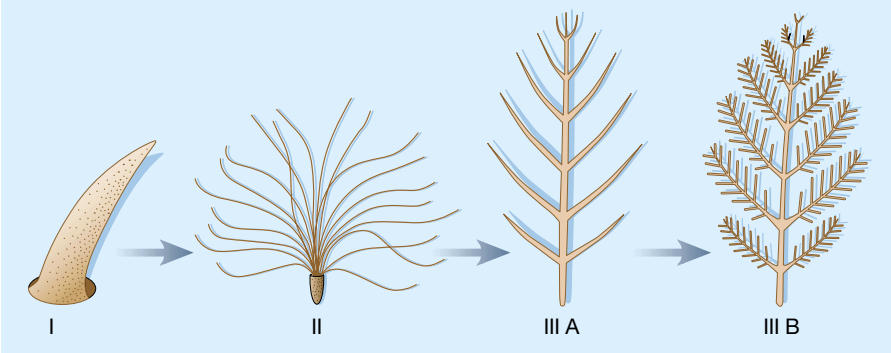

Figure 1 **Hypothesized stages I–III of feather evolution[12]. Stage I of this model assumes an unbranched, hollow filament, which developed from a cylindrical invagination of the epidermis around a papilla. In stage II, a tuft was formed by fusion of several filaments at their bases. Stage III represents the formation of a central rachis and development of serially fused barbs (III A) — to which, at a slightly later stage (III B), secondary barbs (barbules) were added. The two other stages, IV (bipinnate feathers with elaborate barbules and a closed vane) and V (the asymmetrical flight feathers of modern flying birds), are not shown. (Figure redrawn from ref. 5.)**

the Yixian Formation not only further strengthen the case for the theropod–bird connection, but also establish that feathers originated and initially diversified in non-flying non-avian theropod dinosaurs. Feathers pre-date the origin of birds and avian flight. They clearly evolved for some purpose other than flight, perhaps thermal insulation[3] or behavioural display[4] (or both) — functions they retain in present-day birds. ■

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Optics

Holograms of atoms

John Spence

X-ray crystallography can be used to produce three-dimensional images of atoms providing they are arranged periodically. For small atomic clusters or molecules, electron holography could provide even sharper images.

"That's the last potato I'll dig," said the young Ernest Rutherford, perhaps the greatest experimental physicist of the last century, on hearing, on his father's farm in New Zealand, that he'd won a scholarship to the University of Cambridge. And so began a century in which most Nobel prizes in physics and chemistry have been won by scientists trying to figure out the structure or arrangement of atoms by scattering things off them. Calculating structure from scattering patterns is known as 'inversion' — a problem Rutherford solved with help from a mathematician friend. Others who packed their bags for Stockholm included the team that developed methods for determining the structure of proteins from X-ray scattering, and Denis Gabor, who invented holography, a technique for creating three-dimensional images without using a lens.

As they describe in *Physical Review Letters*, a group of Swiss and Italian scientists[1] working at the Trieste X-ray synchrotron has now found a way to apply Gabor's method more effectively to electron scattering patterns, and so have produced strikingly beautiful three-dimensional holographic images of the local arrangement of atoms near the surface of a crystal. The technique Wider *et al.*[1] use is known as internal-source holography because it exploits an internal source of radiation to probe below the surface of structures. The hope is that this method can now be applied to reveal the structure of small atomic clusters that cannot be crystallized for analysis by conventional X-ray crystallography — from biological macromolecules to catalyst particles and nanostructures.

In Gabor's original proposal[2], a small

100 YEARS AGO

Dr. B. Sharp described, at a recent meeting of the Academy of Natural Sciences of Philadelphia, some observations he has made on the contents of the stomachs of the common cod. Several hundred stomachs were opened with the hope of finding shells of gastropods and bivalves. Numerous valuable shells were taken from the cod years ago by Stimpson and Gould on the New England coast, north of Cape Cod, and it was supposed that similar finds would come to light from the cod caught off Nantucket. The stomachs examined were filled almost exclusively with crustaceans and for the most part with species of *Panopeus*. Hermit crabs, without shells, and a few Crepidulae were also seen. Here and there young lobsters were found in the stomachs, occasionally two in one stomach. Dr. Sharp believes that the decrease in quantity of the lobsters, which has been so marked within the past few years, is partly due to their consumption by the cod; and as these have of late greatly increased in numbers, owing to the work of the United States Fish Commission, the lobsters have not been able to keep pace with the increase of their enemies.

From *Nature* 25 April 1901.

50 YEARS AGO

From the news in the Press it might be thought that ordinary administration and, more especially perhaps, forestry administration would be more or less at a standstill in Malaya. The annual report for 1949 of the Federal Forest Administration of the Federation of Malaya, by J. P. Edwards, acting director of forestry, shows the reverse, particularly in the professional and research sides of the work. It is true that the report states that, in connexion with saw milling, "Bandit activity has also caused the sawmills much difficulty. One was burnt down, several more have closed down to avoid being forced to provide supplies to the bandits, others have had lorries destroyed and many find difficulty in persuading their logging gangs to remain at work. The Police, too, are often obliged to clear the forest of loggers so that they are not mistaken for bandits during security operations. In spite of this millers have shown great determination to keep producing and develop their mills. Eleven new mills were planned and five others increased their plant."

From *Nature* 28 April 1951.

external source of radiation illuminates a semi-transparent object, so that a magnified shadow-image (the hologram) appears on a distant screen, much as a child might project rabbit's ears on a wall by making finger-shapes in front of a lamp. Now replace the lamplight with the wavelike electron emitted by an atom just below the surface of an aluminium crystal. The electron can be liberated from the emitter atom by exciting it with an X-ray beam — the photoelectric effect. If you detect the photoelectrons emerging from the crystal surface on a hemispherical screen, then you have the essential arrangement of Wider *et al.*[1] (Fig. 1a). The image of the atoms in the surface layer (the scatterers) is projected by the emitter (source) atom in the layer below. (In fact, because the emitter emits, and the scatterers scatter, in all directions, one obtains a three-dimensional image of all the atoms around the emitter atom.)

Gabor found that the finest detail recoverable from his holograms was about equal to either the wavelength of the radiation or the size of the source — whichever was larger. Wider *et al.* use a wavelength of about 0.04 nanometres, whereas the photoelectron comes from an even smaller region inside an atom, so its wavelength could be shorter still. The small source size enables higher resolution, but makes the emitted electron waves highly uniform (coherent), so that the shadow-image becomes an uninterpretable interference pattern, whose inversion Gabor struggled with unsuccessfully. The idea of using an internal source can be traced back even further. In 1939, Bragg inverted X-ray scattering patterns from crystals containing strongly scattering internal 'source' atoms, by using visible light for the reconstruction[3] — a two-step process similar to Gabor's 1949 holography. More recently Bartell and Ritz[4] used an internal atomic source for electron holography of atoms in a gas.

Interest in photoelectron holography started in the late 1980s when Szöke[5] proposed the internal-source method used by Wider and colleagues[1]. A three-dimensional inversion scheme was introduced by Barton[6] for point-like scatterers, and the first experimental results were obtained by Harp *et al.*[7,8]. You may be wondering why every scattering atom doesn't also act as a source — it does, but because every atom lies among identical atomic surroundings in the periodic structure of the simplest crystals, it produces an identical hologram. For atomic clusters lying on a crystal surface, one can tune the incoming X-rays to excite one particular atomic species, which then alone acts as an emitter. In order for all the clusters to produce identical superimposed holograms, they must all lie in the same orientation, but need not be periodically arranged.

Physicists applying Barton's inversion scheme ran into several problems — notably a 'twin image' problem, which Gabor was famously unable to solve. The twin image is a ghostly inverted image, which appears superimposed on the true image in most inversion schemes. This problem and another involving multiple scattering have been addressed with some success over the past decade by adding together holograms recorded at several electron energies. A third problem arises because the emitting and scattering atoms do not emit pure spherical waves as the inversion schemes assume. Instead, scatterers scatter too strongly in the direction of the incident electron (forward scattering), especially at the higher energies (smaller wavelengths) needed for good resolution.

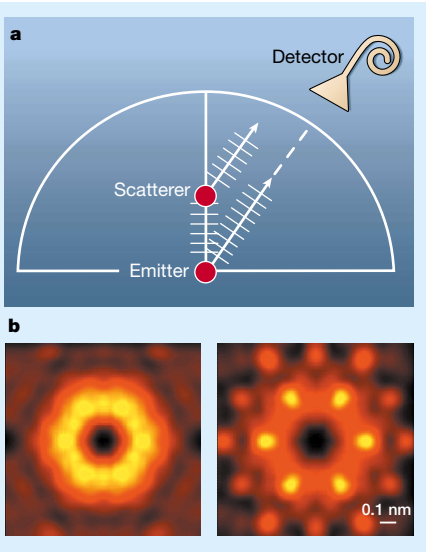

Figure 1 **Looking deeper with internal-source holography.** a, **In their experiment, Wider *et al.*[1] use X-rays to excite an emitter atom below the surface of an aluminium crystal. The emitted photoelectron can reach the detector either directly or by way of a scatterer atom in the surface layer. These two paths produce an interference pattern at the detector (similar to Young's fringes) which can be inverted to provide a three-dimensional image of the atoms around the emitter.** b, **Electron holograms of aluminium crystal (yellow shows highest intensity) from diffraction data collected at ELETTRA, Trieste[1]. The left hologram does not resolve atomic positions well because of forward scattering problems. The emitter atom is in the centre, and appears dark because it cannot image itself. Now, by controlling the angle between the detector and the emitted radiation, Wider *et al.* are able to distinguish nearest and next nearest positions of neighbouring atoms. The right hologram shows 18 atoms lying in one plane of an aluminium crystal. In fact, because of the three-dimensional nature of the holograms, Wider *et al.* can locate all 12 of the emitter atom's nearest neighbours (three above, three below and six in the same plane).**

The achievement of Wider *et al.* is to solve the forward-scattering problem, which they do by adjusting the polarization direction of the incident X-rays, so that the detector is always positioned close to an intensity minimum in the emitter's emission pattern. Practically speaking, the crystal then has to be rotated with respect to the synchrotron and the detector. The result is a more accurate inversion that clearly shows all the surrounding atoms out to much greater distances than previously possible, and in their correct places (Fig. 1b). Forward scattering is a problem when the emitter atom lies further from the detector than the scatterer, as in Fig. 1a. For the more interesting case of a cluster of foreign atoms on a crystal surface (using the top atom as the emitter) this problem doesn't arise, because the electrons must reverse direction to reach the detector. Nevertheless, some adsorbate emitters can lie inside a crystal surface, putting them beyond the scatterer.

These internal-source ideas have spawned other sub-fields[9,10]: X-ray fluorescence holography (XFH) and its time-reversed inverse, IXFH, and Auger fluorescence holography. X-ray fluorescence holograms are excited by synchrotron X-ray beams, and do not suffer from multiple scattering or uneven scattering problems, although the scattering of X-rays by atoms is much weaker (especially for light elements) than for electrons, and radiation damage effects differ. At the very least, these methods provide a starting point on which to base highly accurate calculations of the scattering patterns, and the internal-source method fills a niche between the extended X-ray absorption fine structure technique, which is used to characterize local atomic structure, and crystallography.

Researchers in Berlin, Brookhaven, Grenoble, Berkeley and elsewhere are beginning to apply internal-source methods to locate dopant atoms in semiconductors and alloys, to probe ordering in alloys, quasicrystals and magnetic atoms, and to look at buried interfaces, epitaxial films or macromolecules. Perhaps, with the next generation of brighter synchrotrons, internal-source holography may one day provide three-dimensional holographic movies of chemical processes in action on the atomic scale. If radiation damage doesn't prevent it, we may also see holograms of the membrane proteins, important for drug delivery, which are so difficult to crystallize for analysis by X-ray crystallography. The challenge now is to find ways to prepare arrays of similarly oriented clusters for the new internal-source holographies. ■

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Genome sequencing

A grin without a cat

Paul R. Gilson and Geoffrey I. McFadden

In some types of unicellular algae, the chloroplasts have their own nucleus — a legacy of the time when the chloroplast was a free-living cell. The sequence of the genome in one such nucleus is now revealed.

The electronics industry's mania for miniatures has brought us nifty gadgets such as pocket PCs, handheld televisions and wristwatch phones. But these devices are giants compared with what nature can produce. On page 1091 of this issue[1], Douglas and colleagues present an extraordinary case of genetic miniaturization — the genome sequence of an amazingly small nucleus. The nucleus in question is the so-called nucleomorph of a cryptomonad alga, *Guillardia theta*, and its genome weighs in at a mere 0.55 million base pairs. Compared with the human genome (3,200 million base pairs)[2,3], this nucleomorph sequence is sub-Lilliputian. How can two blueprints be so different?

In fairness, the nucleomorph is not a complete nucleus but a relic, its genome having been distilled to its essence by hundreds of millions of years of enslavement. Nucleomorphs are the highly reduced nuclei of 'endosymbiotic' algal cells that, in the distant past, set up home within unicellular hosts to mutual benefit. Like their hosts, the endosymbionts were eukaryotic, meaning, loosely, that they had a nucleus. Importantly, they were also photosynthetic, feeding their hosts with the products of this chemical reaction — carbohydrates and oxygen.

Such microscopic gardening arrangements are common. But the cryptomonad endosymbiosis belongs to a special category known as secondary endosymbiosis, whereby the photosynthetic captive becomes an integral, enduring part of the host cell. In the case of *G. theta*, the endosymbiont became what is known as a complex chloroplast. Over time — perhaps as many as 600 million years — the nucleus of this endosymbiont lost most of its genes. Nevertheless, Douglas *et al.*[1] show that the tiny vestige, the nucleomorph, is a bona fide nucleus. It has the usual eukaryotic trappings: several linear chromosomes; introns (sections of DNA that interrupt the coding sequence of genes); possible centromeres (the regions on eukaryotic chromosomes to which the chromosome-separating apparatus attaches during cell division); and histones (proteins that are swaddled by DNA). Indeed, the nucleomorph is a fairly typical nucleus but for two features — an impoverished complement of genes, and an almost complete lack of non-coding DNA.

Douglas *et al.* find that this cryptomonad nucleomorph has only 531 genes, whereas humans[2,3] boast at least 31,000. But it is gene density that makes the two genomes so different. Genes make up a mere 1% of the human genome[2,3]. The other 99% — often referred to as junk or non-functional DNA, which has largely unknown functions — may simply be the accumulated clutter of a system with slovenly housekeeping. Nucleomorphs, on the other hand, are the epitome of neatness and compactness. Many of the *G. theta* nucleomorph's genes have few or no spaces between them, and 44 genes even overlap, parsimoniously using both strands of the chromosomes, rather than the usual one[1]. The human genome seems profligate by comparison. Indeed, the entire nucleomorph genome would fit comfortably in one of the many yawning gaps between human genes.

Why are these two genomes so different? Evolutionary forces that shape genome size are not well understood. But we believe that streamlining of the nucleomorph genome is unlikely to be driven solely by natural selection for minimal DNA content. Rather, it may be a result of uncontrolled DNA loss, and it is here that comparison of these two genomes might be enlightening. Unlike other human chromosomes, the Y chromosome has no complementary partner, so it cannot undergo recombination during meiosis (the type of cell division that generates gametes, such as eggs and sperm). Recombination is a process by which mutations — particularly insertions and deletions of sequence — can be weeded out in subsequent generations[4]. The absence of recombination has resulted in wholesale loss of DNA from the Y chromosome, which is now a mere stump with only 50 million base pairs[2,3]. This chromosome persists, however, because it carries a handful of vital genes, in particular the testis-determining factors.

Nucleomorphs do not have sex chromosomes and each of the three chromosomes is thought to be paired[5]. Nevertheless, nucleomorph chromosomes are probably denied the normal opportunity to recombine because cryptomonad endosymbionts do not seem to undergo meiosis followed by genetic exchange with other nucleomorphs[1]. We believe that this genetic isolation and consequent lack of error-correcting mechanisms has caused nucleomorphs to slide into mutational hyperdrive and wholesale DNA loss. But, just as the Y chromosome is maintained because it produces something vital (such as testicles), so too must the nucleomorph genome endure — in this case, because it encodes components of the chloroplast[1].

The interesting question really is not 'How did the nucleomorph genome get so small?', but rather 'Why did it stop where it did?'. Just as the Cheshire Cat in Lewis Carroll's *Alice in Wonderland* faded until only its grin remained, nucleomorphs too appear to have reached an end-point. This applies not only to cryptomonads but also to chlorarachniophyte algae, the nucleomorphs of which also have three chromosomes and similarly small genomes[6]. The nucleomorphs of these two types of algae were derived from different secondary endosymbioses[1,7], so why have their genomes condensed to a similar end-point? Douglas *et al.*[1] offer an attractive explanation.

Gene sequences from the *G. theta* nucleomorph indicate that, as in other eukaryotes, the DNA is wrapped around histone proteins, forming 'chromatin'[1]. However, in contrast to other eukaryotes, nucleomorph chromatin apparently does not condense into higher-order structures during cell division[8]. Douglas *et al.* calculate that uncondensed nucleomorph chromosomes are only just short enough to fit inside a nucleomorph. If the nucleomorph DNA were packaged into fewer than three chromosomes, then those chromosomes would be too large to segregate during cell division. Conversely, if the DNA were separated onto more than three chromosomes, they might be too small to survive[9].

As bonsai versions of the nucleus, nucleomorphs provide important genetic and evolutionary lessons. The path taken by cryptomonads and chlorarachniophytes to obtain chloroplasts, namely by engulfing other algal cells, is well worn. Most phytoplankton — the algal backbone of aquatic food chains — also acquired their chloroplasts in this second-hand way[10]. But in these phytoplankton, all genes have been transferred to the host nucleus from the engulfed nuclei, which have been lost. Nucleomorphs are kept only while they encode something necessary for survival, probably proteins required to operate and maintain the chloroplast.