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High-throughput Imaging as a versatile and unbiased discovery tool

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Light microscopy methods are amongst the most successful tools in modern biomedicine. For decades, they have been instrumental in establishing some of the foundational concepts in biology including the notion that tissues are made up of cells and that cells are compartmentalized in functionally distinct subcellular compartments. Imaging methods have also revealed the functional connections between molecular pathways and the dynamic spatial organization of organisms, organs, tissue, cells, organelles and membrane-free subcellular compartments. In addition, and in contrast to biochemical and molecular biology techniques, microscopy directly visualizes cellular structures in intact, often live, single cells or organisms, thus revealing not only the how, but also the where and when of biological processes.

High-throughput Imaging (HTI) can be loosely defined as the implementation of an automated workflow consisting of liquid handling, microscopy-based image acquisition, image processing and statistical data analysis. Automation allows the systematic study of cell biological processes on a large scale, often involving the analysis of thousands to millions of organisms, cells or subcellular objects per experiment. As such, HTI combines the benefits of traditional microscopy with the capability of testing thousands of experimental conditions and/or detecting rare but important biological events, allowing the unbiased and quantitative study of cell biology. More importantly, by measuring cellular features at the single-object level in a large population of cells or organisms, HTI is also ideally positioned to capture and characterize the inherent population heterogeneity of biological processes. Finally, a major step forward enabled by HTI is the generation of computational models of cellular structure and dynamics that use as input data generated by large-scale imaging.

The applications of HTI are manifold. HTI implementations are highly diverse ranging across biological systems, imaging modalities, probes, and detection methods. The capacity to image a large number of samples enables the execution of chemical or genetic screens, in which any combination of tissue- or cell features that can be visualized by imaging can be utilized as an assay readout; the ability of HTI to collect data on a large number of samples enables the interrogation of rare events by mining the resulting large dataset to extract the few events of interest; given the large number of cellular features per object generated by HTI, this technique has also been used for the systematic phenotypic profiling of collections of compounds or siRNA to infer their targets with as of yet unknown biological function.

The goal of this issue of *Methods* is to capture some of the cutting-edge developments in the field of HTI. The collection of original research papers and technical reviews presented here

is far from representative, but it aims to present a cross-section of some of the emerging HTI tools and applications.

The transformational nature of HTI is evident in the recent foundation of a major research institute centered around HTI. The Allan Institute for Cell Science (AISC), Seattle, will develop and use large-scale HTI pipelines to comprehensively catalogue the location and dynamics of cellular proteins in induced pluripotent stem cells (iPSC) and their derivatives to recapitulate various physiological and pathological developmental states. In the first article of this issue Rick Horwitz, the founding Director of the AISC, outlines the vision, challenges and potential implications of the HTI-centered approach the AISC takes [1]. Of course, all HTI approaches are based on the development of sophisticated automated image acquisition and processing tools to execute and analyze large-scale imaging experiments. A set of original papers and reviews describes some of advanced tools for HTI. Logan, Carpenter and colleagues describe an image analysis approach based on machine learning, which allows the accurate identification of two cell types present in the same sample [2]. This is a major step forward as it is likely that co-cultures of multiple cell types will increasingly be used in HTI approaches as these mixed systems more accurately reflect the heterogeneous nature of most biological environments. Apart from precise detection of individual cells in a sample, the accurate measurement of cellular features is central to HTI data analysis. Gough and colleagues describe an in depth analysis of reproducibility and variability of HTI measurements across individual experiments [3]. They demonstrate that conventional assay metrics are not always sufficient for high-level quality control and they present a workflow for the analysis of measurement heterogeneity in large scale HTI datasets. An essential, albeit sometimes underestimated, component of the HTI workflow is the storage and management of images, analytical data and experimental metadata in an easily accessible and minable format where all these components are directly linked to each other. Li and colleagues from the OMERO (Open Microscopy Environment Remote Object) consortium describe their open-source data management system, highlighting the need in the HTI community for the standardization and distribution of HTI datasets and their related experimental metadata so as to ensure reproducibility, community-wide access and reuse of the results [4]. A major future goal, still in its infancy, is the generation of computational models of cellular structure and dynamics based on HTI data. Murphy describes in his article his cutting-edge work on categorizing cellular patterns generated by HTI and approaches for creation of models of cellular organization [5].

With HTI tools in hand, the range of possible applications is vast. Since many HTI experiments, especially when done at large scale, are logistically complex, they require streamlined, robust primary assays that minimize experimental intervention during the screening process and generate highly reproducible, sensitive read-outs. Larsen et al. describe such an optimized assay for monitoring the trafficking of the mutant Cystic Fibrosis Transmembrane Receptor, the causative agent in Cystic Fibrosis [6]. This assay will enable novel large-scale small molecule screening efforts for the discovery of therapeutic agents for this disease. Another disease-related HTI-based drug screening assay is reported by Kubben et al., who have generated an experimental system to recreate the cellular disease phenotypes characteristic for the premature aging disorder Hutchinson-Gilford Progeria Syndrome [7]. They use the system in a proof-of-principle study to screen a library of FDA-approved drugs

and identify several novel candidate drugs capable of suppressing the cellular phenotypes of this disease. Day et al. describe the development of a single-cell, live-cell assay to measure the kinetics of individual steps of transcription including elongation and RNA processing [8]. They used the assay in a targeted screen and identify compounds that selectively affect particular gene expression steps. While many HTI applications use RNAi or small molecule screens, alternative approaches are similarly feasible. As examples, Hasse et al. report on a versatile system to systematically express fluorescently tagged proteins at large scale in cultured cells to analyze their function and location using HTI [9] and Szafran et al., have developed an HTI pipeline for large scale screening of complex mixtures of antibodies by using a novel supervised machine learning algorithm that can classify each antibody based on immunofluorescence staining features extracted by automated image analysis [10].

While many HTI applications employ cancer-derived cell lines growing in 2D monolayers, it is becoming increasingly clear that more physiologically relevant and 3D cellular systems, or even whole-organism approaches, are needed to fully recapitulate the functional and spatial milieu of many biological processes. Towards this goal, Leha et al. have applied a high-throughput platform to characterize induced pluripotent stem cells (iPSC) to robustly quantify phenotypic diversity in this complex cell population [11]. These approaches will have important practical applications to benchmark iPSCs from different donors and to link phenotypic variability with their different genetic backgrounds as measured by matched genomic datasets. Li et al. discuss the potential of HTI in combination with emerging technologies to generate and analyze 3D organoids, particularly for their use in drug discovery [12]. Puah and Wasser extend these efforts into wholeorganism imaging. They have established a pipeline that combines high-throughput in vivo imaging with gene knockout strategies to identify genes involved in muscle development in *Drosophila* [13]. Finally, Pulak describes tools for automated imaging of zebrafish larvae that may be applied to genetic or small molecule screening approaches [14].

HTI represents a quantum leap in imaging. It enables entirely novel approaches to address previously intractable biological questions and has inspired a wide range of diverse technologies and applications. This is all the more remarkable as HTI is still a young field. We hope the articles in this issue give a flavor of the tremendous possibilities and future potential of HTI in basic and applied research.

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