

Electron tomography

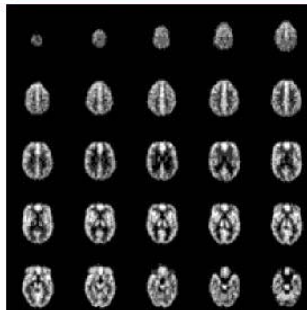
Background

One method of observing a 2-D slice through a 3-D object is ultramicrotomy, which uses a very sharp diamond or glass knife to prepare thin sections - thin enough for TEM. But another approach - computed tomography - uses only virtual sections, which are computed by observing the full 3-D object over a range of observation directions. This method is associated primarily with medical imaging, using X-rays for example.

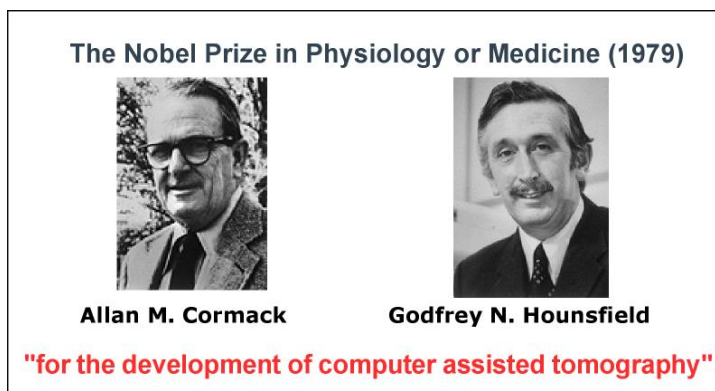
X-ray CT scanner



CT Scan Output

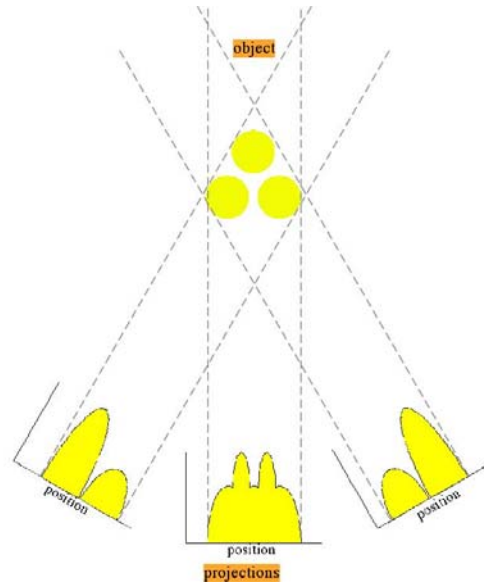


In fact, a Nobel Prize in Physiology or Medicine for the so-called back-projection algorithms that allow the extraction of the 2-D slices was shared by Allan M. Cormack and Godfrey N. Hounsfield in 1979. The transformation for projecting an N -dimensional object into $N-1$ dimensions is primarily attributed to Radon in 1917.



Principles of tomography

Beyond the mathematical demands of tomographic reconstruction, a lot of tomographic imaging of 3-D objects is careful bookkeeping to sort the large data sets required. A tilt series, or collection of 2-D images acquired at different sample tilts, is called an image *stack*. A 2-D samples of the 3-D reconstruction is called a *slice*. It can be easily deduced that, for rotation about a particular axis, every plane normal to that axis is essentially a 3-D slice that can be reconstructed separately (unless other conditions are placed on the reconstruction that related different slices.) So if we just think about what it takes to compute a 2-D slice from a stack of 1-D projections, we can get a handle on how to reconstruct the complete object.



The problem is easiest to approach if we assume the object is amorphous, and the contrast is entirely due to mass-thickness. If coherent diffraction or phase contrast contribute, we cannot directly relate the amount of material along the viewing direction to the intensity.

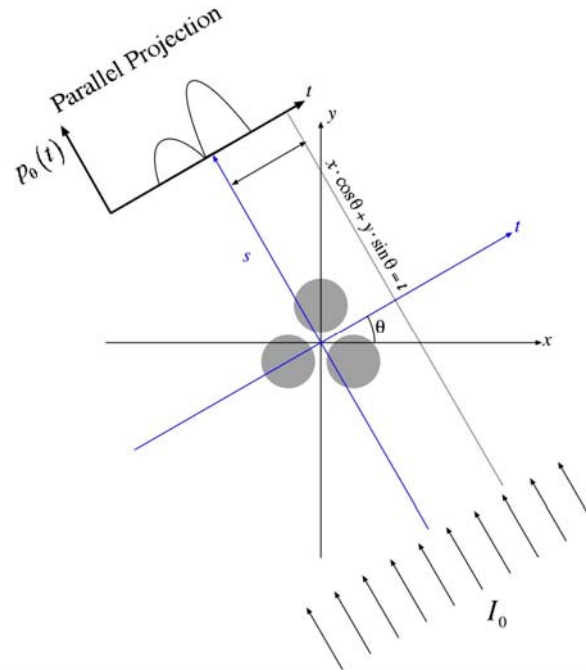
Projection images

At each point in an image of an object, viewed from an angle θ , we are projecting some quantity $f(x, y)$

$$p_{\theta}(t) = \int_s f(x, y) \cdot ds$$

In bright-field TEM, with only mass-thickness contrast present, the projected quantity that is related to the intensity is the attenuation coefficient $\mu(x, y)$. The projection is then related to the intensity by:

$$p_{\theta}(t) = -\ln \left[\frac{I_{\theta}(t)}{I_0} \right] = \int_s \mu(x, y) \cdot ds$$



Fourier-slice theorem (I)

Consider the FT of a 2-D object:

$$F(k_x, k_y) = \mathfrak{F}\{f(x, y)\}$$

Let's take the 1-D slice through the FT along the k_x axis ($k_y = 0$):

$$\begin{aligned} F(k_x, 0) &= \iint_{x, y} f(x, y) e^{2\pi i[k_x x + (0)y]} \cdot dx \cdot dy \\ &= \int_x \left[\int_y f(x, y) \cdot dy \right] e^{2\pi i k_x x} \cdot dx \\ F(k_x, 0) &= \int_x P(x) e^{2\pi i k_x x} \cdot dx \end{aligned}$$

where $P(x)$ is the projection of $f(x, y)$ onto the x-axis ($y = 0$), i.e.:

$$P(x) = \int_y f(x, y) \cdot dy$$

So a 1-D sample along the line $k_y = 0$ of the 2-D FT equals the 1-D FT of the 2-D object projected onto the line $y = 0$. We could say

$$F(k_x, 0) = \mathfrak{F}_{1-D}[p_{\theta=0}(t=x)]$$

The same result would apply in any orientation. If we specify the direct and reciprocal-space components along lines through the origin at angle θ as t and k , respectively, then

$$P_{\theta}(k) = \mathfrak{F}\{p_{\theta}(t)\}$$

This is known as the Fourier-slice theorem:

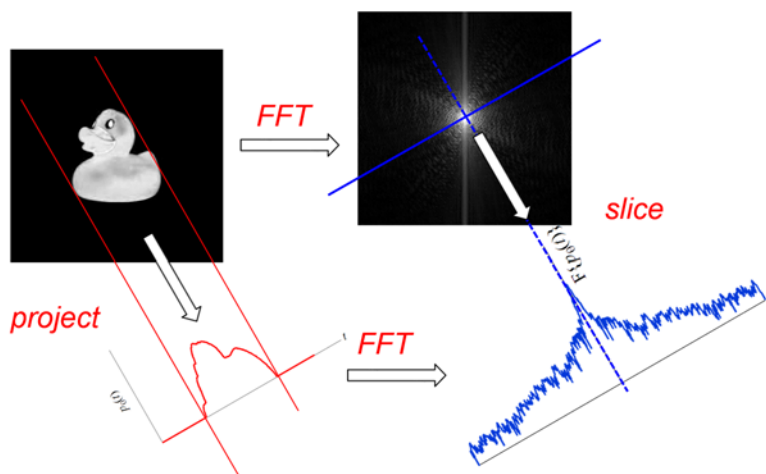
The 2-D FT of each projection is equal to a 2-D slice of the FT of the complete 3-D object

We can use this to build up the full 2-D FT from a collection of 1-D FTs. Then we could invert the 2-D FT to get the original object. Symbolically:

$$\{p_{\theta}(t)\} \rightarrow \{P_{\theta}(k)\} \rightarrow F(k_x, k_y) \rightarrow f(x, y)$$

Fourier-slice theorem (II)

Below is a harmless example of the Fourier-slice theorem:

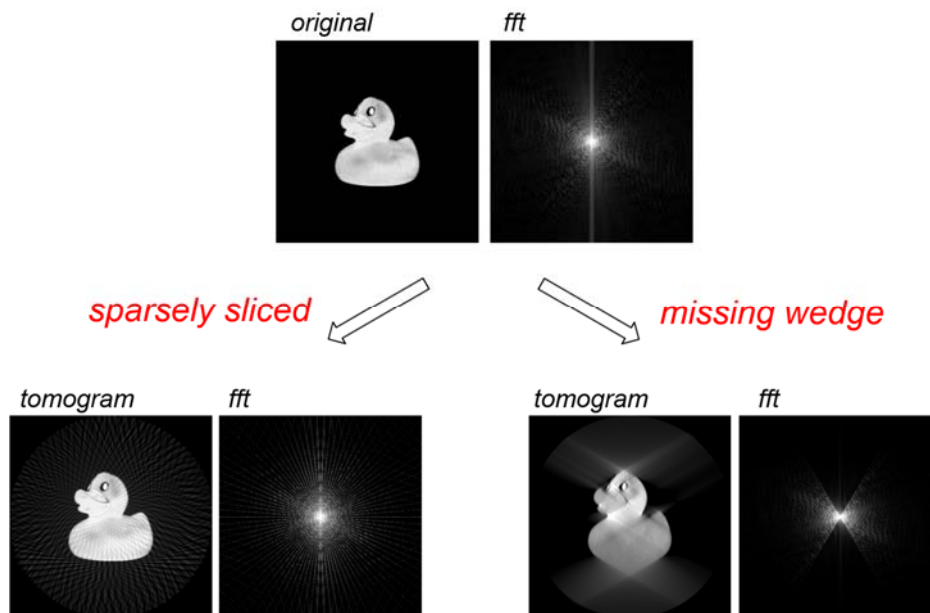


Whether we project the object, then take the 1-D FT of the projection, or sample a 1-D slice through the full, 2-D FT of the object, we get the same result.

Tomogram artifacts

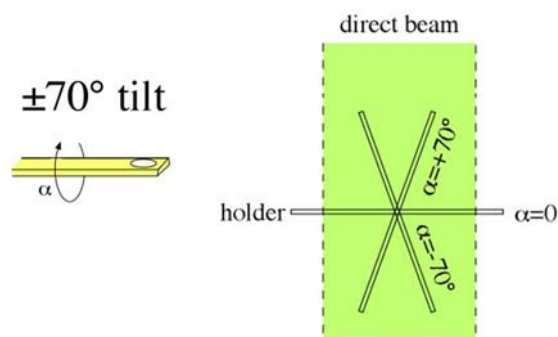
We are often limited by time and computational resources to a sparse sample of the object. For example, rather than acquiring an image every 0.1° we may settle for every 1° . There will be circular segments in the FT between each sampling that will remain zero. When we reconstruct the tomogram, this gives a pinwheel, like halo around the object.

In TEM, we are often limited in the tilt range to less than around $60\text{-}70^\circ$. This leaves an entire missing wedge in reciprocal space. When we reconstruct the tomogram, it shows a lack of clarity along the direction of the missing wedge, essentially due to a complete lack of knowledge about the object in this direction.



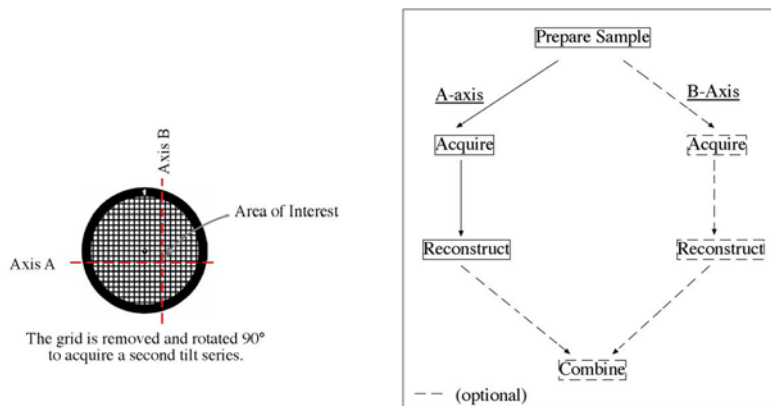
Acquisition and reconstruction

We want to use a TEM stage with a high tilt range for tomography. Usually $\pm 60\text{-}70^\circ$ is about the limit, but sometimes we can get as high as $\pm 80^\circ$. If you can't reach $\pm 60^\circ$ without obstruction, I would say don't bother due the tilt series. We usually try to make the beam as parallel as possible. The stage should be at the eucentric height, so that tilting produces minimal lateral shift of the specimen.



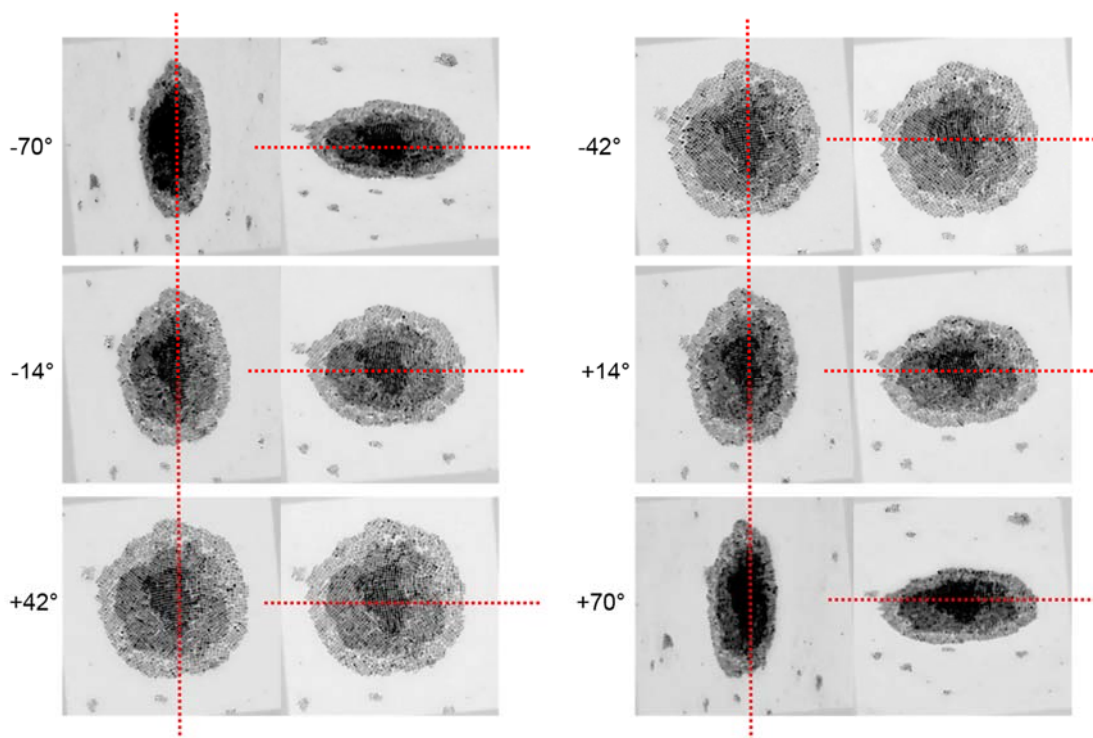
We can't really get around the missing wedge problem in TEM, but we can reduce it somewhat by performing a second tilt series about an axis in the specimen plane, 90° from the first axis. There are stages that help do this rotation in the microscope, but after acquiring a tilt series about the first axis (A), some of us have to actually take the sample out, rotate at as close to 90° as possible, put it back in the TEM, find the same area, then acquire the tilt series about the second axis (B). So it is good idea to take a low-mag image of your area of interest when the first tilt series is done.

Then the question is how to combine the information from these two tilt series. The eTomo program performs reconstructions on the A and B axes separately, then combines these through some kind of averaging. One can imagine other methods, but this one is proven to work well.



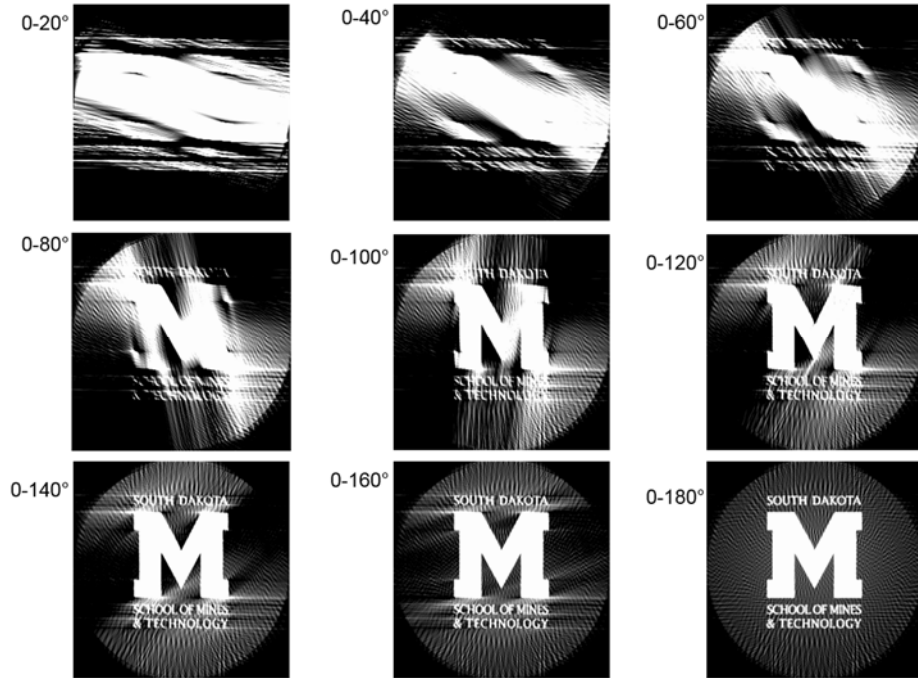
Dual-axis tilt series

An example of a dual-axis tilt series using PbSe nanocubes is shown below. Careful inspection shows that there is some new information gleaned from viewing the specimen over a range of tilt about a second axis that is orthogonal to the first.



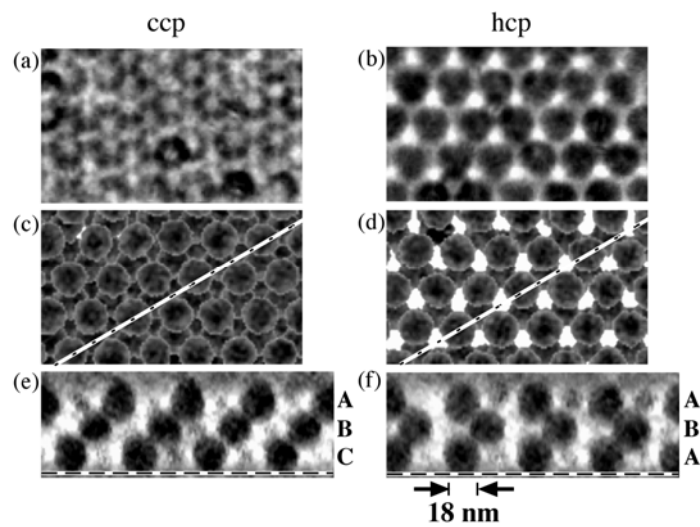
Back projection

To reconstruct an object, we could take the 1-D FT of many projections and piece these together to form the 2-D FT, then inverse transform back to get the reconstruction. Fourier transforming is linear, so we could instead take the 2-D FT formed by placing each 1-D FT separately along its appropriate line in reciprocal space, inverse transforming each of these separate 2-D FTs, then adding up the resulting 2-D images to form the reconstruction. But that is the same as just forming separate images by extending each 1-D projection of the object across the image plane, then adding up all of the resulting images. So there is no need to take any Fourier transforms. We just add up the projected images extended throughout the reconstruction area. This method is called *back projection*.

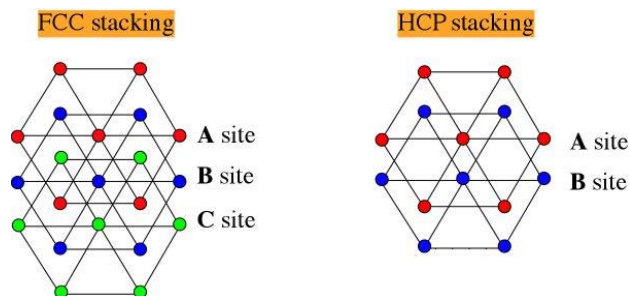


Stacking of In Spheres

I might have to show you a few examples for you to appreciate what tomography can tell us. My best examples all involve materials, rather than biological specimens. Very uniformly-sized, colloidal nanoparticles can order on the TEM grid, if the solvent is allowed to slowly evaporate. When the particles are spheres, we expect a close-packed structure - except there are two close-packed stacking sequences possible: fcc (sometimes called cubic-close packed, ccp) and hcp. In a standard 2-D projection image, it is very hard to deduce the stacking arrangement. But from the tomogram of the same region, we can extract slices through the centers of each plane of spheres, then clearly sort their relative, lateral placement. If the tilt series was acquired over a sufficient angular range, we can even take slices perpendicular to the specimen plane, without too much distortion from the missing wedge effect.

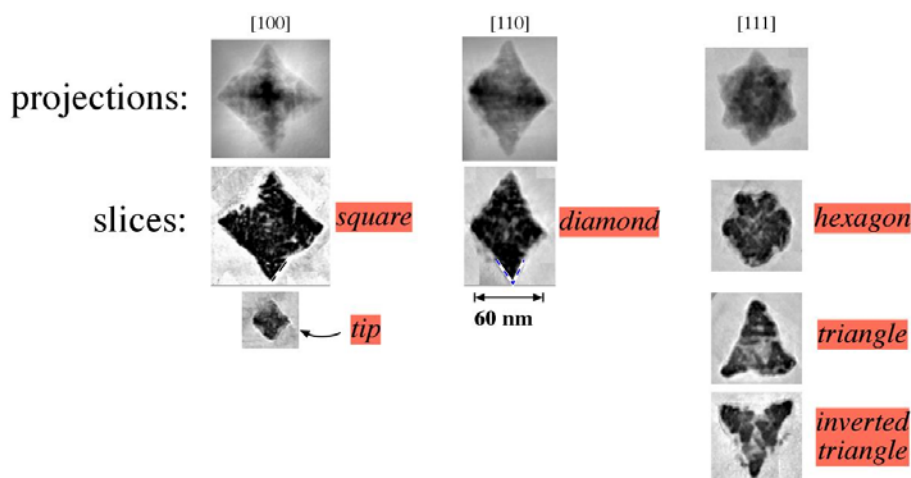


Regions of ABC (ccp) and ACA (hcp) stacking can now be identified.

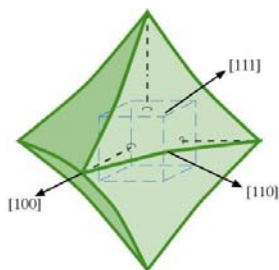


Shape of PbSe octahedron

If a nanoparticle is not too small, and its crystallinity is somewhat imperfect, tilt-series tomography can be used to reconstruct its shape. In fact, from the reconstruction, we can simulate hypothetical images along orientations of the particle that were never directly examined experimentally.

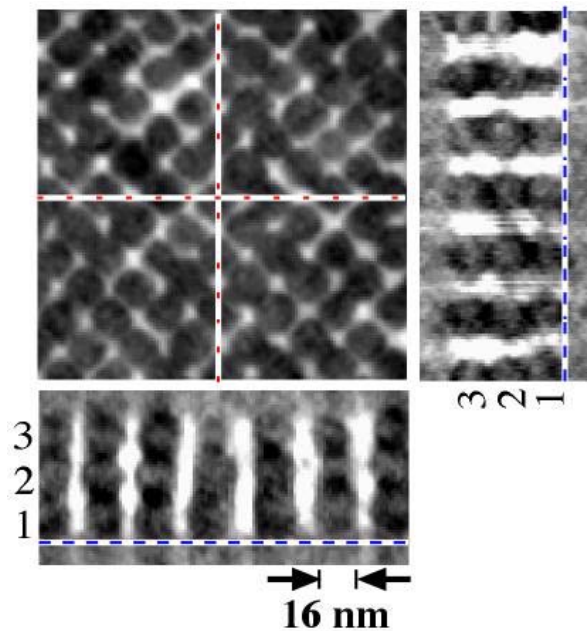


In combination with electron-diffraction or lattice imaging, we can relate the shape to the underlying crystal structure. An example of a PbSe nanoparticle, with size of about 60 nm, is shown below. We confirm that the NP has an octahedral shape and that these points are directed along the $\langle 100 \rangle$ axes. This is consistent with information about its rocksalt structure.



Stacking of PbSe cubes

Bear with me for one more example. The close-packing arrangements of spherical particles is particular to their shape. If the particles are cubes, they can pack closer in a cubic arrangement, as shown below for PbSe. In principle, there could be no gaps between closely packed, monodispersed cubes. The packing is so tight, projections on the bottom and right were taken at 45° from the cube faces to give a little gap between cubes so we can identify their arrangement. The crystallinity is fairly high in these NPs, so there is some glow in the reconstruction, due to diffraction.



In a less densely packed region, we can see that the cubes tend to sit with a flat face on the carbon support, although sometimes a particle will get lodged so that it is resting on an edge.

