



Progress in traction mapping and photomanipulation as input to modeling initiative

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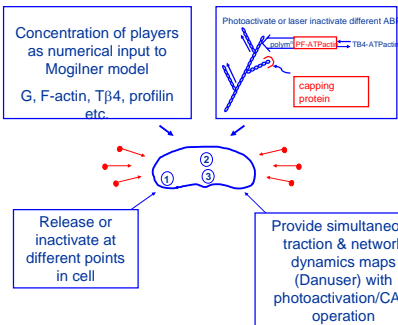
MILESTONE

1. Photoactivate thymosin B-4 in different regions of the keratocyte periphery and simultaneously quantitatively map tractions (collaboration with Mogilner).

- a) Obtain and test caged thymosin β4 in vitro (actin polymerization assay) and in vivo. ✓
- b) Establish conditions that provide sufficient sensitivity to map keratocyte tractions on polyacrylamide substrates. ✓
- c) Establish conditions such that uncaging and subsequent traction mapping may be performed on the same cell. ✓
- d) Input data to the Modeling Initiative and obtain feedback. ✓

- ✓ : work done
- ✓ : work in progress
- ✓ : planned work

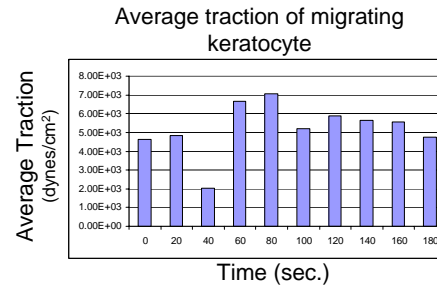
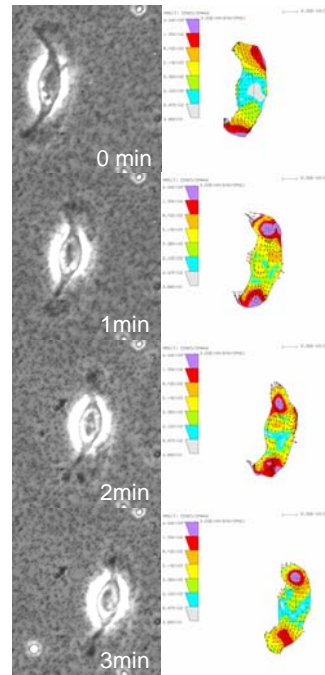
Overall project goals



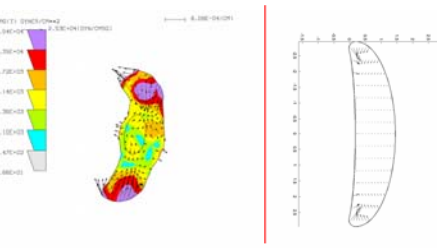
The model system-migrating fish keratocytes

Challenge 1. Settling keratocytes on elastic substrate.

Challenge 2. Design elastic substrate of proper stiffness to map tractions of moving keratocyte



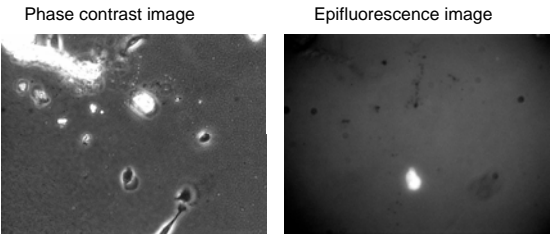
Comparison of experimental tractions of migrating keratocyte (left panel) with modeling results of Alex Mogilner – Modeling Initiative (right panel)



Challenge 3. Introduction of caged molecules into keratocytes migrating on elastic substrate.

Keratocytes on the elastic substrate bead loaded with fluorescein-dextran (60 min. after bead loading)

Caveat: low efficiency loading method, requires concentrated solution of loaded material.



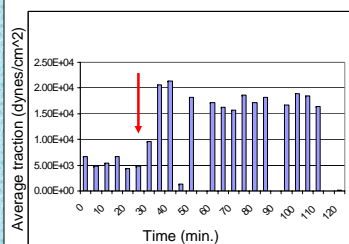
Goals

Find an efficient loading method:

- Microinjection
- Electroporation
- TAT peptide

Ongoing collaborative project (R. Tilghman & T. Parsons)

Goal: investigate the role of focal adhesion kinase (FAK) in the development and maintenance of tension in the cell employing PF-228 - a small molecule inhibitor of FAK

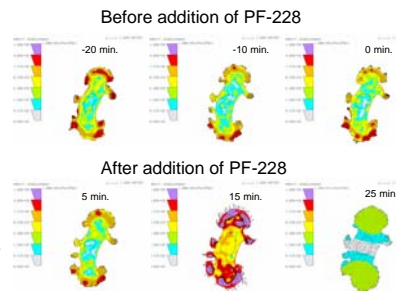


Left side panels:

Average traction of the REF 52 cell after addition of 1 μM of PF-228 drug (red arrow indicates addition of inhibitor).

Right side panels:

Representative figures of tractions and traction magnitudes of the cell before and after addition of 1 μM of PF-228.



Longer term goals

1. concentration of uncaged TB4.
2. Simultaneous network flow (Danuser) and adhesion data (multimode imaging)