X-ray Specs: 3D nano-scale resolution imaging with x-rays

Three-dimensional visualization of a human chromosome using coherent x-ray diffraction, *Phys. Rev. Lett.* **102** 018101 (2009)

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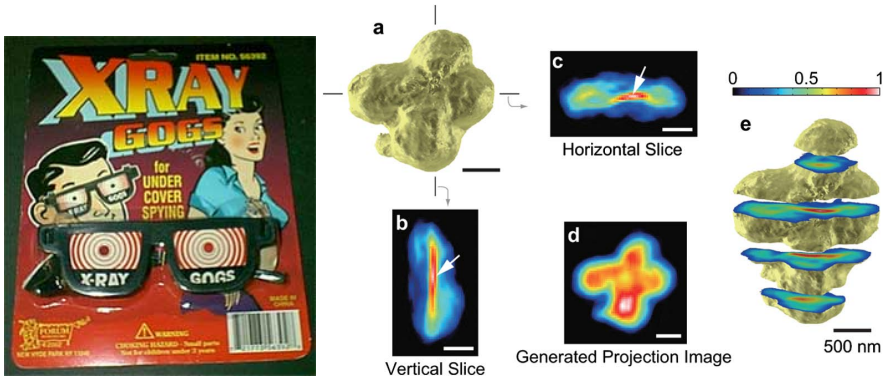

FIG. 1: (left) X-ray specs. (right) A human X-chromosome viewed with real x-ray specs (see below) from [1]

According to Wikipedia, "X-Ray Specs are an American novelty item, purported to allow the user to see through or into solid objects" (http://en.wikipedia.org/wiki/X-Ray_Specs_(novelty)). The recent PRL of Nishino *et al.*[1] reports the results of real x-ray specs (short for spectacles): they have created a full 3D image of a human (appropriately) X-chromosome using lensless diffraction imaging with hard x-rays. The image includes information about the chromosome's internal electron density, realizing the real dream of x-ray specs that allow you to peer inside objects.

This is one chapter in a vibrant field where the dream is to be able to image at the nano, and sub-nano, scale using x-rays. These authors managed 2D images with 38 nm resolution. The 3D images have a somewhat lower 120 nm resolution, presumably due to radiation damage of the sample. This is still a high resolution for 3D phase contrast imaging using any method to reconstruct the image, for example, tomography [2].

Successful imaging methods have a high scientific impact: think of the discoveries made with the first optical microscopes in the 17th Century. However, making a research career by developing imaging methods is a tough business as there are many competing approaches, such as optical near-field microscopes that beat the diffraction limit to get nano-scale images with laser light, electron microscopy, x-ray tomographic and lenseless diffraction imaging approaches, to name a few. It is possible to make 'firsts' but they tend to be highly qualified as here "We report for the first time 3D electron-density mapping of an uncrystallized biological sample using coherent diffraction". There are already (many) 3D images of biological samples using other methods, and 3D images of non-biological samples using coherent diffraction methods [3], for example. Nonetheless, this is clearly an interesting watermark in the field and the images yield useful information about the internal scaffold of the human chromosome. This scaffold was only seen before by staining proteins in the scaffold with fluorescent tags (another potent competitor in the imaging race) and immunoelectron microscopy. The latter two techniques are somewhat perturbative in that electron microscopy of such thick samples tends to involve sectioning the sample and is done in vacuum, and there are always questions about how much you change a biological sample when you attach large fluorophores to its proteins, so there may be a place in the sun for lensless diffraction imaging in biology.

The approach to get the images is to use an intense, coherent, beam of x-rays, in this case of hard 5 keV energy. The beam must be larger than the object to be imaged that is sitting on a nominally non-scattering support. Provided the resulting diffraction pattern satisfies the over-sampling condition [4] it can, in principle, be inverted to get the electron density in real-space, even though the phase information is lost when intensities are measured. To get a 3D image it is necessary to obtain multiple images at different angles. In this case the reconstruction was done by using a standard iterative procedure called the hybrid input-output method [5] where the data are iteratively Fourier transformed between real- and reciprocal-space and the phase and intensity information is updated in each cycle following specific rules. To find the 3D image the reconstruction was done using a 3D transform. The results are impressive, as evident in the figures

in the paper, one of which is reproduced above.

It will be interesting to see how the imaging battles play out. Radiation damage in biological samples is a tough barrier to break-through as dose goes as the inverse FOURTH power of resolution [6]. Rather like the workaround of near-field microscopy that beat the hard barrier of the diffraction limit for optical microscopes, there may be a way around this barrier by using hard-xray free electron lasers whose pulse width is so short that the photon bunch gets in and out before the object blows up. But with this approach, how to get 3D images? Methods to split the x-ray beam may prove useful for getting simultaneous images of the object at different angles [6], but the 3D images in the Nishino paper used 38 images at 2-5 degree steps. This would be a tall order for splitting an XFEL beam! If you can make many identical copies of the object you can also reconstruct the 3D image stroboscopically [7] by imaging (and blowing up) replicas of the object in sequential pulses. These are heroic experiments and these instruments won't be sitting in every biochemist's lab alongside her optical microscope. Still, hopefully X-ray Specs will be a part of future scientists scientific toolkit.

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