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Super-resolution Confocal Live Imaging Microscopy (SCLIM) -- Cutting-edge Technology in Cell Biology*

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***Abstract*—Super-resolution confocal live imaging microscopy (SCLIM) we developed provides amazingly high-speed live cell imaging at high space resolution. With this technology we are now able to observe details of membrane traffic events, including behaviors of small vesicles, cisternal maturation of the Golgi apparatus, and membrane segregation within a compartment.**

I. Introduction

Many questions remain in cell biology as to molecular mechanisms governing a variety of dynamic events in living cells, such as trafficking between membrane organelles (membrane traffic). Live cell imaging is one of the most promising approaches to the precise understanding of these events, and thus, improvement of resolution in space and time has been seriously pursued for a long time.

Membrane traffic is a system connecting a variety of single-membrane-bounded organelles in eukaryotic cells (Fig. 1).

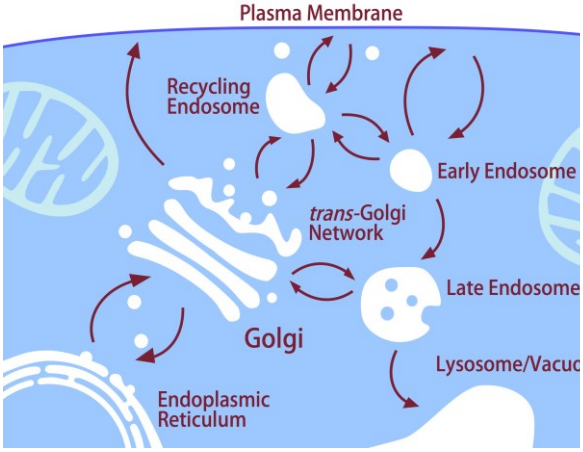

Figure 1. Membrane traffic. Single-membrane-bounded organelles of eukaryotic cells are connected by vesicluar transport processes.

Typically, small vesicles are formed and released from a donor compartment (budding), travel through cytosol (transport), and reach a target compartment to deliver their content (tethering and fusion). By these processes, proteins and other components in one organelle are sorted from each other and correctly targeted to their destinations. The size of vesicles varies, but is usually in the range of 50-150 nm, which is smaller than the diffraction limit of light microscopy.

Some years ago, we decided to challenge this problem and developed a high-performance confocal microscope by combing a high-speed spinning-disk confocal scanner and a high-sensitivity camera system [1]. The high-speed and precise measurements allow over-sampling, which, after deconvolution, turns out to provide extremely high spatial resolution. This simple method has a great advantage in time, and we now call it "super-resolution confocal live imaging microscopy (SCLIM)." In this paper, I will describe the outline of the SCLIM system and its application to understanding molecular mechanisms of membrane traffic.

II. Instrumentation

The original model of our high-speed and high-sensitivity confocal microscope (Fig. 2) was made during the Dynamic-Bio Project of Japan, which was supported by national funds from the Ministry of Economy, Trade and Industry (METI) of Japan and the New Energy and Industrial Technology Development Organization (NEDO) and carried out for 2002-2007. The basic idea was to combine the extremely high-speed spinning-disk confocal scanner (Yokogawa) and the high-quality and high-sensitivity camera system (NHK and Hitachi) [1, 2].

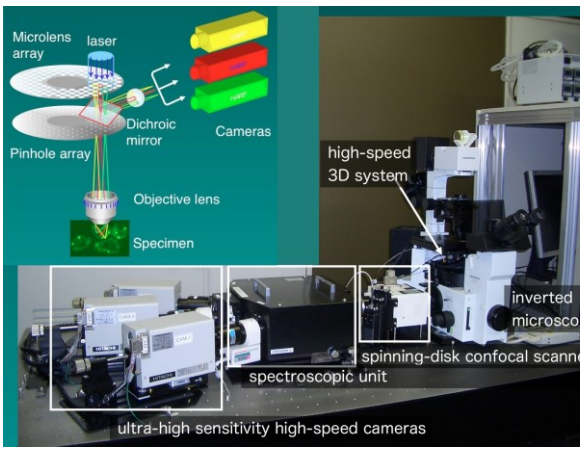

Figure 2. Original model of SCLIM made by the support of METI and NEDO. By combination of a high-speed spinning-disk cofocal scanner (Yokogawa) and high-sensitivity cameras (NHK and Hitachi), a high-performance confocal microscopic system was developed.

The produced system achieved amazing speed performance: 1000 frames per second in 2D, 50-100 Z-stacks per a few

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seconds, at the completely simultaneous two-color separation. Furthermore, we realized that the high quality of data gives rise to a wonderful spatial resolution by data processing. The high-speed and precise measurements of this system allow massive over-sampling. Image processing by deconvolution (maximum entropy method) provides spatial resolution far beyond the diffraction limit. Point spread function is given by measurement of 100 nm fluorescent beads. Upon deconvolution, small vesicles of 50-60-nm diameter and a tubular structure of 50-nm diameter can be clearly captured in a 3D movie [see 6]. This simple method has a superior advantage in time over other super-resolution techniques, and we have given a name "super-resolution confocal live imaging microscopy (SCLIM)" to this system [3, 4]. SCLIM has been further evolved to achieve higher speed by the use of most-recent high sensitivity cameras, and, to our great excitement, enable us now 4-5 color simultaneous observation by custom-made special dichroic mirrors and band-pass filters (Fig. 3).

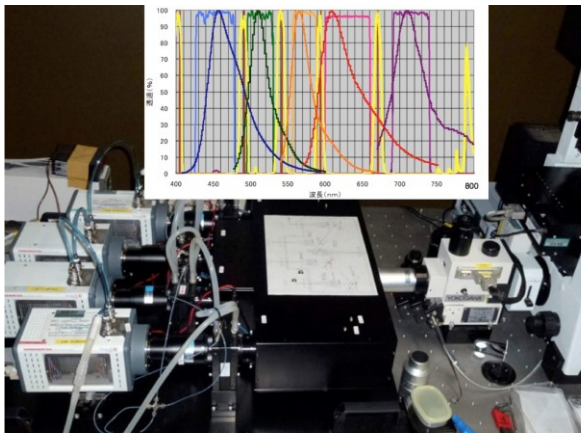

Figure 3. Multicolor SCLIM. Special dichroic mirrors and band-pass filters were designed and produced to separate 4-5 fluoresncent signals simultaneously.

III. Application to Membrane Traffic

We are applying SCLIM to tackle problems of intracellular membrane traffic. We have already challenged a big debate regarding how cargo proteins are transported within the Golgi apparatus, and demonstrated that the yeast Golgi cisternae change their properties over time [2]. This process is called "cisternal maturation" and our data clearly proved its reality and helped end the long-lasting debate [5-7]. We have also shown very dynamic mixing and segregation of Golgi membrane components by virtue of the high spatial resolution. Whether a similar process also takes place in higher eukaryotes, which elaborate more ordered structures of Golgi apparatus, is our next challenge. Using tobacco BY-2 cells, we have successfully analyzed disassembly and assembly processes of the Golgi stacks by live imaging [4].

We are now extending SCLIM to the budding of coat protein complex II (COPII) vesicles on the endoplasmic reticulum (ER) and its interaction with the *cis* Golgi cisternae and proposing an amazing new mechanism. For the *de novo* formation of the Golgi, vesicles derived from the ER are postulated to give rise to a new compartment on the *cis*-Golgi side. SCLIM observation on the relationship between the vesicle budding sites (called ER exit sites) of the ER and the *cis* Golgi cisternae reveals amazing new mechanisms in yeast and plant cells [3, 4]. We will also be able to address controversial post-Golgi events, such as the roles of *trans*-Golgi network, by live imaging.

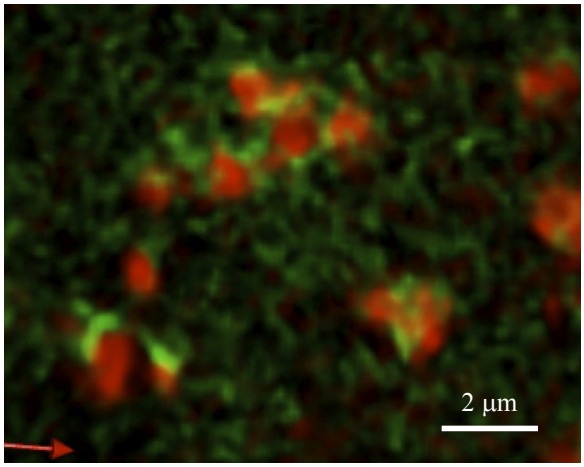

Figure 4. Spatial relationship of ER exit sites (SEC13-YFP) and the *cis*-Golgi (mRFP-SYP31) observed in tobacco BY-2 cells [Ref. 4].

IV. Perspective

Other methods of super-resolution light microscopy have been developed and applied to observe intracellular events, but at the moment most of them require a considerable time for measurement. SCLIM has a clear advantage in speed and is very suitable for live imaging. Completely simultaneous multicolor observation is also a great merit and will be extremely powerful to dissect many events involving directional movement between compartments.

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