Acknowledgements

Ken Frankel   John Spence

8.3.1 creator: Tom Alber
8.3.1 PRT head: Jamie Cate

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Membrane Protein Expression Center II
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Problems and Promises

- radiation damage
- non-isomorphism
- anomalous differences
- the “twin problem”
- postrefinement
- the structure of disorder

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Darwin's Formula

$$I(hkl) = I_{beam}\, r_e^2 \frac{V_{xtal}}{V_{cell}} \frac{\lambda^3 L}{\omega V_{cell}} P\, A\, |F(hkl)|^2$$

I(hkl) - photons/spot (fully-recorded)

I_{beam} - incident (photons/s/m^2)

r_e - classical electron radius (2.818×10^{-15} m)

V_{xtal} - volume of crystal (in m^3)

V_{cell} - volume of unit cell (in m^3)

λ - x-ray wavelength (in meters!)

ω - rotation speed (radians/s)

L - Lorentz factor (speed/speed)

P - polarization factor

$(1+\cos^2(2\theta) - \mathrm{Pfac}\cdot\cos(2\Phi)\sin^2(2\theta))/2$

A - absorption factor

$\exp(-\mu_{xtal}\cdot l_{path})$

F(hkl) - structure amplitude (electrons)

C. G. Darwin (1914)

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Dose Formula

$$\text{dose} \approx I_{beam} \cdot t_{exp} \frac{\lambda^2}{2000}$$

dose	- absorbed energy (Gy)
$\mathbf{I_{beam}}$	- incident (photons/s/μm^2)
$\mathbf{t_{exp}}$	- exposure time (s)
λ	- x-ray wavelength (in Å)

Dose Formula

$$D_{max} \approx I_{beam} \cdot t_{dataset} \frac{\lambda^2}{2000}$$

D_{max} - maximum dose (Gy)

I_{beam} - incident (photons/s/μm^2)

$t_{dataset}$ - accumulated exposure time (s)

λ - x-ray wavelength (in Å)

Darwin's Formula

$$I(hkl) = I_{beam}\, r_e^2 \frac{V_{xtal}}{V_{cell}} \frac{\lambda^3 L}{\omega V_{cell}} P\, A\, |\, F(hkl)\, |^2$$

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$\exp(-\mu_{xtal}\cdot l_{path})$

F(hkl) - structure amplitude (electrons)

C. G. Darwin (1914)

Darwin's Formula

$$I(hkl) = \frac{D_{max}}{t_{dataset}} r_e^2 \frac{V_{xtal}}{V_{cell}} \frac{2 \lambda L}{\omega V_{cell}} P A | F(hkl) |^2$$

Dmax - maximum dose (kGy)

$t_{dataset}$ - accumulated exposure (s)

r_e - classical electron radius (2.818×10^{-15} m)

V_{xtal} - volume of crystal (in m^3)

V_{cell} - volume of unit cell (in m^3)

λ - x-ray wavelength (in meters!)

ω - rotation speed (radians/s)

L - Lorentz factor (speed/speed)

P - polarization factor

$(1+\cos^2(2\theta) - Pfac \cdot \cos(2\Phi)\sin^2(2\theta))/2$

A - absorption factor

$\exp(-\mu_{xtal} \cdot l_{path})$

F(hkl) - structure amplitude (electrons)

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Darwin's Formula

$$I(hkl) = D_{max} r_e^2 \frac{V_{xtal}}{V_{cell}} \frac{2\lambda L}{2\pi V_{cell}} P A |F(hkl)|^2$$

Dmax - maximum dose (kGy)

r_e - classical electron radius (2.818×10^{-15} m)

V_{xtal} - volume of crystal (in m^3)

V_{cell} - volume of unit cell (in m^3)

λ - x-ray wavelength (in meters!)

2π - rotation range (radians)

L - Lorentz factor (speed/speed)

P - polarization factor

$(1+\cos^2(2\theta) - Pfac \cdot \cos(2\Phi)\sin^2(2\theta))/2$

A - absorption factor

$\exp(-\mu_{xtal} \cdot l_{path})$

F(hkl) - structure amplitude (electrons)

C. G. Darwin (1914)

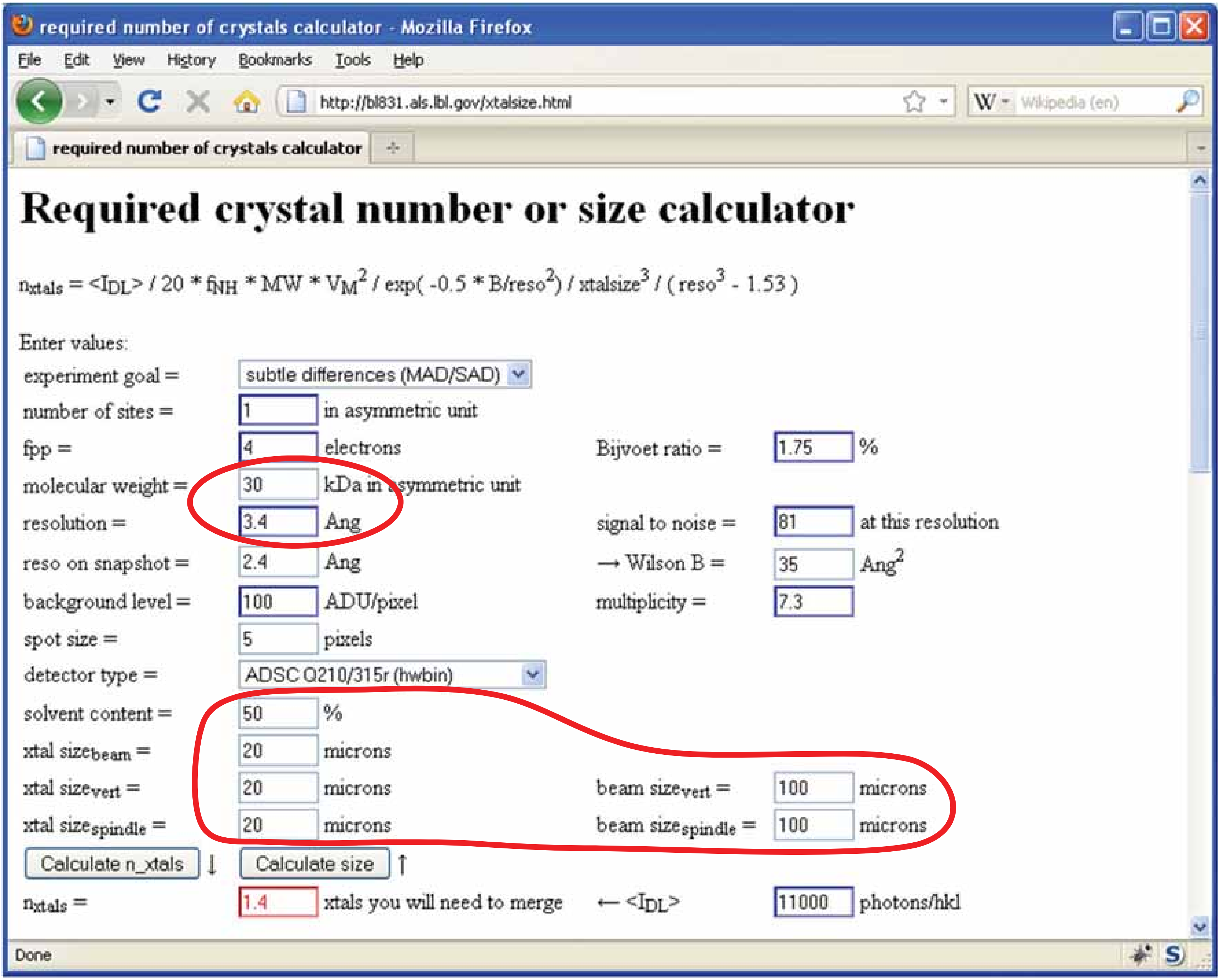
Self-calibrated damage limit

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

Where:

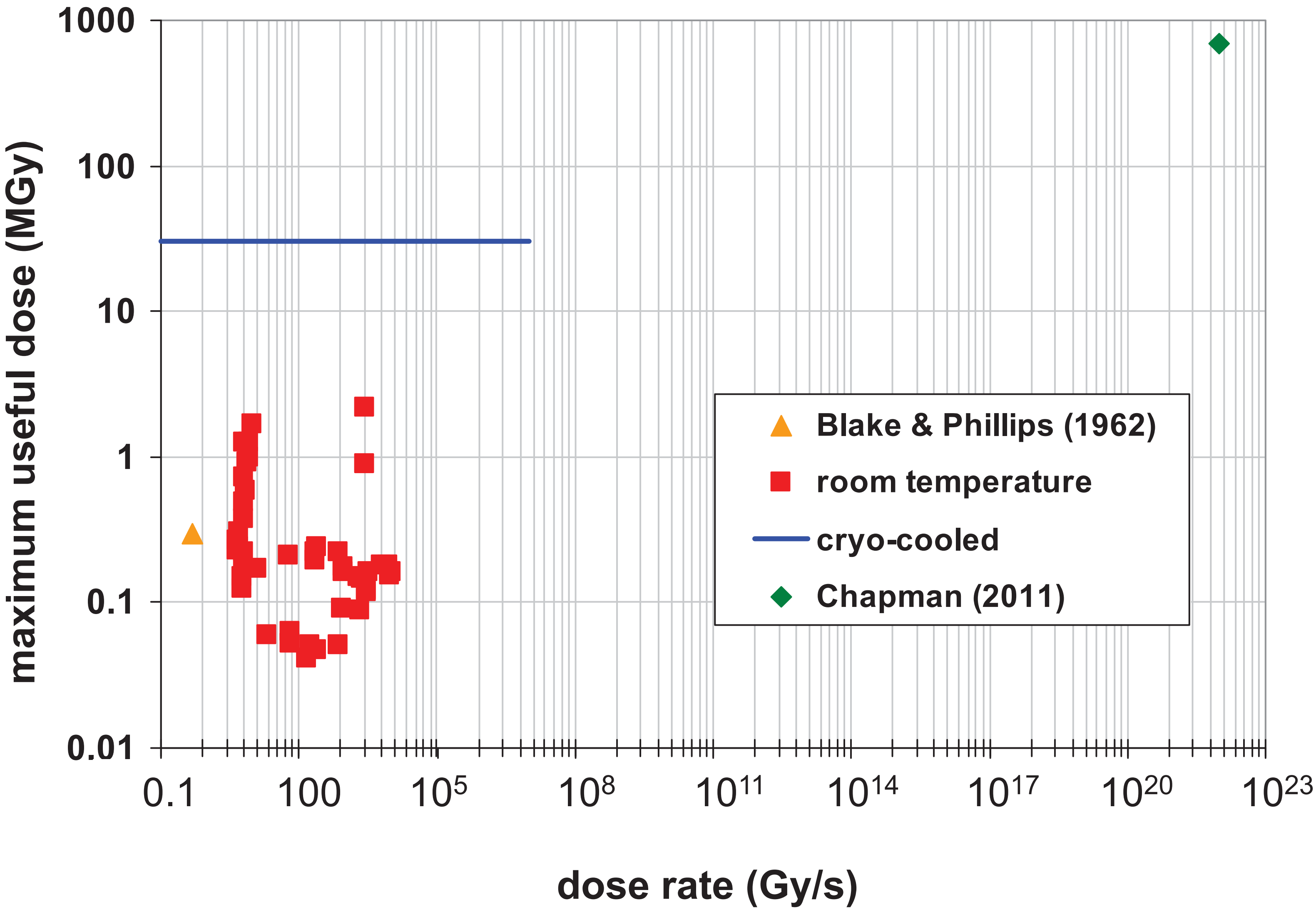
$\langle I \rangle_{DL}$	- average damage-limited intensity (photons/hkl) at a given resolution
10^5	- converting R from μm to m, r_e from m to Å, ρ from g/cm^3 to kg/m^3 and MGy to Gy
r_e	- classical electron radius (2.818 x 10^{-15} m/electron)
h	- Planck's constant (6.626 x 10^{-34} J·s)
c	- speed of light (299792458 m/s)
$\mathrm{f}_{decayed}$	- fractional progress toward completely faded spots at end of data set
ρ	- density of crystal (~1.2 g/cm^3)
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 Å^3/Dalton)
H	- Howells's criterion (10 MGy/Å)
θ	- Bragg angle
$\langle f_a^{\,2} \rangle$	- number-averaged squared structure factor per protein atom (electron^2)
$\langle M_a \rangle$	- number-averaged atomic weight of a protein atom (~7.1 Daltons)
B	- average (Wilson) temperature factor (Å^2)
μ	- attenuation coefficient of sphere material (m^{-1})
μ_{en}	- mass energy-absorption coefficient of sphere material (m^{-1})

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



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

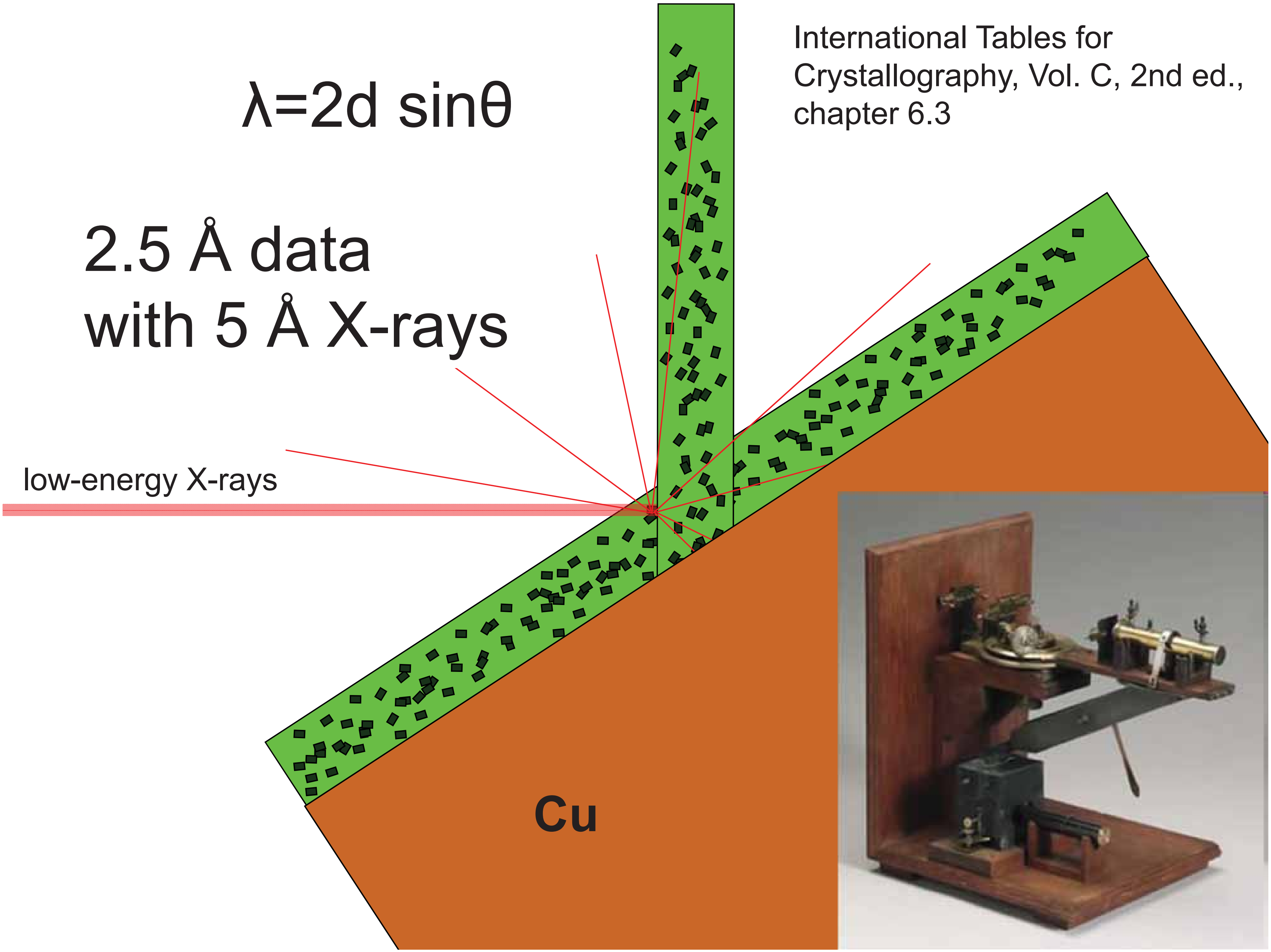
Dose-rate dependence of damage



$\lambda = 2d \sin\theta$

International Tables for Crystallography, Vol. C, 2nd ed., chapter 6.3

2.5 Å data
with 5 Å X-rays



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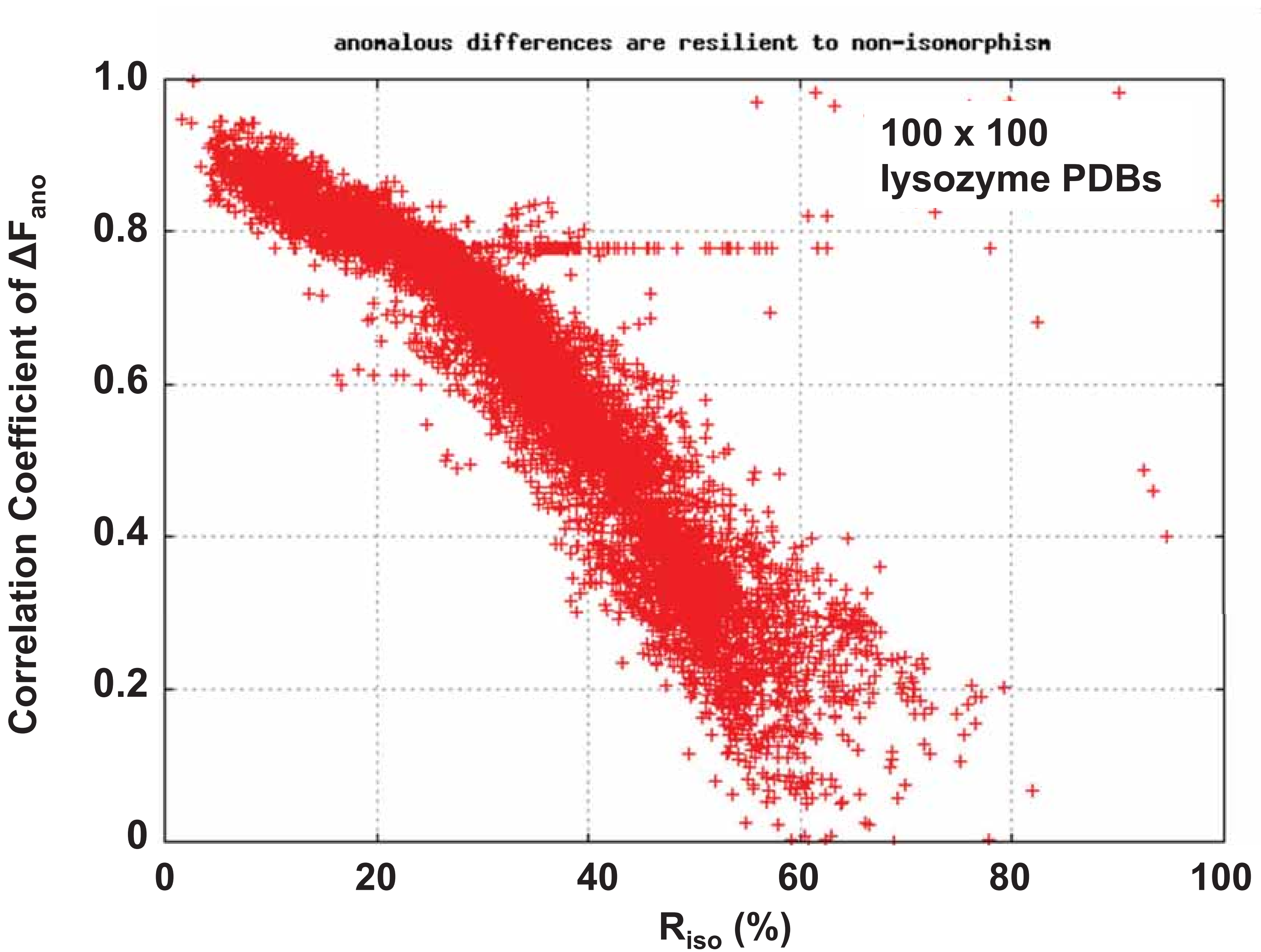
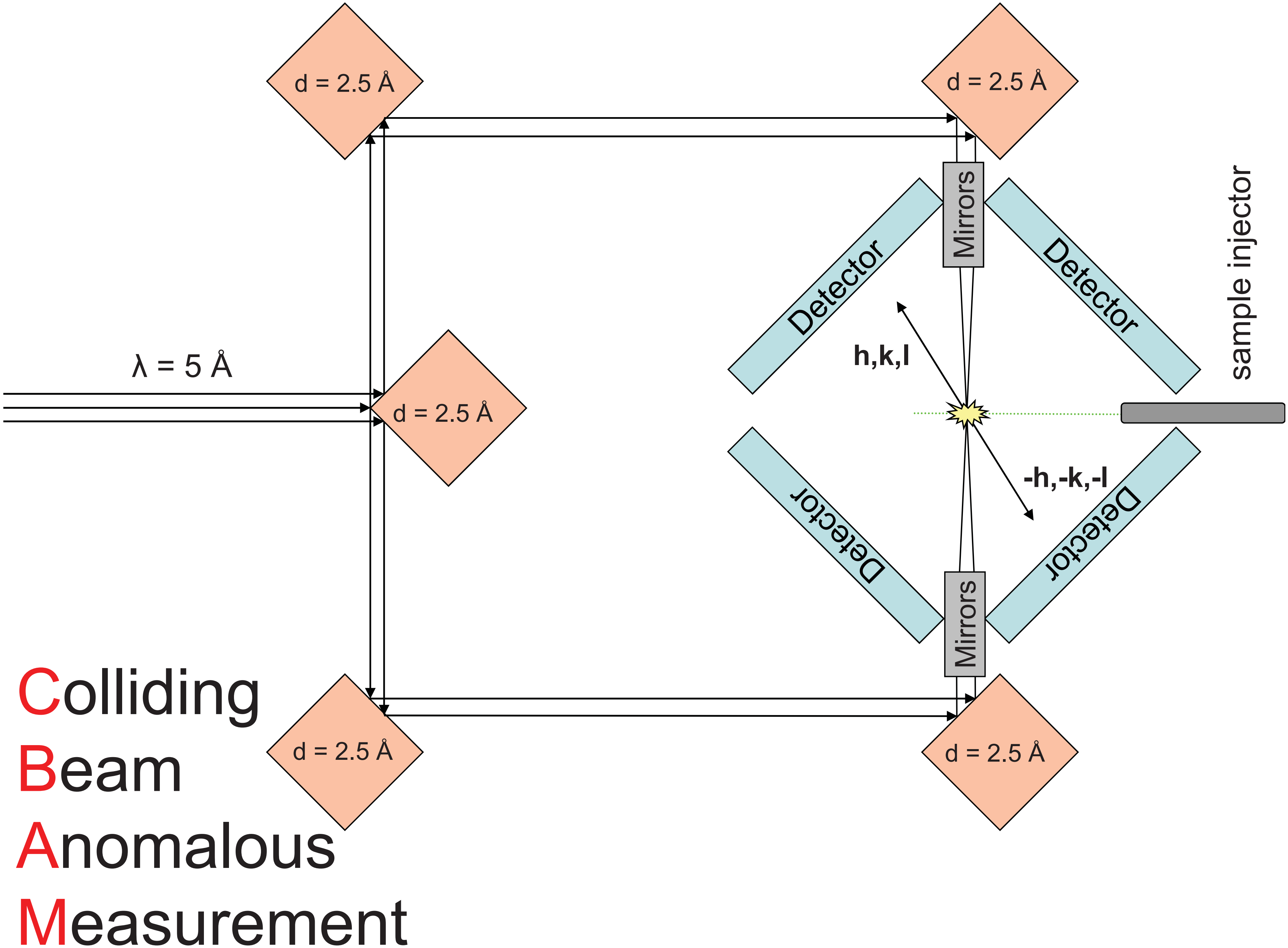
Anomalous differences are resilient to non-isomorphism





λ = 5 Å
d = 2.5 Å
d = 2.5 Å
d = 2.5 Å
d = 2.5 Å
d = 2.5 Å
Mirrors
Mirrors
Detector
Detector
Detector
Detector
h,k,l
-h,-k,-l
sample injector
Colliding
Beam
Anomalous
Measurement

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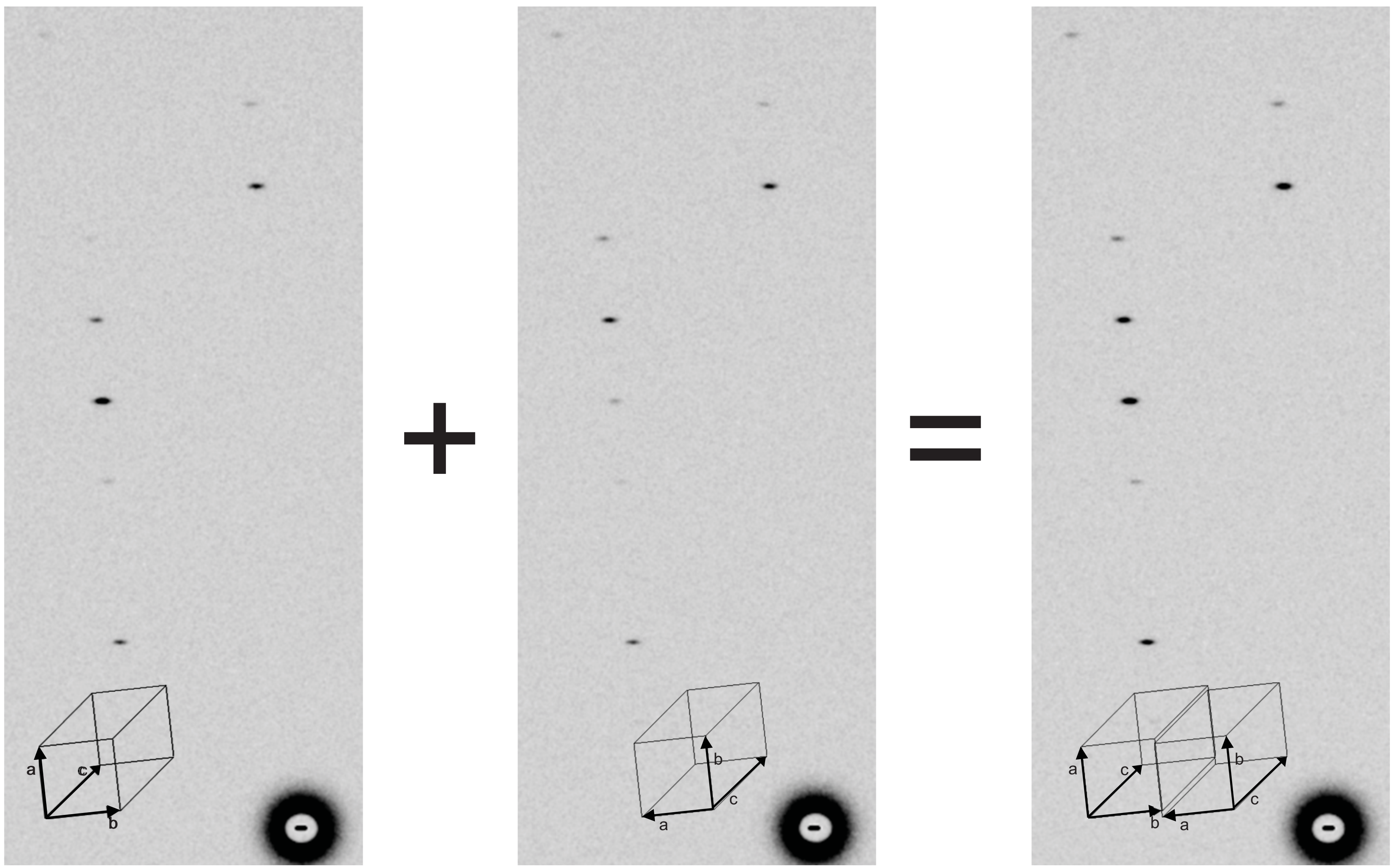
the “twin problem”



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Internal consistency of data

1,850,000 images

112,000 > 10 peaks

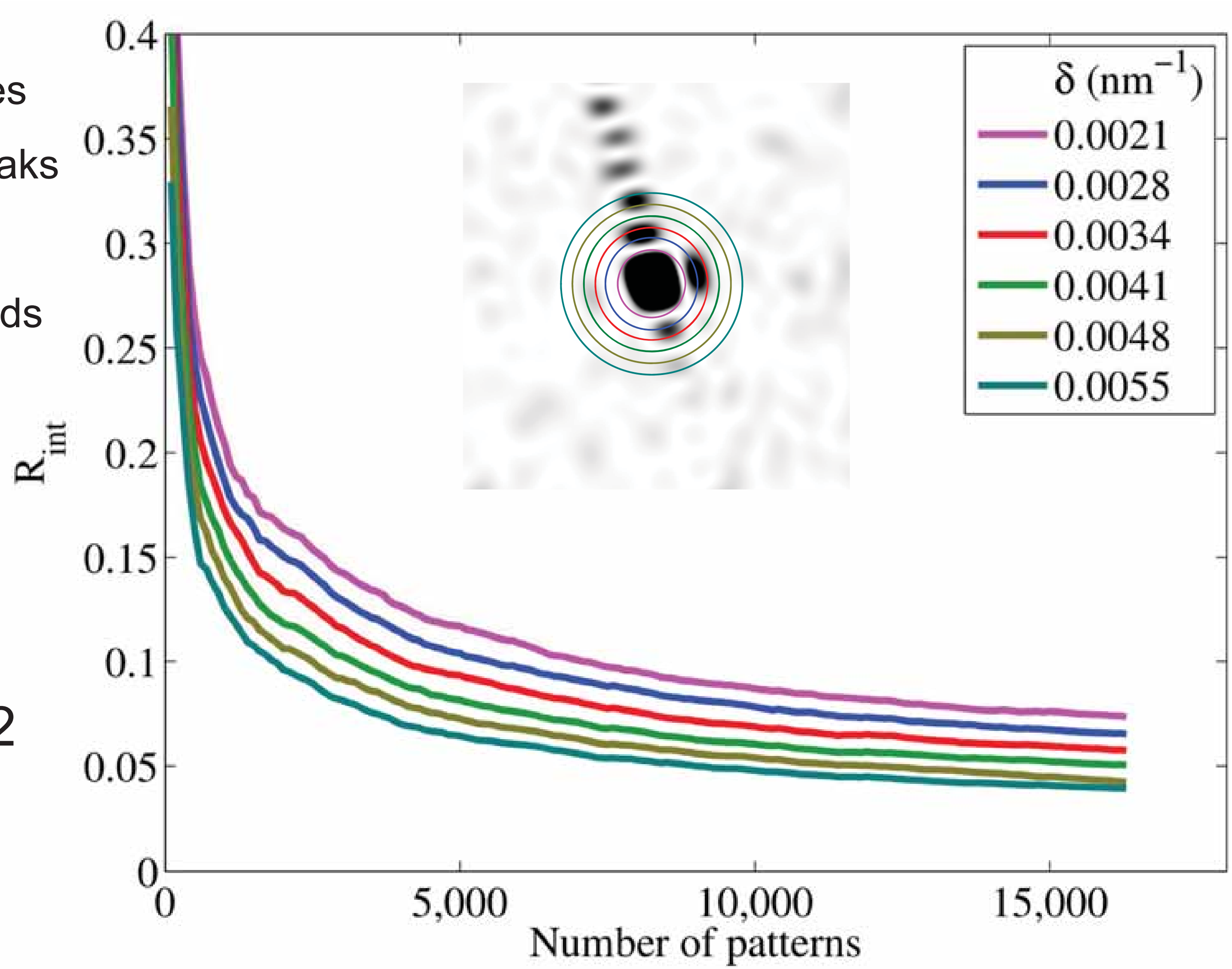
33,000 indexed

16,500 good preds

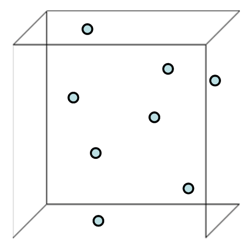
rigid-body:

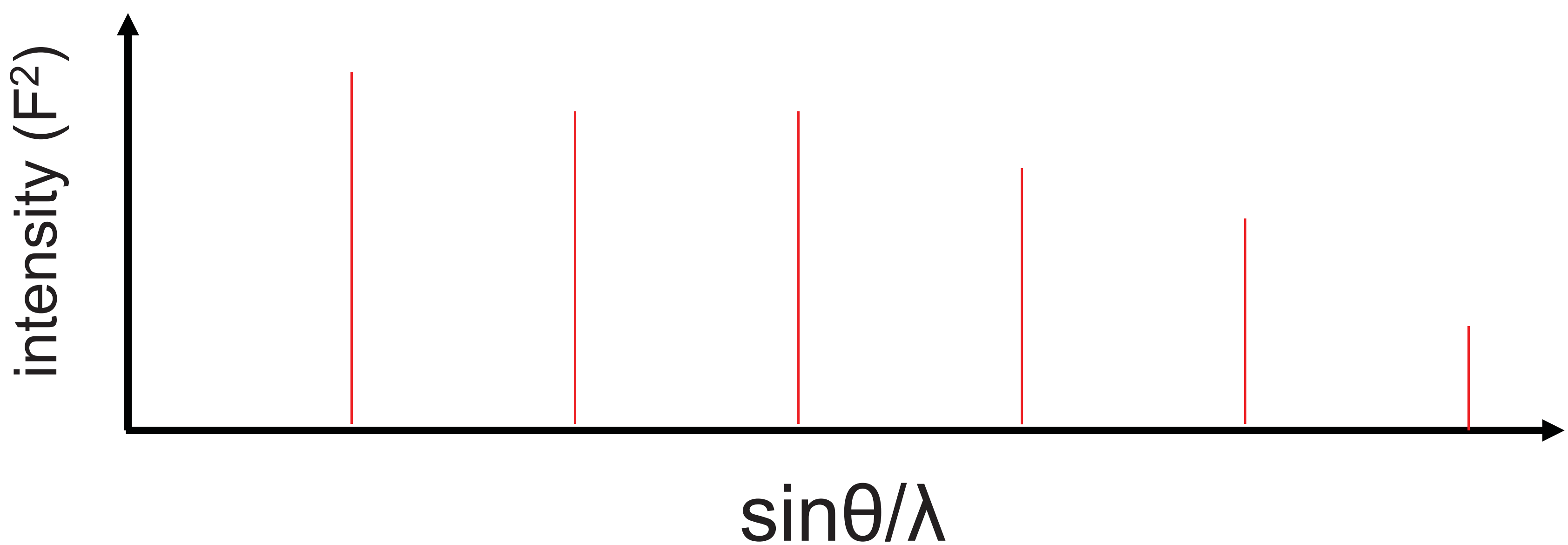
$R_{cryst} = 0.252$

$R_{free} = 0.232$

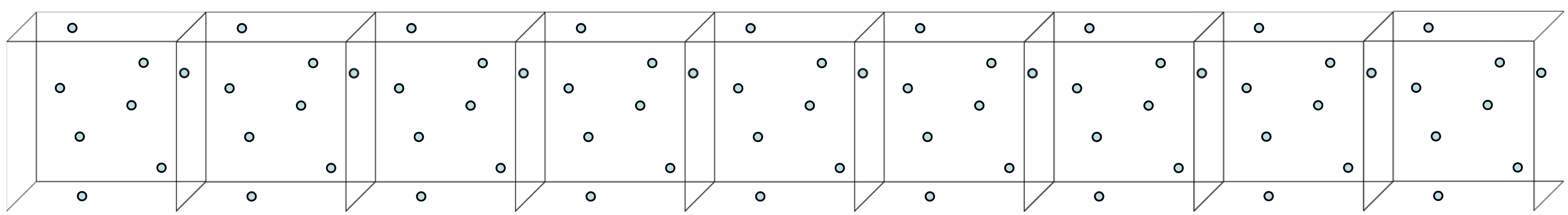


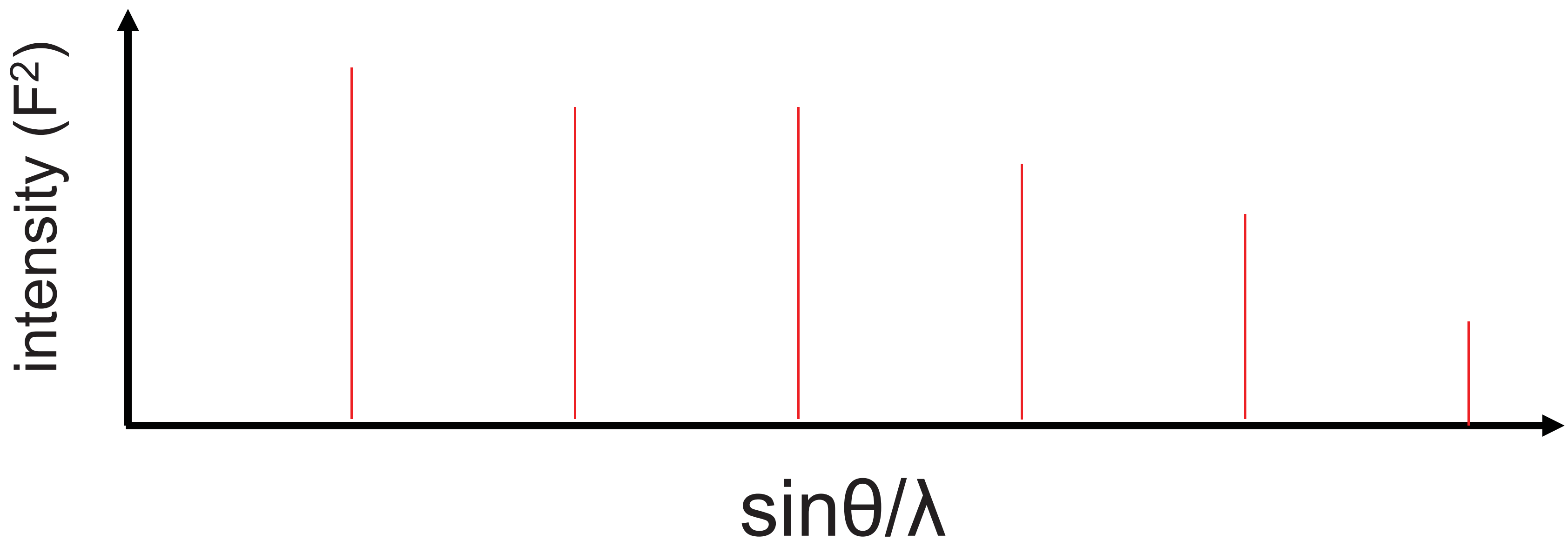
Super-cell formalism for diffuse scatter





Super-cell formalism for diffuse scatter





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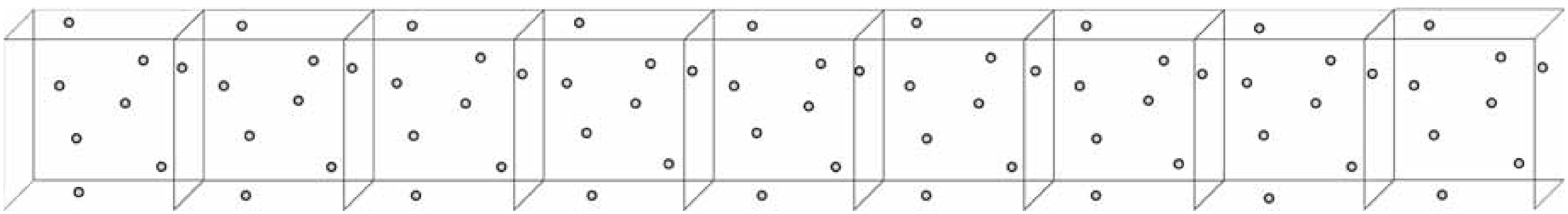

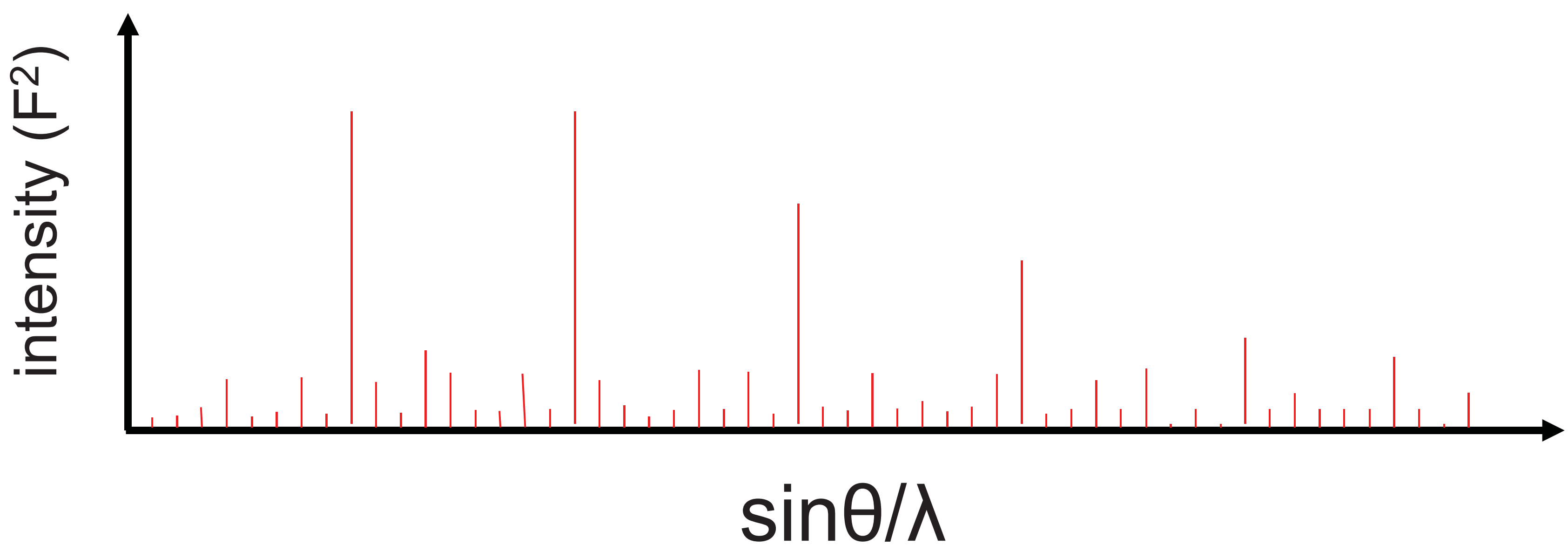

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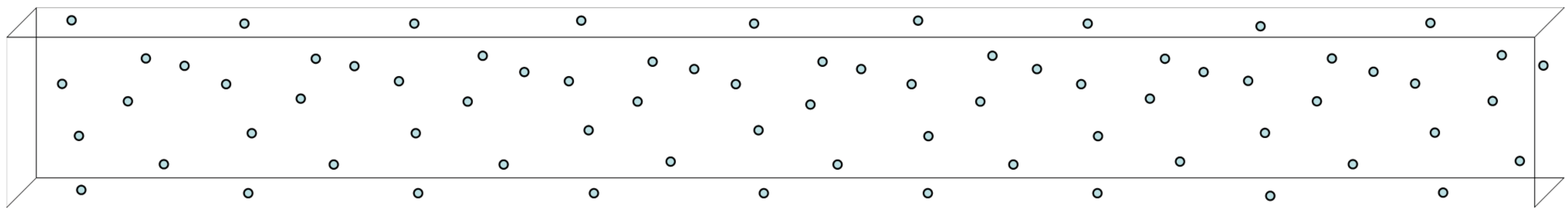

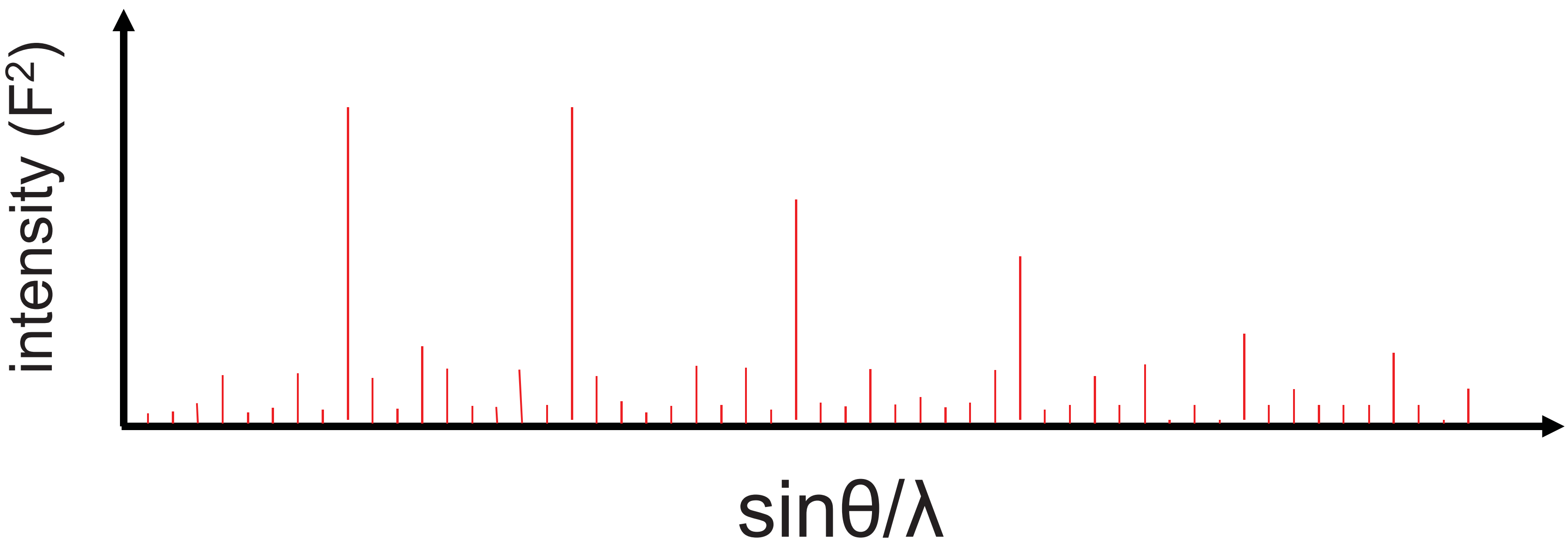

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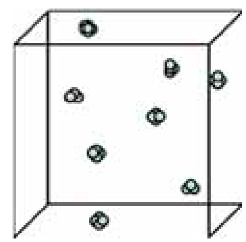
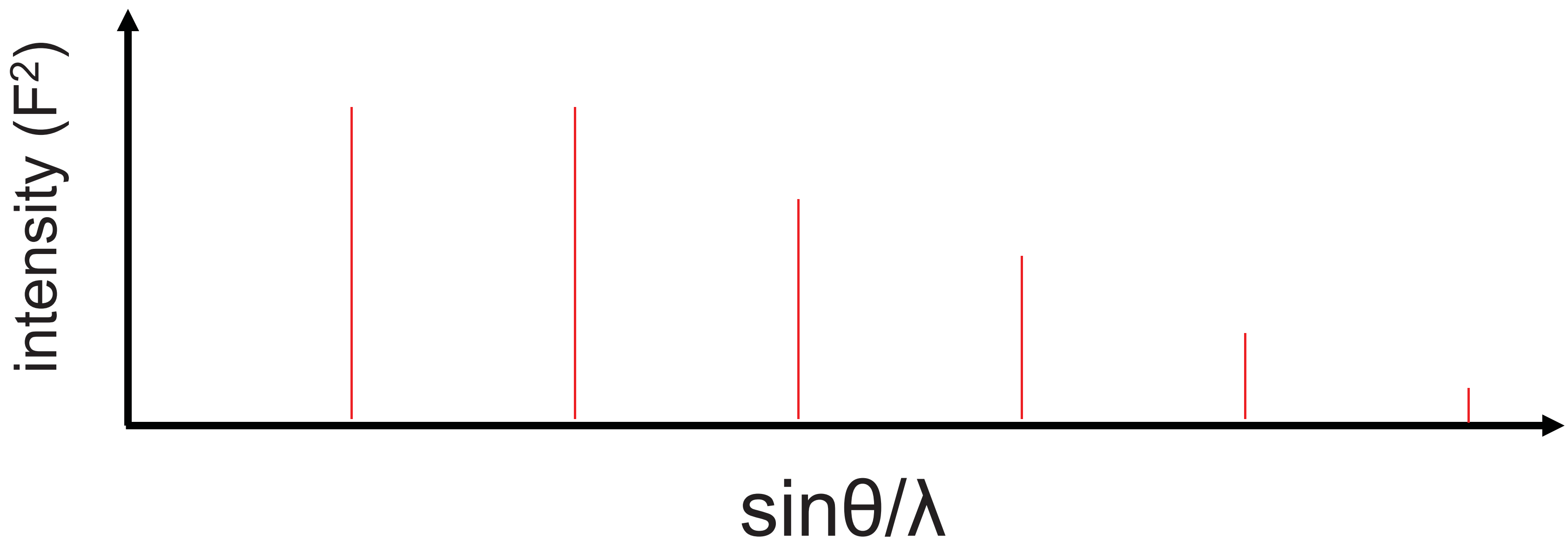
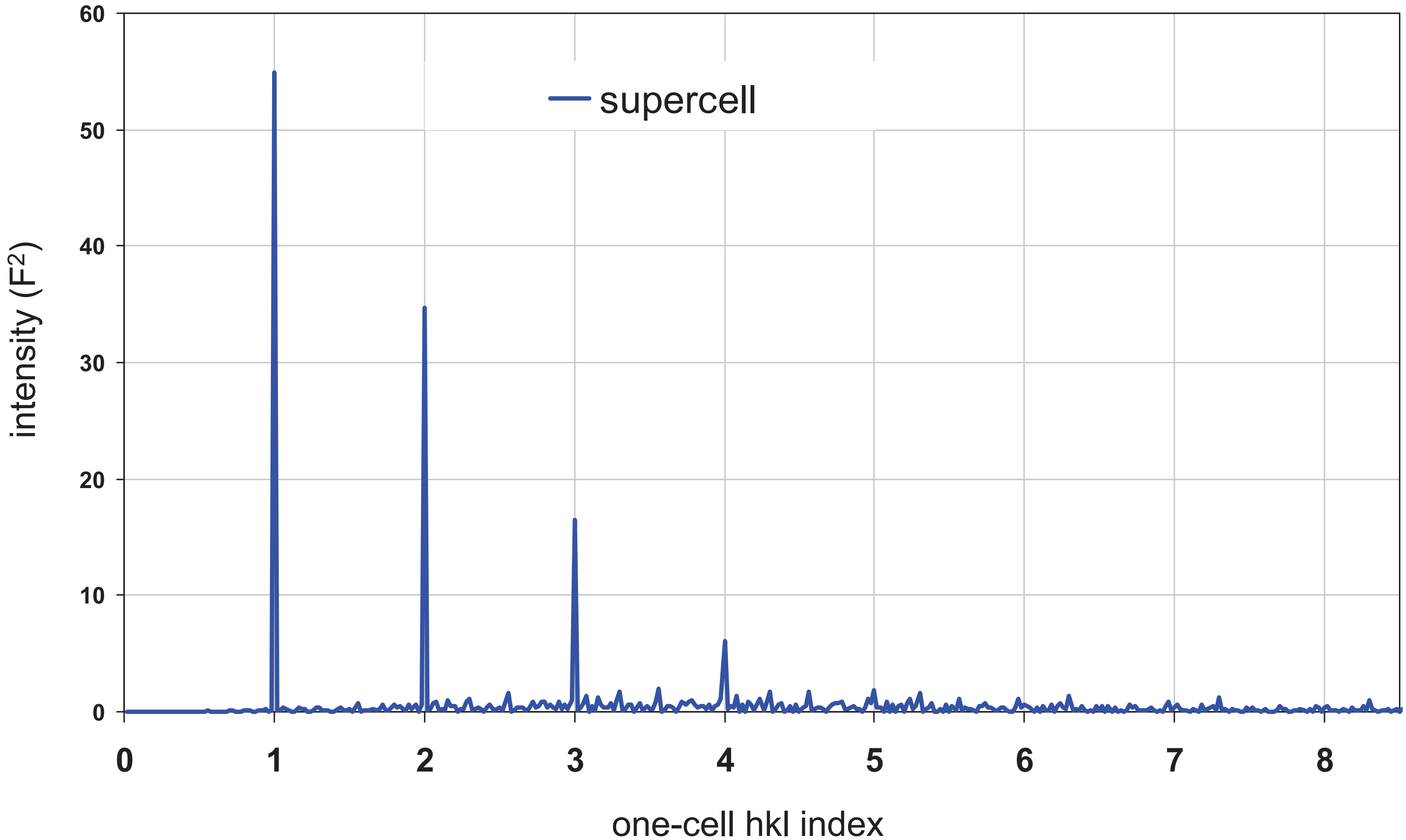




Super-cell formalism for diffuse scatter



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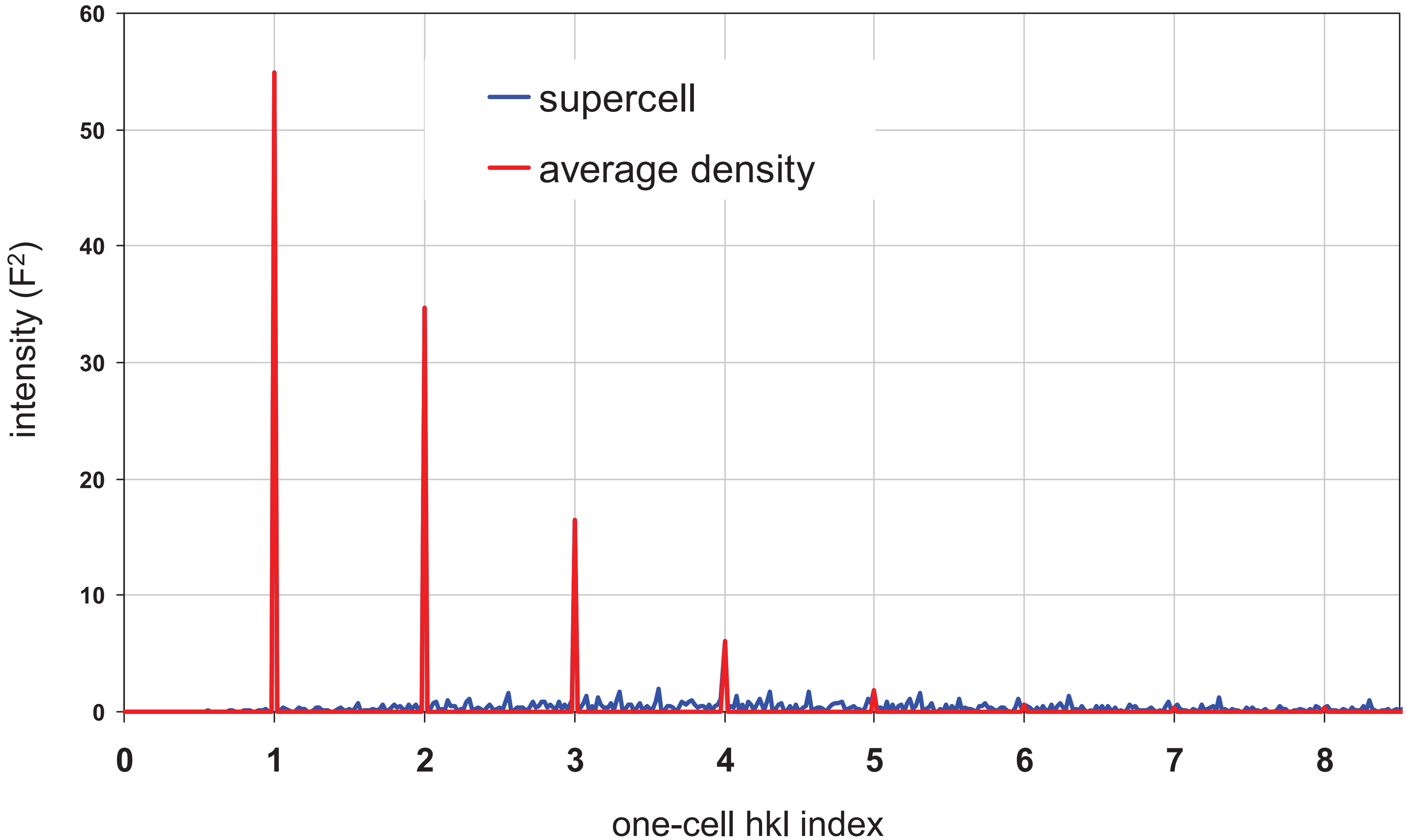
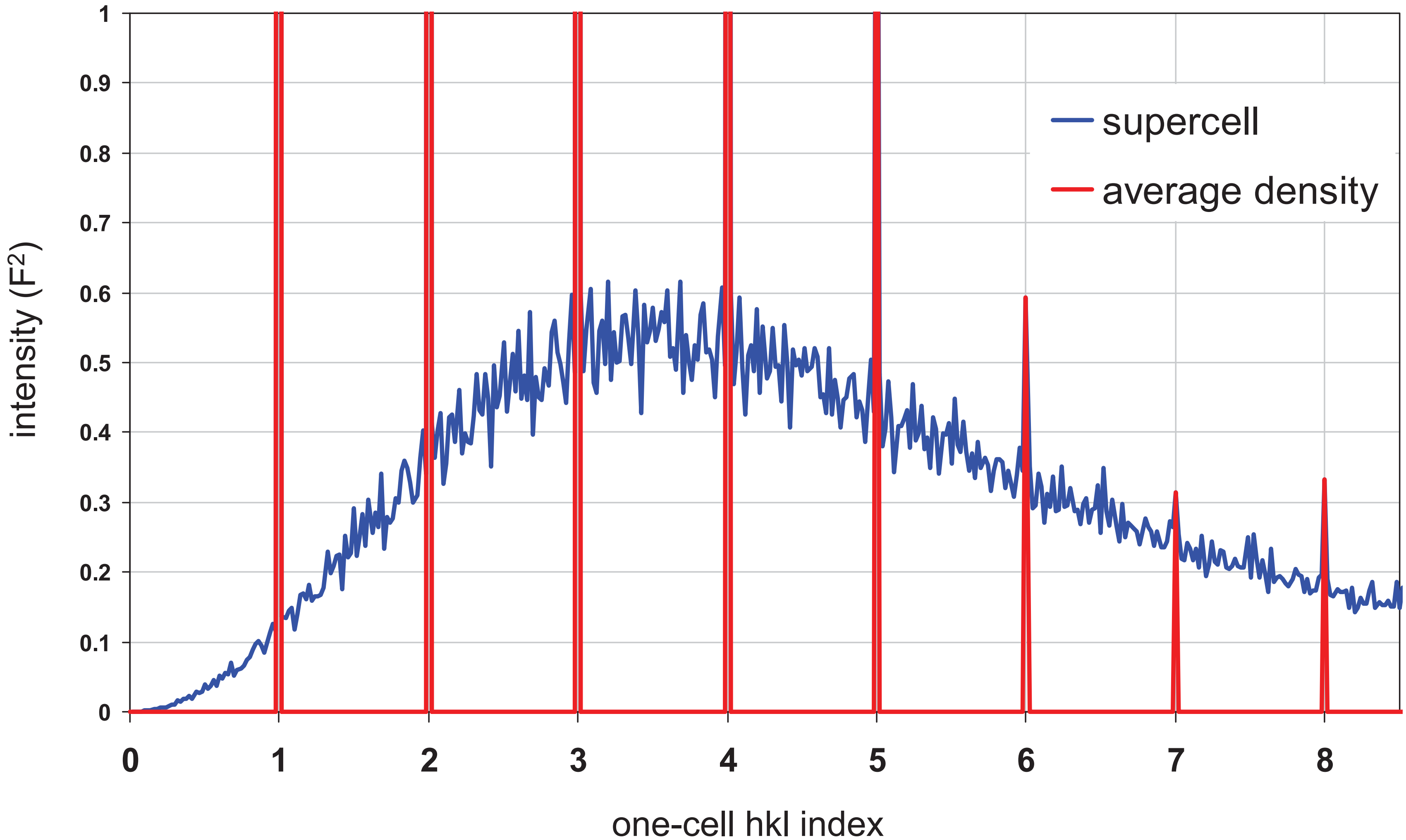


Super-cell formalism for diffuse scatter



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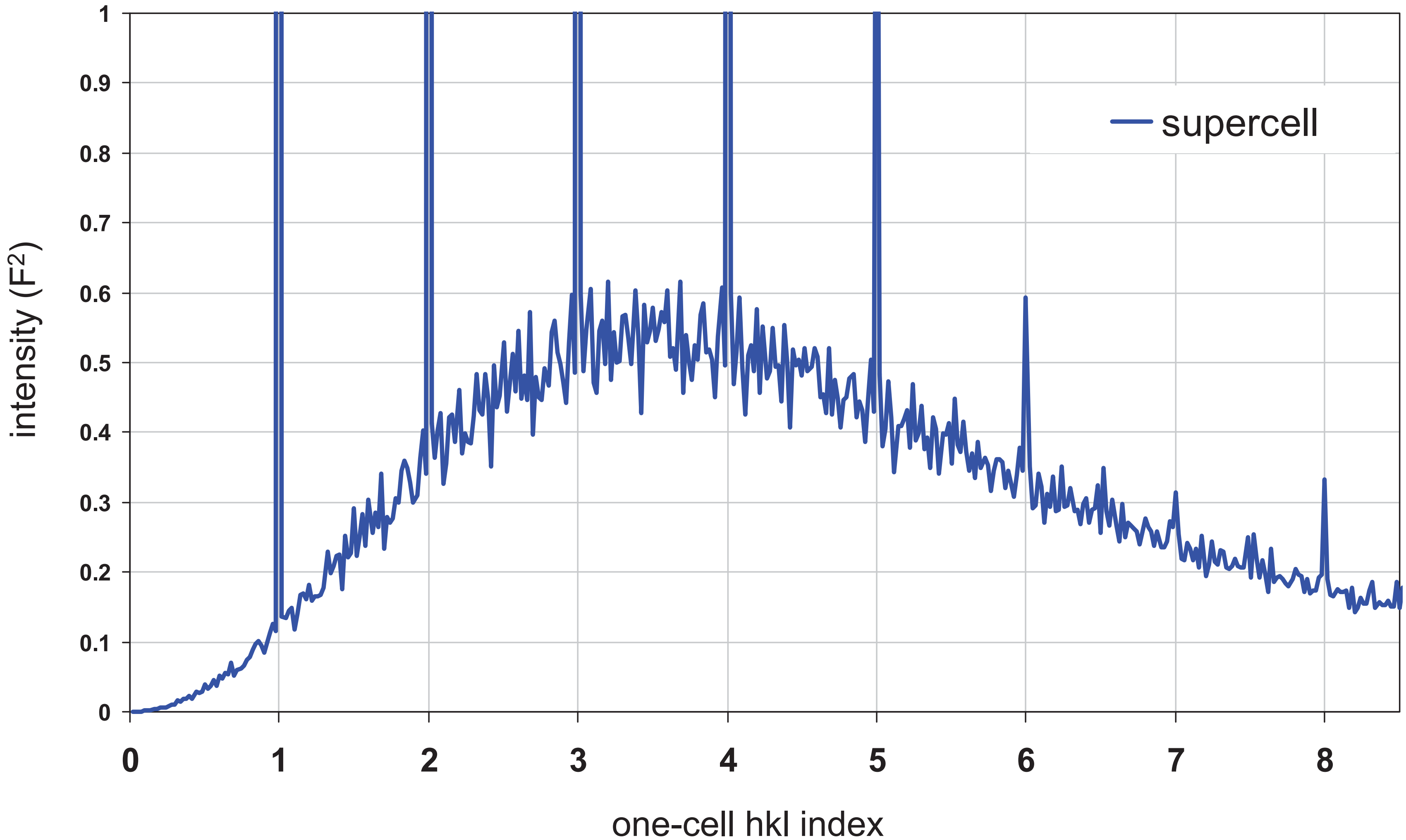

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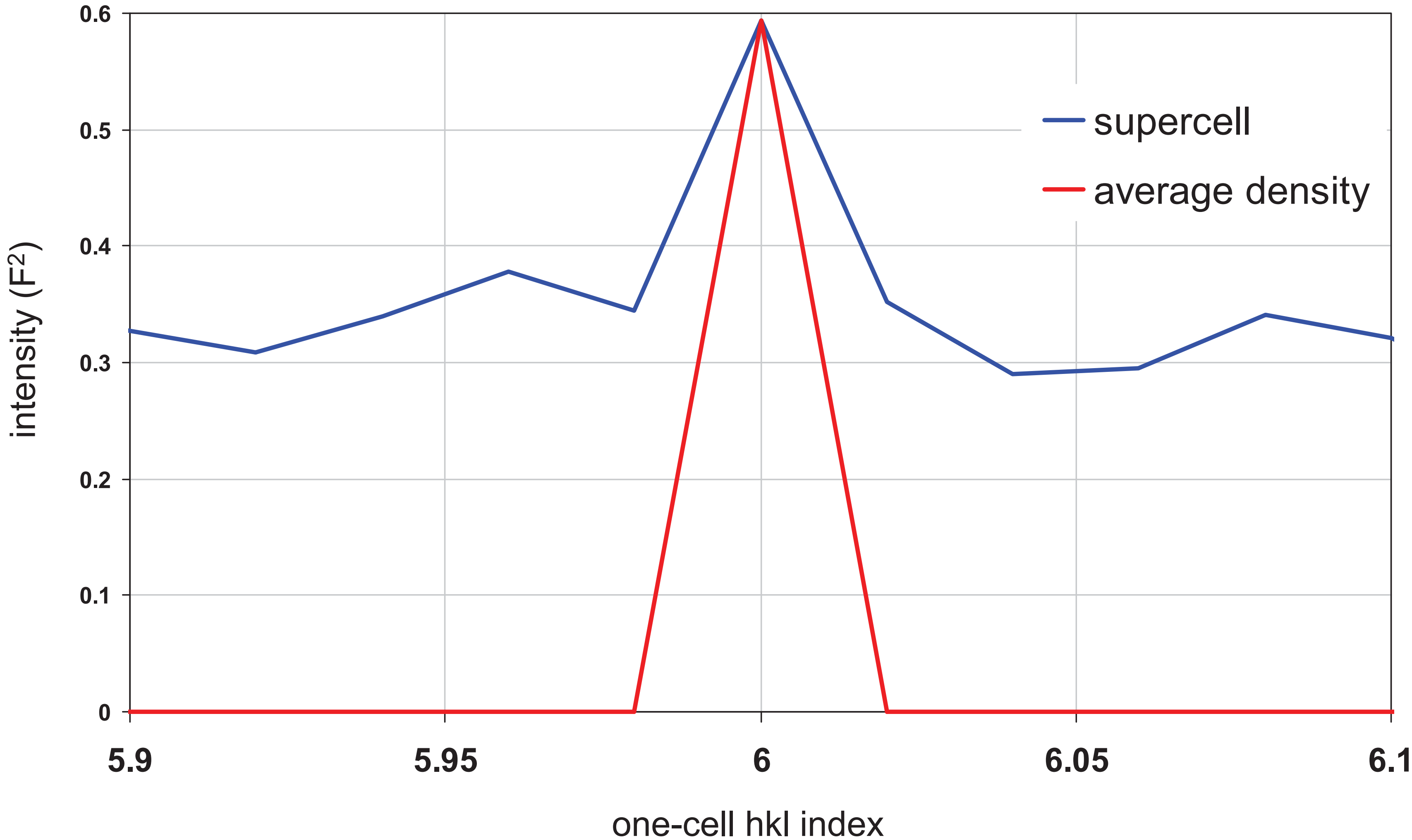

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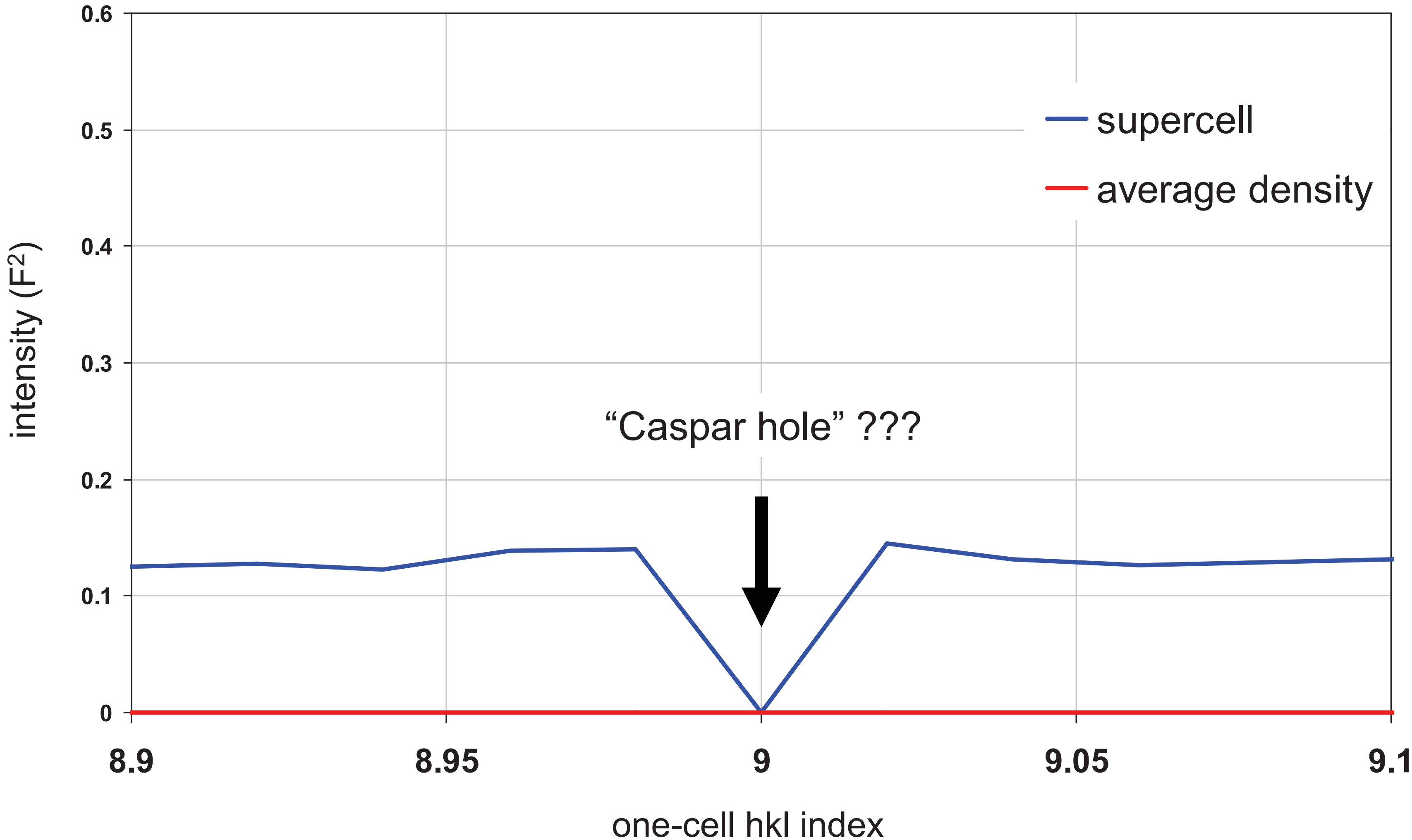
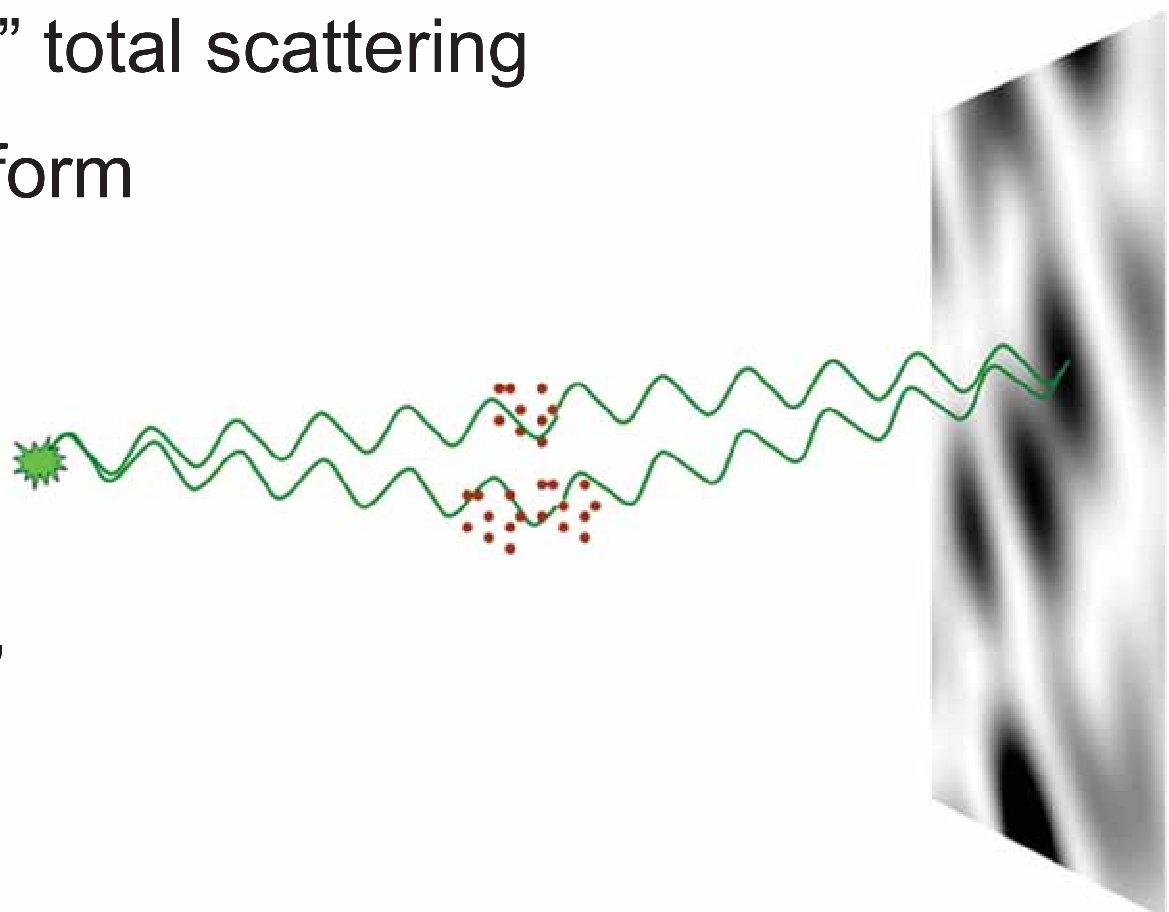


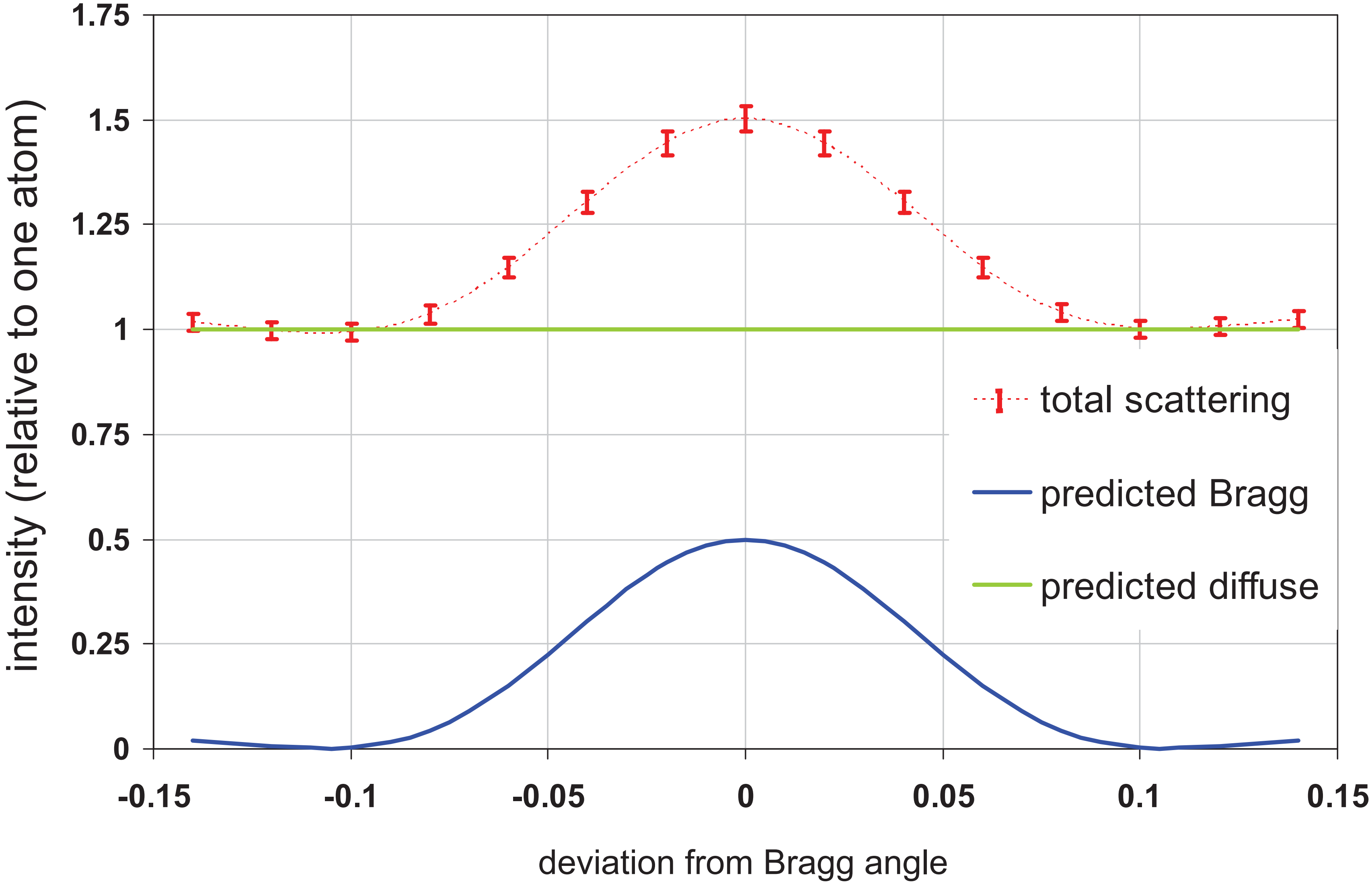
nearBragg program

`http://bl831.als.lbl.gov/~jamesh/nearBragg/`

- "assumption-free" total scattering
- no Fourier Transform
- no unit cells
- no "mosaicity"
- arbitrary "atoms"
- arbitrary "source"
- coherent or not



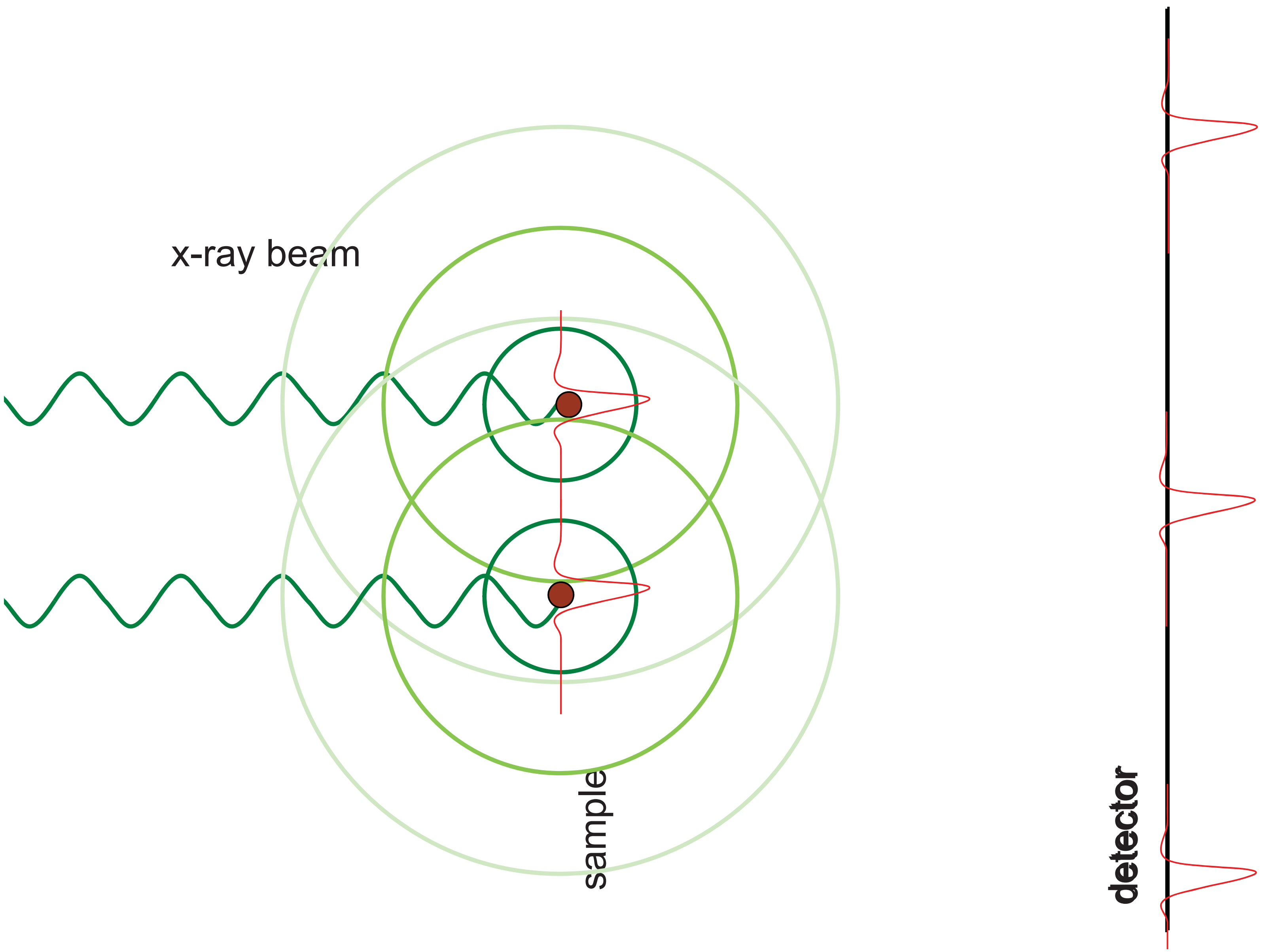
average total scattering from points

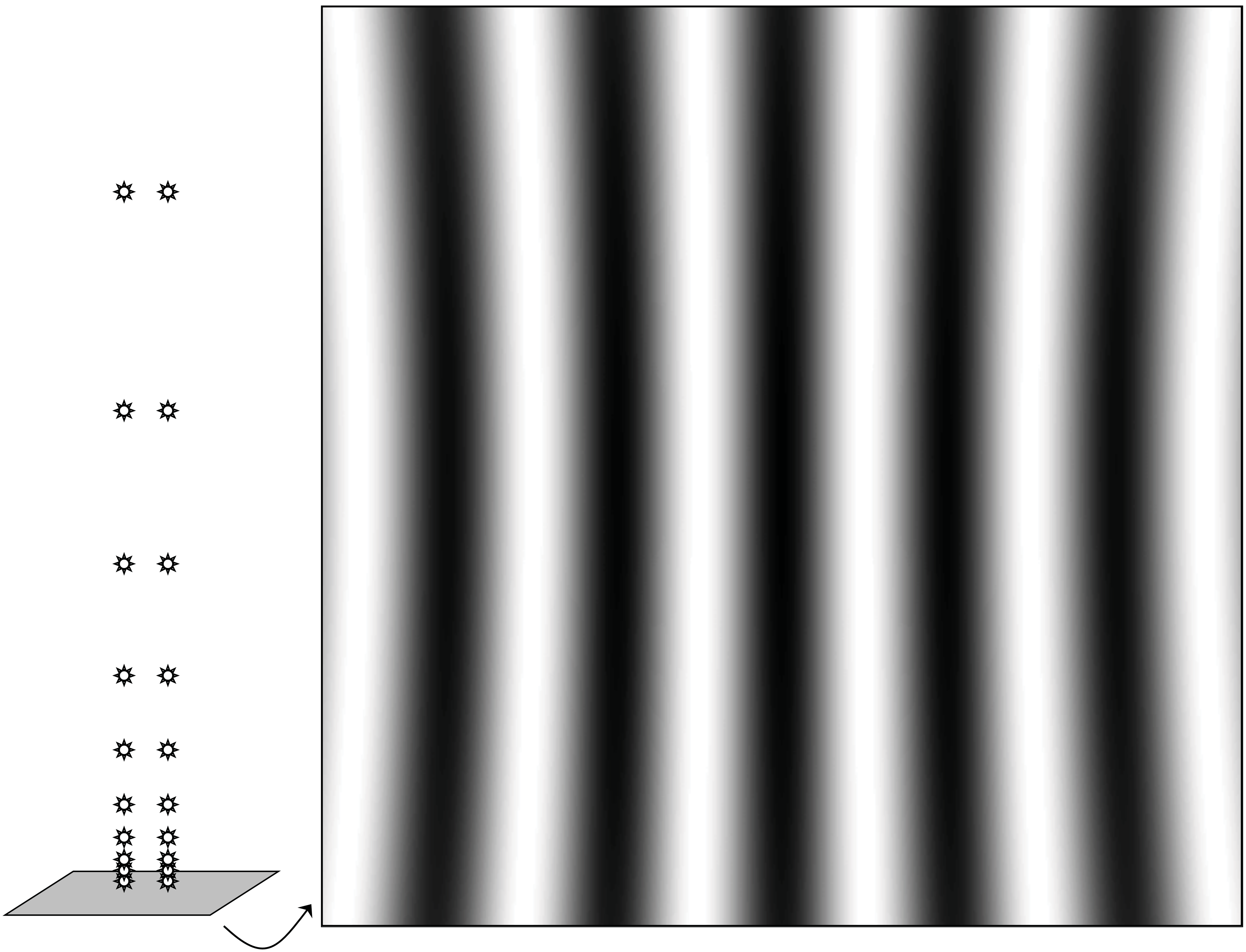


which of these still apply?

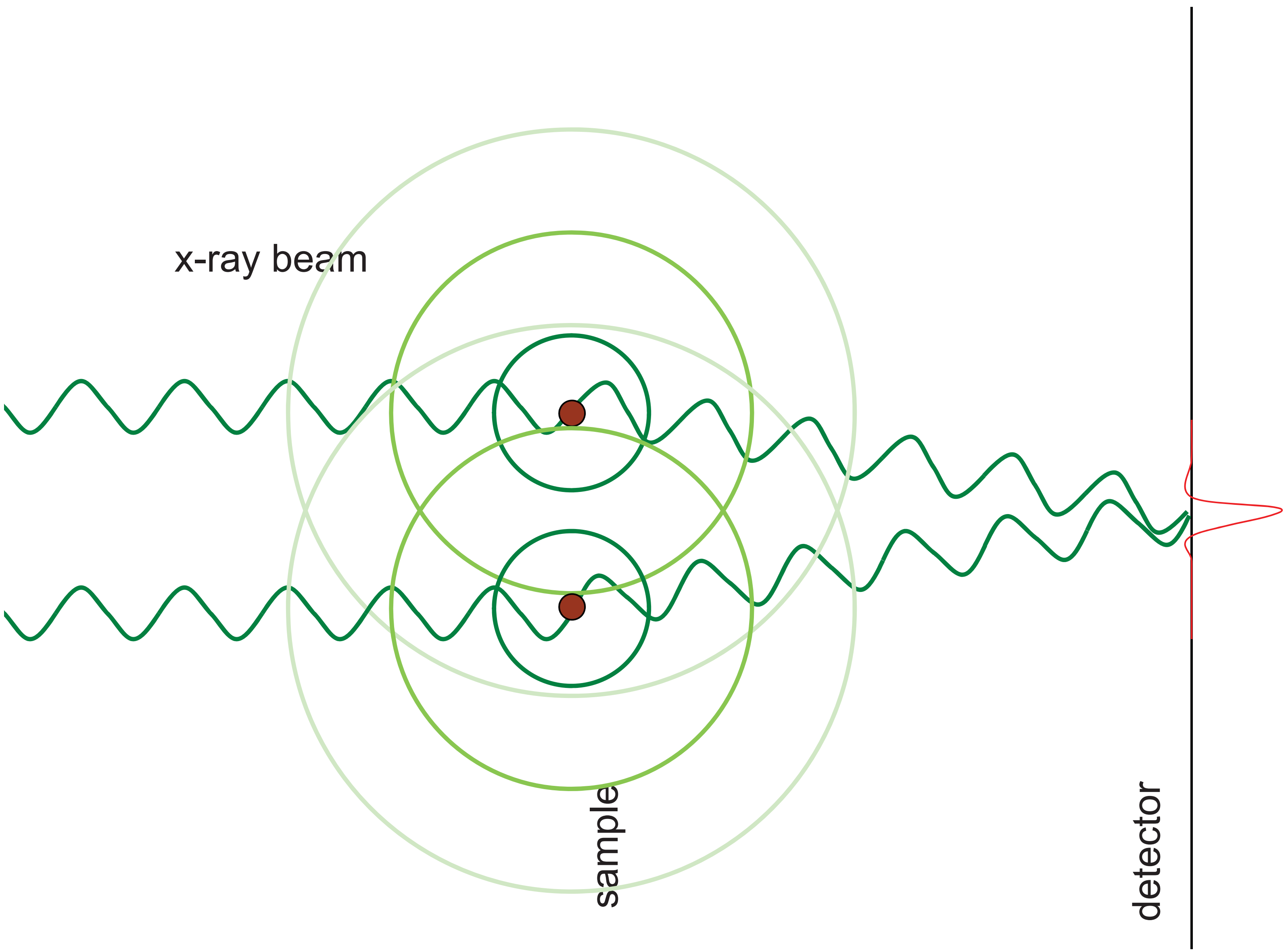
- light is “coherent”
- near-zero divergence
- near-zero dispersion
- crystal cannot rotate
- crystals may be 1 mosaic block
- are small crystals “more perfect”?
- will we see any spots?!

scattering

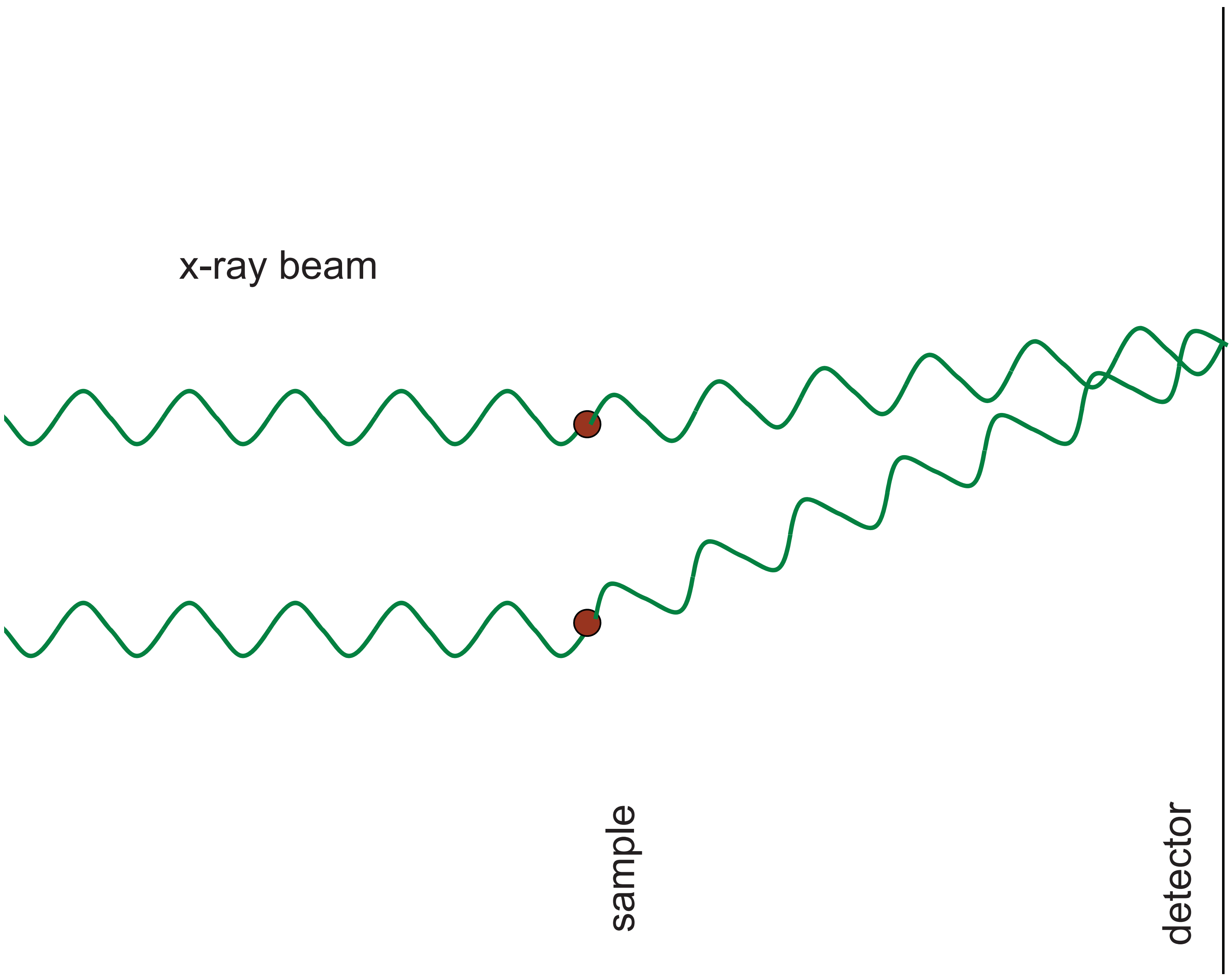




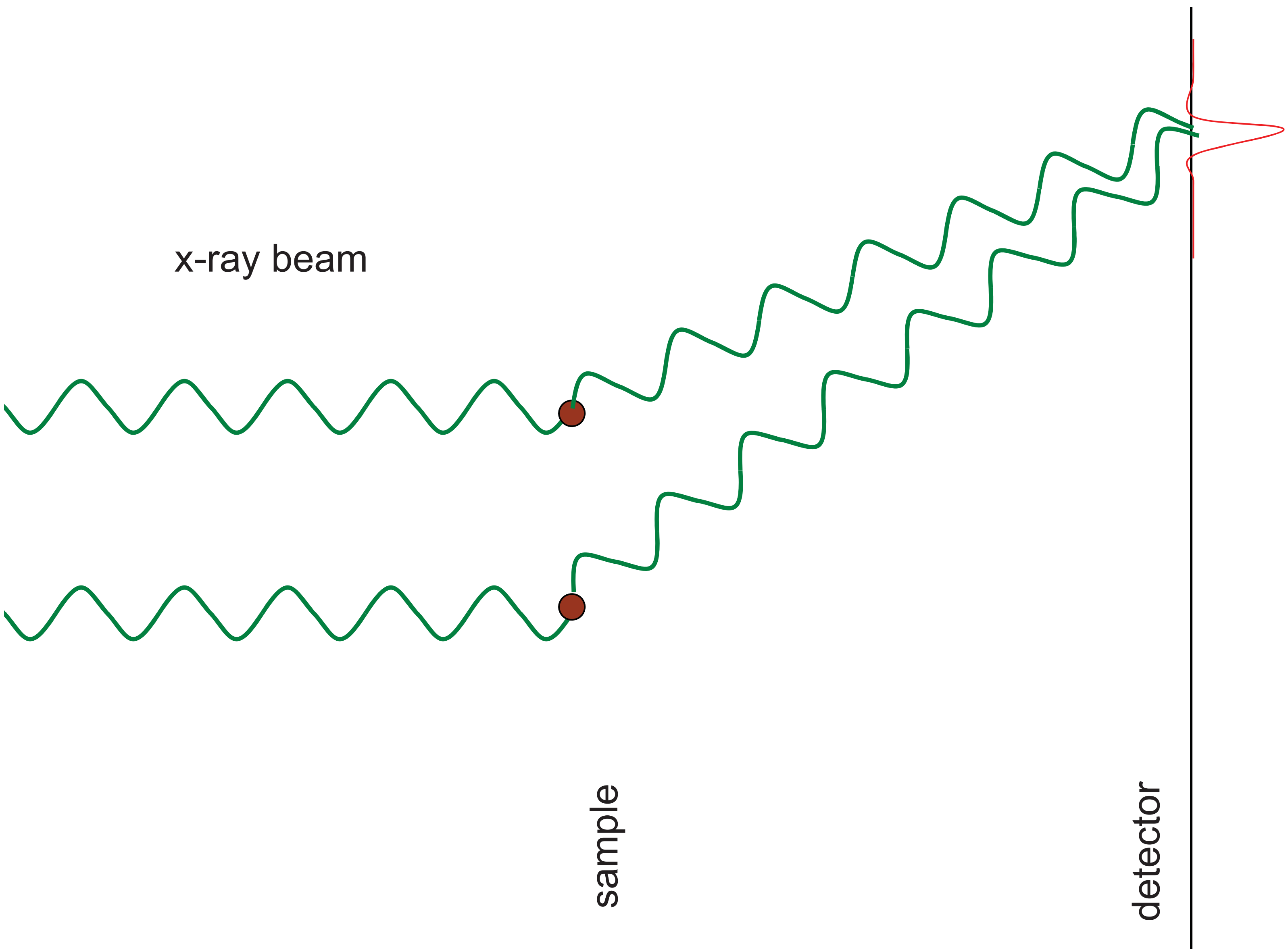
scattering



scattering

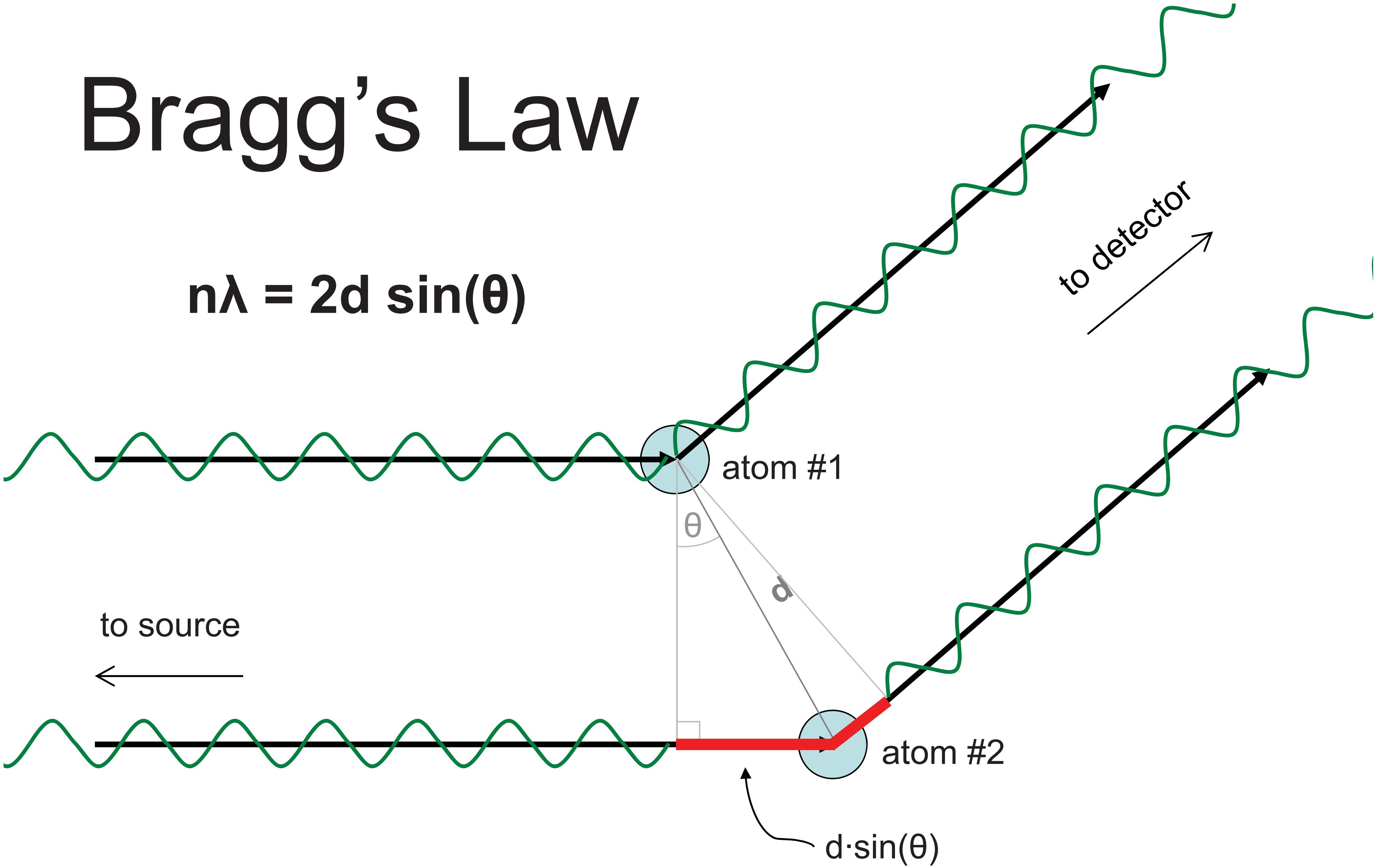


scattering



Bragg's Law

$$n\lambda = 2d \sin(\theta)$$



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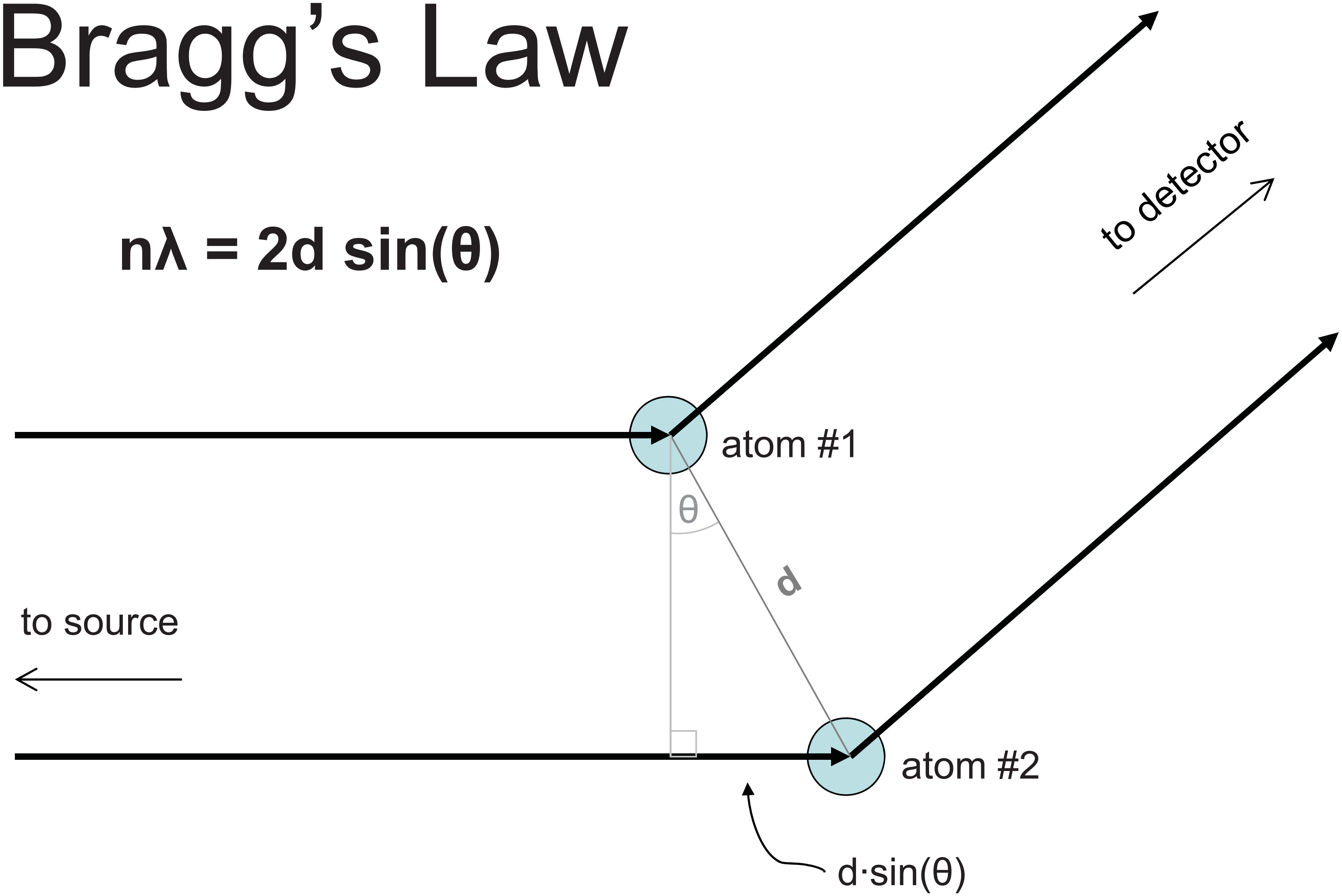
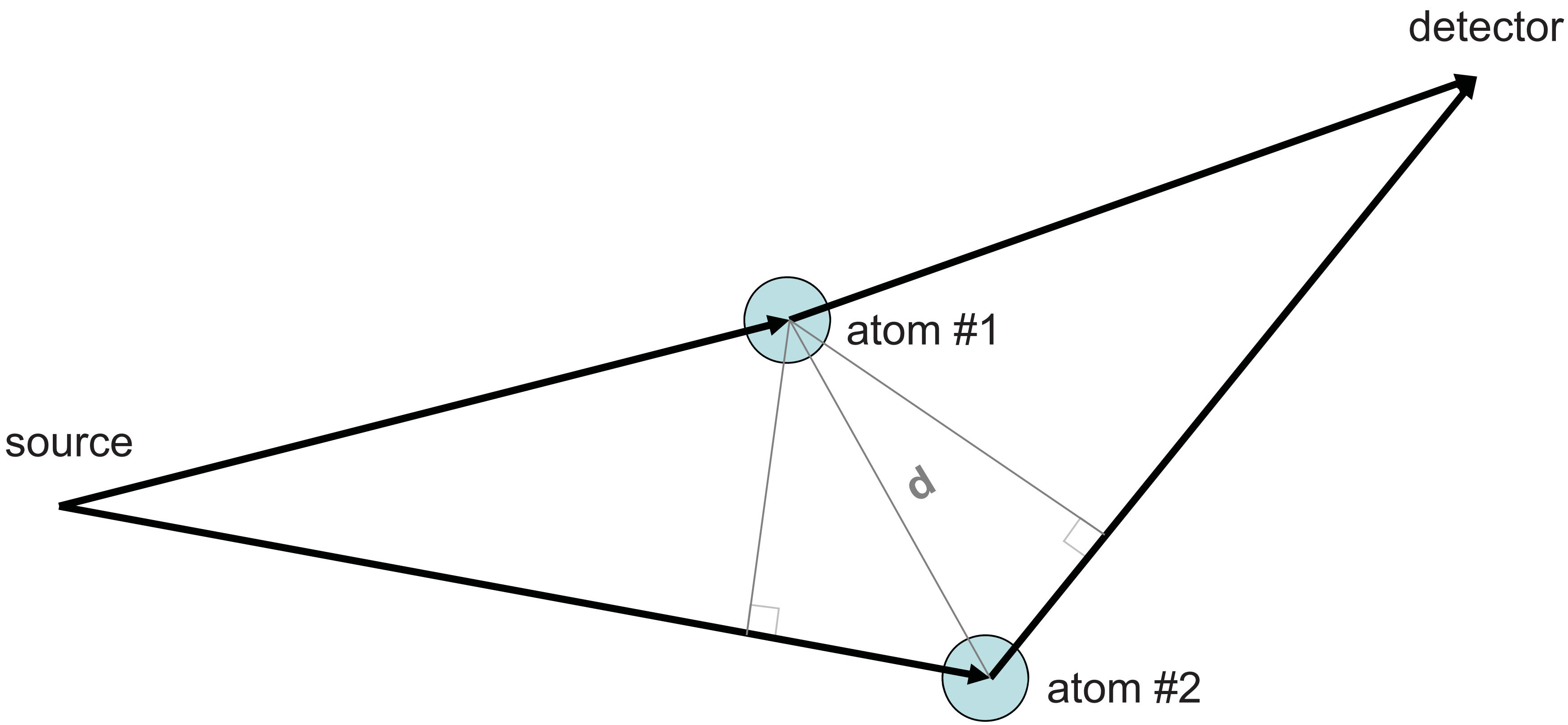


“near”-ly Bragg’s Law



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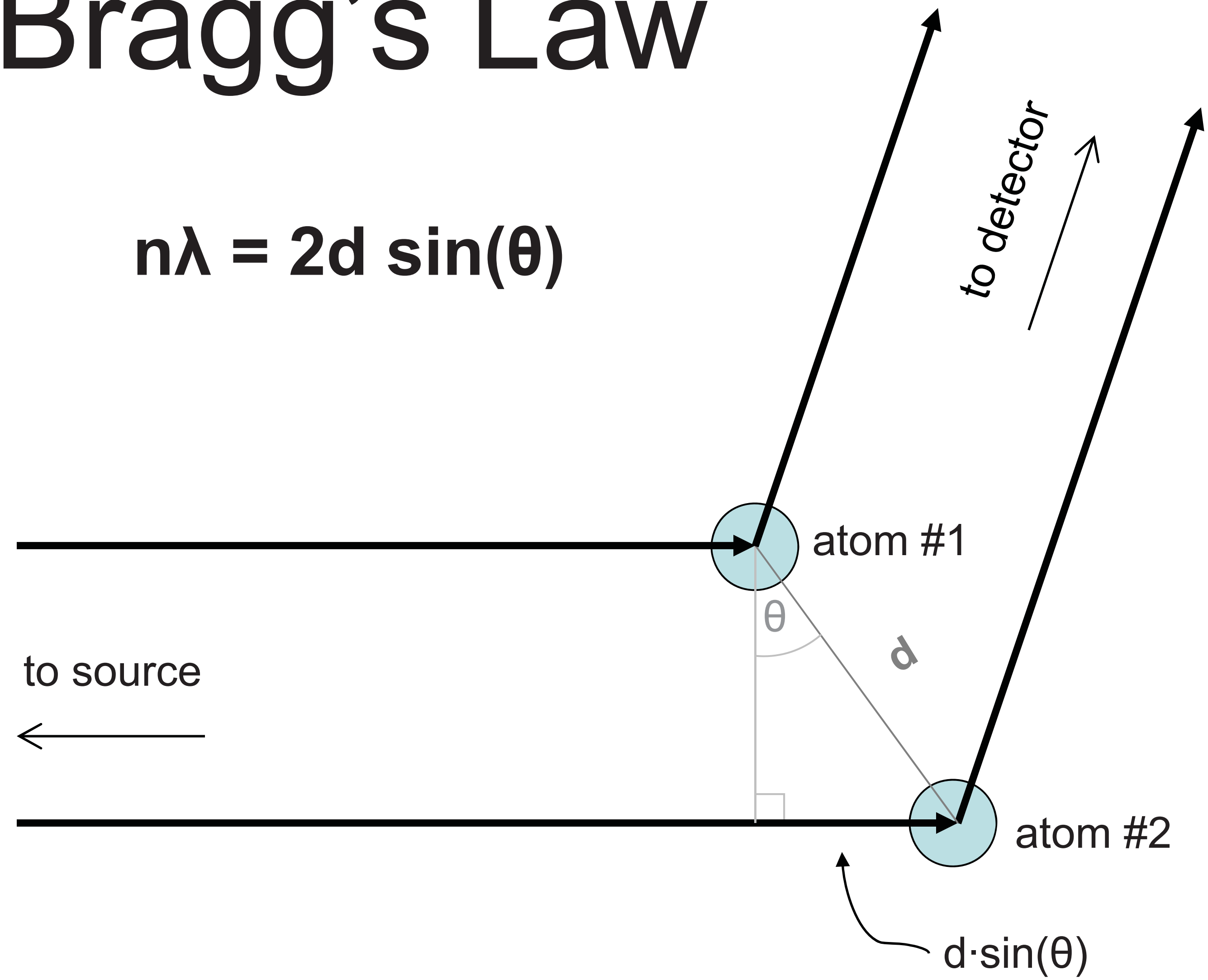
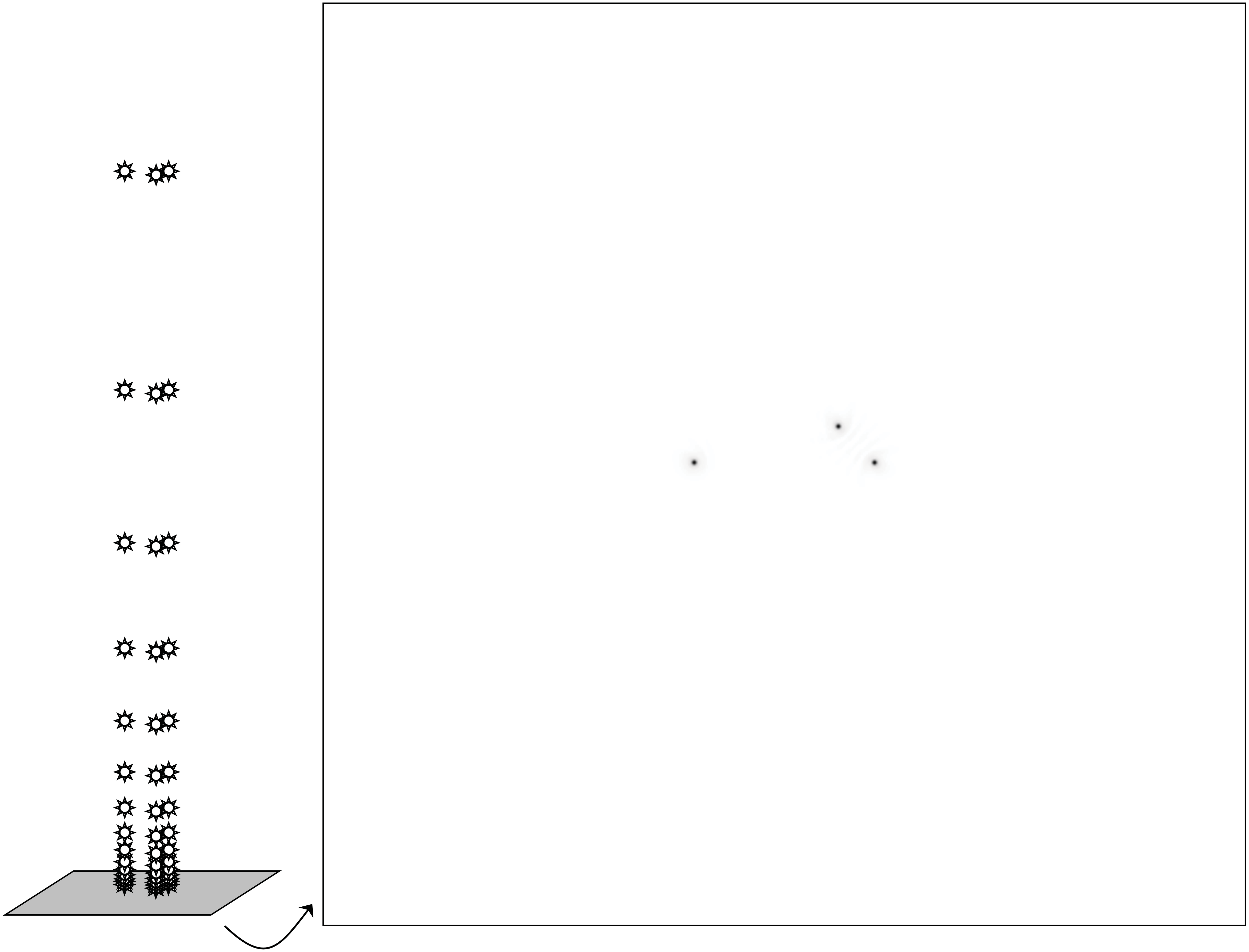
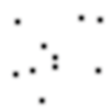




scattering from a structure

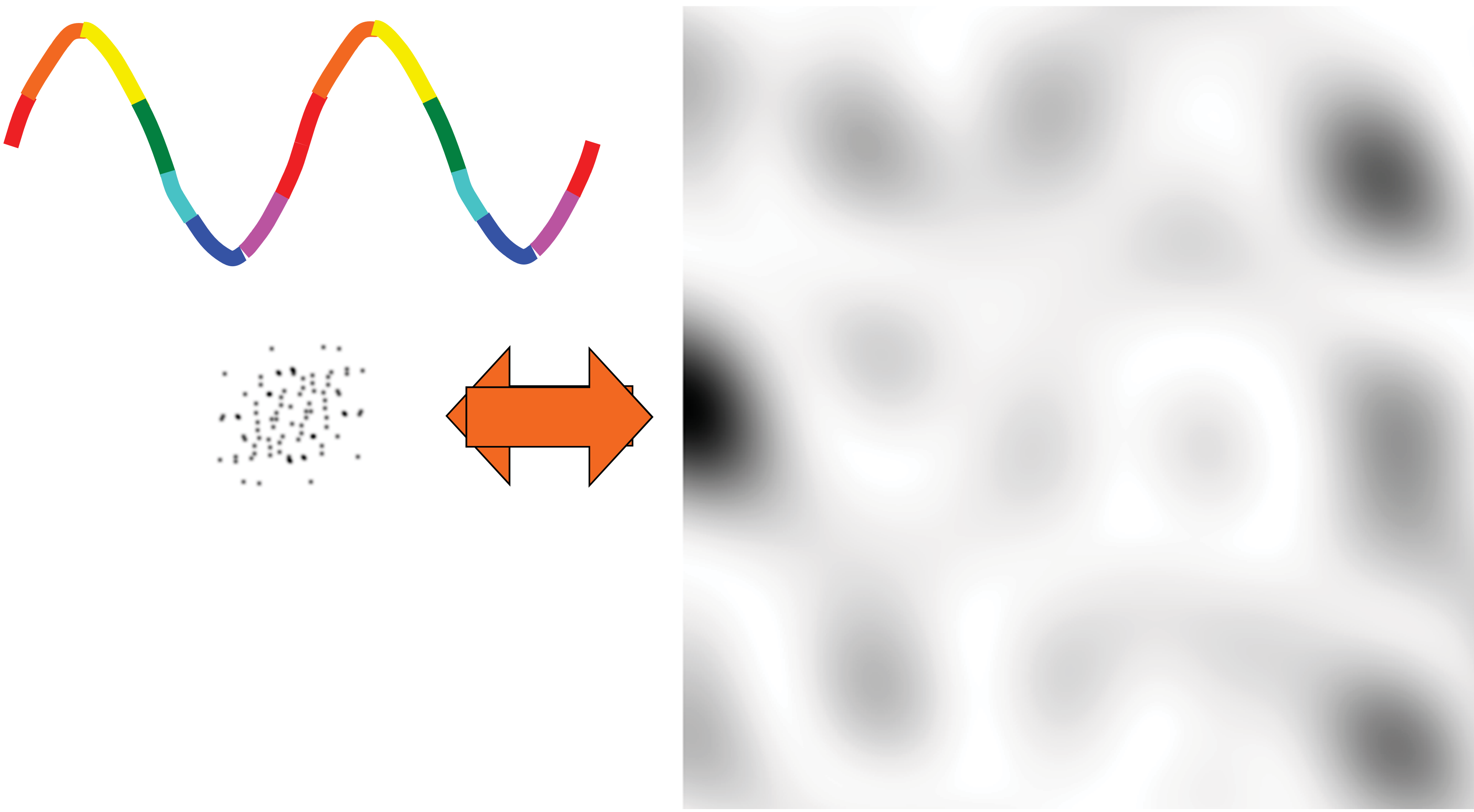
sample



detector

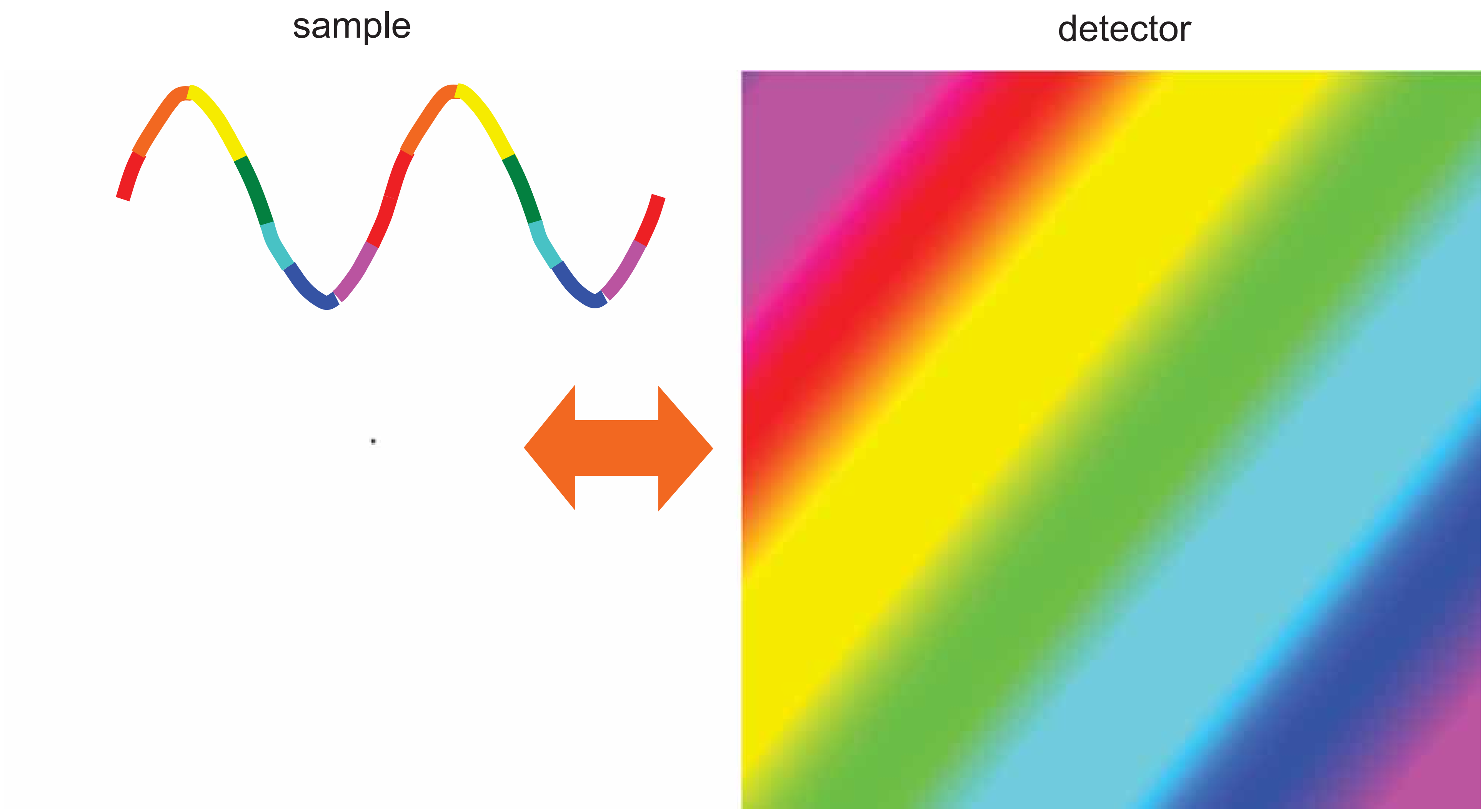
forward inverse Fourier Transform

no phase



inverse Fourier Transform
scattering

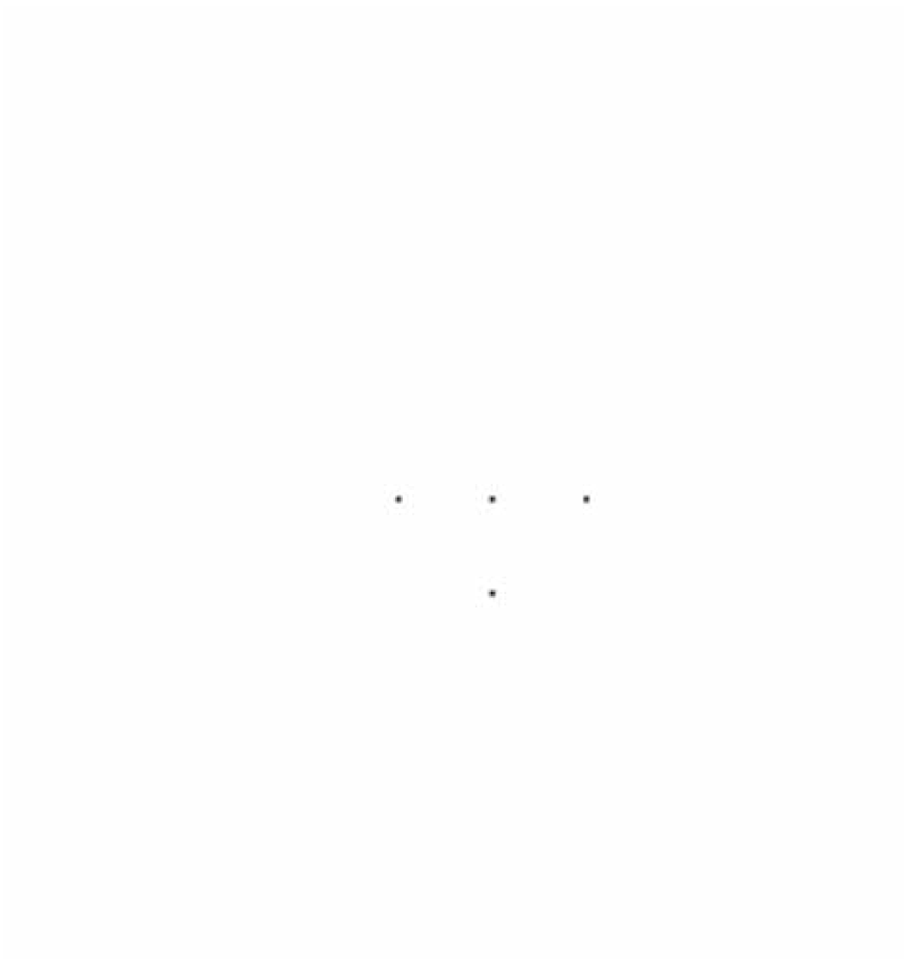
colored by phase



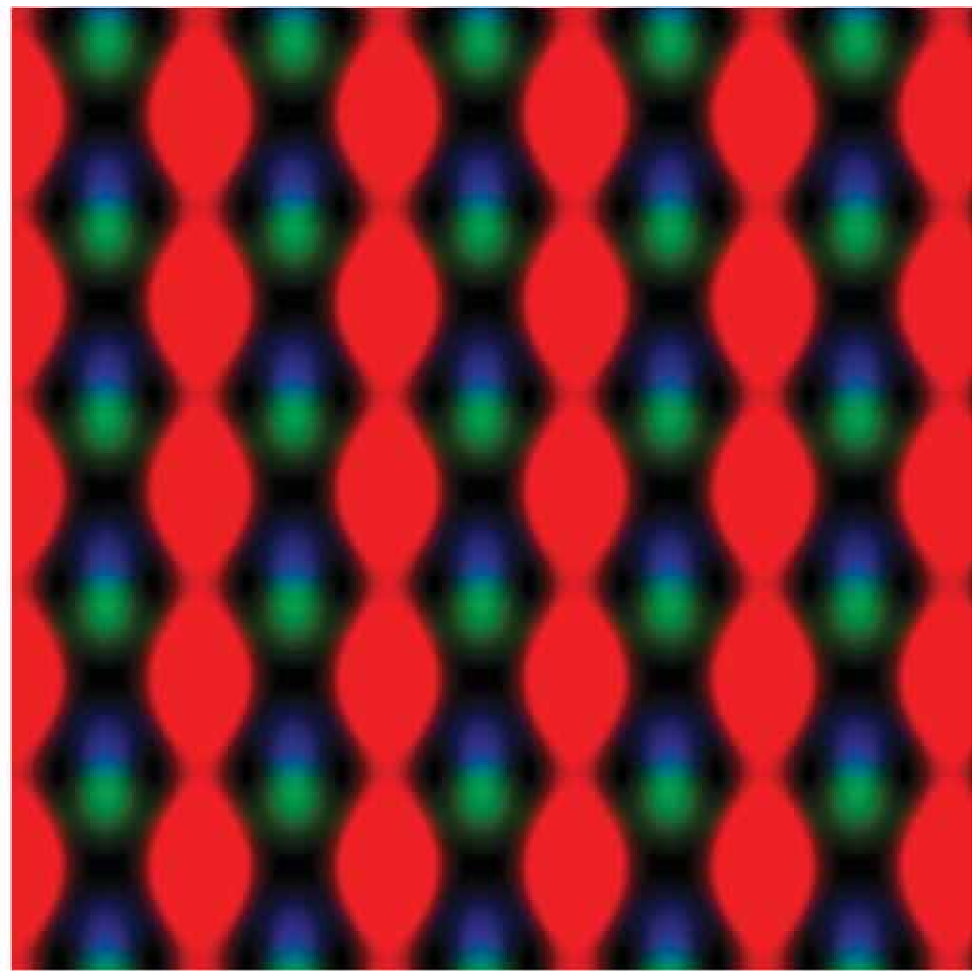
scattering from a lattice

colored by phase

sample

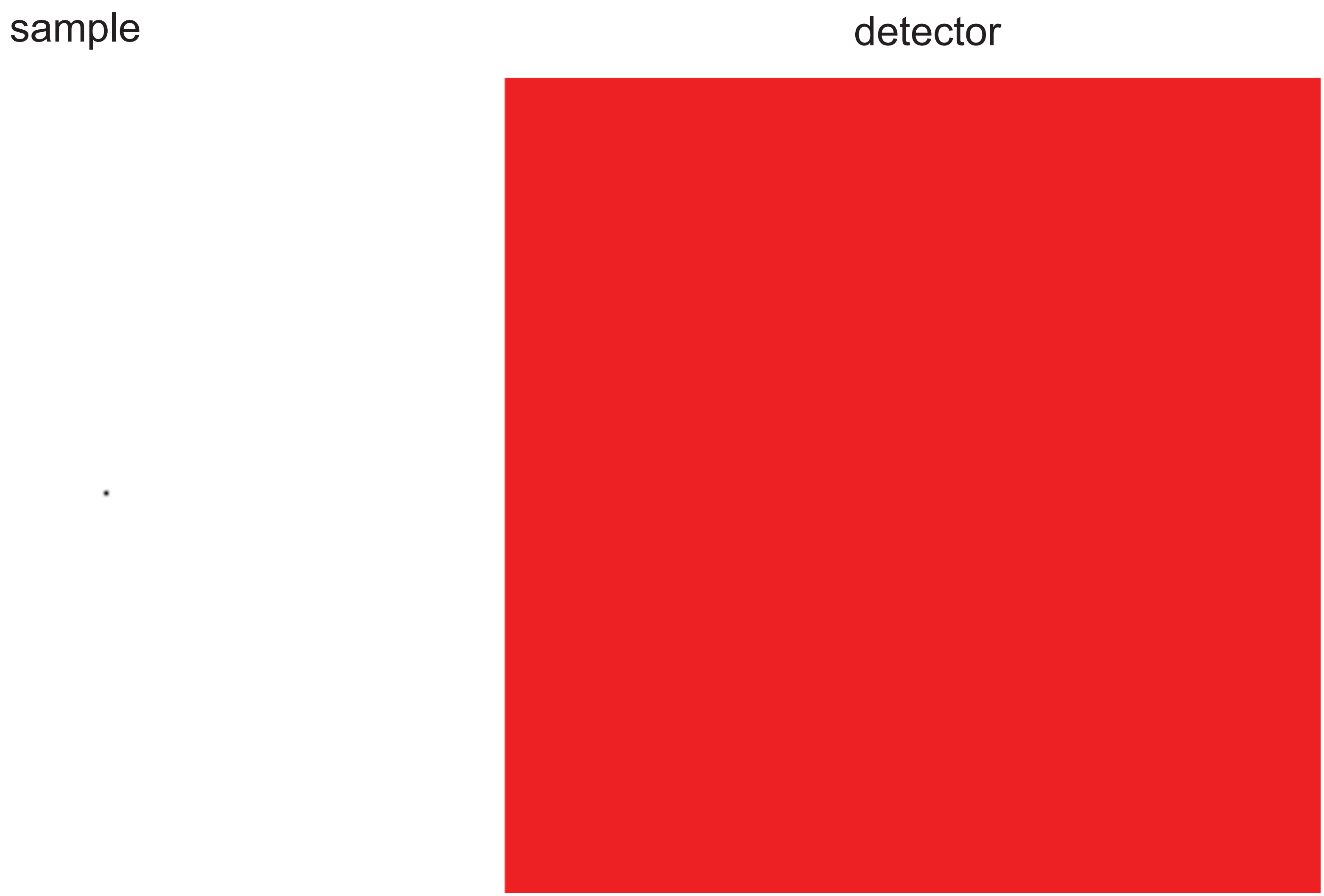


detector



scattering from a lattice

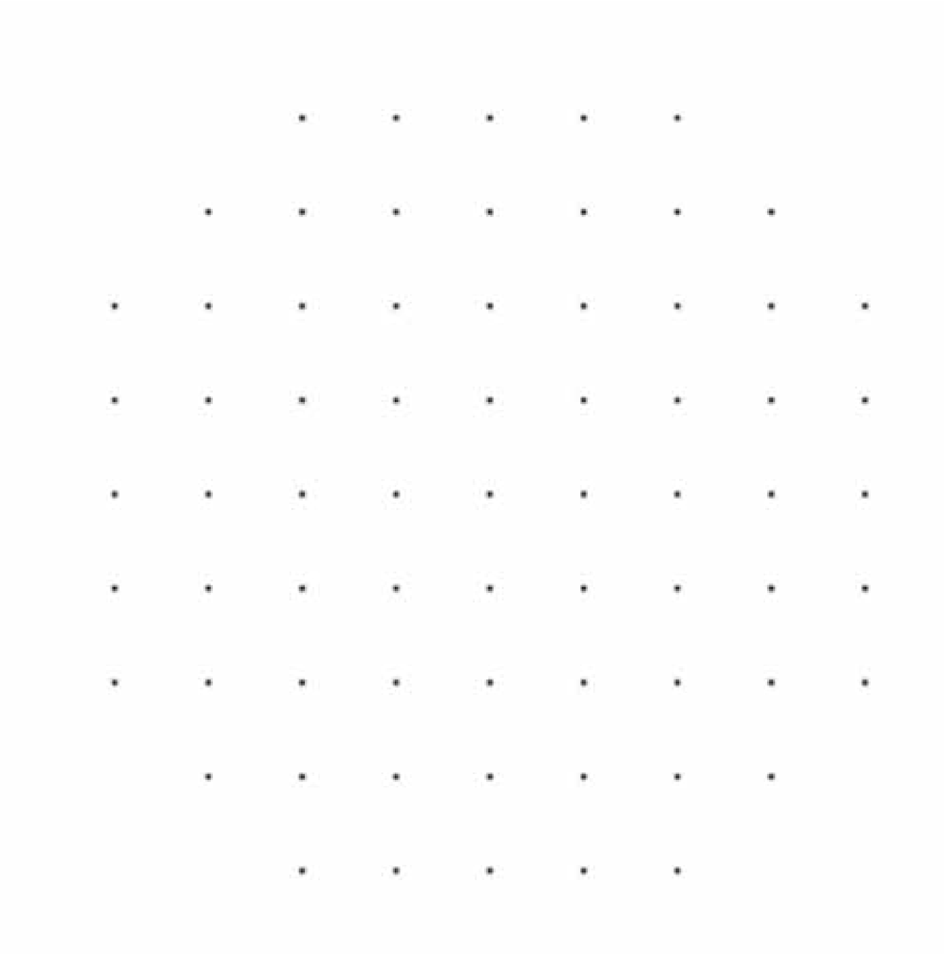
colored by phase



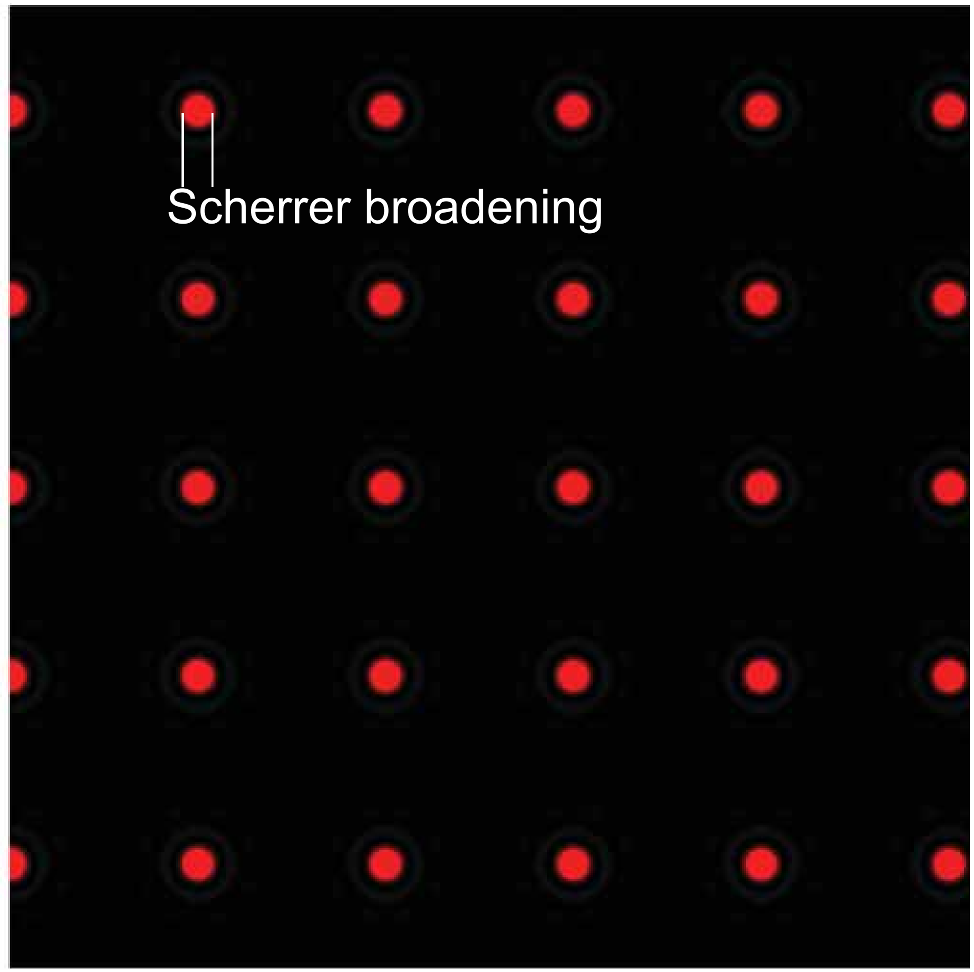
scattering from a lattice

colored by phase

sample



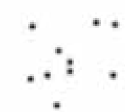
detector



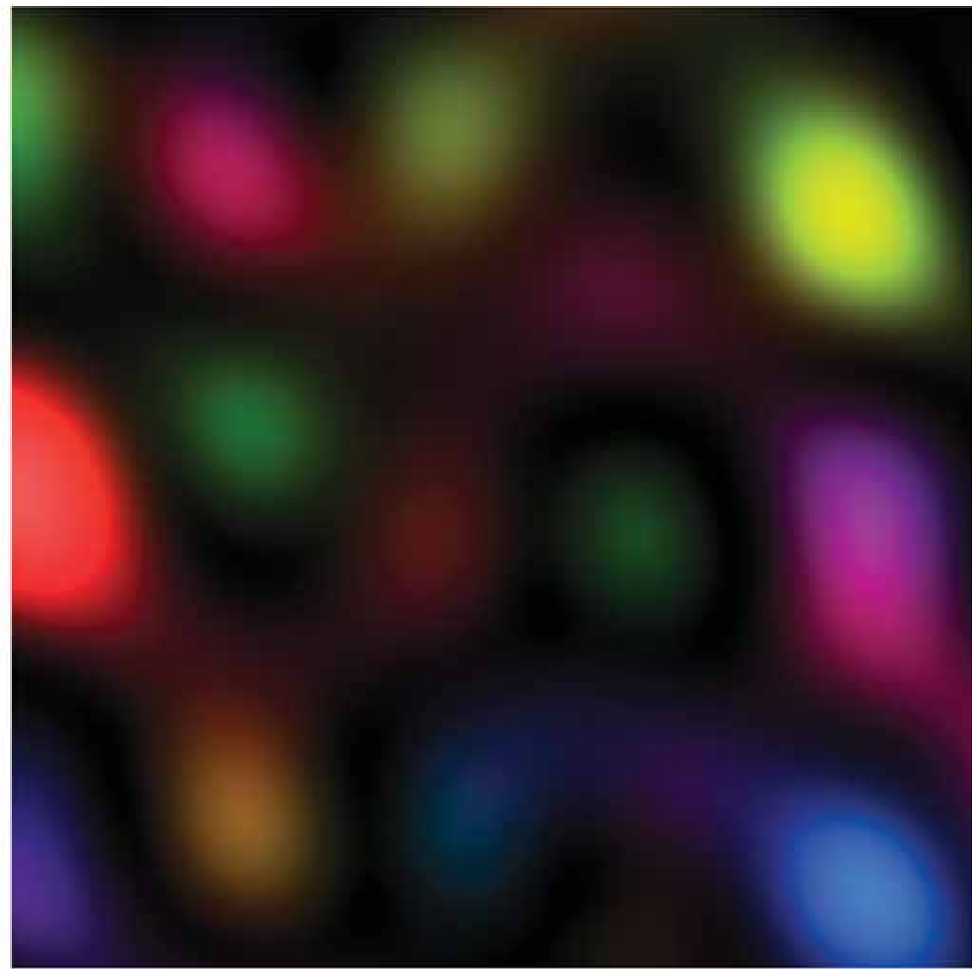
scattering from a crystal structure

colored by phase

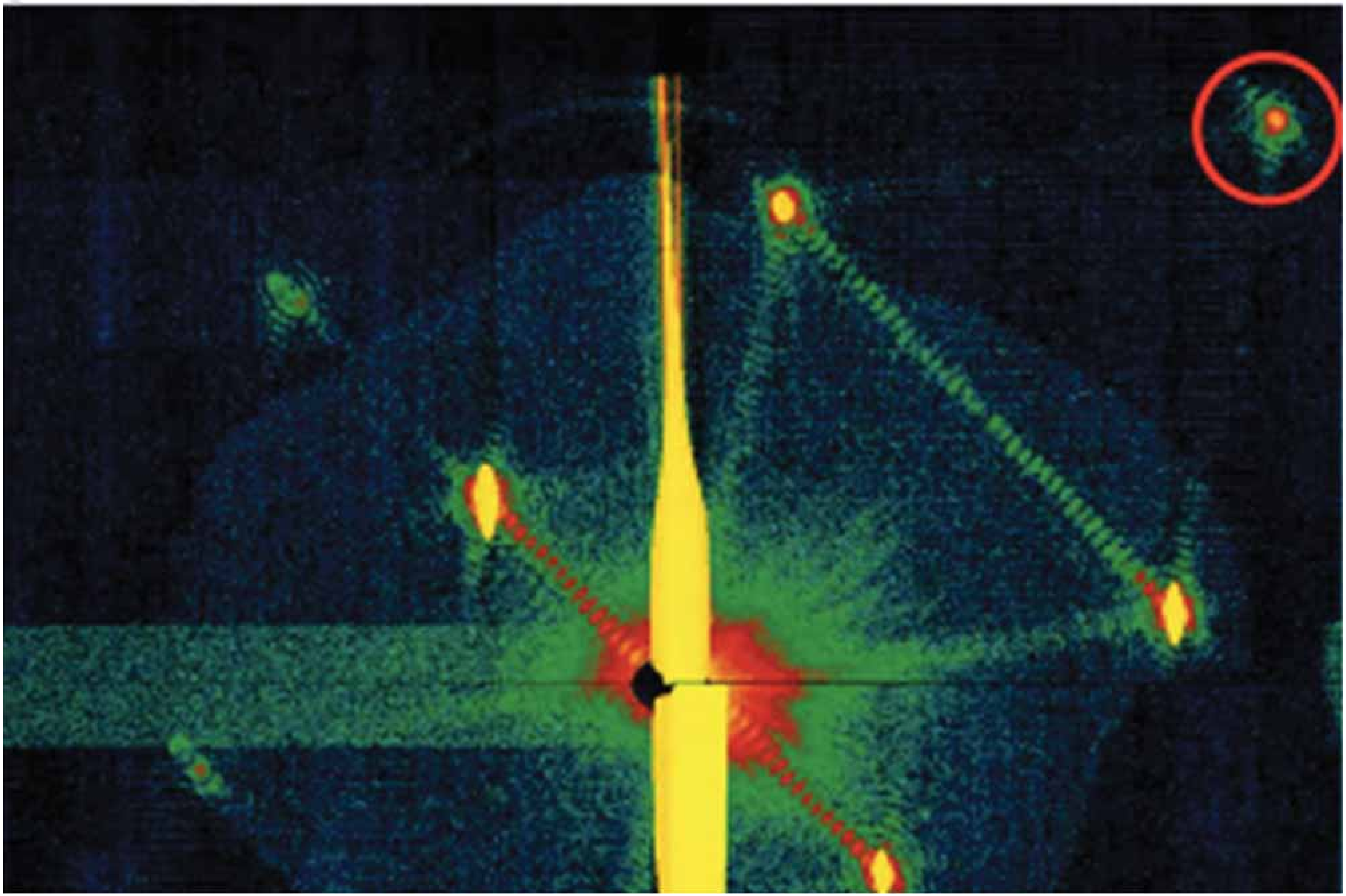
sample



detector



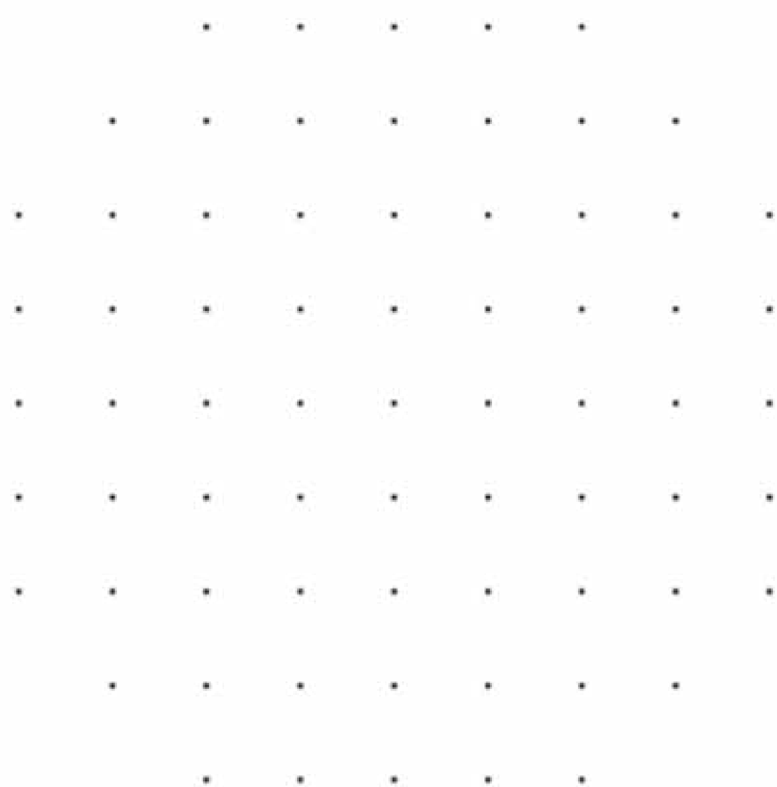
Inter-Bragg spots over-sample unit cell



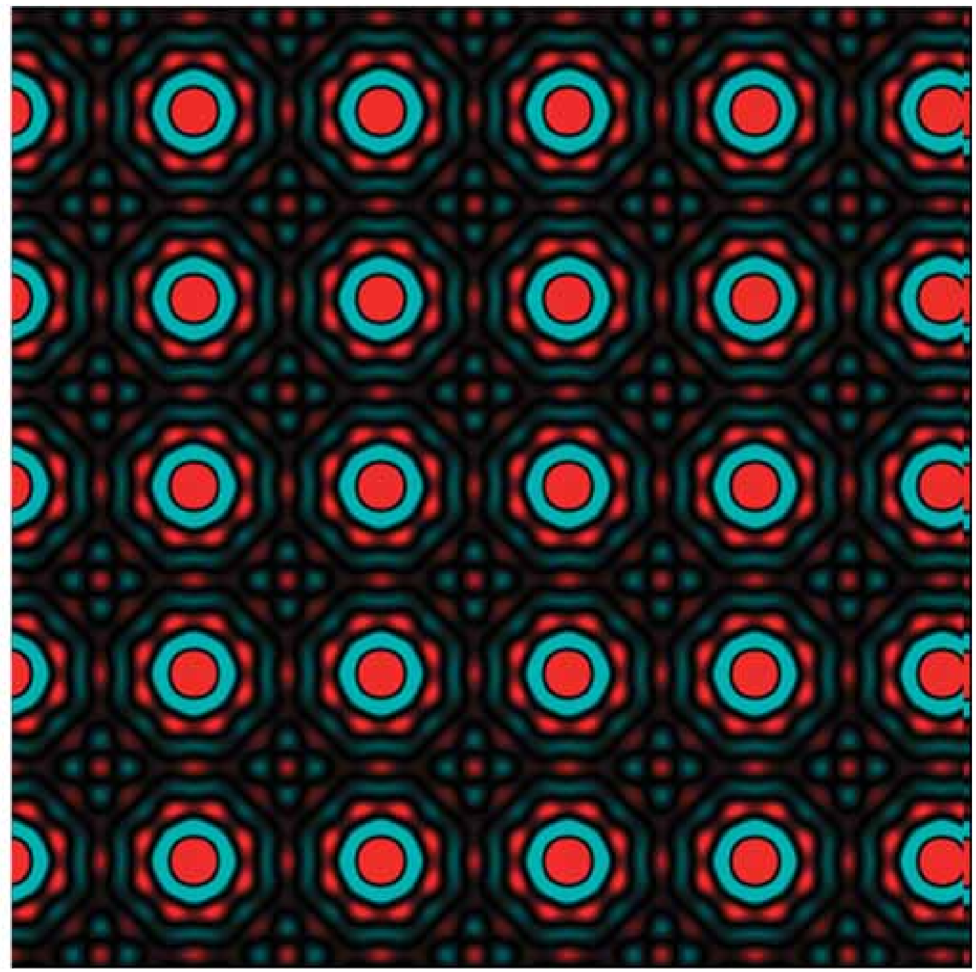
scattering from a lattice

colored by phase

sample



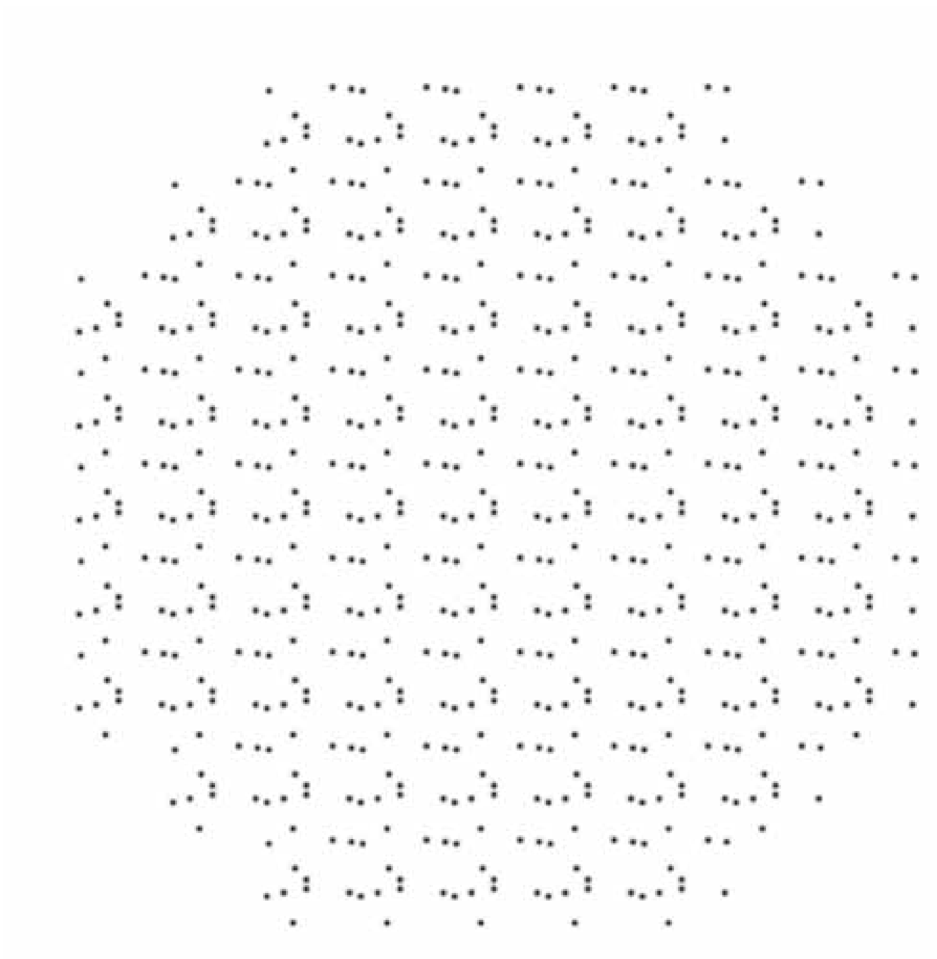
detector



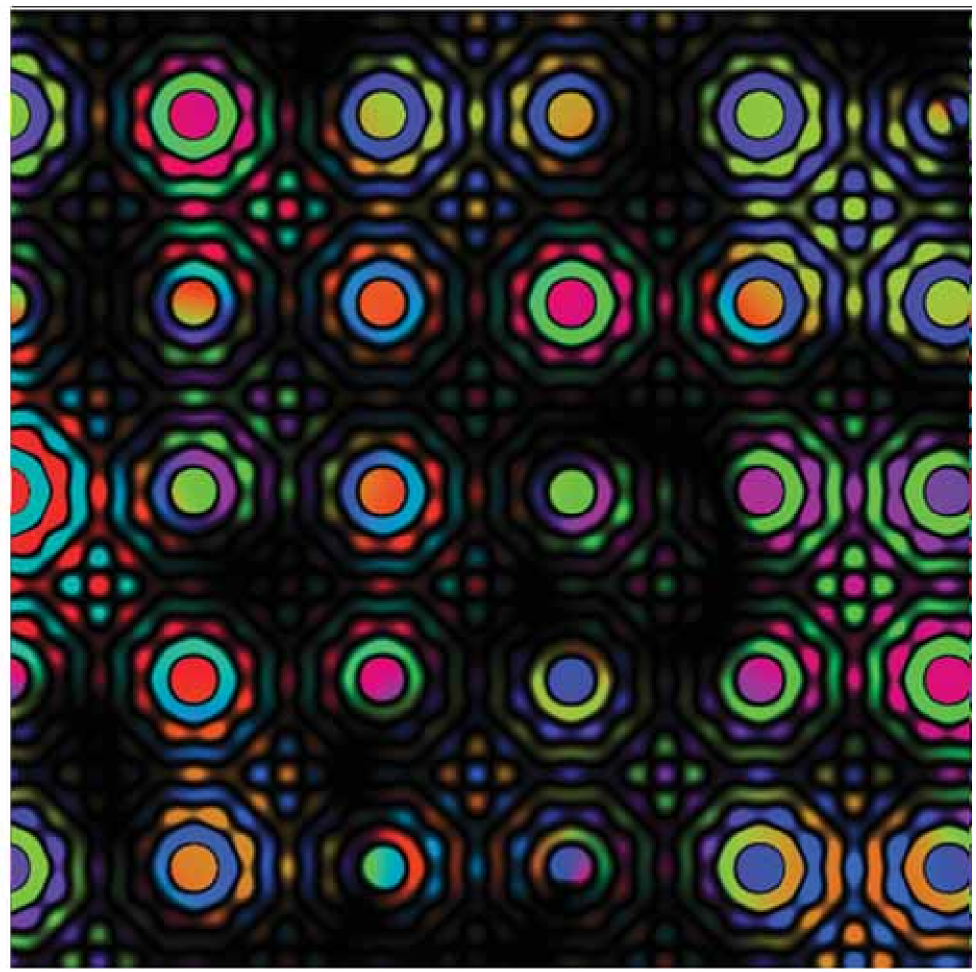
scattering from a crystal structure

colored by phase

sample



detector



scattering from a crystal structure

colored by phase

sample

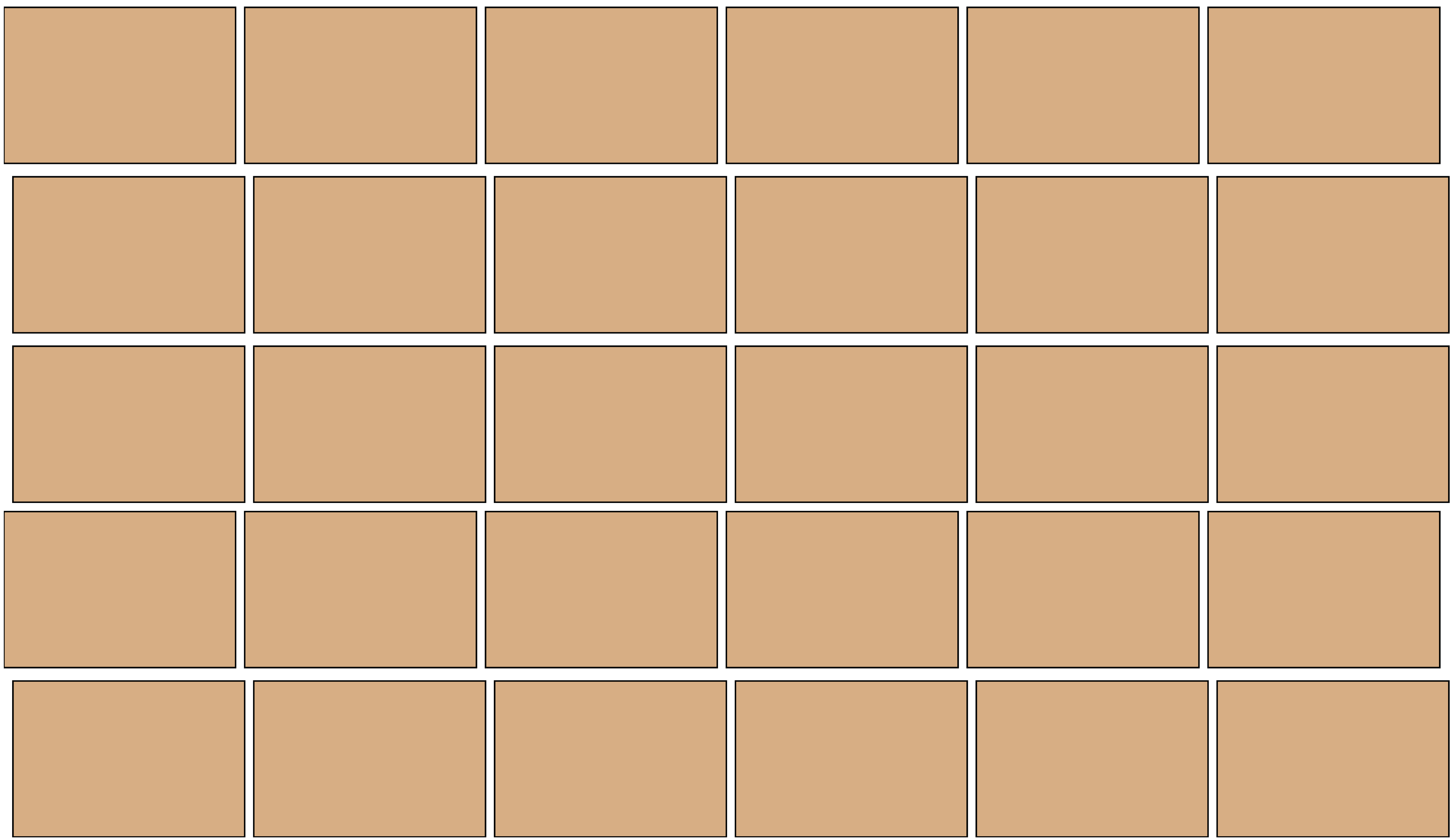
detector

Spence, J. C. H., Kirian, R. A., Wang, X., Weierstall, U., Schmidt, K. E., White, T., Barty, A., Chapman, H. N., Marchesini, S. & Holton, J. (2011)."Phasing of coherent femtosecond X-ray diffraction from size-varying nanocrystals", *Opt. Express* **19**, 2866-2873.

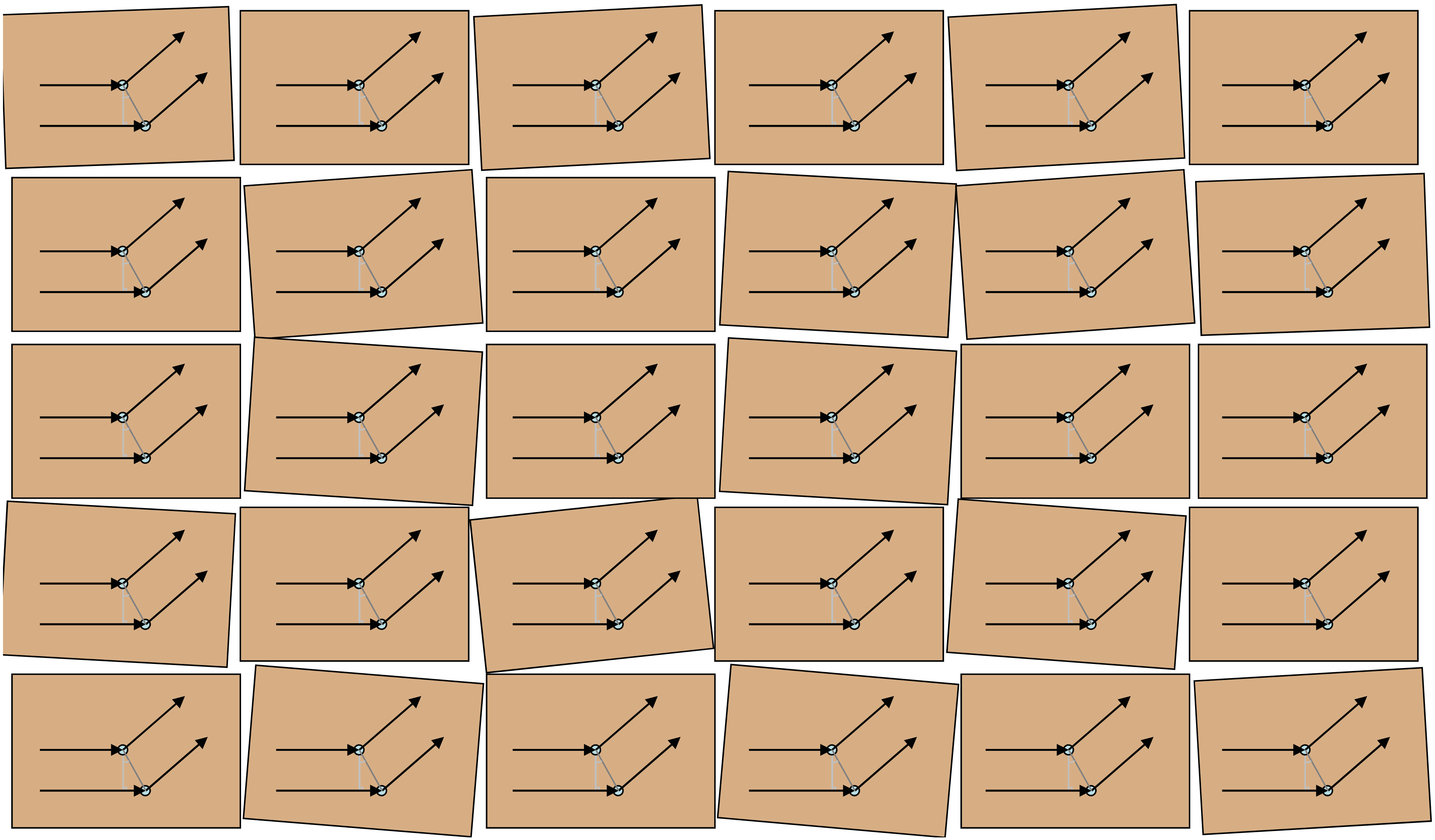
which of these still apply?

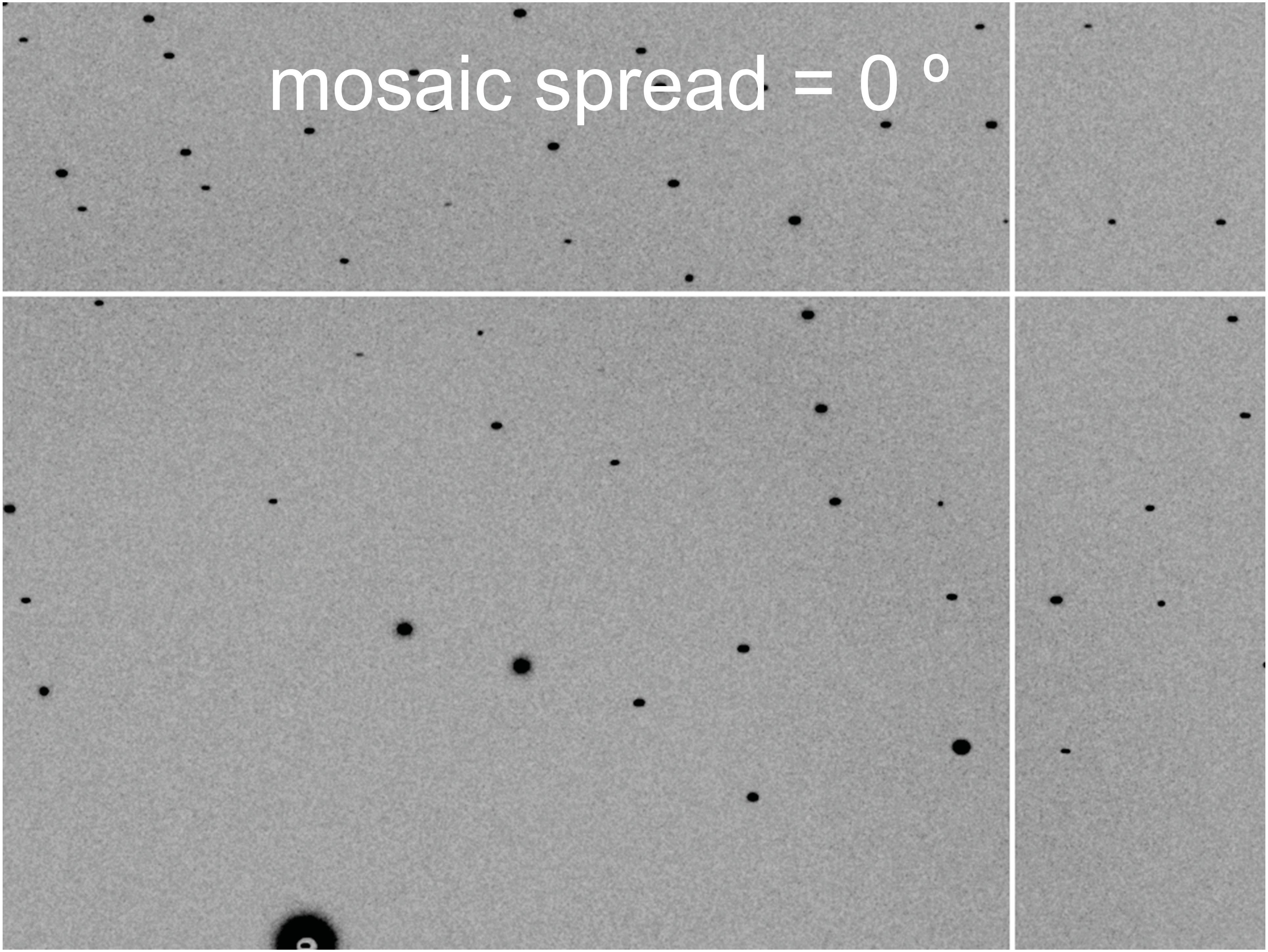
- light is "coherent"
- near-zero divergence
- near-zero dispersion
- crystal cannot rotate
- crystals may be 1 mosaic block
- are small crystals "more perfect"?
- will we see any spots?!

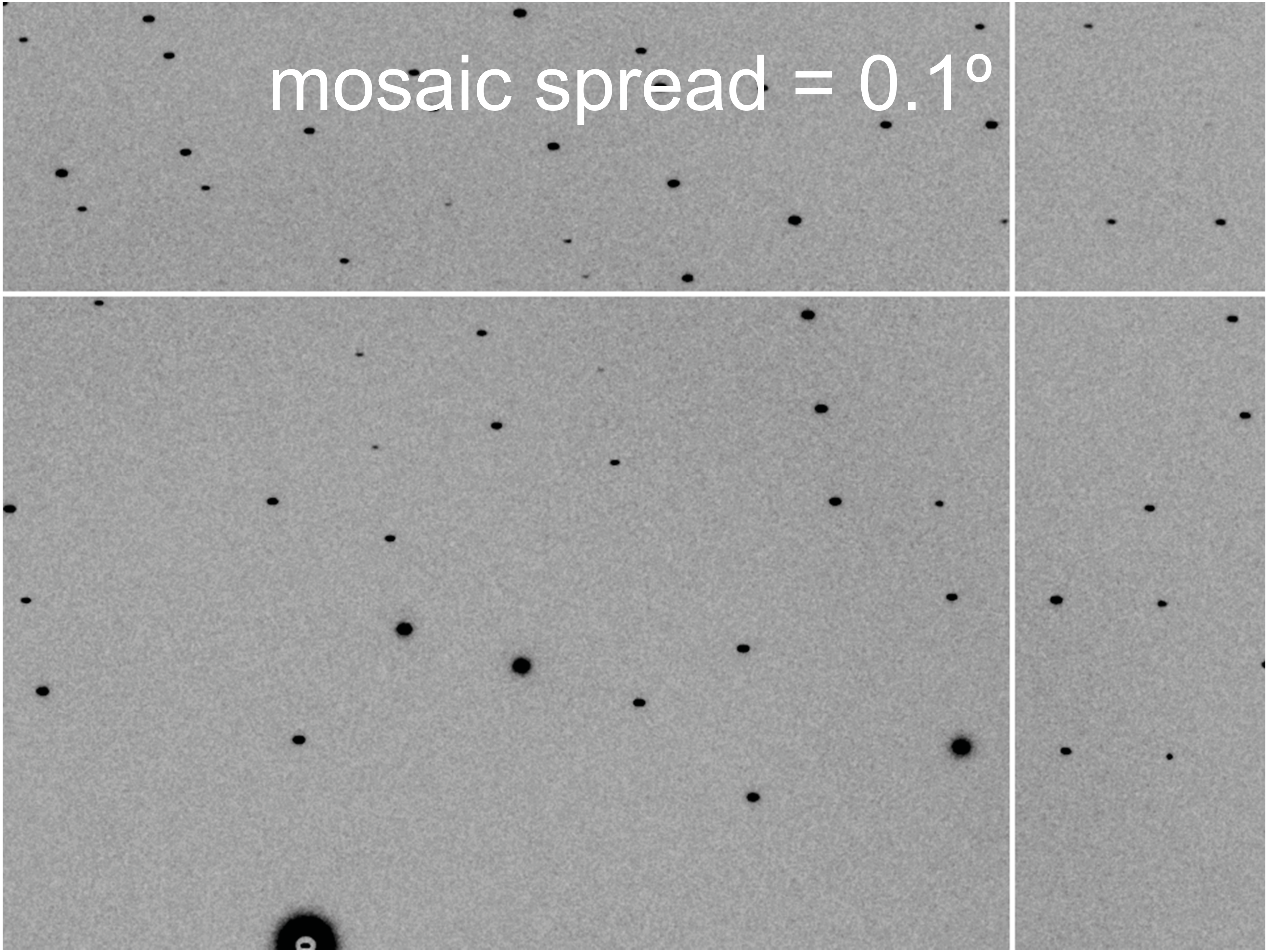
Ewald's "mosaic" picture

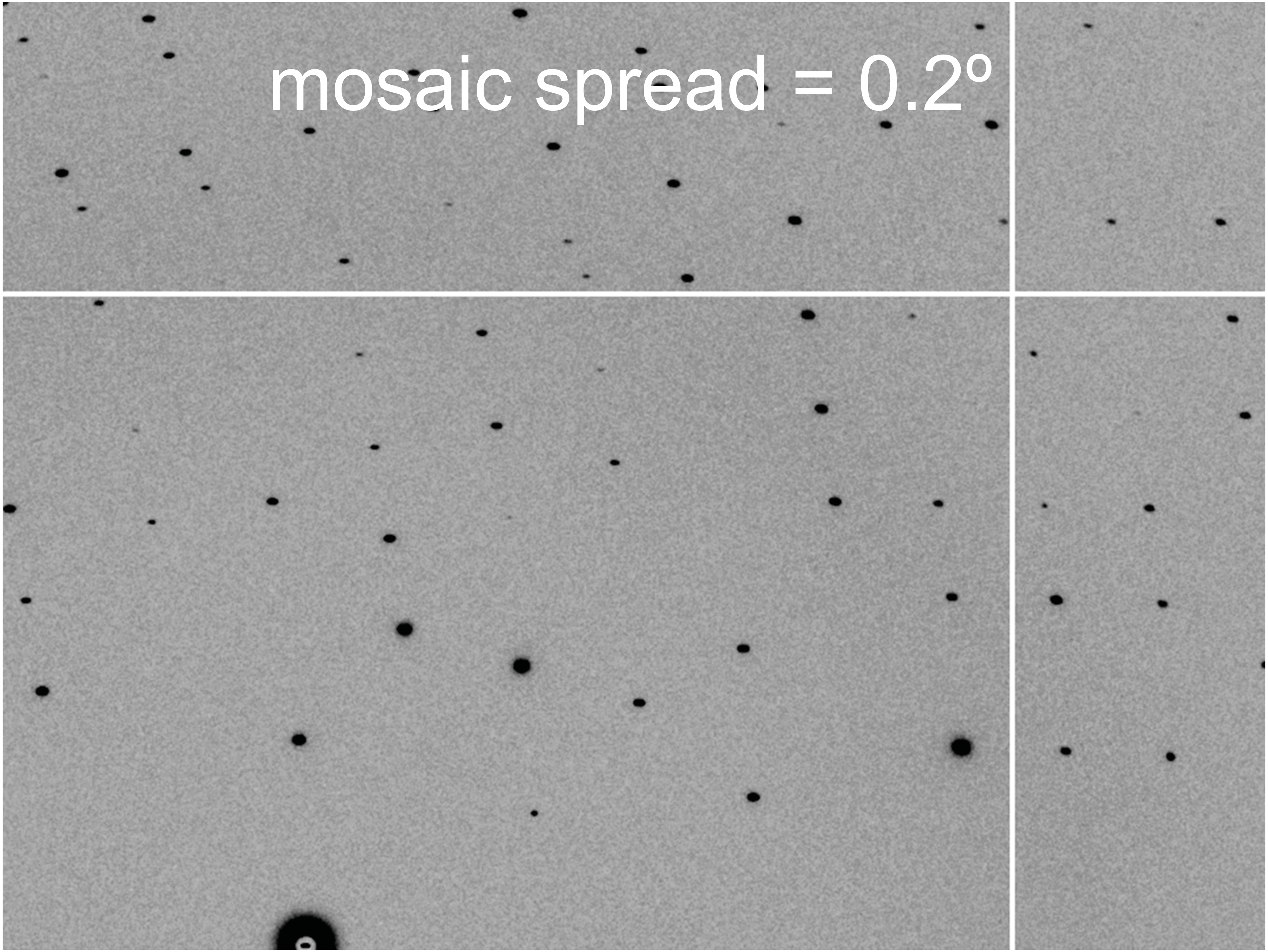


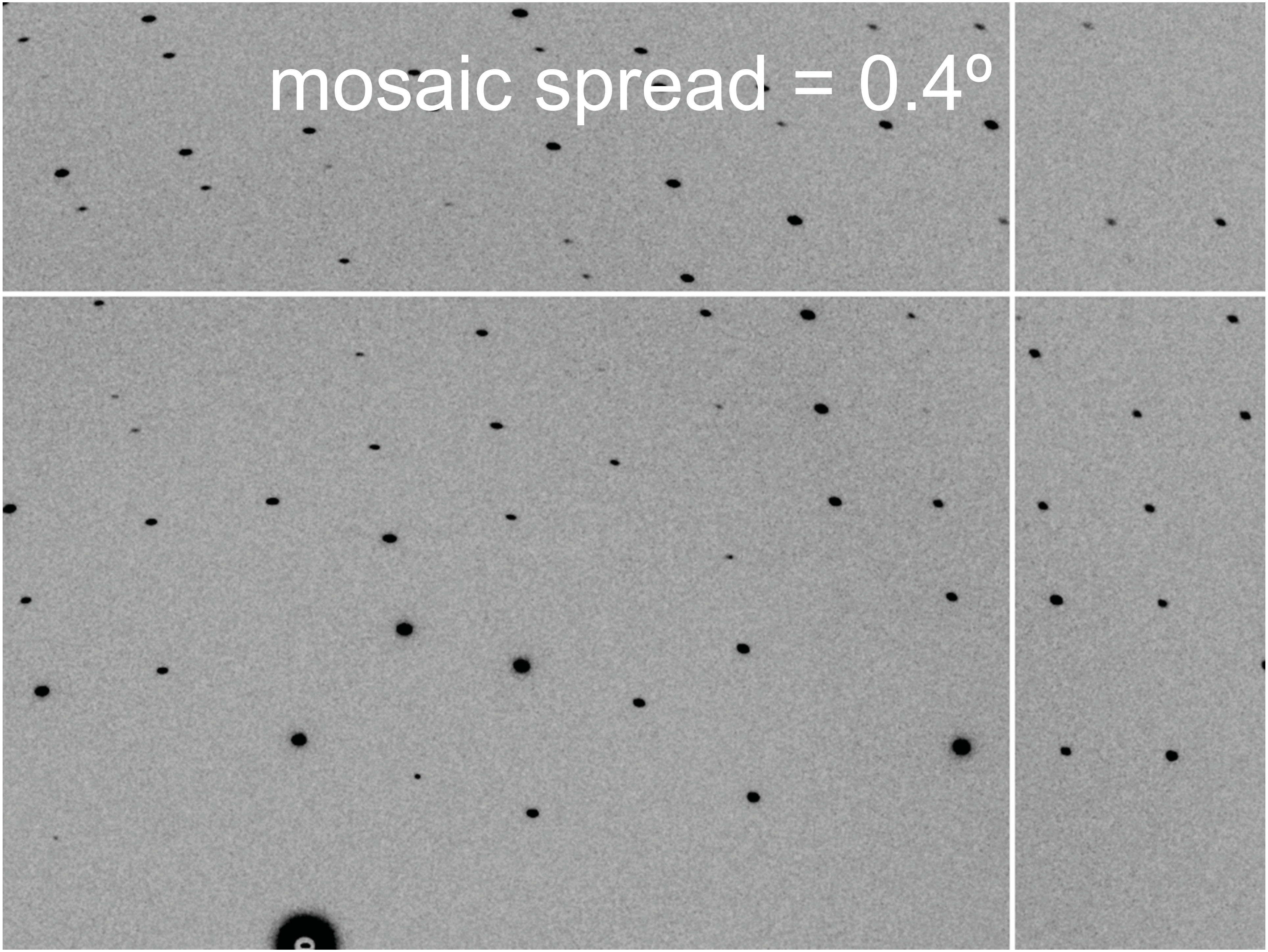
Ewald’s “mosaic” picture

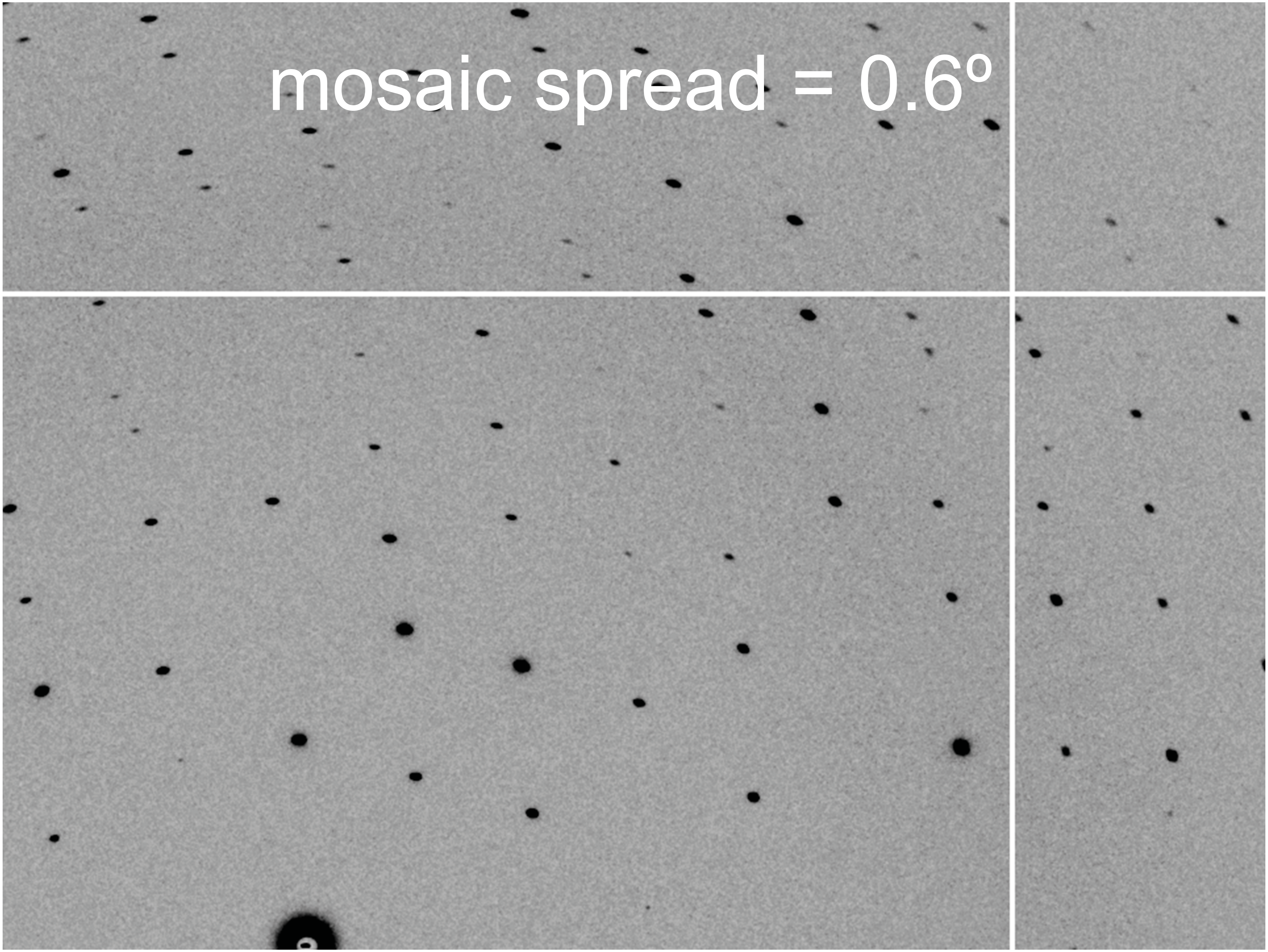


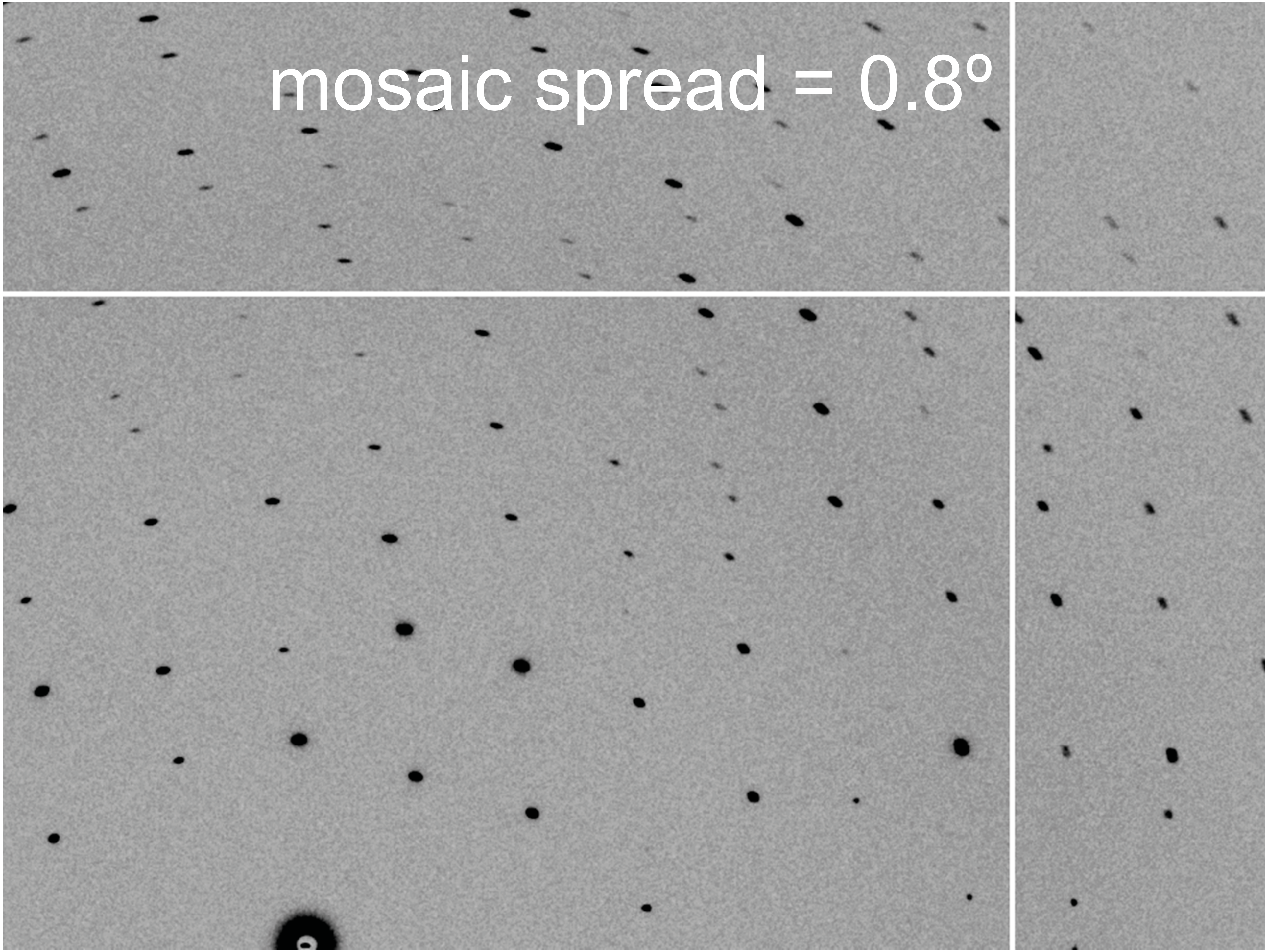

mosaic spread = 0 º

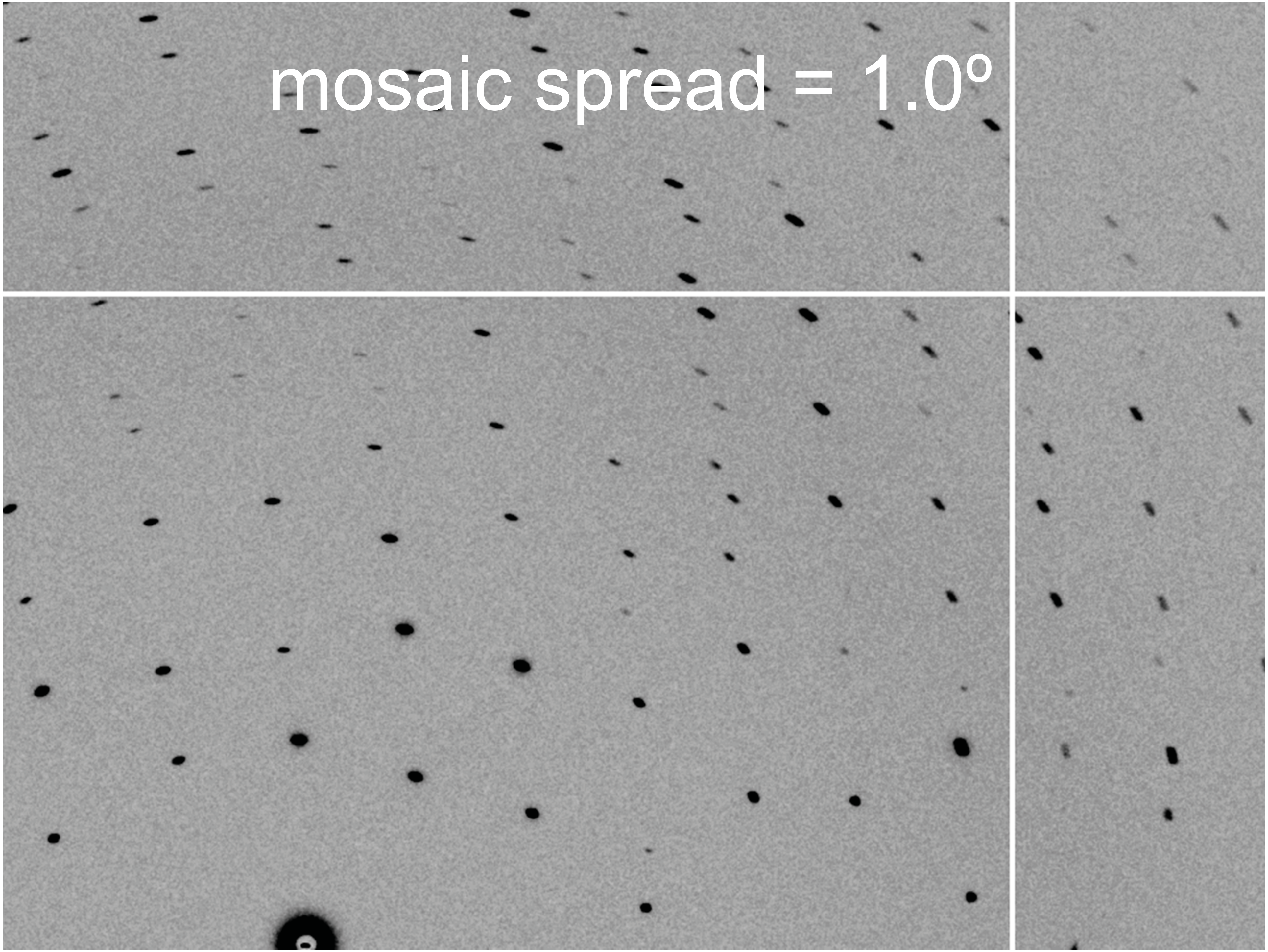

mosaic spread = 0.1º

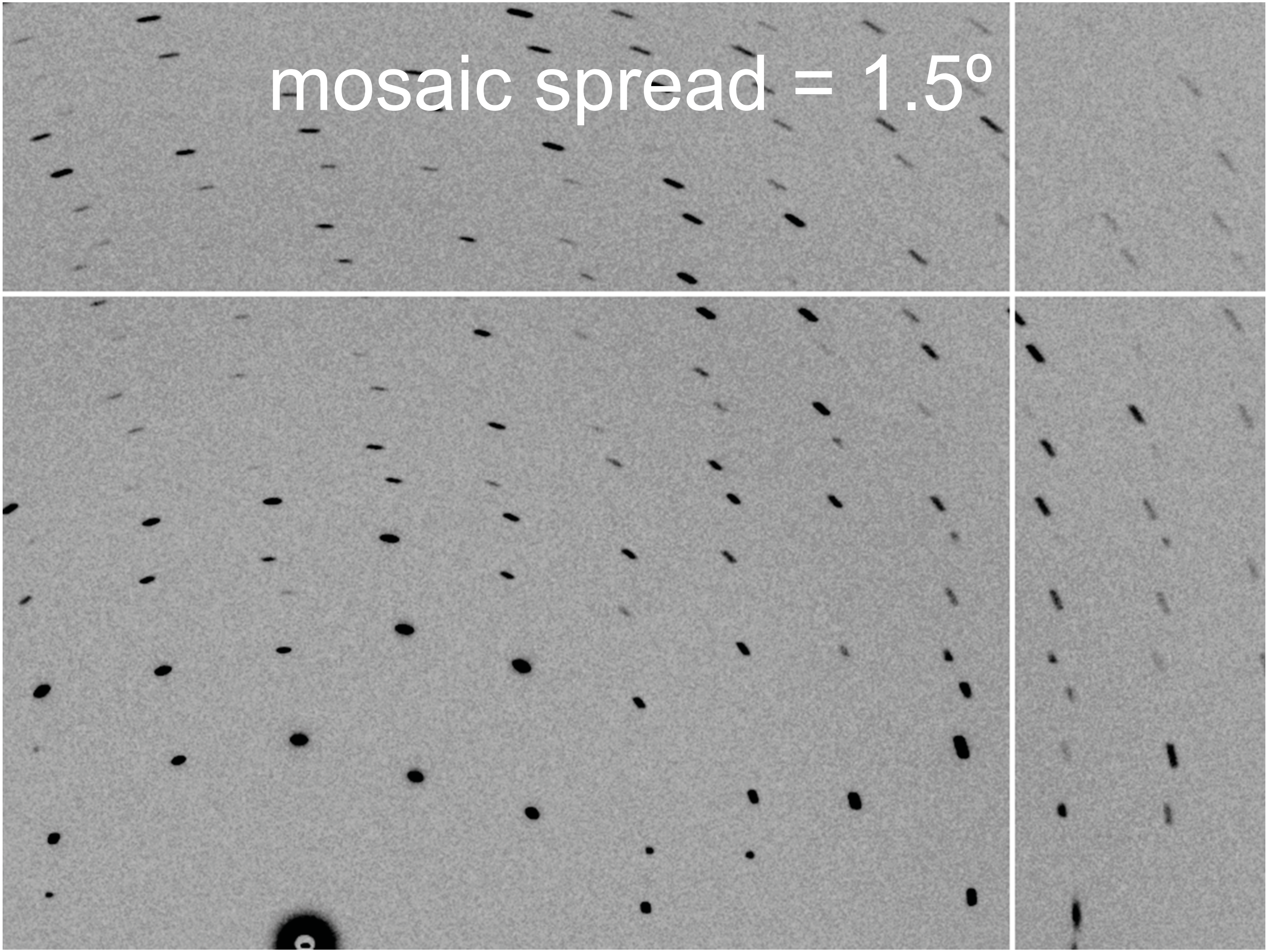

mosaic spread = 0.2º

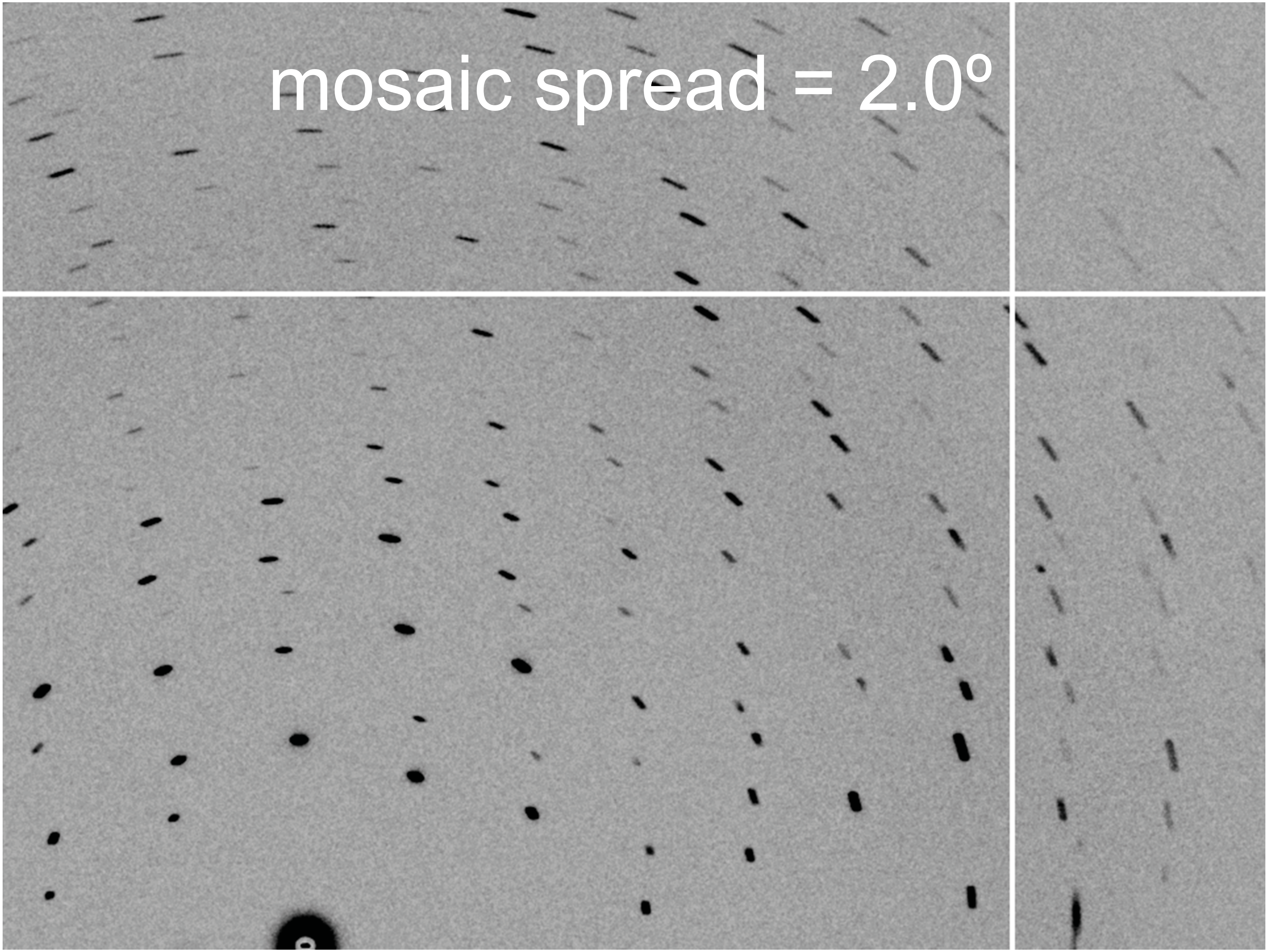

mosaic spread = 0.4º

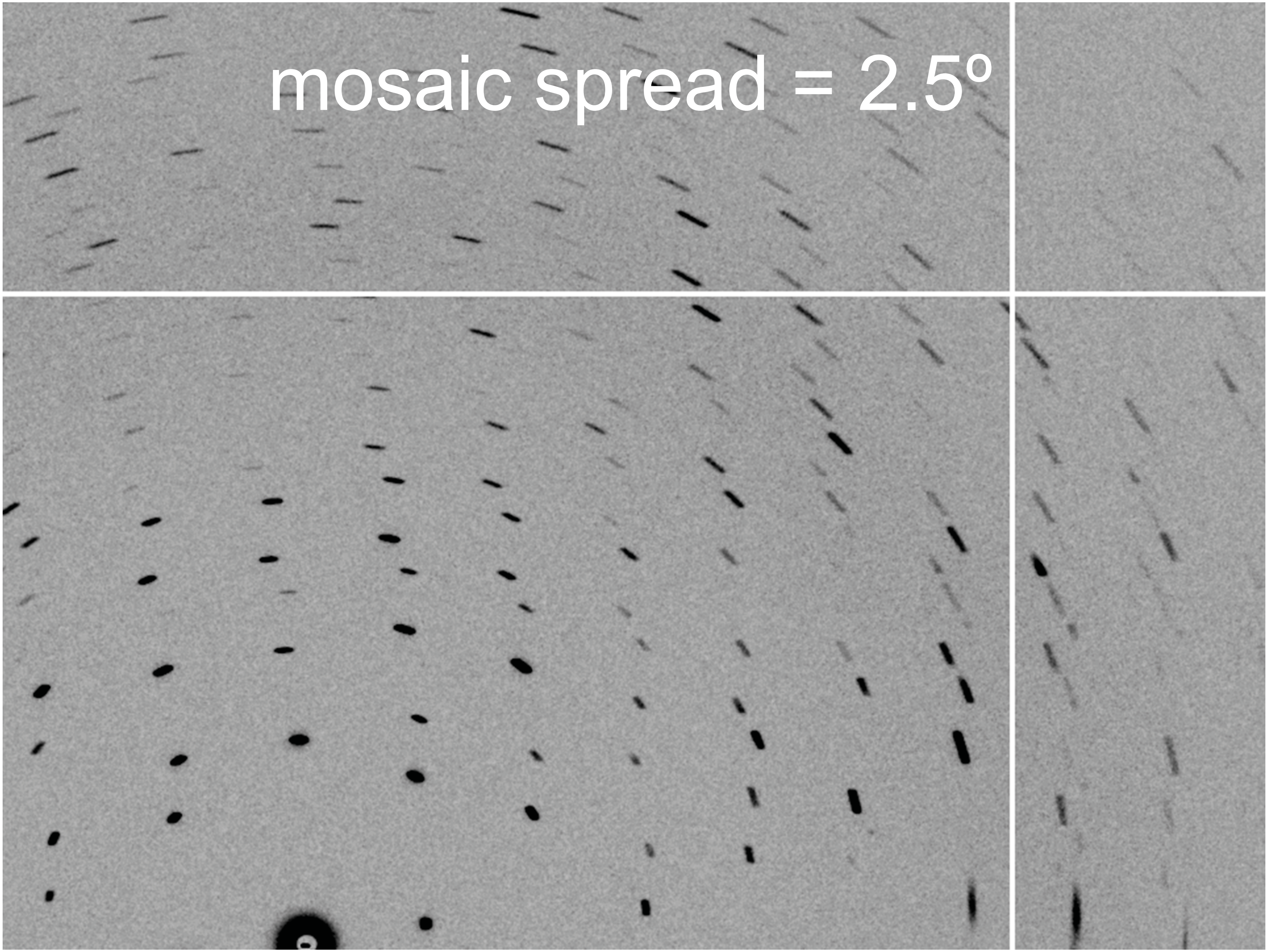

mosaic spread = 0.6º

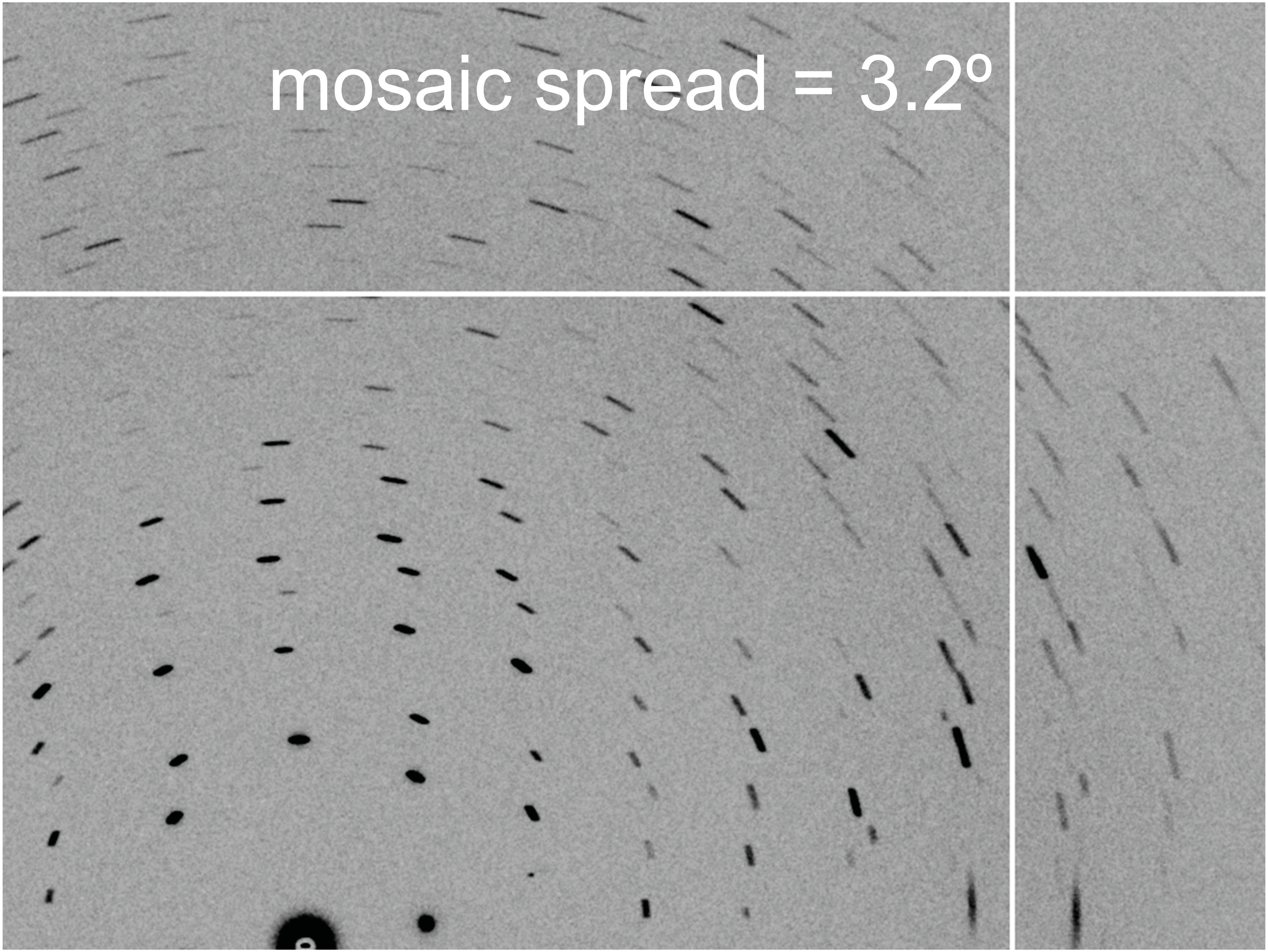

mosaic spread = 0.8º

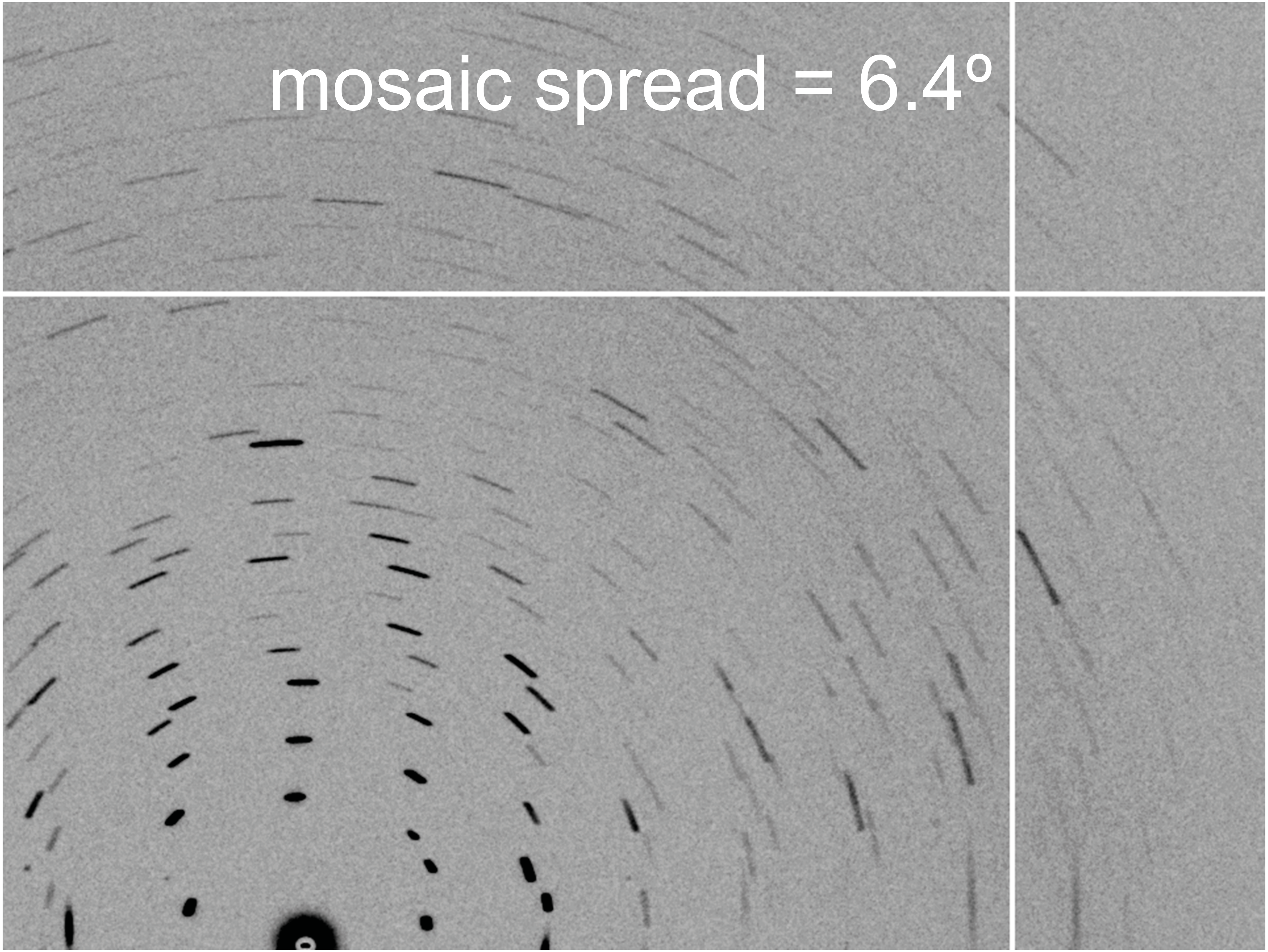

mosaic spread = 1.0º

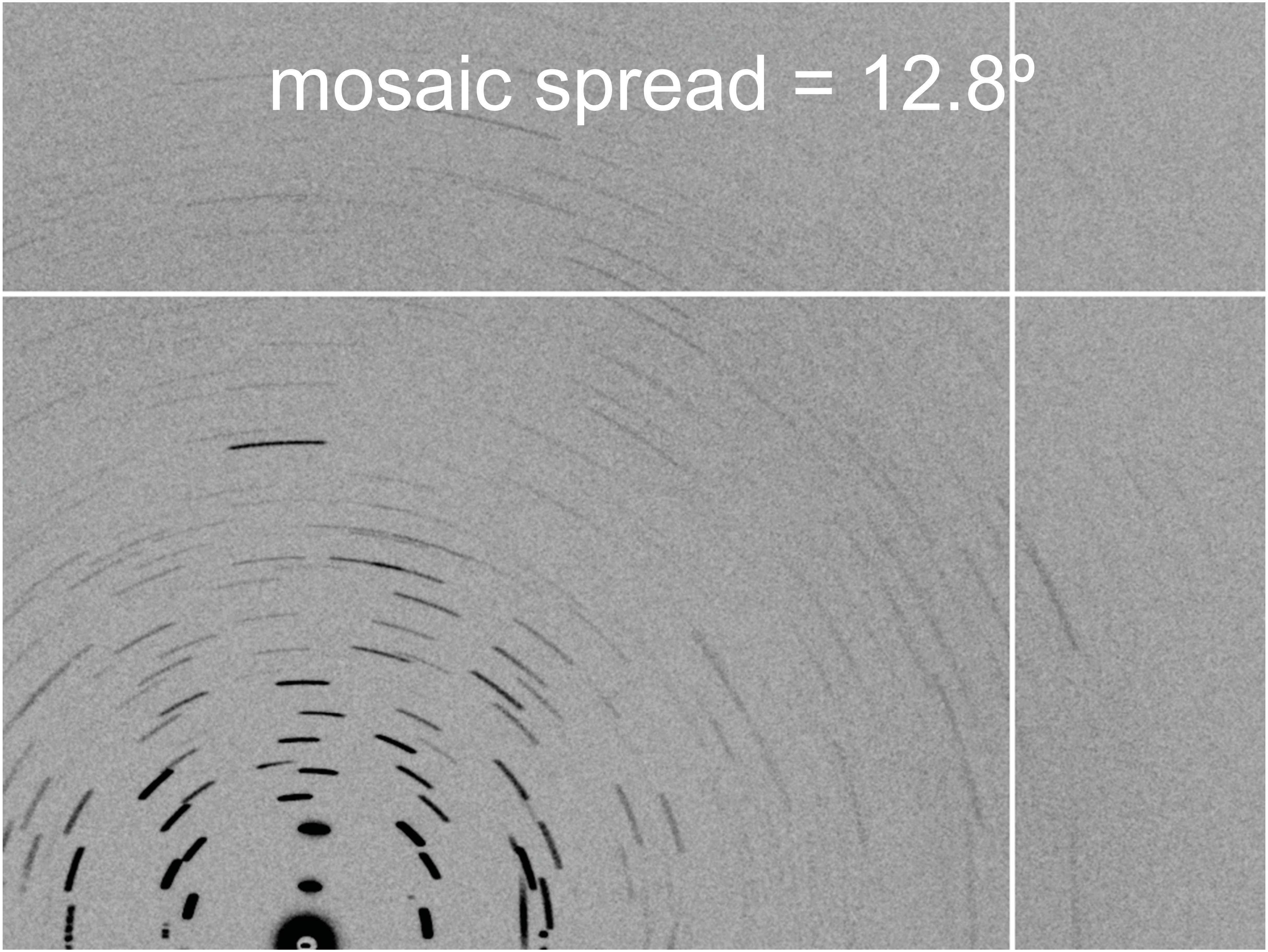

mosaic spread = 1.5º


mosaic spread = 2.0º

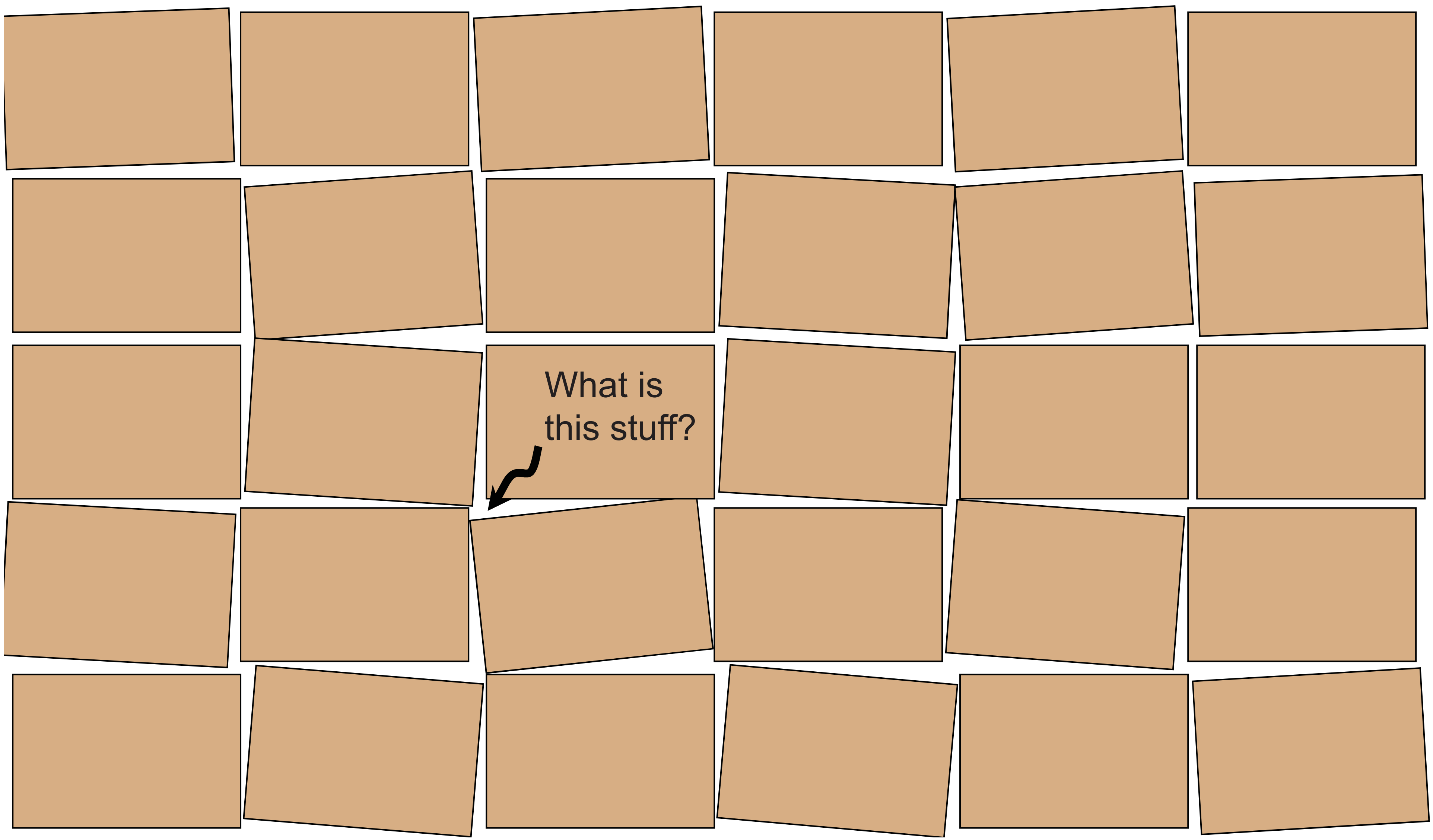

mosaic spread = 2.5º


mosaic spread = 3.2º

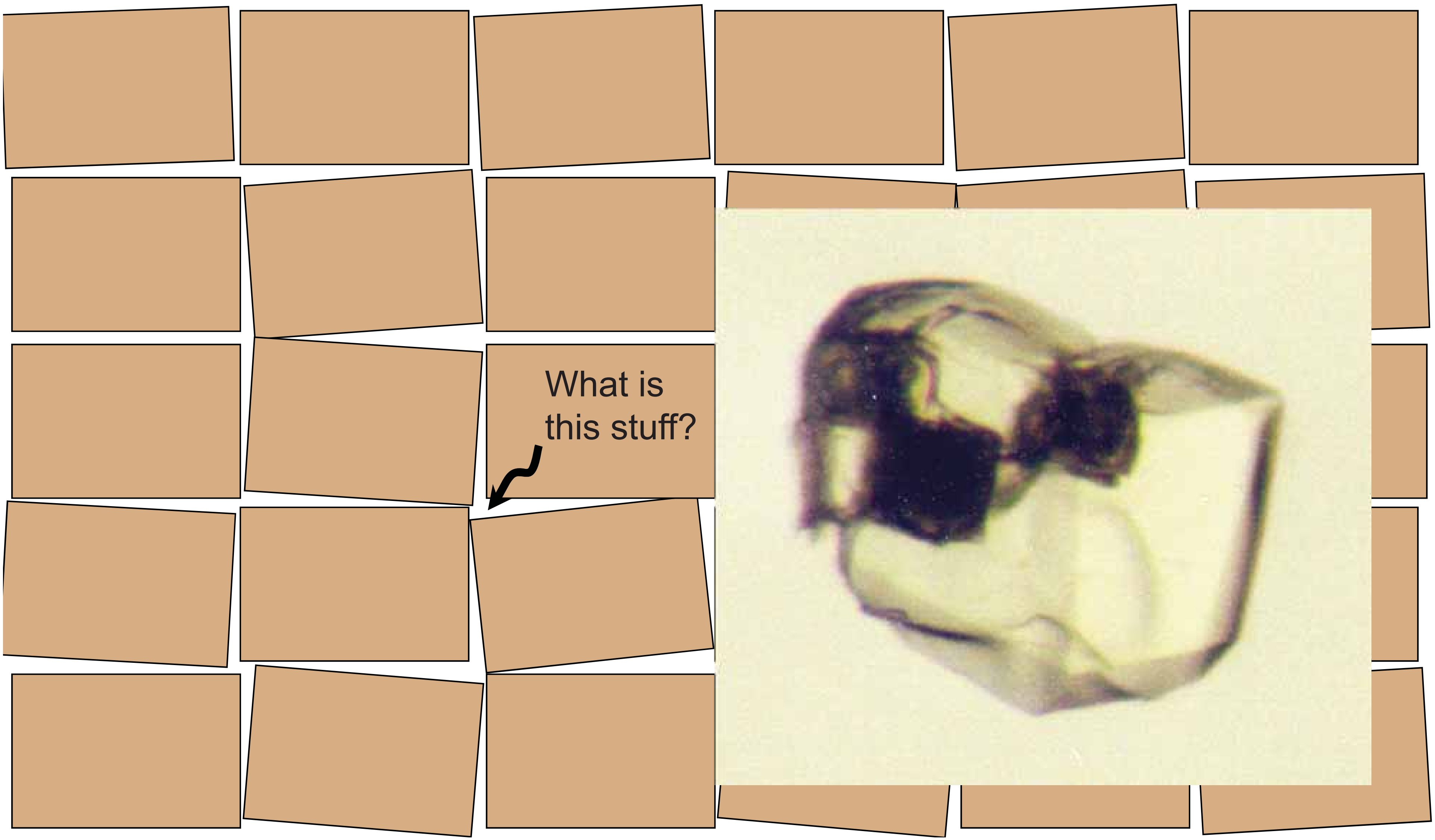

mosaic spread = 6.4º


mosaic spread = 12.8º

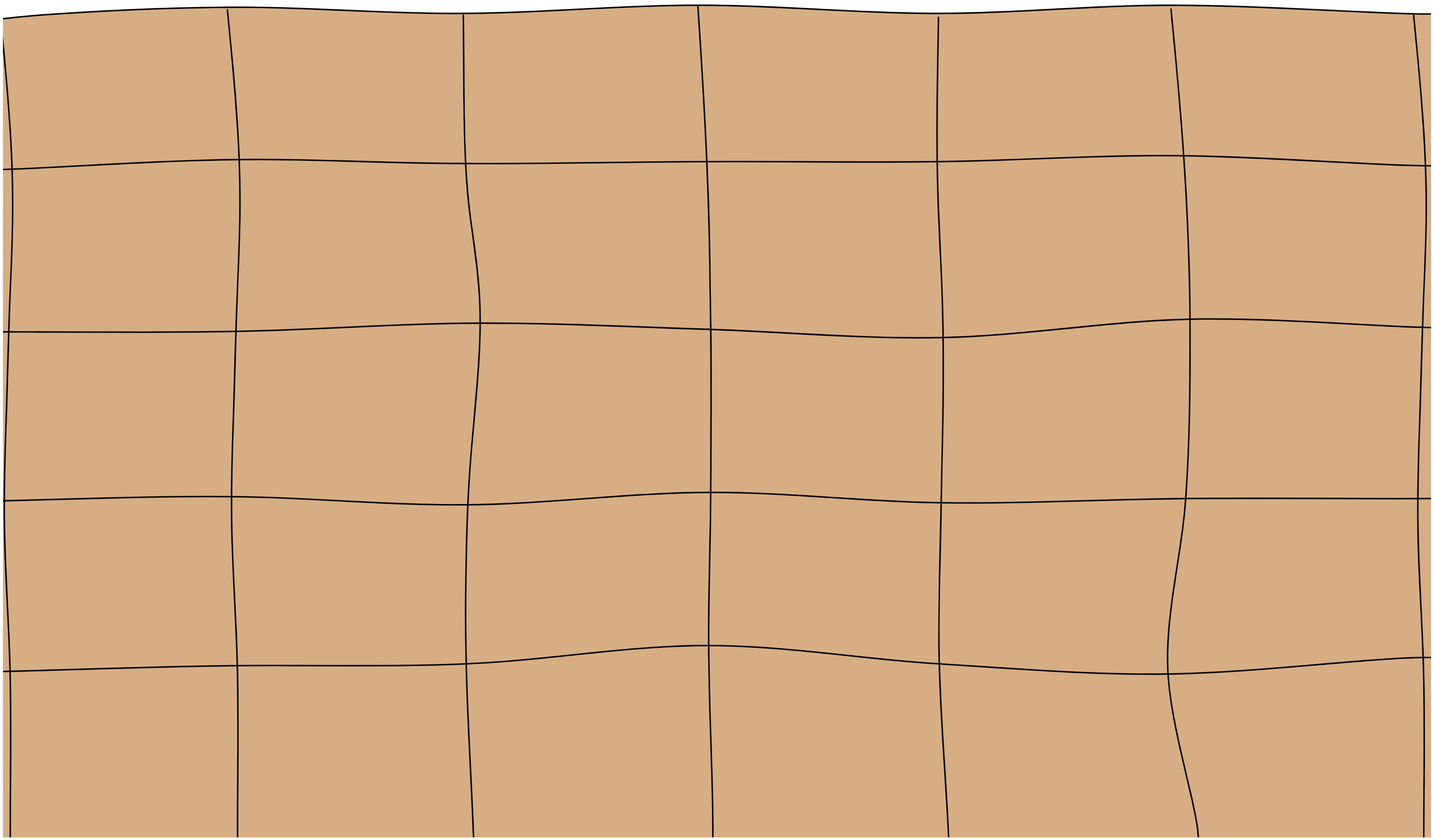
Ewald's “mosaic” picture



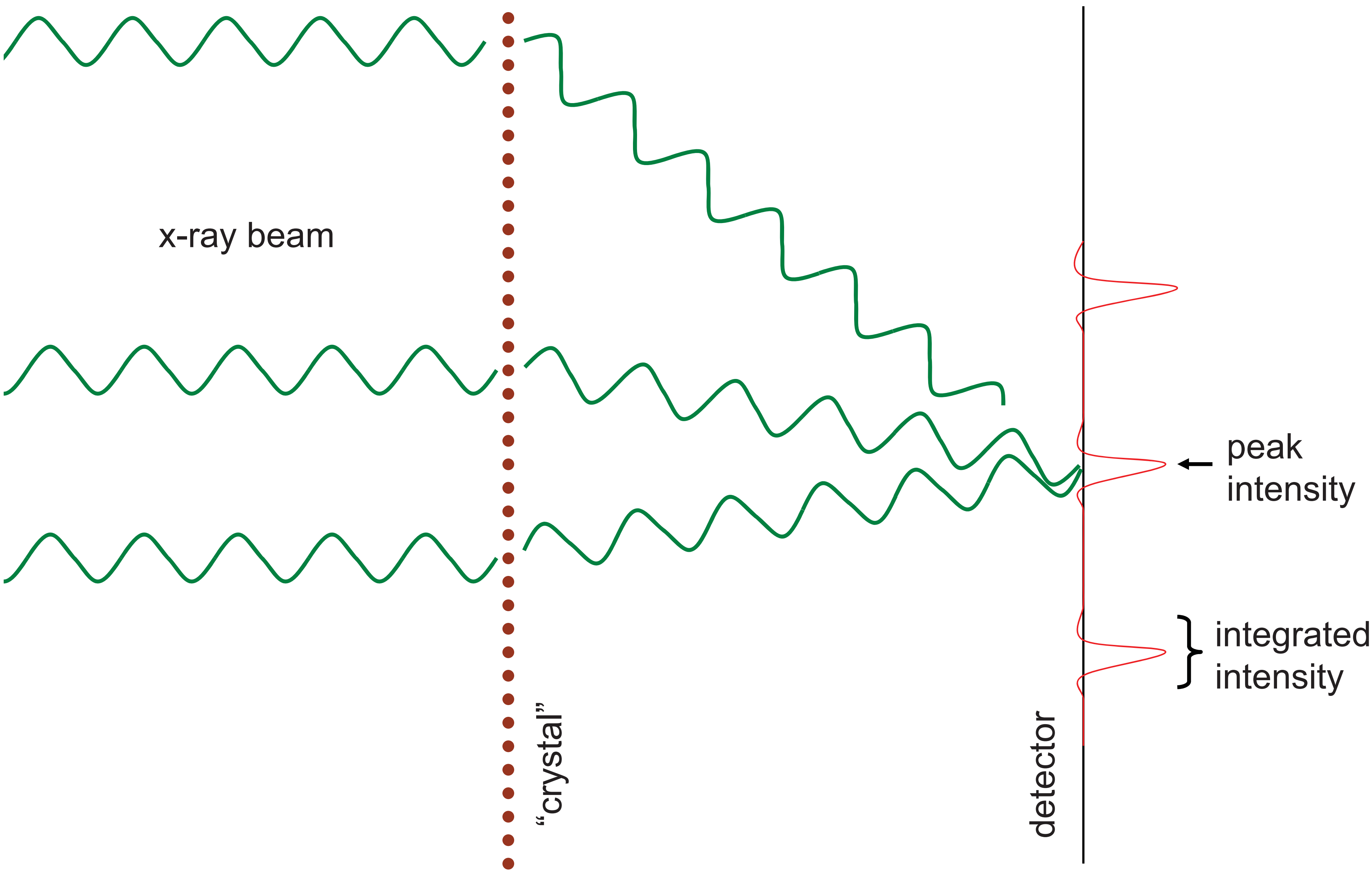
Ewald's "mosaic" picture



Darwin’s original picture

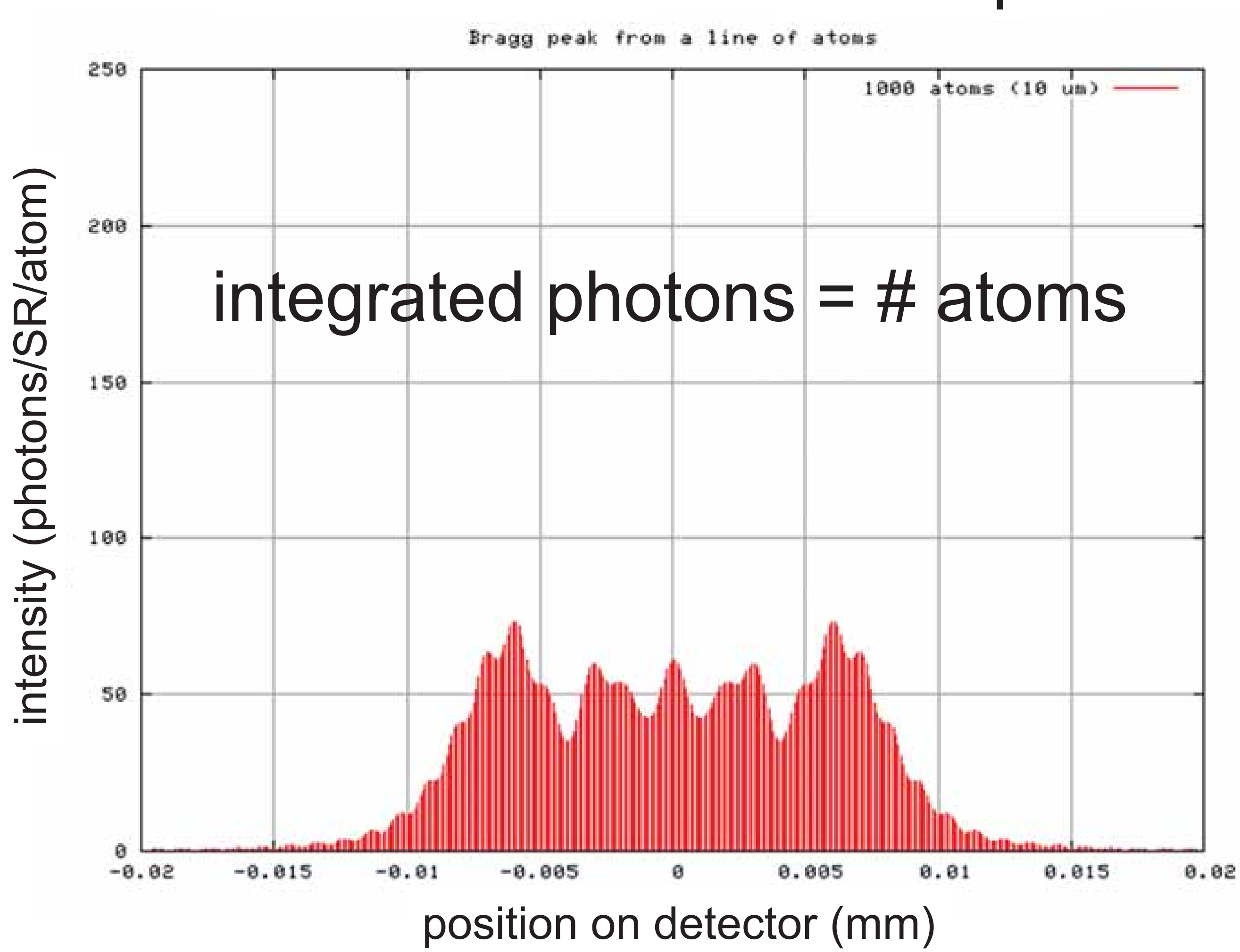


How big is a "mosaic block"

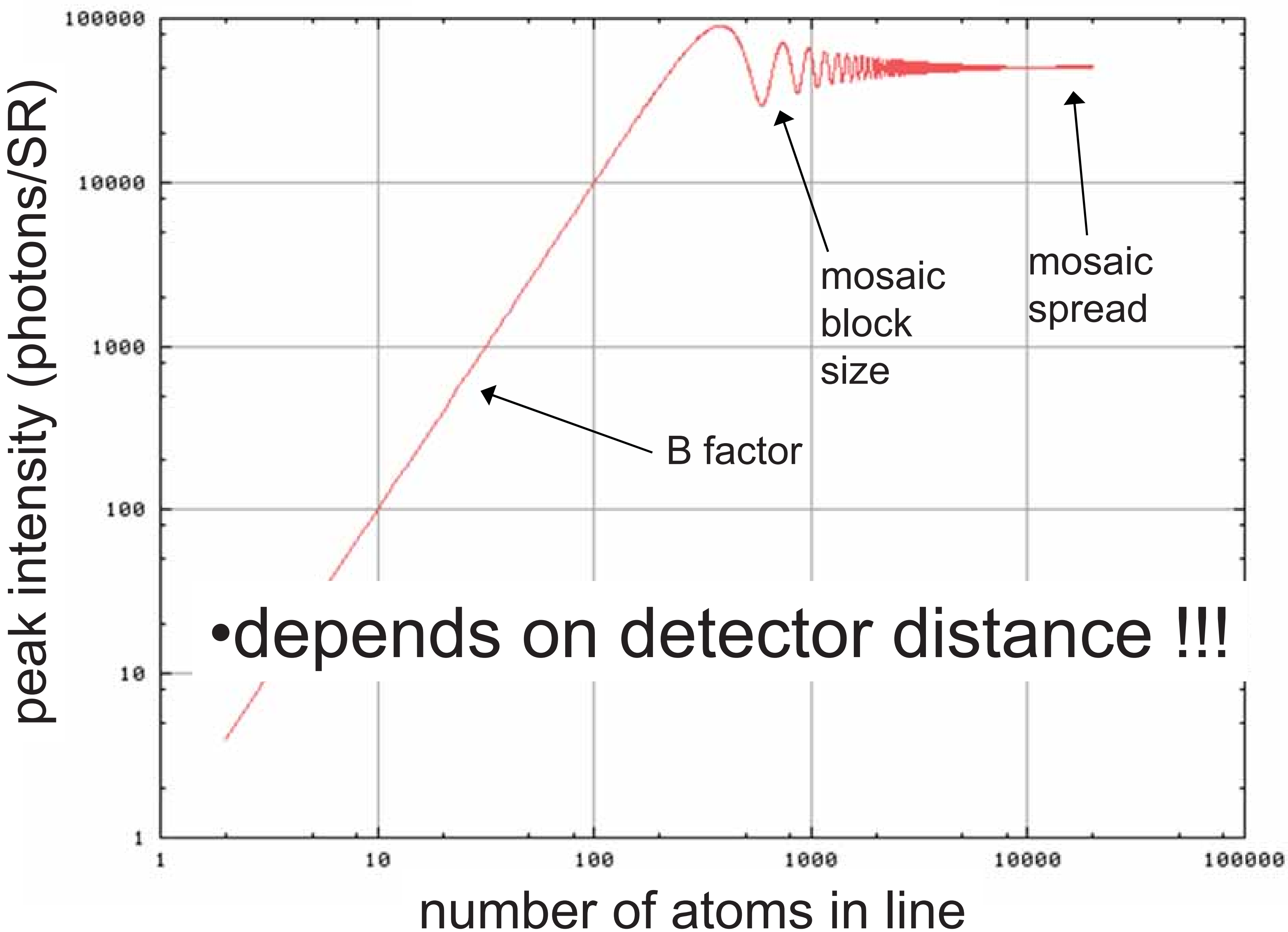


How big is a "mosaic block"

1000 atoms 10 μm



How big is a "mosaic block"



- depends on detector distance !!!

mosaic block = "coherence length"

(*b*) *The amplitude reflected by a plane sheet of atoms :* We shall first consider the amplitude of the wave reflected by an infinite plane sheet of atoms, each of which scatters the incident X-rays.

Suppose A, fig. 15, is the source of the radiation, and let the amplitude of the reflected wave be required at B. Let the plane APB be normal to the plane of atoms, and let AP, PB make equal angles θ with this plane. Then P is such that the distance APB is the shortest distance from A to B *via* the plane. Let M be a point of the plane such that the distance

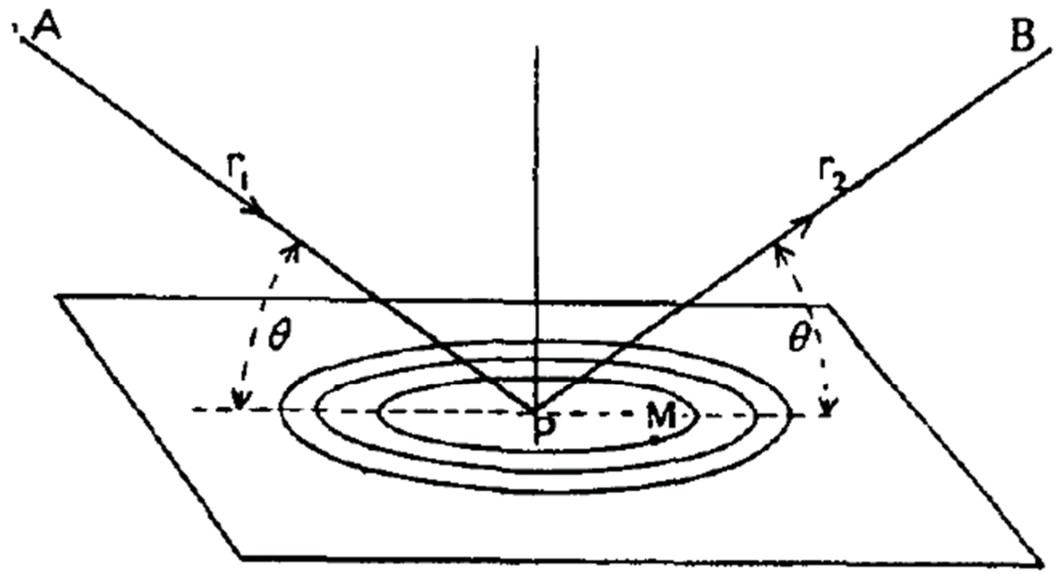
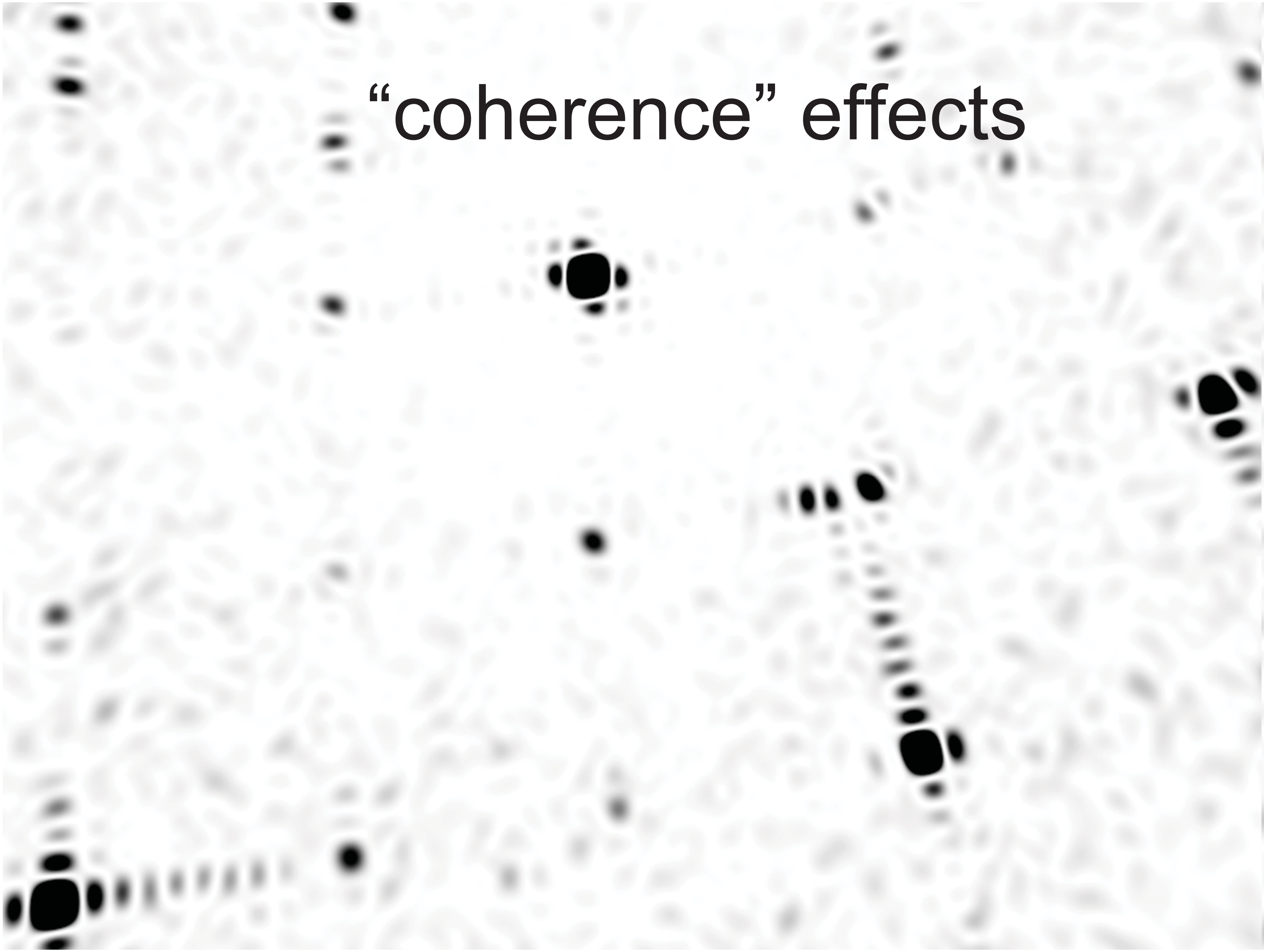


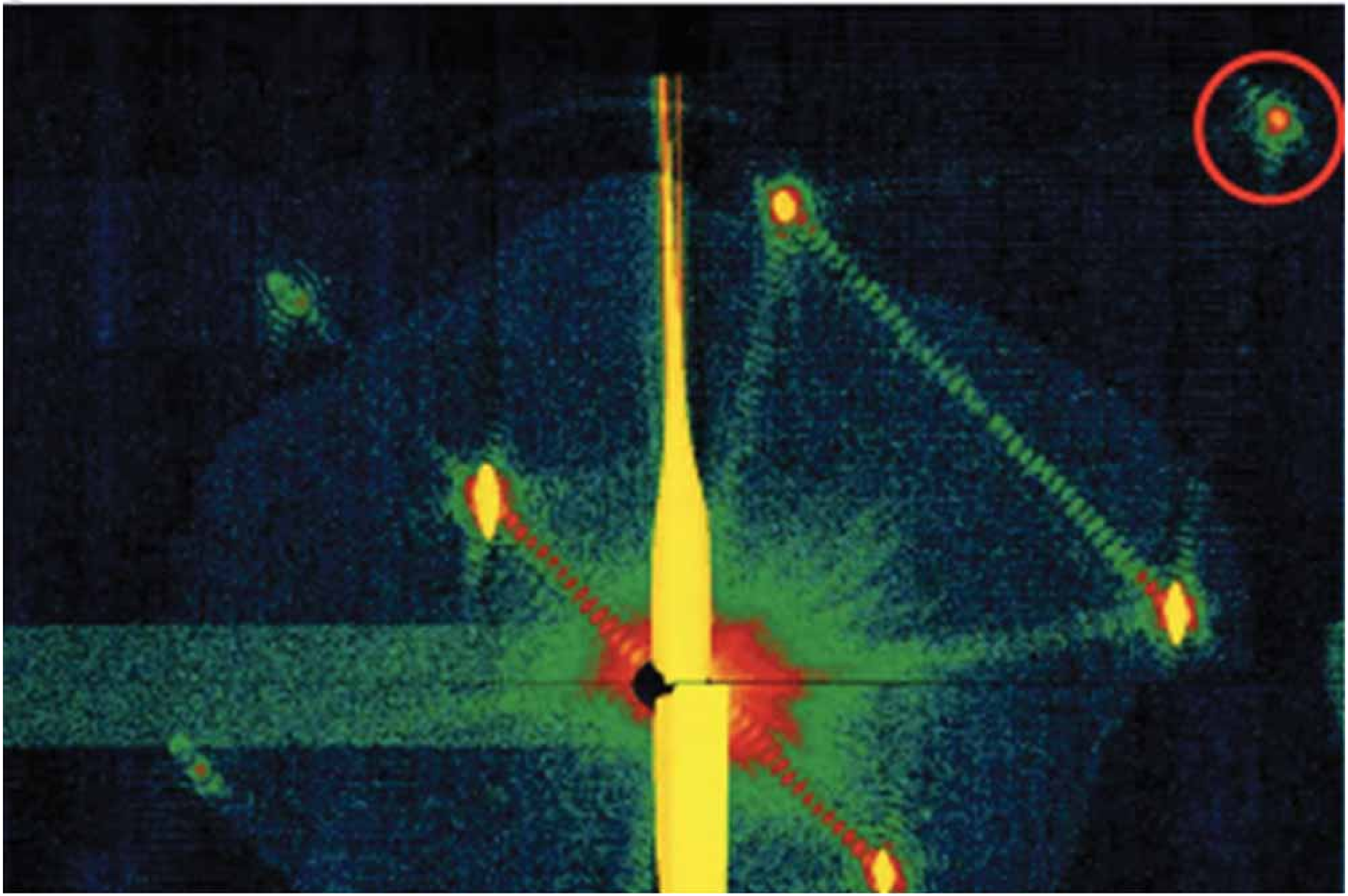
FIG. 15

James R. W. (1962) *Optical Principles of the Diffraction of X rays.* Ox Bow press.

“coherence” effects



Observed “coherence” effects



…what is "incoherence" then?

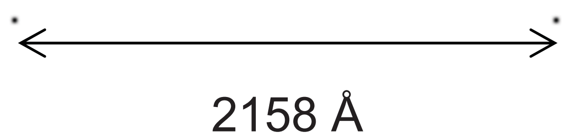
- light is "coherent"
- near-zero divergence
- near-zero dispersion
- crystal cannot rotate
- crystals may be 1 mosaic block
- are small crystals "more perfect"?
- will we see any spots?!

…what is “incoherence” then?

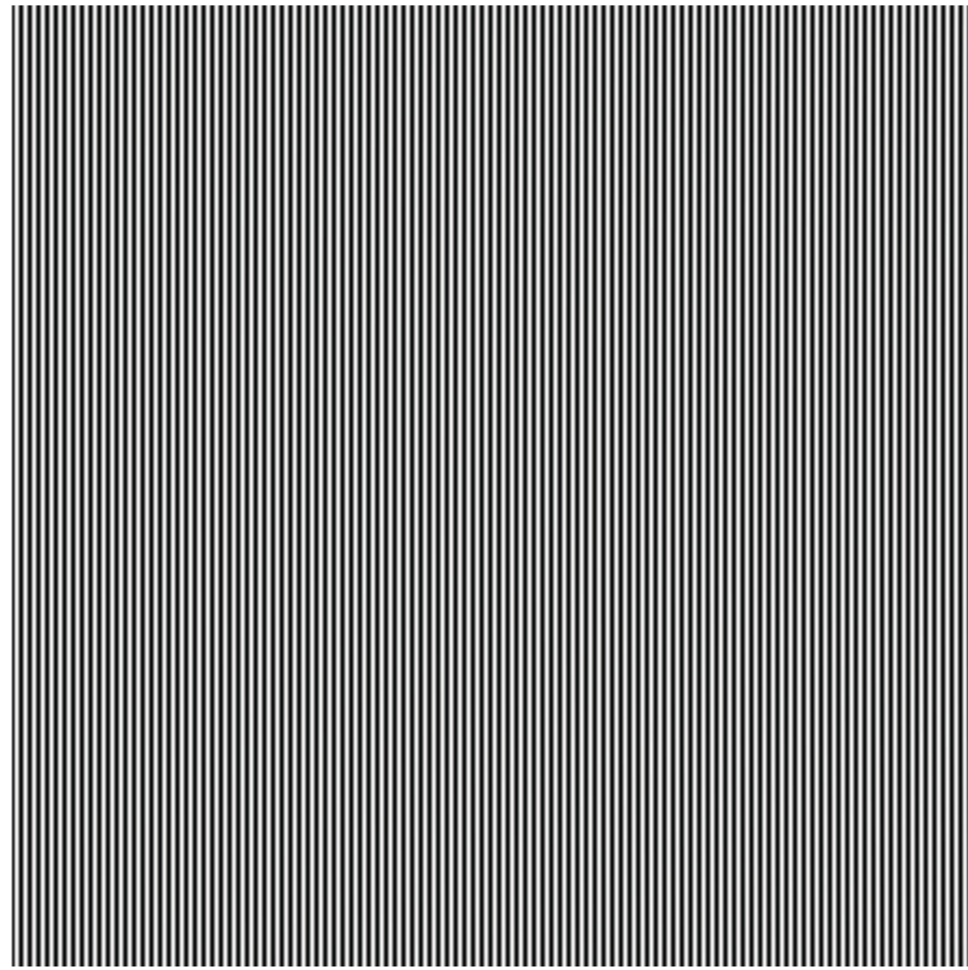
- light is “coherent”
- near-zero divergence
- near-zero dispersion
- crystal cannot rotate
- crystals may be 1 mosaic block
- are small crystals “more perfect”?
- will we see any spots?!

scattering from two atoms

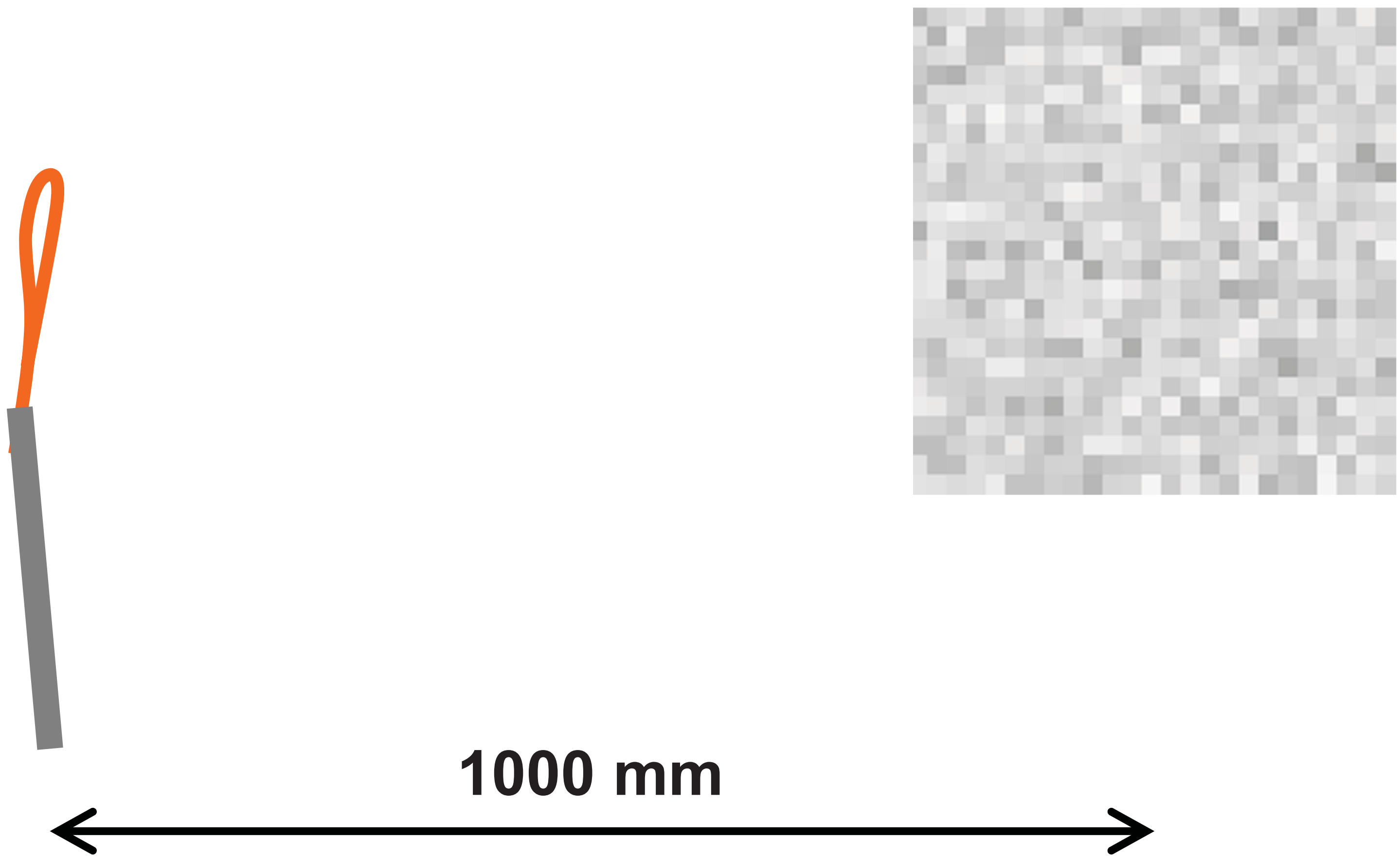
sample



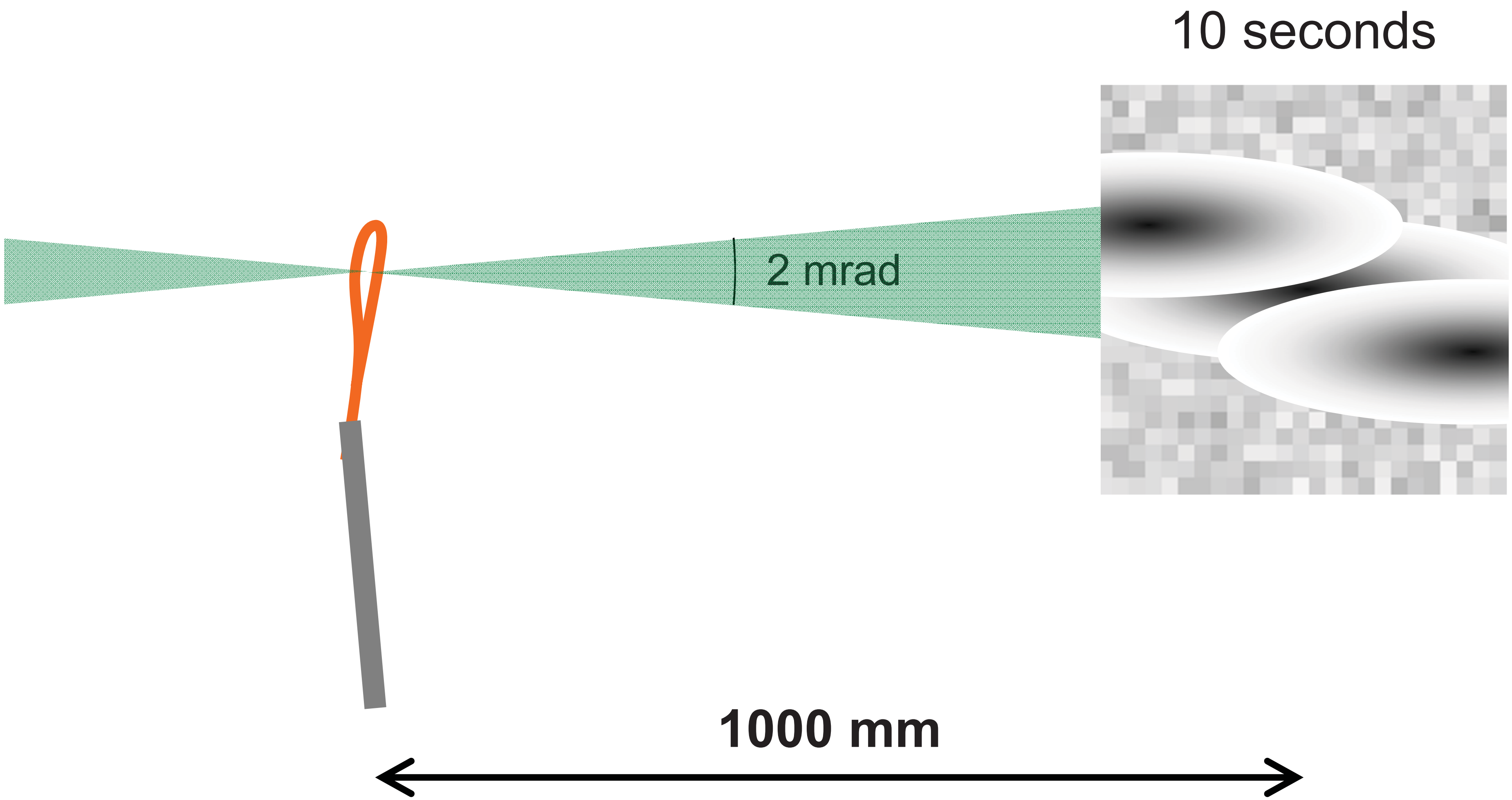
detector



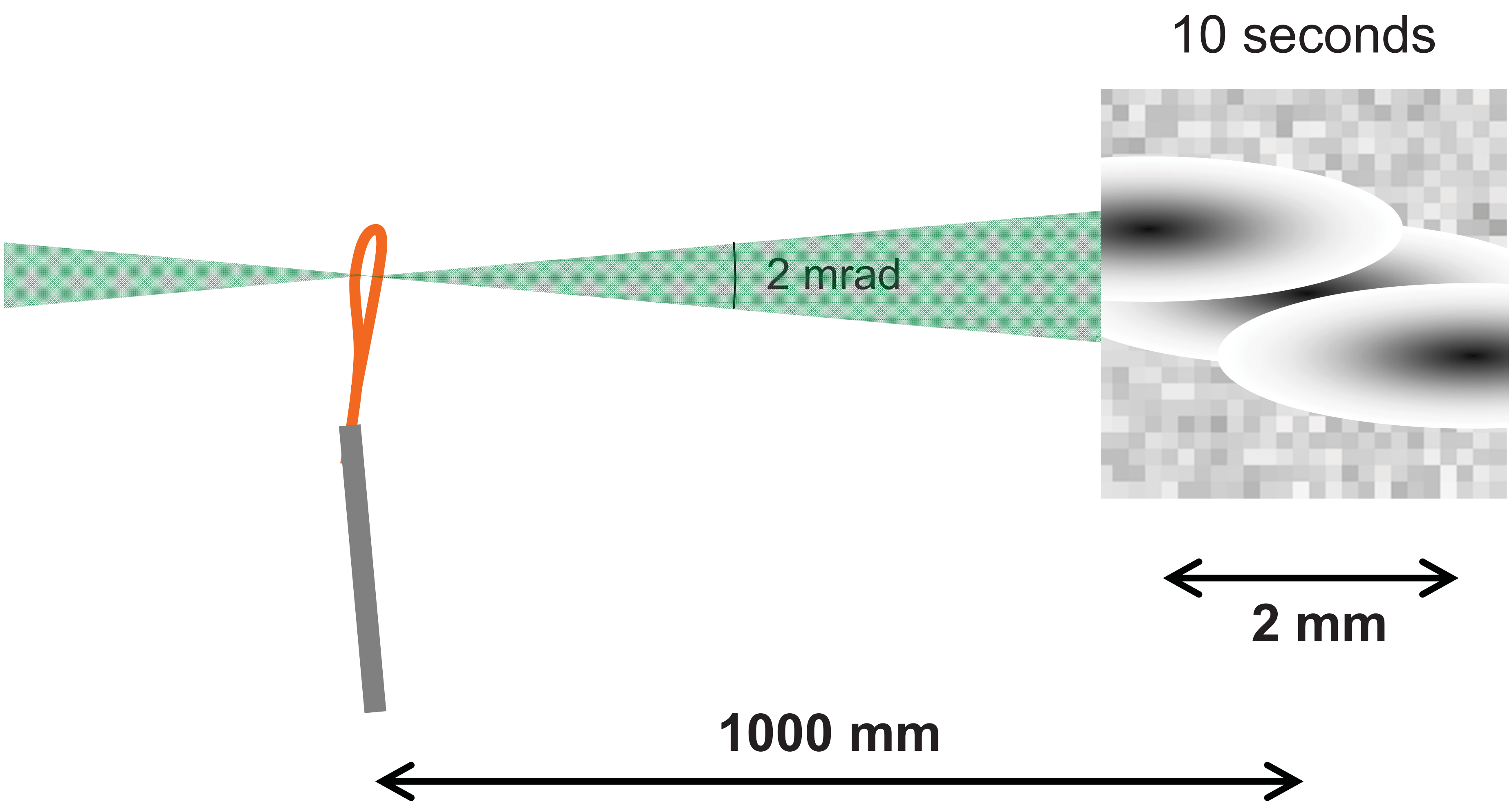
beam divergence



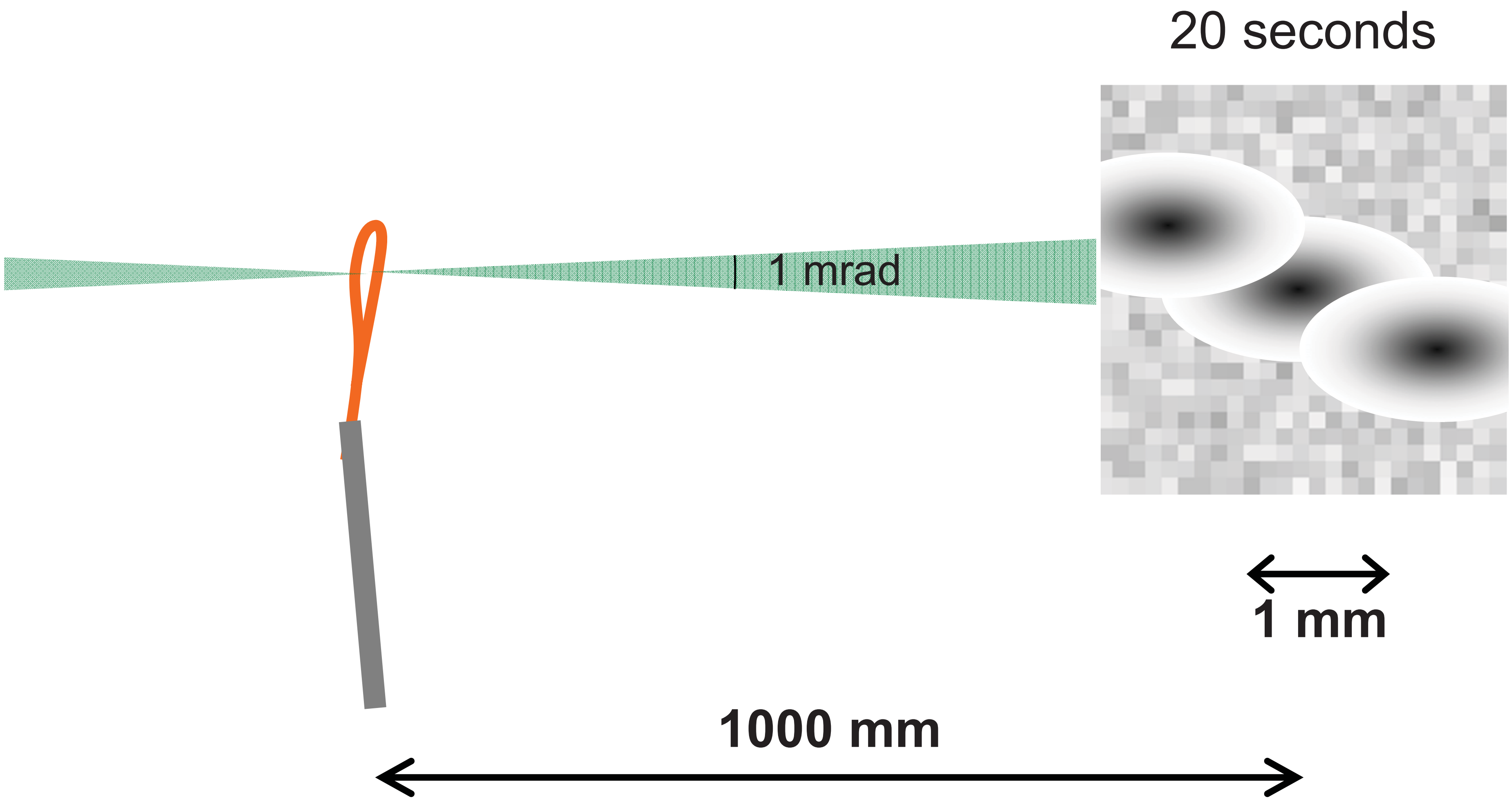
beam divergence



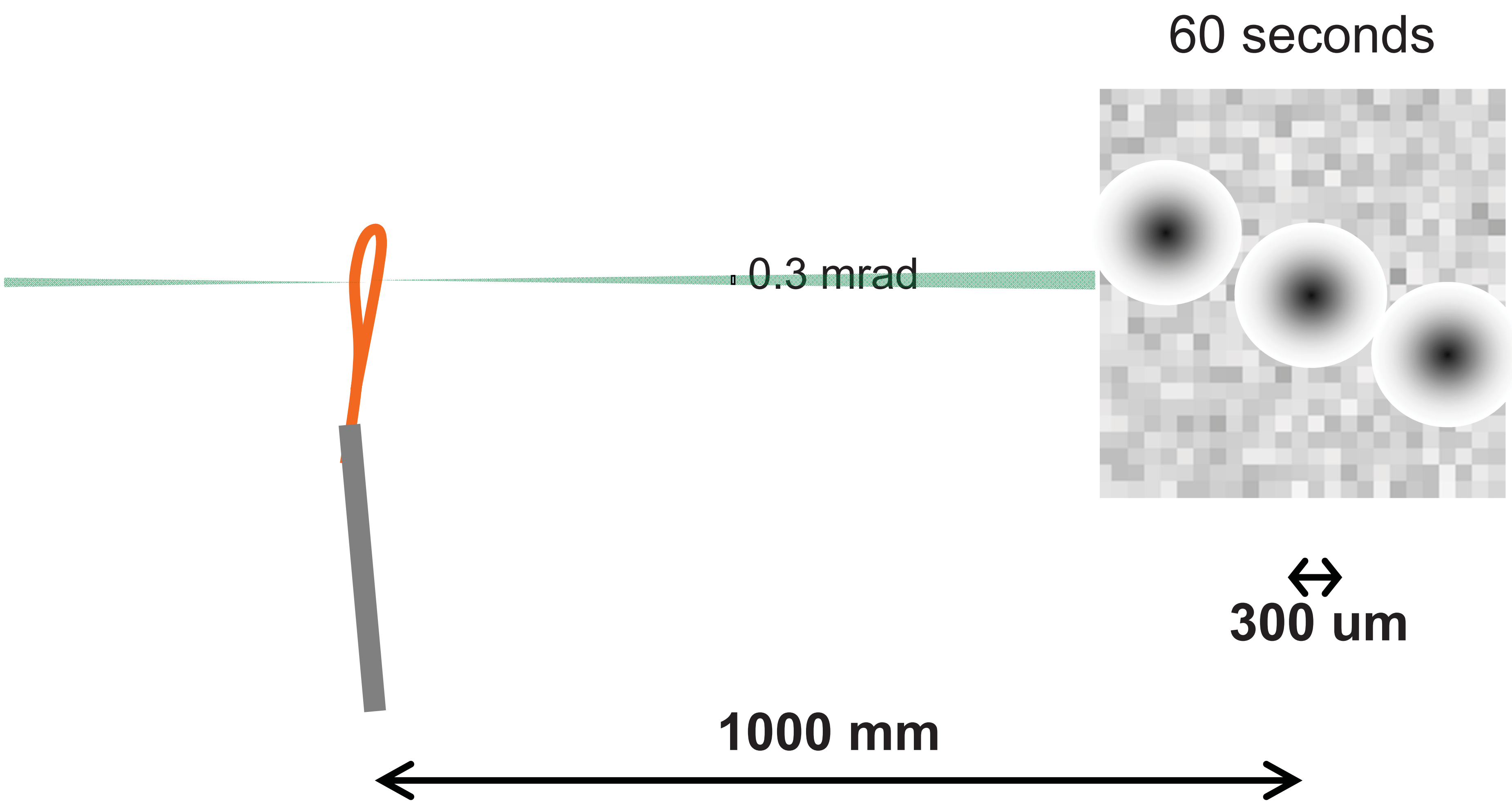
beam divergence



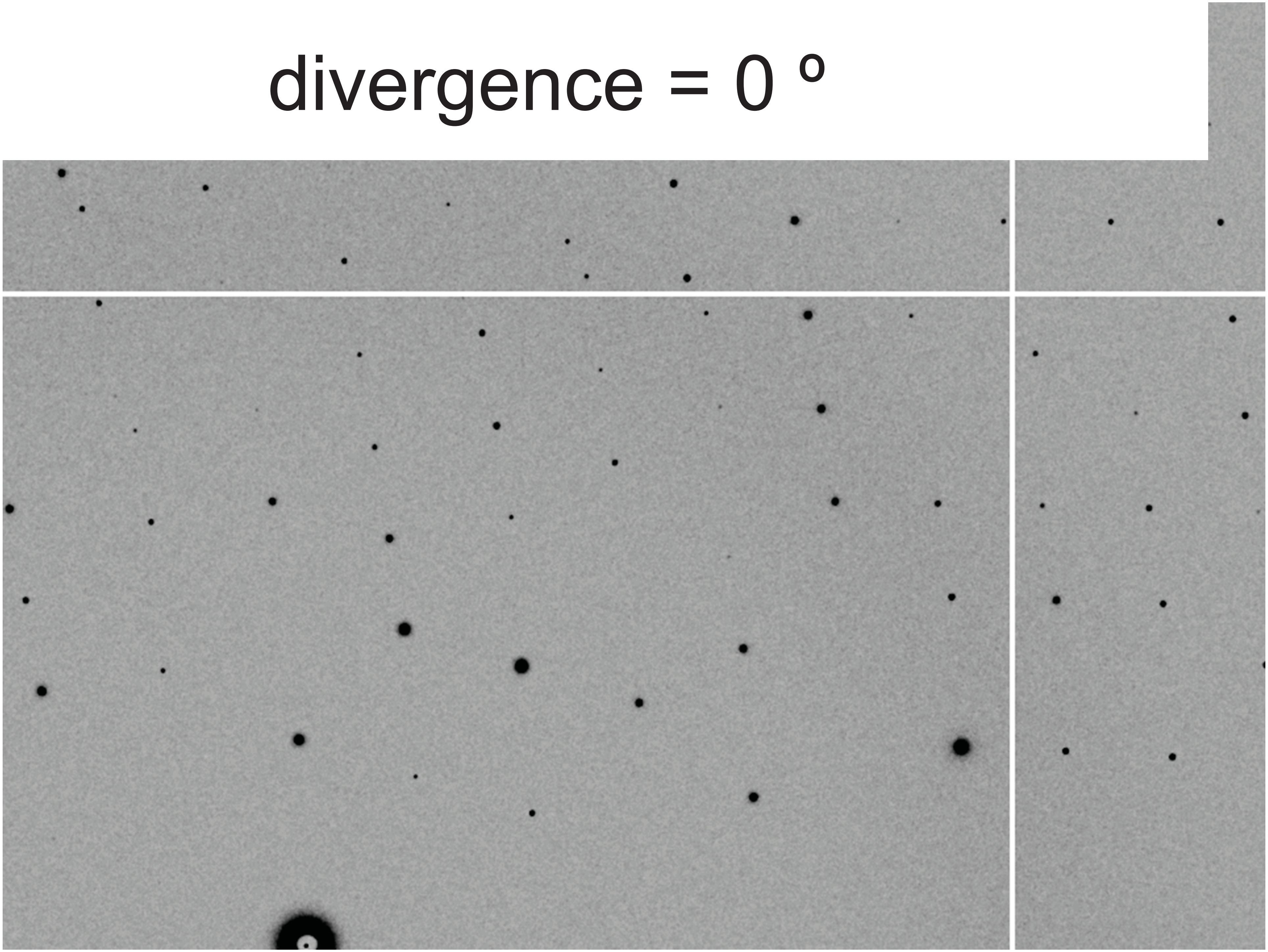
beam divergence



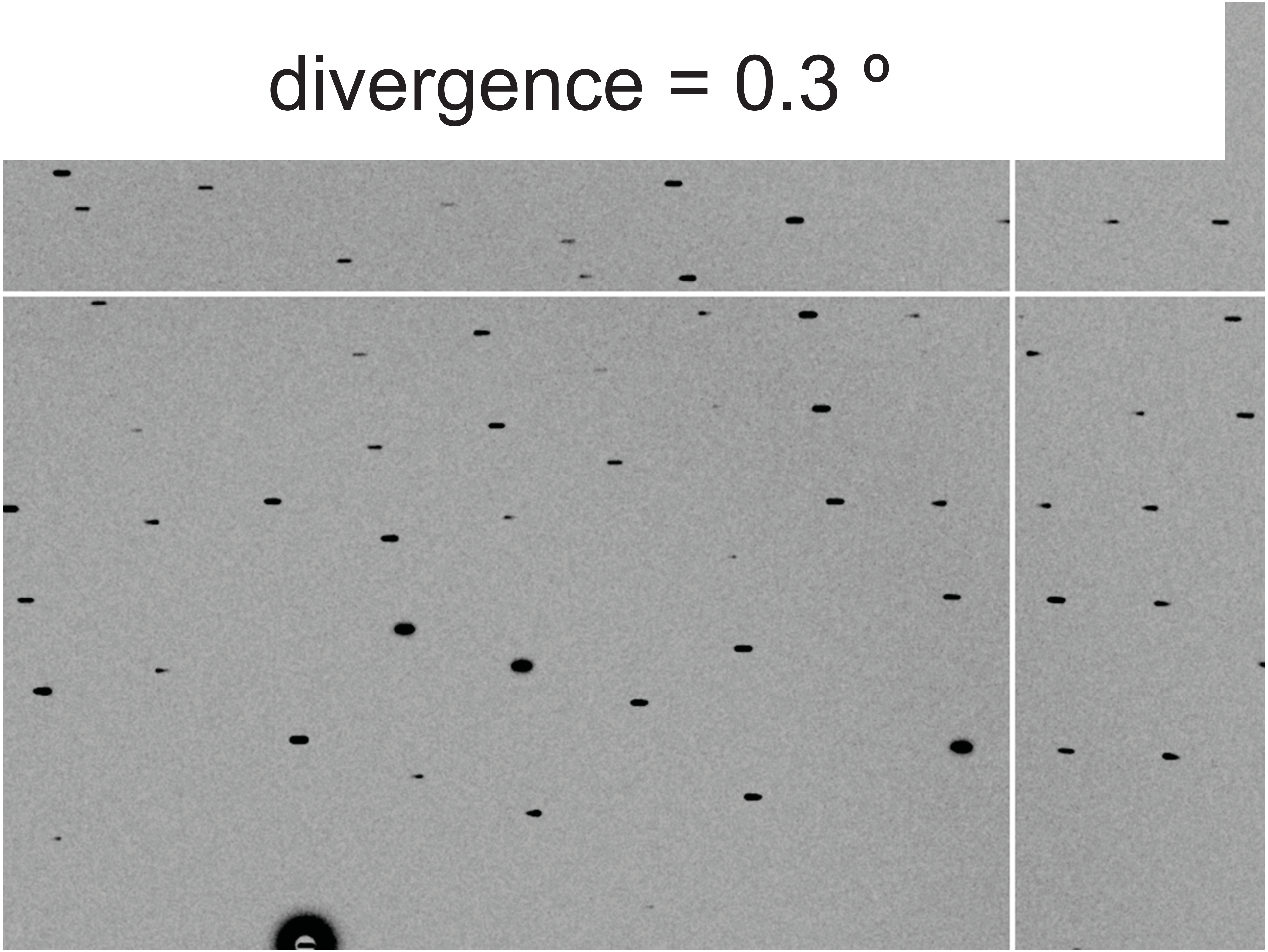
beam divergence



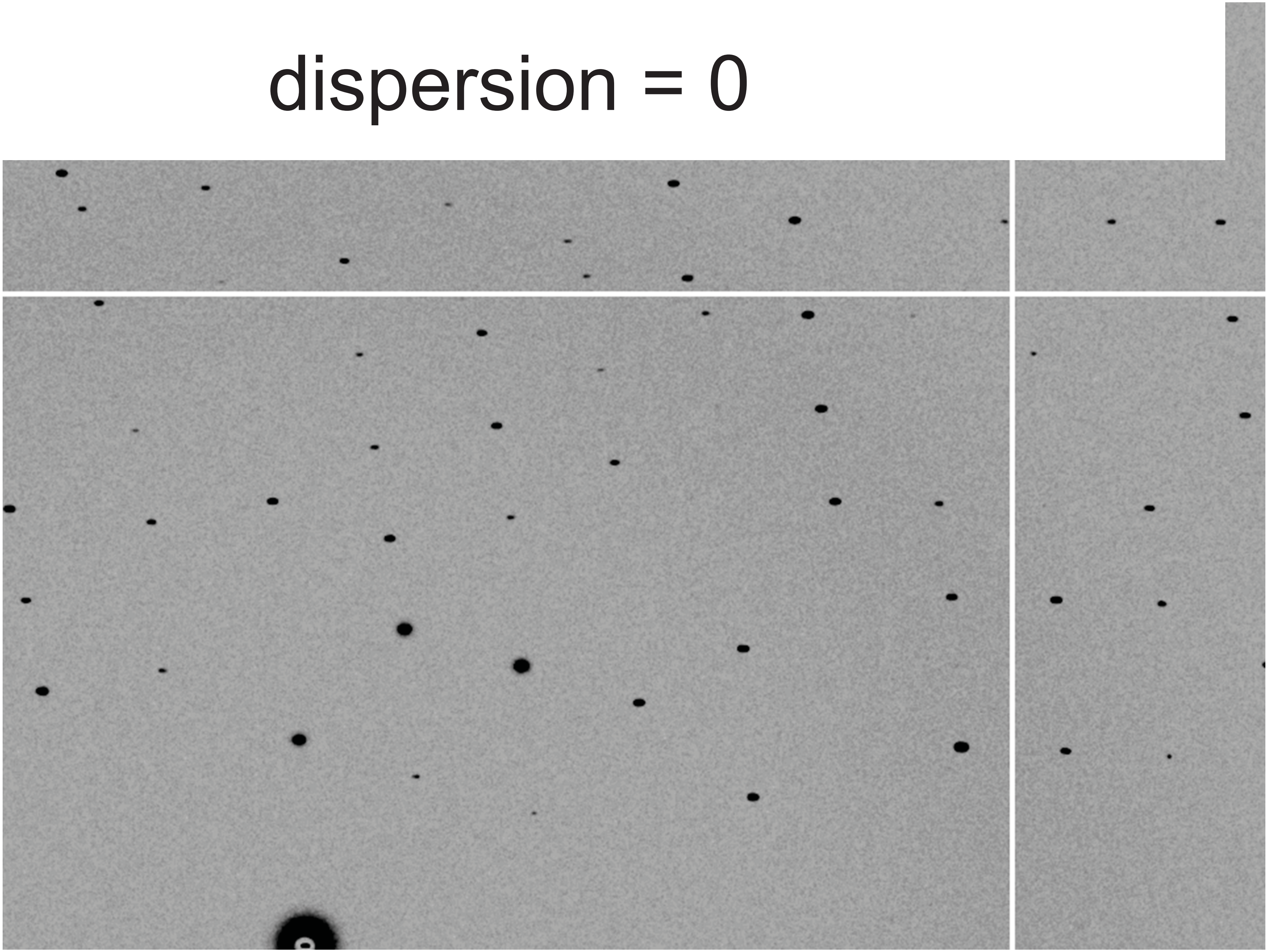
divergence = 0 º



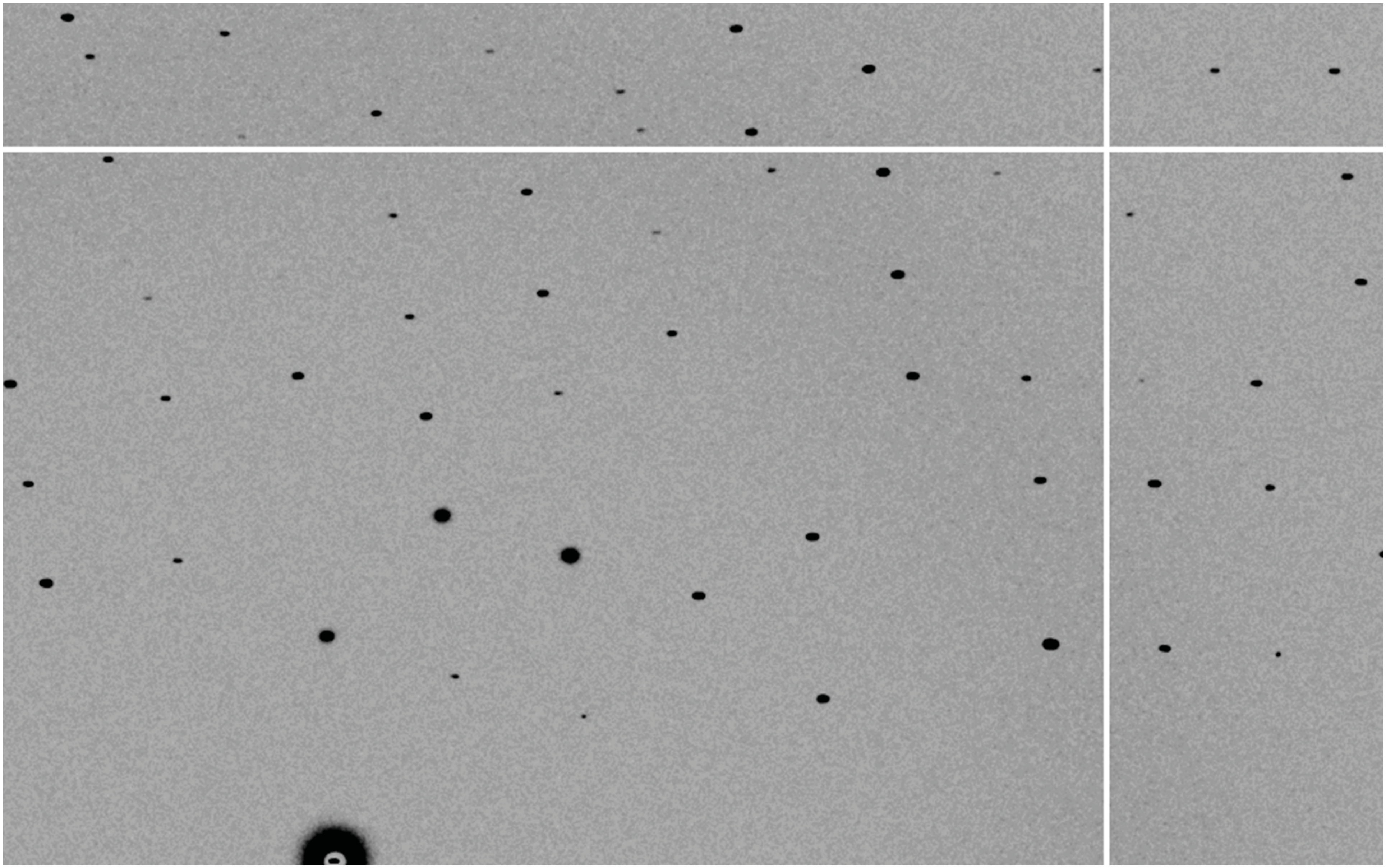
divergence = 0.3 º



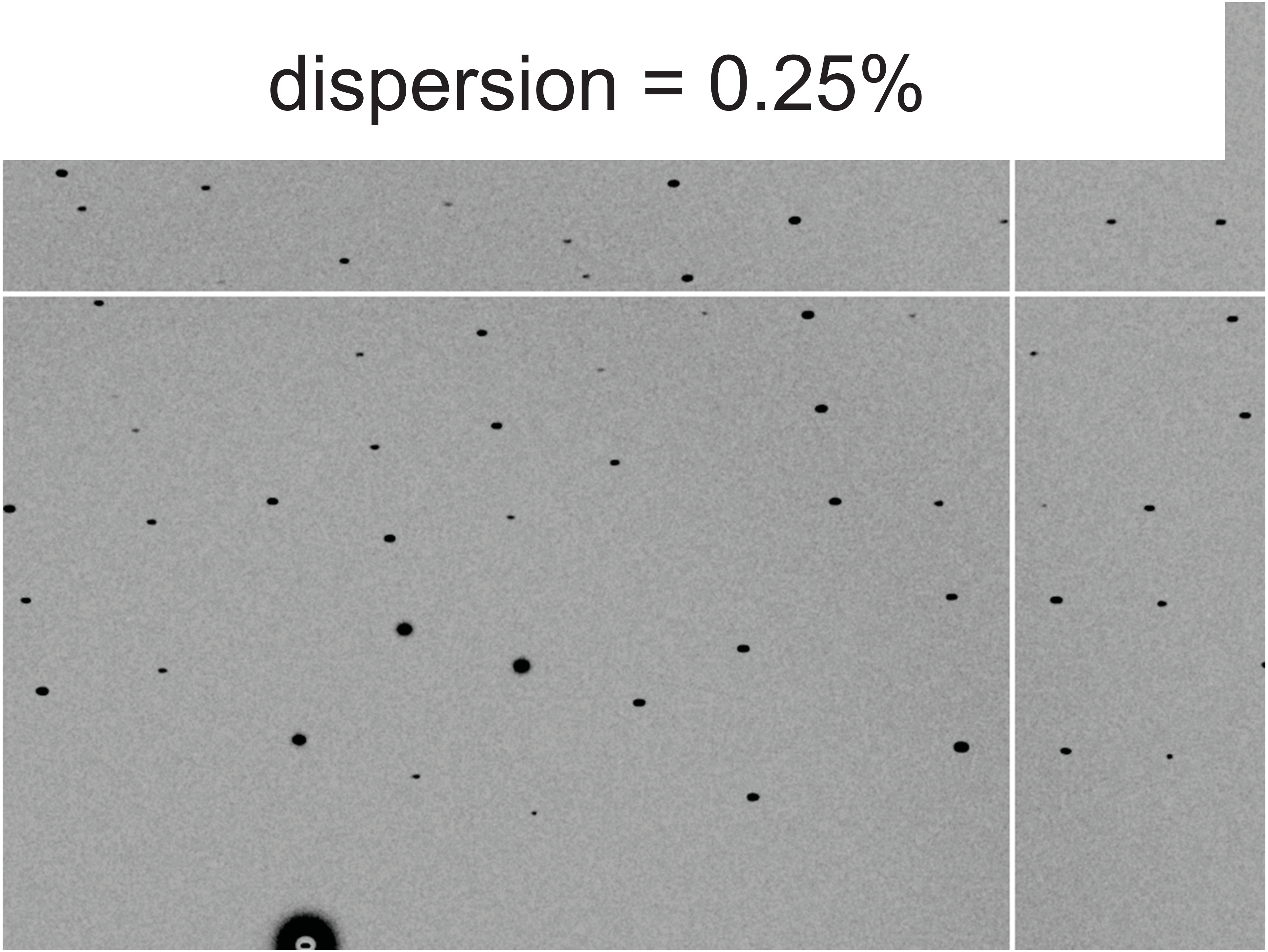
dispersion = 0



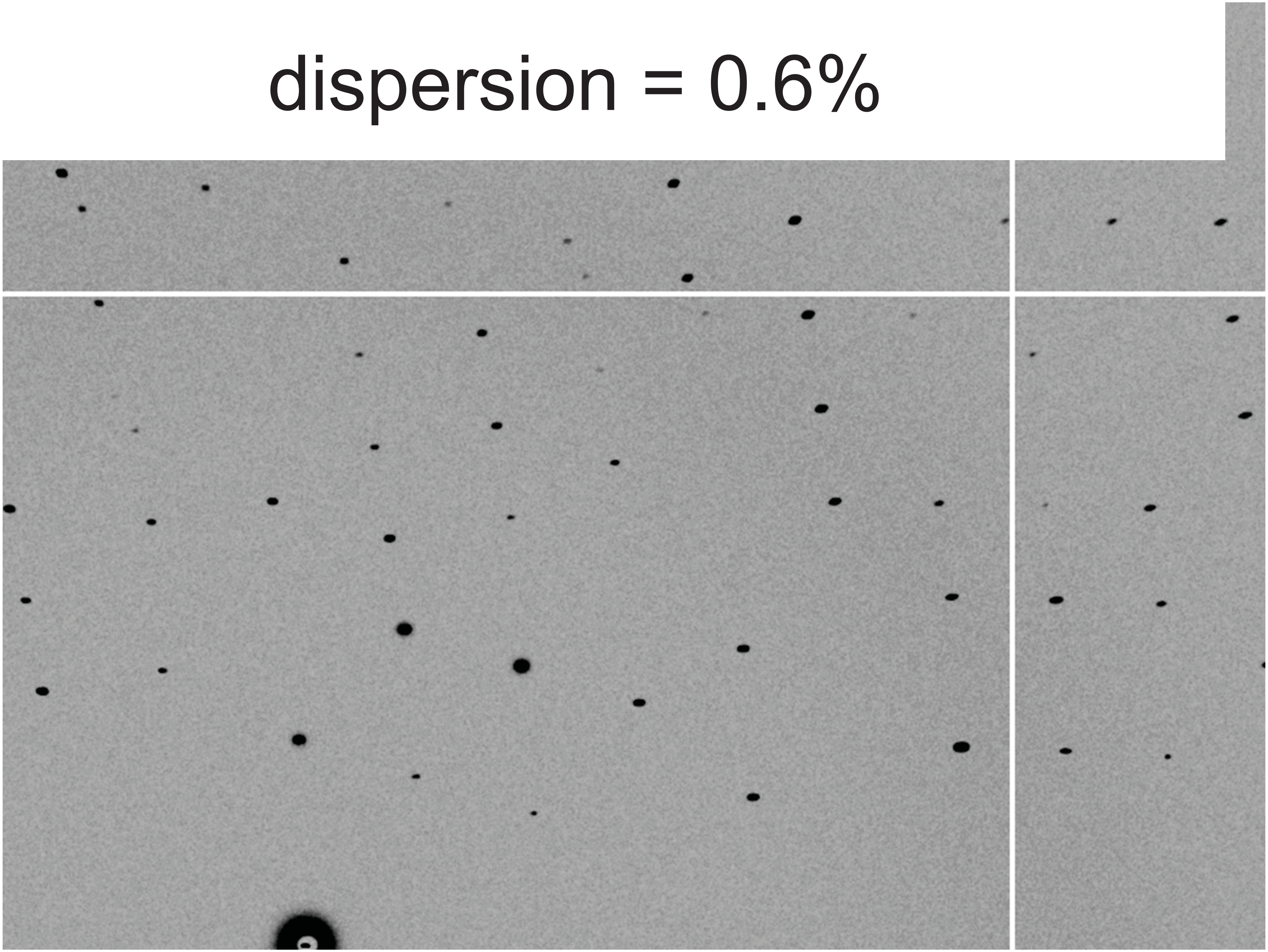
dispersion = 0.014%



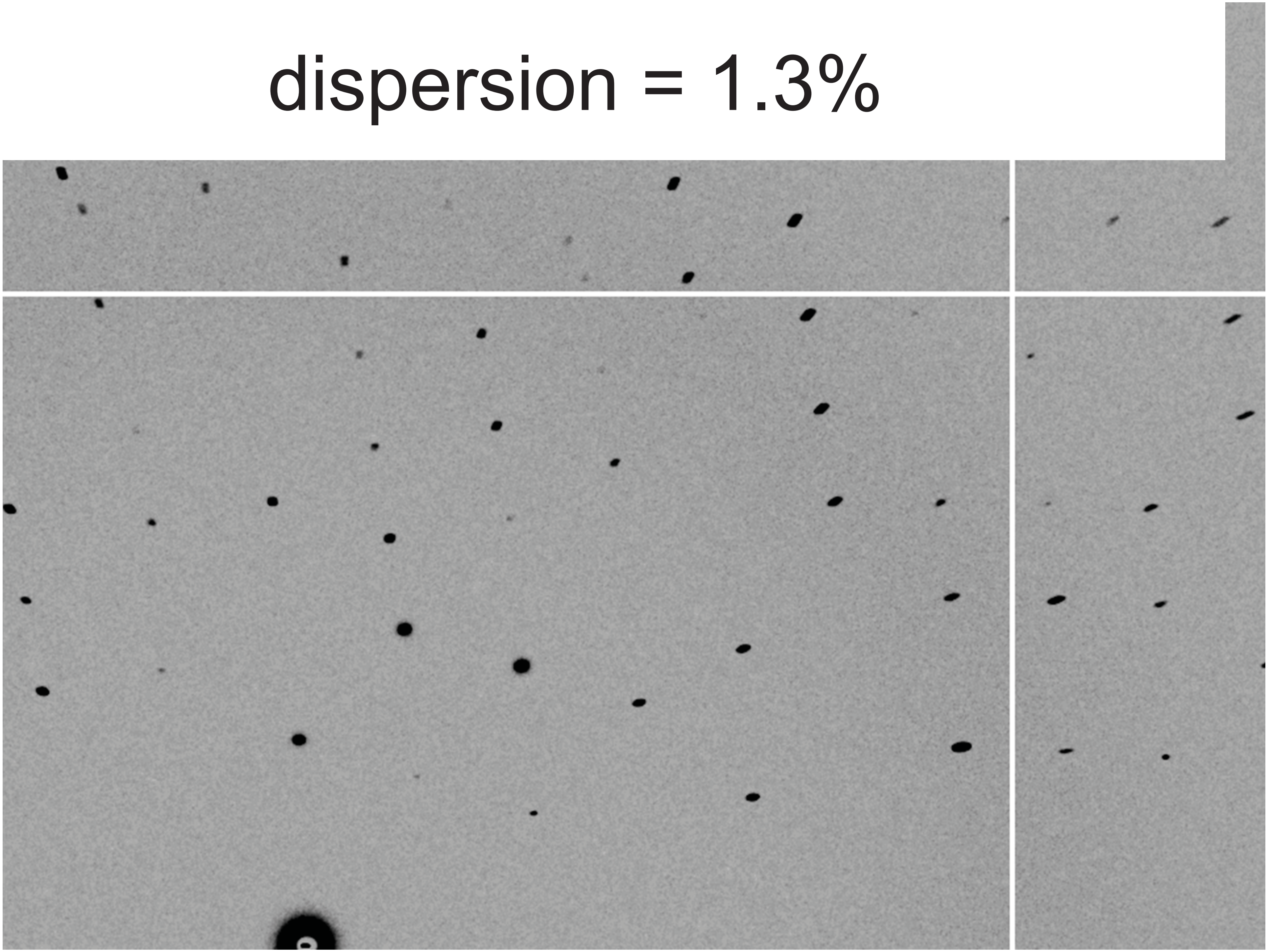
dispersion = 0.25%



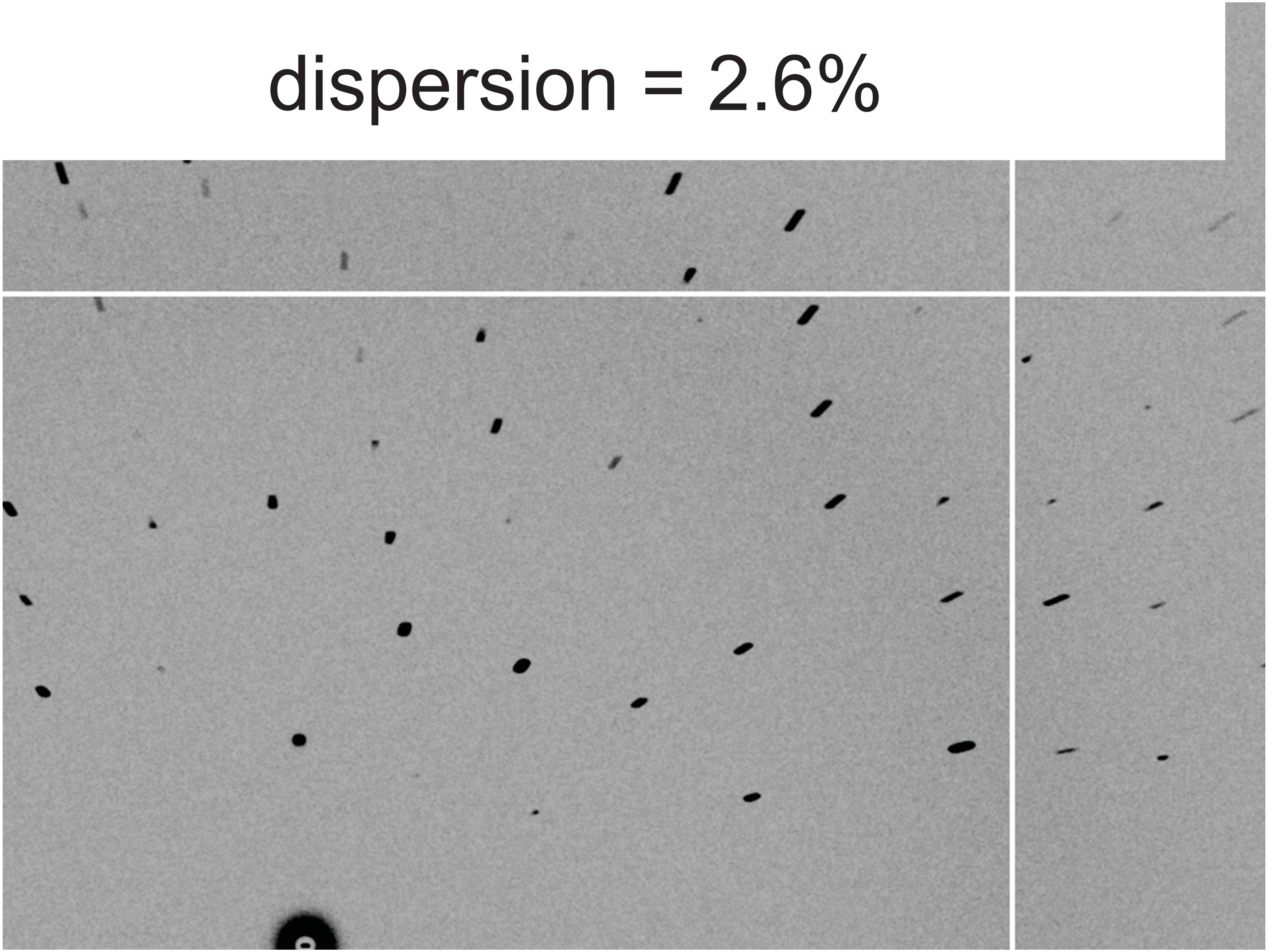
dispersion = 0.6%



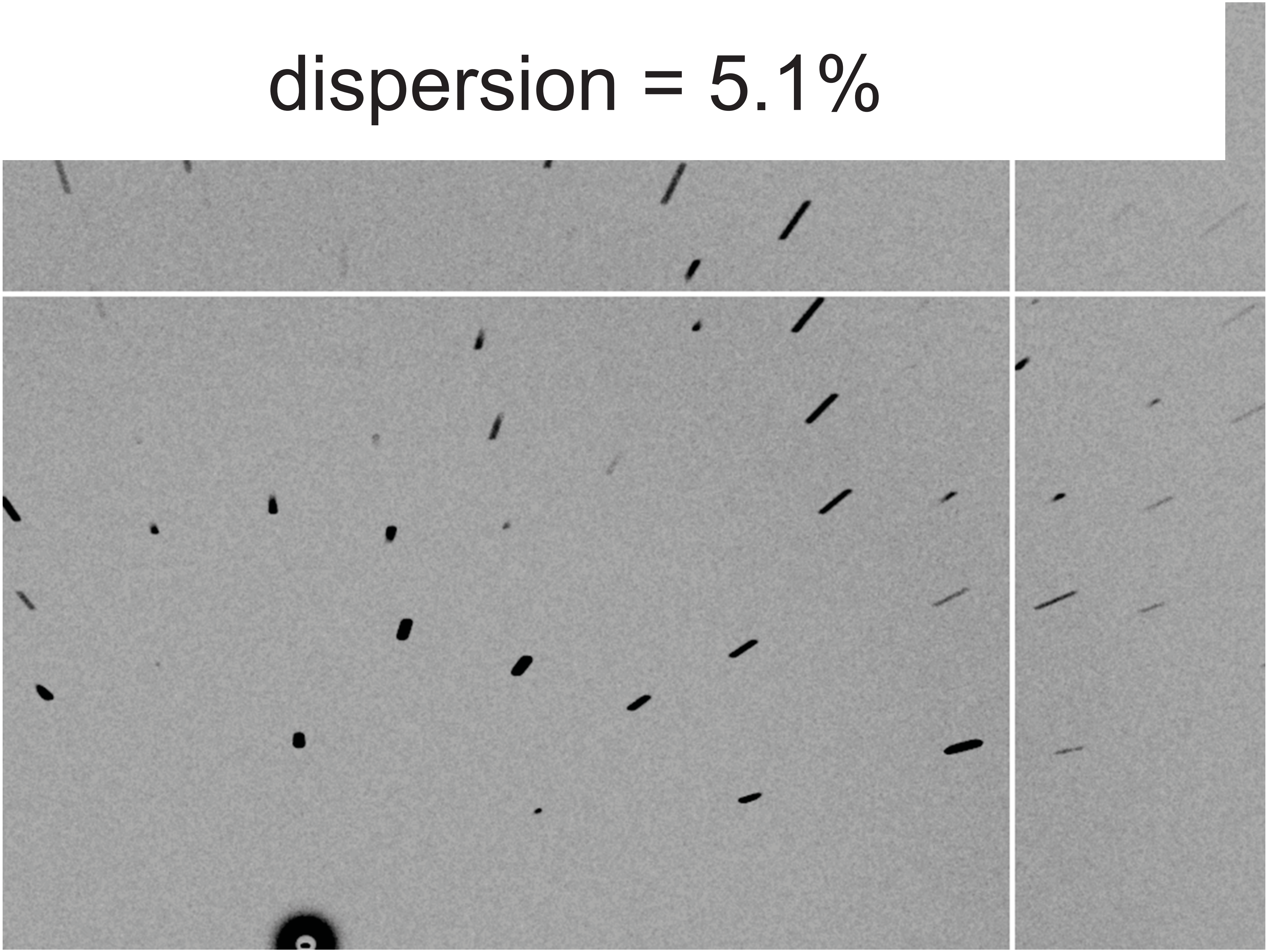
dispersion = 1.3%

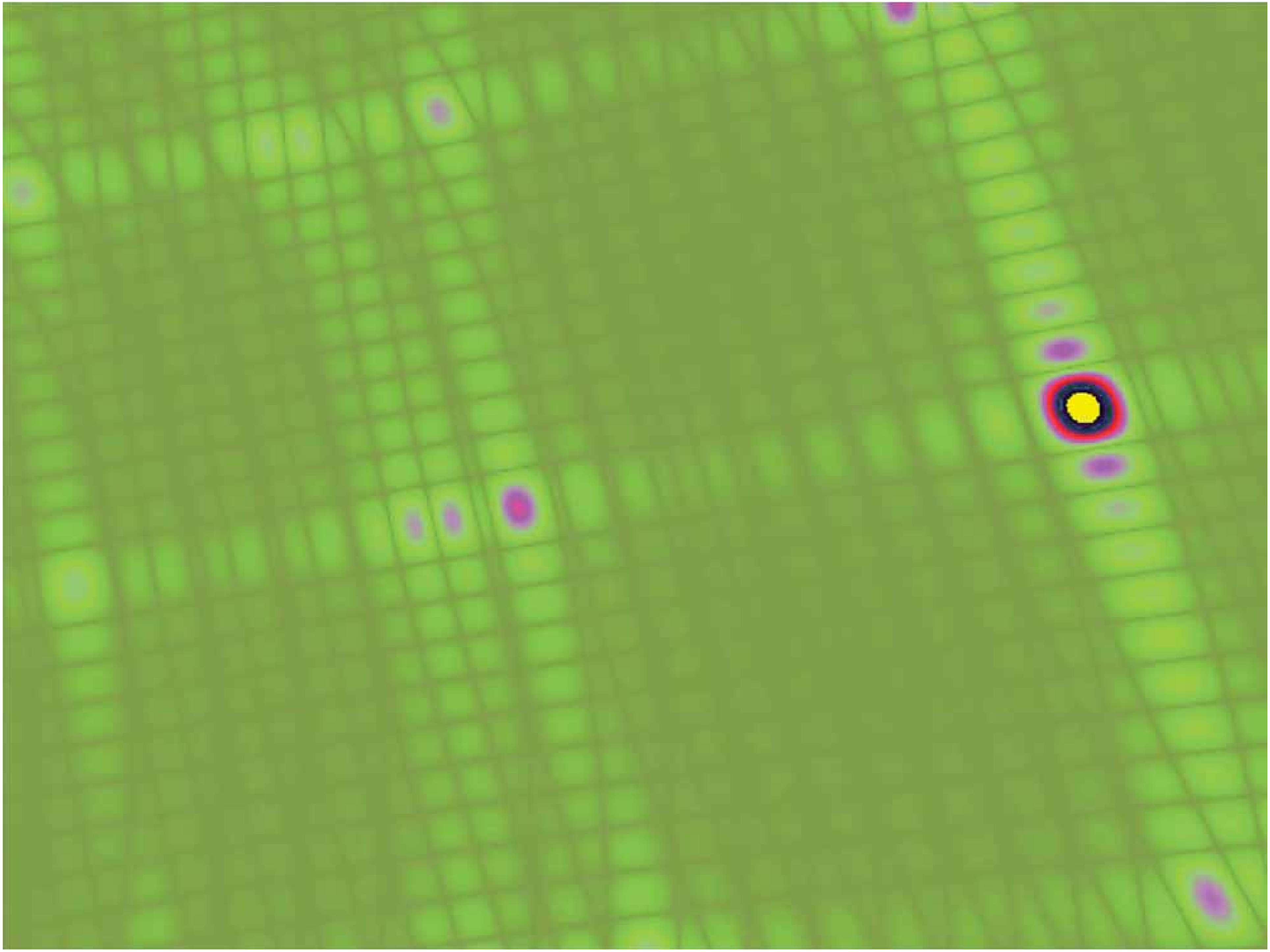


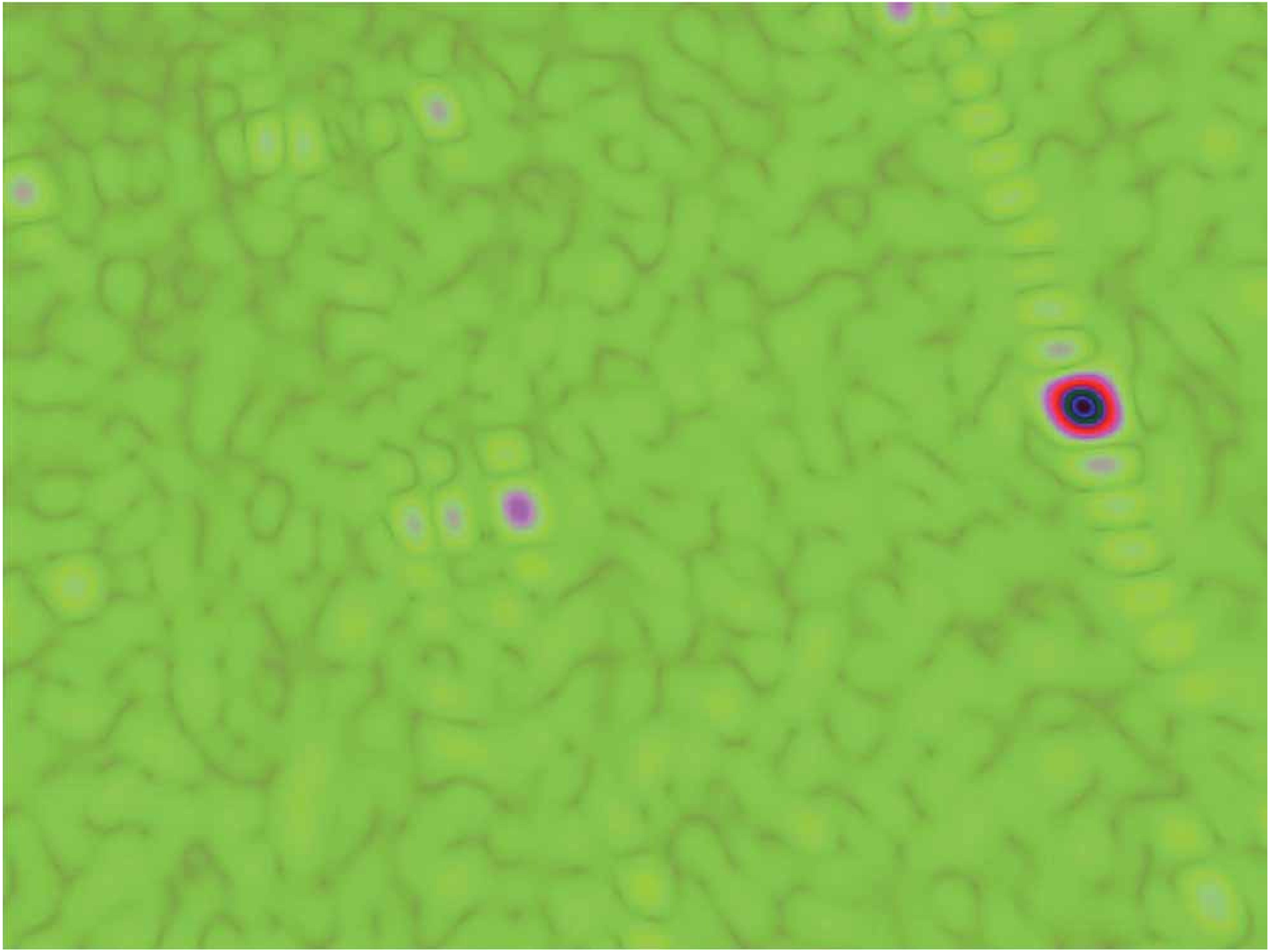
dispersion = 2.6%

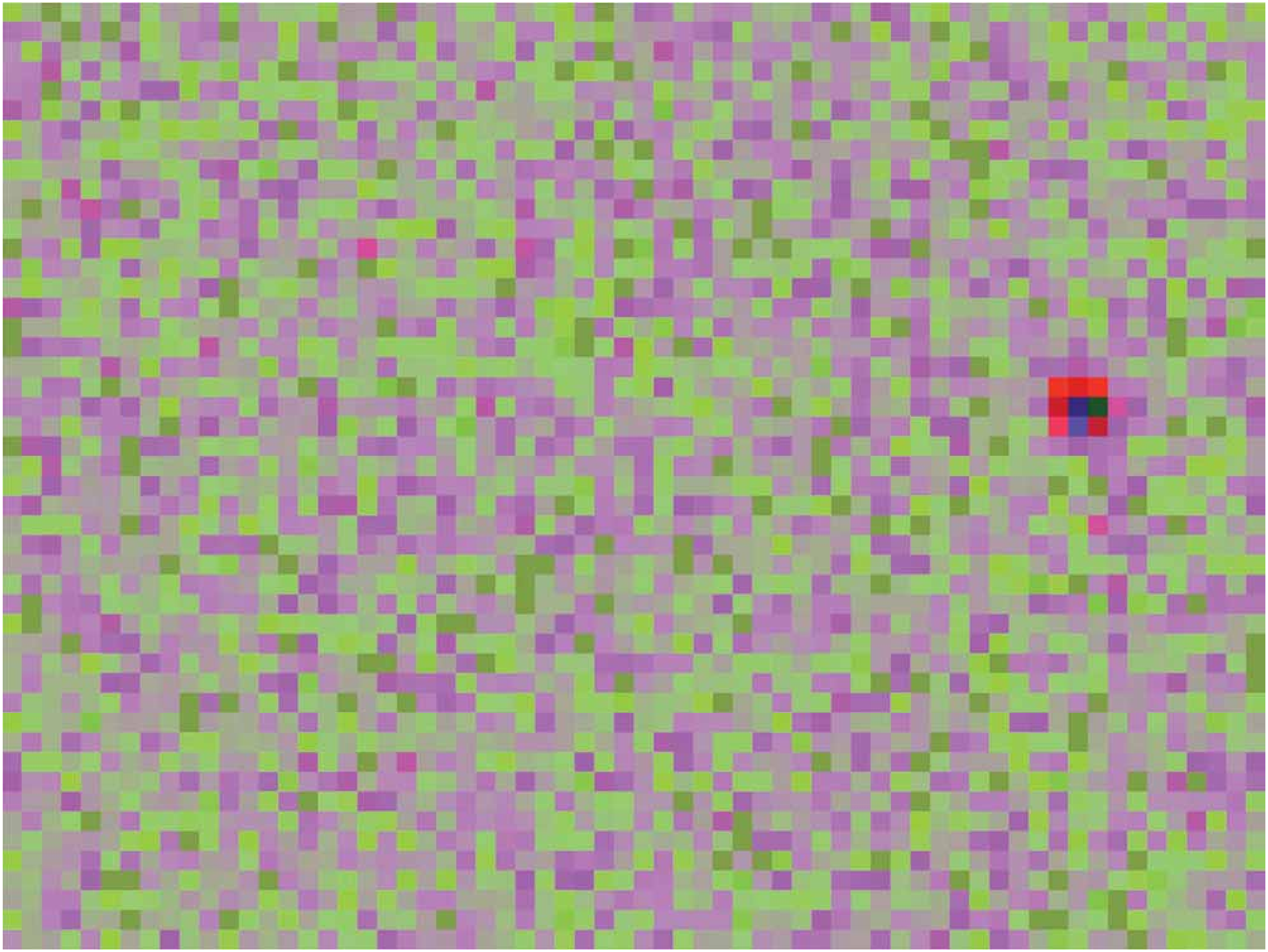


dispersion = 5.1%

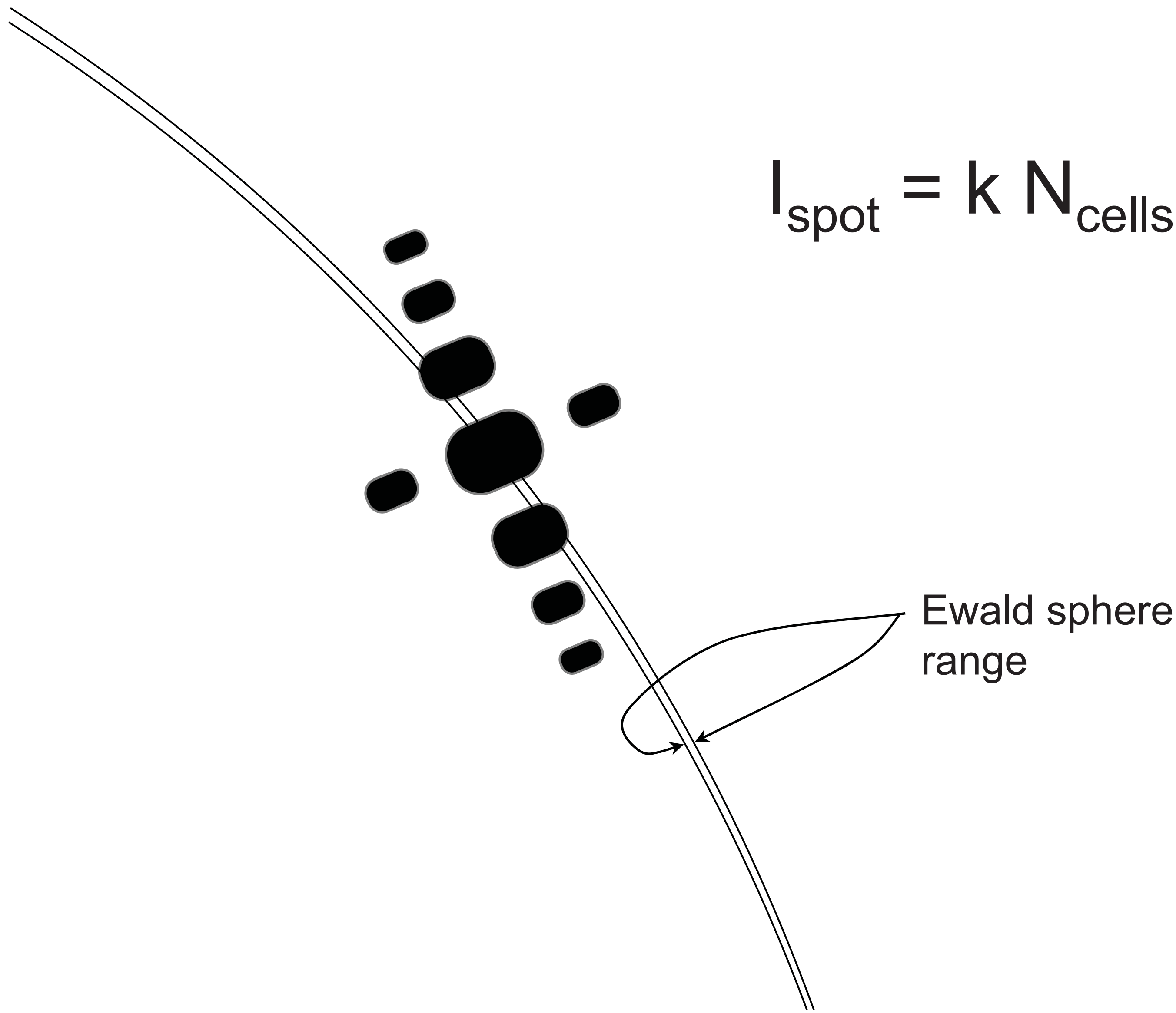






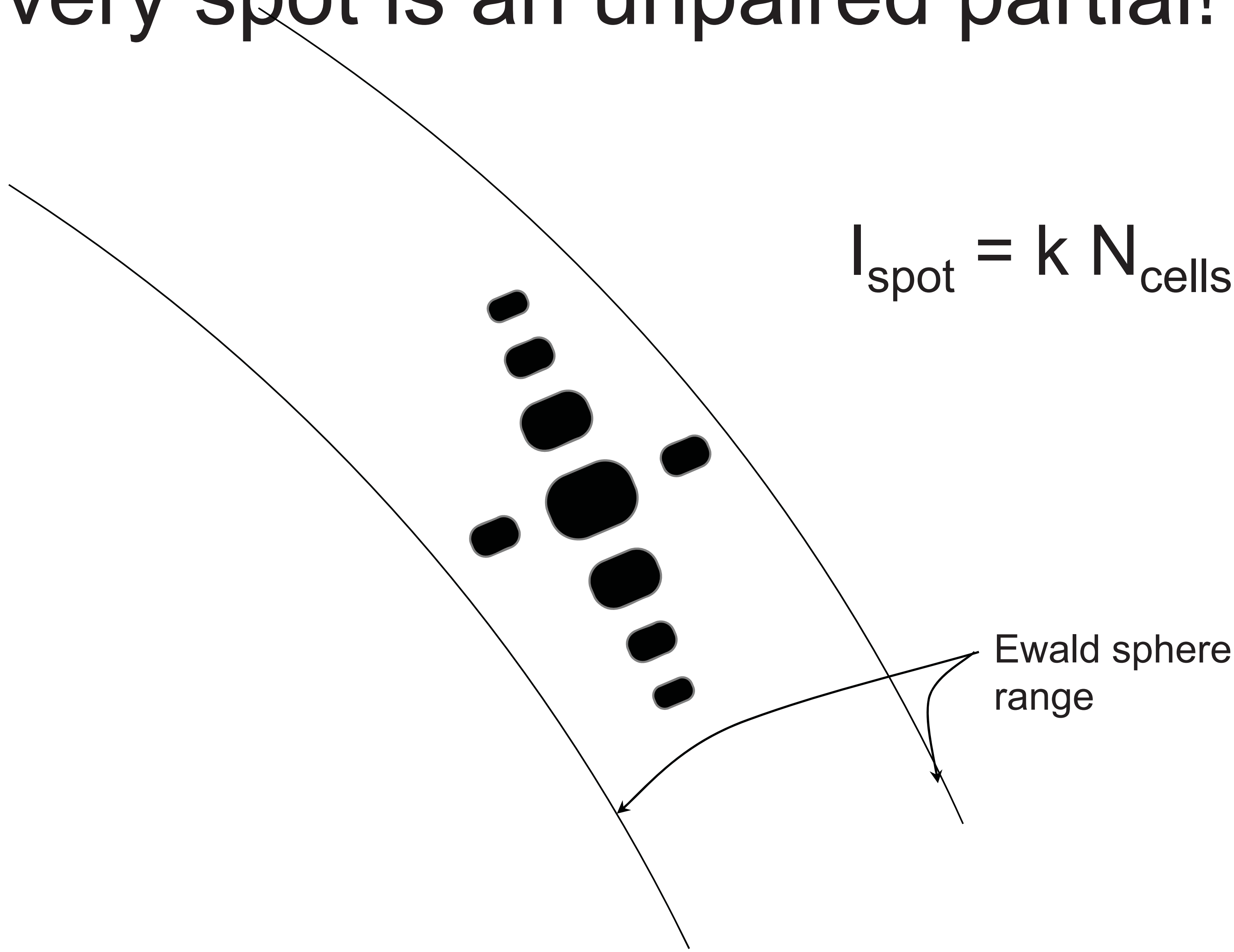


Every spot is an unpaired partial!



$$I_{spot} = k\, N_{cells}^2$$

Every spot is an unpaired partial!



$$I_{spot} = k\,N_{cells}^{1}$$

Woolfson, M. M. (1997). *An introduction to X-ray crystallography*. Ch. 2 & 6

Problems and Promises

- radiation damage
- non-isomorphism
- anomalous differences
- the “twin problem”
- postrefinement
- the structure of disorder

Internal consistency of data

1,850,000 images

112,000 > 10 peaks

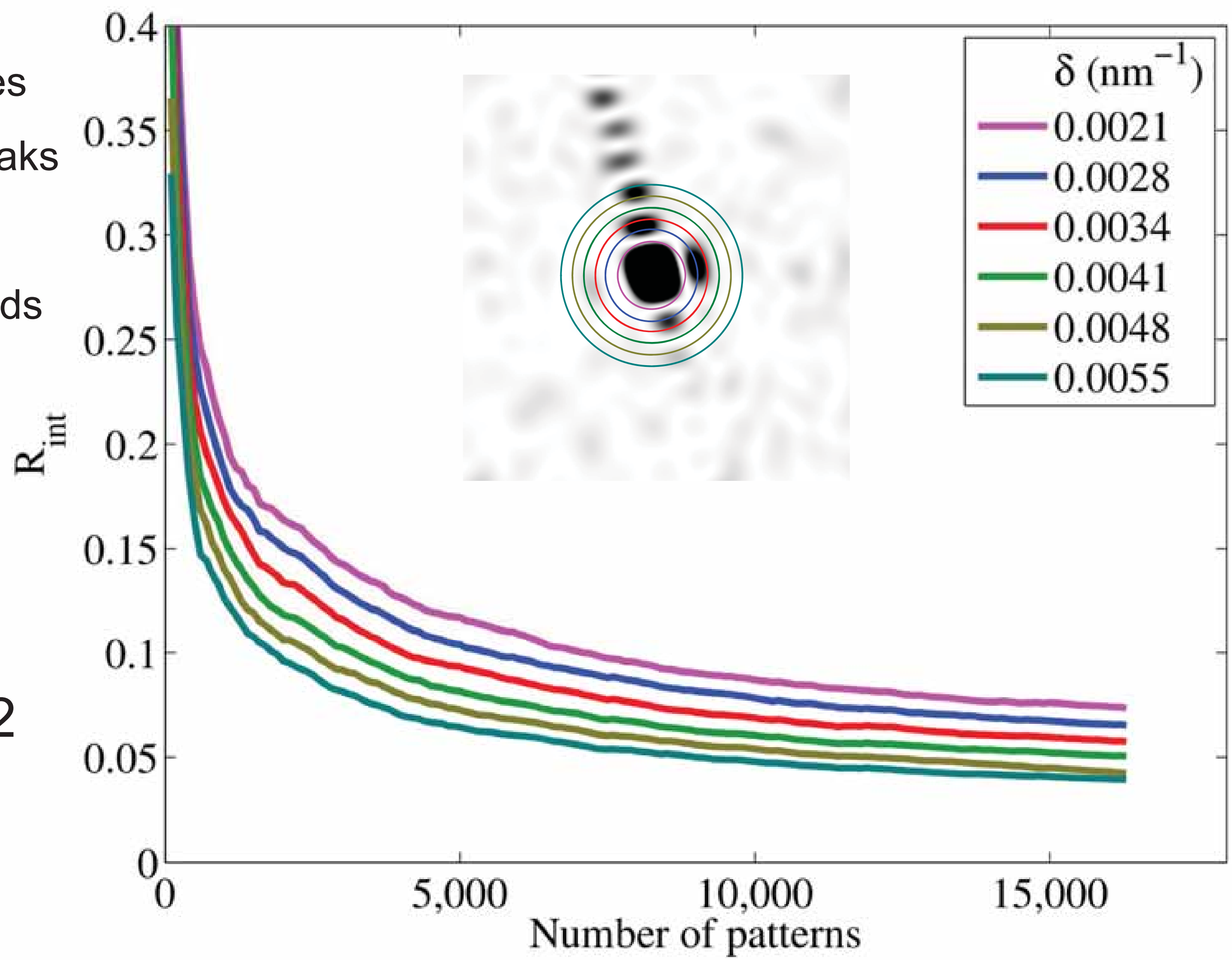
33,000 indexed

16,500 good preds

rigid-body:

$R_{cryst} = 0.252$

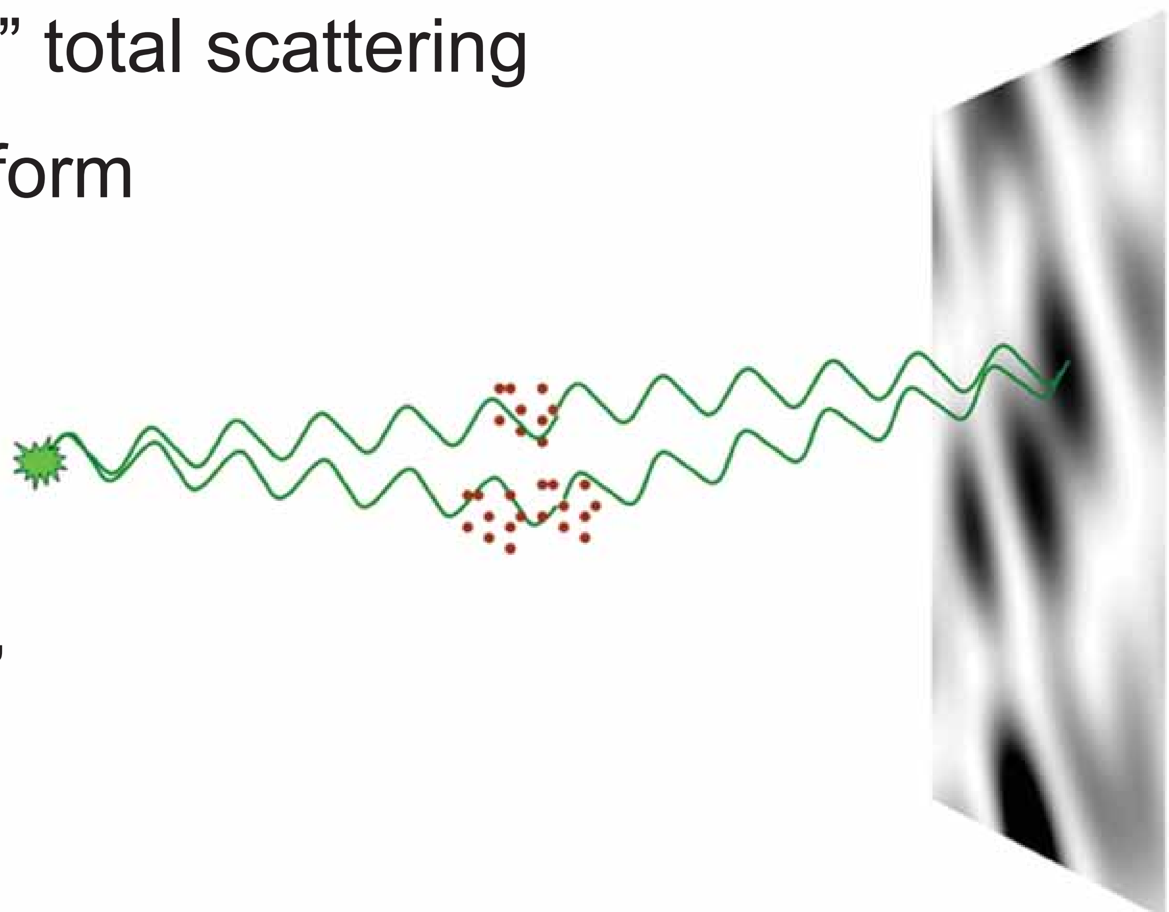
$R_{free} = 0.232$



nearBragg program

http://bl831.als.lbl.gov/~jamesh/nearBragg/

- "assumption-free" total scattering
- no Fourier Transform
- no unit cells
- no "mosaicity"
- arbitrary "atoms"
- arbitrary "source"
- coherent or not



fastBragg program

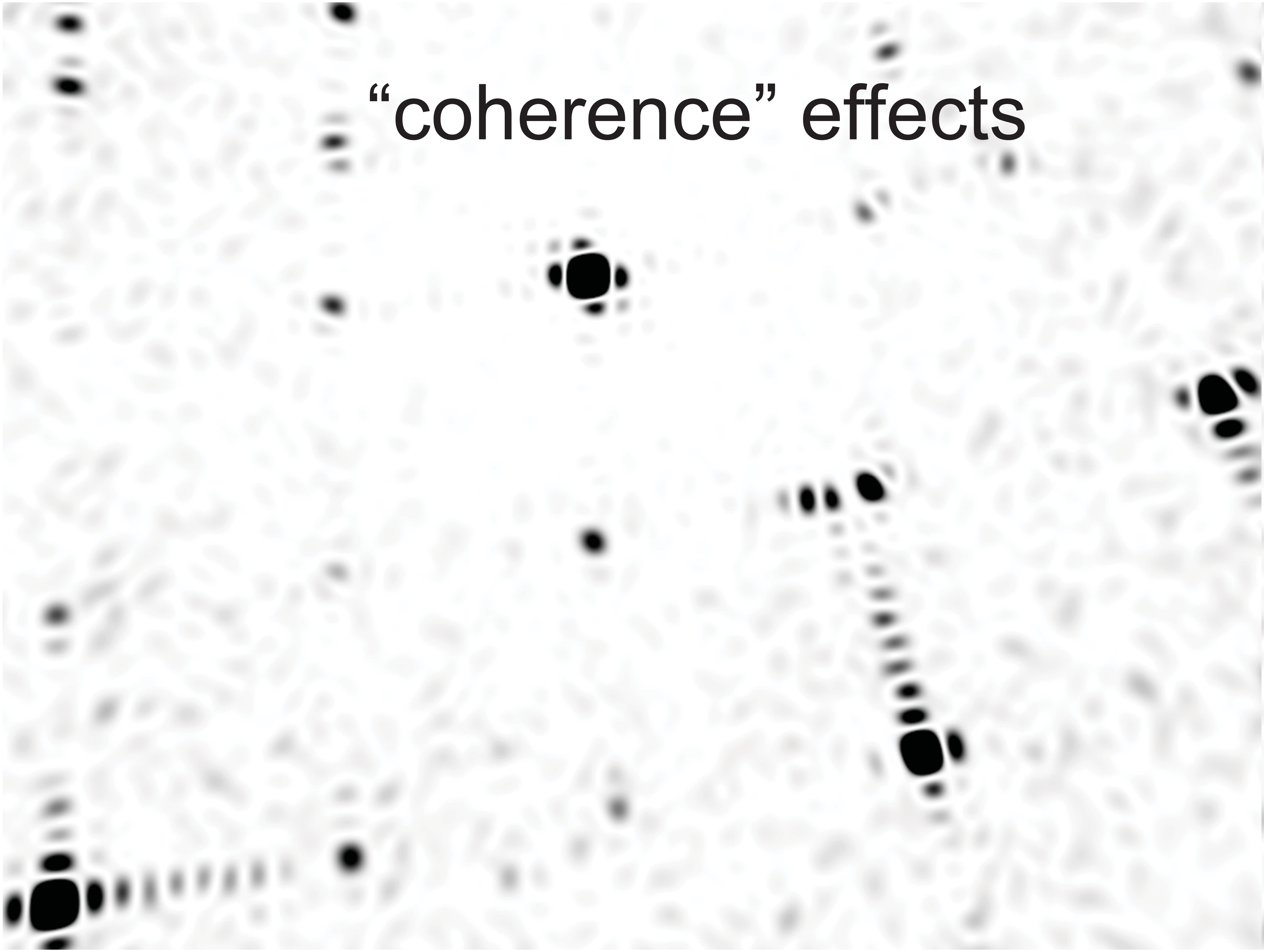
`http://bl831.als.lbl.gov/~jamesh/fastBragg/`

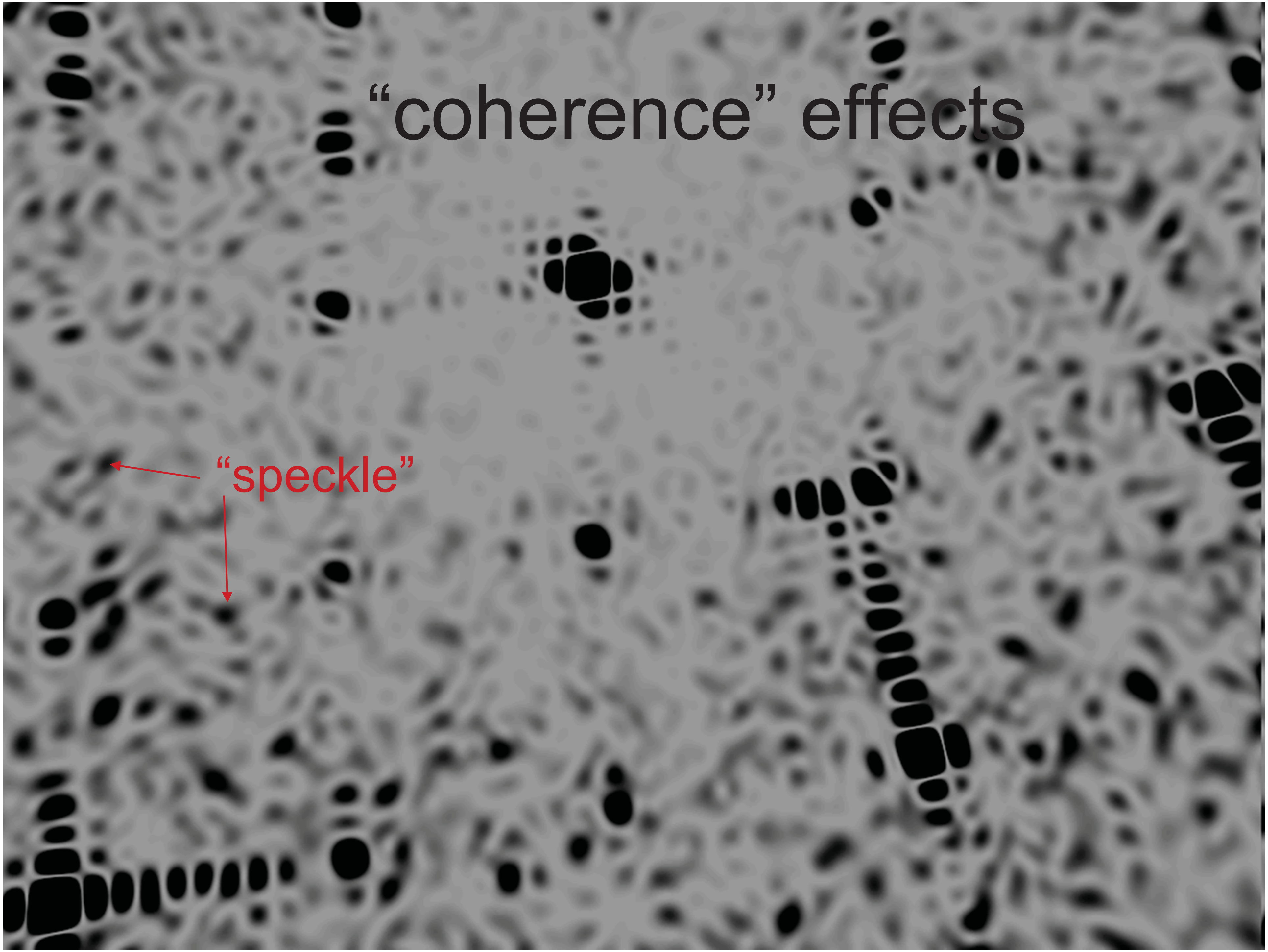
- “total scattering
- Fourier Transform
- unit cells
- no “mosaicity”
- structure factors

Problems and Promises

- radiation damage
- non-isomorphism
- anomalous differences
- the “twin problem”
- postrefinement
- the structure of disorder

“coherence” effects




“coherence” effects
“speckle”

lysozyme: real and reciprocal