Physical resolution limits of single particle 3D imaging with X-rays and electrons

Coherence 2005

Dirk Van Dyck and Sandra Van Aert

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Ultimate goal: imaging of atoms

- Understanding properties of nanostructures
  - biological structures
  - (in)organic materials
  - components
- Matching experiment with theoretical, ab initio calculations
- Modelling
- Predicting and designing nanostructures

What is needed to match experiment with ab initio theory?

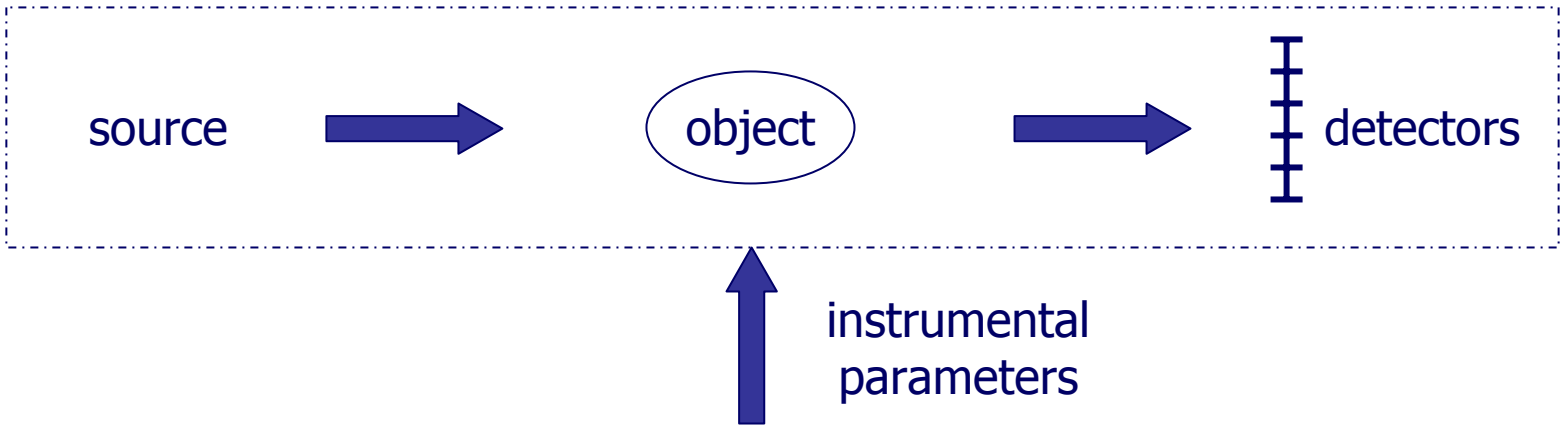
1. Precise atomic positions (±0.001 nm)
2. Complementary information
   - Prior information (e.g. substructures)
   - Local spectroscopic information
     - chemical
     - bonding
     - electronic structure
     - local configuration

How to characterize atomic structures?

- Interaction with particles
  - photons (X-rays)
  - electrons
  - neutrons
  - protons
- Requirements
  - bright coherent sources
  - easy to detect
  - subångstrom wavelength

→ X-rays (XDM, XD) and electrons (EM, ED, EDI)

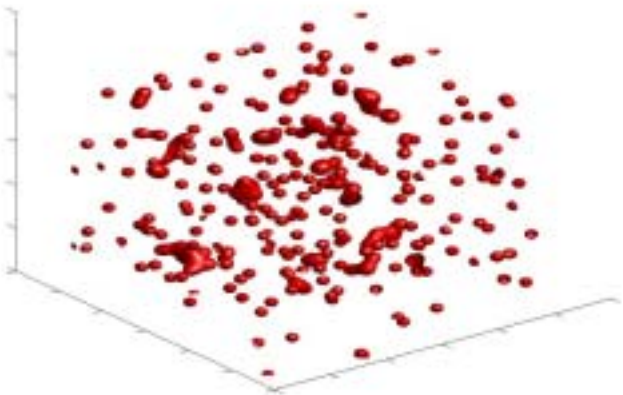
Quantitative experiment



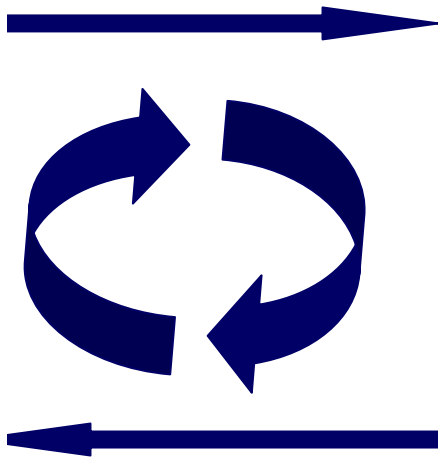
- Detection of individual particles
- Model based fitting

Model based fitting

model space

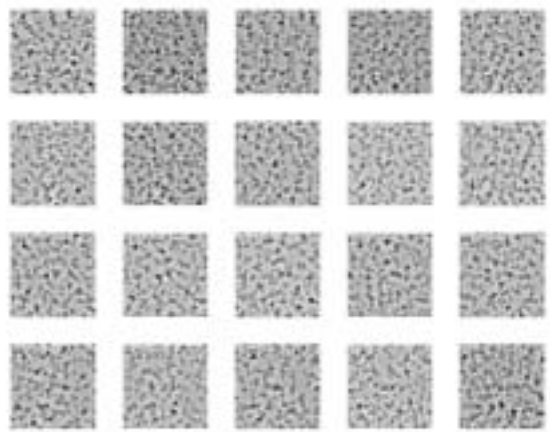


simulation



refinement

experiments



Model based fitting

Observations

n_i
- number of particles hitting detector i (e.g. pixel in CCD)
- stochastic variables

$$\sum_i n_i = N$$

Model

$$E[n_i] = N p_i(\theta_k)$$

p_i probability to hit pixel i

θ_k model parameters
- structural parameters (atomic positions,...)
- instrumental parameters (fixed and tunable)

Model based fitting

Requirements

- The model contains all ingredients needed to perform a simulation (structure, interaction, instrument, detection)
- The model is assumed to be correct
- The experiment is the ensemble of all experiments (focal images, tomographic series, DP,…)
- Only fitting with original experimental data (noise model)

Model based fitting

Maximum likelihood estimator

- Lowest possible error bar (CRLB)
- Unbiased

Likelihood function

$$L = N! \prod_i \frac{p_i^{n_i}(\theta_k)}{n_i!}$$

$$\ln L = \sum_i n_i p_i(\theta_k) + C$$

Maximum likelihood

$$\frac{\partial \ln L}{\partial \theta_k} = 0 \quad \forall k \quad \Rightarrow \text{ estimates } \hat{\theta}_k$$

Error bar on the estimated parameters

σ_i^2 : variance of estimated parameter θ_i

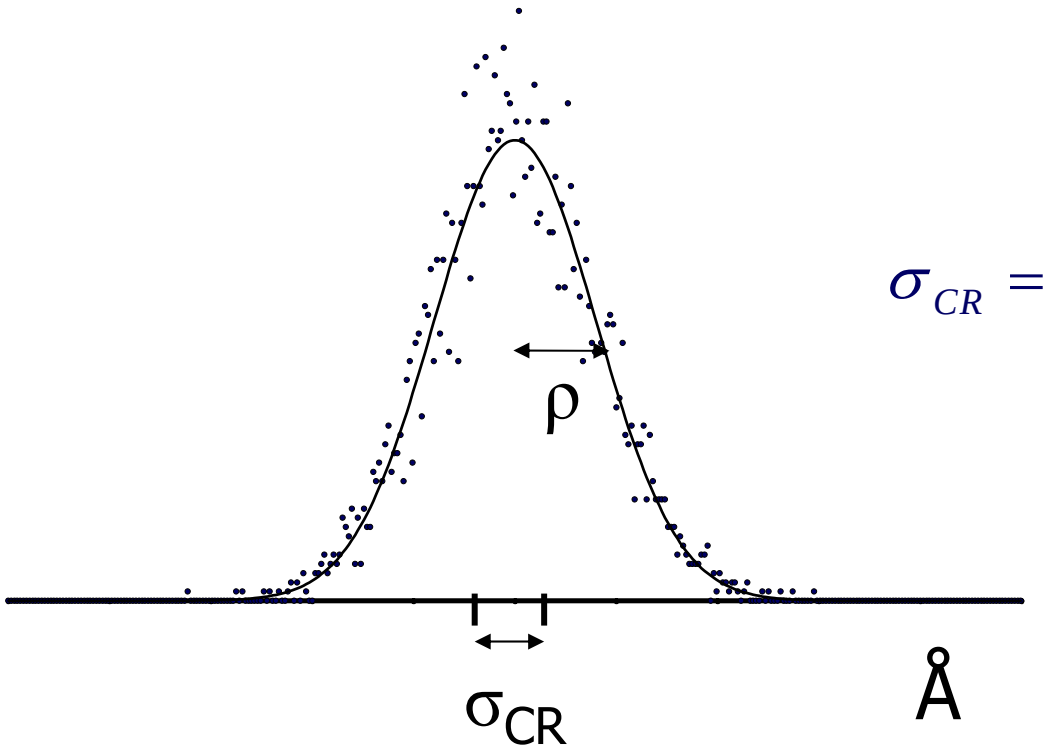
Then

$$\sigma_i^2 \geq -E\left[\frac{\partial^2 \ln L}{\partial\theta_k \partial\theta_l}\right]^{-1}_{ii}$$

→ lower bound on variances σ_i^2 (Cramér-Rao lower bound)

→ can be used for experimental design

Resolution - precision



$$\sigma_{CR} = \frac{\rho}{\sqrt{N}}$$

resolution

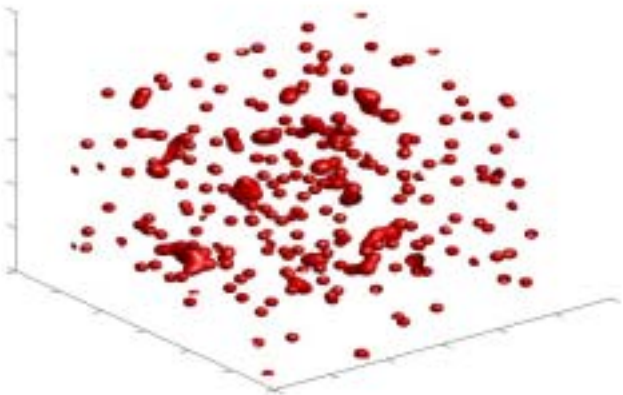
dose

ρ = 1 Å
N= 10000
σ_{CR}= 0.01 Å

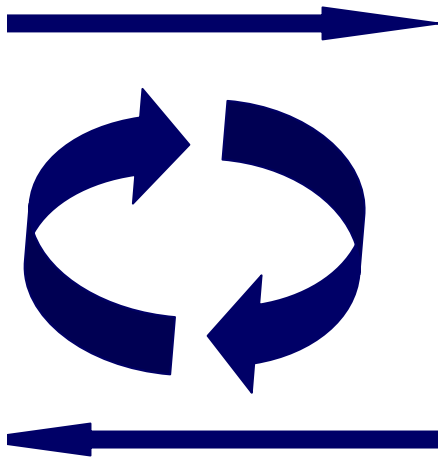
The dose can be distributed over many images (dose fractionation theorem)

Model based fitting

model space

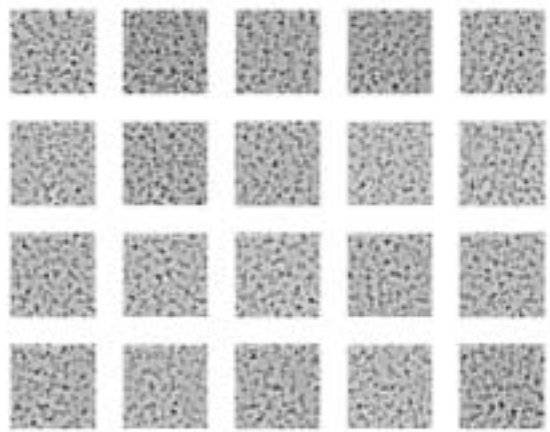


simulation



refinement

experiments



Iteration till best fit

Model based fitting

Problems: - convergence

- local optima

- uniqueness

How to avoid local optima?

1) "Resolving" the structure

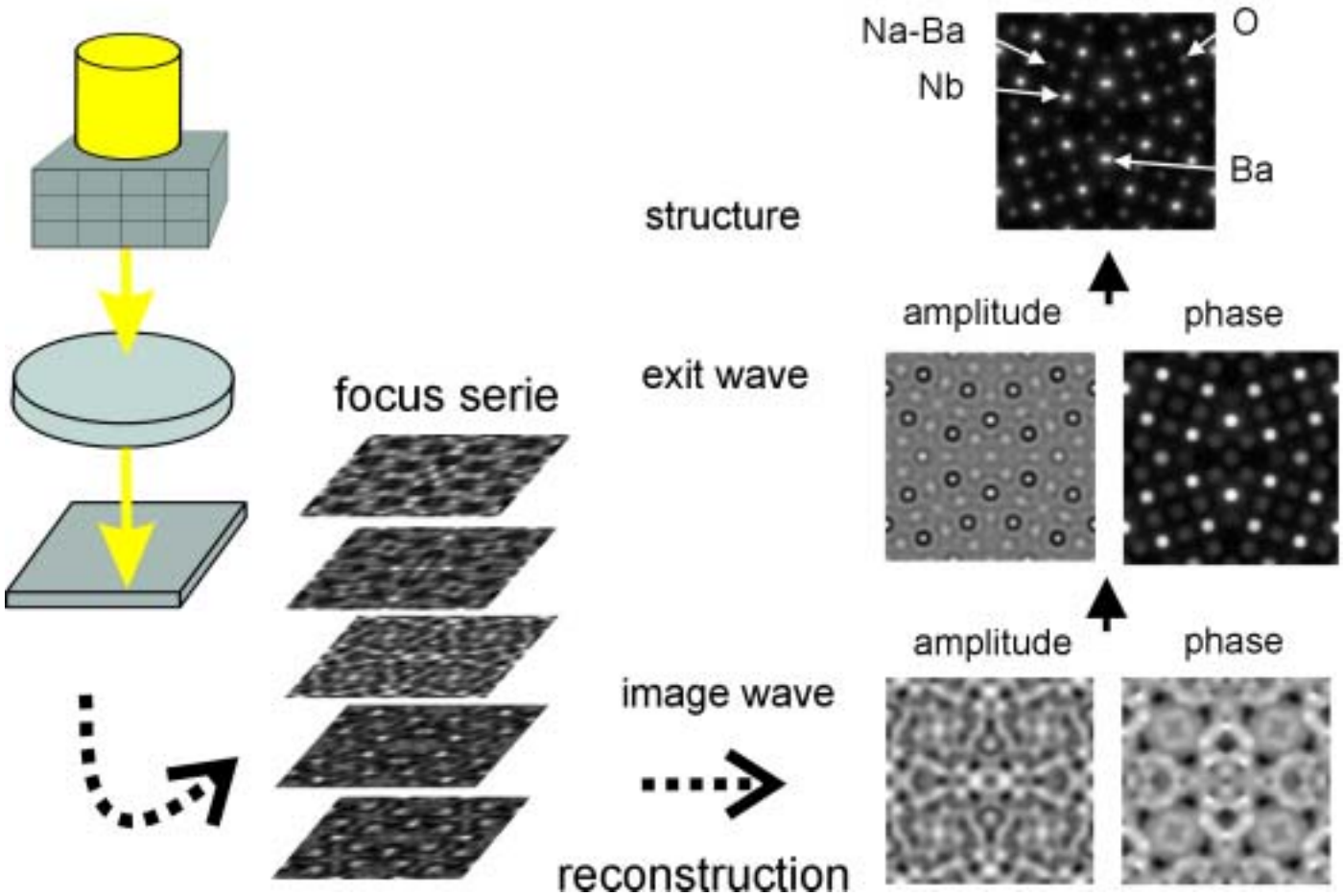
Obtain a good starting structure using general principles

- Direct imaging
- Image reconstruction
- Direct methods (phase constraints)
- Constrained optimisation: hybrid I-O

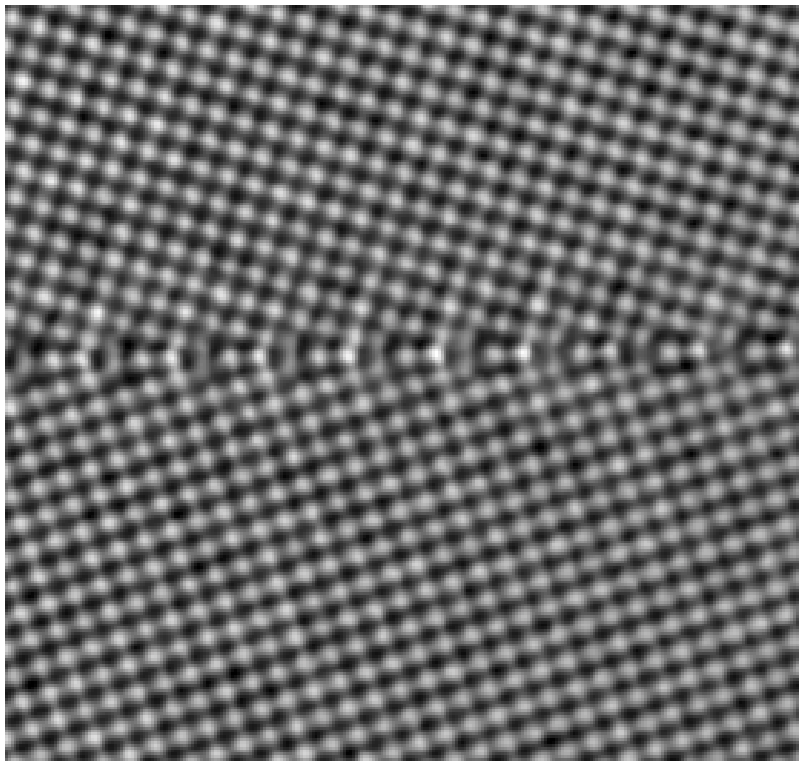
2) "Refining" the structure

Convergence to global optimum (maximum likelihood)

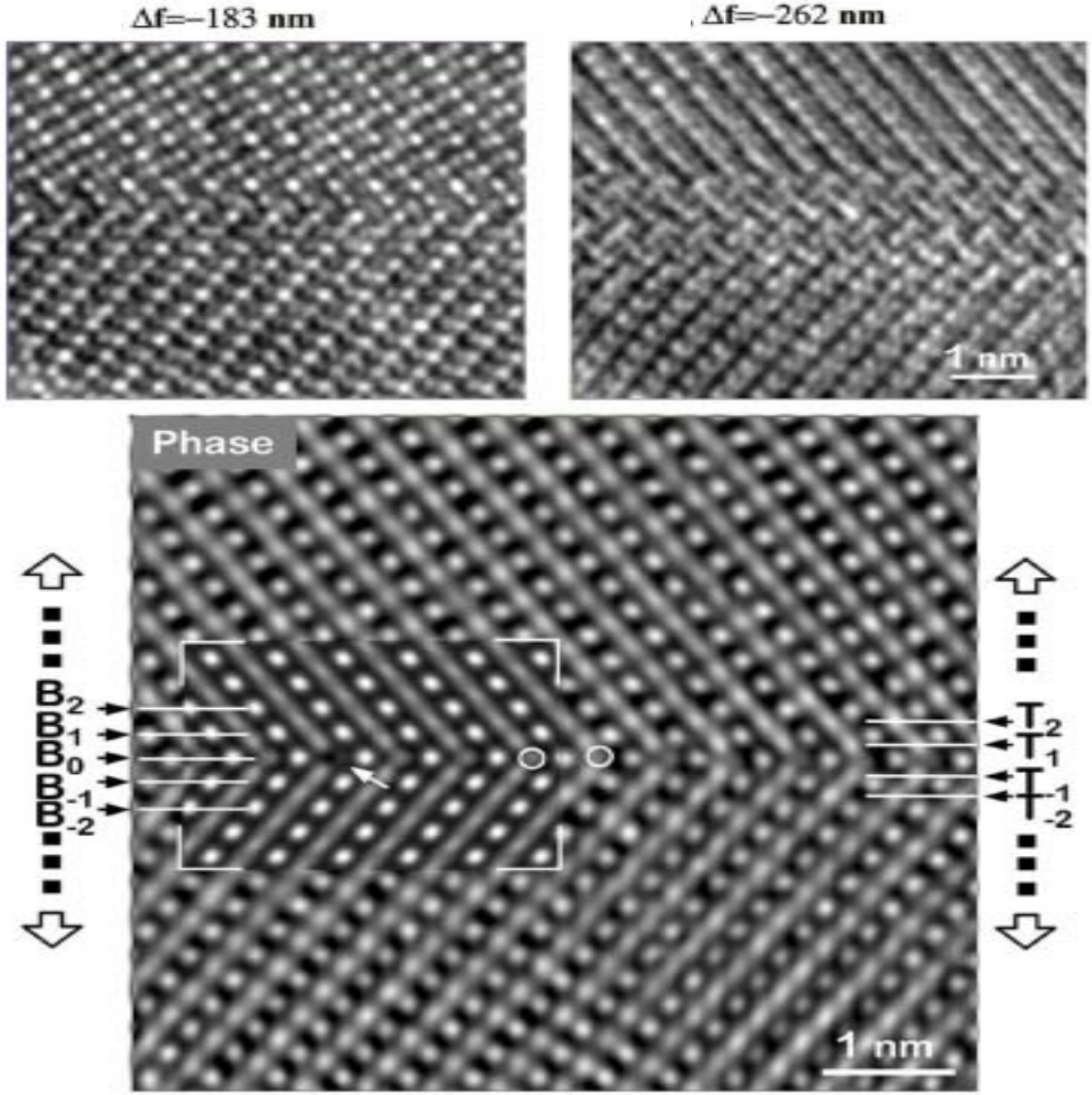
Focus variation reconstruction



Phase of total exit wave
Σ 5 Al: Cu



Courtesy C. Kisielowski (NCEM,Berkeley)



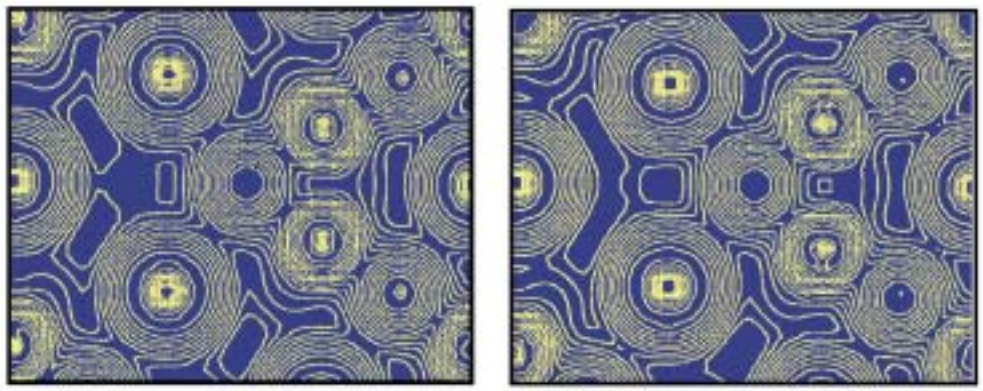
 C.L. Jia and A. Thust, Phys. Rev. Lett. 82 (1999) 5052.

Comparison: Theory & Experiment

Plane Distances at Σ3(111) Twin Boundary

	Ti - Ti [pm]	Ba - Ba [pm]
Geometric	232	232
Experiment	270	216
Theory	267	214

Charge Density from First Principles Calculation:



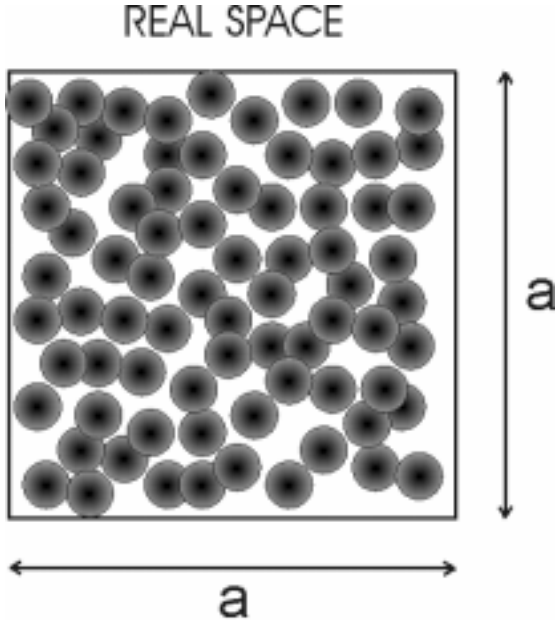
Ideal Geometrical Model Optimized Structure

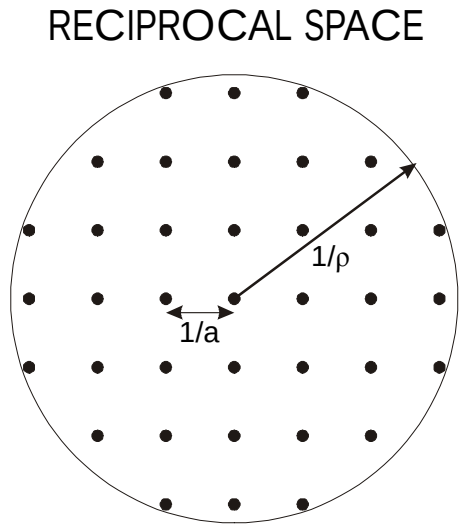
Ti-Ti: 4.5 x 10^{-2} *e* /a.u.^{3} 3 x 10^{-2} *e* /a.u.^{3}

Ba-Ba: 5 x 10^{-3} *e* /a.u.^{3} 9 x 10^{-3} *e* /a.u.^{3}

Uniqueness problem

If number of parameters exceeds information capacity of imaging channel





Requirement: $P < \frac{\pi a^2}{\rho^2}$ or $\frac{P}{a^2} < \frac{3}{\rho^2}$

parameters data

Information content = 3 parameters per unit ρ^2

3D Tomography

parameters $\longrightarrow$ $P < \frac{4}{3}\pi \frac{\frac{1}{\rho^3}}{\frac{1}{a^3}}$ $\longleftarrow$ data

$P/a^3 < 4/\rho^3$

Information content = 4 parameters per ρ^3

2 angstrom resolution sufficient in 3D

HREM of amorphous structures

resolution = 1A

2D: 1.5 atom per A^2 → not resolvable

3D: 1 atom per A^3 → resolvable

Required dose

Rose criterion

Dose

$$D = \frac{\text{imaging particles}}{\text{volume}} = \frac{N}{\rho^3}$$

signal-to-noise ratio

$$SNR = \frac{N}{\sqrt{N}} = \sqrt{N} = \sqrt{D\rho^3}$$

$$D = \frac{4(SNR)^2}{\rho^3}$$

$SNR = 10$

$$D = 400 \text{ particles} / \rho^3$$

Limitations of resolution

$$D > \frac{400}{\rho^3}$$

Sufficient brightness, time, stability of specimen
(inorganic objects)

Resolution limited by the instrument
ultimate resolution = atom

$$D < \frac{400}{\rho^3}$$

- Insufficient brightness, time
- Instability of specimen (radiation damage)
(life-science objects)

"object resolution"

Knock-on damage
(inorganic objects)

Inelastic cross section<elastic cross section

resolution = atom

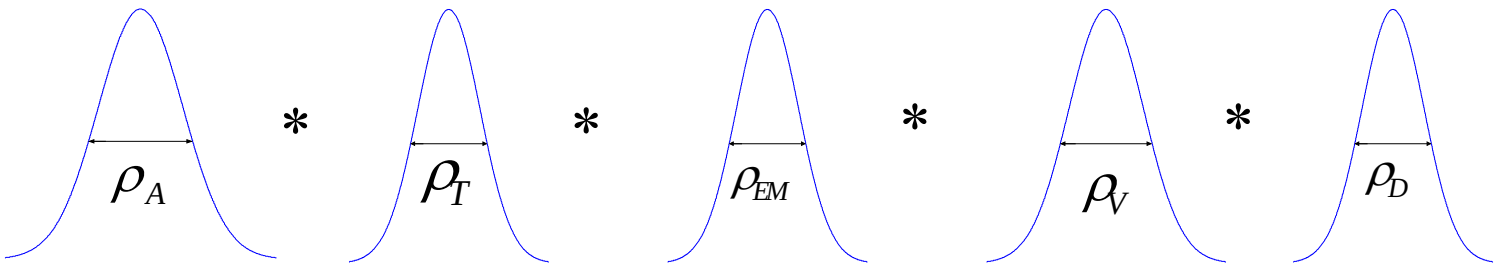
Ionisation damage
(life science objects)

Instrument resolution < object resolution

Atomic resolution EM

Point-spread function:

$$p(r) = p_A(r) * p_T(r) * p_{EM}(r) * p_V(r) * p_D(r)$$



atom potential | thermal motion | electron microscope | environment | detector

$$\rho_R^2 = \rho_A^2 + \rho_T^2 + \rho_{EM}^2 + \rho_V^2 + \rho_D^2$$

$$\boxed{\rho_R \geq \rho_A}$$

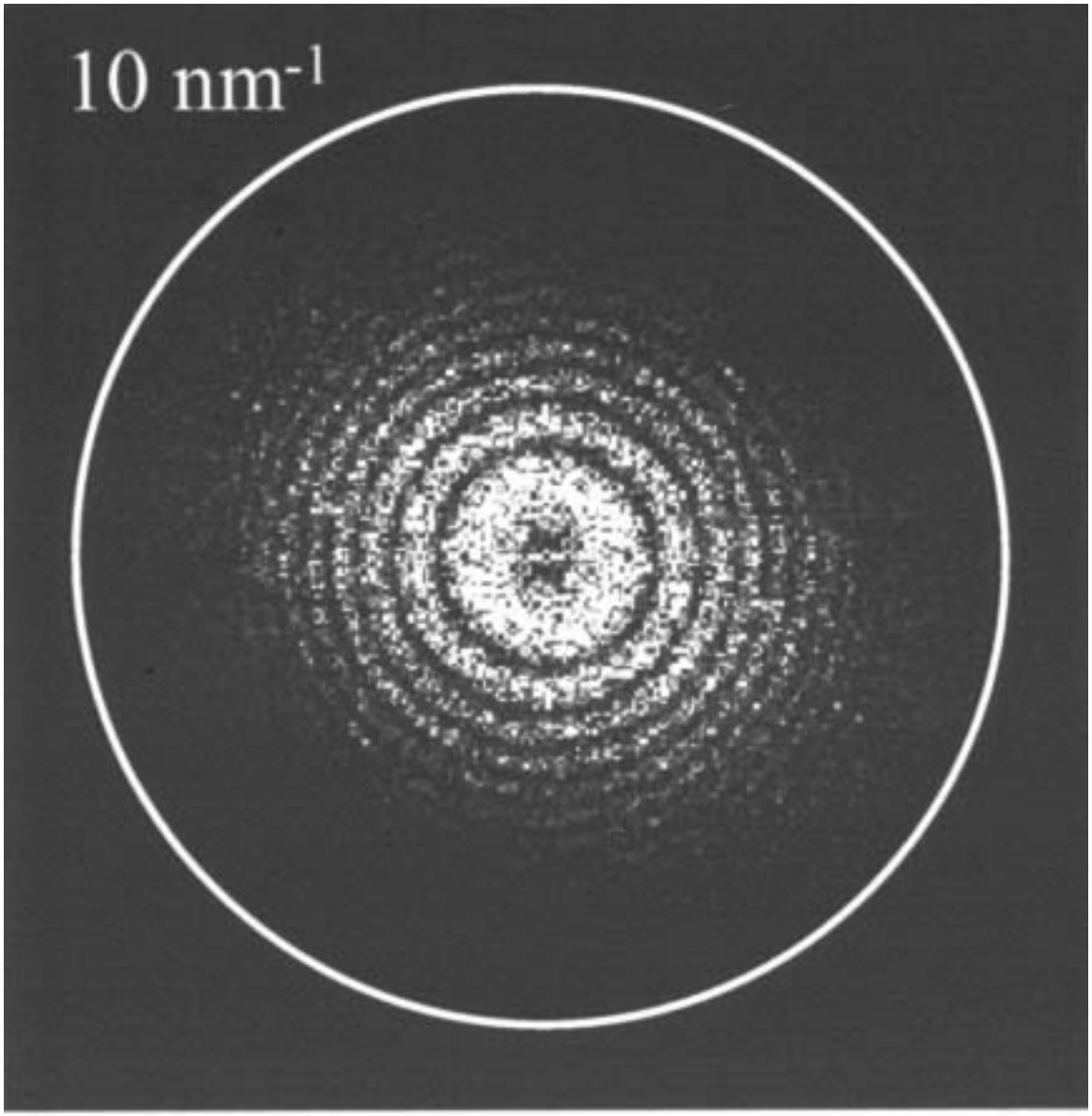
ultimate resolution

=

intrinsic width of the atom

image same information as DP (2x)

Note knock-on damage: cross section smaller than ρ_A



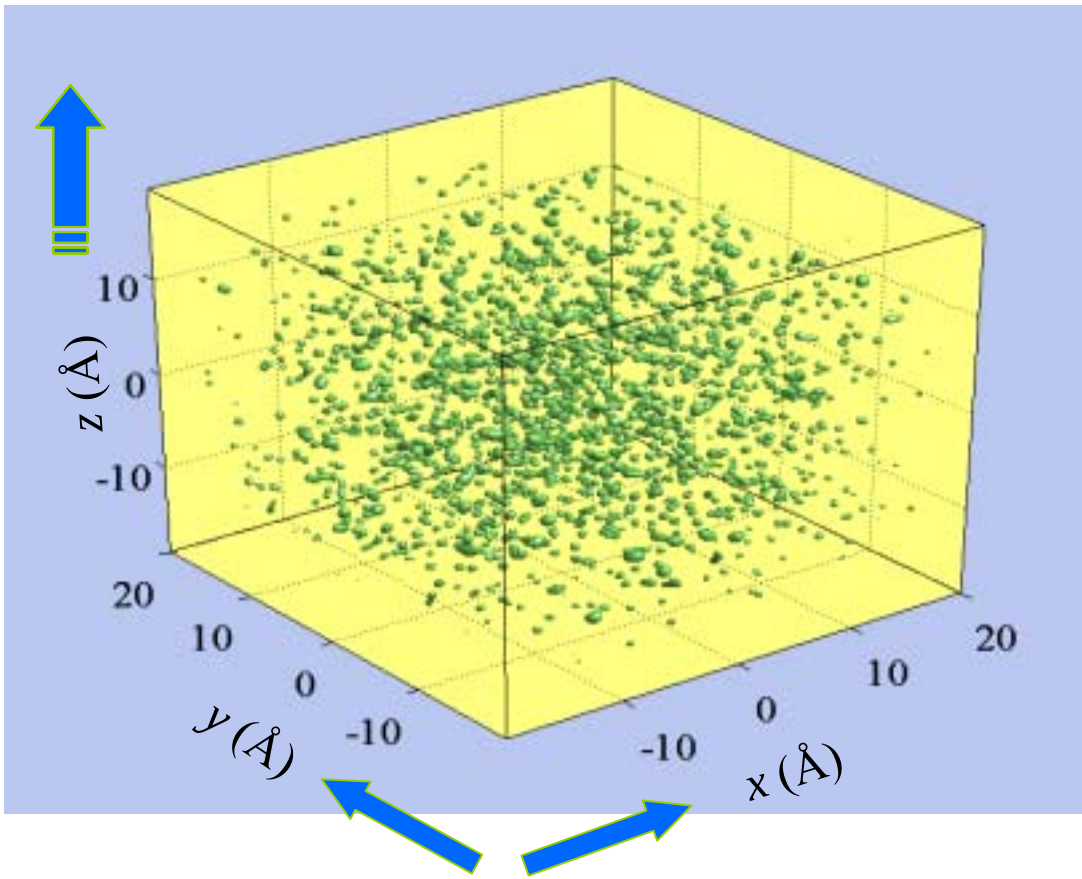
amorphous Ge

Example: amorphous tungsten

Number of atoms used: 1859

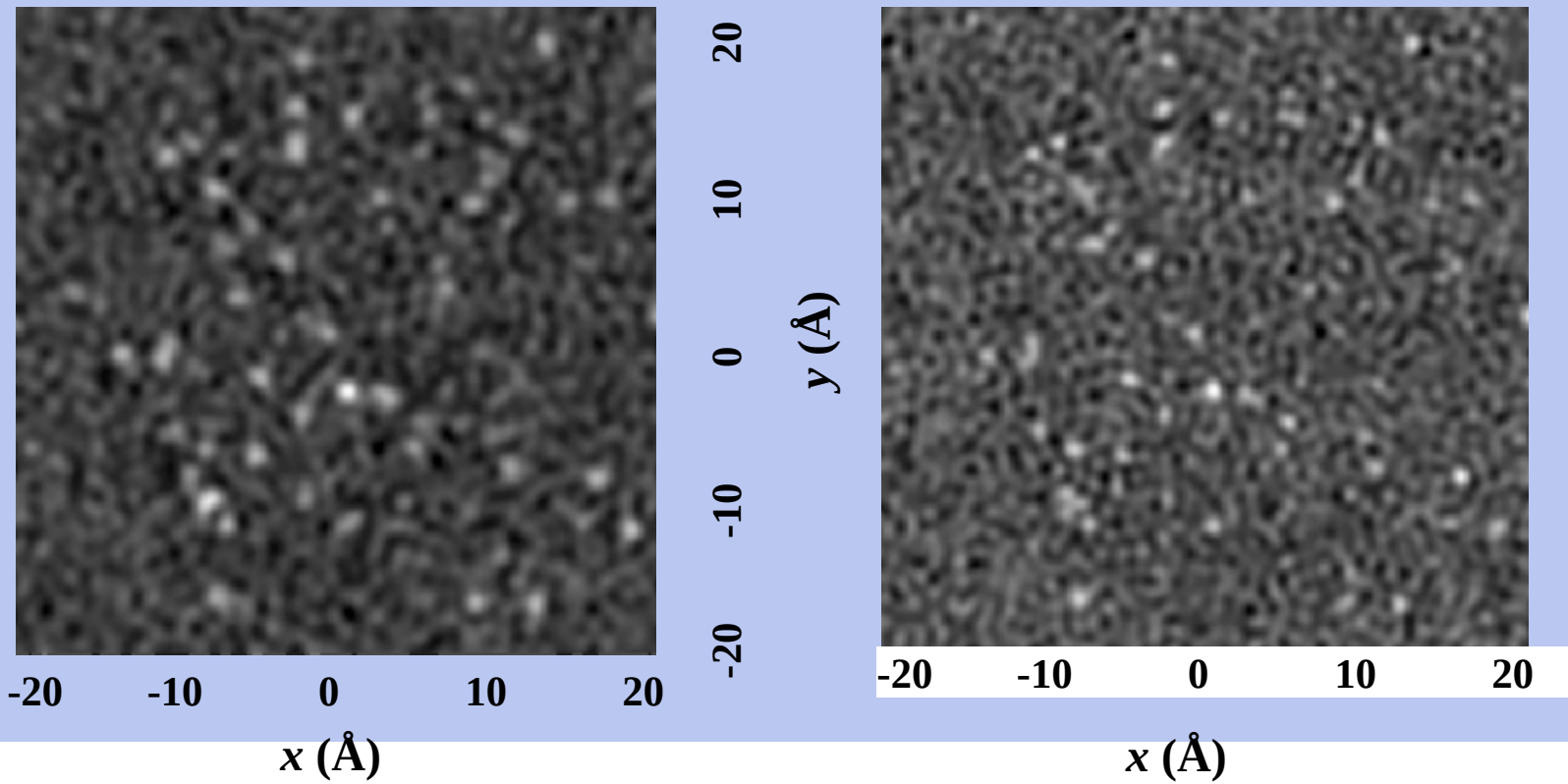
C_s = 0.5 mm E = 300 keV ε = -1.15 Sch

180 images with 1º increment Dose/image = 1.4×10^4 Å^{-2}



C_s = 0.5 mm

Cs = 0.05 mm



Number of atoms used: 1859

C_s = 0.5 mm E = 300 keV ε = -1.15 Sch

180 images with 1º increment Dose/image = 1.4×10^4 Å^{-2}

First atomic-resolution diffractive image reconstruction.

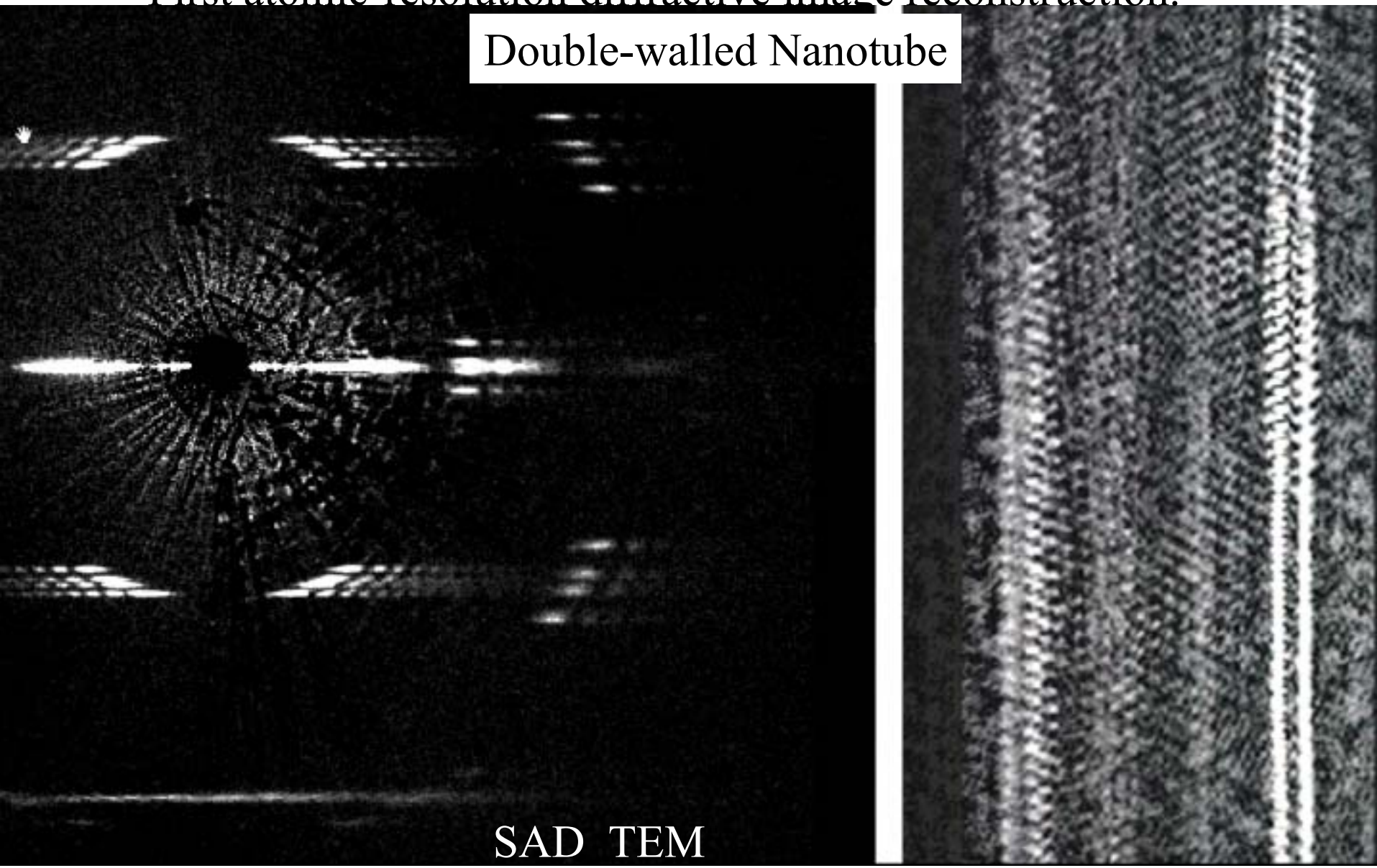
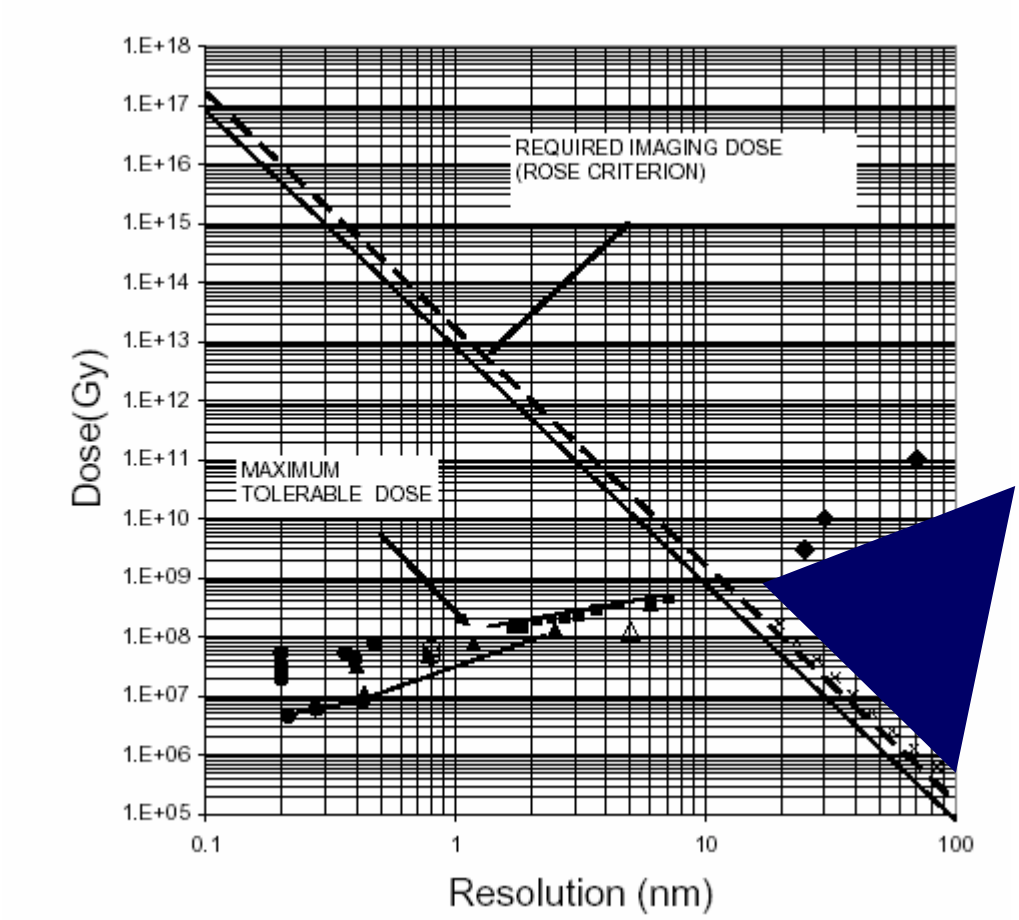


Image reconstructed from electron-diffraction pattern by HiO

J.M.Zuo et al Science 300, 1420 (2003).

Object resolution for life-science objects

Resolution-dose for life-science objects



Region of successful XDM experiments

Howells et al. 2005

How to improve object resolution?

- Radiation damage
  - Electrons better than X-rays
  - Tune energy
  - Cryo protection
  - Averaging over identical objects
  - Inertial imaging (FELS)
- Fitting known substructures
  - Small proteins
  - Alpha helices
  - Beta sheets
- Specimen preparation (FIB, DIP-PEN)

Table 1. *Energy deposited in biological specimens per useful scattering event*

				Neutrons		
				1·8 Å		0·01 Å
	Electrons 80–500 keV	X-rays 1·5 Å	X-rays 30 Å	^{1}H, ^{14}N labelled	^{2}H, ^{15}N labelled (99 %)	
Ratio † (inelastic/elastic) scattering events	3	10	10^{2}–10^{4}	0·080	0·001	10^{-5}
Mechanism of radiation damage	Secondary e^{-} emission	Photoelectric e^{-} emission		^{1}H (n, γ) → ^{2}H ^{14}N (n, p) → ^{14}C and recoil energy	Variety of lower cross-section reactions as well as residual ^{1}H, ^{14}N	
Energy deposited per inelastic event	20 eV	8 keV	400 eV	2 keV*	2 keV*	2 KeV*
Energy deposited per elastic event**	60 eV	80 keV	400 keV	160 eV	2 eV	0·02 eV
Energy deposited relative to electrons						
(inelastic)	1	400	20	100	100	100
(elastic)	1	1000	10000	2·5	0·03	0·0003

† Ratio of inelastic to elastic events for electrons and X-rays are well-known (see, for example, International Tables for crystallography, volume C). The values for neutrons were calculated from neutron scattering cross-sections given by Sears (1992) for the two nuclear reactions $^{1}H(n, \gamma) \rightarrow {}^{2}H$ and ^{14}N (n, p) → ^{14}C, which have similar probabilities for typical protein composition. The black dots in Fig. 2 show where these points have been taken from in the overall curve describing the variation with wavelength.

* The 2 keV estimated energy deposited per neutron inelastic event is the average of the deuteron recoil energy in the first reaction (1·3 keV) and the ^{14}C recoil energy in the second reaction (3 keV). The emitted γ-ray and proton in each case carries away a much larger energy but little of this would be deposited in the specimen. With X-ray and electron inelastic events, energy is deposited by the emitted electrons and secondary electrons respectively.

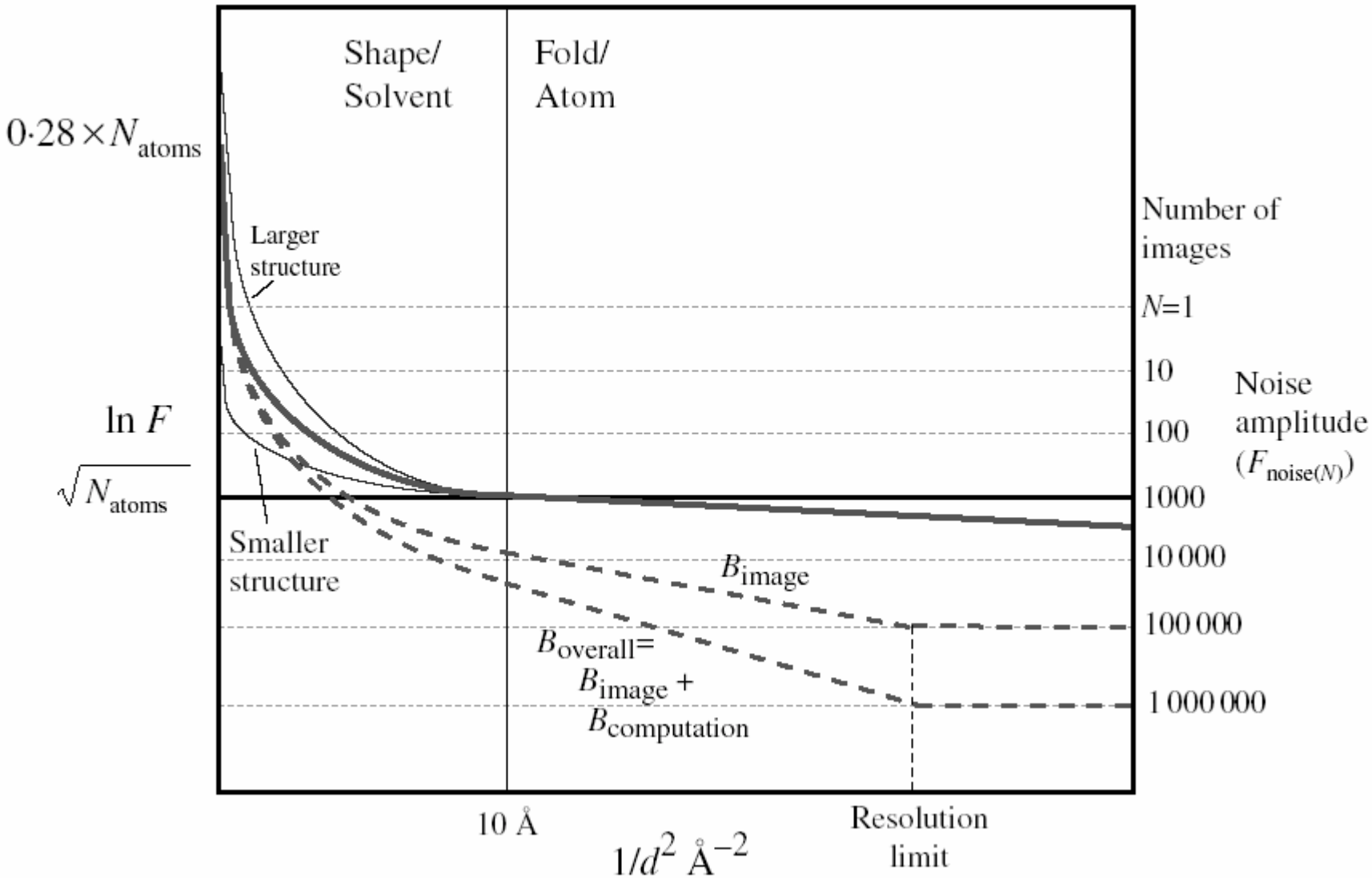
** Row 1 multiplied by Row 3.

Henderson, Quarterly Reviews of Biophysics 28, p. 171 (1995)

Averaging identical objects

$$\rho_A \approx \frac{\rho}{\sqrt{M}}$$

1. Crystalline: natural average, smaller structures
2. 2D, 1D periodic structures
3. Oriented objects (laser)
4. Random objects (minimal size)



Henderson, Quarterly Reviews of Biophysics 37, p. 3 (2004)

Table 1. *2D and 1D structures beyond 10 Å resolution*

Structure	Resolution	Reference
Two-dimensional crystals [high resolution (better than 4 Å)]		
Bacteriorhodopsin p3	3·5 Å, 3·0 Å	Henderson *et al.* (1990), Kimura *et al.* (1997)
Plant LHC-II	3·4 Å	Kühlbrandt *et al.* (1994)
Tubulin dimmer	3·7 Å	Nogales *et al.* (1998)
Aquaporin	3·8 Å, 4·0 Å	Murata *et al.* (2000), Mitra *et al.* (2002)
Two-dimensional crystals [low resolution (better than 9 Å)]		
Bacteriorhodopsin orthorhombic	6·5 Å	Leifer & Henderson (1983)
Deoxycholate bacteriorhodopsin	6·0 Å	Tsygannik & Baldwin (1987)
Halorhodopsin	5·0 Å	Kunji *et al.* (2000)
Porin PhoE	6·0 Å	Jap *et al.* (1991)
Plant photosystem II RC	8·0 Å	Rhee *et al.* (1998)
Yeast H^+-ATPase	8·0 Å	Auer *et al.* (1998)
Gap junction channel	7·5 Å	Unger *et al.* (1999)
Glutathione transferase	6·0 Å	Schmidt-Krey *et al.* (2000)
NhaA Na/H antiporter	7·0 Å	Williams (2000)
Glycerol channel GlpF	6·9 Å	Stahlberg *et al.* (2000)
Rhodopsin, frog p2	7·5 Å	Unger *et al.* (1997)
Rhodopsin, bovine $p22_12_1$	5·5 Å	Krebs *et al.* (2003)
OxlT, oxalic acid transporter	6·5 Å	Hirai *et al.* (2002)
SecYEG complex	8·0 Å	Breyton *et al.* (2002)
EmrE multidrug transporter	7·0 Å	Ubarretxena-Belandia *et al.* (2003)
Helical structures		
Acetylcholine receptor	4·0 Å	Miyazawa *et al.* (2003)
Bacterial flagellum	4·0 Å	Yonekura *et al.* (2003)
Microtubule	8·0 Å	Li *et al.* (2002)
Calcium ATPase	8·0 Å	Zhang *et al.* (1998)
Tobacco mosaic virus	10·0 Å	Jeng *et al.* (1989)

Henderson, Quarterly Reviews of Biophysics 37, p. 3 (2004)

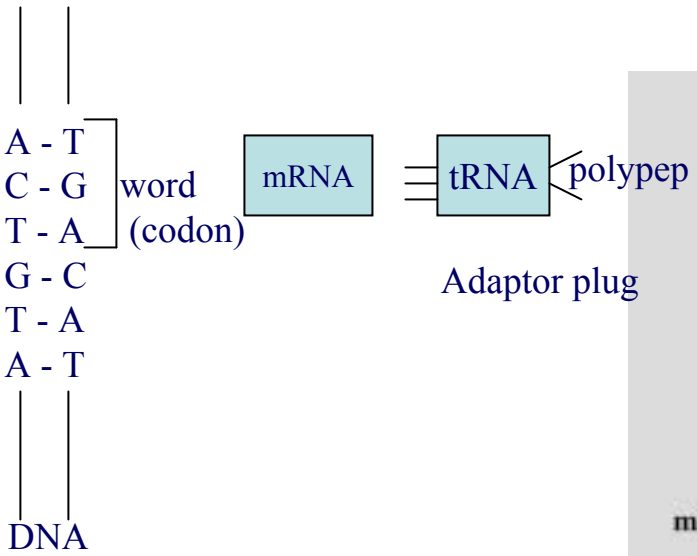
Table 2. *Single particle structures beyond 10 Å resolution*

Structure	MW[a]	Resolution	Reference
Icosahedral structures			
Hepatitis B cores	4·1 MDa	7·4 Å, 9 Å	Böttcher *et al.* (1997), Conway *et al.* (1997)
Herpes virus capsid	192 MDa	8·5 Å	Zhou *et al.* (2000)
Cytoplasmic polyhedrosis virus	>40 MDa	8 Å	Zhou *et al.* (2003)
Semliki Forest virus	48 MDa	9 Å[b]	Mancini *et al.* (2000)
PM2 virus	47 MDa	8·5 Å	Huiskonen *et al.* (In Press)
Sindbis virus	46 MDa	~11 Å	Zhang *et al.* (2002)
Dengue virus	>13 MDa	9·5 Å	Zhang *et al.* (2003a)
Reovirus virions	110 MDa	7·6 Å	Zhang *et al.* (2003b)
Pyruvate dehydrogenase, E2CD	1·5 MDa	8·7 Å	Rosenthal & Henderson (2003)
Rice dwarf virus	53 MDa	6·8 Å	Zhou *et al.* (2001)
Tomato bushy stunt virus	8·9 MDa	5·9 Å[c]	van Heel *et al.* (2000)
P22 bacteriophage mature/shell	50/20 MDa	9·5/8·5 Å	Jiang *et al.* (2003)
Single particles of lower[d] symmetry than icosahedral			
E. coli 70S ribosome	2·5 MDa	11·5 Å, 9 Å	Gabashvili *et al.* (2000), Valle *et al.* (2003)
Bacteriophage SPP1 connectors	1·0 MDa	10 Å	Orlova *et al.* (2003)
50S ribosomal subunit	1·6 MDa	7·5 Å[e]	Matadeen *et al.* (1999)
GroEL	0·8 MDa	8·7 Å, 11·5 Å	Ranson *et al.* (2001), Ludtke *et al.* (2001)

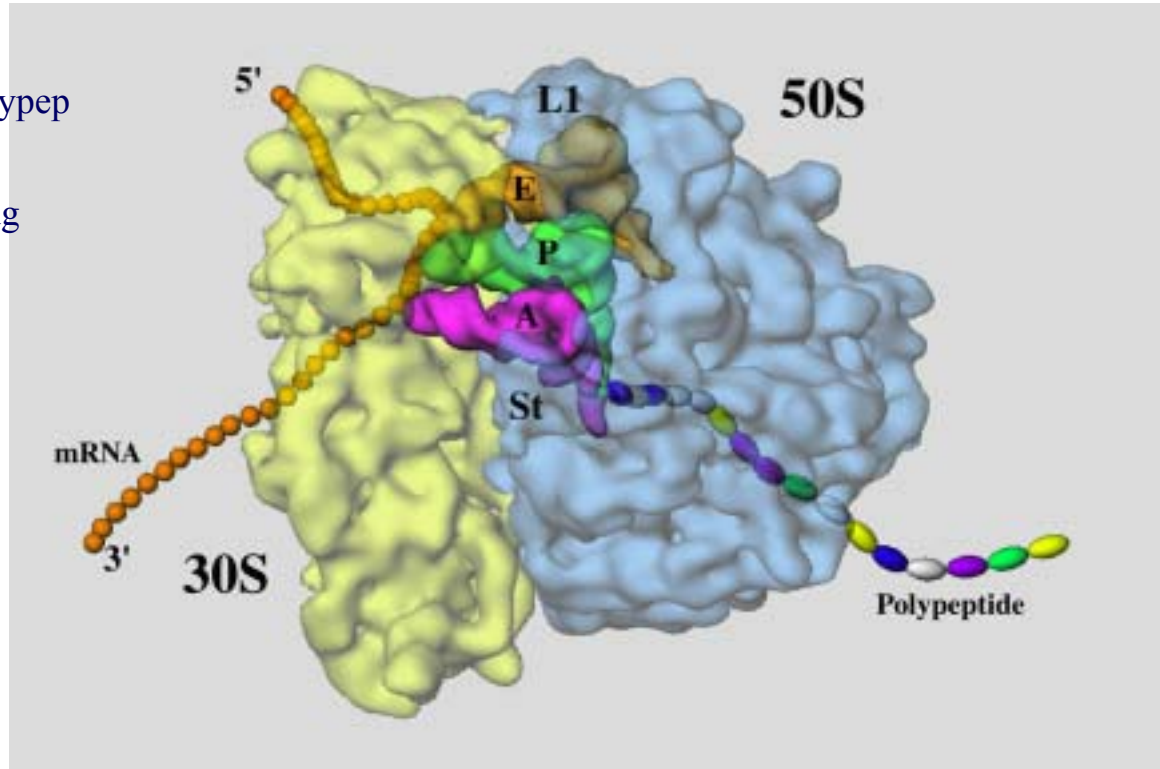
Henderson, Quarterly Reviews of Biophysics 37, p. 3 (2004)

What is the best that has been done by cryo -TEM methods ? (not 2D xtals)

Protein synthesis ("Life itself") in the Ribosome: The ribosome structure determined to 1nm resolution by TEM (tomographic cryomicroscopy). J.Frank et al.



4 nucleic acids
2 (4) base pairs
mRNA reads one side only
3 pairs per word (per amino)
$4^3 = 64$ possibilities per amino
20 amino acids.
n words per gene (protein)



Ribosome width 25nm **(Cell,100, p.537 (2000))**

Experimental e-coli ribsome reconstuction from TEM images of non-crystallised mols in ice. mRNA bring 3-bit codons from DNA. tRNA "adaptors" (E,P,A) have plugs at one end to mRNA codon, at the other to an amino acid, which is added to the polypetide chain as the ribo runs along the mRNA. Chain will fold to become a new protein. (Simplified)

Courtesy J. Spence

- Source brightness
  - X-rays
    - New generation synchrotrons
    - Free Electron Laser Source (FELS)
    - Bosons (NO fundamental limit)
  - Electrons
    - Field emission sources
    - Correctors
    - Fermions: - limit of phase space (still 10^5 off)
      - Coulomb interaction

TABLE 1. Comparison of synchrotron soft X-ray and field-emission electron sources. All values are for 500 eV X-rays, or the 300 keV electron beams which are typically used to study ELNES at around 500 eV. ELNES uses parallel detection, XANES serial.

	ALS Undulator U5	e^- Cold FEG at 300 kV
Brightness	6.9×10^{24} particles /sec /cm^2 /sr /eV (1.1×10^{19} Ph/s /mm^2 /mr^2/ 0.1%BW)*	1.3×10^{29} particles /sec /cm^2 /sr /eV (6×10^9 A/cm^2 /sr.)
Degeneracy δ	18	1.54×10^{-5}
Coherent flux j_c	2.0×10^7 ph/s/0.1%BW	
Energy spread ΔE (un-monochromated).	4.6 eV	0.28 eV
Source size.	307x23 μm	2nm
Resolution of focussing element.	30 nm	0.1 nm
Flux into focussed probe	4.0×10^5 ph/s/0.1%BW	1nA into 1nm diameter. Higher with aberration corrector.

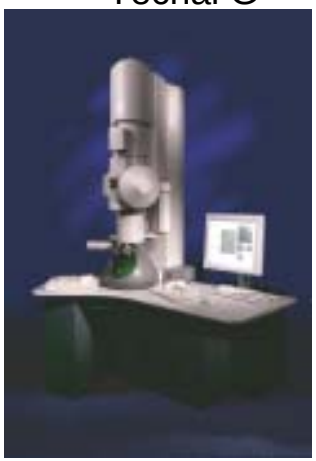
* This is the long time average value. Instantaneous values are about D=100 times greater.

Electron brightness values from Speidel et al Optik 49, 173. Nanotip at RT is 55 times brighter (Qian, Scheinfein, Spence J.Appl Phys.73, p.7041.

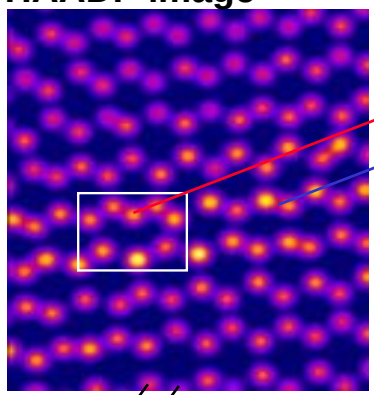
Spence and Howells, Ultramic.93, p.213 (2003)

Scanning Electron Microscopy & HREM & Spectroscopy

A STEM / HRTEM :
Tecnai G^2

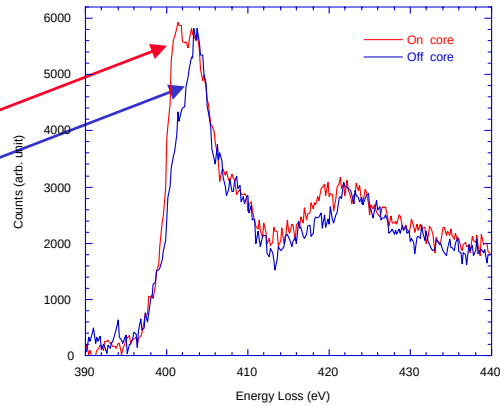


Current technology:
HAADF-image

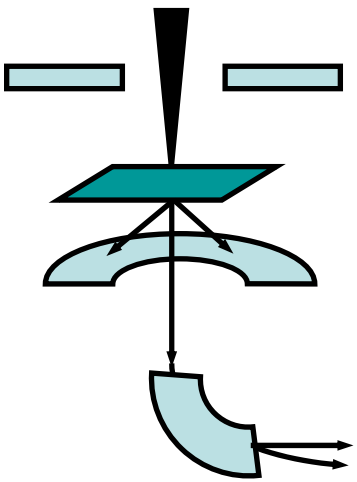


0.2 nm

Local energy spectrum



Dislocation core in GaN [0001]

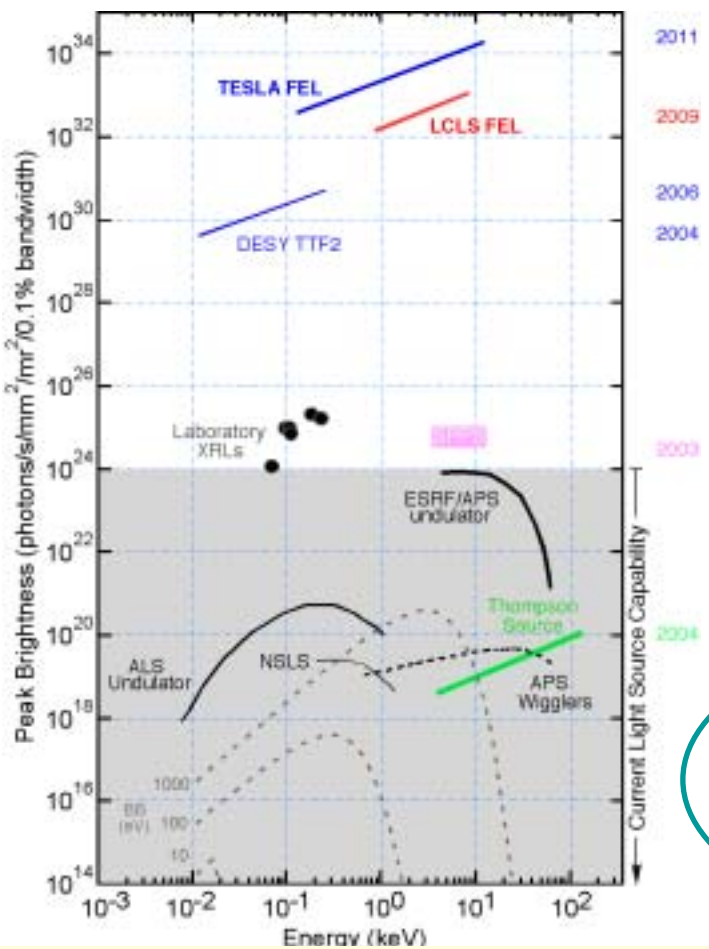


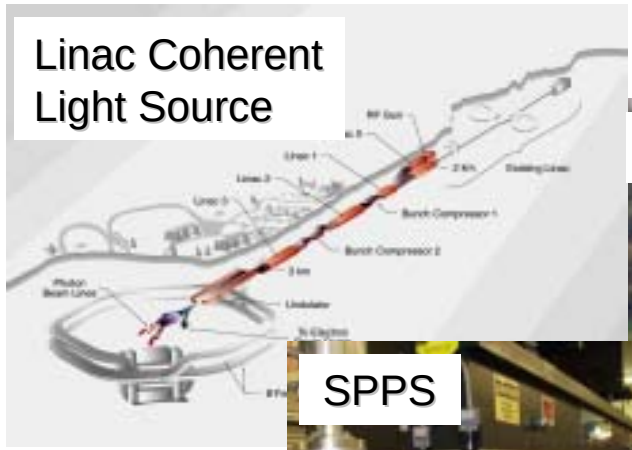
Upgrade to HRTEM/STEM @ NCEM in 2002
First instrument of this kind in the US

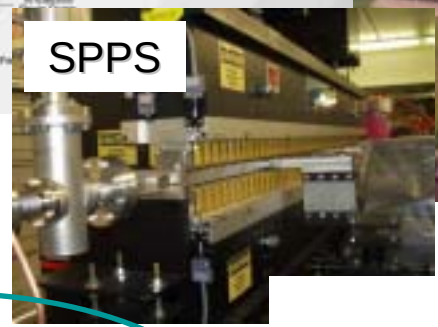
- **Probe size 0.13 nm (currently at NCEM: ~1 nm)**
- **Energy resolution: 200 - 300 meV (currently: ~1eV)**
- **Information Limit : < 0.1 nm @ 200 kV**
- **Phase Contrast & Z-Contrast & Spectroscopy on identical areas**

N. Browning, C. Kisielowski, LDRD, 2002-2003

We are entering a new era in x-ray science

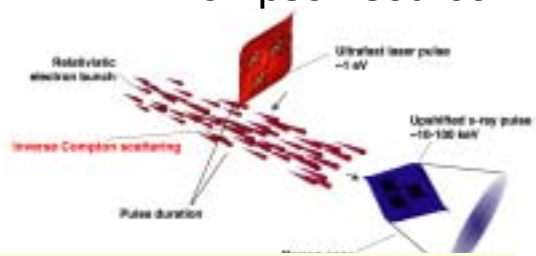






- femtosecond
- 10^{13} photons
- Angstrom wavelength

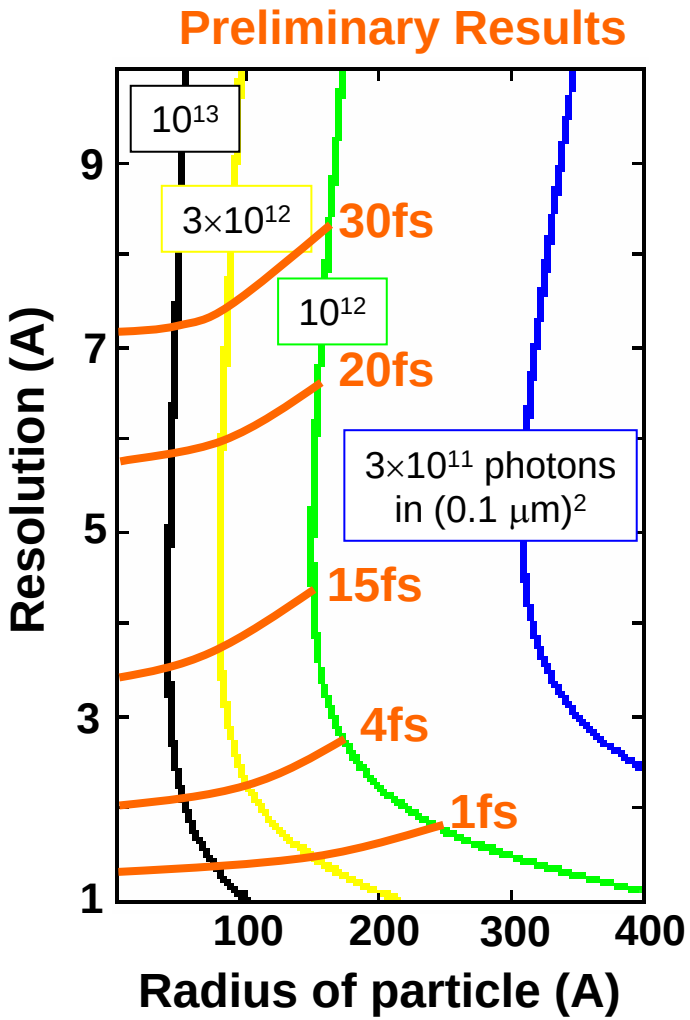
LLNL Thompson source



This short-pulse high-fluence x-ray regime is completely unexplored. What ever we do with these sources will be new science

Courtesy H. Chapman

Combine damage and classification results to determine the required pulse parameters



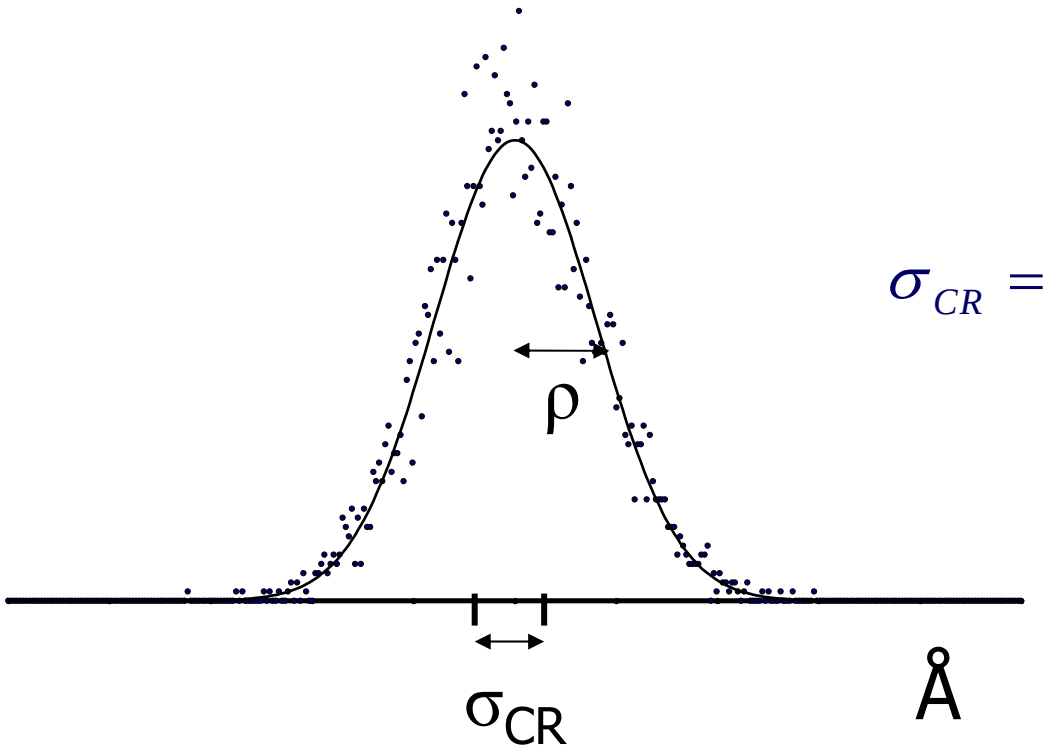
Maximum Pulse Length versus Radius, Resolution, and Sample Size; Limited by Classification and Damage (R_{max} = 0.25)

- Resolution from damage modeling worsens with increasing fluence and increasing pulse length
- For a given radius and fluence, we can determine the maximum pulse length

Atomic resolution can be achieved for optimized XFEL parameters (short pulses, high fluences)

Courtesy H. Chapman

Resolution - precision



$$\sigma_{CR} = \frac{\rho}{\sqrt{N}}$$

resolution

dose

ρ = 1 Å
N= 10000
σ_{CR}= 0.01 Å

EXAMPLE: coherent HREM better than HAADF STEM

Precision ⟷ radiation damage

Precision: Cramér-Rao Lower Bound

$$\sigma_{CR} = \frac{\rho}{\sqrt{\delta \sigma_{el}}}$$

ρ = resolution

δ = incident dose/Å^2

σ_{el} = elastic cross section

Probability for displacive damage of an atom:

$$p = \delta \sigma_{in}$$

σ_{in} = inelastic cross section

Figure of merit ➡

$$\boxed{\sigma_{CR}^2 p = \rho^2 \frac{\sigma_{in}}{\sigma_{el}}}$$

$$\sigma_{el} = \int \left| \frac{2\pi e V_p(\bar{r})}{E\lambda} \right|^2 d\bar{r}$$

$V_p(\bar{r})$ = projected potential
E = incident electron energy
λ = electron wavelength

$$\sigma_{in} = \pi \left(\frac{\alpha}{2E} \right)^2 \left(\frac{T}{\varepsilon} - 1 \right)$$

$$\alpha = \frac{Ze^2}{4\pi\varepsilon_0}$$

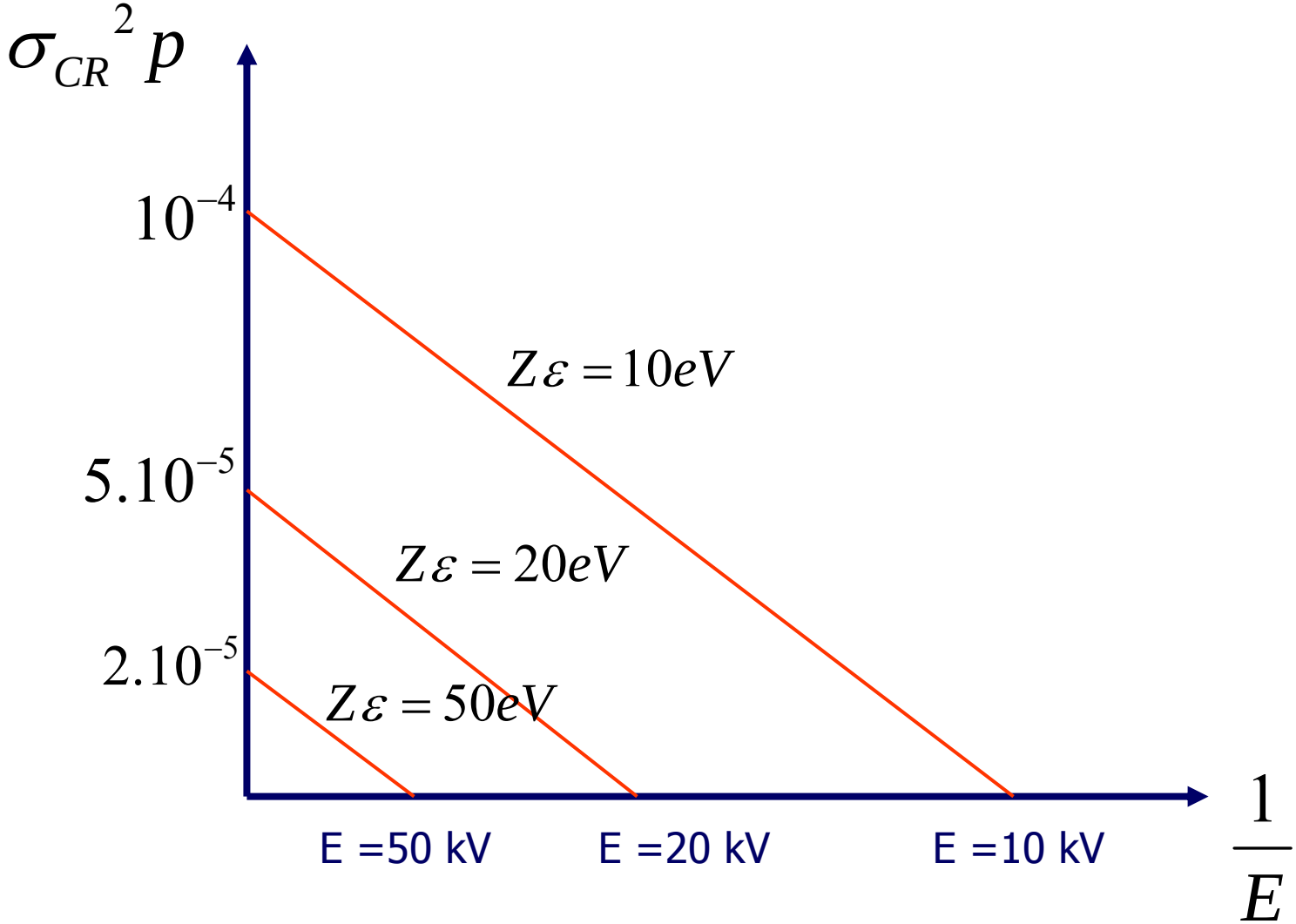
$$T = \frac{4m}{M} E$$

Z = atom number
m = electron mass
M = atom mass
ε = threshold energy for displacive radiation damage

Simple model

$$\sigma_{CR}^2 p = c\left[\frac{1}{1000 Z \varepsilon} - \frac{1}{E}\right]$$

With
Z = atomic number
E = incident energy (eV)
ε = treshold energy for displacement damage



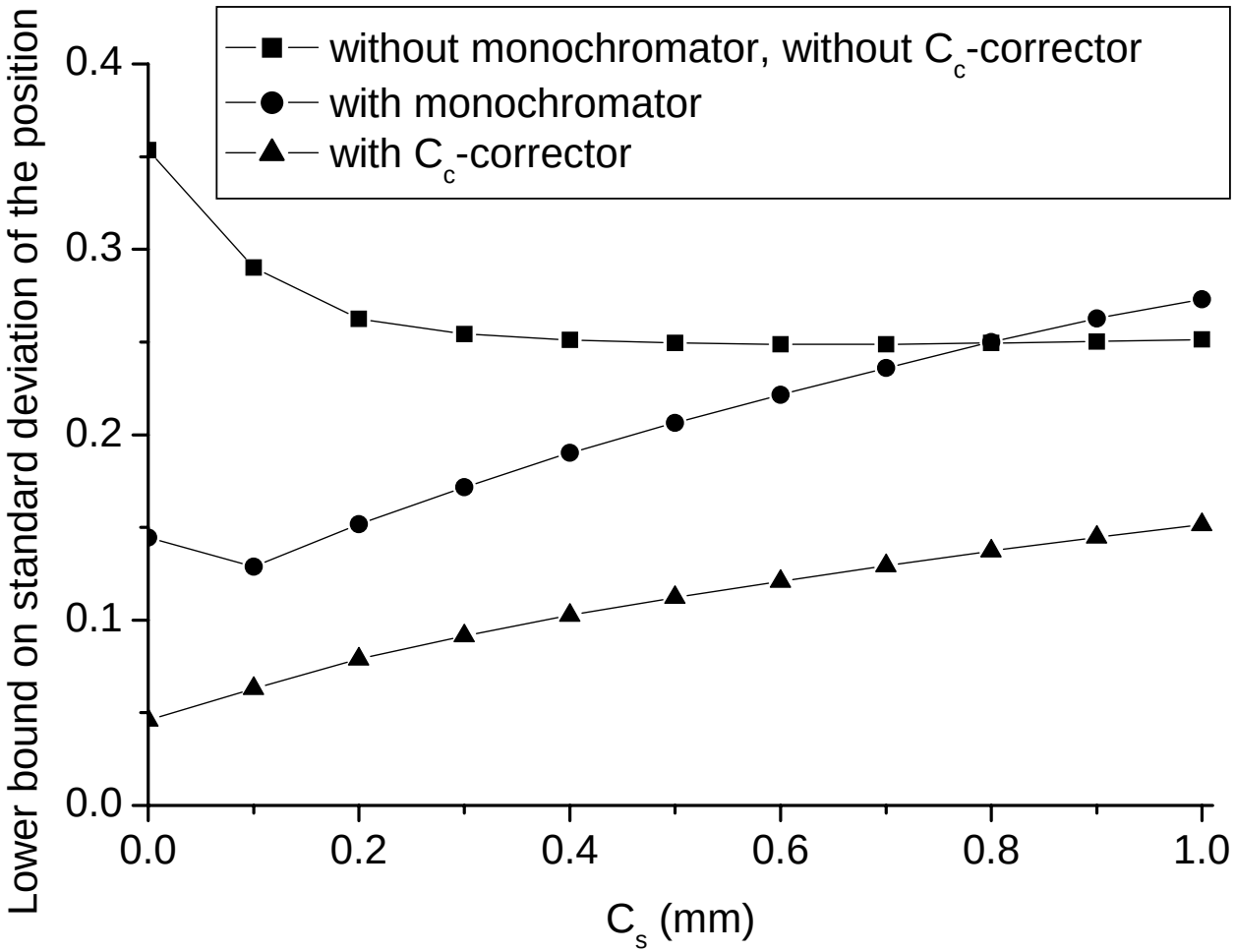
Optimal energy: function of $Z\varepsilon$

Experimental design

At low accelerating voltages:

- Do we need a Cs-corrector?
- Do we need a Cc-corrector?
- Do we need a monochromator?

Precision of a Si atom position as a function of Cs
Accelerating voltage = 50 keV



Conclusion

- Both electron and X-ray methods can ultimately resolve individual atoms.
- Quantitative refinement can yield precisions that are needed for theoretical understanding.