Adaptive optics microscopy: Towards high resolution imaging of the fertilization in living plants

Natl. Inst. Basic Biol. [1], Grad. Univ. Adv. Stud. [2], Natl. Astron. Obs. Jpn. [3],

°Yosuke Tamada[1,2], Masayuki Hattori[1,3]

E-mail: tamada@nibb.ac.jp

Fertilization is the fusion of male and female gametes, such as a sperm and an egg, from the parents. The fused cell is called as a zygote, from which the development of the individual organism starts. The zygote inherits two types of information from the gametes of the parents; genetic and epigenetic information. Genetic information is encoded with four types of nucleotides in genomic DNA, which is stably inherited to next generations. In contrast, the epigenetic information exists as covalent modifications on genomic DNA, and dynamically changes after the fertilization. To understand the dynamics of the epigenetic information, we have produced the fluorescent probes to detect epigenetic modifications on genomic DNA and performed single nucleus live imaging of the epigenetic modifications using optical microscopes during and after the fertilization of the moss *Physcomitrella patens*. However, the images were always quite blurred, because the fertilization occurs inside of the tissue, and the imaging must be performed through several layers of living cells. Living cells contain many kinds of small structures and organelles with different refractive indices. Therefore, the light is severely disturbed when it goes through living cells, and the resultant images are severely degraded. To solve the problem and perform high resolution imaging during the fertilization, we have tried to apply adaptive optics (AO) to the live imaging. AO is the technique developed in astronomy to correct the disturbance on the light caused by atmospheric turbulence and to perform high resolution imaging of astronomical objects using ground-based telescopes. Applying AO to microscope, the disturbance on the light caused by biological structures and organelles will be corrected and high resolution images can be obtained even inside of living cells and tissues. To apply AO to live-cell imaging of *P. patens*, we first analyzed the optical property of living plant cells, and found that chloroplasts are the main source of the disturbance on the light. We then developed the AO microscope to correct the disturbance caused by chloroplasts (Fig. 1), and performed high resolution imaging of the epigenetic modification in living plant cells. In this talk, we will introduce our AO microscope and present the recent data obtained with the microscope. We also summarize recent trend of the research and discuss the future direction.

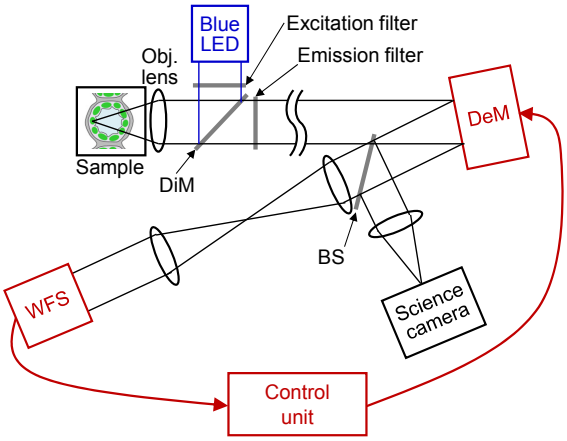

Fig. 1 Our AO fluorescent microscope. Sample (plant cells) is described bigger than the actual size. Some parts of the system is simplified. BS, beam splitter DeM, deformable, mirror; DiM, dichroic mirror; WFS, wavefront sensor.