# People & Ideas

## Shinya Inoué: Lighting the way in microscopy

Inoué's innovations in polarized light microscopy allowed the first documentation of the mitotic spindle in living cells.

When light passes through cells, it is slowed ever so slightly by its encounters with transparent proteins and organelles. The microtubule-based mitotic spindle retards light by a mere 1/100$^{th}$ of its wavelength, creating constructive and destructive interference patterns that can be observed with specialized optics (1, 2).

In the early 1950s, Shinya Inoué used this principle to design a polarized light microscope that could make the first recorded observations of the mitotic spindle in living cells (2). Inoué's continued innovations have revolutionized both microscopes and biological research, resulting in several seminal observations on the biology of living cells (3–6). Now 90 years old, he talked to us from his home near Woods Hole, Massachusetts, where he shared some reminiscences of his past and his hopes for the future of polarized light microscopy.

### FIRST GLIMMER

***What first turned you on to science?***

I think I was always fairly interested in science but not specifically biology until I met Katsuma Dan. He was a biologist who had received his doctorate in Philadelphia and worked at the Marine Biological Laboratory in Woods Hole. But he returned to Japan with his American wife in 1937. In 1941, I was in the first class that he ever taught in Japan, and his approach was entirely different from what I had otherwise experienced in Japanese schools (which involved a lot of rote memorization). Instead of prescribing to us what we had to do in the lab, he would pose various questions and say, "Do you want to try this?" I really decided to go into a career emphasizing biology after I did a project suggested by him in our class at the Musashi Junior College.

PHOTO COURTESY OF CHRISTOPHER INOUÉ

**Inoué preparing to give a seminar in Japan in 2010.**

***I imagine it was difficult to pursue research during the war…***

I didn't get drafted until the last four months, when they just pulled everybody in, so I was able to complete my undergraduate degree. But things were very difficult, especially right after the war. We were very poorly nourished, but, in addition to my parents, Katsuma and Jean Dan actually nursed me back to health at their home in Nagai. Unlike many others, they were supplied with fresh food since, among other things, Jean had helped the local farmers recover half of the airfield (that the Japanese military had usurped from them) by negotiating with General MacArthur's staff. The farmers and fishermen were very grateful and kept on supplying her with fresh food. That's how I regained my strength.

***After the war you emigrated to the US…***

Yes. In 1948 I moved to Princeton, thanks to a fellowship that Jean had found for me. When I arrived, I chose to work with Kenneth Cooper, and he showed me what classical cytologists had done to look at cell division. I found that there was still a lot of controversy as to whether the mitotic spindle even existed and whether the spindle fibers really pulled the chromosomes and so on. So I more or less continued what I had been working on with Katsuma Dan at the Misaki Marine Biological Station after the war but from a different tangent.

### SCINTILLATING OBSERVATION

***At that time people thought the spindle was perhaps just an artifact of fixation…***

Quite a number of people, especially those who were more physiologically oriented, thought that because you could place two drops of fixative in a cytoplasmic gel and get something that looked like the mitotic figure you got after fixing whole cells. And, in fact, quite a number of these "fixation artifacts" were indeed artifacts, but there was also some very good work that showed images resembling what I eventually found in live cells using polarized light. But we had to improve the polarizing microscope before we could make those observations; even the improved polarizing microscope I developed when I was working with Katsuma Dan at Misaki could not bring out the fine detail of these structures.

PHOTO COURTESY OF CHRISTOPHER INOUÉ

**Shinya Inoué**

***How long were you at Princeton before you first made it to Woods Hole?***

The first summer after I came to Princeton, I visited Woods Hole. It was a great experience. Even though it was only four years after the bombing of Hiroshima and Nagasaki, there was very little animosity towards me. And many of the people I met at MBL were friends of Jean and Katsuma Dan, so they introduced me to other people. I learned a great deal, and it was such a friendly and open atmosphere.

We used to work in the basement of an old wooden building that had hosted classes since the 1800s. The general manager, Homer Smith, built a special roof above the microscope benches for us because the building was so old there were all kinds of things falling through the floor above: there was sand coming down, and rain and dish water. But Homer said, "Don't worry. We'll fix it up for you." So that was the atmosphere we were working in.

***Is that where you made your first detailed observations of the mitotic spindle?***

Yes. At Princeton, I had been introduced to the pollen mother cell of the Easter lily. One of the Princeton professors had a

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friend at the University of Pennsylvania who was an expert on this plant, so I went down to Penn and he gave me a few plants at the right developmental stage. It was by working with these cells at Princeton that I got the images that showed spindle fibers most clearly. And it was the movies of those live, dividing cells, which I showed at the Marine Biological Laboratory in Woods Hole, that convinced the skeptics that spindle fibers really existed and that they pulled on the chromosomes and helped with cell division. So that was the first step.

But what was more interesting to me, as I found using marine embryos, was that these fibers and filaments were not static structures. They were very labile—the fibers would fall apart in cold but come right back when you warmed the cell up again, for example. I concluded that these fibers were not like other types of filaments and that they could be made or taken apart by the cell as needed. But it took about 20 years for people to finally isolate the molecule that exhibited those behaviors.

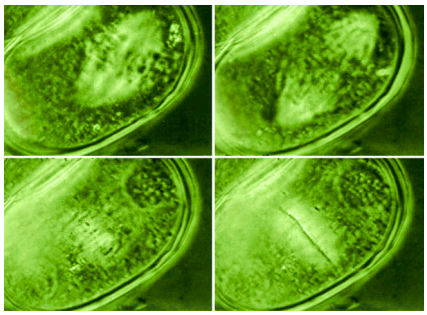

IMAGE COURTESY OF SHINYA INOUÉ, DIGITALLY ENHANCED BY CHRISTOPHER INOUÉ

**Pollen mother cell of the Easter lily, viewed through Inoué's third-generation polarized light microscope, with mitotic spindle fibers clearly visible (false color added).**

## FUTURE VIEW

***How are polarization microscopes being used in cell biology research today?***

They are still in use today, but nowhere near as much as I would like. There are people like Tim Mitchison and my colleagues at MBL who have gotten interested in combining polarization microscopy with fluorescence microscopy. This is a very promising and unique way of finding out how very small numbers of molecules are behaving. It can be used to show changes in the orientation, and even the structure, of molecules.

> "There was still a lot of controversy as to whether the mitotic spindle even existed."

As more people become familiar with the optics of polarized light microscopes, I hope they'll start seeing more uses and a need for improving polarizing microscopes further.

***In your view, what is the strength of the polarizing microscope?***

It has a very high sensitivity that can detect very small numbers of molecules and find out what each molecule is doing, rather than just looking at an assembly of molecules. With fluorescence, you can now pinpoint where even single molecules reside, but you can't tell what they're doing very well. However, by combining fluorescence with polarized light you can tell their organization, their functional changes, and so on. So when it gets down to the really molecular, or submolecular, level of things, I think one needs to go back to using polarized light again.

But there are a number of tricks that one must master to use polarization optics optimally. For one thing, you must be careful to choose the right kinds of cells for these studies. Quite a number of cells have granules that scatter light and interfere with the structures you'd like to observe. We've dealt with that problem by centrifuging cells to get rid of the granules, and the cells behave perfectly normally. But, to do this, you need a certain kind of expertise—somewhat different than with other forms of microscopy.

***Are there any other recent developments in microscopy that have excited you?***

The ability to resolve the location of fluorescence of single molecules or very few molecules that are way below the limit of resolution of the light microscope—that is something very new. What this means is that we are no longer limited to what for over 100 years was thought to be the resolution limit of the light microscope, namely 200 nanometers or two-tenths of a micron. One can actually get down to almost molecular-level distances, and I think that is going to make a huge difference.

Eventually, it may also be possible to use wavelengths way outside the visible spectrum. We're currently limited as to what we can do because we mainly know the properties of electromagnetic waves at certain wavelength ranges. But I think that once the physics improves on this score, we might find some surprising new avenues, too.

***What are you working on now?***

I cannot do much lab work anymore because I lost the use of one eye a few years ago. But I try to help my colleagues develop new uses of polarized light in their microscopes. And, last year, I was able to go back to Japan, which was very nice. My health was just barely okay, but one of my sons took me over. We enjoyed seeing our relatives again, especially my four sisters, who are all in their 80s. I also got to meet with many of my colleagues there, both academic and in industry, who kindly arranged a number of interesting seminars and dinner parties for us. We spent quite some time reminiscing about old times, too. It was very memorable.

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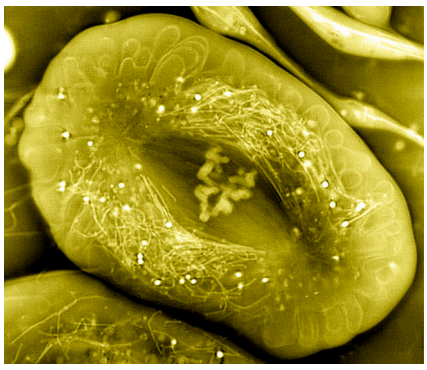

IMAGE COURTESY OF MIKE SHRIBAK AND JIM LAFOUNTAIN, DIGITALLY ENHANCED BY CHRISTOPHER INOUÉ

**A live crane fly cell in anaphase (false color added), seen through a specialized microscope Inoué helped develop.**