



Integrated, multi-scale, spatial–temporal cell biology – A next step in the post genomic era



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ABSTRACT

New microscopic approaches, high-throughput imaging, and gene editing promise major new insights into cellular behaviors. When coupled with genomic and other 'omic information and “mined” for correlations and associations, a new breed of powerful and useful cellular models should emerge. These top down, coarse-grained, and statistical models, in turn, can be used to form hypotheses merging with fine-grained, bottom up mechanistic studies and models that are the back bone of cell biology. The goal of the Allen Institute for Cell Science is to develop the top down approach by developing a high throughput microscopy pipeline that is integrated with modeling, using gene edited hiPS cell lines in various physiological and pathological contexts. The output of these experiments and models will be an “animated” cell, capable of integrating and analyzing image data generated from experiments and models.

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1. Introduction

The emergence and evolution of collaborative activities generating and subsequently analyzing many kinds of large cellular datasets follows in the wake of the enormous success of the human genome project. These “omic” efforts include gene expression profiles, proteomics, metabolomics, lipidomics, glycomics, and epigenomics, among others. Most of these activities first collect and catalog data and then mine for novel associations, developing circuits, pathways and network models for the associations that emerge [1–4]. This useful information, played out most explicitly and robustly with gene expression data, is then used to try to predict cellular behaviors in normal and pathologic contexts.

The jump from genomic, and 'omic data in general, to cellular phenotypes and behaviors is a large and challenging central goal of cell biology and biomedical research that is not yet in sight. Indeed, few studies even try to integrate different kinds of available 'omic information, likely because the data are largely incomplete, of varying quality, or done on different cell types and systems. The phenotypic data are also incomplete, usually focusing on one, or a small number of, activities of particular interest.

There is another, larger issue, however. Live cell microscopy reveals the emerging general theme that transient and localized phenomena drive most cellular behaviors. In this context, most of the 'omic data reflect only a few time points and represent spatial averages across a single cell, or more generally, across a popu-

lation of cells. In most cells, the cellular machinery, and the signaling complexes that regulates it, are polarized. These localized activities are obvious and dramatic in cells like neurons or skeletal muscle that are high polarized morphologically. But the polarity is also apparent in other cell types like fibroblasts or neutrophils. Under migrating conditions, for example, actin polymerization and branching are largely confined the leading edge. Also, organelle organization is similarly polarized with the nucleus, Golgi, MTs, actin filaments, and ER in stereotyped locations [5].

Activity assays using biosensors in living cells reveal not only a polarity but also the transient nature of cellular activities. For example, fluorescent sensors of PIP3 localization show a highly transient and localized accumulation in response to a chemotactic signal [6]. RhoGTPase signaling is likewise highly polarized and transient at the leading edge of migrating cells [7]. The localized and transient character of signals is important, as diseases like cancer often arises from mislocalized or constitutive signaling. In addition to these examples, nanoscale events, like receptor clustering and the formation of signaling complexes, reflects a localized, transient polarization on a molecular scale.

Thus, understanding cellular behaviors requires live cell image data of cellular organization and activities. While many labs are generating this kind of data, it is usually done studying a single organelle, often in a molecule-by-molecule approach. However, each of the molecular machines, as well as the molecular complexes that regulate them, function as “systems”, and the cell can be viewed as an integrated, complex system of these systems, with poorly characterized interactions among the various different

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molecular machines and regulatory complexes. Few microscopy efforts have viewed the cell as such an integrated system and attempted to integrate multiple cellular activities.

2. Microscopy pipelines

A large-scale, high-throughput microscopy program will be required to address the cell as an integrated system. While there are many high-throughput microscopy efforts, particularly in industry, few are devoted to understanding cell behavior, per se. Instead, most are directed toward drug discovery, focusing on high-throughput screens, using robust, relatively large color intensity differences or morphology-based assays with cells growing on microwells. The advantage of these high-throughput, automated approaches is that very large chemical libraries can be screened quickly and reproducibly.

Some of these methods are also used in academic settings not only to screen for drugs, but also to screen RNAi libraries or other genetic perturbations for molecular function in the context of a specific cellular process or organelle, identifying new genes for deeper study. Two early examples are the Cell Migration Consortium (2001–2011; <https://www.cellmigration.org>), which used RNAi screens of adhesion components, kinases and phosphatases for effects on adhesion and migration. Another is the EU-funded MitoCheck (2004–2009, www.mitocheck.org). This project used RNAi to screen all 22,000 human genes in cultured human cells, showing that 600 human genes are involved in mitosis and measuring how their localization changes during the cell cycle. These projects were subsequently integrated into the EU Systems Microscopy Network of Excellence [8].

These and other initial, pioneering and large-scale, high-throughput efforts revealed and addressed some of the challenges of systems microscopy in cell biology [9–14]. They include protein tagging, fluorescence signal to noise, automated analysis of images, the storage, sharing, and integration of image formats, and the analysis, of large, image-based, datasets. These consortia have produced significant initial steps in addressing these challenges. In this regard, the EU imaging community is uniting to set standards in microscopy and imaging. One example is the Open Microscopy Environment (OMERO), a management system for image data. Data mining, in contrast, is still a work in progress, as it requires large amounts of data and insightful analyses to reveal meaningful relationships.

Thus, in the wake of these focused, pioneering efforts, the stage is set to leverage and extend them, initiating large scale efforts that study complex organelles and their integration. This is one of the stimuli behind the Allen Institute for Cell Science.

3. The time is right

In addition to these high-throughput, “systems” microscopy efforts, other threads are conjoining to place live cell imaging for understanding cellular behavior as a focus for cell biology and next step in the post genomic era. Major advances live cell imaging make feasible quantifying the 3D organization and activities of cells as they execute their characteristic behaviors [15,16]. These include the low light damage and rapid data accumulation afforded by advances in spinning disk, structured illumination, light sheet and other microscopies. While not yet available robustly for living cells, superresolution methods could soon become part of the arsenal, pushing the observation limit to both higher resolution and complex 3D cellular environments.

Microscopy, per se, is only part of the challenge. The significant sizes of the image sequences present challenges for their storage, transfer and accessibility, requiring mechanisms for archiving

and short/medium term storage for analysis, particularly for cells imaged in 3-dimensions. Image analysis presents another challenge [17,18]. Most industrial scale image analysis uses relatively simple morphology, feature identification or color intensity parameters that are designed for high throughput screening of chemical libraries or gene inhibition. However, meaningful imaging and analyses for cell biologists will require different kinds of assays and more challenging automated image analyses, using machine learning for segmentation and feature analysis. Fortunately, these assays will also require a relatively modest number of samples – hundreds to a couple of thousand, rather than hundreds of thousands to over a million used in drug screens. Thus, lower throughput can accommodate more complex assays and analyses; nevertheless, while some of the methodology is available, these kinds of studies will likely push the envelope. Finally, the analyses will need to accommodate unusual substrate geometries, 3D configurations, e.g., organoids, and even the unusual properties of some cell types, e.g., epithelial sheets or cardiomyocytes that beat. It is likely that many assays commonly executed on a small scale in the academic laboratory, however, cannot be easily scaled for higher throughput, and other approaches will need to be developed.

Finally, most imaging studies have utilized a wide spectrum of different cell lines due to unusual properties that make them particularly amenable for the activity or process being studied. Recent gene editing and stem cell methods allow observations on human diploid cells that propagate indefinitely, and can be differentiated into a large number of different cell types. Thus, any reagents generated for these studies are useful for a wide spectrum of studies, beyond the intent of the initial study or any one investigator. However, the assays developed for the specialized cells, may not be as easily executed in stem cells.

4. The Allen Institute for Cell Science

The Allen Institute for Cell Science will develop a large-scale, high-throughput imaging platform, i.e., a systems microscopy pipeline, using gene-edited hiPS cells. The overarching goal is to localize key cellular molecular machines and regulatory complexes (MMRCs) and activities and then observe their variation in number and localization and how they change in response to alterations in cellular environment, differentiation, mutation (including disease models), drug treatment, infection with bacteria or viruses, etc. The assays will look at both a general set of MMRCs found in most cells, as well as specific sets found in the specialized or differentiated cell type under investigation. The goal is to develop quantitative data, not only on location of the MMRCs but also on their numbers and dynamics, as well as the numbers and dynamics of key molecules that comprise them. While the MMRCs will be studied individually, the coupling, or interactions, among them will also be studied dynamically and spatially. A key feature is to observe changes in cell organization and activities as cells execute processes like cell division and migration, for example, and in response to perturbations, as this affords the opportunity to identify correlative associations, from which empirical models can be derived.

These data will produce a needed set of quantitative information on cellular organization and dynamics at the “mesoscale” level that can be used to create an integrated, referential visual database showing dynamic relations among MMRCs. While these data in themselves will be novel and informative, their changes with environment and various perturbations will be used for coarse-grained correlative (statistical) modeling, generating new relations and hypotheses for integrated cellular behaviors. These hypotheses, in turn, can be used to develop predictive models for cellular

behavior and their underlying mechanisms. The molecular data on numbers and dynamics can help fine-grained, physical chemical modeling of individual processes to test detailed mechanistic models at the molecular level. In the end, the modeling can be further informed by its coupling with 'omic information. All of the modeling will be done iteratively and integrated with the experimental pipeline.

5. Conclusion

Multi-scale, spatio-temporal cell biology is arguably a major, next step in the postgenomic era, linking 'omic data to the localized, transient activities that drive cellular behaviors. The recent advances in microscopy and image analysis as well as the focused, high throughput live cell microscopy efforts in the US and Europe point to its feasibility and potential. In implementing this approach, the Allen Institute for Cell Science will make all of its models, data, reagents, and methods publicly available. The Institute goal is to use the data and models to create a "cell clinic", with which one can query behaviors of cells in different environments or in cells with disease mutations, for example, which are based either on experiment or computational predictions.

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