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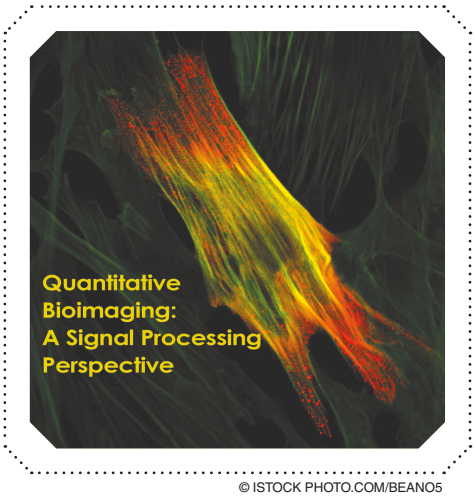
Toward a Morphodynamic Model of the Cell

Signal processing for cell modeling



From a systems biology perspective, the cell is the principal element of information integration. Therefore, understanding the cell in its spatiotemporal context is the key to unraveling many of the still unknown mechanisms of life and disease. This article reviews image processing aspects relevant to the quantification of cell morphology and dynamics. We cover both acquisition (hardware) and analysis (software) related issues, in a multiscale fashion, from the detection of cellular components to the description of the entire cell in relation to its extracellular environment. We then describe ongoing efforts to integrate all this vast and diverse information along with data about the biomechanics of the cell to create a credible model of cell morphology and behavior.

INTRODUCTION

Systems biology [1] is a multilevel approach to the study of biological phenomena that integrates structural and functional information at different levels of spatial (molecular, cellular, tissular, organismal) and temporal resolution. Although many noteworthy ongoing efforts aim at computationally describing the structure, function, and even the development of entire organs [2] and simple organisms [3], the cell remains the principal element of information integration and is the key to the design of higher-order models. The cells emanate signals that collectively determine the fate and evolution of organs and, within the cells, signals are directed that elicit inner mechanisms of protein production, replication, differentiation, and death. Understanding how the cell senses, reacts to, and produces these regulatory signals is the key to explaining the principles of life and disease. This is a daunting task that requires the study of the cell from many different perspectives (morphological, biochemical, mechanical, electrical) accounting for both the temporal and spatial dimensions. Accordingly, numerous efforts today are directed toward the creation of multidimensional morphodynamic models of the cell. Feeding into these models are technologies (hardware) and methods (software) that produce quantitative visual information. The evolution of these methods and technologies poses continuous challenges to the signal processing community. In this article, we review the state of the art of computational and signal processing aspects involved in:

- the development of advanced live cell imaging modalities
- the dynamic tracking of cells and subcellular components
- the estimation of forces exerted between the cell and its local environment
- the integration of "visual" information into credible models of cell behavior.

A brief historical perspective and a discussion of the state of the art of all these fields are presented, along with new challenges that require the involvement of the signal processing community.

IMAGING CELL BEHAVIOR

The first studies of the behavior of living cells date back about 340 years, when the Dutch draper Antoni van Leeuwenhoek decided to turn his interest in lens making to visualizing more interesting objects than his merchandise. In the mid-1670s, possibly inspired by the 1665 groundbreaking publication of Robert Hooke's discovery of plant cells, he was the first to observe micro-organisms, or *little animals* (animalcules) as he called them, in a drop of lake water. The magnification factor of van Leeuwenhoek's single-lens microscopes amounted to a stunning 300×, and the optical resolution of his lenses was already around 1 micron. It took until the 19th century before compound microscopes were developed that surpassed the quality of his microscopes and reached the diffraction limit—roughly half the wavelength of the light—discovered by Ernst Abbe in 1873.

Cells by themselves are fairly transparent and cannot be studied in detail using conventional light microscopy. One trick to improve optical contrast without having to explicitly stain cells is to exploit the fact that when light travels through a medium, it undergoes amplitude and phase changes that are dependent on the properties of the medium. While the human eye is sensitive only to amplitude variations, phase shifts may carry important information about the medium, and can be made visible by conversion to changes in brightness using special optical components. Phase contrast (PC) microscopy [4] was invented in the 1930s by Frits Zernike, which earned him the Nobel Prize in physics in 1953. Unfortunately, the use of this technique is restricted to very thin specimen preparations, and the resulting images suffer from halo artifacts. Another technique invented by Georges Nomarski in the 1950s, is to exploit the interference obtained when recombining two orthogonally polarized and slightly displaced light components after traveling through the specimen. Differential interference contrast (DIC) microscopy [4] yields superior resolution compared to PC microscopy and has excellent optical sectioning capability. However, the effectiveness of DIC is reduced by the specimen's reaction to polarized light. Moreover, the resulting images show typical pseudo-three-dimensional (3-D) artifacts that can be mistakenly interpreted as topographical cell features. Several of the limitations of both PC and DIC microscopy can be avoided by the use of Hoffman modulation contrast microscopy [4], developed by Robert Hoffman in 1975.

These optical contrasting techniques are used particularly in studies that do not require quantification of intracellular components, but that rather aim to characterize the morphodynamics of individual cells or the aggregate migratory behavior of groups of cells. For the study of dynamic processes within a living cell, it is necessary to specifically label the intracellular objects of interest. This has become possible at large by the discovery (1962), gene sequencing and cloning (1992), and expression (1994) of the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* [5]. GFP-labeling enabled the visualization of very specific targets within living cells and opened the door to studying the location and function of intracellular components with unprecedented sensitivity and specificity. This caused a true paradigm shift in biological experimentation [6] to the extent that the inventors of the technique, Osamu Shimomura, Martin Chalfie, and Roger Tsien, were awarded the Nobel Prize in chemistry in 2008. During the 1990s and 2000s, many derivatives of GFP were developed with their own characteristic excitation and emission spectra, which further extended the toolbox of fluorescent labeling [5].

Much of the research in optical microscopy imaging in the past two decades has focused on the development of strategies to break the Abbe resolution limit and achieve "superresolution microscopy." These techniques yield images with a level of detail close to the intrinsic scale of molecular biology. It is especially this endeavor that has led to major new challenges for the signal and image processing community. The most prominent recent examples of microscopy imaging techniques that rely heavily on image processing are photoactivated localization microscopy (PALM) [7] and the related technique of stochastic optical reconstruction microscopy [8]. These exploit the long-known fact that, even though the image of a subresolution particle is diffraction-limited (on the order of hundreds of nanometers), its location can be estimated with much higher accuracy (on the order of nanometers), depending on the signal-to-noise-ratio (SNR) [9]. Instead of acquiring a single image with all labels fluorescing concurrently, by using fluorescent proteins that can be switched on and off, thousands of images of well-separated particles can be acquired and their locations estimated very accurately by particle detection and fitting techniques. The composite image built up from the detections displays very high resolution. An alternative way to acquire better localized images is stimulated emission depletion [10], a nonlinear imaging technique that uses controlled de-excitation of previously excited off-center fluorophores. Another important technique is structured illumination microscopy [11], which computationally combines the images of differently oriented illumination patterns that produce Moiré fringes in the emission, resulting in an image with double resolution in each dimension. Complementary to these developments, improved techniques for imaging intact whole organisms have also been developed in recent years. Selective-plane illumination microscopy (SPIM), for example, in which the specimen is illuminated with a thin sheet of light perpendicular to the direction of observation, has proven to be an extremely valuable technique for long-time observation of embryonic development [12].

UNDERSTANDING THE CELL IN ITS SPATIOTEMPORAL CONTEXT IS KEY TO EXPLAINING MANY OF THE MECHANISMS OF LIFE AND DISEASE.

These and related advanced microscopy imaging techniques have enabled biologists to study the complexity of subresolution

intracellular organelles and the relation of their constituting components down to the molecular level and under physiological conditions in single cells or even developing organisms [13]–[15]. Technical improvements of these fluorescence-based imaging modalities, for instance, taking advantage of the sparsity of the signal being detected [16], go hand-in-hand with new versions of switchable fluorophores that allow nonlinear optical effects to be more efficiently used to push the resolution limit down further. The trends indicate that as imaging techniques become more and more sophisticated, requiring multiple pieces of information to be combined to reconstruct the full image, there is an increasing need for computationally efficient signal and image processing algorithms.

ANALYZING CELL BEHAVIOR

The extraction of biologically relevant information from both classical and novel microscopy imaging modalities requires the use of advanced image processing methods. Here, we will focus on techniques for segmenting and tracking cells and intracellular particles and for estimating cell-matrix tensional forces.

CELL SEGMENTATION AND TRACKING

Accurately defining the boundaries of cells in both static and dynamic images is a classical problem. It has been addressed over the years using a variety of segmentation methods [17]. Traditionally, cell segmentation in high-resolution fluorescence microscopy has been used to establish a spatial reference framework for the quantification of molecular or genetic events inside the cell. Alternatively, low-resolution cell segmentation and tracking, both in fluorescence and brightfield two-dimensional (2-D) microscopy has been applied to study the dynamics of cell populations with an emphasis more on the detection of population changes (e.g., cell mitoses, deaths, fusions) and motility (e.g., organ development, wound healing) than in accurately delineating cell morphology changes. More recently, a growing interest in the mechanobiology of the cell has brought back the focus of the segmentation and tracking field to the accurate delineation of cellular morphology and the changes associated to cell movements on flat substrates, and more recently, in 3-D environments.

There are two main approaches to the problem of cell tracking: tracking by detection and tracking by model evolution. The first approach consists of independently segmenting the cells in all the frames of a video and then, using association methods, tracking each segmented cell in all the video frames. The second approach uses evolution of curves or surfaces, either implicitly or explicitly defined, to track the boundaries of the cells along the entire length of the video. The first approach is more suitable for situations of low spatiotemporal resolution—high cell density, large time step—while the second performs best in high spatiotemporal resolution settings where high segmentation accuracy is required and there are few topological changes. Both paradigms can be enhanced by introducing knowledge of the topology changes (e.g., mitosis, apoptosis, fusion) into the data association (tracking by detection) or evolution (tracking by model evolution) phase of the algorithm.

The most recent tracking by detection methods use relatively simple segmentation approaches, such as wavelet decomposition [18], seeded watersheds [19], [20] or thresholding techniques [21], while investing their efforts in sophisticated association methods, such as minimum-coupled cost flow [18], dynamic programming [19], integer programming [22], or multiple-hypothesis [23] tracking. Some of these association methods implicitly incorporate the detection of topological changes [18], [19] while others include preprocessing detection of mitosis [20] or apoptosis to account for them. The state of the art of the tracking by model evolution paradigm uses the evolution of implicit contours (i.e., level sets) [21], [24] to segment and track individual cells. The principal limitation of these methods is the high computational cost involved in evolving one level set function per cell, by finding the numerical solution of its associated partial differential equation (PDE). To address this point, reducing the computational cost, Dufour et al. [25] use a discrete–parametric-active mesh framework and Maska et al. [26] minimize the original Chan–Vese model without solving any PDE, while evolving one single level set function per frame. Finally, there are complex methods that combine these two paradigms by using a fast level set framework combined with local spatiotemporal association [27].

CHARACTERIZING HOW THE CELL SENSES, REACTS TO, AND PRODUCES REGULATORY SIGNALS IS A DAUNTING TASK THAT REQUIRES THE INTEGRATION OF MORPHOLOGICAL, BIOCHEMICAL, MECHANICAL, AND ELECTRICAL CLUES.

A recurrent problem of the field was the lack of common test data sets and metrics to evaluate the performance of novel and existing algorithms. This prevented a fair and objective evaluation of the segmentation methods leaving the user (normally a noncomputer-proficient biologist) with the decision of choosing between the existing methods, with only the help of complex technical descriptions. In addition, not all published tracking algorithms are publicly available, or they have been released in a format that requires important computer and programming skills. To address this relevant issue, a benchmark for objective evaluation of cell tracking algorithms was recently established [28]. The challenge provides annotated data sets composed of both 2-D and 3-D video microscopy modalities (PC, DIC, fluorescence, confocal), nuclear and cytoplasmic staining, and various cell densities and microscopy resolutions (from high-throughput to high-resolution situations). Realistic simulations of nuclearly stained cells are also provided, for which there is an absolute, unbiased ground truth. The metrics used to compare the algorithms take into account both the accuracy of the segmentation and the accuracy of the tracking (movement and lineage) of the cells.

The outcome of the challenge revealed that the problem of segmenting and tracking cells in microscopy is far from being solved,

especially in the case of cytoplasmic labeled cells or in high-throughput setups (low spatiotemporal resolution and low SNR). In addition, more work needs to be done to segment cells in nonfluorescent microscopy modalities (PC, DIC) where the cellular boundaries present complex gradient patterns or extended artifacts, as well as in novel microscopy techniques such as SPIM.

PARTICLE DETECTION AND TRACKING

Quantitative analysis of healthy cell behavior and how various diseases may alter it often requires the analysis of intracellular dynamic processes. Examples include the motion of proteins or lipids on the cell membrane in relation to cell adhesion and regulation, the dynamics of cytoskeletal filaments involved in cell maintenance and intracellular transport, the interaction of virus particles with the cell machinery, and the intricate molecular processes involved in genome maintenance. Typically, these processes require very large numbers of "particles" (molecules, macromolecular complexes, organelles). In biological experiments, several hundreds to thousands of them are imaged at the same time to allow studying both the characteristic behavior and interaction of individual particles as well as aggregate behavior. Since manual annotation of the image data is infeasible, in addition to being inaccurate, this calls for advanced methods for automatic particle detection and tracking [23], [29]–[33].

QUANTITATIVE ANALYSIS OF HEALTHY CELL BEHAVIOR AND HOW VARIOUS DISEASES MAY ALTER IT OFTEN REQUIRES THE ANALYSIS OF INTRACELLULAR DYNAMIC PROCESSES.

Similar to methods for the analysis of cell dynamic behavior described in the previous section, methods for the tracking of intracellular particles in an image sequence usually consist of two fundamental stages [34]: 1) particle identification within individual image frames and 2) particle association from frame to frame to build trajectories. The goal of the first stage is to distinguish between local image intensity patterns that truly represent particles of interest versus irrelevant image structures and background. Commonly used image analysis methods for this purpose range from simple intensity thresholding, to more advanced linear filtering (in particular, Gaussian and its derivatives) and nonlinear wavelet-based or morphological image processing approaches. After detection, representative coordinates of the underlying particle within its corresponding local image patch are typically estimated by computing the intensity center of mass, by finding the local maximum, or by fitting a theoretical or experimentally obtained intensity model. In the case of spatially well-separated subresolution particles, the ideal model is the point-spread function of the microscope used, which in the case of both widefield and confocal fluorescence microscopy, can be well approximated by a Gaussian [35]. The localization problem is strongly linked to superresolution recovery (SRR) of the underlying true signal. While solid mathematical theory for SRR is now emerging [36], the development of computationally robust and efficient recovery algorithms remains a challenge, especially for multiparticle-tracking applications, where the data usually contains large numbers of (possibly overlapping) diffraction-limited spots drowning in very high levels of Poisson noise. The use of compressed sensing approaches has recently shown promising results in this area [16] and may be further improved by accurate statistical models.

The goal of the second stage in the particle-tracking process is to establish the best possible association of detected particles between image frames. Depending on the density of the particles within the field of view, and whether or not prior knowledge about their dynamic behavior is available, commonly used methods for this purpose range from simple nearest-neighbor linking (connecting each particle in a given frame with the spatially nearest particle in the next frame of the sequence), to more advanced multiframe association schemes, including multiple hypothesis tracking, dynamic programming, and various combinatorial approaches. The use of a motion model is often implemented in the form of Kalman filtering or in the case of nonlinear and non-Gaussian tracking problems, by means of sequential Monte Carlo estimation methods (often confusingly referred to as *particle filtering*). These can be made even more sophisticated by the use of interacting multiple motion models. However, the rise of high-density particle-tracking applications [37] is challenging currently existing methods, increasing the need for dealing with ever-larger amounts of imperfect data. As popular detection and localization methods yield optimal precision and accuracy only in circumstances that are rarely achieved in particle-tracking experiments [38], improved performance can be expected from novel methods that more intimately link the detection, localization, and association aspects of the tracking problem.

Since the early 1990s, many particle-tracking methods have been published based on the mentioned principles. With the increasing encouragement in the field to promote reproducible research, several dozens of software tools implementing these methods have been released [34]. To gain insight into their relative performance in an objective and reproducible manner, an open competition was recently organized [39]. One important finding is that, despite the often-heard claim when a new method is presented in the literature that it beats previous methods, as yet there exists no such thing as a single universal particle-tracking method that works best for all biological experiments. However, overall, certain methods do perform considerably better than others. A shared feature of superiorly performing methods is that they make optimal use of prior knowledge about both the objects of interest and the imaging process, re-emphasizing the importance of domain modeling. Another important finding is that current particle-tracking methods still tend to break down at SNRs representative of typical live-cell fluorescence microscopy imaging experiments. Although the SNR can be easily improved by increasing the illumination level, this has detrimental effects to the cell (photodamage and/or phototoxicity). Thus, in current practice, a careful selection of imaging conditions and analysis methods remains essential.

MEASUREMENT OF FORCES

Mechanobiology is an emerging field at the interface of biology and engineering. It focuses on the processes by which physical forces and cell or tissue mechanics contribute to development, normal physiology and disease. In the early 1920s, Buckminster Fuller first proposed the principle of tensegrity within the realm of architectural design. Tensegrity refers to the stability of a 3-D structure, granted by the opposing equilibrium between a discontinuous set of rigid compression elements and a continuous stabilizing tensile force. It was not until the mid-1970s that the (at that time) Yale undergraduate and current Harvard professor, Donald Ingber, related the behavior of a simple tensegrity stick-and-string model (flat when attached to a flat surface, abruptly becoming rounded when being detached from the flat surface) to the behavior of the cells that he had seen in culture at that semester's cancer lab. Ingber thought that cells might use their recently discovered internal framework, the cytoskeleton, to control their shape, much like a tensegrity structure does by means of a set of compressing elements and force distributing tensile elements [40]. Ingber further reasoned that cells must use their substrate, the extracellular matrix, to anchor themselves. Later on, he proved that mechanical forces exerted at the surface of the cell can be transmitted to the nucleus, resulting in biochemical changes and ultimately genetic changes—causing genes to turn on and off [41].

Understanding the molecular mechanism by which cells sense and respond to physical forces is a major challenge in this field. Traction force microscopy (TFM), a light microscopy technique developed in the mid-1990s [42], can compute traction forces exerted by a cell onto a biomimetic hydrogel substrate. These traction forces are calculated from the displacement of a large number of fluorescent beads embedded in the hydrogel, which in turn can be seen as samples of the deformation field that the forces cause in the substrate. Finally, the traction forces generating the deformations are inferred by direct or inverse methods that work from the expression of the laws of the elasticity of materials. Most of the existing, simplified methods compute the forces exerted on a plane by cells lying flat on a 2-D surface. Legant et al. [43], in a recent breakthrough, estimated traction forces exerted by cells fully encapsulated in a 3-D polymer gel. Using this physiologically relevant model, they discovered that the cells sensed the surrounding gel pulling strongly inward through traction anchors located near the tip of long, thin protruding extensions.

The classical procedure used to recover the forces in 2-D TFM experiments is composed of two steps: first, the displacement of the microbeads is calculated using particle-imaging velocimetry (PIV). Then, the stress field is obtained by considering the substrate as a linear and elastic half-space. The Boussinesq solution of the Green tensor is then computed using Fourier transform traction cytometry (FTTC) [44]. Legant et al. [43] relaxed the half-space constraint and solved the inverse problem within a 3-D geometry using the finite element method (FEM). More recently, the constraint on the linear behavior of the gel has been eliminated by combining multiple nonlinear FEM solutions, thus resulting in higher accuracy in the estimation of the forces [45].

It is clear that there is an urgent need to develop and integrate more efficient, precise, and robust computational methods. In particular, we believe that signal processing could greatly contribute to the technique with 1) robust and accurate cell segmentation algorithms as the ones described in the section "Cell Segmentation and Tracking," 2) sophisticated microbead displacement estimation methods such at the ones described in the section "Particle Detection and Tracking," and 3) fast and robust solutions for the ill-posed problem of recovering the forces (i.e., sparse tensor regularization, sparse reconstruction).

While TFM is well suited for the study of mechanotransduction at the cellular scale, particle-tracking microrheology [46] applies similar approaches to study mechanics at an intracellular scale. In particular, it enables measuring the local viscoelastic properties of the cytoplasm with high spatiotemporal resolution (i.e., nanoscale in seconds intervals). To this end, submicron particles are ballistically injected into the cytoplasm of live cells. After injection, the beads disperse rapidly within the cytoplasm, while being imaged using high-magnification fluorescence microscopy. The random spontaneous movement of the beads is tracked using particle-tracking methods. The trajectories of the cytoplasm-embedded particles are used to compute mean-squared displacements (MSDs). Finally, the time lag-dependent MSDs of the beads are transformed into local estimations of frequency-dependent viscoelastic moduli or the time-dependent creep compliance (deformability) of the cytoplasm. The ongoing efforts to extend the method to 3-D go hand-in-hand with the developments in superresolution microscopy and particle-tracking methods for high-density and low SNR conditions.

MODELING CELL BEHAVIOR

Ultimately, the information obtained using the quantification methods detailed in the section "Analyzing Cell Behavior" should be used to elaborate spatiotemporal models of cell appearance and behavior. The models should not only fit the available data but also lead to new hypotheses that can subsequently be verified experimentally. The truth is that there still remains a long way before a morphodynamic model capturing all the cell's complexity becomes available.

Approaches to modeling cell behavior can be divided into two main categories—top-down and bottom-up—that are being developed independently. Top-down approaches rely primarily on image data starting at the cell level and going down to imaging selected subcellular components trying to infer rules of cell morphology and behavior hidden inside. On the other hand, bottom-up approaches rely primarily on nonimage data (such as bioinformatics databases, signaling pathways, gene expression data, genome sequencing data, measurements of forces, etc.) starting at the level of very basic partial rules of cell behavior and going up to defining more complex rules for specific cellular processes or behavior of small cellular components ultimately leading to the model of how the whole cell works.

TOP-DOWN APPROACHES

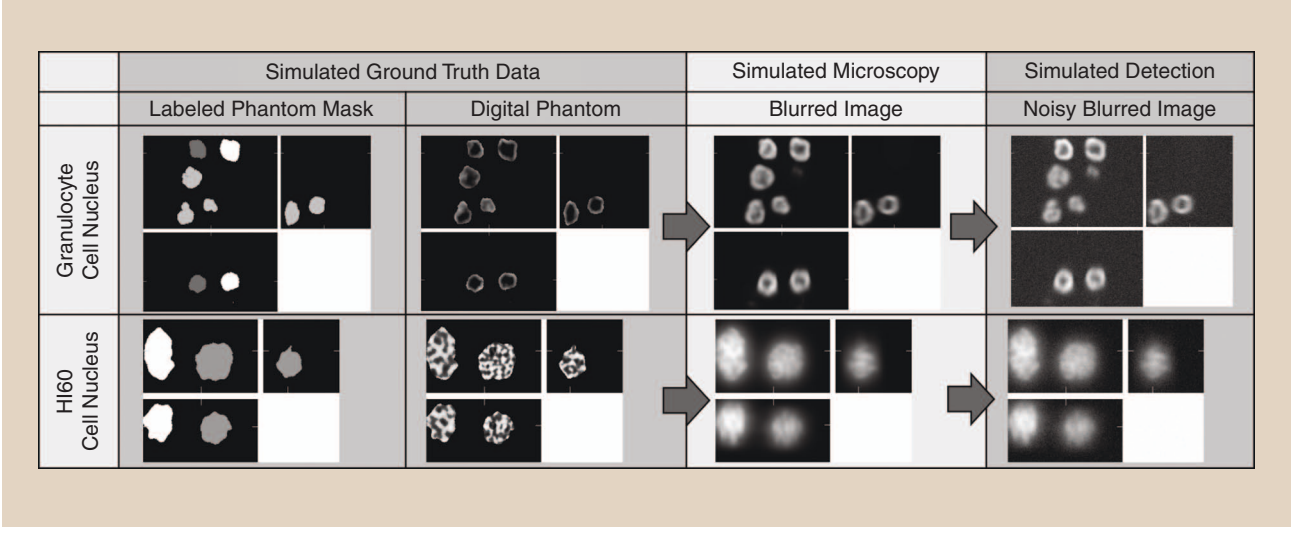
The first models of the cell date back to the 19th century [47], being just rough descriptions based on the limited unspecific observation provided by the microscopes of the time. After oil-immersion lenses became available in the 1870s, people could observe the structure of the membrane, nucleus and cytoplasm (at the time called *protoplasm*). For example, in 1885, C. Rabl [48] published his famous model of the nucleus consisting of nucleoli and chromatin formed by chromosomes. The model even illustrated chromosome behavior during mitosis.

Computers enabled a gradual transition from those original descriptive models based on rather vague verbal explanations of cell components accompanied by drawings to more precisely formulated mathematical models. The earliest mathematical tool to describe cell behavior (interaction of neighboring cells) was the cellular automaton, which became famous in 1970 thanks to John Conway's "Game of Life."

By the end of the 20th century, it became popular to represent cells or cell nuclei using simple mathematical shapes (spheres, ellipsoids, discs, or rods) and create virtual microscopy images that could be used as digital phantoms. These were dedicated to test the limits of image segmentation algorithms to different noise levels, blur degradations, or phantom densities. These digital phantoms enable the comparison of the algorithm results with a known ground truth [28]. Later on, more sophisticated artificial objects were developed: shapes were modeled as randomly deformed spheres or ellipsoids, and texture was added to simulate staining of cell or cell nucleus. There is an abundant work aimed at describing and classifying both subcellular structures and whole cells based on the analysis of protein distribution (i.e., image texture) and morphological descriptors of the cell. This information can be readily incorporated to the digital descriptions of the cell. An excellent review of these machine-learning approaches both from a theoretical and practical point view has been recently presented by Conrad and Gerlich [49].

The virtual microscopy "observation" of these artificial cells also improved: Gaussian blurring was replaced by the convolution with a real point spread function, more noise types were considered (Poisson, Gaussian, dark current, fixed pattern) and imaging artifacts introduced (uneven illumination, depth-related aberrations in 3-D samples, etc.). For a particular cell type, virtual microscopy images can be made almost indistinguishable from reality (not only visually but also based on computed image characteristics) [50]. See Figure 1 for an example of digital phantoms and corresponding virtual microscopy images.

Learning-based cell modeling is another modality that infers algorithmic parameters from training image data by employing supervised or nonsupervised machine-learning techniques [51]. Parameters need not to be just single values but may be also expressed as probability density functions. Learning techniques build either discriminative models dedicated to object classification (of unknown test data) or generative models able to synthetize new artificial images belonging to a given class. Learning-based approaches can be used not only for testing image analysis algorithms but also to characterize the differences between healthy and pathological cells or for structure-function relationship studies.



[FIG1] Modeling cells and their components using 3-D digital phantoms. First, a digital phantom is created for each modeled biological object in the field of view as a synthetic solid object of a precisely defined shape filled with a certain texture. The shape defines the binary mask serving as ground truth segmentation result and the image with texture serves as the input for virtual (simulated) microscopy. The image of the phantom produced by the optical system of a virtual microscope is typically generated by adding blur using convolution with a suitable point spread function. The blurred image is further subject to virtual image detection by adding adequate noise of various types. The noisy blurred images must be indistinguishable from real images of modeled biological objects and can serve for testing performance of image analysis algorithms against known ground truth. Examples of 3-D digital phantoms for two types of cell nuclei are shown. Each 3-D image is shown as a triplet of three mutually orthogonal cuts through the object: xy view (upper left), xz view (bottom), and yz view (right).

Another extended approach is to create a model of a specific cell segmented in a particular image (more precisely a model of its stained components visible in the image data). This approach lacks generalization but can help revealing hidden properties using simulations. For instance, to measure the diffusion coefficient in fluorescence recovery after photobleaching (FRAP), diffusion is simulated on the segmentation-based model [52]. This approximation to the study of biological systems and the learning-based approach presented above are sometimes denoted as image-based systems biology to distinguish them from modeling in computational biology where image data is not considered.

Several software tools for modeling cells and their components have been made publicly available: SIMCEP (2-D digital phantoms), available at http://www.cs.tut.fi/sgn/csb/simcep/; CytoPacq (3-D digital phantoms), available at http://cbia.fi.muni.cz/simulator/; or CellOrganizer (learning-based models), which can be found at http://cellorganizer.org. Moreover, one can use pregenerated benchmark data sets offered on the Web pages of these software packages. The synthetic cell images are still available only for just a few cell types and several cell components but can be generated in large quantities with different levels of noise, various cell densities, and are accompanied by ground truth data. Lately, also time-lapse sequences of such synthetic image data have become available and have been used, for example, in the Cell Tracking Challenge, available at http://www.codesolorzano.com/celltrackingchallenge/.

BOTTOM-UP APPROACHES

Parallel to these black box-modeling efforts, there are attempts to mathematically model the intricacies of the signaling pathways that govern the cellular function of the cell. These models, if properly populated with a complete list of substances (e.g., genes, ribonucleic acids (RNAs), proteins), rules (e.g., transformation of molecular species, reaction kinetics) and cellular spatial or functional compartments, can simulate the molecular machinery of the cell. E-CELL, developed by Tomita et al. [53] is a software environment that simulates the behavior of a cell from the activity of gene sets derived from entire genomes. As a proof of principle, the authors presented a model of a minimal cell based on a subset of genes of *Mycoplasma genitalium*, whose complete 580-kbit genome was sequenced in 1995. This simplified model simulates how proteins interact within the living cell. Specifically, it models how changes in the amount of a protein (by knocking out the corresponding gene or altering its expression level), or the medium (e.g., starving the cell by removing glucose) may affect its behavior (e.g., mitotic rate, probability of entering in apoptosis) and its survival.

These mathematical models are becoming increasingly complex to account for higher organisms and more temporal scales. Simultaneously, computational models [54] are being developed which, instead of representing cell processes with equations, present recipes (algorithms) that mimic natural phenomena. Instead of searching for a mathematical solution to a complicated list of equations, it provides algorithms that steer into different states or configurations of a cell. The rules of navigation are operational, hence the name *executable biology*. In both types of models (mathematical and executable biology), there is a close connection with the experimental image-based data that feeds the models and are used to validate, and when necessary, update them.

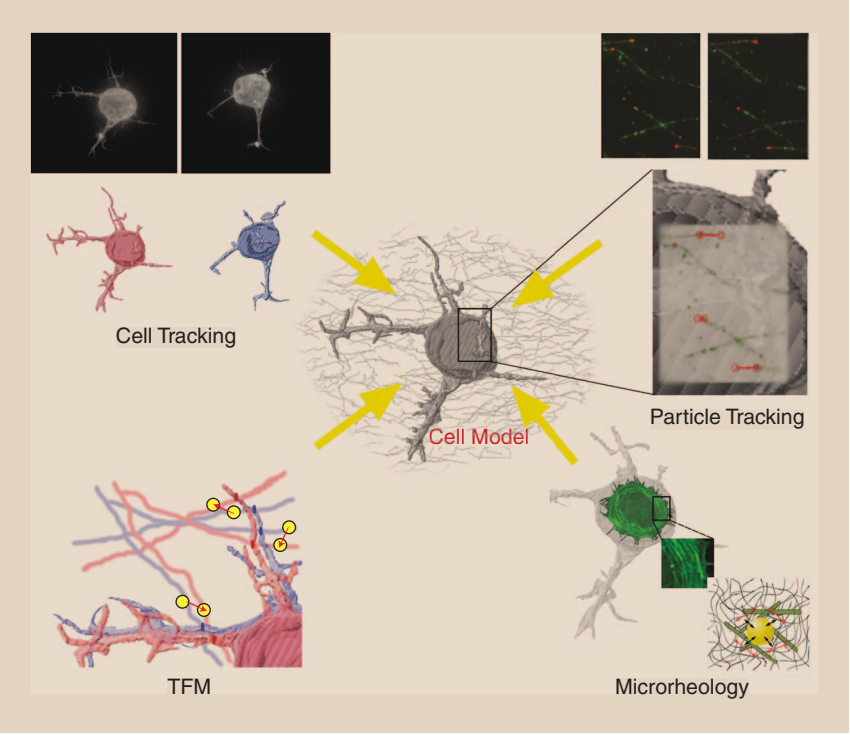
THE ADVENT OF NOVEL IMAGING TECHNIQUES, COUPLED WITH THE USE OF ADVANCED COMPUTATIONAL AND SIGNAL PROCESSING METHODS, HAS OPENED THE DOOR TO UNDERSTANDING MANY CRUCIAL ASPECTS OF THE CELL.

Finally, the recent developments in the field of mechanobiology allow integrating morphological and molecular aspects with the mechanical interactions between the cell and its environment, thus creating mechanical models of the cell. These efforts are fed with information about morphological changes (see the section "Cell Segmentation and Tracking"), traction force and viscosity data (obtained using among others, some of the tools described in the section "Measurement of Forces"), coupled to the trafficking of mechanosensitive and mechanotransductive biomolecules (see the section "Particle Detection and Tracking"). Most of these methods have been used to model cell motility, mostly of cells crawling on a surface [55], from the treadmill of actin that implies persistent front-to-back asymmetry, through a synchronized assembly–disassembly directional process. These models explain the formation and release of lamellas and protrusions, as well as the adhesion-mediated contraction that facilitates the push-and-pull mechanism required for the cell to move on its substrate. Other models focus on mechanosensing in general [56], and on how the mechanical properties of the cell, defined by the composition and structure of its cytoskeleton self-adjust as a reaction to the mechanical properties of the extracellular environment.

FUSION OF AVAILABLE KNOW-HOW

Top-down and bottom-up approaches are complementary to each other. As the coverage of the former ones goes down the scale and deeper into the cell (thanks to the development of imaging techniques enabling observations of subresolution targets with increasing spatial as well as temporal resolution) while the coverage of the latter ones goes up the scale (due to the advances in molecular biology, cellular biochemistry, or the development of high-throughput screening methods), they tend to meet and cover certain cell components or events both from the rules side and from the imaging side. For example, within the MitoCheck project (http://www.mitocheck.org/), systematic analysis of genes and proteins that are required for chromosome segregation and cell division in human cells was performed by inactivating all 22,000 human genes one by one in cultured human cells using RNA interference (RNAi) and recording cellular phenotypes by high-throughput live-cell imaging.

Besides advances in imaging and biotechniques, advances in computer vision and artificial intelligence may also help integrating all pieces of know-how together into a single model of the cell. Besides the ability of learning from image data (as in the case of learning-based modeling methods), they can offer multimodal fusion of knowledge from different sources [57] independent of the application—the data can be related to studying human appearance and behavior in a video sequence as well as studying cell appearance and behavior in a time-lapse series. Multimodal fusion techniques can help integrate pieces of information obtained from different sources depicted in Figure 2, which could be the key for defining an accurate cell model.



[FIG2] A summary of image analysis techniques described in the article. From the top left, counterclockwise: Cell tracking provides dynamic information about morphological changes of a moving cell; TFM calculates tensional forces between a cell and its surrounding environment from the displacement (red arrows) of fluorescence beads (yellow dots) embedded in the extracellular substrate; microrehology informs about the viscoelastic properties of the intracellular space from the microscopic movements (black arrows) of ballistically injected fluorescent nanobeads (yellow dot) under the stress of fibers (green lines), which can be represented as soft and stiff spring series (read arrows); and particle-tracking algorithms provide information about the movement (red arrows) and trafficking of subcellular elements (red dots).

DISCUSSION AND PERSPECTIVE

The cell is an extremely complex machine. Its changing morphology and its dynamic spatial relationship with the surrounding environment depend on the biochemical composition of the latter, internal and external mechanical stresses, electrical signals, gravity, etc. Furthermore, the cell's metabolic production (i.e., its phenotype) is regulated by genetic and epigenetic factors that depend and have an impact on its morphology. Consequently, a faithful model of the cell should take into account the interplay of all these factors in their precise spatiotemporal context.

Needless to say, such a model cannot be based on static observation of the cell, much like the complexity of the universe cannot be explained based on a single snapshot of the skies. The cell is in a particular environment, and the cell is in a precise developmental stage. That explains why the exact genetic content gives rise to such a diverse display of cell types and phenotypes that coexist in a living organism. Therefore, a model of the cell should integrate all the factors involved (e.g., genes, RNAs, molecular/metabolic signaling pathways, structural elements of the cell nucleus and cytoskeleton, forces, biochemical factors) in its precise time and location. Light microscopy, as described in this article, provides visually quantifiable information that feeds into these models. Figure 2 presents a graphic summary of how quantitative image analysis provides information about the cell and its dynamic processes.

Simultaneously visualizing and quantifying all these internal and external players during the entire life of a cell, within its native tissue context is beyond all possible imagination, due to technical and physical limitations. However, the advent of novel imaging techniques, coupled with the use of advanced computational and signal processing methods has opened the door to understanding certain aspects of the cell that can be used to populate a computational model. In this article, we have reviewed the history and the state of the art of both hardware and software that are contributing to this enterprise.

In the hardware arena, the existing microscopy techniques can capture a few events in a relatively limited spatiotemporal framework, mostly in 2-D in vitro setups. The use of synthetic hydrogels of controlled biomechanical properties has recently facilitated the study of cells in more realistic 3-D environments, thus taking full advantage of the sectioning and 3-D imaging capabilities of the diverse flavors of confocal and multidimensional microscopy. Simultaneously, two complementary technological efforts are being pursued. In particular, the development of novel superresolution microscopy methods (especially, those that may work in 3-D and time lapse) and the development of large-scale, whole organ, or whole animal imaging systems, where resolution is sacrificed for the benefit of spatial completeness. Both efforts require significant input from the signal processing community in the areas of efficient data sampling, single-molecule detection, fast sparse-image reconstruction, compression, and data handling.

Software development necessarily follows the advances in image acquisition, thus leading the way to novel 3-D particle and

cell tracking routines. The existence of high-sensitivity sensors has placed the emphasis on the need for fast, optimized tracking methods, while the increasing use of high-throughput systems for big-data analysis pushes toward the development of very robust segmentation and tracking algorithms that may work in low SNR situations. Similarly, the incipient field of mechanotransduction demands novel, more efficient methods for the calculation of cell traction forces, especially in 3-D environments.

Finally, partial, in silico models of the cell, based on simple genetic and molecular approximations are already available. Those are being complemented with morphomechanistic models of cell behavior as it is visualized using an optical microscope of tunable properties. Mechanical models that incorporate the role of forces, and viscoelastic properties in the homeostasis and dynamics of cells are also being developed. These models are far from being complete, and work only as partial descriptions of some cellular processes during limited temporal steps. Furthermore, it remains to be defined how the models of the cells will be incorporated into similarly complex models of both subcellular–molecular models and dynamic models of complete organs or even entire organisms. The tremendous challenge posed to the signal processing and modeling community is how to integrate all the information about the cell—biochemical, structural, and mechanical—into a single unifying, multiscale, and spatiotemporal model that may open the door to the explanation and engineering of life. This, far from being a science fiction exercise, is the goal of the field of synthetic biology. For instance, in what could be considered a breakthrough in the field, Annaluru et al. [58], have reported the synthesis of a functional 272,871–base pair designer eukaryotic chromosome, based on the 316,617–base pair native Saccharomyces cerevisiae chromosome III. The future is indeed here, since the descriptive models of the cell will provide mechanistic information eventually leading to the production of functional cells. The use of this artificial life, properly empowered by bioethical principles, may clear the way to a new era in the field of tissue engineering and regenerative medicine.

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REFERENCES

[1] K. Hiroaki, "Systems biology: A brief overview," *Science*, vol. 295, no. 5560, pp. 1662–1664, 2002.

[2] D. Noble, "Modeling the heart—From genes to cells to the whole organ," *Science*, vol. 295, no. 5560, pp. 1678–1682, 2012.

[3] Y. Setty, "Multi-scale computational modeling of developmental biology," *Bioinformatics*, vol. 28, no. 25, pp. 2022–2028, 2012.

[4] M. W. Davidson and M. Abramowitz. (1999). Optical microscopy. Online technical note. [Online]. Available: http://www.olympusmicro.com/primer/opticalmicroscopy.html

[5] B. N. G. Giepmans, S. R. Adams, M. H. Ellisman, and R. Y. Tsien, "The fluorescent toolbox for assessing protein location and function," *Science*, vol. 312, no. 5771, pp. 217–224, 2006.

[6] C. Vonesch, F. Aguet, J.-L. Vonesch, and M. Unser, "The colored revolution of bioimaging," *IEEE Signal Processing Mag.*, vol. 23, no. 3, pp. 20–31, 2006.

[7] E. Betzig, G. H. Patterson, R. Sougrat, O. W. Lindwasser, S. Olenych, J. S. Bonifacino, M. W. Davidson, J. Lippincott-Schwartz, and H. F. Hess, "Imaging intracellular fluorescent proteins at nanometer resolution," *Science*, vol. 313, no. 5793, pp. 1642–1654, 2006.

[8] B. Huang, W. Wang, M. Bates, and X. Zhuang, "Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy," *Science*, vol. 319, no. 5864, pp. 810–813, 2008.

[9] S. Ram, E. S. Ward, and R. J. Ober, "Beyond Rayleigh's criterion: A resolution measure with application to single-molecule microscopy," *Proc. Natl. Acad. Sci. U.S.A.*, vol. 103, no. 12, pp. 4457–4462, 2006.

[10] S. W. Hell and J. Wichmann, "Breaking the diffraction resolution limit by stimulated emission: Stimulated-emission-depletion fluorescence microscopy," *Opt. Lett.*, vol. 19, no. 11, pp. 780–782, 1994.

[11] M. G. L. Gustafsson, L. Shao, P. M. Carlton, C. J. R. Wang, I. N. Golubovskaya, W. Z. Cande, D. A. Agard, and J. W. Sedat, "Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination," *Biophys. J.*, vol. 94, no. 12, pp. 4957–4970, 2008.

[12] J. Huisken and D. Y. R. Stainier, "Selective plane illumination microscopy techniques in developmental biology," *Development*, vol. 136, no. 12, pp. 1963–1975, June 2009.

[13] M. Lakadamyali, "Super-resolution microscopy: Going live and going fast," *ChemPhysChem*, vol. 15, no. 4, pp. 630–636, 2014.

[14] P. Dedecker, G. C. Mo, T. Dertinger, and J. Zhang, "Widely accessible method for superresolution fluorescence imaging of living systems," *Proc. Natl. Acad. Sci. U.S.A.*, vol. 109, no. 27, pp. 10909–10914, 2012.

[15] S. Abrahamson, J. Chen, B. Hajj, S. Stallinga, A. Y. Katsov, J. Wisniewski, G. Mizuguchi, P. Soule, F. Mueller, C. Dugast Darzacq, C. Wu, C. I. Bargmann, D. A. Agard, M. Dahan, and M. G. Gustafsson, "Fast multicolor 3D imaging using aberration-corrected multifocus microscopy," *Nat. Methods*, vol. 10, no. 1, pp. 60–63, 2013.

[16] L. Zhu, W. Zhang, D. Elnatan, and B. Huang, "Faster STORM using compressed sensing," *Nat. Methods*, vol. 9, no. 7, pp. 721–723, 2012.

[17] E. Meijering, "Cell segmentation: 50 years down the road," *IEEE Signal Processing Mag.*, vol. 29, no. 5, pp. 140–145, 2012.

[18] D. Padfield, J. Rittscher, and B. Roysam, "Coupled minimum-cost flow cell tracking for high-throughput quantitative analysis," *Med. Image Anal.*, vol. 15, no. 4, pp. 650–668, 2011.

[19] K. E. G. Magnusson and J. Jaldén, "A batch algorithm using iterative application of the Viterbi algorithm to track cells and construct cell lineages," in *Proc. 9th IEEE Int. Symp. Biomedical Imaging (ISBI'12)*, Barcelona, Spain, 2012, pp. 382–385.

[20] N. Harder, F. Mora-Bermúdez, W. J. Godiznez, J. Ellenberg, R. Eils, and K. Rohr, "Automated analysis of the mitotic phases of human cells in 3-D fluorescence microscopy," in *Medical Image Computing and Computer-Assisted Intervention*. New York: Springer, 2006, pp. 840–848.

[21] A. Dufour, V. Shinin, S. Tajbakhsh, N. Guillén-Aghion, J. C. Olivo-Marin, and C. Zimmer, "Segmenting and tracking fluorescent cells in dynamic 3-D microscopy with coupled active surfaces," *IEEE Trans. Image Processing,* vol. 14, no. 9, pp. 1396–1410, 2005.

[22] F. Li, X. Zhou, J. Ma, and S. T. C. Wong, "Multiple nuclei tracking using integer programming for quantitative cancer cell cycle analysis," *IEEE Trans. Med. Imaging*, vol. 29, no. 1, pp. 96–105, Jan. 2010.

[23] N. Chenouard, I. Bloch, and J. C. Olivo-Marin, "Multiple hypothesis tracking for cluttered biological image sequences," *IEEE Trans. Pattern Anal. Mach. Intell.*, vol. 35. pp. 2736–2750, Nov. 2013.

[24] O. Dzyubachyk, W. A. van Cappellen, J. Essers, W. J. Niessen, and E. Meijering, "Advanced level-set based cell tracking in time-lapse fluorescence microscopy," *IEEE Trans. Med. Imaging*, vol. 29, no. 3, pp. 852–867, 2010.

[25] A. Dufour, R. Thibeaux, E. Labruyère, N. Guillén, and J. C. Olivo-Marin, "3-D active meshes: Fast discrete deformable models for cell tracking in 3-D time-lapse microscopy," *IEEE Trans. Image Processing*, vol. 20, no. 7, pp. 1925–1937, 2011.

[26] M. Maška, O. Danek, S. Garasa, A. Rouzaut, A. Muñoz-Barrutia, and C. Ortiz-de-Solorzano, "Segmentation and shape tracking of whole fluorescent cells based on the Chan-Vese model," *IEEE Trans. Med. Imaging*, vol. 32, no. 6, pp. 995–1006, 2013.

[27] K. Li, E. D. Miller, M. Chen, T. Kanade, L. E. Weiss, and P. G. Campbell, "Cell population tracking and lineage construction with spatiotemporal context," *Med. Image Anal.*, vol. 12, no. 5, pp. 546–566, 2008.

[28] M. Maska, et al., "A benchmark for comparison of cell tracking algorithms," *Bioinformatics*, vol. 30, no. 11, pp. 1609–1617, 2014.

[29] I. Smal, M. Loog, W. Niessen, and E. Meijering, "Quantitative comparison of spot detection methods in fluorescence microscopy," *IEEE Trans. Med. Imaging*, vol. 29, no. 2, pp. 282–301, 2010.

[30] A. Genovesio, T. Liedl, V. Emiliani, W. J. Parak, M. Coppey-Moisan, and J. C. Olivo-Marin, "Multiple particle tracking in 3-D+t microscopy: Methods and application to the tracking of endocytosed quantum dots," *IEEE Trans. Image Processing*, vol. 15, no. 5, pp. 1062–1070, 2006.

[31] I. Smal, K. Draegestein, N. Galjart, W. Niessen, and E. Meijering, "Particle filtering for multiple object tracking in dynamic fluorescence microscopy images: Application to microtubule growth analysis," *IEEE Trans. Med. Imaging*, vol. 27, no. 6, pp. 789–804, 2008.

[32] K. Jaqaman, D. Loerke, M. Mettlen, H. Kuwata, S. Grinstein, S. L. L. Schmid, and G. Danuser, "Robust single-particle tracking in live cell time-lapse sequences," *Nat. Methods*, vol. 5, no. 8, pp. 695–702, 2008.

[33] W. J. Godinez, M. Lampe, S. Wörz, B. Müller, R. Eils, and K. Rohr, "Deterministic and probabilistic approaches for tracking virus particles in time-lapse fluorescence microscopy image sequences," *Med. Image Anal.*, vol. 13, no. 2, pp. 325–342, 2009.

[34] E. Meijering, O. Dzyubachyk, and I. Smal, "In imaging and spectroscopy analysis of living cells," in *Methods Enzymol.*, vol. 504, P. M. Conn, Ed. New York: Elsevier, 2012, ch. 9, pp. 183–200.

[35] B. Zhang, J. Zerubia, and J.-C. Olivo-Marin, "Gaussian approximations of fluorescence microscope point-spread function models," *Appl. Opt.*, vol. 46, no. 10, pp. 1819–1829, 2007.

[36] E. J. Candès and C. Fernandez-Granda, "Towards a mathematical theory of super-resolution," *Commun. Pure Applied Math.*, vol. 67, no. 6, pp. 906–956, 2014.

[37] J.-B. Sibarita, "High-density single-particle tracking: Quantifying molecule organization and dynamics at the nanoscale," *Histochem. Cell Biol.*, vol. 141, no. 6, pp. 587–595, 2014.

[38] H. Deschout, F. C. Zanacchi, M. Mlodzianoski, A. Diaspro, J. Bewersdorf, S. T. Hess, and K. Braeckmans, "Precisely and accurately localizing single emitters in fluorescence microscopy," *Nat. Methods*, vol. 11, no. 3, pp. 253–266, 2014.

[39] N. Chenouard, et al., "Objective comparison of particle tracking methods," *Nat. Methods*, vol. 11, no. 3, pp. 281–289, 2014.

[40] D. E. Ingber, "Cellular tensegrity: Defining new rules of biological design that govern the cytoskeleton," *J. Cell Sci.*, vol. 104, no. 3, pp. 613–627, 1993.

[41] D. E. Ingber, "Cellular mechanotransduction: Putting all the pieces together again," *FASEB J.*, vol. 220, pp. 811–827, May 2006.

[42] T. Olivier, M. Dembo, and K. Jacobson, "Traction forces in locomoting cells," *Cell Motil. Cytoskeleton*, vol. 31, no. 3, pp. 225–240, 1995.

[43] R. Legant, J. S. Miller, B. L. Blakely, D. M. Cohen, G. M. Genin, and C. S. Chen, "Measurement of mechanical tractions exerted by cells in three-dimensional matrices," *Nat. Methods*, vol. 7, no. 12, pp. 969–971, 2010.

[44] J. Boussinesq, "Application des potentiels á l'étude de l'équlibre et du movement des solides élastiques," Gauthier-Villars, Paris, 1985.

[45] J. Palacio, A. Jorge-Peñas, A. Muñoz-Barrutia, C. Ortiz-de-Solorzano, E. de Juan-Pardo, and J. M. García-Aznar, "Numerical estimation of 3D mechanical forces exerted by cells," *J. Biomech.*, vol. 46, no. 1. pp. 50–55, 2013.

[46] Y. Tseng, T. P. Kole, and D. Wirtz, "Micromechanical mapping of live cells by multiple-particle-tracking micro-rheology," *Biophys. J.*, vol. 83, no. 6. pp. 3162–3176, 2002.

[47] P. Mazzarello, "A unifying concept: The history of cell theory," *Nat. Cell Biol.*, vol. 1, pp. E13–E15, May 1999.

[48] C. Rabl, "Über Zelltheilung," in *Morphologisches Jahrbuch*, vol. 10, C. Gegenbaur, Ed. Leipzig, Germany: Willhelm Engelmann, 1885, pp. 214–330.

[49] C. Conrad and D. W. Gerlich, "Automated microscopy for high-content RNAi screening," *J. Cell Biol.*, vol. 188, no. 4, pp. 453–461, 2010.

[50] D. Svoboda, M. Kozubek, and S. Stejskal, "Generation of digital phantoms of cell nuclei and simulation of image formation in 3D image cytometry," *Cytometry*, vol. 75A, no. 6, pp. 494–509, 2009.

[51] T. E. Buck, J. Li, G. K. Rohde, and R. F. Murphy, "Towards the virtual cell: Automated approaches to building models of subcellular organization 'learned' from microscopy images," *Bioessays*, vol. 34, no. 9, pp. 791–799, 2012.

[52] I. Sbalzarini, "Modeling and simulation of biological systems from image data," *Bioessays*, vol. 35, no. 5, pp. 482–490, 2013.

[53] M. Tomita, K. Hashimoto, K. Takahashi, T. S. Shimizu, Y. Matsuzaki, F. Miyoshi, K. Saito, S. Tanida, K. Yugi, J. C. Venter, and C. A. Hutchison III, "E-CELL: Software environment for whole-cell simulation," *Bioinformatics*, vol. 15, no. 1, pp. 72–84, 1999.

[54] J. Fisher and T. A. Henzinger, "Executable cell biology," *Nat. Biotechnol.*, vol. 25, no. 11, pp. 1239–1249, 2007.

[55] A. Mogilner, "Mathematics of cell motility: Have we got its number?" *J. Math. Biol.*, vol. 58, no. 1–2, pp. 105–134, 2009.

[56] C. Borau, T. Kim, T. Bidone, J. M. García-Aznar, and R. D. Kamm, "Dynamic mechanisms of cell rigidity sensing: Insights from a computational model of actomyosin networks," *PLoS ONE,* vol. 7, no. 11, p. e49174, 2012.

[57] P. K. Atrey, M. A. Hossain, A. El Saddik, and M. S. Kankanhalli, "Multimodal fusion for multimedia analysis: A survey," *Multimedia Syst.*, vol. 16, no. 6, pp. 345–379, 2010.

[58] N. Annaluru, et al., "Total synthesis of a functional designer eukaryotic chromosome," *Science,* vol. 44, no. 6179, pp. 55–58, 2014.