

estimates for the date of that common ancestor, between 1902 and 1921, with 95% confidence intervals ranging no later than 1933. These dates are a little earlier than, but do not differ significantly from, a previous estimate¹ of 1931 from an analysis that did not include the 50-year-old viruses.

The interpretation that HIV-1 was spreading among humans for 60–80 years before AIDS was first recognized should not be surprising. If the epidemic grew roughly exponentially from only one or a few infected individuals around 1910 to the more than 55 million estimated to have been infected by 2007, there were probably only a few thousand HIV-infected individuals by 1960, all in central Africa. Given the diverse array of symptoms characteristic of AIDS, and the often-long asymptomatic period following infection, it is easy to imagine how the nascent epidemic went unrecognized. Conversely, such a low prevalence at that time implies that the Congolese co-authors of the paper² were very lucky to come across this infected sample, even if most infections were concentrated in the area of Léopoldville. But can we trust these sequences?

In work on ancient DNA, contamination is especially problematic, and the work should, if possible, be replicated in other laboratories. For DRC60, independent analyses were performed at the University of Arizona and Northwestern University, Illinois. The sequences obtained were similar, but not identical, exactly as expected when samples come from the diverse set of related viral sequences that — because of the virus's rapid rate of evolution — arise within an infected individual⁶. Furthermore, the distance along the evolutionary tree from the group M ancestor to the ZR59 or DRC60 sequences is much shorter than those between the ancestor and modern strains, consistent with the earlier dates of isolation of ZR59 and DRC60, and confirming that these viruses are indeed old.

Although the ZR59 and DRC60 sequences can show only that two subtypes were present in Léopoldville around 1960, in more recent times the greatest diversity of group M subtypes — as well as many divergent strains that have not been classified — has been found in Kinshasa⁷. So it seems likely that all of the early diversification of HIV-1 group M viruses occurred in the Léopoldville area. Yet the SIV strains most closely related to HIV-1 group M have been found infecting chimpanzees in the southeast corner of Cameroon³, some 700 kilometres away (Fig. 1a). The simplest explanation for how SIV jumped to humans would be through exposure of humans to the blood of chimpanzees butchered locally for bushmeat. So why did the pandemic start in Léopoldville? And, as there must have been many opportunities for such transmission over past millennia, why did the AIDS pandemic not occur until the twentieth century?

The answer may be that, for an AIDS epidemic to get kick-started, HIV-1 needs to

be seeded in a large population centre. But cities of significant size did not exist in central Africa before 1900. Worobey and colleagues² reproduce demographic data showing the rapid growth of cities in west-central Africa during the twentieth century. Léopoldville was not only the largest of these cities, but also a likely destination for a virus escaping from southeast Cameroon. In the early 1900s, the main routes of transportation out of that remote forest region were rivers; those surrounding this area flow south, ultimately draining into the Congo River, and leading to Léopoldville (Fig. 1).

The date estimates of Worobey *et al.* are for an ancestral virus, present in the first individual to give rise to separate transmission chains that still exist today. We may never know how many individuals were infected in the previous transmission chain, the one that led from the person initially infected with SIV

to the progenitor of the current pandemic in humans. This exception aside, we can now paint a remarkably detailed picture of the time and place of origin of HIV-1 group M viruses and their early diversification, and thus of the prehistory of the AIDS pandemic. ■

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APPLIED PHYSICS

Virtues of diamond defects

Michael Romalis

A general method for detecting nuclear magnetic resonance signals from a single molecule has so far been elusive. Magnetic sensors that exploit crystal imperfections in diamond might make such a method a reality.

The phenomenon of nuclear magnetic resonance (NMR), which results from the interaction of the spin of an atomic nucleus with an external magnetic field, has successfully been exploited in such disparate techniques as the structural analysis of molecules (NMR spectroscopy) and structural and functional analysis of the human body (NMR imaging), thus spanning length-scales from ångströms to metres. But these techniques have remained mostly bulk methods, in that they usually require more than a billion spins. Writing in this issue, Maze *et al.*¹ (page 644) and Balasubramanian *et al.*² (page 648) describe a new approach to NMR detection that exploits a single spin associated with a crystal imperfection — a 'nitrogen-vacancy centre' — in diamond to achieve unprecedented magnetic-field sensitivity on the nanometre scale. Crucially, the approach works at room temperature, a key requirement for biological applications.

The ideal imaging technique would be passive, applicable to all materials, and have spatial resolution on the atomic scale. In fact, nature provides us with the means for achieving such imaging. Electrons and many nuclei have a finite magnetic moment associated with their spins, and thus create weak magnetic fields around themselves. All one needs, then, is a magnetic sensor with high enough sensitivity to measure these fields.

Several magnetometry techniques have been considered for this purpose, but they

mostly fall short of the required sensitivity. The sensitivity of existing magnetic-field sensors improves with their characteristic length-scale (r) approximately to the power 3/2 ($r^{3/2}$). But the magnetic field generated by the magnetic moment of a single electron or proton drops with the third power of the distance from the spin (r^{-3}). Thus, the best hope for detecting magnetic fields from single spins comes from sensors on the nanometre scale.

A milestone in this direction was achieved a few years ago when a cantilever with a magnetic tip was used to detect the magnetic field created by the spin of a single electron³. Further improvements to this approach, known as magnetic resonance force microscopy (MRFM), might allow detection of the magnetic field produced by a single nuclear spin, which is a thousand times smaller than that produced by an electron's spin. But MRFM remains a challenging method because it requires a cryogenic environment and prolonged data averaging.

Optical methods involving scattering of light have long been used to study single particles. This is because it is relatively straightforward to detect individual photons. In some atomic systems, the scattering of photons can be made to depend on the direction of the particle's spin, allowing individual spins to be detected. Such methods have been used to detect electron magnetic resonance signals from a single optically active molecule^{4,5}, but they cannot be used

in most samples because they lack convenient optical transitions.

To develop a more general method, it is possible to use one spin with optical readout as a detector of the magnetic fields created by the spins in the sample under analysis (Fig. 1). To achieve sufficient sensitivity, the interaction between spins must persist for a relatively long time. This requires both the detector and the sample spins to have long spin-coherence times, so that their direction is only occasionally perturbed by quantum fluctuations. There are many techniques for achieving long spin-coherence times in atomic systems, even for a single spin that is held, for example, in a laser trap. However, it is generally impossible to bring a sample within nanometres of such spin without disrupting the trapping mechanism.

This is where the properties of nitrogen-vacancy centres in diamond become useful. Recent work⁶ has shown that a spin-coherence time of the order of a millisecond can be achieved in such a solid-state system. Even in diamond nanocrystals that are tens of nanometres across, the spin-coherence time is not dramatically reduced. Furthermore, initialization and detection of the spin's direction can be achieved at room temperature, so a diamond magnetometer can be placed within tens of nanometres of a biologically active sample.

Such long spin-coherence times can only be achieved by periodically flipping the direction of the spin, a technique known as spin echo, which averages out external fluctuations. With this technique, which was first used in nitrogen-vacancy centres for quantum-computing applications⁷, Maze *et al.*¹ describe a magnetometer with a single nitrogen-vacancy centre in both a bulk diamond and a nanocrystal. The magnetometer is sensitive to magnetic fields that oscillate at the frequency of the spin-echo repetition rate, typically in the kilohertz range. After collecting light from the nitrogen-vacancy centre for 100 seconds, Maze and colleagues obtained a magnetic-field sensitivity as low as 3 nanotesla, equal to the magnetic field about 100 nanometres from a single electron, or 10 nanometres from a single proton. This is considerably better than has been achieved with other techniques, such as MRFM, on the nanometre scale.

Balasubramanian *et al.*² operate a nitrogen-vacancy diamond magnetometer placed next to a magnetic tip, creating a strong magnetic-field gradient. Because the magnetometer relies on a single, well-localized spin, its magnetic resonance is not broadened by the steep spatial variation of the magnetic field. Such a high gradient could allow magnetic-resonance imaging with sub-nanometre resolution. As a first step in this direction, Balasubramanian and colleagues locate the position of the nitrogen-vacancy centre itself with a spatial resolution of 5 nanometres by measuring its resonance frequency.

A combination of the techniques developed by Maze *et al.*¹ and Balasubramanian *et al.*² could lead to the detection and imaging of

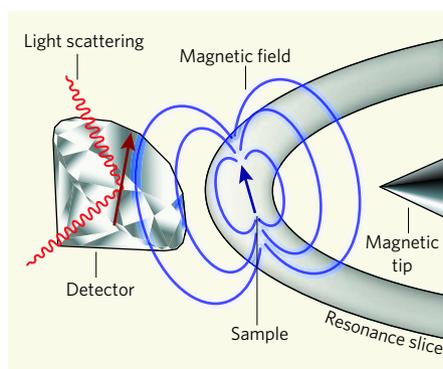


Figure 1 | Optical spin detection using a diamond defect in a nanocrystal. The atomic spin (red arrow) in a crystal defect — a nitrogen-vacancy centre — in a diamond nanoparticle can be manipulated with light. It responds to the magnetic field generated by the spins in a sample, thus changing the light scattering rate. A strong magnetic-field gradient produced by a magnetic tip selects spins with magnetic resonance in a narrow slice of the sample. Maze *et al.*¹ and Balasubramanian *et al.*² have implemented parts of this scheme individually. Integration of these techniques could lead to detection of nuclear magnetic resonance from a single spin.

individual nuclear spins, which would potentially allow the determination of the structure of a single molecule. Crucially, both experiments were performed at room temperature, so biological applications such as the determination of protein structures or detailed imaging of the internal structure of a living cell seem feasible.

Manipulation of spin in diamond is a

fast-developing research area, and many avenues remain to be explored. For example, longer spin-coherence times could be achieved using artificial diamond crystals that have a reduced abundance of the carbon-13 isotope, which creates magnetic fields that perturb the sensor. Another point to note is that the NMR signal on very small length-scales is effectively increased because of quantum fluctuations. As the number of spins (N) decreases, their quantum fluctuations, which scale as $N^{1/2}$, become relatively larger⁸. By resolving individual spins, one can obtain NMR signals that correspond to nearly complete spin polarization even in a low magnetic field, alleviating the current need for strong superconducting magnets in NMR detection, which can polarize only 1 spin in 10^4 . Although integration of nitrogen-vacancy spin-echo techniques with the conditions necessary for NMR remains to be demonstrated, diamond magnetometers seem to provide a promising route towards single-spin NMR detection. ■

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NEUROSCIENCE

Fragile dopamine

David Weinshenker and Stephen T. Warren

Dopamine dysfunction, which is implicated in Parkinson's disease and drug addiction, seems an unlikely culprit in fragile X syndrome. A surprising set of findings means a rethink is required.

Fragile X syndrome is the commonest inherited form of mental retardation, with the patients often also having autism and attention-deficit hyperactivity disorder¹. It is usually caused by the absence of the protein FMRP, which is encoded by *FMR1*, a gene on the X chromosome. Although FMRP function is not well understood, most studies concur that it is a selective RNA-binding protein that modulates the translation of its target messenger RNAs². But this deceptively simple description of FMRP function omits any role for the neurotransmitter dopamine, despite the fact that some of the clinical and behavioural features of fragile X syndrome are reminiscent of dysfunction in dopamine-secreting neurons. Writing in *Neuron*, Wang *et al.*³

elucidate the role of FMRP in modulating dopamine signalling.

Classically, dopamine has been considered an essential mediator of behaviours such as reward-seeking and coordinated movement. Because these brain functions are not always impaired in fragile X syndrome, there has been little reason to link altered dopamine signalling to the aetiology or the manifestation of this disorder. Nevertheless, recent work^{4,5} has expanded and refined dopamine's job description in the brain to encompass more cognitively relevant functions, such as involvement in reward-prediction error, motivation, ability to focus on pertinent environmental stimuli and goal-directed behaviours.

To explore a possible connection between