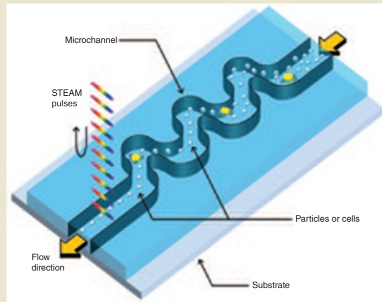
High-speed imaging in a flow



Flow cytometry is one of the most widely used techniques in cell sorting, but its lack of spatial resolution limits its utility for quantifying complex cellular phenotypes. Goda *et al.* present an imaging flow analyzer that can capture microscopic images of single cells at a rate of up to 100,000 cells/s. To achieve this high throughput, the authors combine a technology known as 'serial time-encoded amplified microscopy' with a microfluidic device, which provides uniform flow velocities and cell positioning in the front of an objective lens, and an 'optoelectronic time-stretch image processor' that can analyze the raw data in real time. By screening a heterogeneous budding yeast population, the technique is able to sort cells into different stages of the budding process solely based on cell morphology. The system is also able to detect rare mammalian cells in a blood sample. In a 'spike in' experiment, MCF7 cells were labeled with 1-μm metal beads attached to specific antibodies. The authors show that image-based cell identification is sensitive enough to detect one labeled MCF7 cell in a million white blood cells with a false-discovery rate 100× smaller than conventional fluorescence flow cytometry. (*Proc. Natl. Acad. Sci. USA.* **109**, 11630–11635, 2012) *ME*

Membrane fishing for HIV-neutralizing antibodies

Antibodies capable of neutralizing a range of HIV-1 strains appear in some patients several years after initial infection. Klein *et al.* devise a way to isolate the B cells producing these antiviral antibodies that relies on an epitope displayed on cells. Instead of relying on commonly used HIV-1 envelope soluble-protein baits, the authors use 293T cells displaying a surface HIV-1 protein spike to screen peripheral blood B cells from volunteers infected with HIV-1. Most antibodies isolated by this new method bind to surface-bound but not soluble HIV-1 envelope proteins, suggesting that they would have been missed in screens using soluble baits. Moreover, the newly identified antibodies neutralize HIV-1 strains that are resistant to antibodies isolated some time before from the same volunteers, and seem to bind to a previously unrecognized site on the HIV-1 spike. By revealing the full spectrum of naturally arising HIV-1–neutralizing serologic activity, this strategy may aid the design of vaccine immunogens for HIV-1 and potentially other pathogens. (*J. Exp. Med.* **209**, 1469–1479, 2012) *CB*

Expanding the engineer's toolkit

Synthetic biology promises to enable the design of therapeutics and devices based on modular genetically encoded components, but the number of interoperable parts and circuits remains limited. Now two groups have added much needed parts to realize the potential of precision engineering circuits in human cells. Khalil *et al.* use artificial zinc finger domains, which can be readily engineered to recognize various target DNA sequences, as the basis for a modular, customizable set of synthetic transcription factors (sTFs) and demonstrate how to build and tune these sTFs for higher-order synthetic gene circuits in yeast, a useful testing ground for circuits that might help bridge to studies in human cells. A second group, Wei *et al.*, exploit a family of bacterial pathogen proteins known as effectors that can modulate host cell signaling and dampen immune responses. They use effectors to selectively modulate mitogen-activated, protein kinase–regulated signaling pathways, including those responding to osmolarity in yeast, and show that these effectors can be used to reshape signaling input and output behavior. They use the same effectors as a switch to toggle on and off the activity of human $CD4^+$ T cells in response to a simple drug signal. Control switches like these might find application in adoptive immunotherapy. (*Cell* **150**, 647–658, 2012; *Nature* **488**, 384–388, 2012) *SJ*

Photoactive chemical 'restores' sight

Some inherited degenerative diseases affecting rods and cones irreversibly impair photoreception while leaving the rest of the optical pathway intact. Attempts to restore sight to affected individuals through microchips, gene therapy and even stem cells have had some clinical successes but all pose technical challenges. Now Polosukhina *et al.* demonstrate how a small-molecule photoswitch can restore vision in mutant mice lacking rods and cones. Injecting acrylamide-azobenzene-quaternary ammonium (AAQ), a light-activatable K^+ channel blocker, into the vitreous humor restores pupillary light reflex and light avoidance behavior in blind mice (homozygous for a mutation in cGMP phosphodiesterase-6, causing rods and cones to degenerate by three months of age). AAQ-mediated responses are rapid (median latency of 45 ms), have high spatial resolution (with a field size of 100 μm), and although short wavelength light was most effective, some response was achieved with longer (up to 500 nm) wavelengths. Although AAQ and related molecules may be therapeutic in humans, several issues need addressing. The molecule will need to be modified such that responses can be achieved at appropriate light intensities and wavelengths for the human eye. In addition, as AAQ dissipates within 24 hours, an extended release formulation would be preferred over repeated injections in the eye. (*Neuron* **75**, 271–282, 2012) *LD*

mRNA-bound proteome

Eukaryotic mRNA is bound by a wide array of regulatory proteins, but until recently the full complement of the mRNA-bound proteome has not been systematically characterized. Baltz *et al.* now use photoreactive nucleoside analogs to cross-link proteins and RNA in human embryonic kidney cells and purify the complexes by PolyA-based precipitation. The authors identify close to 800 mRNA-bound proteins using high-resolution quantitative mass spectrometry, one third of which have not been described as RNA binding before and about 15% of which would not have been predicted to interact with RNA. Among the newly identified mRNA binders are proteins that were previously exclusively annotated as transcription factors (e.g., JUN or NXF1). Baltz *et al.* also generate the first transcriptome-wide protein occupation map using the previously published PAR-Clip method. The protein-binding sites have a substantially lower single-nucleotide polymorphism frequency than neighboring sites, suggesting they are under high selection pressure in evolution. Several of the single-nucleotide polymorphisms found in mRNA-protein interaction sites have been associated with disease, lending further support to the idea they are functionally important. (*Mol. Cell* **46**, 674–690, 2012) *ME*

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