International Workshop on Quantitative Biology 2013

Nov. 25th (mon), 2013
Icho-kaikan, Suita Campus
Osaka University

Supported by
CDB, RIKEN JPN

RIKEN Kobe since 2000
Keio University
Dear Colleagues,

International Workshop on Quantitative Biology 2013 focuses on remarkable achievements in measuring, modeling and controlling biological processes, organized by motivated members of Japanese Society for Quantitative Biology with their friends.

Worldwide experts of quantitative biology have been invited to participate in the workshop. This year our new challenge is to set up one common scope for the whole of this workshop instead of dividing sessions into different topics. By putting forward the significance of Temporal Development and Evolution of Biological processes in the wide range of scale - from single molecule, intracellular, cellular network, organs, or individual - we hope to help opening new insights of these biological events and the new insights that may come across attendee’s mind. At the same time, we propose one specific aspect to enjoy our workshop that ‘vivo vs art’: we pay much effort to developing the microscopic tools to gather enough data to solve a biological problem. On the other hand, we have started to synthesize the way to control a living matter with artificial molecular network, also to solve a biological problem. These two directions will meet in the field of Quantitative Biology to solve a problem with inverse or direct strategy.

All talks and poster sessions are sponsored by "Center for Developmental Biology (CDB) meeting” grant of CDB, RIKEN, and Keio University, in Japan.

The talks during the one-day workshop will be in English to help the international audience that would be attending the workshop.

We plan to have poster sessions as well and so we encourage students and young researchers to participate at the workshop.

This special one-day international sessions will be held on the 25th of November 2013, together with the 6th Annual meeting of the Japanese Society for Quantitative Biology in Osaka (22nd - 24th November 2013).

Organizers,
Yoshiyuki Arai (Osaka University)
Viji Mythily Draviam (University of Cambridge)
Miki Ebisuya (CDB, RIKEN)
Akira Funahashi (Keio University)
Noriko Hiroi (Keio University)
Ziya Kalay (Kyoto University)
Rinshi Kasai (Kyoto University)
Chun-Biu Li (Hokkaido University)
Hiroaki Takagi (Nara Medical University)
We greatly appreciate Ms. Yoko Toyama, CDB, RIKEN (Japan), the general affairs section and the supplies section in the school of Science and Technology, Keio University (Japan), for their diligent work in organization of the workshop. Special thanks are due to the group members of Ebisuya Unit (CDB, RIKEN), Funahashi Lab (Keio University) and core-members of Japanese Society for Quantitative Biology for their valuable help in the organisation of the workshop.
The Workshop Venue
Main Hall (Talks)
Meeting Room (Posters)
Reception will be set up at the entrance of main hall, 3F.
Access to Suita Campus

• Train: 15’~30’ east on foot from Kita-Senri, Hankyu Senri Line.
• Monorail: 5’~15’ on foot from Monorail Handai-Byoin-Mae.
• Hankyu Bus: Bus for Handai-Honbu-Mae or Ibaraki-Mihogaoka at Senri-Chuo. Exit at Handai-Honbu-Mae. (5’~15’)
• Kintetsu Bus: Bus for Handai-Honbu-Mae from Ibaraki-Shi, Hankyu Kyoto Line. Exit at Handai-Honbu-Mae. (5’~15’)

Train / Flight connections

Train
• From Shin-Osaka Station
  Subway Midosuji Line to Senri-Chuo, → Monorail. Exit at Handai-Byoin-Mae. (1 hr.)

Flight
• From Kansai International Airport (3 choices)
  (1) JR line to Osaka, → subway Midosuji Line to Senri-Chuo, → Monorail to Handai-Byoin-Mae. (2 hr.)
  (2) Nankai Line to Namba, → subway Midosuji Line to Senri-Chuo, → Monorail to Handai-Byoin-Mae. (2 hr.)
  (3) Airport Bus to Osaka Airport, → Monorail to Handai-Byoin-Mae. (2 hr. 30’)

Domestic & International Airport (Haneda)
• From Osaka Airport (Itami)
  Monorail to Handai-Byoin-Mae. (1 hr.)
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## Abstracts of Posters

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## Introduction of Organizers

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Program - The significance of Temporal Development and Evolution of Biological processes in the wide range of scale -

Opening Remarks (9:50 ~ 10:00)

Session I.
10:00 - 12:00
chair: Miki Ebisuya

[1] Dr. Avigdor Eldar (Tel Aviv University, Israel)
Title: "Social evolution shapes the diversification of bacterial intercellular signaling"

[2] Dr. Naoki Irie (University of Tokyo, Japan)
Title: "Flexibility in early animal developmental system"

[3] Dr. Yu-Chiun Wang (Center for Developmental Biology, RIKEN, Japan)
Title: "Quantitative 4D analyses of epithelial folding during Drosophila gastrulation"

Poster Session I & Lunch Break (12:00 - 13:30)

Short Talk Session
13:30 - 13:45
co-chair: Noriko Hiroi & Akira Funahashi

[1] Masashi Kajita (The University of Tokyo)
Title: "Modeling of Self and Non-self Discrimination by T-cells"

Session II.
13:45 - 15:30
chair: Chun-Biu Li

[1] Dr. Yoshiyuki Arai (Osaka University, Japan)
Title: "Realtime fluorescence and chemiluminescence imaging with optogenetic activation in living cells"

[2] Dr. Hiroaki Takagi (Nara Medical University, Japan)
Title: "Relevance of spontaneous migration to tactic response in Dictyostelium cells"

[3] Dr. Rinshi Kasai (Kyoto University, Japan)
Title: "Reversible dimer formation of G-protein coupled receptor: Quantitative evaluation by a single fluorescent molecule imaging"

Coffee Break (15:30 - 15:50)

Session III.
15:50 - 17:50
chair: Ziya Kalay

[1] Dr. Craig Jolley (Center for Developmental Biology, RIKEN, Japan)
Title: "Mammalian circadian oscillations at the cellular and tissue scales"

[2] Dr. Tomonobu Watanabe (Quantitative Biology Center, RIKEN, Japan)
Title: "Development of fluorescent protein to sense physical parameters"

[3] Dr. Andre Leier (Okinawa Institute of Science and Technology, Japan)
Title: "Simulating diffusion in crowded environments with multifractional Brownian motion"

Closing Remarks (17:50 ~ 18:00)

Free Discussion at the Poster Session Room (18:00 - 20:00)

Venue

Talks: Main Hall
Posters: Meeting Room (Large)

All Workshop Venue in the 3rd floor of Icho-Kaikan.
The Floor Map is in p3 of this booklet.
Opening Remarks

by IWQB2013 organization committee

We thank to all contributors of this workshop with us by giving your talks, posters and your attention to the presentations at here 25th November, Osaka.

Even each session has no specific title, there still will be a focussing point of each session. Session I provides the topics of multicellular level, Session II focuses from single molecule to cellular level dynamics. Session III will give us the other point of view from the environment \textit{in vivo} and the response to the environment of cellular system.

The other two significant achievement of this year’s IWQB is that we will have a short talk by an elected topic among the submitted abstracts by PhD students and PostDocs. Another achievement is that we have succeeded to open an opportunity to publish our papers from Frontiers in Physiology, which is an open access publisher partnered with Nature Publishing Group.

We hope all of you will enjoy much of today’s talks and discussion opportunity, and will use the publication opportunity, too, to make visible your footprint on the field of Quantitative Biology.
Session 1

10:00 - 12:00 at Main Hall

Chair: Miki Ebisuya

Message from Chair:
This session deals with higher order biological processes, such as animal development, evolution, and social behavior. These research topics are normally not quantitative but qualitative because they are too complex to be quantitative. However, the speakers in this session are tackling the complex (and therefore exciting) issues with quantitative methods. The following are the particular complex biological processes that will be covered in the session:

- Evolution of social behaviors in bacteria (1st seminar);
- The relationship between ontogeny and phylogeny (2nd seminar);
- Tissue morphogenesis in Drosophila embryo (3rd seminar).

I hope you will enjoy the session.

Chair
Dr. Miki Ebisuya

Unit Leader
Reconstitutive Developmental Biology, Center Director’s Strategic Program, Center for Developmental Biology, RIKEN
Social evolution shapes the diversification of bacterial intercellular signaling

Avigdor Eldar
Tel Aviv University, Israel

Abstract
Microbial “quorum sensing” (QS) systems, where microbes produce and respond to a signaling molecule, enable cells to sense their local density and coordinate a cooperative response to their environment. QS-dependent cooperation is prone to exploitation by a signal reception mutant that avoids the costs of Quorum-response but rips the cooperative benefits. Cheater null alleles are rare in wild isolates but in contrast, many QS systems show intraspecific allelic divergence of receptor-signal locus in which all alleles are functional but a signaling molecule from one allelic type (known as pherotype) activates its cognate receptor but fails to activate those of other receptor variants in the same species. The phylogeny of the QS locus often deviates from the house-keeping phylogeny, indicating some level of selection for horizontal gene transfer (HGT). It is unclear what evolutionary mechanisms explain the lack of null alleles and the existence and phylogenetic patterns of multiple different pherotypes and how does this evolutionary pattern relate to the cooperative behavior of the cells. In this talk I would present a combination of bioinformatical analysis, theoretical modeling and experiments in the model bacteria /B. subtilis/ that explain this evolutionary pattern. We demonstrate that a /B. subtilis/ reception null mutant is a cheater of the wild-type – in well-mixed environments, it invades into the wild-type population and lead to a general reduction of fitness. The wild-type resist the invasion if the population is spatially structured. When strains from different pherotypes compete, we find that the minority pherotype always invade the majority pherotype and that this invasion will occur also in highly structured environment. Our results can be explained by a model that requires a QS-dependent cooperative behavior. We therefore conclude that QS is a cooperative trait but that the strong structure of the environment prevents the evolution of cheaters, while allowing the evolution of new pherotypes and their rapid spread through horizontal gene transfer.

Keywords:

Dr. Avigdor Eldar
avigdor@gmail.com
Flexibility in early animal developmental system

Naoki Irie
Department of Biological Sciences, the University of Tokyo, Japan

Abstract
Recent advancements in developmental biology highlighted cascade-like molecular mechanisms taking place during embryogenesis, once again supporting our “mechanistic view of life”. On the other hand, apparent discrepancy with this viewpoint was suggested from recent studies in the field of Evo-Devo (Evolutionary Developmental Biology). Taking advantages of state of art technologies, these studies, including ours culminated in tackling the issue of classic argument on how the relationship between ontogeny and phylogeny can be formulated.

Our study demonstrated that the hourglass model best explains the divergence pattern of gene expression profiles between embryos of four vertebrate species (mouse, chicken, xenopus, and zebrafish), and for the first time, identified the stages having the most conserved expression profiles, namely, pharyngular stages. This was further tested with the animal of unique body plan: turtle. Starting from genome sequencing, we will provide our current progress on this project together with discussion.

Keywords: Evo-Devo, hourglass model

Dr. Naoki Irie
irie@biol.s.u-tokyo.ac.jp
Quantitative 4D analyses of epithelial folding during *Drosophila* gastrulation

Zia Khan¹, Yu-Chiun Wang²,³,⁴, Eric F. Wischaus²,³ and Matthias Kaschube⁵

[1] Department of Human Genetics, University of Chicago, Chicago, IL 60637, USA
[2] Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA
[3] The Howard Hughes Medical Institute, Moffett Laboratory 435, Princeton University, Princeton, NJ 08544, USA
[4] Laboratory for Epigenetic Morphogenesis, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan
[5] Frankfurt Institute for Advanced Studies, Faculty of Computer Science and Mathematics, Goethe University, D-60438 Frankfurt am Main Germany

Abstract

Quantitative 3D analyses of cellular dynamics using time-lapse microscopy data have the potential to greatly advance our understanding of complex morphogenetic processes. Yet, obtaining these measurements remains technically challenging due to the intrinsic noise in these data. We developed a new software tool called EDGE4D that automates segmentation, tracking, and quantitative measurement of cellular properties at single cell resolution. EDGE4D introduces several novel algorithmic strategies, such as techniques that leverage the duality between surface meshes and binary image volumes, that allow reliable, systematic, and dynamic analyses of a large number of densely packed membrane-labeled cells, analyses that were previously unattainable by existing methods. We used EDGE4D in a pilot study that tackles the complex cell shape changes and rapid tissue movements that occur during the formation of the dorsal folds in the gastrulating Drosophila embryo. Although the depth of the tissue structure and the poor signal-to-noise imaging conditions render quantitative analysis challenging, EDGE4D successfully confirms known features of this morphogenetic event and uncovers previously undocumented patterns of tissue and cellular dynamics. Furthermore, EDGE4D enables the first 3D, dynamic analysis of cell contact surfaces, allowing us to propose new hypotheses on how internal and external forces might contribute to cell shape changes during dorsal fold formation. EDGE4D addresses a growing need for new generally applicable tools and algorithms for processing and quantitation of 3D time-lapse imaging data. Tools such as EDGE4D hold the promise to transform recent advances in time-lapse imaging technologies into powerful means of biological discovery.

**Keywords**: Automated image processing, 3D reconstruction of membrane-labeled cells, Cell shape change, Epithelial folding, *Drosophila* gastrulation

Dr. Yu-Chiun Wang

yu-chiun.wang@riken.jp
Poster Session I
12:00 - 13:30 at Meeting Room (Large)

Abstracts are listed in page 26 ~ 41
Short Talk Session

13:30 - 13:45 at Main Hall

Co-chairs: Noriko Hiroi & Akira Funahashi

Message from Co-Chairs:
The short talk presentation will be given by an excellent PhD student/PostDoc who has submitted an Abstract for their poster presentation.
We are planning to invite the short talk speaker for submitting one’s paper to Frontiers Research Topic “Quantitative Biology”

Co-Chairs
Dr. Noriko Hiroi
Systems Biology Laboratory,
Biosciences and Informatics Department,
Keio University

Dr. Akira Funahashi
Systems Biology Laboratory,
Biosciences and Informatics Department,
Keio University
Modeling of self and non-self discrimination by T-cells

Masahi Kajita¹, Kazuyuki Aihara¹,² and Tetsuya J Kobayashi¹,²

1. Department of Mathematical Informatics, Graduate School of Information Science and Technology, The University of Tokyo
2. Institute of Industrial Science, University of Tokyo

Abstract

T-cells prevent us from infection or autoimmune disease by the ability to discriminate self and non-self antigens. Self and non-self discrimination is based on the interaction between antigens represented on the surface of antigen presenting cells and T-cell receptors. Research about the structure of the antigens has revealed that self and non-self antigens are almost same in their structures. A parameter that characterizes the difference of antigens is the affinity between an antigen and a T-cell receptor, but this difference is very small because of the similarity in the structure. Therefore T-cell must have the ability to amplify the slight difference in affinities of antigens.

There are some models for molecular discrimination that amplifies the difference of molecules exponentially, but they cannot reproduce several important phenomena. In this research, we develop a novel model that discriminates self and non-self antigens based on a different mechanism. Our model reproduces the phenomena that the previous models cannot reproduce. We also report the several characteristics of this model and the mechanism how the system improves the accuracy of discrimination.

Keywords:

Masashi Kajita

mkajita@sat.t.u-tokyo.ac.jp
Session 2

13:45 - 15:30 at Main Hall

Chair: Chun-Biu Li

Message from Chair:
Recent advanced imaging techniques allow us to probe the real time dynamics of complex biological systems in both the single molecule and cellular levels. This section brings together three experts in the field to inspire new innovations and collaborations that can lead to new line of researches among the participants of this workshop. In particular, the first talk will focus on the development of fluorescent probes for live chemiluminescence imaging; the second talk will discuss how the live imaging of anomalous cell movement can be modeled and quantified; and the last talk will explore the use of single fluorescent-molecule imaging techniques to scrutinize the dimerization of G-protein coupled receptor in the live plasma membrane.

Chair

Dr. Chun-Biu Li

Research Institute for Electronic Science, Hokkaido University, Japan
Realtime fluorescence and chemiluminescence imaging with optogenetic activation in living cells

Yoshiyuki Arai
Osaka University, Japan

Abstract

Keywords: Fluorescent probe, chemiluminescent probe, optogenetic tool, CCD dead time

Dr. Yoshiyuki Arai
araiy@sanken.osaka-u.ac.jp
Relevance of spontaneous migration to tactic response in Dictyostelium cells

Hiroaki Takagi
Nara Medical University, Japan

Abstract
We organized collaborative researches between theory and experiment to answer the function of cellular spontaneous activity by adopting Dictyostelium discoideum. We focused on spontaneous cell migration, applied statistical analysis based on the theory of Brownian movement to cellular trajectories, and identified a generalized Langevin model that can reproduce the revealed statistical nature. Through the numerical simulations and theoretical analysis of this model, we predicted functional significance of fluctuation in cell migration dynamics. Then, we experimentally verified a essential relationship between random cell migration and directional cell migration under DCEF (electrotaxis). Now we apply corresponding analysis to spontaneous migration of immune T cell, in order to examine the generality of the view.

Keywords: cell migration, anomalous diffusion, electrotaxis, Dictyostelium

Dr. Hiroaki Takagi
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Reversible dimer formation of G-protein coupled receptor: Quantitative evaluation by a single fluorescent molecule imaging

Rinshi Kasai
Institute for Frontier Medical Sciences, Kyoto University, Japan

Abstract

G-protein coupled receptor, or GPCR is one of the biggest protein families in our human body. For the past few decades, the GPCR dimerization has been discussed because of its importance regarding the signal tunings and divergences. However, there have been only few reports quantitatively showing a GPCR dimerization, resulting in ongoing controversies. Here, we wanted to resolve this controversy by applying the single fluorescent-molecule imaging technique to visualize each individual dimerization in the live plasma membrane. By directly observing GPCR dimers, their physiological properties were evaluated in terms of their dynamics. As a result of our observations of some class A GPCRs, it has eventually become clear that both monomers and dimers exist at the same time, and each dimer is transiently formed and fallen apart within a certain period, around 100 milliseconds. In this presentation, I’m going to introduce our recent progress.

Keywords:

Dr. Rinshi Kasai

rkasai@frontier.kyoto-u.ac.jp
Session 3

15:50 - 17:50 at Main Hall

Chair: Ziya Kalay

Message from Chair:
This section focuses on recent advances in understanding biological systems at the molecular and tissue level, via modeling and experimental methods. In the first talk, we will hear about the multiscale modeling of the mammalian circadian clock, from the dynamics of gene expression in individual neurons to macroscopic patterns in the brain tissue. In the second talk, development of novel probes with superior capabilities of sensing the structure and dynamics of water in the intracellular environment will be presented. Lastly, recent theoretical and computational findings on the diffusion of intracellular particles in the presence of molecular crowding and physical obstacles will be discussed.

Chair
Dr. Ziya Kalay

Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Japan
Mammalian circadian oscillations at the cellular and tissue scales

Craig Jolley
Center for Developmental Biology, RIKEN, Japan

Abstract

In many organisms, behavior and physiology follow an autonomous 24-hour rhythm driven by a circadian clock. In mammals, cellular oscillations are driven by transcriptional and post-translational feedback loops. In the suprachiasmatic nucleus (SCN), a specialized center in the anterior hypothalamus, the noisy oscillations of individual neurons are coupled to generate robust circadian rhythms that drive organismal behavior.

Understanding SCN function is an inherently multi-scale problem: rhythms arise from molecular interactions within and between cells, but the important outputs are at the level of the complete tissue, which contains ~20,000 neurons. We have employed a variety of different modeling approaches to understand both the detailed phase relationships of oscillating components within individual cells and the collective behavior of the oscillating tissue.

At the single-cell level, we have developed a model in which oscillations are driven by three clock-controlled elements (CCEs) – promoter sequence elements that are bound by circadian transcription factors and give the target gene a place on the circadian schedule. Depending on which CCE sequences are present a gene will be expressed in the morning (E/E'-box), in the daytime (D-box), or in the evening (RRE); intermediate expression phases can be generated by combinatorial regulation. We used a differential evolution algorithm to identify parameter values yielding an optimal agreement with mRNA/protein expression phases derived from qPCR and quantitative Western blotting, followed by Monte Carlo sampling to determine the sensitivity of model fitting to different parameter combinations. The model outputs agree well with experimental evidence, and allow us to make novel predictions that will be tested in future experiments.

At the tissue level, we are interested in how spatiotemporal “waves” of circadian gene expression arise in a heterogeneous population of SCN neurons. The SCN is known to employ a wide variety of different chemical signals, and we are building a coarse-grained model that incorporates the effects of different chemical signals. Our goal is to assess the impact of different intercellular signals on the stability, entrainability, and phase heterogeneity of the tissue-level clock.

Keywords: Circadian clock, intercellular coupling, optimization, dynamical systems

Dr. Craig Jolley
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Development of fluorescent protein to sense physical parameters

Tomonobu Watanabe
Quantitative Biology Center, RIKEN, Japan

Abstract
Fluorescent protein-based indicators for intracellular environment conditions such as pH and ion concentrations are commonly used to study the status and dynamics of living cells. Despite being an important factor in many biological processes, the development of an indicator for the physicochemical state of water, such as pressure, and hydrophobicity, however, has been neglected. We here found a novel mutation that dramatically enhances the sensitivity to the state of water of the yellow fluorescent protein (YFP). In this symposium, we would like to present you the developmental strategy, proof of concept and experimental demonstrations of our fluorescent proteins.

Keywords: Fluorescent protein, pressure measurement, protein-crowding, live imaging

Dr. Tomonobu Watanabe
tomowatanabe@riken.jp
Simulating diffusion in crowded environments with multifractional Brownian motion

Andre Leier

Okinawa Institute of Science and Technology, Japan

Abstract

Diffusion of molecules within cells has many times been measured and characterized as highly anomalous. Even though the underlying mechanisms are not entirely understood, excluded volume effects inside the crowded cell have already been identified as one cause for subdiffusion. Aside from experimental studies, computer simulations have been used to deepen our understanding of anomalous diffusion. In the past, the focus has been largely on continuous time random walks (CTRWs), but recent work has suggested that fractional Brownian motion (FBM) may be a better descriptor of diffusion in crowded environments. FBM is driven by a Gaussian process with zero-mean and a covariance function that depends on the so-called Hurst exponent H. A natural generalization of FBM is achieved by replacing H by a time-dependent Hölder function H(t) leading to multifractional Brownian motion (MFBM).

Here, we present results from a recent study [1] using FBM and MFBM to simulate diffusion of a tracked particle in the presence of crowding molecules and physical obstacles. Particle tracking data, mimicking experimental data, was first generated with an off-lattice particle simulator. We then attempted to obtain computationally significantly less expensive (M)FBM paths that match the statistical properties given by mean-square displacement (MSD) and time averaged MSD of our sample data. While diffusion around immovable obstacles can be reasonably characterized by a single Hurst exponent, diffusion in the presence of crowding molecules seems to exhibit multifractional properties in form of a different short and long-time behaviour.

References

Closing Remarks

at Main Hall
17:50 - 18:00

by IWQB2013 organizer committee

We thank everyone who supported the organisation of this workshop, specially for Ms. Toyama, CDB RIKEN, and the laboratory members of Ebisuya Unit, CDB, RIKEN and Funahashi Laboratory, Keio University. Also we would like to express our gratefulness to the core-members who cooperate to organise this workshop constantly.

We will have a free discussion time at the Large meeting room, the poster session venue, with some nibbles to enhance your activity.

Thank you to all for joining us and we hope will see each other soon for new collaboration opportunities, at the other conference, and more, and will keep in touch for exchanging useful information on our research progress.
Poster Session II & Free Discussion
18:00 - 20:00 at Meeting Room (Large)

Abstracts are listed in page 26 ~ 41
Poster Abstracts (1 ~ 16)

Poster Session I  12:00 - 13:30 at Meeting Room (Large)
Selected Short Talk 13:30 - 13:45 at Main Hall
Poster Session II & Free Discussion 18:00 - 20:00 at Meeting Room (Large)
Development of microfluidic system for high resolution control of long-term cell culturing environment

Takumi Hiraiwa¹, Tadamasa Kimura¹, Yuma Takenaka¹, Ryo Tanamoto¹, Hiroki Ota⁴, Hiroshi Kimura³, Yoshihiro Taguchi², Norihisa Miki², Yoshinori Matsumoto¹, Kotaro Oka¹, Akira Funahashi¹, and Noriko Hiroi¹

1. School of Fundamental Science and Technology, Keio University
2. School of Integrated Design Engineering, Keio University
3. Department of Mechanical Engineering, Tokai University
4. Electrical Engineering, University of California, Berkeley

Abstract

Both inter- and intracellular environment can be characterized with inhomogeneity of various parameters. These inhomogeneity lead us insights into the behaviors of the biological phenomena. To elucidate the mechanisms of organizing these biological inhomogeneities or localization is significant to understand complex biological phenomena. However, to analyse the mechanism by bulky experimental systems conquers difficulties in principle. Therefore, development of a suitable system to analyse the mechanisms experimentally is required. We have been fabricating reusable Cell Culturing Device, designed for single cells and cellular network analysis. This is the first success of combination of Microcontact Printing (µCP) and Vacuum Device. In contrast to the earlier works, this device has following advantage: (i) cells stay within the micropatterns for long enough duration to achieve local activation of cells or cellular networks (12 hrs < ~), (ii) the displacement of laminar flow from the boundary of two fluids in Vacuum Device keeps smaller than the diameter of a cell (e.g. ±5.92 µm at 20 µl/min), (iii) all components of our device except micropatterned substrate are reusable for further analyses. The success of the combination of above techniques provided a controllable environment for the local activation of single cell, colony or cellular network. Our device allows to exhibit the different responses induced with the various conditions in a single observation sight at exactly the same time point.
Development of High-speed 3D imaging system with electrically tunable lenses for deeper probing of subcellular structures

Yuichiro Nakai¹, Naoka Tamura², Takumi Hiraiwa³, Takahiro Okuhara³, Viji Draviam², Akira Funahashi¹,³ and Noriko Hiroi¹,³

1. Department of Biosciences and Technology, Keio University, 3-14-1, Hiyoshi, Kohoku-ward, Yokohama, Japan
2. Department of Genetics, University of Cambridge, Downing street, Cambridge CB2 3EH, United Kingdom
3. Center of Biosciences and Technology, Keio University, 3-14-1, Hiyoshi, Kohoku-ward, Yokohama, Japan

Abstract

Microtubules play important roles in spindle formation, chromosome segregation and spindle orientation during cell division. Understanding the dynamics of microtubules during the proliferation process is absolutely essential for explaining how microtubule targeting drugs work and for understanding mechanisms of tumorigenesis and microtubule dysregulation triggered human diseases.

We are developing the high-speed 3D (3-dimensional) imaging system with electrically tunable lenses to analyse the dynamics of plusTips by 3D particle tracking. The plusTip of the microtubule is the most dynamic end. There exists some tools of both optical and computational nature for 2D plusTip tracking. Because a cell in mitotic phases is rounded, they require z-stack analyses for the precise tracking of plusTips in 3D space.

However, current microscopic systems are not sufficient to take z-stack images with high-resolution both in time and z-axis at the same time. We solved this problem by attaching an electrically tunable lens on an objective lens of inverted microscope. We developed a software to control the lens and succeeded to control the focus of the lens.

In future, we will take time-course images with z-stacks to analyse the 3D tracking of plusTips in a mitotic cells. Our effort will not only be useful for studying microtubule behaviour but also for probing other sub-cellular organelles in deeper parts of the dividing cell.

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Mathematical models of RNP-mediated synthetic translational systems for precise control of the differentiation of iPS cells

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2. Center for iPS Cell Research and Application, Kyoto University

Abstract

Current strategy of controlling the differentiation process of iPS cells depends on the empirical techniques based on microscopic observations. However, because of the dispersion of the states of iPS cells, efficient induction of differentiation is difficult to achieve by current strategy. Here we exhibit the strategy to induce aimed differentiation of iPS cells by applying RNP-mediated synthetic translational systems. Our synthetic translational systems detect the cellular state automatically, and will provide appropriate stimuli for differentiation at the each stage of the cell, respectively. To design a desired synthetic translational system, we developed some mathematical models which can simulate the behavior of its dynamics in a cell. We designed and simulated the behavior of ON/OFF switch circuits, sequential circuit and oscillation circuit. Our model will provide useful information to produce appropriate synthetic translational system and induce neuronal cells or platelet cells effectively.

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Detection and Analyses of Intracellular Distribution of Temperature

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Abstract

Many of biochemical models assume in vitro reaction environment, which is homogeneous in space and in temperature. However, in vivo reaction environment is supposed to be inhomogeneous both in space and in temperature. To develop in vivo oriented modeling with taking into account such intracellular inhomogeneity has potential to represent the behaviors of biological systems more precisely in the wider range of biological event. We incorporated CdSe Q-Dot into human neuroblastoma cell line, SK-N-SH, to detect the intracellular distribution of temperature. We used confocal microscope with spectrophotomater to achieve spatially high-resolution observation. The detected distribution of temperature were adopted into a mathematical model of long-term memory [1] to analyse how the temperature distribution could affect the high-order function of brain such as long-term memory. At the same time, we performed single particle tracking of Q-Dot with STED in order to estimate the viscosity inside fine spaces, and found the correlation with the mechanism of long-term memory. Our results suggest that inhomogeneous intracellular temperature could affect the high-order function of human brain, and could affect the other physiological functions of living systems.


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Development of Semi-Auto nuclei identification system from fluorescent 4D embryonic images

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Abstract

Successful infertility treatment is around 10% of total clinical example. One of the cause of the difficulty for achievement is supposed to the low quality of embryo. In the previous studies, full-automated image analyzing software was applied to find out the marker of the quality of embryo. The software can find the correlation between fluorescent 4D microscopic images and the ability of successful offspring. However, the identified positions of nuclei were unstable dependent on the experimental condition, as the result the quality of embryo was unreliable to use in quantitative manner. We modified the full-automated system to find out the center of each nuclei in an embryo as the system can produce multi-candidates of the center of nuclei in an embryo. We improved the accuracy of segmentation by including intercellular distance and the cell size. Our system allows users to choose the plausible candidate manually by its user interface. The semi-automated system is supposed to enhance the accuracy of identification of nuclei in embryos. High-precision of identification of cellular positions is the basic requirement to calculate quantitative parameters, such as the rate of cell proliferation, and finally to achieve 4D tracking for mapping cell-lineage.

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A theoretical investigation of protein folding funnel from protein structures

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Abstract

The protein folding funnel has been considered as the essential origin of the spontaneously folding of proteins. The protein folding funnel has never sufficiently confirmed in investigations based on protein structure. Recently the protein folding was also explained on the basis of the Markov state model. The relation between the two pictures based on the folding funnel and the Markov state model, however, are not clear. To gain insight into the protein folding funnel and its relation to the Markov state model, we theoretically consider energy Markov state model and reconstruct folding funnel from the protein structure. Our result shows a funnel structure of the energy Markov state model at a folding temperature and a network structure crossover of the energy Markov state model with changes in the temperature.

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Modeling of self and non-self discrimination by T-cells

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Abstract

T-cells prevent us from infection or autoimmune disease by the ability to discriminate self and non-self antigens. Self and non-self discrimination is based on the interaction between antigens represented on the surface of antigen presenting cells and T-cell receptors. Research about the structure of the antigens has revealed that self and non-self antigens are almost same in their structures. A parameter that characterizes the difference of antigens is the affinity between an antigen and a T-cell receptor, but this difference is very small because of the similarity in the structure. Therefore T-cell must have the ability to amplify the slight difference in affinities of antigens.

There are some models for molecular discrimination that amplifies the difference of molecules exponentially, but they cannot reproduce several important phenomena. In this research, we develop a novel model that discriminates self and non-self antigens based on a different mechanism. Our model reproduces the phenomena that the previous models cannot reproduce. We also report the several characteristics of this model and the mechanism how the system improves the accuracy of discrimination.

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Observing the rotational diffusion of nanodiamonds with arbitrary nitrogen vacancy center configurations

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Abstract

We present theoretical results on the relationship between the rotational diffusion coefficient of a nanodiamond undergoing Brownian motion and the configuration of NVCs contained in the particle. Through exact calculations and simulations, we obtain the fluorescence intensity autocorrelation function that is measured in optically detected magnetic resonance experiments conducted at single particle level. We relate the autocorrelation function to the rotational diffusion coefficient and discuss the influence of different nitrogen vacancy center configurations on the outcome of measurements. We believe that our results can be useful in interpreting observations on nanodiamonds that contain multiple nitrogen vacancy centers.

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Reinforcement Learning Model based on DNA Strand Displacement

Rizki Mardian, Kosuke Sekiyama, Toshio Fukuda
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Abstract
In order to develop a molecular robot made from DNA, there are various things have to be considered. In addition to the design of the molecular body itself, the information processing system that is solely based on DNA reaction has to be designed as well. In this research, a toehold mediated DNA strand displacement and branch migration mechanism is treated as methodology to deliver a computation above DNA substrate. A decision making problem has been selected as the main focus to deliver a DNA-based agent that is capable to do action-taking under dynamical environment. To approach the solution, a self-organizing method, namely Artificial Immune System is chosen as the computational algorithm. This nature-inspired metaphor has successfully solved many problems in machine learning, robotics, etc. In this work, we would like to achieve the same functionality above the biological counterparts of the similar applications. Moreover, we also would like to develop the capability of learning from unknown configuration of environments, so that DNA agent become more robust, can always optimize its action, can recover from any error happen during the delivery of task, and so on. To approach this we observe the self-organizing and dynamics of Immune Network based algorithm and to implement about DNA-only reaction mechanism.

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Growth rate dependent effect on gene expression under environmental perturbation

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Abstract

Cell growth rate reflects the physiological state and largely relies on gene expression in response to the living environments. The relationship in common between growth rate and gene expression was unclarified. Here, the growth rate coordinated changes in gene expression was discovered, by analyzing the exponentially growing Escherichia coli cells cultured in multiple defined environments, varied in osmotic pressure, temperature and starved status. Gene expression analyses showed that the total 3740 genes across the genome could be simply divided into three clusters (C1–3), accompanied by a generic trend of the growth rate coordinated transcriptional changes. The directions of transcriptional changes in C1 showed an environmental specificity, whereas, those in C2 and C3 were commonly correlated to the growth rates in a negative and positive manner, respectively. The three clusters exhibited the differentiation in gene function along with the task division in gene regulation. Growth rate correlated transcriptional changes with reversed direction and distinguished duty among the three clusters indicated the homeostasis in transcriptome, balancing the total expression cost for the sustainable life in new habitats.

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Decision making under fluctuated environments: get over with a social group or alone

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Abstract

Cooperation is general behavior in populations from microbe to human. There are two types of the way to cooperation: altruistic cooperation among kin (not required repayment) and reciprocal cooperation among non-kin (required repayment). However it is unknown whether group of altruistic cooperation rule can evolve into group of reciprocal rule. We built up a hypothesis that altruistic cooperation can evolve at non-kin group if there is any chance that altruistic actor retrieve paying cost. The social amoeba Dictyostelium discoideum is suitable system for validating this hypothesis. Unicellular amoeba feeds on bacteria, but when starved, 10000-100000 cells synchronously aggregate and differentiate to form a multicellular fruiting body. Approximately 20% of the cells differentiate into a non-viable stalk (later starver) that altruistically support the remaining cells that become viable spores (earlier starver). We hypothesize that when starvation progress directionally, later starvers pay altruistic cost by differentiating into stalk, however when nutrition recovered on the way, later starvers are advantageous to resume feeding rapidly (retrieve cooperative cost). To investigate this hypothesis, at first, we analyzed the effect of adding bacteria to the differentiating cells. We found that (1) cells synchronously aggregate and then several hours after second aggregation were observed using dark field microscopy observation. This result indicates that clonal cells could divide two heterochronic-differentiating subpopulations. In addition, (2) part of fruiting bodies that almost completed differentiation, it was considered to be irreversible, could re-start differentiation with immolating the scaffold stalk cells. Our results may have possibility that cells have collective bet-hedging strategy, and the social games sometimes were "reset" after decided victory (become spore) or defeat (stalk). We will discuss the possible avenues of future studies, we are investigating the contribution of such responses to adaptation and evolution.

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Assessing uncertainly in model parameters based on sparse experimental data

Maciej Swat\textsuperscript{2}, Akira Funahashi\textsuperscript{1} and Noriko Hiroi\textsuperscript{1}

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2. European Bioinformatics Institute, Cambridge, UK

Abstract

To perform the parametric identification of mathematical models of biological event, we face sparse experimental data, which contains complex nonlinear dynamics. This fact makes difficult to parameterize and analyze uncertainty of the model. Here we introduce a scheme of parameter identification to achieve the necessary knowledge of the model and to find the necessary experimental conditions by using our original experimental data and a published model [1](Figure 1A). The model exhibits bimodality, also has not yet performed parameter fitting to time course data. We calibrated model parameters with Generalized Least Squares (GLS) method, after that performed both Local and Global Sensitivity Analysis (LSA and GSA) followed by parameter ranking, also evaluated the Fisher Information Matrix in the parameter space, finally checked the model characteristics by performing bifurcation analysis, which tells us the fundamental characteristics of model behavior before and after parameter fitting to experimental data(Figure 1B and C). These analyses provided us the evidence how sparse experimental data can be useful to change a mathematical model closer to physiological condition.


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Data driven findings of function-dependent fluctuations and fluctuation - response relation of gene expressions in plants

Akinori Awazu, Atsushi Nagano
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Abstract
In several organisms, non-negligible fluctuations of the gene expression levels have often been observed among individuals with completely the same genome under the same environment. However, the meanings of such phenotype fluctuations have not yet been clarified enough while some conjectures were proposed (B. Lehner and K. Kaneko (2011)).
In this study, we analyze the microarray data of plants such as Arabidopsis thaliana and Rice to unveil the meanings of such fluctuations. We found the magnitude of the fluctuations of gene expression levels among individuals with the same genotype tends to depend on the functions of genes. We also found the high positive correlation between such fluctuations and responses, where genes showing large variations of expression levels against the environmental changes tend to exhibit large phenotype fluctuations under the uniform environment.

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Biological significance of heterogeneity in iPS cells by single cell transcriptome analysis

Masaki Nomura, Masahiro Nakamura, Naoki Amano, Yuji Hashimoto, Chihiro Okada, Sayaka Arai, Sachiko Ando, Yumi Inoue and Akira Watanabe

Dept. of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto University

Abstract

In cultured colony, cells are in their own state. In order to capture cellular states faithfully, we introduced single-cell RNA-seq and quantified the mRNA expression levels of 48 iPS 201B7 cells. In our data, we found that the pluripotent genes, NANOG, POU5F1 and SOX2, lowly expressed and highly fluctuated. Applying a hierarchical clustering analysis, cells are separated into clusters according to their mRNA expression patterns, where we found that two of them are associated with cell cycle. Then, we investigated how the variability of NANOG expression level is related with the distribution of the cellular states. The partial clustering analysis [1] unveiled that the cellular states fork at the low NANOG expression level. We are also interested in co-expressing genes with NANOG. To identify candidate genes, we did persistent homology analysis [2] in addition to correlation analysis. We found that the persistent homology analysis caught interesting gene expression pathways that correlation analysis dropped off. Finally, our neighboring gene analysis revealed that some neighboring genes share fluctuations some of which are located [3] near the boundaries found in a Hi-C experiment. This result suggests that cis-regulatory regions and high order chromatin architecture may contribute to gene expression fluctuations.


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Aggregation of Swarm Molecular Robotics in Cell Culture Medium

Wibowo Adi, Kosuke Sekiyama

Micro-Nano System Engineering Department, School of Engineering, Nagoya University

Abstract

Intelligent drug device for diagnosis and also therapy for specific areas and specific disease is one of issues in drug delivery system. In advance, drug delivery system must ability for biosensing in the biological environment and also can control the release system of drug only for specific target. Development of nano scale of robotics system with have ability to interact with bimolecular system is one of solution in this issues. Deoxyribonucleic acid (DNA) is one of biomolecular material and also DNA is a perfect polymer for creating devices on the nano scale. Prior to deliver the DNA molecular robotics in biological environment, it’s required testing the possibility of molecular robotics behavior. One of the behaviors is aggregation with other robot to increase the drug volume at target cell. In this poster, we show an aggregation testing of molecular robotics in cell culture medium that can be used to maintain cell in tissue culture. The aggregation was between two kinds of double strand DNA as assume as two robot with single strand DNA as mediator. We incubated two experiment in 4 Hours and overnight in DMEM growth media (10% FBS, 2% penicillin/ streptomycin, 37 °C, 5% CO2).
Design and Implementation of CellDesigner

Akira Funahashi\(^1\), Yukiko Matsuoka\(^2,3\), Samik Ghosh\(^2\), Noriko Hiroi\(^1\) and Hiroaki Kitano\(^2\)

1. Department of Biosciences and Informatics, Keio University
2. The Systems Biology Institute (SBI)
3. JST ERATO KAWAOKA Infection-induced Host Responses

Abstract

Understanding of the logic and dynamics of gene-regulatory and biochemical networks is a major challenge of systems biology. To facilitate this research topic, we have developed a modeling/simulating tool called CellDesigner. CellDesigner primarily has capabilities to visualize, model, and simulate gene-regulatory and biochemical networks. Two major characteristics embedded in CellDesigner boost its usability to create/import/export models:

1. A solidly defined and comprehensive graphical representation (systems biology graphical notation) of network models and
2. Systems Biology Markup Language (SBML) as a model-describing basis, which function as inter-tool media to import/export SBML-based models.

In addition, since its initial release in 2004, we have extended various capabilities of CellDesigner. For example, we integrated third party Garuda enabled simulation/analysis software packages. CellDesigner also supports simulation and parameter search, supported by integration with SBML ODE Solver, enabling users to simulate through our sophisticated graphical user interface. Users can also browse and modify existing models by referring to existing databases directly through CellDesigner. Those extended functions empower CellDesigner as not only a modeling/simulating tool but also an integrated analysis suite. CellDesigner is implemented in Java and thus supports various platforms (i.e., Windows, Linux, and MacOS X). CellDesigner is freely available via our Web site.

Fig.1 Screenshot of CellDesigner 4.4β (Left: Simulation example, Right: Integration with Garuda modules)

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Yoshiyuki Arai

Assistant Professor
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The institute of scientific and industrial research,
Osaka University, Japan

He started to develop his career in Single molecule Biophysics and Cellular dynamics, and currently works in Biophysics area. His research interest includes developing novel optical microscopy, microfluidic technology, single molecule imaging, image processing and spying minority of biological phenomena.

Most recent publications are;

Viji M. Draviam

Group leader
Department of Genetics, University of Cambridge, UK

Her research group uses a combination of molecular, biochemical, cell biological and modelling techniques to learn about how microtubules are controlled to ensure proper cell division. Because cell division defects can drive aneuploidy and tissue disorganisation in cancers, her research findings are relevant to unsolved problems in both fundamental and clinical biology. Her current research interests include automated single-cell image analysis, microtubule behaviour and regulation, chromosome segregation and spindle positioning in human cells.

For a recent review on her group's work, please see:

Miki Ebisuya

Unit Leader
Riken Center for Developmental Biology, Japan

She received her PhD degree in Life science from Kyoto University (Japan, 2009), where she found and analyzed the transcriptional ripple effect, co-upregulation of the transcription of neighboring genes. Then she got interested in synthetic biology, and started her own group. Her current research interest is to reconstitute developmental mechanisms in cultured cells.

Akira Funahashi

Associate Professor
Systems Biology Laboratory,
Department of Biosciences and Informatics,
Keio University, Japan

He received his BE, ME, and PhD degrees in Computer Science from Keio University, Japan, in 1995, 1997, and 2000 respectively. He was a Research Fellow of the Japan Society of the Promotion of Science (DC1) from 1997 to 2000, and a Research Associate in the Department of Information Technology, Mie University, Japan, from 2000 to 2002. He then joined Kitano Symbiotic Systems Project, JST and The Systems Biology Institute as a researcher before moving to Keio University in 2007. His research interests include the area of systems biology, computational biology, interconnection network and parallel processing.

Recent articles;

**Chun-Biu Li**

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Recent articles;
Hiroaki Takagi

Senior Assistant Professor
Department of Physics, School of Medicine, Nara Medical University, Japan

He started his theoretical research on nonlinear dynamics and complex systems, and received his PhD from University of Tokyo, Japan (2002). He was a researcher in the field of single-molecular biophysics at Osaka University, extensively collaborated with experimentalists, and then moved to Nara Medical University. He targeted single-molecule and single-cell measurements, pursuing functional mechanism of molecular dynamics and cellular information processing.

Recent articles:

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