Pattern formation in sol media using branched DNA motifs

Hiroshi Ogata[1]
hiroshi.ogata.t2@dc.tohoku.ac.jp

Keita Abe[1]
keita.abe.t7@dc.tohoku.ac.jp

Ibuki Kawamata[1,2]
ibuki.kawamata@tohoku.ac.jp

Satoshi Murata[1]
satoshi.murata.a4@tohoku.ac.jp

[1] Department of Robotics, School of Engineering, Tohoku University, 6-6-01, AramakiAzaAoba, Aoba, Sendai, Miyagi, 980-0845, Japan
[2] Natural Science Division, Faculty of Core Research, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan

Keywords: Reaction-diffusion system, Self-assembly, Physical pattern formation, DNA hydrogel

Pattern formation in reaction-diffusion systems is a phenomenon in which molecules diffuse while reacting, autonomously forming heterogeneous concentration distributions of chemical species [1]. Pattern formation is well known as a mechanism of morphogenesis in biological systems, such as embryogenesis in Drosophila and generation of patterns on the skin surface [2]. If we can artificially reconstruct this mechanism, we can construct macroscale molecular systems that far exceed the size of molecules. The technology for constructing macro-scale molecular systems is expected to be applied to artificial organs in the future.

So far, we developed an autonomous linear pattern formation method using a reaction-diffusion system of DNA in hydrogel media [3,4], in which a steep concentration distribution of chemical species (DNA) forms a pattern and showed that it can be successfully cascaded. As a next step, we consider here the possibility of pattern materialization by adding heterogeneity of physical and mechanical properties to the distribution of the chemical species formed.

The proposed reaction-diffusion system forms patterns by polymerization of branched DNA motifs (X motifs) in a highly viscous sol medium [5]. In this system, the four single-stranded DNAs that comprise the branched DNA motif are divided into two groups that do not polymerize into each other and diffuse from sources on either side of the sol reaction field. DNA motifs are formed at equidistant positions where these DNAs meet, and the motifs polymerize in a position-specific manner to form a DNA hydrogel.

Fluorescence microscopy showed that a linear pattern (bisector pattern between sources) was formed even in the sol medium. The fluorescence recovery inside and outside the reaction area was measured to quantitatively evaluate the heterogeneity of the diffusion coefficient. More surprisingly, we observed that the once formed linear patterns gradually wave in the sol medium. The cause of this phenomenon is currently under investigation.

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Force measurement of kinesin-propelled microtubules in swarming using electromagnetic tweezers

Mst Rubaya Rashid[1]
rubaya.rashid1993@gmail.com
Mousumi Akter[2]
akter80mousumi@gmail.com
Arif Md. Rashedul Kabir[2]
kabir@sci.hokudai.ac.jp
Jakia Jannat Keya[2]
jakiajannatk@gmail.com
Kazuki Sada[1,2]
sadatcm@sci.hokudai.ac.jp
Akira Kakugo[1,2]
kakugo@sci.hokudai.ac.jp

[1] Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo, Japan
[2] Faculty of Science, Hokkaido University, Sapporo, 060-0810, Japan

Keywords: Microtubule, Kinesin, DNA conjugation, Swarm formation, Force of swarm, Magnetic bead, Electromagnetic tweezers

The Swarming of living organisms such as fishes, birds, and ants is a fascinating display of coordinated behavior in nature (1). Swarming provides the organisms with several advantages such as parallelism, robustness, flexibility, and permits exhibiting emergent functions by the organisms (2). Such attractive features of the swarming of living beings have motivated researchers working in material science, and engineering to utilize the swarming of self-propelled objects in artificial environments for sophisticated applications (3, 4). Recently our group has successfully demonstrated the swarming of a large number of kinesin-propelled microtubules (MTs), a self-propelled biomolecular machine, where the swarms of MTs exhibited translational and rotational motion upon consumption of chemical energy of ATP hydrolysis by kinesins (5). The swarming of MTs with rotational and translational motion holds great potential as their motion can be harnessed to perform work for nanotechnological applications, e. g. in molecular machines or devices, molecular robotics, etc. To ensure real-life applications of the MT swarms it is a prerequisite to quantify the amount of work that can be harnessed from the swarms. In this work, we attempt to quantify the force associated with the swarming of MTs driven by kinesins. Azide-labeled MTs were prepared from azide-labeled tubulin using the polymerizing agent guanosine triphosphate. Two complementary DNA sequences were used to modify two sets of azide-MTs by azide-alkyne cycloaddition reaction and the magnetic beads were modified with one of the DNAs. Swarming of the DNA-modified MTs was performed on a kinesin-coated glass surface and observed by a fluorescence microscope. A circular-shaped swarm was formed by the MTs, and the bead was loaded by the swarm. An electromagnetic tweezer (EMTw) was used to apply electromagnetic force (EMF) on magnetic bead-attached rotational swarms. The force of the circular-shaped MT swarms was successfully determined from its velocity by applying EMF. The force increases linearly with increasing the size of the circular swarm. This increase in force arises from the higher number of active kinesins driving the larger swarms. Such estimation of the force of the swarm of MTs will widen its application in nanotechnology as well as in robotics.

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Order Parameter Analysis of Microtubule Motility Dynamics

Ma Chen[1,3]
ma.c.ab@m.titech.ac.jp

Masayuki Yamamura[1]
my@c.titech.ac.jp

Mousumi Akter[2,3]
Akther80mousumi@gmail.com

Akira Kakugo [2,3]
kakugo@sci.hokudai.ac.jp

Gutmann Gregory Spence[3]
gutmann@molecular-robotics.co.jp

Akihiko Konagaya[3]
konagaya@molecular-robotics.co.jp

[1] School of Computing, Tokyo Institute of Technology
[2] Dept. Chemistry, Physical Chemistry, Hokkaido University
[3] Molecular Robotics Research Institute, Co., Ltd.

Keywords: Molecular Robotics, Microtubule Motility Dynamics, Order Parameter Analysis

Microtubules often form streams at high density, which glide in the same directions on Kinesin coated glass surface in microtubule motility assay. At higher density of microtubules, they tend to move together (snuggling) to avoid collision and overriding of microtubules. As a result, microtubule groups emerge motion patterns showing straight, curved or wave like trajectories.

In order to analyze the microtubule group motion patterns, we applied dense and sparse optical flow (DOF and SOF) methods followed by polarity analysis with order parameter. In case of DOF, the polarity analysis revealed overall difference of microtubule motility dynamics depending to microtubule concentration, to some extent.

In order to identify the emergence of motion patterns, SOF method was attempted to the same video data as used in the SOF method. In this presentation, we will discuss the limitation of conventional order parameter definition when dealing with parallel and anti-parallel movement of microtubule dynamics.

Deep Learning Detection of Giant Vesicles Using a Synthetic Data Set of Confocal Fluorescence Microscopy Images

Soichiro Hiroi[1,2]
hiroi-soichiro1729@g.ecc.u-tokyo.ac.jp

Taro Toyota[1,3]
cttoyota@mail.ecc.u-tokyo.ac.jp

Zhang Yuhui[2]
zhang@molecular-robotics.co.jp

Akihiko Konagaya[2]
konagaya@molecular-robotics.co.jp

[1] Graduate School of Arts and Science, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan
[2] Molecular Robotics Research Institute, Co., Ltd., W304, TITECH Yokohama Venture Plaza, 4259-3, Nagatsuta, Yokohama Midori-ku, Kanagawa, 226-8510, Japan
[3] Universal Biology Institute, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

Keywords: Confocal fluorescence microscopy, Giant vesicle, Deep learning

Confocal laser scanning microscopy has the capability of acquiring cross-section images by removing fluorescence generated outside the focal plane to be observed. This means that we can construct a three-dimensional (3D) image of tiny structures that would be difficult to be physically sectioned by acquiring multiple images in which the focal plane position is continuously changed. Therefore, it has been widely used in many scientific fields and is a common method to visualize the 3D morphology of cell-sized closed lipid bilayer membranes called giant vesicles (GVs).

On the other hand, virtual reality (VR) visualization of microscopic images[1] is useful for the observation of molecular assemblies in which 3D morphology is important. GV is one of those molecular assemblies whose morphology is susceptible to surrounding disturbances[2]. However, when confocal fluorescence microscopy images of GVs are captured from the top to the bottom while changing the focal plane, the GVs shift their centroid position due to advection or fluctuation including Brownian motion, and the 3D structure of freely floating GVs could not be correctly reconstructed.

Therefore, in order to correct the position of each focal plane prior to VR-ization, we conceived the idea of detecting each GV using an object detection method based on deep learning. Deep learning is a part of machine learning methods and several deep learning frameworks have been applied to object detection tasks and achieved great results[3]. In this study, to aim at VR-ization, we developed a synthetic model of confocal fluoresce microscopy images of GVs. Furthermore, using the teacher data images generated by the synthetic models, we constructed a GV detect model with transfer learning based on YOLO, one of the object detection models by convolutional neural networks.

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DNA AFM images in Super-resolution by VR system and Deep Learning

Hu Xiaoran[1,3]
hu.x.ab@m.titech.ac.jp

Masayuki Yamamura[1]
my@c.titech.ac.jp

Qing Liu[2]
r412989@kansai-u.ac.jp

Akinori Kuzuya[2,3]
kuzuya@kansai-u.ac.jp

Gutmann Gregory Spence[3]
gutmann@molecular-robotics.co.jp

Akihiko Konagaya[3]
konagaya@molecular-robotics.co.jp

[1] School of Computing, Tokyo Institute of Technology
[2] Dept. Chemistry and Materials Engineering, Kansai University
[3] Molecular Robotics Research Institute, Co., Ltd.

Keywords: Molecular Robotics, Super Resolution, Atomic Force Microscope Images, Deep Learning

Atomic force microscopy (AFM) is one of the most popular imaging and characterization techniques, which has the advantage of analyzing many types of surfaces in nano-scale. Many kinds of research have been done the studies on DNA dynamics and structures with the help of AFM, so far. However, AFM's tip radius and resolution are still not enough to imaging results of DNA nano structures, losing the vital information of DNA double strand structure like major grooves and minor grooves. This study presents a super-resolution model combining VR molecular model and deep learning to visualize major and minor grooves from real DNA AFM images. Applying the model, the reconstruction resolution can be beyond the limits set by tip radius and original imaging resolution. To build high-resolution AFM images, VR DNA molecular model[1] is built to obtain simulated DNA strands, and AFM imaging is simulated to build simulated AFM images. Tip radius of AFM probe and image resolution are the parameters in the AFM imaging process to obtain different images by the same simulated DNA model. Then deep learning method based on Cycle GAN[2] is used to build a super-resolution network to construct detailed information for DNA images. Finally, real AFM images are used to test the performance of the model and determine the valid parameters in the image processing section.

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A 3D hydrodynamic ocean model simulation and its data analyses targeting waters off Niihama in Seto Inland Sea, Japan

Masanori Niwano[1] **Yoshihide Matoba**[1]
niwanom@sc.sumitomo-chem.co.jp

Fumio Horiguchi[2] **Yuriko Ishikawa**[2]

[1] Sumitomo Chemical Co., Ltd., 3-1-98 Kasugade-naka, Konohana-ku, Osaka 554-8558, Japan
[2] National Institute of Advanced Industrial Science and Technology, 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan

Keywords: hydrodynamic model, Seto Inland Sea, Niihama, tidal residual current, water transparency, harmonic analyses

Toward a final goal for developing a model of chemical fate and its aquatic organism effect, a high-resolution hydrodynamic ocean model [1] was applied to waters off Niihama in the middle of the Seto Inland Sea (SIS), Japan. A heating formula depending on distribution of water transparency was newly incorporated into the model. Using this model, tidal current, temperature and salinity were calculated in the areas covering the middle of SIS, and then the behaviors at the waters off Niihama were simulated in detail by using results of the first calculation. The simulation was performed in 2016 summer when the effects of the chemicals to aquatic organisms seem highly concerned due to a suppressed convective mixing. The model accurately reproduced spatial and temporal variations of tidal current, water temperature and salinity observed at the waters off Niihama as well as the mid SIS [2-3]. Results from the simulation demonstrated that the waters off Niihama experienced fast water exchange by tidal residual currents enhanced at steep bathymetry and large vertical water displacement due to propagation of waves generated there in comparison with the middle of the SIS.

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