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Descriptive no more: The dawn of high-throughput microscopy

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Abstract

The next revolution in microscopy is upon us: it is high-throughput imaging (HTI). In HTI large numbers of images from many samples are acquired and analyzed. This has become possible due to the confluence of dramatic progress in microscope engineering enabling efficient image collection and the availability of high computing power for data analysis. As recently exemplified by Neumann, Ellenberg and colleagues, combining HTI with genome-wide RNA interference-based gene-knockdown technology offers a powerful approach for unbiased discovery of cellular mechanisms.

Microscopy – the cell biologist’s favorite tool

Microscopy has always been the cell biologist’s favorite tool. Cell Theory, which posited in the 1600s that all living things are made of individual cells, was developed entirely based on observations by light microscopy which revealed the presence of distinct entities, later called cells, in tissues and organisms. Until the mid 1900s light microscopy was the major tool in cell biology and led to a detailed description of cellular structure and cellular processes such as the complex behavior of chromosomes during cell division (Fig. 1A). A quantum leap occurred in the early 1970s with the development of staining methods using fluorescently marked antibodies [1]. This allowed for the first time to probe the localization of not only morphologically defined cellular structures but of specific proteins. In parallel, powerful electron microscopy approaches including immunolabelling techniques to detect individual proteins emerged. The next breakthrough in microscopy occurred with the development of confocal imaging which dramatically increased the resolution of observation and revealed unprecedented details of cellular structure ([2]; Fig. 1B). Light microscopy further gained ground by a revolution in digital imaging including the emergence of high-sensitivity cameras to enhance the power of conventional light-microscopy. Then in the 1990s, the discovery and application of the green fluorescent protein changed the landscape of cellular imaging once again by enabling tracking of proteins in living cells and by providing a tool to study the dynamics of proteins in their natural context of the living cells [3]. These revolutionary innovations did not merely represent technological progress, but each brought with it a wave of transforming insights into cellular mechanisms.

Despite their transformative impact, imaging methods have suffered from one major drawback: they are descriptive! They allow observation of individual proteins in their cellular environment but functional questions about a protein’s behavior can only be asked if one has information as to what pathways it may be part of. Unbiased discovery of novel pathways using imaging approaches has been difficult. This is a severe limitation in our cell biological arsenal. However, we are about to overcome this roadblock. We are in the midst of the next frontier in imaging

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which is the use of high-throughput imaging [4], particularly in combination with RNA interference (RNAi)-based knockdown technology, for the unbiased discovery of cellular mechanisms. Proof that such approaches are feasible is a recent paper by Ellenberg and colleagues [5] describing the largest HTI screen to date.

The mother of HTI screens

The goal of the study by Neumann *et al.* [5] was to identify new genes involved in cell division. To do so, they silenced 21,000 protein-coding human genes using RNAi and monitored the effect of each by automated high-throughput time-lapse microscopy of ~19 million cell divisions by imaging fluorescently labeled chromosomes in a HeLa cell line stably expressing the histone H2B tagged with the green fluorescent protein. This yielded about 19 terabytes of imaging data. This exceptionally large dataset was then computationally subjected to automated classification using a morphology recognition program based on supervised machine learning [6] to distinguish various mitotic phenotypes. This allowed classification of chromosome configurations into distinct morphological classes, such as “mitotic arrest/delay” or “binuclear” and “polylobed” classes. Using this approach, a first screen identified 1,249 genes involved in mitosis. Of those, 572 hits were confirmed and validated based on consistent phenotypes with two or more independent RNAis and complementation assays [7], to exclude off-target effects. Strikingly, the function of more than half of the identified genes had not previously been linked to mitosis, increasing substantially the number of known proteins involved in cell division.

Despite this success the study by Neumann *et al.* also highlights the limits of current HTI screens. Although a number of putative novel mitotic components were identified, primary screening data usually provide little biological or mechanistic insight. It is easy, and tempting, to identify intriguing novel components of a cellular process. But to ensure that these are true players in a given process, each HTI hit needs to be followed up by more traditional, focused studies to delineate its function. In the case of the mitotic screen, a follow-up study by Hutchins *et al.* [8] has already analyzed the subcellular localizations and protein interactions of a large fraction of identified mitotic genes. In doing so, they characterized around 100 protein complexes, using large-scale protein localization studies and tandem-affinity purification-mass spectrometry of tagged genes on bacterial artificial chromosomes stably expressed at near physiological levels in human tissue culture cells. Importantly, many identified interactors were found to have previously unknown functions in chromosome segregation, demonstrating that despite the power of the method, current HTI-based RNAi screens are generally not exhaustive.

The power of image analysis

Although the workflow used in this study is not novel [9], the complexity of combining several individual steps in a robust working platform for a genome-wide live-imaging screen and the sheer volume of data are impressive. In a crucial step, the authors computed the relative order of phenotypic events deduced from the phenotype classes, generating not just a static phenotype read-out, but a dynamic event-order map for each hit. This step provides a temporal signature of mitotic phenotypes for all siRNAs, revealing patterns of interrelations between the different mitotic perturbations. For example, the event order map for the “mitotic delay/arrest” class showed that this phenotype is transient and occurs first, leading in most cases to secondary phenotypes such as cell death or “polylobed” nuclei indicative of aberrant chromosome segregation. Use of such even maps is a powerful tool that can be applied to any biological system with distinct temporal phenotypic deviations, revealing their interplay and temporal coupling. Moreover, combining this information with the severity of the observed phenotype for each siRNA, one can sort hits into clusters of genes with common phenotypic profiles, with the aim of predicting groups of genes with similar functions in mitosis. Similarly, predictions

for the role of genes located within a cluster can be made based on the function of other known genes found in the same cluster.

The future of HTI

Despite the grandiose scale of the reported screen, this is just the tip of the iceberg, and there is much more to be discovered. Considering the >200 different cell types in a human body, it will be interesting to compare the importance of mitotic components and other pathways in various different specialized cell types. Furthermore, in addition to the protein-coding genome, it will be an important task to extend HTI studies to the whole non-protein coding genome [10].

There is no doubt that a multitude of similarly large-scale studies will follow. To truly harvest the full benefit of such large data sets, it will be important to combine the results into common databases and overlay them with other platforms to form an integrated comprehensive geno-/phenotyping forum that will also include features like gene expression profiles, protein-protein/protein-DNA interactions and single nucleotide polymorphisms in both normal and patient cells (Fig. 2). Such comprehensive databases will provide a powerful basis for systems biology approaches. To achieve this, biologists, computer scientists and researchers from various other fields need to work closely together to ensure an easily accessible and standardized forum with high compatibility between databases. The Neumann *et al.* dataset is freely available to the scientific community via the Mitocheck Consortium website (www.mitocheck.org) but in the future, ensuring full availability of data from HTI screens, will require combined efforts of the scientific community and funding agencies to facilitate the archiving of large imaging datasets. This will become increasingly important as imaging datasets are growing exponentially and imaging screens will become more common. The hope is that large funding agencies mandate deposition of HTI datasets into a central archive akin to the standards now applied to DNA sequence or microarray expression data. Until funding agencies are prepared to do so, the burden to ensure data accessibility falls onto the shoulders of authors and publishers. A laudable example of a publicly available imaging database is the DataViewer developed by *The Journal of Cell Biology* (<http://jcb-dataviewer.rupress.org>;) amongst others [11,12].

HTI will have a profound impact on the future of cell biology, including in the area of drug discovery and medicine (Box 1). One of the most challenging next moves will be the application of similar HTI screens to dissect other major cellular pathways such as cytoplasmic vesicles trafficking, cell migration, cytoskeletal dynamics and nuclear functions. Some such screens at various scales have already been undertaken [13–24]. One of the major, and most exciting, steps will be the use of HTI in live, rather than fixed cells as is currently the norm. Numerous cell lines exist in which cellular components of various pathways are tagged by fluorescent proteins, and the imaging tools for such challenging endeavors are currently being implemented. Combining these tools with an automated screening pipeline will be a milestone on the way to systematically, and in an unbiased fashion, decipher gene function in an exhaustive manner and eventually unravel the mysteries of cell behavior.

Box 1

The impact of HTI – from cell biology to clinical research

Apart from deciphering cell biological pathways and signaling networks, HTI screens have implications for translational medical applications. Controlled cell cycle progression is essential for normal cell behavior, and misregulation of this process plays a crucial role in numerous diseases such as cancer. Accordingly, many oncogenes and tumor suppressors that function in this pathway have successfully been targeted by cancer drugs. HTI approaches can be used to investigate these mechanisms *in vivo* for example to explore drug

resistance and off-target effects of clinically used drugs. Knowledge of the precise mechanism of action of cancer drugs would be a ground-breaking step forward in the development of improved drug derivatives. Obviously, HTI screens targeting other cellular processes such as protein secretion, viral infection, protein trafficking and degradation, cell migration or apoptosis will yield similarly important insights into the fundamental cell biology providing the basis for development of diagnostic and therapeutic approaches.

HTI is of particular interest in the medical arena since it can be applied to clinically relevant samples such as tissues from disease animal models, patient tissues arrays, patient cell lines and patient-derived iPS cells and their differentiation along various lineages. HTI approaches will be useful in defining the cell biology of disease mechanisms and discovery, development and improvement of drugs.

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Figure 1. The evolution of depicting mitosis

(A) Drawings of mitotic stages in newt cells described by Walther Fleming in the late 1800s [25]. (B) Micrographs of mitosis in fixed PtK1 cells revealed by antibody stainings and fluorescence microscopy (actin, red; microtubules, green; centrosomes (gamma-tubulin), magenta; and DNA, blue (courtesy of Dr Alexey Khodjakov)

Figure 2. From HTI screening to comprehensive databases

HTI screening together with validation and follow-up experiments make up the experimental part of HTI screening (upper grey panel). The organization of the resulting primary information into a comprehensive database requires an interdisciplinary approach (lower grey panel).