

## Local Instrumentation Guide Radboud Flowcytometry Technology Center

### Instrumentation Overview:

Location Laboratory Hematology (LH) M379 4.148 Route 475

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#### Beckman Coulter Gallios

1. 3 Lasers (405, 488, and 642 nm), all lasers are parallel ( time-delayed) on this instrument
2. 10 Fluorescent detectors
3. Fully digital >20 bit dynamic range in area
4. Kaluza 1.0
5. Carrousel 32 tubes
6. Characterization and QC of the instrument is performed using Flowcheck, Flowset and Duke (6-peak) Calibration beads on weekly basis.

Instrument Name		Instrument Description	
Laser (Excitation)	Detector Name	Emission Capture (nm)	Example Fluors
488-Blue	FSC		
488-Blue	SSC		
488-Blue	FL 1	525/40	FITC, Alexa488, GFP
488-Blue	FL 2	575/30	PE
488-Blue	FL 3	620/30	ECD, PI, PE-A610, PE-CF594
488-Blue	FL 4	675/20	PE-Cy5.5, 7AAD, PerCP-Cy5.5
488-Blue	FL 5	755LP	PE-Cy7, PE-Alexa750
405-Violet	FL 9	450/40	DAPI, V450, BV421, Sytox Blue, PBSE, efluor 450
405-Violet	FL 10	550/40	V500, BV510, KO
642-Red	FL 6	660/20	APC, Alexa647
642-Red	FL 7	725/20	APC-Alexa700, Alexa 700, DRAQ 7
642-Red	FL 8	755LP	APC-Cy7, APC-Alexa750, APC-H7, efluor 780
Brief instrument summary: Company: Beckman Coulter Model: Gallios 10-color Similar instruments: Navios Special considerations: Sensitive instrument			

Beckman Coulter FC500

1. 2 Lasers (488, 640 nm), lasers are co-linear on this instrument
2. 5 Fluorescent detectors
3. Electronics is analog
4. CXP
5. Differential pressure, carousel 32 tubes
6. Characterization and QC of the instrument is performed using Flowcheck, Flowset and Duke (6-peak) Calibration beads on weekly basis.

Instrument Name		Instrument Description		
Laser (Excitation)	Detector Name	Emission Capture (nm)	Example Fluors	
488-Blue	FSC			
488-Blue	SSC			
488-Blue	FL 1	525	FITC, Alexa488, GFP	
488-Blue	FL 2	575	PE	
488-Blue	FL 3	620 SP	ECD, PI, PE-A610, PECF594	
488-Blue, 640-Red	FL 4	675	APC, PE-Cy5.5, 7AAD, PerCP-Cy5.5	
488-Blue, 640-Red	FL 5	755LP	PE-Cy7, PE-Alexa750, APC-A750	
Brief instrument summary: Company: Beckman Coulter Model: FC500 Similar instruments: Special considerations: Red laser shutter is not standard open				

**Instrumentation Overview:**

Location Tumor Immunology, M850.05 Route 278

Contact; [Tjiske.Duiveman@radboudumc.nl](mailto:Tjiske.Duiveman@radboudumc.nl)

**Flow Cytometry Analysis Instruments**

**BD FACS Lyric**

1. 3 Lasers (405, 488, 640 nm), lasers are parallel on this instrument
2. 10 Fluorescent detectors
3. Electronics is digital
4. BD FACSuite software
5. Differential pressure
6. Characterization and QC of the instrument is performed using BD CS&T beads and BD FACSuite software on a daily basis.

Instrument Name		Instrument Description	
Laser (Excitation)	Detector Name	Emission Capture (nm)	Example Fluors
488-Blue	FSC		
488-Blue	SSC		
488-Blue	Blue E 527/32	511-543	FITC, Alexa488, GFP
488-Blue	Blue D 586/42	565-607	PE
488-Blue	Blue B 700/54	673-727	PE-Cy5, PerCP, PerCP-Cy5.5
488-Blue	Blue A 783/56	755-811	PE-Cy7, PE-Alexa750
405-Violet	Violet E 448/45	425-470	DAPI, V450, BV421
405-Violet	Violet D 528/45	505-550	V500, BV510
405-Violet	Violet C 606/36	588-624	BV605
633-Red	Red C 660/10	655-665	APC, Alexa647
633-Red	Red B 720/30	705-735	APC-R700, Alexa700
633-Red	Red A 783/56	755-811	APC-Cy7, APC-Alexa750, APC-H7
Brief instrument summary: Company: BD Biosciences Model: FACSLyric Similar instruments: Same layout as the FACSVerse and MACSQuant and two additional colors (1 on Violet and one on Red). Special considerations: The Lyric will be upgraded with 2 additional colors on Violet once these colors are IVD.			

Beckman Coulter CyAn

1. 3 Lasers (488, 561, and 643 nm), all lasers are parallel on this instrument
2. 9 Fluorescent detectors, in practice only 8 colors since PE is measured using 2 different detectors.
3. Electronics digital
4. Summit 4.3
5. Differential pressure
6. Characterization and QC of the instrument is performed using 6 peak beads.

Instrument Name		Instrument Description	
Laser (Excitation)	Detector Name	Emission Capture (nm)	Example Fluors
488-Blue	FSC		
488-Blue	SSC	488/10	
488-Blue	FL1	530/40	FITC, GFP, A488, CFSE
488-Blue	FL2	585/30	PE
488-Blue	FL3	613/20	ECD, PE-A610, Qdot605
488-Blue	FL4	680/30	PE-Cy5, PerCP, 7AAD, Qdot655
488-Blue	FL5	750LP	PE-Cy7, PE-A750
633-Red	FL8	665/20	APC, Alexa647
633-Red	FL9	730/45	Alexa700
633-Red	FL6	755LP	APC-Cy7, APC-H7, eFluor780
561-Yellow	FL7	613/20 (688/32)	PE, mCherry, dsRed After filter change: PE-Cy5, PE-Cy7, mPlum
Brief instrument summary: Company: Beckman Coulter Model: CyAn Similar instruments: Special considerations: Standard 405 nm laser has been replaced for a 561 nm laser. Therefore it's possible to measure FITC and PE without compensation.			

**BD FACS Verse**

1. 3 Lasers (405, 488, and 640 nm), all lasers are parallel on this instrument
2. 8 Fluorescent detectors
3. Fully digital >18 bit dynamic range in area with 32 bit floating point signal processing
4. BD FACSuite
5. Differential pressure
6. Characterization and QC of the instrument is performed using BD CS&T beads and BD FACSuite software on a daily basis.

Instrument Name		Instrument Description		
Laser (Excitation)	Detector Name	Emission Capture (nm)	Example Fluors	
488-Blue	FSC			
488-Blue	SSC			
488-Blue	Blue E 527/32	511-543	FITC, Alexa488, GFP	
488-Blue	Blue D 586/42	565-607	PE	
488-Blue	Blue B 700/54	673-727	PE-Cy5, PerCP, PerCP-Cy5.5	
488-Blue	Blue A 783/56	755-811	PE-Cy7, PE-Alexa750	
405-Violet	Violet B 448/45	425-470	DAPI, V450, BV421	
405-Violet	Violet A 528/45	505-550	V500, BV510	
633-Red	Red B 660/10	655-665	APC, Alexa647	
633-Red	Red A 783-56	755-811	APC-Cy7, APC-Alexa750, APC-H7	
Brief instrument summary: Company: BD Biosciences Model: FACSVerser Similar instruments: Same layout as the MACSQuant and on both machines it's possible to use the plate reader Special considerations:				

**Milteni MACSQuant**

1. 3 Lasers (405, 488, and 640 nm), all lasers are parallel on this instrument
2. 8 Fluorescent detectors
3. Fully digital >18 bit dynamic range in area with 32 bit floating point signal processing
4. MACSQuantify software 2.6
5. Syringe driven sample injection
6. Characterization and QC of the instrument is performed using MACSQuant Calibration beads and MACSQuantify software on a daily basis.

Instrument Name		Instrument Description		
Laser (Excitation)	Detector Name	Emission Capture (nm)	Example Fluors	
488-Blue	FSC			
488-Blue	SSC			
488-Blue	B1	525/50	FITC, Alexa488, GFP	
488-Blue	B2	585/40	PE	
488-Blue	B3	655-730	PE-Cy5, PerCP, PerCP-Cy5.5	
488-Blue	B4	750LP	PE-Cy7, PE-Alexa750	
405-Violet	V1	450/50	DAPI, V450, BV421	
405-Violet	V2	525/50	V500, BV510	
633-Red	R1	655-730	APC, Alexa647	
633-Red	R2	750LP	APC-Cy7, APC-Alexa750, APC-H7	
Brief instrument summary: Company: Miltenyi Biotec Model: MACSQuant analyzer 10 Similar instruments: Same layout as the FACSVerse and on both machines it's possible to use a plate reader. Special considerations: Possible to use for cell counting without the need for Truecount tubes.				