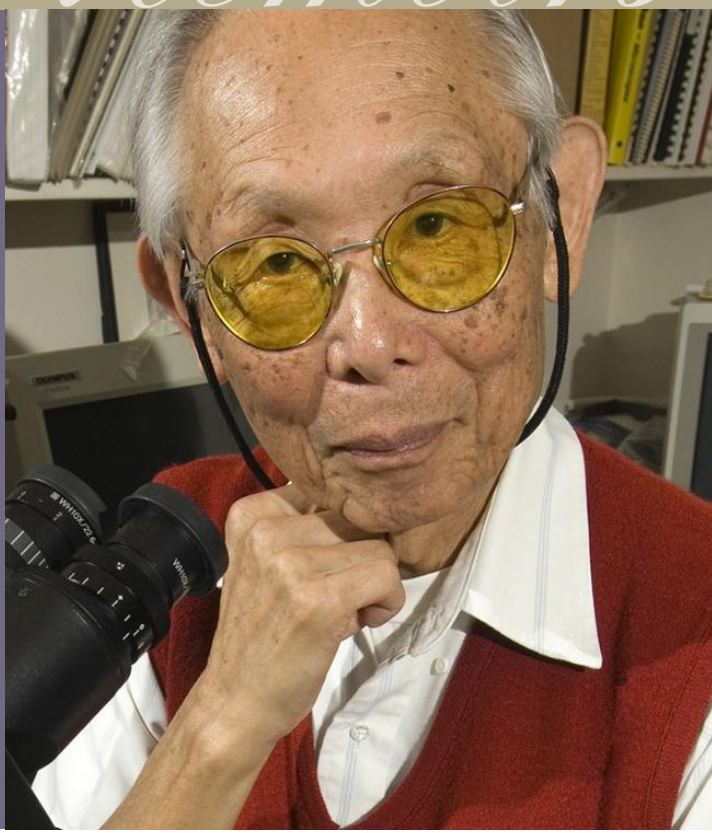
Shinya Inoué

1921–2019

BIOGRAPHICAL *Memoirs*



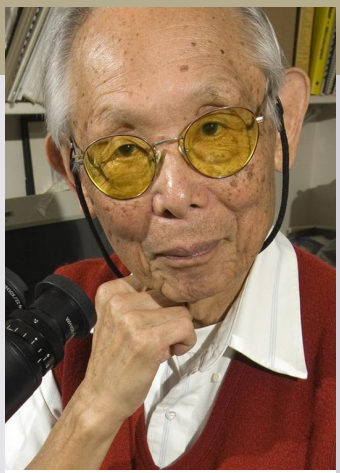
A Biographical Memoir by
J. Richard McIntosh,
Nancy and Edward Salmon,
Paul Maddox,
and Tim Mitchison

NATIONAL ACADEMY OF SCIENCES

SHINYA **INOUÉ**

January 5, 1921–September 30, 2019

Elected to the NAS, 1993



Photography by Tom Kleindinst/Marine Biological Laboratory

By J. Richard McIntosh, Nancy and Edward Salmon, Paul Maddox, and Tim Mitchison

Shinya Inoué is most widely known for his work on mitosis in marine eggs and plant cells, for which he used polarized light to detect and measure the birefringence caused by spindle microtubules. He was one of the early students of mitosis to employ this kind of microscopy,[1] and with colleagues he invented a rectifier that corrected the complex perturbations of plane polarized light induced by lenses with high curvature and numerical aperture.[2] This device allowed him to achieve images that were simultaneously of high spatial resolution and high signal-to-noise ratio. The resulting views of spindles in action were of unprecedented sensitivity and revealed aspects of spindle behavior that had never before been seen.[3] Inoué was also a strong believer in the importance of quantitative measurement. He and his students used his special microscopes to assess the magnitude of spindle birefringence under a variety of experimental perturbations, demonstrating that the spindle was a dynamic structure whose characteristics at steady state were readily altered by environmental changes, such as temperature, hydrostatic pressure, or the presence of spindle-poisoning chemicals.[4,5,6] These observations became the foundation for his concept of spindle mechanism, the idea that the assembly and disassembly of biological polymers could do mechanical work.[7]

Shinya Inoué was born in London on January 5, 1921, the son of a widely traveled Japanese diplomat. He lived in China, the United States, and Australia before moving to Japan for high school and college. During his undergraduate work with Katsuma Dan at the University of Tokyo, he developed an interest in mitosis and polarization microscopy, receiving his degree in 1944. After World War II, he came to the United States to study biology at Princeton University, earning a Ph.D. in 1951.

Inoué's accomplishments include four U.S. patents for his microscopes and more than 100 scientific papers, many of which are included in *The Collected Works of Shinya Inoué:*

Microscopes, Living Cells, and Dynamic Molecules (2008). He also authored the book *Video Microscopy* (1986). In recognition of these contributions, he received several awards: the Order of the Sacred Treasure, Gold Rays with Neck Ribbon Award from the Government of Japan; the International Prize for Biology from the Japan Society for the Promotion of Science; membership in the National Academy of Sciences and the American Academy of Arts and Sciences; the Distinguished Scientist Award from the Microscopy Society of America; and the E. B. Wilson Award from the American Society for Cell Biology.

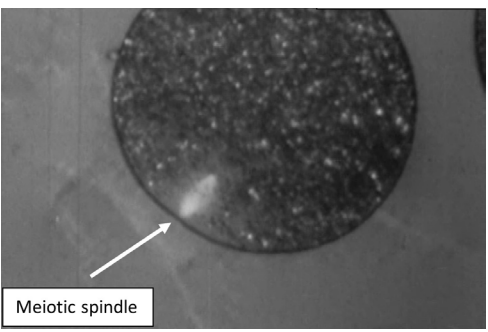
Inoue receiving award. (Photo credit Eve Inoue.)

This biographical memoir of Inoué's life and achievements fails to convey his value to cell biology and his impact on the field. Although he was not the first person to use a polarization microscope to visualize spindle fibers in living cells,[8,9] his work with this instrument was of the highest quality. He pursued the study of spindle birefringence with a care and rigor that allowed him to learn important aspects of spindle dynamics and eventually the behavior of the microtubules that are responsible for most of a spindle's optical properties. It was both the rigor of his approach to cellular imaging and his gentle but insistent approach to scientific pedagogy that let him make his most important contributions. After completing his doctoral training at Princeton University in 1959, Inoué took a position at Dartmouth Medical School, which was then hiring young scientists of admirable quality who shared scientific interests: Andrew Szent-Gyorgyi, a student of muscle myosin, and Gordon Ellis, Robert Kane, Hidemi Sato and Inoué, all of whom studied mitosis. During this period, Inoué trained Arthur Forer in both microscopy and the study of mitosis, leading to seminal papers about the effects of ultraviolet microbeam irradiation on spindle birefringence and subsequent chromosome motions.[10] However, this group disbanded when the medical school discontinued support for this type of basic science in 1966. Szent-Gyorgyi went to Brandeis University, and Kane to the University Hawaii, where he could obtain large volumes of sea urchin eggs for his studies of spindle biochemistry. The other three students of mitosis went to the Department of Biophysics at the University of Pennsylvania (Penn), with Inoué serving as chair.

At Penn, these scholars collaborated with great effect. Ellis developed tools for the micromanipulation of small objects in cells, so he could learn about the forces that cells exert

on organelles, with a special focus on meiotic chromosomes. Sato continued his work with polarization optics to study spindle birefringence under diverse experimental conditions. Inoué developed tools that allowed careful observation of spindle physiology when conditions, such as ambient temperature, were altered. It was this work that allowed Inoué and Sato to develop and refine their model for cell motility by labile association of molecules.[11] Inoué's work attracted several graduate students, allowing him to pursue the effects of temperature,[12] pressure,[13] and colchicine,[14] a well-known spindle poison, on the stability of the spindle and its birefringence. The papers published by Inoué's students were commonly single author; Inoué put himself in the background, even when his intellectual and technical contributions were significant.

During this period, Inoué's student, Edward D. "Ted" Salmon developed a chamber that could exert high hydrostatic pressure on a sample of living cells while their birefringence was simultaneously being measured, a difficult accomplishment because of strain-induced birefringence in the chamber's transparent top and bottom. At about this time, Inoué and Salmon began spending summers at the Marine Biological Laboratories (MBL) in Woods Hole, Massachusetts. This famous laboratory provided an excellent working environment. Marine organisms were available in large quantities and provided eggs, zygotes, and embryos, excellent sources of experimentally controllable mitotic cells. Some oocytes, such as those of the marine worm Chetopterous, contain meiotic spindles arrested in metaphase to await fertilization. These comparatively stable spindle structures are attached to the cell cortex near the cell surface, where they are comparatively easy to image and allow for ready measurements of chromosome position and spindle length during experimental perturbation. Salmon and Inoué used these eggs in specially designed chambers to show that as pressure increased and spindle birefringence decreased, the chromosomes moved to the spindle poles.[15]



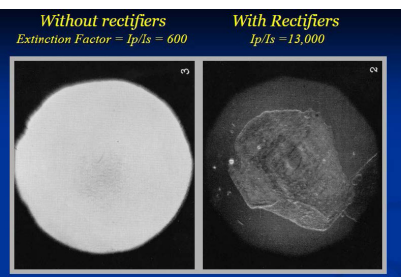
Chetopterous egg viewed with polarization optics.
(Photo credit E.D. Salmon/ Univ. North Carolina.)

In 1982, Inoué resigned his position at Penn to devote full time to research at Woods Hole. In collaboration with Rudolf Oldenbourg, he continued to improve microscopes for polarization microscopy, and he also became interested in differential interference

contrast imaging. Inoué's stature as a microscopist allowed him to interact constructively with many of the manufacturers of the best light microscopes; they, in turn, became interested in supporting a course at the MBL in analytical and quantitative light microscopy. The first such course was offered in 1981; it brought state of the art microscopes and cameras to Woods Hole, allowing technicians from the companies to contribute to the education of the lucky students who participated. This course continues to this day, and the MBL courses in physiology and developmental biology have also benefited from corporate participation. Inoué's standing as a microscopist has led to the high-level education of generations of students at the MBL summer programs. Additionally, the exposure given to the companies has improved their sales as well as the quality of the optical technologies available to biologists.

Inoue at work with one of his novel research microscopes. (Photo credit MBL/ Woods Hole.)

Several of Inoué's scientific colleagues at Woods Hole brought expertise and approaches that complemented his own, enhancing the contributions that this thoughtful man was able to make. Rudolf Oldenbourg and Inoué developed the LC-Polescope, a polarization microscope that uses numerous innovations to make a commercially available instrument with the best-available sensitivity and resolution.[16] Ted Salmon had been trained initially as an engineer, so he understood electronics and computing as well as optics. Ted's influence led Inoué to experiment with television cameras and image processing as tools for enhancing microscopy.



Images showing the value of rectified polarization optics. (Photo credit Paul Maddox/ Univ. North Carolina.)

A remarkable collaboration between Inoué's group and Robert D. Allen, a cell biologist who early on recognized the importance of differential interference contrast (DIC) optics,[17] led to the realization that the video cameras then commercially available could improve image signal-to-noise and contrast to the point that single microtubules became visible. This technical advance led to the discovery of kinesin by Ron Vale in Michael Sheetz' lab.[18] The work was an early example of research that is now widespread, using the visualization of individual polymers, rather than bulk properties such as viscosity or light scattering, to examine protein behavior in vitro.

The impact of this change has been career-changing for hundreds of scientists and has had a major impact on study of the cytoskeleton. In this context, it is surprising that Inoué never espoused fluorescence optics, though he and collaborators at Woods Hole were the first to put a cooled CCD camera on a spinning disk confocal microscope,[19] making an instrument that has since become widely used for the study of fluorescent proteins in living cells. Moreover, Inoué contributed to our understanding of the structure of the green fluorescence protein.[20] His own avoidance of fluorescent labels was perhaps a result of his sense of data purity. He was not satisfied with the study of protein dynamics if the molecule under study had been chemically or genetically modified by the addition of a fluorophore, an intuition that may prove to be more insightful than many of us who have used fluorescent proteins would like to believe!

The equipment for electronic imaging available in the 1980s and 1990s included analog control of important image parameters, but at this time the hard- and software for fast and economical image digitization and processing was developing rapidly. Inoué's son, Ted, wrote code for video image processing at his father's request. Eventually, he formed a company to develop and support image processing software that provided more subtle, varied, and extensive image processing than was allowed by the knobs on an analog device. Inoué recognized the tremendous value of video image processing and supported it through his teaching at Woods Hole summer courses, the books he wrote and published, and the example of his own work. He was awarded the title of Distinguished Scientist by the MBL in 1986 and continued to work there until his death in 2019 at the age of 98. He is survived by his wife, Sylvia (McCandless) Inoué, five children, six grandchildren, and one great-grandchild. Inoué's gentlemanly approach to teaching and to science itself had a significant impact on generations of students who came through the MBL. He and his contributions will be valued for years to come.

As Oldenbourg commented:

> *For the last 30 years, I had the great fortune to work with Shinya Inoué, who was an exacting and demanding, yet patient and always generous mentor, who taught by example, combining a passion both for creating tools and applying them to reveal the mysteries of life. Inoué not only was an outstanding scientist, but he was universally respected for his kind and thoughtful ways, for his humanity, and his attention to personal relationships.*

REFERENCES

1. Inoué, S. 1953. Polarization optical studies of the mitotic spindle. I. The demonstration of spindle fibers in living cells. *Chromosoma* 5:487–500.

2. Inoué, S., and W. L. Hyde. 1957. Studies on depolarization of light at microscope lens surfaces. II. The simultaneous realization of high resolution and high sensitivity with the polarizing microscope. *J. Biophys. Biochem. Cytol*. 3:831–838.

3. Inoué, S., and A. Bajer. 1961. Birefringence in endosperm mitosis. *Chromosoma* 12:48–63.

4. Salmon, E. D. 1975. Pressure-induced depolymerization of spindle microtubules. I. Changes in birefringence and spindle length. *J. Cell Biol.* 65:603–614.

5. Fuseler, J. W. 1975. Temperature dependence of anaphase chromosome velocity and microtubule depolymerization. *J. Cell Biol.* 67:789–800.

6. Inoué, S., J. Fuseler, E. D. Salmon, and G. W. Ellis. 1975. Functional organization of mitotic microtubules. Physical chemistry of the in vivo equilibrium system. *Biophys. J*. 15:725–744.

7. Inoué, S., and H. Sato. 1967. Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. *J. Gen. Physiol. Suppl*. 50:259–92.

8. Schmidt, F. O. 1939. The ultrastructure of protoplasmic constituents. *Physiol. Rev*.19:270–302.

9. Hughes, A. F., and M. M. Swann. 1948. Anaphase movements in the living cell. *J. Exp. Biol*. 25:45–72.

10. Forer, A. 1965. Local reduction of spindle fiber birefringence in living Nephrotoma suturalis (Loew) spermatocytes induced by ultraviolet microbeam irradiation. *J. Cell Biol.* Suppl. 25:95–117.

11. Inoué, S., and H. Sato. 1967. See Ref. 7.

12. Fuseler, J. W. 1975. See Ref. 5.

13. Salmon, E. D. 1975. See Ref. 4.

14. Inoué, S., J. Fuseler, E. D. Salmon, and G. W. Ellis. 1975. Functional organization of mitotic microtubules. Physical chemistry of the in vivo equilibrium system. *Biophys. J*. 15:725–744.

15. Salmon, E.D. 1975. See Ref. 4.

16. Oldenbourg, R. 2007. Analysis of microtubule dynamics by polarized light. *Methods Mol. Med*. 137:111–123.

17. Allen, R. D., N. S. Allen, and J. L. Travis. 1981. Video-enhanced contrast, differential interference contrast (AVEC-DIC) microscopy: A new method capable of analyzing microtubule-related motility in the reticulopodial network of *Allogromia laticollaris. Cell Motil*. 1:291–302.

18. Vale, R. D., T. S. Reese, and M. P. Sheetz. 1985. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* 42:39–50.

19. Maddox, P., et al. 1999. Dynamic confocal imaging of mitochondria in swimming *Tetrahymena* and of microtubule poleward flux in Xenopus extract spindles. *Biol. Bull.* 197:263–265.

20. Inoué, S., et al. 2002. Fluorescence polarization of green fluorescence protein. *Proc. Natl. Acad. Sci. U. S. A*. 99:4272–4277.

SELECTED BIBLIOGRAPHY

1953 Polarization optical studies of the mitotic spindle. I. The demonstration of spindle fibers in living cells. *Chromosoma* 5(5):487–500.

1957 With W. L. Hyde. Studies on depolarization of light at microscope lens surfaces. II. The simultaneous realization of high resolution and high sensitivity with the polarizing microscope. *J. Biophys. Biochem. Cytol*. 3(6):831–838.

1958 With H. Kubota. Diffraction anomaly in polarizing microscopes. *Nature* 182:1725–1726.

1961 With A. Bajer. Birefringence in endosperm mitosis. *Chromosoma* 12:48–63.

1967 With H. Sato. Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. *J. Gen. Physiol. Suppl*. 50(6):259–292.

1970 With J. Aronson. Reversal by light of the action of N-methyl N-desacetyl colchicine on mitosis. *J. Cell Biol.* 45(2):470–477.

1974 With G. G. Borisy and D. P. Kiehart. Growth and lability of Chaetopterus oocyte mitotic spindles isolated in the presence of porcine brain tubulin. *J. Cell Biol.* 62(1):175–184.

1975 With J. Fuseler, E. D. Salmon, and G. W. Ellis. Functional organization of mitotic microtubules. Physical chemistry of the in vivo equilibrium system. *Biophys. J*. 15(7):725–744.

With H. Sato and G. W. Ellis. Microtubular origin of mitotic spindle form birefringence. Demonstration of the applicability of Wiener's equation. *J. Cell Biol.* 67(3):501–517.

1981 Video image processing greatly enhances contrast, quality, and speed in polarization-based microscopy. *J. Cell Biol.* 89(2):346–356.

1986 With G. W. Ellis and T. Inoué. Computer-aided light microscopy. *Soc. Gen. Physiol. Ser.* 40:15–30.

1987 Video microscopy of living cells and dynamic molecular assemblies. *Appl. Opt.* 26(16):3219-3225.

1989 Imaging of unresolved objects, superresolution, and precision of distance measurement with video microscopy. *Methods Cell Biol*. 30:85–112.

1990 Dynamics of mitosis and cleavage. *Ann. N. Y. Acad. Sci.* 582:1–14.

1995 With E. D. Salmon. Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol. Biol. Cell.* 6(12):1619-1640.

1997 With K. Spring. *Video Microscopy: The Fundamentals*. New York: Plenum Press.

1998 With R. Oldenbourg. Microtubule dynamics in mitotic spindle displayed by polarized light microscopy. *Mol. Biol. Cell.* 9(7):1603–1607.

1999 With P. T. Tran, P. Maddox, and F. Chang. Dynamic confocal imaging of interphase and mitotic microtubules in the fission yeast, S. pombe. *Biol. Bull.* 197(2):262–263.

2001 With R. A Knudson et al. Centrifuge polarizing microscope. I. Rationale, design and instrument performance. *J. Microsc*. 201(Pt. 3):341–356.

2002 With O. Shimomura, M. Goda, M. Shribak, and P. T. Tran. Fluorescence polarization of green fluorescence protein. *Proc. Natl. Acad. Sci. U. S. A*. 99(7):4272–4277.

2008 Microtubule dynamics in cell division: Exploring living cells with polarized light microscopy. *Ann. Rev. Cell Dev. Biol*. 24:1–28.

With M. Shribak, J. LaFountain, and D. Biggs. Orientation-independent differential interference contrast microscopy and its combination with an orientation-independent polarization system. *J. Biomed. Opt*. 13(1):014011.

Published since 1877, *Biographical Memoirs* are brief biographies of deceased National Academy of Sciences members, written by those who knew them or their work. These biographies provide personal and scholarly views of America's most distinguished researchers and a biographical history of U.S. science. *Biographical Memoirs* are freely available online at www.nasonline.org/memoirs.