Guest Editorial

I. Background and Motivation

UNDERSTANDING the structure and architectural dynamics of the complex cellular and molecular machinery driving living organisms has become the prime target of biological research in the postgenomic era. The social and economic relevance of these efforts follows from the fact that detailed knowledge of the spatial and temporal relationships of cells and molecules in the context of specific physiological functions can be harnessed to improve health and well-being. It seems likely, therefore, that this knowledge will become an increasingly important factor in future human health care. Evidently, images and image sequences play a key role in obtaining this knowledge.

Over the past two decades, enormous progress has been made in the development of microscopy imaging hardware and methodology to visualize cells and molecules with high specificity. Advances in fluorescence microscopy have been especially noteworthy, including the development of the laser scanning confocal microscope, the advent of CCD cameras for digital image acquisition, the development of methods for using naturally fluorescent proteins (notably the green fluorescent protein), and for engineering a host of derived fluorescent probes. All of these developments have led to an explosive increase in the acquisition of digital-image data in biological studies.

There is now a growing consensus that sophisticated computational methods are necessary not only to handle the growing rate at which images are acquired, but, more importantly, to provide a level of sensitivity and objectivity that human observers cannot match. Efficient and robust image analysis tools generating accurate and reproducible quantitative results are desperately needed in support of high-throughput biological research. The high variability of the image data in biological imaging, as opposed to medical imaging with its highly standardized image-acquisition protocols, poses a huge challenge to the image-processing and computer-vision community.

Despite an increasing number of praiseworthy efforts in applying computational methods to biological image data, the field is still very much in its infancy. In many biological research labs, the application of computational tools is often limited to low-level image signal manipulation, while the extraction of biologically meaningful information from the image data is still done manually. A possible explanation of this fact is that it takes biologists and computer scientists working closely together to develop and successfully apply automated biological image analysis tools: Different algorithms need to be developed and constantly adapted to specific tasks, requiring substantial domain knowledge. Our motivation for this Special Issue was to stimulate the interaction between researchers from both communities by presenting some of the cutting-edge work currently being done in the field and by revealing the challenges that still lie ahead.

II. Facts and Figures

The idea of putting together a Special Issue on Molecular and Cellular Bioimaging was first put forward by the Editor-in-Chief in September 2003. By April 2004, the outline and planning of this Special Issue was established, and the first call for papers was distributed through the internet. In the subsequent months, the announcement also appeared in print in several IEEE transactions. Between June and December 2004, 66 manuscripts in total were submitted for inclusion in this Special Issue. Following this overwhelming response, the Guest Editors decided to focus this Special Issue primarily on the computational analysis of electron and optical microscopy image data, thereby favoring papers contributing to the state-of-the-art in image analysis methodology, as well as presenting thorough experimental results, possibly leading to new biologically useful insights. The 46 papers that were found to be most relevant to this Special Issue were sent out for review. Each of the papers was reviewed by three or four experts in the fields of computer vision and quantitative biology. By the end of March 2005, the first review round was completed and all authors were notified of the decision. In May 2005, following a round of revisions, 15 papers were finally selected for inclusion.

III. Scanning the Issue

The papers included in this Special Issue provide good coverage of the field and are illustrative of the variety of issues encountered in molecular and cellular biological imaging, ranging from image acquisition, to image processing, to image analysis.

One of the most critical issues that must be dealt with in fluorescence microscope image acquisition is the fact that the number of photons that can be acquired is often a limiting factor in designing experiments and interpreting results. Lidke *et al*. describe a careful analysis of the influence of photon statistics on a still-developing microscopy method, fluorescence anisotropy imaging. This method can use resonance energy transfer between pairs of identical fluorophores to measure the average distance between them. The low signals usually obtained require tradeoffs between resolution in space, time, or probe anisotropy, and this very practical paper presents an automated procedure for achieving a desired balance between them.

Continuing on the theme of photon limitations, the paper by Merryman and Kovacevic presents an elegant idea how real-time multiscale image processing can be used to increase the efficiency of image acquisition in laser scanning confocal microscopy. They propose an adaptive point-sampling strategy. Image regions with no or little information remain down-sampled, while regions with significant information are identified

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and subjected to increasingly finer sampling. Interestingly, they validate their system by showing that classifiers of location patterns of subcellular structures perform equally well on adaptively sampled images as on full-resolution images. The proposed concept is not only relevant for minimizing the scanning time of single images but can readily be applied to robotic microscopy for the efficient acquisition of large mosaics of multiple fields of view.

Deconvolution represents an area of image processing where computational methods are well established. The paper by Ralston *et al*. proposes a new deconvolution algorithm for optical coherence tomography, a relatively unconventional imaging modality in molecular and cellular bioimaging. It indicates how prior knowledge of the optical system and signal analysis must be combined to increase the resolution of complex imagery and to prepare it for subsequent visual or computational information extraction.

The segmentation of images into regions containing single cells is a long-standing and critical problem that needs to be addressed again as each new imaging modality is developed. The paper by Luck *et al*. describes an automated method for segmenting nuclei in confocal reflectance images obtained by endoscopy. Their approach uses image enhancement by anisotropic median diffusion, Gaussian Markov random fields to model interactions between adjacent nuclei, and a Bayesian classification framework.

Automation of chromosome classification is a challenging problem that has been worked on for over 25 years. The paper by Wu *et al*. describes approaches to addressing this problem using 2-D images that provide improved classification accuracy compared to the current benchmark 1-D method. Automated analysis of fluorescent *in situ* hybridization (FISH) experiments is another domain where computational image analysis has been successfully applied for a long time. The paper by Raimondo *et al*. describes the state of the art in this application domain and presents an interesting expansion to the statistical classification of the image data.

Fernandez-Gonzalez *et al*. describe a tool for the quantitative spatial analysis of complex cell populations, with first applications in mammary gland tissue analysis. A refined version of the relative neighborhood graph is used to establish vicinity relationships between cells, which yields a faithful model of tissue topology. The authors introduce measures computed from this graph representation of the data to quantitatively investigate the presence of spatial patterns within a single-cell population, or the relationship between the spatial distributions of multiple populations, at multiple scales.

The paper by Bartesaghi *et al*. presents an image segmentation technique for the quantitative analysis of 3-D electron tomograms. The segmentation task is formulated as an energy minimization problem, carried out fully automatically in a spherically transformed representation of the image data around manually indicated points of interest. The authors demonstrate the potential of the technique for the analysis of HIV particles and selected subcellular compartments. Since each segmented particle is represented by an implicit function, a variety of geometrical features can easily be derived subsequently. The technique may, therefore, be an important tool in establishing a correlation between these features and the progression of viral infections.

An automatic technique for the segmentation and structural analysis of 3-D-reconstructed cryo-electron microscopy images of icosahedral viruses is presented in the paper by Yu and Bajaj. The technique builds on the fast marching algorithm and incorporates knowledge about the global and local symmetries of the viruses to correctly segment the boundaries of the asymmetric subunits involved. With more and more virus maps available at near atomic resolution, it becomes increasingly important to be able to study these subunits. First results on model data demonstrate the potential of the technique for such studies.

Generally, automated segmentation algorithms are based on one or more parameters in order to be able to cope with biological variability. Usually, these parameters are to be set by the user, which may sometimes have a large effect on the results, making them still subjective. The paper by Abdul-Karim *et al*. presents an interesting approach to automate the selection of parameter settings, with first applications to automated neurite and vessel tracing. This allows nonexperts to use the algorithms without knowledge of the underlying algorithms, increasing the objectivity of the results and allowing developers to modify the algorithms while maintaining a consistent interface.

Although fluorescence microscopy has been used for many decades to study the patterns of proteins and other macromolecules within cells, analysis has traditionally been done by visual inspection. However, experiments within the last few years have demonstrated that the patterns characteristic of major subcellular organelles and structures can be recognized with accuracy significantly better than visual analysis. The paper by Zhao *et al*. describes a significant advance in which images of a protein localized to more than one organelle can be broken down into its component patterns. This capability will be critical to systematic characterization of patterns on a proteome-wide basis, since proteins are often localized to more than one organelle.

The paper by Ning *et al*. addresses a similar classification problem: the automated analysis of embryo phenotypes. The authors describe a framework for automated analysis of the state of a nematode embryo in its early development, based on the characteristics of its image. Applications of such cell state classification schemes are numerous, from the discovery of gene functions to the systematic screening of drug effects on embryo behavior.

This Special Issue concludes with three papers on the very important problem of object tracking and motion analysis, at both the cellular and the molecular level. The paper by Sage *et al*. describes software for finding the optimal track of a single fluorescence particle through an entire time series of images. The approach is used to demonstrate differences in mobility between yeast telomeres. The paper by Bonneau *et al*. also addresses tracking of single particles, using a variant of the fast marching method to simultaneously find minimal paths for many individual particles. Finally, the paper by Dufour *et al*. presents a robust and fully automatic segmentation and tracking method based on multiple active surfaces, enabling quantitative analyses of cellular shape and motion from dynamic 3-D microscopy image data, even at very low signal-to-noise ratios.

IV. Looking Ahead

Notwithstanding the impressive advances in many aspects of biological imaging in the past decades, biological research has only just begun to scratch the surface of the cellular and molecular secrets of life. As new biological hypotheses will continue to be posed and to be verified, new biological image analysis tools will need to be devised and validated, too. Given the many open issues in biological image enhancement, image segmentation, texture analysis, object detection, tracking, and classification, the future for our community looks bright. The unraveling of the molecular mechanisms of life is one of the most exciting scientific endeavors of the 21st Century, and it seems not too daring to predict that, within the next decade, image data analysis will take over the role of gene sequence analysis as the number one informatics task in molecular and cellular biology.

An important issue in this process is the rapid dissemination and exchange not only of new biological knowledge, but also of the technical details of the image analysis tools involved in obtaining this knowledge. For several reasons, some of the most advanced methods for cellular and molecular image analysis are buried in the small print of the materials and methods sections of biological journal papers, making them virtually inaccessible to others. With the present Special Issue, the IEEE TRANSACTIONS ON IMAGE PROCESSING makes an effort to expose the challenges in molecular and cellular bioimaging to the computer science community. Recently, the scope of the journal was broadened with a new section that explicitly welcomes image processing and analysis applications in cellular and molecular bioimaging. Together with the Editorial Board, we hope that this Special Issue stimulates a continuous influx of high-quality manuscripts in this area. In closing, we express our sincere thanks to the reviewers, whose feedback was invaluable in shaping this Special Issue.

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Dr. Danuser currently serves on the editorial boards of the *Biophysical Journal* and the IEEE TRANSACTIONS ON IMAGE PROCESSING. His work has been recognized by several awards, particularly his efforts in combining cell biology with engineering and computer science approaches.