Recent Advances in Fluorescent Labeling Techniques for Fluorescence Microscopy

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Tremendous progress in recent computer-controlled systems for fluorescence and laser-confocal microscopy has provided us with powerful tools to visualize and analyze molecular events in the cells. Various fluorescent staining and labeling techniques have also been developed to be used with these powerful instruments. Fluorescent proteins such as green fluorescent protein (GFP) allow us to directly label particular proteins of interest in living cells. This technique has been extended over a large area of cell biology, and a variety of fluorescent protein-derived techniques have been developed to visualize the functions and conditions of the molecules within living cells. In this review, we summarize the techniques for fluorescent staining and labeling for recent fluorescence microscopy.

Key words: fluorescence microscopy, fluorescent labeling, immunofluorescent staining, fluorescent dyes, fluorescent proteins

I. Introduction

Since biology entered the new post-genome era, the function of each gene product in the cell has attracted researchers’ interests. The development of techniques of fluorescent protein (FP) labeling and recent computer-controlled systems for fluorescence and laser-confocal microscopes has also boosted our eagerness to see directly the behavior of particular proteins of interest in living cells [17, 26]. New techniques for fluorescent labeling of molecules have been devised depending on such requirements to see directly into the molecules in living cells. Live-cell imaging techniques make it possible to see the molecular basis of a variety of functions in the cell [12, 20]. Originally, fluorescence microscopy itself was a powerful technique to visualize the location of particular molecules in the cell [30, 31]. The combination of new techniques for fluorescent labeling and staining with advanced fluorescence microscopy has provided us with new tools to analyze the dynamic behavior of cellular molecules.

Before the emergence of GFP-labeling technique, the advantage of fluorescence microscopy was the detailed mapping of the molecules in cells and tissues by multiple fluorescent staining. After the advent of GFP-labeling, fluorescence microscopes including laser-confocal microscopes have been actively involved in elucidating the molecular functions in the cell as follows: 1) live-cell imaging for the molecular dynamics by FP-fusion probes [12]; 2) the analyses of the activity or status of signal transduction by visualizing the binding of proteins with other molecules or their folding in itself by fluorescence resonance energy transfer (FRET) microscopy [9, 18] or fluorescence correlation spectroscopy [19]; 3) the analyses of movement and trafficking of particular proteins by fluorescence recovery after photobleaching (FRAP) techniques [35]; 4) the analyses of turnover of proteins by employing molecular tags that specifically bind the particular membrane-permeable dyes [4]. In this review, we summarize the techniques for fluorescent staining and labeling for recent fluorescence microscopy.

II. Overview

The techniques of fluorescent labeling of molecules are divided broadly into two categories: the conventional fluorescent staining and the molecular tagging by introducing the genes of FPs or specific binding motifs for fluorochromes by genetic engineering. Conventional fluorescent staining includes the immunostaining using the fluoro-chrome-labeled specific antibodies and chemical staining.
using the fluorochrome-labeled chemical reagents that specifically react with the target molecules in the cell. The techniques for molecular labeling with FPs by genetic engineering do not need the staining procedure for microscopy, hence we can easily see the behavior of molecules of interest in living cells. All the other labeling techniques require the procedures of fluorescent staining prior to microscopic examination. Moreover, immunofluorescence staining usually requires fixation procedures and is not suitable for live-cell imaging.

III. Fluorescent Staining Techniques Using the Fluorescent-labeled Molecular Probes to Detect the Particular Biomolecules, Structures, or Molecular Events in the Cell

Immunofluorescence staining

Immunofluorescence microscopy is a versatile procedure and is able to detect any biomolecules in the cell so far as the specific antibodies are provided in advance [16]. To stain intracellular biomolecules, permeabilization of plasma membrane or thin-sectioning of samples is required for the easy access of antibody molecules to the target molecules in the cell. The technique generally requires the fixation procedure of tissues and cells prior to the staining, hence is unsuitable for live-imaging. Specific antibodies for the particular molecules are the key issues in this method. One can either purchase them from manufacturers, order to have them made, or make them by oneself. Two methods, i.e., direct and indirect immunofluorescent staining have been widely used. In the direct-method, the fluorochrome-labeled antibodies are used as probes. The indirect method, on the other hand, uses non-labeled antibodies for the particular biomolecules as the primary antibodies. The fluorochrome-labeled secondary antibodies are used for fluorescent

| Table 1. Fluorochromes for immunofluorescence microscopy |
|----------------------------------------------------------|
| **Fluorochromes** | **excitation (nm)** | **emission (nm)** |
| AMCA | 347 | 445 |
| Alexa Fluor 350 | 345 | 440 |
| Alexa Fluor 488 | 488 | 520 |
| Cy2 | 492 | 510 |
| FITC | 496 | 518 |
| Bodipy-FL | 503 | 511 |
| TRITC | 544 | 572 |
| Cy3 | 550 | 570 |
| LRSC | 572 | 590 |
| Rhodamine Red-X | 570 | 590 |
| Texas Red | 596 | 620 |
| Cy5 | 650 | 670 |
| Alexa Fluor 647 | 650 | 668 |

AMCA (aminomethylcoumarin acetic acid), Cy2 (cyanine), FITC (fluorescein isothiocyanate), TRITC (tetramethylrhodamine isothiocyanate), Cy3 (indocarbocyanine), LRSC (lissamine rhodamine sulfonyl chloride), Cy5 (indodicarbocyanine).

Fig. 1. a. Laser confocal image for SGLT1 and ZO-1 of confluent MDCK cells. Green signal indicates the GFP fluorescence of SGLT1-GFP, and red signal indicates the immunofluorescent labeling of ZO-1. Bar=10 µm. Reproduced and modified from [29]. b. Immunofluorescence image of rat kidney with anti-aquaporin 2 antibody (red), anti-aquaporin 3 antibody (green), and DAPI (blue). Fluorescence image was merged with corresponding differential interference-contrast (DIC) image. Bar=100 µm. c. Immunofluorescent staining image of HSC-4 cells with γ-tubulin antibody (red), Golgi 58K (green), and TO-PRO-3 (blue). The Golgi apparatus is labeled by Golgi 58K. Golgi 58K is also localized to one (arrow) of two dots of the centrosome [8]. Bar=10 µm.
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labeling of the primary antibodies. Since the various fluorochrome-labeled secondary antibodies are commercially provided (Table 1), one can choose the proper antibodies by considering the properties of fluorochromes and the animal species of the primary antibodies. The direct method is simple and easy as long as the fluorochrome-labeled antibodies are available. Zenon method is a modification of the direct method, and has a wide range of applications especially for multi-color labeling [21]. As to sensitivity, the indirect method is better than the direct method. In the indirect method, multi-color-staining can be carried out by the combination of secondary antibodies coupled with different fluorochromes (Fig. 1) [15, 29, 32]. Special caution should be paid to the possible cross-reactivity of antibodies to the unintended molecules in the tissue cells [14].

**Fluorescence in situ hybridization (FISH)**

Antisense single-stranded DNAs for the particular genes or sequences are labeled with proper fluorochromes and used as the molecular probes. The fluorescent-labeled probes hybridize with the complementary RNA or DNA molecules in the cells and are used to detect the particular gene expression in the cells or the number of copies and their localization of particular genes or sequences in chromosomes or nuclei.

**Chemical fluorescent probes**

Cellular structures such as nucleus, plasma membrane, and cytoskeletons can be visualized with chemical reagents that bind to the biomolecules specific to particular structures. When the reagents have fluorescent chromophores or are coupled with fluorochromes, the cellular structures can be seen by fluorescence microscopy. Various fluorescent probes for staining particular cellular structures are commercially available as shown in Table 2. You can choose suitable ones for your experiments depending on the character of the probes. You can also choose fluorochromes in a variety of aspects such as wavelength (excitation and emission), photobleaching speed, and permeability in the cellular membrane. In the case of nuclear staining, the specificity of fluorescent probes to DNA is important because most of the DNA-binding fluorescent probes also bind RNAs (Fig. 2, Table 3) [13, 27, 28]. You have to choose the DNA-specific dyes, or otherwise, RNase pretreatment is required. Phalloidin is a toxic substance from *Amanita phalloides*, commonly known as the death cap poisonous mushroom. Fluorochrome-coupled phalloidin specifically decorates F-actin in the cell. Chemical fluorescent probes are easy to use and are best suited for counter-staining. Cell permeable fluorescent dyes such as Hoechst dyes are also useful for live-cell imaging.

**Calcium indicators**

Fluorescent probes that exhibit a spectral response upon Ca$^{2+}$ binding have enabled us to directly visualize changes of intracellular Ca$^{2+}$ concentrations in living cells by

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**Table 2. Chemical fluorescent probes for counter-staining**

| target            | Ex/Em (nm) | cell permeability |
|-------------------|------------|-------------------|
| FITC-phalloidin   | actin fiber| 496/516 impermeant|
| Rhodamine-phalloidin| actin fiber| 554/573 impermeant|
| MitoTrackers      | mitochondria| * permeant |
| Rhodamine 123     | mitochondria| 507/529 permeant |
| DiOC$_6$          | ER+mitochondria| 484/501 permeant|
| ER-Trackers       | ER         | 374/575 permeant |
| LysoTrackers      | lysosome   | ** permeant |
| NBD C$_6$-ceramide | Golgi apparatus | 466/536 permeant |

* There are five color-variants of MitoTrackers of green (490/516), orange (551/576), red (578/599), far red (581/644), and deep red (644/665).
** There are four color-variants of LysoTrackers of blue (373/422), green (504/511), yellow (465/535), and red (577/590).

**Table 3. Chemical fluorescent dyes for nuclear staining**

| target     | Ex/Em (nm) | DNA | RNA | cell permeability   |
|------------|------------|-----|-----|---------------------|
| DAPI       | 358/461    | +++ | −   | semi-permeant       |
| Hoechst 33258 | 352/461    | +++ | −   | permeant            |
| BO-PRO-1   | 462/509    | +   | ++  | impermeant          |
| YO-PRO-1   | 491/509    | +++ | +   | impermeant          |
| SYBR Green 1 | 494/512    | ++  | −   | impermeant          |
| PicoGreen  | 502/523    | +   | −   | impermeant          |
| SYTOX Green| 504/523    | +++ | −   | impermeant          |
| TO-PRO-1   | 515/531    | +   | +   | impermeant          |
| POPO-3     | 534/570    | +++ | +++ | impermeant          |
| PI         | 535/617    | +++ | ++  | impermeant          |
| YO-PRO-3   | 612/631    | +++ | ±   | impermeant          |
| TO-TO-3    | 642/660    | ++  | +++ | impermeant          |
| TO-PRO-3   | 642/661    | +++ | ±   | impermeant          |

DAPI (4’,6-diamidino-2-phenylindole), PI (propidium iodide). Reproduced and modified from [13].
fluorescence microscopy [33]. Most of calcium indicators are derivatives of the Ca\(^{2+}\) chelators such as EGTA, APTRA and BAPTA. Acetoxymethyl esters of the calcium indicators are permeable to cellular membranes and passively taken up into the cytoplasm of living cells, where they are cleaved to cell-impermeant products by intracellular esterases.

**IV. Direct Fluorescent Labeling of Particular Gene Products by Genetically Engineered Molecular Tags**

Particular gene products can be labeled directly by genetic engineering with the molecular tags such as FPs. They can also be tagged with certain amino acid sequences that specifically bind to the particular cell-permeable fluorescent dyes. FP-fusion gene products are detected after the transformation of the cells. In the case of non-fluorescent molecular tags, incubation with specific fluorescent dyes

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Fig. 2. Laser confocal images of MDCK cells with various fluorescent dyes for nucleic acid. Bar=10 µm. Reproduced and modified from [13].
and subsequent washing procedure are needed prior to observation.

**FP-fusion proteins**

FPs such as GFP have made it possible to see not only the localization per se but also the dynamics of the labeled molecules in the cell, allowing the development of new ways to analyze the functional behavior of proteins of interest in living cells [5, 11, 29]. This technique is also applicable to the functional motifs or domains of proteins as noted in the accompanying sections. Labeling with FPs is carried out by genetic engineering including recombinant DNA technology. Many FPs have been identified and engineered as shown in Table 4. We can choose suitable ones for the specific experiments by taking into account the wavelength (excitation and emission), the quantum-yield that correlates to the signal intensity, and the degree of polymerization (monomer, dimer or tetramer) [25]. For example, monomeric FPs are suitable for labeling when one wants to see the tracking of biomolecules. Although dimeric and tetrameric FPs emit relatively strong signals, multimeric FPs have a tendency to affect the behavior of biomolecules by the artificial clumping of labeled molecules. The behavior of FP-labeled molecules thus is sometimes different from that of endogenous ones. Moreover, tagging with monomeric FPs cannot completely exclude the possibility of adverse effects on the behavior and/or localization of fusion proteins. The localization and behavior of the non-labeled endogenous proteins should be checked by immunofluorescent staining to avoid possible artifacts.

**Molecular tags for specific cell-permeable fluorescent dyes**

Just like FP-tagging, tagging with proper amino acid sequences that specifically bind to the particular cell-permeable fluorescent dyes by genetic engineering is also useful to see the behavior and localization of particular molecules in living cells. Two types of molecular tag systems for specific cell-permeable fluorescent dyes are commercially available (Table 5). Tetracysteine tag (TC-Tag: formerly Lumio tag) consists of the six-amino acid sequence of Cys-Cys-Pro-Gly-Cys-Cys- and specifically binds to the cell permeable labeling reagents of FLAsH-EDT₂ and ReAsH-EDT₂. FLAsH-EDT₂ and ReAsH-EDT₂ labeling reagents become strongly fluorescent (green and red, respectively) only upon binding to the TC-Tag, allowing specific detection of tagged proteins. In the case of TC-Tag, however, the procedures of staining and washing prior to the observation are necessary. Although the labeling-reagent for the TC-Tag itself is not fluorescent, the analogues that are present in the serum bind to the reagent and become fluorescent and disturb the examination of specific labeling, making the washing procedure necessary. Pretreatment of culture medium with a non-fluorescent and cell-impermeable reagent that binds to such endogenous analogues could eliminate the necessity of washing procedures.

The HaloTag protein, a genetically engineered derivative of a hydrolase, is also used as a molecular tag. Since the HaloTag protein is large as FPs (297 amino acid residues), utmost care should be taken as to possible artifacts just like the FP tagging. Five types of Halo-ligands of cell-permeable fluorescent dyes are available as shown Table 5.

Unlike the FPs, the tags themselves do not have their own fluorescence in either case. Elaborate experiments such as pulse-chase labeling using a single fluorescent dye or time-sequential pulse-labeling by different-colored fluorescent dyes for the same tag are possible. These pulse-labeling techniques have enabled us to analyze the turnover of a particular protein or the directivity of protein distribution [4].

**Table 4.** Fluorescent proteins [2, 7, 20, 22]

| FP                | Ex/Em (nm) | molar absorptivity | quantum yield | polymerization |
|-------------------|------------|--------------------|---------------|---------------|
| EBFP              | 380/440    | 29,000             | 0.31          | monomer       |
| ECFP              | 433/475    | 32,500             | 0.40          | monomer       |
| Midoriishi-Cyan1  | 472/495    | 27,250             | 0.90          | monomer       |
| AmCyan1           | 458/489    | 40,000             | 0.24          | tetramer      |
| EGFP              | 488/509    | 56,000             | 0.60          | monomer       |
| Azami-Green       | 492/505    | 72,300             | 0.67          | tetramer      |
| mAzami-Green1     | 492/505    | 55,000             | 0.74          | monomer       |
| ZsGreen1          | 493/505    | 35,600             | 0.63          | tetramer      |
| EYFP              | 514/527    | 83,400             | 0.61          | monomer       |
| Venus             | 515/528    | 92,200             | 0.57          | monomer       |
| ZsYellow          | 529/539    | 20,200             | 0.42          | tetramer      |
| Kusabira-Orange1  | 548/561    | 73,700             | 0.45          | dimer         |
| mKusabira-Orange1 | 548/559    | 51,600             | 0.60          | monomer       |
| DsRed2            | 563/582    | 43,800             | 0.55          | tetramer      |
| mRFP1             | 584/607    | 44,000             | 0.25          | monomer       |
| HcRed1            | 588/618    | 20,000             | 0.02          | dimer         |

Intensities of FPs are in proportion to the product of molar absorptivity and quantum yield.
Functional motifs and domains of proteins to visualize cellular structures and molecular functions

A variety of functional motifs and domains of proteins have been known and can be used to design probes to visualize particular cellular structures such as the plasma membrane, mitochondria, cell nucleus, Golgi apparatus, and endoplasmic reticulum by FP-tagging (Table 6). Visualization of the particular cellular structures is useful for the purpose of counter-staining of living cells. Lipid-second-messengers such as phosphatidylinositol (PtdIns) derivatives can also be visualized by the probes of FP-tagged functional motifs or domains in living cells (Table 6). For example, the pleckstrin-homology domain (PH domain) of AKT (protein kinase B) specifically binds to phosphatidylinositol-(3,4,5)-trisphosphate (PtdIns(3,4,5)P$_3$) that is the product of PI3-kinase. The FP-tagged AKT-PH probe can then detect the active-site for PI3-kinase in living cells (Fig. 3). Since the second messengers are transiently formed and localized at the specific sites in cells during the course of signal transduction, live-cell imaging by FP-tagged probes is a powerful tool for the study of signal transduction mechanisms.

V. Prospects for Fluorescent Labeling

Tagging by FPs has provided us with a great means to analyze the molecular behavior and functions of living cells. Tagging with FPs, however, sometimes has an adverse effect on the behavior of tagged molecules by altering their original molecular properties. Such effects can be minimized by the use of small-sized tags such as TC-Tag that consists of only 6 amino acids. Combined use of such labeling procedures with conventional immunofluorescent methods will remain powerful tools in examining the cellular functions.

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VII. References

1. Andresen, M., Schmitz-Salue, R. and Jakobs, S. (2004) Short tetracysteine tags to beta-tubulin demonstrate the significance of
small labels for live cell imaging. *Mol. Biol. Cell* 15; 5616–5622.

2. Campbell, R. E., Tour, O., Palmer, A. E., Steinbach, P. A., Baird, G. S., Zacharias, D. A. and Tsien, R. Y. (2002) A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. U.S.A* 99; 7787–7882.

3. Carrasco, S. and Merida, I. (2004) Diacylglycerol-dependent binding recruits PKC0 and RasGRP1 C1 domains to specific subcellular localizations in living T lymphocytes. *Mol. Biol. Cell* 15; 2932–2942.

4. Gaietta, G., Deerinck, T. J., Adams, S. R., Bouwer, J., Tour, O., Laird, D. W., Sosinsky, G. E., Tsien, R. Y. and Ellisman, M. H. (2002) Multicolor and electron microscopic imaging of connexin trafficking. *Science* 296; 503–507.

5. Giepmans, B. N. G., Adams, S. R., Ellisman, M. H. and Tsien, R. Y. (2006) The fluorescent toolbox for assessing protein location and function. *Science* 312; 217–224.

6. Griffin, B. A., Adams, S. R. and Tsien, R. Y. (1999) Specific covalent labeling of recombinant protein molecules inside live cells. *Science* 281; 269–272.

7. Gurskaya, N. G., Fradkov, A. F., Terskikh, A., Mikhail, V. V., Matz, M. V., Labas, Y. A., Martynov, V. L., Yanuşevich, Y. G., Konstantin, A., Lukyanov, K. A. and Lukyanov, S. A. (2001) GFP-like chloroproteins as a source of far-red fluorescent proteins. *FEBS Lett.* 507; 16–20.

8. Hagiwara, H., Tajika, Y., Matsu tsuki, T., Suzuki, T., Aoki, T. and Takata, K. (2006) Localization of Golgi S58 protein (formiminotransferase cycloaddemase) to the centrosome. *Histochem. Cell Biol.* 126; 251–259.

9. Kurokawa, K., Takaya, A., Terai, K., Fujioka, A. and Matsuda, M. (2004) Visualizing the signal transduction pathways in living cells with GFP-based FRET probes. *Acta Histochem. Cytochem.* 37; 347–355.

10. Los, G. E., Learish, R., Karassina, N., Zimpich, C., McDougall, M. G., Encell, L. P., Friedman-Ohana, R., Wood, M., Vidugiris, G., Zimmerman, K., Otto, P., Berstock, S., Klaubert, D. H. and Wood, K. (2006) HaloTag technology: cell imaging and protein analysis. *Cell Notes* 14; 10–14.

11. Masukawa, K., Sakai, N., Ohmori, S., Shirai, Y. and Saito, N. (2006) Spatiotemporal analysis of the molecular interaction between PICK1 and PKC. *Acta Histochem. Cytochem.* 39; 173–181.

12. Matsu suka-Tokita, K., Takeuchi, M., Ichihara, M., Mikuriya, K. and Nakano, A. (2006) Live imaging of yeast Golgi cisternal maturation. *Nature* 441; 1007–1010.

13. Matsuzaki, T., Suzuki, T., Fujikura, K. and Takata, K. (1997) Nuclear staining for live confocal microscopy. *Acta Histochem. Cytochem.* 30; 309–314.

14. Matsuzaki, T., Suzuki, T., Tanaka, S. and Tanaka, K. (2000) An anti-peptide antibody that recognized unexpected protein—A case report. *Acta Histochem. Cytochem.* 33; 361–365.

15. Matsuzaki, T., Ablimit, A., Tajika, Y., Suzuki, T., Aoki, T., Hagiwara, H. and Takata, K. (2005) Water channel aquaporin 1 (AQP1) is present in the perineurium and perichondrium. *Acta Histochem. Cytochem.* 38; 37–42.

16. Miller, D. M. and Shakes, D. C. (1995) Immuno-fluorescence microscopy. *Method. Cell Biol.* 48; 365–394.

17. Miyawaki, A. (2003) Visualization of the spatial and temporal dynamics of intracellular signaling. *Dev. Cell* 9; 295–305.

18. Mochizuki, N., Yamashita, S., Kurokawa, K., Ohba, Y., Nagai, T., Miyawaki, A. and Matsuda, M. (2001) Spatio-temporal images of growth-factor-induced activation of Ras and Rap1. *Nature* 411; 1065–1068.

19. Muto, T., Saito, K., Tamura, M. and Kinjo, M. (2002) Microenvironment analysis in squid axons using fluorescence correlation spectroscopy and laser scanning microscopy. *Acta Histochem.* 35; 87–91.

20. Nagai, T., Iba ta, K., Park, E. S., Kubota, M., Mikoshiba, K. and Miyawaki, A. (2002) A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nature Biotech.* 20; 87–90.

21. Petrovic, S., Barone, S., Xu, J., Conforti, L., Ma, L., Kujala, M., Kere, J. and Soleimani, M. (2004) SLC26A7: a basolateral Cl-/HCO3- exchanger specific to intercalated cells of the outer medullary collecting duct. *Am. J. Physiol. Renal Physiol.* 286; 161–169.

22. Richards, B., Zharkikh, L., Hsu, F., Dunn, C., Amb, A. and Teng, D. H. F. (2002) Stable expression of Anthozoa fluorescent proteins in mammalian cells. *Cytometry* 48; 106–112.

23. Sakai, D., Suzuki, T., Osumi, N. and Nakano, A. (2006) Cooperative action of Sox9, Snail2 and PKA signaling in early neural crest development. *Development* 133; 1323–1333.

24. Schwer, B., Ren, S., Pietschmann, T., Kartenbeck, J., Kaelicke, K., Bartenschlager, R., Yen, T. S. B. and Ott, M. (2004) Targeting of hepatitis C virus core protein to mitochondria through a novel C-terminal localization motif. *J. Viral.* 78; 7958–7968.

25. Shaner, N. C., Steinbach, P. A. and Tsien, R. Y. (2005) A guide to choosing fluorescent proteins. *Nature Method.* 2; 905–909.

26. Sluder, G. and Wolf, D. E. (2007) Digital microscopy. Methods in Cell Biology Vol. 81, Academic Press.

27. Suzuki, T., Fujikura, K., Higashiyama, T. and Takata, K. (1997) DNA staining for fluorescence and laser confocal microscopy. *J. Histochem. Cytochem.* 35; 49–53.

28. Suzuki, T., Matsu tsuki, T. and Takata, K. (1998) Fluorescence counter-staining of cell nuclear DNA for multi-color laser confocal microscopy. *Acta Histochem. Cytochem.* 31; 297–301.

29. Suzuki, T., Matsu tsuki, T., Hagiwara, H., Aoki, T., Tajika-Takahashi, Y. and Takata, K. (2006) Apical localization of sodium-dependent glucose transporter SGLT1 is maintained by cholesteroleand microtubules. *Acta Histochem. Cytochem.* 39; 155–161.

30. Takata, K., Hirano, H. and Kasahara, M. (1997) Transport of glucose across the blood-tissue barriers. *Int. Rev. Cytol.* 172; 1–53.

31. Takata, K., Matsu tsuki, T. and Tajika, Y. (2004) Aquaporins: water channel proteins of the cell membrane. *Prog. Histochem. Cytochem.* 39; 1–83.

32. Takata, K., Matsu tsuki, T., Tajika, Y., Ablimit, A., Suzuki, T., Aoki, T. and Hagiwara, H. (2005) Aquaporin water channels in the kidney. *Acta Histochem. Cytochem.* 38; 199–207.

33. Tanaka, H. and Takamatsu, T. (2003) Spatiotemporal visualization of intracelluar Ca2+ in living heart muscle cells viewed by confocal laser scanning microscopy. *Acta Histochem. Cytochem.* 36; 193–204.

34. Värnai, P. and Balla, T. (2007) Visualization and manipulation of phosphoinositide dynamics in live cells using engineered protein domains. *Pflugers Arch. Eur. J. Physiol.* 455; 69–82.

35. Ward, T. H., Polishchuk, R. S., Caplan, S., Hirschberg, K. and Lippincott-Schwartz, J. (2001) Maintenance of Golgi structure and function depends on the integrity of ER export. *J. Cell Biol.* 155; 557–570.

36. Zhang, L., Joshi, A. K. and Smith, S. (2003) Cloning, expression, characterization, and interaction of two components of a human mitochondrial fatty acid synthase: malonyltransferase and acyl carrier protein. *J. Biol. Chem.* 278; 40067–40074.