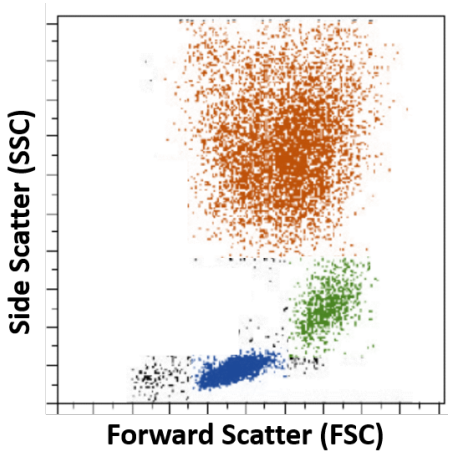
Increasing Access to Hematological Cancer Care
for the Middle of the Pyramid with a Microscopy-Based Approach



Shivang R. Dave, PhD Jason Tucker-Schwartz, PhD Elfar Adalsteinsson, PhD (PI)

NIH POCTRN Symposium
June 9th, 2016

Disclosure

Intellectual property filed and owned by:

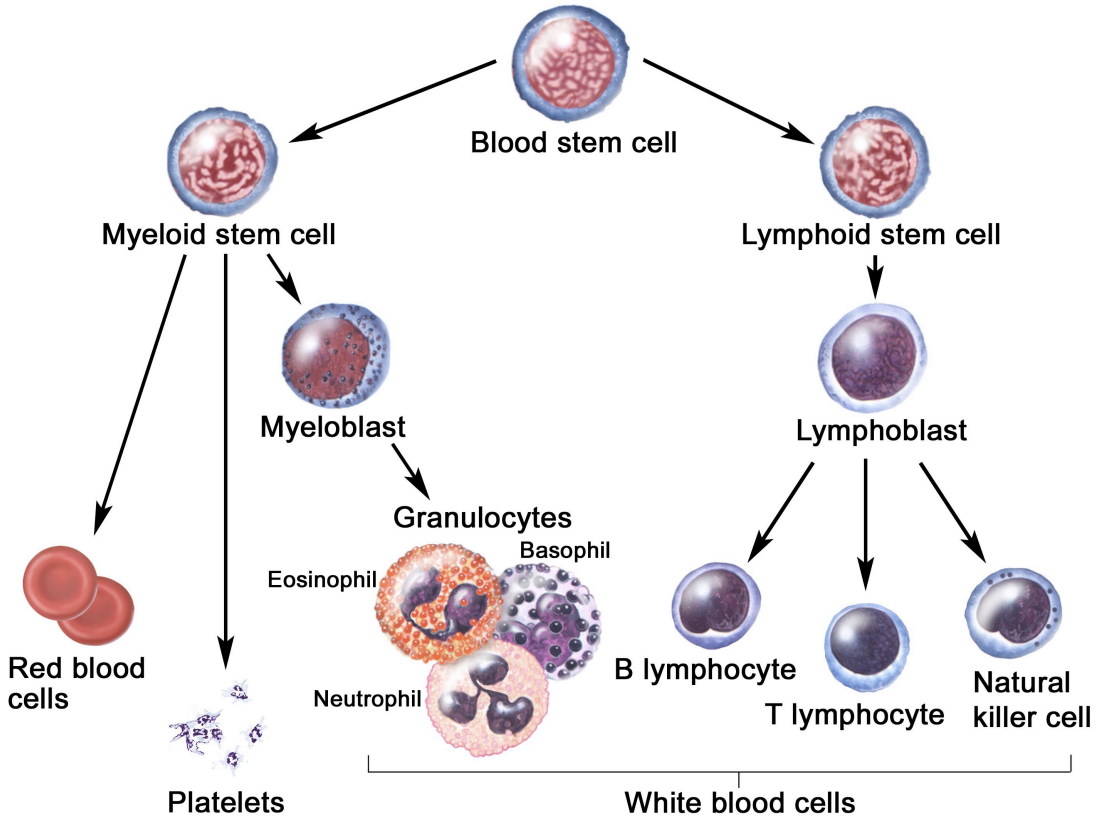
- Previous work (3 patent applications, **SRD**)
- Work funded by this grant (1 patent application, **SRD, JTS**)

IP portfolio was licensed by:

SRD provides technical advice to:

Leukemia & lymphoma are common and varied

Prevalence: **1.2M** Incidence: **171K/year** Mortality: **>58k/year**



Need to distinguish cell type and stage

http://www.cancer.gov/images/cdr/live/CDR526538.jpg
Leukemia & Lymphoma Society. Fact 2014; National Cancer Network. Guidelines. 2014

Outcomes have improved

Generally due to better access, detection, classification, and treatments

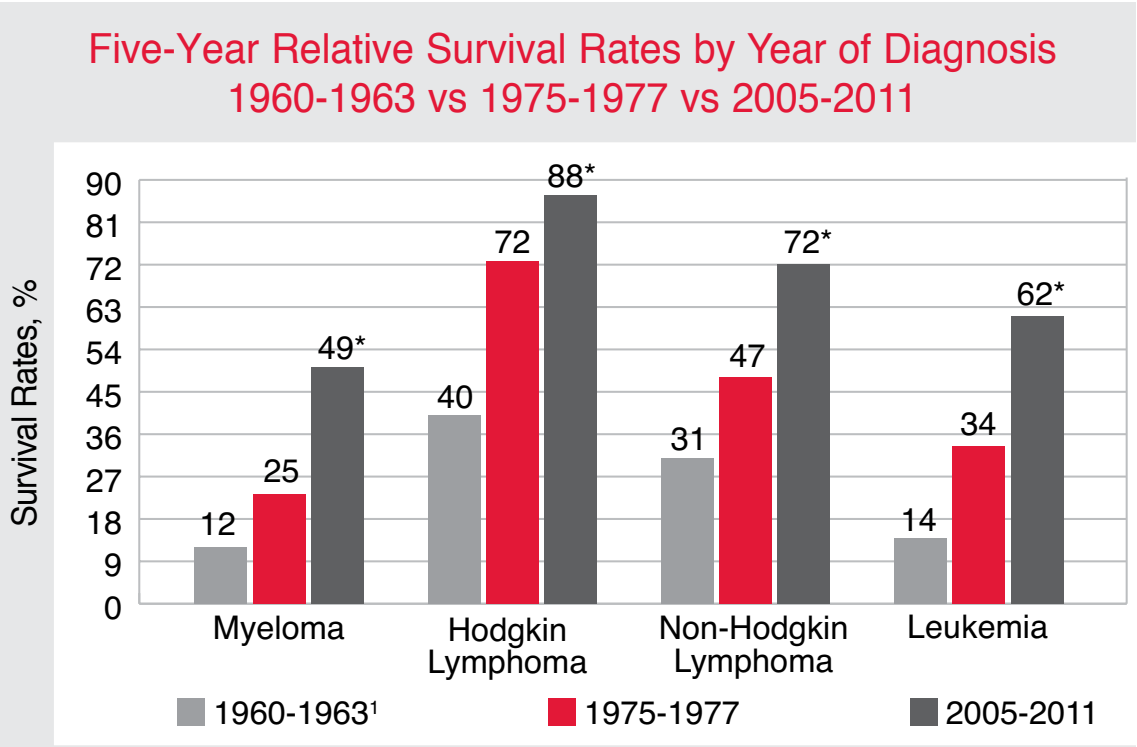


Figure 2. Source: *SEER (Surveillance, Epidemiology and End Results) Cancer Statistics Review, 1975-2011.* National Cancer Institute; 2015.

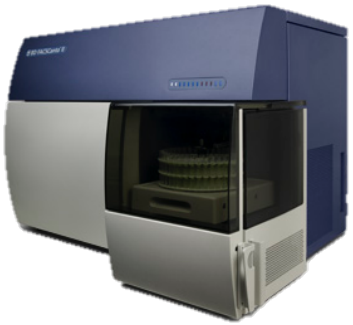
*The difference in rates between 1975-1977 and 2005-2011 is statistically significant ($P<.05$).

[1]Survival rate among whites (only data available).

Cell classification with flow cytometry

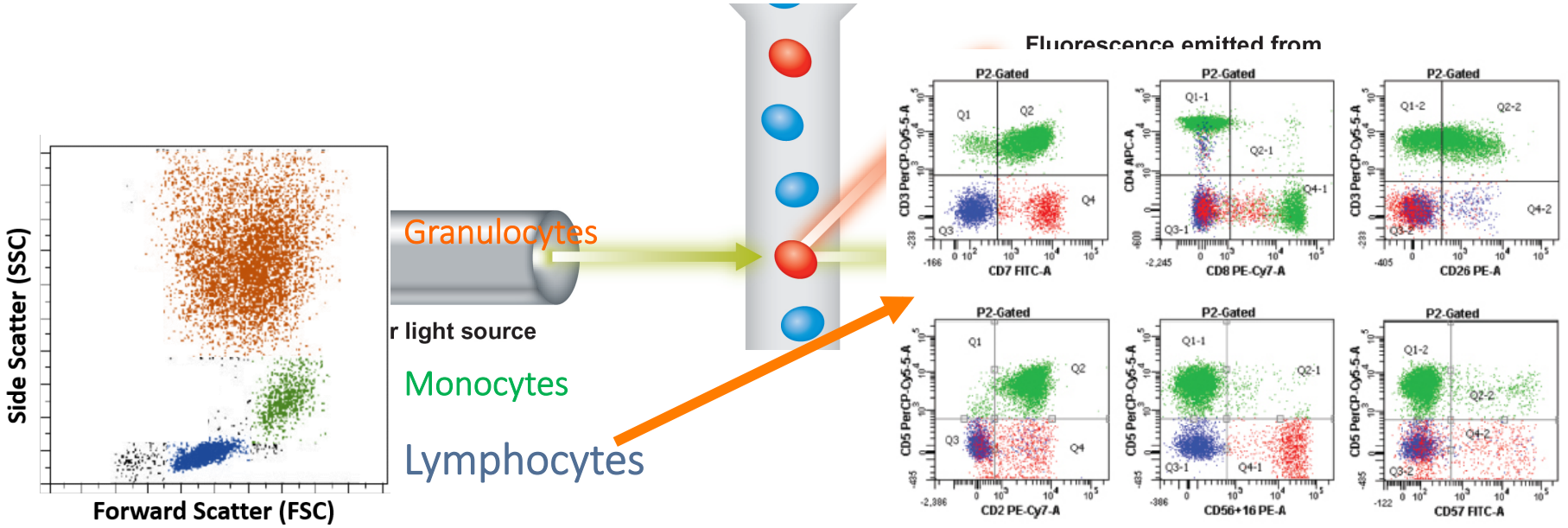
Features

- High-throughput
- Quantitative
- Multi-parameter



Impact (USA)

- >1.2M L/L samples/year
- >$700M clinical testing

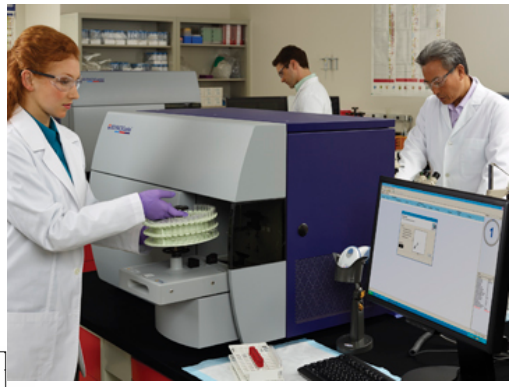


Flow cytometry: Principles and clinical applications in Hematology. Clinical Chemistry 46:8(B) 1221E1229, 2000

Economics of flow cytometry impacts access
particularly **access**, **detection**, and **classification**

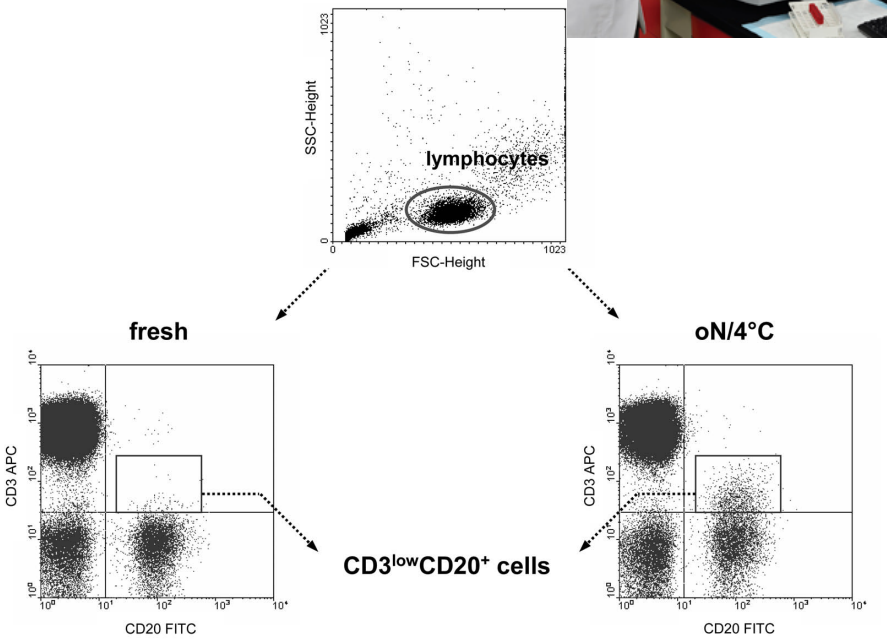
Costs

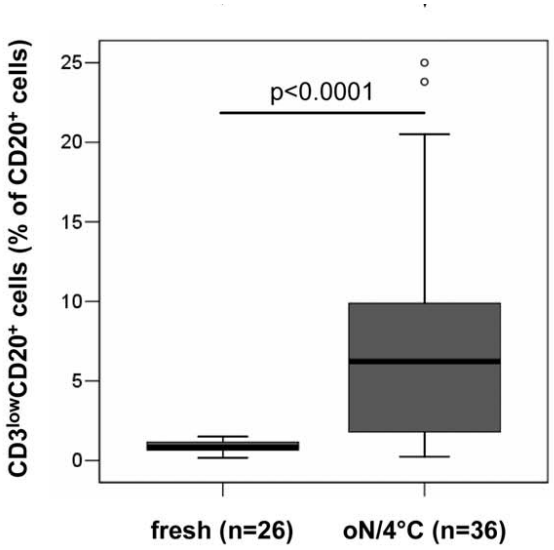
- Instrument cost
- Maintenance
- QA requirements



Limited deployment*

- 1,200 hospitals with
- 4,000 hospitals without
- Out-sourced offsite



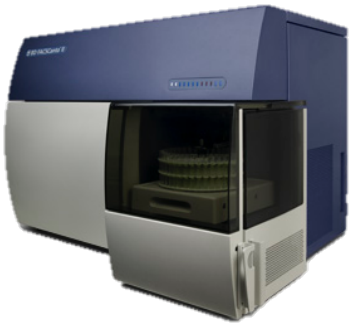


Mobs et. Al. (2014) CD3-Positive B Cells: A Storage-Dependent Phenomenon. PLoS ONE 9(10): e110138.

Hematology. Clinical Chemistry 46:8(B) 1221E1229, 2000
Amer. Hospital Assoc. 2011 Statistics
Ekong Immunolog. Methods 164 1993

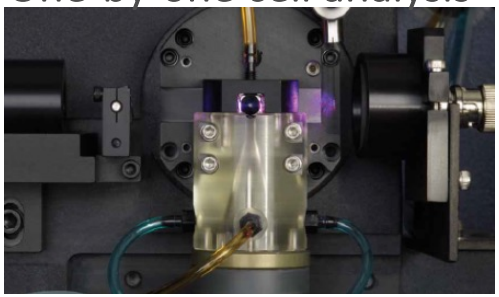
High-throughput classification drives complexity

serial cell analysis and multi-parameter detection reduce access

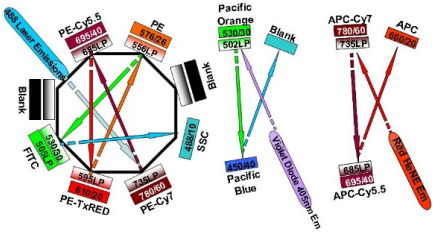


Laser illumination

Fluidics System:
One-by-one cell analysis

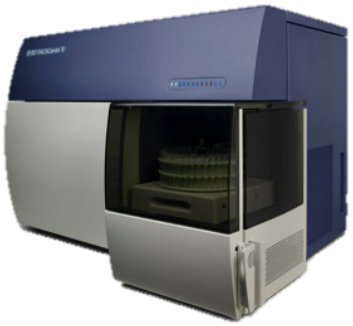


Multichannel (8+)
signal detection



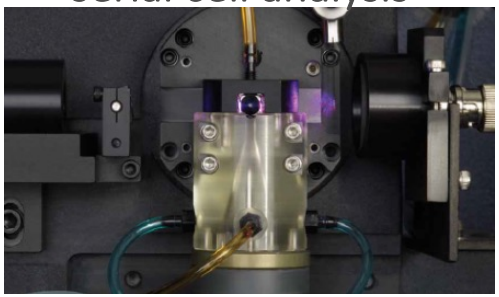
Microscopy: democratize flow data?

replacing the complexity and removing the flow

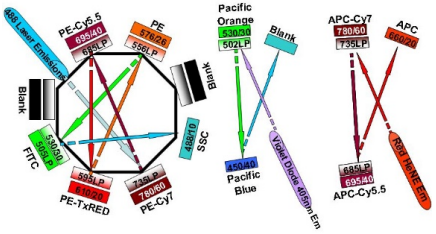


Laser illumination

Fluidics System:
serial cell analysis



Multichannel (8+)
signal detection

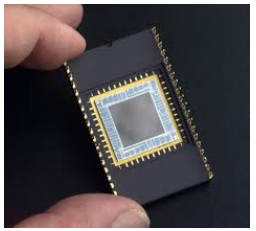


Advances in illuminators, sensors, and image processing enable

Cost-effective, sensitive, high throughput, and quantitative imaging

Stable, affordable,
high power LEDs

Flow-less
imaging sample

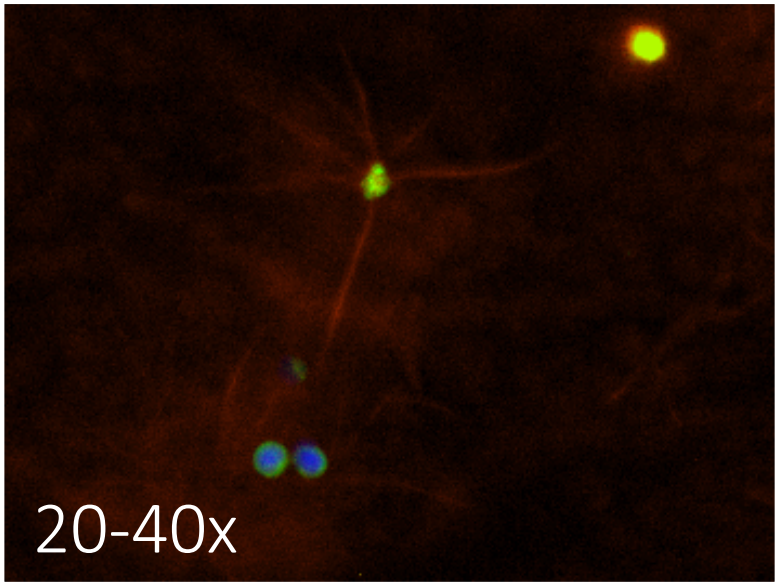


Single detector

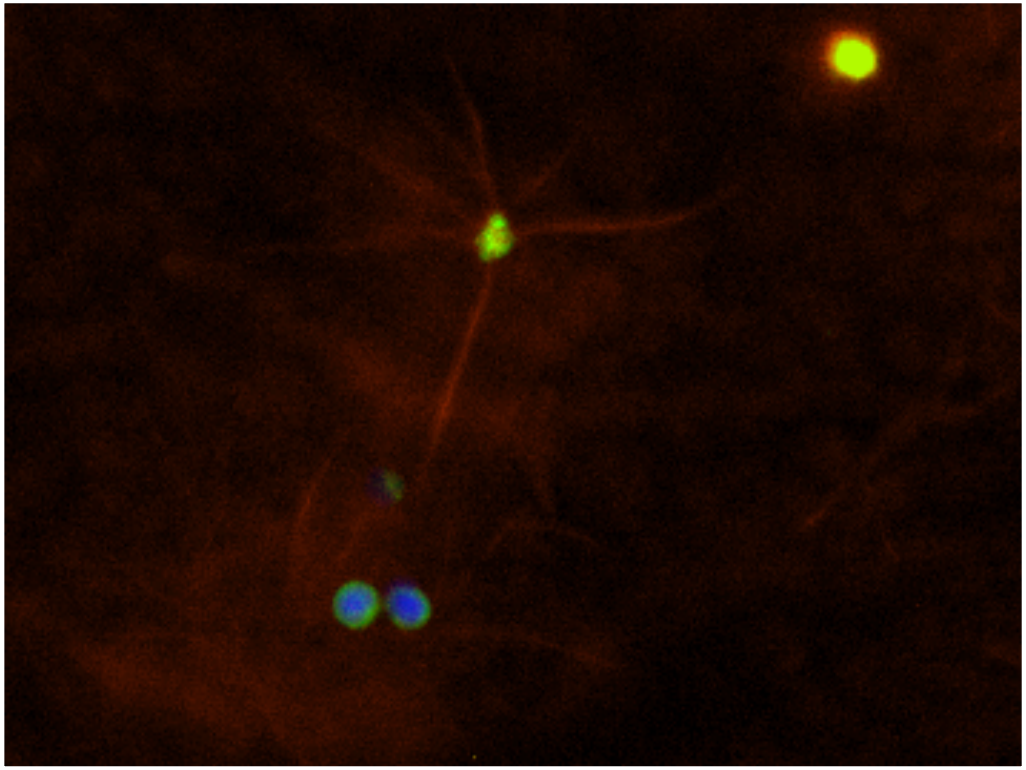
Cellular astronomy: democratizing flow data

zooming out from traditional microscopy and towards flow cytometry

Traditional microscopy



Cellular astronomy



Shapiro, "Cellular Astronomy" – a foreseeable future in cytometry. 2004

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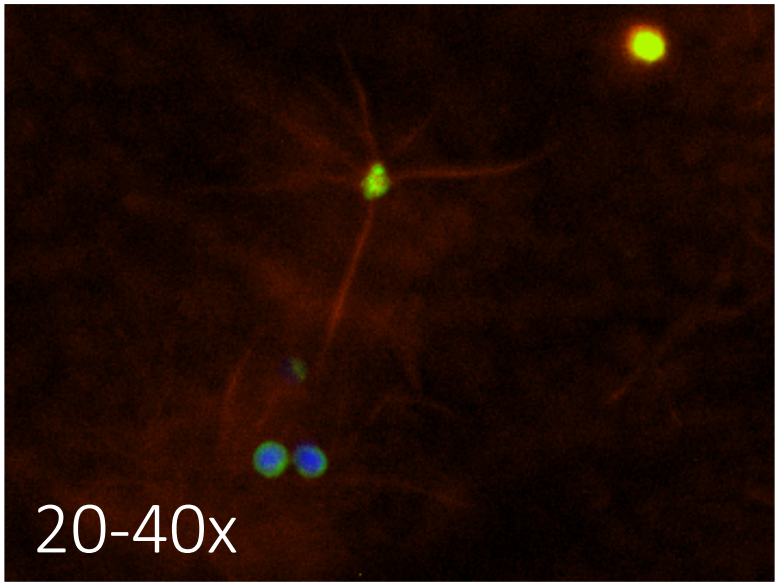
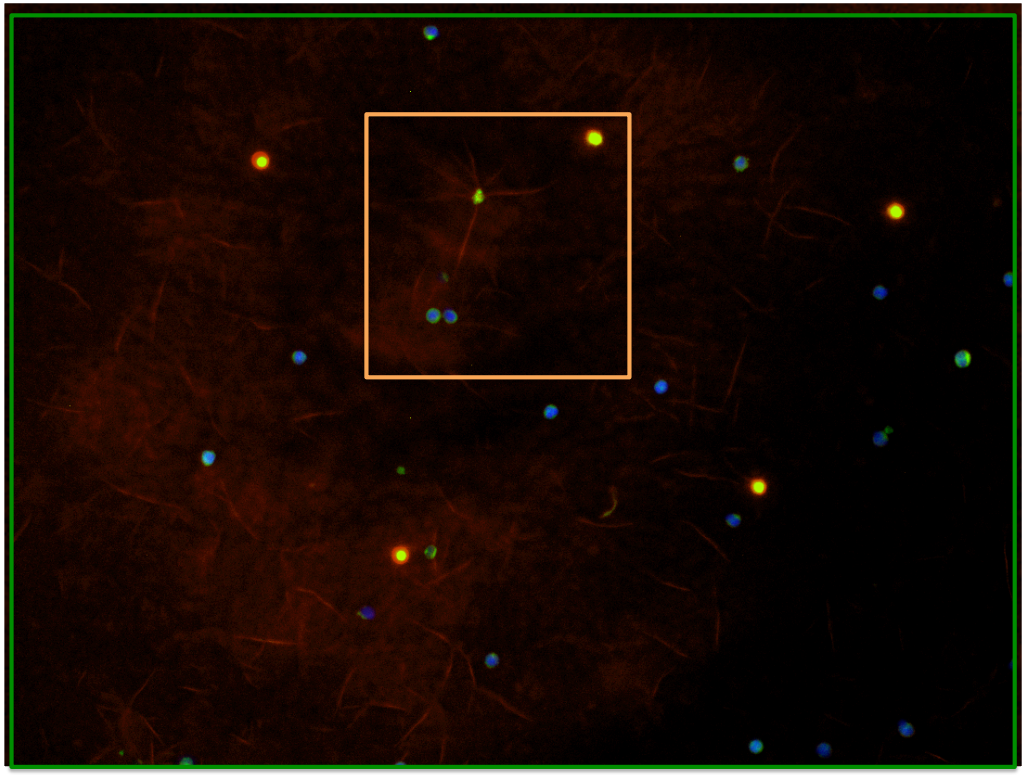
zooming out from traditional microscopy and towards flow cytometry

Traditional microscopy



Cellular astronomy



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zooming out from traditional microscopy and towards flow cytometry

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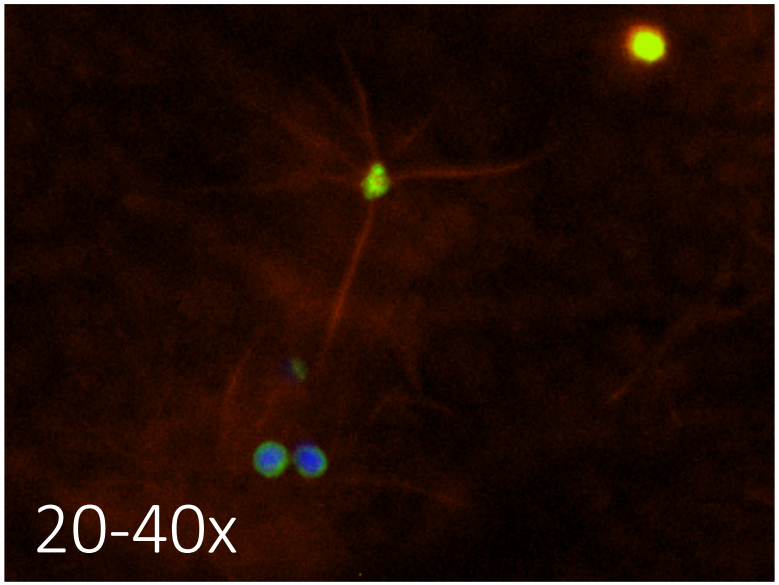
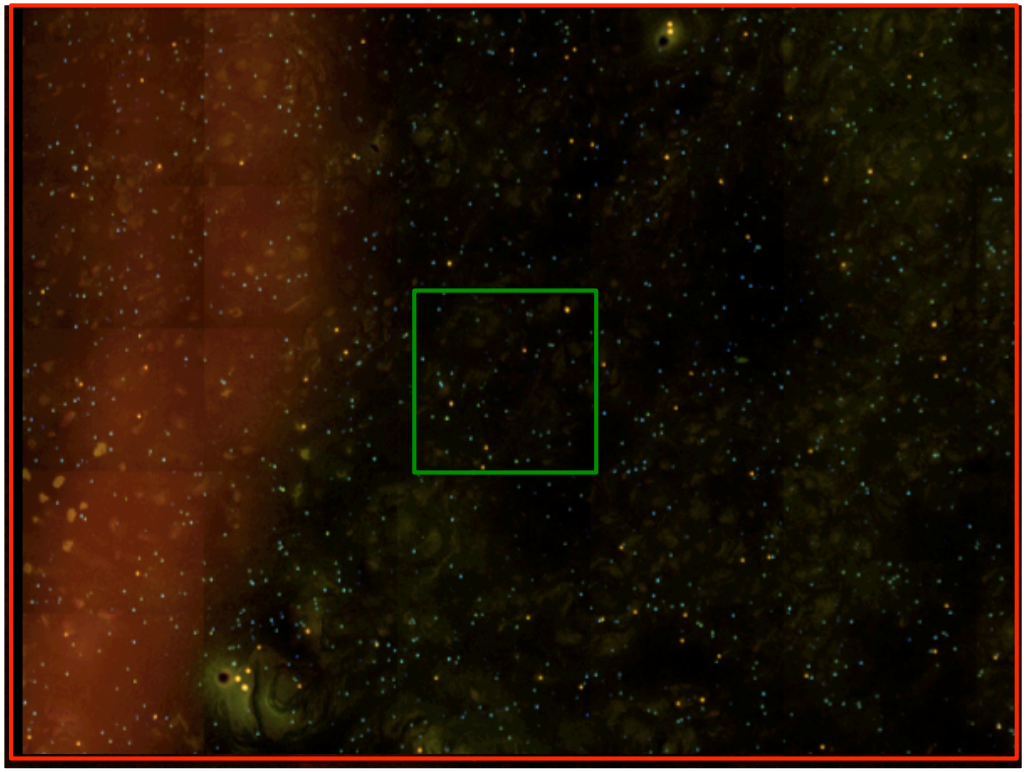


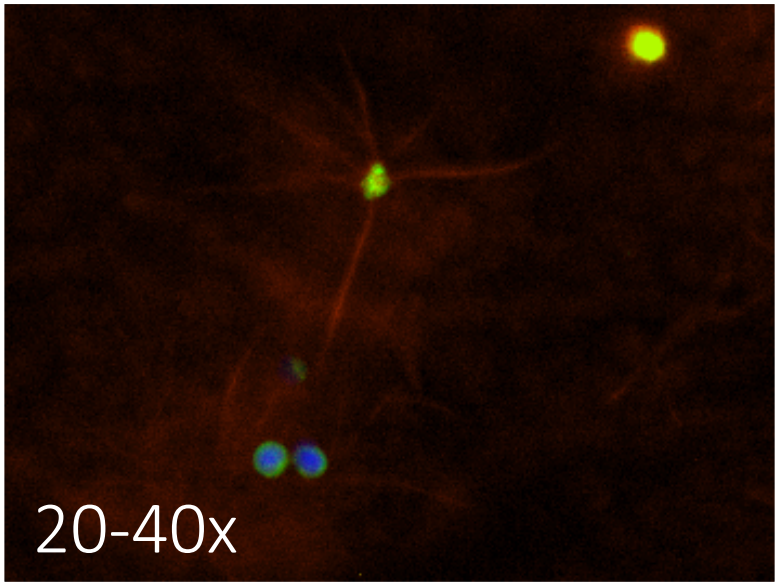
Cellular astronomy



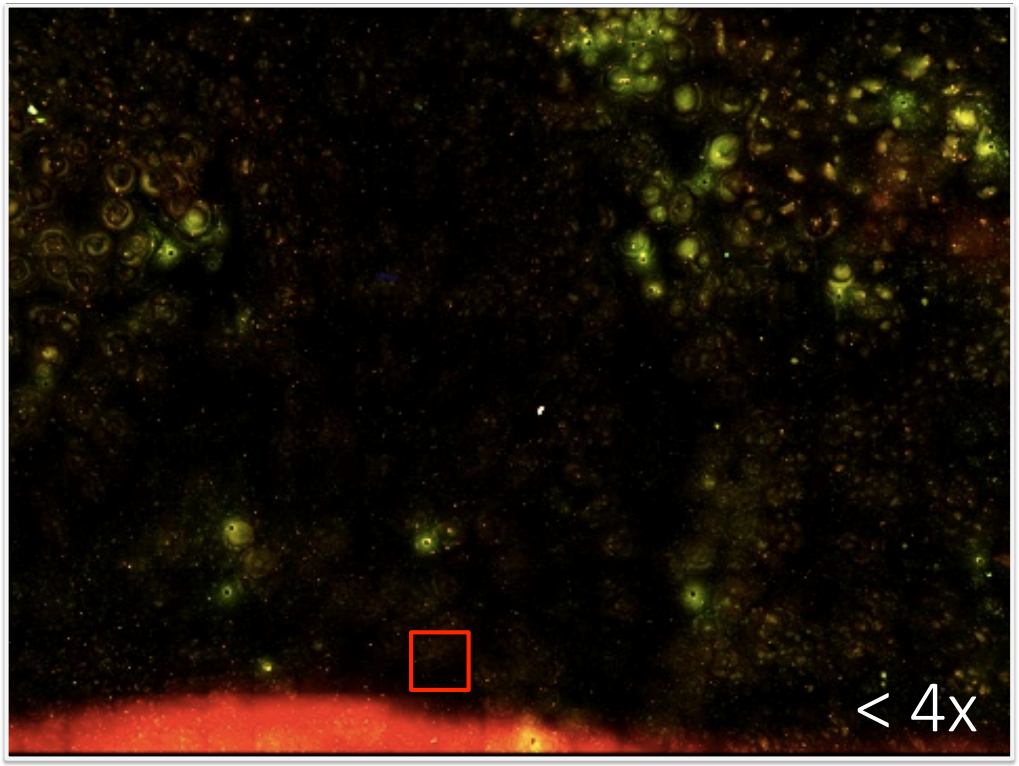
Cellular astronomy: democratizing flow data

zooming out from traditional microscopy and towards flow cytometry

Traditional microscopy



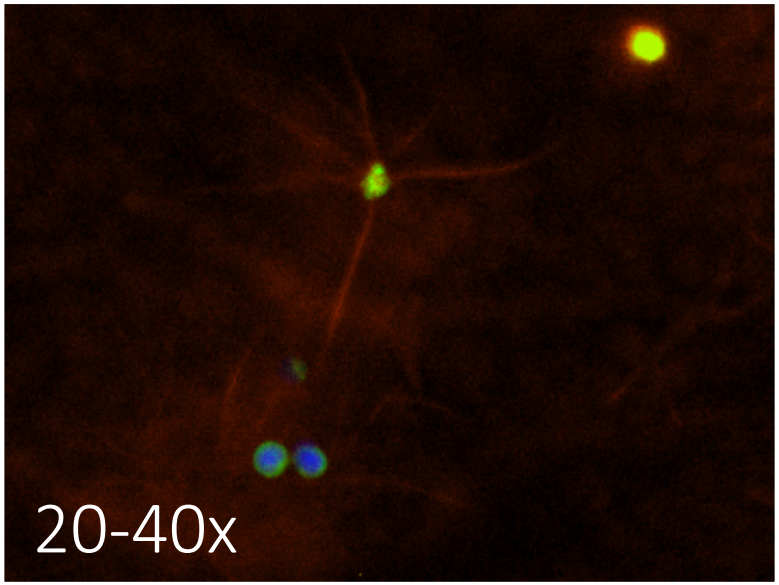
Cellular astronomy



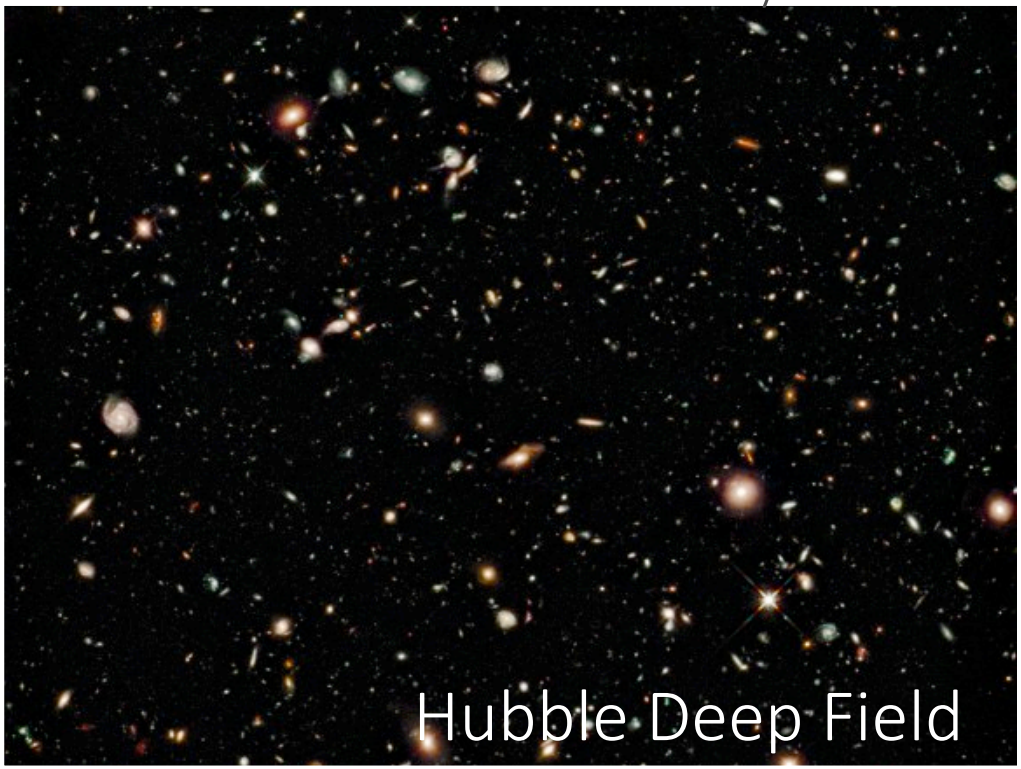
Cellular astronomy: democratizing flow data

zooming out from traditional microscopy and towards flow cytometry

Traditional microscopy



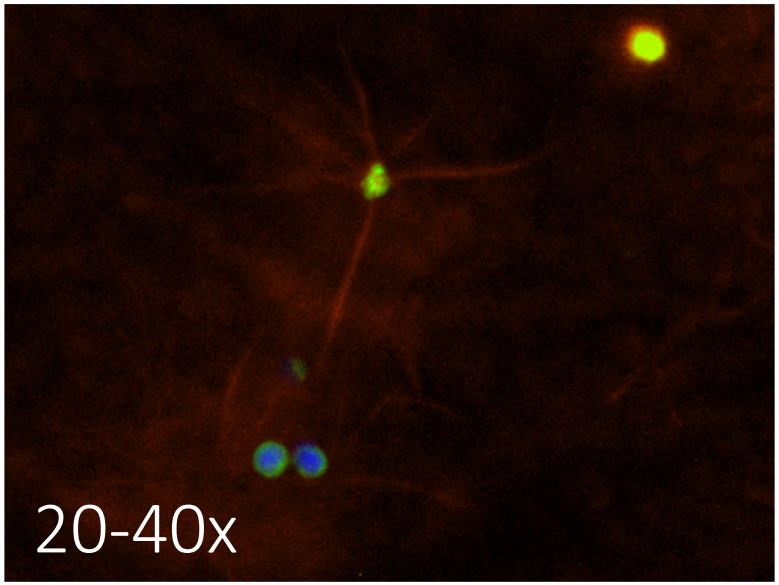
Cellular astronomy



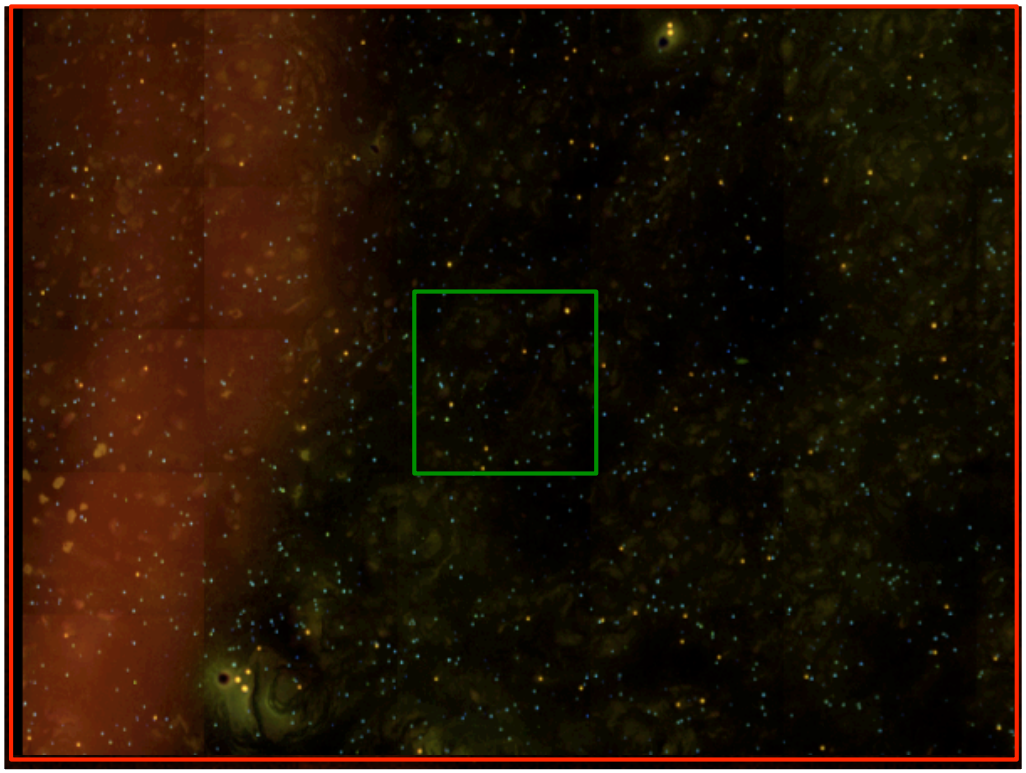
Cellular astronomy: democratizing flow data

zooming out from traditional microscopy and towards flow cytometry

Traditional microscopy

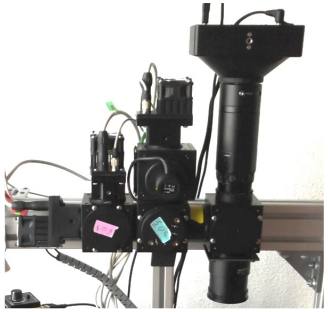


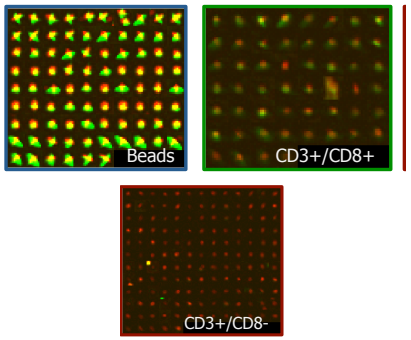
Cellular astronomy



Cost-effective, potential for high-throughput, but can it be quantitative?

Steps towards cell astronomy to increase access





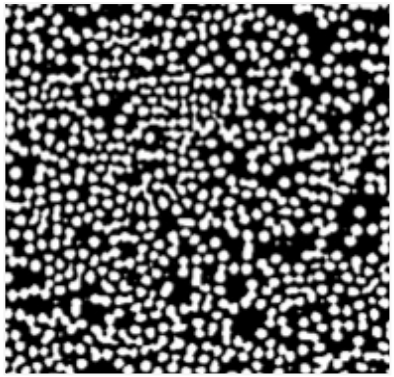
Remaining bottleneck

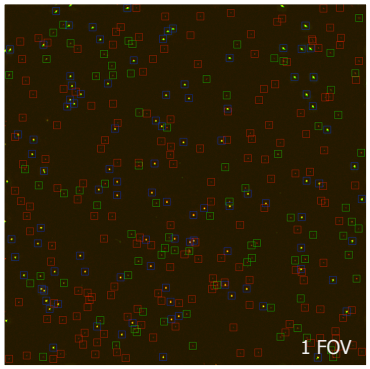
High-density Sample Preparation

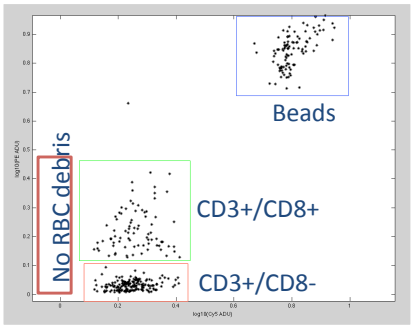
Low-magnification epi-fluorescence

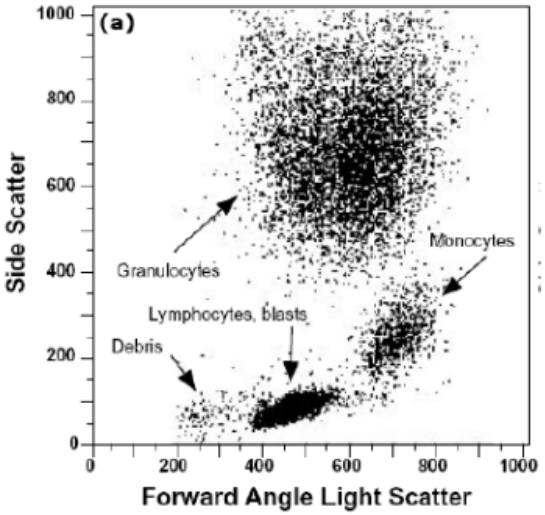
Quantitative Image Analysis

Scatter-based Leukocyte gating









Remaining step towards cell astronomy

Remaining bottleneck

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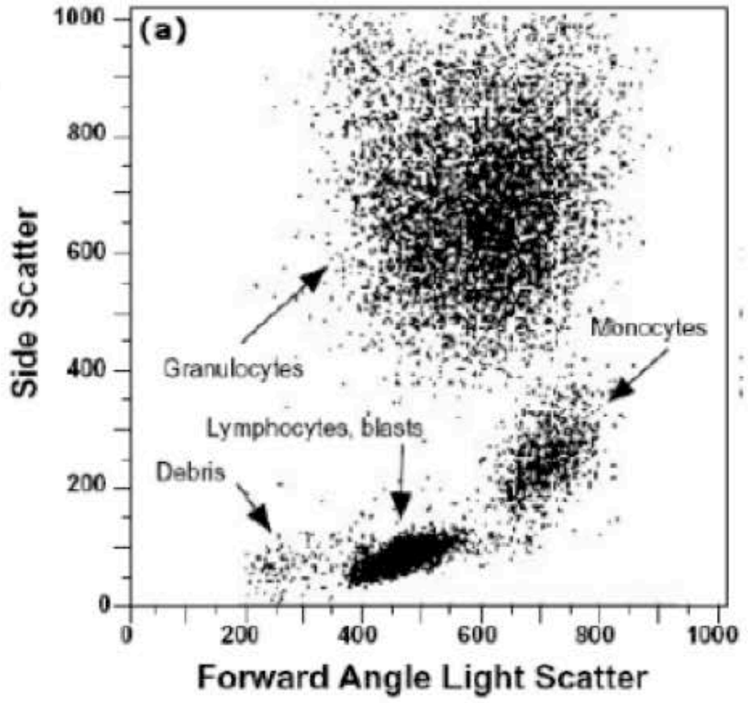
Scatter-based Leukocyte gating



Massachusetts Institute of Technology

Madrid-MIT m+Visión CONSORTIUM

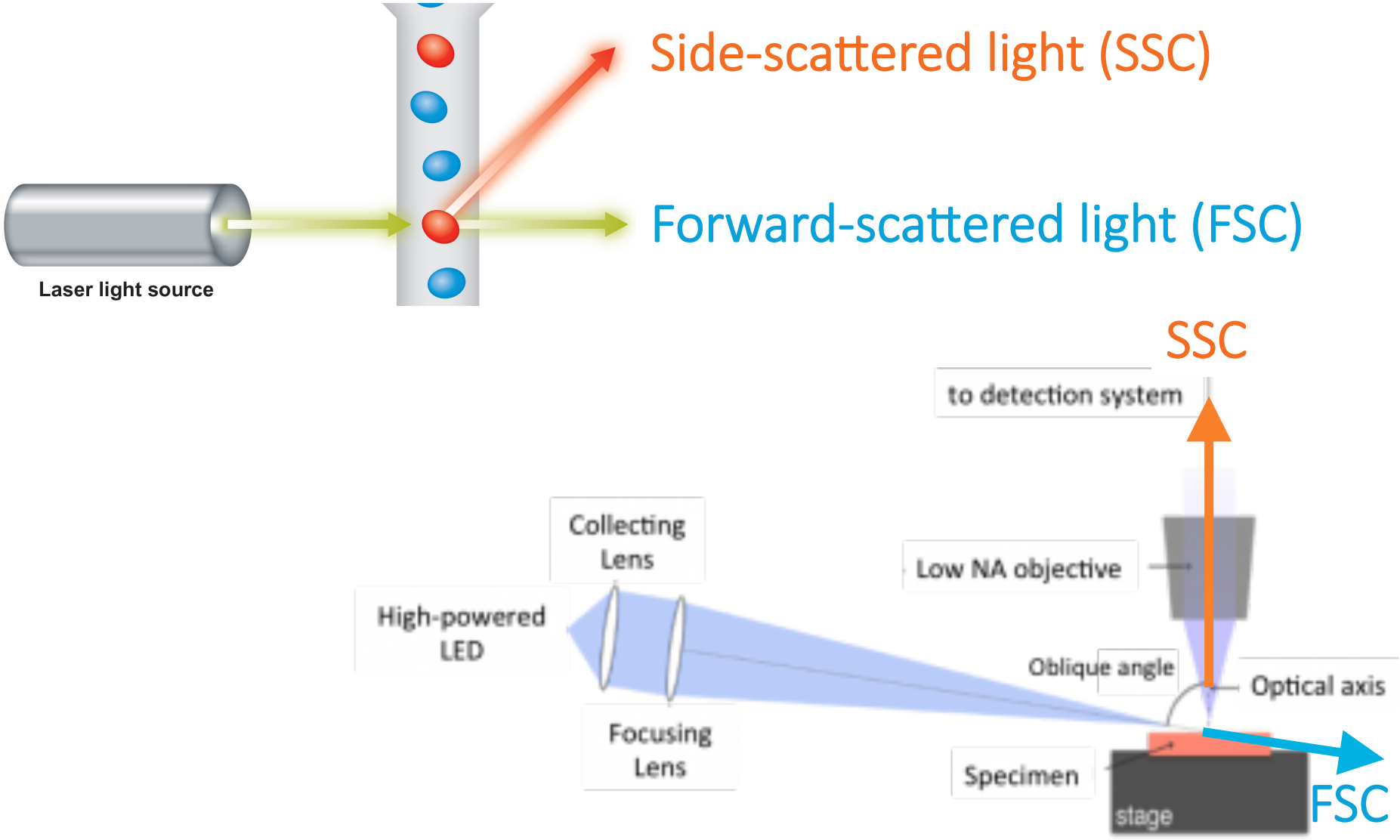
Innovation, Leadership, Impact

La Suma de Todos

Comunidad de Madrid

fundación madrid+d para el conocimiento

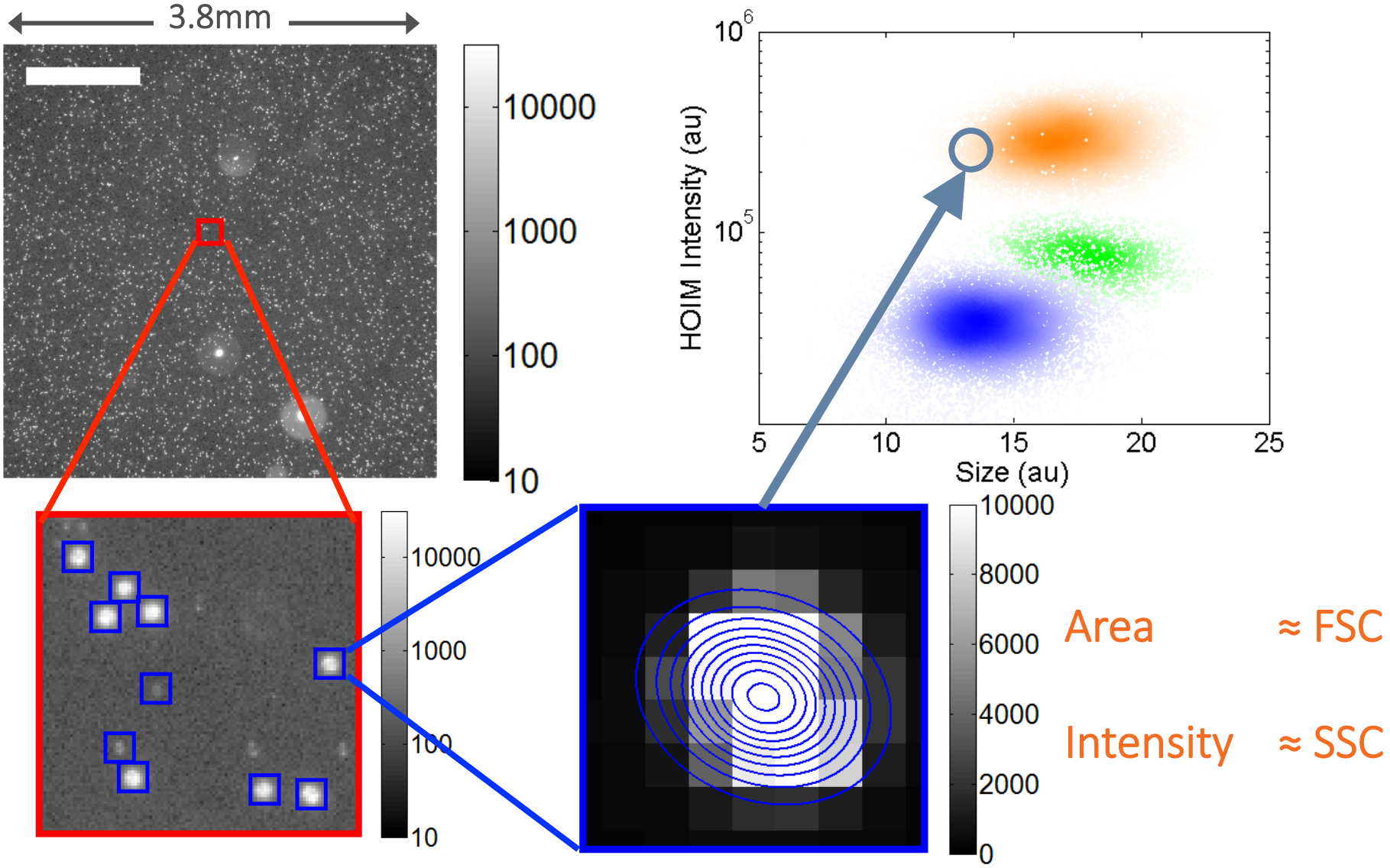
Highly oblique illumination microscopy (HOIM)



HOIM uses modular illumination hardware

Compatible with a broad set of microscopes

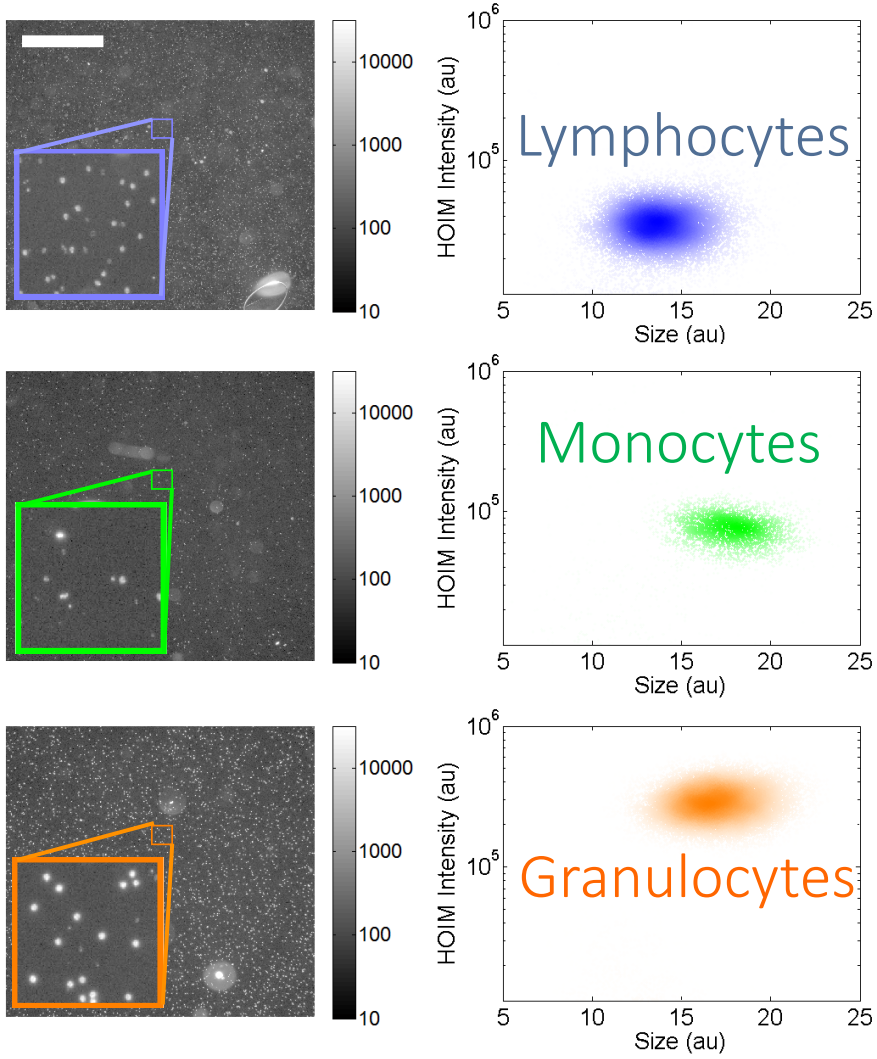
Fit the detected event to obtain scatter analogs



Area ≈ FSC

Intensity ≈ SSC

HOIM recapitulates individual WBC populations
using **sorted** stabilized human control WBCs

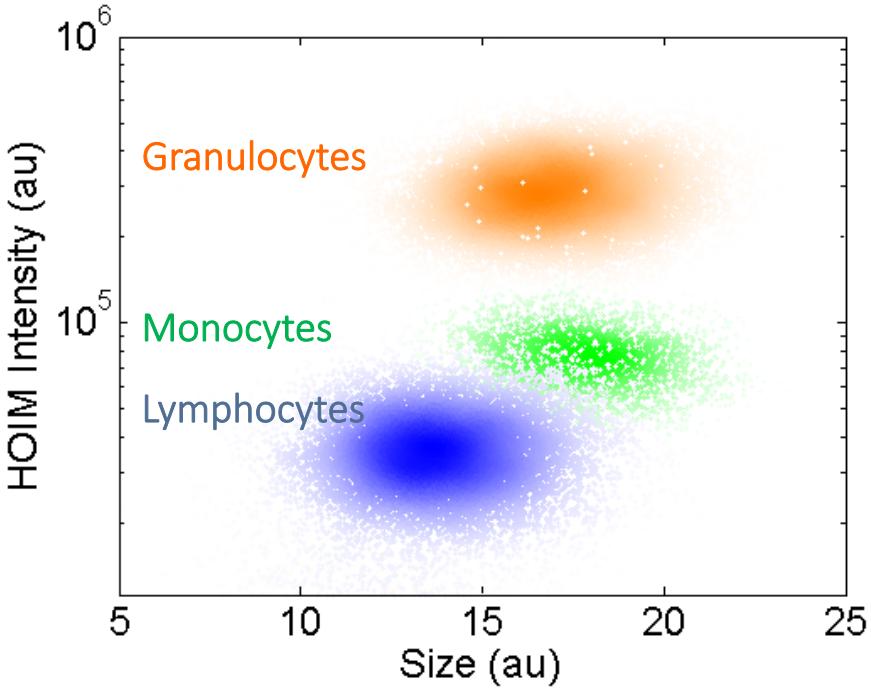


Sorted lymphocytes, monocytes, and granulocytes have distinguishable size and HOIM signal intensity

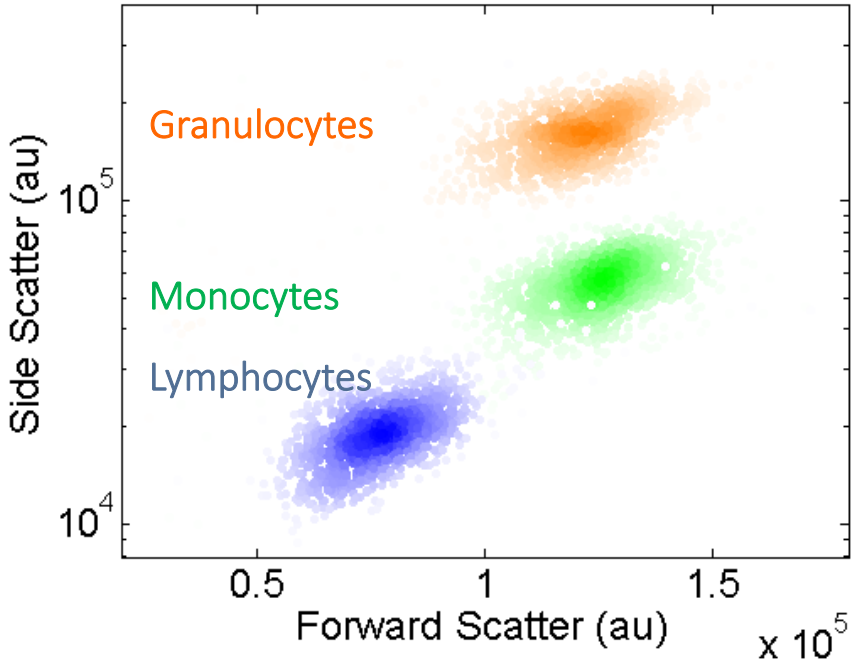
HOIM qualitatively resembles flow data

adding together the data from **sorted** stabilized human control WBCs

HOIM



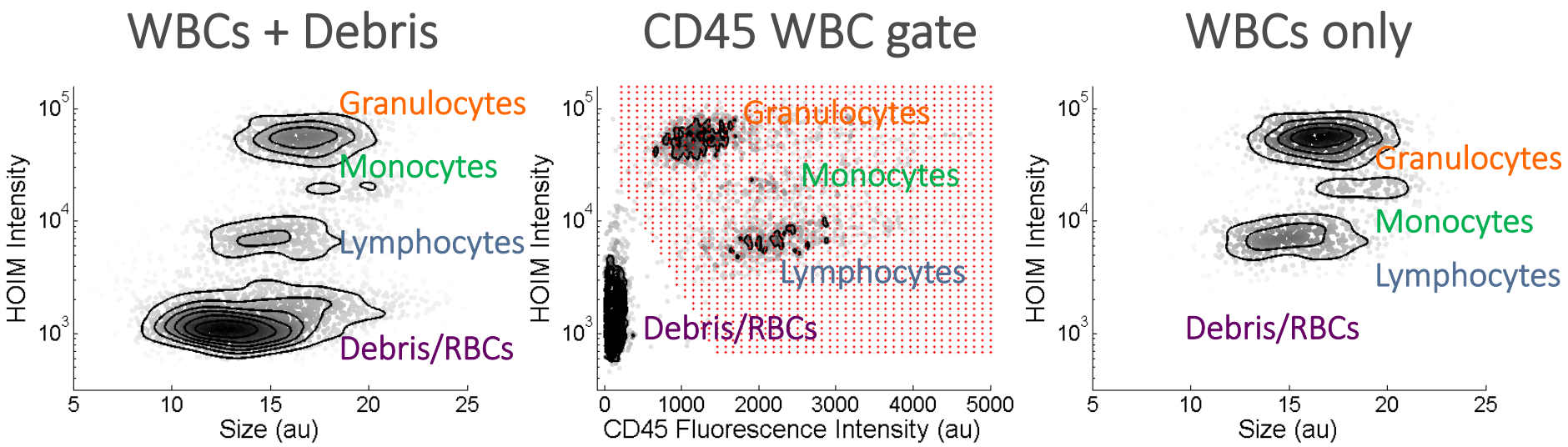
Flow cytometer



HOIM size and intensity data replicate
forward and side scatter flow cytometry data

Gating out RBCs with HOIM and fluorescence

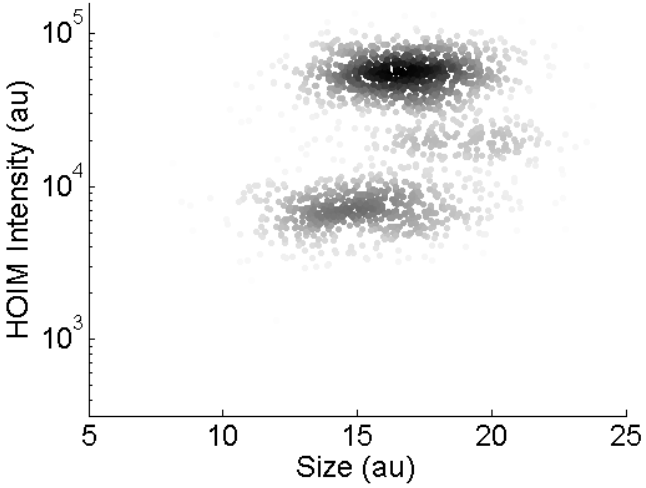
using **unsorted lysed** stabilized human control samples

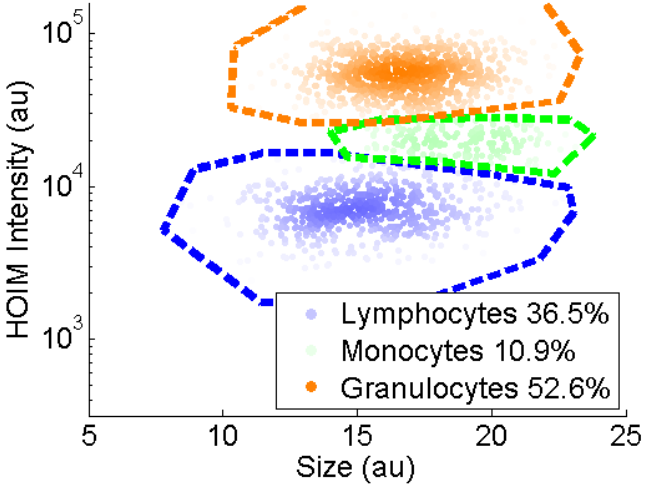


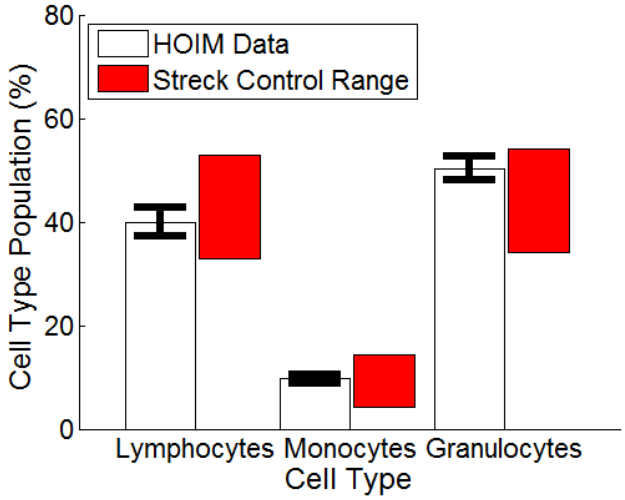
HOIM size and intensity data can be obtained on unsorted lysed samples and used in clinical workflow to isolate WBC populations

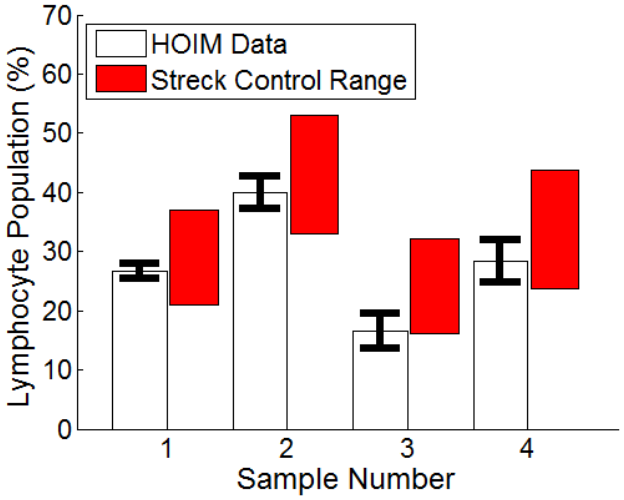
Analyzing HOIM performance w/ manual gating

using **unsorted lysed** stabilized human control samples









Lymphocyte population ratios correlate with the values supplied by the sample manufacturer ($r^2 = 0.98$)

Conclusions: Scatter-based WBC discrimination
using HOIM and fitting in the cellular astronomy regime

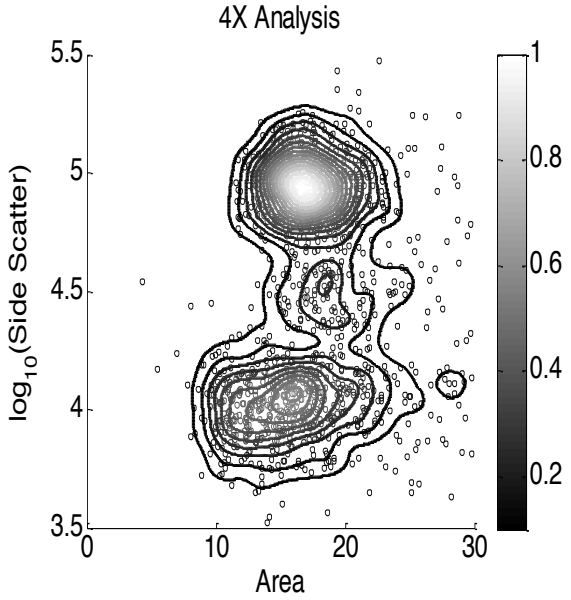
- Detect and quantify scattering signals from cells at 4x magnification
- Accurately classify leukocytes into 3 subpopulations
- Accurately quantify 3 leukocyte subpopulations

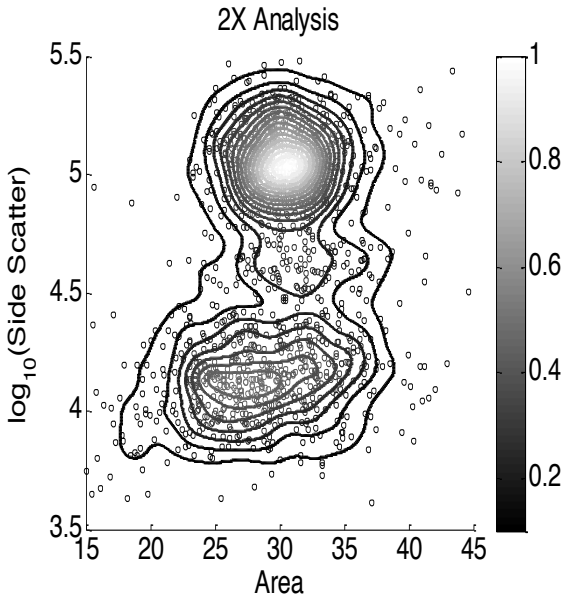
- Outstanding concern:
  - Leukemia/lymphoma applications require >8 color fluorescence
  - Work underway by licensor of the IP

Future directions *and unpublished work*

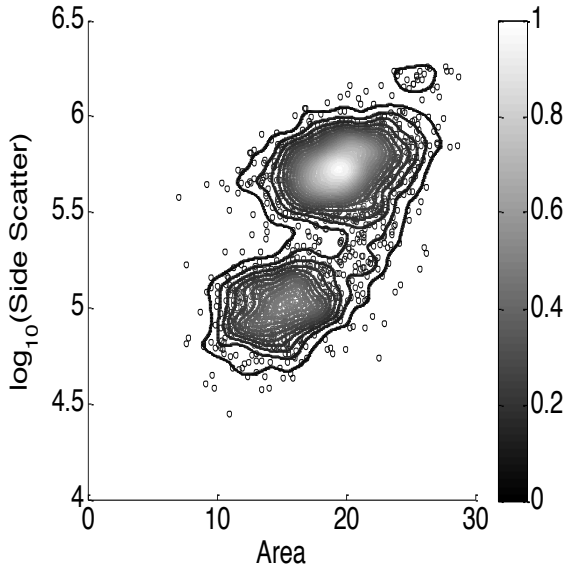
- Dynamic imaging of leukocytes / RBCs
- Different substrates
- Effects of cell packing fraction

HOIM at 2x magnification





HOIM w/ human samples



Outcomes & broader impacts

CFTCC grant

- Technical proof-of-concept
- Manuscript (submitted)
- Provisional patent application
    - Licensing and commercial development underway

Translation of a complete cell astronomy platform

- 5 year effort to develop and de-risk the whole system
- SSC was the final step to compel an industrial partner to license
- Development in industry moves us closer to clinical impact

Acknowledgements – Team Cell

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Madrid-MIT m+VISIÓN CONSORTIUM
Innovation, Leadership, Impact

Gray Lab for space

Massachusetts Institute of Technology

Shivang R. Dave, PhD

Jason Tucker-Schwartz, PhD

Prof. Elfar Adalsteinsson, PhD **(PI)**

Prof. Norberto Malpica, PhD
Esteban Pardo

Universidad Rey Juan Carlos

Prof. Peter Bryant, PhD
Daniel Luk, MBA

ie business school

Prof. Ben Vakoc, PhD

MGH 1811

Peter Hansen, PhD
Petra Krauledat, PhD

PLASMONIC NANO PARTICLE RESEARCH