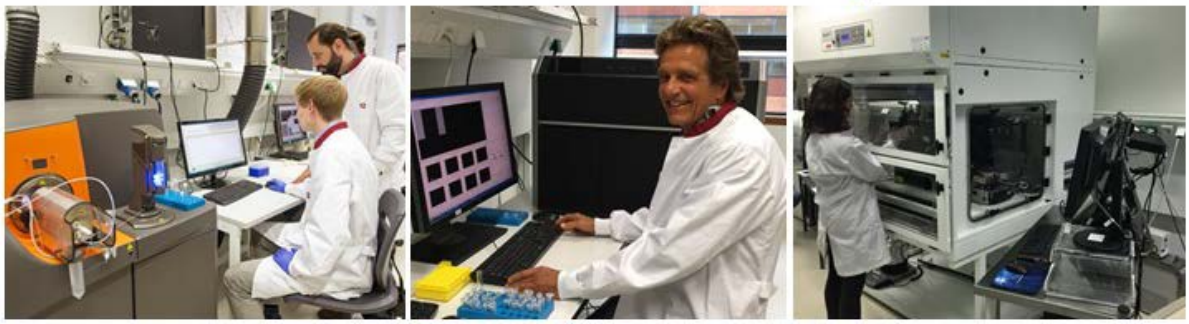
3rd Advanced Cytometry Course



Practical information

Dates:	13-17 May, 2019
Time:	9:00 – 18:00, drinks after 17:00 on Friday 17th May
Target audience:	PhD students, post-docs, and technicians (Max. 30 participants)
Credits:	1.5 ECTS (42 hours)
Location:	O|2 Lab Building, De Boelelaan 1108, 1081 HZ Amsterdam
Costs:	Free (50 EUR cancellation penalty) Documentation is provided free of charge Lunch and coffee are not provided
Registration	Please, fill in this form before May 1st, 2019

Supporting Institutes

- Amsterdam Infection & Immunity Institute.
- Cancer Center Amsterdam.
- Oncology Graduate School.

Organization & Contact

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Sponsors

ThermoFisher, Miltenyi Biotec, OOA

Why an advanced course in cytometry?

Cytometry has become a fundamental technology that enables application 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 both fluorescence- and mass-based detection methods that are revolutionizing the field. 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.

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.

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

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. We emphasize on providing a wide overview of the different modalities of cytometry currently available and all specialized applications that can be of used for the 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 three types of sessions; interactive classroom teaching, data analysis sessions, and lab demonstrations. For the data analysis sessions, attendants are expected to use their own laptops equipped with the latest version of R Studio[1] and the freely available software cytosplore[2] (www.cytosplore.org). We will pay special attention to the R packages flowcore[3] and cytofkit[4].

[1] Download software here https://www.rstudio.com/, see also this useful tutorial https://www.statmethods.net/r-tutorial/index.html

[2] It can be downloaded here: and this is the original publication: T. Höllt, *et al*. Cytosplore: Interactive Immune Cell Phenotyping for Large Single-Cell Datasets. Computer Graphics Forum (Proceedings of EuroVis), 35(3): pp. 171—180, 2016.

[3] Flowcore can be downloaded for installation here, it contains a tutorial.

[4] Cytofkit can be downloaded for installation here, it contains a tutorial.

Specific Learning Objectives

1. 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.

2. 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.

3. 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.

4. 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.

5. 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.

6. Non-antibody based applications. Multiple applications exist that take advantage of fluorescent molecules to measure cell biology phenomena such as cell division, redox estate, lysosome stability, caspase activation, etc.

7. 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.

8. Standardization and normalization in cytometry. Standardization of immunophenotyping requires careful attention to reagents, sample handling, instrument setup, and data analysis, and is essential for successful cross-study and cross-center comparison of data. Flow cytometry datasets from clinical trials generate very large datasets and are usually highly standardized, focusing on endpoints that are well defined *a priori*. Staining variability of individual makers is not uncommon and complicates manual gating, requiring the analyst to adapt gates for each sample, which is unwieldy for large datasets. It can lead to unreliable measurements, especially if a template-gating approach is used without further correction to the gates.

9. 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.

10. 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.

11. 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.

12. CyTOF. 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.

13. 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.

14. New trends in cytometry: High-throughput avidity-based analysis and sorting. AFS is the first platform able to directly measure the strength of interaction, or avidity, between cells with specific targets and sort cells of interest, in a high throughput and label-free manner. The essence of AFS lies in a glass microfluidic chip with a piezo element that generates resonant acoustic waves. These resonant acoustic waves are used to exert forces on cells.

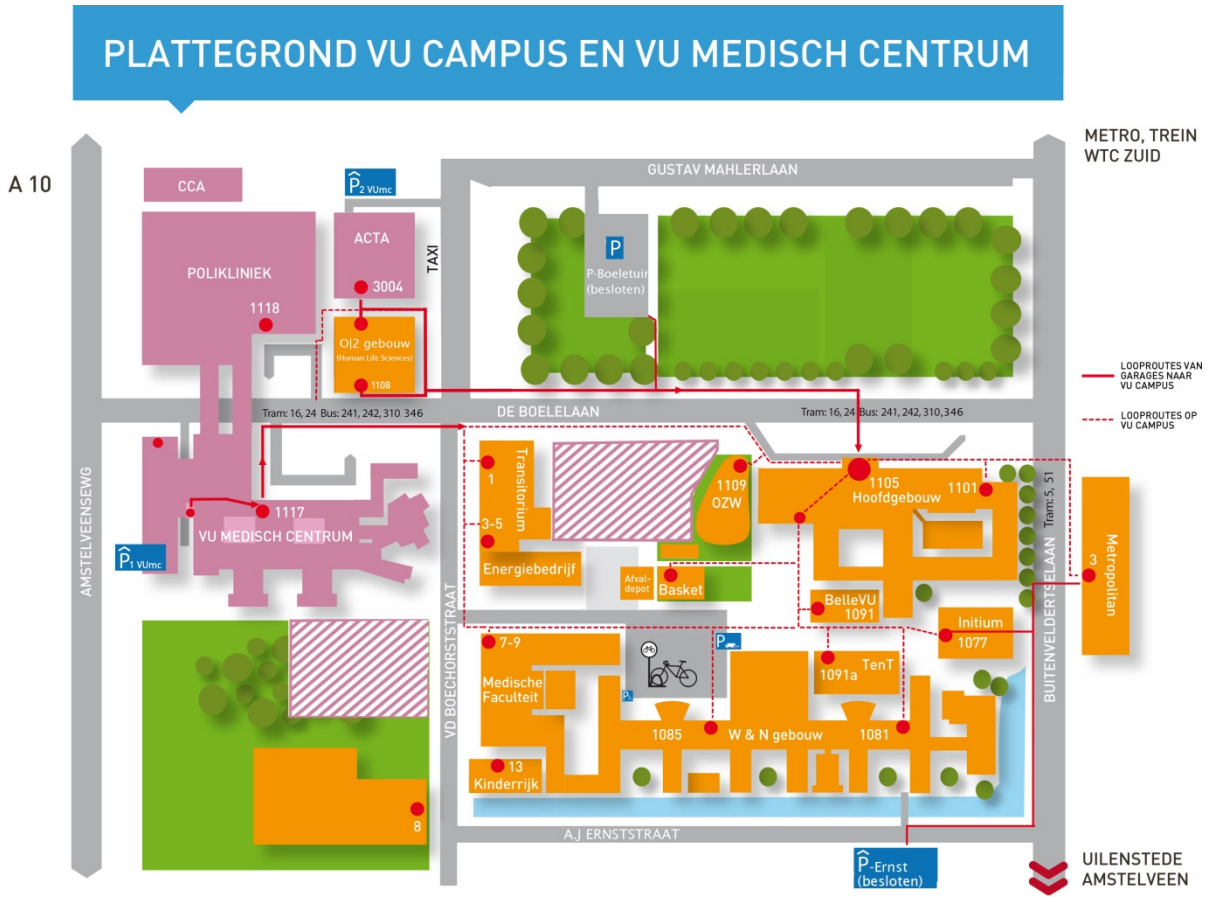
15. Analysis and sorting of extracellular vesicles (EVs). Blood and other body fluids contains EVs originating from a variety of cell types that may contain very valuable clinical information. The selection or development of a single EVs detection method requires knowledge on the physical properties of EVs.

16. Data analysis. From standard gating to multidimensional analysis, cytometry is moving towards the field of cytomics and computational sciences. We will cover from standard characterization of the most frequent immune and hematological subpopulations through to the latest algorithms in high dimensional cytometry, with a special focus on open source tools and data presentation strategies. Participants are requested to install R Studio as well as cytosplore and familiarize themselves with these software packages prior to this course.

17. Lab demonstrations. We will split the class in groups of 5 in order to be able to follow an in depth demonstration in a smaller setting and allowing personalized feedback. There will be 5 lab demonstrations, as detailed in the schedule (next page).

Location of the course

The course will take place at the O|2 Lab Building (De Boelelaan 1108, 1081 HZ Amsterdam), at the VU campus:



Reaching the O|2 Lab Building is easy:

- By public transport:
  - Amsterdam Zuid NS station (approx. five-minute walk)
  - Metro line 50, Amstelveenseweg stop (approx. five-minute walk)
  - Metro line 51, De Boelelaan/VU Amsterdam stop (approx. five-minute walk)
  - Sneltram (fast tram) 5, De Boelelaan/VU Amsterdam stop (approx. five-minute walk)
  - Tram line 16 VUmc stop (approx. one-minute walk)
  - Bus lines 62, 166, 171, 176, 242, 310 and 346, VUmc stop (approx. one-minute walk)
  - Bus lines 142, 170 and 172, De Boelelaan stop (approx. five-minute walk)
- By car: The O|2 Lab Building is accessible from all directions via the A10 Amsterdam ring road. Follow the ring road to the S108 (Oud-Zuid/Buitenveldert/Olympic stadium) exit. At the end of the slip road turn left onto Amstelveenseweg (in the direction of VUmc/Zuidas/Amsterdamse bos/Amstelveen). Follow the signs to the P2/VUmc car park. You can park in the P2 car park on Gustav Mahlerlaan. The entrance is located next to the ACTA building (see map). Parking fees are €1 per 17 minutes, or €30 for a day ticket. You can find information and parking options near VU Amsterdam on www.parkerenbijvu.nl. The lift or stairs lead you to the O|2 Lab Building's staff entrance on the side of the ACTA building.