Japan Academy Prize to:

Atsushi Miyawaki
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for "Technological Innovations in Bioimaging for a Better Understanding of the Spatiotemporal Regulation of Cellular Signaling Mechanisms"

Outline of the work:

In 1962, which is around the time Dr. Atsushi Miyawaki was born, Dr. Osamu Shimomura discovered and purified the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*. In 1992, the jellyfish GFP gene was cloned, causing a revolution in fluorescence labeling technology in cell biology. The use of genetic engineering techniques enabled the fluorescence labeling of cells, subcellular organelles, and biomolecules. In 1999, new fluorescent proteins were cloned from coral animals, and at the turn of the century, the genome structures of various life forms were elucidated, with players in cellular function and biomolecules gathering together. With a growing demand for visualization techniques to understand how biomolecules behave in live cells, expectations were high for innovations in fluorescence imaging technology and the spread of such technology. The dynamic behavior of biomolecules is crucial in understanding the mechanisms of cell proliferation, differentiation, and canceration, and the field itself has attracted the attention of the pharmaceutical industry, in particular.

The intersection of biochemistry, optical physics, and imagination by a few prescient individuals revolutionized the life sciences by opening the optical spectrum to spatiotemporal interrogation. In the 1980s, Dr. Miyawaki developed the first framework to import the physical science concept of fluorescence resonance energy transfer (FRET) to biological imaging. Inspired by the cloning of GFP in 1992, he pioneered the application of GFP-based FRET with Dr. Roger Tsien in 1997. Further, he helped to discover and develop a panoply of GFP variants that could serve as FRET donors and acceptors, of which cameleon was the prototype for the future proliferation of genetically-encoded Ca^{2+} indicators (GECIs) that are now ubiquitous in life sciences research. Although Ca^{2+} imaging has been popular since around 1985, the classical indicators used were mostly synthetic compounds, and thus, there were limitations in the type of cell that could be analyzed and the method that could be employed. The combination of FRET with genetic engineering created the first research tool for visualizing molecular interactions and structural changes in live cells in real time.

In a quest for new fluorescent proteins in nature, Dr. Miyawaki conducted molecular cloning from a variety of life forms with the aim of elucidating their physical properties and developing new modes of bioimaging technology. Since 2002, he pioneered the field of "light-operated technologies" using photoconvertible or photochromic FPs; for example, creating the first pairings

called Kaede and Dronpa and elucidating their photonic and molecular mechanisms. In 2006, Dr. Miyawaki invented Keima that shows the largest Stokes shift, which is the difference between the peak excitation and peak emission wavelengths, of any FP discovered to date.

The novel probes for cell functions that Dr. Miyawaki and his colleagues designed include Fucci, GEPRA, UnaG, mt-Keima, and mito-SRAI. Fucci is a cell-cycle probe that uses the cell-cycle degradation machinery of ubiquitin oscillators. Fucci in cells and transgenic animals have provided a wealth of information about the proliferation and differentiation of eukaryotic cells, including studies on stem cells and neurogenesis. GEPRA, which is a genetically-encoded probe for retinoic acid, enables the visualization of endogenous gradients of the morphogen retinoic acid in zebrafish embryos, whereas UnaG is a natural fluorescent sensor for bilirubin isolated from eel muscle. This highly sensitive method for measuring bilirubin, which at high levels causes jaundice and kernicterus, may contribute to improving global health. Both mt-Keima and mito-SRAI are now widely used to monitor mitochondrial phagy, a process, which is linked to Parkinson's disease.

Recently, Dr. Miyawaki and his group performed directed evolution of firefly luciferase using a red-shifted and highly deliverable luciferin analog to establish a fully engineered bioluminescence system called AkaBLI. This remarkable system can produce a 100 to 1,000-fold brighter emission *in vivo* than conventional systems, thereby allowing the non-invasive visualization of small numbers of cells inside the deep organs of freely moving animals.

Finally, Dr. Miyawaki and colleagues helped to drive a renaissance in tissue clearing technology. In 2011, his group reported the Sca*l*e technology, which is a method for optically clearing fixed brain samples. In the following years, a new field emerged based on variations of this tissue clearing method. His group recently developed a variation called Sca*l*eS that enables large-scale three-dimensional tissue reconstruction, including the brains of patients with Alzheimer's disease.

These discoveries and advances demonstrate that Dr. Miyawaki is a leading innovator at the cutting edge of life sciences optical imaging technology through his formative efforts in the original research of bioimaging technology and the development of applied technology that has proguced a substantially pervasive efect.

List of Main Publications

1. Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N. and Mikoshiba, K. (1989) Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400. Nature **342**, 32–33.
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