CINA

CINA
Cellular Imaging
and Nano Analytics

SpotScan TEM *vs.* STEM

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Biozentrum, University Basel

Switzerland

BIOZENTRUM
Universität Basel
The Center for Molecular Life Sciences

Uni Basel

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

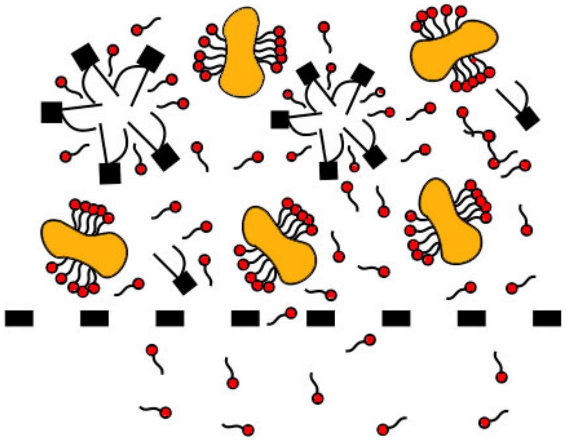
Bob Glaeser, Discussion Leader

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-spotters, what else? These additional features are expensive! Speakers are charged to give a critical review of:

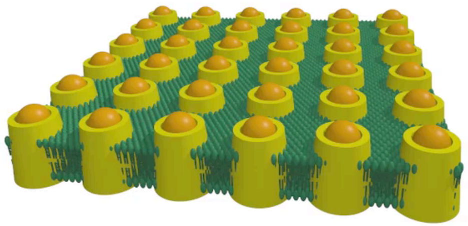
Are they worth it? What do they get us? What does the future hold?

Membrane Protein 2D Crystallization by Dialysis

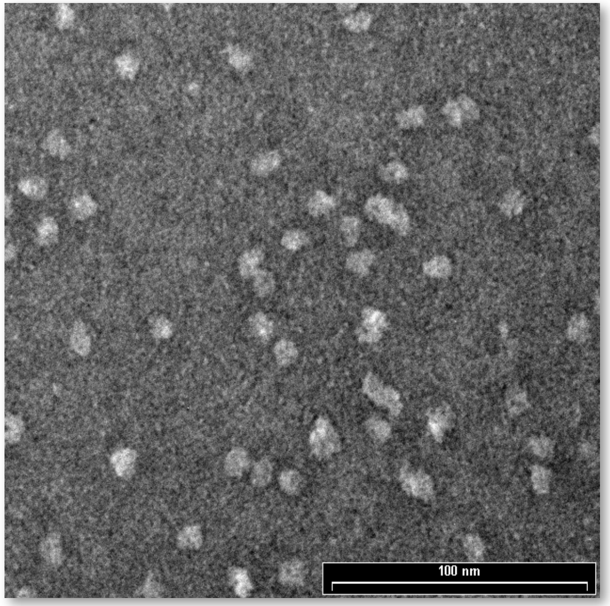


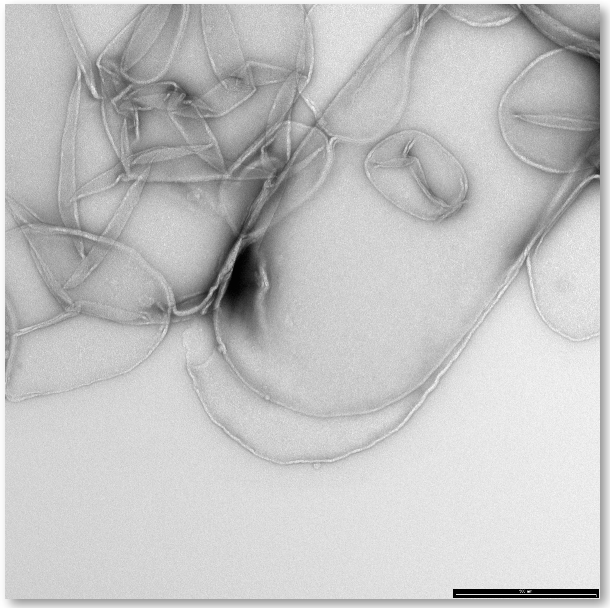
Detergent removal

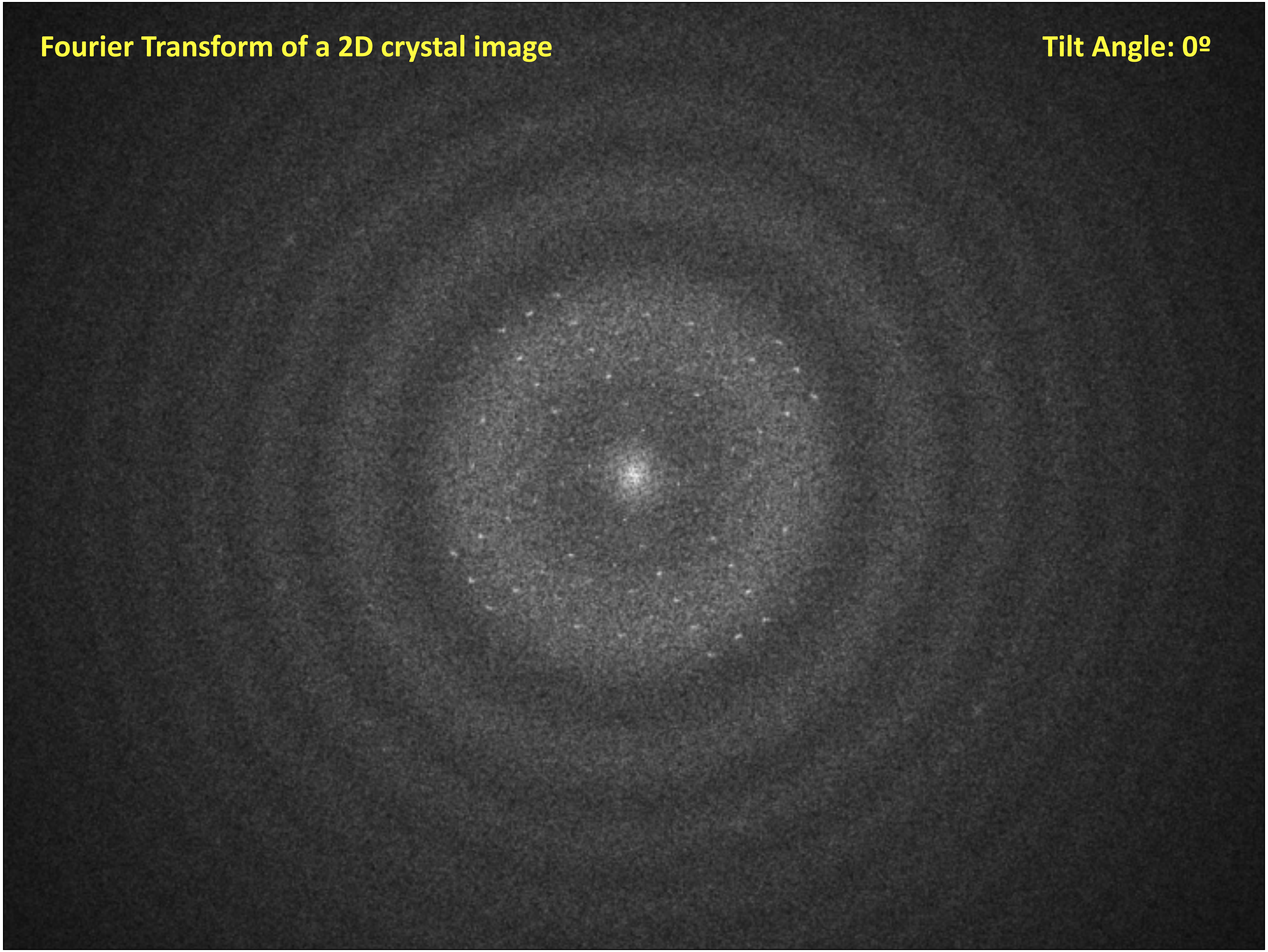
2D crystal formation



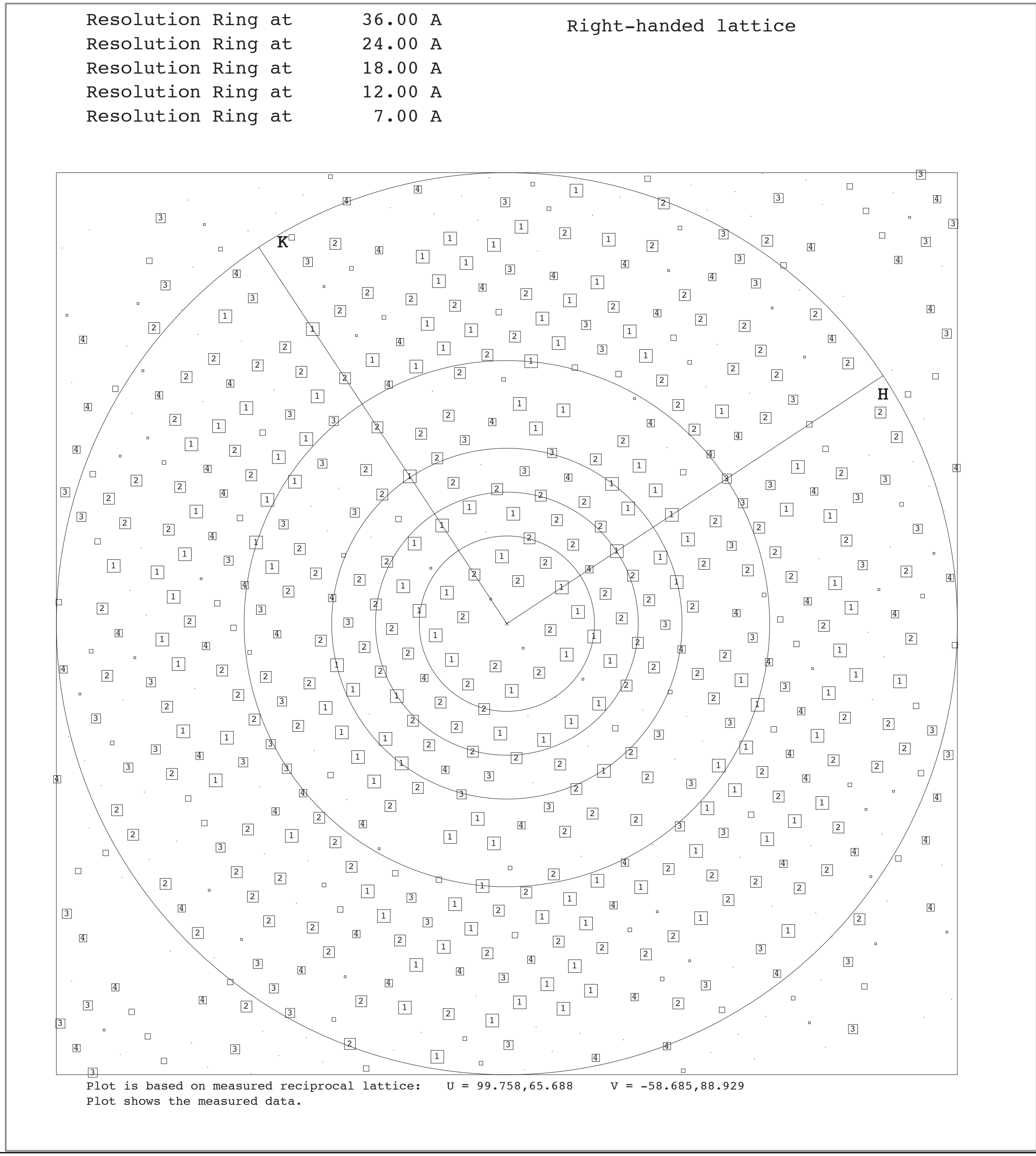
2D crystal





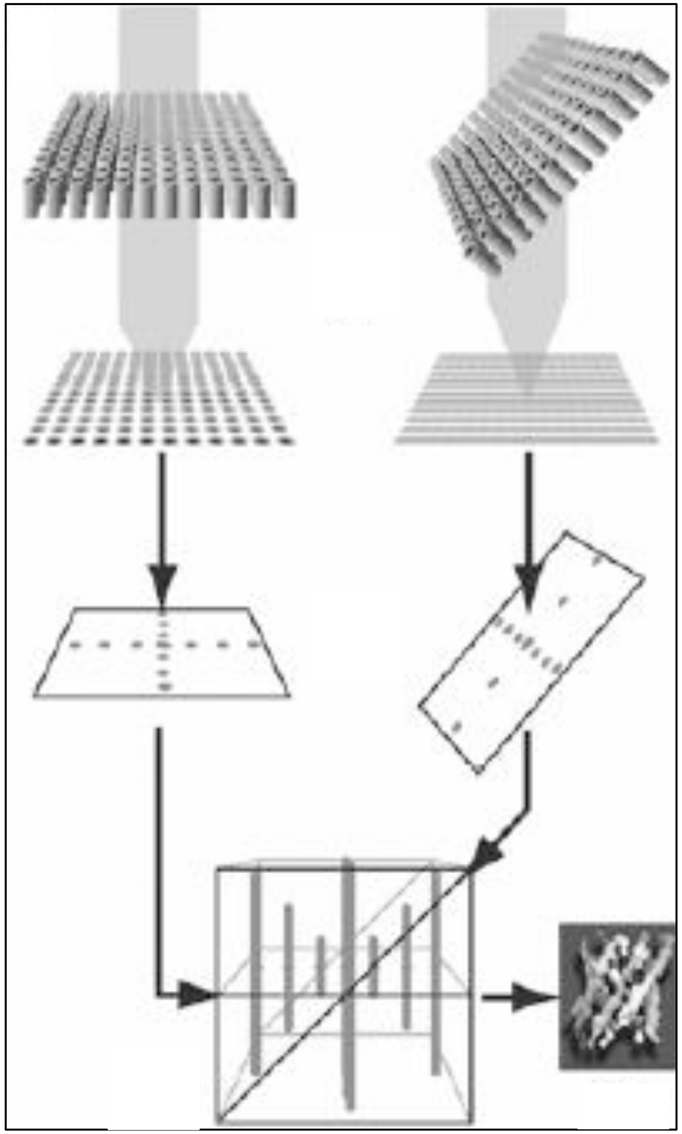

Fourier Transform of a 2D crystal image
Tilt Angle: 0º

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

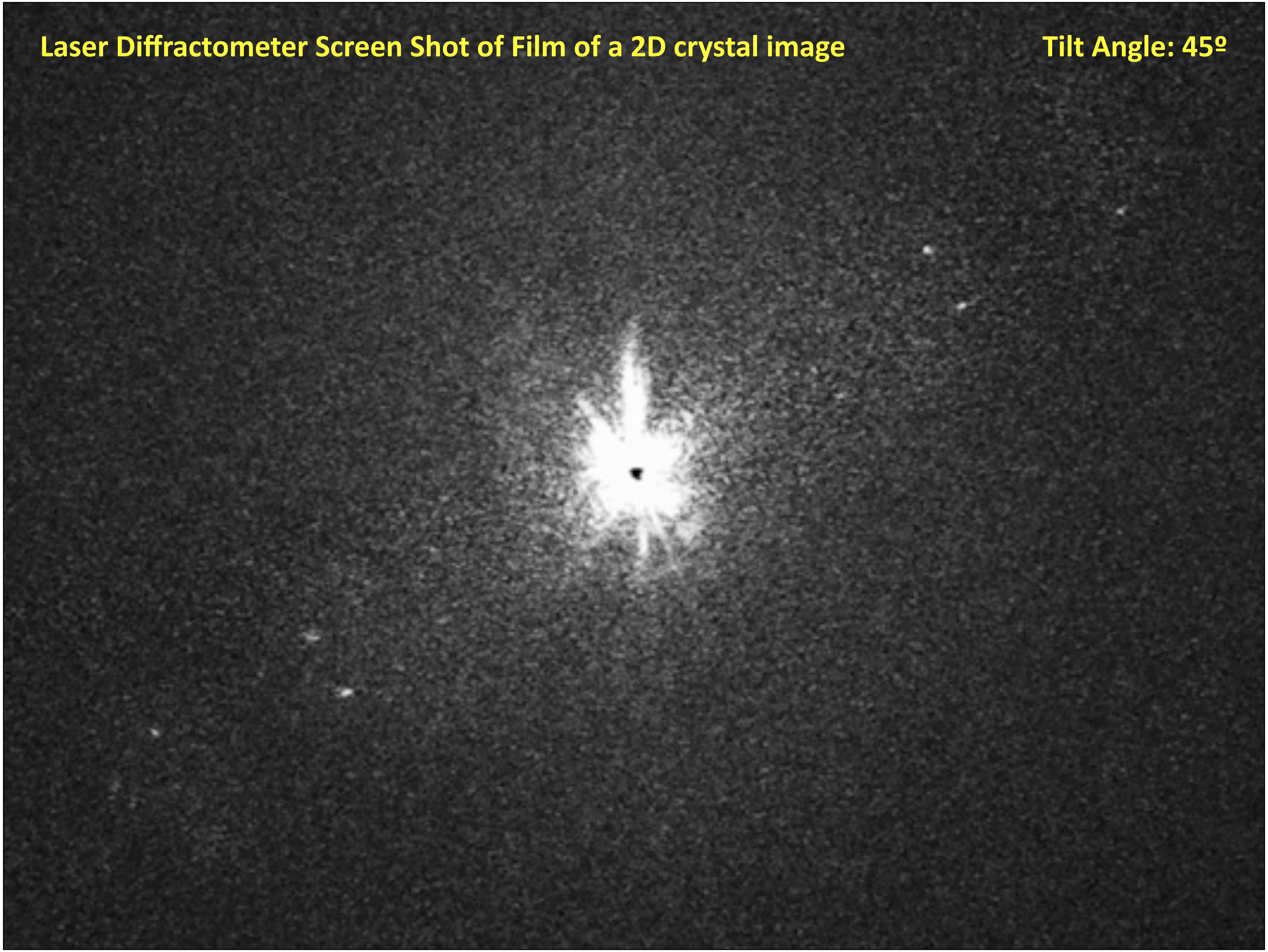


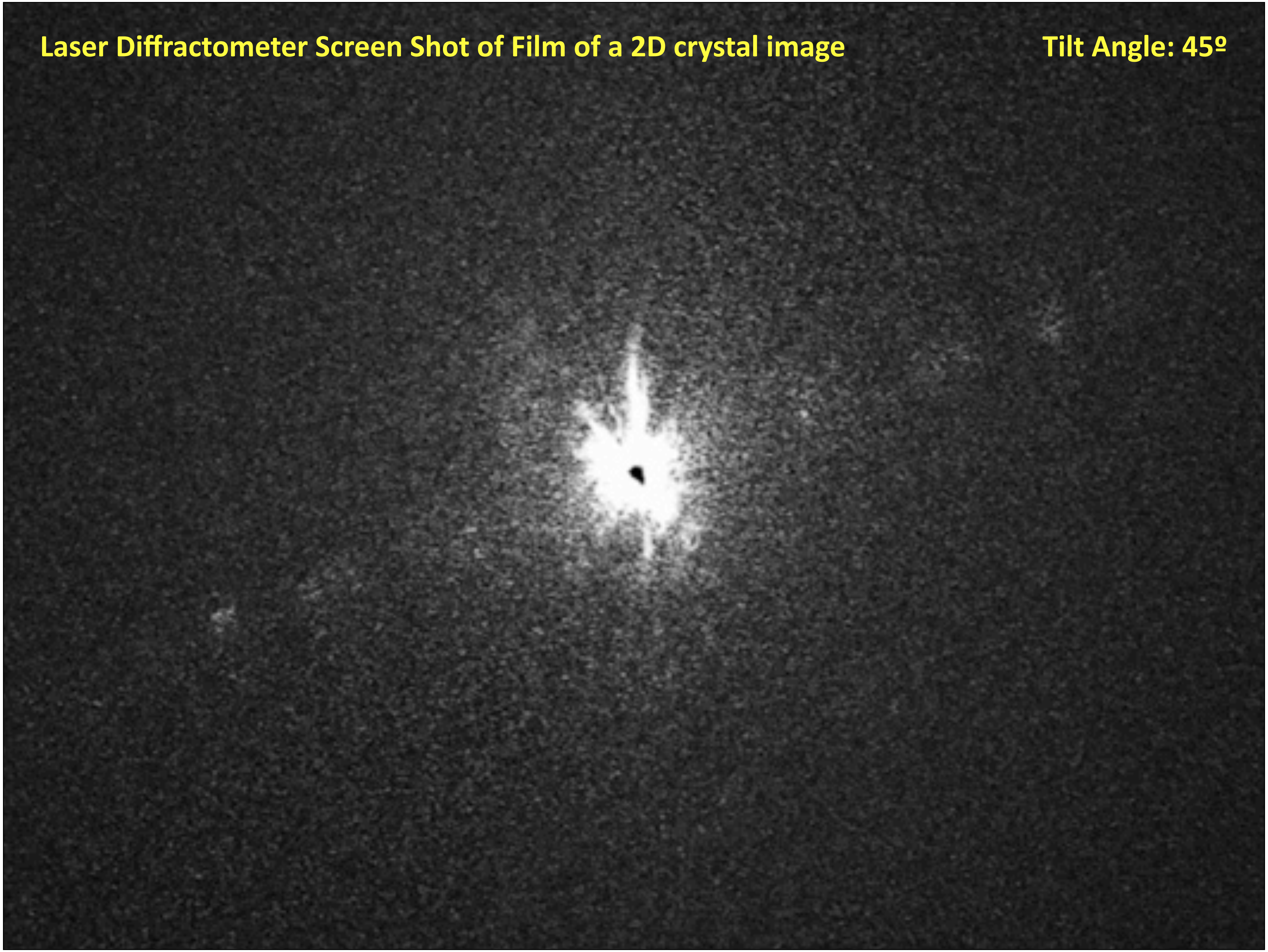
Electron crystallography: The third dimension

Tilting: Filling the Fourier space



www.iimcb.gov.pl/itn/research_method.html

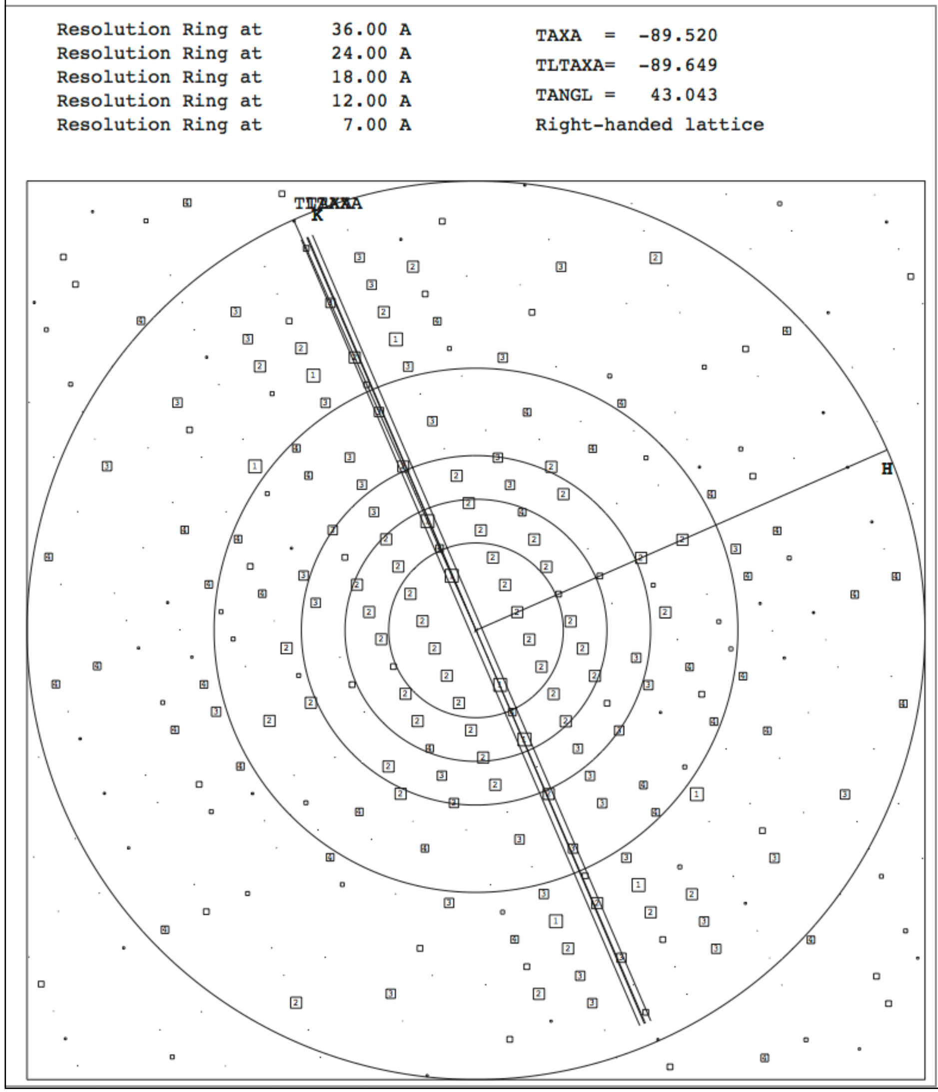

Laser Diffractometer Screen Shot of Film of a 2D crystal image
Tilt Angle: 45º


Laser Diffractometer Screen Shot of Film of a 2D crystal image
Tilt Angle: 45º

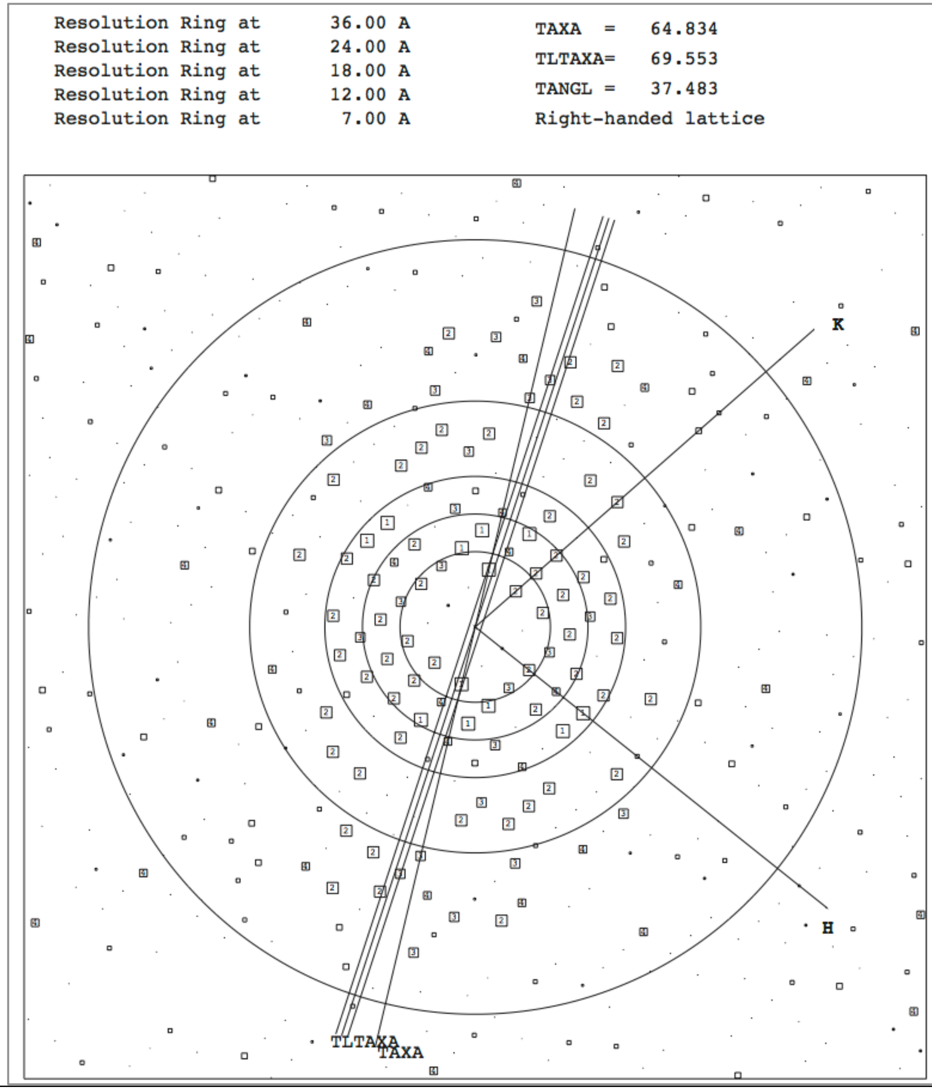
IQ plots (~FFT) of tilted 2D crystals

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

45 degree tilt

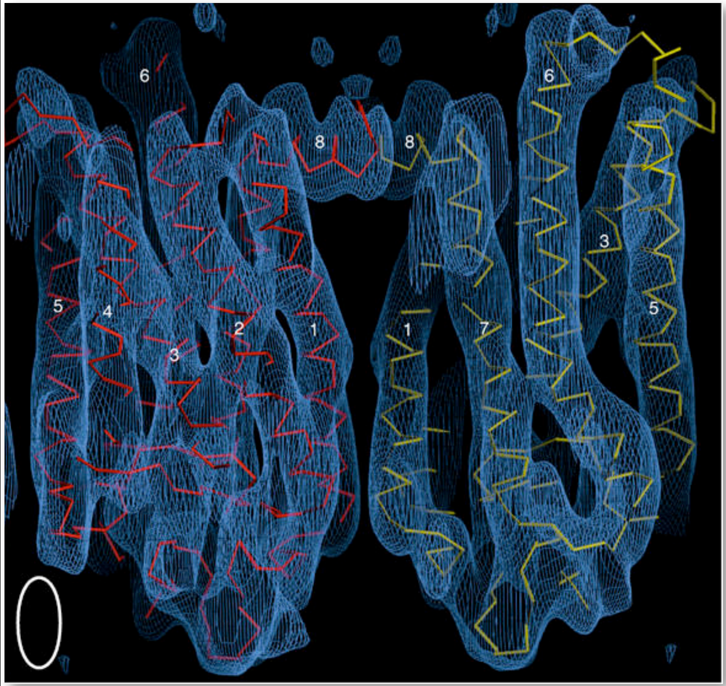


40 degree tilt



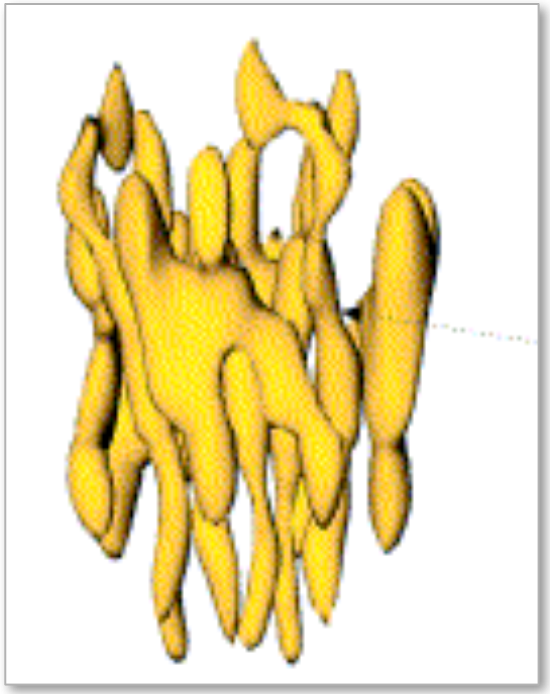
The third dimension: x,y / z Anisotropy

Metarhodopsin: 5Å vs. 12Å



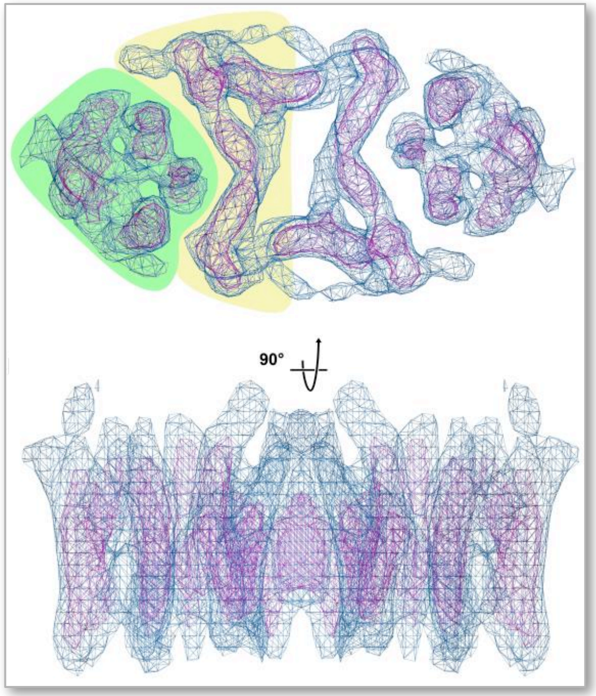
Ruprecht *et al* (2004)**, EMBO J.** 23 (18), 3609-3620

Glutathion Trans.: 5Å vs. 15Å



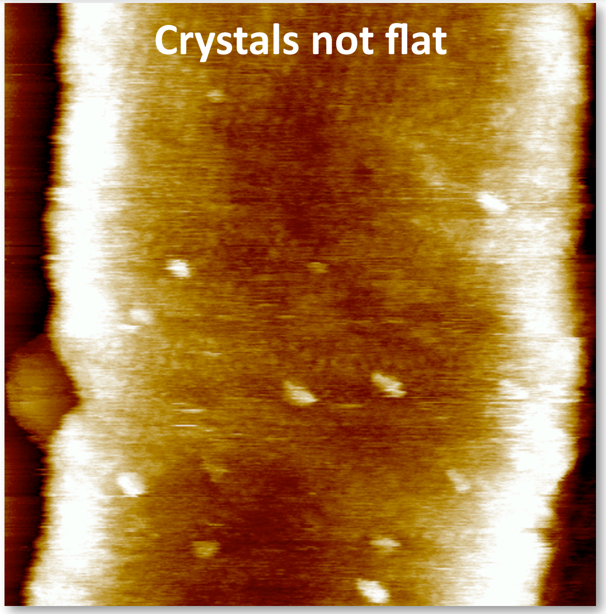
Holm *et al* (2002)**, *Biochim. Biophys. Act.*** 1594, 276-85

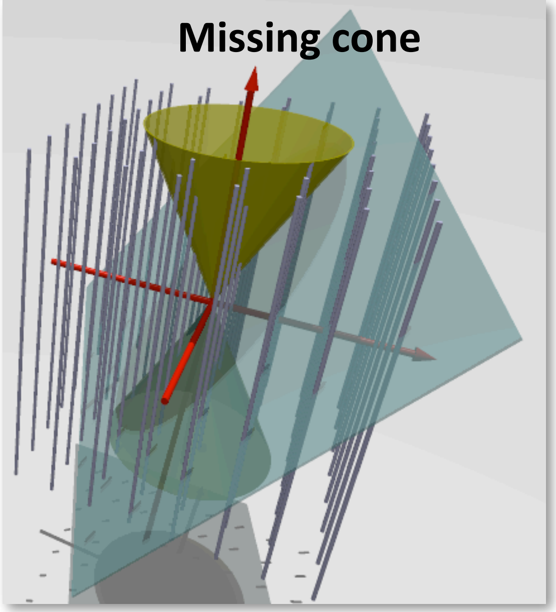
NhAP1: 6Å vs. 15Å

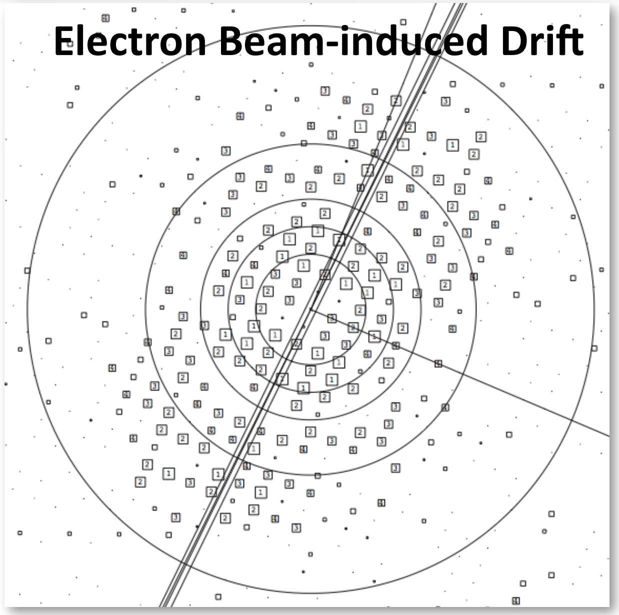


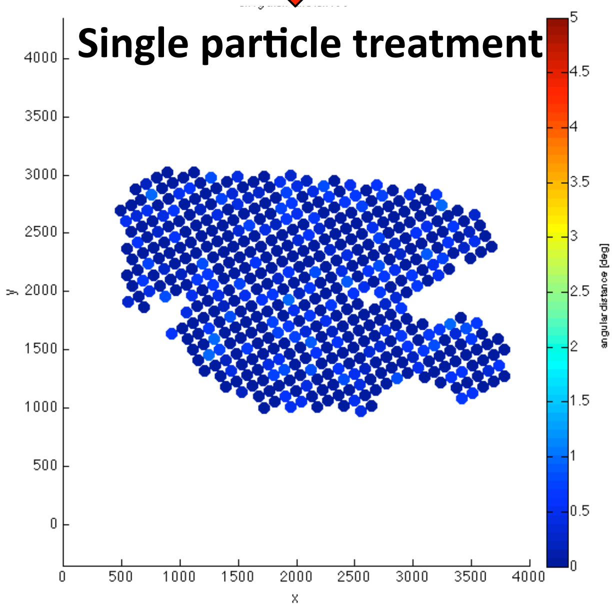
Goswami *et al* (2011)**, *EMBO J.*** 30(2) 439-449

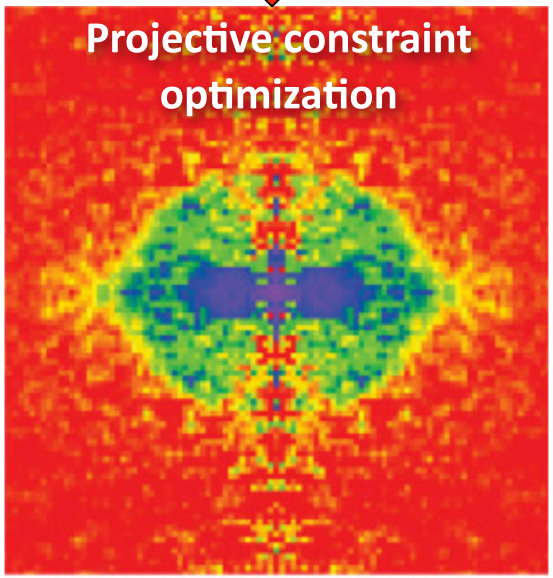
x,y / z Anisotropy: Reasons & Solutions



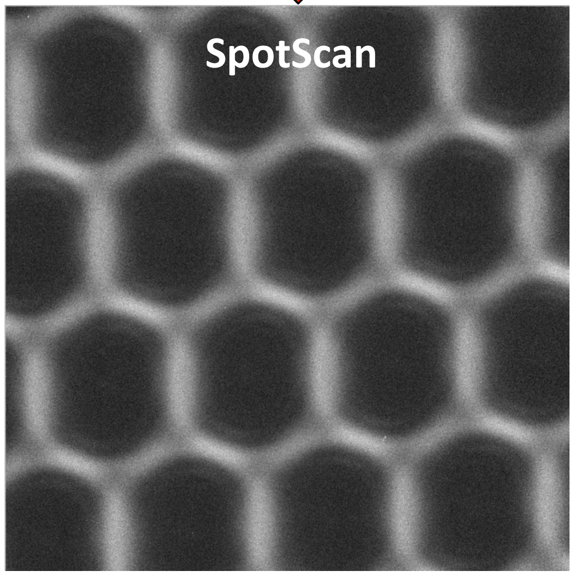








Gipson *et al* ***Phys Rev.E*** 84 011916 (2011)

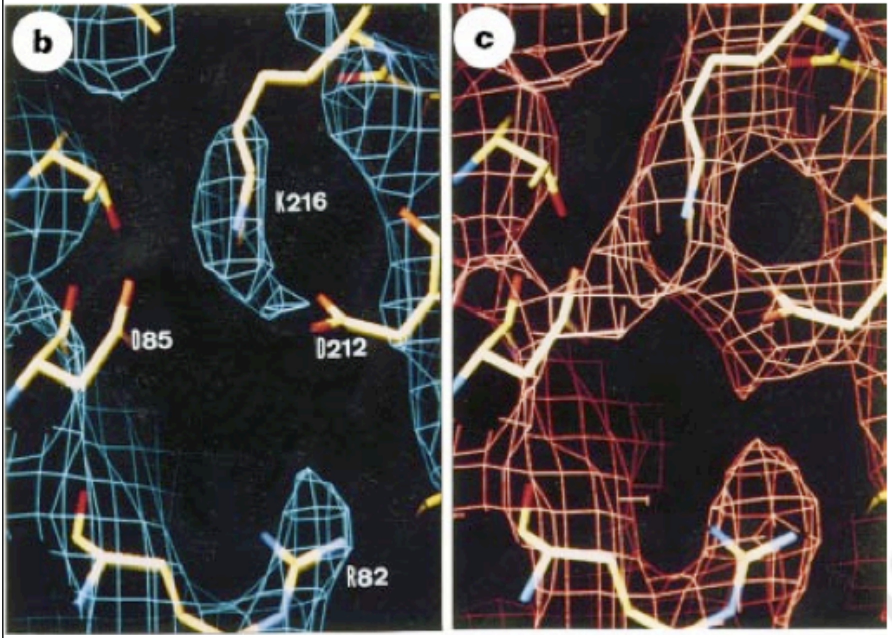


Downing, ***Science*** 251(4989), 53-59 (1991)

Projective Constraint Optimization

Bacteriorhodopsin, Schiffbase region
(data from Mitsuoka / Fujiyoshi)

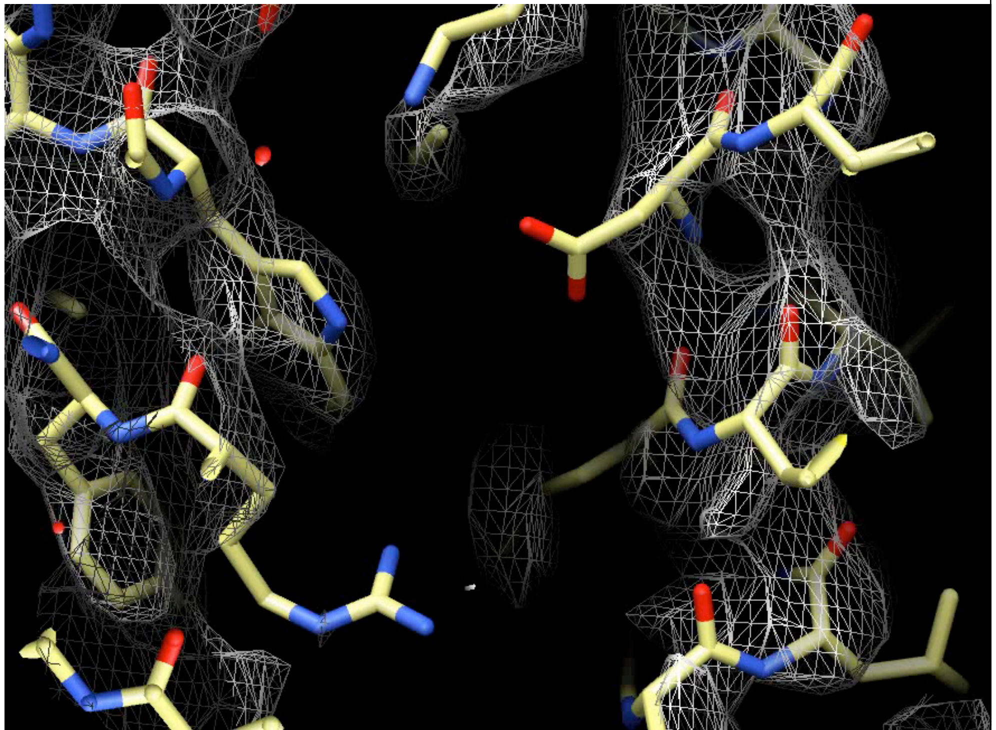
Overlaid atomic model: 1BRR
Essen et al 1998
(2.8 Å, XRD with lipids)



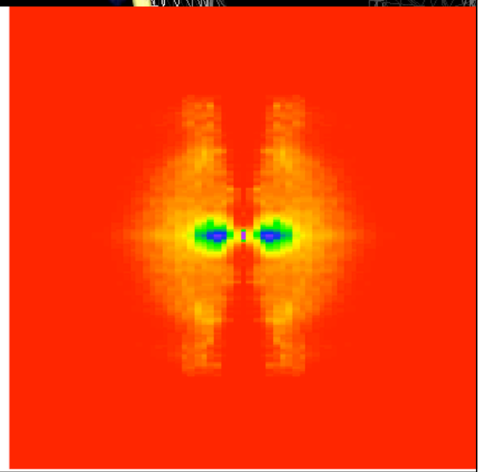
Total dataset

Only 7 - 3 Å

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

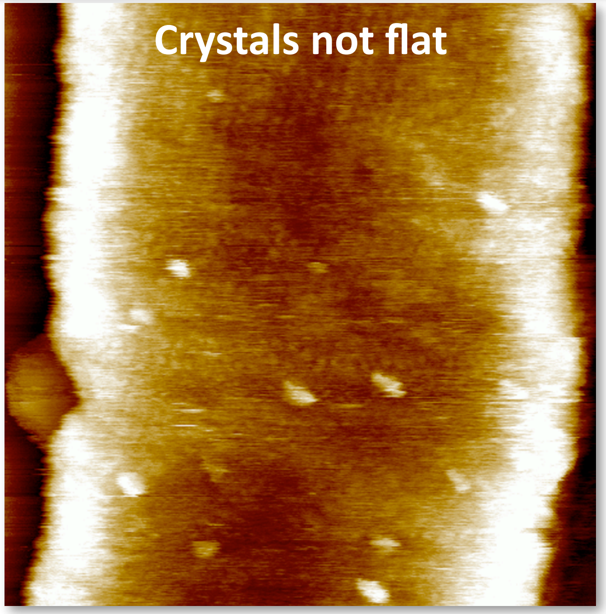


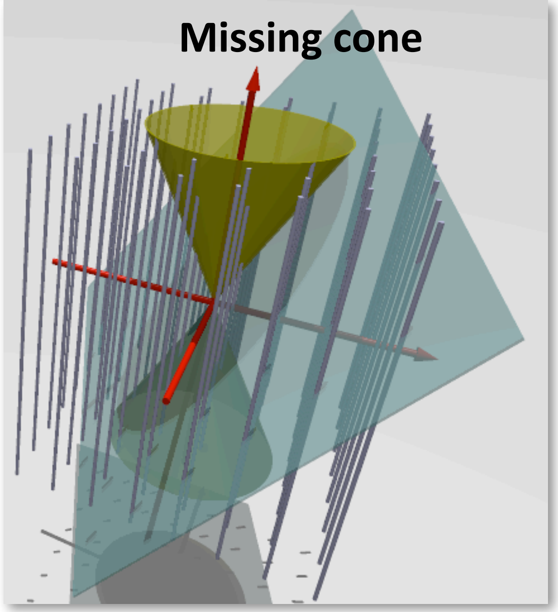
PCO: 100 initial rounds,
followed by 30 rounds
of edge detection

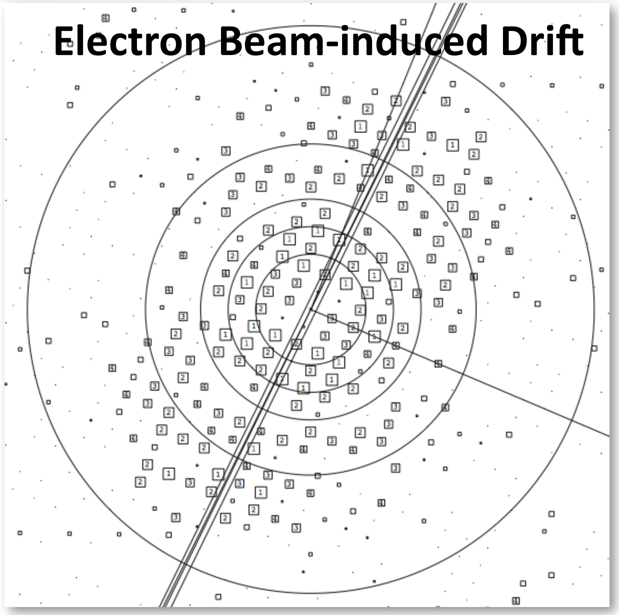


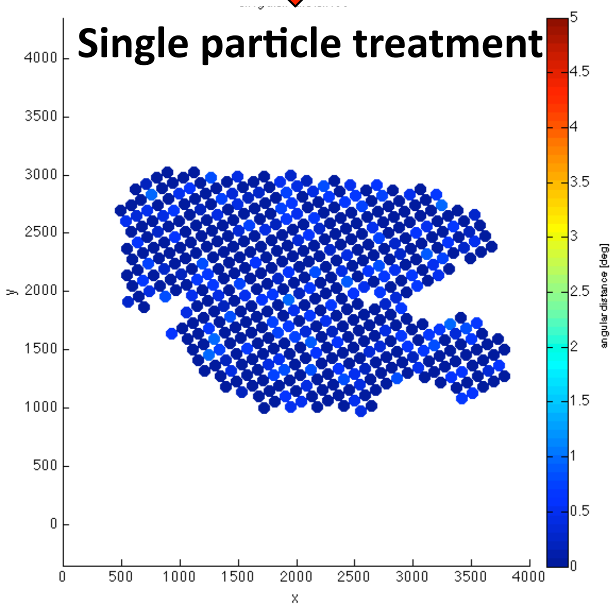
Gipson *et al* ***Phys Rev.E*** 84 011916 (2011)

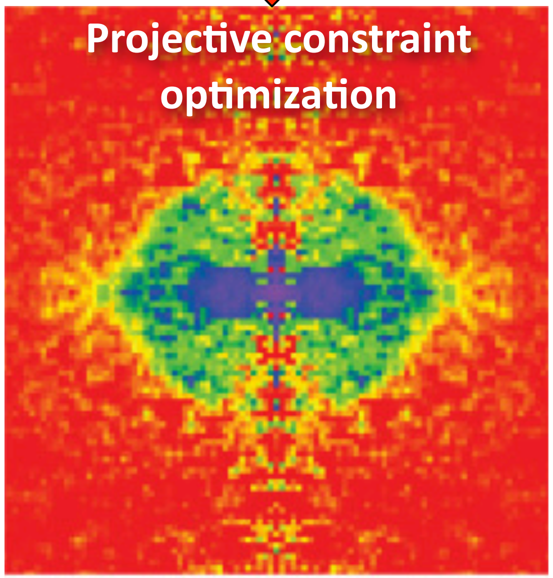
x,y / z Anisotropy: Reasons & Solutions











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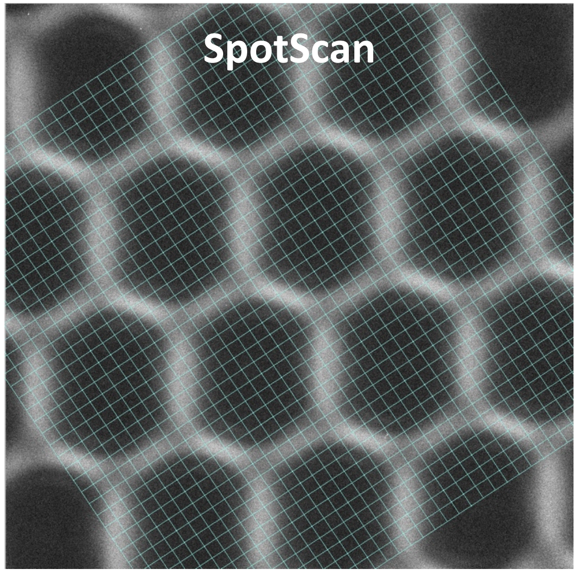
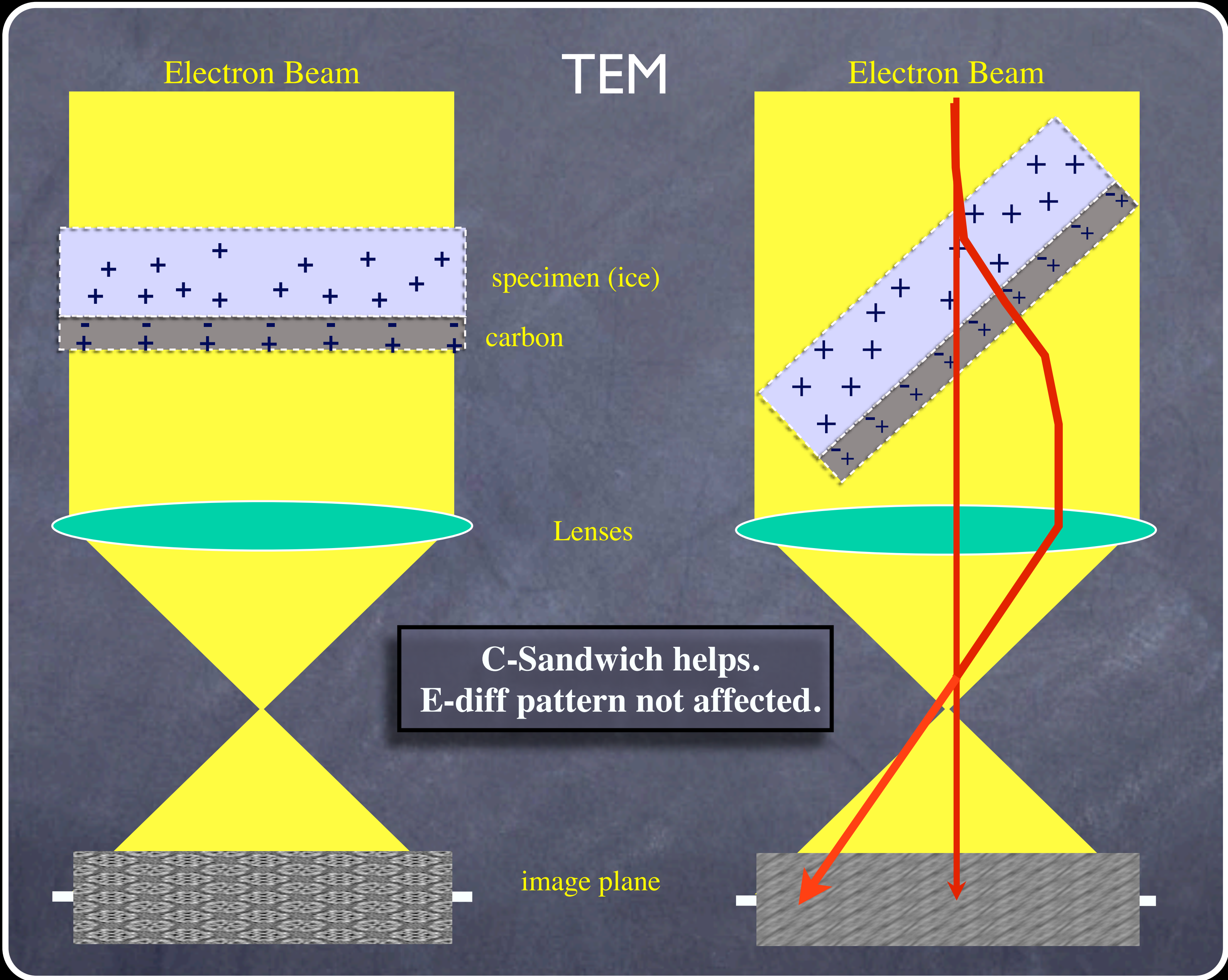


Downing, ***Science*** 251(4989), 53-59 (1991)

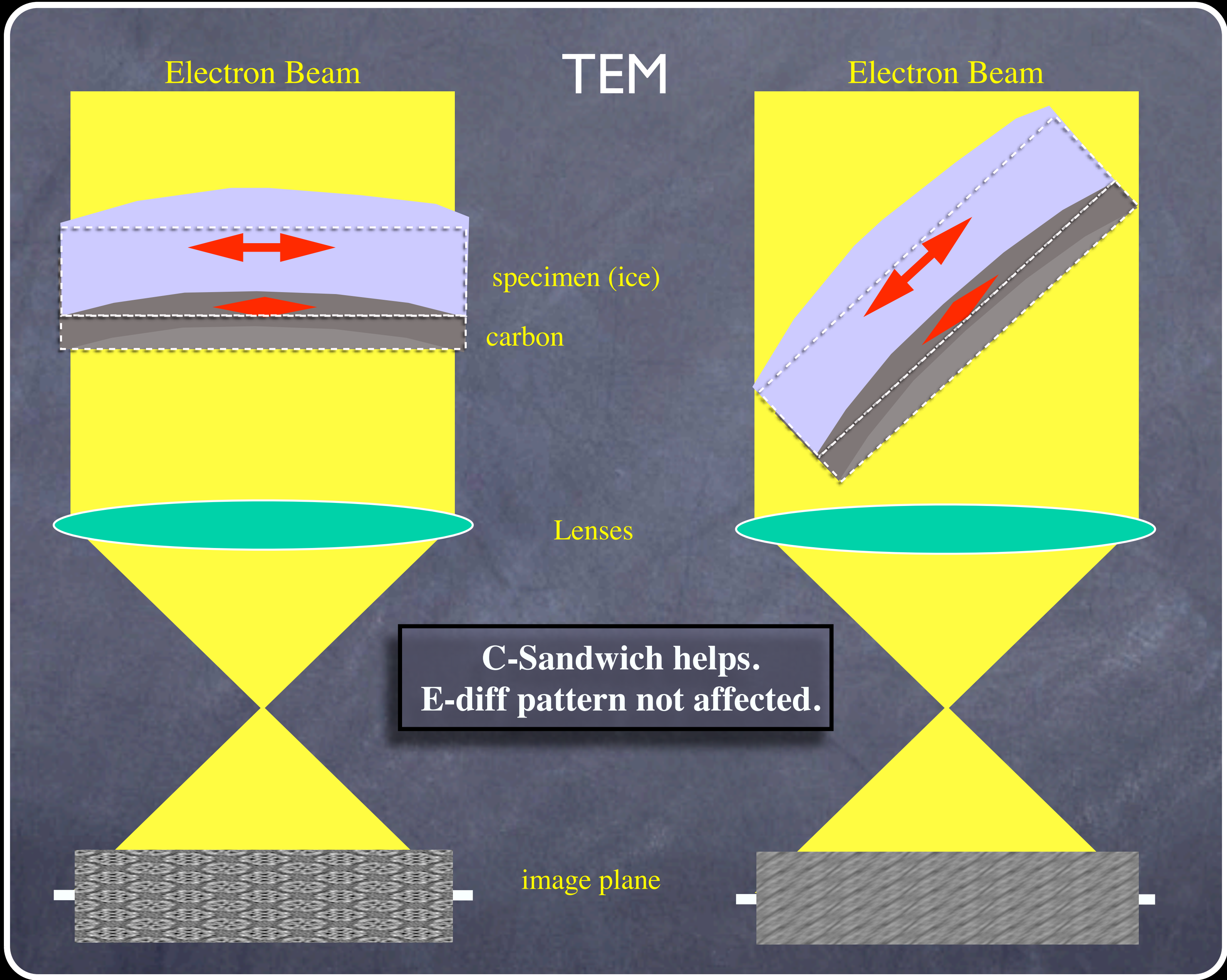
Cryo-EM of tilted samples: *Beam-induced resolution loss*

Is it charging?



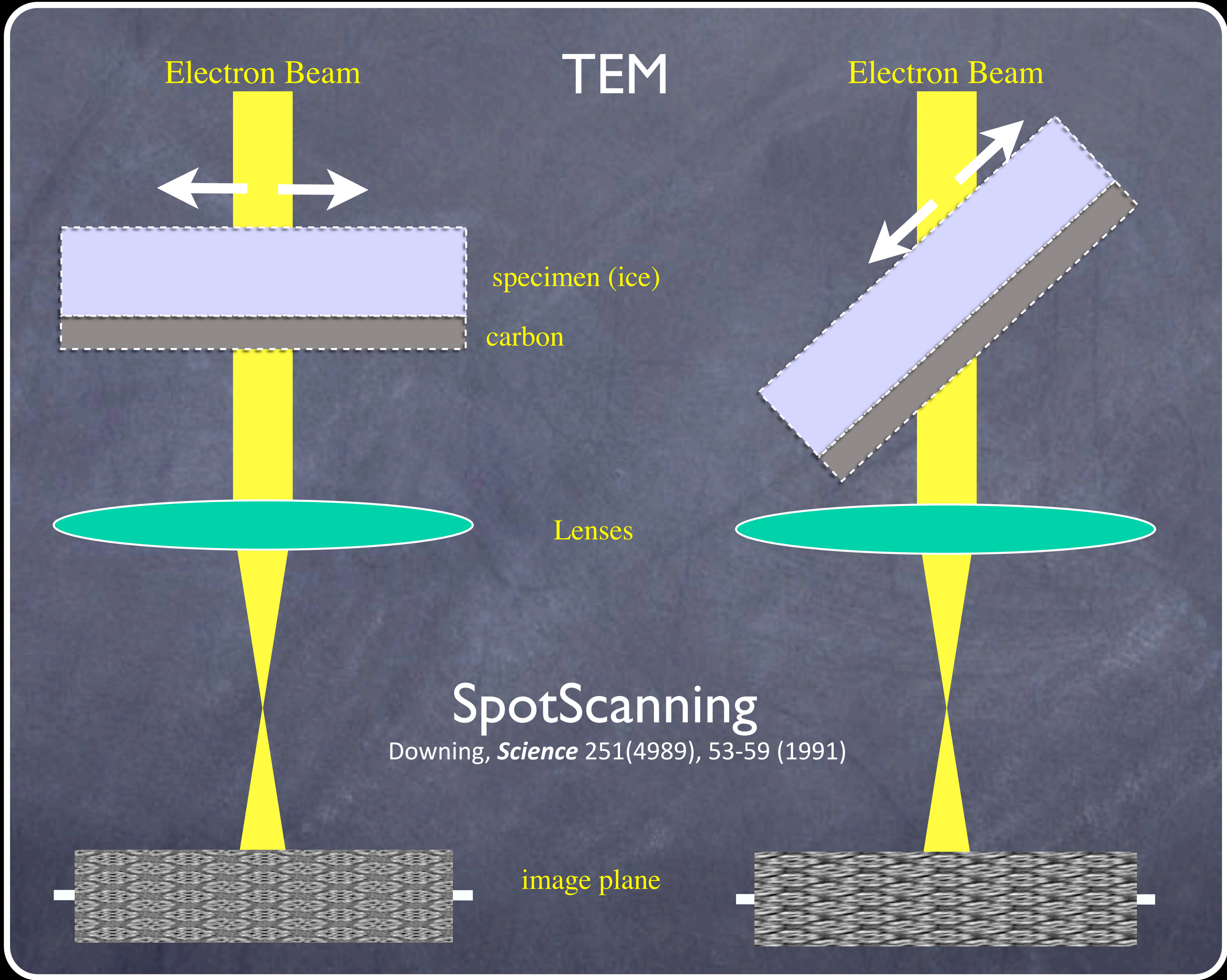
Cryo-EM of tilted samples: *Beam-induced resolution loss*

Or is it physical movement?



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

Anyway, SpotScan helps in both cases.

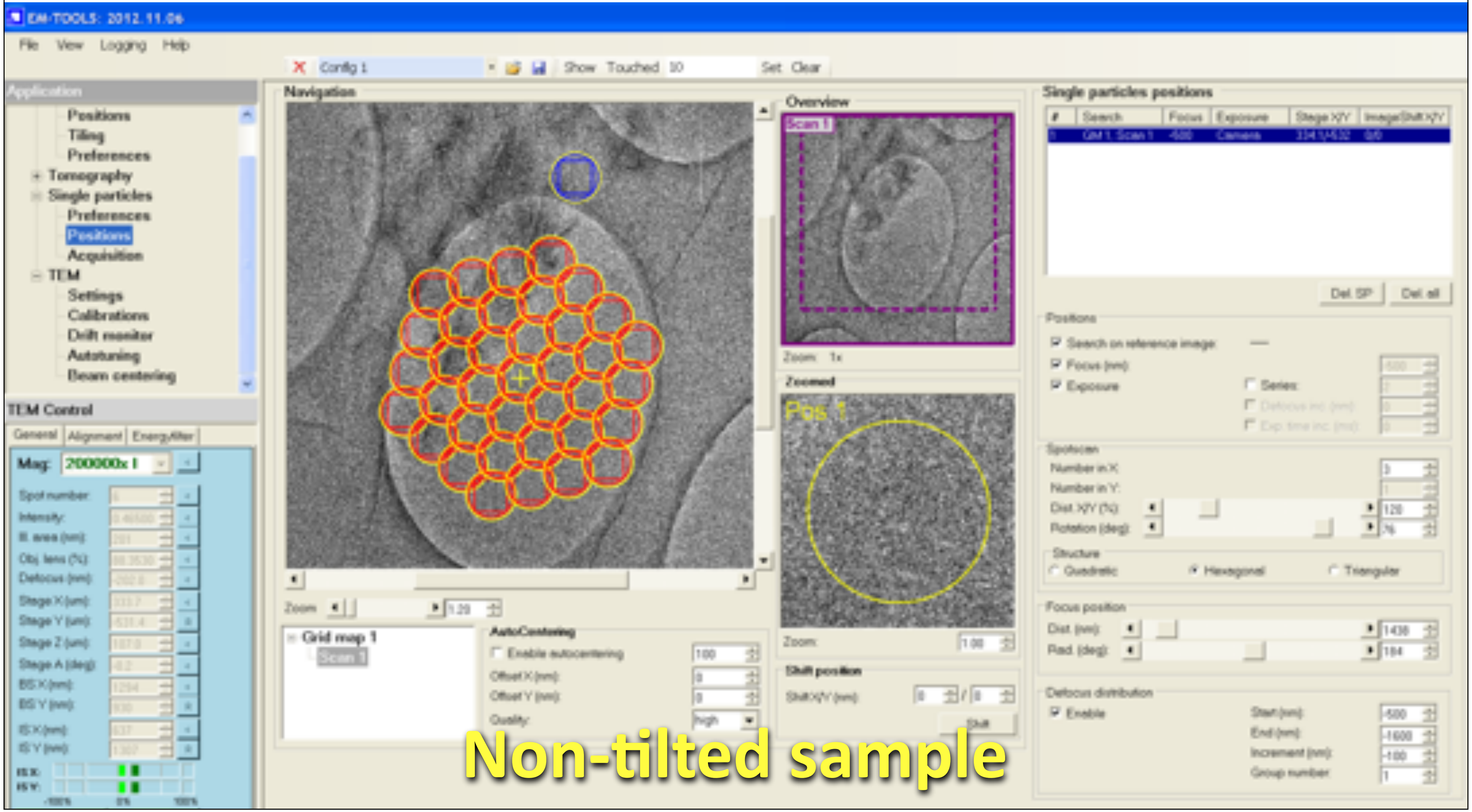


Camera-based SpotScaning

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.



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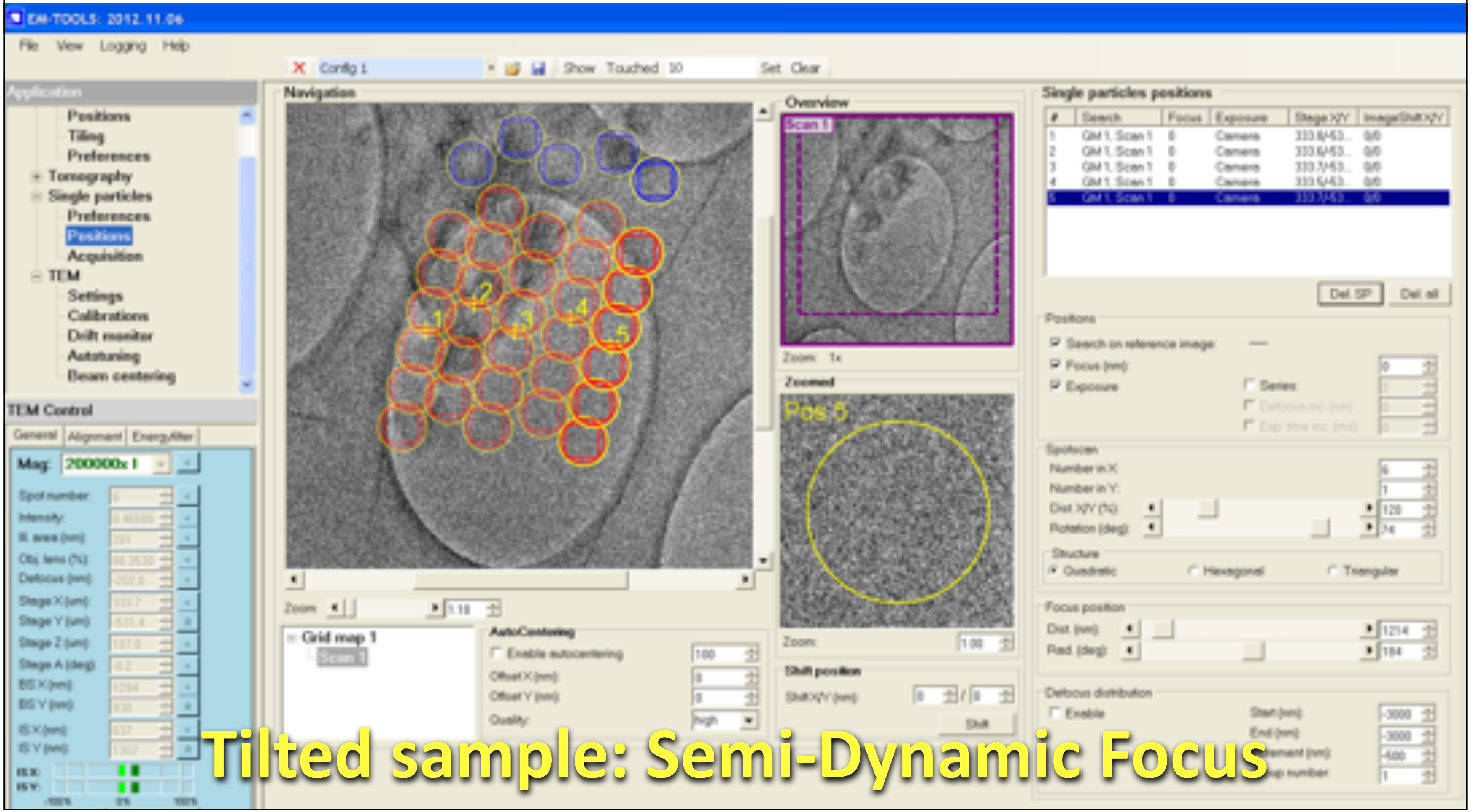
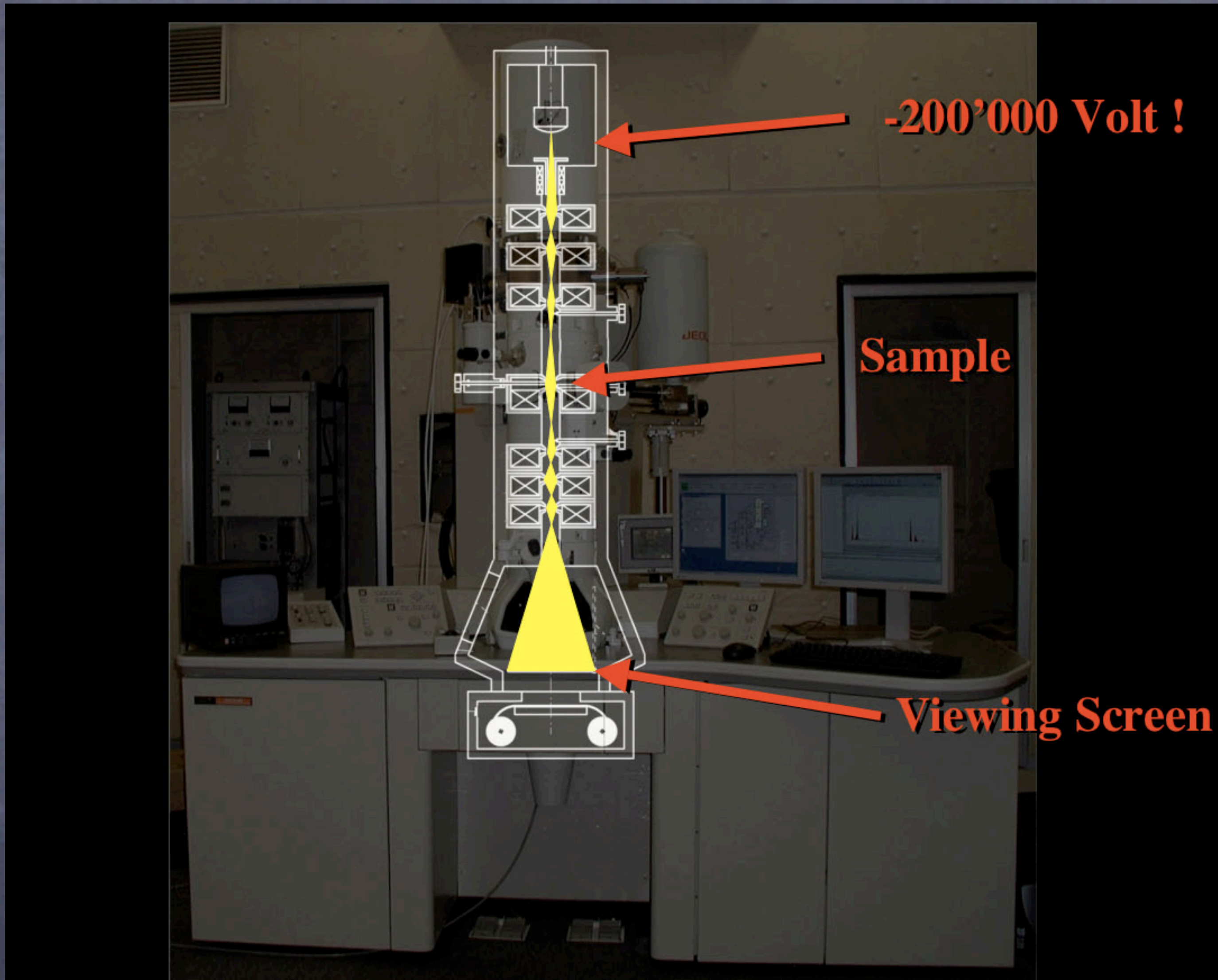
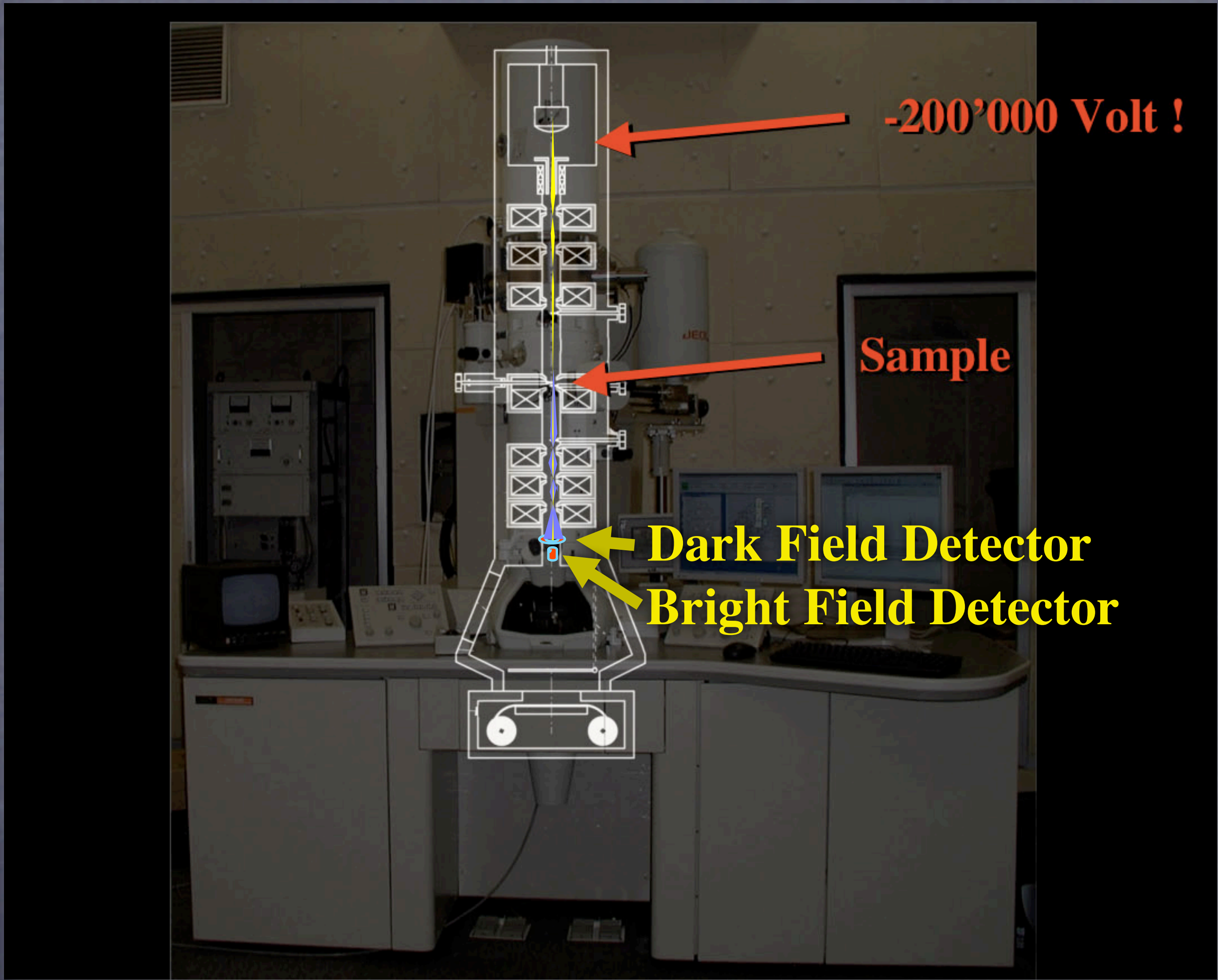


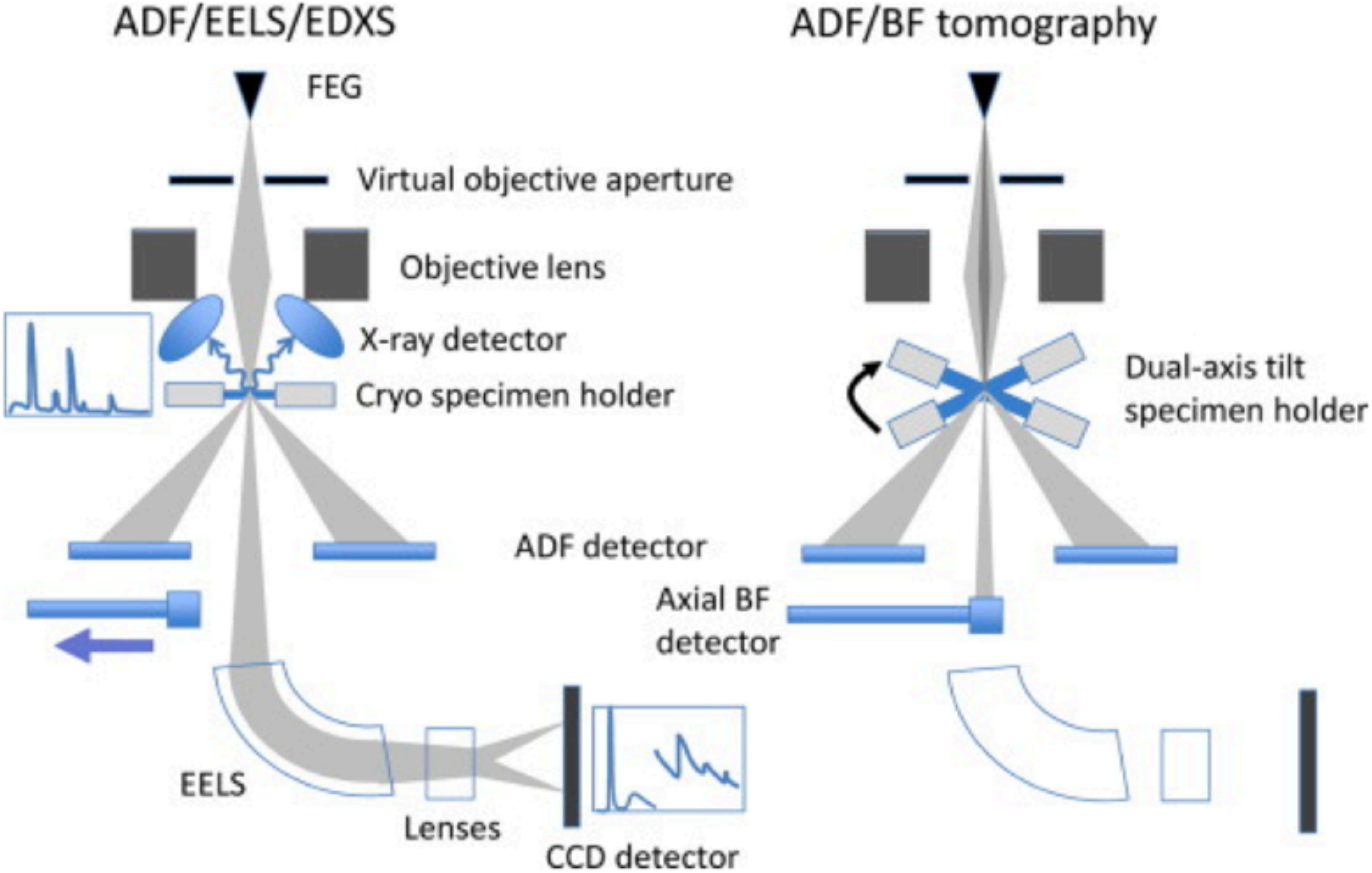
Transmission Electron Microscope



Scanning Transmission Electron Microscope



STEM applications for biological specimens

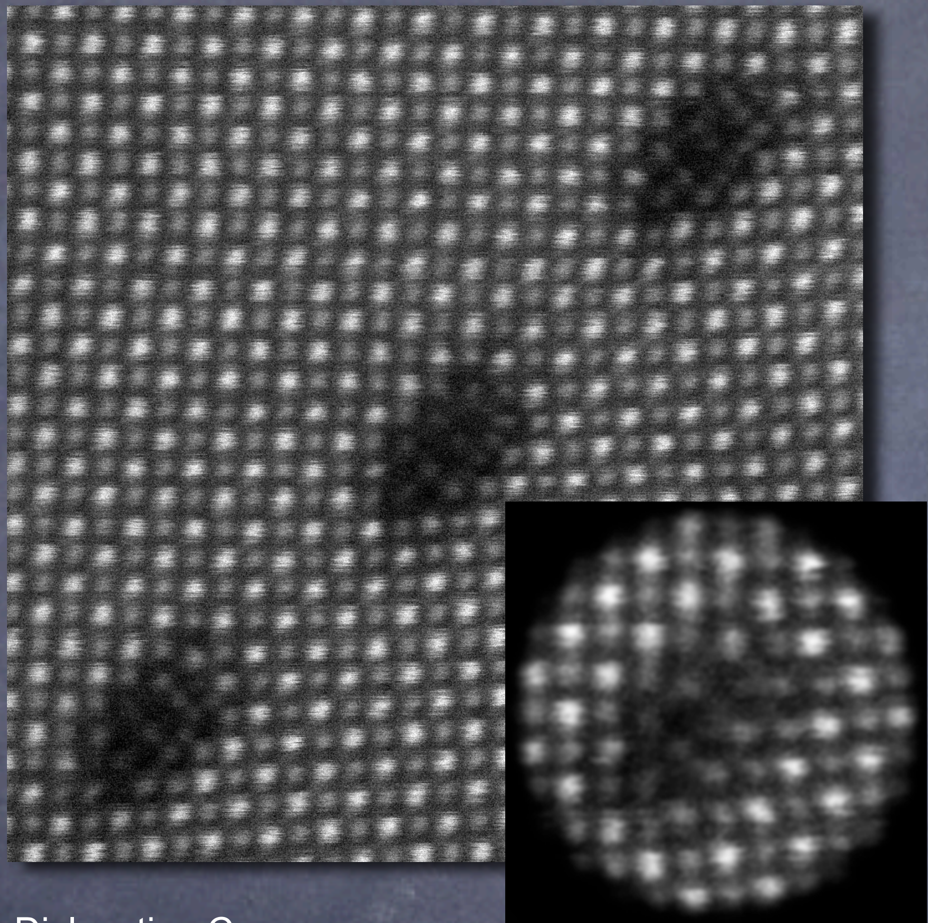


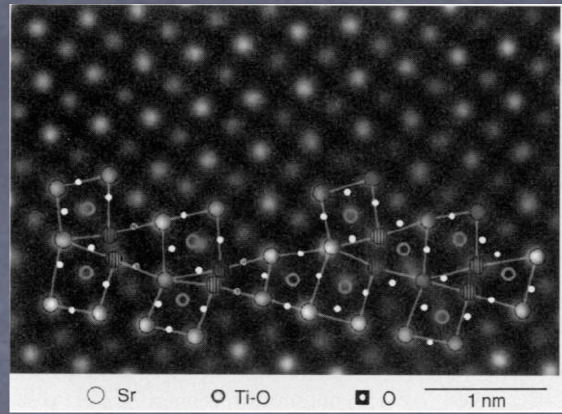
Sousa & Leapman, Ultramicroscopy (2012)

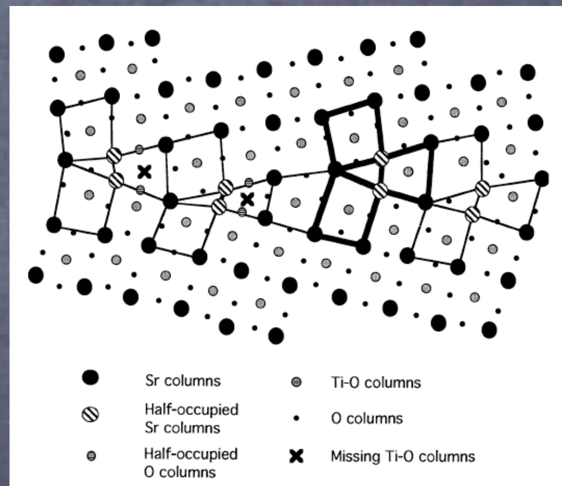
Cs-corrected HAADF STEM (Z-contrast) gives highest resolution.

STEM of $SrTiO_3$

Resolution < 1Å







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

Dislocation Cores
with Nigel Browning

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

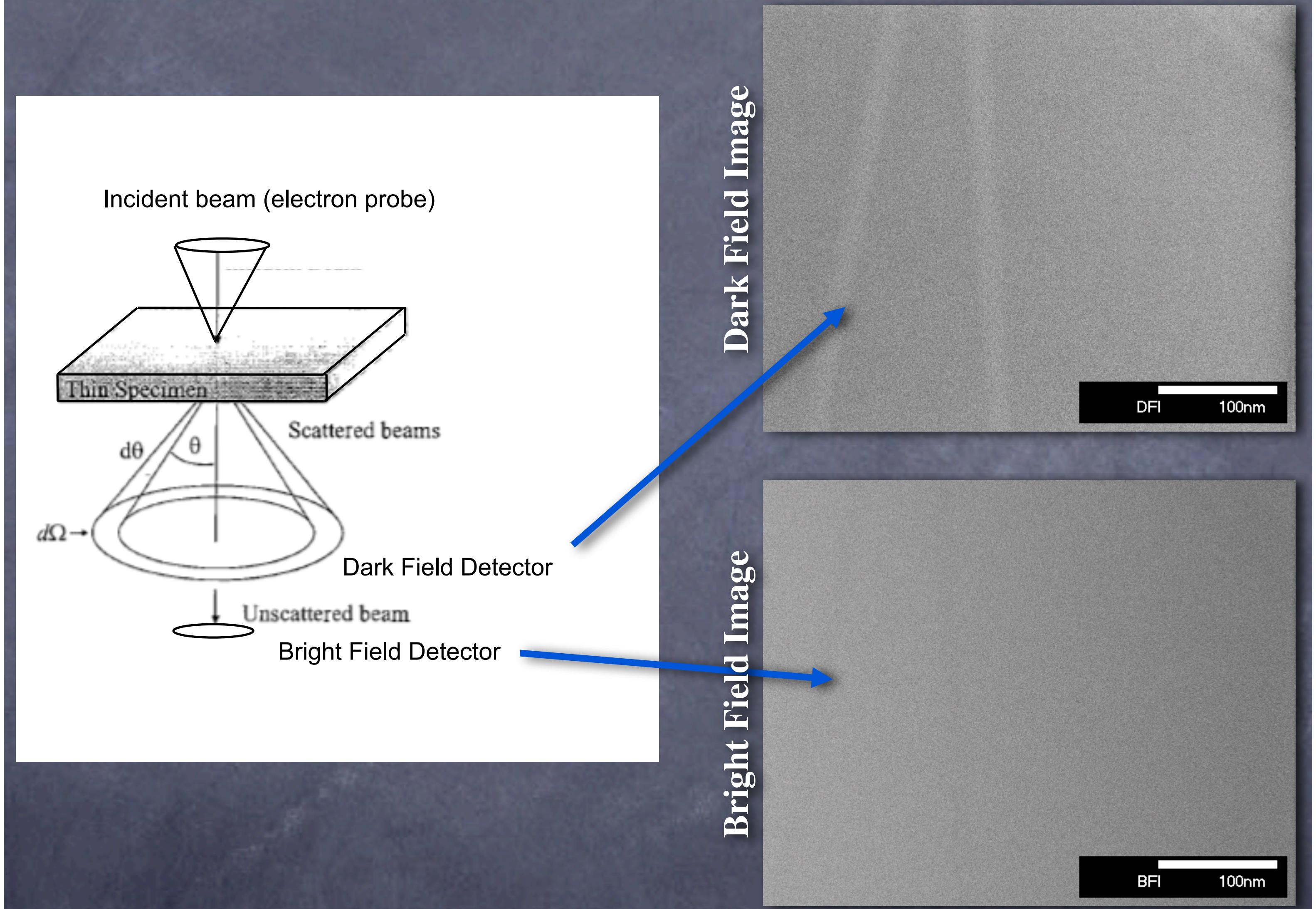
McGibbon et al. Science 226 (1994) 102

Atomic structure model for a 25° [001] symmetric tilt grain boundary in $SrTiO_3$

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

(but good for Mass Measurements)

TMV in vitreous ice



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STEM has no apparent resolution loss on tilted samples.

Here:

- **Gatan 626 holder**
- **TMV in vitreous ice**
- **Tilt pair: 0º, 45º**

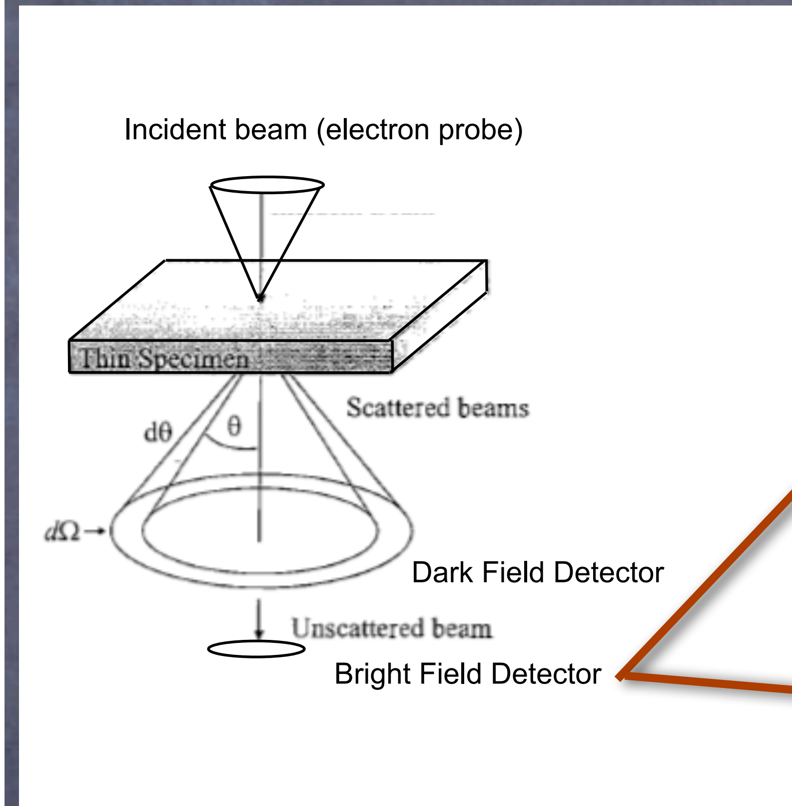
High resolution ✓

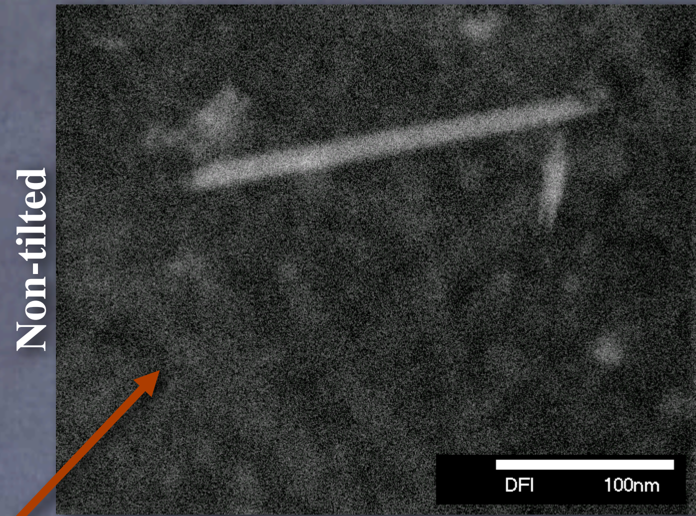
Tilted samples

Low dose

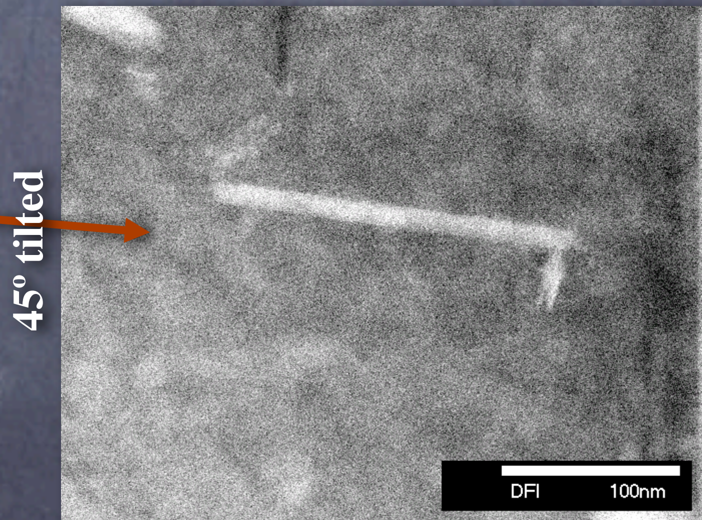
High contrast

TMV in vitreous ice (heavily contaminated)





Both are Dark Field images



STEM can be done at low dose.

Here:

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

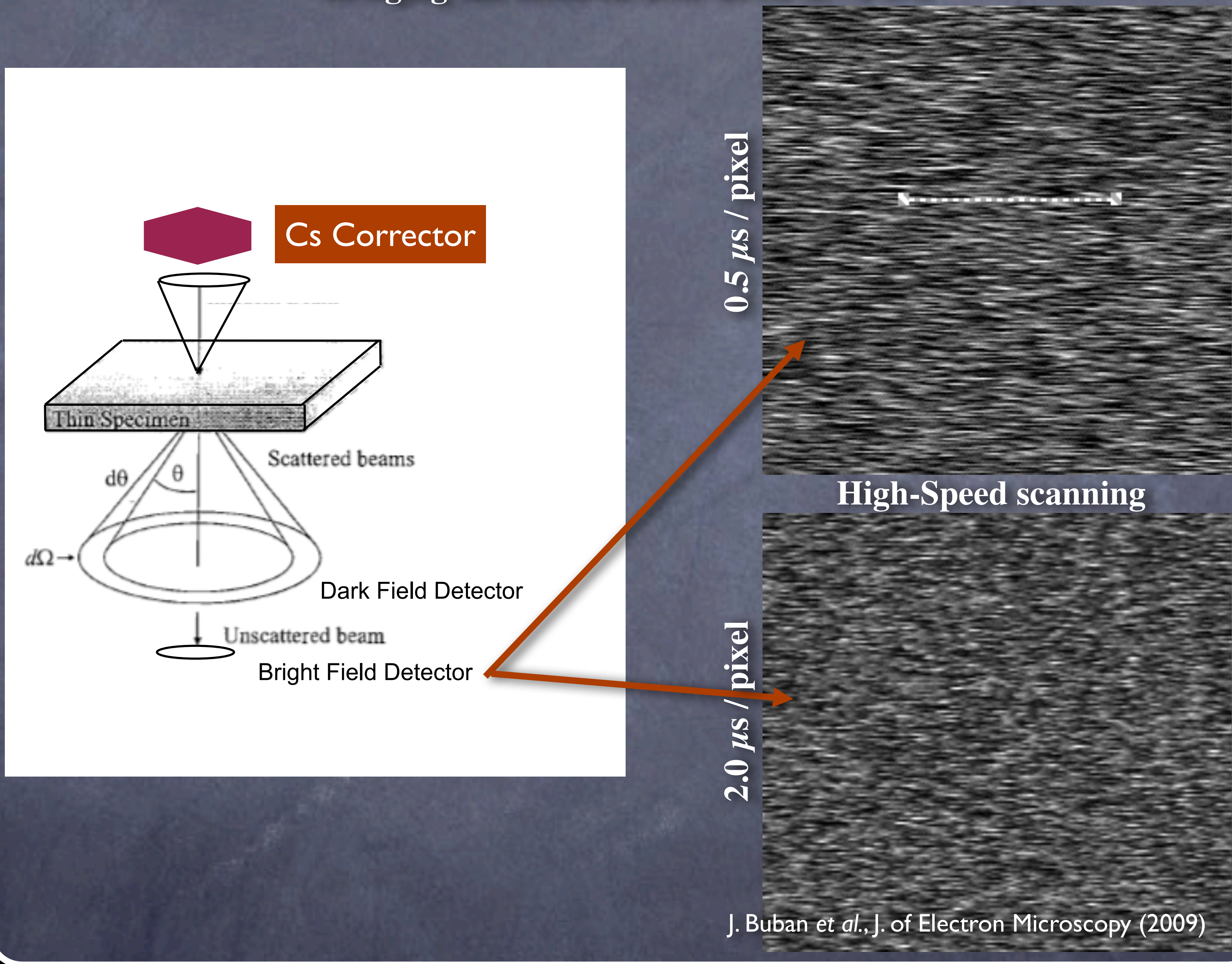
High resolution ✓

Tilted samples ✓

Low dose

High contrast

Bringing the electron dose down to ~1 e/Å^2



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

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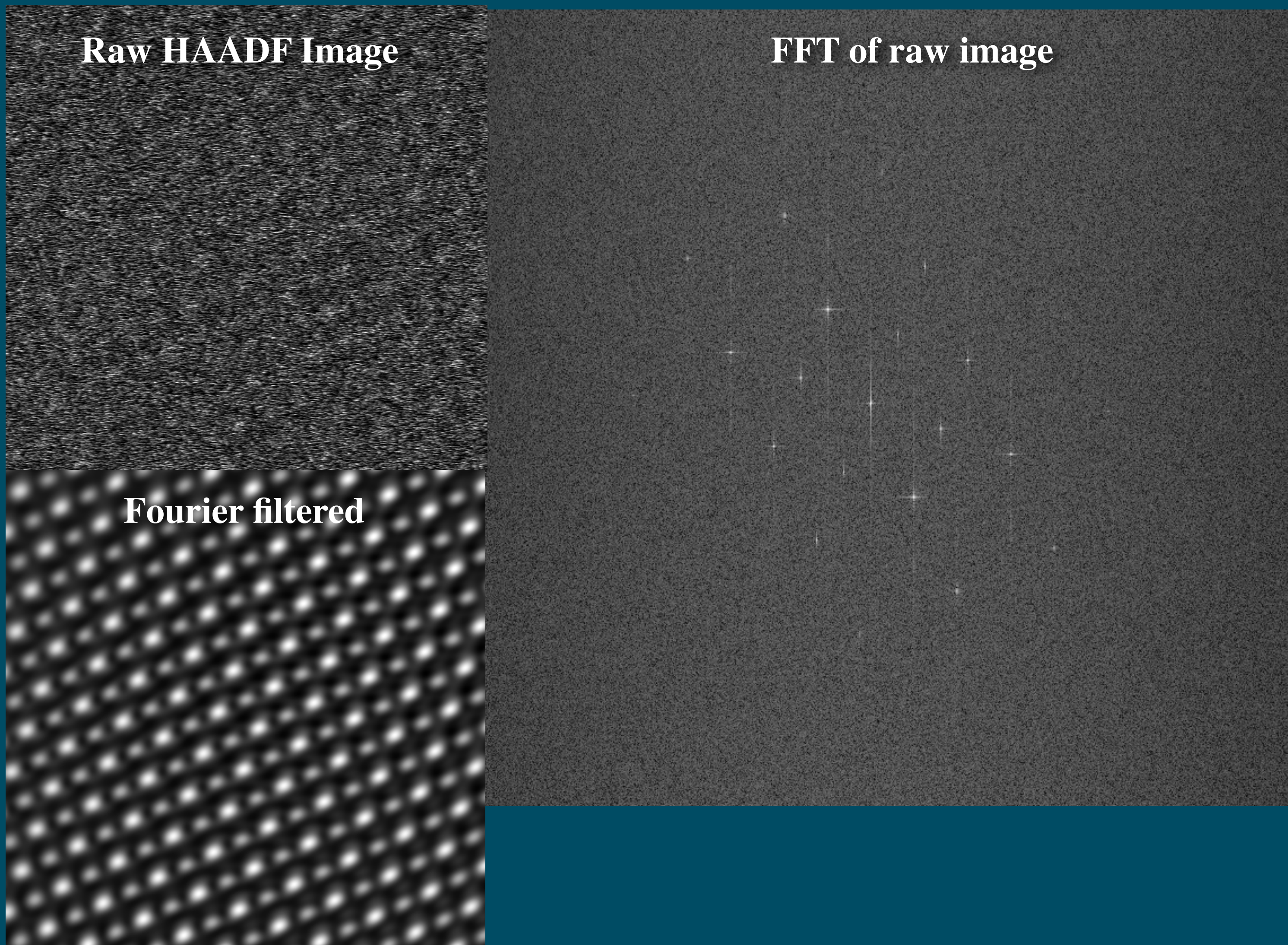
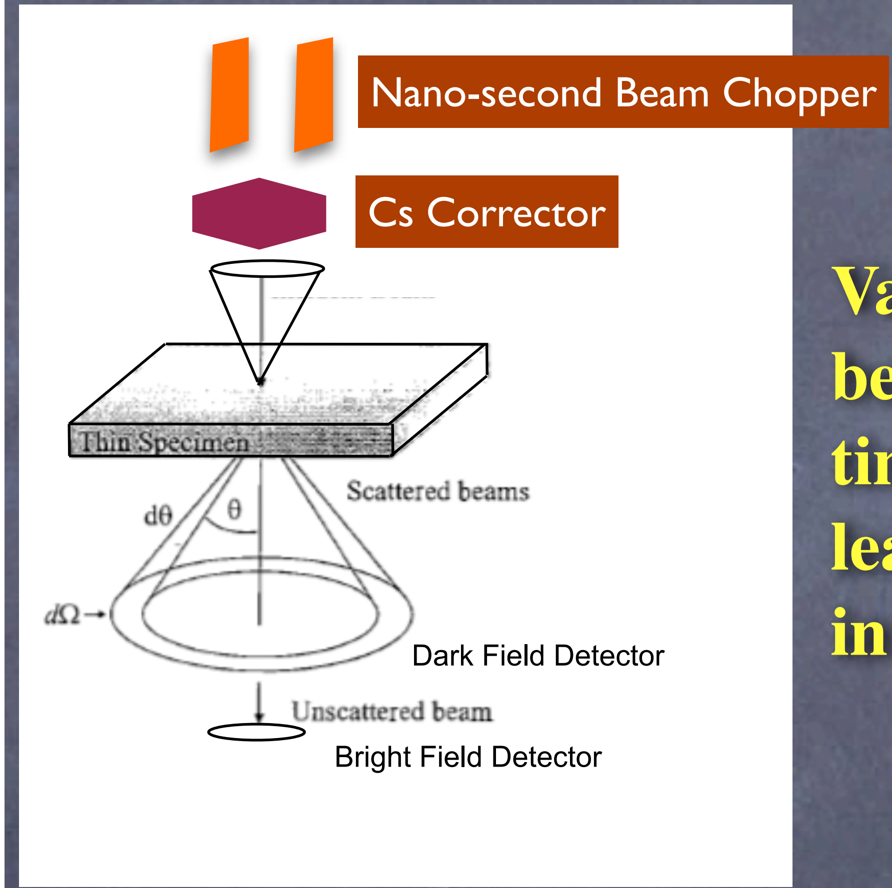


High-resolution STEM on beam-sensitive samples: The need for a **Beam Dimmer**

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

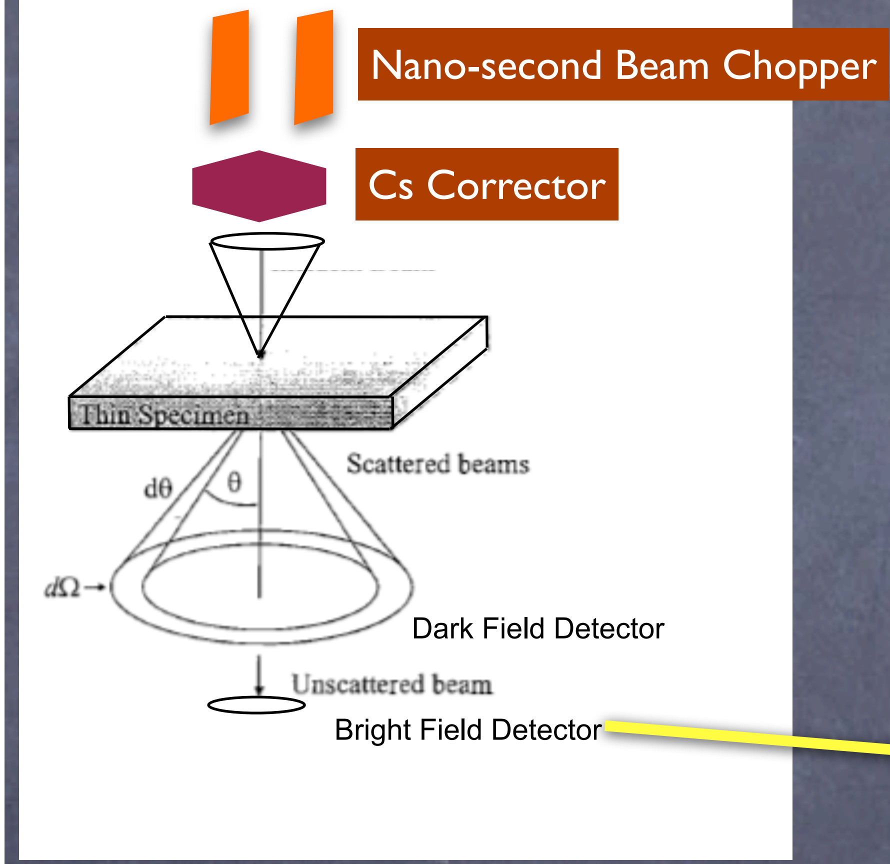


Vary On/Off ratio of beam in nano-second time range, while leaving everything else in the (S)TEM the same

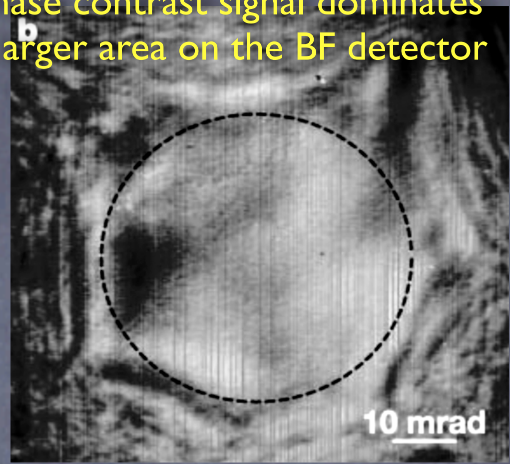
Phase-Contrast in Cs-Corrected BF STEM

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

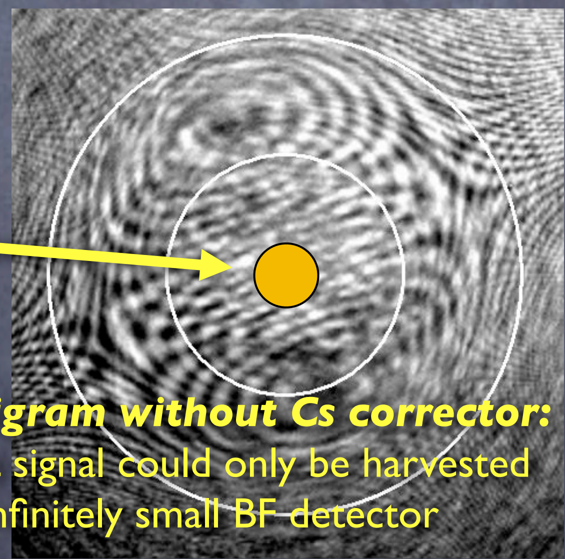
Sweet Spot in Ronchigram increases with Cs correction



STEM Ronchigram with Cs corrector:
Phase contrast signal dominates
a larger area on the BF detector



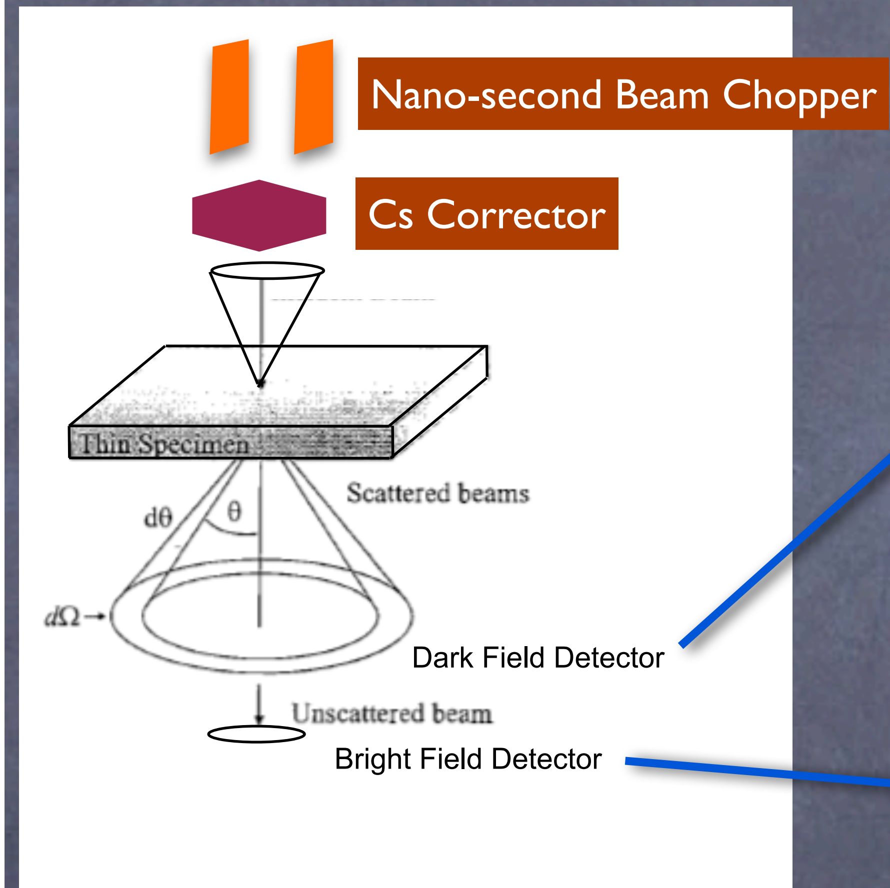
Batson et al., Nature (2002)

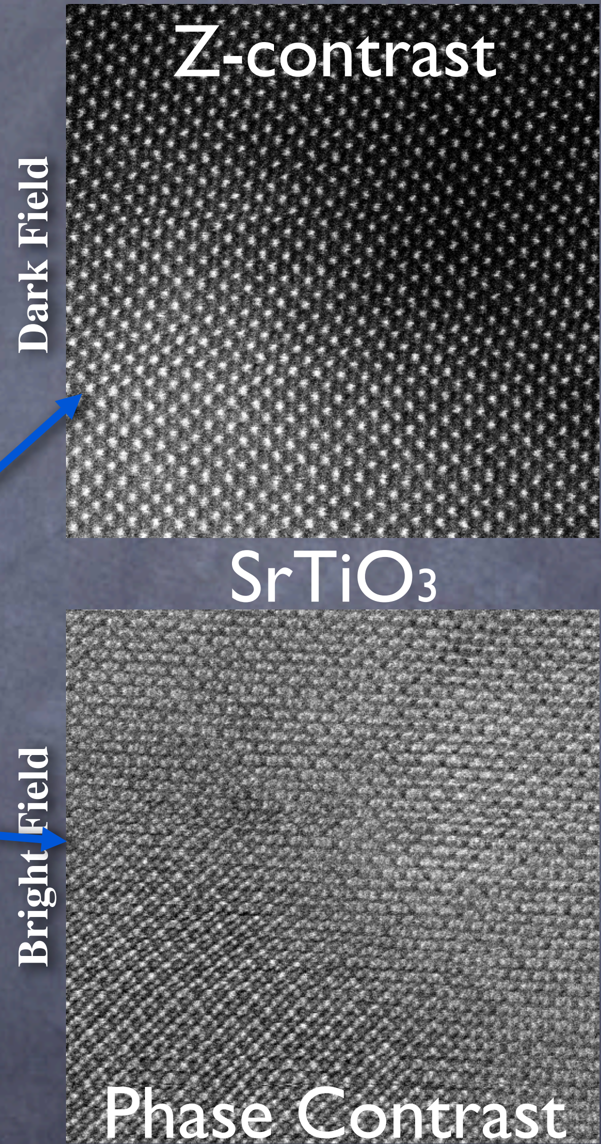


STEM Ronchigram without Cs corrector:
Phase contrast signal could only be harvested
with an infinitely small BF detector

Phase-Contrast in Cs-Corrected BF STEM

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

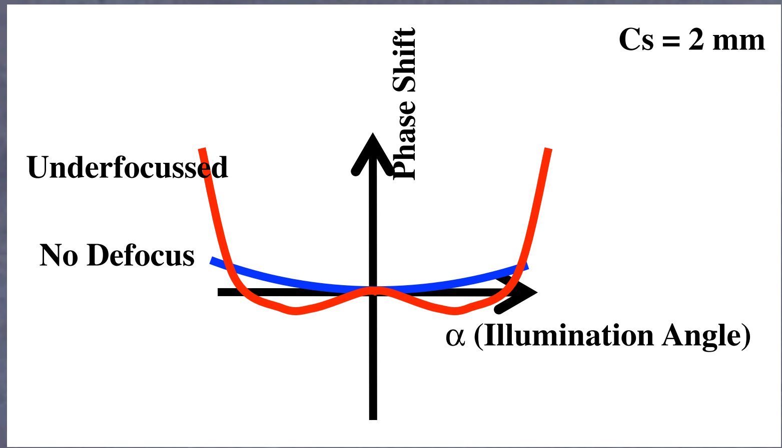


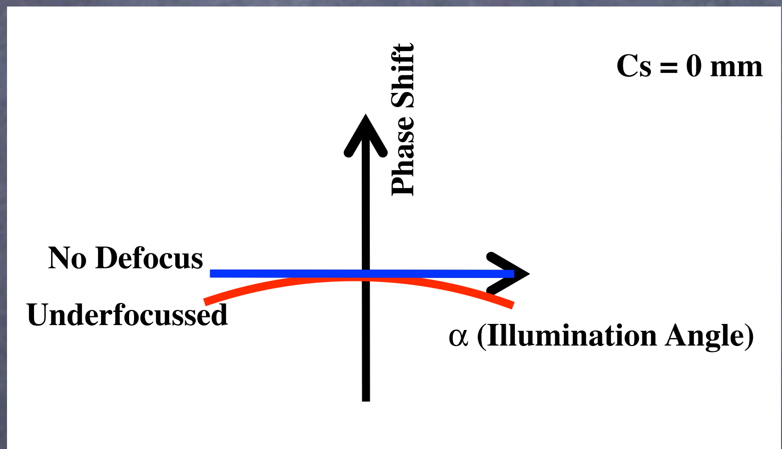


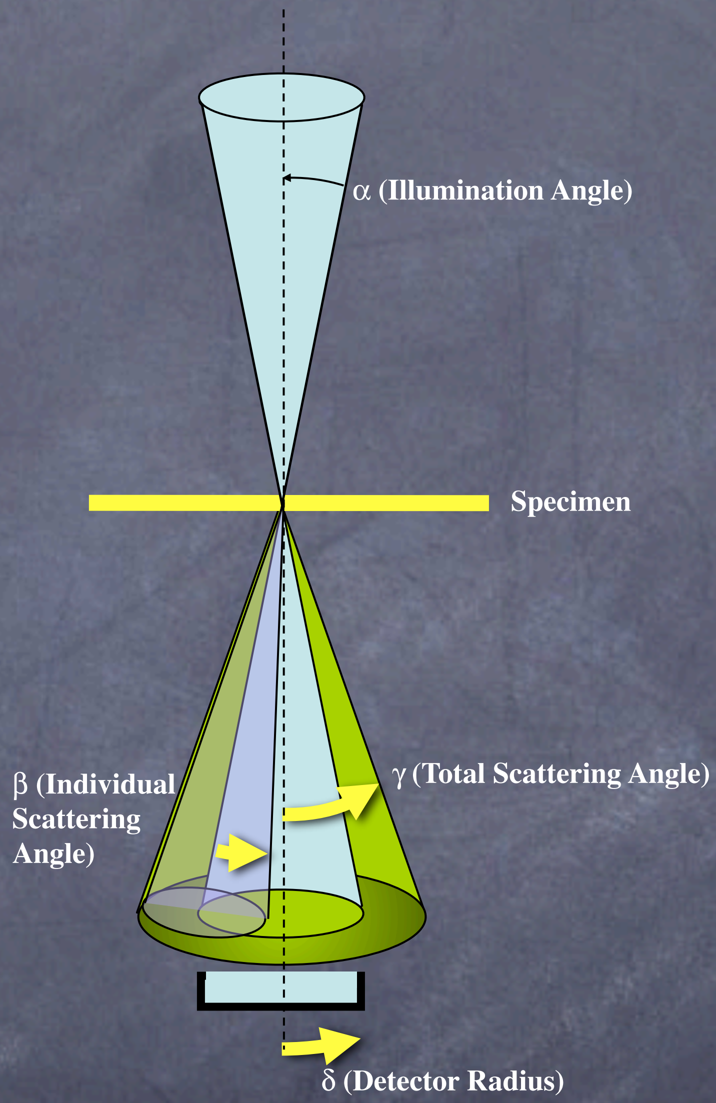
- High resolution ✓
- Tilted samples ✓
- Low dose ✓
- High contrast

with: J. Buban, Nigel Browning,

Phase-Shift in STEM illumination







High resolution ✓

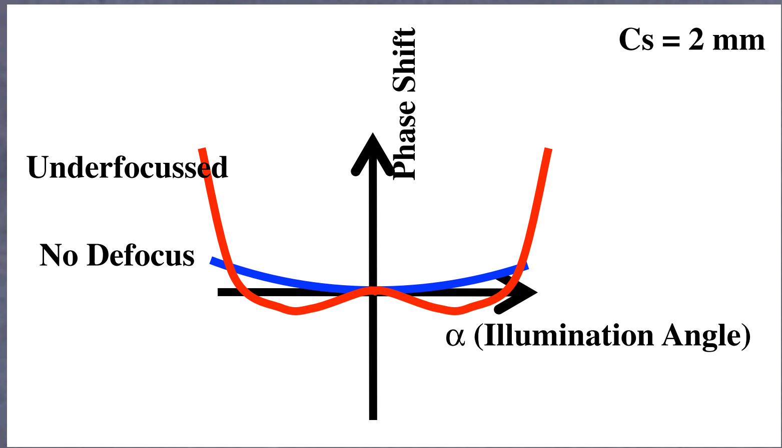
Tilted samples ✓

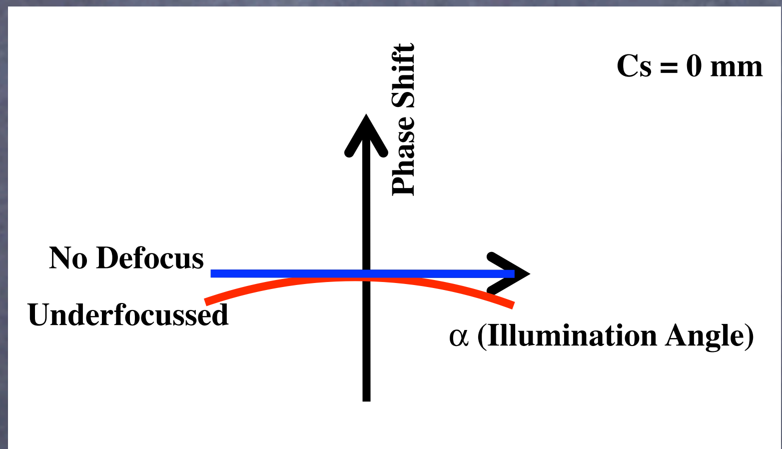
Low dose ✓

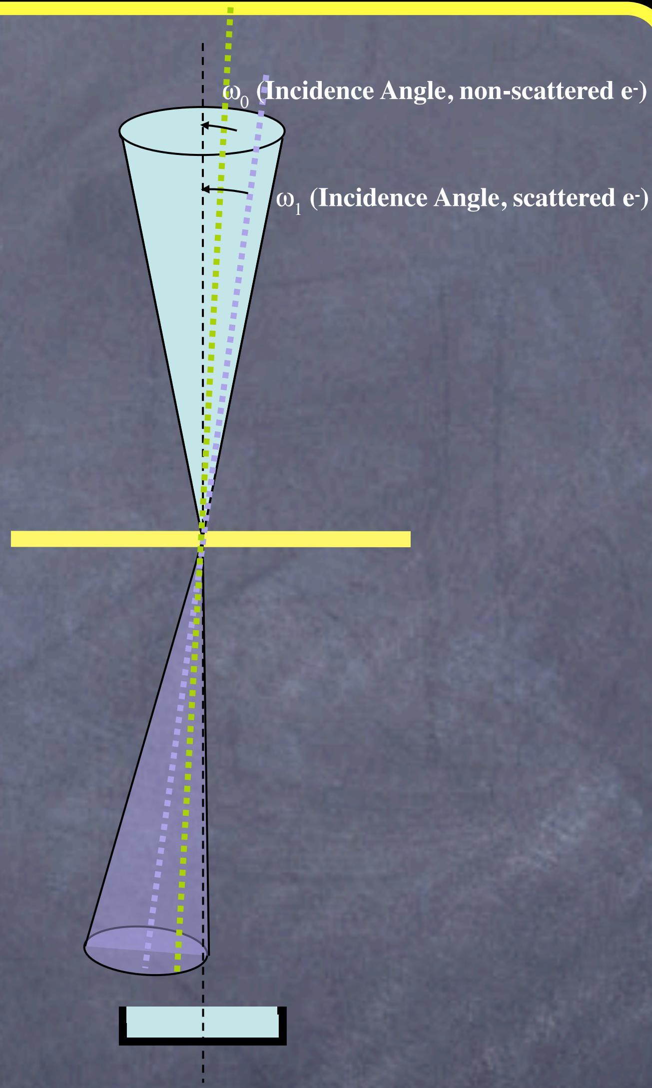
High contrast

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

Interference contrast between non-scattered and scattered electrons







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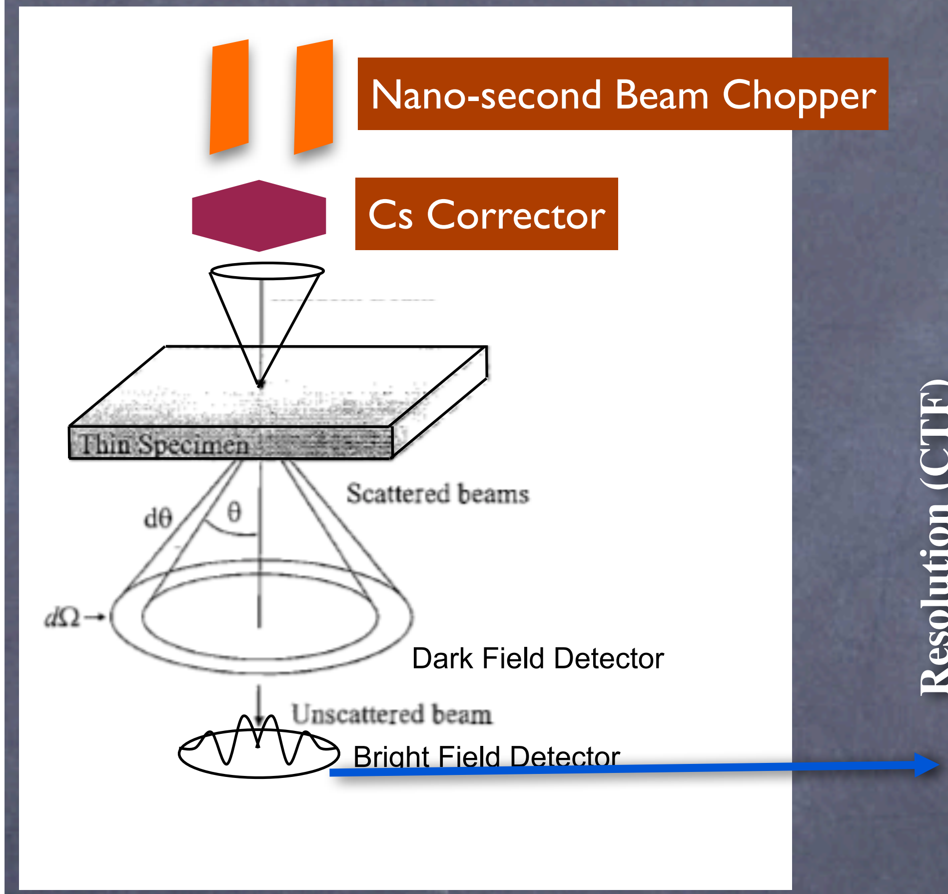
- Tilted samples ✓
- Low dose ✓
- High contrast

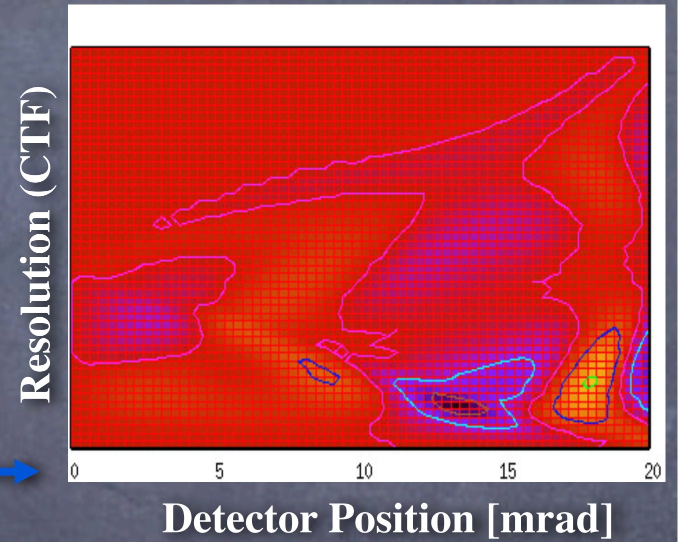
(see also: Hammel & Rose, Ultramic., 1995)

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)

N·H
ELSEVIER

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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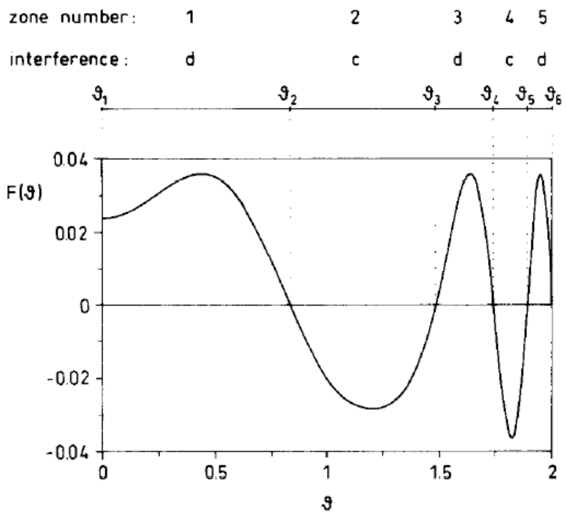

Fig. 2. Normalized current density fluctuations in the image of a weak point scatterer for a defocus Δ = 1.45 and an objective aperture angle $\vartheta_0 = 2.0$. The zeros ϑ_1, ..., ϑ_6 separate the regions of destructive "d" interference from those of constructive "c" interference.

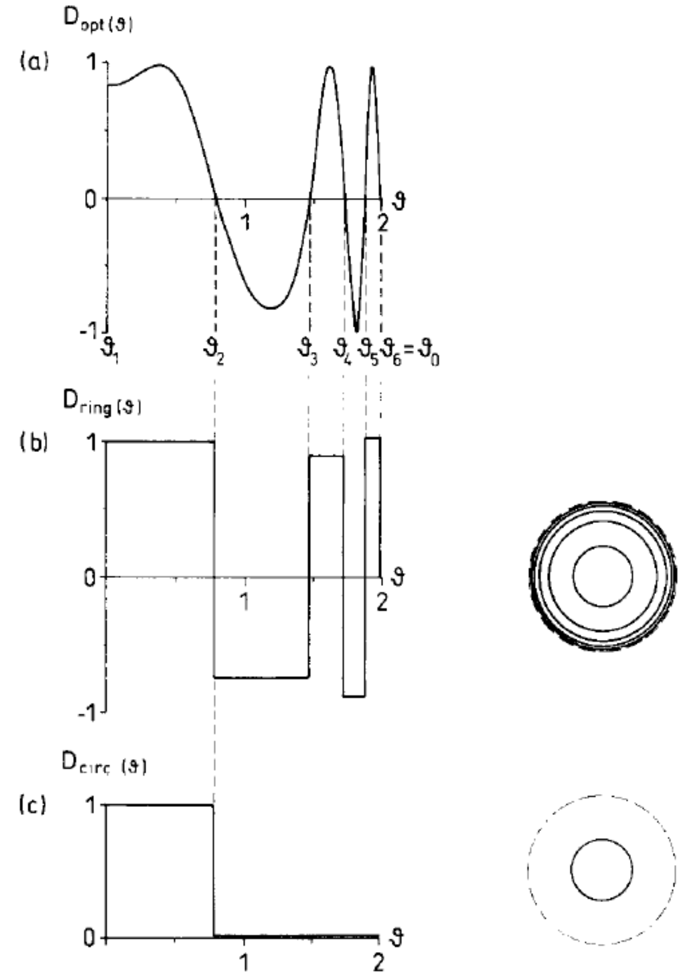

Fig. 4. (a) Detector function D_{opt} of the ideal detector obtained for a normalized defocus Δ = 1.4 and an objective aperture angle $\vartheta_0 = 2.0$. This function indicates how the signal, recorded at an angular distance ϑ, should be weighted in order to yield optimum signal-to-noise ratio in the 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 ($t/\text{pixel} \geq 4\ \mu s$) 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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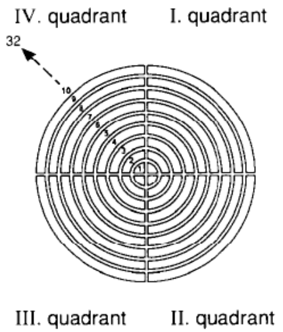


Fig. 4. Schematic drawing of the inner part of the detector layout.

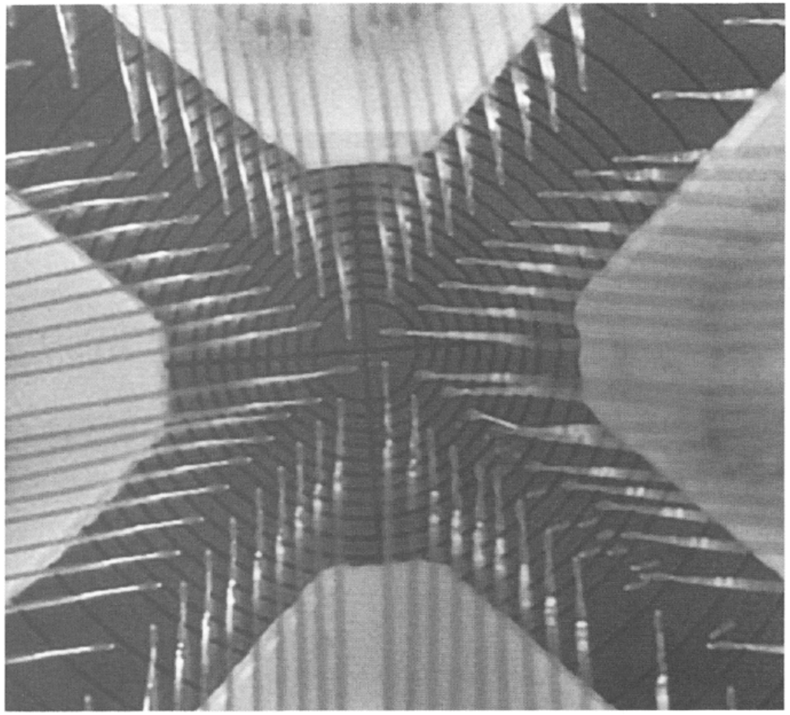


Fig. 6. Photograph of the inner part of the multichannel detector. The shape of the individual channels can be observed as rings split into quadrants. The diameter of the inner disk is 260 μm.

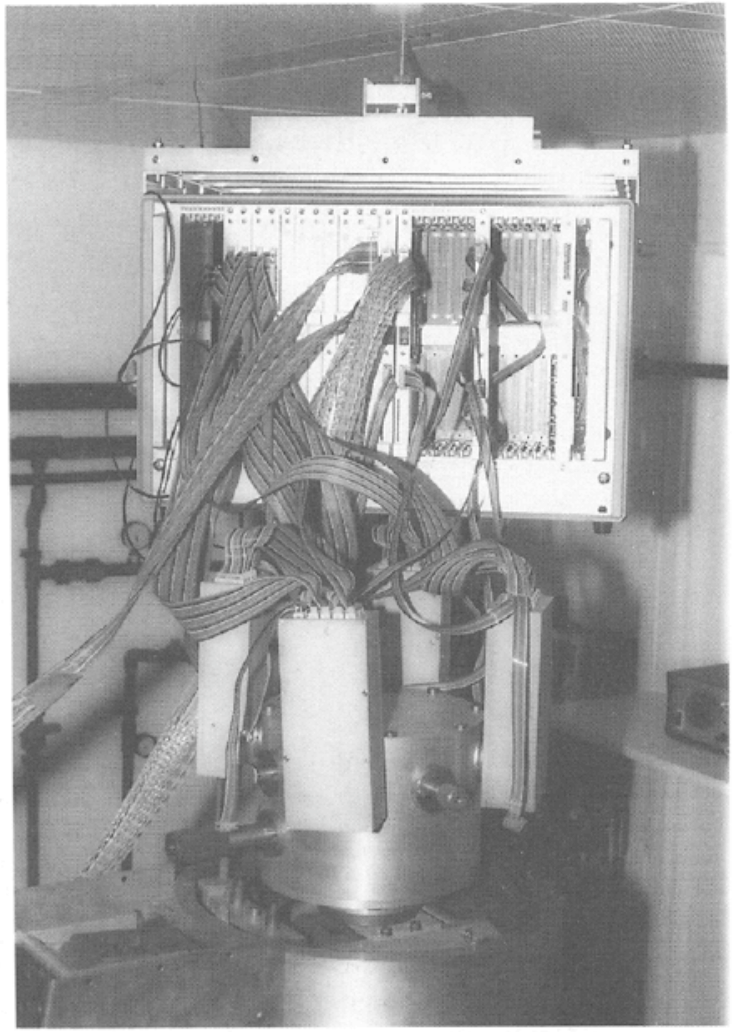
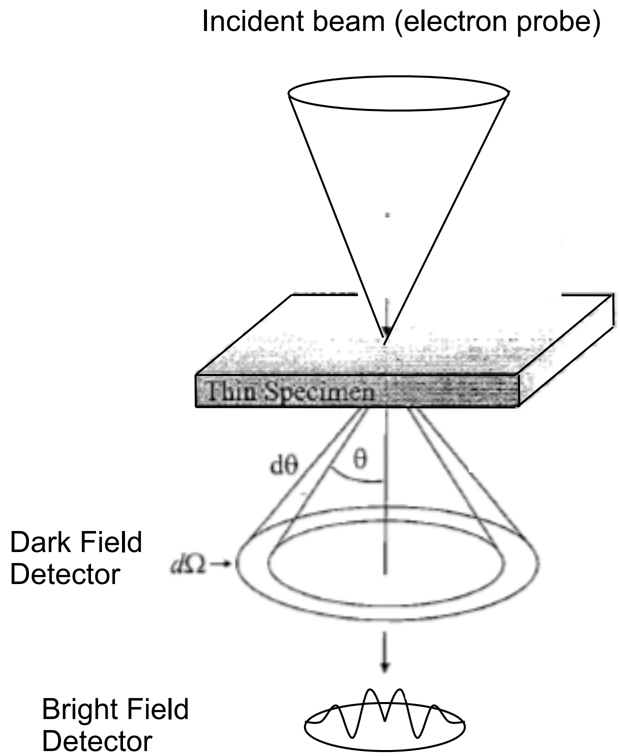
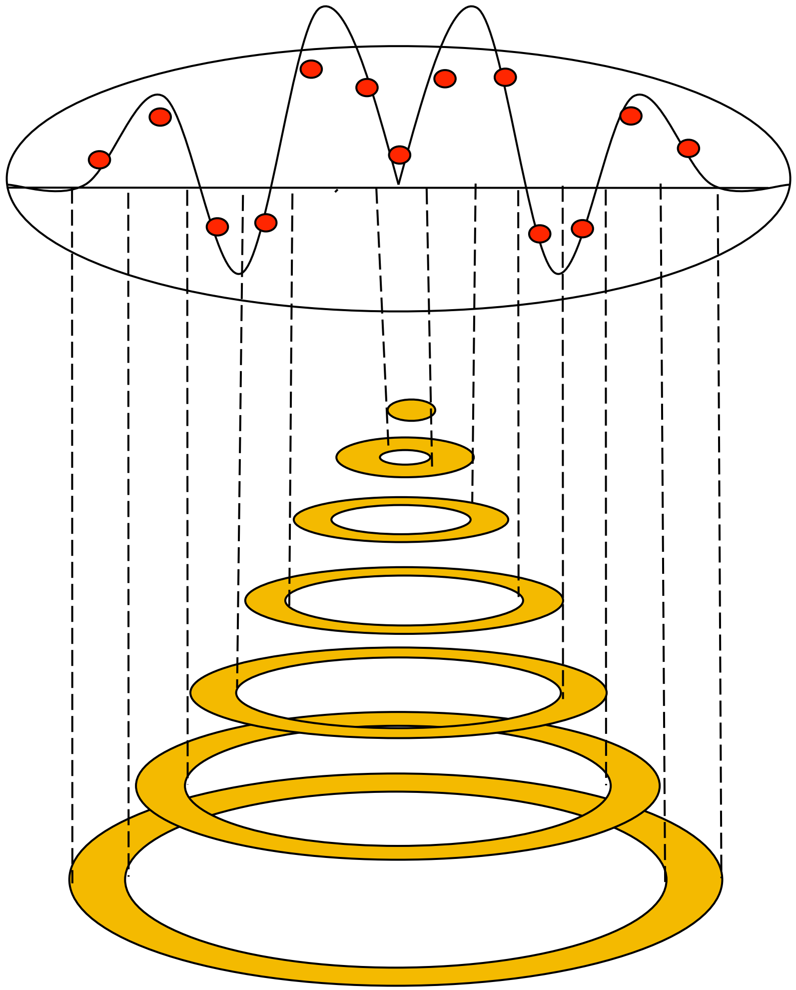


Fig. 2. Photograph of the upper part of the cryo-STEM column showing the housing of the multichannel detector and the attached four housings of the preamplifiers and discriminators.

Proposed Multi-Ring Detector
(UC Davis, 2007)





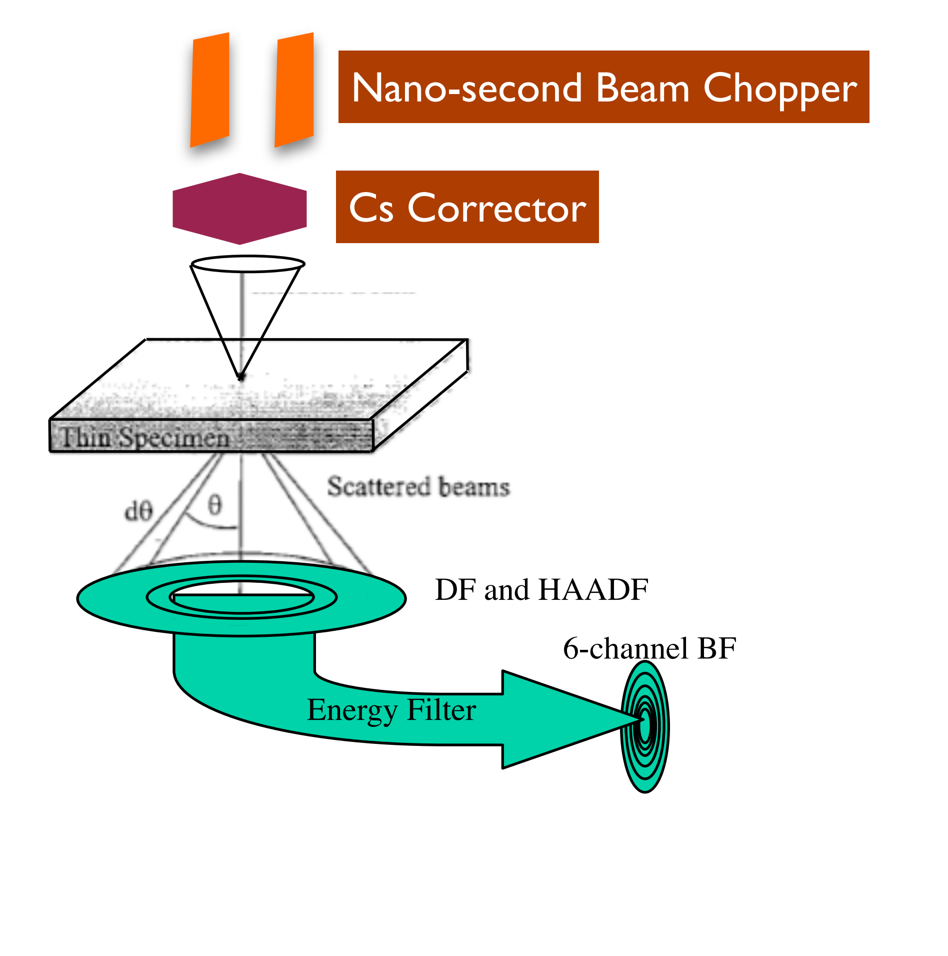
Segmented Ring Detector Array

James Buban, Nigel Browning, Quentin Ramasse, Hui-Ting Chou, Henning Stahlberg, UC Davis, 2007

Proposed Phase-Contrast BioSTEM (2008)

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

Low-dose cryo-STEM of tilted biological specimens



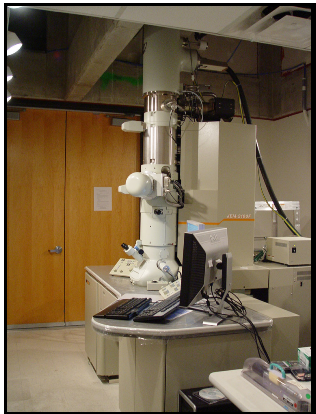
Tilted Samples:
Precise computer control, online feedback

Low-dose:
Fast Electronics (1 μs / pixel)
Beam Chopper

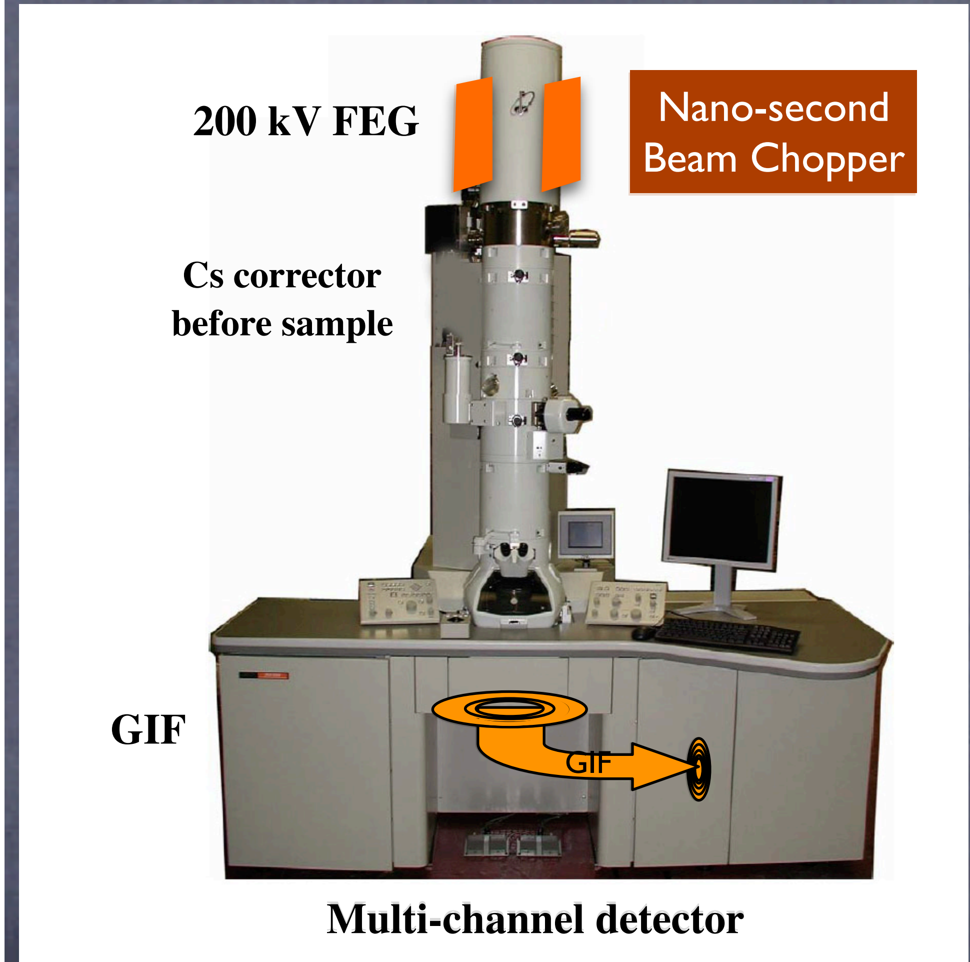
Phase Contrast:
Aberration correction

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

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



Proposed Phase-Contrast BioSTEM (2009)



Tilted Samples:
Precise computer control, online feedback

Low-dose:
Fast Electronics (1 μs / 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

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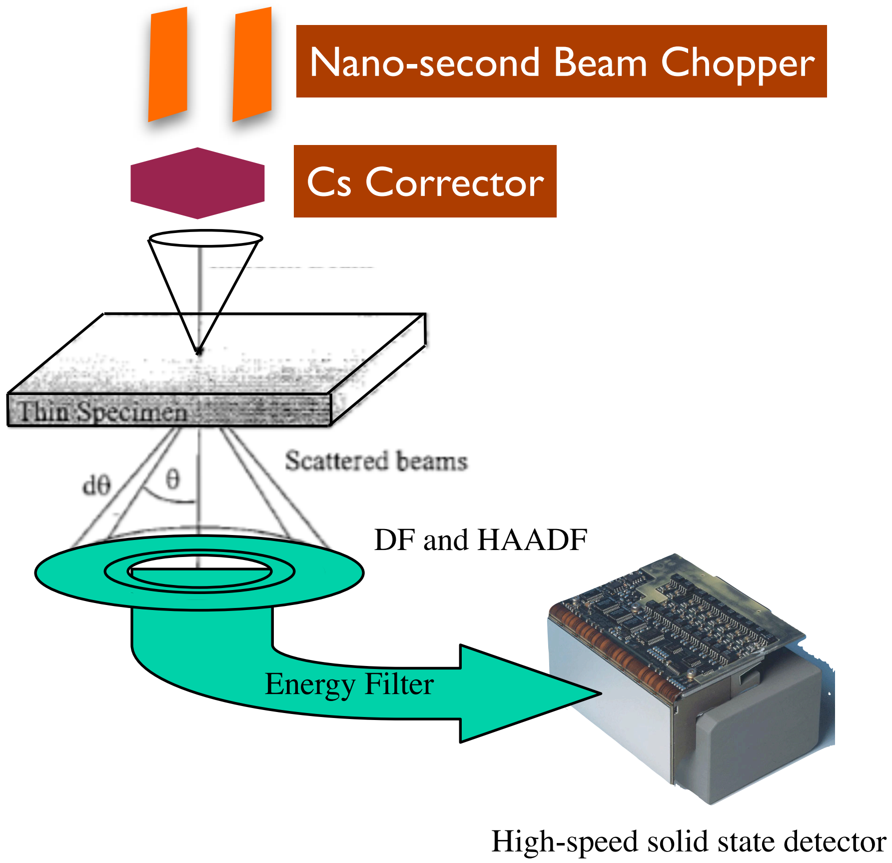
Proposed Phase-Contrast BioSTEM (2010)

High resolution ✓

Tilted samples ✓

Low dose ✓

Phase contrast ✓



Tilted Samples:
Precise computer control, online feedback

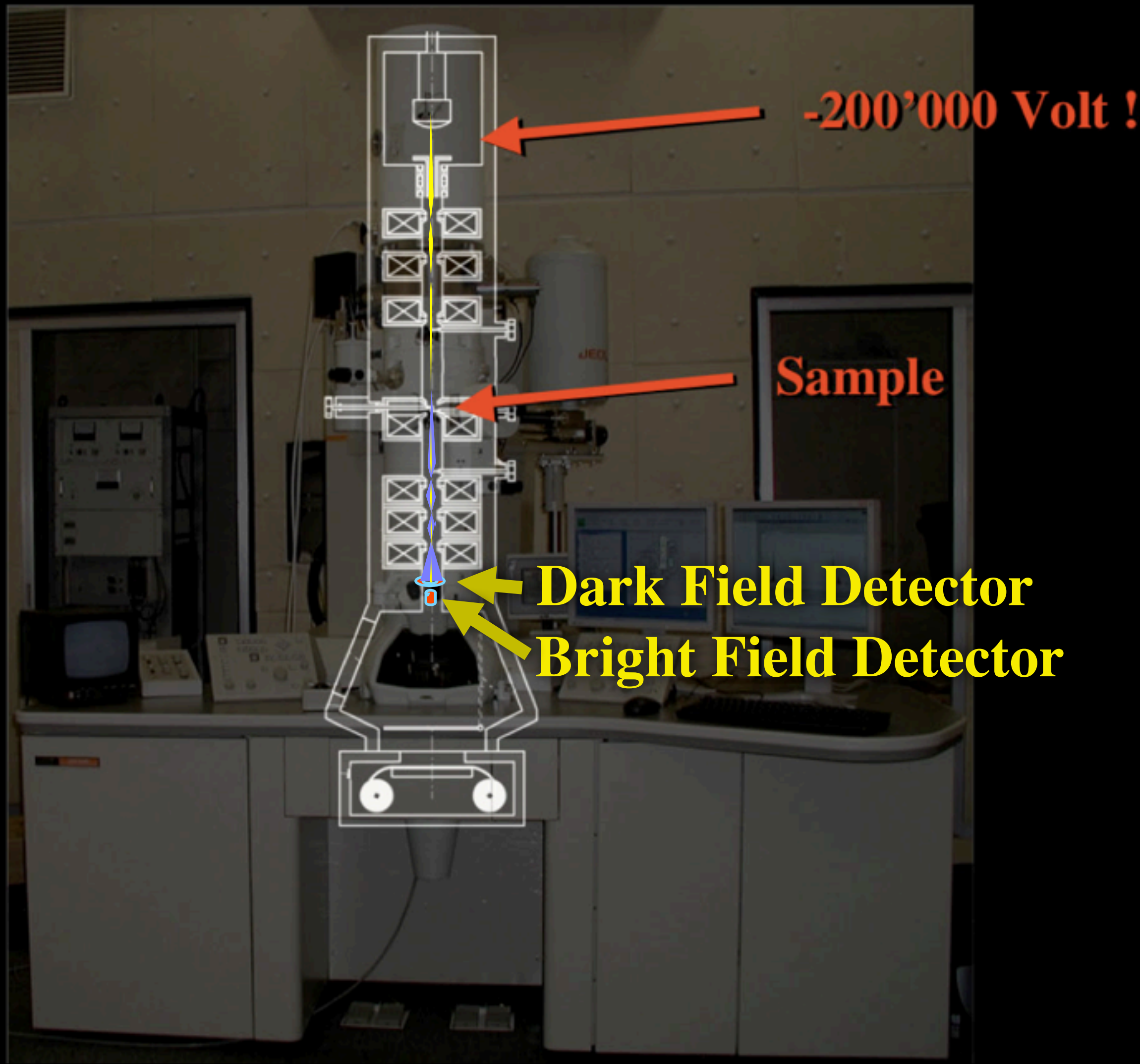
Low-dose:
Fast Electronics (1 μs / pixel)
Beam Chopper

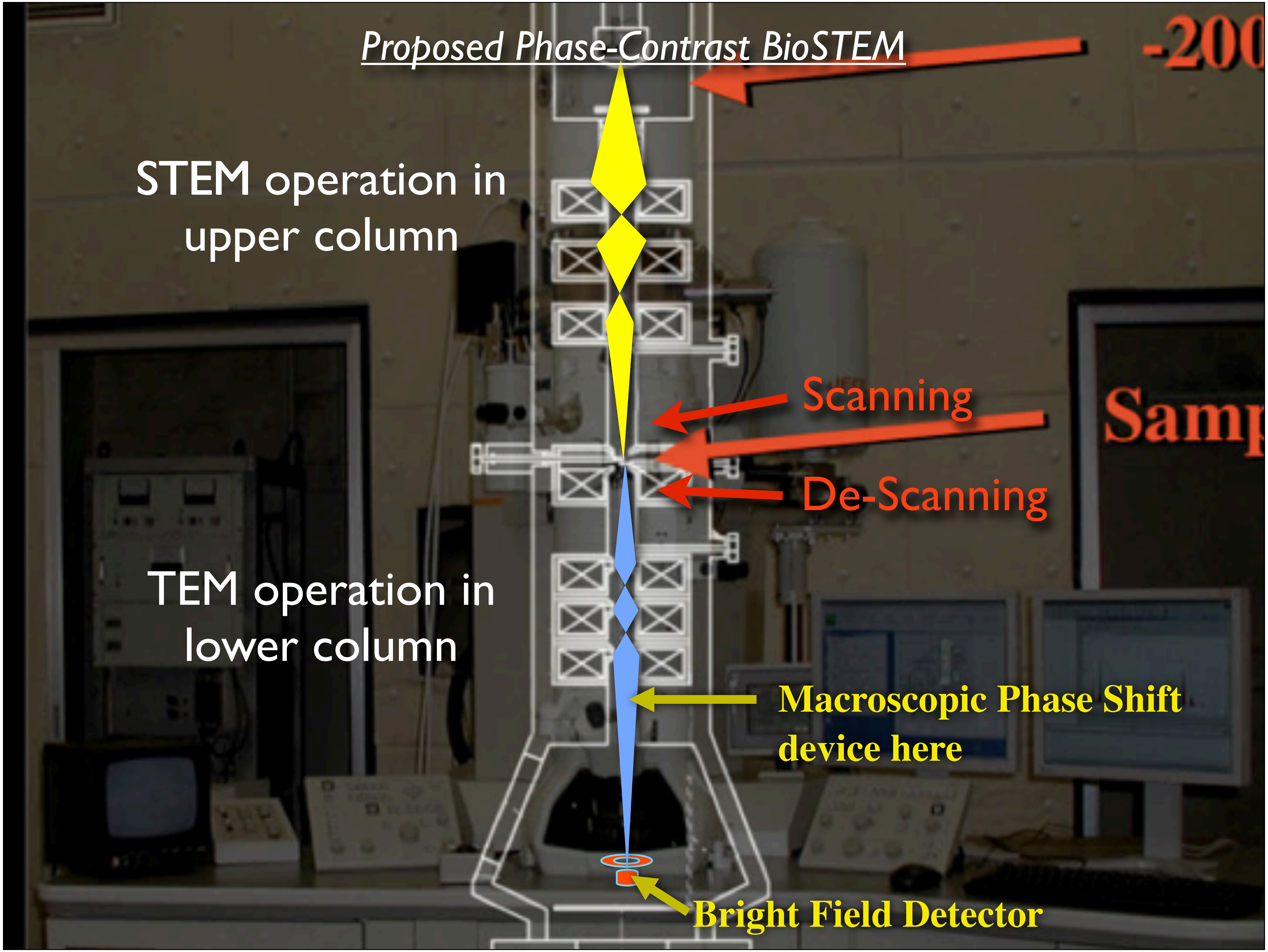
Phase Contrast:
Aberration correction

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
Scanning
Samp
De-Scanning
TEM operation in lower column
Macroscopic Phase Shift device here
Bright Field Detector

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 descan-STEM
  - Pre-illumination
  - ***Filming the sample during TEM imaging***

CINA Center for Cellular Imaging and Nano Analytics

Uni Basel

Basel, Switzerland

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C-CINA.org

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Sebastian Scherer

Hui-Ting Chou (UCD/Harvard)
Quentin Ramasse (LBNL)
James Buban (UCD)
Nigel Browning (UCD/PNNL)