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Authors

Zhao, Xiang

Guo, Su

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Methods for Visualizing Intracellular Organelles

Xiang Zhao[1,2], **Su Guo**[1,2,3]

[1]Chan Zuckerberg Biohub, San Francisco, USA

[2]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, USA

[3]Programs in Human Genetics and Biological Sciences, University of California, San Francisco, San Francisco, CA, USA

EDITORIAL TEXT:

All multicellular organisms on earth, including animals and plants, evolved from unicellular eukaryotes ~1.7 billion years ago[1]. Distinct from primitive prokaryotes, eukaryotic cells have a membrane-bound nucleus and many membrane-enclosed organelles within their cytoplasm, including mitochondria, the Golgi apparatus, the endoplasmic reticulum (ER), lysosomes, and vesicles. These organelles form compartments in which specialized biological functions and highly efficient metabolic activities are localized. Due to the limited resolution of light microscopy, most intracellular organelles contain functional structures that fall under the light diffraction limit, making their visualization challenging[2]. Innovative microscopic and biological labeling techniques are beginning to enable the high-resolution visualization of ultra-fine architectures and the *in vivo* dynamics of organelles. This collection brings together recent methods that have successfully labeled and imaged different intracellular organelles across different species and, thus, have furthered our understanding of the spatiotemporal regulation of organelle biology.

In recent decades, the development of fluorescent sensors and the labeling of proteins in living cells have greatly facilitated our understanding of the dynamics of intracellular networks, signal transduction, and cell–cell interactions. By using advanced inorganic synthetic methods, fluorescent semiconductor nanocrystals with nano-scale size, also known as quantum dots or QD, have been invented as a new class of optical reporters. Normally, QD particles are within 2–10 nm in size and have high photosensitivity, stability, and low cytotoxicity to cells. QD particles have emerged as alternatives to organic dyes and fluorescent proteins for imaging intracellular processes at high resolution. In the protocol by Aishwarya et al., the authors describe QD-mediated immunolabeling followed by imaging with transmission electron microscopy (TEM)[3]. By performing antibody-targeted QD labeling and imaging under TEM at super-resolution, the authors successfully visualized

Corresponding authors: Xiang Zhao (xiang.zhao@czbiohub.org); Su Guo (su.guo@ucsf.edu).

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DISCLOSURES:
The authors have nothing to disclose.

Sigmar1 localization on multiple cell organelles in adult mouse heart cells and highlighted its enrichment on the lysosomes and the mitochondrial membranes. In addition to emphasizing the high resolution and high sensitivity of QD-TEM, the authors also mention several drawbacks of the methods. For instance, the osmium tetroxide used in QD-mediated immunolabeling irreversibly destroys the properties of the specimen, and the optimization of the fixation and antigen unmask steps is a key yet challenging element to ensure high-quality images.

Aside from QD, many other different dyes have been successfully applied for labeling organelles in live cells. Those methods have facilitated the live imaging of the transformation or translocation process of cell organelles *in vivo.* Sometimes, multiple organelle-specific dyes are used for visualizing different types of organelles simultaneously. In the protocol by Liu, Li et al.[4], the authors provide a detailed protocol for the live staining and imaging of both mitochondria and lysosomes in mouse embryonic fibroblast (MEF) cells by using two different dyes: MitoTracker Green and LysoTracker Red[4]. In this study, both dyes showed high fluorescent sensitivity and stability specific to mitochondria and lysosomes, respectively. Moreover, the authors successfully observed and recorded the critical *in vivo* cellular process of mitophagy induced by carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) in MEF cells using the protocol. In the discussion, the authors mention that the protocol can be applied with different cell lines or cells from tissues by optimizing the dye concentration and staining time.

By making use of reporter constructs composed of organelle-specific proteins fused to fluorescent tags, one can observe *in vivo* organelle dynamics and expression patterns without immunolabeling. In the protocol by Liu, Zhu et al.[5], the authors provide a detailed protocol for imaging most types of subcellular organelles in the living budding yeast, *Saccharomyces cerevisiae*[5]. For beginners working with budding yeast, this protocol is helpful for learning how to identify protein subcellular localization and track organelles of interest using time-lapse imaging. A limitation of the protocol is that most of the constructs used in the protocol cannot be used for labeling organelles in other species. For different types of eukaryotic cells, organelle-specific patterns of fluorescent proteins would still need to be validated by antibody immunostaining.

An antibody uptake assay was first developed for the specific live labeling of endosomal Notch/Delta signaling in the sensory organ precursors of the *Drosophila* notum[6]. Due to the dosage-sensitive nature of Notch/Delta signaling, expressing a reporter construct inside cells is not suitable for visualizing Notch/Delta signaling *in vivo*, since an extra copy of the reporter-fused gene would interrupt the normal signaling and, in turn, the cell fate. In the protocol by Zhao and Guo[7], the authors apply the antibody uptake assay for imaging Notch/Delta endosomal trafficking in the radial glia progenitors of the developing zebrafish brain[7]. In the protocol, after microinjecting anti-DeltaD-Atto647N conjugated antibody precisely into the 24 hpf (hours post fertilization) embryonic hindbrain ventricle, the whole process of endosomal trafficking is recorded using the internalized anti-DeltaD-Atto647N antibody throughout the mitosis of radial glia progenitors. In the study, it was found that the antibody uptake assay did not interfere with Notch/Delta signaling. This protocol can be used to

visualize other membrane ligand-receptor signaling, given an antibody to the extracellular domain of the protein of interest is available.

Recent developments in expansion microscopy (ExM), involving physically expanding the sample within a swellable hydrogel, have enabled conventional confocal microscopes to be used for super-resolution imaging. Since its invention, ExM has been tested with many different types of biological samples[8]. One main challenge of ExM is the fluorescent signal loss during the polymerization and digestion steps. The recently developed label-retention ExM has solved the problem with trifunctional anchors[9]. In the protocol by Park et al.[10], the authors describe how to perform label-retention ExM (LR-ExM) with a set of trifunctional anchors[10]. The protocol improves expansion microscopy imaging not only by preventing signal loss but also by promoting high-efficiency labeling with SNAP or CLIP tags. In their study, the authors successfully demonstrated multicolor LR-ExM for a variety of subcellular structures and achieved single-xmolecular resolution when combining LR-ExM with super-resolution stochastic optical reconstruction microscopy (STORM).

With the fast development of super-resolution microscopy and the advent of novel fluorescent labeling techniques, we can anticipate further improvements in the methods and protocols for visualizing intracellular organelles at an unprecedented resolution. These efforts are poised to bring new breakthroughs in understanding the dynamics and functions of intracellular organelles.

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