DEPARTMENT OF MEDICINE, HUDDINGE

H7F6085, Advanced Flow Cytometry, Experimental Design and Analysis, 3 credits (hec)

Avancerad flödescytometri, experimentell design och analys, 3 högskolepoäng

Third-cycle level / Forskarnivå

Approval

This syllabus was approved by The Committee for Doctoral Education on 2025-09-07, and is valid from spring semester 2026.

Responsible department

Department of Medicine, Huddinge, Faculty of Medicine

Contributing department/s

Department of Microbiology, Tumor and Cell Biology

Prerequisite courses, or equivalent

The applicant must have prior theoretical and practical experience in flow cytometry, including prior training in their lab and/or local core facility. Preference will be given to PhD students with current or planned research projects where advanced flow cytometry represents a critical technology platform. Applications must contain the following 2 parts: 1) short description of the applicants prior theoretical and practical knowledge in flow cytometry, including specific instruments they have worked on and how much (number of hours), and 2) a brief description of the scientific aim of their own flow cytometry projects (maximum ½ page). Applications will only be considered when both parts are included. Researchers who have not used flow cytometry or do not have an active/planned flow cytometry project are advised to acquire prior basic and practical knowledge and apply for the course the next year. The PhD course “Flow cytometry: From theory to application” is not a prerequisite but forms a good basis for this PhD Course.

Purpose & Intended learning outcomes

Purpose

To enable PhD students and researchers, who are working with flow cytometry as a critical part of their research project, to become proficient in designing and analysing advanced flow cytometry experiments, including interpreting and reporting data. Both conventional and spectral flow cytometry will be covered in the course.

The course is NOT aimed at training students to operate a flow cytometer or to practically prepare samples. Rather the focus is to teach principles of experimental design and setup as well as data analysis, interpretation and reporting, that are applicable to multiple instrument platforms and experimental applications.

Intended learning outcomes:

After having successfully completed the course, participants will be able to independently apply flow cytometry in their research. Specifically, they will be able to:

1. **Understand and explain the theoretical principles of flow cytometry**, including fluorescence, spectral overlap and compensation in multicolor experiments, and detection technologies.
2. **Describe and evaluate instrument design and configuration** for conventional and spectral flow cytometry, including lasers, detectors, and optical paths.
3. **Design and optimize complex multicolor experiments** (10+ colors), including panel design, selection of appropriate controls, sample preparation, and troubleshooting experimental issues.
4. **Analyze flow cytometry experiments, and interpret and visualize flow cytometry data** using appropriate software, applying accurate gating strategies, statistical approaches, and quality control measures.
5. **Identify limitations and potential pitfalls of flow cytometry methods**, including sample quality issues, reagent limitations, and common sources of artefacts.
6. **Critically read and evaluate research papers in which flow cytometry is applied**, assessing experimental design, data quality, and the validity of conclusions.
7. **Report and communicate flow cytometry results effectively** in line with standards (e.g., MiFlowCyt and other formats), including preparing figures, tables, and documentation for publications and presentations.

Course content

The course content covers the entire process of producing and interpreting scientific results for an experiment requiring flow cytometry: formulation of the scientific question, reagent panel design including controls, sample preparation, choice of instrument and settings for acquisition, data storage, data analysis, preparation of figures and text for publication, ethics.

The course is focused on the experimental design of advanced multiparametric (10++ parameters) flow cytometry performed on conventional and spectral flow cytometers. Students will get theoretical exposition to and opportunity for hands-on data analysis of specific applications in advanced flow cytometry such as immune-phenotyping, phospho-flow, imaging flow cytometry and small particle analysis.

While the course, in its outline, does not include wet-lab experiments, it is designed so that during the course the students can apply their acquired knowledge using their OWN samples and equipment. They will receive feedback on experimental design, data quality, and post-

acquisition data analysis, which will be practiced using existing datasets.

Forms of teaching and learning

Theoretical concepts will be presented in in-class lectures and video tutorials, with the possibility for in-depth discussion in smaller groups. During the course, the students will work in teams to solve smaller assignments and present results to the class. The students will get raw data sets from already acquired flow cytometry experiments to individually and in groups analyze the data in available post-acquisition software. Formative feedback will be provided throughout the course on individual and group hand-in assignments and data analysis exercises. Student learning will also be assessed/self-assessed using quizzes.

Language of instruction

The course is given in English

Grading scale

Pass (G) /Fail (U)

Compulsory components & forms of assessment

Compulsory components

Attendance to all sessions is compulsory. Any absence must be reported to the course leader in advance by e-mail. Absence from any part of the course (lectures, discussion sessions and examination) is generally not accepted but could in exceptional cases be compensated for by a written additional assignment to ensure the learning outcomes of the day have been reached. If it is not possible to compensate, the student will be given the chance to complete the course by attending the missing sessions the following year.

Forms of assessment

The final mark (pass or fail) will depend on student participation in in-class activities (group work and presentations), individual quizzes and practical assignments, and the final individual written report. The final written report will be assigned individually and will involve producing publication-ready data analysis with associated methodology and data reporting in line with best practice guidelines for publication of flow cytometry data.

Course literature

Recommended literature and resources

Lee JA, Spidlen J, Boyce K, Cai J, Crosbie N, Dalphin M, Furlong J, Gasparetto M, Goldberg M, Goralczyk EM, Hyun B, Jansen K, Kollmann T, Kong M, Leif R, McWeeney S, Moloshok TD, Moore W, Nolan G, Nolan J, Nikolich-Zugich J, Parrish D, Purcell B, Qian Y, Selvaraj B, Smith C, Tchuvatkina O, Wertheimer A, Wilkinson P, Wilson C, Wood J, Zigon R;

International Society for Advancement of Cytometry Data Standards Task Force; Scheuermann RH, Brinkman RR. MIFlowCyt: the minimum information about a Flow Cytometry Experiment. Cytometry A. 2008 Oct;73(10):926-30. doi: 10.1002/cyto.a.20623. PMID: 18752282; PMCID: PMC2773297.

Spidlen J, Breuer K, Brinkman R. Preparing a Minimum Information about a Flow Cytometry Experiment (MIFlowCyt) compliant manuscript using the International Society for Advancement of Cytometry (ISAC) FCS file repository (FlowRepository.org). Curr Protoc Cytom. 2012 Jul;Chapter 10:Unit 10.18. doi: 10.1002/0471142956.cy1018s61. PMID: 22752950.

https://voices.uchicago.edu/ucflow/spectral-flow-cytometry/

https://flow.ucsf.edu/protocols

https://flow.ucsf.edu/education-library