3-3 Fluorescence Microscopy for Live Cell Imaging

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Communications within the cell are accomplished by the coordinated interactions of molecular components. Observation of such molecular interactions in living cells is essential in understanding cellular communications. Toward this end, we have developed microscope systems for imaging intracellular molecular components in living cells. Here we introduce unique features of these microscope systems and their applications.

Keywords
Bio-molecular communications, Fluorescence microscopy, Dynamics, Three-dimensional imaging

1 Preface

Since modalities of communication in the modern society are changing rapidly, it becomes necessary to develop a flexible and dynamic communication system which replaces the traditional static communication system. In the natural world, such flexible and dynamic communication system has already been realized and operated in living organisms. To learn such excellent functions of living organisms, it is essential to study and understand their communication system. For this purpose, our Cell Biology Group has developed fluorescence microscope systems which are capable of observing molecular behaviors within the living cells. By using the different instruments and methods, it becomes possible to follow dynamics of particular intracellular molecular components in the living state. It is also possible to observe multiple components when they are stained with different colors. In this report, we will introduce these imaging instruments and methods which were developed as our research projects in Cell Biology Group.

2 Live cell imaging by fluorescence microscopy

As a high-resolution microscopy, electron microscope would be a choice in general. Although the electron microscope provide high resolution, it will only allow us to observe fixed specimens. To continuously observe the changes of the particular molecules in living cells, we have adopted fluorescence microscopy. By using fluorescent staining, the particular molecules with high molecular specificity can be stained with different colors. Since their cytotoxicity are relatively mild, there is merit on the use of fluorescence microscopy to enable us to observe the living biological specimen. This is in contrast with electron microscopy which requires the specimen to be fixed and dehydrated. Even though the cytotoxicity are relatively mild during fluorescence staining and observation, there were quite different problems to keep the cells alive and to continuously observe them in comparison with handling the fixed specimens. Therefore, it is necessary to develop imaging methods for fluorescence microscopy which
are capable of observing living cells. To solve various problems associated with fluorescence staining and observation, we have developed several different instruments, and succeeded in continuously observing the molecular behaviors within living cells. We will further discuss instrumentations and applications of these microscope systems.

2.1 Multi-color three-dimensional fluorescence microscope system

This imaging apparatus was developed to enable three-dimensional observation of living cells with a maximum of four-color staining as a three-dimensional specimen for several days. To do three-dimensional observation of multi-color fluorescent stains for long periods, their cytotoxicity to cells and the focal plane drift due to temperature changes become major problems in imaging. To solve these problems, we introduced special considerations in instrumentations with accurate temperature control, as well as considerations in observation conditions optimized for living cells. Main hardware consists of wide-field fluorescence microscope, cooled CCD image detector, computer for image capture and equipment control, and a custom-made temperature control room that contains the whole microscope (Fig.1). Furthermore, the fluorescence microscope is equipped with a computerized filter exchanger for multi-color stains, a focus controller for three-dimensional observation, an XY-motorized stage for specimen movement, and shutters for fluorescent illumination and the CCD detector. For more details of this apparatus, refer to the literatures [1][2]. This microscope system is user-friendly for fluorescence observation of living cells (Fig. 2). Furthermore, it is also possible to obtain images with high resolution by computational deconvolution image processing [3].

2.2 Spectral imaging fluorescence microscope system

The main feature of this apparatus is its ability to measure fluorescence spectrum of the image. By using this apparatus, it is possible to continuously observe the fluorescence spectrum of the image of living cells [4]. By using a fluorescent probe which can detect chemical changes within the biological specimen as fluorescence spectrum, it is possible to image chemical reactions within living cells. As a fluorescent probe that measures calcium concentration within the biological specimen using FRET (Fluorescence Resonance Energy Transfer) which occurs between CFP and YFP, for example, the so-called “Cameleon” has been developed [5]. With this probe, it is possible to measure calcium concentration within cells as the fluorescence spectrum changes [6][7]. As a hardware, a confocal microscope with spectroscopy is provided with tempera-
ture control devices for live cell observation. Water-cooling Kr laser (413 nm) is equipped to effectively excite CFP without exciting YFP. Therefore, FRET can be measured using CFP and YFP. When two fluorescent molecules exist within a distance of less than several nanometers and with their dipole moments aligned, FRET occurs and can be useful in measuring molecular interactions (Fig. 4). In addition to FRET, this apparatus can be used for FRAP (Fluorescence Recovery After Photobleaching). In this method, protein molecules stained with the fluorescent dye are photobleached in a small area of the cell; by measuring recovery of fluorescence in the bleached area, mobility of the fluorescent protein can be measured [8].

2.3 Spinning-disk confocal fluorescence microscope system with a dual view detector

This apparatus was developed as an imaging system specialized for measurement of FRET which occurs between CFP and YFP. A commercial fluorescence microscope is equipped with a spinning-disk confocal unit of the Nipkow disk type (Yokogawa Electric, Inc). The emitted fluorescence are split into two light paths for CFP and YFP through a dual-view spectroscopy unit and simultaneously detected on split areas of a single CCD. As an excitation light source, a semiconductor laser of 430 nm optimum to excite CFP is used. The ratio of CFP and YFP fluorescence intensities is computed for measuring FRET. This apparatus is suitable for time-lapse measurement of FRET change within living cells. FRET measurements can detect within living cells, providing an opportunity of imaging molecular communications within the living cell.

3 Conclusion

The development of live cell imaging technology is a top priority for the 21st century. The live cell imaging technology developed by Cell Biology Group has been highly appreciated in the world because of its high level of technology. Through this technology,
we can promote understanding of living cells and bio-molecular communications. With those understandings as premises, new communication media can be developed by following the examples of living organisms. In this report, we introduced the imaging technology developed by Cell Biology Group with a focus on instruments. For the detailed academic achievements, refer to our Web page of Cell Biology Group [9].

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