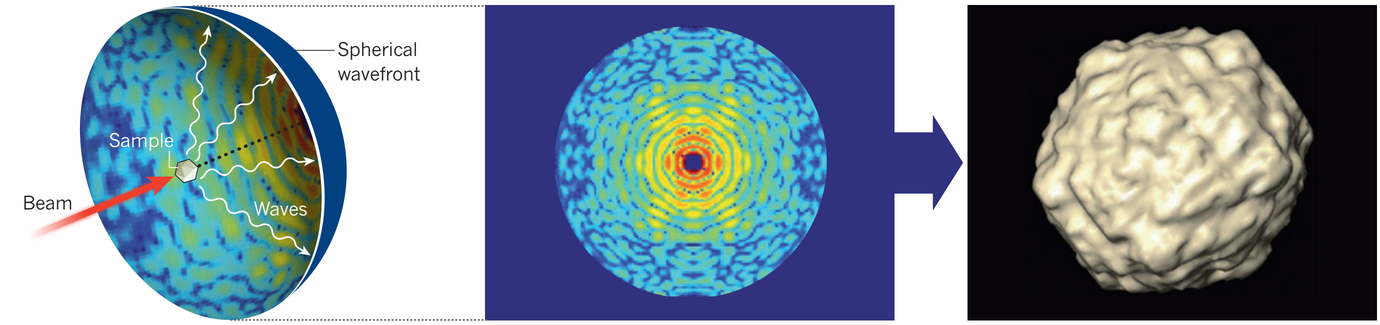
A SHORT CUT TO THE THIRD DIMENSION?

Ankylography promised to elucidate three-dimensional (3D) structures from data gathered on a curved, two-dimensional (2D) surface.

1 A high-powered beam is fired at the sample, which then scatters waves in all directions.

2 The scattered waves produce a 2D pattern (in which colour represents intensity).

3 This is used to decode the 3D structure of the original sample — a macromolecule.



IMAGING

Three-dimensional technique on trial

Critics take a hard look at ankylography, a proposed method for revealing molecular structures from single pictures.

BY EUGENIE SAMUEL REICH

Imagine developing a detailed and accurate three-dimensional model from a single two-dimensional photograph. That is the promise of ankylography[1], a technique that, according to its creators, could reveal the structure of scientifically important subjects such as complex proteins that can be glimpsed only once before they are destroyed by the high-powered lasers used to image them.

But rather than sparking a revolution in imaging, the idea has raised objections from researchers who say that it amounts to pulling a three-dimensional rabbit out of a two-dimensional hat. Now, critics have weighed in with two technical comments[2,3], putting ankylographers on the defensive.

"The imaging principles and proposed methodology of ankylography are flawed, bringing into question its applications," one group of commenters writes[3].

Ankylography was developed by John Miao, a physicist at the University of California, Los Angeles, and his colleagues. It uses the spherical wavefront formed when radiation scatters off an object (see 'A short cut to the third dimension?'). Most current imaging technologies assume that this wavefront is roughly flat where it meets the imaging detector — a reasonable assumption for a detector that intercepts only a very small fraction of the sphere. Miao and his colleagues collected waves across a larger angle, from different depths of the object. They argued that this larger sample could be reconstructed into an accurate three-dimensional (3D) image using only very basic assumptions. They derived the name of their technique from the Greek *ankylos*, or 'curved', and *graphein*, or 'writing'.

"The claim is amazingly attractive, to take a single projection and do three-dimensional reconstruction," says Ge Wang, a biomedical imager at the Virginia Polytechnic Institute and State University in Blacksburg, and an author of one of the critical comments[3].

Ankylography seemed to be a boon for an emerging X-ray technology called diffract and destroy, which would use X-ray lasers — online or under construction at around a dozen sites worldwide — to take images of objects using a dose of radiation high enough to destroy them, forming an image from the X-rays scattered in the few dozen femtoseconds (10^{-15} seconds) before the object explodes. The ideal application would be imaging biological samples such as proteins, viruses, cells or tissue that are very sensitive to radiation. Ankylography would allow researchers to extrapolate 3D structures.

Despite the appeal of ankylography, many researchers were perplexed by what they see as a violation of the basic physical principle that you cannot get complete, 3D information from a single flat picture. In particular, the picture will provide incomplete information about the interior of a subject, and critics argue that many possible 3D structures could generate the same image. Pierre Thibault, a physicist at the Technical University of Munich in Germany, who challenged Miao's work even before it was published[4], explains that the curvature of the wave scattered from an object contains some 3D information, like the blurriness produced by a camera with a limited depth of field, but not enough to build up an accurate 3D image.

Miao is unable to comment, because a response to the criticism is still under review. However, his research group has placed all the computer code used to make his ankylographic reconstructions on a website so that others can test it (go.nature.com/gd6dvo). Miao has since made clear that the technique does not work on objects larger than 15 × 15 × 15 volume pixels, a size dependent on the resolution of the imaging technology. "I can't think of any object that exists in nature that we care about that it would work for," says Stefano Marchesini, a physicist at Lawrence Berkeley National Laboratory in Berkeley, California, who has analysed ankylography.

Even if ankylography can be made to work for objects larger than the current range, it is likely that more than one image would be needed to generate a high enough signal-to-noise ratio to see fine detail, says John Spence, an expert in diffract-and-destroy imaging at Arizona State University in Tempe.

Without ankylography, X-ray lasers can still be used to take two-dimensional snapshots of a series of subjects from various angles, building up an average 3D image. This would work for identical objects such as viruses or proteins, but not for dissimilar objects such as cells.

Felipe Maia, a physicist at Lawrence Berkeley, says that the ankylography controversy has triggered a useful discussion about the limitations of 3D imaging. Wang agrees; he has been inspired to propose an alternative technique[3], in which waves of different energies are collected and used to derive some of the 3D information that a flat image lacks. "There's been some positive influence," he says. ■

1. Raines, K. S. *et al. Nature* **463**, 214–217 (2010).
2. Wei, H. *Nature* http://dx.doi.org/10.1038/nature10634 (2011).
3. Wang, G., Yu, H., Cong, W. & Katsevich, A. *Nature* http://dx.doi.org/10.1038/nature10635 (2011).
4. Thibault, P. Preprint available at http://arxiv.org/abs/0909.1643 (2009).