Where do things stand? We know the factor that controls limb formation, but not the detailed mechanism. But these three recent studies provide new insights toward the ultimate goal of a unified framework for understanding how information in a single morphogenetic signal leads ultimately to the generation of a complex pattern.

References
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The marriage of scanning x-ray microscopy with diffractive imaging provides a route to dramatic increases in spatial resolution.

**Focus on X-ray Diffraction**

Henry N. Chapman

X-ray microscopy fills the “resolution gap” between light microscopy and electron microscopy. The high penetration of x-rays in matter, however, makes the production of high-resolution lenses challenging. On page 379 of this issue, Thibault et al. (1) sidestep this problem by recording high-angle (and hence high-resolution) diffraction patterns in the microscope at each point along the specimen. This information is then used to increase the resolution of the image by more than a factor of four. Unlike “lensless” diffractive imaging methods such as crystallography and coherent diffractive imaging (2), the scanning diffraction microscopy of Thibault et al. offers a general instrument to obtain images, zoom into regions of interest, and build up large fields of view of specimens, within minutes rather than hours.

Scanning diffraction microscopy, or ptychography, was first developed for the scanning transmission electron microscope (STEM) (3). The x-ray counterpart, a STXM, is conceptually the same. The x-ray beam is focused onto the sample via a lens, and the transmission is measured in a large-area, single-element detector (see the figure). The image is built up by plotting the transmission as a function of the sample position, as it is rastered across the beam: The smaller the beam focus, the finer the features that can be resolved. If the large-area detector is pixelated, an entire angularly resolved diffraction pattern may be recorded at each point of the scan. Plotting the sum of all detector pixels as a function of beam position simply provides the standard transmission image, but the diffraction pattern contains much more information than that. For example, the pattern can be recorded to high angles, revealing information from features that are much smaller than the beam size. To use this information, we must numerically transport the beams back to the image plane, which is only possible if the phases of the diffracted intensities are also known. This is the “phase problem” of crystallography and other lensless techniques.

Progress has been made in solving the phase problem for a single diffraction pattern recorded from a nonperiodic object (2). Such methods, referred to as coherent diffractive imaging (CDI), have achieved better than 10-nm resolution (4). The complete complex-valued wave scattered from the object is recovered, giving phase contrast and a way to overcome depth-of-focus limitations (5). Although ideal for ultrafast single-shot imaging (6), recovering the phases in the diffraction patterns is computationally intensive and requires specific geometric constraints to be satisfied in order to obtain a solution.

For scanning diffraction microscopy, the phase problem becomes tractable. A thin periodic sample will diffract a plane wave into discrete directions (or diffracted orders). For a focused beam consisting of an angular range of plane waves, each diffracted order will now be a disc (dashed white circles in the figure), given by the angular extent (or numerical aperture of the lens). The intensities of the diffracted orders are the same as in the case of illumination with a plane wave, except where they overlap. In these regions of overlap, the

Improved imaging. (Left) Image creation in the scanning transmission x-ray microscope. Simulated images show the improvement of resolution demonstrated by Thibault et al. when the angularly resolved transmitted diffraction pattern is used (lower right), as compared to the case where total transmission is plotted as a function of beam position (upper right).

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diffracted orders interfere and the intensity depends on the relative phase of the two diffracted orders. It is the folding of one diffraction order onto another that encodes the phase information. As in Gabor holography and “keyhole diffraction” (7), there is an ambiguity in the sign of the phase, which scanning diffraction microscopy can resolve from how the diffraction pattern changes as the sample is moved relative to the beam.

The amount of information in the series of diffraction patterns is highly redundant, and many methods to obtain high-resolution images have been proposed and tried over the past 20 years by John Rodenburg and collaborators (3). I demonstrated the technique with soft x-rays, recording a total of 4096 diffraction patterns for a single image (8), and was delighted in how direct and straightforward it was to obtain phase and amplitude images. However, the long readout time of the charge-coupled device detector between each diffraction pattern exposure discouraged further research.

Two key developments have created the breakthrough demonstrated by Thibault et al. The first is the Pilatus detector (9), a large-area detector using advances from high-energy physics experiments. Each pixel is activated independently and counts up the charge as the photons arrive, speeding up the detector and eliminating noise. The second is the adaptation of the iterative methods from CDI (10, 11), which are stable when applied to the redundant ptychographic data sets.

These two improvements should be implemented at every STXM at synchrotrons worldwide. Doing so will be revolutionary, allowing desktop computers to overcome x-ray optical limitations to reach resolutions below 10 nm. This is not to say that work in x-ray optics should cease. On the contrary, focusing the x-ray beam to dimensions less than the unit cell size of a macromolecular crystal may indeed be feasible for hard x-rays (12). In this case, with thin crystals it will be possible to overlap the Bragg peaks and read out their phases, thereby achieving atom-level-resolution x-ray microscopy.

References

Matthew Huber

A Hotter Greenhouse?

Scientists have long been puzzled by the fact that mid-to-high latitude continental interiors and the poles in the Eocene (55 to 34 Million years ago) were much warmer than today, without freezing winters (1), while tropical sea surface temperatures (SSTs) were apparently near-modern (2, 3). Mechanisms proposed by climate modelers to maintain high-latitude warmth require substantial tropical temperature increases (4, 5). The implication is that fundamental gaps remain in our understanding of climate dynamics. Many hypotheses have been advanced to resolve this paradox: Paleoclimate proxies require reinterpretation, boundary conditions need improvement, or a major mechanism is missing from climate models. However, no proposal has led to a simple, general solution. A resolution might be in sight based on efforts to develop better climate proxies and multiproxy Eocene records. But this resolution may present new challenges.

Most of what we know about Eocene SSTs comes from records of the oxygen isotopic composition ([δ¹⁸O]) of planktonic foraminiferal calcite shells that reflect the temperature of shell formation and other factors (3, 5). Reconstructing temperatures using [δ¹⁸O] of foraminifera requires knowing or assuming the δ¹⁸O of the seawater in which they grew. This composition changes globally as terrestrial ice volume varies, and regionally with changes in net evaporation. Estimated evaporation is, in turn, affected by SST. Interpretation is complicated by ambiguity about the depth range at which foraminifera were calcifying and by seasonal biases.

Even more troubling, the δ¹⁸O of planktonic foraminiferal shells may be diagenetically altered once they reach the seafloor (that is, in the early phases of burial, secondary calcite can precipitate from the much colder sediment pore-water fluids onto or into the shell itself). Alteration can push SST estimates toward cooler (bottom water) values (6). Alteration is enhanced by increased rates of pore-water flow and increased burial temperature and pressure (6). Thus, the best preservation is expected from relatively impermeable sediments that have not been buried deeply or exposed to high temperatures.

Pearson et al. (7) sought and found records from clay-rich regions in the hope of identifying whether previous records were biased. In a series of pioneering studies (7–10), they recovered foraminifera from shallowly buried impermeable clays in several regions, most notably in Tanzania. When viewed under a light microscope, the recovered foraminifera were translucent or “glassy,” as modern samples are, unlike the “frosty” or “chalky” appearance of typical foraminifera used in most previous analyses. Under a scanning electron microscope, the frosty shells were revealed as recrystallized. Analysis of δ¹⁸O in the glassy foraminifera indicated temperatures warmer by 5° to 10°C than previous reconstructions (8). The implications are that the shells in many reconstructions from open-ocean sediments were altered and that much of the original isotopic SST signal had been overprinted by cold deep water trends. Acceptance of this viewpoint is, however, not universal (11, 12). Focus has shifted to using other proxies, such as Mg/Ca and TEX₈₆₀ to develop independent SST records.

The Mg/Ca ratio of foraminiferal shells is a function of temperature and offers a paleoclimate record independent of seawater δ¹⁸O. However, the exact relationship is species-specific; depth, seasonality, and alkalinity have influences, the global value of seawater Mg/Ca must be measured or modeled as it varied in time, and Mg/Ca values may also be diagenetically altered, although perhaps to a lesser degree than δ¹⁸O (8).