internalization, Selwood and colleagues confirmed that SMoC entry routes are diverse, and that the addition of geminin cargo influenced the mode of delivery into the cell.

Whatever the mechanism, the goal of designing a nonpeptidic protein-transduction agent allowing the internalization of a biologically active agent was successfully attained in this work. This is a very important finding for pharmacology. It might also trigger additional research by attracting the attention of chemists, promoting the discovery of new mimics and the development of efficient strategies to reach intracellular targets.

There is, however, a large amount of work ahead. It remains to be seen whether SMoCs are active *in vivo*, or whether they are toxic or mutagenic. Additionally, it will be interesting to study their distribution *in vivo*, in particular whether they can reach the nervous system, and to study different modes of administration, particularly oral administration as favored by the pharmaceutical industry. It will also be important to be able to send these new agents to the appropriate cells *in vivo* and, once in the cells, into the appropriate compartments—not an easy task. It can be anticipated, however, that in addition to gene therapies based primarily on the use of viral vectors, transduction peptides and their small-molecule mimics will complete our pharmacological toolbox.

COMPETING INTERESTS STATEMENT
The author declares that he has no competing financial interests.

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Software opens the door to quantitative imaging

Anne E Carpenter

Cellular imaging by fluorescence microscopy is becoming simultaneously higher-throughput and more quantitative as researchers develop integrated systems for image acquisition and analysis.

For hundreds of years, biologists have examined cell and tissue samples by microscopy, gradually progressing from sketching their observations (often beautiful, but subjective) to capturing images by film photography. The work of Brent and colleagues[1] described in this issue of *Nature Methods* is an example of another gradual but monumental change in biology: the shift toward automated digital image acquisition by robotic microscopy, coupled with automated image analysis. The successful integration of these steps allows quantitative analysis and mathematical modeling of processes that previously were relegated to qualitative and subjective observation. These new methods are helping to provide a firm foundation for systems biology.

Typically, if a difference in cellular appearance is not immediately obvious to the human eye by microscopy, it is assumed there is no visual difference. Particularly industrious researchers with a critically important research question might nevertheless invest effort to check for differences more objectively by making quantitative measurements manually (for example. cell counting or cell-size measurement) if the number of samples is manageable. Alternatively, certain cell features can be measured on a single-cell basis by flow cytometry, but the same individual cells cannot be analyzed over time, and certain cellular features cannot be measured by flow cytometry (for example, morphology, localization), especially in non-suspension cells. An increasingly appealing alternative, therefore, is to collect per-cell measurements by automated image cytometry as described in the article by Gordon *et al.*[1] (**Fig. 1**).

It is clear that for many studies single-cell quantitative analysis is critical for making meaningful biological conclusions because even clonal populations of cells can vary substantially. Progress has been made recently in cataloging and understanding the sources of variation, which include differences in cell-cycle position, stochastic variations in gene expression, the preexisting amounts of proteins and metabolites in each cell, and microenvironment differences (for example, cell-cell contacts or local differences in substrate or medium).

For example, in a study using some of the methods presented by Gordon *et al.* and analyzing thousands of yeast cells over time, Brent and colleagues found that expression noise is a minimal source of the existing cell-to-cell variation, whereas the pathway capacity (the capacity of individual cells to transmit signals through the pathway) and expression capacity (the capacity to express proteins from genes) are the main sources of variation[1,2]. Other laboratories are pursuing this question as well and are carefully quantifying sources of noise in cellular systems[3,4]. The recent demonstration that individual molecules can be counted, under appropriate experimental conditions[5,6], indicates that precise quantitative systems biology models are on the horizon.

The ease with which quantitative biological data can be obtained using such methods is highlighted by the application of a suite of methods, which Gordon *et al.* describe, to the analysis of molecular maturation and degradation rates in unperturbed yeast cells. Their measurement of the maturation rates of fluorescent proteins on a per-cell basis was remarkably consistent regardless of the protein expression level or the physiological state of the cell, supporting the hypothesis that maturation requires intramolecular interactions only. This, together with other measurements and assumptions of the properties of the fluorescent proteins and cells, allowed Gordon *et al.* to take a single snapshot of a field of view of cells expressing different molecular variants to compare mRNA or protein degradation rates between two species. They confirmed the validity of

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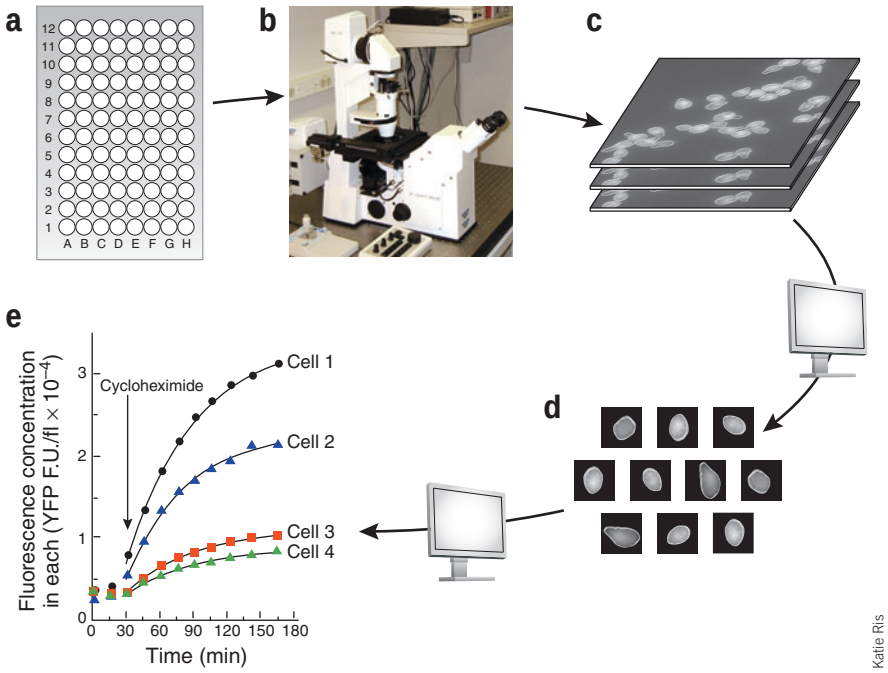

Figure 1 | Microscope-based cytometry. (**a**) Cells are seeded in 96-well plates. (**b**) The wells are imaged using an automated microscope and autofocus software. (**c**) Automated imaging generates a series of images for a single field of view in each well over time. (**d**) The images are processed by image-analysis software, Cell-ID, which identifies and tracks individual cells, and measures total cell fluorescence. (**e**) The fluorescence imaging data are used for quantitative analysis of per-cell protein levels over time, in this case for *Saccharomyces cerevisiae* cells.

the new methodology using conventional experiments.

Image cytometry is clearly poised to be a new staple of the modern biological laboratory. Although commercial integrated systems for automated cell-image acquisition and analysis have existed for the past decade, they often are not flexible enough for most basic research, including work in the aforementioned laboratories. As a result, most researchers piece together various components as needed. A tremendous amount of work must often be expended, therefore, to get a complete, quantitative analysis system functioning. Many laboratories have yielded dramatic biological insights in high-throughput experiments by investing in such efforts[7–9].

Fortunately, this work is becoming easier as many pieces of this puzzle are now available as a result of open-source, academic efforts. They range from prototype to well-developed, but over time their continued individual development and integration, if supported by funding agencies, should yield a fully automated, fully open-source system. This includes software packages for the control of microscope hardware (for example, μManager, http://www.micro-manager.org/), the storage and manipulation of images (for example, Open Microscopy Environment, http://openmicroscopy.org/), the quantitative analysis of images (for example, ImageJ, http://rsb.info.nih.gov/ij, and CellProfiler, http://www.cellprofiler.org), and the exploration of per-cell measurements (for example, Physics Analysis Workstation, http://paw.web.cern.ch/paw, and CellVisualizer, http://www.cellvisualizer.org).

In practical terms, this growing availability of free software advances the pace of science because researchers can test the software immediately rather than first investing time estimating its utility based on product literature, justifying its cost to supervisors and navigating purchasing departments. Furthermore, and often overlooked, free software levels the playing field for researchers with less funding, including younger researchers and researchers in resource-poor nations or institutions.

Yet the free availability of the source code goes beyond economic considerations. Access to the underlying methods is not only key for understanding and interpreting the data—it often provides a basis for improvement and advancement by other researchers, preventing redundant custom programming and moving research forward that much more quickly.

Admittedly, the software presented by Gordon *et al.* is not a breakthrough in terms of its algorithms, nor in immediate broad applicability to a wide range of cell types or research questions. The real advance is in the validation of an integrated system capable of revealing valuable biological knowledge. This work is an illustration of what we hope will become a host of research projects using high-throughput cell-image acquisition and analysis methods that are free and open-source.

COMPETING INTERESTS STATEMENT
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