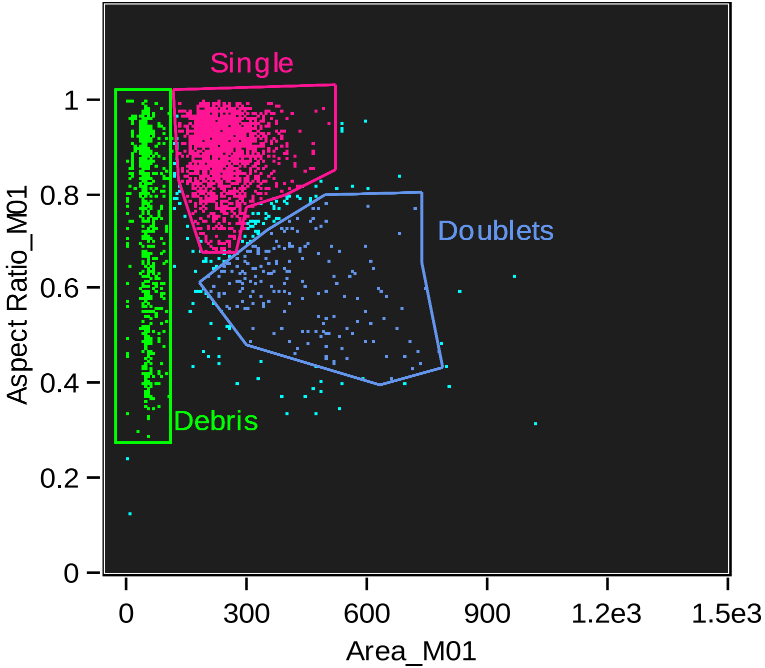
Luminex

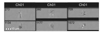
complexity simplified.

量化成像流式细胞仪

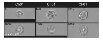
2021年9月9日星期四

视觉验证 圈门不再靠猜

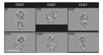




Debris

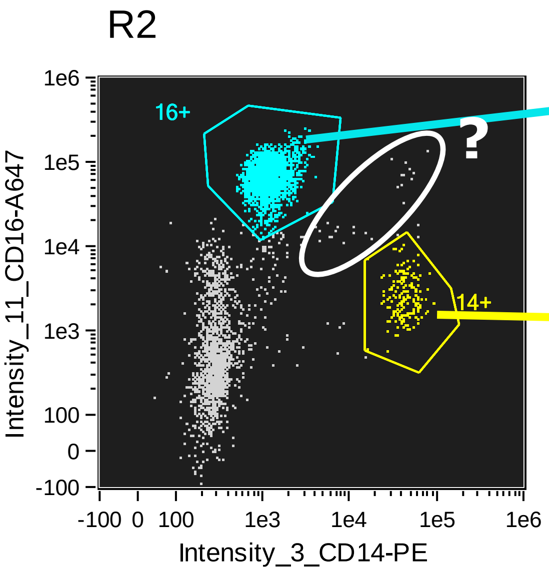


Singlet



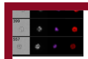
Doublets

视觉验证 圈门不再靠猜



中性粒细胞

BF SSC DAPI CD16

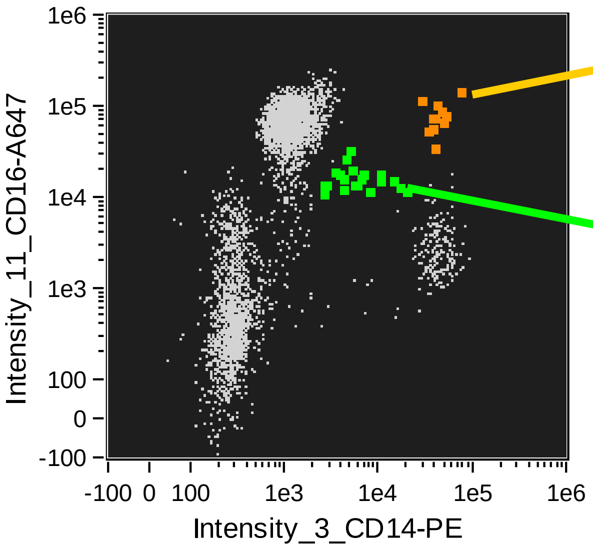


单核细胞

BF SSC DAPI CD14

眼见为实，不再迷惑

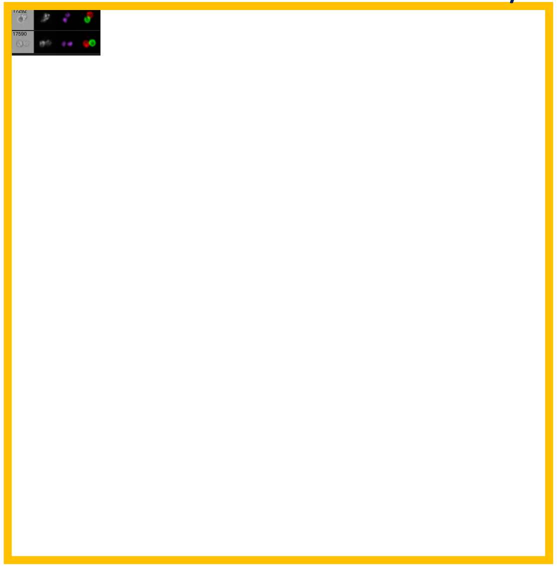
R2



CD14+ CD16+ populations

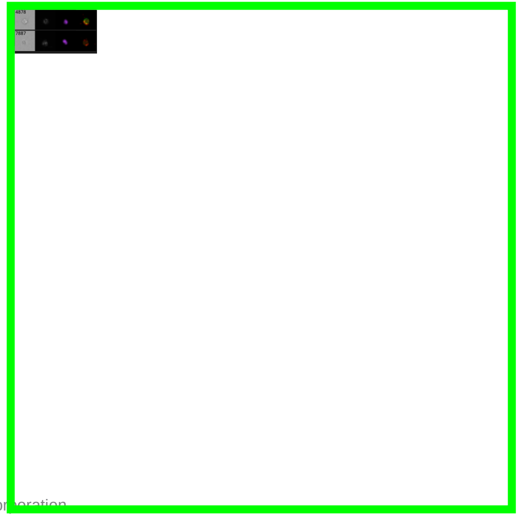
14+16+ Bright

BF SSC DAPI CD14/16

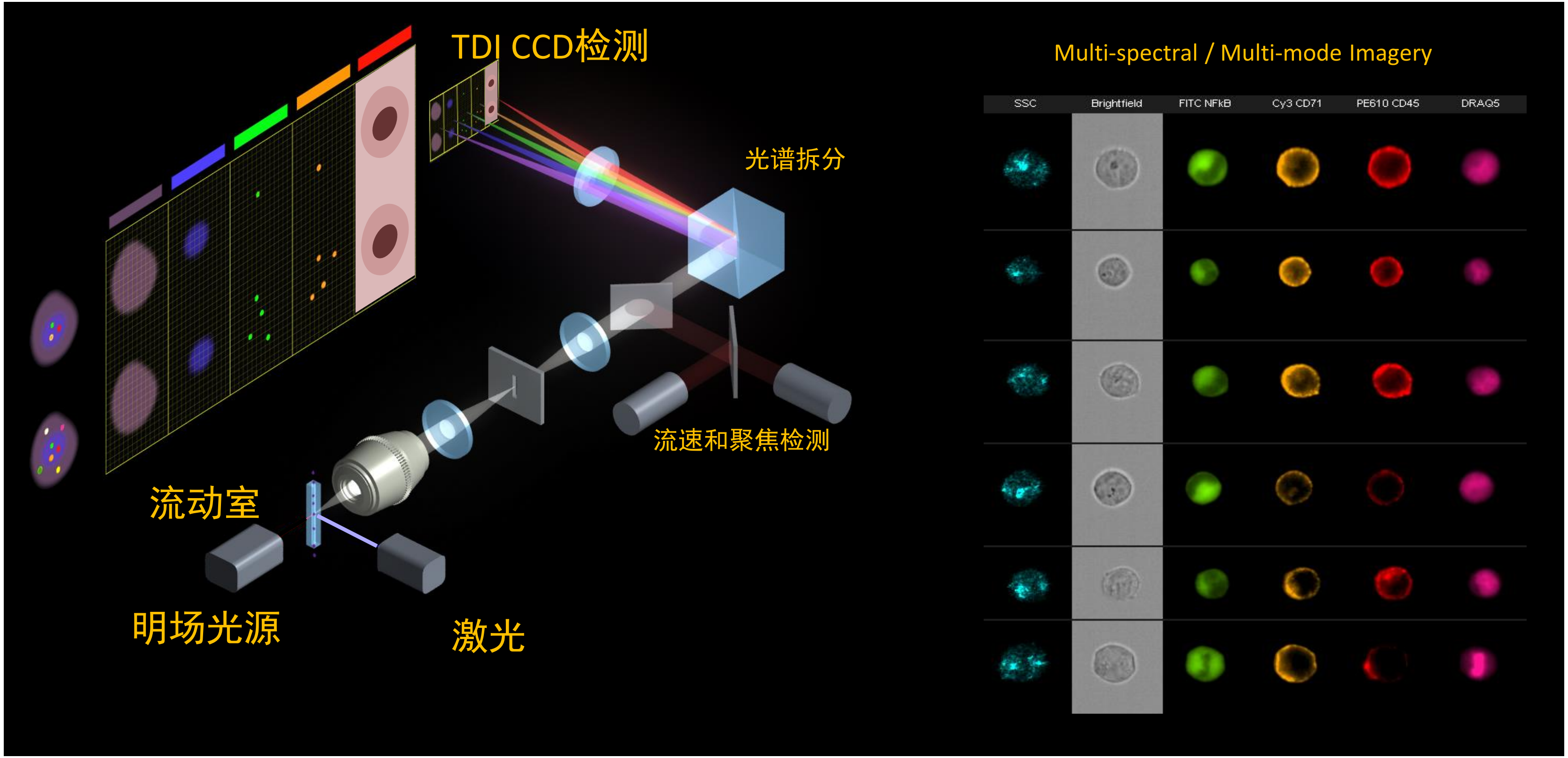


14+16+ Dim

BF SSC DAPI CD14/16



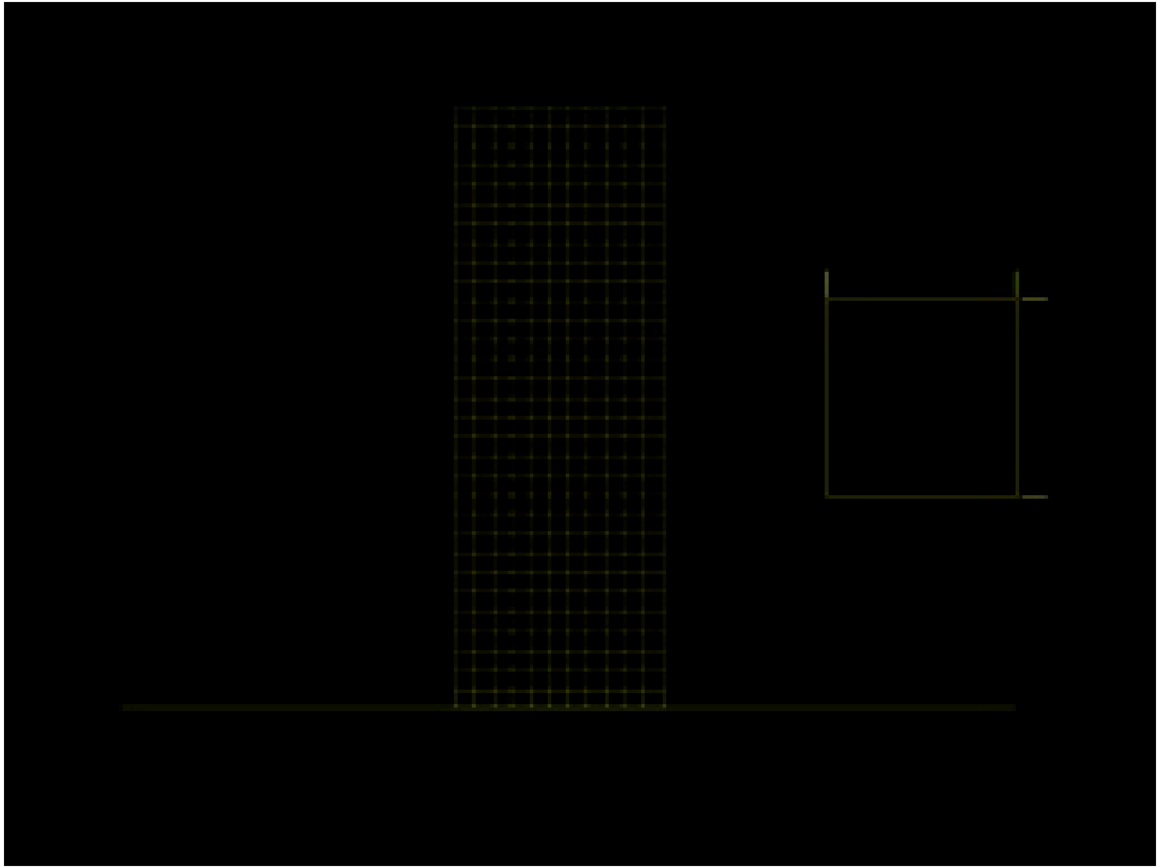
运行原理：1个细胞12张图像



专利的 TDI CCD 技术提供卓越的灵敏度

时间延迟积分（TDI）

- Amnis 核心技术
- 增加的光子收集时间以提供了卓越的灵敏度
- 细胞同步生成聚焦图像



检测通道

Spectral Imaging Bands and Applicable Dyes

CHANNEL 1 430-480 nm	CHANNEL 2 505-560 nm	CHANNEL 3 560-595 nm	CHANNEL 4 595-642 nm	CHANNEL 5 642-740 nm	CHANNEL 6 740-800 nm	CHANNEL 7 430-505 nm	CHANNEL 8 505-560 nm	CHANNEL 9 560-595 nm	CHANNEL 10 595-642 nm	CHANNEL 11 642-740 nm	CHANNEL 12 740-800 nm
	FITC	DsRed	7-AAD	PerCP	PE-Cy7	DAPI	Alexa Fluor 430	Qdot 565	Qdot 605	Qdot 705	Qdot 800
	GFP	Dil	PE-Texas Red (ECD)	PerCP-Cy5.5	PE-Alexa Fluor 750	Hoechst 33258	Pacific Orange	Qdot 585	Qdot 625	eFluor 650	APC-Cy7
	YFP	Cy3	PE-Alexa Fluor 610	PE-Alexa Fluor 647		CFP	Cascade Yellow		eFluor 605	Nile Blue	APC-Alexa Fluor 750
	Acridine Orange	R-phycoerythrin	Propidium Iodide	PE-Alexa Fluor 680		Alexa Fluor 405	Lucifer Yellow			APC	APC-H7
	Alexa Fluor 488	OFP	Spectrum Orange	PE-Cy5		Marina Blue	Qdot 525			APC-Cy5.5	APC-eFluor780
	Alexa Fluor 500	Alexa Fluor 546	MitoTracker Red	PE-Cy5.5		Pacific Blue	Qdot 545			DyLight 649	DyLight 750
Brightfield	Alexa Fluor 514	Alexa Fluor 555	LysoTracker Red	DRAQ5	Darkfield (SSC)	Cascade Blue		Brightfield		MitoTracker Deep Red	
	SYTO	DyLight 549	RFP	Nile Blue		LIVE/DEAD Violet				Alexa Fluor 647	
	Spectrum Green	Calcium Orange	mCherry			DyLight 405				Alexa Fluor 660	
	LysoTracker Green		Alexa Fluor 568			eFluor 450				Alexa Fluor 680	
	DyeCycle Green		Alexa Fluor 594			Spectrum Aqua				DRAQ5	
	Calcium Green-1		Alex Fluor 610							Cy5	
	MitoTracker Green		DyLight 594							Cy5.5	
	DyLight 488		Texas Red								

Excitation Lasers: 405 nm | 488 nm | 561 nm | 642 nm

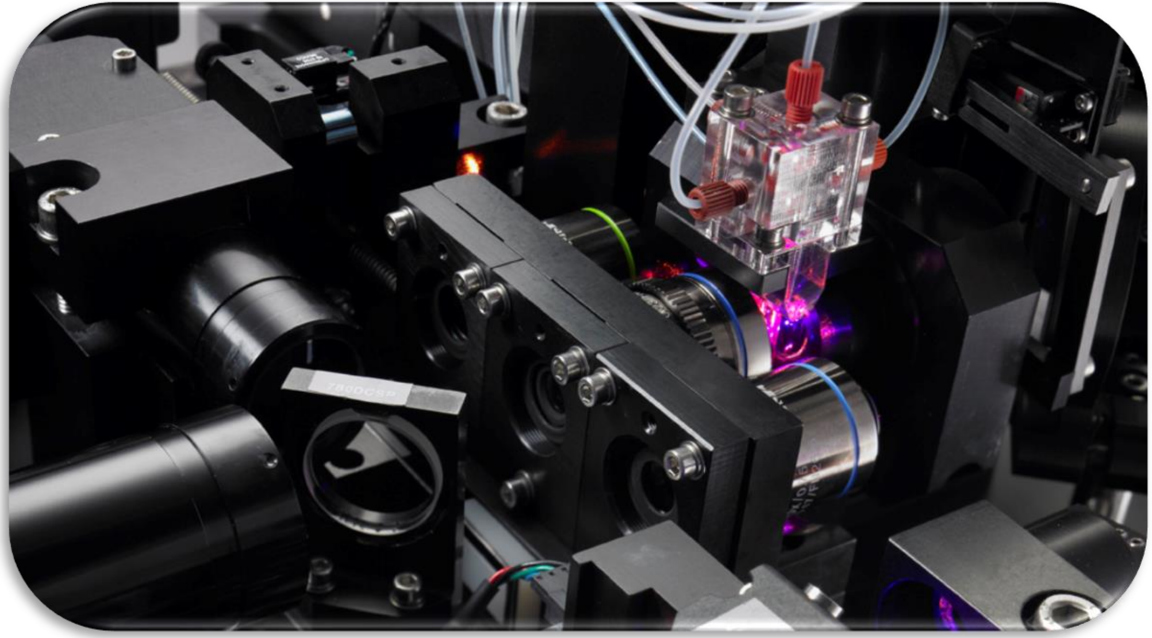
Darkfield (SSC) Laser: 785 nm

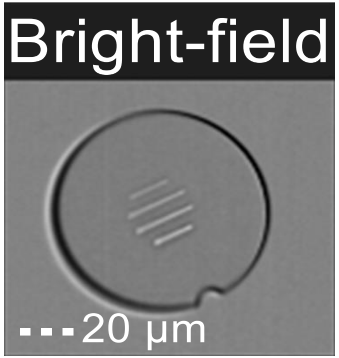
642nm Laser
(AF647, Cy5, APC, APCCy7)

785nm laser (SSC)

多种放大倍数

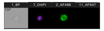
性能特征	放大倍数		
	40X	60X	20X
数值孔径	0.75	0.9	0.5
像素大小	0.5 × 0.5 µm	0.3 × 0.3 µm	1.0 × 1.0 µm
视野大小	60 × 128 µm	40 × 170 µm	120 × 256 µm



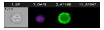


80 µm SU8 particle (20x)

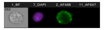
Jurkat cells (~ 11µm diameter)



20x



40x

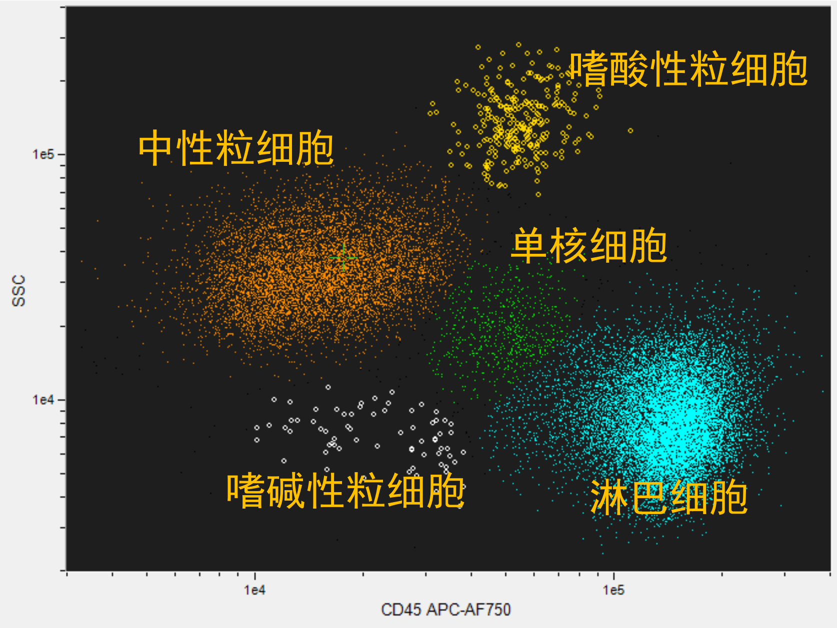


60x

异源细胞群分群更细 WBC分群

实验设计

- 取全血细胞
- 裂解红细胞
- 标记 anti-CD45
- SSC 通道激光设置
- 获取5000细胞
- 分析 CD45 vs. SSC

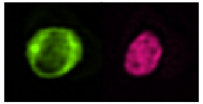


专设785nm激光器用于SSC检测，打造独一无二的超高细胞群分辨力

Luminex. complexity simplified. |

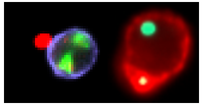
For Research Use Only.

量化成像流式细胞术



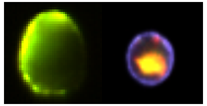
Cell signaling:

NFkB/NFAT/Foxo3核转位



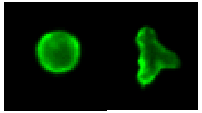
Internalization & phagocytosis

CpGB内吞， 吞噬细胞吞噬病原体



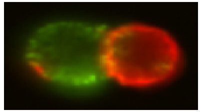
Intracellular co-localization

配体运输到溶酶体



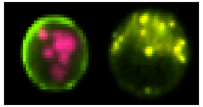
Shape change & chemotaxis

单核细胞形变， FDCP细胞分化



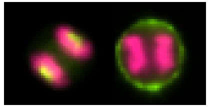
Cell-cell interaction

免疫突触、胞间结合



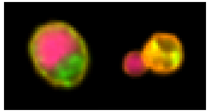
Cell death & autophagy

凋亡、DNA损伤、自噬



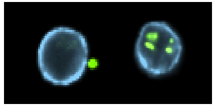
Cell cycle & mitosis

形态学鉴别分裂期



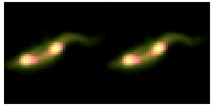
Stem cell biology

红系细胞分化



Microbiology

外周血单个核细胞对细菌的吞噬

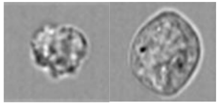


Parasitology

锥体虫、疟原虫感染

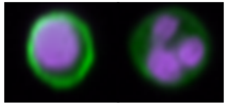
 |

每通道超百个分析参数



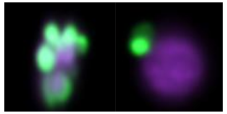
Size features are in microns:

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



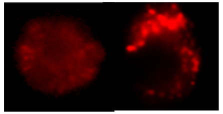
Shape features define the mask shape, units vary with the feature:

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



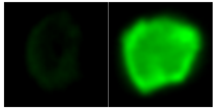
Location features are in X,Y pixel coordinates:

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



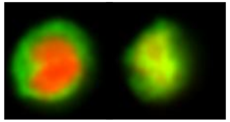
Texture features determine local intensity variations in images:

Bright Detail Intensity R3; Bright Detail Intensity R7; Contrast; Gradient Max; Gradient RMS; Modulation; Spot Count; Std Dev; and the Haralick (H) texture features H-Contrast; H-Correlation; H-Energy; H-Entropy; H-Homogeneity; and H-Variance



Signal Strength features are measured in counts:

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



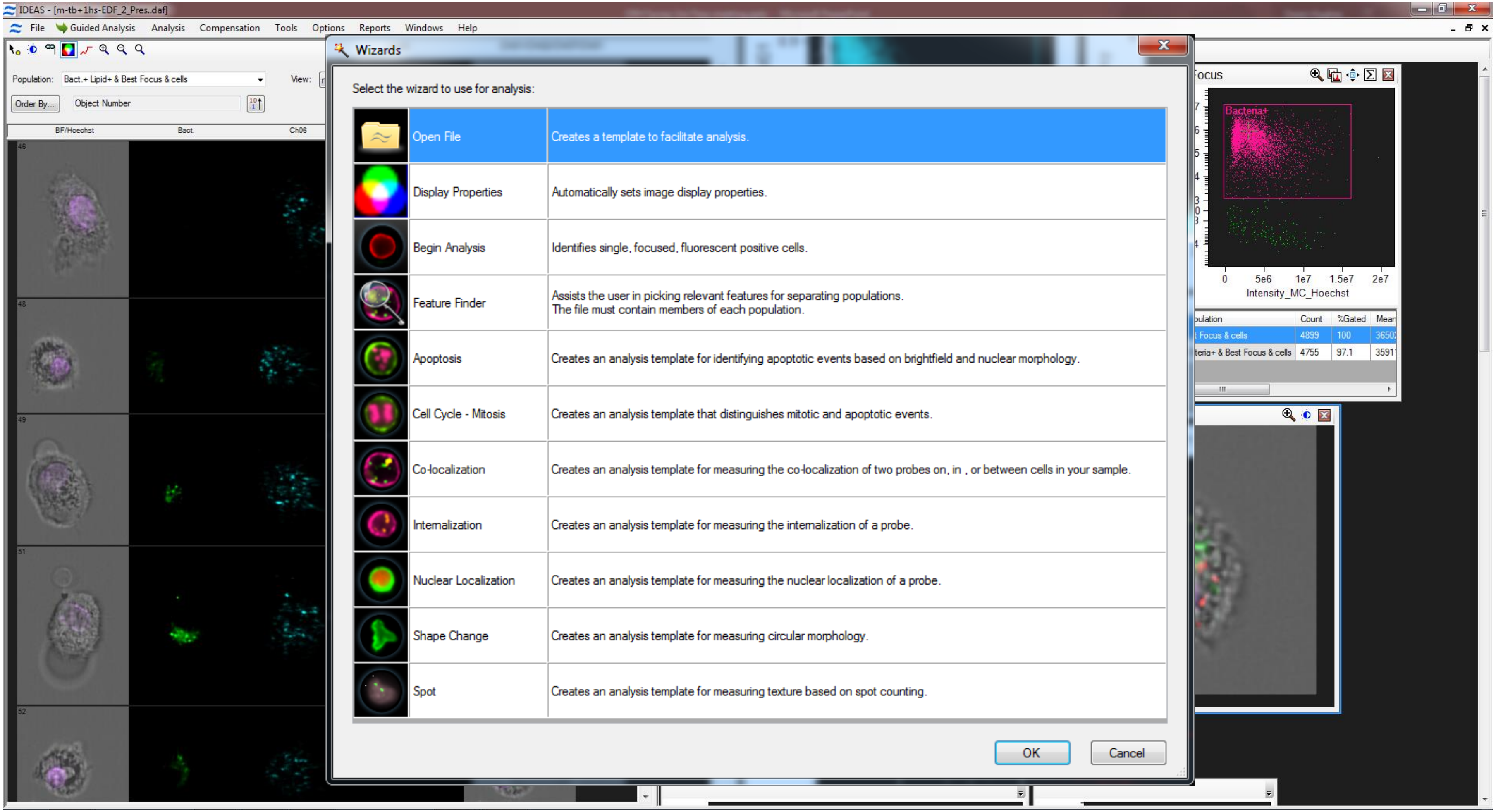
Comparison features quantify intensity differences between masks or pixels:

Intensity Concentration Ratio; Internalization; Similarity; Bright Detail Similarity R3, Bright Detail Similarity 3Way

System features do not require a mask:

Camera Line Number; Camera Timer; Flow Speed; Object Number; Objects per second; Objects per ml and Time

图像数据挖掘分析软件： IDEAS



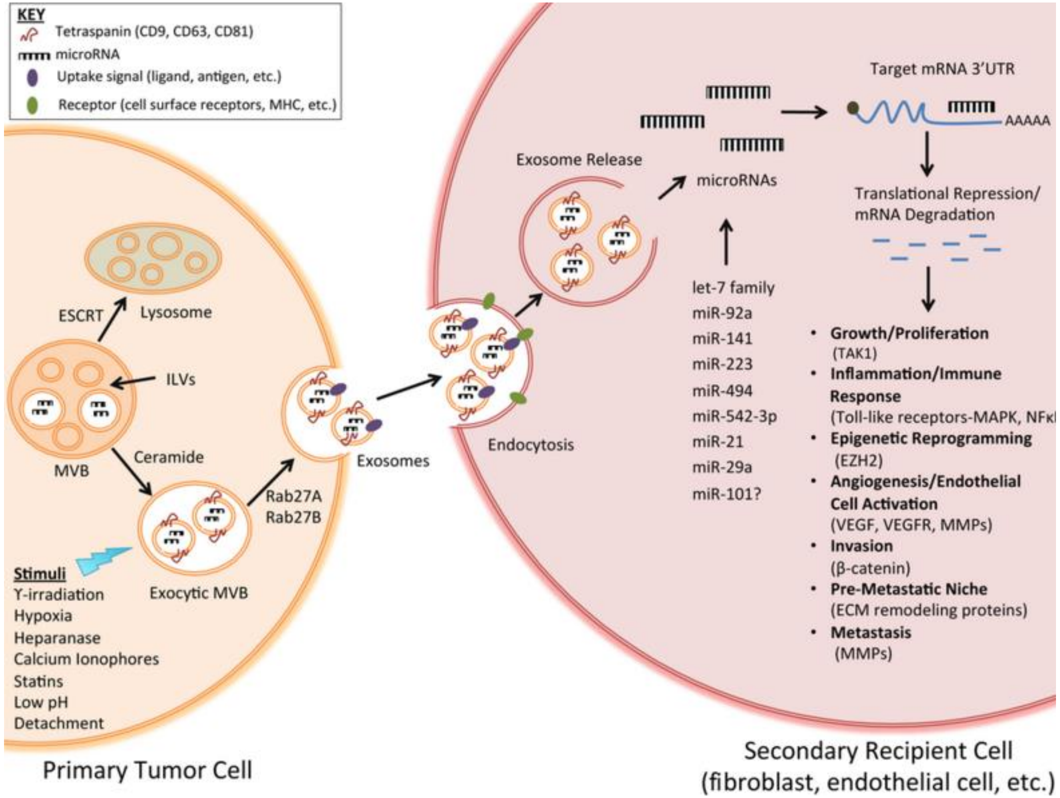
更简单操作流程

- 全自动校正：
  - ✓ 激光器
  - ✓ 对焦
  - ✓ 明场
  - ✓ 光谱
  - ✓ 暗场
  - ✓ 同步
  - ✓ 液流
  - ✓ 成像
- 10分钟完成校正
- 全自动清洗流程
- 自动补偿
- 引导式软件分析
- 内置自动筛选分析参数模式，简化分析过程

Image Stream MKII

微粒研究

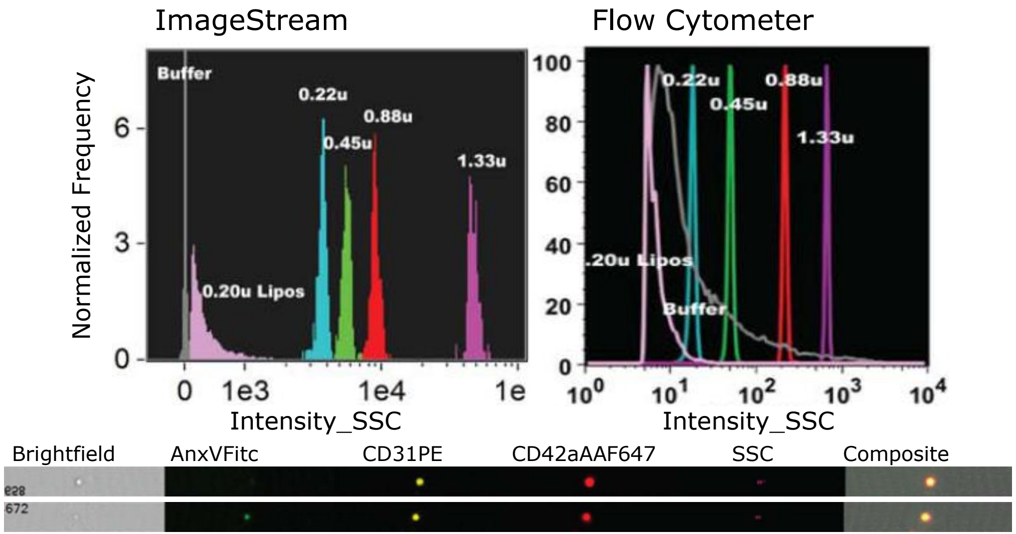
- 外泌体、微囊泡、凋亡小体等
- 50 – 150 nm
- 胞吞、内化等



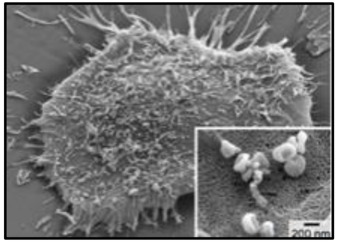
Intercellular Communication by Exosome-Derived microRNAs in Cancer
Bethany N. Hannafon and Wei-Qun Ding
Pol Arch Med Wewn. 2015;125(5):370-80. Epub 2015 May 15.

外泌体检测

- 难点:
- PMT的噪音背景
- 抗体的非特异性结合
- 溶液中的一些小颗粒干扰



Imaging flow cytometry elucidates limitations of microparticle analysis by conventional flow cytometry
Erdbrügger, U., Rudy, C. K., E. Etter, M., Dryden, K. A., Yeager, M., Klibanov, A. L. and Lannigan, J. (2014). Cytometry, 85: 756–770. doi: 10.1002/cyto.a.22494

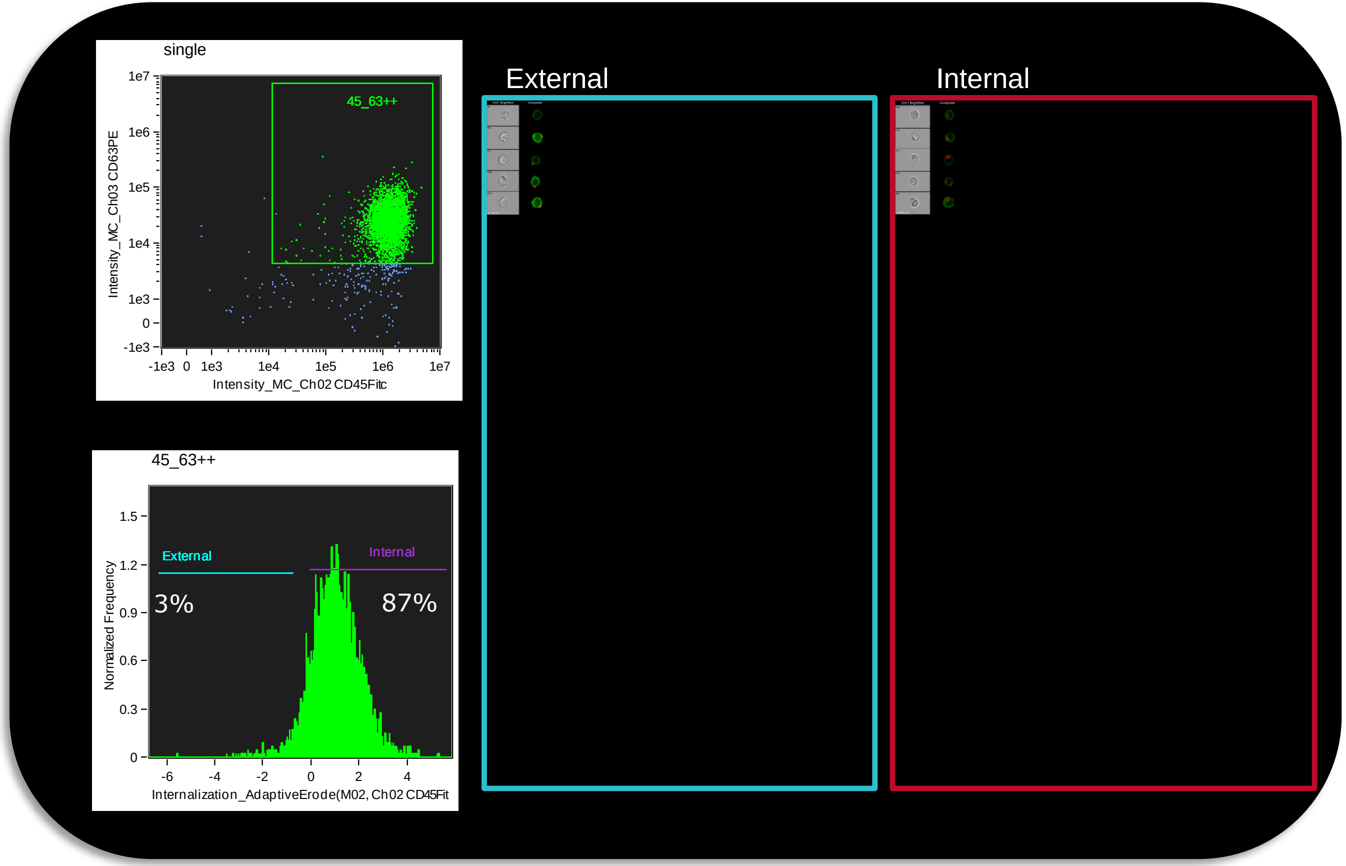


SEM of hGlioblastoma cell
Releasing exosomes.

Hakho Lee: Center For Systems Biology

MKII：Jurkat内吞CD63^{+}EVs

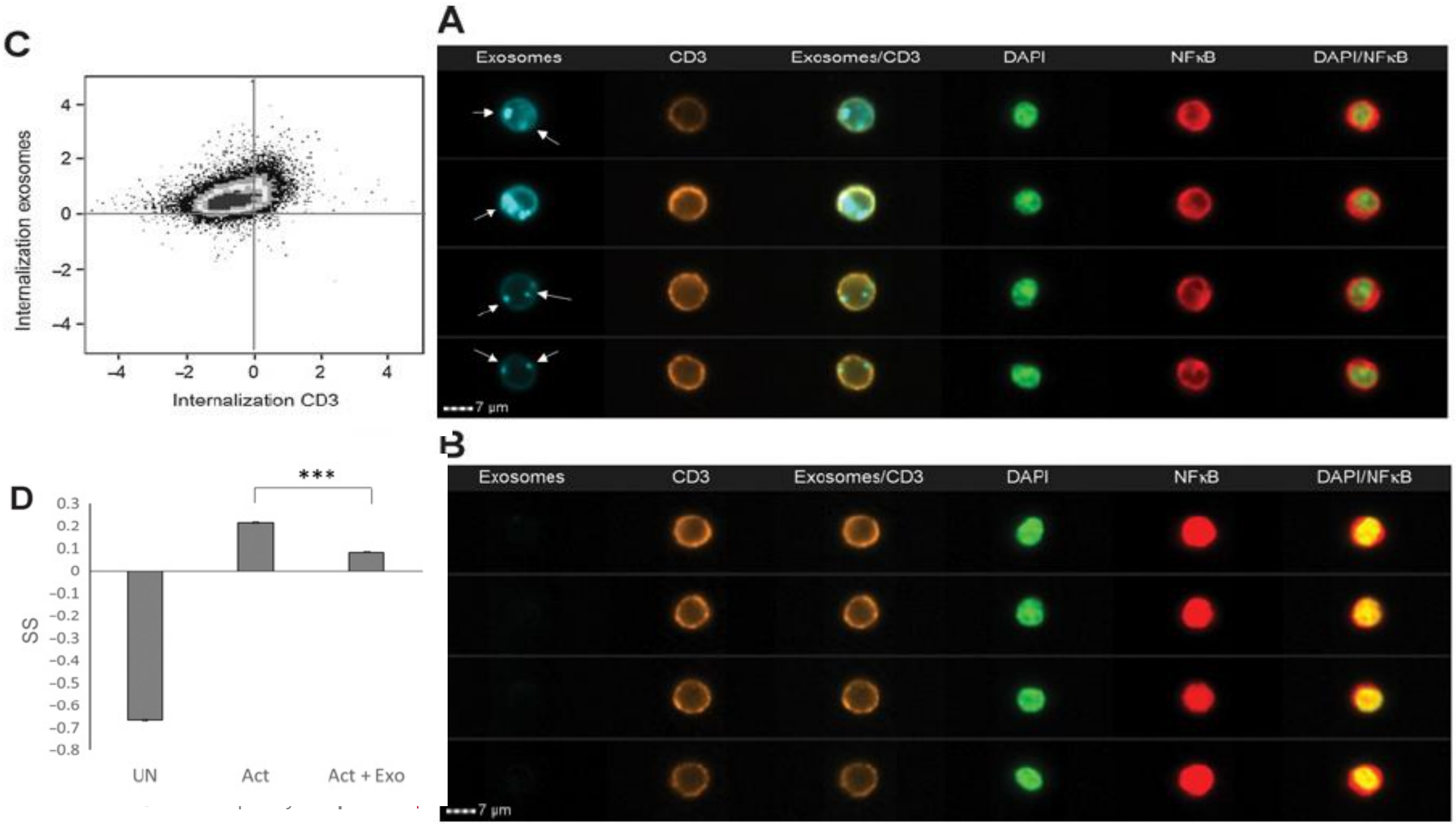
- CD63^{+}外泌体是一种关键的细胞通讯机制，与各种炎症、心血管、代谢、神经退行性疾病和癌症有关
- 使用Amicon Ultra-0.5 ml小颗粒浓缩器纯化CD63^{+}外泌体，并用PE标记
- 将CD63^{+} PE外泌体加入Jurkat细胞中孵育3小时，1%PFA固定
- ImageStream上60倍成像，收集10000个细胞，并对其内化水平进行量化



Cancer Immunol Res. 2018 Feb;6(2):236-247. doi: 10.1158/2326-6066.CIR-17-0113. Epub 2018 Jan 4.

Exosomes Associated with Human Ovarian Tumors Harbor a Reversible Checkpoint of T-cell Responses.

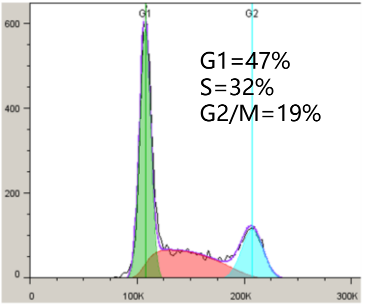
Shenoy GN[1], Loyall J[1], Maguire O[2], Iyer V[3], Kelleher RJ Jr[1], Minderman H[2], Wallace PK[4], Odunsi K[5], Balu-Iyer SV[3], Bankert RB[6].

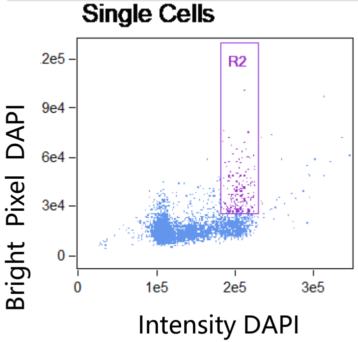


白色箭头所示是被内吞的外泌体

人卵巢瘤的腹水中的外泌体会抑制T细胞的一些信号激活，比如NFkB的激活
A.培养时加入外泌体的T细胞没有发生NFkB转位
B.没有加入外泌体的T细胞发生了核转位

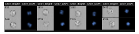
细胞周期分析





Prophase

Metaphase



Anaphase

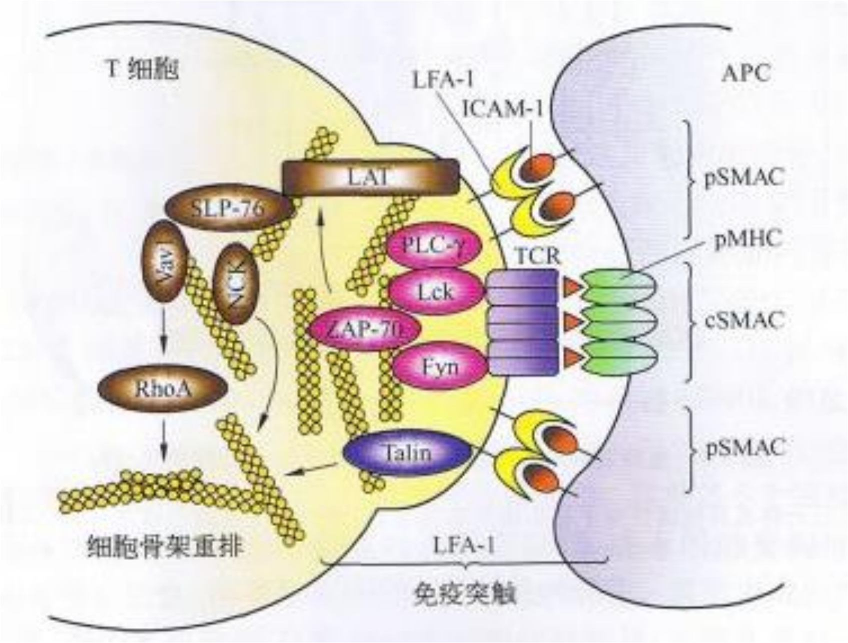
Telophase

时期	G0/G1期	S期	G2/M期	前期	中期	后期	末期	凋亡
细胞比例（%）	48.1	25.2	15.1	0.4	0.5	0.05	0.04	0.5

量化成像流式独有应用 细胞间相互作用

T细胞和抗原递呈细胞APC间免疫突触的形成

免疫突触的形成是T细胞识别抗原 、增殖和活化的关键步骤，是机体细胞免疫应答的重要组成部分。



量化成像流式独有应用 细胞间相互作用

T细胞和抗原递呈细胞APC间免疫突触的形成

将APC和T细胞按照1：1混合

↓

固定、打孔

↓

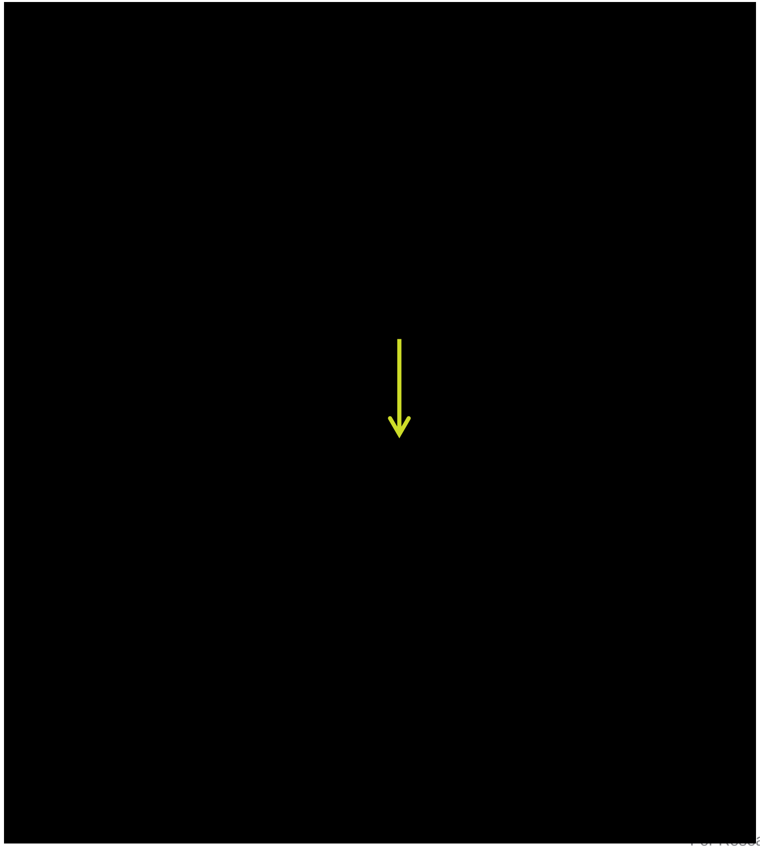
用CD3-PE-TR标记T细胞
用CD19-AF488标记APC细胞
用phalloinin-AF647标记Actin

↓

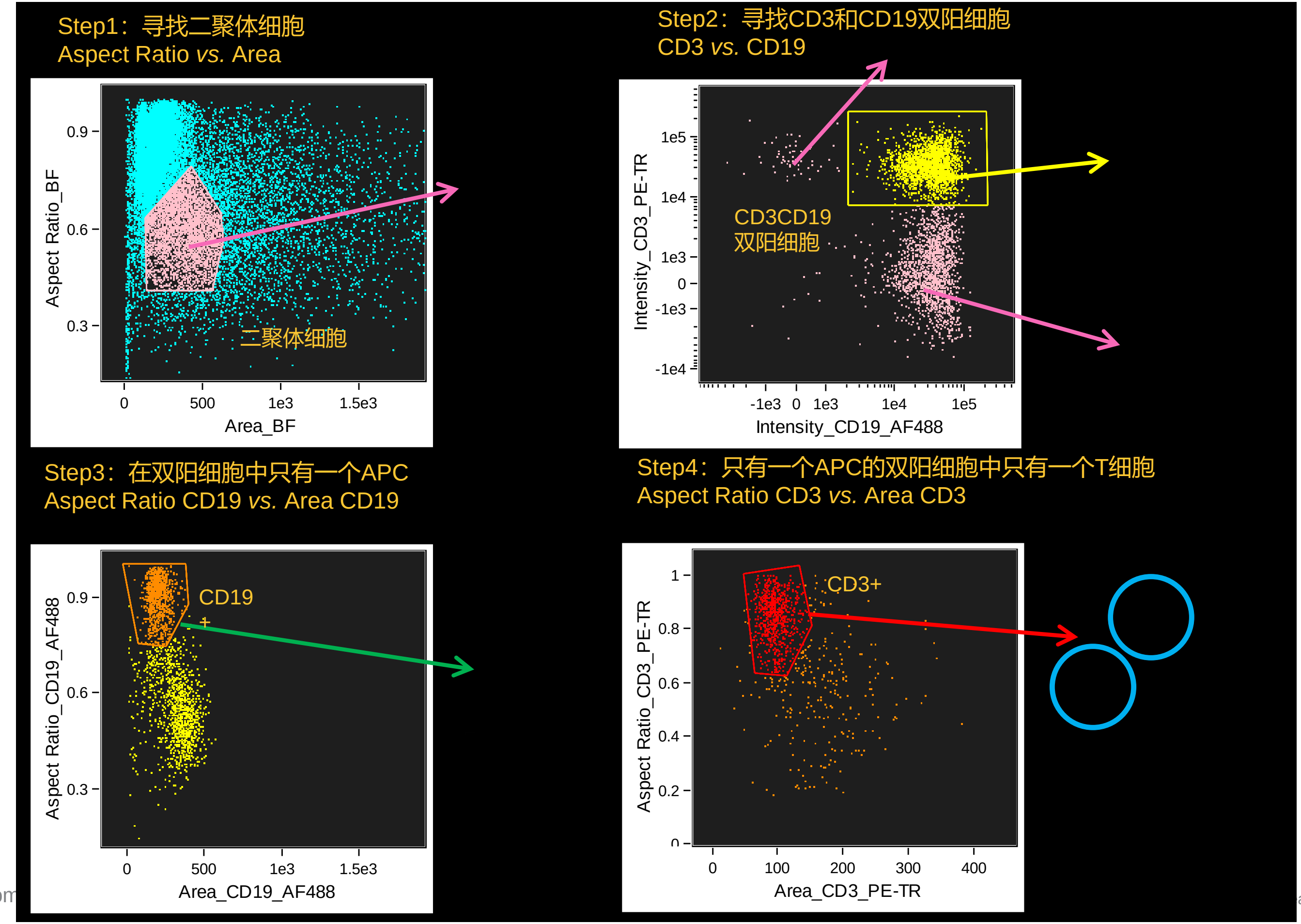
收集30000个细胞数据

↓

定量分析出现免疫突触的几率

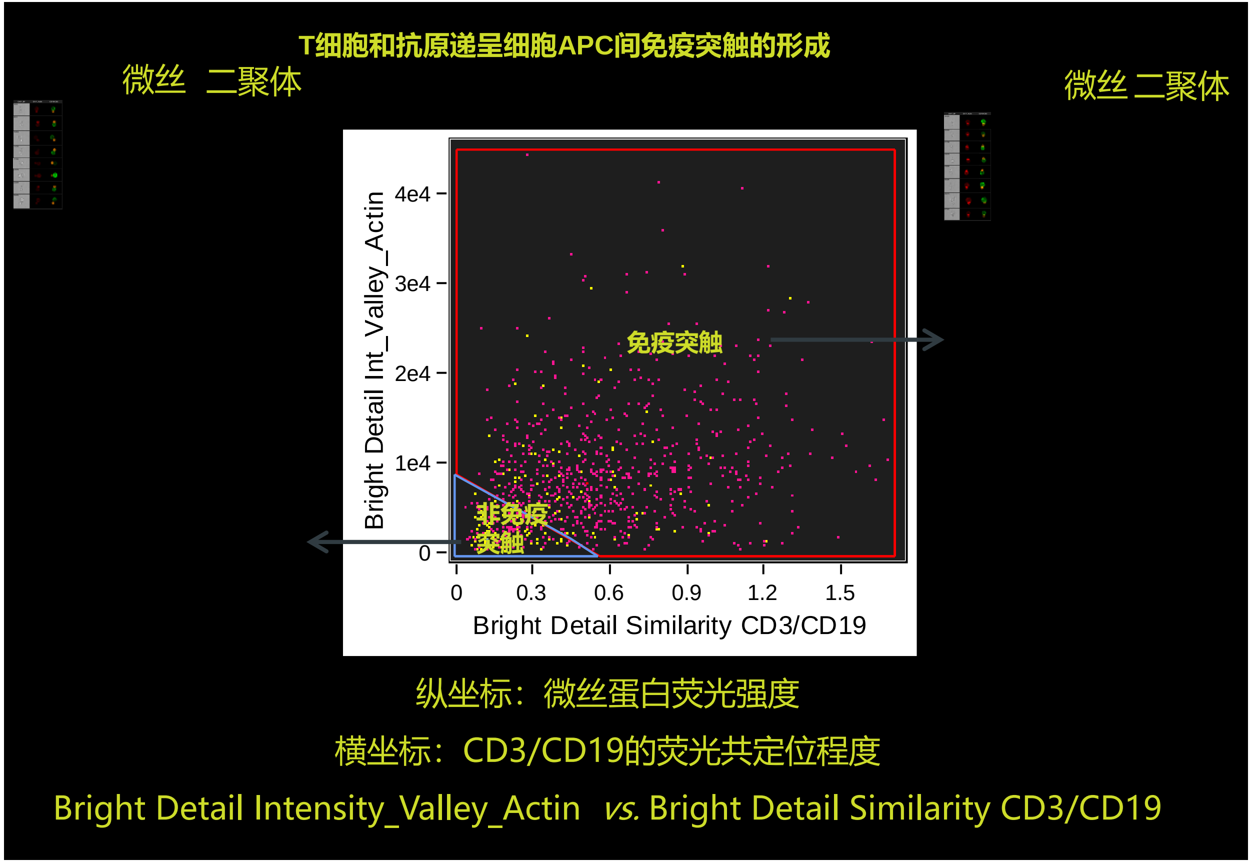


圈门策略



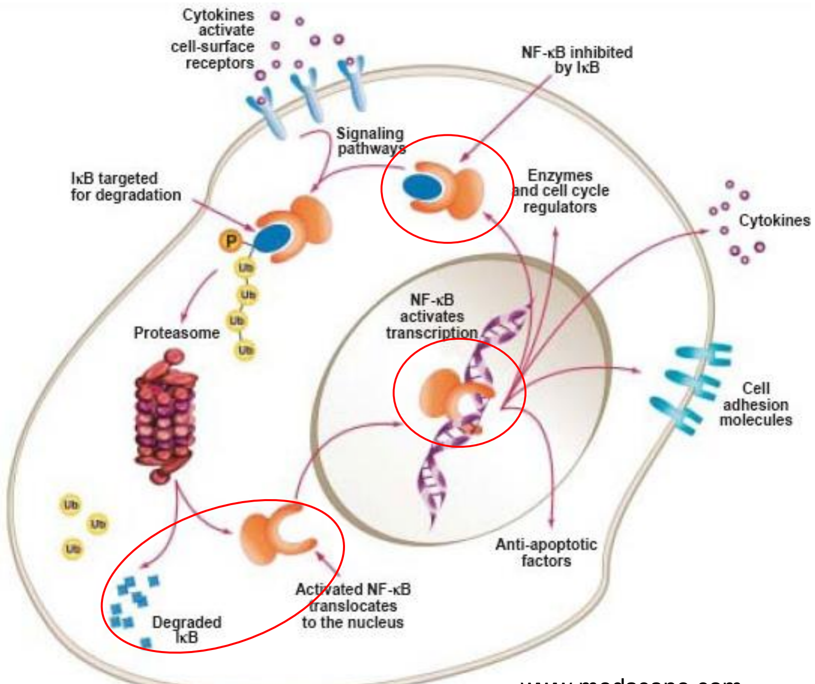
Step1：寻找二聚体细胞
Aspect Ratio vs. Area
Aspect Ratio_BF
Area_BF
二聚体细胞
Step2：寻找CD3和CD19双阳细胞
CD3 vs. CD19
Intensity_CD3_PE-TR
Intensity_CD19_AF488
CD3CD19
双阳细胞
Step3：在双阳细胞中只有一个APC
Aspect Ratio CD19 vs. Area CD19
Aspect Ratio_CD19_AF488
Area_CD19_AF488
CD19
+
Step4：只有一个APC的双阳细胞中只有一个T细胞
Aspect Ratio CD3 vs. Area CD3
Aspect Ratio_CD3_PE-TR
Area_CD3_PE-TR
CD3+

量化成像流式独有应用 细胞间相互作用



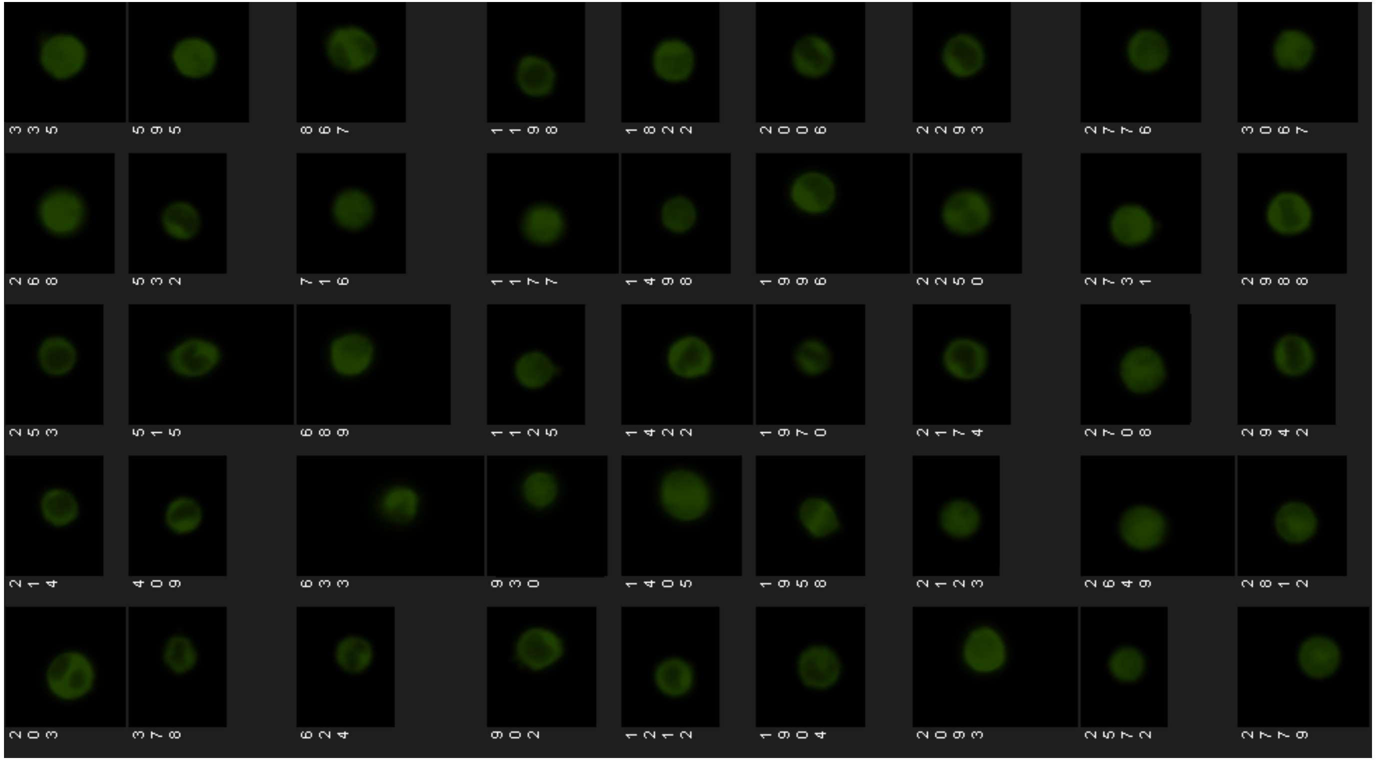
蛋白质转位程度的定量分析

NF-κB是一类重要的转录因子，能够在多种组织中激活不同基因的表达，
与慢性和急性炎症，自身免疫性疾病及多种癌症的发生存在着联系。

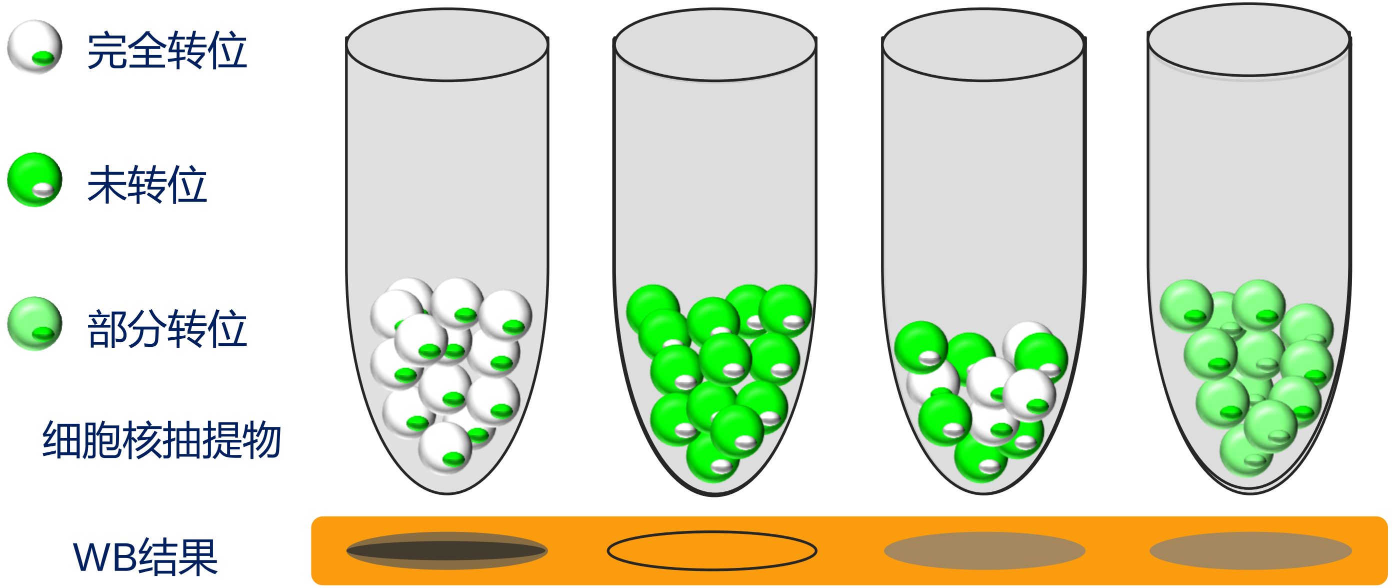


www.medscape.com

传统方法—荧光显微镜



传统方法——Western Blotting



不适合分析混合细胞群；操作复杂、耗时长、不稳定；半定量

全血细胞中NFkB的转位

LPS刺激全血

↓

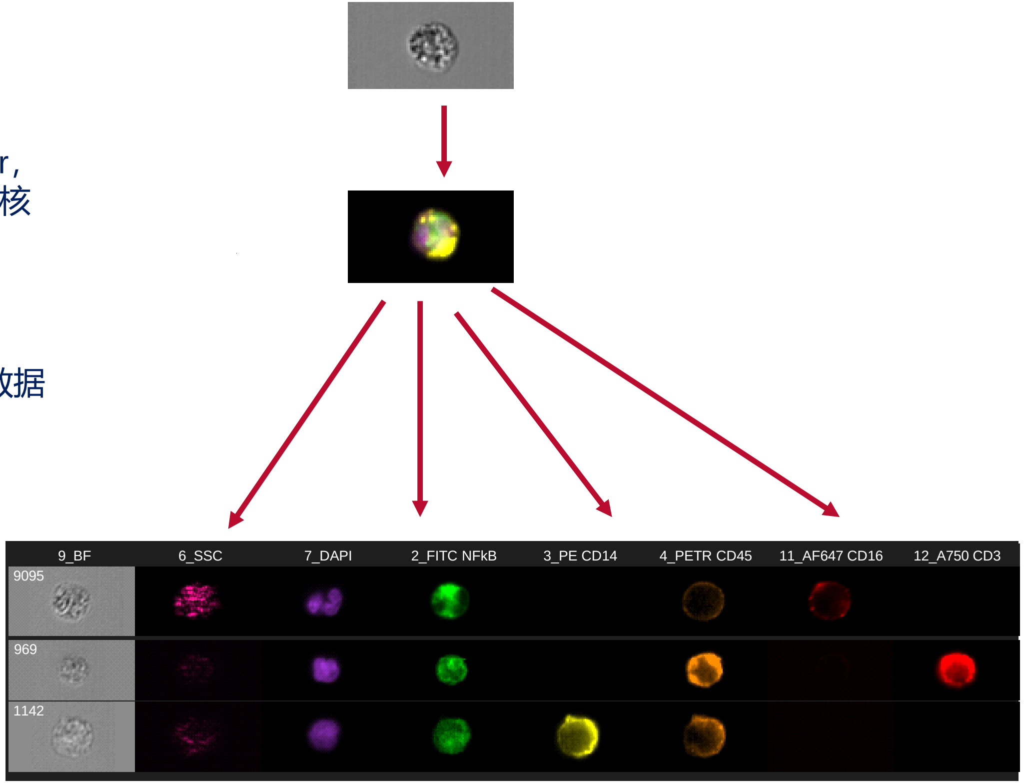
4色标记白细胞表面Marker，
FITC标记NFkB， DAPI染核

↓

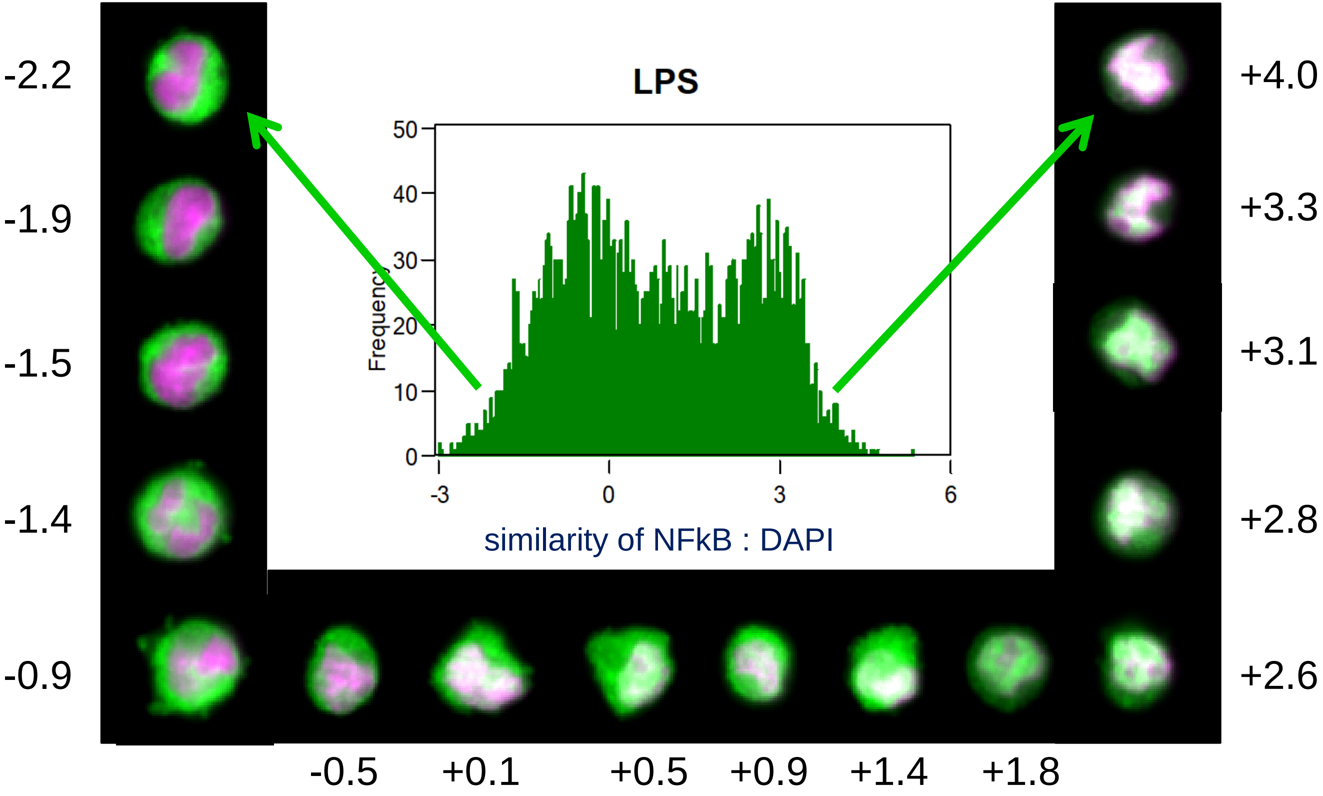
用Amnis收集20000个细胞数据

↓

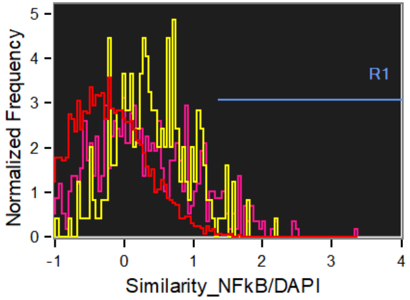
量化分析NFkB在
细胞内的定位



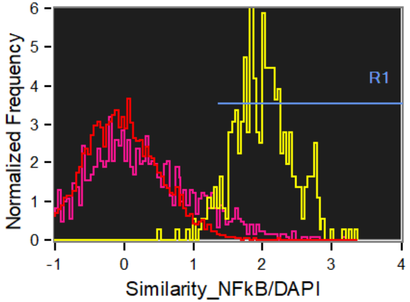
利用Similarity衡量全血单核细胞NFkB转位



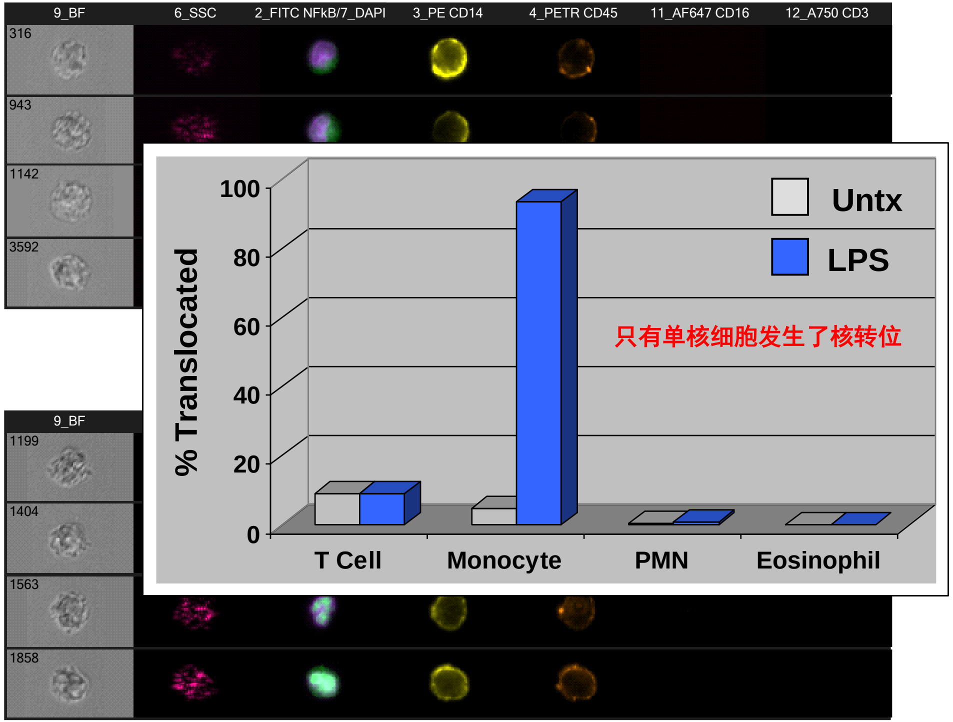
单核, 中性粒, T细胞



对照组

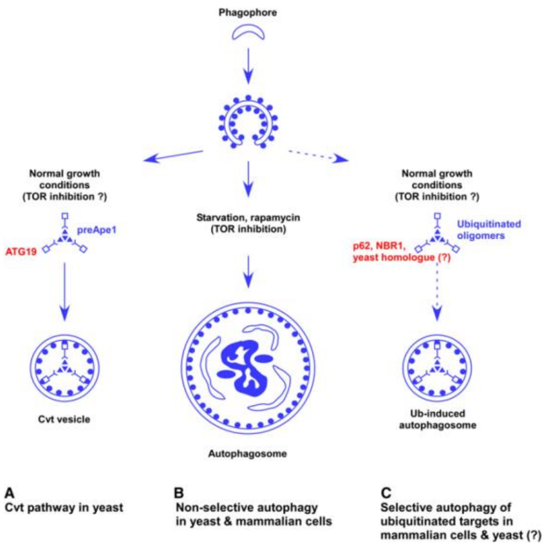


LPS刺激组

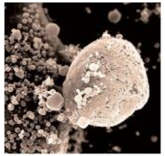


量化分析自噬程度

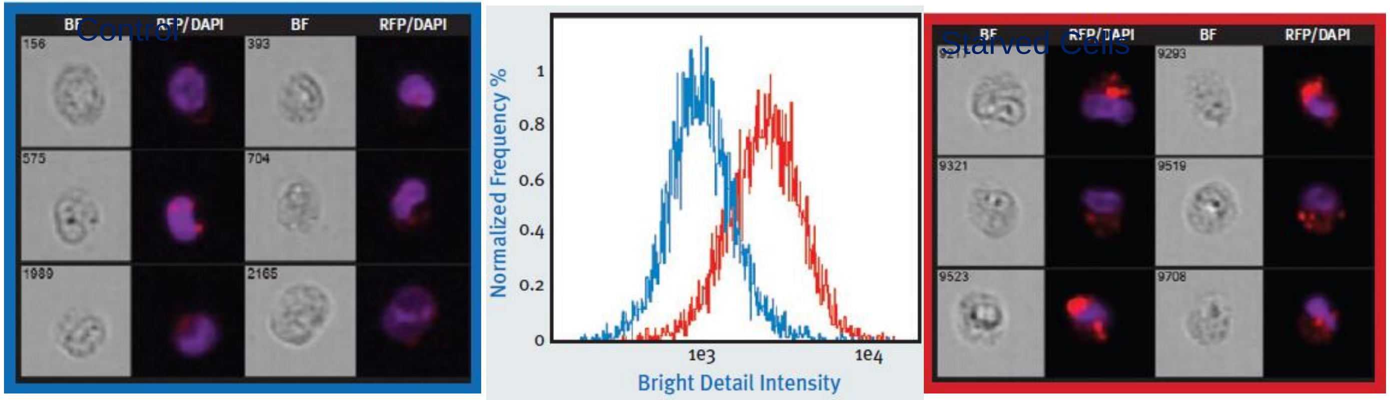
自噬 (autophagy)
由 Ashford 和 Porter 在 1962 年发现细胞内有“自己吃自己”的现象后提出的，是指从粗面内质网的无核糖体附着区脱落的双层膜包裹部分胞质和细胞内需降解的细胞器、蛋白质等成分形成自噬体 (autophagosome) ，并与溶酶体融合形成自噬溶酶体，降解其所包裹的内容物，以实现细胞本身的代谢需要和某些细胞器的更新。



引发自噬的因素
- ✓ 饥饿
- ✓ 生理压力
- ✓ 药物
- ✓ 感染

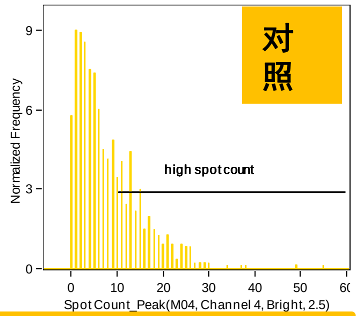


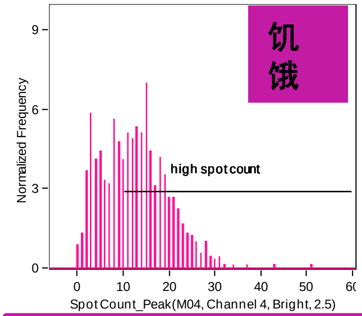
饥饿引起的自噬现象



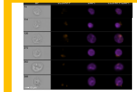
Histograms for the control non-starved cells and starved cells are shown, along with representative images of the selected regions. The brightfield image and a composite of the RPF-LC3 (red) and DAPI (purple) nuclear dye are shown. The starved cells with more LC3 puncta have higher BDI values.

自噬的定量分析：Spot Count

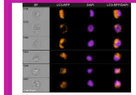




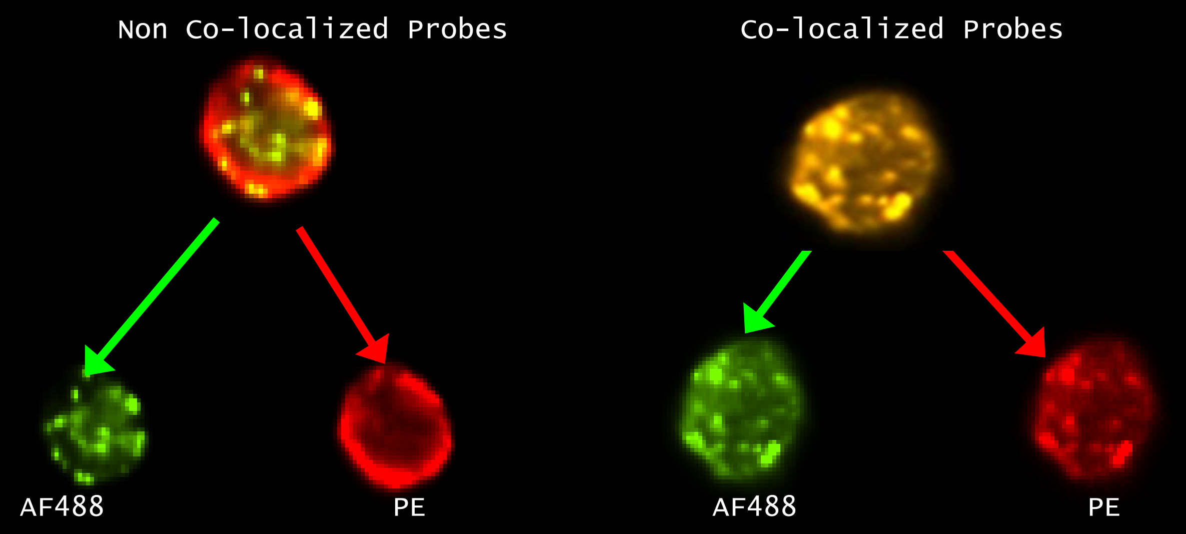
Low spot count



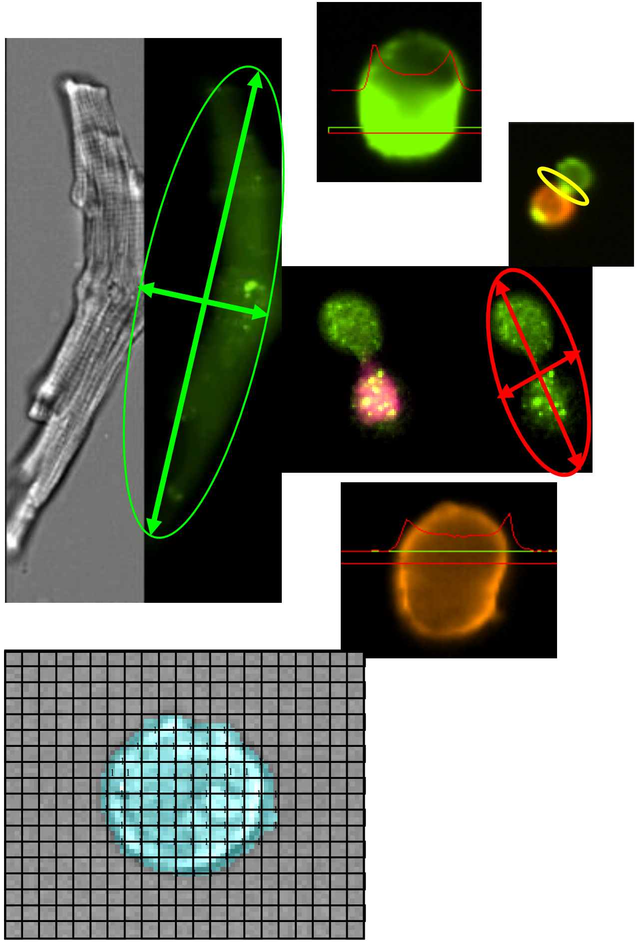
High spot count



自噬的定量分析：Bright Detail Similarity



Degree of probe co-localization is measured by performing cross-correlation analysis of the small bright regions of pairs of images of the same cell



量化成像流式独有应用 细胞形态学变化

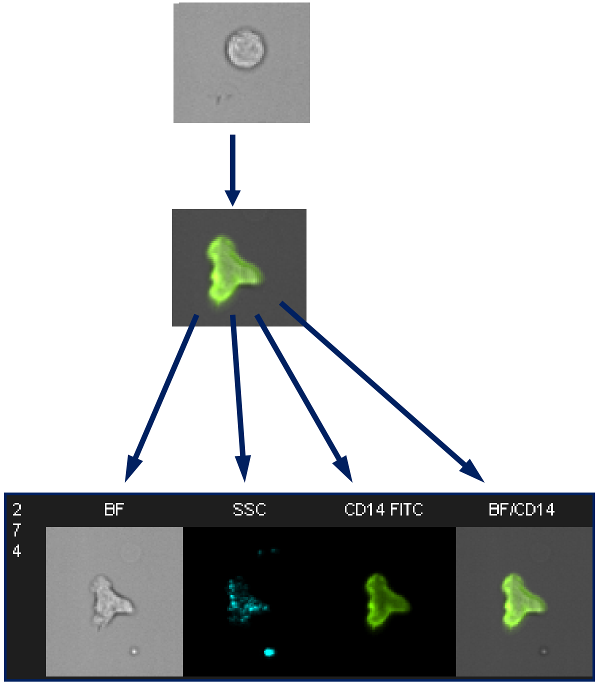
化学诱剂MCP-1引起的外周血中单核细胞形变

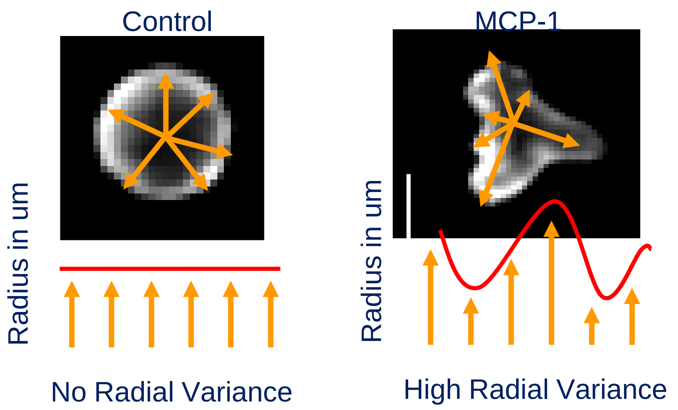
MCP-1刺激人的外周血细胞

FITC anti-CD14标记细胞膜、去红

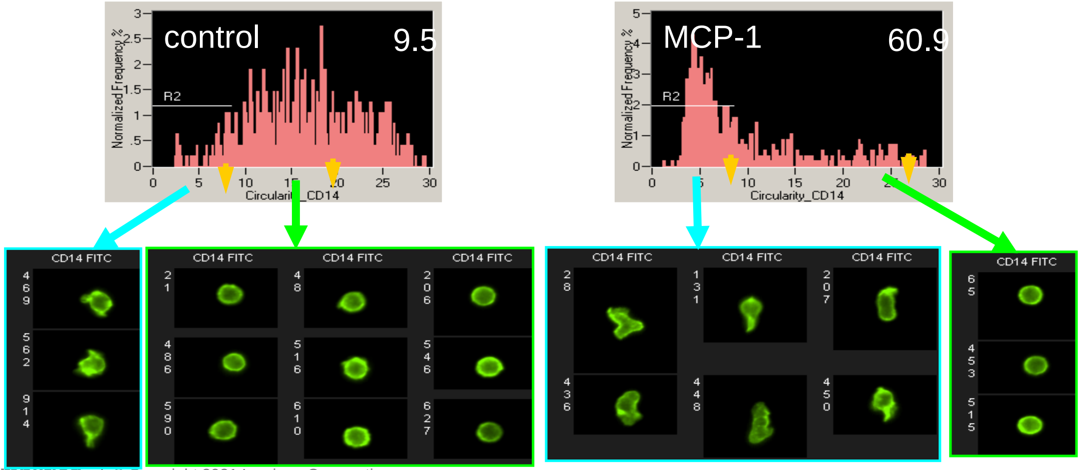
量化成像流式收集5000个细胞

用参数Circularity定量分析形变

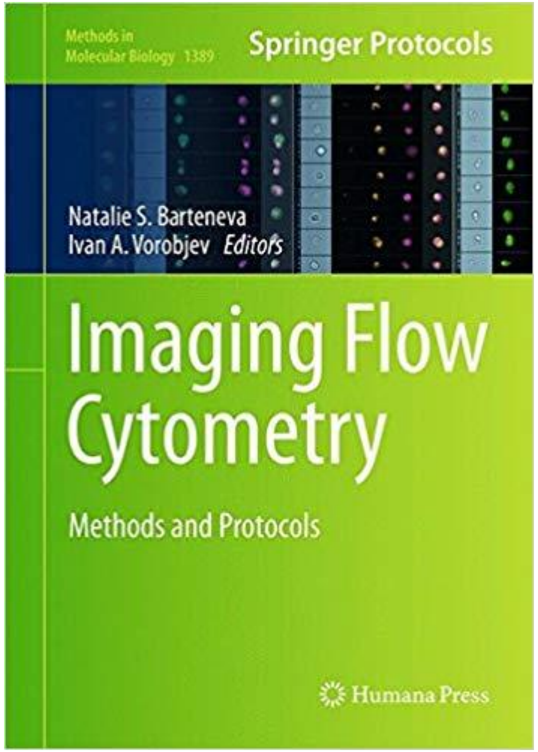




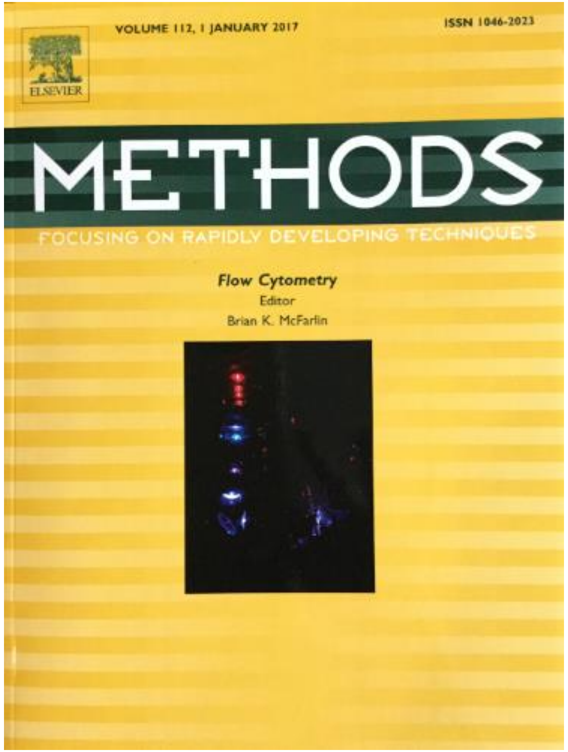
Circularity =
平均半径/径向变化



Journal of Immunological Methods



Methods In Molecular Biology



Methods, Volume 112

And numerous webinars, application notes and application scientists also available!

肝脏和细胞因子人源化可改善模型鼠体内人红细胞的循环

HEMATOLOGY

Combined liver–cytokine humanization comes to the rescue of circulating human red blood cells

Yuanbin Song[1,2,3]*, Liang Shan[4,5]*†, Rana Gbyli[1,2]*, Wei Liu[1,2], Till Strowig[4,6], Amisha Patel[1,2], Xiaoying Fu[1,2,7], Xiaman Wang[1,2,8], Mina L. Xu[9], Yimeng Gao[1,2], Ashley Qin[1,2], Emanuela M. Bruscia[10], Toma Tebaldi[1,2,11], Giulia Biancon[1,2], Padmavathi Mamillapalli[1,2], David Urbonas[4], Elizabeth Eynon[4], David G. Gonzalez[12], Jie Chen[4], Diane S. Krause[2,9,13], Jonathan Alderman[4], Stephanie Halene[1,2]†‡, Richard A. Flavell[4,14]†‡

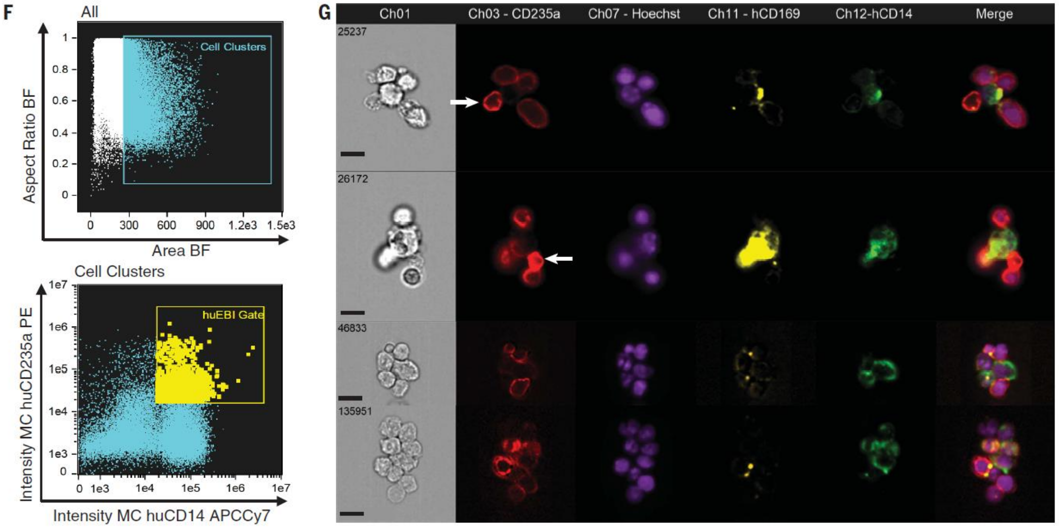
Song *et al.*, *Science* **371**, 1019–1025 (2021)   5 March 2021

肝脏和细胞因子人源化可改善模型鼠体内人红细胞的循环

- **MISTRG**小鼠模型：表达人巨噬细胞集落刺激因子(M-CSF)、人白介素3(IL-3)/粒细胞-巨噬细胞集落刺激因子(GM-CSF)、人信号调节蛋白α(SIRPa)、人血小板生成素(TPO)及人红细胞生成素(EPO)基因，但不表达重组激活基因2(RAG2)或白介素2受体γ(IL2Rg)的小鼠

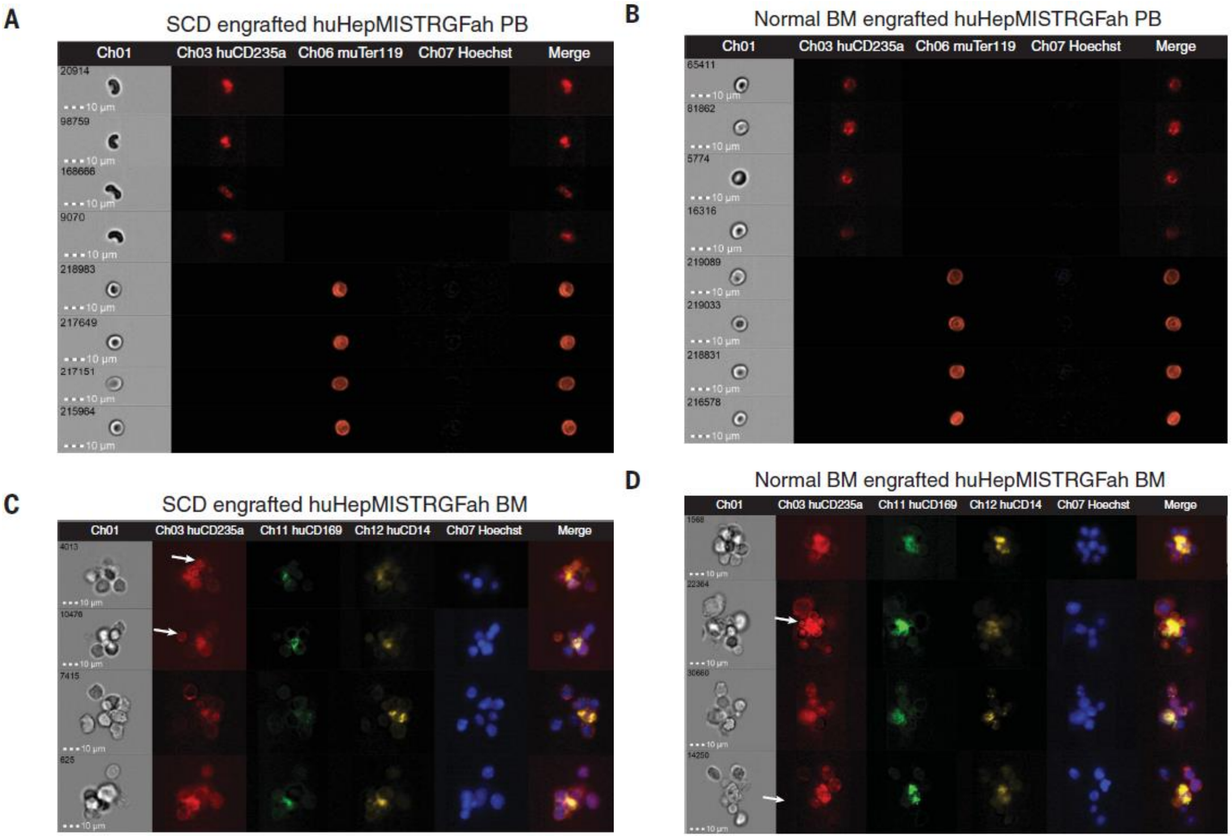
- **huHepMISTRGFah**小鼠模型：在**MISTRG**小鼠模型的基础上利用**CRISPR-Cas9**技术去除了**Fah**基因**,**同时通过脾内注射人的肝脏细胞，停用**NTBC**，导致模型鼠的肝细胞衰竭死亡，人的肝细胞生长，获得肝脏和细胞因子人源化的小鼠模型

肝脏和细胞因子人源化可改善模型鼠体内人红细胞的循环



ImageStream(40X)鉴定了**huHep**模型鼠骨髓中的**EBIs,** 具有与人类**EBIs**同样的属性，**huCD169+huCD14+**的中央的巨噬细胞被不同大小的**huCD235a+**的红细胞包围，包括有核的和无核的红细胞

肝脏和细胞因子人源化可改善模型鼠体内人红细胞的循环



利用**huHepMISTRGFah**模型鼠建立**SCD**疾病模型：进行骨髓中镰刀状红细胞以及**EBI**的鉴定

A和**B**图是对两组小鼠中的镰刀状红细胞的鉴定

C和**D**是对两组小鼠中**EBIs**的鉴定

巨噬细胞通过gp130促进心肌增殖和心脏再生

MK II IF: 23.603

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Gp130 Controls Cardiomyocyte Proliferation and Heart Regeneration

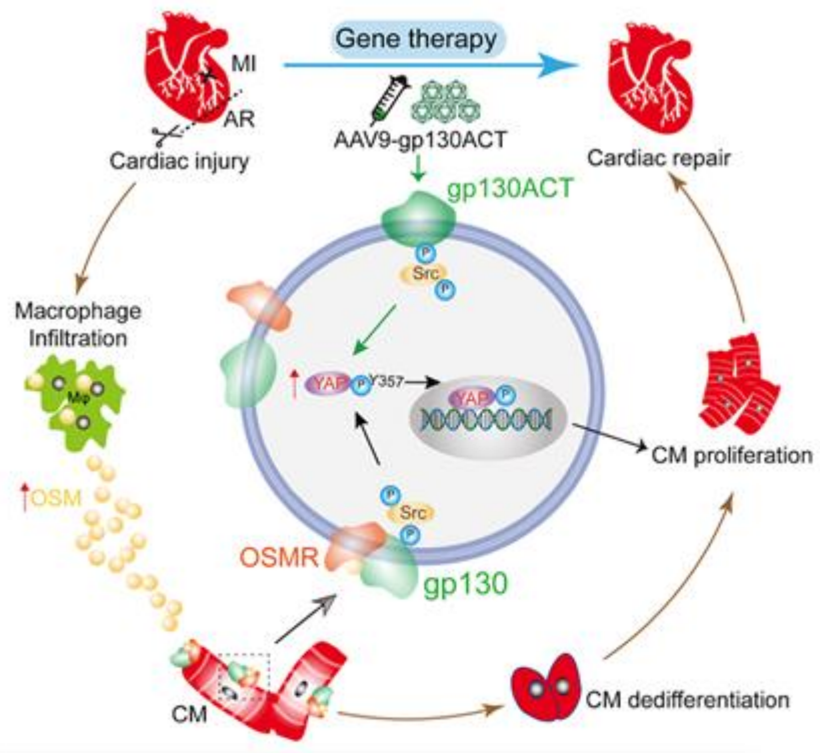
Yandong Li, Jie Feng, Shen Song, Haotong Li, Huijun Yang, Bin Zhou, Yan Li, Zhang Yue, Hong Lian, Lihui Liu, Shengshou Hu, and Yu Nie

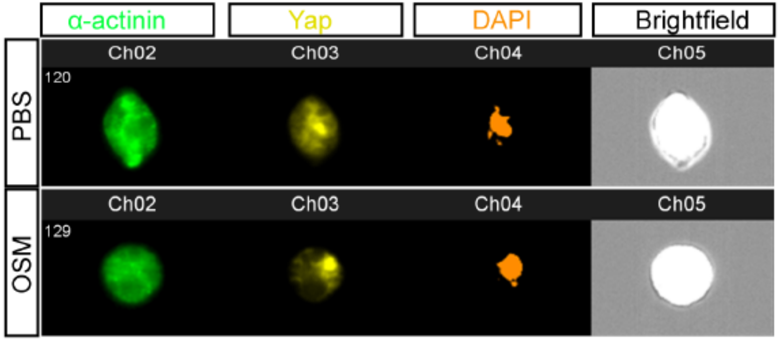
Originally published 30 Jun 2020 | https://doi.org/10.1161/CIRCULATIONAHA.119.044484 | Circulation. 2020;0

- 巨噬细胞在调节哺乳动物心肌再生中起到了重要的作用。巨噬细胞可以通过分泌炎症因子，激活gp130受体，促进心肌细胞增殖和心脏再生，为心血管疾病的治疗提供了新的干预靶点。该研究于2020年6月30日在线发表于 Circulation 杂志。该论文第一作者是北京协和医学院2015级博士生李衍冬，通讯作者是胡盛寿院士和聂宇教授。

巨噬细胞通过gp130促进心肌增殖和心脏再生

MK II IF: 23.603





OSM处理后Yap在细胞核内浓度升高，提示Yap参与了gp130激活后促进心肌细胞增殖的调控

抑瘤素M（OSM）通过OSMR/gp130异源二聚体受体，作用于心肌细胞，促进小鼠心肌细胞的增殖和心脏的再生。gp130磷酸化可以调节Yap蛋白入核，从而发挥促进心肌细胞增殖的作用，gp130激活Yap是通过Src蛋白磷酸化实现的。

氯化对细胞激活、毒素释放和蓝藻降解的影响

IF: 10.652

Impact of chlorination on cell activation, toxin release and degradation of cyanobacteria of development and maintenance stage

中国科学院城市环境与健康重点实验室

Xi Li, Sheng Chen, Jie Zeng, Kassim Chabi, Weijun Song, Xuanxuan Xian, Xin Yu

氯是饮用水处理厂处理蓝藻的常用氧化剂。在天然淡水中，蓝藻水华是一个连续的过程，主要包括发展和维持阶段。文章比较了两个阶段的细胞特性及氯化对细胞失活、毒素去向的影响。结果表明，两个阶段的微囊藻都保持了较高的光合能力，在氯化过程中，这两个阶段的微囊藻都可以通过破坏膜的完整性而被完全灭活。

古菌中病毒介导的细胞体积增大和不对称分裂

MK II IF: 9.412

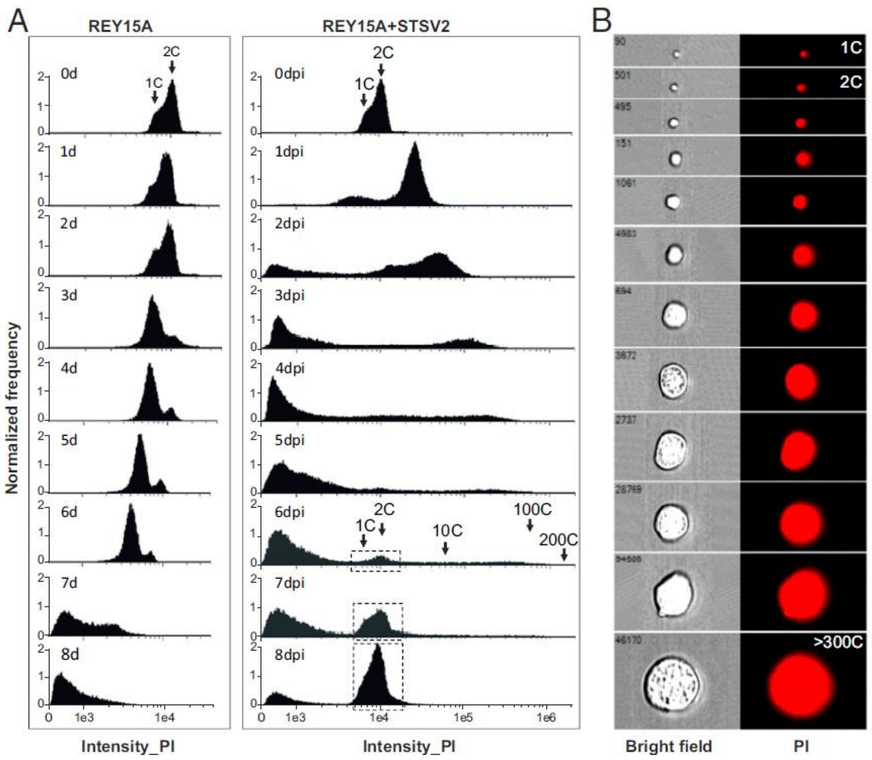
Virus-induced cell gigantism and asymmetric cell division in archaea

Junfeng Liu[a,b], Virginija Cvirkaite-Krupovic[a], Diana P. Baquero[a], Yunfeng Yang[b], Qi Zhang[c], Yulong Shen[b,1], and Mart Krupovic[a,1]

[a]Archaeal Virology Unit, Institut Pasteur, 75015 Paris, France; [b]CRISPR and Archaea Biology Research Center, State Key Laboratory of Microbial Technology, Microbial Technology Institute, Shandong University, 266237 Qingdao, China; and [c]Faculty of Life Science and Technology, Kunming University of Science and Technology, 650500 Kunming, China

Edited by Alice Telesnitsky, University of Michigan Medical School, Ann Arbor, MI, and accepted by Editorial Board Member Michael F. Summers February 25, 2021 (received for review October 28, 2020)

古菌中病毒介导的细胞体积增大和不对称分裂



以嗜酸热古菌冰岛硫化叶菌REY15A和非裂解性纺锤状病毒STSV2为材料,研究古菌中病毒对宿主细胞分裂和细胞周期的影响以及宿主在细胞水平上如何获取CRISPR免疫能力。

当REY15A细胞感染STSV2后，细胞周期被阻滞在DNA复制期 (S期)。此时DNA可以正常复制，但细胞分裂过程受到抑制，从而导致感染后的细胞体积变得巨大，细胞直径最大可达正常细胞20倍左右，体积可达正常细胞的8000倍左右。这些大细胞被用作“制造工厂”以进行病毒的大量增殖。

自噬通过促进Sirt3的表达来减缓造血衰老

MK II IF: 7.238

Received: 25 June 2019 | Revised: 24 November 2019 | Accepted: 25 January 2020
DOI: 10.1111/acel.13114

ORIGINAL ARTICLE

Aging Cell WILEY

Deterioration of hematopoietic autophagy is linked to osteoporosis

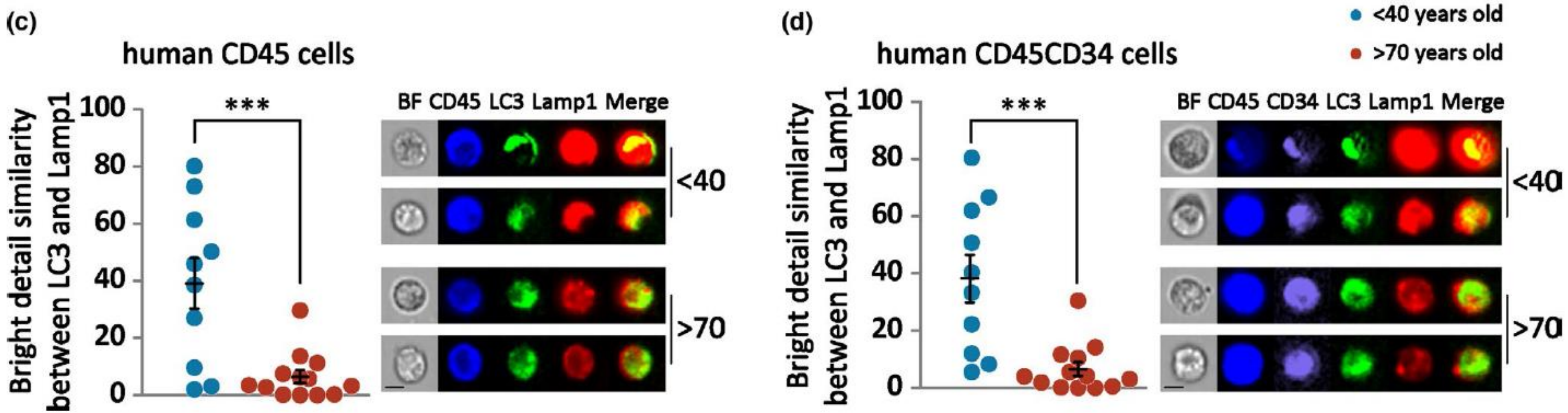
苏州大学附属第一医院国家血液病临床研究中心

Ye Yuan[1,2] | Yixuan Fang[3,4,5] | Lingjiang Zhu[3] | Yue Gu[3] | Lei Li[3] | Jiawei Qian[3] | Ruijin Zhao[3] | Peng Zhang[1,2] | Jian Li[1,2] | Hui Zhang[1,2] | Na Yuan[3,4,5] | Suping Zhang[3,4,5] | Quanhong Ma[5] | Jianrong Wang[3,4,5] | Youjia Xu[1,2]

[1]Department of Orthopaedics, the Second Affiliated Hospital of Soochow University, Suzhou, China
[2]Osteoporosis Institute of Soochow University, Suzhou, China
[3]Research Center for Non-medical Healthcare of Soochow University & Beijing Yaozhongtang, Cyrus Tang Medical Institute, Soochow University School of Medicine, Suzhou, China
[4]Hematology Center, Cyrus Tang Medical Institute, Soochow University School of Medicine, Suzhou, China
[5]Collaborative Innovation Center of Hematology, Jiangsu Institute of Hematology, Institute of Blood and Marrow Transplantation, Institute of Neuroscience, Key Laboratory of Stem Cells and Biomedical, Materials of Jiangsu Province and Chinese Ministry of Science and Technology, State Key Laboratory of Radiation Medicine and Radioprotection, Soochow University School of Medicine, Suzhou, China

自噬通过促进Sirt3的表达来减缓造血衰老

MK II IF: 7.238



ImageStream定量检测两个年龄组人骨髓原代造血细胞(CD45)、HSC富集造血细胞(CD45CD34)中的基础自噬水平。左图为统计数据。右，细胞的代表性图像，单染（CD45—蓝色；CD34—紫色；LC3—绿色；Lamp1—红色）或两种标记物染色（LC3和Lamp1合并）。

成像流式观察LC3和溶酶体标记物Lamp1，用于测量两个不同年龄人群在自噬溶酶体形成期间造血自噬的活性。结果显示，与青年组相比，两种细胞的基础自噬水平均有明显下降

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