NEWSLETTER SPRING 2023

FOCUS ON TECHNOLOGIES

WHAT IS CRYO-FLUORESCENCE MICROSCOPY GOOD FOR?

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Recently, Imaging Methods Core Facility (IMCF) at BIOCEV upgraded their upright confocal microscope Leica TCS SP8 with a cryo module. This upgrade allows observation of deeply frozen samples in their near-native state. The microscope can be operated in both, wide-field or confocal mode, depending on the sample's needs. The confocal modality brings several advantages, like optical sectioning, background suppression, and spectrally tunable detection range. Cryo-fluorescent microscopes (cryoFM) enable observing samples only with dry objectives, which despite using the highest possible numerical aperture 0.9 at 50x magnification slightly lowers the spatial resolution. On the other hand cryo-conditions offer the benefit of long exposure times due to very low photobleaching rates.

CryoFM is becoming more and more popular in the field of correlative light and electron microscopy (CryoCLEM, Fig. 1). Localization of some transient or rare cellular structures or phenotypes directly in electron microscope can be difficult and time-consuming. To overcome this problem, the structure of interest can be labeled with fluorescent markers, localized in cryoFM, and examined with cryoEM. This technique thus allows scientists to combine both, molecular specificity and ultrastructure in the near-native state while avoiding several artifacts induced by traditional chemical fixation.

Another promising application can be found in the research of photosystems I a II (PSI and PSII) in photosynthetic organisms. To this date, the quantification of relative abundance of PSI to PSII was mainly determined in a cuvette with cryo-spectroscopy, as spectrally distinguishable auto-fluorescence of PSI and PSII is quenched at room temperature. Cryo-fluorescence microscopy with spectrally tunable detection extends this approach by providing a valuable information on spatial distribution of the PSI and PSII specific signals with sub-cellular resolution (Fig. 2), which enables to get deeper understanding on physiology of switching between PSI and PSII photosynthetic routes.

If you find cryoFM useful for your research, please do not hesitate to reach out to us at imcf@natur.cuni.cz. For more information, you may visit our website **https://imcf.natur.cuni.cz/** or follow us on Twitter **@IMCF_BIOCEV**.

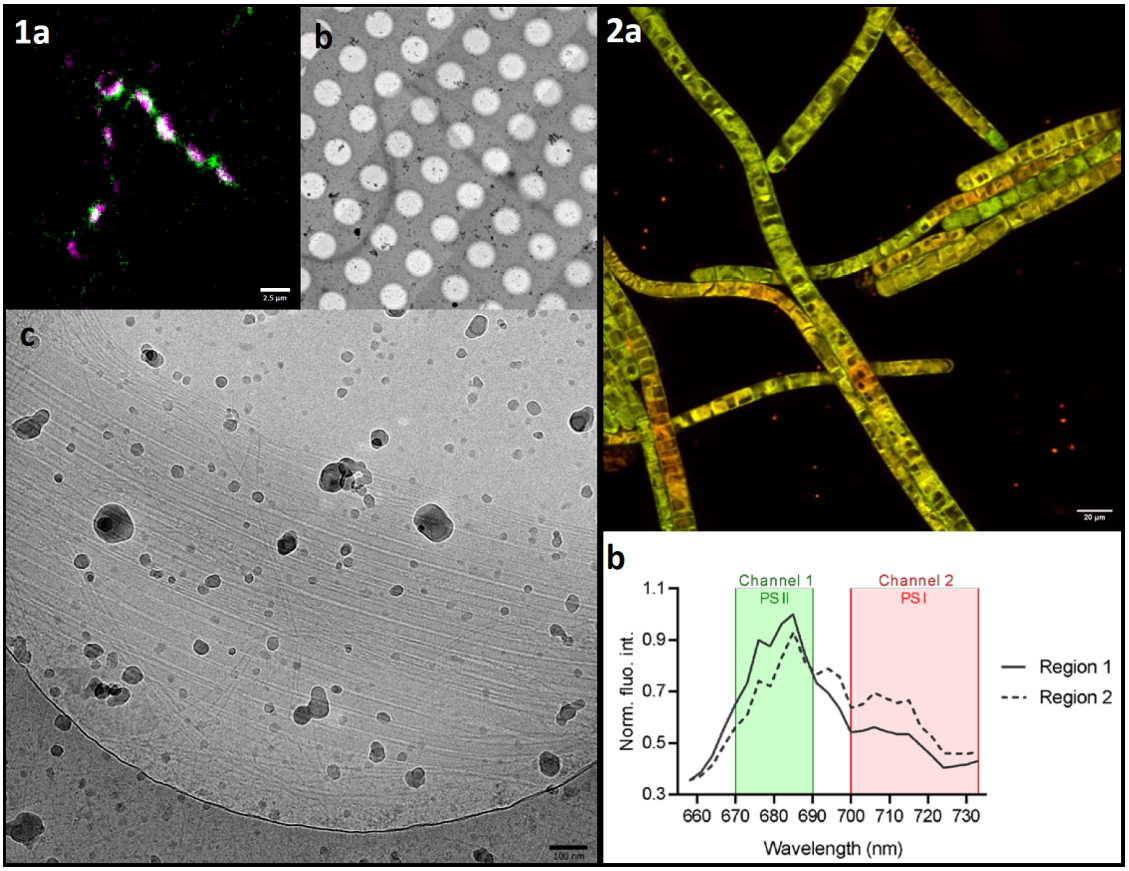

Figure **1a+b)** Correlative CryoFM and CryoEM images of protein complex composed of microtubules, actin filaments and CKAP5 protein. Scalebar = 2.5 µm **1c)** High-resolution CryoEM image of the complex structure at 30k magnification with visible microtubules and actin filaments. Scalebar = 100 nm. In collaboration with J. Sabó (Institute of Biotechnology, CAS). **2a)** Illustrative two-channel (1:green, 2:red) composite image of cyanobacteria filaments shows heterogeneity of PSI:PSII (Ch2:Ch1) fluorescence intensity ratios within filaments. Scalebar = 20 µm **2b)** Emission spectra of cyanobacteria at two distinct regions with different distributions of photosystems (PS) I and II (405 nm excitation). Channels 1 and 2 specified in ranges 670-690 nm and 700-733 nm, respectively, are optimal for imaging of PS I and PS II specific autofluorescence. In collaboration with M. Eichner (Institute of Microbiology, CAS).

HIGHLIGHTS OF USER RESULTS

A 30-YEAR FOLLOW-UP OF A PRENATAL BIRTH COHORT WITH A WITHIN-SUBJECT DESIGN NEUROIMAGING COMPONENT IN YOUNG ADULTHOOD REVEALS PREDICTORS OF ACCELERATED BRAIN AGING

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Maternal mental health problems during pregnancy are associated with altered neurodevelopment in the offspring but the long-term relationship between these prenatal risk factors and offspring brain structure in adulthood remains incompletely understood due to a paucity of longitudinal studies.

We conducted a 30-year follow-up of prenatal birth cohort with a within-subject design neuroimaging component in young adulthood and evaluated the relationship between exposure to maternal depression in utero and offspring brain age in the third decade of life and the role of recent stressful life events as potential moderators of this relationship. Thanks to the Multimodal and Functional Neuroimaging Laboratory (MAFIL) at CEITEC, Masaryk University, Brno, Czech Republic, we acquired MRI data at two timepoints - in the early 20s and late 20s - and calculated the gap between estimated neuroanatomical vs. chronological age at each MRI session (BrainAGE) as well as the pace of aging between the two MRI sessions. Greater maternal depression during pregnancy predicted larger BrainAGE in both the early and late 20s and the stability of the relationships was also supported by the lack of interactions with recent stress. In contrast, more recent stress was associated with greater pace of aging between the two MRI sessions.

Klara Mareckova at the MAFIL, CEITEC facility

Association of maternal depression during pregnancy and recent stress with brain age among adult offspring
Klara Mareckova, Radek Marecek, Martin Jani, Lenka Zackova, Lenka Andryskova, Milan Brazdil, Yuliya S. Nikolova, *JAMA Network Open*, 2023, 6(1).
doi: **10.1001/jamanetworkopen.2022.54581**

UPCOMING EVENT

UPCOMING EDUCATIONAL ACTIVITIES (SPRING - SUMMER)

PROCESSING AND ANALYSIS OF MICROSCOPIC IMAGES IN BIOMEDICINE

Practical course | April 17-21, 2023 | IMG, Prague

CRYO-IMAGING OF BIOLOGICAL SAMPLES

Course | April 24-26, 2023 | UK, BIOCEV, Vestec

NEURAL NETWORKS IN BIOIMAGE ANALYSIS

Course | May 9-11, 2023 | UK, BIOCEV, Vestec

INTRODUCTION TO METHODS OF PROTEIN STRUCTURE ANALYSIS BY CRYO-ELECTRON MICROSCOPY

Course | May 15-19, 2023 | UK, BIOCEV, Vestec

TECHNICAL ASPECTS OF EXPERIMENTS USING ADVANCED LIGHT MICROSCOPY

Course | May 16-18, 2023 | IPHYS, Prague

BIOLOGICAL SPECIMENS IN ELECTRON MICROSCOPES

Course | June 12-16, 2023 | LEM BC, České Budějovice

FUNDAMENTALS OF LIGHT MICROSCOPY

Course | June 13-15, 2023 | CEITEC MU, Brno

IMAGE ANALYSIS AND DATA PROCESSING IN SUPERRESOLUTION MICROSCOPY

Course | August 21-25 2023 | Viničná MCF, Prague

IMGLIB2 AND BIGDATAVIEWER ECOSYSTEM WORKSHOP - EFFICIENT HANDLING OF LARGE BIOIMAGING DATA WITH FIJI

Course | Summer 2023 | CEITEC MU, Brno

ENTRY-LEVEL SUPER-RESOLUTION MICROSCOPY

Course | Summer 2023 | IEM, Prague

USEFUL LINKS

Czech-Bioimaging website

Czech-Bioimaging – pro veřejnost

Euro-BioImaging website

Velké výzkumné infrastruktury v České republice

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www.czech-bioimaging.cz