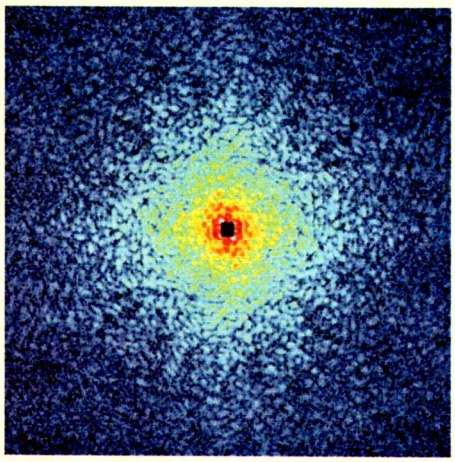
A three-dimensional x-ray image of a single pair of human chromosomes

X-ray crystallography routinely yields the structures of proteins and other biomolecules with 0.1-nm resolution. But how does one take a similarly detailed look at something far bigger—a chromosome, say, or a cell nucleus? Such objects don't crystallize because no two individuals have the same shape. And at a micron or so in size, they're too thick for electron microscopy. Electrons either get stuck in the object or scatter too many times on their way out to form a clear image.



X rays, of course, pass through whole animals, not just single cells. For the past decade or so, groups around the world have been developing a technique called x-ray diffraction microscopy for imaging single, uncrystallized samples.

The first biological application of XDM appeared in 2003. Jianwei Miao, who's now at UCLA, and his collaborators obtained two-dimensional images of single *Escherichia coli* bacteria with 30-nm resolution.[1] To boost the scattered signal, Miao had to stain the bacteria beforehand with a manganese salt. Staining doesn't damage the sample, but it biases the image because only certain amino acids stain readily. Two years later David Shapiro of Stony Brook University and his collaborators demonstrated that XDM could yield 2D images of single yeast cells without staining.[2]

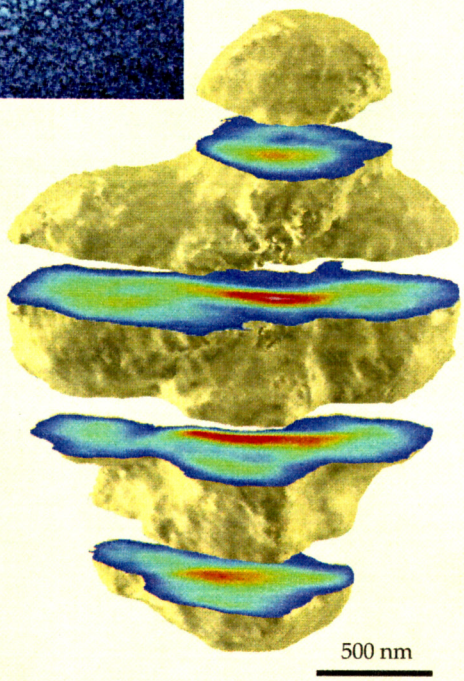
Now, extending XDM's steady progress, Yoshinori Nishino of the SPring-8 synchrotron in Sayo, Japan, and his collaborators have created 3D images of pairs of unstained human chromosomes.[3] The result not only represents a new XDM milestone, it's also biologically significant: Nishino's images reveal that a human chromosome in its most compact state has a dense axial backbone.

In XDM, coherent, monochromatic x rays scatter off electrons in the sample's constituent atoms to form a continuous diffraction pattern, like the one shown above on the left. The pattern's features embody the length scales of substructures present in the sample but not the substructures' locations. As in all diffractive imaging, obtaining those locations—and therefore deriving the structure itself—requires knowing the phases of the scattered x rays.

The detectors that record x-ray diffraction patterns can't measure those phases directly, a shortcoming that gives rise to crystallography's famous phase problem. In 1952 David Sayre suggested a solution that would later be applied to XDM. The all-important phases, Sayre realized, are not lost, but reside in the continuous diffraction pattern. When the pattern is sampled at a fine enough spatial frequency, the phases can be extracted and the structure derived by using a computer algorithm that iterates back and forth between real and reciprocal space. The first demonstration of XDM was performed by Miao and colleagues in 1999; it used an algorithm devised in 1978 by James Fienup (see PHYSICS TODAY, October 1999, page 9).

Human chromosomes provide an apt testbed for XDM. At its most basic structural level, a chromosome consists of a strand of negatively charged DNA. To hold the information needed to encode a genome, the strand would stretch to about a meter in length. But to fit inside a cell's nucleus and to enable gene transcription to proceed in an orderly way, DNA must be compacted. Positively charged proteins called histones help DNA fold and coil into a hierarchy of ever-denser structures that culminates in mitotic chromosomes, the paired form that appears during cell division. A mitotic chromosome is typically a few microns long. Pairs of them can be seen with an optical microscope. Their surface can be mapped with atomic force microscopy, but their internal structure is not fully known.

The figure below shows the SPring-8 team's 3D image of a pair of mitotic human chromosomes joined at their middles in an X-like shape. The arms of the chromosomes have dense, axial backbones, suggesting a high level of packing.



In principle, the resolution of XDM is limited only by the x rays' wavelength. In practice, x rays would damage and deform the sample long before the signal was strong enough to yield the limiting resolution. The SPring-8 team obtained the same 30 nm resolution in 2D—as Miao and Shapiro did for their pioneering images. But for the 3D image, which required combining exposures made at 38 different viewing angles, the resolution was 120 nm.

Radiation damage is unavoidable but it can be mitigated. Encasing a sample in ice not only boosts radiation resistance, it also preserves—or comes close to preserving—the sample's natural, hydrated state. Freezing a sample risks introducing spurious signals from ice crystallites, but it could yield a 3D resolution of 10 nm.

Radiation damage can also be made irrelevant. In 2006, Henry Chapman, Janos Hajdu, and their collaborators resolved a single object with a free-electron laser. The FEL's intense pulse destroyed the sample, but not before photons had scattered off the atoms and carried away structure-determining information. (See PHYSICS TODAY, January 2007, page 19.) Chapman and Hajdu's demonstration used extreme UV photons, which could not have resolved a chromosome. That barrier will fall when SLAC's Linac Coherent Light Source and other x-ray FELs come on line next year.

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References

1. J. Miao et al., *Proc. Natl. Acad. Sci. USA* **100**, 110 (2003).
2. D. Shapiro et al., *Proc. Natl. Acad. Sci. USA* **102**, 15343 (2005).
3. Y. Nishino et al., *Phys. Rev. Lett.* **102**, 018101 (2009).