IMAGING TECHNIQUES

A record-breaking microscope

An electron microscope has been developed that produces images at higher resolution than conventional approaches can achieve, and is suitable for studying fragile materials that can be damaged by electron beams. SEE ARTICLE P.343

JOHN RODENBURG

On page 343, Jiang *et al.*[1] report the highest-magnification image ever obtained using a transmission electron microscope. The image reveals the atoms in a two-dimensional self-supporting sheet of a semiconductor, and has a resolution of 0.39 ångströms; for comparison, most atoms are about 2–4 Å in diameter. The technique might eventually allow 2D materials to be examined with unprecedented precision, providing insight into this burgeoning class of useful compounds. It might also lead to the development of a method that can image individual atoms in 3D objects.

To generate their image, the authors used a method called ptychography (the 'p' is silent) in which radiation — in this case, electrons — is passed through a specimen to produce many 2D diffraction patterns. The basic principle of the technique was proposed almost 50 years ago by the physicist Walter Hoppe, who reasoned that there should be enough information in the diffraction data to work backwards to produce an image of the diffracting object[2]. However, it was many years before computer algorithms were developed that could do this reverse calculation easily and reliably[3,4]. The pictures produced by ptychographic methods are generated using a computer from a vast amount of indirect scattering data. An important advantage of this approach over conventional microscopy is that it can surpass the resolution limit imposed by lens imaging. In fact, it can work without any lenses at all[5].

Over the past ten years, ptychography has been widely used for microscopy in the X-ray[4], extreme ultraviolet[6] and visible-light[7,8] regions of the electromagnetic spectrum. It has also been used with some success with electrons, but Jiang and colleagues are the first to show that it can surpass the resolution obtained by the best electron lenses. For the technique to work, every electron must be counted almost perfectly, but the scattering patterns contain both extremely bright and extremely dark regions (they are said to have a high dynamic range), which makes it difficult to count every electron without any errors. To complicate matters further, the experiment must be done as quickly as possible to fulfil other experimental constraints, which means that about 1,000 diffraction patterns must be recorded every second.

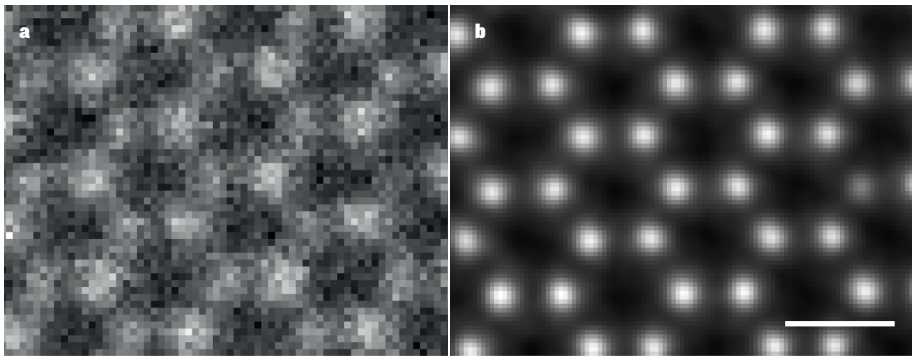

Figure 1 | Improving the resolution of electron microscopy. **a**, This image of a sheet of molybdenum disulfide was obtained using annular dark-field electron microscopy — the conventional method for obtaining extremely high-resolution images of samples. **b**, Jiang *et al.*[1] report an electron microscope that works by analysing the diffraction patterns of electrons that have been transmitted through a sample (a technique known as ptychography). This method provides the best resolution yet reported for an electron microscope. Here, the atoms in the sheet of molybdenum disulfide are much clearer. Scale bar, 3 ångströms.

Speed, accuracy and dynamic range are conflicting performance parameters in any electron detector — achieving them all is difficult. All previous electron detectors have compromised on one or more of these properties. The authors' main achievement is to implement a detector that can handle such demanding specifications.

Remarkably, Jiang *et al.* gave themselves a huge handicap with regard to beating the resolution record. For any given microscope lens, the best resolution is achieved by using the shortest possible wavelength of the radiation or electron beams concerned. However, the authors used relatively low-energy electrons, which have twice the wavelength of those used in the highest-resolution lens-based microscopes[9,10]. Using low-energy electrons for microscopy is good because it greatly reduces the damage inflicted on the specimen by the electrons. But in this case, it also meant that the resolution of the lens used by Jiang and colleagues was reduced by a factor of two. To beat the resolution record, the authors had to process a particular subset of the ptychographic diffraction data (the high-angle data), thereby obtaining an image with a resolution 2.5 times better than would otherwise have been possible.

Achieving high resolution is not the whole story, however. Anyone with poor eyesight knows that they need as much light as possible if they want to read small print. This is because there is an intimate relationship between resolution, contrast and the amount of light illuminating the object. If the small print is light grey, not black, then the contrast is low, and even more light is needed to read it. The same principle is true for an electron microscope.

Jiang and colleagues used ptychography to work out how a particular property of the electron waves, known as the phase, changes as the waves pass through an object. This information can be used to produce images that have strong contrast — even for specimens that contain atoms of low atomic number, which are difficult to detect with conventional electron-microscopy methods that offer very high resolution. The authors therefore needed relatively few electrons to generate their images compared with other state-of-the-art techniques, such as annular dark-field electron microscopy (Fig. 1). So not only did they use low-energy electrons without compromising resolution, but they also used many fewer electrons than other techniques do, further reducing the damage done to the sample.

Perhaps the most striking feature of Jiang and co-workers' image is not the atoms themselves but the enormous gaps between them. The average bond lengths in a material can be measured in a bulk sample by using all sorts of diffraction and spectroscopic methods, but the authors' image provides an extremely precise measurement of the lengths of the bonds between individual pairs of atoms, which are sensitive to the atoms' local bonding

environment. But are ultrahigh-resolution images of gaps between atoms useful for anything else?

I think the answer lies in the big success story of X-ray ptychography: tomography[4], a technique in which lots of 2D images of a transparent object are acquired as it is rotated, so that a 3D image can be built up. Phase information is an ideal imaging signal for this technique. But when images are taken through a solid object, the resolution needs to be as high as possible to distinguish features lying on the top surface from those at the bottom, many of which will seem (when seen in projection) to be laterally close to one another.

Jiang *et al.* tested the resolution of their electron microscope by putting two layers of atoms on top of one another and measuring the minimum apparent lateral distance between atoms in different layers, some of which were almost overlapping. In my view, this test demonstrates that their instrument could potentially be used for tomography. In theory, such imaging of multiple layers is not limited to crystalline 2D materials and could be used for any complicated, non-crystalline structure. Unfortunately, for thicker objects, the electron waves would scatter so strongly that they would spread out and re-interfere with each other in complicated ways, which would make it even harder — although in theory not impossible — to work out the structure.

Perhaps the take-home message of this work is not so much the record resolution, or its applications to 2D materials, but the fact that it will provide a way of precisely imaging the 3D bonding of every individual atom in a solid volume of matter, while using a minimal flux of damaging electrons. Indeed, the authors allude to this enticing possibility in their conclusions, suggesting that the next step is to use their remarkable detector for tomography. The aim would then be to solve the exact 3D atomic structures of solids that have no long-range order, such as nanocrystalline materials, glasses and amorphous metals, for which we must currently infer structures from averaged bulk measurements. ■

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CARDIOVASCULAR BIOLOGY

Cells stop dividing to become arteries

An analysis of gene-expression patterns in single cells provides detailed insights into the developmental processes that lead to maturation of the coronary arteries. SEE ARTICLE P.356

ARNDT F. SIEKMANN

The human heart pumps between about 5 and 20 litres of blood through the body every minute[1]. To receive enough oxygen to fulfil this tremendous task, heart-muscle cells need their own blood supply. This is provided by specialized blood vessels, including coronary arteries. Defects in these arteries can lead to coronary heart disease and even heart attack[2,3]. Understanding how coronary arteries form during embryonic development is therefore of great interest, because such knowledge might help in developing strategies to prevent or treat coronary heart disease. On page 356, Su *et al.*[4] provide a detailed picture of the sequence of events that leads to coronary artery development.

The cells that generate coronary arteries originate from various regions of the embryo, including a sac-like structure called the sinus venosus that adjoins the embryonic heart[5,6]. From these sites, the cells invade the heart's muscle-cell layer. Here, they form an immature blood-vessel network called a plexus that is subsequently remodelled into functional arteries and veins.

Su and colleagues set out to investigate how cells from the sinus venosus develop into coronary arteries, using single-cell RNA sequencing (scRNA-seq) — a technique that enables precise identification of the genes being expressed in each cell of a tissue[7]. Gene-expression patterns change during tissue differentiation, for example as sinus venosus cells mature into coronary arteries. Comparison of the gene-expression patterns for individual cells of a given type can therefore reveal the cells' relationships to one another.

The authors extracted single endothelial cells, which make up the inner lining of blood vessels, from the hearts of mouse embryos at a developmental time point just before coronary artery formation. They reasoned that, at this embryonic stage, they would obtain cells at the various stages leading to coronary artery maturation, including sinus venosus and plexus cells. They then used bioinformatics to investigate the lineage relationships between these cells.

It has been thought that the remodelling of

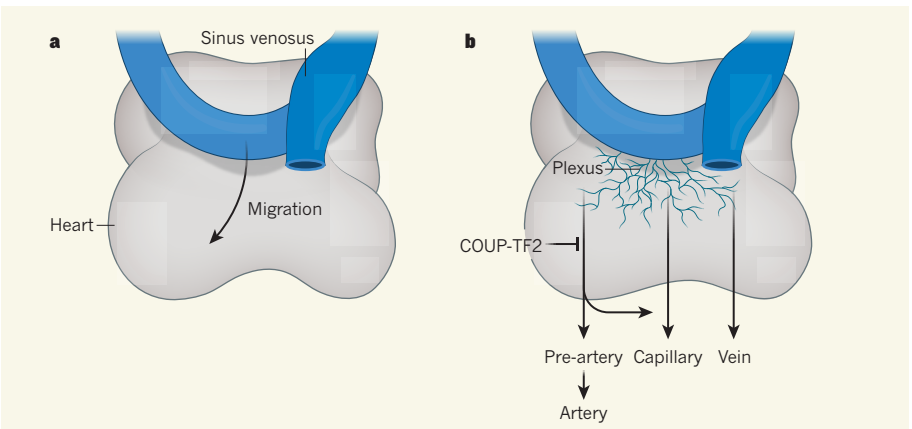

Figure 1 | Coronary artery development starts early. **a**, During the development of mouse embryos, cells from a sac-like structure called the sinus venosus migrate into the muscle-cell layer of the heart. **b**, There, they give rise to an immature blood-vessel network (a plexus), which will be remodelled to form arteries, veins and capillaries. Su *et al.*[4] have shown that a subpopulation of immature plexus cells, which the authors dub pre-artery cells, have a gene-expression profile that is characteristic of mature arteries. The transcription factor COUP-TF2 prevents plexus cells from adopting this profile. Pre-artery cells predominantly give rise to mature coronary artery cells, although a few become part of capillaries instead. (Figure adapted from Fig. 4h of ref. 4.)