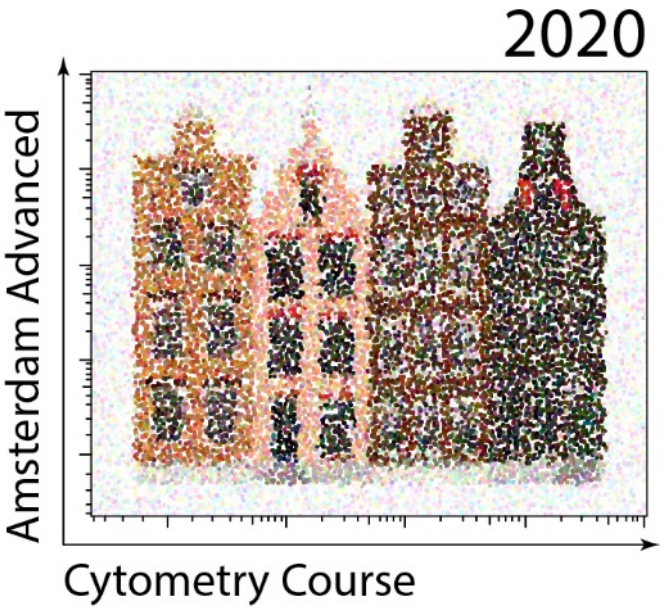


# *Focus on Spectral Cytometry*

**Practical information**

| | |
|---|---|
| Date: | 30th October, 2020 |
| Time: | 9:00 – 18:00 |
| Target audience: | PhD students (a restricted number of post-docs, and technicians will be selected (Max. 30 participants) |
| PhD Credits: | 1.5 ECTS (42 hours, in 5 blocks throughout October 2020 to May 2021) |
| Location: | Online via Zoom |
| Costs: | Free (50 EUR cancellation penalty)<br>Documentation is provided free of charge |
| Registration | Fill in the form (https://goo.gl/forms/JOOQVeL4IOrFVQcZ2) before October, 25th |

**Supported by** Cytek Biosciences, Amsterdam Infection & Immunity Institute, Cancer Center Amsterdam, and the Oncology Graduate School.

**Organization & Contact**

**Microscopy & Cytometry Core Facility**
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**Why an advanced course in cytometry?**

Cytometry has become a fundamental technology that enables scientific applications in multiple disciplines including fundamental (Cell Biology), translational (Immunology) and clinical research (Hematology and Oncology). The last 10 years have witnessed an explosion in the development of fluorescence- and mass-based detection methods that are revolutionizing the field. Recently, genomic cytometry and immunophenotyping on tissue have been added to the arsenal of possibilities within most advanced core facilities. An integral part of the responsibilities of cytometry core facilities is to provide educational support and technical training to facilitate access to cytometry-based methods, in order to ultimately ensure the generation of reproducible quality data and an optimal use of existing resources.

Dr. De Biasi discuses about multicolor panel design (*1st Advanced Cytometry Course, November 2017)*

**General Learning Objectives**

This is a comprehensive course that covers the fundamentals of flow cytometry analysis and sorting, as well as mass cytometry in a lecture format supplemented by practical lab and data analysis sessions. The course is designed to gain in depth knowledge on general technical aspects of the different types of cytometers available in the market and how the different components and their configuration influence data acquisition. Special emphasis is placed on basic concepts of lasers and fluorescence emission, how to properly set up a cytometer for standardized performance, how to optimally prepare cells for a cytometry experiment, the principle of FACS-based cell sorting, and other specialized applications, including data analysis.

Interactive sessions on data analysis will build up upon basic concepts on compensation, data processing and gating and will focus on the analysis of medium-large multicolor panels. Basic understanding of flow cytometry packages is a not prerequisite, but will help a great deal. Our aim is to provide a wide overview of the different modalities of cytometry currently available and all specialized applications that can be used by PhD students in the fields of Immunology, Hematology, and Oncology. The course will conclude with a feedback session with cytometry experts where a selected group of students will present special cases and discuss important aspects of their own PhD projects.

Drinks and feedback session with cytometry experts Dr. Epron, Dr. De Biasi, and Dr. van Gassen
*1st Advanced Cytometry Course, November 2017*

**Format**

The course consists of four types of sessions; interactive classroom teaching, panel design workgroups, data analysis sessions, and lab demonstrations. For the data analysis sessions, attendants may use their own laptops equipped with a demo version of FCS Express.

**Note**: Because of restrictions imposed by the COVID-19 crisis, we are not able to offer a classroom-based course. Instead, we have set up an online program split over several focused sessions that covers the most important aspects of the course, with the exception of the live demos. Attendants that would like to receive a training for the usage of an instrument after the course may do so via making a training request on PPMS at https://ppms.eu/vumc/areq/?pf=3

**Course Schedule, FOCUSED SESSION ON SPECTRAL CYTOMETRY**

| | | |
|---|---|---|
| 8:30 – 9:00 | Welcome and round of introduction to the course | J. J. Garcia-Vallejo |
| 9:00 – 10:00 | (1) What is spectral flow cytometry? | S. van Bockstael |
| 10:00 – 11:00 | (2) Principles of spectral unmixing | S. van Bockstael |
| 11:00 – 11:15 | Break | |
| 11:15 – 12:00 | (3) Panel design | L. Ferrer-Font |
| 12:00 – 13:00 | (4) Panel optimization | L. Ferrer-Font |
| 13:00 – 14:00 | Lunch | |
| 14:00 – 15:00 | (5) Experimental design | A. Paul |
| 15:00 – 15:45 | (6) Data QC | A. Paul |
| 15:45 – 16:00 | Break | |
| 16:00 – 16:45 | (7) Implementing spectral cytometry in a research project | A. Belkina |
| 16:45 – 17:15 | (8) Q&A Session | A. Belkina |
| 17:15 – 17:30 | (9) Course evaluation | J. J. Garcia-Vallejo |

**Specific learning objectives:**

(1) What is spectral flow cytometry? Review of basic concepts on lasers, fluorochromes, and fluorescence. How does spectral flow cytometry compares to conventional cytometry (detector usage). Differences in the workflow, expected differences in sensitivity, number of parameters, impact on spread. Technical fundaments (instrument architecture basics).

(2) Principles of spectral unmixing. Algorithm used. Autofluorescence, different strategies. Unmixing vs compensation. What to expect with a normal unmixing. Live example.

(3) Panel design. Information needed prior to start (markers needed for biological insight, expression pattern, gating wishes). Available tools to guide panel design (including demo), What is spreading error, what is the difference with spillover, what is similarity and complexity, what is brightness. Fluorochrome-marker assignation rules. Panel quality check in silico vs experimentally.

(4) Panel optimization. Titrations and staining conditions. Reference controls, how to choose them, how to prepare them. Tips and tricks on instrument usage.

(5) Experimental design. What are special applications for spectral flow cytometry and how to make the best of them. What are necessary controls. How to QC the controls. What to keep in mind for long studies (multiple batches).

(6) Data QC and analysis strategies. How to check for unmixing accuracy. What are the most frequent errors found and how to troubleshoot them. What to show to prove that data is perfect. How to continue afterwards (general strategies).

(7) Personal experiences during the implementation of spectral flow cytometry in an academic research context. There will be a Q&A session focused on do's and don'ts in spectral flow cytometry.

**Faculty:**

- Juan J. Garcia Vallejo, MD, PhD, MBA. Associate Professor and Scientific Director of the Microscopy and Cytometry Core Facility at the Amsterdam UMC – Location VUmc (Amsterdam, The Netherlands).
- Sebastiaan van Bockstael, PhD. Technical Application Scientist, Cytek Biosciences.
- Laura Ferrer-Font, PhD. Post-doctoral fellow at Malaghan Institute of Medical Research (Wellington, New Zealand)
- Liesbeth Paul, PhD. Technical Application Scientist, Cytek Biosciences.
- Anna C. Belkina, MD, PhD. Assistant Professor of Pathology and Laboratory Medicine and Director of the Flow Cytometry Core Facility at Boston University School of Medicine (Boston, MA, USA)

**Recommended reading:**


- Ferrer-Font L, Pellefigues C, Mayer JU, Small SJ, Jaimes MC, Price KM. Panel Design and Optimization for High-Dimensional Immunophenotyping Assays Using Spectral Flow Cytometry. *Curr Protoc Cytom*. 2020; 92 (1): e70. doi:10.1002/cpcy.70
- Park LM, Lannigan J, Jaimes MC. OMIP-069: Forty-Color Full Spectrum Flow Cytometry Panel for Deep Immunophenotyping of Major Cell Subsets in Human Peripheral Blood. *Cytometry A*. 2020;10.1002/cyto.a.24213. doi:10.1002/cyto.a.24213
- Nolan JP, Condello D. Spectral flow cytometry. *Curr Protoc Cytom*. 2013; Chapter 1: Unit1.27. doi:10.1002/0471142956.cy0127s63

**General Learning Objectives**

The course will focus on the following aspects:

- **Instrumentation and cytometry modalities.** There are a number of technical requirements necessary for the analysis of cells in flow cytometry: A light illumination system, a detection system, and a fluidics system. The diverse modalities of these parts will be discussed with a focus on how their technical specifications determine the measurement.
- **Reagents.** The great majority of applications in cytometry require the use of antibodies. We will discuss the importance of antibody validation as well as tips and tricks for antibody-dependent applications. In addition, we will also discuss multiple applications that take advantage of fluorescent molecules to measure cell biology phenomena such as cell division, redox estate, lysosome stability, caspase activation, etc.
- **Lasers, fluorescence, and fluorochromes.** The most extended cytometry applications are based on the property of fluorochromes and fluorescent proteins of emitting photons when excited with the appropriate wavelength. We will focus on the basic principles of fluorescence for a better understanding of crucial concepts in cytometry, such as fluorescence cross-talk and compensation, but also a good understanding of what signal intensity means.
- **Sample preparation.** Next to instrument set up and the optimization of acquisition, the most important aspect of a successful cytometry experiment is an optimal preparation of the sample, irrespective of whether the cells of interest grow in suspension or need to be isolated from a solid tissue.
- **Instrument optimization strategies.** To obtain high quality cytometry data, a well-optimized instrument is required. Here, we will focus on how to set up the minimum voltage required for cytometry, that is, an ideal minimal voltage will amplify dim signal above background, but is not so high that the signal exceeds the upper range of detector linearity.
- **Spectral analysis.** Spectral cytometry is gaining popularity because of its enhanced possibilities for multicolor analysis and panel design. We will pay special attention to this new technology with a focus on the fundaments of spectral un-mixing, the possibilities within the available palette of fluorochromes, and the implications for data analysis.
- **Multicolor panel design.** The development of new fluorochromes and the design of instruments able to measure dozens of dyes simultaneously is recently having an enormous impact on the size of the average FACS panel. The successful development of such panel requires following a strict rules which will be carefully discussed and demonstrated here. This session will consist of a lecture on the principles of panel design and an assignment in groups, where the participants will be requested to design a panel based. All the necessary materials will be provided in class.
- **Rare event analysis.** The ability to accurately detect and analyze rare cells in a cell population is critical, not only for the study of disease progression but also for our understanding of key pathways in normal development. Applications include stem cells, circulating endothelial cells, circulating tumor cells, and residual disease cells. Thanks to technological advances in instrumentation and better detection reagents and more sophisticated analysis strategies, identifying as little as 0.0001% rare cells at frequencies is possible.

- **Compensation, electronic noise and data spread.** One of the limitations of fluorochromes is their often overlapping spectra and cross-excitation. Also, signal acquisition and properties of light filters and photomultipliers contributes to introducing data errors that are important to identify and avoid. We aim to provide the fundaments to understand the sources or error in cytometry, with a focus in the concept of spreading error and how to avoid it.
- **Controls, reproducibility, and troubleshooting.** A critical aspect of a successful cytometry experiment is the selection of the most appropriate antibody clones, their conjugation, characterization, and titration. We will go through the basics of antibody technology and provide a solid workflow for antibody usage in cytometry.
- **Mass cytometry.** Mass cytometry is becoming a widespread technology that brings multicolor cytometry to higher levels of multidimensionality without the burden of compensation. An overview of the technology will be provided with an special attention to human immunomonitoring applications and a lab demonstration.
- **Imaging Flow Cytometry.** Imaging Flow Cytometer combines the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insights of microscopy. This unique combination enables a broad range of applications that would be impossible using either technique alone.
- **Genomic Cytometry.** Genomic Cytometry is new field that is evolving pushed by the rapid developments in single cell RNAseq-based methods. Just like fluorescent, mass and imaging cytometry uses light, mass or images respectively to measure cells, genomic cytometry uses nucleotide sequences to measure various attributes of cells one at a time.
- **Immunophenotyping on tissue**. Multiplexed immunohistochemistry and multicolor immunofluorescence microscopy enable the characterization of tissue cellular heterogeneity with a resolution and capabilities matching that of flow cytometers but while preserving spatial information.
- **Microbial cytometry**. In microbiology, flow cytometry permits the reliable and rapid detection of single or multiple microbes and can provide information about their distribution within cell populations. Flow cytometry may also lead to a faster means of viability counting of microorganisms while at the same time enabling a better understanding of all bacterial cells within a given population.
- **Data analysis.** From standard gating to multidimensional analysis, cytometry is moving towards the field of cytomics and computational sciences. We will cover from standard gating and operations through to the latest algorithms in high dimensional cytometry. This session will be provided by Andrea Valle, from De Novo software. Participants are requested to install a demo version of FCS Express and familiarize themselves with this software packages prior to the course.