Automating Life-on-a-chip for Biomedical & Ecogenomic Applications

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NIH NHGRI
Centers of Excellence in Genomic Science (CEGS)

Microscale Life Sciences Center (MLSC)
• University of Washington & Fred Hutchinson Cancer Research Center
• Started August 2001

Goal: Develop microscale modules to measure multiple parameters in living cells in real time to correlate cellular events with genomic information

“Life-on-a-chip”
MLSC Investigators
Build the Interface Between
Biomedical Applications & Technology

Cookson (UW/Micro/LabMed/Pyroptosis)
Hockenbery (FHCRC-UW/Mol. Oncology)
Lidstrom (UW/Microbiology/Metabolism)
Reid (FHCRC/Neoplastic progression/Cancer Biology)

Böhringer (UW/ElecEngr/MEMS)
Burgess (UW/Chem/Detection)
Dovichi (UW/Chem/Separations)
Hartshorn (Brandeis/LATE-PCR)
Holl (ASU/ElecEngr/Microfluidics)
Jen (UW/Matl Science / Sensors)
Meldrum (ASU/Biodesign)
Parviz (UW/ElecEngr/Nanotech)
Wangh (BrandeisLATE-PCR)

Applications drive innovations in technology development

Technologies enable biologists to push frontiers & answer new questions
MLSC Goal: Understanding, Predicting, and Diagnosing Cell Function/Dysfunction

• Challenge
  △ Inherent heterogeneity of cell populations [Raser & O’Shea 2005]
    – Gene expression
    – Phenotype
  △ Heterogeneity at cellular level underlies transitions to disease states
    – Cancer
    – Inflammatory response-linked diseases

• Approach
  △ Microscale technology for dynamic, real-time, multi-parameter analysis of single cells
  △ Apply this technology to fundamental problems of biology and health for early diagnosis and treatment
MLSC Vision: Single Cell to *In Vivo* Analysis of the Live/Die Decision

**Phase 1**
Single Cell in Isolation

**Phase 2**
Cell-Cell Interactions

**Phase 3**
Tissue

**Phase 4**
*In Vivo*

MLSC (years 01-10)

- Global response to live/die stimuli
- Cell signaling factors
- In live/die decision
- Clonal evolution
- Influence of genomic variation
- Genomic fingerprints of *in vivo* progression at the single cell level
- Epidemiology
- Clinical research
Common Theme:
Cell Damage & Cell Death Pathways

Normal Cell

Death Stimulus

Apoptosis

Pyroptosis

Dying Cells

Dead Cells

Loss of Membrane Integrity

Others?

DNA damage

Inflammatory cytokines

Others?

Inflammatory contents

Inflammatory contents

Inflammatory contents

Apoptotic bodies

Autophagic vacuoles

Organellar swelling

Pyroptosis

Caspase-1

IL-1β

DNA damage

Inflammatory contents

Necrosis

Inflammatory contents

Acroptotic necrosis

Cell Damage & Cell Death Pathways

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Acroptotic necrosis
Disease States

- Disease states represented by suite of problems related to cell death include
  - Cancer
  - Heart disease
  - Stroke
  - Inflammatory bowel disease

- Inflammation is key to a variety of diseases
Approach

• Single-cell & cell-cell analysis to dissect mechanisms involved in live/die decisions

• Two model systems
  ▲ Proinflammatory cell death or pyroptosis (mouse macrophage)

 ▲ Neoplastic progression of Barrett’s esophagus (human epithelial cells)
Cellular Heterogeneity: Genomic
Barrett’s epithelial cells – chromosome 17

Normal

Abnormal

Green=centromere
Red=arm with p53

Brian Reid, Tom Paulson, Carissa Sanchez
Heterogeneity within Barrett’s Crypts by FISH

# of centromere spots
# of 17p spots
# of observations

- 2,2 (39)
- 2,3 (9)
- 3,2 (3)
- 3,3 (2)
- ≥4,2 (2)
- ≥3,≥3 (1)
- 1,1 (4)
- ≤1,2 (6)
- 2,≤1 (5)
Cellular Response Questions
linking genomics to phenotype

- Biochemical Cues
- Infecting Agent Cues
- Temporal Cues

- Metabolic Networks: Global response to environmental change?
- Infection: Global changes in agent & host?
- Cancer & Aging: How does the genome change over time?

- Understand dynamics of cell function
- Use for diagnosis and therapeutics
Stimulus → Response of Barrett’s Epithelial Cells

Cellular Heterogeneity of Single Cells

- To analyze crypts (from patients during progression)
- To analyze abnormal cell lines derived from patients

- Proposed experiment: determine kinetics of change in multiple parameters (genomic, genetic, physiological) during and after exposure to an agent (acid, bile, gastrin) that induces oxidative damage, in single cells

- Measure:
  - Δ Respiration rate (mitochondrial function)
  - Δ Mitochondrial membrane potential (mitochondrial function)
  - Δ Viability
  - Δ Reactive oxygen species
  - Δ Cell membrane integrity
  - Δ Nuclear DNA content
  - Δ Loss of heterozygosity for key markers
  - Δ Specific genome rearrangements
Multiparameter Measurements
Physiological, genetic, genomic, proteomic, transcriptomic

1. **O₂** uptake (respiration rate)
   - mitochondrial function
   - activity/latency
   - screen metabolic capabilities

2. **Multiwavelength fluorescence** /
   **Electronic detection**
   - membrane potential
   - membrane integrity
   - ion gradients
   - substrate utilization
   - DNA content
   - surface markers

3. RT-, q-, linear, & conventional PCR
   - expression of targeted genes
   - loss of heterozygosity (LOH)
   - gene copy number alterations

4. Single cell proteomics
   - proteomics fingerprints
   - link between protein profiles
   & cell death/life

5. Single cell transcriptomics
   - global gene expression
   - correlate transcripts & proteins
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Real-time Measurements
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Post-fixation Measurements
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General Configuration

\[ x(t), y(t), z(t) \]

\( \{I_B(t_i), I_C(t_i), I_G(t_i), I_Y(t_i), I_R(t_i), V(t_i)\} \)

\( t \in [t_0, t_f; \Delta t_f] \)

\( j \in [1, N_{chamber}] \)

Media+Cells

Media

Media+A

Media+B

Rinse Buffer

O\_2 Sensor

Lid

Lid control

O\_2 Sensor

Stage/Focus Control

Database

Storage

Multispectral and Time-domain Imaging

Microwell Array

Waste

T = 37 °C

5% CO\_2

T = 37 °C

5% CO\_2
Microwell Sensor Array Chip

Microwell array with sensor rings

[Holl, McQuaide, Meldrum et al.]
Oxygen consumption – five cells (A549 – human lung epithelial cells) #1

Hoechst 33342

[Dragavon, McQuaide, Holl, Molter, Meldrum]
Oxygen consumption – single cell (A549 – human lung epithelial cells) #2

Hoechst 33342

Propidium Iodide (PI)

[Dragavon, McQuaide, Holl, Molter, Meldrum]
Current Chip Layout

- All microwells seeded simultaneously (random seeding)
- Can sequentially perform oxygen consumption measurements on multiple locations
Living Cell Array (LCA)

[Holl, Meldrum]
Sequential Drawdowns

- 2 Locations
- 1 chip
- 4 sequential repetitions
- Mouse macrophage
MLSC Living Cell Array Analysis Cassette

- 11 independently addressable cell arrays
- 100-400 cells per cassette depending on configuration
- Provision for optical and electrical interrogation of individual cells
Platform Architecture

37 °C, Fluidics/Miniature Robotics Enclosure

- Linear lid closure
- z-stage actuator
- Replacable lid assembly
- Auxiliary electronics PCB daughterboard (option)
- On-board electronics PCB (option)
- Electrical interface (standard)

xy-Microwell lid stage

Compliance layer

Lid technology
1. O₂ barrier
2. O₂ permeable

Retractable 5% CO₂ chamber lid

Reagent Waste

37 °C, Objective chamber temperature control enclosure

4 mm
Platform Architecture

37 °C Microscope enclosure, above custom xy-platen, and below, in the objective turret chamber.

Front panel access for reagent cassette, analysis cassette, and lid rack insertion.
Loading of Cells in LCA Wells

- Random seeding of cells in wells
- Patterned protein assisted random seeding
- Microfluidic row assisted loading
- Deterministic active loading
  - Microeddy trap
  - Capillary sampling and placement of cells of interest using
    - Manual proof-of-principle (already being done with yeast and in Dovichi systems)
    - Automated machine vision system (planned)
Trapping with Acoustic Streaming

- coverslip with piezo
- channel layer
- microscope slide

Audible frequencies (~1000 Hz)

B. Lutz, D. Schwartz, S. McQuaide, M. Lidstrom, D. Meldrum
Microeddy Trapping System

- Cylinders: 100 μm diameter
- Microspheres: 20 μm diameter
- Fluid oscillation + steady flow

 steady flow
Digital Confocal Microscopy for a Living Cellular Array Scanner

Joseph Chao, Mark Holl, Deirdre Meldrum
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Enabling Technology
Rapid Microfabrication of 3D Structures

- Structures developed using a CAD program
- Structures easily fabricated in 3D
- Reporters may be incorporated into structure

[Cody Young, Alex Jen]
Electronic Sensor for Single Cells

- Direct (label-free) detection of nanomolecule in volume occupied by single cell
  - Semiconducting nanowire sensor
    - conduction change upon binding of target molecule
  
- Successfully detected ions: (H+), proteins (streptavidin), small DNA fragments, and single base-pair mismatches in short DNA strands (12 bases long)

[Babak Parviz, et al.]
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Reproducibility LATE-PCR SNP genotyping with a single Molecular Wire probe for the three possible genotypes for the rs858521 SNP site near TP53 on human chromosome 17.

Pyrograms generated from LATE-PCR prepared single cells. Plots A-C show homozygous wild-type, heterozygous and homozygous mutant sequences for the IVS 110 mutation in the human β-globin gene.

[Larry Wangh, Jesse Salk, Brian Reid, Carissa Sanchez, Deirdre Meldrum]
Two-Dimensional CE for Single Cell Proteomics

Norm Dovichi
Fully automated 2-D electrophoresis
single MCF-7 cells

Norm Dovichi
High throughput analysis

multiple capillary interface

High throughput analysis

5X MECC separation of Barrett's esophagus proteins
Self-assembled low-cost arrays

[Babak Parviz]
Self-assembled low-cost arrays

Electronic, photonic, and other devices integrated on flexible transparent plastic

To this date: 10000 devices 95 % yield

[Babak Parviz]
LEDs On Substrate

• Successful fabrication of LEDs
• Emission wavelength 688nm
• ~300 microns in diameter

[Kim, Parviz, Meldrum]
Extensions of “Life-on-a-chip”

• Tools that enable
  △ Understanding of how systems work
    – Living cells
    – Cell-to-cell interactions
    – Populations of cells
  △ Disease monitoring and diagnosis
  △ Screening for drug discovery
  △ Cancer and aging studies
  △ Exploration
    – Microbial populations in the ocean via ecogenomic sensors
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Biological Sensors

Ginger Armbrust
What are ecogenomic sensors?

Biological sensors that detect

Who is there?
How many are there?
What are they doing?
Where are future technologies headed?

• Sensors and sensor platforms for continuous real-time measurements in time and space
Extending Life-on-a-chip to the oceans

Microwell array with sensor rings

[McQuaide, Holl, Meldrum]
Initial Tests with the R/V Thompson & Underwater Robot, Jason – September 2005
Materials Tested

• Borosilicate glass with platinum porphyrin polymer
  Borosilicate glass with platinum porphyrin beads
  (melted onto surface)
• Fused Silica with platinum porphyrin polymer
  Fused Silica with platinum porphyrin beads
  (melted onto surface)
• Sapphire with both platinum porphyrin polymer and
  platinum porphyrin beads (melted onto surface) at
  different locations on same sample
• Experiment
  △ Samples placed in diffuse flow site, 25-30 degrees C, at Endeavor
  Ridge in NE Pacific for 1 year
Retrieved Samples with the R/V Atlantis & Alvin
1 year later – September 2006
Alvin - submersible
Alvin on the seafloor – 2200 m deep
Alvin on the seafloor – 2200 m deep
Samples from Alvin
2200 m deep, ~25 degrees C, 1 year
Post-submersion photos of samples: Fused silica and sapphire
Post-submersion photos of borosilicate chips with wells
Samples with pt porphyrin beads had no evidence of fluorescence after 1 year submerged in the diffuse flow site, BUT...
Borosilicate + polymer
still there after 1 year!

[McQuaide, Holl, Meldrum]
Sapphire + polymer
also still there after 1 year!

[McQuaide, Holl, Meldrum]
Ocean Observatories Initiative

**Basic Infrastructure:**
Network providing high bandwidth communications and electrical power

**Three primary components:**
- Global-scale moored buoy systems
- Regional-scale seafloor fiber optic cable system
- Coastal observatories

**Cyberinfrastructure** will allow users to remotely control their instruments, perform in-situ experiments, construct virtual observatories, and access data in near-real-time

The OOI will provide the ability to investigate processes at the scales at which they occur
Three components - NSF's Ocean Observatory Program

Global Network of Permanent and Relocatable Deep-Sea Buoys

Regional (Plate-Scale) Ocean Observatory

Expanded Network of Coastal Observatories
NEPTUNE-Canada
Regional Cabled Observatory - OOI

A OCEAN OBSERVATORY AT THE SCALE OF A TECTONIC PLATE

• 2000 Miles of electro-optical cable
• A network of submarine laboratories
• Next Gen Internet to the sea floor 10’s Gb/sec
• 100 Kw Power on Grid
• Robotic interaction at speed of light
• 30 yr life-time

John Delaney
Deb Kelley
Sensorbot Scenario
Turbulent the sea
Stretching across to Sado
The Milky Way

--- Basho
Acknowledgments

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  http://www.life-on-a-chip.org

- NEPTUNE project for ecogenomics research
  http://www.neptune.washington.edu

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