

»» Imaging through automation

Automated imaging has the power to transform microscopy into a more quantitative technique with new capabilities.

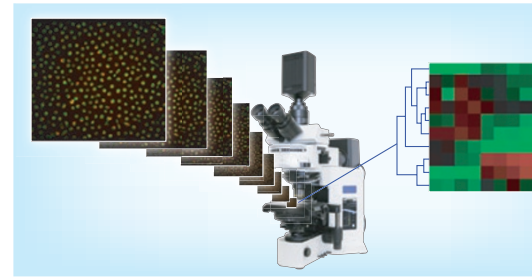
Microscopic imaging has historically been a powerful but laborious and descriptive method. When Antonie van Leeuwenhoek first observed microorganisms in the 1600s and Santiago Ramón y Cajal discerned the fine structure of the nervous system more than 200 years later, these scientists spent long hours observing and drawing what they saw. Modern day scientists—with their sophisticated microscopes and CCD cameras—can instantly record more detailed observations, but for most biologists, the main objective is still to obtain descriptive, representative images of the phenomenon under observation.

Today's computers and the sophisticated algorithms they can run are beginning to

change the game, though. These developments make it possible for a computer to take control of most of the imaging process and have the power to transform microscopy from a mostly descriptive method to a more quantitative technique that is capable of making observations that human observers could never match.

This gradual revolution is already occurring in several applications. Large-scale automated imaging screens of cells in culture or small model organisms are capable of detecting very rare or subtle phenotypes that would be missed by human observers. Similarly, image analysis algorithms can recognize, categorize and track cellular phenotypes in hundreds or even thousands of cells at once—something that is beyond the abilities of even the most highly trained microscopist. Even applications that are possible without automation, such as measurements of gene expression in large numbers of cells, whether *in vivo* or *in vitro*, can be much more efficient and quantitative when they are automated.

Automated microscopy methods do



Automated acquisition of many images of cells, followed by computer analysis, allows quantitative assessment of cellular phenotypes.

require substantial technical know-how to correctly design and use the necessary algorithms, and care must be taken when evaluating the results. These techniques are gradually becoming more widespread, though, and efforts are underway to make them more accessible to a wider range of users. Caution is warranted to ensure that novices—and even experts—use these methods properly, but the exciting possibilities these methods provide make them well worth watching. **Daniel Evanko**

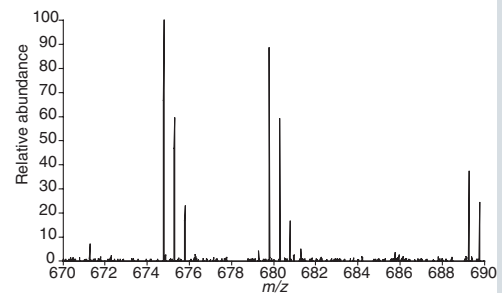
»» Quantitative mass spectrometry

Quantitative mass spectrometry-based proteomics is now being applied on a large scale to address interesting biological questions.

Mass spectrometry has made protein identification fairly routine and is now widely used in proteomic studies. But to really understand the biology of complex systems, one also needs information about protein expression levels. Unfortunately, mass spectrometry is not inherently quantitative.

Therefore, over the last decade, proteomics researchers have devised a series of stable-isotope labeling strategies to obtain quantitative information. These methods have been touted for investigating the effects of knockdown, of an inhibitor, or of the environment on a proteome; for comparing two disease states; for determining the stoichiometry of proteins in a large complex; or even for monitoring changes to a proteome over time.

Made possible also by recent advances in instrumentation and bioinformatics



The 'SILAC mouse': metabolic isotope labeling now allows quantitative comparison of the proteomes of knockout mice.

analysis, several noteworthy proteomics studies have been reported over just the last year, in particular using metabolic labeling techniques such as the SILAC (stable isotope labeling by amino acids in cell culture) method invented by Matthias Mann's group in 2002. In 2008, Mann's group and others showed, to give a few examples, that SILAC can be used to isotope-label a whole mouse, investigate the dynamics of phosphorylation across the cell cycle, study the effects of microRNA on cellular proteomes, and compare the proteomes of haploid and diploid yeast.

Absolute quantification of protein levels, however, can only be achieved by

spiking a digested proteomic sample with known amounts of synthetic, isotope-labeled peptides, a concept first introduced by Steven Gygi's group in 2003. However, until isotope-labeled proteotypic peptides (those most likely to be consistently detected by the finicky mass spectrometer) representing all proteins are available, absolute quantification on a proteomic scale is a far-away dream.

In the near future, look for further exciting applications using metabolic isotope labeling. The stage is now set for researchers to use mass spectrometry to answer challenging biological questions on a proteomic scale. **Allison Doerr**