INNOVATION

High-throughput fluorescence microscopy for systems biology

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Abstract | In this post-genomic era, we need to define gene function on a genome-wide scale for model organisms and humans. The fundamental unit of biological processes is the cell. Among the most powerful tools to assay such processes in the physiological context of intact living cells are fluorescence microscopy and related imaging techniques. To enable these techniques to be applied to functional genomics experiments, fluorescence microscopy is making the transition to a quantitative and high-throughput technology.

The information in complete genome sequences[1] and the identification and systematic cloning of human cDNAs are providing us with the challenging opportunity to analyse the complexity of biological processes on a large scale, with the goal of reaching a more complete description of their molecular regulation. For this purpose, high-throughput techniques — such as protein analysis by mass spectrometry, or expression and transcription profiling by protein or DNA microarrays — have been developed and successfully applied to diverse biological questions. However, despite their great usefulness, these techniques cannot provide adequate temporal or spatial resolution and, most importantly, they do not directly show whether the identified molecules have a functional role in the cellular process that is under investigation.

Fluorescence-based imaging assays in intact living cells overcome these limitations because they can probe the function of macromolecules in their natural environment with exquisite and ever increasing spatial and temporal resolution[2–4]. Fluorescence-imaging assays, in principle, also have the potential to be applied to large-scale analyses, and simple assays have already been applied to cell-biological problems in high-throughput fluorescence-microscopy experiments[5,6]. Standardized reagents that interfere with cellular functions and high-throughput transfection methods, such as cell arrays[7,8], are becoming available. Using these, large-scale fluorescence imaging at single-cell or even subcellular resolution can be combined with genome-wide RNA interference (RNAi) approaches[9,10], small-molecule-based perturbations[11] or overexpression strategies[8] to reveal comprehensively the regulatory networks that underlie the functions of intact cells.

Carrying out functional high-throughput microscope-based experiments that can provide data for such systems-biology questions is currently still a challenge. It requires automation and the precise coordination of various steps in an integrated workflow (FIG. 1). In this article, we highlight recent developments in high-throughput fluorescence microscopy and focus on future requirements for imaging and image analysis, which are currently two of the most important areas of new technology development.

Assays: live cells are the future

The key to any high-throughput fluorescence-microscopy approach is the development of an appropriate imaging assay that specifically reads out the biological function of interest and is robust enough to provide reproducible, quantitative data using high-throughput image acquisition and analysis. Large-scale projects using high-throughput microscopy that have been reported so far almost exclusively used fixed-cell assays (see, for example, BOX 1 for a detailed example). Unfortunately, such endpoint experiments do not provide any temporal information and results might be misinterpreted if, for example, the final state of the examined cells is an indirect consequence of a number of sequentially occurring events. Experiments using live-cell assays (for example, REF. 10) and high-throughput time-lapse microscopy can overcome this problem, and they provide much more detailed phenotypic information than fixed-cell assays.

However, the high-throughput automated fluorescence imaging of biological processes in living cells is currently technically challenging (see later), and requires robust and simple fluorescent labelling techniques. The fluorescent reporters need to be specific, they must interfere as little as possible with the biological process being visualized and they must not perturb the global physiological conditions of the cells. Most importantly, the labelling and detection procedures have to be quantitative and highly reproducible so that different experiments that have been carried out at different times can be compared, and so that image data can be evaluated using an automated phenotypic analysis — an absolute requirement for large-scale projects (see later). Numerous green fluorescent protein (GFP)-based protein markers for a vast array of cellular functions have been successfully developed in the past years for manual, single live-cell experiments (see, for example, REFS 2,4,13–16). Monoclonal cell lines that stably express one or more of such proteins that are tagged with spectral variants of GFP will be one way to develop robust assays for high-throughput fluorescence-microscopy experiments in living cells. In addition to enabling the detection of the dynamic spatial distribution of the respective fusion proteins, GFP-based reporters have also been the basis for more sophisticated assays that monitor protein interactions, enzyme activities, pH, cyclic AMP or Ca^{2+} concentrations in living cells (reviewed, for example, in REFS 17–19). Although such reporters have so far only been used in single-cell experiments, they have the potential to be used in high-throughput fluorescence-microscopy experiments once the appropriate hardware and software for data acquisition and analysis are in place.

High-throughput imaging

High-throughput fluorescence-microscopy experiments comprise at least five independent steps — sample preparation, image acquisition, data handling, image analysis and data mining combined with bioinformatic modelling — and these steps need to be tightly coordinated to sustain a rapid flow of work and data (FIG. 1). Therefore, any image-acquisition system that is used for large-scale experiments has to meet requirements that are different from those for traditional fluorescence microscopes. High-throughput imaging systems should ideally be compatible with many fluorescence-based assays and they should be completely adapted in terms of both hardware and software for fully automated operation.

Several automated fluorescence-microscope-based image-acquisition systems are already available on the market (TABLES 1,2). However, because such commercial systems are often designed and optimized for highly specific and/or ultra high-throughput applications, they are limited in regards to their adaptation to new and more complex assays or are difficult to use with live cells. To overcome these limitations, commercially available high-end microscopes have been automated to increase throughput (see, for example, REF. 20) and new automated systems with an open software architecture that allows users to add their own modules have been developed (see, for example, REF. 21). Only a few applications of high-throughput microscopy in intact living cells using either simple time-lapse transmitted light or fluorescence microscopy have been reported[10] (reviewed in REFS 11,12). This is due to the fact that the high-throughput imaging of living cells, although much more powerful compared to work in fixed cells, is still a demanding problem. For example, it requires stable temperature and tissue-culture conditions on the microscope. Furthermore, if the different samples in a multi-well dish or cell microarray are imaged in a time-resolved manner, either the time resolution of each individual live-cell experiment or the sample sizes that can be imaged on one microscope becomes limited.

A further drawback of the currently available high-throughput imaging systems is that once the image acquisition has started, little or no modification of the experiment is possible. This is especially problematic for live-cell studies, in which variations in the expression levels of the fluorescent markers frequently necessitate online image analysis so that acquisition parameters can be adjusted to optimum levels between the capture of images. Similarly, in applications in which only specialized cells are of interest (for example, mitotic cells), online analysis of the image data with subsequent feedback to image acquisition would enable the imaging system to focus the acquisition on only those objects of interest. Improving and accelerating online image-analysis procedures and microscope-control hardware is necessary to overcome these hurdles. Once possible, this would open new avenues for much more complex, and more informative, automated microscopy strategies with high-throughput capabilities. So, it might become possible to analyse living cells using high-throughput approaches to determine intermolecular interactions using fluorescence resonance energy transfer (FRET)[17,22] techniques, or molecule dynamics using fluorescence recovery after photobleaching (FRAP)[23–25] or fluorescence correlation spectroscopy (FCS)[26,27] (FIG. 1). These imaging techniques have been shown to be extremely powerful in combination with

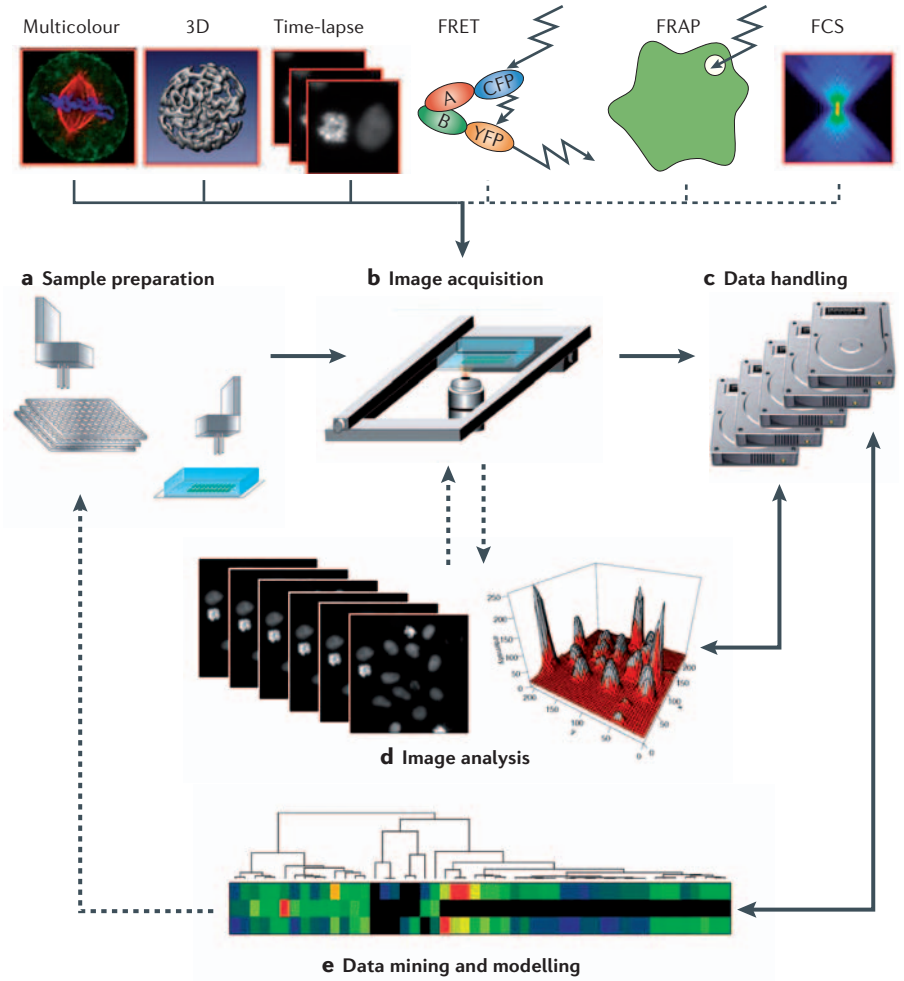
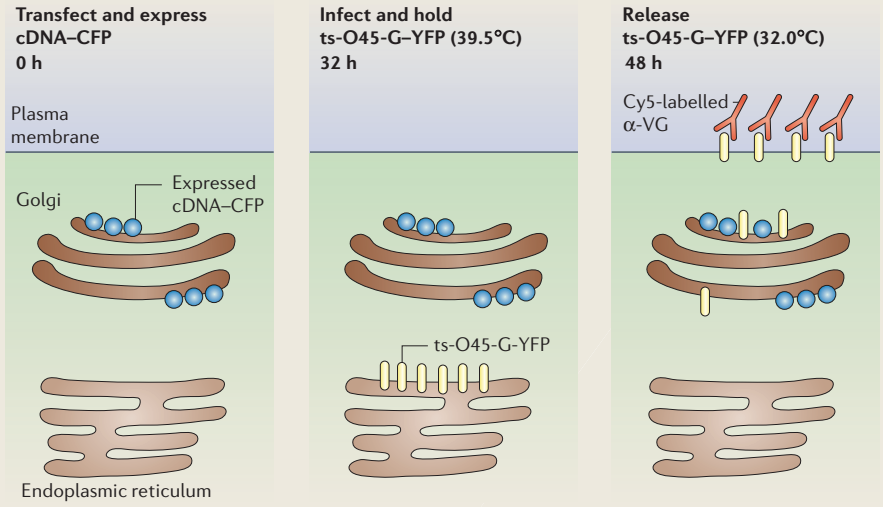


Figure 1 | **The steps in a high-throughput fluorescence-microscopy experiment. a** | Sample preparation, including cell transfection and fluorescent labelling for imaging, is typically conducted in multi-well dishes using robotics (see, for example, REF. 9) or transfected cell microarrays[39]. **b** | Image acquisition for samples in fixed or living cells is carried out using automated microscopes (see TABLE 1 for a selection of commercially available systems). Images can be acquired in different modes, for example, in multicolour, in three dimensions (3D), using time-lapse techniques, by detecting fluorescence resonance energy transfer (FRET), by detecting fluorescence recovery after photobleaching (FRAP), or by using fluorescence correlation spectroscopy (FCS). **c** | Image data can easily exceed several terabytes and therefore require specialized software and hardware for data handling. **d** | Central to this approach is automated image analysis, which needs to be developed or adjusted for each assay system and is currently one of the biggest challenges in this field. **e** | Last, data are integrated and mined to formulate models of the system under investigation. Dashed lines indicate interactions that are not presently implemented in high-throughput fluorescence microscopy. Developments along these lines will considerably improve the technology in the future. CFP, cyan fluorescent protein; YFP, yellow fluorescent protein.

Box 1 | **Assays for high-throughput fluorescence microscopy**



Assays for high-throughput fluorescence imaging need to be designed in such a way that sample preparation on a large scale can be done automatically, image acquisition is fast and quantitative, and image data can be analysed automatically. Along these lines, several assays have been developed in recent years for small-compound screening projects (reviewed in REFS 12,36,37). Only very recently have assays been developed and successfully applied in medium-to-large-size microscopy screening applications to identify genes that are involved in key cellular processes (see, for example, REF. 9). Here, we describe one representative assay in fixed cells that we used to screen a library of fluorescently-tagged cDNAs for a function in protein secretion using an overexpression approach in mammalian cells[8] (see figure).

Cells were transfected with cyan fluorescent protein (CFP)-tagged cDNAs from the library. After 32 hours, these cells were infected with adenovirus that encoded a yellow fluorescent protein (YFP)-tagged temperature-sensitive mutant of the vesicular stomatitis virus G protein (ts-O45-G)[38] — a marker of membrane transport through the secretory pathway. The cells were then incubated at the restrictive temperature of 39.5°C, so that ts-O45-G–YFP accumulated in the endoplasmic reticulum. After a further 16 hours, the temperature was shifted to the permissive temperature of 32.0°C to induce the transport of ts-O45-G–YFP to the plasma membrane, where it was detected following fixation by immunostaining using a fluorescently (Cy5) labelled monoclonal antibody (α-VG). So, the relative amount of total ts-O45-G–YFP that was transported to the plasma membrane could be determined by calculating the ratio of the Cy5-labelled antibody and the YFP signals (see also FIG. 2). Measuring transport to the plasma membrane on the basis of the Cy5/YFP signal ratio makes this assay robust, and errors that occur during image acquisition — such as minor focus shifts or variations in the expression of the transport marker — are compensated for because these shifts occur equally for both of the signals. This assay can be used with minor modifications to score for the effects of protein suppression by RNA interference or inhibition by small molecules.

GFP technology in single-cell experiments because they provide quantitative data on the dynamics of biochemical reactions in living cells[2,15,17]. However, none of them have so far been successfully applied to more systematic studies using high-throughput microscopy.

Image analysis

High-throughput fluorescence microscopy quickly generates large amounts of digital image data from standardized live-cell or fixed-cell imaging assays. Genome-wide RNAi screens, for example, in which several images or movie sequences are acquired to document the phenotype for each suppressed gene in a genome generate from several tens of thousands to up to millions of images in a single screen. To handle such volumes of data (up to several tens of terabytes), high-throughput imaging experiments have to be coupled to automatic, computerized image-processing methods to score the assay and to annotate the data in a truly quantitative and unbiased fashion. The result of the image analysis is a numerical profile of the assay, which can be as simple as a ratio of the measurements acquired in two different channels (BOX 1; FIG. 2) or as complex as a detailed phenotypic signature (see later; FIG. 3). Image processing delivers standardized parameters that are amenable to thorough statistical evaluation and further bioinformatic analysis and modelling, an essential property for use in systems biology. Also, automatic phenotyping relieves a bottleneck in high-throughput microscopy because automated microscopes can generate data at a much higher rate than it can be annotated and evaluated manually. Currently, automated image processing for high-throughput data sets is in its infancy, and for kinetic data from living cells, in particular, almost no suitable tools exist. Consequently, we foresee much further development in this area, including, as a first step, making existing algorithms computationally much more efficient to allow the processing of large-scale data sets in a reasonable time. In general, on the basis of existing concepts from single-cell experiments, automated image-processing routines for high-throughput images can be divided into the three steps that are outlined below.

Pre-processing. The first task of image processing is to find the biological object(s) of interest in an image, which can contain one or many cells. Depending on the cellular assay, the object of interest could be the whole cell or subcellular organelles/structures that are labelled with an appropriate fluorescence marker (BOX 1). Pre-processing methods are well developed for single-image processing and they can involve the filtering of noise and the normalization of intensities. They typically end with a segmentation step, in which the boundaries of the object(s) of interest are defined (FIGS 2,3).

Quantitative analysis and classification. After the objects have been segmented, they need to be analysed according to the needs of the high-throughput imaging assay. For each image in the fixed-cell assay that is described in BOX 1, it was sufficient just to identify the cells that were expressing cyan fluorescent protein above a certain intensity and then to calculate the relative amount of marker-protein secretion by dividing the signals from two further fluorescence channels (FIG. 2). Such straightforward intensity-based ratio measurements are robust and quantitative, and can be used for various functional assays.

In the more complex case of a live-cell phenotypic assay to identify new gene functions (FIG. 3), cells have to be classified into relevant phenotypic categories — for example, 'dead cell', 'live cell', 'mitotic cell' or 'interphase cell'. Although methods for recognizing cellular morphologies in single images have been reported[28,29], they are not yet commonly applied to large-scale data, and the pattern recognition of cellular morphologies, with all of their inherent biological

cell-to-cell variability, is still in its infancy. A classification decision can be simple, for example, if it is based on the size or the fluorescence intensity of the object, but it can also be more complex if many or only subtly different classes have to be recognized. When many or subtly different classes must be recognized, a useful approach is to extract a long list of numerical parameters, or 'features', that describe the shape and texture, as well as other derived characteristics such as the pixel-intensity statistics, of each object. The software then computes a collection, or 'vector', of values for each object that can be based on several hundreds of different features[28,30].

On the basis of these feature vectors, a classification algorithm can be trained to discriminate between phenotypes. This training is based on manually annotated images that contain many example objects for each phenotypic class. The software then systematically selects the most powerful features that together discriminate between the different classes, and it determines boundaries for feature values that allow a decision to be made. In our experience, such feature-based classification systems can reach high accuracies that are comparable to those of human annotators for single static images. However, an inherent problem is that such classifiers that are obtained by supervised learning only recognize phenotypes that they were trained to recognize and therefore require retraining if novel phenotypes arise in an experiment. Developing software that automatically learns and defines new classes with similar feature properties would represent a big step forward for this powerful approach, and this is now feasible because large-scale data sets of sufficient quality are becoming available.

Analysing movies: tracking and more. For live-cell high-throughput assays, the classification of single images should ideally not be the only step in the analysis because it ignores the large amount of kinetic information that is contained in time-resolved data. As there are so far almost no time-resolved large-scale data sets, there are even fewer suitable analysis tools for large-scale kinetic imaging data. To make use of temporal information about individual cells or subcellular organelles, these structures have to be connected or 'tracked' throughout all of the single frames of a movie. Similar to pre-processing, tracking algorithms are well established for single-cell experiments, but they have not been adapted or used for high-throughput data. Tracking can work directly on the segmented images, and it will connect objects in time on the basis of criteria such as the speed of motion, the shape of the trajectory and the possibility of the objects splitting (cell division) or merging (cell fusion). Once tracks have been computed using an appropriate algorithm, the tracks and object classification can be combined to yield a powerful description of the phenotypic evolution of cells. For example, tracking can aid classification decisions by looking at the history of a particular cell (a cell that was classified as 'dead' is unlikely to become 'live' again at a later time) and, vice versa, the correct classification can aid tracking (if two different cells are as likely to be connected by a tracking algorithm to a cell in a preceding image, the more similarly classified cell will be chosen). Tracking is only one way of processing time-resolved image data sets. Time adds another dimension to multidimensional spatial images, so many spatial algorithms, such as noise filtering or interpolating between the edges of objects to create three-dimensional boundaries, can also be applied in time. This is an important area for future innovative developments in image processing.

In its entirety, advanced image processing allows the automatic and quantitative scoring of high-throughput imaging assays and is therefore an integral part of assay development. Importantly, image processing has become sufficiently powerful in recognizing cells of interest such that it also has the potential to replace human involvement in carrying out complex experiments or in providing online feedback regarding the way in which images are acquired during large-scale experiments. Although this has not yet been accomplished, it holds the promise of making automated microscopes that are as versatile and powerful as an experienced microscopist. For example, rather than tracking cells after a movie is acquired, the cell of interest can be located by image processing after each frame of a movie is taken, and the spatial information can then be used to move the stage of the fully motorized microscope so that the cell will be perfectly centred in the next image[20]. With the appropriate microscope, a cell could not only be centred, but, once identified, a FRET

Table 1 | **Selection of commercial high-throughput microscope systems**

Name of instrument	Manufacturer*	Microscope features	Applicable to live-cell imaging?
IN Cell Analyzer 3000	GE Healthcare	Laser-scanning confocal	Yes
Opera	Evotec Technologies	Nipkow-disk confocal	No
Pathway HT	BD Biosciences	Nipkow-disk confocal	Yes
Scan^R	Olympus	Widefield	Yes
KineticScan	Cellomics	Widefield	Yes
ArrayScan	Cellomics	Widefield	No
Discovery-1	Molecular Devices	Widefield	No
ImageXpress	Molecular Devices	Widefield	No
cellWoRx	Applied Precision	Widefield	No
Acumen Explorer	TTP LabTech	Laser-scanning non-confocal	No
iCyte	CompuCyte	Laser-scanning non-confocal	No
Cell Lab IC 100	Beckman Coulter	Widefield	No

*See the Manufacturer information for links to the manufacturers. In laser-scanning microscopes, a single laser spot scans the specimen to generate an image. In Nipkow-disk microscopes, multiple spots scan the specimen in parallel, which allows faster image-acquisition rates. Confocal systems achieve optical sectioning using the confocal principle (for more details, see REF. 40). Widefield and non-confocal microscopes illuminate the entire specimen at once and do not produce optical sections.

Table 2 | **Selection of image-analysis software**

Name of software	Manufacturer*	Applicable to high-throughout imaging?
Cellenger	Definiens	Yes
Cell Profiler	Open source	Yes
BioConductor	Open source	Yes

*See the Manufacturer information for a link to the manufacturer (open source is publicly available code). Cellenger and Cell Profiler have both been used in high-throughput imaging studies (see REFS 9,41, respectively), whereas BioConductor remains to be applied.

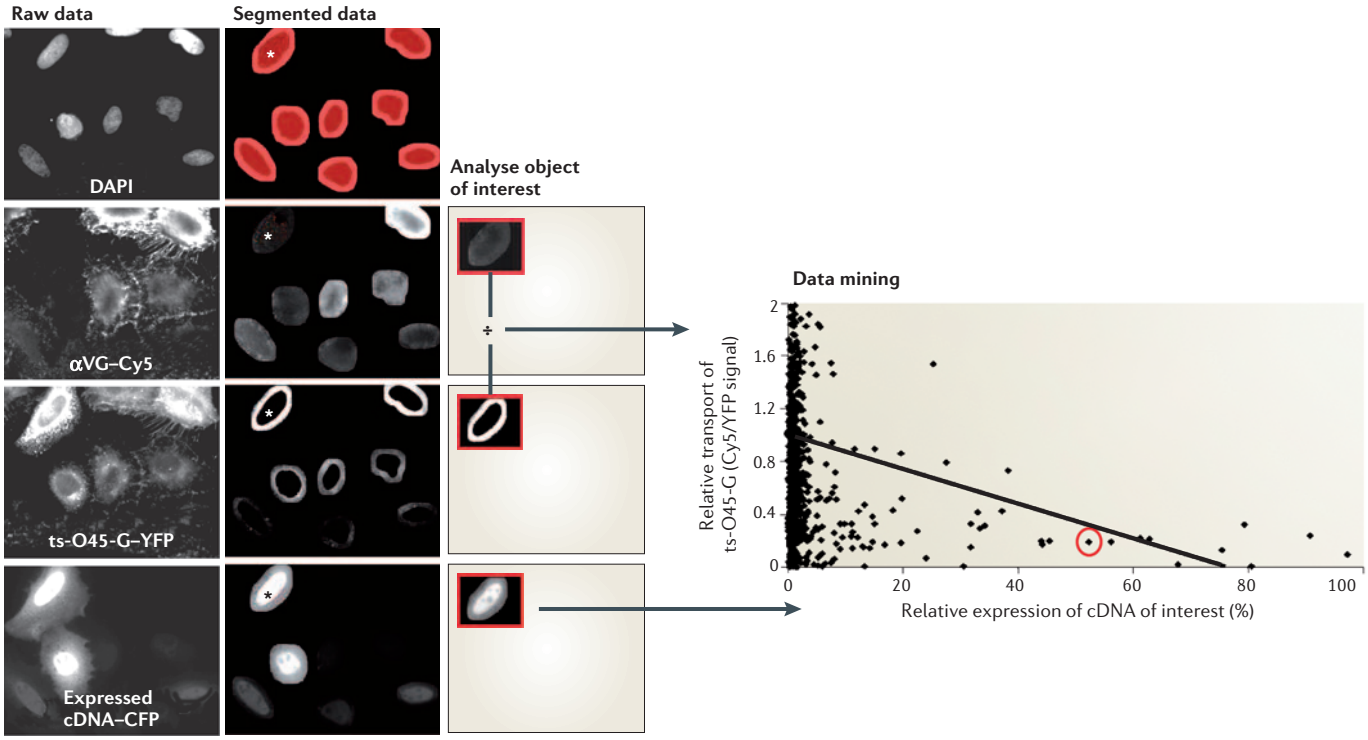


Figure 2 | **Image analysis of high-throughput image data from fixed cells.** The multichannel immunofluorescence data from the secretion assay described in BOX 1 are shown. In this assay, a library of fluorescently-tagged cDNAs was screened for a function in protein secretion using an overexpression approach in fixed mammalian cells. Raw data were first segmented in the DAPI channel to identify single cells by their nuclei (DAPI stains DNA), and an area around the nucleus was defined as cytoplasm belonging to each cell. On the basis of the intensity of an overexpressed cyan fluorescent protein (CFP)-tagged cDNA, a cell of interest was chosen for further analysis (marked by asterisks). The mean CFP intensity above the background in the combined nuclear and cytoplasmic area is a measure of the expression level of the cDNA being tested. The mean intensity above the background in the combined nuclear and cytoplasmic area for the secretion reporter protein ts-O45-G (temperature-sensitive mutant of the vesicular stomatitis virus G protein) was detected at the plasma membrane by a fluorescently labelled monoclonal antibody (α-VG–Cy5). To calculate the relative amount of protein that had been transported to the plasma membrane, this intensity was divided by the mean above background intensity in the cytoplasmic area of the cellular yellow fluorescent protein (YFP)-tagged ts-O45-G protein that was detected in any cellular compartment. Data for many cells were then mined by plotting the relative transport of ts-O45-G versus the relative expression level of the cDNA of interest, and this produced a clear correlation between transport inhibition and high expression (the red circle highlights a data point from a single cell). Such a cDNA would have been scored as a transport inhibitor in this assay. The graph in this figure is reproduced with permission from REF. 8 © (2004) Cold Spring Harbor Laboratory Press.

measurement could be taken, a short FCS trace could be acquired to measure diffusion or a FRAP experiment could be carried out to study protein dynamics — all in a fully automated and high-throughput mode. We speculate that such 'intelligent microscopes' will become more and more powerful in the years to come.

Data mining and quality control

The results of image analysis are derived from a single experiment — for example, a single RNAi gene knockdown, gene overexpression or inhibitor treatment — in a large-scale data set that is generated from many (tens of thousands) of such experiments. The parameters that are computed by image analysis can come from many cells in single or numerous static images per experiment, or even from time-resolved three-dimensional data in live-cell assays. The data mining of these parameters has two functions.

First, it should allow a decision to be made about whether a particular experiment scored as a 'hit' in the assay used. This is determined by comparing the results of the experiment with those from internal negative control experiments that were carried out under the same conditions. If the results of the experiment are different to those for the negative controls in a statistically significant way, the experiment scores in the assay. It is very important to use an appropriate statistical evaluation because parameters that are derived from processing live-cell images can be noisy and multidimensional. Hits can either simply be flagged for further analysis or ranked according to their difference from the controls.

The second function of data mining is that it allows a quantitative comparison of all of the experiments in a high-throughput data set. This is achieved by assembling all of the measured significant differences from the control experiments into a comprehensive quantitative signature, which is also referred to as a 'profile' or 'phenoprint'[31] of each experiment. The phenoprint consists of at least one parameter (for example, the relative transport ratio for the secretion assay; BOX 1; FIG. 2) but can comprise a panel of parameters (for example, different biological classes for phenotyping; FIG. 3). These parameters can be used to compare and cluster experiments on the basis of similarities. Similar to expression profiling, phenoprints for many experiments can be visually displayed as heat maps (FIG. 3), which immediately reveal similarities between experiments.

In addition to scoring and phenoprinting experiments, data mining has an equally important function in the quality control of high-throughput imaging experiments. Automatically generated data only deliver useful information if the quality of each

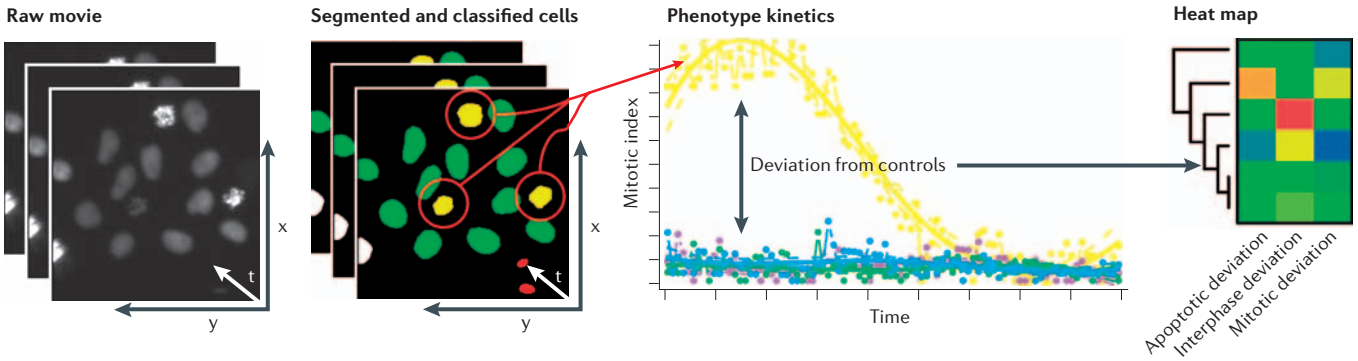


Figure 3 | **Image analysis of high-throughput image data from live cells.** The figure shows single-channel time-lapse data that were acquired in an RNA-interference experiment to screen genes for a role in cell division[31]. Each image in the raw data was segmented to identify individual cell nuclei that were labelled by histone–green-fluorescent-protein, and each cell was then assigned to a cell-cycle stage using an automatic classification algorithm. The example shows 3 prometaphase cells (yellow), 11 interphase cells (green) and 1 pair of anaphase chromosomes (red), as well as 1 cell that could not be analysed at the edge of the image (white). For all of the images in the time-lapse movie, this yields the percentage of cells in the different cell-cycle stages — for example, the index of mitotic cells (yellow curve in the mitotic-index plot), which can be plotted over time to describe the phenotype kinetics. If the index deviated from control experiments (blue, green and purple in the mitotic-index plot), this difference was plotted in a heat map (scaled from small deviations in blue to large deviations in red). The heat map serves to compare deviations in different phenotypic classes (columns) for one experiment (row) and to compare these phenotypic signatures between different experiments (six experiments/rows are shown), which can be clustered by similarity (see the dendrogram to the left of the heat map). The two right-hand panels of this figure were modified with permission from REF. 31 © (2006) Macmillan Magazines Ltd.

experiment is of the highest standard technically possible. To this end, internal negative and positive controls of the cellular assay are crucial to define the acceptable standard response and reproducibility of the assay. Mining the quantitative parameters that were generated by the image processing of control experiments allows boundaries of acceptable assay performance to be set using statistical methods and confidence. Only this allows us then to accept and score real experiments, because the assay has been shown to work over the expected dynamic range.

Conclusions

High-throughput fluorescence microscopy is starting to revolutionize the way we do cell biology. Experiments that were typically carried out manually in single cells and that addressed one gene or protein at a time for many months can now be done for whole genomes in a matter of days or weeks. In addition, more and more advanced fluorescence-microscopy techniques will become available for high-throughput imaging, because image processing can be used to make automatic microscopes not only high-throughput but also intelligent. For example, we predict that defining the genes that affect a certain cellular function using microscopy-based RNAi screening will become a routine experiment for many molecular-cell-biology laboratories in the next few years. Furthermore, even higher content measurements will be developed and applied in a high-throughput mode, such as the systematic and quantitative biophysical characterization of libraries of physiologically expressed GFP fusion proteins in live cells[32,33]. Examples of such measurements include protein diffusion (determined using FRAP and FCS), protein interactions (determined using FRET), or subcellular localization and concentration throughout the cell cycle[34] (determined using four-dimensional imaging). These parameters, if carefully quantified and standardized, will constitute the data backbone of comprehensive predictive models of cell function in systems biology[35].

Although it has enormous promise, high-throughput imaging still poses serious challenges for our future research because the required hardware and software are still being developed. The transition to living cells and truly quantitative large-scale data have also not yet happened. Systems-biology projects that are based on high-throughput imaging will require an interdisciplinary team of robotics and optics engineers, cell biologists, image-processing specialists, bioinformaticians and statisticians. There is a strong need for the development of intelligent, more automated and sensitive imaging equipment as well as image-analysis and experiment-control software. There are also increasing IT and bioinformatics demands for data management and analysis. Producing, handling and storing many terabytes of data require image databases, for which there are currently no suitable solutions. Processing millions of single images with computation-intensive algorithms is currently only feasible on computer clusters and therefore requires access to an advanced IT infrastructure. At the same time, comparing several large-scale experiments will require standardized data and annotation formats that the community needs to develop.

In our opinion, one of the exciting perspectives for high-throughput imaging is that we will be able to carry out large-scale and high-content cell-based assays rapidly enough to allow different large-scale perturbation approaches to be combined to create powerful and comprehensive tools for functional genomics approaches. One example is to use the overexpression of genome-coverage cDNA libraries and RNAi screening to achieve comprehensive gene discovery in standardized human cell-culture systems in a manner that is similar to the loss- and gain-of-function screens that were formerly only possible in truly genetic systems such as *Saccharomyces cerevisiae* or *Drosophila melanogaster*. With this being just one of many exciting possibilities, we predict that high-throughput fluorescence microscopy will be a key technology for systems biology in mammalian organisms.

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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OPINION

Metabolic cycles as an underlying basis of biological oscillations

Benjamin P. Tu and Steven L. McKnight

Abstract | The evolutionary origins of periodic phenomena in biology, such as the circadian cycle, the hibernation cycle and the sleep–wake cycle, remain a mystery. We discuss the concept of temporal compartmentalization of metabolism that takes place during such cycles, and suggest that cyclic changes in a cell's metabolic state might be a fundamental driving force for such biological oscillations.

The simplest of cells and the most complex of organisms rely on food and nutrients to fuel intricate metabolic programmes that enable survival, growth and reproductive proliferation. The set of metabolic reactions that is required for life is vast and complex. Individual cells execute thousands of biochemical reactions — some are intrinsically anabolic or catabolic, some are seemingly incompatible and others even produce toxic or mutagenic by-products. How does an organism perform the many metabolic reactions that are demanded of it without dire or futile consequences?

Spatial compartmentalization is one mode of dealing with this dilemma. At the single-cell level, organelles serve as specialized compartments that are dedicated to executing specific metabolic functions. For example, mitochondria represent the site of oxidative phosphorylation, peroxisomes perform fatty-acid oxidation and the endoplasmic reticulum facilitates protein oxidation. By confining particular reactions within organelles, disparate chemical reactions are insulated from the bulk environment and can proceed under locally optimized conditions. In complex metazoan animals, spatial compartmentalization also occurs more macroscopically within tissues and organs. For example, the liver can be gluconeogenic when other organs are glycolytic.