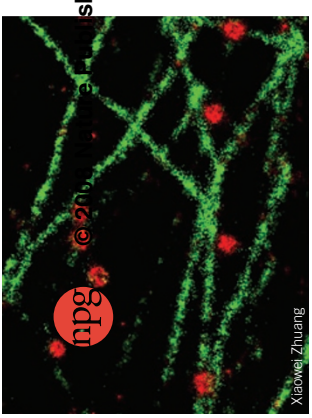
Methods on the cusp of profoundly impacting their field, areas in which methodological developments are sorely needed and wild wishes: here is our (incomplete) selection of methods to watch in future years.

Seeing fluorescence at super-resolution

Fluorescence microscopy is being transformed by methods to improve its resolving power.

After the grandfather of modern microscopy Ernst Abbe formulated the theories that revolutionized modern microscope design, the imposed limits on the spatial resolution were considered inviolable. Over the past decade clever microscopists have devised ways to break these limits in fluorescence microscopy, but the need for specialized equipment inhibited uptake of super-resolution optical techniques. In the past two years the situation has changed dramatically.



Stochastic optical reconstruction microscopy (STORM) image of microtubules and clathrin-coated pits.

First, it is now possible to purchase a microscope from a major optical company that can implement one of these techniques such as stimulated emission depletion (STED) or 4Pi microscopy. Second, several groups have described methods for super-resolution fluorescence imaging that only require a commonly available microscope, specialized but easily obtainable fluorescent labels, and clever microscope control routines and data processing.

These methods operate by stochastically switching individual fluorophore labels back and forth between fluorescent and nonfluorescent states or between two different colors. This allows the precise location of individual fluorophores to be determined. The methods can theoretically provide the exact location of every labeled protein in the cell at resolutions close to the size of proteins themselves.

Whereas the first reports of these methods used fixed samples and a single label, 2007 saw the development of multicolor versions and application to living cells. We look forward to continued development of these stochastic imaging techniques to further improve their performance and simplify their implementation. Even methods like STED can be and have been simplified, and methods based on new technologies with unique capabilities are likely to be developed.

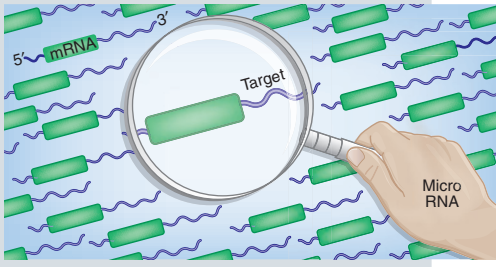
Super-resolution optical imaging is still only providing a trickle of novel biological results, but this situation should change quickly. **Daniel Evanko**

Experimental micro-matchmaking

Needed: large scale experimental validation of predicted microRNA targets.

The importance of microRNAs—short noncoding RNAs that inhibit mRNA expression—is as big as their size is small. They are thought to regulate at least 30% of all human genes and have a crucial role in normal and disease development. The relatively easy part in studying microRNAs is to find new ones and to establish their profiles in a cell or tissue using techniques such as microarrays, reverse-transcriptase PCR and sequencing. The challenging part is to match a microRNA to its mRNA target, as the interaction is not only based on the complementarity of the sequence but on many other factors.

Given the much larger number of mRNAs compared to microRNAs, the most efficient way to find a match is by computational means. Several researchers have developed algorithms relying not only on sequence but on other factors such as evolutionary conservation, sequences surrounding the binding site and, most recently, mRNA structure and accessibility as well as expression levels of both mRNA and micro RNA. The real crux of the matter, though, is the experimental validation of these predicted targets. Currently the wet-lab validation of an *in silico* target is still cumbersome. What is needed is a large-scale assay that allows rapid and definitive identification of an mRNA targeted by a microRNA. Various efforts toward that goal are under way, some making use of the microRNA-mRNA pair *in vivo* by using the microRNA as a primer and extending the sequence further (*J. Mol. Biol.* **358**, 983–986; 2006), others trying to isolate and characterize the microRNA-mRNA-ribonucleoprotein complex (*RNA* **13**, 1198–1204; 2007). Although these assays allow validation of specific targets, they are not at a scale yet that will allow rapid and comprehensive validation of all microRNA targets—and to go from large scale *in silico* predictions to large-scale *in vitro* validation will be a welcome breakthrough in the field of microRNA biology. **Nicole Rusk**



MicroRNA finds its targets.

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