Acknowledgements

Pavol Janowski David Cerutti David Case
Jaime Fraser Terry Lang Ho Leung-Ng

8.3.1 creator: Tom Alber
8.3.1 PRT head: Jamie Cate

Center for Structure of Membrane Proteins
Membrane Protein Expression Center II
Center for HIV Accessory and Regulatory Complexes

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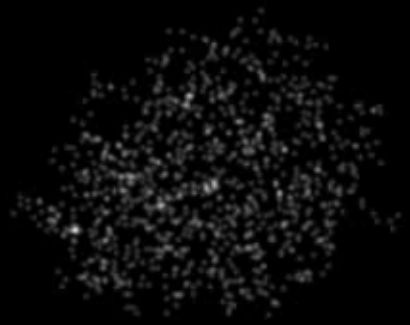
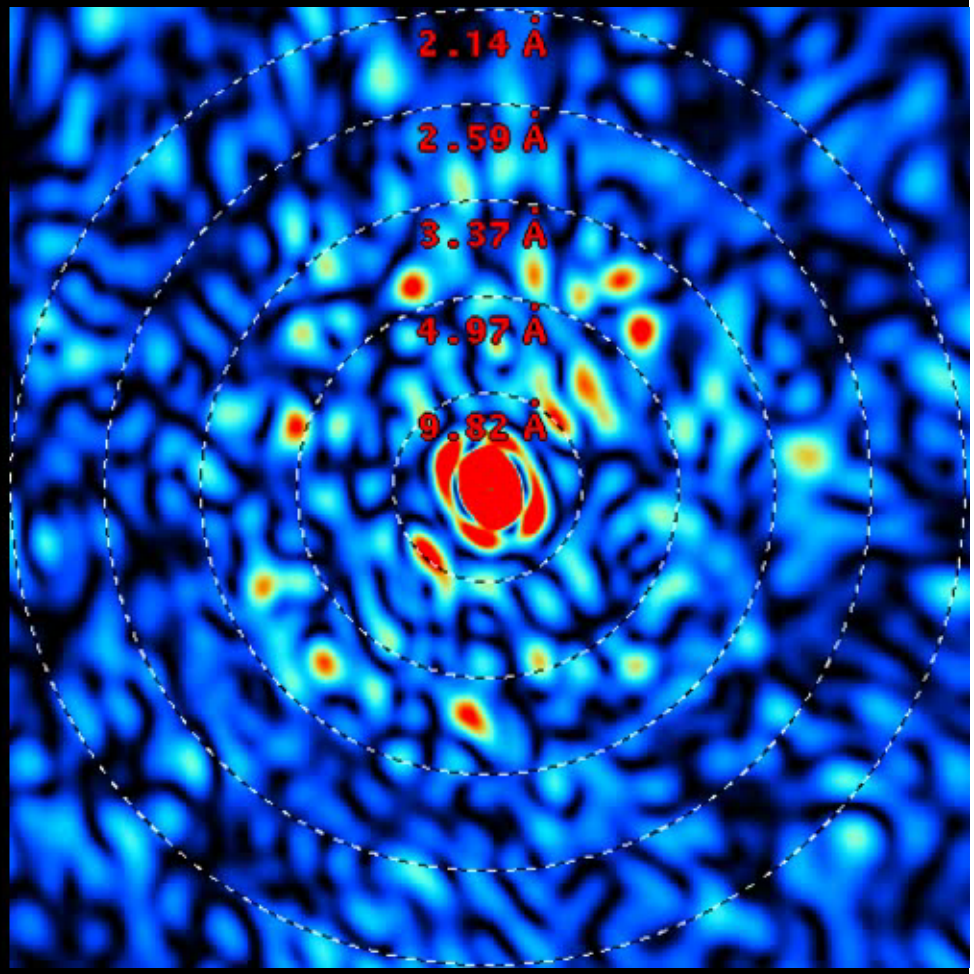
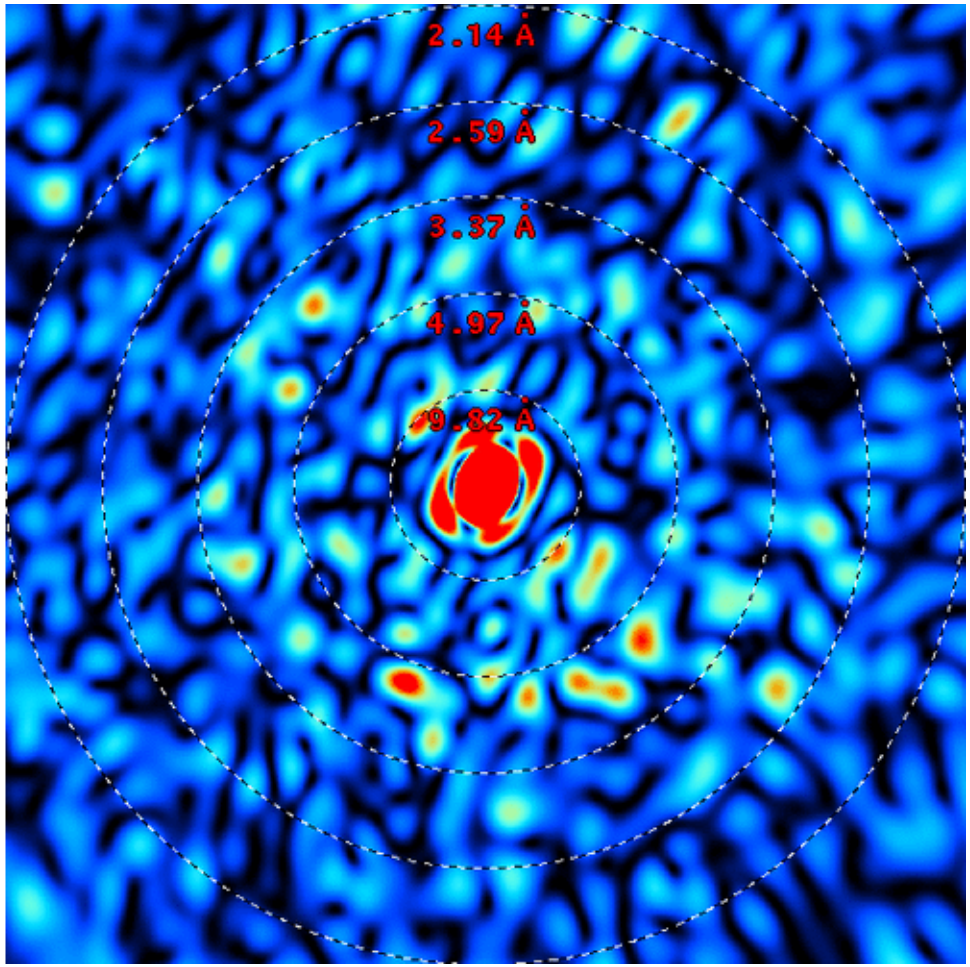
lysozyme: real and reciprocal

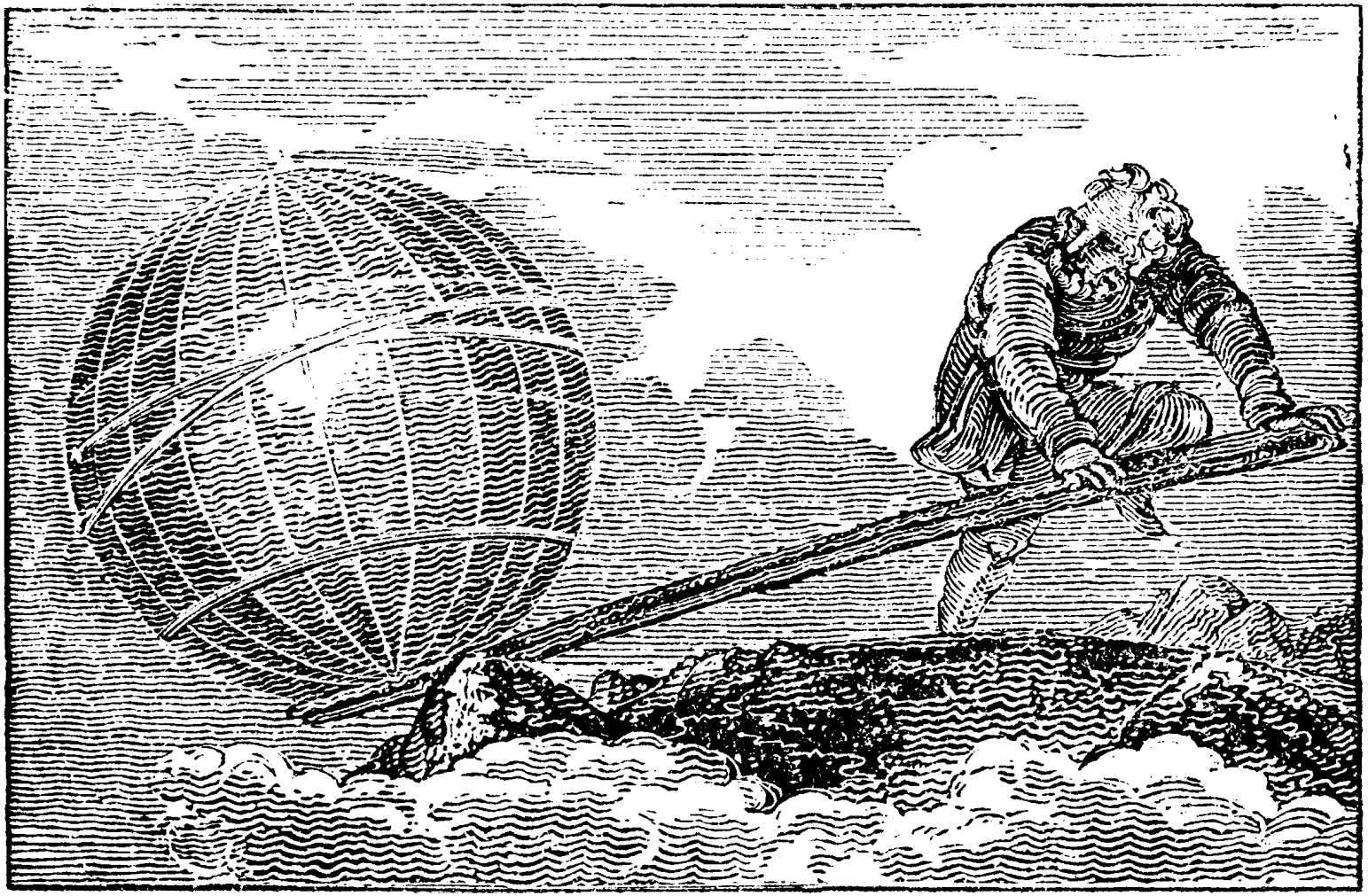




lysozyme: thermal motion



Anything is possible … with the right tools.

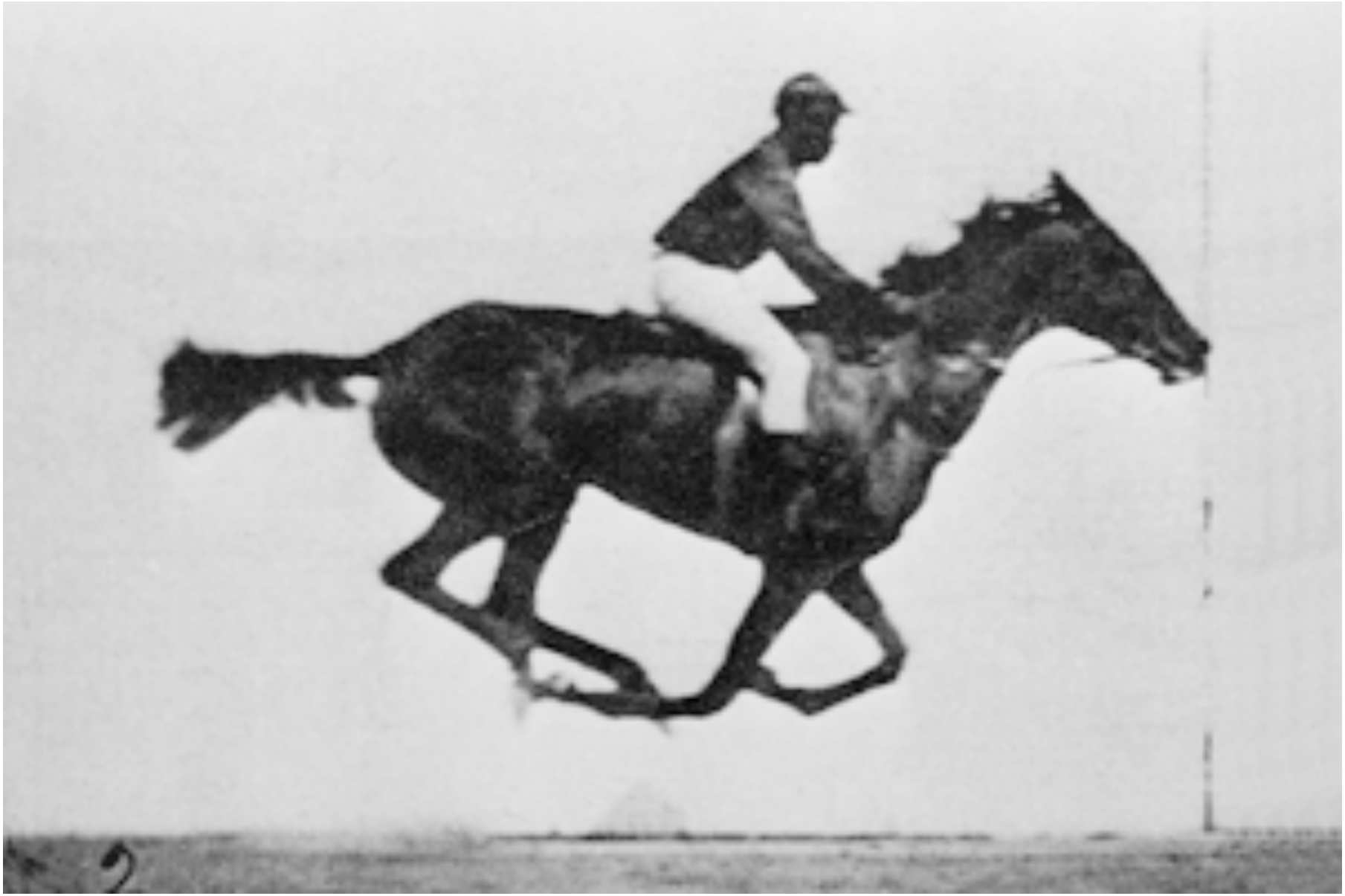


AD 1872

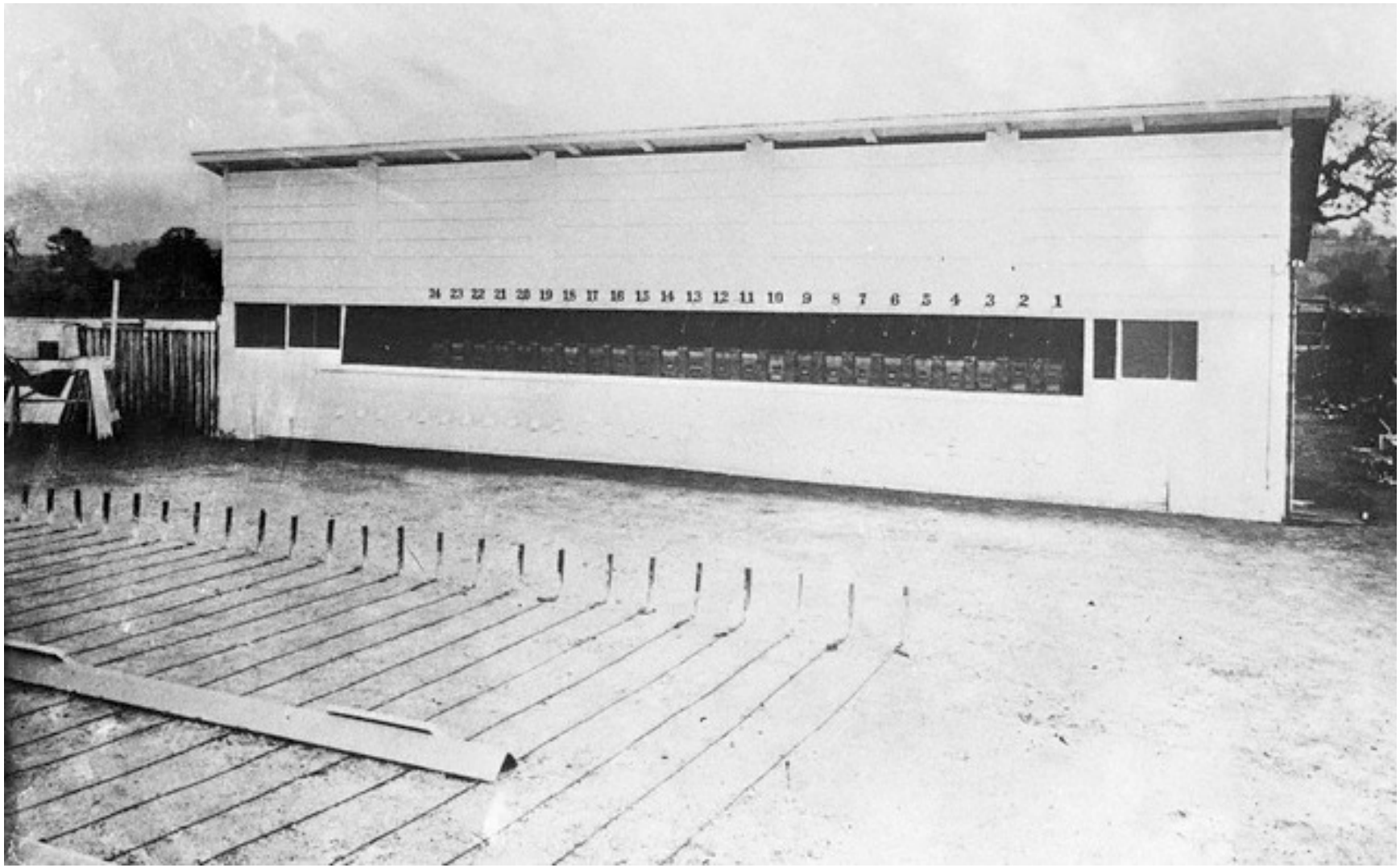
Big Question:

When a horse gallops, is there ever a moment where all four hooves leave the ground?

Muybridge’s galloping horse (1878)

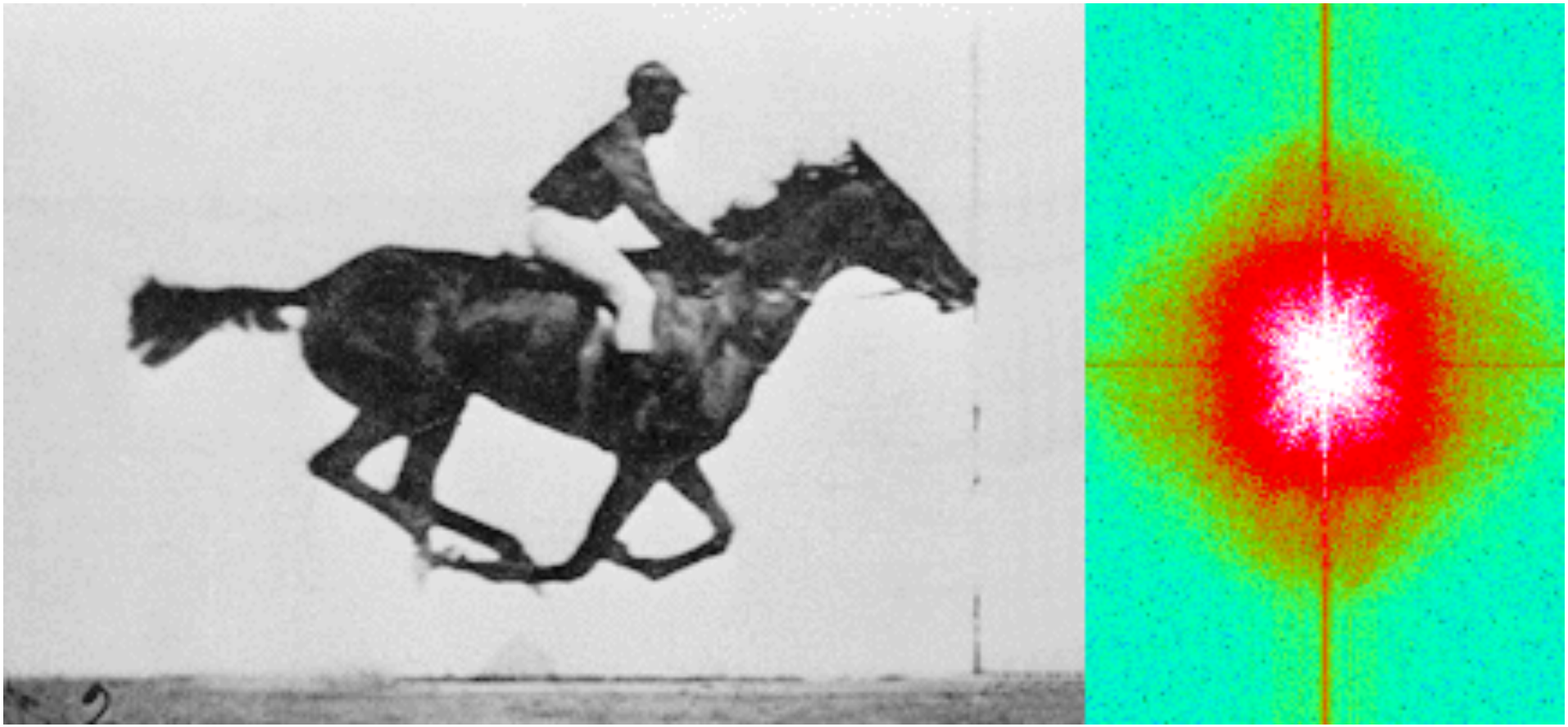


Muybridge’s multi-camera



Muybridge's galloping horse (1878)

"Time-resolved" diffraction

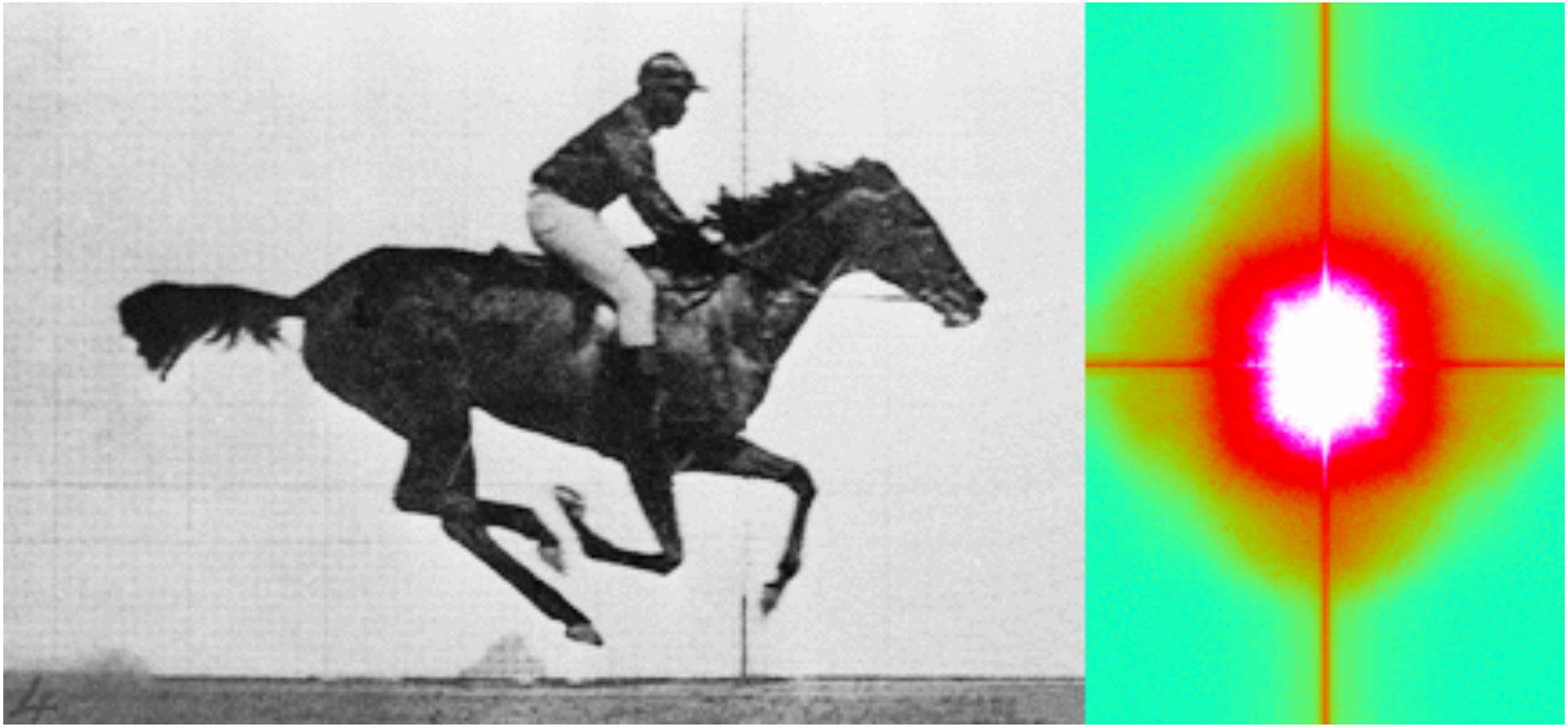


Real space

reciprocal

Muybridge’s galloping horse (1878)

Average intensity

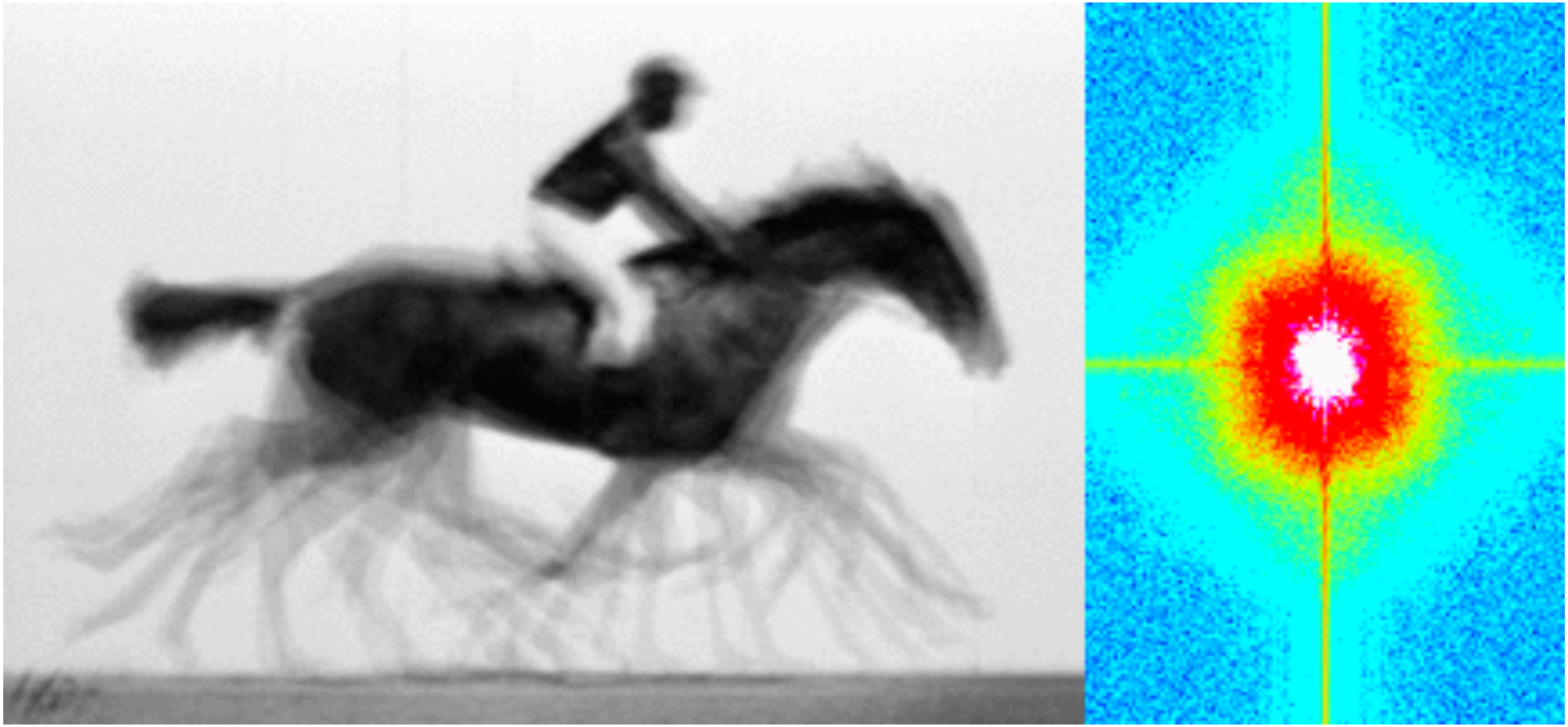


Real space

reciprocal

Muybridge’s galloping horse (1878)

Average electron density

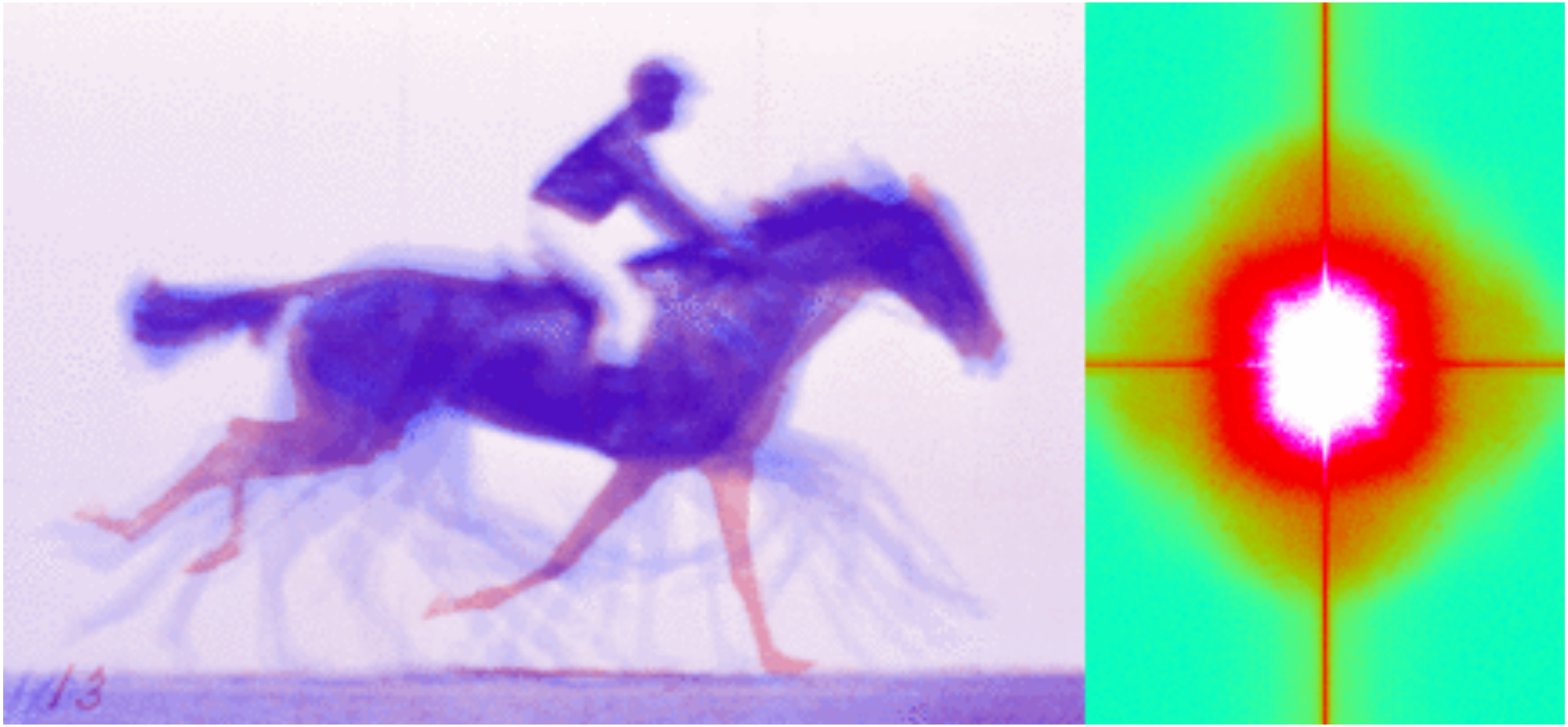


Real space

reciprocal

Muybridge’s galloping horse (1878)

Sum(intensity) – Sum(density) = diffuse scatter

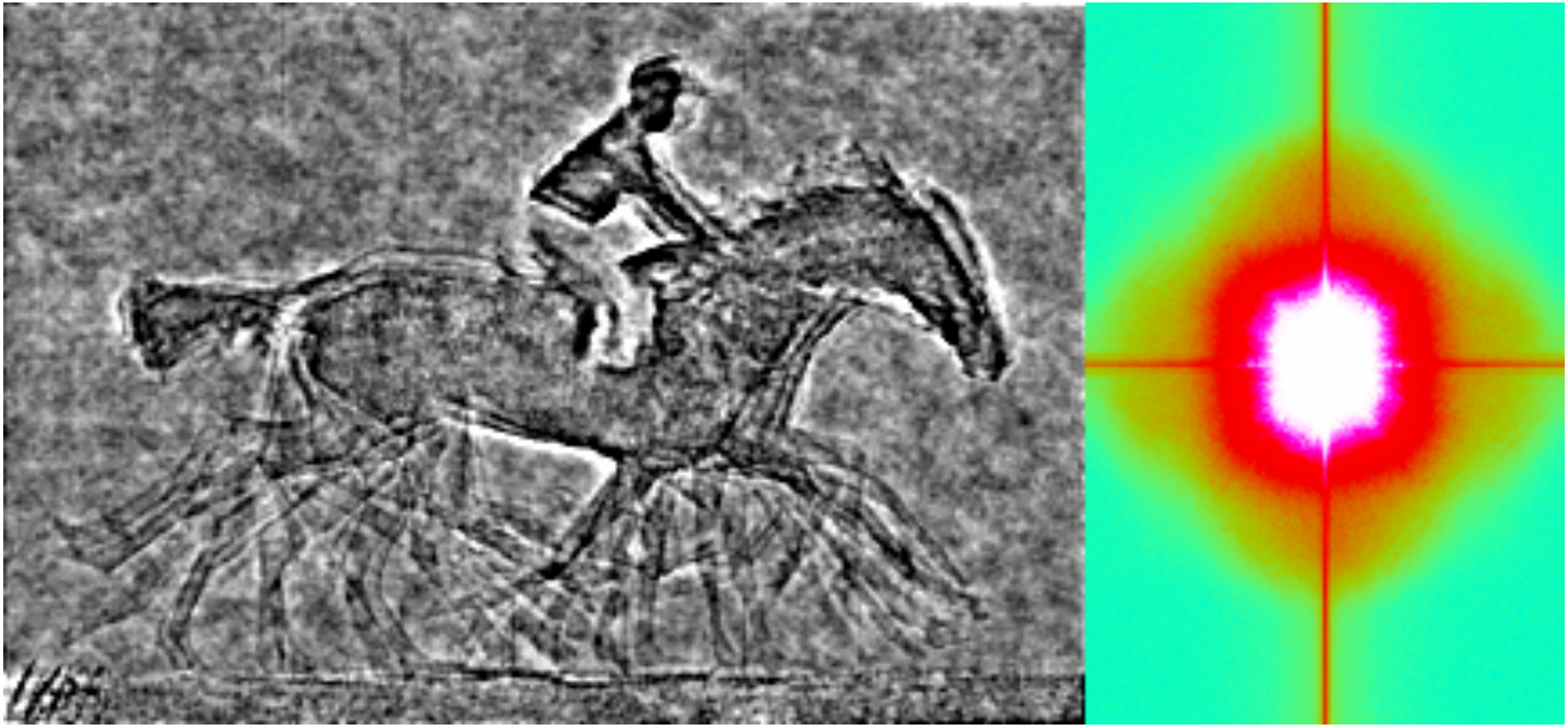


Real space

reciprocal

Muybridge's galloping horse (1878)

$F_{incoh} - F_{coherent}$ with density phases

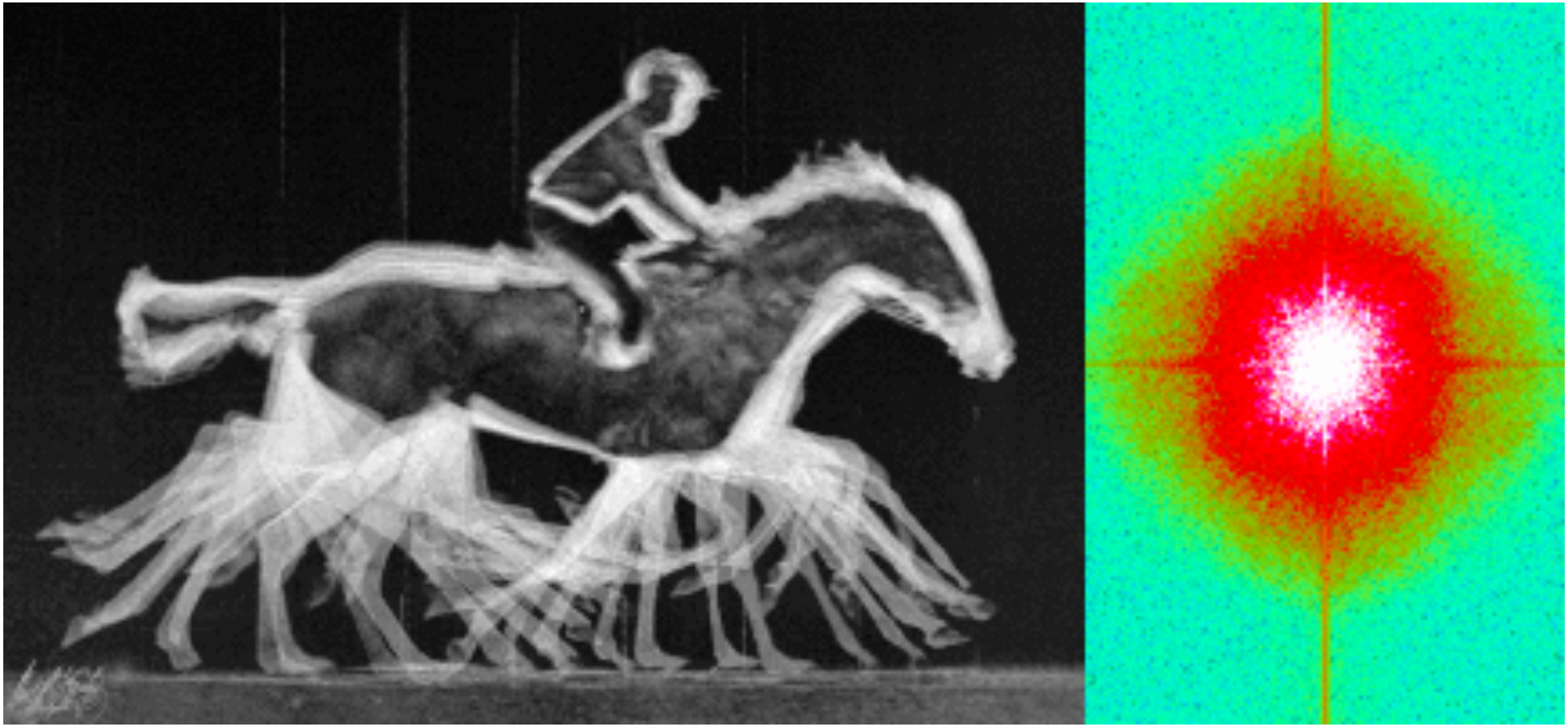


Real space

reciprocal

Muybridge’s galloping horse (1878)

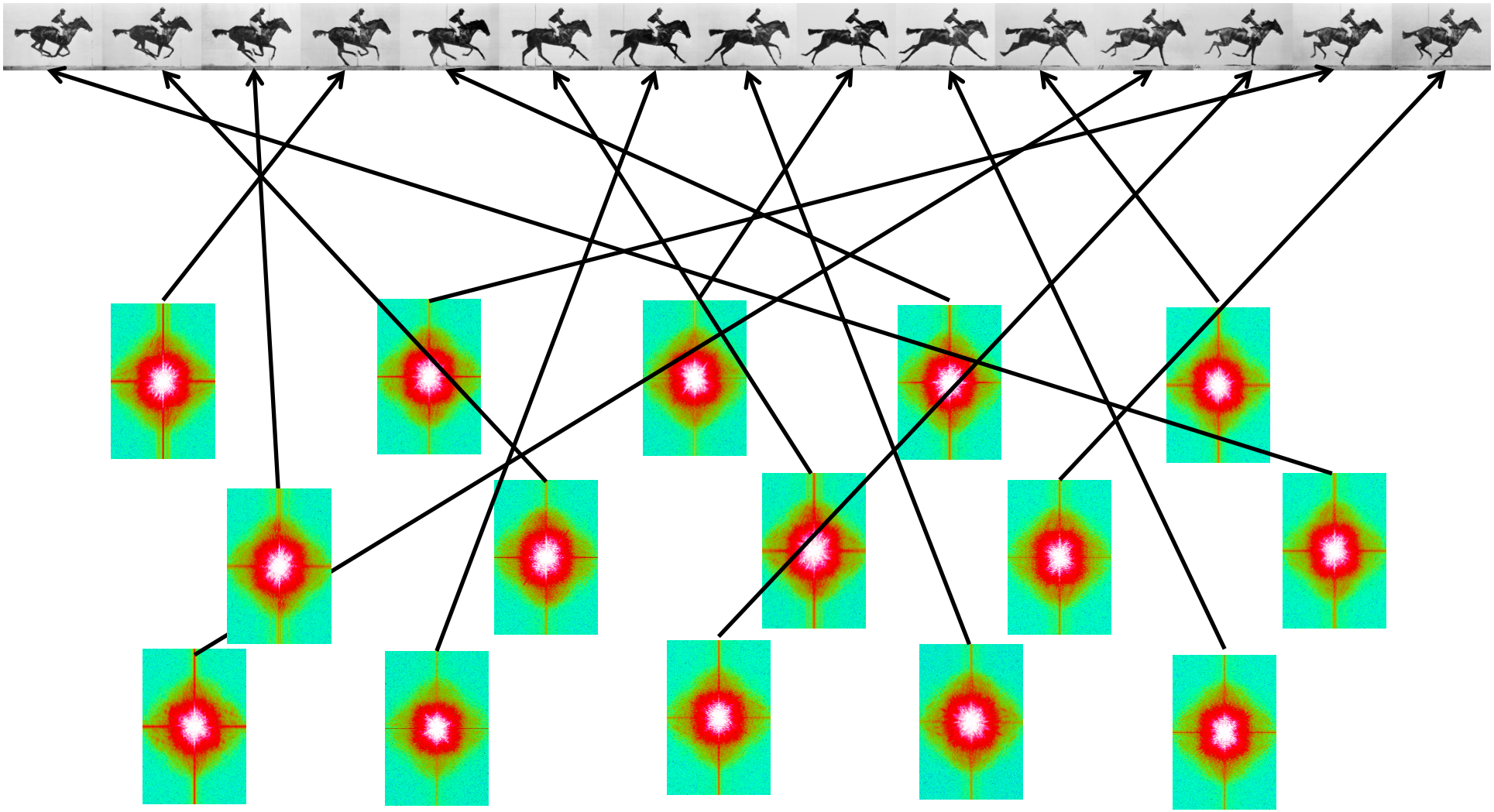
RMS variation in density



Real space

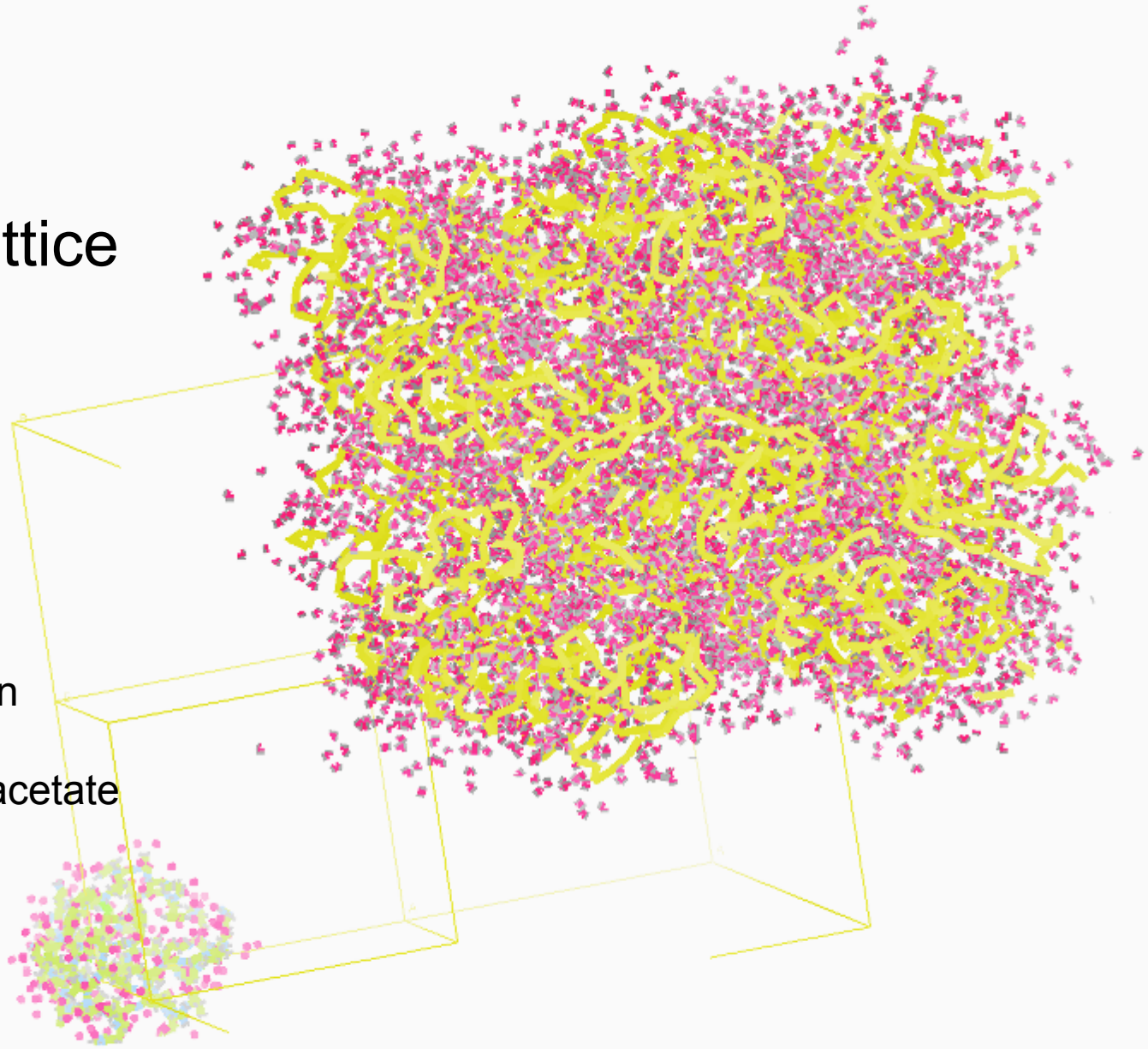
reciprocal

Supporting a model with data



Molecular Dynamics Simulation

using **real**
crystal's lattice



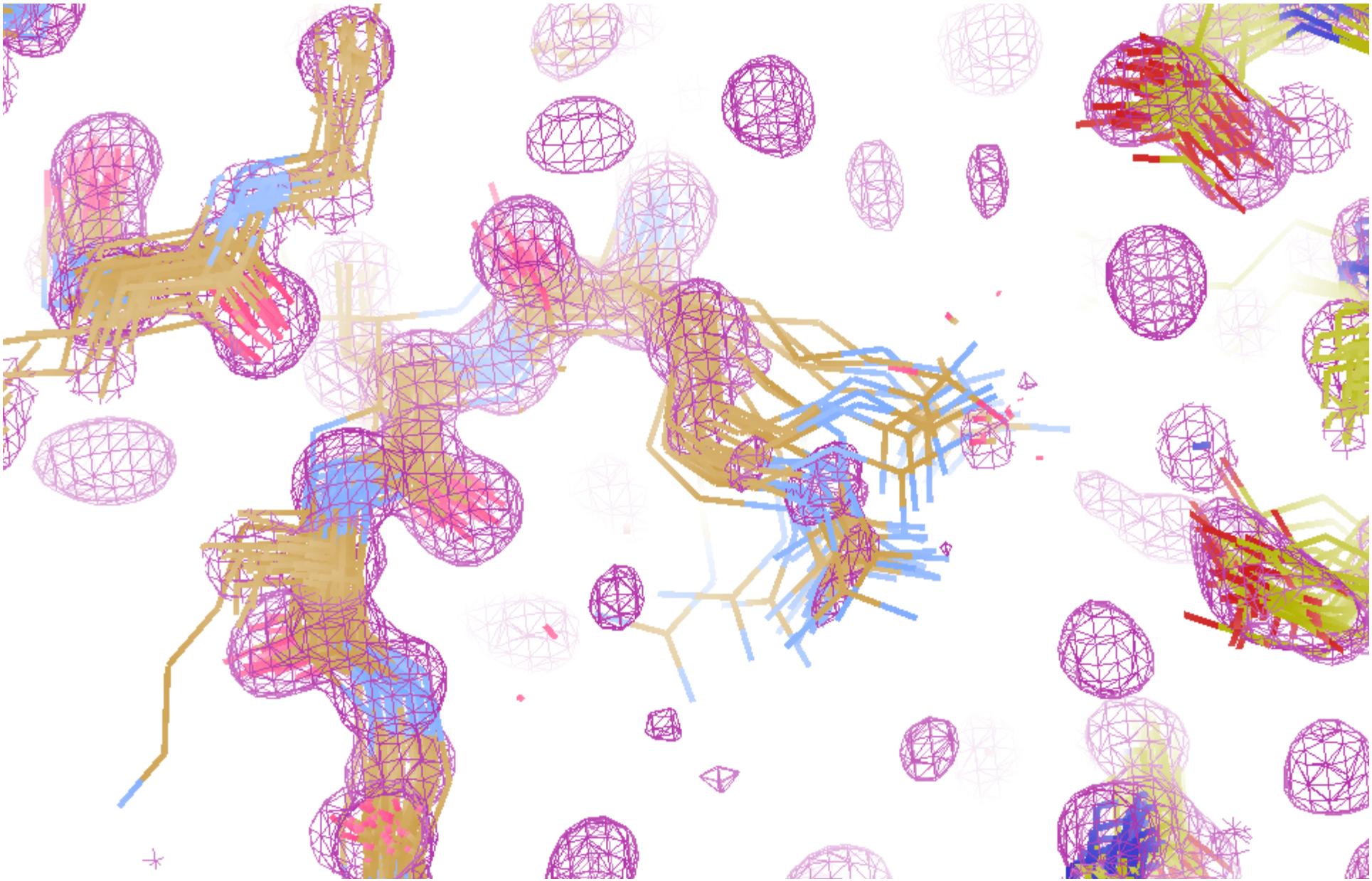
1aho
Scorpion toxin

0.96 Å resolution
64 residues
Solvent: H_20 + acetate

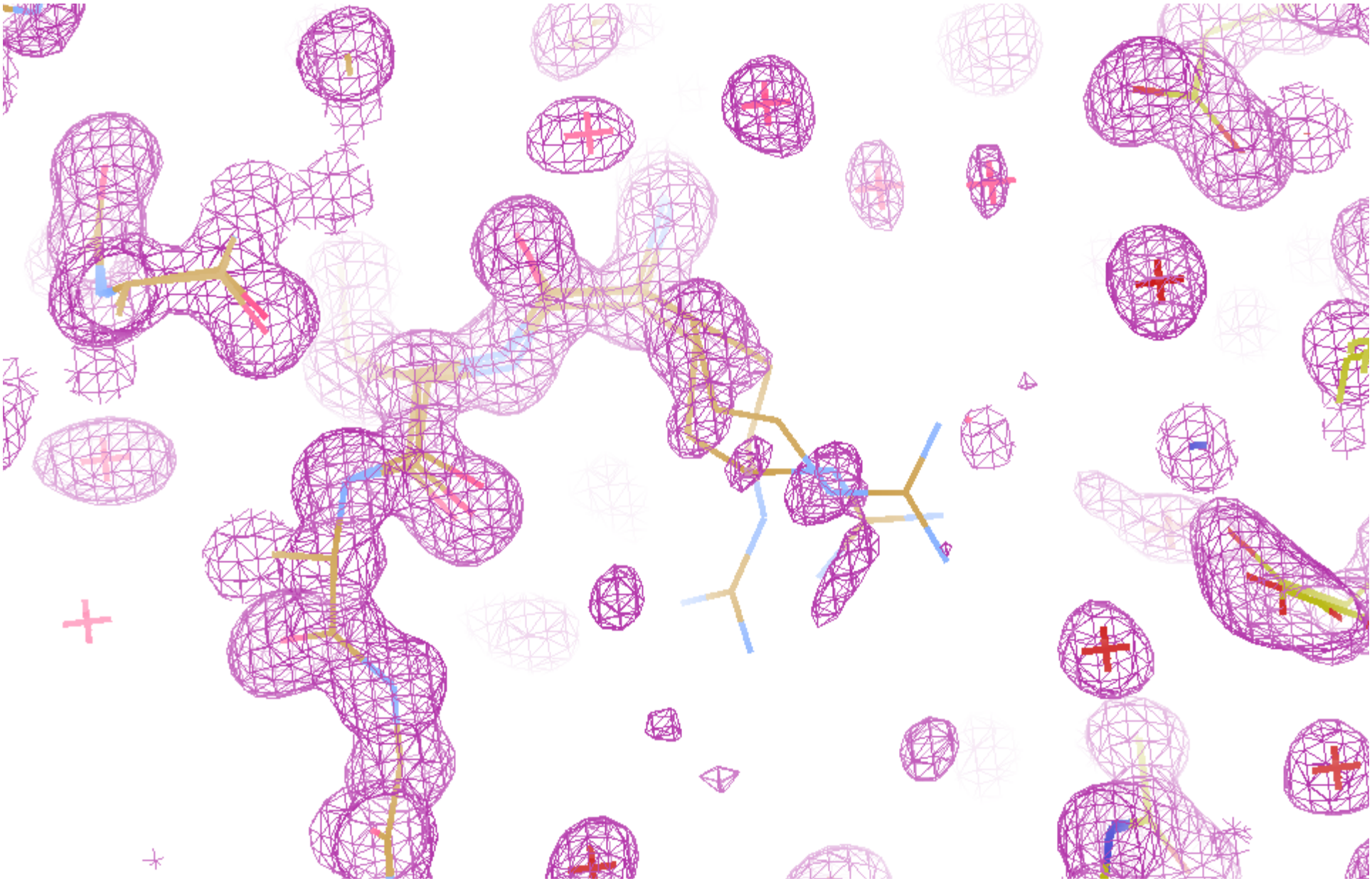
Cerutti *et al.* (2010).*J. Phys. Chem. B* **114**, 12811-12824.

30 conformers from 24,000

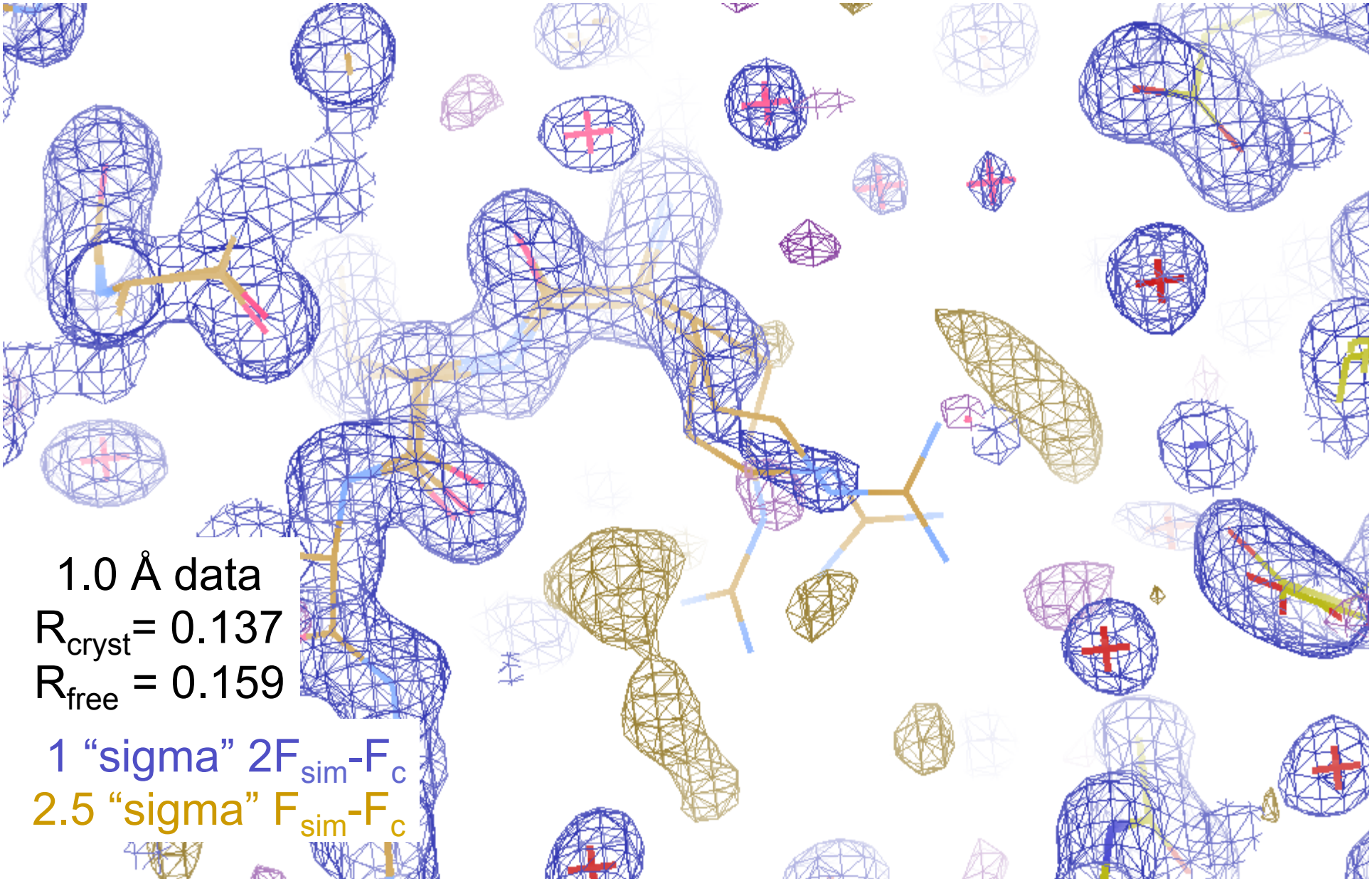
Electron density from 24,000 conformers



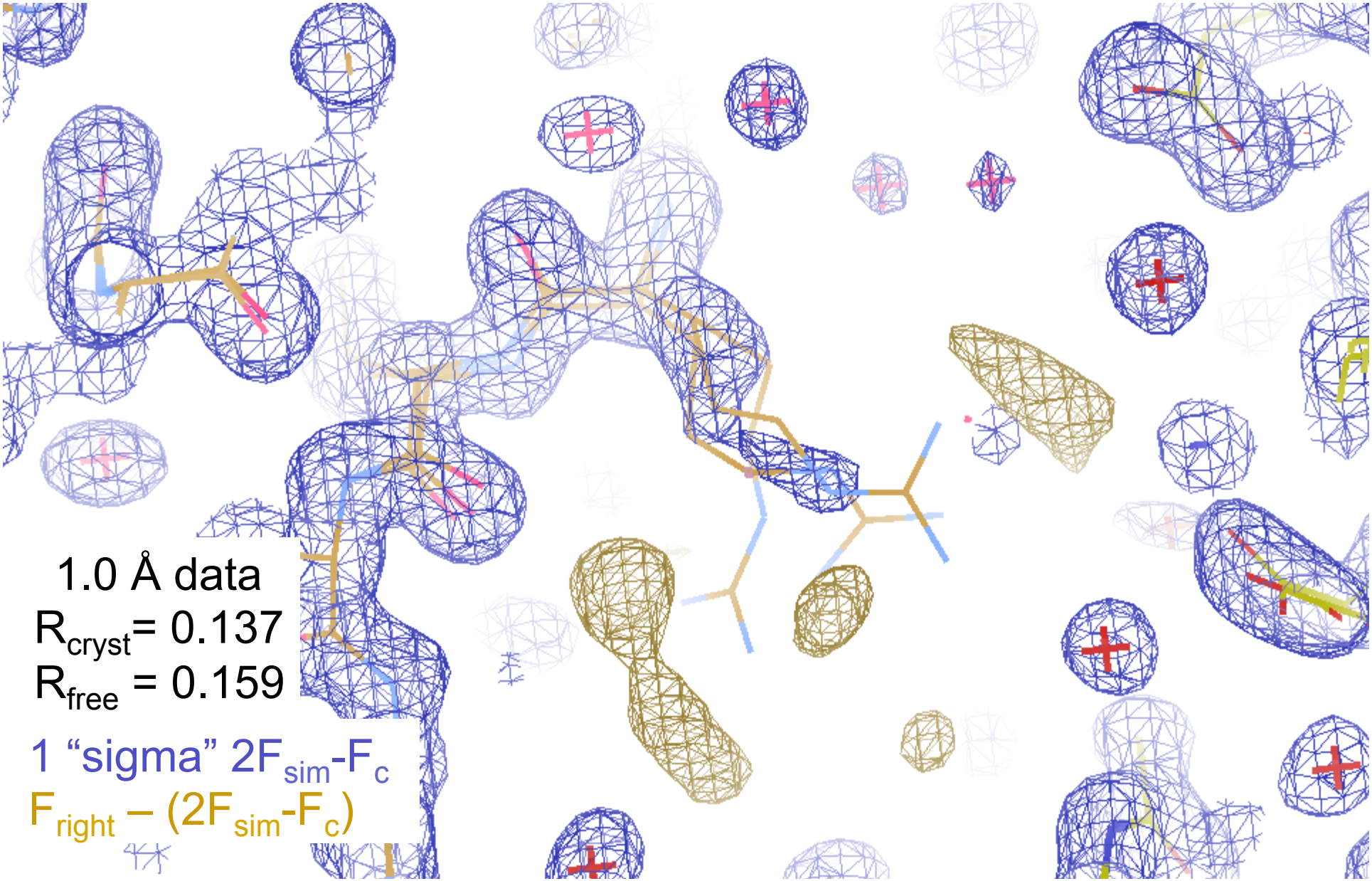
Electron density from 24,000 conformers



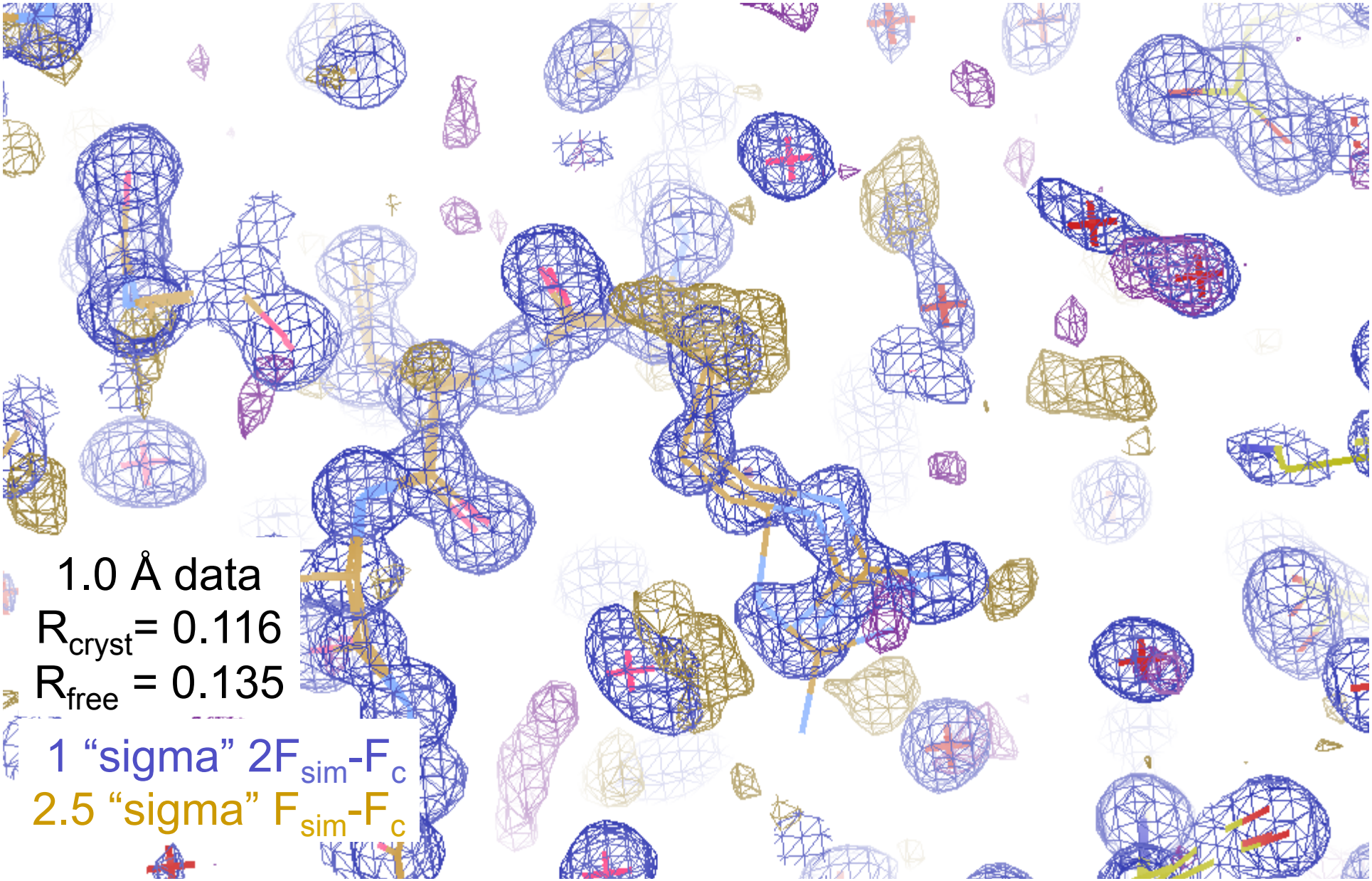
$2F_{sim}$-F_{calc} and F_{sim}-F_{calc} maps



$2F_{sim}$-F_{calc} and (F_{sim} Φ_{sim}) - (F_{calc} Φ_{calc}) maps

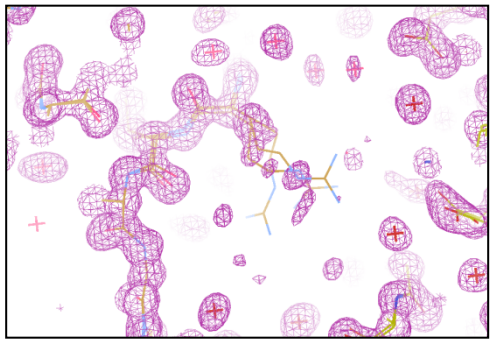


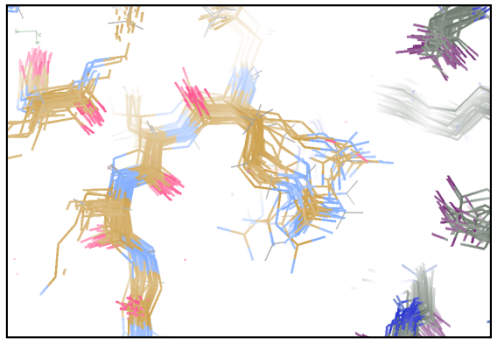
Regular model with real data!



Molecular Dynamics vs Observation

1aho 64-residue scorpion toxin in water to 1.0 Å resolution





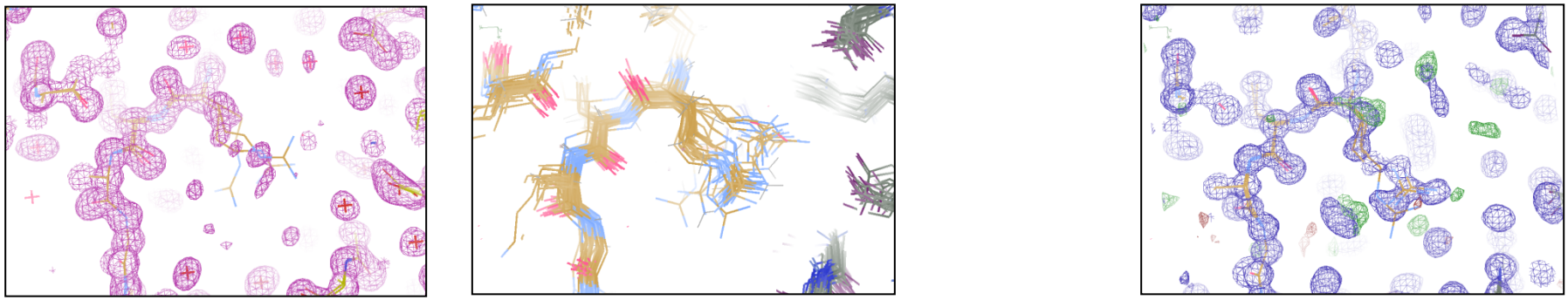
refined_vs_Fsim.pdb

F_{calc} F_{sim}

R_{cryst}= 0.137

Molecular Dynamics vs Observation

1aho 64-residue scorpion toxin in water to 1.0 Å resolution



refined_vs_Fsim.pdb

1aho.cif

1aho.pdb

F_{calc} F_{sim} F_{obs} F_{calc}

R_{cryst}= 0.137

R_{free} = 0.69

R_{cryst}= 0.116

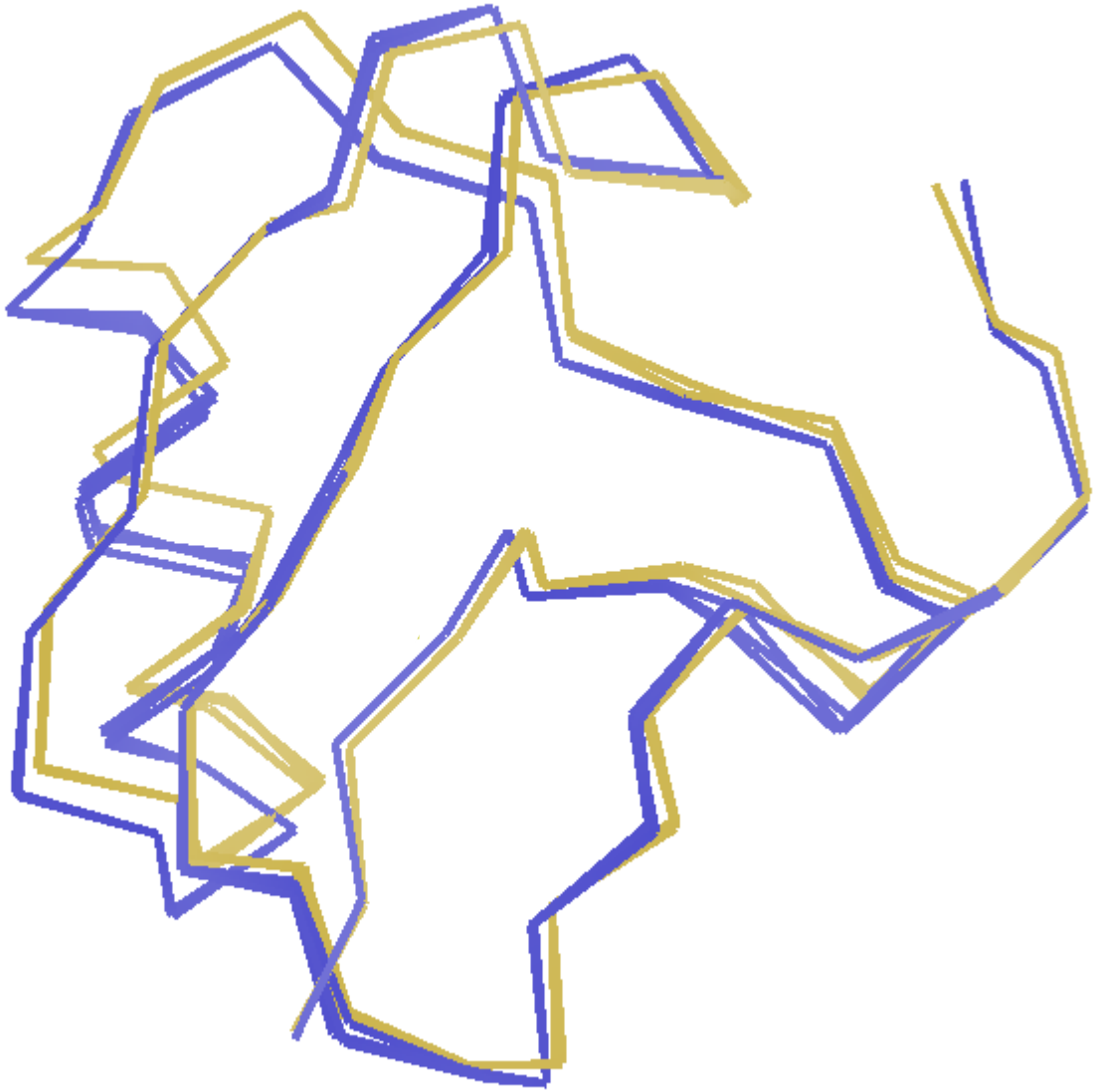
R_{free} = 0.48 to 4 Å

rmsd = 1.05 Å

LSQ rmsd = 0.43Å

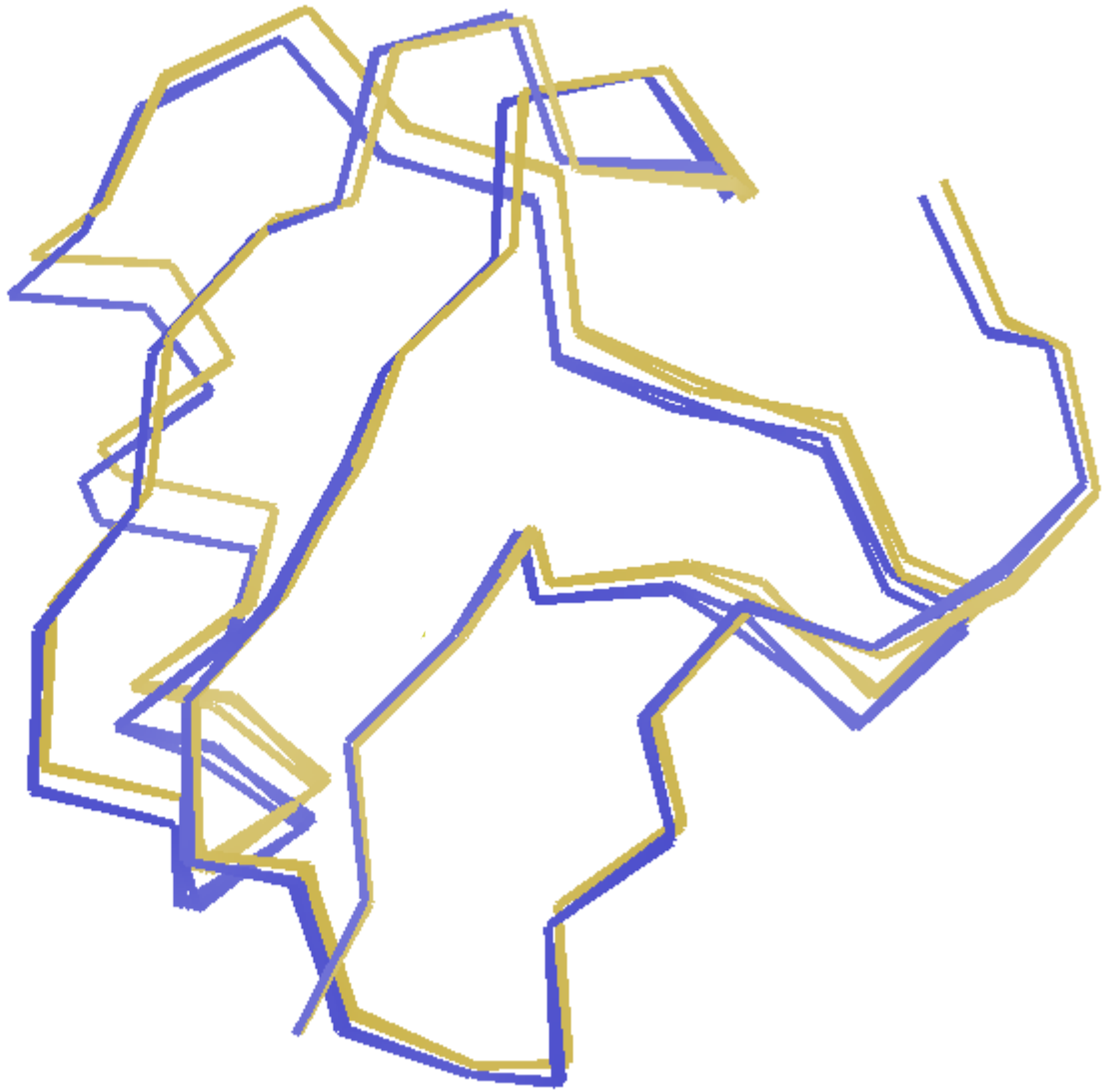
Molecular Dynamics vs Observation

RMSD
1.05 Å

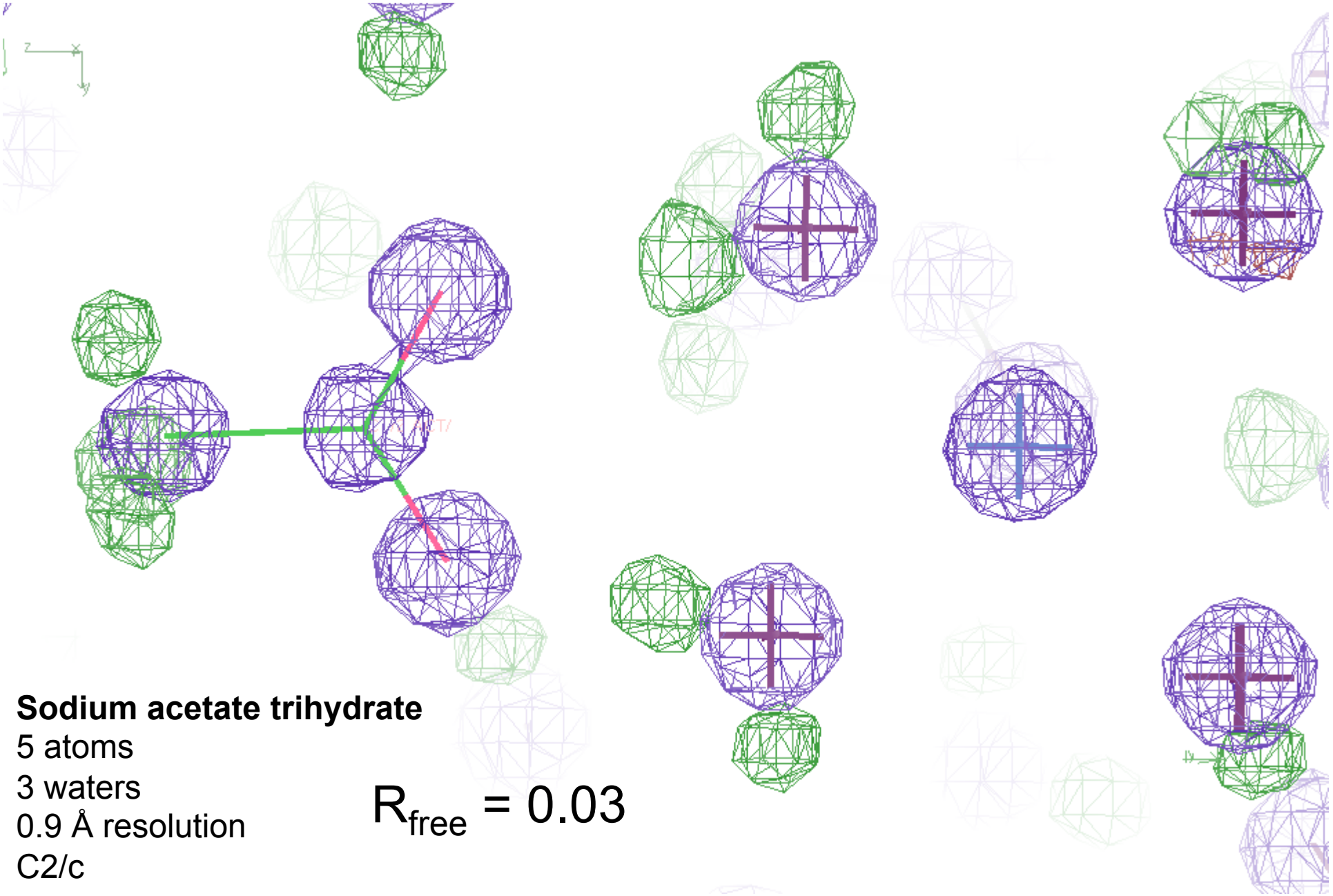


Molecular Dynamics vs Observation

RMSD
0.45 Å
aligned



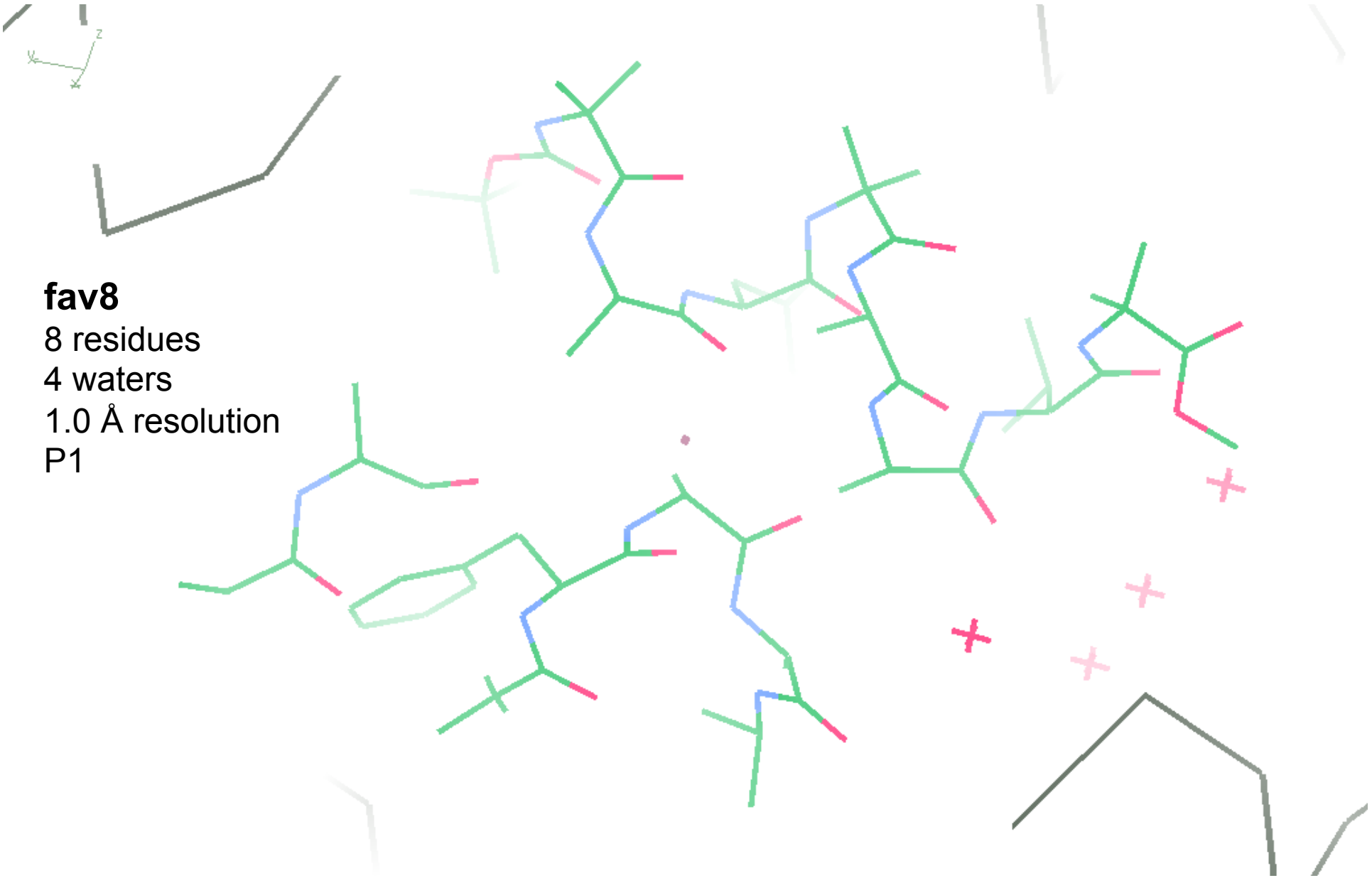
Molecular Dynamics vs Observation



Sodium acetate trihydrate
5 atoms
3 waters
0.9 Å resolution
C2/c

R_{free} = 0.03

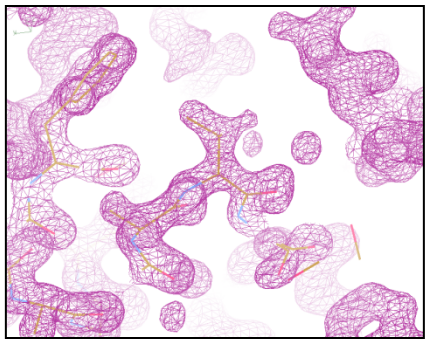
Molecular Dynamics vs Observation

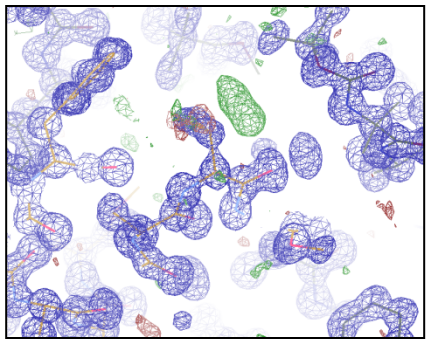


fav8
8 residues
4 waters
1.0 Å resolution
P1

Molecular Dynamics vs Observation

"fav8" 8-residue aromatic peptide with 4 waters to 1.0 Å resolution





refined_vs_Fsim.pdb

fav8.fcf

fav8.cif

F_{calc} F_{sim} F_{obs} F_{calc}

R_{cryst}= 0.13 R_{free}= 0.23 R_{cryst}= 0.16

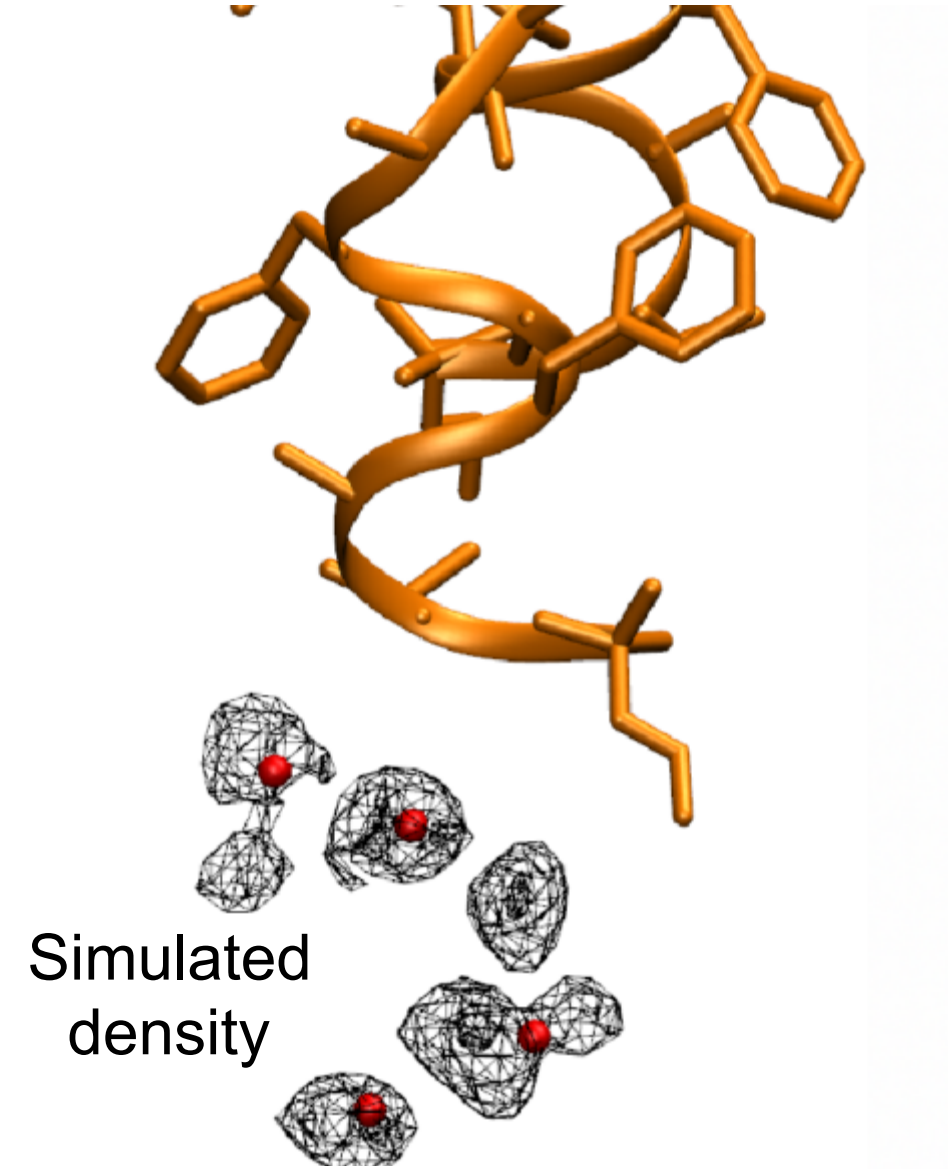
R_{free} = 0.20 to 2Å

R1 = 0.041
With 4σ cutoff!

rmsd = 0.20 Å

LSQ rmsd = 0.091Å & 0.15 Å

MD-predicted water structure



Janowski *et al* (2013) JACS **135**, 7938-7948

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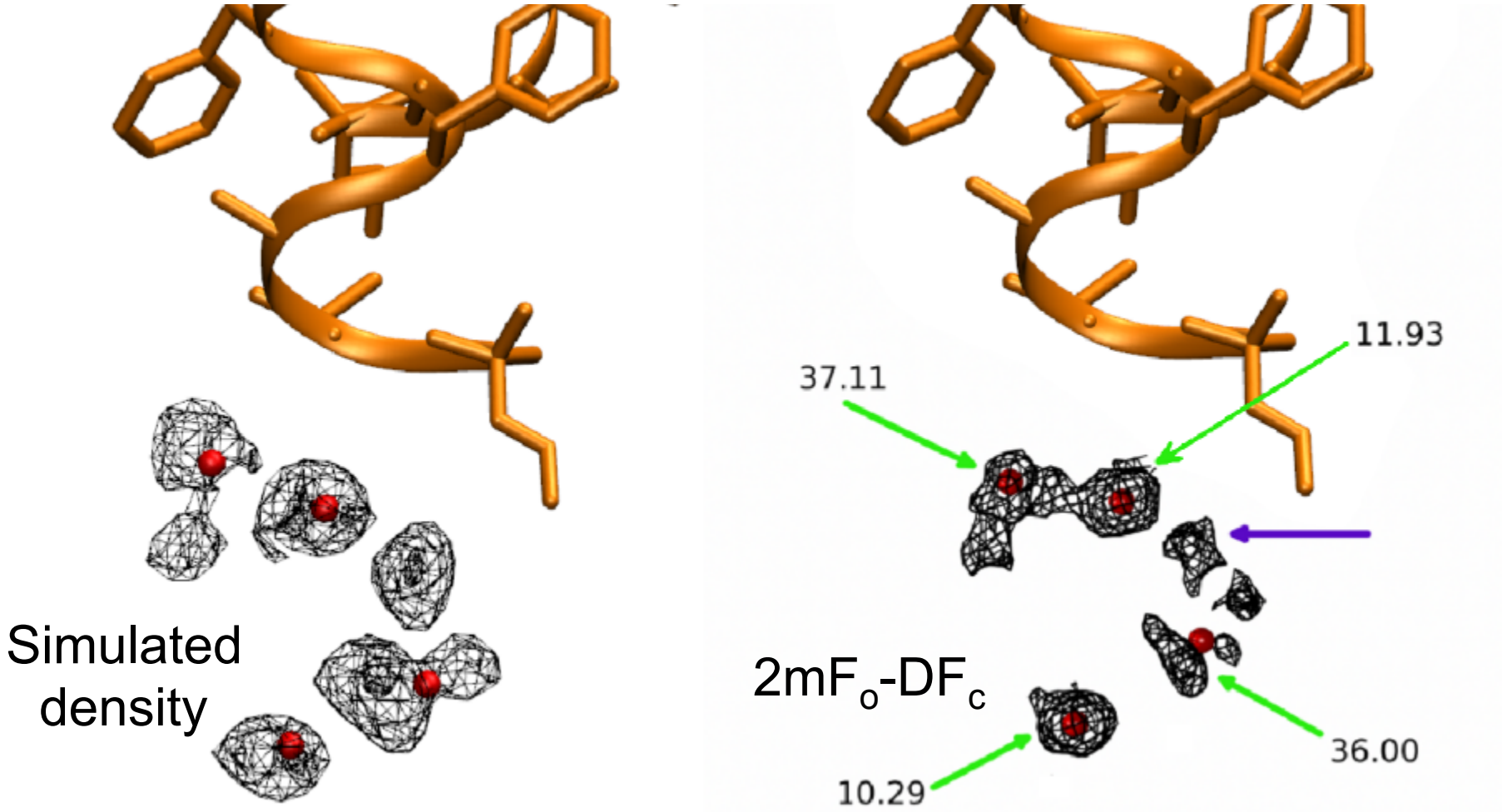
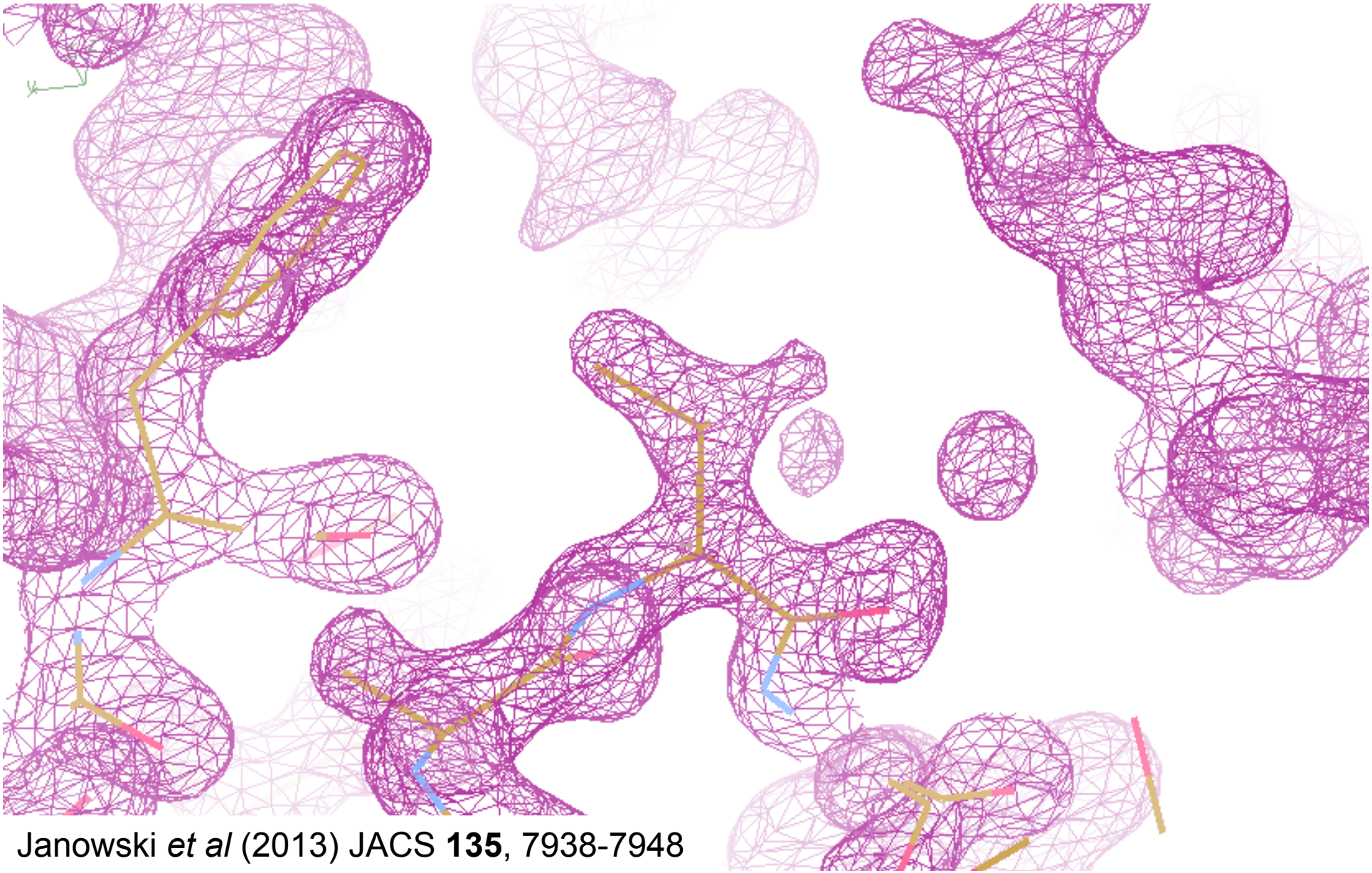


Janowski *et al* (2013) JACS **135**, 7938-7948

MD-predicted Val rotamer



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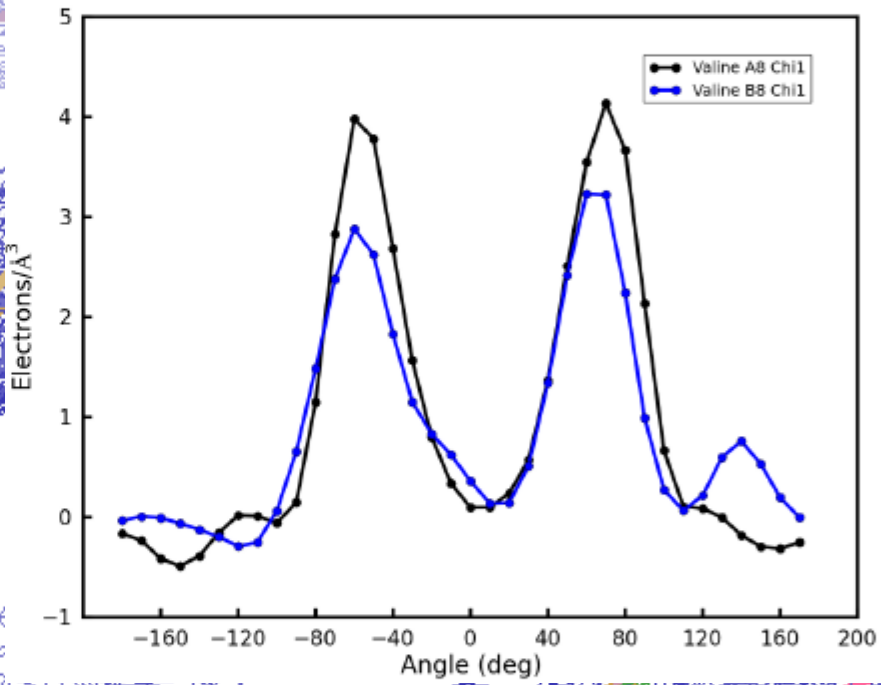
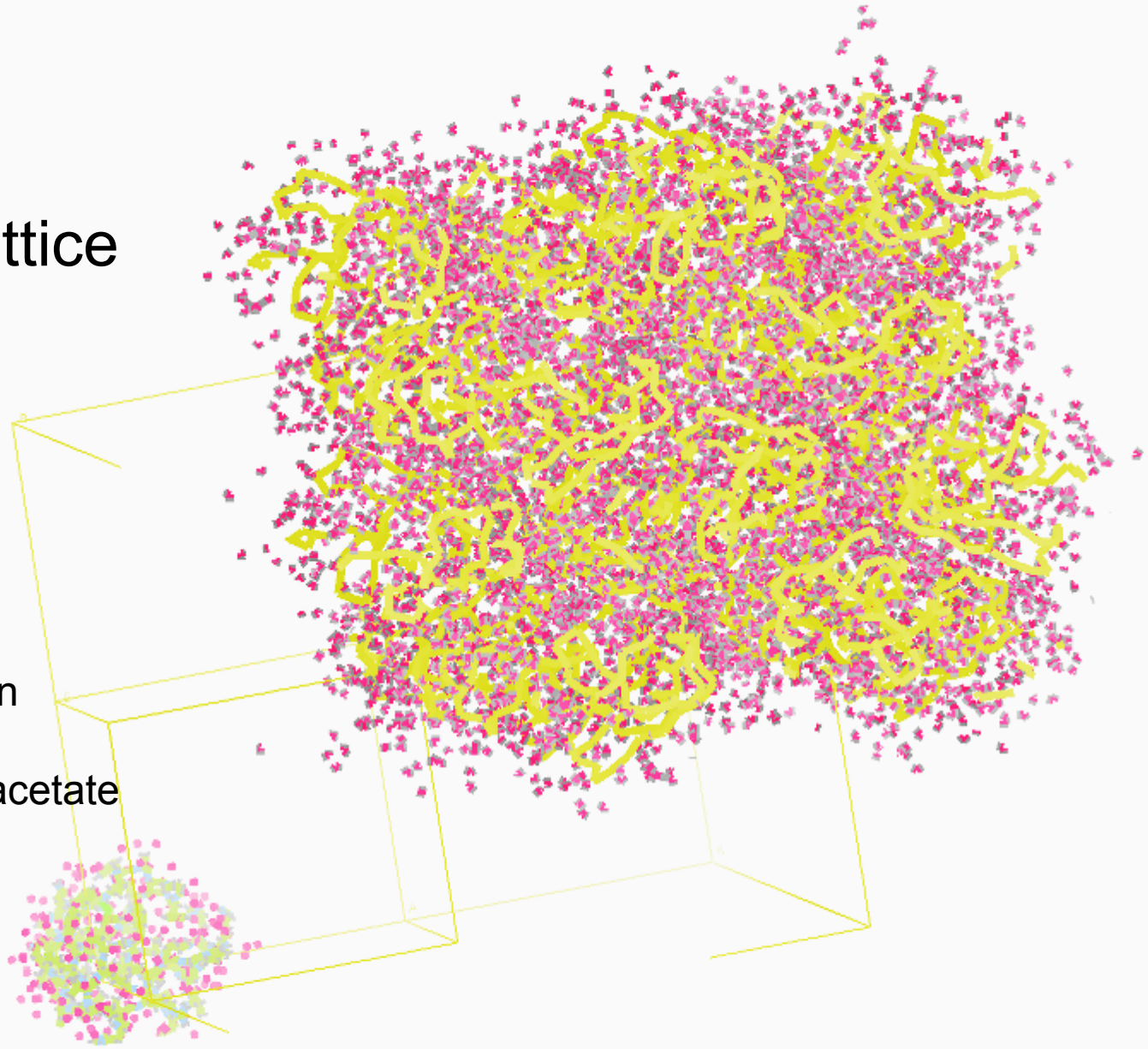


confs	R_{work}	R_{free}
one	4.11%	5.84%
A & B	3.89%	5.53%

Janowski *et al* (2013) JACS **135**, 7938-7948

Molecular Dynamics Simulation

using **real**
crystal's lattice



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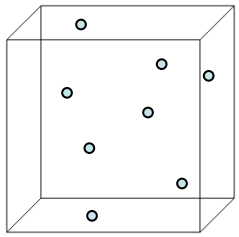
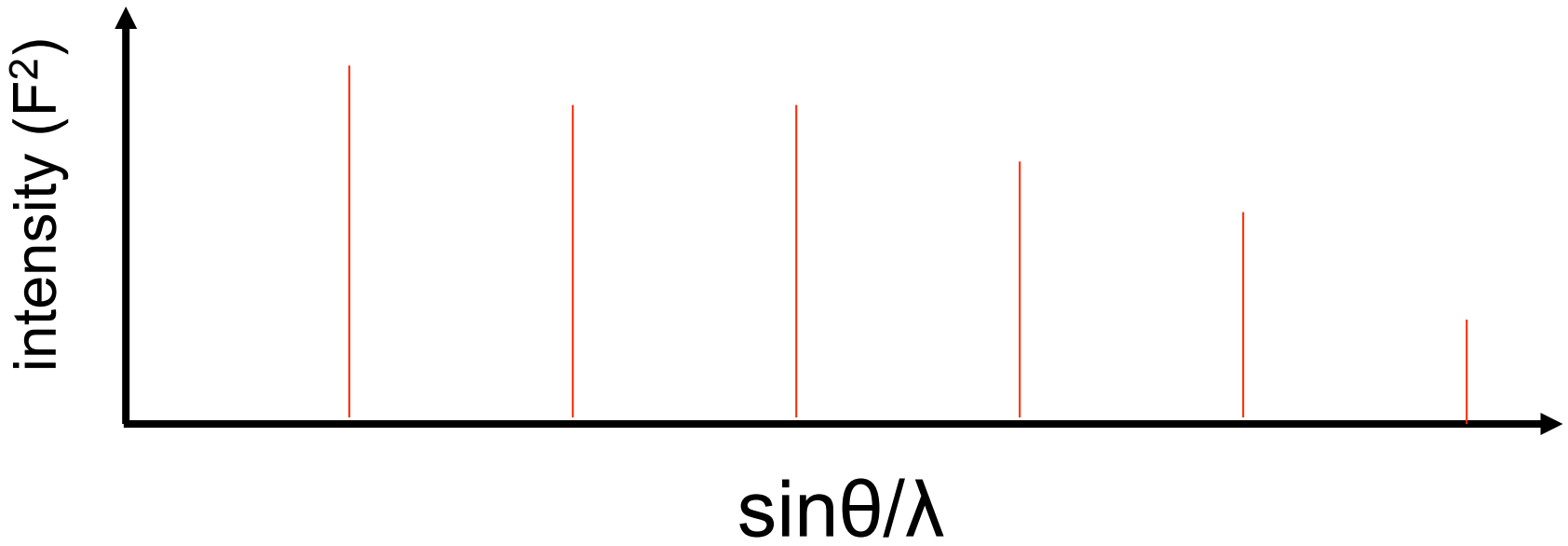
Super-cell formalism for diffuse scatter





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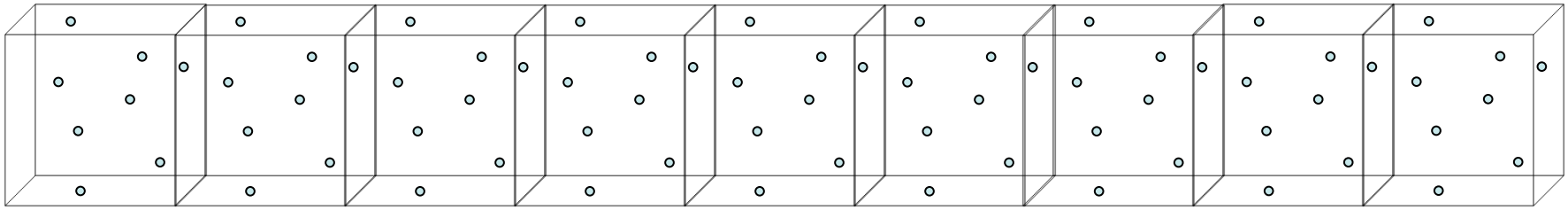

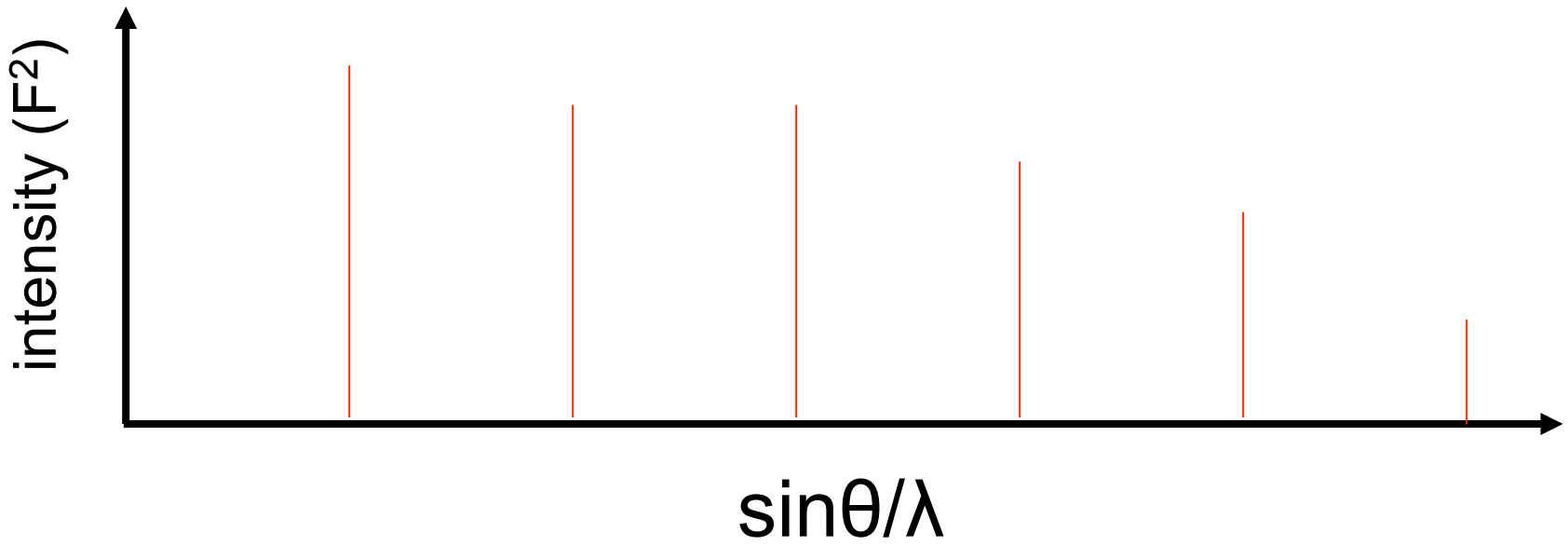

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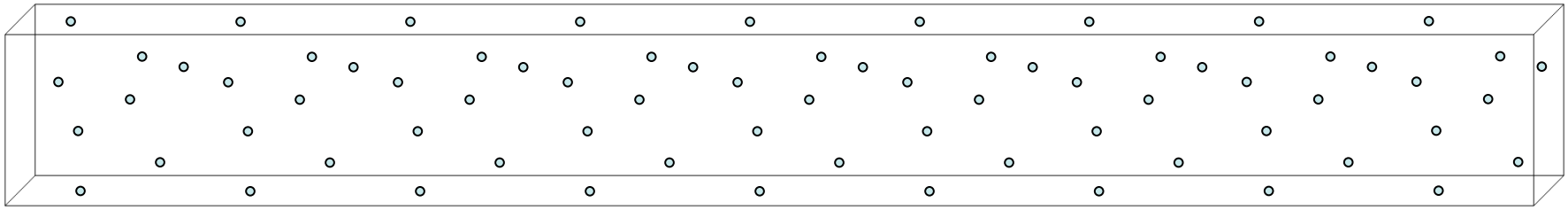

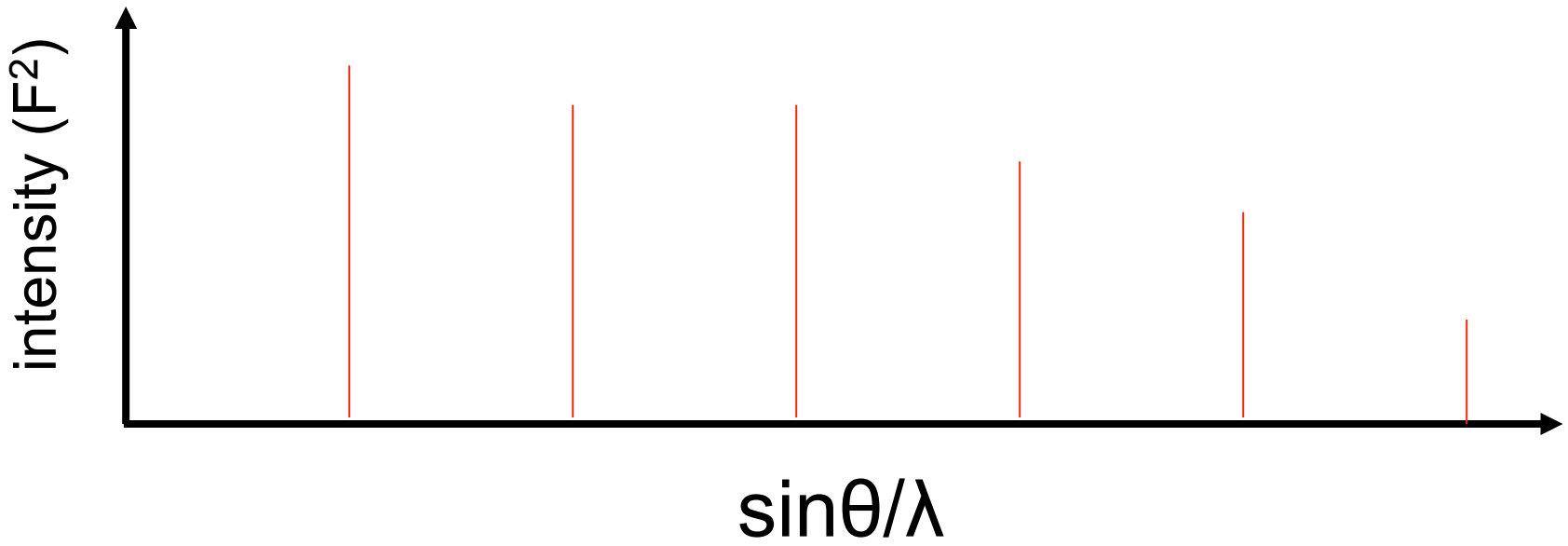

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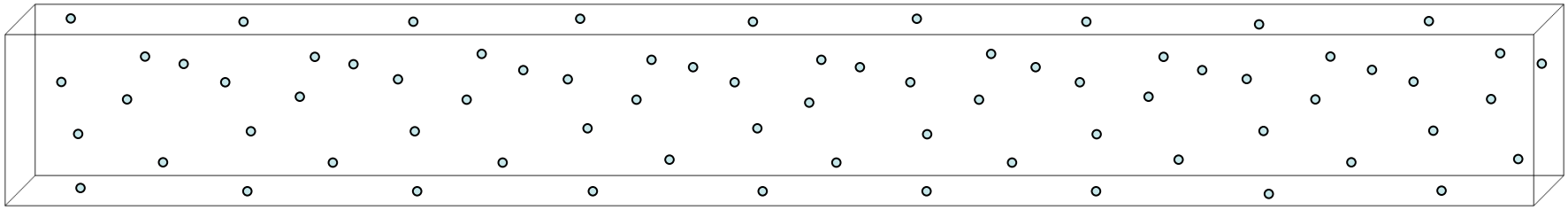

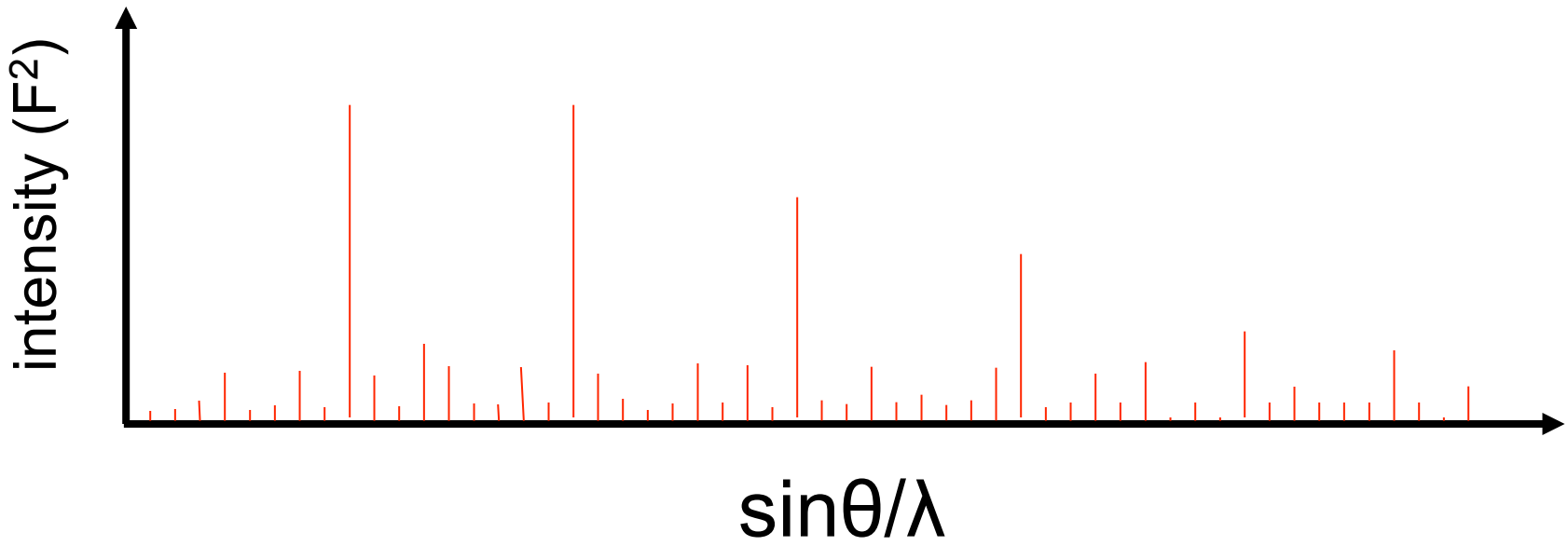

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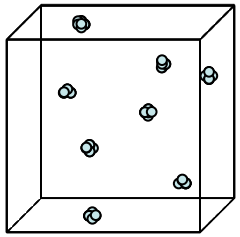
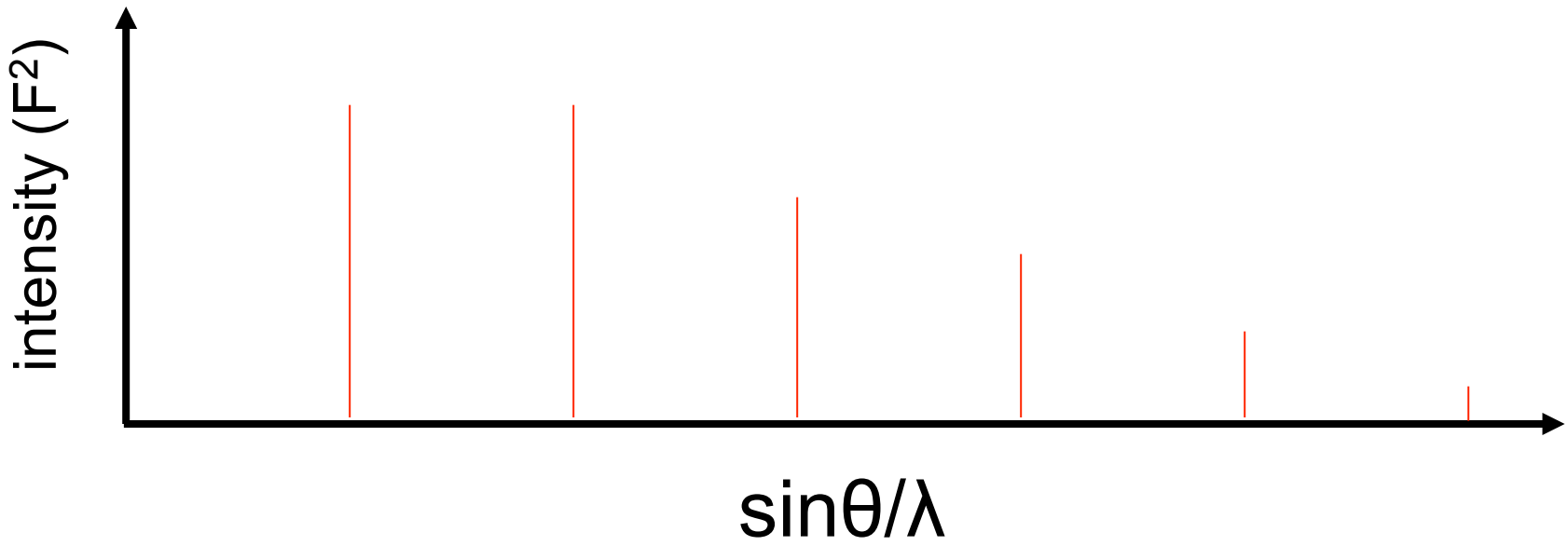
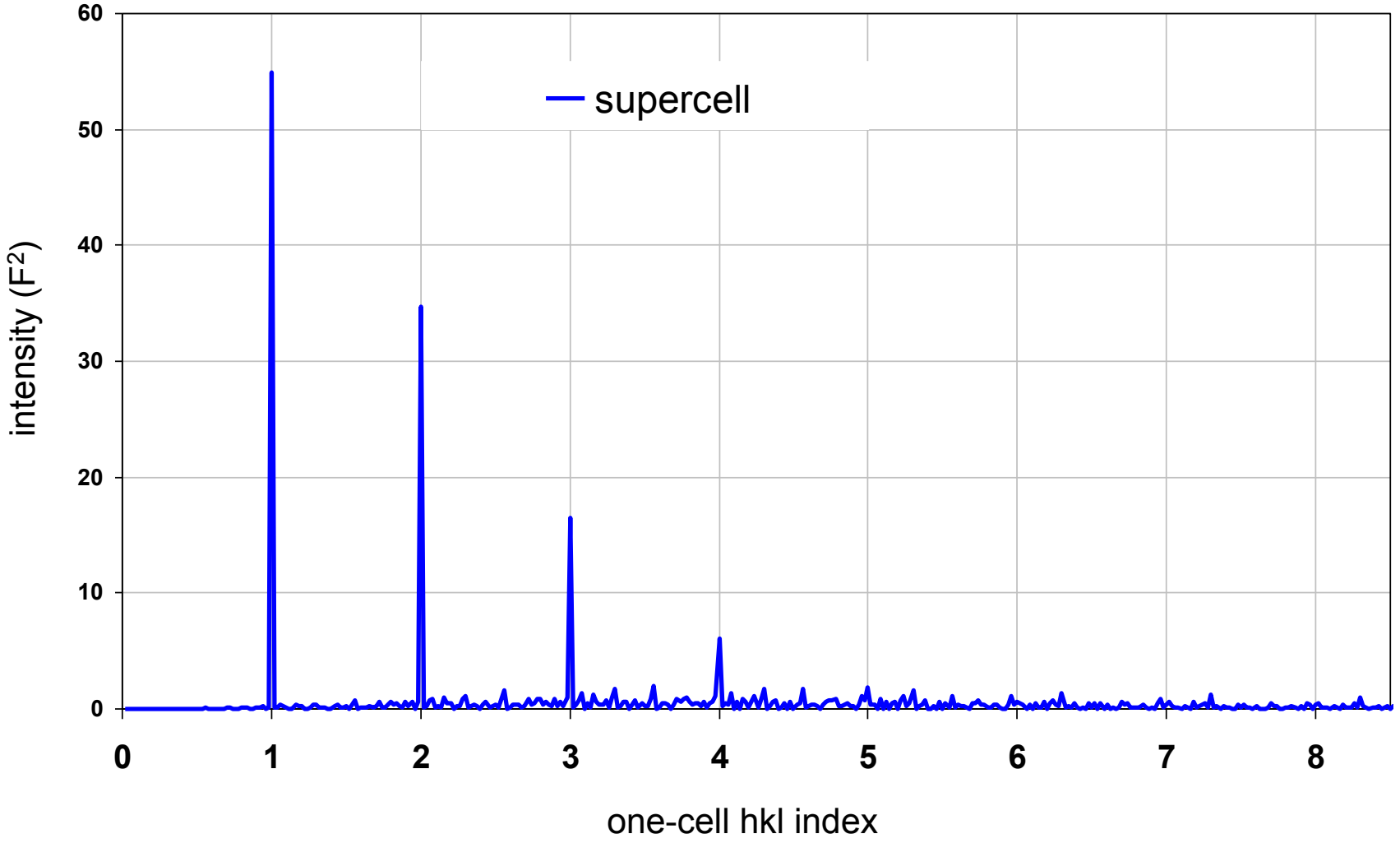




Super-cell formalism for diffuse scatter



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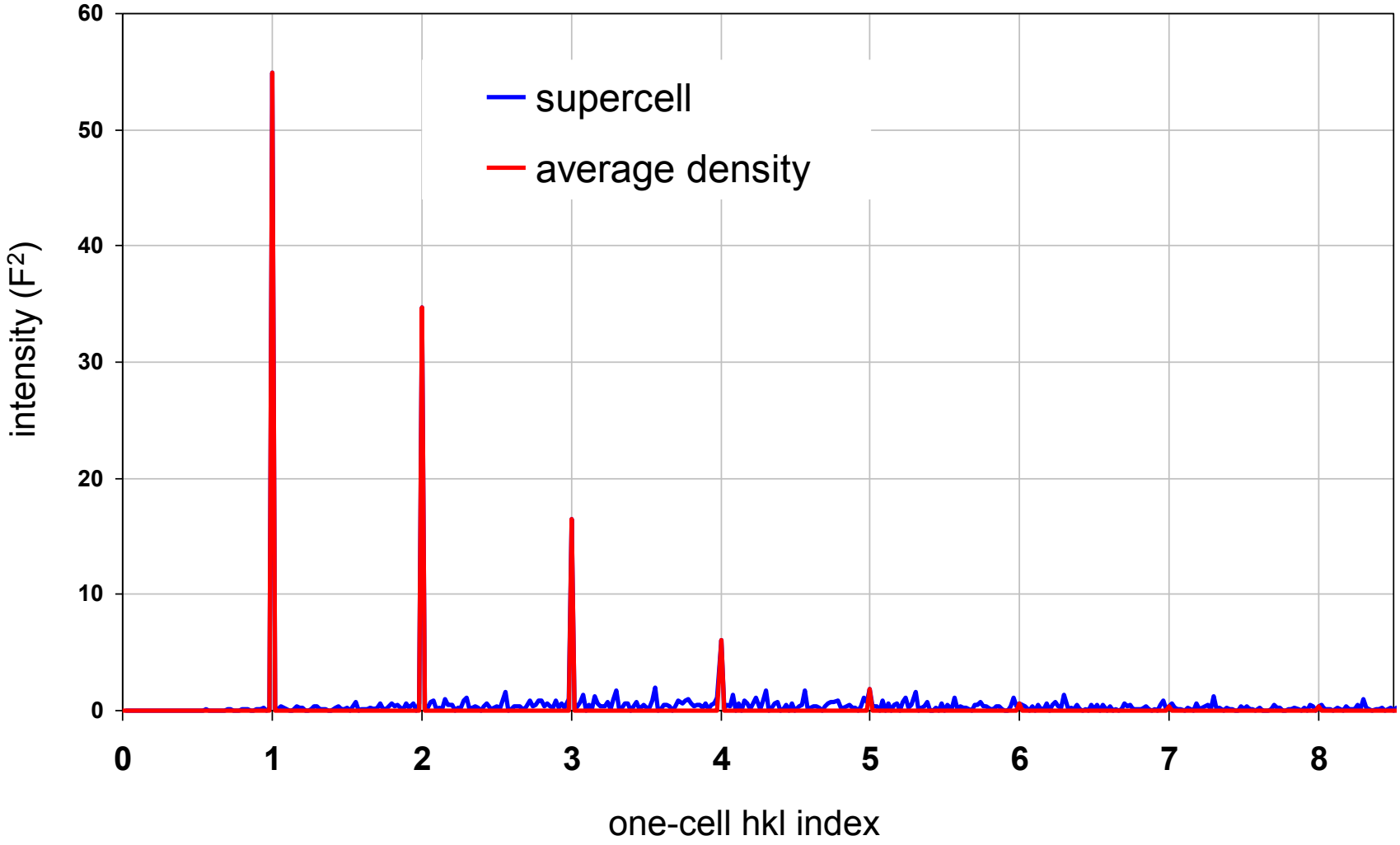
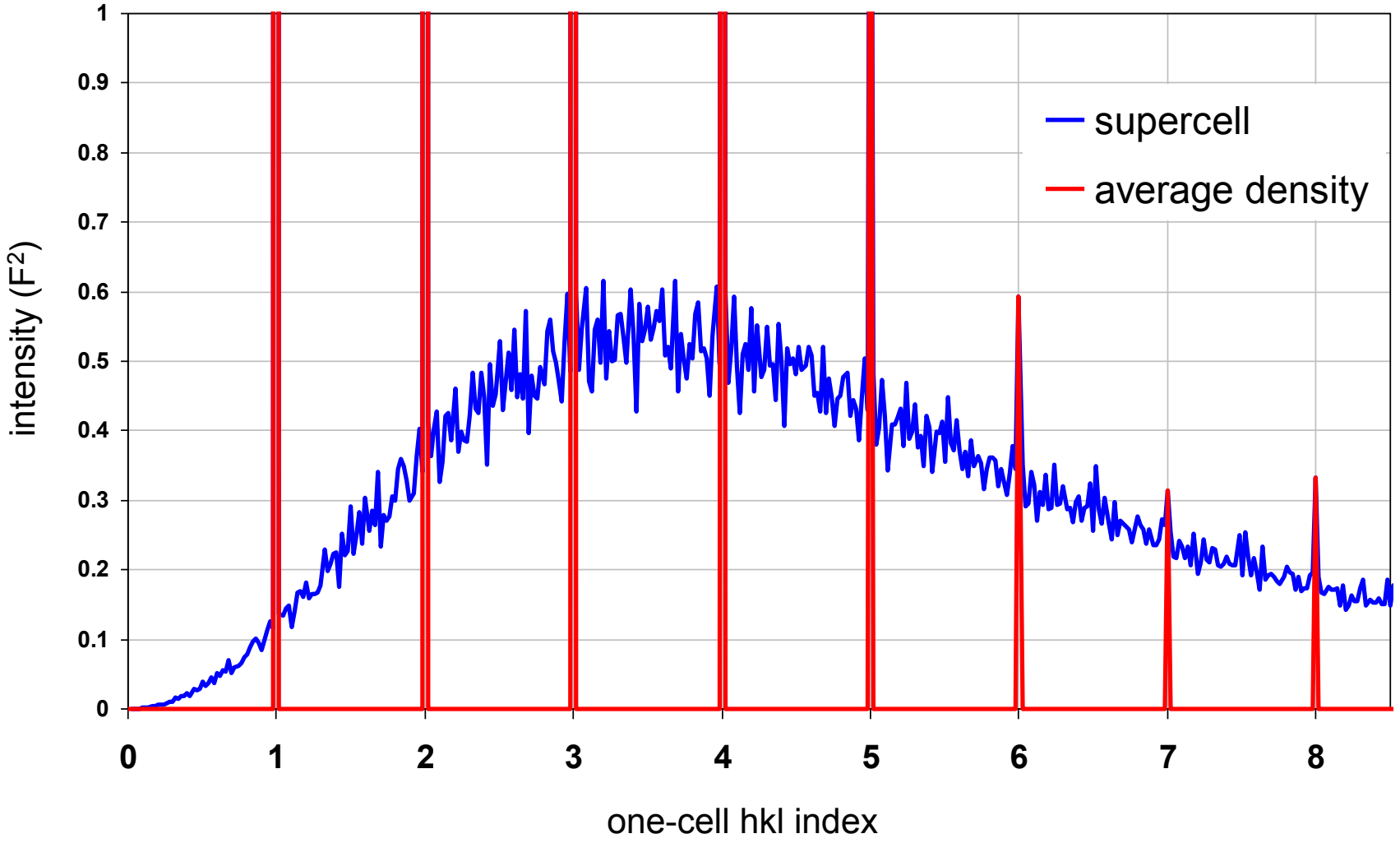


Super-cell formalism for diffuse scatter



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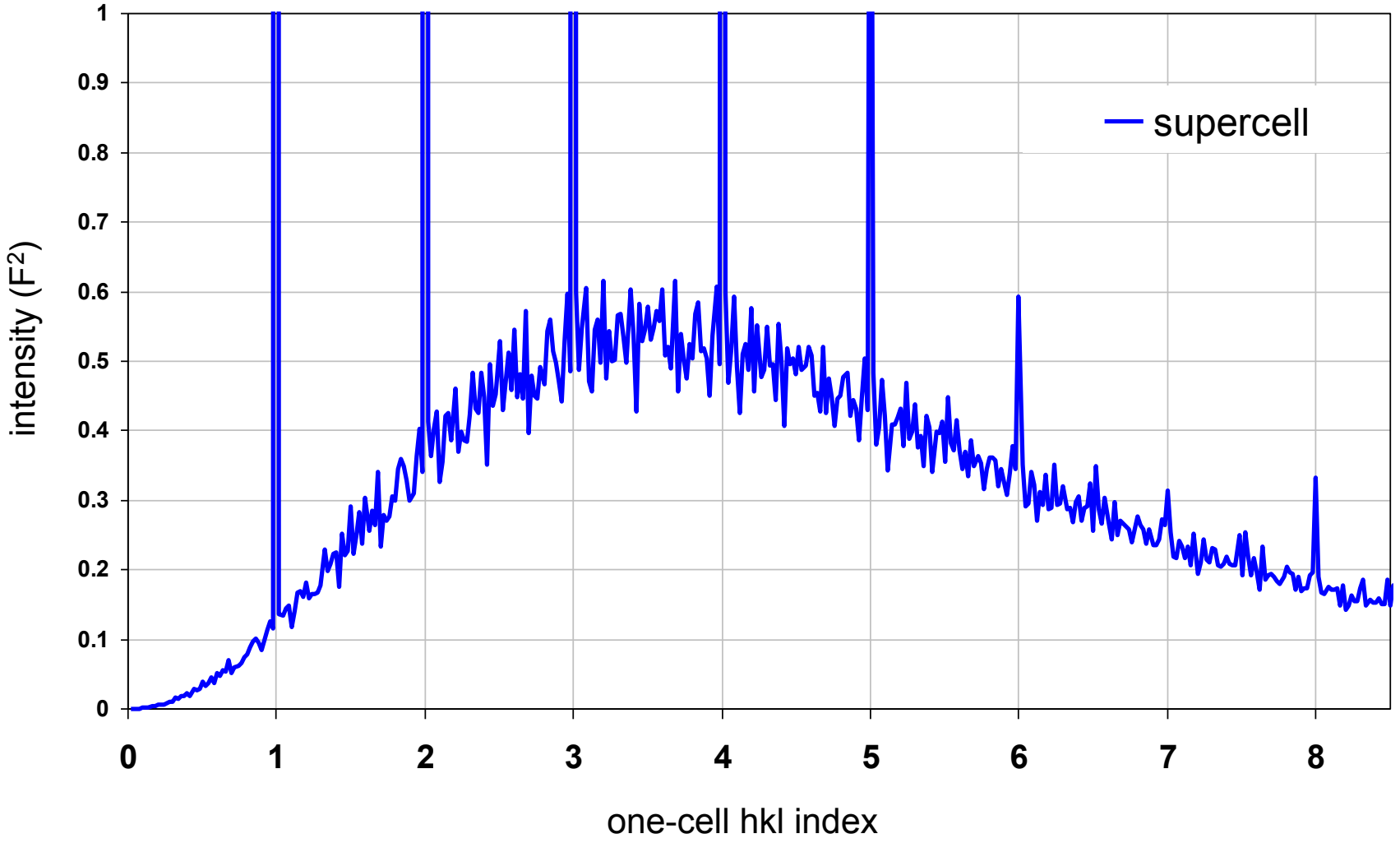

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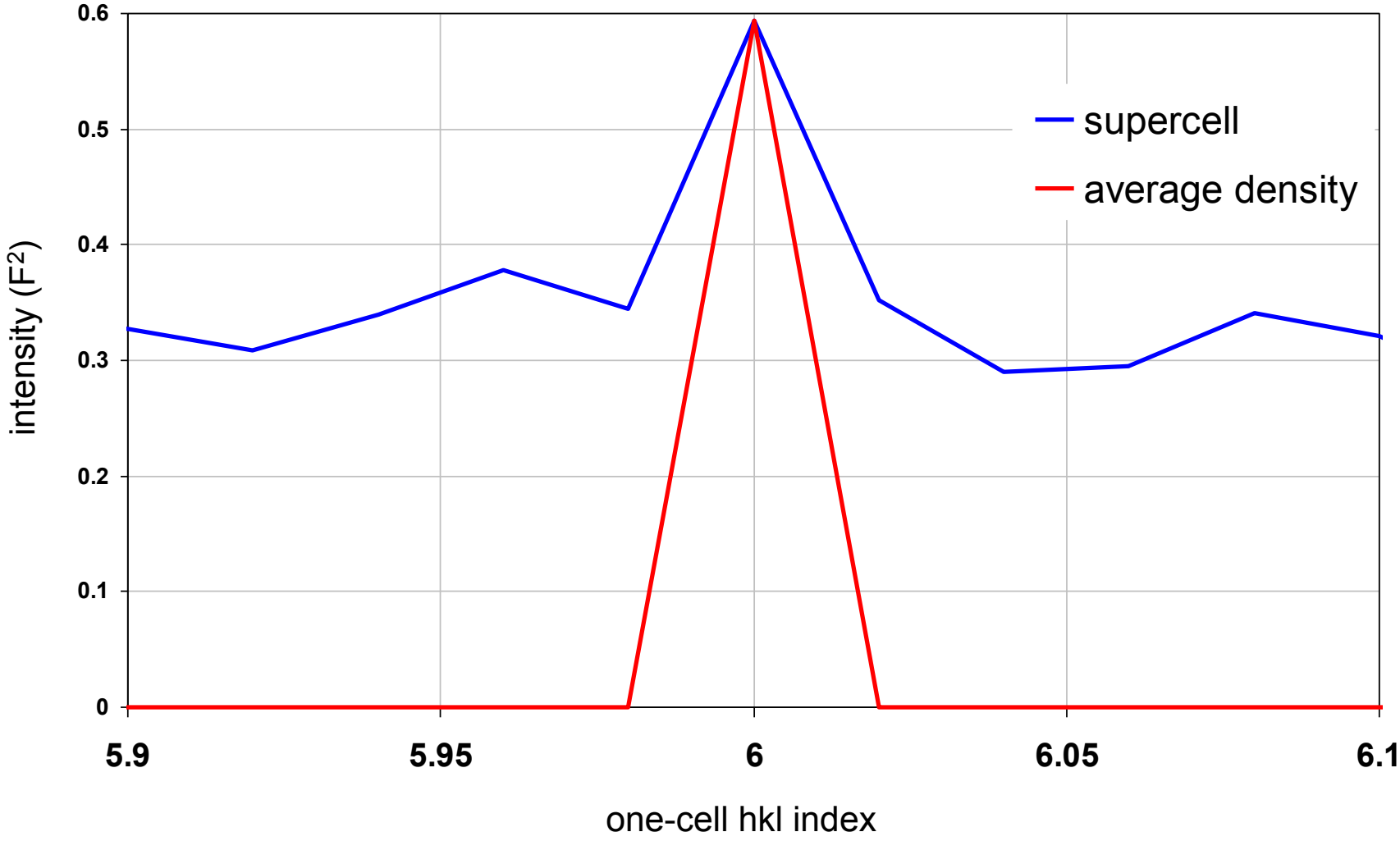

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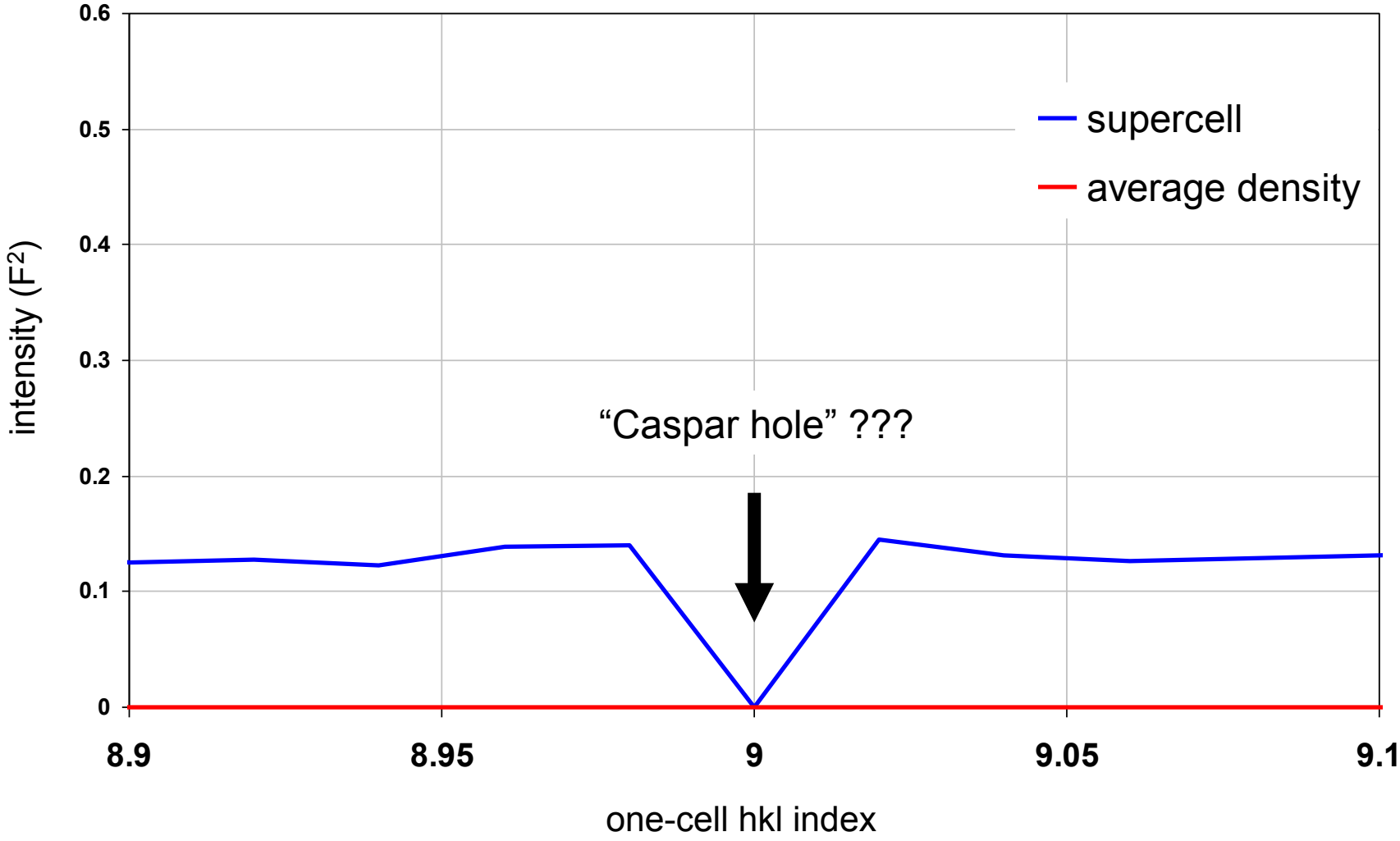
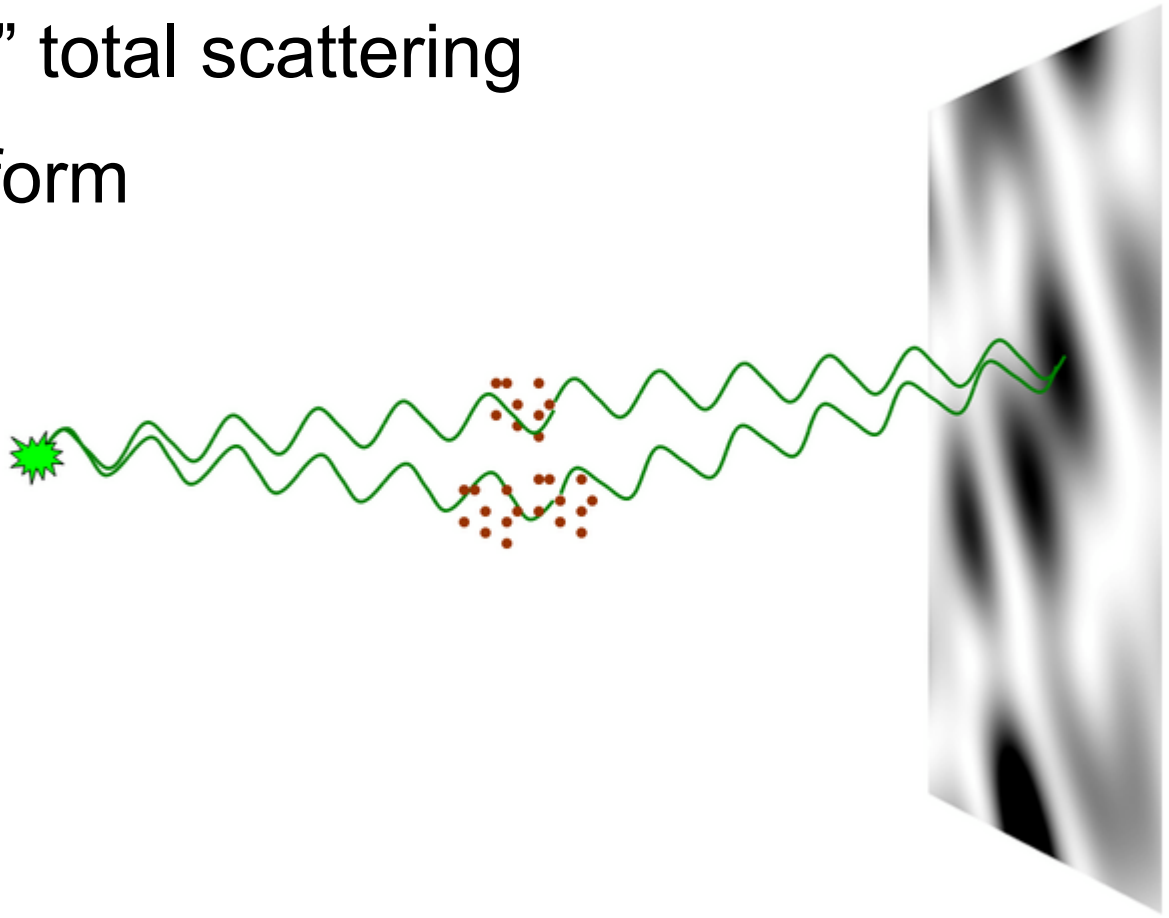
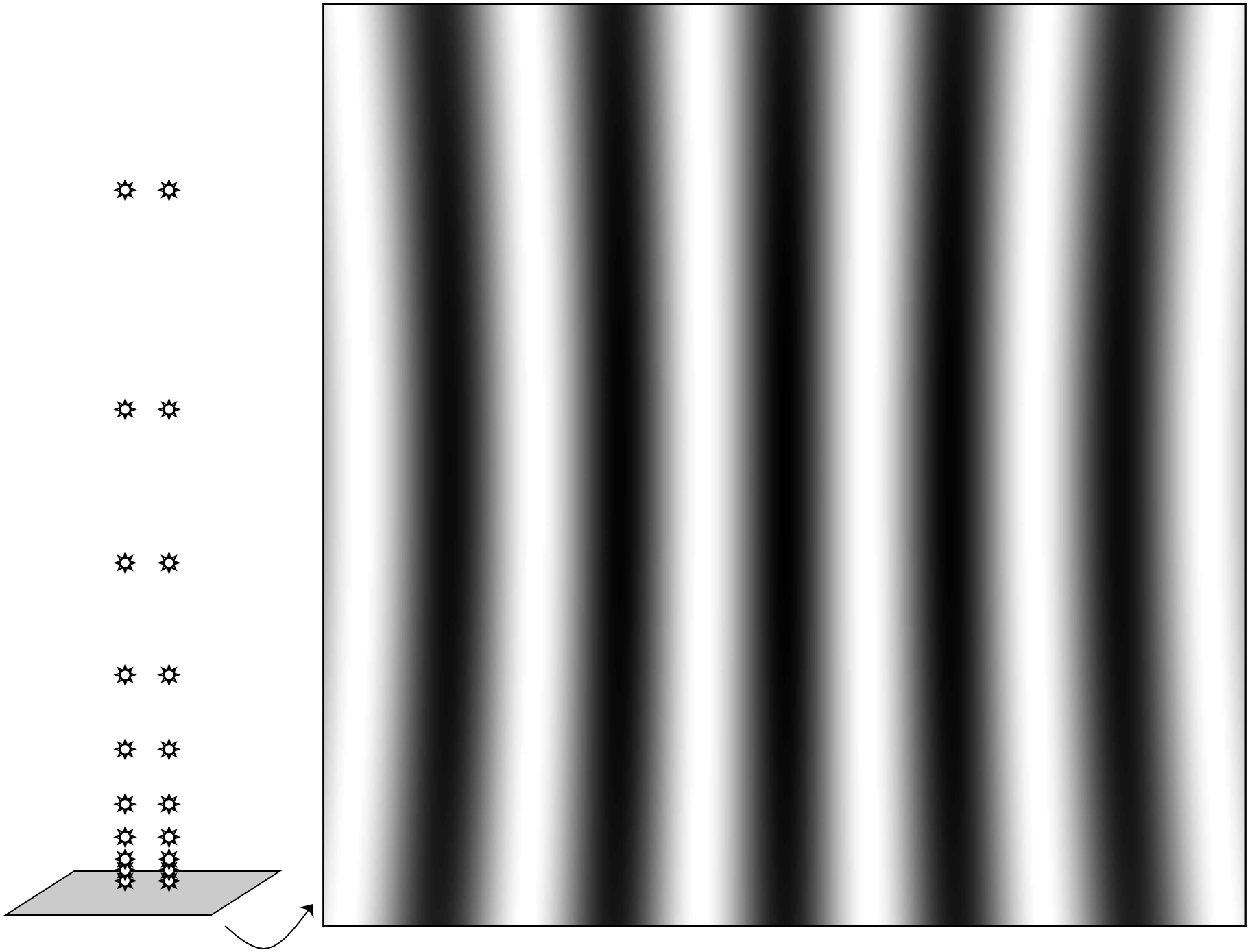


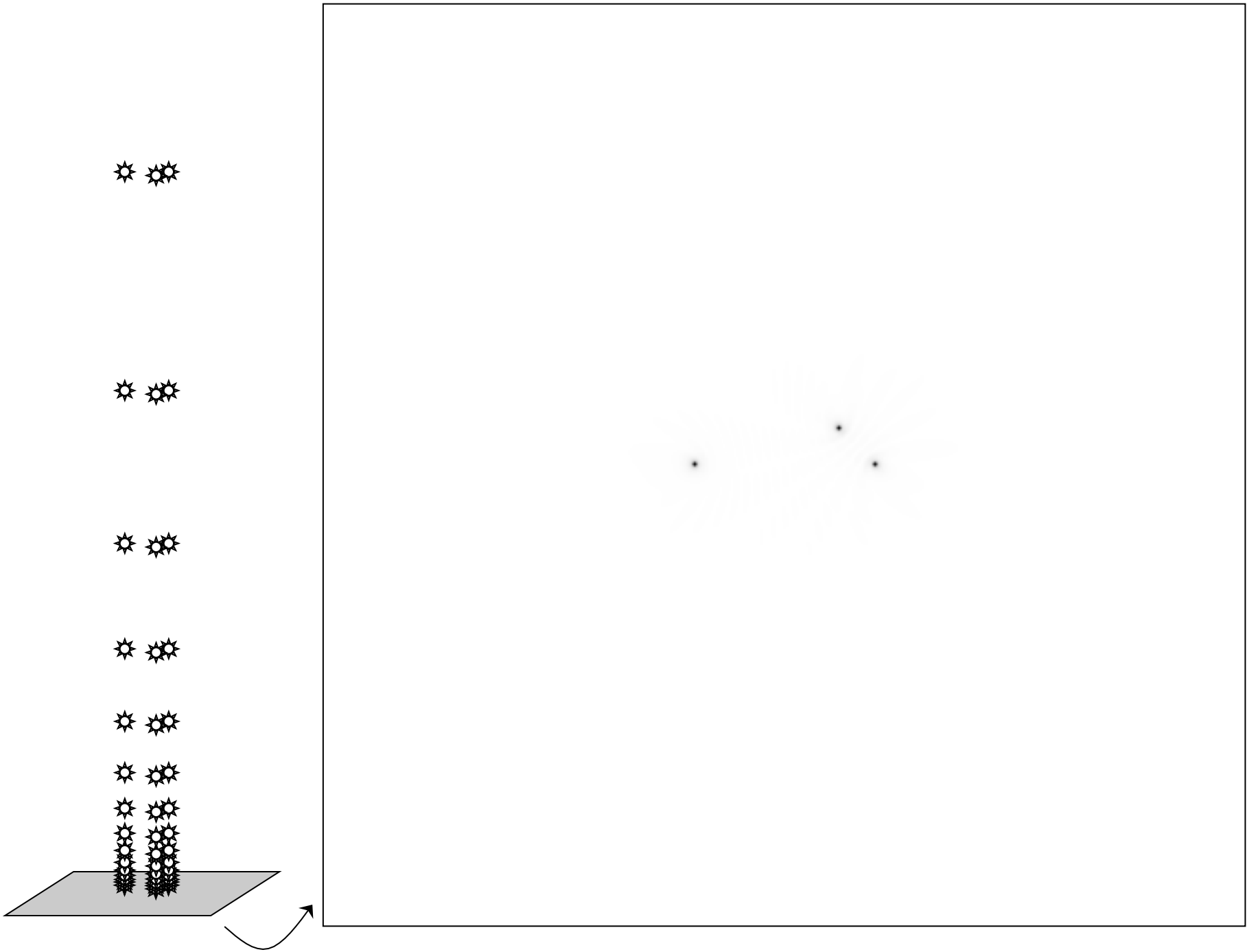
nearBragg program

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

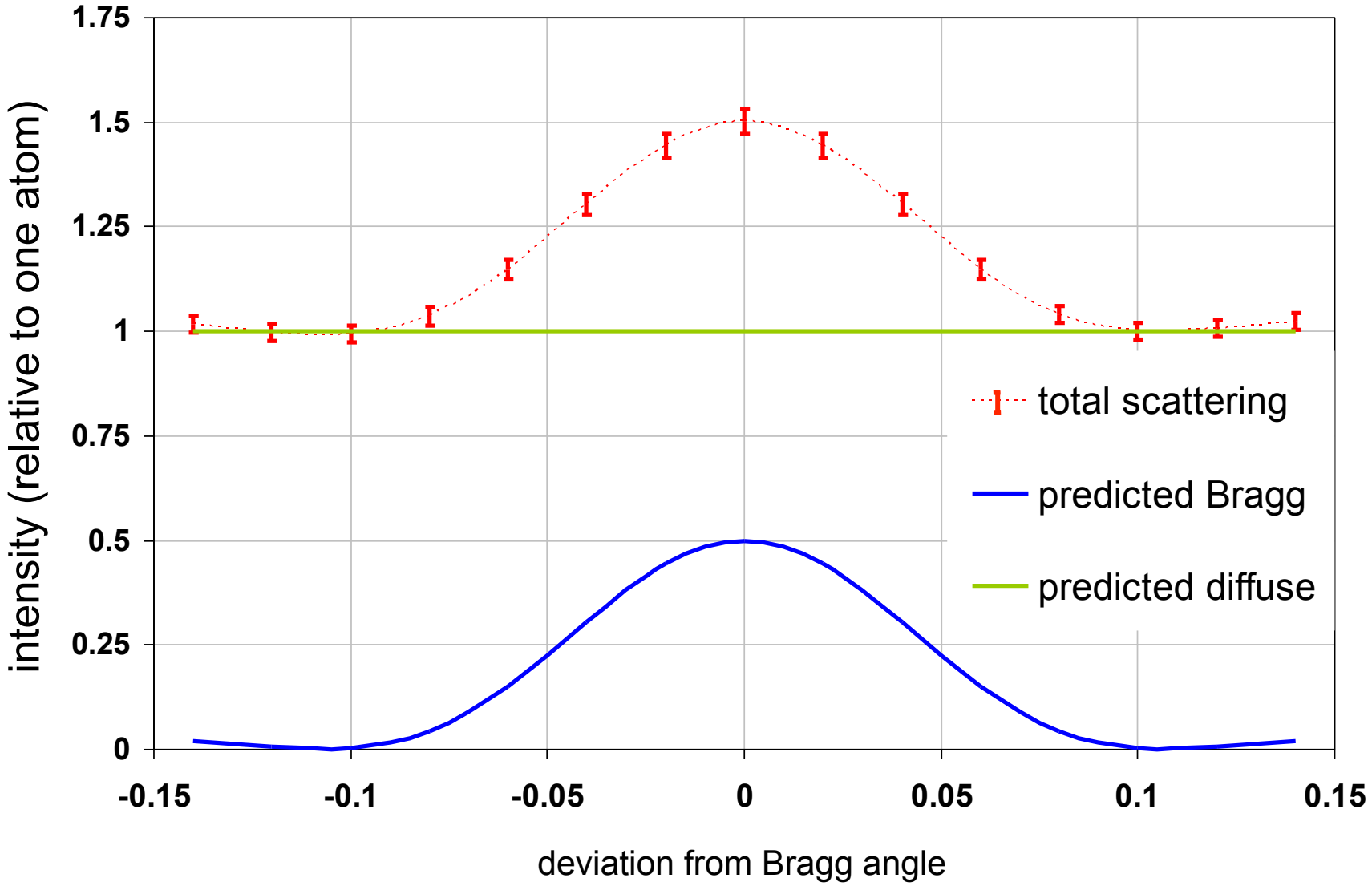
- "assumption-free" total scattering
- no Fourier Transform
- no unit cells
- no "mosaicity"
- arbitrary "atoms"
- arbitrary "source"
- coherent or not



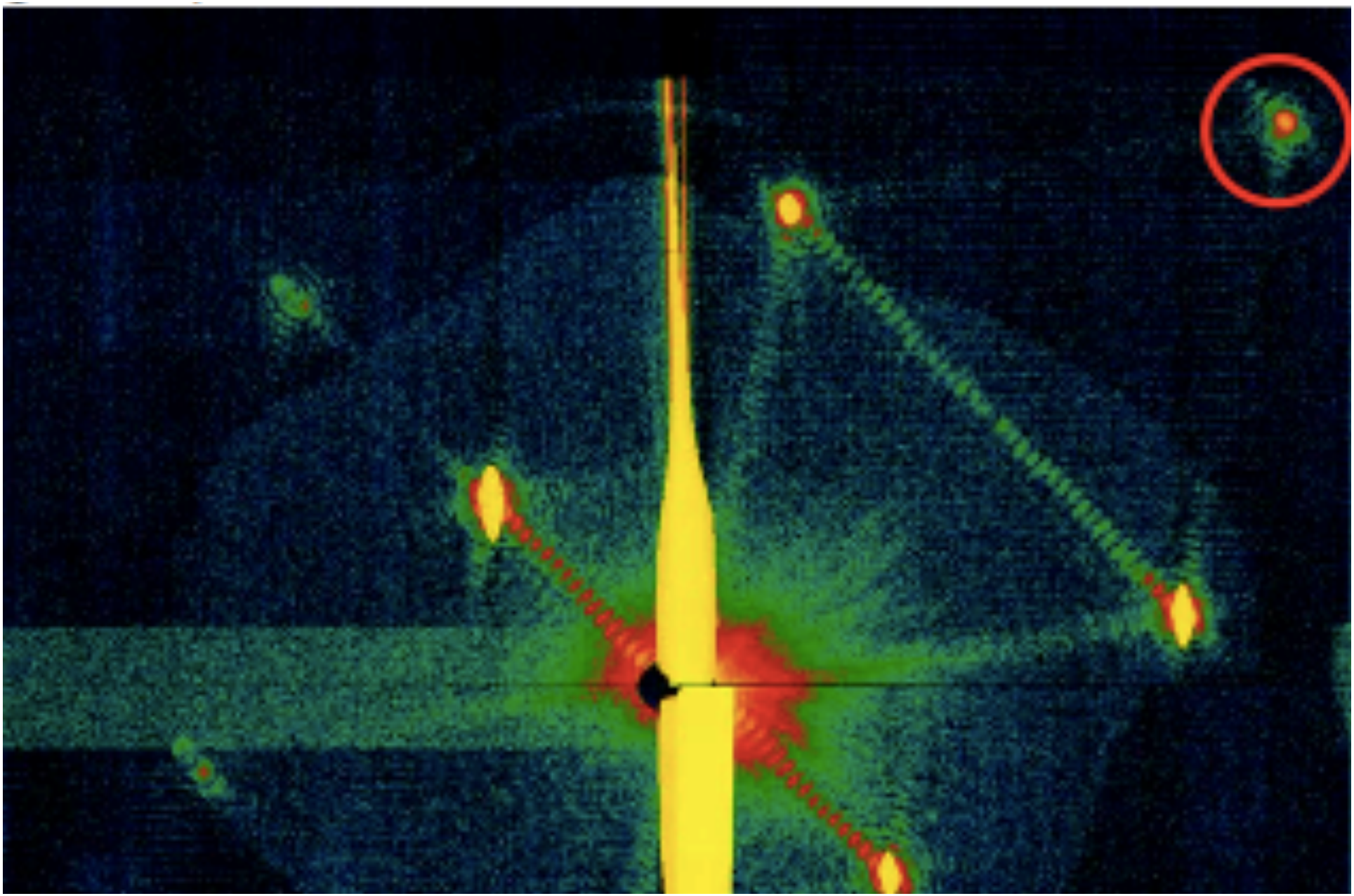


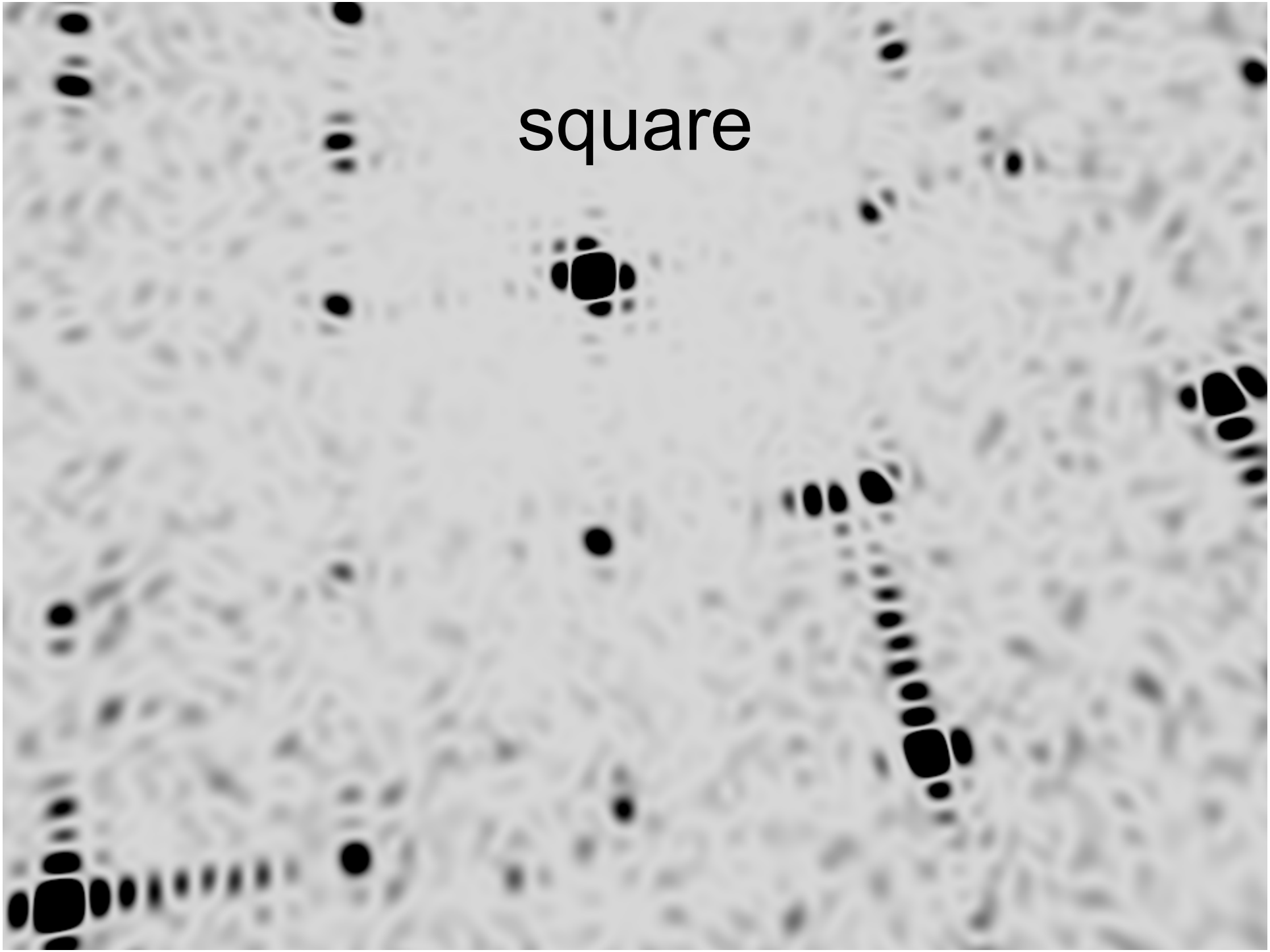


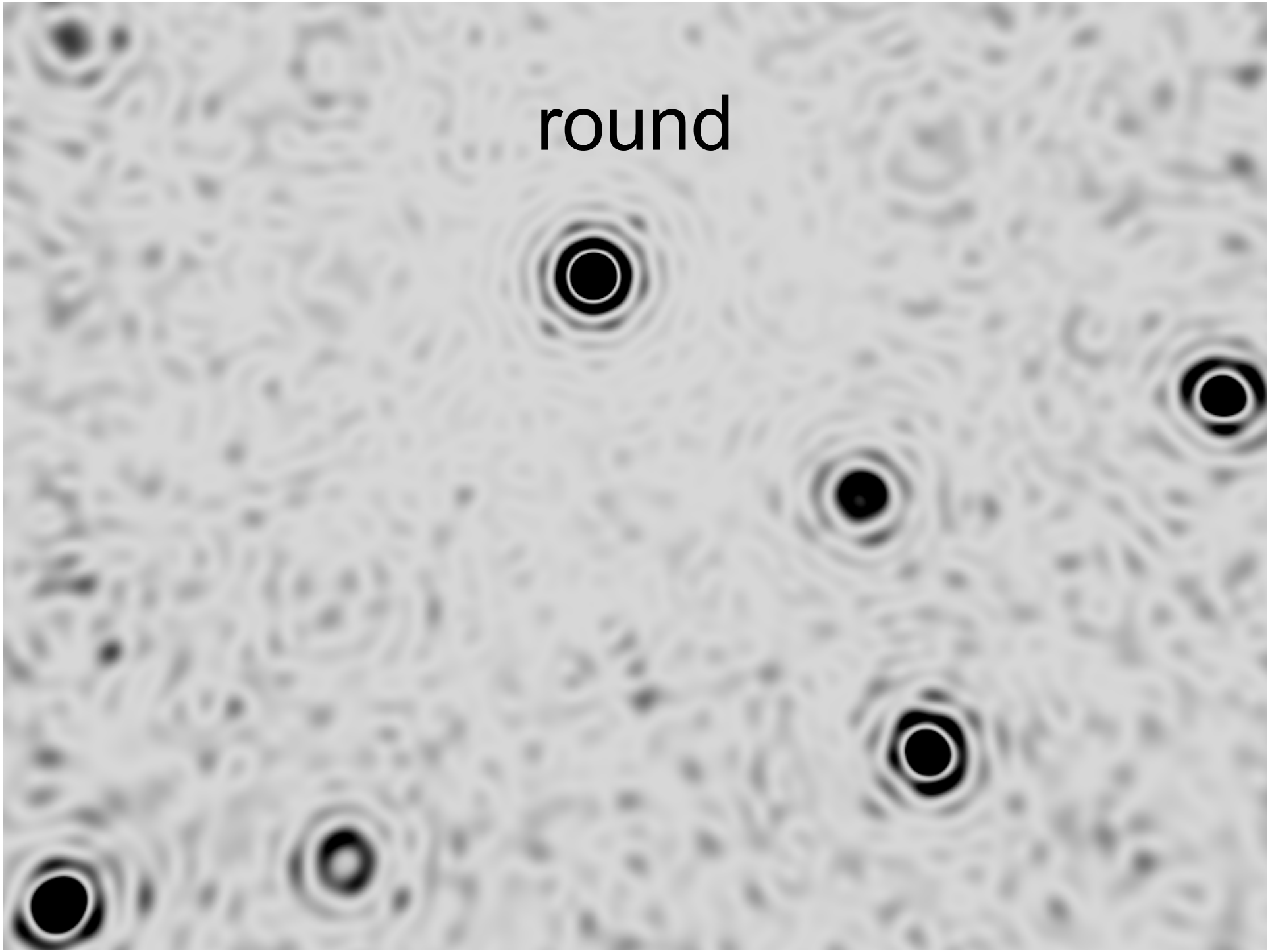
average total scattering from points



Inter-Bragg spots over-sample unit cell



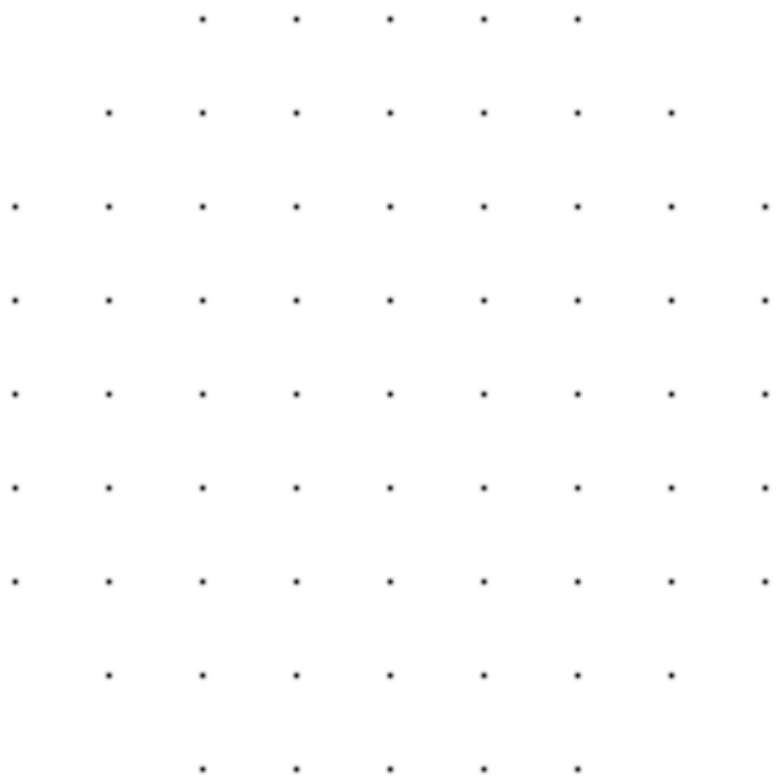

square


round

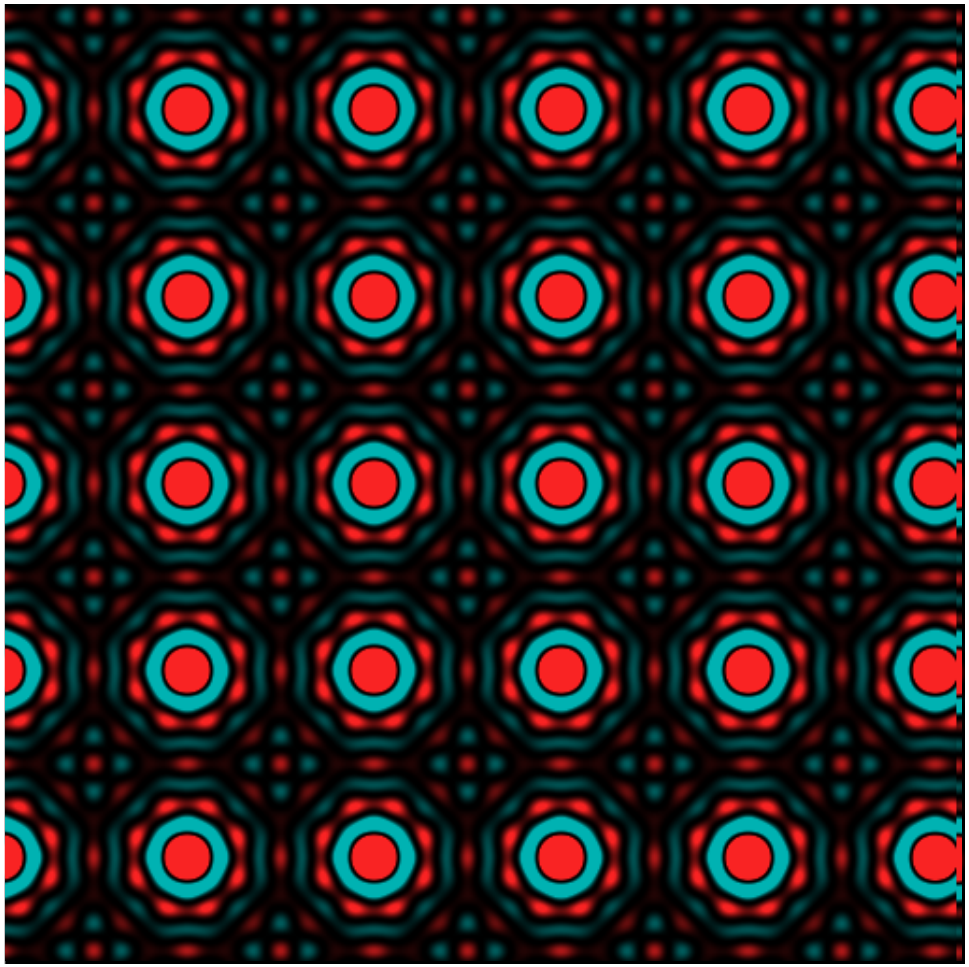
scattering from a lattice

colored by phase

sample



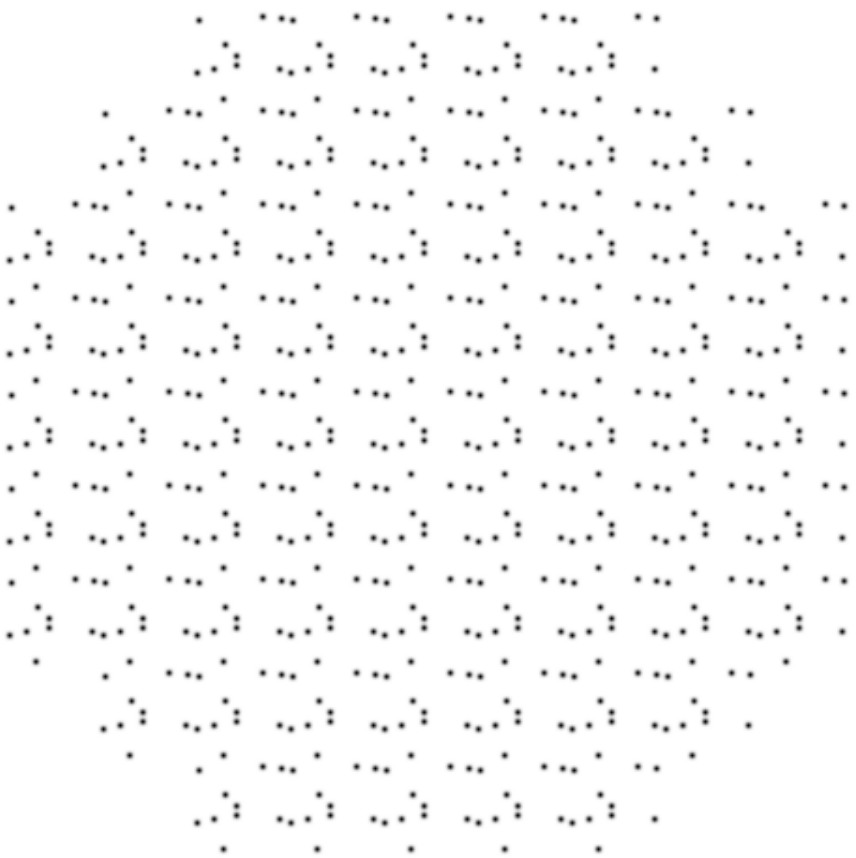
detector



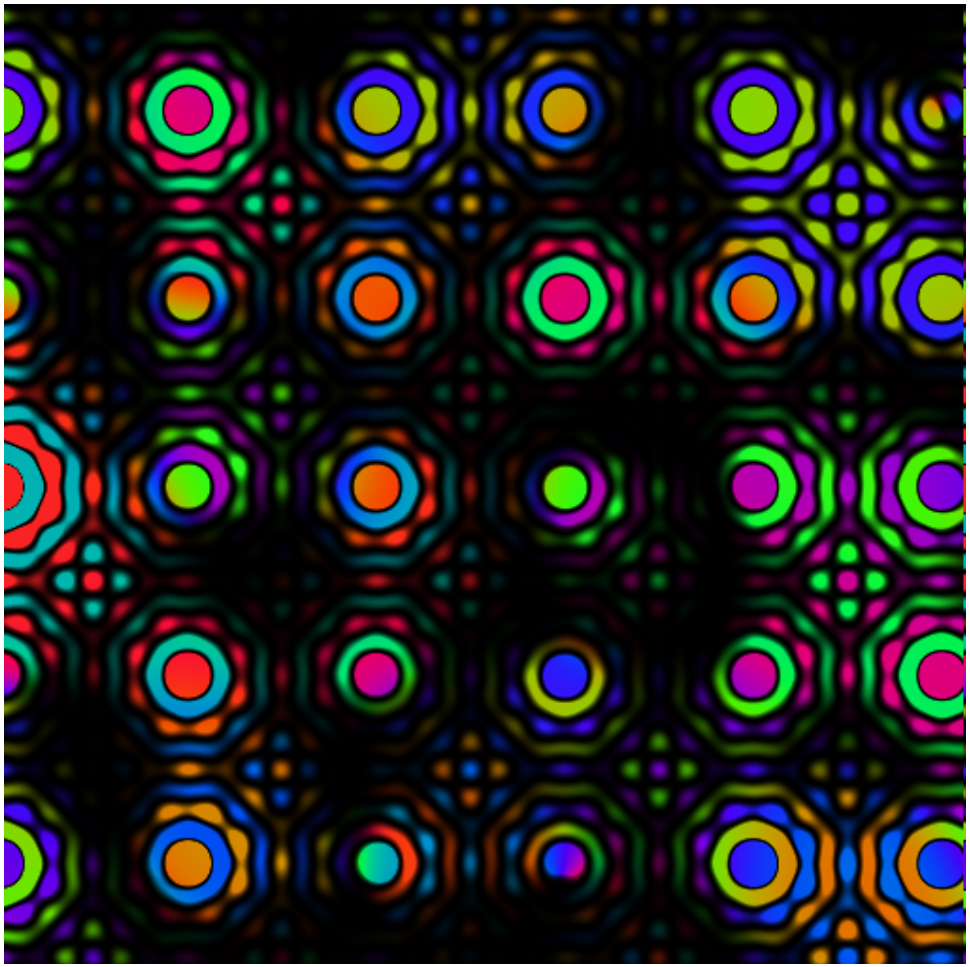
scattering from a crystal structure

colored by phase

sample



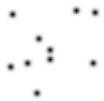
detector



scattering from a crystal structure

colored by phase

sample

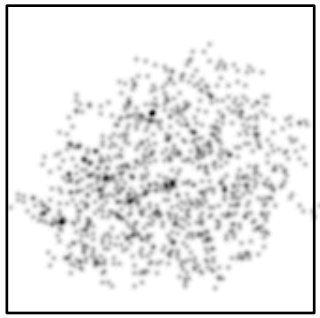


detector

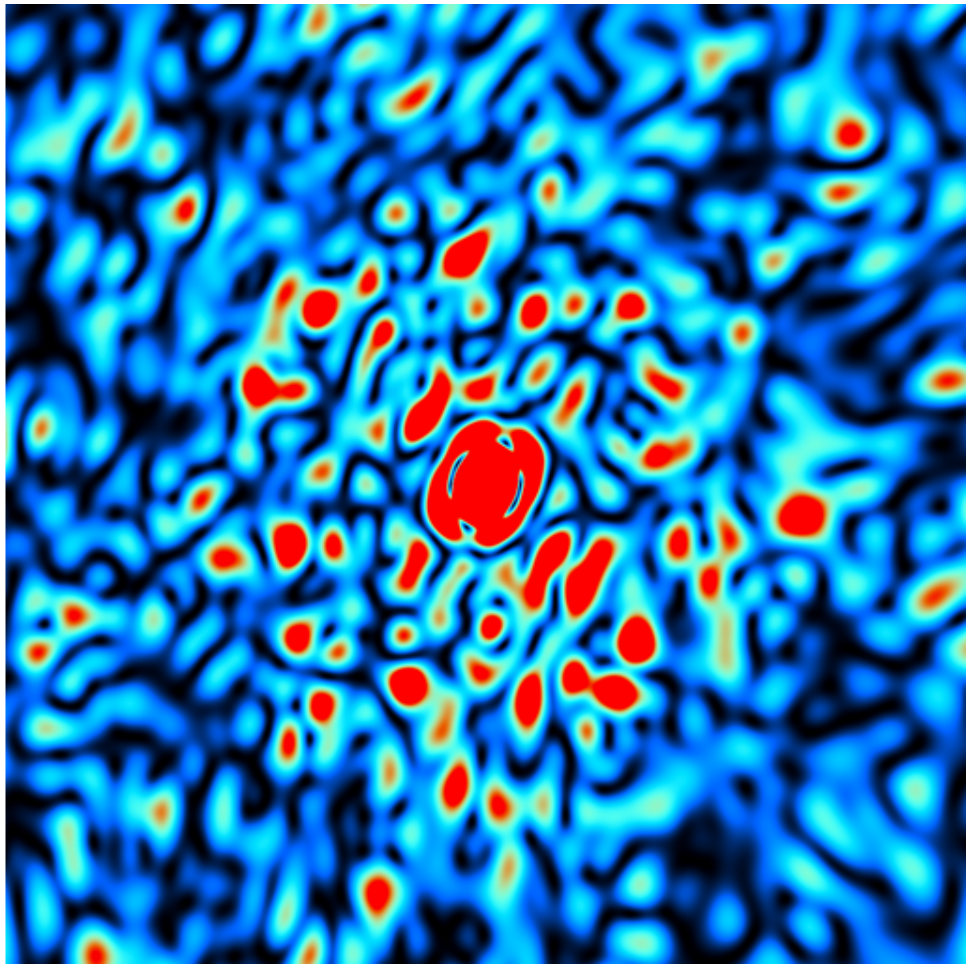
scattering from a crystal structure

False color intensity

sample



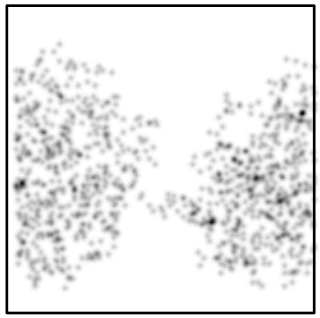
detector



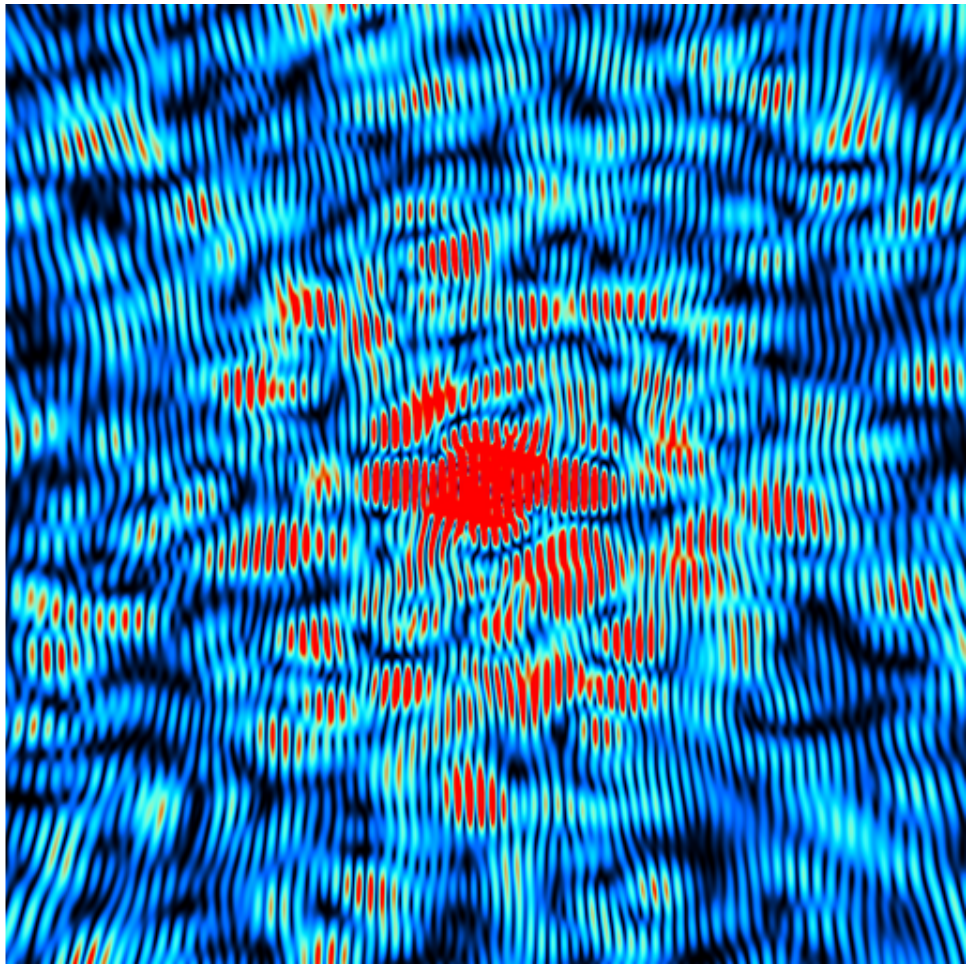
scattering from a crystal structure

False color intensity

sample



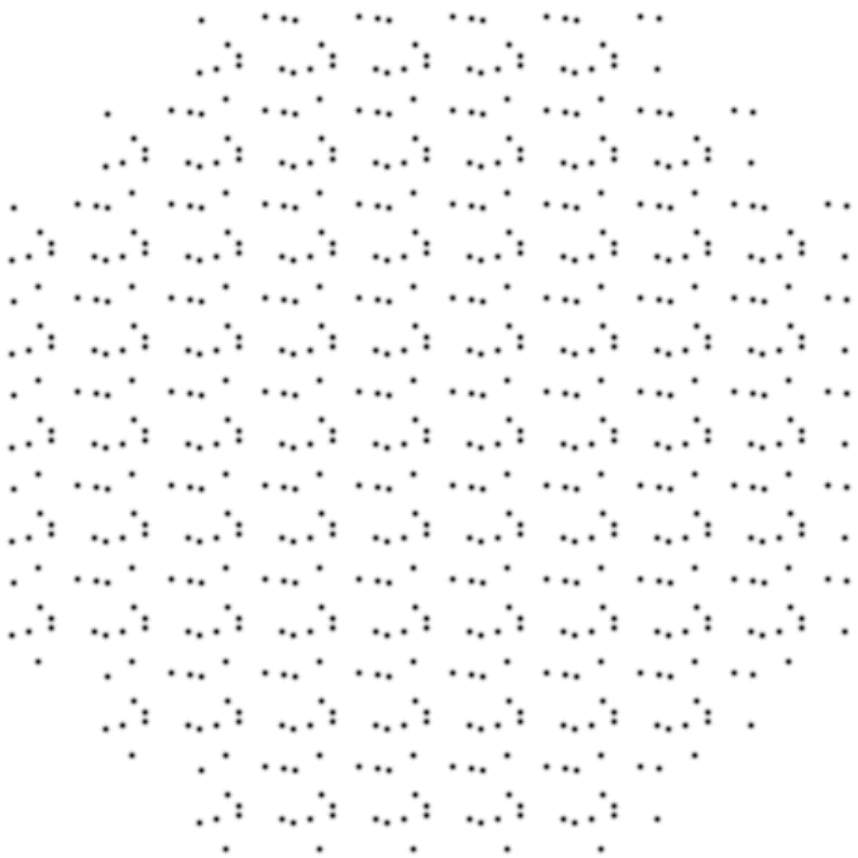
detector



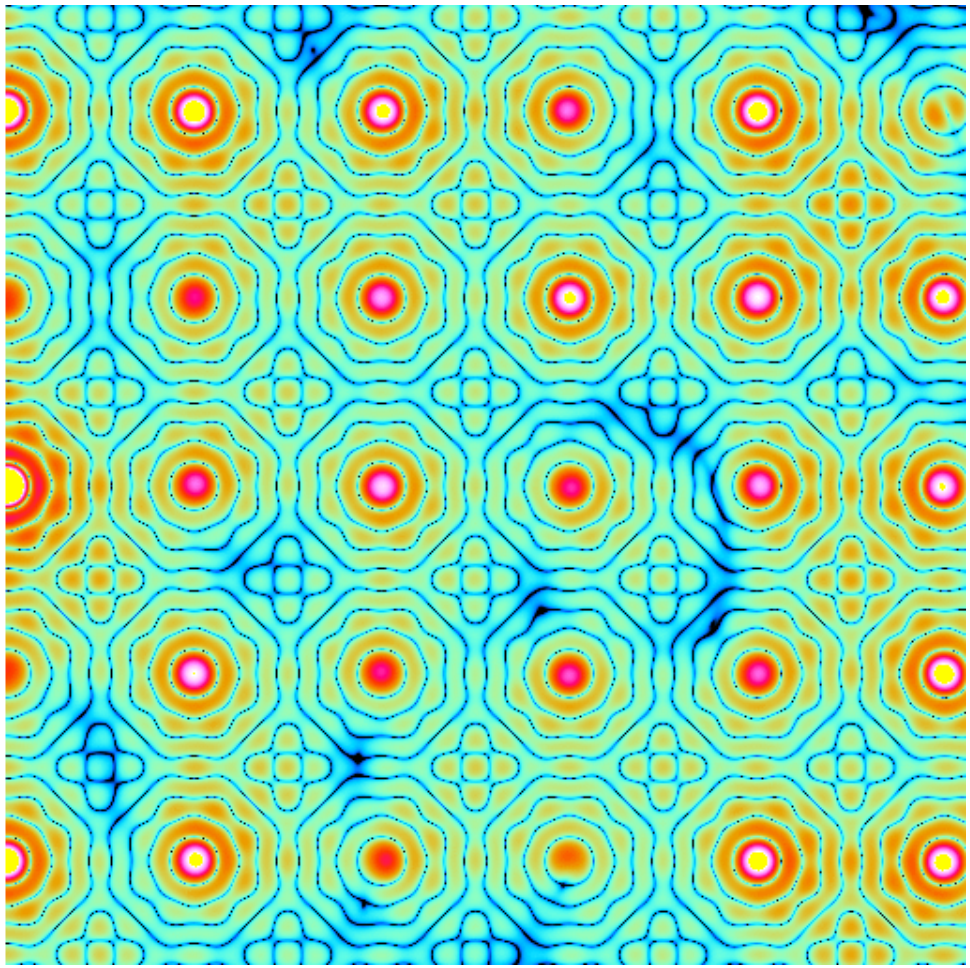
scattering from a crystal structure

False color intensity

sample



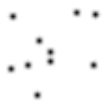
detector



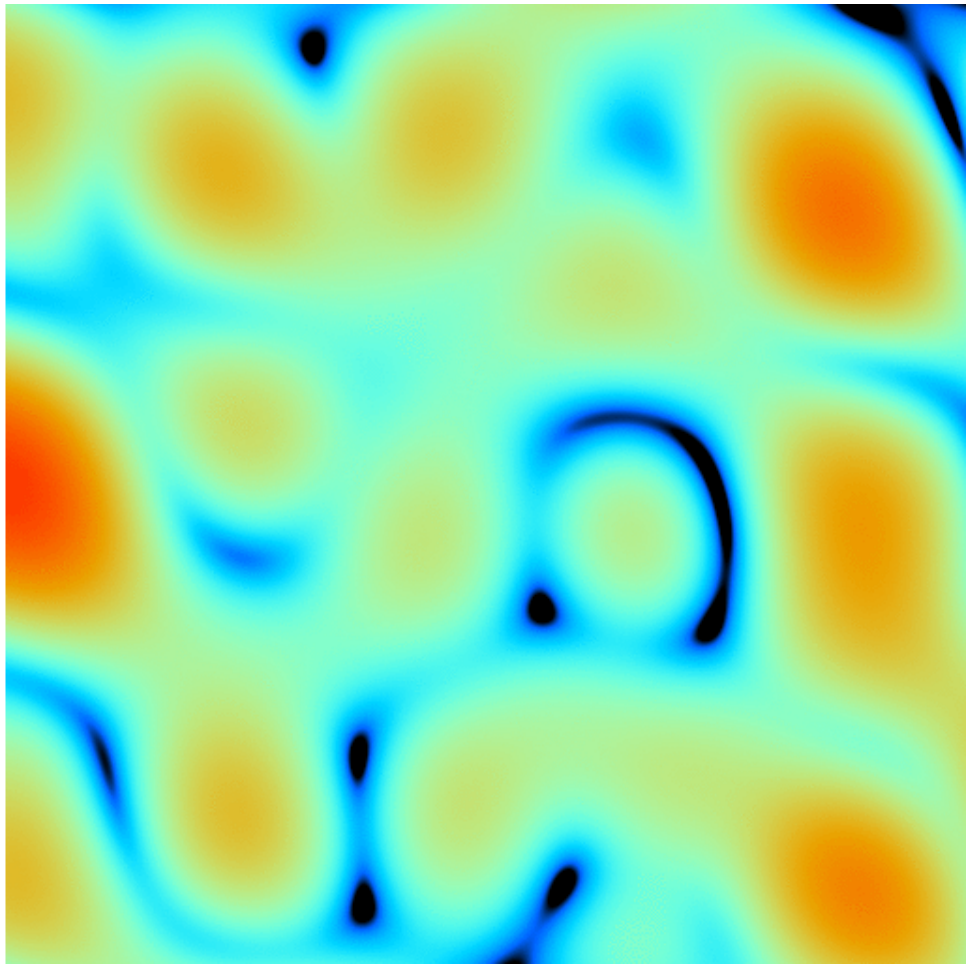
scattering from a crystal structure

False color intensity

sample



detector

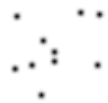


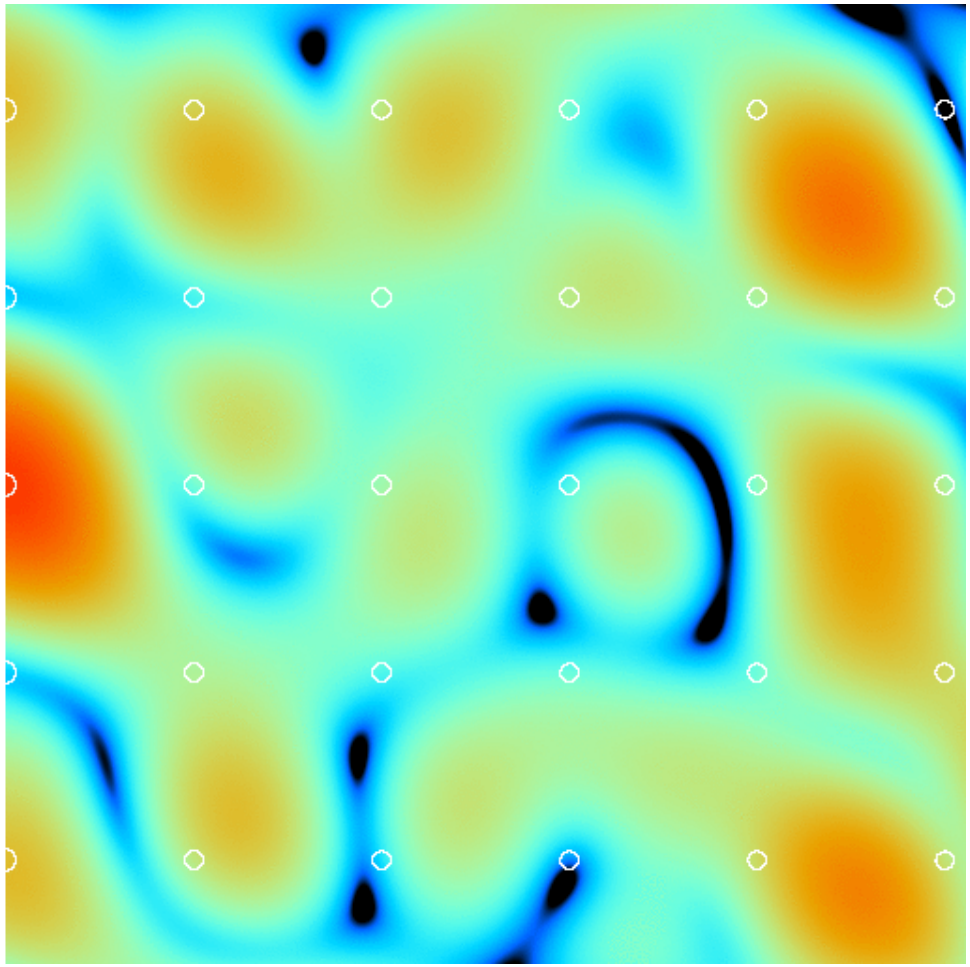
scattering from a crystal structure

False color intensity

sample

detector

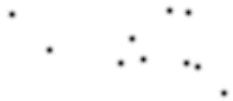




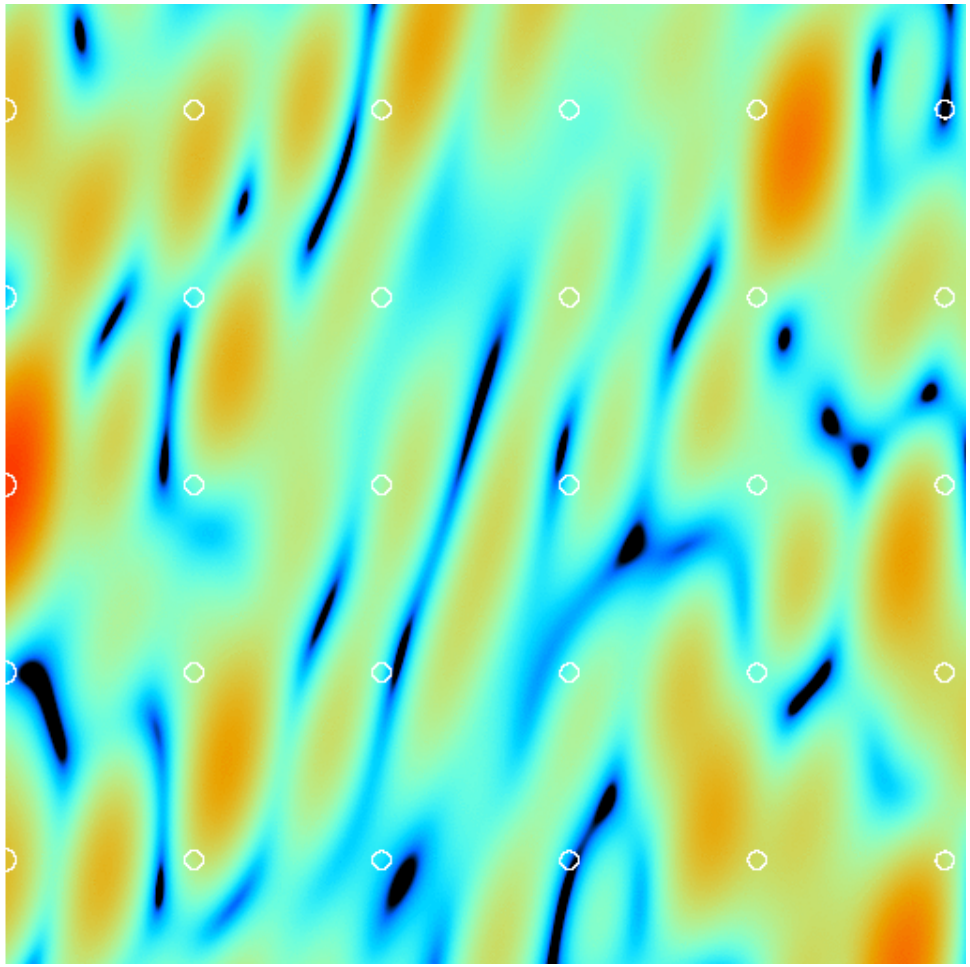
scattering from a crystal structure

False color intensity

sample



detector

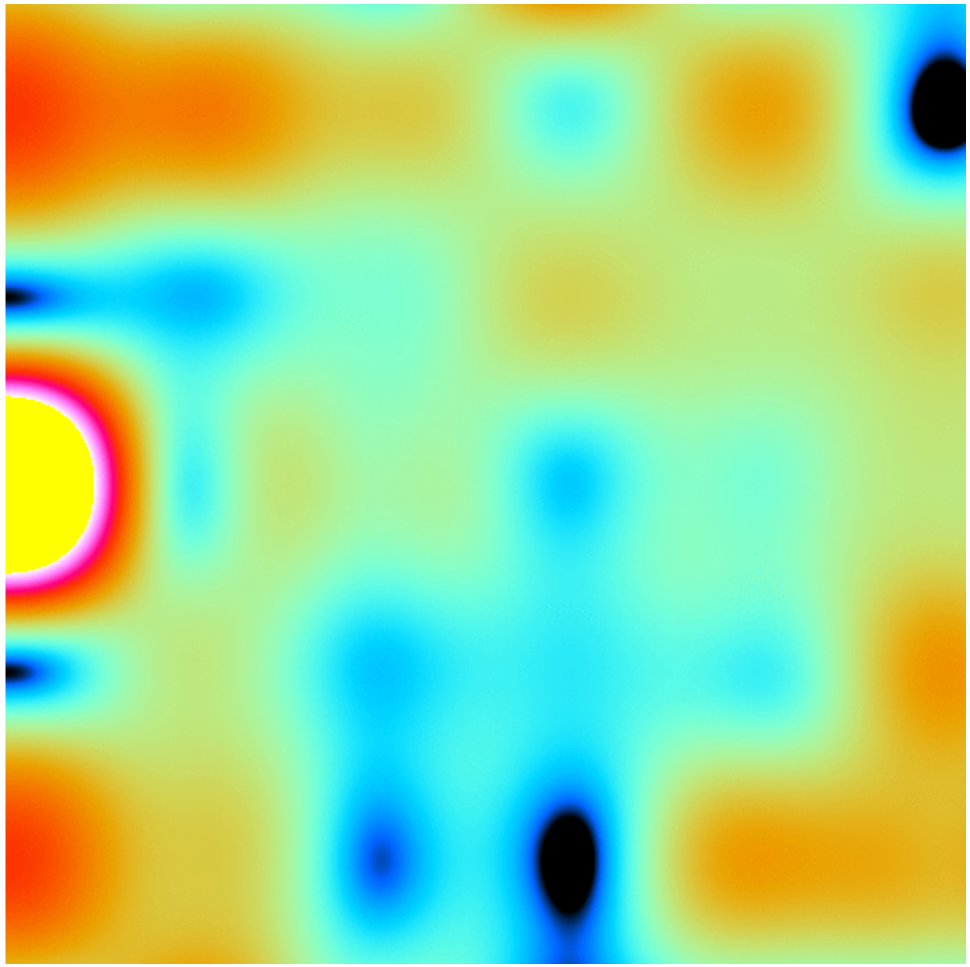


scattering from a crystal structure

False color intensity

sample

detector

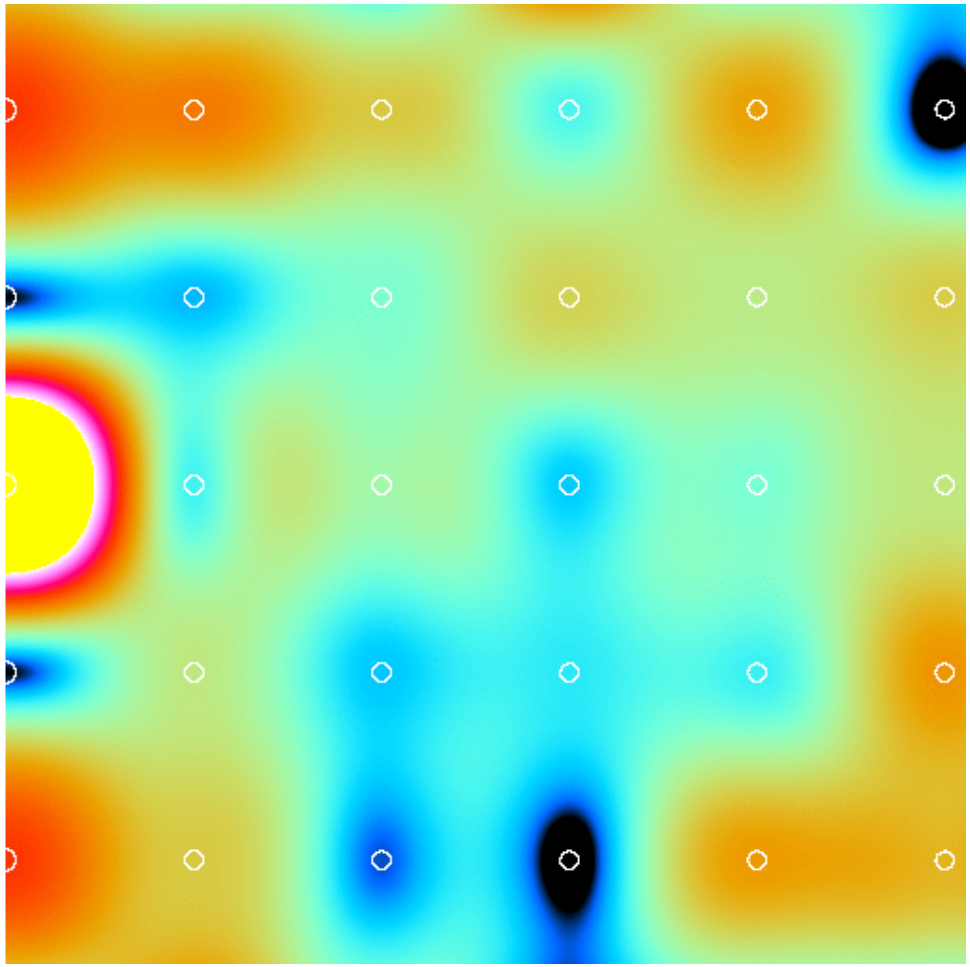


scattering from a crystal structure

False color intensity

sample

detector

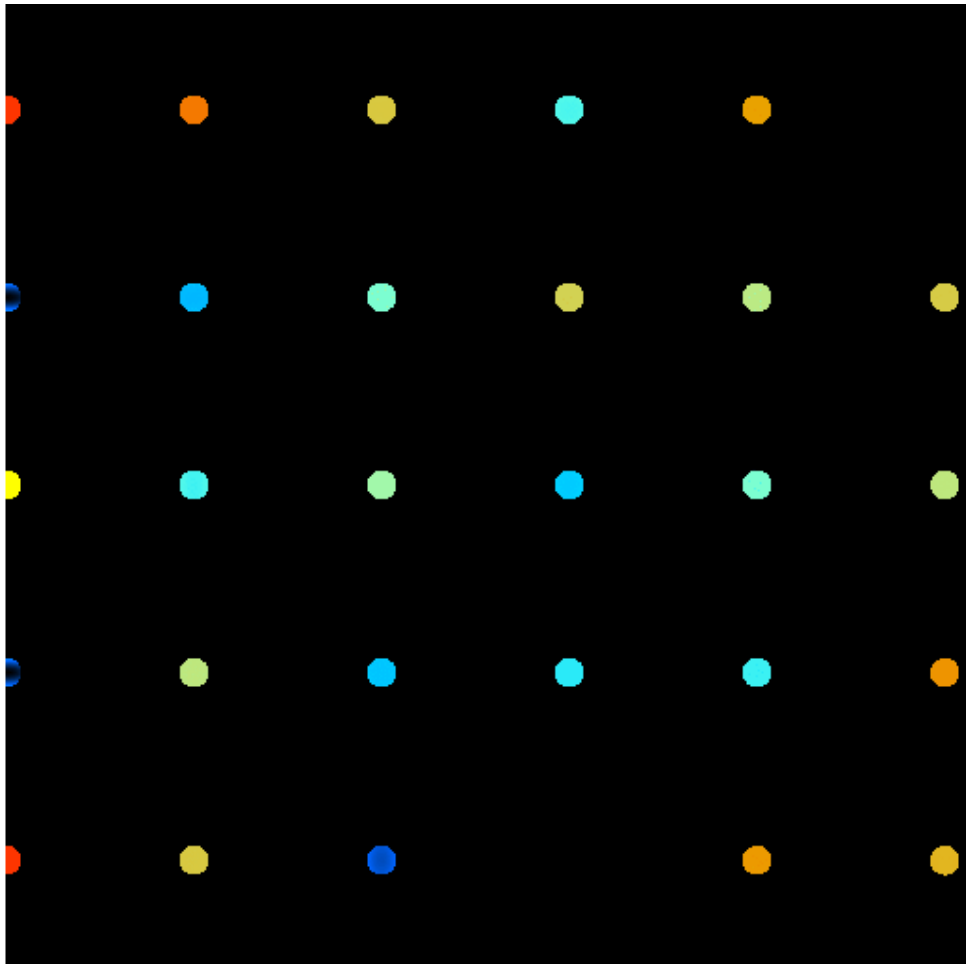


scattering from a crystal structure

False color intensity

sample

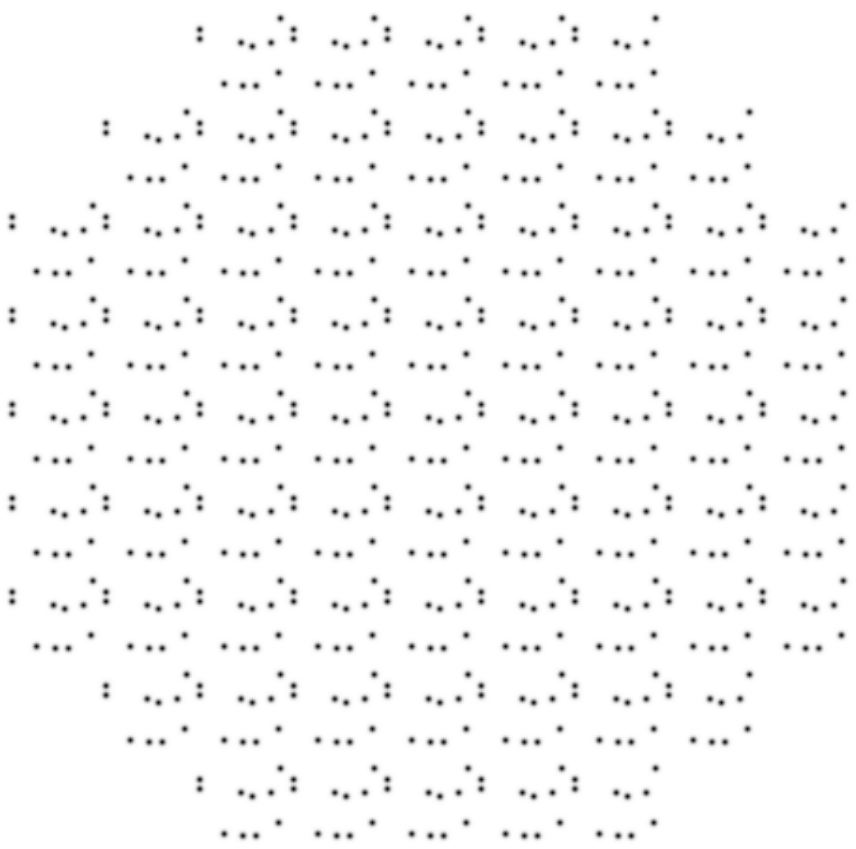
detector



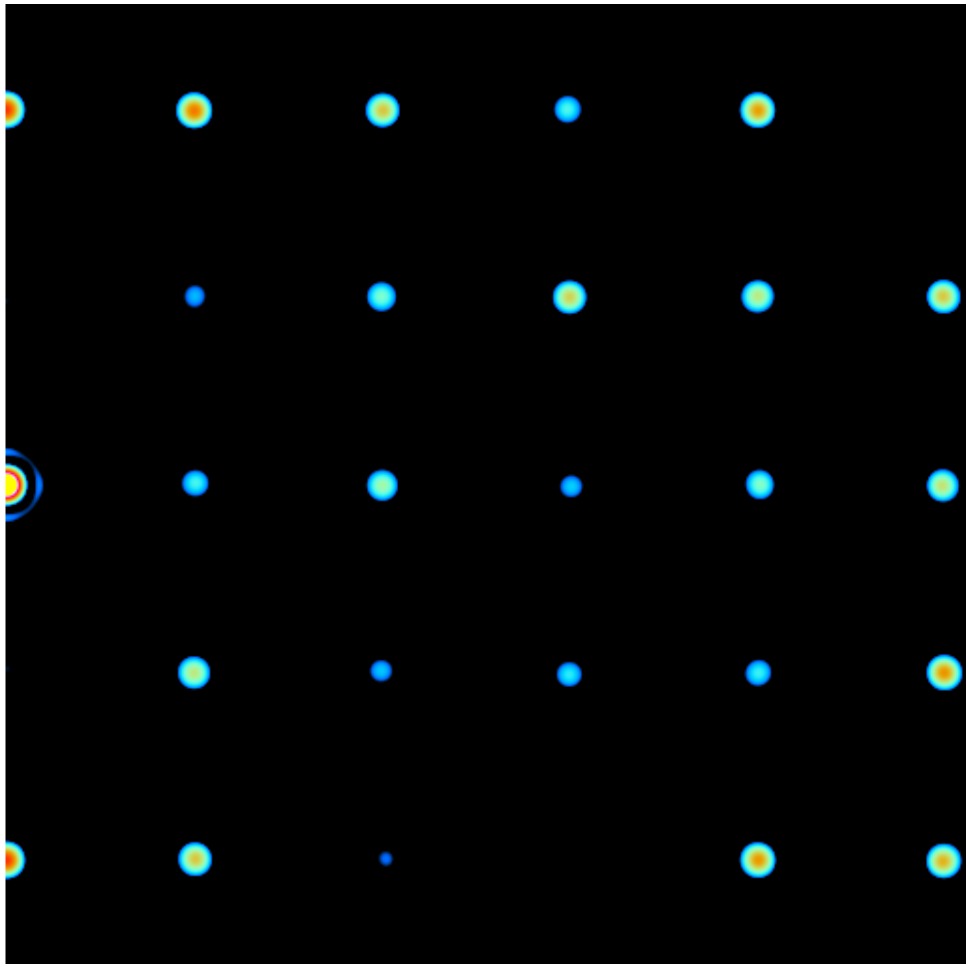
scattering from a crystal structure

False color intensity

sample



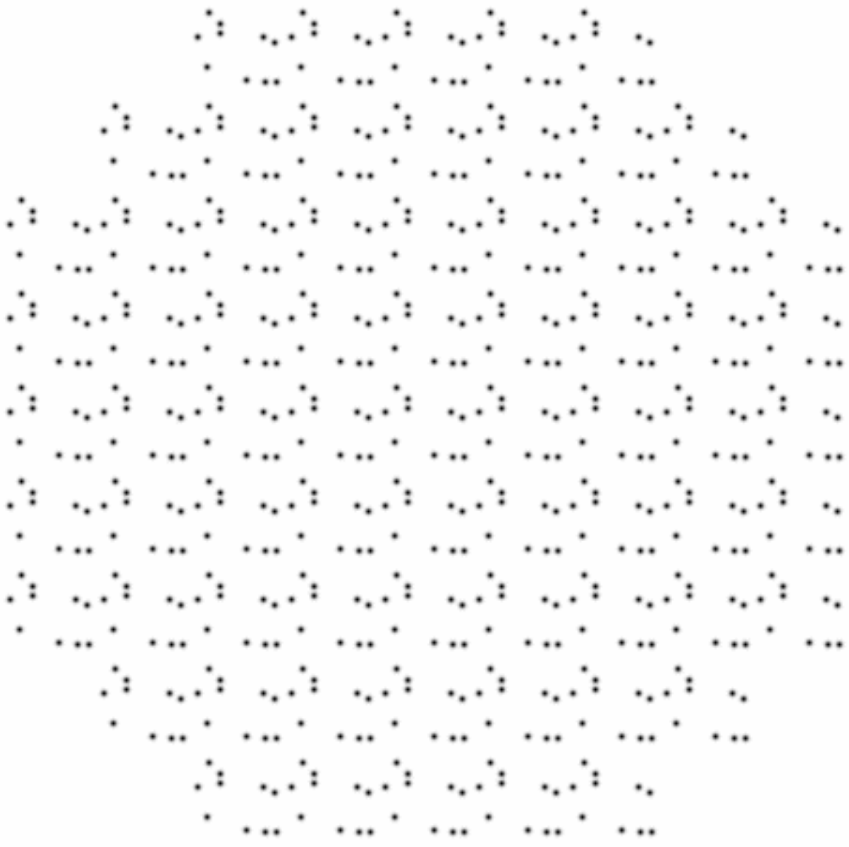
detector



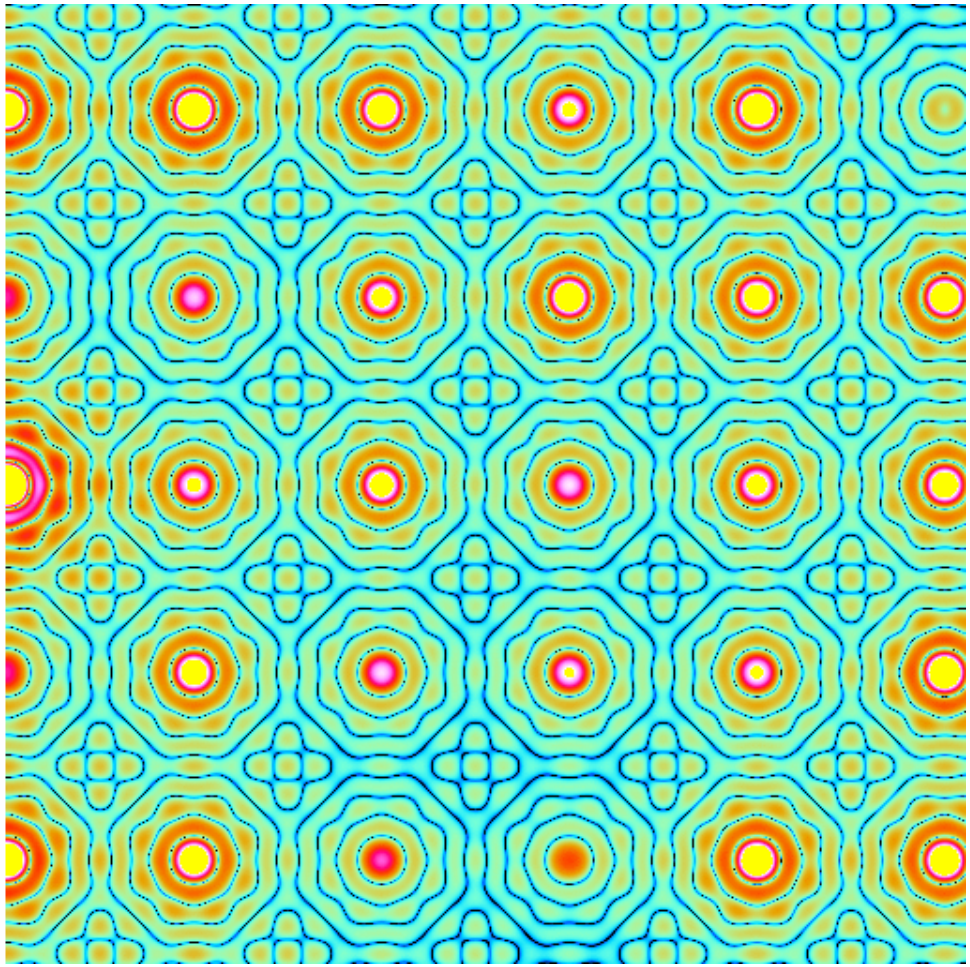
scattering from a crystal structure

False color intensity

sample



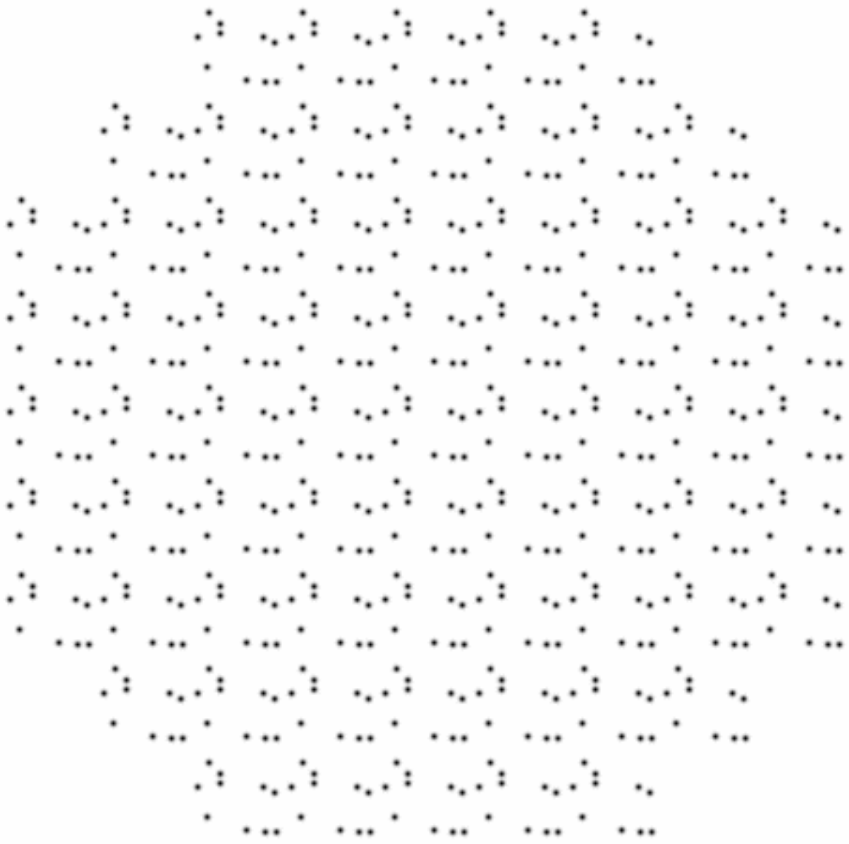
detector



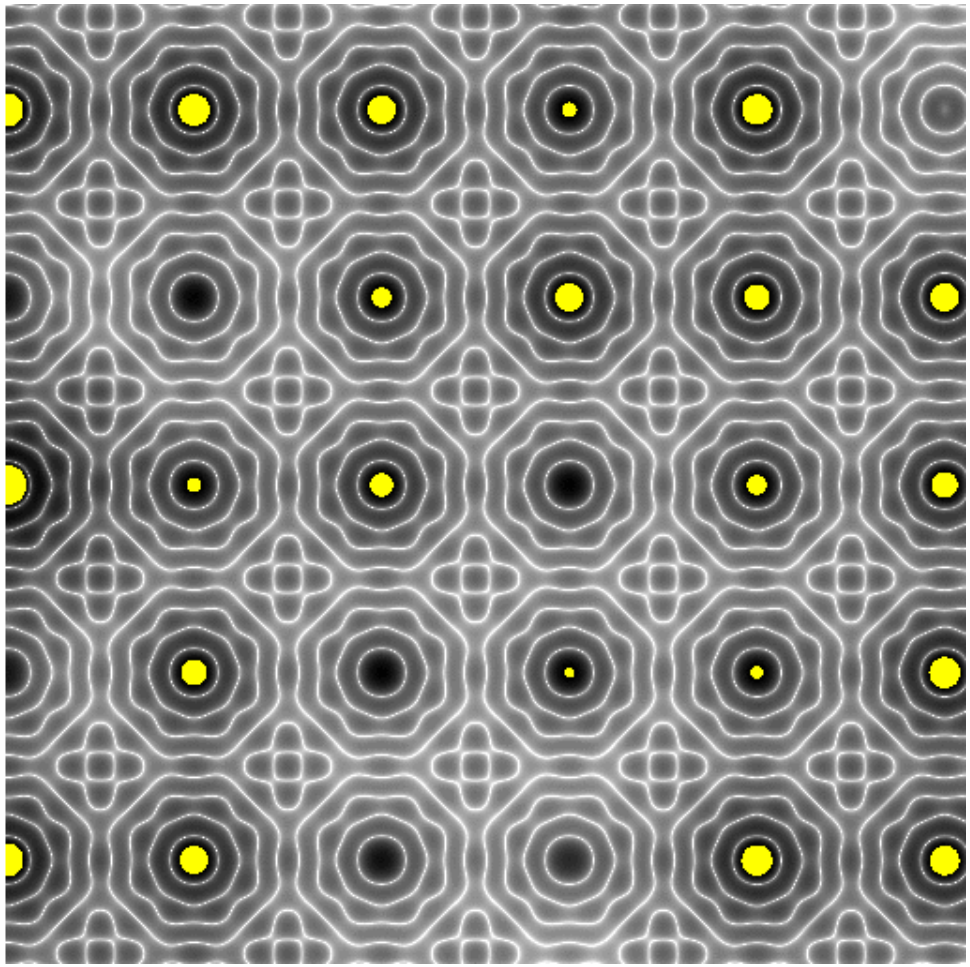
scattering from a crystal structure

False color intensity

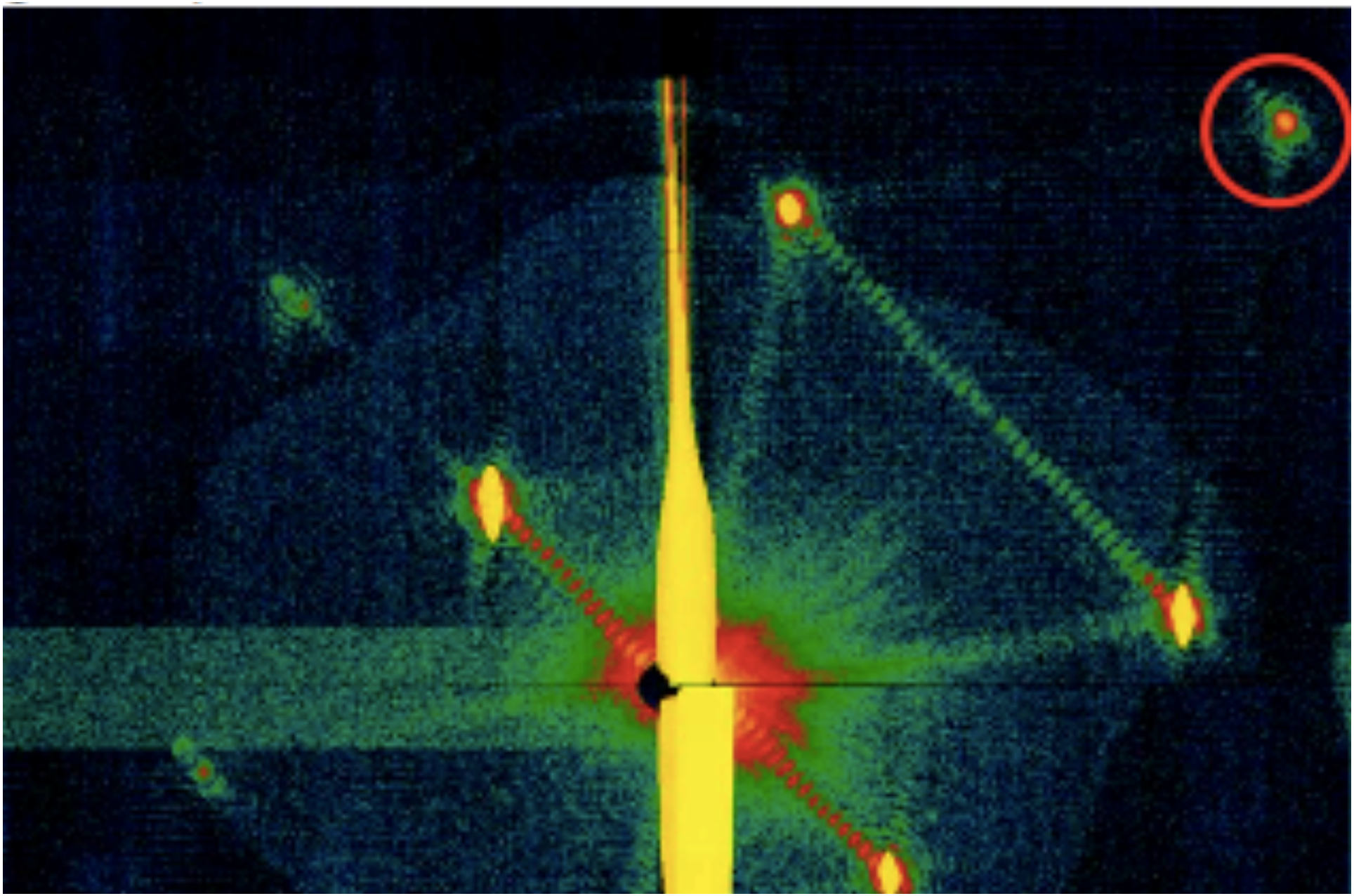
sample



detector



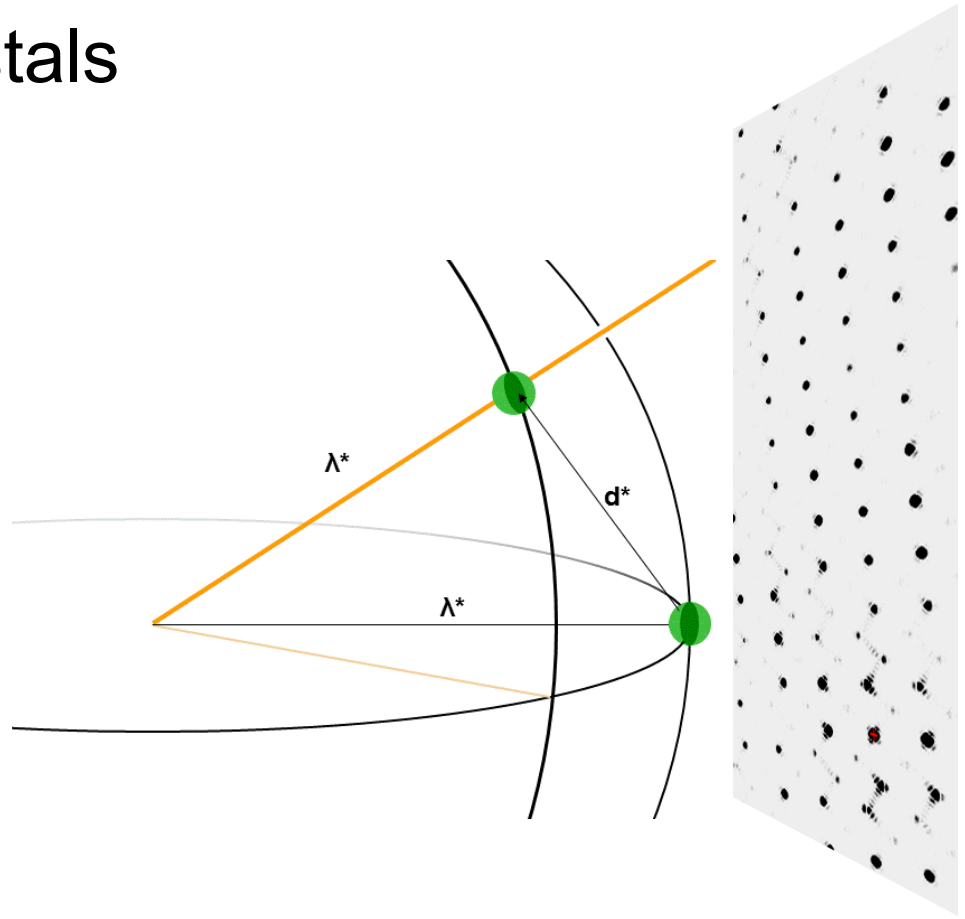
Inter-Bragg spots over-sample unit cell?

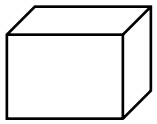


fastBragg program

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

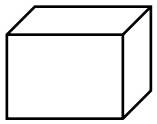
- Optimized for nanocrystals
- Square or round
- Takes h,k,l and F
- Supports 1 unit cell
- no “mosaicity”
- arbitrary “source”
- arbitrary “phi” steps





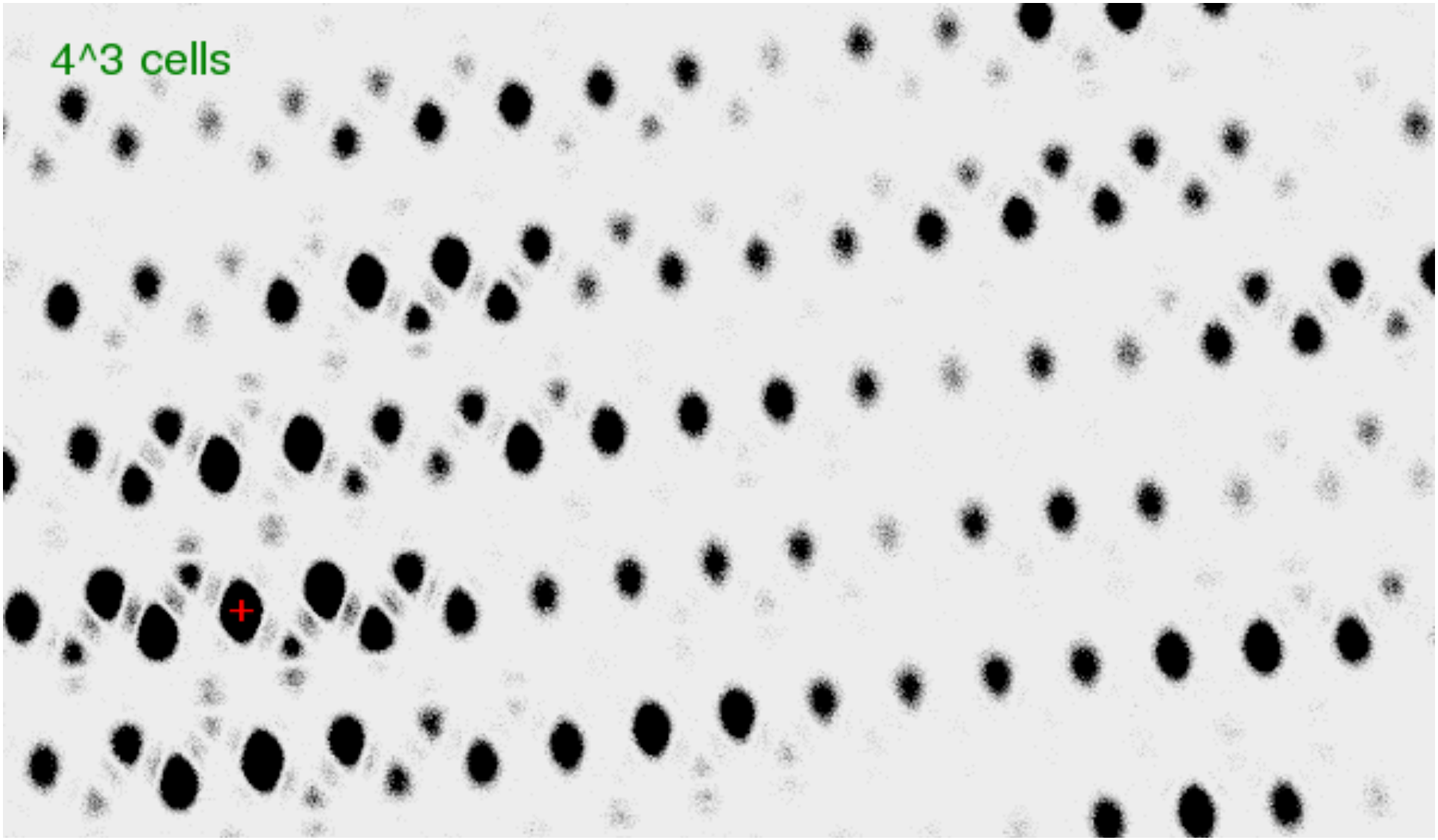
fastBragg program

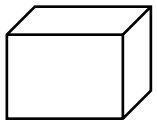
http://bl831.als.lbl.gov/~jamesh/fastBragg/



fastBragg program

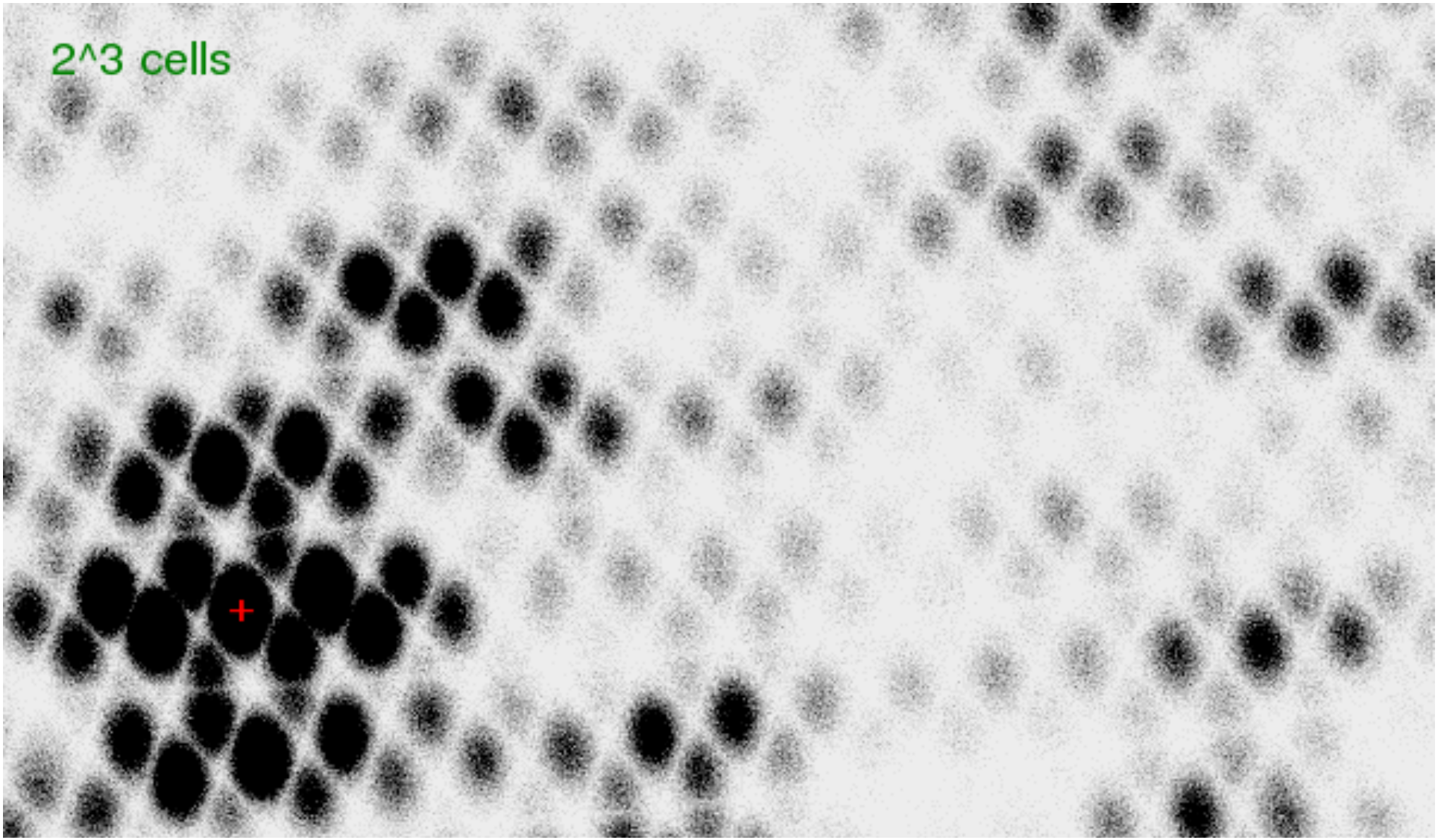
http://bl831.als.lbl.gov/~jamesh/fastBragg/

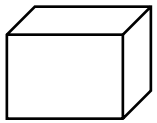




fastBragg program

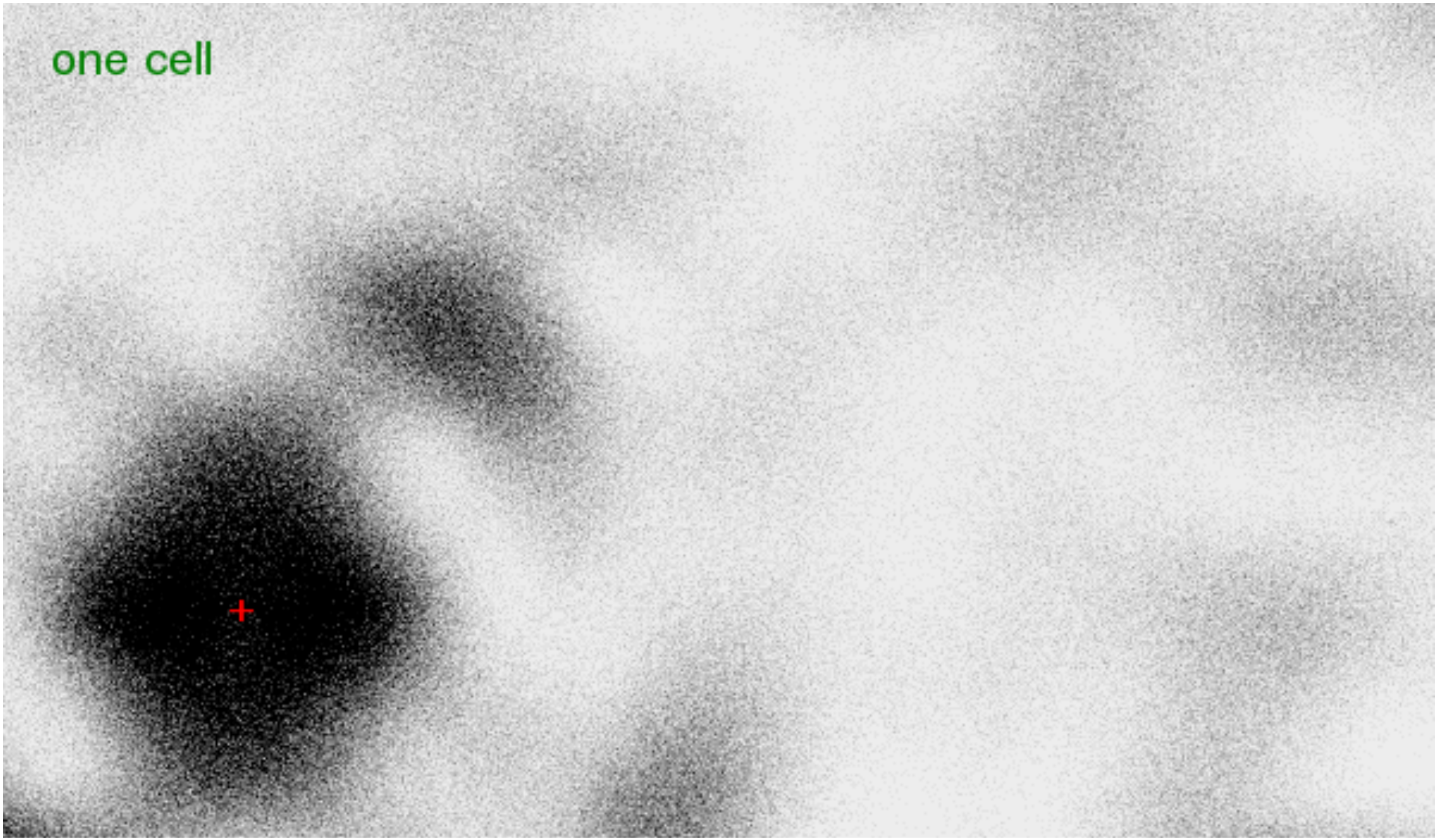
http://bl831.als.lbl.gov/~jamesh/fastBragg/



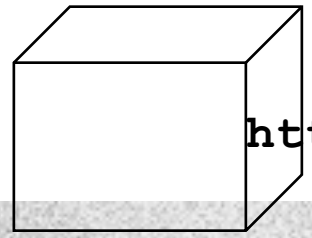


fastBragg program

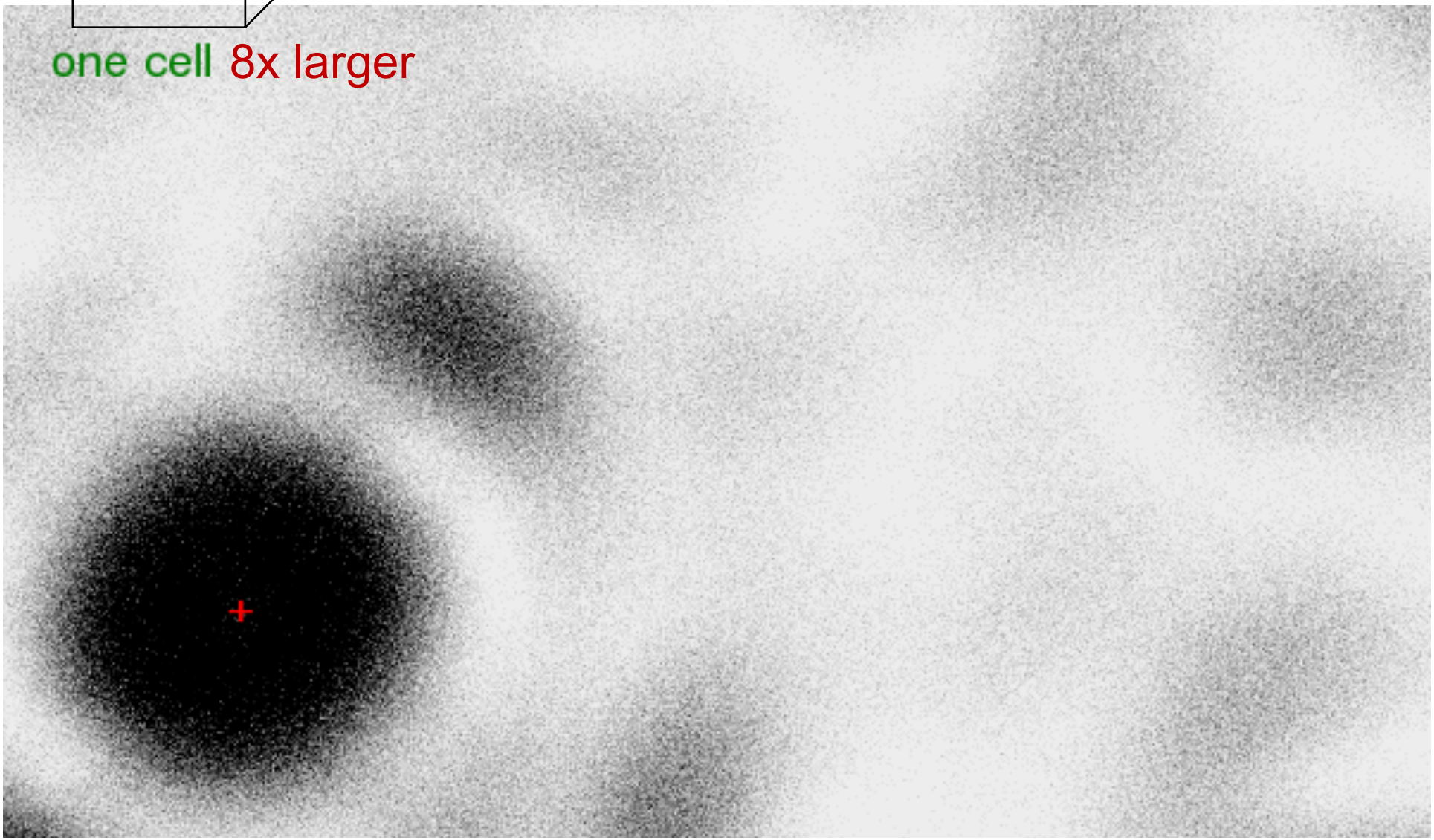
http://bl831.als.lbl.gov/~jamesh/fastBragg/



fastBragg program

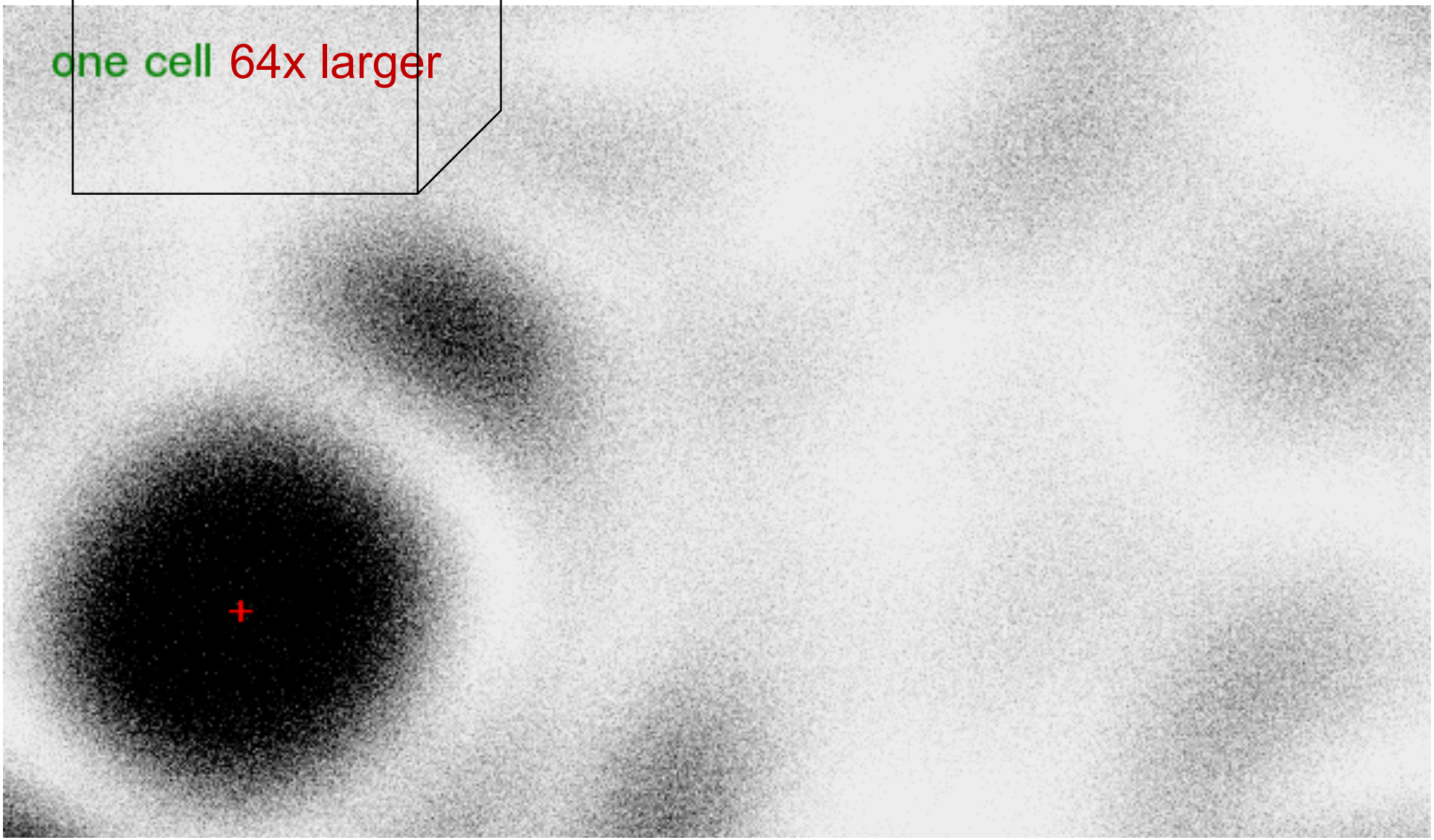


http://bl831.als.lbl.gov/~jamesh/fastBragg/



fastBragg program

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



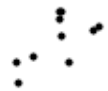
Total scattering from a structure

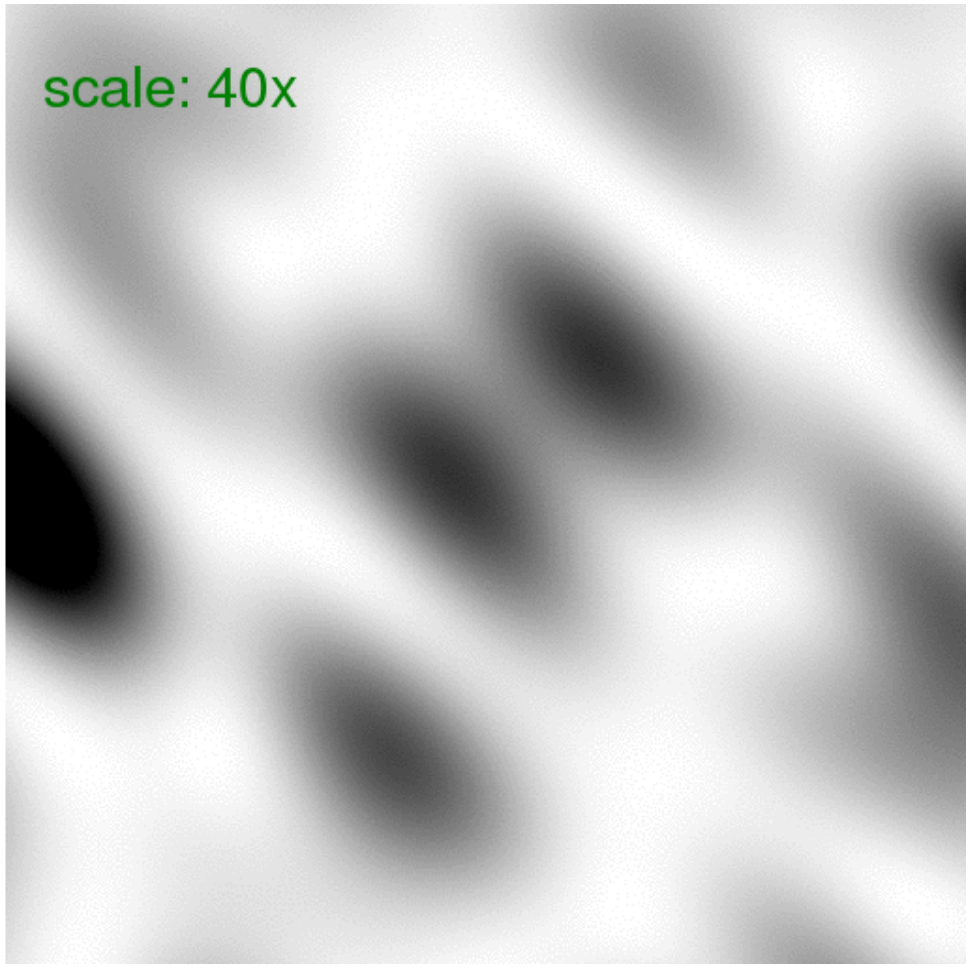
Move one atom

sample

detector

A





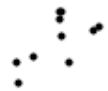
Total scattering from a structure

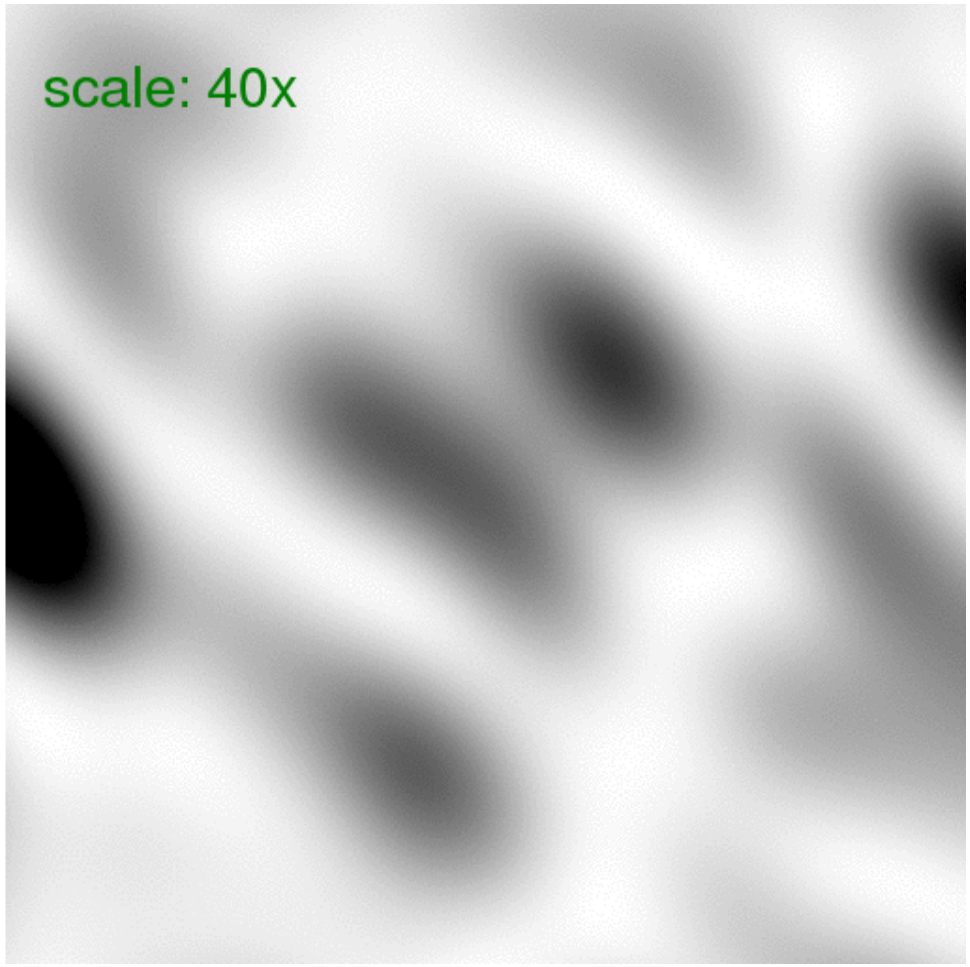
Intensity sum: $F_1^2+F_2^2$

sample

detector

A





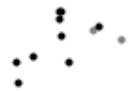
Total scattering from a structure

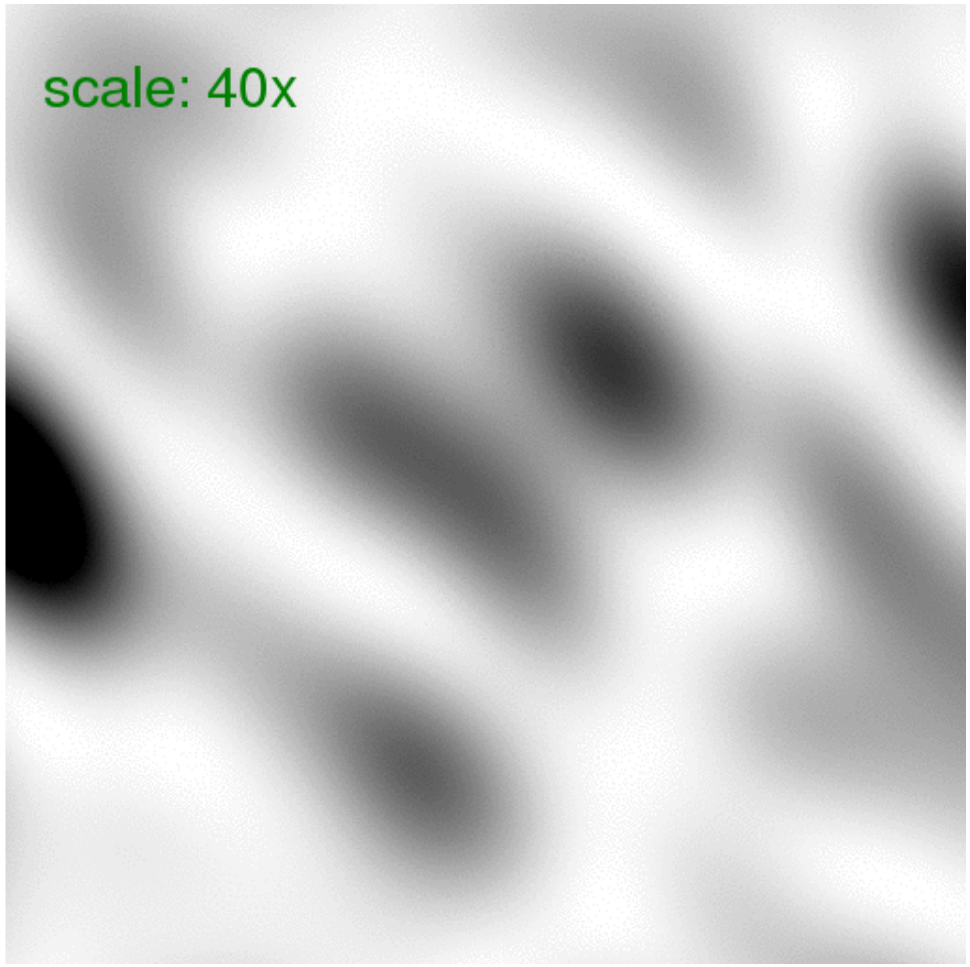
Coherent sum: $(F_1+F_2)^2$

sample

detector

AB





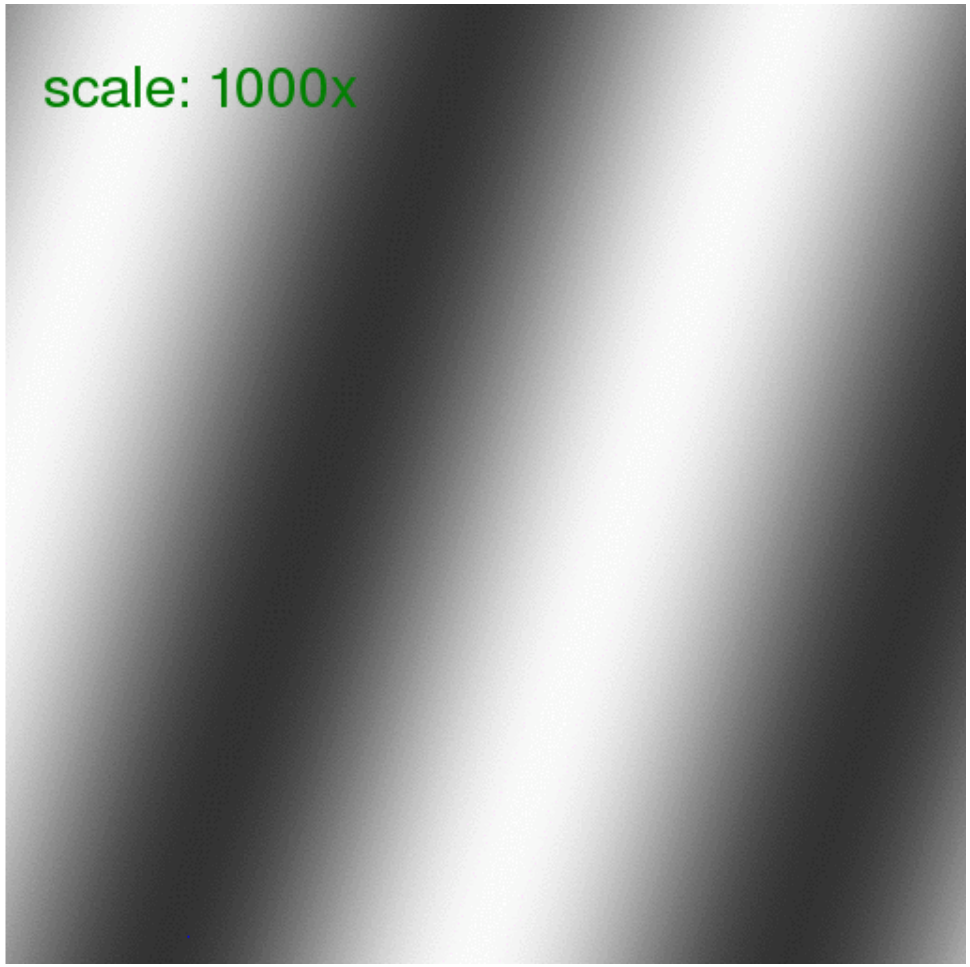
Total scattering from a structure

Incoherent – coherent difference: $(F_1^2+F_2^2) - (F_1+F_2)^2$

sample

detector

A



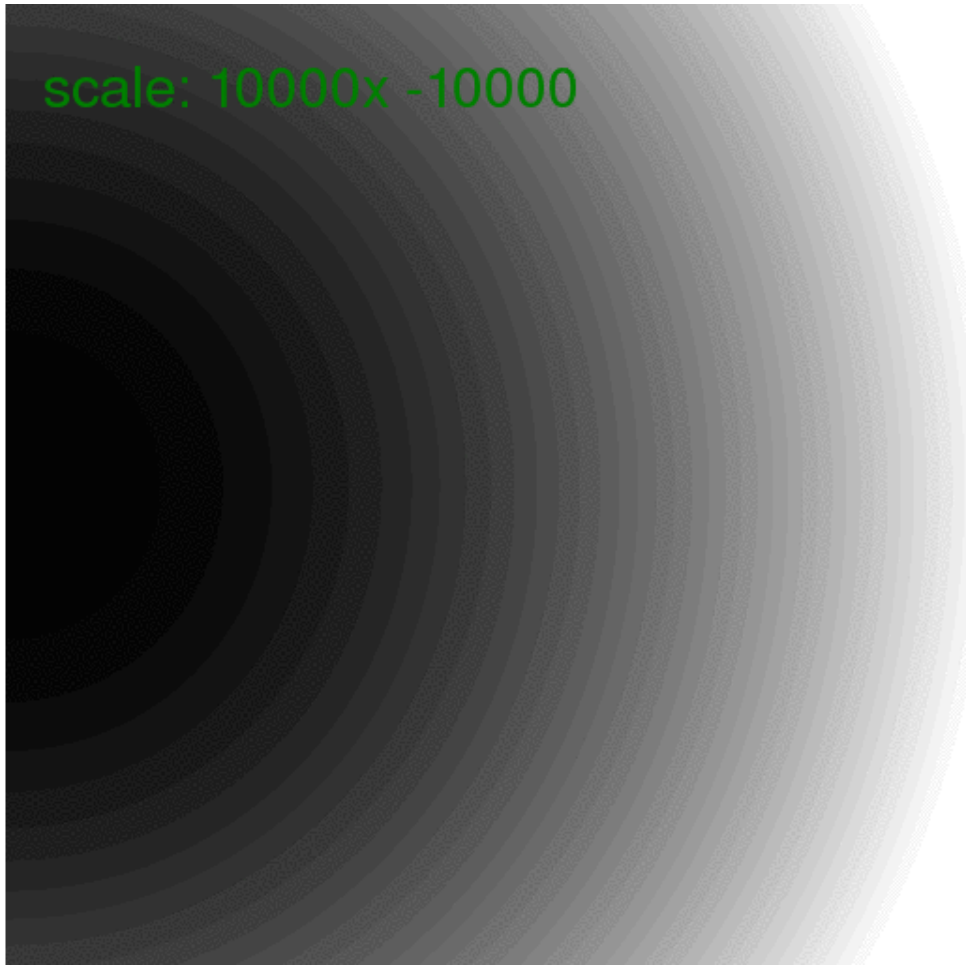
Total scattering from one atom

Intensity sum: $F_1^2+F_2^2$

sample

detector

A



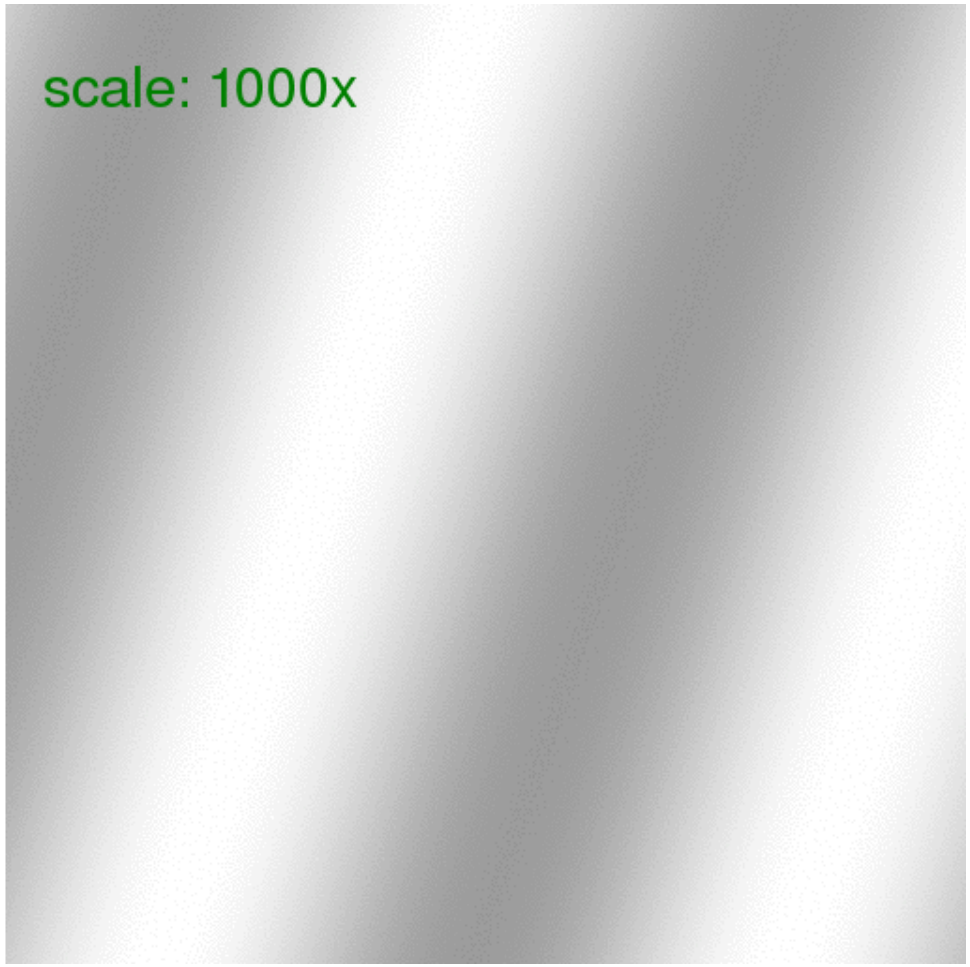
Total scattering from two ½ atoms

Coherent sum: $(F_1+F_2)^2$

sample

detector

AB



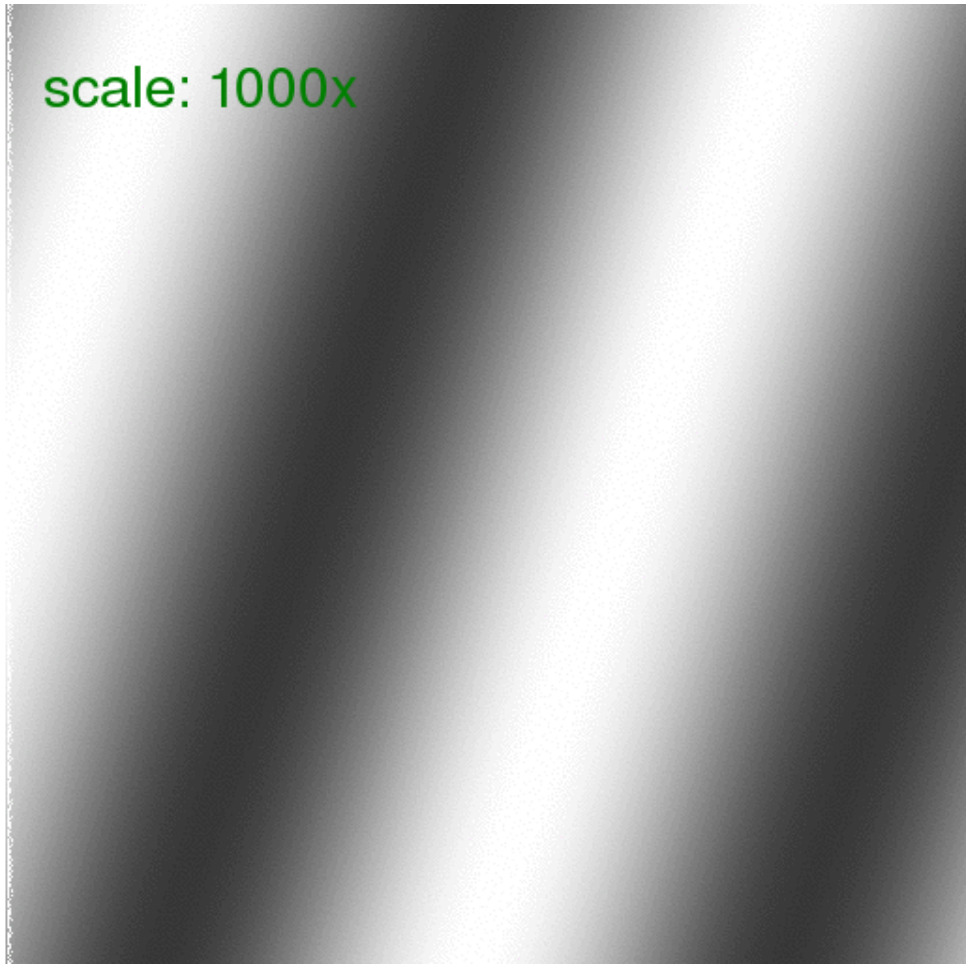
Total scattering from one atom

Incoherent – coherent difference: $(F_1^2+F_2^2) - (F_1+F_2)^2$

sample

detector

A



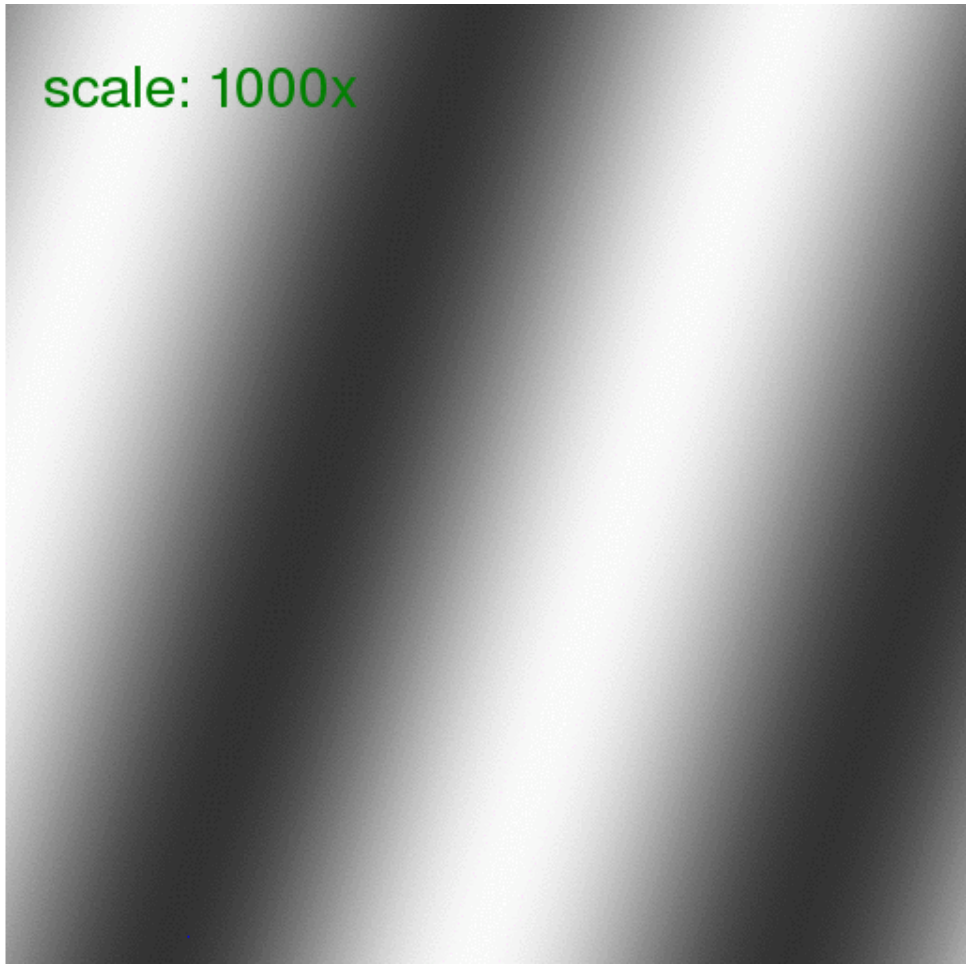
Total scattering from a structure

Incoherent – coherent difference: $(F_1^2+F_2^2) - (F_1+F_2)^2$

sample

detector

A

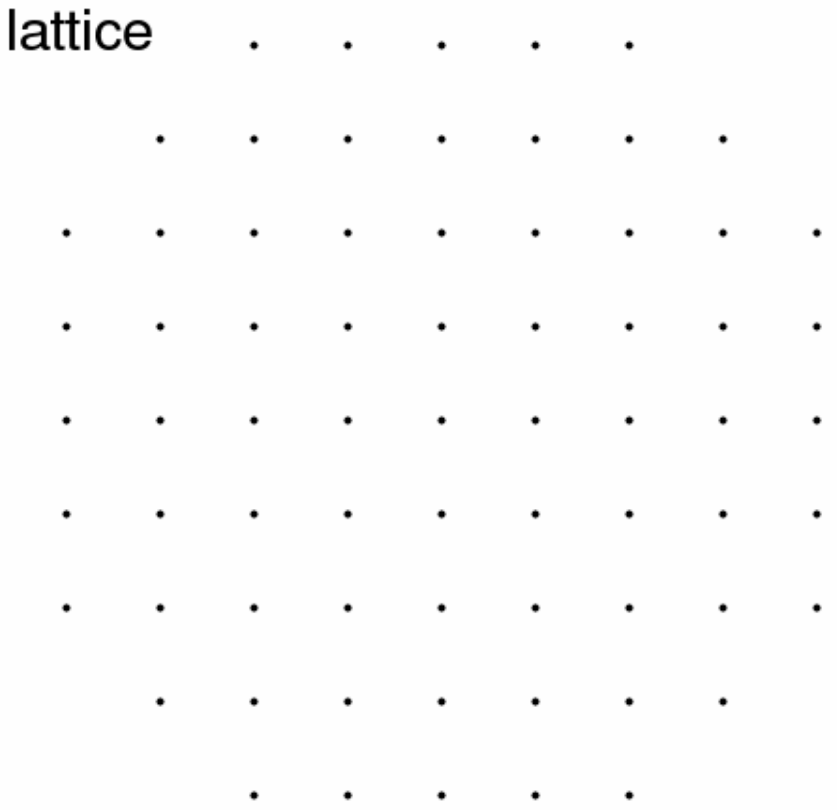


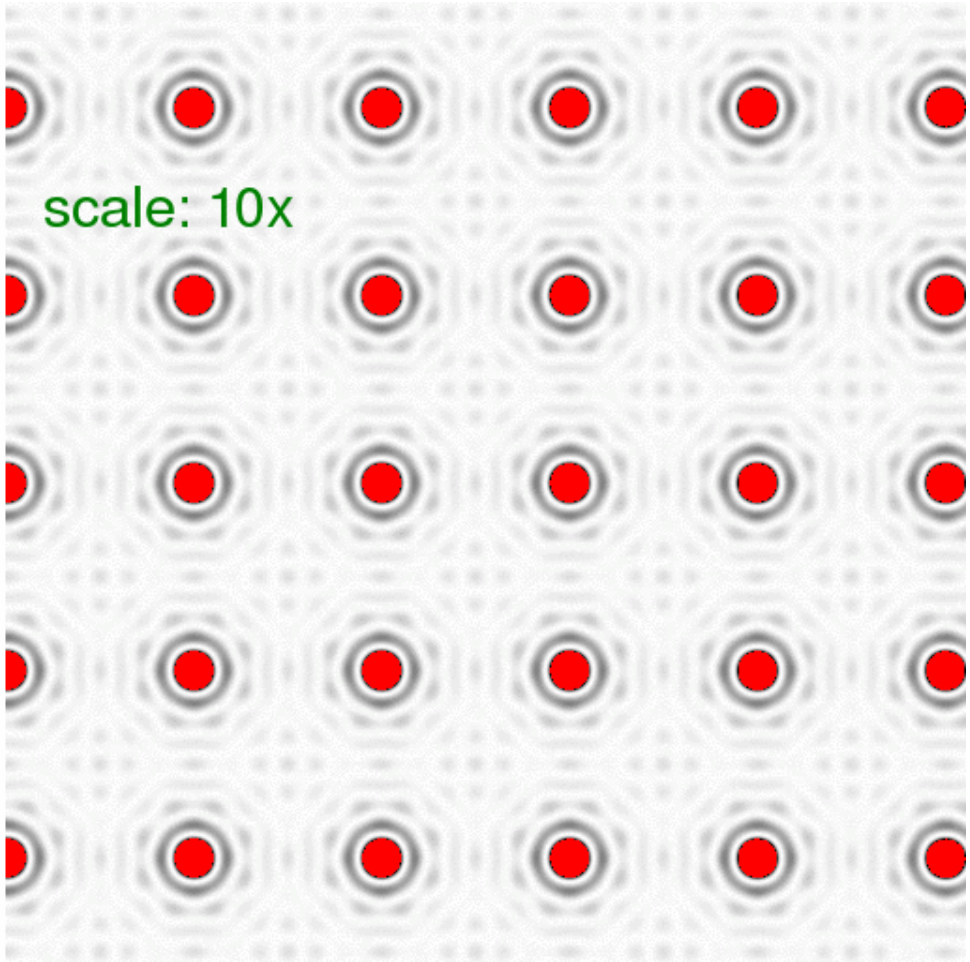
Total scattering from a lattice

Intensity sum: $F_1^2+F_2^2$

sample

detector





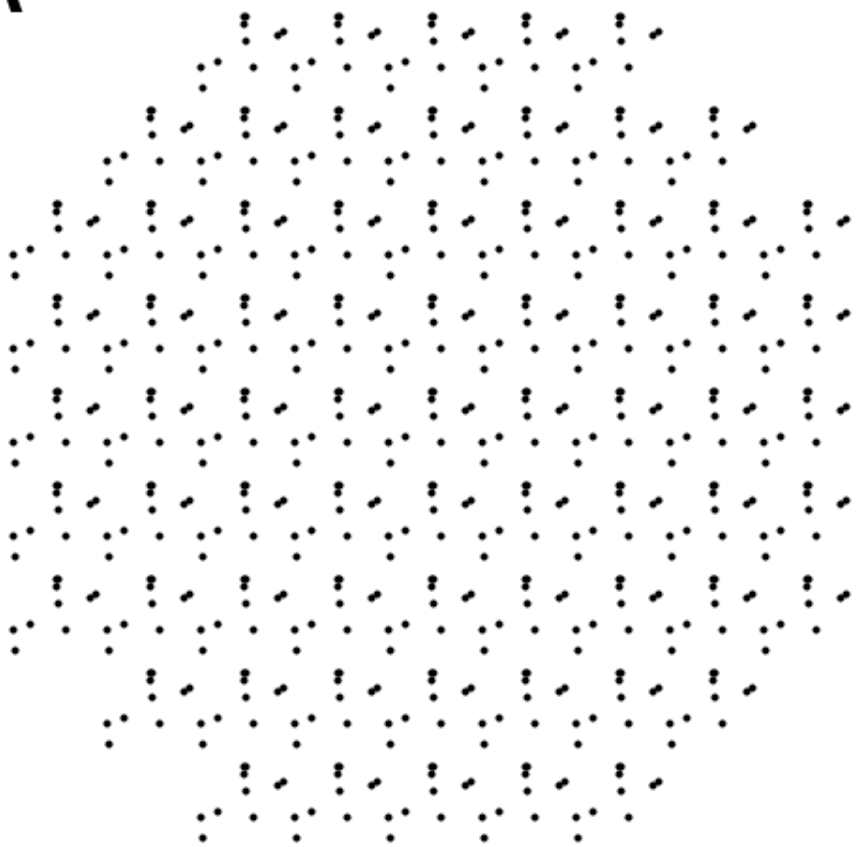
Total scattering from a crystal

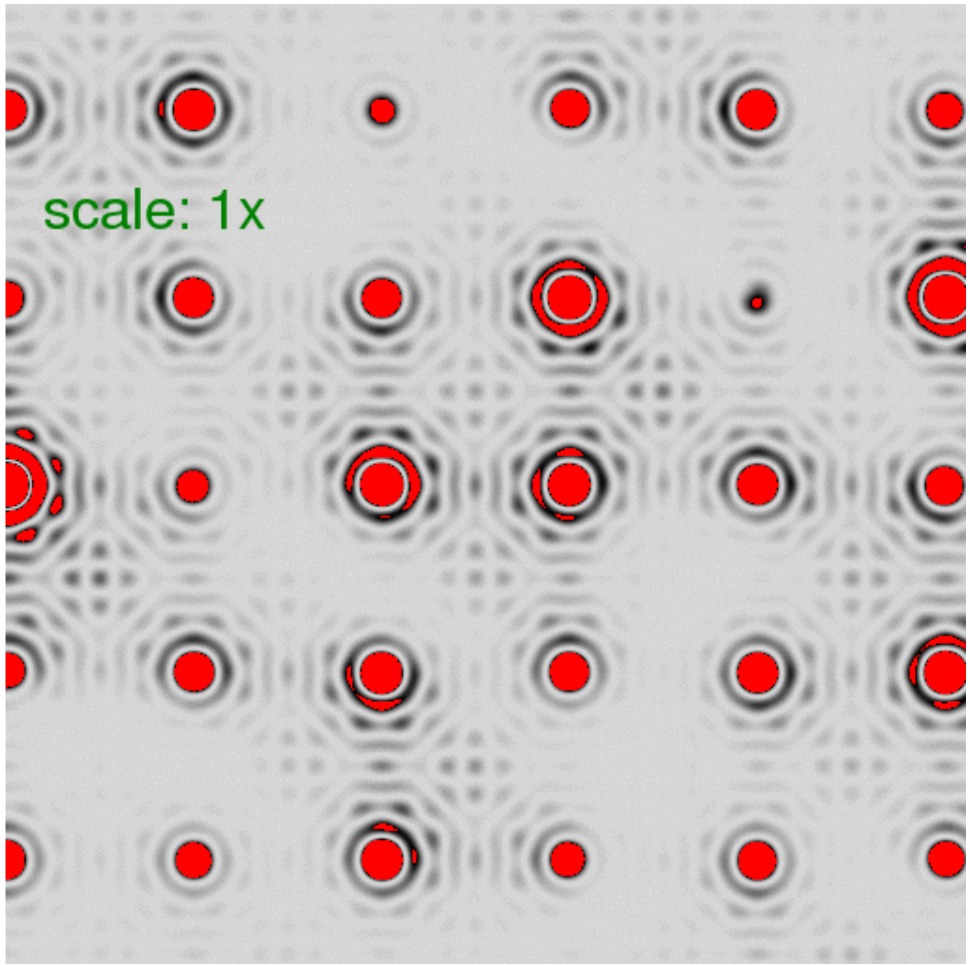
Intensity sum: $F_1^2+F_2^2$

sample

detector

A



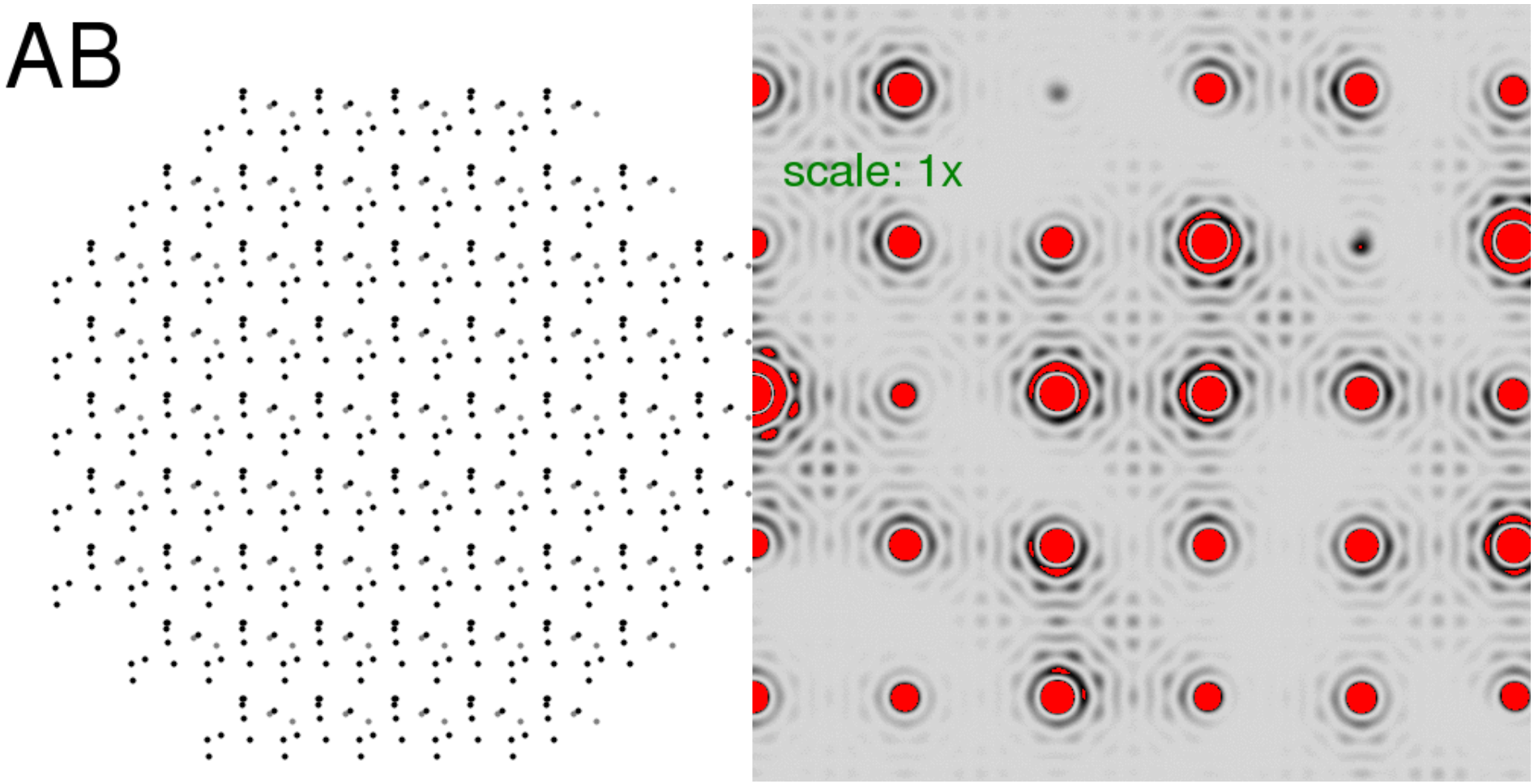


Total scattering from a crystal

Coherent sum: $(F_1+F_2)^2$

sample

detector

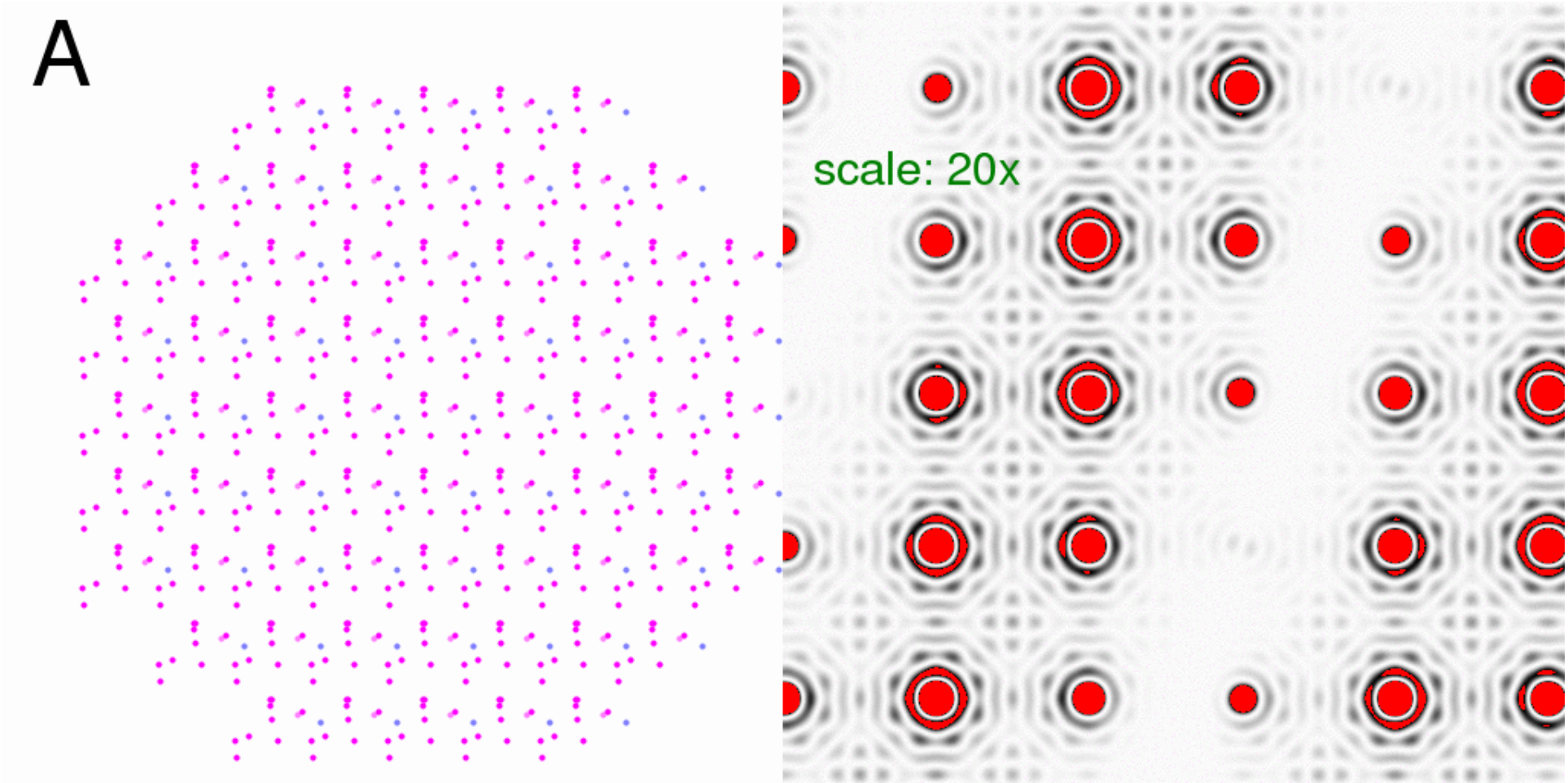


Total scattering from a crystal

Incoherent – coherent difference: $(F_1^2+F_2^2) - (F_1+F_2)^2$

sample

detector

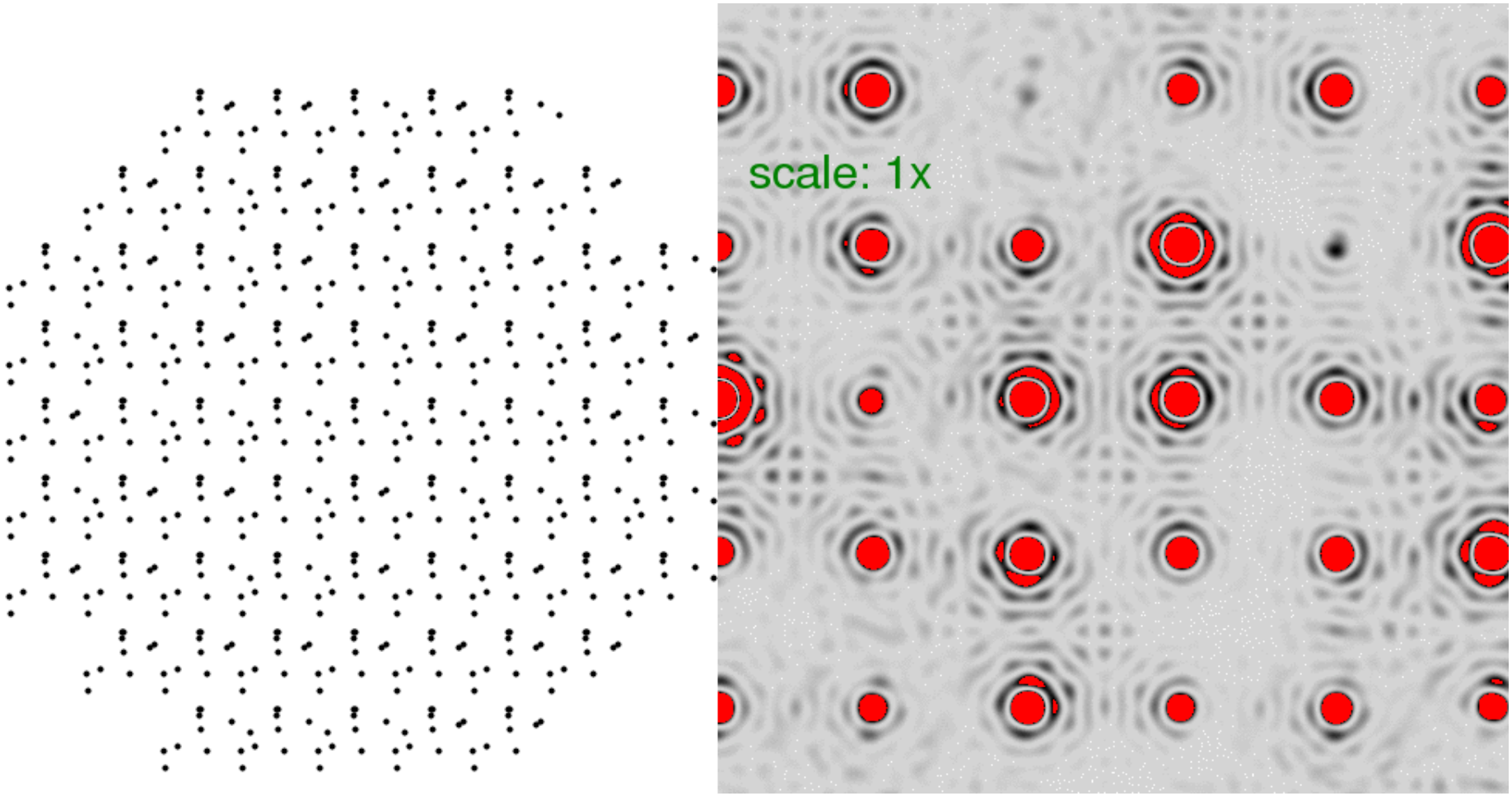


Total scattering from a crystal

Intensity: random fluctuations

sample

detector

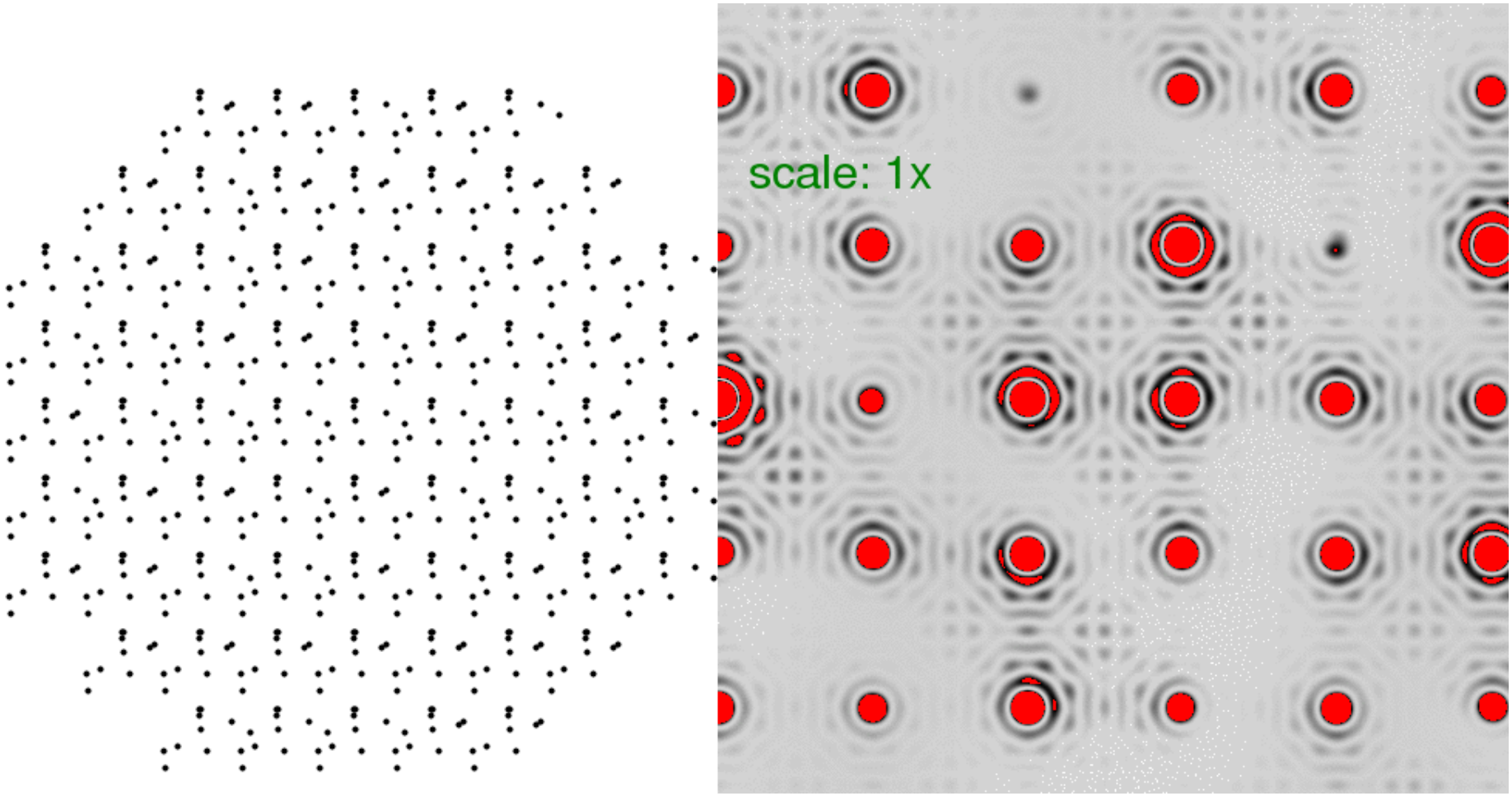


Total scattering from a crystal

Intensity sum: $F_1^2+F_2^2+F_3^2+F_4^2+\ldots$

sample

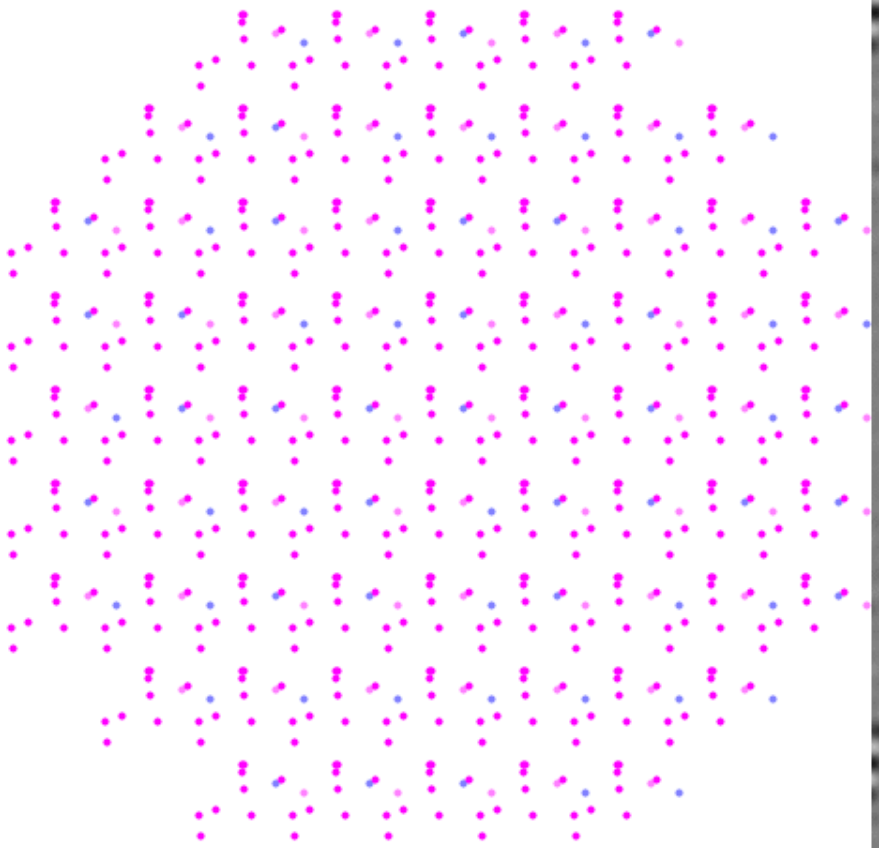
detector



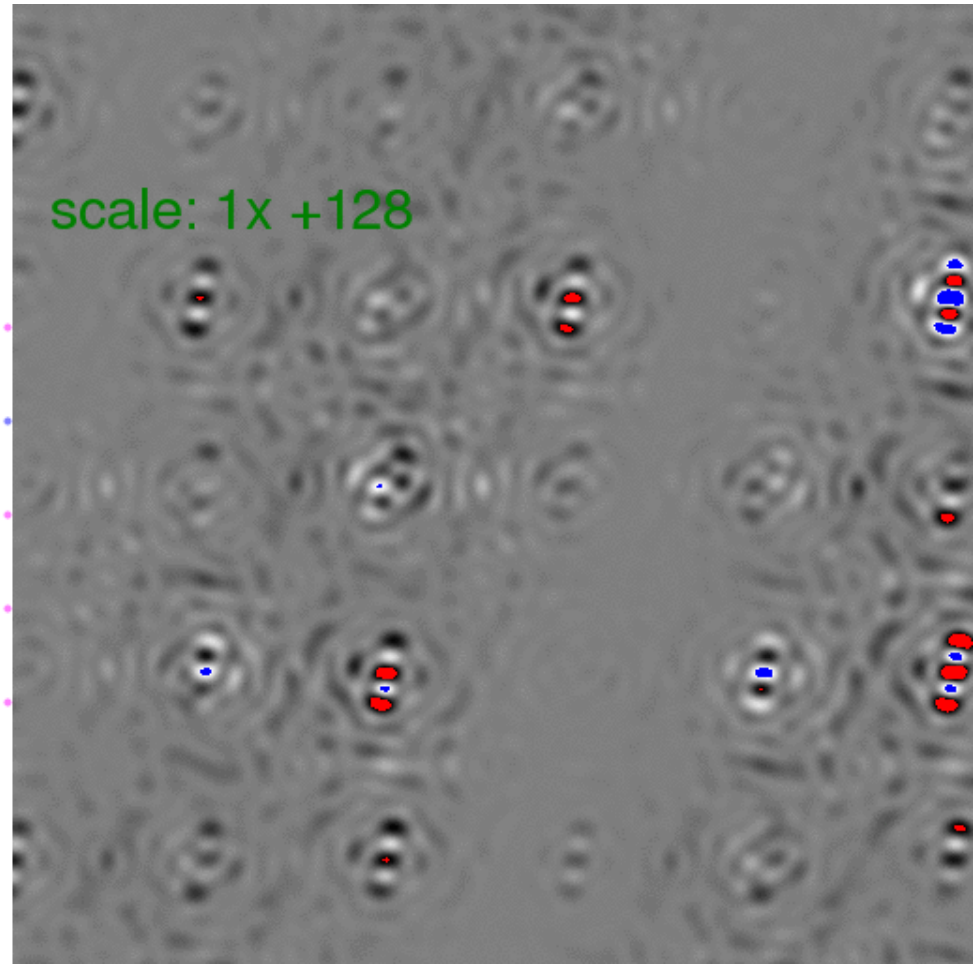
Total scattering from a crystal

Random fluctuations - Coherent average

sample



detector



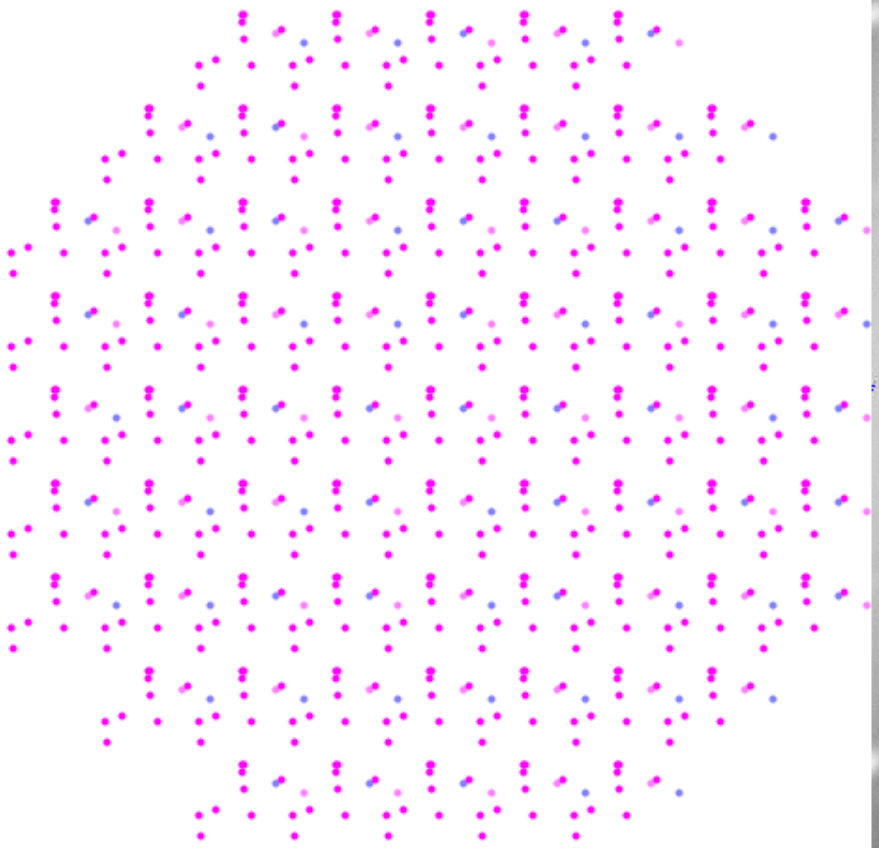
Total scattering from a crystal

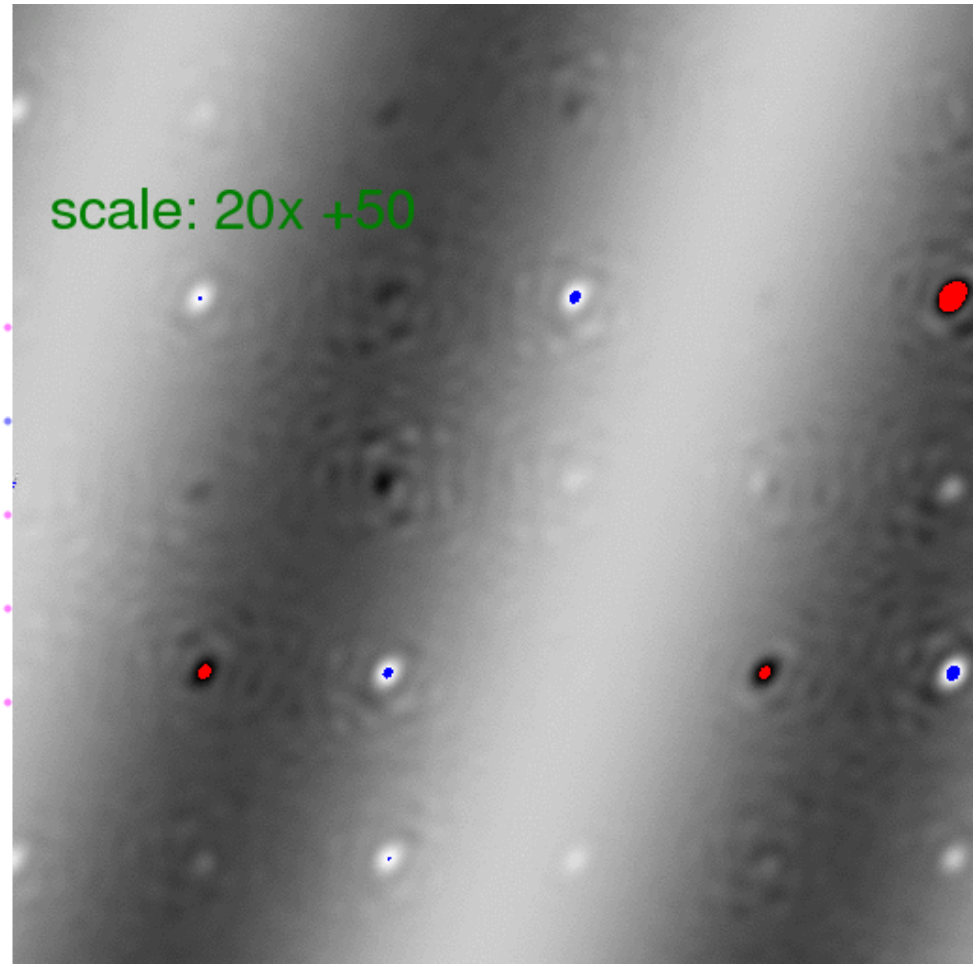
Incoherent-coherent difference:

$(F_1^2+F_2^2+F_3^2+F_4^2+\ldots) - (F_1+F_2+F_3+F_4+\ldots)^2$

sample

detector





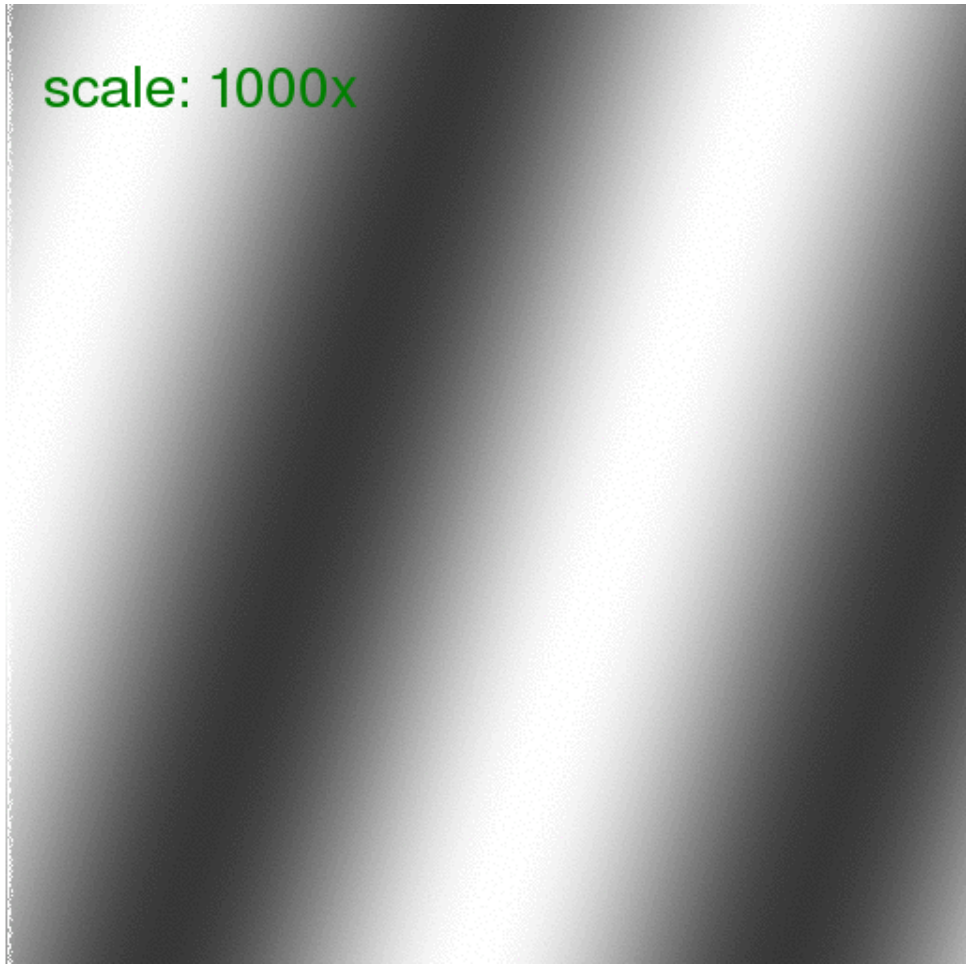
Total scattering from one atom

Incoherent – coherent difference: $(F_1^2+F_2^2) - (F_1+F_2)^2$

sample

detector

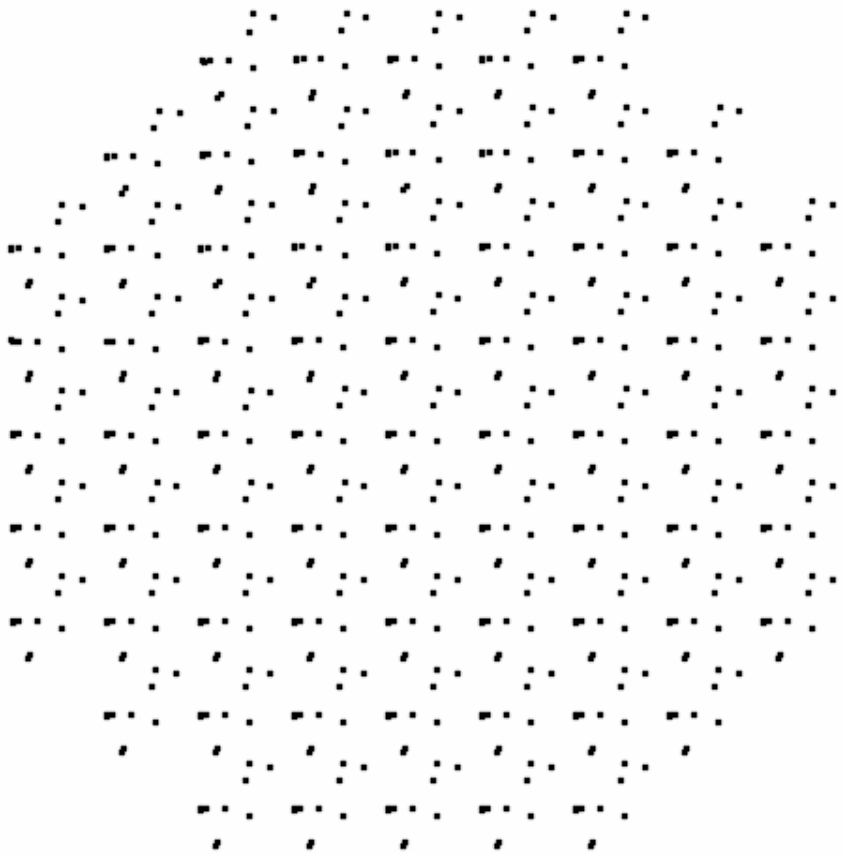
A



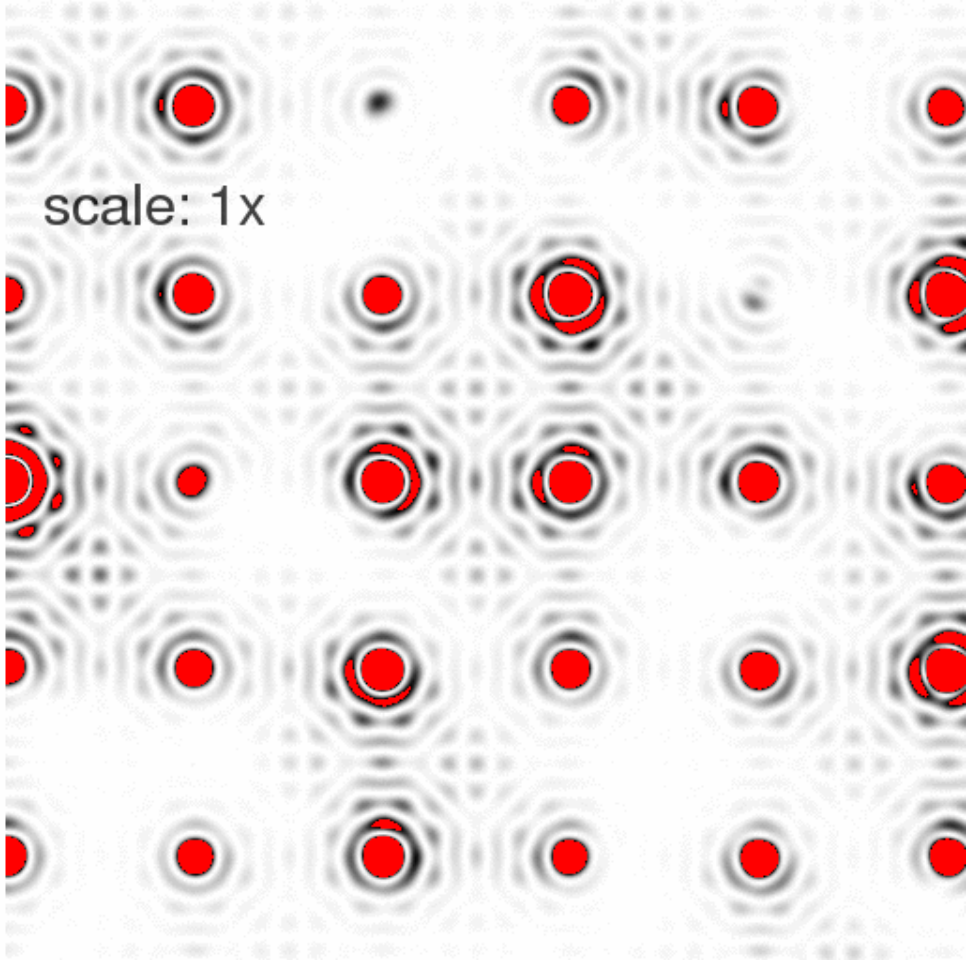
Total scattering from a crystal

"Dilatation" movements

sample



detector



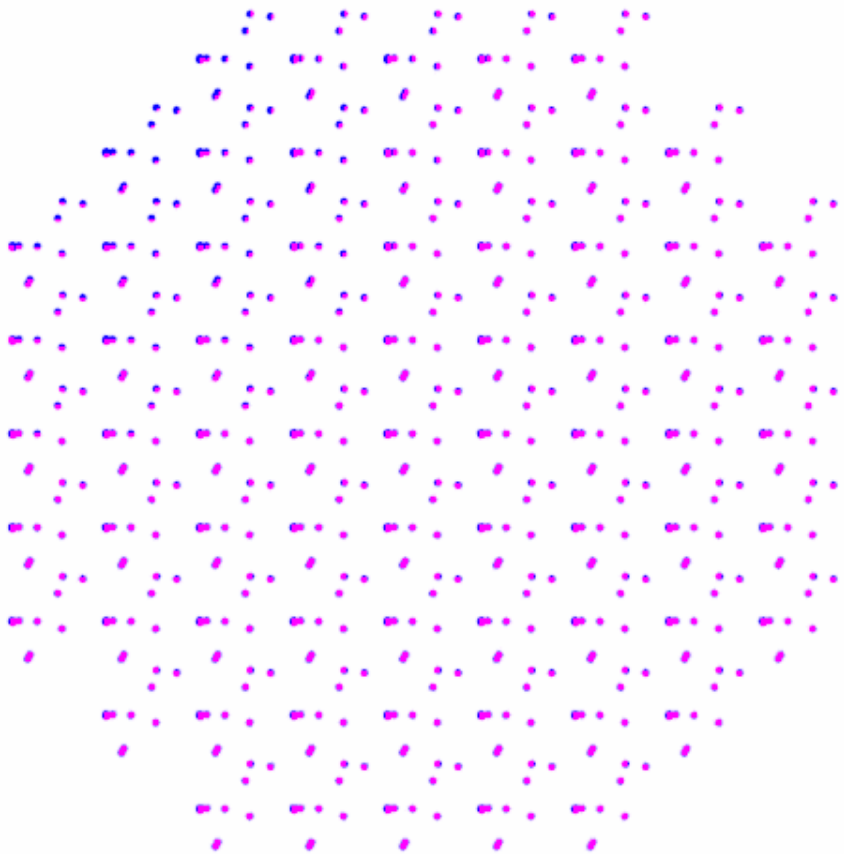
Total scattering from a crystal

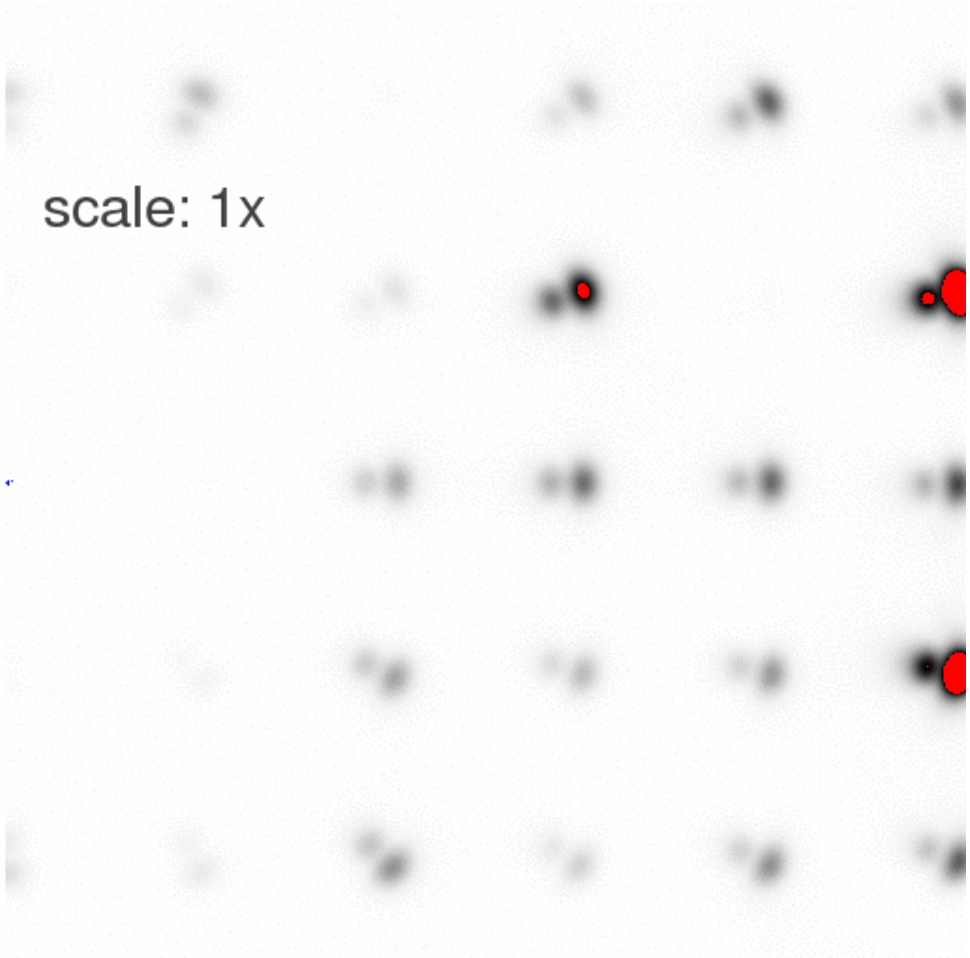
Incoherent-coherent difference:

$(F_1^2+F_2^2+F_3^2+F_4^2+\ldots) - (F_1+F_2+F_3+F_4+\ldots)^2$

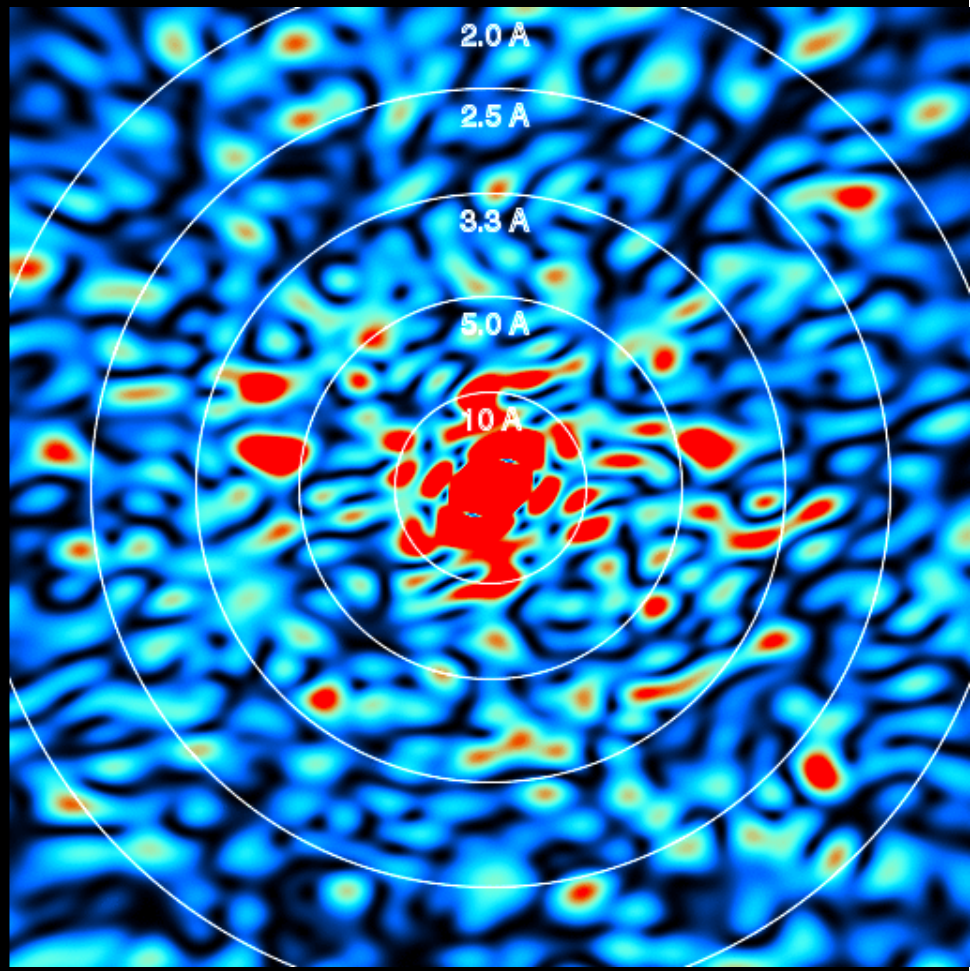
sample

detector



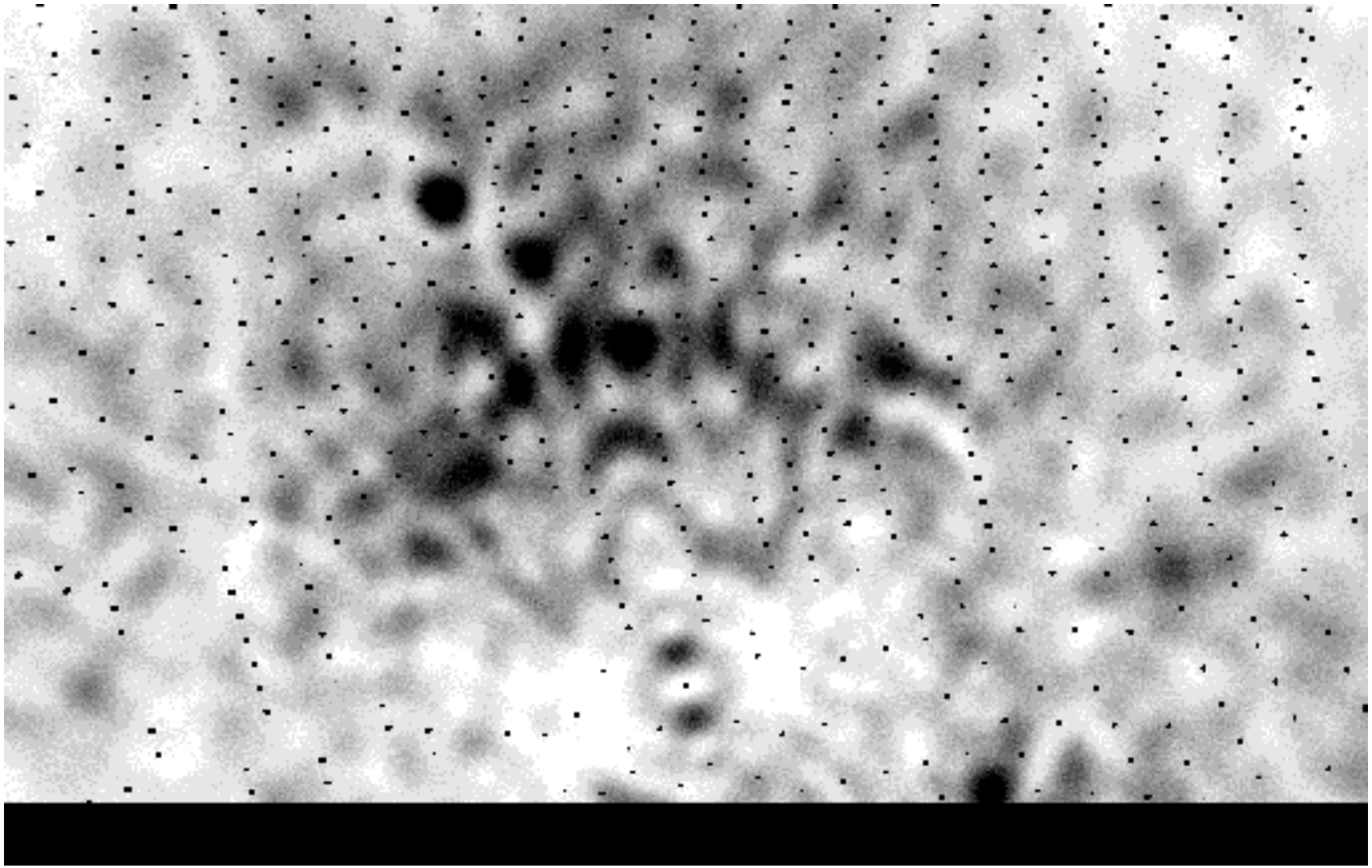


lysozyme: breathing motion



Simulation: but can you use it?

http://bl831.als.lbl.gov/example_data_sets/Illuin/lyso_DS

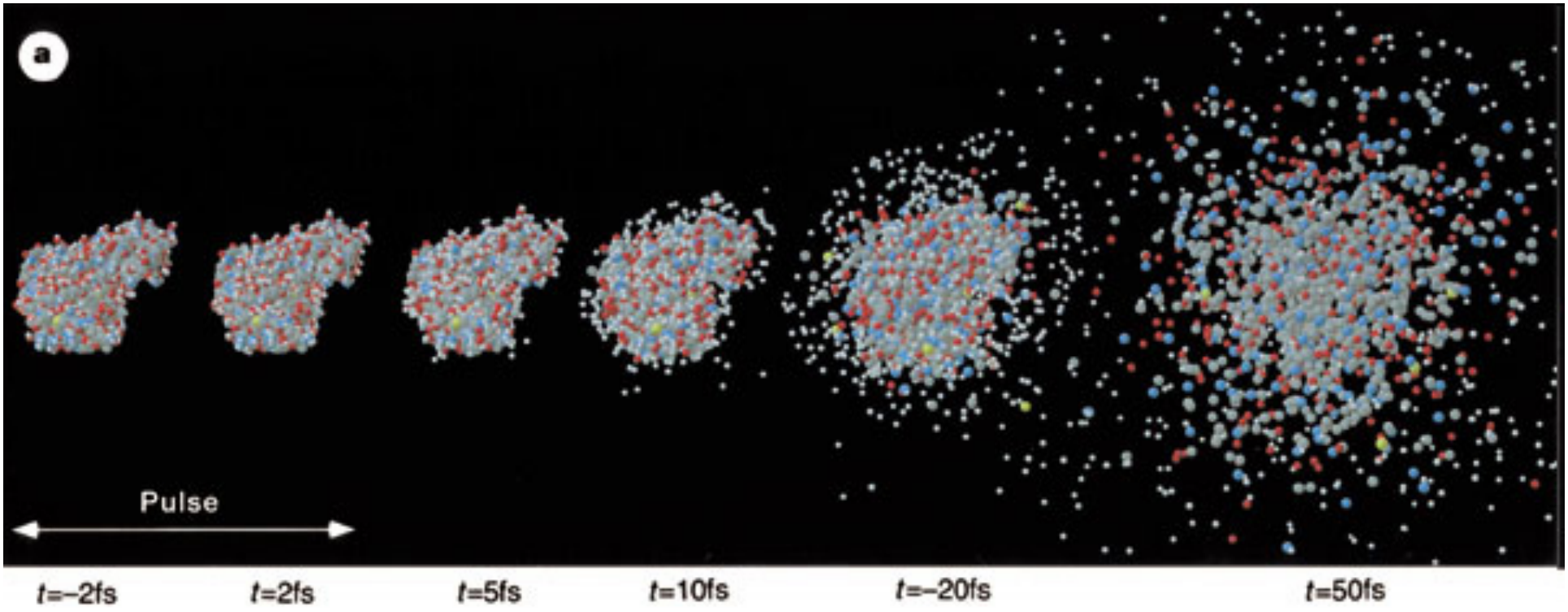


Summary

- Inter-Bragg stuff = xtal surface
- Beware the Caspar hole
- Spot – background = avg density
- If we can't get the spots right … ?

http://bl831.als.lbl.gov/~jamesh/powerpoint/ALS_UM_2013.ppt

Promise of Single-molecule imaging



R. Neutze *et al.,* Nature **406**, pp. 752-757 (2000)