biological motors for use in the molecular manufacturing of nanoelectronic circuits.

Biochemists generally have to break open and extract the contents of cells to study reactions of interest, often after having to synchronize the cells' activity to get a high enough concentration of the molecules of interest in the same state. But now, thanks to a handful of pioneering research groups, it is becoming possible to study individual protein, DNA or RNA molecules in live cells, in real time, and to follow their interactions and kinetics with nanometre-scale spatial precision and millisecond time resolution.

One at a time

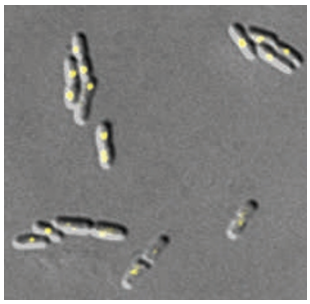
Chemical biologist Sunney Xie and his team at Harvard University in Cambridge, Massachusetts, have developed methods for observing the expression of individual protein molecules. After initially testing them in bacterial cells, the group is now extending the techniques to mammalian cells. This approach is useful for detecting proteins expressed at low copy number, at just a few copies per gene, such as transcription factors, which would be undetectable using conventional proteomics methods.

The great advantage of the single-molecule method is that individual proteins are detected as they are synthesized in an individual cell. One of Xie's techniques detects the expression of proteins genetically tagged with the fast-maturing yellow fluorescent protein Venus. The appearance of the protein, observed as a series of fluorescent bursts that can be quantified, indicates that transcription and translation are taking place.

Rapid strobing illumination enables the observation of fast binding and unbinding of fluorescently tagged proteins to immobile components in the cell and of interactions between fluorescent proteins in the cytoplasm. "Like photographic images of a bullet going through an apple, we can detect fluorescent proteins in the cytoplasm. We can play with the pulse width to determine the dynamics and where the proteins are," says Xie.

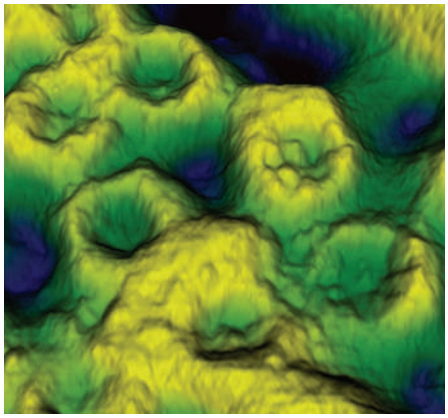
This technology could be extended to a high-throughput system, according to Xie's postdoc Nir Friedman. "You could put cells on a large chip with many chambers and do these kinds of measurements on a large scale."

The fluorescent protein fusion could be done with a DNA library in order to detect the expression of proteins of unknown function. "Although you need to target specifically, the advantage is that you can look at live cells in real time and with single-molecule sensitivity," Friedman adds.



J. ELF & G.-W. LI, HARVARD UNIV.

One at a time: single fluorescently labeled proteins binding at one site per *Escherichia coli* chromosome.



B. WINDOFFER, UNIV. MUNSTER/JPK

A 3D image of a nuclear pore complex.

Nanotechnology seems destined to leave a lasting legacy for cell biology with a host of innovative new technologies. With continuing efforts to combine existing technologies in novel ways, and to create new ones, the possibilities for gaining new insights through nanoscale cell manipulation are increasing rapidly. Nanopatterning and nanotopography are techniques that are, as yet, practised by only a handful of specialists, but the equipment and software are fast becoming available commercially. This trend towards the increasing use of nanotechnology is pushing the very boundaries of cell biology. ■

Hayley M. Birch and Julie Clayton are science writers based in Bristol, UK.

DOWN TO THE LETTER

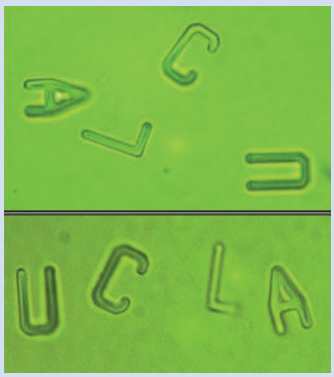
Nanoprobes come in all shapes and sizes. In the latest advance in probe engineering, chemists, physicists and engineers at the University of California, Los Angeles, are pooling their resources to perfect a method for mass-producing novel fluorescent microparticles. The nature of these particles can be so precisely controlled that researchers have been experimenting by creating entire alphabets that can be manipulated with optical tweezers, raising the intriguing possibility of playing nano-scrabble.

These so-called LithoParticles are sculpted by electron-beam lithography, directed by the same computer-aided design (CAD) software used by architects. "E-beam writing is a serial process," says Thomas Mason, who leads the group. "Each letter is written one at a time, so it's not very good for mass production. However, once the mask is made, it can be used over and over again in a special optical-projection printer. We use a mask made by E-beam lithography to expose resist-coated wafers to patterned ultraviolet light. A different projection-printing device — an optical lithography system known as a stepper — is used to mass-produce many particles in parallel." The Ultratech XLS stepper has a lens weighing over 90 kilograms and its own heating and air-conditioning systems to control thermal expansion. The same technology could be used to mass-produce particles with feature sizes as small as 30 nm.

The potential implications for cell biology are huge. Such accuracy of design, coupled with high fidelity on a mass scale, means researchers could soon be supplied with solutions of probes tailored to their specific needs, as neatly demonstrated by Mason's 'alphabet soup'.

Nanoprobes are being increasingly used in the emerging field of bio-microrheology, which examines transport processes within living cells, and in investigating the mechanical properties of cellular components. Nanoparticles introduced by ballistic injection have revealed how the cytoplasm of human umbilical vein endothelial cells undergoes elastic changes in response to growth factors. But the approach could be expanded to investigate the cell's response to all manner of different shapes. "Tracking how differently shaped particles move and rotate inside cells may provide a wealth of information about life cycles and internal cytoplasmic transport in different cell types," says Mason. "You could also use these probes to study how cells respond to various external stimuli. For instance, particles that have many long 'arms' may behave very differently to the compact spheres and quantum dots that are currently available."

UCLA is currently applying to patent their technology and are involved in discussions with commercial partners. Mason is already speculating about building functional nanomachines — including motors, pumps and entire engines — which could be sent to probe even further into the workings of the cell. H.M.B.



J. N. WILKING/T. G. MASON/UCLA

Under the spell: nano-alphabet.