the bound biliverdin in order to decrease its nonradiative decay, so that absorbed 700-nm photons can be emitted through fluorescence."

Using saturation mutagenesis and DNA shuffling to vary the protein residues around the biliverdin chromophore, Shu, Tsien and their colleagues evolved a bright, photostable and monomeric infrared fluorescent protein mutant, with an excitation maximum of 684 nm and an emission maximum of 708 nm. When they expressed the mutant in the livers of mice via an adenoviral vector that specifically targets the liver, they observed strong infrared fluorescence.

Besides *in vivo* imaging applications, infrared fluorescent proteins will also be useful for cell-based imaging. "Infrared fluorescent proteins provide another color well-separated from those of existing fluorescent proteins," notes Shu. Furthermore, cell autofluorescence in the infrared region of the spectrum is nearly nonexistent, thus providing clearer images. Additionally, infrared fluorescent proteins may find application in fluorescence resonance energy transfer (FRET).

Infrared fluorescent proteins also need not be limited to being engineered from the phytochrome scaffold from *D. radiodurans*. There are many bacterial phytochrome sequences available, with absorption maxima varying from 650 to 750 nm. "Therefore," says Shu, "they are potential candidates for engineering blue- or red-shifted infrared fluorescent proteins, which then may be used for multicolor imaging and as FRET donors or acceptors for *in vivo* FRET imaging."

Allison Doerr

RESEARCH PAPERS

Shu, X. *et al.* Mammalian expression of infrared fluorescent proteins engineered from a bacterial phytochrome. *Science* **324**, 804–807 (2009).

of Queensland in Australia to create efficient software code to carry out the statistical analysis. They incorporated the code into a user-friendly interface, the FLAME software.

The Mesirov and De Jager groups tested FLAME with data sets derived from peripheral mononuclear blood cells. Using two-step clustering with multiple surface markers, they isolated an important regulatory T-cell population that consists of only 0.81% of the total number of blood cells. However, FLAME does more than identify subpopulations. Aided by another mathematical approach called bipartite graph matching, FLAME can be used to detect changes to the surface marker profile of specific cell subpopulations in distinct states. The researchers demonstrated this by matching cell populations before and after T-cell stimulation and visualizing phosphorylation and other marker shifts.

"The FLAME output is much richer than the old way of looking at flow-cytometric data," said De Jager. As a neurologist, he plans to use FLAME to discover biomarkers for neurodegenerative diseases, such as multiple sclerosis. Meanwhile, Mesirov's group is tackling more complex data, such as the nonconvex distributions, to examine biological systems by mathematical description. Also, by adding FLAME to the Gene Pattern genomic software package developed by the Mesirov group, researchers will be able to compare flow-cytometric data to other high-throughput data, according to Mesirov.

Wayne Peng

RESEARCH PAPERS

Pyne, S. *et al.* Automated high-dimensional flow cytometric data analysis. *Proc. Natl. Acad. Sci. USA* **106**, 8519–8524 (2009).

NEWS IN BRIEF

GENE TRANSFER

Transgenic marmosets express EGFP

Nonhuman primate models of human disease would be extremely valuable for biomedical research. By injecting marmoset embryos with a lentiviral vector, Sasaki *et al.* have now generated transgenic marmosets that express enhanced GFP (EGFP). Besides expressing EGFP in somatic cells, two of the transgenic marmosets expressed EGFP in germ cells, and one transgenic male passed the 'green' gene onto his healthy offspring.

Sasaki, E. *et al. Nature* **459**, 523–527 (2009).

GENOMICS

Sequencing pools of tens of thousands

In current multiplexing strategies for high-throughput sequencing, a molecular barcode is appended to each sample, allowing the parallel analysis of dozens of specimens. Erlich *et al.* now expand this multiplexing capability by three orders of magnitude with a smart pooling approach. They assign barcodes to pools, rather than to individual specimens, and identify each sample by decoding the pooling pattern. This approach allowed them to sequence and decode a pool of 20,000 different artificial microRNAs.

Erlich, Y. *et al. Genome Res.* advance online publication (15 May 2009).

CHEMICAL BIOLOGY

Unnatural DNA bases put to the test

The creation of unnatural but functional DNA base pairs has been a long-standing goal in chemical biology. Several unnatural base pairs have been developed and tested *in vitro*, where they have been accepted by DNA polymerases. Delaney *et al.* now describe the first test of unnatural base pairs in living cells. They demonstrated that two size-expanded base pairs were efficiently read by the replication machinery in *Escherichia coli*.

Delaney, J.C. *et al. Angew. Chem. Int. Ed.* **48**, 4524–4527 (2009).

BIOINFORMATICS

A tool to find regulatory elements in RNA

Regulation of gene expression occurs at many different levels, including RNA stability. Foat and Stormo now present a computational approach to identify secondary structure–defined *cis*-regulatory elements (SCREs) in mRNAs, by modeling their effect on mRNA levels as measured by microarrays. They applied their algorithm, named StructRED, to all stem-loops 9–12 nucleotides in length and recovered the known binding specificities of two RNA binding proteins.

Foat, B.C. & Stormo, G.D. *Mol. Sys. Biol.* **5**, 268 (2009).

NANOTECHNOLOGY

Quantum dots without blinking

Wang *et al.* describe the synthesis of quantum dots that exhibit continuous photoluminescence. Other groups have developed various strategies to suppress blinking, but none have completely eliminated blinking. Wang *et al.* achieved this by making core nanocrystals of CdZnSe coated with a semiconductor shell of ZnSe. Though these nanocrystals have not yet been tested in biological applications, the fact that they do not blink makes them promising for use in single-molecule experiments.

Wang, X. *et al. Nature* **459**, 686–689 (2009).