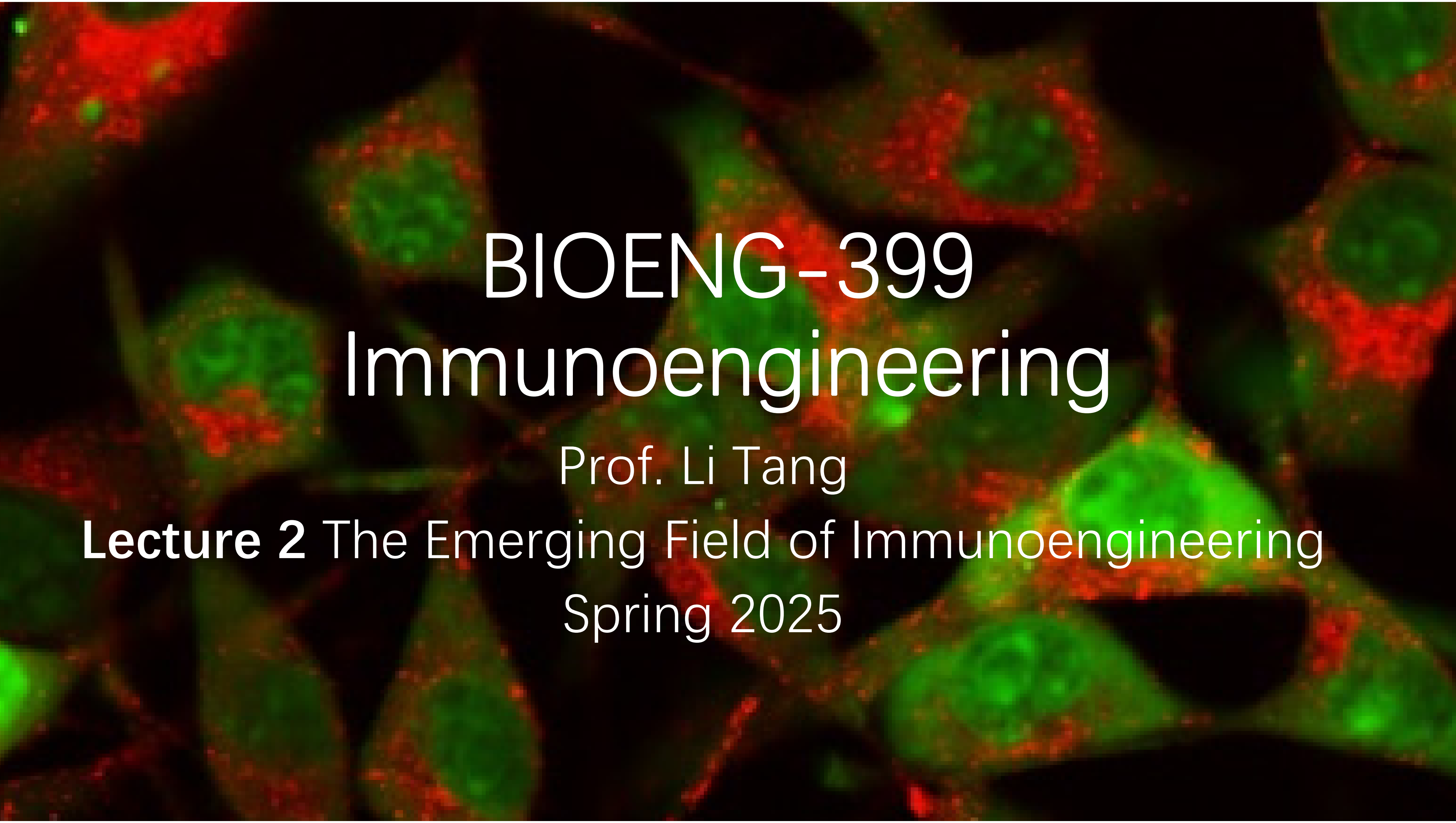
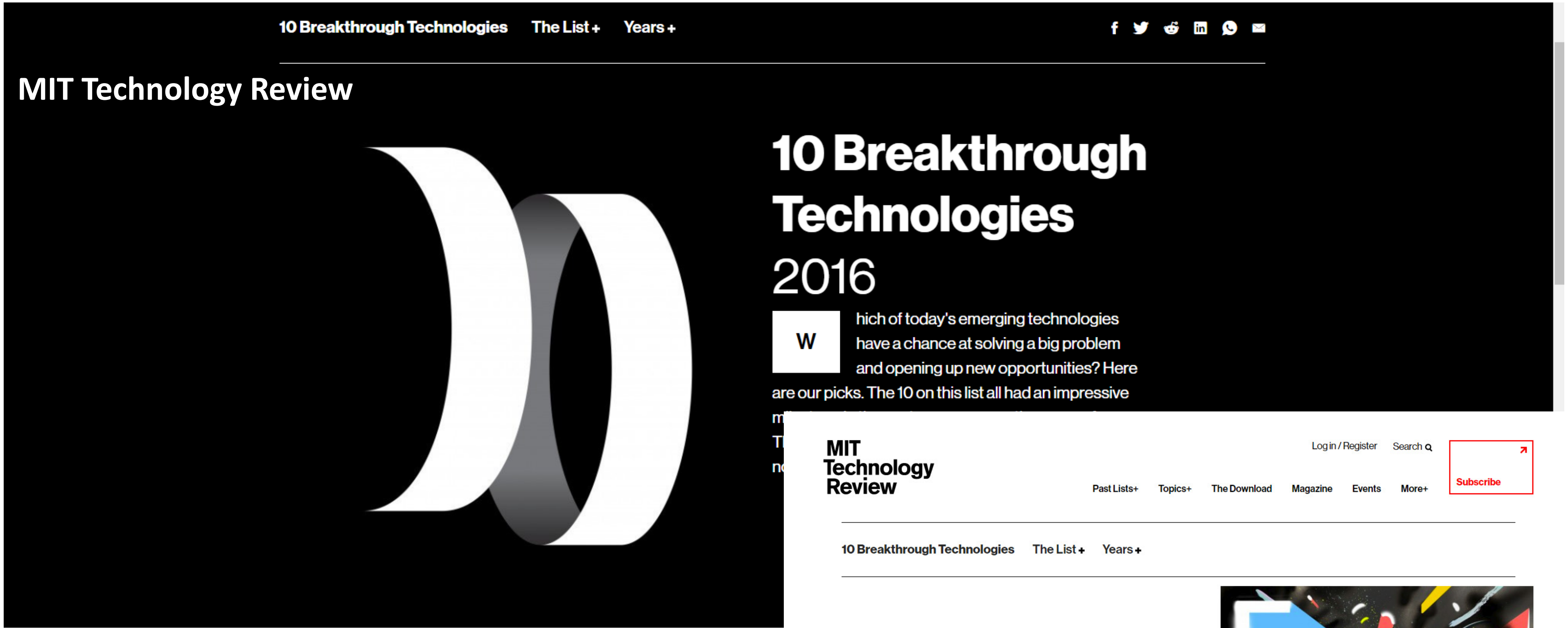


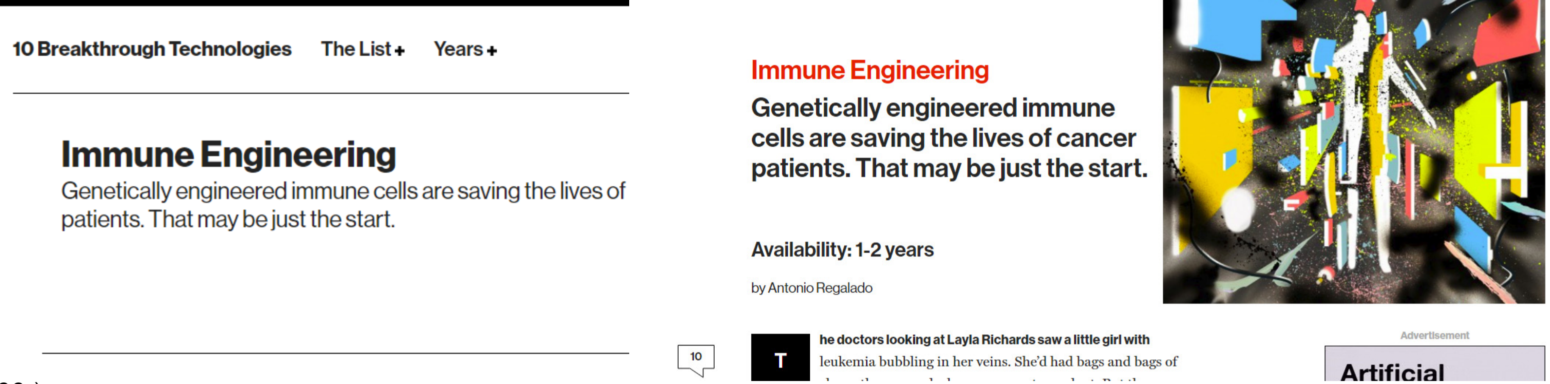
BIOENG-399 Immunoengineering

Prof. Li Tang

Lecture 2 The Emerging Field of Immunoengineering

Spring 2025





Aug 30, 2017
Novartis receives first ever FDA approval for a CAR-T cell therapy

MIT Technology Review (founded in 1899)
https://www.technologyreview.com/lists/technologies/2016/

Immunoengineering: Interfacing Immunology(Engineering) with Engineering(Immunology)

Engineering is the application of scientific knowledge and mathematical methods to practical purposes of the design, analysis, or operation of structures, machines, or systems.

Immunology is a branch of biology that covers the study of immune systems in all organisms.

What are exactly the interactions between engineering and immunology?

Engineering disciplines have developed from fundamental sciences

Mechanical Engineering | Chemical Engineering | Electrical Engineering | ……

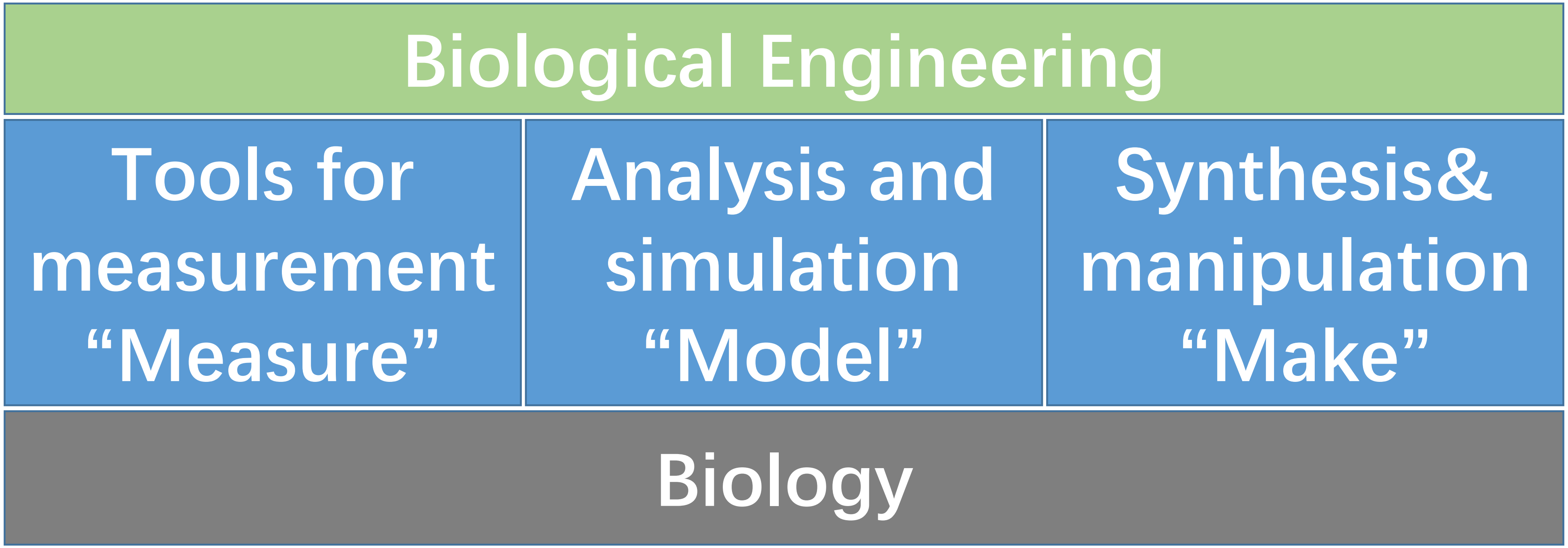
Mechanical Engineering

Tools for measurement “Measure”	Analysis and simulation “Model”	Synthesis& manipulation “Make”

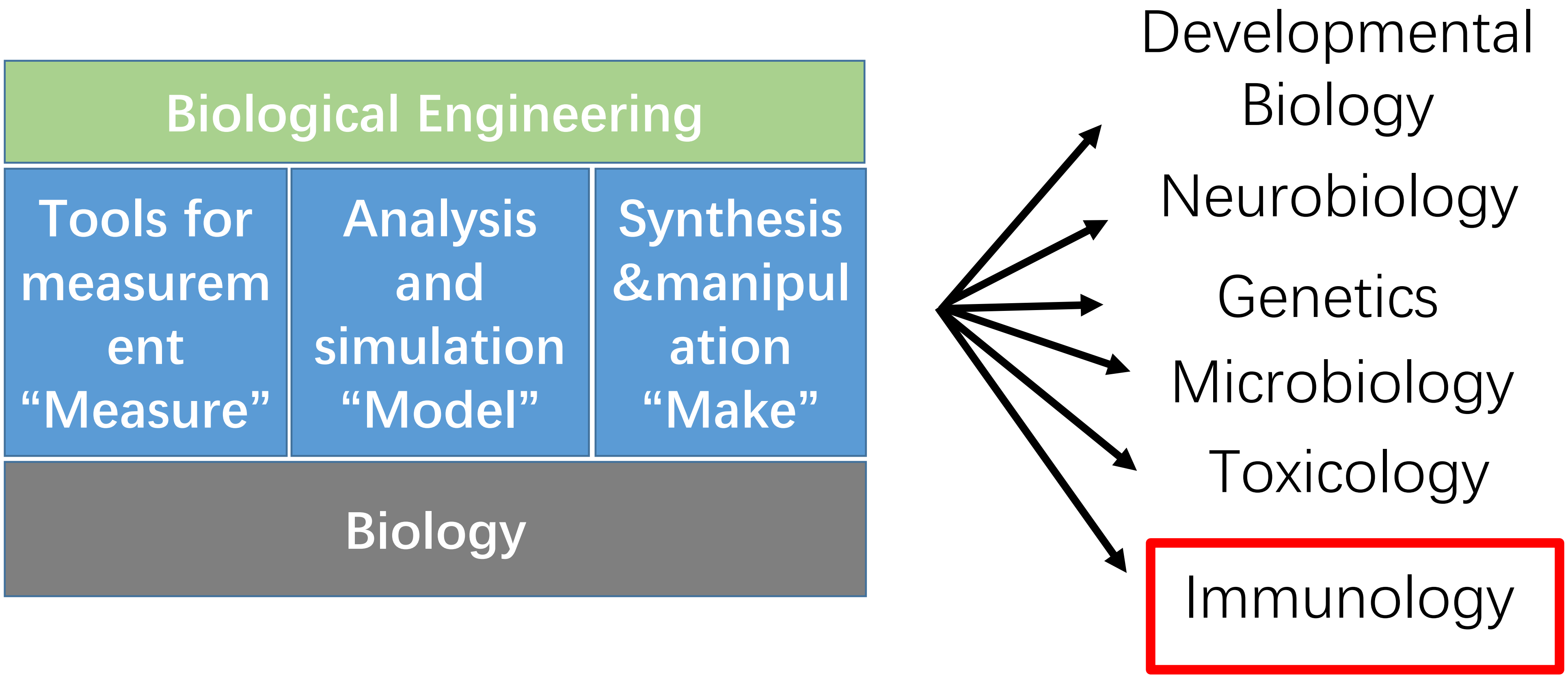
Physics

Biological Engineering: Application of a new engineering

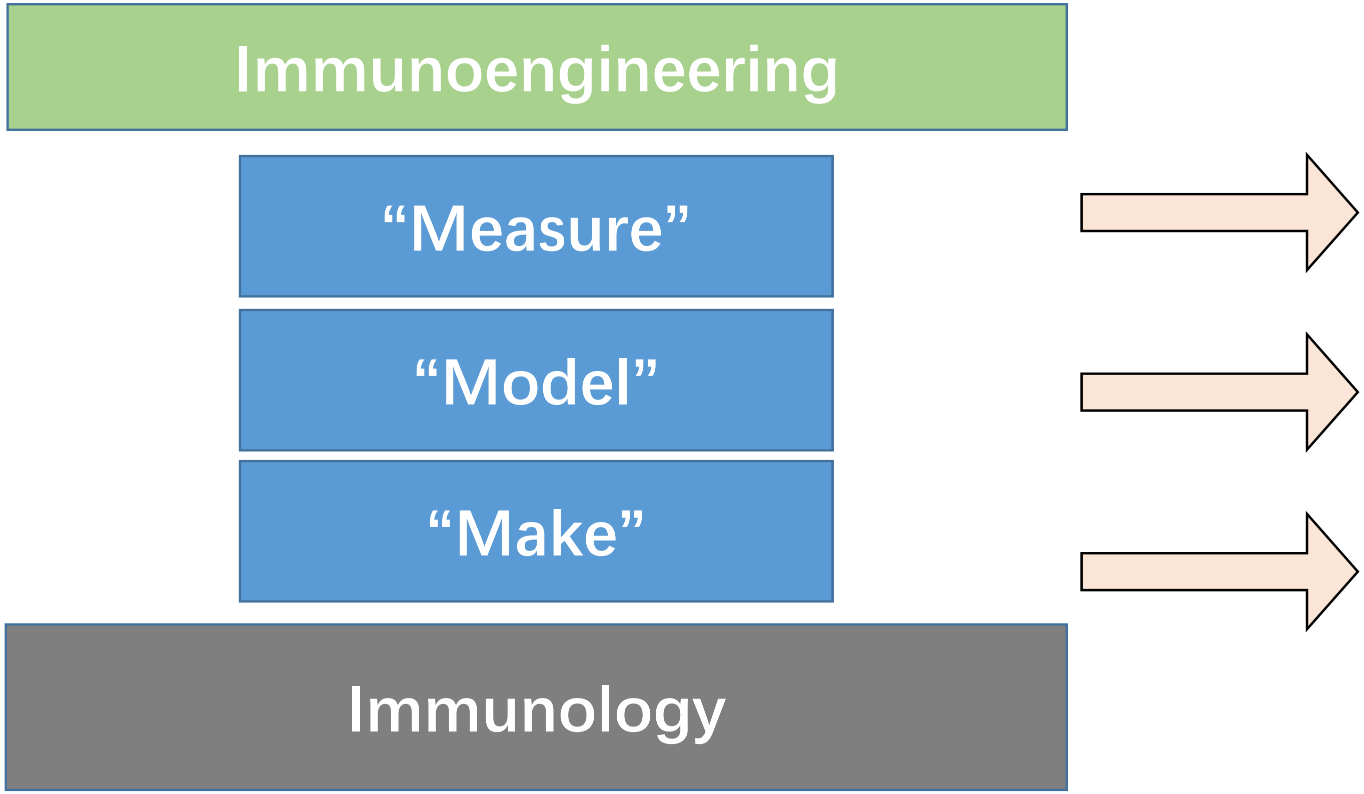
Mechanical Engineering | Chemical Engineering | Electrical Engineering | ……



Biological Engineering:
Application of a new engineering



Immunoengineering:
Application of a new engineering



Measuring and characterizing immune functions

Bioengineering approaches to model the immune systems

Manipulating immune system; designing new immuno-therapeutics

Modified from slides of Prof. Darrell Irvine (MIT)

What are the interactions between engineering and immunology?

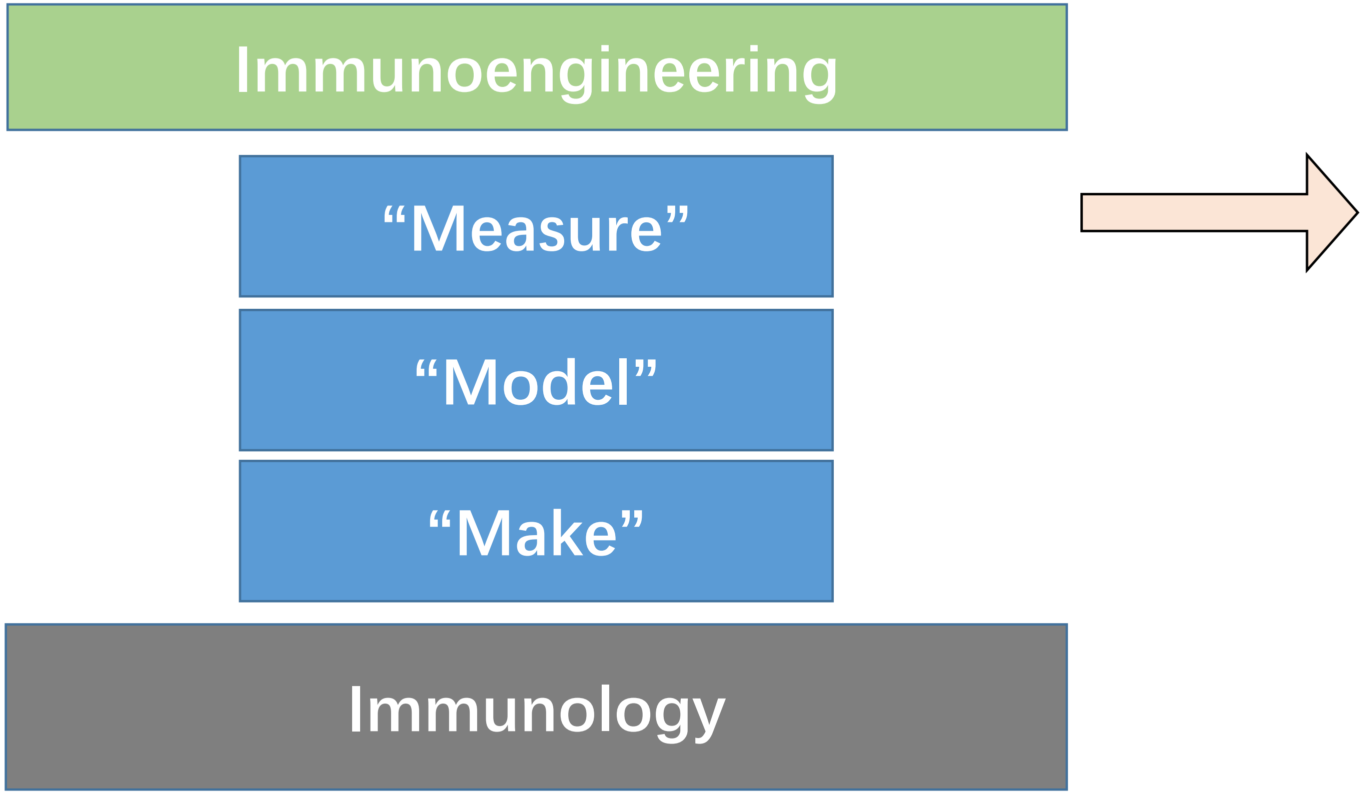
- Engineering helps us understand immunology
- Engineering allows us control immune response

Mechanical engineering
Electrical engineering
Materials Engineering
Nano-engineering
Chemical engineering
Tissue engineering
Systems Biology and Engineering
Protein Engineering
Genetic Engineering
Metabolic Engineering
….

↔

Immunology

Immunoengineering:
Application of a new engineering



Conventional and new techniques to characterize immune systems

Modified from slides of Prof. Darrell Irvine (MIT)

Several key technologies have become cornerstones of modern immunological analysis

- –Monoclonal antibodies
- –ELISA, ELISPOT -> sensitive detection using mAbs
- –Flow cytometry: multidimensional analysis
- –pMHC tetramers: directly visualize antigen-specific T cells
- –Mouse models

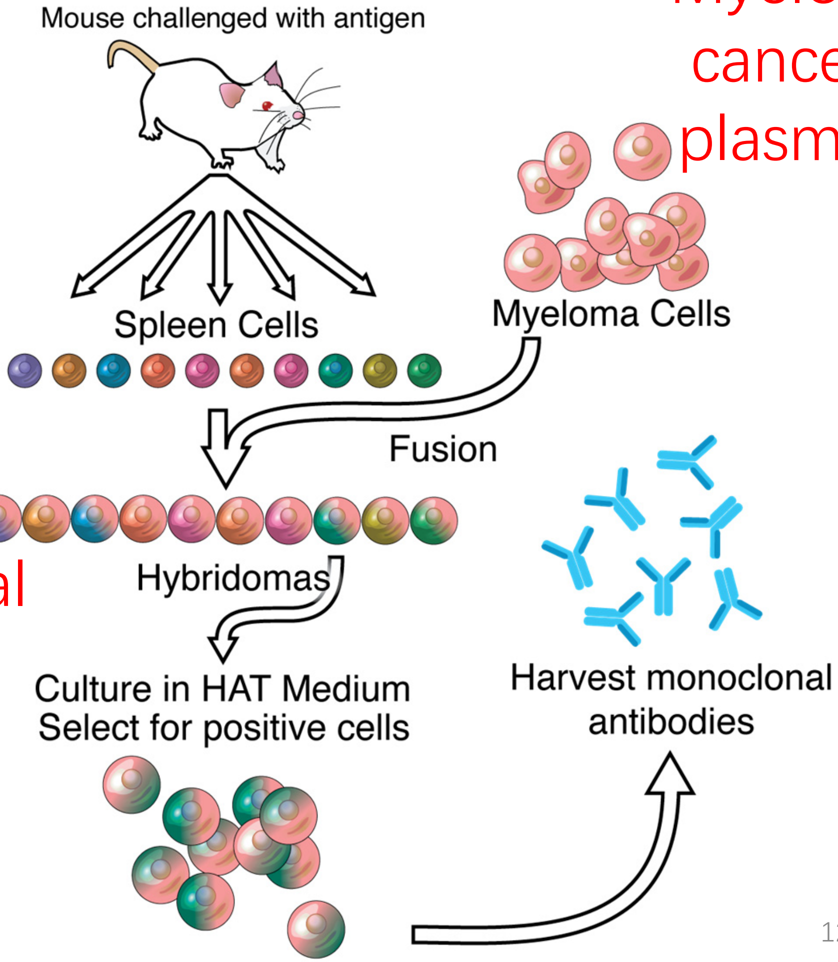
A first transformative tool: Hybridoma technology – the generation of monoclonal antibodies

Recognition that the immune system of animal models could be used to generate **highly sensitive and specific reagents** for analyzing biological systems

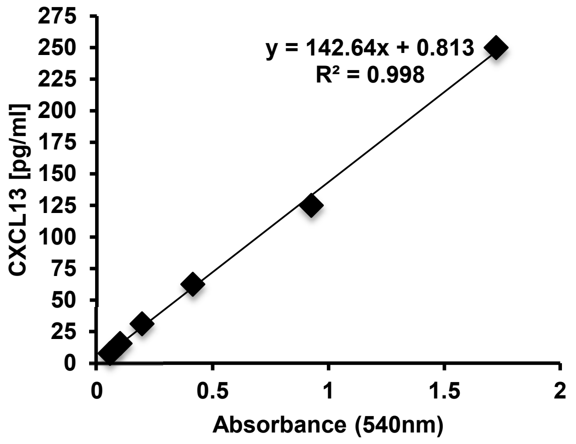
Cesar Milstein and **Georges Köhler** at the time of their nobel prize award in 1984

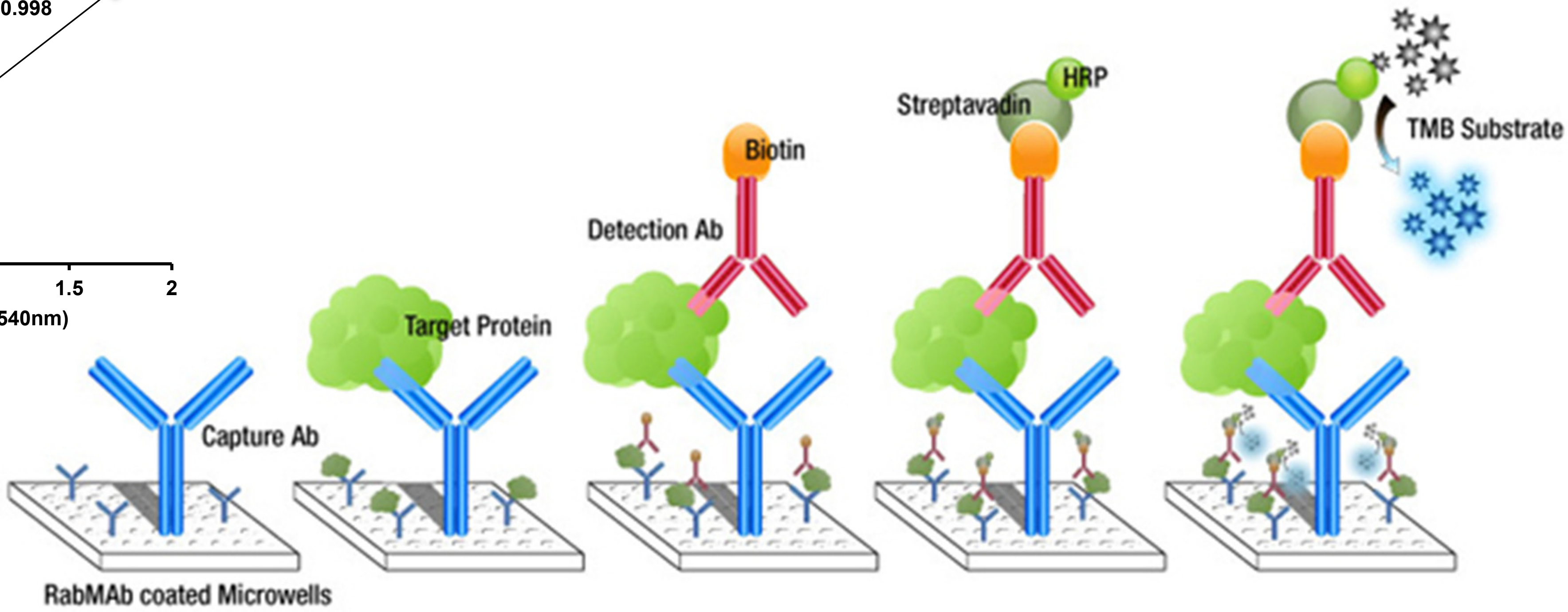
Myeloma: a cancerous plasma cell

Immortal growth



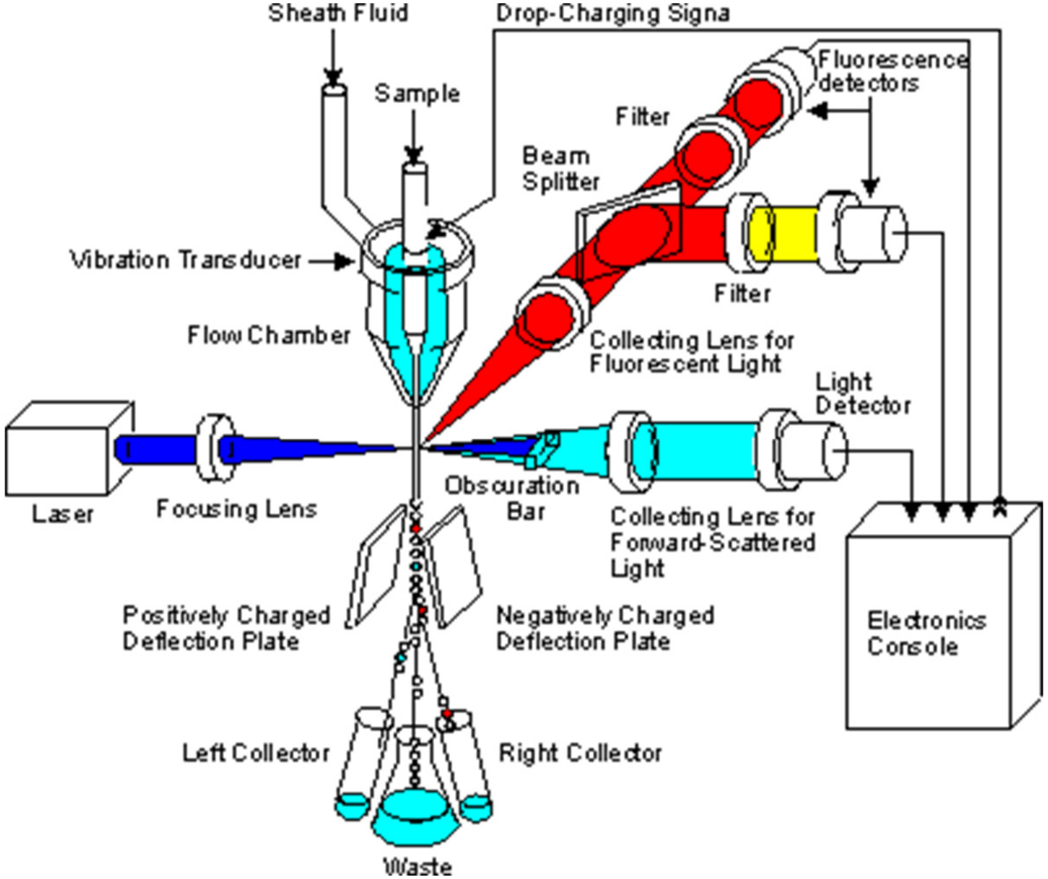
Putting monoclonal antibodies to work: Enzyme-linked immunoassay (ELISA)

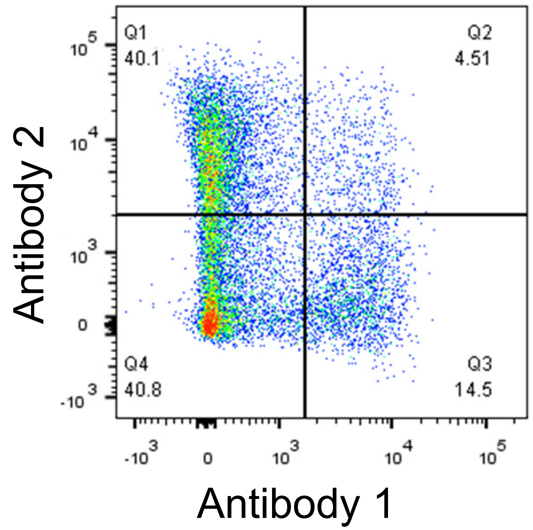




Epitomics.com

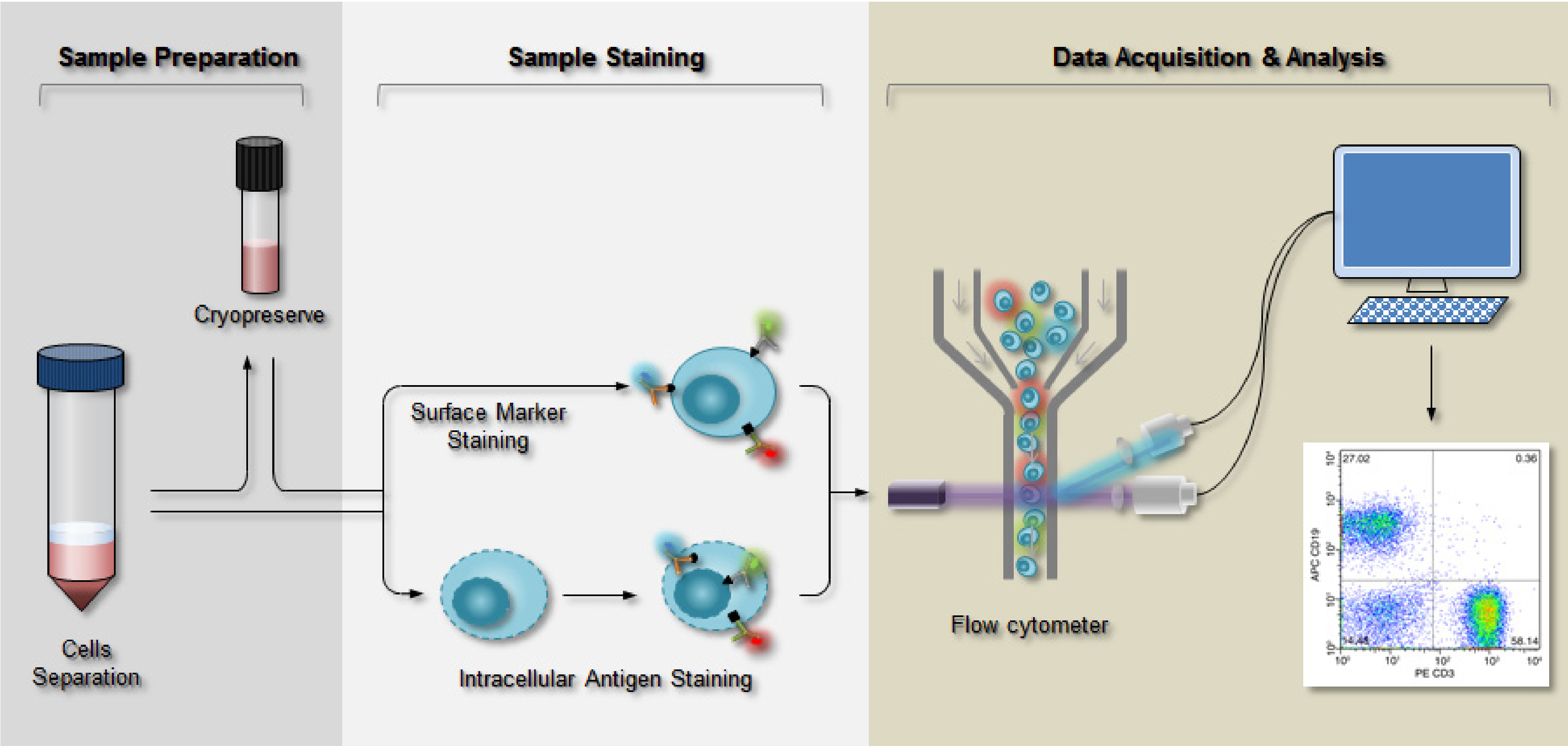
Flow cytometry





http://olomouc.ueb.cas.cz/book/export/html/18

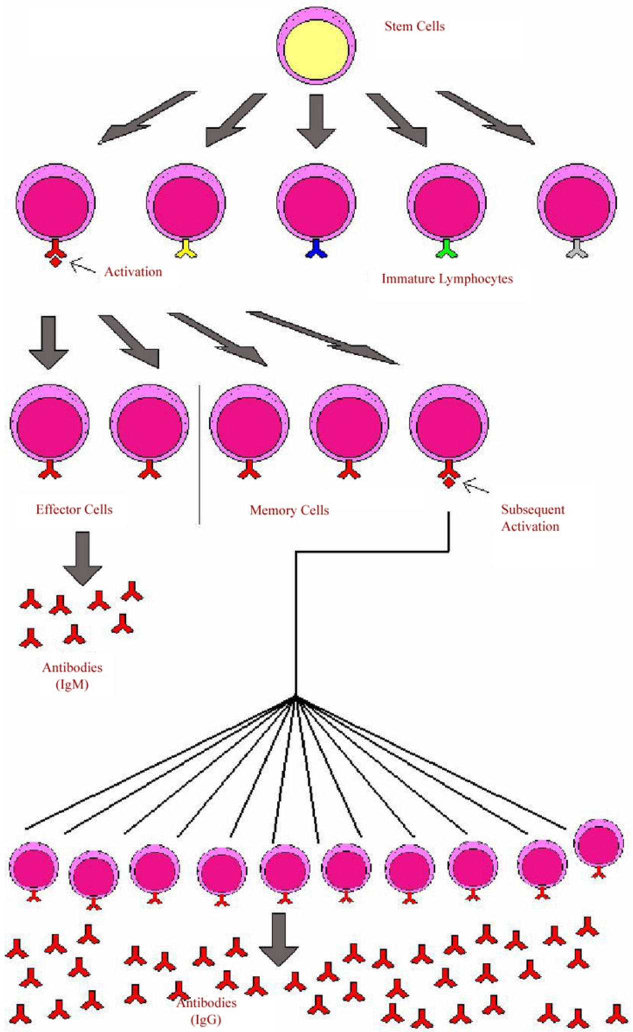
Flow cytometry



http://www.sinobiological.com/flow-cytometry-fcm-facs-protocol.html

THE CLONAL IMMUNE SYSTEM

B-cells:



http://doctor-jones.co.uk/Immunology/Tutorial/Clonal%20Selection%20Theory%20-%20larger%20image.jpg

T-cells:

- 10^{12} total T cells in adult human
- 25-100 million distinct clones
- Only several thousand T cells at most respond to any individual antigen (von Andrian and Mackay 2000)

- Precursor frequency of antigen-specific cells:

$CD8^+$ T cells: estimated at 1 in 200,000 cells specific for any given antigen (**0.0005%** antigen-specific cells), but can expand up to 10,000-fold

Arstila et al. Science 286, 958 (1999)
Blattman et al. J. Exp. Med. 195, 657 (2002)

Identifying antigen-specific T-cells

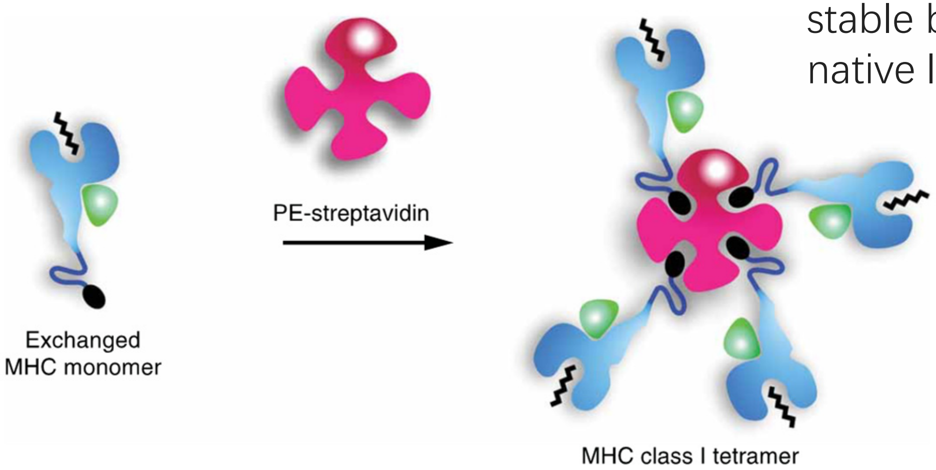
Peptide-MHC and TCR interaction

KD of TCR binding to pMHC:

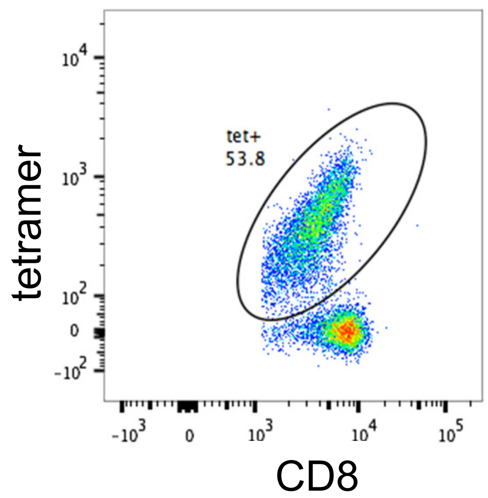
- CD8 TCRs: 10 nM – 1 μM
- CD4 TCRs: 1-10 μM

Original paper:
John D. Altman; Paul A. H. Moss; Philip J. R. Goulder; Dan H. Barouch; Michael G. McHeyzer-Williams; John I. Bell; Andrew J. McMichael; Mark M. Davis. (1996) "Phenotypic Analysis of Antigen-Specific T Lymphocytes". Science. 274 (5284): 94–96.

Using multivalency to achieve stable binding of TCRs to their native ligands

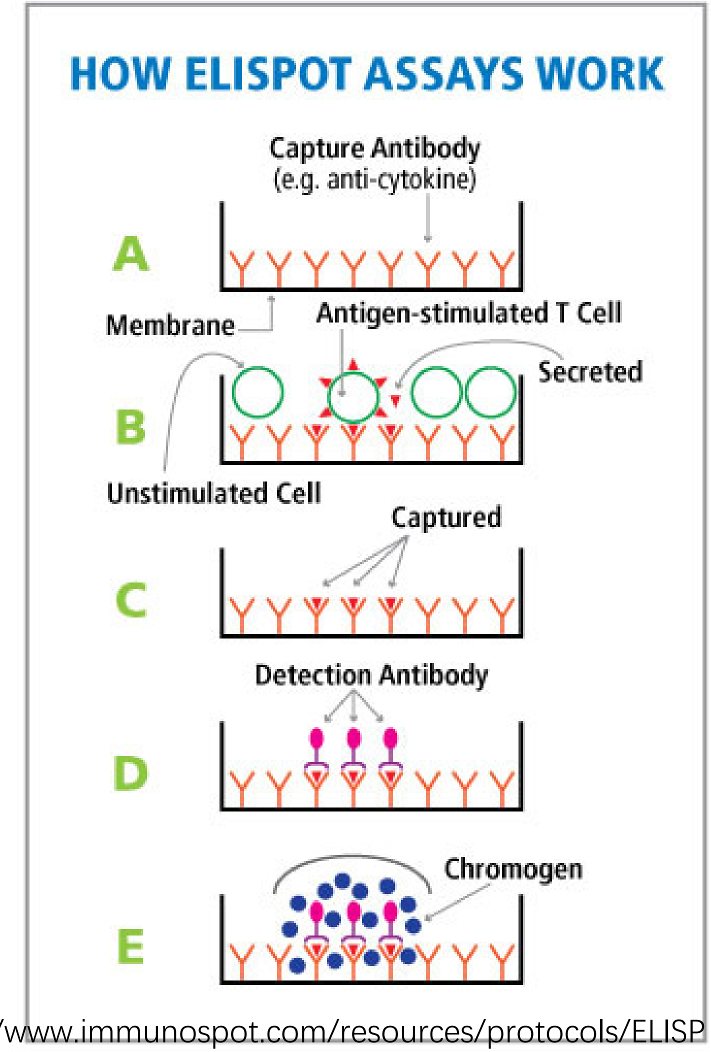


Nature Protocols **1**, 1120 - 1132 (2006)



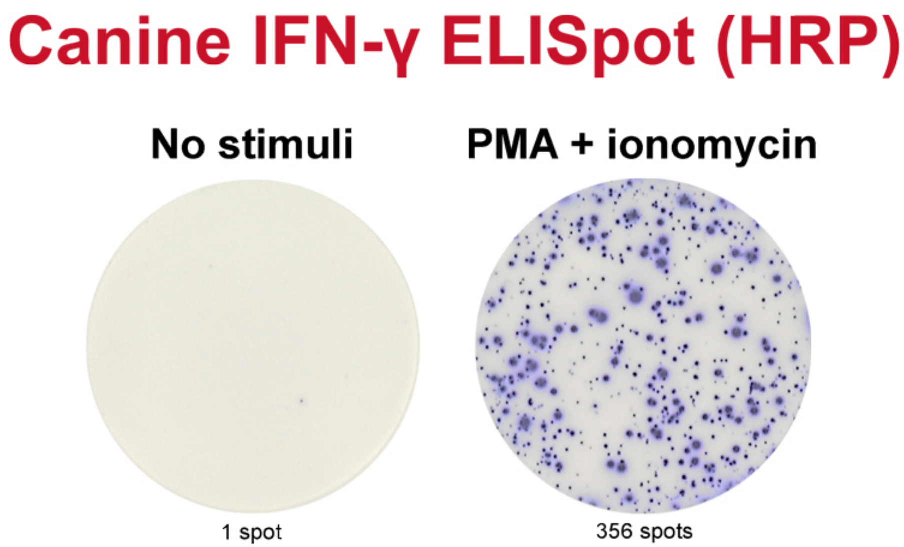
Limit of detection:
~0.1-0.2% among T cells
(1 in 1,000)

Identifying antigen-specific T-cells/B-cells



http://www.immunospot.com/resources/protocols/ELISPOT-protocol.htm

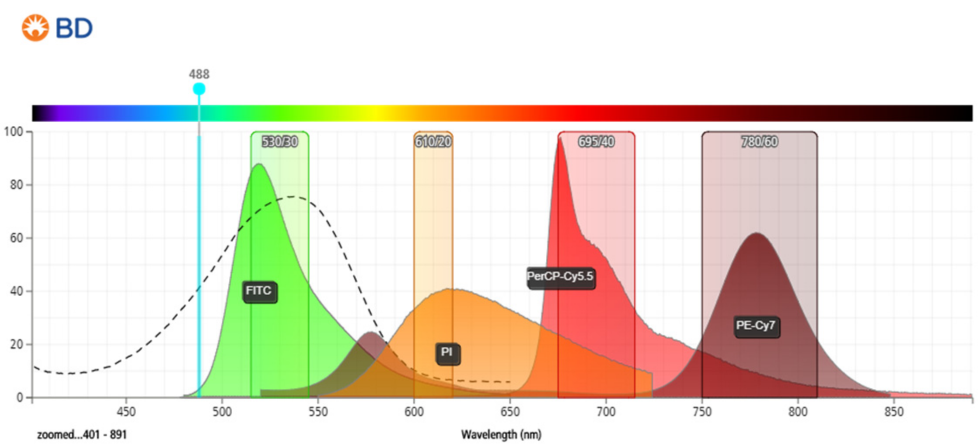
Ezyme-linked immunospot assay (ELISPOT)

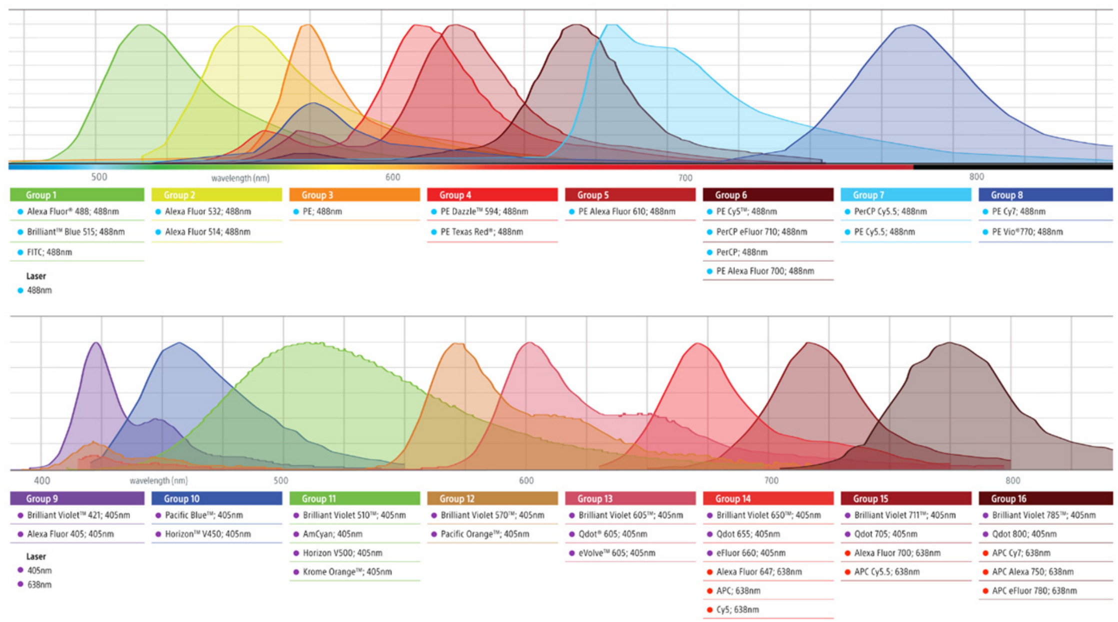


https://www.mabtech.com/knowledge-center/assay-principles/elispot-assay-principle/elispot-images

Limit of detection:
~0.0025% among assayed cells
(25 in 1,000,000)

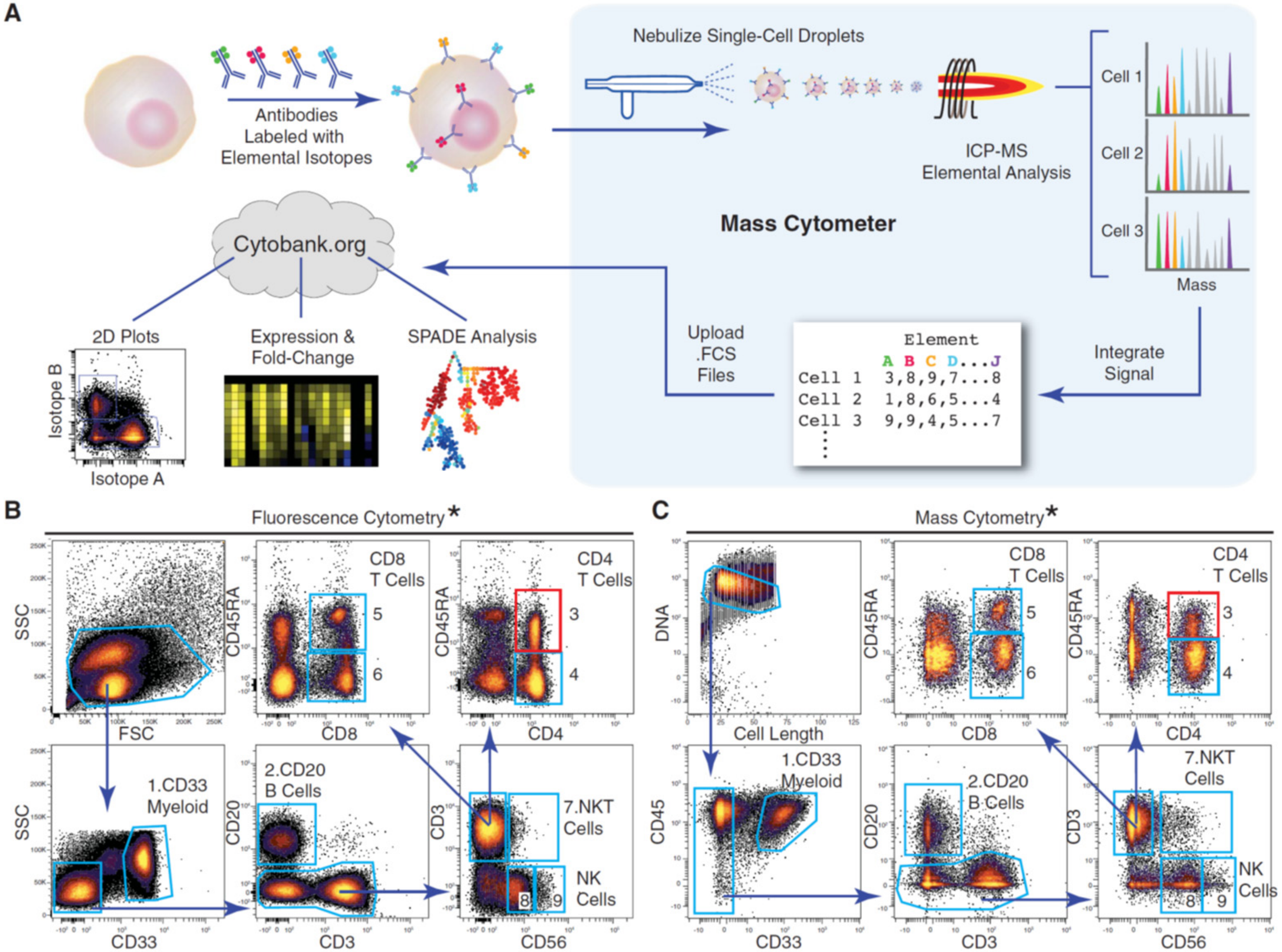
Flow cytometry: an intrinsic limitation





https://www.sonybiotechnology.com/us/instruments/sp6800/instruments-spectral/

New development: Single cell Mass Cytometry



Workflow summary of mass cytometry analysis:

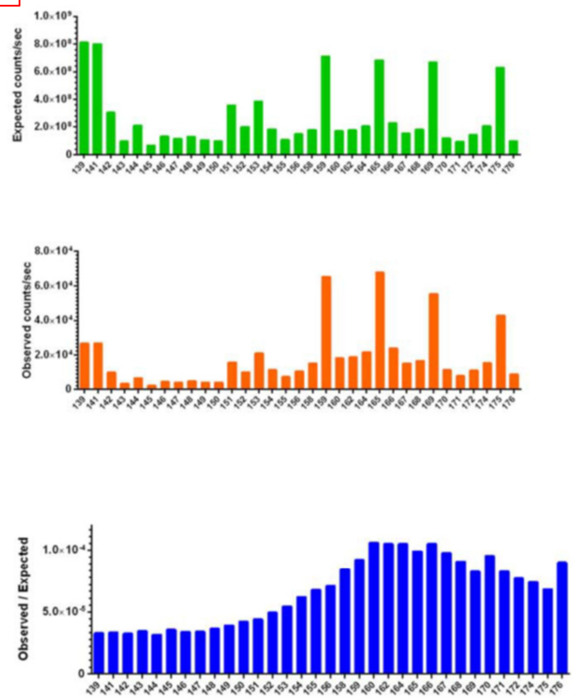
- Cells are stained with epitope-specific antibodies conjugated to transition element isotope reporters, each with a different mass.
- Cells are nebulized into single-cell droplets, and an elemental mass spectrum is acquired for each.
- The integrated elemental reporter signals for each cell can then be analyzed by using traditional flow cytometry methods
- as well as more advanced approaches such as heat maps of induced phosphorylation and tree plots.

Science 06 May 2011: Vol. 332, Issue 6030, pp. 687-696

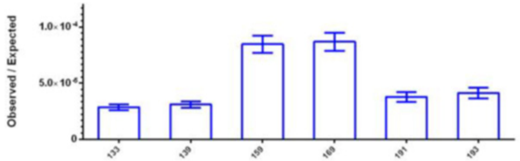
Bendall et al. Nature Biotechnology 30, 639–647 (2012)

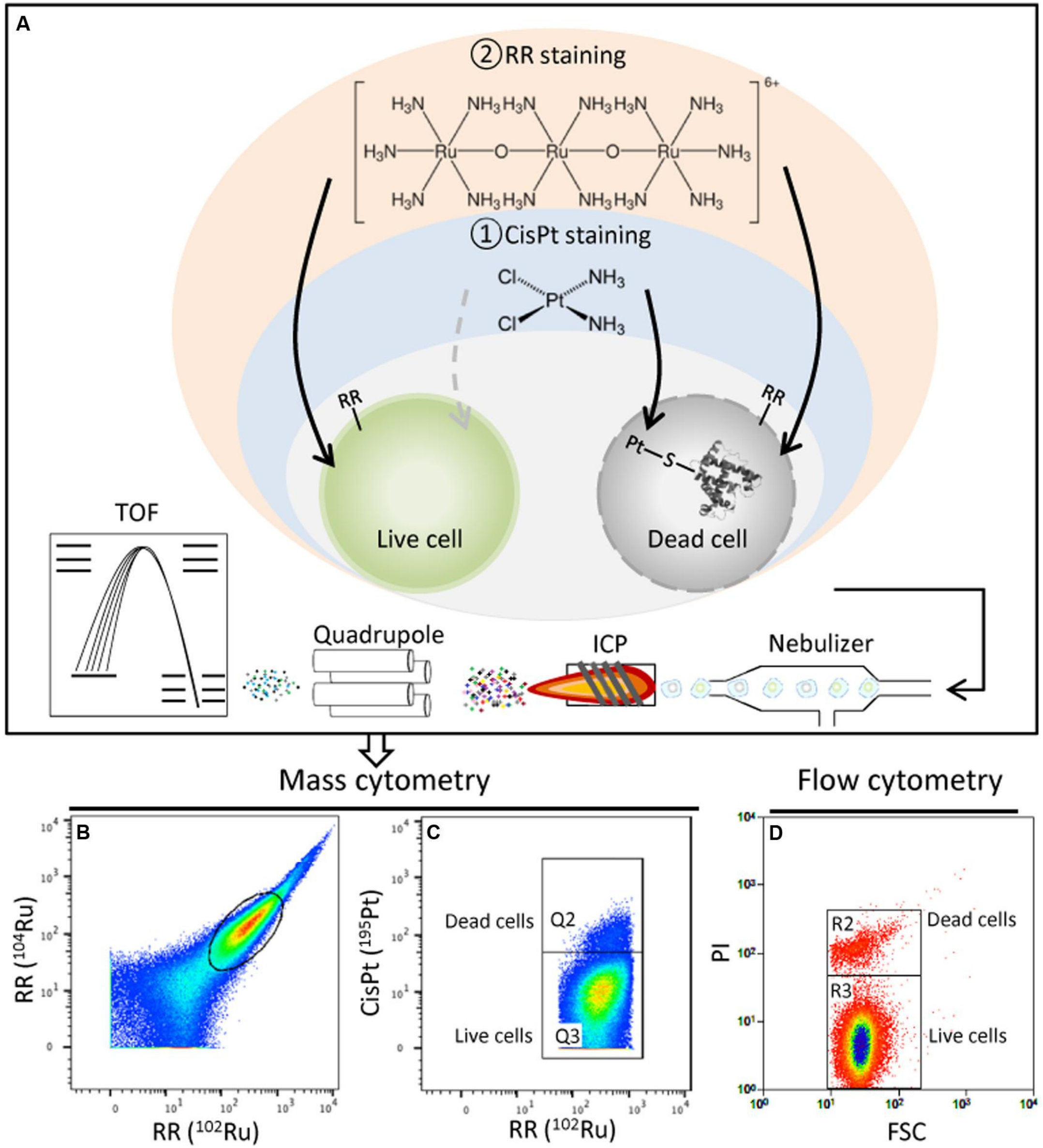
This slide is not required.

Element	Isotope	% NA	Detection Channel
La	139	99.91	139
Ce	140	88.45	140
Pr	141	100.00	141
Ce	142	11.11	142
Nd	142	27.20	
Nd	143	12.18	143
Nd	144	23.80	144
Sm	144	3.07	
Nd	145	8.30	145
Nd	146	17.19	146
Sm	147	14.99	147
Nd	148	5.76	148
Sm	148	11.24	
Sm	149	13.82	149
Nd	150	5.64	150
Sm	150	7.38	
Eu	151	47.80	151
Gd	152	0.20	152
Sm	152	26.75	
Eu	153	52.20	153
Gd	154	2.18	154
Sm	154	22.75	
Gd	155	14.80	155
Gd	156	20.47	156
Dy	156	0.06	
Gd	157	15.65	157
Gd	158	24.84	158
Dy	158	0.10	
Tb	159	100.00	159
Gd	160	21.86	160
Dy	160	2.34	
Dy	161	18.91	161
Dy	162	25.50	162
Er	162	0.14	
Dy	163	24.90	163
Dy	164	28.18	164
Er	164	1.61	
Ho	165	100.00	165
Er	166	33.60	166
Er	167	22.95	167
Yb	168	0.13	168
Er	168	26.80	
Tm	169	100.00	169
Yb	170	3.05	170
Er	170	14.90	
Yb	171	14.30	171
Yb	172	21.90	172
Yb	173	16.13	173
Yb	174	31.80	174
Lu	175	97.41	175
Yb	176	12.70	176
Lu	176	2.59	



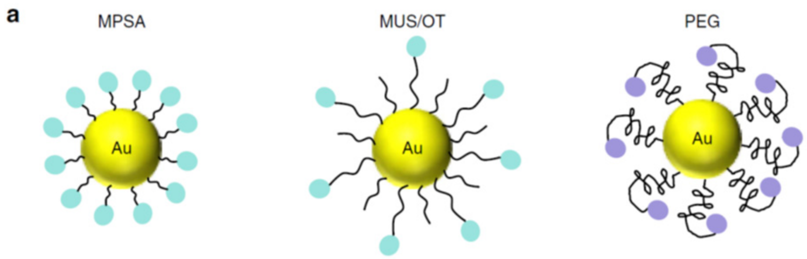
C



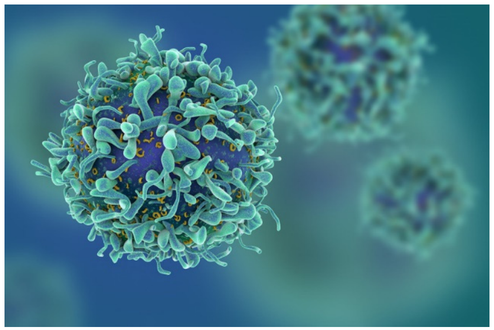


Cytometry Part A 87A: 357368, 2015

How to study the interactions between immune cells and metal particles?



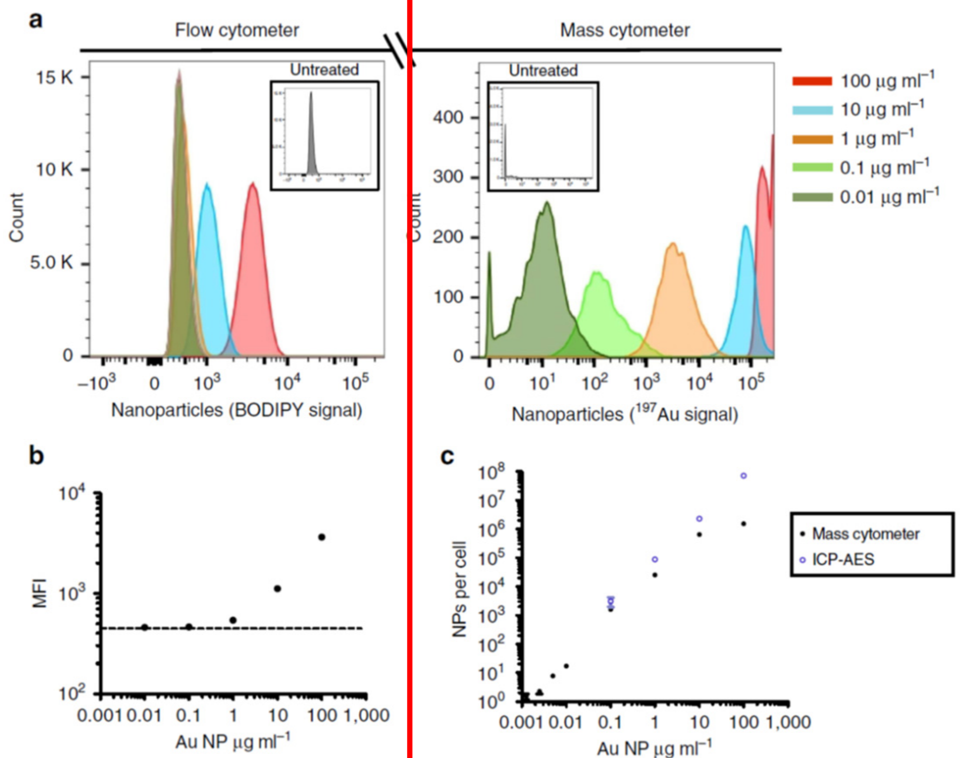
T cell



Gold/Atomic mass
196.96657 u

Conventional flow cytometry

Mass cytometry



detection limit: 1.5×10^{6} particles/cell

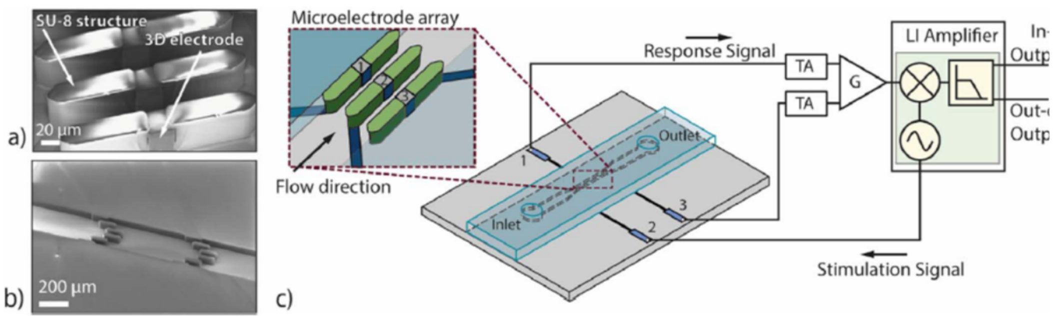
detection limit: 4.2 particles/cell

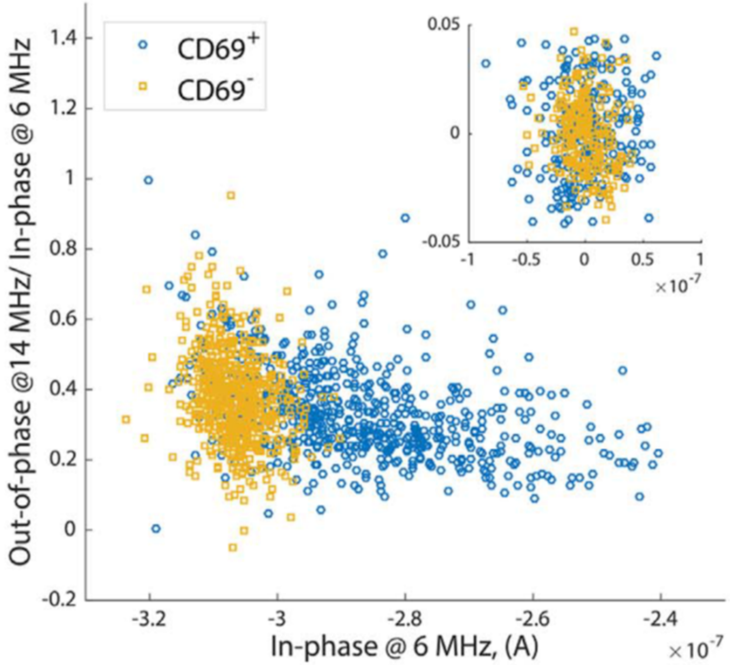
https://www.mskcc.org/blog/t-cell-science-behind-immunotherapy
Yang, Y. S., et al. (2017). Nature Communications 8: 14069.

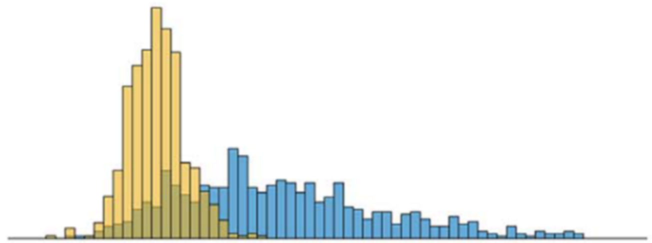
New development: Label-free identification of activated T cells

– Based on electronic properties of cells
– impedance signature was used to differentiate inactivated and activated CD8+ T cells
– It relates to the size, morphology, and structure of plasma membrane of cells

Carlotta Guiducci





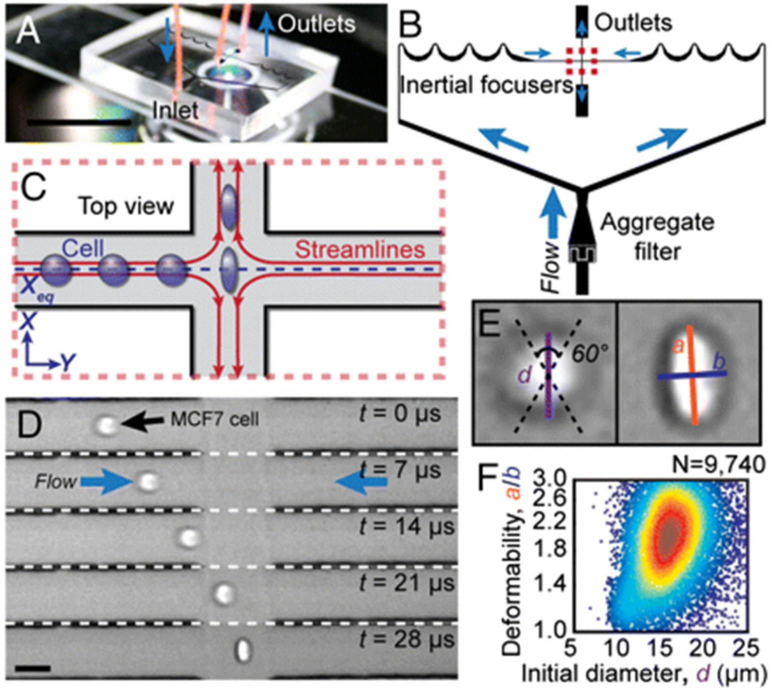


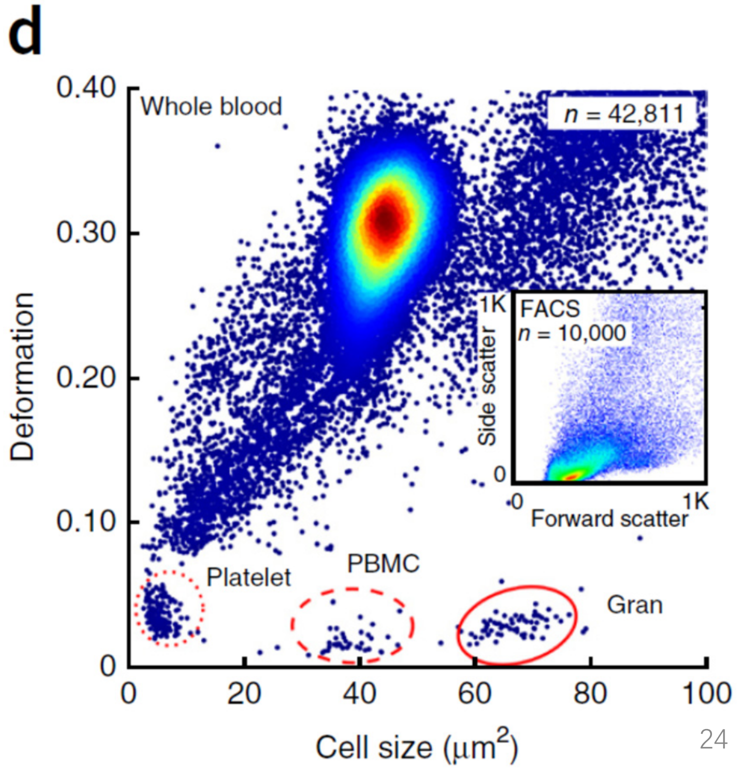
Rollo, E., et al. (2017). "Label-free identification of activated T lymphocytes through tridimensional microsensors on chip." Biosensors & Bioelectronics **94**: 193-199.

New development: Real-time deformability cytometry

- Based on mechanical properties of cells
- Size and deformability signature of different cell populations

- red dotted (platelets)
- dashed (peripheral blood mononucleated cells, PBMC)
- solid lines (granulocytes, gran)

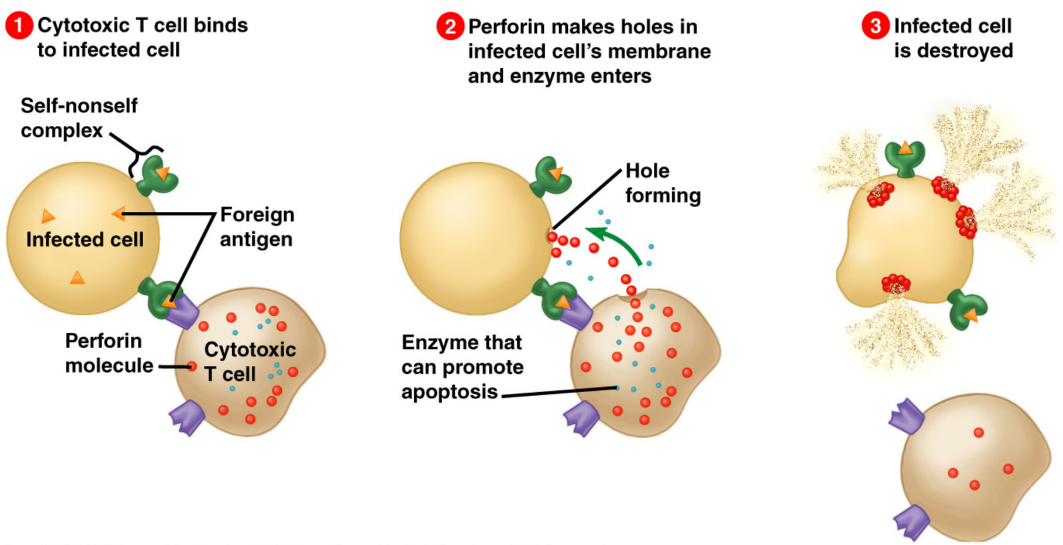




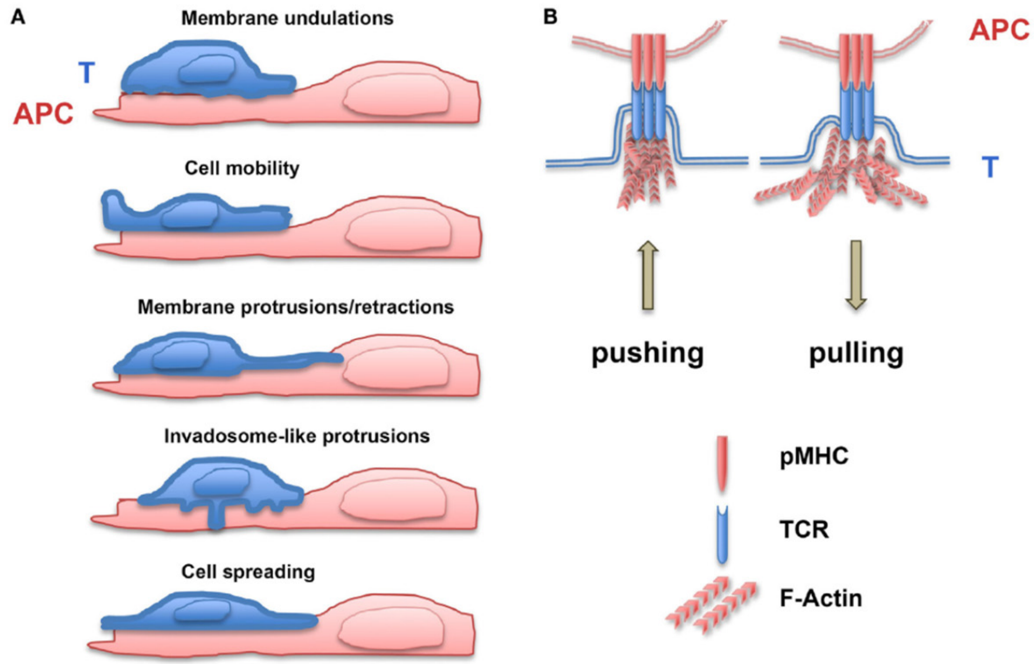
PNAS 2012 May, 109 (20) 7630-7635; Nature Methods 12, pages199–202 (2015)

Does mechanical force play a role in immune system?

– CTL killing is mediated by pore formation reagents

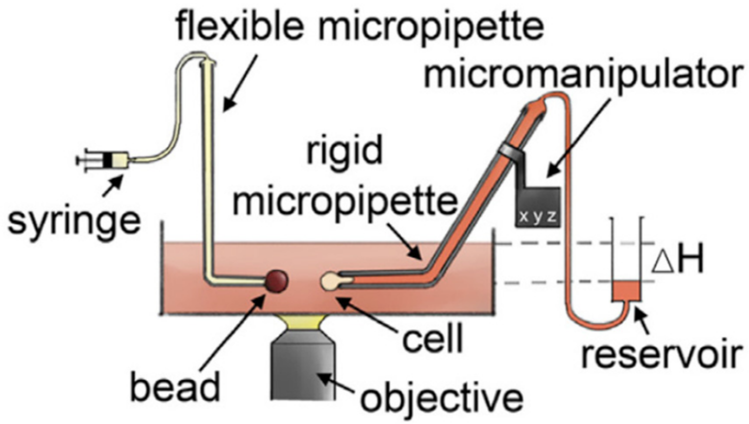


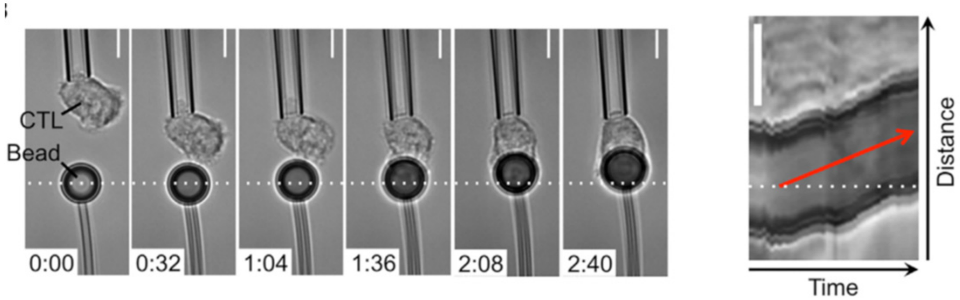
–And also, force exertion at the immunological synapse

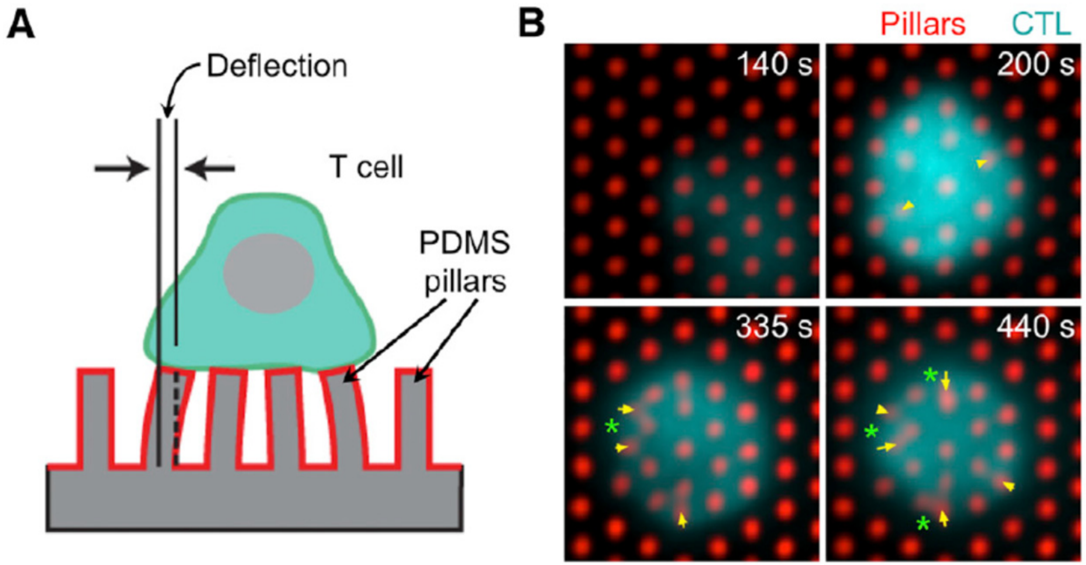


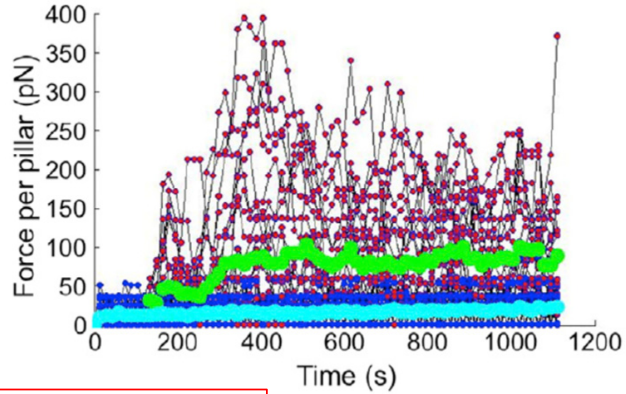
Hivroz, C. and M. Saitakis (2016). "Biophysical Aspects of T Lymphocyte Activation at the Immune Synapse." Frontiers in immunology 7(46).

How to characterize the mechanical force in immune system?







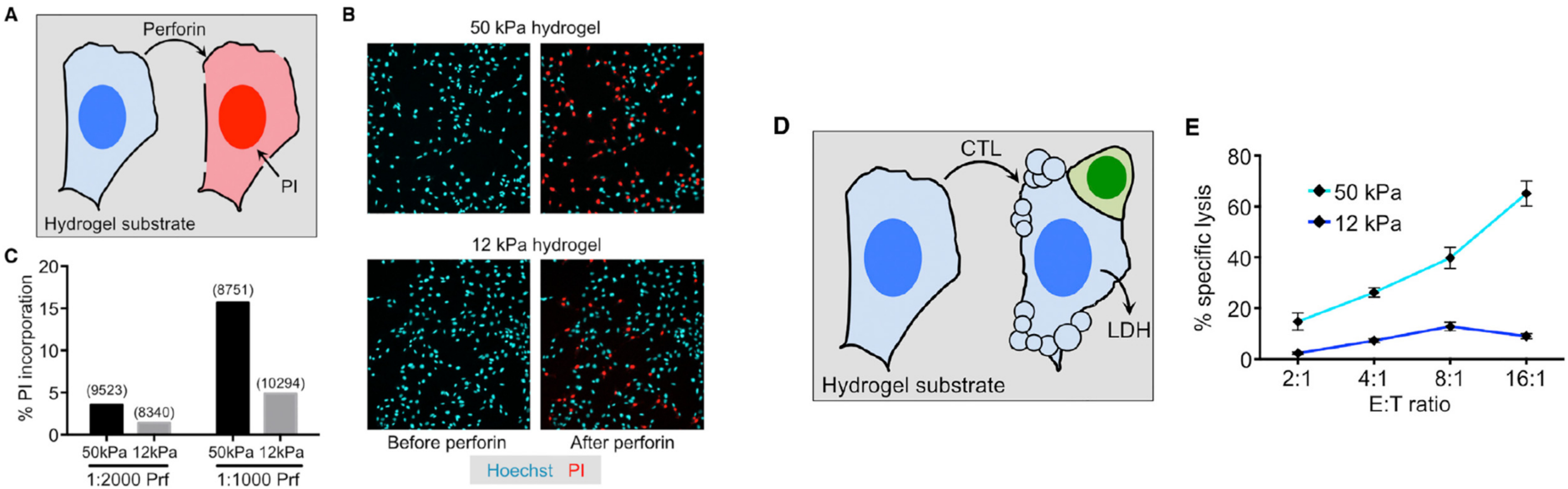


Basu, R., et al. (2016). "Cytotoxic T Cells Use Mechanical Force to Potentiate Target Cell Killing." Cell 165(1): 100-110.

This slide is not required.

How to prove the mechanical force help in killing?

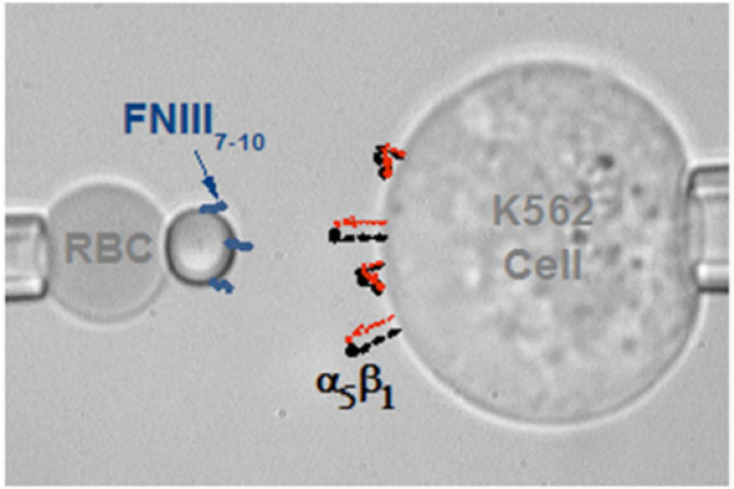
This slide is not required.

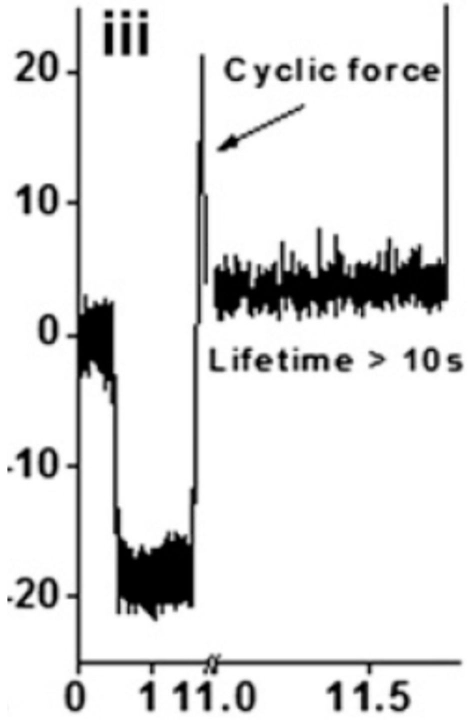


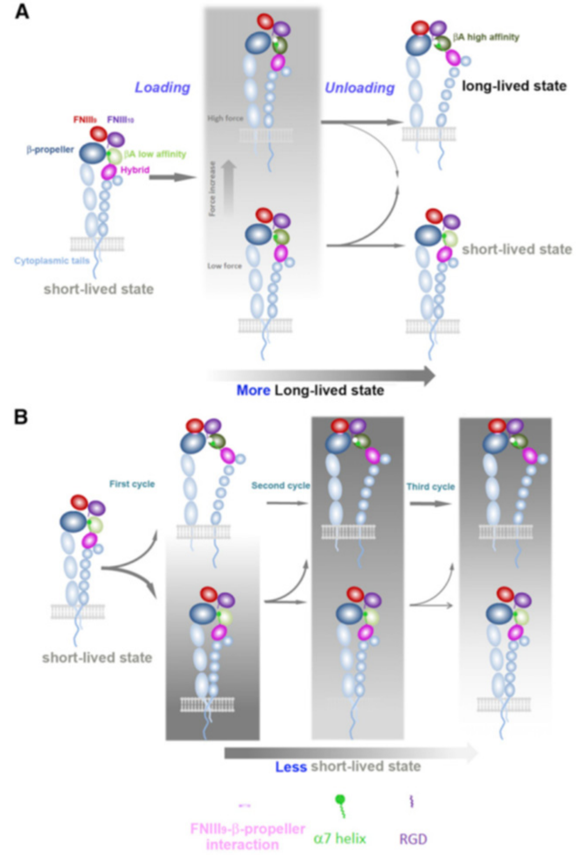
Basu, R., et al. (2016). "Cytotoxic T Cells Use Mechanical Force to Potentiate Target Cell Killing." Cell 165(1): 100-110.

Does immune cell respond to mechanical force?

A



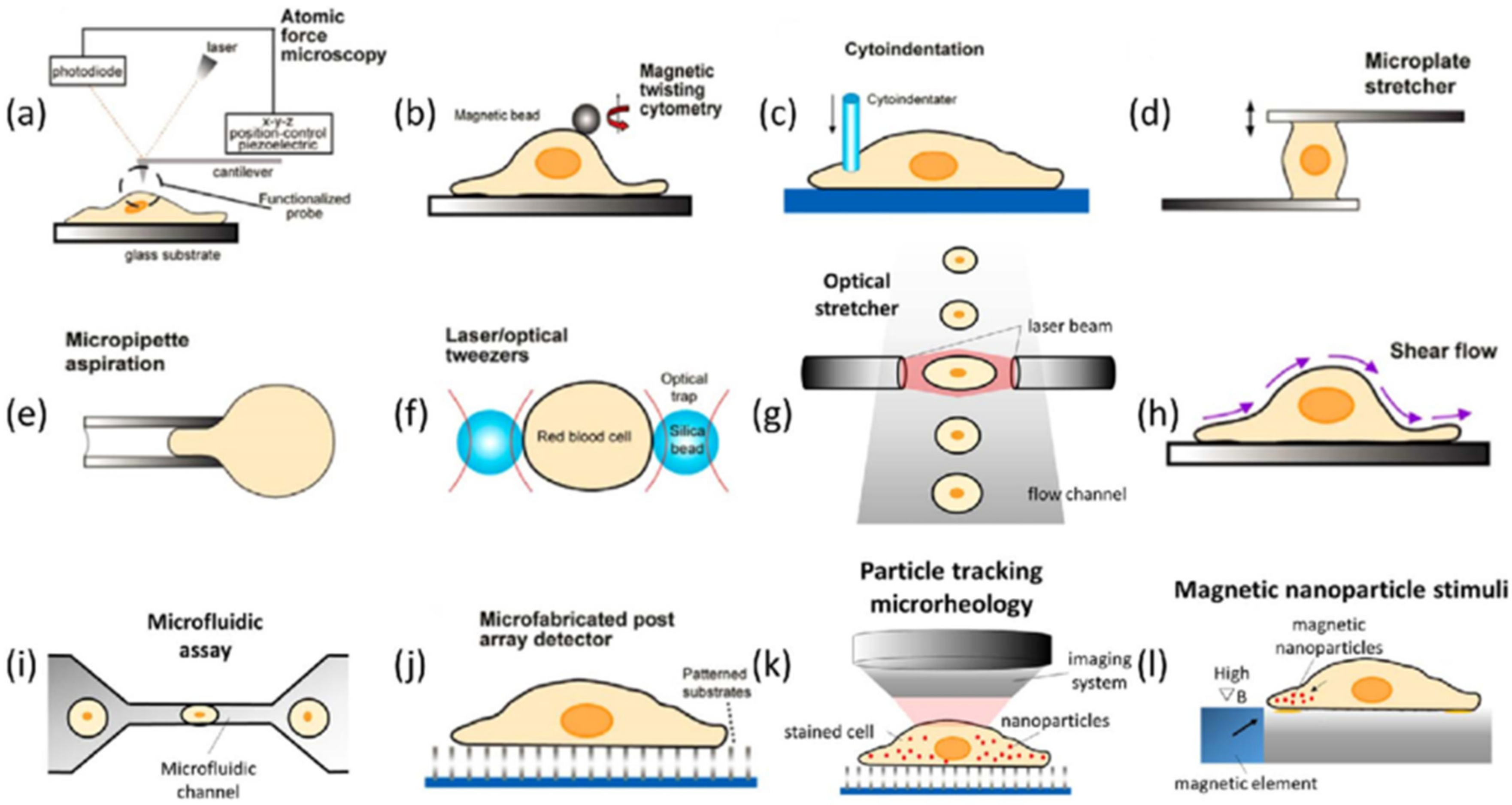




This slide is not required.

Kong, F., et al. (2013). "Cyclic mechanical reinforcement of integrin-ligand interactions." Molecular Cell 49(6): 1060-1068.

Techniques to characterize the mechanical force of cells

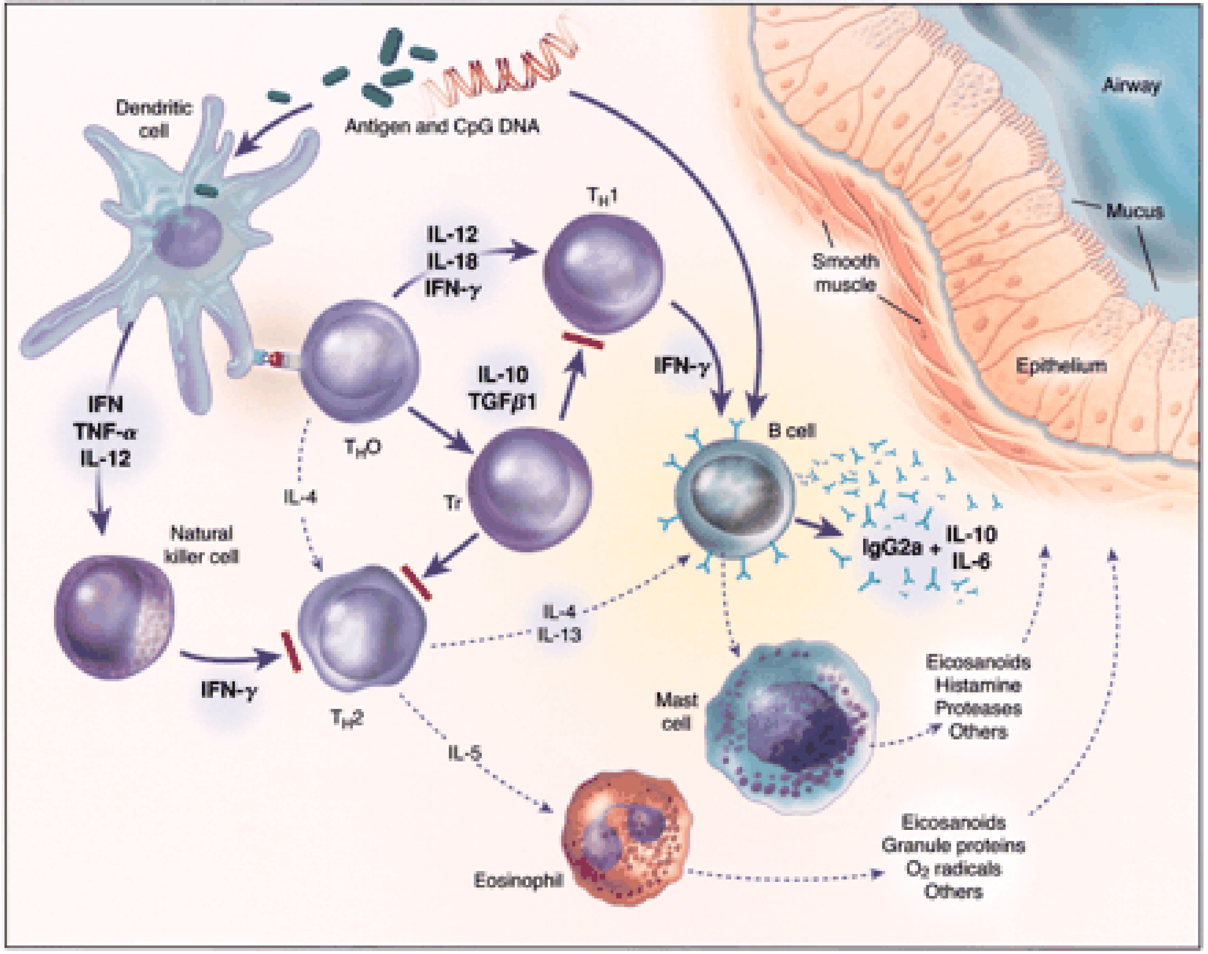


Micro and Nano-Scale Technologies for Cell Mechanics
https://doi.org/10.5772/59379

Major techniques for cancer cell mechanics study. (a) Atomic force spectroscopy; (b) magnetic twisting cytometry; (c) cytoindentation; (d) microplate stretcher; (e) micropipette aspiration; (f) laser/optical tweezers; (g) optical stretcher; (h) shear flow; (i) microfluidic assay; (j) microfabricated post array; (k) particle tracking microrheology; (l) magnetic nanoparticle-based stimuli

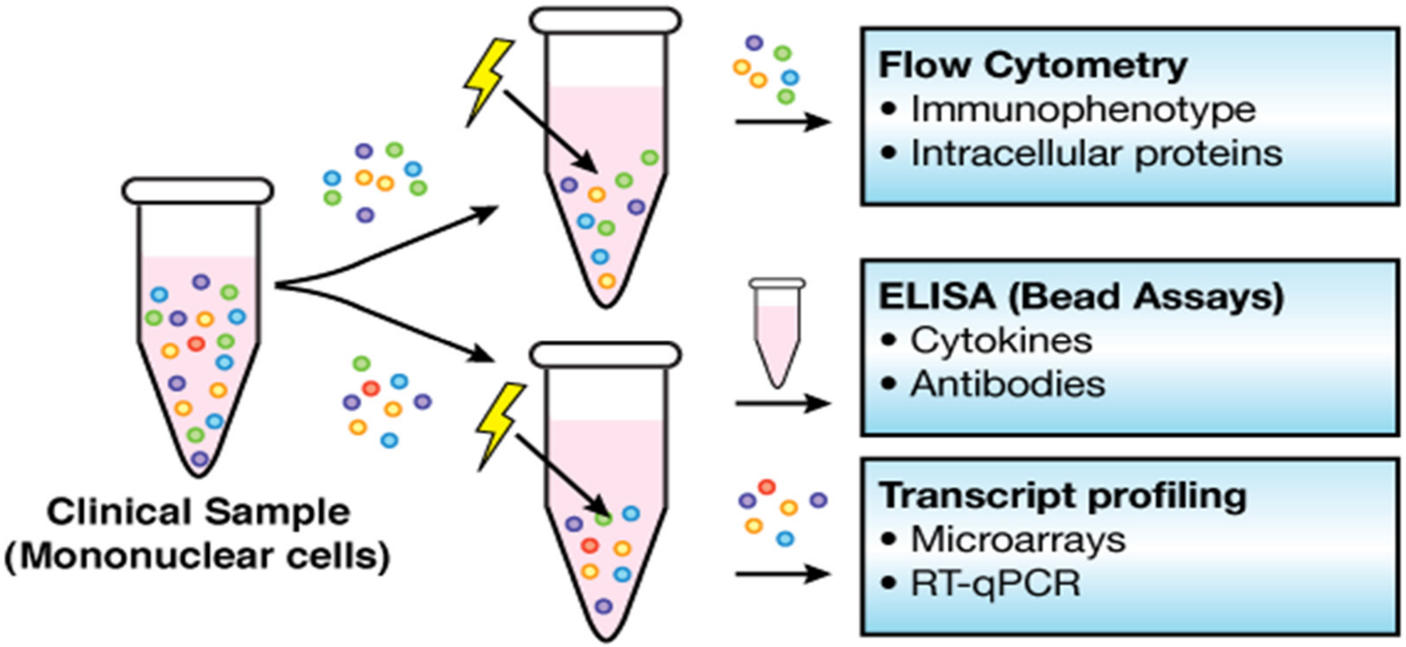
This slide is not required.

The immune system is highly complex



Silverman, E. S. (2003). Immunostimulatory DNA for Asthma: Better than Eating Dirt. American Journal of Respiratory Cell and Molecular Biology, 28(6), 645–647. doi:10.1165/rcmb.F268

How do we analyze the state of this network?



segregated nature of assays - cannot link result from one measurement to another at the single-cell level

cannot probe kinetics of processes within a single cell

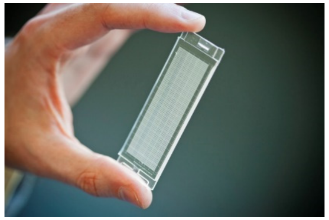
destructive assays, limit ability to work with small cell samples

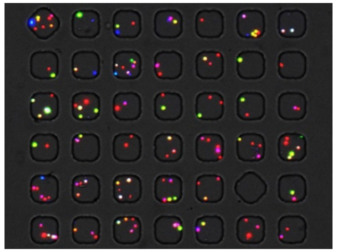
Is it possible to do all together?

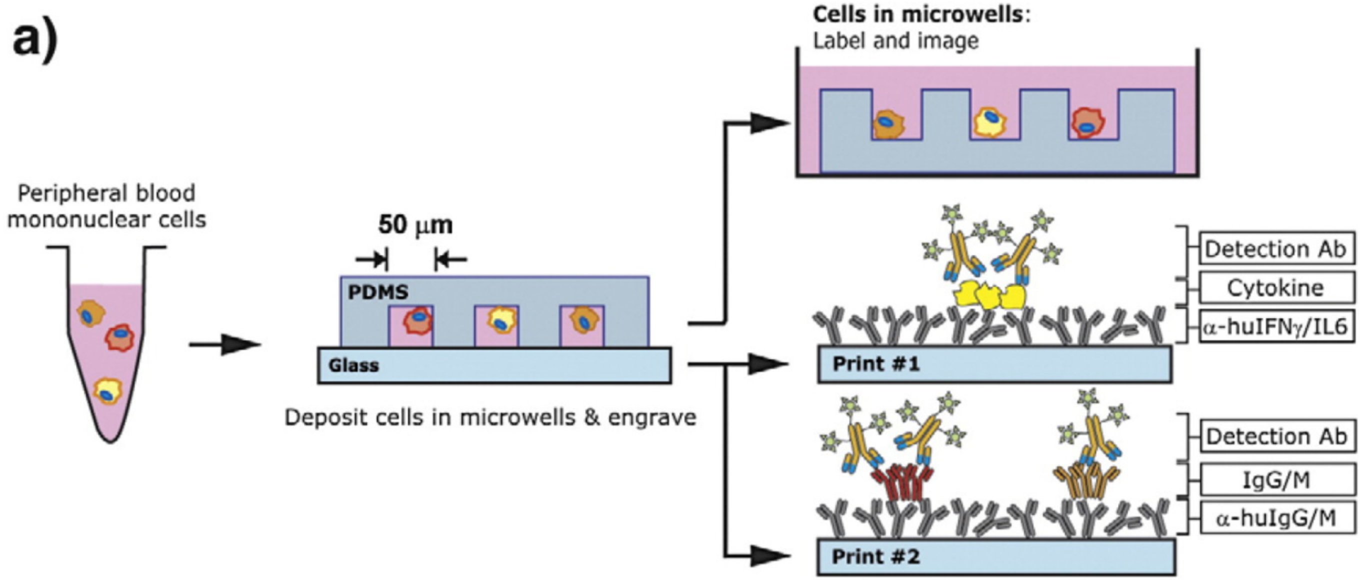
Microengraving: a strategy to capture quantitative, kinetic information from live cells

Chris Love, MIT

Array of microwells







Microengraving with human PBMCs for concurrent detection of antibodies and cytokines. (a) Schematic illustration of the microengraving method for detection of multiple secreted products from human PBMCs. PBMCs, suspended in media, are deposited onto a large array (~20×50 mm2) of microwells (~0.1 nL each) molded on a poly(dimethylsiloxane) slab, and allowed to settle from suspension in the microwells at a density of~1 cell/well. The cells adhere loosely to the bottoms of each well. Excess cells are rinsed off the surface of the array, and the microwells are then inverted onto a glass slide coated with a specific capture reagent (e.g., anticytokine). After an incubation period (~1 h), the microwells are removed and applied to a second glass slide coated with a different capture reagent (e.g., anti-IgG and anti-IgM). The resulting microarrays are interrogated with fluorescently labeled reagents for detection and laser-based fluorescence scanners. After printing, the cells in the wells can be stained in situ for subsequent imaging by immunofluorescence.

http://news.mit.edu/2015/faculty-profile-christopher-love-0617

Love, J. C., Ronan, J. L., Grotenbreg, G. M., van der Veen, A. G., & Ploegh, H. L. Nature Biotechnology, 24(6), 703–707 (2006)
Bradshaw, E. M., Kent, S. C., Tripuraneni, V., Orban, T., Ploegh, H. L., Hafler, D. A., & Love, J. C. Clinical Immunology, 129(1), 10–18 (2008)

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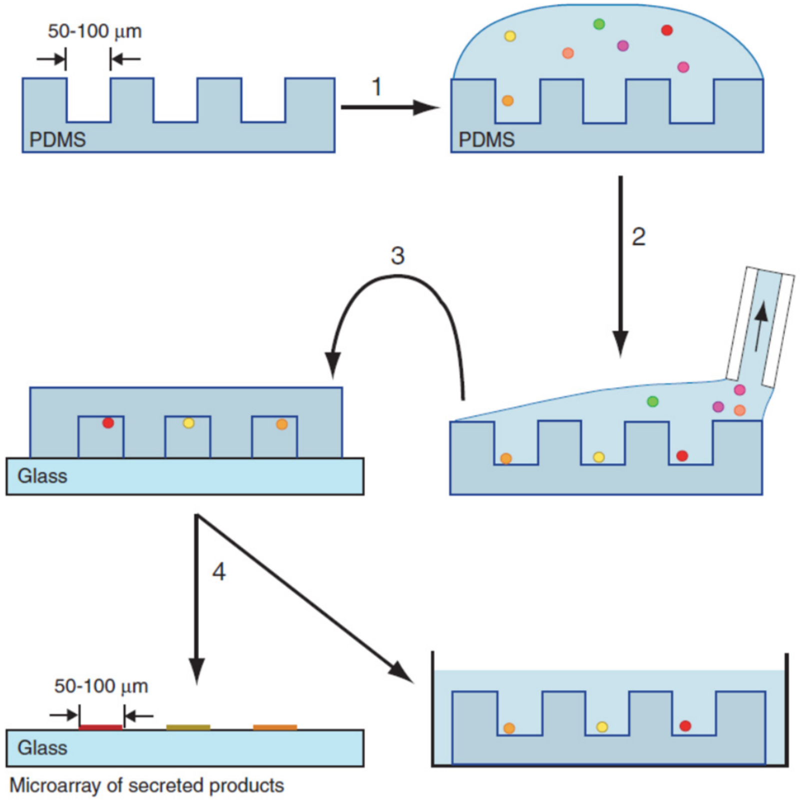
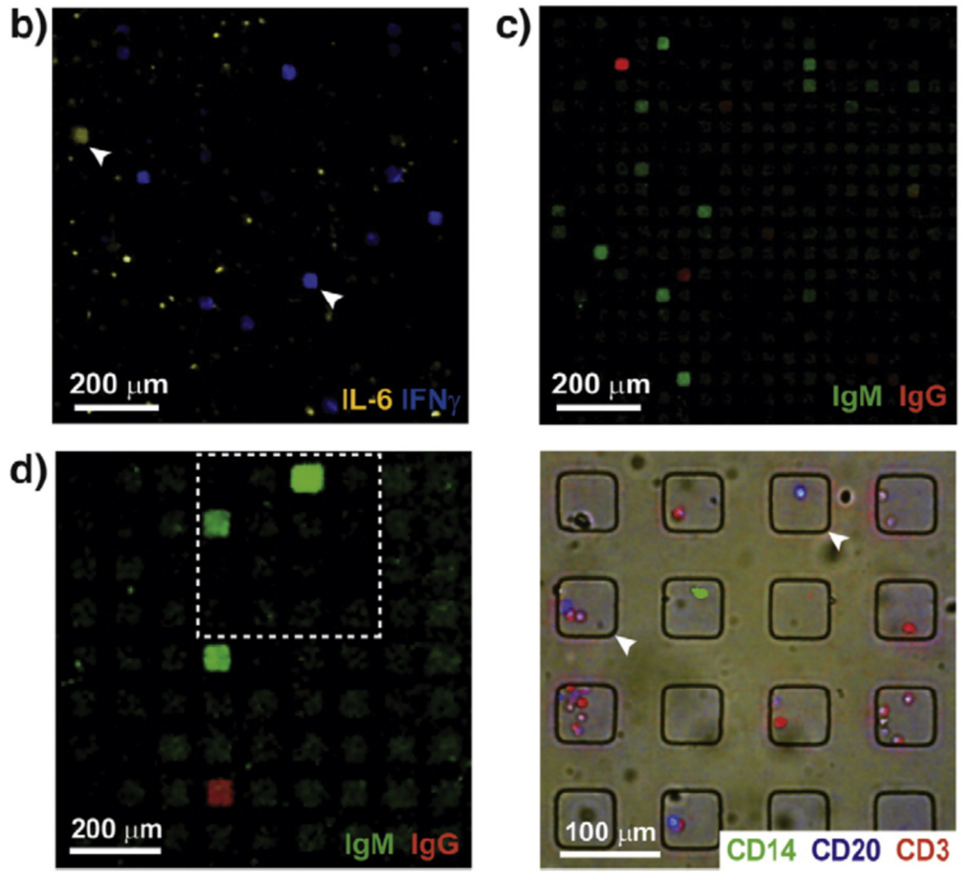
Microengraving: a strategy to capture quantitative, kinetic information from live cells





Bradshaw, E. M., Kent, S. C., Tripuraneni, V., Orban, T., Ploegh, H. L., Hafler, D. A., & Love, J. C. Clinical Immunology, 129(1), 10–18 (2008)

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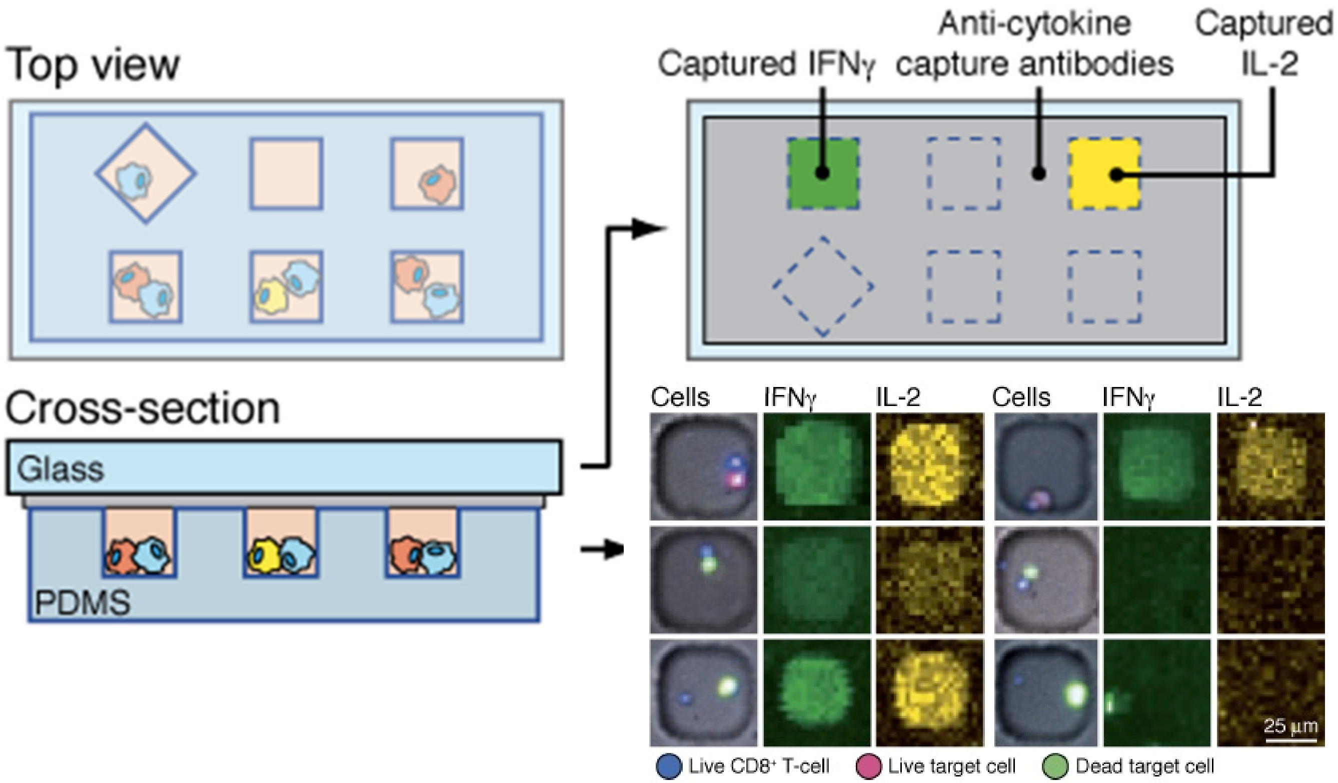

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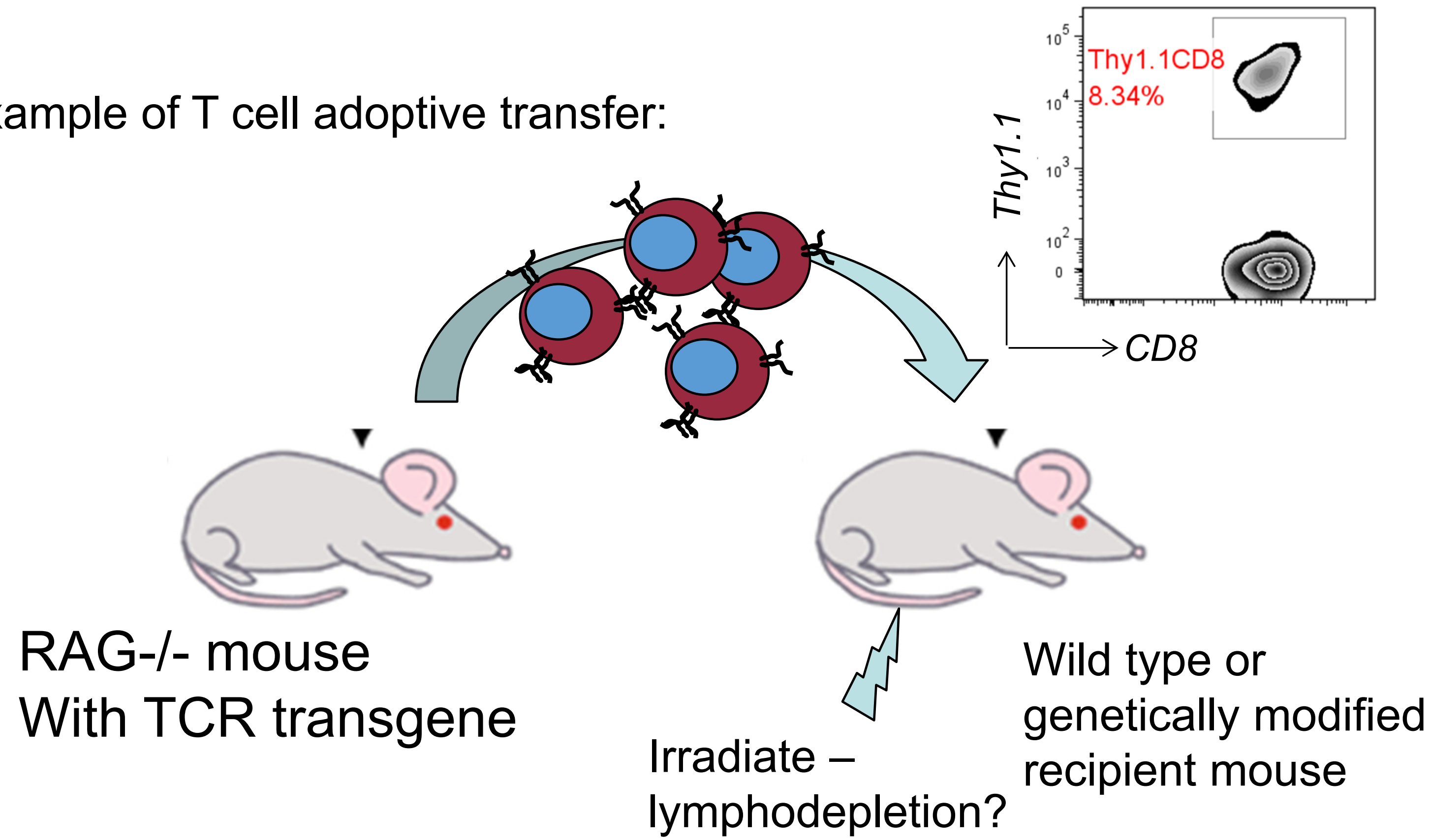
N. Varadarajan, B. Juelg et al., J. Clin. Invest. (2011)

Using its mobile nature to genetically manipulate the immune system: adoptive transfer models

Example of T cell adoptive transfer:



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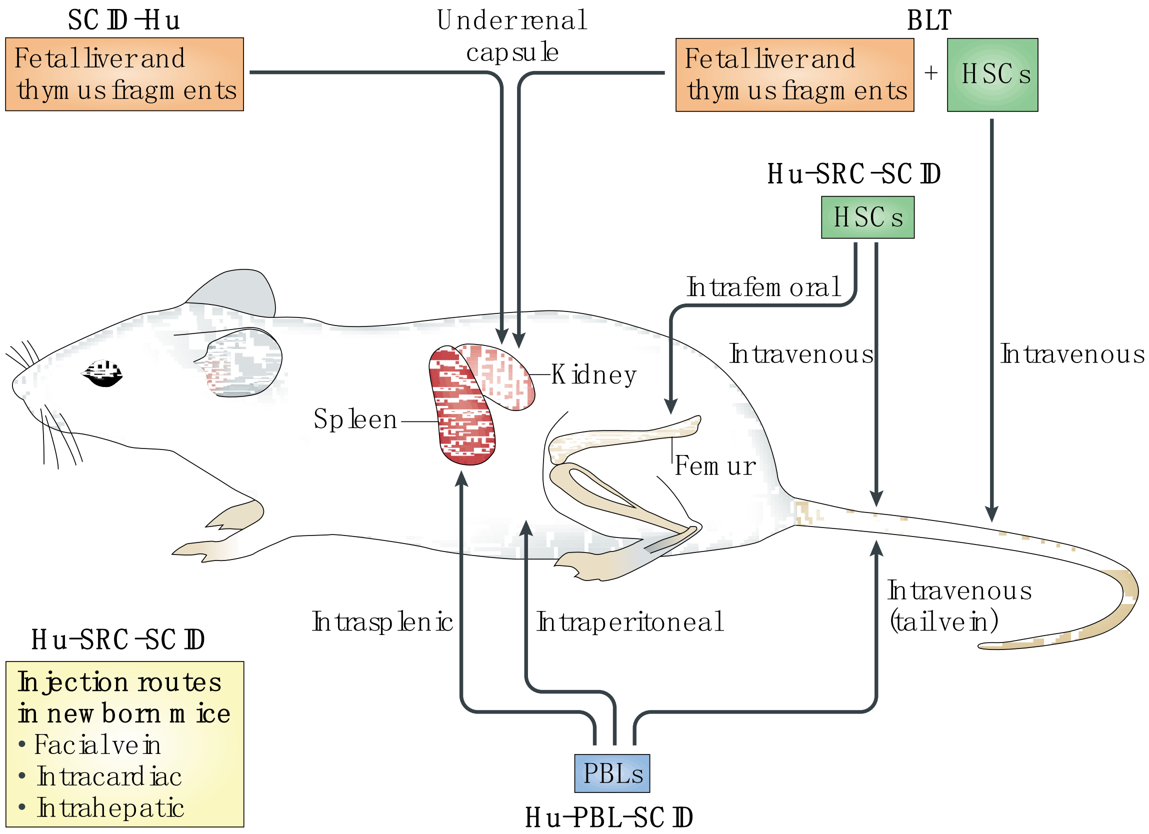
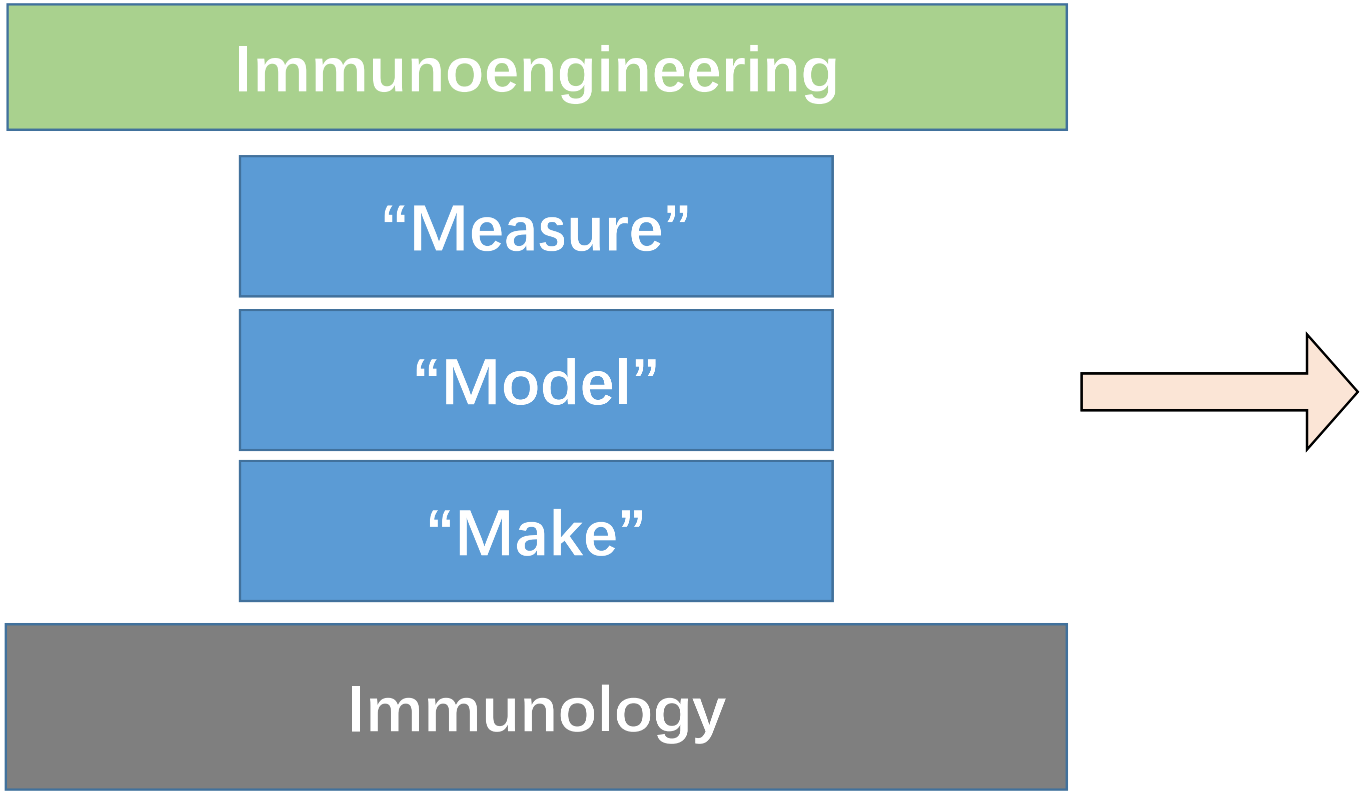
Humanized mice



Shultz, L. D., Brehm, M. A., Garcia-Martinez, J. V., & Greiner, D. L. (2012). *Nature Reviews Immunology*, *12*(11), 786–798. http://doi.org/doi:10.1038/nri3311

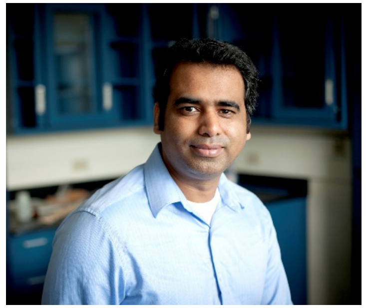
Immunoengineering:
Application of a new engineering



Engineering artificial immune systems

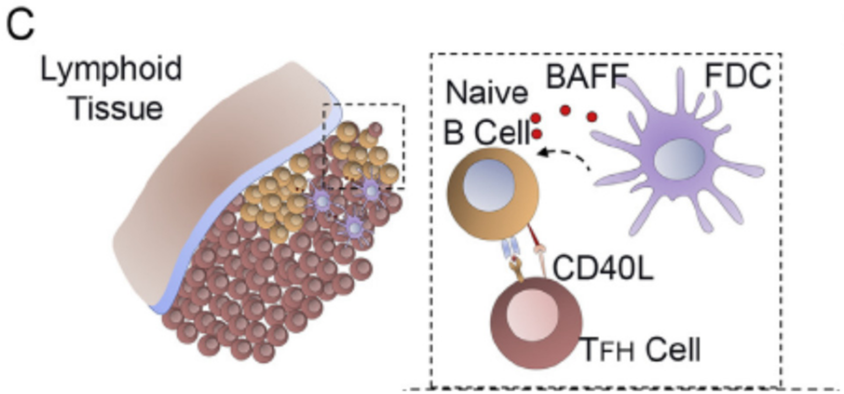
Modified from slides of Prof. Darrell Irvine (MIT)

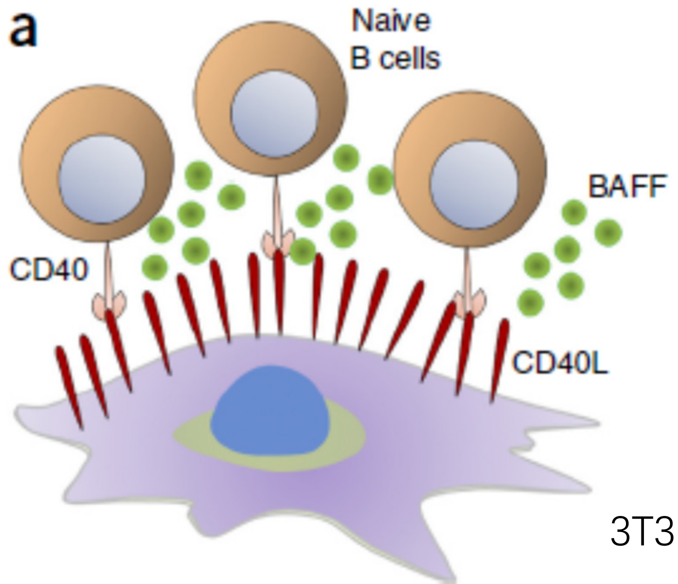
Engineering artificial immune systems --Lymph node



Ankur Singh (Georgia Tech)

BAFF: a B cell activating factor



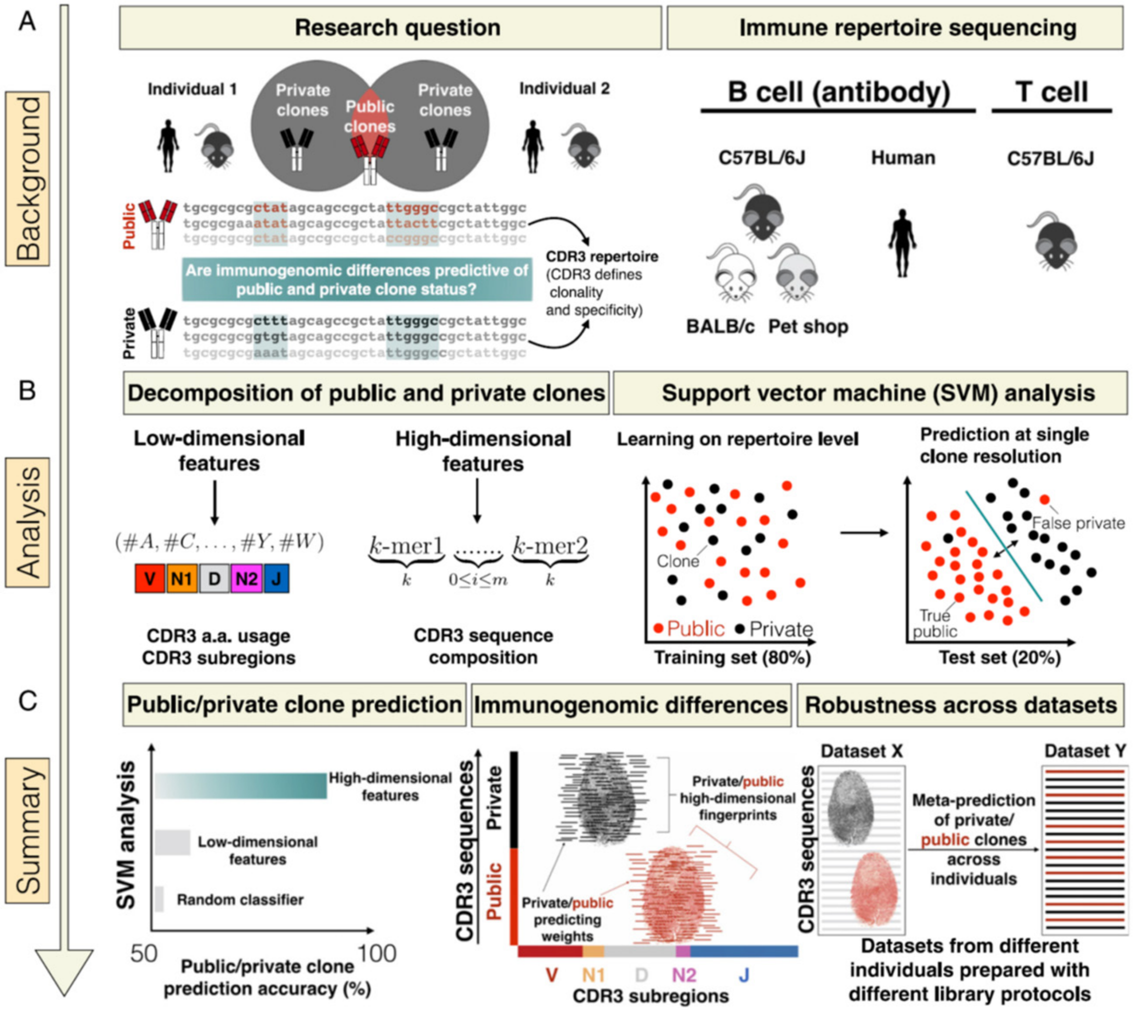


3T3 fibroblasts

the BALB/c 3T3 fibroblasts are stably transduced with **CD40L and BAFF** (hereafter we refer to this transgenic cell line as 40LB), and function as a substitute for TFH cells and FDCs.

Biomaterials 63 (2015) 24-34;
Nature Protocols volume12, pages168–182 (2017)

Modeling the immune system: systems biology approaches

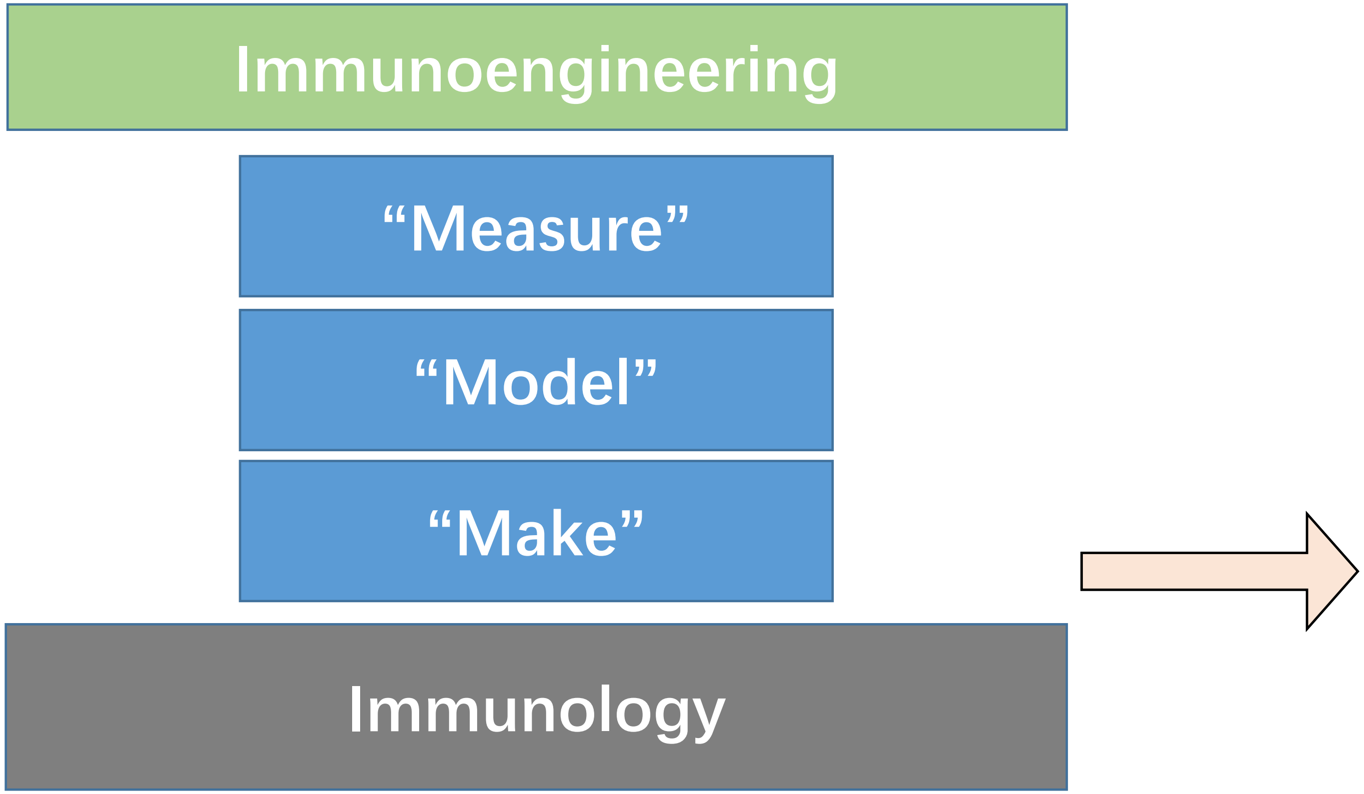


Sai Reddy, ETH

This slide is not required.

J Immunol September 18, 2017, ji1700594; DOI: https://doi.org/10.4049/jimmunol.1700594

Immunoengineering:
Application of a new engineering



Mechanical engineering
Electrical engineering
Tissue engineering
Systems Biology and Engineering
Protein Engineering
Genetic Engineering
Materials Engineering
Nano-engineering
Chemical engineering
Metabolic Engineering
….

Manipulating immune system; designing new immuno-therapeutics

Modified from slides of Prof. Darrell Irvine (MIT)