Life-on-a-Chip

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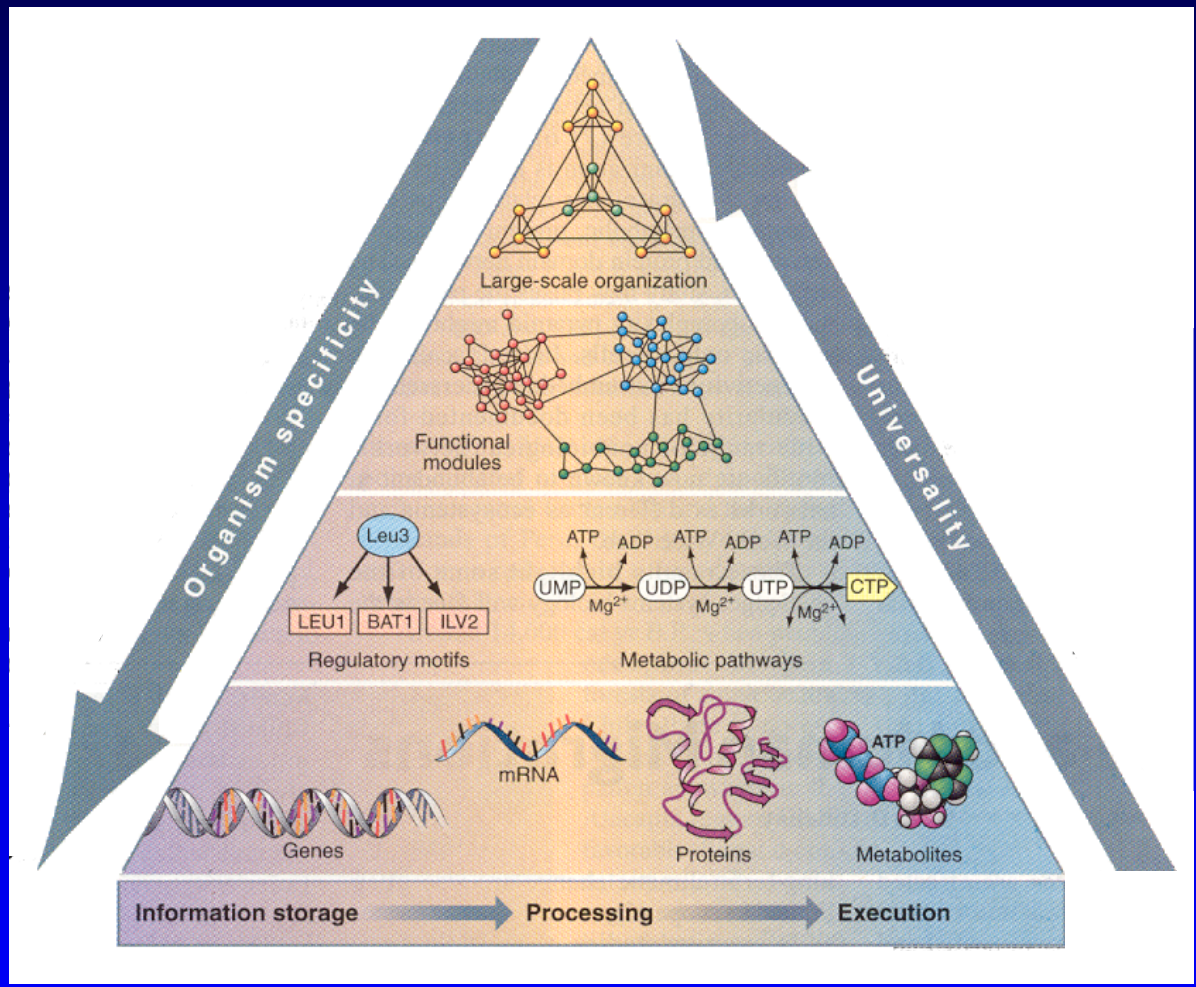
University of Washington

NAE Regional Symposium

March 13, 2003

Life's Complexity Pyramid

Comprehend complexity, versatility & robustness of living systems?

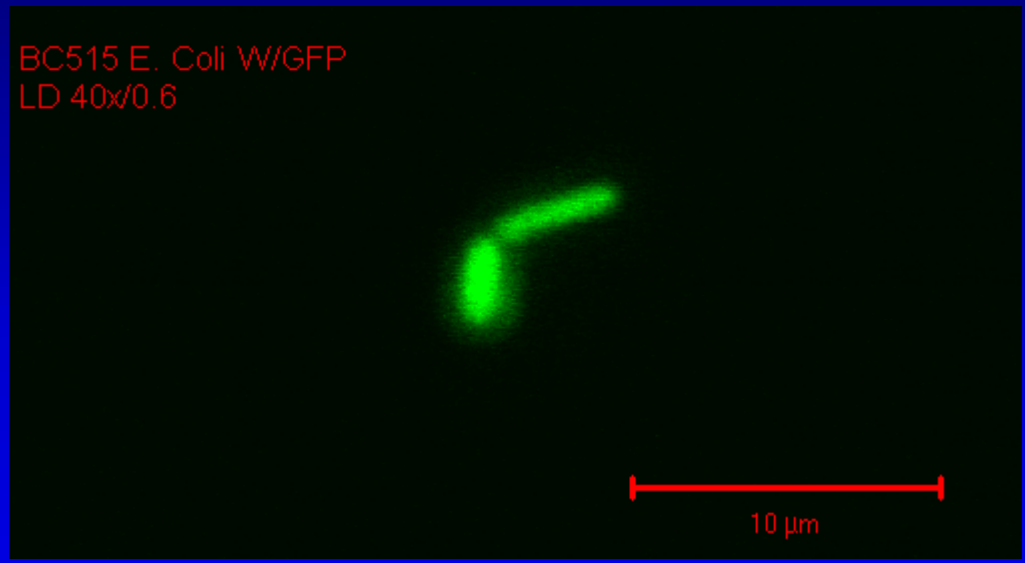


[Z. Oltvai & A-L Barabási 2002 Science 298, 763-764.)]

A single cell…

To understand how a system functions, we must first examine how individual components dynamically interact during operation:

- What is the voltage on each signal line?
- How are the signals encoded?
- How can we stabilize the voltage against noise and external fluctuations?
- How do the circuits react when a malfunction occurs in the system?
- What are the design principles and possible circuit patterns?
- How can we modify them to improve system performance?



[H. Kitano (2002) Science 295:1662-1664.]

CEGS
Centers of Excellence in Genomic Science

- New Centers funded by NIH NHGRI
- Microscale Life Sciences Center (MLSC) CEGS at University of Washington

- Started August 2001
- Focus on microscale and life-on-a-chip studies in:
  - Proteomics
  - Metabolic Networks
  - Cancer biology
  - Viral pathogenesis
  - Bacterial pathogenesis

MLSC

Application needs dri… Foundation technologies innovation in mod… pport system design and system leve… and integration development activities

Outline

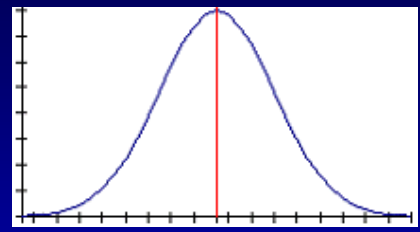
- Goals of MLSC
- Organization
- Motivation and Application Areas
  - Technology Development

MLSC Goals

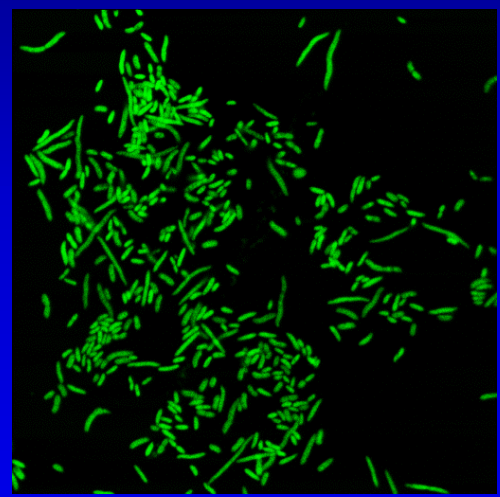
- **Functional genomics, a cell at a time**
  - Δ **Enabling technology**
  - Δ **Fundamental applications**
- **Measure multiple parameters in individual living cells in real-time to correlate cellular events with genomic information.**
  - Δ **Develop microsystems for analyzing complex cellular processes.**
  - Δ **Apply these systems to specific biological problems:**
    - – **Proteomics**
    - – **Metabolic networks**
    - – **Cancer biology**
    - – **Viral pathogenesis**
    - – **Bacterial pathogenesis**

Why Individual Cells?

- **Populations provide averages**



- Heterogeneity cannot be assessed in bulk cultures

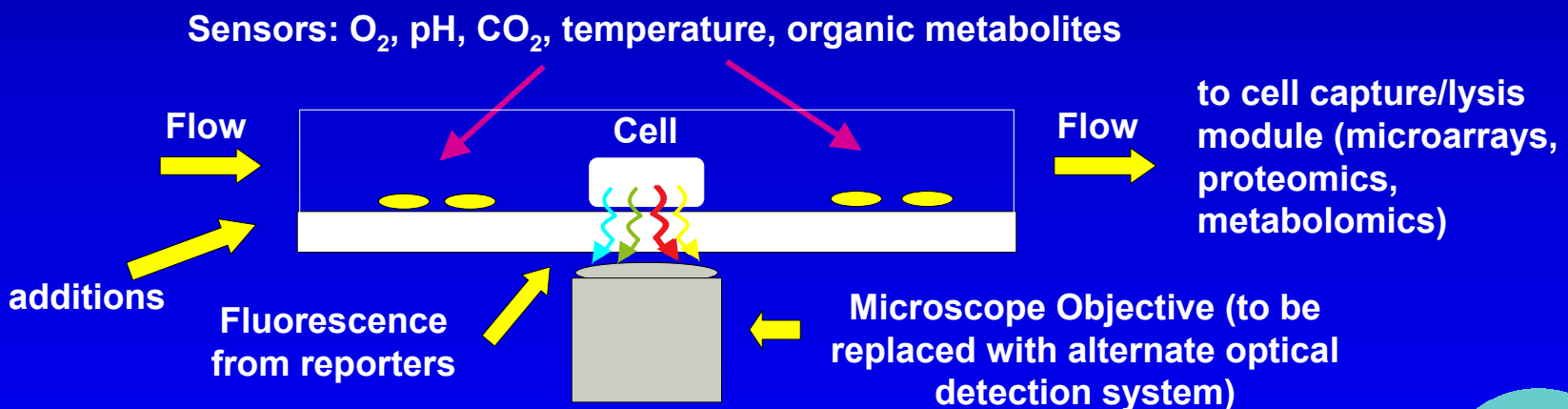


- What is biological noise?
- Is it significant in complex biological events?

Single Cell Pathway: Biological Wish List

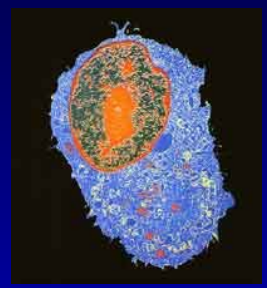
Measure complex biological events in multiple, living single cells in real time

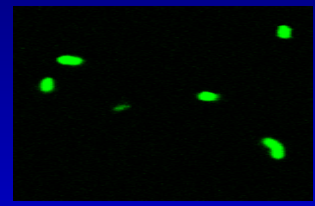
- **Monitor cell function (e.g. respiration rates)**
- **Monitor expression of central genes**
- **Measure outcomes (e.g. metabolic product, protein/receptor expression, alteration of cell physiology or morphology)**



Single Cell Pathway: Common Needs

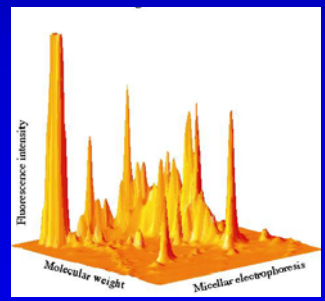
- Measure events in single cells under stressed and non-stressed conditions





- Use fluorescent reporters to monitor gene expression

- Harvest cells after data-gathering phase for single-cell proteomics



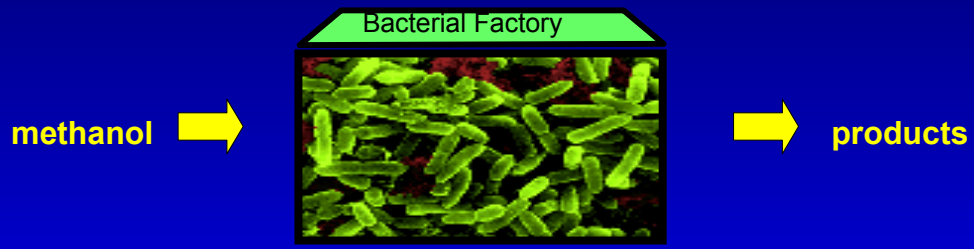
Biological Questions

- Single Cell Pathway
  - Δ Metabolic Networks
  - Δ Viral Pathogenesis
  - Δ Bacterial Pathogenesis
- Proteomics
- Cancer Biology

Metabolic Networks (Lidstrom Group)

- **Goal:** understand and manipulate central metabolism in a bacterium that grows on methanol

- **Application:** develop environmentally-benign chemical production systems



Methylobacterium extorquens AM1

- **Problem requiring single cell analysis:** metabolic heterogeneity

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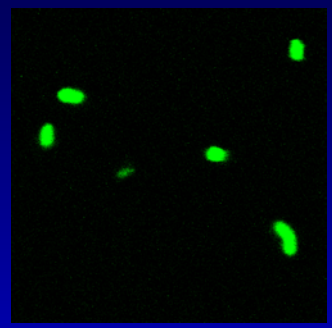
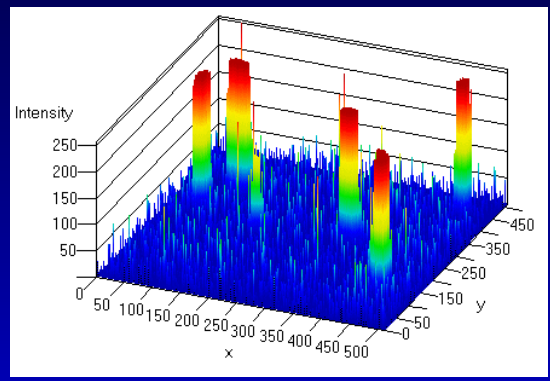
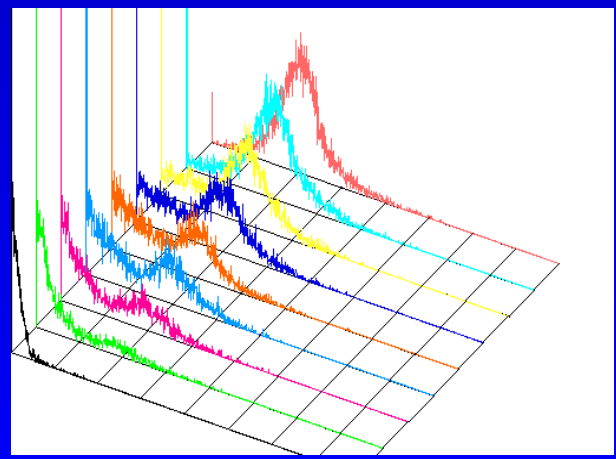
Accomplishments: Metabolic Networks

- Fusion constructs made to GFP and YFP



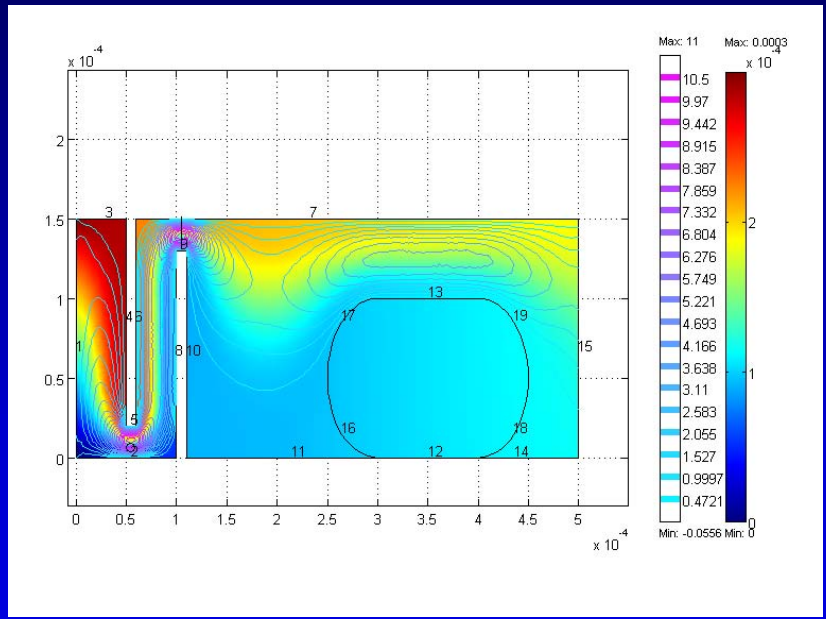


- Populations analyzed by Fluorescence-Activated Cell Sorting (FACS)



Metabolic Networks: Progress

- Cells modeled in environmental chamber with respect to nutrient consumption, mixing, and flow rate



Next step:
Analyze multiple metabolic modules in individual cells

[Kelly FitzGerald]

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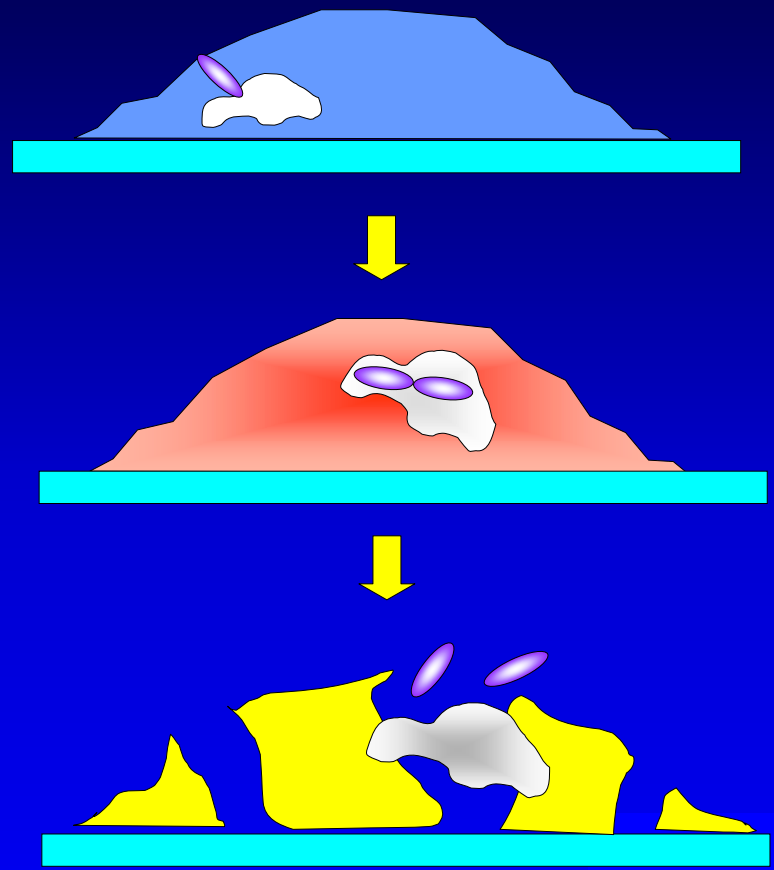
Bacterial Pathogenesis: (Cookson Group)

- **Goal:** understand interactions of *Salmonella* with host macrophages, including inflammatory cell death (pyroptosis)

- **Applications:**
  - Brain: stroke, neurodegeneration
  - Heart: myocardial infarction
  - Multiple organs: autoimmune diseases
  - Infections, inflammatory processes

- **Problem requiring single cell analysis:**
  Heterogeneity in infected macrophages



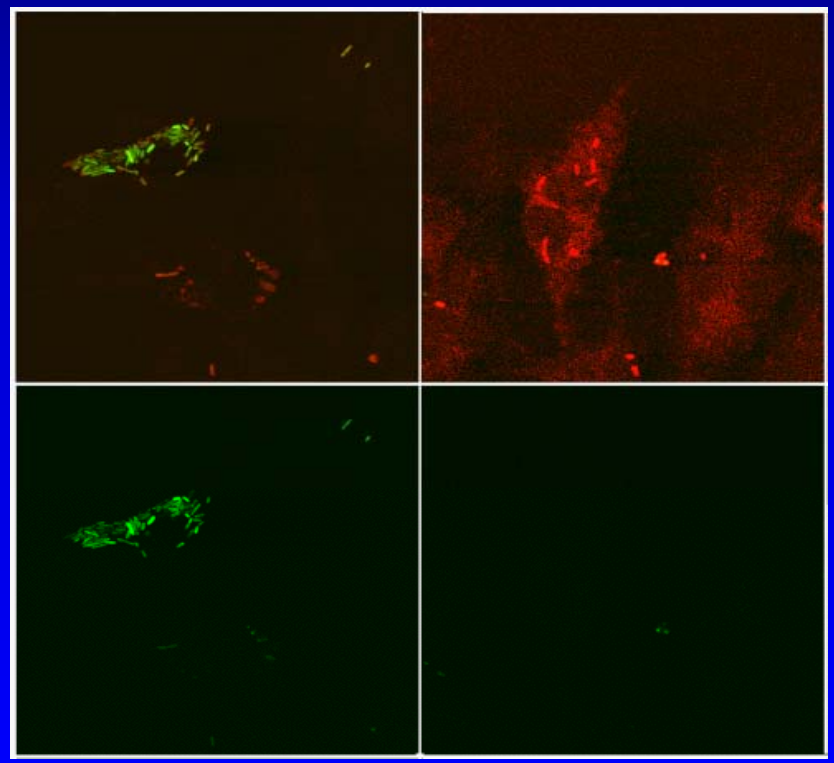
Accomplishments: Bacterial Pathogenesis

Gene expression inside macrophages: *fliC* is not expressed, contrary to results from bulk experiments

Promoter driving GFP: constitutive *fliC*

GFP + Texas Red signal

GFP signal



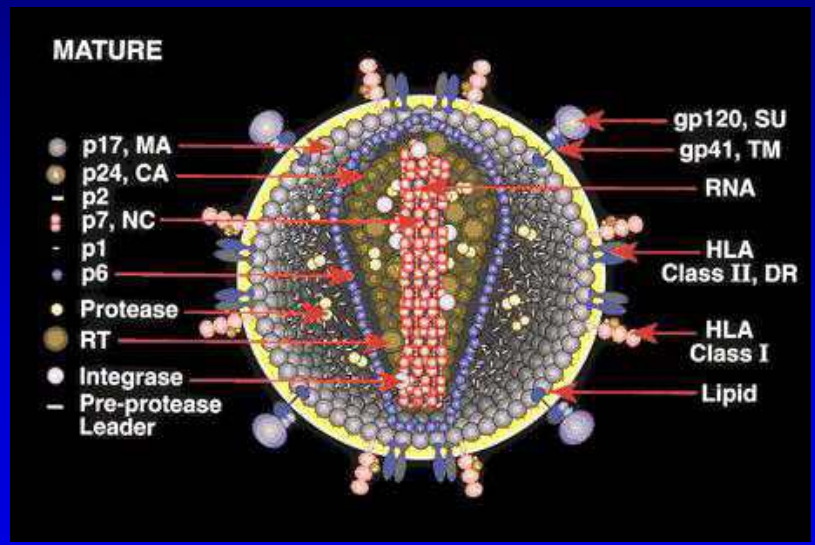
Viral Pathogenesis: (Mullins/Mittler Group)

Goal: understand effects of HIV-1 infection on target cells *in vitro* and *ex vivo*

Applications: find new targets for drug and vaccine development

Why single cell analysis: heterogeneity of infected cell populations, fewer than 1:100 cells infected *in vivo*

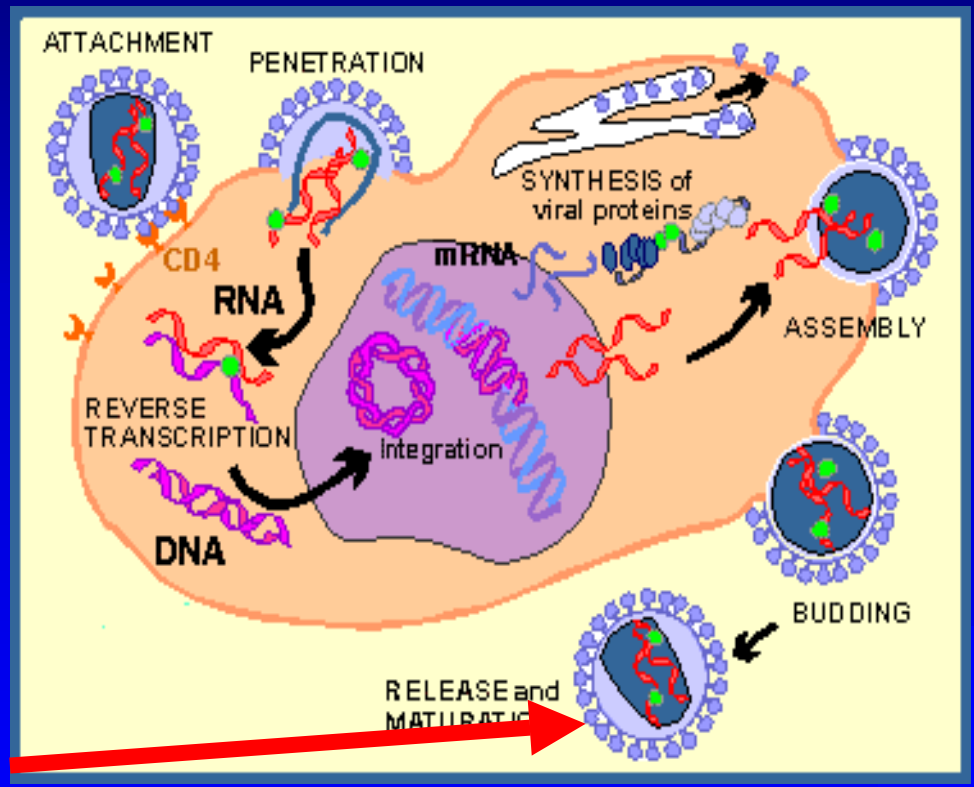
HIV-1 virion structure



Viral Pathogenesis: HIV-1 infection of T cells

Dissect the impact of HIV infection on gene and protein expression in naïve and memory T-cells

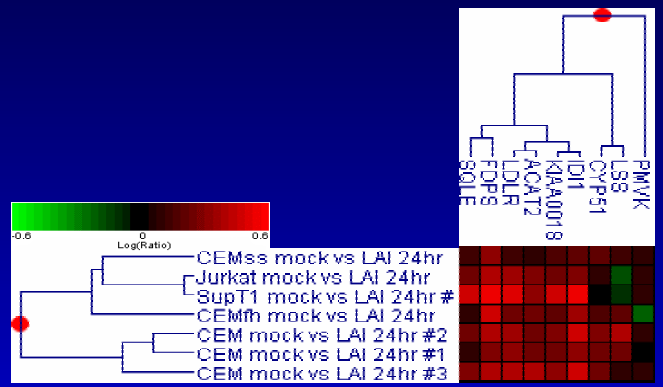
- Microarray analysis in bulk culture
- Single cell proteomics to screen marker proteins (e.g., p24)
- Initial focus on T-cell lines *in vitro*
- Long-term goal is to assess rare infected T-cells in *ex vivo* samples

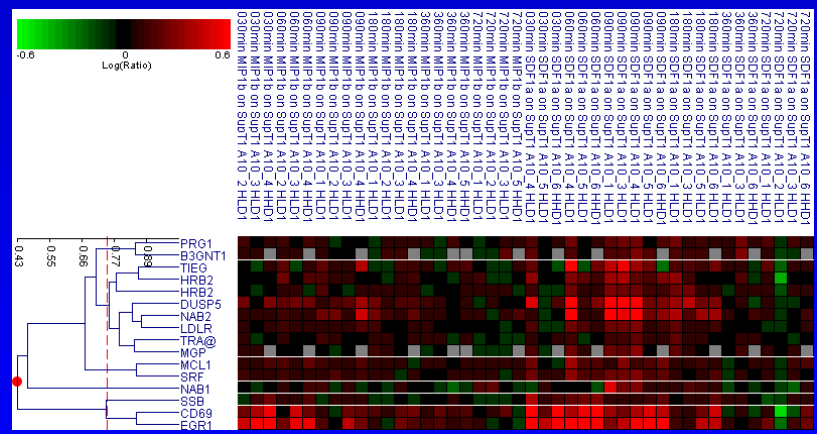


p24 capsid protein

Accomplishments: Viral Pathogenesis

- Microarray analysis showed increased expression of genes responsible for cholesterol uptake and biosynthesis in HIV-1 infected cells
- MIP-1α and SDF-1α induce expression of early response genes





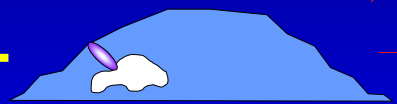
Next steps: proteomics in bulk and single cells

Single Cell Pathway: Expected Outcomes

Understand and manipulate cellular metabolism

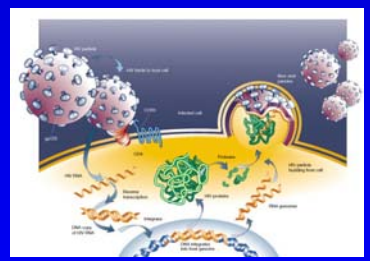
Address response at the single cell level

Understand pyroptosis



Assess heterogeneity in stressed and non-stressed cells

Understand HIV-1 infection



Study infection events at the single cell level

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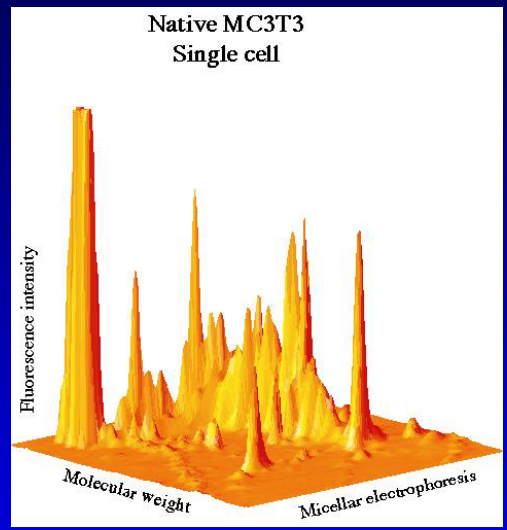
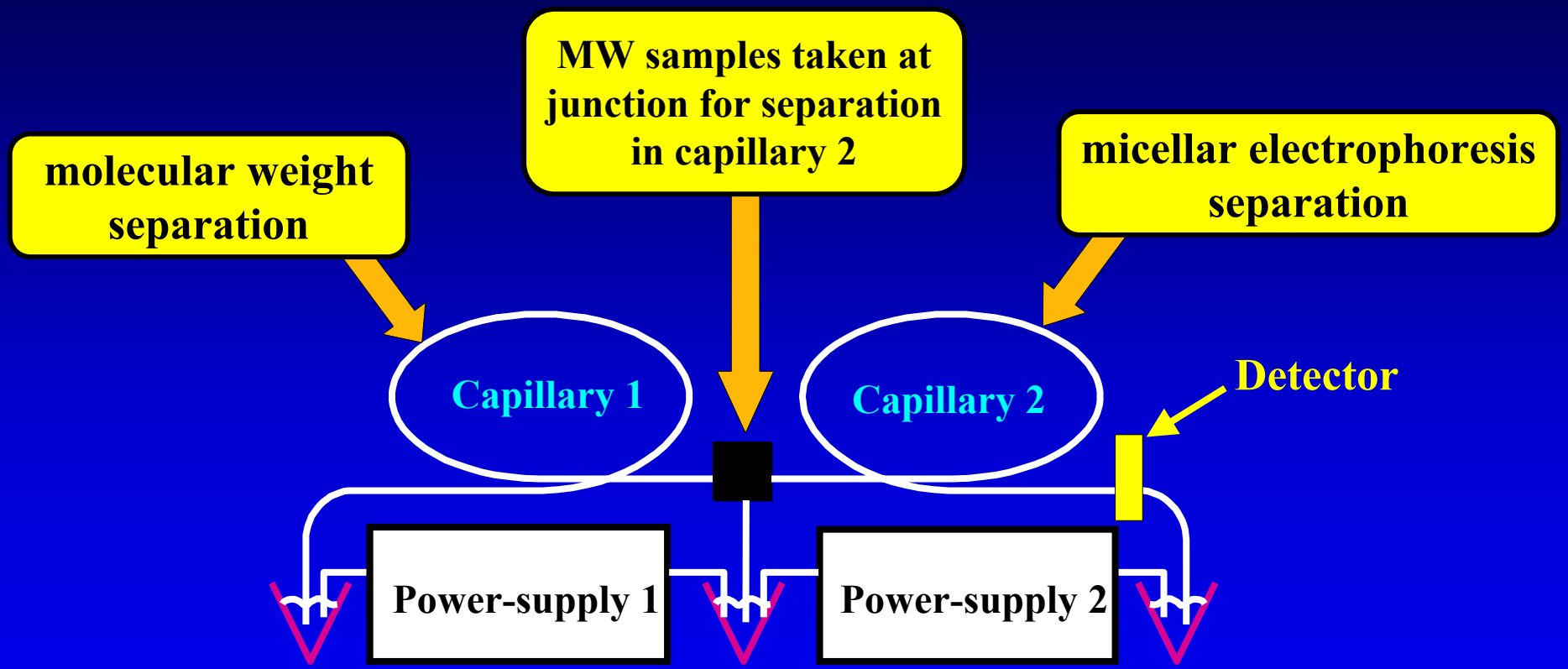
Proteomics (Dovichi Group)

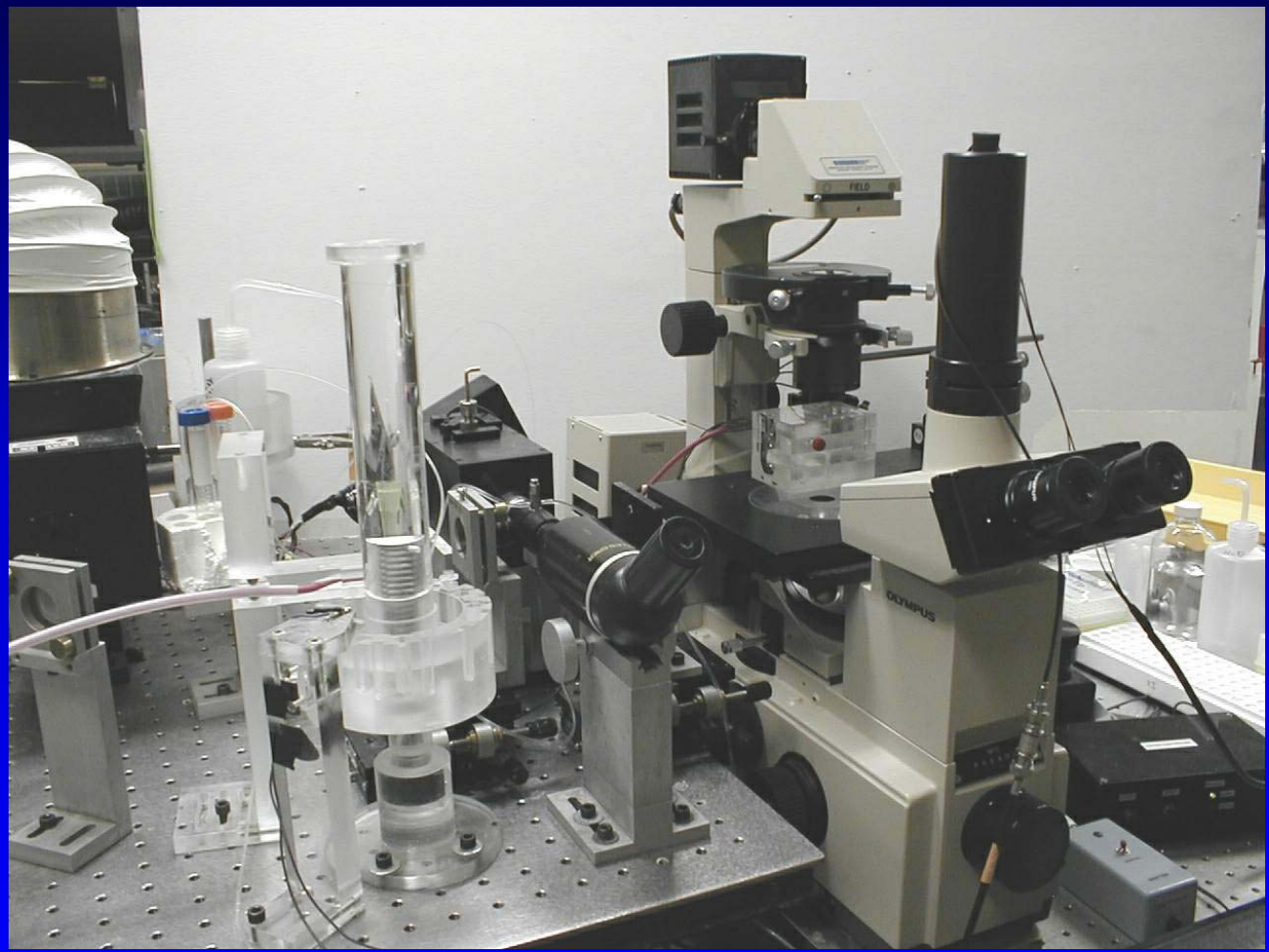
- **Goal:** Monitor very low level protein expression in single cells
  - What does it mean to say that a protein is not expressed?
    - Either no copies are present
    - Or analytical sensitivity is not sufficient to detect protein
  - How does variation in protein expression modulate cellular function?
    - Stochastic variation in transcription regulator expression
- **Application:** Understand regulatory circuits at the single cell level
  - Monitor variation in expression with cell cycle
  - Observe cell-to-cell heterogeneity in expression



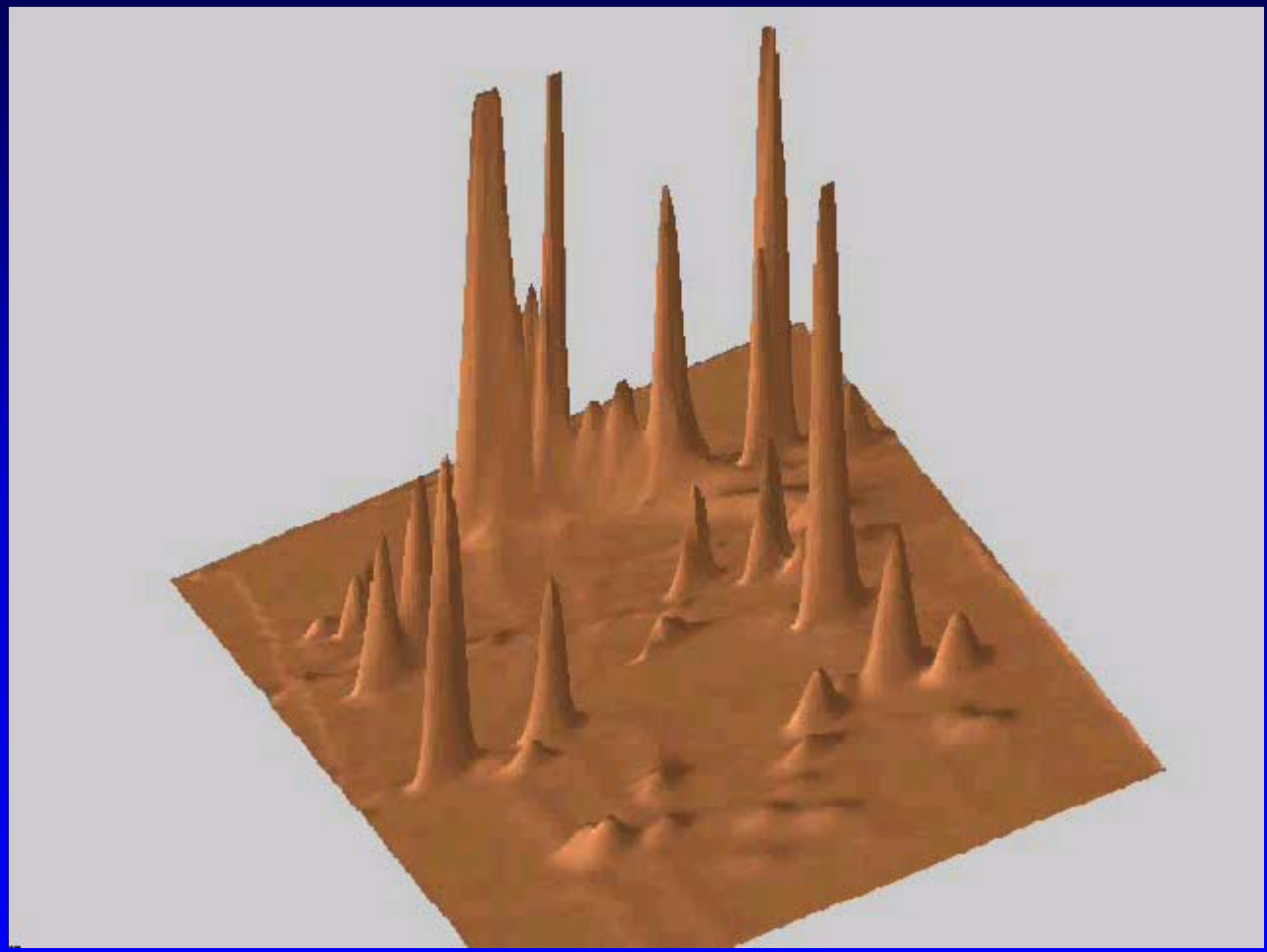
Comprehensive 2-D Capillary Electrophoresis



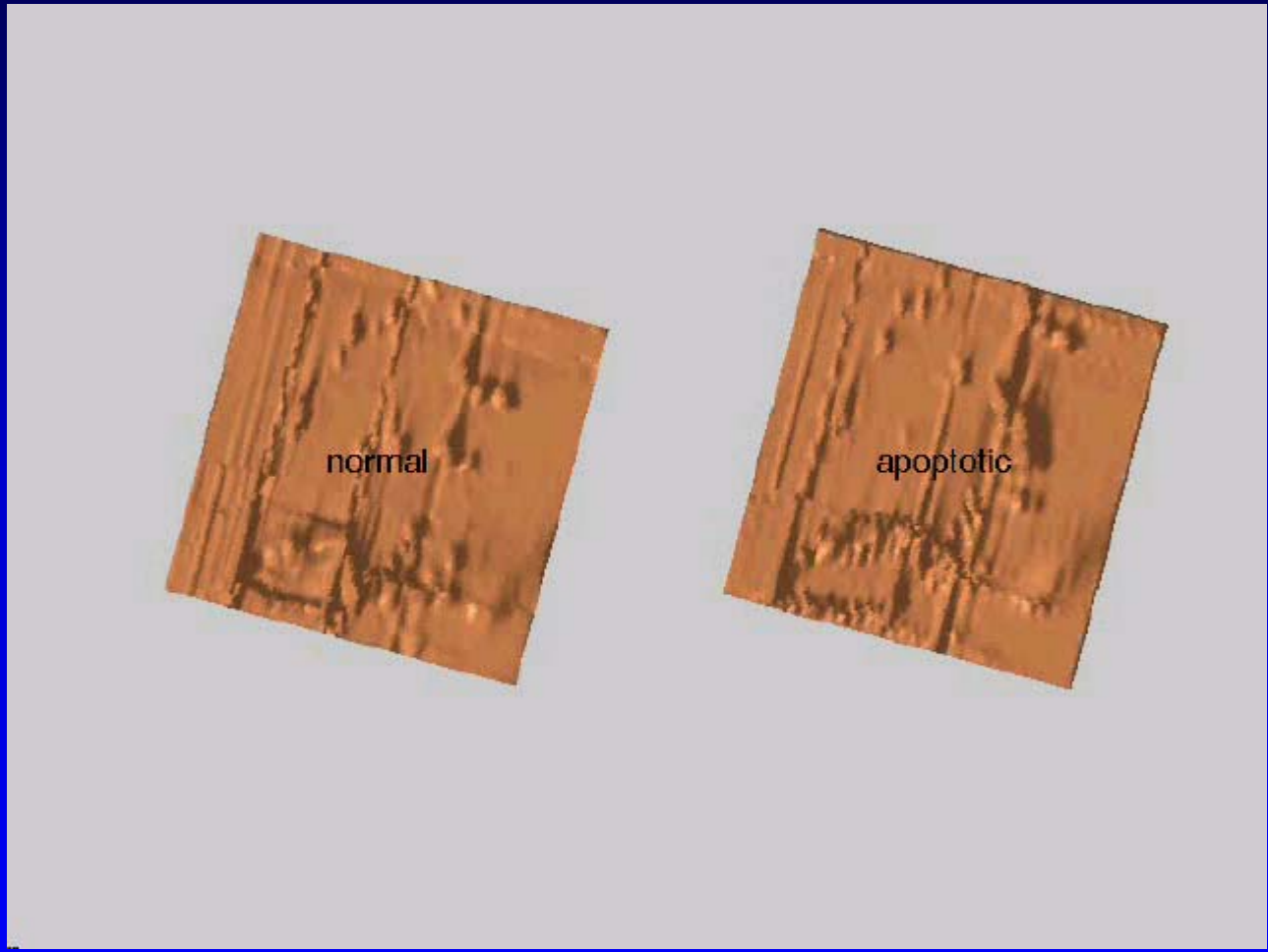
2-D Capillary Electrophoresis Instrument



Fully automated 2-D electrophoresis HT29 cell extract

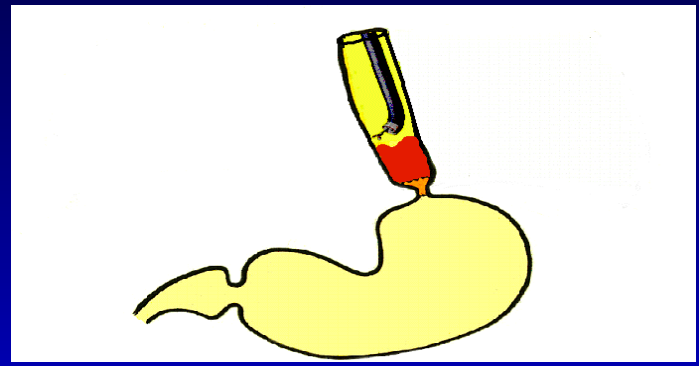


Fully automated 2-D electrophoresis single MCF-7 cells



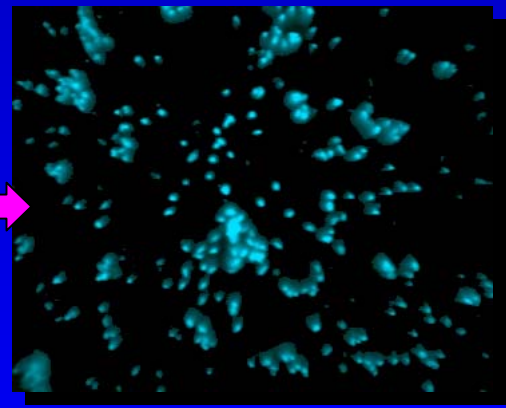
Cancer Diagnostics (Reid Group)

- **Goal:** Automated biopsy screening
  - Δ Decrease biopsy sample size
  - Δ Decrease sample processing time
- **Application:** Predict disease & stratify risk



Barrett's Esophagus

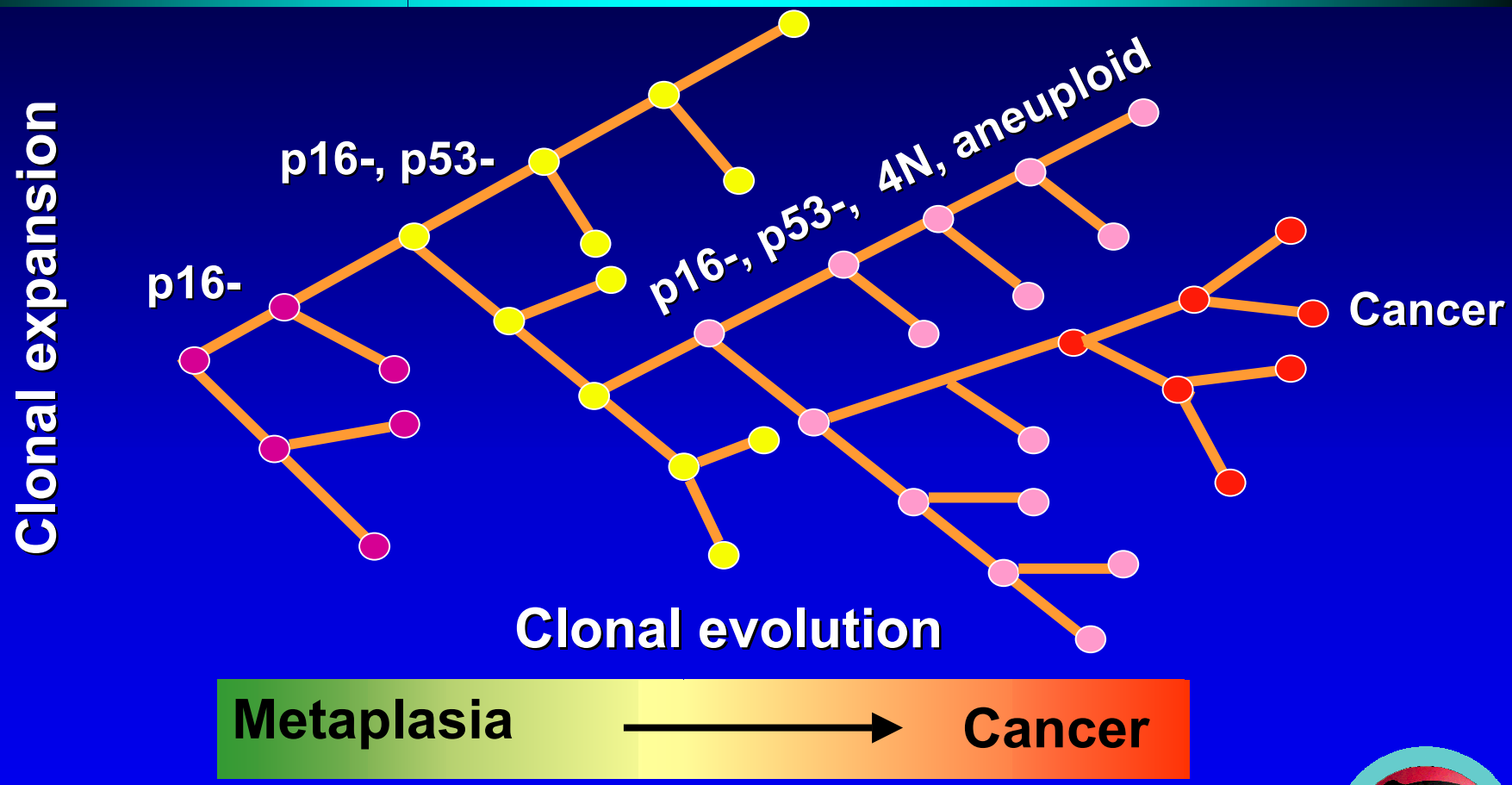
Tissue disaggregation



Single cell suspension of biopsy

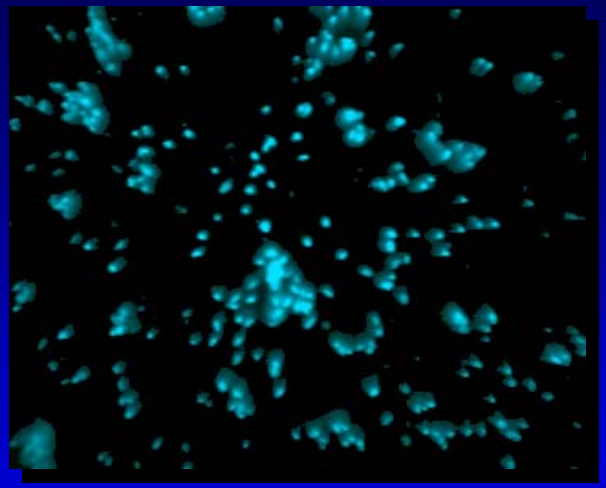
Barrett's Esophagus

Molecular Predictors of Progression

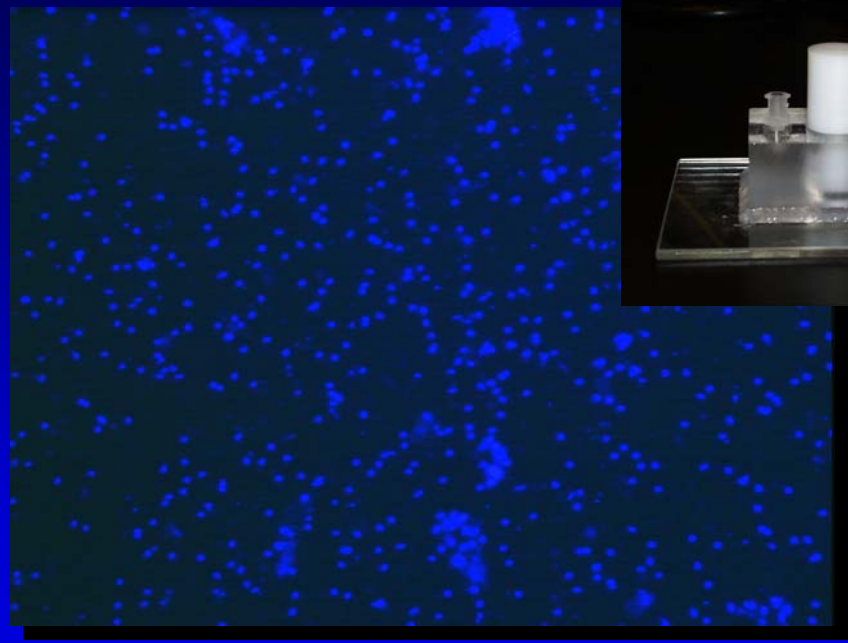


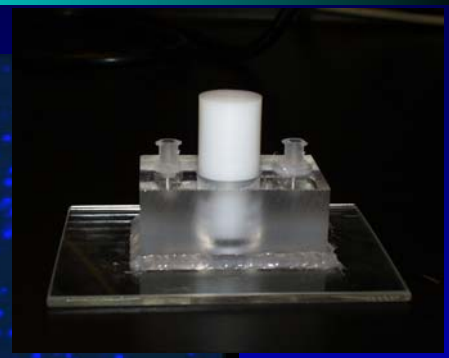
Wong et al, Cancer Research 2001; 61:8284
Reid et al, Am J Gastroenterol 2001; 96:2839
Rabinovitch et al, Am J Gastroentrerol 2001; 96:3071
Teodori et al, Cytometry 1998; 34:254

Results and Comparison



Hand-minced biopsy
after 40 micron filtration

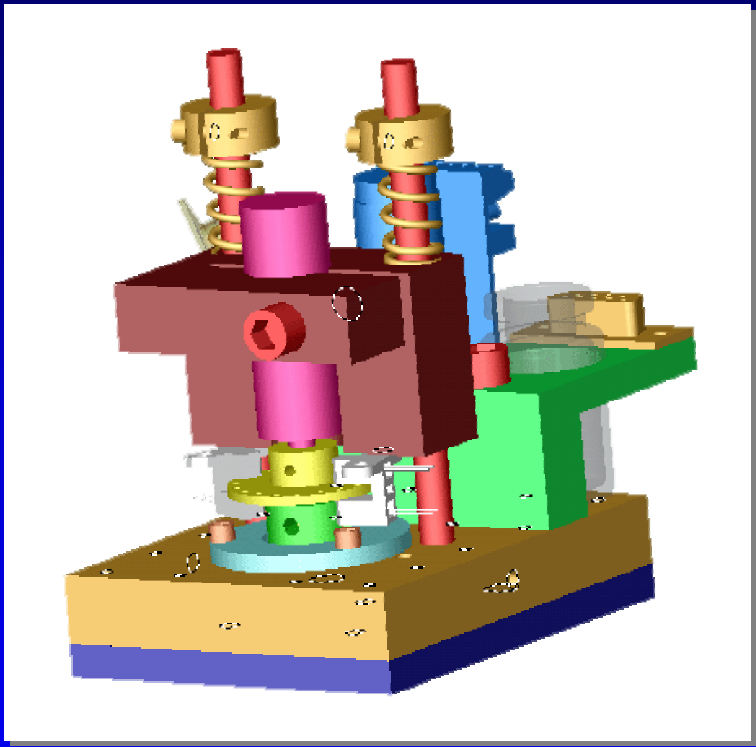




Results of sample disaggregation
on test platform *without* filtering

Test platform disaggregation yields a single cell suspension comparable to the traditional method, with less clumping

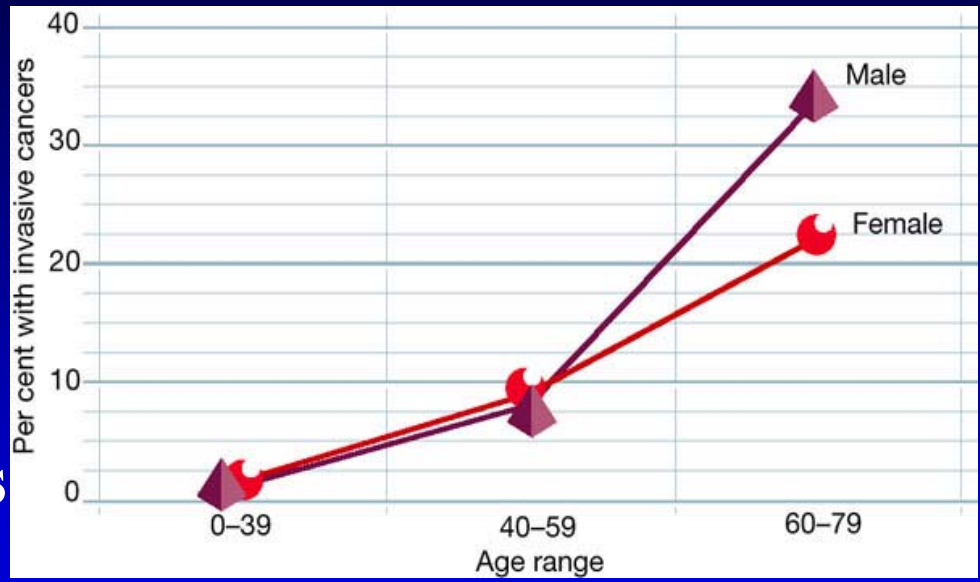
Future Directions



- Develop a fully automated and motorized tissue disaggregation platform
  - Further testing
  - Clinical use
- MEMS grinders currently under development

Study of Aging & Correlation to Cancer (Gottschling Group)

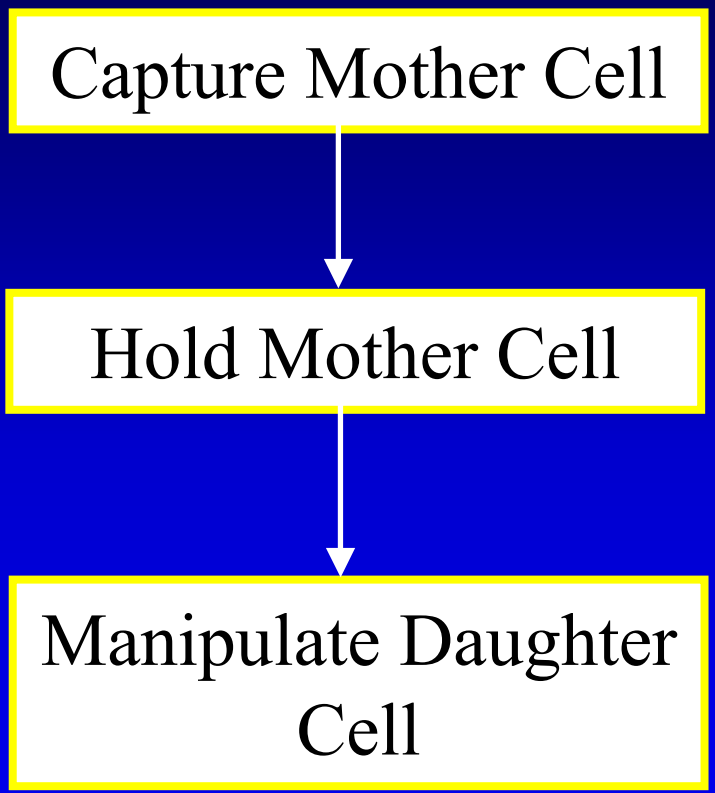
- **Background:** % invasive cancers in humans increases with age
- **Goal:** provide high-throughput, automated partitioning of yeast daughter cells for studies
  - Δ Current methods require sleepless nights in Seattle!



[DePinho, *Nature* 408, 248-254 (2000)]

- **Application:** study yeast as model organism (Gottschling Lab)
  - Δ Shown that frequencies of chromosome loss & recombination in yeast genome increases with age
  - Δ New study is to analyze LOH with increasing age in yeast

Automation of Yeast for Aging/Cancer Studies



- **Side channel & valve capture single cell**
- **Bond cell wall to channel surface**
- **Series of channels & valves to deposit daughter cells onto agar plate**

[Koschwanez, Holl, Meldrum]

Outline

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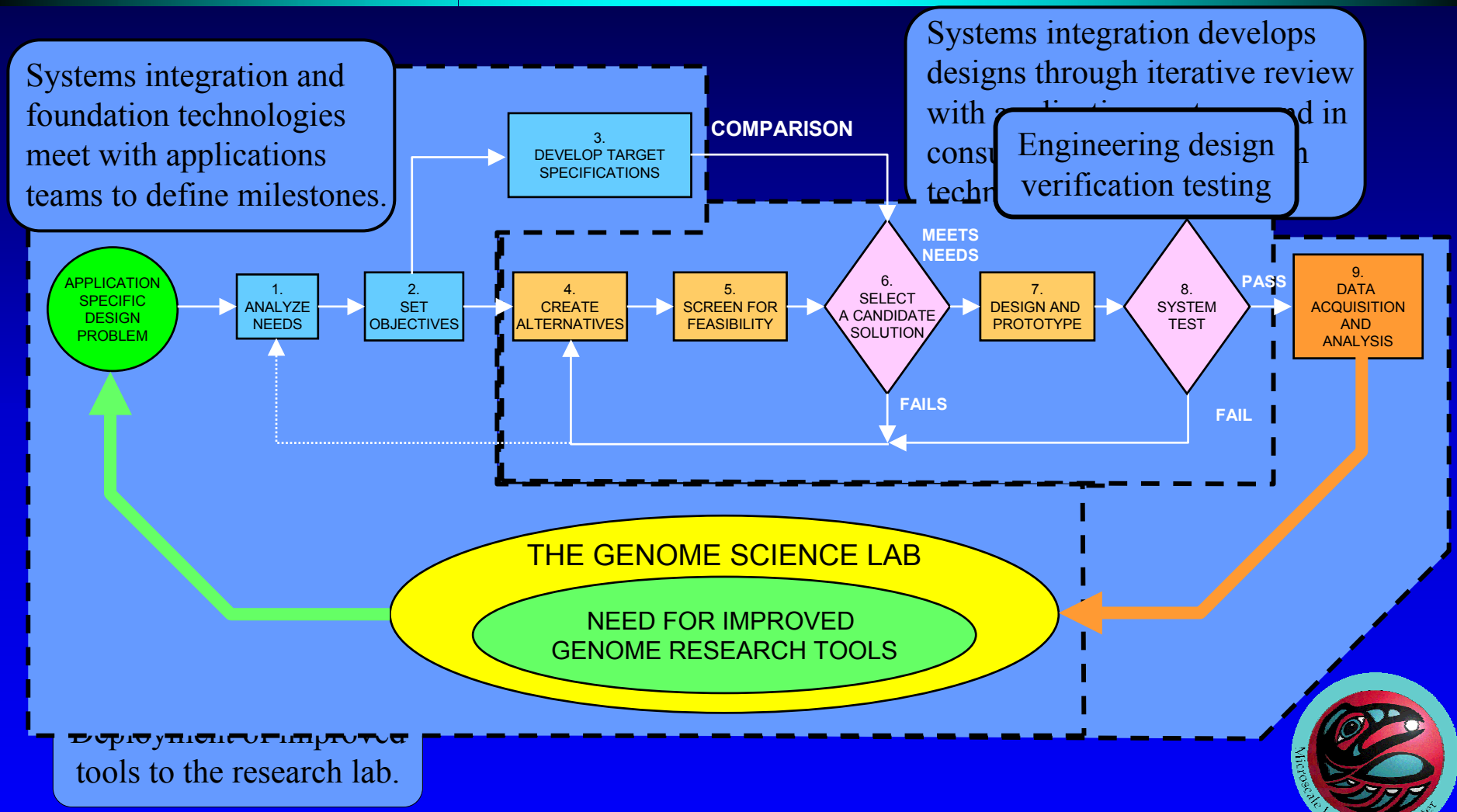
- Technology Development

Application/Module Development Process



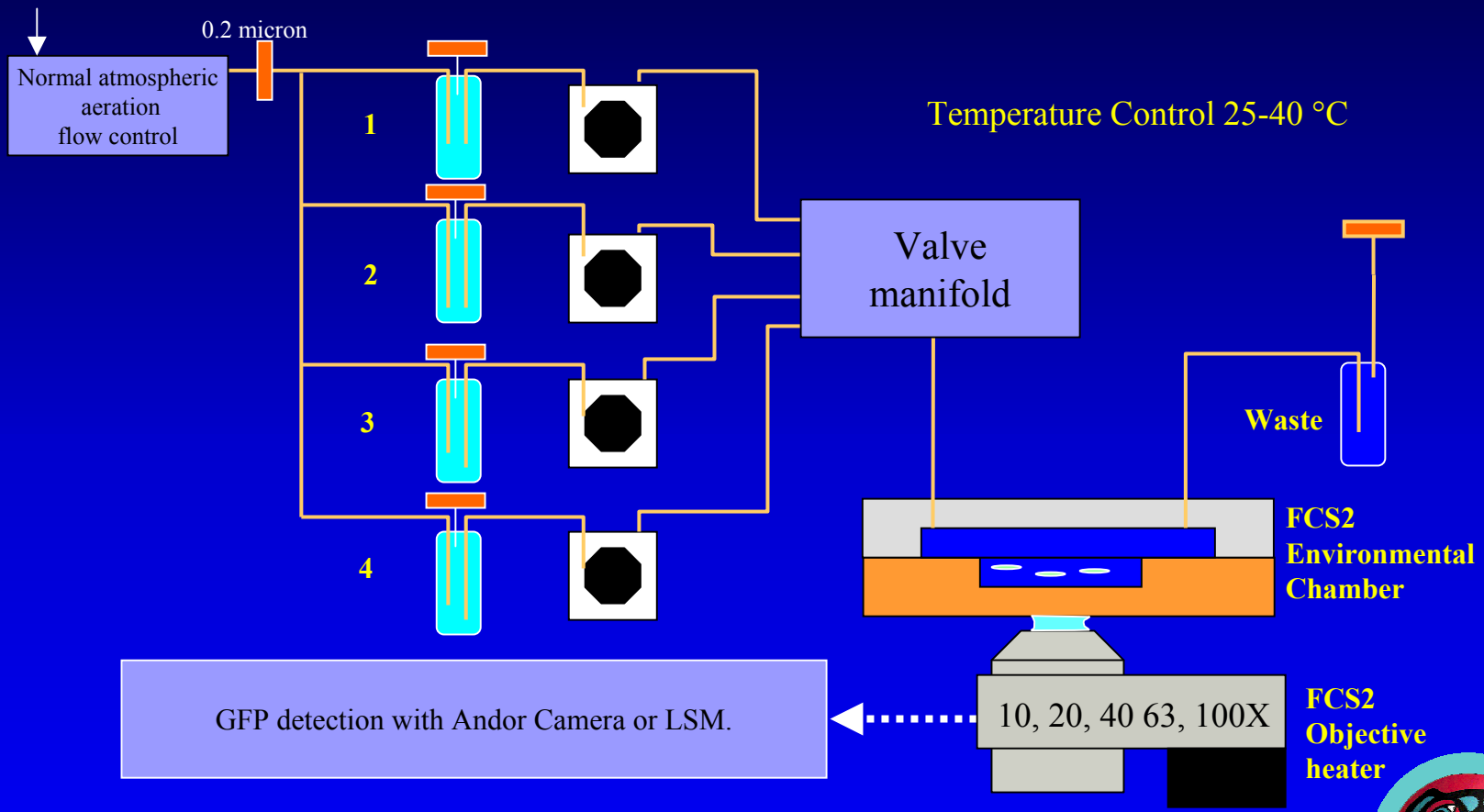
Foundation Technologies Needed to Support Applications

- Automated microfluidic handling and sorting of cells
- Highly sensitive detection small molecules, and proteins
- **Integrated microsystems** platform for the maintenance of living cells (eukaryotic and prokaryotic)
- Integrated controls and data handling system

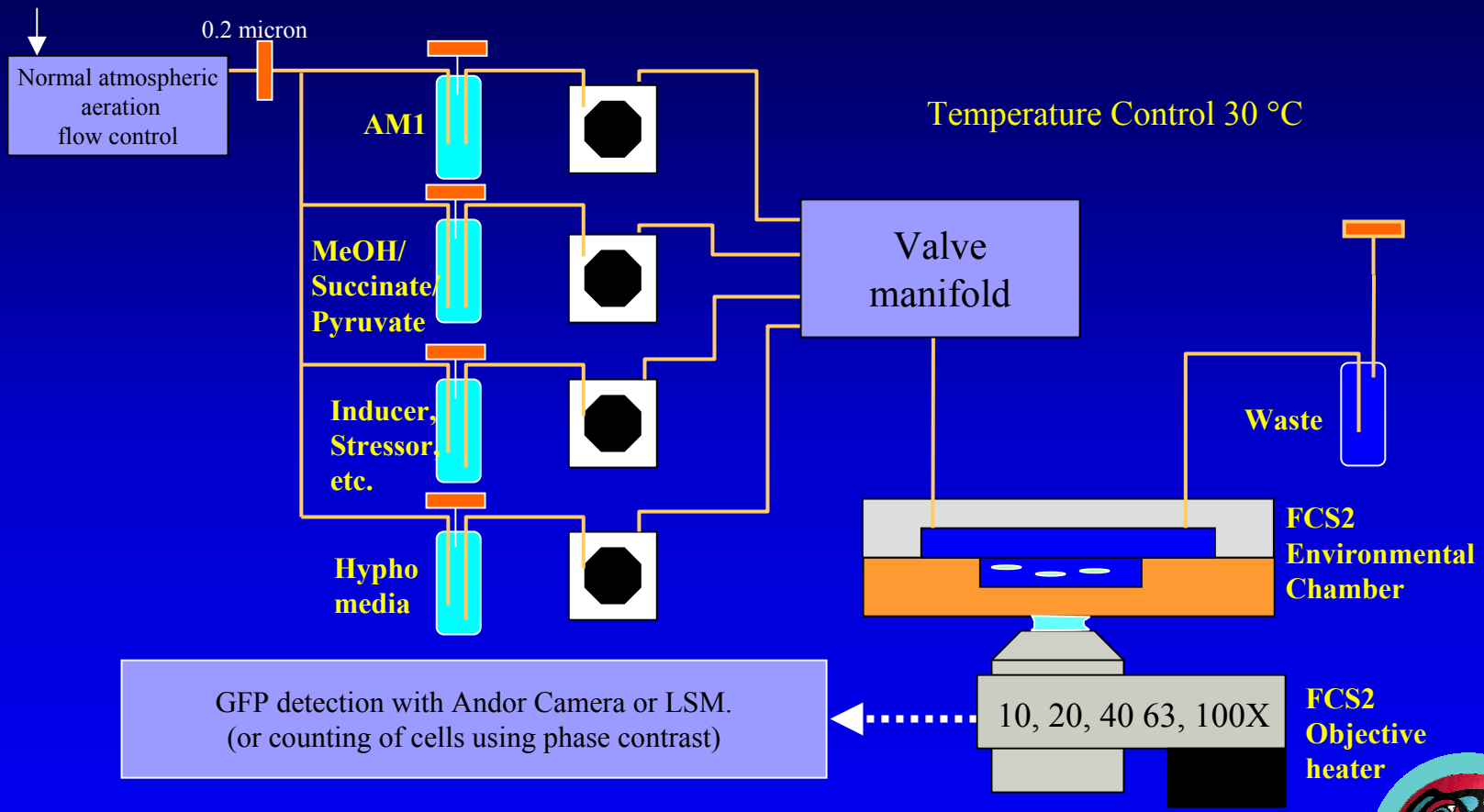
Team Expertise

- Genomics and Proteomics
- Microbiology, Biochemistry, Analytical Chemistry
- Microfluidic device design test and analysis
- MEMS modules / processes
- Detection
- Nanotechnology
- Sensor Fusion
- Automation/Systems integration

Initial Generic Setup

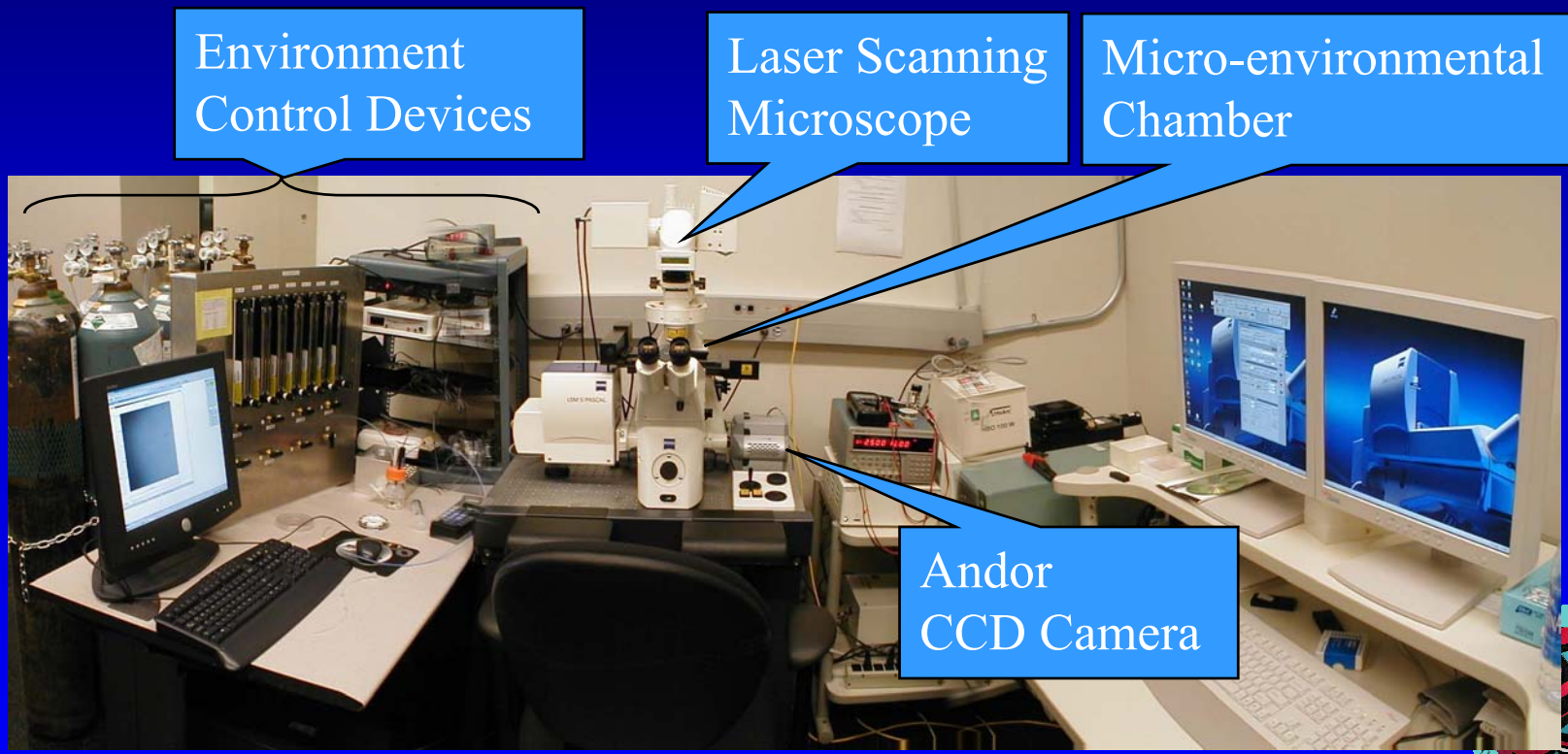


Lidstrom Lab AM1 Setup



Laser Scanning Confocal Microscope

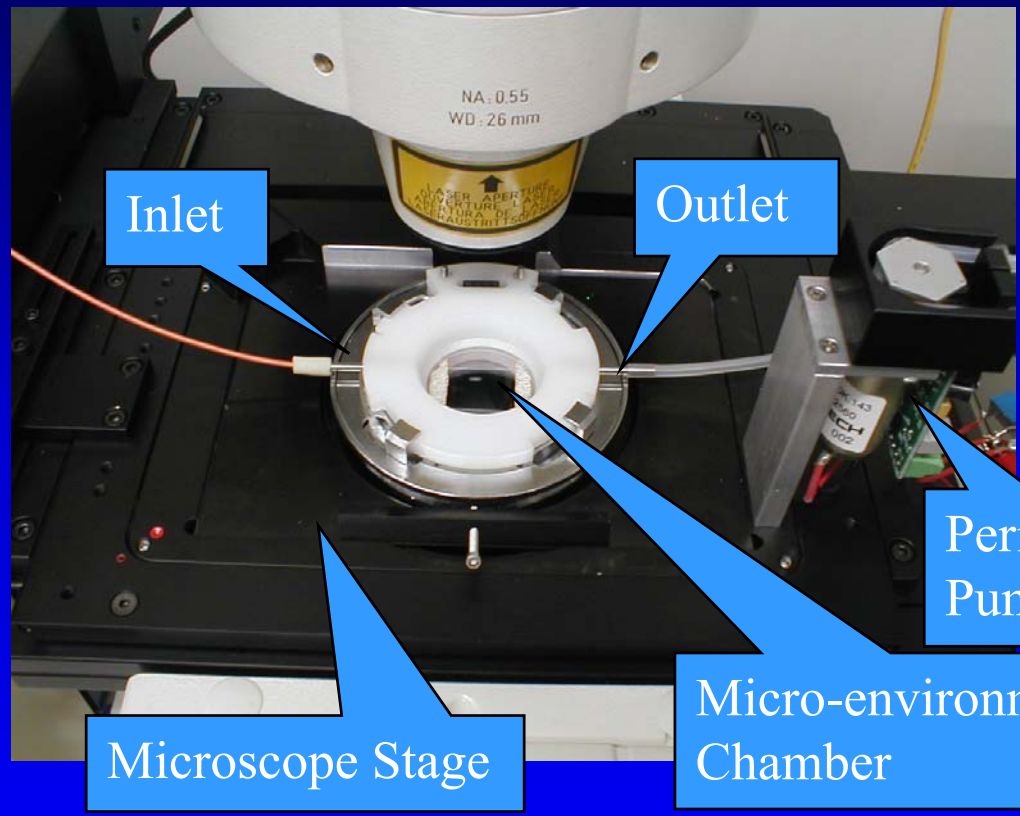
Overview of Setup



Micro-Environmental Chamber

Advantages:

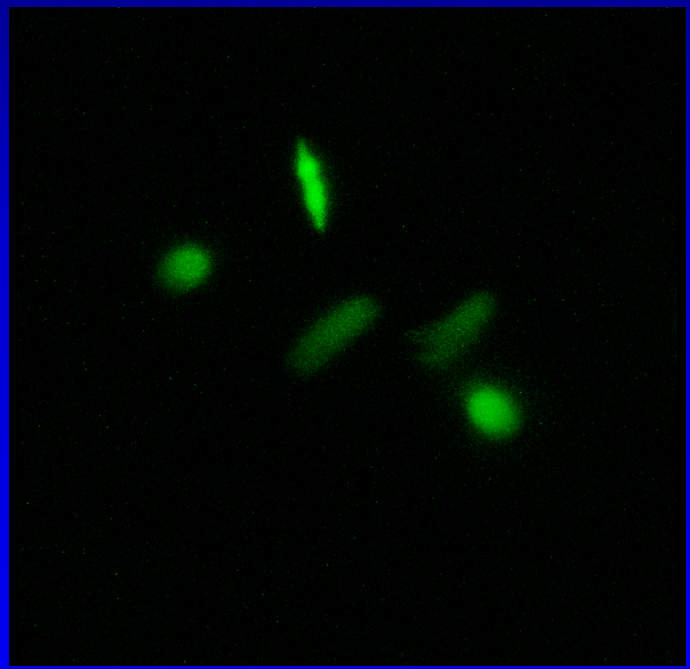
- Ability to control media concentration and temperature in a closed space.
- Compatibility with microscopes and microfluidic devices.



3D Confocal imaging

3D image of *M. extorquens* AM1 cells

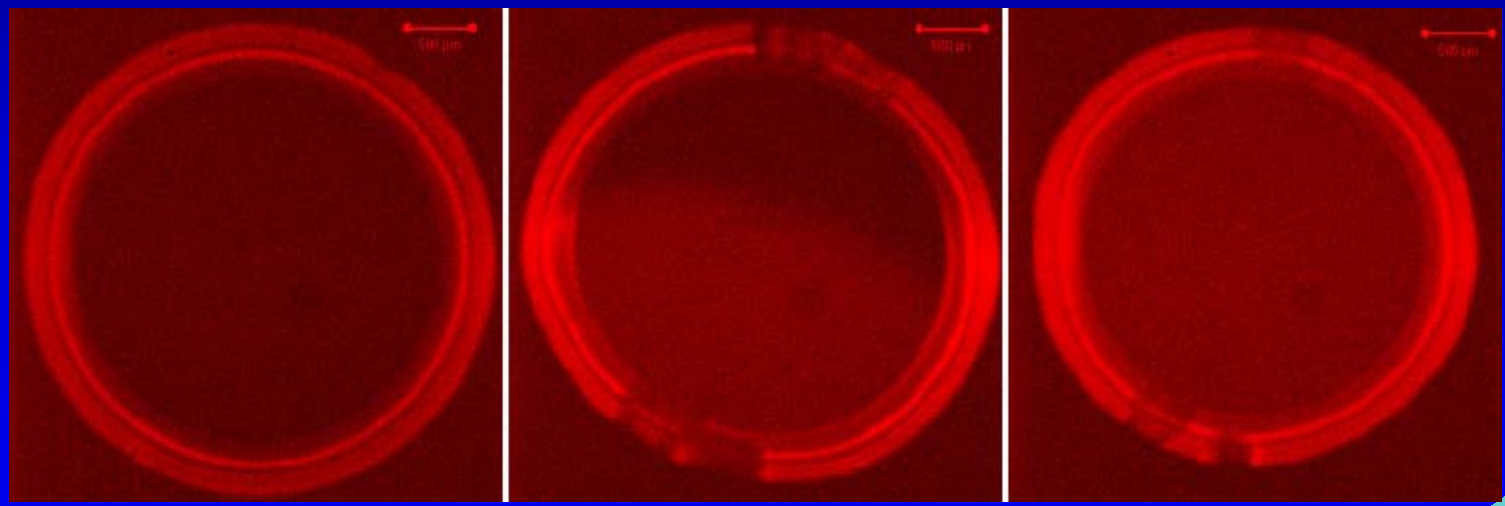
- Rod shape five bacteria with length < 5 μm.
- Green fluorescence detection.



O_2 Sensing

Simulation of oxygen consumption

- Low concentration of O_2 causes high phosphorescence intensity of polyporphyrin Pt(TFPP) thin films.
- Na_2SO_3 in the lower-left part of the water droplet.
- Emission intensity increased by 60% after 60 seconds—indicates less O_2 .



Before introducing Na_2SO_3

10 seconds after introducing Na_2SO_3

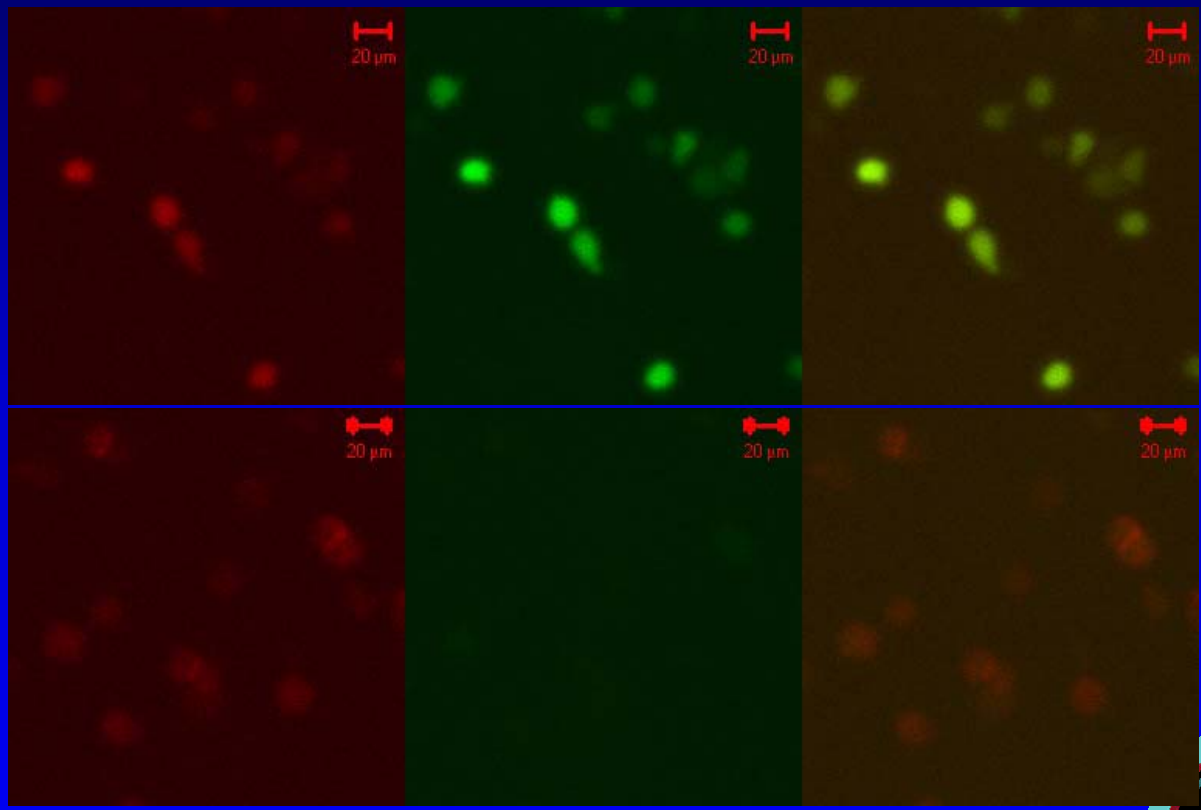
60 seconds after introducing Na_2SO_3

Fluorescence Detection

The detection of protease expression of HIV-infection of macrophages with CD4 antibodies

HIV-infected group. The GFP expression is turned on.

Mock group

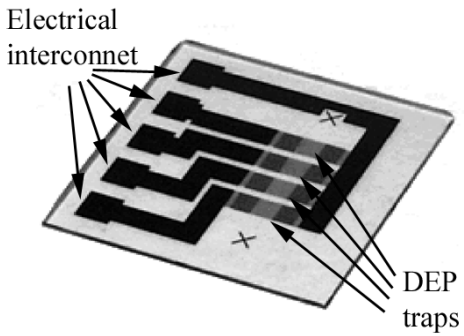


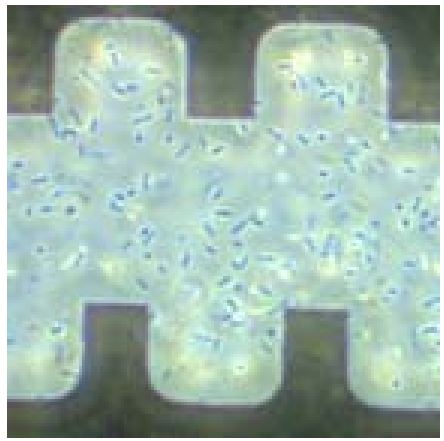
PE fluorescence **GFP fluorescence** **Combination of PE and GFP**

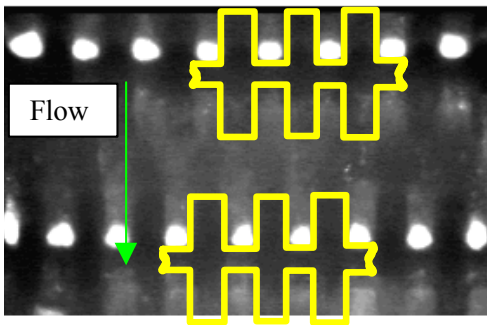
Technology Development

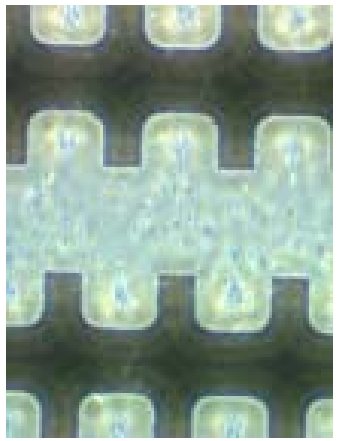
- **MEMS**
  - Cell size separation microsystem
  - Electroimpedance spectroscopy
  - Future technologies
- **Detection**

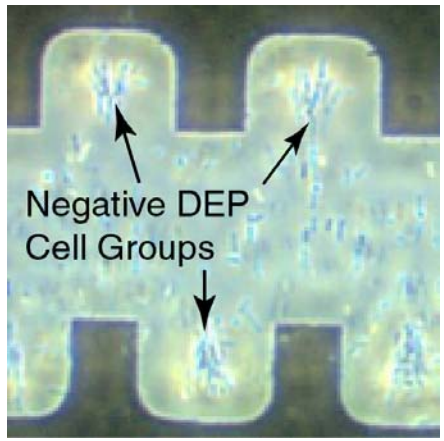
Manipulation of DNA and Cells (Meldrum Group)







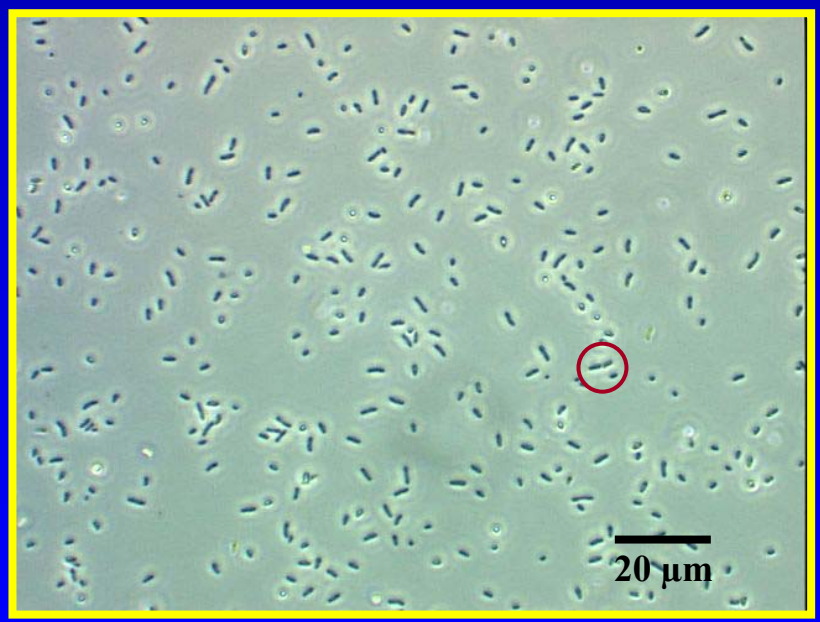




(Crippen, Holl, Kosar, Meldrum)

Cell Size Separation Microsystem

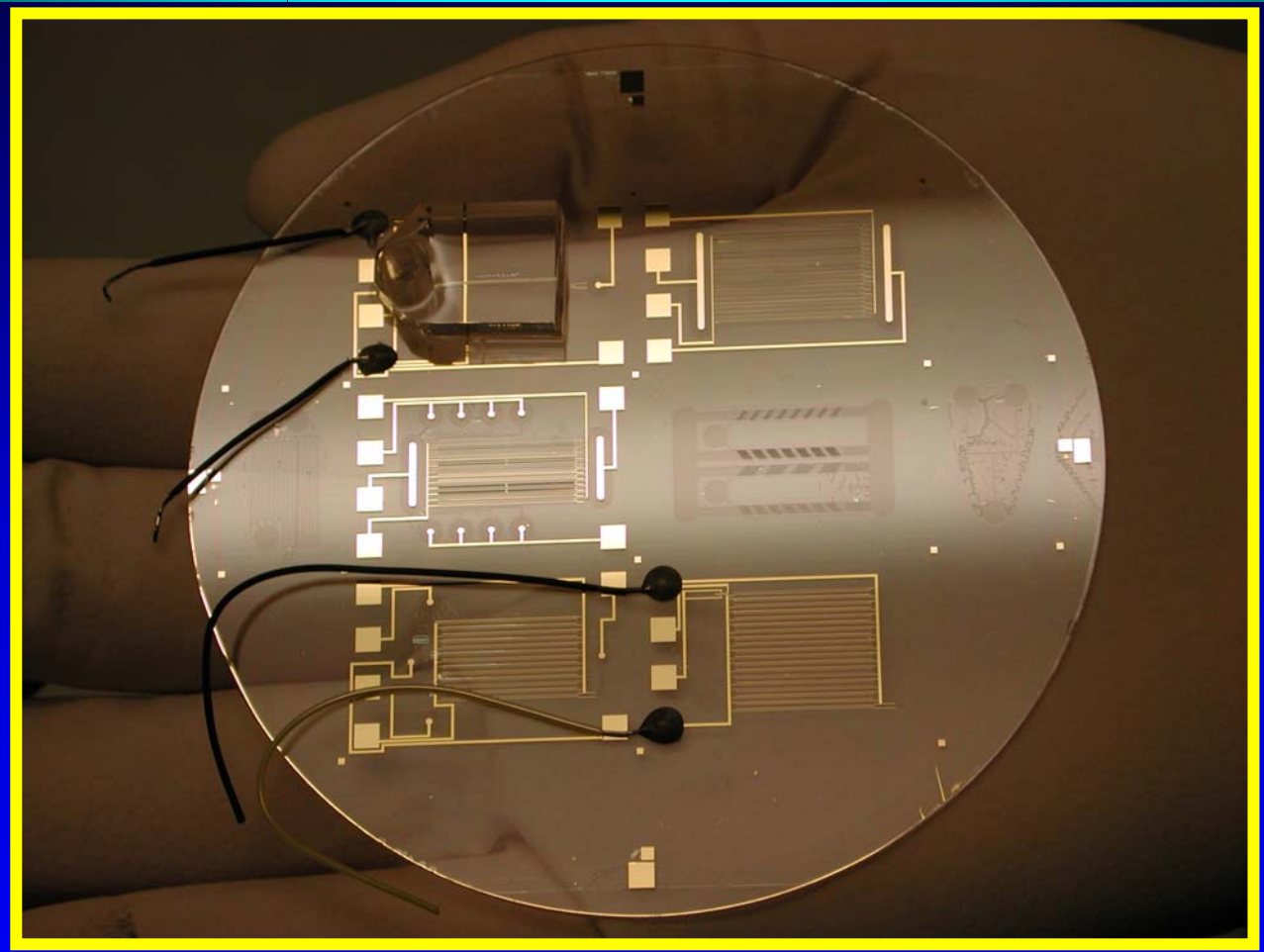
- Model system: *M. extorquens* AM1
- Rod-shaped: ~ 1 µm diameter, ~ 3-6 µm length
- Grow lengthwise
- Divide in the middle about every 6 hr
- Age ∝ Length



Phase Contrast Image of AM1 Cells

(Kosar, Holl, Lidstrom, Meldrum)

Microfabricated Devices for Cell Size Separation



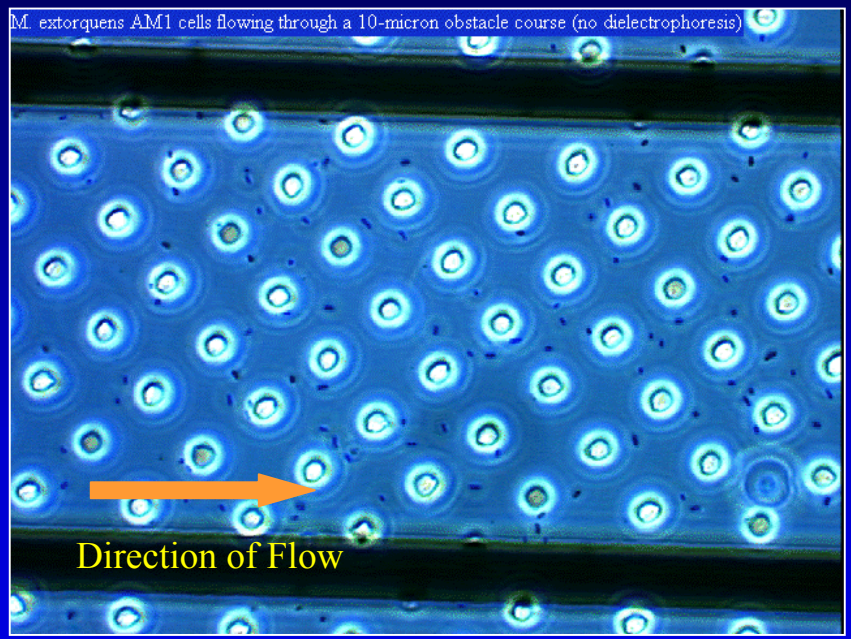
(Kosar, Holl, Meldrum)

Electrokinetic alignment of AM1 cells inside a microchannel while flowing (10V p2p, 100kHz)

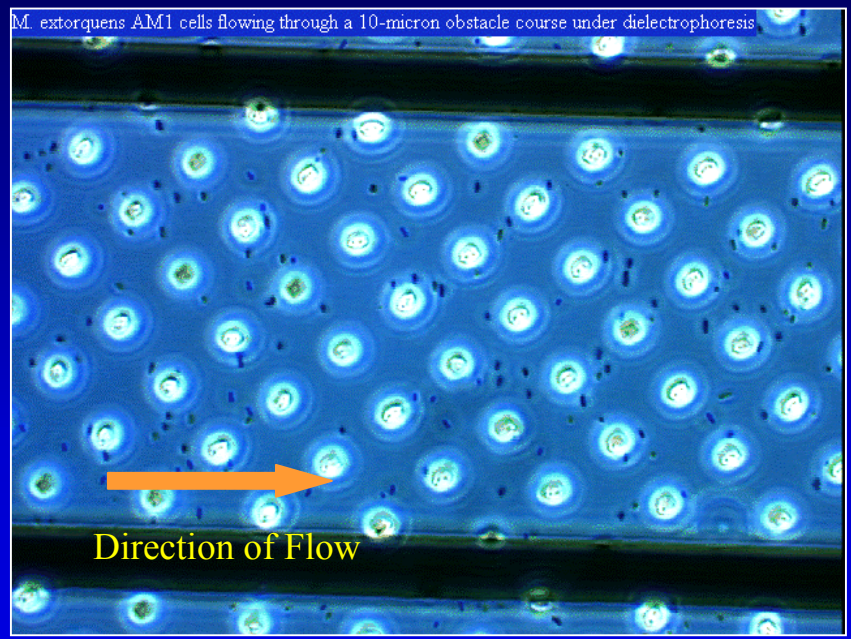
(Kosar, Holl, Meldrum)

Manipulation of *M. extorquens* AM1 using DEP

Random Orientation



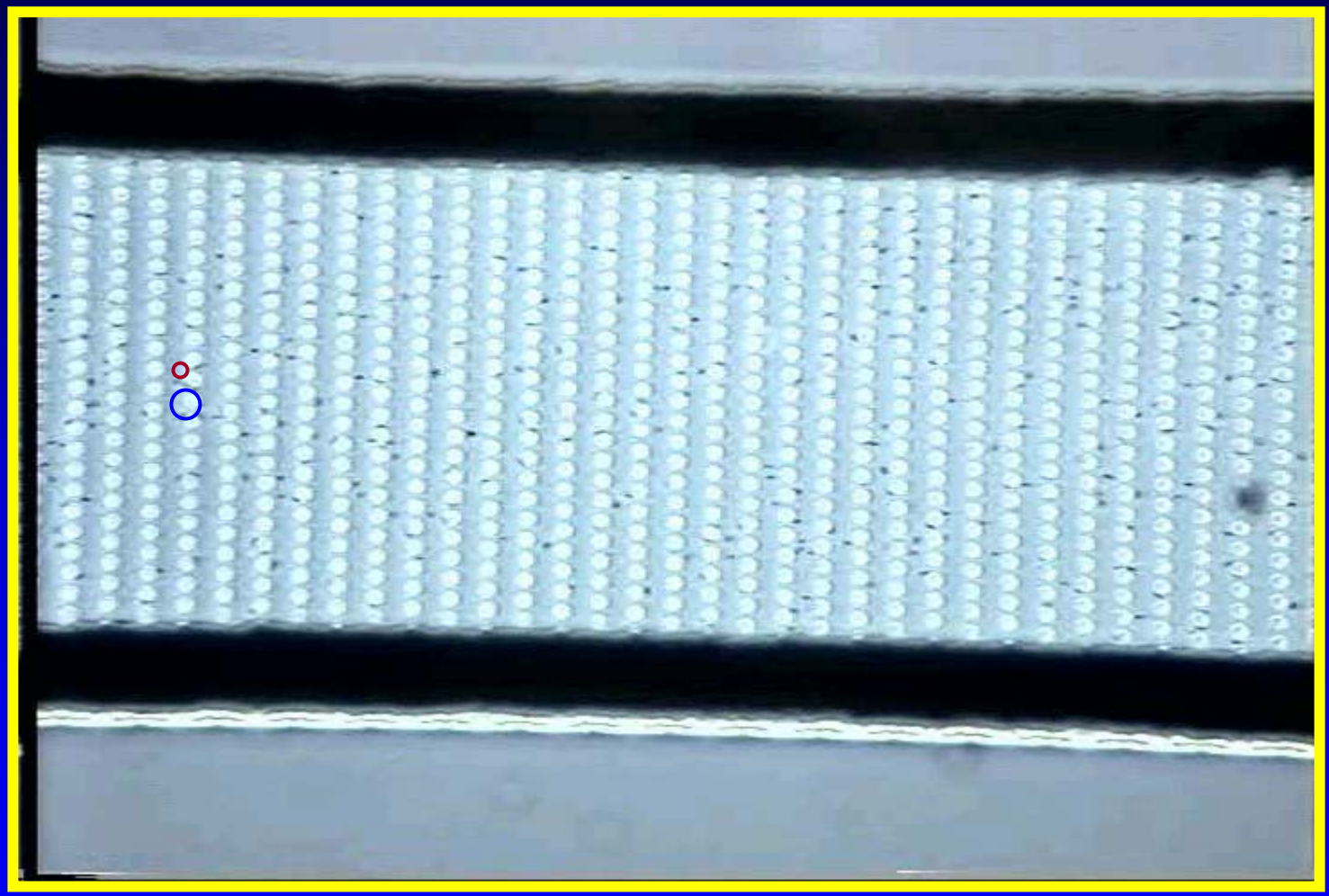
Aligned with Electric Field



10 um diameter obstacles with 10 um separations
10 um deep channel (PDMS on glass)
16 V peak-to-peak 1 MHz sinusoidal signal

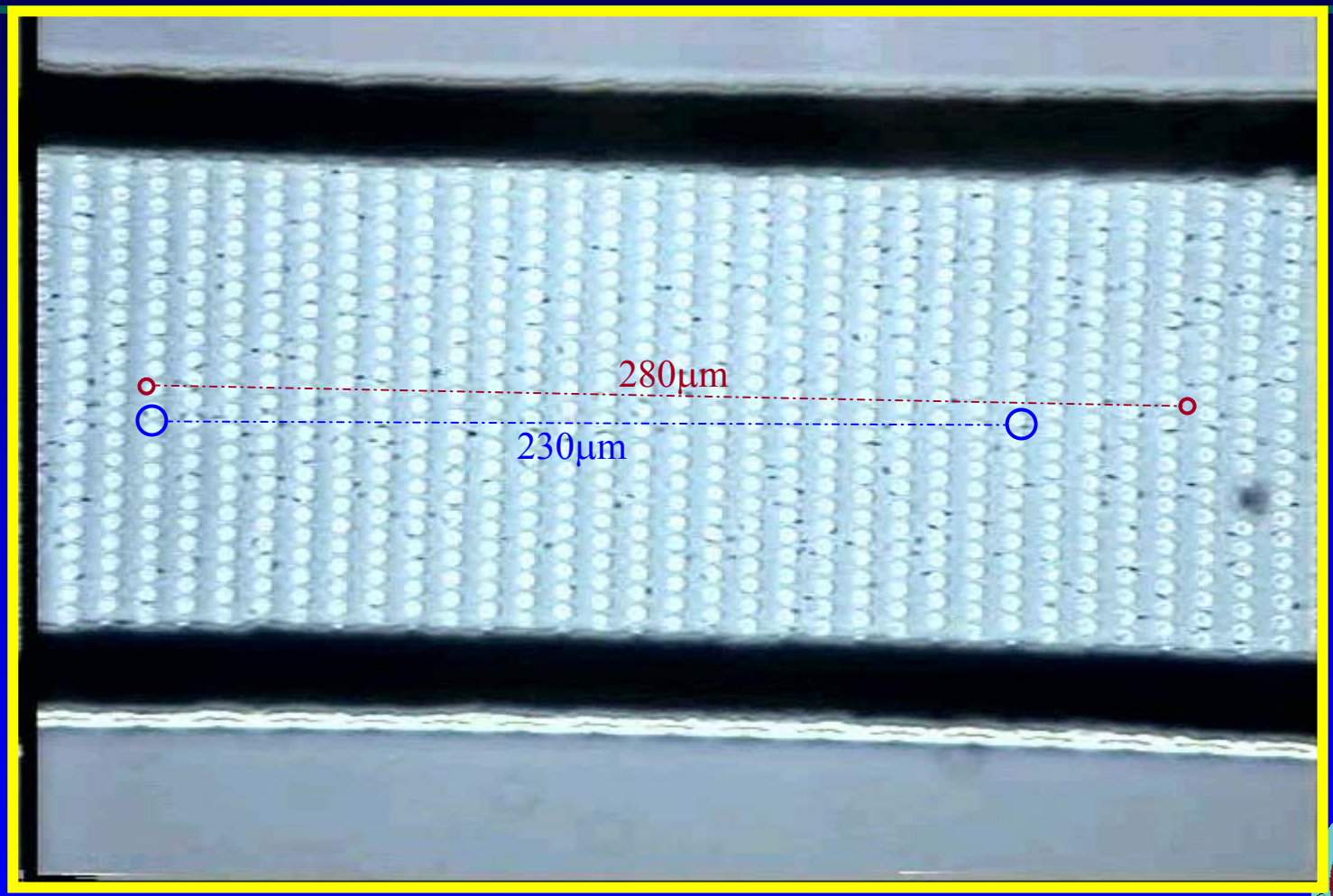
(Kosar, Holl, Lidstrom, Meldrum)

Results: Separation by Size



(Kosar, Holl, Meldrum)

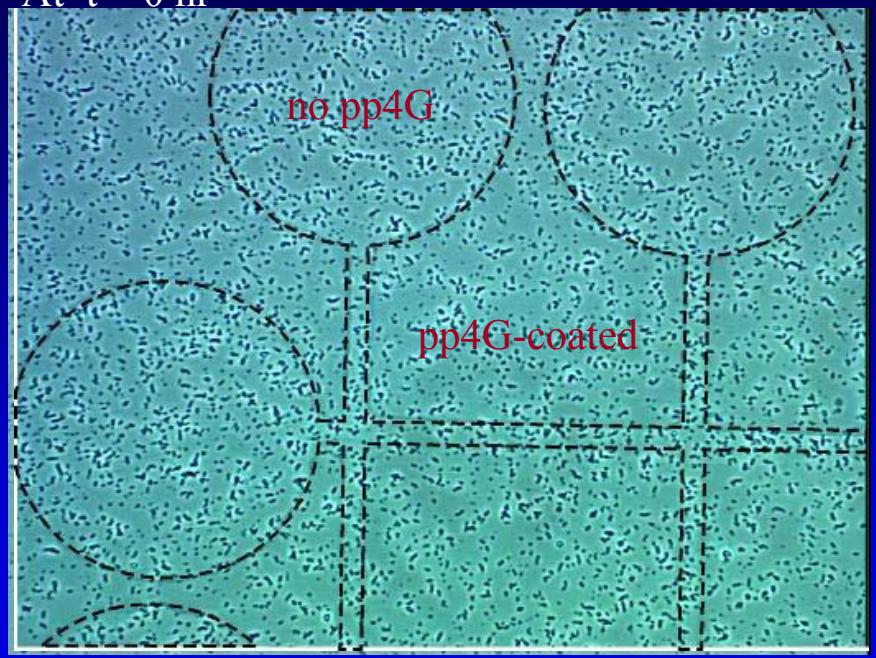
Results: Separation by Size



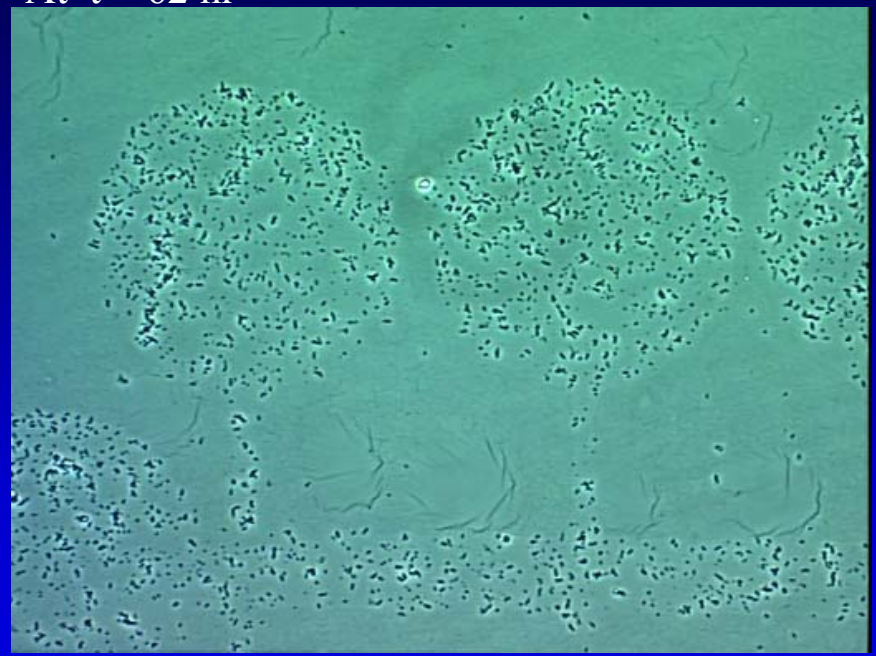
(Kosar, Holl, Meldrum)

Problem: Cell Attachment (Biofilms)

At t = 0 hr



At t = 62 hr



Potential solution:

Micropattern pp4G (plasma polymerized tetraglyne) coating on glass

PEG-like non-fouling surface

(Kosar, Hanein, Lipscomb, Ratner, Böhringer, Holl, Meldrum)

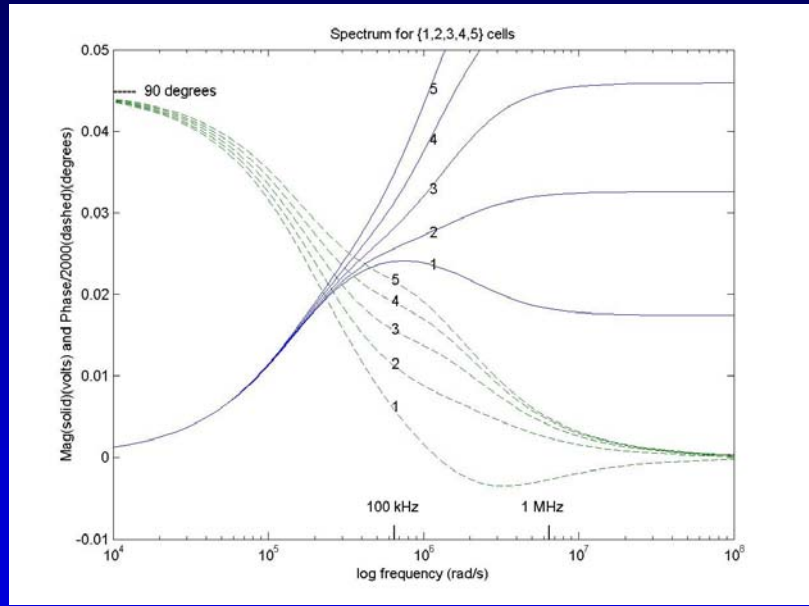
Real-time detection of cells using electroimpedance spectroscopy (Seriburi/Phillips/Holl/Meldrum)

- **Why Electroimpedance Spectroscopy?**
  - Δ Detection and characterization of cells in real-time [1,2,3]
  - Δ Fast, direct, minimally-invasive method
  - Δ Easy fabricated and integrated MEMS device
- **Simulations and preliminary measurements indicate adequate sensitivity for categorizing cells types and properties**

1. S. Gawad et al., MicroTAS 2002, pp.649-651.
2. S. Gawad et al., Lab on a Chip, vol. 1, 2001, pp. 76-82
3. H. Edward Ayliffe et al., J. of MEMS, vol. 8, 1999, pp. 50-57

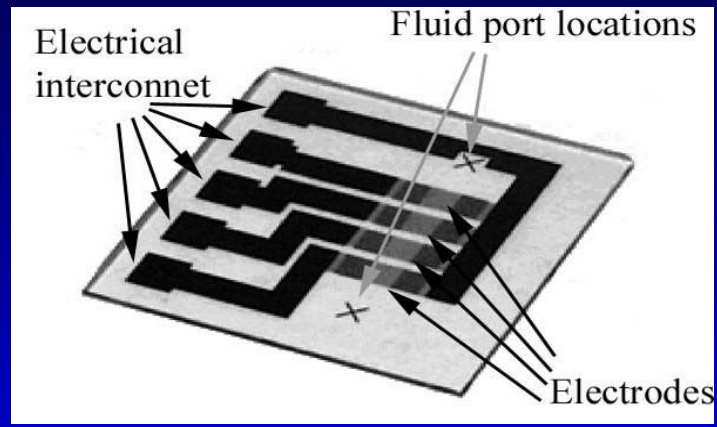
Real-time detection of cells using electroimpedance spectroscopy

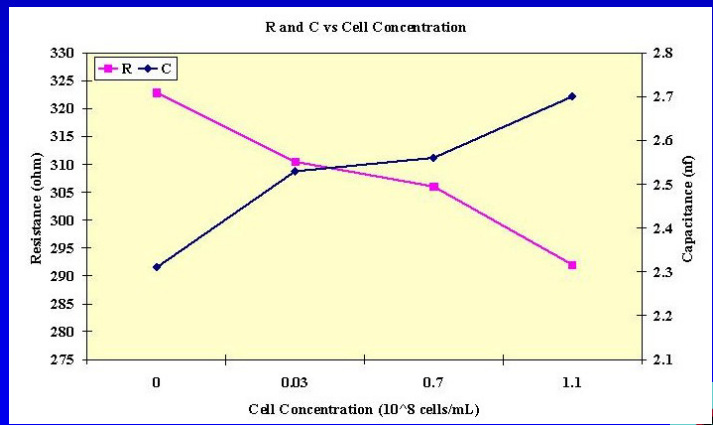
- Simulations indicate that cell sizes, cell types, and the number of cells will be detectable with this system
- Simulations show optimal frequency applied should be in kHz to MHz range



Real-time detection of cells using electroimpedance spectroscopy

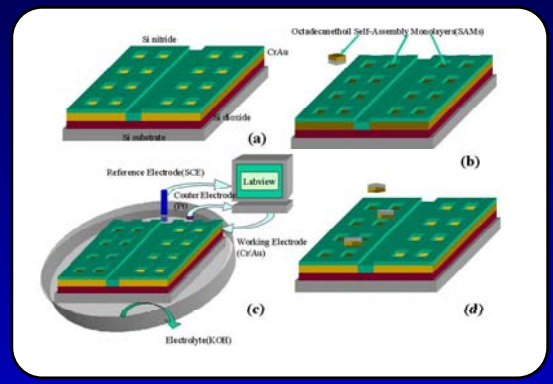
- Microfabricated interdigitated gold electrodes on glass with a PDMS (Polydimethylsiloxane) fluid channel was used
- Results show that impedance magnitude and phase varied with cell concentrations



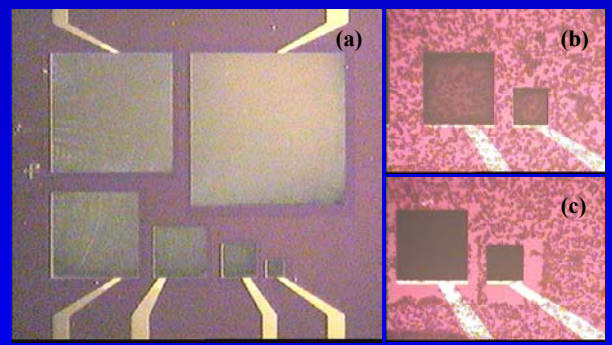


Future MEMS Technologies (Böhringer Group)

- **Microfluidic Breadboard: MEMS modules and components for rapidly adaptable and reconfigurable microsystems**
- **Self-assembly as enabling technology for integration of heterogeneous microdevices - electrochemical modulation of surface hydrophobicity**
- **Integration of biocompatible materials into MEMS processes**



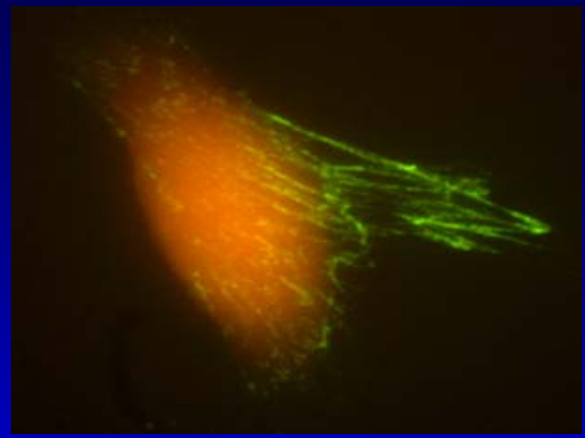
(Böhringer, Xiong, Wang, Hanein et al.)



(Böhringer, Hanein, Pan, et al.)

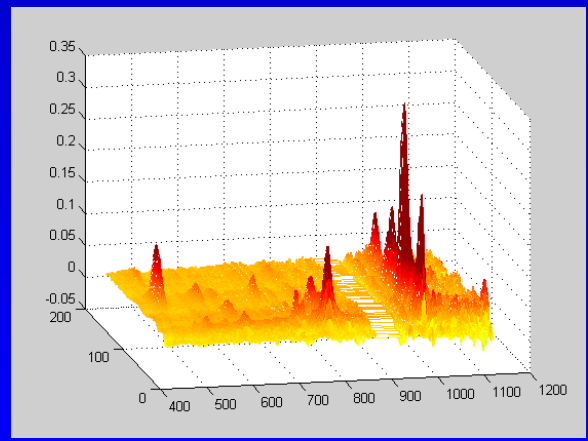
Detection

- **Multiwavelength fluorescence detection in a microsystem (Vogel Group)**
  - Δ **Phase 1: use confocal microscopy**
  - Δ **Phase 2: incorporate miniaturized system into microsystems**



Gretchen Baneyx, Loren Baugh, Viola Vogel, *Proc. Natl. Acad. Sci. USA* 98 (2001) 14464

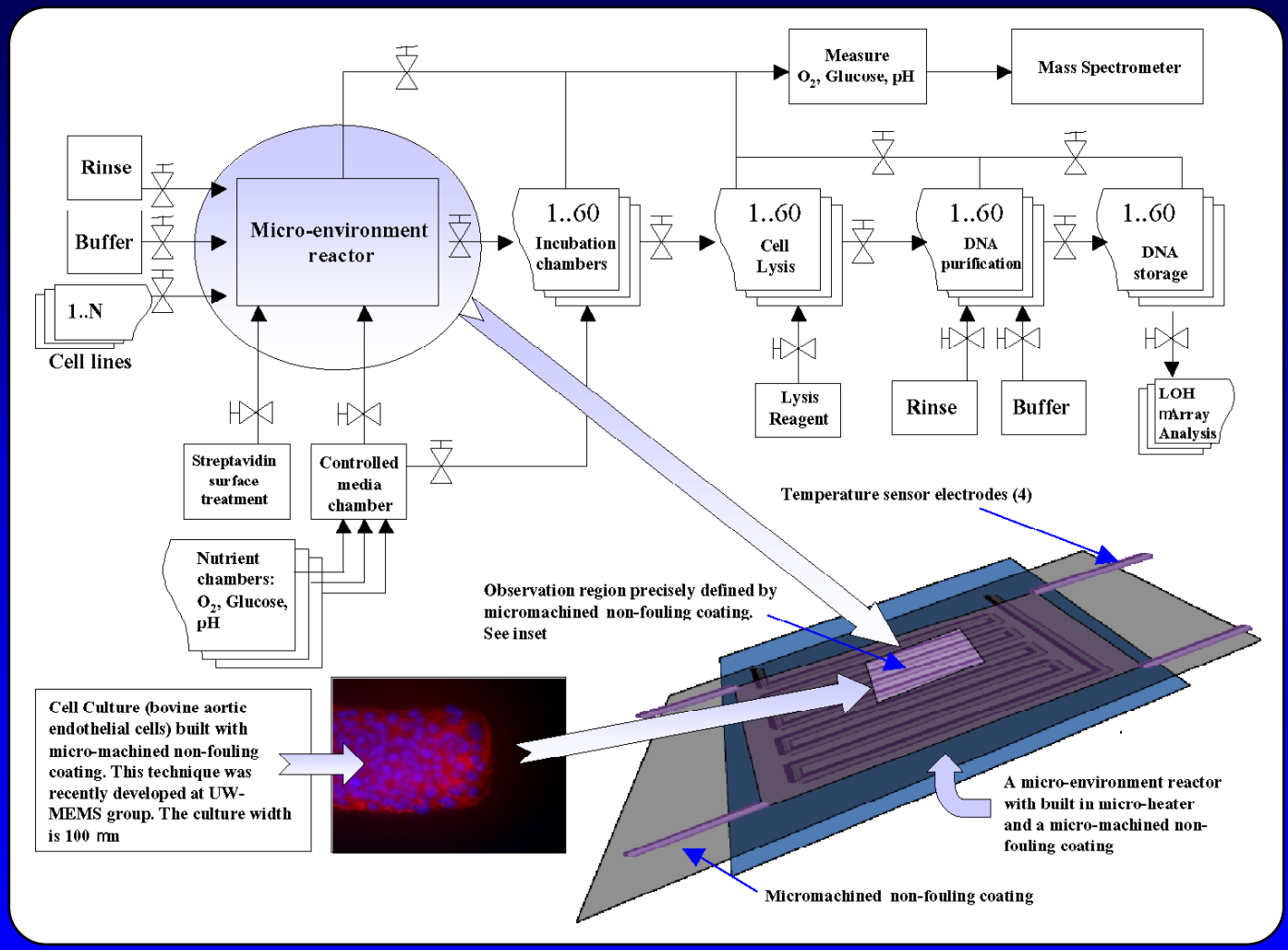
- **Ultra-sensitive (ppm-ppb) detection of small molecules as metabolic indicators (Burgess Group)**
  - Δ **Raman waveguide system**



(Burgess, Marquardt, et al., CPAC)

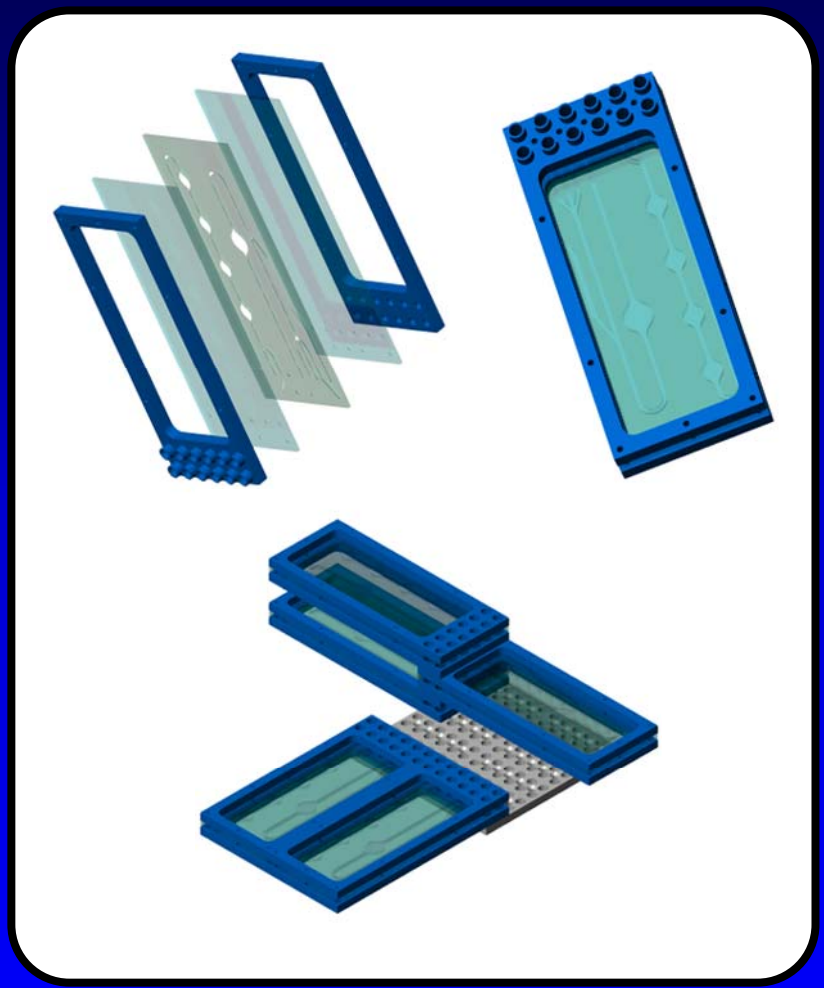
Example MEMS Module

(Microsystem for Study of *Saccharomyces cerevisiae* Growth and Progeny LOH Expression)



(Holl, Hanein, Gottschling, Meldrum)

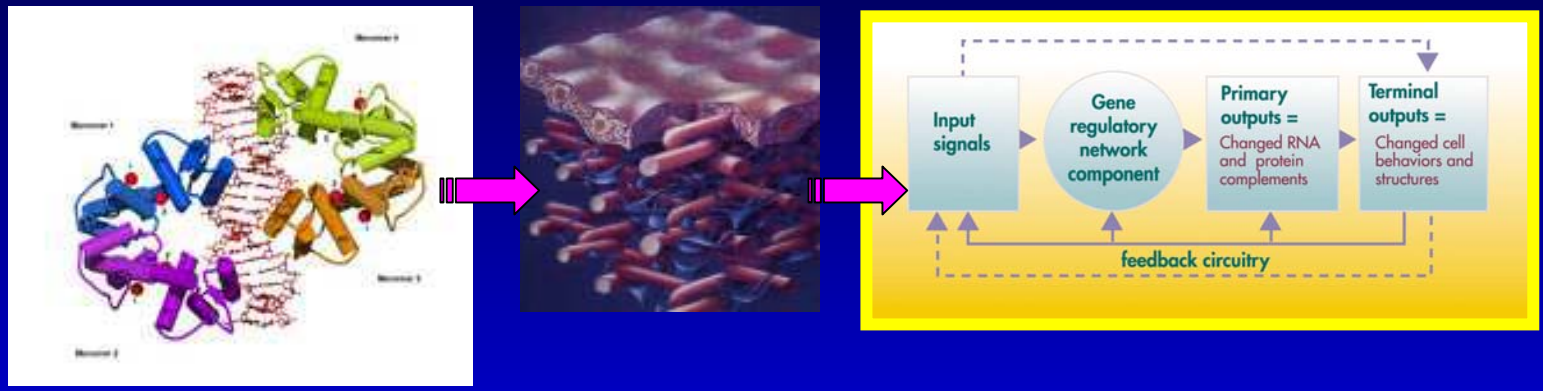
One of Several Modular Microsystem Integration Strategies ("Legome" [J. Doyle])



- Fluidic and electrical connectivity
- Mechanical probe access
- Optical access
- Thermal access
- Magnetic probe access
- Flexible
- Easily manufactured
- Rapid turnaround
- Low cost

New Course Winter 2003
EE546 Biology & Genomics for Engineers

(Advanced Topics in Control System Theory)



From molecules to cells/tissues to systems

- for students with no biology background
- learn about biology from an engineering perspective
- combine problem-based learning, computational analysis and hands-on laboratory exercises

Microscale Life Sciences Center

Acknowledgments

- NIH National Human Genome Research Institute for funding the MLSC CEGS and ACAPELLA Advanced Development
- Faculty, staff, post-docs, graduate students, and undergraduate students that participate in this research

<http://www.life-on-a-chip.washington.edu>