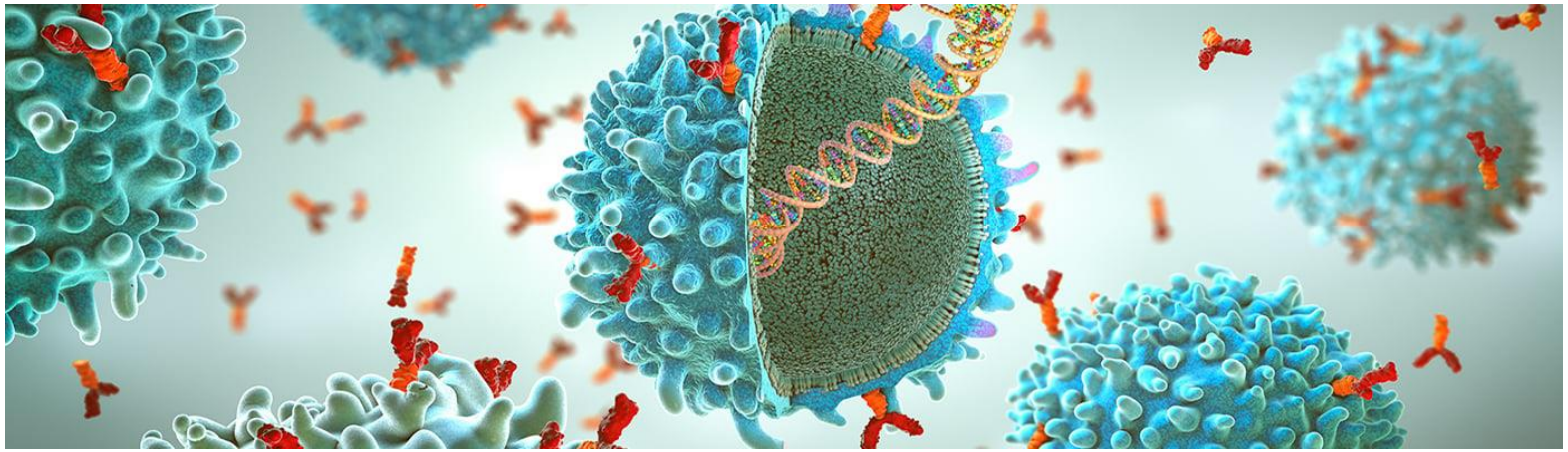
# Foundations in Cancer Engineering



**Lecture 1:** Introduction & Instrumentation for Biomedical Research

September 14, 2026

Daniel A. Heller, PhD

Member, Molecular Pharmacology Program, Sloan Kettering Institute

Memorial Sloan Kettering Cancer Center

# Outline

- A Theory of Cancer Engineering
- Problems in Need of Cancer Engineering
- Core Knowledge of Cancer Engineering and Some Rationale
- Feedback and Possible Next Steps
- Engineering Tools for Biology Research

# A Theory of Cancer Engineering

- A Theory of Cancer Engineering
- Problems in Need of Cancer Engineering
- Core Knowledge of Cancer Engineering and Some Rationale
- Feedback, Advice, Next Steps
- Engineering Tools for Biology Research

# Why Create this (Sub-)Discipline?

# A Perspective Piece:

Cancer Cell

CellPress

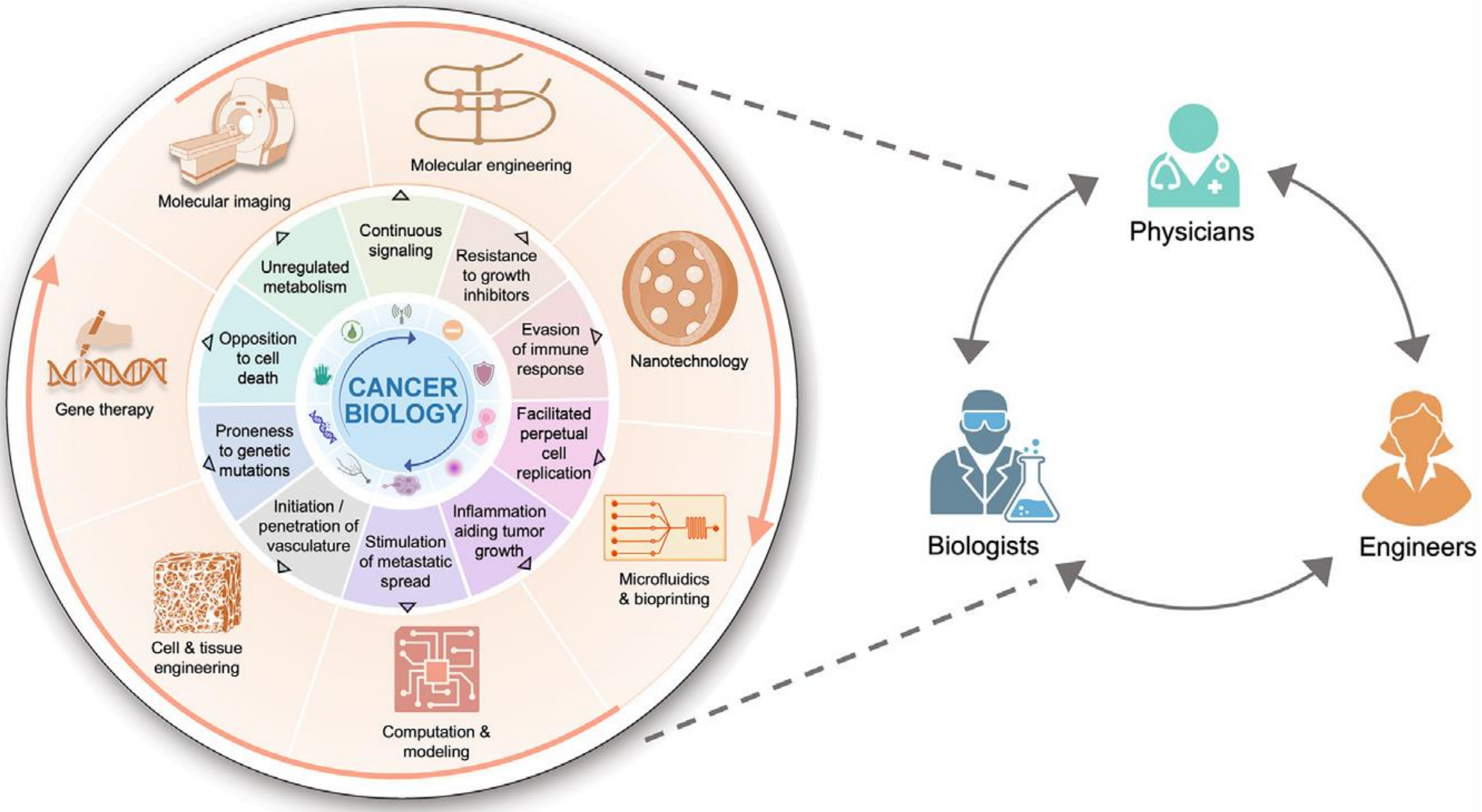
Commentary

## Engineering focusing on cancer

Kayvan R. Keshari,[1,2,4,5,*] Daniel A. Heller,[1,4,5,*] Rostislav Boltyanskiy,[5] Hedvig Hricak,[1,2,4,5] Thomas Magaldi,[4] and Michael Overholtzer[3,4,5,*]

[1]Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[2]Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[3]Cell Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[4]Gerstner Sloan Kettering School for Biomedical Sciences, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[5]Center for Molecular Imaging and Bioengineering, Memorial Sloan Kettering Cancer Center, New York, NY, USA
*Correspondence: rahimikk@mskcc.org (K.R.K.), hellerd@mskcc.org (D.A.H.), overholm1@mskcc.org (M.O.)
https://doi.org/10.1016/j.ccell.2024.04.013

# Cancer Engineering




"A schematic representation of the elements of cancer engineering that are built on the needs and unique features of cancer biology" *Cancer Cell* (2024), https://doi.org/10.1016/j.ccell.2024.04.013

# Some Potential Types/Goals of Technologies in Cancer Engineering

- Therapeutic technologies
- Diagnostic technologies
- Tools/methods for cancer biology research
- Tools/methods for clinical research
- Tools/methods for drug discovery

# Some Disciplines Not Often Included in Bioengineering/Biomedical Engineering

- Pharmacology
- Drug discovery and development
- Biomarker discovery
- Clinical diagnostics
- Imaging probes
- Chemical biology

# Another Perspective: Engineering and Biology are Verbs

- Engineer:
  - A tool
  - A method
  - A technology

- Biology:
  - To study
  - To ask a question
  - To assess a therapeutic action

# Some Problems in Need of Cancer Engineering

- A Theory of Cancer Engineering
- Problems in Need of Cancer Engineering
- Core Knowledge of Cancer Engineering and Some Rationale
- Feedback, Advice, Next Steps
- Engineering Tools for Biology Research

# Better Cancer Models

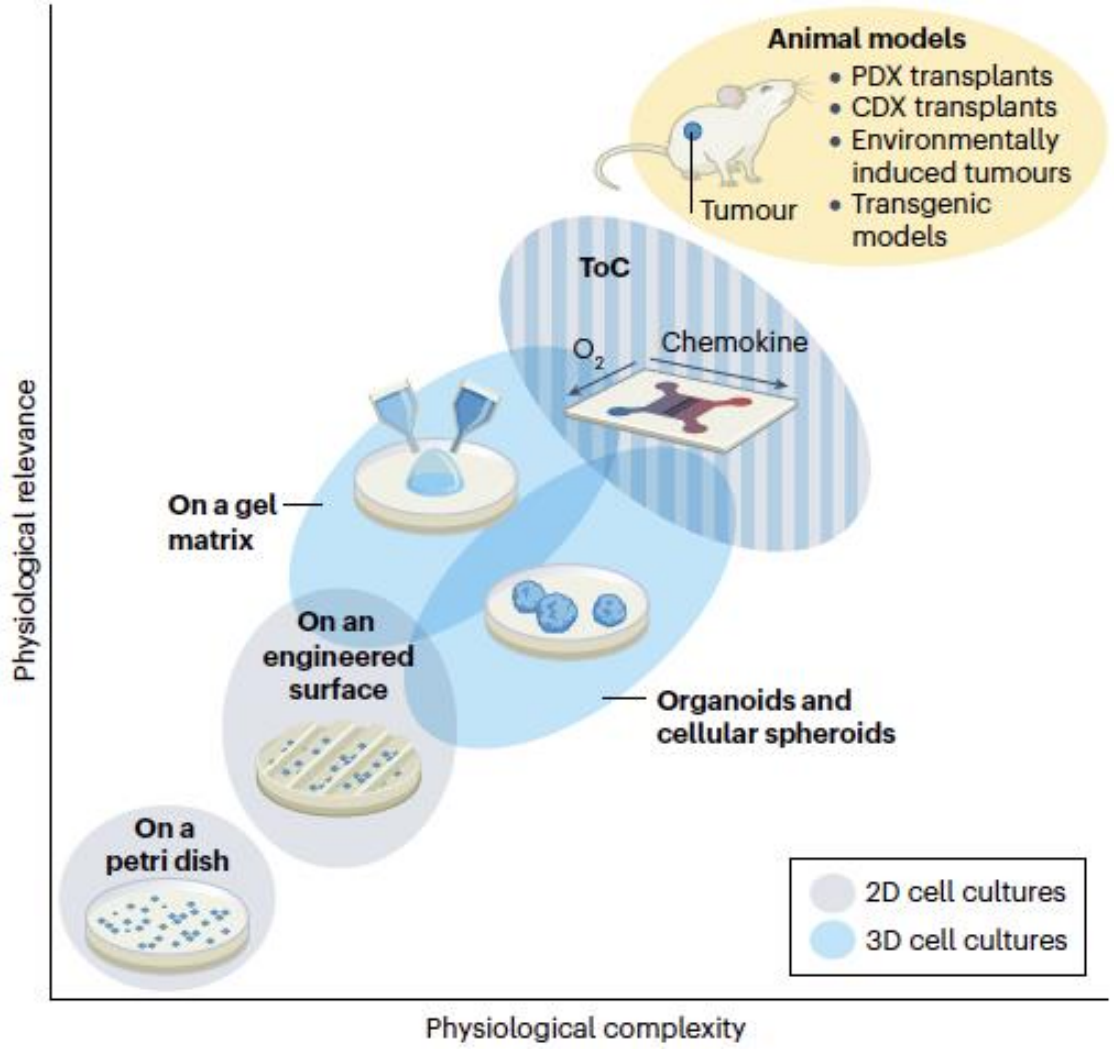
- Many in vivo cancer models do not represent the human cancers well, including:
  - Tumor microenvironment (vasculature, stroma, immune microenvironment)
    - Leaky vasculature
    - Types/ratios of stroma/immune cells present

- 2D cell culture models lack many features of tumors that can be improved/modeled:
  - Mechanical properties (substrate, flow of blood/lymph)
  - Cell types present
  - Nutrient/oxygen gradients

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8212826/

# Using Engineering Approaches for Cancer Modeling (Some Examples)

- Surface mechanical properties
- Shear forces
- Chemical gradients
- Tumor spheroids (cancer cells)
- Organoids (multiple cell types)
- Cell-derived xenograft (CDX)
- Patient-derived xenograft (PDX)
- Organoid-based xenograft
- Transgenic mouse models




Kim et al., Nat Rev Cancer (2023)

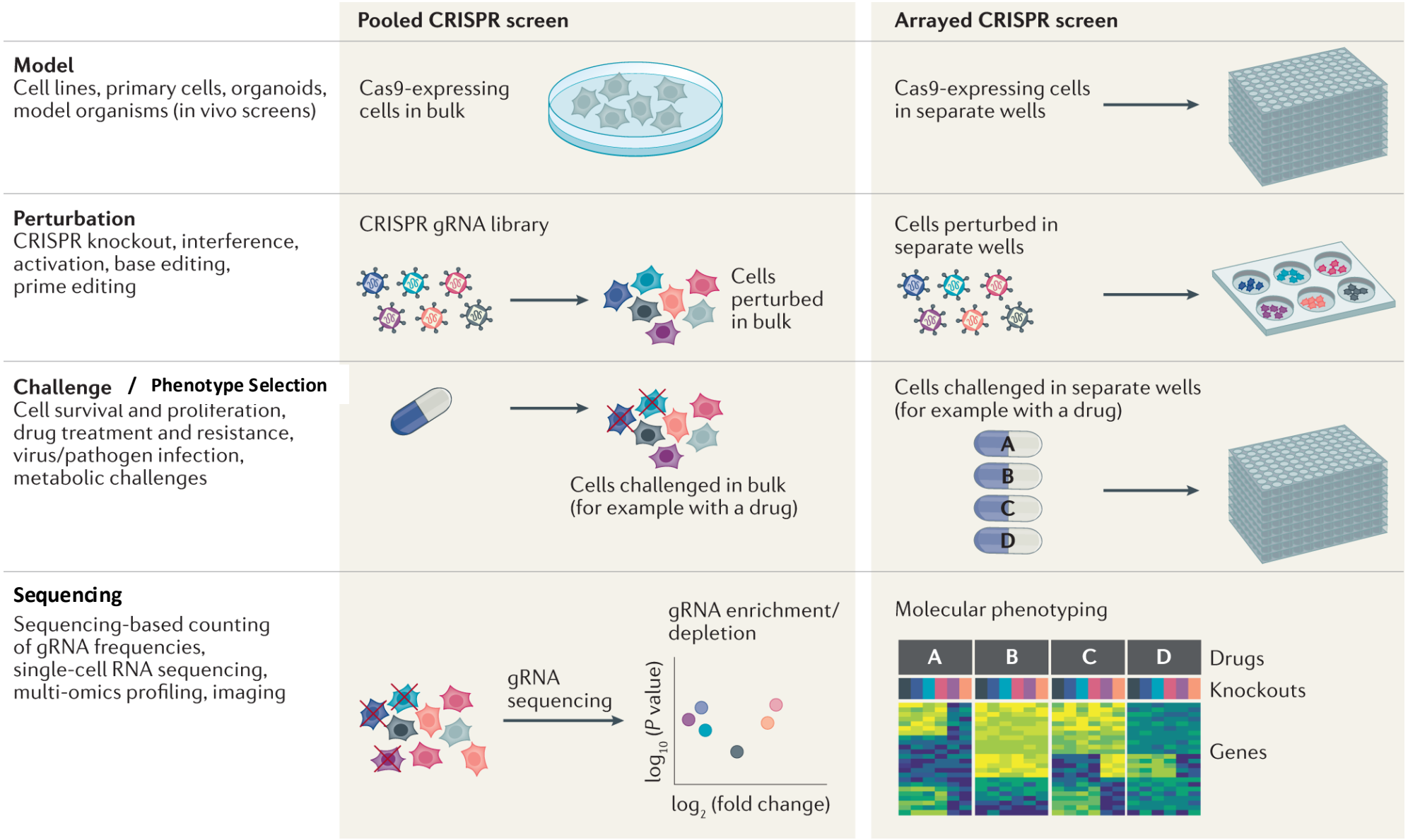
# Measuring Analytes in Situ

- Detecting nutrients, lipids, chemokines, cytokines, hormones, etc.
- Quantifying amounts and changes, dynamics, and gradients
- Measuring in different locations of the tumor (microenvironment), and other areas of the body (regional, distant organs, immune system, blood, etc.)
- For studying disease biology as well as for tumor detection (screening, diagnosis, prognosis, for improving decisions about treatment).

# Improved Bench/Mechanistic Biology/Drug Discovery Methods

- Assays for disease/mechanistic targets
- High-throughput assays for drug/activity screening
    - Tumor cell death
    - Tumor proliferation
    - Target engagement
    - Modulation of a pathway
    - Changes in metabolism

# Example: CRISPR Screen Readouts/Phenotyping




Bock, C., Datlinger, *et al.* High-content CRISPR screening. *Nat Rev Methods Primers* **2**, 8 (2022). https://doi.org/10.1038/s43586-021-00093-4

# Earlier Cancer Detection/Screening, Better Treatment Decisions

- Minimally-invasive tests
- Inexpensive, rapid detection for cancer screening
- Better diagnostic/prognostic biomarkers
- Biomarkers that help decisions about cancer treatment
  - How to tell when a particular drug/immunotherapy might work for a patient?

# Core Knowledge in Cancer Engineering and Some Rationale

- A Theory of Cancer Engineering
- Problems in Need of Cancer Engineering
- Core Knowledge of Cancer Engineering and Some Rationale
- Feedback, Advice, Next Steps
- Engineering Tools for Biology Research

# Core Knowledge in Cancer Engineering and Some Rationale

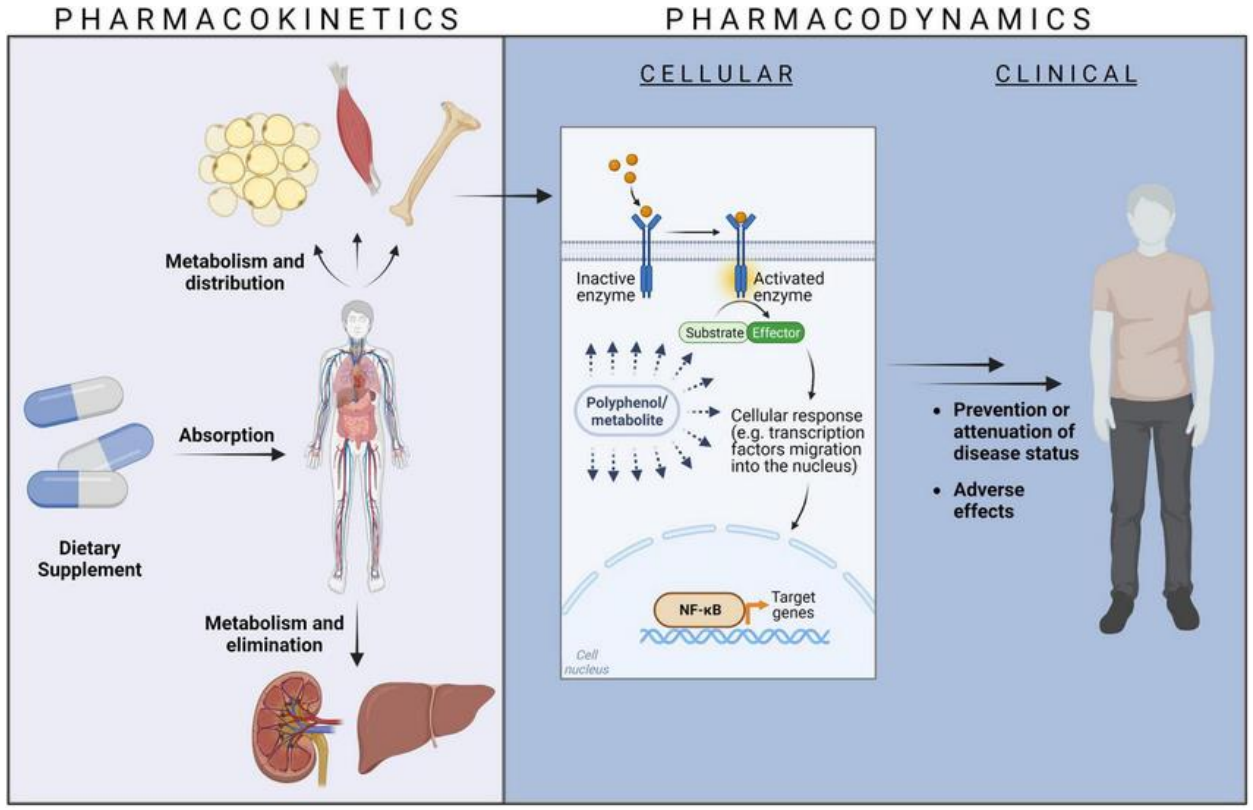
- Foundations in Cancer Engineering
- Imaging
- Genetic Engineering (including protein, cell, and tissue engineering)

# Pharmacology

**Pharmacology** is the science of drugs and medications, including a substance's origin, composition, pharmacokinetics, pharmacodynamics, therapeutic use, and toxicology.

Pharmacology is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function. If substances have medicinal properties, they are considered pharmaceuticals.

=> Very important for not only drug development, but also imaging agents, any molecule that could have an effect on a cell, animal, or person, for therapy or for research.

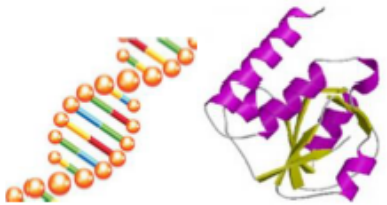



https://en.wikipedia.org/wiki/Pharmacology

DOI:10.3389/fnut.2024.1389422

# Drug Development

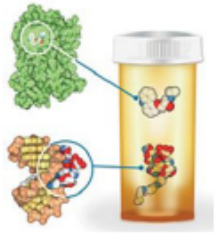


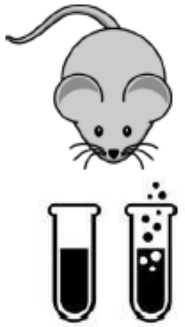
Gene



Protein Target



Lead Identification and Optimization



Non-Clinical Safety

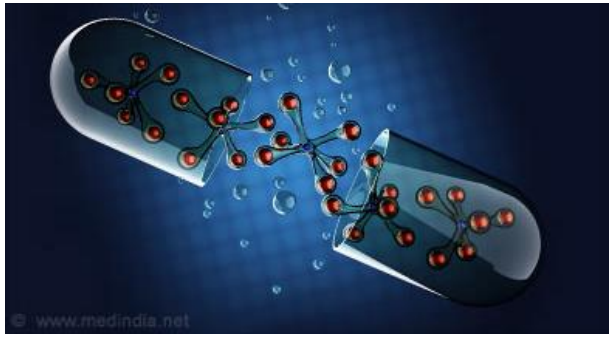


Clinical Development



# Drugs: Small Molecules and Biologics

- Organic Compounds  (chemotherapies + precision medicines)
  - Cytotoxic drugs
  - Inhibitors
  - Degraders




- Biologics (proteins)
  - Antibody inhibitors
  - Antibody-drug conjugates
  - Molecular imaging probes
  - Enzyme replacement therapy



# Clinical Research & Early-Phase Clinical Development

Memorial Sloan Kettering Cancer Center

Sloan Kettering Institute | Research | Education & Training | Careers | MSK Direct | Search

Make an Appointment | Learn About Cancer & Treatment | For Physicians | About Us | MSK MyChart | Donate Now

< Cancer Clinical Trials

## Find a Clinical Trial

English | Share

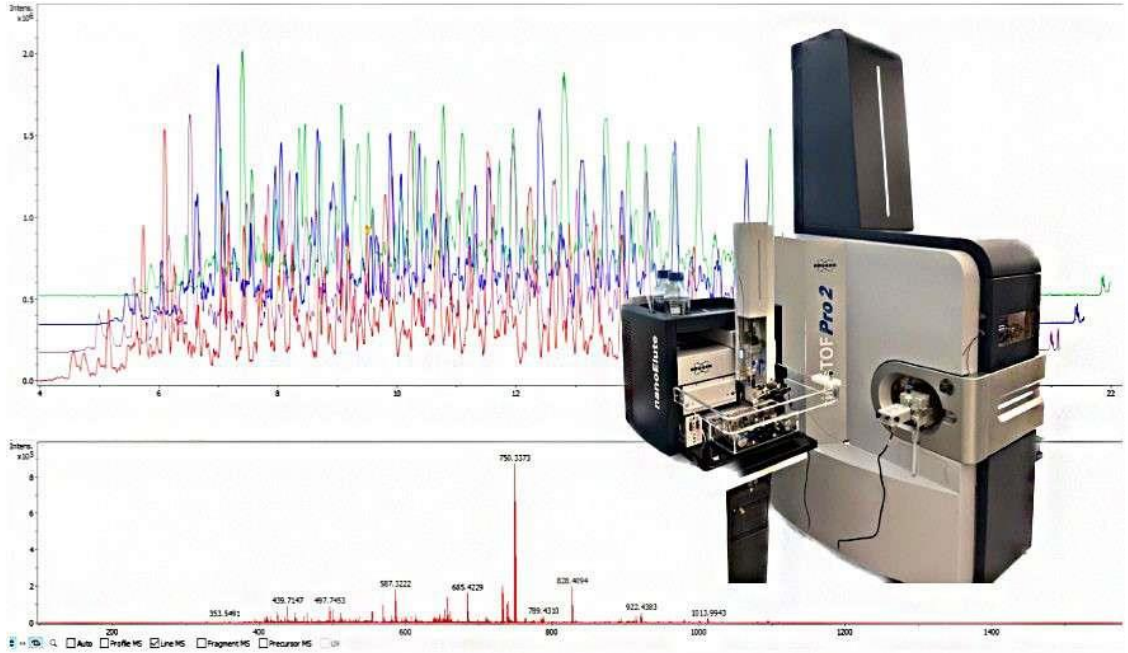
At any time Memorial Sloan Kettering Cancer Center is conducting hundreds of clinical trials to improve care for many types of cancer. Use the tool below to browse our clinical trials that are currently enrolling new patients. Each listing explains the purpose of the trial, the trial's eligibility criteria, and how to get more information.

The list below includes clinical trials for adult cancers. Please visit our pediatric cancer care section to find a pediatric clinical trial.
Memorial Sloan Kettering offers language assistance services for those who prefer to receive health information in another language. Learn more about our language assistance program here.



# Mass Spectrometry

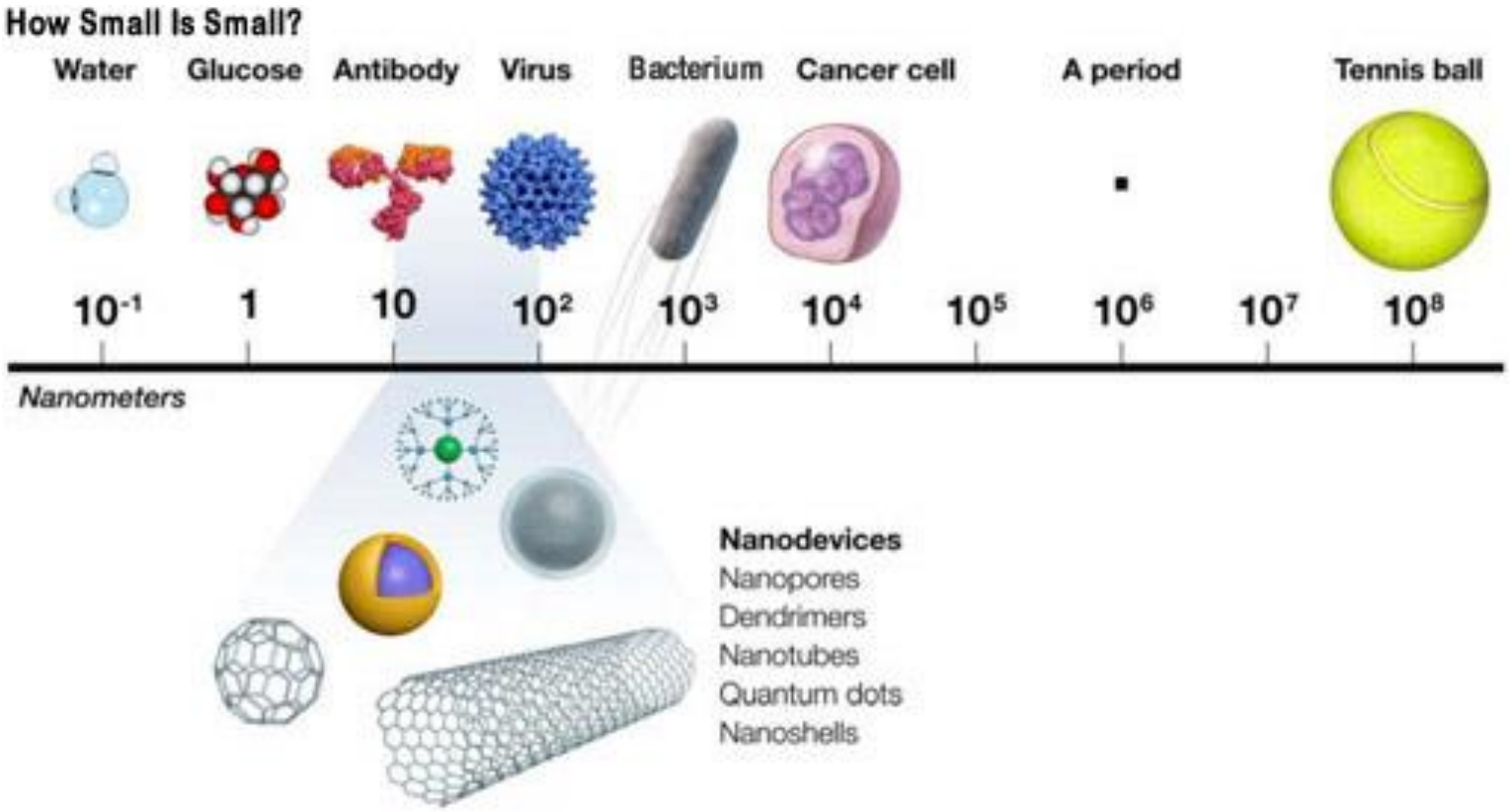
- Important for:
  - Chemistry and drug development
  - Pharmacology (measuring drug concentration and metabolism)
  - Drug delivery (quantifying drugs in different tissues)
  - Proteomics
    - Basic biological discovery
    - Target discovery
    - Diagnostics




https://wertheim.scripps.ufl.edu/cores-and-technologies/mass-spectrometry-and-proteomics/

# Nanotechnology

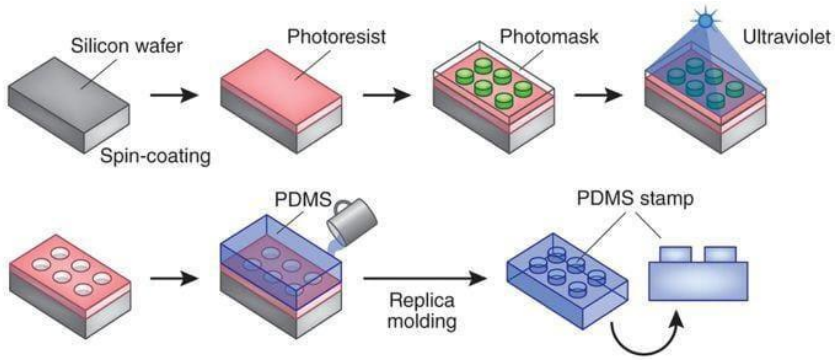
- Important for:
  - Drug delivery
  - Imaging
  - Sensors
  - (Biological measurement)

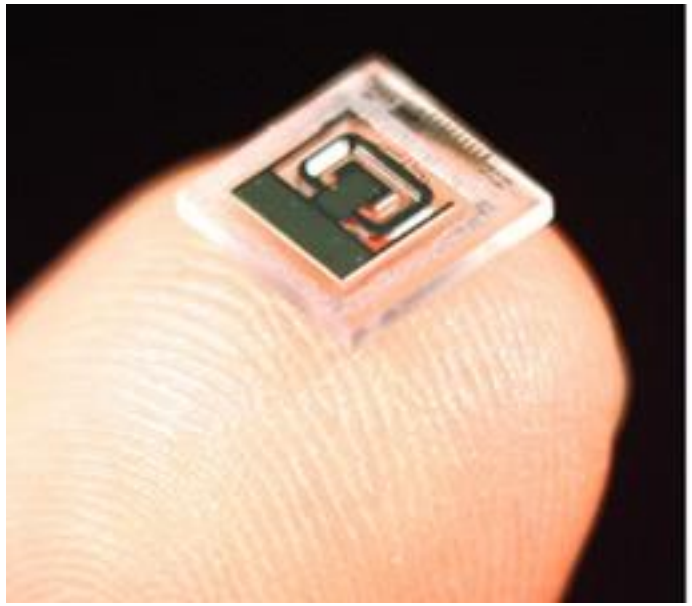


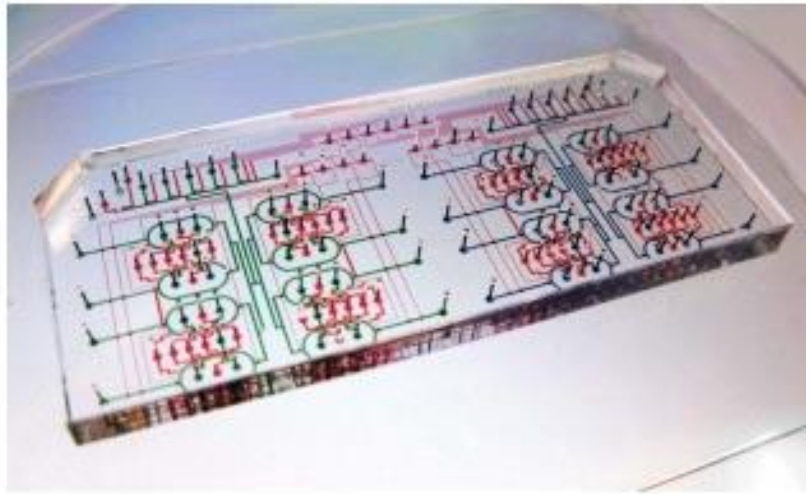
# Drug Delivery

The goal of **drug delivery** is to control/alter the pharmacokinetics of a drug. Drug delivery systems may be medical devices, excipients (inactive ingredients/vehicle), drug carriers (like nanoparticles).

# Fabrication and Fluidics





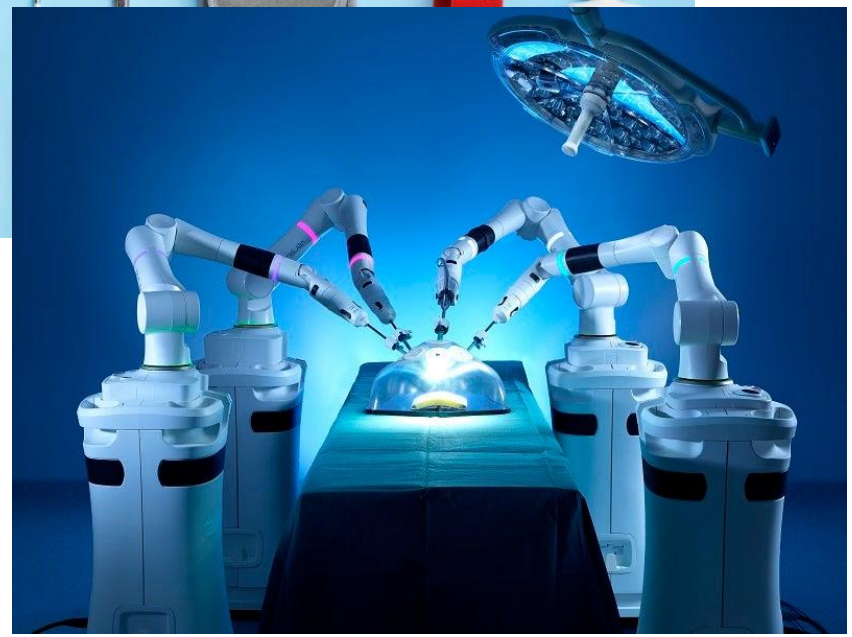
**Microfluidic Devices:**
Assays/microarrays
Control of cell environment
Single cell measurements
Drug screening
Drug (protein) delivery

https://www.advancedsciencenews.com/the-success-of-plasma-for-microfabrication/

# Medical Devices

- Surgical devices
- Medical implants
- Drug delivery devices
- Anesthesia
- Measurement devices
- Point-of-care diagnostics





https://highpowervtls.com/2024/06/the-different-classes-of-reusable-medical-devices/
https://www.medicaldevice-network.com/features/da-vinci-surgical-robot-competitors/

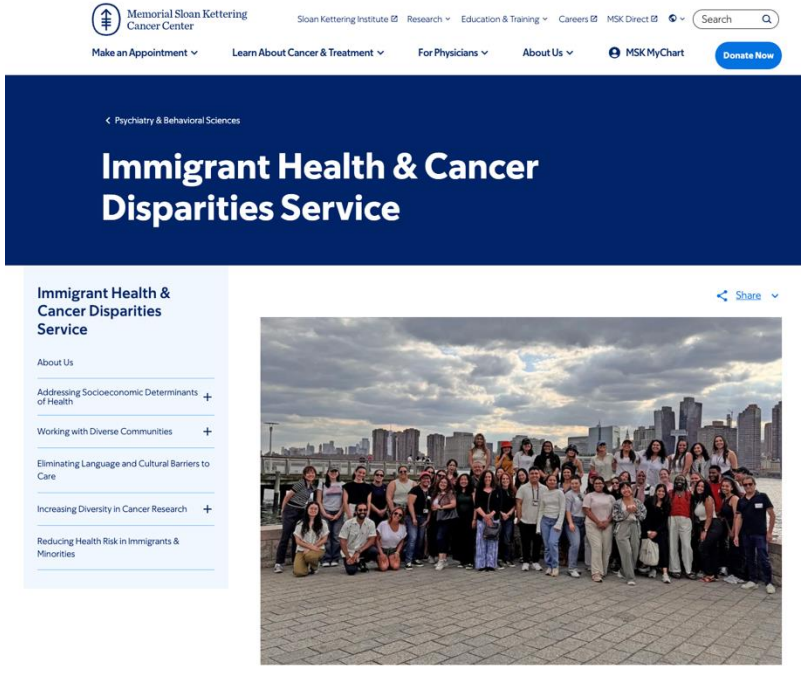
# Laboratory Diagnostics and Biomarkers

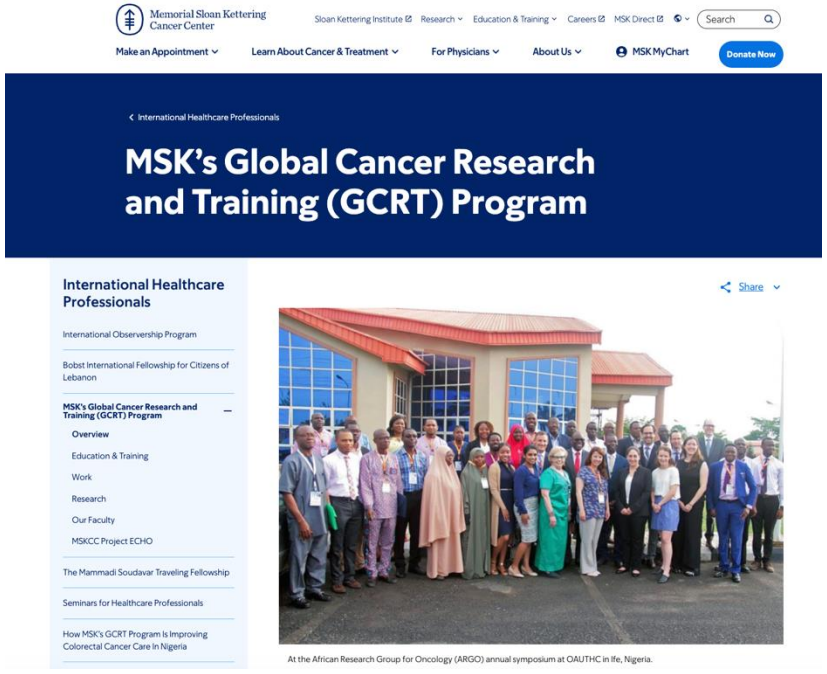
- Laboratory medicine = information from blood (and other body fluids)
  - Chemistry
  - Hematology
  - Immunology
  - Microbiology
  - Transfusion medicine
  - Toxicology
  - Molecular diagnostics
- Biomarkers = molecules that give information about health and disease



# Global Health and Underrepresented Populations

- Disparities - cancer diagnosis and treatment in:
  - Rural communities in the US
  - Cities in the US
  - Developing countries

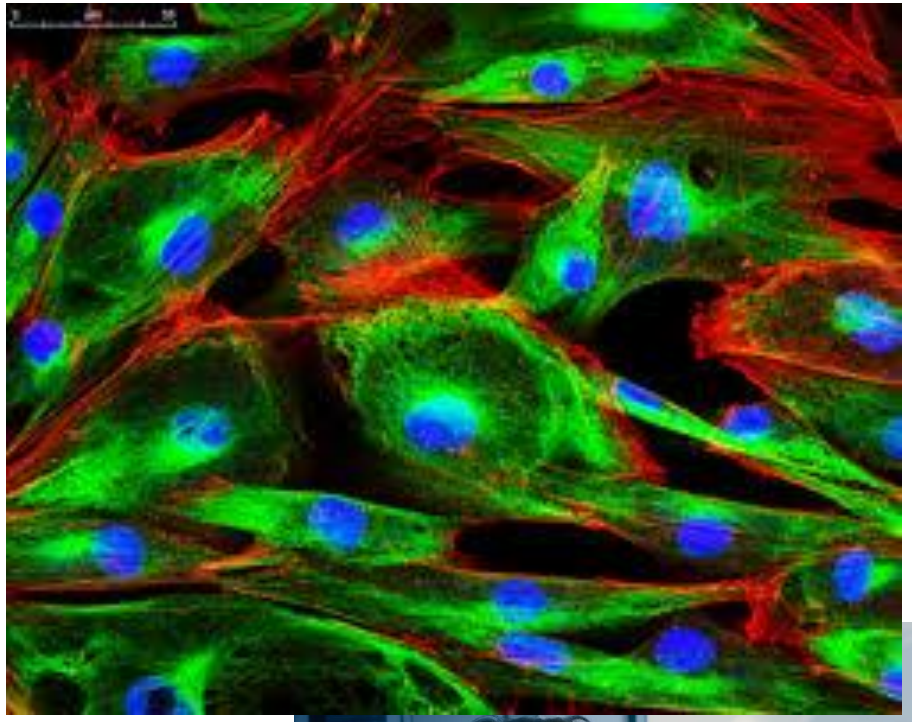


# Imaging

- Pathology
- Cells
- Animals
- Whole body

- Optical imaging
- Pathology
- Nuclear imaging
- MRI
- PET
- Theranostics
- Molecular imaging probes





https://www.edmundoptics.com/
https://www.aps.org/careers/advice/medical-clinical-physicist

# Genetic Engineering

- Gene engineering methods
  - CRISPR/CAS9
- Genome editing and chromosomal engineering
- Human genetics
- Genetic screening



https://www.pirbright.ac.uk/engage-with-us/science-case-studies/genetic-engineering

# Protein, Cell and Tissue Engineering

(Genetic Engineering Course)

- Protein engineering – designing proteins that do what you want
- Stem cell engineering – making cells that can differentiate into desired cell types
  - Induced pluripotent stem cells (iPSCs)
- Cell therapy engineering
  - CAR T cells
- Tissue engineering
  - Organoids
  - Cancer models



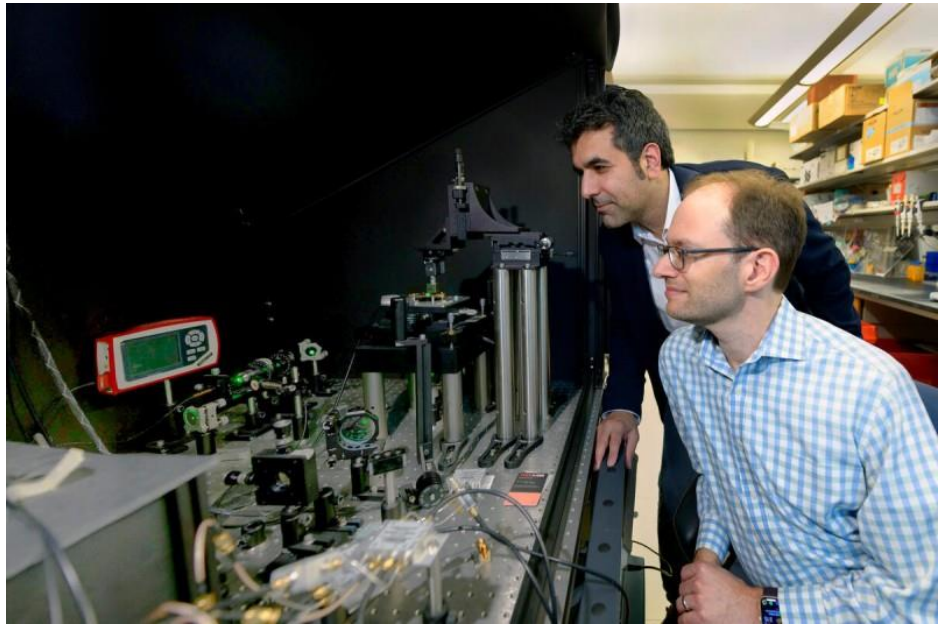
https://www.quantamagazine.org/an-ethical-future-for-brain-organoids-takes-shape-20200123/

# Feedback, Advice, Next Steps

- A Theory of Cancer Engineering
- Problems in Need of Cancer Engineering
- Core Knowledge of Cancer Engineering and Some Rationale
- Feedback, Advice, Next Steps
- Engineering Tools for Biology Research

# Feedback

- Cancer Engineering is a work in progress:
  - As a discipline
  - As a graduate program



# Questions About Cancer Engineering as a New Discipline

- What is important for cancer engineers to learn?
- What is (and what is not) part of cancer engineering?
- Where is cancer engineering headed as technologies develop: therapeutics, genetic engineering, diagnostics, AI?

# Some General Thoughts/Advice

- Learn about the MSK mission and the cancer patient journey
  - Meet physicians, patients, patient advocates, etc.
- Learn about the many different types of jobs and people who work here
  - In science, clinical, administrative, etc. roles
- Attend seminars outside of your field
- Don’t be shy about telling others about your research
- Make at least the first 30% of your seminar accessible to people outside of your field

# Some General Thoughts/Advice

- Learn about the opportunities and events at MSK/the Tri-I
  - Courses in: Drug development, entrepreneurship, biostatistics, etc. etc.
  - Lectures in science (seminars/works in progress) and medicine (grand rounds)
  - Continuing medical education (CME) activities
  - Entrepreneurship and company-related opportunities
  - Outreach opportunities

- Learn about opportunities and events in the NYC Area
  - New York Academies of Science/Medicine
  - NYU, Mount Sinai, Columbia, etc.
  - Outreach and entrepreneurship activities

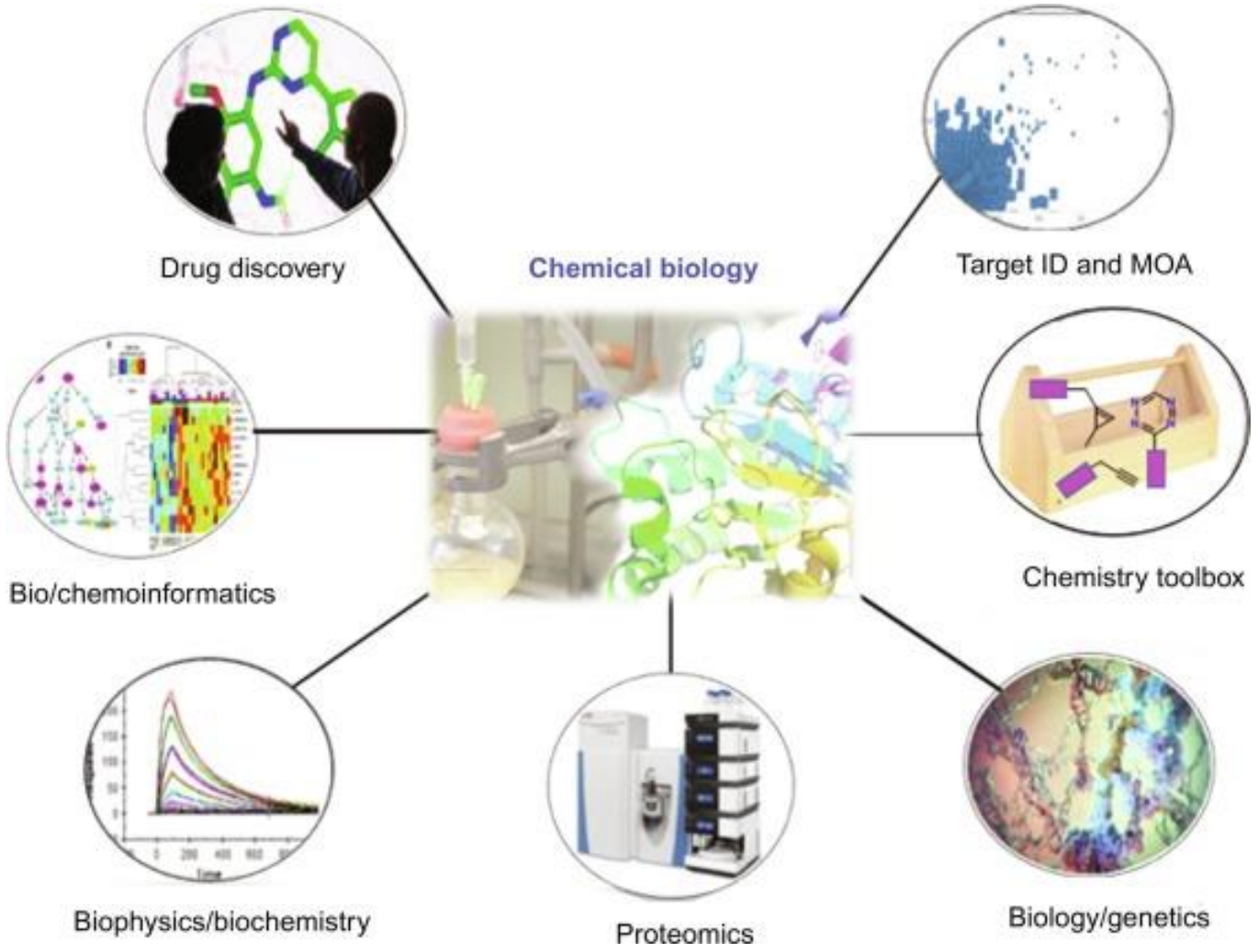
# Engineering Tools for Biology Research

- A Theory of Cancer Engineering
- Problems in Need of Cancer Engineering
- Core Knowledge of Cancer Engineering and Some Rationale
- Feedback, Advice, Next Steps
- Engineering Tools for Biology Research

# Chemical Biology

The application of chemical techniques, analysis, and often small molecules produced through synthetic chemistry, to the study and manipulation of biological systems.

An important department at MSK whose faculty often develop novel therapeutics.




M. Paola Castaldi, et al., Annual Reports in Medicinal Chemistry 2017

nature chemical biology

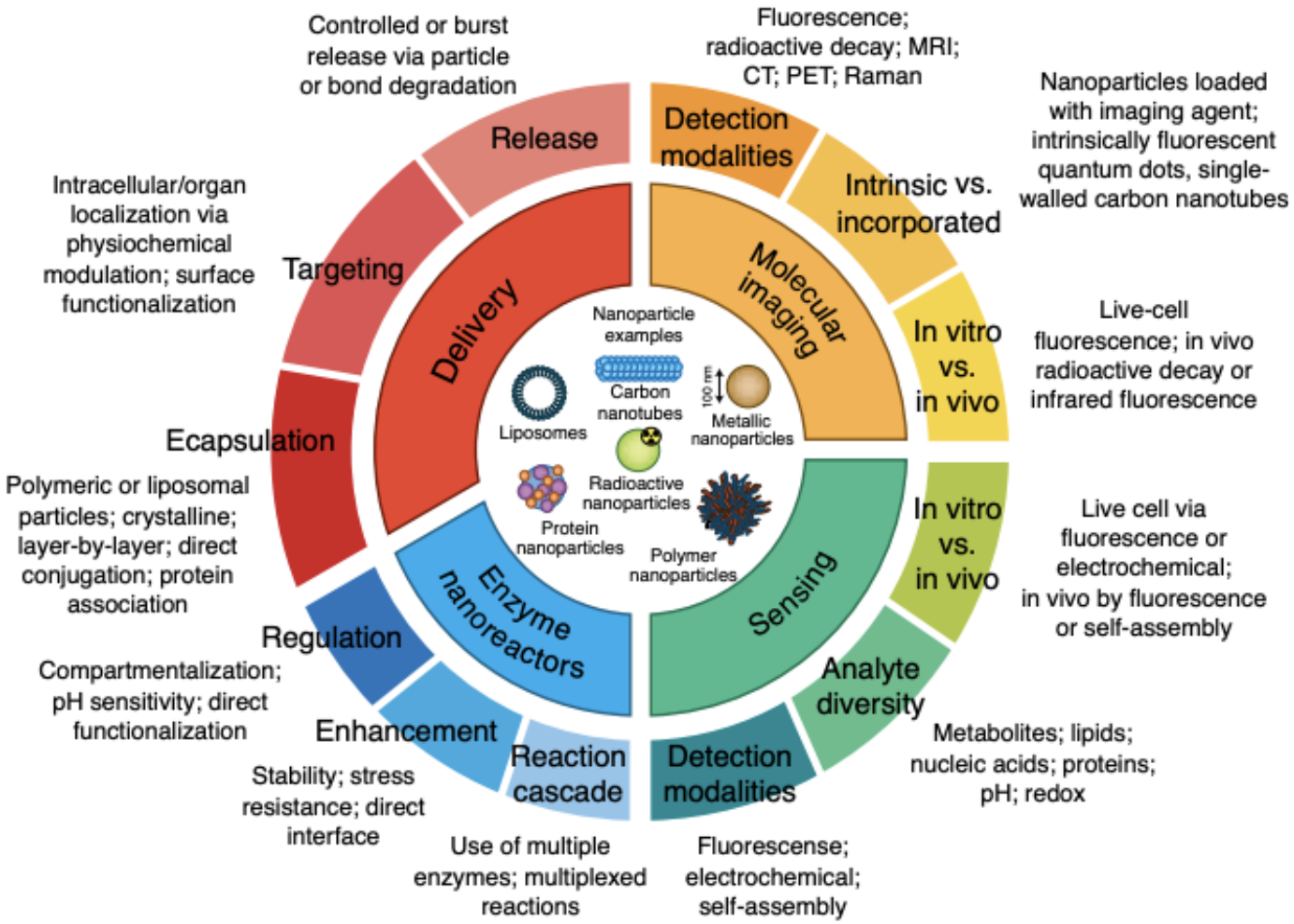
PERSPECTIVE

https://doi.org/10.1038/s41589-020-00690-6

Check for updates

**=> Nanochemical Biology**

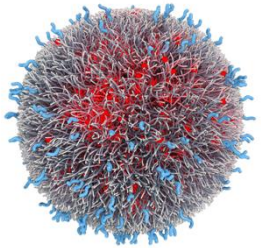
# Harnessing nanotechnology to expand the toolbox of chemical biology




Williams, *Nat Chem Biol*, 2021

# What is the definition of a nanoparticle/nanomaterial?

A nanoparticle/material must have at least one dimension with a size of:



**a.) 1 – 10 nm**

**b.) 1 – 100 nm**

**c.) 10 – 1000 nm**

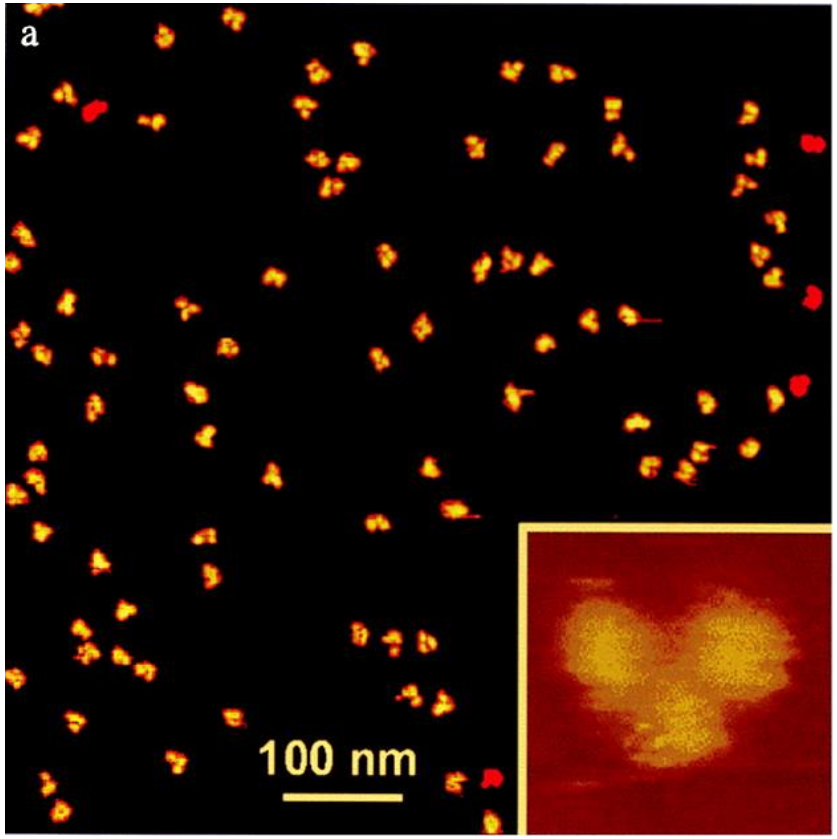
**d.) 1 – 1000 nm**

# Nano: Why Should You Care?

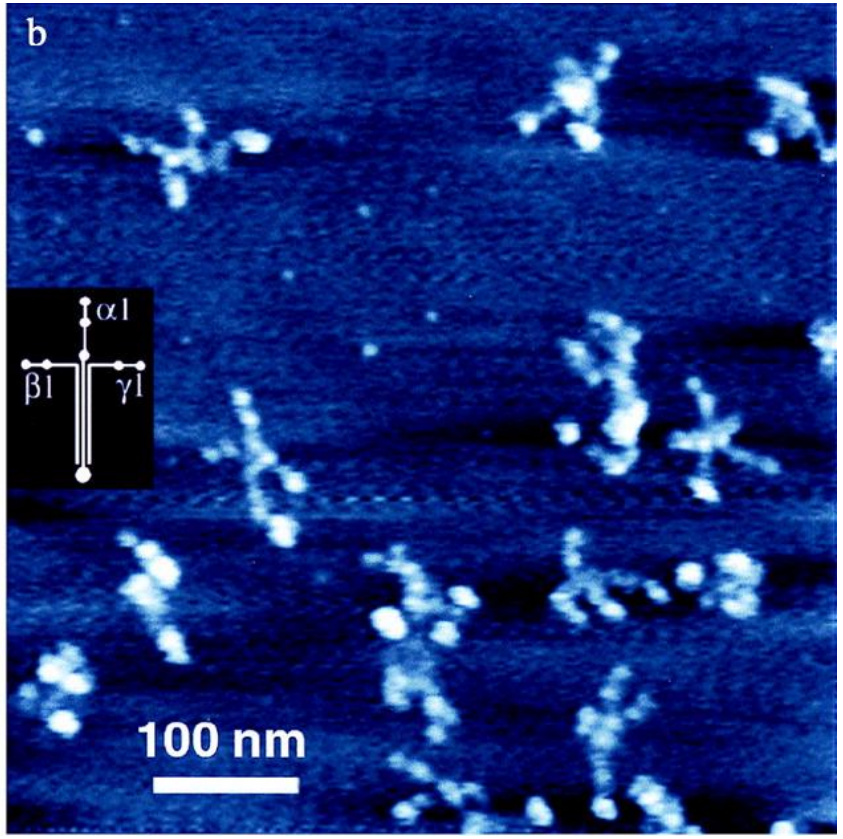
## Proteins are nanoparticles

Atomic Force Microscopy

**IgG Antibody**

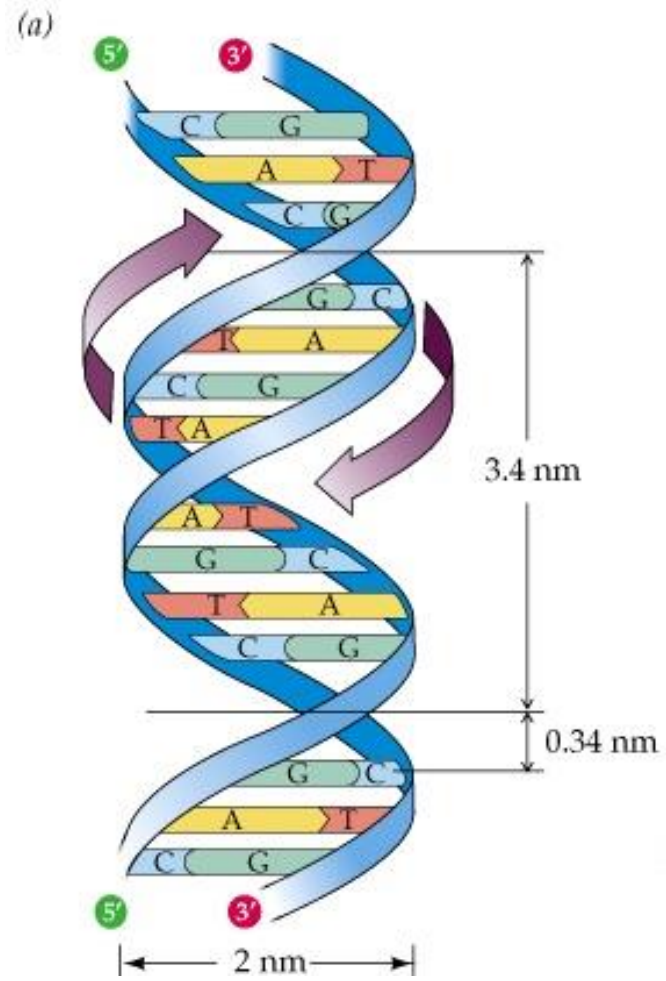



**Laminin-1**

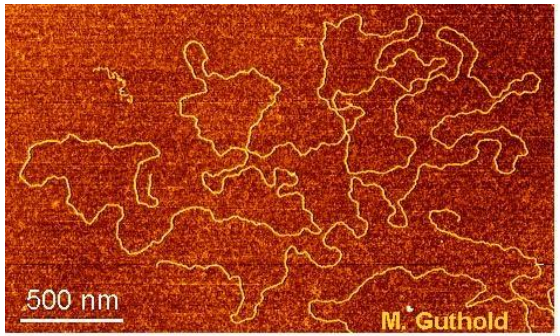



Helen G. Hansma. Varieties of imaging wih scanning probe microscopes PNAS 1999 96 (26) 14678-14680; doi:10.1073/pnas.96.26.14678

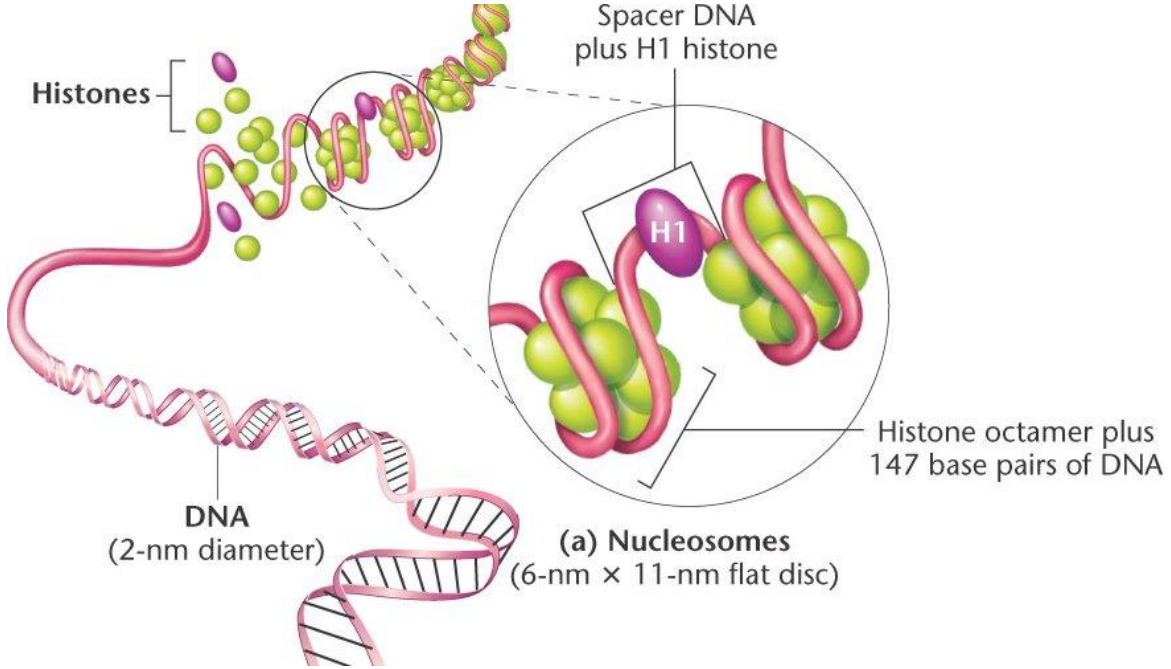
# Biomacromolecules are Nanoscale



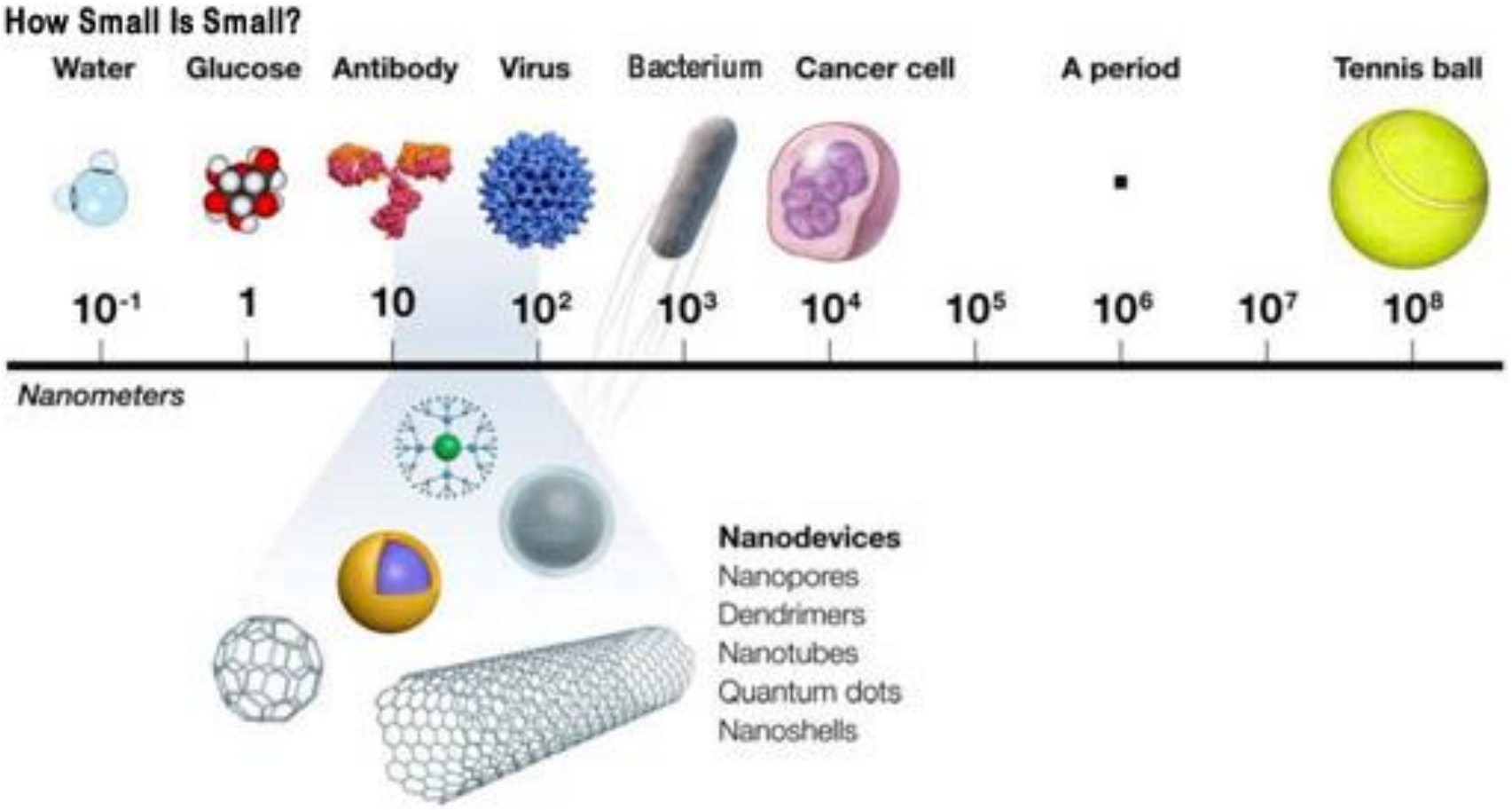

## DNA is a Nanoribbon




λ-DNA



# Nanoscale is Bio-Scale




David W. Piston, Gert-Jan Kremers, Fluorescent protein FRET: the good, the bad and the ugly, Trends in Biochemical Sciences, Volume 32, Issue 9, September 2007, Pages 407-414, ISSN 0968-0004, 10.1016/j.tibs.2007.08.003.

# Nanotechnology: Measurement Techniques

How to measure/characterize nano-sized (bio) materials?

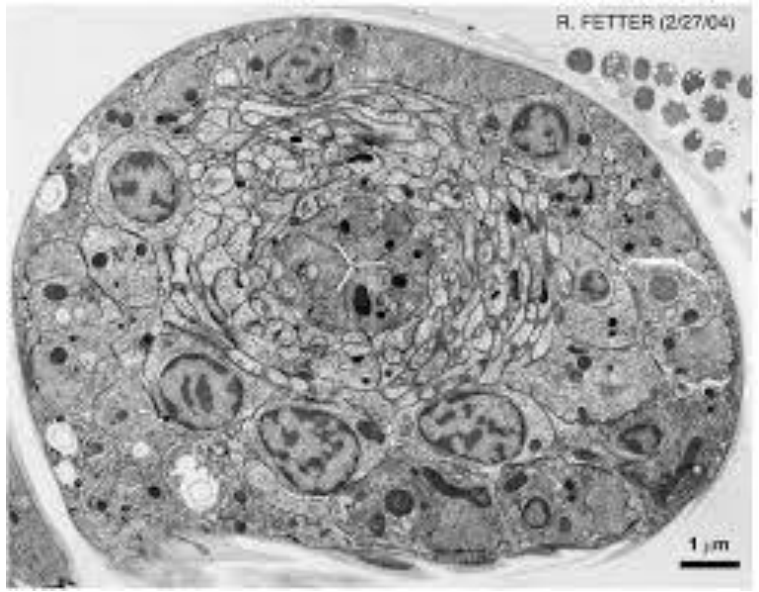
**Techniques Include:**
Transmission Electron Microscopy
Scanning Electron Microscopy
Atomic Force Microscopy
Dynamic Light Scattering
Zeta Potential Measurement
Thermogravimetric Analysis

# Transmission Electron Microscopy

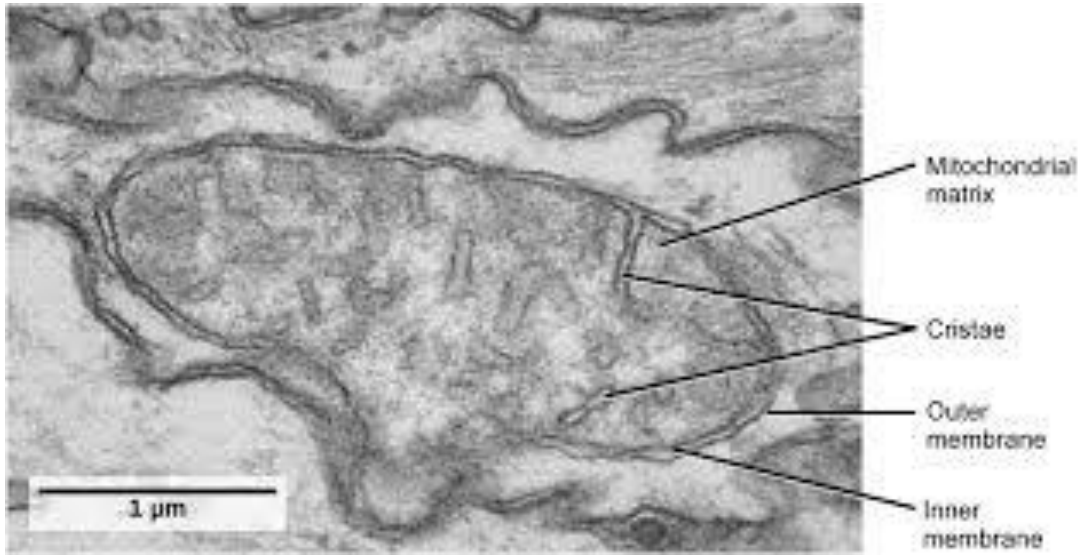
Electron beam is transmitted through an ultra-thin specimen.

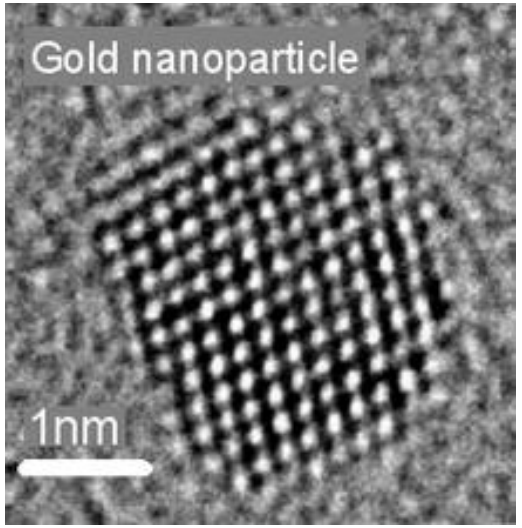
Specimen absorbs electrons to create contrast.

Resolution down to 0.5 Ångströms (50 pm)





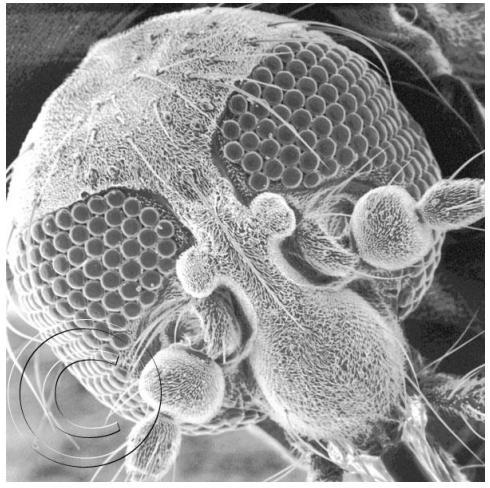
# Scanning Electron Microscopy

Electron beam hits sample, creating secondary electrons emitted by atoms in the sample.

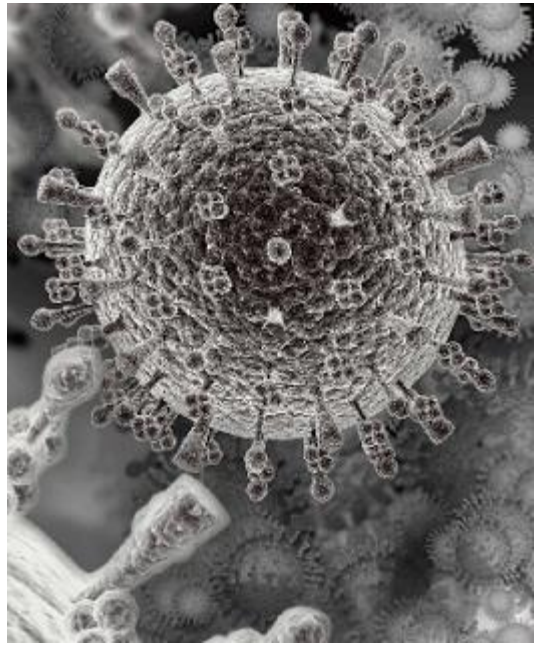
Specimen should be electrically conductive or can be coated with an ultra-thin layer of gold.

Resolution down to 1 nanometer

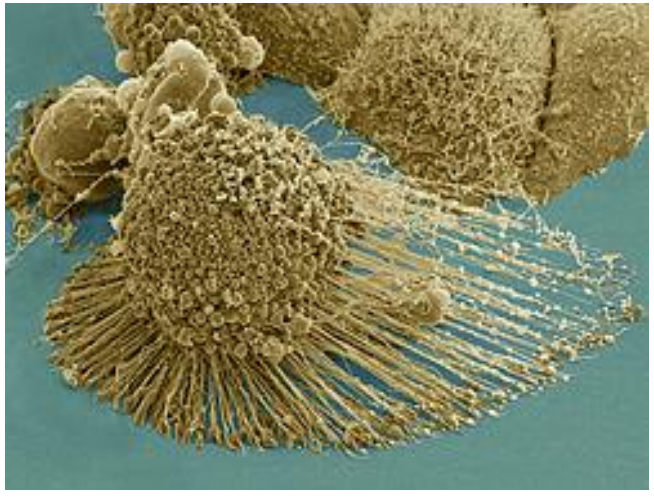
Culicoides biting midge

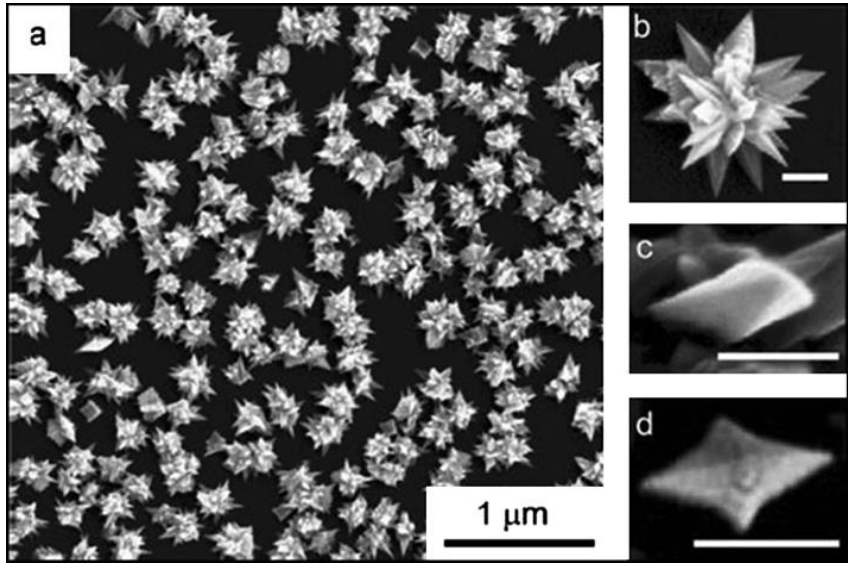


Avian Flu Virus



Apoptotic HeLa Cell



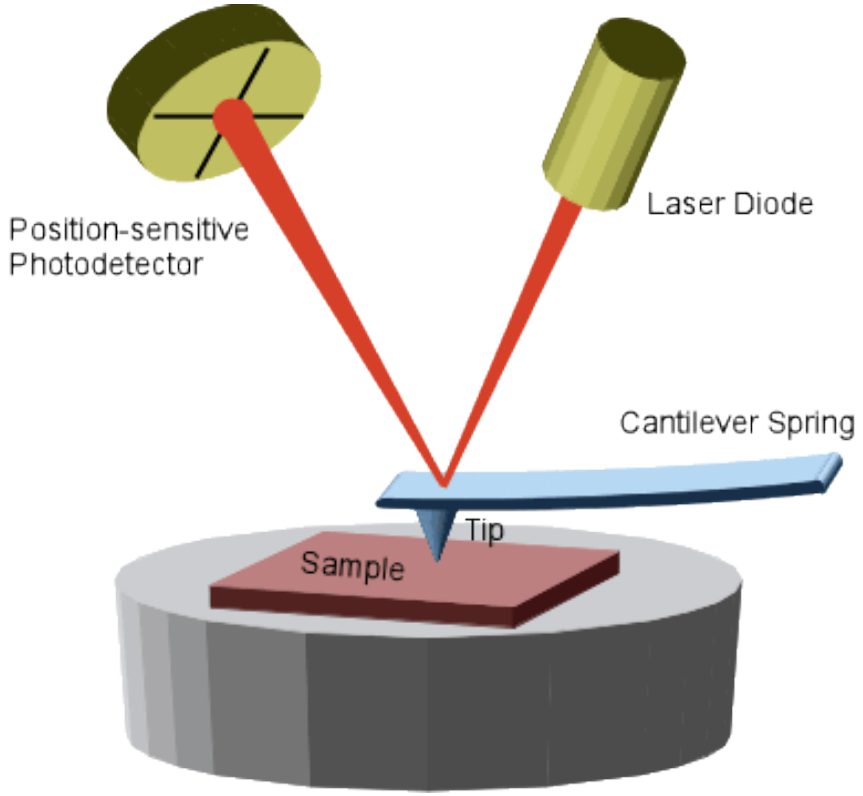



Platinum "Nanothorns"

# Atomic Force Microscopy

**Resolution down to 0.1 nm (in z direction)**
Images surface topology
Measures binding affinity
Measures elastic modulus

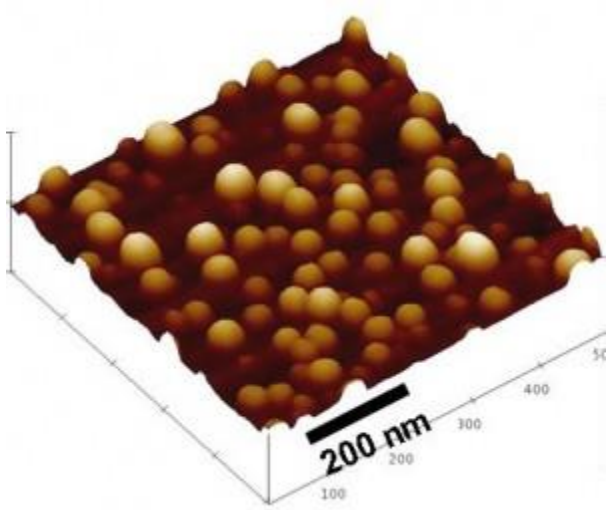
Imaging possible in native conditions
(in buffers/on live cells)

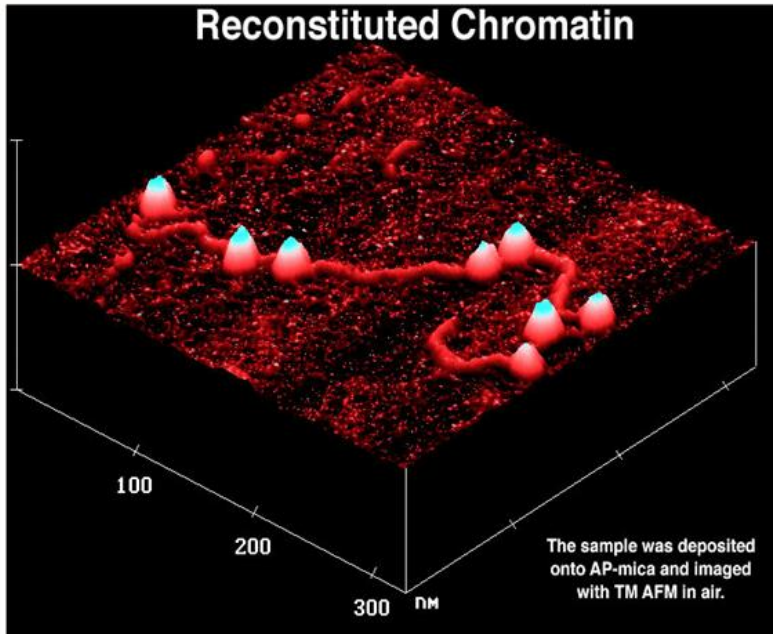



**Principle:**
Cantilever with a nano-sized tip is dragged over a surface.
Laser reflection off of cantilever records changes in height.

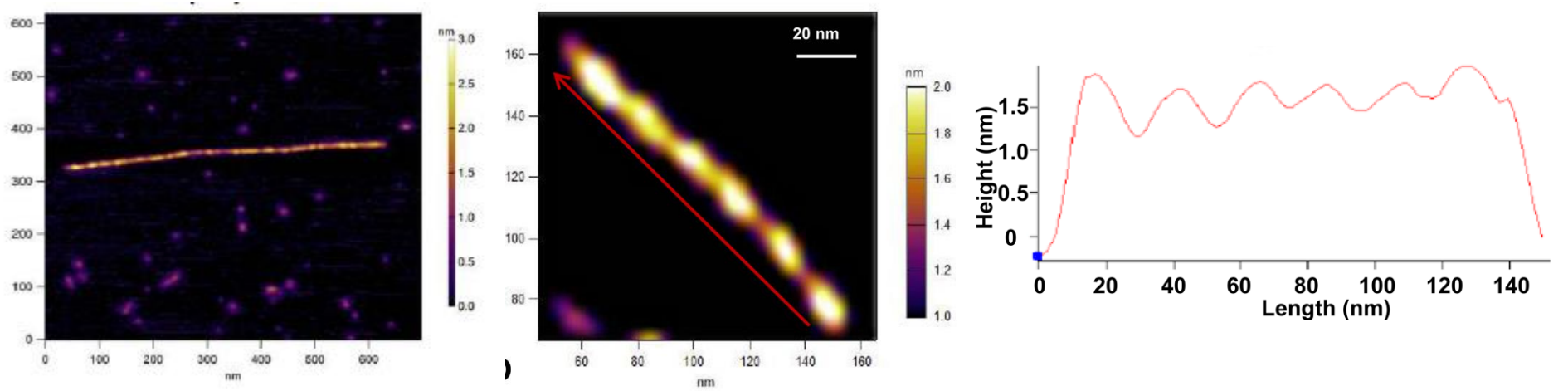
# Atomic Force Microscopy

## Gold Nanoparticles on a Silk Film



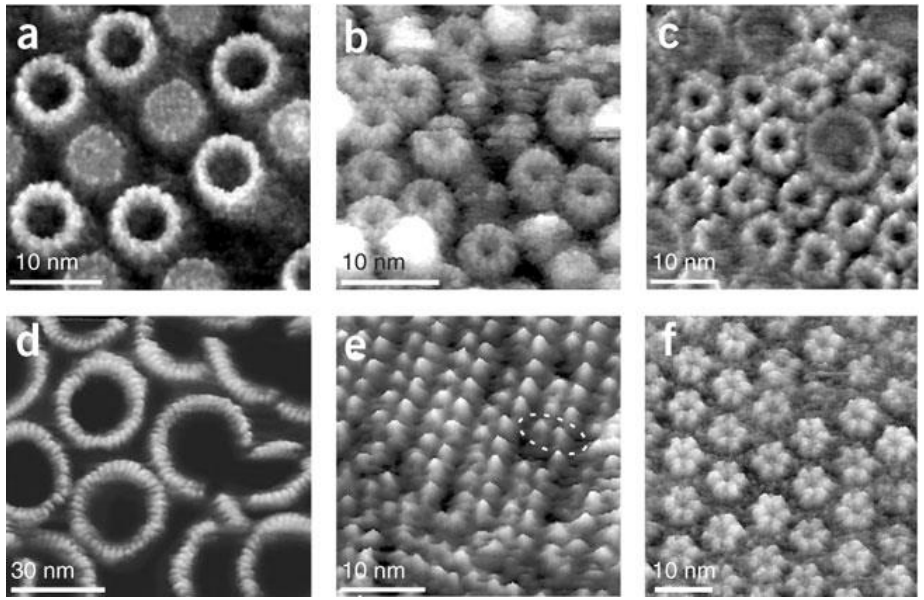




## Polymer-Encapsulated Carbon Nanotubes




Budhathoki-Uprety, et. al., *JACS*, 2014

# AFM Images Native Proteins

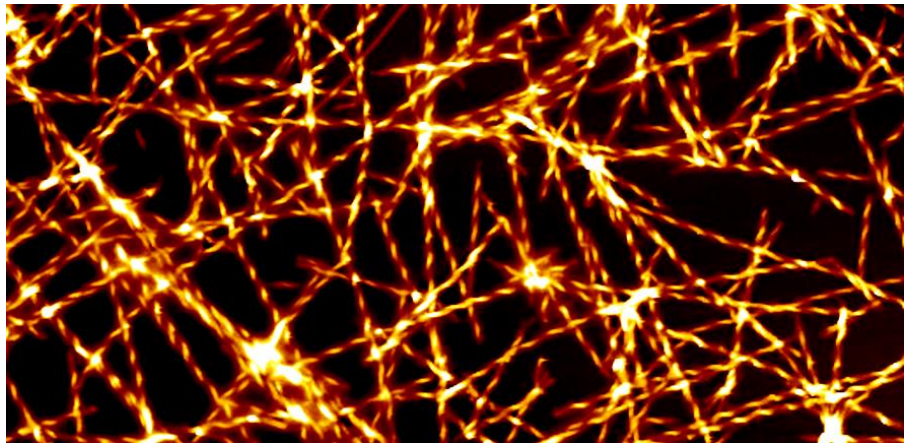



## Membrane Proteins

(**a**) Ion-driven rotors from spinach chloroplast and (**b**) *Illyobacter tartaricus* FoF1-ATP synthase[26, 27]. (**c**) High-light-adapted native photosynthetic membrane from *Rhodospirillum photometricum*[16]. (**d**) Perfringolysin O pore complexes[14]. (**e**) Dimeric bovine rhodopsin (dotted ellipse) in native disc membranes[28]. (**f**) Extracellular surface of gap junction hemichannels[29]. Contact mode AFM topographs were recorded in buffer solution at forces of 100 pN or less

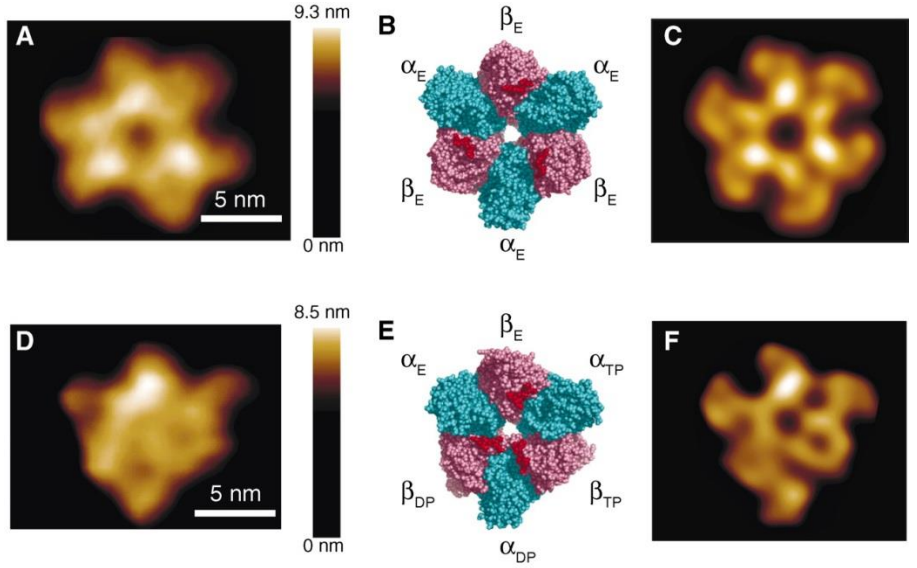
Müller, et. al. *Nature Protocols* **2**, 2191 - 2197 (2007)

## Amyloid Fibrils



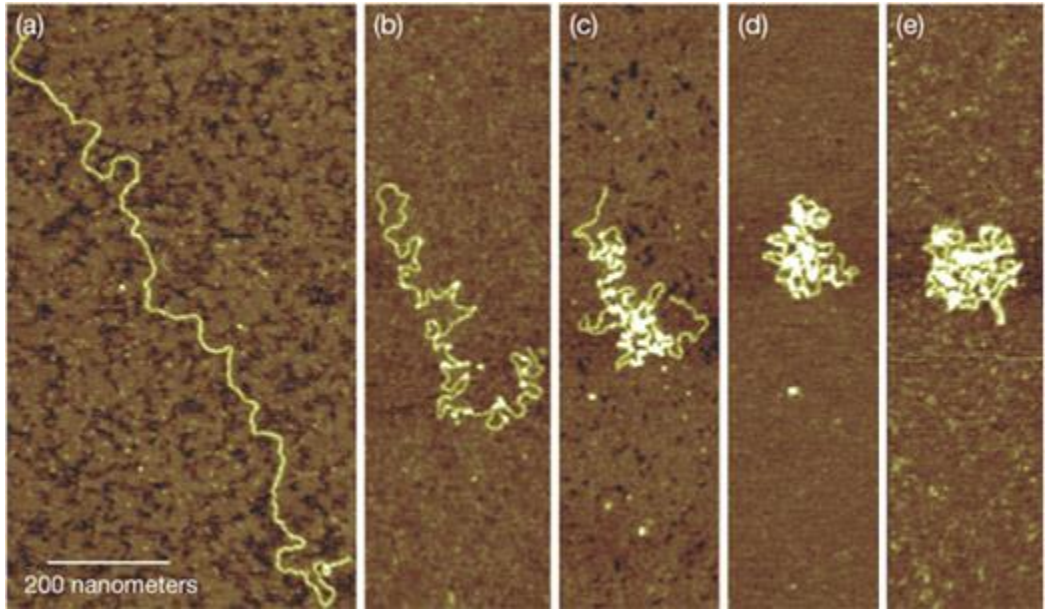
Knowles, Cambridge University

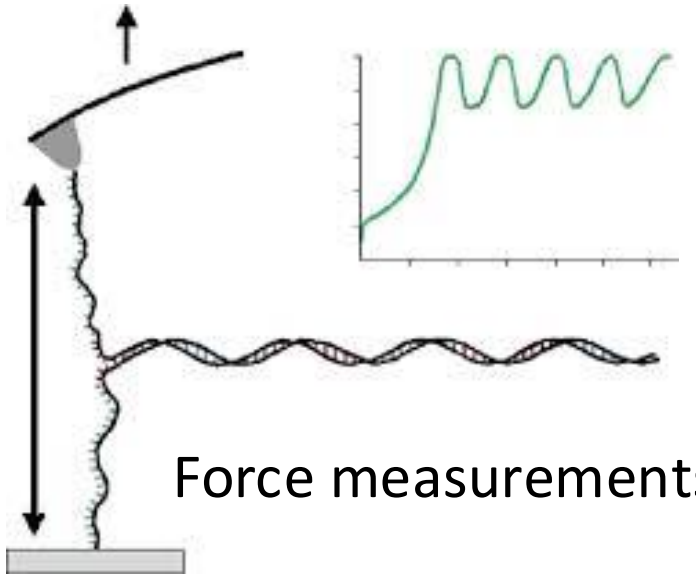
## $F_1$-ATPase



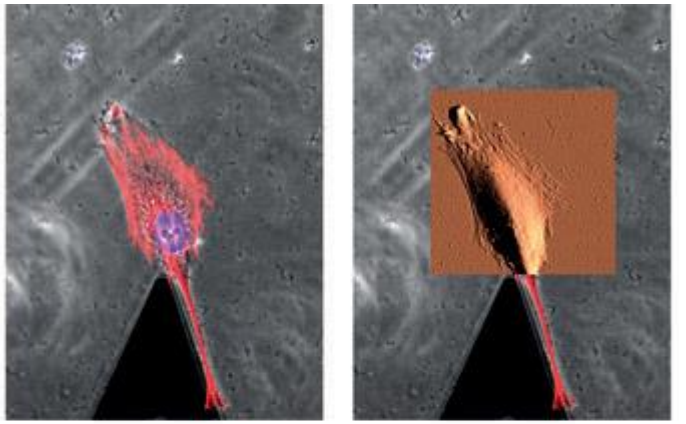

Science 5 August 2011: vol. 333 no. 6043 755-758

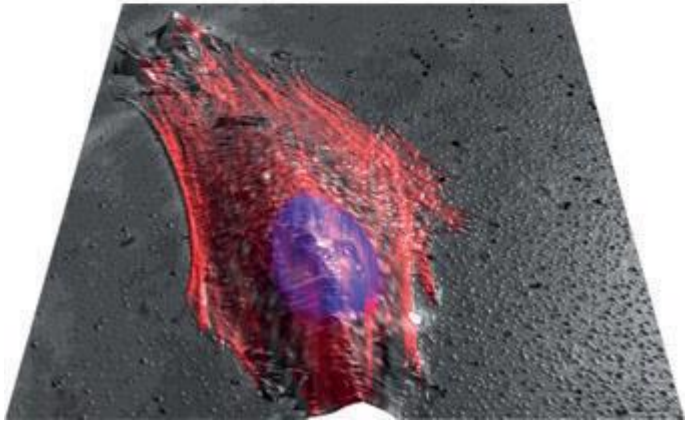
# AFM Can Measure Force and Live Cell Mechanics



Force measurements





**Samples:**

Nanomaterials

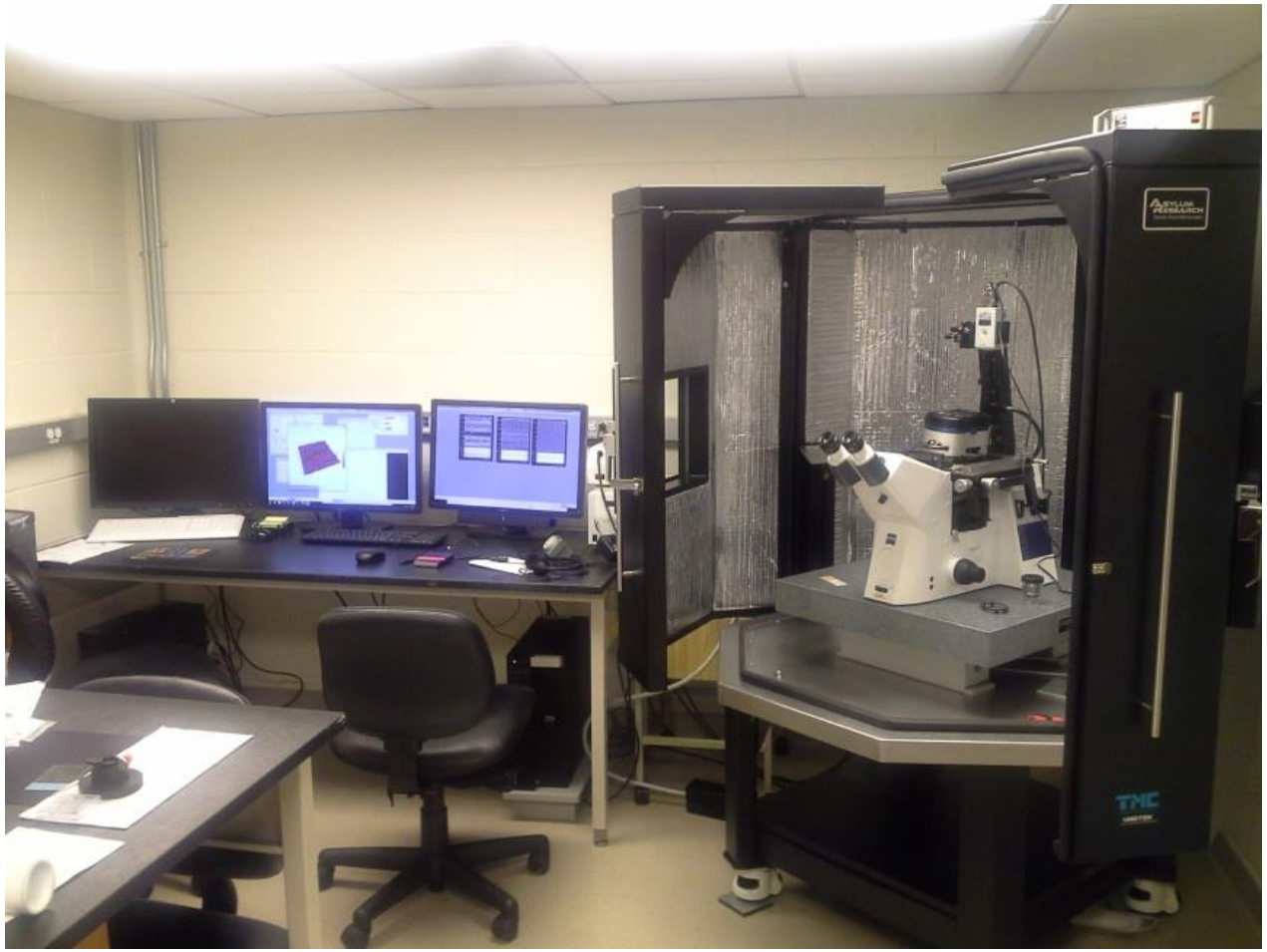
Single biomolecules

Live cells

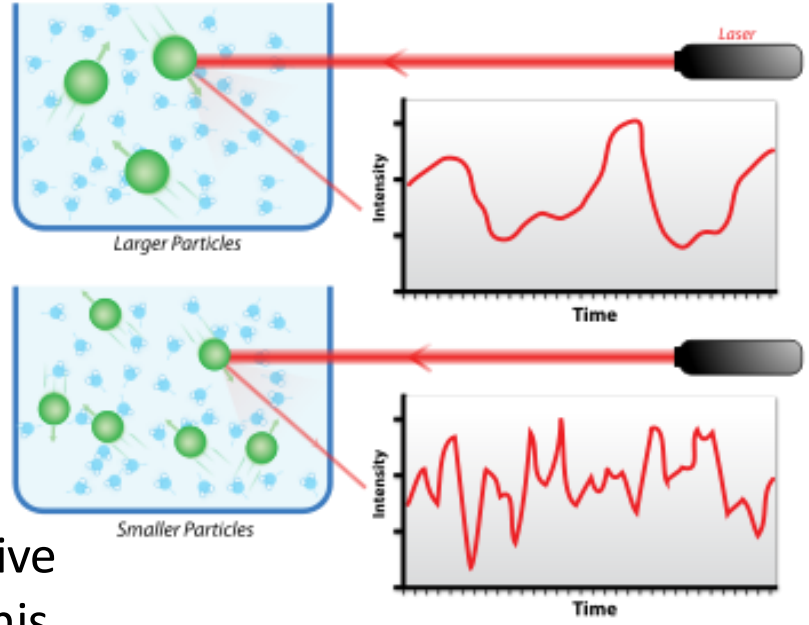
# Atomic Force Microscopy @ the Tri-I

## Asylum Instruments Bio AFM 3D

MSKCC Molecular Cytology Core Facility
Zuckerman C4 Basement



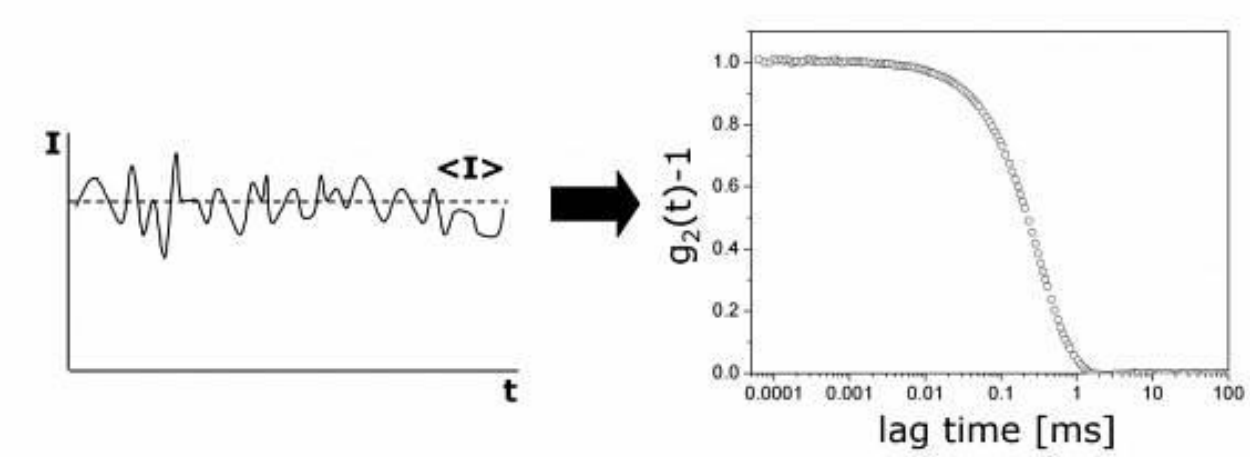
# Dynamic Light Scattering

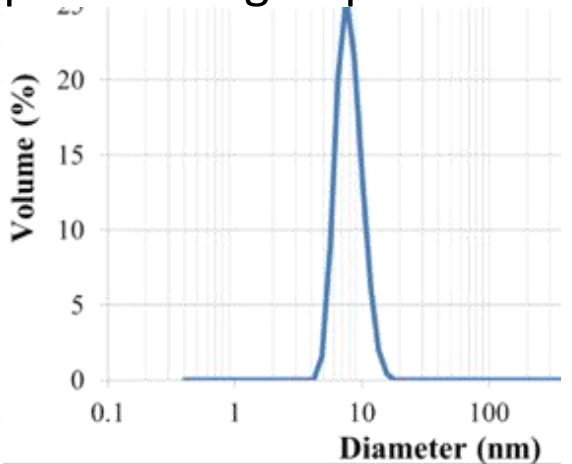



**Measures:**
Particle size: ~0.3 nm – 8 µm
Molecular weight: ~$1x10^3$ – $2x10^7$ Da
Zeta potential: (particle surface charge)

**Principle:**
Particles in solution scatter light. This light then undergoes either constructive or destructive interference by the surrounding particles. By this intensity fluctuation, information is contained about the time scale of movement of the scatterers.

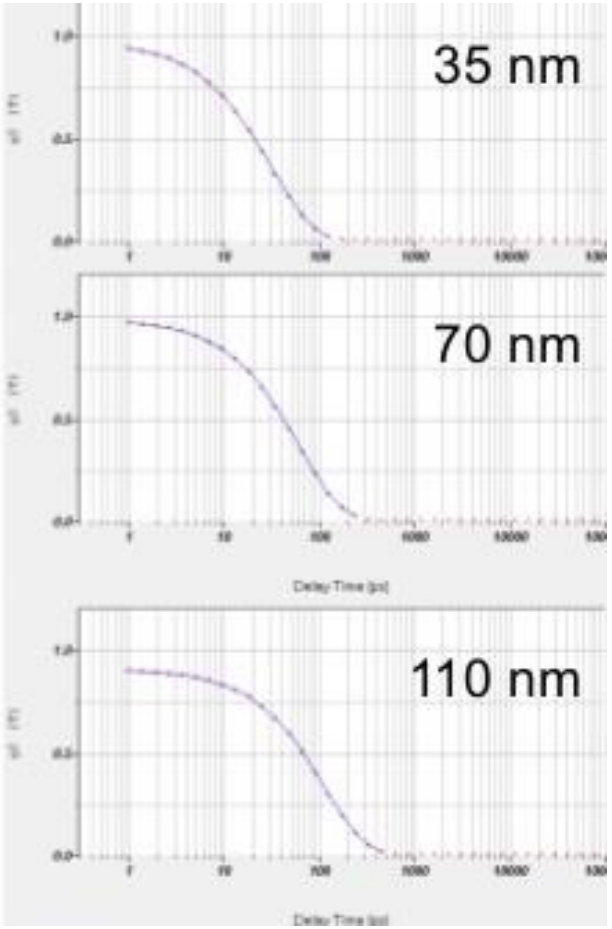
**Data produced:**
Autocorrelation function, which is then fitted to equations to give particle size



# Dynamic Light Scattering Cont'd

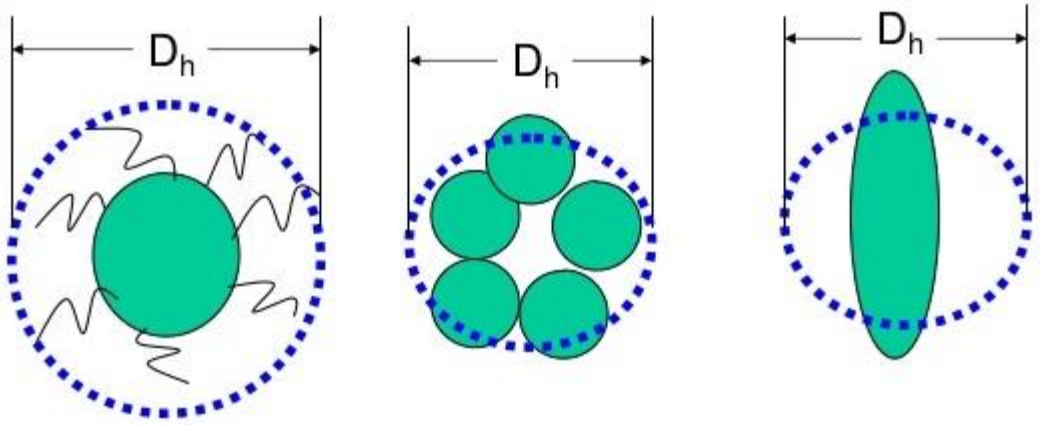
**Autocorrelation Function Data of Particles with Different Sizes**




**DLS measures the Hydrodynamic Radius of the Particle**

Stokes radius, Stokes-Einstein radius, aka hydrodynamic radius $R_H$, is the radius of a hard sphere that diffuses at the same rate as the molecule.

- DLS gives the diameter of a sphere that moves (diffuses) the same way as your sample.



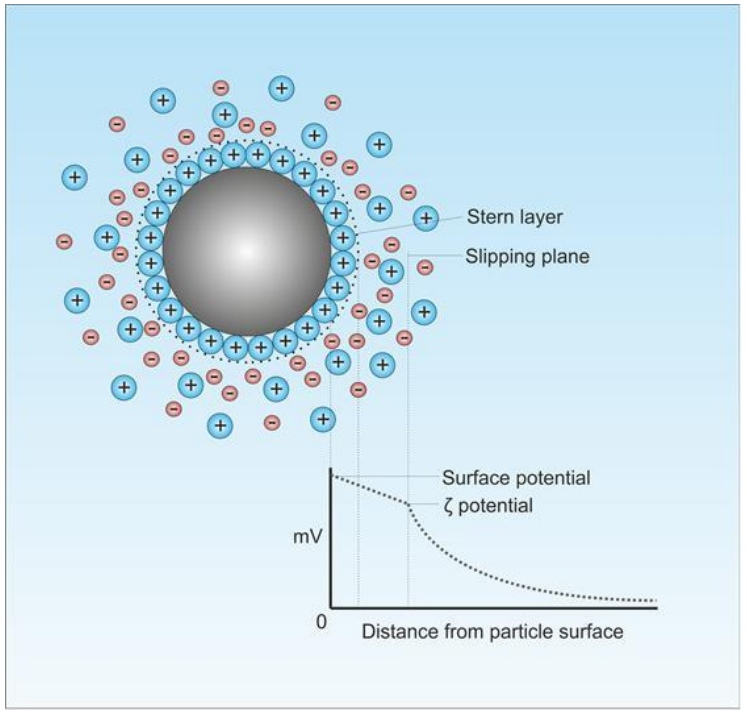
# Zeta Potential

**Zeta potential (*ζ-potential)* is a measure of electrostatic potential on the surface of a particle** (colloid). It is thought of as the **overall charge** of a particle. It can help predict the long-term stability of a particle in solution.

More precisely, zeta potential is the electric potential at the slipping plane—the plane that separates mobile fluid from fluid that is attached to the particle.

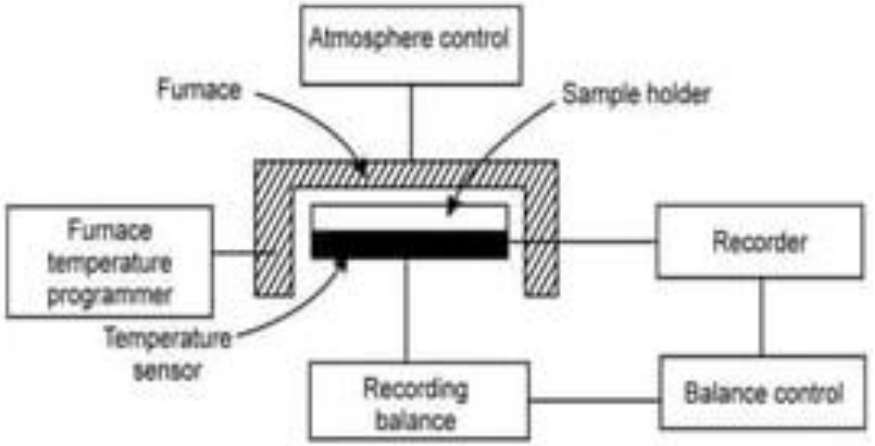
Zeta potential of a particle can be positive or negative, just as the overall charge of the nanoparticle can be positive or negative. The larger the absolute magnitude, the better the stability of the particle.

| Zeta potential [mV] | Stability behavior of the colloid |
|---|---|
| from 0 to ±5, | Rapid coagulation or flocculation |
| from ±10 to ±30 | Incipient instability |
| from ±30 to ±40 | Moderate stability |
| from ±40 to ±60 | Good stability |
| more than ±61 | Excellent stability |



# Thermogravimetric Analysis

- Thermogravimetric analysis (TGA) is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature.
- TGA measures **thermal decomposition**, or **thermolysis**: chemical decomposition of a substance caused by heat. The decomposition temperature of a substance is the temperature at which the substance chemically decomposes. The reaction is usually endothermic as heat is required to break chemical bonds in the compound undergoing decomposition.

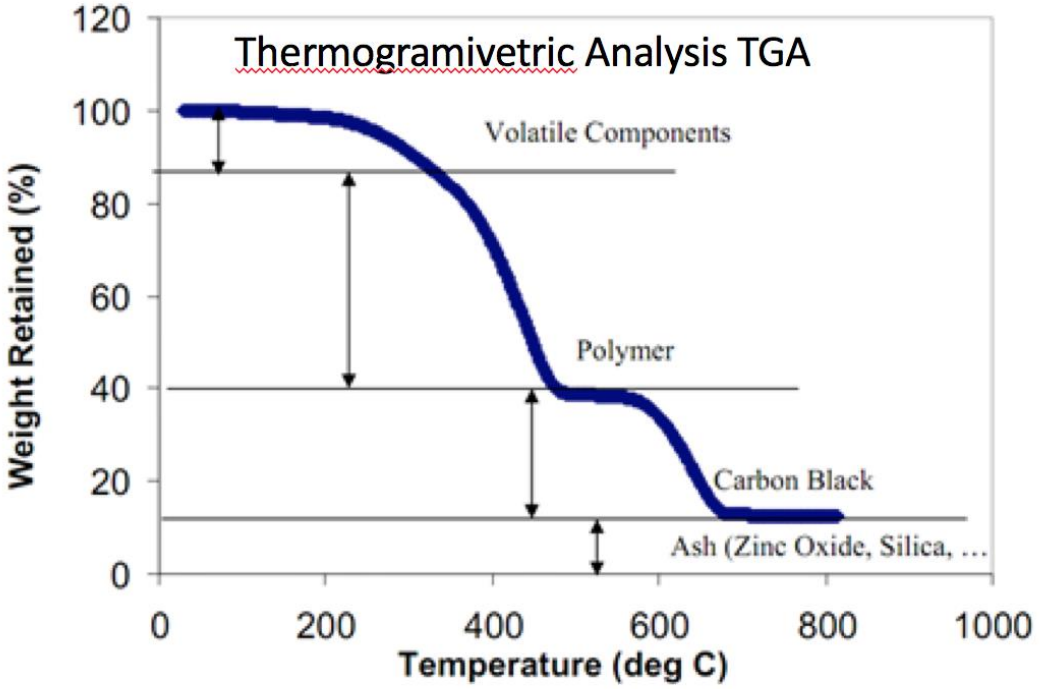



https://chem.libretexts.org/Courses/Franklin_and_Marshall_College/Introduction_to_Materials_Characterization__CHM_412_Collaborative_Text/Thermal_Analysis/Thermogravimetric_analysis_(TGA)

# Thermogravimetric Analysis

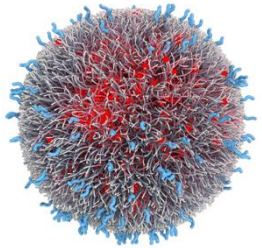
•Dynamic TGA - Temperature continues to increase over time as mass is recorded. This allows simulataneous identification of how much gas is removed and the temperature at which it occurs.

•Used in the characterization of crystalline and amorphous pharmaceutical materials, and many other applications.




https://chem.libretexts.org/Courses/Franklin_and_Marshall_College/Introduction_to_Materials_Characterization__CHM_412_Collaborative_Text/Thermal_Analysis/Thermogravimetric_analysis_(TGA)

**If you want to image a nanoparticle <u>inside</u> of a cell, which technique(s) could you use?**

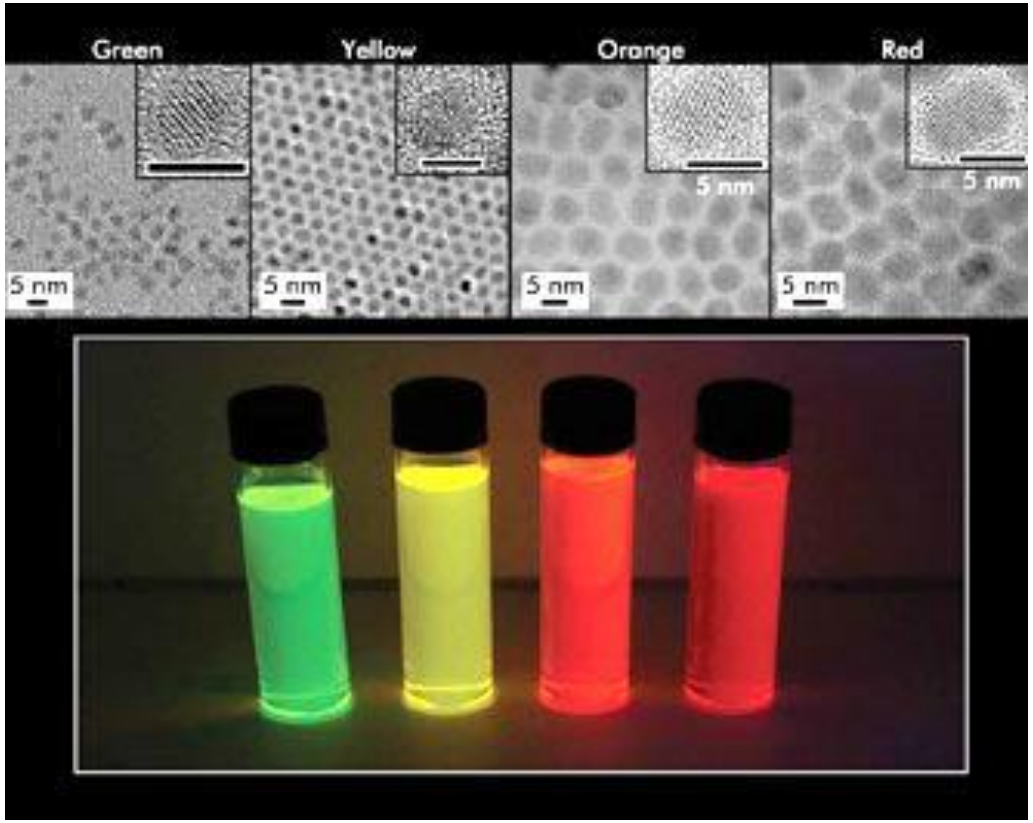


a) Transmission Electron Microscopy
b) Scanning Electron Microscopy
c) Atomic Force Microscopy
d) Dynamic Light Scattering
e) Zeta Potential Measurement

# Nanomaterials – What is Unique?

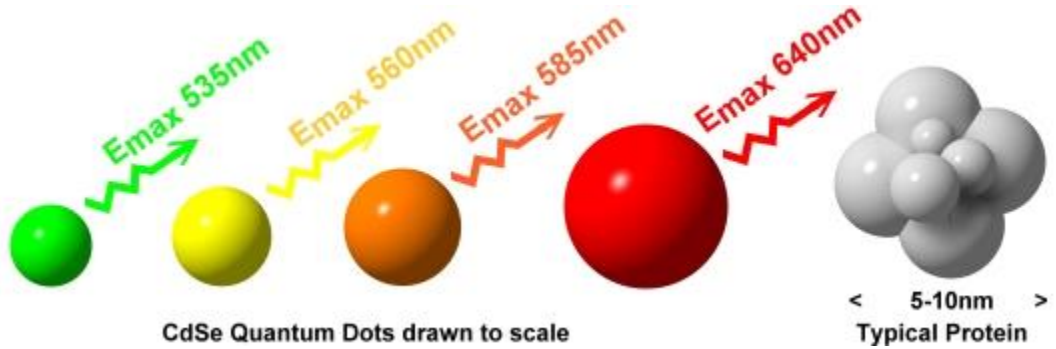
**Quantum size effect**: electronic properties of certain solids are altered with great reductions in particle size.

Quantum dots: Emission wavelength is size-dependent

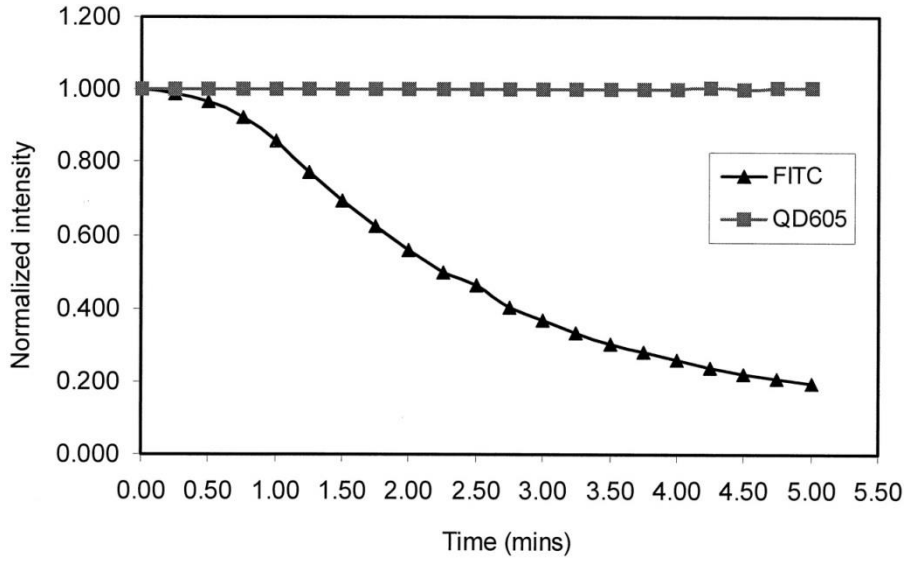


# Quantum Dots

Emission wavelength is size-dependent

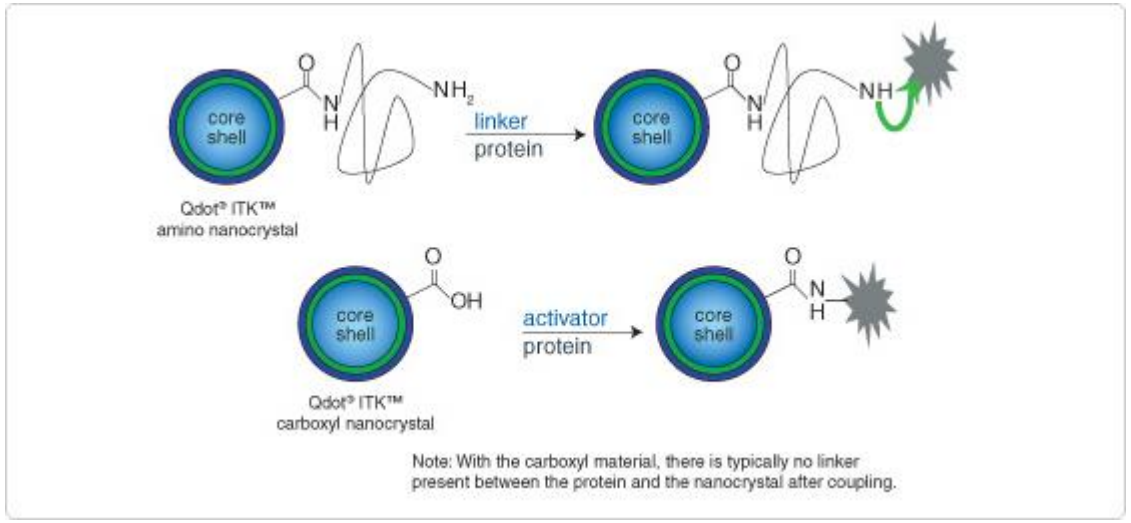



Highly Photostable

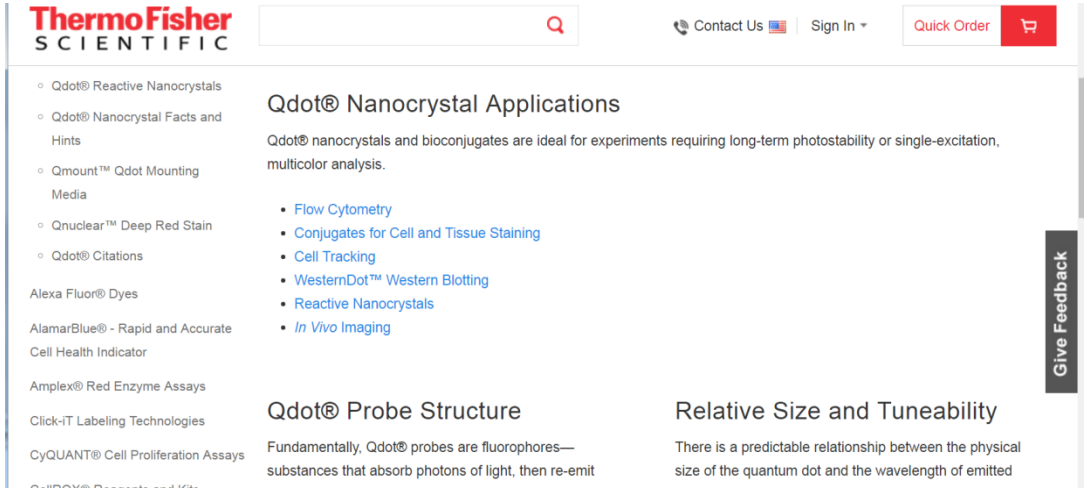


# Quantum Dots are Versatile Nanoprobes

Can be linked to proteins, etc.

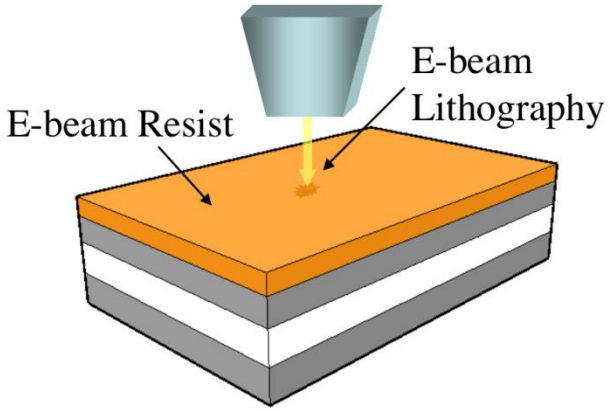


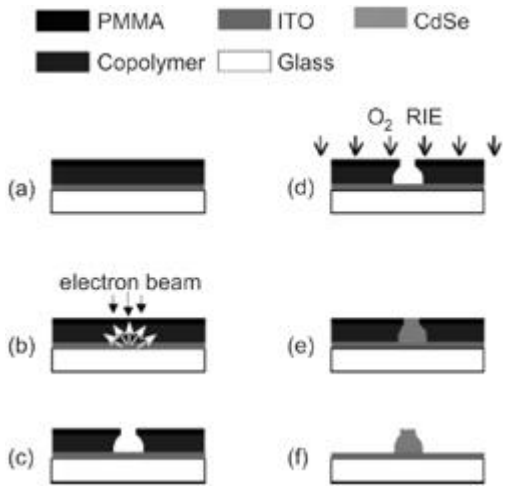

Commercially available:



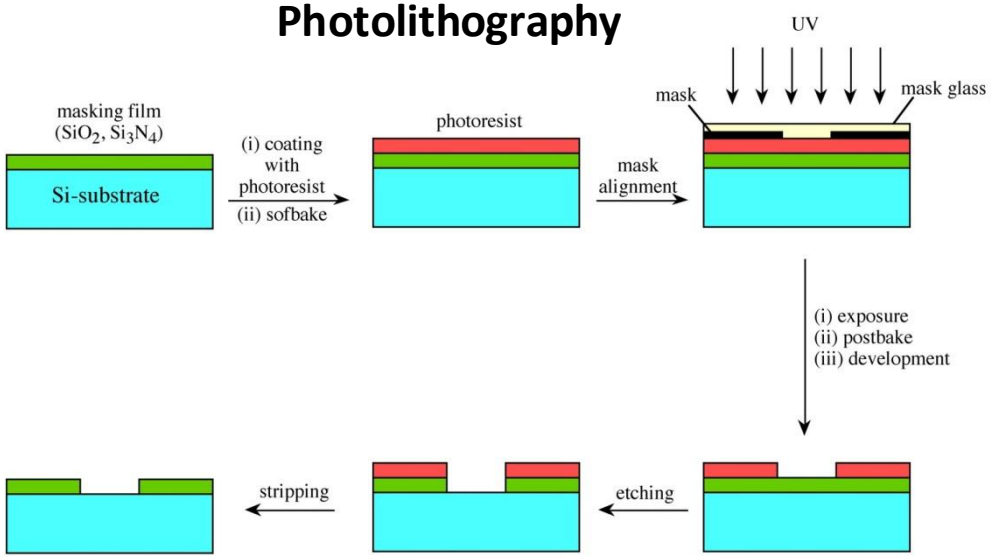
# Microlithography, Nanolithography, and Micro/Nanoprinting

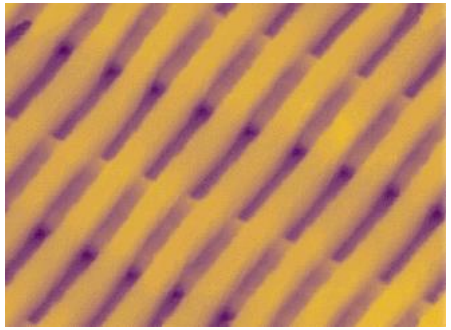
## E-beam Lithography

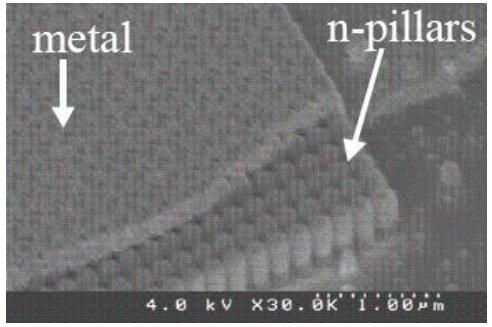


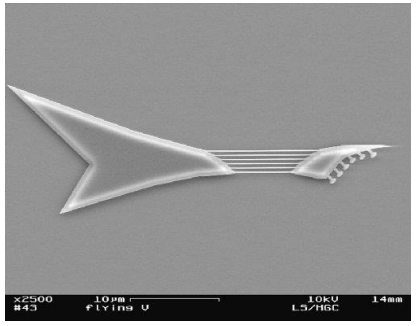



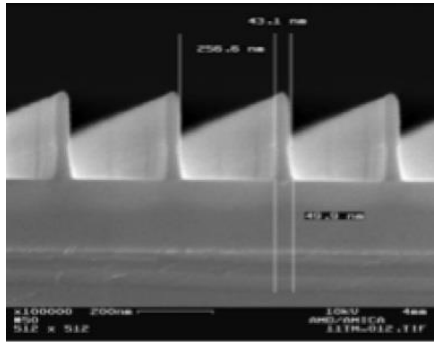

## Photolithography



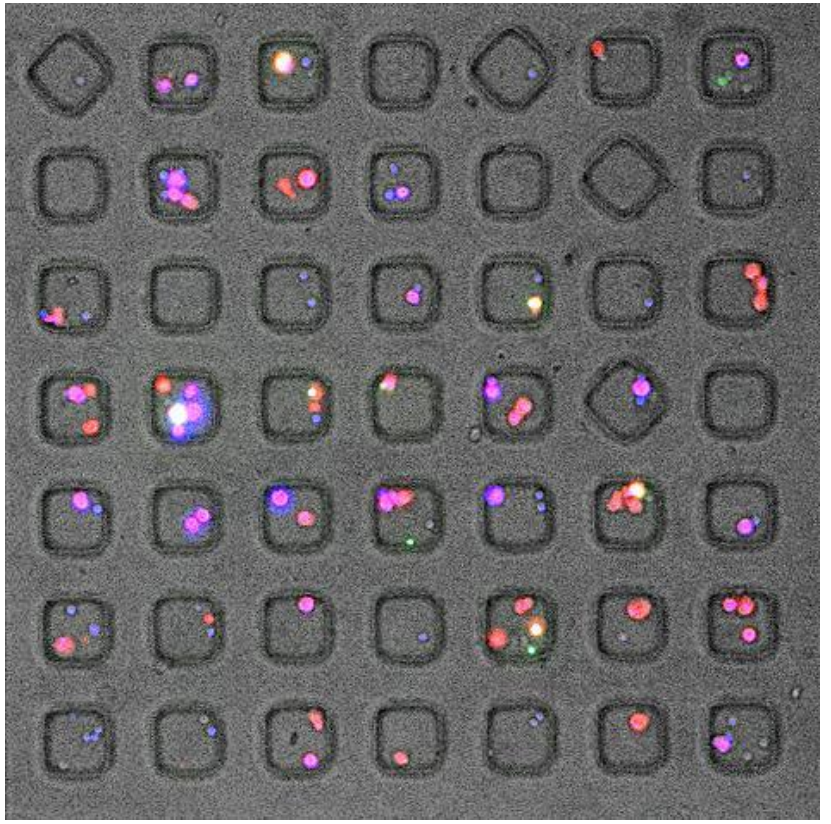




# Lithography for Single-Cell Analysis

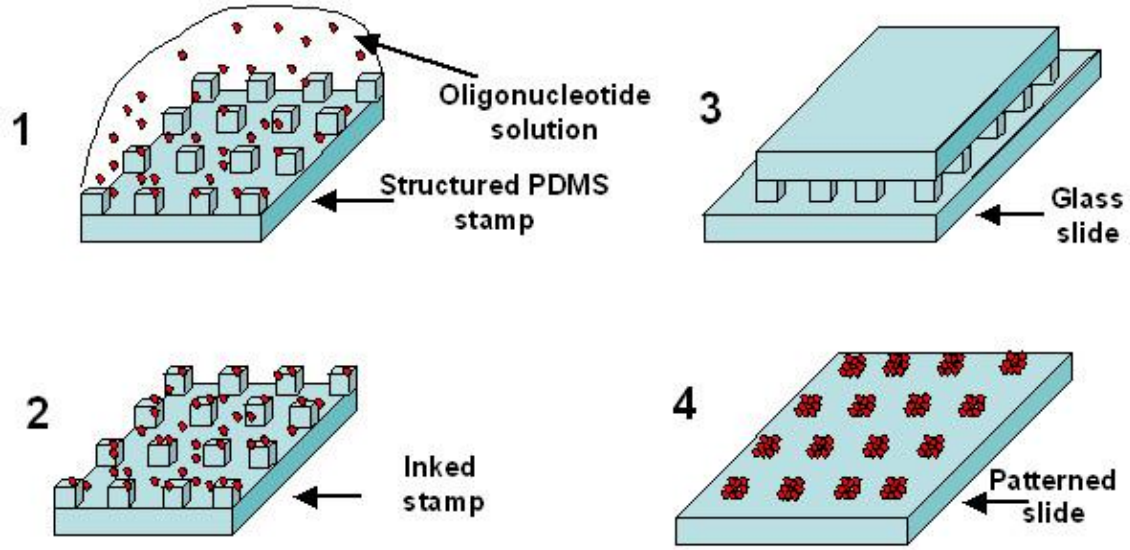
Useful for measuring differences among populations, screening of phenotypes



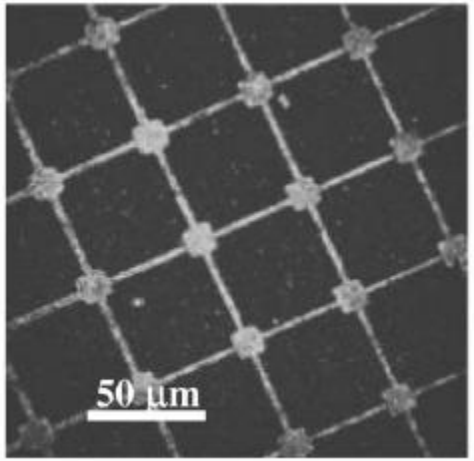
A high-throughput single-cell analysis of human $CD8^+$ T cell functions reveals discordance for cytokine secretion and cytolysis

Varadarajan, et. al. *J Clin Invest.* 2011;121(11):4322–4331. doi:10.1172/JCI58653.

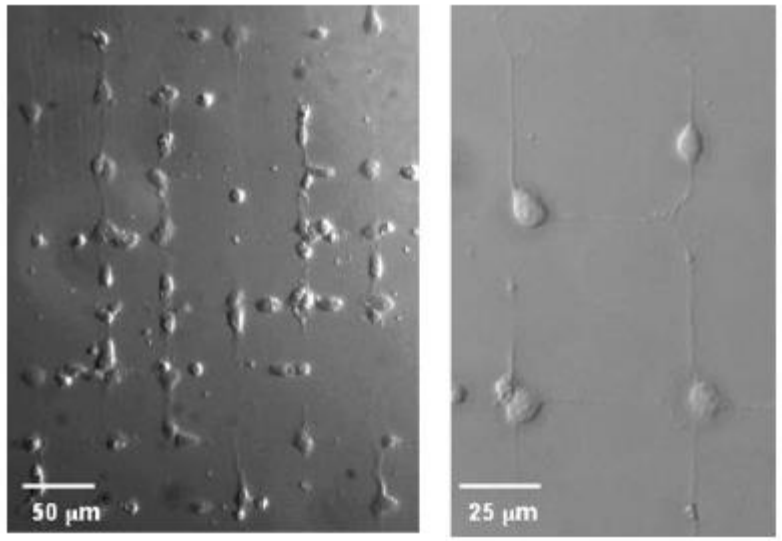
# Microcontact Printing

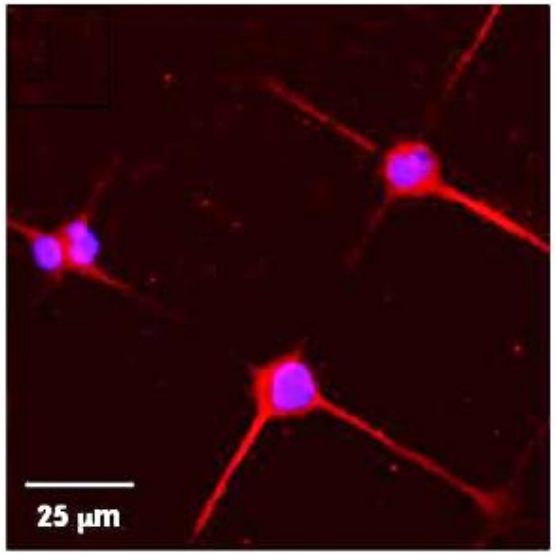



Patterned IKVAV peptide




Neurons migrate to patterned peptide




Heller, et. al. Biomaterials (2005) 26, 883-889.

# Microfluidic Devices: Lab on a Chip

**Use nanoliters of fluid for many applications:**

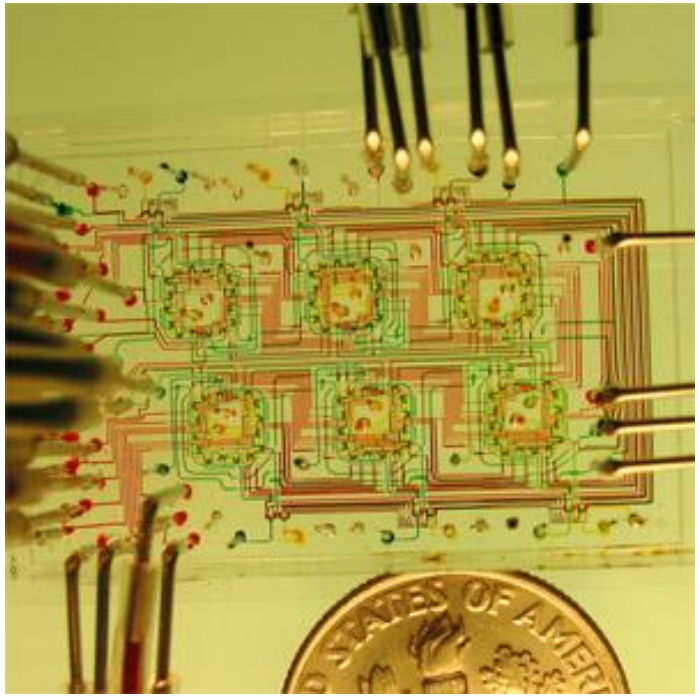
Assays/microarrays

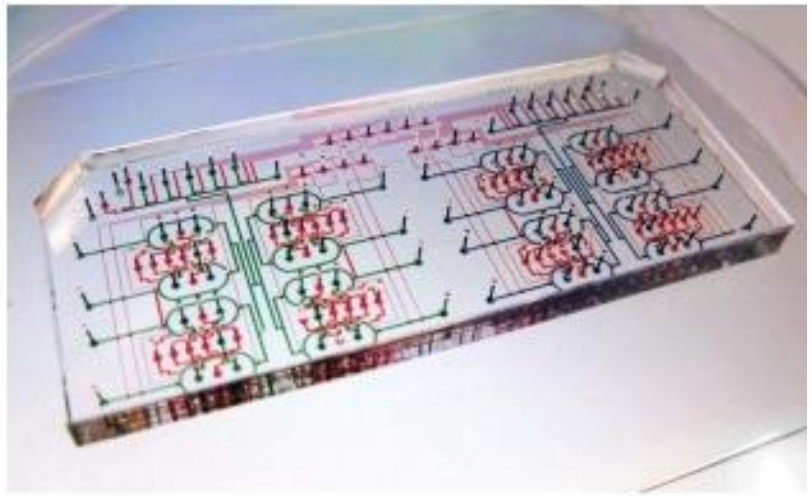
Control of cell environment

Single cell measurements

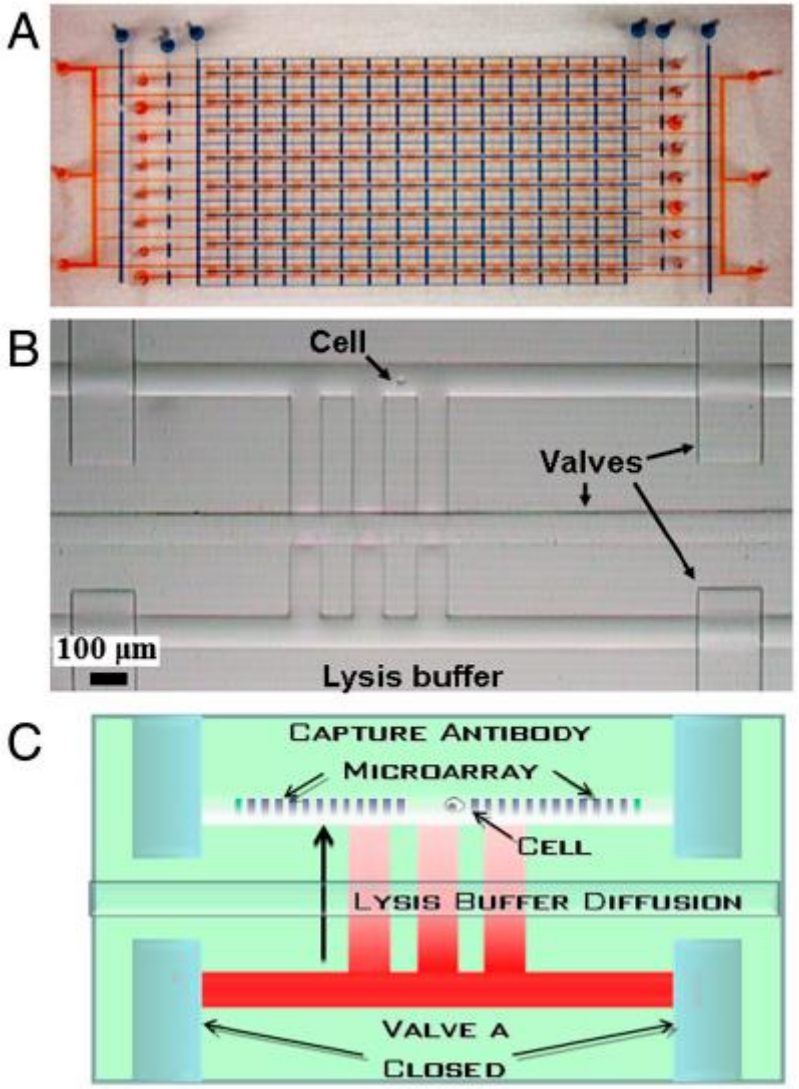
Drug screening

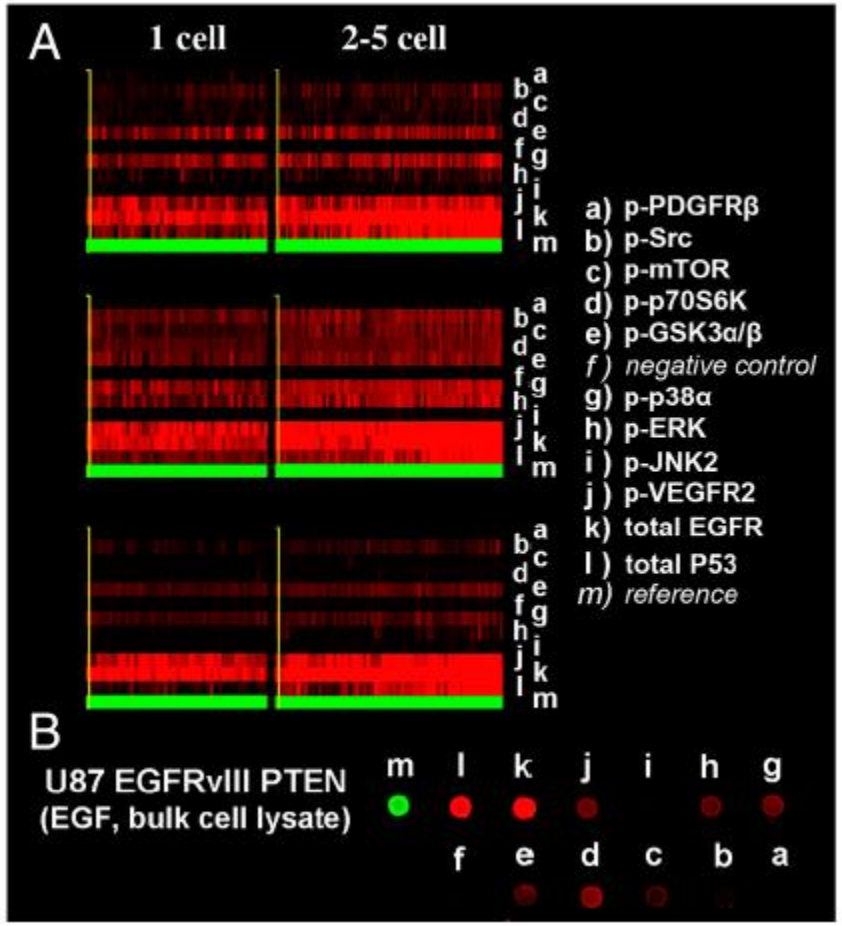
Drug (protein) delivery





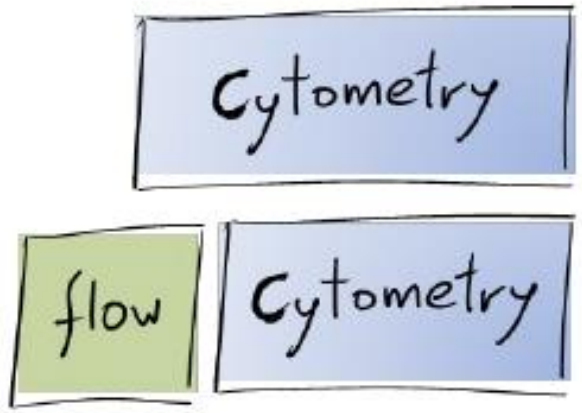
# Microfluidic Device Application: Single-Cell Microarray




Shi, et. al. PNAS, 2011

# Flow Cytometry




Measurement of
physical and chemical properties of cells.

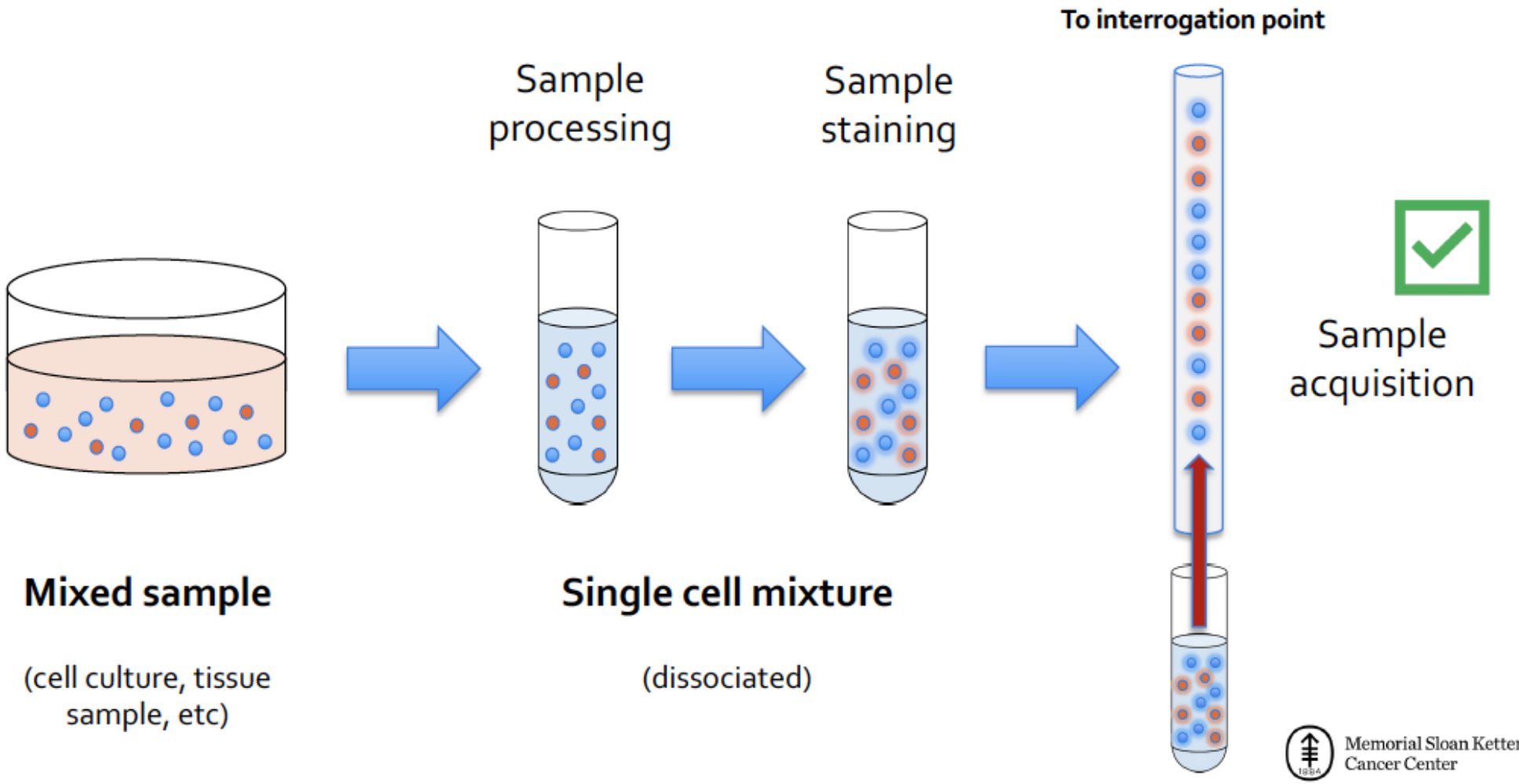
Characterization of cells
flowing in a stream of fluid

## What is **Flow Cytometry** used for?

Flow cytometry is typically used to count large numbers of cells or particles based on size, internal compexity, phenotype, cellular state, cell function, DNA content, gene expression, and to quantify these same cellular properties at a single-cell level.

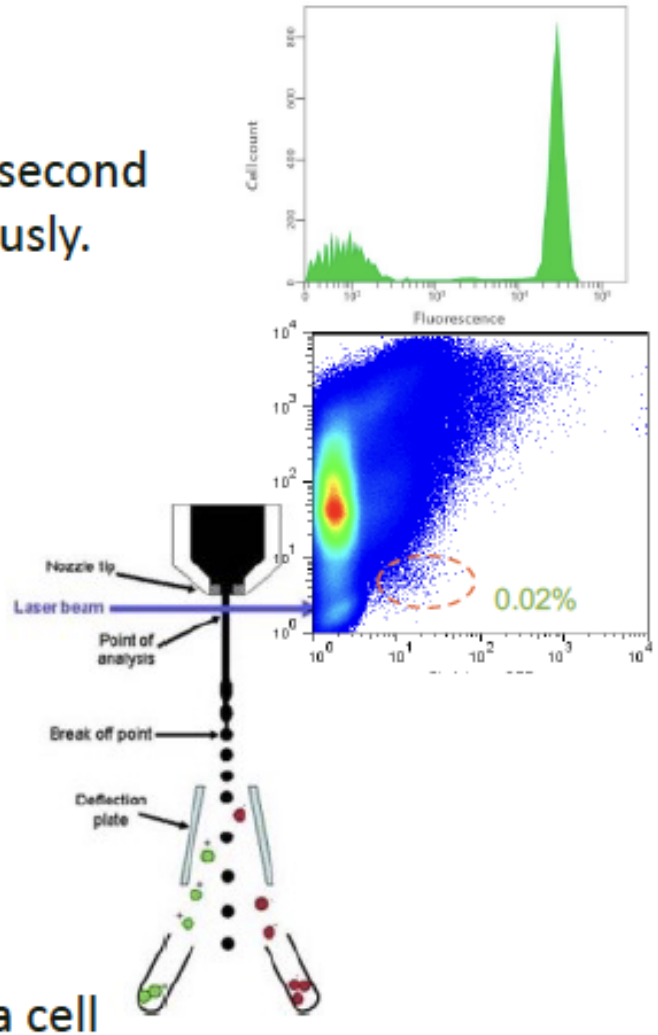
Flow Cytometry is not equivalent to FACS: **F**luorescence **A**ctivated **C**ell **S**orting

# Flow Cytometry Lights Up Your Cells



# Why Use Flow Cytometry?

- Identifying and quantifying cells
  - Measure thousands of cells/particles per second
  - Measure multiple parameters simultaneously.
- Detection of extremely rare populations
- Cell sorting
  - High purity, speed, and yield.

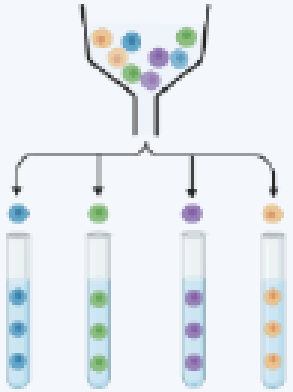



## **Flow Cytometry** does not...

- Locate where a certain component is within a cell
- Measure distribution of cellular components
- Measure cell morphology

# FLOW CYTOMETRY APPLICATIONS

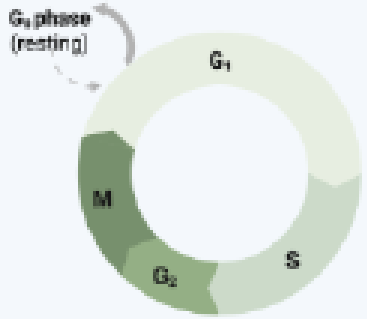
## CELL SORTING



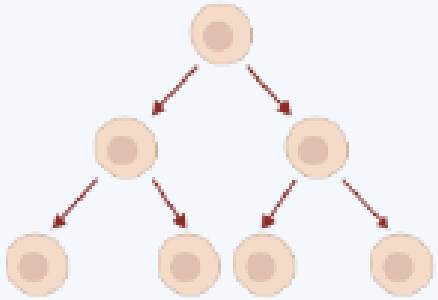
## IMMUNOPHENOTYPING



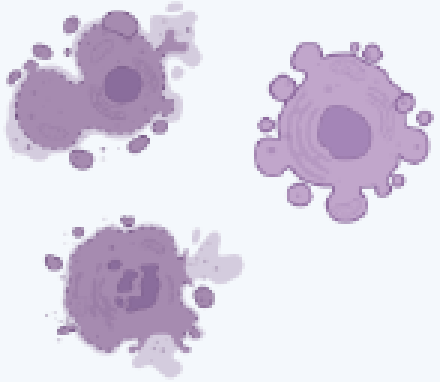
## CELL CYCLE



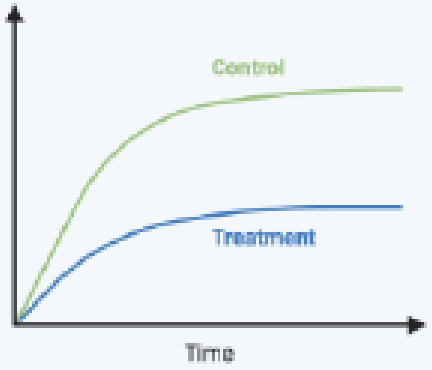

## CELL PROLIFERATION



## CELL DEATH

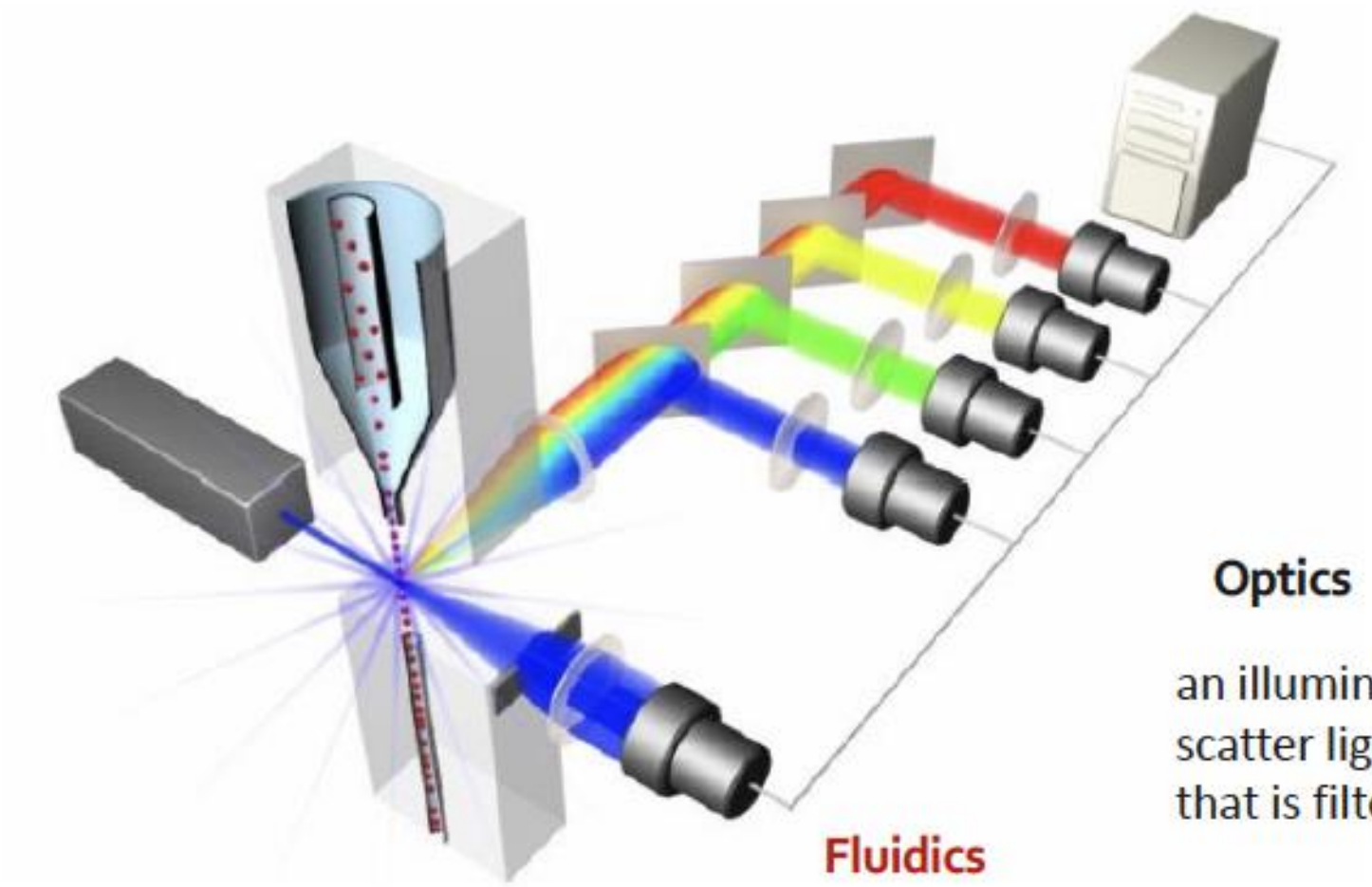


## KINETIC ANALYSIS




….and many more

# Anatomy of a Flow Cytometer



**Electronics**

converted to digital values
that are processed
and analyzed in a computer.

**Optics**

an illuminated volume where they
scatter light and emit fluorescence
that is filtered, collected and...

**Fluidics**

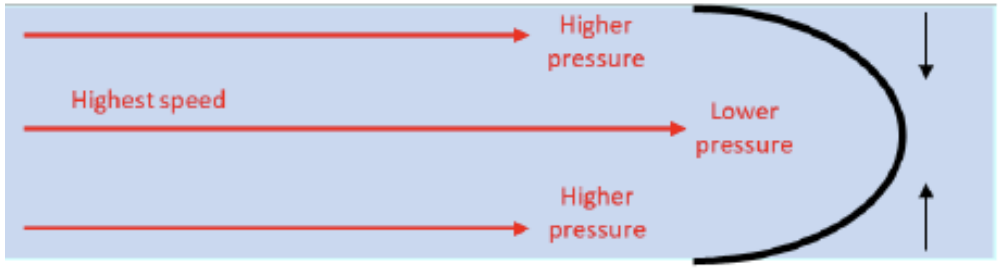
Cells in suspension
flow in single file through...

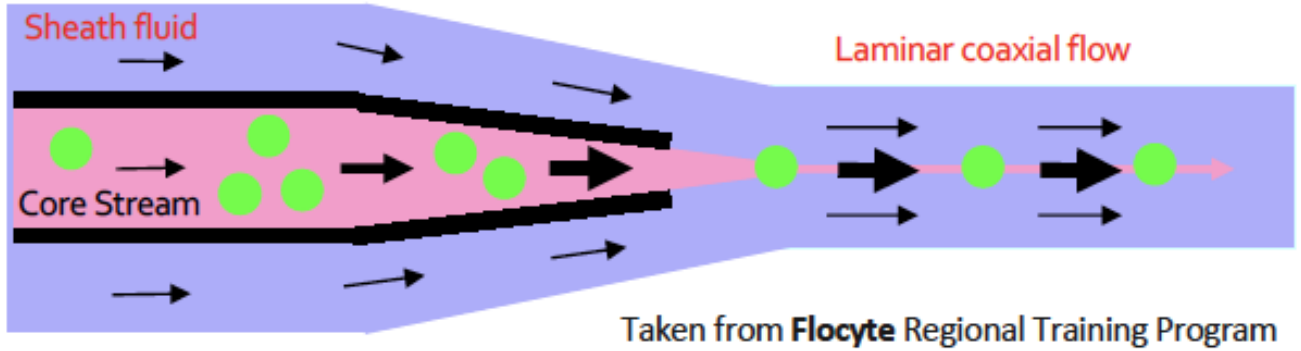
# Hydrodynamic Focusing: Getting Cells To Line Up Single-File

How does hydrodynamic focusing happen?

**The same way that planes fly - pressure manipulation**

Bernoulli's principle in flow cytometry



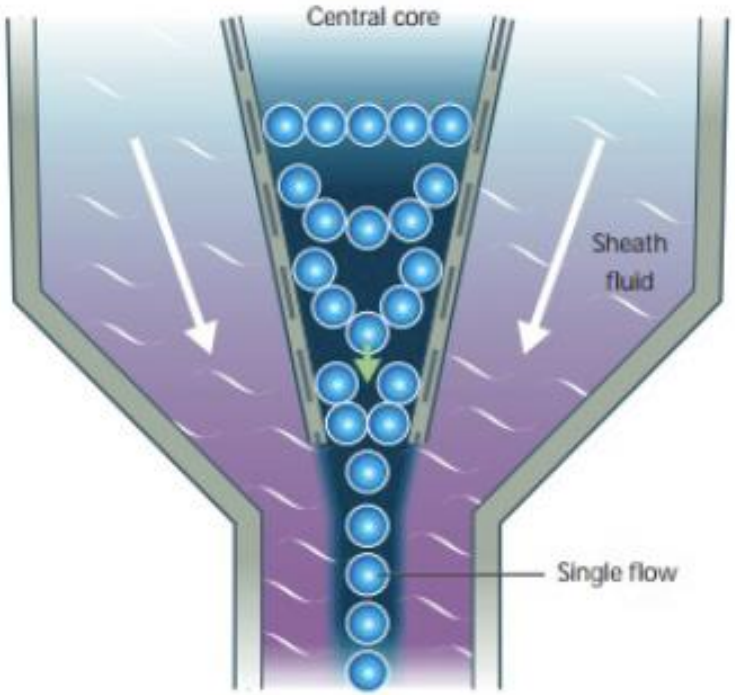



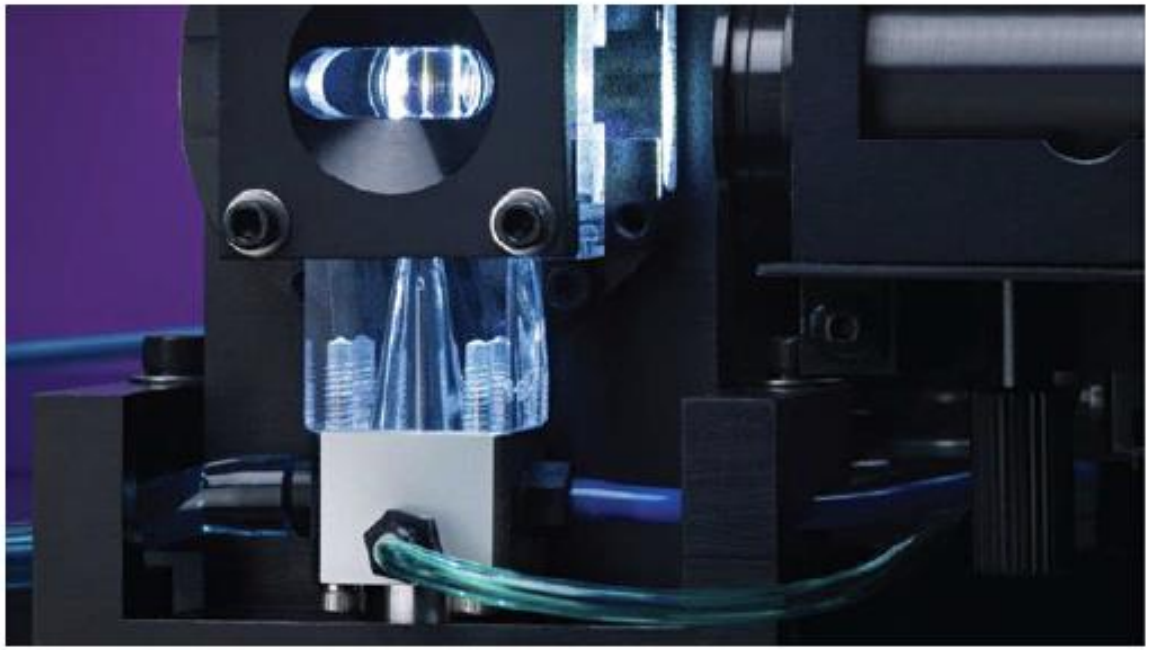

Taken from **Flocyte** Regional Training Program

**By forcing our sample through a smaller area (cuvette/nozzle), we increase its velocity (lowering the internal pressure) and cause the sample stream to compress**

# Hydrodynamic Focusing: Getting Cells To Line Up Single-File

- The fluidics system consists of a central core through which the sample fluid is injected
- As the sheath fluid moves, it creates a drag effect that causes the sample in the central core to narrow.
- This creates a single line of particles and is called hydrodynamic focusing.



MSKCC Flow Cytometry Core Facility

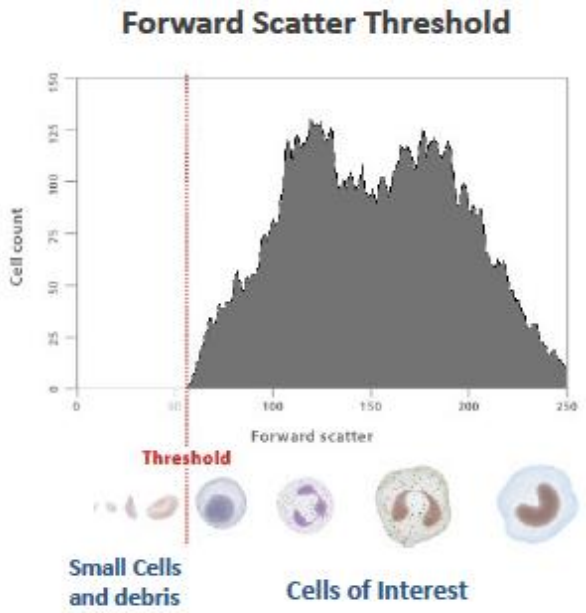
# Scattered Light Measured by Flow Cytometry

**Forward Scatter (FSC)**

- Collected at 0°
- Diffraction of light as photons pass through cell
- Influenced by Size

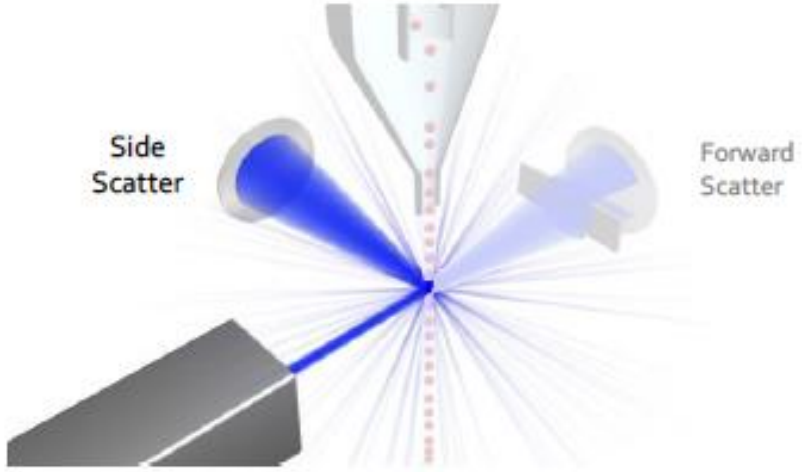
Depends on:
- Size
- Δ Diffractive index
- Presence of material with strong absortion at the Illumination wavelength

FSC follows non-monotonically with size

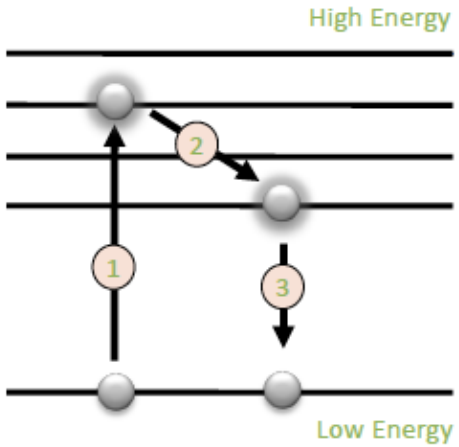


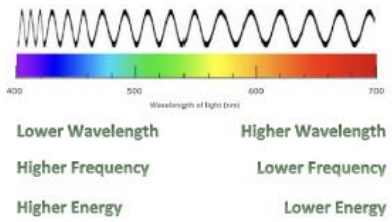

**Side Scatter (SSC)**

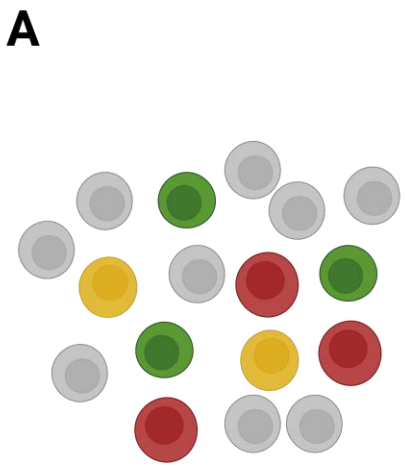
- Collected at 90°
- Reflection of light off cellular structures
- Measures relative "granularity"

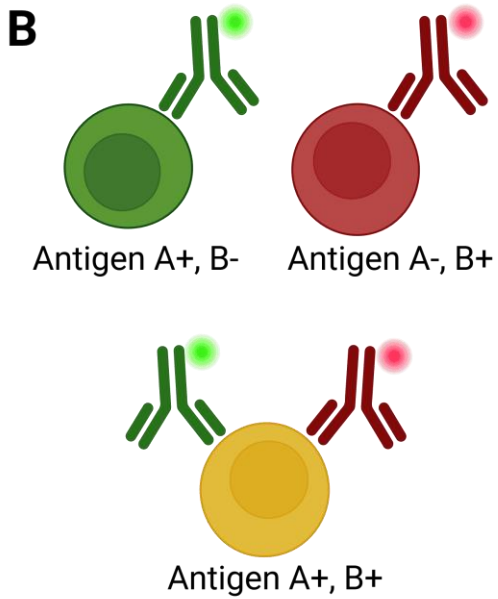


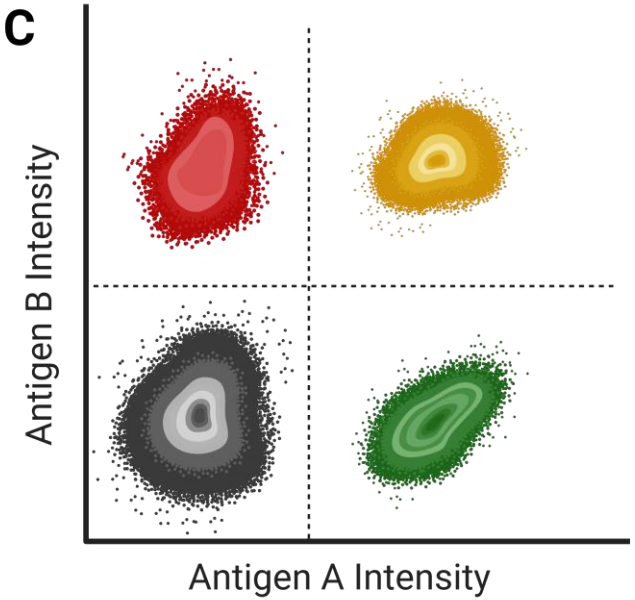
# Fluorescence of (Multiple) Fluorescent Dyes
## (Usually Bound to Antibodies for Your Targets of Interest)



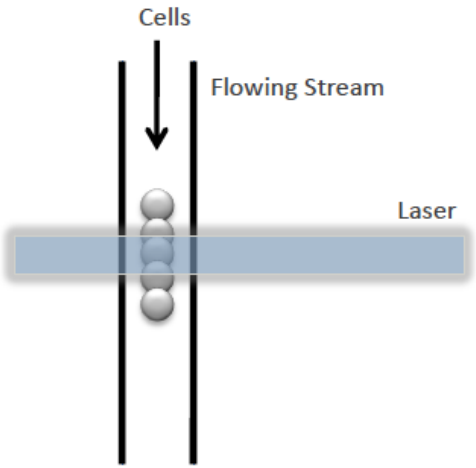



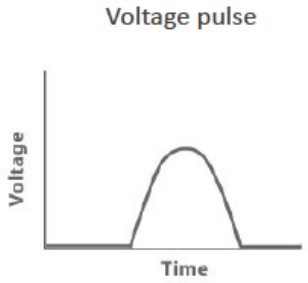



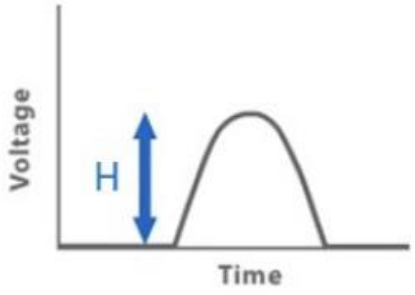



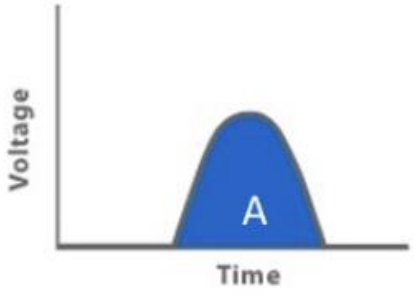
# The Raw Data from Flow Cytometry: Pulses



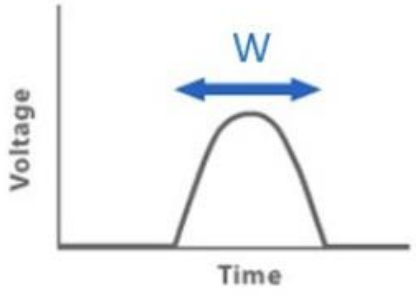




H: Pulse height




A: Pulse area




W: Pulse width

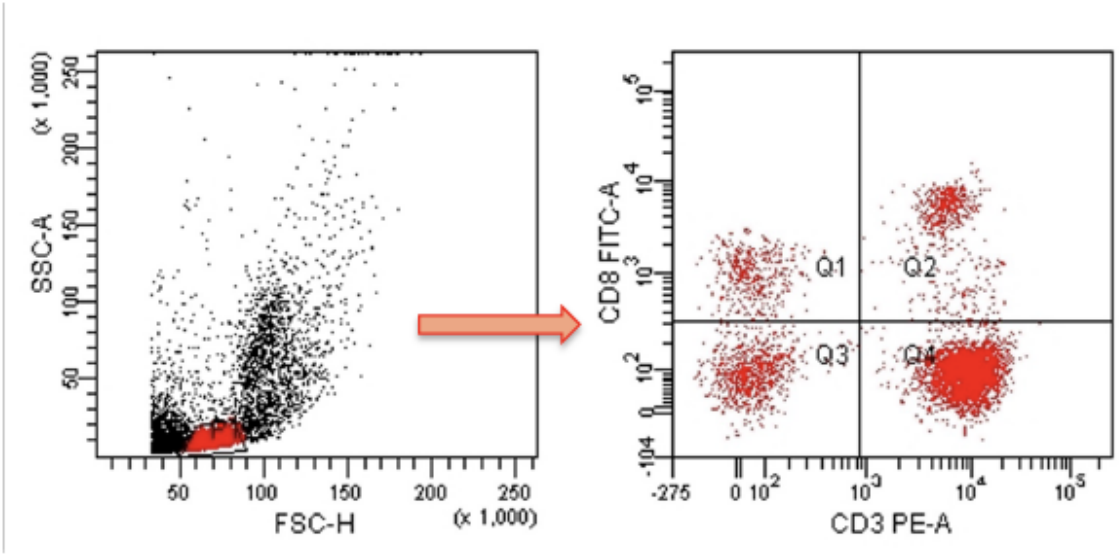
Pulse area gives us the more accurate measurement of our emission intensity.

# The Raw Data

- Cell passes through a laser
- Light is scattered, fluorescence is emitted
- For each cell we get a number for each detector

| | FSC | | | SSC | | FL1 | | FL2 | | FLX |
|---|---|---|---|---|---|---|---|---|---|---|
| | FSC-H | FSC-A | FSC-W | SSC-H | SSC-A | FL1-H | FL1-A | FL2-H | ... | FLX-H |
| Cell 1 | 50 | 52 | 21 | 45 | 46 | 2010 | 2000 | 102 | | 45 |
| Cell 2 | 55 | 60 | 33 | 47 | 49 | 1877 | 1867 | 55 | | 47 |
| Cell 3 | 67 | 63 | 36 | 56 | 55 | 2144 | 2134 | 212 | | 56 |
| ... | | | | | | | | | | |
| Cell n | 87 | 89 | 45 | 54 | 52 | 2331 | 2321 | 44 | | 54 |

# Gating

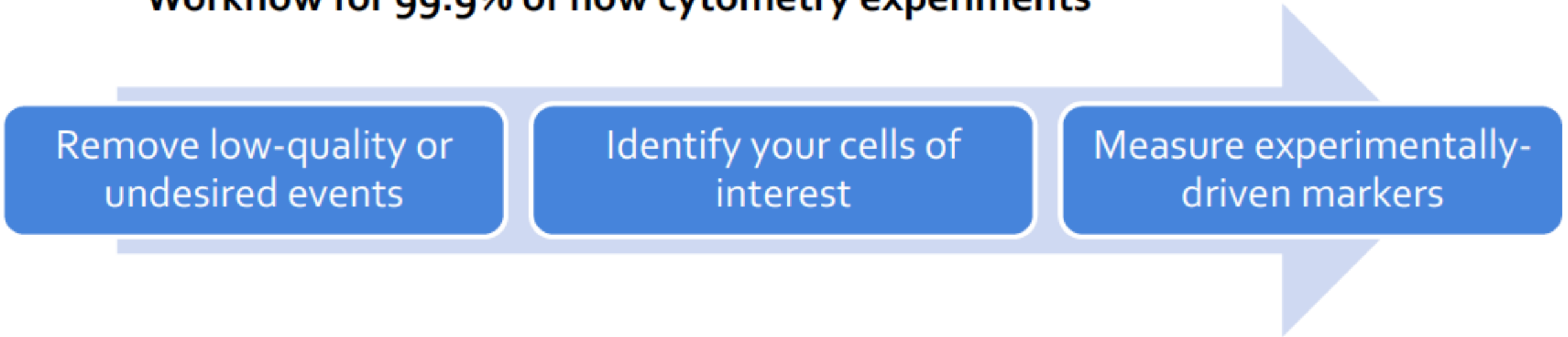



| Population | #Events | %Parent | %Total | CD8 FITC Median | $CD_3$ PE Median |
|---|---|---|---|---|---|
| P1 | 46,835 | 73.0 | 73.0 | 642 | 7,000 |
| Q1 | 4,332 | 9.2 | 6.8 | 1,104 | 65 |
| Q2 | 5,216 | 11.1 | 8.1 | 4,180 | 6,699 |
| Q3 | 7,519 | 16.1 | 11.7 | 97 | 54 |
| Q4 | 29,768 | 63.6 | 46.4 | 92 | 9,816 |
| P2 | 34,887 | 74.5 | 54.4 | 702 | 9,375 |

- **Standard Gating Steps**
- **Time gate:** Removes fluidic clogs, bubbles, or flow instabilities at the start or end of a run.
- **Debris exclusion:** Uses Forward Scatter (FSC) and Side Scatter (SSC) to filter out small cell fragments and dead cells.
- **Doublet discrimination:** Compares pulse area (FSC-A) to height (FSC-H) or width (FSC-W) to isolate single cells from cell clumps.
- **Viability gate:** Uses a live/dead stain to exclude dead cells that take up the fluorescent dye.
- **Population marker gating:** Uses targeted fluorescent antibodies to identify specific cell types, such as T-cells or B-cells. [1, 2, 3, 4, 5, 6]

https://nanocellect.com/blog/flow-cytometry-gating-everything-you-need-to-know/

## Workflow for 99.9% of flow cytometry experiments

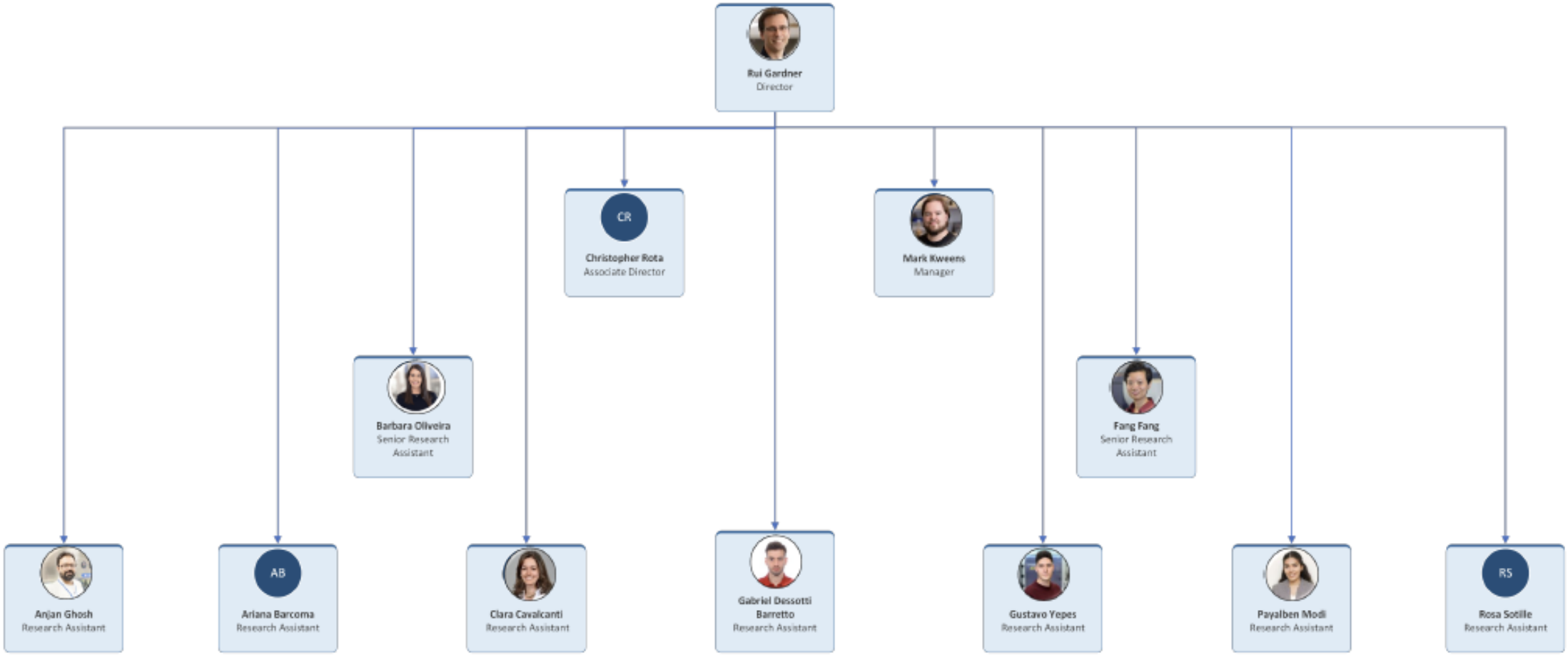



- Flow abnormalities
- Doublets
- Dead cells
- Unwanted cells ("dump")

- FSC/SSC profile
- Cell type-specific markers
- Intrinsic tags (e.g fluorescent proteins)

- Receptor-ligand pairs
- Intracellular signaling factors
- Cell cycle markers
- Etc, etc

# Flow Cytometry Core: A Team to Help



# Core Facilities at MSKCC (and the Tri-I)

- ~30 professionally-run core facilities at MSKCC
- (and some additional non-overlapping facilities Weill Cornell and Rockefeller University)
- Like having the expertise of hundreds of people who can help you (ask nicely).

https://www.mskcc.org/research-areas/core-facilities