

Trends in Tools and Strategies for Quantifying Biological Response

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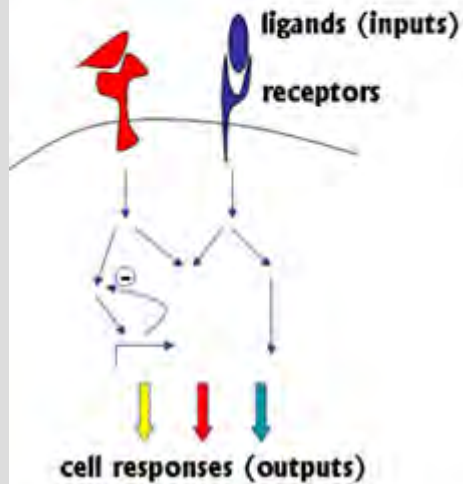
Workshop on In Vitro Analyses of Cell/Scaffold Products
December 7, 2007

Progression from fundamental understanding to tissue engineering

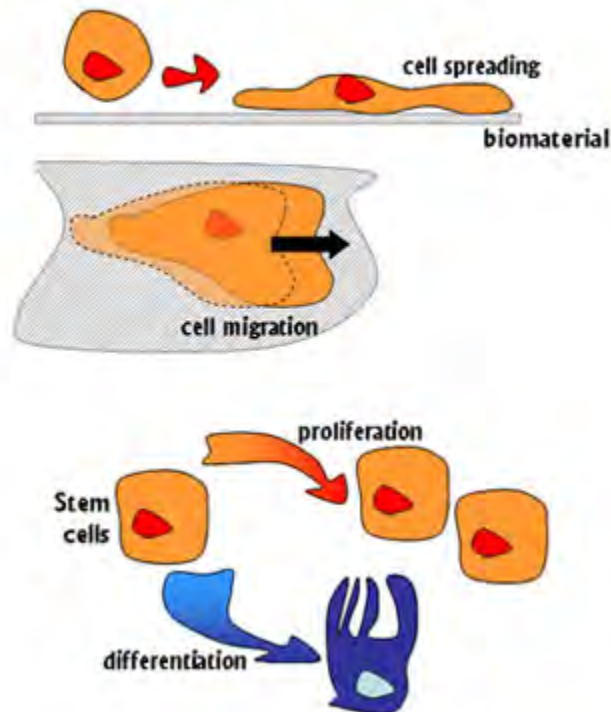
Based on molecular mechanisms...

Topology (+) Dynamics

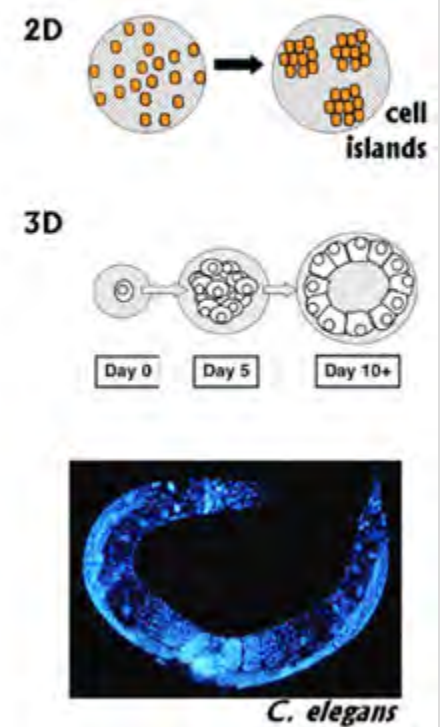
Signaling and Genetic regulatory networks



...to manipulate predictably mammalian cell behaviors...



... to engineer higher-order structure and function.



Tissue Engineering and mechanistic understanding

- MATES Strategic Plan:

‘..describes how contributions from the physical, chemical, biological and computational sciences and engineering will provide the tools by which better understanding and control of biological systems will be achieved.’

- ASTM International: Committee F04.46 Cell Signaling:

- Directing cells** to migrate, differentiate, and assemble in a desired fashion may **require understanding of the intracellular pathways** that control the response of cells to a complex environment.

- Accurate and quantitative measurements of cell signaling biomarkers**, aided by reference materials and standards

- would aid R&D (development of hypotheses, prediction of outcome, comparison of data over time).

- Would aid regulatory, and QA/QC (by providing predictive capabilities for determining safety, efficacy).

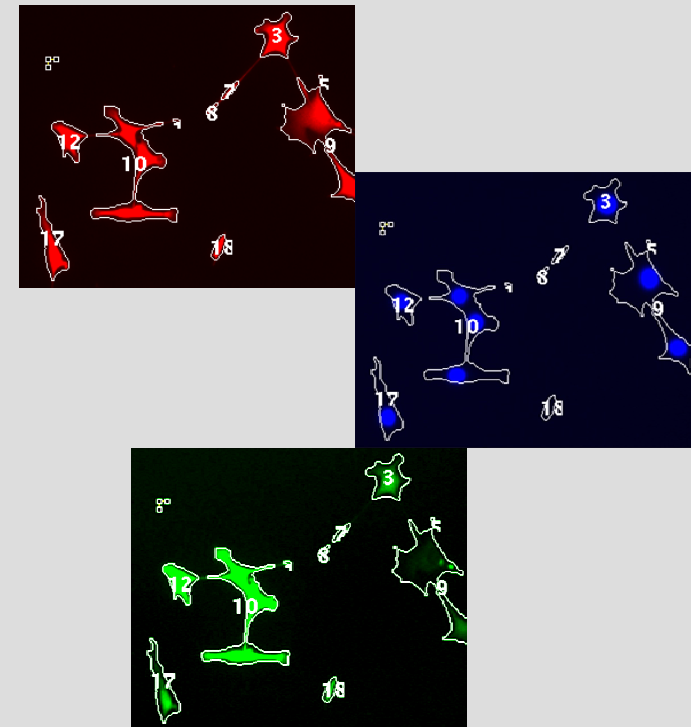
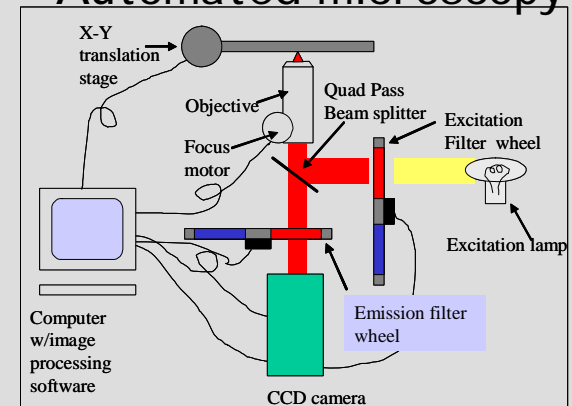
- Standardized assays would facilitate **pooling of data**.

- More than one biomarker may be needed to evaluate a response.***

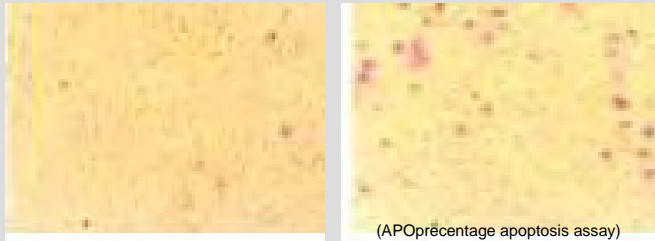
Quantitative Imaging for mechanistic understanding

- *In vitro* imaging of cells has the potential to provide large amounts of quantitative molecular scale information.
- Simultaneous evaluation of multiple response parameters.
- Evaluate different kinds of parameters from same image.
- Temporal responses in live cells.
- Temporal and spatial relationships between parameters.
- Temporal control of conditions for testing molecular level mechanisms.

Automated microscopy



Quantitative Imaging of Cells - Statistical Analysis

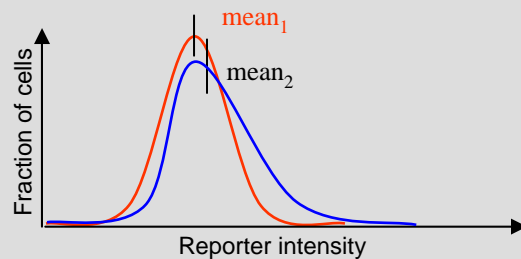


● -without drug

● -with drug

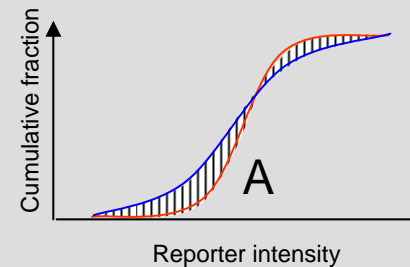
Example: The most potent assay response to a cytotoxic drug only occurs in small fraction of cells. (APOpercentage apoptosis assay)

Measured Response Histogram
Only small difference in mean value!



Replot same data

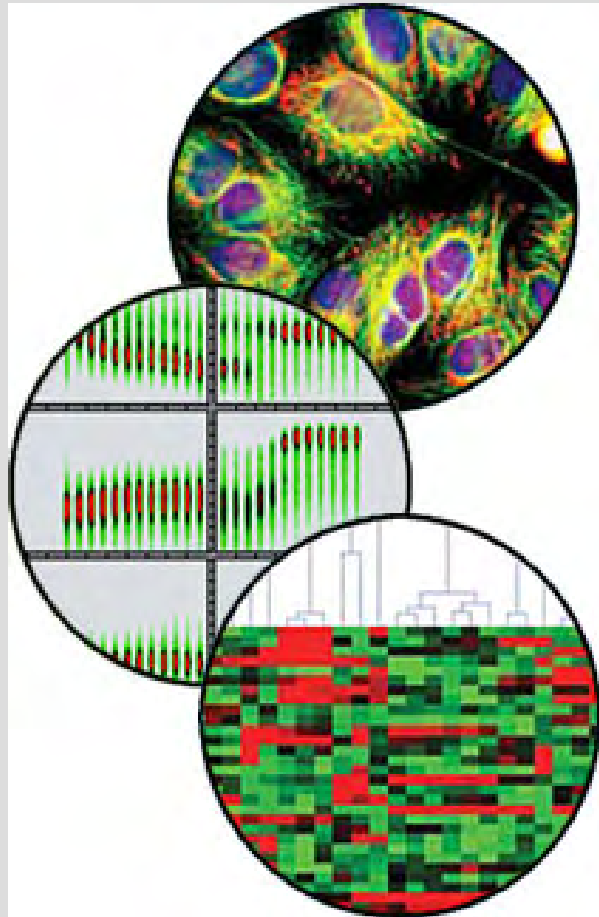
Cumulative Distribution
Large difference in Cum. Dist. metrics!



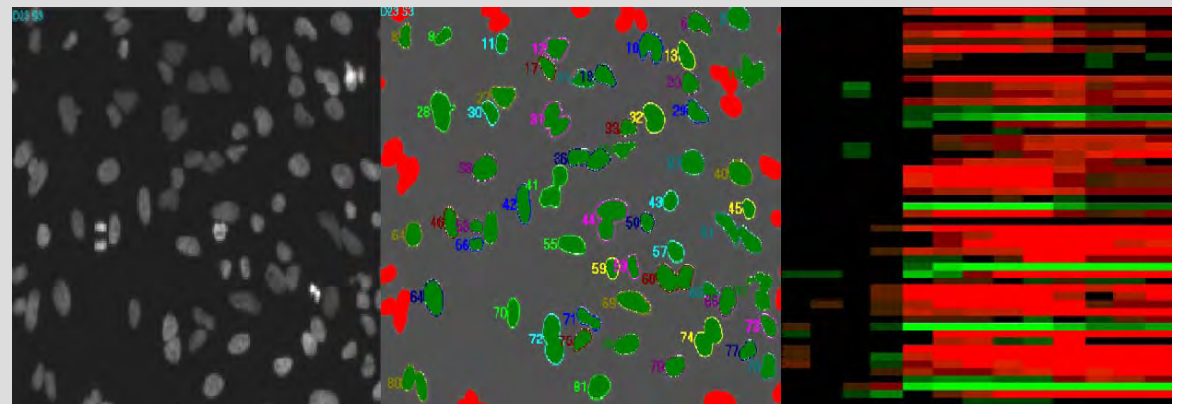
- **Distribution statistics may reveal early time points changes or predictor events that are not visible with traditional “mean well value” statistics!**
- **Distribution statistics can often be more sensitive to higher order moments (i.e. changes in the tails).**
- **Cell-by-cell analysis offers a mechanistic view of action**

More than one biomarker may be needed to evaluate a response...and what are appropriate biomarkers?

High content cell imaging for mechanistic understanding

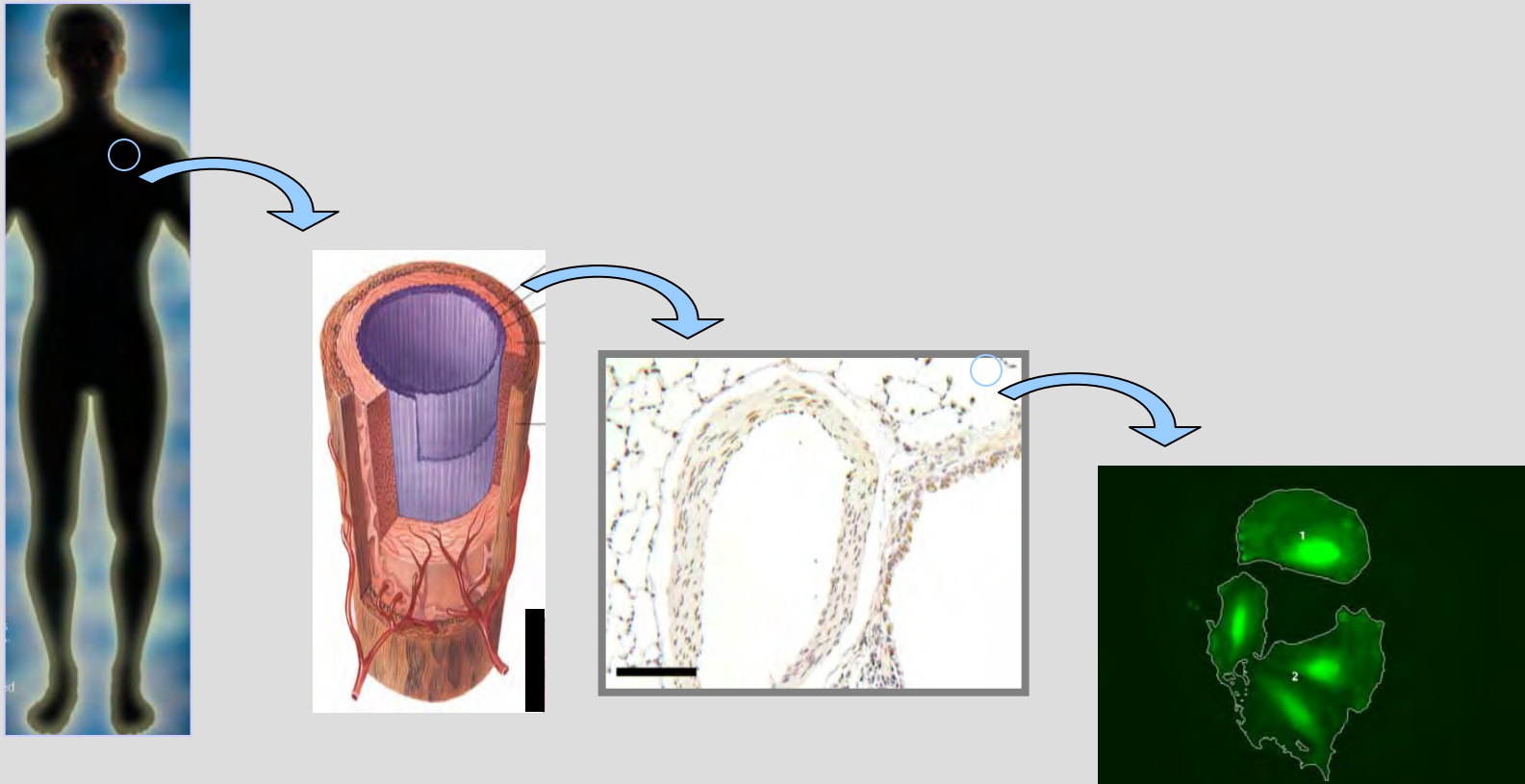


Cellumen, Inc.



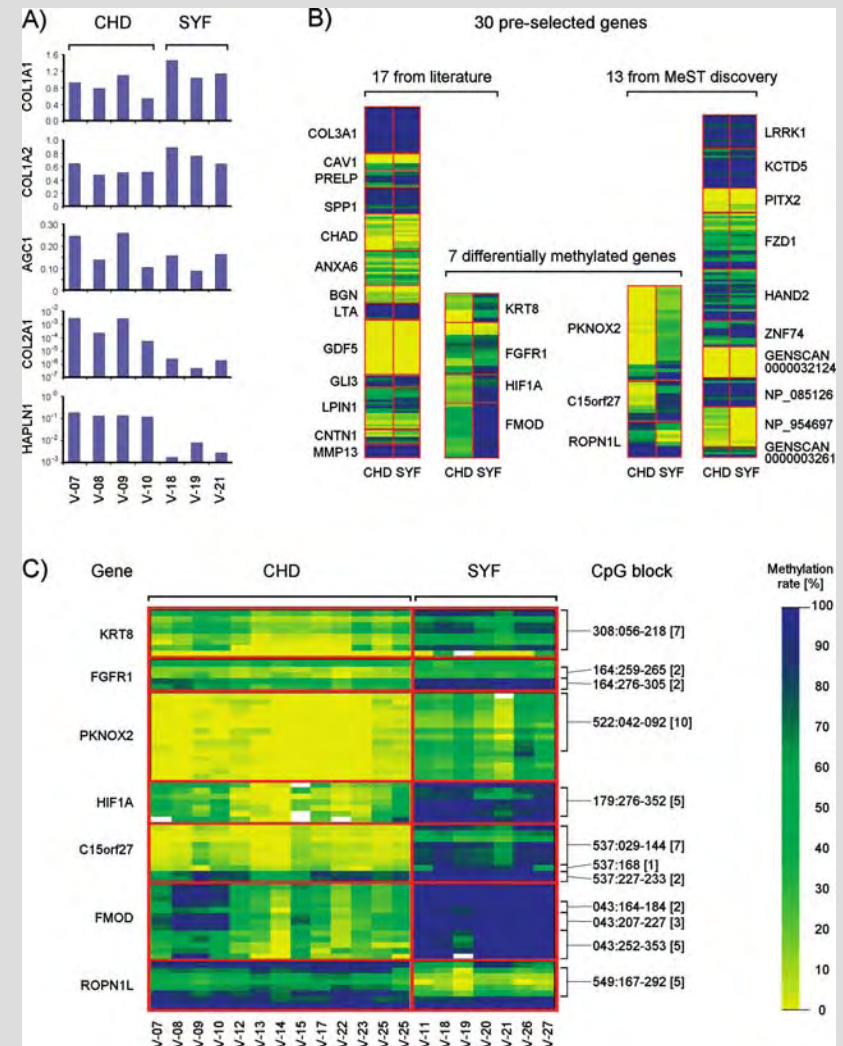
Lani Wu lab

The scales of interest for product evaluation



More than one biomarker may be needed to evaluate a response...and what are appropriate biomarkers?

- Cartilage generation for replacement therapies by expansion of cells from cartilage biopsies
- Challenge: contamination by other cell types (e.g., fibroblast-like cells), which can overgrow the desired cells
- DNA methylation analysis as a quality assessment tool; identified 7 potent discriminators
- Training set: cell type classifier that could discriminate chondrocytes from synovial membrane derived cells



DNA Methylation Analysis as Novel Tool for Quality Control in Regenerative Medicine
RAPKO et al TISSUE ENGINEERING, Volume 13 2007

Proteomics for evaluation of tissue engineering

- Protein expression varies widely in different parts of its body and in response to stage of an organism or cell's life cycle, and in response to environmental conditions.
- In contrast to the genome, the proteome is dynamic.
- The proteome is a useful tool for characterising cells and tissues of interest, and has a significant role to play in Tissue Engineering.

-Biomaterials and Tissue Engineering Centre of Industrial Collaboration Ltd (Assoc with School of Mechanical Engineering, University of Leeds).

Proteomics will probably be important to evaluate long-term health of the patient.

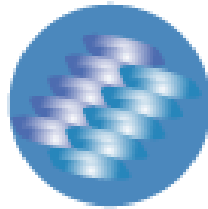


Interactions among proteins encoded by the yeast genome, and profiles of transverse sections of rat brain showing different protein signals.

Personalized medicine and systems biology: components of tissue engineering?



McGowan Institute for Regenerative Medicine
A program of the University of Pittsburgh and UPMC



WINDBER RESEARCH INSTITUTE
PITTSBURGH TISSUE ENGINEERING INITIATIVE
REGENERATIVE MEDICINE PARTNERSHIP

We believe that **broad clinical implementation of cellular therapy will require a patient specific understanding** of how gene expression controls cell behavior.
.....(Researchers will) **explore the genome and proteome of engineered tissues. Bioinformatics and tissue engineering will be combined to advance the frontiers of regenerative medicine.**

A large parameter space for tissue engineering.

