

**Figure 1 | Role of LL37 in psoriasis.** In response to microbial infection or tissue injury, LL37 is produced locally in the skin, where its normal functions include antimicrobial activity and aiding in wound-healing. Lande *et al.*<sup>1</sup> show that LL37 also forms complexes with self DNA that is released from dying cells. In normal skin, these DNA-LL37 complexes probably remain undetected and inconsequential. But in the presence of plasmacytoid dendritic cells (pDCs), which accumulate in the skin lesions of patients with psoriasis, these complexes trigger strong interferon- $\alpha$  (IFN- $\alpha$ ) production, which is known to perpetuate the disease<sup>10</sup>.

break tolerance and signal the production of IFN- $\alpha$  (Fig. 1). These findings considerably advance our understanding of psoriasis, and could provide insight into other biological and pathological processes.

But how do the three elements of this response — self DNA, LL37 and pDCs — converge? A high turnover of keratinocytes in the psoriatic lesions, and the associated release of DNA from dying cells, means that high concentrations of human DNA are on hand at the disease site. The high local levels of LL37 can be explained by the pathways that induce its production — infection and tissue injury, which are also known to precede psoriasis onset. Yet the reason for pDC accumulation, a hallmark of psoriasis, is unknown. Could it be that  $\beta$ -defensin 2, the other antimicrobial peptide highly expressed in this disease, attracts pDCs to or retains them in psoriatic lesions? This peptide is known<sup>10</sup> to attract cells that express the chemokine receptor CCR6, and so may recruit CCR6-bearing pDCs<sup>11</sup>. However, as both LL37 and  $\beta$ -defensin 2 are increased in normal skin responses, additional factors specifically associated with psoriasis might lead to an especially robust induction of  $\beta$ -defensin 2 or an exaggerated pDC response.

The deleterious activity of LL37 in psoriatic lesions, as observed by Lande and colleagues, unveils an apparent paradox. It has been shown<sup>12</sup> that microbial stimulation of macrophage cells causes increased expression of the genes encoding the vitamin-D receptor and the enzyme vitamin-D1 hydroxylase. This, in turn, leads to vitamin-D-mediated induction of LL37, which helps macrophages to kill

pathogens. If the molecular signalling pathways in skin keratinocytes and macrophages were similar, one might imagine that application of vitamin D to psoriatic lesions would increase LL37 concentrations and perhaps exacerbate the disease. However, the opposite is true. Vitamin-D3 analogues are mainstay drugs for psoriasis<sup>2</sup>.

Possible explanations for this puzzle may include tissue-specific effects of vitamin D, altered responses to vitamin D depending on the cytokine milieu in the lesions, and the already maximal induction of LL37 in skin lesions before therapy. One would certainly expect that several biological effects of vitamin D, beyond increasing LL37 expression, may trump its possible effects on LL37. Investigation of this apparent paradox may lead to even more effective therapies for psoriasis.

Abnormal levels of LL37 in the skin have also been linked to other human diseases, including a skin disorder of unknown cause called rosacea<sup>13</sup>, and the allergic skin disorder atopic dermatitis, which is associated with susceptibility to skin infections<sup>4,14</sup>. These findings, together with those of Lande *et al.*<sup>1</sup>, highlight the importance of tightly controlled LL37 expression for healthy skin. Moreover, that LL37 can bind to self DNA and subsequently activate pDCs may mark a seminal discovery in the field of autoimmunity in general. Earlier work<sup>15</sup> had shown that recognition of complexes of self DNA and antibodies underlies autoimmunity. Thus, complexes of self DNA with components of the immune system, both innate (LL37) and adaptive (antibody), are involved

in the perpetuation of autoimmune diseases. Once in complex, self-DNA molecules become strongly immunostimulatory by engaging crucial molecular signalling pathways mediated by Toll-like receptors. The physiological role, if any, of such immunomodulatory complexes remains to be discovered.

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## IMAGING TECHNOLOGY

# Harmonic pictures in a flash

John Spence

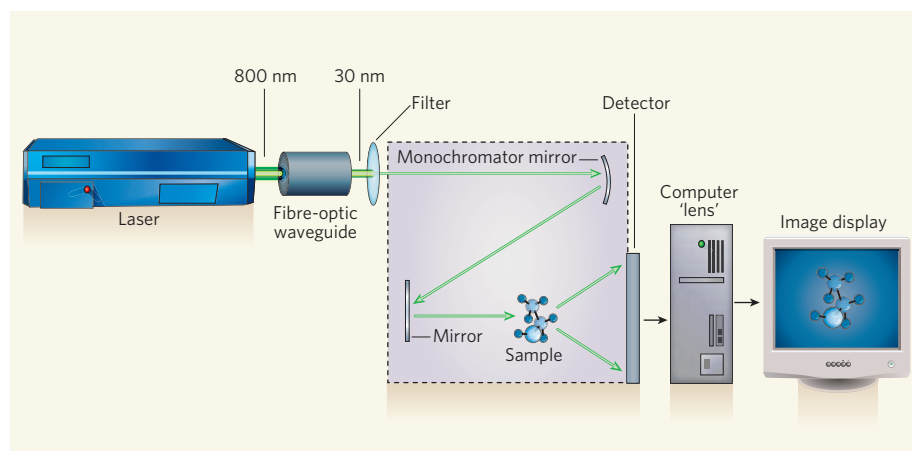
**Making films of atomic-scale processes as they happen makes huge demands on any imaging system. One approach combines the advantages of pulsed laser harmonics and computerized image reconstruction.**

To make a movie of molecular rearrangements, the criteria are strict. Enough of the illuminating beam should be scattered off the imaged object during each frame to form an image (at least one particle for each pixel), so the beam must be very intense and have a high scattering probability. This scattering should also be mostly elastic, and not transfer too much damaging energy to the sample. But most crucially, the imaging camera should have a frame speed of a few femtoseconds, coupled with atomic-scale spatial resolution.

These dual demands rule out most conventional imaging techniques. Optical lasers, for example, can offer the right sort of speed (a single period of laser light lasts about 2.5 fs), but they fall down on spatial resolution. (Spatial

resolution is generally limited to about the wavelength of the probe, and the wavelength of optical light lies in the region of 400–700 nanometres.) Conversely, electrons have a small enough wavelength, but lack the requisite speed owing to the added complication of their charge interactions. Meanwhile, X-rays are limited by the aberrations and fabrication difficulties of the ‘zone-plate’ lenses that focus them.

Writing in *Physical Review Letters*, Sandberg *et al.*<sup>1</sup> report taking a valuable step towards circumventing these problems. They combine recent breakthroughs in lensless imaging — which avoids the problems associated with lens aberrations by using a computer for image reconstruction — with advances in laser-driven X-ray generation to overcome the problem of



**Figure 1 | Fast pictures without lenses.** Sandberg *et al.*<sup>1</sup> convert laser light at an optical wavelength into 30-nm-wavelength extreme ultraviolet pulses in an argon-filled fibre-optic waveguide with an inner diameter of 150  $\mu\text{m}$ . The resulting fast, directed 'high-harmonic' beam has a diameter of 25  $\mu\text{m}$  that is further reduced in intensity (by several orders of magnitude) by filters and a monochromator. The authors get around the need for low-wavelength lenses (which are prone to large aberrations) by using an iterative phase-retrieval computer algorithm to extract the maximum information from the scattered light.

spatial resolution, while preserving the laser's innate speed.

This advance is just the latest act in a fascinating story of the replacement of lenses by computers in imaging technology. The origins lie in the realms of signal processing, X-ray crystallography and electron microscopy<sup>2</sup>, and the breakthrough for X-rays came in 1999, with the first non-holographic reconstruction by numerical means of an image made by scattering X-rays from a non-periodic sample<sup>3</sup>. The current state-of-the-art<sup>4</sup> fast, lensless imaging technique uses radiation produced by a free-electron laser at a synchrotron facility to make, in a single shot, images with a temporal resolution of 25 fs and a spatial resolution of 90 nm.

The secret behind all these techniques is an iterative phase-retrieval algorithm<sup>2</sup>. Iterative phase retrieval is one answer (various forms of holography and X-ray crystallography use other approaches) to the notorious 'phase problem' — that all detectors record only the intensity of the radiation that impinges on them, throwing away the phase information. Under suitable experimental conditions, however, this phase information is encoded in the intensity, and may be recovered if the intensity is sampled correctly. The algorithms iterate between the image and the scattering pattern (which are related by a mathematical operation known as a Fourier transform), while imposing known information, such as the approximate boundary of the object, on each. The great strength of such an algorithm is that it can be implemented for any type of imaging particle of any wavelength. Each particle interacts differently with a sample, and so can potentially provide new information about it. On the downside, such algorithms introduce constraints on the sample geometry, and coherence and aberrations in the illuminating wavefield become important.

Sandberg *et al.*<sup>1</sup> generate very 'soft' X-rays

(actually, extreme ultraviolet radiation) with a wavelength of about 30 nm by scattering intense pulses of infrared laser light of a much longer wavelength (800 nm) on gas atoms (Fig. 1). These X-rays scatter from the object, and are combined into an image with 214-nm resolution using a phase-retrieval algorithm. The imaging technique exploits high-frequency harmonics produced when laser light of energy  $hw$  ( $w$  is the laser frequency and  $h$  is Planck's constant) passes through a nonlinear medium. An atomic electron in the medium absorbs  $n$  laser photons before spitting out a single high-energy photon of  $n$  times the energy (and a similarly increased frequency  $nw$ ), but the same properties of phase coherence and pulse duration as the driving laser.

Classically, we can think of the atomic electron being initially ejected by the laser pulse, before being returned to the atom during the second half of the laser cycle when the electric field reverses direction. The resulting acceleration produces radiation (bremsstrahlung) at the high-harmonic frequency. Importantly, this beam of radiation is directed forwards, and its phase coherence and conversion efficiency are greatly enhanced if generated inside a waveguide. High harmonics extending into soft X-ray frequencies were first observed<sup>5</sup> in 1988, and used 5 years ago for holographic imaging with a resolution of about 10  $\mu\text{m}$  (ref. 6).

Might a high-harmonic technique such as that of Sandberg's group one day provide competition for the large synchrotron particle accelerators currently used for molecular crystallography and the like? Synchrotrons provide tunable radiation with wavelengths from tens of nanometres to less than a tenth of a nanometre by collecting the bremsstrahlung from high-energy electrons accelerated over an optimized path. The wavelengths at which the high-harmonic technique is viable are continuing to fall (a collisional X-ray laser

of wavelength 13 nm seeded by high-harmonic radiation is on the cards), pulsing rates are increasing and pulse duration is decreasing. A similar scheme using laser standing waves to undulate electron beams produces tunable, directed 35-kiloelectronvolt bremsstrahlung X-rays (which have wavelengths of a few hundredths of a nanometre). This would be useful for protein crystallography, but the apparatus occupies a room rather than a table. Then there is wakefield acceleration, in which laser pulses running through a plasma are used to accelerate electrons to giga-electronvolt energies over a few centimetres.

This cornucopia of techniques is starting to produce viable competitors of synchrotrons in the effort to obtain higher-resolution, faster images. But the competition between the alternative techniques is intense. Sandberg and colleagues' method<sup>1</sup>, although promising, has some way to go. Its spatial resolution is still not sharp enough to see atoms, and the images required one and a half hours' exposure time with continuous 25-fs pulsing, owing to losses in the optics. One way around this problem for inorganic samples, in which repetitive processes such as electronic excitation and atomic motion can be triggered by another synchronized laser, is 'stroboscopic' imaging, which builds up pictures at different instants during the repeated cycle. In this case, fewer scattered particles are needed in each pulse, because many noisy images can be added together. A moving picture can thus be constructed by varying the delay between images. With biological samples, the limiting factor for this technique is the radiation damage caused by high exposure.

Despite these outstanding problems, lensless imaging in biology and materials science using electrons, neutrons and X-rays spans a wide and increasing array of techniques and capabilities. It is still early days — atoms were first seen<sup>7</sup> in the field-ion microscope in 1951, and soon after with electron microscopy, whereas atomic-resolution lensless images of a single carbon nanotube were first reconstructed<sup>8</sup> in 2003. One viable proposal for a non-damaging, sub-femtosecond, atomic-scale imaging technique based on self-diffraction of high-harmonic electrons from laser-aligned gas molecules already exists<sup>9</sup>. That is indeed a goal worth striving for. ■

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