Cellular Imaging Laboratory

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Research

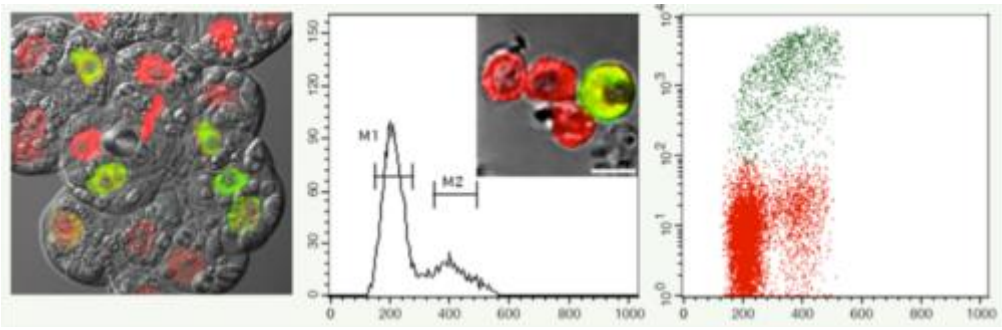
At the Cellular Imaging Laboratory, we are strongly committed to the development and application of advanced imaging, fluorescent labeling and biochemical techniques and approaches that will enable us to understand the complex organization within and between cells. Our recent achievements include (i) the application of the fast and robust ethynyldeoxyuridine-based replication assay for plant cells, (ii) the discovery of novel blue fluorescent dyes for in vivo detection, and (iii) 3D analysis of lipid droplets and high efficiency oligonucleotide-directed gene editing monitored by mutant GFP and fluorescence imaging methods.

Our modern imaging center is equipped with confocal laser scanning and spinning disc microscopes, fluorescence and stereo microscopes, a laser microdissection microscope, and powerful image analysis computers with imaging software. With these modern microscopes we can perform protein localization and mobility analyses, three dimensional, time course dynamic analyses of live cells, tissues and organisms. Thanks to the new imaging techniques and the development of new fluorescent dyes and proteins, today's biological and medical research has increasingly become dependent on microscopy and image analysis. It is now possible to specifically label virtually any molecule and directly probe its function in live cells by light microscopy.

This ability to visualize the dynamics of proteins in vesicles, organelles, cells and tissue has begun to provide new insights into how cells function in health and disease. Such work yields unique mechanistic insight by directly illustrating the complex spatial-temporal dynamics of fundamental cellular processes such as mitosis, morphogenesis, polarization, embryonic development, membrane trafficking and cytoskeleton dynamics. Many of these processes are highly dynamic and are challenging to image by traditional means. In this aim, we are strongly committed to the development and application of optical imaging, fluorescent labeling and biochemical techniques that will enable us and others to understand the complex organization within and between cells.

Microscopy and cytometry application of a new cell proliferation assay

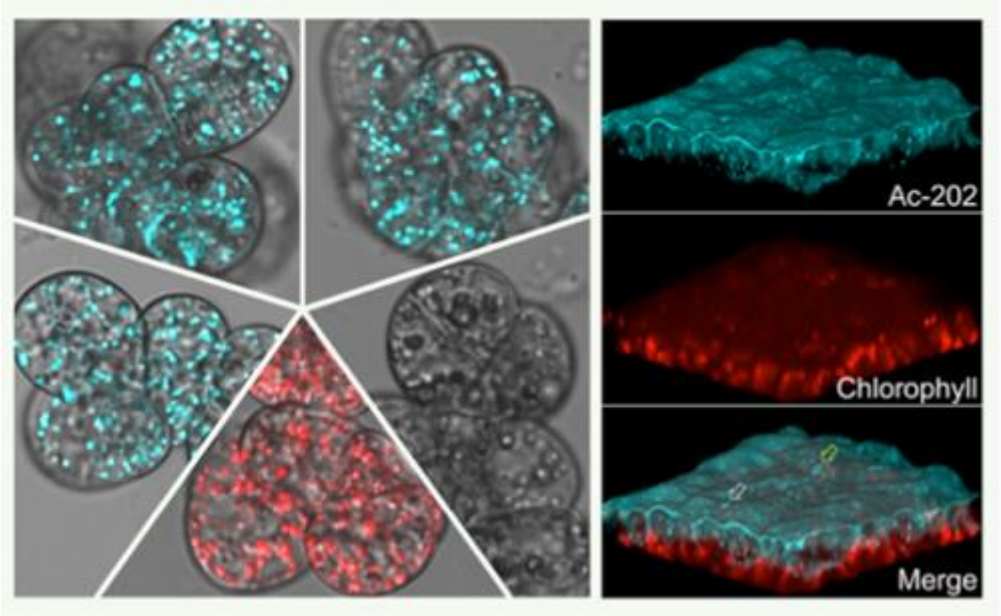
Labeling, detection and quantification of cells in the S-phase (DNA synthesis) of cell cycle progression are crucial in characterizing the cellular responses to various treatments and genetic modifications. Bromo-deoxyuridine (BrdU) labeling of cells followed by antibody staining is the standard method for detecting cells in the S-phase. Antibody detection of BrdU involves harsh treatments or nuclease digestion to facilitate epitope access. Moreover, in plants cells, cell wall digestion is also necessary. These steps could interfere with cellular morphology, and are time-consuming. We have optimized the recently developed ethynyl-deoxyuridine (EdU) method on plant cell cultures and seed-derived roots as well as on isolated plant nuclei using confocal laser scanning microscopy and flow cytometry (Kotogany et al., 2010, Ayaydin et al., 2011, Kuntam and Ayaydin, 2015)



Microscopy and cytometry application of EdU-based replication assay

Novel fluorescent dyes for plant lipid droplet imaging

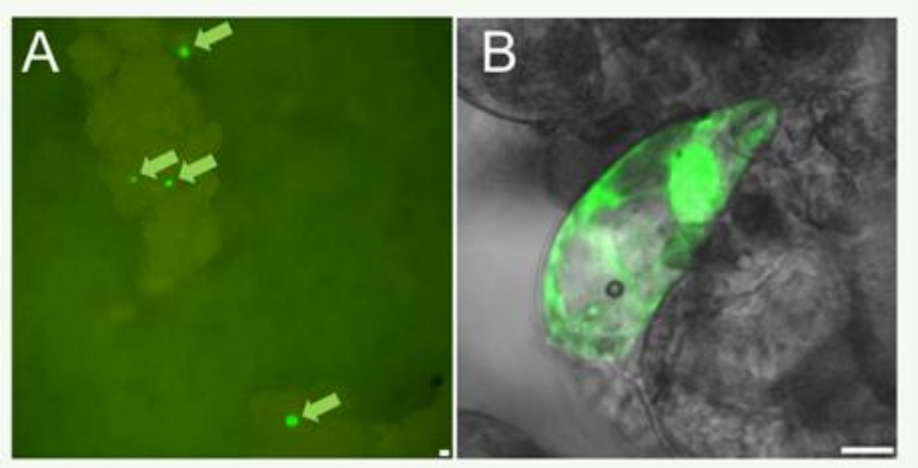
Plant lipid droplets (LDs), similar to their yeast and mammalian counterparts, are highly dynamic. Their proteomic analysis has revealed that a host of proteins reside on their surface with varying composition under different conditions. However, a lot remains to be uncovered in the area of LD protein and lipid composition as well as LD transport, mechanism of protein targeting, assembly and regulation. Live cell analysis is thus required to unravel the dynamic regulation of this important organelle. We have recently reported the development and characterization of novel fluorochromes as markers for LDs in living plant cells that emit in the blue range hence presenting flexibility during multicolor imaging. (Kuntam et al., 2015)



Discovery of novel fluorescent dyes for plant lipid droplet imaging

High efficiency oligonucleotide-directed gene editing

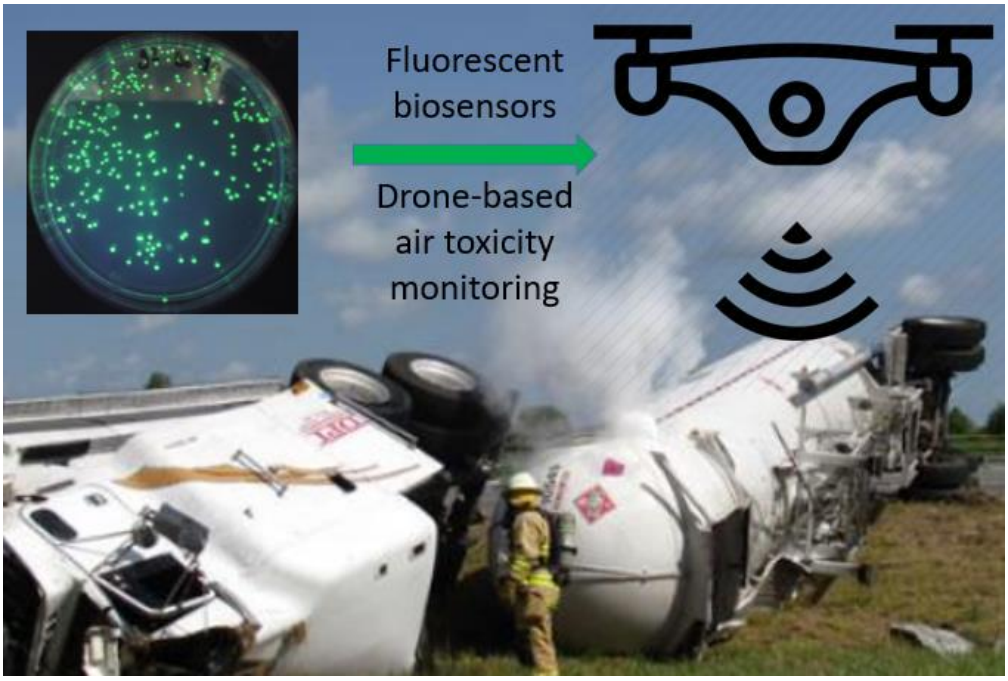
Targeted genome editing has been developed as an alternative to classical mutation breeding and transgenic (GMO) methods to improve crop plants. The Oligonucleotide Directed Mutagenesis (ODM) as Targeted Nucleotide Exchange (TNE) by single stranded DNA oligonucleotides (SDOs) attracts special attention for use in both basic science and plant breeding. On the other hand, one of the major limitations of this technique is the low frequency of TNE events. Using a mutant version of green fluorescent protein and chromatin modifying agents, recently, in collaboration with Prof. Dénes Dudits (Institute of Plant Biology, BRC, HAS, Szeged), we have achieved significantly increased frequency of TNE on cultured maize cells. We predict our results will increase the use and applicability of this new gene editing technique, which in turn may lead to crops with improved characteristics not imparted by introduction of foreign DNA sequences. (Tiricz et al, 2015)



Restoration of GFP fluorescence by targeted nucleotide exchange

Drone-integrated air pollution monitoring using fluorescent biosensor bacteria

Industrial activities necessitate the production of toxic chemicals for various purposes. These compounds are of different hazard levels, some being highly toxic. They are not only present in our surroundings during their manufacturing but also during their storage and transportation and can pose significant health hazard via respiratory pathway, contact through skin or by ingestion. Thus, the ability to monitor continuously their environmental levels with high sensitivity and accuracy is of key importance. In our recently initiated bilateral project (2019, NKFI TR-NN_17 Hungary-Turkey), we aim to develop a highly sensitive biosensor based toxic chemical detection platform and a flying device (drone) capable of monitoring these chemicals instantaneously, continuously over a prolonged time and over a large geographical area. Such a device will be outperforming the sensitivity and speed of detection of currently available devices, by being highly sensitive and permitting instantaneous data collection and transmission at the same time of sensing. In our laboratory, we have already successfully miniaturized a fluorescence detection system ready to be integrated into a drone for sensitive detection of toxic chemicals using biosensor bacteria.

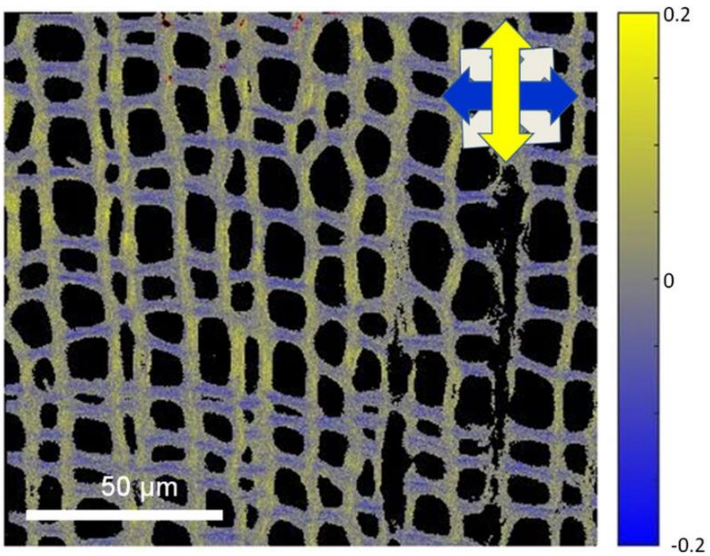


Drone-integrated pollution monitoring using fluorescent biosensor bacteria

Differential-Polarization Laserscanning Microscopy

DP microscopy provides 2D or 3D maps of the most important physical parameters characteristic of the anisotropic molecular architecture of specimens. This is achieved by measuring different spectropolarimetric quantities, pixel-by-pixel or voxel-by-voxel. Our knowledge about the anisotropic architecture of microscopic specimens is very far from being complete, especially in biology. Numerous examples demonstrate that various forms of anisotropy are present in highly organized molecular assemblies, organelles, cells and tissues. Detailed characterization requires microspectropolarimetric tools, which justifes our R&D efforts to combine the techniques of advanced microscopy and spectropolarimetry,

Our DP-LSMs have been used in a number of collaborative works. The birefringence of chloroplast thylakoid membranes revealed unexpectedly large local LB values – leading to the discovery that anisotropic structures with high LBs are amenable for micromanipulation with polarized beam of laser tweezers. We have imaged the domain structure of human lymphocyte cells – these results are of interest with regards to immunology. Our FDLD-imaging of actinbased macroassemblies led to the recognition of the role of some proteins in organizing specific cellular (O-ring canal) structures – these data advanced our understanding of processes of cell development. High-precision FDLD measurements were performed on plant tissues in order to better understand the architecture of cellulose in cell walls upon environmental or mechanical stresses – this technique is suitable to monitor the processes of enzymatic or chemicallyinduced decomposition of cellulose, a rich energy source. The stability of human amyloids was indicated to depend on the supercoiling of protein aggregates – this is of interest in the research of neurodegenerative diseases. By combining microscopic and macroscopic DP techniques, we revealed the orientation of pigment dipoles in self-assembled nanorods of porphyrin derivatives – this type of structures might be utilized in photocatalytic systems.



FDLD image of Ginkgo biloba tissue recorded using our DP-equipped RCM

Selected publications

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