PowerPoint File available:

http://bl831.als.lbl.gov/
~jamesh/powerpoint/
IGBMC_SvN_2016.pptx

Acknowledgements

Robert Stroud James Fraser John Spence
Chris Nielsen Clemens Schulze-Briese Aina Cohen Ana Gonzalez

UCSF LBNL SLAC

ALS 8.3.1 creator: Tom Alber

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Integrated Diffraction Analysis Technologies (IDAT)

Plexxikon, Inc.

M D Anderson CRC

Synchrotron Radiation Structural Biology Resource (SLAC)

The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences, Materials Sciences Division, of the US Department of Energy under contract No. DE-AC02-05CH11231 at Lawrence Berkeley National Laboratory.

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

The “success rate” of structure determination

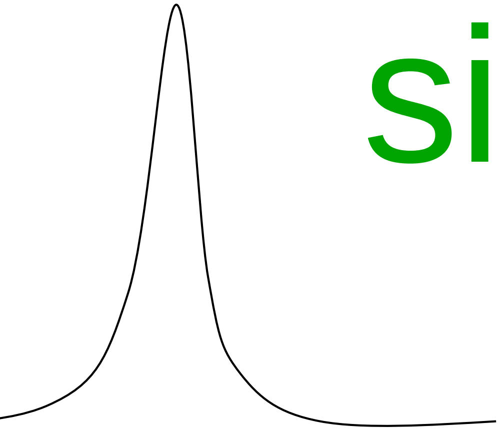
100 s/dataset

200 days/year

~150 beamlines

~26,000,000 datasets/year

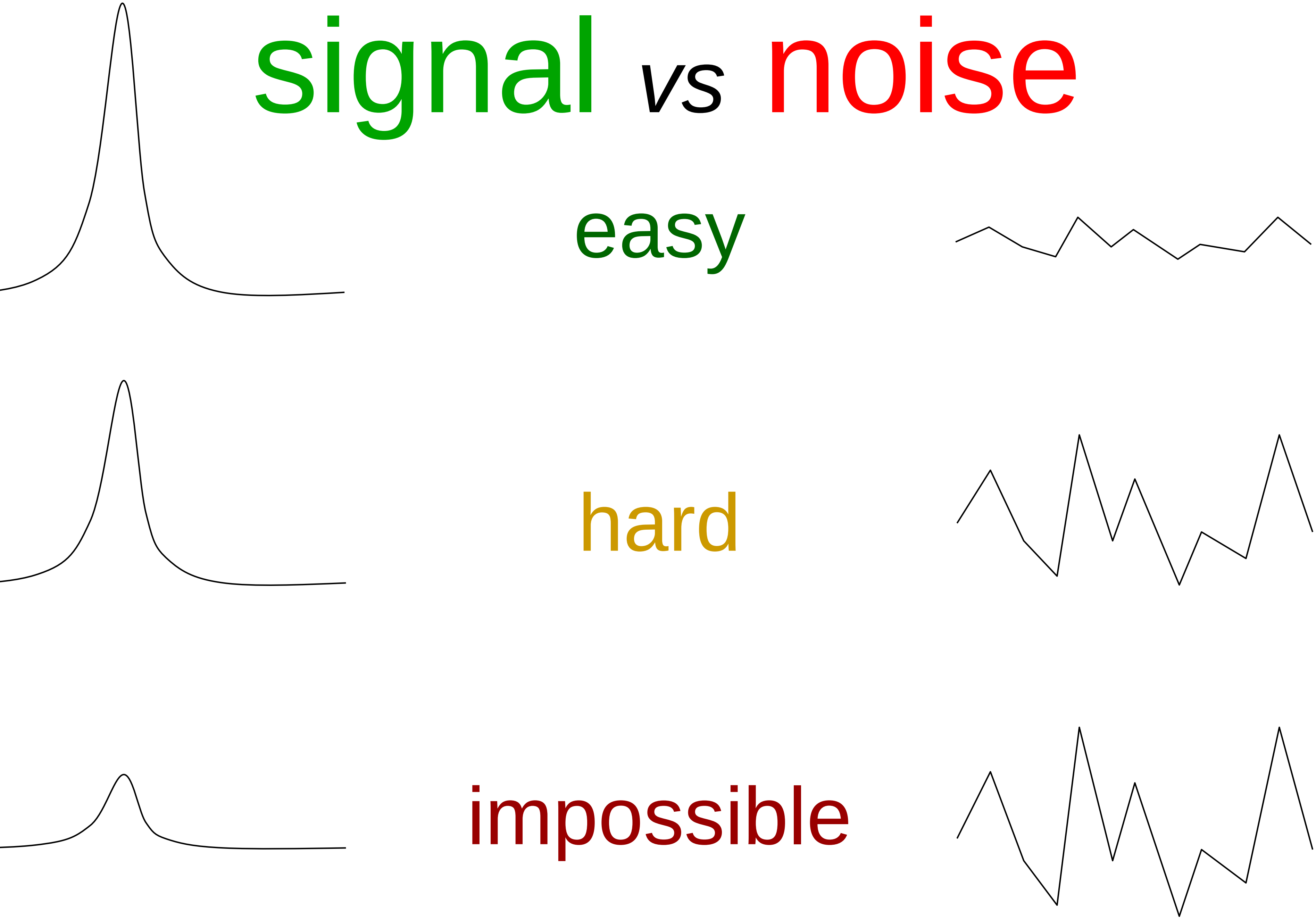
9333 PDBs in 2015

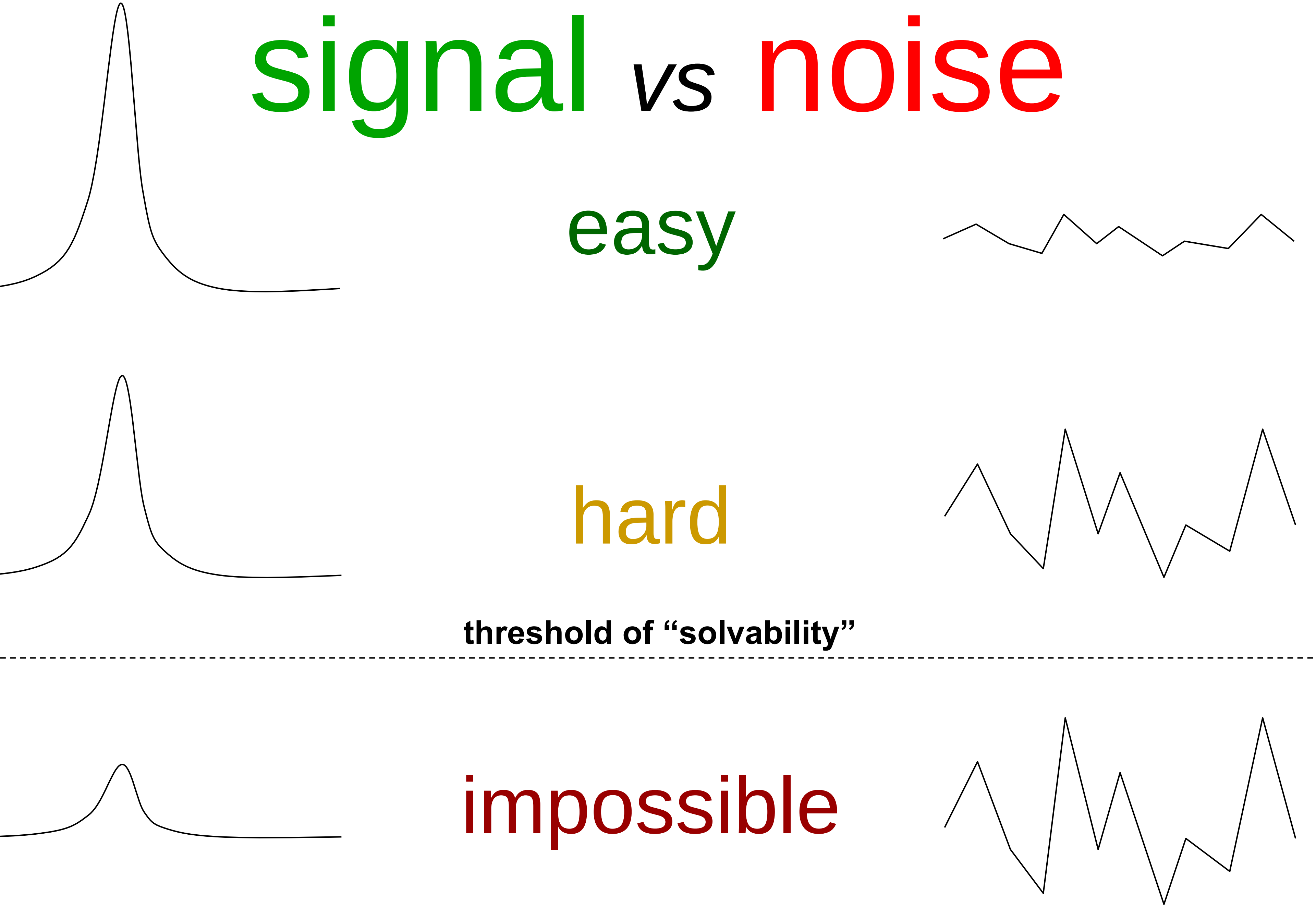


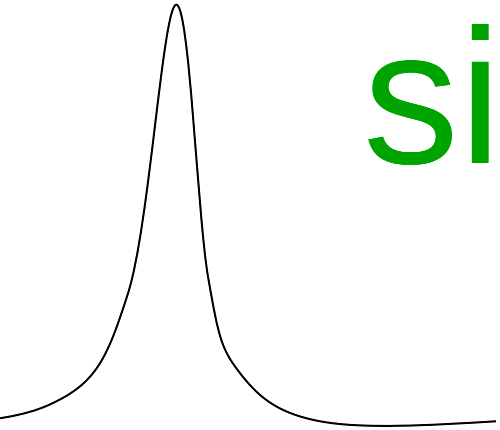
signal

“If you don’t have
good data,
then you have
no data at all.”

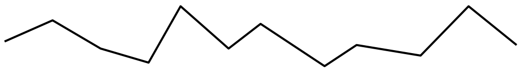
-Sung-Hou Kim


signal vs noise
easy
hard
impossible


signal *vs* noise
easy
hard
threshold of “solvability”
impossible



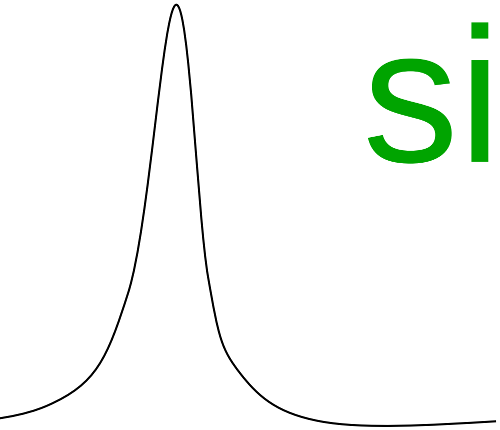
signal / noise = $\sqrt{\text{photons}}$?

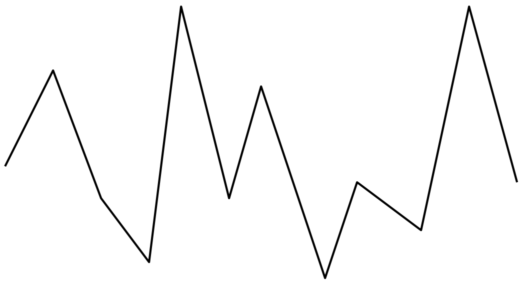


Time

	none	sqrt	proportional
none	CCD Read-out	Photon counting	Detector calibration attenuation partiality Non-isomorphism Radiation damage
1/sqrt			Beam flicker
1/prop.			Shutter jitter Sample vibration Pile-up

signal *vs* noise





"If you don't have good data, then you must learn statistics."

-James Holton

Adding noise

Adding noise

$$1 + 1 = 1.4$$

Adding noise

1 + 1 = 1.4

$$\sigma_{total}^2 = \sigma_1^2 + \sigma_2^2$$

Adding noise

$$1^2 + 1^2 = 1.4^2$$

$$\sigma_{total}^2 = \sigma_1^2 + \sigma_2^2$$

Adding noise

$$1^2 + 1^2 = 1.4^2$$

$$3^2 + 1^2 = 3.2^2$$

$$\sigma_{total}^2 = \sigma_1^2 + \sigma_2^2$$

Adding noise

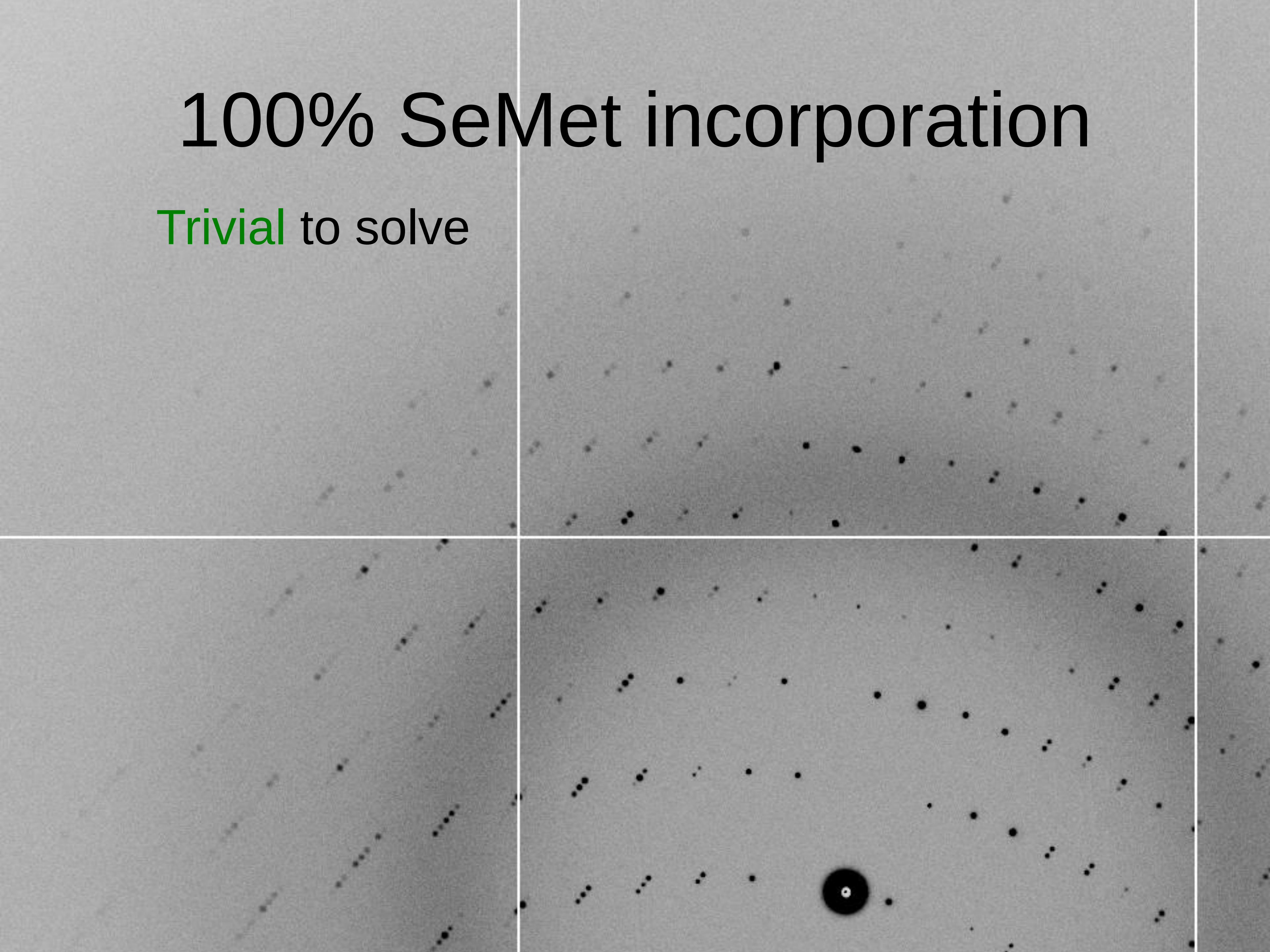
$$1^2 + 1^2 = 1.4^2$$

$$3^2 + 1^2 = 3.2^2$$

$$10^2 + 1^2 = 10.05^2$$

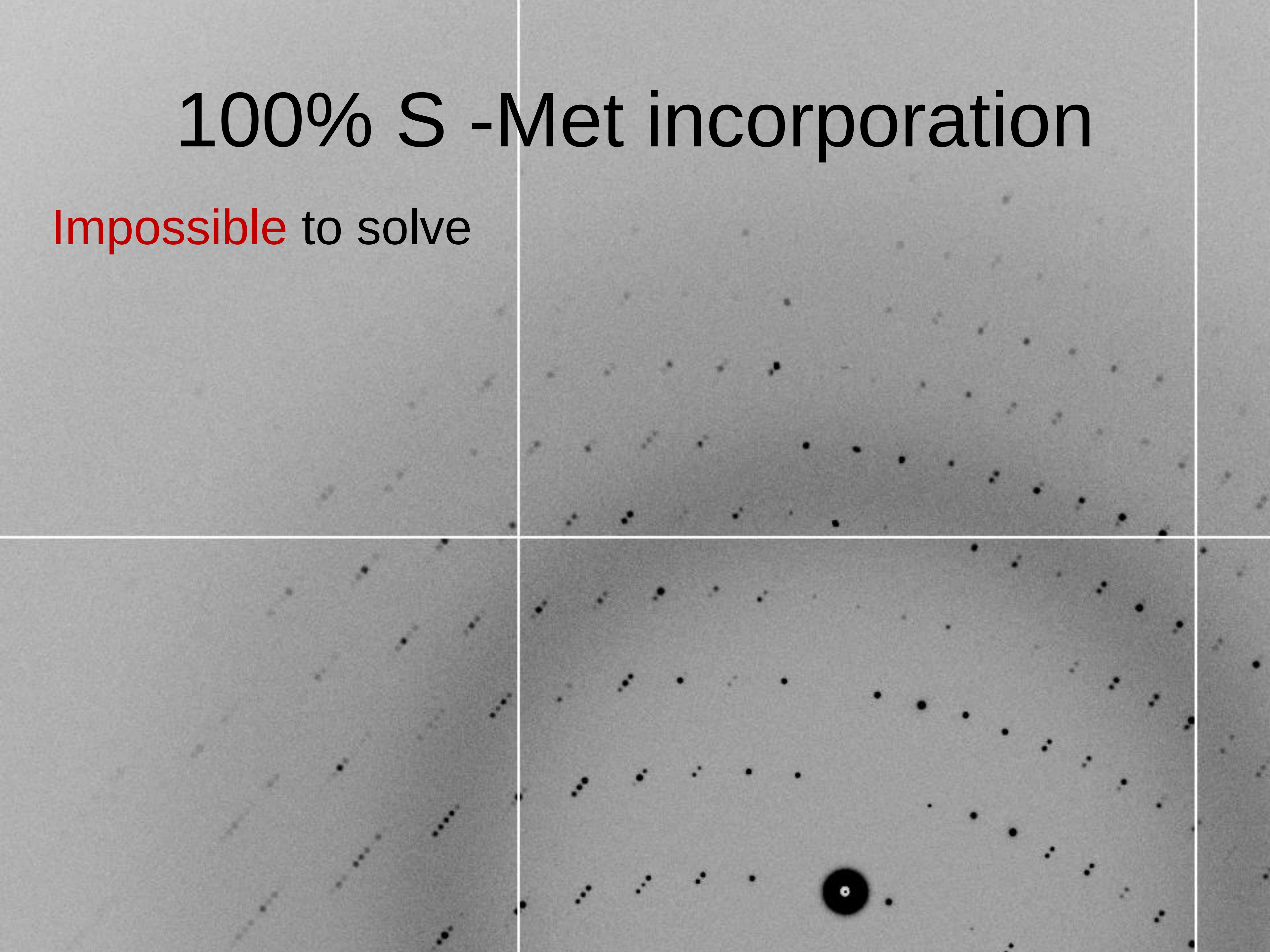
100% SeMet incorporation

Trivial to solve

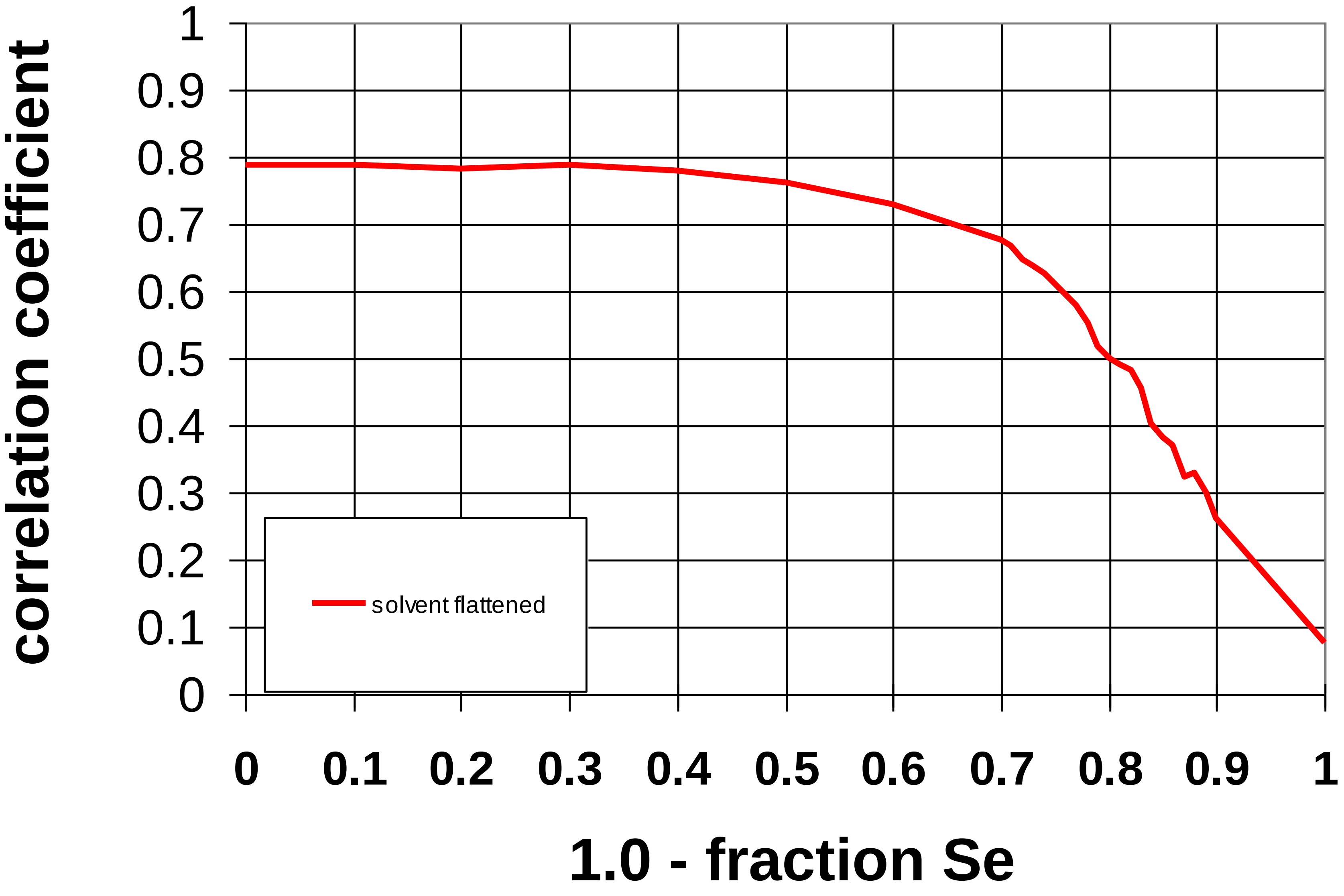


100% S -Met incorporation

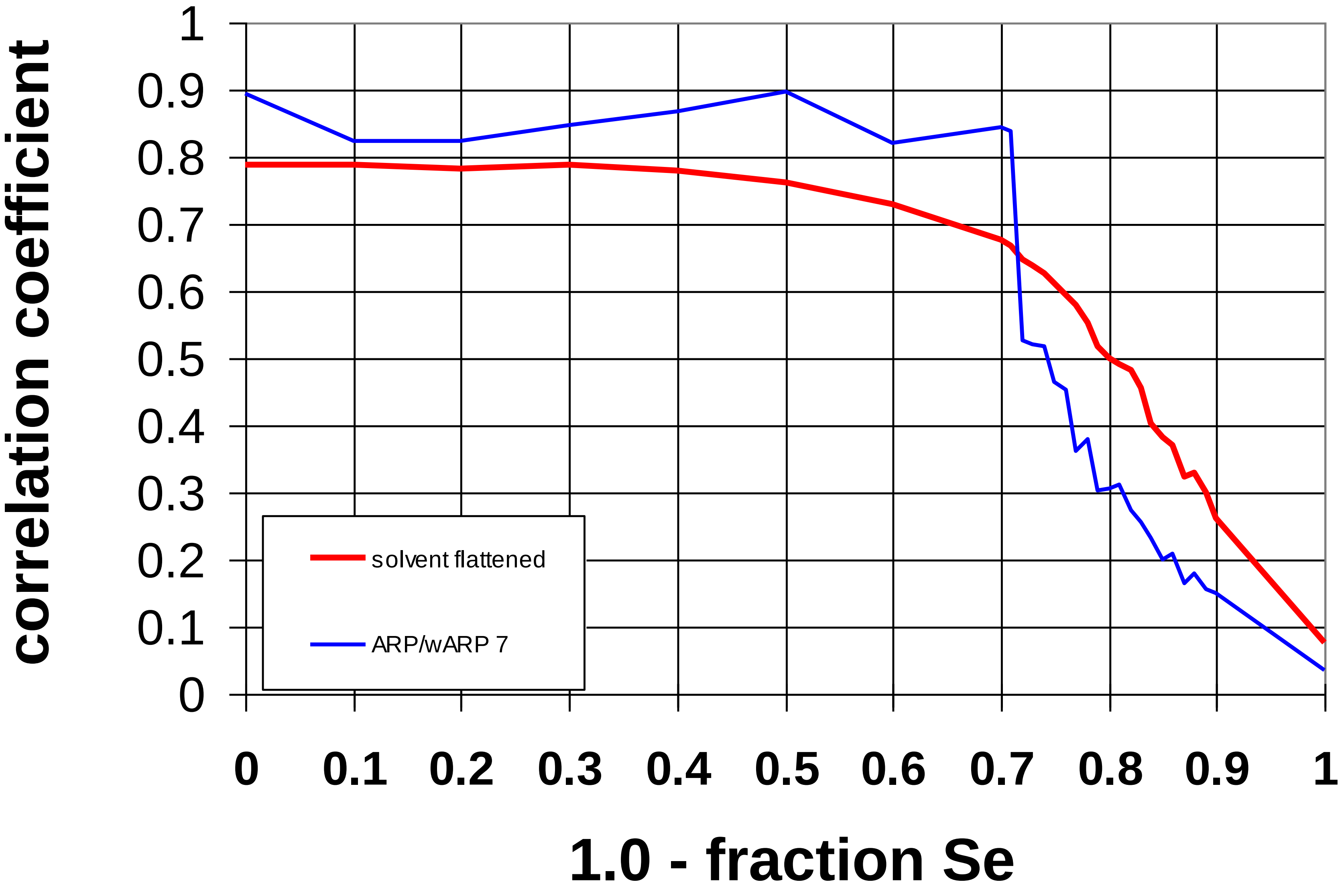
Impossible to solve



Phase Problem: Se vs S



Phase Problem: Se vs S



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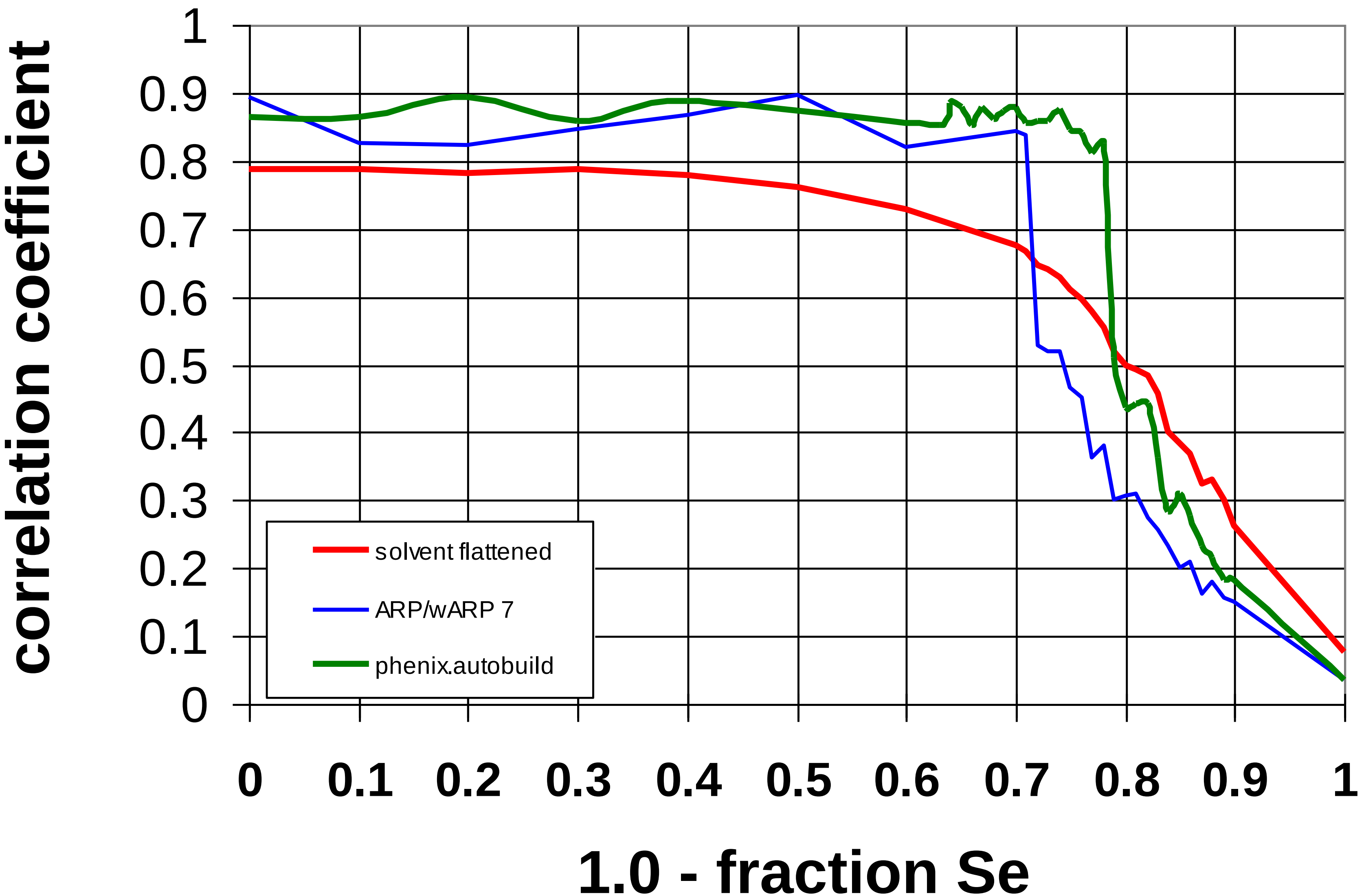
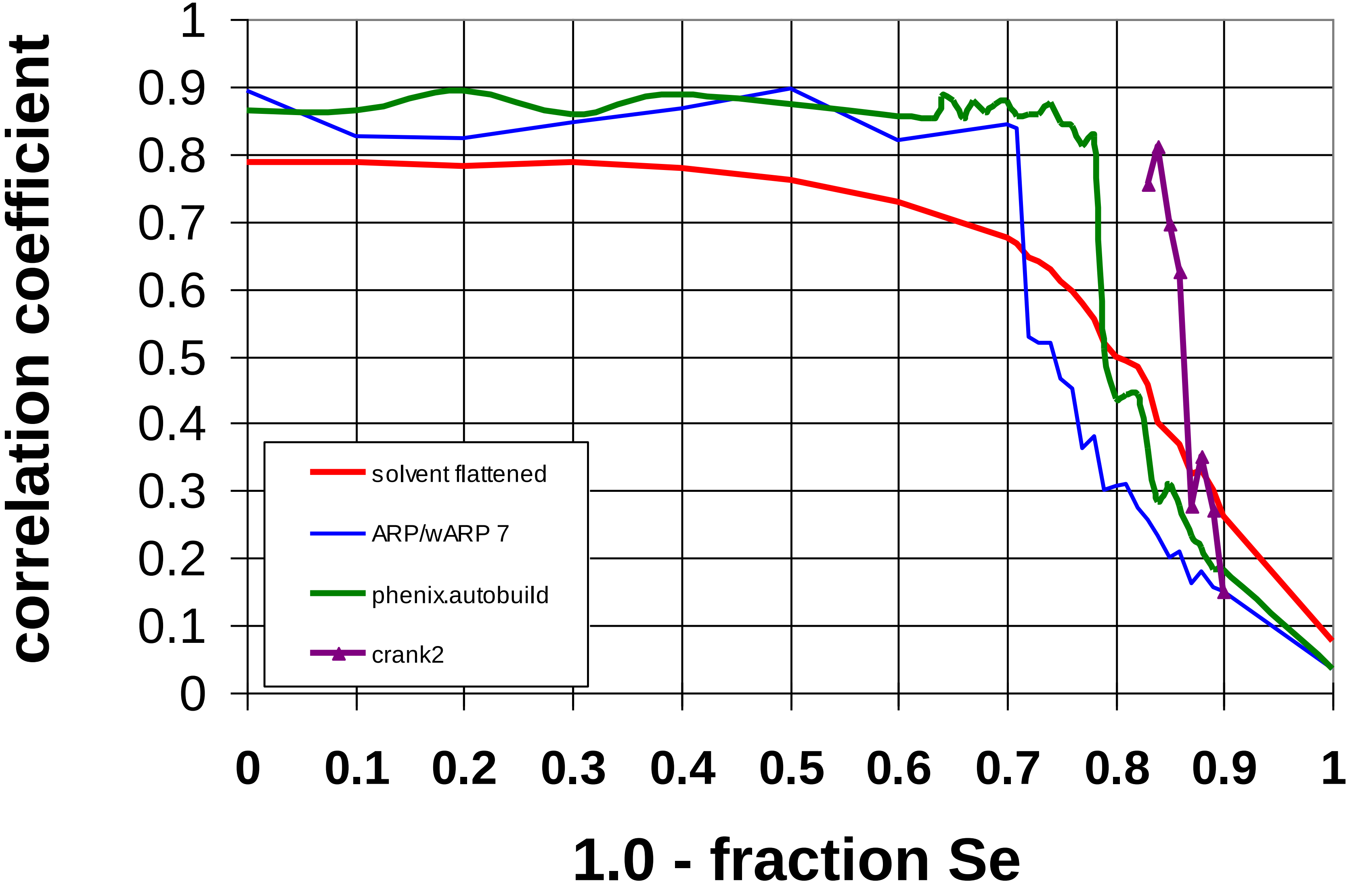
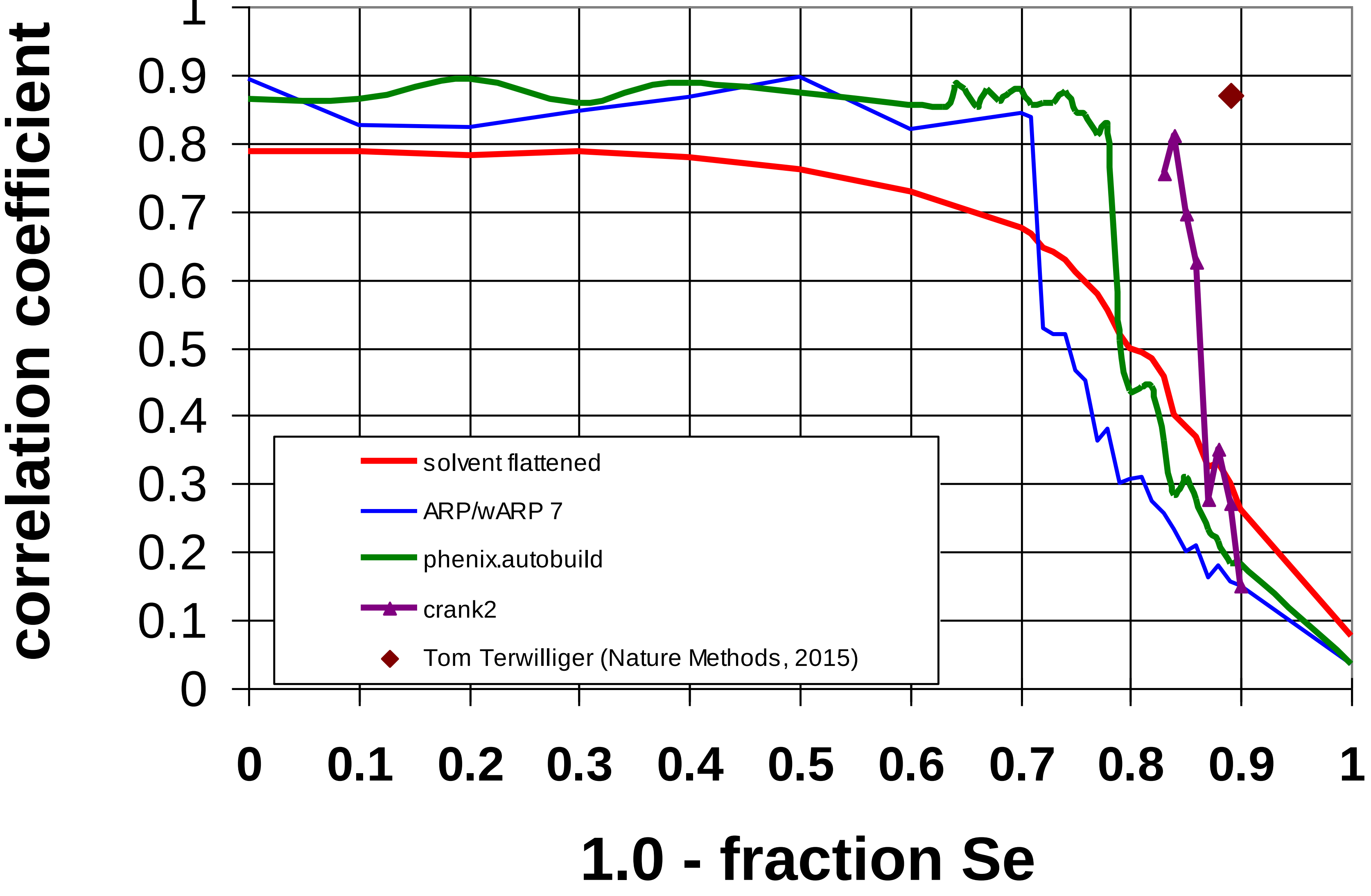


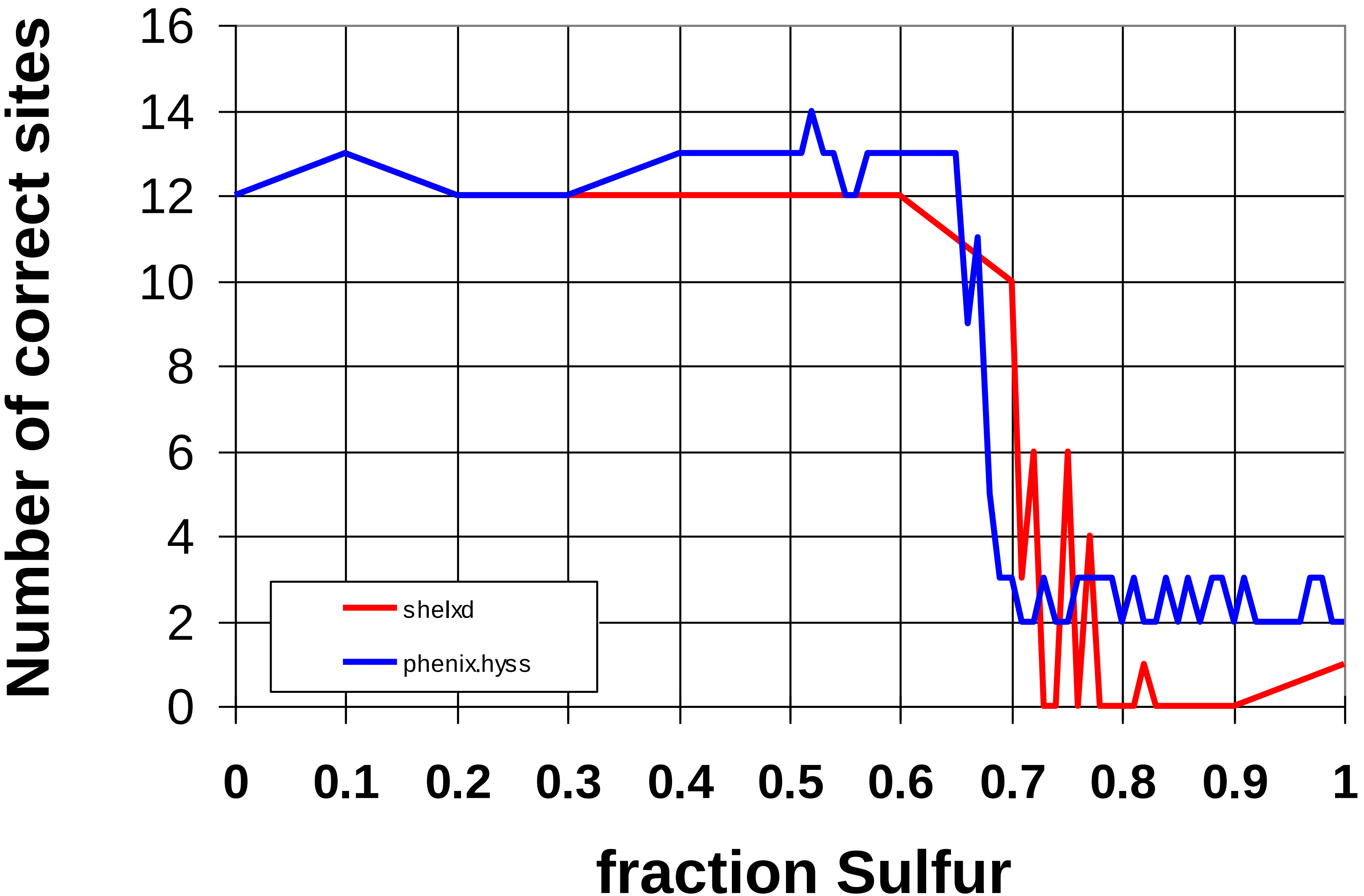
Phase Problem: Se vs S



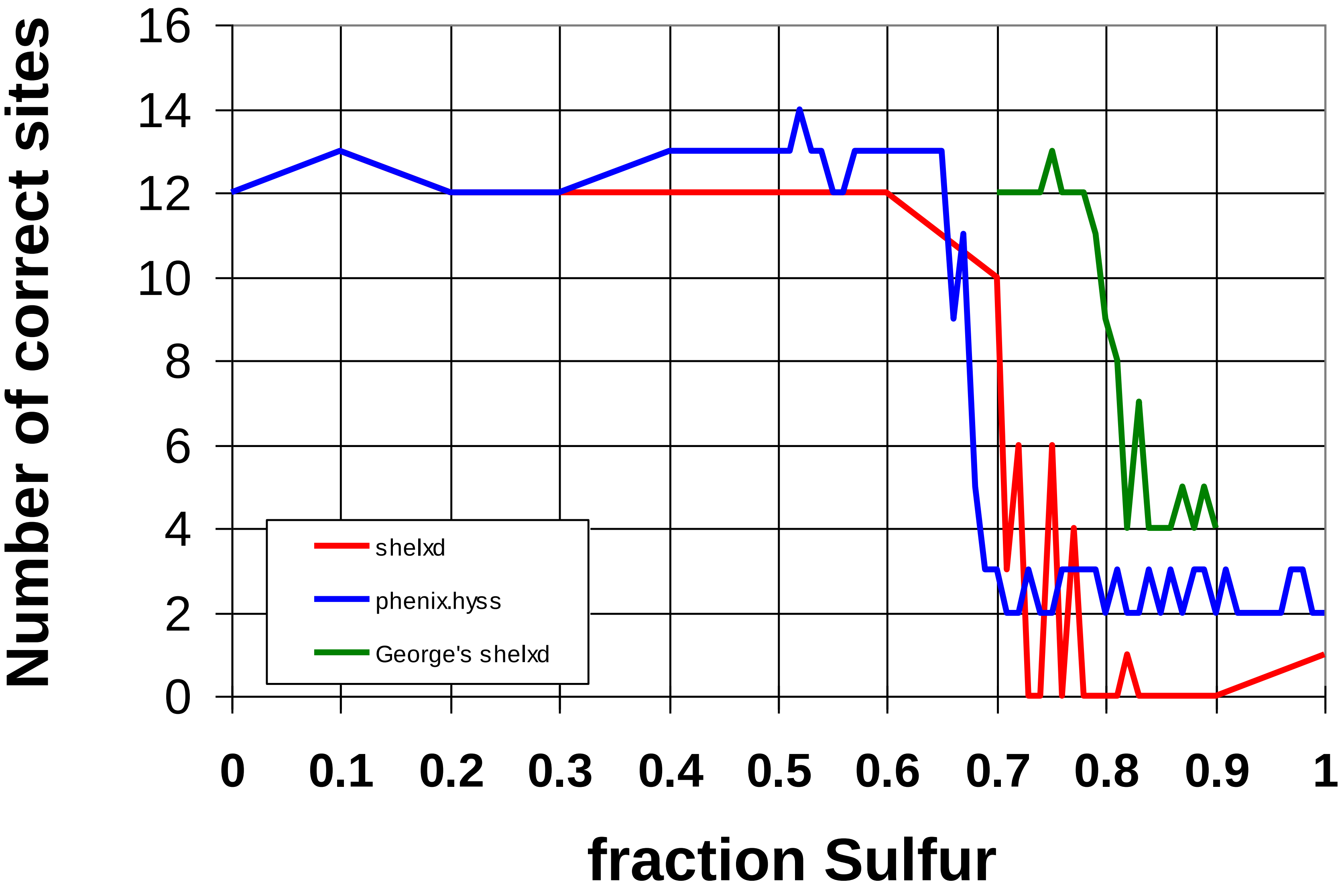
Phase Problem: Se vs S



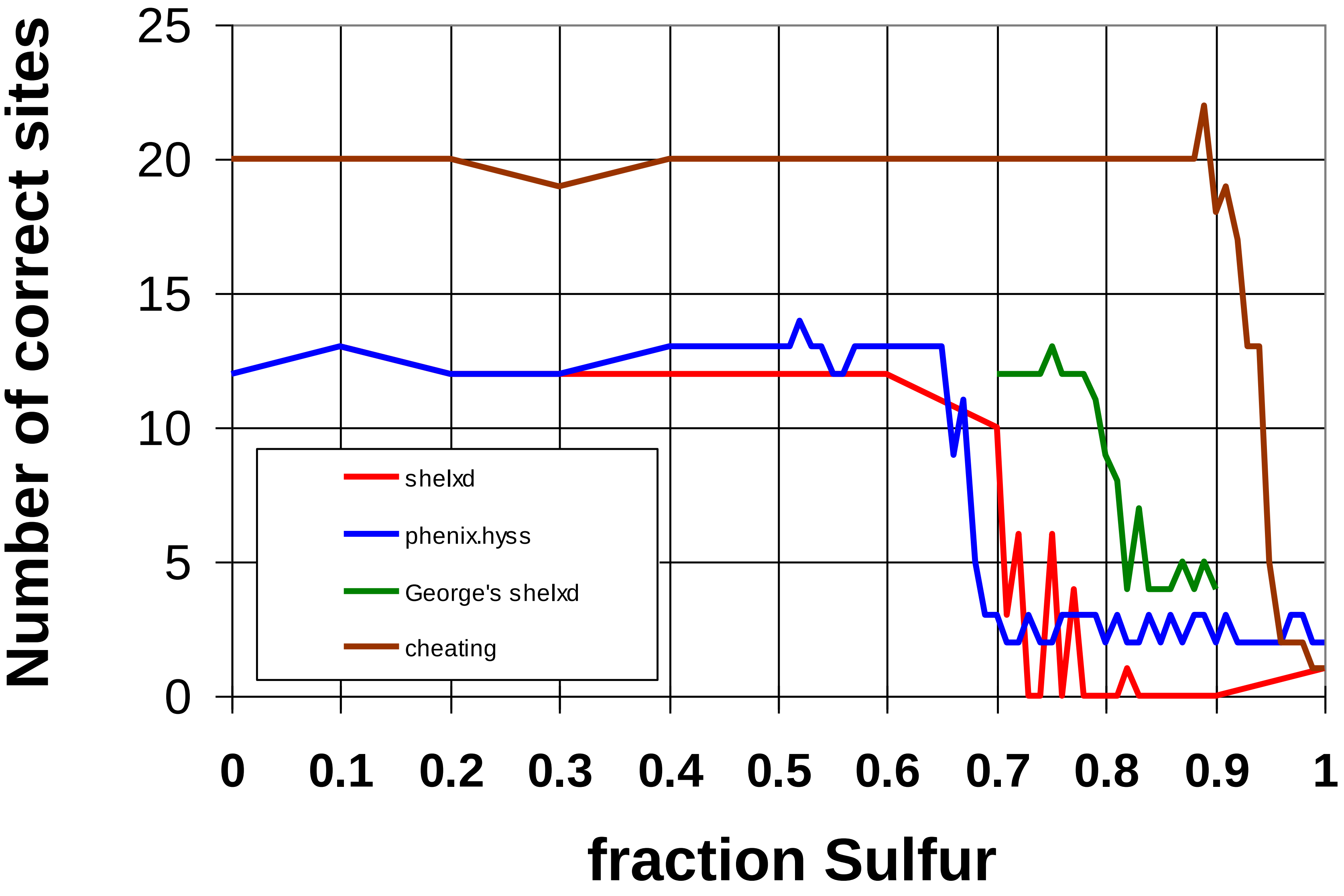
Finding sites



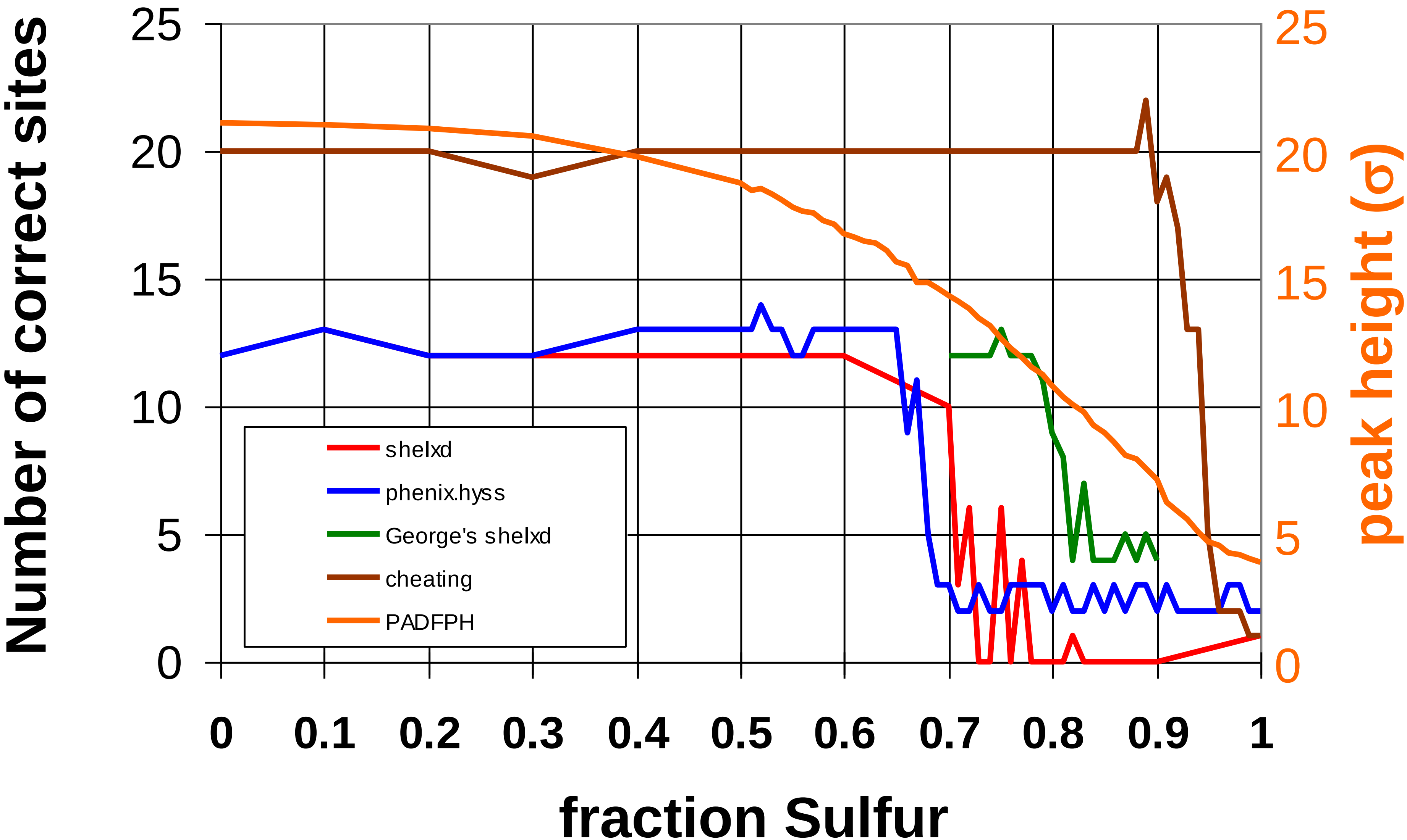
Finding sites

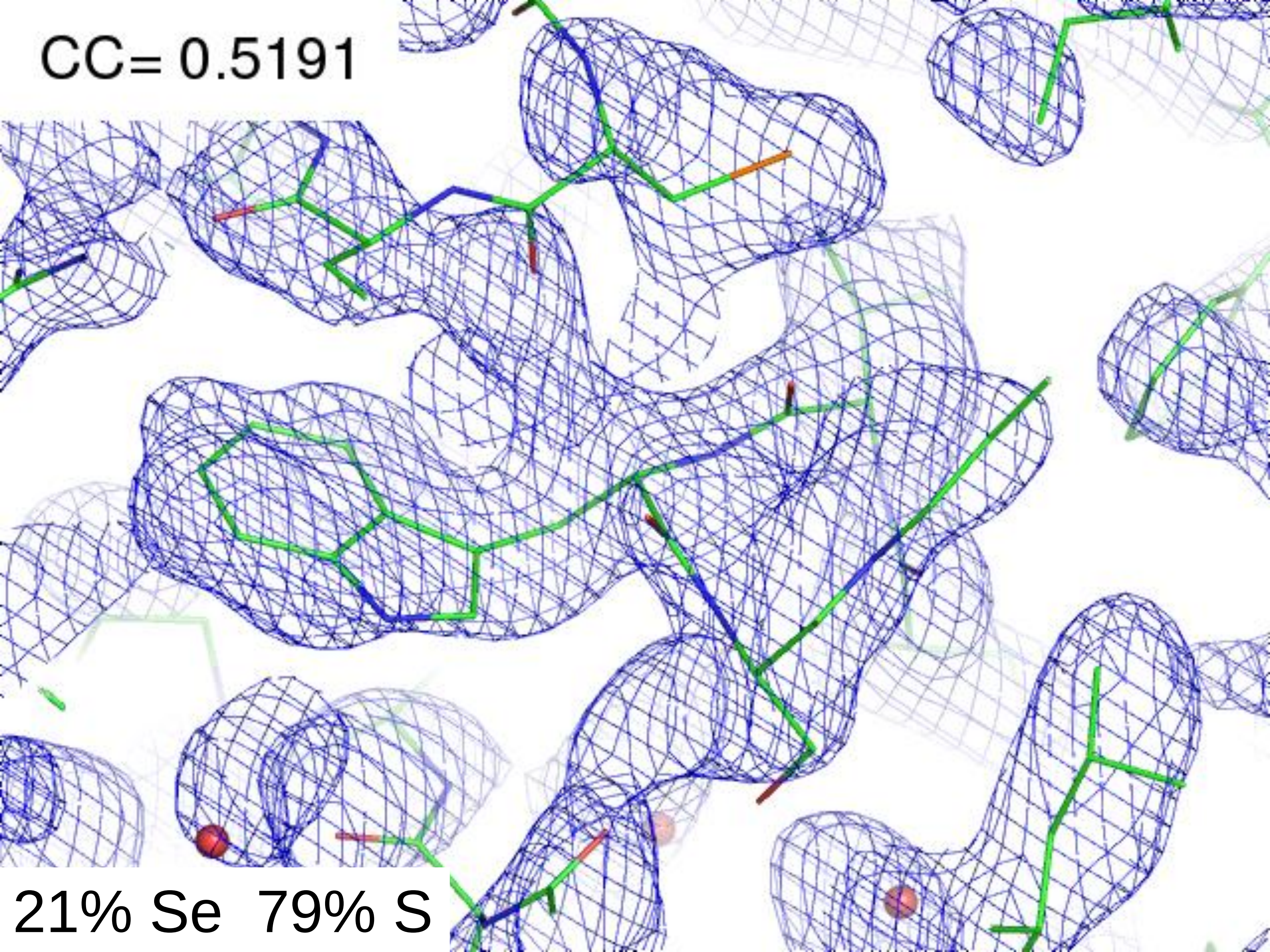


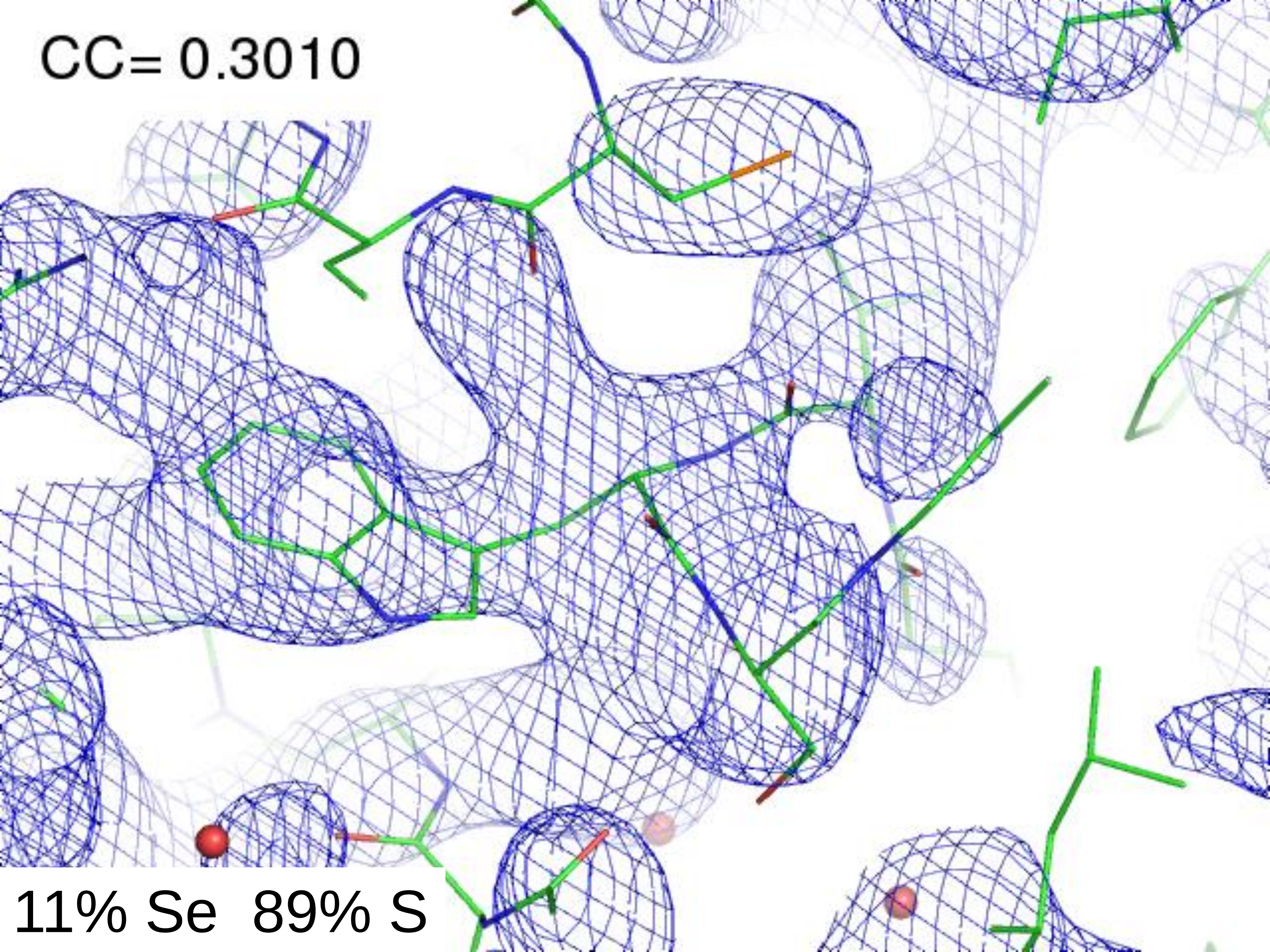
Finding sites



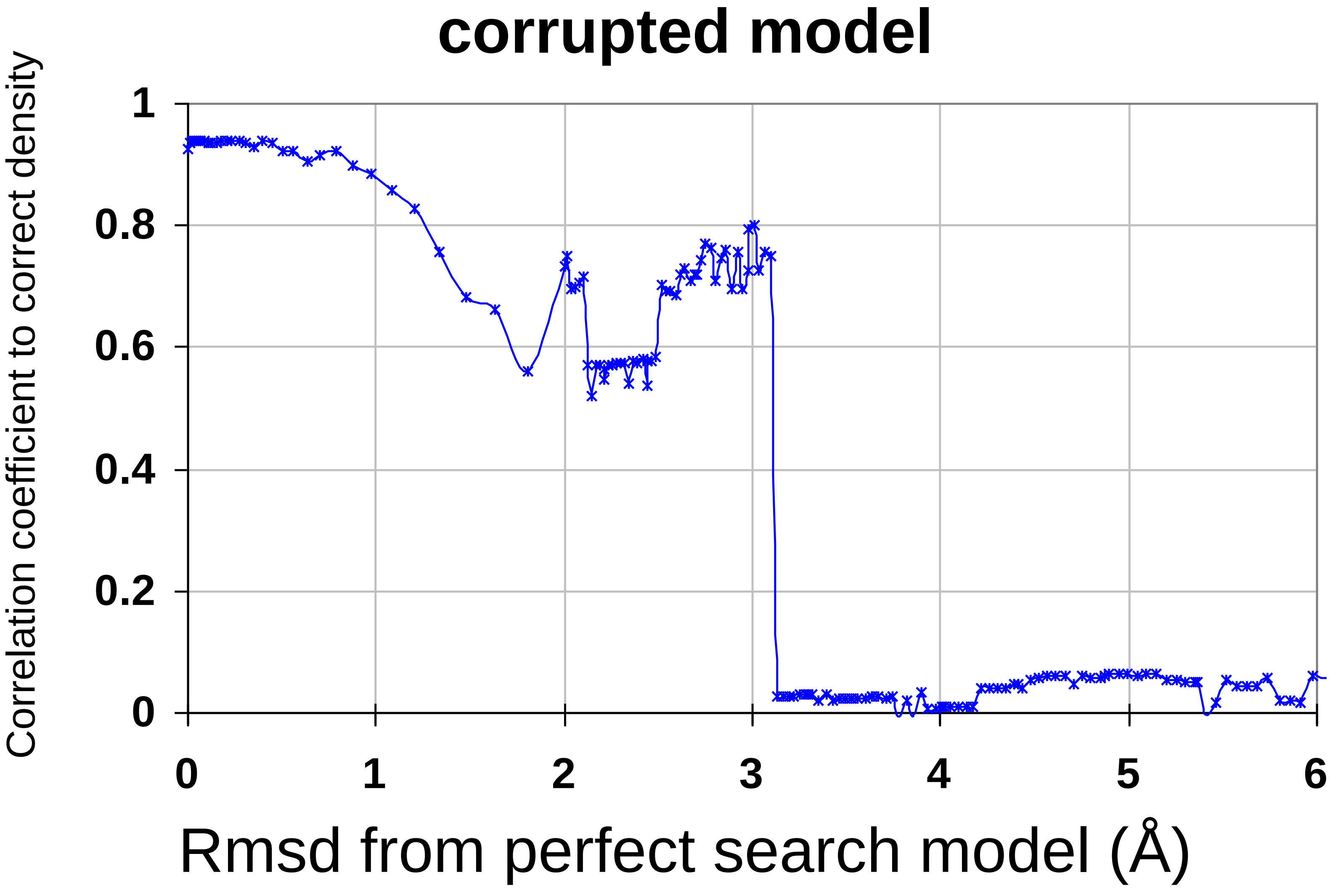
Finding sites




CC= 0.5191
21% Se 79% S


CC= 0.3010
11% Se 89% S

MR simulation



The transitions are sharp!

How can we predict success/failure?

Know Thy Experiment

Basic Principles

"Hell, there are NO RULES here - we're trying to accomplish something."

Thomas A. Edison – inventor

"You've got to have an ASSAY."

Arthur Kornberg – Nobel Laureate

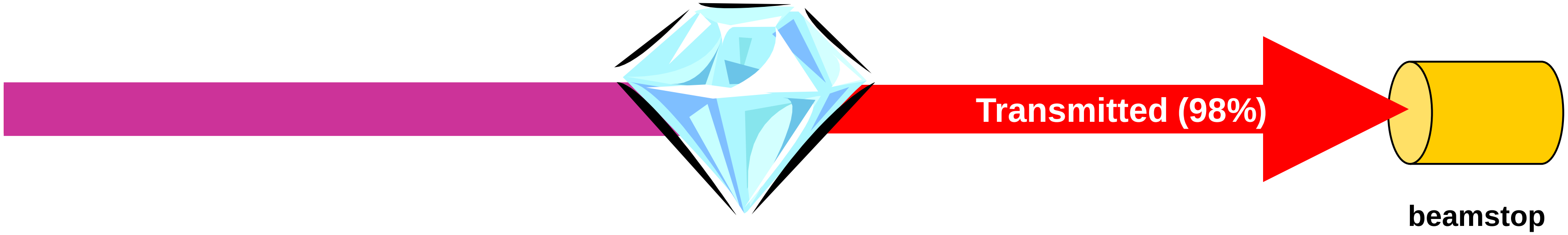
"Control, control, you must learn CONTROL!"

Yoda – Jedi Master

Where do photons go?

Protein

1A x-rays



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Protein

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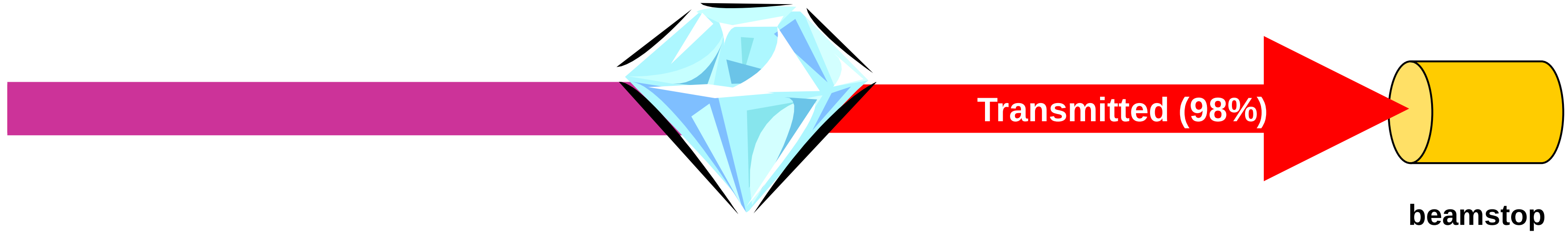
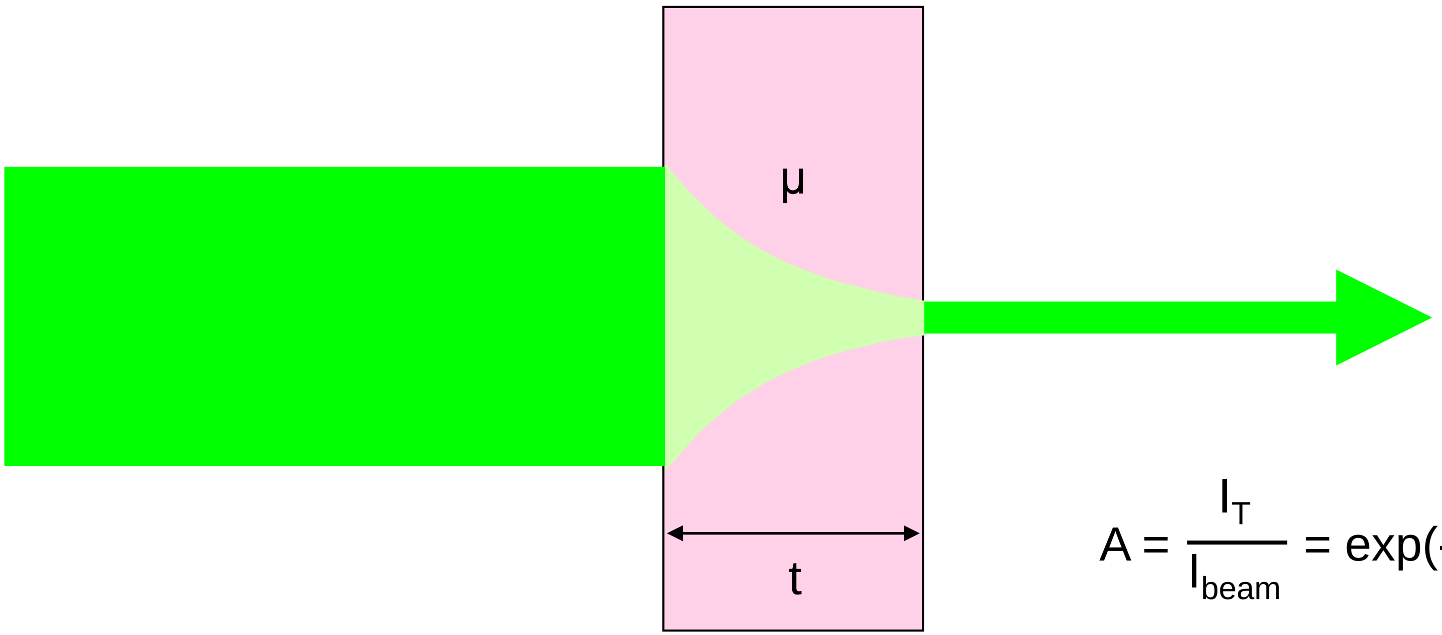


2% total → error < 2%

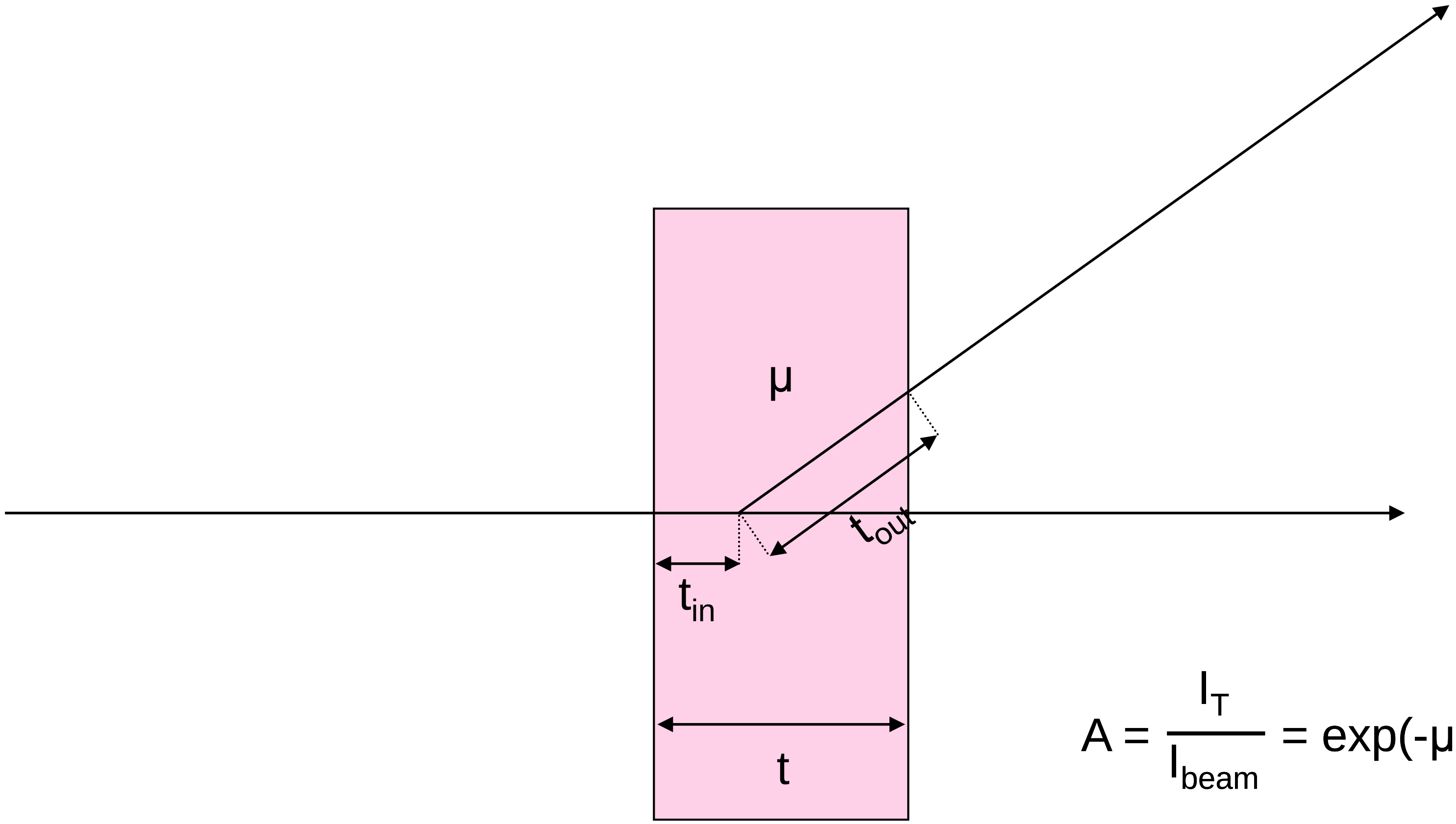
attenuation factor



$$A = \frac{I_T}{I_{beam}} = \exp(-\mu t)$$

Bouguer, P. (1729). *Essai d'optique sur la gradation de la lumière*.
Lambert, J. H. (1760). *Photometria: sive De mensura et gradibus luminis, colorum et umbrae*. E. Klett.
Beer, A. (1852)."Bestimmung der Absorption des rothen Lichts in farbigen Flüssigkeiten", *Ann. Phys. Chem* **86**, 78-90.

attenuation factor



$$A = \frac{I_T}{I_{beam}} = \exp(-\mu(t_{in} + t_{out}))$$

Bouguer, P. (1729). *Essai d'optique sur la gradation de la lumière*.
Lambert, J. H. (1760). *Photometria: sive De mensura et gradibus luminis, colorum et umbrae*. E. Klett.
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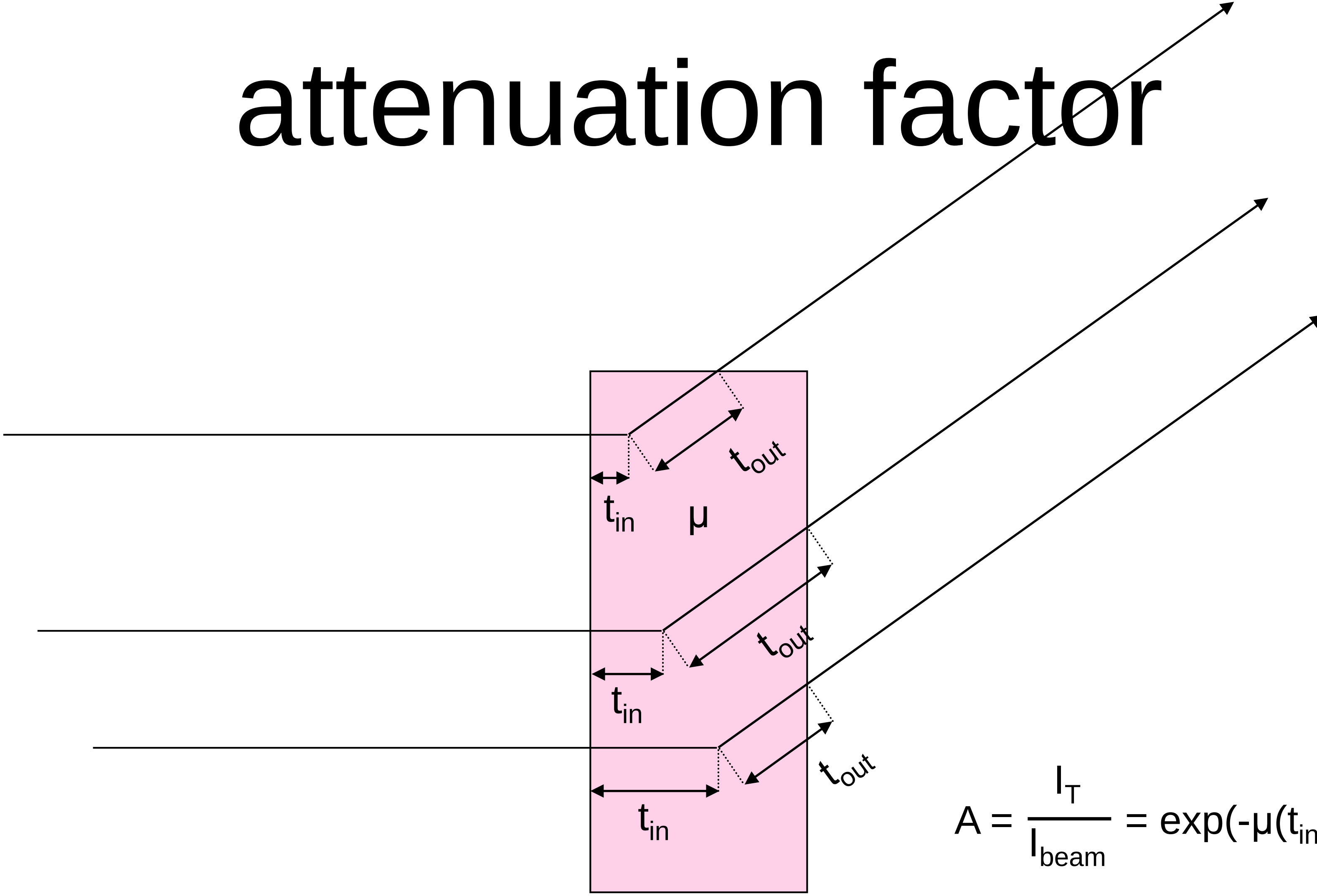

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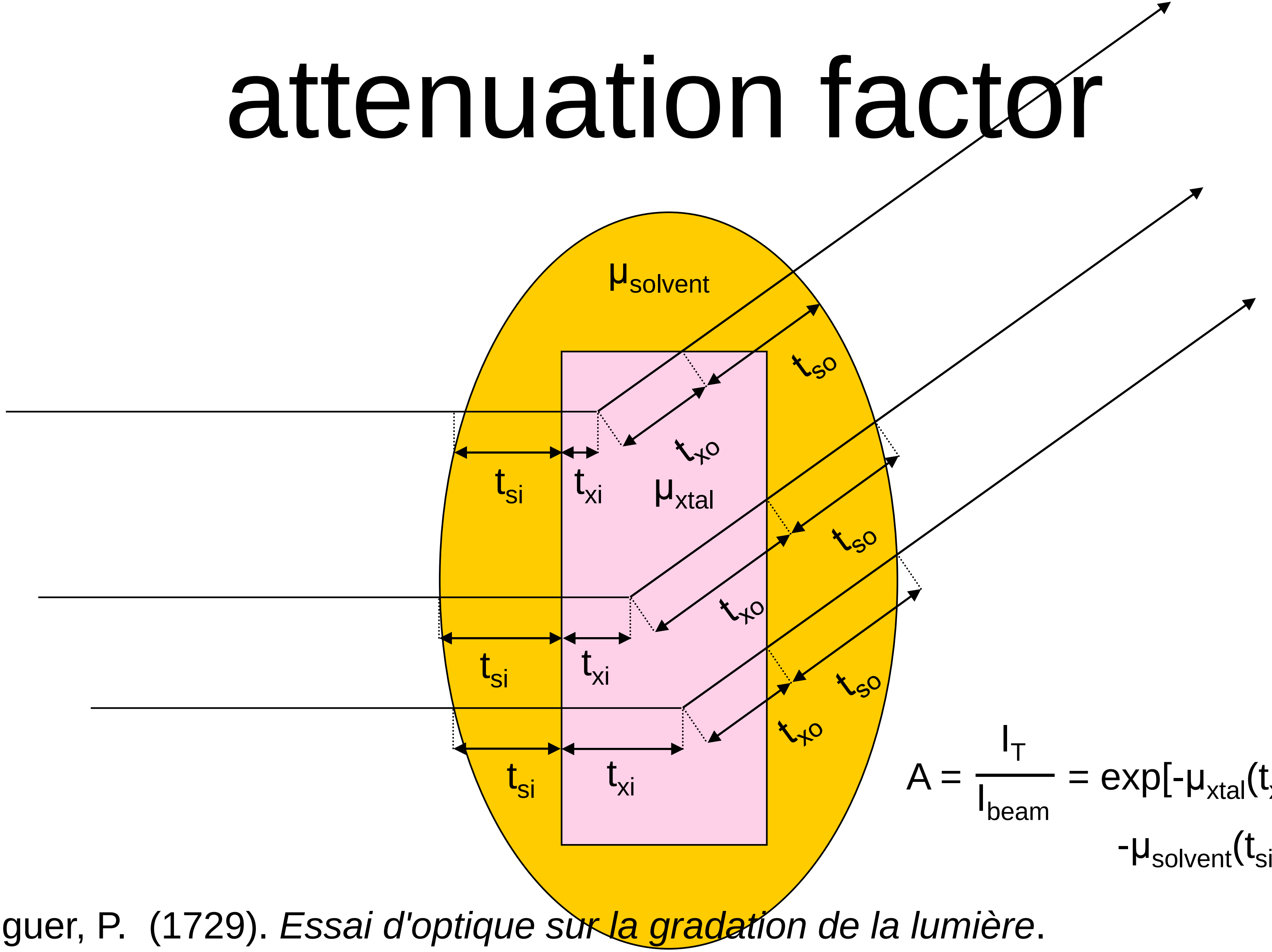
attenuation factor



$$A = \frac{I_T}{I_{beam}} = \exp[-\mu_{xtal}(t_{xi} + t_{xo}) - \mu_{solvent}(t_{si} + t_{so})]$$

Bouguer, P. (1729). *Essai d'optique sur la gradation de la lumière*.
Lambert, J. H. (1760). *Photometria: sive De mensura et gradibus luminis, colorum et umbrae*. E. Klett.
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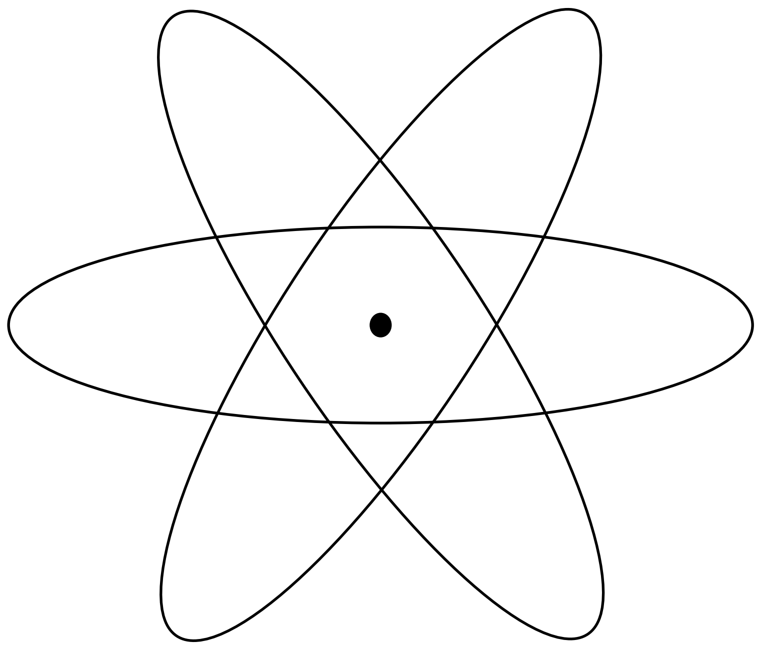
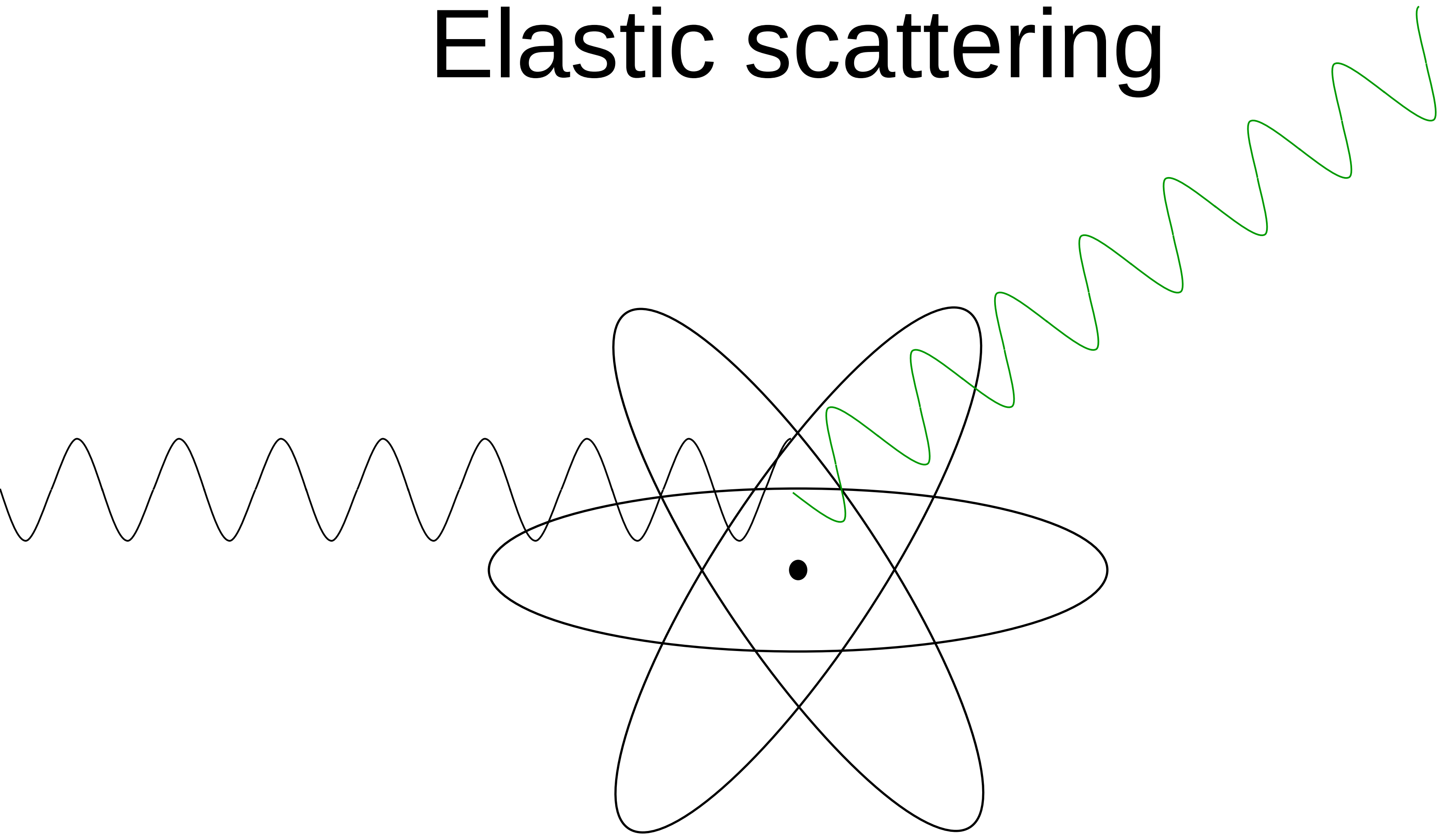
Run Strategy

Elastic scattering

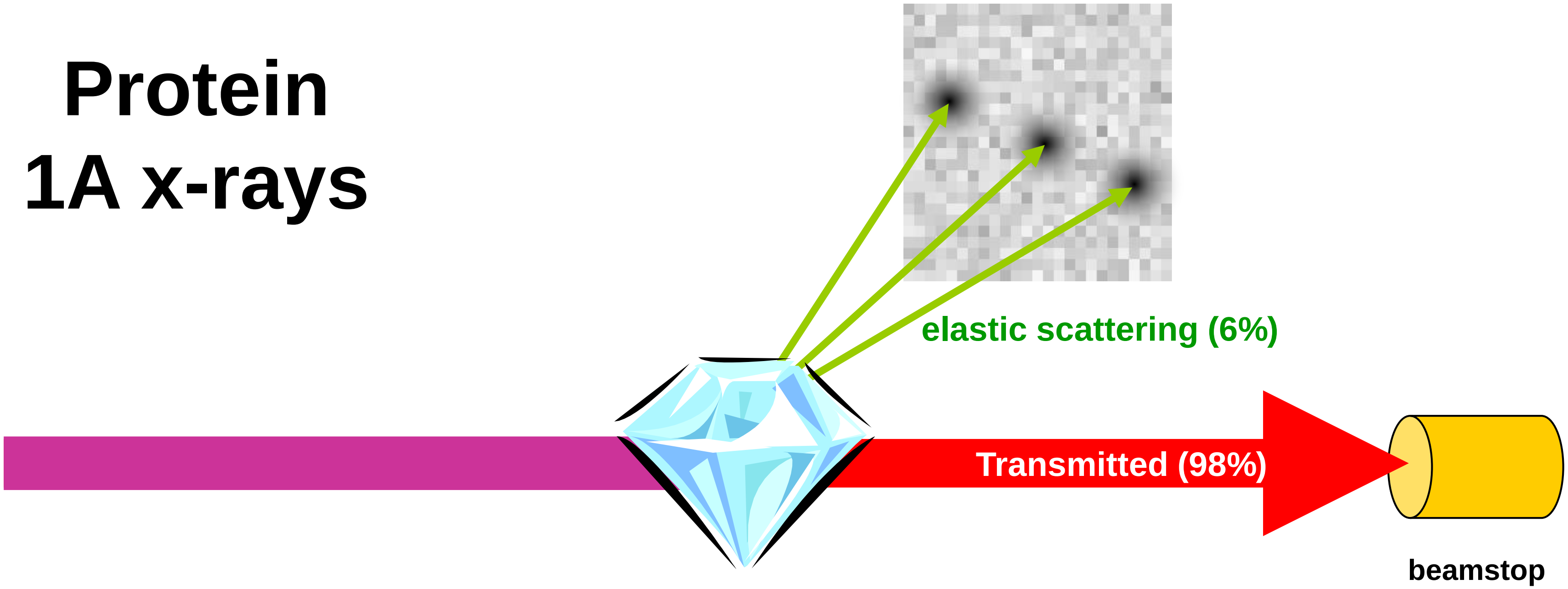


Elastic scattering

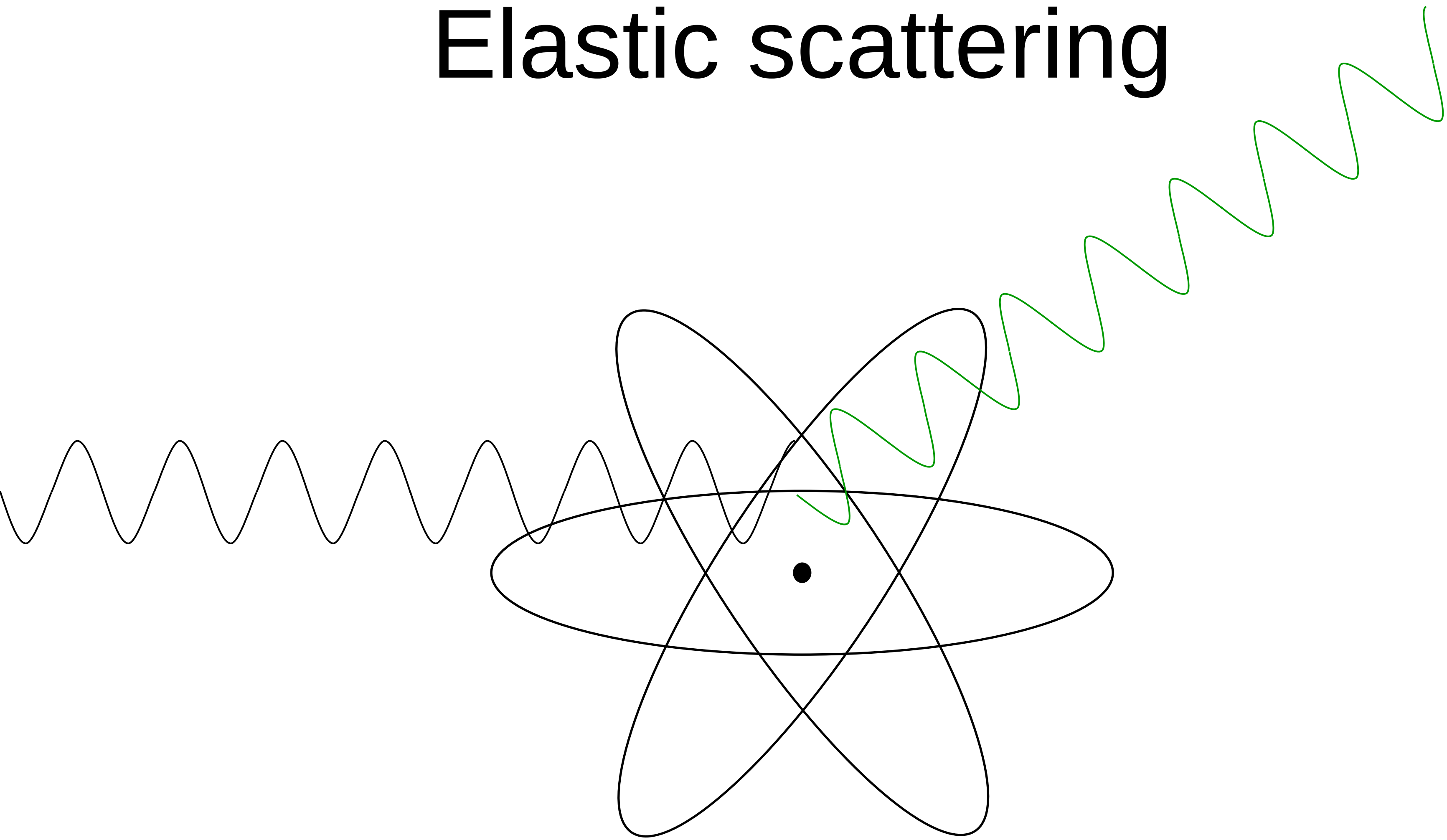


Where do photons go?

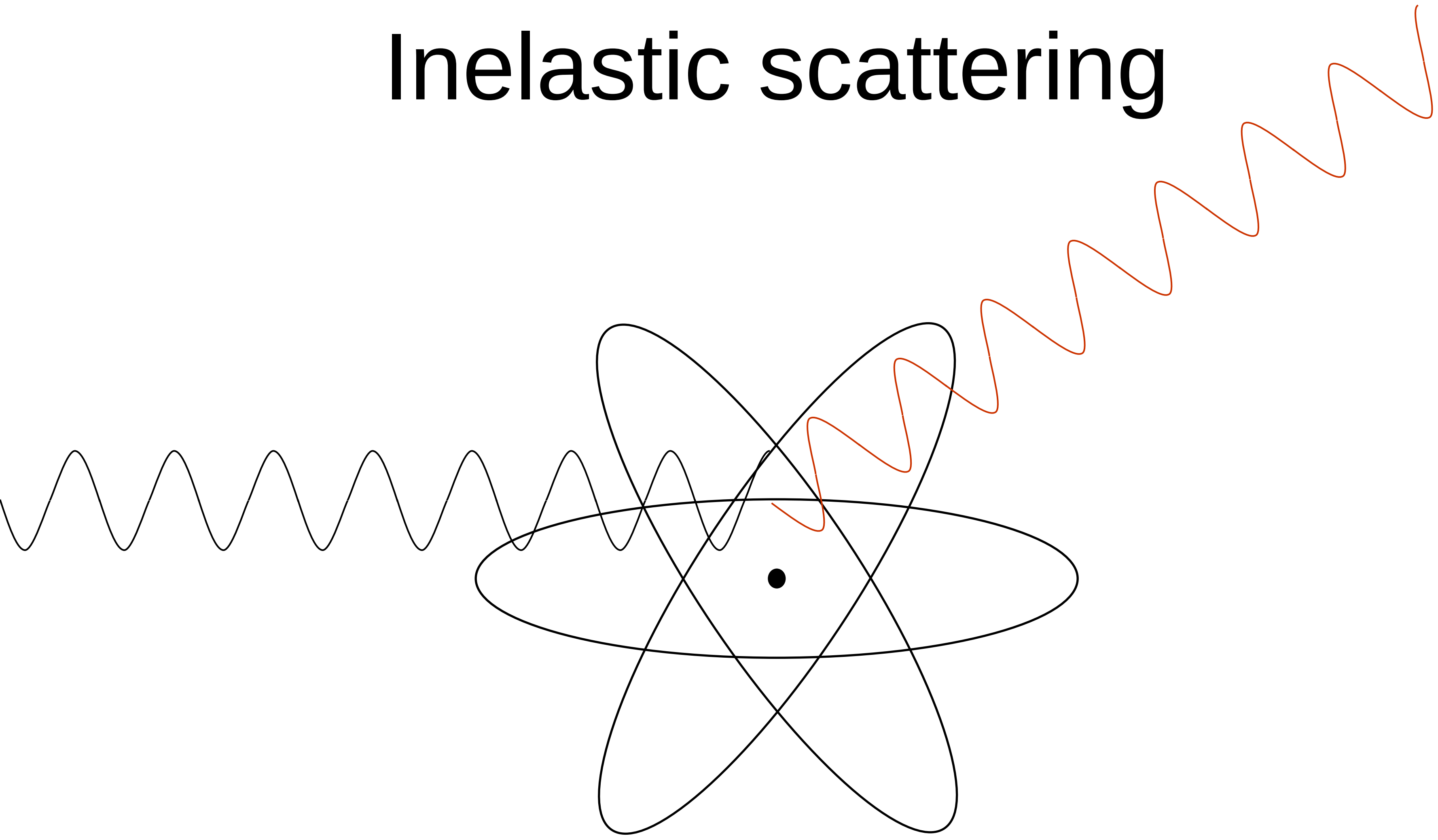
Protein
1A x-rays



Elastic scattering

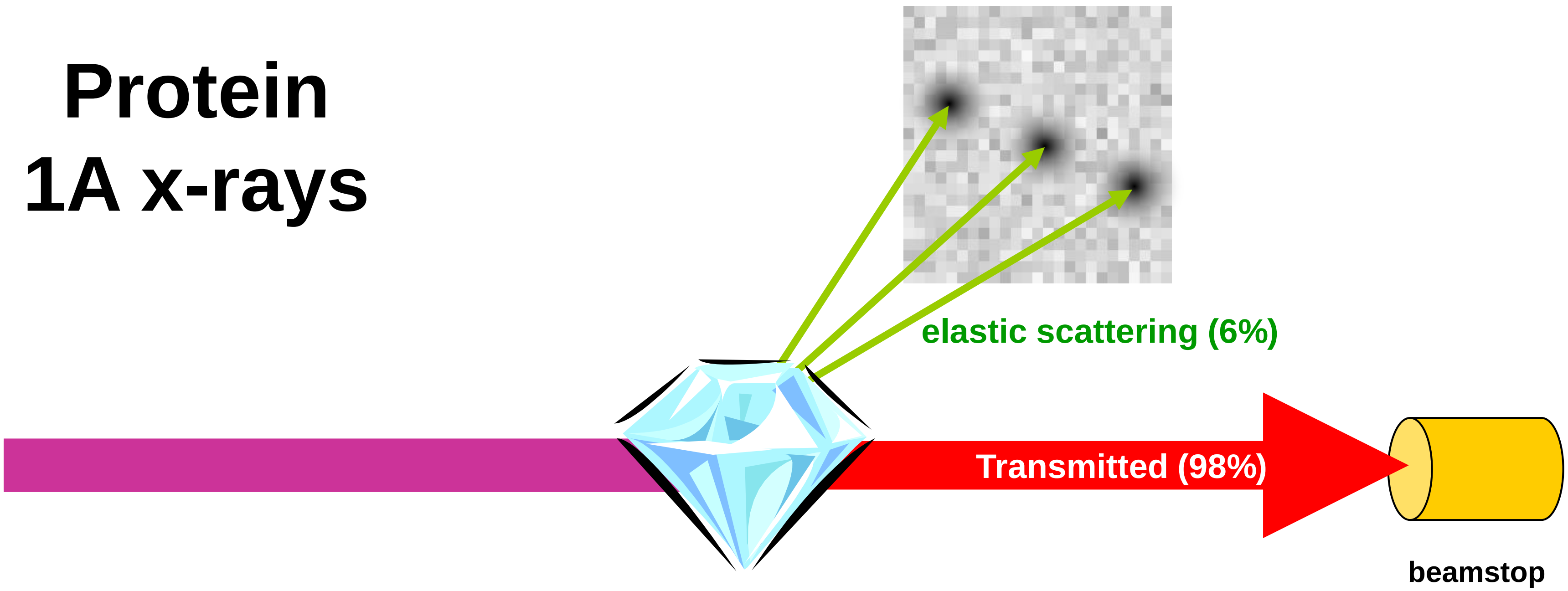


Inelastic scattering



Where do photons go?

Protein
1A x-rays



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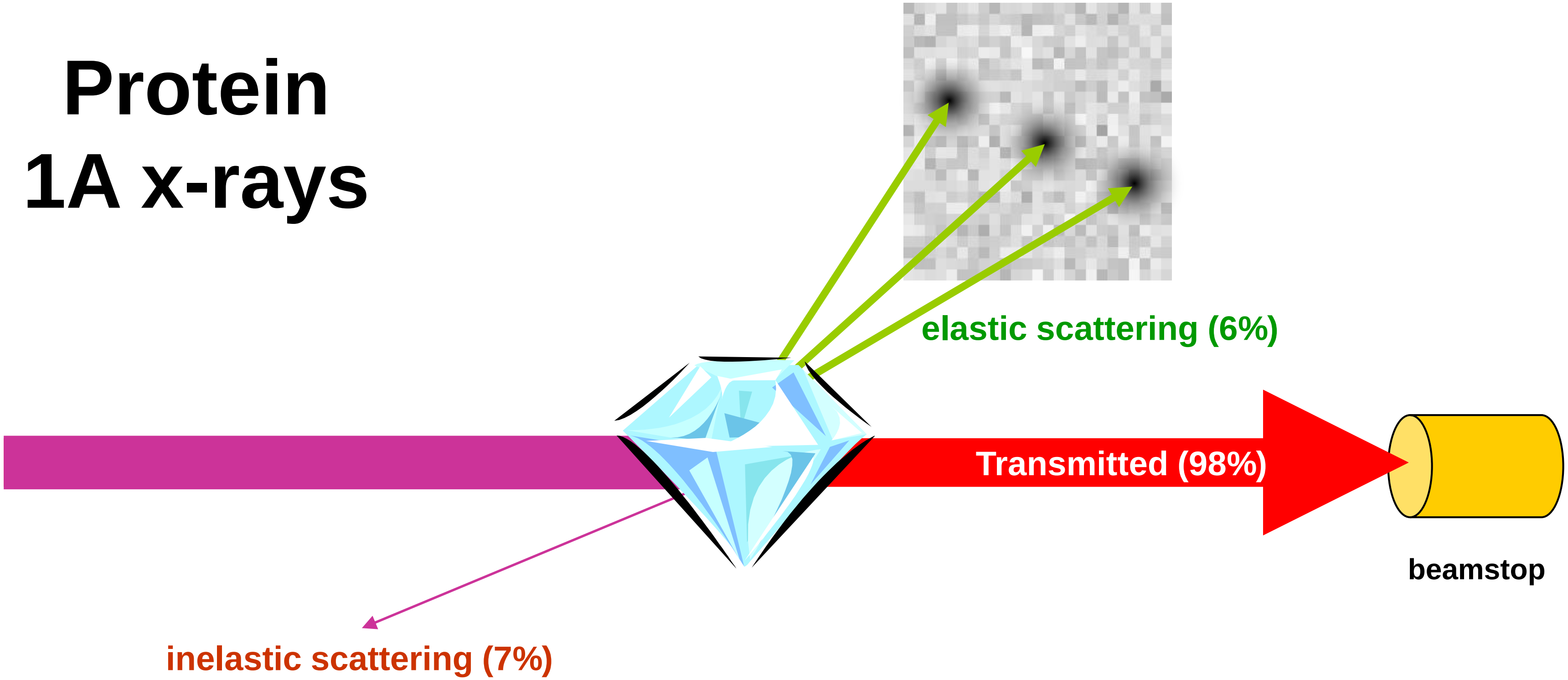

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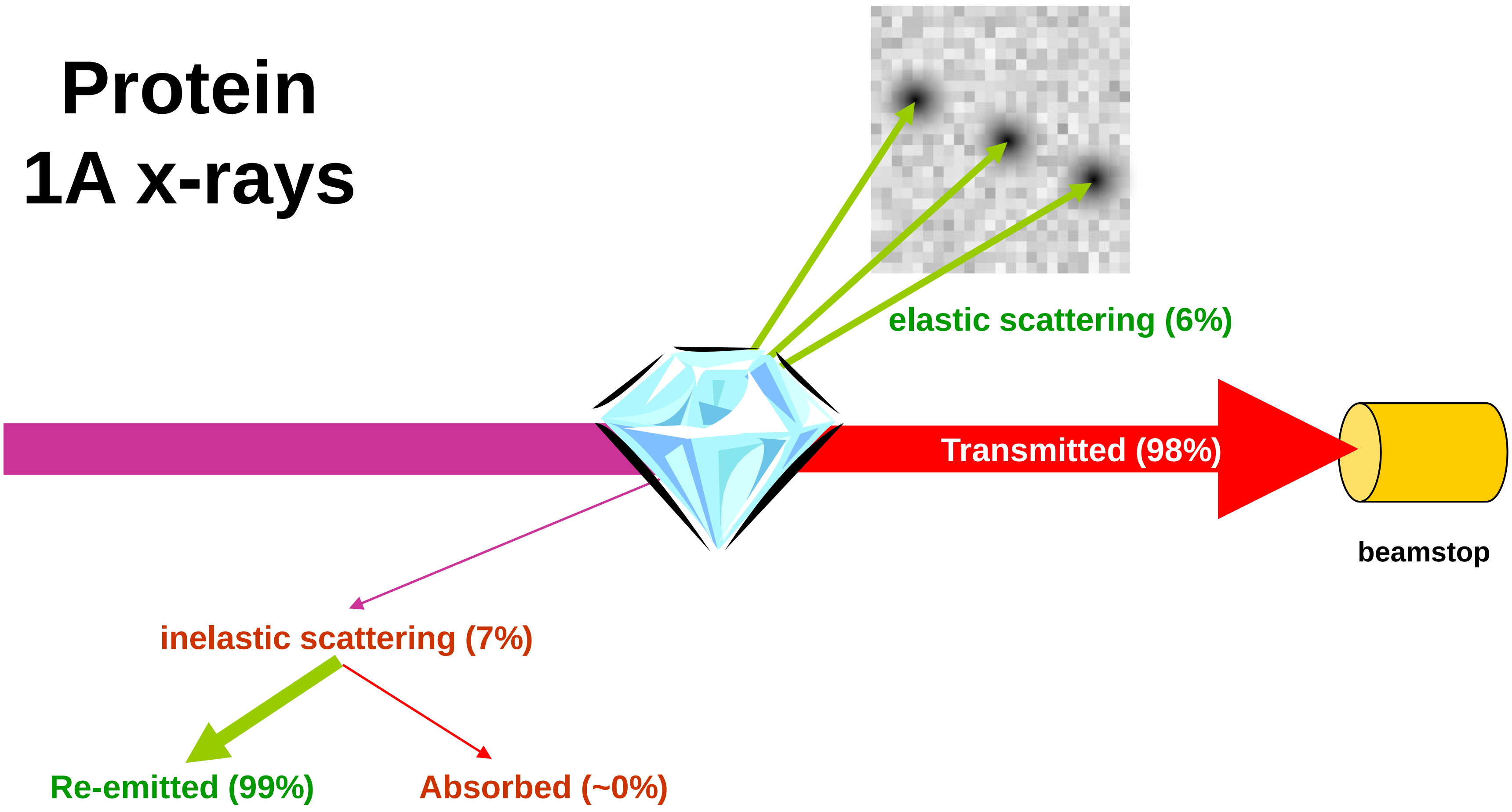
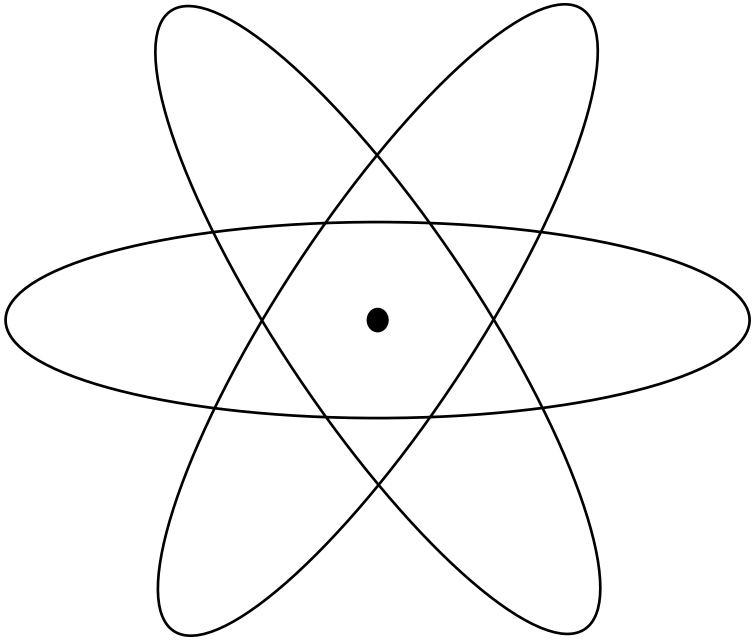
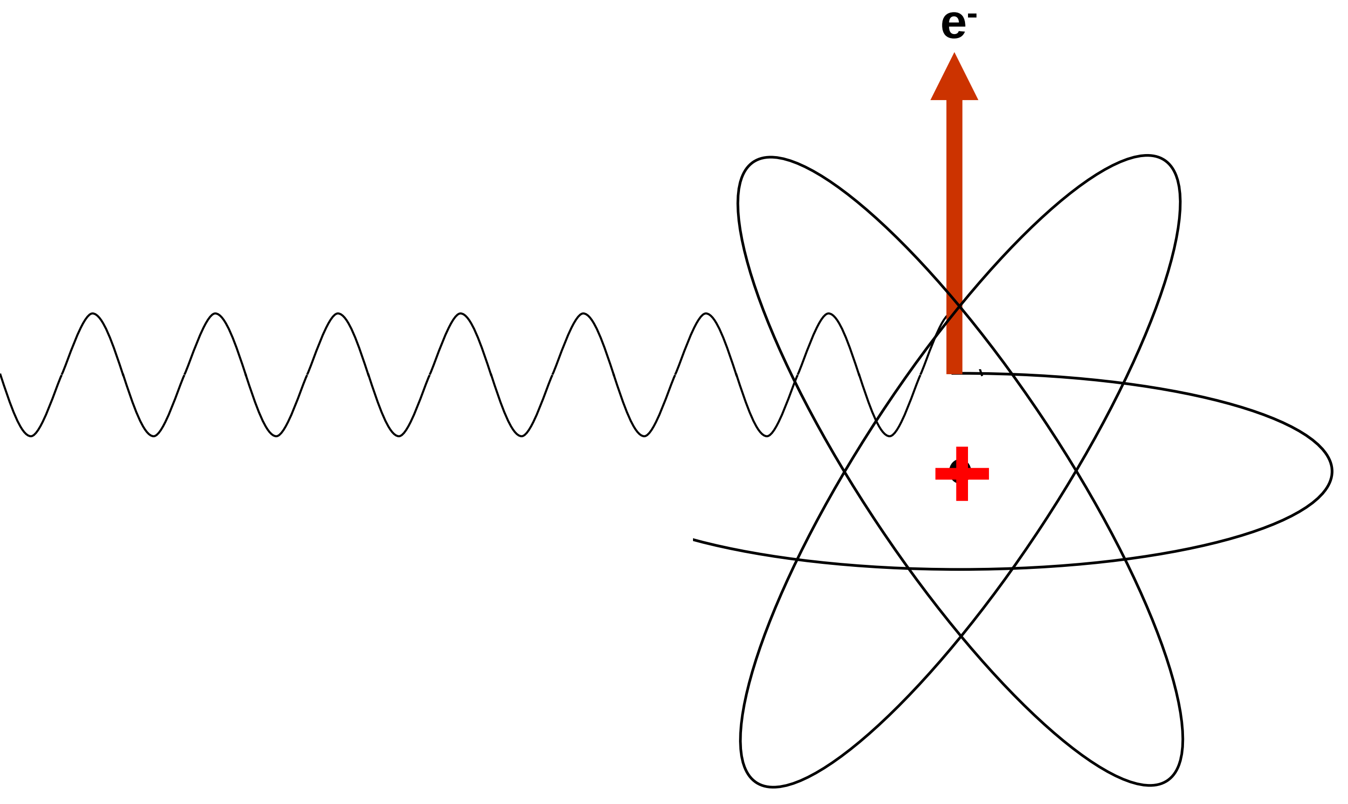


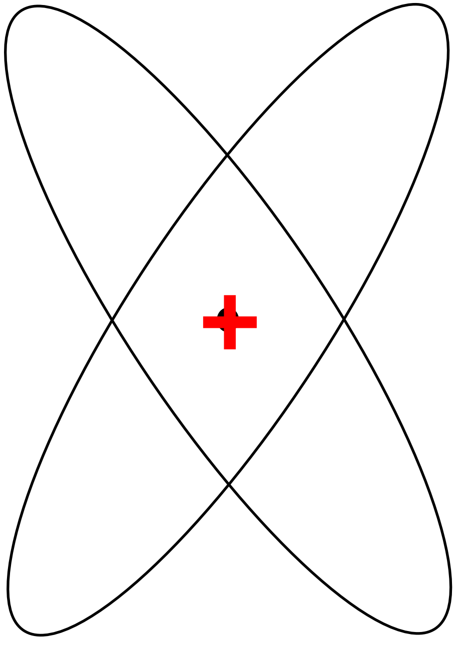
Photoelectric absorption



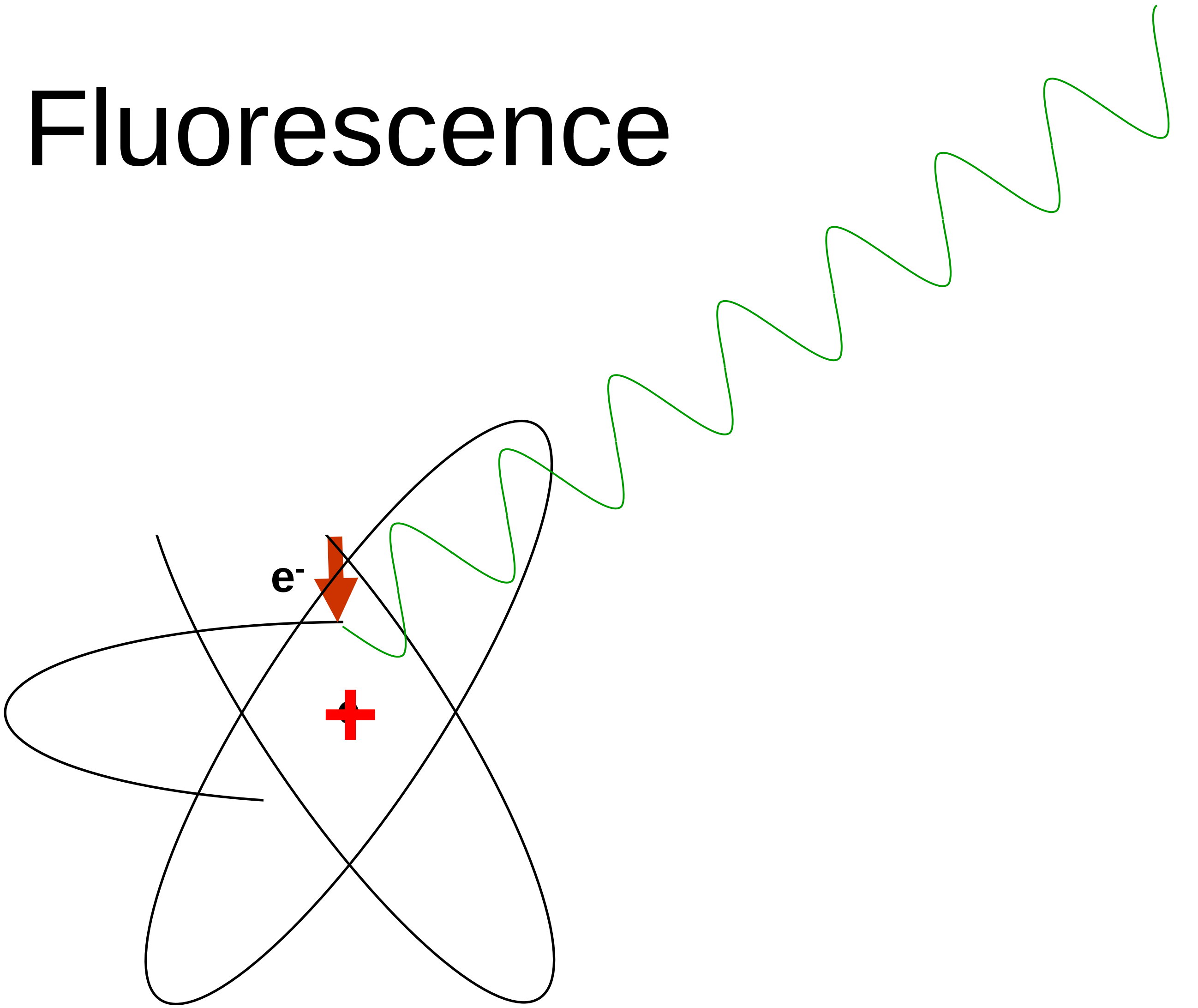
Photoelectric absorption



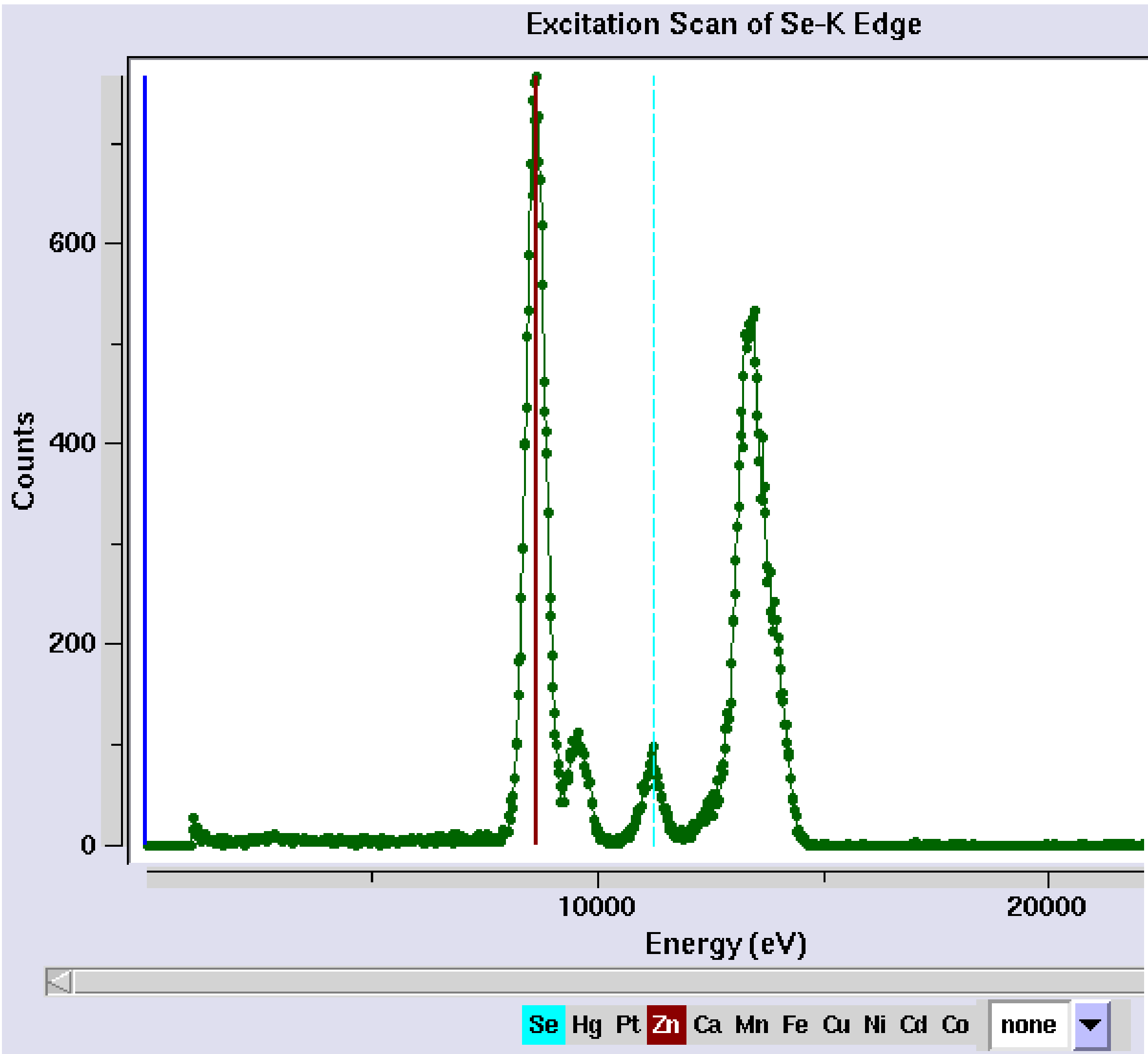
Fluorescence

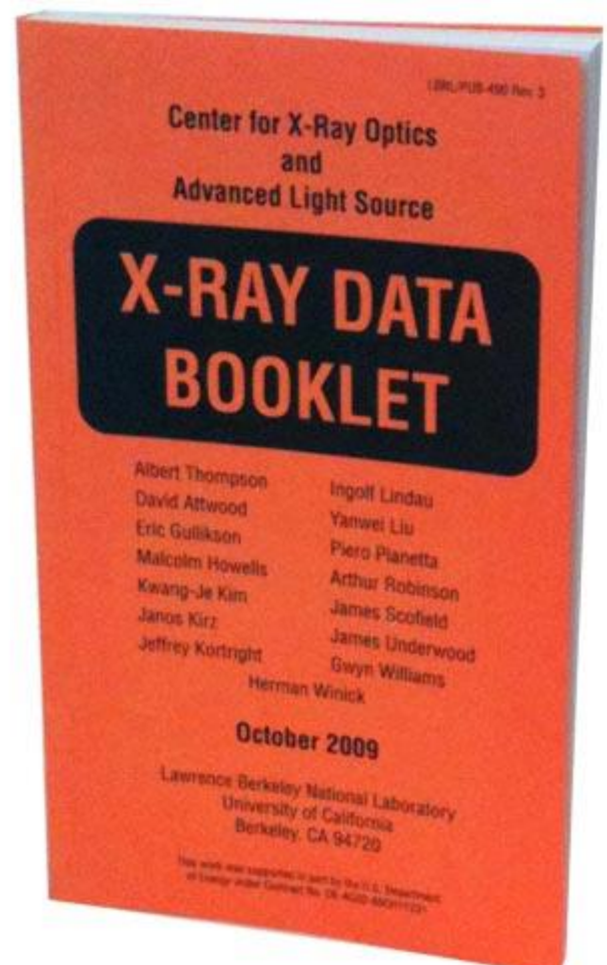


Fluorescence



Metal identification





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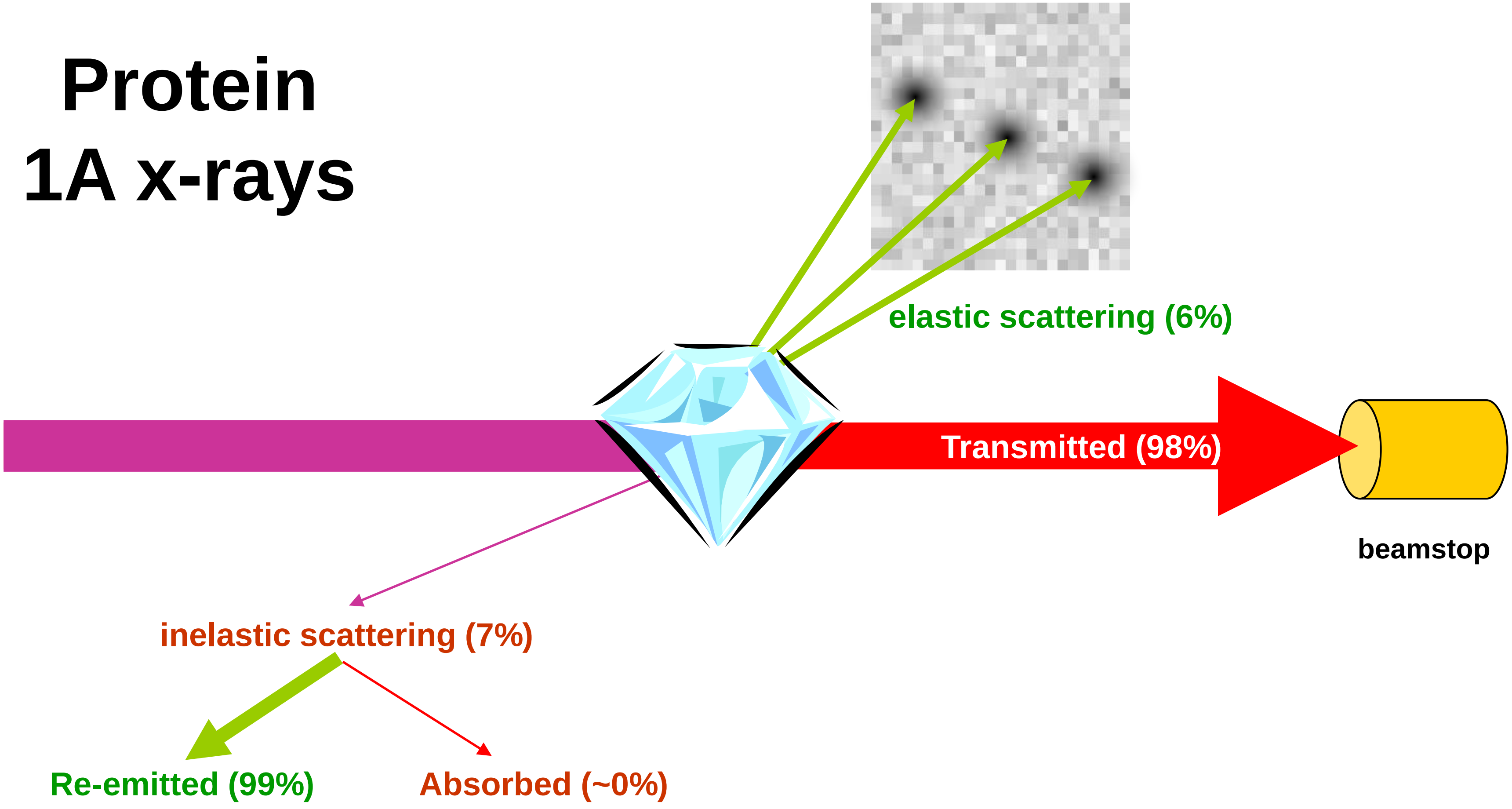

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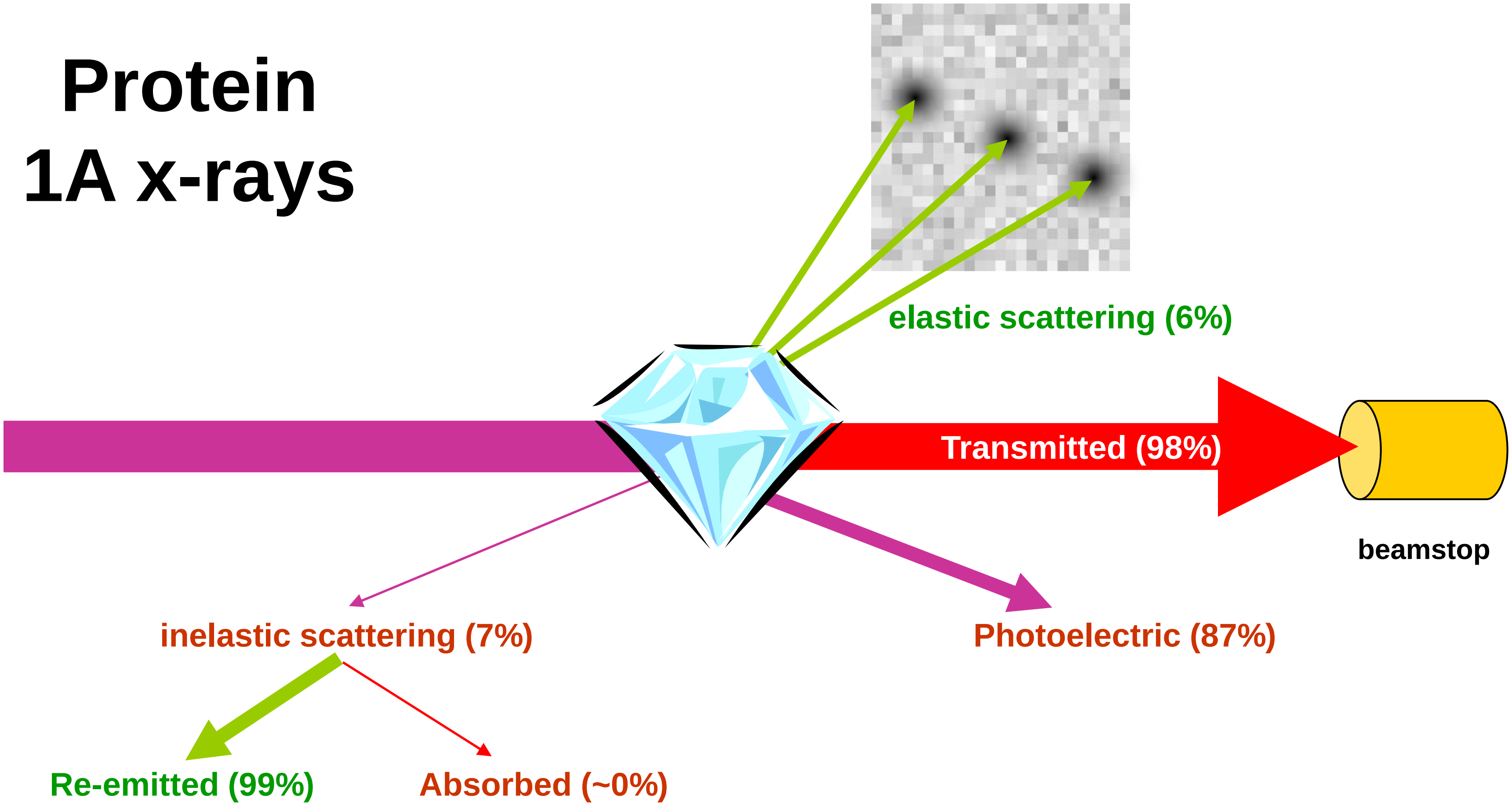

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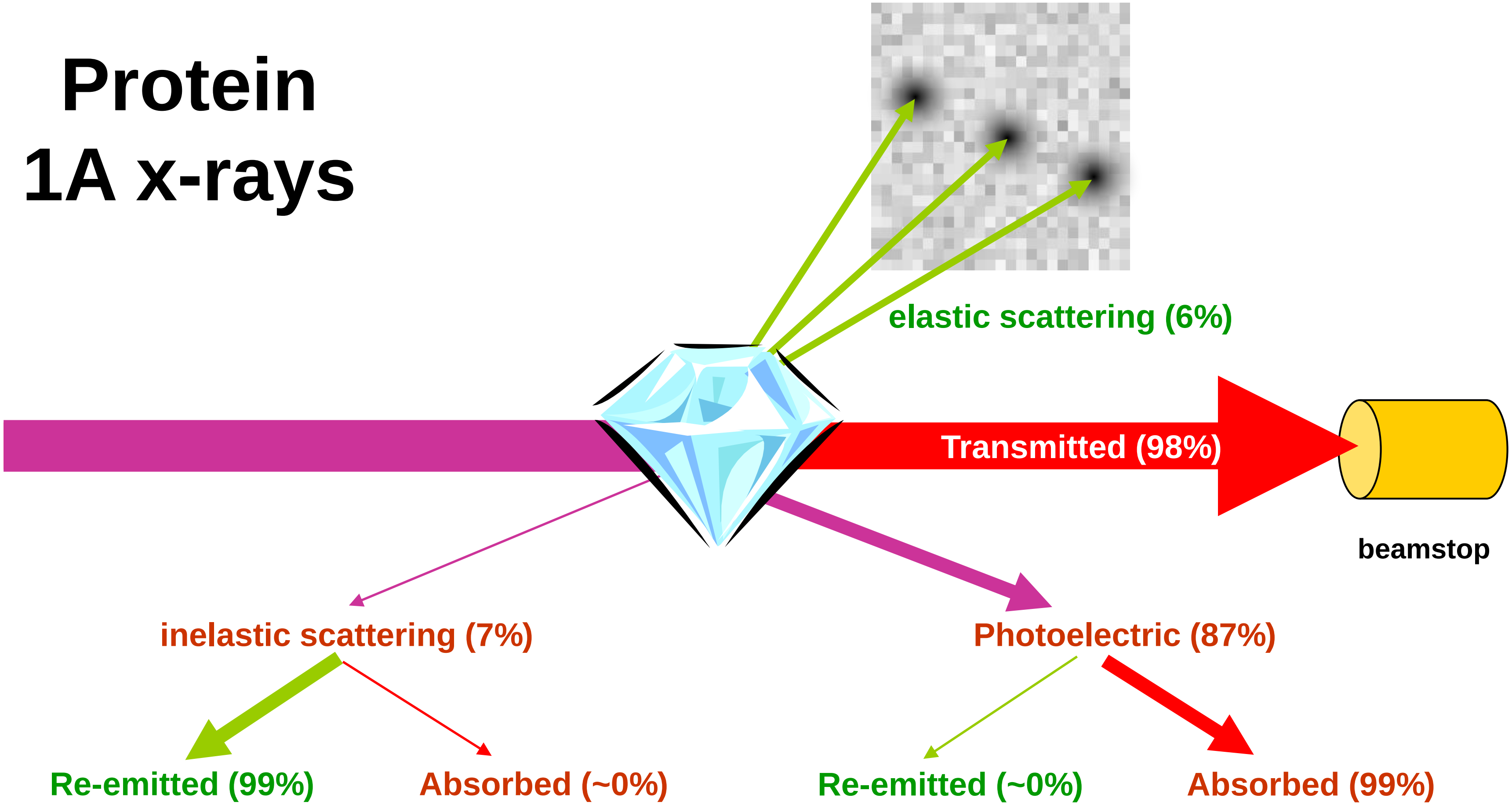
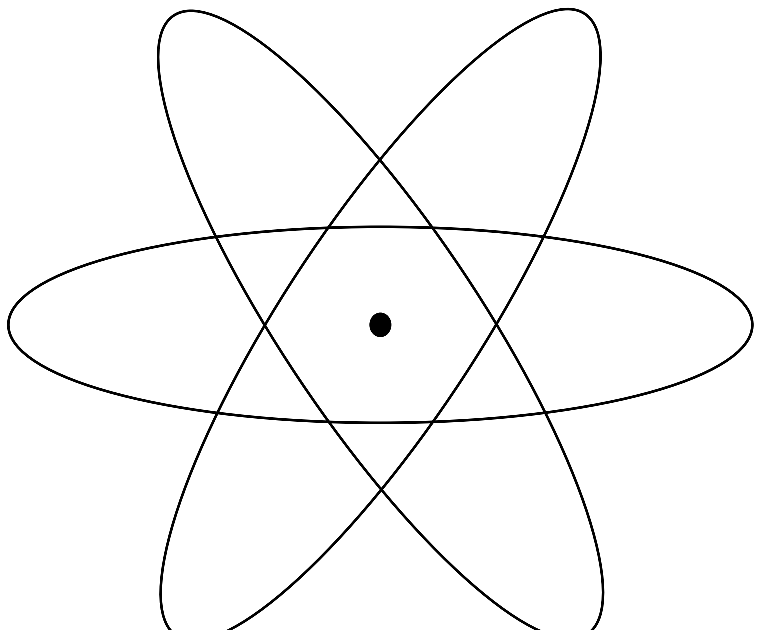
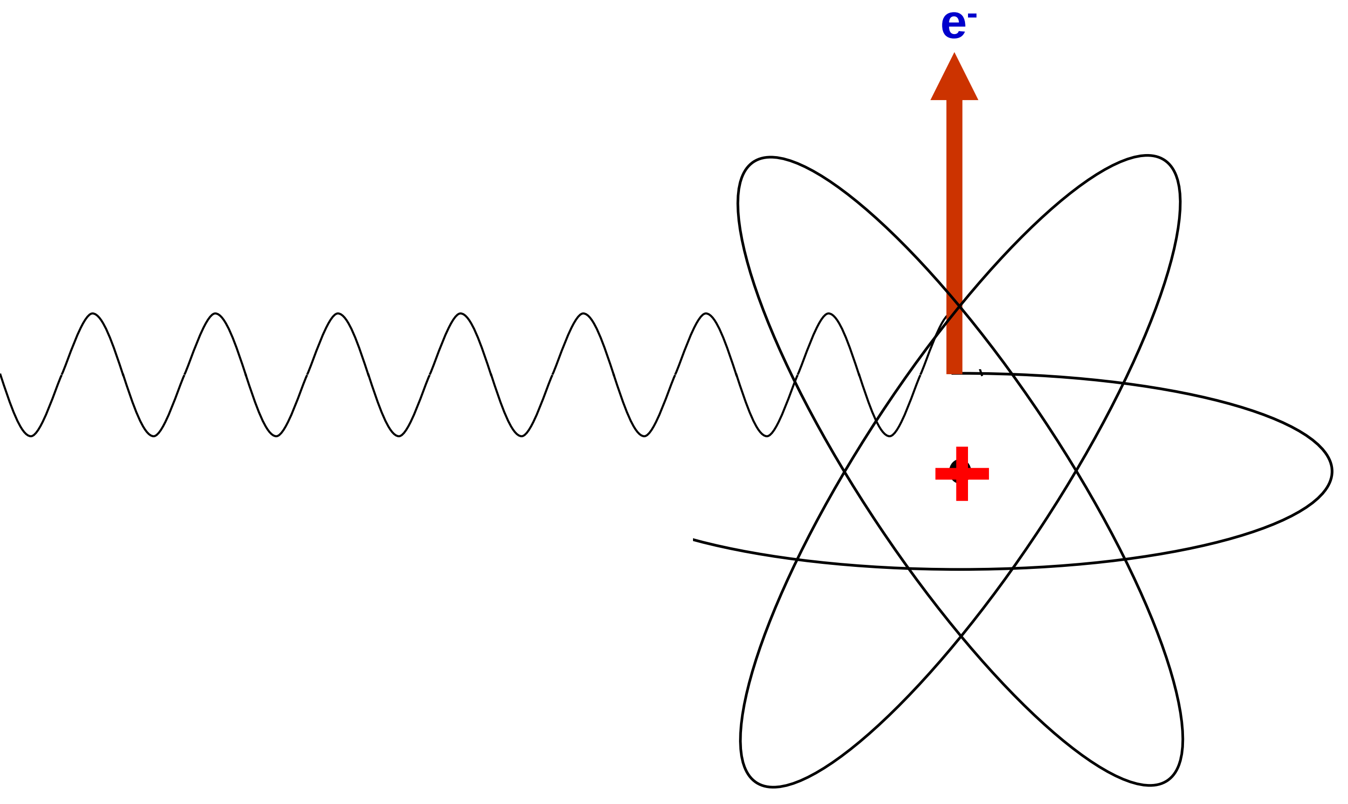


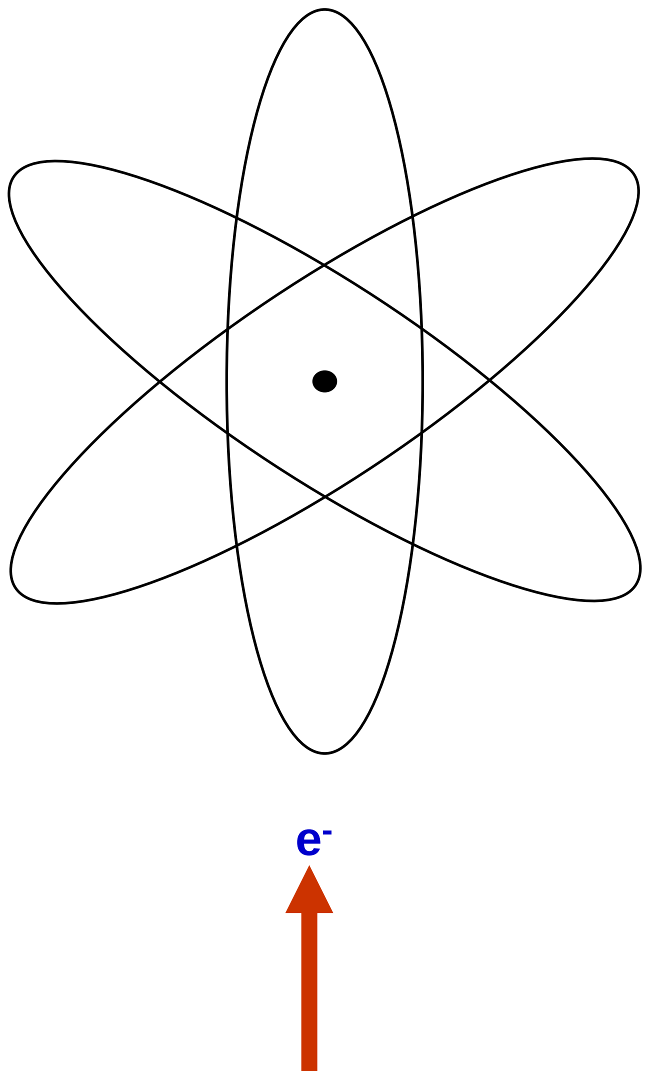
Photoelectric absorption



Photoelectric absorption



Secondary ionization



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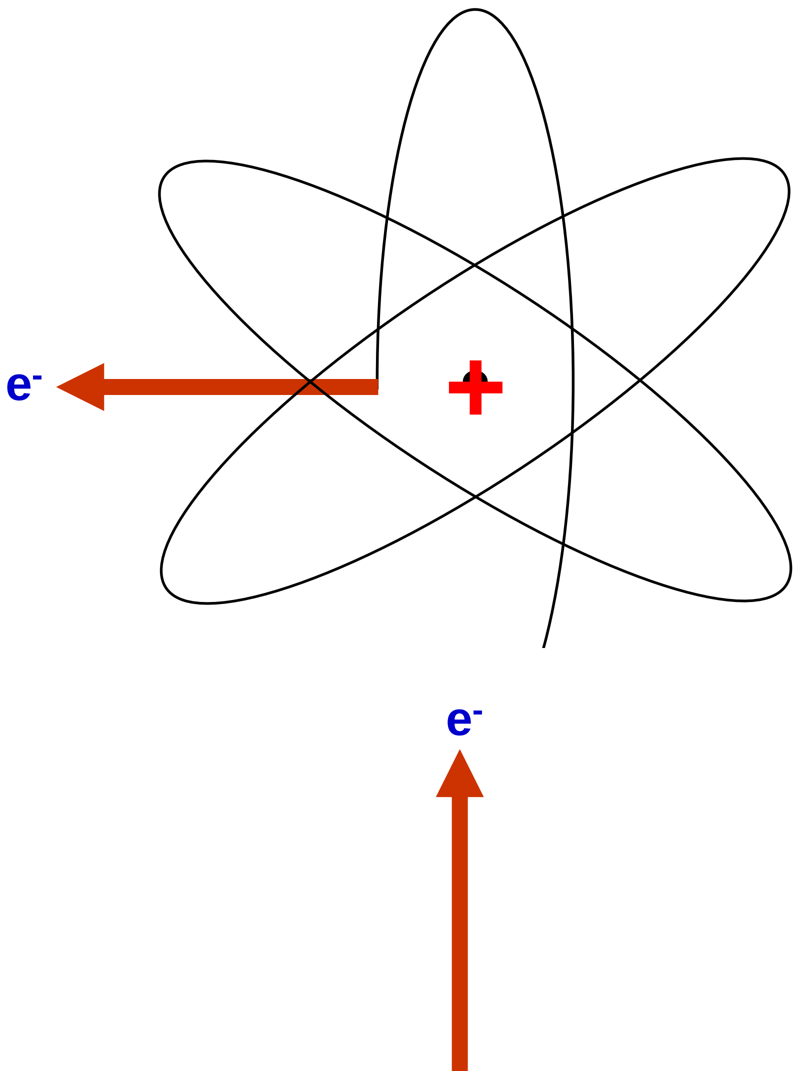
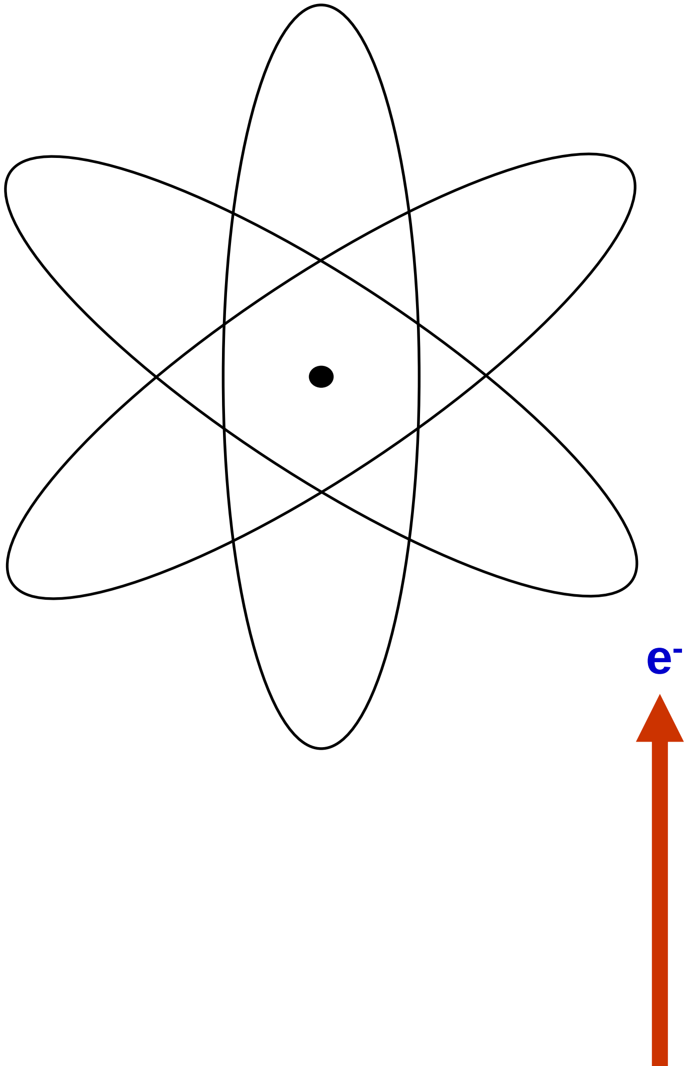


Excitation



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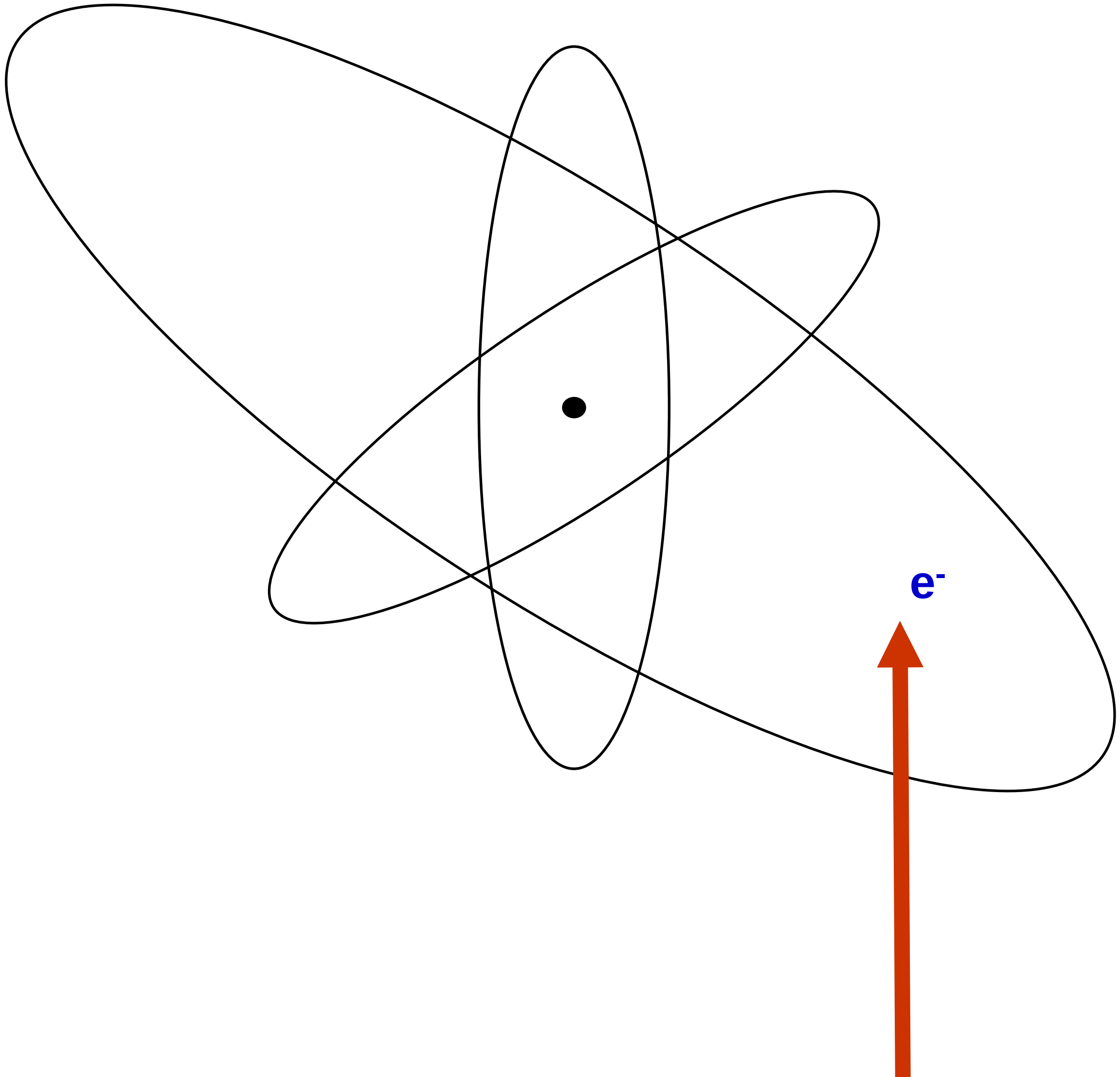

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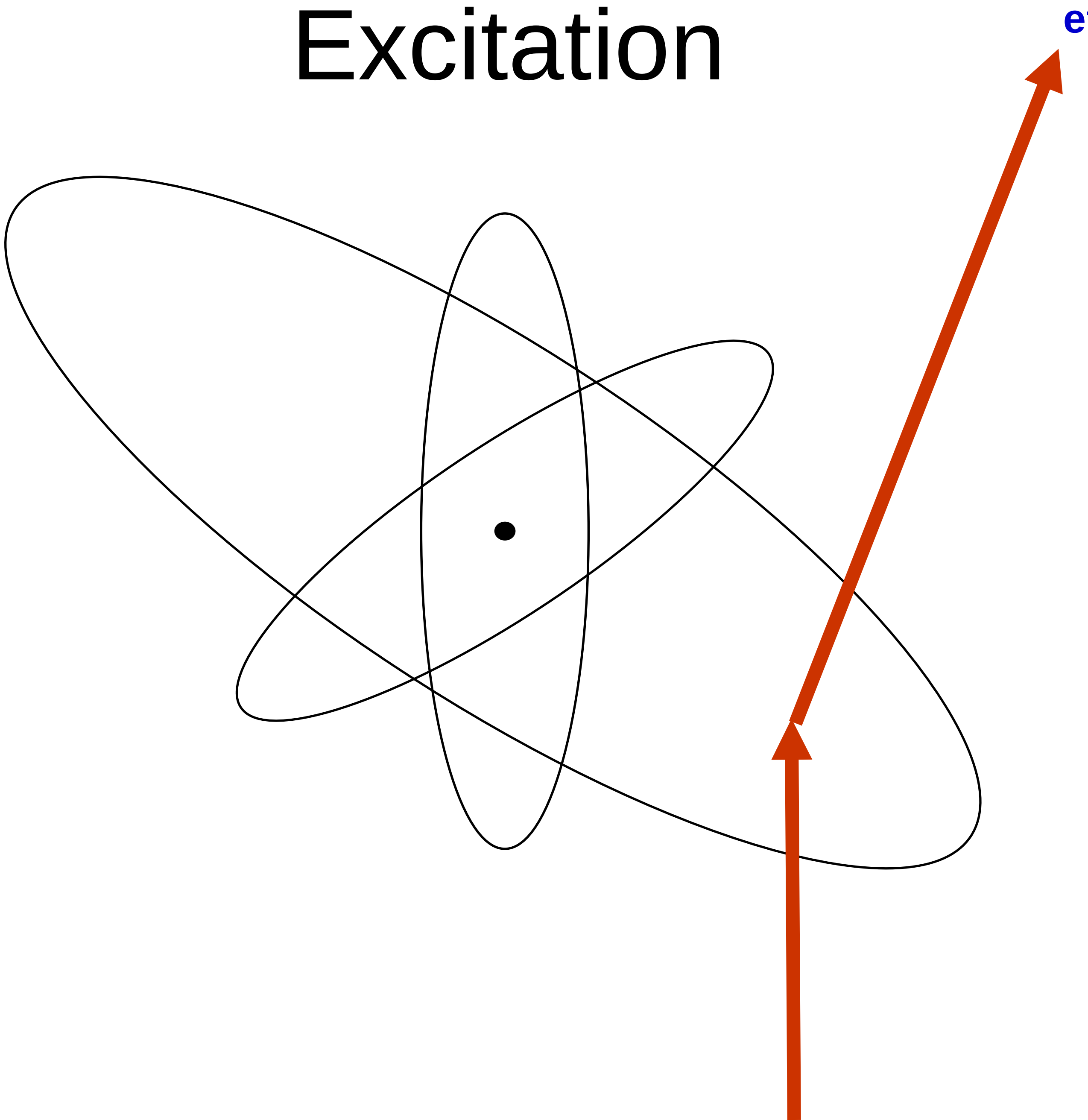

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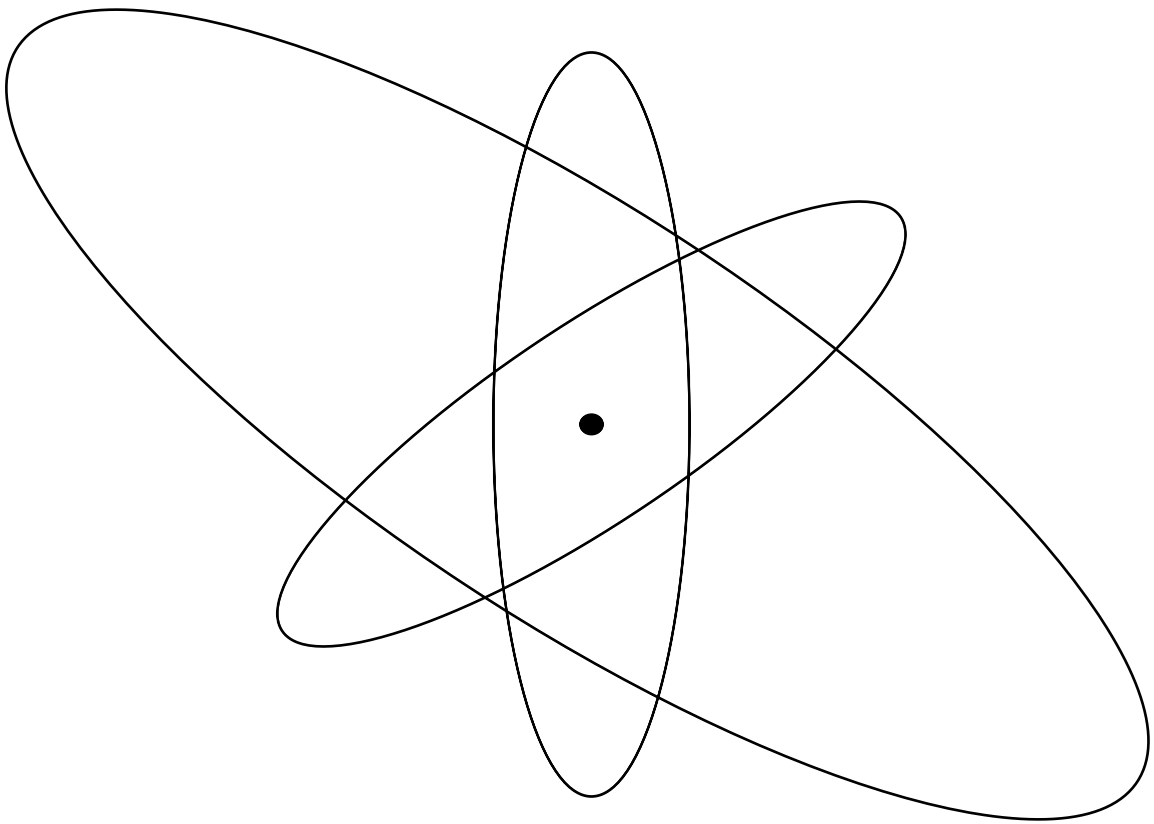
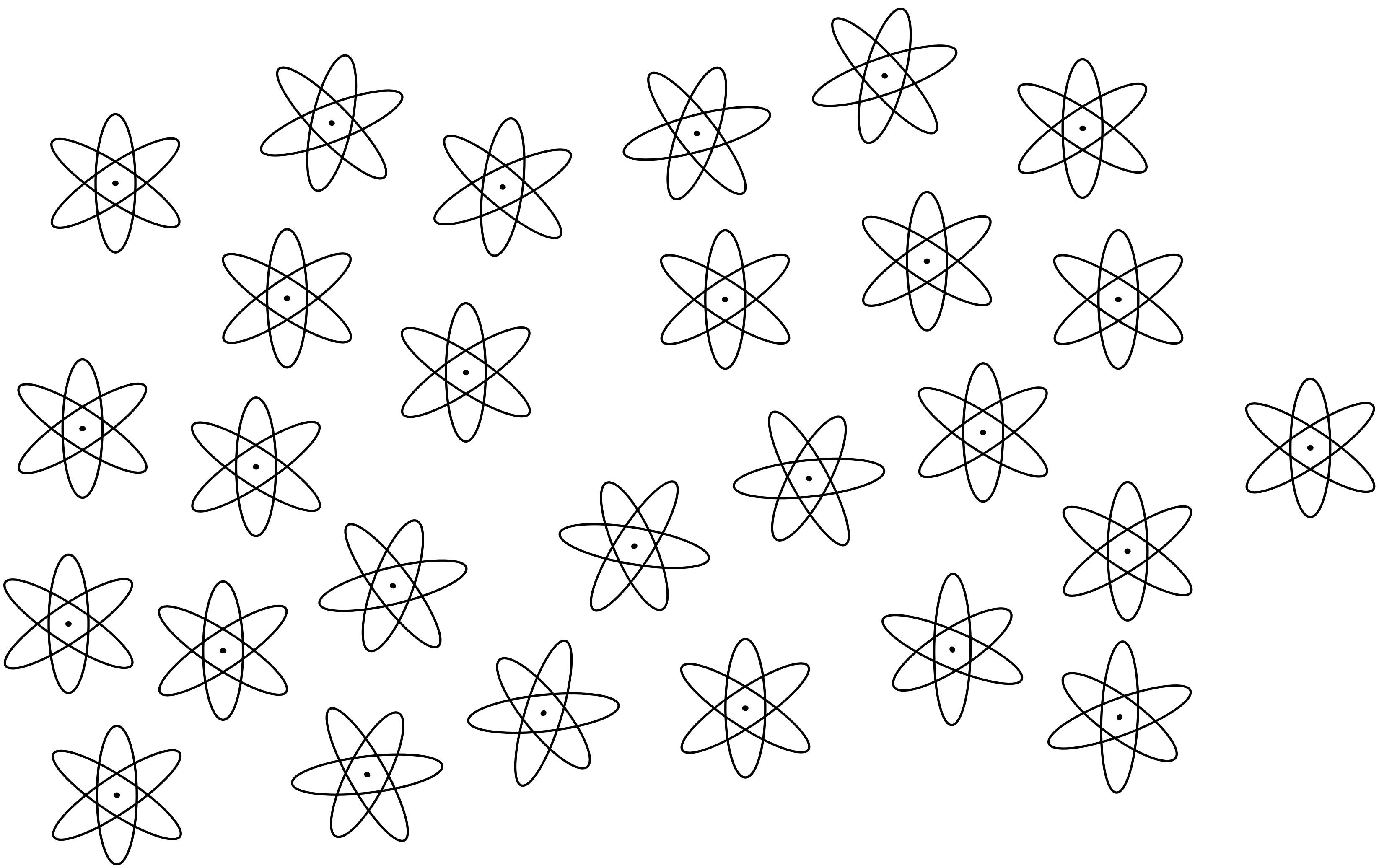


Sample atoms



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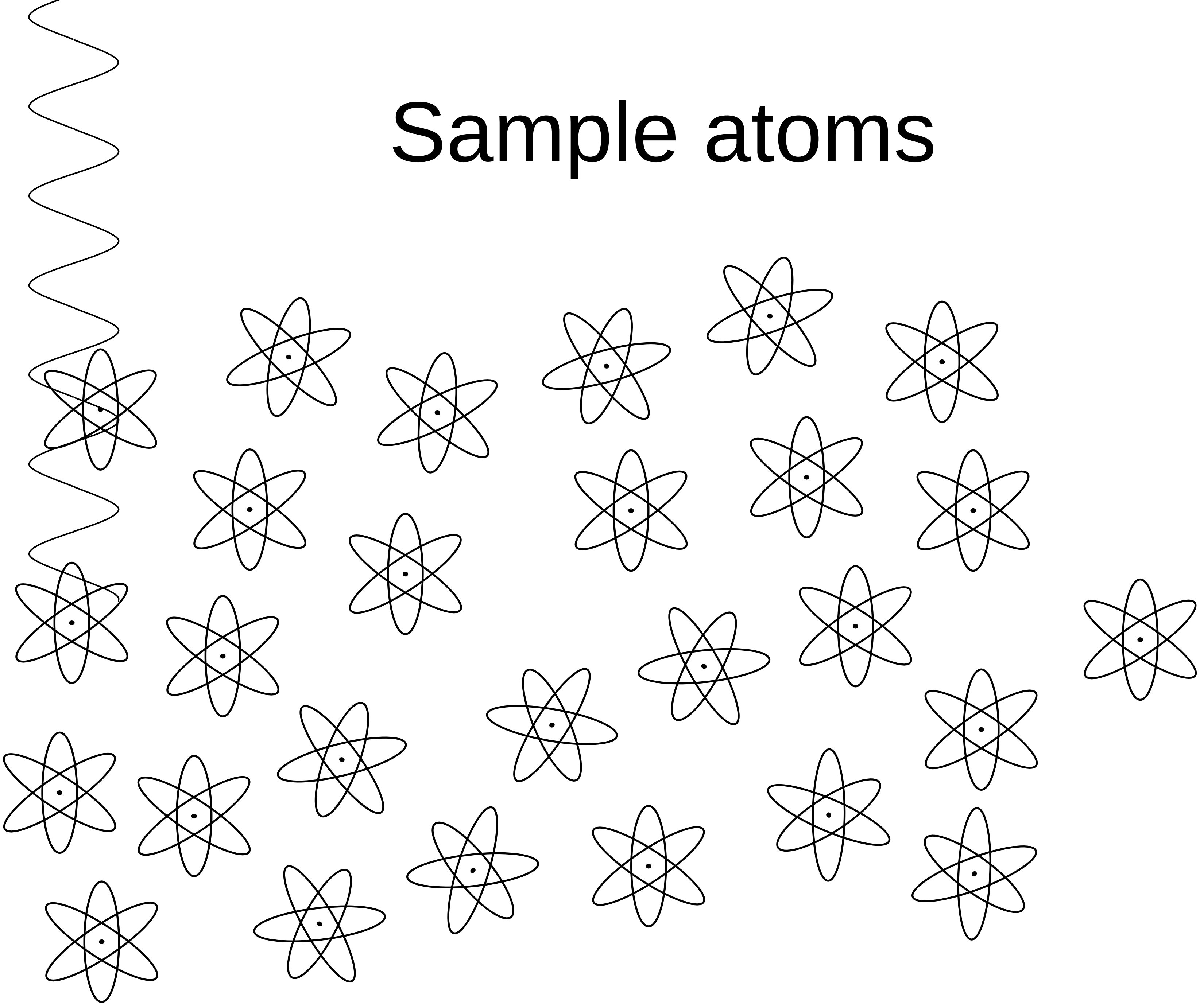
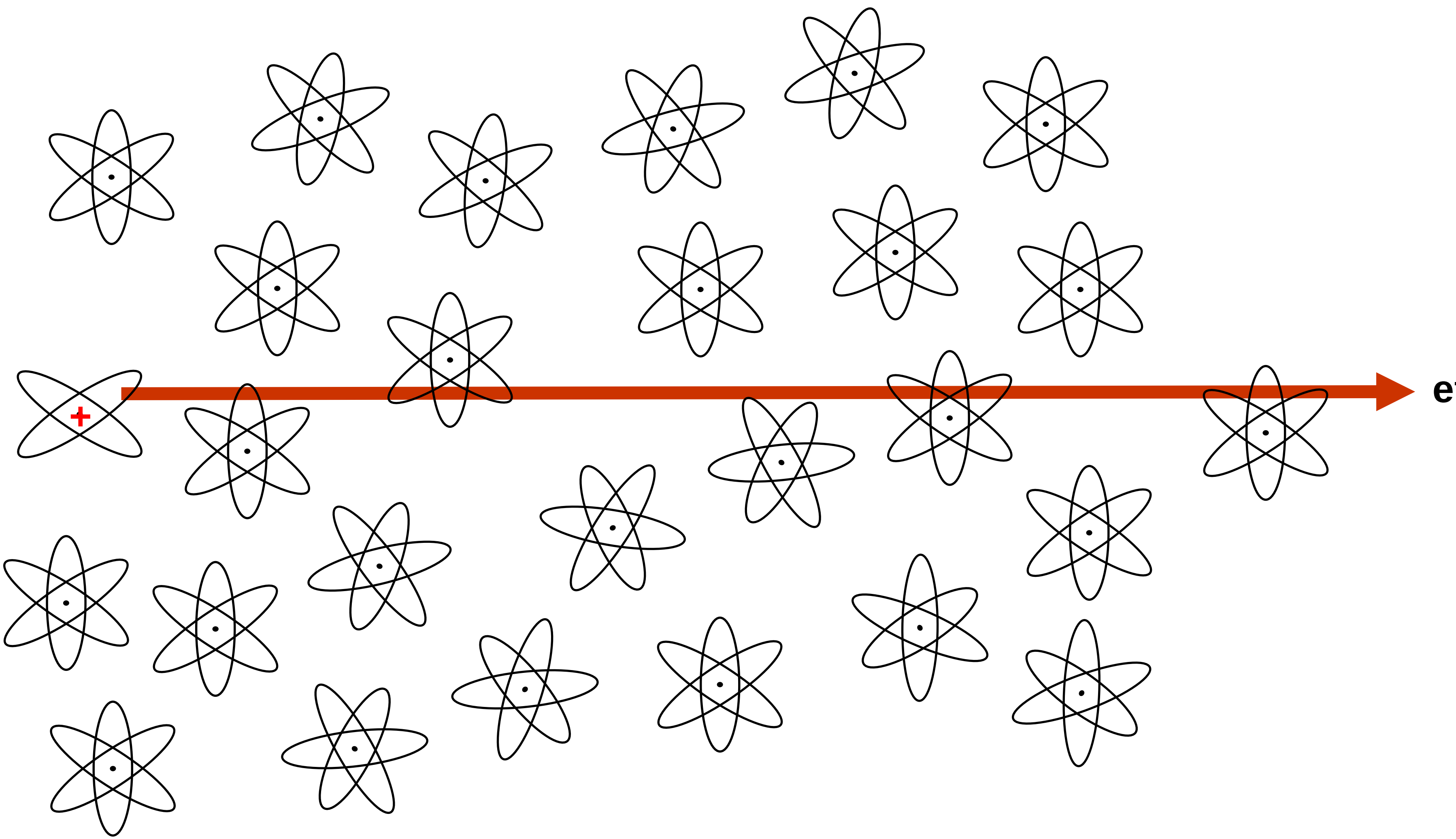
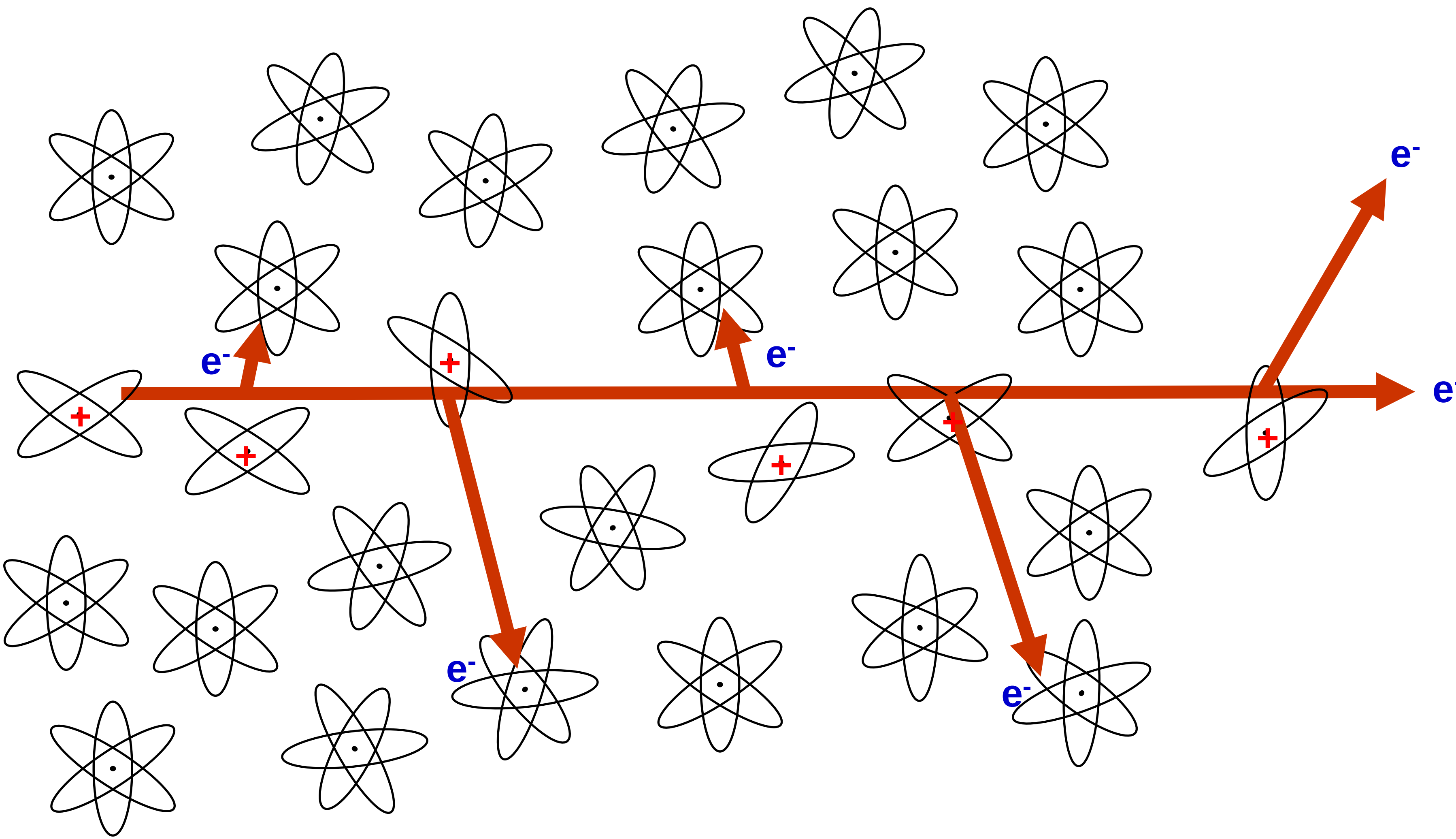


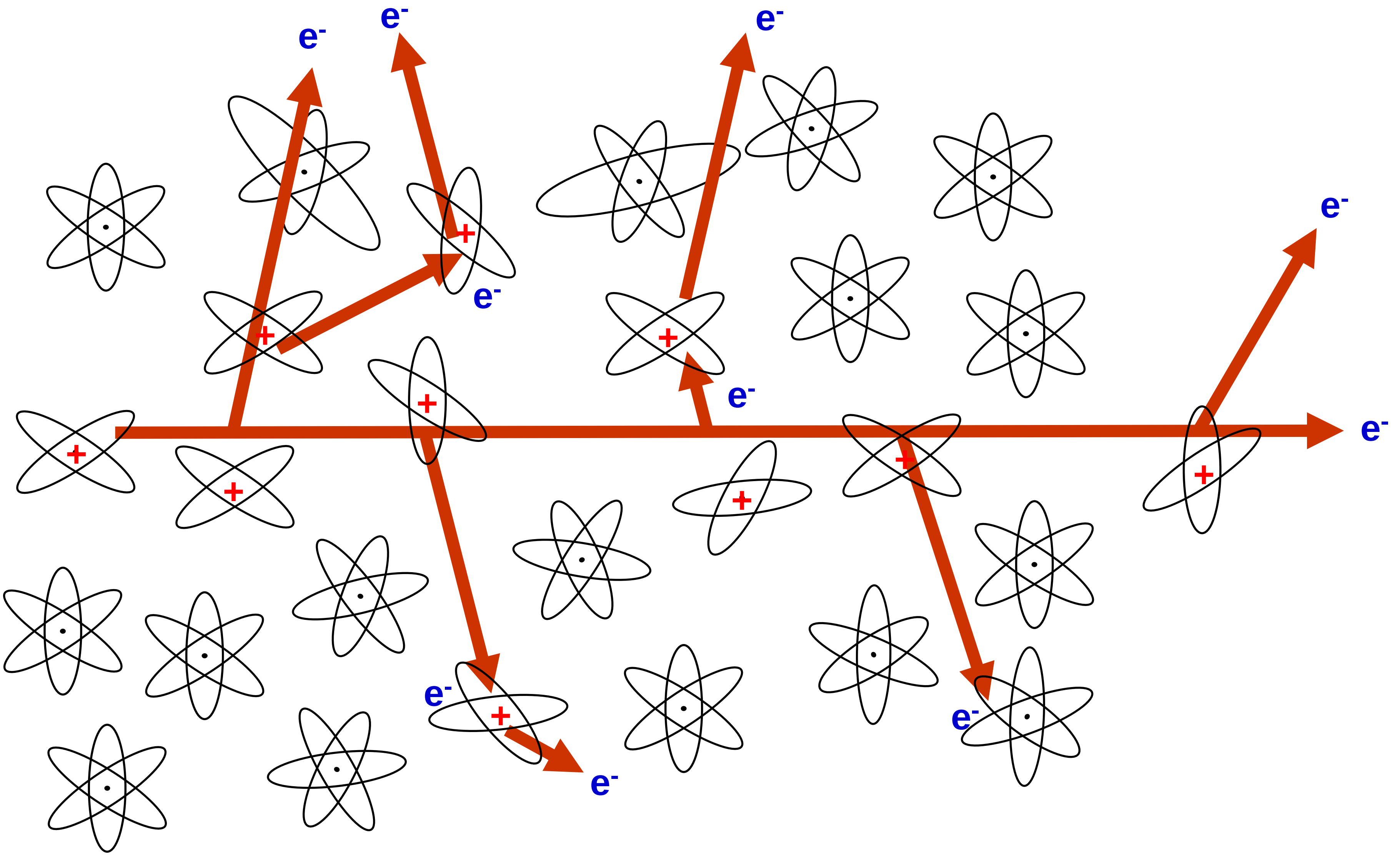
Primary ionization



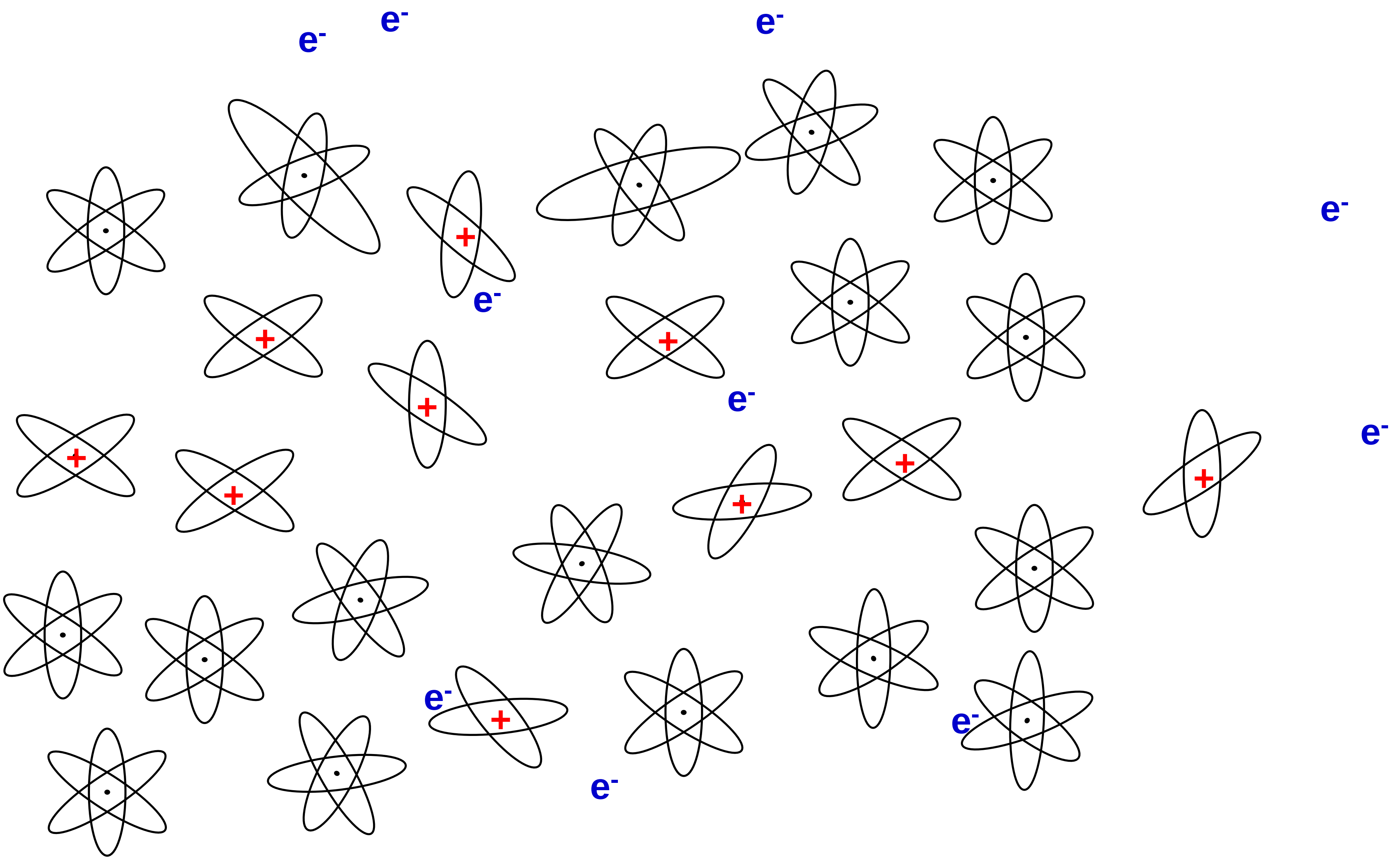
Secondary ionizations



Ionization track

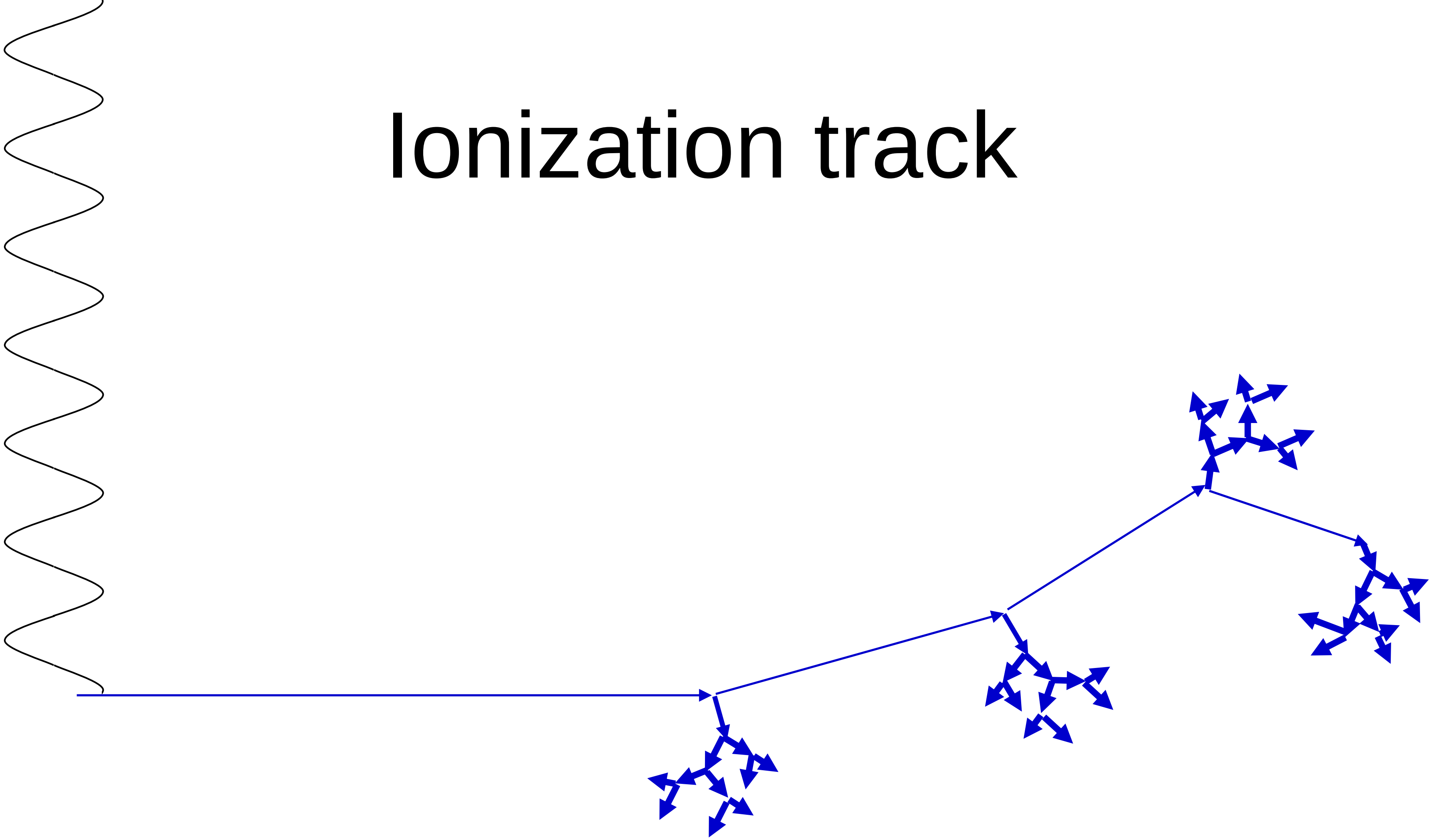


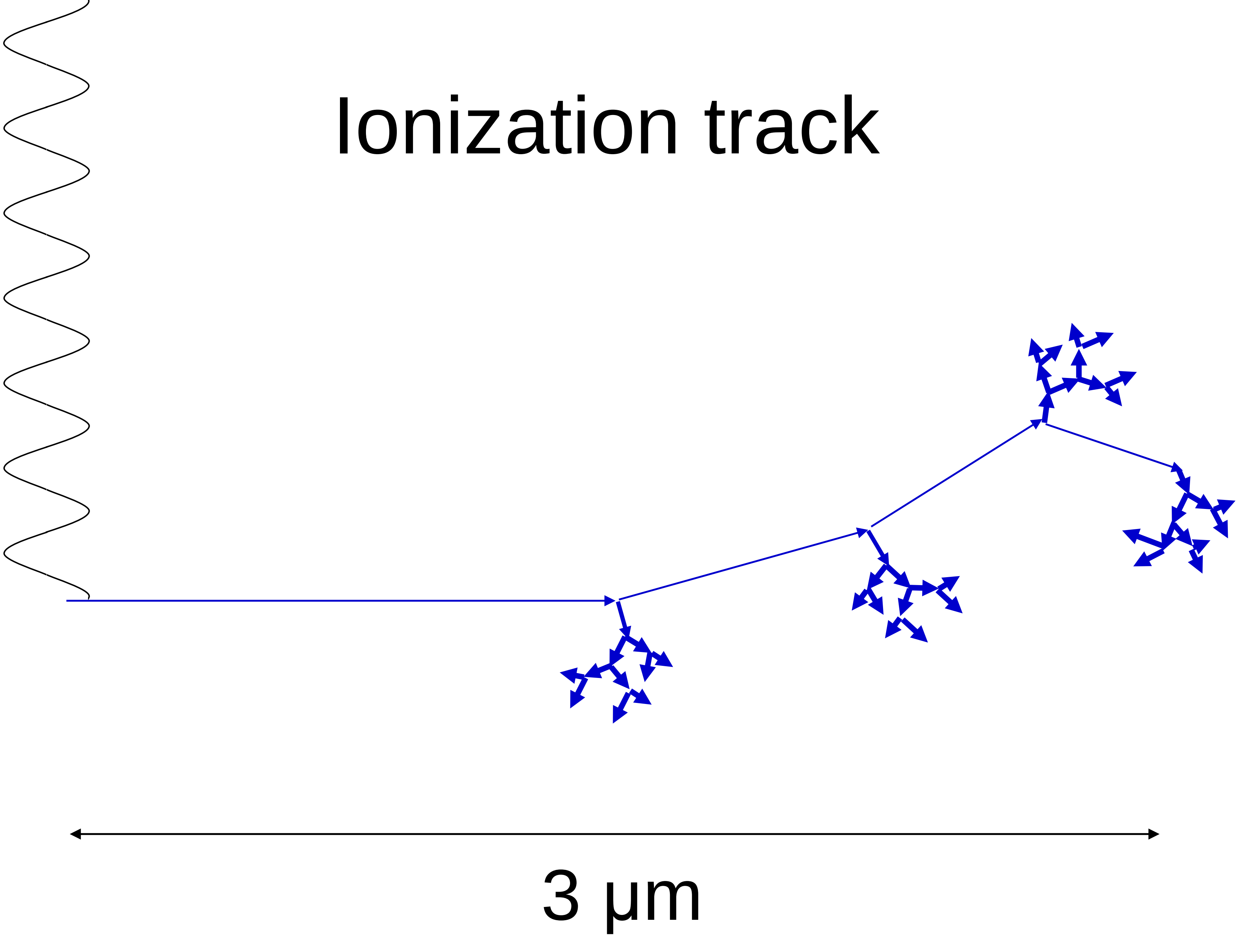
Ionization track



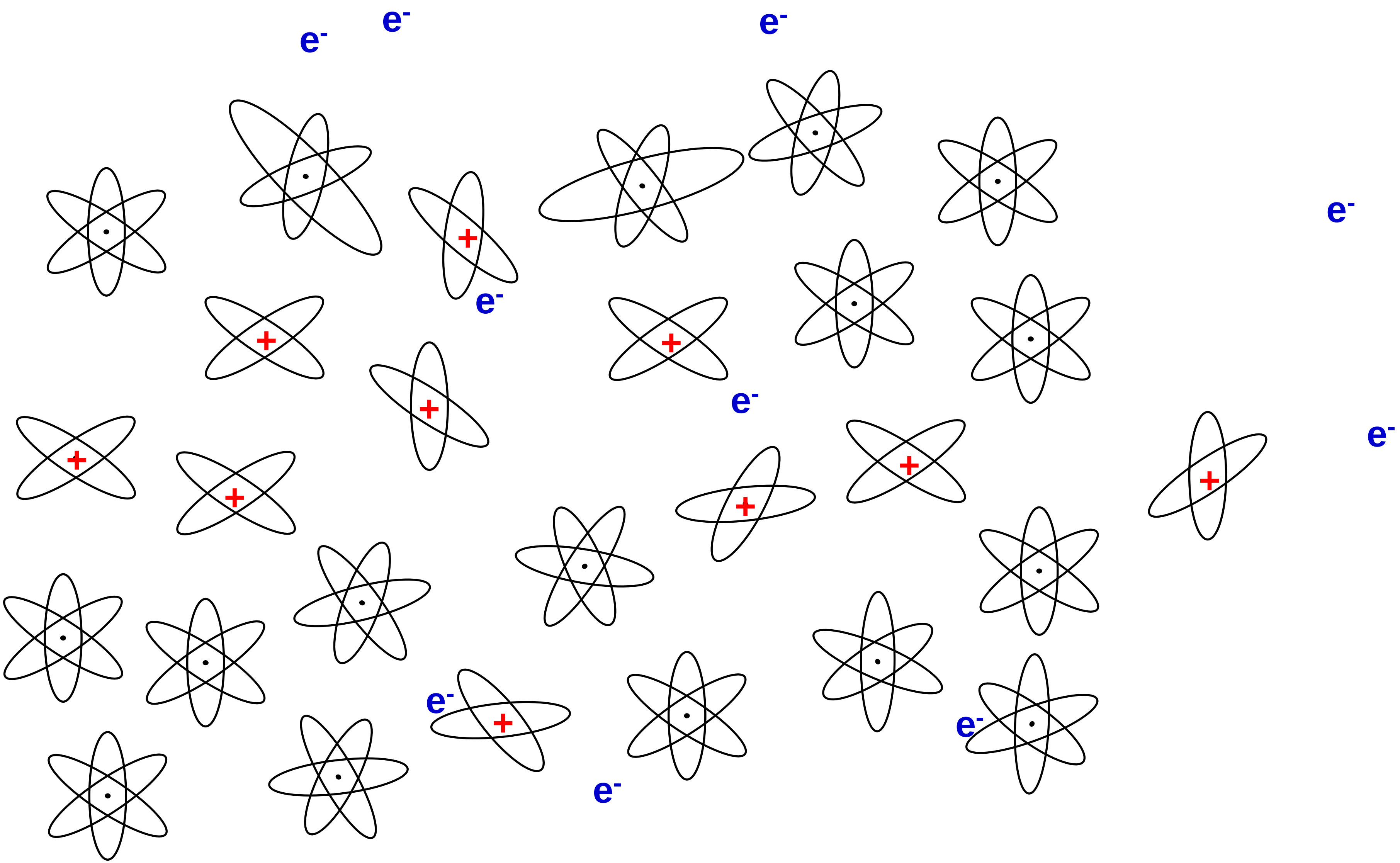
Ionization track

Ionization track

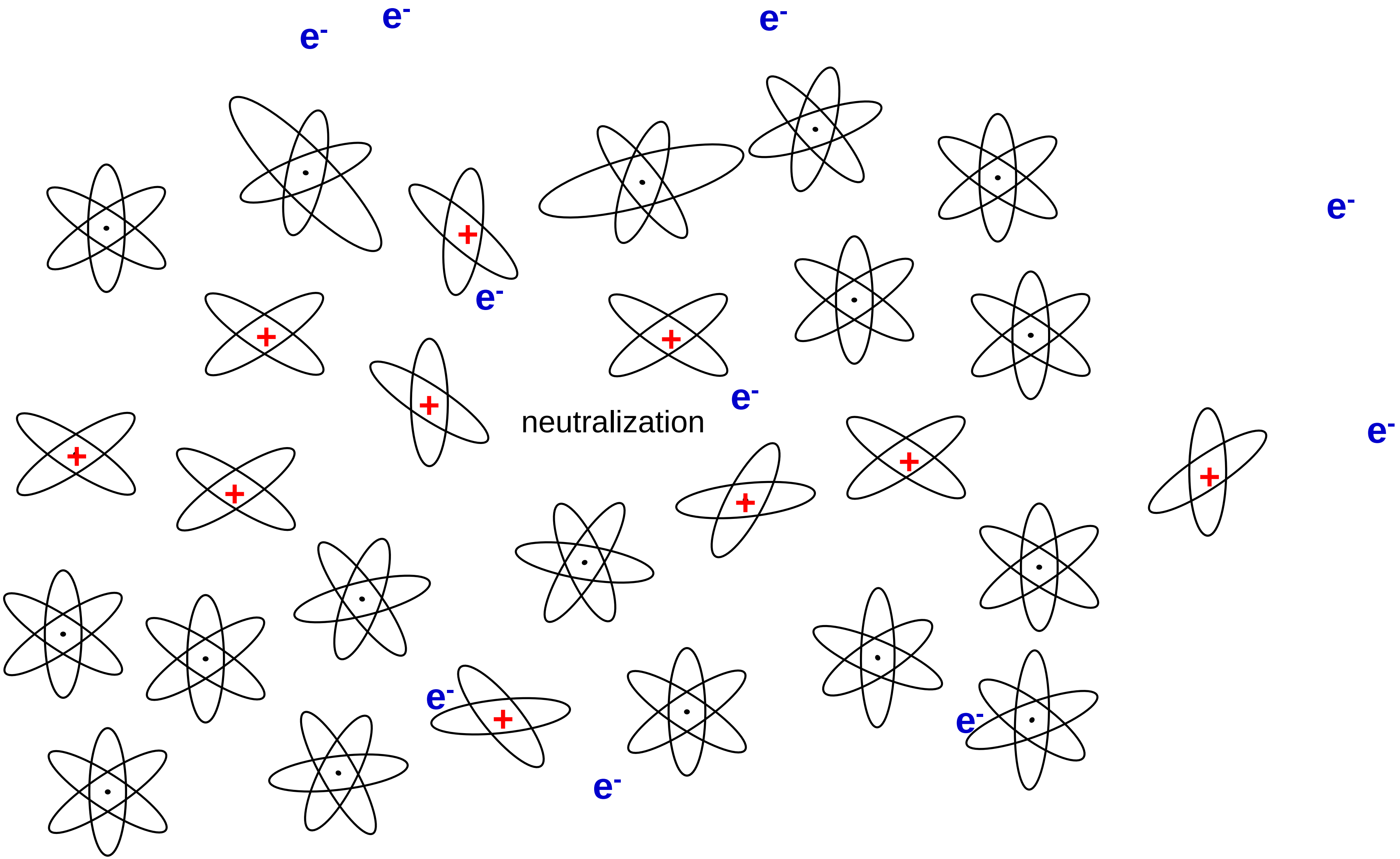



Ionization track
3 µm

initial effects



initial effects



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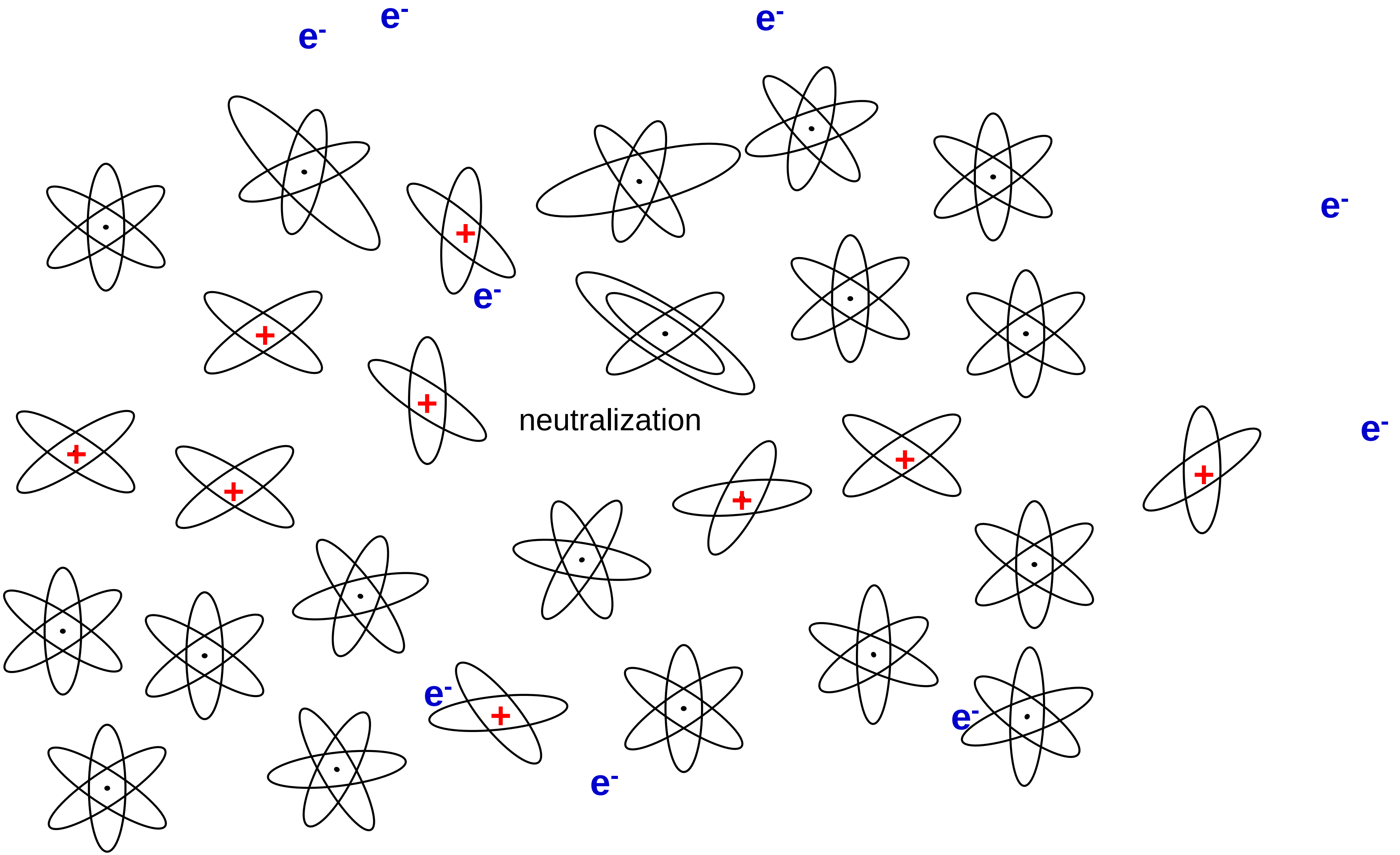
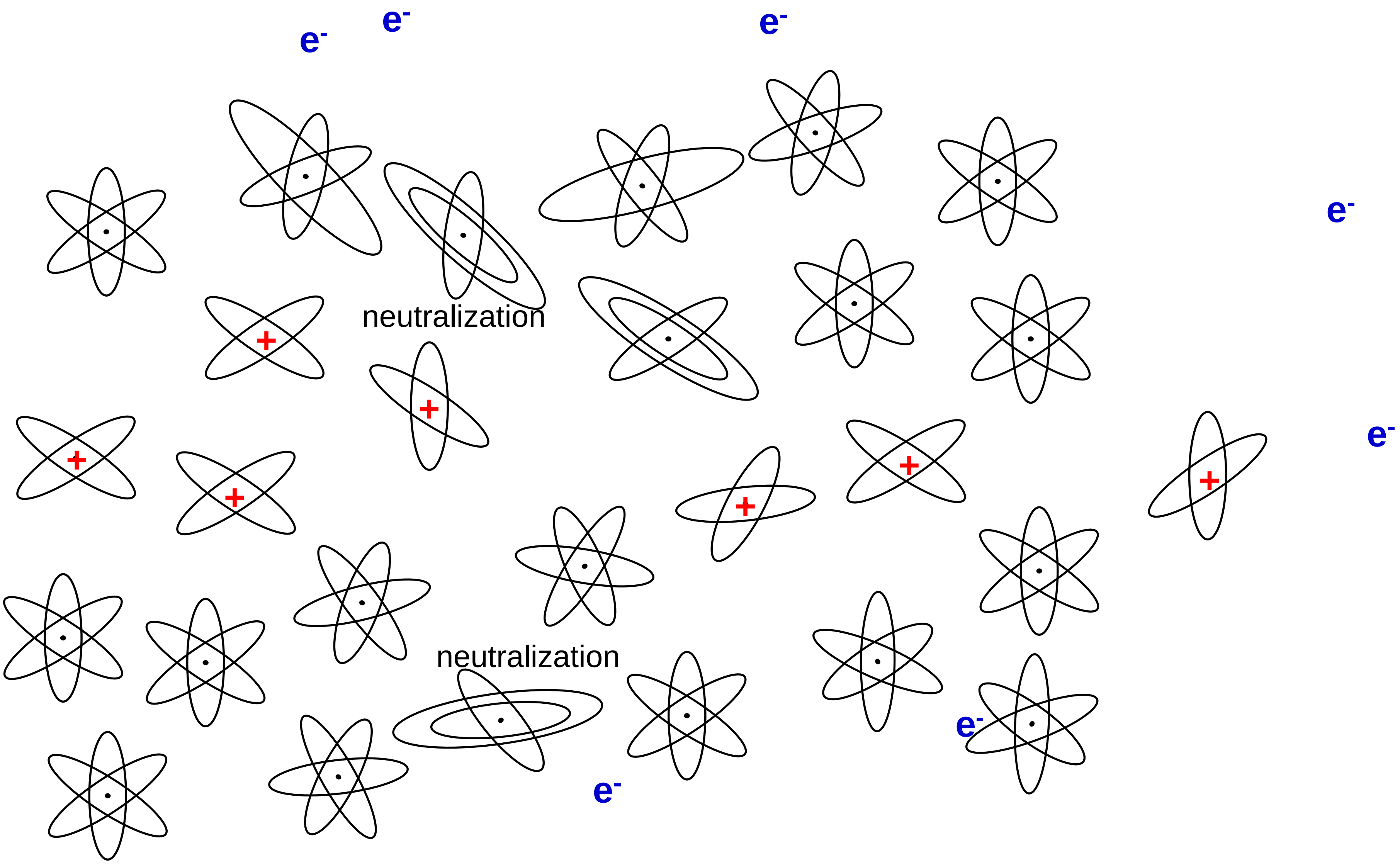
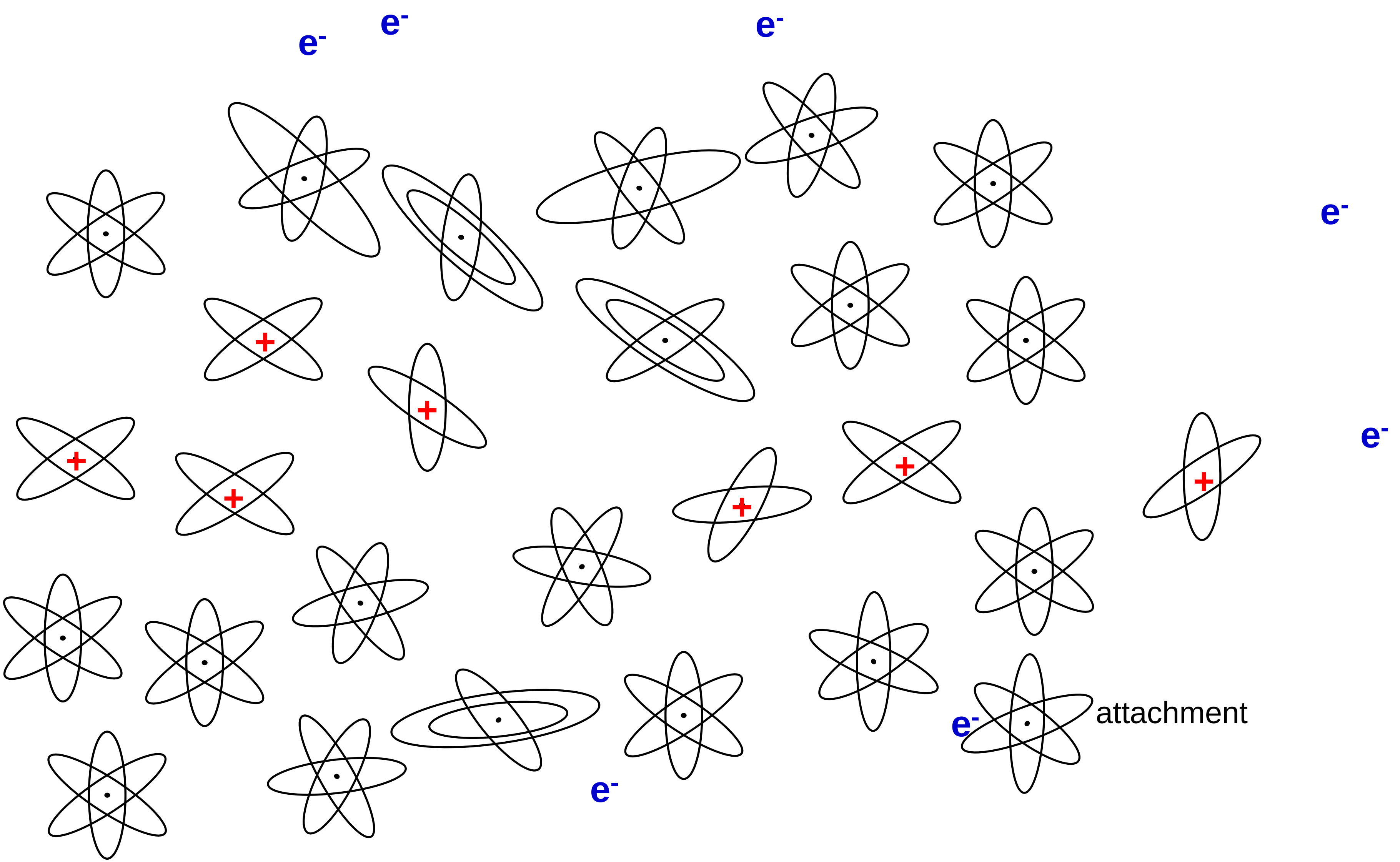


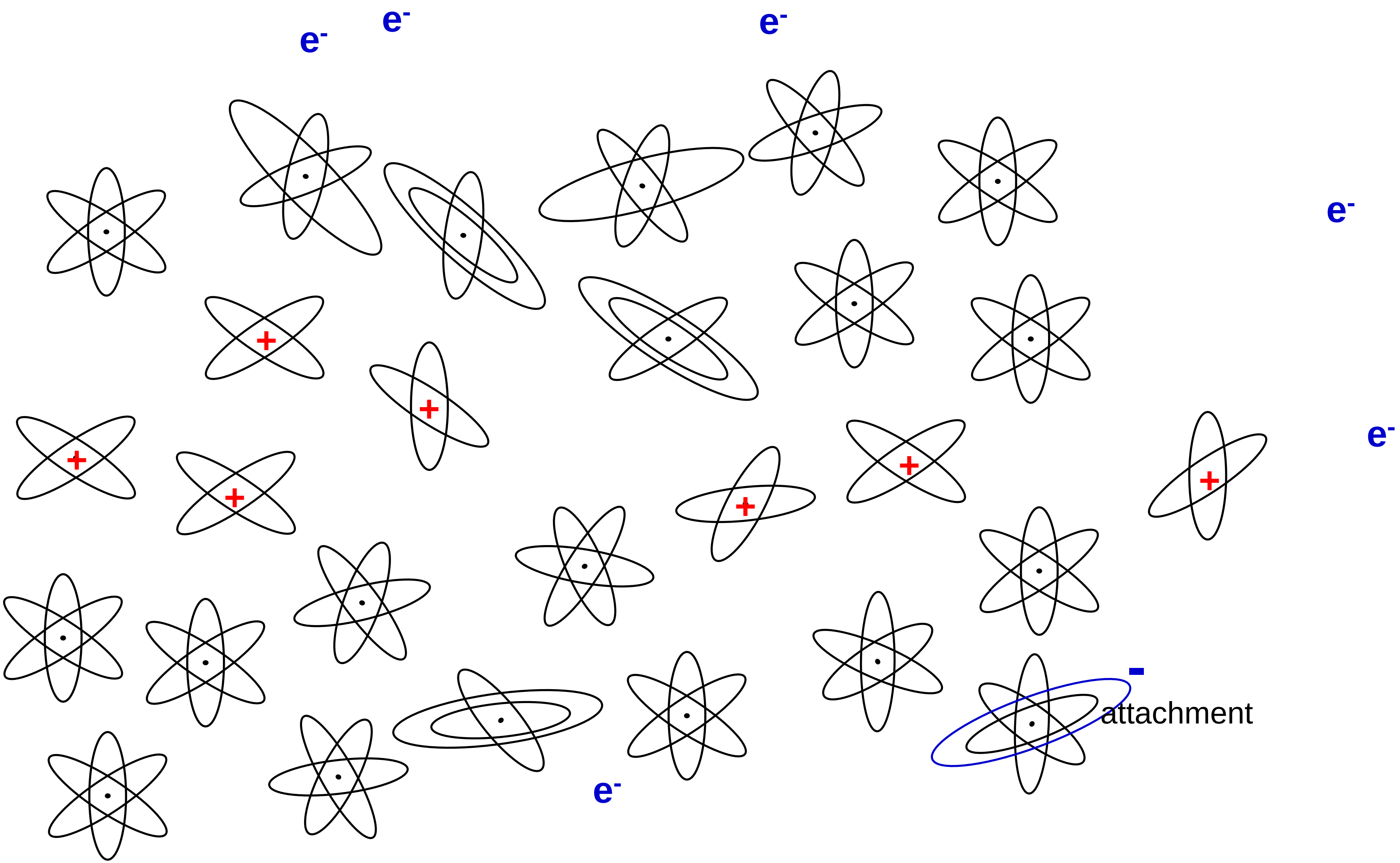
initial effects



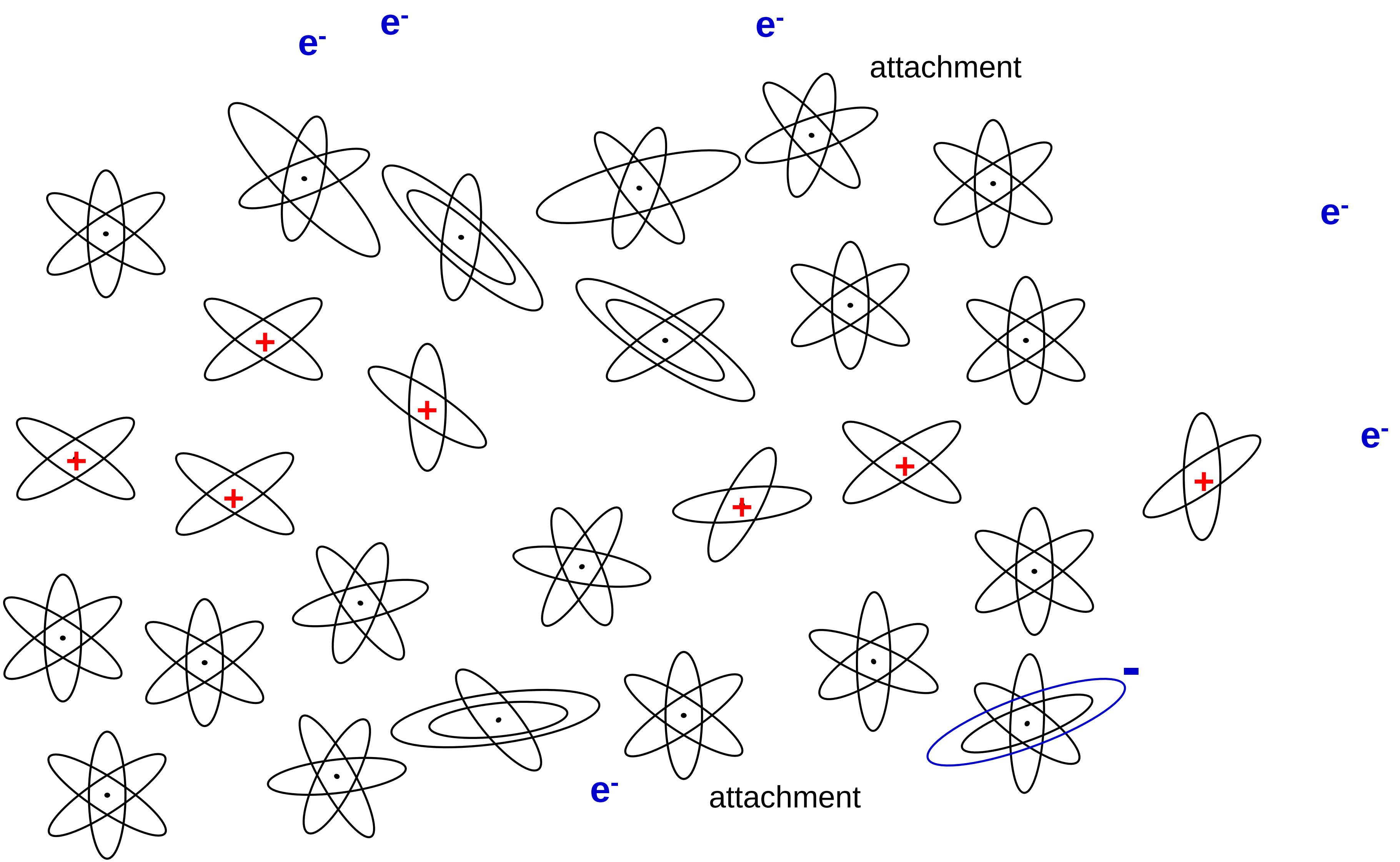
initial effects



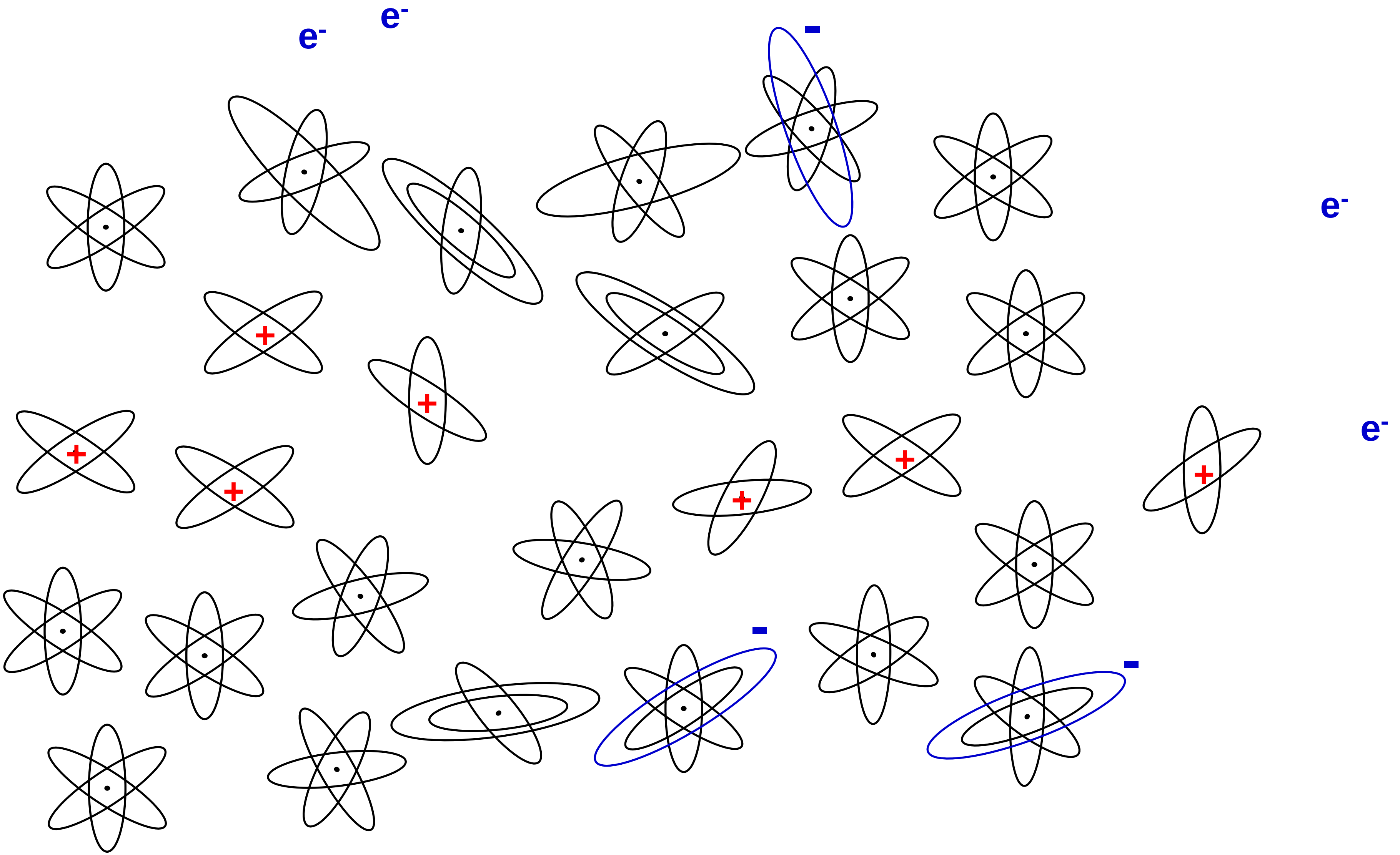
initial effects



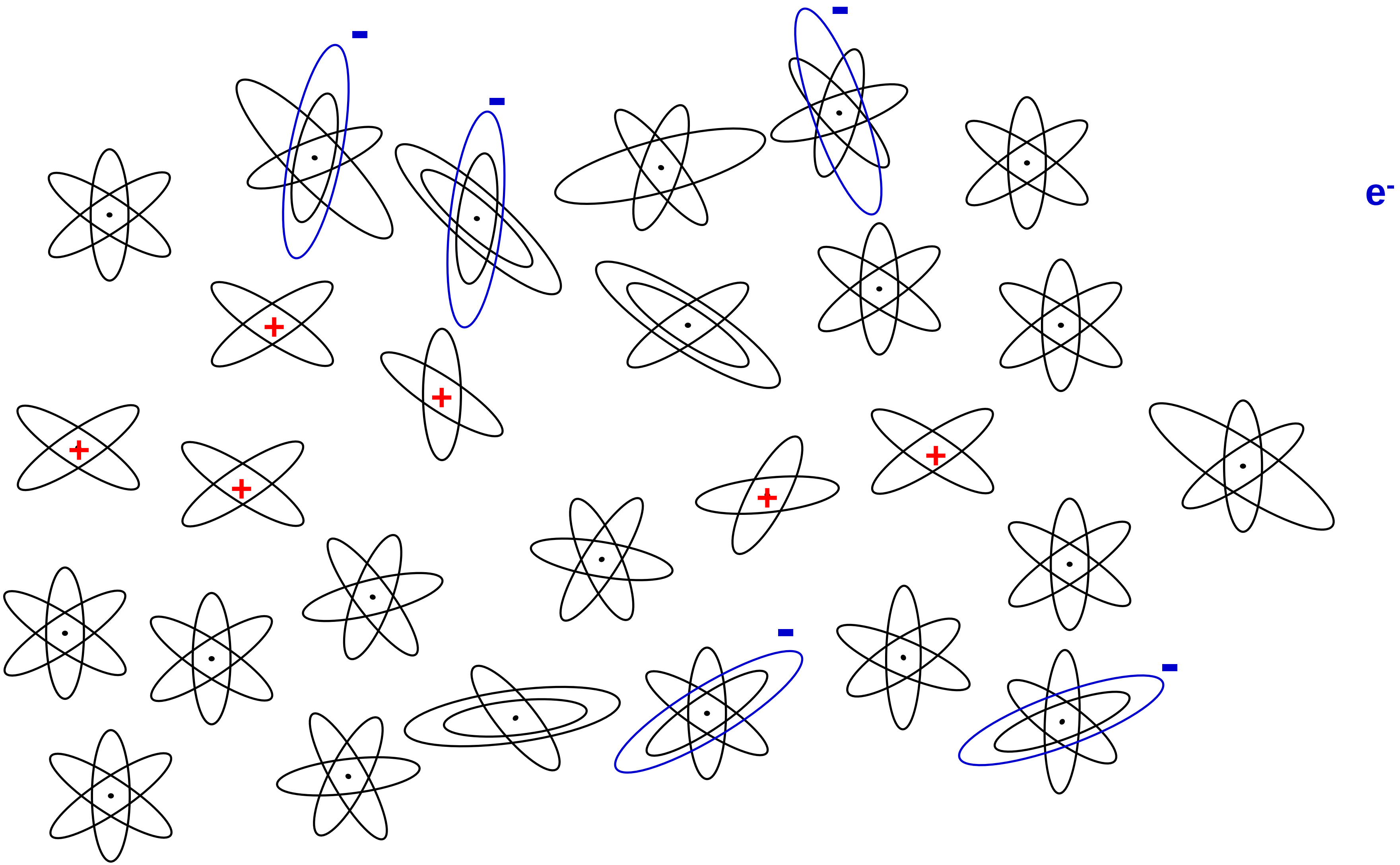
initial effects



initial effects



initial effects



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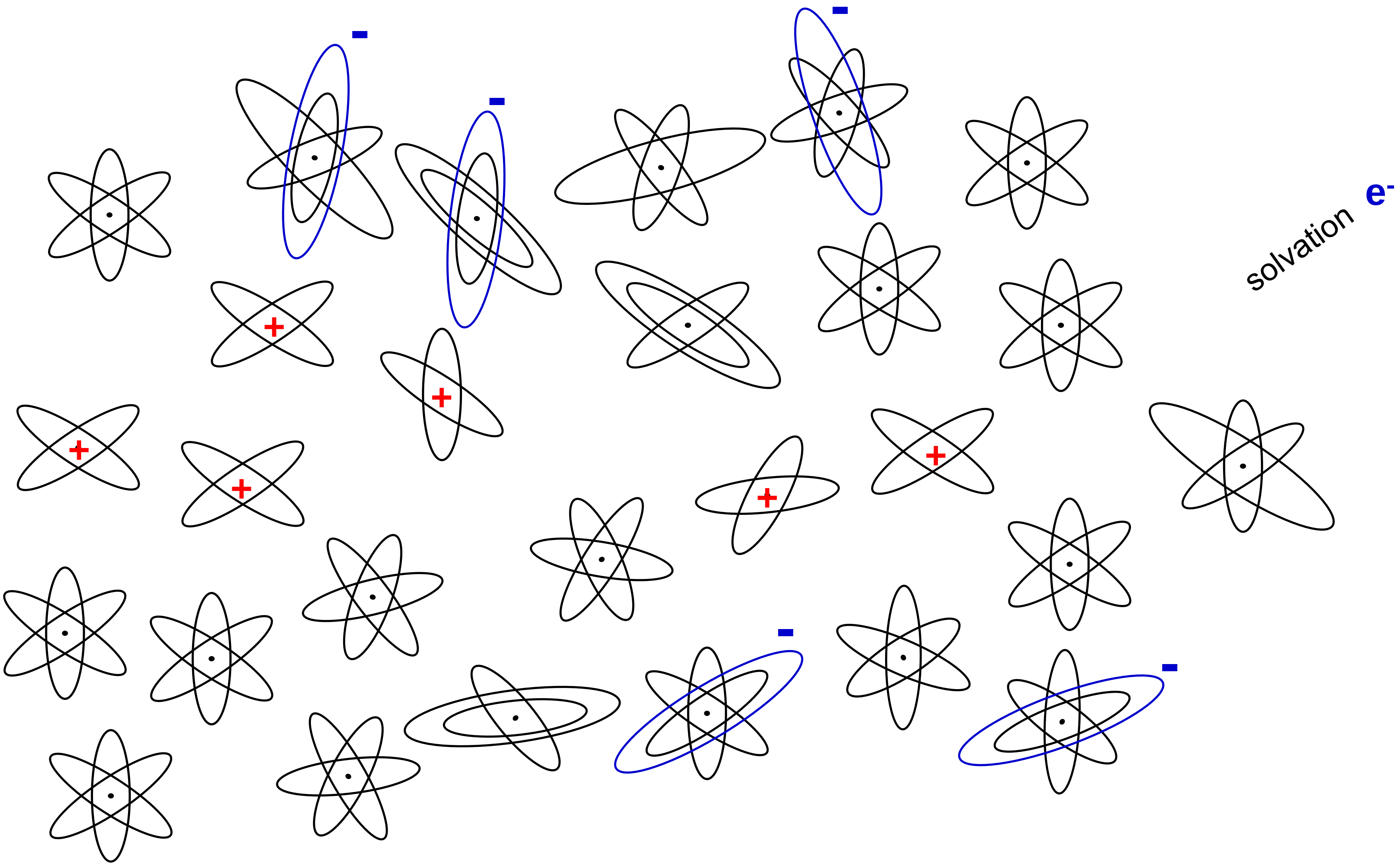
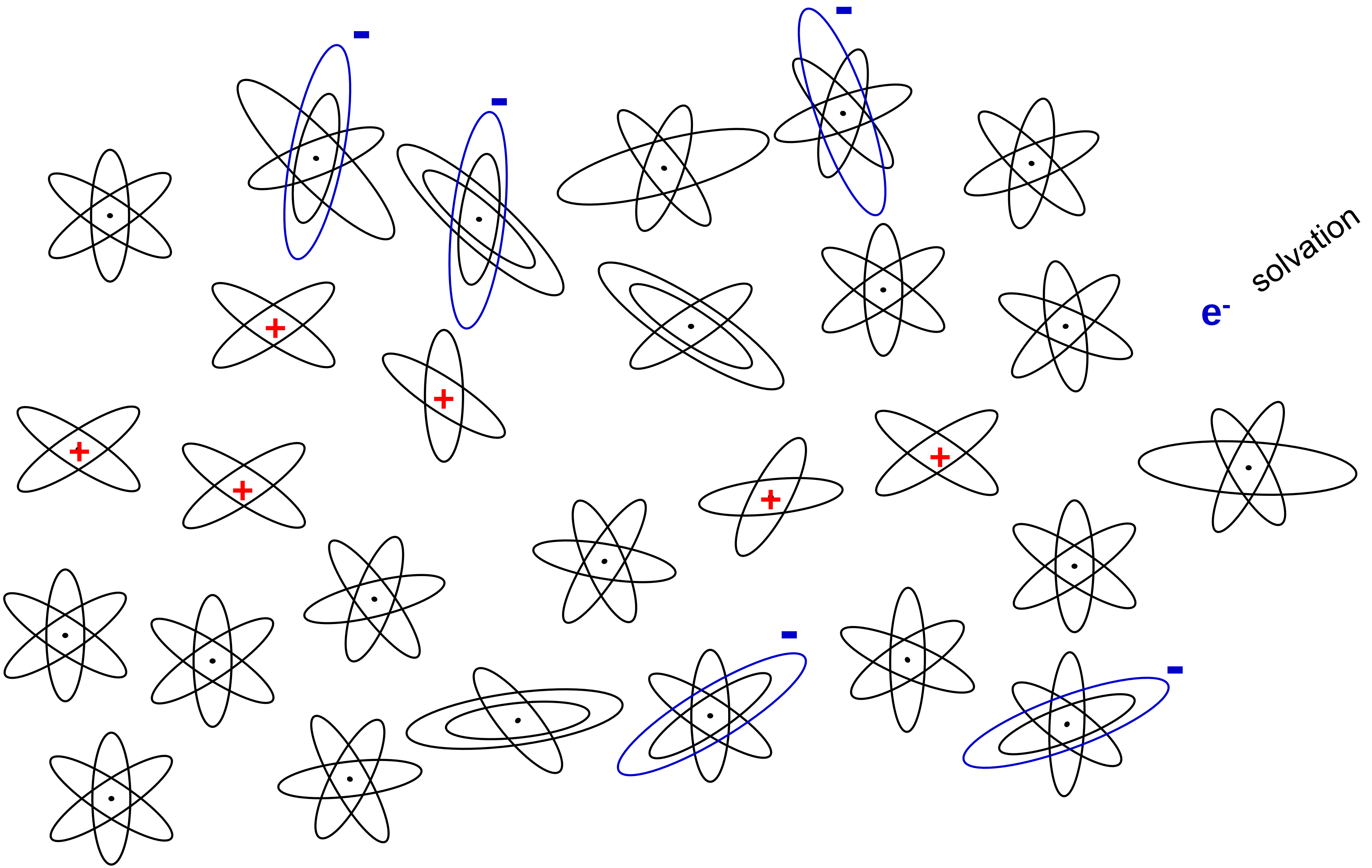
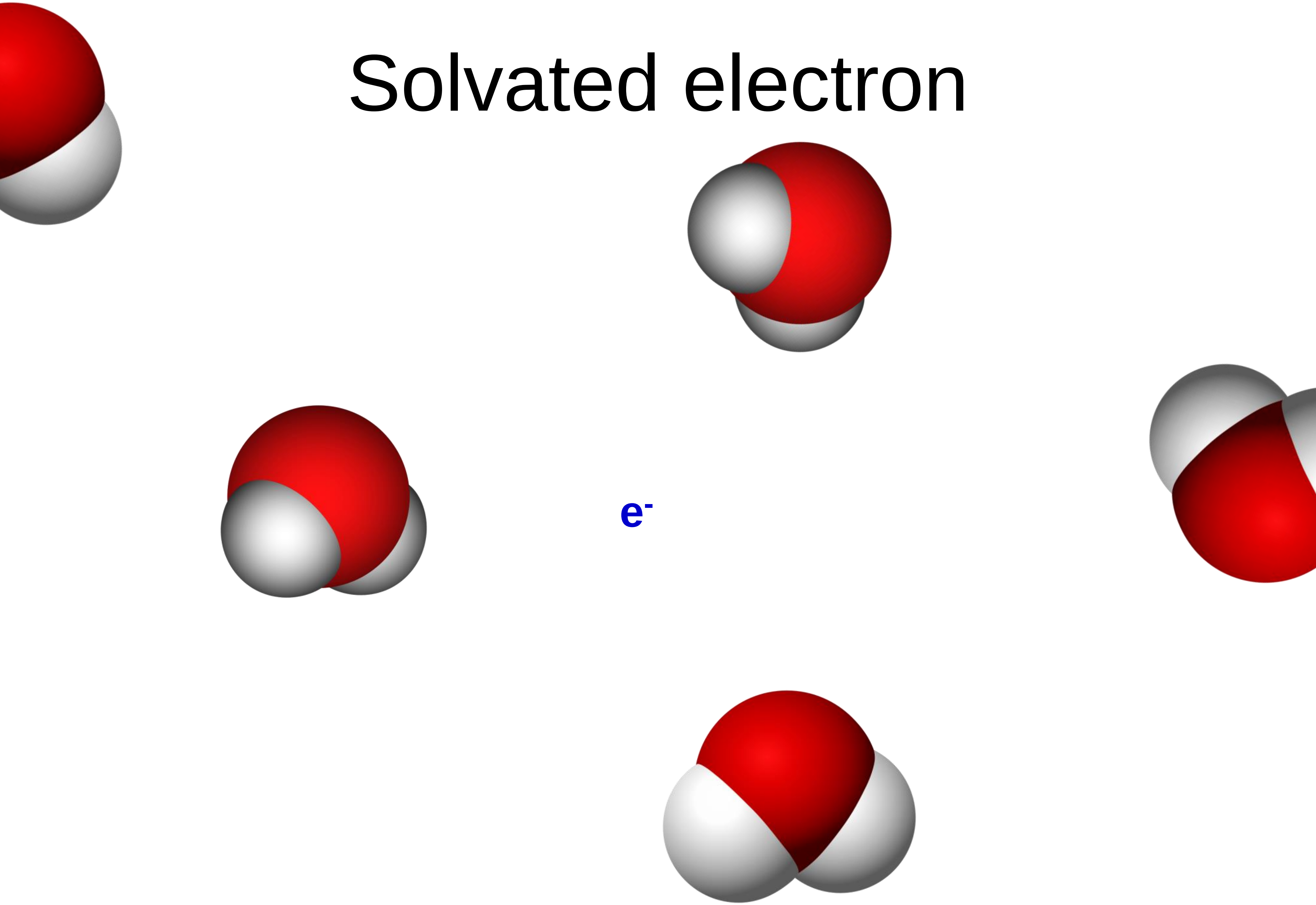


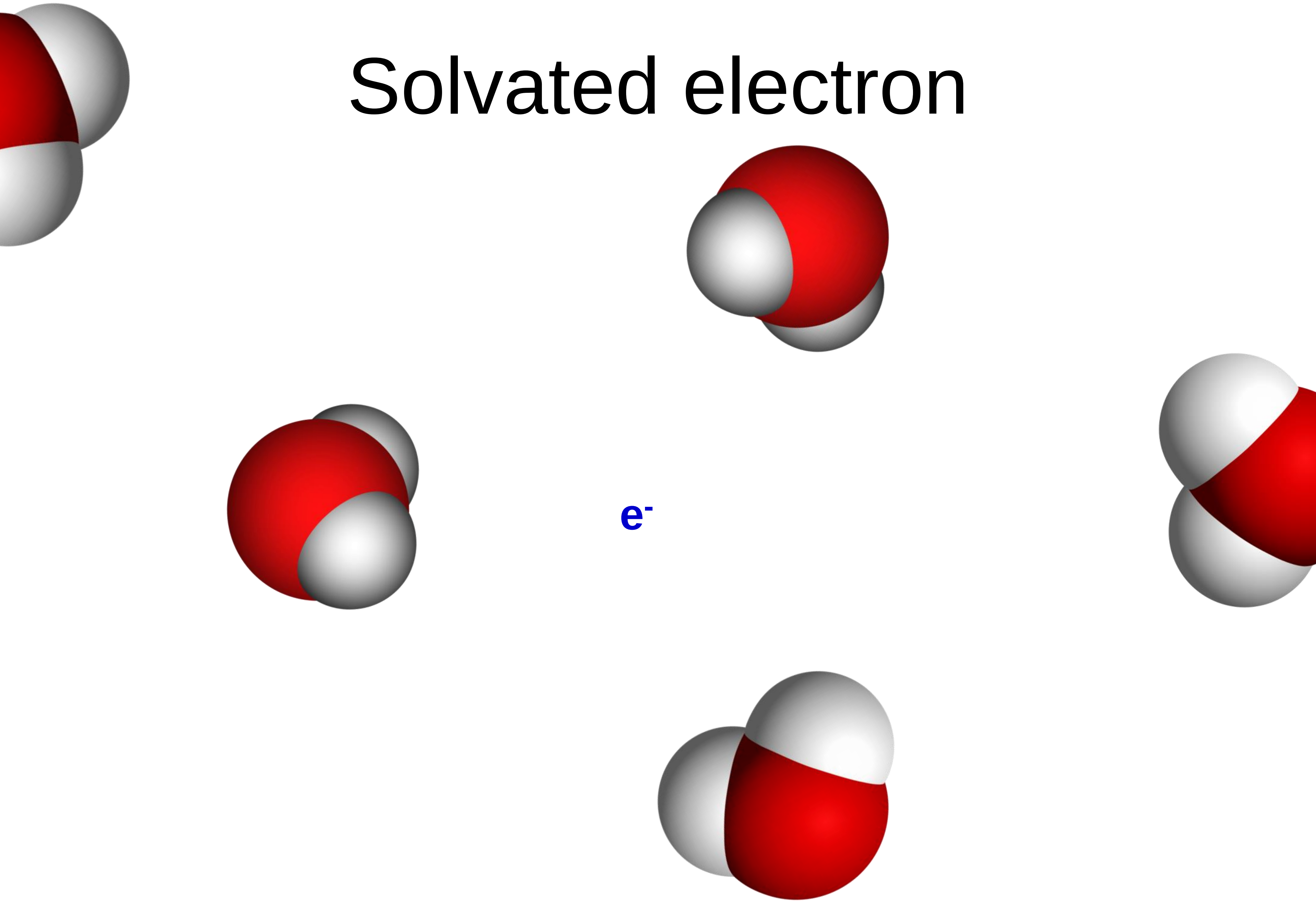
initial effects

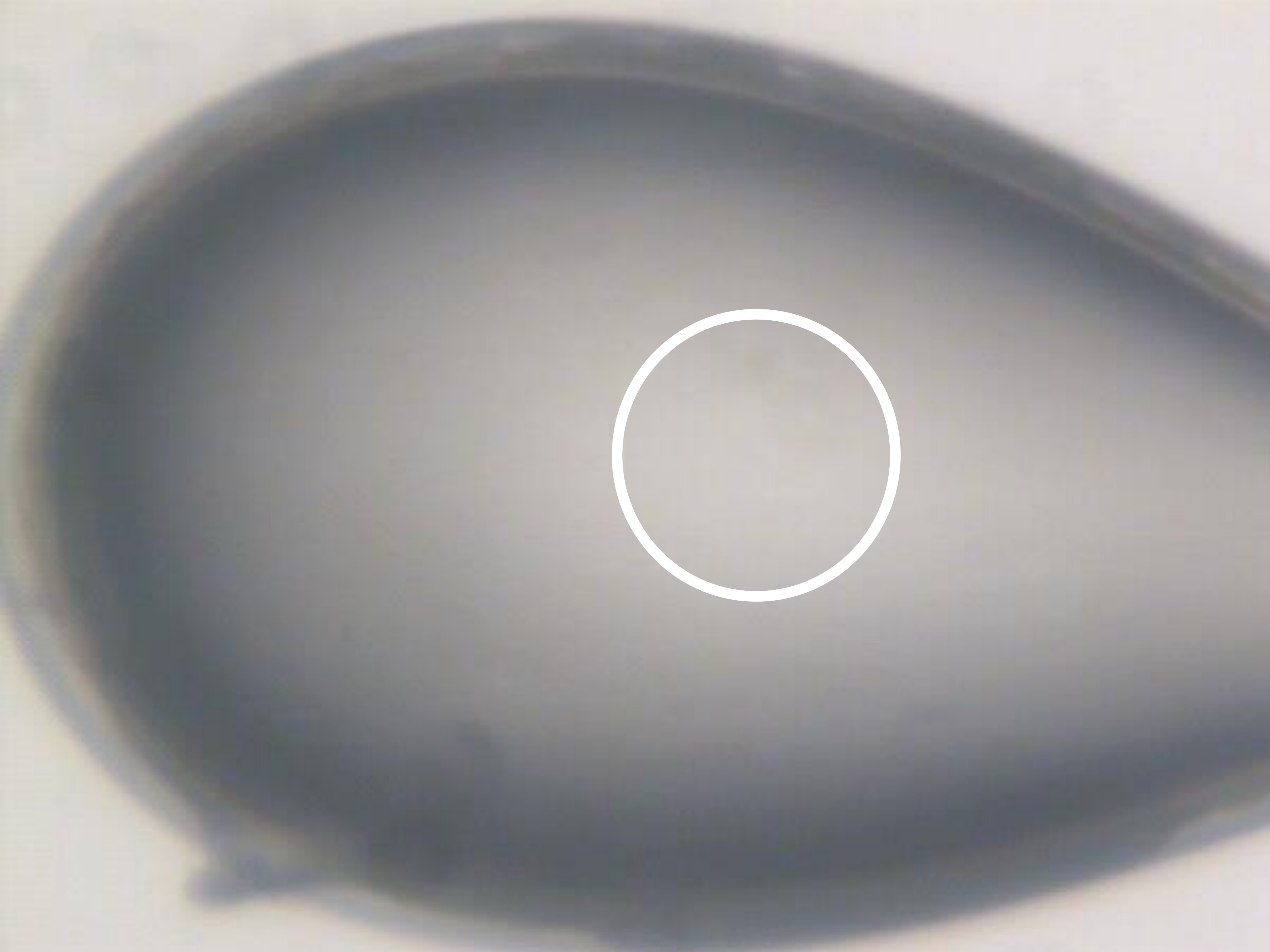


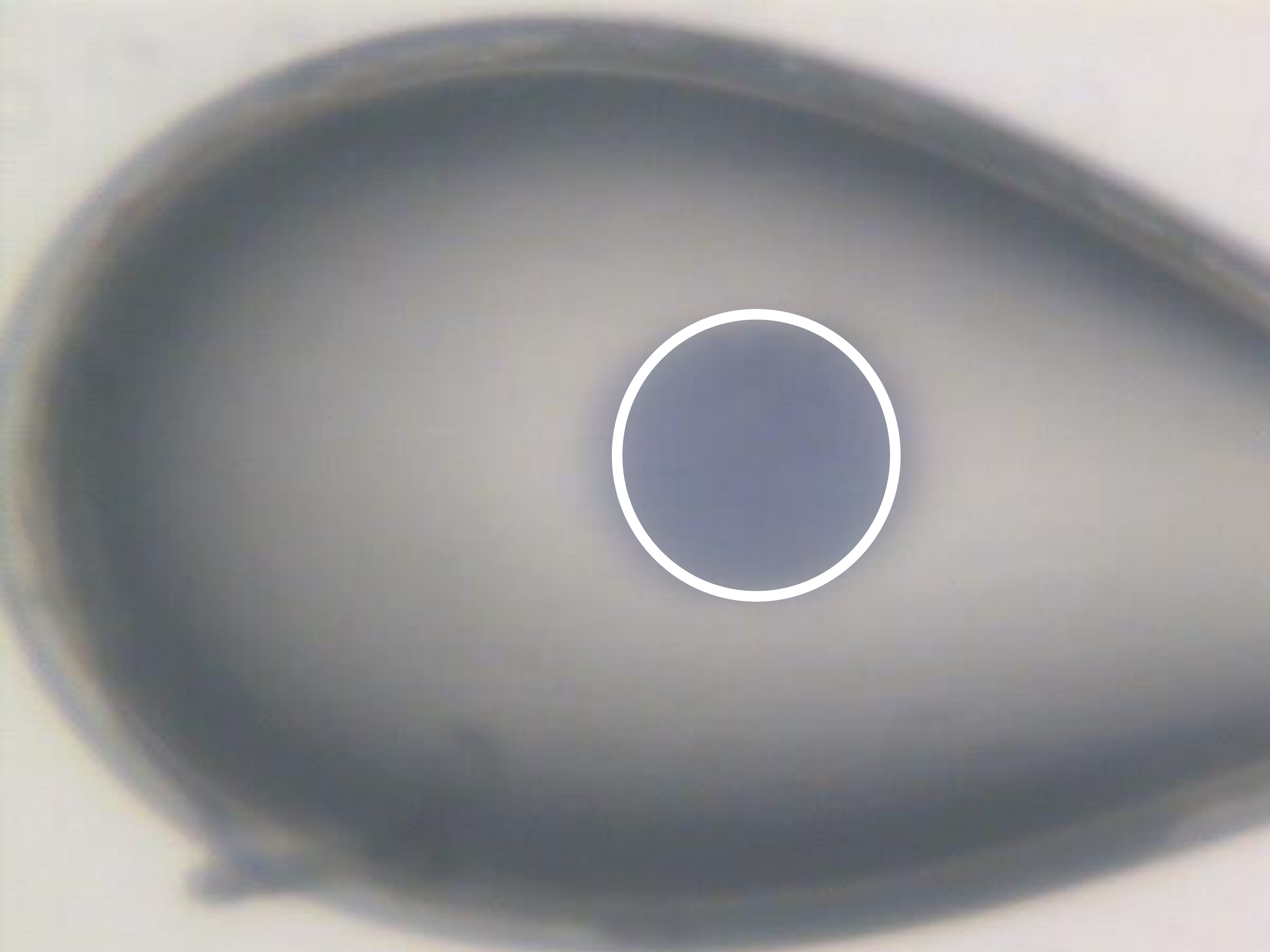
Solvated electron



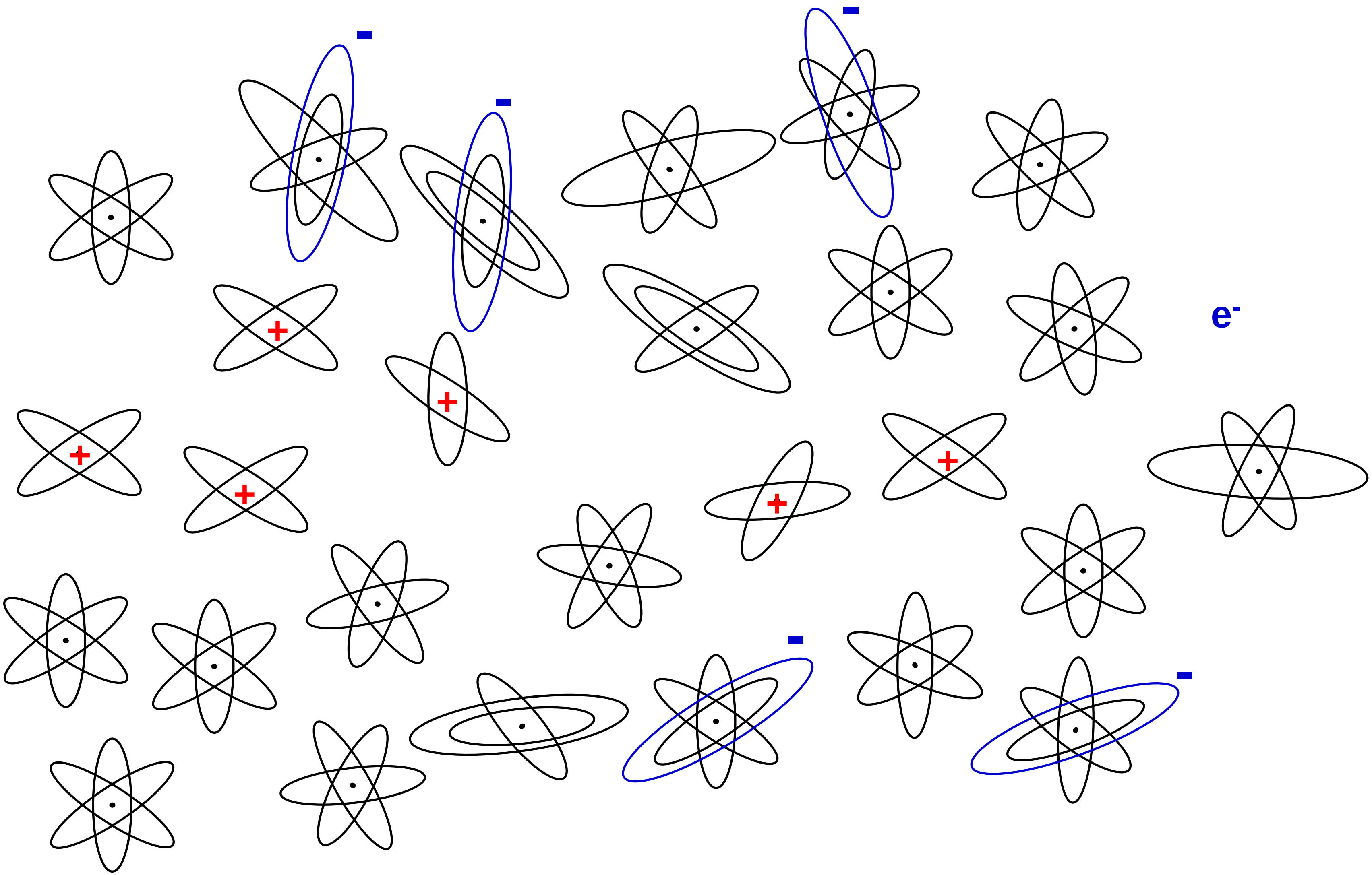
Solvated electron



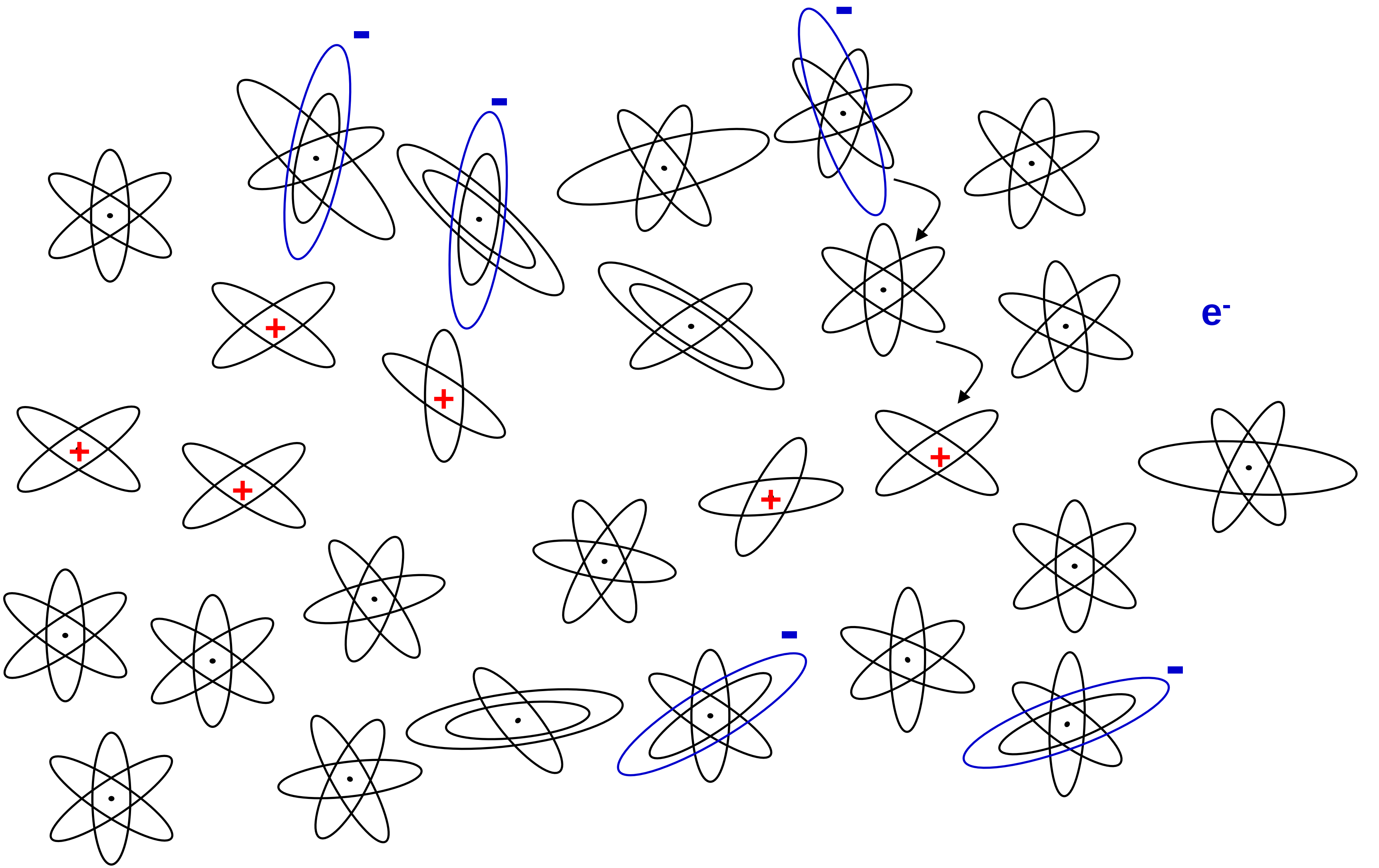




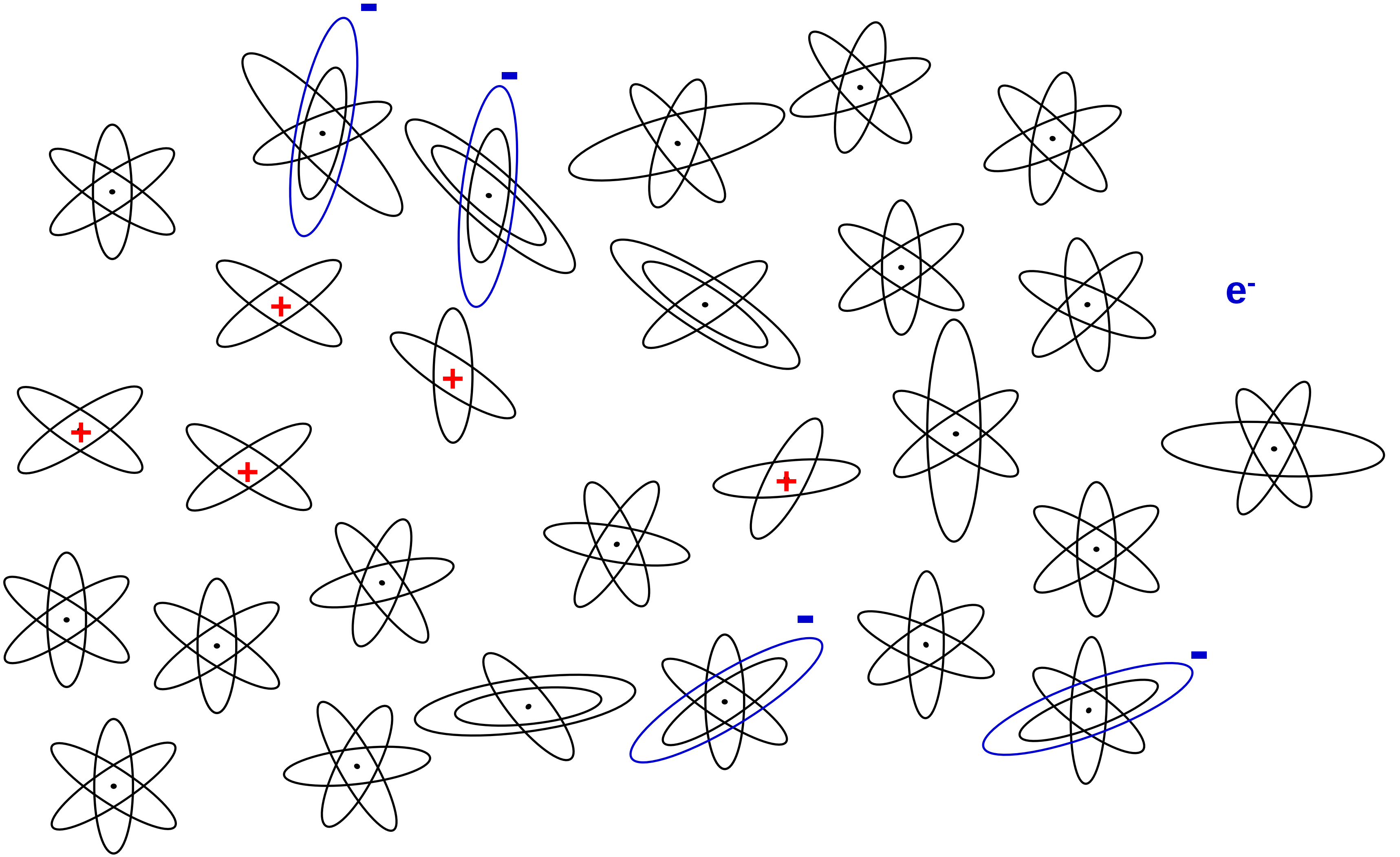
initial effects



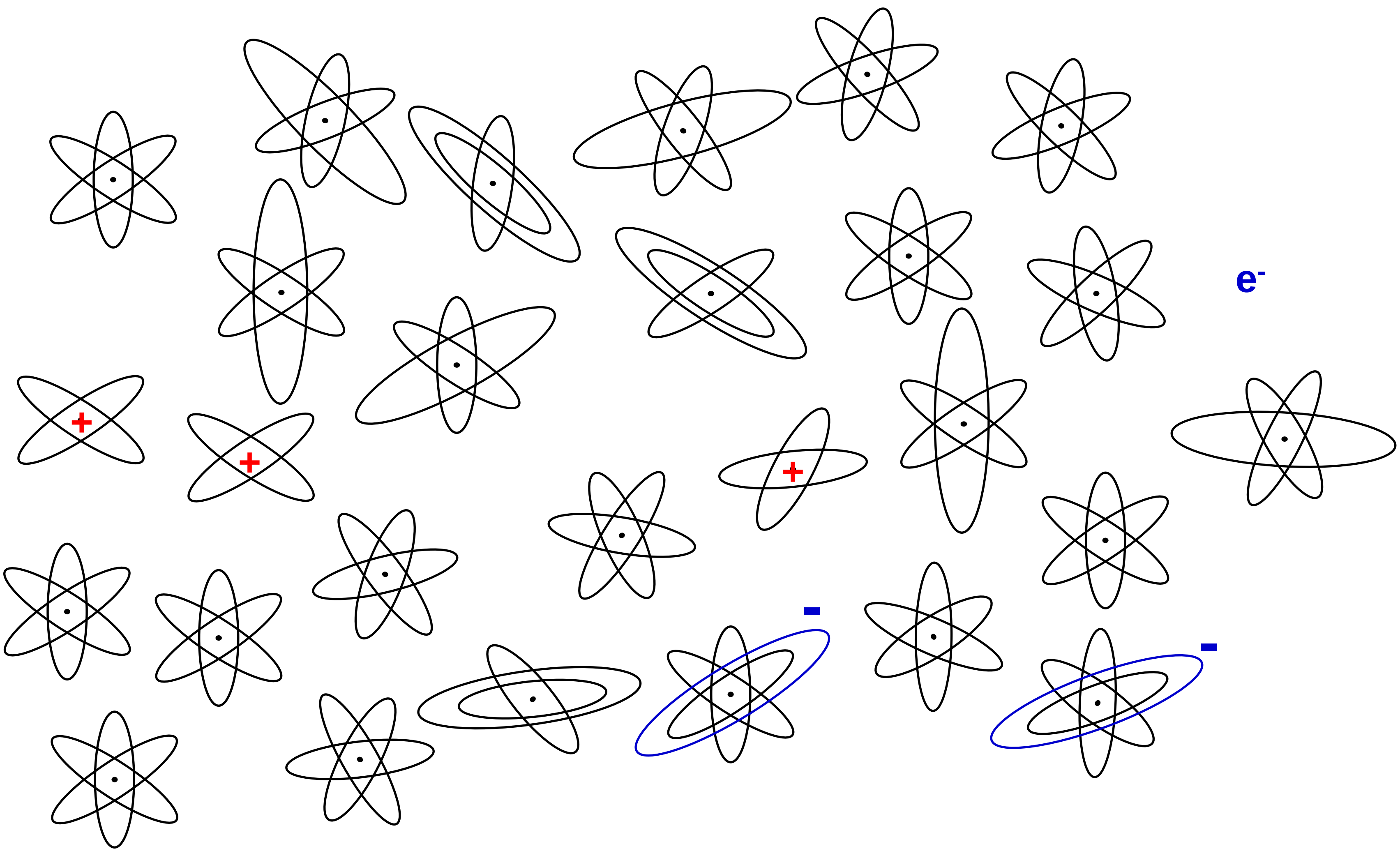
initial effects



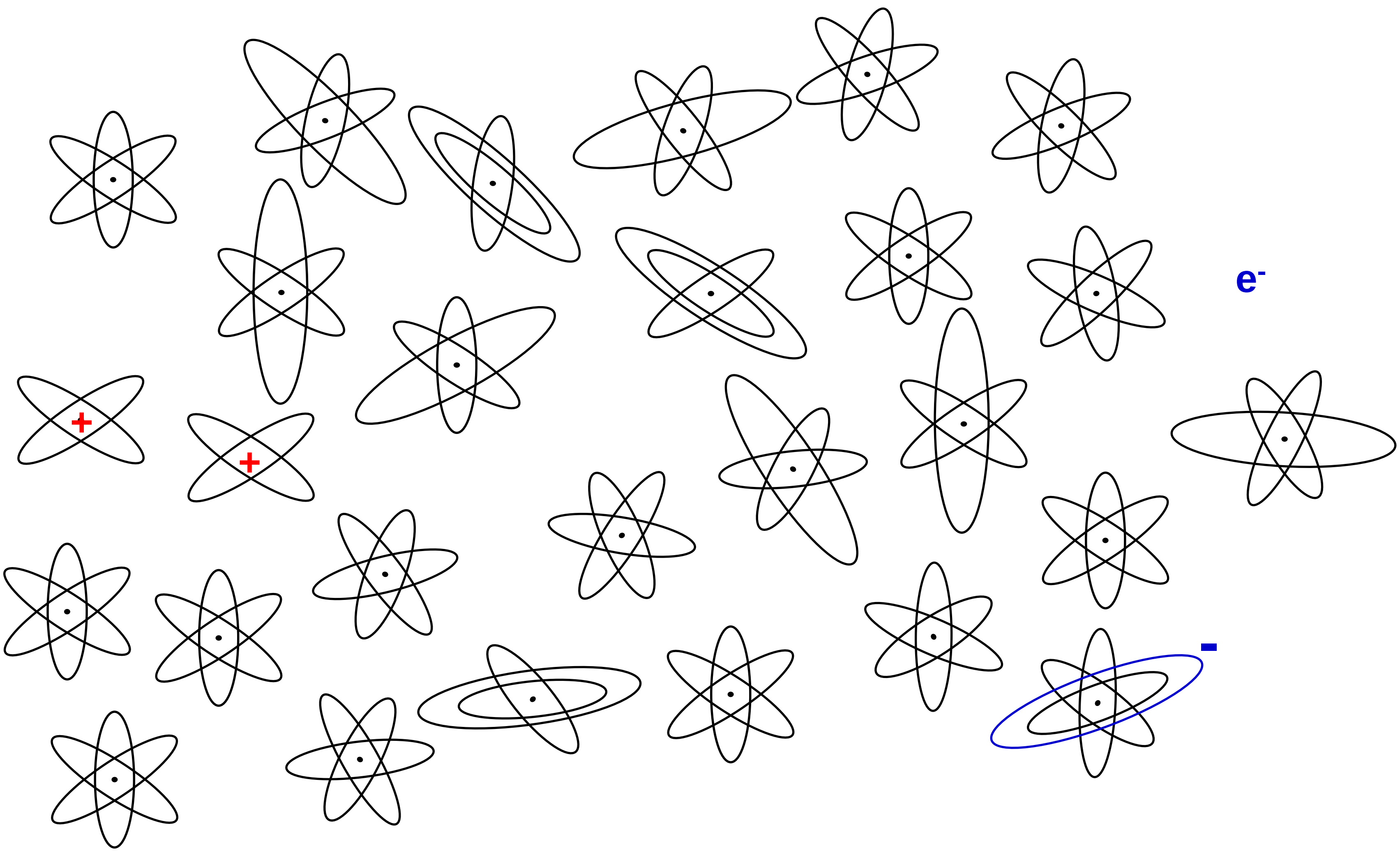
initial effects



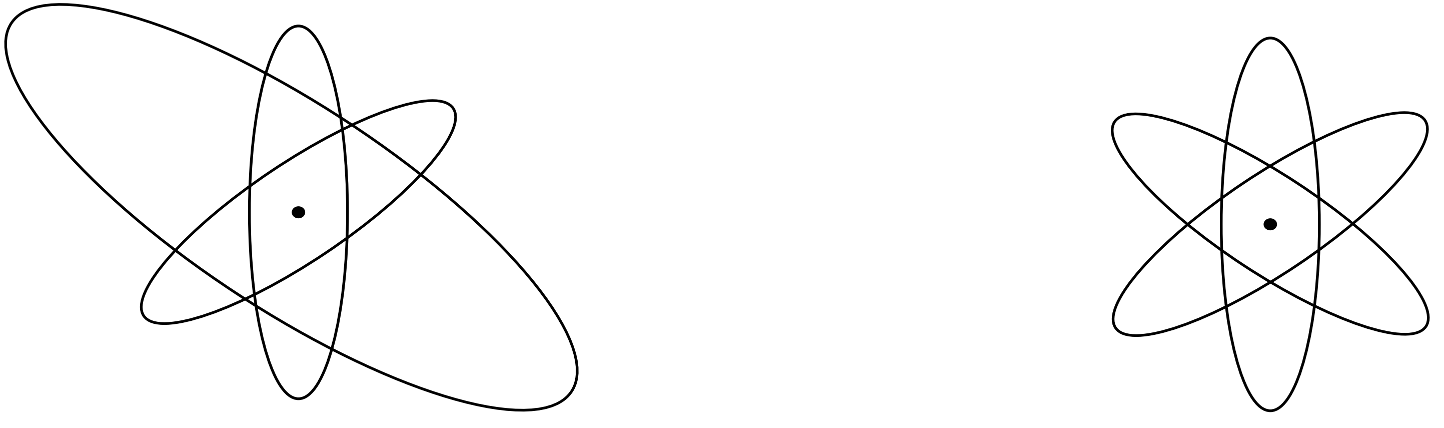
initial effects



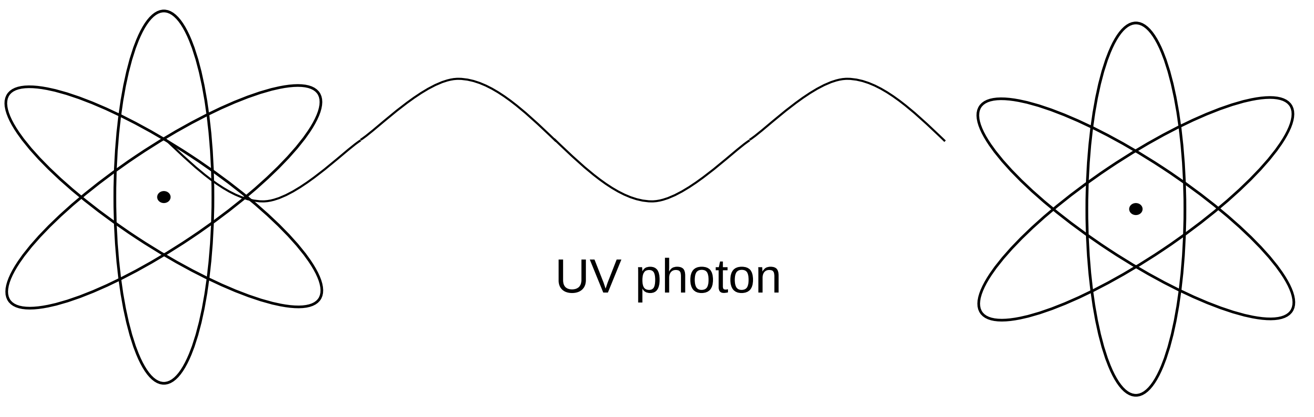
initial effects



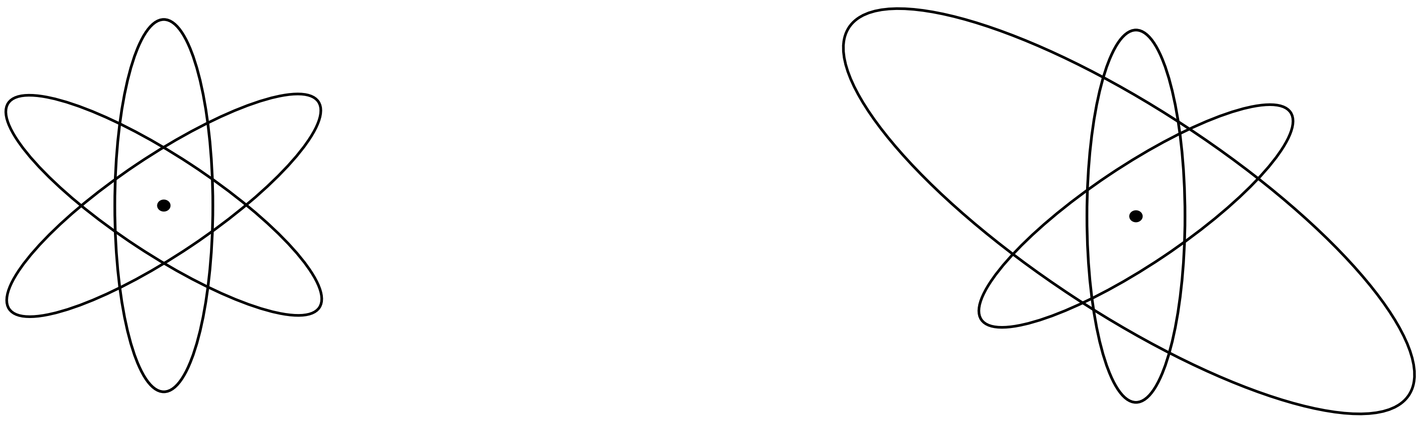
Excitation transfer



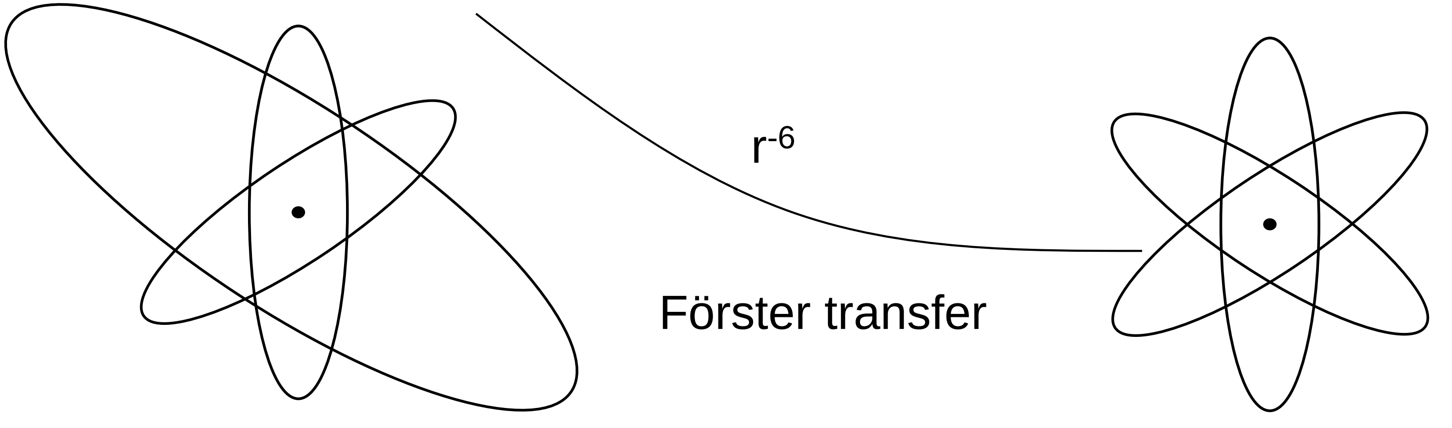
Excitation transfer



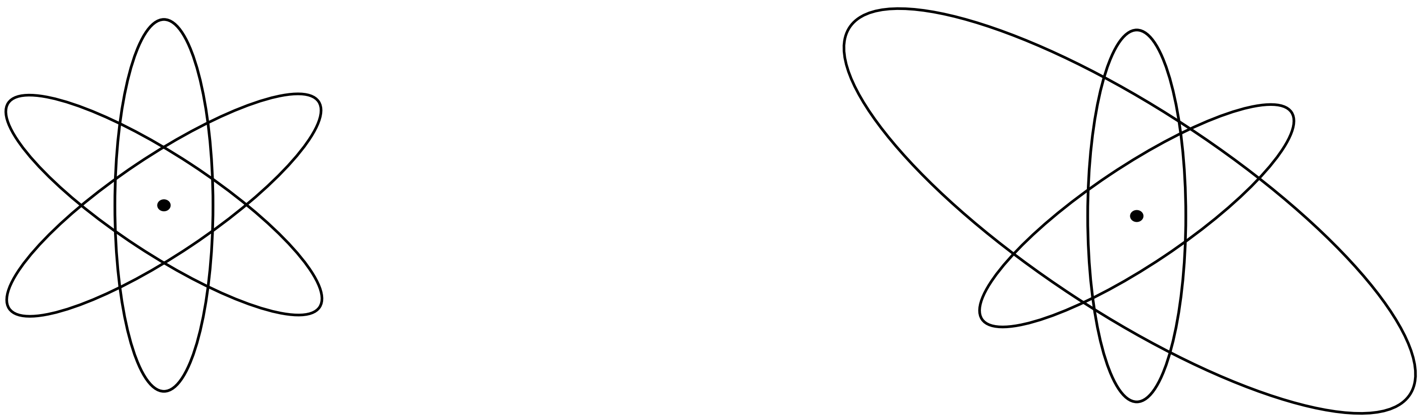
Excitation transfer



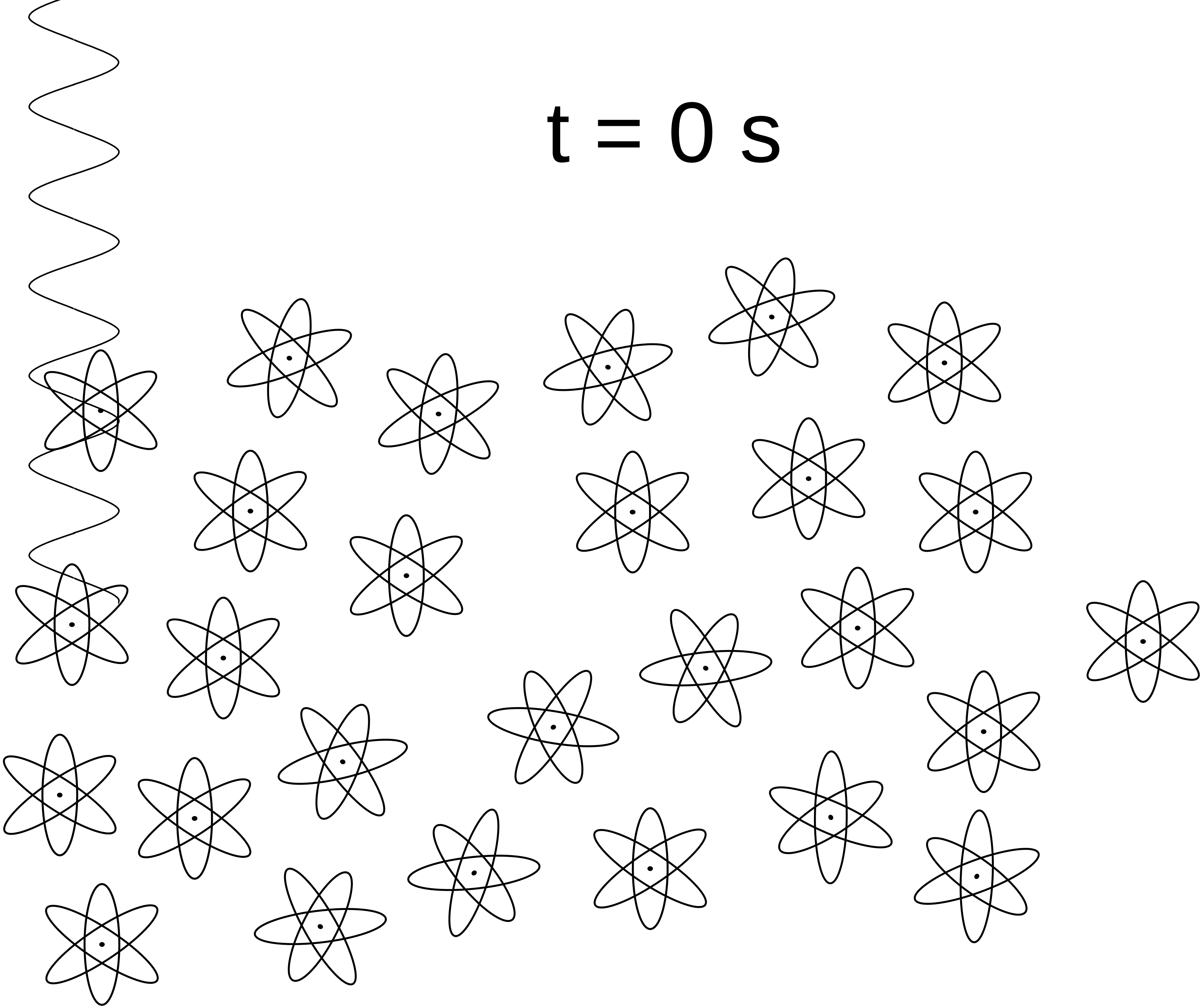
Excitation transfer



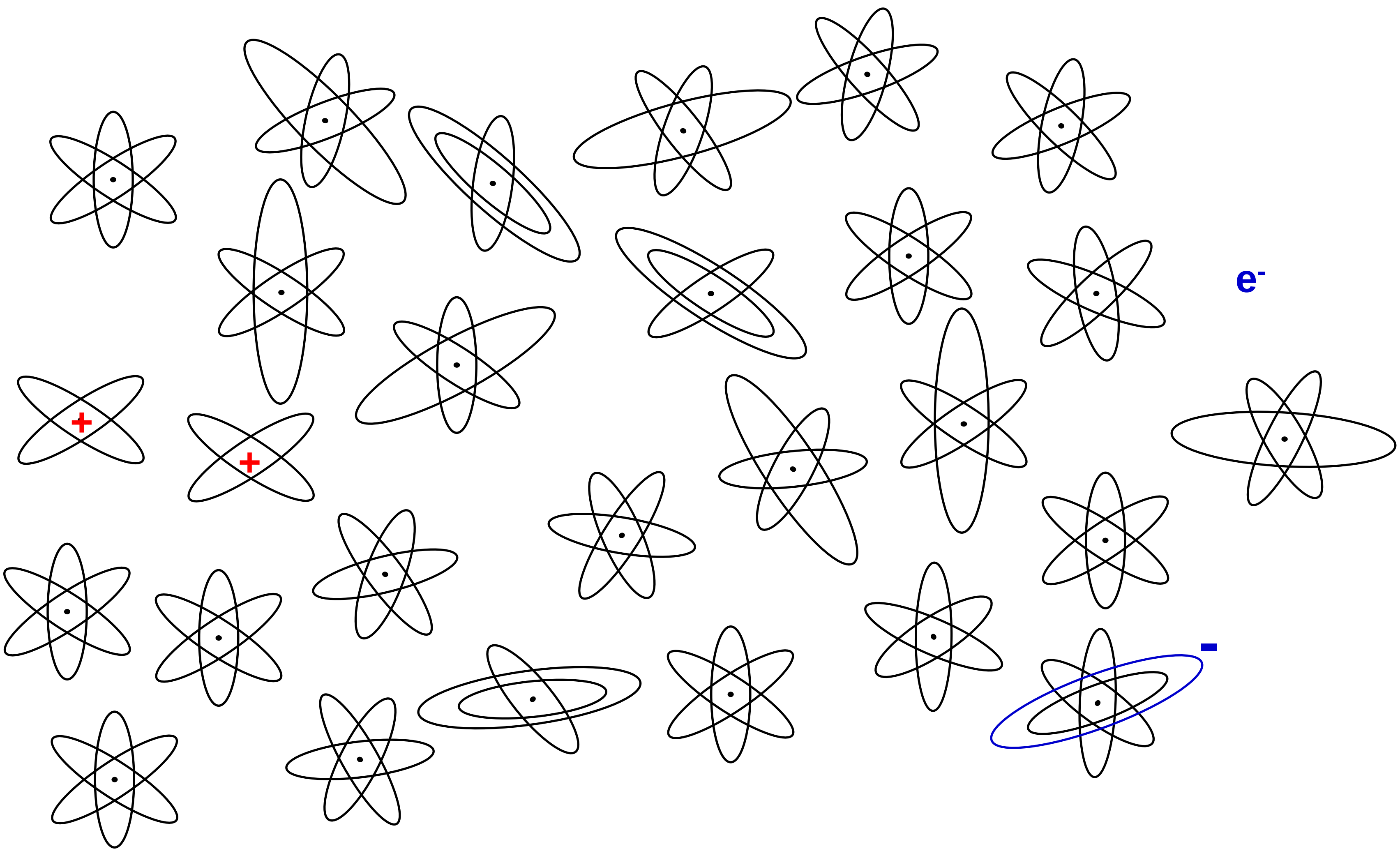
Excitation transfer



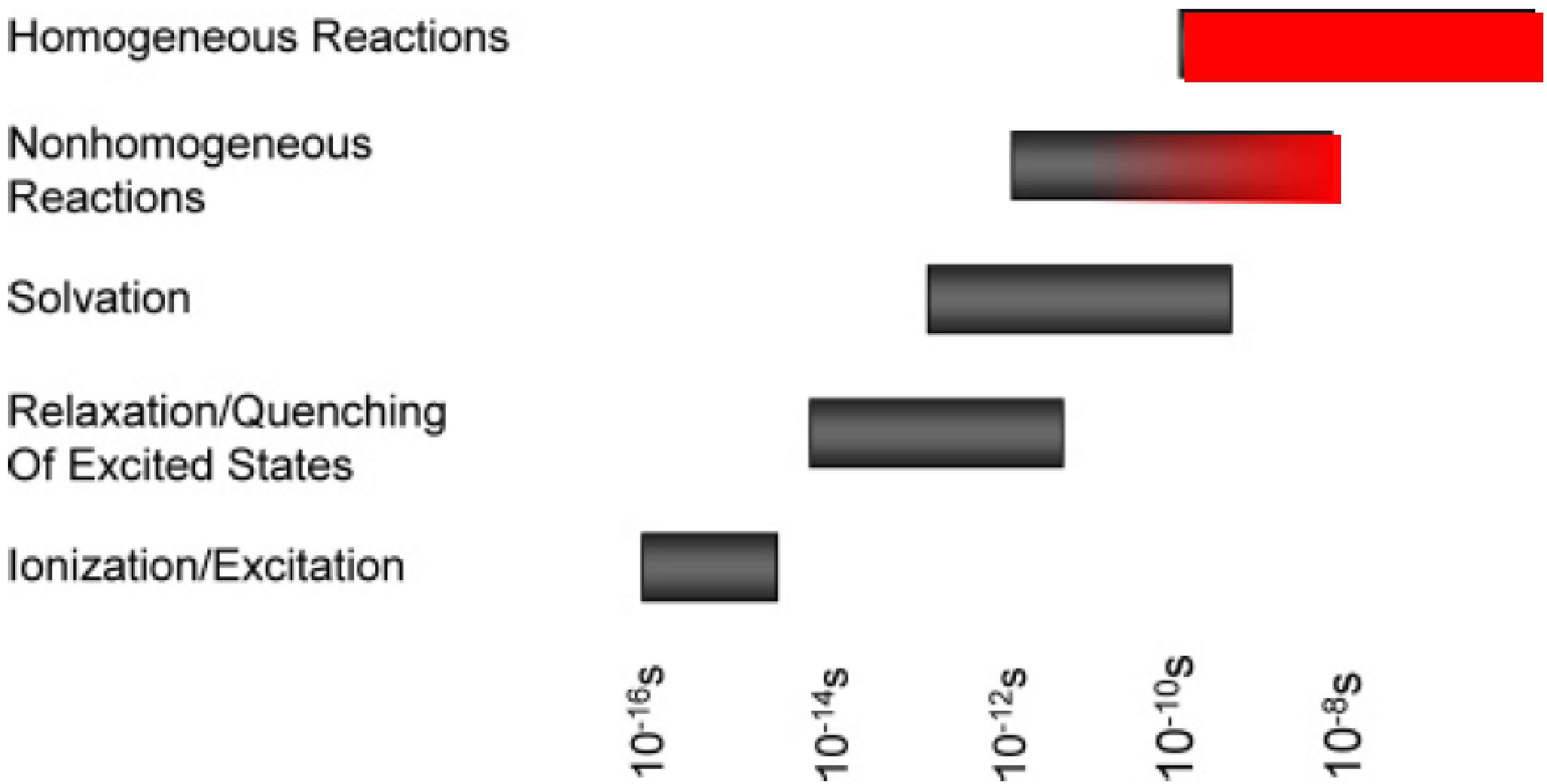
t = 0 s



$t \approx 10^{-14}$ s

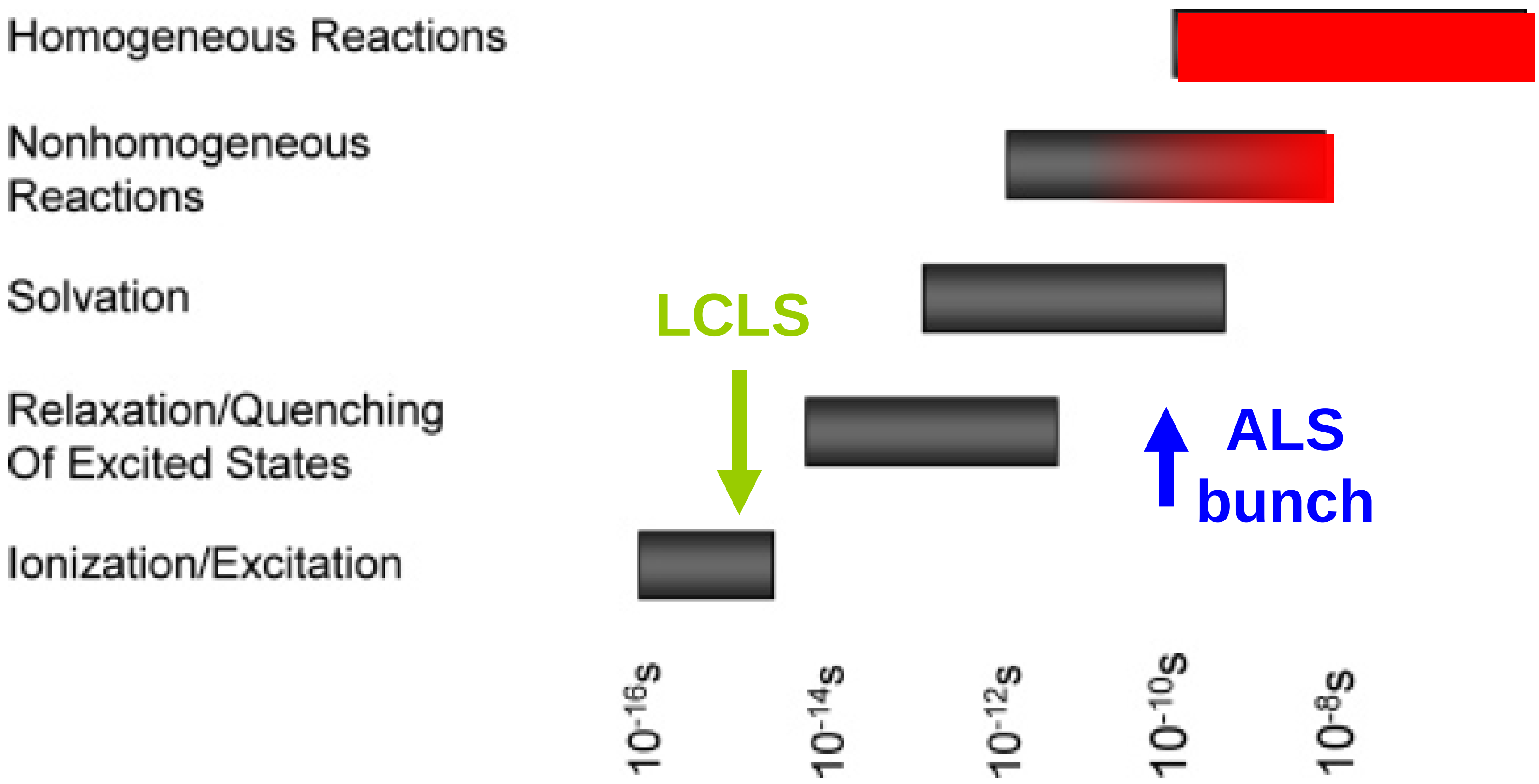


Timescales of radiation damage



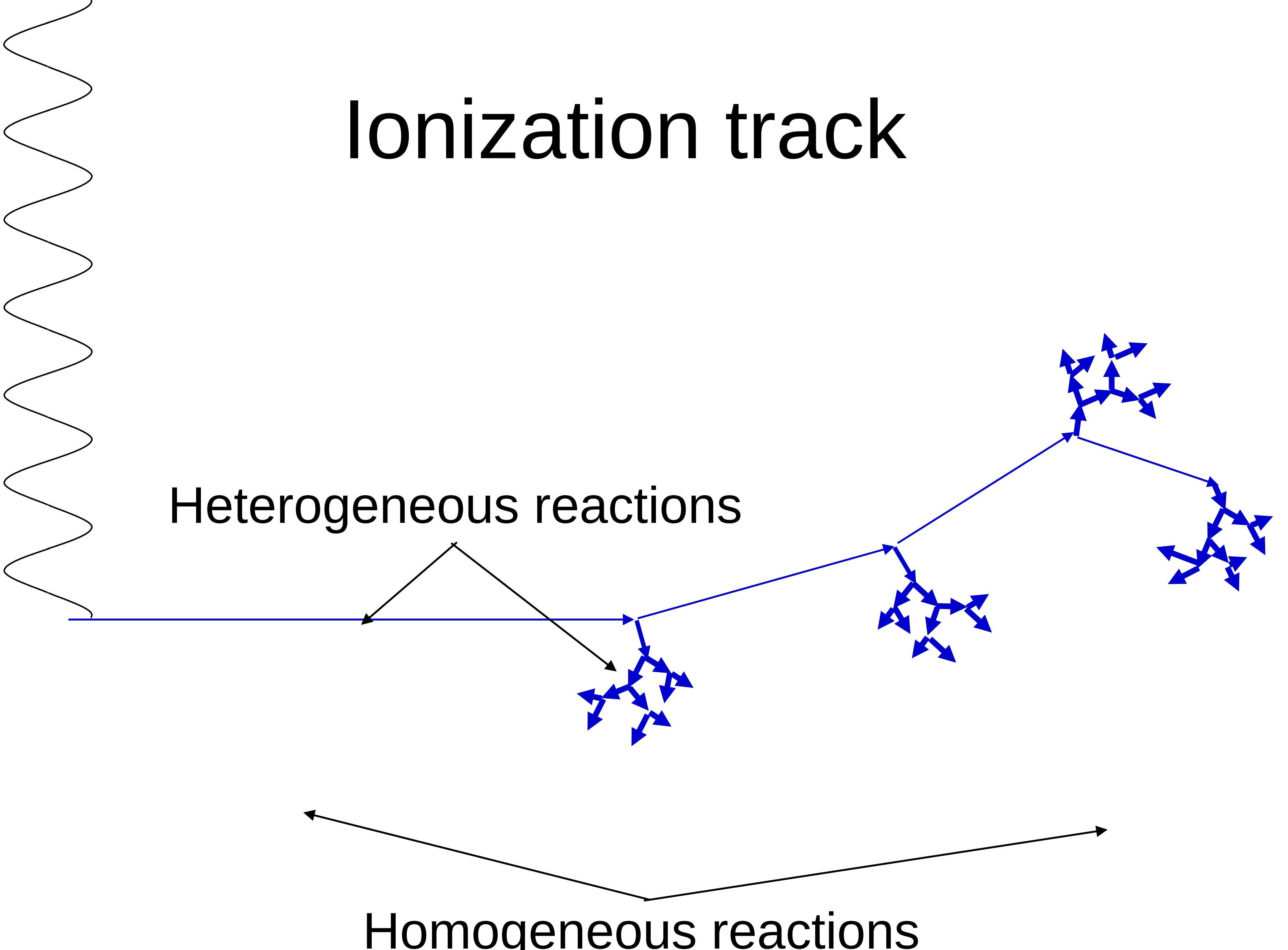
Garret et. al. (2005) *Chem. Rev*. **105**, 355-389

Timescales of radiation damage



Garret et. al. (2005) *Chem. Rev*. **105**, 355-389

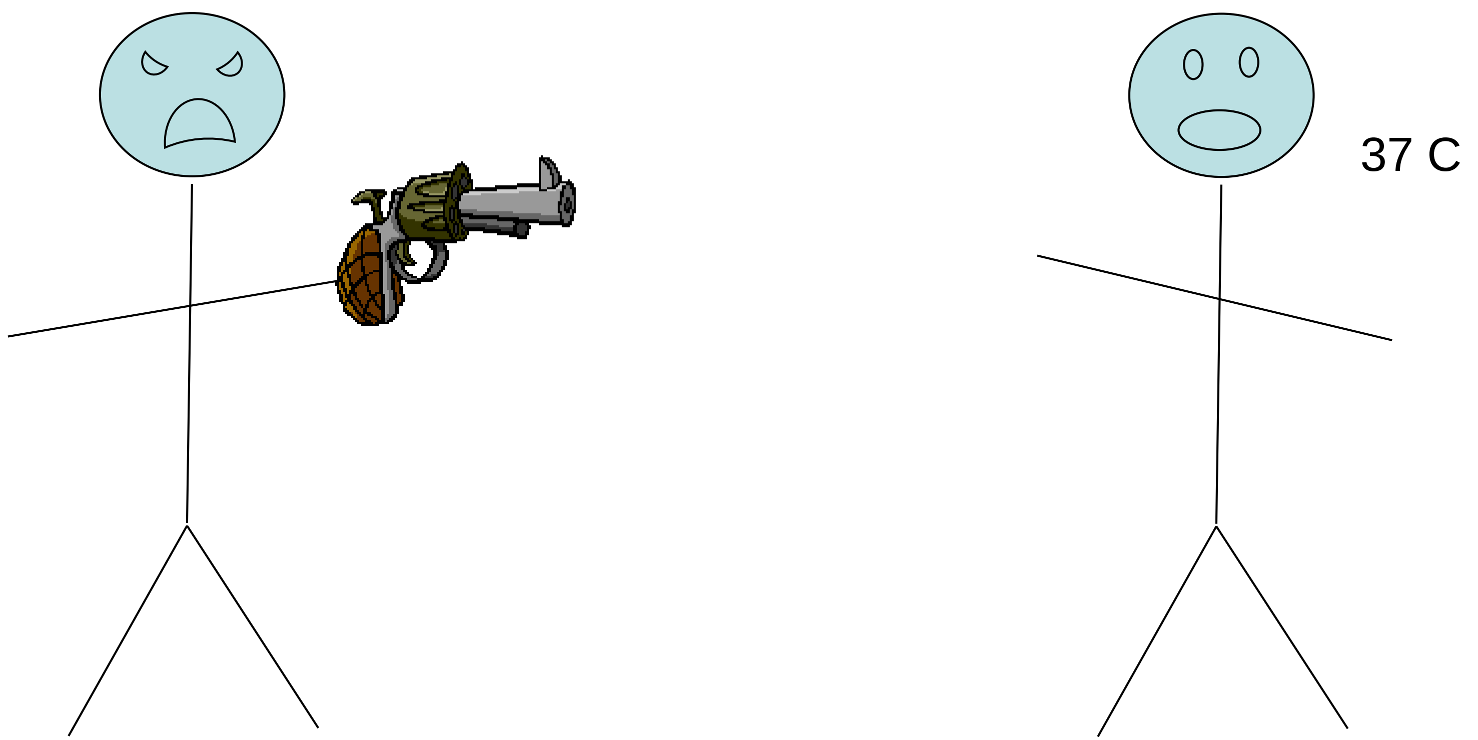
Ionization track



Rough values of energy quanta

1 MeV	100 GJ/mol	Medical radiation therapy
100 keV	10 GJ/mol	Medical imaging
10 keV	1 GJ/mol	X-ray crystallography
1 keV	100 MJ/mol	S and P K-edges
100 eV	10 MJ/mol	“water window”
10 eV	1 MJ/mol	C≡C bond
1 eV	100 kJ/mol	C-C bond, visible light
100 meV	10 kJ/mol	hydrogen bond
10 meV	1 kJ/mol	heat (~300 K)

Energy Transfer Analogy



Damage is done by dose (MGy)

proportional to photons/area

not time

not heat

Sliz P, Harrison SC & Rosenbaum G (2003). *Structure* **11**, 13-19.
Garman EF & McSweeney SM (2006). *J. Sync. Rad.* **14**, 1-3.
Owen RL, Rudino-Pinera E & Garman EF (2006). *PNAS* **103**, 4912-4917.
Leiros et al. (2006). *Acta Cryst. D* **62**, 125-132.
Holton JM (2007). *J. Synch Rad.* **14**, 51-72.

what the is a MGy?

$1\ MGy = 10^6\ J/kg$

http://bl831.als.lbl.gov/

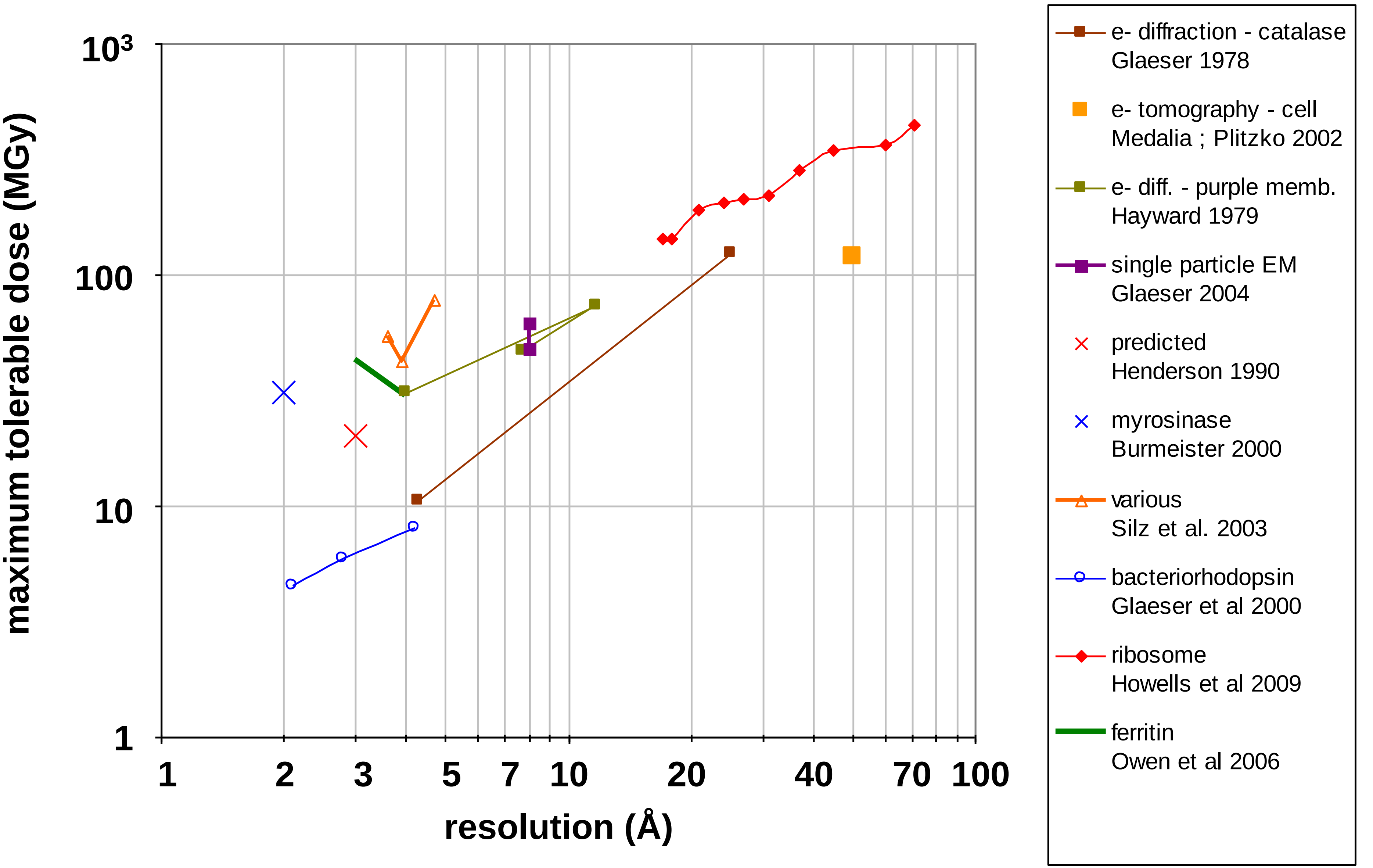
damage_rates.pdf

Holton J. M. (2009) *J. Synchrotron Rad.* **16** 133-42

How long will my crystal last?

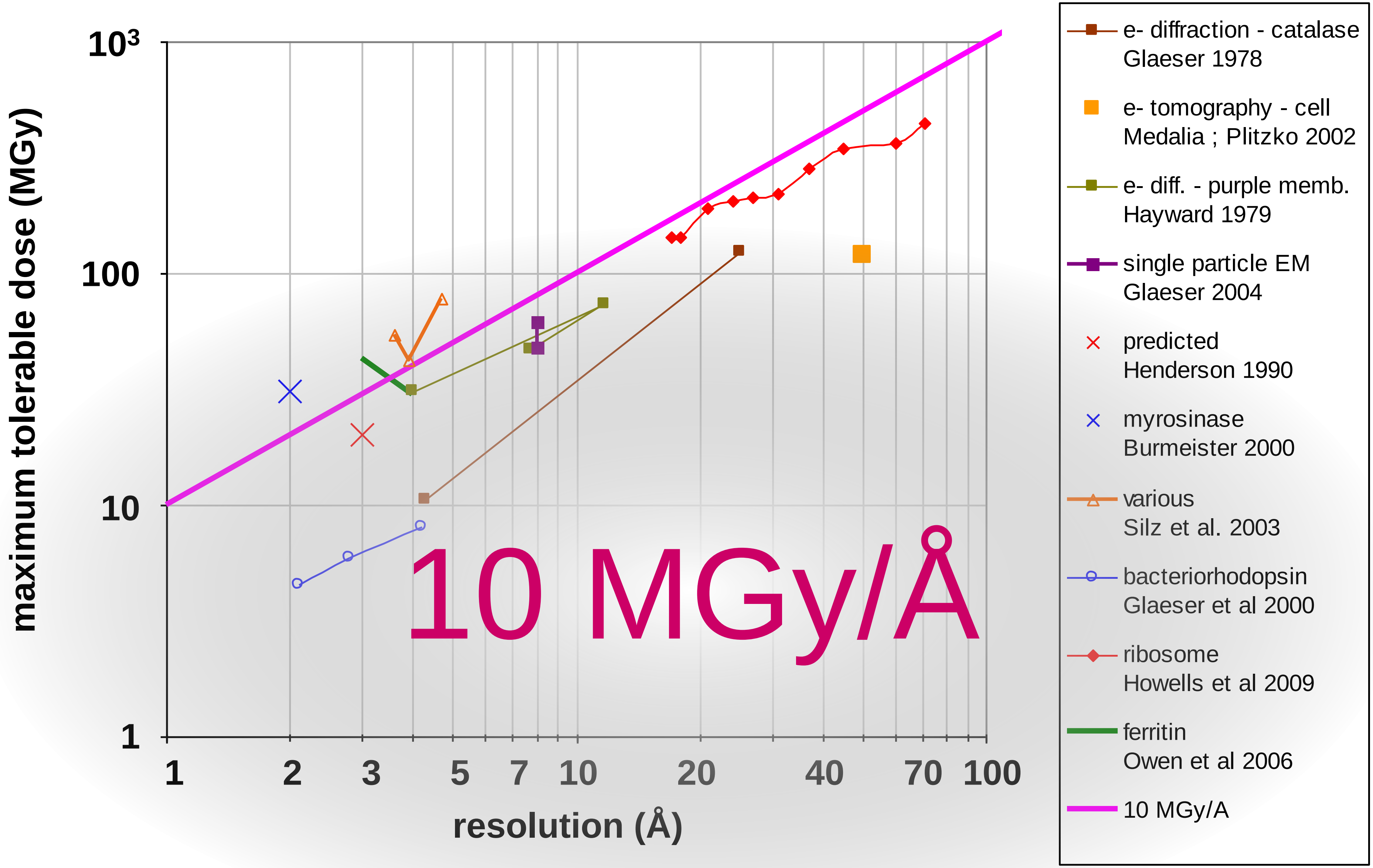
synch	line	type	flux ph/s	beamsize µm	flux density ph/µm2/s	dose rate	max xtal lifetime	min site lifetime
ALS	4.2.2	MAD	2.2e11	75x80	3.7e+07	27.3 kGy/s	18 m	73 s
ALS	5.0.1	mono	1.6e11	100	2.0e+07	10.4 kGy/s	48 m	3.2 m
ALS	5.0.2	MAD	8e11	100	1.0e+08	51.8 kGy/s	9.6 m	39 s
ALS	5.0.3	mono	1.7e11	100	2.2e+07	11 kGy/s	45 m	3 m
ALS	8.2.1	MAD	1.8e11	100	2.3e+07	11.7 kGy/s	43 m	2.9 m
ALS	8.2.2	MAD	2.3e11	100	2.9e+07	14.9 kGy/s	34 m	2.2 m
ALS	8.3.1	MAD	9e11	70	2.3e+08	119 kGy/s	4.2 m	17 s
ALS	8.3.1	typical	6e11	70	1.6e+08	115 kGy/s	4.3 m	17 s
ALS	12.3.1	MAD	1.8e11	100	2.3e+07	11.7 kGy/s	43 m	2.9 m
ALS	12.3.1	ML	4.0e12	100	5.1e+08	513 kGy/s	58 s	3.9 s
APS	8-BM	MAD	1e11	200	2.5e+06	1.27 kGy/s	6.6 h	26 m
APS	14-BM-C	mono	5.8e10	200	1.4e+06	738 Gy/s	11 h	45 m
APS	14-BM-D	MAD	3.3e9	200	8.2e+04	42 Gy/s	8.3 d	13 h
APS	14-ID-B	MAD	6.0e10	200	1.5e+06	763 Gy/s	11 h	44 m
APS	17-BM	MAD	1.1e11	200	2.8e+06	1.4 kGy/s	6 h	24 m
APS	17-ID	MAD	2.3e11	200	5.8e+06	2.93 kGy/s	2.8 h	11 m
APS	19-BM	MAD	2.0e11	70x60	4.8e+07	24.2 kGy/s	21 m	83 s
APS	19-ID	MAD	1.3e13	80x40	4.1e+09	2.07 MGy/s	15 s	0.97 s
APS	19-ID	typical	5.5e11	100x100	5.5e+07	28 kGy/s	18 m	71 s
APS	22-BM	MAD	7e12	80x40	2.2e+09	1.23 MGy/s	24 s	1.6 s
APS	22-ID	MAD	7e12	80x40	2.2e+09	1.23 MGy/s	24 s	1.6 s
APS	22-ID	typical	1.5e12	80	2.3e+08	119 kGy/s	4.2 m	17 s
APS	23-ID-B	MAD	1e13	75x25	5.3e+09	3.01 MGy/s	10 s	0.66 s
APS	23-ID	typical	1.5e12	80	2.3e+08	119 kGy/s	4.2 m	17 s
APS	24-ID-C	MAD	1.3e13	20x60	1.1e+10	5.23 MGy/s	5.7 s	0.38 s
APS	24-ID-E	MAD	0.5e13	20x100	2.5e+09	1.19 MGy/s	25 s	1.7 s
APS	31-ID	MAD	2e12	70	4.1e+08	194 kGy/s	2.6 m	10 s

resolution dependence of global damage



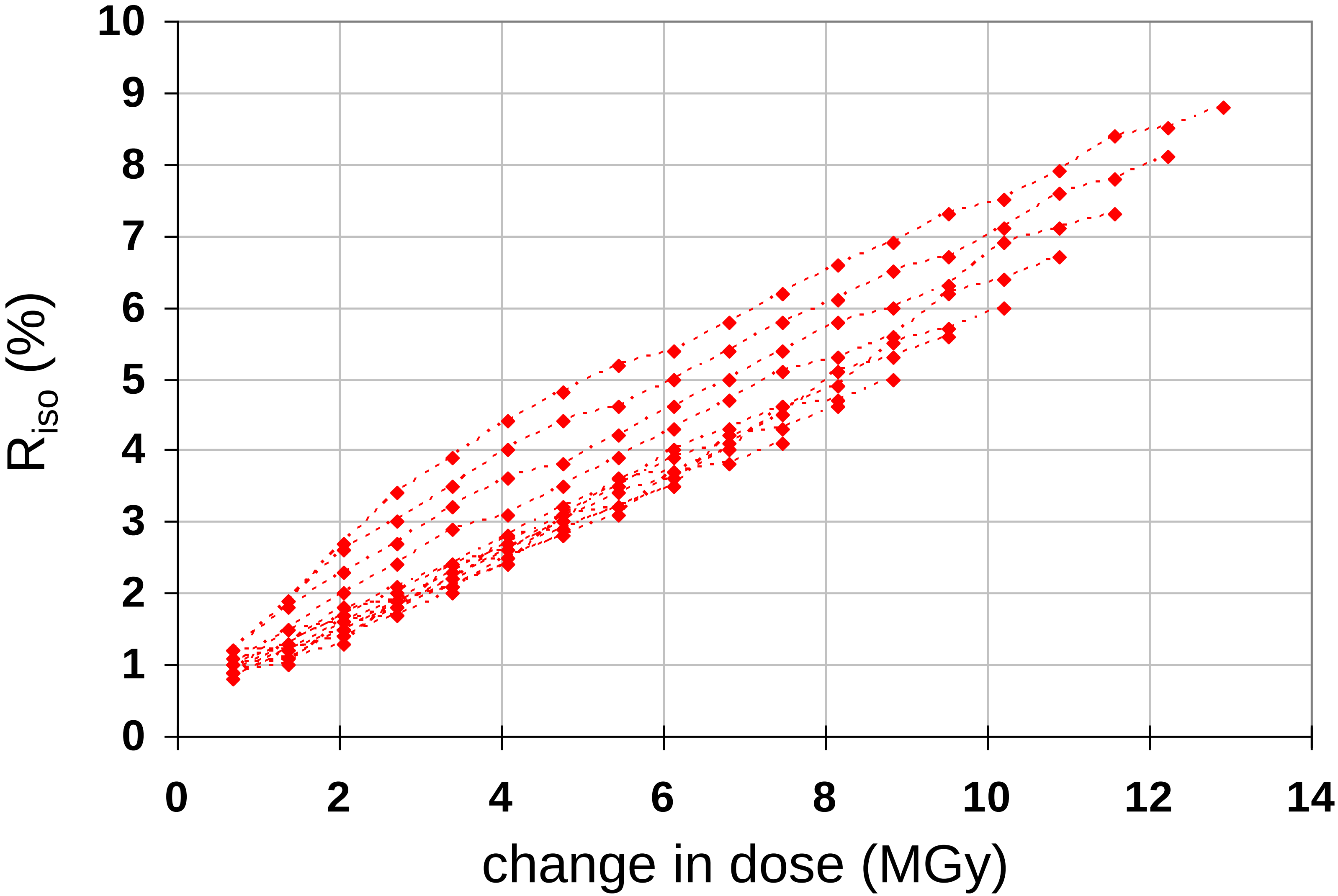
Howells *et al.* (2009) *J. Electron. Spectrosc. Relat. Phenom.* **170** 4-12

resolution dependence of global damage



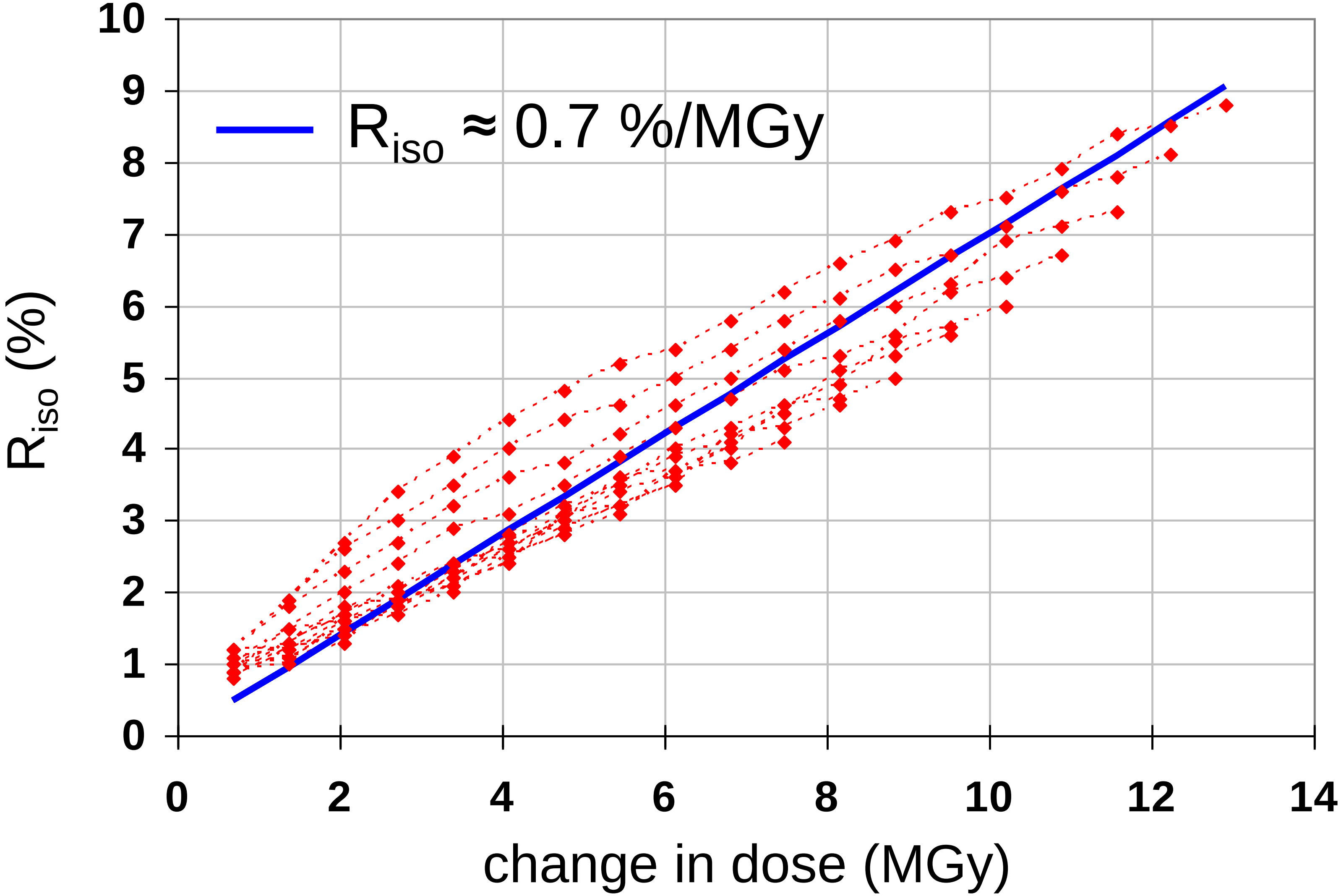
Howells *et al.* (2009) *J. Electron. Spectrosc. Relat. Phenom.* **170** 4-12

R_{iso} vs dose



data taken from Banumathi, *et al.* (2004) *Acta Cryst. D* **60**, 1085-1093.

R_{iso} vs dose



data taken from Banumathi, *et al.* (2004) *Acta Cryst. D* **60**, 1085-1093.

Dose-doubling concentration

molar, at the Se edge based on RADDOSE

Na	44
Mg	27
P	9
S	7
Cl	5
K	3.3
Ca	2.7
Fe	1.0
Cu	0.67
Zn	0.58

As	0.44
Se	0.42
Br	2.9
I	0.47
Gd	0.21
Ta	0.13
Pt	0.17
Au	0.16
Hg	0.16
U	0.23

Dose-doubling concentration

molar, at the Se edge based on RADDOSE

Na	44	As	0.44
Mg	27	Se	0.42
P	9	Br	2.9
S	7	I	0.47
Cl	5	Gd	0.21
K	3.3	Ta	0.13
Ca	2.7	Pt	0.17
Fe	1.0	Au	0.16
Cu	0.67	Hg	0.16
Zn	0.58	U	0.23

Specific Damage World Records

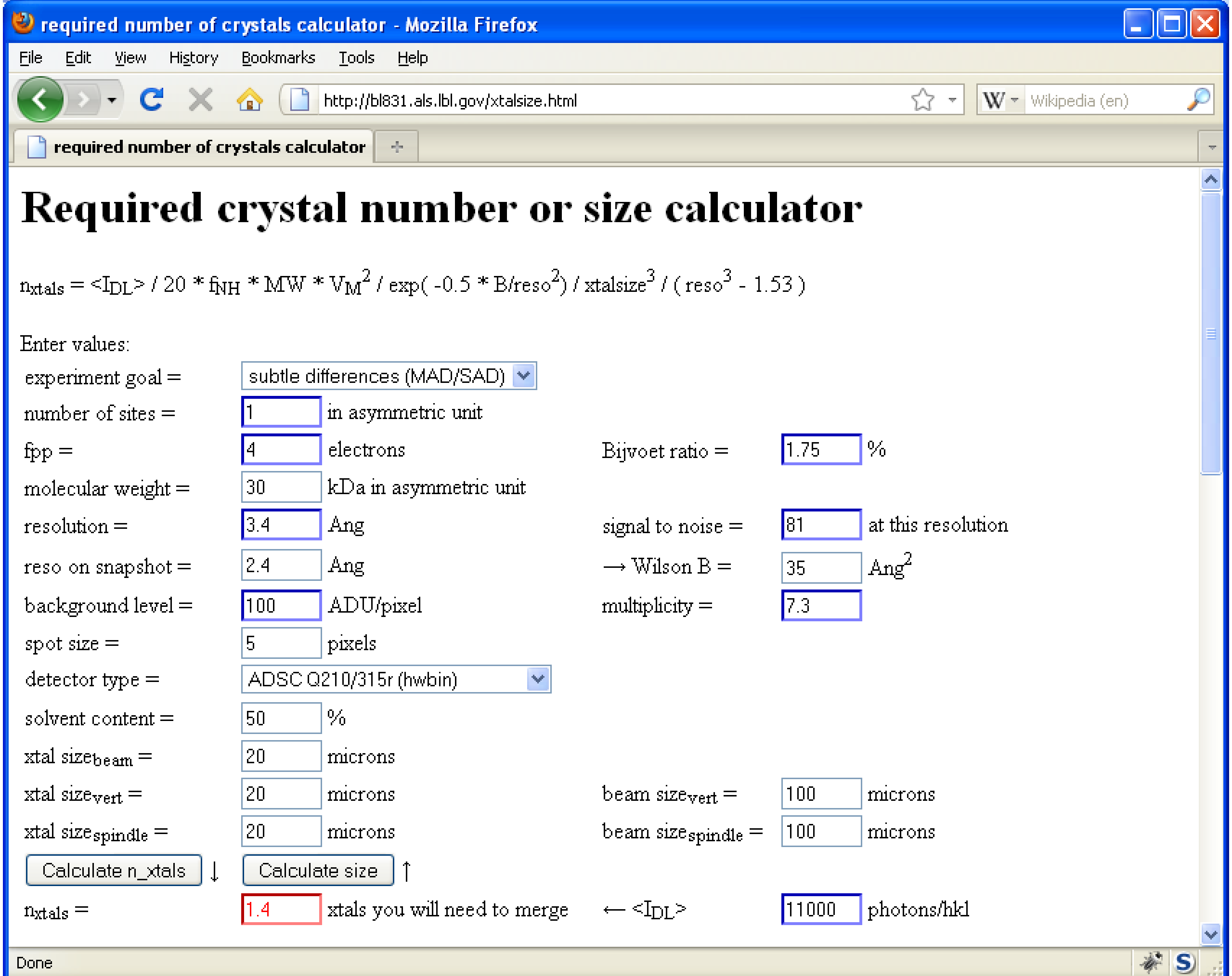
MGy	reaction	reference
~45	global damage	Owen *et al.* (2006)
5	Se-Met	Holton (2007)
4	Hg-S	Ramagopal *et al.* (2004)
3	S-S	Murray *et al.* (2002)
1	Br-RNA	Olieric *et al.* (2007)
?	Cl-C	???
0.5	Mn in PS II	Yano *et al.* (2005)
0.02	Fe in myoglobin	Denisov *et al.* (2007)

Holton (2009) *J. Synchrotron Rad.* **16** 133-42 expected crystal lifetime calcul...

bl831.als.lbl.gov/xtallife.html

expected crystal lifetime calculator

source =	APS		22-ID		
full flux =	7.0e+12	photons/s			
attenuation =	0	%	transmittance =	100	%
beam size$_{horiz}$ =	40.0	microns	beam size$_{vert}$ =	80.0	microns
wavelength =	1	Ang	k_{dose} =	2000	photons/micron^2/Gy
dose rate =	1.1e+6	Gy/s			
experiment goal =	high resolution (cryo)				
resolution =	3	Ang			
dose limit =	30	MGy			
exposure time =	1	seconds/image			
xtal size$_{horiz}$ =	50	microns	xtal size$_{vert}$ =	50	microns
translation during dataset =	0	microns	rotisserie factor	1	disable warnings
max images =	28	at damage limit			
inverse beam =	no				
number of wavelengths =	1				
images/wedge =	28				



Holton & Frankel (2010) *Acta D* **66** 393-408.

The number of photons you get before a given crystal dies

is **independent** of data collection time

1 $um^3 = 10^6$ photons

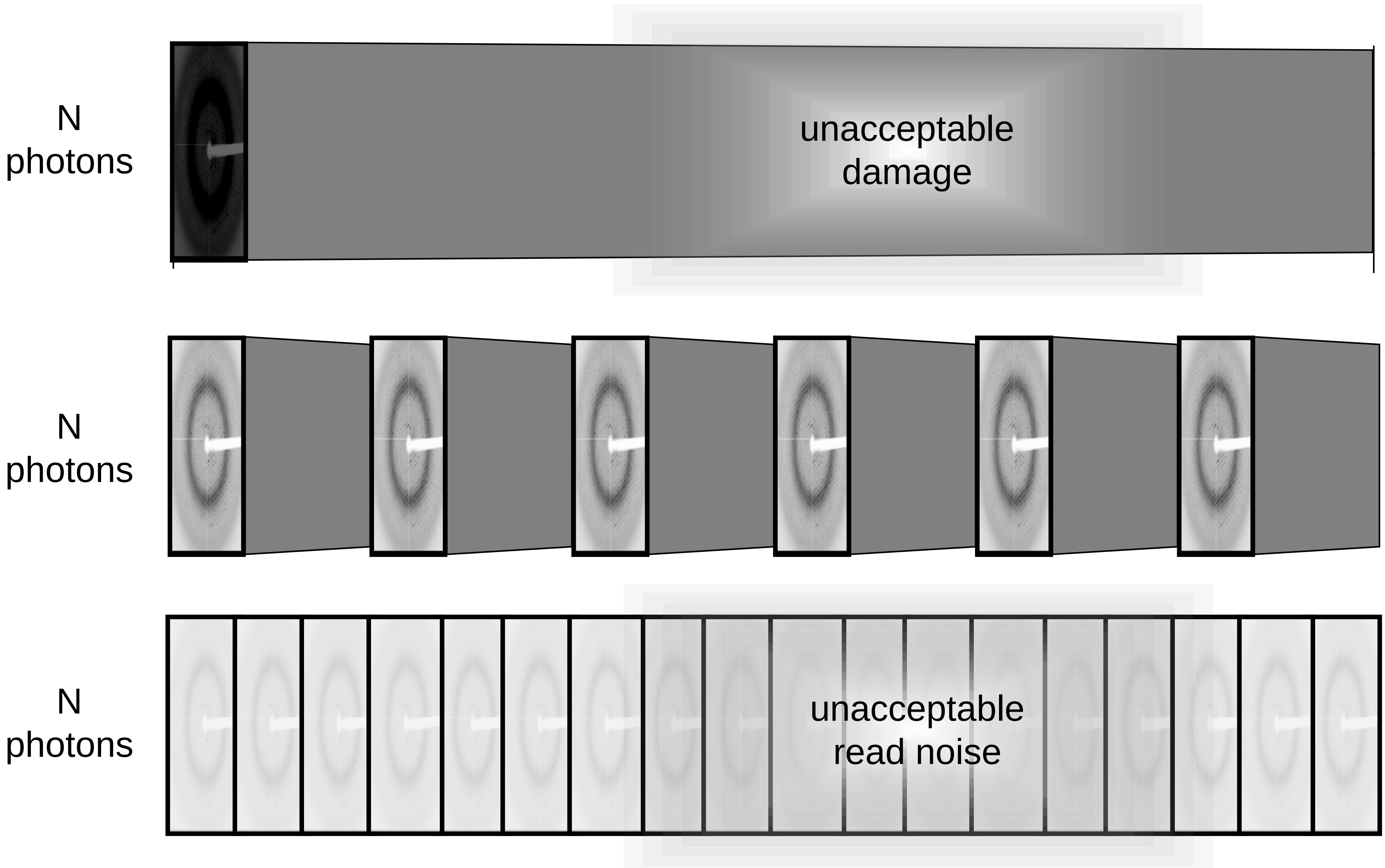
http://www.nist.gov/pml/data/xcom/

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

Dose slicing



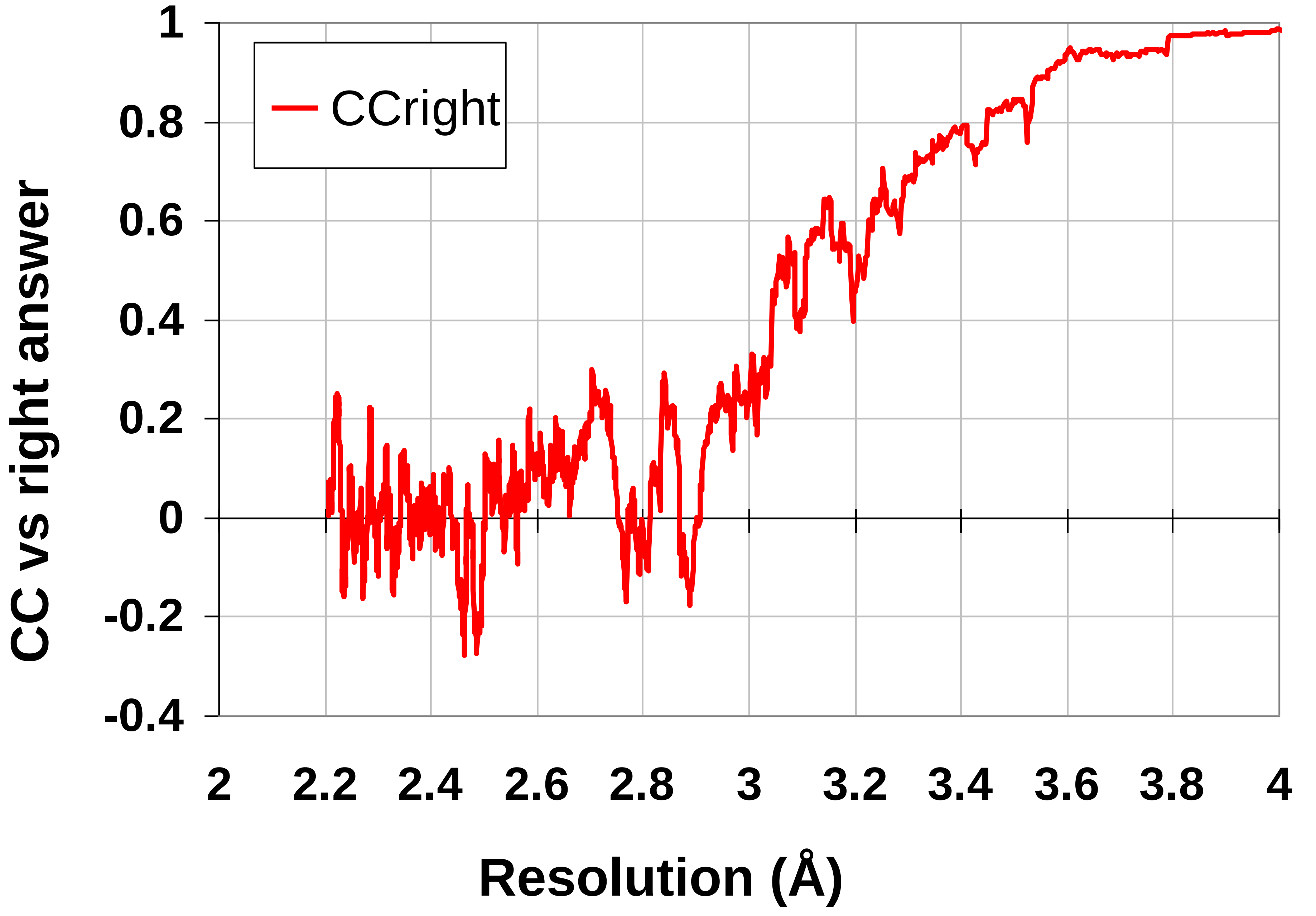
What if…

- You only have a few small crystals?

  Should you:

  a) Collect 360° from each?
  b) Collect 10° from each at 36x exposure?
  c) Glue 36 xtals together, then collect 360° ?
  d) Glue together and do 12960° ?

“true” resolution limit



“true” resolution vs strategy

processing procedure	Dose slice collection scenario			
	fine	coarse	glue	gluefine
XDS/XSCALE	3.03874	3.02970	3.0270	3.02948
XDS/aimless	3.13351	3.03531	3.02638	3.05292
XDS/noscale	3.01449	3.03048	3.02576	3.31013

301,640,334 photons

Self-calibrated damage limit

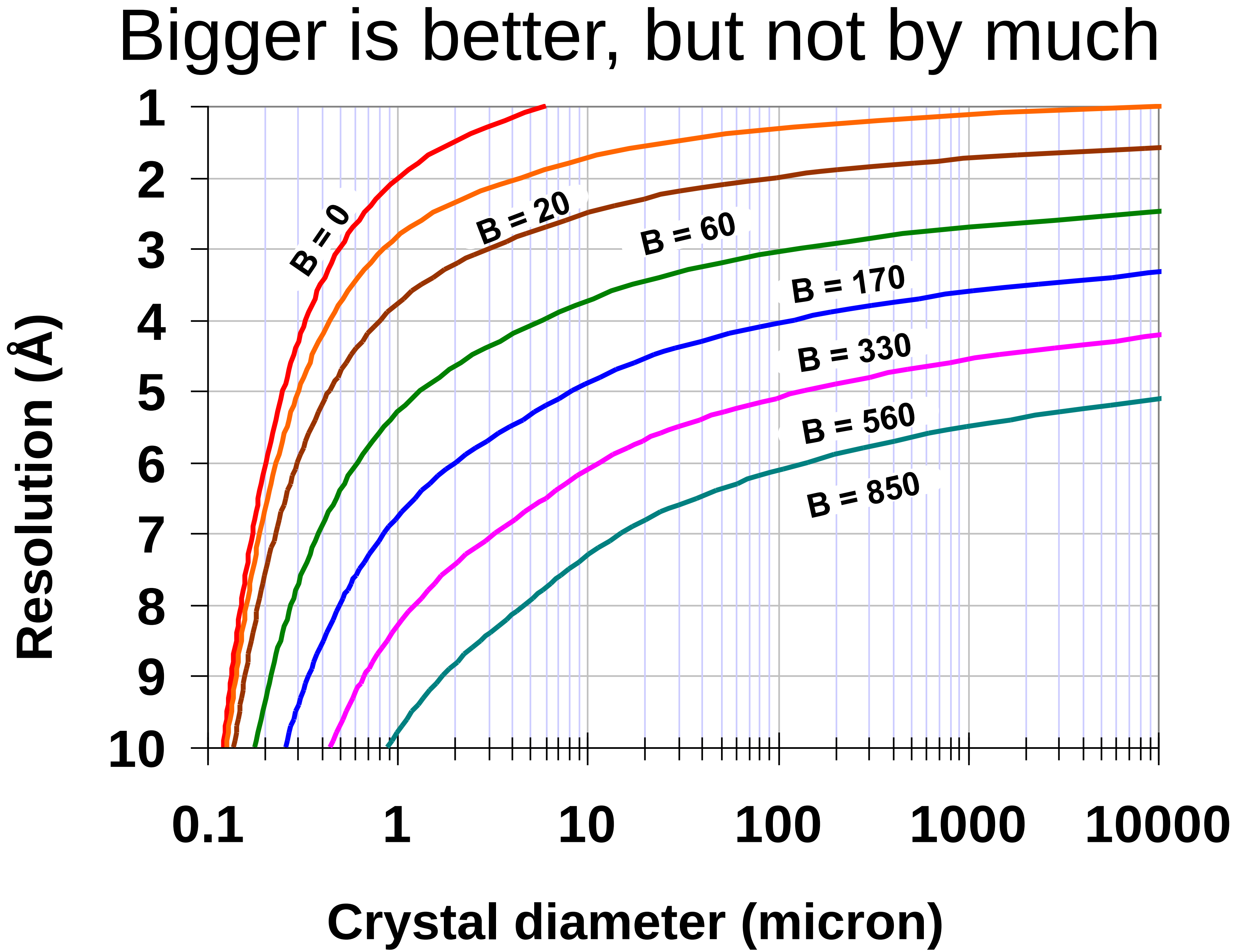
$$\langle I \rangle_{DL} = \frac{2\pi}{9}\frac{10^5 r_e^{\,2}}{hc}\frac{f_{decayed}\,\rho R^4 \lambda^4}{f_{NH}\, n_{ASU} M_r V_M^{\,2}}\frac{0.5\lambda H}{\ln(2)\sin\theta}\frac{T_{sphere}(\,2\theta\,,\mu,R)}{\left(1-T_{sphere}(0,\mu_{en},R)\right)}\frac{(3+\cos 4\theta)}{\sin\theta}\frac{\langle f_a^{\,2}\rangle}{\langle M_a\rangle}\exp\left(-2B\left(\frac{\sin\theta}{\lambda}\right)^2\right)$$

Where:

- $\langle I \rangle_{DL}$ - average damage-limited intensity (photons/hkl) at a given resolution
- 10^5 - converting R from μm to m, r_e from m to Å, ρ from g/cm³ to kg/m³ and MGy to Gy
- r_e - classical electron radius (2.818×10^{-15} m/electron)
- h - Planck's constant (6.626×10^{-34} J·s)
- c - speed of light (299792458 m/s)
- $f_{decayed}$ - fractional progress toward completely faded spots at end of data set
- ρ - density of crystal (~1.2 g/cm³)
- R - radius of the spherical crystal (μm)
- λ - X-ray wavelength (Å)
- f_{NH} - the Nave & Hill (2005) dose capture fraction (1 for large crystals)
- n_{ASU} - number of proteins in the asymmetric unit
- M_r - molecular weight of the protein (Daltons or g/mol)
- V_M - Matthews's coefficient (~2.4 Å³/Dalton)
- H - Howells's criterion (10 MGy/Å)
- θ - Bragg angle
- $\langle f_a^{\,2}\rangle$ - number-averaged squared structure factor per protein atom (electron²)
- $\langle M_a\rangle$ - number-averaged atomic weight of a protein atom (~7.1 Daltons)
- B - average (Wilson) temperature factor (Å²)
- μ - attenuation coefficient of sphere material (m⁻¹)
- μ_{en} - mass energy-absorption coefficient of sphere material (m⁻¹)

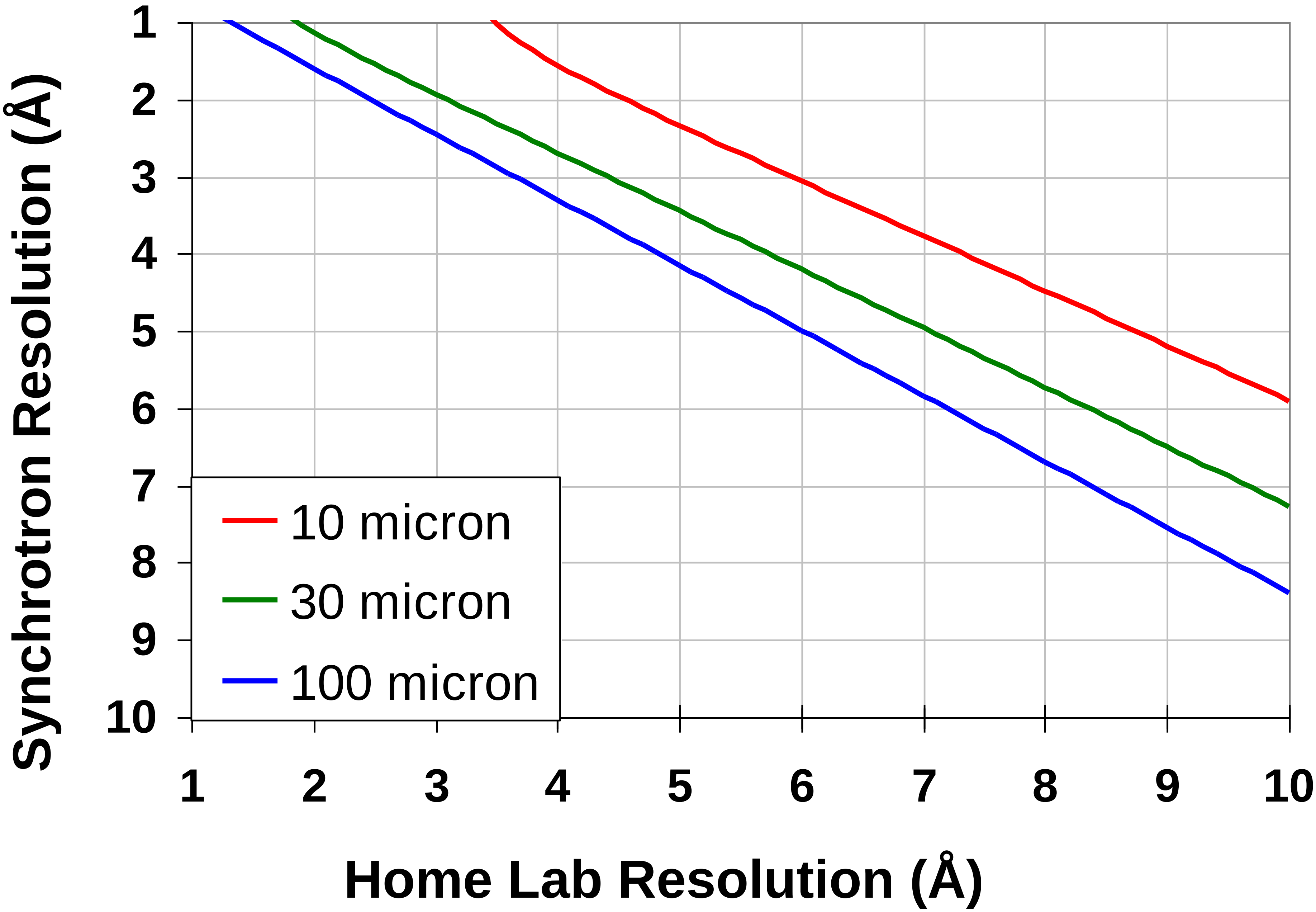
No flux
No symmetry

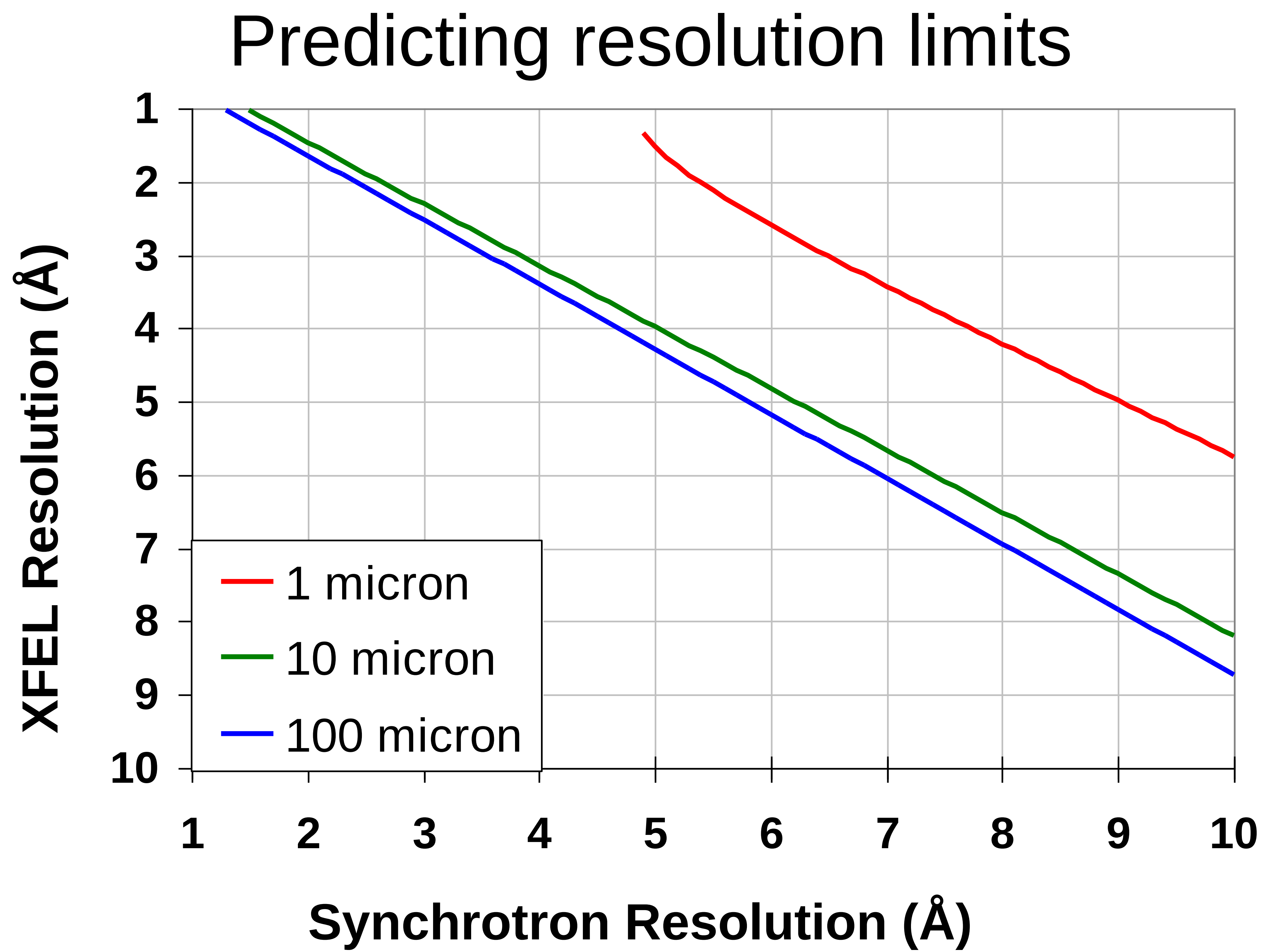
Holton & Frankel (2010) *Acta D* **66** 393-408.



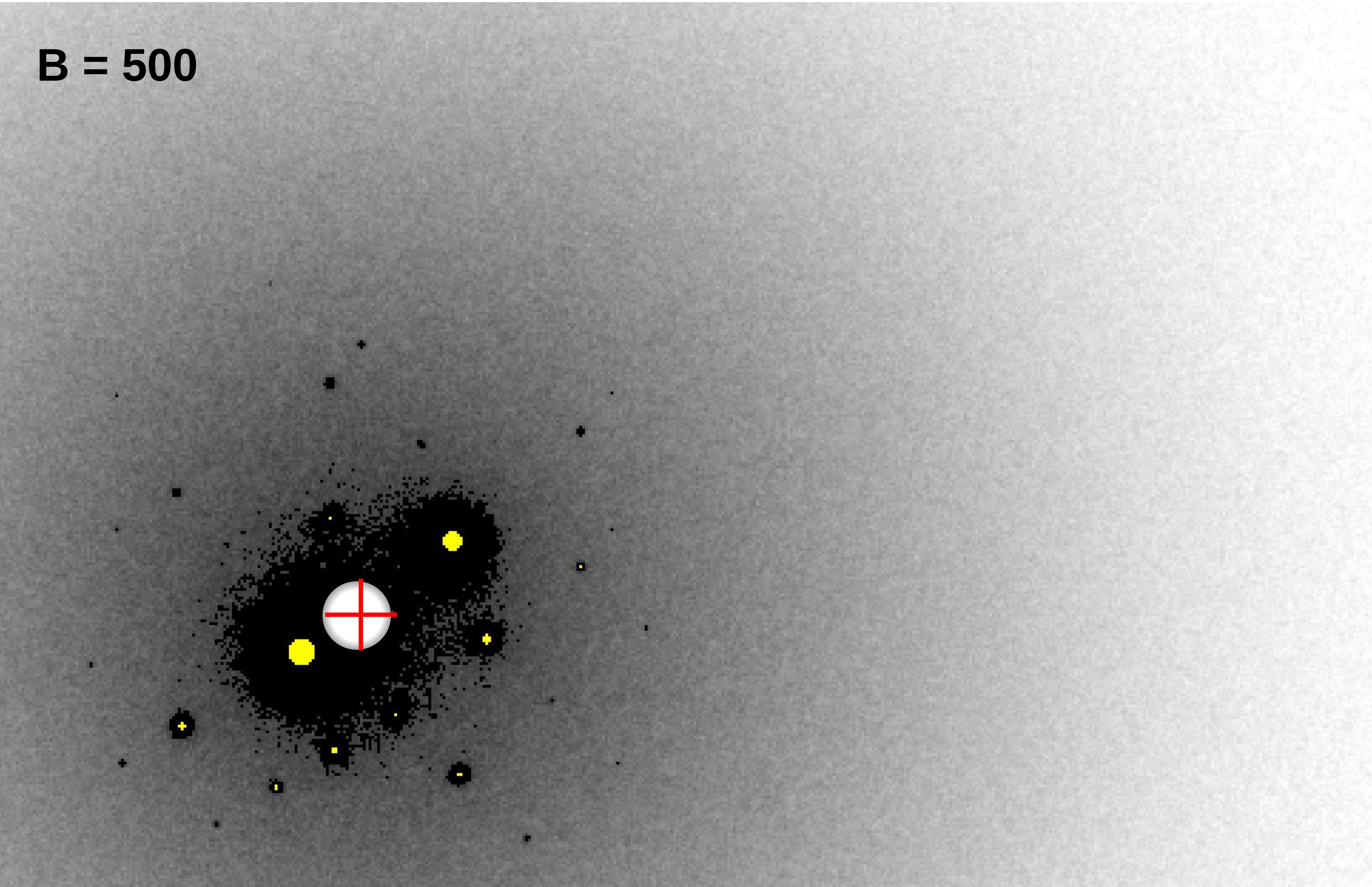
Bigger is better, but not by much
Resolution (Å)
1
2
3
4
5
6
7
8
9
10
0.1
1
10
100
1000
10000
Crystal diameter (micron)
B = 0
B = 20
B = 60
B = 170
B = 330
B = 560
B = 850

Predicting resolution limits

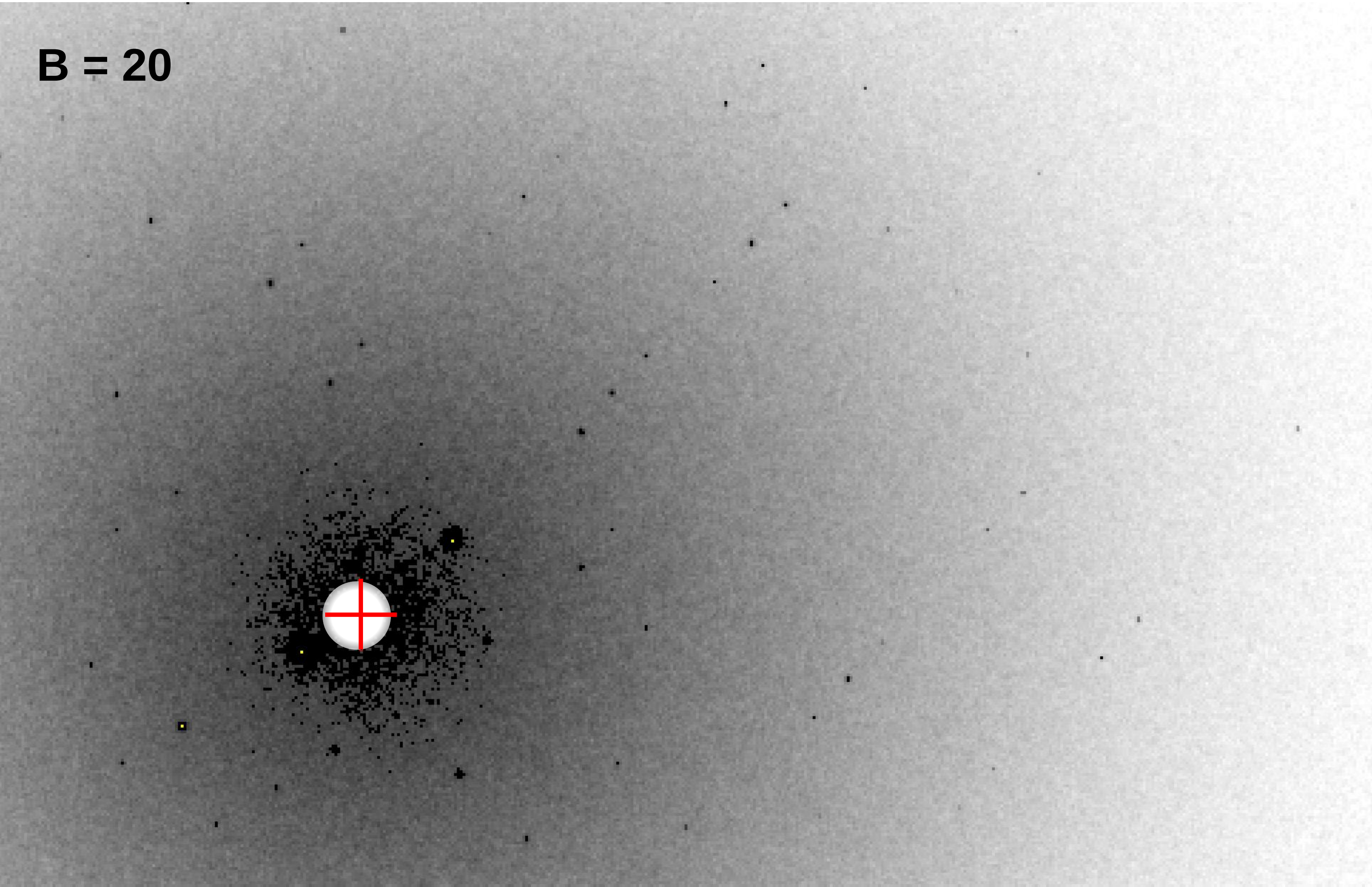



Predicting resolution limits
XFEL Resolution (Å)
Synchrotron Resolution (Å)
1
2
3
4
5
6
7
8
9
10
1 micron
10 micron
100 micron

B factor from image analysis



B factor from image analysis



At the beamline…

- Resolution

  problem: background

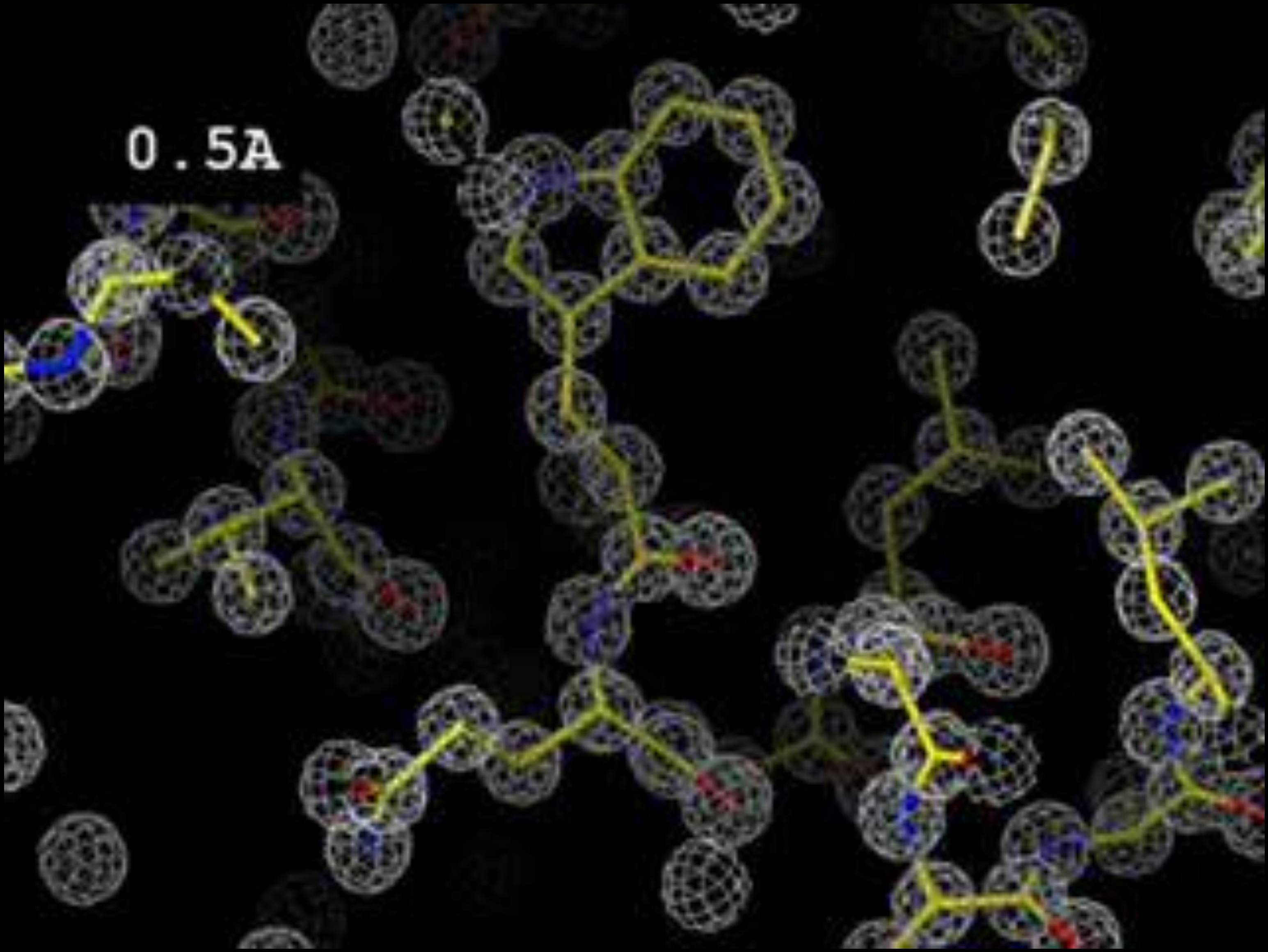
  solution: use as few pixels as possible

- Phases

  problem: fractional errors

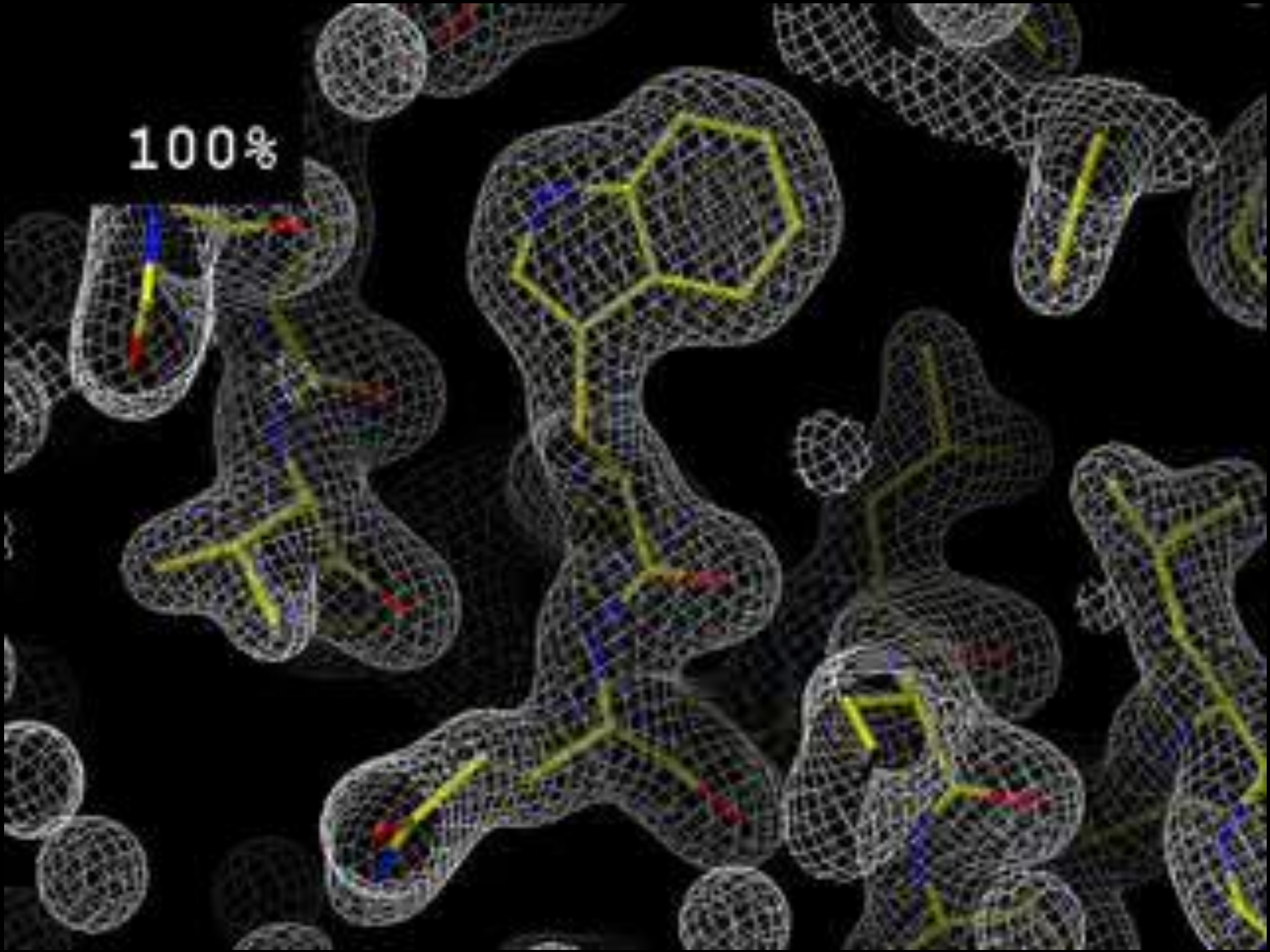
  solution: use as many pixels as possible

Resolution



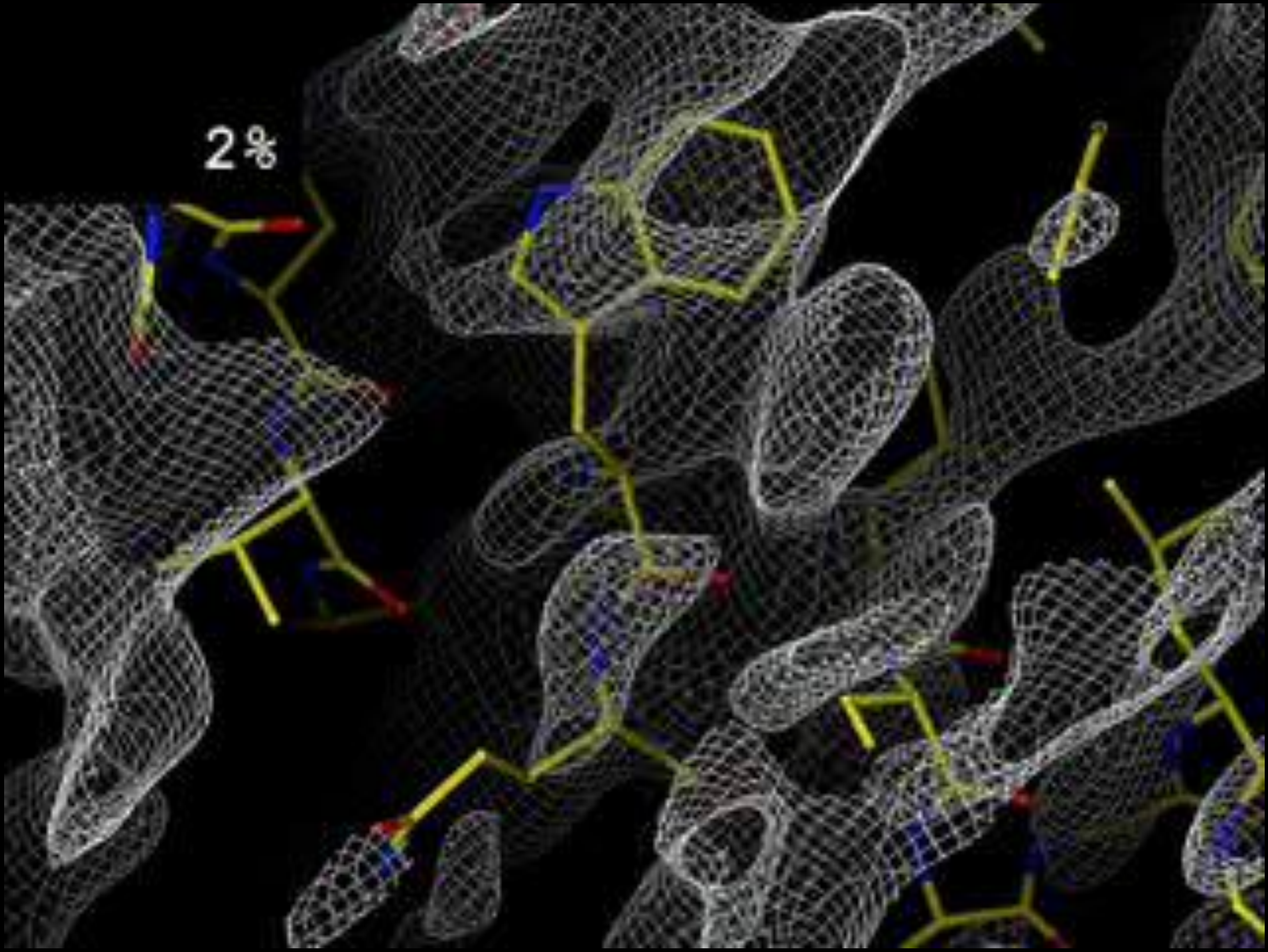
http://bl831.als.lbl.gov/~jamesh/movies/resolution.mpeg

Completeness: random deletion



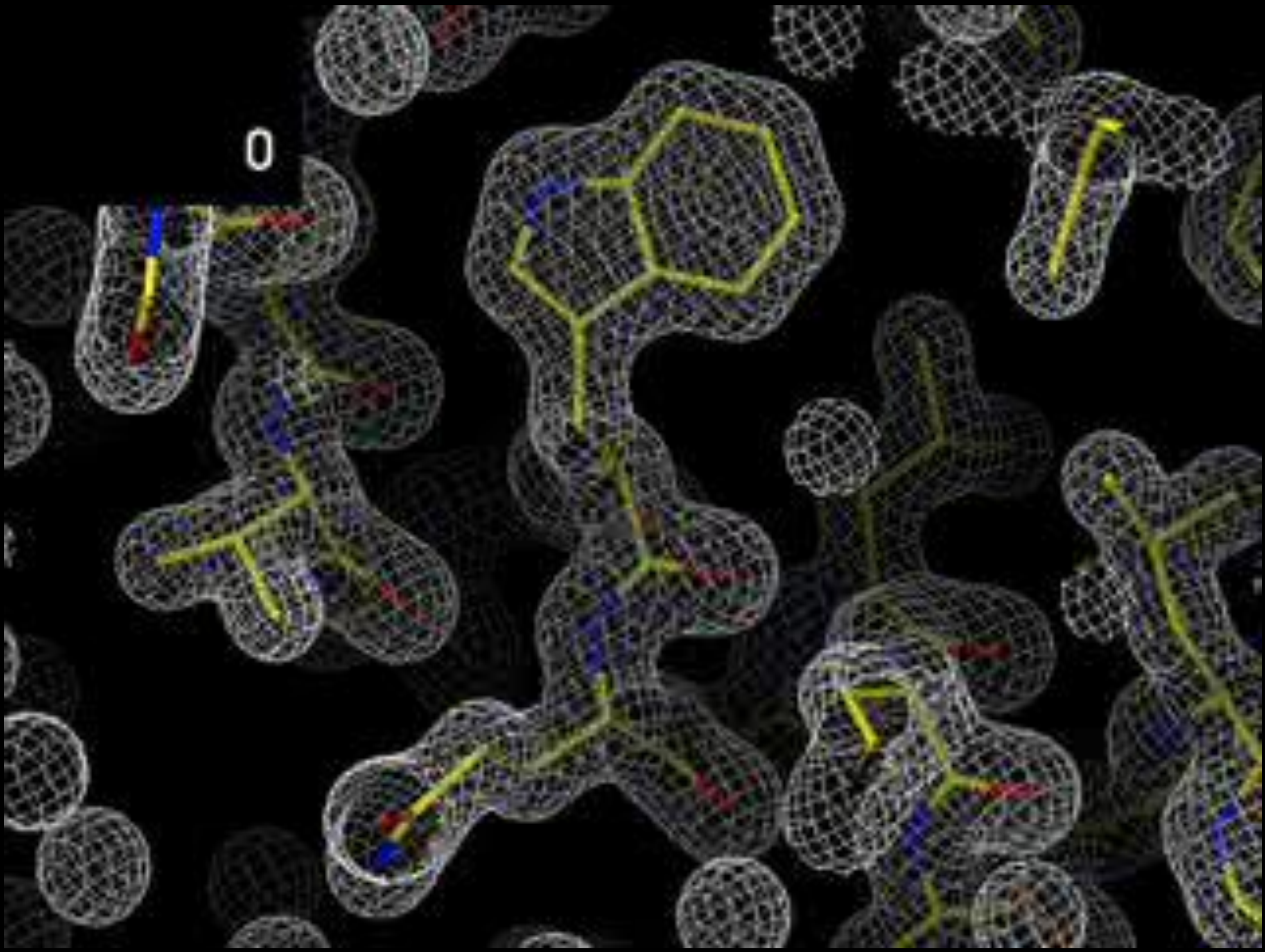
http://bl831.als.lbl.gov/~jamesh/movies/completeness.mpeg

Completeness: missing wedge



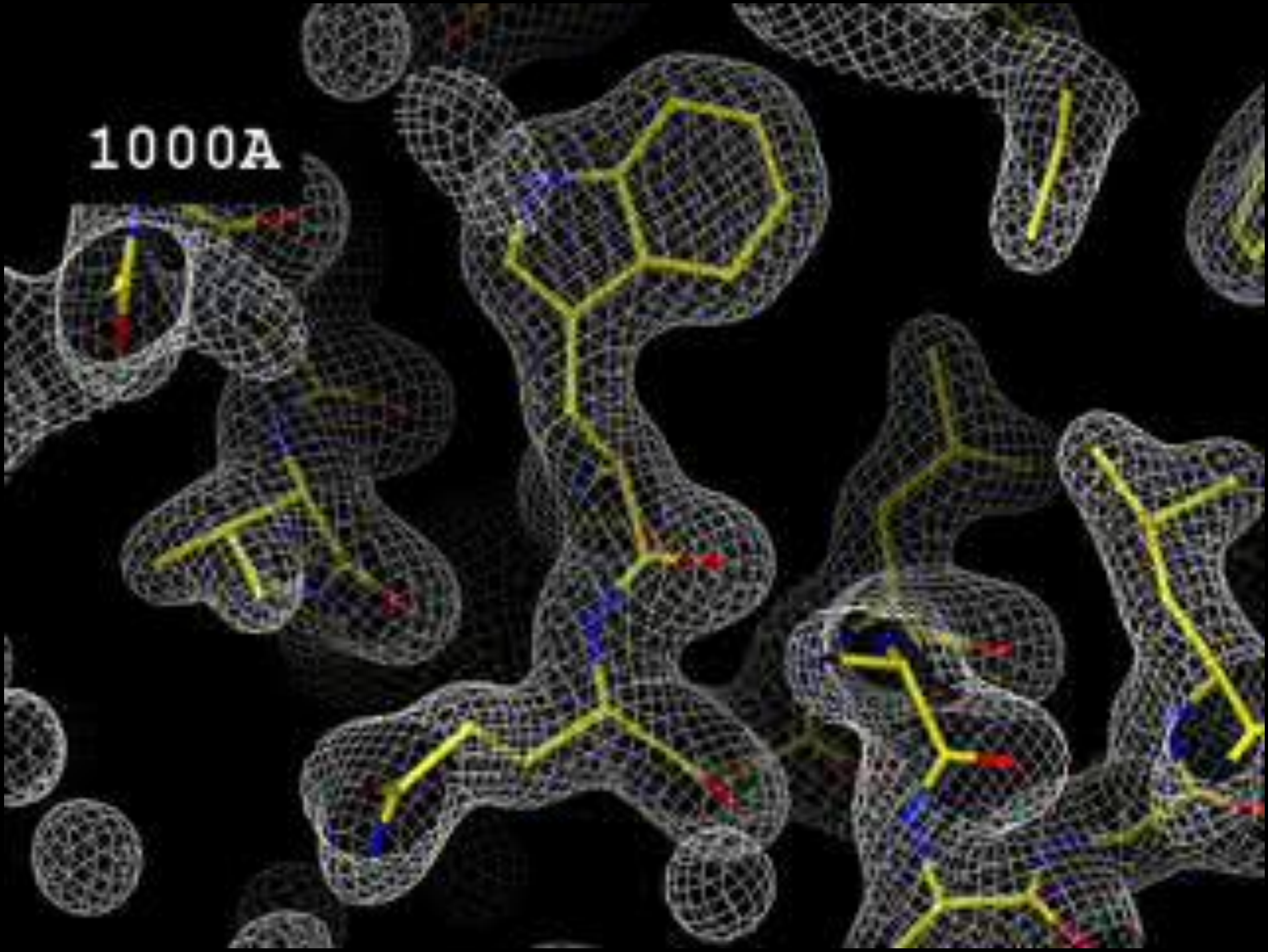
http://bl831.als.lbl.gov/~jamesh/movies/osc.mpeg

Overloads



http://bl831.als.lbl.gov/~jamesh/movies/overloads.mpeg

Resolution: low-angle cutoff



http://bl831.als.lbl.gov/~jamesh/movies/lo_cut.mpeg

The truth about x-ray beams

Term	units	significance
Flux	photons/s	duration of experiment
Beam Size	μm	match to crystal
Divergence	mrad	spot size vs distance
Wavelength	Å	resolution and absorption
Dispersion	$\Delta\lambda/\lambda$	spot size
Flux density	ph/s/area	scattering/damage rate
Fluence	ph/area	scattering/damage

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Flux density	ph/s/area	scattering/damage rate
Fluence	ph/area	scattering/damage

The truth about x-ray beams

quantity	**units**	**home source**	**MX2**
flux	Photons/second	2.5×10^9	2×10^{12}
exposure	seconds	400	1

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

The truth about x-ray beams

quantity	**units**	**home source**	**MX2**
flux	Photons/second	2.5×10^9	2×10^{12}
exposure	seconds	400	1
Dispersion	wavelength range / wavelength	0.2% ($K\alpha_1$ - $K\alpha_2$)	0.014% (Si111)
Divergence	milliRadian	4.8	1.8 (h) 0.18 (v)
Beam size	microns	100	24 (h) 12 (v)
Spectral brightness	Photons/s/mm^2/mR^2/0.1%BW	5.4×10^9	1.5×10^{17}

The truth about x-ray beams

quantity	**units**	**home source**	**MX2**
flux	Photons/second	72 x 10^6	2 x 10^{12}
exposure	time	4 hours	1 second
Dispersion	wavelength range / wavelength	0.2% ($K\alpha_1$ - $K\alpha_2$)	0.014% (Si111)
Divergence	milliRadian	4.8	1.8 (h) 0.18 (v)
Beam size	microns	17	24 (h) 12 (v)
Spectral brightness	Photons/s/mm^2/ mR^2/0.1%BW	5.4 x 10^9	1.5 x 10^{17}

The truth about x-ray beams

quantity	**units**	**home source**	**MX2**
flux	Photons/second	1×10^6	2×10^{12}
exposure	time	10 days	1 second
Dispersion	wavelength range / wavelength	0.2% ($K\alpha_1$ - $K\alpha_2$)	0.014% (Si111)
Divergence	milliRadian	0.6	1.8 (h) 0.18 (v)
Beam size	microns	17	24 (h) 12 (v)
Spectral brightness	$Photons/s/mm^2/mR^2/0.1\%BW$	5.4×10^9	1.5×10^{17}

The truth about x-ray beams

quantity	**units**	**home source**	**MX2**
flux	Photons/second	8×10^4	2×10^{12}
exposure	time	5 months	1 second
Dispersion	wavelength range / wavelength	0.014% (Si111)	0.014% (Si111)
Divergence	milliRadian	0.6	1.8 (h) 0.18 (v)
Beam size	microns	17	24 (h) 12 (v)
Spectral brightness	Photons/s/mm^2/ mR^2/0.1%BW	5.4×10^9	1.5×10^{17}

The truth about x-ray beams

Term	units	significance
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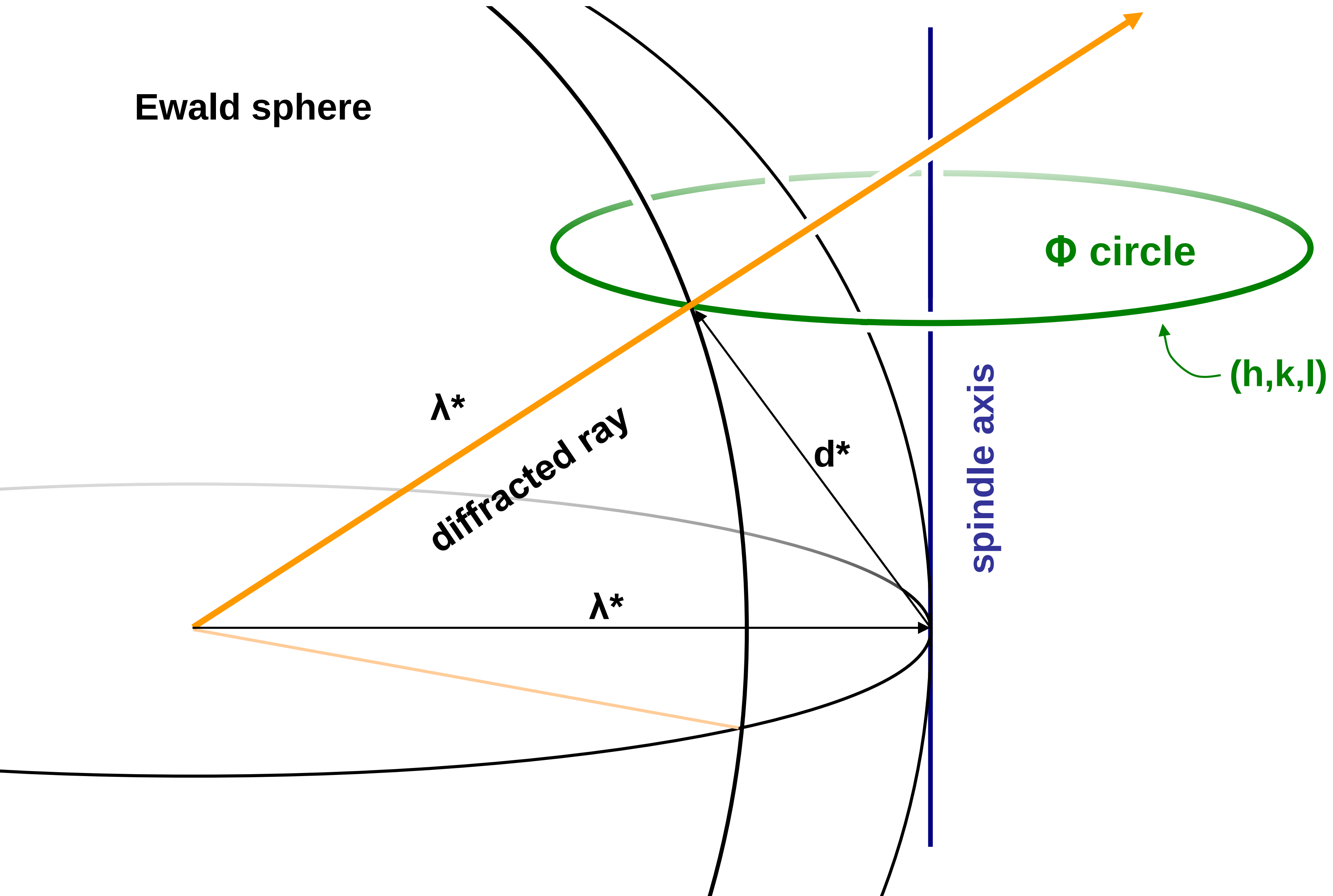
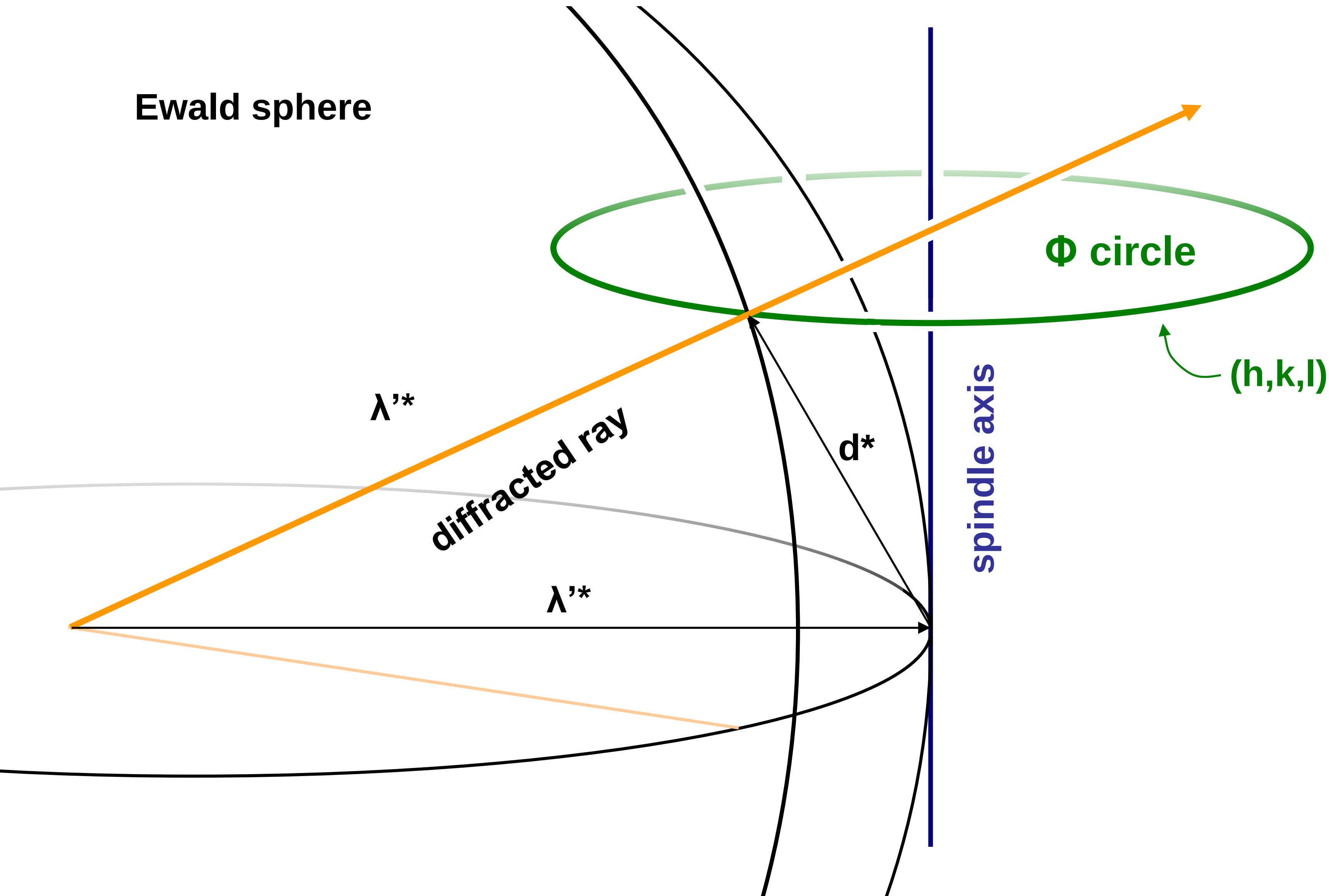
Beam Size	μm	match to crystal
Divergence	mrad	spot size vs distance
Wavelength	Å	resolution and absorption
Dispersion	Δλ/λ	spot size
Flux density	ph/s/area	scattering/damage rate
Fluence	ph/area	scattering/damage

spectral dispersion



spectral dispersion



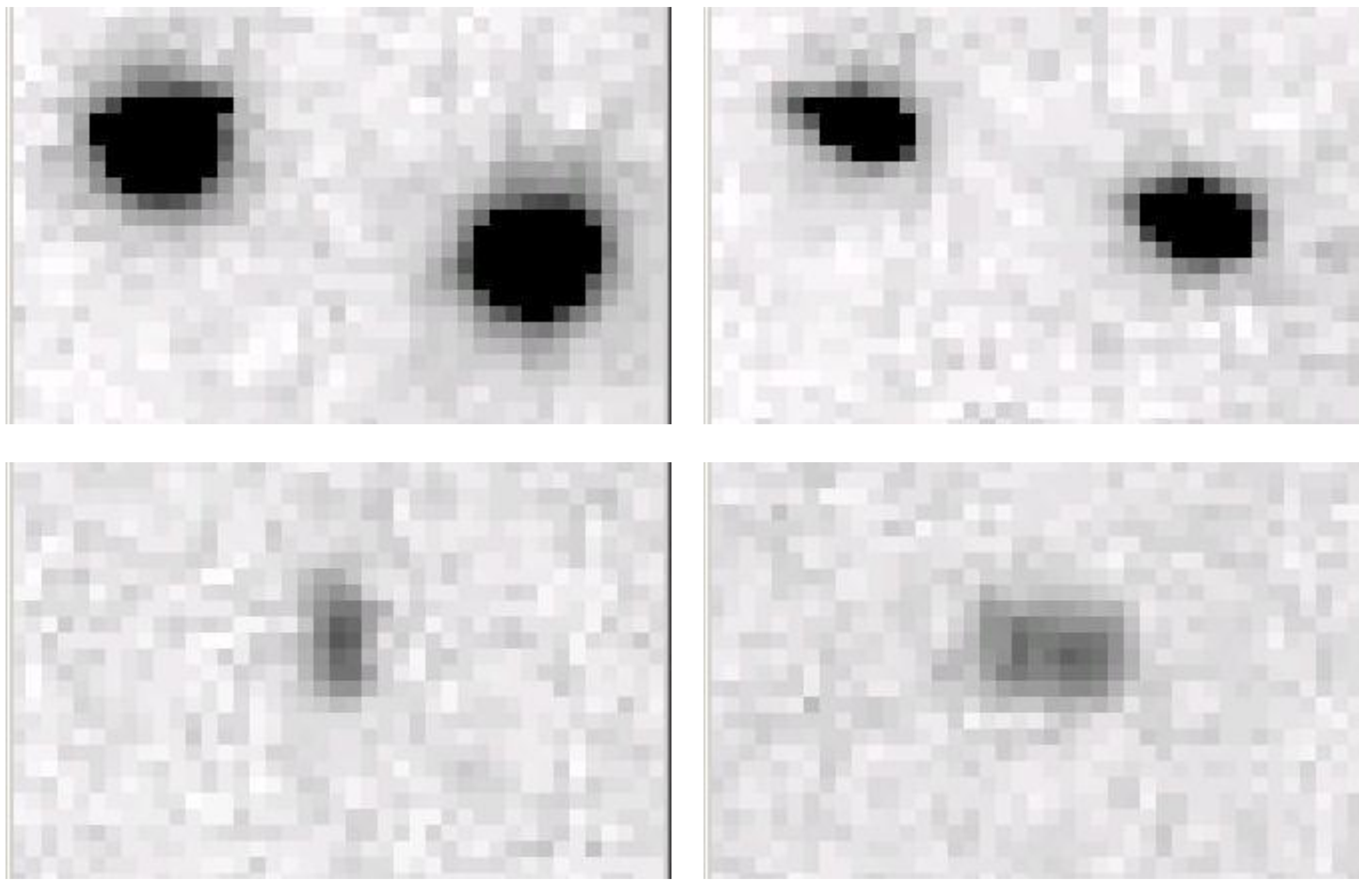
Si(111) vs multilayers

0.014% Si(111) — spectral dispersion — 1% multilayer

4 Å

resolution

1.9 Å



Decisions, Decisions, Decisions

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- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
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Run Strategy

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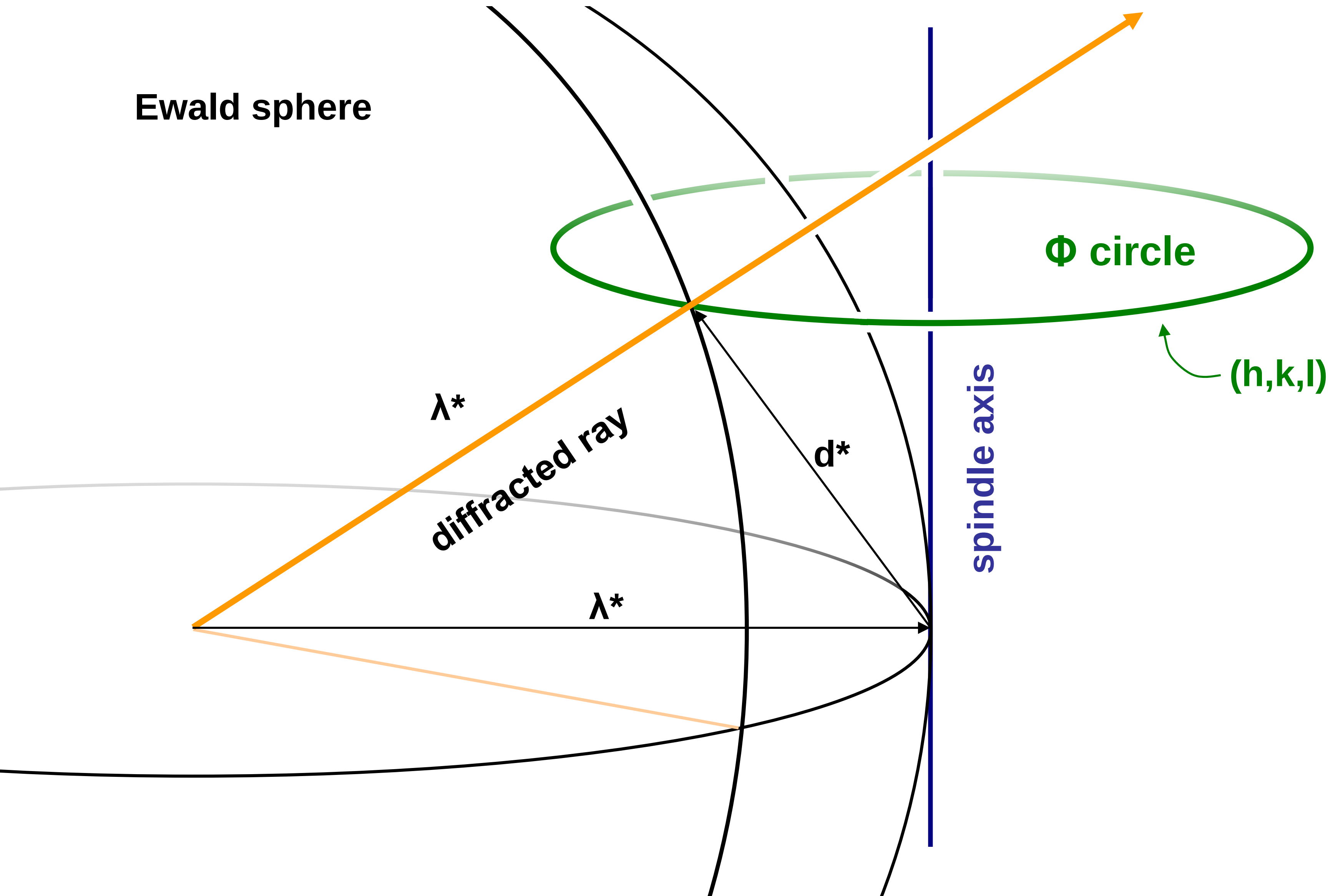
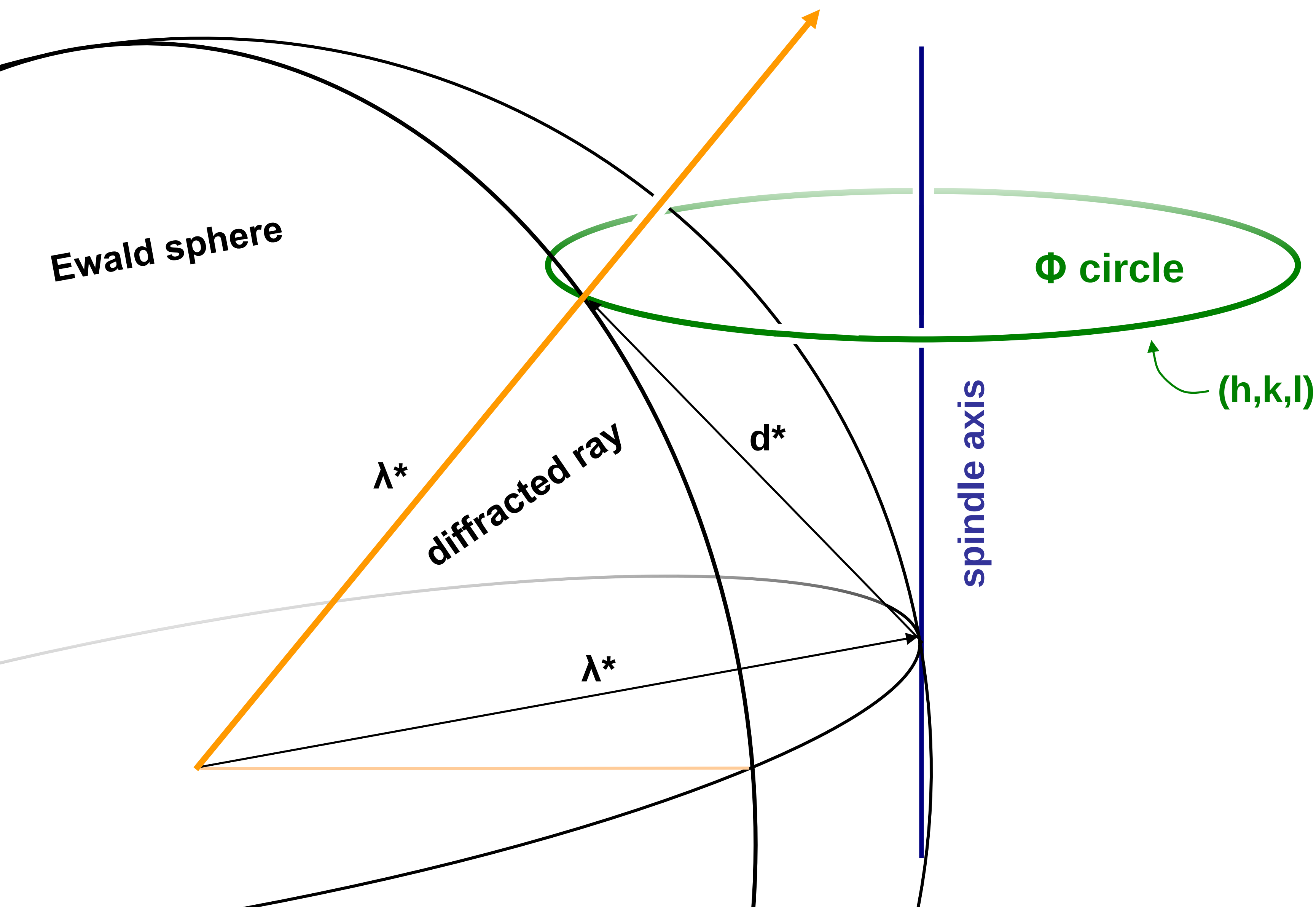
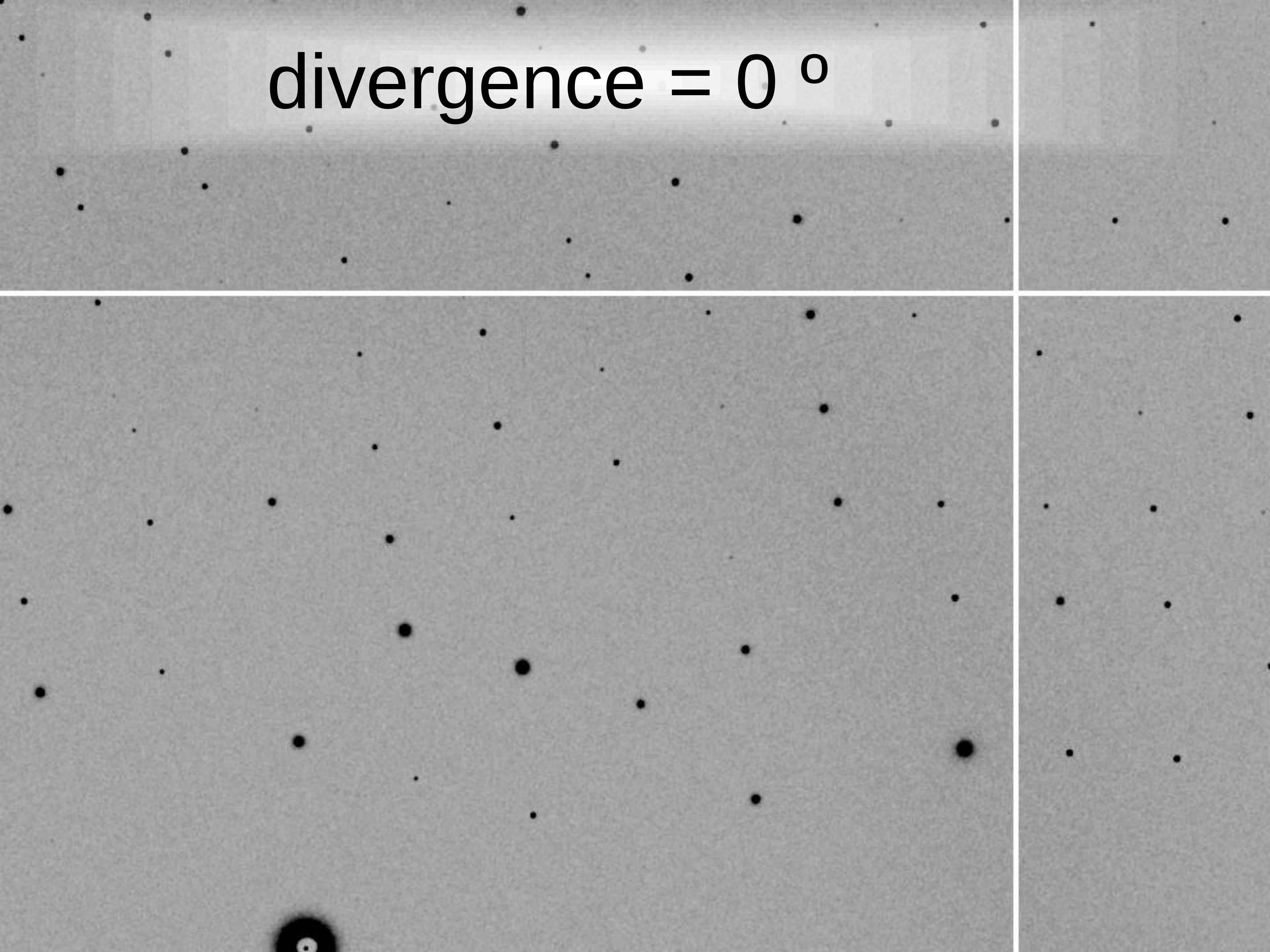
Flux density	ph/s/area	scattering/damage rate
Fluence	ph/area	scattering/damage

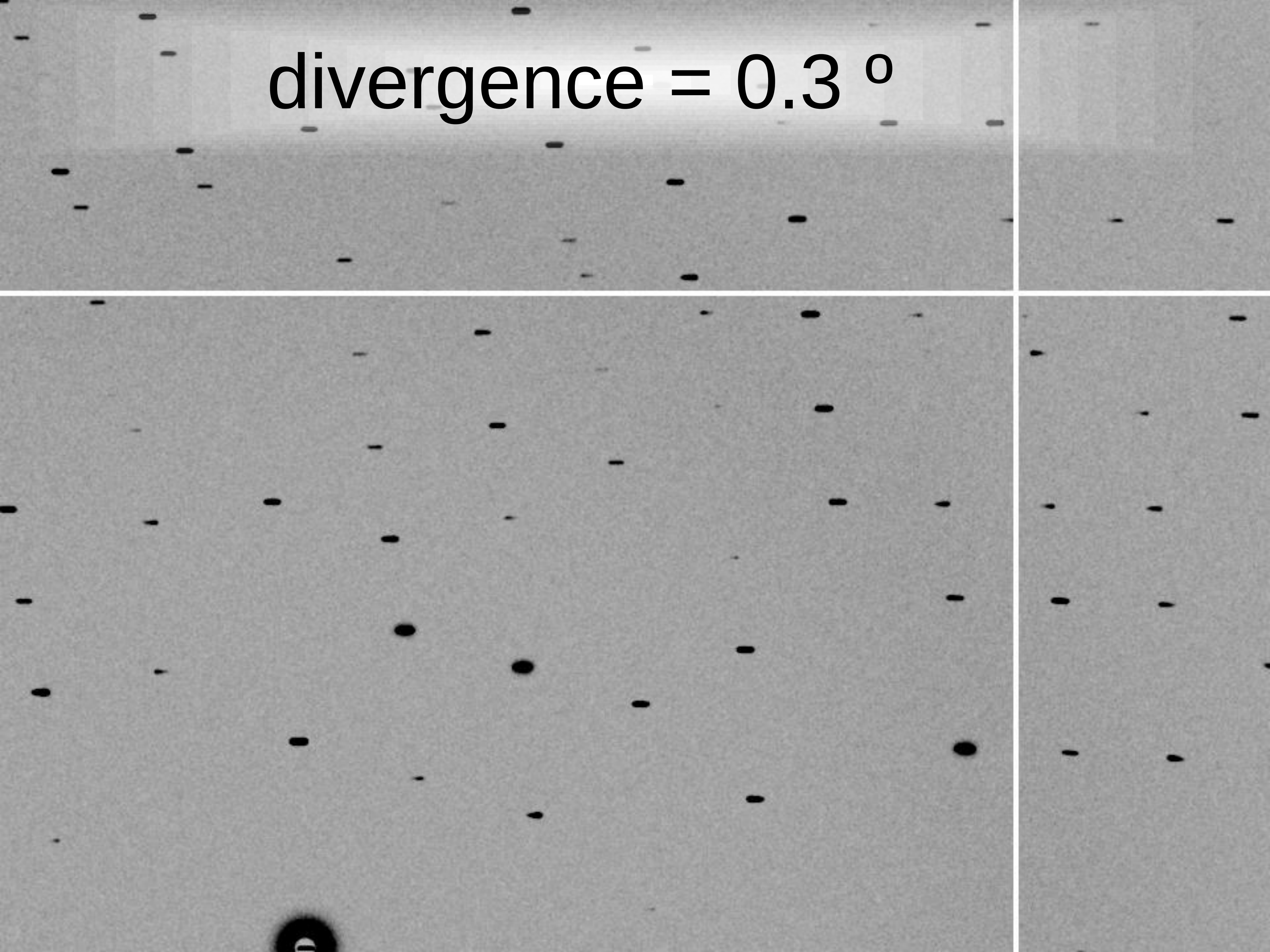
beam divergence



beam divergence



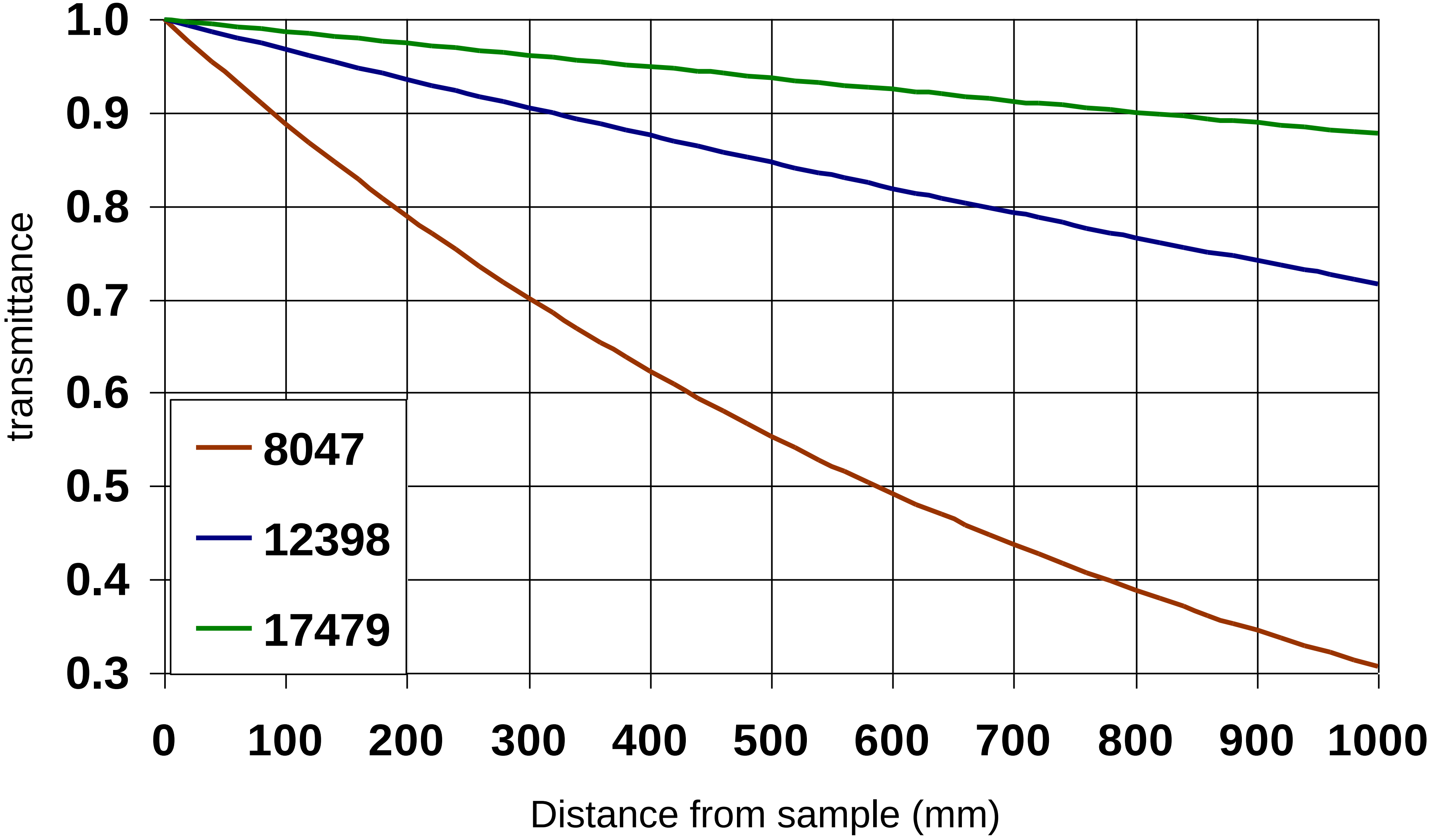

divergence = 0 º


divergence = 0.3 º

The truth about x-ray beams

Term	units	significance
Flux	photons/s	duration of experiment
Beam Size	μm	match to crystal
Divergence	mrad	spot size vs distance
Wavelength	Å	resolution and absorption
Dispersion	$\Delta\lambda/\lambda$	spot size
Flux density	ph/s/area	scattering/damage rate
Fluence	ph/area	scattering/damage

Air absorption



The truth about x-ray beams

Term	units	significance
Flux	photons/s	duration of experiment
Beam Size	μm	match to crystal
Divergence	mrad	spot size vs distance
Wavelength	Å	resolution and absorption
Dispersion	$\Delta\lambda/\lambda$	spot size
Flux density	ph/s/area	scattering/damage rate
Fluence	ph/area	scattering/damage

Decisions, Decisions, Decisions

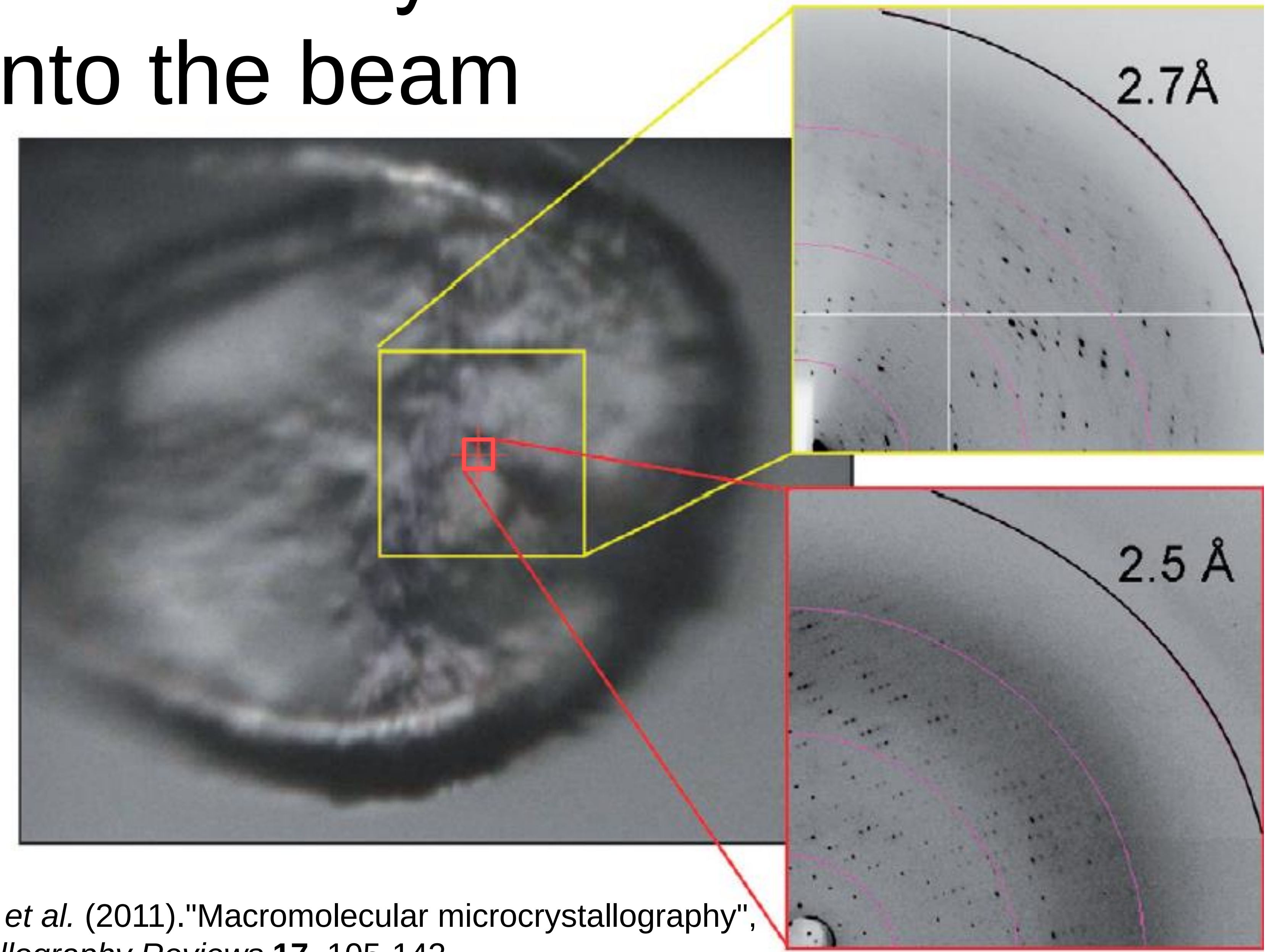
- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

beam size vs xtal size

1. Put your crystal into the beam

Put the "crystal" into the beam



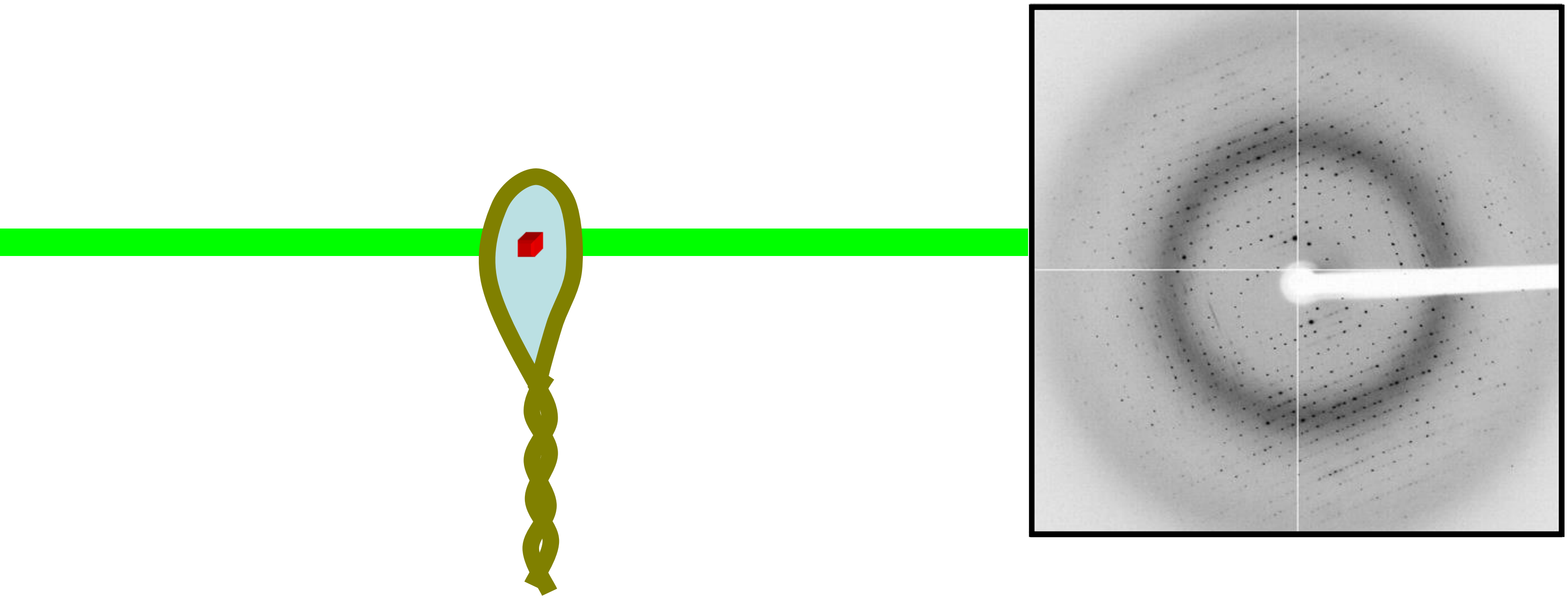
Evans *et al.* (2011)."Macromolecular microcrystallography", *Crystallography Reviews* **17**, 105-142.

“crystal” = thing you want to shoot

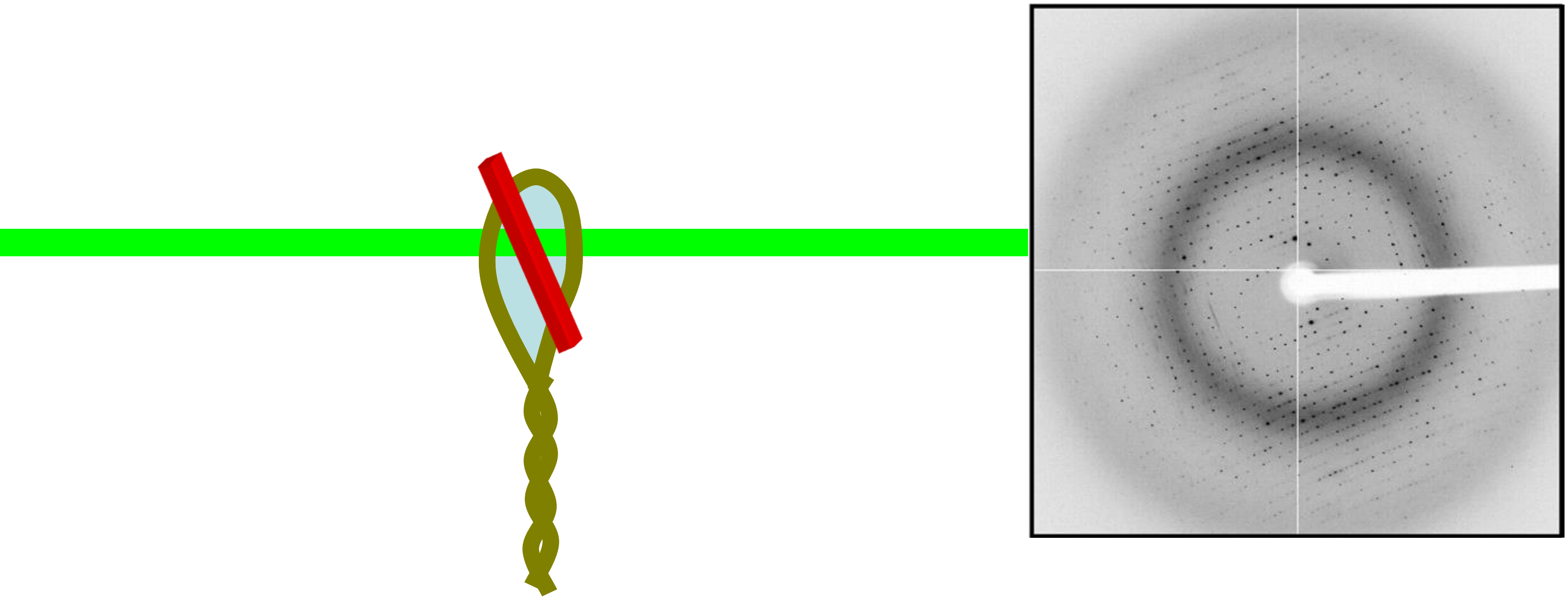
beam size vs xtal size

1. Put your crystal into the beam
2. Shoot the whole crystal

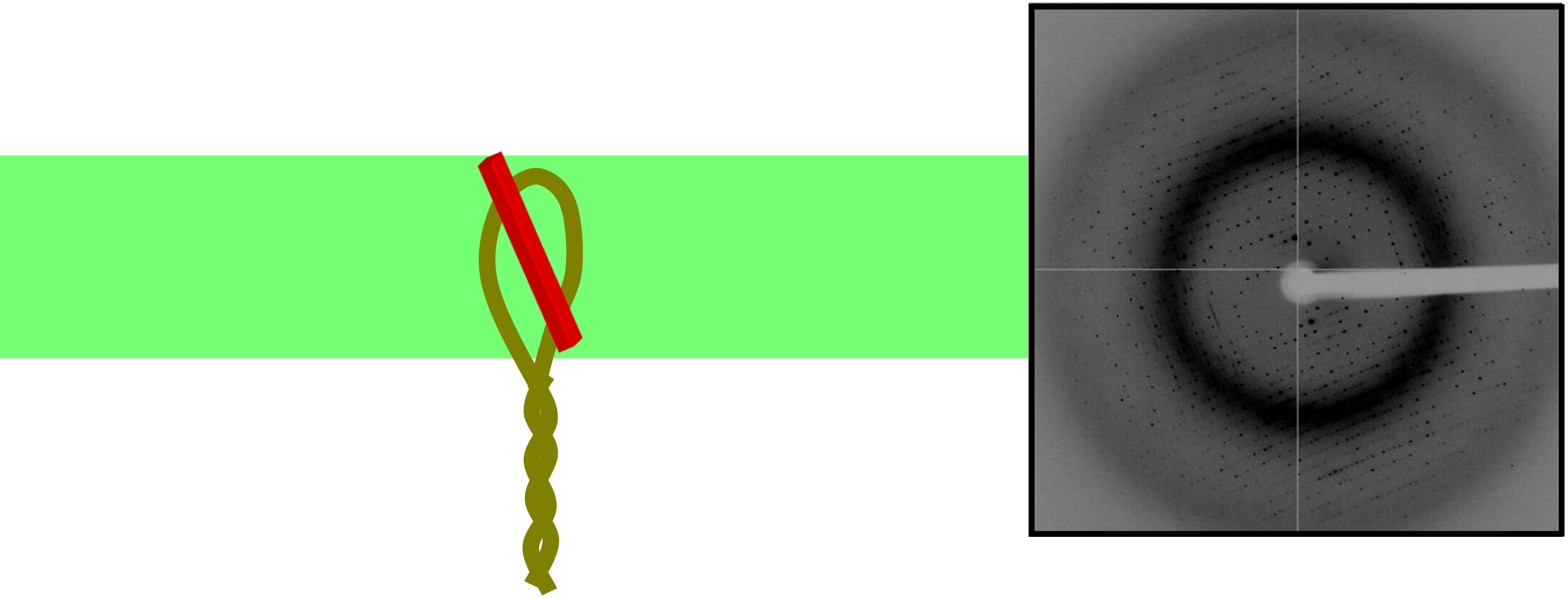
shoot the whole crystal



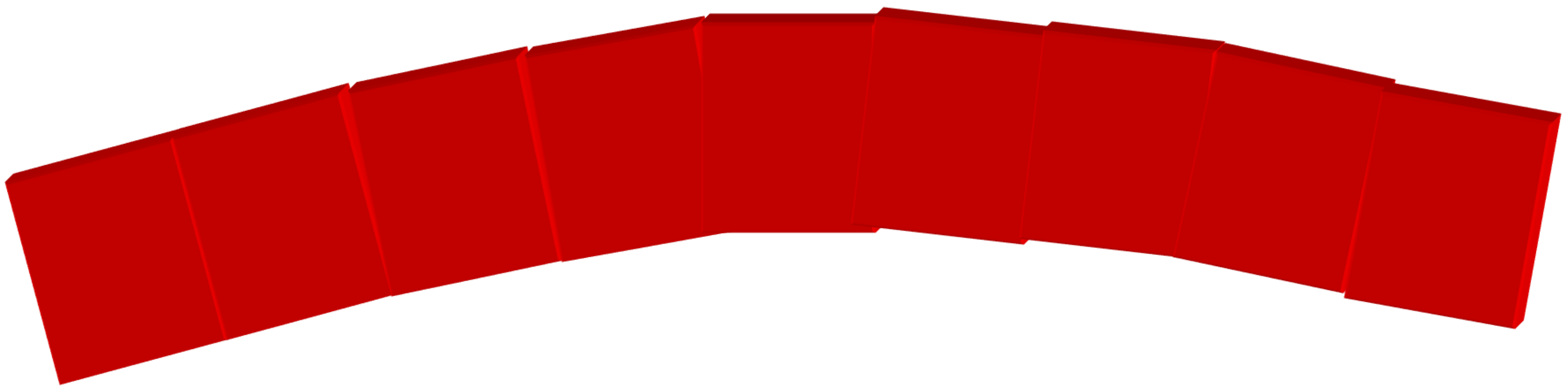
shoot the whole crystal



shoot the whole crystal

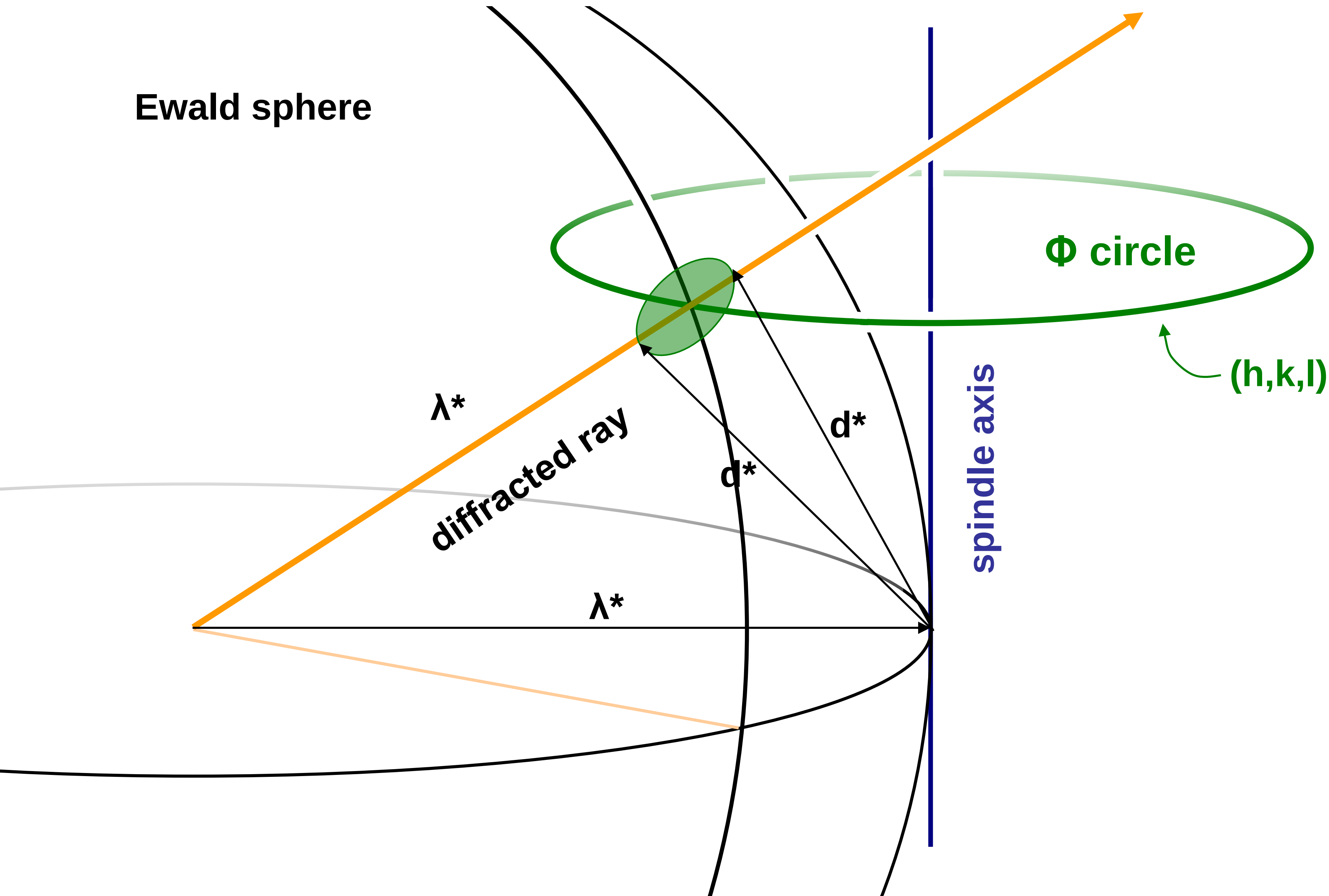


How many crystals do you see?

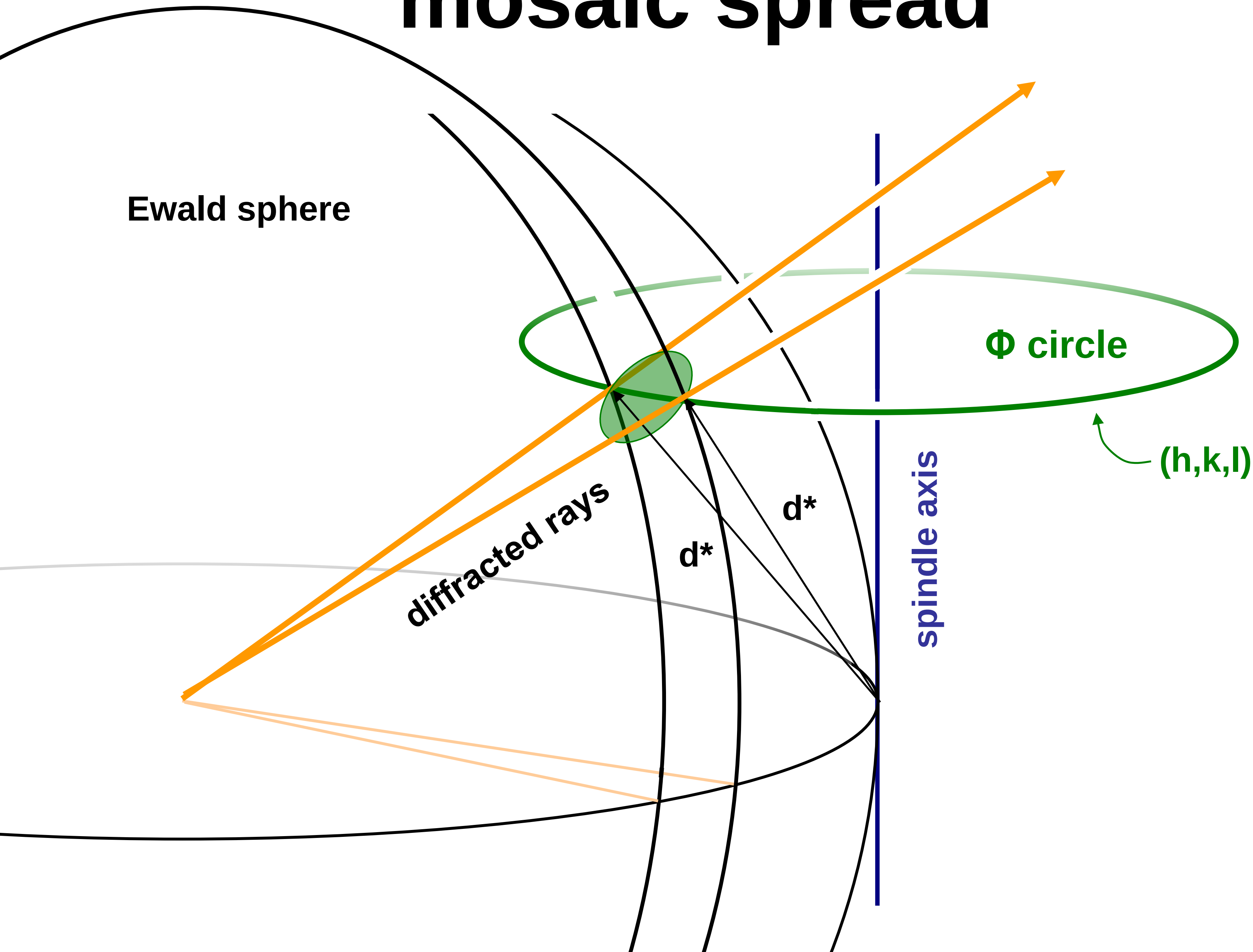


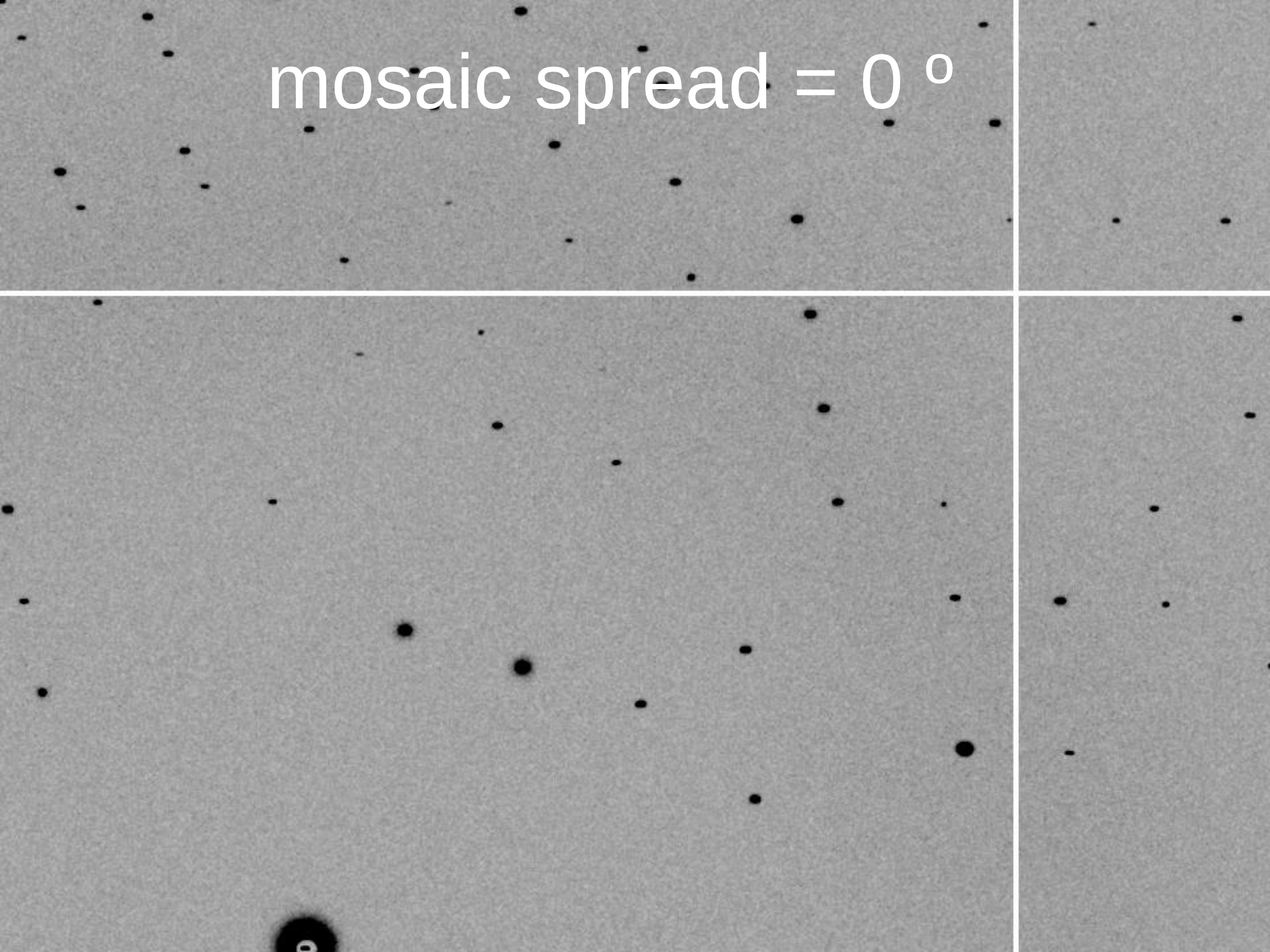
Shoot the "**crystal"** (singular)

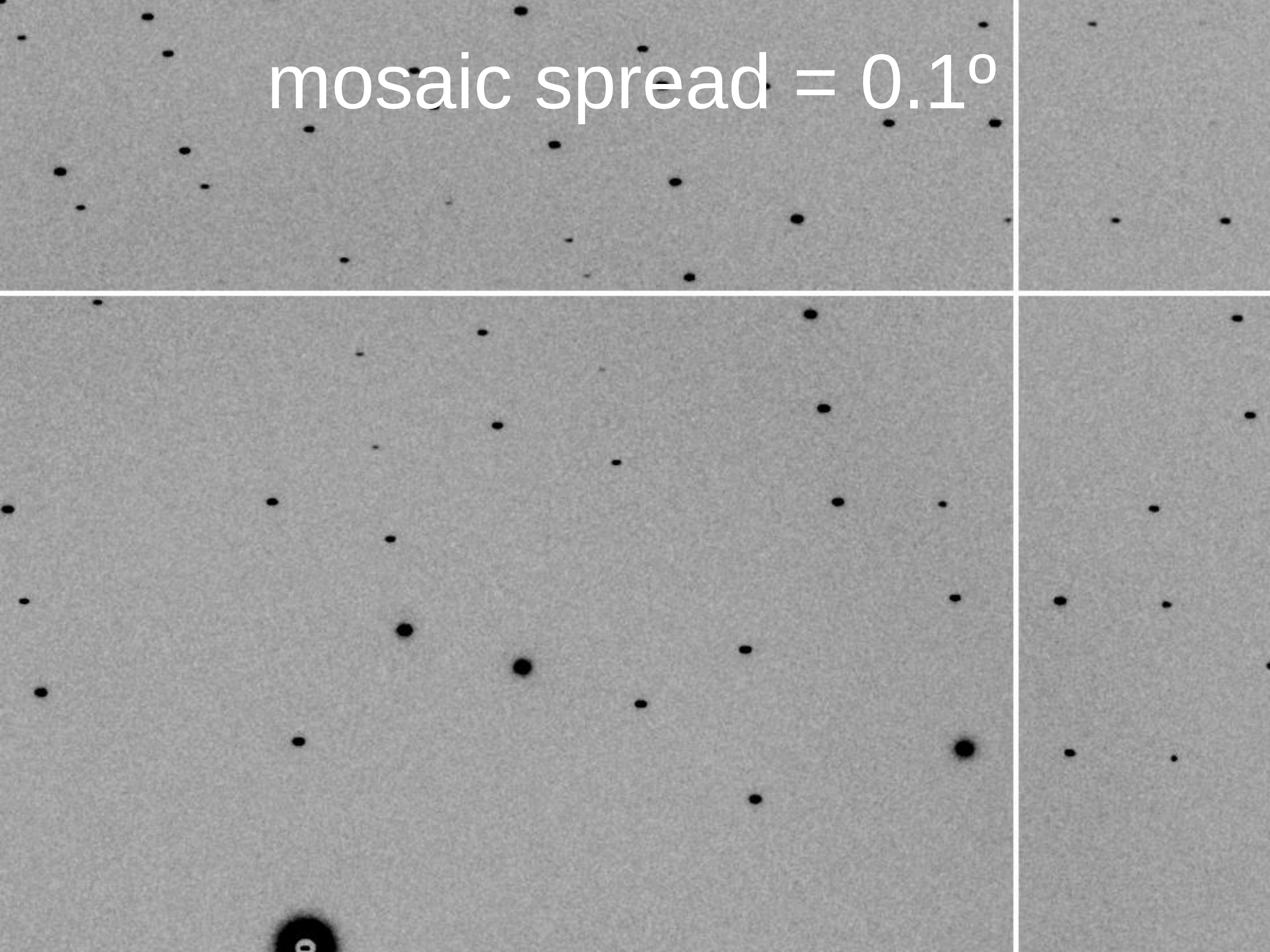
mosaic spread

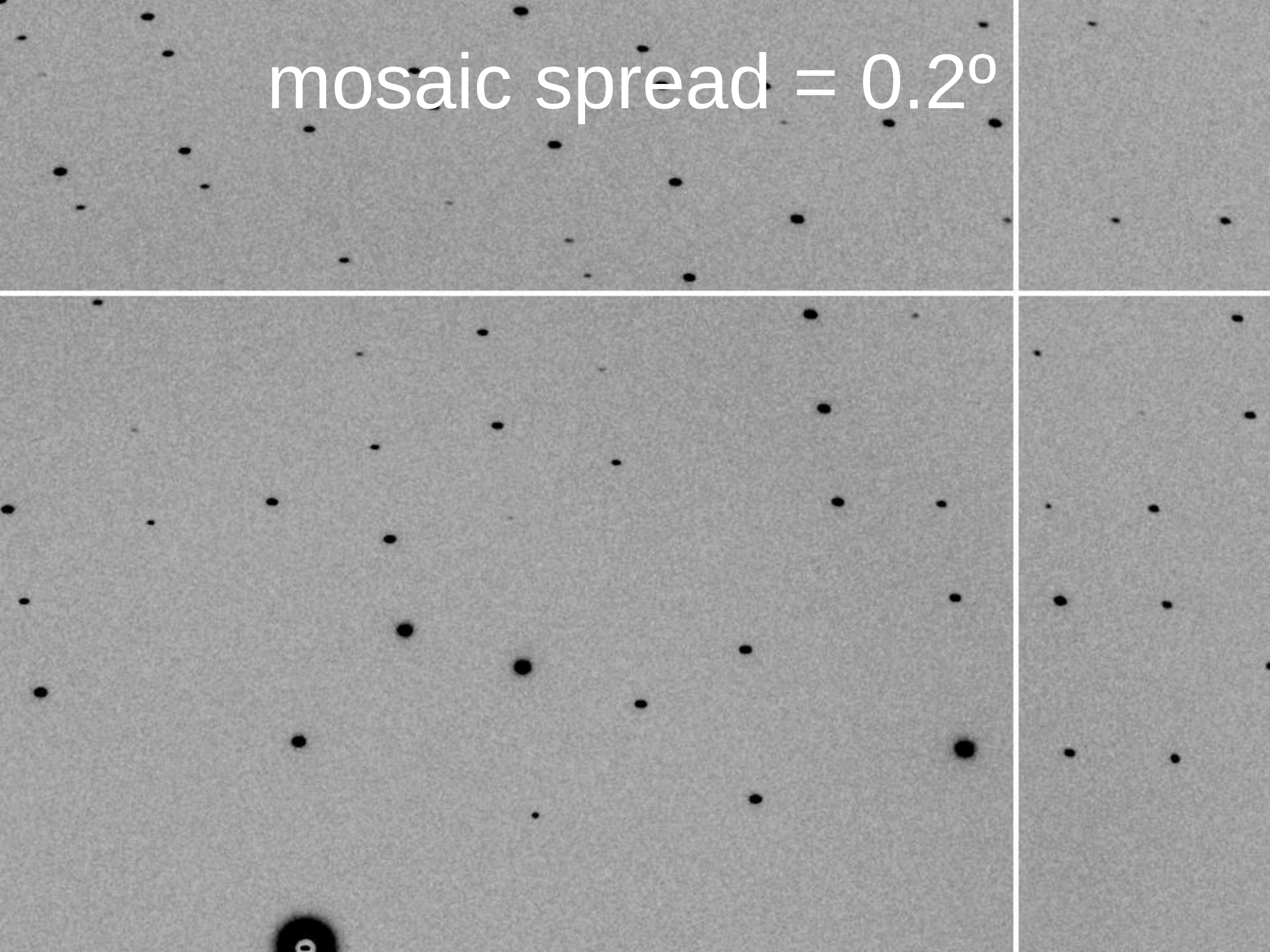


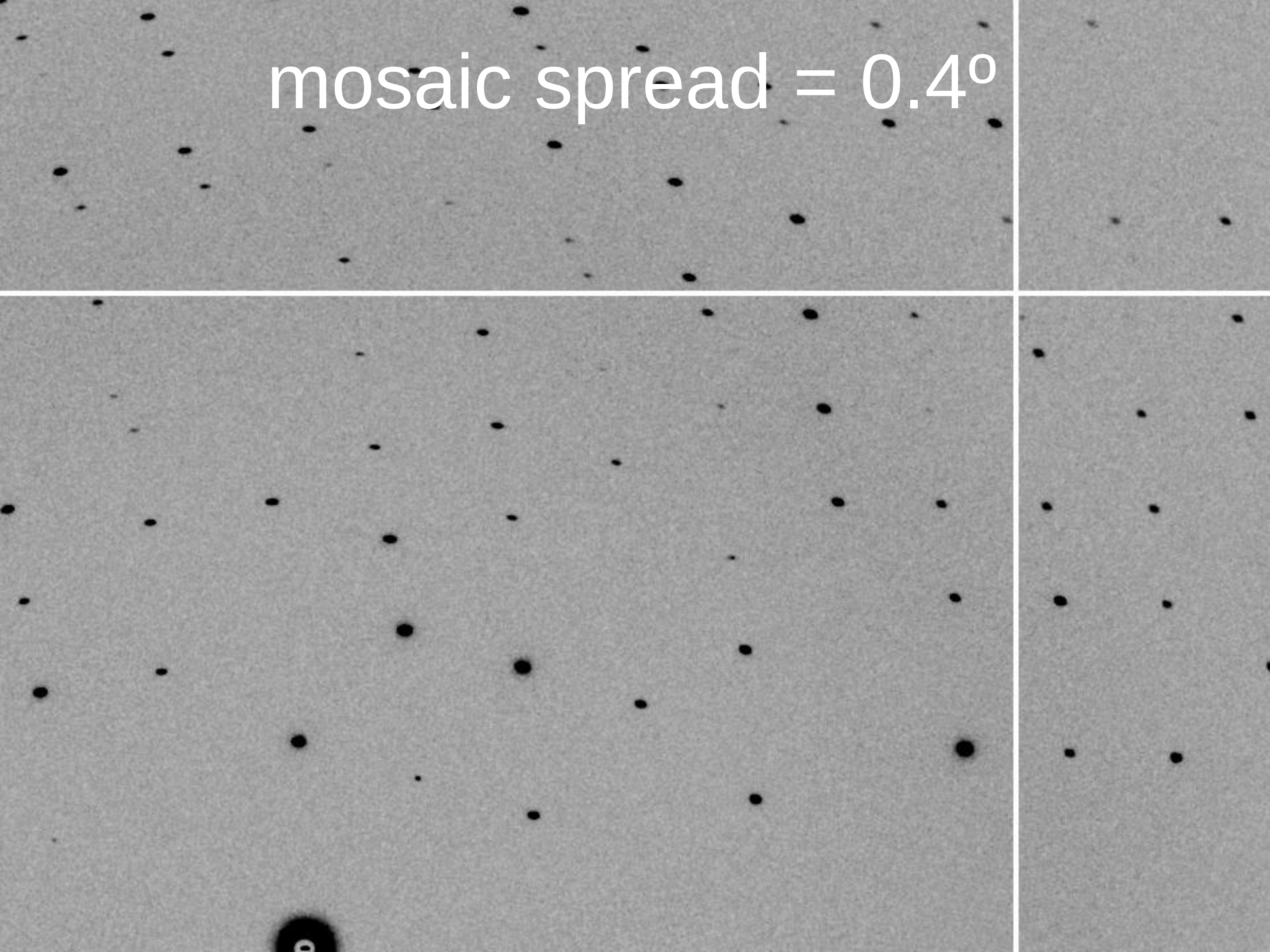
mosaic spread

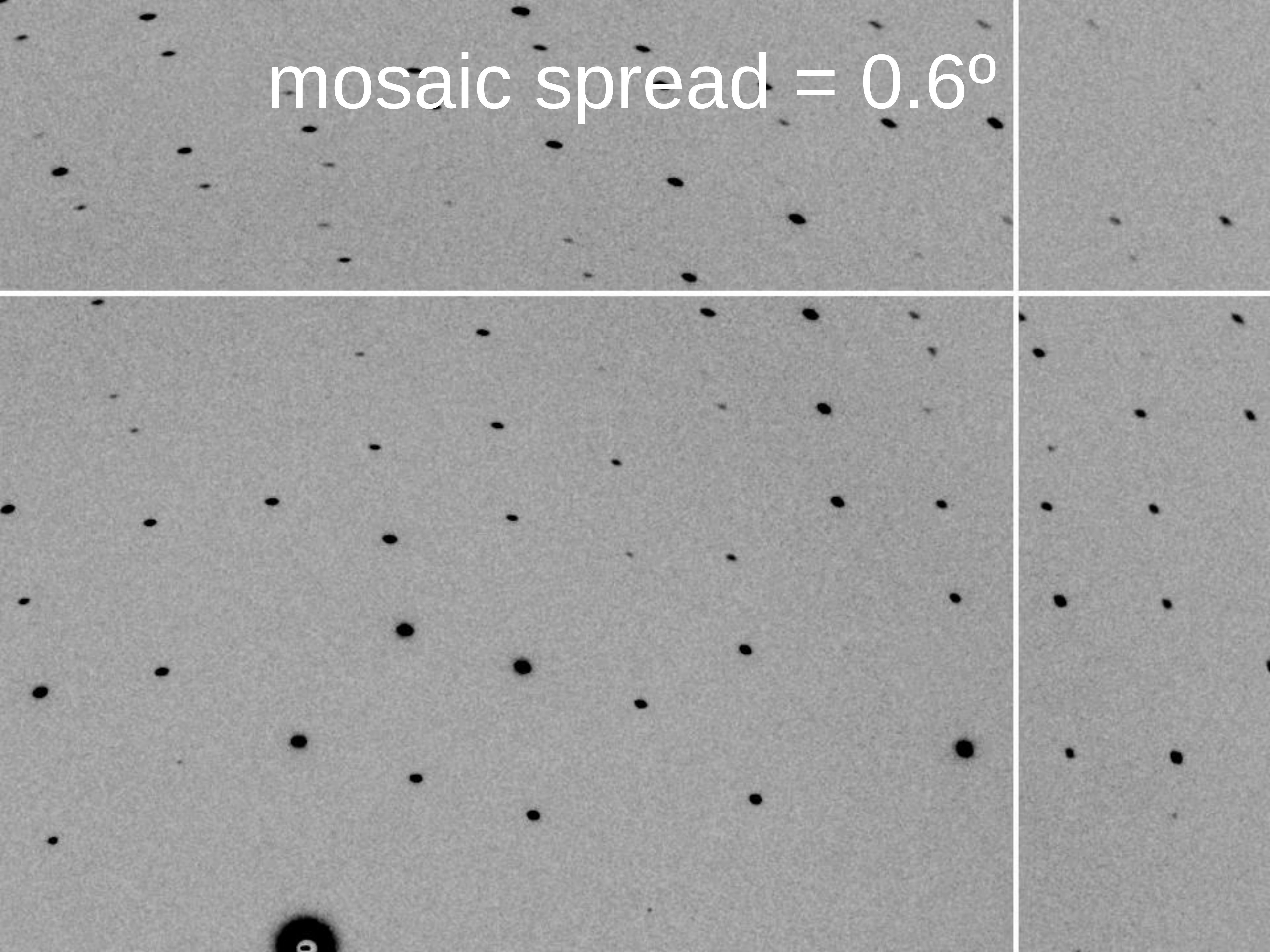


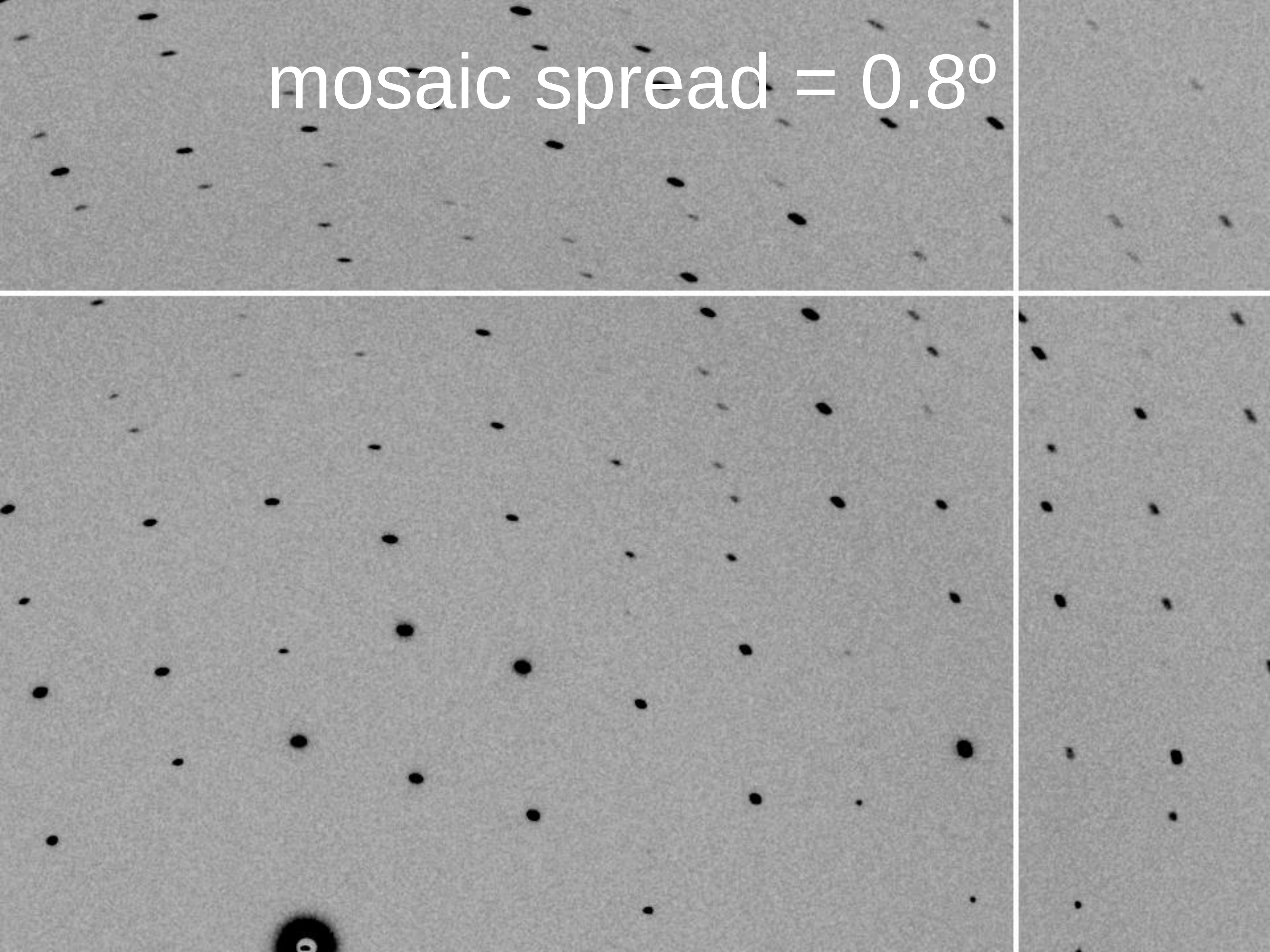

mosaic spread = 0 º

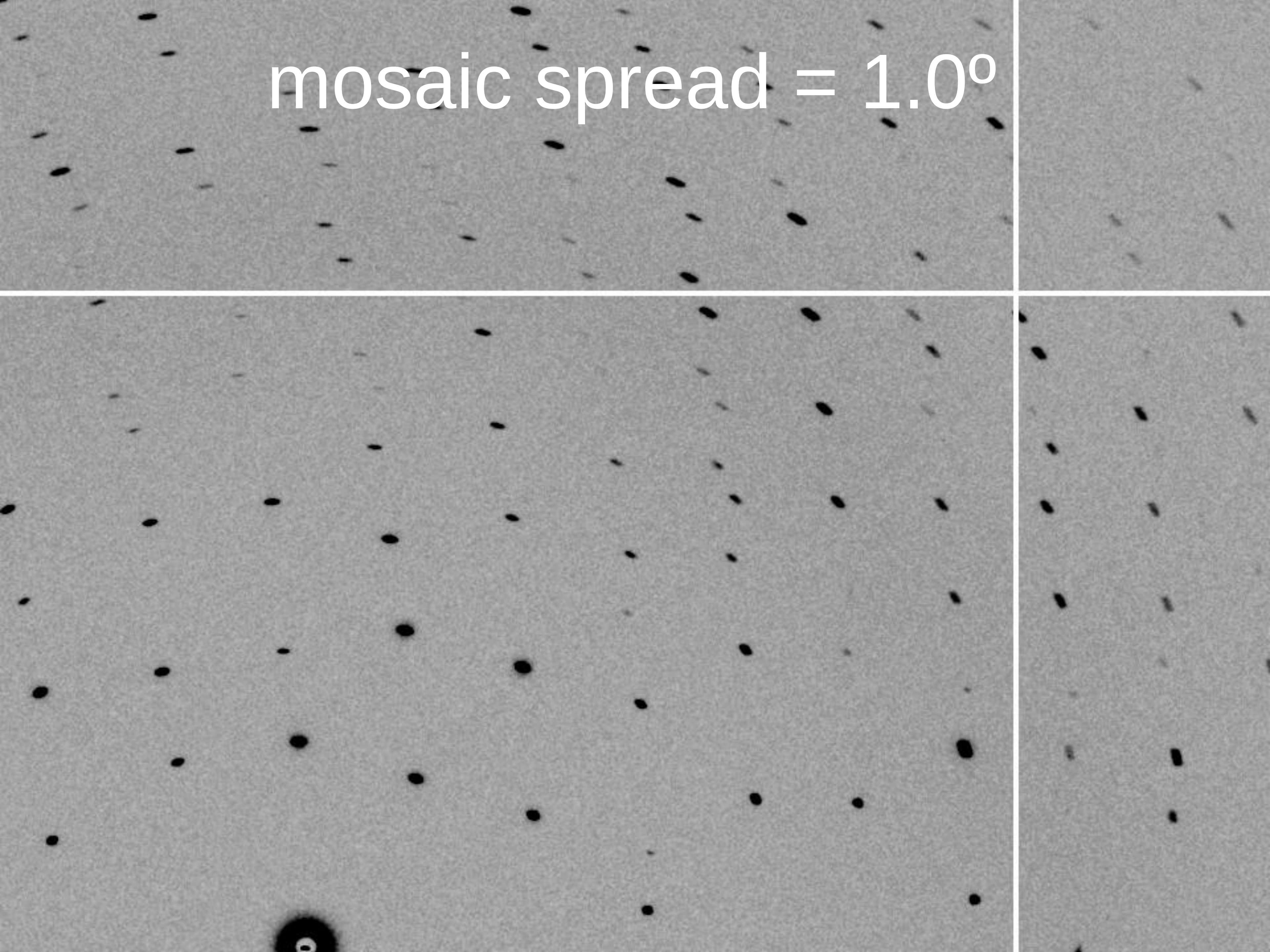

mosaic spread = 0.1º

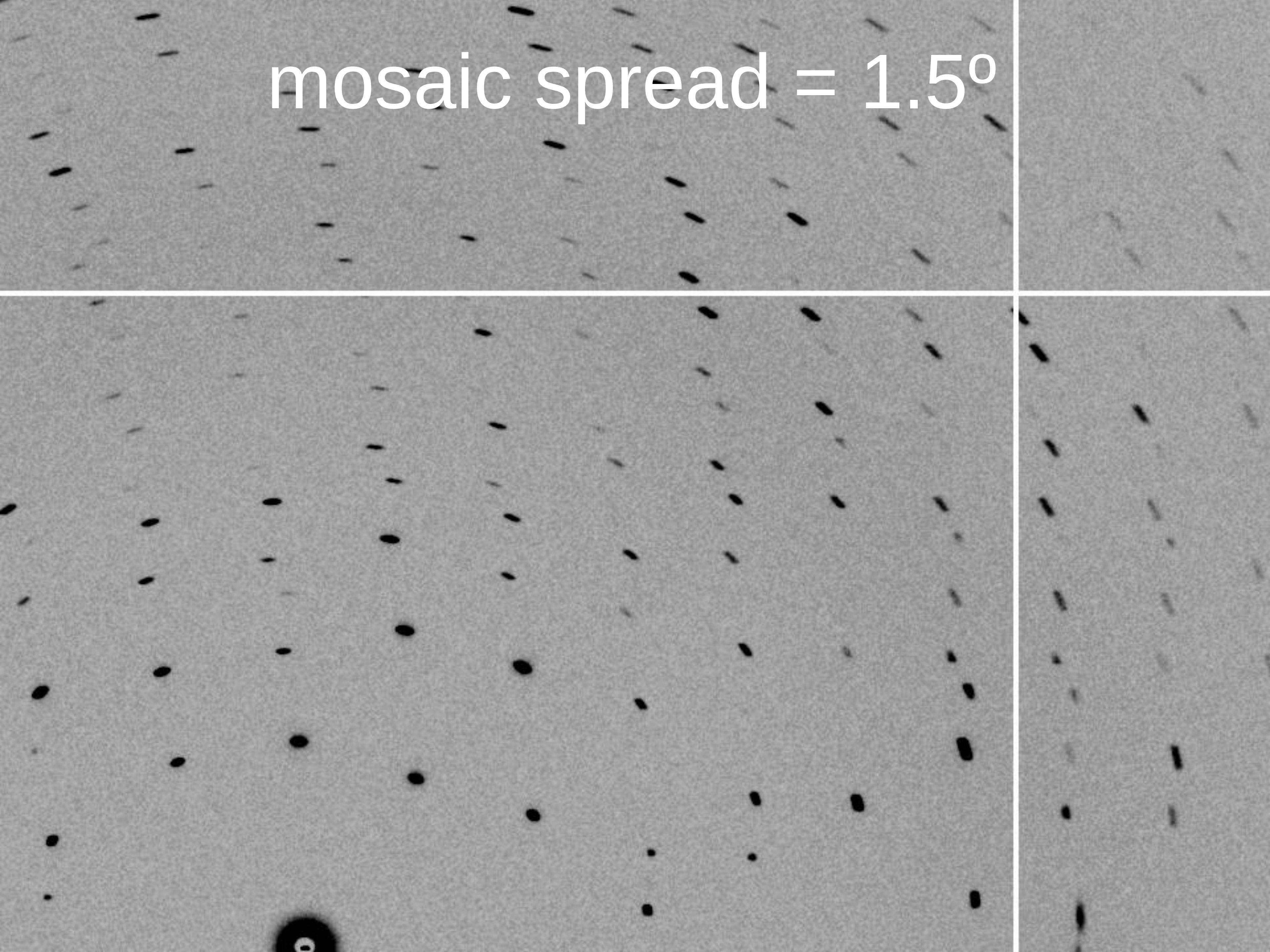

mosaic spread = 0.2º

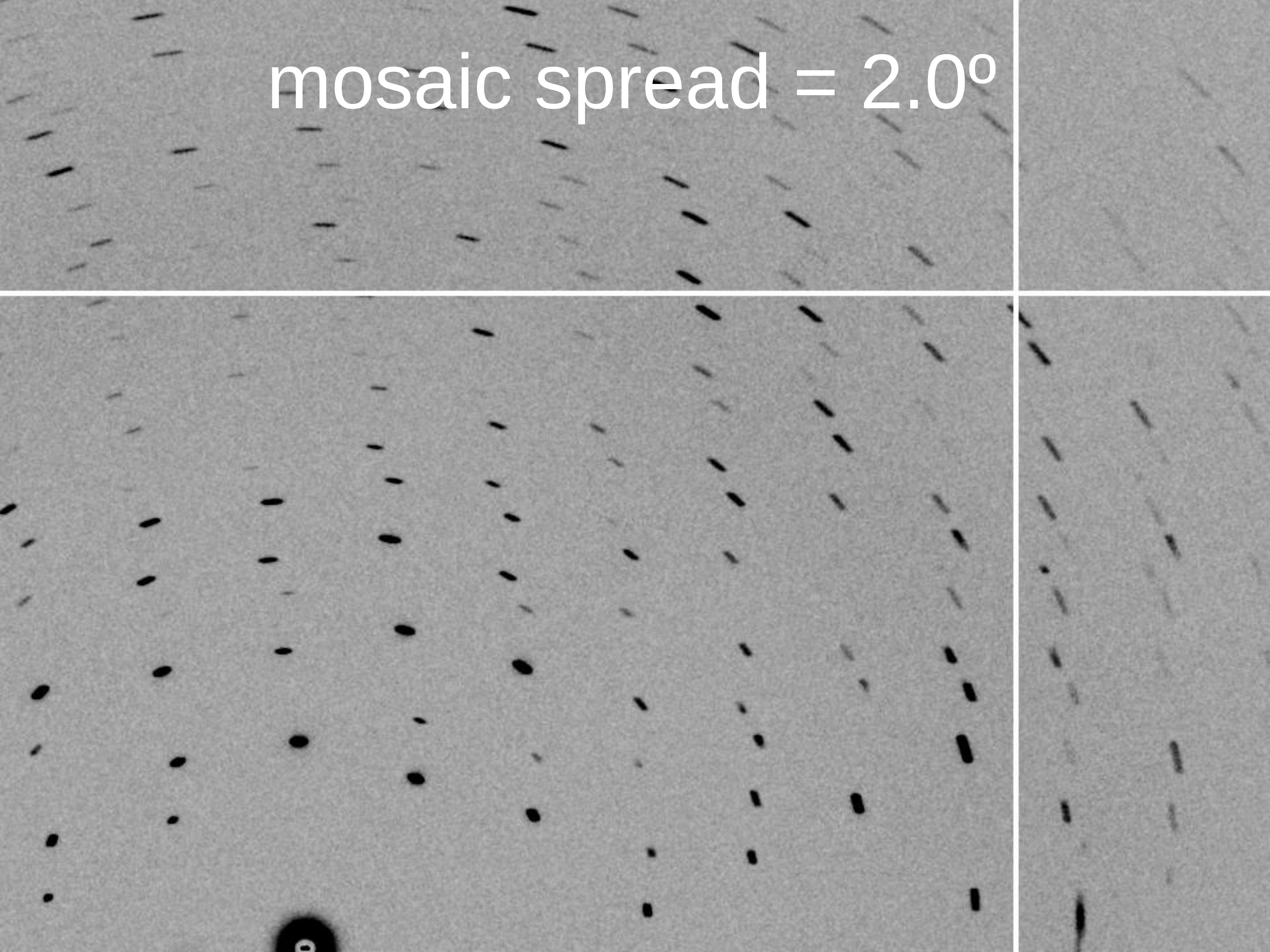

mosaic spread = 0.4º

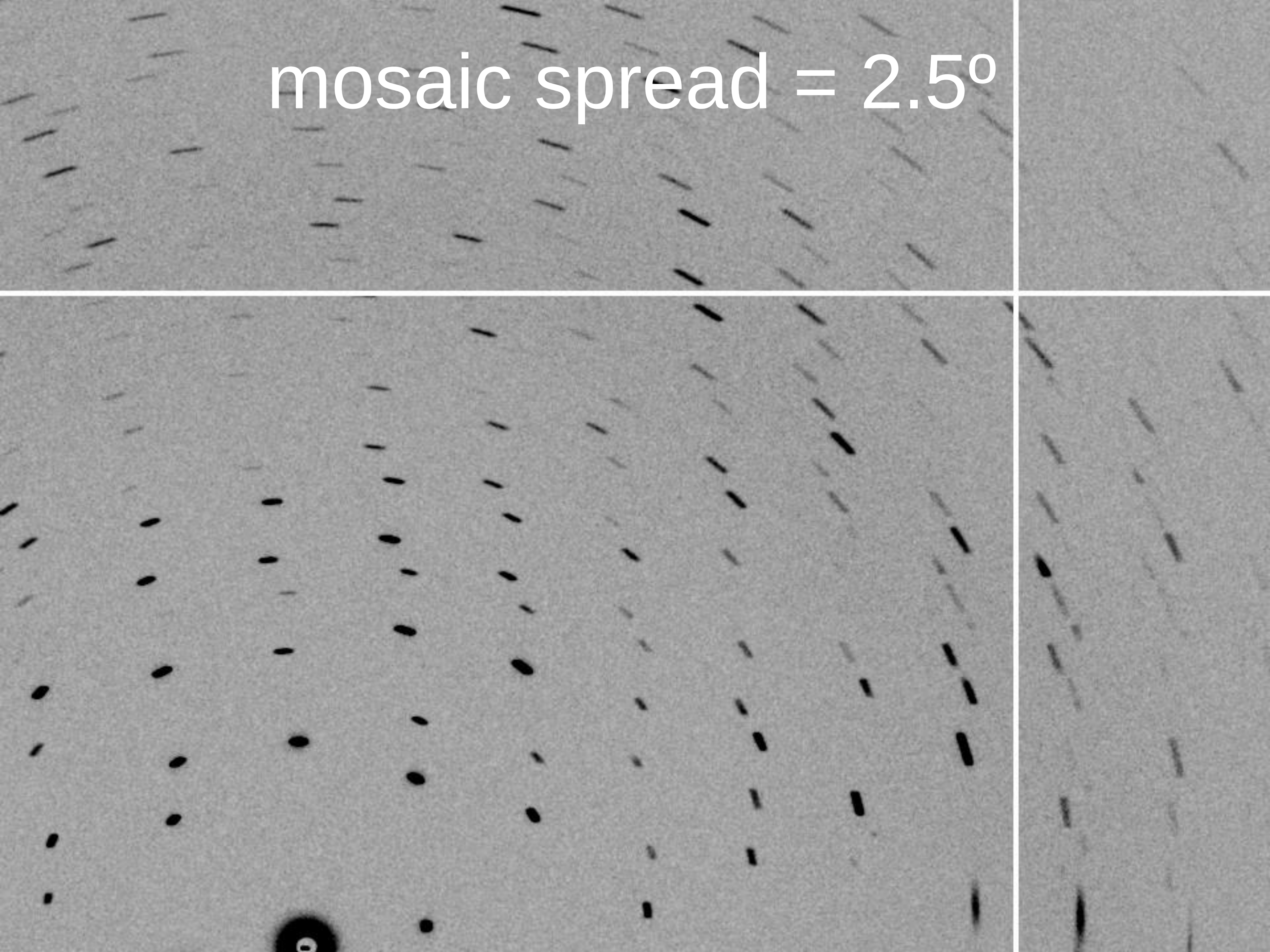

mosaic spread = 0.6º

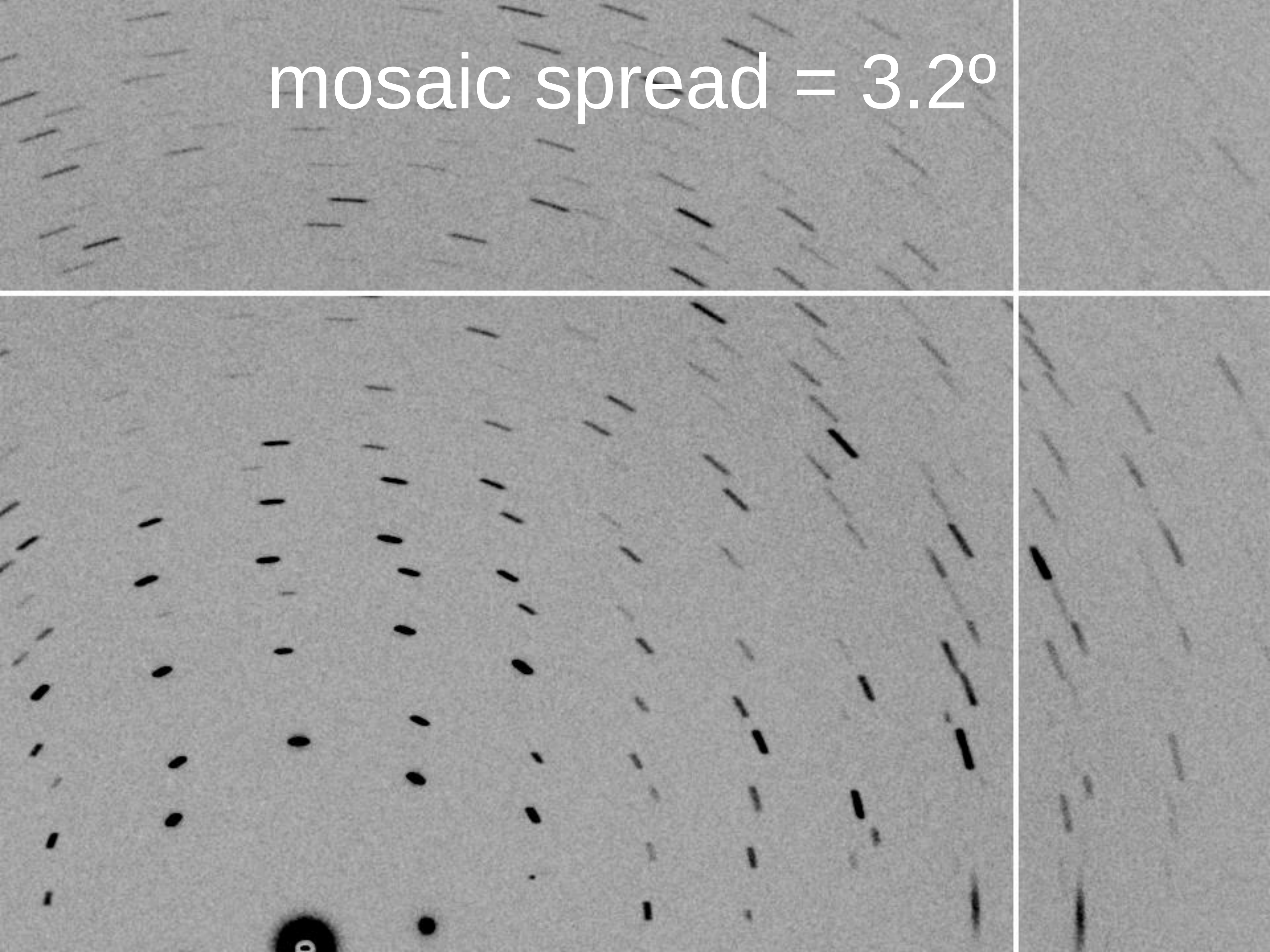

mosaic spread = 0.8º

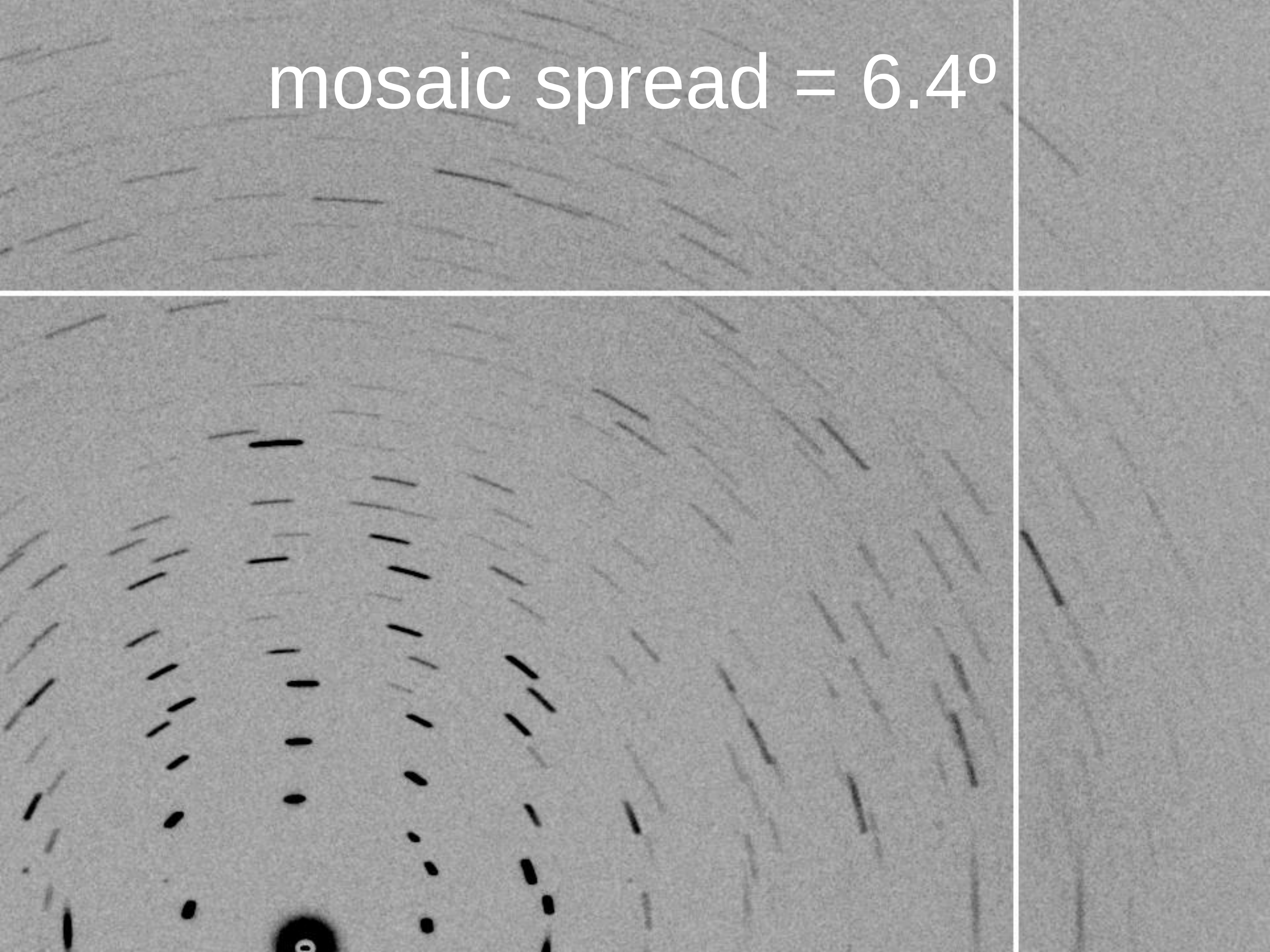

mosaic spread = 1.0º

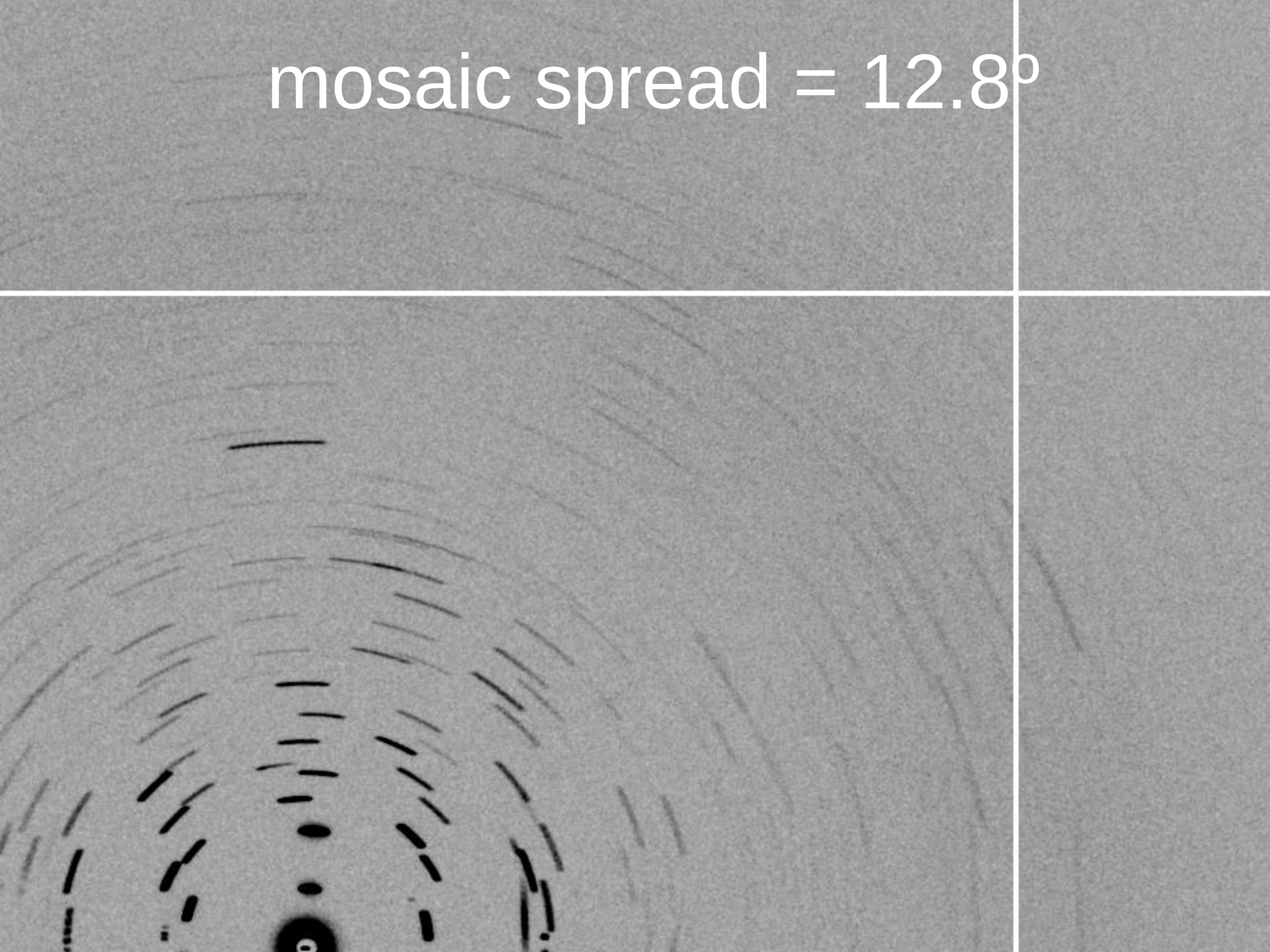

mosaic spread = 1.5º

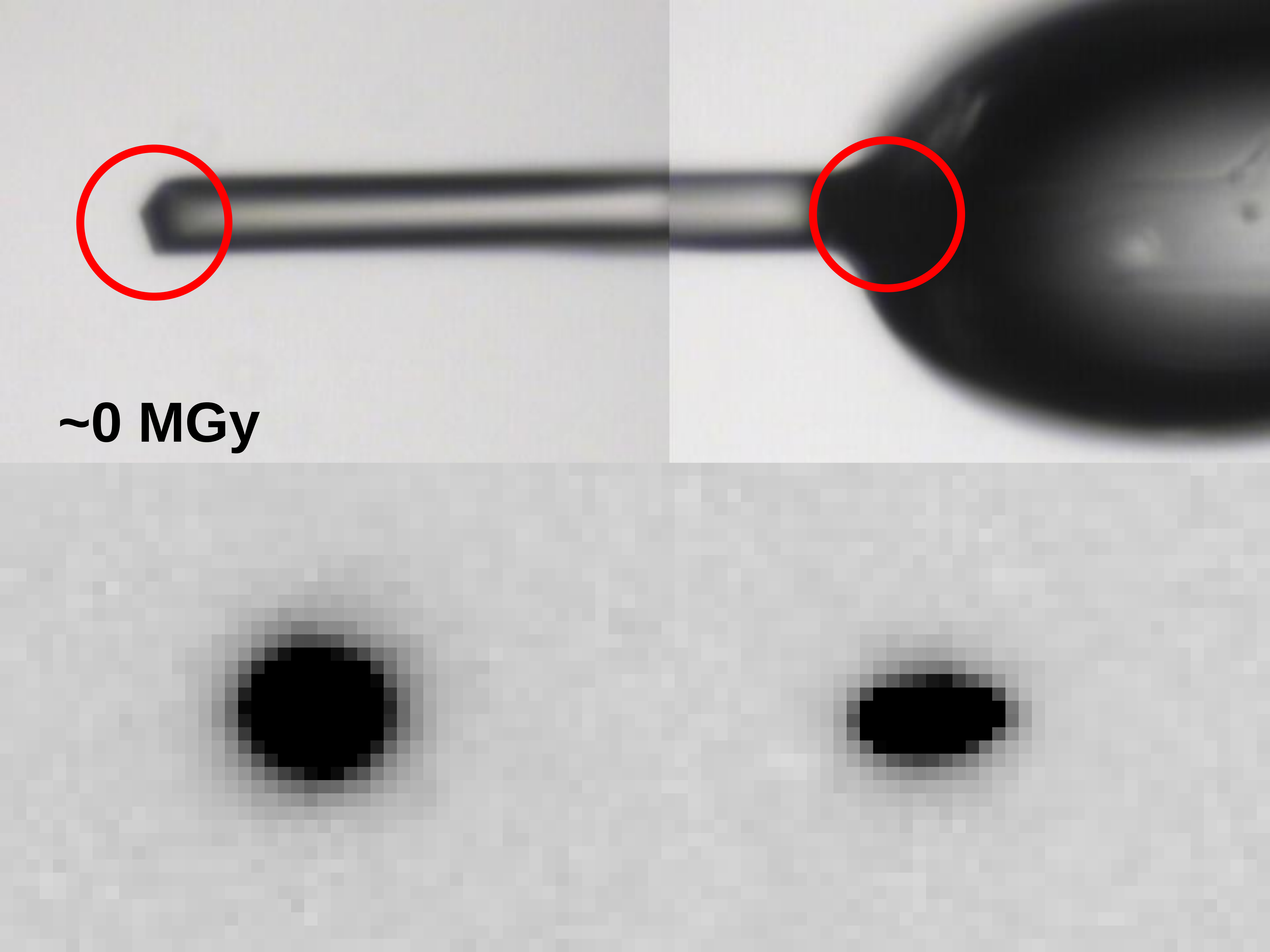

mosaic spread = 2.0º

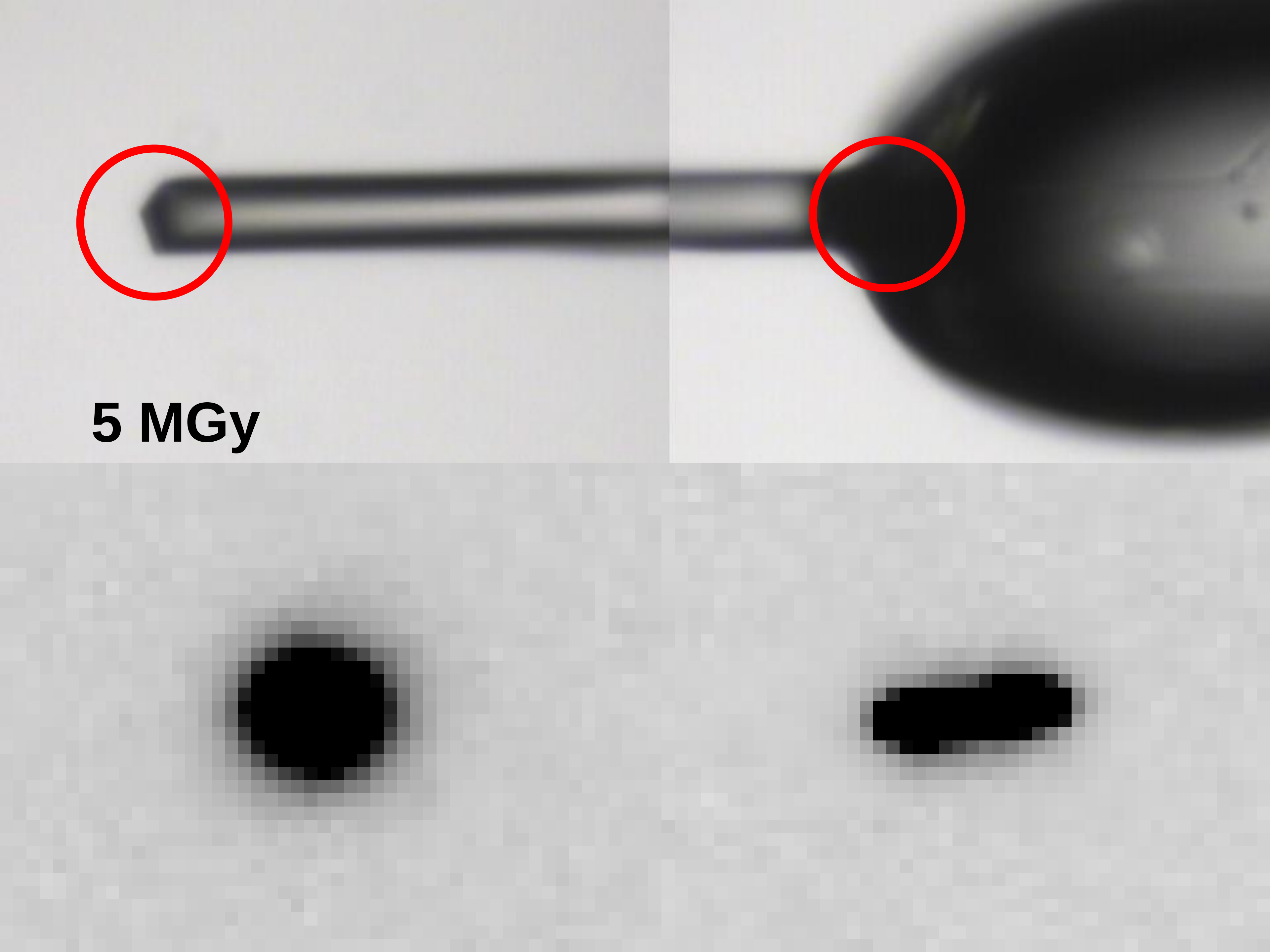

mosaic spread = 2.5º

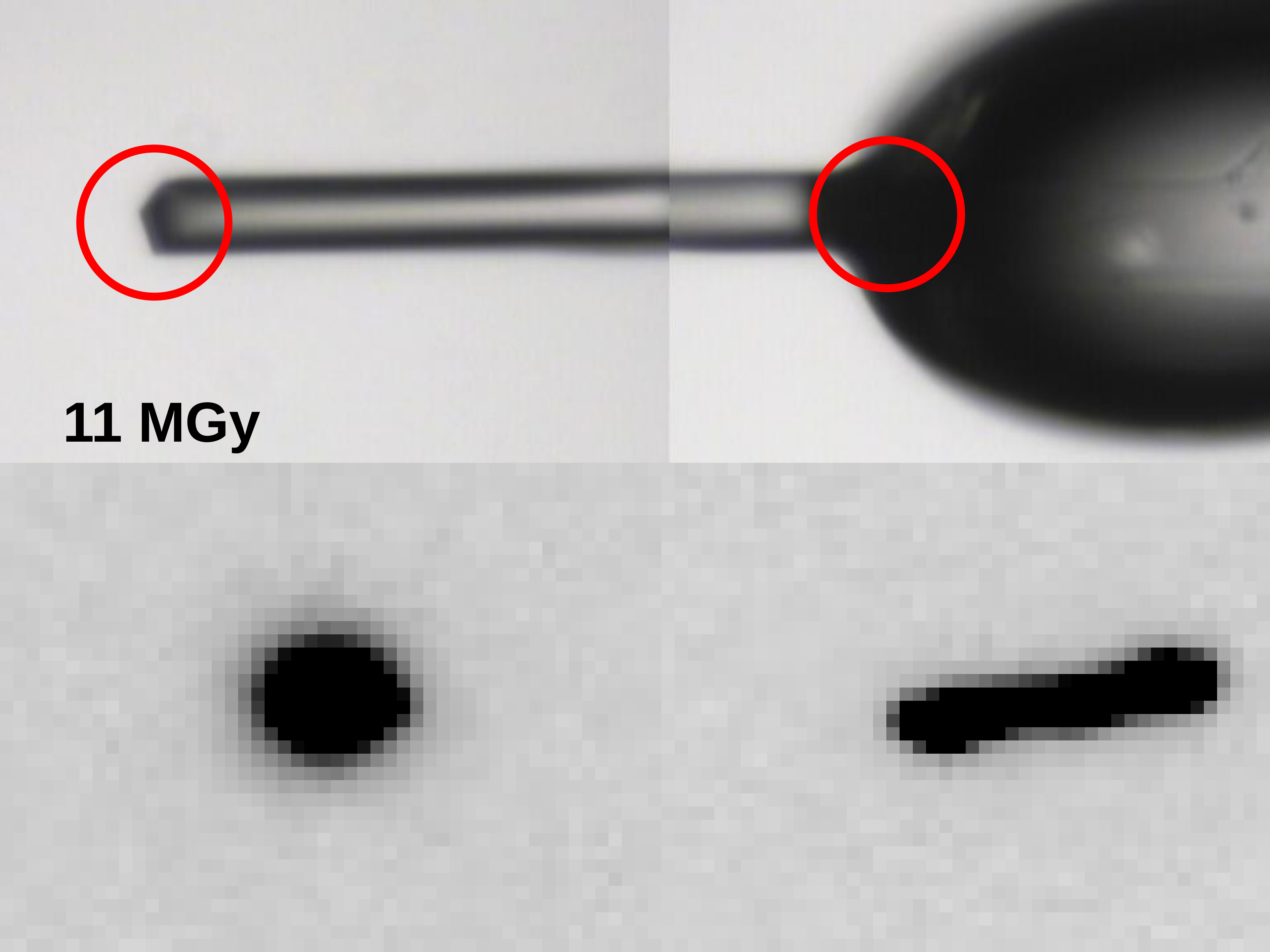

mosaic spread = 3.2º

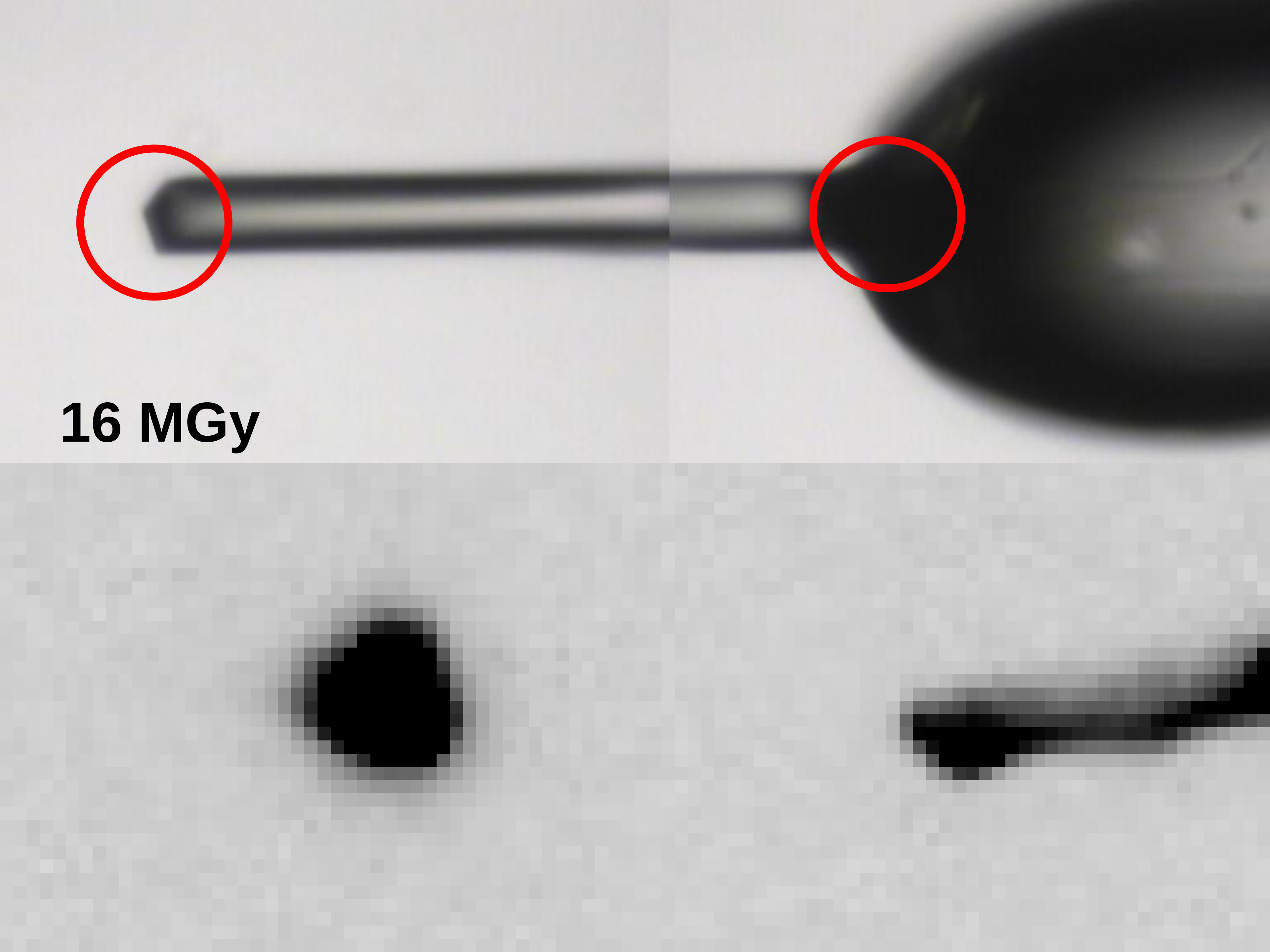

mosaic spread = 6.4º

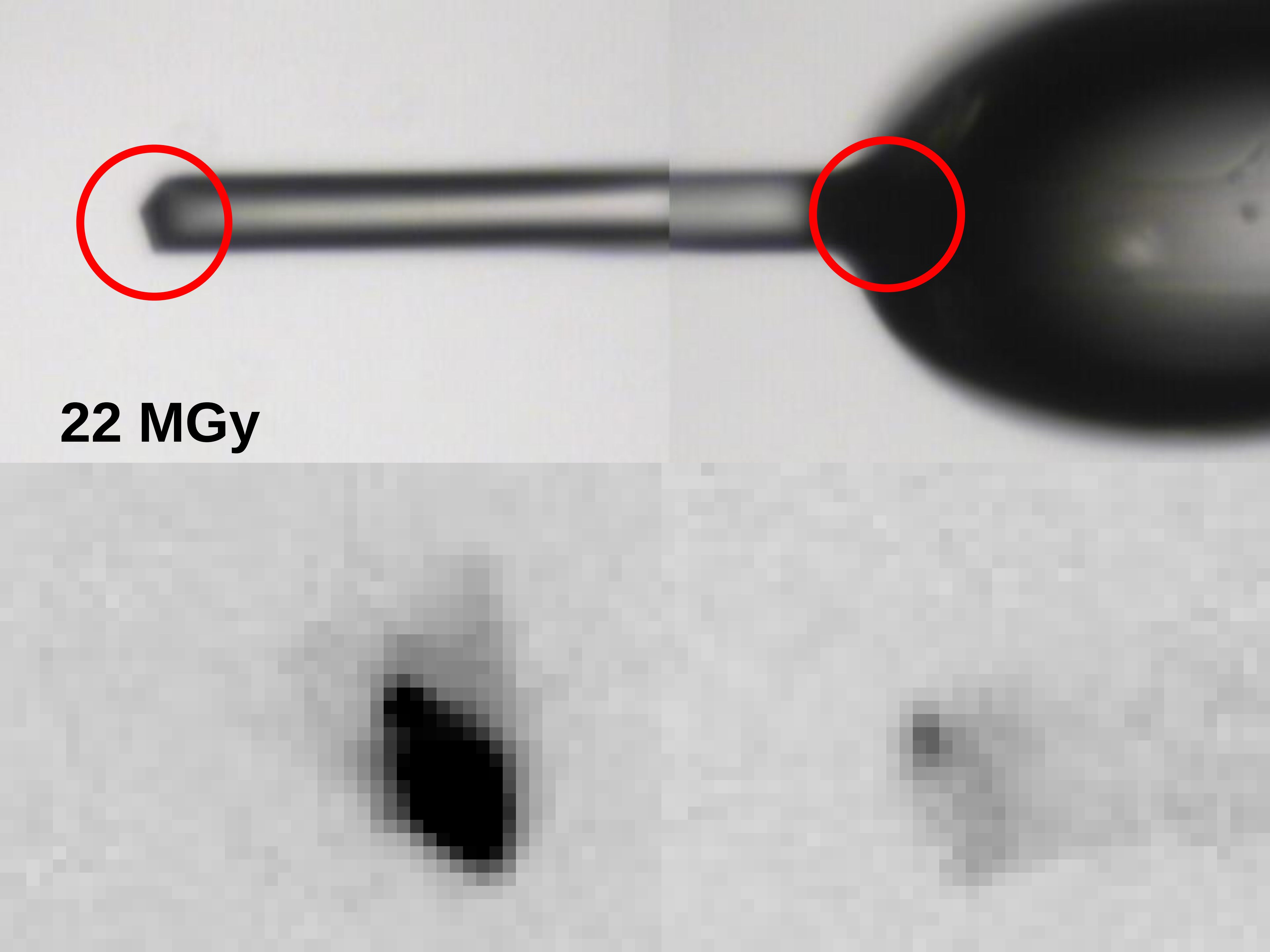

mosaic spread = 12.8º

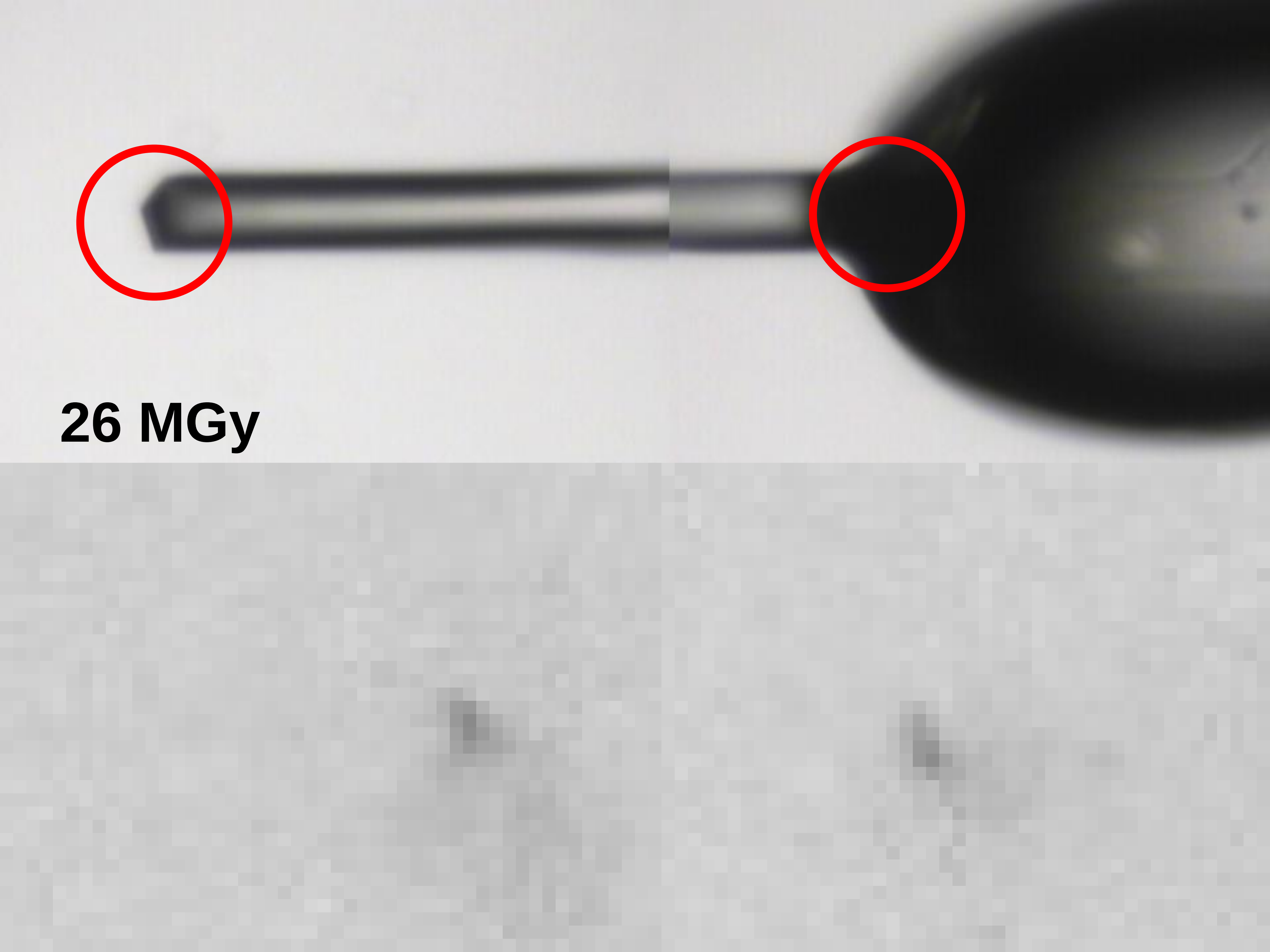

~0 MGy


5 MGy


11 MGy

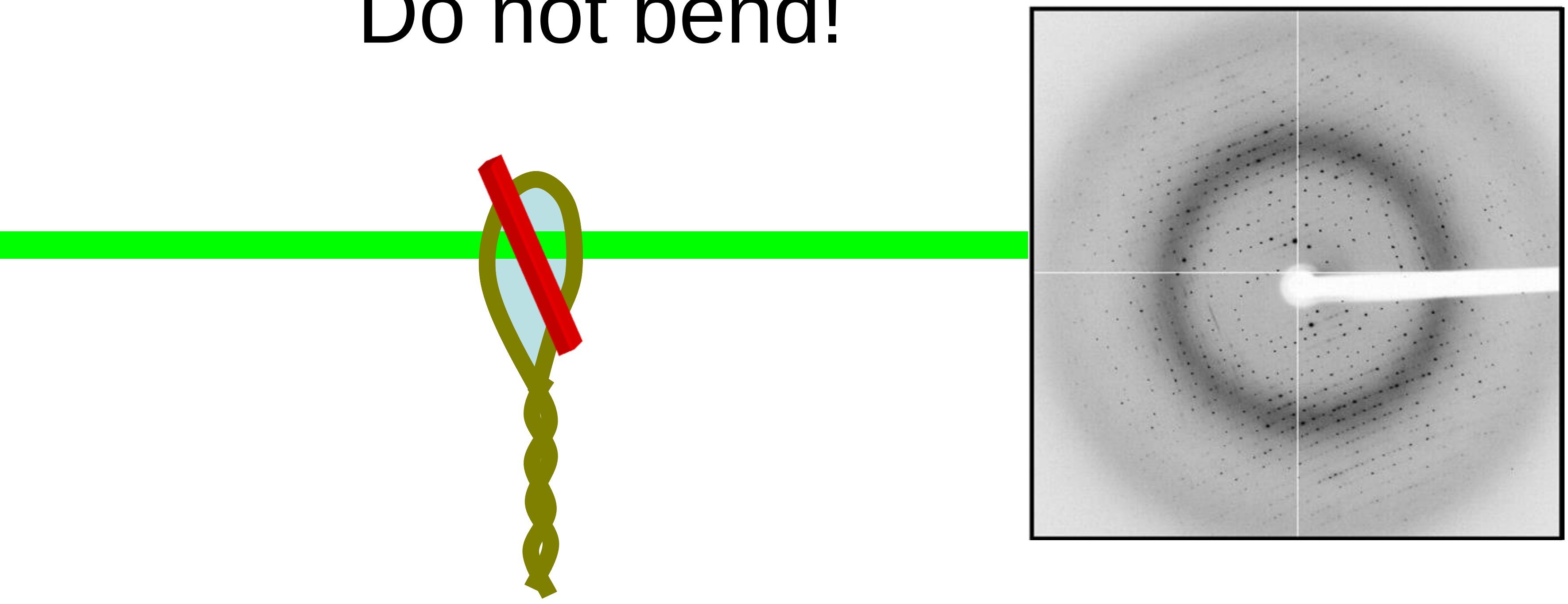

16 MGy


22 MGy

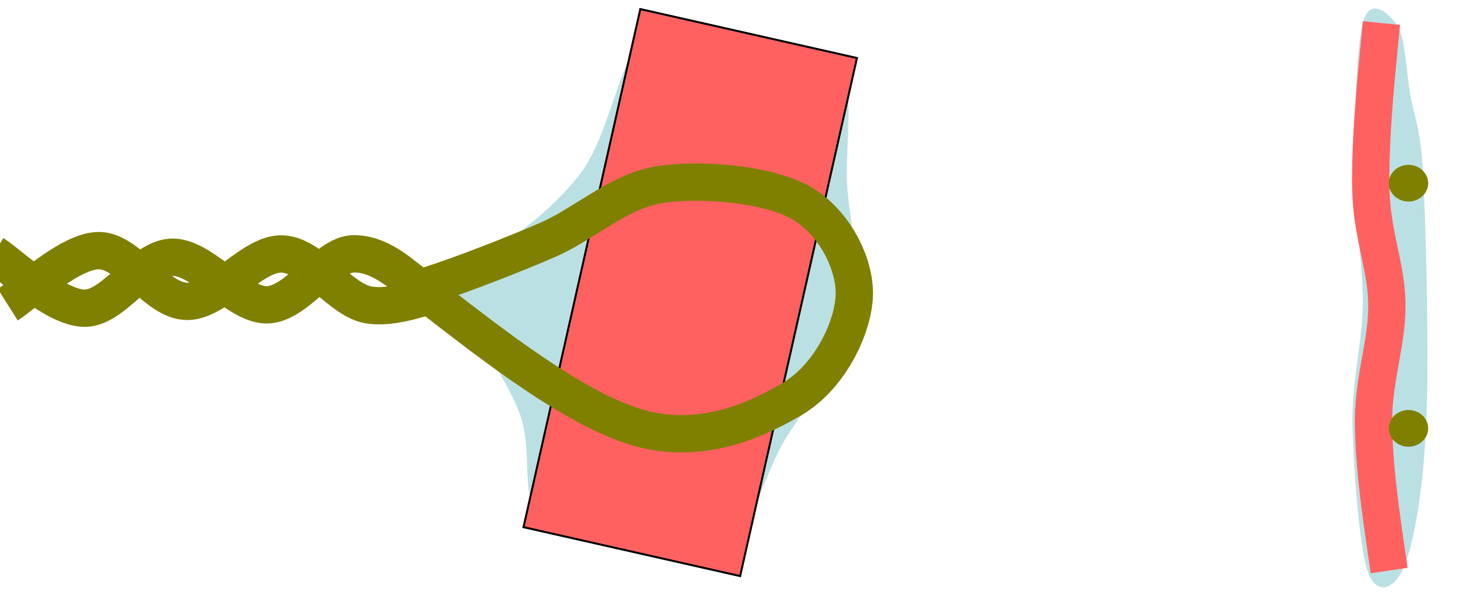

26 MGy

shoot the whole crystal

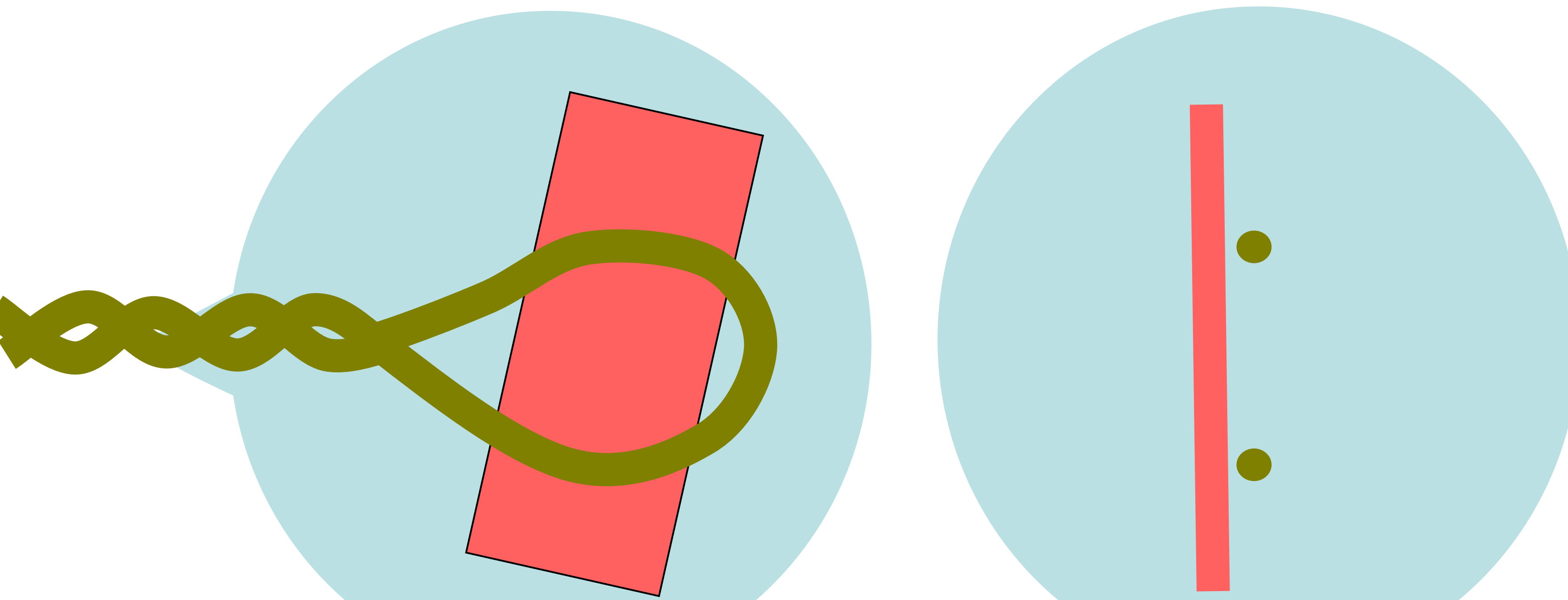
Do not bend!



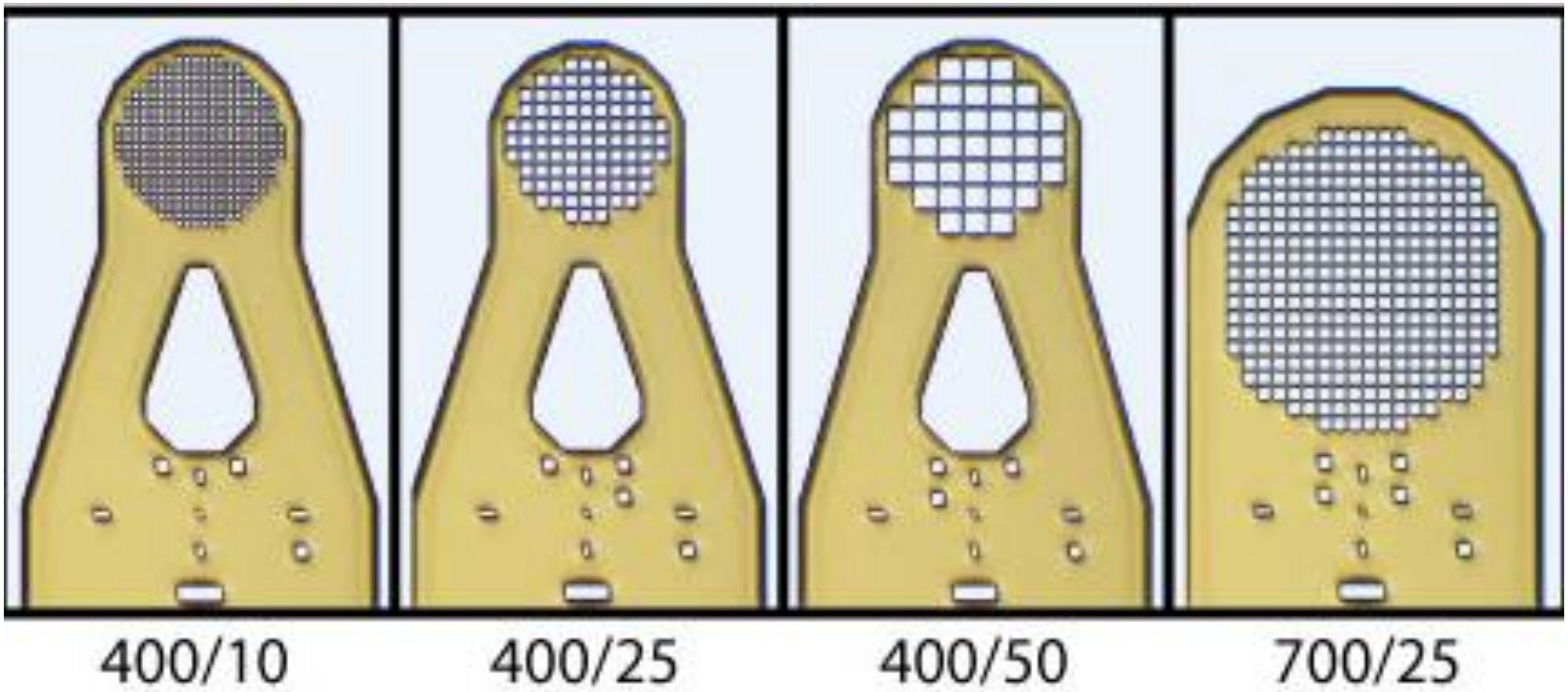
platy crystals



platy crystals

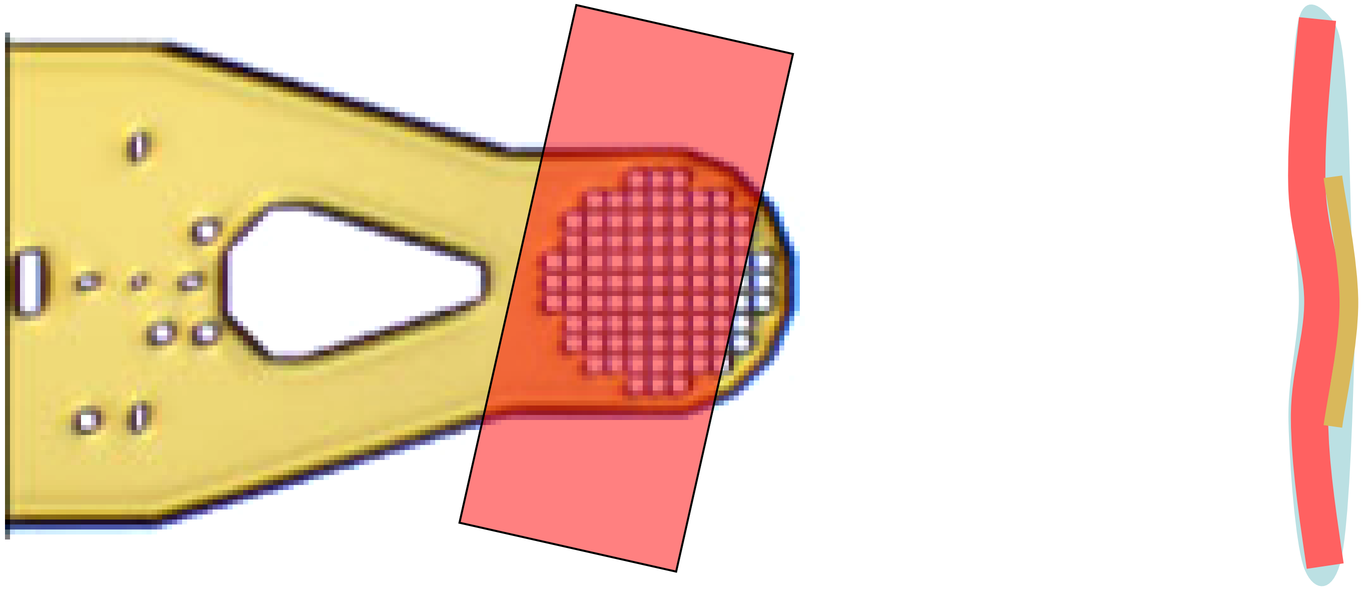


MiTeGen MicroMesh grids

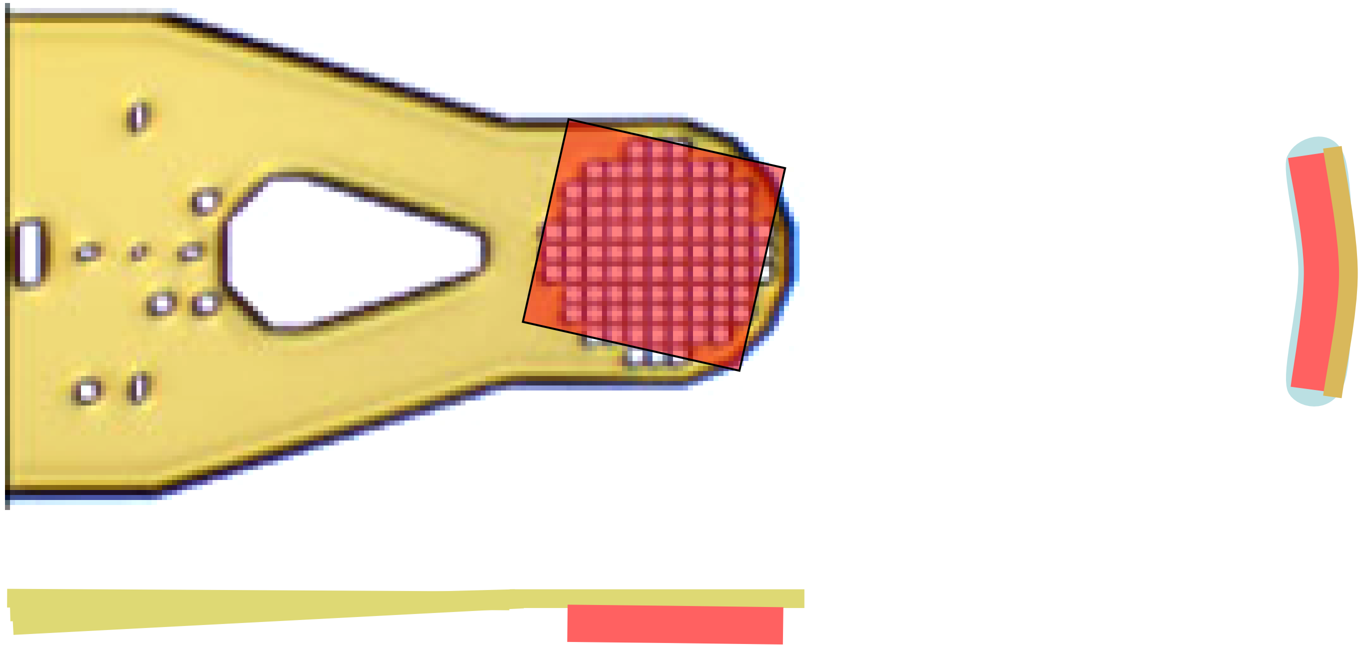


http://www.mitegen.com/products/micromeshes/micromeshes.shtml

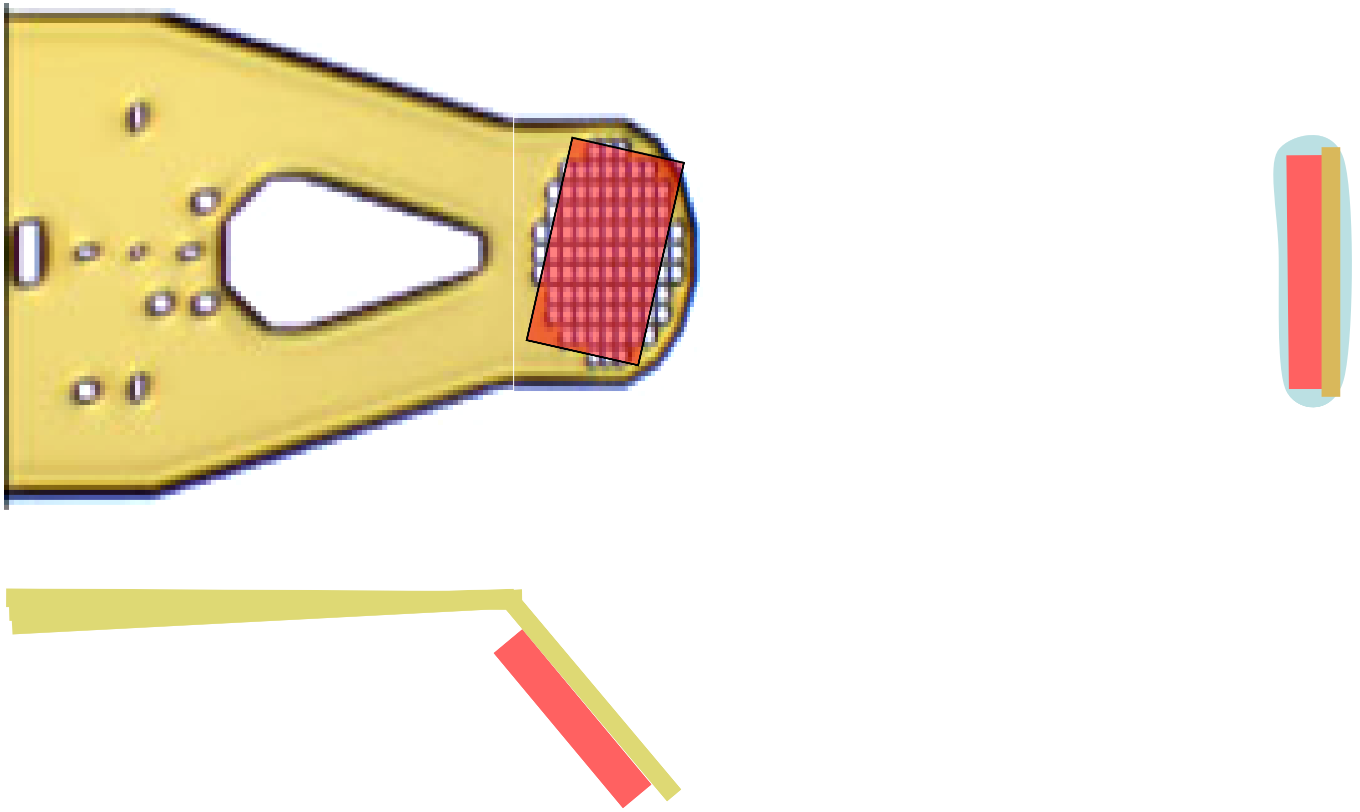
platy crystals



platy crystals



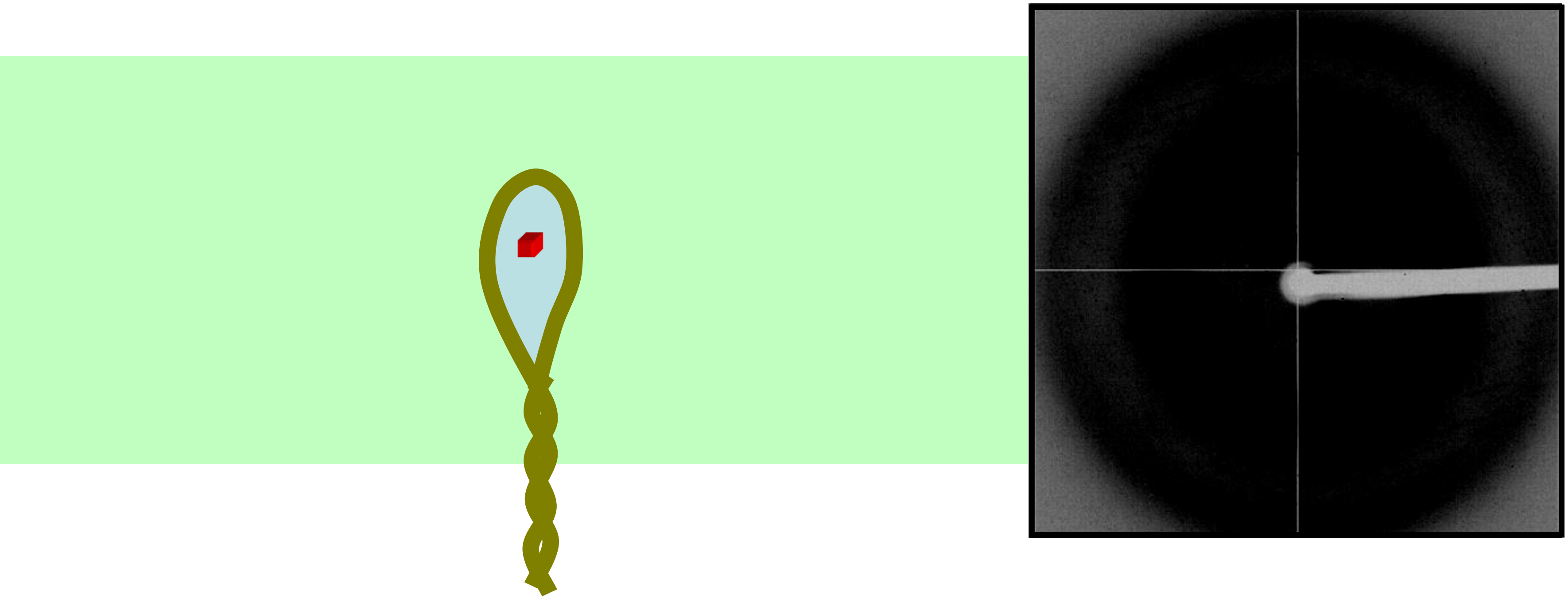
platy crystals



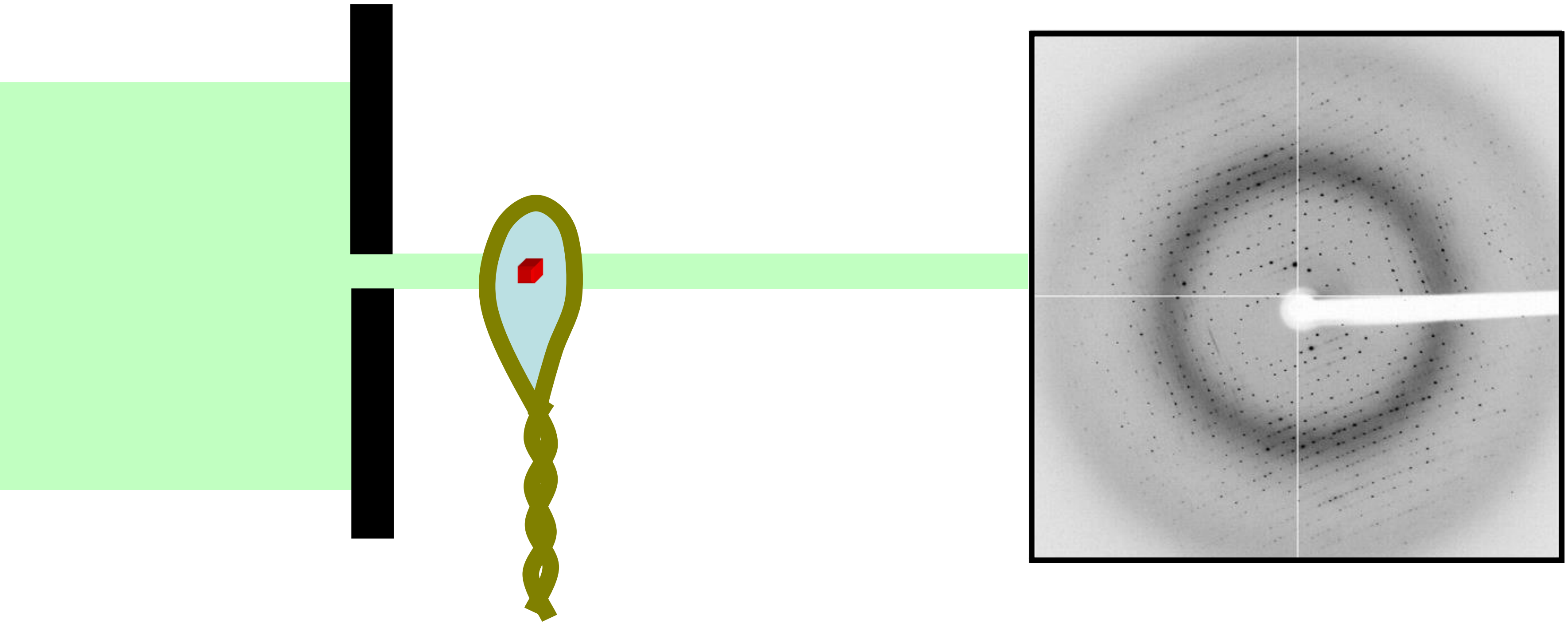
beam size vs xtal size

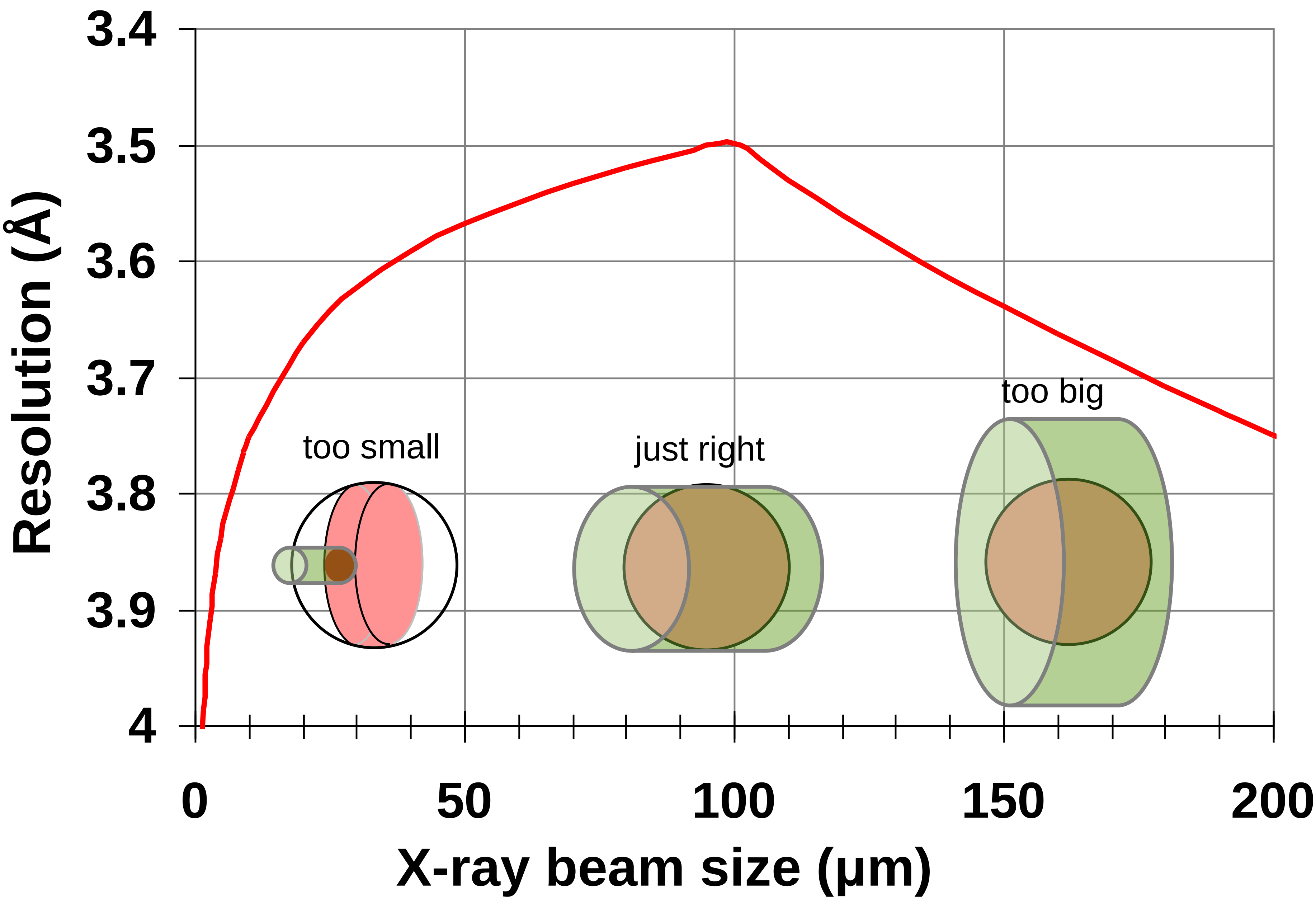
1. Put your crystal into the beam
2. Shoot the whole crystal
3. Shoot nothing but the crystal

shoot nothing but the crystal



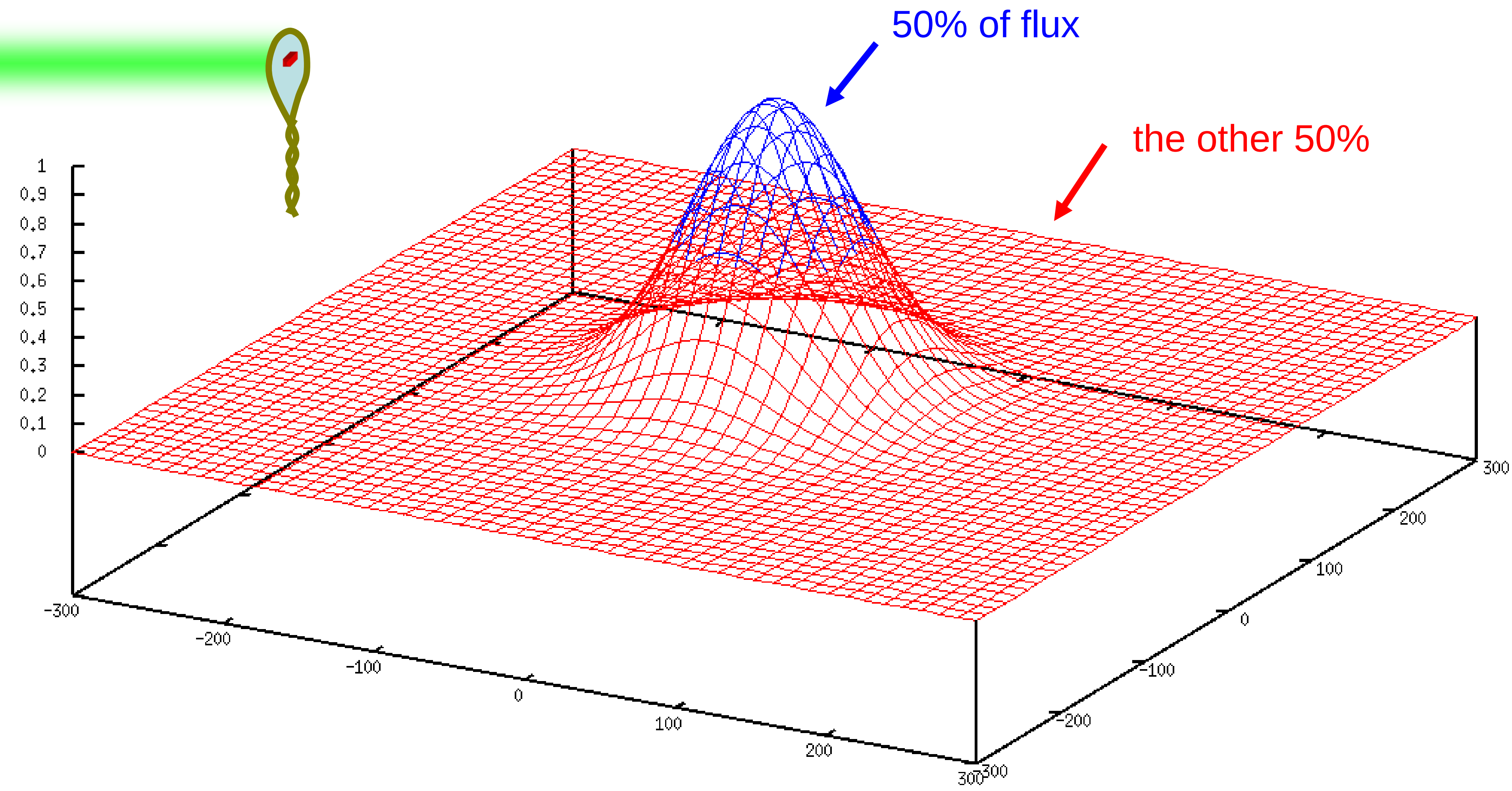
shoot nothing but the crystal



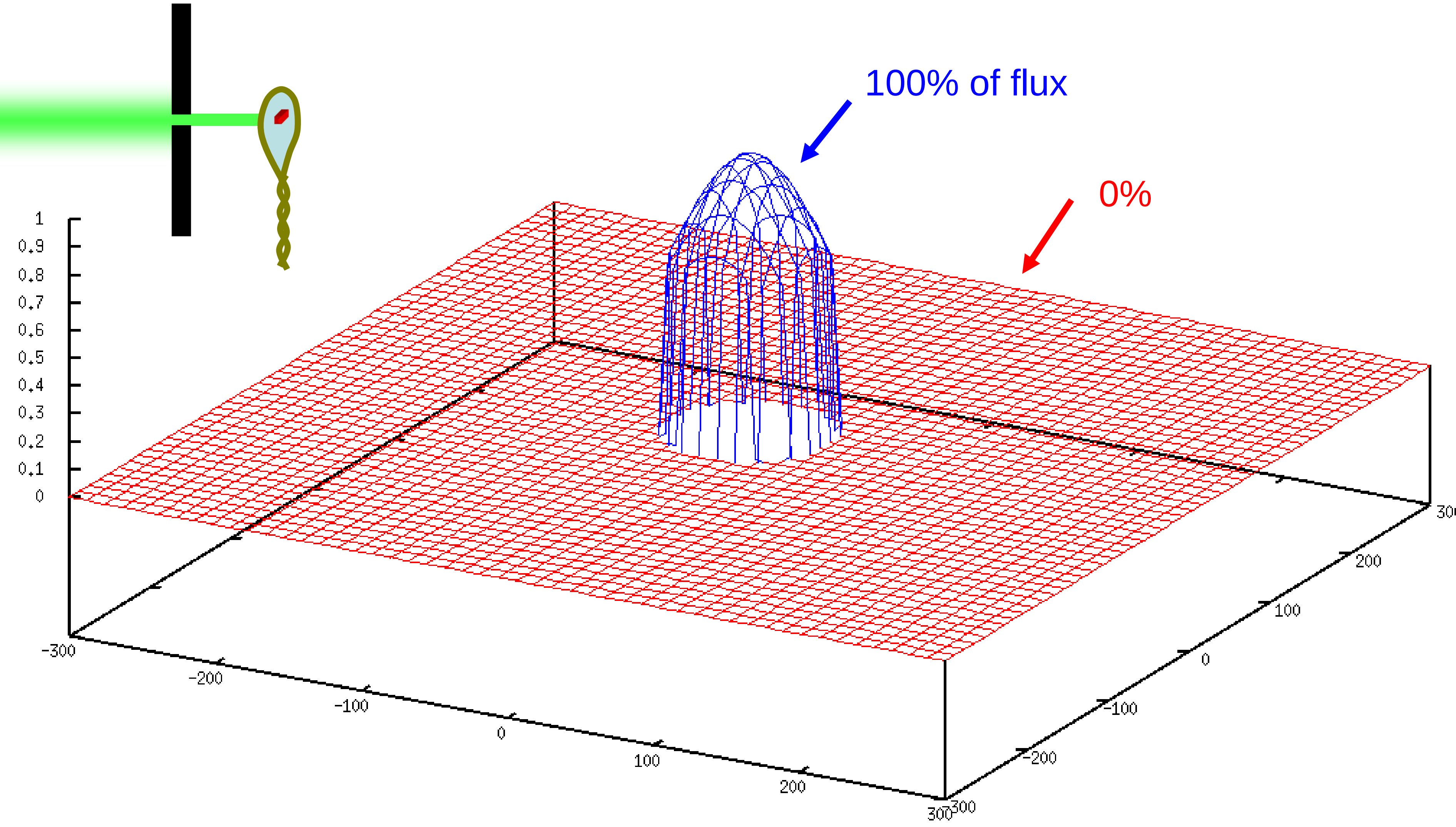


3.4
3.5
3.6
3.7
3.8
3.9
4
Resolution (Å)
too small
just right
too big
0
50
100
150
200
X-ray beam size (μm)

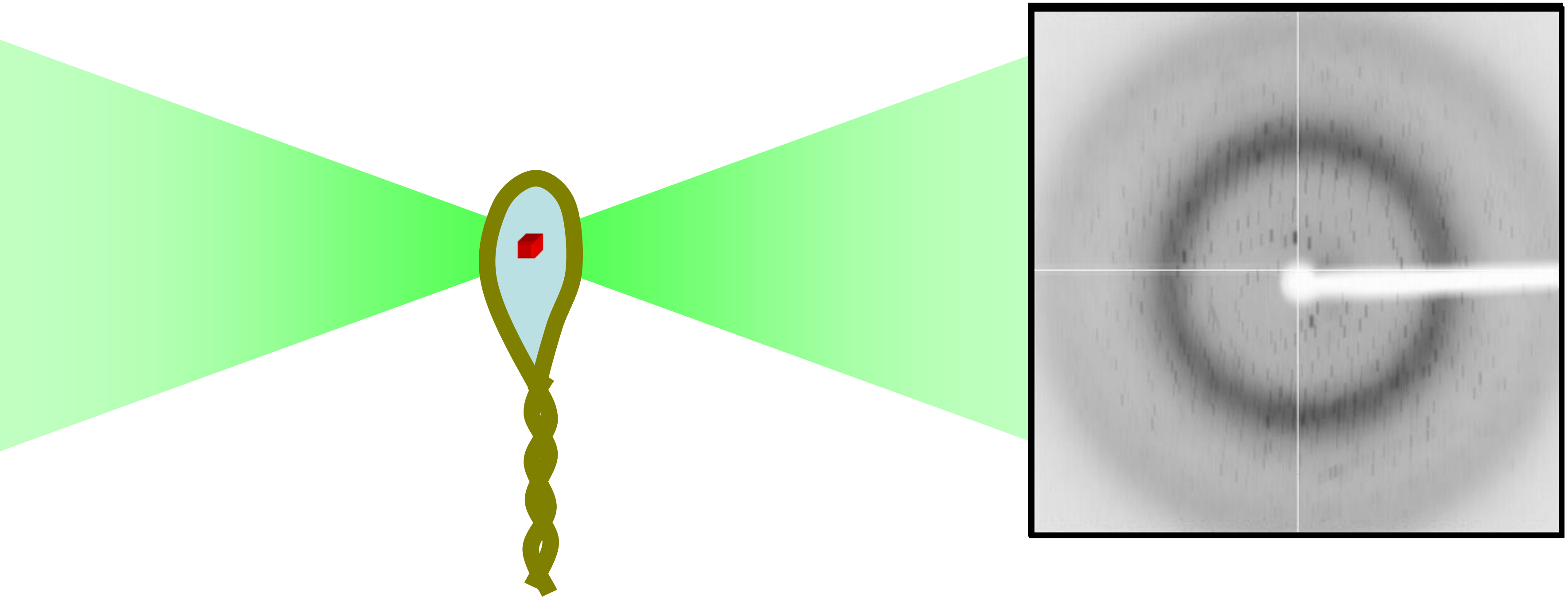
Gaussian beam



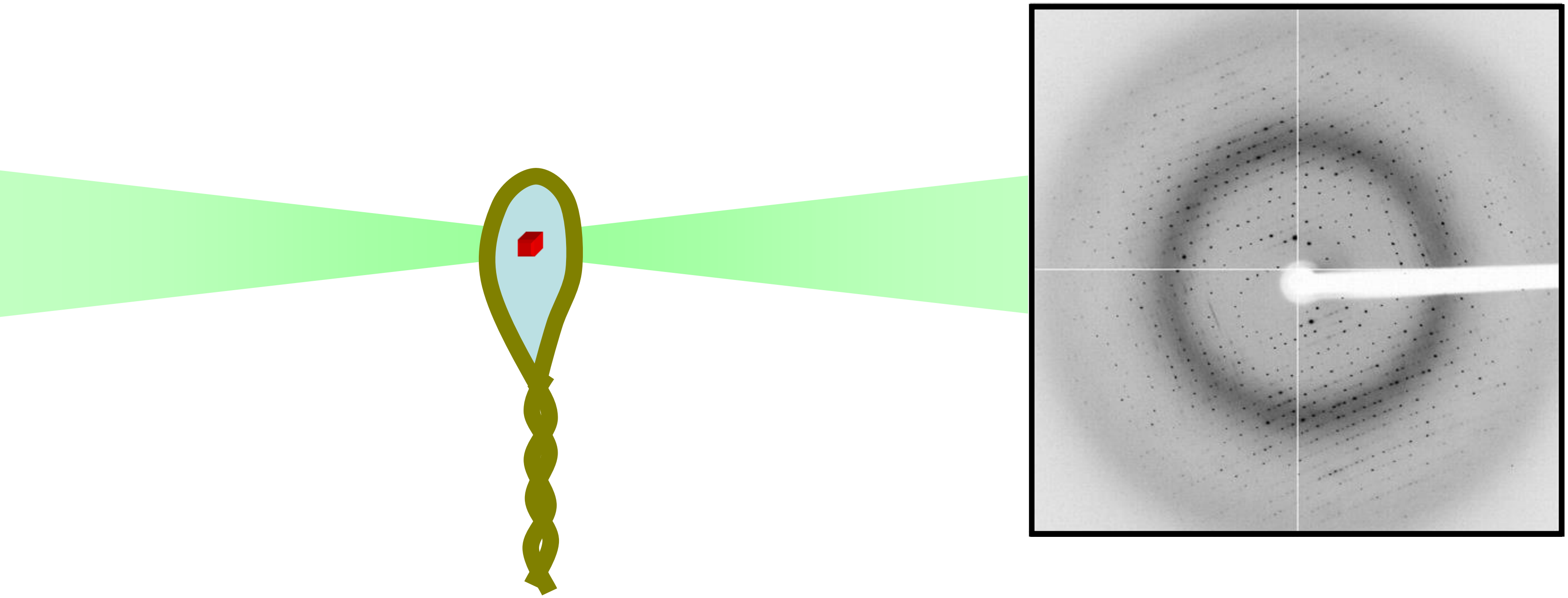
Collimated beam



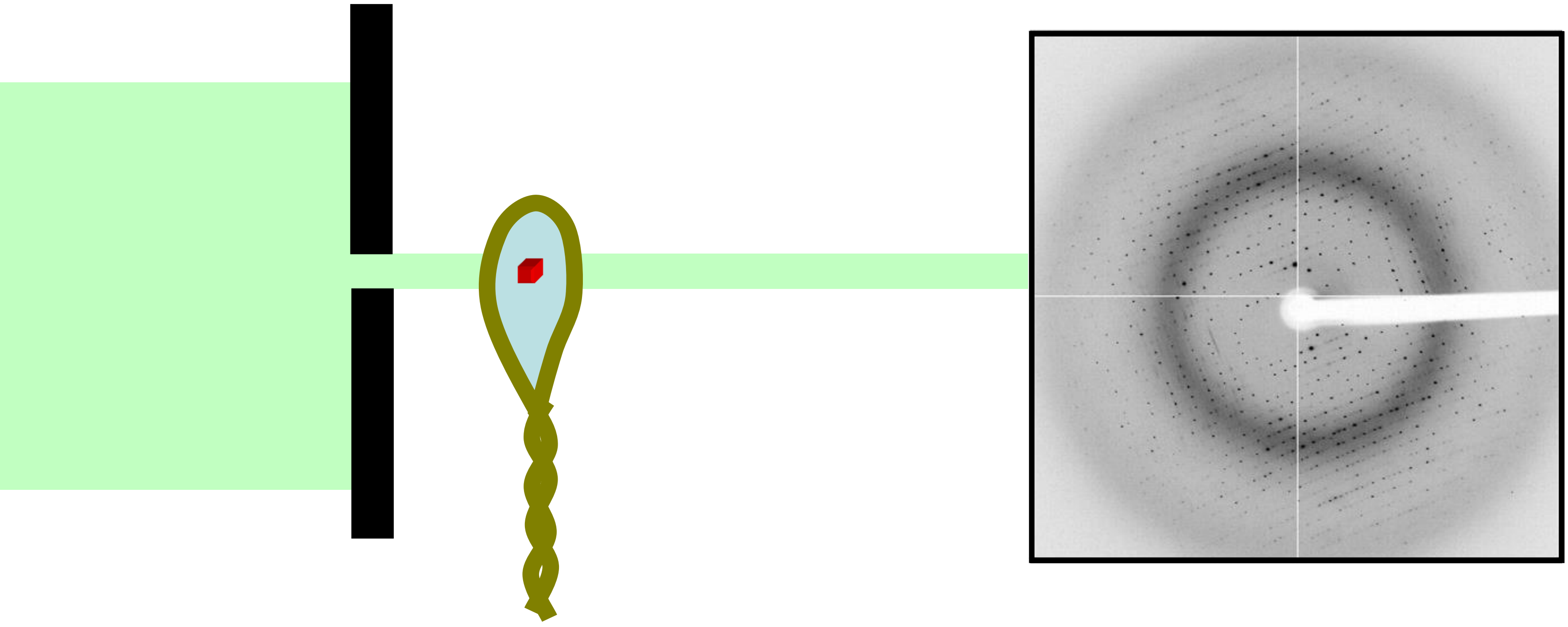
shoot nothing but the crystal



shoot nothing but the crystal



shoot nothing but the crystal



X-ray scattering “rules”:

Optimum: support thickness = crystal thickness

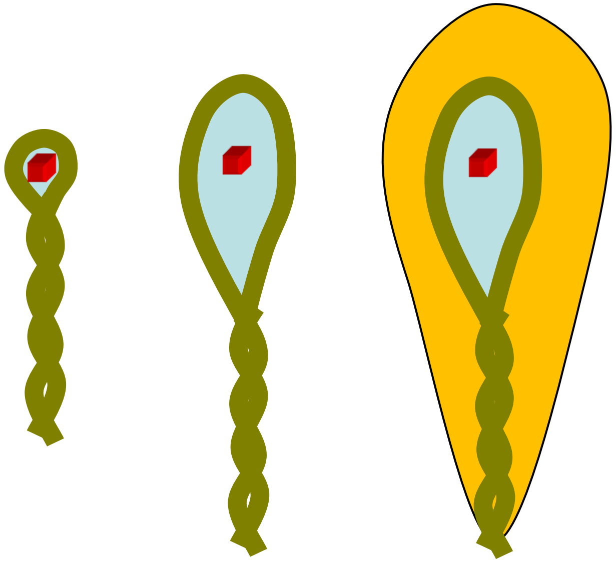
1 μm crystal ≈ 1 μm water

≈ 1 μm plastic

≈ 0.1 μm glass

≈ 1000 μm air

~ 50 mm He



≈ 370 mm sat water vapor @ 4C

beam size vs xtal size

1. Put your crystal into the beam
2. Shoot the whole crystal
3. Shoot nothing but the crystal
4. Back off!

Mosquito tips cost $0.15 each

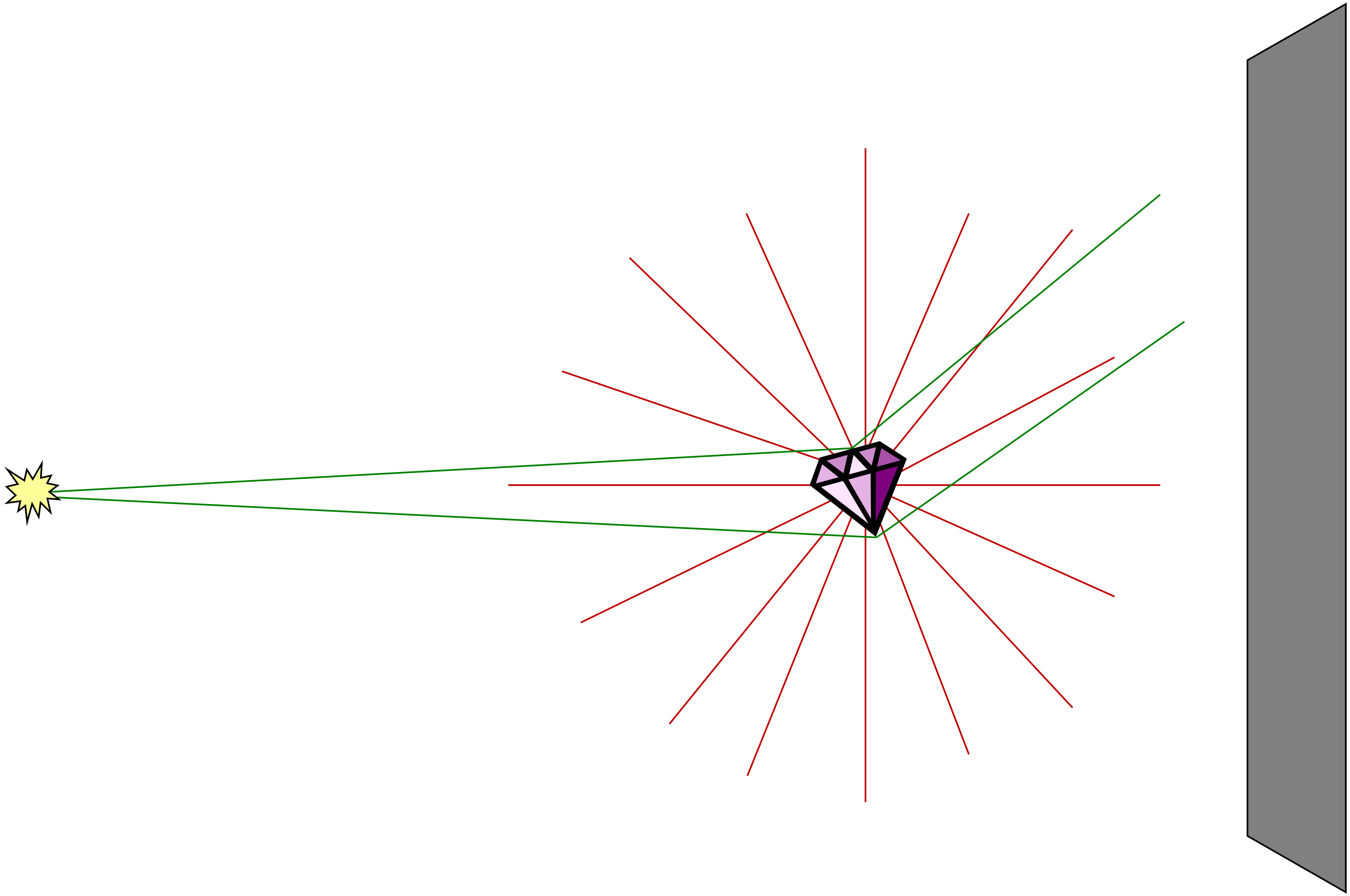
So do Pilatus pixels!

We bought 6,224,001

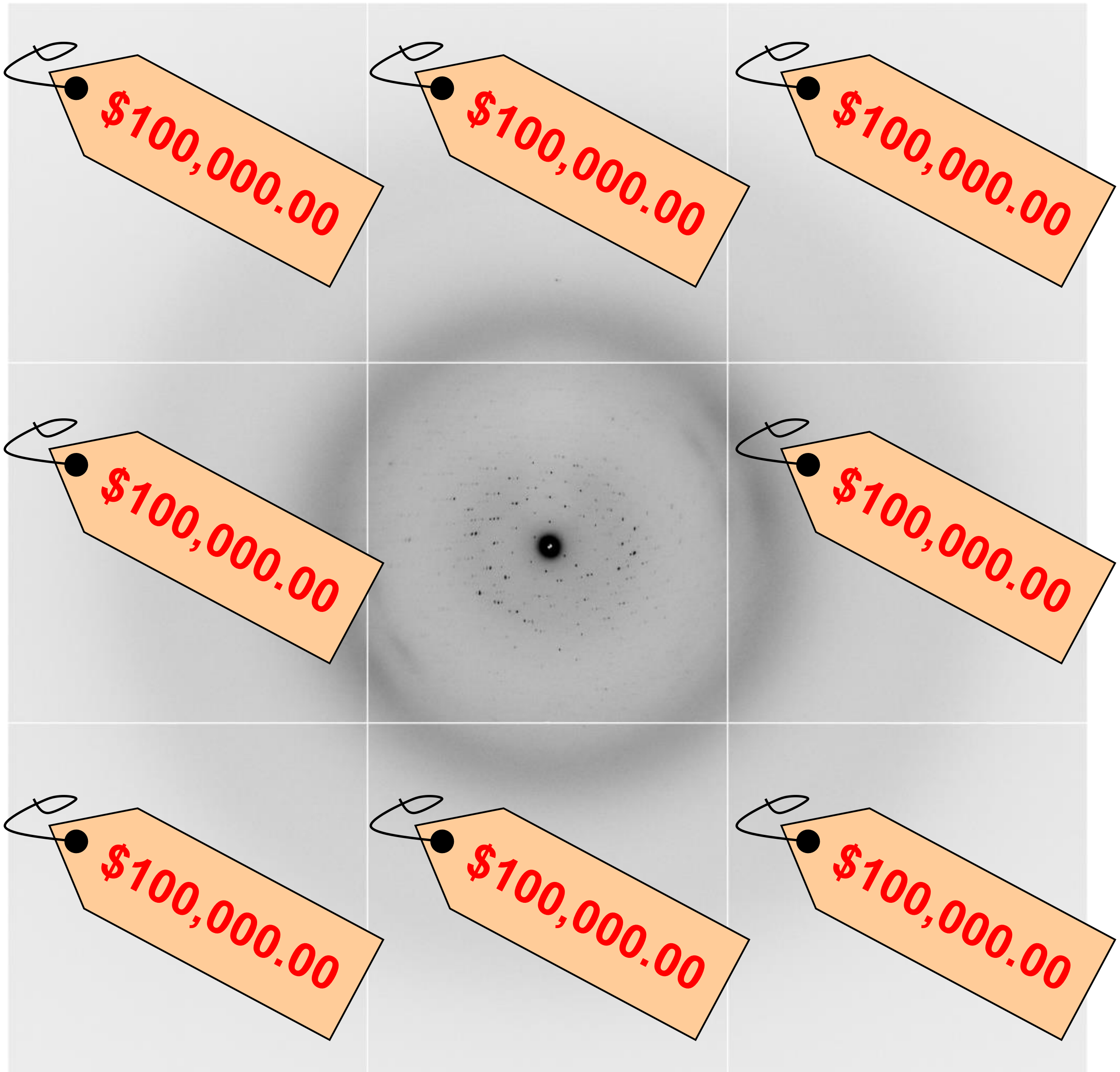
Use them!

(they are reusable)

Background scattering



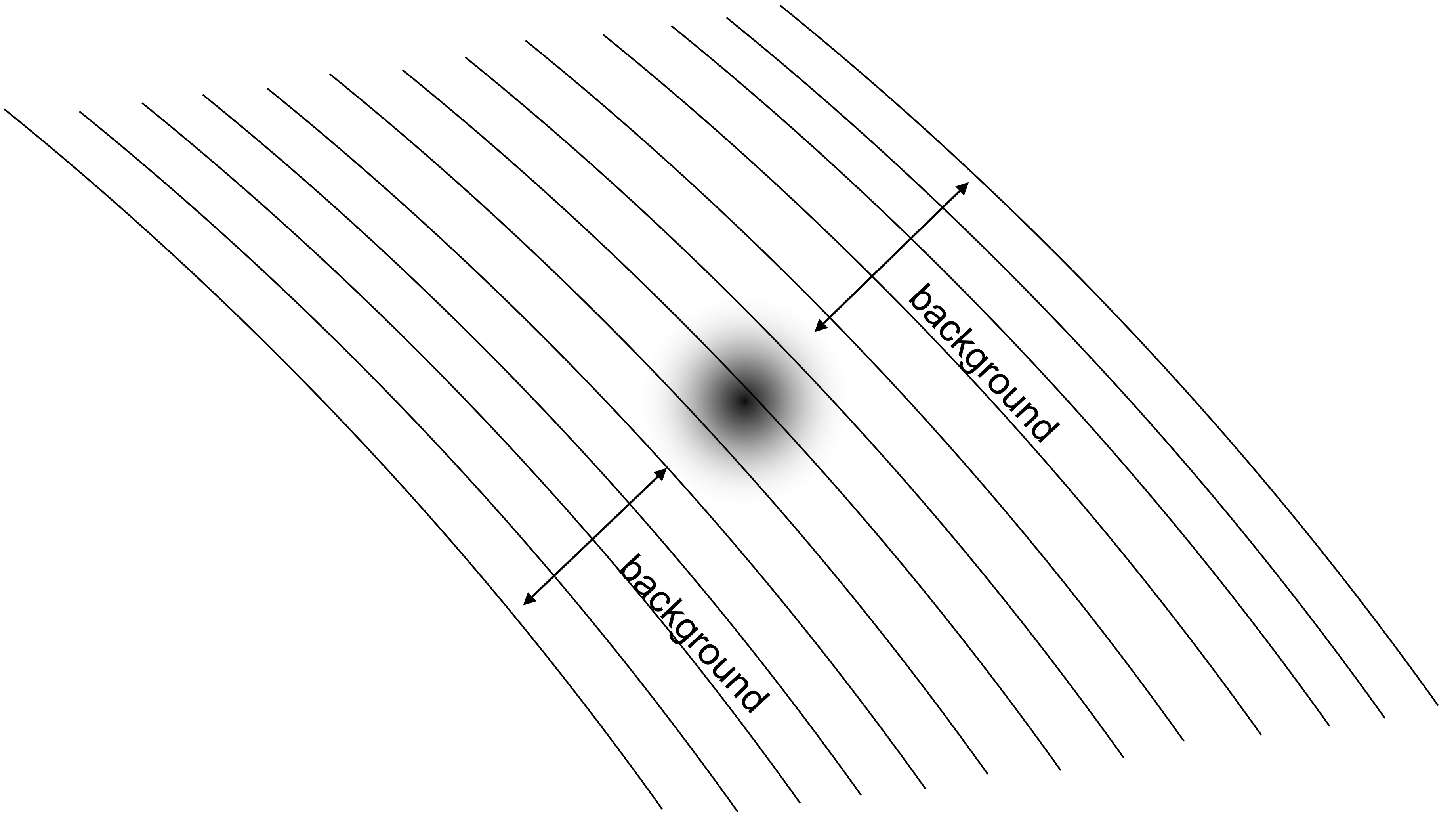
Background scattering



real estate is expensive

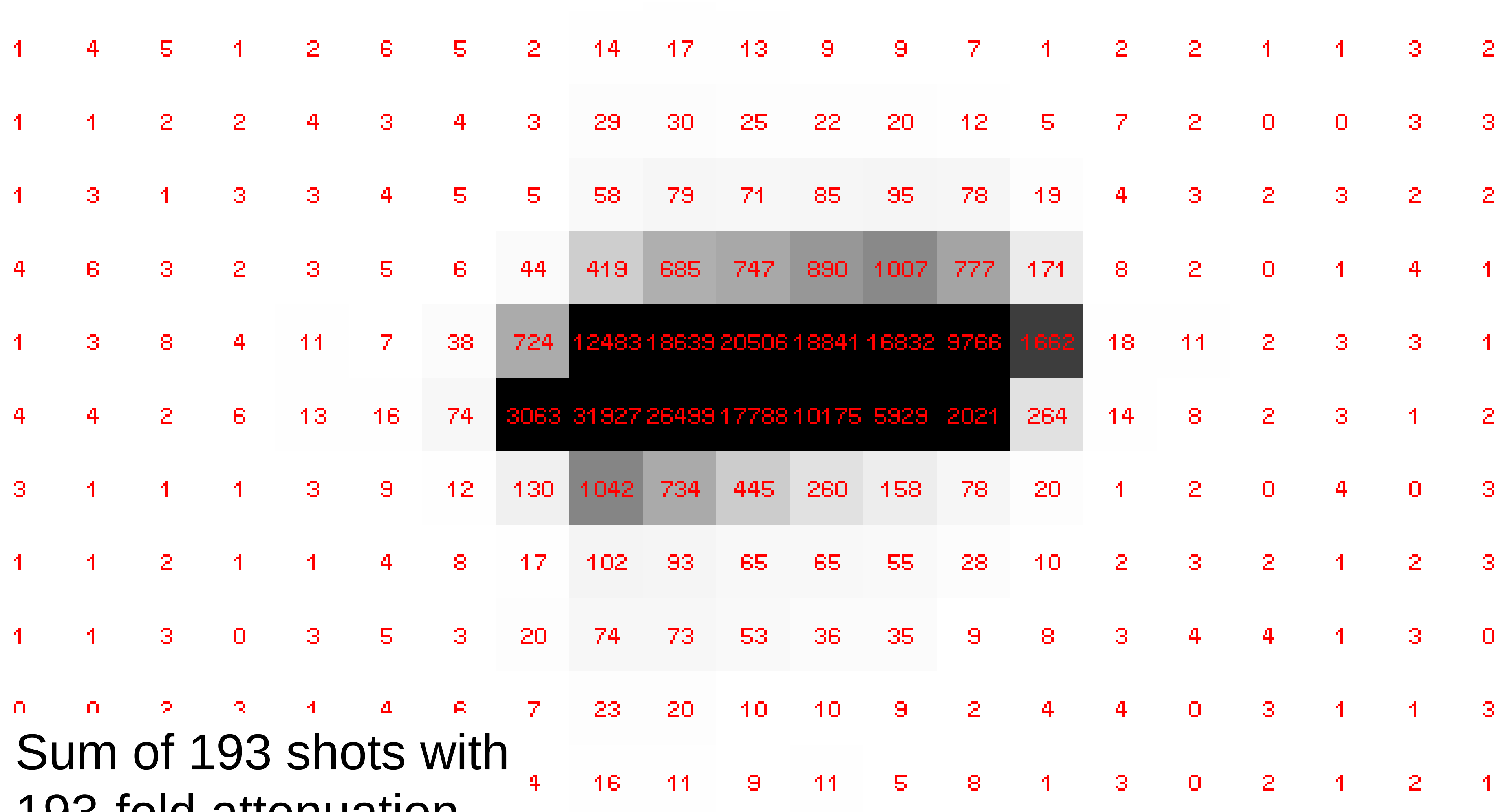
use it!

Fine Slicing



Pflugrath, J. W. (1999)."The finer things in X-ray diffraction data collection", *Acta Cryst. D* **55**, 1718-1725.

Pilatus pile-up for RT MX?
same photons, different speeds



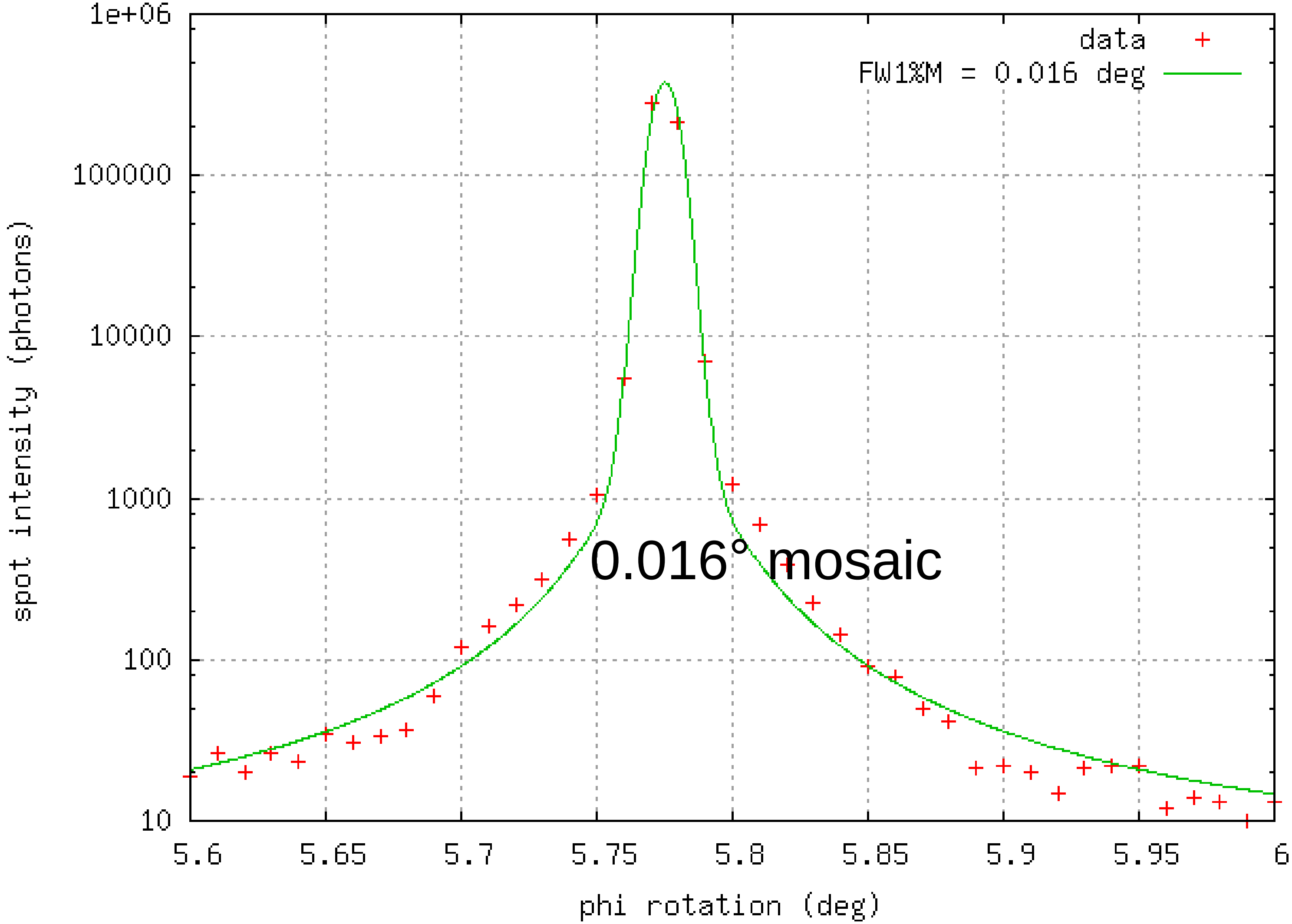
Sum of 193 shots with
193-fold attenuation

Pilatus pile-up for RT MX?
same photons, different speeds

1	0	0	1	2	2	3	5	22	23	14	16	13	9	4	2	3	4	2	2	2
1	2	2	2	4	1	2	10	37	38	37	31	31	18	4	5	2	4	1	1	2
1	3	0	2	2	2	3	15	88	105	101	83	106	95	19	4	2	3	2	4	1
4	3	1	1	2	9	14	60	585	930	980	1014	1115	893	226	5	2	0	1	2	3
1	2	4	6	4	14	66	738	1680	1557	1398	1278	1306	1174	1052	31	9	7	7	5	2
5	1	2	6	7	17	84	1198	1452	1340	1281	1305	1442	1094	211	13	6	2	4	4	5
5	2	4	5	4	3	14	132	1112	831	480	255	147	71	14	5	4	6	3	3	0
2	0	2	2	4	1	5	21	137	147	94	109	53	31	5	4	1	1	4	3	1
0	3	2	2	5	6	6	17	108	104	64	48	39	12	5	3	4	2	4	0	0
1	3	2	2	2	1	2	4	17	19	10	16	9	6	2	3	3	2	0	6	1
					1	0	3	15	12	5	7	4	4	1	2	1	2	0	0	1

1 shot with
no attenuation

Pilatus pile-up for RT MX?
same photons, different speeds

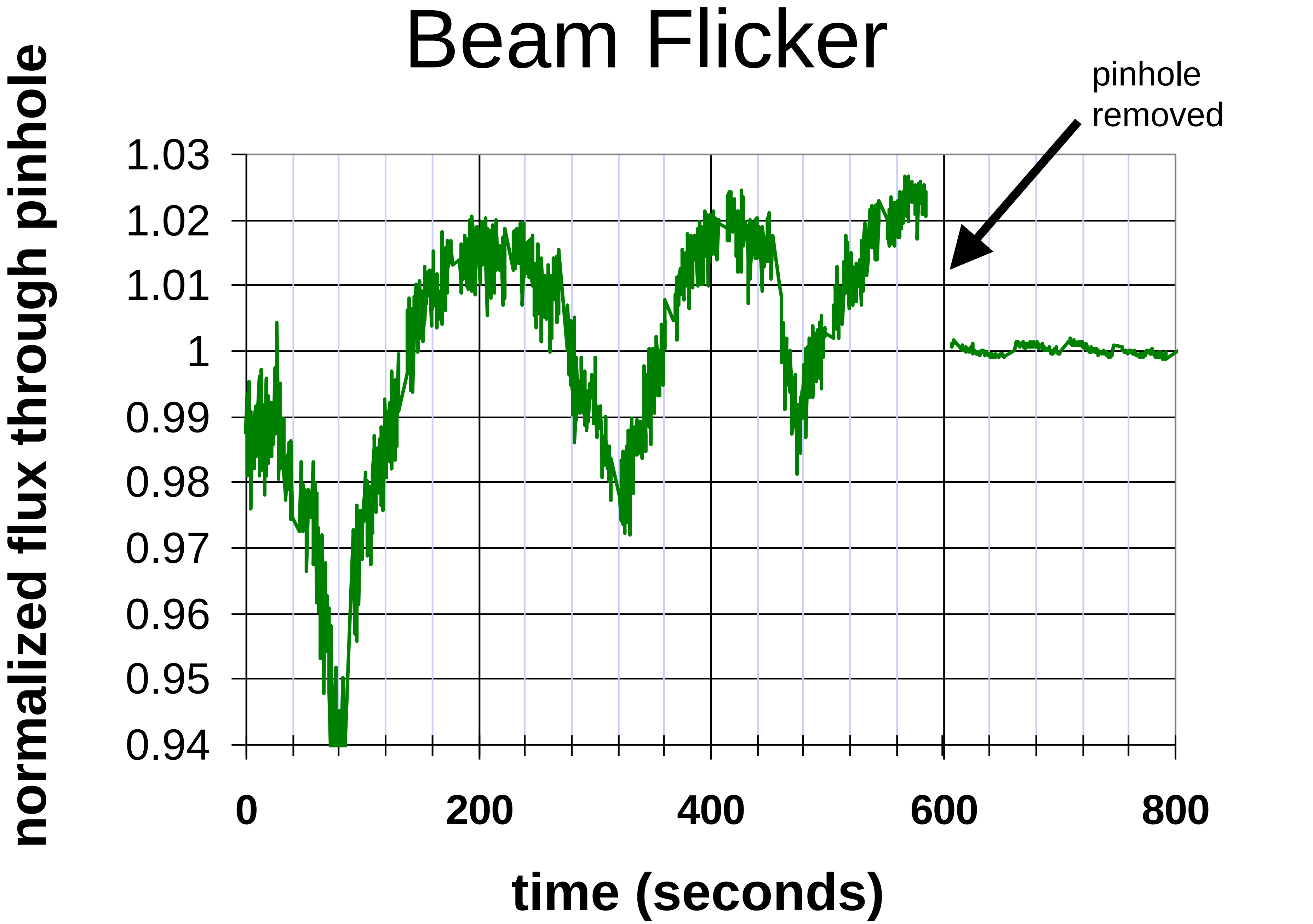


Classes of error in MX

Dependence on signal

Time

	none	sqrt	proportional
none	CCD Read-out	Photon counting	Detector calibration attenuation partiality Non-isomorphism Radiation damage
1/sqrt			Beam flicker
1/prop.			Shutter jitter Sample vibration Pile-up



Beam Flicker
pinhole
removed
normalized flux through pinhole
1.03
1.02
1.01
1
0.99
0.98
0.97
0.96
0.95
0.94
0
200
400
600
800
time (seconds)

This is an Aperture!

Classes of error in MX

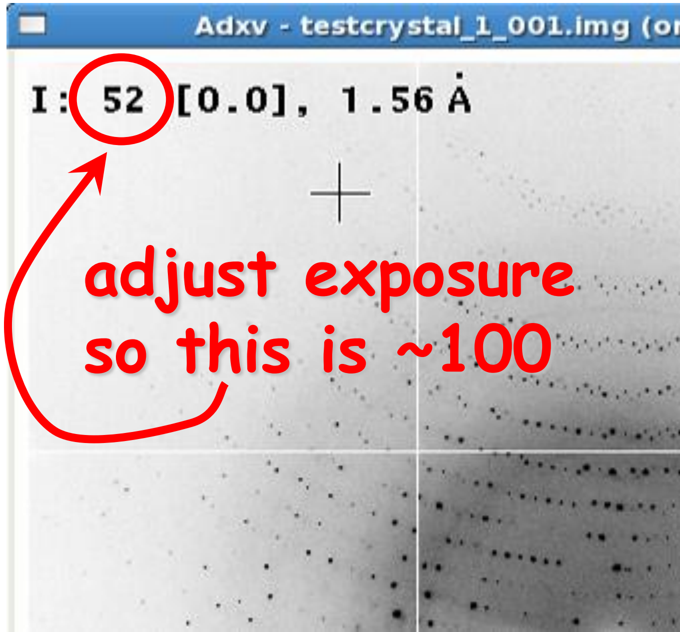
Dependence on signal

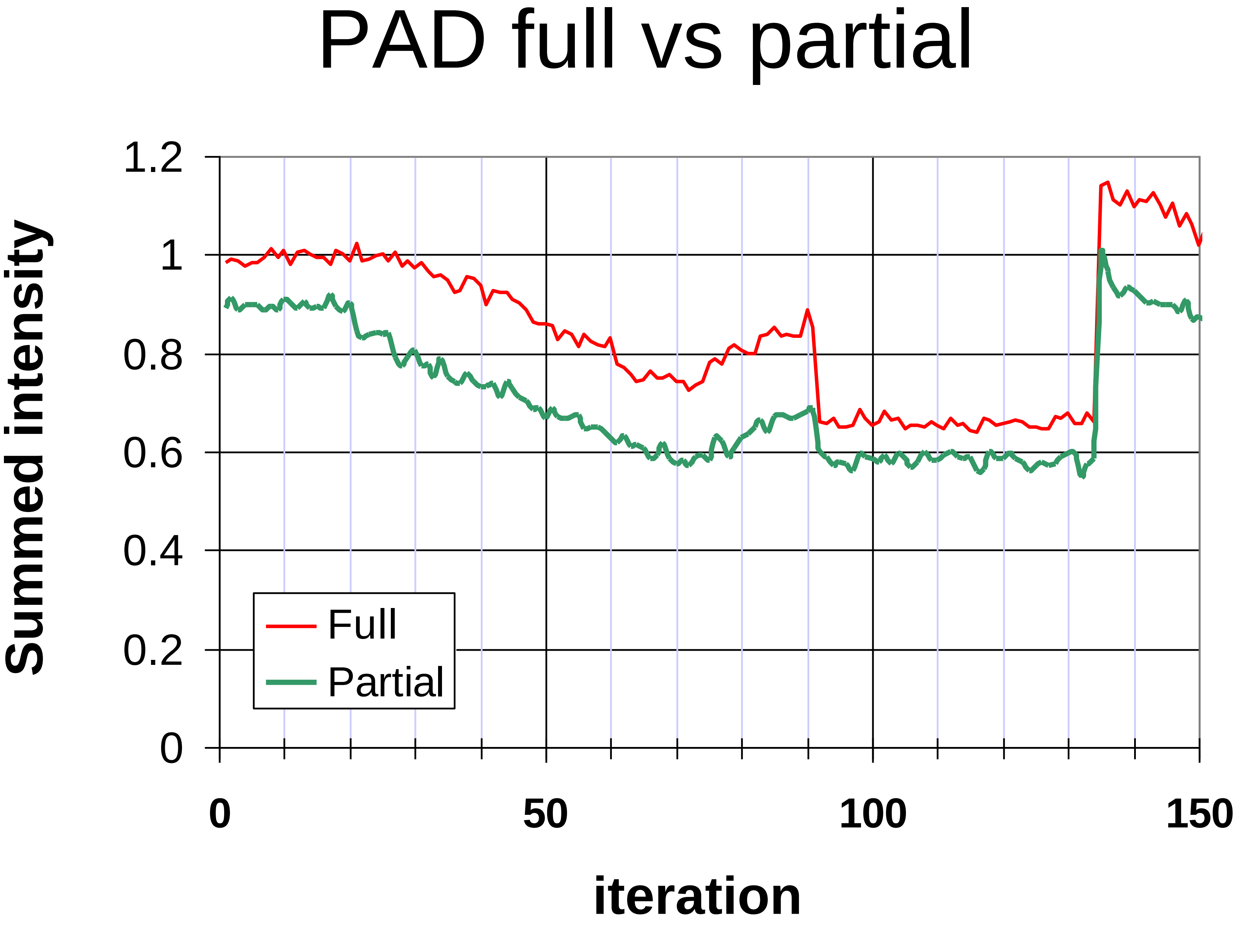
Time	none	sqrt	proportional
none	CCD Read-out	Photon counting	Detector calibration attenuation partiality Non-isomorphism Radiation damage
1/sqrt			Beam flicker
1/prop.			Shutter jitter Sample vibration Pile-up

Optimal exposure time
(faint spots on CCD)

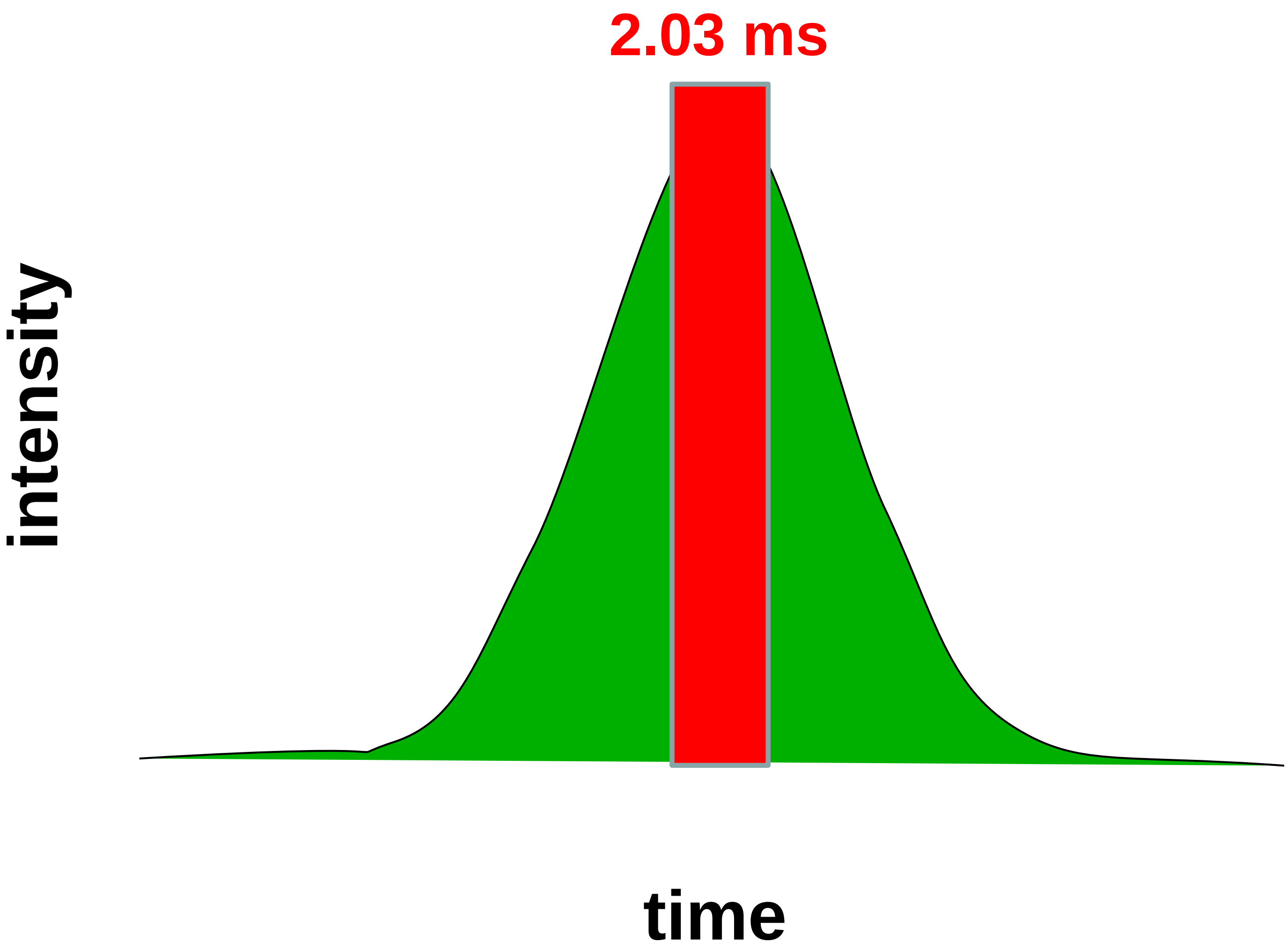
$$t_{hr} = t_{ref} \frac{10 \cdot m \cdot \sigma_0^{\,2}}{gain \cdot \left(bg_{ref} - bg_0\right)}$$

t_{hr}	Optimal exposure time for data set (s)
t_{ref}	exposure time of reference image (s)
bg_{ref}	background level near weak spots on reference image (ADU)
bg_0	ADC offset of detector (ADU)
bg_{hr}	optimal background level (via t_{hr})
σ_0	rms read-out noise (ADU)
gain	ADU/photon
m	multiplicity of data set (including partials)




PAD full vs partial
Summed intensity
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
iteration
Full
Partial

PAD full vs partial



Optimal exposure time

(faint spots on PAD)

$$t_{hr} > t_{ro} / 5\%$$

t_{hr} Optimal exposure time (s)

t_{ro} read-out time (s)

2.03 ms on PILATUS3 S

3 μs on EIGER

Optimal exposure time

(anomalous on PAD)

$$t_{ano} > t_{ro} \frac{< F >}{< \Delta F >}$$

t_{ano} Optimal exposure time (s) **0.2 s**

t_{ro} read-out time (s)

2.03 ms on PILATUS3 S

3 μs on EIGER

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

How to avoid time-dependent error?

Attenu-wait!

Apply 10x attenuation

Expose 10x longer

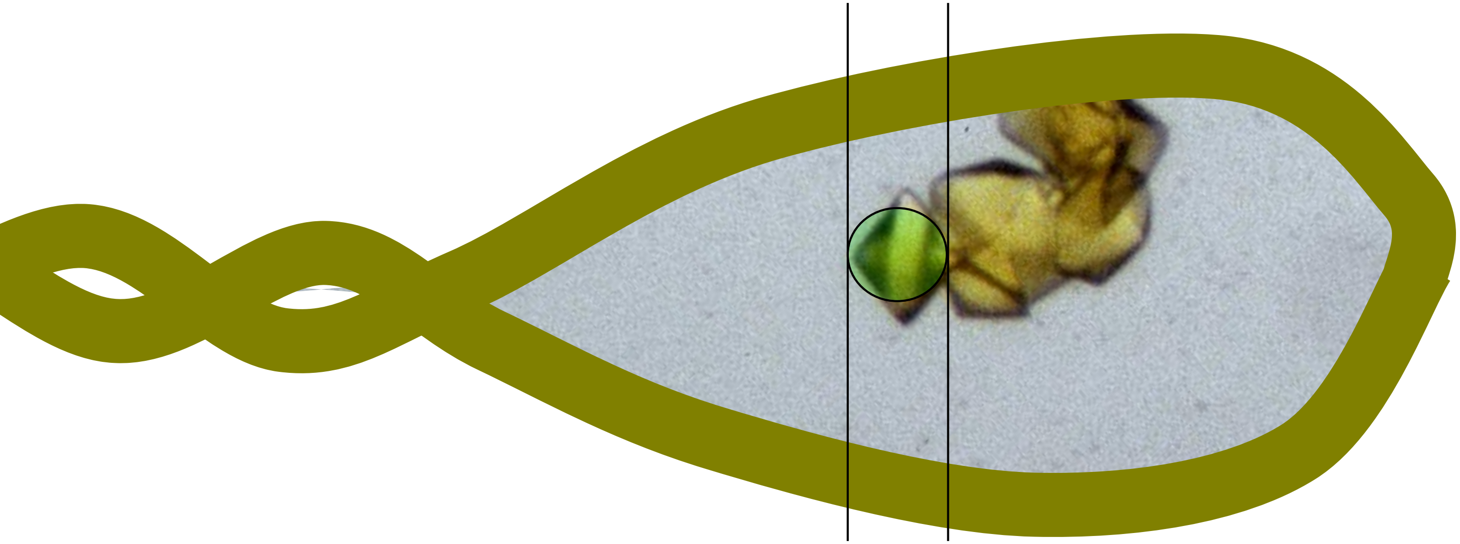
Number of photons should be the same!

Damage will also be the same!

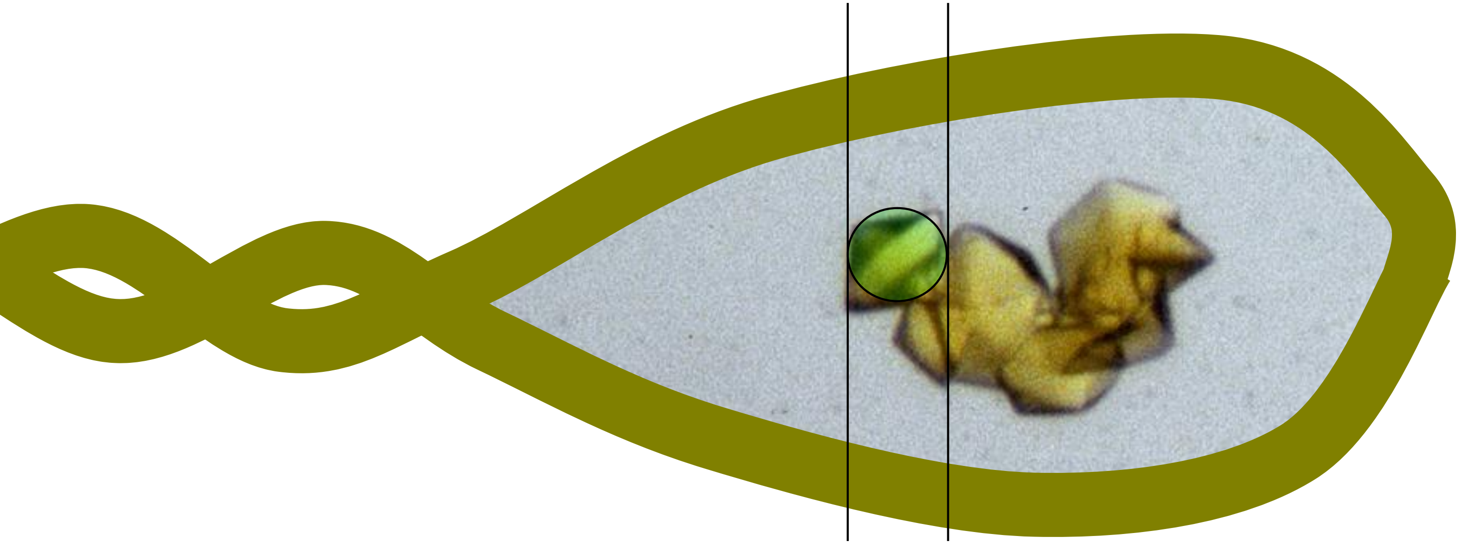
beam size vs xtal size

1. Put your crystal into the beam
2. Shoot the whole crystal
3. Shoot nothing but the crystal
4. Back off!
5. The crystal must rotate

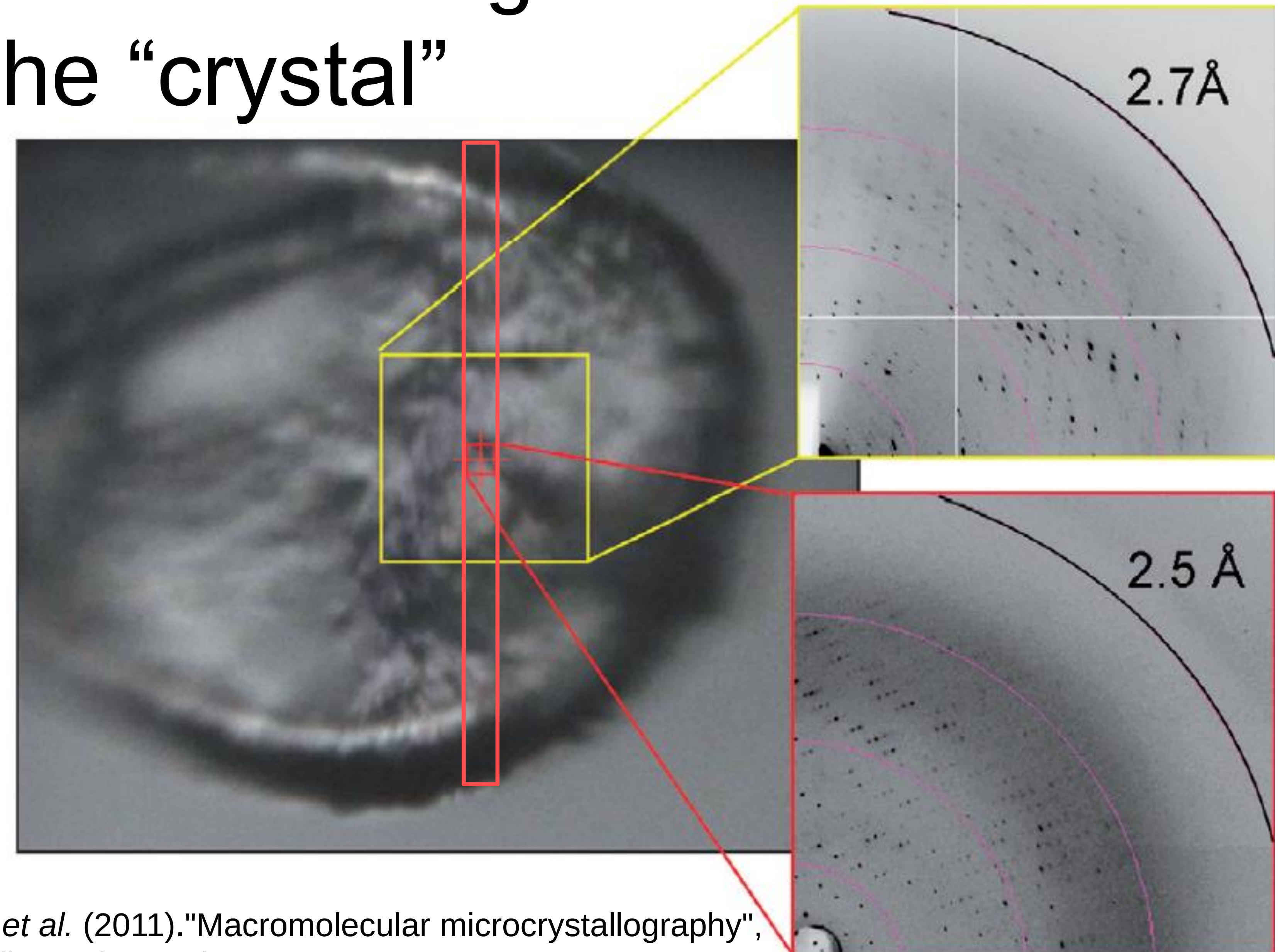
The crystal rotates!



The crystal rotates!



Shoot nothing but the "crystal"



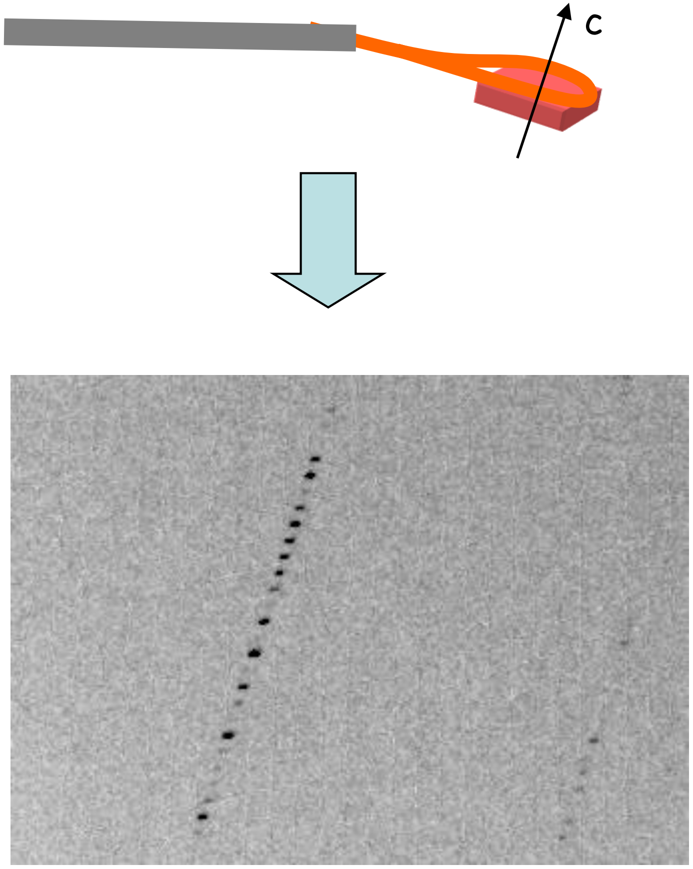
Evans *et al.* (2011)."Macromolecular microcrystallography", *Crystallography Reviews* **17**, 105-142.

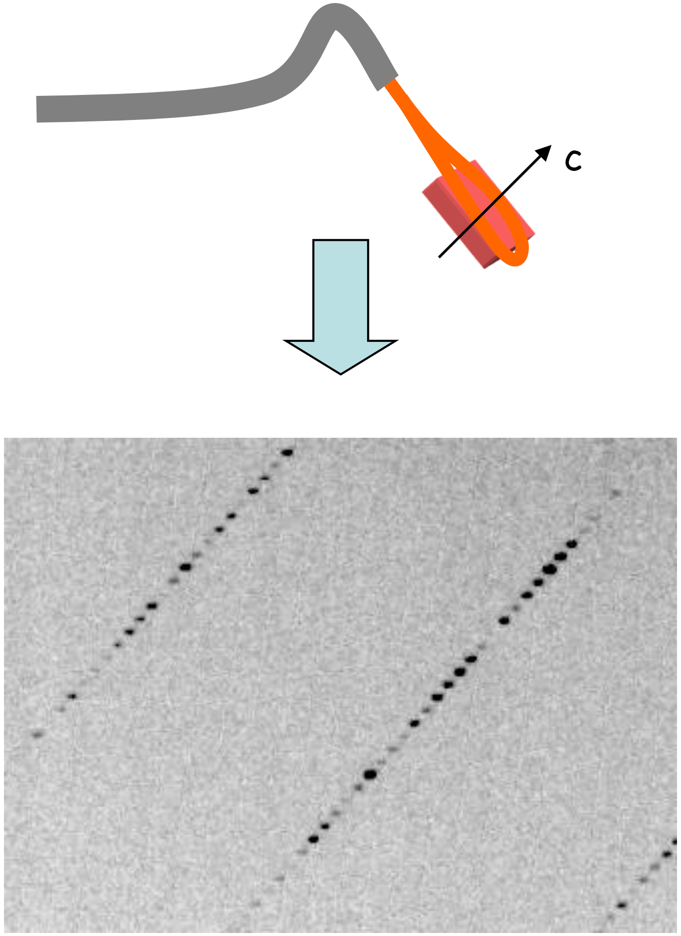
Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

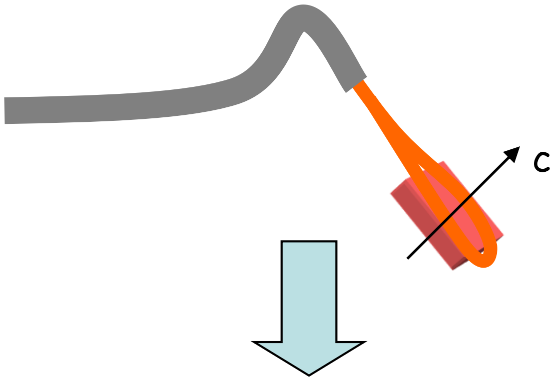
Run Strategy

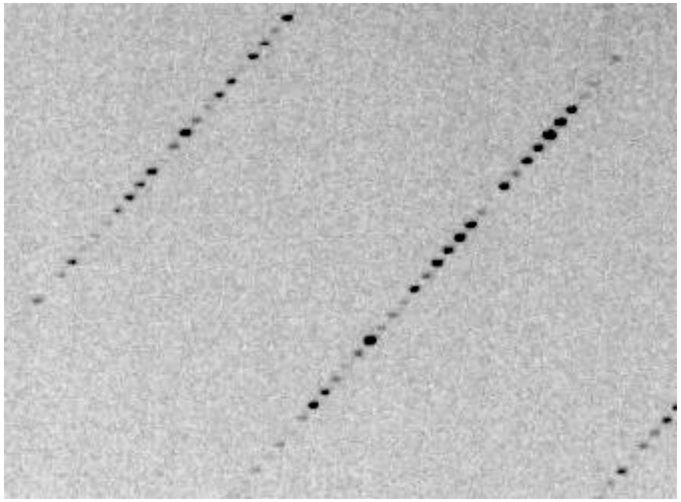
avoiding overlaps





avoiding overlaps





SmarAct's "SmarGon" Commercialized PRIGo

At the beamline…

- Resolution

  problem: background

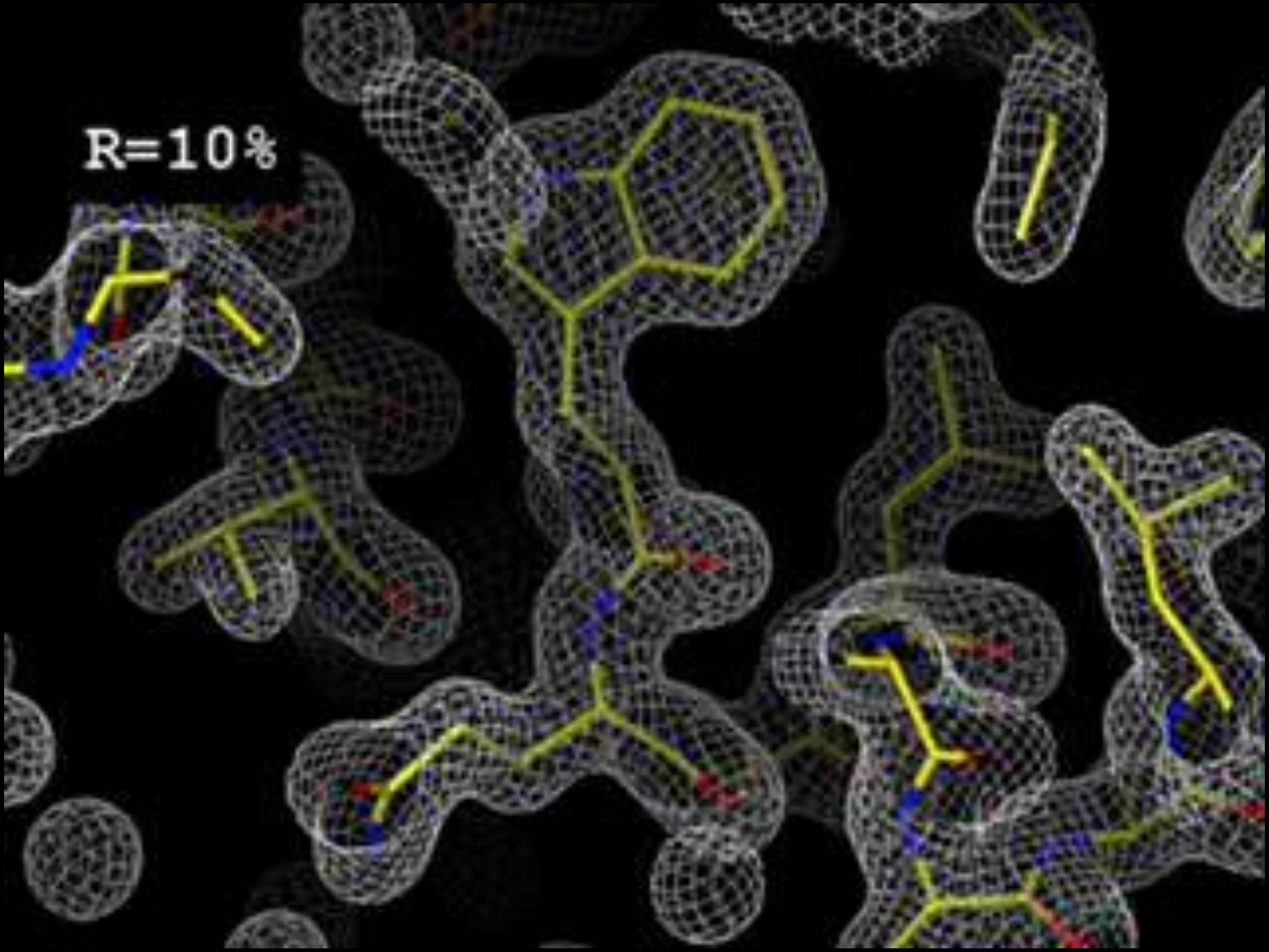
  solution: use as few pixels as possible

- Phases

  problem: fractional errors

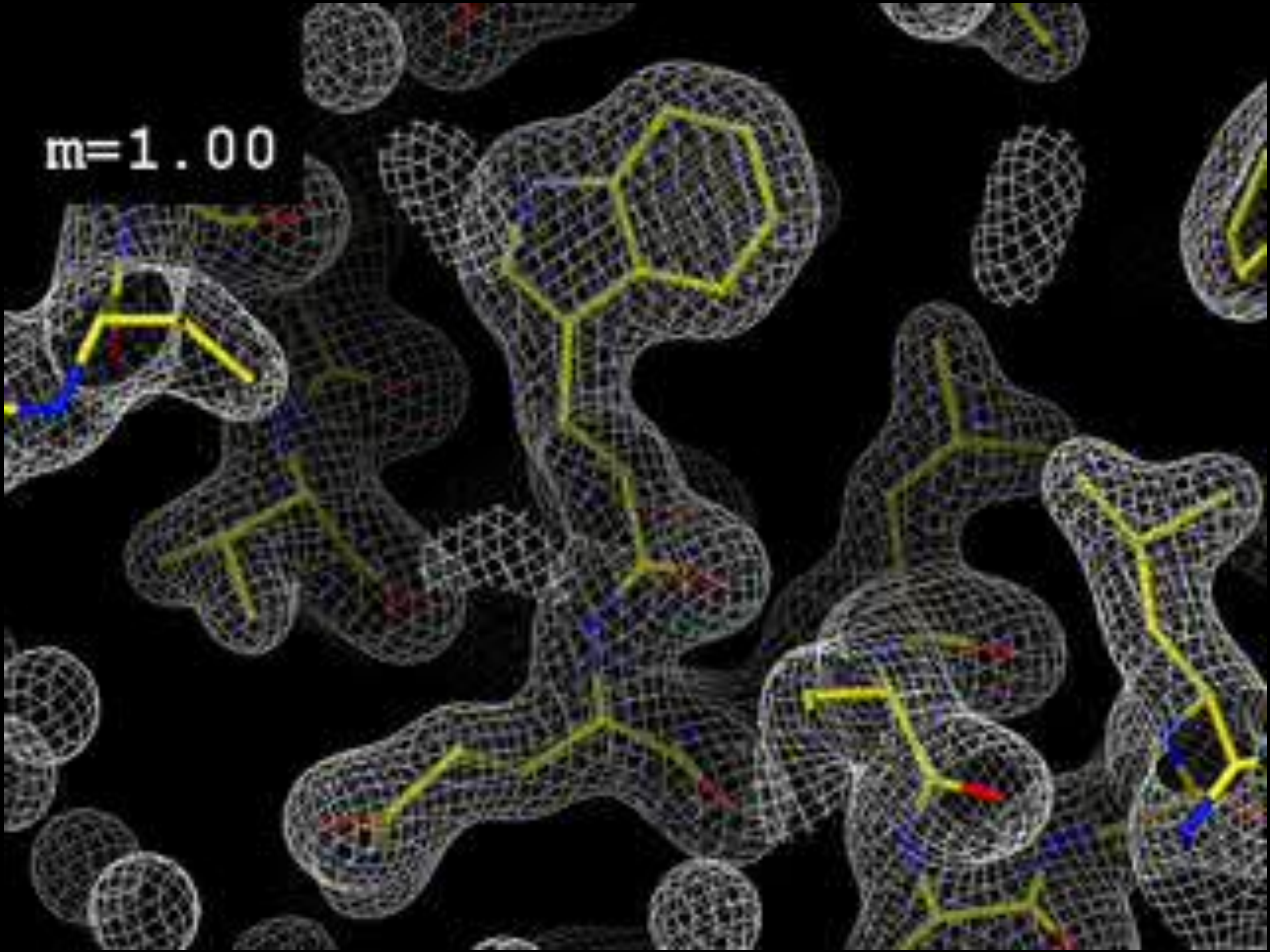
  solution: use as many pixels as possible

R-factor



http://bl831.als.lbl.gov/~jamesh/movies/rfactor.mpeg

Figure of Merit



http://bl831.als.lbl.gov/~jamesh/movies/dephase.mpeg

anomalous signal

$$\frac{\Delta F}{F} \approx 1.2\, f'' \sqrt{\frac{\#\ \text{sites}}{\text{MW (Da)}}}$$

World record!

$\Delta F/F = 0.5\%$

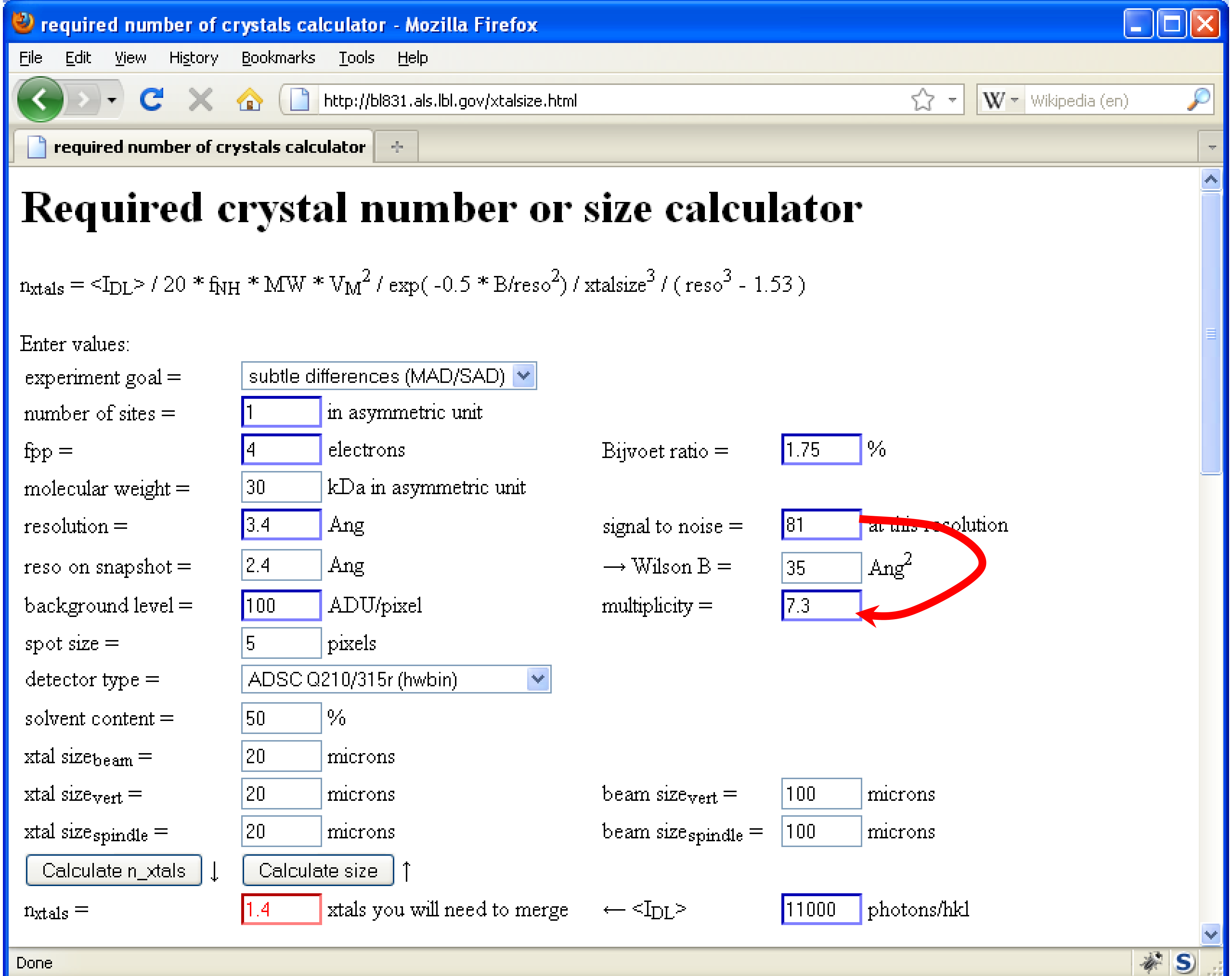
Wang, Dauter & Dauter (2006) *Acta Cryst. D* **62**, 1475-1483.

Crick, F. H. C. & Magdoff, B. S. (1956) *Acta Crystallogr.* **9**, 901-908.

Hendrickson, W. A. & Teeter, M. M. (1981) *Nature* **290**, 107-113.

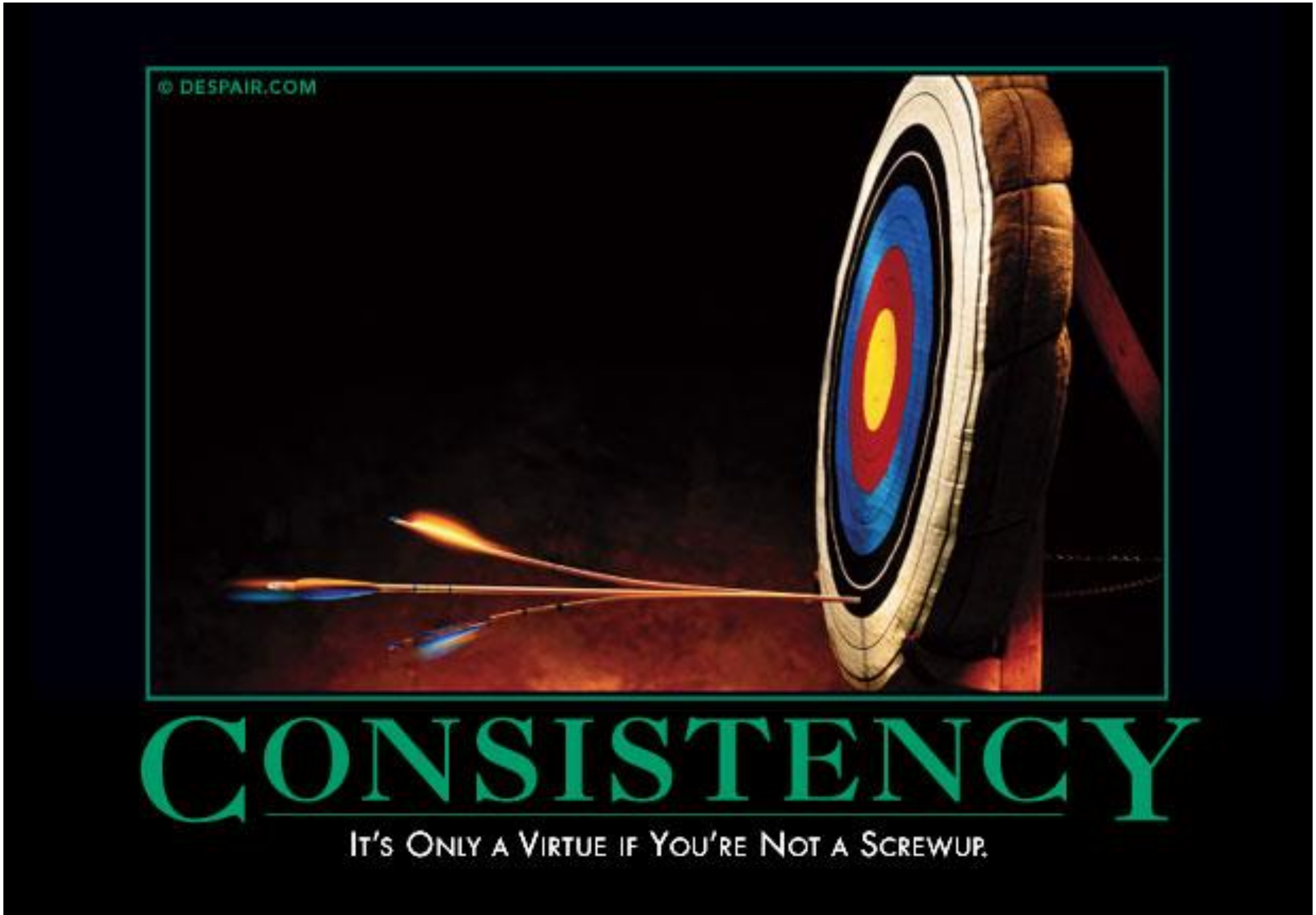
Fractional error

$$\text{mult} > \left(\frac{\sim 3\%}{\langle \Delta F/F \rangle}\right)^2$$



Holton & Frankel (2010) *Acta D* **66** 393-408.

Systematic error does not “average out”



Can you count to 1,000,000 ?

Theoretically: $$\frac{\text{sqrt}(1{,}000{,}000)}{1{,}000{,}000} = 0.1\%$$

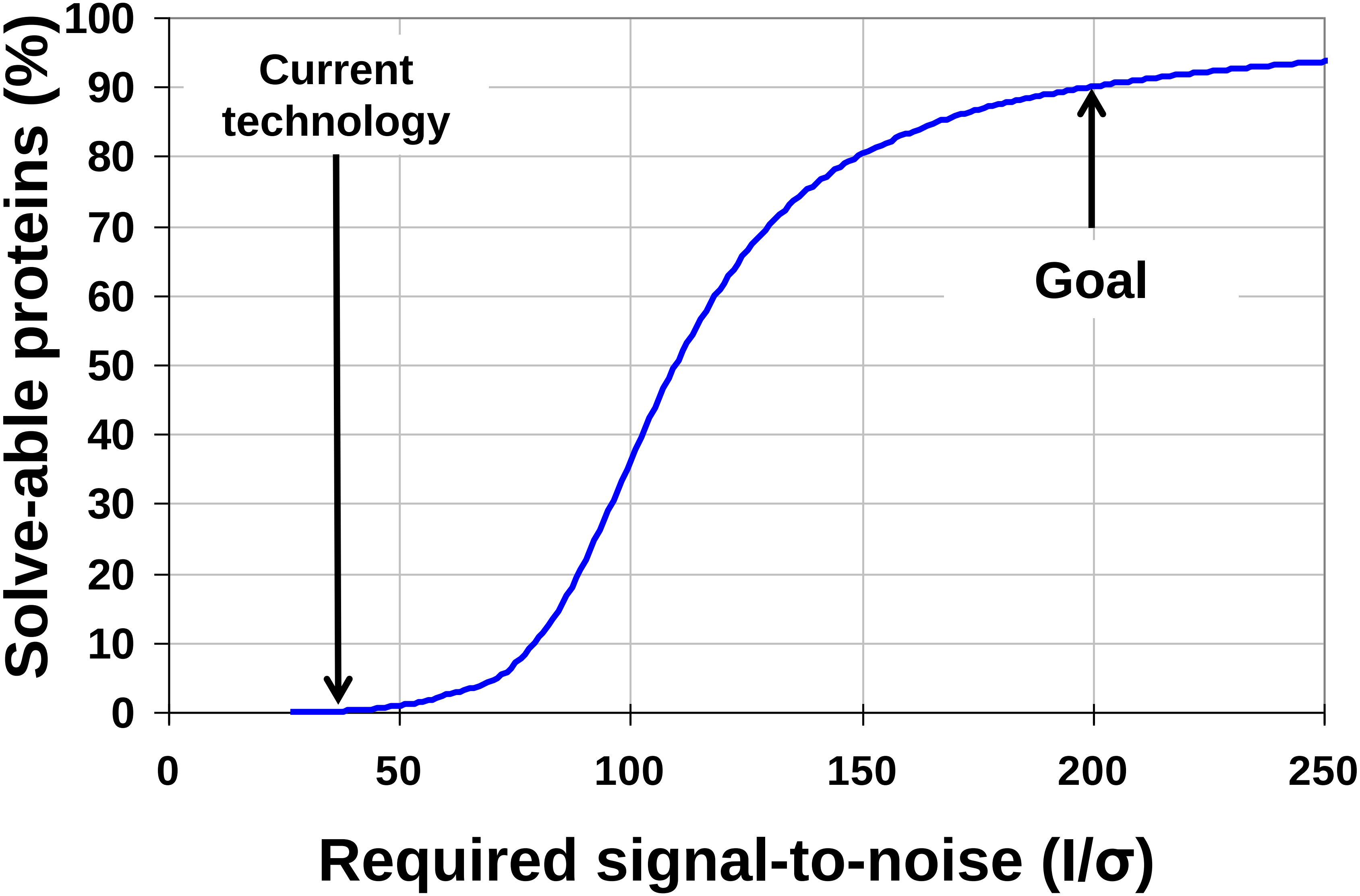
$R_{meas} \approx 0.1\%$? → ISa = 1000

In reality: ISa ~ 33 → $R_{meas} = \approx 3\%$

$$\frac{\text{sqrt}(1{,}000)}{1{,}000} = 3\%$$

$> 1000 \frac{\text{photon}}{\text{spot}}$ is a waste!

Threshold of a revolution in phasing



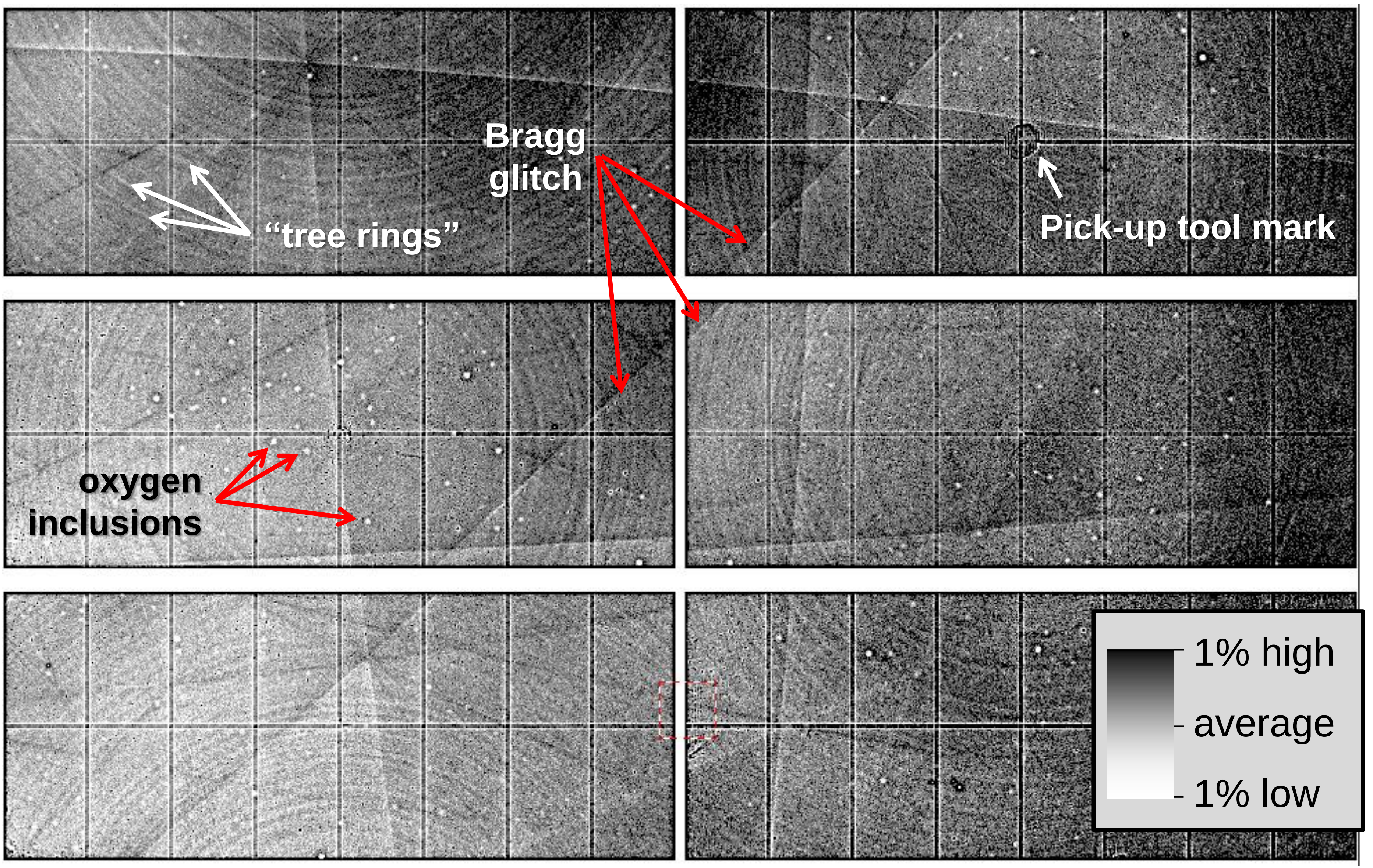
Threshold of a revolution in phasing

Source of error	realistic simulation
Photon counting	+
Shutter jitter	+
Beam flicker	+
Sample absorption	+
Radiation damage	+
Imperfect spindle	+
vignette	+
Corner correction	+
SHSSS	+
R_{meas} (∞-10 Å)	2.8%
I/σ asymptotic	26.8

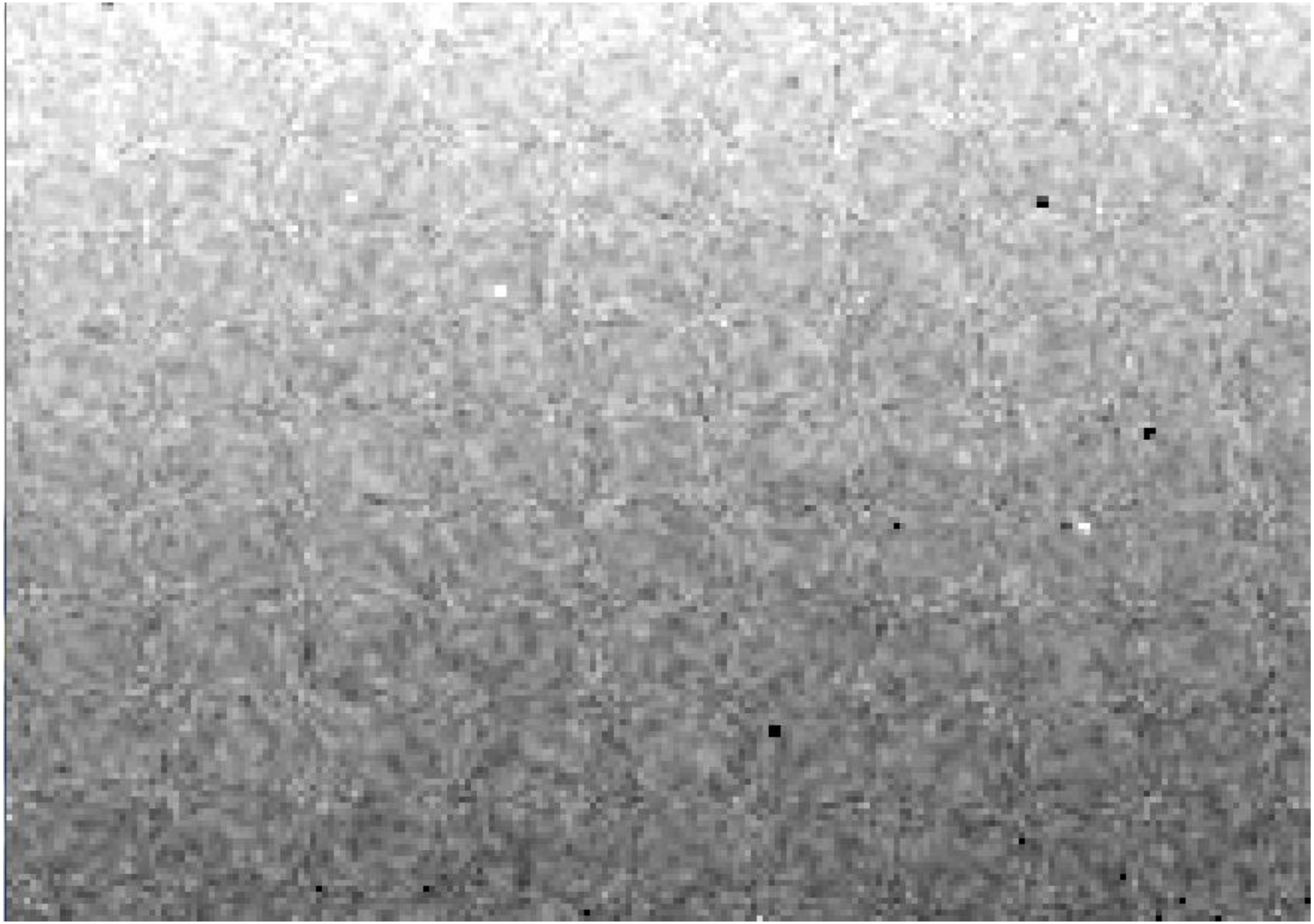
Holton *et al* (2014) "R-factor gap", *FEBS Journal* **281**,

Pilatus: subtract smooth baseline

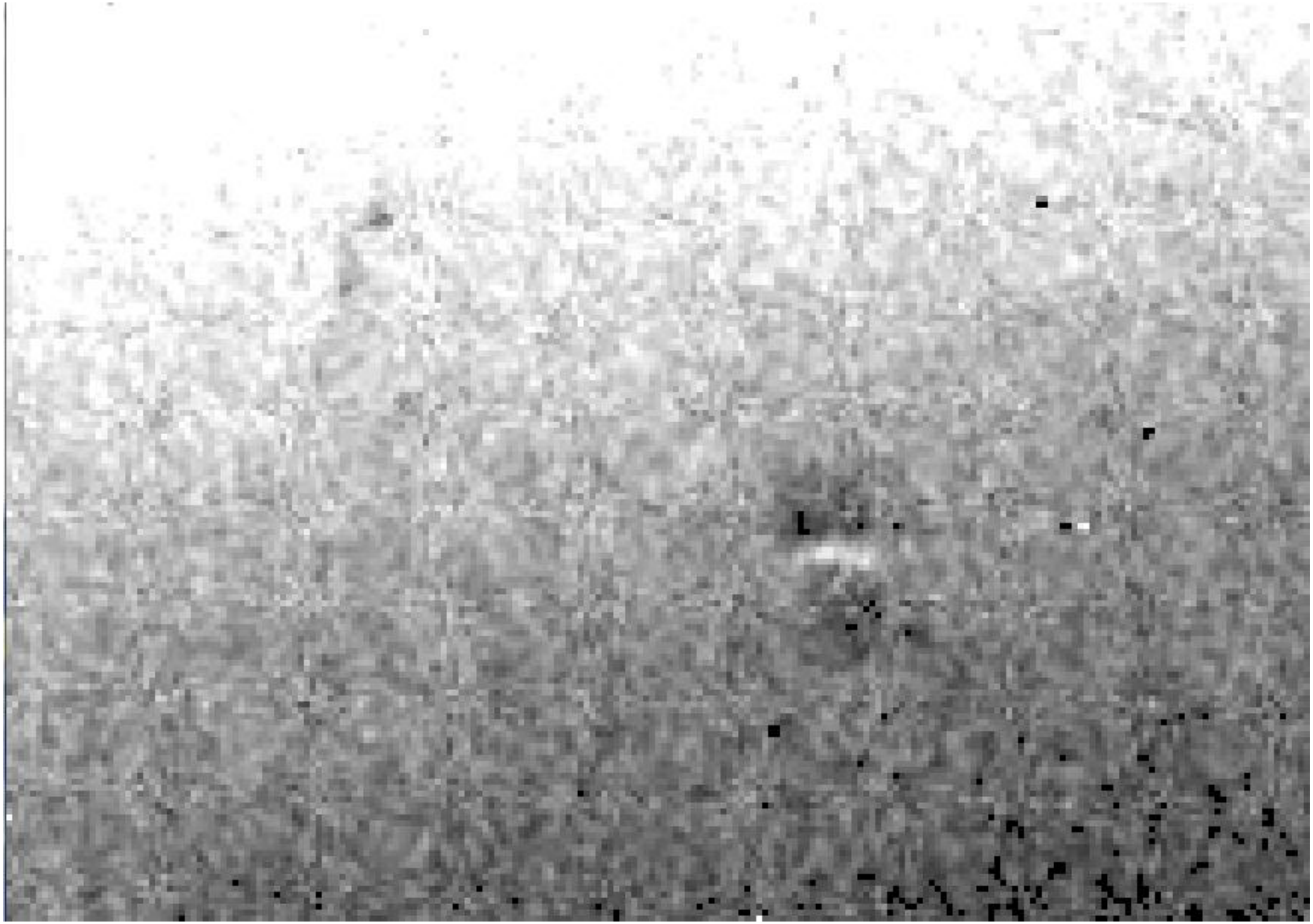
~3x10^5 photon/pixel



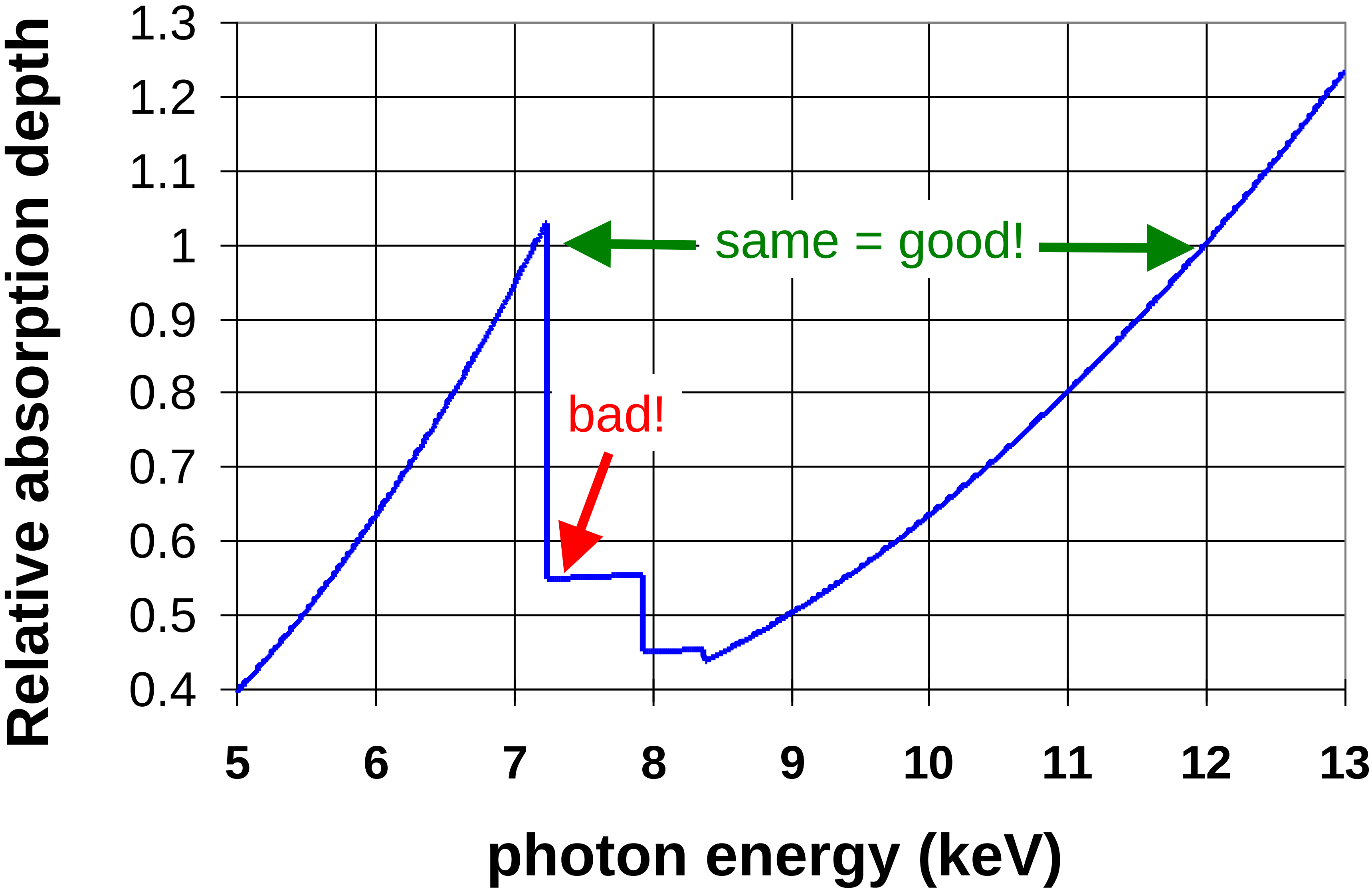
CCD calibration: 7235 eV



CCD calibration: 7247 eV



Gadox calibration vs energy



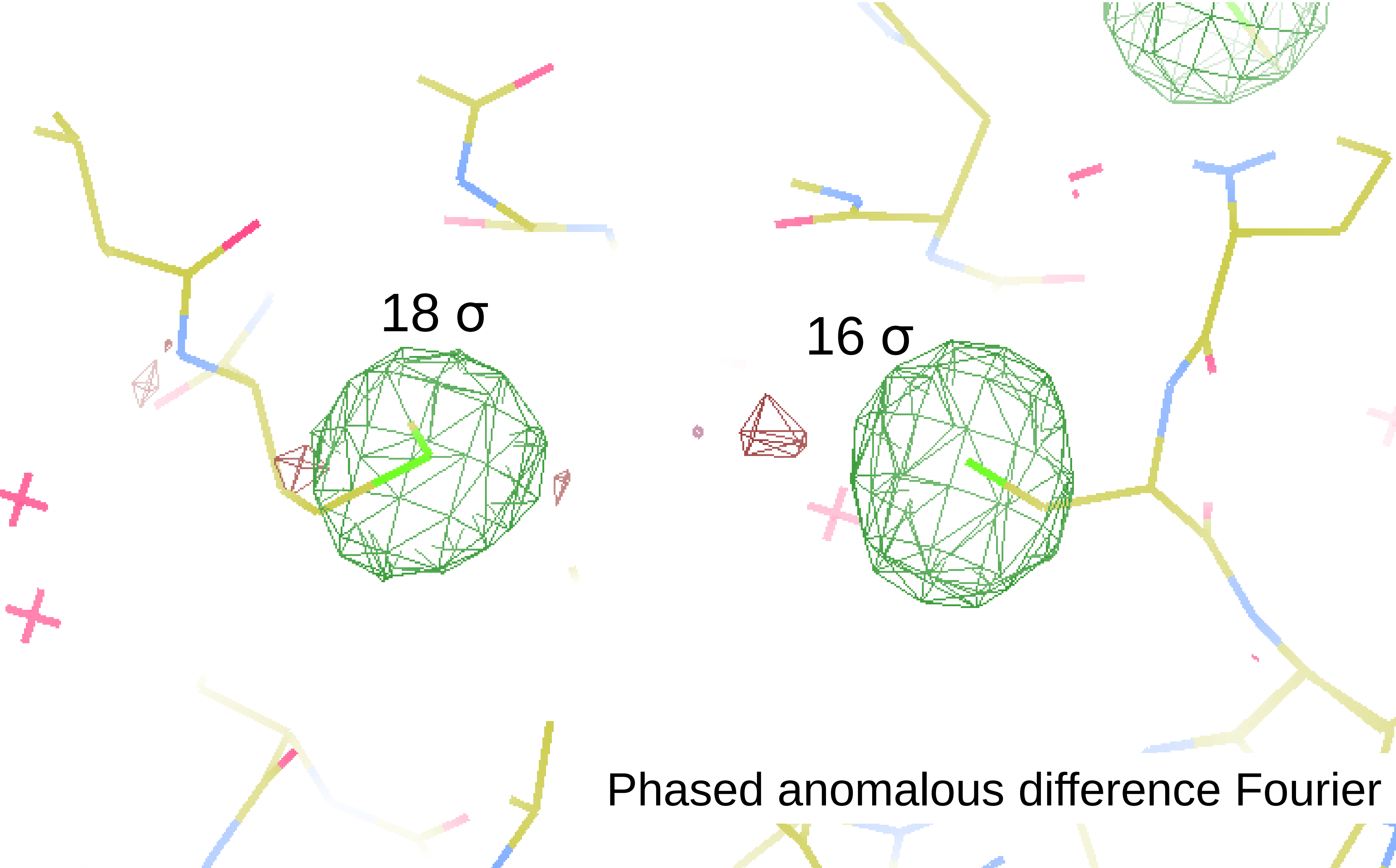
Data collection parameters:

- 16 crystals
- 360° each, inverse beam
- 7235 eV photon energy
- < 1 MGy per xtal
- Australian Synchrotron MX1
  - 35 kGy/s into 100 μm x 100 μm

140-fold multiplicity: 16 crystals, 360° each, inverse beam, 7235 eV

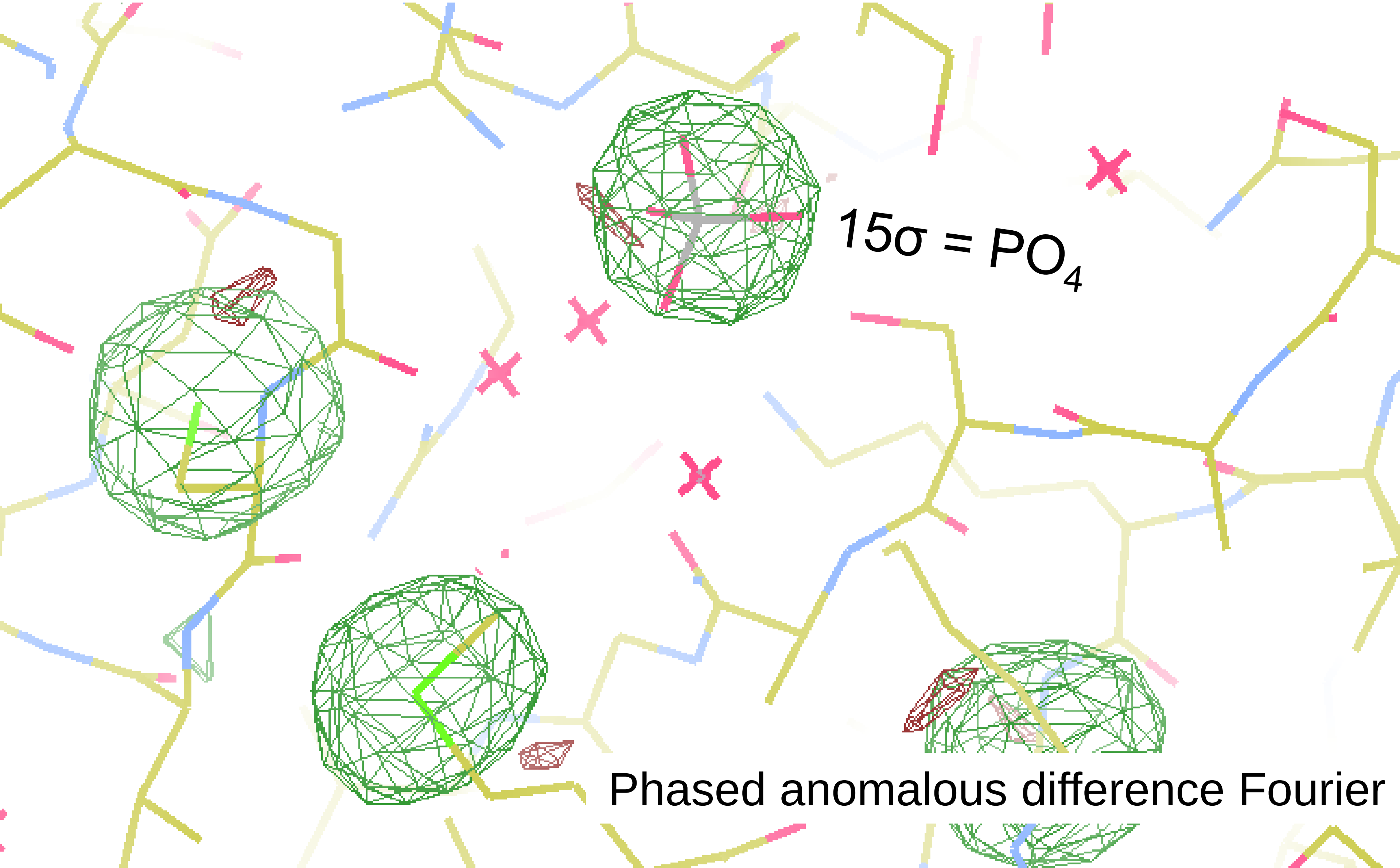
RESOLUTION LIMIT	COMPLETENESS OF DATA	R-FACTOR observed	I/SIGMA	R-meas	CC(1/2)	Anomal Corr	SigAno	Nano
9.17	99.1%	3.9%	257.47	3.9%	100.0*	91*	5.024	450
6.49	100.0%	5.2%	214.33	5.2%	100.0*	86*	3.836	882
5.30	100.0%	7.2%	165.13	7.2%	100.0*	76*	3.257	1175
4.59	100.0%	7.2%	175.42	7.3%	100.0*	67*	2.589	1403
4.10	99.9%	7.7%	174.13	7.7%	100.0*	59*	2.264	1594
3.74	99.9%	9.4%	143.09	9.4%	100.0*	49*	1.953	1783
3.47	100.0%	11.2%	120.17	11.2%	100.0*	39*	1.696	1942
3.24	100.0%	14.1%	91.14	14.1%	100.0*	30*	1.333	2103
3.06	99.9%	19.5%	65.79	19.5%	100.0*	23*	1.117	2214
2.90	99.9%	29.0%	44.85	29.1%	99.9*	17*	1.008	2369
2.77	99.9%	40.5%	32.58	40.6%	99.8*	11*	0.901	2493
2.65	99.9%	52.8%	25.16	52.9%	99.8*	10*	0.866	2605
2.54	100.0%	67.4%	19.47	67.6%	99.6*	2	0.804	2705
2.45	100.0%	88.9%	14.58	89.2%	99.2*	4	0.831	2859
2.37	100.0%	109.3%	9.97	109.7%	98.1*	5	0.829	2925
2.29	100.0%	138.2%	6.87	138.9%	96.1*	1	0.760	3037
2.22	100.0%	197.1%	4.03	198.6%	83.5*	-1	0.721	3159
2.16	100.0%	227.3%	2.41	230.8%	46.9*	-1	0.677	3224
2.10	61.2%	154.4%	1.28	163.6%	47.0*	-2	0.660	1999
2.05	47.9%	170.1%	0.68	196.5%	25.7*	3	0.629	1578
total	93.3%	15.7%	54.30	15.8%	100.0*	12*	1.217	42499

140-fold multiplicity

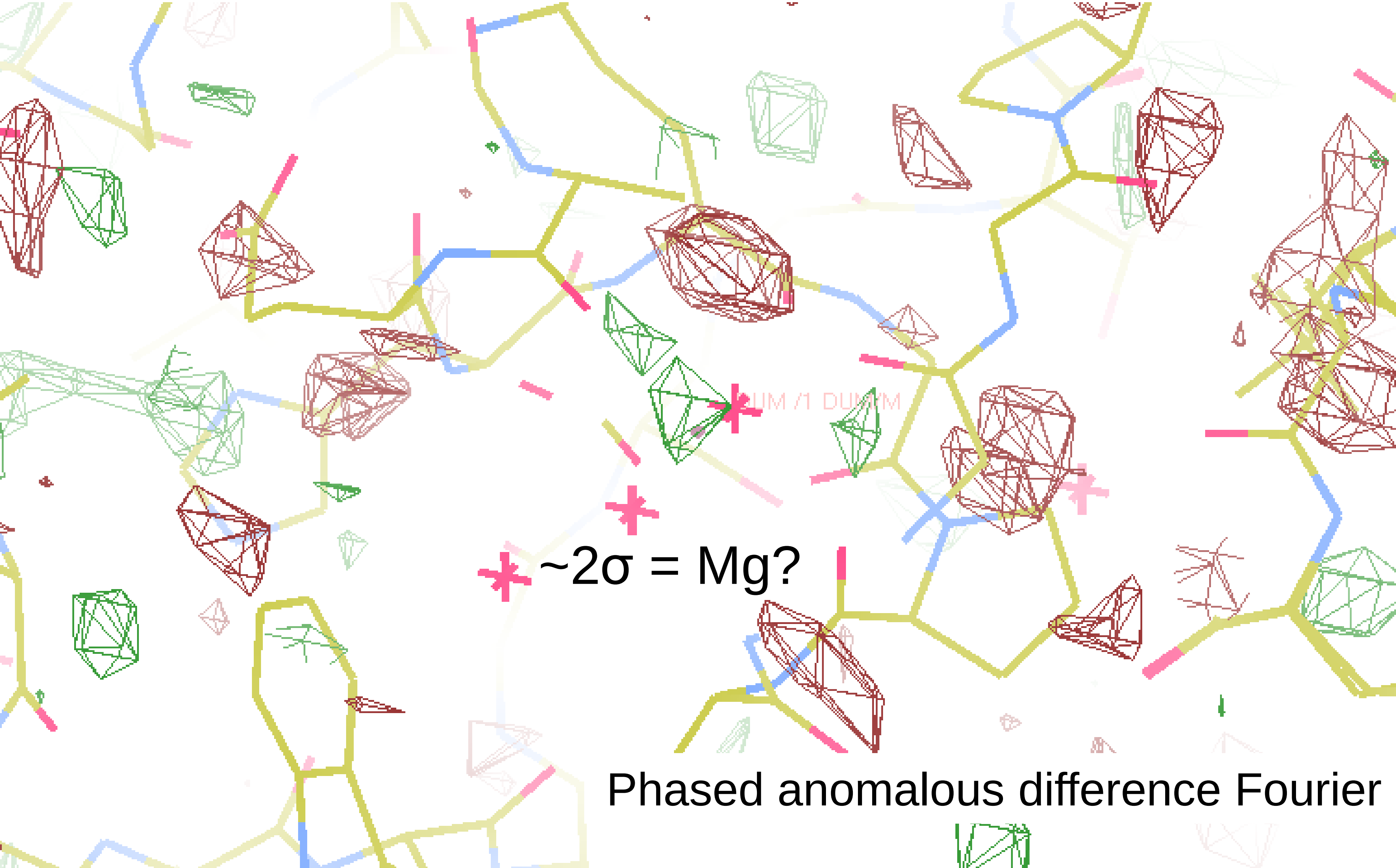


Phased anomalous difference Fourier

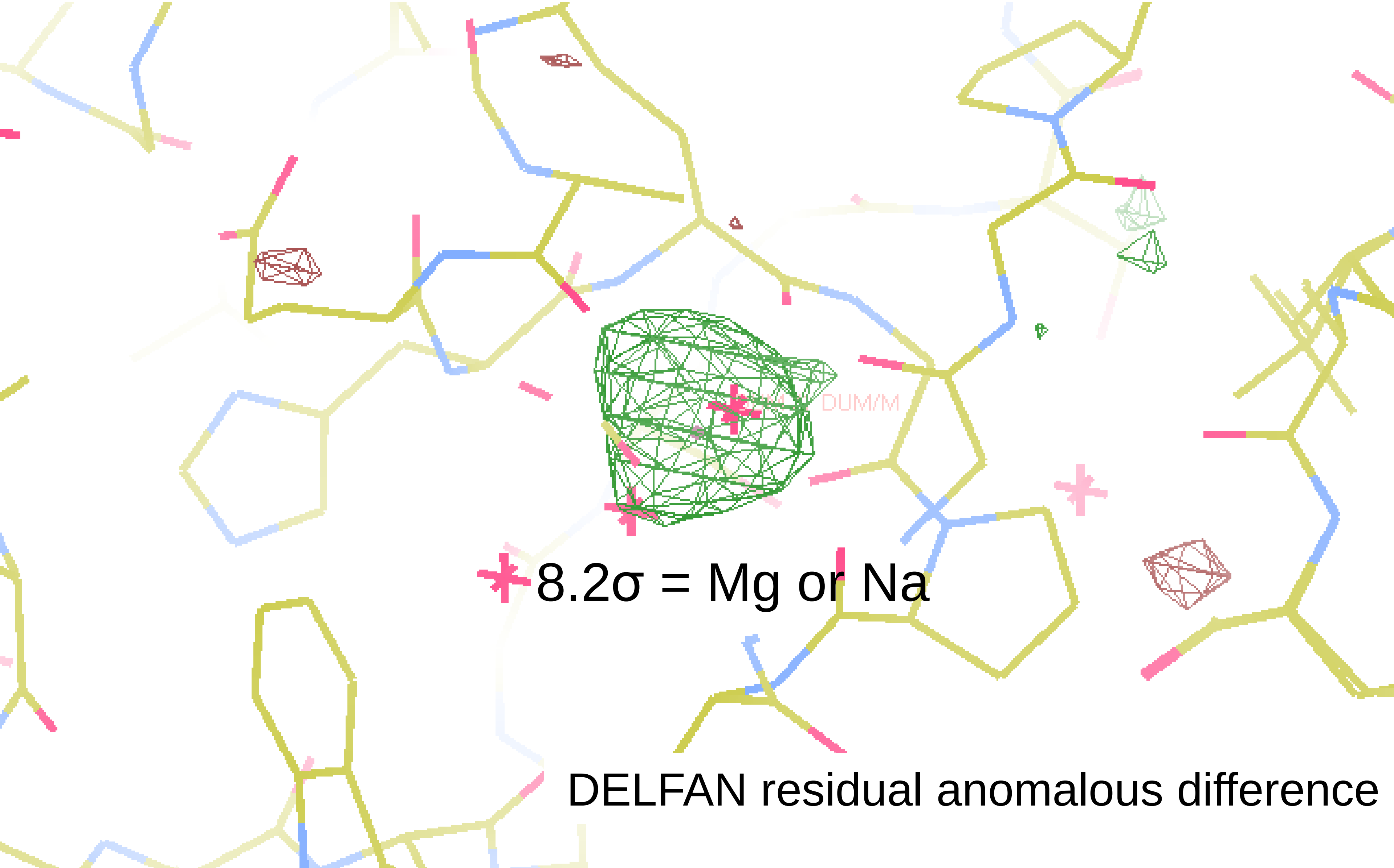
140-fold multiplicity



140-fold multiplicity



140-fold multiplicity



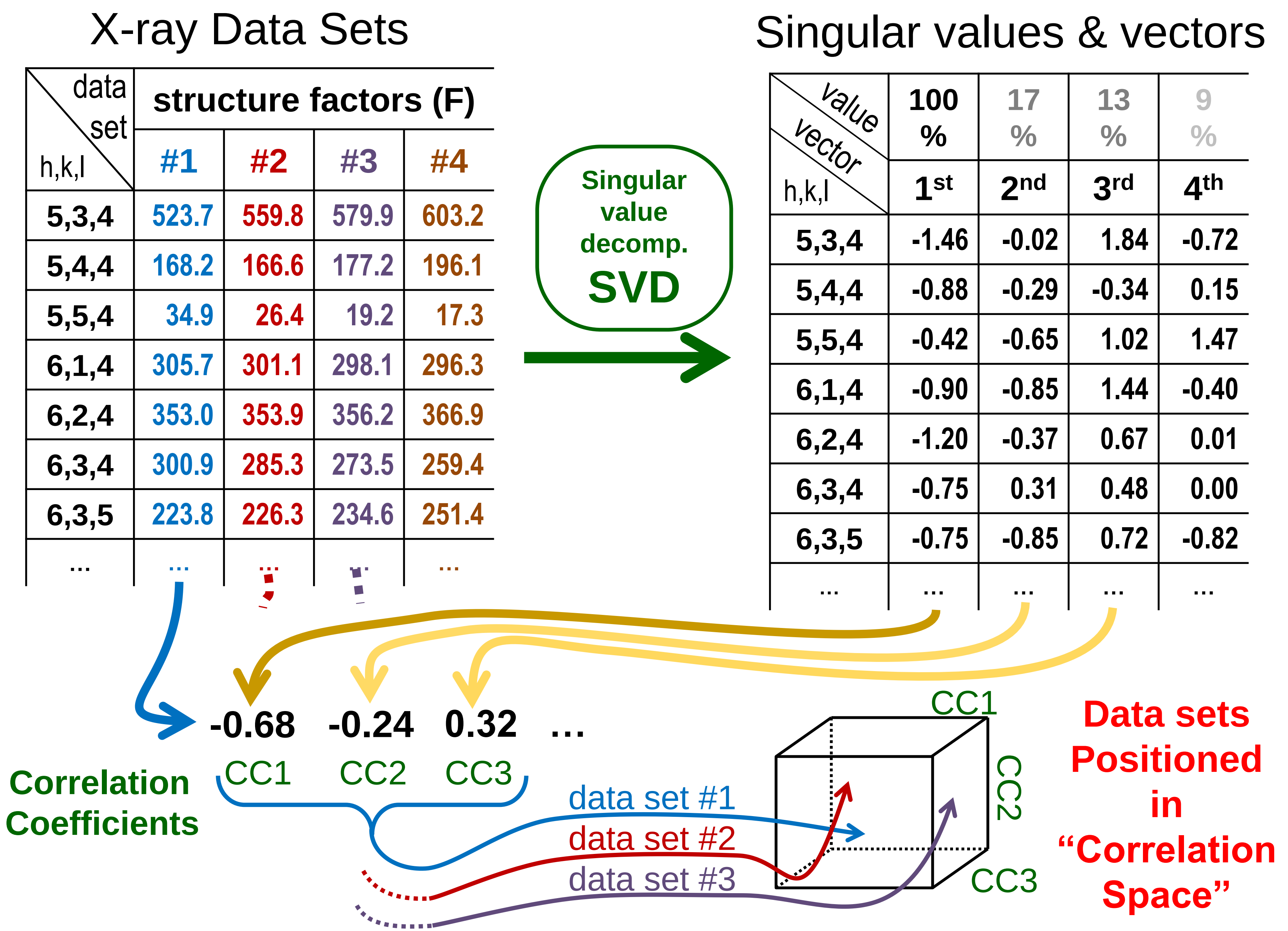
Why doesn’t everyone do this?

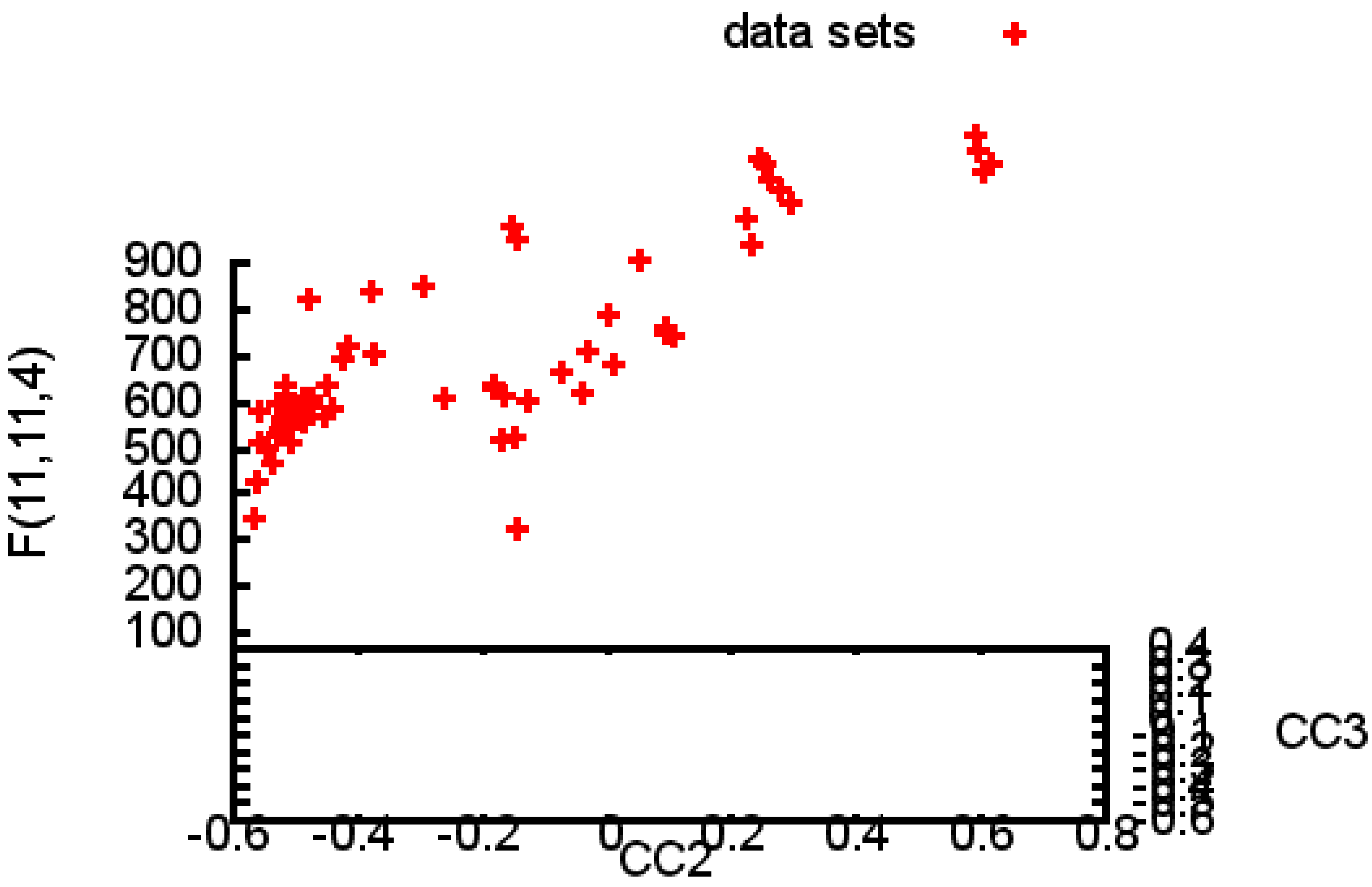
non-isomorphism

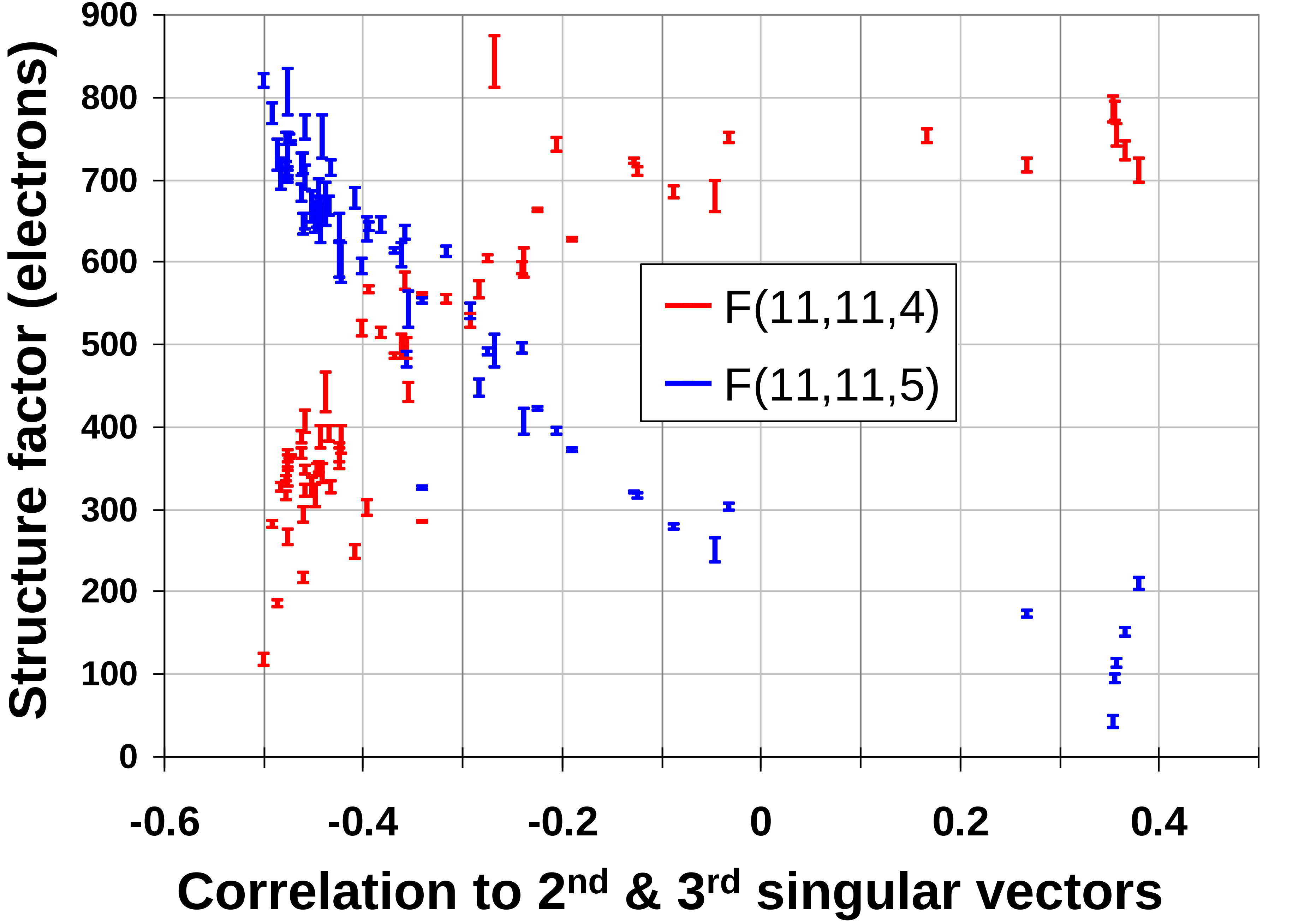
Non-isomorphism in lysozyme

RMSD = 0.18 Å   Δcell = 0.7 % R_{iso} = 44.5%

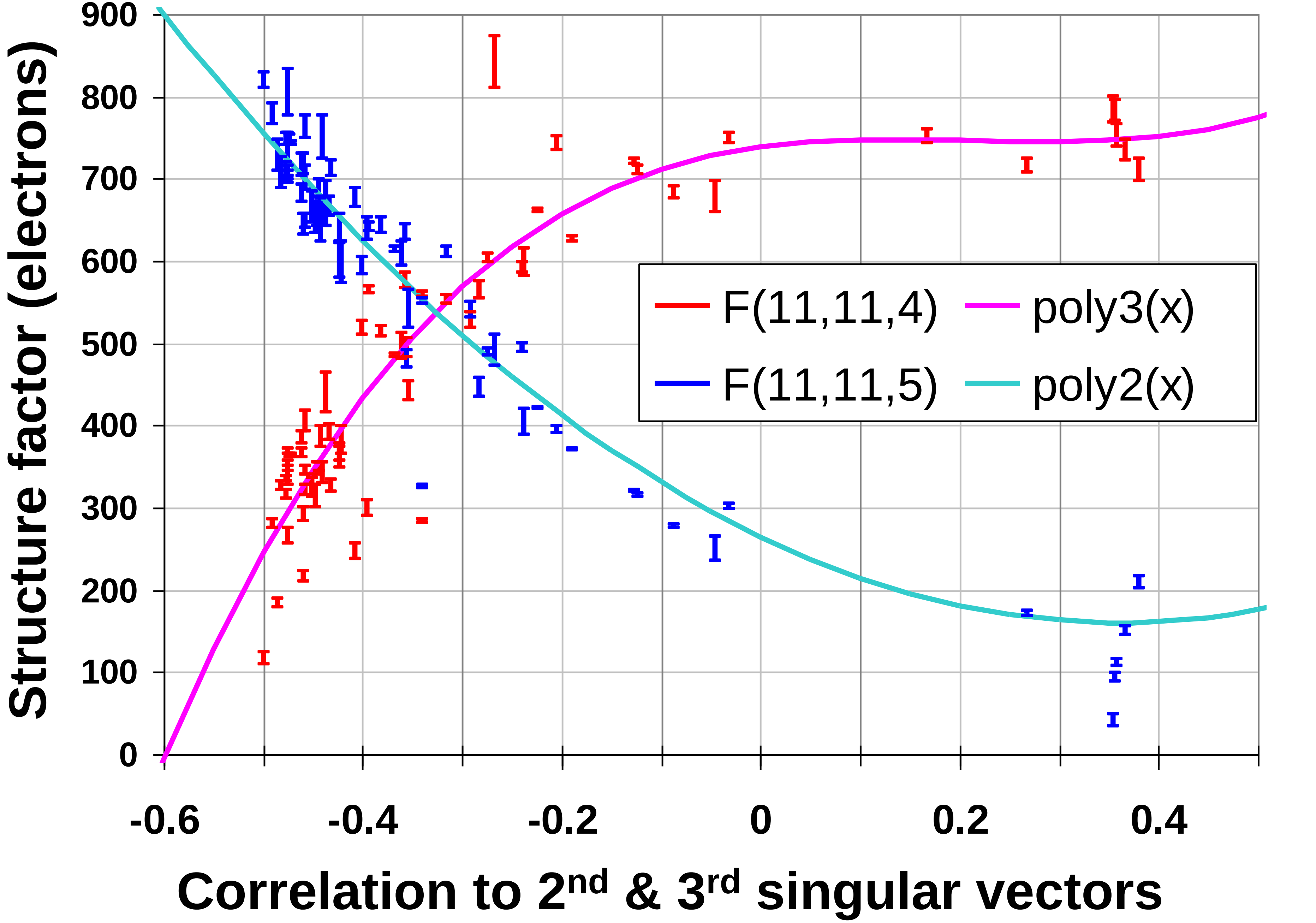
RH: 84.2%
vs 71.9%


X-ray Data Sets
data set
h,k,l
structure factors (F)
#1 #2 #3 #4
5,3,4 523.7 559.8 579.9 603.2
5,4,4 168.2 166.6 177.2 196.1
5,5,4 34.9 26.4 19.2 17.3
6,1,4 305.7 301.1 298.1 296.3
6,2,4 353.0 353.9 356.2 366.9
6,3,4 300.9 285.3 273.5 259.4
6,3,5 223.8 226.3 234.6 251.4
... ...
Singular value decomp.
SVD
Singular values & vectors
value
vector
h,k,l
100 % 17 % 13 % 9 %
1st 2nd 3rd 4th
5,3,4 -1.46 -0.02 1.84 -0.72
5,4,4 -0.88 -0.29 -0.34 0.15
5,5,4 -0.42 -0.65 1.02 1.47
6,1,4 -0.90 -0.85 1.44 -0.40
6,2,4 -1.20 -0.37 0.67 0.01
6,3,4 -0.75 0.31 0.48 0.00
6,3,5 -0.75 -0.85 0.72 -0.82
... ...
-0.68 -0.24 0.32 ...
CC1 CC2 CC3
Correlation Coefficients
data set #1
data set #2
data set #3
CC1
CC2
CC3
Data sets Positioned in “Correlation Space”

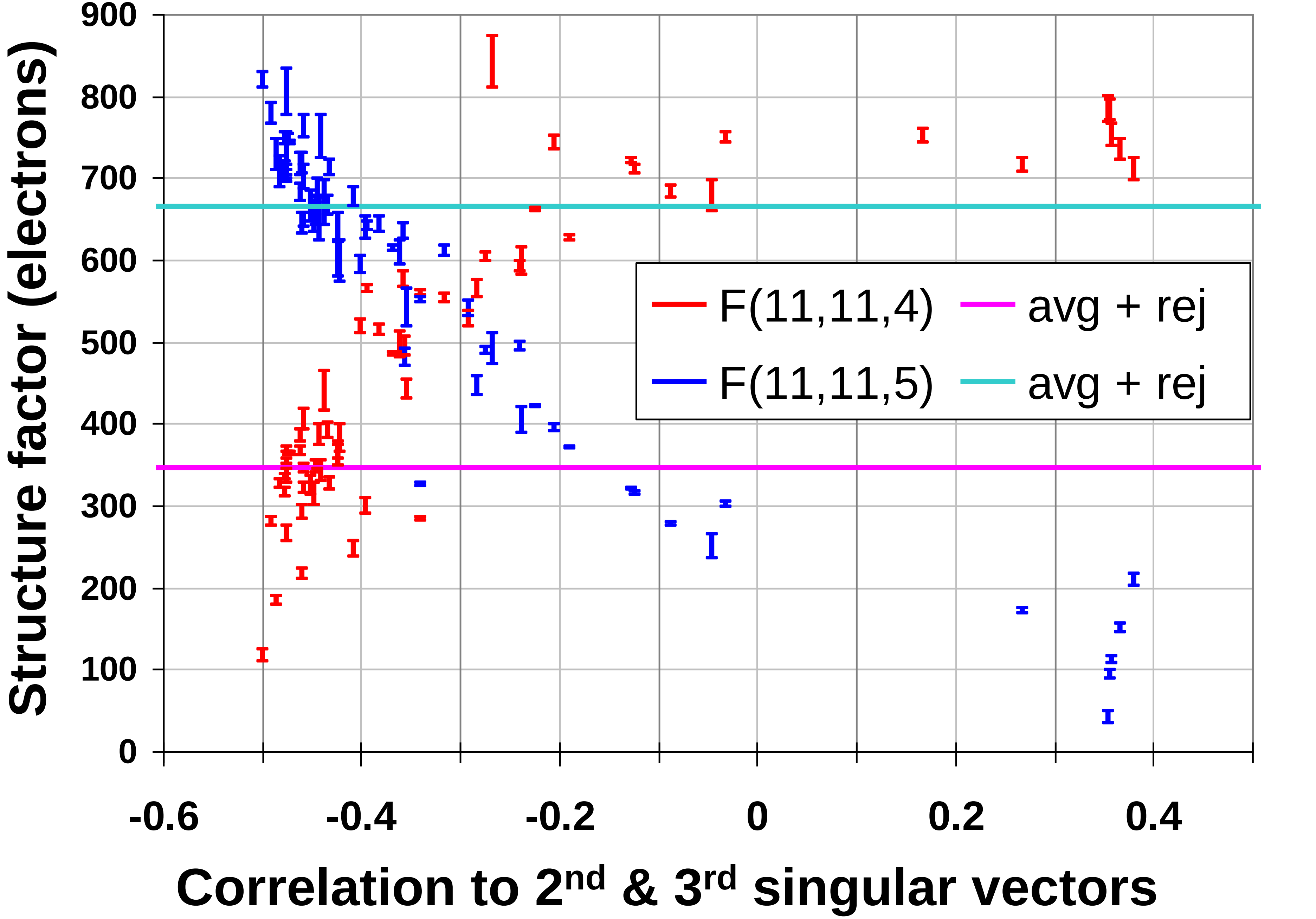

data sets
F(11,11,4)
900
800
700
600
500
400
300
200
100
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
CC2
CC3



Structure factor (electrons)
900
800
700
600
500
400
300
200
100
0
-0.6
-0.4
-0.2
0
0.2
0.4
Correlation to 2nd & 3rd singular vectors
F(11,11,4)
F(11,11,5)

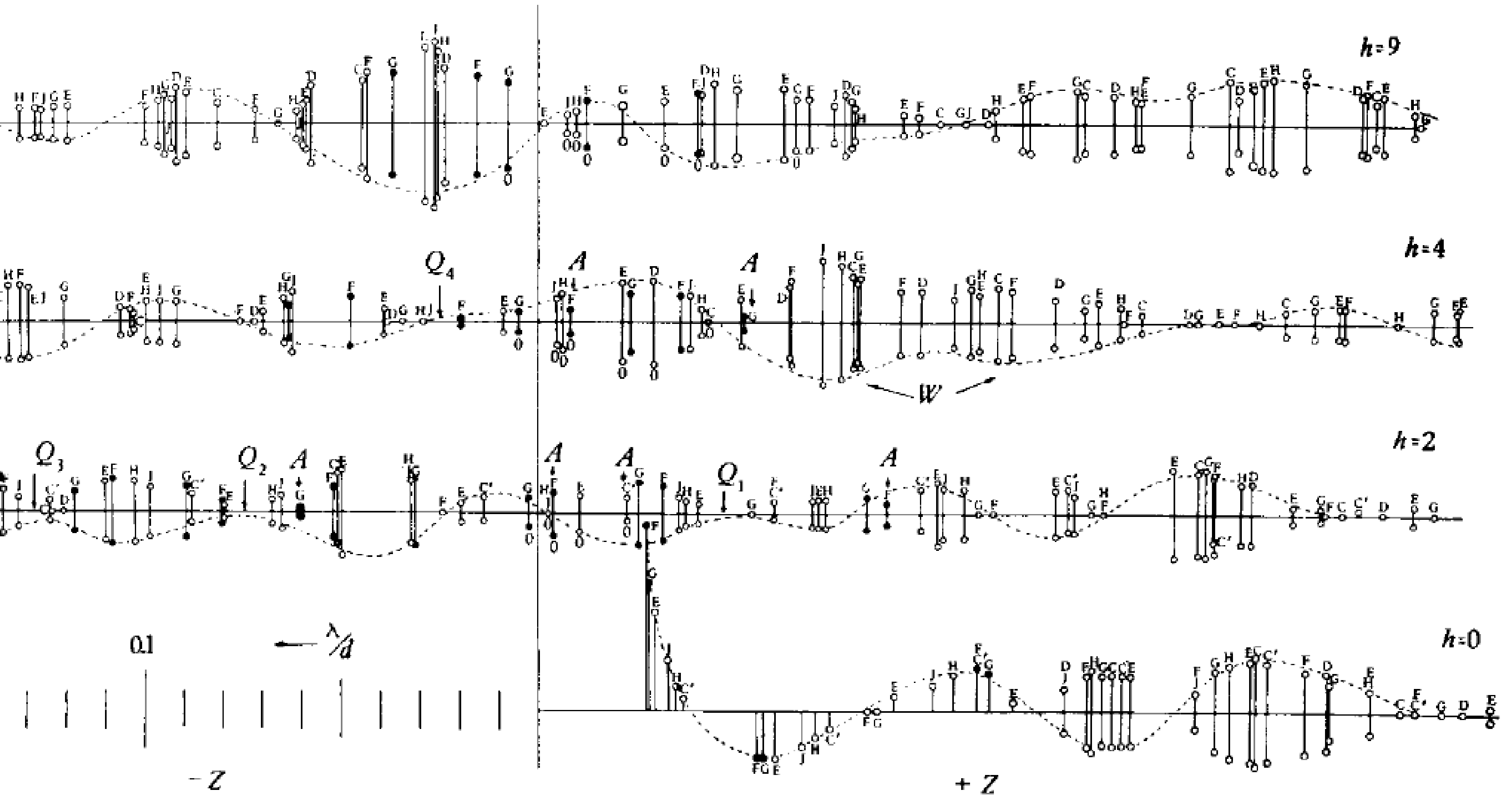


Structure factor (electrons)
900
800
700
600
500
400
300
200
100
0
-0.6
-0.4
-0.2
0
0.2
0.4
Correlation to 2nd & 3rd singular vectors
F(11,11,4)
F(11,11,5)
poly3(x)
poly2(x)



Structure factor (electrons)
900
800
700
600
500
400
300
200
100
0
-0.6
-0.4
-0.2
0
0.2
0.4
Correlation to 2nd & 3rd singular vectors
F(11,11,4)
avg + rej
F(11,11,5)
avg + rej

Bragg's "minimum wavelength principle"

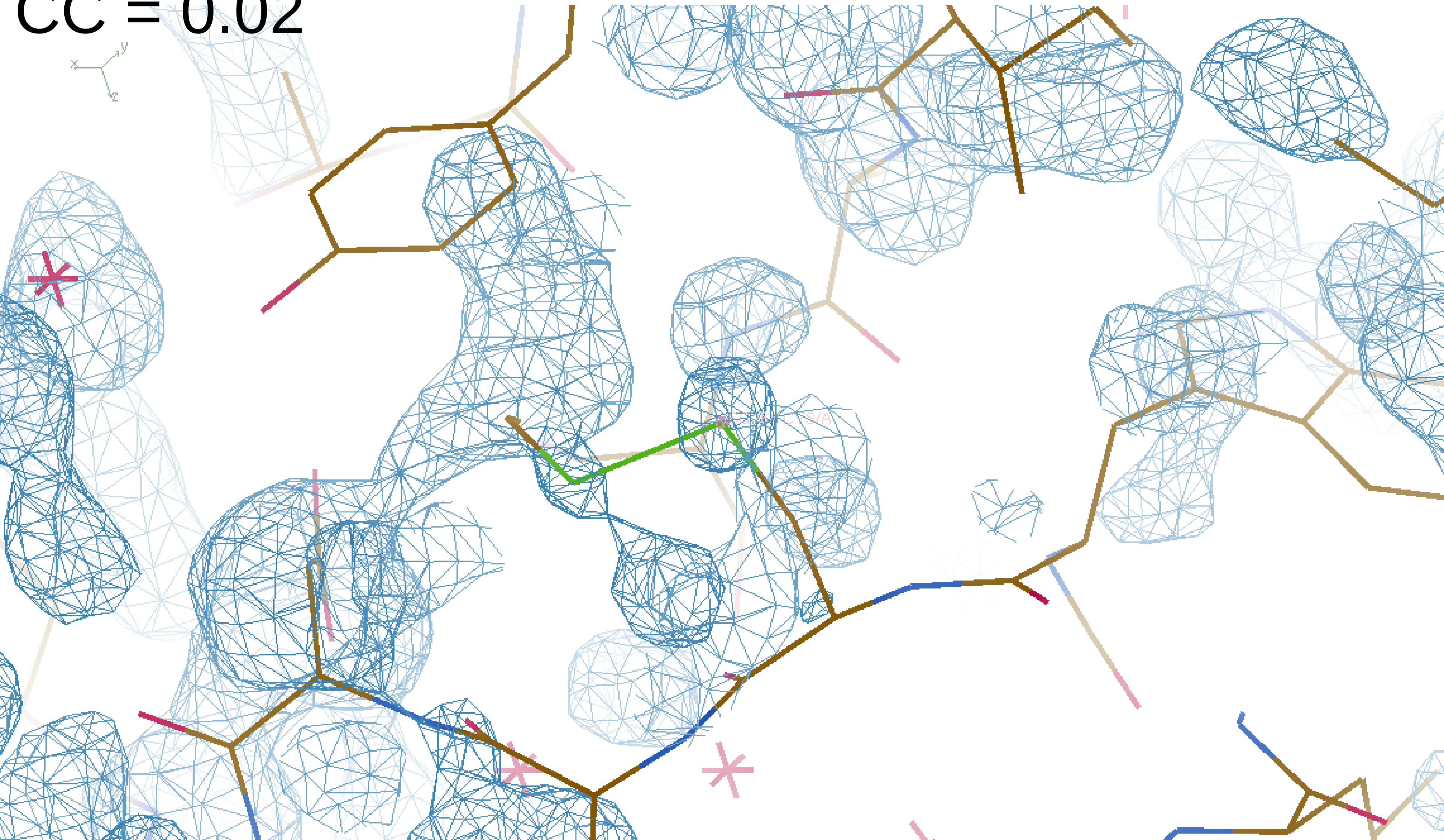


Perutz (1985). "Early Days of Cryst…" *Methods in Enzymology*, Vol. 114, 3-18.
Bragg & Perutz (1952)."external form haemoglobin I", *Acta Cryst.* **5**, 277-283.

DMMULTI – fake data

4 deg rotation: 8 “xtals”: before

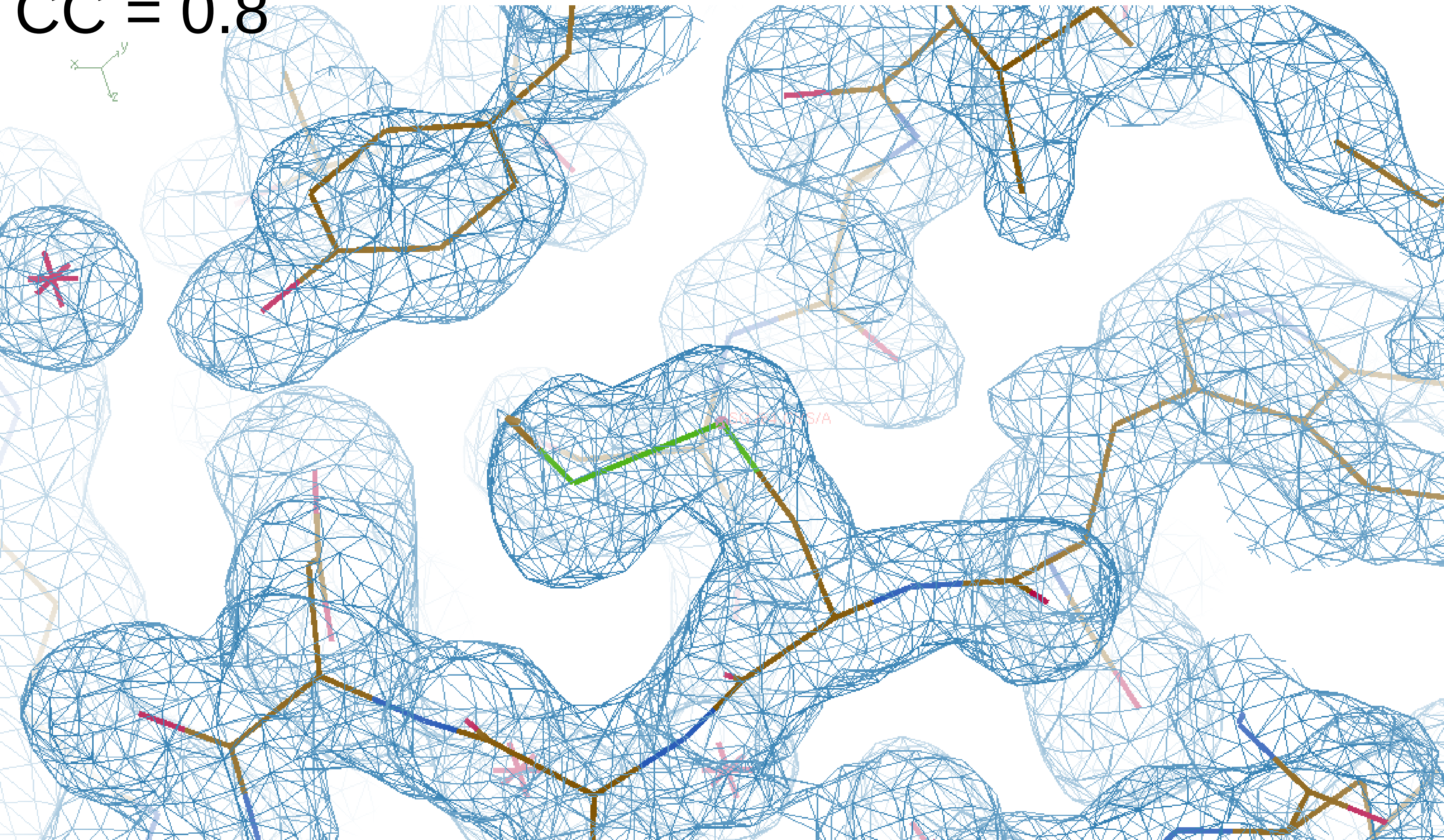
CC = 0.02



DMMULTI – fake data

4 deg rotation: 8 “xtals”: after

CC = 0.8



Suggested anomalous protocol:

1. 360° in < 5 MGy
2. move detector
3. 4X exposure
4. goto 2

2 wavelengths are better than 1

- (peak + inf)/2, and remote

MAD, not M-SAD!

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

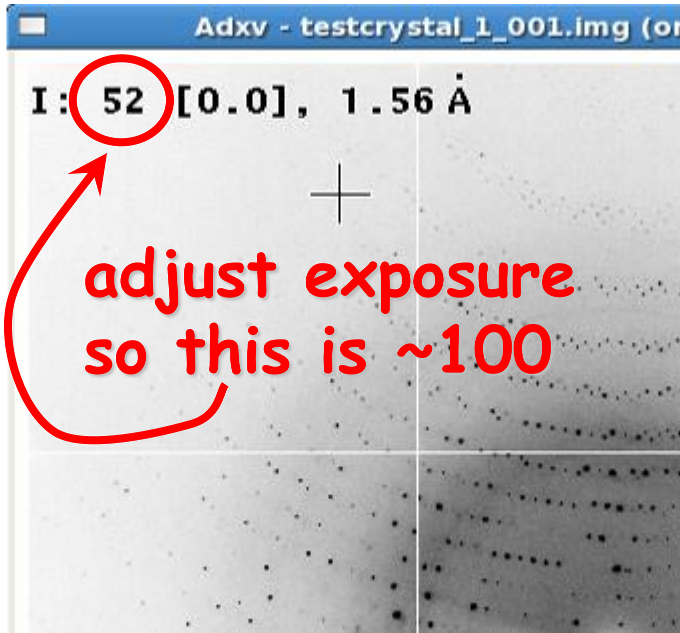
Run Strategy

Optimal exposure time

(CCD)

$$t_{hr} = t_{ref} \frac{10 \cdot m \cdot \sigma_0^{\ 2}}{gain \cdot \left(bg_{ref} - bg_0 \right)}$$

t_{hr}	Optimal exposure time for data set (s)
t_{ref}	exposure time of reference image (s)
bg_{ref}	background level near weak spots on reference image (ADU)
bg_0	ADC offset of detector (ADU)
bg_{hr}	optimal background level (via t_{hr})
σ_0	rms read-out noise (ADU)
$gain$	ADU/photon
m	multiplicity of data set (including partials)



Optimal exposure time

(PAD)

$$t_{hr} > 100 \times t_{ro}$$

t_{hr} Optimal exposure time for data set (s)

t_{ro} read-out time (s)

2.03 ms on PILATUS3 S

3 μs on EIGER

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

expected crystal lifetime calcul... ×

bl831.als.lbl.gov/xtallife.html

Search

expected crystal lifetime calculator

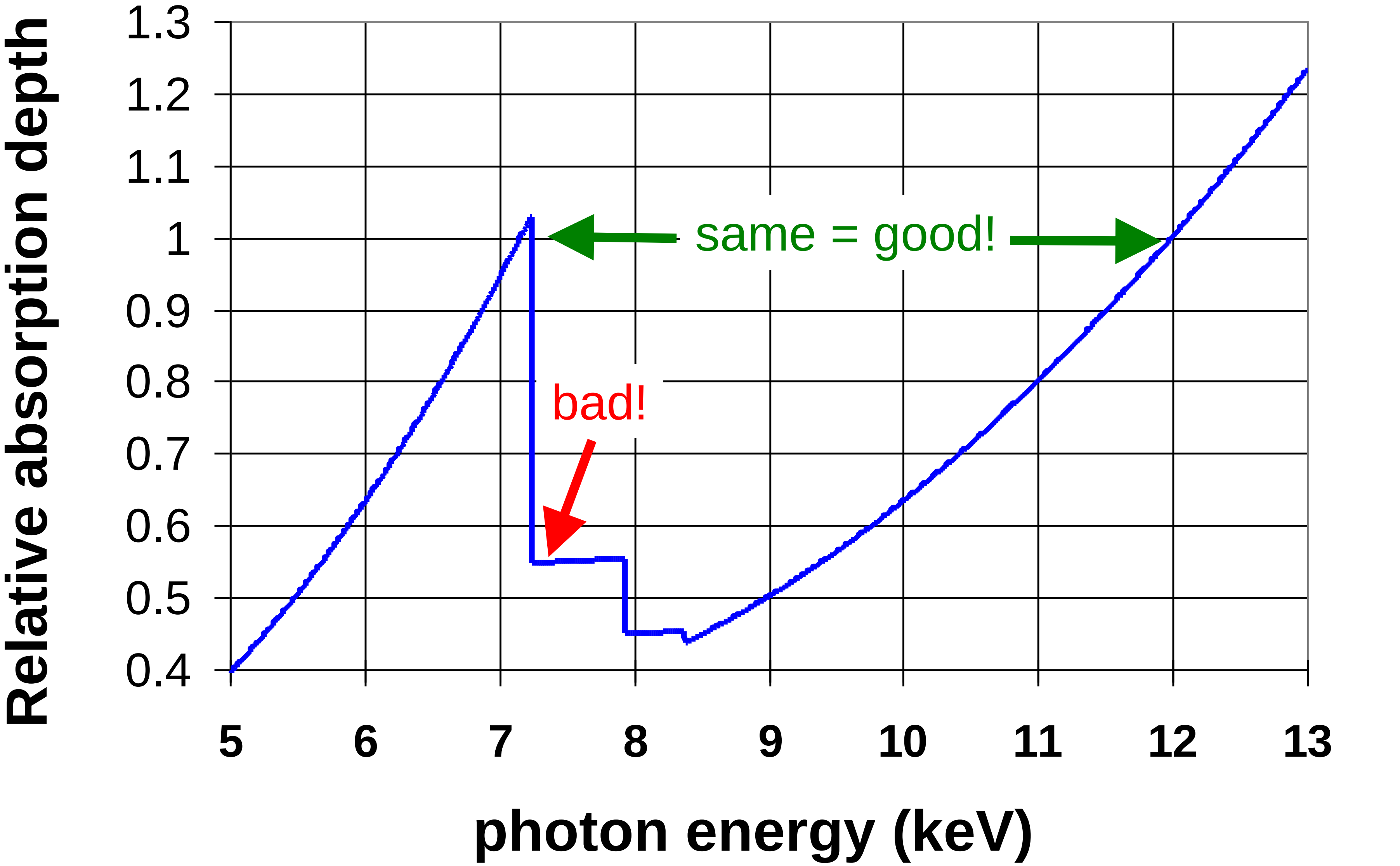
source =	APS		22-ID		
full flux =	7.0e+12	photons/s			
attenuation =	0	%	transmittance =	100	%
beam $size_{horiz}$ =	40.0	microns	beam $size_{vert}$ =	80.0	microns
wavelength =	1	Ang	k_{dose} =	2000	$photons/micron^2/Gy$
dose rate =	1.1e+6	Gy/s			
experiment goal =	high resolution (cryo)				
resolution =	3	Ang			
dose limit =	30	MGy			
exposure time =	1	seconds/image			
xtal $size_{horiz}$ =	50	microns	xtal $size_{vert}$ =	50	microns
translation during dataset =	0	microns	rotisserie factor	1	☐ disable warnings
max images =	28	at damage limit			
inverse beam =	no				
number of wavelengths =	1				
images/wedge =	28				

Decisions, Decisions, Decisions

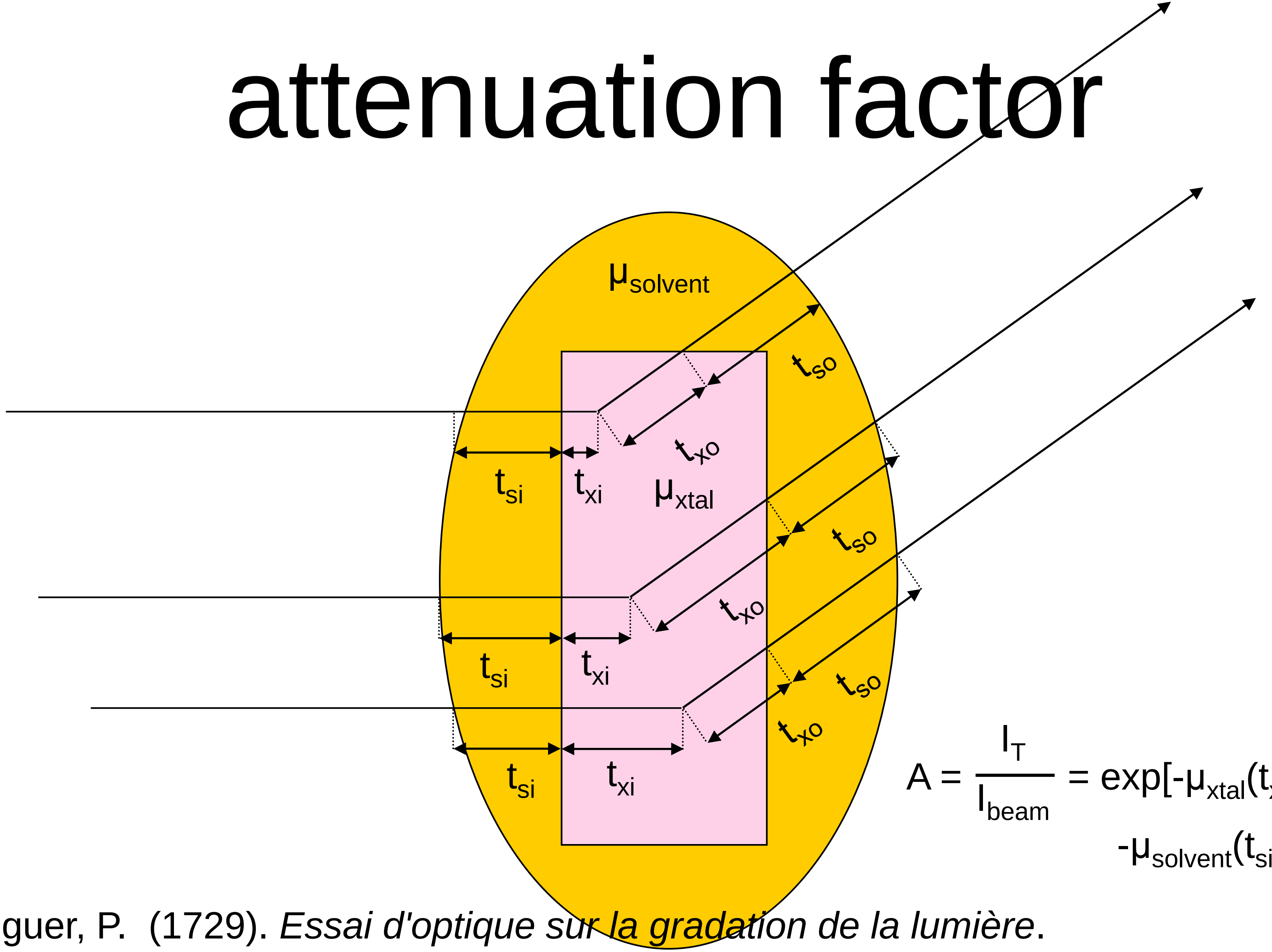
- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

Gadox calibration vs energy



attenuation factor



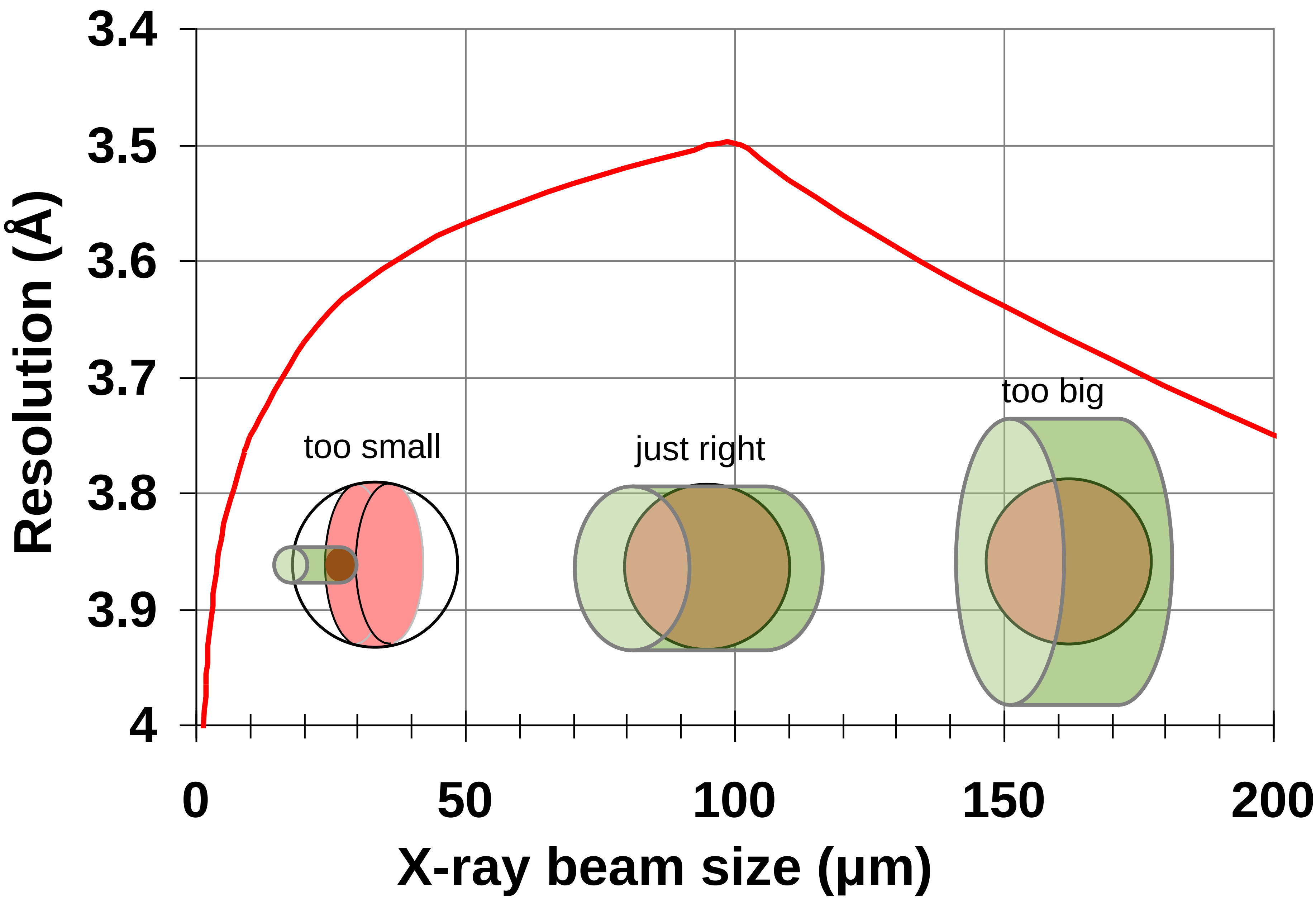
$$A = \frac{I_T}{I_{beam}} = \exp[-\mu_{xtal}(t_{xi} + t_{xo}) - \mu_{solvent}(t_{si} + t_{so})]$$

Bouguer, P. (1729). *Essai d'optique sur la gradation de la lumière*.
Lambert, J. H. (1760). *Photometria: sive De mensura et gradibus luminis, colorum et umbrae*. E. Klett.
Beer, A. (1852)."Bestimmung der Absorption des rothen Lichts in farbigen Flüssigkeiten", *Ann. Phys. Chem* **86**, 78-90.

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy



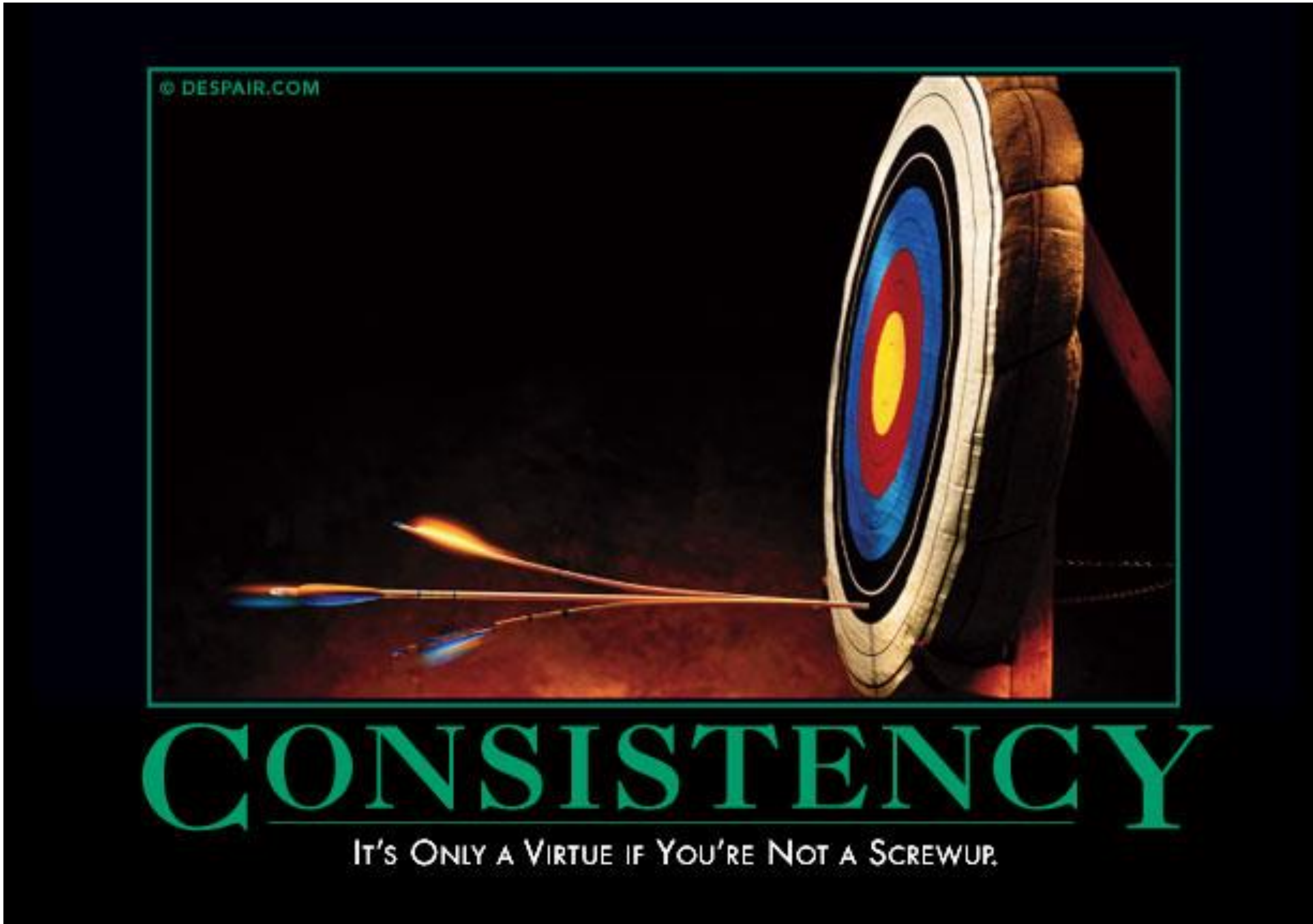
Resolution (Å)
3.4
3.5
3.6
3.7
3.8
3.9
4
too small
just right
too big
0
50
100
150
200
X-ray beam size (µm)

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

Systematic error does not "average out"



Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

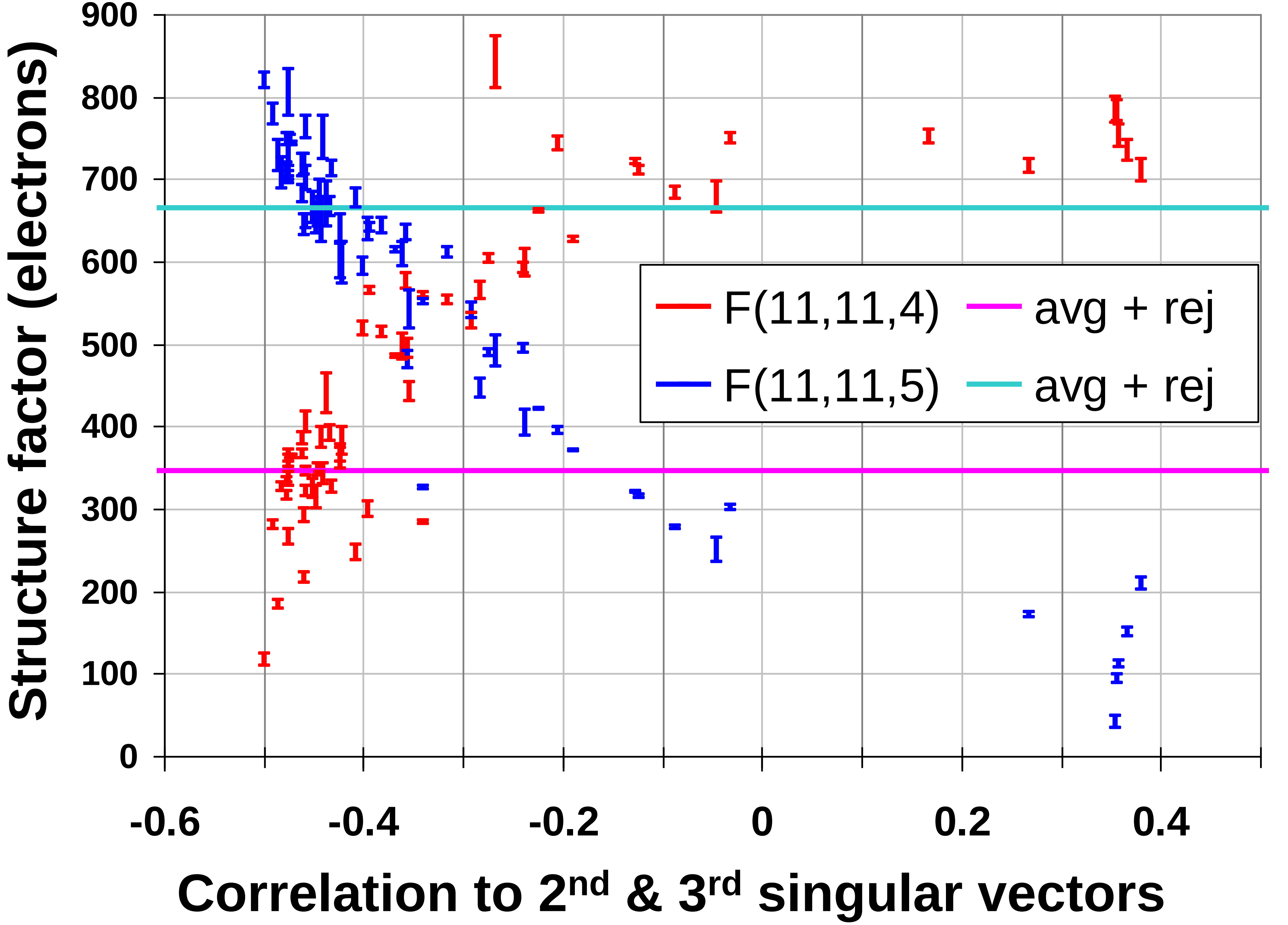
Run Strategy

SmarAct's
"SmarGon"
Commercialized
PRIGo

Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy


900
800
700
600
500
400
300
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0
Structure factor (electrons)
-0.6
-0.4
-0.2
0
0.2
0.4
Correlation to 2nd & 3rd singular vectors
F(11,11,4)
avg + rej
F(11,11,5)
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Decisions, Decisions, Decisions

- Exposure time
- Number of images
- Wavelengths
- Beam: size, flux, divergence, bandpass
- Inverse beam?
- Kappa? - overlaps
- Multiple crystals? - non-isomorphism

Run Strategy

URL Summary

http://bl831.als.lbl.gov/~jamesh/powerpoint/IGBMC_SvN_2016.pptx

http://bl831.als.lbl.gov/xtalsize.html

http://bl831.als.lbl.gov/xtallife.html

100 ADU/pixel

10 µm for lysozyme

~3% error per spot, 1%/MGy

7235 eV for S-SAD on CCDs

“Attenu-wait” & dose slicing

CCP4: aimless log

```
$TABLE:  Analysis against resolution, XDSdataset:
$GRAPHS:I/sigma, Mean Mn(I)/sd(Mn(I)):0|0.216023x0|137.14:2,13,14:
:Rmerge, Rfull, Rmeas, Rpim v Resolution:0|0.216023x0|1.70834:2,4,5,6,7:
:Average I, RMSdeviation and Sd:0|0.216023x0|1650.8:2,10,11,12:
:Fractional bias:0|0.216023x0|0:2,15:
$$
```

N	1/d^2	Dmid	Rmrg	Rfull	Rcum	Rmeas	Rpim	Nmeas	AvI	RMSdev	sd	I/RMS	Mn(I/sd)	Frc
1	0.0064	12.55	0.020	0.020	0.020	0.021	0.006	13115	1651	57	42	29.2	137.1	
2	0.0191	7.24	0.027	0.027	0.024	0.028	0.008	24753	1171	47	42	25.0	105.2	-
3	0.0318	5.61	0.038	0.038	0.029	0.040	0.012	32197	857	46	43	18.4	79.6	-
4	0.0445	4.74	0.034	0.034	0.031	0.035	0.010	37743	1212	57	53	21.4	91.2	-
5	0.0572	4.18	0.036	0.036	0.032	0.038	0.011	42642	1181	59	57	19.9	83.8	-
6	0.0699	3.78	0.049	0.049	0.036	0.052	0.015	47224	883	59	57	15.1	65.1	-
7	0.0826	3.48	0.065	0.065	0.040	0.068	0.020	51052	685	59	58	11.7	50.9	-
8	0.0953	3.24	0.096	0.096	0.045	0.100	0.029	54636	448	56	56	8.0	35.0	-
9	0.1080	3.04	0.151	0.151	0.050	0.158	0.046	58072	268	53	53	5.1	22.7	-
10	0.1207	2.88	0.229	0.229	0.056	0.240	0.070	60731	171	51	51	3.3	15.4	-
11	0.1334	2.74	0.314	0.314	0.063	0.329	0.097	63807	125	51	51	2.4	11.3	-
12	0.1461	2.62	0.406	0.406	0.070	0.425	0.125	66241	98	51	52	1.9	8.7	-
13	0.1588	2.51	0.537	0.537	0.078	0.562	0.166	68272	76	53	53	1.4	6.5	-
14	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

CCP4: aimless log

```
$TABLE:  Correlations CC(1/2) within dataset, XDSdataset:
$GRAPHS: Anom & Imean CCs v resolution:0|0.216023x0|1:2,4,7:
: RMS correlation ratio :0|0.216023x0|2.20344:2,6:
$$
```

N	1/d^2	Dmid	CCanom	Nanom	RCRanom	CC1/2	NImean	$
1	0.0064	12.55	0.659	499	2.203	1.000	669	
2	0.0191	7.24	0.550	975	1.853	1.000	1155	
3	0.0318	5.61	0.527	1295	1.798	1.000	1479	
16	0.1970	2.25	0.037	2123	1.038	0.711	2275	
17	0.2097	2.18	0.043	1682	1.044	0.460	1877	

XDS: CORRECT.LP or XSCALE.LP

ION OMPARED	I/SIGMA	R-meas	CC(1/2)	Anomal Corr	SigAno
3018	33.77	2.3%	99.9*	21*	1.012
4585	22.56	3.6%	99.8*	9	0.914
5327	19.99	4.0%	99.7*	9	0.859
6094	12.27	6.8%	99.3*	-1	0.784
7068	6.01	14.2%	97.8*	-2	0.799
8185	3.10	29.4%	88.8*	-4	0.776
8981	1.90	48.8%	75.9*	2	0.765
5991	1.14	87.3%	53.5*	-2	0.722
2520	0.59	170.4%	21.9*	4	0.693
51769	8.97	9.5%	99.5*	2	0.804

XDS: CORRECT.LP or XSCALE.LP

```
**************************************************************
   CORRECTION PARAMETERS FOR THE STANDARD ERROR OF REFLECTION I
 *************************************************************


The variance v0(I) of the intensity I obtained from counting st
replaced by v(I)=a*(v0(I)+b*I^2). The model parameters a, b are
minimize the discrepancies between v(I) and the variance estima
sample statistics of symmetry related reflections. This model i
an asymptotic limit ISa=1/SQRT(a*b) for the highest I/Sigma(I)
experimental setup can produce (Diederichs (2010) Acta Cryst D6

     a          b             ISa
3.806E+00  1.080E-04     49.32
```