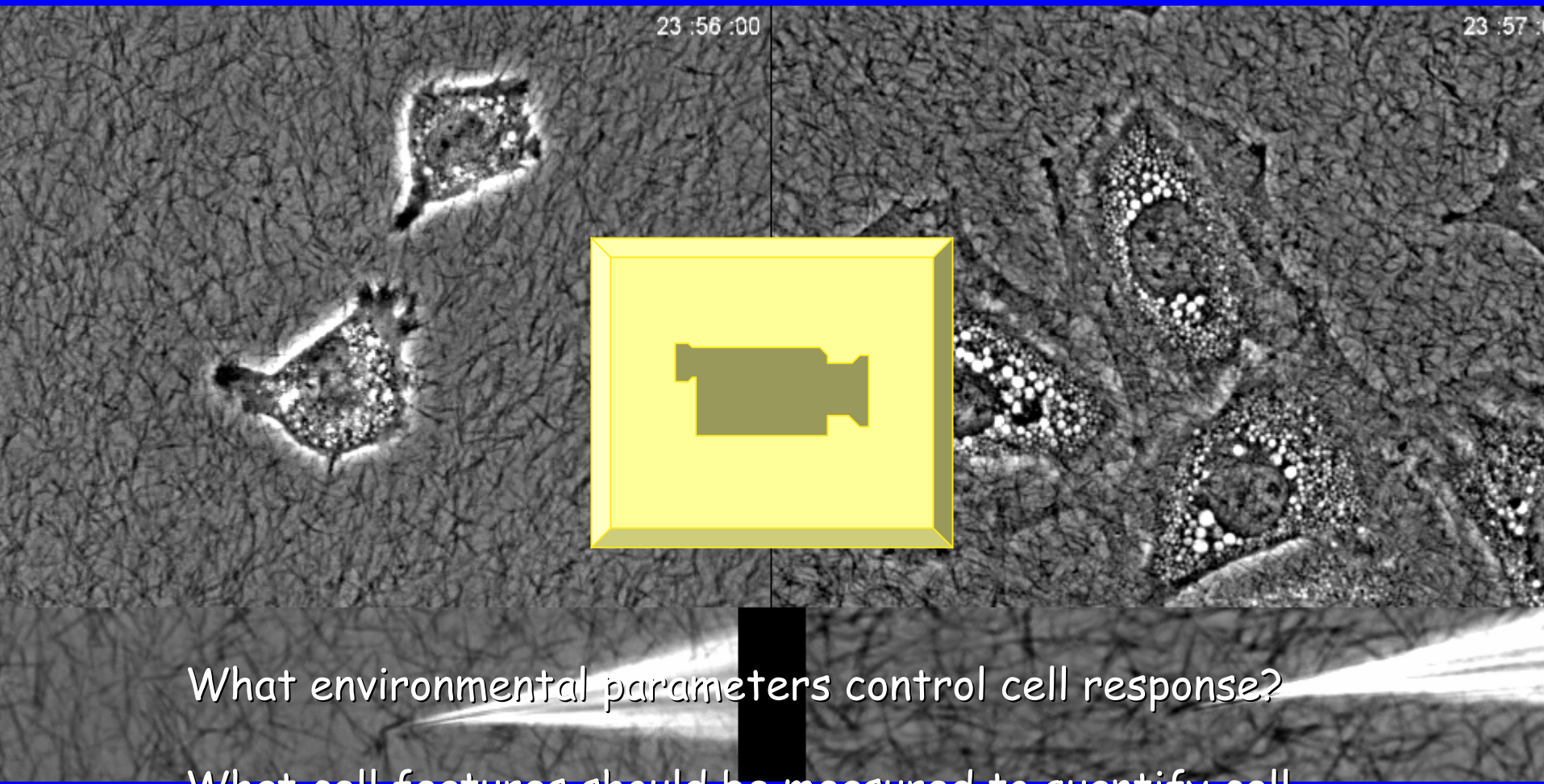
Metrology Infrastructure for Innovation in Cell-based Technologies

- Drug Discovery
- Cell, Tissue and Gene Therapies
- Cytotoxicity (environmental, defense, agricultural)
- Diagnostics / Pathology
- Personalized Medicine
- Systems Biology

NIST VCAT
September, 2006
Boulder CO

Anne Plant
Chemical Science and Technology Laboratory

What is the problem and why is it hard?

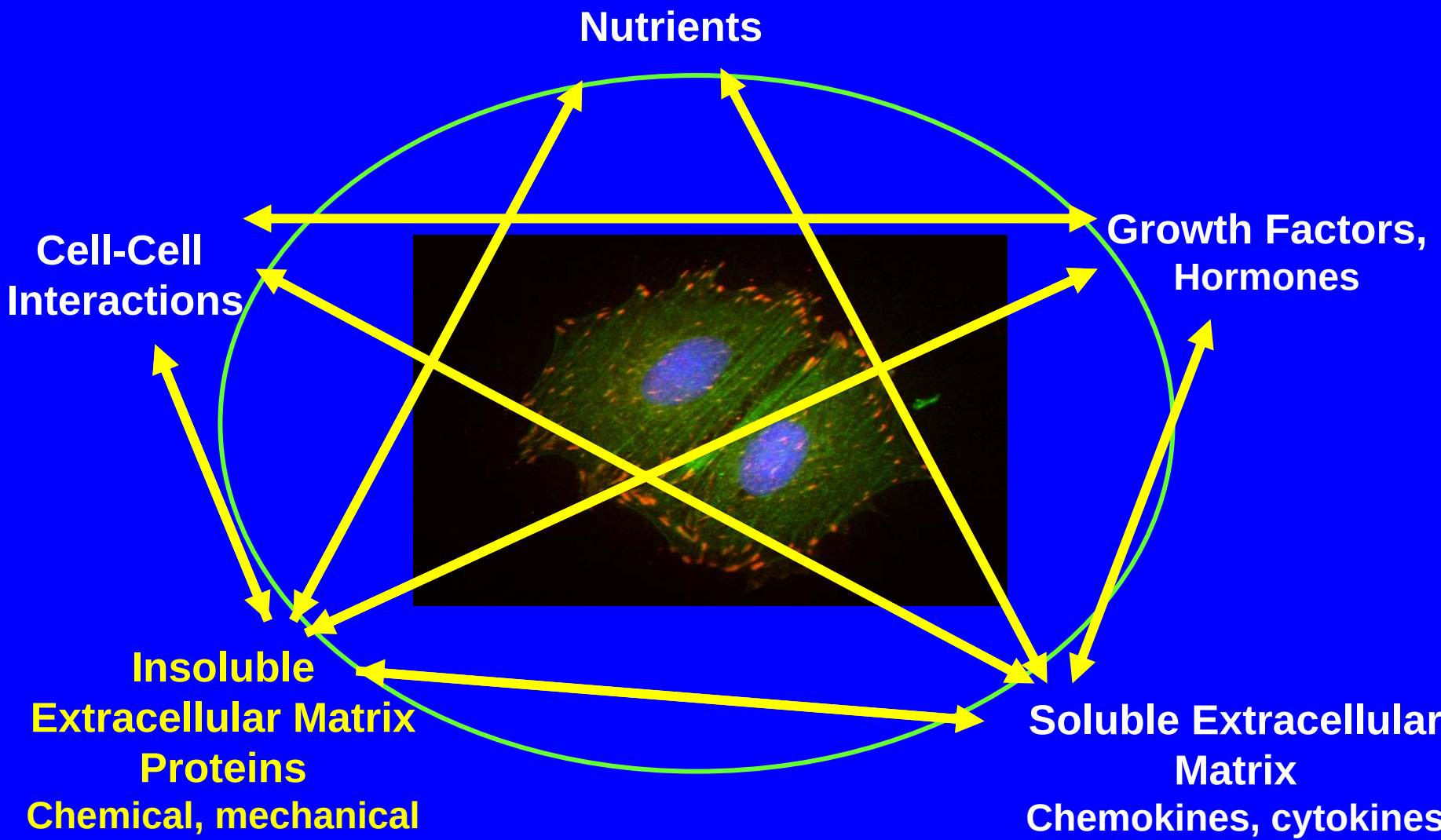


What environmental parameters control cell response?

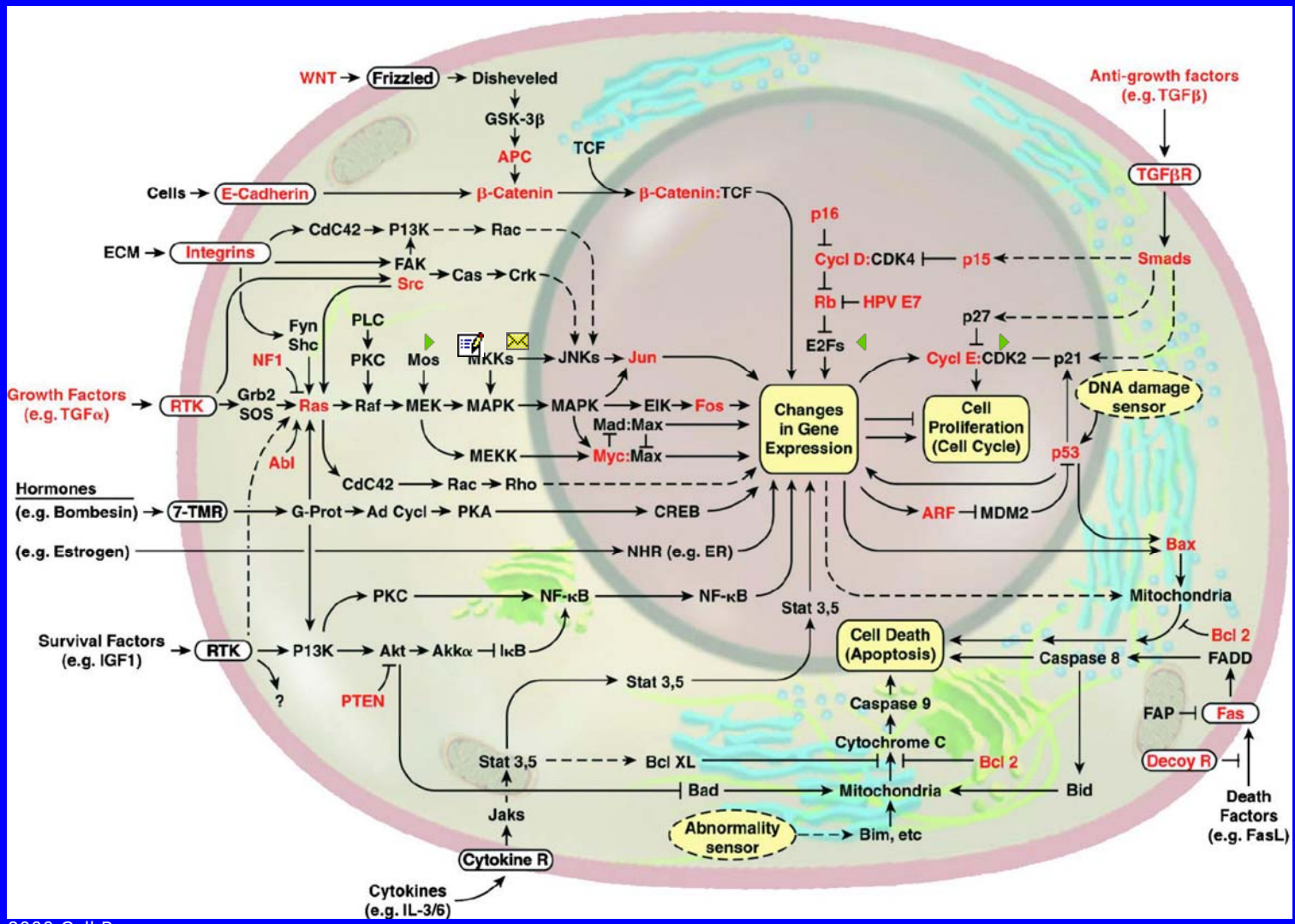
What cell features should be measured to quantify cell response?

What is the inherent variability in the biological response relative to the measurement uncertainty?

Complexity of Cell Response



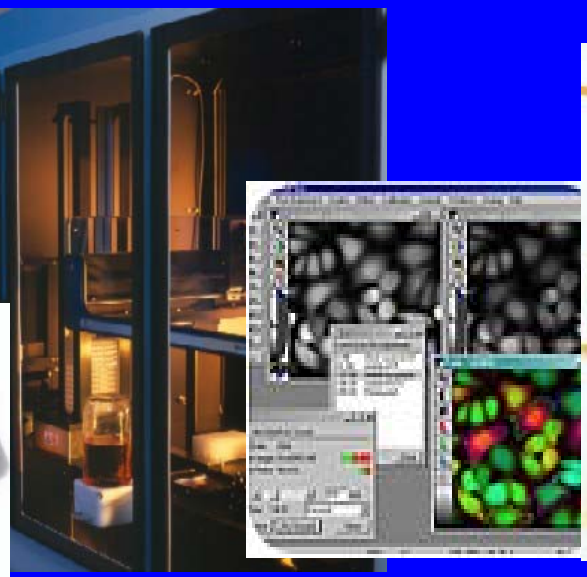
Complexity of intracellular signaling



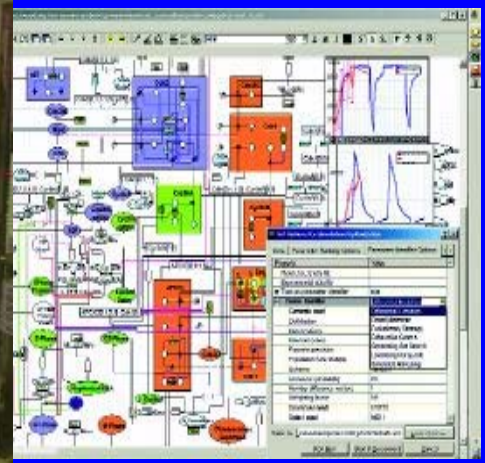
The Hallmarks of Cancer
Douglas Hanahan and Robert A. Weinberg

How are cell measurements approached today? **Drug Discovery**

High Content Screening: Cell-based imaging for rapid accumulation of multi-parametric data for model development



IN SILICO model of the cell cycle by Gene Network Sciences



Multi-well plates

High-throughput data collection

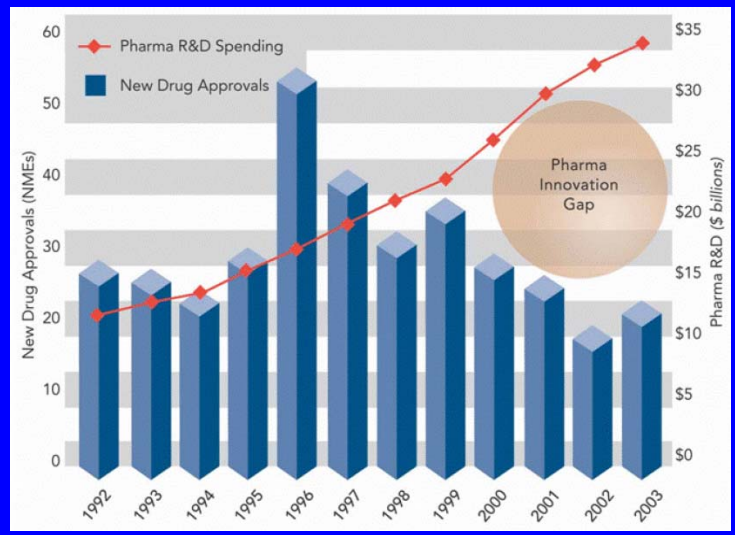
Image data

Large datasets

Intracellular pathway models

Magnitude of the problem Drug Discovery

- 92% Failure in clinical trials
- Failures due to toxicity/adverse effects cost $2B/yr
- 14yr product to market (R&D lead times/FDA approval)



Drug Costs Nearing $2 Billion, Warns Lilly Executive

By Kevin Davies, Bio-IT World

August 11, 2006 | BOSTON - The head of science and technology at Eli Lilly & Co. warned that the cost of producing a successful drug could top $2 billion by 2010 unless the pharma industry can identify new and better ways to improve efficiency and effectiveness of drug discovery and clinical trials.

Steven Paul, executive vice president at Lilly, issued his dire warning in the final keynote at the 2006 Drug Discovery Technology conference in Boston on Wednesday.

Key Assumptions-Part 1 of 3

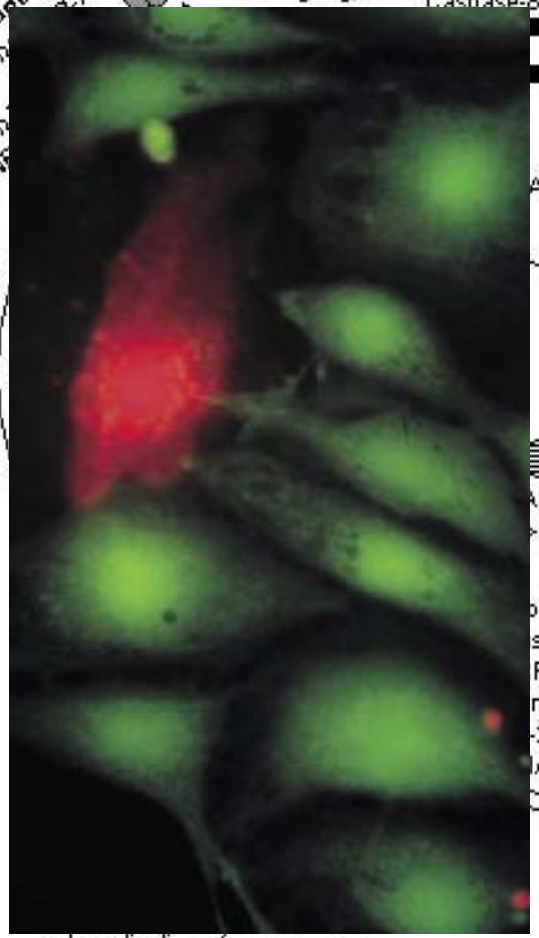
- The assay development **focus** should be on ***in vitro* assays**
  - *In vitro* responses are **less variable** than *in vivo* assays
  - *In vitro* responses have a **greater dynamic** range than *in vivo* assays

David C. Kaslow M.D.
Chief Scientific Officer
Vical Incorporated

Vical

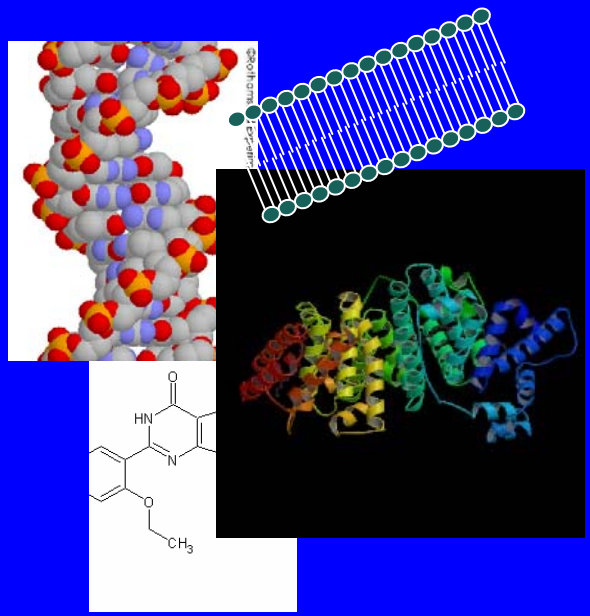
How are cell measurements approached today? **Cytotoxicity**

Example: nanoparticles



externalization of phosphatidylserine, membrane blebbing or budding, formation of apoptotic bodies...)

What is the challenge and why is it hard?



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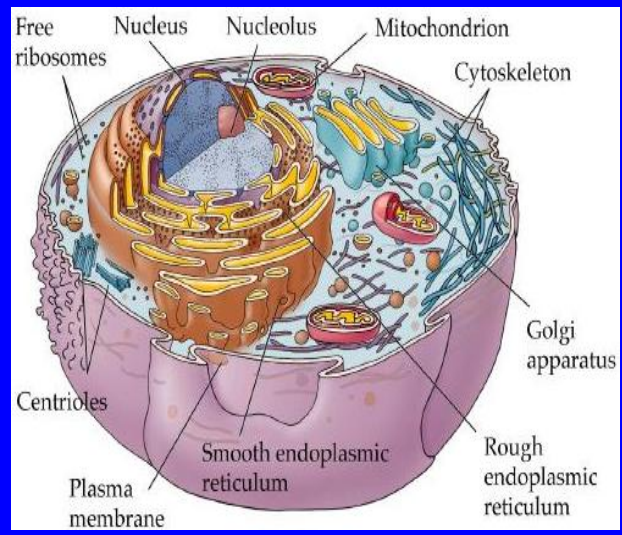


Physical Components:

DNA, proteins, lipids

Biological systems take advantage of:

Compartmentalization, spatial organization, stochastic effects, dynamic interactions, complex signaling pathways

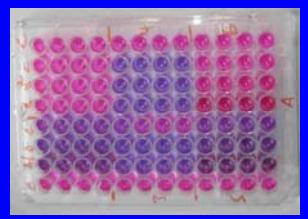
- Many cellular components and their integration
- Lots of data requires high throughput
- Spatial and temporal

Why NIST?

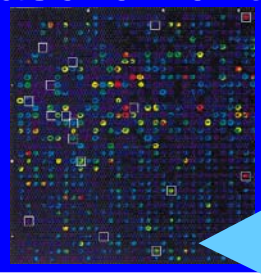
WTEC Panel Report on Assessment of International Research / Development in Systems Biology October, 2005

- Federal Agencies investing ~$140M/yr (Largely on Modeling)

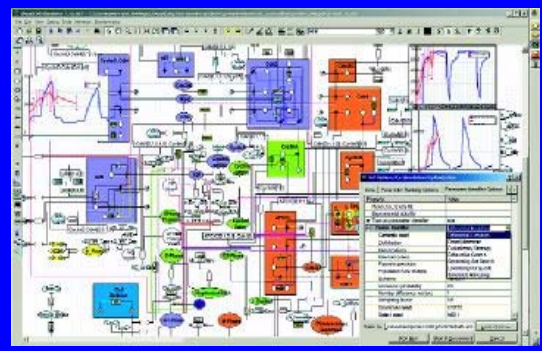
(Traditional)
Experimental Methods



(Inadequate)
Measurements



Models

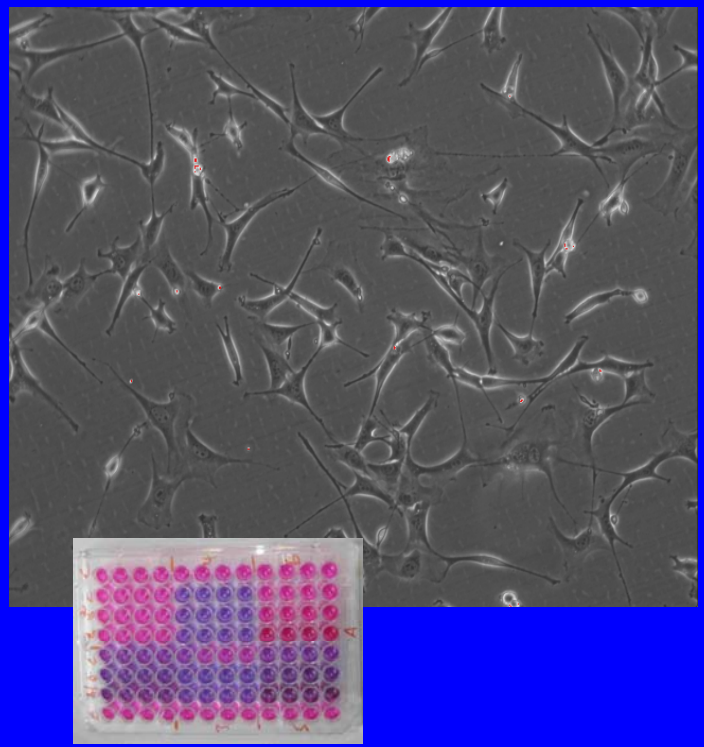


Improved Measurement Technology
Standards
Protocols
Validation
Error analysis

Role for NIST

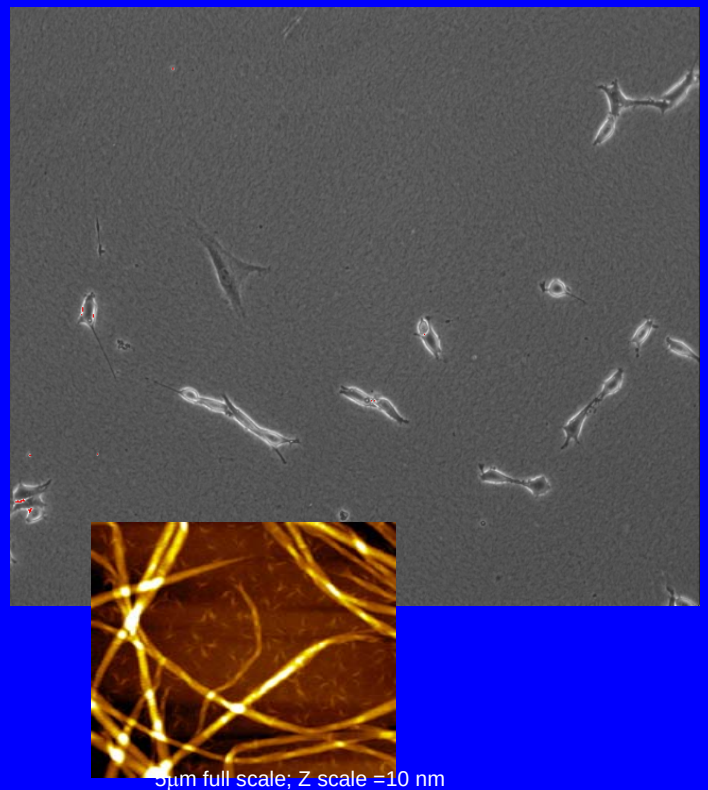
Appropriate and Controlled Cell Environments

NIH 3T3 fibroblasts on polystyrene



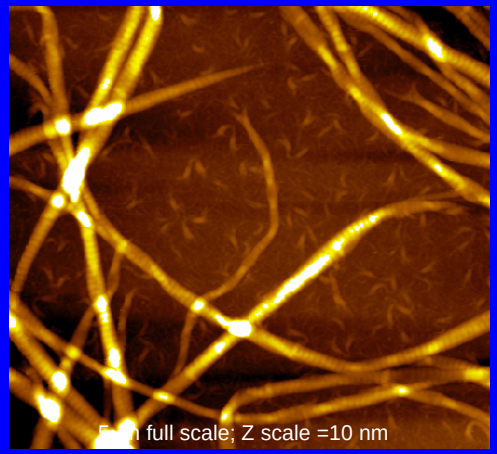
(traditional environment)

NIH 3T3 fibroblasts on the ECM protein, collagen

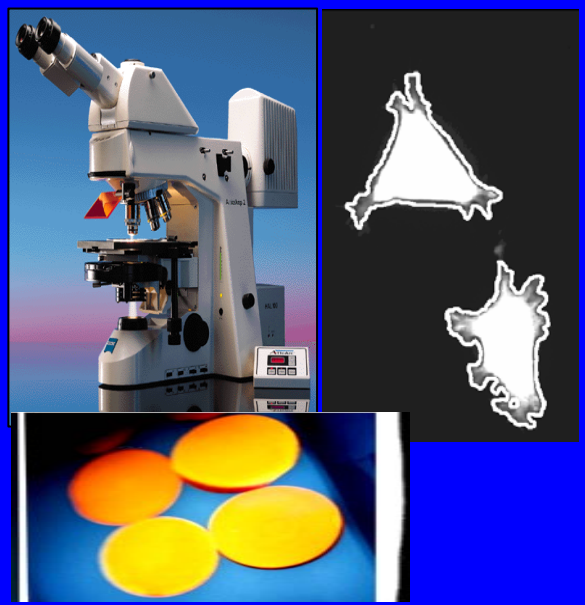


(more physiologically relevant)

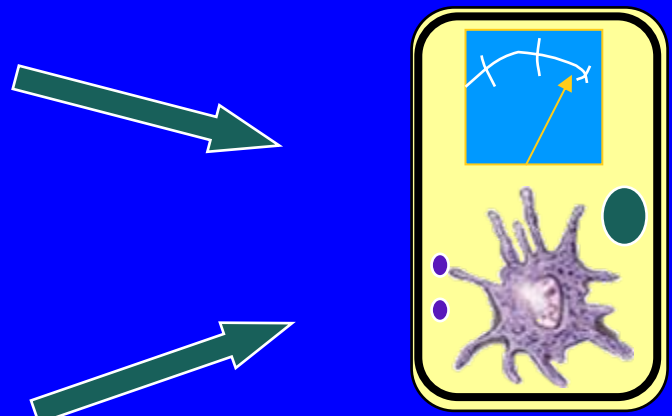
Quantitative Cell Measurements



Controlled cell environments *Reproducible surfaces of extracellular matrix (ECM) proteins*



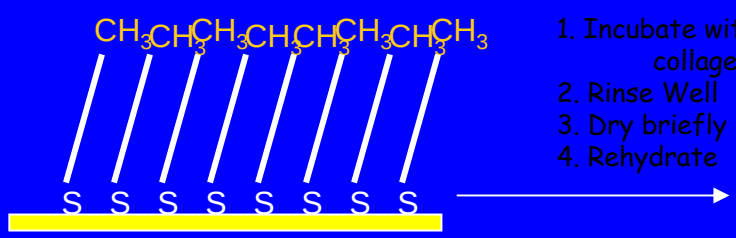
Quantitative fluorescence microscopy *Materials and protocols for accuracy and reproducibility*



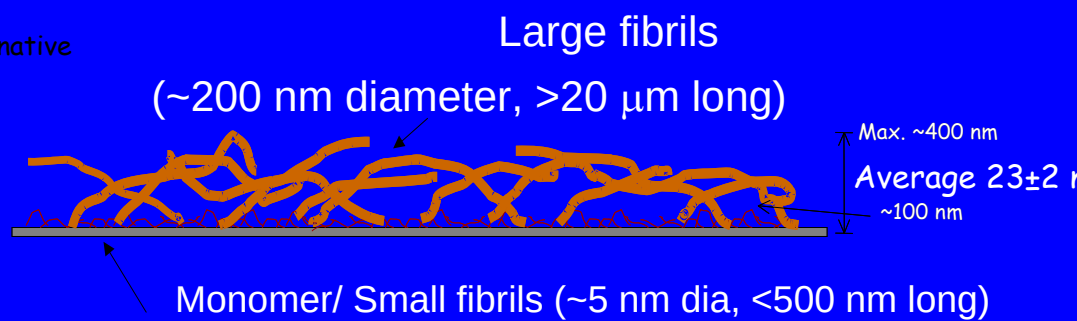
Determine cell response under known and reproducible conditions using quantitative methods

Thin Films of Type 1 Collagen

- **Alkanethiol ($C_{16}SH$) self-assembled monolayer on translucent Au (~50nm)**



1. Incubate with native collagen
2. Rinse Well
3. Dry briefly
4. Rehydrate



Advantages:

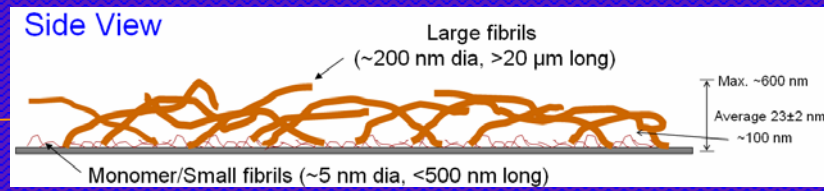
1. **Highly reproducible and spatially homogeneous.**
2. **Can be characterized and verified with surface analysis techniques.**
3. **Very robust and easy to use.**
4. **Excellent optical properties for microscopy**

Ellipsometry
IR spectroscopy
Surface Plasmon Resonance
Microscopy
Neutron Reflectivity

Mechanical Properties

Collagen Gels compared to Thin Films

Collagen Thin Films are less than 0.001 the Thickness of a Collagen Gel



Collagen and Cells: *Are thin films of collagen equivalent to thick gels?*

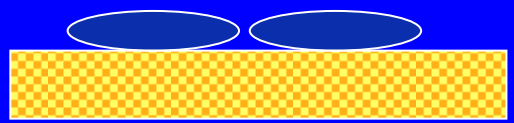
Normal Condition-
Growth Arrested Phenotype

- *β1 integrin*
- *Slow Cell Division*
- *Not well spread*
- *Poorly organized cytoskeleton*

Native Fibrillar Collagen

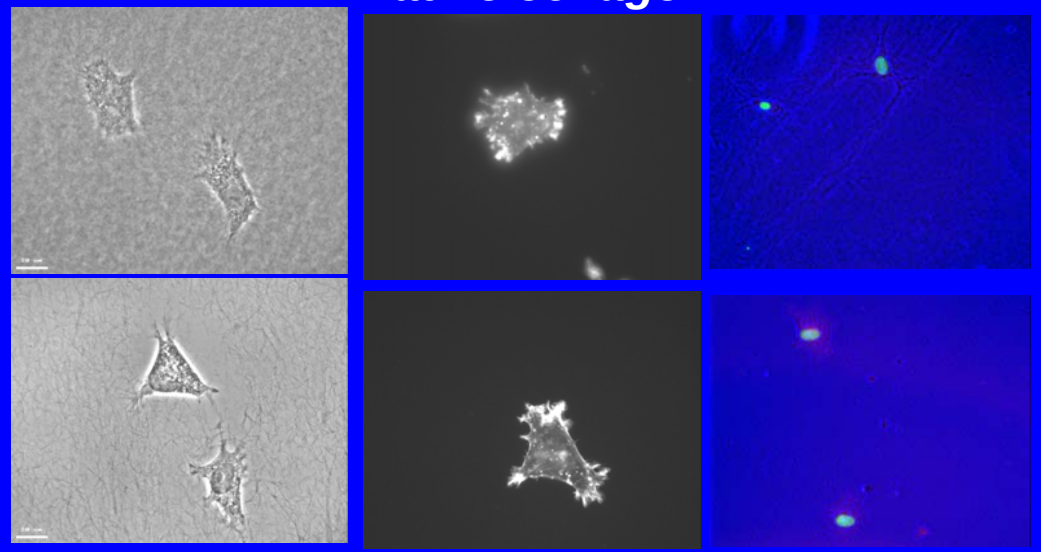
Wound Healing Response
Proliferative Phenotype

- *β3 integrin*
- *Rapid Cell Division*
- *More spread*
- *Strong actin stress fibers*
- *Express Tenascin-C*

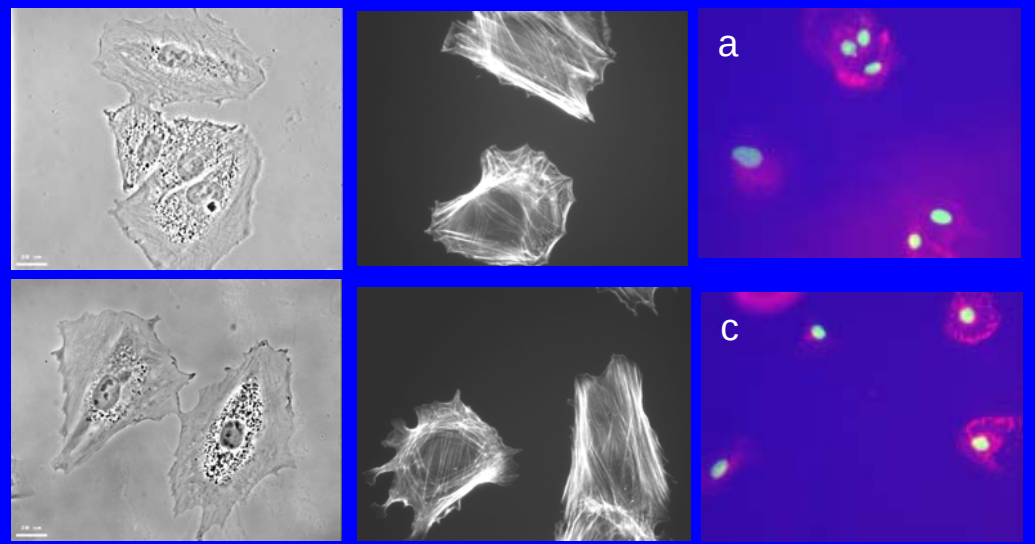


Denatured Collagen

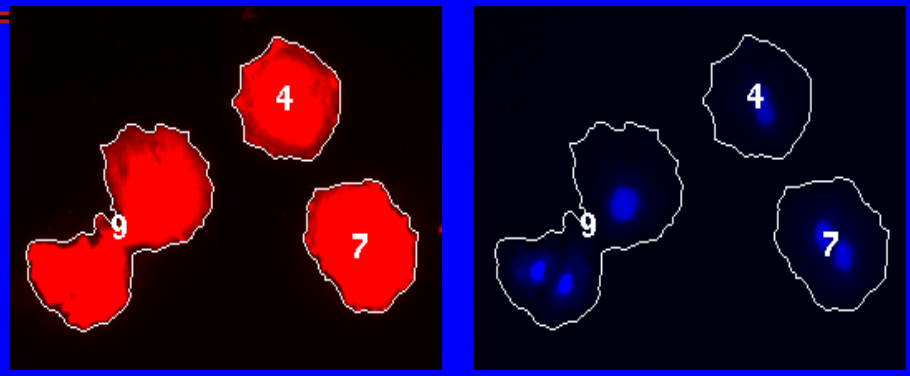
Native collagen

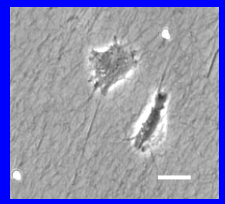


Denatured collagen

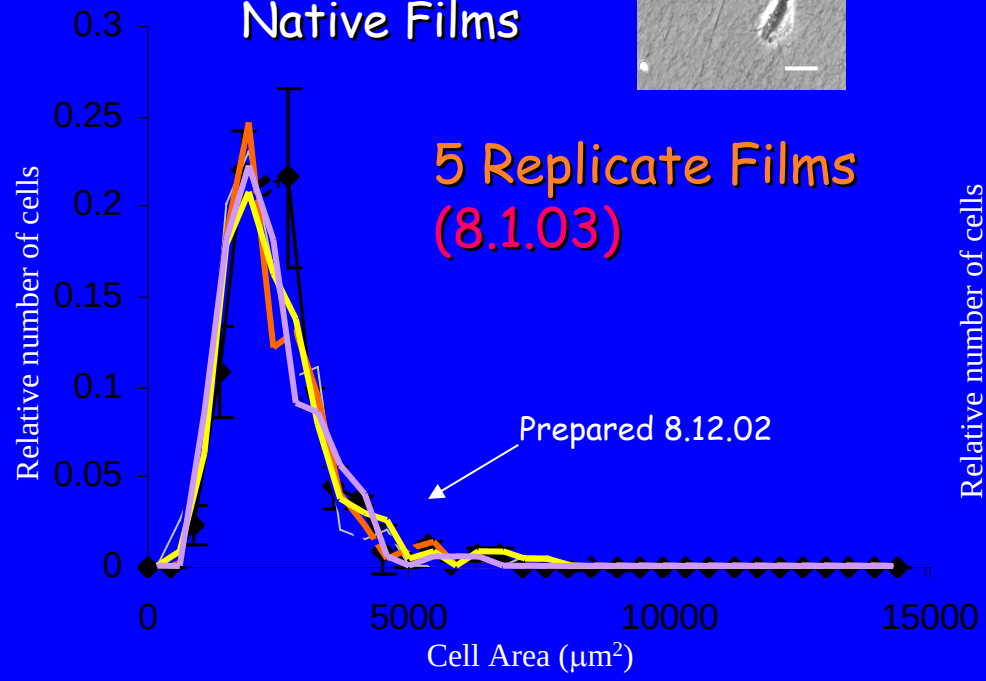


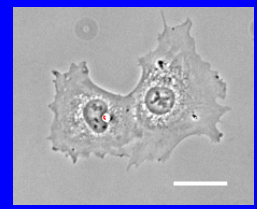
Quantification of cell morphology is highly reproducible





Native Films





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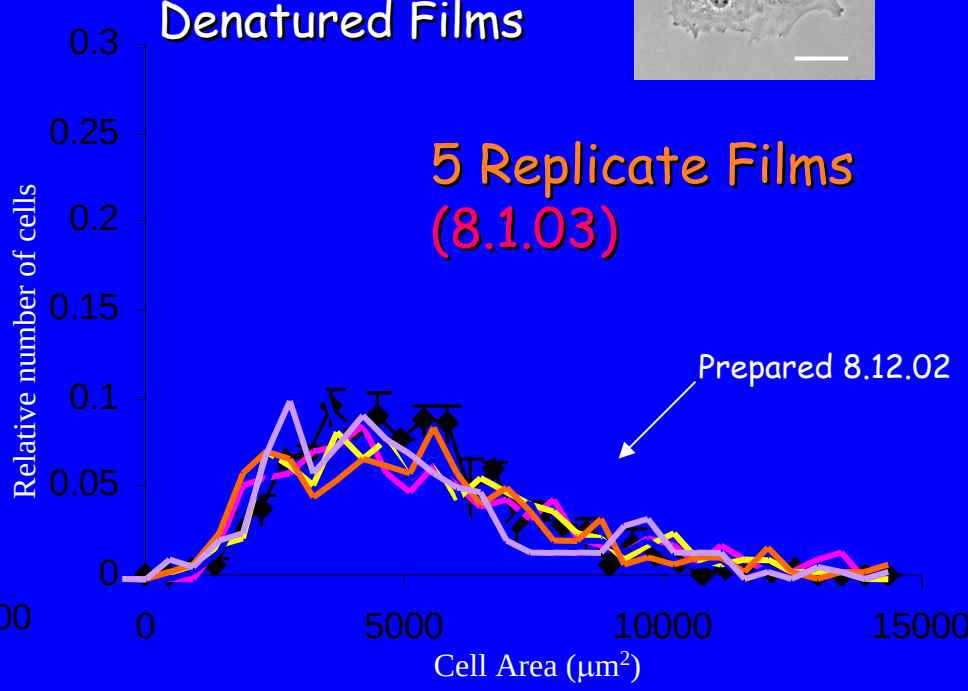
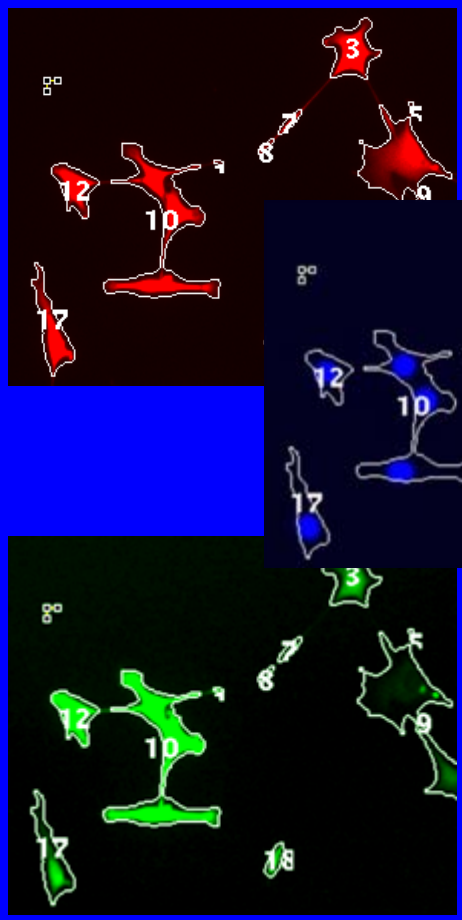
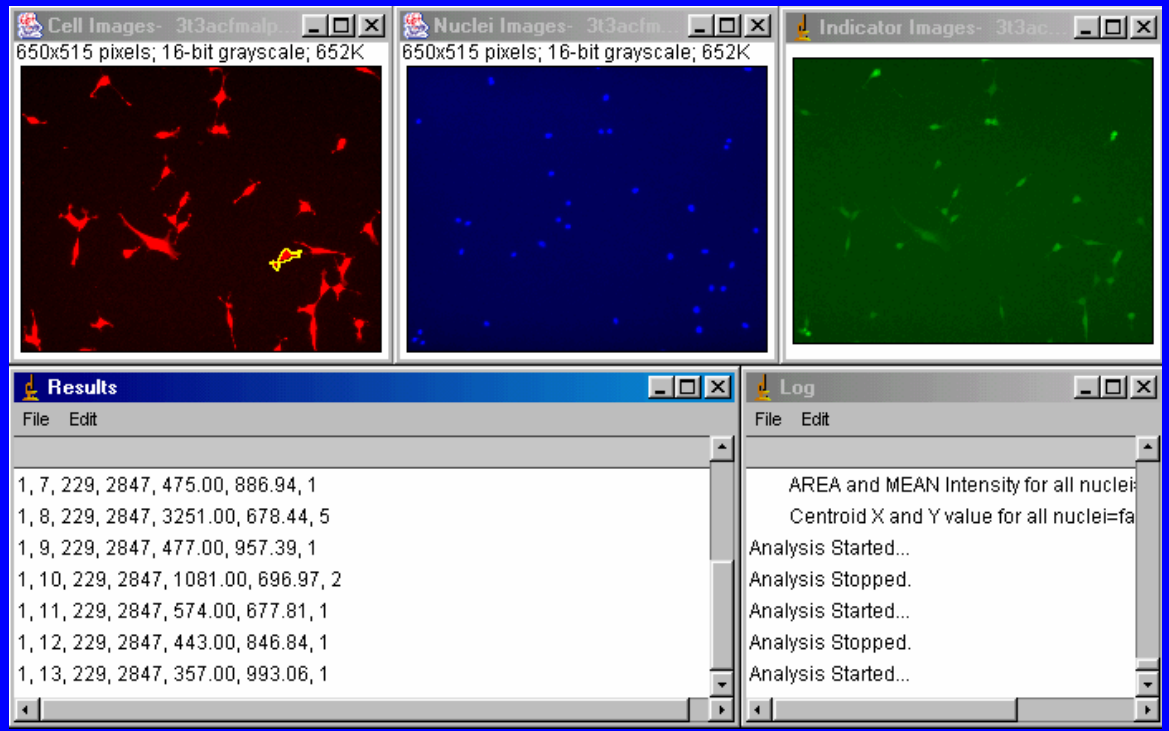


Quantifying Cells: Automated Cell Image Analysis





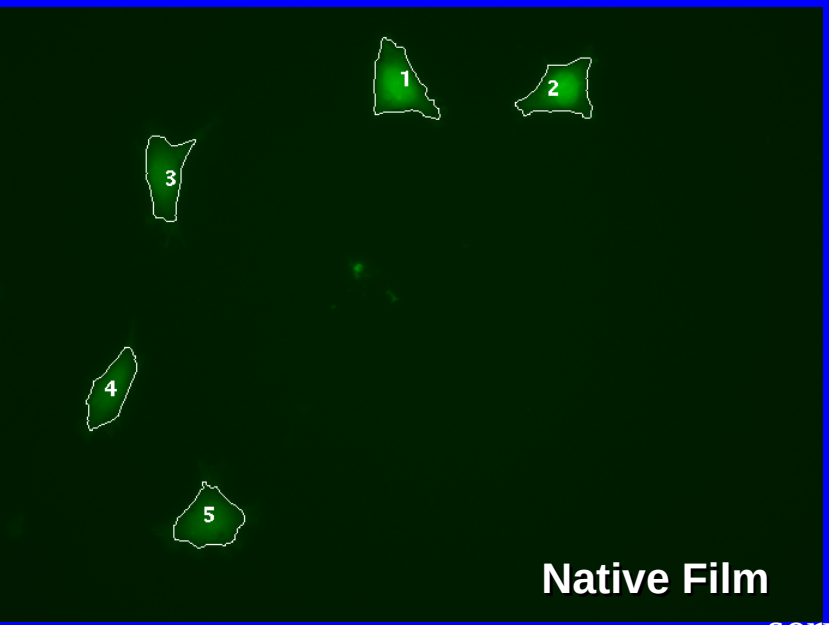
Measurement Tool Development

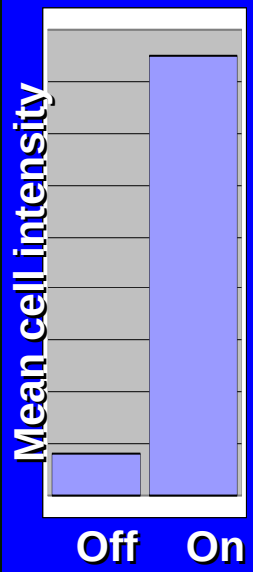
- Novel fluorescent stains
- Image analysis algorithms
- Intensity normalization

Quantifying GFP-Tenascin Expression

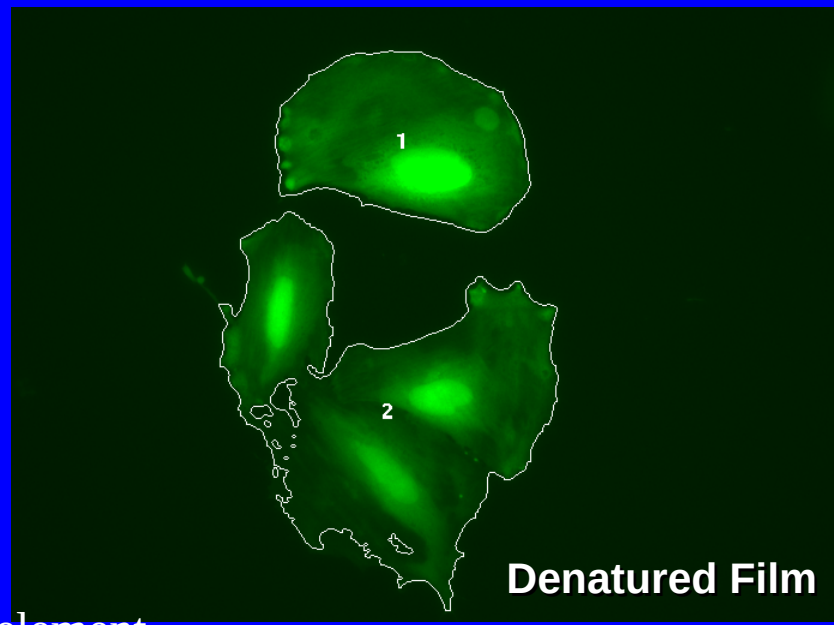
Cell fluorescence ∝ GFP expression ∝ Tenascin expression

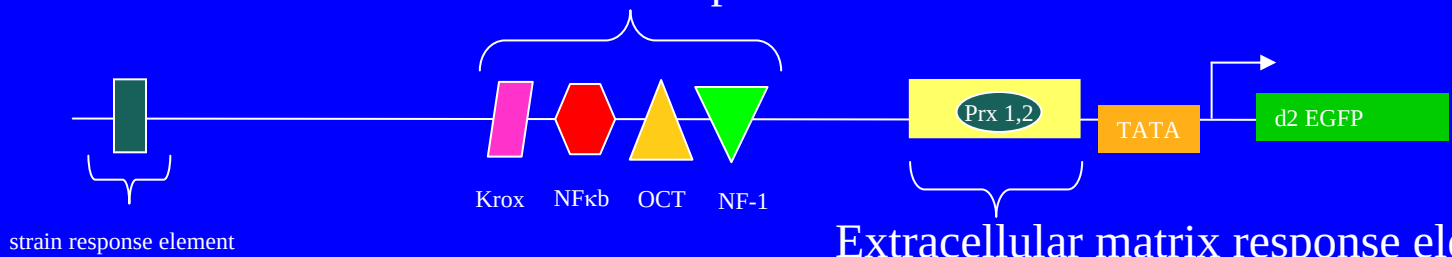
Non-Proliferative





Proliferative

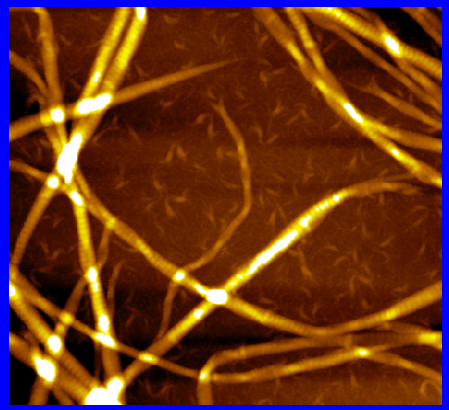




Quantitative Cell Biology

Known materials/ conditions

Allows comparison of responses



Collagen reference extracellular matrix film. 5μm full scale; Z scale =10 nm

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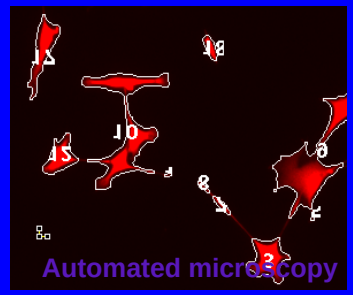
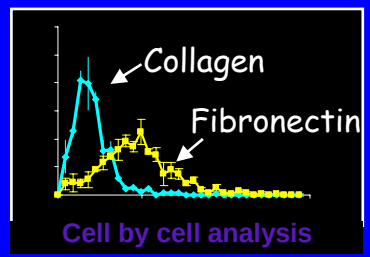
Quantitative Image Analysis





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Improved Biological Applications

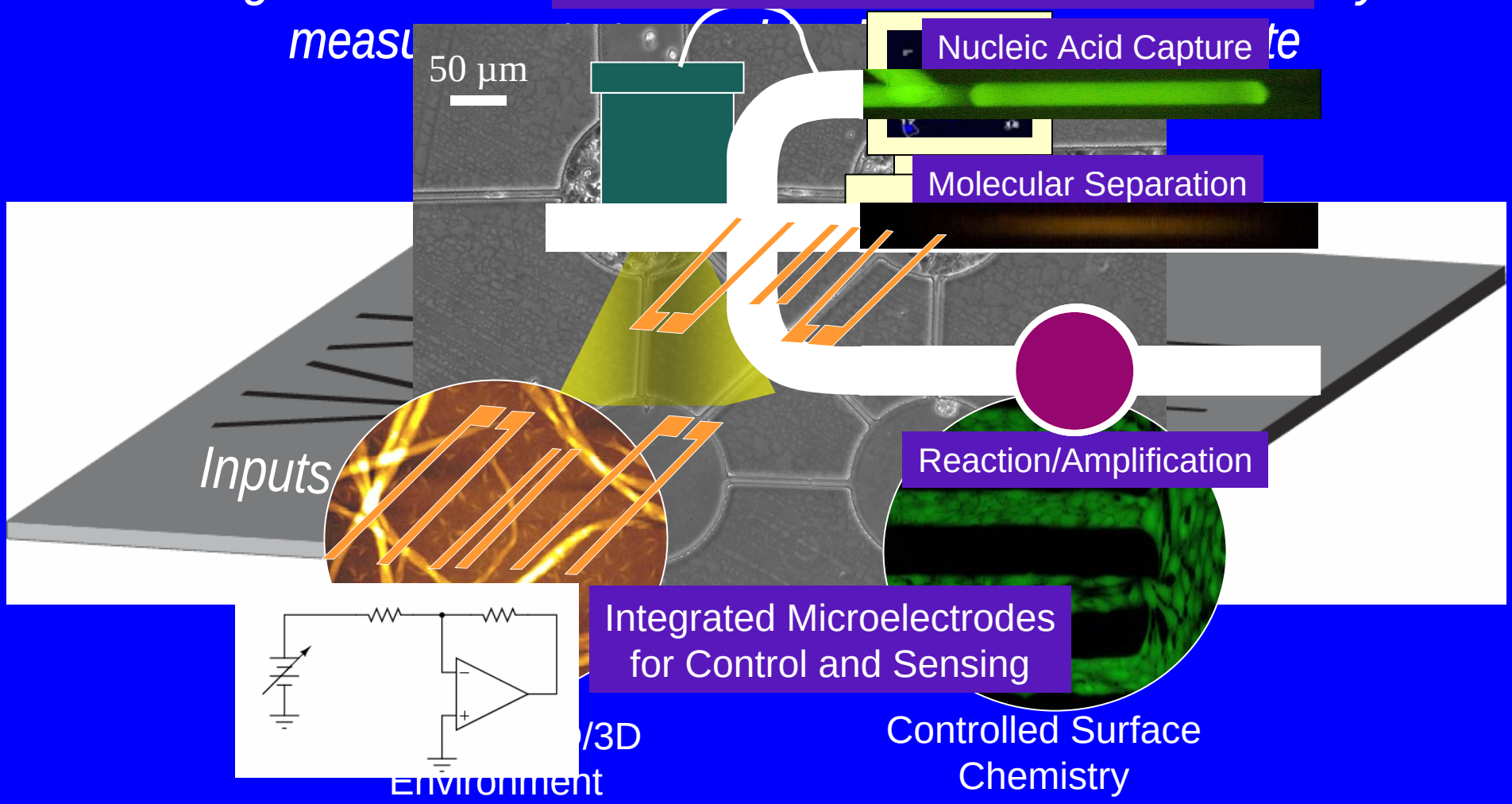
- **Robust protocols**
- **Comparable data**
- **Bioinformatics**
- **Quality control**

- **Drug screening**
  - **Soc for Biomolecular Screening**
- **Tissue Engineering**
  - **ASTM International**
- **Cytotoxicity**
  - **MUSC/Hollings Marine Lab**

Future

High throughput Microfluidic Control and Integration

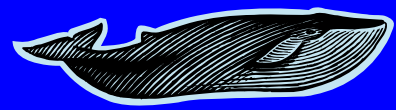
Combining micro- and n… …alytical measu… …te



Future

High throughput for multiparametric data and Cell Signature for Cytotoxicity

Impact: Environment, regulation/disposal, health, security, biotechnology



Test Conditions

Environmental Contaminant

Drug Candidate

Nanoparticles

Controlled Parameters

Cell Type

Cell Age

Species

Physical Measurements

Assay

Reactive Oxygen
Mitochondrial
Fluorescent Probes
Stress

Genetic Changes
Mutation
PCR & Sequencing
Single Molecules

Oxygen Uptake
Metabolic Rate
Electrochemistry

Cellular Signature

Future

Cell-based measurements: rapid accumulation of multi-parametric data from many laboratories for model development

This requires comparable data.
What limits comparability of data?

- **Poorly controlled, analyzed and documented experimental conditions**
  Poor inter- and intra- laboratory reproducibility
  Unknown comparability of data

- **No reference methods for validating image analysis**
  Insufficient statistical characterization
  No image data exchange formats

- **Databases are not interoperable**
  No determination of quality of the data
  No standards for data storage, including metadata

Future

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CONCLUSION: Understanding complex biological systems will require a lot of *comparable* data

High-throughput, quantitative data.

Measurement methodologies and protocols.

Better control of the experimental parameter space.

Robust extracellular matrix materials.