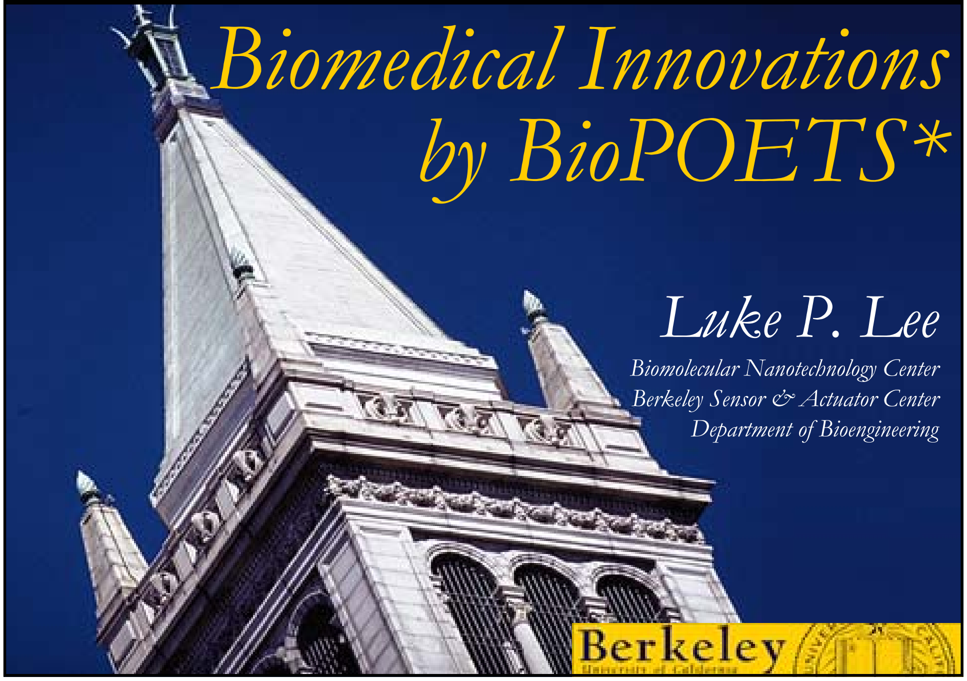
**BioPOETS*: Biologically-inspired Photonics-Optofluidics-Electronics Technology & Science*

*Biomedical Innovations by BioPOETS**



Luke P. Lee

Biomolecular Nanotechnology Center
Berkeley Sensor & Actuator Center
Department of Bioengineering

Acknowledgments

- Graduate & Post-doctoral Researchers :

Dino Di Carlo (Harvard), Paul Hung, Michelle Khine (UCM), Sunghoon Kwon (SNU), Poorya Sabounchi, Yitao Long (SWU), David Breslauer, Mimi Zhang, Jeonggi Seo (PARC), Yeonho Choi, Christy Trinkle (UK), Nikolas Chronis (UM), Kihun Jeong (KAIST), Megan Dueck, Hansang Cho, Yolanda Zhang, Teawook Kang, Jay Kim (Iowa State), Cristian Ionescu-Zanetti, Franklin Kim (NU), Yangku Choi (KAIST), Dukhyun Choi, Eunice Lee, Ben Ross, Gang Liu, Philip Lee, Adrian Lau, J. Tanner Nevill, Robert Szema, Yu Lu (Intel), Liz Wu

- Undergraduate Researchers

Ryan Cooper, Todd Fong, Angelee Kumar, Tao-yang Chen, Andrea Fils, Albert Mach, Brian Lee, Shalini Indrakanti, Selena Wong, Irene Sinn

- Collaborators:

Erin O'Shea @ Harvard, Lily Jan @ UCSF, Gabor Somorjai @ UCB, Precision Biology @ Intel, Yang Dan @ UCB, Paul Alivisatos @ UCB, Wilhelm Krek @ ETH Zurich, Matthias Peter @ ETH Zurich, Markus Stoffel @ ETH Zurich

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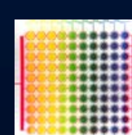


Outline

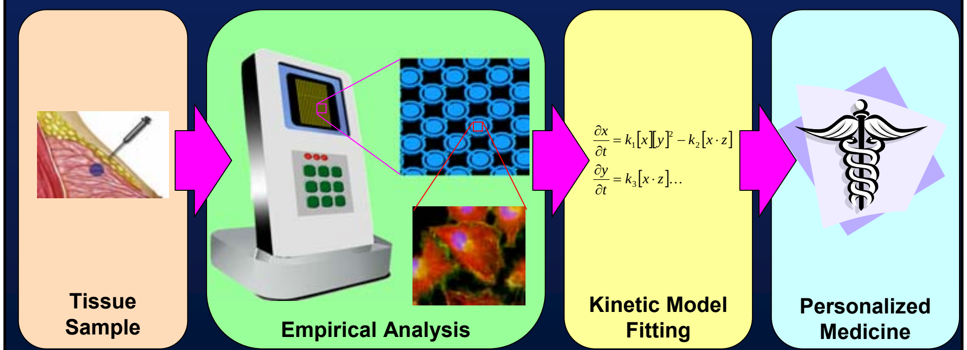
- Motivations
- Biophotonics inspired by Nature
- Biologically-inspired Optics
- Biologically-inspired Fluidics
- *Biologically-inspired Electronics*
- Summary

http://biopoets.berkeley.edu

*Quantitative Biomedicine by BioPOETS**

**Biomolecular Photonics-Optofluidics-Electronics Technology & Science*

Quantitative Biomedical Science



Fundamental Concepts:

- *Rapid collection of large experimental data sets*
- *Intelligent consolidation of quantitative values*

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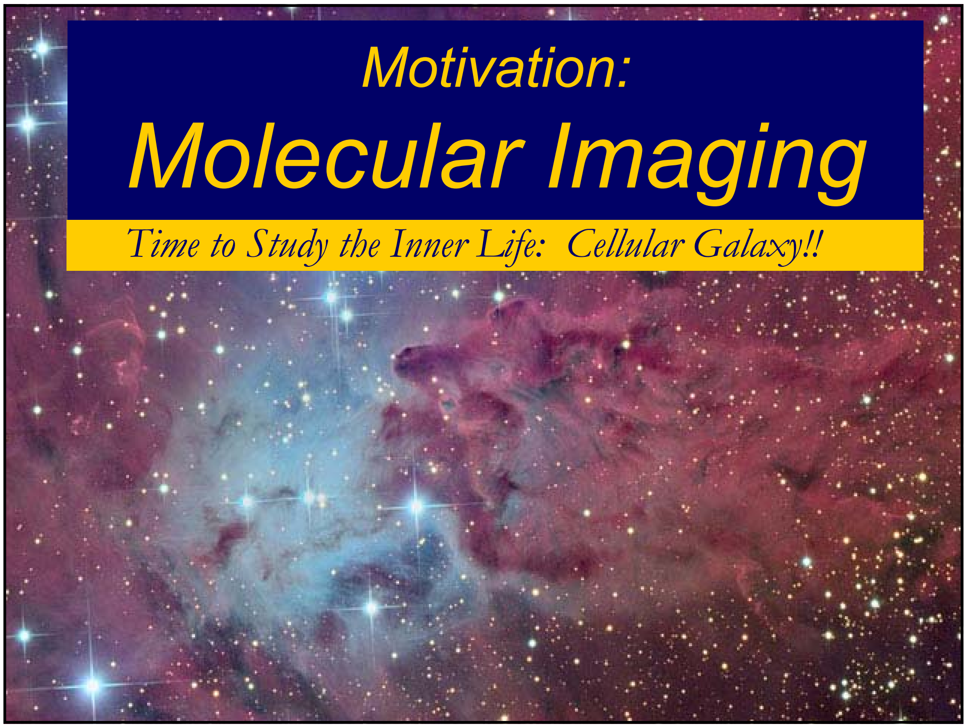
Nano-Biophotonics Inspired by Nature

for Cellular Galaxy Biophysics and Imaging

Motivation:

Molecular Imaging

Time to Study the Inner Life: Cellular Galaxy!!



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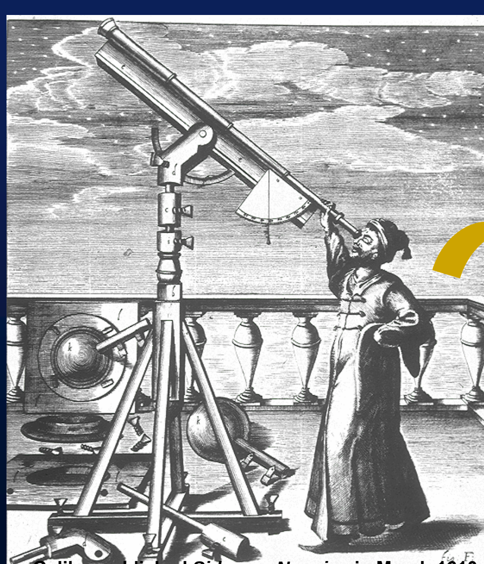
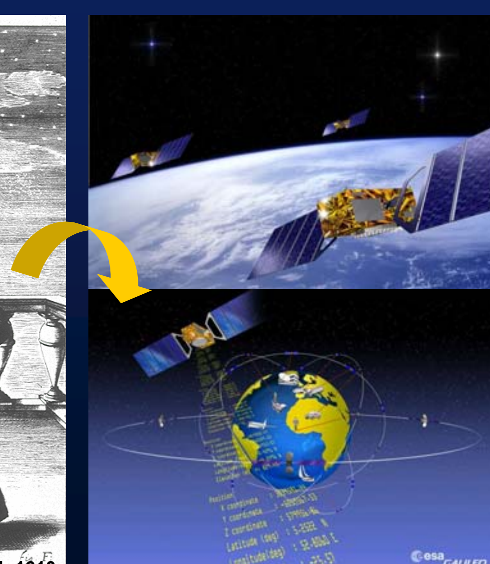
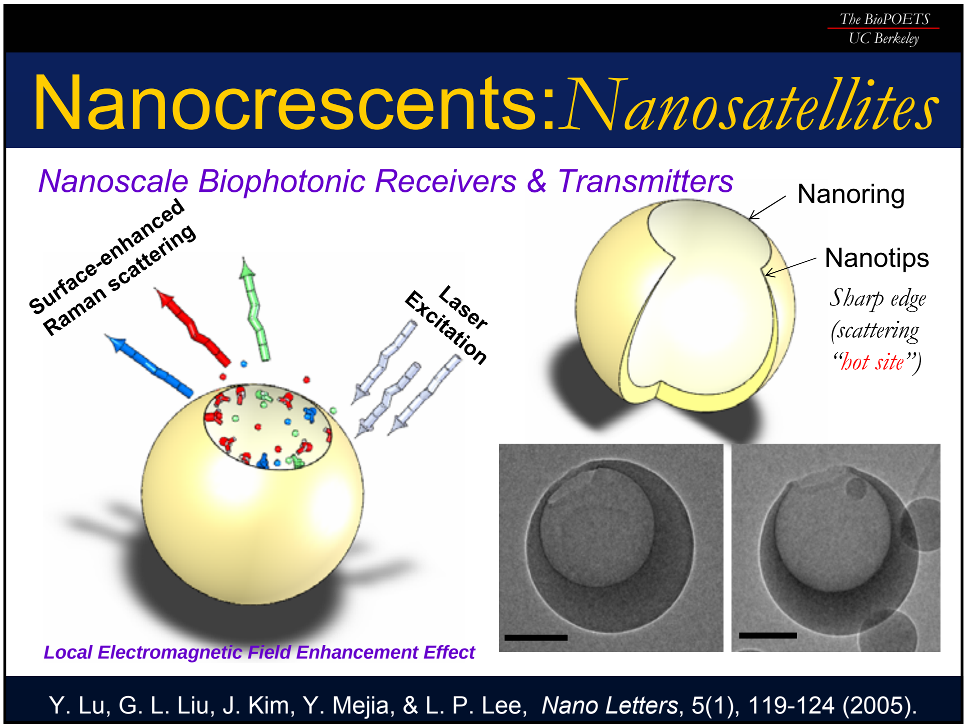
From Telescope to Satellite Telescope



Galileo published *Sidereus Nuncius* in March 1610




The BioPOETS
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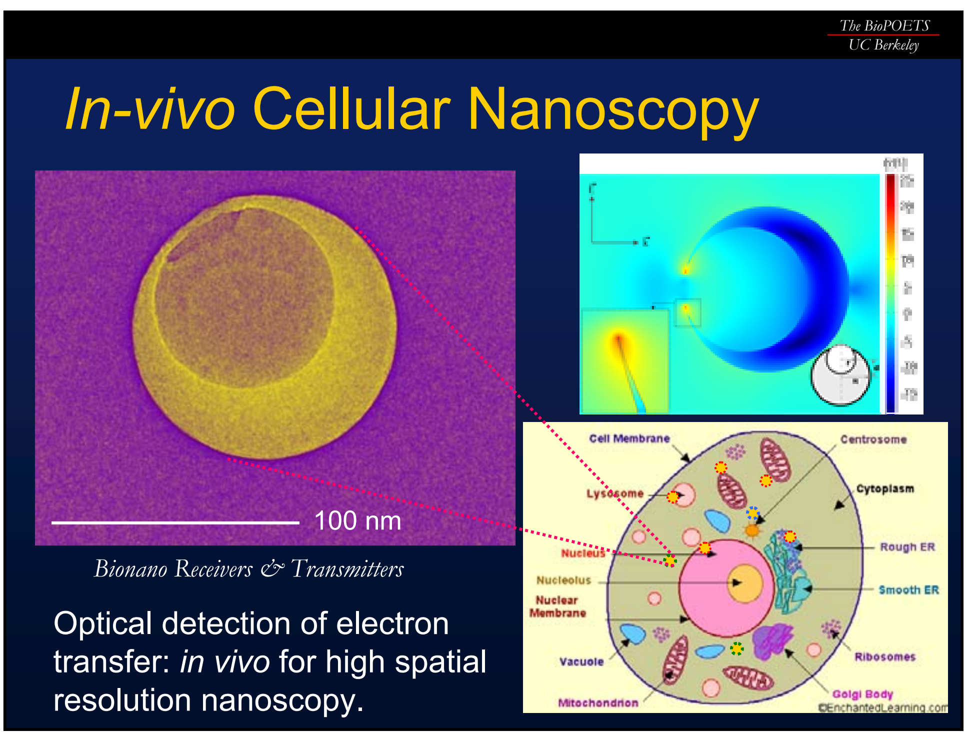
Nanocrescents: Nanosatellites
Nanoscale Biophotonic Receivers & Transmitters
Surface-enhanced Raman scattering
Laser Excitation
Nanoring
Nanotips
Sharp edge (scattering "hot site")
Local Electromagnetic Field Enhancement Effect
Y. Lu, G. L. Liu, J. Kim, Y. Mejia, & L. P. Lee, Nano Letters, 5(1), 119-124 (2005).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In-vivo Cellular Nanoscopy
100 nm
Bionano Receivers & Transmitters
Optical detection of electron transfer: in vivo for high spatial resolution nanoscopy.
Cell Membrane
Centrosome
Cytoplasm
Lysosome
Rough ER
Smooth ER
Nucleus
Nucleolus
Nuclear Membrane
Vacuole
Ribosomes
Golgi Body
Mitochondrion
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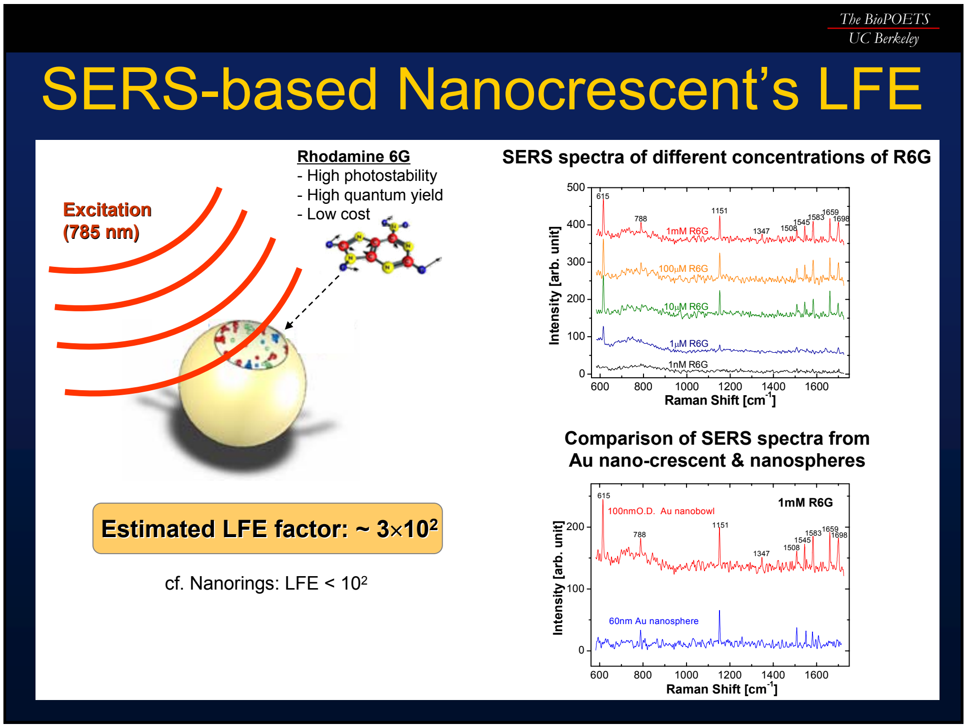
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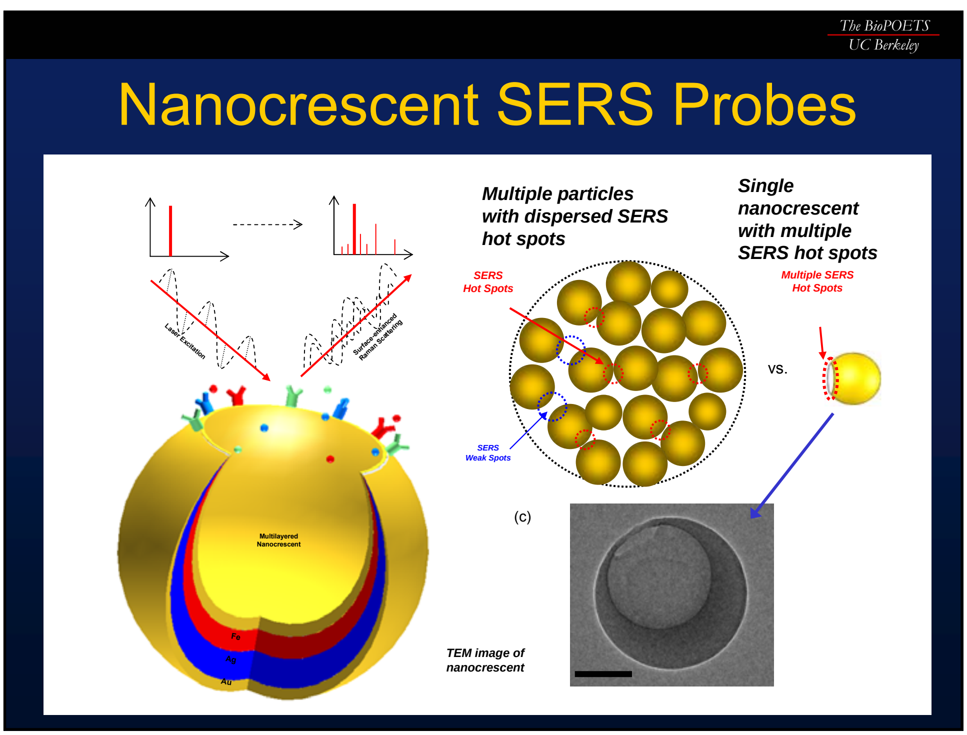
SERS-based Nanocrescent's LFE
Excitation
(785 nm)
Rhodamine 6G
- High photostability
- High quantum yield
- Low cost
Estimated LFE factor: ~ 3×10²
cf. Nanorings: LFE < 10²
SERS spectra of different concentrations of R6G
Intensity [arb. unit]
Raman Shift [cm⁻¹]
1mM R6G
100μM R6G
10μM R6G
1μM R6G
1nM R6G
Comparison of SERS spectra from
Au nano-crescent & nanospheres
1mM R6G
100nmO.D. Au nanobowl
60nm Au nanosphere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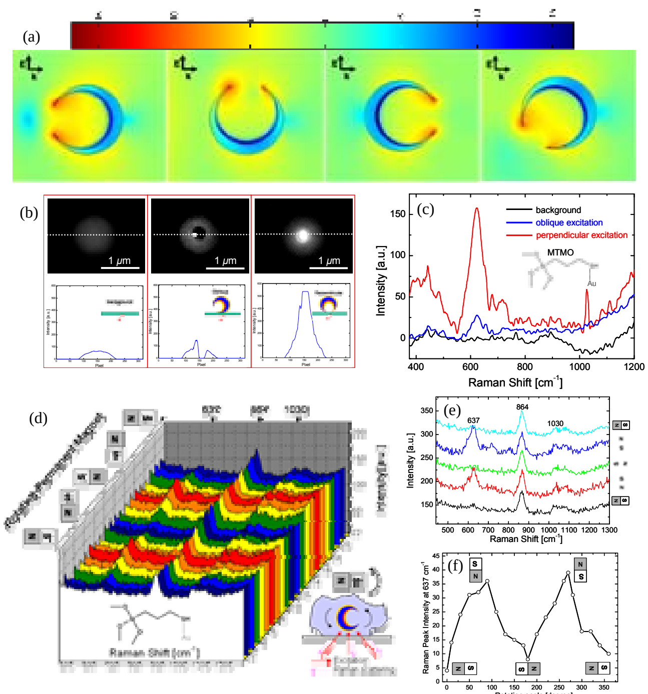
Nanocrescent SERS Probes
Multiple particles
with dispersed SERS
hot spots
SERS
Hot Spots
SERS
Weak Spots
Single
nanocrescent
with multiple
SERS hot spots
Multiple SERS
Hot Spots
vs.
Multilayered
Nanocrescent
(c)
TEM image of
nanocrescent

Magnetic Nano-Crescent SERS

G. L. Liu, Y. Lu, J. Kim, J. C. Doll, and L. P. Lee

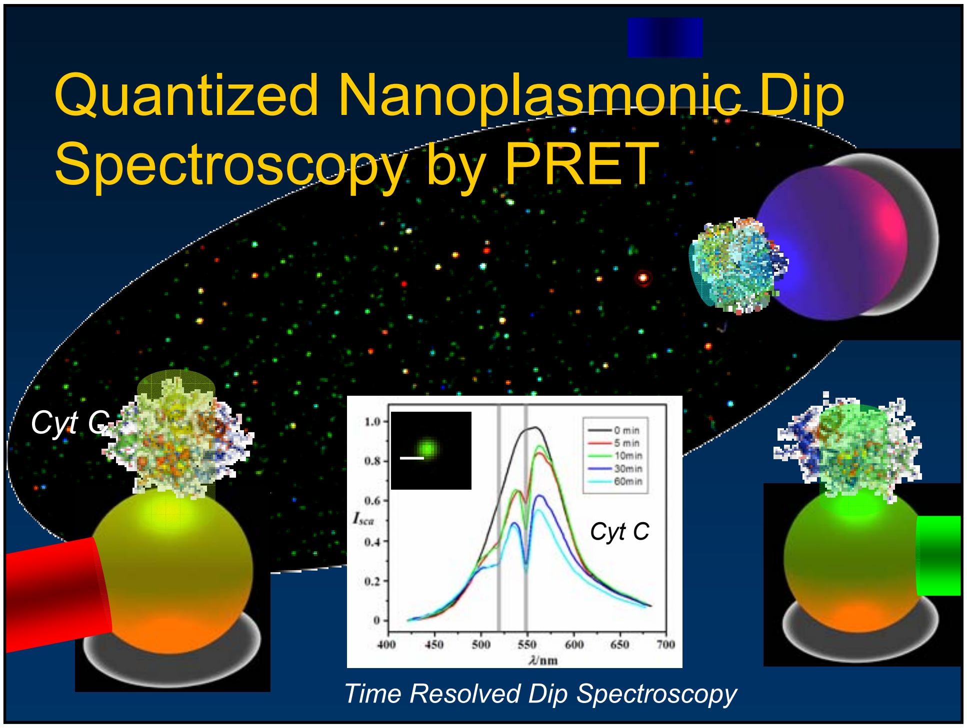
Advanced Materials (2005)

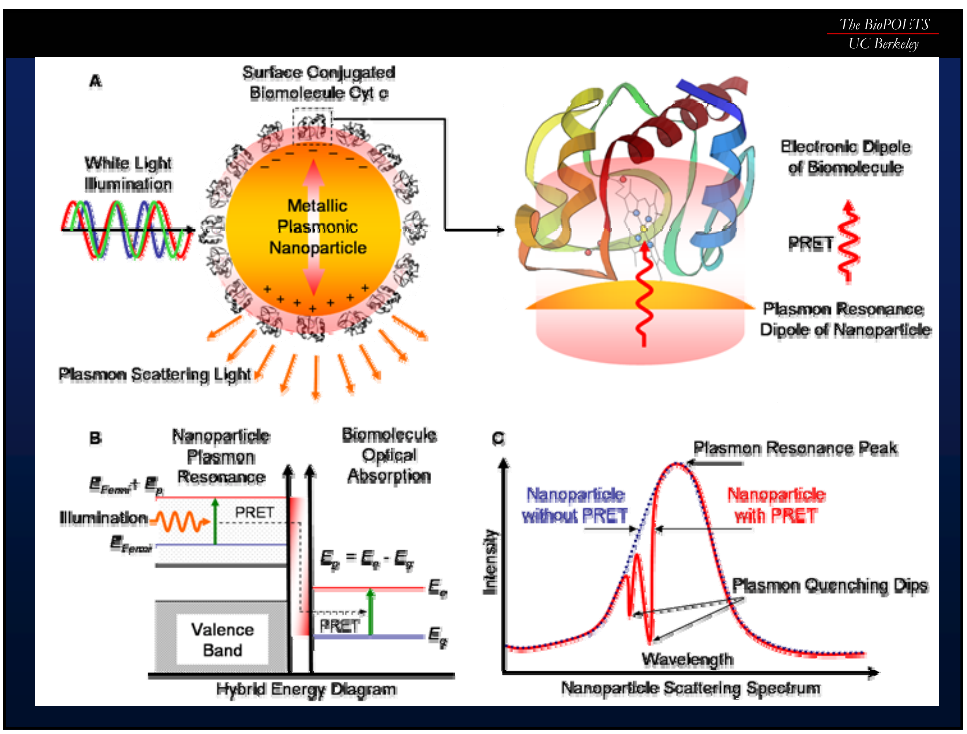


Plasmonic Resonance Energy Transfer (PRET)

Nanospectroscopic Imaging

G. Liu, Y. Long, Y. Choi, T. Kang, and L. P. Lee (*Nature Methods, 2007*)


Quantized Nanoplasmonic Dip Spectroscopy by PRET
Cyt C
Cyt C
0 min
5 min
10min
30min
60min
Time Resolved Dip Spectroscopy


The BioPOETS
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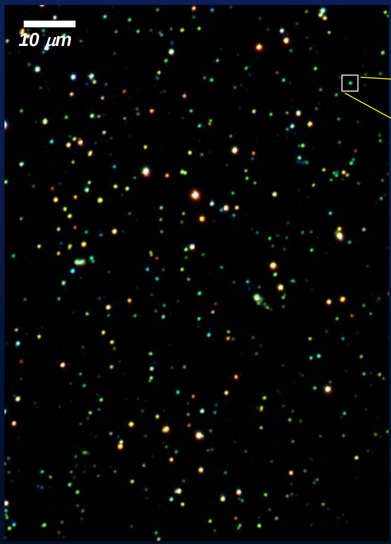
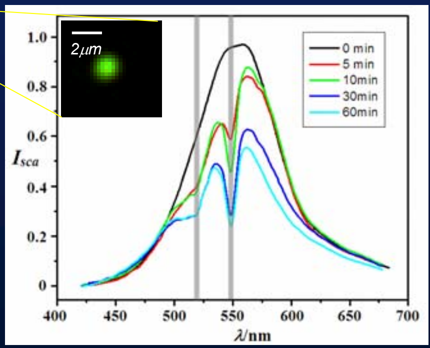
A
Surface Conjugated Biomolecule Cyt c
White Light Illumination
Metallic Plasmonic Nanoparticle
Plasmon Scattering Light
Electronic Dipole of Biomolecule
PRET
Plasmon Resonance Dipole of Nanoparticle
B
Nanoparticle Plasmon Resonance
Biomolecule Optical Absorption
Illumination
PRET
Valence Band
Hybrid Energy Diagram
C
Plasmon Resonance Peak
Nanoparticle without PRET
Nanoparticle with PRET
Plasmon Quenching Dips
Intensity
Wavelength
Nanoparticle Scattering Spectrum

Molecular Dynamics of Cyt c



Time resolved dip change



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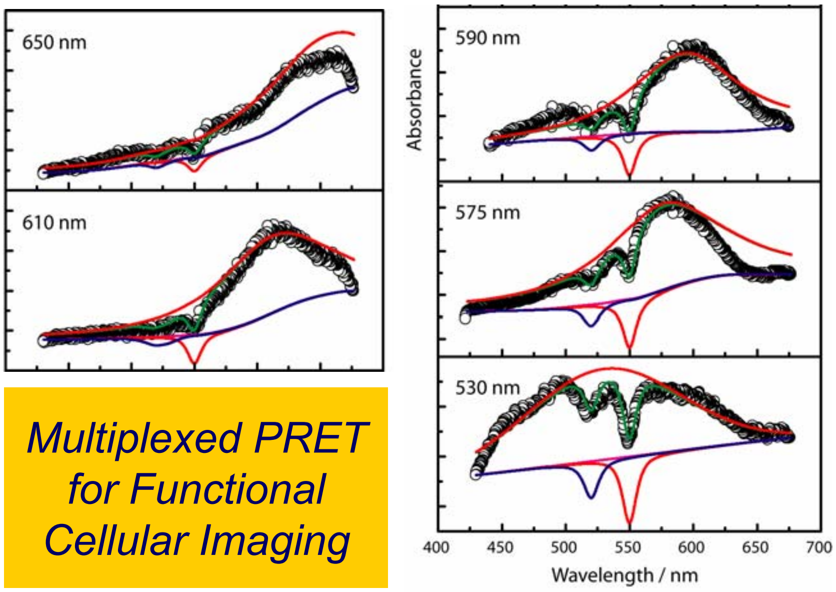
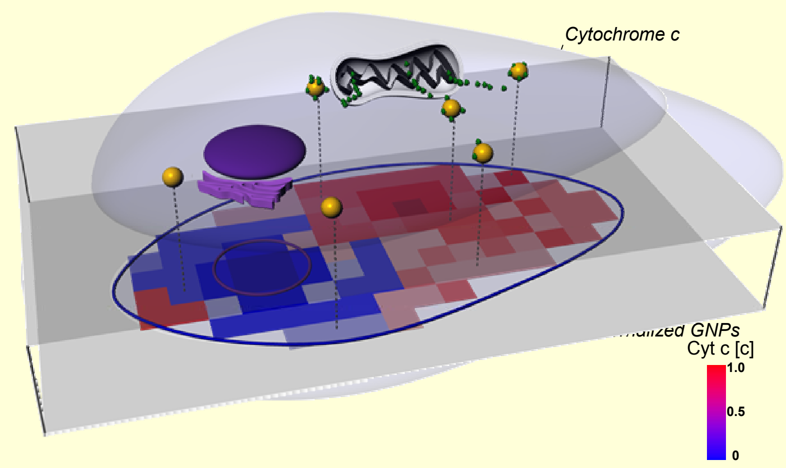


Multiplexed PRET for Functional Cellular Imaging

PRET Nanospectroscopic Imaging

Spatially Resolved in-vivo Cellular Imaging

Mapping of Cellular Processing: Apoptosis Dynamics



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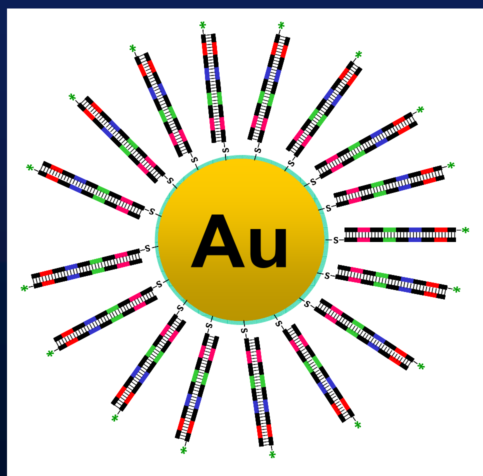
Dynamic Molecular Ruler

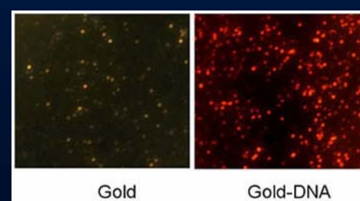
for Measuring Nuclease Activity & DNA Footprinting

G. L. Liu *et al.* (*Nature Nanotechnology, 2006)*

Mechanism of Plasmon Resonance Wavelength Shift *by Nucleolytic Reactions*

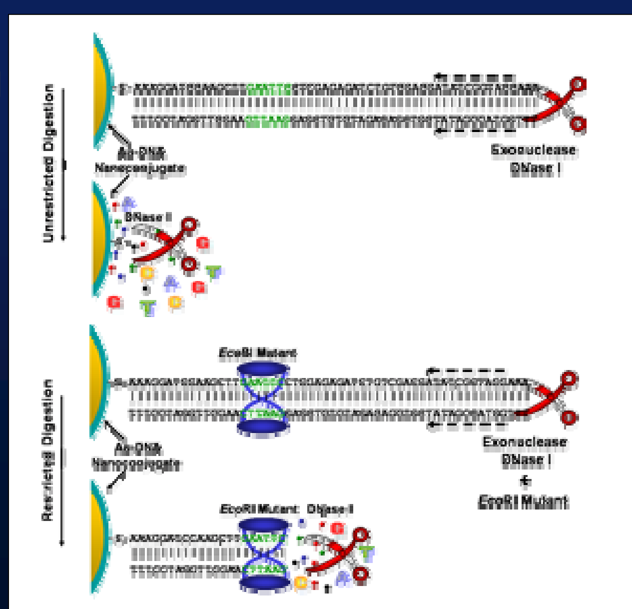


1. Effective size change after DNA digestion.
2. Dielectric constant of dsDNA is dependent on its length (Langevin model).
3. Ionic condensation condition change due to the DNA length change



DNA length ↓ ⟶ negative charges ↓ ⟶ PR Shift

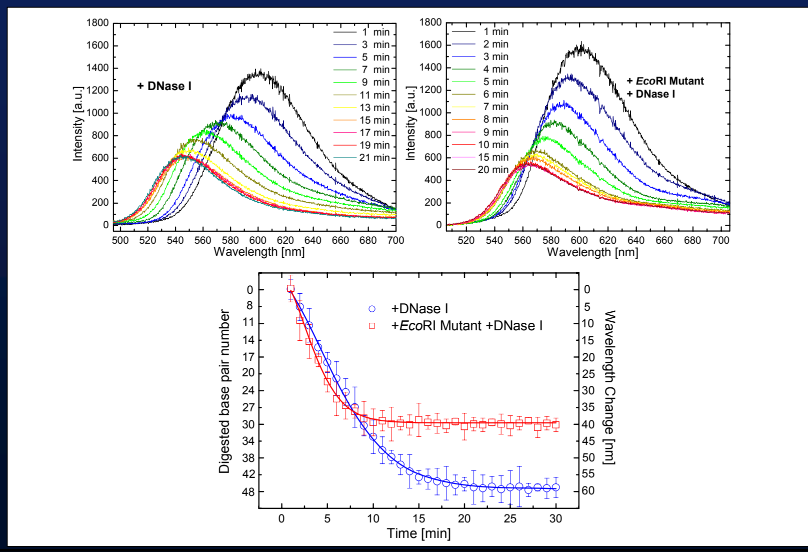
DNA Footprinting *of the Binding Position of EcoRI Mutant*



Nature Nanotechnology (2006)

Plasmon Resonance Detection

of EcoRI Mutant Position on the dsDNA



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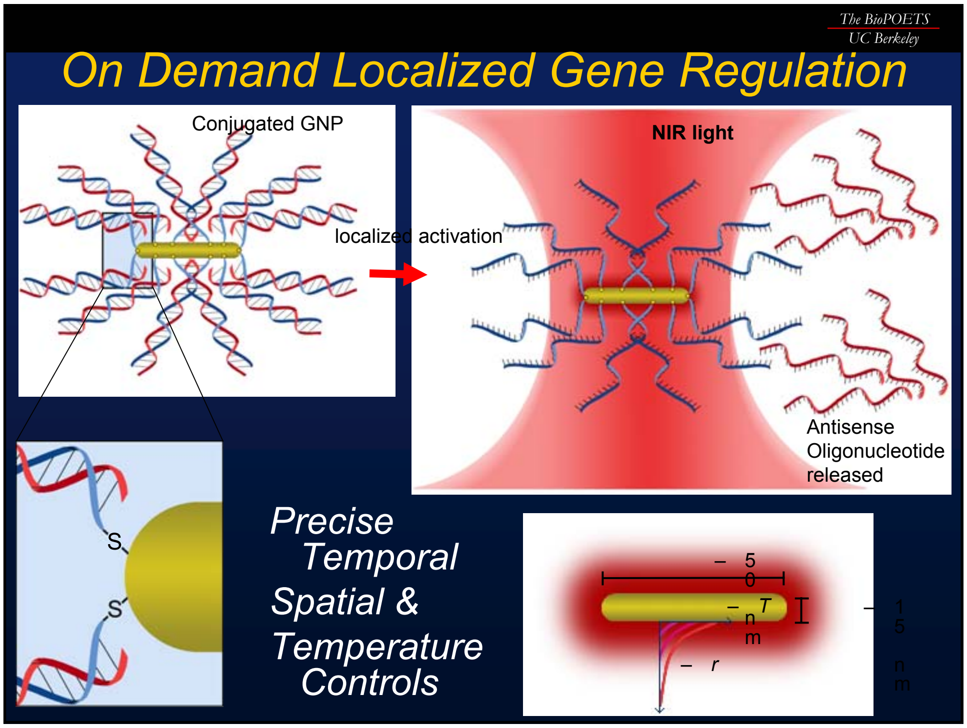
Oligonucleotides on a Nanoplasmonic Carrier Optical Switch (ONCOS)

Precise Temporal and Spatial Controls of Localized Gene Regulation and Protein Translation

Eunice S. Lee, Gang Liu, Franklin Kim, Yitao Long, and Luke P. Lee
(unpublished)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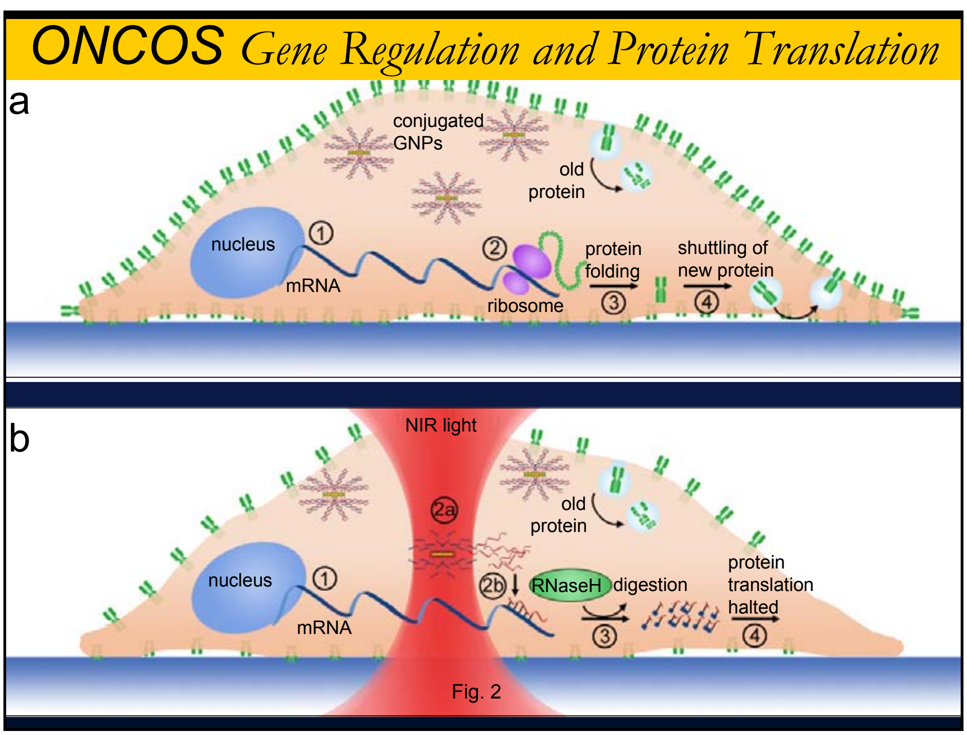
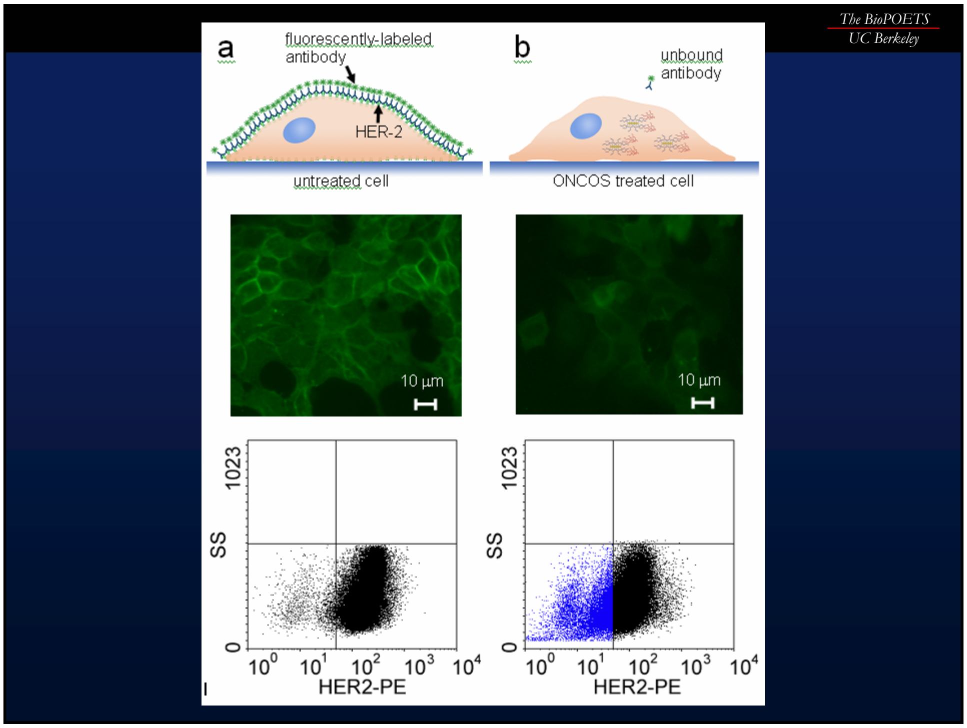
On Demand Localized Gene Regulation
Conjugated GNP
localized activation
NIR light
S
S
Antisense Oligonucleotide released
Precise
Temporal
Spatial &
Temperature
Controls


ONCOS Gene Regulation and Protein Translation
a
conjugated GNPs
old protein
nucleus
mRNA
ribosome
protein folding
shuttling of new protein
b
NIR light
old protein
nucleus
mRNA
RNaseH
digestion
protein translation halted
Fig. 2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a
fluorescently-labeled antibody
HER-2
untreated cell
b
unbound antibody
ONCOS treated cell
10 μm
10 μm
SS
1023
0
10^0 10^1 10^2 10^3 10^4
HER2-PE
SS
1023
0
10^0 10^1 10^2 10^3 10^4
HER2-PE


Time to Study the Inner Life
via Quantum Nanoplasmonics: Nanospectroscopic Imaging

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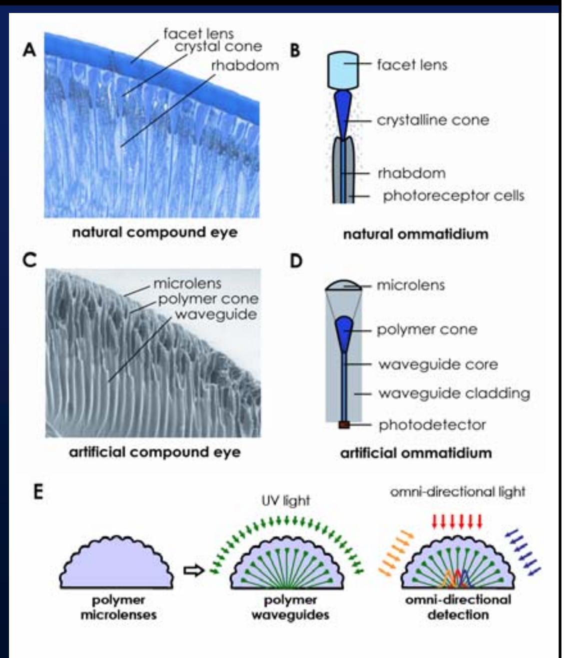
Biologically-inspired Optical Systems

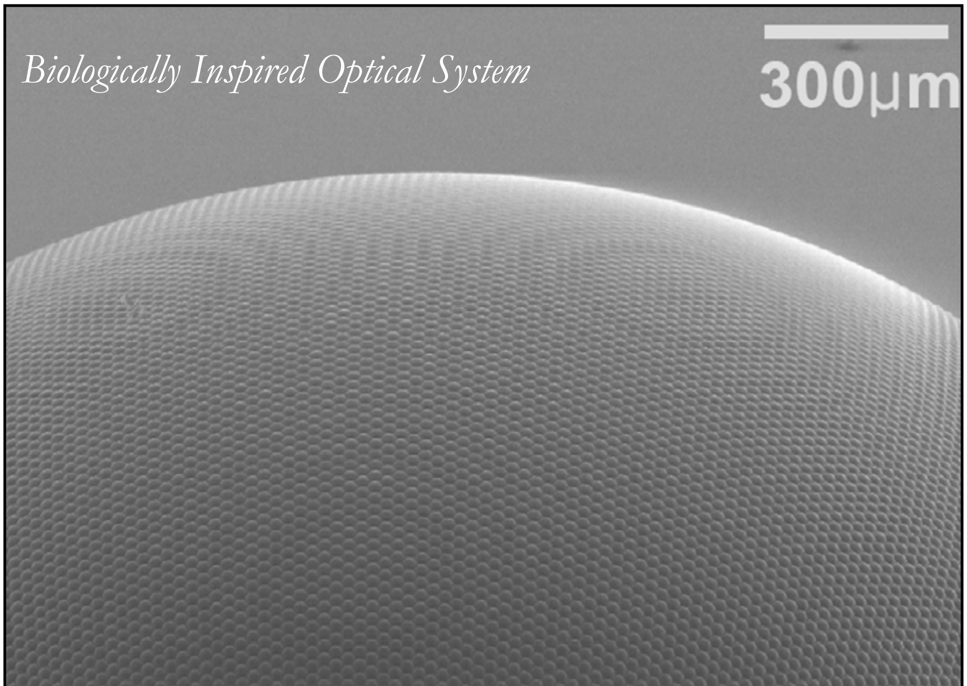
for Biological microprocesor controls, automations, and imaging

Learn from Nature: *Watch out carefully*

Artificial Compound Eye

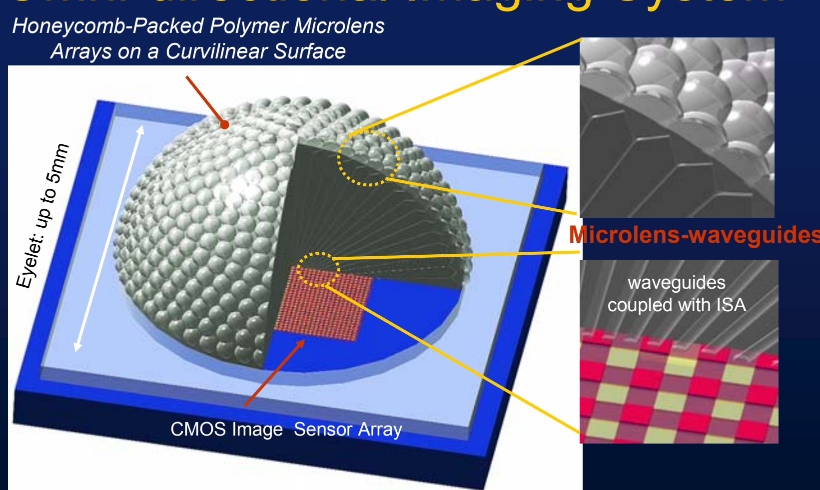
Jeong *et al*, "Biologically Inspired Artificial Compound Eyes," *Science*, 312, 557-561 (2006)





Ki-Hun Jeong, "Biologically Inspired Artificial Compound Eyes," *Science, vol.* 312, p. 557-561 (2006)

Omni-directional Imaging System



Potential applications:
Handheld miniaturized Imaging System for wide field-of-view, Endoscopic imaging system, Omni-directional sensor array for surveillance detection.

Biologically-inspired Fluidic ICs

for Quantitative Cell Biology and Quantitative Medicine on Chip

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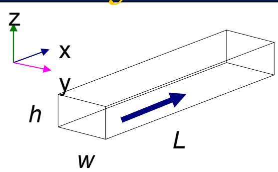
Cellular BASICs*

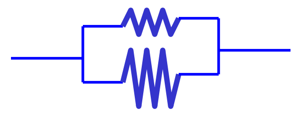
*Biological Application Specific Integrated Circuits

BASIC #1 Integrated Microfluidic Patch-clamp Array Chip
BASIC #2 Single Cell Electroporation Chip
BASIC #3 High-density Single Cell Analysis Chip
BASIC #4 Dynamic Cell Culture Chip for Systems Biology
BASIC #5 Cell-cell Communication Chip
BASIC #6 Cell Lysing Devices for Sample Preparation
BASIC #7 Biomimetic Cell Sorting Microfluidic Devices
BASIC #8 Micro PALM for Cell Manipulations
BASIC #9 Integrated Cell Culture & Lysing & Harvesting
BASIC #10 Biomimetic Artificial Livers on a Chip
BASIC #11 Biofluidic Self-assembly of Spheroids on a Chip

http://biopoets.berkeley.edu

Basics of BASICs: Fluidic Resistance





Pressure Driven Navier-Stokes

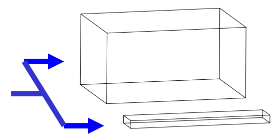
$$\rho\left(d_t v + (v \bullet \nabla) v\right) = -\nabla P + \eta \nabla^2 v$$

$\nabla P = \eta \nabla^2 v$ Time, translation invarience at low Re

$$v = f(y, z, h, w, \Delta P, \eta, \rho)$$

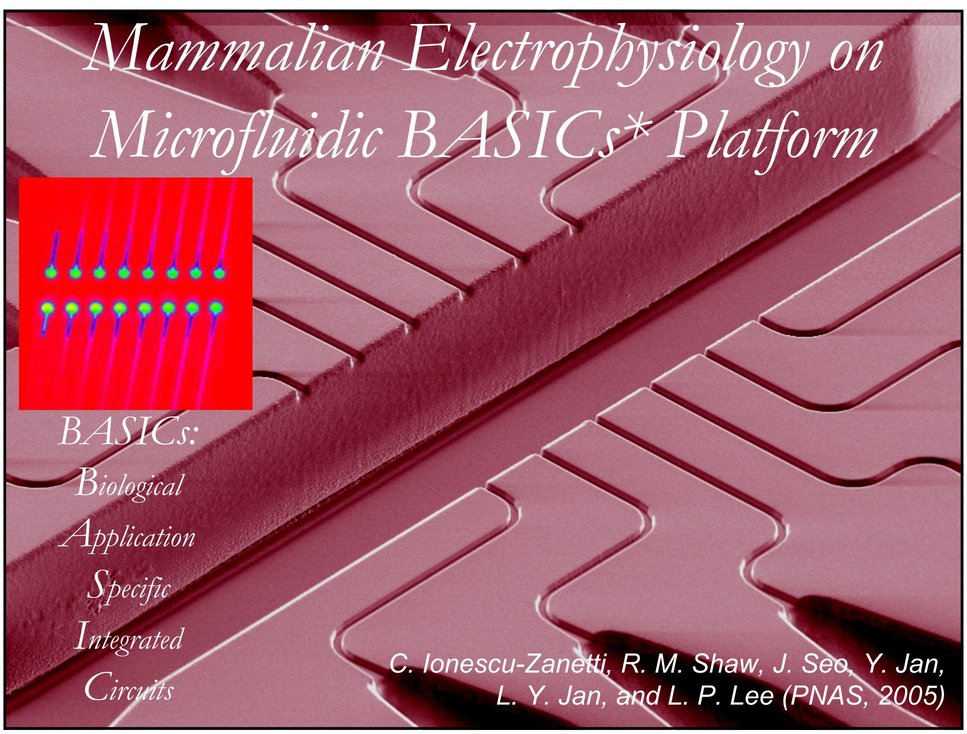
$$Q = f(L, h, w, \Delta P, \eta, \rho) = \frac{\Delta P}{R}$$

$$R_{rect} \cong \frac{12\eta L}{1 - 0.63(h/w)} \frac{1}{h^3 w}$$



Cross Section	*R/L* *(Pa s/m³)*
2x5 µm	$4.0*10^{20}$
50x50 µm	$7.5*10^{14}$

$R_1/R_2 = 5.4*10^5$



BASIC #3

High-density Single Cell Analysis Chip

http://biopoems.berkeley.edu

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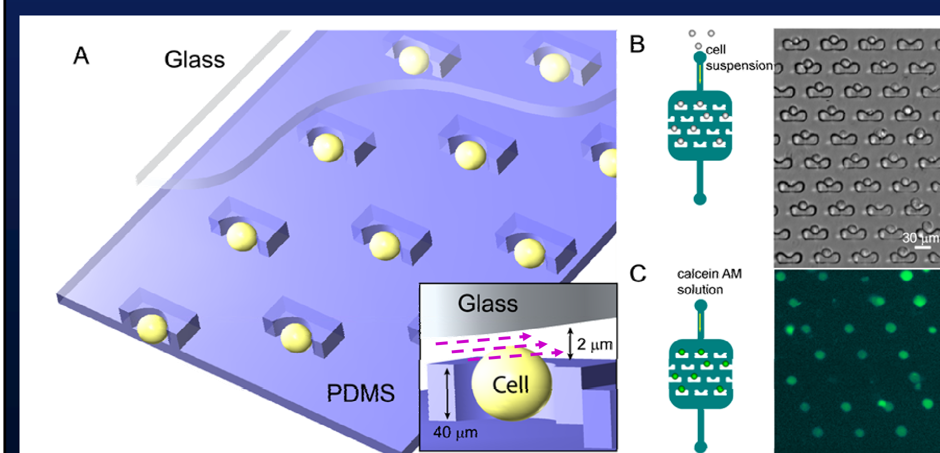
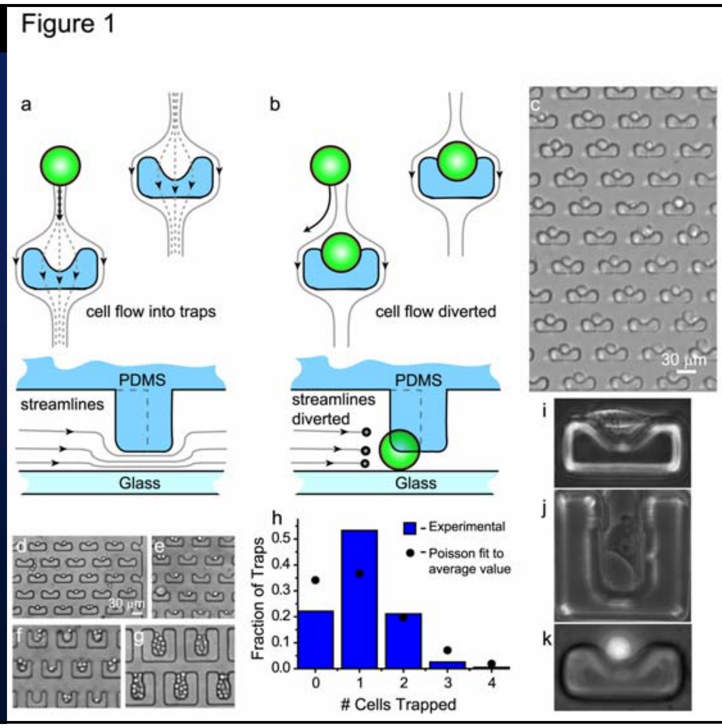
BASIC#3: High density Single Cell Array via *Hydrodynamic Single Cell Tweezers*





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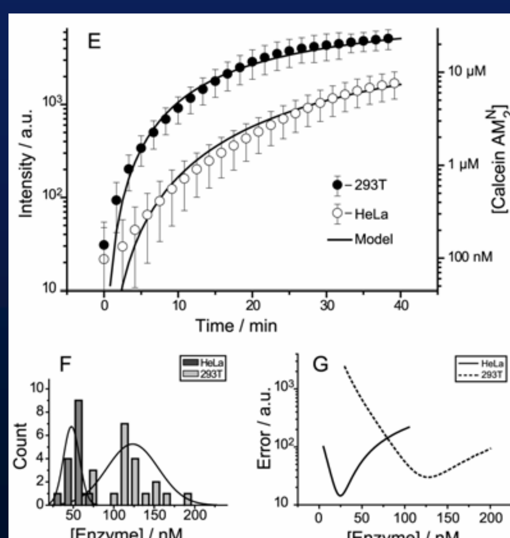
Carboxylesterase Kinetics

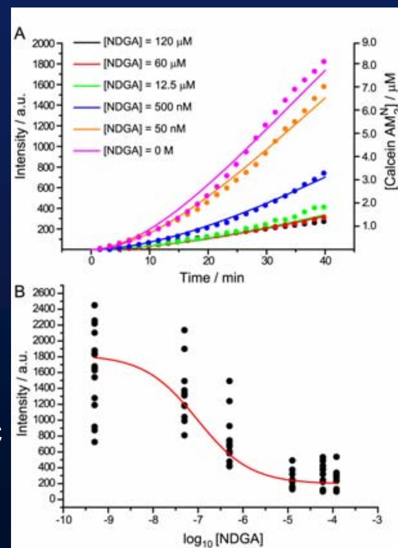
- Probed with Calcein AM – fluorogenic substrate
- Two different cell types:
  - Human cervical carcinoma vs. human kidney cells
- *Key result:*
  - Statistically larger esterase activity in kidney cell line.
  - Means: 50 nM vs. 125 nM

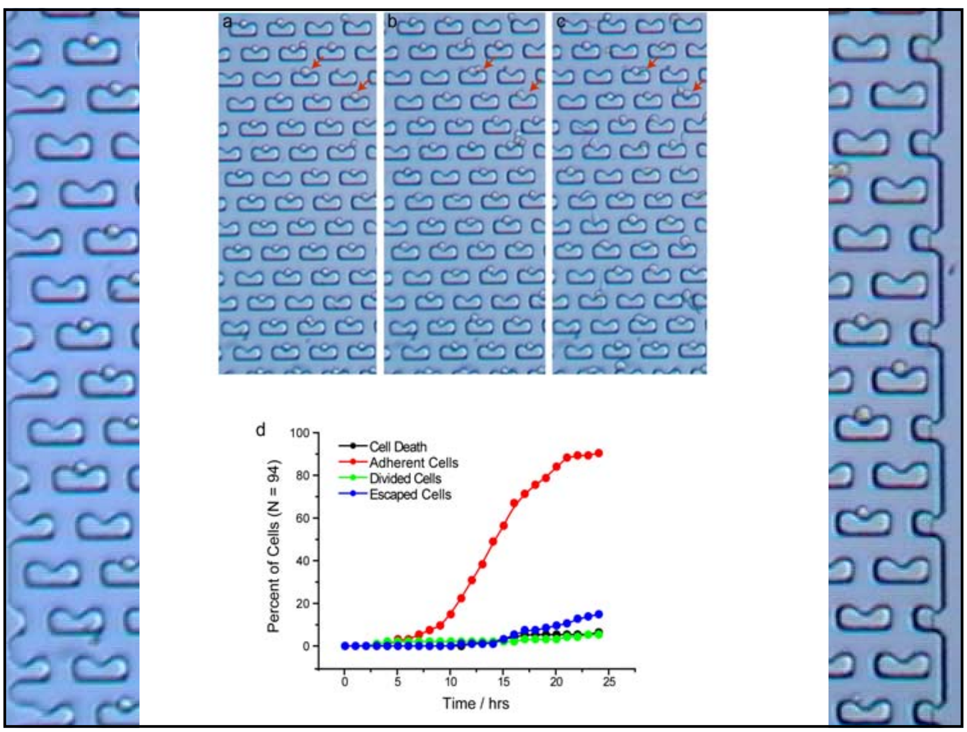


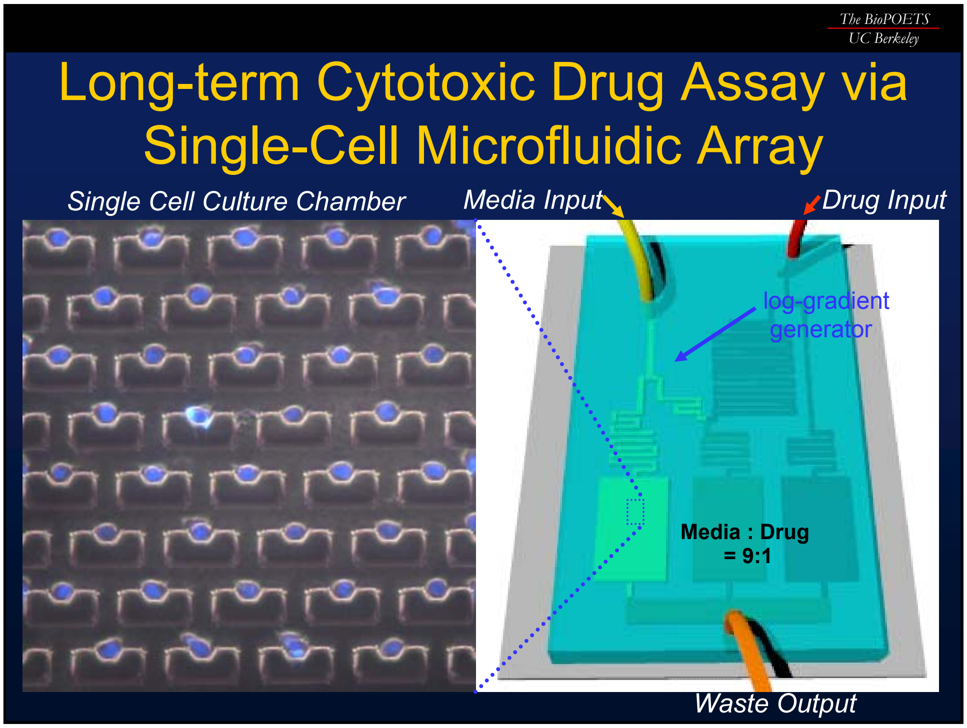
NDGA Inhibition

Key results:

- IC_{50} of 233 nM for carboxylesterase and isozyme inhibition
- 20 nM of 50 nM total activity is not inhibited by NDGA.
- *Future:* Various fluorogenic substrates having different enzyme specificity.



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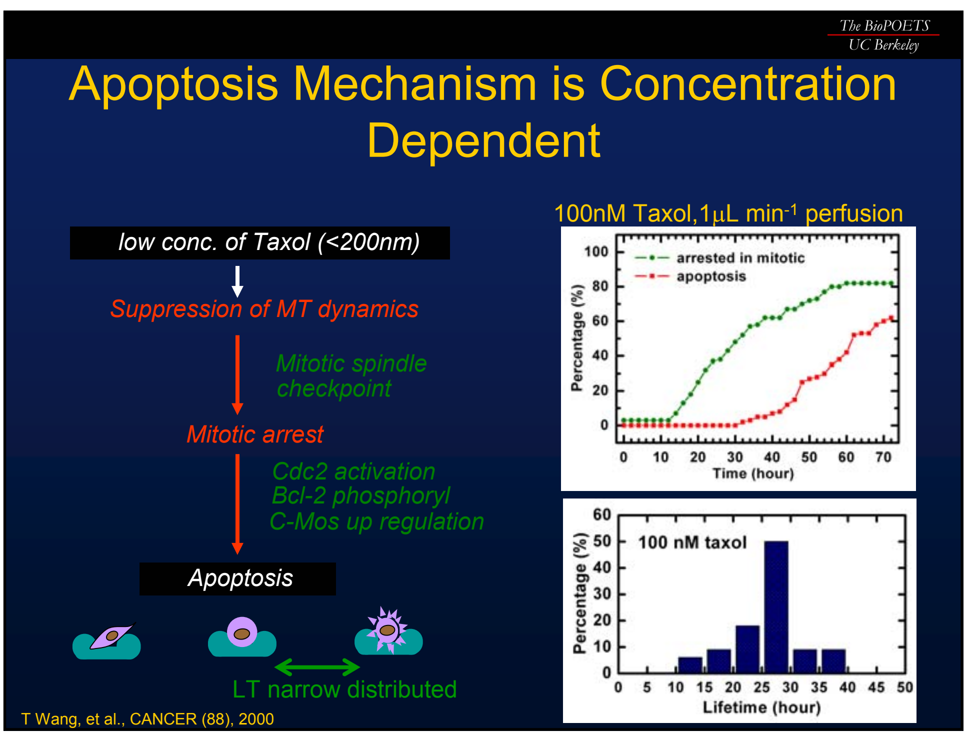
Long-term Cytotoxic Drug Assay via Single-Cell Microfluidic Array
Single Cell Culture Chamber
Media Input
Drug Input
log-gradient generator
Media : Drug = 9:1
Waste Output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Apoptosis Mechanism is Concentration Dependent
low conc. of Taxol (<200nm)
Suppression of MT dynamics
Mitotic spindle checkpoint
Mitotic arrest
Cdc2 activation
Bcl-2 phosphoryl
C-Mos up regulation
Apoptosis
LT narrow distributed
100nM Taxol,1μL min-1 perfusion
arrested in mitotic
apoptosis
Percentage (%)
Time (hour)
100 nM taxol
Lifetime (hour)
T Wang, et al., CANCER (88), 2000

Apoptosis Mechanism is Concentration Dependent

High conc. of Taxol (0.2-30μm)

↓

Massive MT damage

↙ ↘

JNK activation *Cdc2, Cdks*

↘ ↙

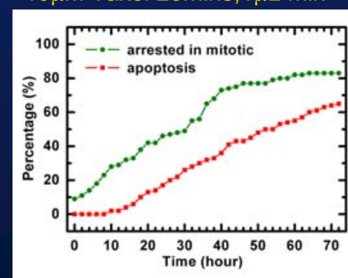
Caspase activation

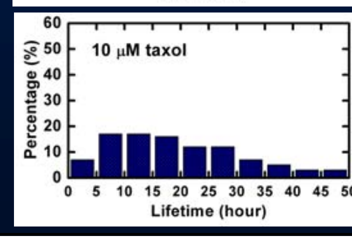
↓

Apoptosis

LT dispersed

10μM Taxol 20mins,1μL min^{-1}





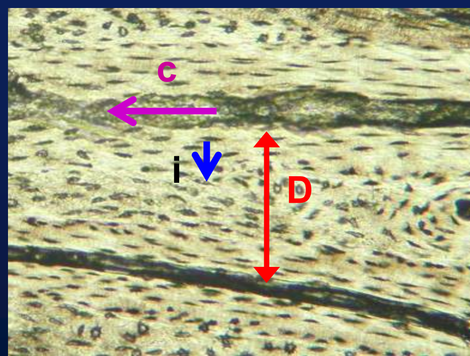
T Wang, et al., CANCER (88), 2000

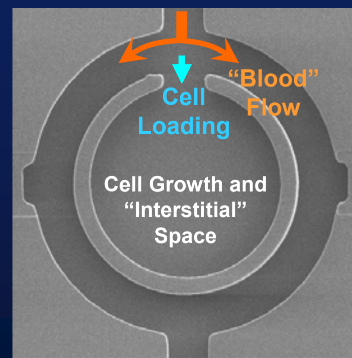
Cultural Revolutions

for Physiologically Relevant Cell Culture Array

Creating Dynamic Cell Culture Systems

Biomimetic *Physiological Microenvironment*



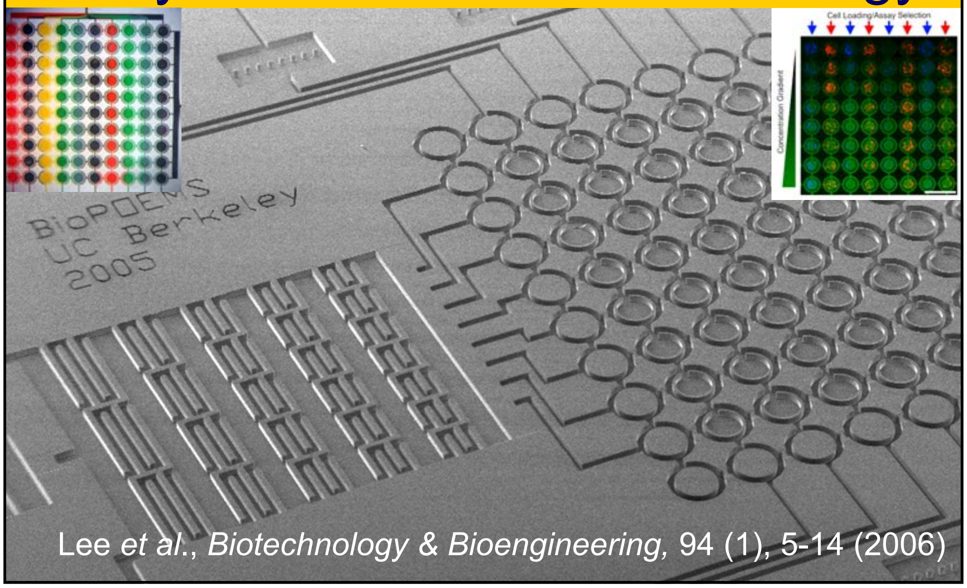


	Tissue	Microfluidic
Size (D)	100-300 µm	50-1000 µm
Circulatory Flow (c)	700 µm/s	80-4,000 µm/s
Interstitial Flow (i)	0.1 µm/s	0.08-4 µm/s
Extracellular Matrix	Complex	Surface Coating

Cell Culture Biotechnology

	Microtiter Plate	CSTR Bioreactor	Microfluidic Bioreactor
Volume	50 µl	2 L	3 nl
Cell Density (v/v)	5%	<10%	40-80%
Medium Turnover	3 days	0.5-3 days	2-120 sec
Throughput	1-384	1	64-1024

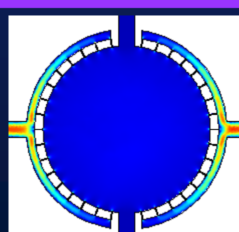
Nanoliter Scale Microbioreactor Array for Quantitative Cell Biology



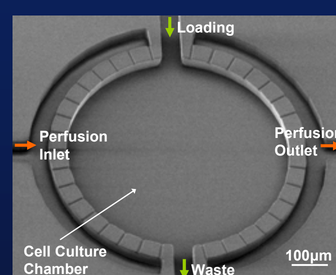
Lee *et al.*, *Biotechnology & Bioengineering,* 94 (1), 5-14 (2006)

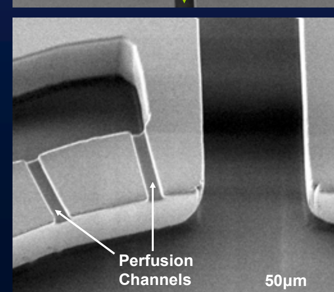
Design for Uniform Fluid Velocity Profile

High Aspect Ratio between the Perfusion Channels & Cell Culture Chamber



With different flow rates applied from the perfusion inlet, shear stresses inside the chamber can be adjusted. This would potentially be beneficial for study the responses of cells under various shear conditions.





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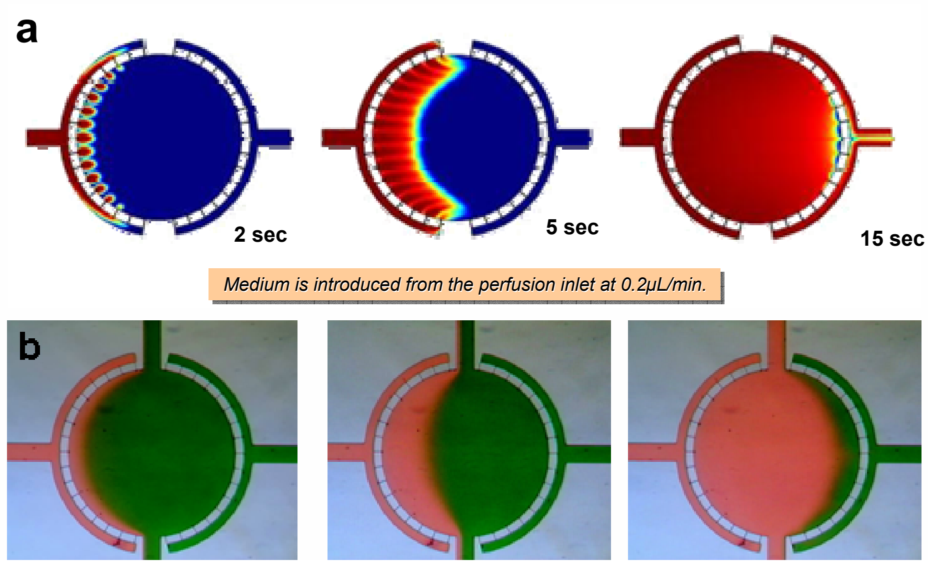
Uniform Mass Transfer



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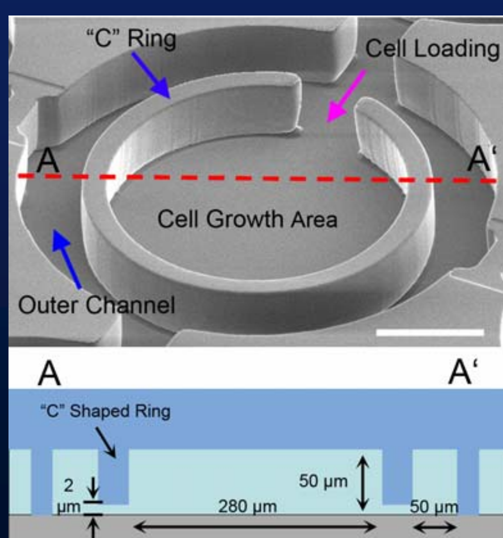
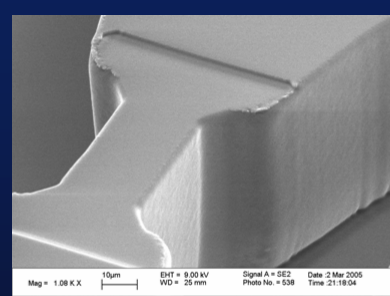
Device Fabrication

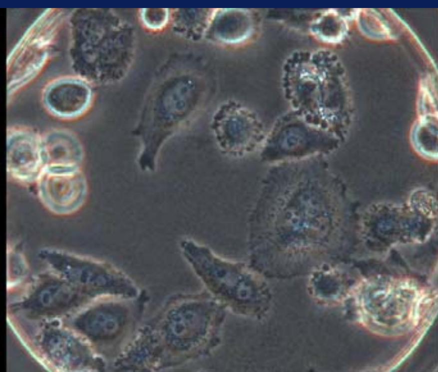




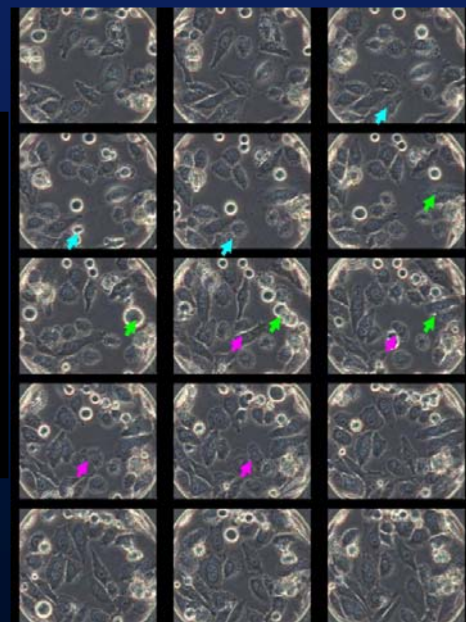
Key Features:

- Biocompatible
- Rapid Processing Time
- 1.5-250 μm PR Available
- Optically Transparent

Cell Growth



Cell Growth (HeLa, 30 min/frame, 38 hours)



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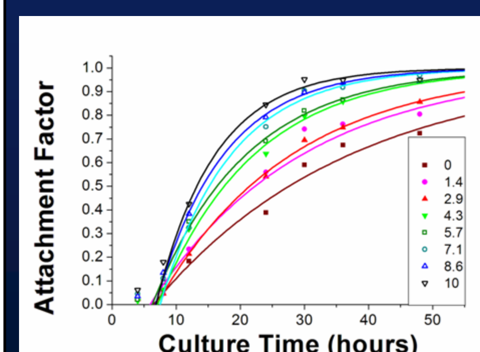
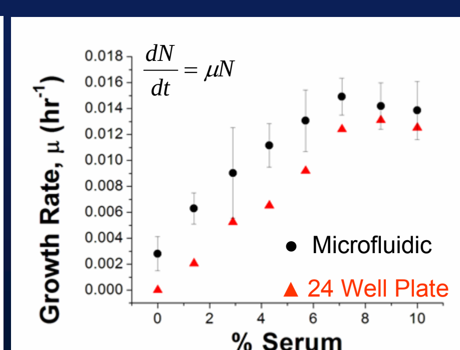
Quantitative Data



Cell Attachment Kinetics



Cell Growth Rate

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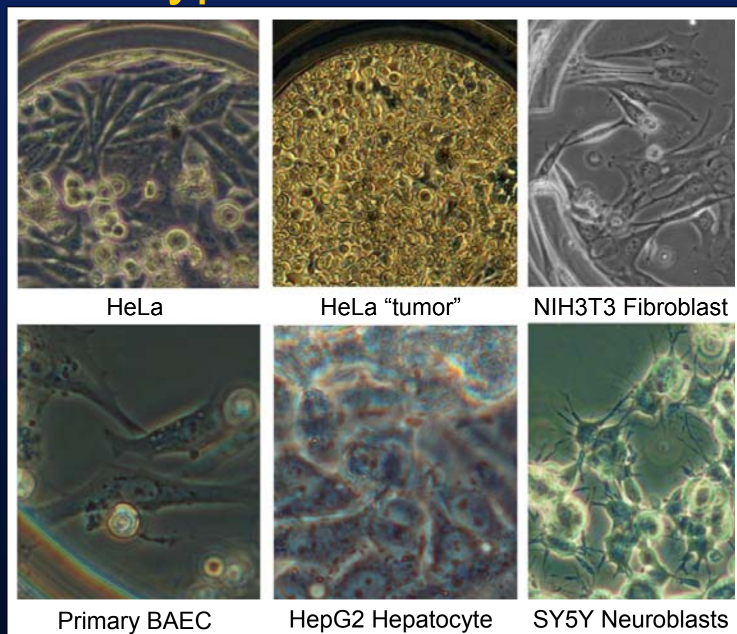
Cell Types Cultured



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Physiologically-inspired Artificial Liver Sinusoids

Lee et al., Biotechnology and Bioengineering 97, 1340 (2007)

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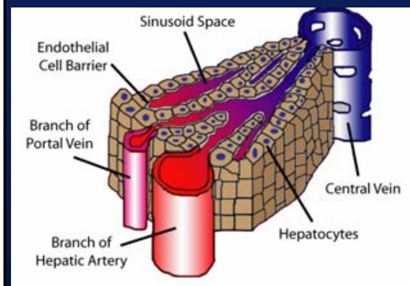
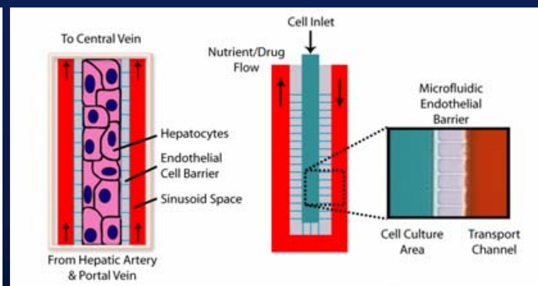
Liver Micro-architecture





- Sinusoid space transports blood to hepatocytes
- Lined with fenestrated endothelial barrier
- Hepatocytes form extensive cell-cell contact

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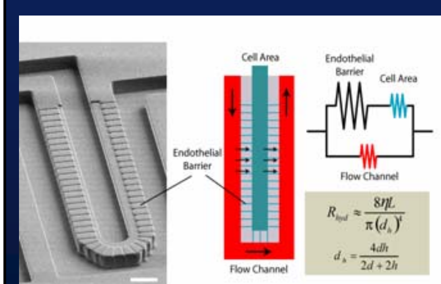
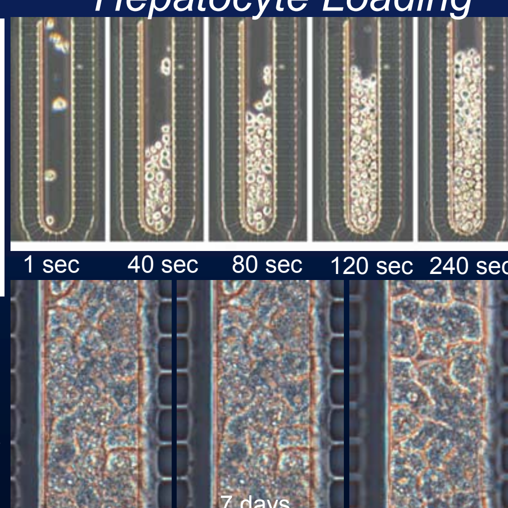
Microfluidic Artificial Liver Sinusoid

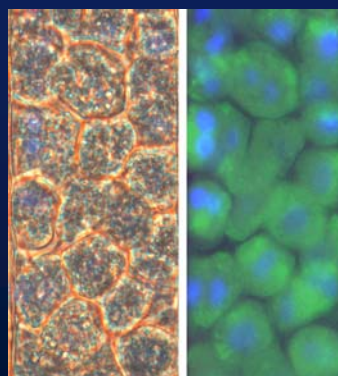
Hepatocyte Loading



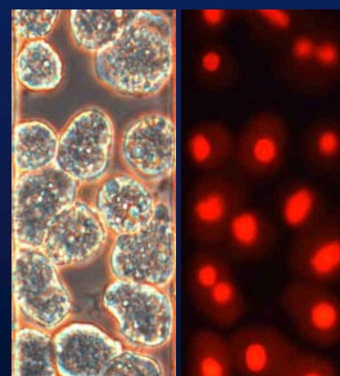


- Microfluidic endothelial barrier
- High density hepatocyte culture
- Continuous flow mass transport

Effect of Hepatocyte Density



High density = happy cells



Low density = dead cells

- Microfluidic culture without ECM coating
- “Spheroid” effect previously documented

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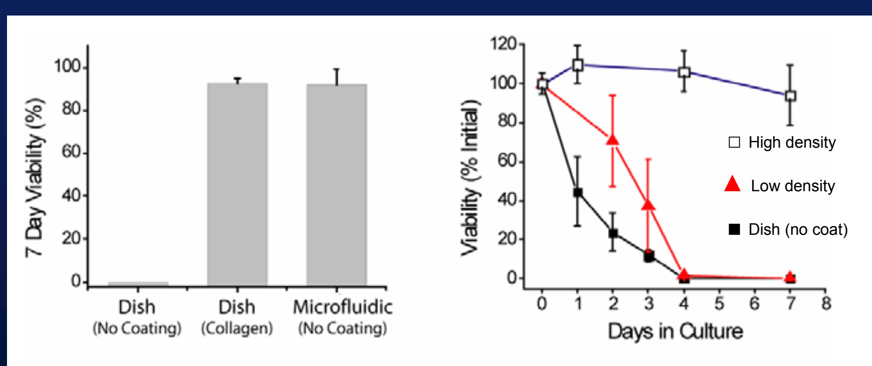
Quantitative Results



- Collagen coating required for dish based hepatocyte culture
- High density loading can rescue viability in absence of ECM coating

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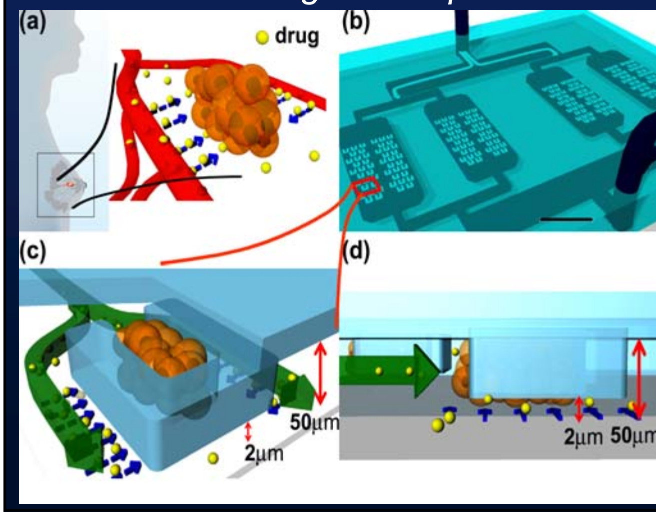
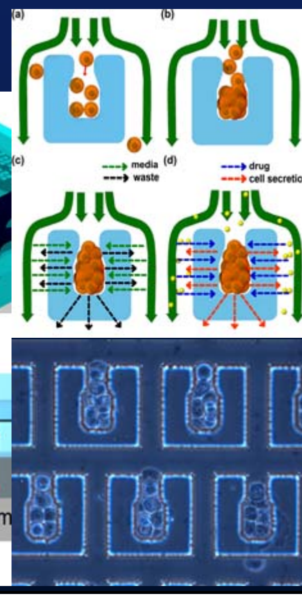
Microfluidic Assembly of Spheroids

"Tumor factory" to Accelerate Cancer Drug Development





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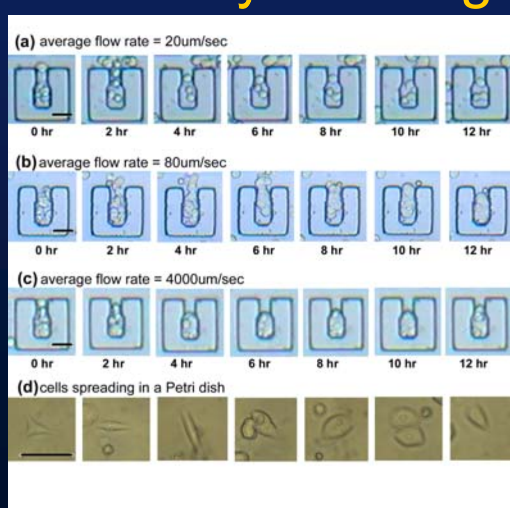
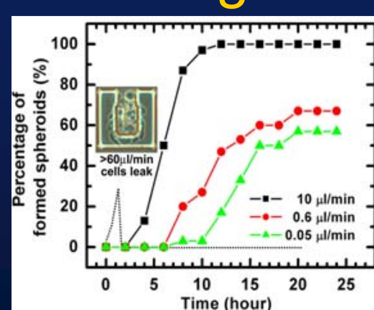
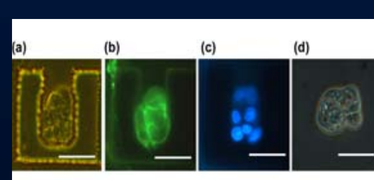
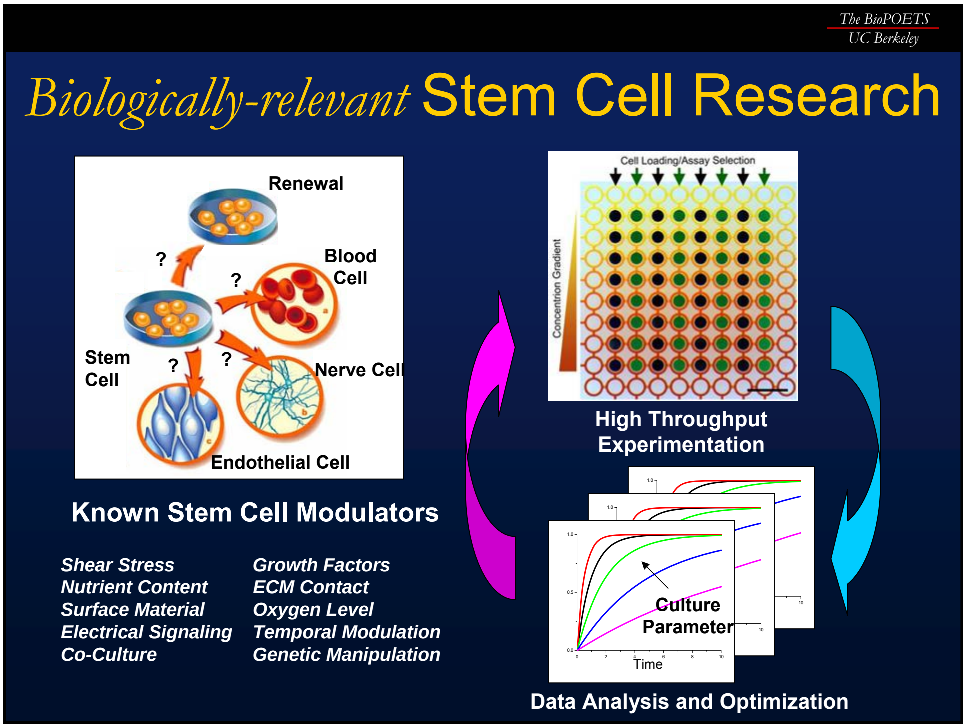
Effects of Flow Rate in Spheroids Assembly for Drug Screening





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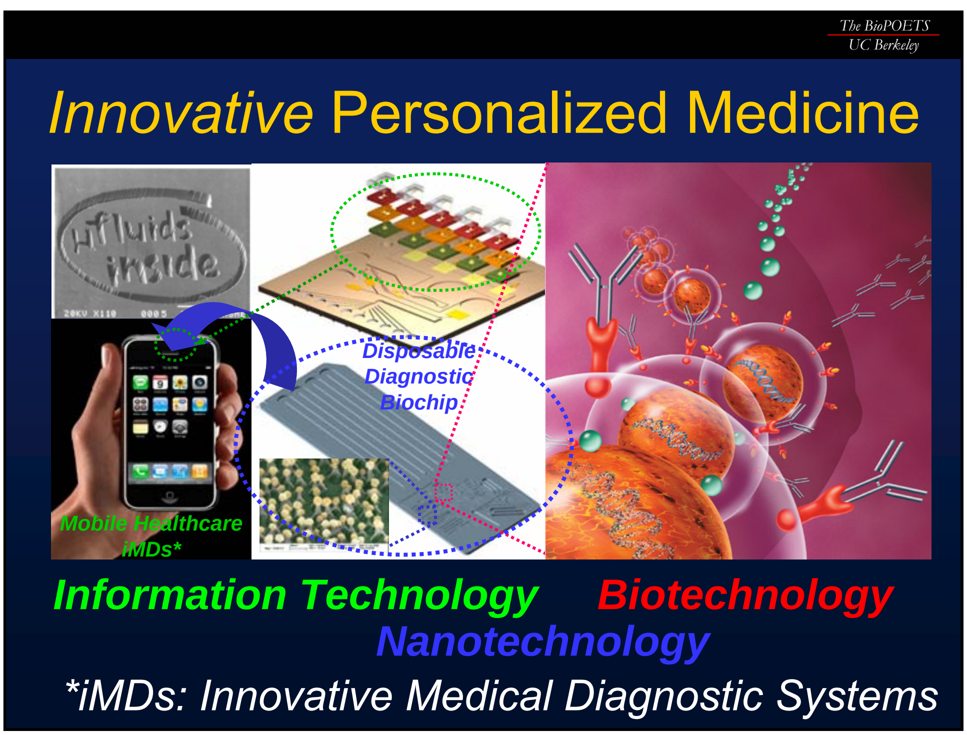
Biologically-relevant Stem Cell Research
Renewal
Blood Cell
Stem Cell
Nerve Cell
Endothelial Cell
?
?
?
?
Known Stem Cell Modulators
Shear Stress
Nutrient Content
Surface Material
Electrical Signaling
Co-Culture
Growth Factors
ECM Contact
Oxygen Level
Temporal Modulation
Genetic Manipulation
Cell Loading/Assay Selection
Concentration Gradient
High Throughput Experimentation
Culture Parameter
Time
Data Analysis and Optimization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Innovative Personalized Medicine
μfluids inside
Disposable Diagnostic Biochip
Mobile Healthcare iMDs*
Information Technology
Biotechnology
Nanotechnology
*iMDs: Innovative Medical Diagnostic Systems

Summary

- Biologically-inspired photonics and optical systems are being developed for innovative healthcare systems.
- Cellular BioASICs are being developed for quantitative biology & medicine.
- Quantum nanoplasmonic molecular probes, molecular ruler, ONCOS (gene regulator & protein expression controller) are developed for molecular/cellular imaging, and quantitative *in vivo* biology.
- High-content Integrated Quantitative Molecular Diagnostic (iQMD) system can be created for future preventive, personalized medicine, and integrated health & environmental monitoring systems.