopsin: 5Å vs. 12Å



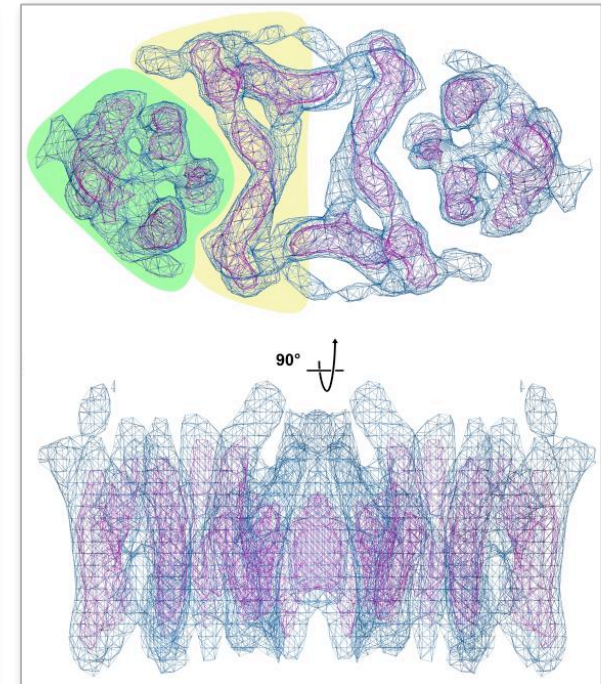
Ruprecht *et al* (2004), *EMBO J.* 23 (18), 3609-3620

Glutathion Trans.: 5Å vs. 15Å



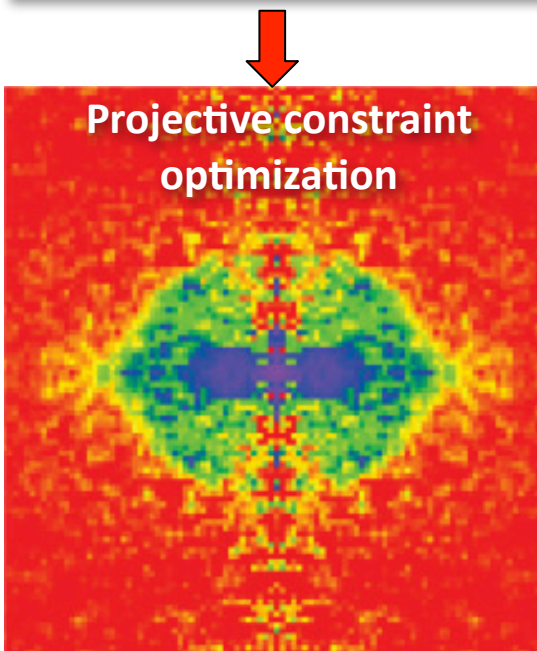
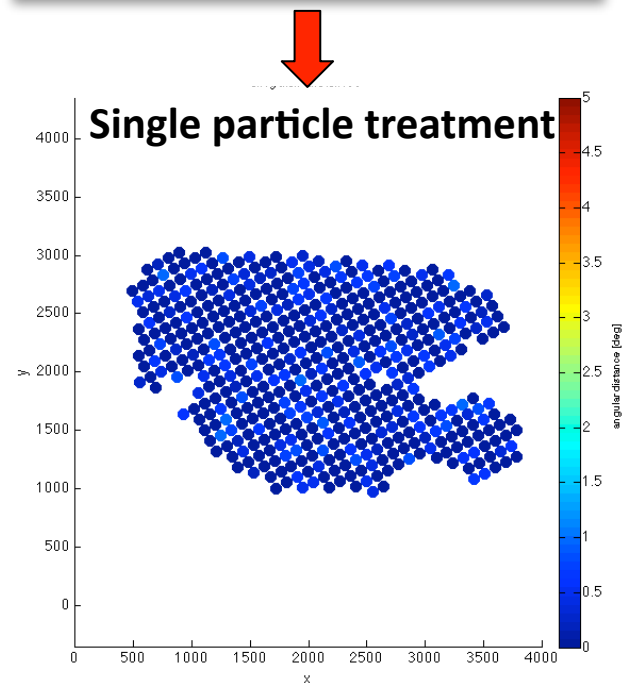
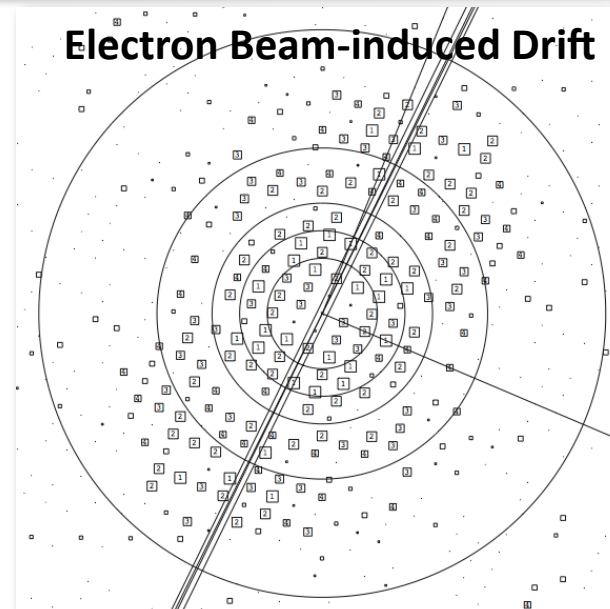
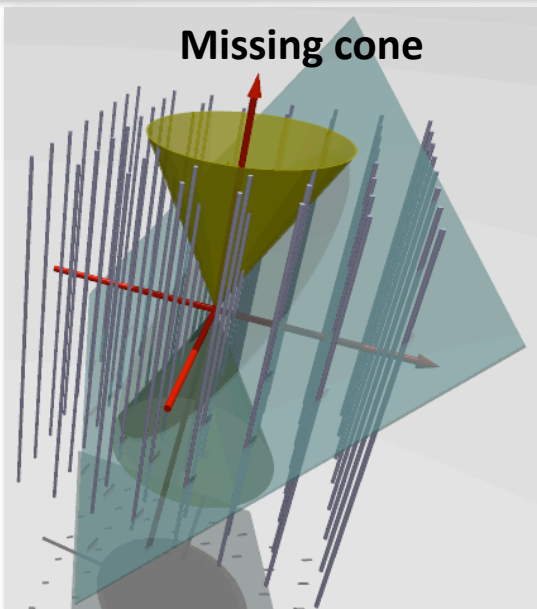
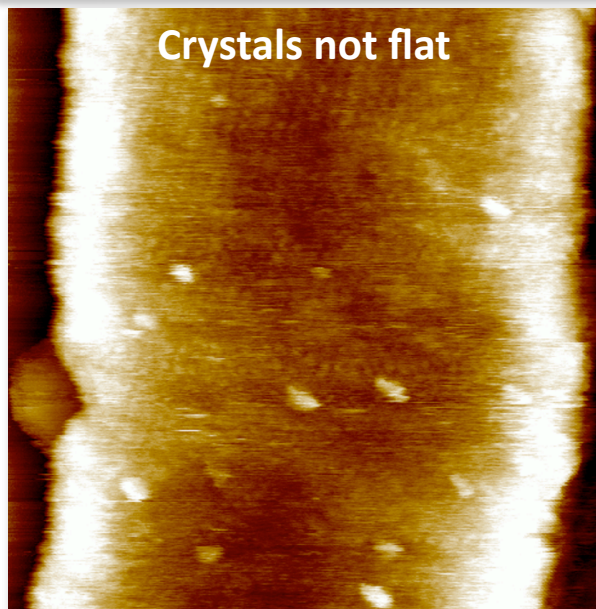
Holm *et al* (2002), *Biochim. Biophys. Act.* 1594, 276-85

NhAP1: 6Å vs. 15Å

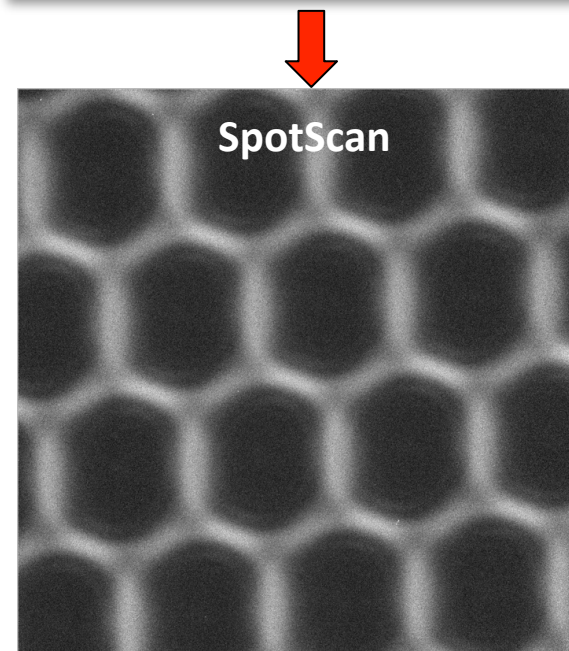


Goswami *et al* (2011), *EMBO J.* 30(2) 439-449

x,y / z Anisotropy: Reasons & Solutions



Gipson et al *Phys Rev.E* 84 011916 (2011)

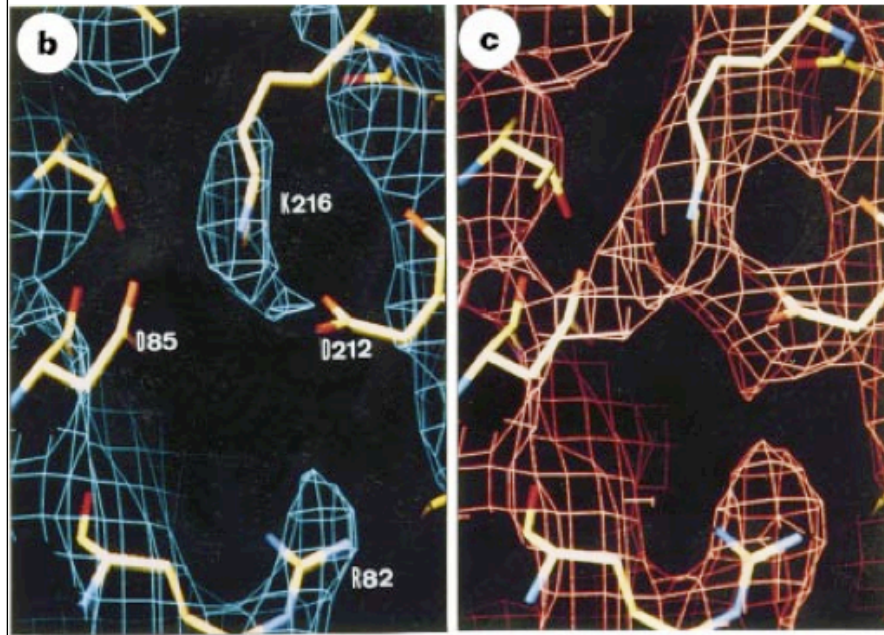


Downing, *Science* 251(4989), 53-59 (1991)

Projective Constraint Optimization

Overlaid atomic model: IBRR
Essen et al 1998
(2.8 Å, XRD with lipids)

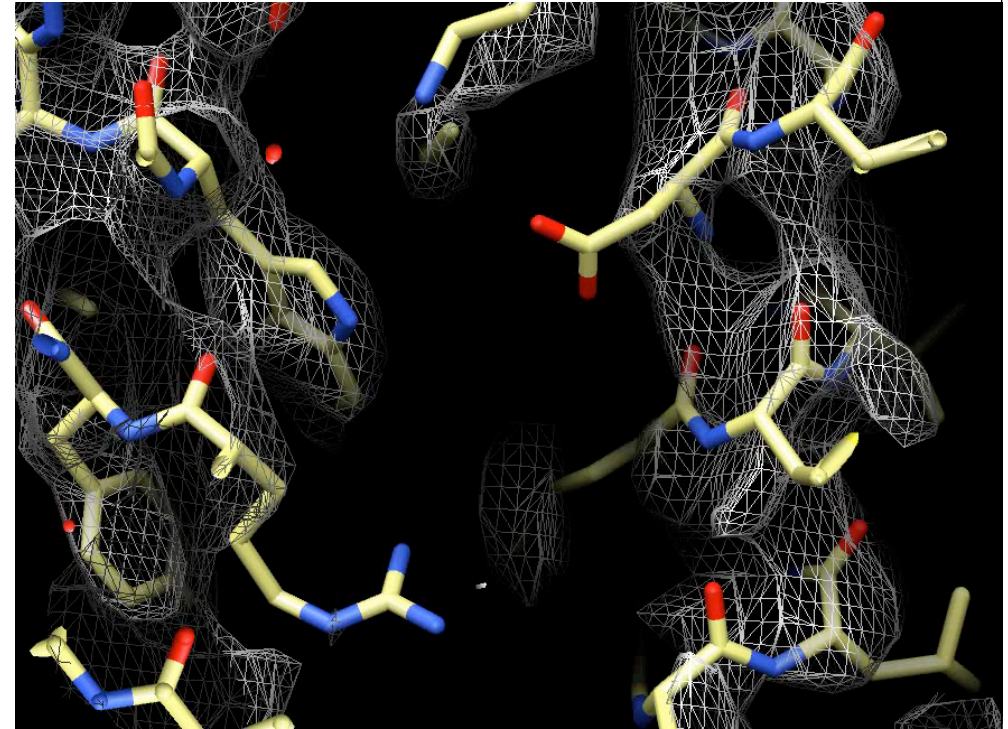
Bacteriorhodopsin, Schiffbase region
(data from Mitsuoka / Fujiyoshi)



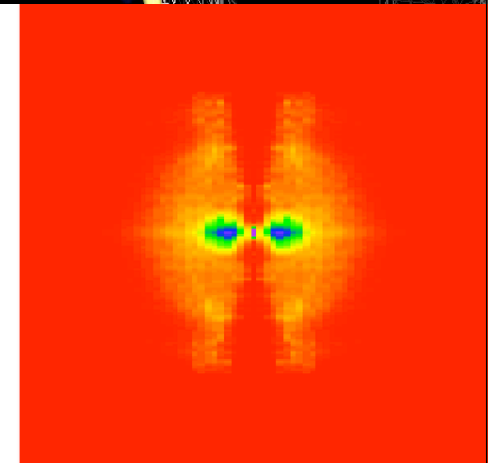
Total dataset

Only 7 - 3 Å

Kimura et al, 1997
(3.0 Å, electron crystallography)

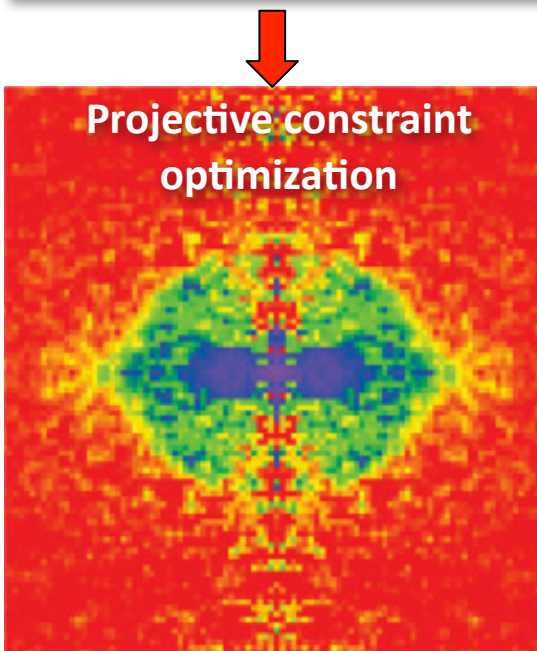
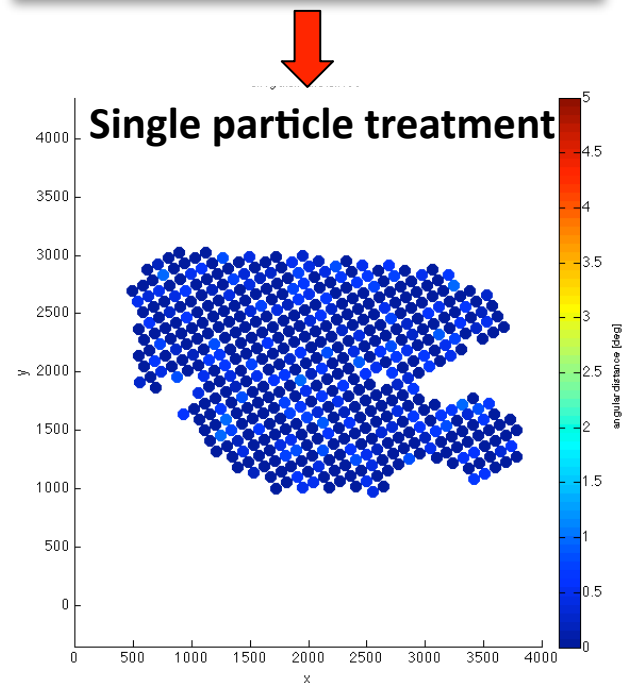
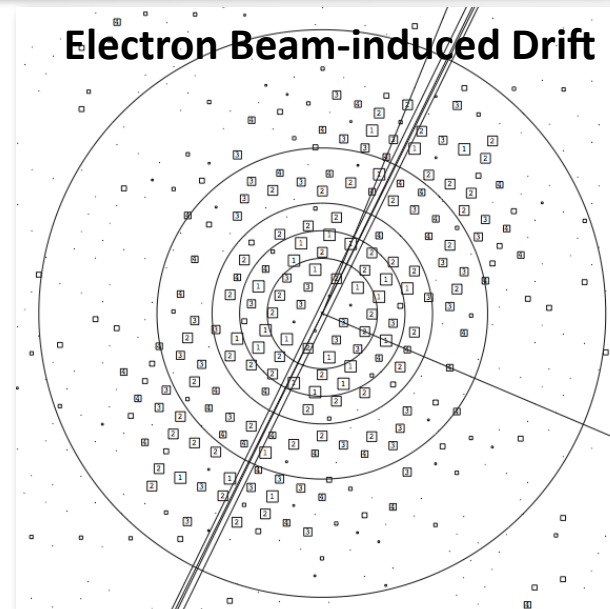
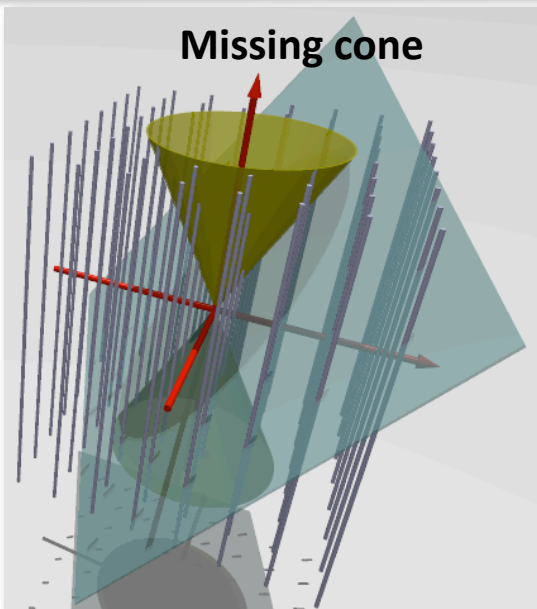
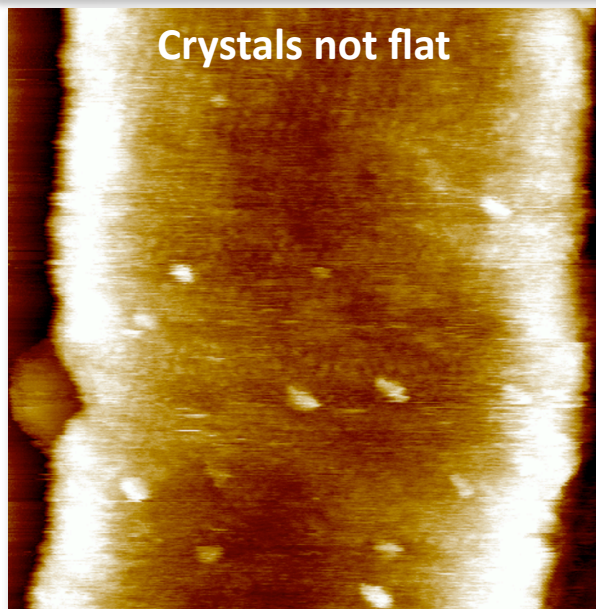


PCO: 100 initial rounds,
followed by 30 rounds
of edge detection

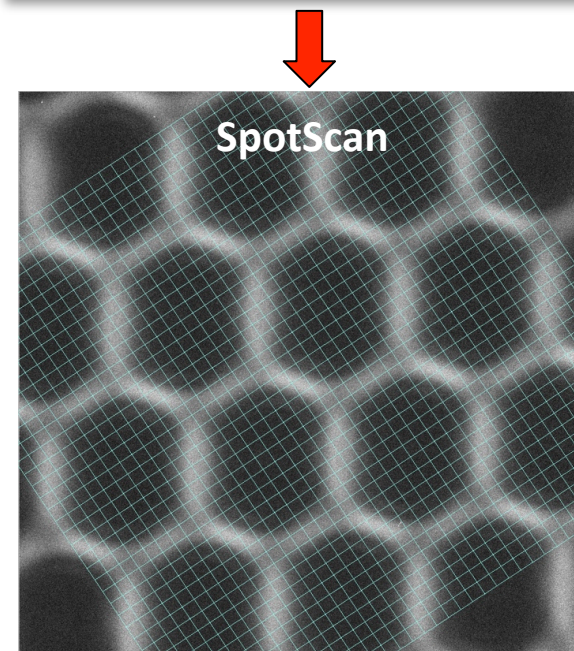


Gipson et al *Phys Rev.E* 84 011916 (2011)

x,y / z Anisotropy: Reasons & Solutions



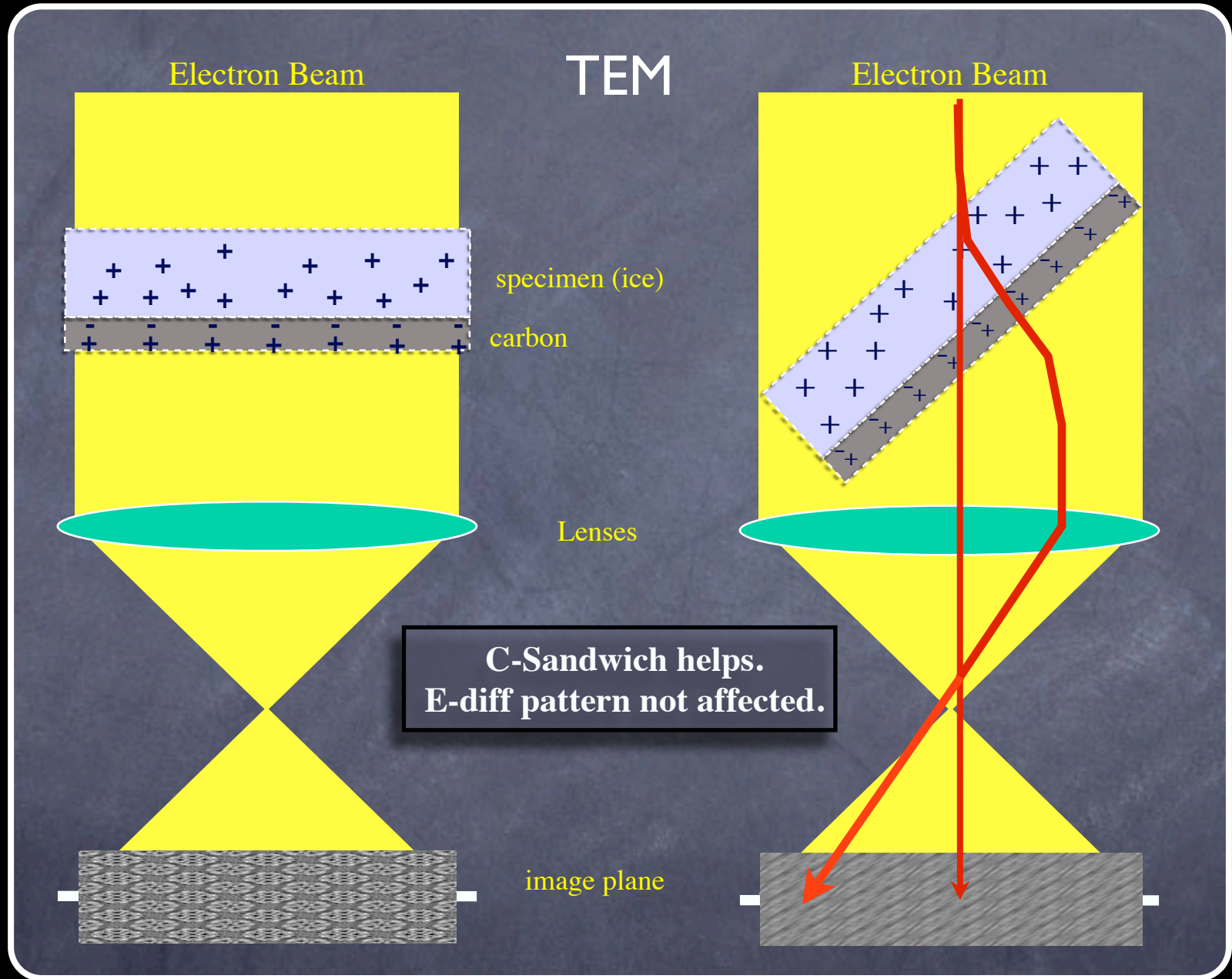
Gipson *et al* *Phys Rev.E* 84 011916 (2011)



Downing, *Science* 251(4989), 53-59 (1991)

Cryo-EM of tilted samples: Beam-induced resolution loss

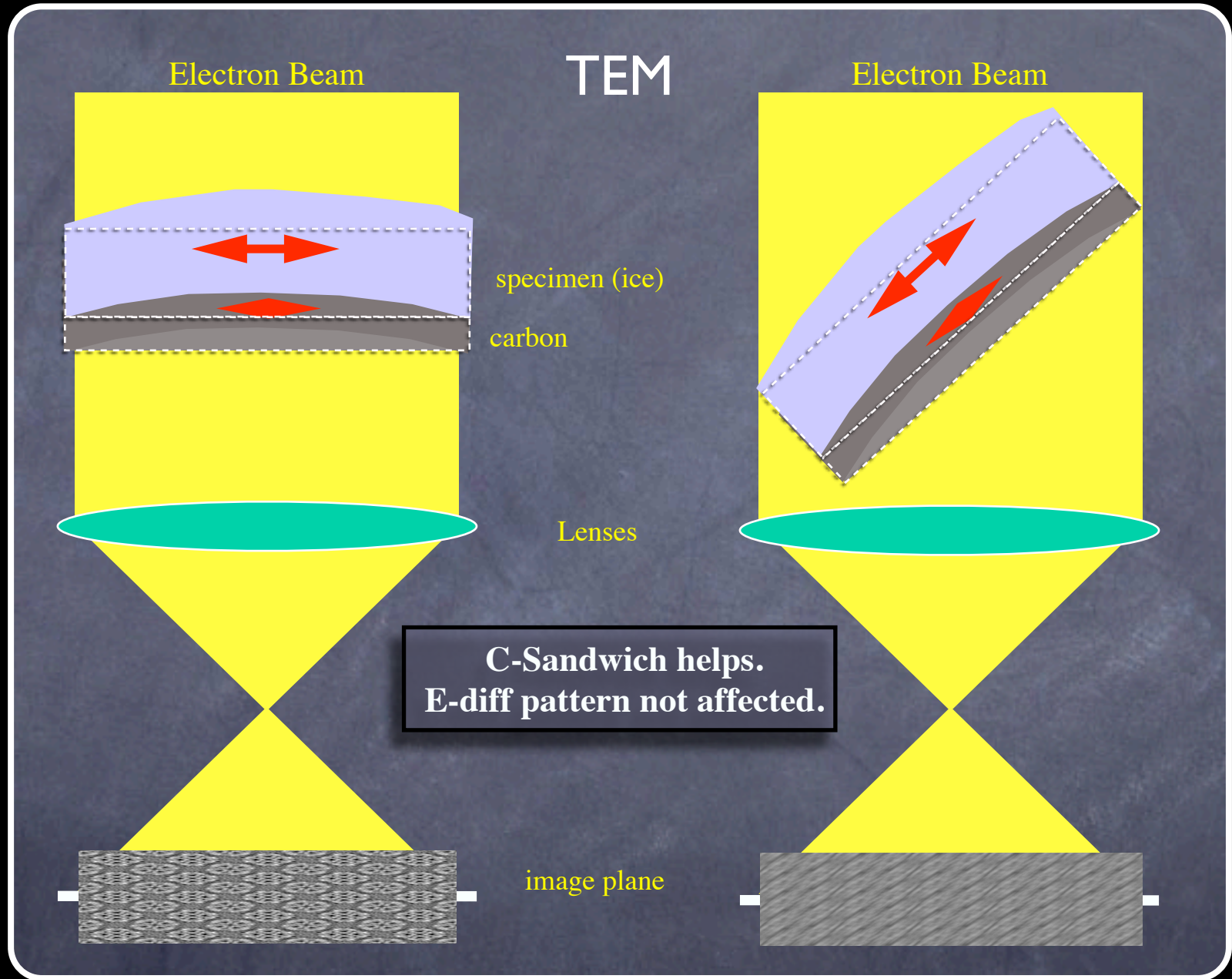
Is it charging?



C-Sandwich helps.
E-diff pattern not affected.

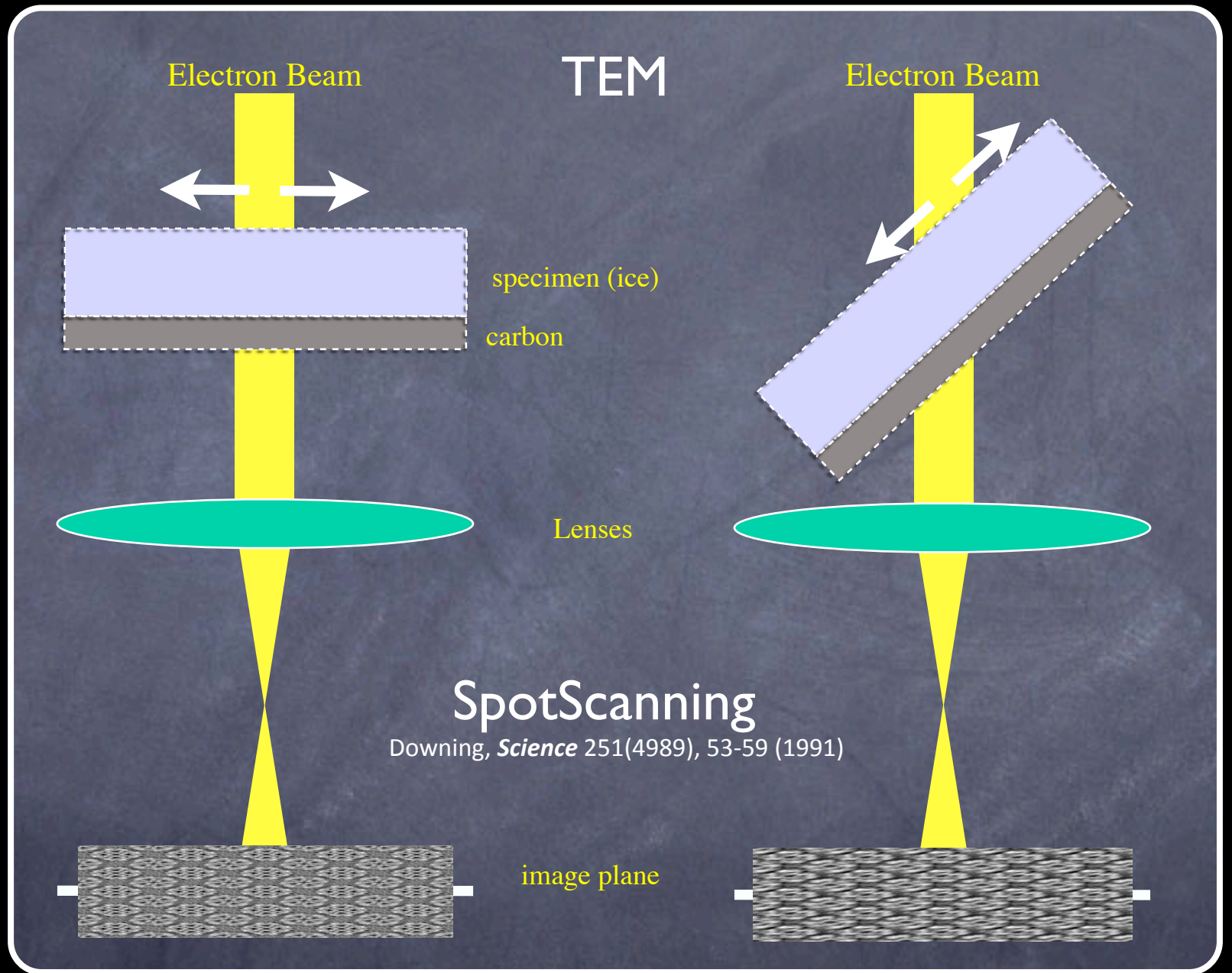
Cryo-EM of tilted samples: Beam-induced resolution loss

Or is it
physical
movement?



Cryo-EM of tilted samples: Beam-induced resolution loss

Anyway,
SpotScan
helps in
both cases.



Camera-based SpotScanning

Here: EM-TOOLS, TVIPS,
using a F416 CMOS camera

Proposed Approach:

- Every SpotScan spot is recorded as an individual CMOS image, which is 2x2 or 4x4 binned
- Recorded spot images may be CTF corrected individually. (*Beam-tilt* ?)
- Spots may then be merged into one large output image.

The screenshot displays the EM-TOOLS software interface. The main window shows a TEM image with a grid of red and yellow circles overlaid, representing the spot scanning pattern. A blue circle highlights a specific spot. The interface includes several panels:

- Navigation:** Shows the main TEM image with a grid of spots.
- Overview:** Shows a zoomed-in view of the selected spot, labeled "Scan 1".
- Zoomed:** Shows a further zoomed-in view of the selected spot, labeled "Pos 1".
- Single particles positions:** A table with columns: #, Search, Focus, Exposure, Stage X/Y, Image Shift X/Y. The first row contains: 1, Grid 1, Scan 1, -400, Camera, 114.1432, 00.
- Positions:** Includes checkboxes for "Search on reference image", "Focus (nm)", and "Exposure".
- Spotscan:** Includes input fields for "Number in X", "Number in Y", "Dist. X/Y (%)", and "Rotation (deg)".
- Structure:** Radio buttons for "Quadratic", "Hexagonal", and "Triangular".
- Focus position:** Includes input fields for "Dist. (nm)" and "Rad. (deg)".
- Defocus distribution:** Includes checkboxes for "Enable" and input fields for "Start (nm)", "End (nm)", "Increment (nm)", and "Group number".

The TEM Control panel on the left shows the following settings:

- Mag: 200000x 1
- Spot number: 1
- Intensity: 0.40000
- Obj. lens (V): 80.0000
- Defocus (nm): -200.0
- Stage X (nm): 111.7
- Stage Y (nm): 621.4
- Stage Z (nm): 187.0
- Stage A (deg): 0.0
- ES X (nm): 1254
- ES Y (nm): 930
- IS X (nm): 137
- IS Y (nm): 1307

The AutoCentering panel shows the following settings:

- Enable autocentering:
- Offset X (nm): 0
- Offset Y (nm): 0
- Quality: High

The text "Non-tilted sample" is overlaid in yellow at the bottom of the image.

Camera-based SpotScanning

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using a F416 CMOS camera

Proposed Approach:

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- Spots may then be merged into one large output image.

The screenshot displays the EM-TOOLS software interface. The main window shows a TEM image with a grid of spots. The spots are numbered 1 through 5. The interface includes a navigation panel on the left, a central image area, and a right-hand control panel. The control panel includes a table for single particles positions, a 'Positions' section with search and focus options, a 'Spotscan' section with grid parameters, and a 'Focus position' section with distance and rotation controls.

#	Search	Focus	Exposure	Stage X/Y	ImageDist X/Y
1	GM 1, Scan 1	0	Camera	333.6/453.0	0/0
2	GM 1, Scan 1	0	Camera	333.6/453.0	0/0
3	GM 1, Scan 1	0	Camera	333.6/453.0	0/0
4	GM 1, Scan 1	0	Camera	333.6/453.0	0/0
5	GM 1, Scan 1	0	Camera	333.6/453.0	0/0

Positions

- Search on reference image
- Focus (µm): 0
- Exposure
- Series
- Defocus inc. (µm)
- Exp. time inc. (µm)

Spotscan

- Number in X: 6
- Number in Y: 1
- Dist. X/Y (%): 120
- Rotation (deg): 74
- Structure: Quadratic Hexagonal Triangular

Focus position

- Dist. (µm): 1214
- Rad. (deg): 184

Defocus distribution

- Enable
- Start (µm): -3000
- End (µm): -3000
- Increment (µm): -400
- Exp. number: 1

TEM Control

General Alignment Energy Filter

Mag: 200000x 1

Spot number: 1

Intensity: 0.40000

Obj. lens (µm): 101

Obj. lens (V): 80.000

Defocus (µm): 200.0

Stage X (µm): 333.7

Stage Y (µm): 453.4

Stage Z (µm): 187.0

Stage A (deg): 8.0

ES X (µm): 1254

ES Y (µm): 184

ES X (µm): 137

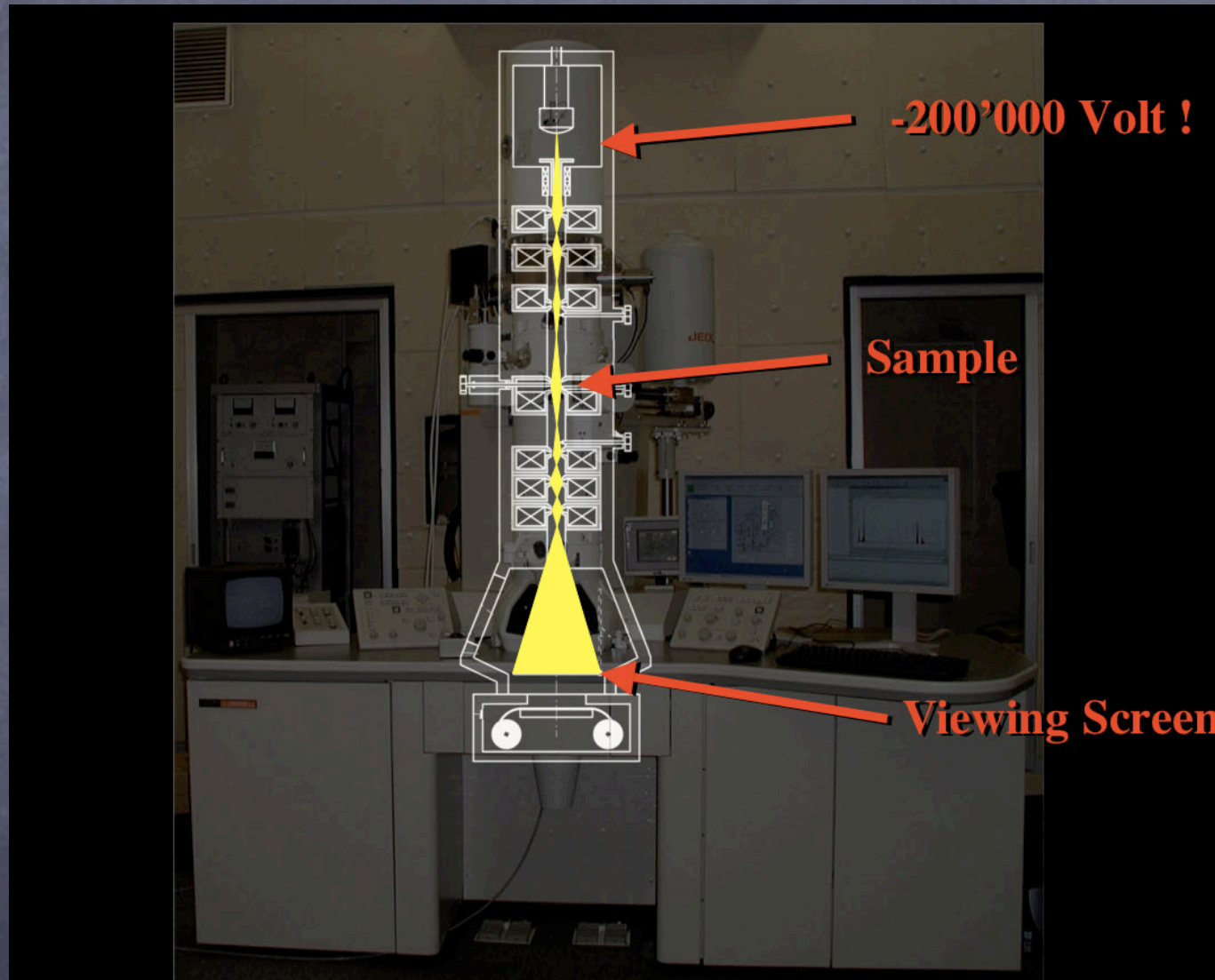
ES Y (µm): 187

ES X: 100%

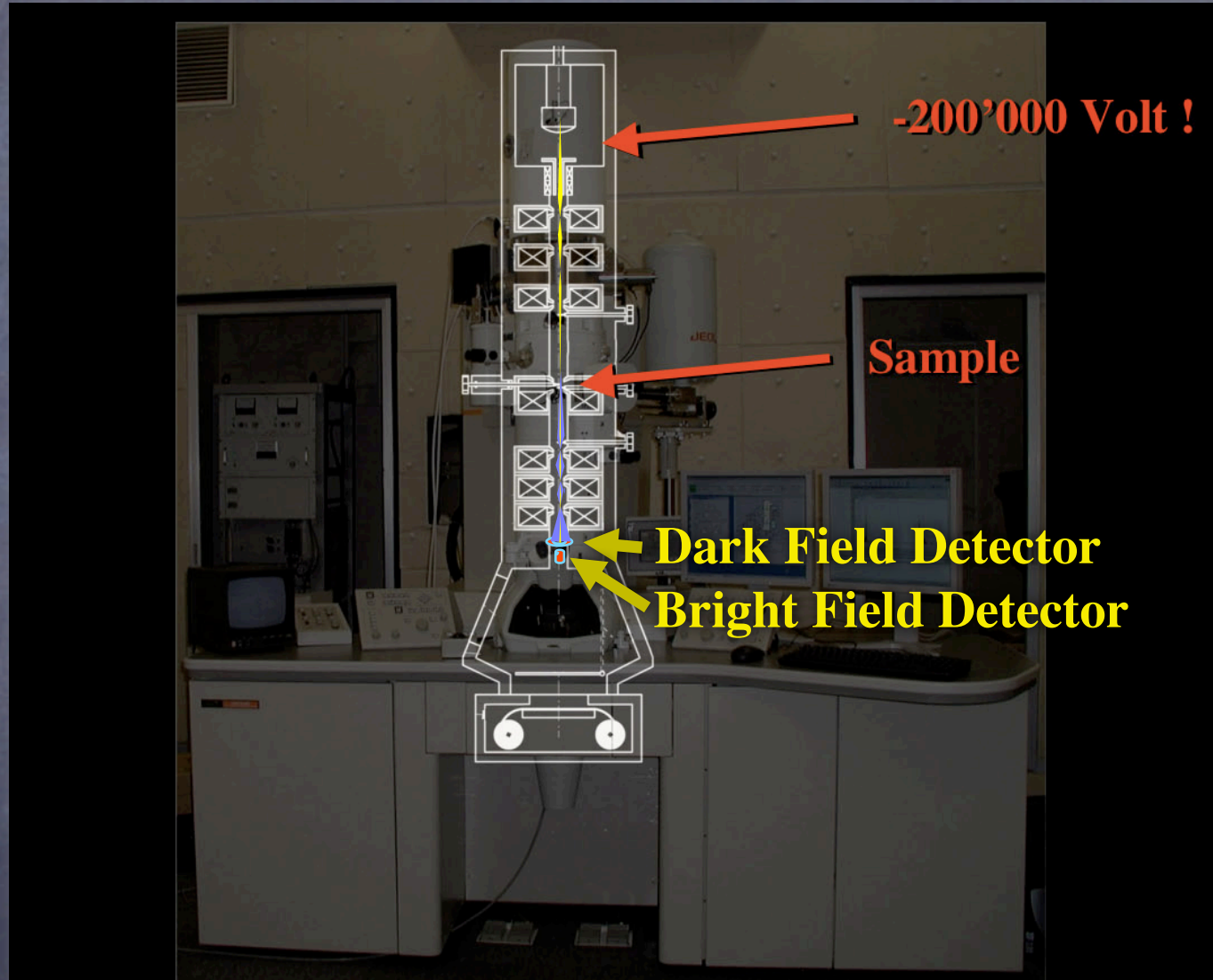
ES Y: 100%

Tilted sample: Semi-Dynamic Focus

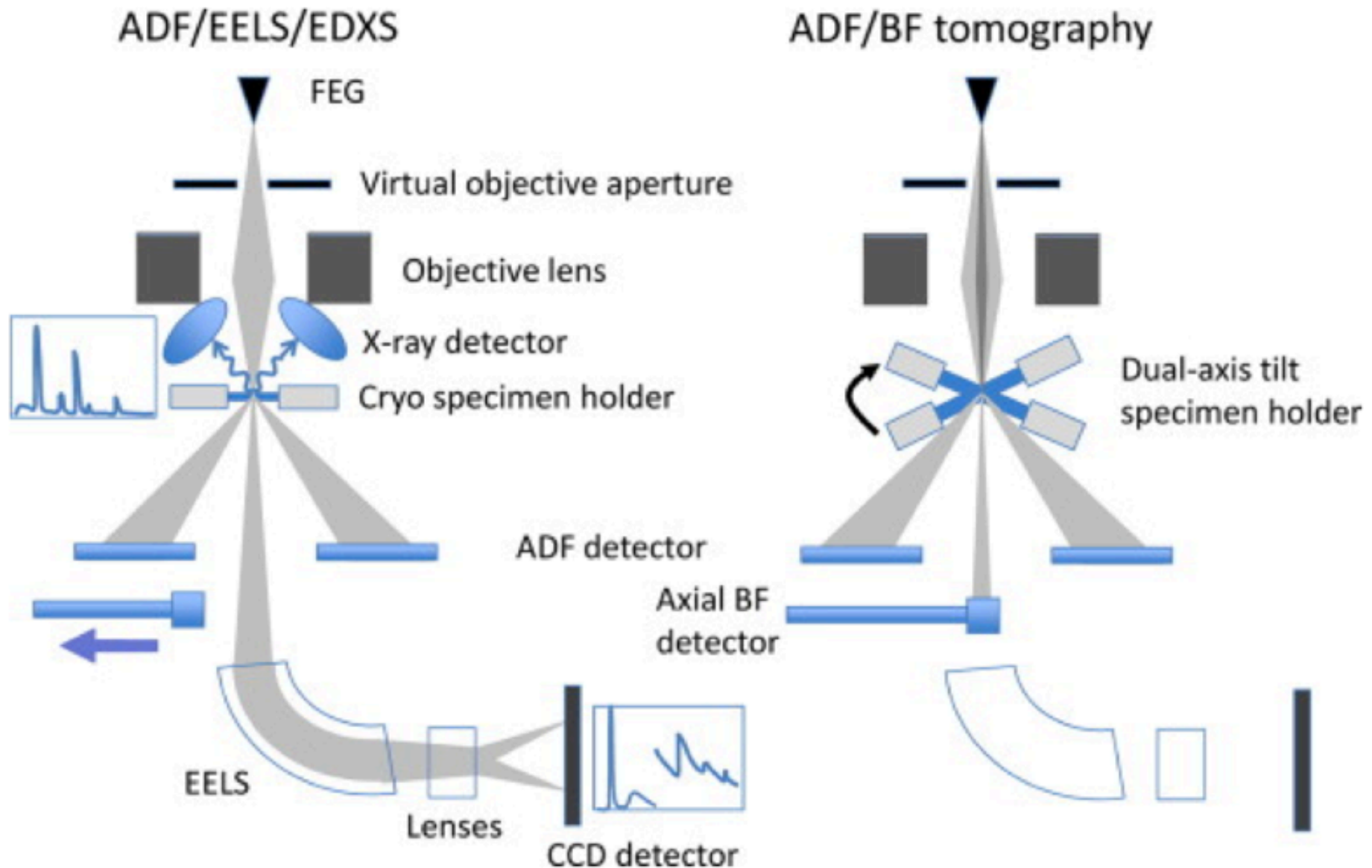
Transmission Electron Microscope



Scanning Transmission Electron Microscope



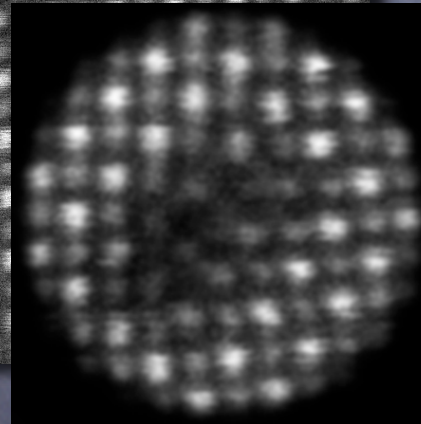
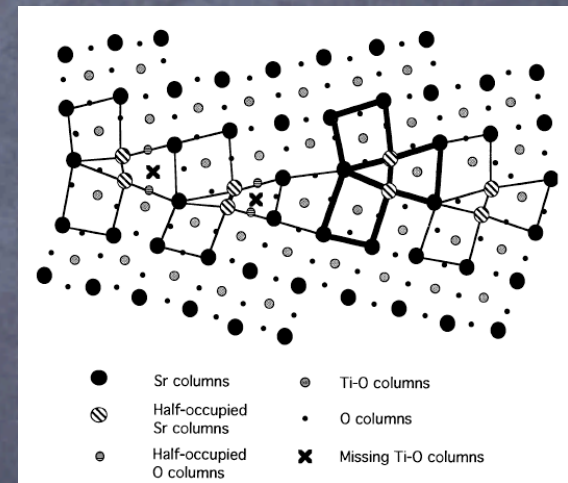
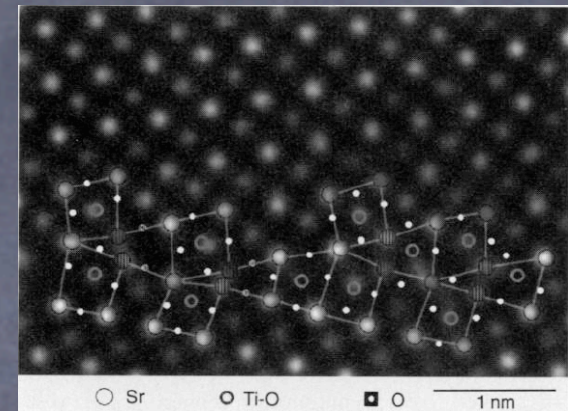
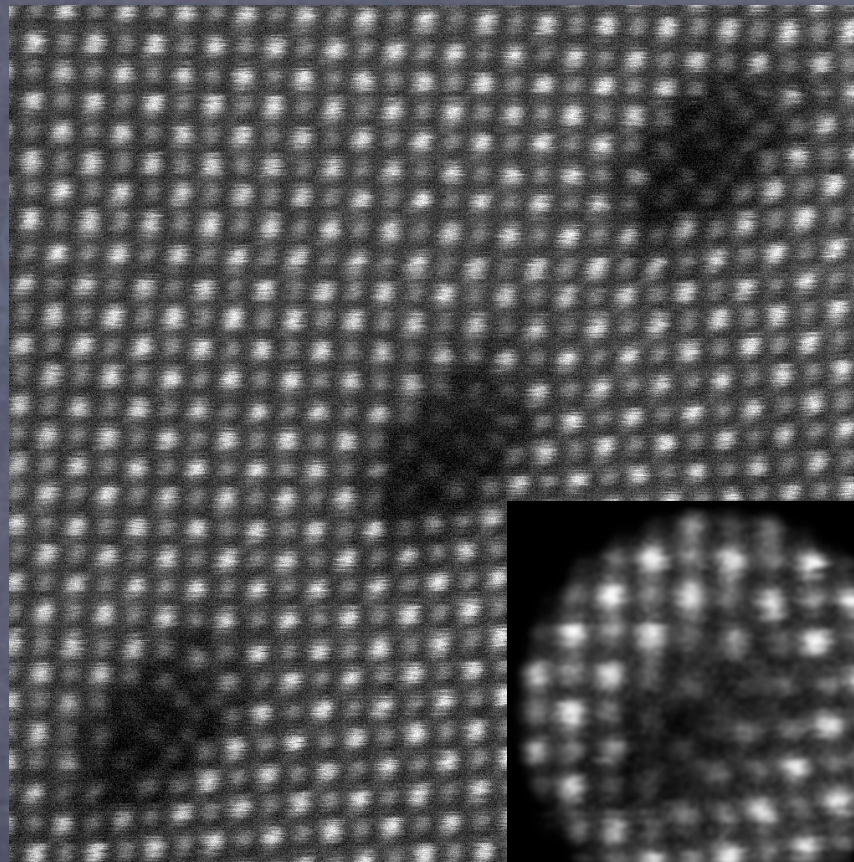
STEM applications for biological specimens



Cs-corrected HAADF STEM (Z-contrast) gives highest resolution.

STEM of SrTiO₃

Resolution < 1 Å



Dislocation Cores
with Nigel Browning

Buban et al. J. of Electron Microscopy, 2009)

McGibbon et al. Science 226 (1994) 102

Atomic structure model for a 25° [001] symmetric tilt grain boundary in SrTiO₃

High resolution ✓

Tilted samples

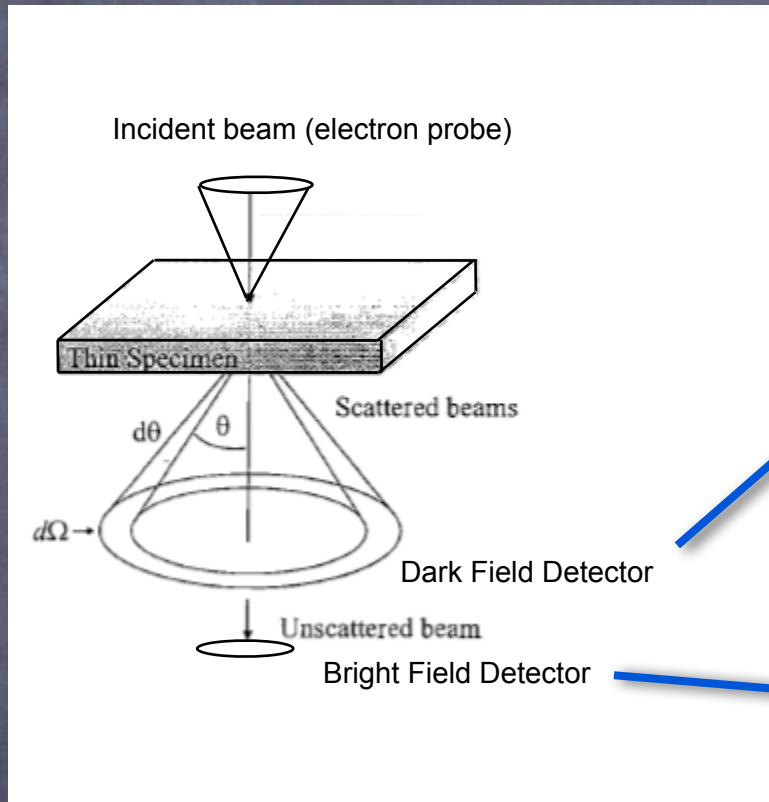
Low dose

High contrast

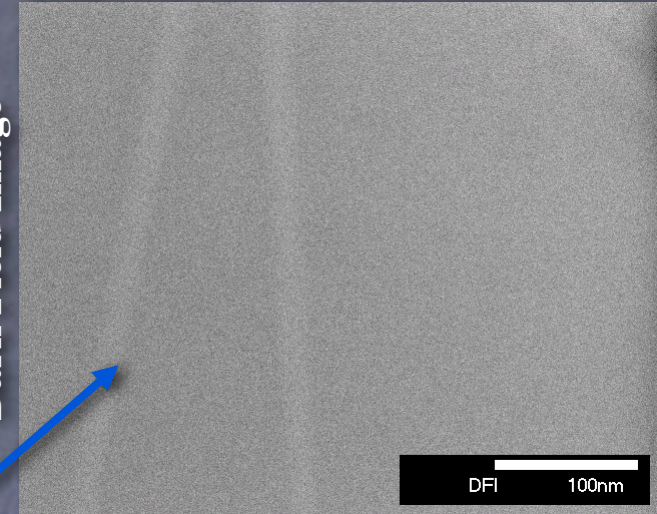
Conventional STEM for high-resolution Structural Biology is **not useful**.

(but good for Mass Measurements)

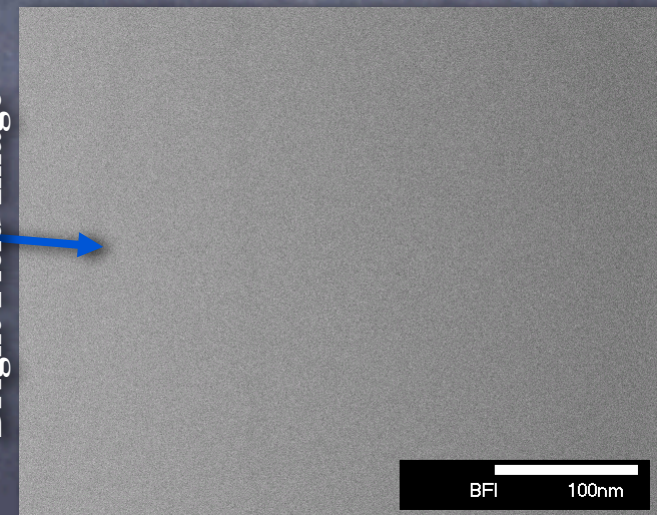
TMV in vitreous ice



Dark Field Image



Bright Field Image



- High resolution ✓
- Tilted samples
- Low dose
- High contrast

STEM has no apparent resolution loss on tilted samples.

Here:

- Gatan 626 holder
- TMV in vitreous ice
- Tilt pair: $0^\circ, 45^\circ$

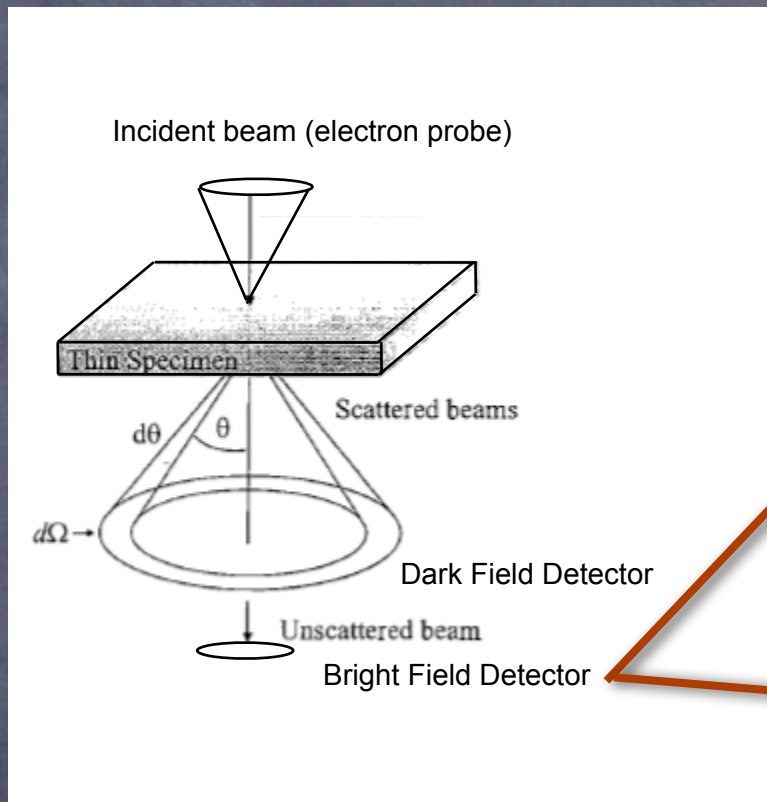
High resolution ✓

Tilted samples

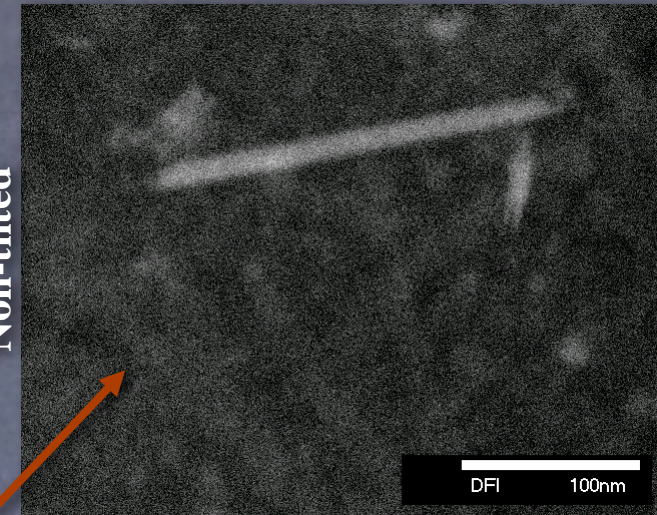
Low dose

High contrast

TMV in vitreous ice (heavily contaminated)

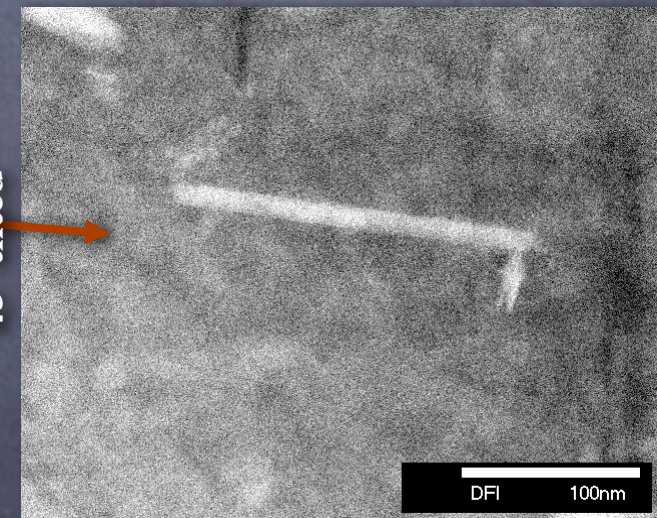


Non-tilted



Both are Dark Field images

45° tilted



STEM can be done at low dose.

Here:

- Very short dwell time
- Lowered extraction voltage on FEG
- Use Cs correctors as gun stigmators

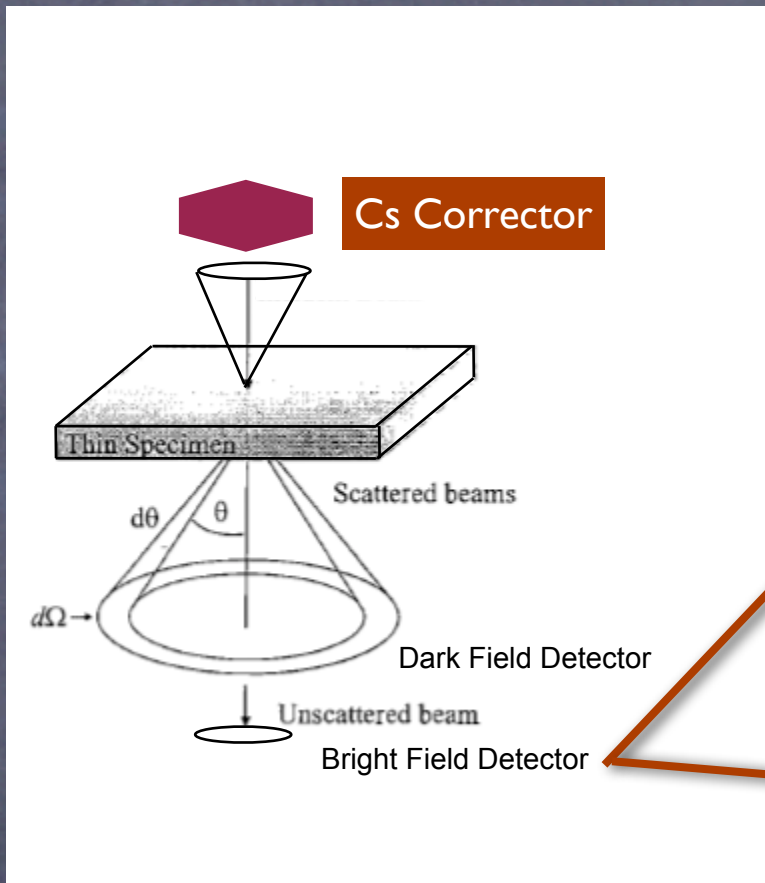
High resolution ✓

Tilted samples ✓

Low dose

High contrast

Bringing the electron dose down to $\sim 1 \text{ e}/\text{\AA}^2$



0.5 μs / pixel

High-Speed scanning

2.0 μs / pixel

STEM can be done at low dose.

Here:

- Very short dwell time
- Lowered extraction voltage on FEG
- Use Cs correctors as gun stigmators

High resolution ✓

Tilted samples ✓

Low dose

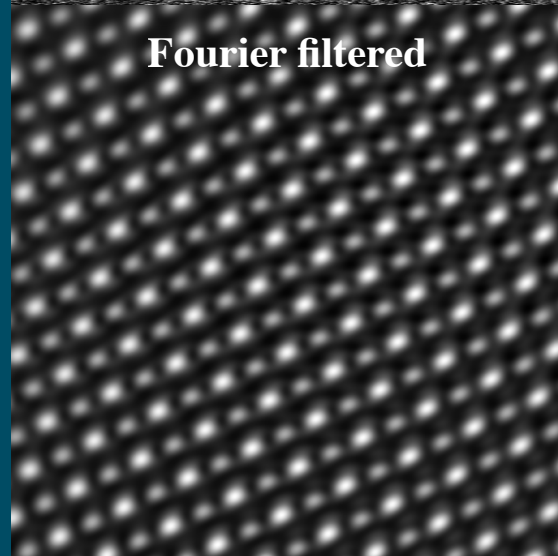
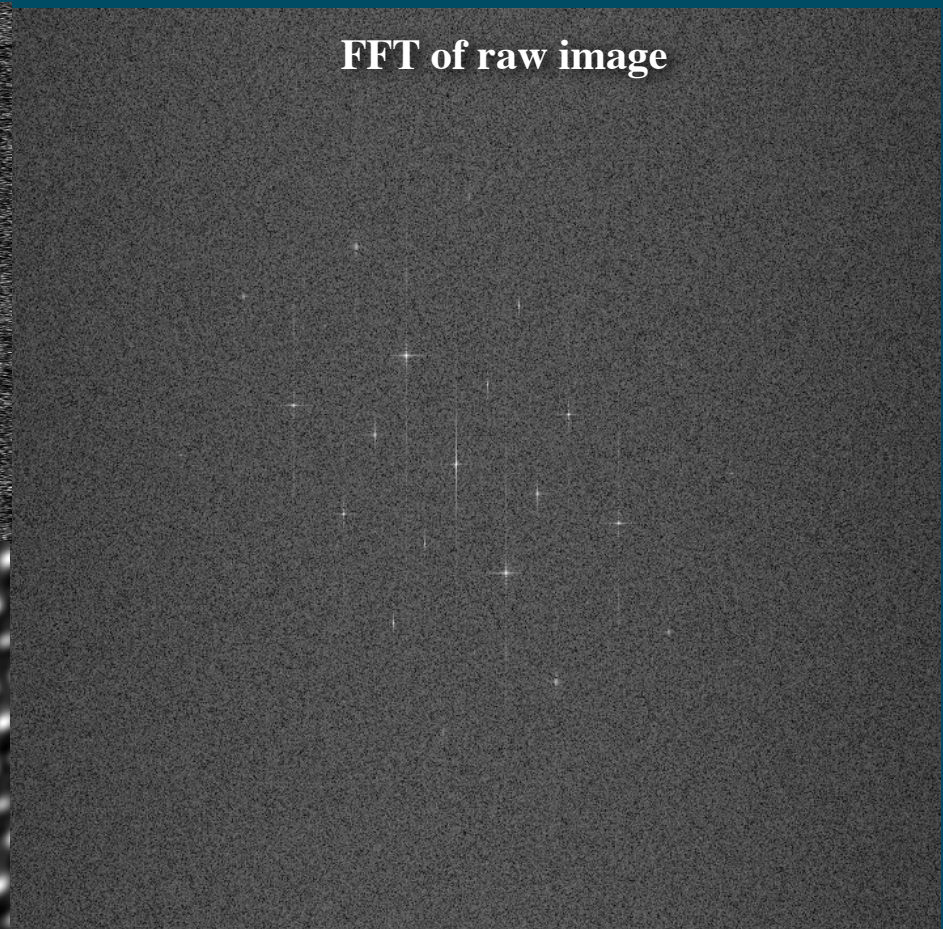
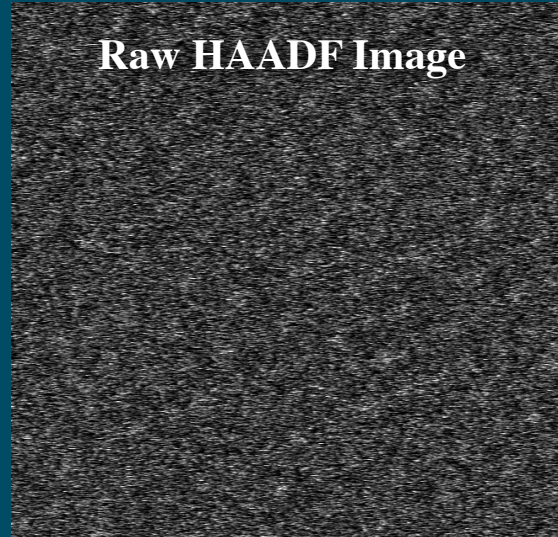
High contrast

Bringing the electron dose down to $\sim 1 \text{ e}/\text{\AA}^2$

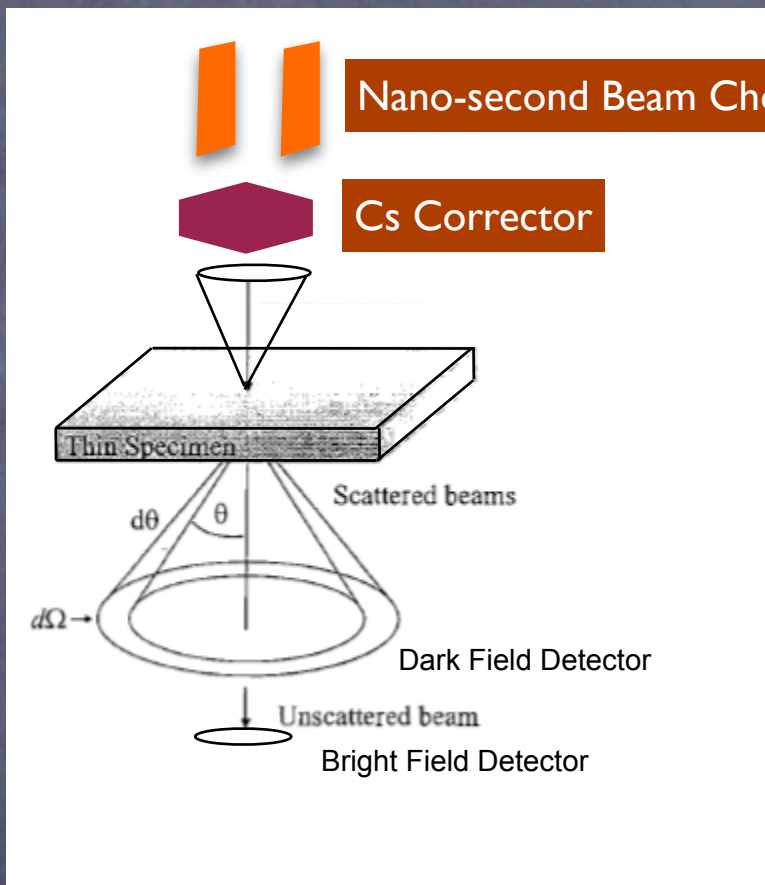
Raw HAADF Image

FFT of raw image

Fourier filtered



High-resolution STEM on beam-sensitive samples: The need for a **Beam Dimmer**



- High resolution ✓
- Tilted samples ✓
- Low dose
- High contrast

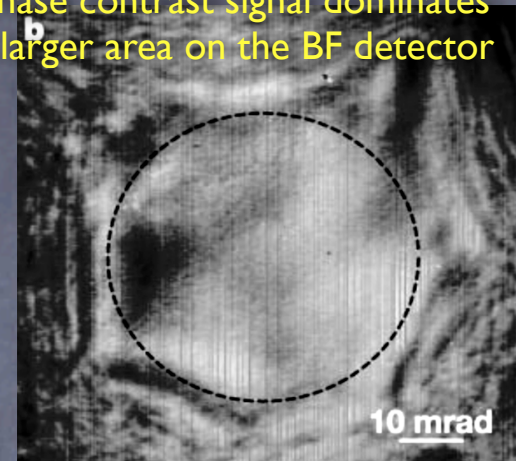
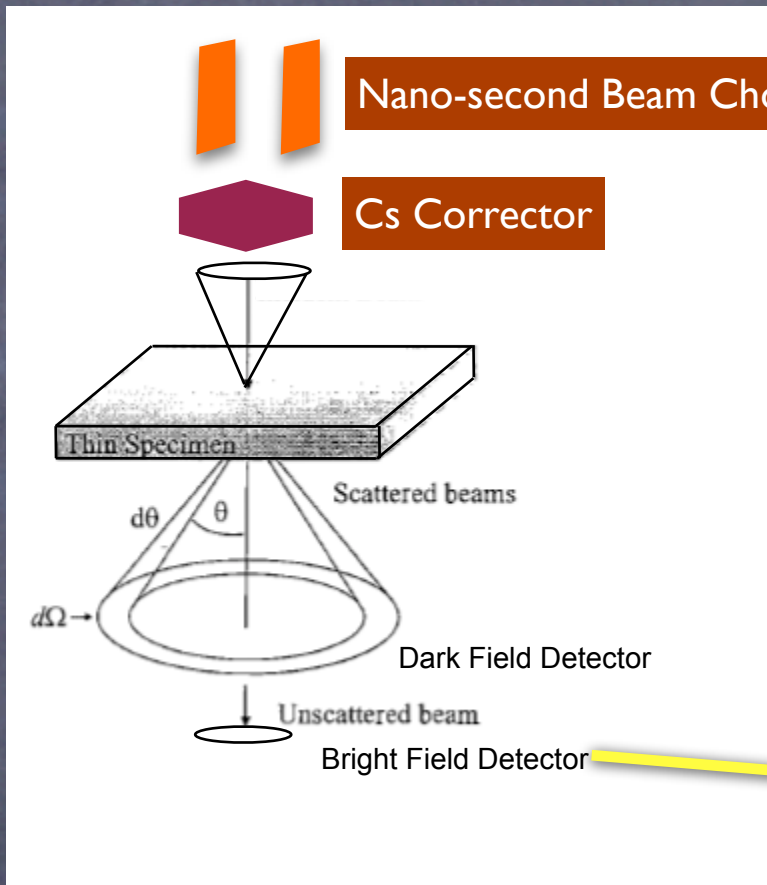
Vary On/Off ratio of beam in nano-second time range, while leaving everything else in the (S)TEM the same

Phase-Contrast in Cs-Corrected BF STEM

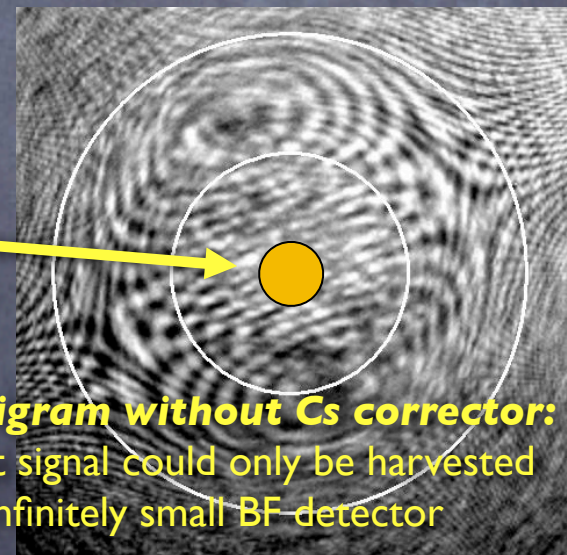
Sweet Spot in Ronchigram increases with Cs correction

STEM Ronchigram with Cs corrector:

Phase contrast signal dominates a larger area on the BF detector



Batson et al., Nature (2002)



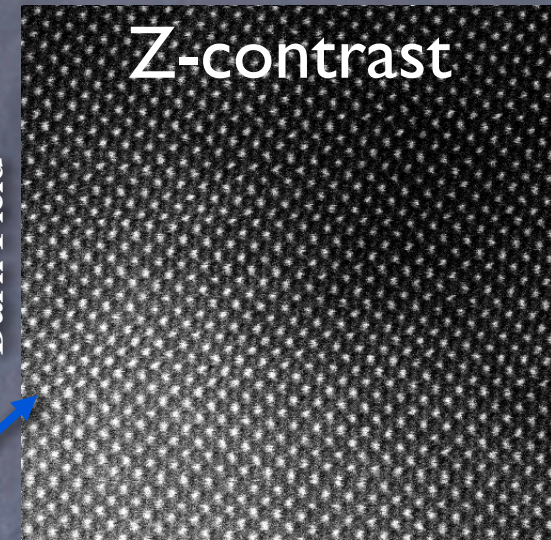
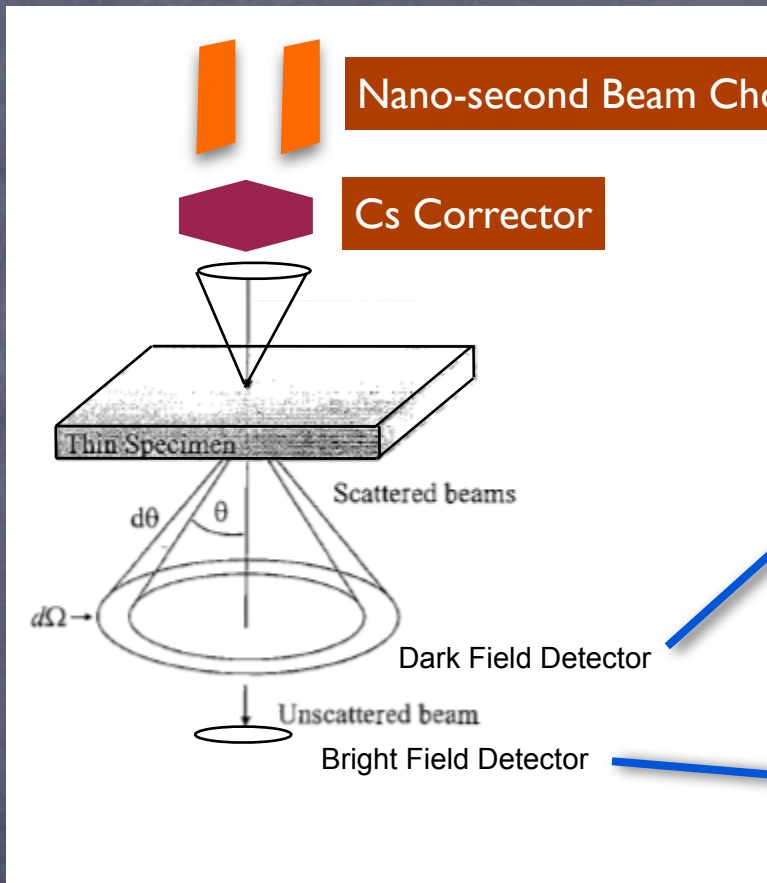
STEM Ronchigram without Cs corrector:

Phase contrast signal could only be harvested with an infinitely small BF detector

- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- High contrast

Phase-Contrast in Cs-Corrected BF STEM

BF image variation upon thickness or defocus change -> Phase Contrast



Dark Field

SrTiO₃

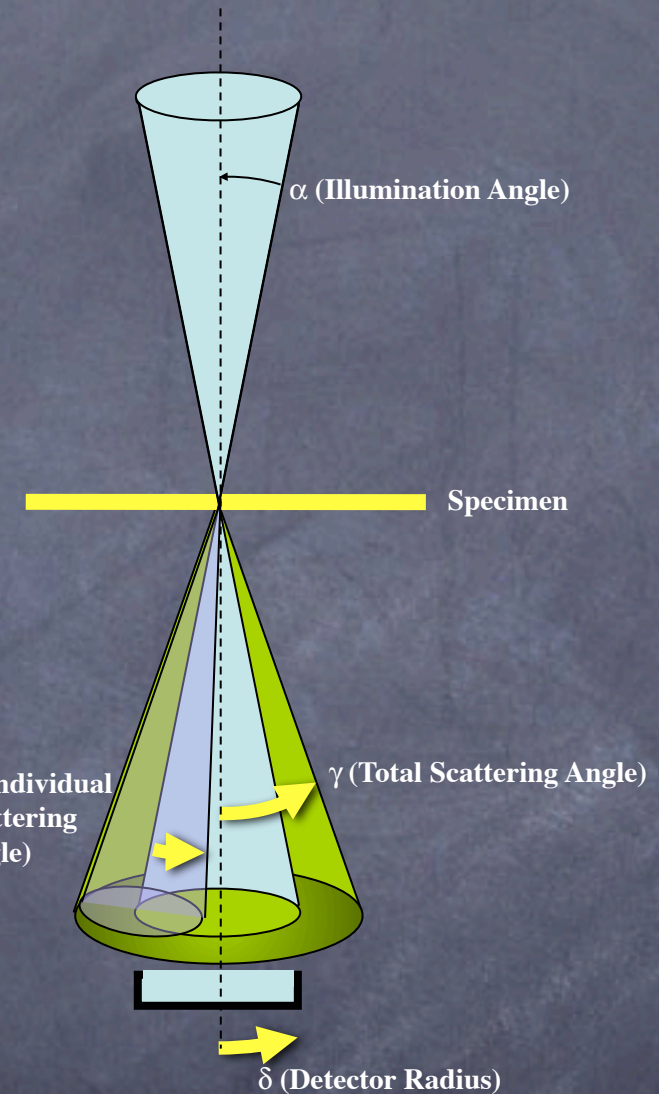
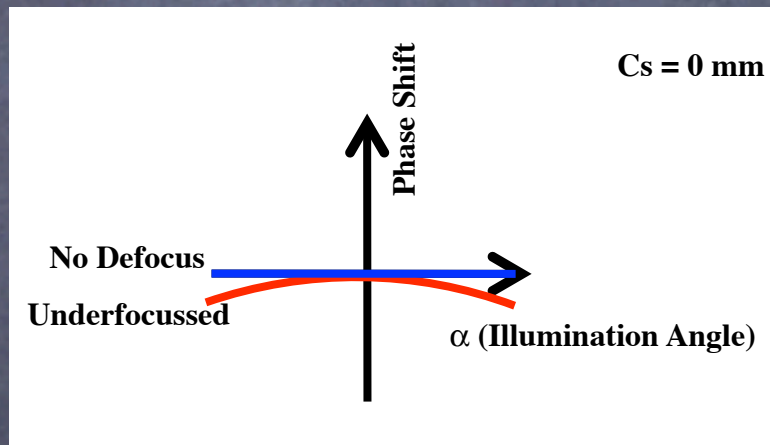
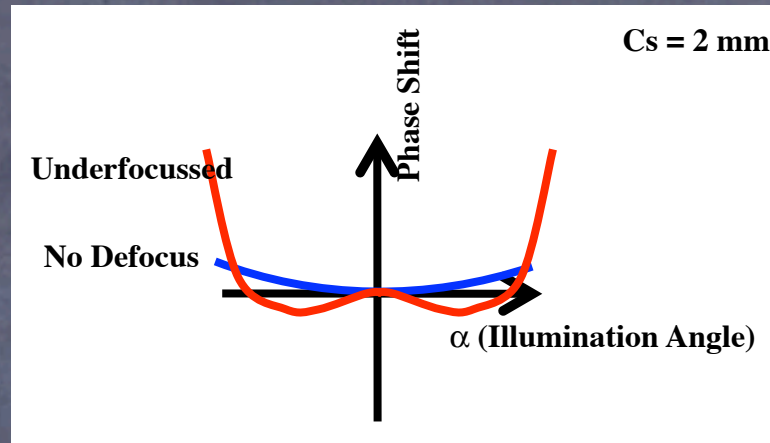


Bright Field

- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- High contrast

with: J. Buban, Nigel Browning,

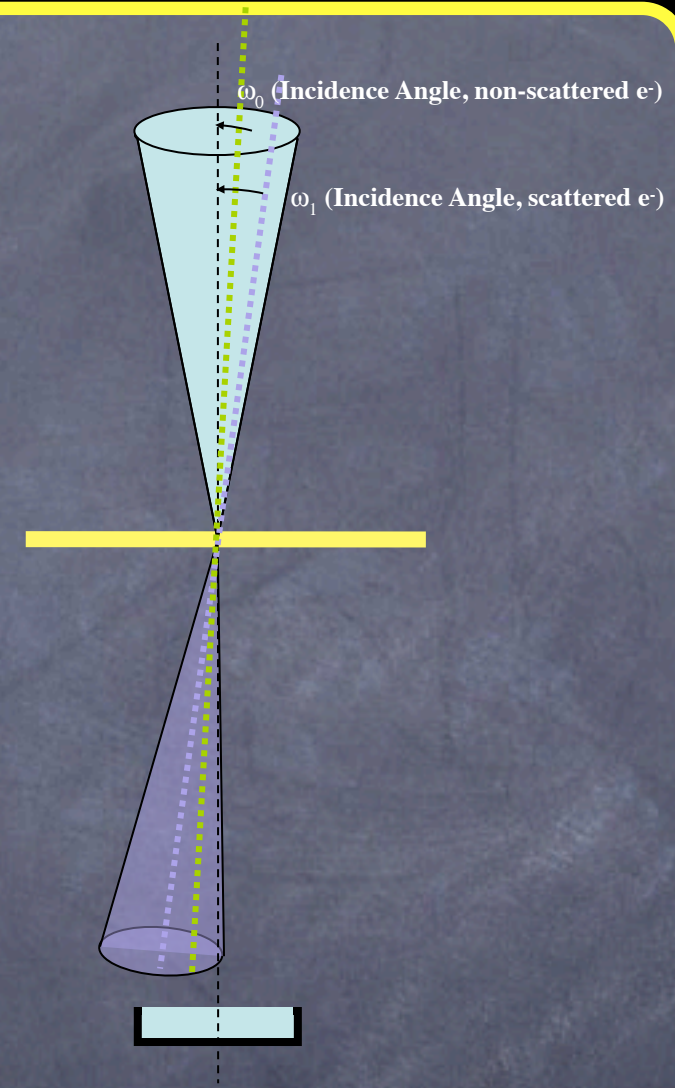
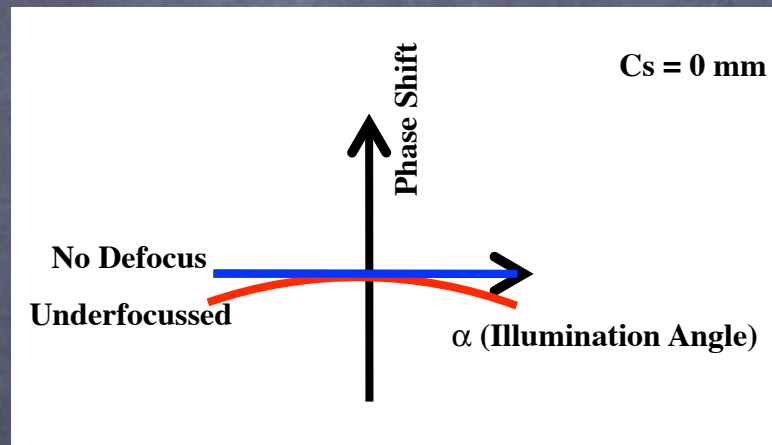
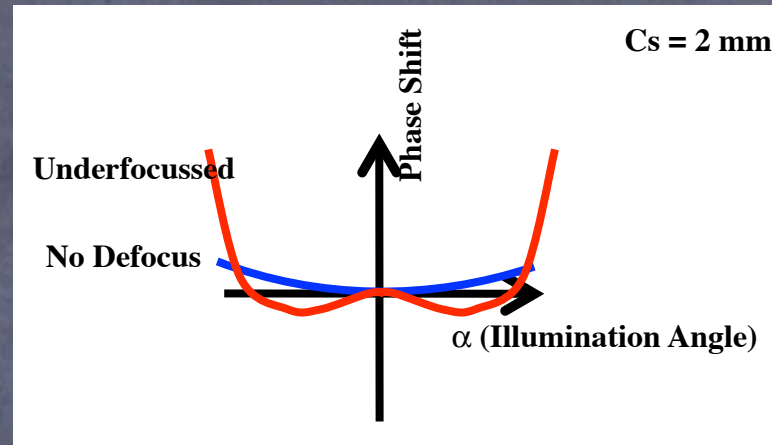
Phase-Shift in STEM illumination



- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- High contrast

(see also: Hammel & Rose, Ultramic., 1995)

Interference contrast between non-scattered and scattered electrons

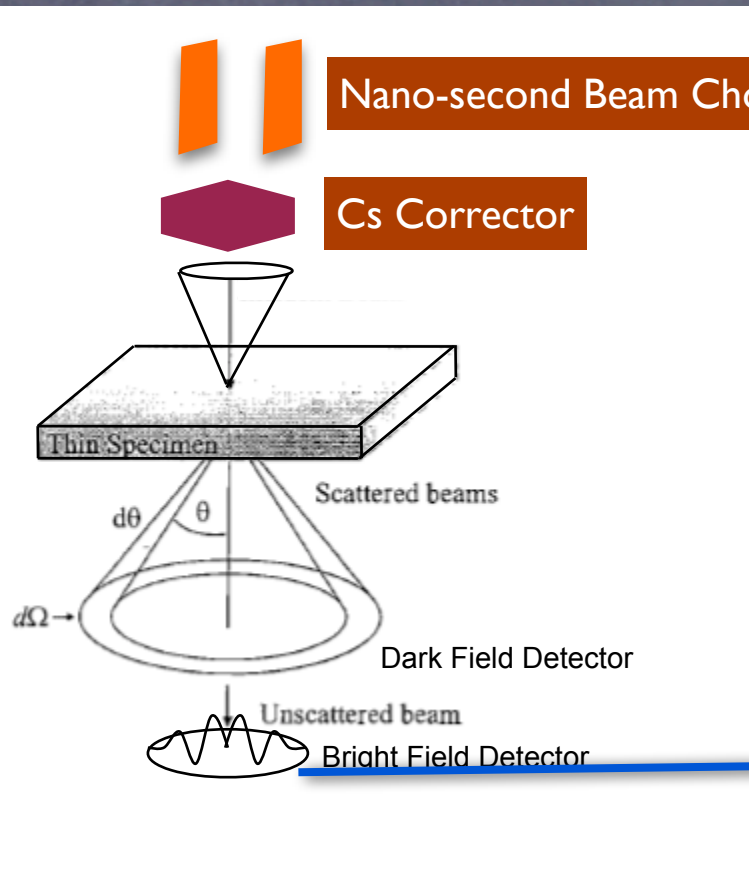


- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- High contrast

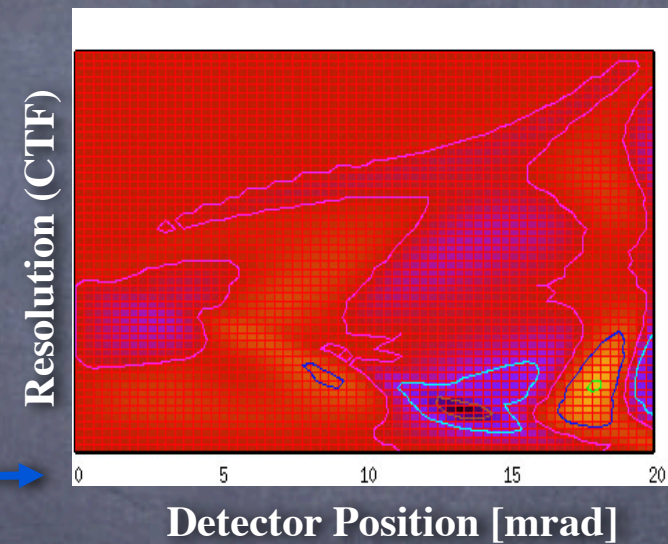
(see also: Hammel & Rose, Ultramic., 1995)

Phase-Contrast in Cs-Corrected BF STEM

- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- High contrast



Detector-position dependent Contrast Transfer Function (Simulation)



Def=-40nm, Cs=0.2mm

(see also: Hammel & Rose, Ultramic., 1995)



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Ultramicroscopy 58 (1995) 403–415

ultramicroscopy

Optimum rotationally symmetric detector configurations for phase-contrast imaging in scanning transmission electron microscopy

M. Hammel, H. Rose

Institut für Angewandte Physik, Technische Hochschule Darmstadt, Hochschulstrasse 6, D-64289 Darmstadt, Germany

Received 7 October 1994; in final form 2 January 1995

Abstract

A configured STEM detector yields simultaneously several signals which can be arbitrarily combined. We have calculated the optimum annular subdivision of the detector which maximizes the signal-to-noise ratio in the image of weak scatterers. The optimum detector doubles the signal-to-noise ratio as compared to the conventional STEM detector, increases the resolving power of the instrument and enhances the contrast of strong scatterers located on a supporting foil.

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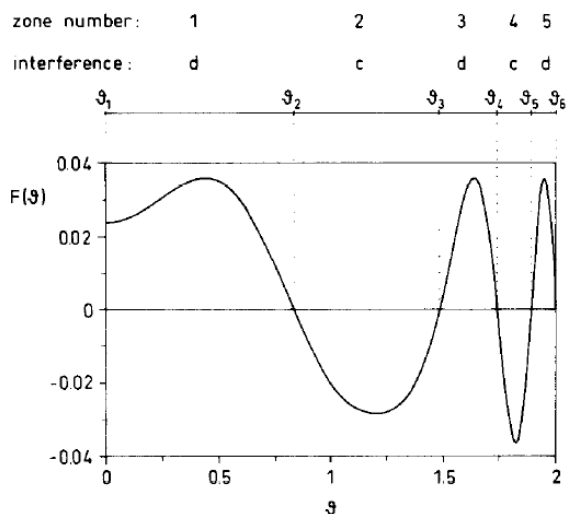


Fig. 2. Normalized current density fluctuations in the image of a weak point scatterer for a defocus $\Delta = 1.45$ and an objective aperture angle $\vartheta_0 = 2.0$. The zeros $\vartheta_1, \dots, \vartheta_6$ separate the regions of destructive "d" interference from those of constructive "c" interference.

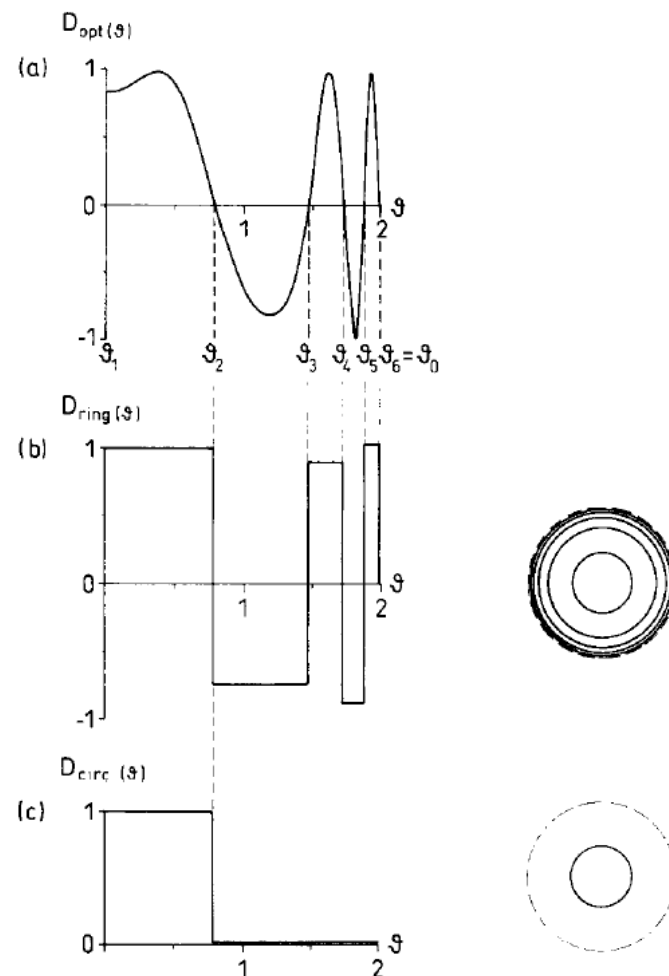


Fig. 4. (a) Detector function D_{opt} of the ideal detector obtained for a normalized defocus $\Delta = 1.4$ and an objective aperture angle $\vartheta_0 = 2.0$. This function indicates how the signal, recorded at an angular distance ϑ , should be weighted in order to yield optimum signal-to-noise ratio in the image of a weak point scatterer. (b) Realistic substitute of the ideal detector function D_{opt} . The weighting factors c_k of the partial signals are listed in Table 2. (c) Conventional configuration for the same imaging parameters. The geometries of the ring and the conventional detector are depicted on the right hand side.



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Ultramicroscopy 54 (1994) 41–59

ultramicroscopy

A versatile, software configurable multichannel STEM detector for angle-resolved imaging

M. Haider ^a, A. Epstein ^a, P. Jarron ^b, C. Boulin ^a

^a *EMBL, Meyerhofstrasse 1, Postfach 102209, D-69012 Heidelberg, Germany*

^b *CERN, Geneva, Switzerland*

(Received 29 September 1993; in final form 18 January 1994)

Abstract

A new type of STEM detector has been developed and tested. This detector consists of 30 rings which are split in quadrants (= 120 channels) and it allows further advantage to be taken of the image formation principle of STEM: the scattered electrons can be recorded separately according to their scattering angles and eight images can be acquired in parallel. Because of its electron counting capability, this detector is very well suited to analytical and quantitative applications of STEM (e.g. absolute mass determination and Z-contrast). The various channels of the detector can be combined by software in order to obtain images with the desired detector acceptance angles. The minimal time per pixel ($t/\text{pixel} \geq 4 \mu\text{s}$) is approximately as short as in the case of conventional single-channel detectors.

A versatile, software configurable multichannel STEM detector for angle-resolved imaging

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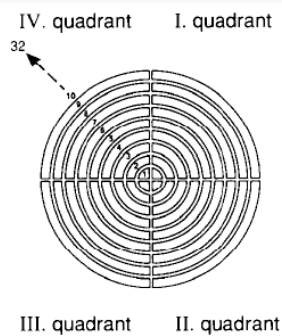


Fig. 4. Schematic drawing of the inner part of the detector layout.

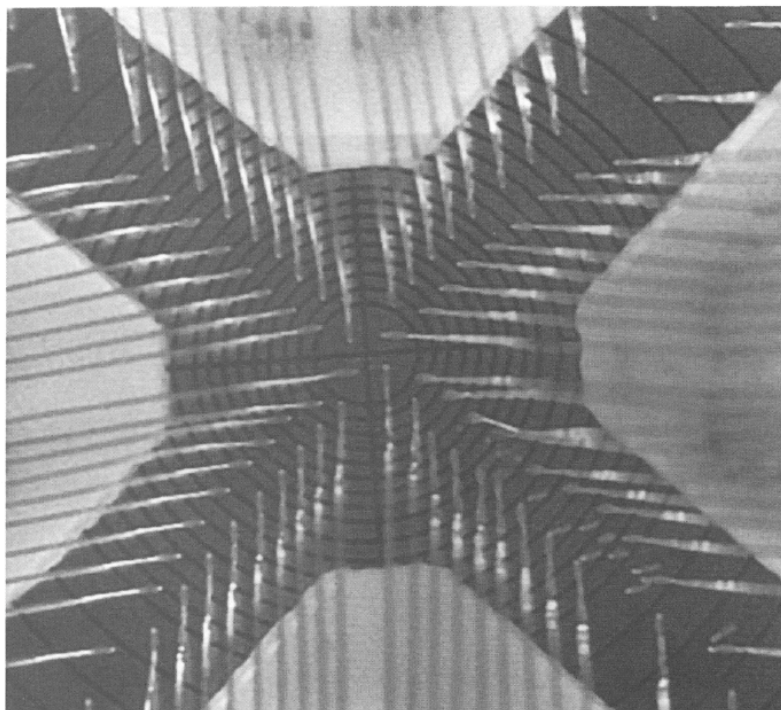


Fig. 6. Photograph of the inner part of the multichannel detector. The shape of the individual channels can be observed as rings split into quadrants. The diameter of the inner disk is 260 μm .

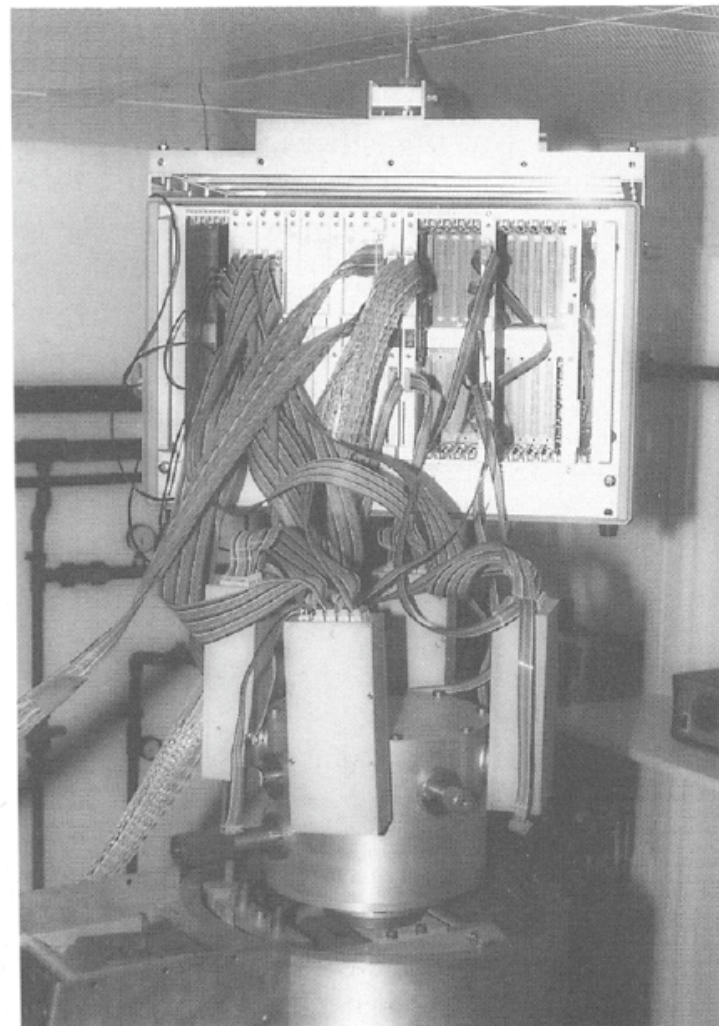
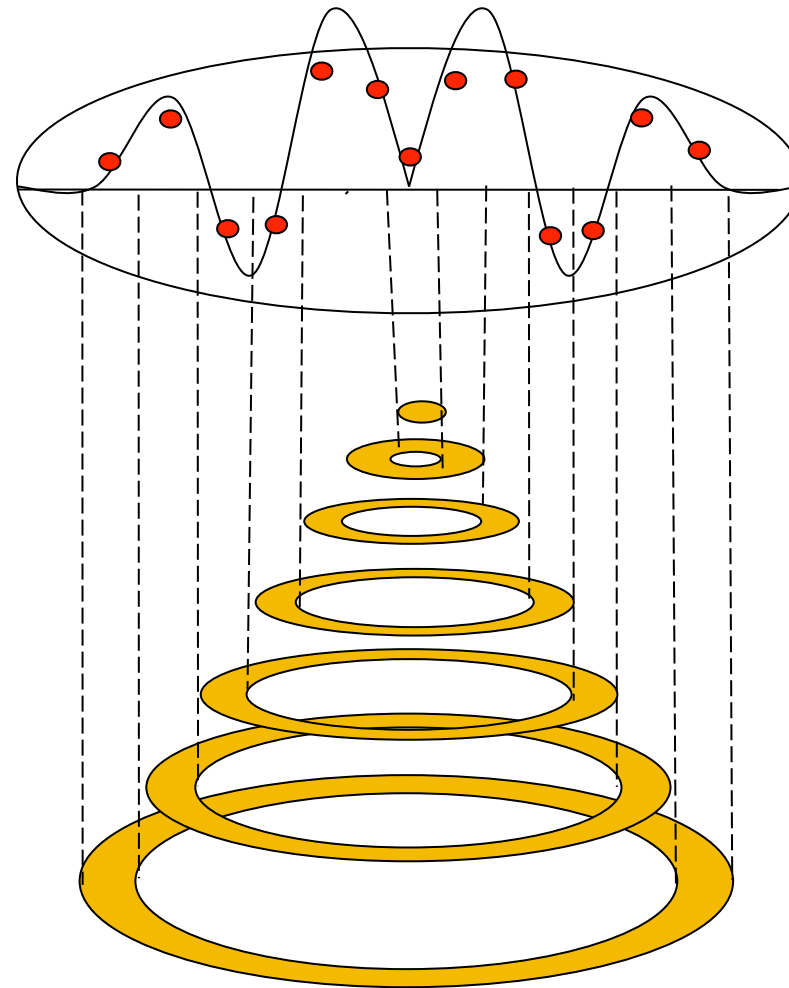
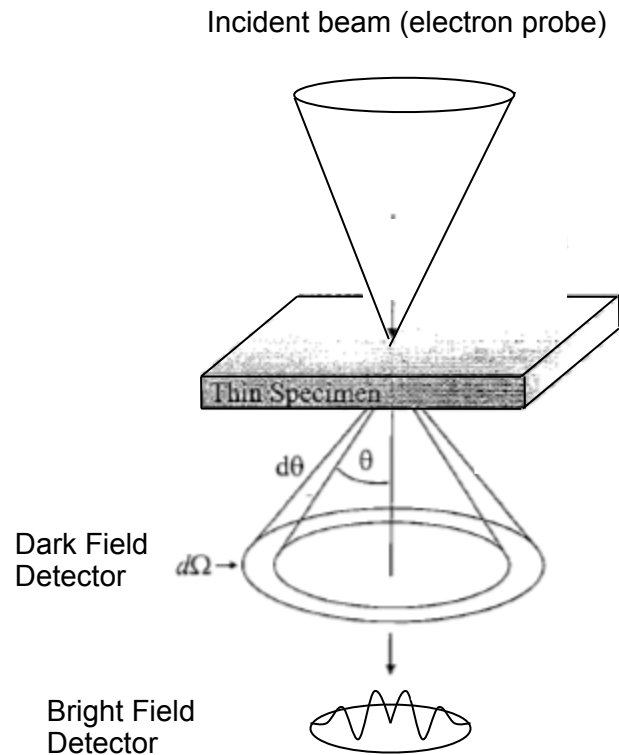


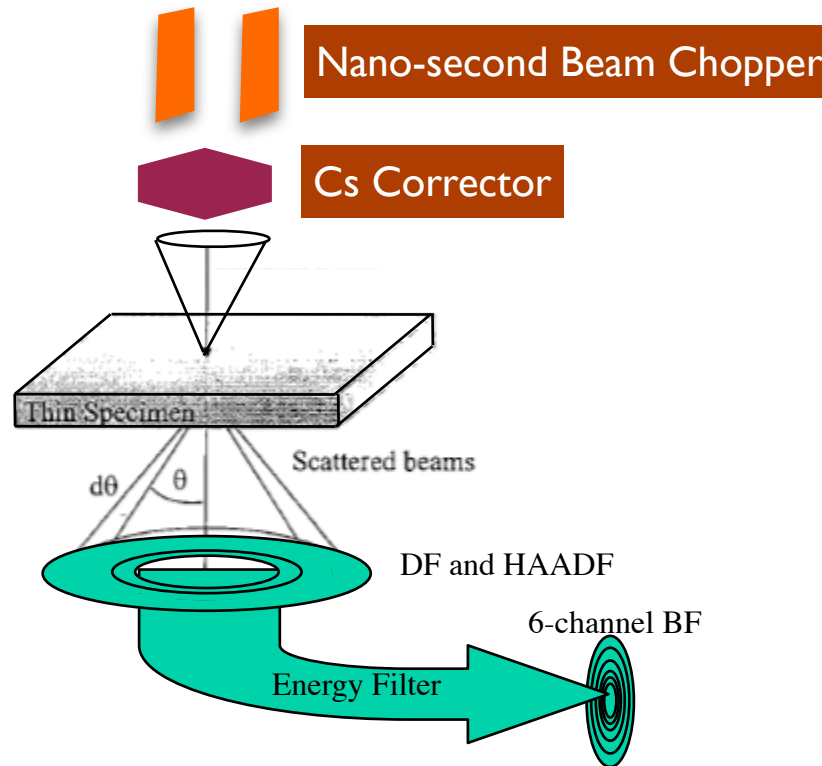
Fig. 2. Photograph of the upper part of the cryo-STEM column showing the housing of the multichannel detector and the attached four housings of the preamplifiers and discriminators.

Proposed Multi-Ring Detector (UC Davis, 2007)



Proposed Phase-Contrast BioSTEM (2008)

Low-dose cryo-STEM of tilted biological specimens



Tilted Samples:
Precise computer control,
online feedback

Low-dose:
Fast Electronics ($1 \mu\text{s} / \text{pixel}$)
Beam Chopper

Phase Contrast:
Aberration correction

Data Collection:
8k x 8k images,
8-channel detector system
(HAADF, DF, 6 BF channels)

- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- Phase contrast ✓

Proposed Phase-Contrast BioSTEM (2009)

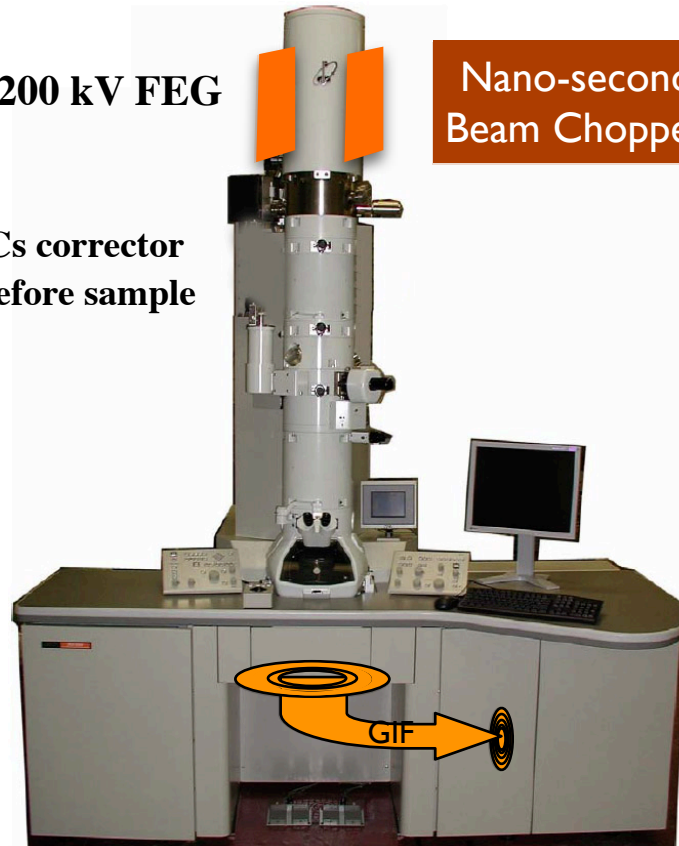


200 kV FEG

Nano-second
Beam Chopper

Cs corrector
before sample

GIF



Multi-channel detector

Tilted Samples:
Precise computer control,
online feedback

Low-dose:
Fast Electronics ($1 \mu\text{s}$ / pixel)
Beam Chopper

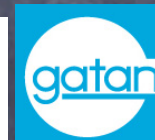
Phase Contrast:
Aberration correction

Data Collection:
8k x 8k images,
8-channel detector system
(HAADF, DF, 6 BF channels)

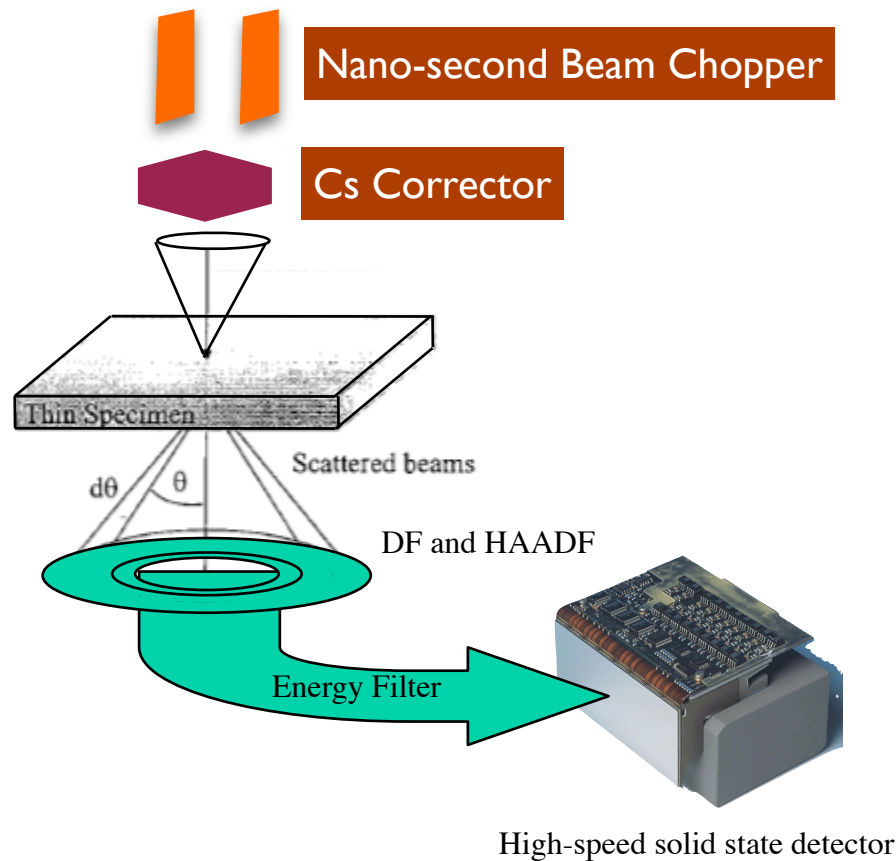
- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- Phase contrast ✓

James Buban, Nigel Browning, Quentin Ramasse, Hui-Ting Chou, Henning Stahlberg

JEOL
Stability · Performance · Productivity



Proposed Phase-Contrast BioSTEM (2010)



- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- Phase contrast ✓

Tilted Samples:
Precise computer control,
online feedback

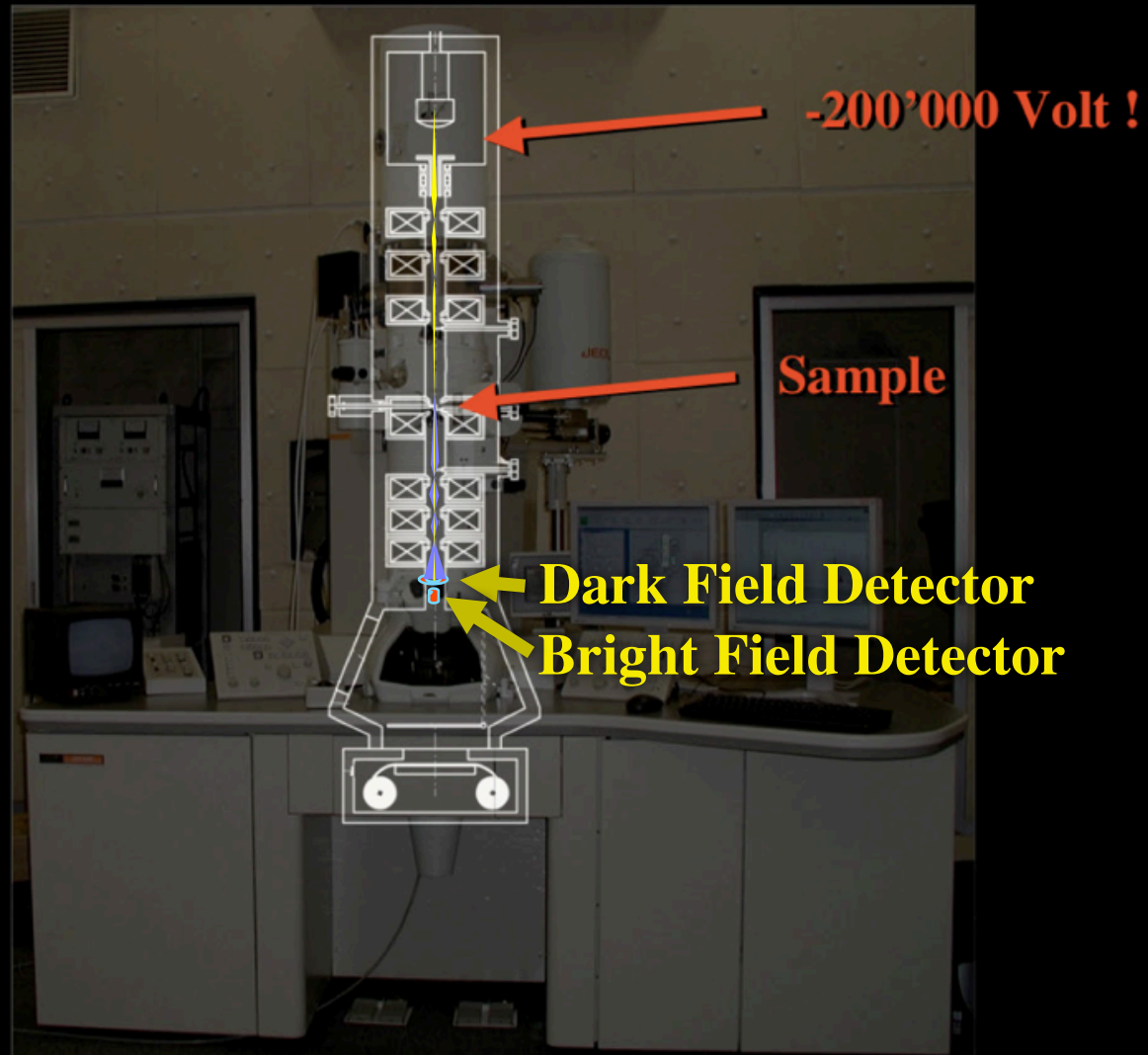
Low-dose:
Fast Electronics ($1 \mu\text{s} / \text{pixel}$)
Beam Chopper

Phase Contrast:
Aberration correction

Data Collection:
8k x 8k images,
multi-channel detector system
(HAADF, DF, Pilatus)

Proposed Phase-Contrast BioSTEM

STEM



Proposed Phase-Contrast BioSTEM

-200

STEM operation in upper column

TEM operation in lower column

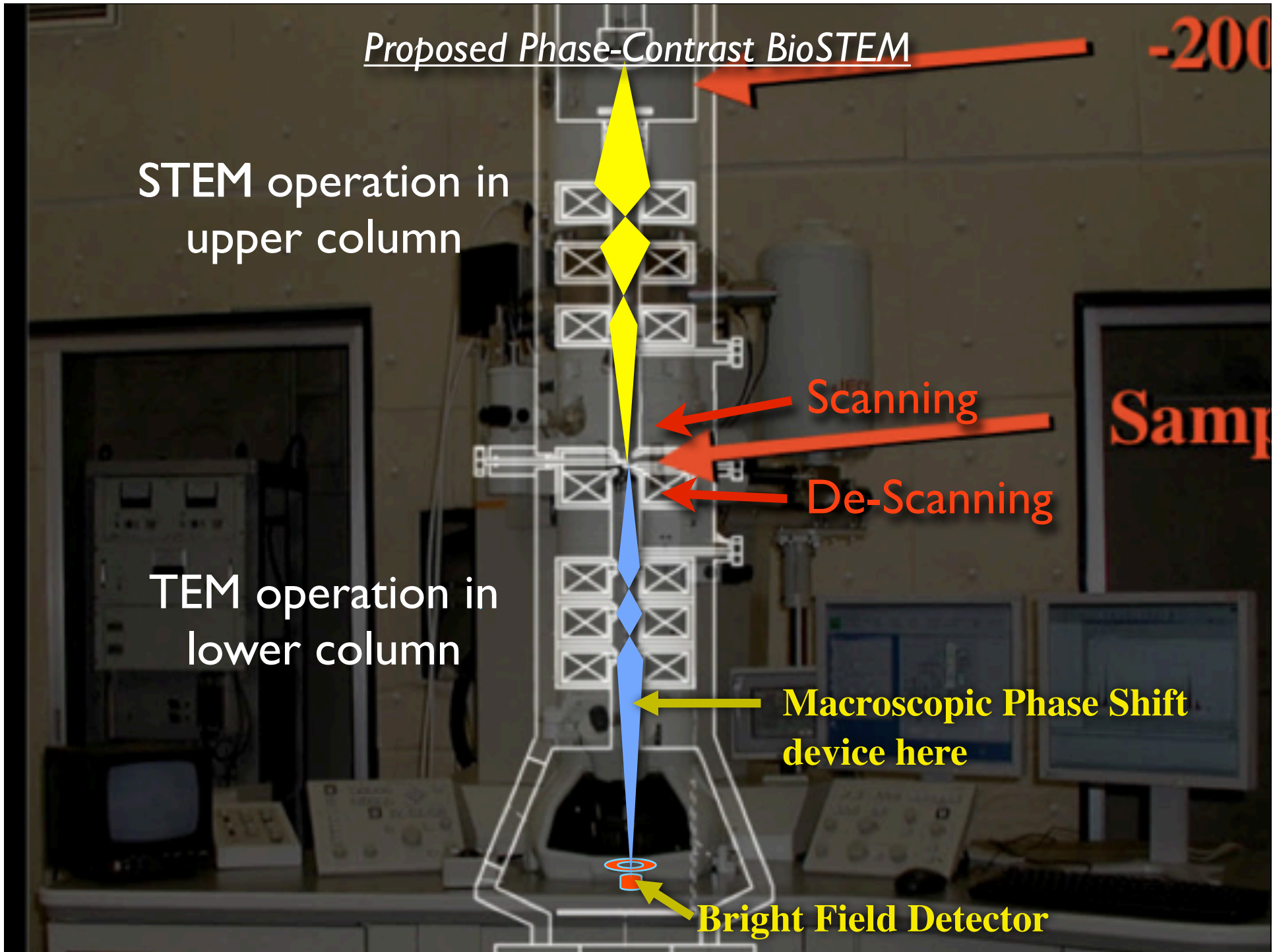
Scanning

De-Scanning

Macroscopic Phase Shift device here

Bright Field Detector

Sample

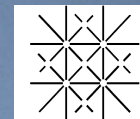


Summary

- The Problem:
 - Imaging tilted specimens suffers from beam-induced resolution loss
- The Cause:
 - Physical specimen movement (and charging?)
- Solutions:
 - Symmetric sample preparation: Carbon sandwich
 - SpotScan TEM imaging
 - STEM
 - Beam chopper for low dose.
 - Phase contrast is required. Possible avenues (?):
 - Multi-channel detector on Cs-corrected STEM
 - Phase plate on with BF-detector-focussed descanned-STEM
 - Pre-illumination
 - **Filming the sample during TEM imaging**



Center for Cellular Imaging and Nano Analytics



Uni Basel

Basel, Switzerland

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C-CINA.org

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