



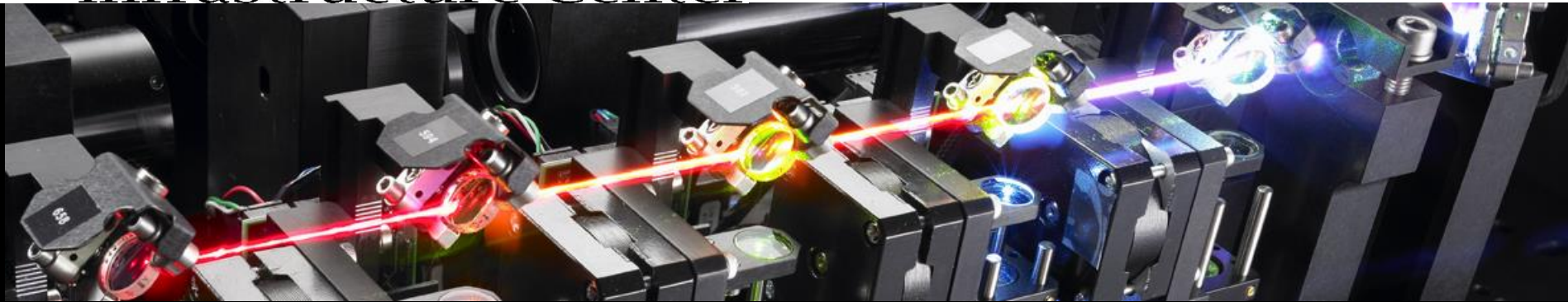
LS&E

Infrastructure Center



הטכניון
מכון טכנולוגי
לישראל

Technion
Israel Institute
of Technology



Imaging Flow Cytometry (IFC)

Dr. Aviv Lutaty

Head of Flow Cytometry Unit

e-mail: Avivlutaty@Technion.ac.il

Tel: 04-8293676

LS&E site: <https://lse.technion.ac.il/>



Imaging Flow Cytometry - Outline

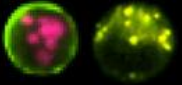
➤ How it works

➤ Applications:

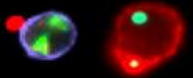
- Cell signaling



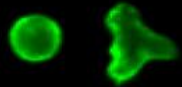
- Apoptosis



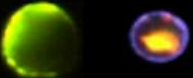
- Internalization



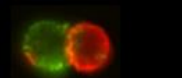
- Shape change



- Co-localization



- Cell-cell interactions



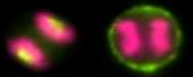
- Microbiology



- Oceanography



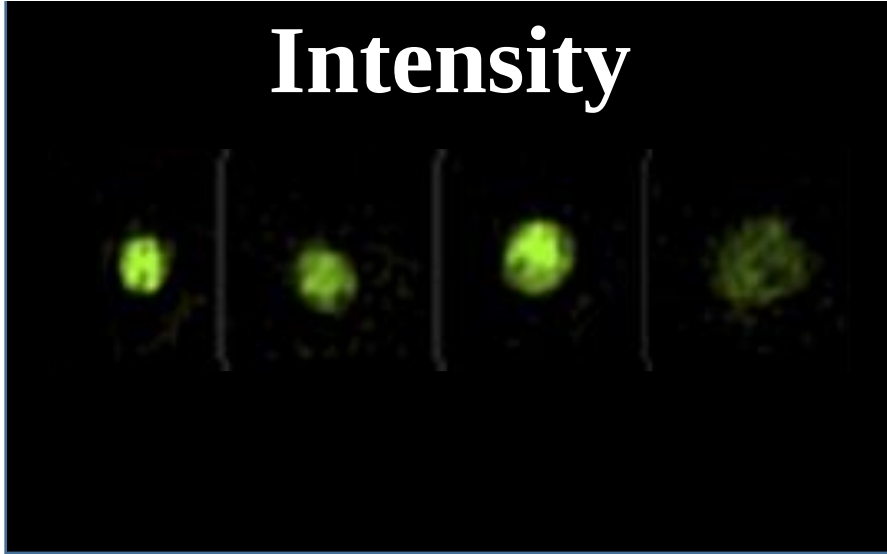
- Cell cycle



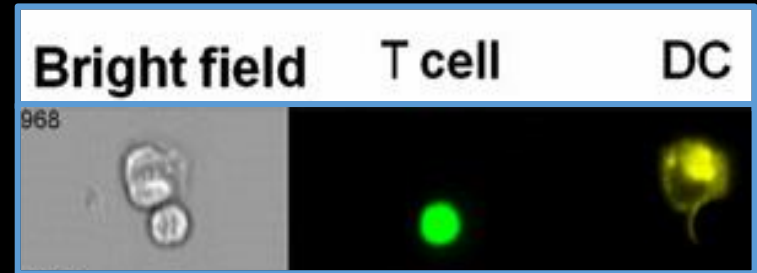
➤ Planning your experiment

Imaging Flow Cytometry

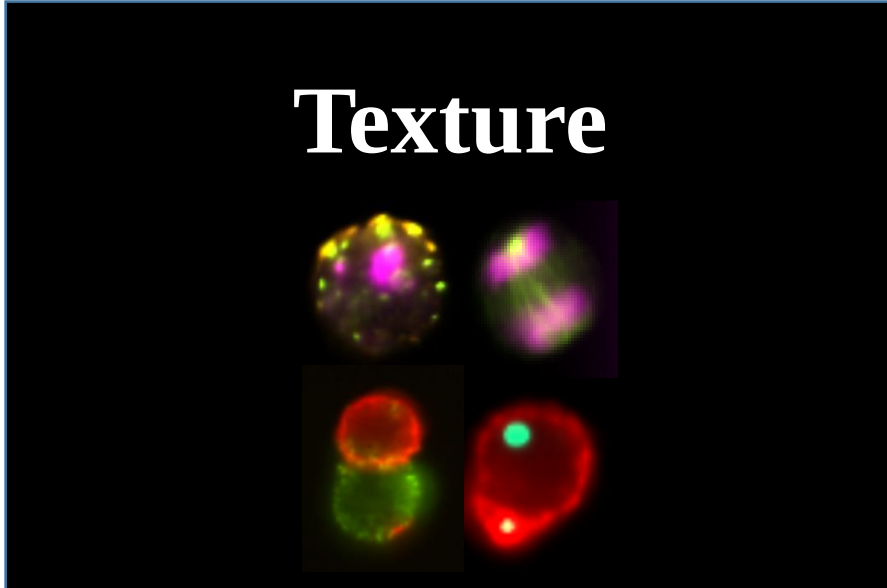
Intensity



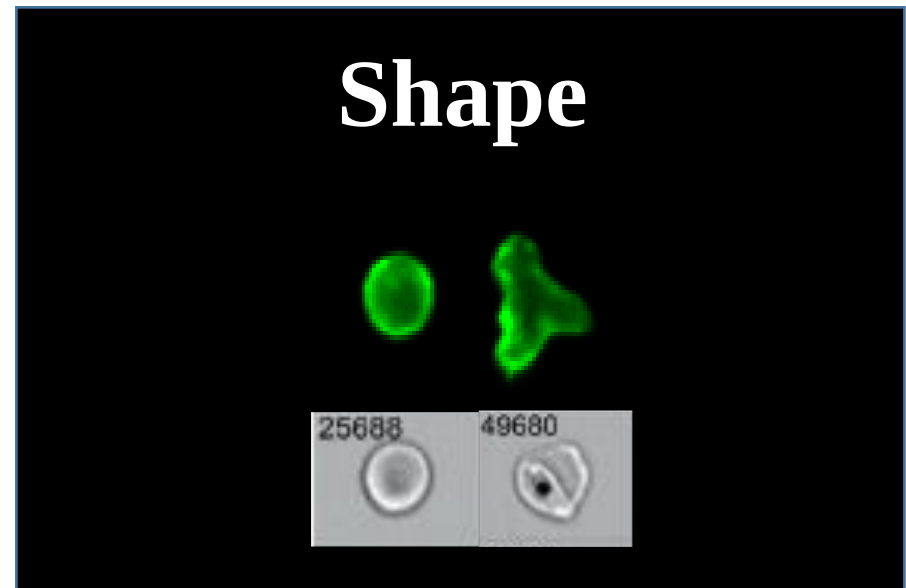
Size



Texture



Shape



ImageStream X mark II – Amnis®

Lasers:

405, 488, 561, 642

6 Channels:

435-505 nm

505-560 nm

560-595 nm

595-642 nm

642-745 nm

745-780 nm

Operation formats:

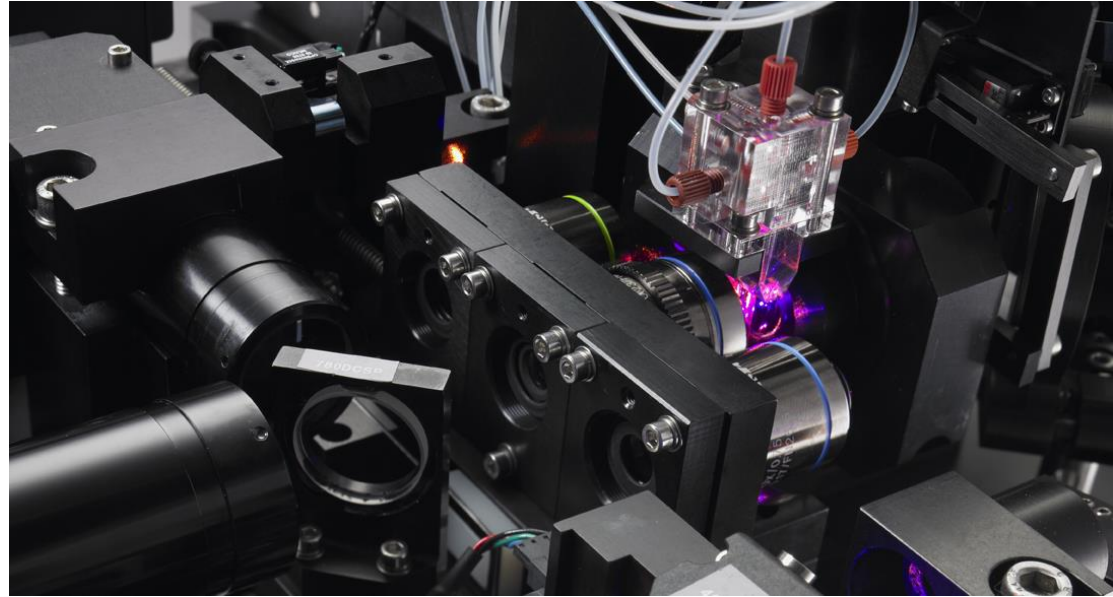
Eppendorf tubes

Automated - 96 well plates

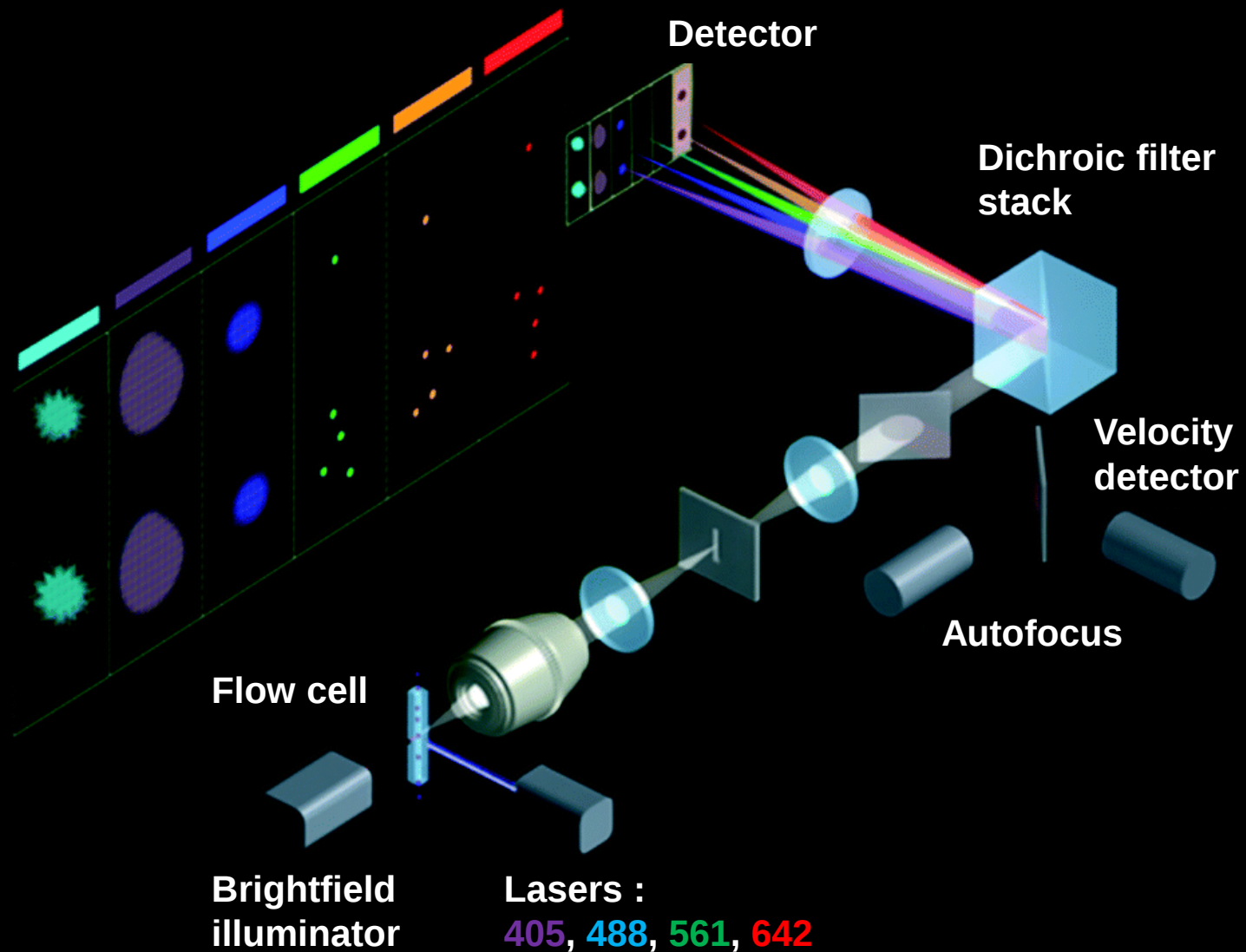


ImageStream X mark II – Amnis®

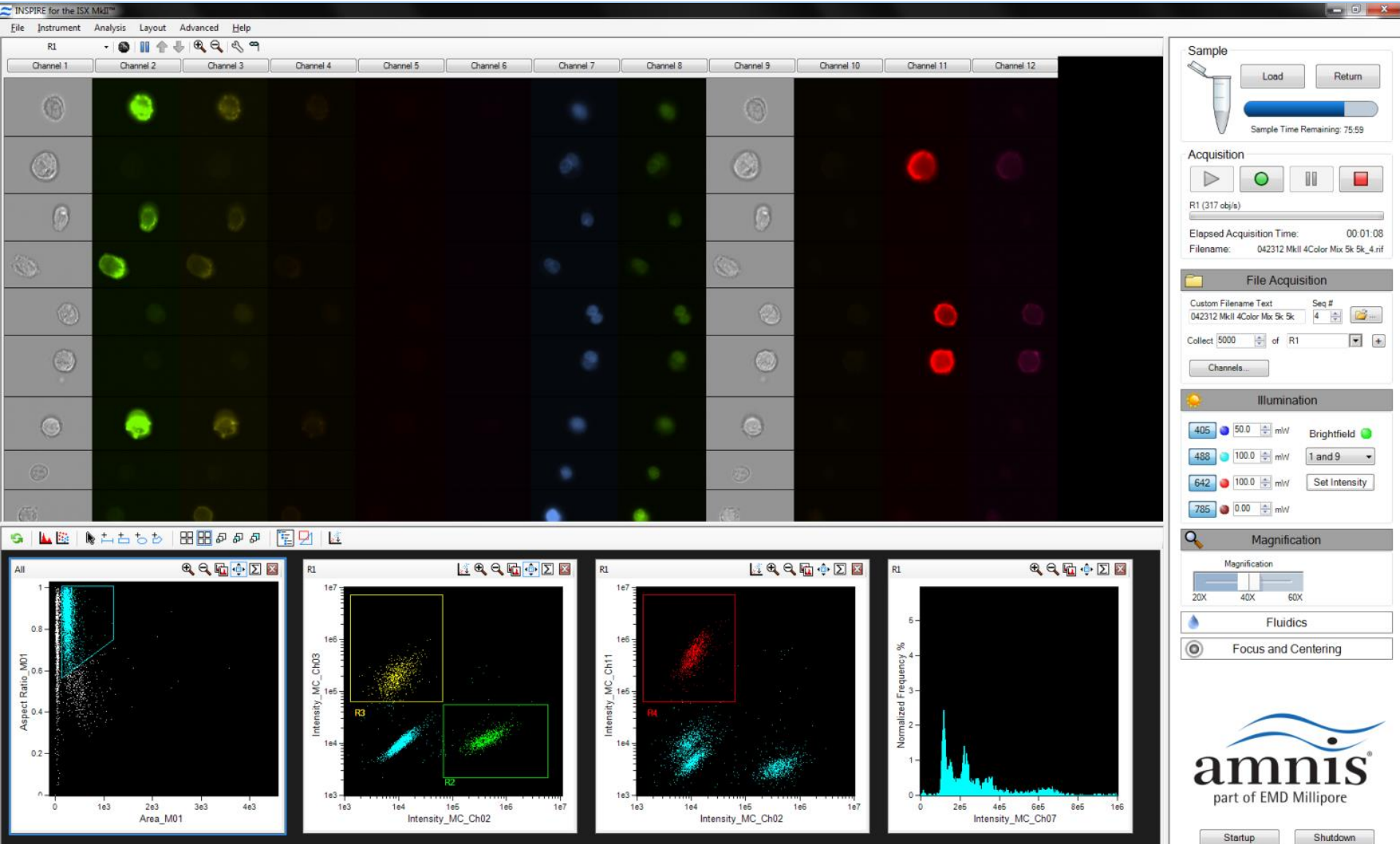
- **40x objective;**
60um field of view
0.5um per pixel
4um depth of field
0.75 NA.
- **20x objective;**
120um field of view
1um per pixel
8um depth of field
0.75NA
- **60x objective;**
40um field of view
0.33um per pixel
2.5um depth of field
0.9NA.



ImageStream – Operation layout

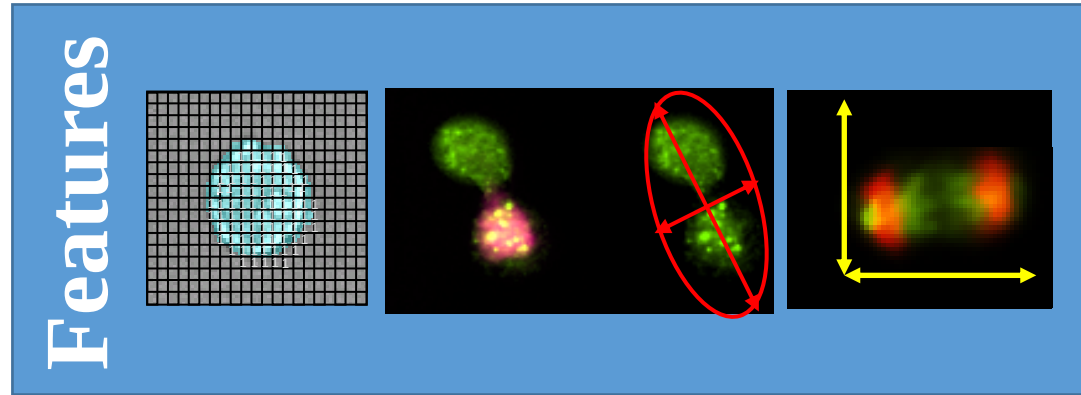


INSPIRE: Instrument Controller



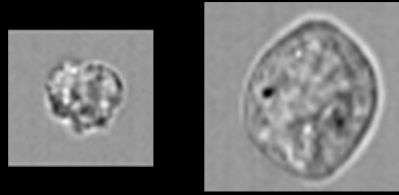
IDEAS® Analysis Software

- High content **morphometric analysis** of tens of thousands of images
- “**Automated feature finder**” quickly finds the best feature for analysis
- **User defined features** for advanced image-based discrimination of cells
- **Application wizards** for validated protocols



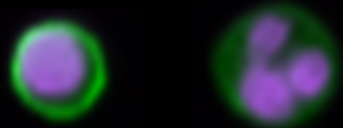
Feature Categories

Size features



Area; Diameter; Major Axis; Minor Axis; Major Axis Intensity; Minor Axis Intensity; Perimeter; Thickness Max and Min; Spot Area Min; Width; Height; Length

Shape features



Aspect Ratio; Aspect Ratio Intensity; Circularity; Compactness; Elongatedness; Lobe Count; Shape Ratio; Symmetry 2,3,4

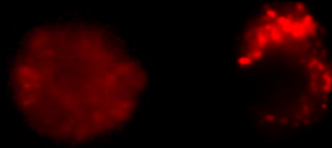
Location features



Angle; Angle Intensity; Centroid X; Centroid Y; Centroid X/Y Intensity; Δ Centroid X; Δ Centroid Y; Δ Centroid XY; Max Contour position; Spot Distance Min; Valley X and Valley Y

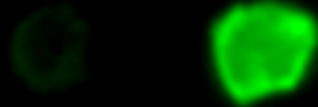
Feature Categories

Texture features



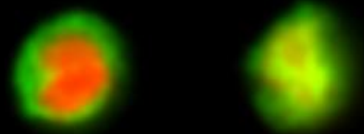
Bright R3; Bright Detail Intensity R7; Contrast; Gradient Max; Gradient RMS; Modulation; Spot Count; StDetail Intensity d thDev; and e Haralick (H) texture features H-Contrast; H-Correlation; H-Energy; H-Entropy; H-Homogeneity; and H-Variance

Signal Strength



Bkgd Mean; Bkgd StdDev; Intensity; Raw Intensity; Raw Max Pixel; Raw Min Pixel; Raw Mean Pixel; Raw Median Pixel; Max Pixel; Min Pixel; Mean Pixel; Median Pixel; Saturation Count; Saturation Percent; Spot Intensity Min

Comparison features



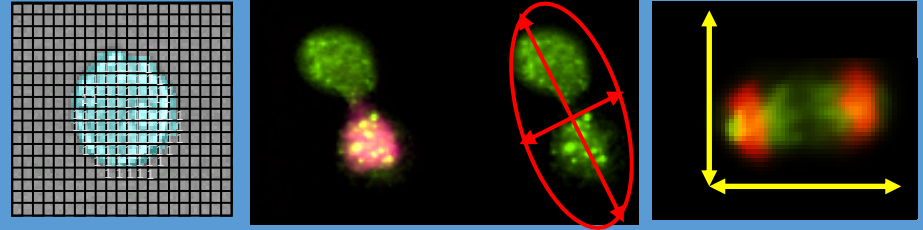
Intensity Concentration Ratio; Internalization; Similarity; Bright Detail Similarity R3

Combined features

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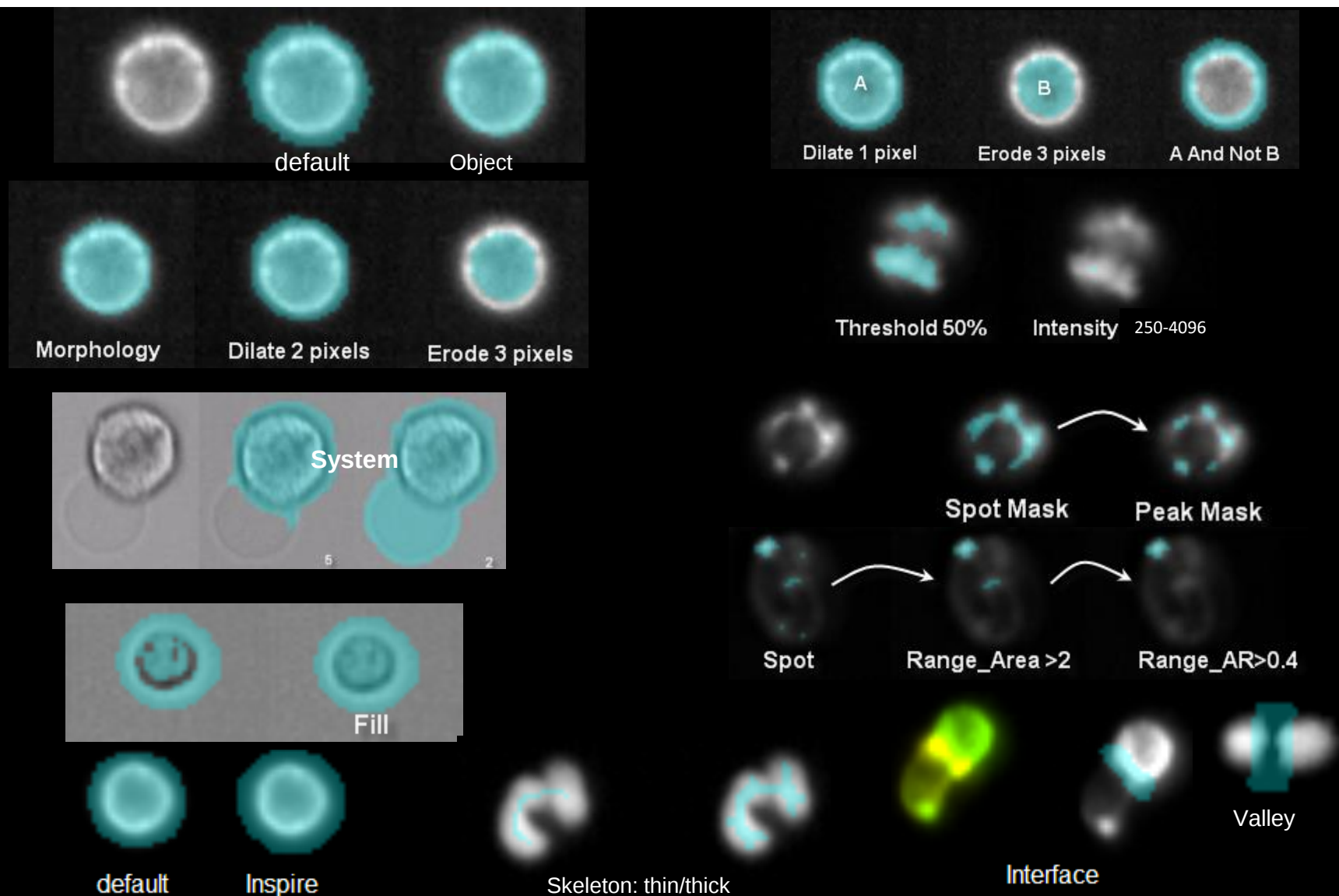
Features



Masks



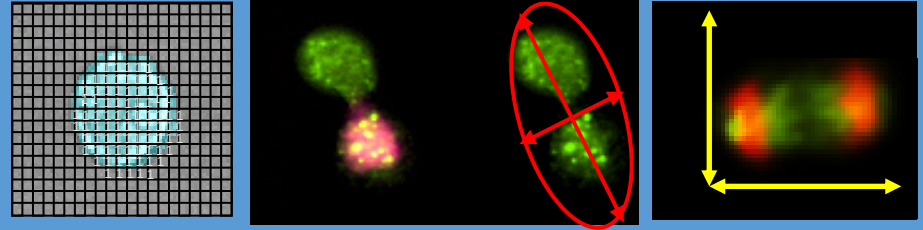
Masks Define a Region of Interest



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Features



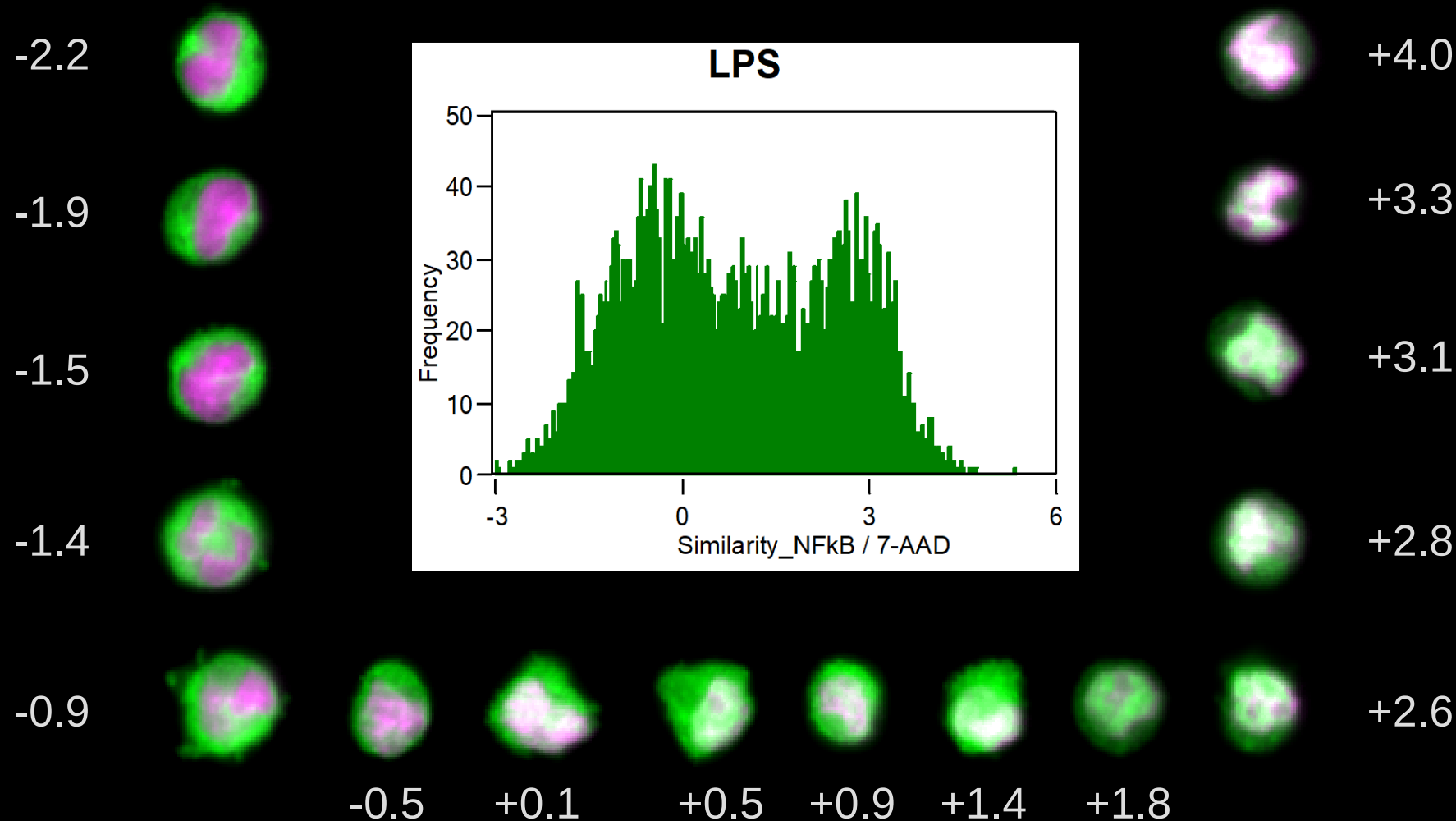
Masks



Wizards

	Internalization		Apoptosis
	Nuclear Localization		Cell Cycle - Mitosis
	Shape Change		Co-localization

ImageStream Quantitation: How do I measure what I see?



Finding the best translocation analysis

Incubate cells with / without LPS for 1 hr

Fix and perm cells. Label NFkB with FITC and stain nuclei with DRAQ5

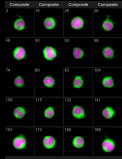
Collect large event image files on the ImageStream

Quantify nuclear translocation of NFkB

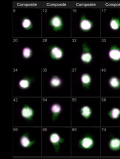
Which feature is best at distinguishing the samples?

Truth sets: untreated vs LPS-treated cells

Untreated

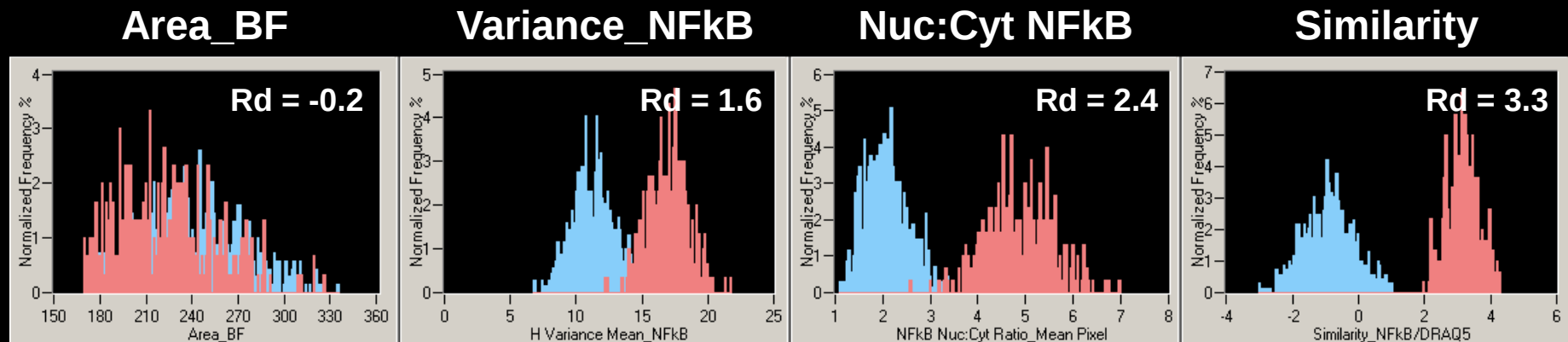


LPS-Treated



Finding the best translocation analysis

Feature finder wizard helps you find the features for distinguishing the populations.



Feature		Untreated		Positive Control	
Name	Category	Mean	Std. Dev.	Mean	Std. Dev.
Area_Brightfield	Size	237.7	35.0	223.7	32.9
H Variance Mean_NFkB	Texture	11.5	1.8	17.0	1.5
NFkB Nuc:Cyt Ratio_Mean Pixel	Comparison	2.1	0.5	4.9	0.7
Similarity_NFkB/DRAQ5	Comparison	-0.9	0.8	3.1	0.5

Rd - statistical measure of discrimination

Fisher's Discriminant ratio (Rd)

- Measure of the statistical separation a feature provides between two populations (1&2) using their means and standard deviations:

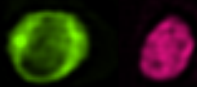
$$\text{Rd} = (\text{Mean}_1 - \text{Mean}_2) / (\text{StdDev}_1 + \text{StdDev}_2)$$

- Populations 1&2 can be:
 - Different samples
 - Different hand-picked truth sets
 - Same set of cells analyzed with different features

ImageStream – Applications

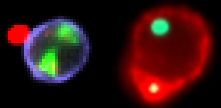
Field of Study

Example from Imagestream



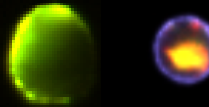
Cell signaling

NFkB Translocation, HIV induced NFAT, FoxP3 localization



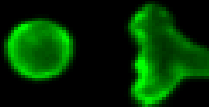
Internalization

CpGB, phagocytosis of Bacteria by monocytes



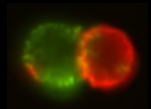
Co-localization

Ligand colocalization to lysosomes



Shape change

MCP-1 activation of monocytes, Differentiation of FDCP cells



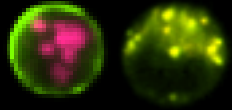
Cell-cell interaction

Immune synapse formation, NFkB activation from T-cell APC conjugation

ImageStream – Applications

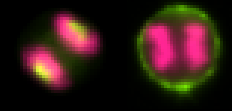
Field of Study

Example from Imagestream



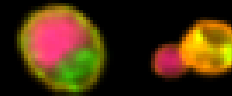
Cell death &
autophagy

Apoptosis, nuclear fragmentation,
caspase3 activation



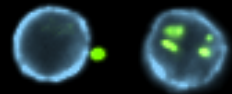
Cell cycle & mitosis

Morphological classification of mitosis



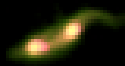
Stem cell biology

Erythroid differentiation



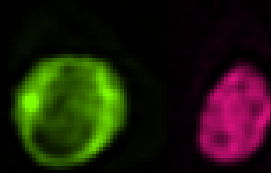
Microbiology

Bacterial phagocytosis in PBMC

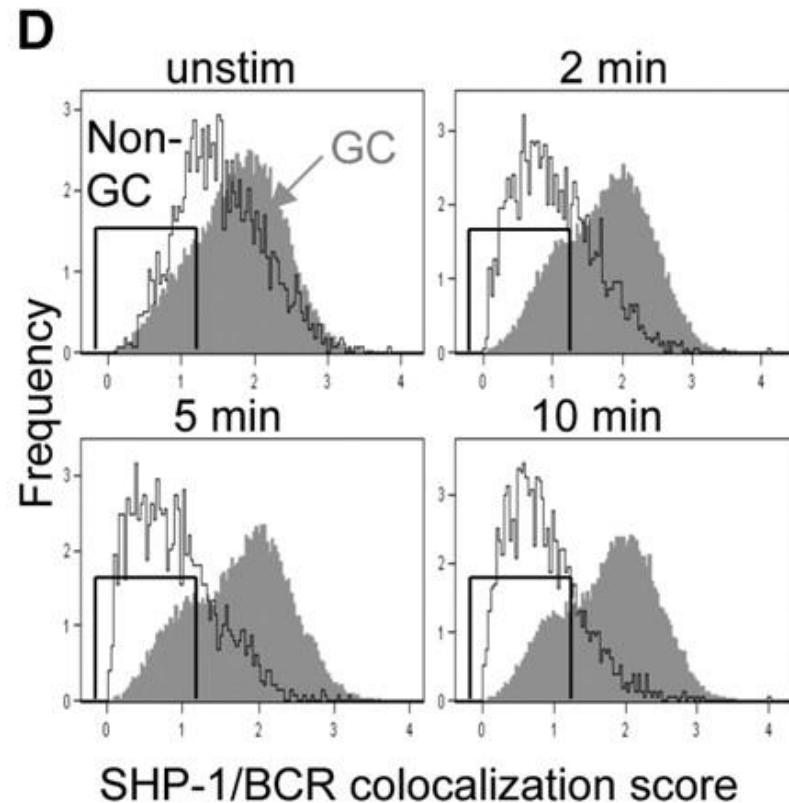
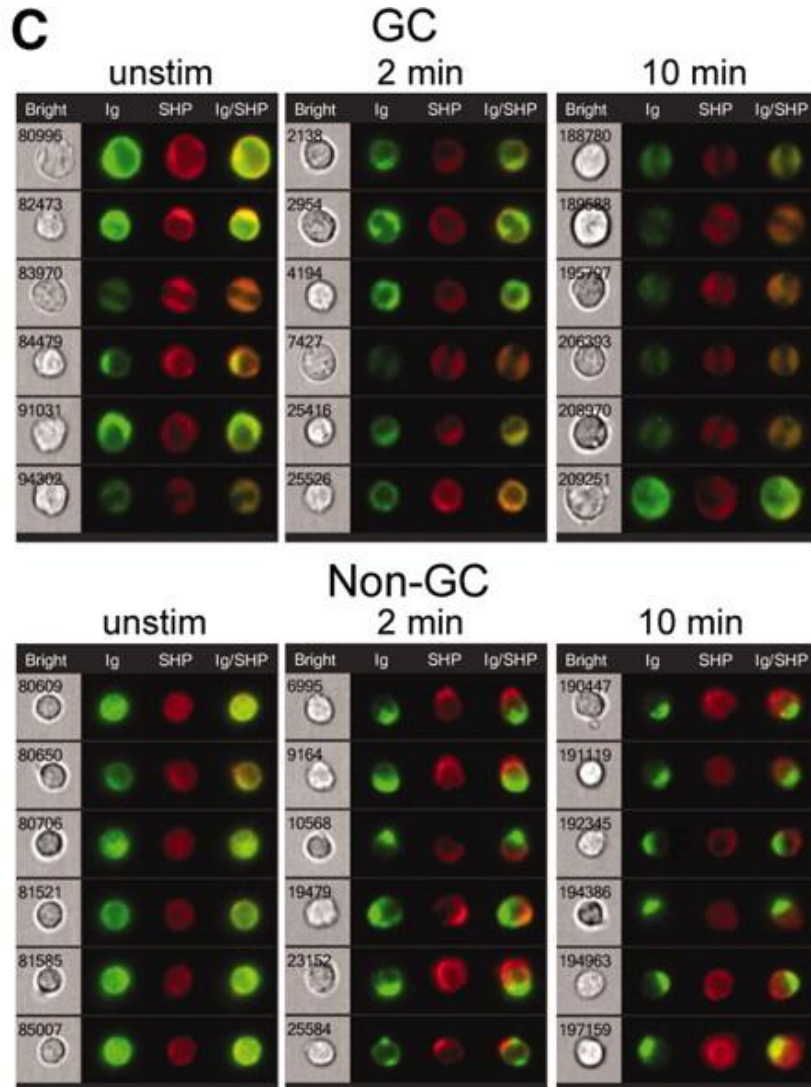


Parasitology

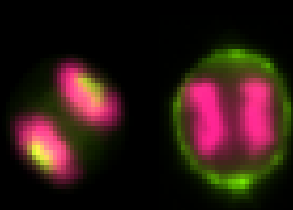
Babesia infection in RBCs



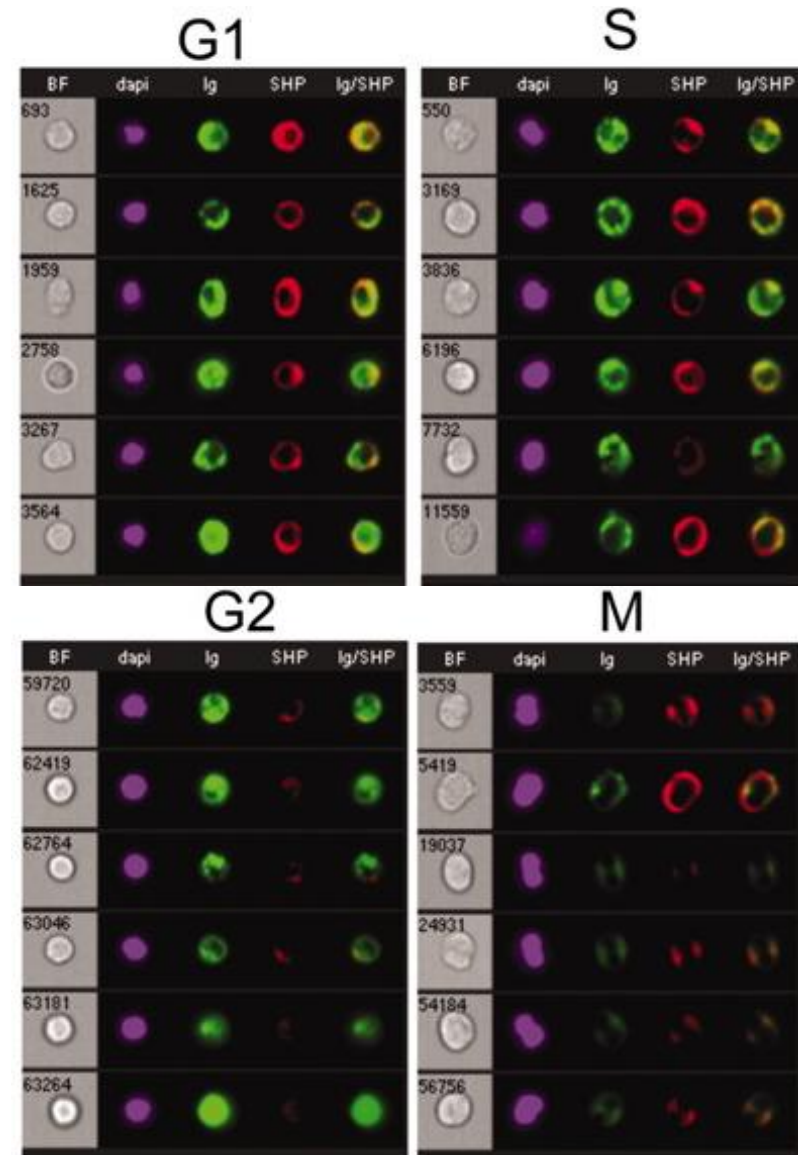
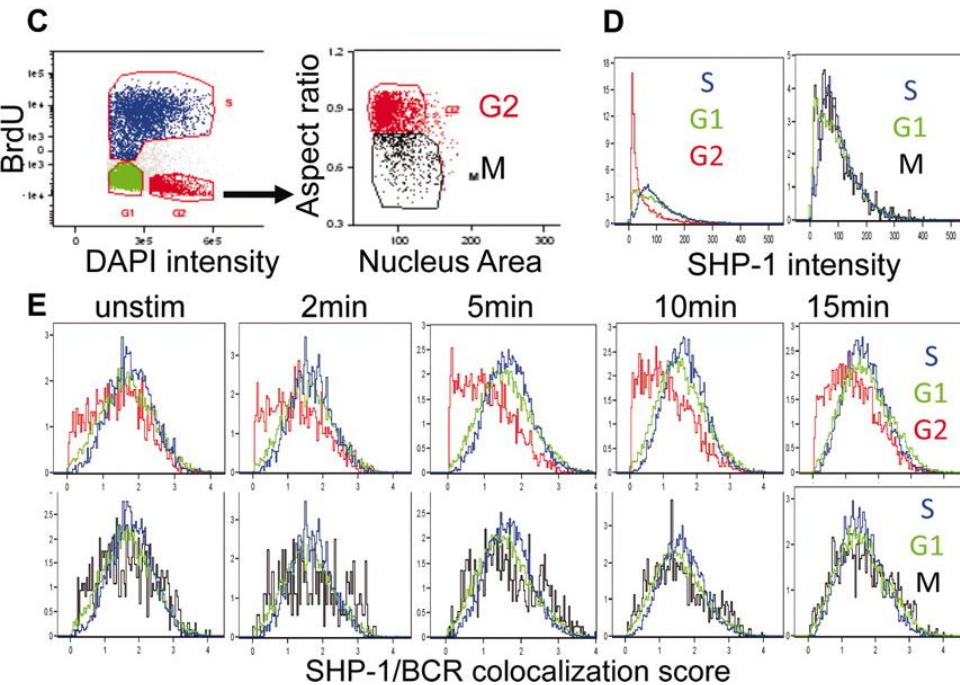
B cell receptor signal transduction



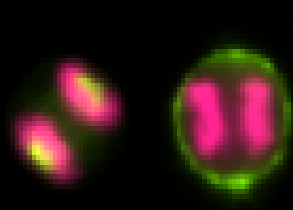
Cell Cycle



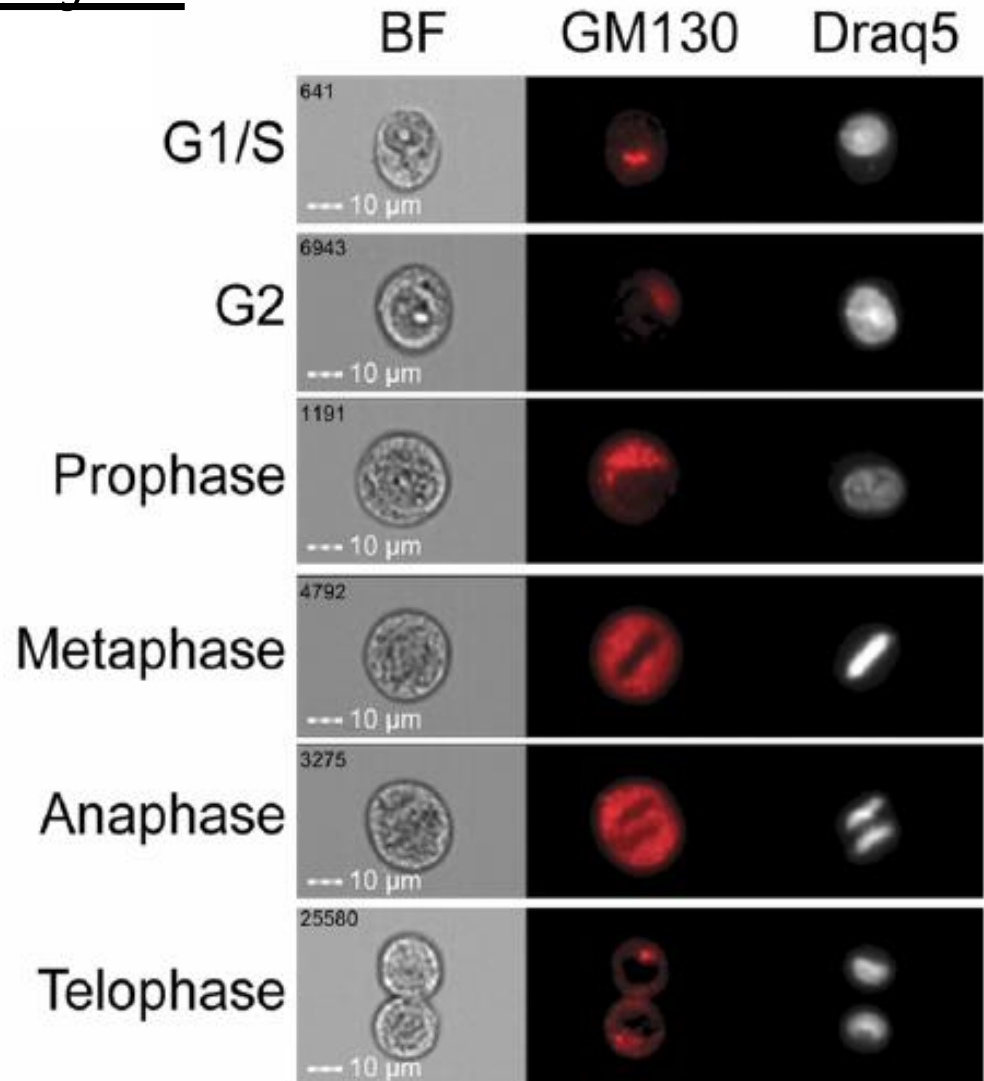
B cell receptor signal transduction



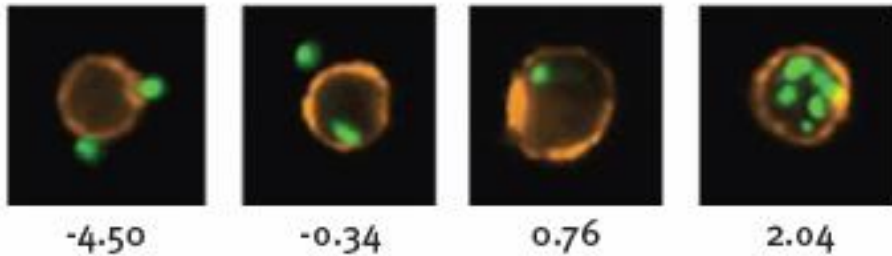
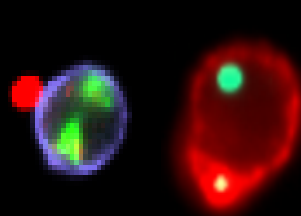
Cell Cycle



Golgi structure during cell cycle

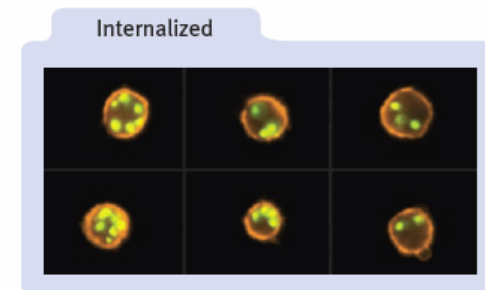
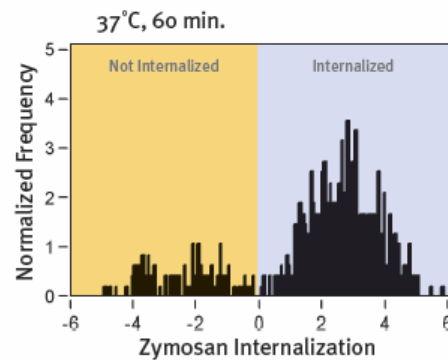
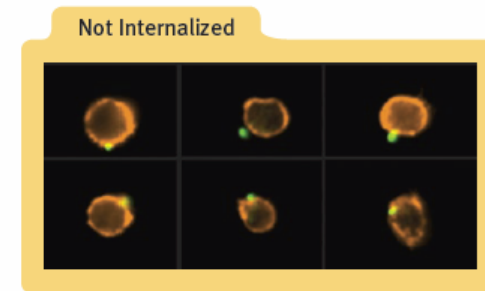
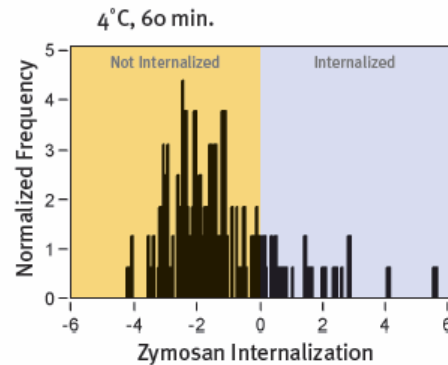


Internalization



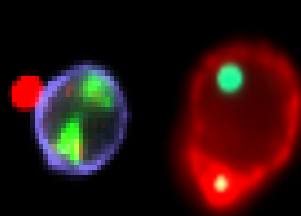
Internalization score

Phagocytosis by murine macrophages



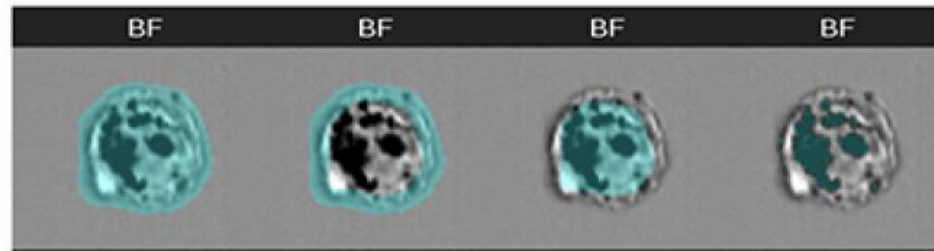
Phagocytosis of FITC-labeled Zymosan particles (green) by RAW cells (orange)

Internalization

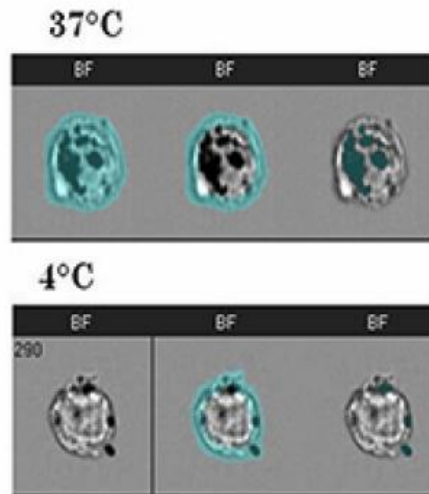
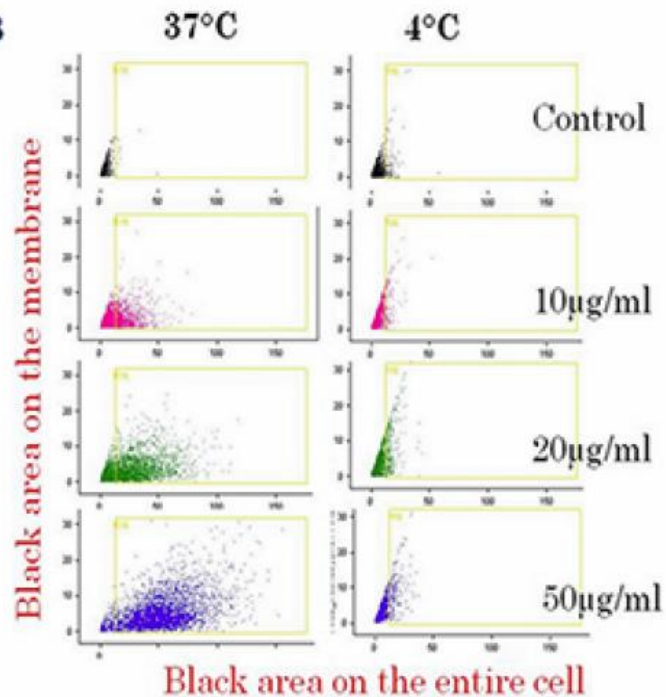


Carbon nanotubes uptake

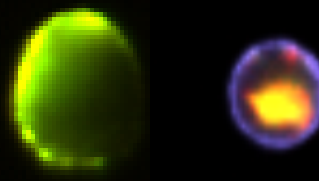
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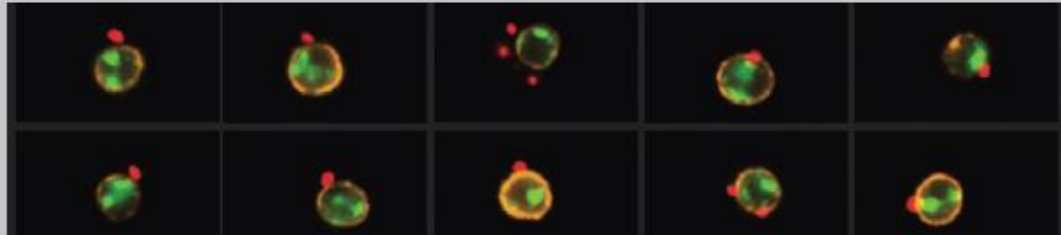
B



Co-localization



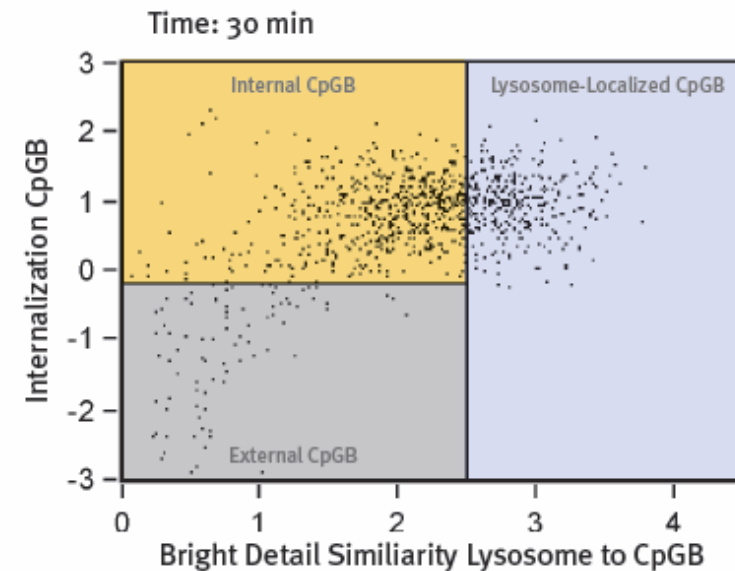
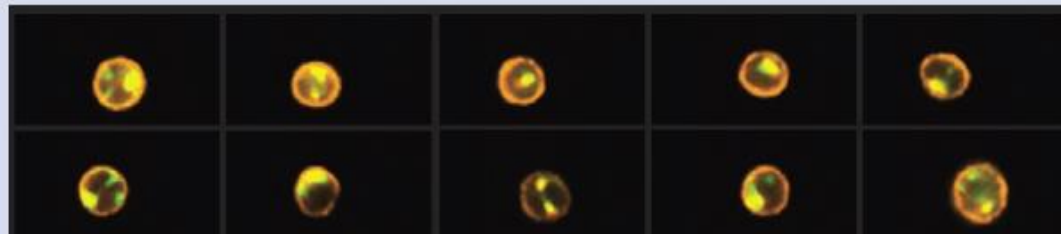
External CpGB



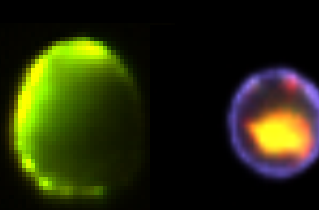
Internal CpGB



Lysosome-Localized CpGB

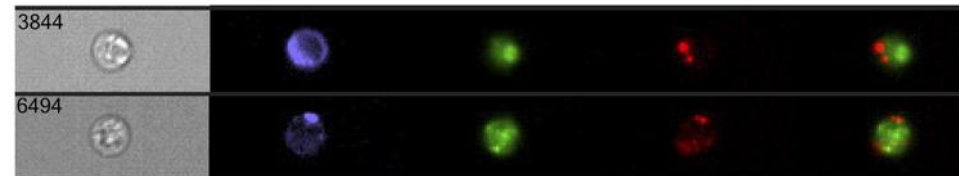


Co-localization

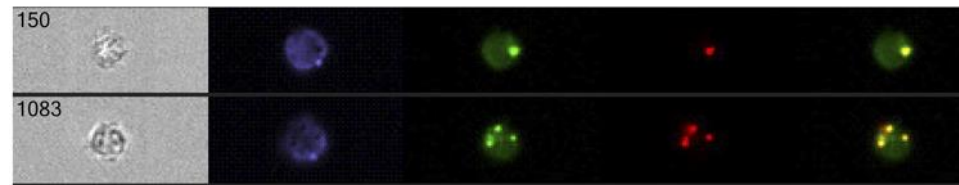


Immune cluster localization within monocytes

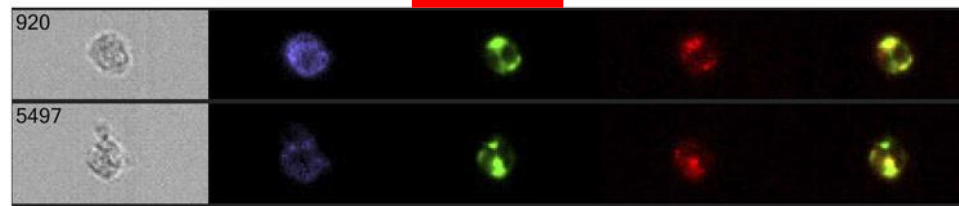
No C1q CD14 **CD71** SLE IC CD71/IC



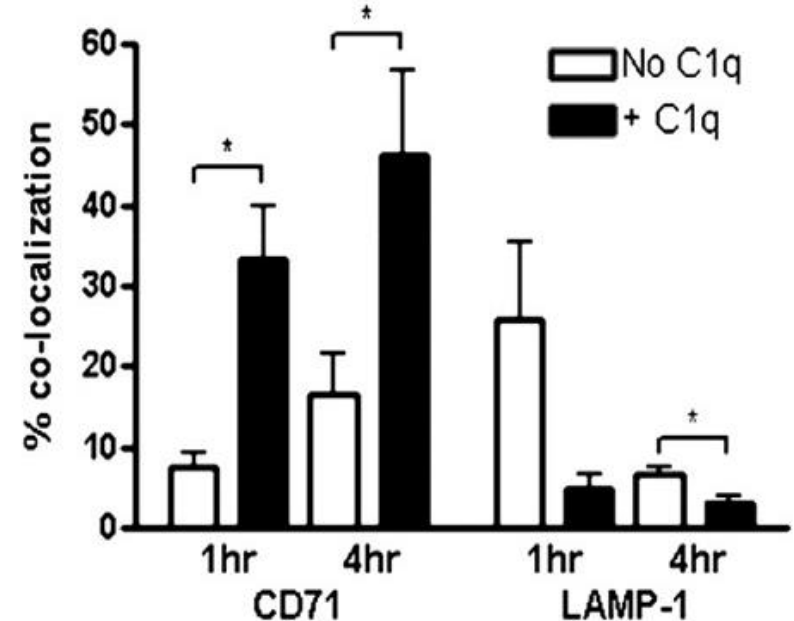
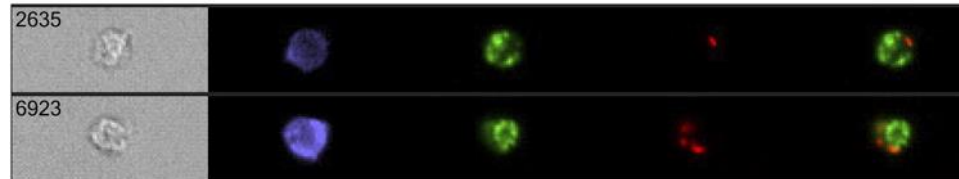
+ C1q



No C1q CD14 **LAMP-1** SLE IC LAMP-1/IC

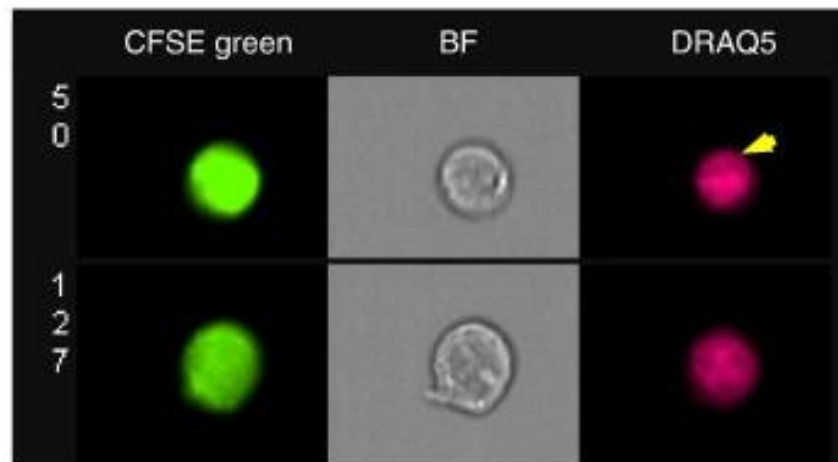


+ C1q

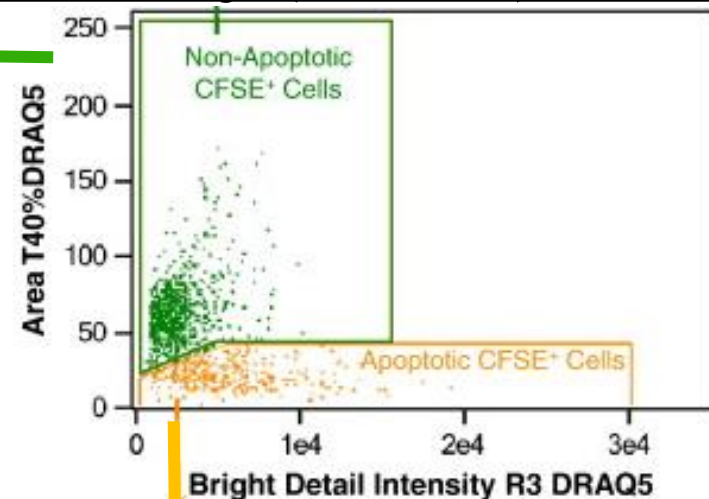


Cell death & Autophagy

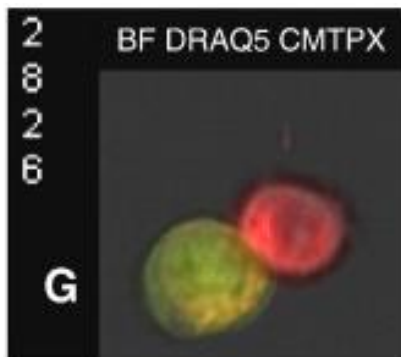
Ab Dependent Cell mediated Cytotoxicity (ADCC) activity



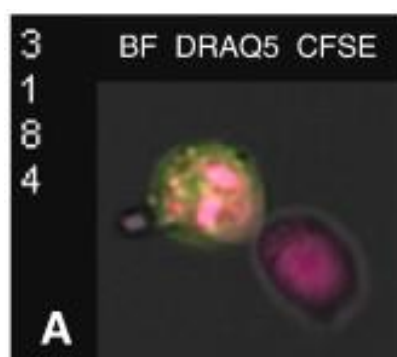
Non-Apoptotic CFSE⁺ Cells



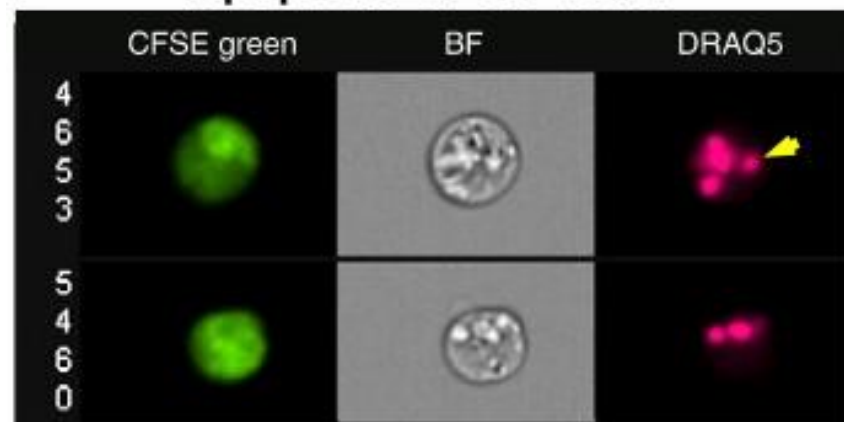
Non-apoptotic
Target Cell



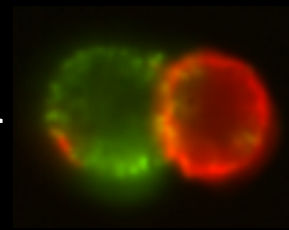
Apoptotic
Target Cell



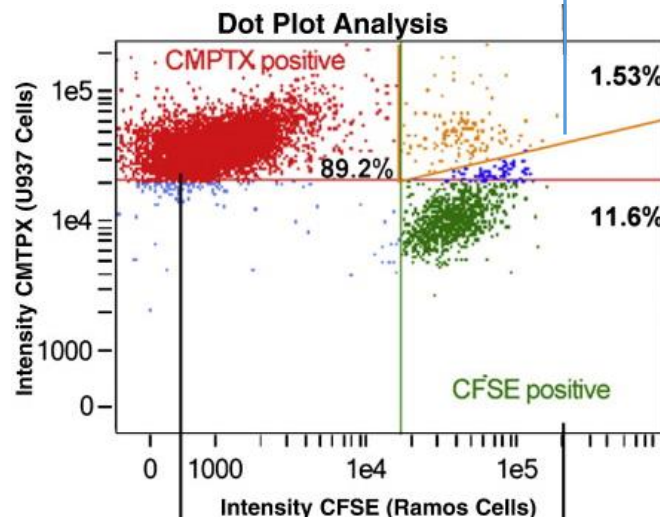
Apoptotic CFSE⁺ Cells



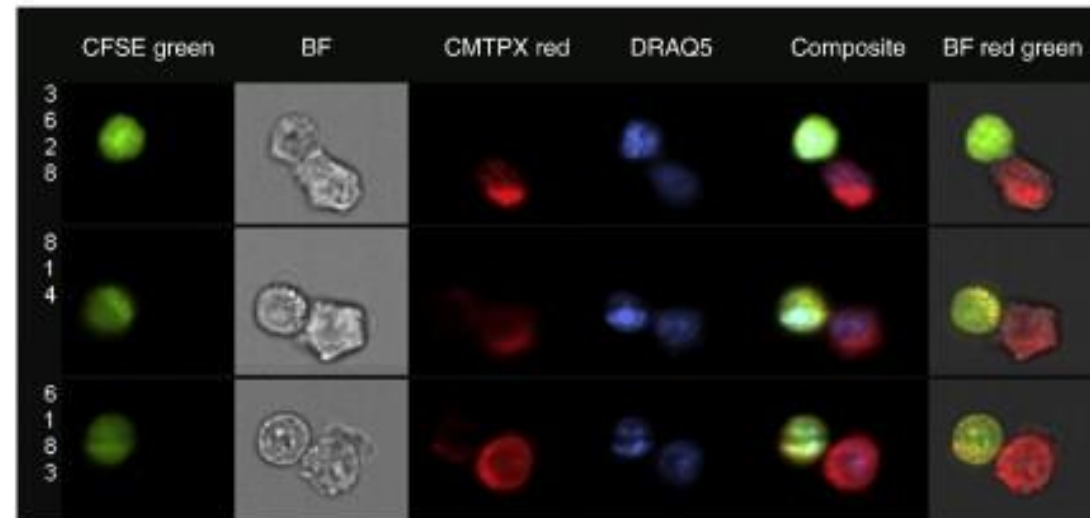
Cell-cell interaction



Ab Dependent Cell mediated Cytotoxicity (ADCC) activity



Double Positive Events



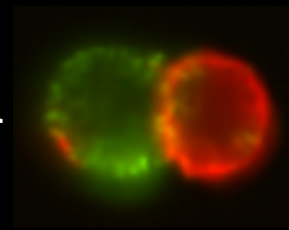
CMTPX+ Events



CFSE+ Events



Cell-cell interaction

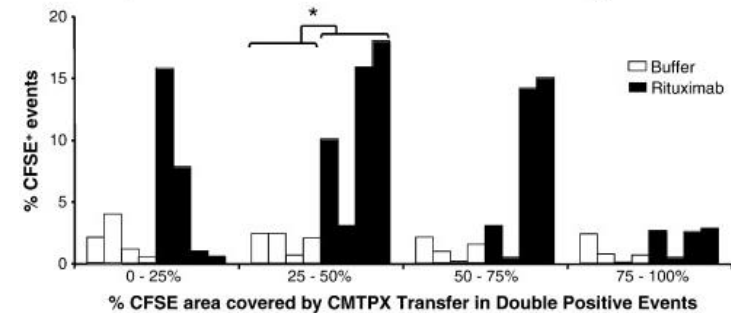


Ab Dependent Cell mediated Cytotoxicity (ADCC) activity

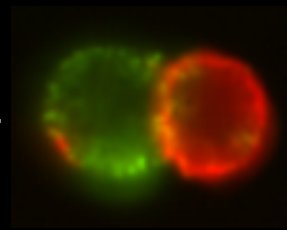
Imagery of Double Positive Events



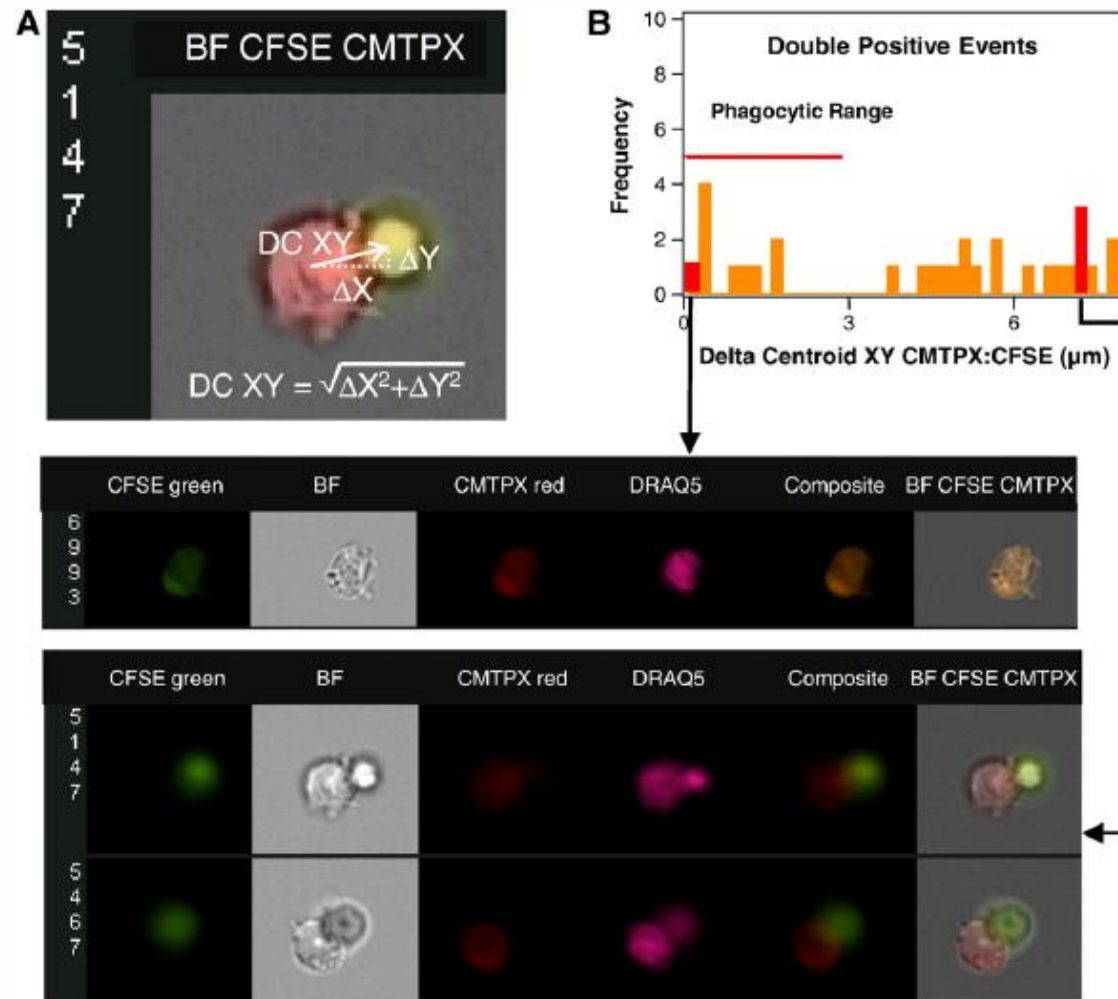
Quantification of CMTX Transfer to Target Cells



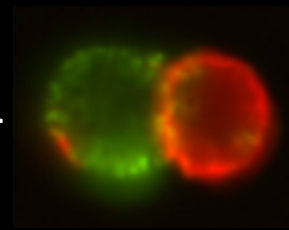
Cell-cell interaction



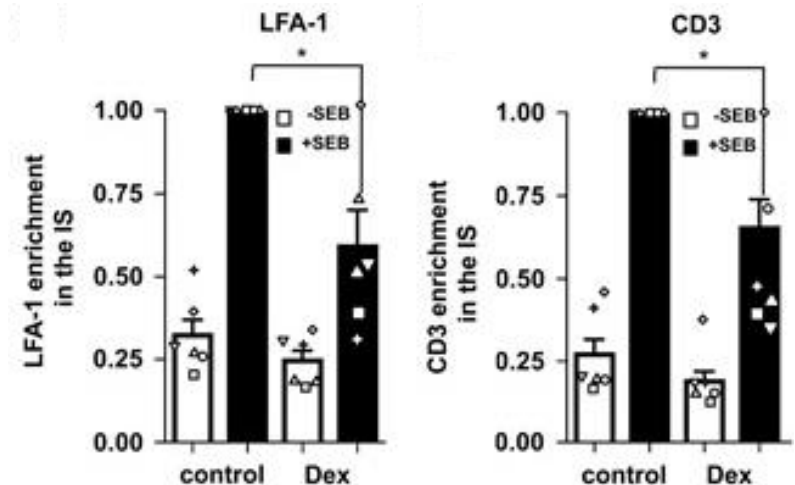
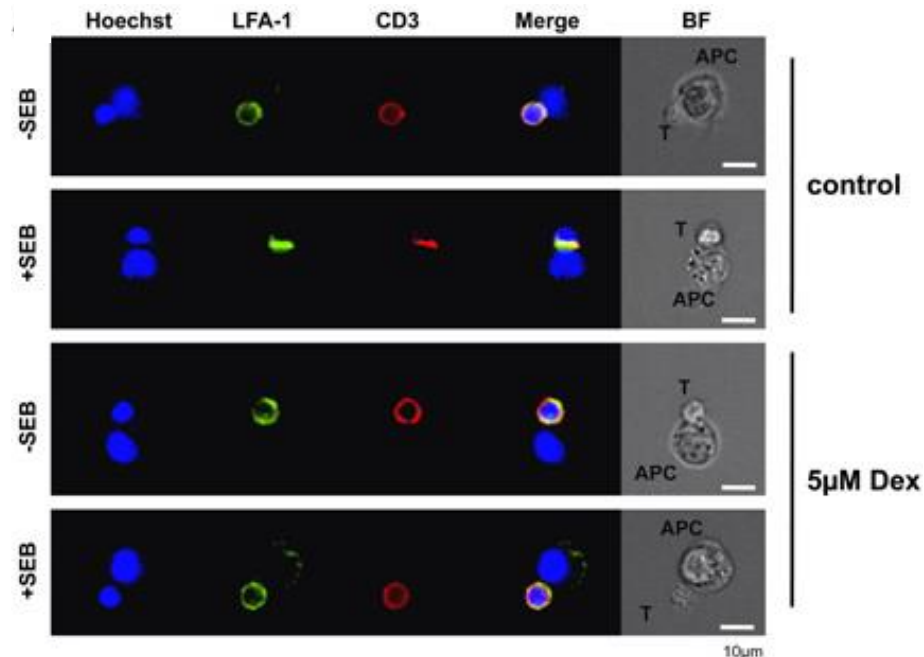
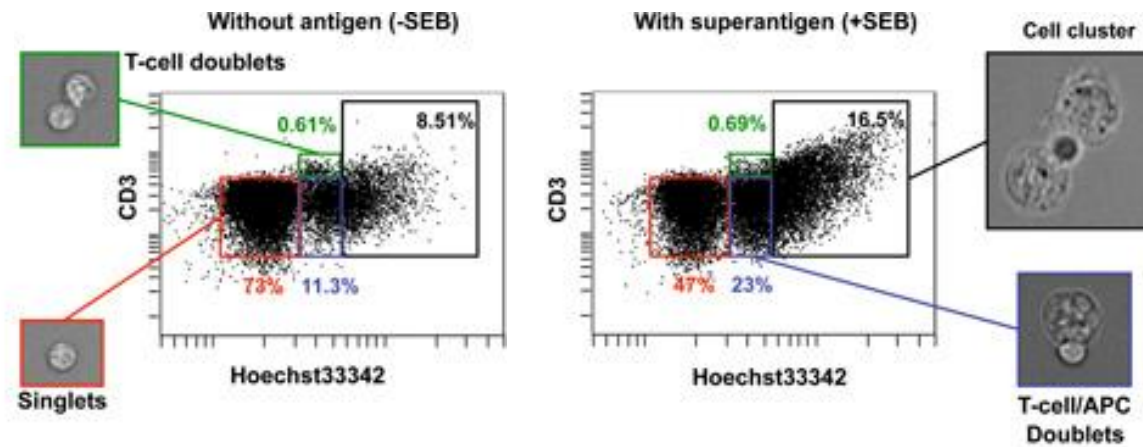
Ab Dependent Cell mediated Cytotoxicity (ADCC) activity



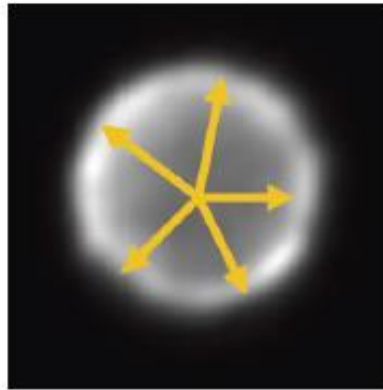
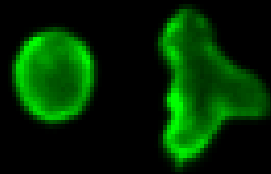
Cell-cell interaction



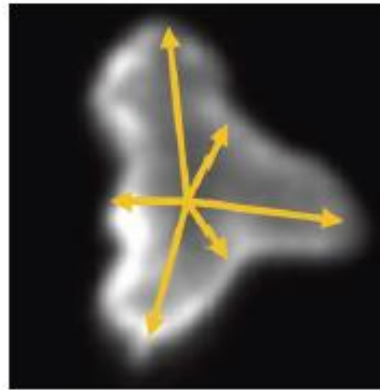
Immunosuppressive dexamethasone in primary T cells



Shape change



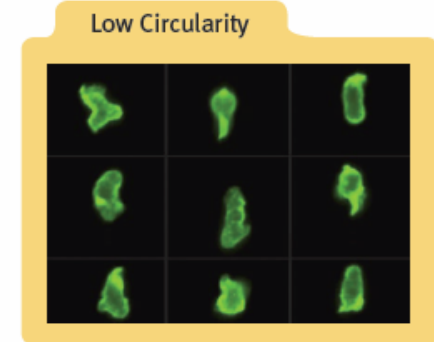
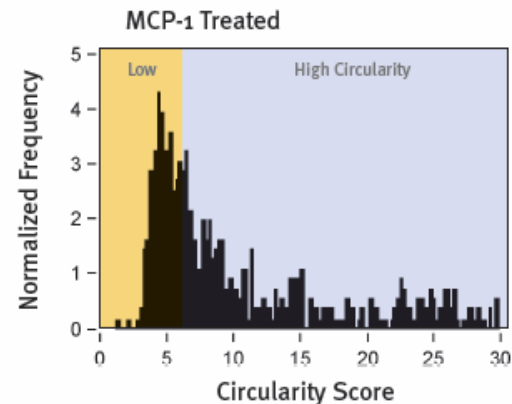
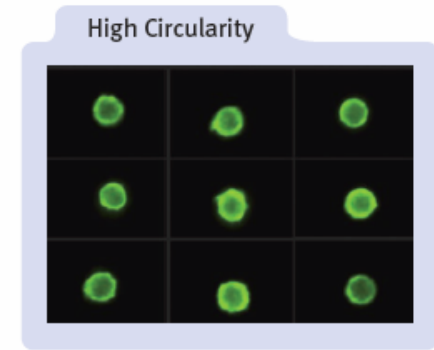
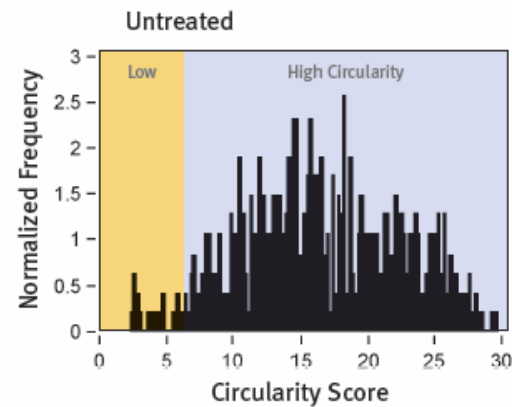
23.8



4.6

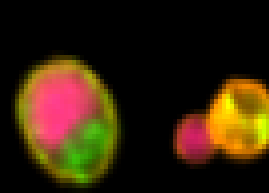
Circularity score

Shape Change in Primary Monocytes



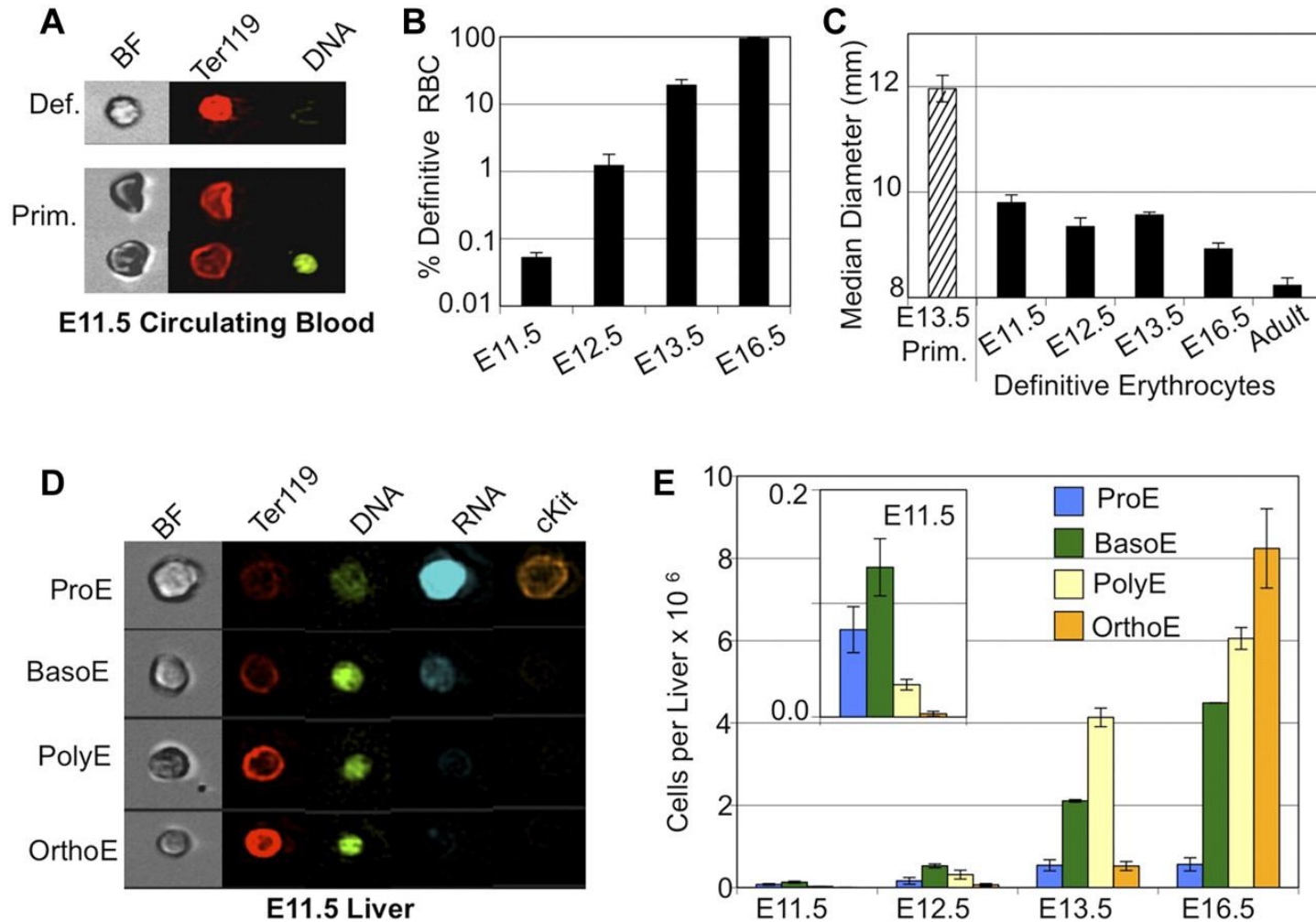
Chemoattractant MCP-1 induces monocyte shape change and migration to sites of inflammation

Stem cell biology



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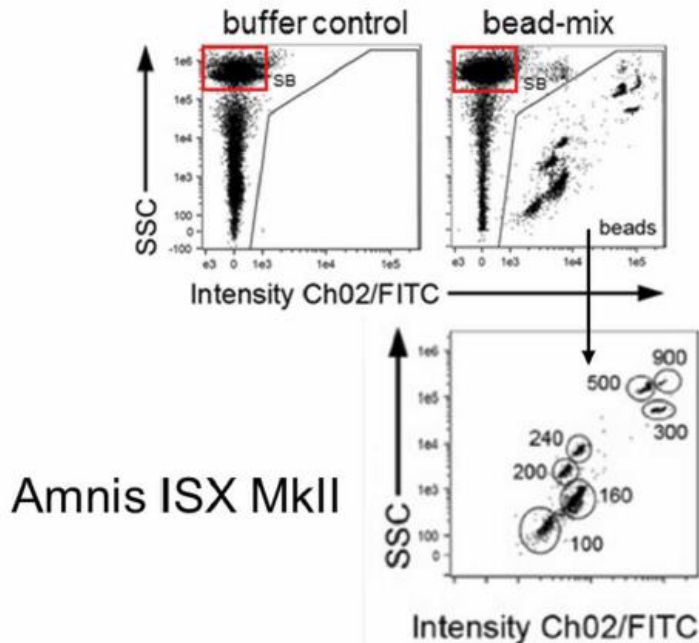
Erythroid differentiation



McGarth KE. (2011), *Blood*

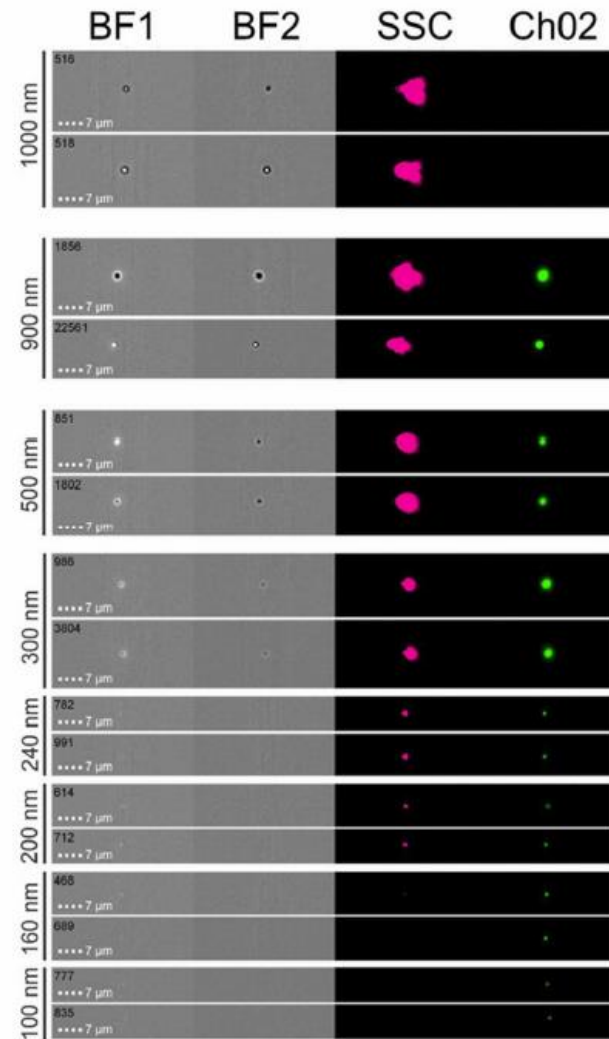
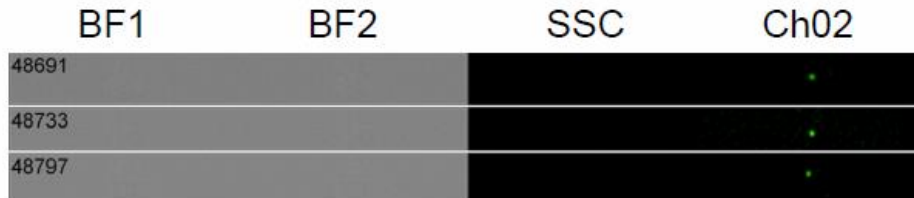
Extracellular vesicles

Submicron-Sized Polystyrene Beads as Calibrators



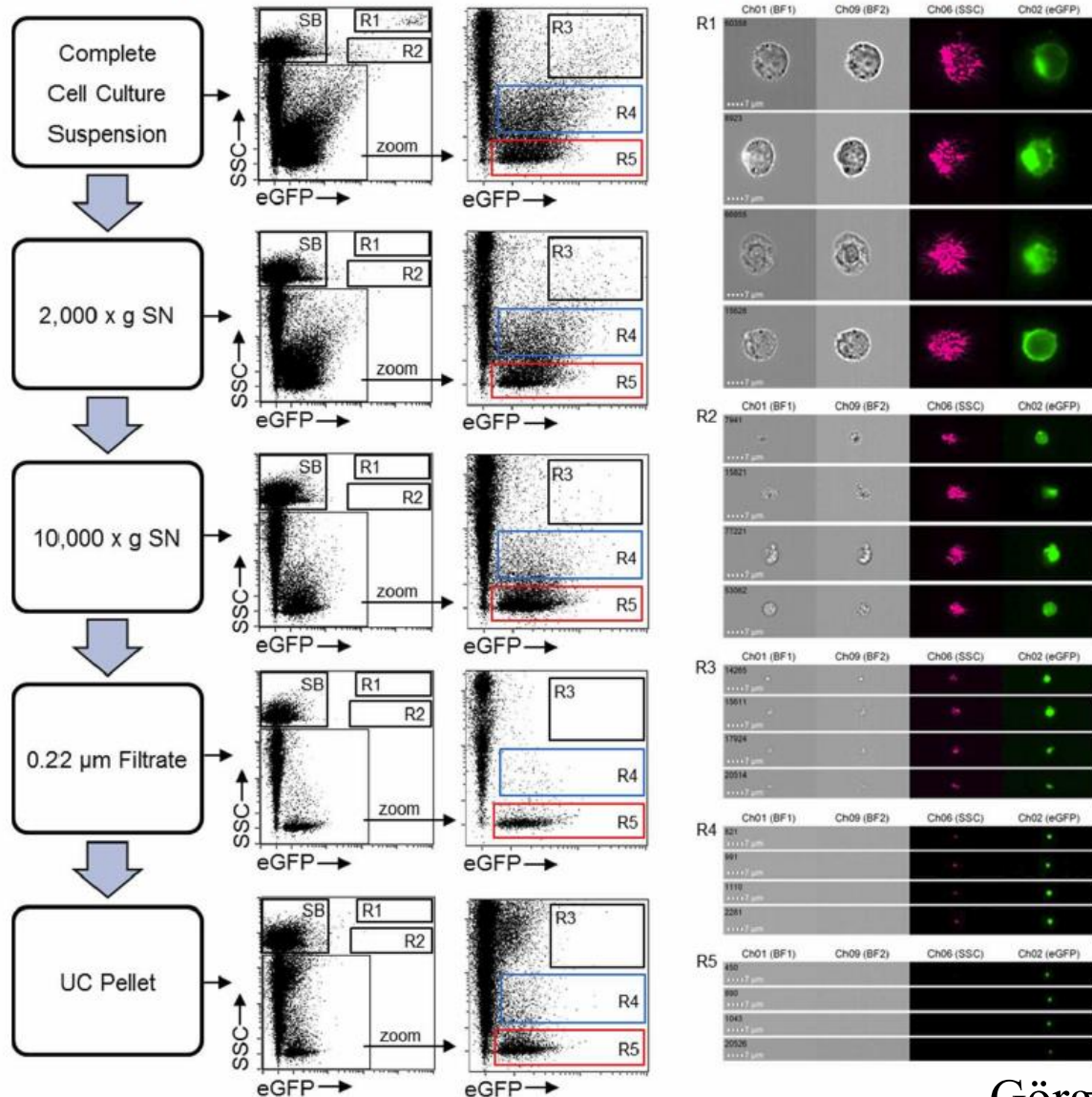
Amnis ISX MkII

24 nm beads (Thermo Scientific)

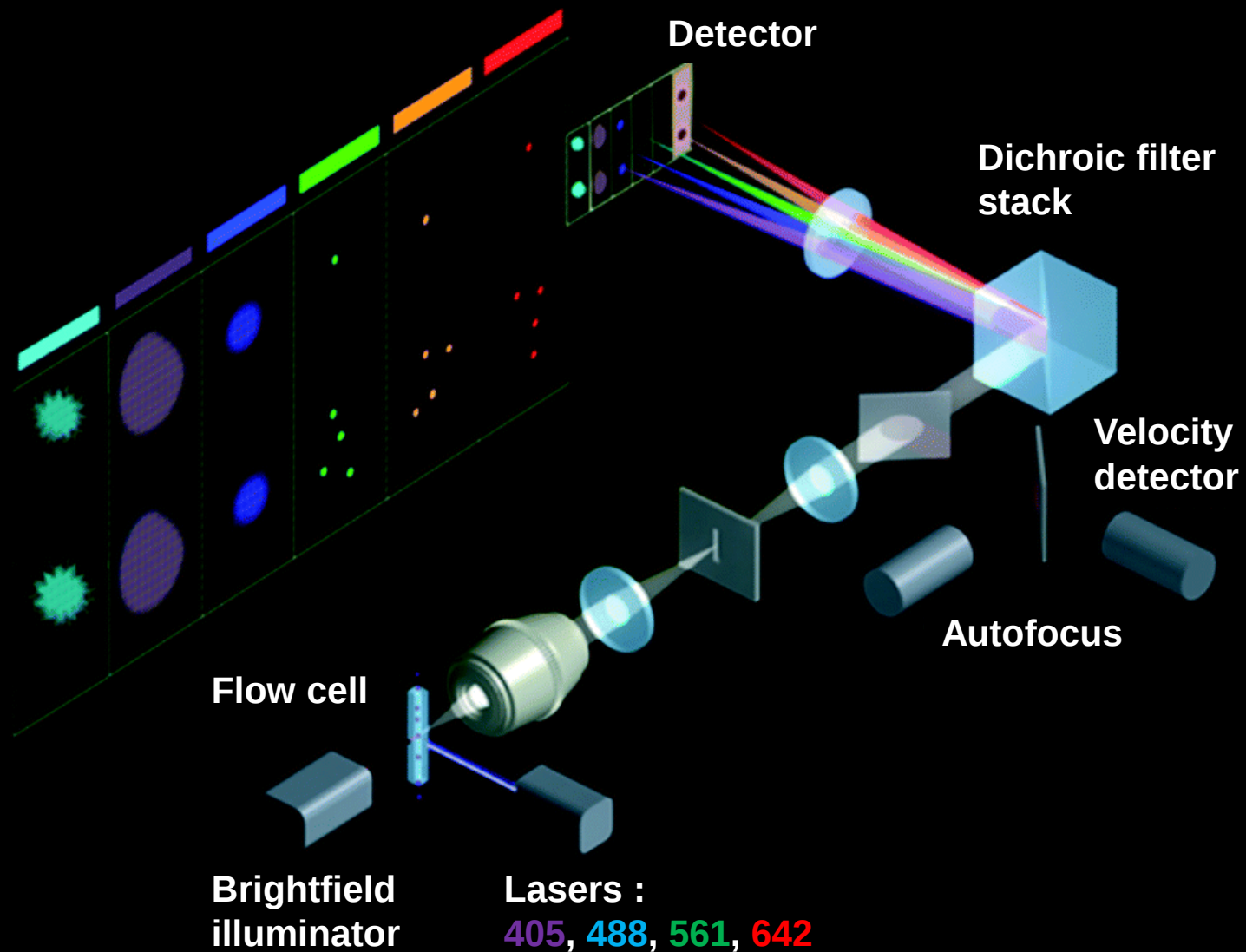


Extracellular vesicles

Re-Evaluation of Exosome Isolation Protocols



Planning your experiment



Planning your experiment

		Excitation laser (nm)				
Ch	Band	405	488	561	642	785
1	435-505	DAPI, BV421, Hoechst, PacBlue, CascadeBlue, eFluor450, DyLight405, CFP, LIVE/DEAD Violet				
2	505-560	BV510, PacOrange, Cascade Yellow, AF430, eFluor525, QD525	FITC, AF488, GFP, YFP, DyLight488, PKH67, Syto13, LysoTrackerGreen, MitoTrackerGreen			
3	560-595	QD565, QD585, eFluor565	PE, PKH26, DSRRed, mOrange, Sytox Orange, Cy3	PE, AF546, Cy3, DyLight550, PKH25, DSRRed		
4	595-642	QD625, eFluor625, BV605	PE-TexRed, PI, RFP, QD625, eFluor625	AF568, Cy3, PE-TexRed, TexRed, AF610, RFP, mCherry, PI		
5	642-745	QD705, eFluor700, BV711	PE-Cy5, PE-AF647, 7AAD, PerCP, PerCP-Cy5.5, DRAQ5, QD705	PE-Cy5, PE-AF647, DRAQ5, 7AAD	APC, AF647, AF660, AF680, APC, Cy5, DyLight649, PE-AF647, PE-Cy5, DRAQ5, PerCP, , PerCP-Cy5.5	
6	745-780	QD800, BV786	PE-Cy7, PE-AF750, QD800	PE-Cy7, PE-AF750	APC-Cy7, APC-AF750, APC-H7, Cy7, AF750, PE-Cy7, PE-AF750	SSC



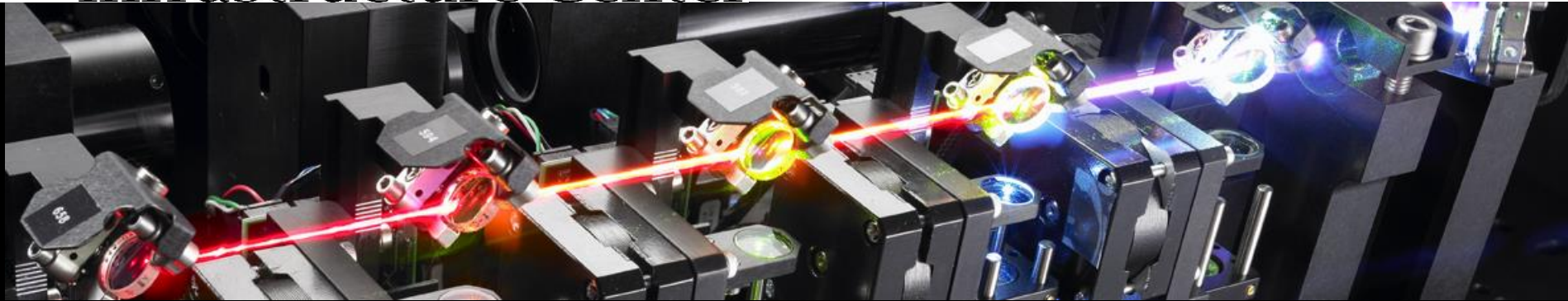
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- *FCS Express 6*

Thank You!!!

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LS&E site: <https://lse.technion.ac.il/>