Computational Biology

Bir Bhanu Prue Talbot *Editors*

Video Bioinformatics

From Live Imaging to Knowledge







Computational Biology

Volume 22

Editors-in-Chief

Andreas Dress CAS-MPG Partner Institute for Computational Biology, Shanghai, China

Michal Linial Hebrew University of Jerusalem, Jerusalem, Israel

Olga Troyanskaya Princeton University, Princeton, NJ, USA

Martin Vingron Max Planck Institute for Molecular Genetics, Berlin, Germany

Editorial Board

Robert Giegerich, University of Bielefeld, Bielefeld, Germany Janet Kelso, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany Gene Myers, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany Pauel A. Paueren, University of Celifornia, San Diago, CA, USA

Pavel A. Pevzner, University of California, San Diego, CA, USA

Advisory Board

Gordon Crippen, University of Michigan, Ann Arbor, MI, USA Joe Felsenstein, University of Washington, Seattle, WA, USA Dan Gusfield, University of California, Davis, CA, USA Sorin Istrail, Brown University, Providence, RI, USA Thomas Lengauer, Max Planck Institute for Computer Science, Saarbrücken, Germany Marcella McClure, Montana State University, Bozeman, MO, USA Martin Nowak, Harvard University, Cambridge, MA, USA David Sankoff, University of Ottawa, Ottawa, ON, Canada Ron Shamir, Tel Aviv University, Tel Aviv, Israel Mike Steel, University of Canterbury, Christchurch, New Zealand Gary Stormo, Washington University in St. Louis, St. Louis, MO, USA Simon Tavaré, University of Cambridge, Cambridge, UK Tandy Warnow, University of Texas, Austin, TX, USA Lonnie Welch, Ohio University, Athens, OH, USA The *Computational Biology* series publishes the very latest, high-quality research devoted to specific issues in computer-assisted analysis of biological data. The main emphasis is on current scientific developments and innovative techniques in computational biology (bioinformatics), bringing to light methods from mathematics, statistics and computer science that directly address biological problems currently under investigation.

The series offers publications that present the state-of-the-art regarding the problems in question; show computational biology/bioinformatics methods at work; and finally discuss anticipated demands regarding developments in future methodology. Titles can range from focused monographs, to undergraduate and graduate textbooks, and professional text/reference works.

More information about this series at http://www.springer.com/series/5769

Bir Bhanu · Prue Talbot Editors

Video Bioinformatics

From Live Imaging to Knowledge





Editors Bir Bhanu University of California Riverside, CA USA

Prue Talbot University of California Riverside, CA USA

ISSN 1568-2684 Computational Biology ISBN 978-3-319-23723-7 DOI 10.1007/978-3-319-23724-4

ISBN 978-3-319-23724-4 (eBook)

Library of Congress Control Number: 2015950871

Springer Cham Heidelberg New York Dordrecht London

© Springer International Publishing Switzerland 2015

© Springer International Publishing Switzerland (outside the USA) 2015 for Chapter 9

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

The recent advances in high-throughput technologies for functional genomics and proteomics have revolutionized our understanding of living processes. However, these technologies, for the most part, are limited to a *snapshot analysis* of biological processes that are by nature *continuous and dynamic*. Modern visual microscopy enables video imaging of cellular and molecular dynamic events and provides unprecedented opportunities to understand how spatiotemporal dynamic processes work in a cellular and multicellular system. The application of these technologies is becoming a mainstay of the biological sciences worldwide. To gain a more mechanistic and systematic understanding of biological processes, we need to elucidate cellular and molecular dynamic processes and events.

Video Bioinformatics as defined by the first author (BB) is concerned with the automated processing, analysis, understanding, data mining, visualization, query-based retrieval/storage of biological spatiotemporal events/data and knowledge extracted from microscopic videos. It integrates expertise from the life sciences, computer science and engineering to enable breakthrough capabilities in understanding continuous biological processes. The video bioinformatics information related to spatiotemporal dynamics of specific molecules/cells and their interactions in conjunction with genome sequences are essential to understand how genomes create cells, how cells constitute organisms, and how errant cells cause disease.

Currently, new imaging instrumentation and devices perform *live video imaging* to image molecules and subcellular structures in living cells and collect biological videos for on-line/off-line processing. We can now see and study the complex molecular machinery responsible for the formation of new cells. Multiple imaging modalities can provide 2D to 5D (3D space, time, frequency/wavelength) data since we can image 2D/3D objects for seconds to months and at many different wavelengths. However, data processing and analysis (informatics) techniques for handling biological images/videos have lagged significantly and they are at their infancy. There are several reasons for this, such as the complexity of biological videos which are more challenging than the structured medical data, and the lack of

interdisciplinary research at the intersection of life sciences and engineering and computer science.

We already are at a point where researchers are overwhelmed by myriads of high-quality videos without proper tools for their organization, analysis, and interpretation. This is the main reason why video data are currently underutilized. We believe that the next major advance in imaging of biological samples will come from advances in the automated analysis of multi-dimensional images. Having tools that enable processes to be studied rapidly and conveniently over time will, like Hooke's light microscope and Ruska's electron microscope, open up a new world of analysis to biologists, scientists, and engineers.

This interdisciplinary book on *Video Bioinformatics* presents computational techniques for the solution of biological problems of significant current interest such as 2D/3D live imaging, mild-traumatic brain injury, human embryonic stem cells, growth of pollen tubes, cell tracking, cell trafficking, etc. The analytical approaches presented here will enable the study of biological processes in 5D in large video sequences and databases. These computational techniques will provide greater sensitivity, objectivity, and repeatability of biological experiments. This will make it possible for massive volumes of video data to be analyzed efficiently, and many of the fundamental questions in life sciences and informatics be answered. The book provides examples of these challenges for video understanding of cell dynamics by developing innovative techniques. Multiple imaging modalities at varying spatial and temporal resolutions are used in conjunction with computational methods for video mining and knowledge discovery.

The book deals with many of the aspects of the video bioinformatics as defined above. Most of the chapters that follow represent the work that was completed as part of an NSF-funded IGERT program in Video Bioinformatics at the University of California in Riverside. Several of the chapters deal with work that keynote speakers presented at retreats sponsored by this program (Chaps. 14 and 16). Most other chapters are work done by IGERT Ph.D. fellows who were selected to participate in this program. The program emphasizes an interdisciplinary approach to data analysis with graduate students from engineering and life sciences being paired to work together as teams. These resulting chapters would likely never have been produced without cooperation between these two distinct disciplines and demonstrate the power of this type in interdisciplinary cooperation.

We appreciate the suggestions, support feedback and encouragement received from the IGERT faculty, IGERT fellows and NSF IGERT Program Directors Richard Tankersley, M.K. Ramasubramanian, Vikram Jaswal, Holly K. Given, and Carol Stoel. Authors would like to thank Dean Reza Abbaschian, Dean Joe Childers, Dallas Rabenstein, David Eastman, Victor Rodgers, Zhenbiao Yang, Vassilis Tsotras, Dimitri Morikis, Aaron Seitz, Jiayu Liao, David Carter, Jerry Schultz, Lisa Kohne, Bill Bingham, Mitch Boretz, Jhon Gonzalez, Michael Caputo, Michael Dang and Benjamin Davis for their support and help with the IGERT program. Authors would also like to thank Atena Zahedi for the sketch shown on the inside title page. Further, the authors would like to thank Simon Rees and Wayne Wheeler of Springer and Priyadarshini Senthilkumar (Scientific Publishing Preface

Services) for their efforts related with the publication of this book. The first author (BB) would like to acknowledge the support from National Science Foundation grants DGE 0903667 video bioinformatics, CNS 1330110 distributed sensing, learning and control, IIS 0905671 video data mining, IIS 0915270 performance prediction, CCF 0727129 bio-inspired computation, and DBI 0641076 morphological databases. The second author (PT) would like to acknowledge support from the Tobacco Related Disease Research Program of California (18XT-0167; 19XT-0151; 20XT-0118; 22RT-0217), the California Institute of Regenerative Medicine (CL1-00508), and NIH (R01 DA036493; R21 DA037365).

Riverside, CA, USA June 2015 Bir Bhanu Prue Talbot

List of Video Links with QR Codes

Software for Using QR Codes:

In order to scan a QR Code you must install a QR Code reader app on your smartphone. You can download an app on Google Play (Android Market), Blackberry AppWorld, App Store (iOS/iPhone), or Windows Phone Marketplace.

- Most QR scanning apps are free. Any app that can read barcodes should be able to process QR codes.
- Recommended for Android: Google Goggles Version 1.9.4
- Recommended for iOS: Quick Scan-QR Code Reader Version 1.1.5

Hyperlinks and QR Codes for each Video Referenced in the Book

IGERT on Video Bioinformatics YouTube Channel: https://www.youtube.com/channel/UCX9tYZRm-mGwhg6866FWizA

Chapter 6: Video 1: https://www.youtube.com/watch?v=3ylu3oHZEC4



Video 2: https://www.youtube.com/watch?v=sfNhuK9JSxA



Chapter 8: Video 1: https://www.youtube.com/watch?v=dQTkldB8lNk



Video 2: https://www.youtube.com/watch?v=NgJZ49xgev8



Video 3: https://www.youtube.com/watch?v=plb_CInFoEY



Video 4: https://www.youtube.com/watch?v=tHJ5JDcpkWY



Chapter 9: Video 1: https://www.youtube.com/watch?v=wN1F_K_2vsg



Video 2: https://www.youtube.com/watch?v=INrVYlemvCY



Video 3: https://www.youtube.com/watch?v=xUcU3HpiMwI



Video 4: https://www.youtube.com/watch?v=RAiqZtK80Dw



Video 5: https://www.youtube.com/watch?v=hYayvIvuzOM



Video 6: https://www.youtube.com/watch?v=KLaFIhkovPI



Video 7: https://www.youtube.com/watch?v=Orw_lUs8KFI



Video 8: https://www.youtube.com/watch?v=M-asAiwiLnM



Video 9: https://www.youtube.com/watch?v=OHD0j74qbk0



Video 10: https://www.youtube.com/watch?v=aZlbfqfDDyg



Video 11: https://www.youtube.com/watch?v=OTBt1WBqqGw



Chapter 11: Video 1: https://www.youtube.com/watch?v=l-Sb2iqzw1Y



Chapter 12: Video 1: https://www.youtube.com/watch?v=jvdL7L4nQIs



Chapter 15: Video 1: https://www.youtube.com/watch?v=pxg14SdvNbE



Video 2: https://www.youtube.com/watch?v=NnPHrGxJ7j0



Chapter 18: Video 1: https://www.youtube.com/watch?v=6KZc6e_EuCg



Contents

Part	t I Video Bioinformatics: An Introduction	
1	Live Imaging and Video Bioinformatics Bir Bhanu and Prue Talbot	3
2	Video Bioinformatics Methods for Analyzing Cell Dynamics:A SurveyNirmalya Ghosh	13
Part	t II Organismal Dynamics: Analyzing Brain Injury and Disease	
3	High- and Low-Level Contextual Modeling for the Detection of Mild Traumatic Brain Injury Anthony Bianchi, Bir Bhanu and Andre Obenaus	59
4	Automated Identification of Injury Dynamics After NeonatalHypoxia-Ischemia.Nirmalya Ghosh, Stephen Ashwal and Andre Obenaus	77
5	A Real-Time Analysis of Traumatic Brain Injury from T2 Weighted Magnetic Resonance Images Using a Symmetry-Based Algorithm Ehsan T. Esfahani, Devin W. McBride, Somayeh B. Shafiei and Andre Obenaus	99
6	Visualizing Cortical Tissue Optical Changes During Seizure Activity with Optical Coherence Tomography	119

Part III Dynamics of Stem Cells

7	Bio-Inspired Segmentation and Detection Methods for Human Embryonic Stem Cells Benjamin X. Guan, Bir Bhanu, Prue Talbot and Jo-Hao Weng	135
8	A Video Bioinformatics Method to Quantify Cell Spreading and Its Application to Cells Treated with Rho-Associated Protein Kinase and Blebbistatin Nikki Jo-Hao Weng, Rattapol Phandthong and Prue Talbot	151
9	Evaluation of Dynamic Cell Processes and Behavior Using Video Bioinformatics Tools Sabrina C. Lin, Henry Yip, Rattapol Phandthong, Barbara Davis and Prue Talbot	167
Part	t IV Dynamic Processes in Plant and Fungal Systems	
10	Video Bioinformatics: A New Dimension in QuantifyingPlant Cell Dynamics.Nolan Ung and Natasha V. Raikhel	189
11	Understanding Growth of Pollen Tube in Video Asongu L. Tambo, Bir Bhanu, Nan Luo and Zhenbiao Yang	201
12	Automatic Image Analysis Pipeline for Studying Growthin ArabidopsisKatya Mkrtchyan, Anirban Chakraborty, Min Liuand Amit Roy-Chowdhury	215
13	Quantitative Analyses Using Video Bioinformatics and Image Analysis Tools During Growth and Development in the Multicellular Fungus <i>Neurospora crassa</i> Ilva E. Cabrera, Asongu L. Tambo, Alberto C. Cruz, Benjamin X. Guan, Bir Bhanu and Katherine A. Borkovich	237
Part	t V Dynamics of Intracellular Molecules	
14	Quantification of the Dynamics of DNA Repair to Ionizing Radiation via Colocalization of 53BP1 and yH2AX Torsten Groesser, Gerald V. Fontenay, Ju Han, Hang Chang, Janice Pluth and Bahram Parvin	253
15	A Method to Regulate Cofilin Transport Using Optogenetics and Live Video Analysis Atena Zahedi, Vincent On and Iryna Ethell	265

Contents

Part VI Software, Systems and Databases

16	Integrated 5-D Cell Tracking and Linked Analytics in the FARSIGHT Open Source Toolkit Amine Merouane, Arunachalam Narayanaswamy and Badrinath Roysam	283
17	Video Bioinformatics Databases and Software	313
18	Understanding of the Biological Process of Nonverbal Communication: Facial Emotion and Expression Recognition Alberto C. Cruz, B. Bhanu and N.S. Thakoor	329
19	Identification and Retrieval of Moth Images Based on WingPatterns.Linan Feng, Bir Bhanu and John Heraty	349
Ind	ex	371

List of Figures

Figure 2.1	Conceptual diagram of a typical video bioinformatics	
E' 0.0	system	15
Figure 2.2	Interdependency of different low and midlevel image	17
	processing modules in bioinformatics	17
Figure 2.3	Different types of extraction, recombination,	
	and selection methods for static image features	
	and interrelations between them	23
Figure 2.4	Major pattern recognition techniques utilized in cellu-	
	lar and tissue classification in biological datasets	34
Figure 2.5	Bioinformatics modules in large-scale biological	
	dynamic databases that are often distributed across	
	the globe and datamined by complex content-based	
	queries over the Internet	42
Figure 3.1	Graphical representation of the Bayesian network	
	showing the dependencies of random variables.	
	Intuitive definition of distributions: A-anatomical	
	constraints, L_1 , L_2 —central location of injury for	
	injury one and two, H_I —time since the first event,	
	H_2 —time between first and second injury, Q —volume	
	(quantity) of injury with time, S_I , S_2 —spread for	
	first and second injury, M-max operator,	
	V—visual information, I—estimated injury.	
	Where $I = 1$ is absolute certainty of injury	
	and $I = 0$ is absolute certainty of NABM	
	(normal appearing brain matter)	62
Figure 3.2	A typical example of the fusion between visual and	
	contextual models in a rat mTBI. In this example,	
	the rat experienced two mTBIs several days apart	
	to opposite brain hemispheres. Top Probability	
	map after pSVM. Middle Probability map from	
	the contextual model. Bottom Fusion of the contextual	

	and visual models. Note that this illustrates that the	
	contextual model for repeated injuries progress	
	at different rates (compare nemispheres,	
F '	where the right herisphere was injured first)	00
Figure 3.3	Experimental design. a illustration of the mIBI	
	locations for the first (<i>right</i>) and second (<i>left</i>) injuries.	
	b Experimental mTBI and neuroimaging timeline.	
	A single mTBI was induced to the <i>right</i> cortex on day	
	0 (denoted as an *) in all animals. A second mTBI	
	was induced to the left cortex at either 3 or 7 days later	
	(*). MR imaging was performed 1 day post-first (1d),	
	1 day post-last (4 day rmTBI 3 day, 8 day rmTBI	
	7 day) and 14 day post-injury (17 day rmTBI 3 day,	
	21 day rmTBI 7 day) (red circles)	67
Figure 3.4	a ROC plot with multiple contextual inputs.	
	Results were evaluated after thresholding the output	
	probability map. b Dice plot with multiple contextual	
	inputs. The peak of the Dice curve (maximum dice	
	coefficient) is used to threshold the output probability	
	map. Legends in (b) are the same as in $(a) \dots \dots \dots$	68
Figure 3.5	Example outputs from the thresholded probability	
	maps (for single and repeated mTBI) with varying	
	levels of contextual input. The thresholds were selected	
	at the highest point on the dice curve for each	
	respective level of context. Each row is a coronal slice,	
	where the <i>top</i> is from a single mTBI and the <i>bottom</i> is	
	from a repeated mTBI. The colors represent the	
	following: Green true positive, Teal true negative,	
	<i>Red</i> false positive, <i>Brown</i> false negative	69
Figure 3.6	Overview of the proposed system where context	
	information is sent from one classifier to the next	
	at each time point. This information is then propagated	
	to the next time point	70
Figure 3.7	a Illustration of the proximity feature. V is the	
	observed voxel and the feature is the average	
	probability of the regions (R_1, R_2, R_3) . b Illustration	
	of the distance features. V is the observed voxel and	
	an example feature is the average probability	
	between P_1 and P_2 along the 45° ray	71
Figure 3.8	a Example MPM estimate. b Corresponding PMED	
	feature. Note that the values become more negative	
	toward the center of the object and more positive	
	farther away from the object	72

Figure 3.9	Dice coefficients after thresholding the posterior probability map at the end of each cascade of classifier (i.e., at each time point). This is the average of all the tests in the leave one out validation	72
Figure 3.10	Qualitative results of the proposed approach using dynamic and static contextual features. Each coronal slice is from a separate volume. <i>Color code: yellow =</i> true positive, <i>black =</i> true negative,	15
Figure 4.1	 orange = false negative, brown = false positive Comparative HII lesion dynamics results between HRS, SIRG and MWS computational methods. a The ischemic lesion detected (<i>red-border</i>) by manual and compared methods from representative serial T2 data are similar. b Lesion volumes at later time points are similar, but there are clear differences at the earlier imaging time points between computational performances for lesion location are compared for sensitivity, specificity, and similarity. HRS and SIRG performances were similar while 	74
Figure 4.2	MWS was not as accurate in all respects Long term lesion dynamics from serial T2 weighted MRI data: HRS detected lesion volumes closely matched with the ground-truth of manually derived results. Lesion volume initially decreases at 4–7 days post HII (see the initial sharp drop in injury volume) and then gradually increases and reaches its final size by 15–18 weeks. Note that, HRS includes ventricular hypertrophy (5–10 %) in late imaging time-points, which human observers typically	82
Figure 4.3	Spatiotemporal evolution of ischemic core-penumbra. a Temporal HRS detections of ischemic core (<i>red</i>) and penumbra (<i>blue</i>) from serial T2-weighted MRI at representative brain depth (from anterior-to-posterior scan) demonstrate evolving anatomical involvement over time. b HRS extracted volumetric lesion components identifies the proportional compositions of core-penumbra within the entire lesion over time post HII. Large proportion of salvageable penumbra is quickly converted into non-salvageable necrotic core by 7 days post HII.	83
Figure 4.4	Effect of hypothermia treatment in core-penumbra (CP) evolution. Hypothermia or normothermia	05

	(control group) treatment was applied for 24 h	
	immediately post HII and serial DWI data	
	was acquired to evaluate efficacy of HT therapy.	
	a HRS derived core-penumbra superimposed on DWI	
	data at 0 and 24 h clearly demonstrate that HT	
	restricted lesion expansion. Comparative HRS/DC	
	result in b mild and c moderate injury show delayed	
	injury progression in HT group with both severities	
	until 72 h when volume of HII lesion (and ischemic	
	core) rapidly increases and thus overall	
	reducing notential salvageability	88
Figure 4.5	Therapeutic effect of stem cells implanted in con-	00
I iguie 4.5	tralateral cortex 3 days post HIL a HRS derived	
	ischemic core (rad) and penumbra (blue) in serial	
	T2 weighted MDL date demonstrate martial restriction	
	of III emerging as implemented NSC migrates from	
	of HII expansion as implanted NSC migrates from	
	injection site (<i>aownwara yellow arrow</i>) to the tissues	
	close to HII (rightward yellow arrow). b Volumetric	
	summary of core-penumbra evolution suggests	
	presence of penumbra even 7–17 days post HII	
	(4–14 days post NSC implantation)	90
Figure 4.6	Stem cell detection using Susceptibility weighted	
	imaging (SWI). a SWI data at different brain levels	
	(at a single imaging time-point) visualized Fe-labeled	
	NSCs (hypointensity regions) implanted in contralat-	
	eral ventricles and migrated to the lesion (right) site	
	though cerebrospinal fluid. HRS could detect small	
	NSC regions that, in future studies can be tracked in	
	serial SWI data to monitor NSC activity. b Superim-	
	posed NSC locations derived by HRS from serial	
	T2-data (in Figure 4.5) demonstrated a clear migration	
	of NSC from injection site in contralateral cortex	
	towards lesion location—even as early	
	as 4d post implantation.	92
Figure 5.1	Representative T2-weighted MR images and	
8	pseudo-code for the automatic algorithm. a Represen-	
	tative Sham (surgery, no TBI) and a repetitive TBL	
	The MR images were obtained 1 day after the first	
	(1st TBI) and the second (2nd TBI) injury	
	The injured hemisphere (<i>dotted line</i> , sham)	
	is expanded to illustrate the manual ROI that were	
	used to delineate the brain injury lacion. After the first	
	TRI there was primarily adams present but after the	
	and TDI there appeared significant amounts of his d	
	2nd 1 bi there appeared significant amounts of blood	

	(see also Figure 5.5). b Pseudo code for our automated	
	algorithm that is composed of two primary	
	components, skull stripping (see Figure 5.2) followed	
	by lesion detection (see Figure 5.3)	103
Figure 5.2	Primary skull stripping components. Level set initial-	
U	ization: a inputs for skull stripping included	
	both T2-weighted MR images and the quantitative	
	T2 map (T2 values, ms), b Foreground separations.	
	c Morphological cleaning composed of cumulative	
	roundness index (CRI) and delineation of the brain	
	bounding box d 2D results were validated using 3D	
	connectivity testing Level set algorithm: here the final	
	brain area is found for each slice and then summed	
	to identify the brain volume in 3D (see manuscript	
	for additional details)	107
Figure 5.3	Lesion detection components for naive 1st TBL1 day	107
i iguie 515	post injury and 2nd TBL 3 days post injury examples	
	a Firstly, the algorithm identifies the axis of symmetry \mathbf{a}	
	b Seed point selections for edema and blood using	
	asymmetry c Region growing/shrinking based on the	
	intensity of asymmetry for edema and blood, d Final	
	lesion area(s) of brain tissues that contain edema and	
	blood. <i>Black line</i> demarcates the brain bound found	
	from skull stripping	109
Figure 5.4	Region growing/shrinking. The seeds identified (see	
8	Figure 5.3) from the asymmetry undergo diffusion of	
	T2 asymmetric values for region growing/shrinking for	
	either blood or edema. This example is from a TBI	
	animal. The <i>black bar</i> on the color bar is the	
	threshold	112
Figure 5.5	Comparison of lesion visualization. Temporal (1, 3 and	
C	14 days post injury) of repetitive TBI and sham	
	animals where compared to naive animals in which no	
	lesion was identified. Both T2 maps and T2-weighted	
	image (T2WI) illustrate the raw data with the results of	
	our automated algorithm shown in the two <i>right</i>	
	<i>panels</i> . As can be seen, the algorithm allows accurate	
	demarcation of evolving blood and edema in brain	
	tissues that were validated against the gold-standard	
	manual lesion detection	113
Figure 6.1	Comparison of resolution and imaging depth for	
-	functional magnetic resonance imaging (fMRI),	
	ultrasound, OCT, and confocal microscopy	120
Figure 6.2	Michelson interferometer with a beam splitter	121

Figure 6.3	a SD-OCT System Diagram: superluminecent diodes	
	vanometers (gm) grating (gr) Image of a mouse skull	
	with raster scanning pattern for volume data acquisi-	
	tion represented by solid and dashed arrows b dB	
	gravesale OCT image: thinned skull (s) cerebral cortex	
	(atx) a compute collection (a), coole here $0.5 \text{ mm} \cdot a 2D.4 \times a$	
	(c(x), corpus canosum (cc), scale bar. 0.5 mm. c 5D 4 ~	100
Eiser 6.4	2 × 2 mm volume rendering of 200 grayscale images	123
Figure 0.4	A-line preprocessing schematic. a Fringe magnitude	
	with respect to the pixel array for one A-line.	
	b Wavelength array W with respect to wavenumber	
	used to correct wavenumber assignment in each pixel	
	in (a). IP: Interpolation step of A with respect to B.	
	c Resulting dB intensity A-line with depth (mirror	
	image removed). d Resulting fringe magnitude plot	
	post-IP with correct wavenumber assignment. FFT:	
	Fast Fourier Transform	124
Figure 6.5	Normalized average intensity (N) from an ROI plotted	
	over time. The three arrows from <i>left</i> to <i>right</i> represent	
	saline injection, PTZ injection, and initiation of full	
	seizure, respectively. The shaded region is the 2SD	
	of the 10 min baseline	126
Figure 6.6	SD-OCT grayscale intensity volume $(4 \times 2 \times 2 \text{ mm})$	
	of mouse cortical tissue (left) and its fOCT volume	
	(48 min) (right). Color bar represents ratio	
	values \pm 50 % away from a 0 % change at baseline	128
Figure 6.7	MIP frames of cortical volumes. Numbers are time	
-	(min) of volume. 0-32: Control, 4-56: Seizure activity,	
	64: post-overdose. Color is scaled to the color bar in	
	Figure 6.6 and represents ratio values ± 50 % away	
	from a 0 % change at baseline. Scale bar 1 mm.	
	See Media 1: https://www.youtube.com/watch?v=	
	3vlu3oHZEC4	129
Figure 6.8	Frames of cortical tissue volumes with functional maps	
C	applied. Volumes are $4 \times 2 \times 2$ mm Numbers are time	
	(min) of volume. 0–32: Control. 40–56: Seizure	
	activity, 64: post-overdose. <i>Color bar</i> percent change	
	from baseline (0 %) saturating at ± 50 %. Scale bar 1	
	mm See Media 2: https://www.youtube.com/watch?y=	
	sfNhuK9ISxA	130
Figure 7.1	a Unattached single stem cell: h substrate-attached	150
i iguie 7.1	single stem cell: c dynamically blebbing stem cell.	
	d apontotically blebbing stem cell: e apontotic stem	
	call	136
		130

List of Figures

Figure 7.2	 a Image of hESCs taken with 10× objective; b Image of hESCs taken with 20× objective; c Image 	
	of hESCs taken with 40× objective	137
Figure 7.3	True positive rate versus false positive rate (ROC	
-	curves) (Note the red squares are the optimal results	
	of the proposed method)	145
Figure 7.4	A sample of results of unattached single stem cell	
	detection approach	146
Figure 7.5	a – c Median filter-induced texture-based method results	
-	for $10\times$, $20\times$, and $40\times$ images respectively;	
	(d - f) entropy-based k-means method results	
	for 10^{\times} , 20^{\times} , and 40^{\times} images, respectively;	
	(g-i) gradient magnitude distribution-based method	
	results for 10^{\times} , 20^{\times} , and 40^{\times} images, respectively	147
Figure 8.1	Comparison of CL-Quant segmentation protocol and	
e	ground truth. hESC were plated in mTeSR medium in	
	a 35 mm dish and incubated in a BioStation IM for 4 h.	
	a Phase contrast images modified with ImageJ to	
	remove text labels and the same images with masks	
	applied using the professional CL-Ouant protocol.	
	b Graph showing cell area (spreading) in pixels for	
	CL-Ouant derived data and the ground truth. The areas	
	obtained from the two methods were in good agree-	
	ment	156
Figure 8.2	Cell area (spreading) was successfully masked by the	
8	professional CL-Quant protocol in different experi-	
	mental conditions, hESC were treated with ROCK	
	inhibitors (Y27632 and H1152) or blebbistatin.	
	incubated in a BioStation IM for 4 h, and imaged at 1	
	min intervals. Phase contrast images and the corre-	
	sponding masked images are shown for hESC treated	
	with: a control medium b Y27632 c H1152	
	and d blebbistatin	158
Figure 83	The morphology and spreading of hESC was affected	150
I igule 0.5	by treatment with ROCK inhibitors (Y27632 and	
	H1152) and blebbistatin in two experiments. Spreading	
	was measured using the professional CL_Quant pro-	
	tocol a Phase contrast images from the first experi-	
	ment showed that treated cells were morphologically	
	different than the control b The rate of spreading and	
	the fold increase in spread area was greater in V27622	
	and blabbistatin tracted calls then in controls. a Dhace	
	contrast images of control and treated cells in the	
	contrast images of control and treated cells in the	
	second experiment snowed morphological changes in	

	the treated groups. d The fold increase in spread area was greater in the treated cells than in the controls in the second experiment; however, the effect of Y27632 was not as great as previously seen. Data in (b) and (d) are plotted as a percentage of the area in the first frame. Each point is the mean \pm the SEM	159
Figure 8.4	Comparison of the professional and user-generated cell spreading protocols. a Phase contrast micrographs of hESC treated with Y27632 and the corresponding masks created with the professional and user-generated protocols. b Comparison of ground truth to area (spreading) data obtained with the professional proto- col in control and treated groups. c Comparison of ground truth to area (spreading) data obtained with the user generated protocol in control and treated groups	160
Figure 8.5	Differences in cell morphology showing why treated hESC are more difficult to segment than control cells. a Phase contrast image of hESC colonies taken at 60 min of incubation. Segmentation of the image in "(a)" created with the user-generated protocol (b) and the professional protocol (c). d Phase contrast image of hESC colonies treated with Y27632 for 60 min. The cells have many thin surface projections not present on controls. Segmentation of the image in "(c)" with the user-generated protocol (e) and the professional protocol (f)	160
Figure 9.1	Growth of hiPSC colonies over 48 h. a–c Phase contrast images of control iPSC colonies at various times during growth. d–f The same control images segmented using a CL-Quant protocol developed in our lab. g–i Phase contrast images of smoke treated iPSC colonies at various times during growth. j–l The same treatment group images masked using the same CL-Quant protocol as applied to control colonies. m Graph of control and treated cells showing growth rate. Data are means and standard errors of three	101
Figure 9.2	experiments. <i>CN</i> control	172

Figure 9.3	showing confluency rate. Data are means and standard errors of three experiments. <i>CN</i> control	173
	image of a growing hESC colony during migration.	
	b Masked phase contrast image of a shrinking hESC	
	colony during migration. c Masked phase contrast	
	image of a dying hESC colony during migration. d ,	
	e Graphs showing total displacement/distance traveled	
	for each control and treated colonies. All CL-Quant	
	masking and tracking of colonies were done by	
	applying a tracking recipe developed by our lab. CN	
	control	175
Figure 9.4	Diagram of Ibidi gap closure culture inserts	176
Figure 9.5	Gap closure for mNSC and NTERA2 cells. a – c Phase	
	contrast images of mNSC at various 3 times during gap	
	closure. d – f The same images after segmentation using	
	a protocol developed in our lab with CL-Quant	
	software. g, h Graph showing rate of gap closure for	
	control (<i>blue</i>) and treated NTERA2 cells (<i>red</i>) and the	
	corresponding ground-truth (<i>dotted lines</i>) obtained	
	using imageJ. I Graph of miNSC migration by mon-	
	itoring percent of gap closure over 44 n. J Graph of	
	NIERA2 cell migration by monitoring percent of gap	
	closure over 44 n. Data are means and standard errors	177
	Of three experiments.	1//
Figure 9.6	Production of reactive oxygen species in hPF.	
	a–a Merged phase contrast and fluorescent images at	
	be with MitoSox Bod a Crark showing fluoressance	
	intensity in control and treated cells over time. CN	
	control	170
Figure 0.7	Quantification of neurons in neural differentiation	1/9
Figure 9.7	assay a c Phase contrast images of mNSC at various	
	times during incubation $d_f CL_Ouant software$	
	masking of mNSC phase contrast images to identify	
	the neurons within each frame a Graph showing	
	quantification results obtained using the CL-Quant	
	software was similar to the ground-truth obtained using	
	the Image I software	181
Figure 10.1	Pattern recognition of membrane compartments in leaf	101
	epidermal tissue at cellular and subcellular resolution	
	Merged confocal laser microscopic images show	
	Arabidopsis leaf epidermal cells. Top section	
	GFP-2xFYVE plants were imaged at 403 magnifica-	
	- r	

	٠	٠	٠
XXV	1	1	1

	 tion (scale bar = 20 mm) and images analyzed by the endomembrane script. <i>Bottom section</i> GFP-PEN1 plants were imaged at 203 magnification (scale bar = 50 mm) and images analyzed by the plasma membrane microdomain script. a and b, Merged pseudo images. c and d, Recognition of epidermal cells is shown by <i>colored lines</i>. e, Recognition of GFP-2xFYVE-labeled endosomal compartments is shown by <i>colored circles</i>. f, Recognition of (b). Graminis induced GFP-PEN1 accumulation beneath attempted fungal entry sites 	
	(indicated by <i>arrowheads</i>) is shown by <i>colored circles</i> . Color coding is random, different colors indicate	
Figure 10.2	individual cells compartments [25] Cell wall stained by Propidium iodide: a Raw data of a	196
	segmentation (from local minima) shown in random color. d Final segmentation result. e Classification of the segments. f Manually generated ground-truth for	
	segmentation and classification (modified from Liu	
Figure 10.3	et al. [32])	197
	of the same tracking result from a cell tagged with GFP-Rab6. a 3D trajectories of the detected objects. The colour indicates the time window when an object was detected. The z projection of the first 3D image of the time-series is shown below the trajectories to visualize the cell morphology (modified from Racine	
Figure 11.1	et al. [35])	197
Figure 11.2	part of the process	203
	F-Actin network and stops the transportation of	

	ROPGEF. With diminished localized concentration of ROPGEF, global conversion of ROP1-GTP into	
F '	ROP1-GDP prevails	205
Figure 11.3	Representation of ROPI-GIP activity as a 2-state	
	Markov process. The transition from	
	the start of executoric which leads to growth	206
Eigura 11 4	Images showing the results of tin tracking for two	200
Figure 11.4	avage showing the results of up tracking for two	
	The number of images in each row denotes the number	
	of detected growth cycles. Each image shows the initial	
	tin shape (<i>wellow</i>), the models estimate of the final tin	
	shape (<i>genow</i>), the models estimate of the final up shape (<i>genow</i>) and the observed tip shape (<i>red</i>). The	
	first experiment shows straight growth while the	
	second shows both straight and turning tin behavior	
	Please see Supplemental Materials for a video	210
Figure 11 5	ROC plot of average accuracy of predicted shape as	210
rigule 11.5	nixel acceptance threshold increases from 0 to 10	
	pixels (0–0.58 µm) <i>Error bars</i> indicate standard	
	deviation Statistics are obtained for approximately 64	
	predicted shapes from experiment 1 in Fig. 11.4. Over	
	90 % accuracy is achieved within 0.1 µm (5.43 % of	
	cell radius)	211
Figure 11.6	Elongation trend in the pollen tube shown in Fig-	
8	ure 11.4, row 1. The <i>blue curve</i> is the cumulative sum	
	of the measured affine parameter for elongation (b) and	
	the <i>red curve (dotted)</i> shows the fit of Eq. (11.6) to the	
	data for each growth cycle (between <i>vertical bars</i>).	
	Curve agreement indicates that the Gompertz function	
	is suitable for explaining observed cell wall dynam-	
	ics	211
Figure 12.1	The general workflow in the image analysis pipeline	217
Figure 12.2	Registration methodology in sparse confocal image	
	stacks-a SAM located at the tip Arabidopsis shoot,	
	b raw images taken at consecutive time instances,	
	c segmented images after applying watershed seg-	
	mentation, d estimation of the corresponding landmark	
	point pairs, and e bottom image is registered to the top	
	image (the same color arrows represent the same	
	cell)	219
Figure 12.3	a , d The local graphs G_1 and G_2 at time t and $t + 1$ with	
	the central cells C and C' , respectively, and clockwise	
	ordered neighboring cell vertices N_1, \ldots, N_6 and	
	N'_1, \ldots, N'_6 , b , c two enlarged triangle subgraphs with	

		indicated features N_{i_1}, N_{i_2} — i_1 th and i_2 th neighboring cells of C, N'_1, N'_2 — i_1 th and i_2 th neighboring cells of	
		$C', \theta_{N_i}, c_{N_i}, (t)$ —angle between $\overline{N_i, C}$ and $\overline{N_i, C}$.	
		$\theta_{N_1,C,N_2}(t)$ and $\overline{N'_1,C'_2}(t)$ and $\overline{N'_2,C'_2}(t)$	
		$V_{j_1}, C, N_{j_2}(t)$ and $V_{j_1} = 0$ and $V_{j_2} = 0$, $V_{j_1}(t)$, $I_{aver}(t)$ preighbor edge lengths $I_{aver}(t+1)$	
		$U_{C,N_{i_2}}(t) = \text{heighton edge lengths}, \ U_{C',N_{j_1}}(t+1),$	
		$l_{C',N'_{j_1}}(t+1)$ —edge lengths, $A_{N_{i_1}}(t), A_{N_{i_2}}(t)$ —areas	
		of the cells $N_{i_1}, N_{i_2}, A_{N'_{j_1}}(t+1), A_{N'_{j_2}}(t+1)$ —areas	
		of the cells N'_{j_1}, N'_{j_2}	220
Figure	12.4	a Raw consecutive images (the same color arrows	
		represent the same cells) and tracking results obtained	
		b without registration c with MIRIT registration,	
		a with manual registration, and e with proposed	
		automatic registration. The same colors represent the	
		consecutive images g Length of cell lineages	222
Figure	12.5	The overall diagram of the adaptive cell segmentation	
Ingule	12.0	and tracking scheme.	223
Figure	12.6	Optimization Scheme. a Schematic showing how to	
U		integrate the spatial and temporal trackers for 4D	
		image stacks. b Adaptive segmentation and tracking	
		scheme for a certain image slices S_k^t (the <i>k</i> th slice at the	
		<i>t</i> time point)	225
Figure	12.7	The segmentation and tracking results using adaptive	
т.	10.0	method	227
Figure	12.8	The segmentation and tracking results in 3D stacks at	
		selected time instances. The segmented cells snown in	
		and fourth slices) represent same cells	227
Figure	12.0	Visualization of the AOVT-based 3D reconstruction of	221
Ingule	12.7	SAM cell cluster a Visualization of the 3D recon-	
		structed structure of a cluster of around 220 closely	
		packed cells using convex polyhedron approximations	
		of the densely clustered data points for each cell, as	
		obtained from the proposed 3D reconstruction scheme,	
		b a subset of cells from the same tissue	232
Figure	12.10	Reconstruction of a cluster of cells using Euclidean	
		distance-based Voronoi tessellation and the proposed	
		AQVT for comparison of the 3D reconstruction	
		accuracy. a Segmented and tracked cell slices for a	
		cluster of 52 cells from the L1 and L2 layers of SAM.	
		A dense confocal image stack is subsampled at	
		a z-resolution of 1.35 μ m to mimic the 'z-sparsity'	

	observed in a typical Live-Imaging scenario. The slices belonging to the same cell are marked with the same number to show the tracking results. b 3D recon- structed structure for a subset of these cells when reconstructed using the Euclidean distance-based Voronoi Tessellation. c The AQVT-based reconstruc-	
Figure 13.1	tion result for the same cell cluster	233
Figure 13.2	cycle Automated analysis of hyphal cell compartments. (<i>Left</i>) Image of hyphae stained with Calcoflour-white.	239
Figure 12.2	(<i>Right</i>) Demonstration of automatic analysis software results before selection of <i>Neurospora</i> regions	242
Figure 13.5	midline of the hypha over time. The <i>green dashed line</i> is the smoothed measurements from the <i>solid blue line</i> . The smoothed measurements were obtained with a moving average. b Rate of change in length of the hypha over time (velocity). The results show that	
Figure 13.4	hyphal growth is oscillating over time Conidia images. Original image (<i>left</i>) and after being	244
Figure 13.5	Halo intensity histogram. The halo intensity distribu- tion is located at the <i>right</i> of the histogram, while the background distribution is on the <i>left</i> . As the result, the halo stands out as part of detected cell regions by	245
Figure 14.1	Multifactorial experimental design and associated images can be visualized through the web. Each row corresponds to thumbnail views of samples prepared and imaged with the same set of experimental factors	240
Figure 14.2	a An example of γ H2AX and 53BP1 co-localization on a cell-by-cell basis. <i>Blue</i> is the DAPI (nuclear) stained channel. <i>Green</i> corresponds to γ H2AX, and red corresponds to 53BP1. b Each nucleus and the	251
Figure 14.3	Steps in delineating clumps of nuclei	255 257
Figure 14.4	Detection of foci on a cell-by-cell basis for two nuclei	258

Figure 14.5	Representative phenotypic signatures of the DNA	
	repair sites, as a function of time, indicates that the	
	repair mechanism is typically complete within the first	
	15 h following the radiation exposure	260
Figure 14.6	a Amount of colocalization of 53BP1 (source channel)	
	and yH2AX (target channel) foci in 2D cultures of	
	stationary MCF10A cells after iron ion exposure over	
	time. The amount of foci overlap is given in paren-	
	thesis. Data points represent the average of three	
	independent experiments. b Amount of colocalization	
	of 53BP1 (search channel) and vH2AX (target chan-	
	nel) foci in 2D cultures of cycling MCF10A cells after	
	iron ion exposure over time. Amount of foci overlap is	
	given in parenthesis. Data points represent the average	
	of two independent experiments. c Amount of	
	co-localization of 53BP1 (source channel) and γ -	
	H2AX (target channel) foci in 2D cultures of stationary	
	MCF10A cells after iron ion exposure over time for	
	50 % (0.5) foci overlap. Error bars represent the	
	standard deviation of three independent experiments	261
Figure 15.1	Cofilin Modulation in Dendritic Spines. The activity of	
	cofilin can be regulated by altering its phosphorylation	
	state: (1) NMDAR-mediated Ca^{2+} influx, which acti-	
	vates calcineurin and dephosphorylates cofilin by SSH;	
	(2) Rac1-PAK1-LIMK signaling cascade downstream	
	of Ephrin/EphB receptors, which phosphorylates	
	cofilin; and (3) activation via a light-controllable	
	PA-Rac1. Pathway (1, green) can lead to F-actin	
	severing and loss and/or reorganization of dendritic	
	spines, or formation of cofilin-actin aggregates called	
	rods. Pathways (2, red) and (3) are proposed to lead to	
	polymerization of F-actin and stabilization of spines	267
Figure 15.2	Experimental schematics of cell culture, transfection,	
	and photoactivation methods of the hippocampal	
	neurons. a Primary hippocampal neurons were isolated	
	from the hippocampus of embryonic day 15-16	
	(E15-16) mice, dissociated enzymatically, plated onto	
	poly-D-lysine and laminin-plated glass coverslips, and	
	allowed to mature in culture for 1-4 weeks. b The	
	cultures were transfected at 14 days in vitro (DIV)	
	using calcium phosphate method to overexpress	
	PA-Rac and GFP-tagged-wt-Cofilin (shown in green).	
	The live changes in cofilin localization were recorded	
	by tracking the GFP signal, while simultaneously	