

Amnis[®] ImageStream[®]X Mk II

Microscopy in Flow

08.11.2022

Claudia Müller

Inflammation and Tumormodel

Amnis® ImageStream® X Mk II

Microscopy in Flow



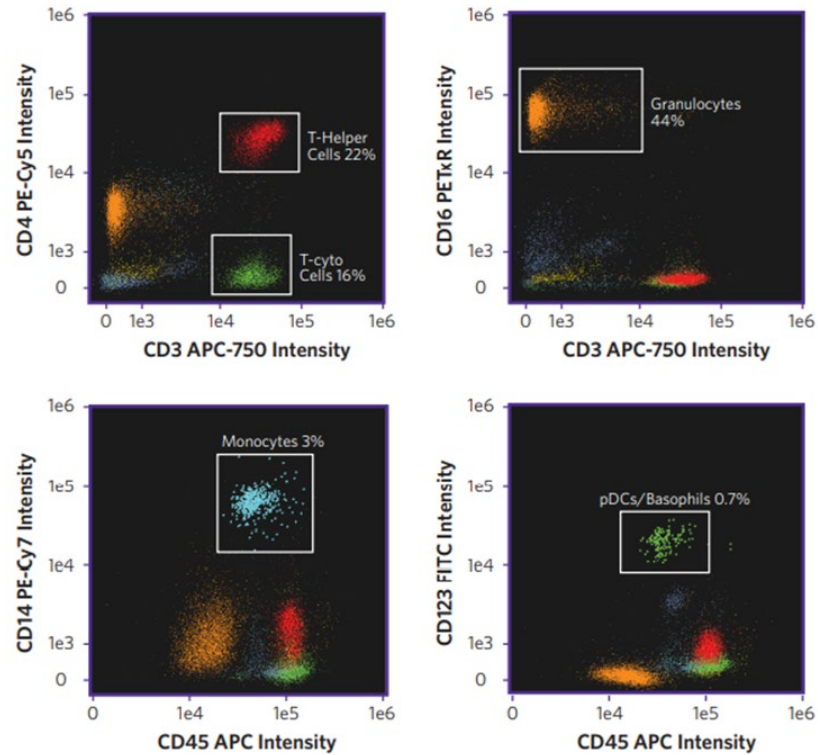
- First generation of imaging flow cytometry was introduced in 2005
- Bridge the gap between flow cytometry and microscopy

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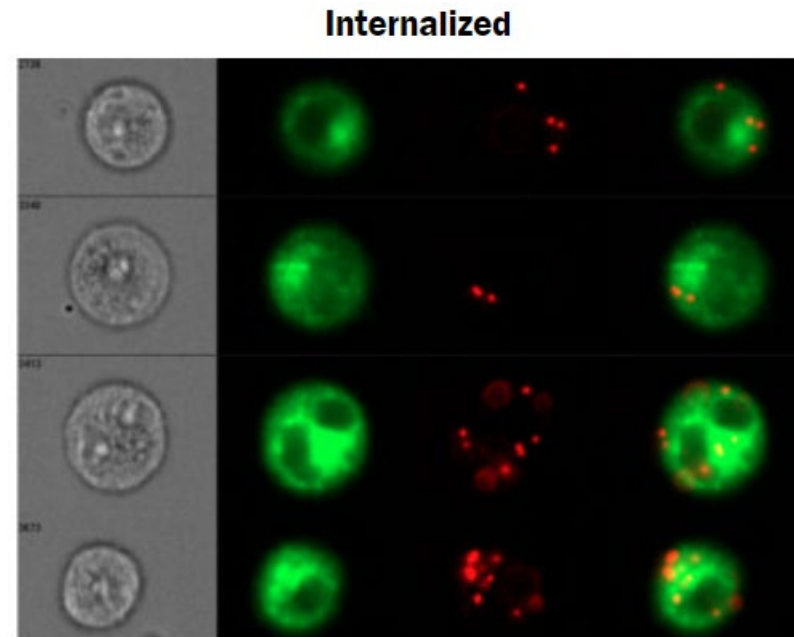
Microscopy in Flow

Combination of flow cytometry and microscopy

flow cytometry plots



morphology of the cells and localization of the fluorescence signal (surface vs intracellular)



[Instrumentation \(unimelb.edu.au\)](http://Instrumentation.unimelb.edu.au)

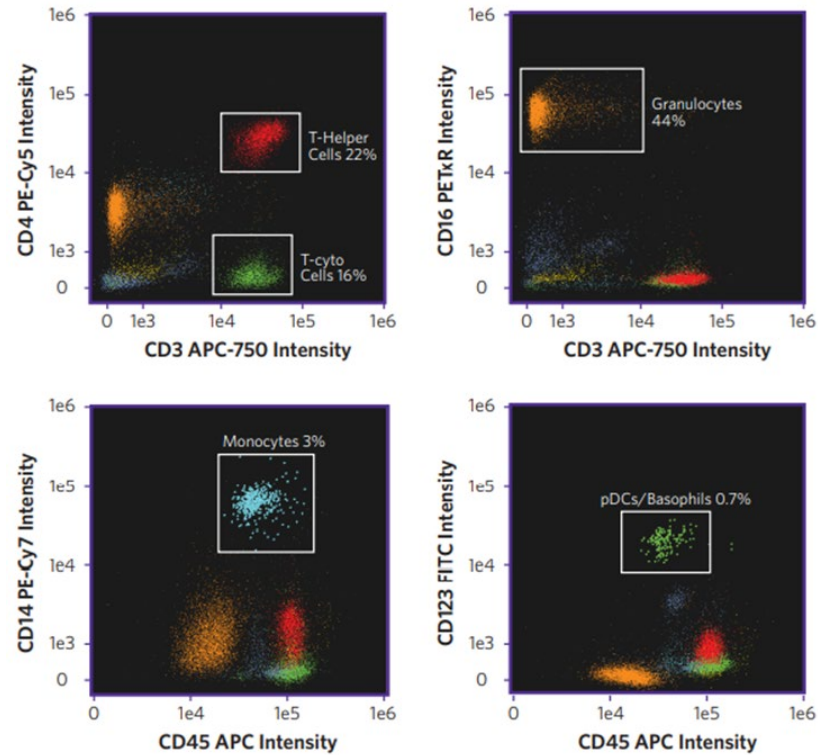
<https://www.luminexcorp.com/eu/amnis-imagestream-imaging-flow-cytometer/>

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Microscopy in Flow

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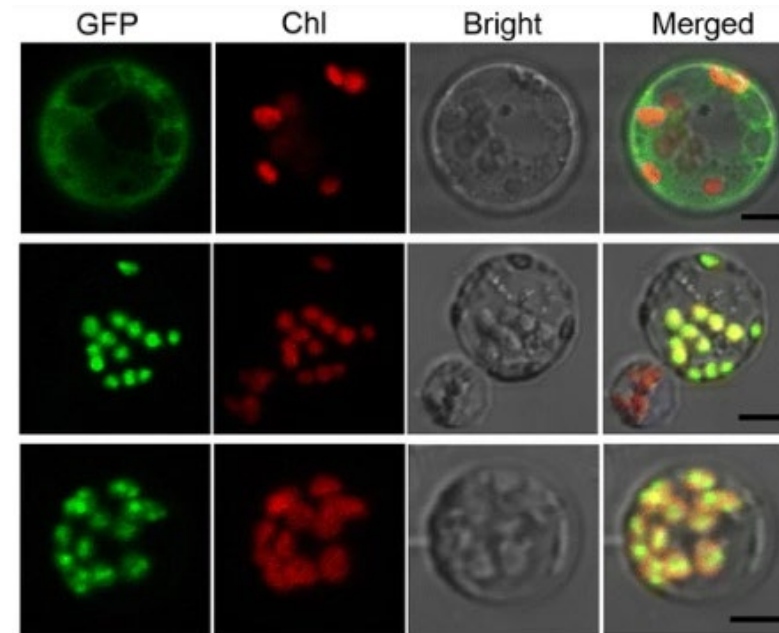


Figure 5 | A Point Mutation of Magnesium Chelatase OsCHL1 Gene Dampens the Interaction Between CHL1 and CHLD Subunits in Rice | SpringerLink

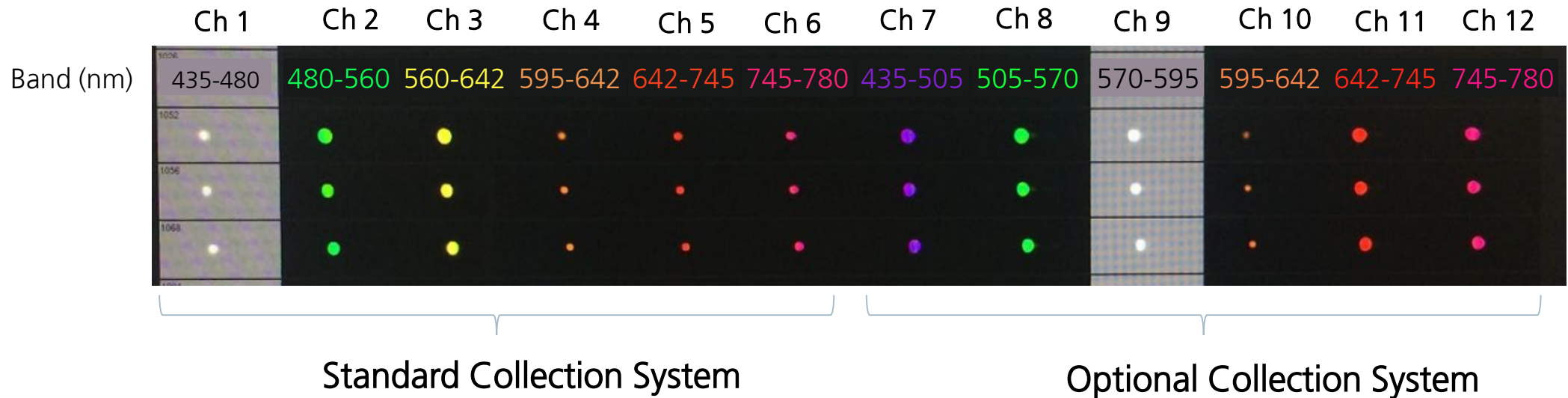
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Microscopy in Flow



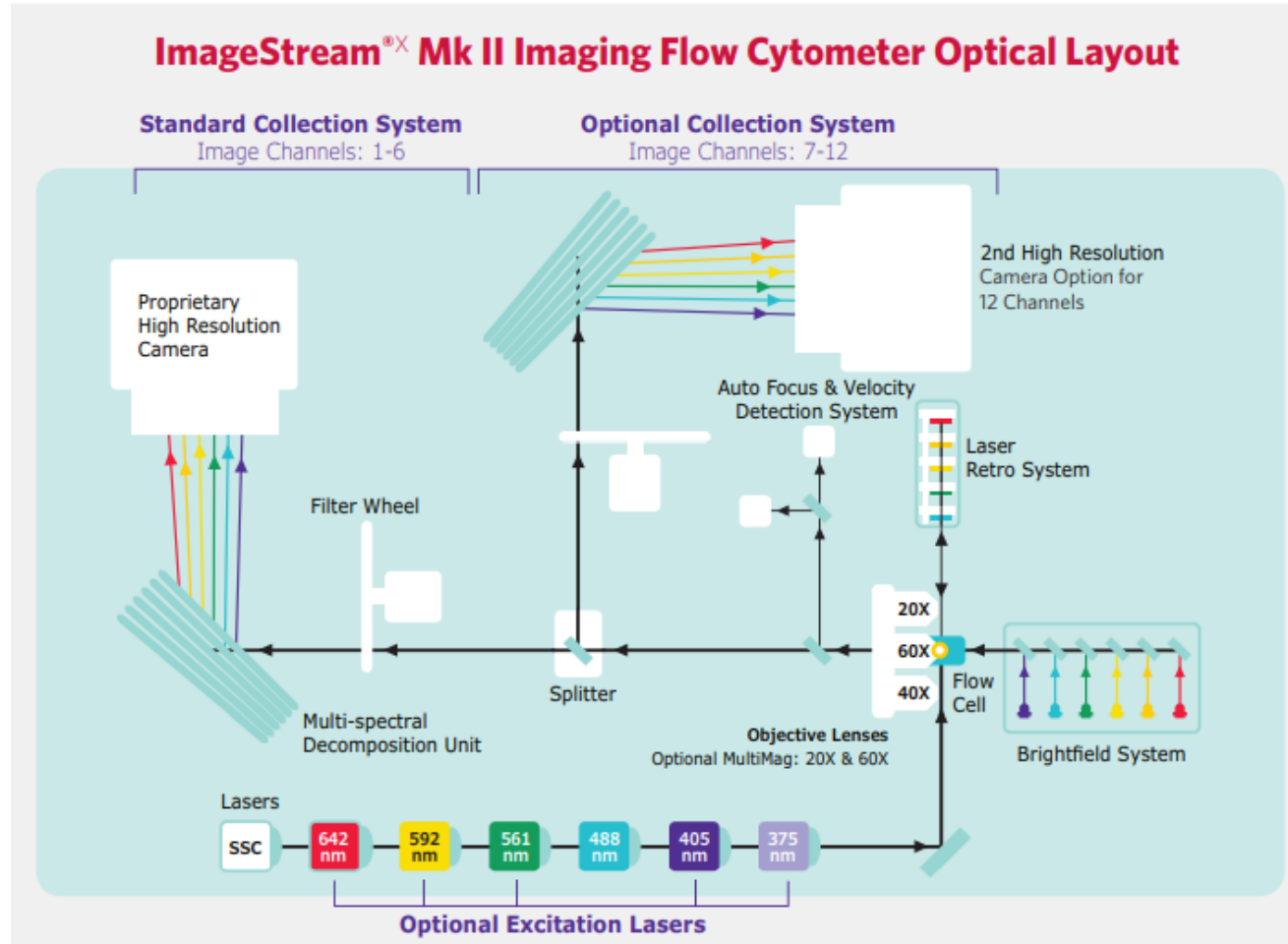
- 3 Laser: 405 nm | 488 nm | 648 nm
- 12 channels; Brightfield and SSC channel



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Microscopy in Flow



- Two different CCD cameras
- Arrangement of the cameras makes it possible to measure up to 12 images

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Lasers and Example Dyes

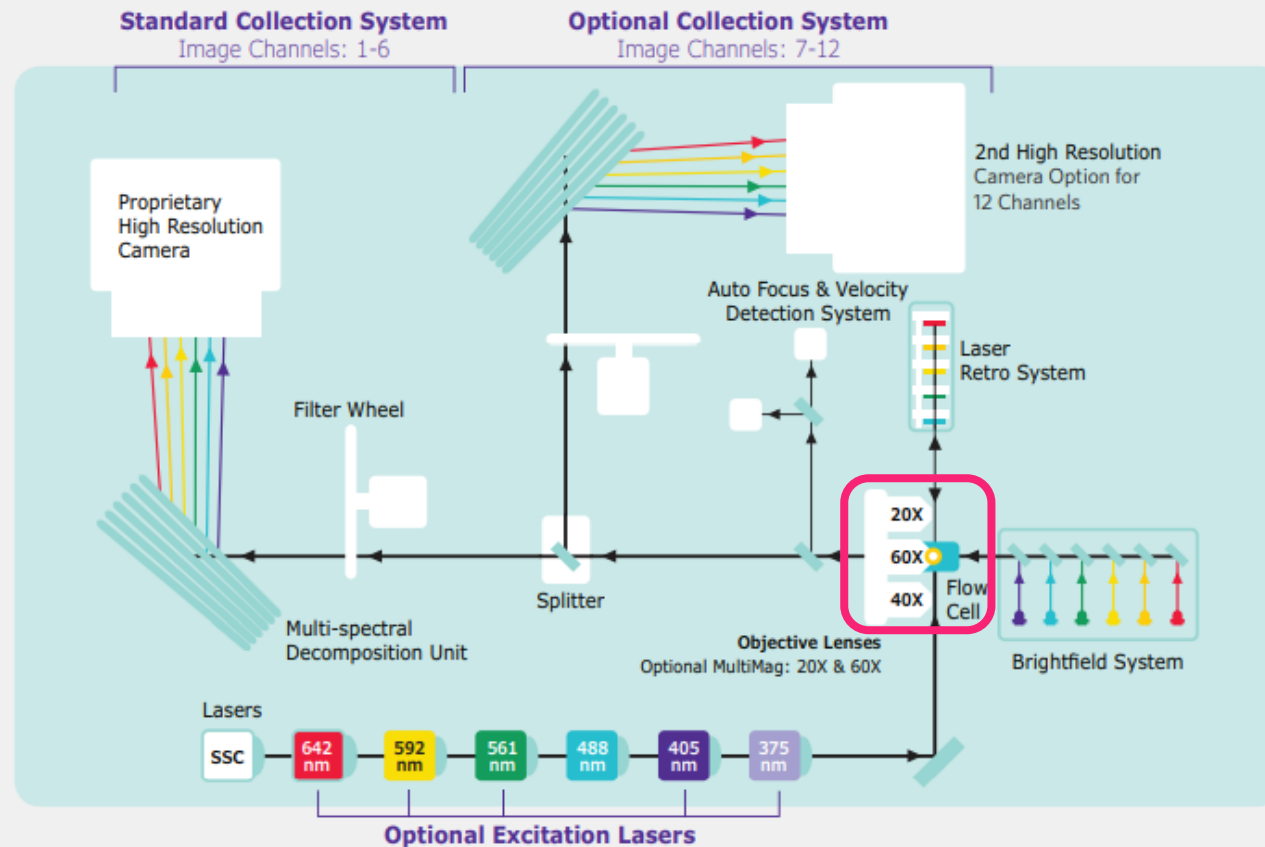
		Excitation Laser (nm)						
Ch	Band (nm)	375	405	488	561	642	785	
Camera 1	1	435-480 (457/45)	Ch1/Ch9 BF or *DAPI, *Hoechst, Zombie-Aqua, BV421, AF350, PB	Brightfield (BF)				
	2	480-560 (528/65)	*BV510, Pac Orange, QD525		FITC, AF488, DyLight488, GFP, YFP, Syto13			
	3	560-595 (577/35)	eFluor565, *QD565, QD585		PE, Cy3, DsRed	PE, AF546, DsRed, tdTomato, Cy3		
	4	595-642 (610/30)	Ch4/Ch10 BF or BV605, eFluor625, *QD625		Ch4/Ch10 BF or *PE-TexRed, *PI, RFP, *eFluor625	Ch4/Ch10 BF or *AF568, *DyLight594, *PE-TexRed, RFP, mCherry		
	5	642-745 (702/85)	BV711, eFluor700, *QD705		*PerCP-Cy5.5, *PE-Cy5, *PE-AF647, *7AAD, *Draq5	*PE-Cy5, *PE-AF647, *7AAD,		
	6	745-780 (762/35)	BV786, *QD800		*PE-Cy7, *PE-AF750	*PE-Cy7, *PE-AF750	SSC	
Camera 2	7	435-505 (457/45)	*DAPI, BV421, AF405, PB, DyLight405, CFP, LiveDead-violet					
	8	505-570 (537/65)		*BV510, AF430, QD525, PacOrange				
	9	570-595 (582/25)	Ch1/Ch9 BF or *QD565, QD585, eFluor570	Brightfield (BF)				
	10	595-642 (610/30)	Ch4/Ch10 BF or BV605, eFluor625, QD625					
	11	642-745 (702/85)	BV711, eFluor700, *QD705	AF647, APC, Cy5, DyLight649, *Draq5, *PE-AF647, *PE-Cy5, *PerCP-Cy5.5				
	12	745-780 (762/35)	BV786, *QD800	APC-Cy7, APC-AF750, APC-H7, PE-Cy7, eFluor750, DyLight750				SSC

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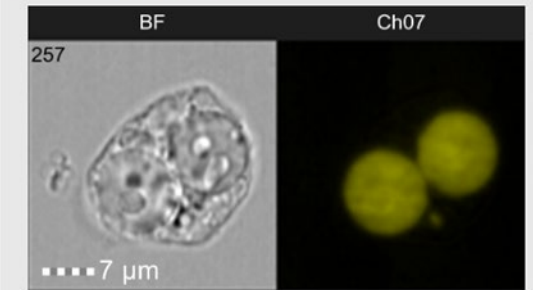
Microscopy in Flow

ImageStream® X Mk II Imaging Flow Cytometer Optical Layout

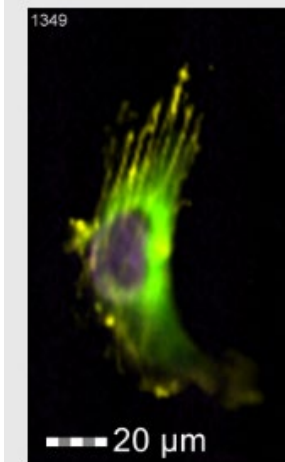


The Right Magnification for Your Work

60X magnification for higher resolution



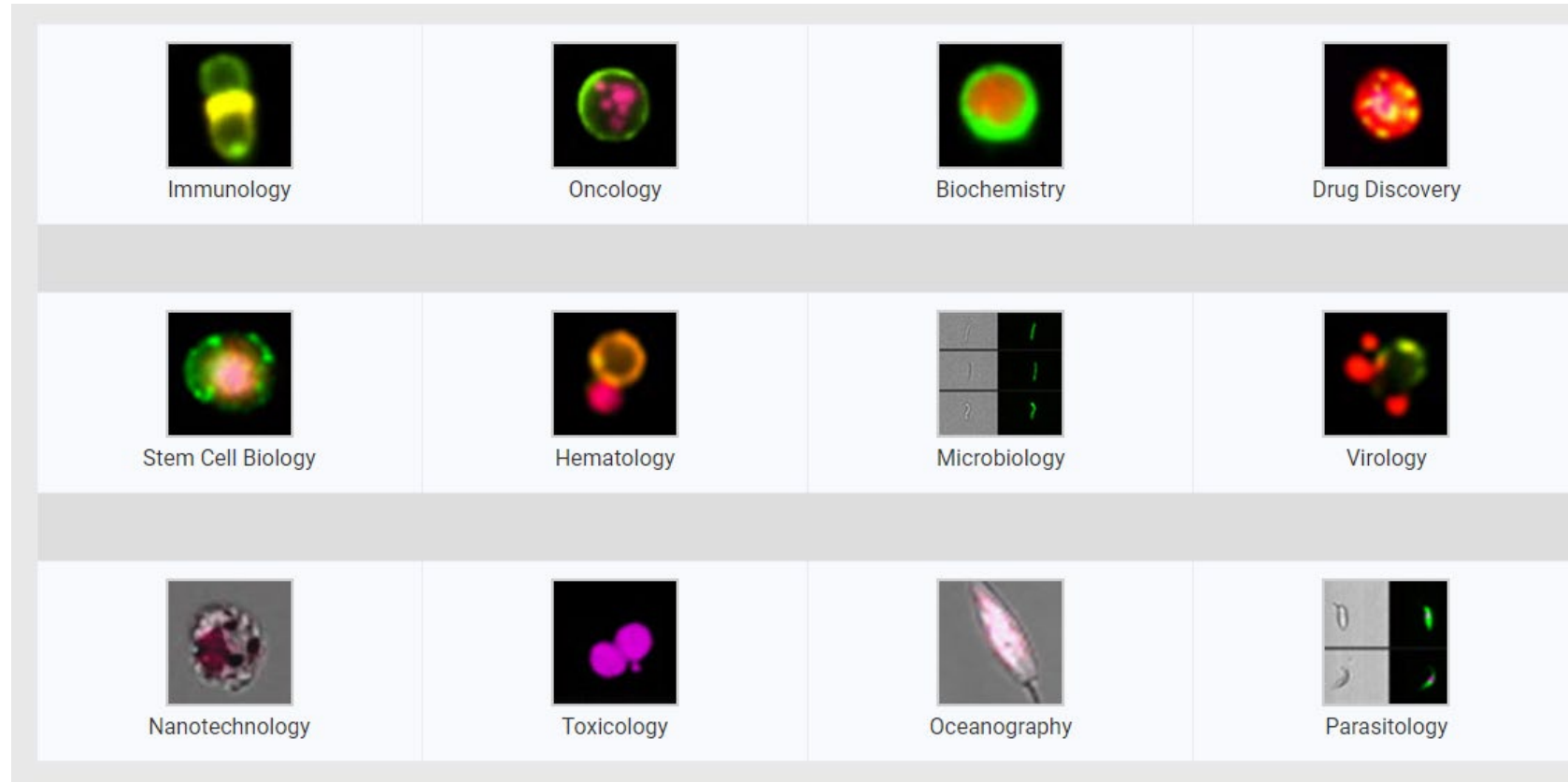
20X magnification for large cells



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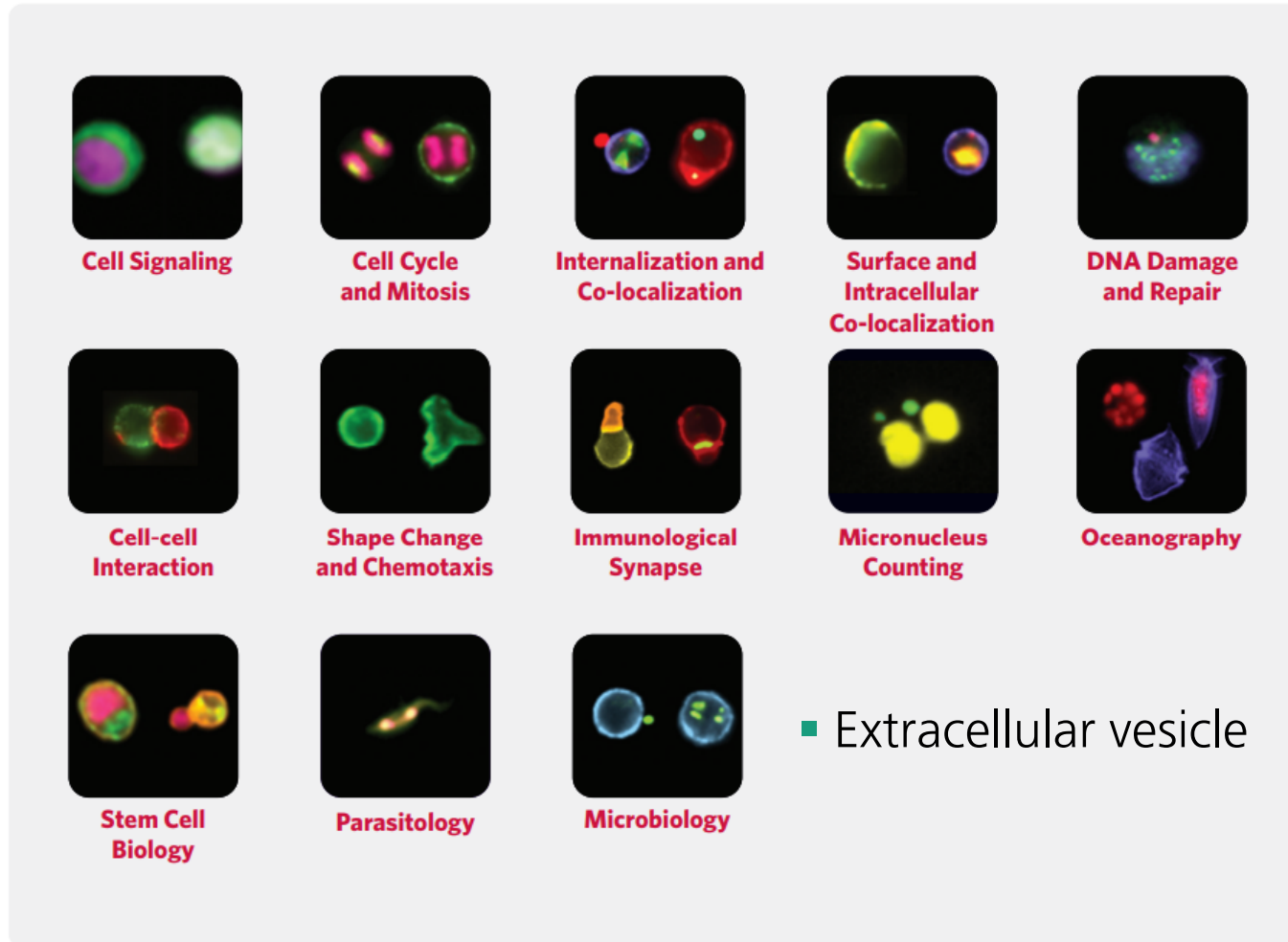
Research Areas



<https://www.luminexcorp.com/eu/amnis-imagestream-imaging-flow-cytometer/>

Amnis® ImageStream® X Mk II

Research Areas



Cell Signaling

Cell Cycle and Mitosis

Internalization and Co-localization

Surface and Intracellular Co-localization

DNA Damage and Repair

Cell-cell Interaction

Shape Change and Chemotaxis

Immunological Synapse

Micronucleus Counting

Oceanography

Stem Cell Biology

Parasitology

Microbiology

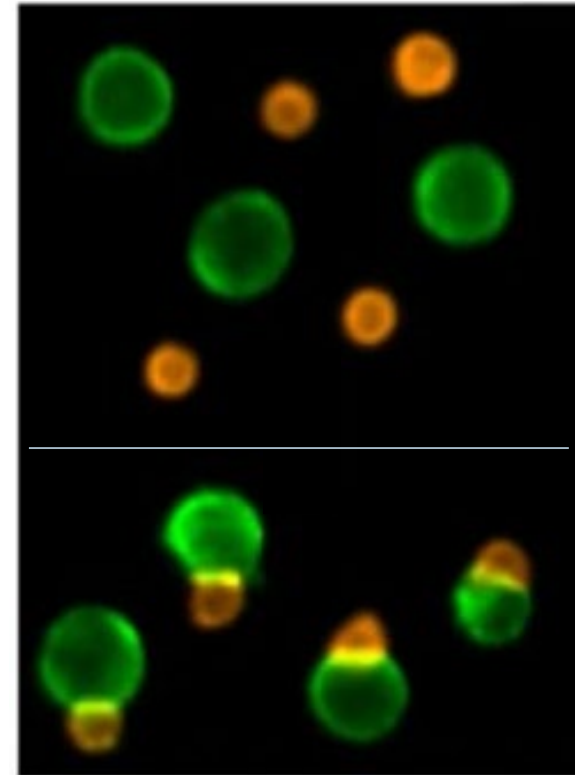
- Extracellular vesicle

<https://www.luminexcorp.com/eu/amnis-imagestream-imaging-flow-cytometer/>

Immunological Synapse

- Activated Antigen Presenting Cells (APCs):
- T cells (isolated from human blood)
 - Mixed APCs and T cells 1:1 for 45 min
 - Fixed and permeabilized cells
 - Labeld T cells and APCs

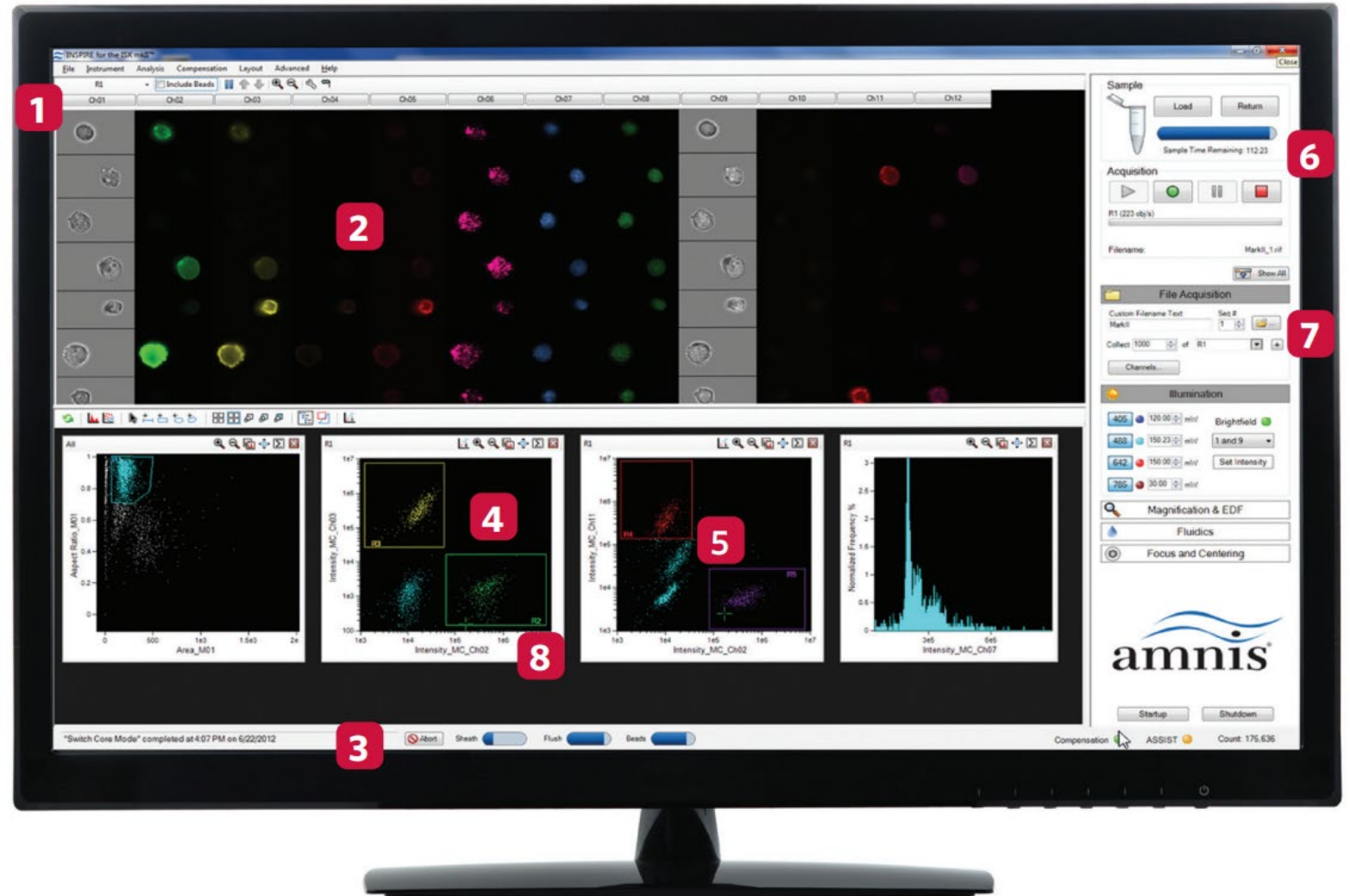
Co-culture experiment → e.g. Tumor cells and CARs



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Microscopy in Flow

INSPIRE™



1 → Population viewer

2 → Image Gallery

3 → Instrument Status

4 **5** → Flow cytometry plots/histograms

6 → Efficient Sample Handling

7 → Acquisition

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Microscopy in Flow

IDEAS® Software

- 1 → Population viewer
- 2 → Image for every dot
- 3 → Graphical Population
- 4 → Statistics
- 5 → Flexible image display
- 6 → Graph what you see



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Microscopy in Flow



- it is possible to image populations of rare cells at frequencies at or below 1:100,000
- 20 – 200 μl volume with a cell concentration of 1×10^7 cells per ml

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Microscopy in Flow



Our device is very sad at the moment
because only few people play with it!

If you are interested to play with the device,
contact me !

Thank you for your interest!

Claudia Müller

Entzündungs- und Tumormodelle

Abteilung Präklinische Entwicklung und Validierung

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