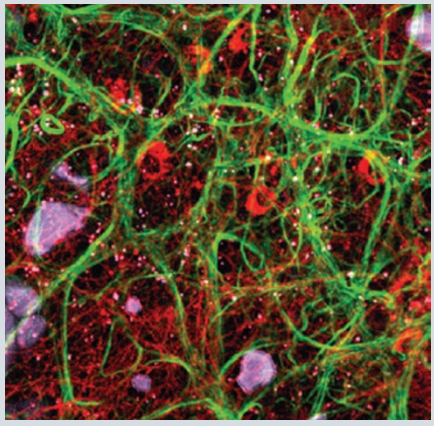
Label-free microscopy

New methods to coax signals from unlabeled biological molecules may finally fulfill the promise of practical label-free microscopy with molecular specificity.

It would be difficult to deny that recent developments in fluorescent probes—most notably the development of fluorescent proteins—have resulted in spectacular growth in the use of light microscopy in the life sciences. But the enormous benefits provided by these probes for observing the location and dynamics of biomolecules are offset by the fact that they invariably require the attachment of the probe to the biomolecule of interest. Unfortunately, if the goal is to image endogenous molecules *in vivo*, labeling may not be feasible. Furthermore, labels may perturb the function of biomolecules, particularly small ones.

Label-free microscopy methods that rely on a variety of different photophysical processes to generate light signals from biological molecules have been around for many years, but they have seen limited use for answering biological questions. Two-photon microscopy can detect some prevalent autofluorescent cellular species.



Label-free image produced by the older techniques of two-photon and second- and third-harmonic-generation microscopy.

Second- and third-harmonic-generation methods can distinguish fibrillar structures and lipid bodies. Raman microscopy can detect specific types of chemical bonds and is capable of determining the chemical makeup and abundance of lipids, but its use is hindered by high background.

About ten years ago, the development of coherent anti-Stokes Raman scattering (CARS) microscopy by Sunney Xie's group significantly improved the Raman signal and the usability of the method, but identification of specific molecules was still challenging. In the past two years, however, Xie and colleagues have reported promising new developments in label-free imaging that make it clearly a method to watch.

At the end of 2008, Xie's group reported a new Raman-based imaging method that overcomes some of the problems with CARS microscopy. Their stimulated Raman scattering (SRS) microscopy makes it easier to identify specific target molecules and provides very high sensitivity. The relatively poor molecular specificity of the label-free methods in general has been one of their major drawbacks compared to fluorescent labeling. SRS promises to help bridge this divide while being particularly amenable to smaller biological molecules like lipids, which have proven difficult to productively tag with fluorescent labels. And in 2009 the Xie group developed a different label-free imaging method, based on stimulated emission, that coaxes light from nonfluorescent molecules.

Although none of these methods will replace fluorescence microscopy or fulfill the needs of every label-free imaging experiment, it appears that we are reaching a turning point in label-free microscopy that holds great promise for the future of these methods. **Daniel Evanko**

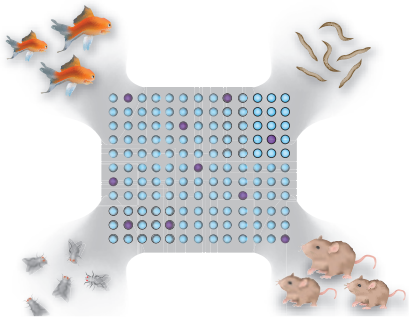
High-throughput phenotyping

Automated methods to score phenotypes in model organisms continue to develop and will permit previously inaccessible areas of biology to be probed.

Once you have the genome of your favorite creature sequenced, genome manipulation tools developed, and libraries for gain- or loss-of-function studies generated, then what? Especially if your inclinations run to genome-wide studies, you need a good, fast, reliable way to identify the alterations in phenotype that result from modulating or eliminating genes. This is also true for classical mutagenesis or forward-genetic studies: the likelihood of identifying interesting mutants depends on the scaleability of the phenotypic readout and the precision with which it reports on the process of interest.

Although many large-scale studies have been performed manually over the last decades, either through effective design of the phenotyping assay or thanks simply to the sheer doggedness of the scientists involved, there is little doubt that the development of automated or semiautomated phenotyping approaches could enable studies that have previously not been possible.

Indeed, several researchers are coopting technological developments in other fields and bringing them to bear on the phenotyping of model organisms. In the pages of *Nature Methods* alone, such strategies have ranged from the use of new (or newly applied) instruments that vastly increase the rate at which organisms can be evaluated to the application of computer vision–based tracking methods to monitor phenotypes that are too complex to be reasonably scored by human observers. Light-microscopic imaging of cell positions and lineages, and of fluorescent reporter gene expression—all within the context of the living organism—are also becoming



High-throughput phenotyping of model organisms

amenable to automated approaches, which should permit analyses of growth and development that, without large-scale datasets, would be difficult to achieve.

It will be of interest to watch as these methods are put to work and refined in response to problems that may arise. And we predict that entirely new approaches are still in the making. Surely all the interesting phenotypes have not been studied yet. **Natalie de Souza**

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