001-2-2076 - **Practical Environmental Cytometry 2.5 creduts**
Lecturer: Dr. Nina Kamennaya

Five-day intensive course: 26.02-02.03.2023, 9:00 – 17:00

The course goal
Provide theoretical basis and practical training for cytometric separation of target cells from both solid and liquid samples; systematically analyse credible applications of cytometric techniques in the environmental research.

Topics covered during the course
Essential basics of cytometry:
- Cell morphology, ultrastructural contrasts and staining
- (Auto)fluorescence
- Characterization of cells using microscopy (up-right/inverted microscopes, BF, PH, DIC, epifluorescence)
- Visualization of cell compartments and components (size, shape, granularity, general and specific stains)
- Characterization of cell populations using flow cytometry (fluidics, optics, data acquisition and processing: light scatter and fluorescence)
- Basics of flow cytometry (choice of lasers, trigger, threshold, types of data plot, cross-talk)

Applications of cytometry in molecular environmental research:
- Visualization of target cells: identification of individual cells, characterization of specific populations, revealing rare populations
- Enrichment of target cells for downstream molecular analyses
- Strengths and weaknesses of different cell separation approaches
- Applications of laser capture microdissection (LCM) and flow cytometric sorting (FCS) in molecular environmental research

Different modes of sorting strategies:
- LCM: catapulting vs gravity collection strategy
- Demonstration of gravity-based LCM: collection, excision or removal of specific cells/components from a solid sample
- FCS: jet-in-air, mechanical, magnetic or electric field-based sorting methods
- Demonstration of mechanical and jet-in-air FCS instruments: cell cycle, phagocytosis, separation of overlapping populations

Design of LCM- and FCS-based experiments:
- Step-by-step experimental design
- Collection and interpretation of multidimentional data sets – sorting logics
- Preparation for practical work – independent work in small groups

Practical work – cytometric separation of target cells in aquatic and solid samples
- Isolation of target cells for molecular and physiological analyses
- Analysis of sorted cells and secondary separation to increase sorting purity

Audience
Up to 12 students
MSc and PhD in Environmental Microbiology, Biotechnology, Hydrology and Water Quality, and Sustainability and Climate Change

Grading
The final grade will be based on active participation in a class (20%), 3 short quizzes (30%) and the assignment performance level (50%).

Course literature:

Shapiro, H.M. (2003). Practical Flow Cytometry, 4th edition. John Wiley & Sons, Inc.
Laser-capture microdissection: opening the microscopic frontier to molecular analysis. *Trends in Genetics*, 14(7), 272-276.
Day, R. C., Grossniklaus, U., & Macknight, R. C. (2005). Be more specific! Laser-assisted microdissection of plant cells. *Trends in Plant Science*, 10(8), 397-406.
Klitgaard, K., Mølbak, L., Jensen, T. K., Fredrik Lindboe, C., & Boye, M. (2005). Laser capture microdissection of bacterial cells targeted by fluorescence in situ hybridization. *Biotechniques*, 39(6), 864-868.
Gloess, S., Grossart, H. P., Allgaier, M., Ratering, S., & Hupfer, M. (2008). Use of laser microdissection for phylogenetic characterization of polyphosphate-accumulating bacteria. *Applied and Environmental Microbiology*, 74(13), 4231-4235.
Simone, N. L., Bonner, R. F., Gillespie, J. W., Emmert-Buck, M. R., & Liotta, L. A. (1998).