Emerging Biomedical Technologies at the Micro and Nano Levels

High-throughput cell imaging and nanotechnology are emerging technologies that exhibit great potential for disease diagnosis, prognosis, and treatment. We briefly introduce these emerging technologies and discuss challenging open research issues to fully realize the potentials of these new tools.

ADVANCES IN LIFE SCIENCE RESEARCH

Throughout the history of medicine, many advances have been driven by important technological innovations. For example, the invention of the X-ray machine, more than a century ago, revolutionized medicine and pioneered the use of noninvasive imaging in the diagnosis and detection of diseases. The invention of the microscope essentially redefined the fields of pathology and microbiology. In the past few decades, an explosion in technological innovations has had an immense impact on both biomedical research and clinical practice. Tremendous strides have been made with the aid of new technological inventions, such as recombinant DNA, DNA sequencing, magnetic resonance imaging (MRI), polymerase chain reaction (PCR), monoclonal antibodies, and so on.

Life science research in the past few years has witnessed equally revolutionary progress driven by high-throughput measurement technologies for biomolecules and cellular systems. Recently, life science researchers have been adopting a new integrated approach. This approach has naturally led to the emergence of the field of systems biology, focused on achieving a system-level understanding of biological units on a variety of physical scales, as shown in Figure 1 [1]. In this postgenomic era, researchers will access, model, analyze, and mine vast volumes and multiple types of data on various scales. New computer-based approaches or algorithmic-based approaches must tackle these challenges, in the areas of functional genomics, comparative genomics, proteomics, metabolomics, pathway analysis, and systems biology.

TECHNIQUES AT THE MICRO/NANO LEVELS

HIGH-THROUGHPUT CELL IMAGING

As the pharmaceutical industry is faced with an escalation in the amount of time and money required for drug discovery and development, there is a growing need to increase confidence in early stage targets, improve lead selection, and reduce late-stage attribution. An equally important issue is how to choose the best therapeutics from an increasingly large drug arsenal for patients with various conditions. In addition to microarray technologies [2]–[6], another promising technology for improving such decision-making is cellular imaging-based assays. This technology allows the functional analysis of target and pathway modulation in cells. High-throughput screening methods, such as transcriptional and proteomic profiling, are discussed in [7] and [8]. There are many types of high-throughput cellular data, capturing DNA polymorphism, gene activation, mRNAs, proteins, metabolites, calcium flux, cytotoxicity,
and proliferation. Processing this data has contributed substantially to the understanding of disease processes and drug actions. None of this data, however, can capture the important spatial and temporal information contained within functional cellular proteins.

To harness the power of this new high-throughput spatial and temporal information, we need to increase data acquisition throughput and to process and quantitate large amounts of image data effectively. By using automated microscopy (shown in Figure 2), combined with imaging techniques that show protein locations, cell structures, and physiological states of cells, researchers can increasingly observe system biology events such as multiple cellular events and intracellular events in individual cells [9], [10]. Quantitative cytological information available at early stages in the drug discovery process improves our understanding of drug targets and compound leads. However, the image informatics tools that are required to automate, quantify, and analyze cellular information on such a large scale have not yet been developed to complement the high-throughput imaging technologies that have already been adopted. The availability of the informatics tools would enable the close coupling of automated microscopy and image analysis with biostatistical and data mining techniques to provide an integrated system biologic approach to studying cells, the basic units of life. These informatics tools would also have many exciting applications in the life and health sciences.

Compounds that affect spatial arrangement of signaling proteins or cellular structures can provide important information about biological processes and therapeutic interventions. To identify such compounds using automated fluorescence microscopy is part of the “forward chemical genetics” approach [11], [12]. Effort is being directed towards high-throughput, image-based screening of large chemical libraries. Currently, about a dozen commercial vendors offer automated fluorescence imagers. Most, however, are assembled using off-the-shelf components. Their prices range from hundreds of thousands to over US$1 million for the high-end products. More than 20 cellular imaging assays have been developed and screened via our in-house compound libraries, consisting of approximately 100,000 commercial and 100,000 diversity-oriented synthesis (DOS) compounds (refer to Table 1). Please also refer to http://iccb.med.harvard.edu/screening/compound_libraries/index.htm for details on the compound library.

The major advantage offered by imaging is that it allows us to score changes in individual cells within a population of hundreds or thousands of cells, rather than simply finding cell population averages, as traditional means allow. This potentially enhances our ability to identify cell changes.

**NANOTECHNOLOGY**

To successfully detect diseases like cancer at their earliest stages, scientists often have to detect molecular changes in tissues or organs even when they occur only in a small percentage of cells. This means that nanotechnology tools must be extremely sensitive and specific. The potential for nanostructures to enter and analyze single cells suggests that they could meet this challenge. In addition, many nanotechnology tools will make it possible for clinicians to run tests without physically altering cells or tissues taken from a patient. This is important because the number of samples that clinicians use to screen for disease is often limited. Scientists would like to perform tests without altering cells, so they can be used again if further tests are needed. Reduction in the size of tools means that many tests could be run on a single small device. This would make screening faster and more cost-efficient than is possible with the current generation of tools.

Here, we list a specific set of nanotechnology techniques for disease management.

- **Cantilever** [13]: The cantilever is one of the nanotechnology tools with the potential to aid in disease diagnosis. Nanoscale cantilevers are based on a simple mechanical concept: disparate materials bond together, causing a mechanical reaction. When disease-associated molecules bind to cantilevers, changes in the surface tension of the molecules cause the cantilevers to bend.

- **Nanopores** [14]: The nanopore device is another nanotechnology tool for reading the genetic code of single strands of DNA. Nanopores are tiny holes that connect two liquid compartments that are positively charged at one side and negatively charged at the...
We began our work by measuring high-throughput cell imaging research issues. Here we discuss a number of open discussions to 200 pounds in a concentration ranging from 0.1 pM were treated with a certain compound HeLa cells grown in 384 well plates in serial dilution (see Figure 3). A panel of 60 known bioactive compounds, splicesome, and cell-cycle-regulated changes in three cellular markers: DNA, RNA, and cell-cycle-regulated changes in vivo. We measured the response of cell pathways to drugs (compounds), can be mined for hypotheses concerning cell responses at the system level. We believe that cytological profiling using this type of image informatics approach is a fast and effective method of gathering broad, quantitative phenotypic information from individual cultured cells. We can use it to categorize drug mechanisms, and similar methods should be useful for annotating RNAi screens and profiling cancer phenotypes. Cytological profiling provides information complementary to existing high-throughput profiling techniques, such as transcript and proteomic profiling. It also has the advantage of requiring far fewer cells per data point, and it is faster and cheaper when a limited number of fluorescent probes are employed.

One application of high-throughput cellular imaging for system biology is cytological profiling. Profiling studies typically have two outputs, namely, identifying similarities at the global level and generating mechanistic hypotheses using particular subsets of measurements. Our cytological data, which measures the response of cell pathways to drugs (compounds), can be mined for hypotheses concerning cell responses at the system level. We believe that cytological profiling using this type of image informatics approach is a fast and effective method of gathering broad, quantitative phenotypic information from individual cultured cells. We can use it to categorize drug mechanisms, and similar methods should be useful for annotating RNAi screens and profiling cancer phenotypes. Cytological profiling provides information complementary to existing high-throughput profiling techniques, such as transcript and proteomic profiling. It also has the advantage of requiring far fewer cells per data point, and it is faster and cheaper when a limited number of fluorescent probes are employed.

Discussions
Here we discuss a number of open research issues.

High-Throughput Cell Imaging
We began our work by measuring changes in three cellular markers: DNA, splicesome, and cell-cycle-regulated protein anillin. They were compared to a panel of 60 known bioactive compounds in serial dilution (see Figure 3). HeLa cells grown in 384 well plates were treated with a certain compound in a concentration ranging from 0.1 pM to 200 μM for 20 hours. The cells were then fixed and stained with antibodies to show the above three biomarkers simultaneously using different fluorescent dyes. The image data were then recorded using an autoscope equipped with a 20× lens and DAPI/FITC/TxRed filter set. Nine data points were recorded for each condition, and approximately 300 GB of image data were collected.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Titrations</th>
<th>Time Points</th>
<th>Replicates</th>
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<tr>
<td>60</td>
<td>16</td>
<td>2</td>
<td>2</td>
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</table>

0.1 pM – 200 μM 2 hr – 20 hr

<table>
<thead>
<tr>
<th>Stains</th>
<th>Descriptors</th>
<th>Images</th>
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<tbody>
<tr>
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<td>2 – 10</td>
<td>~2000</td>
</tr>
<tr>
<td>Anillin</td>
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<td>8</td>
<td></td>
</tr>
<tr>
<td>SC35</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Giantin</td>
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= 491,520,000 to 2,457,600,000 or over half a billion measurements at least over 2 billion if more descriptions are used.

[FIG3] Experimental setup for cytological profiling of drugs.

Nanotechnology
We proposed the use of relatively inert carbon nanotube structures to induce a cytotoxic radical activity cascade for cancer treatment. The research is carried out in the following steps:

By analyzing the quantum states of nanotubes under certain magnetic or electrical field conditions, we can determine the possibility that electrons in carbon nanotubes can transfer energy. These conditions can also excite the ground state radical (triplet) O₂ into its nonradical excited single state. We are also investigating the effect of phonon energy transfer into other biologically available compounds that exhibit cytotoxic effects.

We are exploring means to excite nanotubes remotely to induce a cytotoxic cascade. This would allow the activation of our cytotoxic agent (nanotubes) even in the deepest or most inaccessible tumor sites.

We are also developing a method to deliver our cytotoxic nanotubes to specific tumor sites. This will be absolutely necessary as there will be no way to form highly localized magnetic fields within an organism. In the presence of our activating magnetic field, all of our nanotubes will become cytotoxic; thus, we need to ensure that they are localized to the tumor site.

One application of high-throughput cellular imaging for system biology is cytological profiling. Profiling studies typically have two outputs, namely, identifying similarities at the global level and generating mechanistic hypotheses using particular subsets of measurements. Our cytological data, which measures the response of cell pathways to drugs (compounds), can be mined for hypotheses concerning cell responses at the system level. We believe that cytological profiling using this type of image informatics approach is a fast and effective method of gathering broad, quantitative phenotypic information from individual cultured cells. We can use it to categorize drug mechanisms, and similar methods should be useful for annotating RNAi screens and profiling cancer phenotypes. Cytological profiling provides information complementary to existing high-throughput profiling techniques, such as transcript and proteomic profiling. It also has the advantage of requiring far fewer cells per data point, and it is faster and cheaper when a limited number of fluorescent probes are employed.

In this article, we have briefly reviewed two emerging technologies for studying biomedical systems on the micro and nano scales. High-throughput imaging technologies have fundamentally changed the way in which we perform
biological research. Designing nanoparticles to penetrate the microstructures inside cells and perform multiple tasks may significantly impact disease diagnosis, prognosis, and treatment. To realize this goal will require further research into issues of the toxicity, sensitivity, and specificity of such nanodevices in human bodies.

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REFERENCES