

FLOW CYTOMETRY COURSE 2023

Program

9h00-9h45: Introduction to Flow Cytometry (Erwin Wijnands)

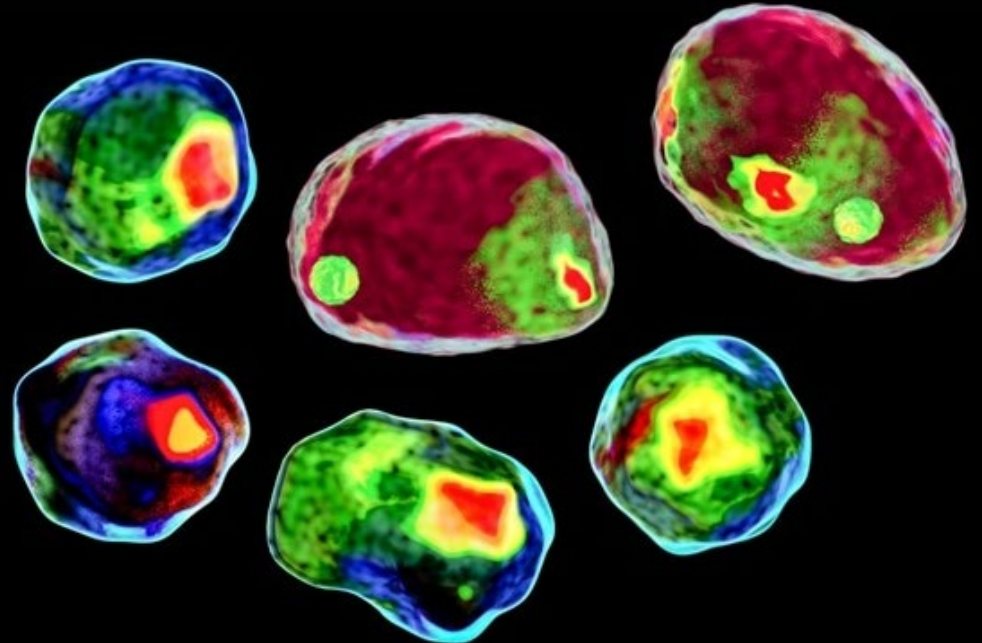
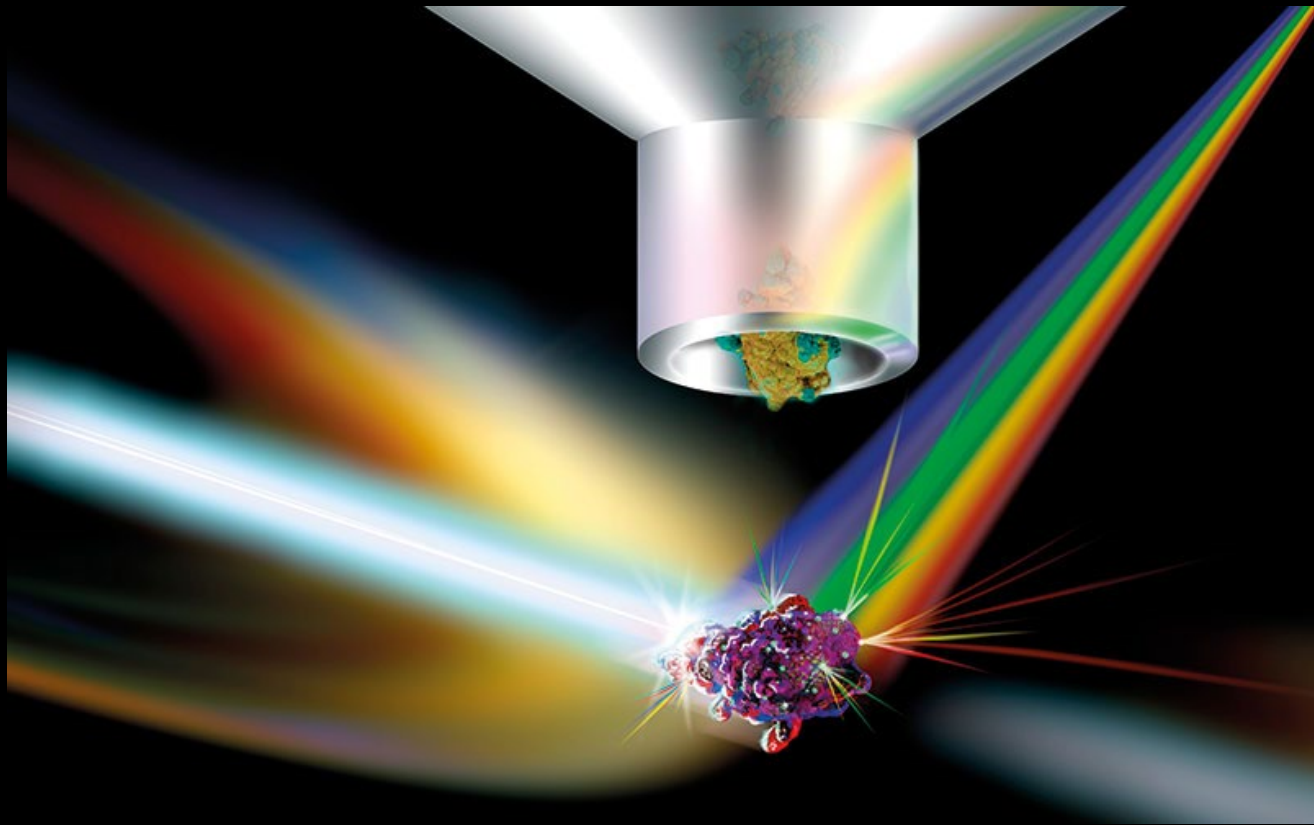
9h45-10h30: Experimental design: Instrument, Panel, Controls (Lieve Temmerman)

10h30-11h00: break

11h00-11h30: Applications and Fluorescence activated cell sorting (Erwin Wijnands)

11h30-12h00: Flow Cytometry in Maastricht: instruments and practicalities (Kristiaan Wouters)

12h00-12h30: break



**FLOW CYTOMETRY
In Maastricht**

BD FACS Canto II



BD FACS Canto

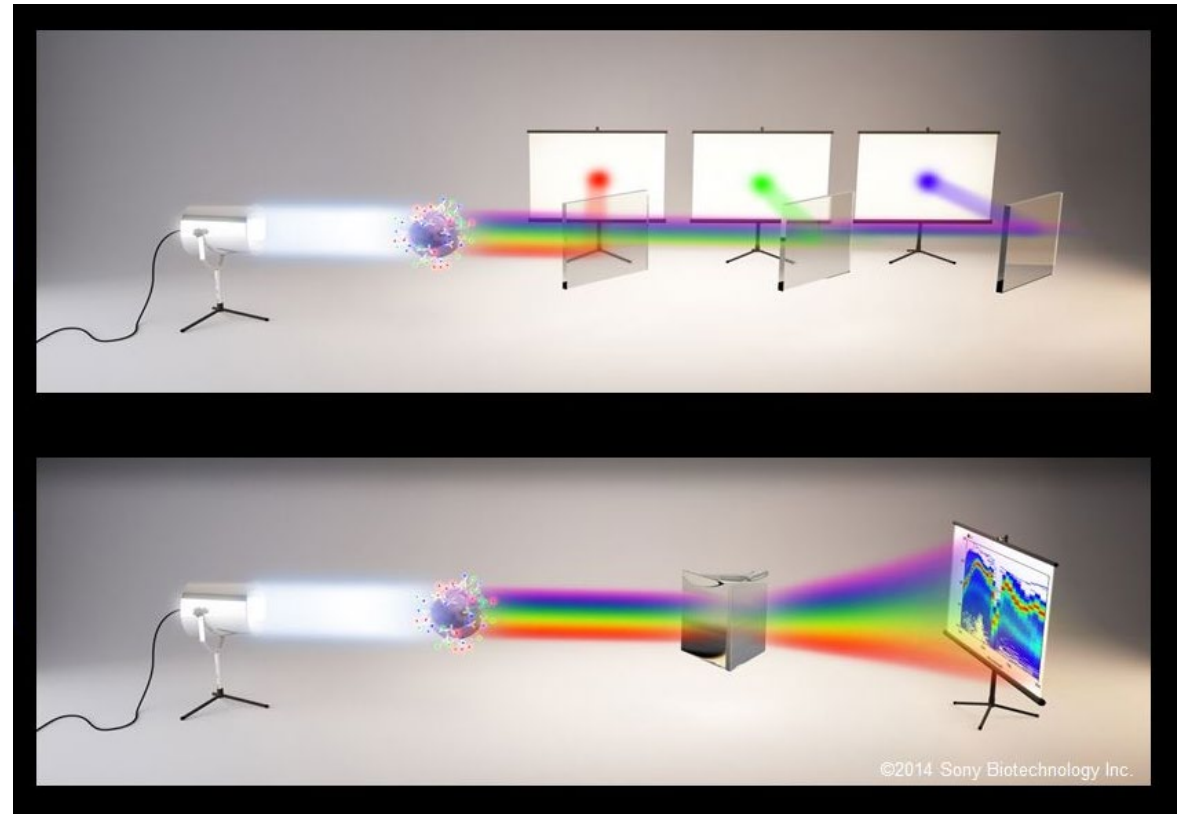
- 3 laser
- 8 colors

Laser	PMT	LP	BP	Fluorochromes
488	A	735	780/60	PE-Cy7
	B	685	710/50	PE-Cy5.5 PerCp
	D	556	585/42	PE
	E	520	530/30	FITC A488
633	A	735	780/60	APC-Cy7 APC-H7
	B	-	660/20	APC A647
405	A	750	510/50	V500 BV510
	B	-	450/40	Pacific blue Hoechst Dapi BV421

fccf.cdl@mumc.nl

lynn.janssen@maastrichtuniversity.nl/w.germeraad@maastrichtuniversity.nl

Cytek Aurora



Booking (all flow cytometers)

<https://maastricht-fc.calpendo.com/>

The screenshot displays the Calpendo booking interface for MUMC+ Flow Cytometry. The browser address bar shows the URL <https://maastricht-fc.calpendo.com/#calendar>. The page header includes logos for Maastricht University and Maastricht UMC+, the Calpendo logo (Version 10.1.20), and the text "UM-MUMC+ Flow Cytometry". The main heading is "UM-MUMC+Flow Cytometry Bookings" with "Version 10.1.20" below it. Two forms are highlighted with blue boxes: a login form on the left with fields for "Username:" and "Password:", a "Login" button, and a "Register new user" button; and a "New User Registration" form on the right with a "Local" button, "Exprodo" and "Maastricht University" authentication options, and a "Cancel" button. The Calpendo LITE logo is centered at the bottom of the interface.

You need to provide a budgetnumber and the name of the budgetholder to the manager of the cytometer (by email).

Calendars Templates Bookings Projects Search Admin Help

All | kwouters | [Change Password](#) | [Settings](#) | [Sign out](#)

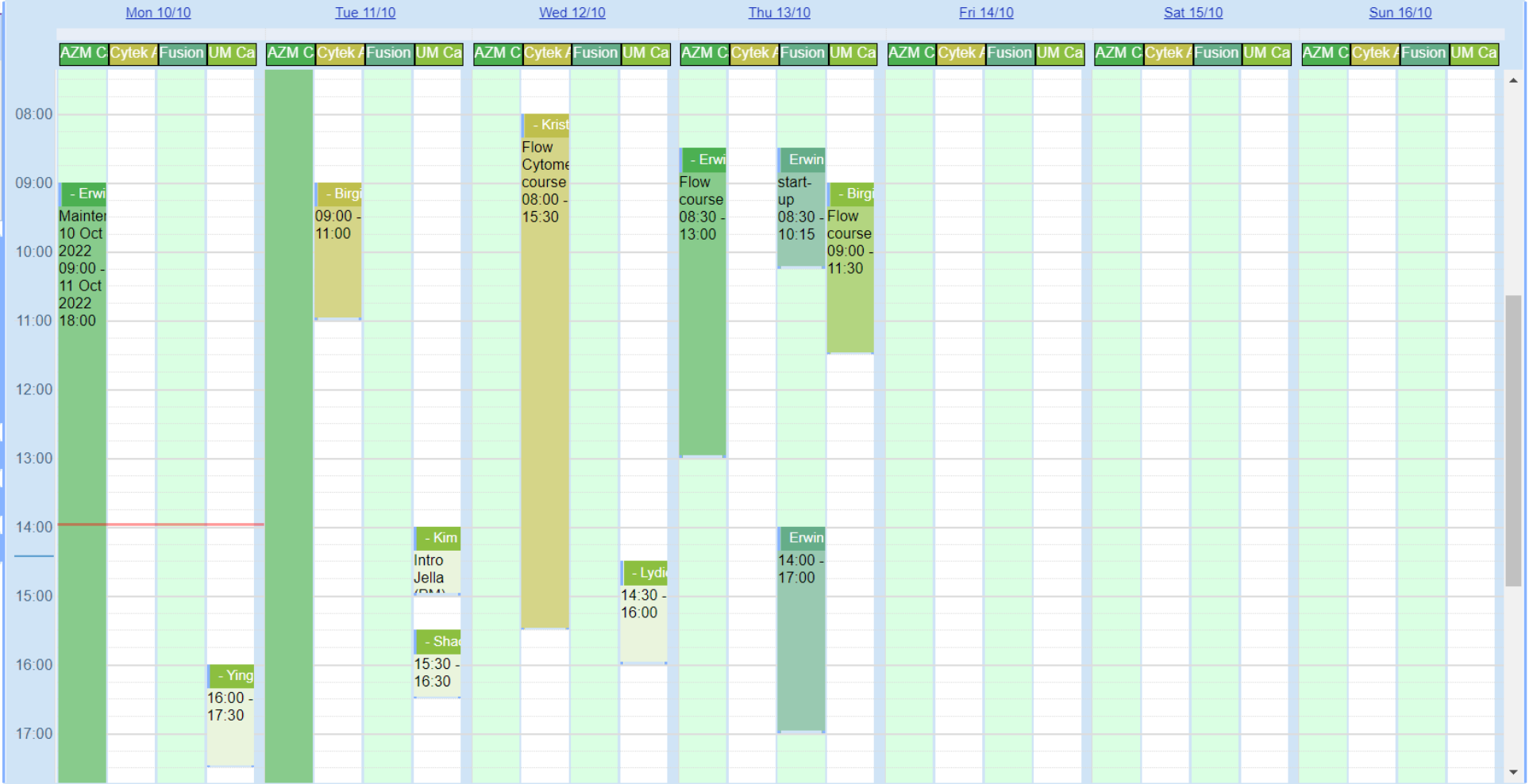
Calpendo LITE Week 41 Today Refresh Printable View

Day Week Month Horizontal Vertical

<<	<	Oct	>	>>		
M	T	W	T	F	S	S
26	27	28	29	30	1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31	1	2	3	4	5	6

- ▼ Resources
- AZM Canto
 - Cytek Aurora
 - Cytek analysis station
 - Fusion Sorter
 - UM Canto
- Bookmarks

- ▶ Templates
- ▶ Status
- ▶ Filter



Check technical information

Maastricht University | Maastricht UMC+ | Calpendo Version 10.1.8

Calendars Templates Bookings Projects Search Help


Calpendo LITE Week 43 Today Refresh Printable View

Mon 25/10 Tue 26/10 Wed 27/10 Thu 28/10

AZM Can Cytek Aur Fusion S UM Cant AZM Can Cytek Aur Fusion S UM Cant AZM Can Cytek Aur Fusion S UM Cant AZM Can Cytek Aur Fusion S UM Cant

AZM
BD FACS Canto II

- 3 laser
- 8 colours



Laser	PMT	LP	BP	Fluorchromes
488	A	735	780/60	PE-Cy7
	B	685	710/50	PE-Cy5.5
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633	A	735	780/60	APC-Cy7 APC-H7
	B	-	660/20	APC A647
405	A	750	510/50	V500 BV510
	B	-	450/40	Pacific blue Hoechst Dapi BV421

Send email to erwin.wijnands@mumc.nl for an introduction

Create booking

The screenshot displays the Calpendo software interface for Maastricht University and Maastricht UMC+. The main view is a calendar for Week 41 (October 10-14, 2022). A 'New Booking' dialog box is open, showing the following details:

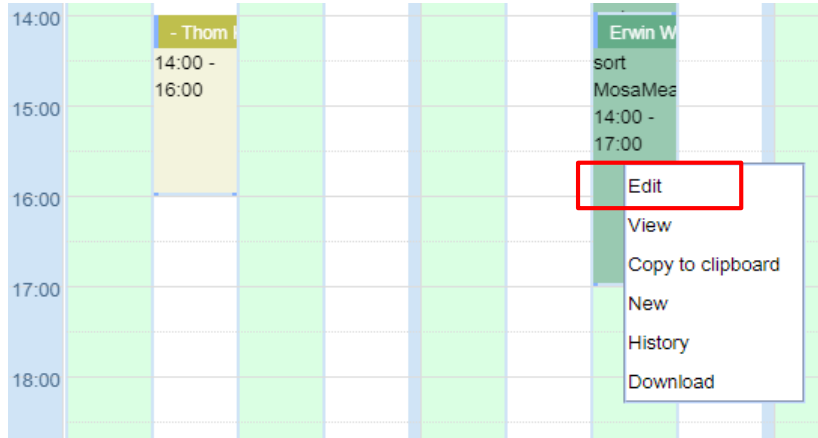
- Resource: UM Canto
- Owner: kwouters (Kristiaan Wouters)
- Project: **None** (highlighted in yellow)
- Status: Best Possible
- From: 12 Oct 2022 10:45
- To: 12 Oct 2022 11:45
- GMO Information: (Empty)
- Repeat: No repeat
- Reminders: Send reminder email, Notice Period: 30 minutes
- Send reminders to: Booker, Booking owner, Project owner, Project users
- Resource Problem: (Empty)

A dropdown menu is open next to the Project field, listing various project names such as (B. Meis), (C. Formica), (C. Tomasina MERLN), (Denise Consten (RT)), (E. Prades Sagarra PM), (G. Dole Trans Immuno), (J. Hounjet (RT)), (Janine van Elissen), (Joel Beaumont (RT)), (K. Kampen RT), (K. Wouters Trans Immuno), (L. Barbeau RT), (Lorena Giuranno (RT)), and (Lynn Janssen).

A yellow warning banner at the top right of the calendar area states: "No project has been selected (but one is required for UM Canto)".



Edit booking



Dialog box titled "Edit Booking 20211005.114358.0.1".

Resource: Fusion Sorter

Owner: erwijnands (Erwin Wijjnands)

Status: Approved

From: 26 Oct 2021 14:00 All day

To: 26 Oct 2021 17:00 duration 180

GMO Information: sort MosaMeat

Repeat: No repeat

Reminders: Send reminder email

Resource Problem:

Buttons: Update Booking, Cancel Booking, Cancel Changes

Bookings are invoiced based on the registered time in Calpendo!! If you used less time than booked, then update your specific booking and click on the Update booking button. **This adjustment is only possible within 3 days after the booking-date!**

Cytek Analysis station

Maastricht University | Maastricht UMC+

Calendars Templates Bookings Projects Search Admin Help

Calendar

All

Analysis stations

Facility Instruments

do LITE

Week 43 Today Refresh

Mon 25/10

AZM Can Cytek Aul Fusion S UM Cantr AZ

08:00

09:00

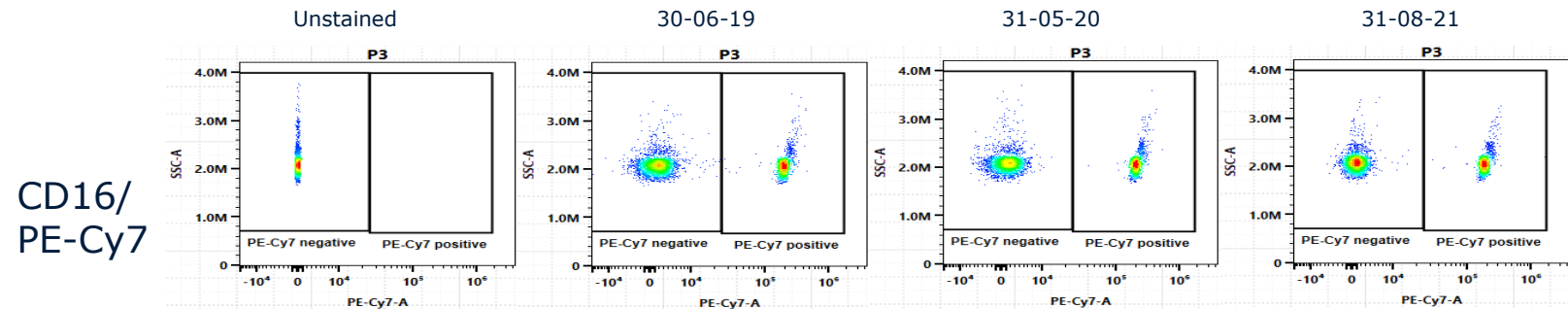
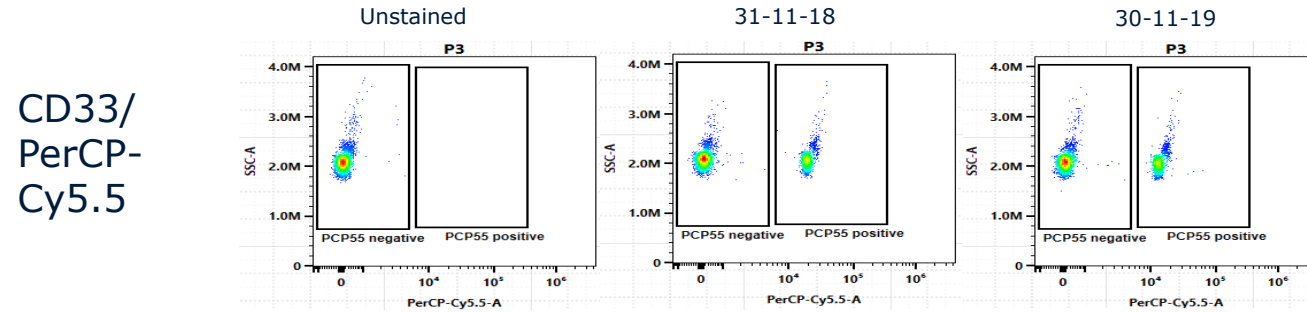
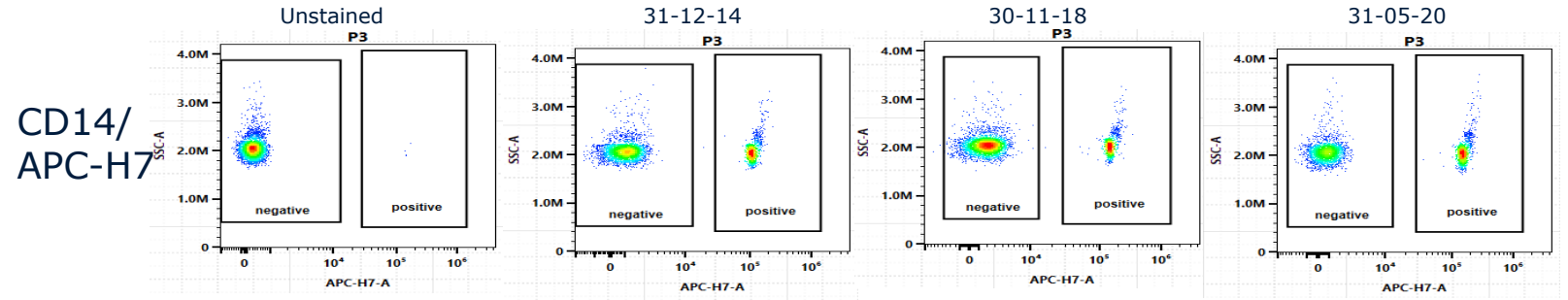
How can we help you?

- Help getting started
- Theoretical support
 - Collaborative
 - Service
- Antibody libraries
 - Outdated antibodies clinic
 - Own stocks
 - Antibody library

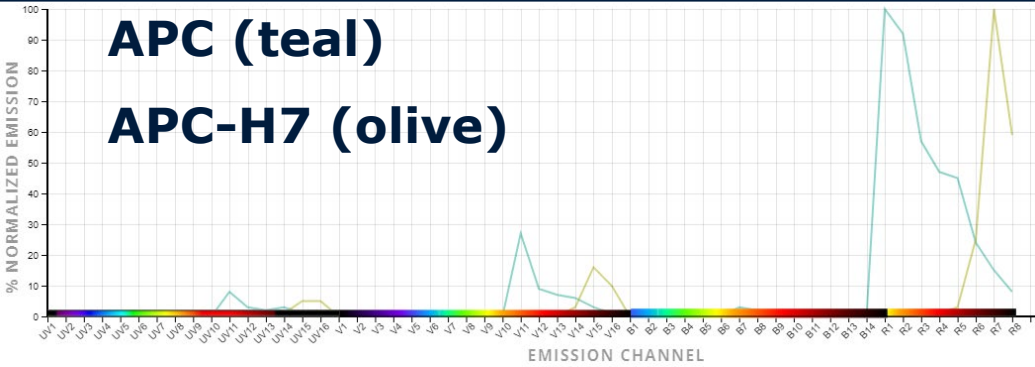
Outdated Abs

Positive counts and MFI

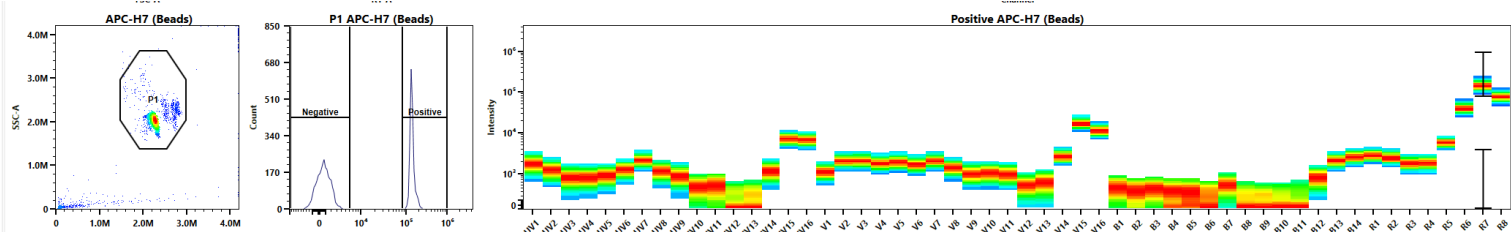
Stain	Expiry Date	Positive (count/%)	MFI
CD14/ APC-H7	Unstained	4 (0.08%)	0
	31-12-14	1820 (36.4%)	111803
	30-11-18	1491 (29.81%)	154178
	31-05-20	1898 (43.03%)	155627
CD33/ PerCP- Cy5.5	Unstained	0 (0%)	0
	30-11-18	1971 (39.44%)	20252
	30-11-19	1981 (39.64%)	14116
CD16/ PE-Cy7	Unstained	0 (0%)	0
	30-06-19	1976 (39.56%)	198114
	31-05-20	2042 (40.82%)	206266
	31-08-21	2044 (40.9%)	185708



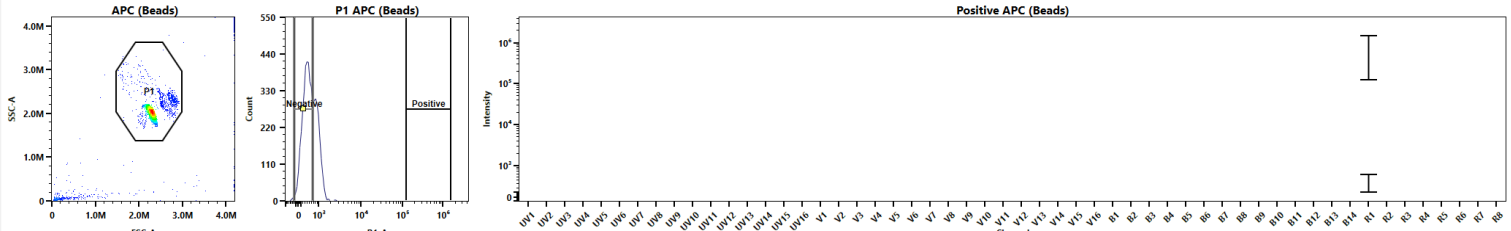
Outdated APC-H7



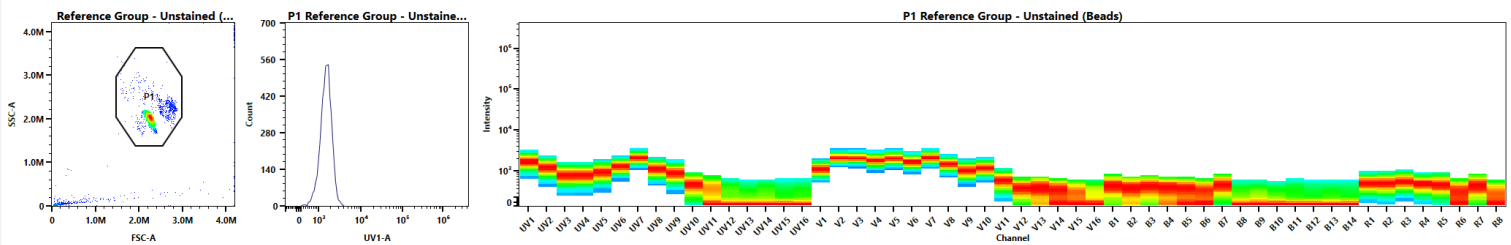
APC-H7



APC



AutoF



Antibody library

- For panel design
- For small scale experiments
- Buy antibodies (antibody sets) per sample/ μ l

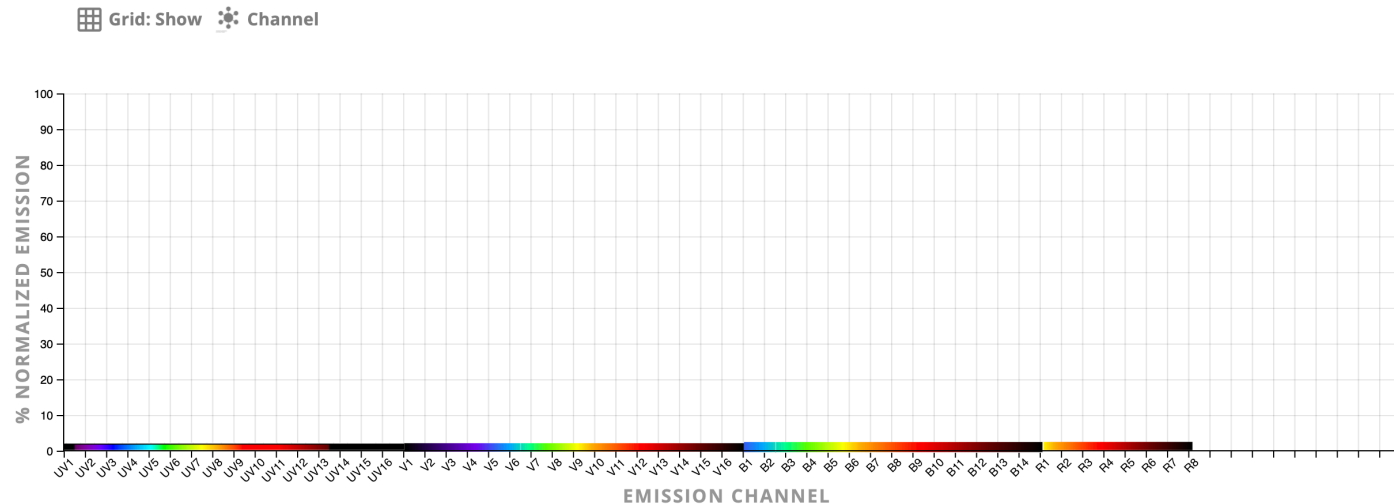
Species	Antigen	Fluorophore	Clone	Company	Catalog #	Price	Price/microl
Mouse	CD19	BUV395	145-2C11	BD	563557	261,09	1,04
Mouse	CD3	BUV496	1D3	BD	612955	268,72	1,07
Mouse	CD11c	BUV563	HL3	BD	749091	380	1,52

Antibody library: backbone panel

PBMCs

Uncommon labels to provide with room for your favorite marker(s) of interest

- CD3 (T)
- CD19 (B)
- CD56 (NK)
- CD66b (granulo)
- HLA-DR (CD74)
- CD16 (non-classical mono)
- CD14 (classical mono)
- CD4
- CD8
- Zombie (dead)



Configuration

4L 16UV-16V-14B-8R

Fluorochrome

APC

Search by peak channel

Double click Fluorochromes to add

APC

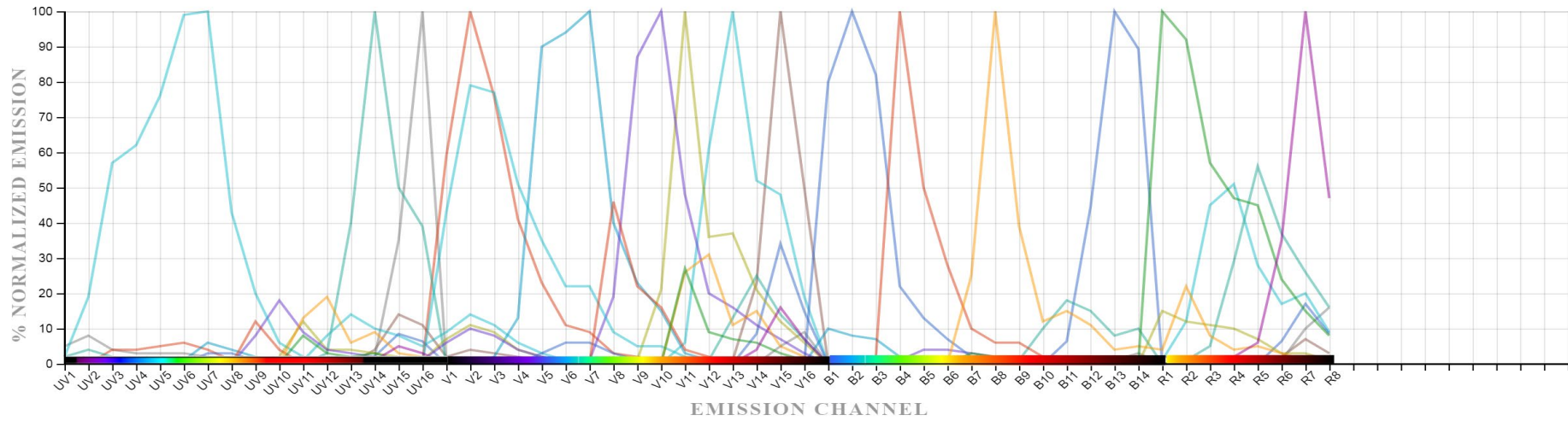
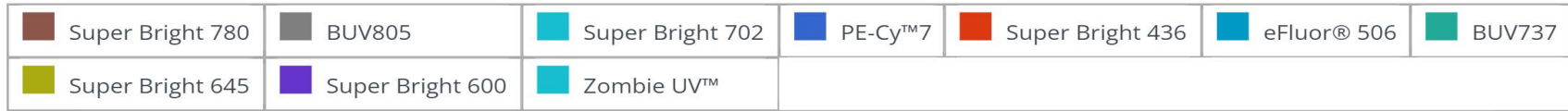
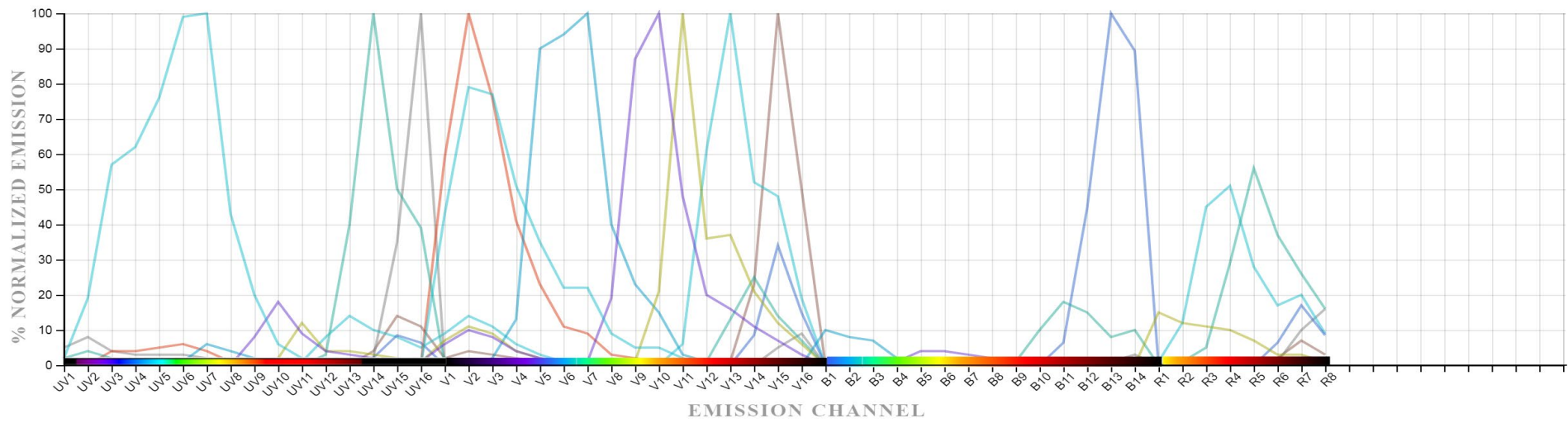
APC-Alexa Fluor® 700

APC-Alexa Fluor® 750

Tags

0 Tags Added

Drag a tag to rearrange the order



Adding common fluorophores to the backbone

Similarity™ Indices

Configuration: 4L 16UV-16V-14B-8R

Super Bright 780	1									
BUV805	0.21	1								
Super Bright 702	0.4	0.09	1							
PE-Cy7	0.3	0.12	0.12	1						
Super Bright 436	0.05	0	0.13	0	1					
eFluor 506	0.01	0	0.03	0	0.19	1				
BUV737	0.24	0.4	0.41	0.22	0	0	1			
Super Bright 645	0.16	0.03	0.52	0.04	0.14	0.06	0.15	1		
Super Bright 600	0.07	0.01	0.19	0.02	0.12	0.19	0.05	0.5	1	
Zombie UV	0.03	0.05	0.08	0	0.63	0.24	0.02	0.09	0.1	1
	Super Bright 780	BUV805	Super Bright 702	PE-Cy7	Super Bright 436	eFluor 506	BUV737	Super Bright 645	Super Bright 600	Zombie UV

Complexity™ Index: 3.54

Backbone alone

Similarity™ Indices

Configuration: 4L 16UV-16V-14B-8R

Super Bright 780	1																				
BUV805	0.21	1																			
Super Bright 702	0.4	0.09	1																		
PE-Cy7	0.3	0.12	0.12	1																	
Super Bright 436	0.05	0	0.13	0	1																
eFluor 506	0.01	0	0.03	0	0.19	1															
BUV737	0.24	0.4	0.41	0.22	0	0	1														
Super Bright 645	0.16	0.03	0.52	0.04	0.14	0.06	0.15	1													
Super Bright 600	0.07	0.01	0.19	0.02	0.12	0.19	0.05	0.5	1												
Zombie UV	0.03	0.05	0.08	0	0.63	0.24	0.02	0.09	0.1	1											
FITC	0	0	0	0	0.01	0.14	0	0	0.02	0.03	1										
PE	0	0	0.01	0.01	0.01	0.13	0	0.05	0.28	0.04	0.17	1									
PerCP	0.07	0.03	0.27	0.1	0.01	0.02	0.17	0.34	0.14	0	0.01	0.07	1								
APC	0.04	0.03	0.38	0.03	0	0	0.25	0.38	0.07	0	0	0.01	0.24	1							
APC-Cy7	0.22	0.19	0.24	0.19	0	0	0.33	0.07	0.01	0	0	0	0.04	0.19	1						
	Super Bright 780	BUV805	Super Bright 702	PE-Cy7	Super Bright 436	eFluor 506	BUV737	Super Bright 645	Super Bright 600	Zombie UV	FITC	PE	PerCP	APC	APC-Cy7						

Complexity™ Index: 3.7

Backbone + common fluorophores

Complexity index 3,54

3,7

Cytek Aurora: real world data – multiple markers

UV				Violet				Blue				Red			
D	C/BW (nm)	Fluor	Antigen	D	C/BW (nm)	Fluor	Antigen	D	C/BW (nm)	Fluor	Antigen	D	C/BW (nm)	Fluor	Antigen
UV1	372/15														
UV2	387/15	BUV395	CD45RA												
UV3	427/15			V1	428/15	BV421	CCR7								
UV4	443/15			V2	443/15	Saprk V423									
UV5	458/15	UV450	ZOMBIE UV	V3	458/15	PacBlue	CD16								
UV6	473/15			V4	473/15	BV480									
UV7	514/28	BUV496	CD4	V5	508/20			B1	508/20	BB515					
				V6	525/17	BV510	CD45RO	B2	525/17	FITC	CD57				
UV8	542/28			V7	542/17	Spark Vio 538		B3	542/17	Spark Blue550	CD14				
UV9	581/31	BUV563	CD27	V8	581/19	BV570		B4	581/19	PE	CD25				
				V9	598/20			B5	598/20						
UV10	612/31	BUV615		V10	615/20	BV605	CXCR4	B6	615/20	PE-Dazzle	CD29				
UV11	664/27	BUV661		V11	664/27	BV650	CD56	B7	660/17	PE/Fire640		R1	660/17	APC	CCR6
								B8	678/18	PE-Cy5	CXCR3	R2	678/18	AF647	
UV12	691/28			V12	692/28			B9	697/19	PerCP-Cy5.5	CD66b	R3	697/19	SparkNIR 685	CD19
UV13	720/29			V13	720/29	BV711	CD28	B10	717/20	PE/Fire700		R4	717/20	Alexa700	CD3
								B11	738/21			R5	738/21		
UV14	750/30	BUV737	CD62L	V14	750/30	BV750	CD11b	B12	760/23			R6	760/23	Zombie NIR	
														APC-Cy7/APC-Fire750	
UV15	780/30			V15	780/30	BV785	CD127	B13	783/23	PE-Cy7	CCR4	R7	783/23		CCR5
UV16	812/34	BUV805	CD8	V16	812/34			B14	812/34	PE/Fire 810	HLA-DR	R8	812/34	APC-Fire810	CD38

Currently: 25-marker panel

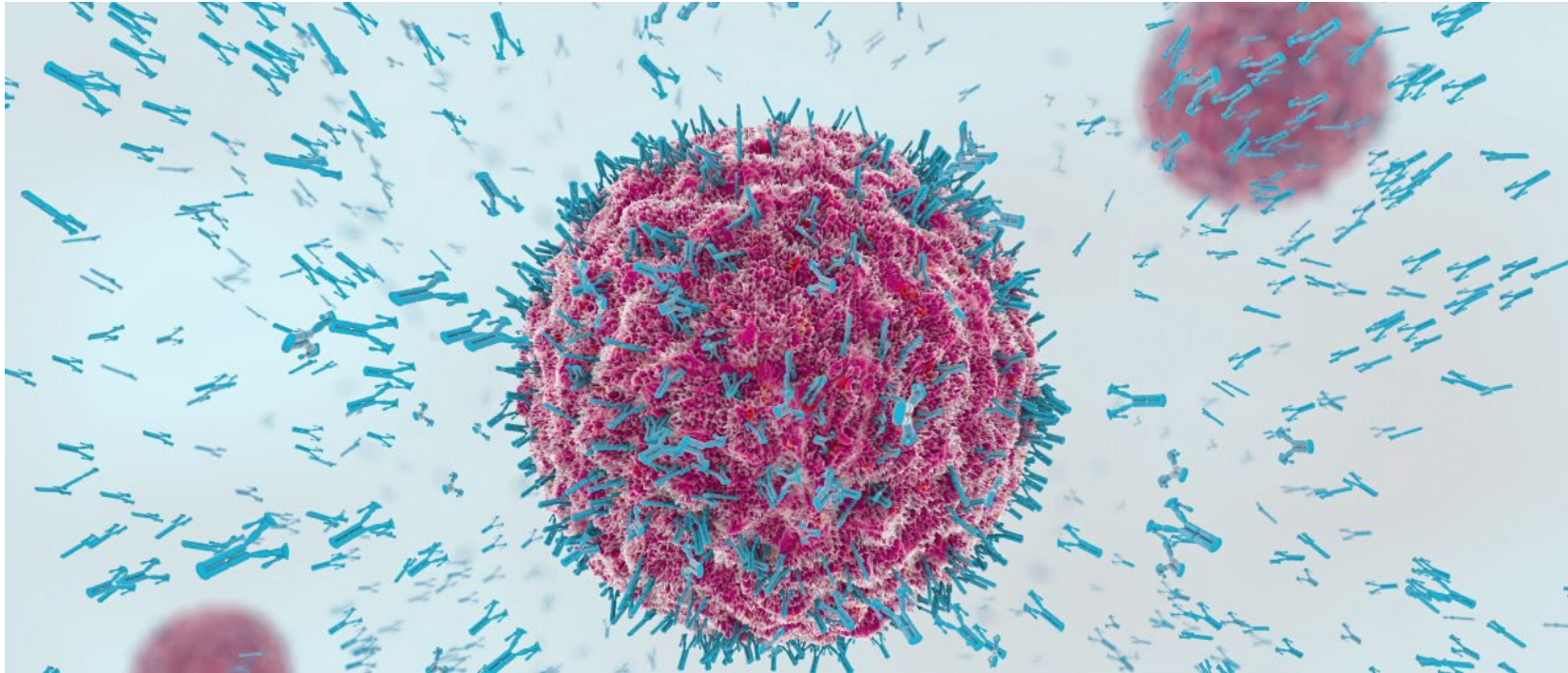
Hands-on session

Group schedule

12u30-13h15	Aurora Demo 1		Canto Demo 1	
13u15-14h00	Aurora Demo 2	Aurora analysis 1	Canto Demo 2	Canto Analysis 1
14h00-14h45	Aurora Demo 3	Aurora analysis 2	Canto Demo 3	Canto Analysis 2
14h45-15h00	BREAK	BREAK	BREAK	BREAK
15h00-15h45	Aurora Demo 4	Aurora analysis 3	Canto Demo 4	Canto Analysis 3
15h45-16h30	Aurora Demo 5	Aurora analysis 4	Canto Demo 5	Canto Analysis 4
16h30-17h15		Aurora analysis 5		Canto Analysis 5

	Room number
CANTO Demo	Hospital 5th floor 5.E1.018
CANTO Analysis	Int Med UNS50 room H5.301
Aurora Demo	Int Med UNS50 room H5.307
Aurora Analysis	Int Med UNS50 room H5.307

Questions?



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lieve.temmerman@mumc.nl
kristiaan.wouters@maastrichtuniversity.nl

www.maastrichtuniversity.nl/teaching-group-immunology