

Unraveling what it means to be alive

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Dive into BDR's intriguing research

Building microscopes?



Y (Yakushiji) ▶ Wow, this looks cool. What is this?

K (Kaneshiro) ▶ It's a laser.

Y ▶ You can actually build a laser?

K ▶ Of course. Lasers were developed by humans after all. If you take the outer cover off of commercial lasers, they will look like something similar to this.

It's like resonator, with the incident light (i.e. light that hits a surface) being amplified through its reflection between these mirrors. Be careful, it's dangerous to touch. This is not mine, by the way.

Y ▶ Oh. (Oops! I thought this was his.) Which one is your work, then?

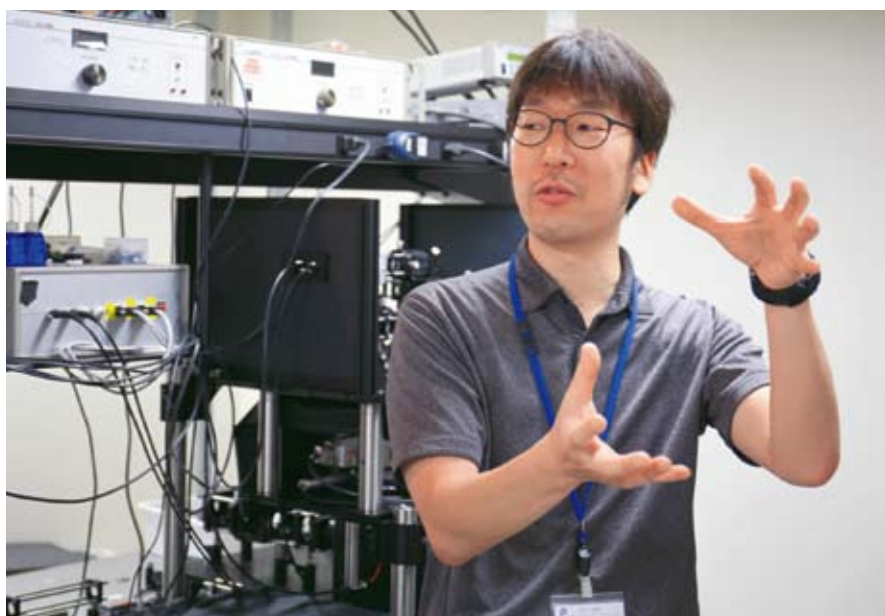
K ▶ This microscope here. It is built based on the theory of Second Harmonic Generation.



Teamwork of specialists from different fields propels biological research

Building microscopes?

This interview took place in Dr. Kaneshiro's lab. I was greeted by the sight of bare lasers and a lot of lenses. I could already sense that this was going to be a different interview than the previous ones...



Dr. Jun-ichi Kaneshiro

Technical Scientist in Laboratory for Comprehensive Bioimaging. He majored in solid state physics at Waseda University. Yearning to break new ground, he moved to RIKEN after finishing his thesis. He currently is developing an instrument dedicated for observing cell division and differentiation inside a mouse embryo. His hobbies include gaming, castles, and watching movies.



Hideki Yakushiji

Business developer based in Kobe. He has a broad background in areas such as analytical chemistry, optics, biotechnology and IT. He is involved in a widerange of activities to assist in commercializing technologies and ideas born from academia, including setting up opportunities for idea sharing, finding investors, and strategic planning.

Y ▶ (He has already lost me...)

K ▶ Light waves that come out (of the microscope) are doubled in frequency, hence it's called "second harmonic generation" or SHG for short.

Y ▶ Why is it doubled?

K ▶ It's generated from the interaction between light and material. Although it is not precisely the same, one analogy might be sound waves, and imagine a higher octave tone coming out. Only certain materials can generate this SHG. Examples of biological materials that can generate SHG include cytoskeleton, microtubule, actin, myosin, collagen and muscle in organisms, or cellulose and fibers in plants. Crystallized proteins can also generate SHG.

Y ▶ What about kidney stones?

K ▶ Hahaha, probably.

If they are materials that can generate SHG, we can observe them in a label-free manner.

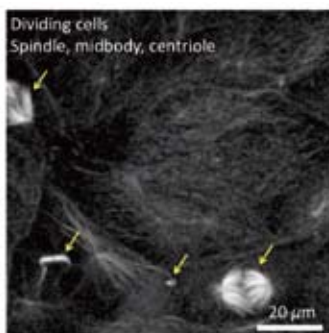
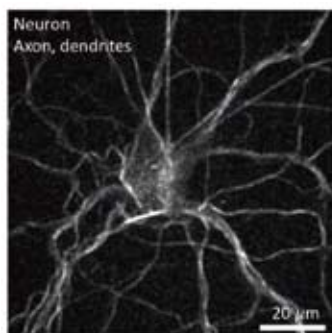
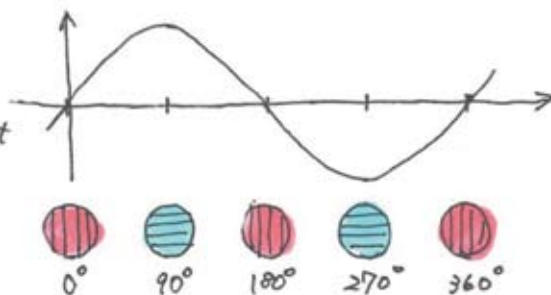
Y ▶ So, do you mean that those materials can be observed without the use of any labeling technique? Is it possible to distinguish between myosin and collagen, for example?

K ▶ SHG light reflects the structure of materials. To make a long story short, SHG is polarized light. If you change the angle of polarization of the incident light and place an analyzer (another polarizer) on the detecting side, you will see that the intensity of observed light will vary depending on the specimen structure. We can repeat this for each molecule, which will allow us to distinguish

When polarizer is



Intensity of detected light depends on the angle of analyzer.



the different molecules.

Y►Okay...

K►When you rotate the polarizer, it produces a beautiful sine curve, and this pattern will vary depending on the molecule or structure.

It takes a long time...

K►This is a neuron with its axon and dendrites, and the other image shows the spindles and other structures that appear during cell division. This is showing three stages of cell division—the beginning, middle and late stages. We can observe similar structures inside a cell.

This figure shows single microtubules that were extracted from a cell and observed in a label-free manner.

Y►Label-free? That's quite an achievement, isn't it?

K►We observed two patterns of polarization dependency, indicating that microtubules have two different structures. This is an old work of mine—it took six years to publish.

Y►It does take time indeed.

K►It was quite a tough job. In particular, it was difficult to try observing a single microtubule with SHG.

Y►What were the most difficult points?

K►First, we cannot amplify the signal since we are not using any labelling techniques. After close examination, we determined that the glass slide used to place the microtubules on produced some background noise. So we stopped using ordinary glass.

Y►Glass?

K►Yes. Ordinary glass contains some impurities, probably to adjust its refractive index. We decided to use quartz glass, which produces less noise and allowed us to obtain this image.

Physical quantity reveals characteristics

Y►I don't understand what this formula is trying to illustrate.

$$P_i^{(2\omega)} = \epsilon_0 \sum_{j,k=1}^3 \chi_{ijk}^{(2)} E_j^{(\omega)} E_k^{(\omega)}$$

K► χ (kai) represents SHG characteristics and E represents the electric field. This is nonlinear optics. This formula says that when two photons combine into one, it results in energy being doubled and in turn the light wavelength being halved.

Y►I don't think I'm following...

K►There has been more progress made recently, and we now know that if you narrow the field of observation, we can even track time-dependent structural changes.

Y►Is that possible even in a living cell?

K►Yes, it is. The recent trend is what's called "all-optical." It's a movement in which scientists are trying to do everything by using optical techniques. Optics have many advantages; for example, we can observe the inside of a cell without having to dissect it.

Y►It's good that no labelling techniques are needed with SHG.

K►That's right. Our lab has been discussing how this technology can be further applied to biology.

In fact, when we talk to biologists, we receive a lot of interest on our work. We have also worked with them to test different samples.

Specialists get together to create new science

Y►What are you going to develop next using SHG?

K►Oh, I am working on something different right now.

Y►Really?

K►I'm currently developing a lightsheet microscope to track individual cells within a mouse embryo during early development.

Y►Ah, that's why you are developing a lightsheet microscope!

K►Yes. It will be a microscope used specifically for observing early-stage mouse embryos.

One of the important aspects of this project is that we are working with a team of specialists in other fields. For example, I don't know much about biology, but I can build microscopes, and vice versa. It is also not easy to obtain a clear image just by taking a picture with a microscope.

Y►Really?

K►Since it is difficult to obtain a perfect, clear image using only a microscope, we need to modify the shooting techniques and deconvolve the obtained images. If we are unable to do this, then it will be quite difficult to track all cells simultaneously. This is the reason why many informatics researchers are included as authors on recent papers related to imaging technology.

Y►It is great that you are able to combine the strengths of different experts to make advancements.

K►It is becoming more and more difficult to do research solely on your own or within one lab.

This type of project was probably not very commonly seen in the past. But, our current project and team is going well so far.

Y►So even life science research now requires teamwork.

I'm looking forward to seeing something you publish in the future. Thank you very much.



POSTSCRIPT

There was a time when I myself fiddled around with lenses and mirrors, so listening to his research brought a sense of nostalgia over me. But, as I never mastered the craft enough to build a lightsheet microscope, I was filled with a renewed sense of respect for the work scientists do. I also learned that this work does not easily lead to publications, and that there are new trends in this field.

Read other interviews



01

Analyzing chromatin structure in more detail than ever before

A powerful method that can pinpoint the positions and orientations of individual nucleosomes—one of the building blocks of chromatin, which contains the genetic material of a cell—has been demonstrated on yeast by Yuichi Taniguchi of the Lab for Cell Systems Control and his team. Their technique will help researchers to explore the relationship between chromatin structure and function.

Ohno M, Ando T, Priest DG, et al. *Cell* 176. 520-534.e25 (2019)

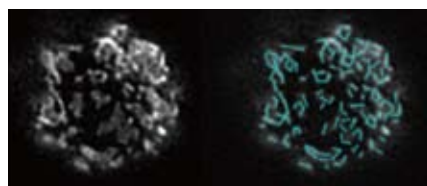


02

A new model for exploring cell polarity

Kalyn Kono and Fumio Matsuzaki of the Lab for Cell Asymmetry, and other collaborators in BDR have found an intrinsic way to induce cell polarity—the asymmetry observed in the shape, structure, or organization of cells—in fruit fly cells. This method provides a useful means for scientists to study the real-time dynamics that occur during cell polarization on a micrometer scale.

Kono K, Yoshiura S, Fujita I, et al. *eLife* 8. e45559 (2019)



03

How chromosomes change their shape during cell differentiation

Mammalian chromosomes are comprised of megabase-sized globular units called topologically associating domains (TADs), and multiple TADs assemble to form what are called A and B subnuclear compartments. TADs containing many active genes form A compartments, while TADs with few or no active genes form B compartments.

A team led by Ichiro Hiratani of the Lab for Developmental Epigenetics showed that A/B compartment changes represent physical movements of TADs within the 3D nuclear space. The study also demonstrated that changes in chromosome conformation preceded changes in DNA-based transactions such as gene expression and DNA replication, entertaining the possibility of predicting DNA transactions based on preceding changes in chromosome structures.

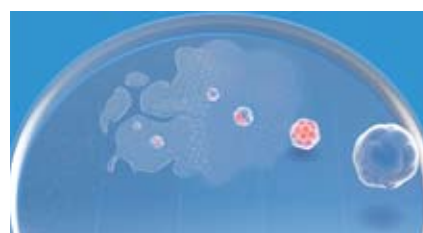
Miura H, Takahashi S, Poonperm R, et al. *Nat Genet* 51. 1356-1368 (2019)

04

Implantable 3D blastocyst-like embryonic structure generated from mouse stem cells

Cody Kime of the Lab for Retinal Regeneration and his collaborators have generated 3D blastocyst-like structures from pluripotent stem cells. The study shows that the blastocyst-like structures very closely resemble actual blastocysts, and even induce proper changes in the uterus after being implanted in pseudo-pregnant mice.

Kime C, Kiyonari H, Ohtsuka S, et al. *Stem Cell Rep* 13. 485-498 (2019)



05

Doubling down on boron to make complex organic molecules

A team led by Takamitsu Hosoya of the Lab for Chemical Biology has developed a user-friendly method to form sought-after diborylalkenes by attaching boron groups to organic molecules. These diborylalkenes are stepping stones to generate many valuable compounds, including pharmaceutical drugs and medical imaging probes for positron emission tomography (PET) scans.

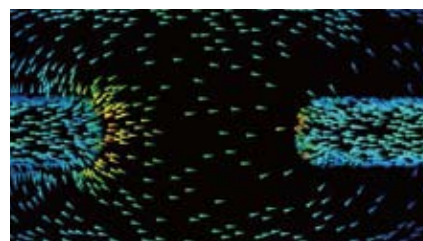
Uetake Y, Isoda M, Niwa T, Hosoya T. *Org Lett* 21. 4933-4938 (2019)

06

Analyzing the metabolites produced by single motile cells

Yo Tanaka and Nobutoshi Ota of the Lab for Integrated Biodevice and their collaborators fabricated a microfluidic device that can trap up to about 30 individual motile cells at one time so that their metabolism can be monitored using an analytic technique known as Raman microscopy. They demonstrated the device's potential by using it to track the production of a metabolite called paramylon in highly motile single algal cells.

Ota N, Yonamine Y, Asai T, et al. *Anal Chem* 91. 9631-9639 (2019)



07

Cyborg-like microchip valve driven by earthworm muscle

Yo Tanaka and his team (Lab for Integrated Biodevice) have developed the first microchip valve powered by living cells. The earthworm muscle tissue used in their system to allowed for a high contractile force that could be sustained for minutes, and unlike electrically controlled valves, did not require any external power source such as batteries.

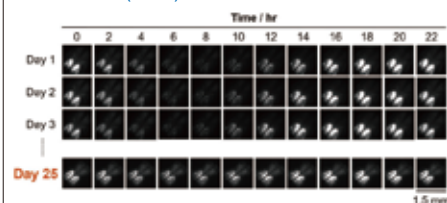
Tanaka Y, Funano SI, Noguchi Y, et al. *Sci Rep* 9. 8042 (2019)

08

Brain tissue kept alive for weeks on an artificial membrane

Nobutoshi Ota and Yo Tanaka (Lab for Integrated Biodevice) have developed a new system for keeping tissue viable for long-term study once transferred from an animal to a culture medium. The new system uses a microfluidic device that can keep tissue from both drying out and from drowning in fluid. A proof-of-concept experiment showed that tissue explanted from the mouse brain remained viable after almost one month in culture, much longer than is possible with other microfluidic culturing methods, and also much simpler.

Ota N, Kanda GN, Moriguchi H, et al. *Anal Sci* 35. 1141-1147 (2019)

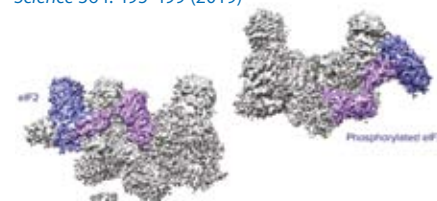


09

How phosphorylation of eIF2 reduces protein synthesis

When cells are subject to conditions of stress, they will stop production of proteins to concentrate their resources on processes more necessary for survival. A study by Takuhiro Ito and Kazuhiro Kashiwagi of the Lab for Translation Structural Biology reveals how a small modification to eukaryotic translation initiation factor 2 (eIF2), a key molecule for initiating translation of messenger RNA into proteins, can shut down a cell's protein-producing machinery when the cell is stressed. This finding could help to find new treatments for neurodegenerative diseases and traumatic brain injury.

Kashiwagi K, Yokoyama T, Nishimoto M, et al. *Science* 364. 495-499 (2019)



Researcher Spotlight

BDR Team Leader

Li-Kun Phng

Laboratory for Vascular Morphogenesis

Interviewed by Emily Bian

(University of Wisconsin-Madison Intern)

Li-Kun

Falling into a career path in research

You can read more about her research at the BDR website:
<https://www.bdr.riken.jp/en/research/labs/phng-lk/index.html>

Q► How did you get started in research?

A► It just progressed from interests, really. I went to school in the UK, and at the time I was interested in biology, so I studied a biology related subject—pharmacology. But after spending one year as an internship student in a pharmaceutical company in Switzerland, I realized that it wasn't the type of research I wanted to do. I then did a Master's degree in Life Sciences; it was quite general but I enjoyed it a lot. It was a one-year research-based course that allowed me to go to three different labs that work on three different research topics. I subsequently decided to pursue a PhD in Life Sciences and was awarded a Graduate Studentship position in Cancer Research UK London Research Institute (now part of The Crick Institute). I have been on this path ever since.

Q► Do you ever find that you need to go above and beyond to prove yourself as a female team leader in the research industry?

A► I spent many years working in Europe and there, especially in biology, you see more representation by women. When I came to Japan, I was very shocked to see fewer women in research. Although there are women working in labs as students and postdocs, the number drops off drastically at PI positions. This is of course a global problem, but it is much more prominent in Japan. I therefore cannot help but wonder what is being done to recruit and retain women in these positions in BDR. I think that more action needs to be taken as there will otherwise be a brain drain. To be honest, it is quite isolating to be one of the very few women team leaders.

As for whether or not I have to stand up more as one of

the few female team leaders to prove my worth, I do not know because, honestly, this is still a new role for me and everything is a challenge! I just try to do my best...

Q► Do you find any difficulties working in Japan as a foreigner?

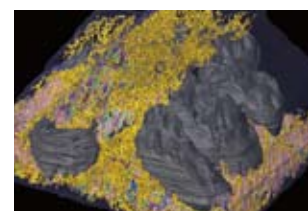
A► I didn't come to Japan knowing much or any Japanese so it is quite challenging to work and live here. However, the great thing about BDR is that most of the science is conducted in English. I know that the administration in the Promotion Office takes a lot of time to translate documents into English, and I really do appreciate it. My assistant also does a great job acting as a mediator between my lab and the rest of the institute (and Japan). Although there is a lot of language support in BDR, one should not expect it in other institutions in Japan, as the previous Japanese lab I worked at was nearly all in Japanese.

Another challenge that I faced was cultural differences. However, this was resolved with time.

Q► Can you explain a little bit about the research you are currently doing?

A► My lab aims to understand how blood vessels are formed. We are interested in understanding how blood vessels are shaped and patterned into a hierarchical network of arteries, veins and capillaries of different sizes as this is essential for optimal blood perfusion. We use the zebrafish as our model system. As the embryos are optically transparent, we can easily perform live imaging to visualize and investigate the behavior of endothelial cells, the basic building blocks of blood vessels, to understand the process of the blood vessel development.

On the cover!



Inside view of a cell captured by electron microscopy. Structure in grey is the nucleus, and structures in yellow and other colors are mitochondria. Mitochondria show wide variation in sizes and shapes, and are crammed tightly in the cell. Image: Lab for Cell Field Structure

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