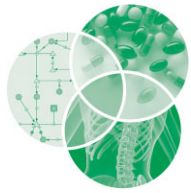
Advanced Review



Bioimage informatics for understanding spatiotemporal dynamics of cellular processes

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The inner environment of the cell is highly dynamic and heterogeneous yet exquisitely organized. Successful completion of cellular processes within this environment depends on the right molecules or molecular complexes to function at the right place at the right time. Understanding spatiotemporal behaviors of cellular processes is therefore essential to understanding their molecular mechanisms at the systems level. These behaviors are usually visualized and recorded using imaging techniques. However, to infer from them systems-level molecular mechanisms, computational analysis and understanding of recorded image data is crucial, not only for acquiring quantitative behavior measurements but also for comprehending complex interactions among the molecules or molecular complexes involved. The technology of computational analysis and understanding of biological images is often referred to simply as *bioimage informatics*. This article introduces fundamentals of bioimage informatics for understanding spatiotemporal dynamics of cellular processes and reviews recent advances on this topic. Basic bioimage informatics concepts and techniques for characterizing spatiotemporal cell dynamics are introduced first. Studies on specific cellular processes such as cell migration and signal transduction are then used as examples to analyze and summarize recent advances, with the focus on transforming quantitative measurements of spatiotemporal cellular behaviors into knowledge of underlying molecular mechanisms. Despite the advances made, substantial technological challenges remain, especially in representation of spatiotemporal cellular behaviors and inference of systems-level molecular mechanisms. These challenges are briefly discussed. Overall, understanding spatiotemporal cell dynamics will provide critical insights into how specific cellular processes as well as the entire inner cellular environment are dynamically organized and regulated.

How to cite this article:
WIREs Syst Biol Med 2013, 5:367–380. doi: 10.1002/wsbm.1214

INTRODUCTION

The inner environment of the cell is highly dynamic, heterogeneous, and crowded.[1,2] But overall an exquisitely organized architecture is maintained. Within this environment, successful completion of cellular processes ranging from signal transduction to cell division depends on the right molecules or molecular complexes to be at the right place at the right time to deliver their functions.[3,4] This is the case even for comparatively simple prokaryotic cells, which were assumed to have limited internal organization. As an example, extensive studies of *Caulobacter crescentus*, a bacterial species, have established that successful execution of its different cellular processes all depends on proper localization of specific proteins to specific sites.[5,6] Chemotaxis of this bacterium, for example, requires localization of chemoreceptors

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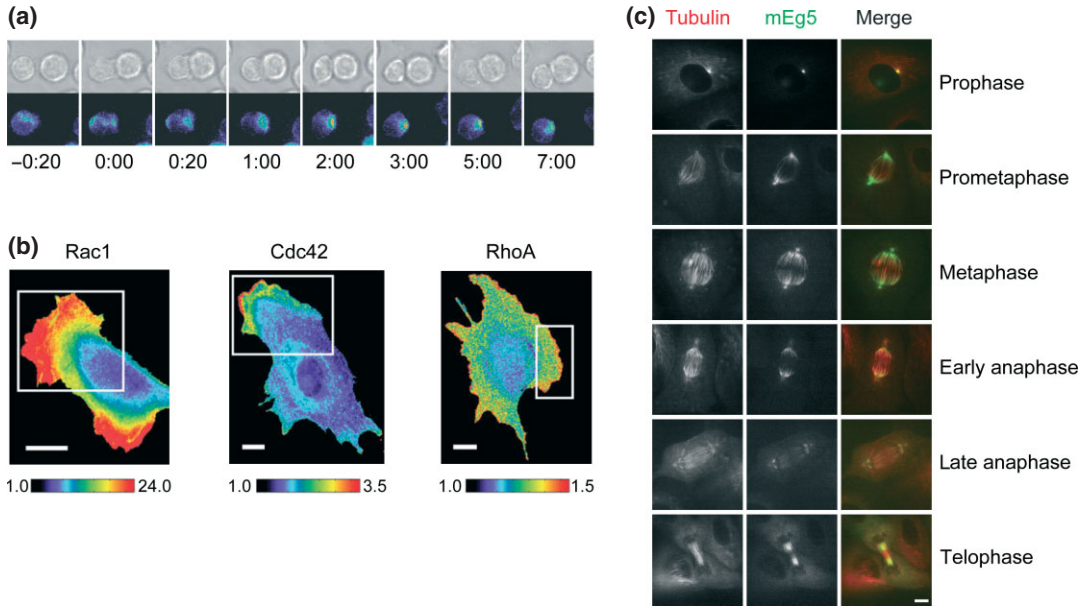

FIGURE 1 | Spatiotemporal dynamics patterns of different cellular processes. (a) Upper row: phase-contrast images of a T cell (left) activated by contact with an antigen-present cell (right). Lower row: activities of fluorescently labeled LAT (linker of activated T cells), one of the many signaling molecules involved in the activation of the T cell.[10] (Reprinted with permission from Ref 10. Copyright 2009 AAAS). (b) Activities of Rho GTPase Rac1, Cdc42, and RhoA visualized by fluorescence biosensors in protruding mouse embryonic fibroblasts. Activity levels are encoded in colors.[11] Scale bars: 20 μm. (Reprinted with permission from Ref 11. Copyright 2009 Nature Publishing Group). (c) Reorganization of mitotic motor Eg5 (green) relative to the spindle microtubule network (red) at different stages of mitosis of LLC-PK1 cells.[12] Scale bar: 10 μm. (Reprinted with permission from Ref 12. Copyright 2012 ASCB)

from the MCP (methyl-accepting chemotaxis proteins) superfamily to its flagellum-bearing pole,[6,7] whereas division of this bacterium requires localization of cytokinesis-associated proteins such as ZapA to its midsection FtsZ ring.[6] Importantly, localization of these proteins is not static but dynamically regulated in precise synchronization with the progression of their associated cellular processes. Dynamic localization of these proteins is often controlled by upstream signaling cues and accomplished through diffusion-and-capture.[6] Together, these studies in bacterial cells demonstrate that spatiotemporal cell dynamics is both an integral part and an important outcome of complex systems-level organization and regulation of cellular processes.[8]

Compared to prokaryotic cells, eukaryotic cells use different or similar but more sophisticated mechanisms for their dynamic internal organization. Membrane bound organelles, for example, are used by eukaryotic cells for compartmentation of their cellular processes, a mechanism that prokaryotic cells lack. Active intracellular transport, which enables dynamic reorganization of the inner cellular environment, is functionally and mechanistically much more advanced in eukaryotic cells.[9] Consequently, cellular processes in eukaryotic cells often exhibit complex spatiotemporal dynamics patterns, which can be visualized at high resolution and with molecular specificity using fluorescence imaging techniques. Figure 1 shows several specific examples from processes such as signal transduction in T cell activation[10] (Figure 1(a)). Rho GTPase activity coordination in cell protrusion[11] (Figure 1(b)), and mitotic motor kinesin-5 reorganization in cell division[12] (Figure 1(c)). These patterns contain critical information of systems-level organization and regulation of their associated cellular processes. So far, however, our understanding of these patterns remains very limited.

Fluorescence imaging is the tool of choice for visualizing and recording spatiotemporal dynamics of cellular processes. Advances in areas such as super-resolution microscopy,[13–15] single-molecule techniques,[16] and fluorescence biosensor techniques[17,18] have significantly improved imaging resolution, sensitivity, specificity, and multiplexity and provided exciting opportunities for studying spatiotemporal behaviors of cellular processes. However, to understand these behaviors at the mechanistic and systems level, computational analysis and understanding of recorded image data is crucial, not only for acquiring quantitative behavior measurements but also for understanding how they result from complex

interactions among the molecules or molecular complexes involved. The technology of computational analysis and understanding of biological images is often referred to simply as *bioimage informatics*.[19–22] Understanding complex spatiotemporal behaviors of cellular processes is one of its key applications.

This article introduces fundamentals of bioimage informatics for understanding spatiotemporal dynamics of cellular processes and reviews recent advances on this topic. It aims to provide useful and up-to-date information to readers who are interested in understanding spatiotemporal behaviors of cellular processes as well as readers who are interested in developing related technologies. It starts with a brief introduction to basic concepts and techniques of bioimage informatics for quantitative characterization of spatiotemporal cellular behaviors. It then uses several specific examples to review recent technological advances. Systems-level studies of spatiotemporal behaviors of cellular processes represent a new direction in biological research. Despite the advances made, significant technological challenges remain, especially in representations of spatiotemporal cellular behavior and in inference of systems-level molecular mechanisms from such representations. These challenges are briefly discussed. The focus of this review is exclusively on biological processes within single cells. Spatiotemporal dynamics of biological processes at other scales, such as multicellular processes in development and regeneration, is also important but is beyond the scope of this review. The rest of this article is organized as follows: Section *Background* introduces related basic bioimage informatics concepts. Section *Characterization of Spatiotemporal Dynamics of Cellular Processes* summarizes bioimage informatics techniques for characterizing spatiotemporal cell dynamics. Section *Towards Systems-Level Mechanistic Understanding of Spatiotemporal Dynamics of Cellular Processes* uses two case studies to review recent advances toward system-level mechanistic understanding of spatiotemporal cell dynamics. Section *Discussion* concludes with a brief discussion of key remaining challenges.

BACKGROUND

Quantitative analysis of biological images started shortly after digital microscopy images became available in 1960s.[22] However, it is over the last decade that development of biological image analysis and understanding techniques significantly accelerated and that *bioimage informatics* emerged as a new technology.[19,20,22] This development is driven by the synergy of several key factors, including

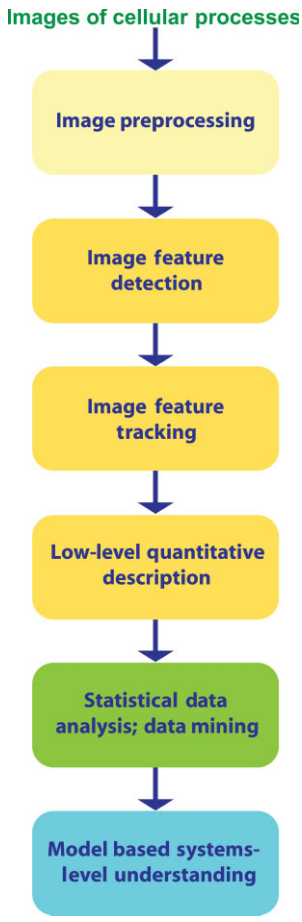

FIGURE 2 | General procedure of using bioimage informatics techniques to study spatiotemporal cell dynamics.

increased accessibility to microscopes for digital image recording; significant advances in general image analysis and understanding techniques; and decreased cost of computation. But most importantly, it is driven by the need to quantitatively understand complex spatiotemporal behaviors of biological processes at large scales and systems levels, which is not feasible by qualitative or manual analysis.

This section introduces basic bioimage informatics concepts for understanding spatiotemporal cell dynamics. The general procedure of using bioimage informatics techniques to study spatiotemporal dynamics of cellular processes is outlined (Figure 2). This is followed by a classification of cellular process images as different types of images require different bioimage informatics techniques for analysis (see Section *Characterization of Spatiotemporal Dynamics of Cellular Processes*). For users of bioimage informatics techniques, it is of critical importance to ensure accuracy and precision in image data analysis and to prevent analysis artifacts. The topic of data analysis

quality control in bioimage informatics is briefly discussed. Because of space constraints, technical details often have to be left out but can be found in the references provided.

Using Bioimage Informatics Techniques to Study Spatiotemporal Cell Dynamics

Figure 2 outlines the general procedure of utilizing bioimage informatics techniques to study spatiotemporal dynamics of cellular processes. Recorded images of cellular processes are usually preprocessed prior to analysis. For example, image noise can be suppressed using *image filtering* techniques.[23–25] As another example, specimen drift, a problem commonly encountered in live imaging of cellular processes, often must be corrected using *image registration* techniques to avoid artifacts in motion analysis.[26] After preprocessing, image features, which are image objects carrying useful information, are identified using *feature extraction* techniques.[27] The extracted image features represent different cellular structures. For example, fluorescently labeled molecules are identified as *particles* if they are spatially well separated (Figure 3). As another example, cells shown in Figure 1 are usually identified as *regions*.

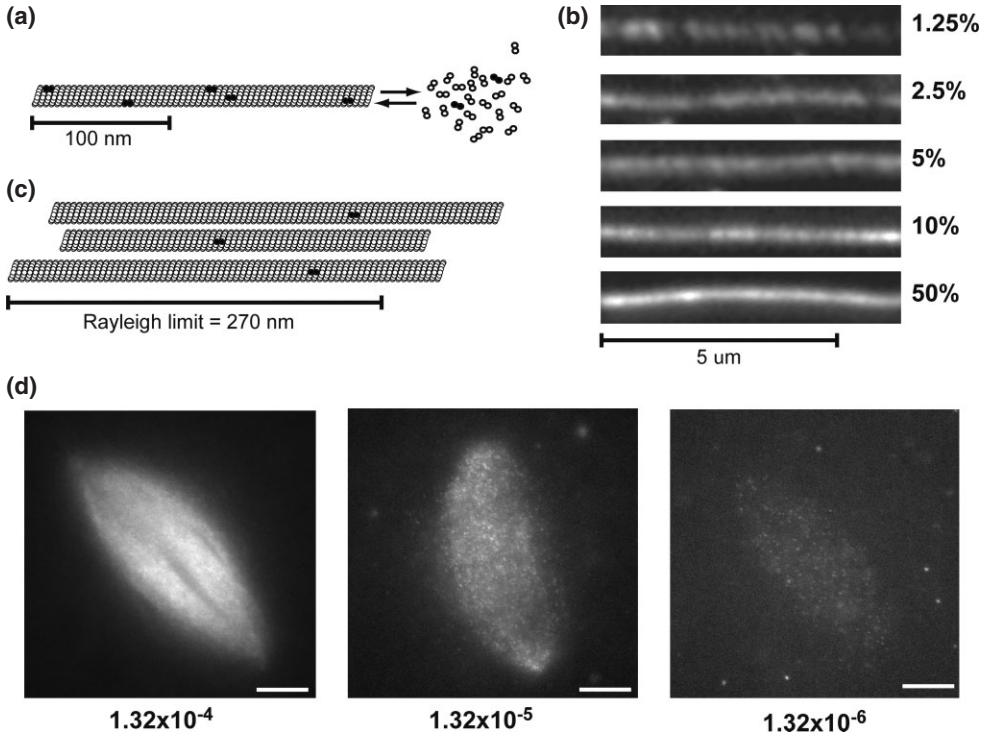

FIGURE 3 | Fluorescent speckle microscopy: an example of single particle images versus continuous region images. Fluorescent speckle microscopy is an imaging technique that is often used for high-resolution visualization of dynamics of the cytoskeleton.[31] It provides a good example for illustrating the differences between single particle images and continuous region images. Here, the basic principle of FSM is explained based on its application in microtubule imaging. (a) To visualize individual microtubules *in vitro*, fluorescently labeled tubulin subunits (solid black) are added to the solution. These subunits are randomly incorporated into individual microtubules undergoing polymerization. (b) When the fraction of labeled tubulin (indicated to the right of each image) relative to the total tubulin pool is low (e.g., 1.25–2.5%), individual labeled tubulin subunits appear as separated particles after being incorporated into the microtubule. This produces a single particle image with speckle-like appearance (b, upper two images), hence the name of fluorescence speckle microscopy.[30] When the fraction of labeled tubulin is increased, individuals labeled tubulins become spatially closer to each other after being incorporated. Eventually, when the fraction of labeled tubulin is very high (e.g., 10–50%), individual labeled tubulin subunits can no longer be resolved readily following incorporation. This produces a continuous region image (b, lower three images). (Reprinted with permission from Ref 31 Copyright 2006 Annual Reviews). (c) and (d) Following the same principle for single microtubules, when multiple microtubules are adjacent to each other, even lower fractions of labeled tubulin [indicated at the bottom of each image in (d)] are required to produce single particle images. This is because labeled tubulin subunits from different microtubules may fall within a distance smaller than the Rayleigh limit of image resolution and become unresolvable, as illustrated in (c). This observation is confirmed experimentally in microtubule networks of *Xenopus* egg extract spindles, an *in vitro* model for studying cell division, as shown in (d). Compared to the continuous region image (d: left panel), much lower fractions of labeled tubulin are required to produce single particle images (d: middle and right panels). Imaging low fractions of labeled tubulin is more challenging because substantially longer exposure is required. Scale bars: 10 μm.

Dynamic changes of these features in their properties, such as positions, shapes, image intensities, can be followed using *feature tracking* techniques, which provide their *trajectories,* that is, spatial and temporal histories.[23,28] From these trajectories, simple quantitative *descriptors*, such as velocities and travel distances in the case of individual moving molecules, can be calculated. Together, these trajectories and descriptors provide a dataset of quantitative measurements for characterizing cellular processes of interest.

Substantial amount of quantitative measurement data is usually required to fully characterize complex spatiotemporal behaviors of cellular processes. Consequently, statistical techniques are required to ensure accuracy, precision, reliability, and sensitivity in data analysis. In addition, data mining techniques are often used to search for valuable but hidden information.[29] Overall, however, this process is analytic rather than synthetic in nature. To understand spatiotemporal dynamics of cellular processes, it is essential to transform quantitative measurements of these processes into knowledge of their molecular mechanisms. This is a synthetic process because models must be constructed to represent molecular mechanisms. This process will be discussed in detail using specific examples in Section *Towards Systems-Level Mechanistic Understanding of Spatiotemporal Dynamics of Cellular Processes*.

A Classification of Cellular Process Images

Different cellular processes and imaging techniques can produce images with very different properties. In general, however, these images can be classified into two categories (Figure 3). Images in the first category are referred to as *single particle images* in this review. Primary features of these images are spatially separated points or particles, which are images of fluorescently labeled structures smaller than the imaging resolution limit. Under the Rayleigh criterion, this limit is ~200 nm and is defined by $\frac{0.61\cdot\lambda}{NA}$, where λ is the excitation wavelength and NA is the numerical aperture of the microscope objective lens. Images in the second category are referred to as *continuous region images*. Primary features of these images are regions,[23] which are images of fluorescently labeled structures that are spatially connected. Differences between these two types of images are explained in detail in Figure 3 using Fluorescent Speckle Microscopy[30,31] as a specific example. This classification is important because different bioimage informatics techniques are required to analyze images in each category. Some of these techniques are introduced in Section *Characterization of Spatiotemporal Dynamics of Cellular Processes*.

For quantitative characterization of spatiotemporal dynamics of cellular processes, single particle images are usually preferred as they provide higher spatiotemporal resolutions than continuous region images. This is because individual particles serve as sensitive local reporters of cell dynamics. Their activities can be followed with high resolution using single particle tracking techniques (see Section *Characterization of Spatiotemporal Dynamics of Cellular Processes*). This, for example, makes it possible to detect spatial and temporal heterogeneity of cellular behaviors, which is an important advantage. However, as illustrated in Figure 3, generating single particle images is often technically more challenging, and spatiotemporal dynamics of cellular processes may not be fully represented if particles are too sparsely distributed.

Continuous region images provide lower spatiotemporal resolutions mainly due to their lower contrast. To understand this, it is useful to think about the theoretical limit case of a region with completely uniform image intensities. Under such a condition, it is not possible to detect any movement within this region due to a complete lack of contrast. In practice, of course, continuous region images cannot be completely uniform in image intensities and thus will provide some level of contrast. Furthermore, specialized imaging techniques such as photoactivation (PA) and fluorescence recovery after photobleaching (FRAP) can be used to directly generate image contrast.[32] An important advantage of continuous region images is that they are more convenient to produce.[31] A variety of computational image analysis techniques have been developed to analyze continuous region images, as will be introduced in Section *Characterization of Spatiotemporal Dynamics of Cellular Processes*. Overall, however, performance of these techniques is sensitive to image contrast levels. It is worth noting that recent advances in super-resolution imaging techniques have significantly improved the resolution of light microscopy. Under the right conditions, these techniques are capable of generating single particle images of specimens that would otherwise produce continuous region images under conventional fluorescence microscopy.

Quality Control in Bioimage Informatics

For users of bioimage informatics techniques, it is of critical importance to ensure accuracy and precision in image data analysis and to prevent

analysis artifacts, especially when a wide variety of analysis methods are integrated (Figure 2). Because these requirements are shared by applications in other related areas such as computer vision[24] and medical image analysis,[33] a wide range of well characterized methods have been developed in those areas for verification and benchmarking of image analysis techniques.[34] Many of these techniques can be readily used in bioimage informatics applications. On the other hand, specialized quality control techniques have also been developed specifically for bioimage informatics applications. An example of such techniques for benchmarking performance of fluorescent particle detection algorithms can be found in Ref 35.

For most bioimage informatics techniques, their inputs and outputs are well defined. Consequently, their performance can be readily characterized using defined quantitative measures. In comparison, it is often more difficult to identify analysis artifacts. In the context of using bioimage informatics techniques to study spatiotemporal behaviors of cellular processes, analysis artifacts are false observations that are generated in the data analysis process. Analysis artifacts can be caused by a variety of factors, such as erroneous imaging settings or limitations of selected data analysis techniques. Although there are perhaps no guaranteed solutions, analysis artifacts can be detected and prevented through quality control practices, such as comparing results under different image settings, from different analysis methods, and under different controlled experimental conditions.

CHARACTERIZATION OF SPATIOTEMPORAL DYNAMICS OF CELLULAR PROCESSES

Quantitative characterization of spatiotemporal behaviors of cellular processes is the first step toward understanding their molecular mechanisms. Image feature tracking techniques are an important class of bioimage informatics techniques that serve this purpose by following cellular behaviors in recorded images and reporting their trajectories. On the basis of these trajectories, cellular processes can be characterized at different scales. This section starts with an introduction to image feature tracking techniques for single particle images. Application of these techniques is demonstrated using an example of analyzing spatiotemporal dynamics of microtubules and molecular motor kinesin-5 in the mitotic spindle. Continuous region image based feature tracking techniques are also briefly introduced.

Single Particle Image-Based Characterization Techniques

As discussed previously, fluorescently labeled cellular structures smaller than the resolution limit of light microscopy appear as individual particles when they are spatially well separated. These particles serve as sensitive local reporters of spatiotemporal cell dynamics. Their activities can be followed using *single particle tracking* techniques[28,36,37] (Figure 4). Recovered trajectories of these particles can then be used to characterize and analyze local and global behaviors of cellular processes.

Recovery of single particle trajectories

Single particle tracking has been used broadly in biological studies.[12,36,38,39] The recovered trajectory of a particle typically records its positions and intensities over time. For example, in the case of a particle in a time-lapse series of 2D images, its trajectory can be represented by a time series $\{(x_1^k, y_1^k; I_1^k), (x_2^k, y_2^k; I_2^k), \ldots, (x_j^k, y_j^k; I_j^k), \ldots, (x_N^k, y_N^k; I_N^k)\}$ where (x_j^k, y_j^k) and I_j^k denote the Cartesian coordinate and intensity, respectively, of this kth numbered particle in the jth image frame, and N is the total number of image frames. As outlined in Figure 2, particle tracking is preceded by image preprocessing and particle detection. Individual particles can be localized up to sub-pixel accuracy by fitting their images using the point spread function of the microscope used.[35,40] This technique is standard in super-resolution imaging.[14,15] After detection of individual particles, single particle tracking algorithms establish correspondence between particles within different image frames, i.e. at different time points, so that complete particle trajectories can be recovered. Detailed examples are illustrated in Figure 4 and described in the next section.

Single particle tracking techniques have been studied extensively. Early approaches tried to solve the problem using optimal graph assignment[41] or statistical data association.[42] These approaches often cannot follow complex movement of large numbers of particles, which is common in biological applications. This is mainly because of their high-computational complexity and their limitations in handling events such as particle appearance and disappearance as well as merging and splitting. Recently, significant progress has been made in overcoming these limitations. To handle events such as particle appearance/disappearance and merging/splitting, graph based algorithms based on the framework of multiple hypothesis testing have been developed.[43–45] These algorithms can effectively follow complex particle movement events but may not be suitable for tracking large numbers (e.g., >1000)

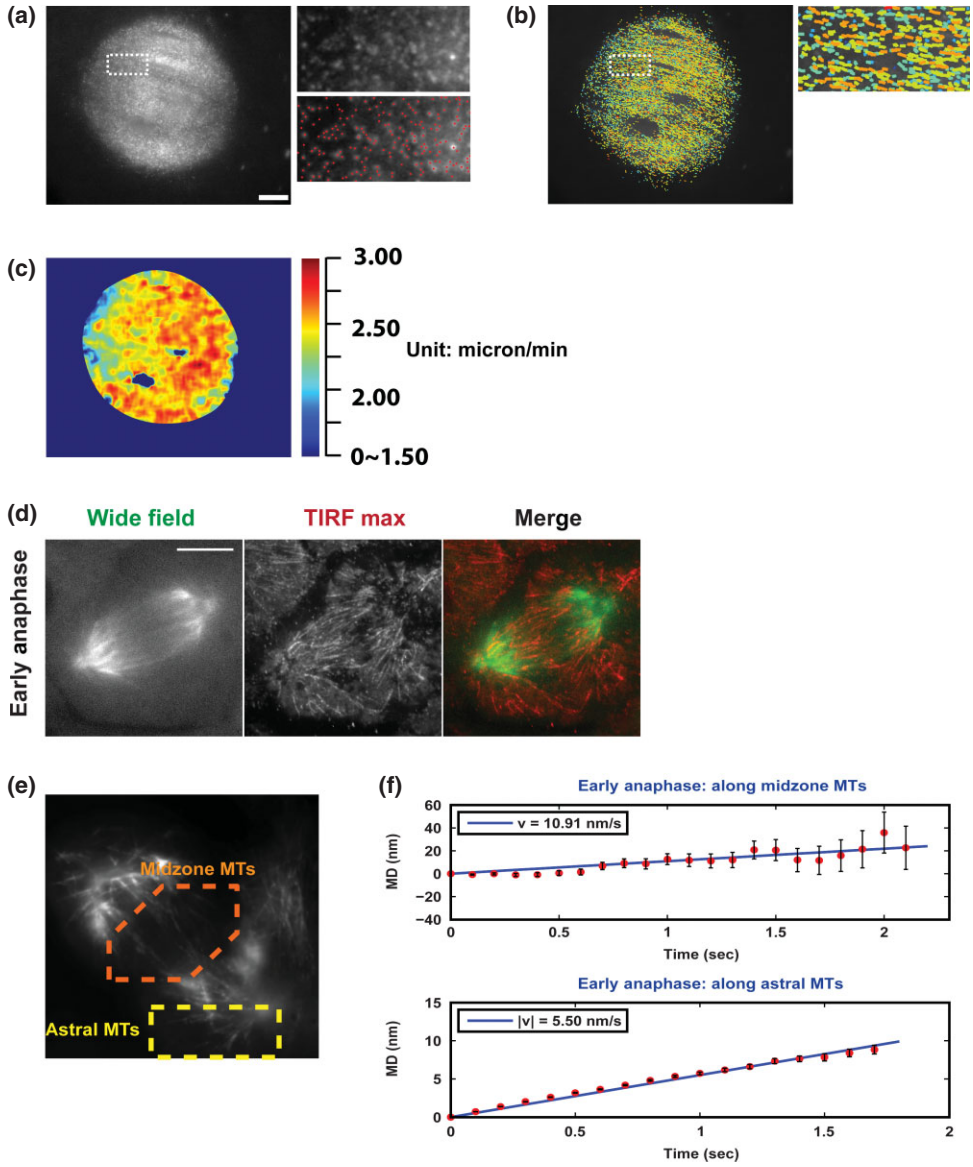

FIGURE 4 | Characterizing spatiotemporal dynamics of the mitotic spindle using single particle tracking. (a) Left panel: an X-rhodamine tubulin speckle image of a Xenopus egg extract spindle. The dotted rectangular region is magnified in the right upper panel. Right lower panel: tubulin speckles detected by software are marked in red and overlaid onto the original image. Scale bar: 10 μm. (b) Left panel: speckle trajectories recovered using single particle tracking. Right panel: trajectories within the dotted rectangular region. Warmer colors indicate faster speckle velocities. (c) Global distribution of speckle movement velocities with the spindle. (a–c: Reprinted with permission from Ref 53. Copyright 2008 Rockefeller University Press). (d) Kinesin-5 distribution in early anaphase spindles of LLC-PK1 cells visualized by fluorescence microscopy. Left panel: kinesin-5 visualized using wide field imaging, which produces a low-contrast continuous region image. Middle panel: kinesin-5 visualized using TIRF, which produces a single particle image. Right panel: overlay. TIRF imaging provides significantly improved image contrast. Scale bar: 10 μm. (e) Identified regions of midzone microtubules and astral microtubules. (f) Velocities of kinesin5 along midzone and astral microtubules are determined using linear regression of kinesin-5 particle mean displacement (MD), calculated from individual kinesin-5 trajectories. (d–f: Reprinted with permission from Ref 12. Copyright 2012 ASCB)

of particles because of their high computational complexity. Another important class of algorithms first recover pieces of trajectories, often referred to as tracklets, and then linked them into full trajectories in postprocessing.[46,47] These algorithms have lower computational complexity and can effectively track large numbers of particles. However, they are less effective in resolving complex particle movement events. For all these algorithms, the specific cellular processes under investigation often impose

constraints on particle movement. Such constraints can be incorporated into the proposed single particle tracking algorithms in forms such as prior probability distributions for improved performance. Comprehensive reviews of single particle tracking techniques are provided in Refs 38,48.

Characterizing local spatiotemporal dynamics of cellular processes

From recovered particle trajectories, simple quantitative descriptors can be calculated to characterize local spatiotemporal dynamics of cellular processes at the single particle level. For example, if a particle undergoes directed motion, with its trajectory represented by $\{(x_1^k, y_1^k; I_1^k), (x_2^k, y_2^k; I_2^k), \ldots, (x_j^k, y_j^k; I_j^k), \ldots, (x_N^k, y_N^k; I_N^k)\}$, its behavior can be characterized using it average velocity, defined as $v^k = \frac{1}{(N-1)\cdot T}\sum_{i=2}^{N}\sqrt{(x_i^k - x_{i-1}^k)^2 + (y_i^k - y_{i-1}^k)^2}$ where T is the time interval between two consecutive frames. In the case of a particle undergoing diffusive motion, its behavior can be characterized using the relation of its mean-square displacement (MSD) with respect to time.[49,50] A particle undergoing pure diffusion satisfies the linear relation of $\mathrm{MSD}(t) = 2Dt$ where D is its diffusion coefficient. When its diffusion is superimposed with local flow, its MSD follows $\mathrm{MSD}(t) = 2Dt + v^2t^2$ where v is the flow velocity. When its diffusion is locally confined, its MSD follows $\mathrm{MSD}(t) = \mathrm{MSD}(\infty)[1 - \mathrm{e}^{-t/\tau}]$ where τ is a constant representing constraint. This type of single particle analysis is used broadly in biological studies to characterize local spatiotemporal behavior of cellular processes.[12,39,51–53]

The simple MSD based analysis described above implicitly assumes that the particle has the same diffusion constant in different regions. This may not be true in the heterogeneous intracellular environment. Recently, a hidden Markov model based approach was proposed to overcome this limitation and was used successfully to study the different diffusion behaviors of LFA-1 particle within different regions of the dynamic actin cytoskeleton.[54,55] For particles undergoing directed movement but switch between different velocity modes, statistical clustering analysis was used to identify their velocity modes[51,56] and probabilities of switching between these modes were estimated using hidden Markov models.

Characterizing global spatiotemporal dynamics of cellular processes

Spatiotemporal dynamics of cellular processes can be characterized at the global level by analyzing behaviors of particle populations within the selected region of interest. An important class of analysis is to examine the global distribution patterns of particles. A variety of statistical analysis tools have been developed for this purpose.[57,58] Another important class of analysis is to detect spatial heterogeneity and patterns. This often requires a partition of the selected region of interest into smaller units to characterize spatiotemporal dynamics within each unit.[59] A simple example is given in Ref 53. Overall, bioimage informatics techniques for representing global spatiotemporal dynamics of cellular processes remain very limited.

Example: characterizing spatiotemporal dynamics of the mitotic spindle using single particle tracking

The mitotic spindle is a complex molecular machine assembled from dynamic microtubules and molecular motors.[4] Its function is to reliably segregate replicated chromosomes during cell division. Spindles assembled in *Xenopus* egg extracts[60] (Figure 4(a)) provide a powerful *in vitro* model system to study mitotic spindle structure and function. Spatiotemporal dynamics of spindle microtubules can be visualized with high resolution using Fluorescent Speckle Microscopy[30,31] (Figures 3(d) and 4(a)). It reveals, for example, that tubulin subunits of spindle microtubules move toward spindle poles, a phenomenon called microtubule flux. Single particle tracking techniques can be used to follow spatiotemporal dynamics of spindle microtubules, such as movement of labeled tubulins[53] (Figure 4(b)) or growing microtubule tips.[61] On the basis of trajectories of tracked tubulins, local and global properties of spindle microtubule flex can be analyzed (Figure 4(c)). For example, it was found that locally individual neighboring microtubules slide relative to each other,[52] and that globally tubulin movement is spatially heterogeneous and slows down near spindle poles[53] (Figure 4(c)).

Molecular motor kinesin-5 is another important component of the mitotic spindle, serving essential roles in its formation, organization, and function.[4] Understanding spatiotemporal dynamics of kinesin-5 is important to understanding spindle architecture and chromosome segregation. Early studies of kinesin-5 concluded that spindle kinesin-5 is largely stationary.[62] This conclusion was challenged by a later study using photoactivation and fluorescence recovery after photobleaching techniques, which revealed active transport of spindle kinesin-5.[63] However, the continuous region images produced in the study did not have sufficient resolution to examine whether kinesin-5 dynamics varies in different spindle regions. This question was answered in a recent study that combines high-resolution

imaging with single particle tracking of kinesin-5[12] (Figure 4(d)). It found that kinesin-5 movement varies spatially between midzone microtubules and astral microtubules (Figure 4(e) and (f)). It also found that kinesin-5 movement on astral microtubules changes directions at different stages of cell division, indicating that kinesin-5 distribution is tightly regulated in synchronization with the progression of mitosis.[12] Together, these studies provide a representative example of the power of bioimage informatics techniques in understanding complex spatiotemporal dynamics of cellular processes.

Continuous Region Image-Based Characterization Techniques

Despite their lower spatiotemporal resolutions, continuous regions images are more convenient to generate experimentally. In comparison, generation of single particle images often requires precise control of fluorescent labeling and image collection, as illustrated by the example of Fluorescent Speckle Microscopy[30,31] (Figure 3). When there is sufficient image contrast, movement within continuous regions of fluorescence signals can be tracked using a variety of template-based techniques.[24,25,64–66] These techniques require image intensity distributions with the region of interest to have some level of stability to allow selection of templates. Output of these techniques is typically the vector field of movement within the region analyzed. Further analysis can be performed on this vector field. For example, its divergence can be used to infer local assembly and disassembly of cellular components if mass conservation is assumed. This technique was used to determine actin assembly and disassembly at the leading edge of migrating keratocytes.[67]

TOWARDS SYSTEMS-LEVEL MECHANISTIC UNDERSTANDING OF SPATIOTEMPORAL DYNAMICS OF CELLULAR PROCESSES

The example in the previous section shows how bioimage informatics techniques can be used to analyze spatiotemporal dynamics of the mitotic spindle. Although the analysis provided important insights into the underlying molecular mechanisms, it could not fully account for the spatiotemporal dynamics observed. Specifically, for example, the analysis revealed spatial heterogeneity of spindle microtubule movement but could not answer the question of what molecular mechanism generates this heterogeneity. To answer this question would require systems-level understanding of interactions among the many molecules or molecular complexes involved.

The ultimate goal of understanding spatiotemporal dynamics of cellular processes is to understand at the systems level the underlying molecular mechanisms. Here, two studies on cell protrusion[11] and signal introduction[68] are selected as examples to review recent advances toward achieving this goal. Different from the previous example in which different components of the mitotic spindle were analyzed separately, the first study analyzed how three Rho GTPase RhoA, Rac1, and Cdc42 work together in space and time to regulate cell protrusion.[11] The main limitation of this study, however, is that it did not provide a systems-level model that can fully account for the spatiotemporal dynamics of GTPase activities observed. The second study analyzed how signals propagate in the cAMP/PKA/B-Raf/MAPK pathway in space and time to regulate processes such as synaptic plasticity.[68] Different from the first study, a systems-level quantitative model was developed in the second study, which made it possible to use computer simulation to fully recapitulate the spatiotemporal dynamics of signal propagation. It is worth noting that images in both studies belong to the category of continuous region images.

Example 1: Spatiotemporal Coordination of Rho GTPase Activities in Cell Protrusion

Rho GTPase RhoA, Rac1, and Cdc42 are key regulators of actin cytoskeleton dynamics.[70] To understand how they work together in space and time to regulate cell protrusion, an essential step in cell migration,[71] their activities in mouse embryonic fibroblasts were visualized using fluorescence biosensors[11] (Figure 1(b)). Protrusion and retraction of the cell edge were quantitatively characterized using bioimage informatics techniques such as edge detection and edge tracking and represented using morphodynamic activity maps[11] (Figure 5(a)). In a similar fashion, activities of RhoA, Rac1 and Cdc42 along the cell edge were also represented using morphodynamic maps.[11]

To analyze temporal relations between RhoA, Rac1, and Cde42, correlation coefficients between protrusion/retraction rates and GTPase activity levels along the cell edge were calculated under different time shifts of the morphodynamic maps. The time shift that resulted in the highest positive correlation coefficient was interpreted as representing the actual temporal separation between cell edge movement and

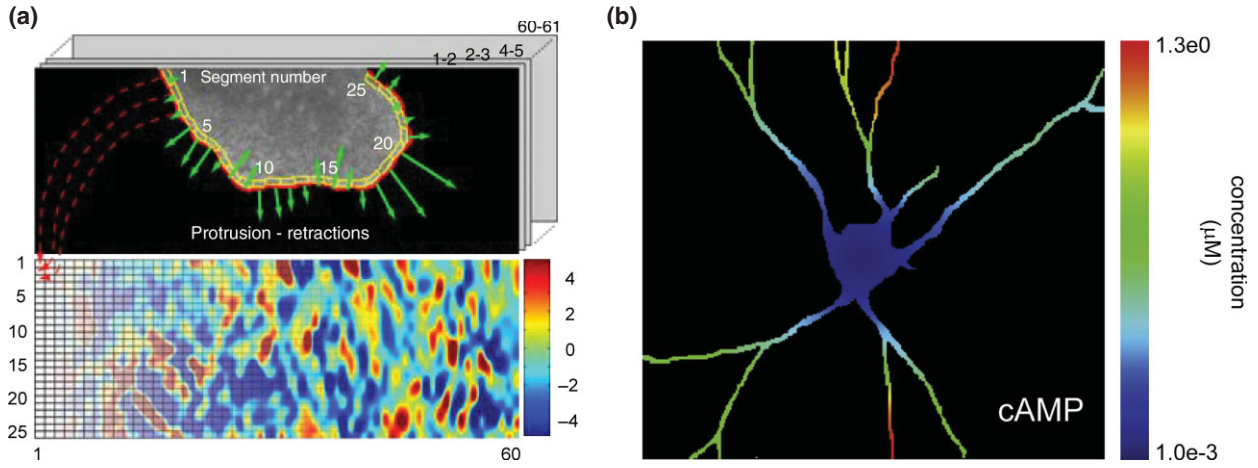

FIGURE 5 | Systems-level representation of spatiotemporal dynamics of cell protrusion and signal transduction. (a) Spatiotemporal dynamics of cell protrusion is represented using a morphodynamic map. Upper panel: to generate the map, a series of sampling windows are defined along the cell edge. Lower panel: protrusion or retraction of cell edge within each sampling windows was tracked using bioimage informatics techniques. Protrusion/retraction activities over the entire edge is quantitatively represented in color-coding in a morphodynamic map. The vertical axis of the map corresponds to the series of sampling window. The horizontal axis of the map corresponds to time. Each column of the map represents in color-coding protrusion or retraction rates within the entire series of sampling windows at a given time, whereas each row represents protrusion or retraction rates within a specific sampling window along the cell edge over the entire duration analyzed. (Reprinted with permission from Ref 69. Copyright 2006 Cell Press). (b) Computer simulated spatial distribution of cAMP concentrations in neurites of actual geometry at a given time point. (Reprinted with permission from Ref 68. Copyright 2008 Cell Press)

GTPase activity activation. This analysis revealed, for example, that RhoA was activated in synchronization with the initiation of cell edge protrusion whereas Cdc42 and Rac1 were activated with a time delay of around 40 s. In a similar fashion, spatial correlation coefficients were calculated between protrusion/retraction rates at the cell edge and GTPase activity activation at different distances from the cell edge. The analysis revealed, for example, that RhoA was activated at the cell edge while Cdc42 and Rac1 were activated at around 2 μm behind. Overall, the study provides important insights into how RhoA, Rac1, and Cdc42 coordinate in time and space to regulate cell protrusion.

The study has its limitations, which are representative of current bioimage informatics techniques in understanding spatiotemporal cell dynamics. A limitation of correlation analysis is that it cannot differentiate different relations between the GTPases studied. Biological images often have low signal-to-noise ratios (SNRs). Correlation analysis is a valuable tool because it is capable of analyzing activities of RhoA, Rac1, and Cde42 under low SNRs. However different relations between these GTPase may result in the same correlation coefficients. In particular, conventional correlation analysis cannot identify causal relations, although it may be possible to overcome this limitation using more sophisticated techniques such as Granger's causality analysis.[72,73] Another limitation of the study is that it cannot account for the overall spatiotemporal patterns of the GTPase activities. For example, it is unclear how high levels of GTPases at the edge were maintained (Figure 1(b)). These spatial distribution patterns, which may be mediated by active transport, passive diffusion, or both, contain important information about the overall organization of GTPase activities. To understand these patters requires systems-level quantitative models of activities of these GTPases. An example of such models is given in the next study.

Example 2: Spatiotemporal Signal Transduction in the cAMP/PKA/B-Raf/MAPK Pathway

Activation and deactivation as well as production and degradation of signaling molecules are often spatially heterogeneous. This heterogeneity may hold the key to understanding why the same signaling pathway can produce very different physiological outcomes. This study analyzed how signals initiated by β-adrenergic receptor propagate in space and time through the cAMP/PKA/B-Raf/MAPK pathway in neurons.[68] Specifically, it combined computer simulation with experiments to analyze the formation and propagation of microdomains, which are small dynamic intracellular regions with elevated levels

of signaling molecules[68] (Figure 5(b)). Interactions among the signaling molecules were fully modeled using partial differential equations with boundary geometry taken from actual neurons. The study found that the formation and dynamic changes of microdomains are controlled by four factors, including cell geometry, intracellular localization, reaction network topology, and reaction rates. It also found that cell geometry controls the formation of microdomains while the propagation of microdomains is controlled by network topology, specifically negative feedback, of the signaling pathway. It is unknown, however, whether these microdomains also form in non-polarized cells, which do not have the constrained geometry of neurites.

This study differs from the previous study on GTPase activities in that it fully modeled reactions among the signaling molecules as well as cell geometry. This makes it possible to understand at the systems level spatiotemporal patterns of the signaling molecules investigated. This study demonstrates the important role of comprehensive mechanistic models in transforming quantitative measurements of cellular behaviors into understanding of systems-level molecular mechanisms.

DISCUSSION

Significant advances have been made over the past decade in using bioimage informatics techniques to understand spatiotemporal dynamics of cellular processes. For example, advances in single particle tracking techniques have made it possible to study highly complex spatiotemporal behaviors of cellular processes.[46,51] System-level analysis and modeling of signal transduction have started to reveal how information is propagated and integrated within the intracellular environment.[11,68] It is also clear, however, that we have only started to understand some of the general principles governing spatiotemporal dynamics of cellular processes. Overall, systems-level studies of spatiotemporal behaviors of cellular processes represent a new direction in biological research. Despite the advances made, significant technological challenges remain. Some of these challenges are discussed here.

First, systems-level characterization and representation of spatiotemporal cellular behaviors remain a challenge. For example, cells usually have different shapes. It remains difficult to compare spatiotemporal dynamics patterns of cellular processes under different geometry. A possible solution may be to compute an average cell shape[74] and then map the spatiotemporal patterns of individual cells in different shapes to the mean shape for normalization. However, a key challenge is to avoid biases and artifacts in this mapping process. Another challenge is to effectively characterize and represent inherent variations of cellular processes in different cells under the same experimental condition.[22,75] Overall, a fundamental but open question is: how to represent spatiotemporal behaviors of cellular processes to best support understanding of their molecular mechanisms?

Second, to effectively model the dynamic and heterogeneous inner cellular environment in which cellular processes are completed remains a challenge. Spatiotemporal behaviors of cellular processes may be mediated by active transport as well as passive diffusion and may depend strongly on the dynamic cytoskeleton. Another factor to be considered in modeling is the scale. Local and global spatiotemporal behaviors of cellular processes can provide very different information. Model-based computer simulation is an important solution to these problems.[76–78] Complex models, however, can make result interpretation difficult.

Third, spatiotemporal behaviors of cellular processes result from complex interactions among the molecules or molecular complexes involved. To infer molecular mechanisms of these interactions from observed behaviors is often equivalent to solving an inverse problem, which tends to be ill-posed. To overcome this challenge, it is necessary to impose additional constraints from existing knowledge of the cellular processes under investigation. However, such constraints often are very different and specific for different cellular processes. A significant challenge is to develop widely applicable techniques to address this problem.

ACKNOWLEDGMENTS

I am grateful to my many colleagues for valuable discussions and inputs. I apologize to authors whose work cannot be cited due to space limitations. This work was supported in part by Samuel and Emma Winters Foundation grant 1030558, NSF grants MCB-1052660 and DBI-1052925, and NSF Faculty Early Career Award DBI-1149494.

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FURTHER READING

Systematic research on image analysis and understanding techniques started mostly in 1960s in areas such as digital image processing and computer vision.[24,25] Later, research in areas such as medical image analysis[33] also provided a wide variety of image analysis techniques based on medical applications. Together, these areas have made and continue to make substantial contributions to the development of bioimage informatics techniques. However, bioimage informatics is a new and emerging technology because of its unique applications and distinct properties of biological images.[19–22]

Detailed descriptions of bioimage informatics techniques introduced in this review can be found in the references provided. Although currently there are no specialized books on bioimage informatics, related technical background can be found in the references given below.

A general introduction to bioimage informatics can be found in Refs 19–22. A detailed introduction to computational techniques for analyzing dynamic fluorescence images can be found in Ref 23.

A general introduction to fluorescence imaging techniques can be found in Refs 79 and 80. Superresolution imaging techniques were reviewed in Refs 13–15.

A focused treatment of biological image analysis techniques is given in the edited volume: Rittscher J, Machiraju R, Wong STC. *Microscopy Image Analysis for Life Science Applications*. Artech House Publishers; 2008.

Many image analysis techniques used in bioimage informatics were initially developed for computer vision and medical image analysis applications. There are many excellent references on computer vision techniques, including Refs 24, 25, and 33.

Spatiotemporal dynamics of several basic cellular processes was reviewed in a special collection of articles on spatial biology in the journal *Science*.[3,6,81,82]. Systems-level analysis of spatiotemporal dynamics of bacterial cellular processes was reviewed in Refs 8, 83 and 84. Stochastic computer simulation of spatiotemporal cell dynamics was introduced in Refs 76 and 77.

Up-to-date information on latest bioimage informatics research can be found in proceedings of related conference. Some of these conferences are listed at: http://www.bioimageinformatics.org/ and http://www.biomedicalimaging.org.

