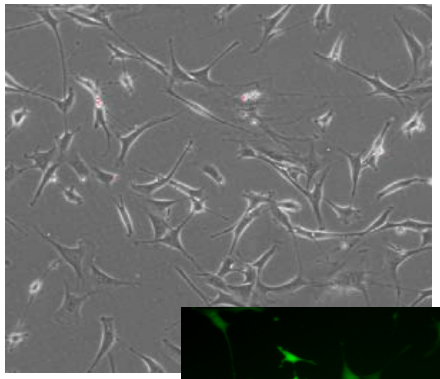
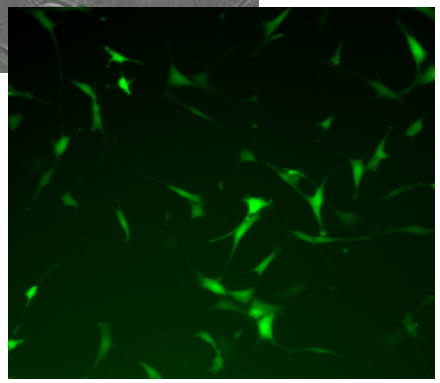
useR! 2010, Gaithersburg, MD
July 21, 2010

NIST National Institute of Standards and Technology

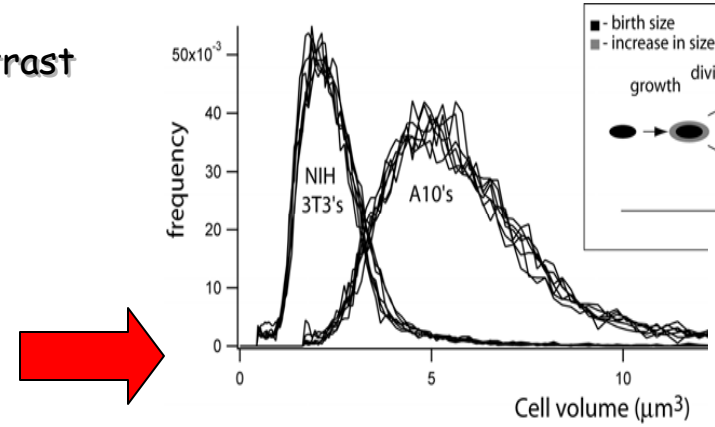
Quantitative Cell Biology at NIST:
Statistical Analysis of Cell Population Data

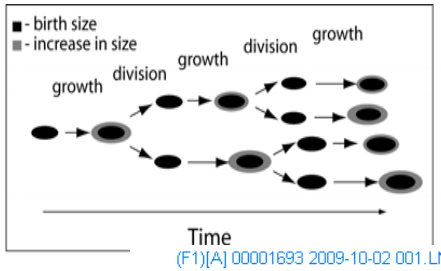


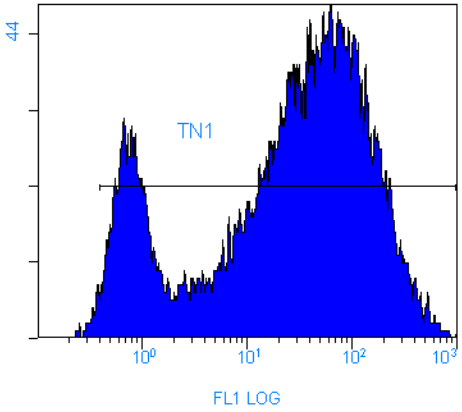
phase contrast



fluorescent protein



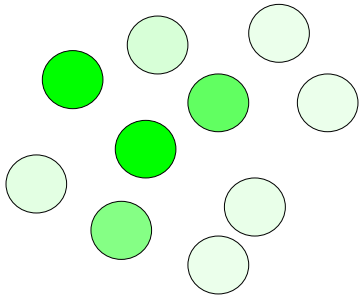




Michael Halter, Daniel R. Sisan, Katharine M. Mullen, Z. Q. John Lu
NIST, Gaithersburg, MD

Cell population distributions

Biomarkers



Physical parameters
(cell size, shape, volume)
Protein expression levels
others…

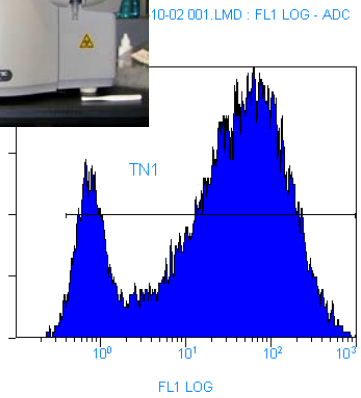
Indicate Cell Status

- Proliferation
- Differentiation
- Apoptosis
- Inflammation
- Remodeling

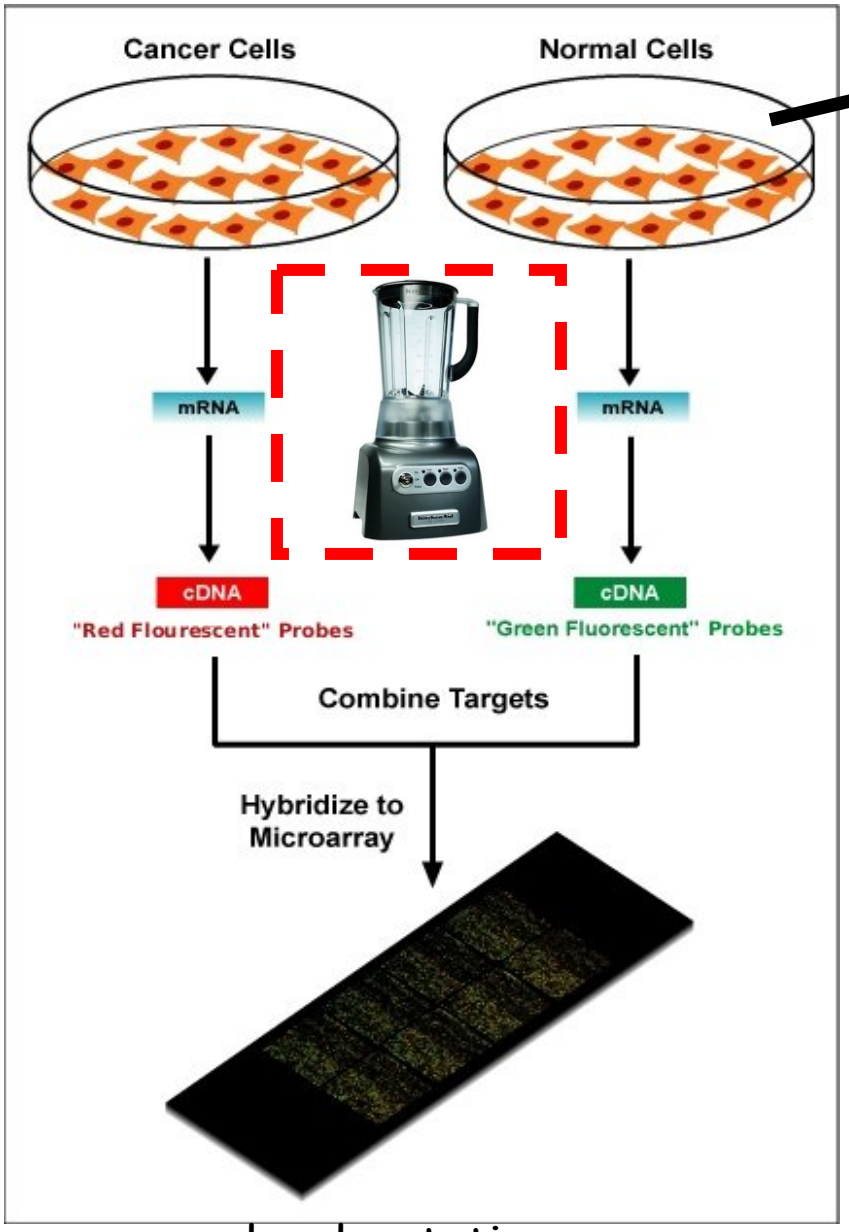
Measurement tools

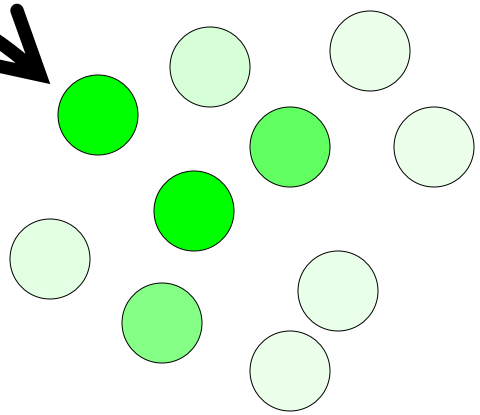
Automated Microscopy

Flow Cytometry



Single cell analysis

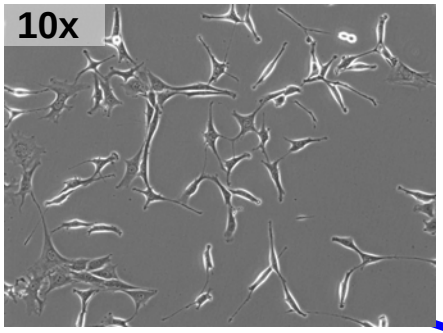


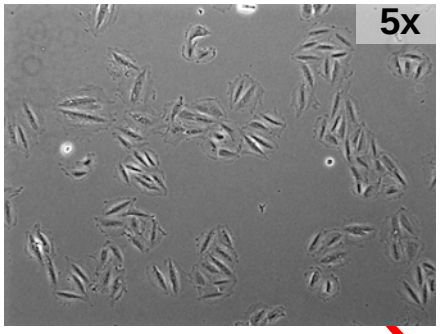


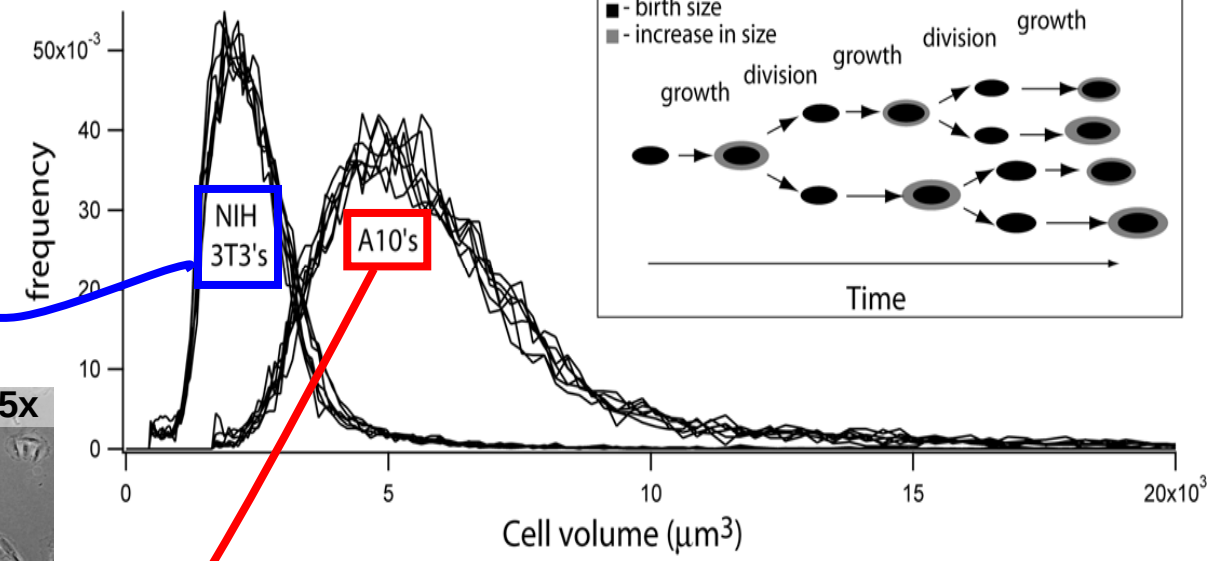
Biological variability
Subpopulation information

www.molecularstation.com

Volume distributions of cultured cells

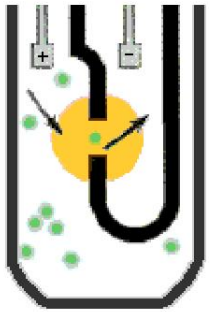






- Electronic cell volume measurements have been around for 50 years.
- Provides data describing the distribution of cell volumes.
- This distribution appears to be stable in expanding cells

The Coulter Principle

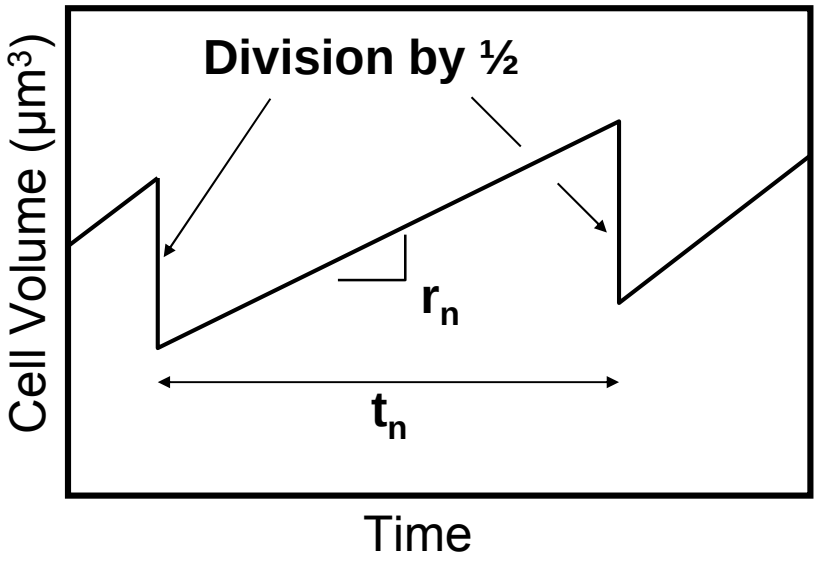


Halter et al., (2009) J. Theor. Biol. 257, 124

Cell growth/division is reflected in cell volumes

MODEL ASSUMPTIONS:

- Volume changes at a constant rate for individual cells during the cell cycle (Conlon and Raff, J. of Biol., 2003)
- Individual cells can have different growth rates. The population of cells exhibits a normal distribution of growth rates
- At division, each cell divides exactly in half
- Cell cycle times are normally distributed



$$\lambda(V) = A \cdot \int_0^{\infty} 2^{\frac{-\tau}{t}} \cdot \left(\frac{1}{\sqrt{\frac{1}{3}(r \cdot \sigma_t)^2 + \frac{1}{3}(t \cdot \sigma_r)^2 + (\tau \cdot \sigma_r)^2} \cdot \sqrt{2\pi}} \cdot e^{\frac{-(V - r \cdot (t + \tau))^2}{2 \cdot \left(\frac{1}{3}(r \cdot \sigma_t)^2 + \frac{1}{3}(t \cdot \sigma_r)^2 + (\tau \cdot \sigma_r)^2 \right)}} \right) \cdot \left(\Omega(|\tau - t|) \right) d\tau$$

Can be used to estimate the growth rate, r (μm³/h), from a distribution and known cell cycle time, t (h).

Halter et al., (2009) J. Theor. Biol. 257, 124

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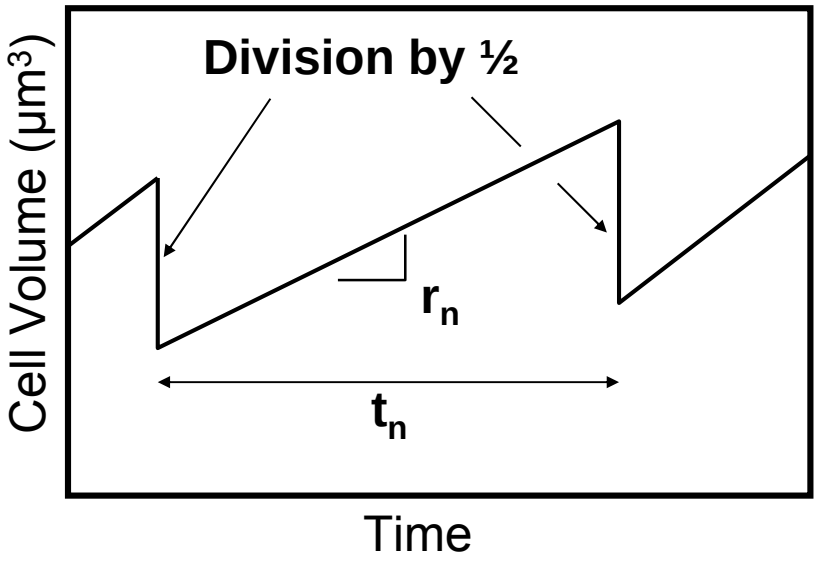

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Relates the volume distribution to the noise in the growth and division processes

Halter et al., (2009) J. Theor. Biol. 257, 124

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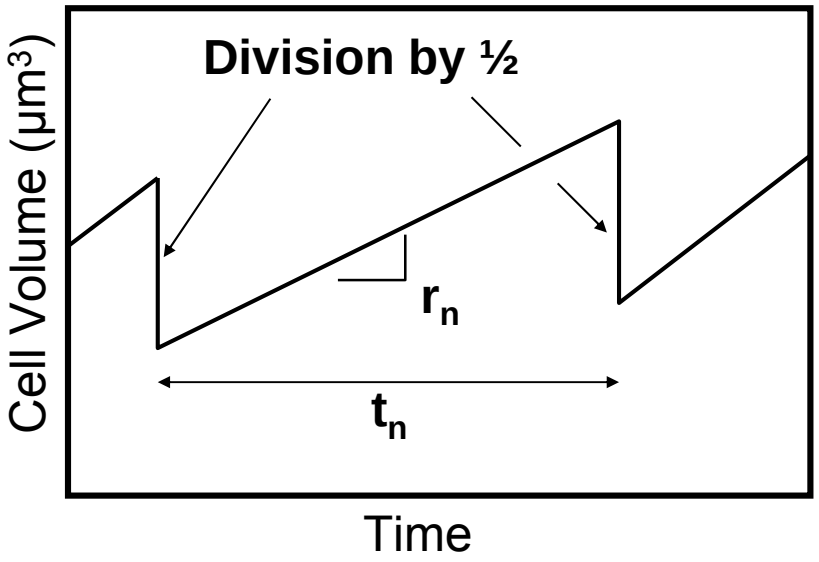

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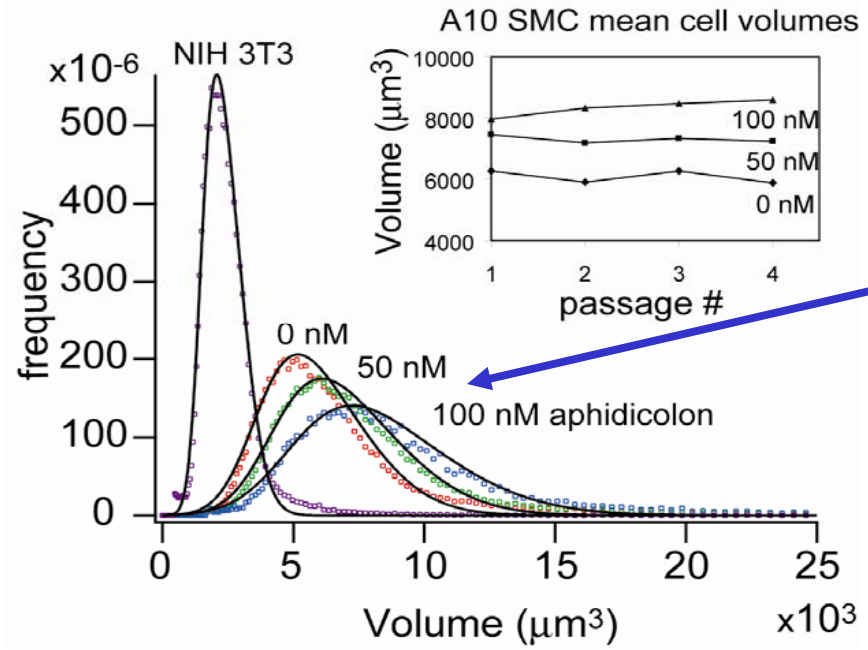
Provides a mechanistic understanding of the observed distribution and increases confidence in the measurement.

Halter et al., (2009) J. Theor. Biol. 257, 124

Cell growth/division is reflected in cell volumes



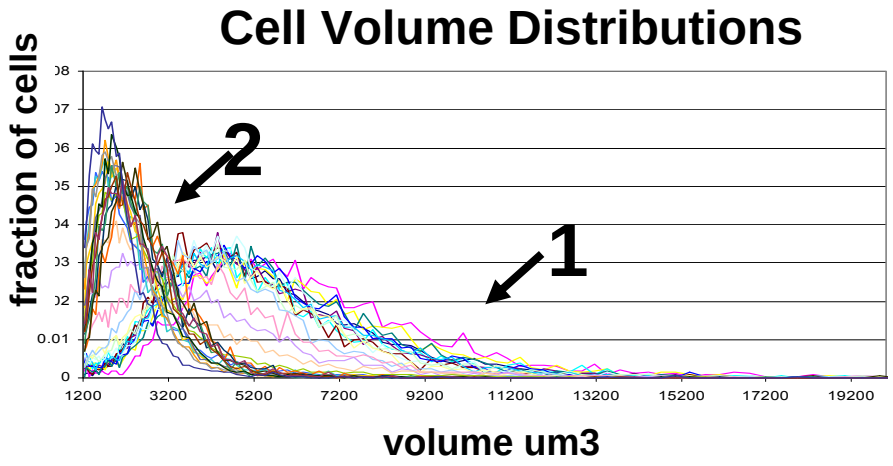
Use of a drug to increase cell cycle time

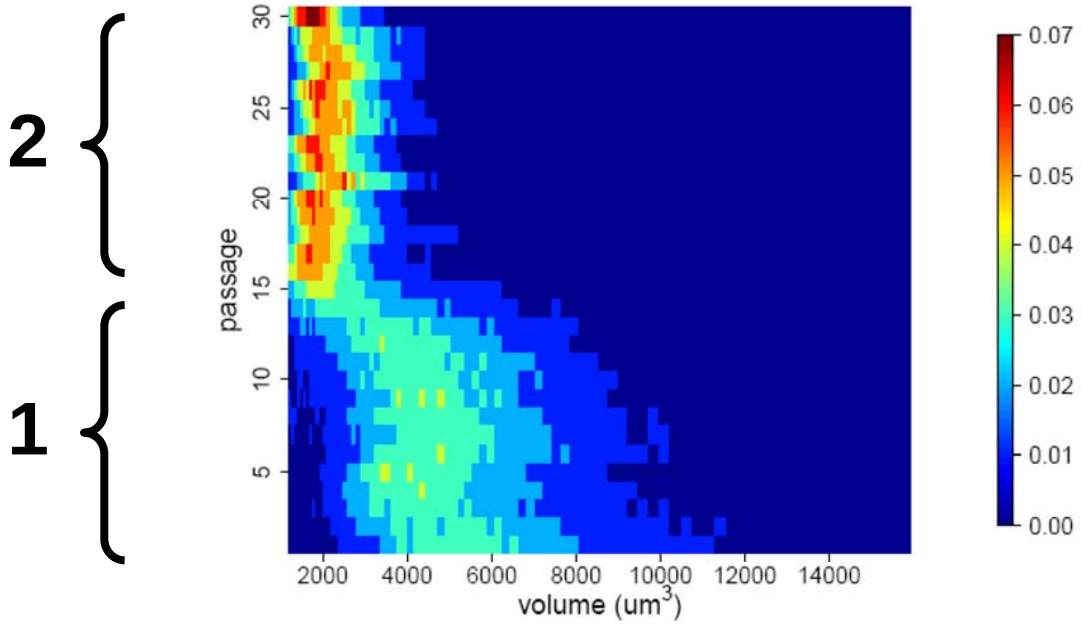
Aphidicolon	Mean Generation Time
0 nm	29 h
50 nm	36 h
100 nm	50 h

Cell type[a]	t (h)	σ_t (h)	r (µm³/h)	σ_r (µm³/h)	$(\sigma_r/r)^e$
NIH 3T3	19.5 ± 0.7	5.9	87 ± 4	29 ± 6	0.34 ± 0.07
A10	29 ± 3	8.7	140 ± 20	52 ± 7	0.37 ± 0.07
A10 (50 nM)	36 ± 3	10.8	139 ± 15	57 ± 6	0.41 ± 0.06
A10 (100 nM)	50 ± 8	15	115 ± 23	51 ± 10	0.43 ± 0.12

CellVolumeDist: R package for fitting cell volume data

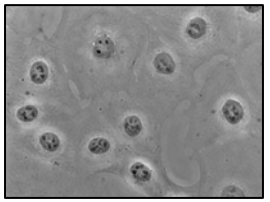
Dolphin Lung Endothelial Cells



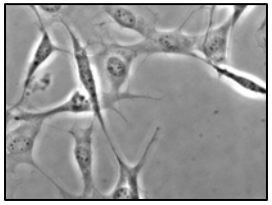


Morphology

1 round shape



2 spindly shape



- Cell culture changes phenotype after 25 passages

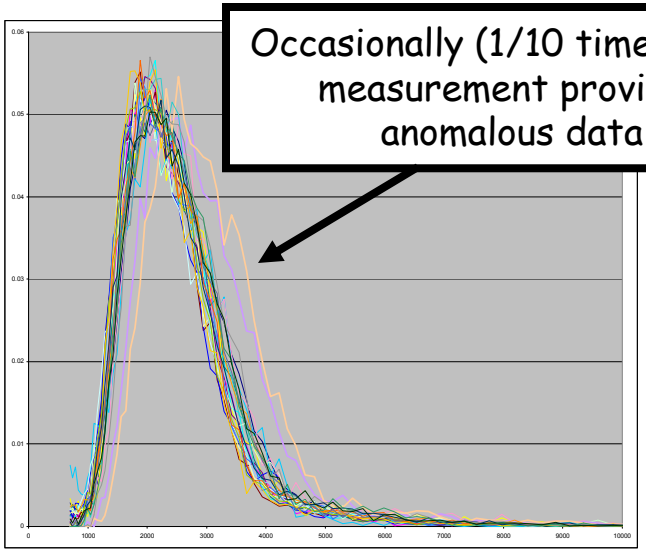
AL Mancia, et al, in preparation, collaboration with Hollings Marine Laboratory, SC

Many volume distributions

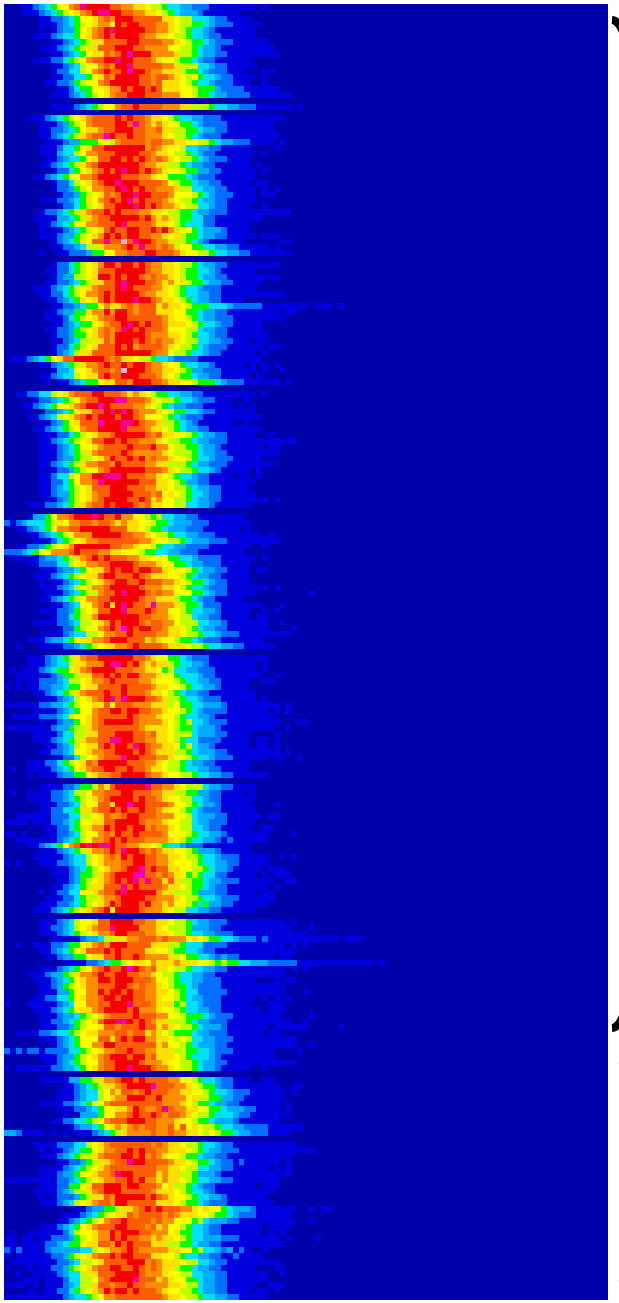
NIH 3T3 cell line

(12/28/06 – 11/7/09)

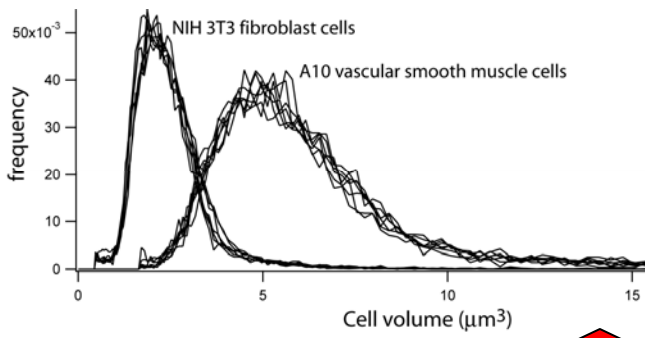
- >200 passages
- 11 thaws
- How to separate 'measurement variability' from true changes in culture behavior?

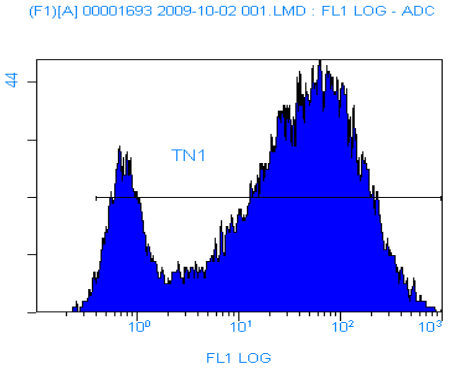


Occasionally (1/10 times?) the measurement provides anomalous data

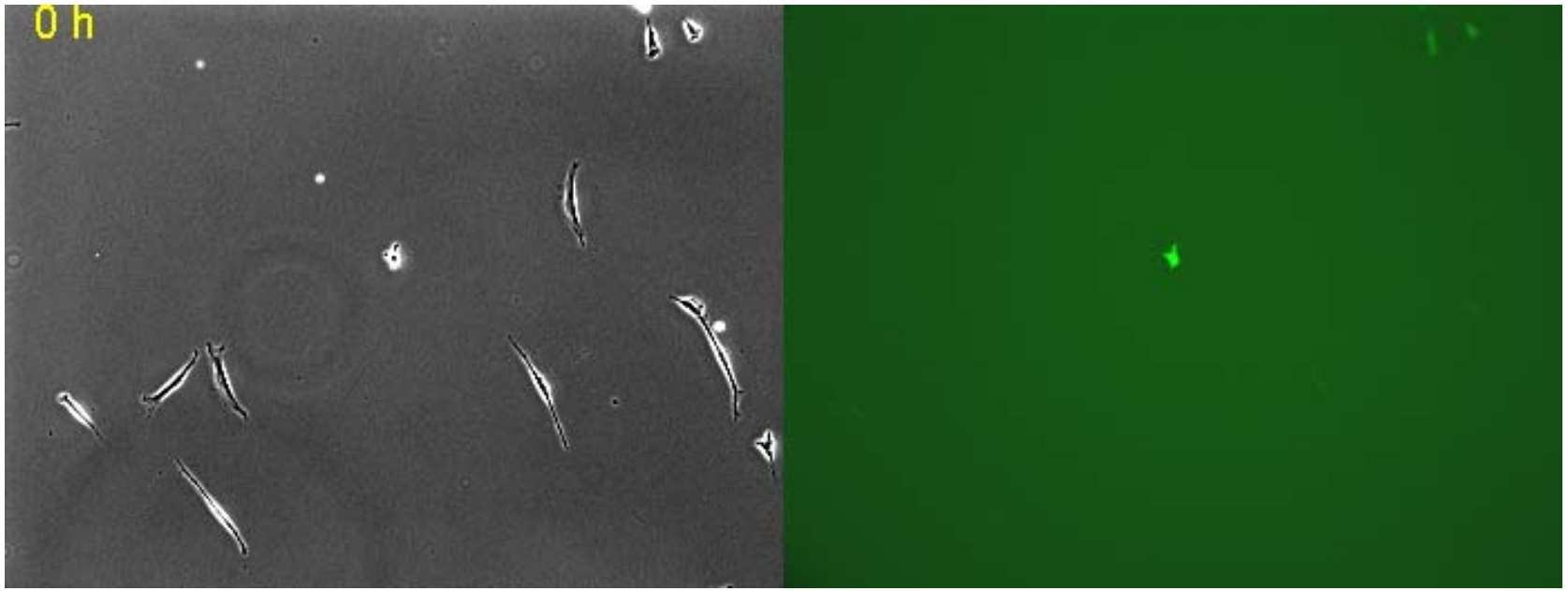


Relating cellular dynamics to the observed distribution





Cells are dynamic:

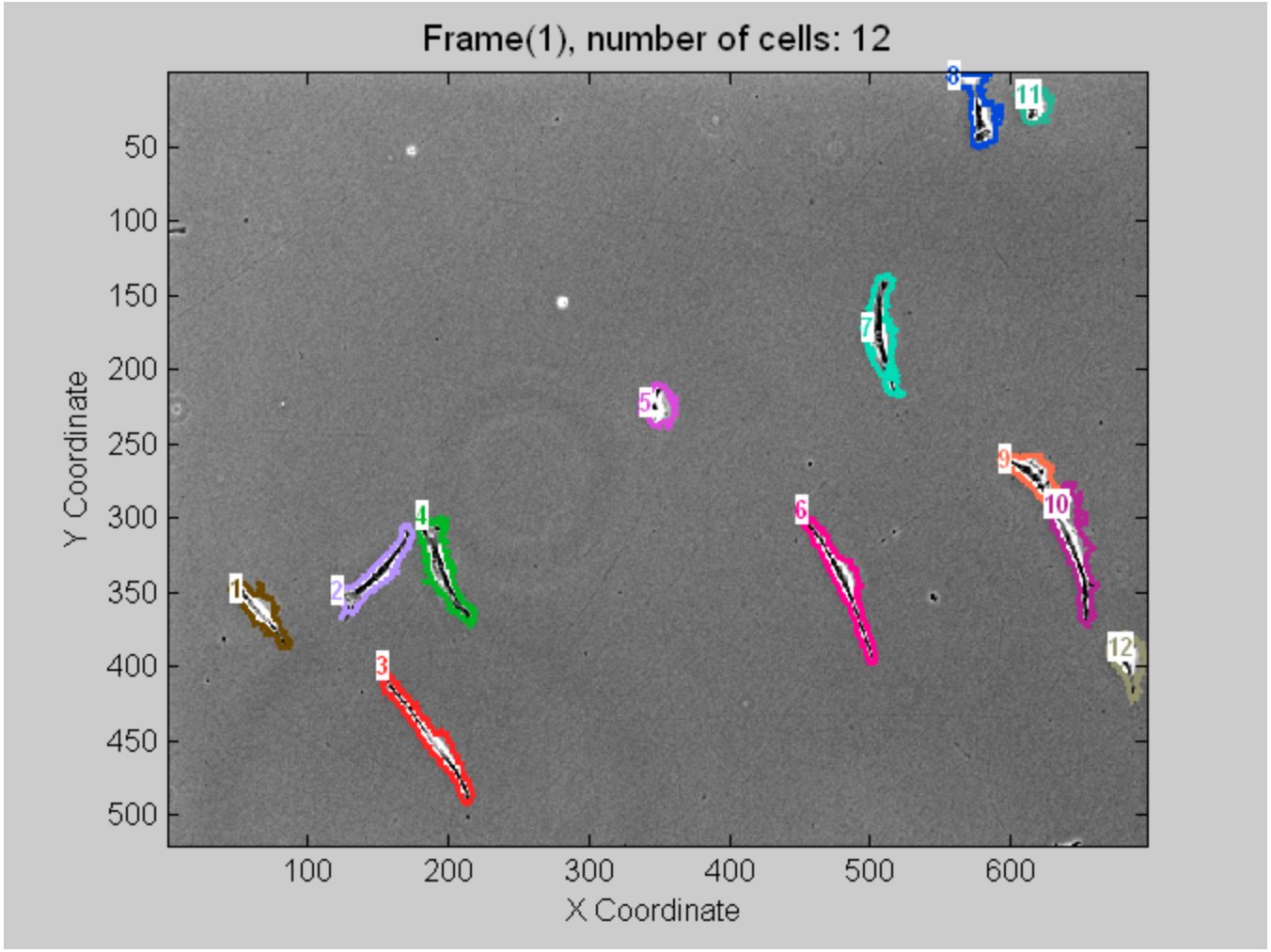


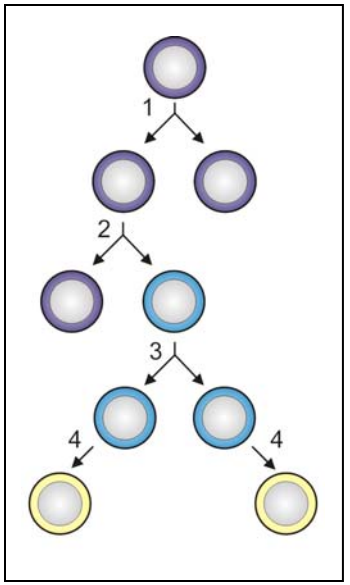
movie

Obtaining quantitative, dynamic cellular data

NIST National Institute of Standards and Technology

How does a steady state distribution arise?





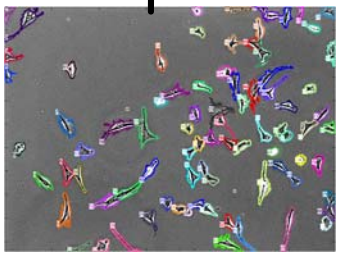
Need to derive quantitative data from live cell images to understand biological variability

movie

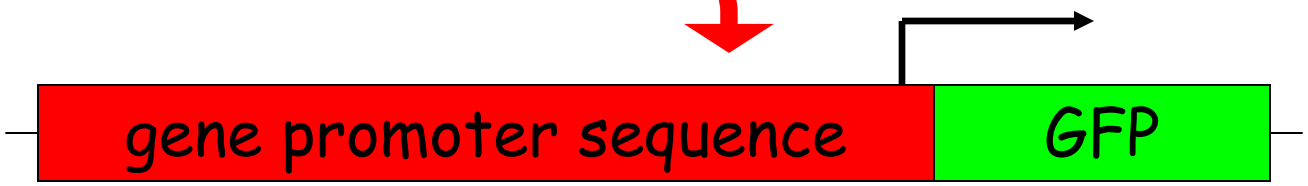
Image analysis tools developed by J. Chalfoun, A. Dima, A. Cardone, NIST

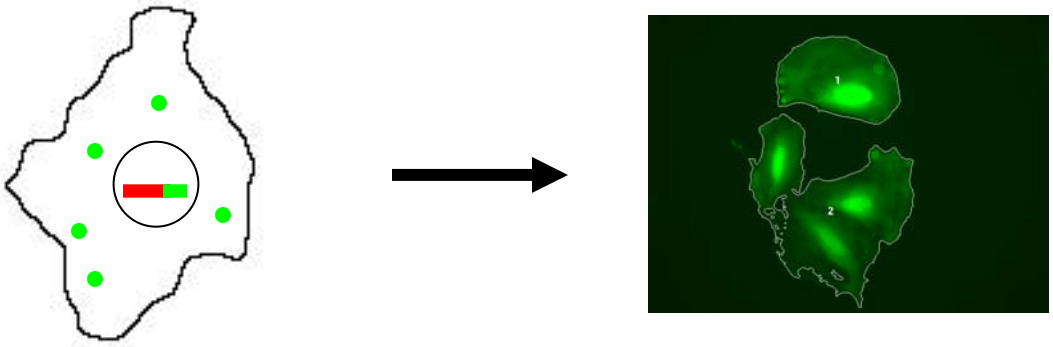
Tenascin-C gene reporter cell lines

Apply image analysis to GFP reporters



Full Tenascin-C promoter

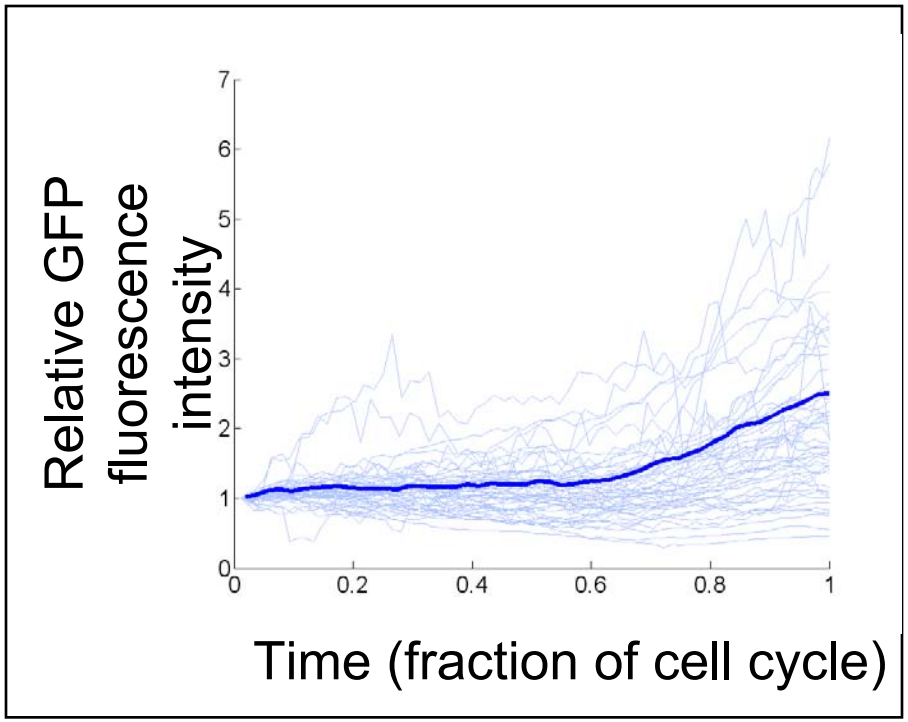


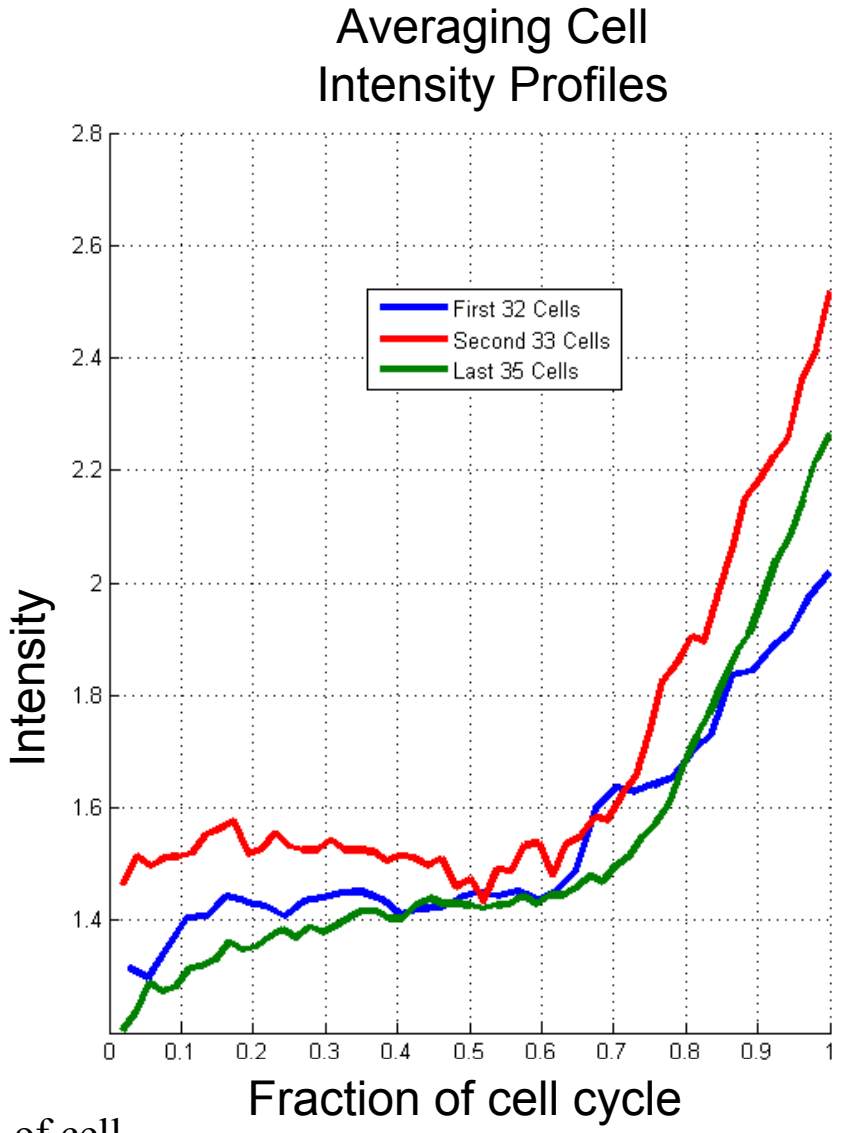


- Single cell clones from NIH-3T3 cell population transfected with a **destabilized** EGFP reporter (PEST sequence, reported ~2 hr half-life)

CONCEPT: GFP is produced when gene is active

Single cell GFP intensities over time indicate tenascin-C regulation is coupled to the cell cycle



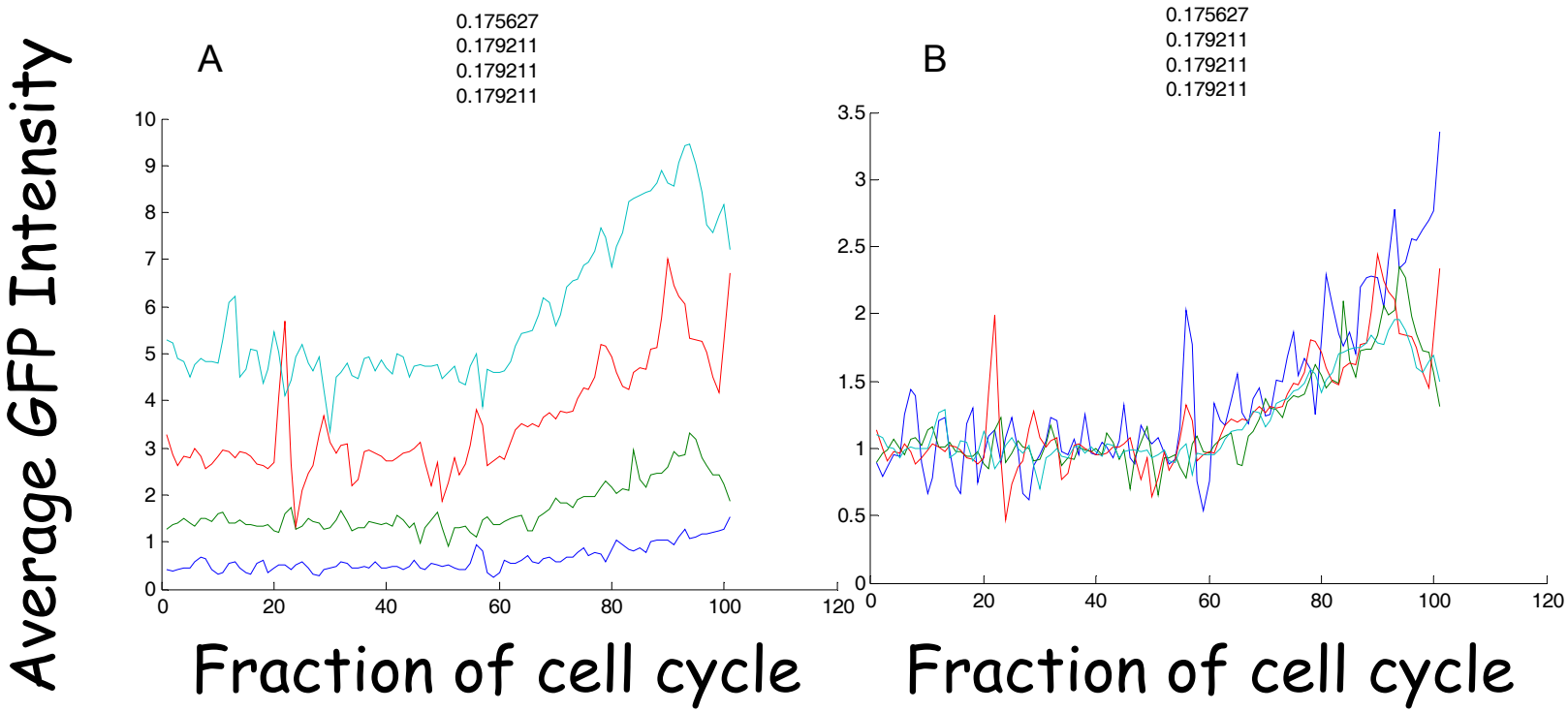


Normalizing the intensity data and averaging over >30 cells suggests that tenascin-C production is upregulated before division and is directly coupled to cell cycle progression

relative GFP between daughter cells after division; length of cell cycle vs. TNC expression; TNC expression vs. parent cell expression

Halter et al., submitted

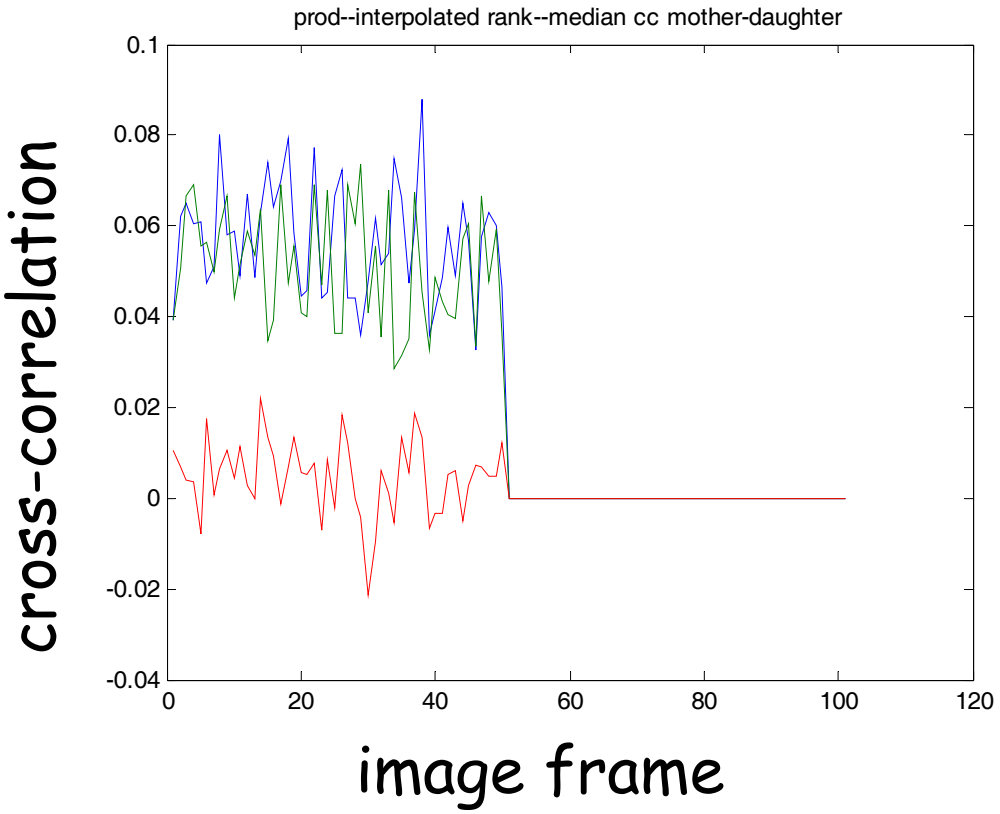
Upregulation of gene promoter activity

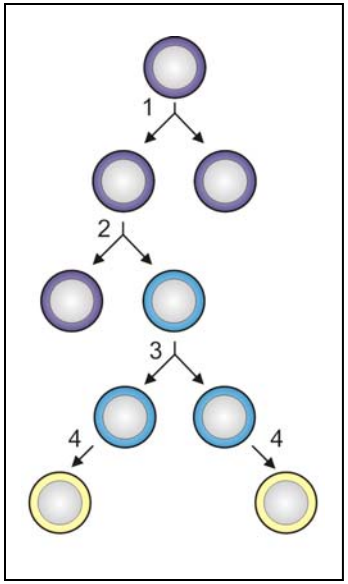


Upregulation of gene promoter activity is proportional to initial activity

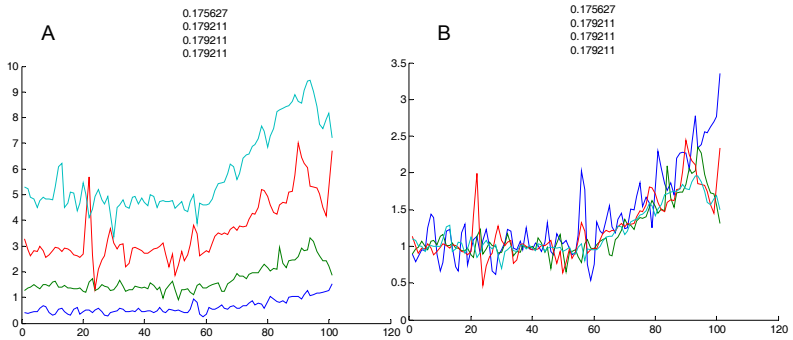
Halter et al., submitted

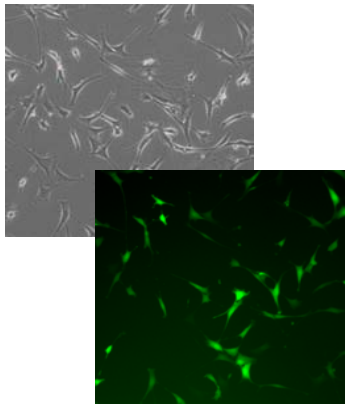
Cross-correlation in GFP expression between sister cell pairs and parent daughter pairs





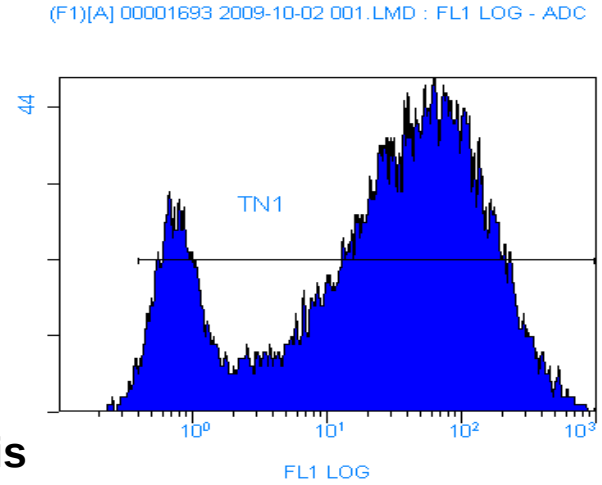
Relating cellular dynamics to the observed distribution

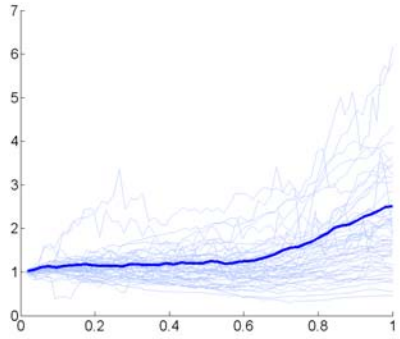


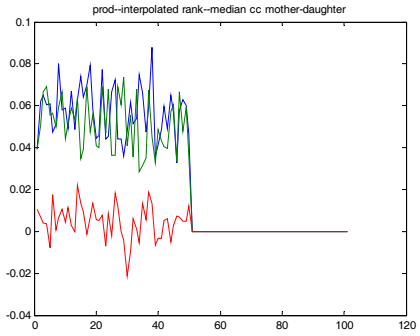


?

statistical analysis and modeling







Summary

- Developed a model for the distribution of cell volumes that parameterizes the cell cycle times and growth rates of cells
- Using live cell imaging and image analysis tools to montior the processes in cells the underly the biological variability
- Need models that relate the dynamic cellular processes to the measured distributions

Other acknowledgements:

John Elliott, Anne Plant, Cell Systems Science group at NIST