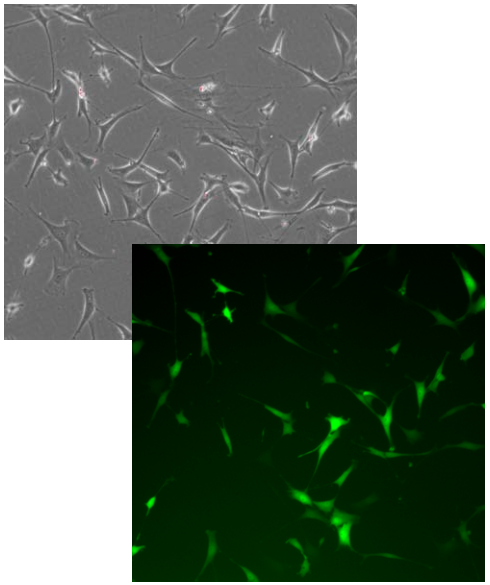
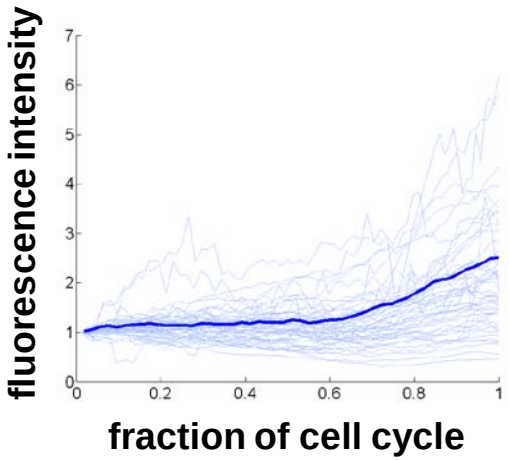
October 6th, 2009

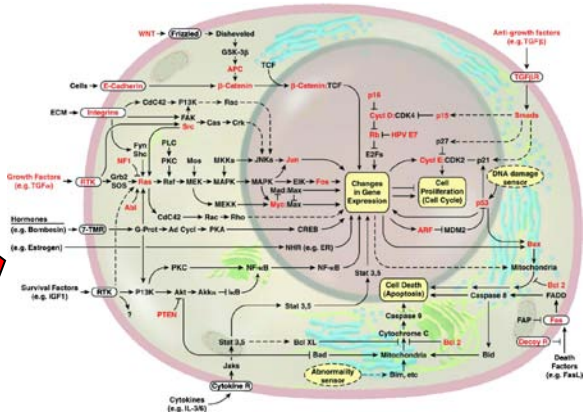
Maryland TEDCO Technology-Development-Corporation

NIST National Institute of Standards and Technology

Use of live cell microscopy to follow the temporal regulation of gene expression





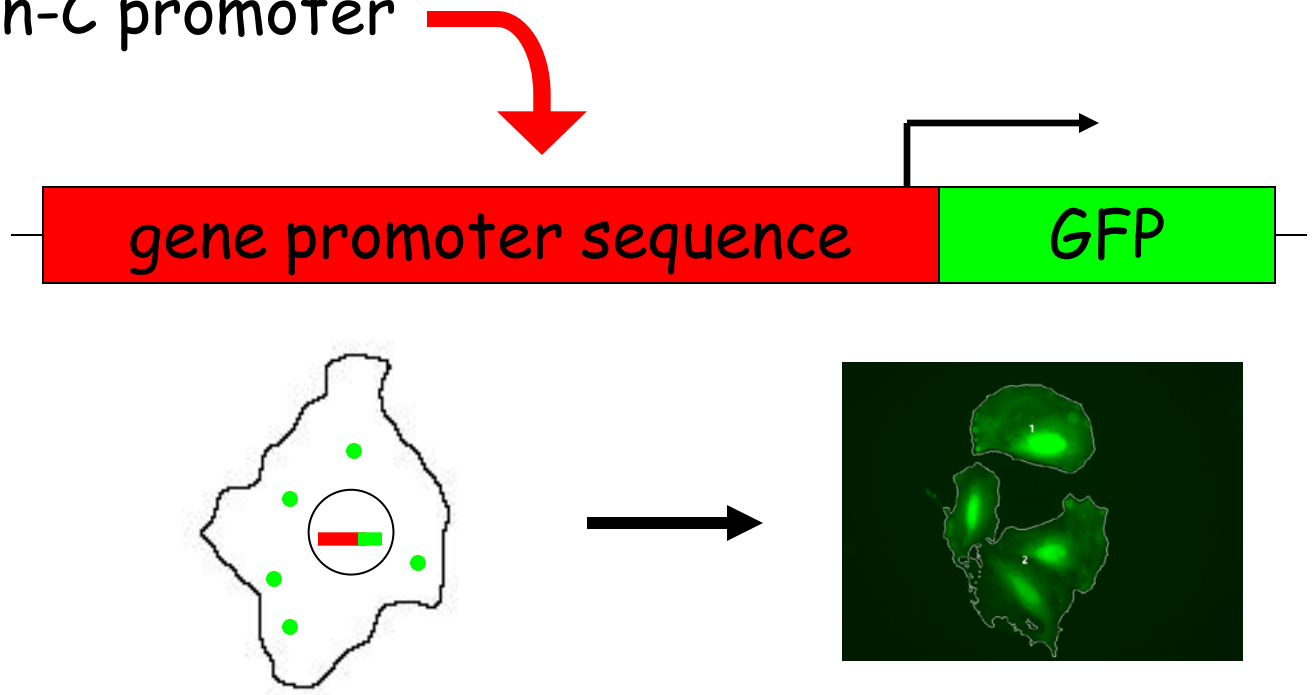


The Hallmarks of Cancer
Douglas Hanahan and Robert A. Weinberg

Michael Halter, Joe Chalfoun, Antonio Cardone, Benjamin L. Stottrup, Alex Tona, Alden A. Dima, Anne L. Plant, John T. Elliott
Cell Systems Science Group, Biochemical Sciences, CSTL, NIST

Tenascin-C gene reporter cell lines

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

Tenascin-C (TNC) regulation and role in disease

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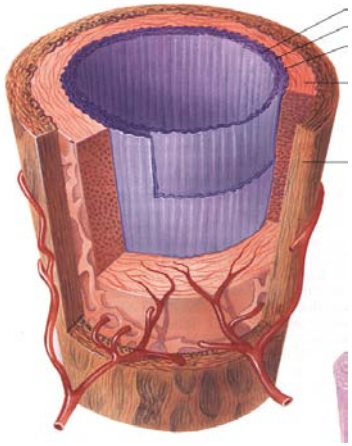
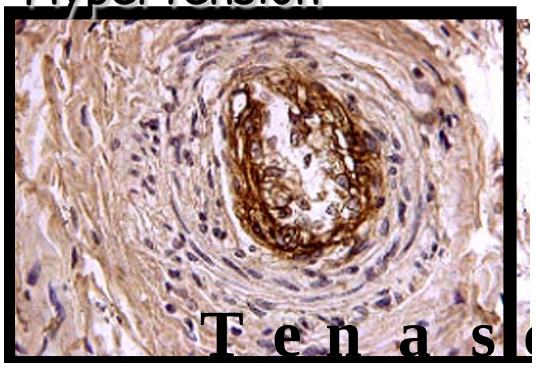
Normal Artery

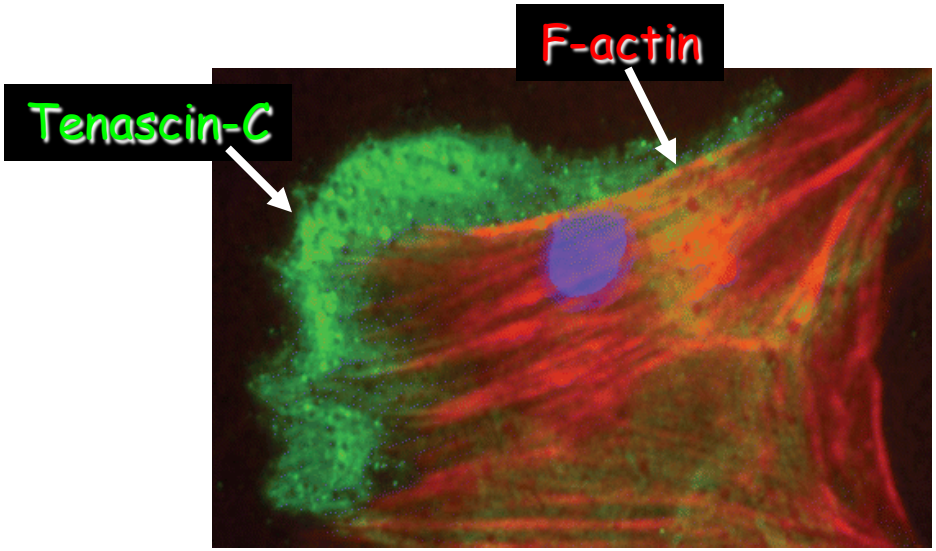


Artery Blocked from Hypertension



Courtesy of Peter Jones, UCHSC

TNC expression associated with:
- Mechanics
- ECM/integrins
- Proliferation
- Migration…



(Chapados et al., *Circulation Research.* 2006;99:837.)

Tenascin-C GFP reporter cells

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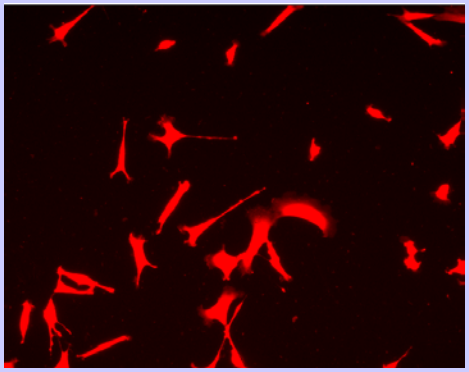
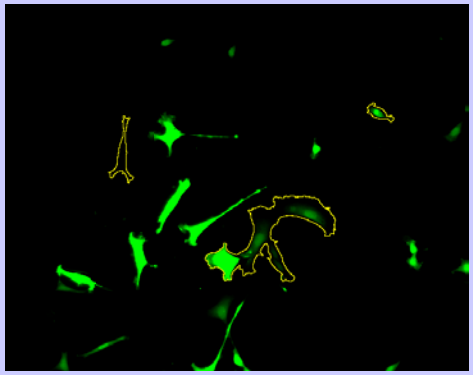
Fluorescence Microscopy

Cell Body Stain



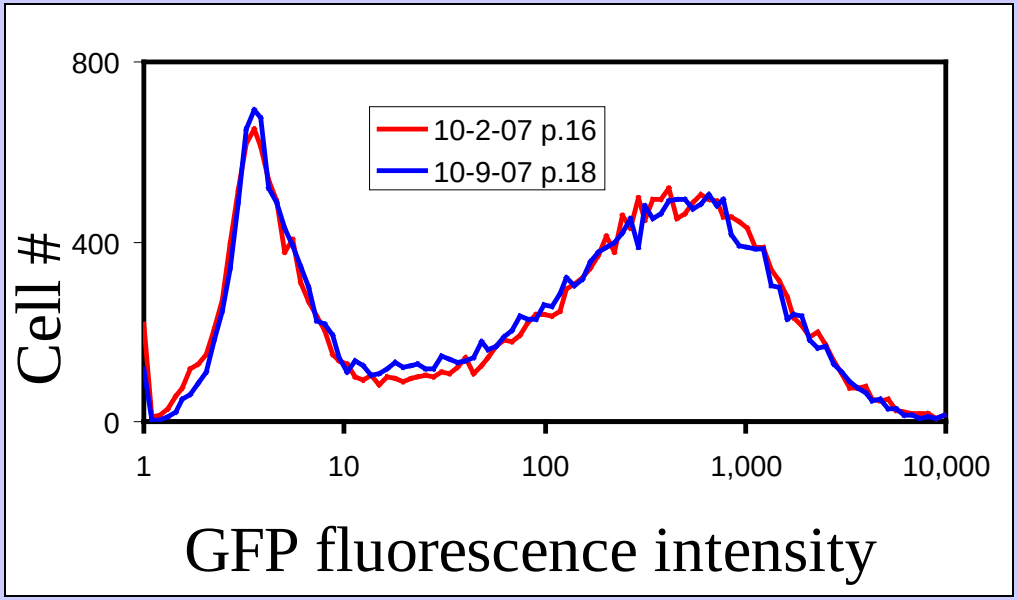
GFP Fluorescence



- Correlations between spread area and GFP intensity
- Gene expression responses to different ECM conditions

Langenbach et al, BMC-Biotech, 2006

Flow Cytometry

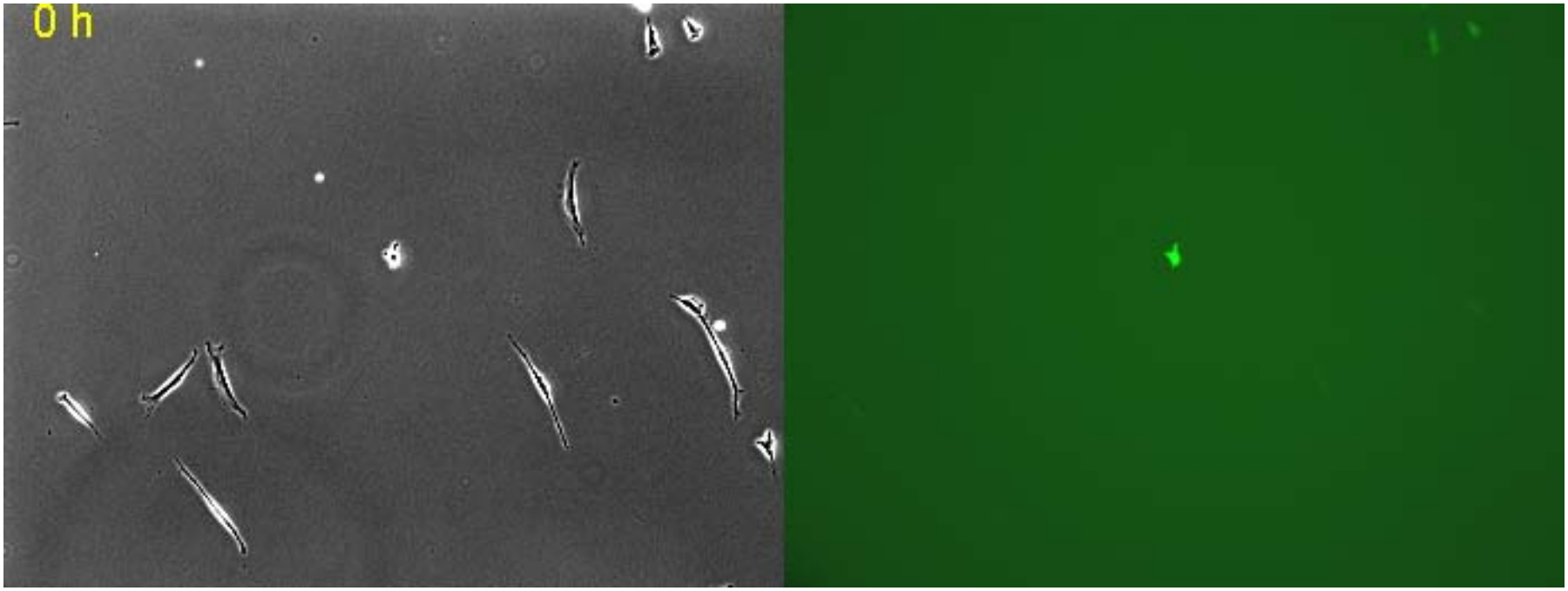


GFP intensity distributions are stable in culture (gene activity varies over 3-orders of magnitude?)

GFP Intensity=
Transcription+Translation+Folding+Degradation

What are the dynamics of cell behavior?

Challenges to quantifying live cell images

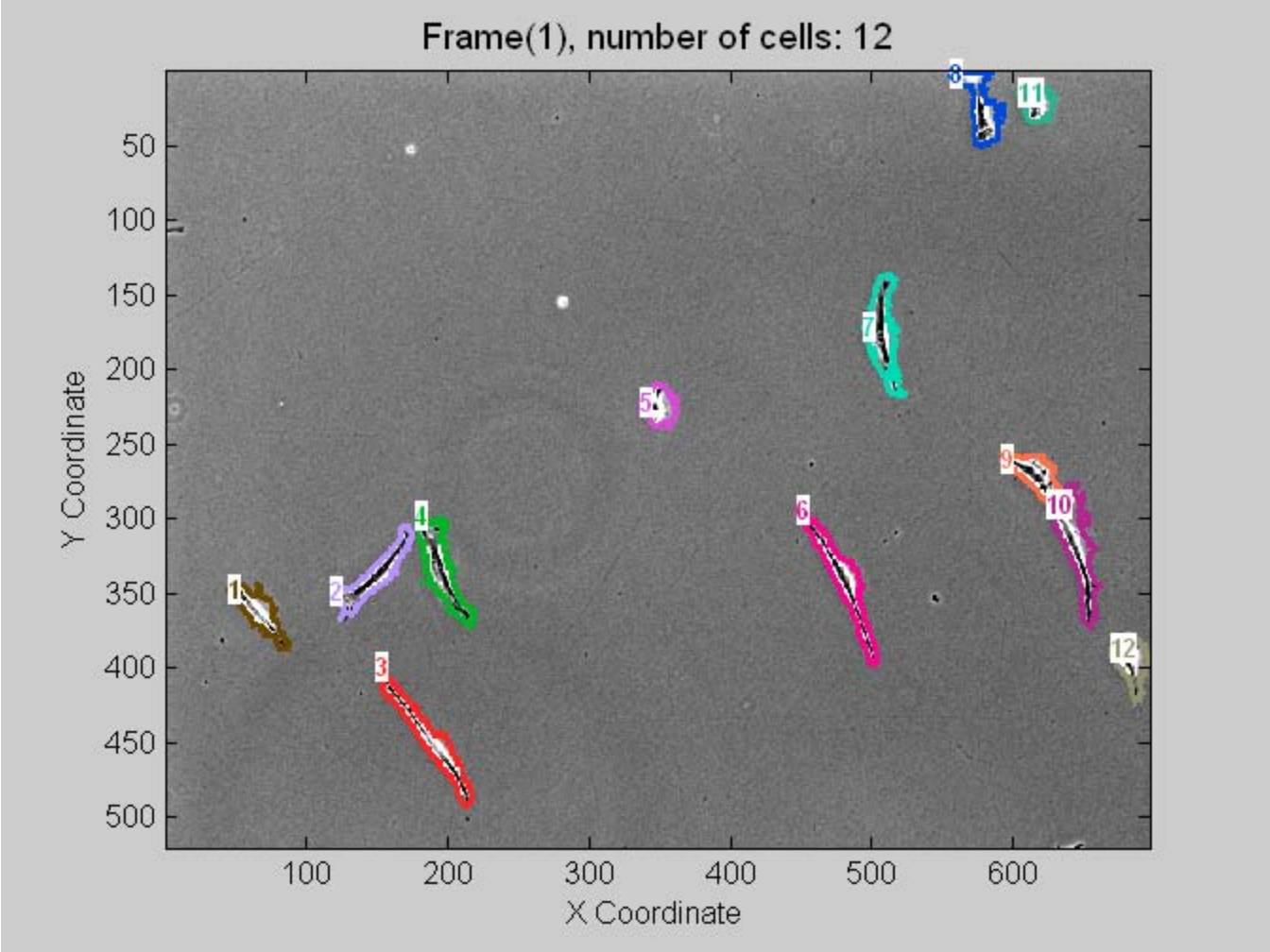


Movie: >62 hours, phase contrast on left, GFP fluorescence on right

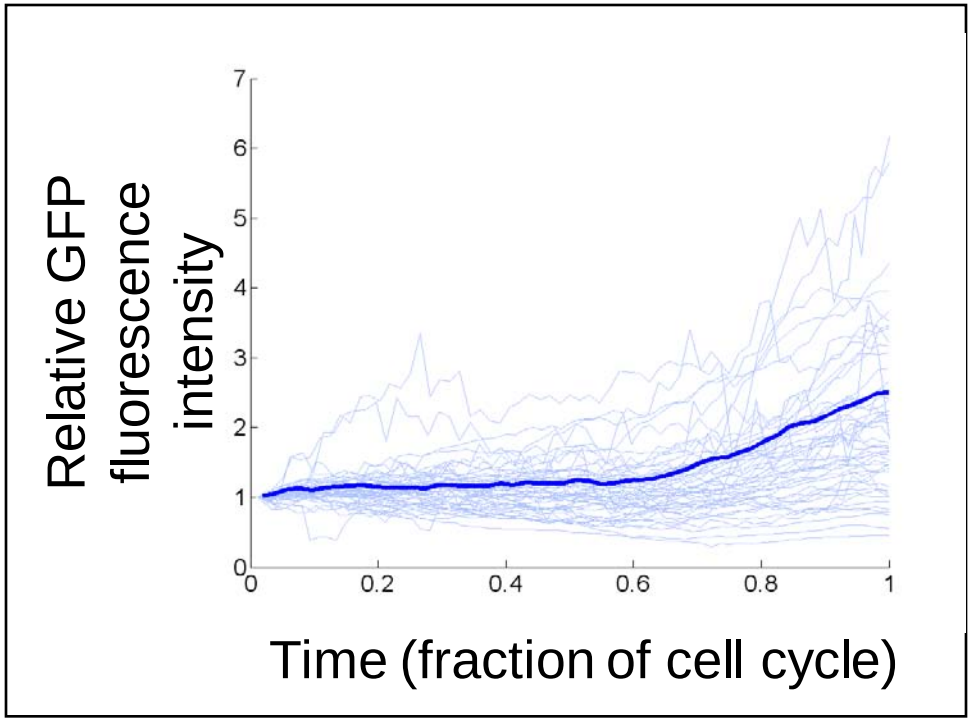
Challenges
- Cell segmentation
- Tracking, mitotosis, edge cells (leave the field)

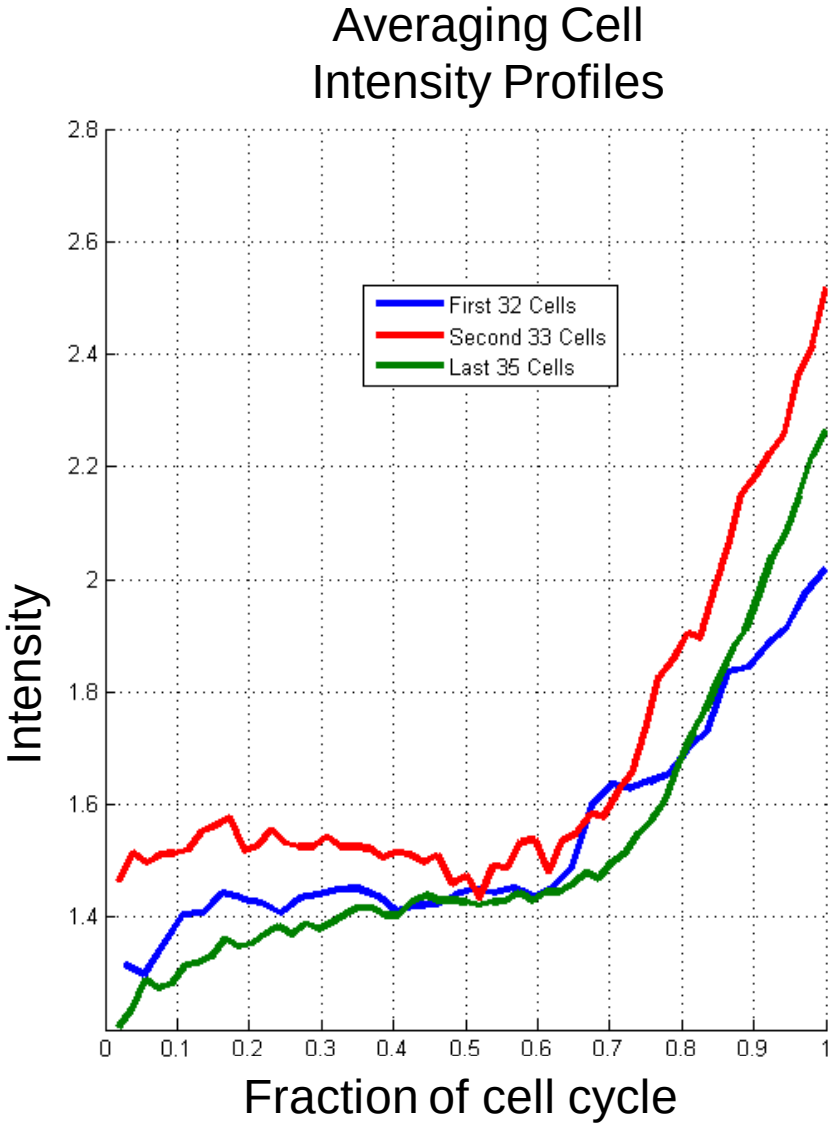
36 fields @ 15 min intervals

Live cell segmentation and tracking



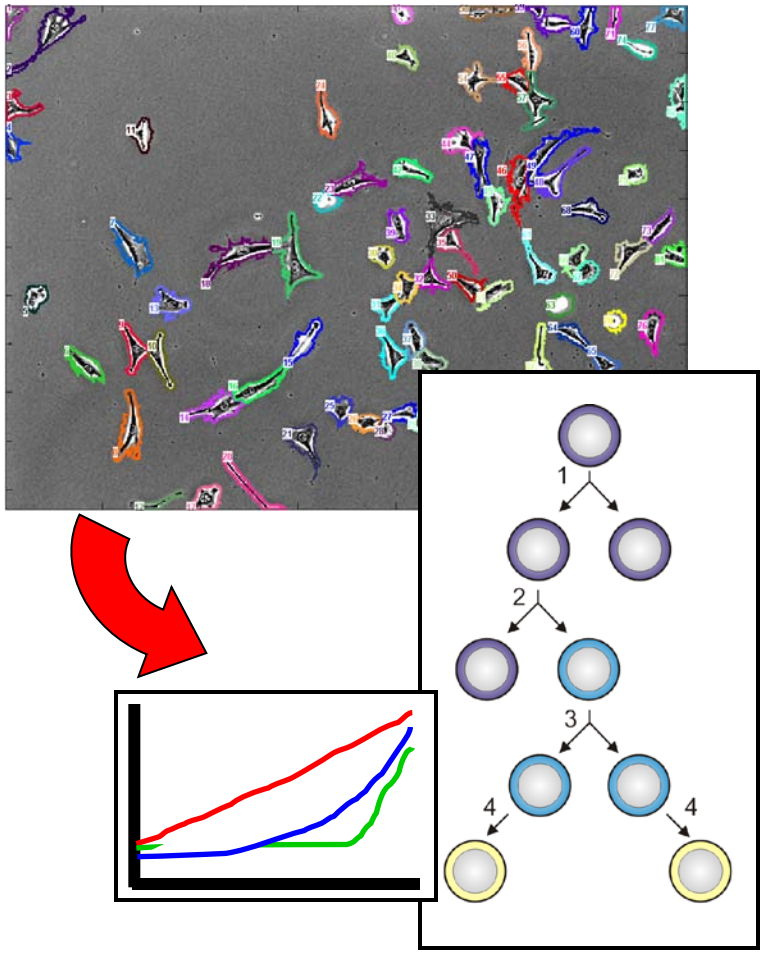
Single cell GFP intensities over time indicate tenascin-C regulation is coupled to the cell cycle





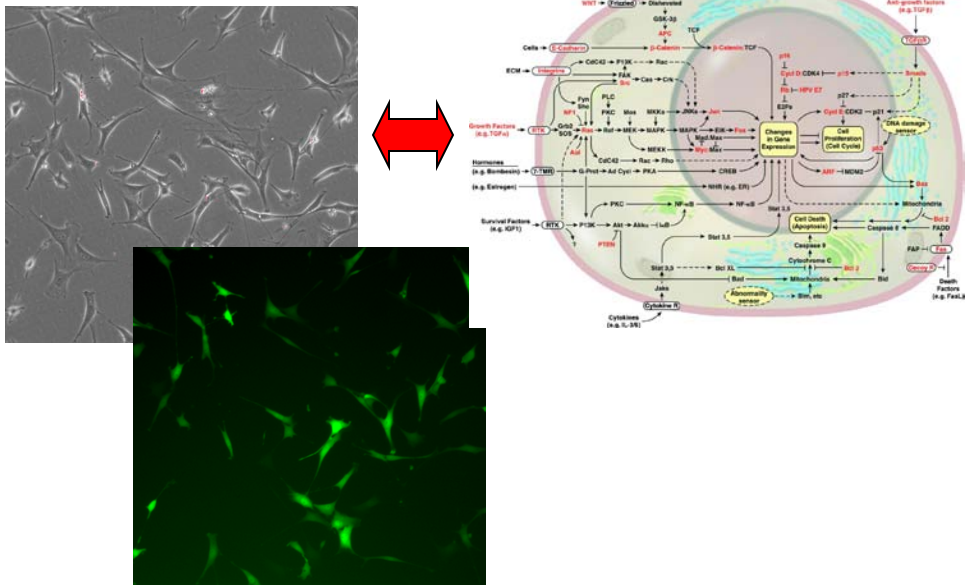
Normalizing the intensity data and averaging over >30 cells suggests that tenascin-C production is upregulated before division and is directly coupled to cell cycle progression

Applications of quantitative live cell image data



- Quantify GFP intensities changes with time
- Examine correlation between phenotype (migrate, division time) and gene expression
- Understand (stem cell) lineage progression and epigenetic gene regulation

Acknowledgements:



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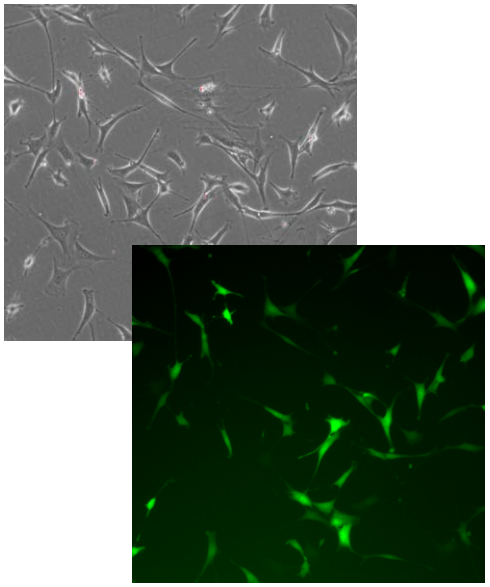
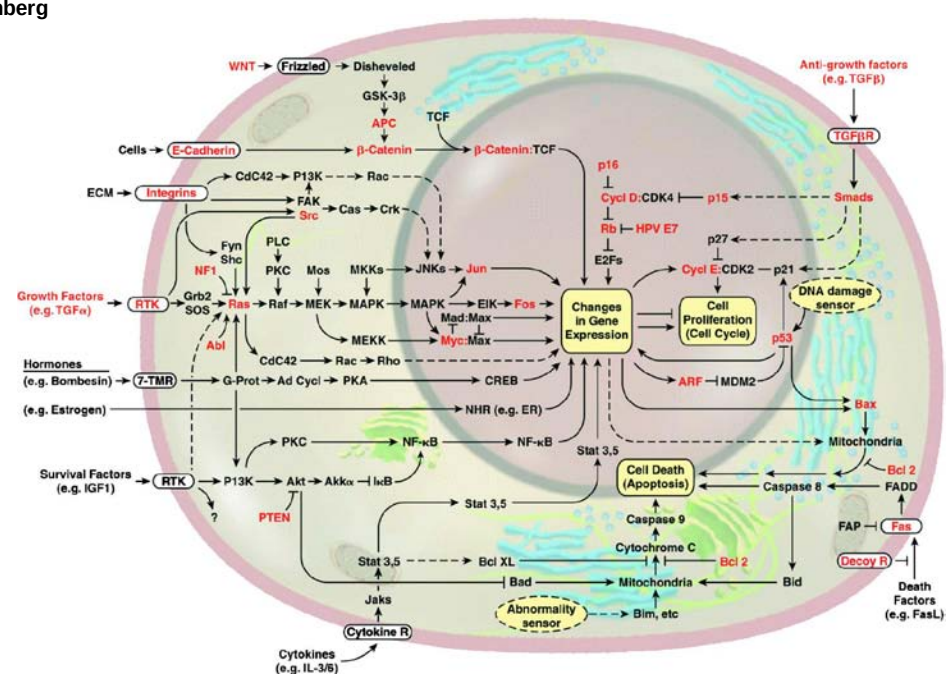
Live Cell Image Analysis

- Joe Chalfoun (segmentation, tracking)
- Marcin Kociolek (segmentation)
- Alden Dima (segmentation, tracking)
- Antonio Cardone (segmentation, tracking)
- Ben Stottrup (manual segmentation, Augsburg College, MN)

Understanding cell state with quantitative live cell imaging

The Hallmarks of Cancer
Douglas Hanahan and Robert A. Weinberg

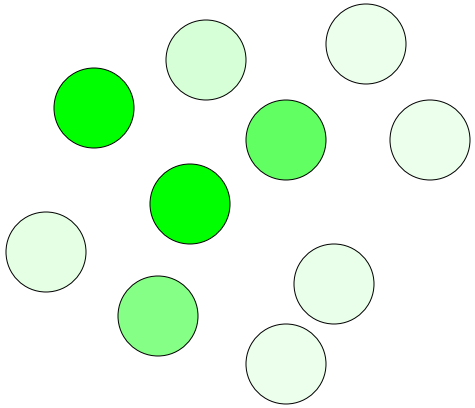




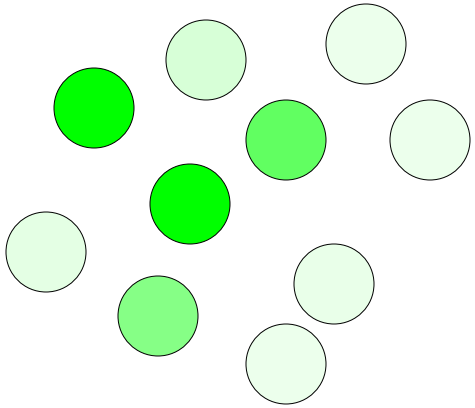
Gain pathway knowledge:

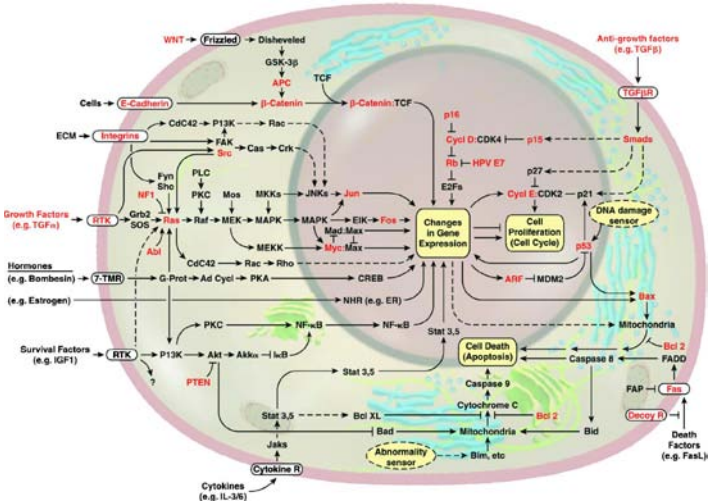
- mechanistic understanding of disease
- identification/validation of cellular biomarkers
- therapeutic intervention, drug discovery, toxicity

Single cell analysis



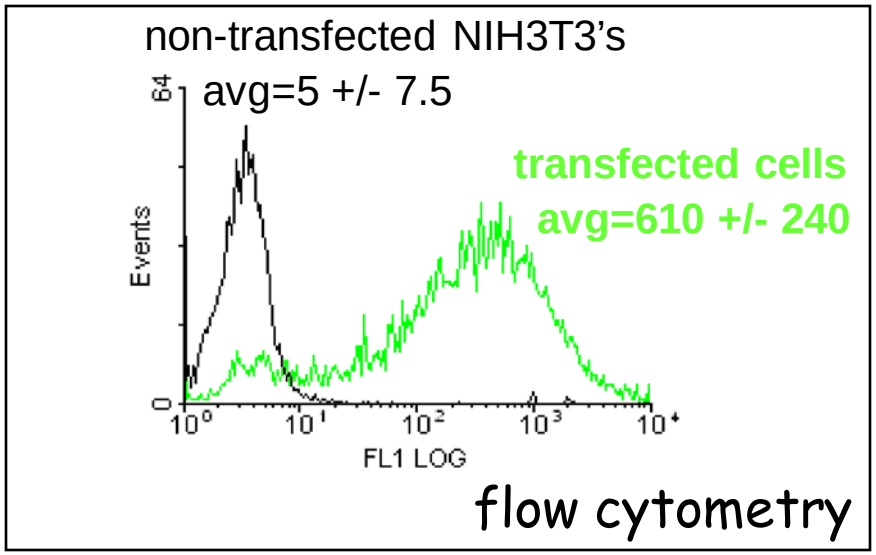
Average expression



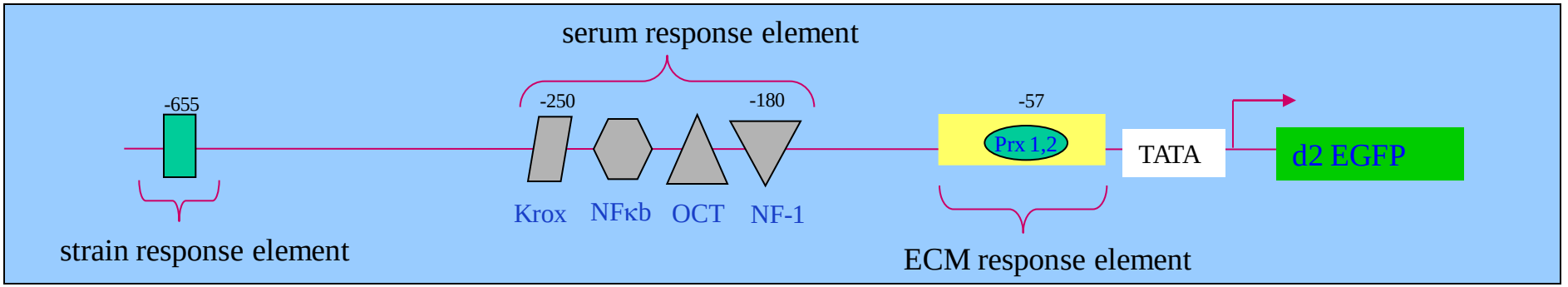


Biological variability
Subpopulation information

Cell intensities

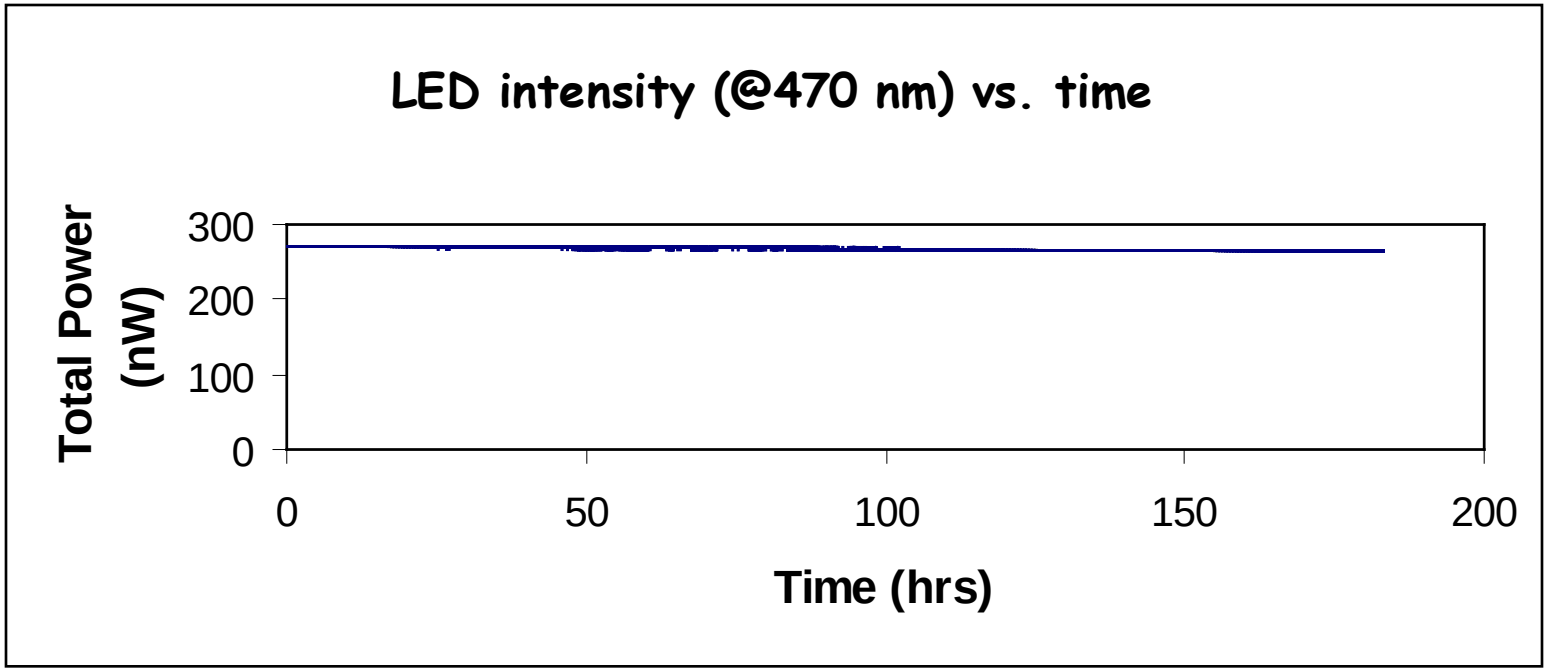


The Tenascin-C promoter



- Tenascin-C is an extracellular matrix protein
- Promoter sequence is ~4kBases with a number of transcription factor binding sites
- Gene activity is upregulated during development, wound healing and in some cancers and is often correlated with cell spreading and proliferation *in vitro*

LED illumination stability



LED intensity measured over 7 days

Tenascin-C: an ECM protein

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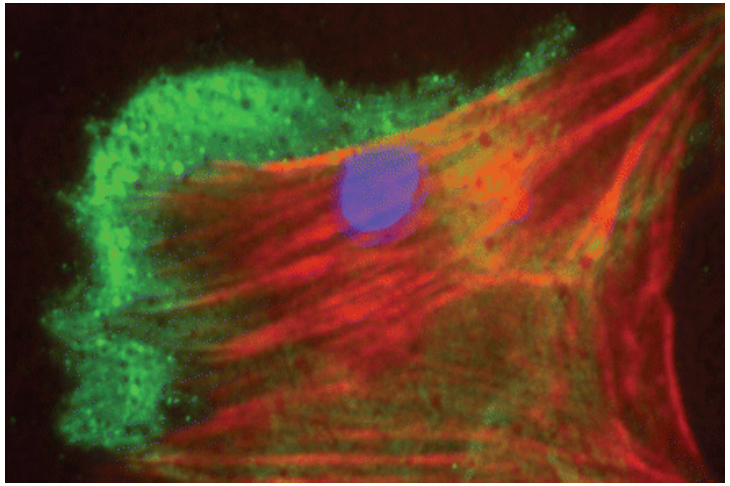
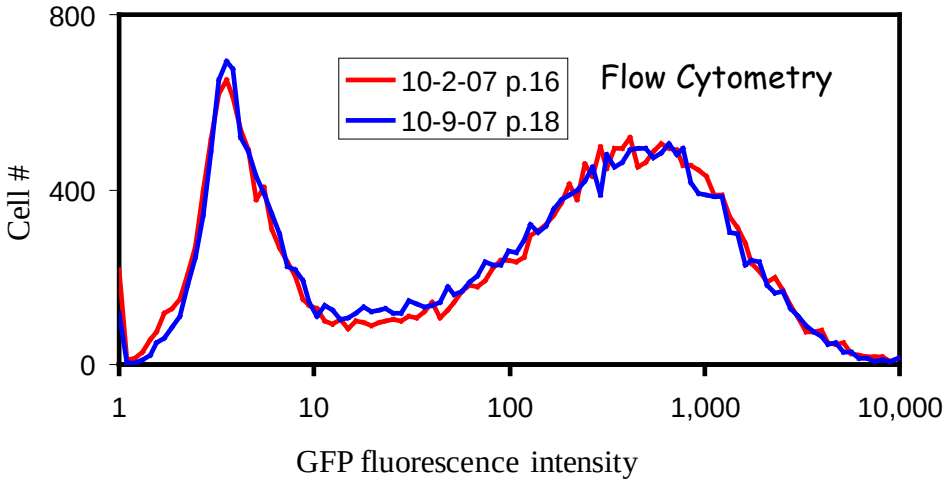
- TNC levels are high during embryogenesis, but almost absent during normal postnatal life with some basal expression detectable in tendons and ligaments only.



(Chapados et al., *Circulation Research.* 2006;99:837.)

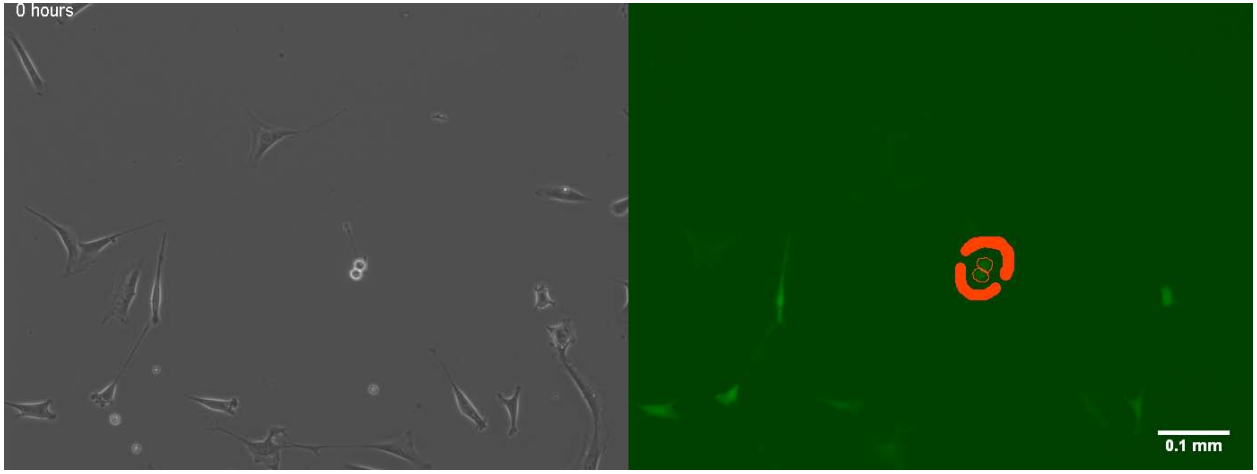
- Tenascin-C expression is upregulated during inflammation, wound healing, and in many cancers
- Tenascin-C is thought to have anti-adhesive properties and play a role in signaling
- The expression of TNC is often correlated with cell spreading

Quantify GFP intensities from live cells



What gene regulation (or other?) processes give rise to the stable distribution of GFP intensities with large variability?

We use live cell imaging and image analysis to quantify the dynamics of GFP expression driven by the tenascin-C promoter



Movie: 65 hours, phase contrast on left, GFP on right