

## Stress Fiber remodeling in Living Cells

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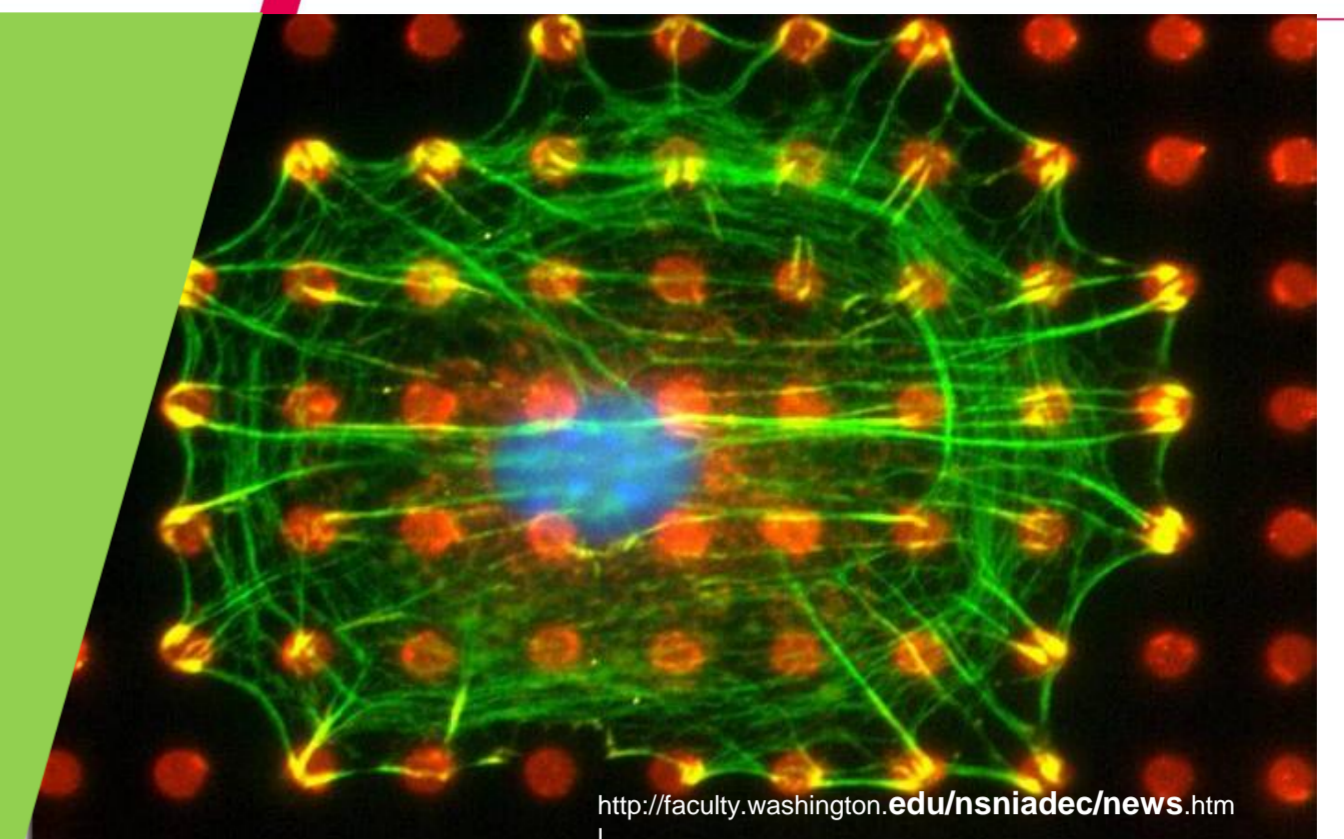
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# Stress fiber remodeling in living cells

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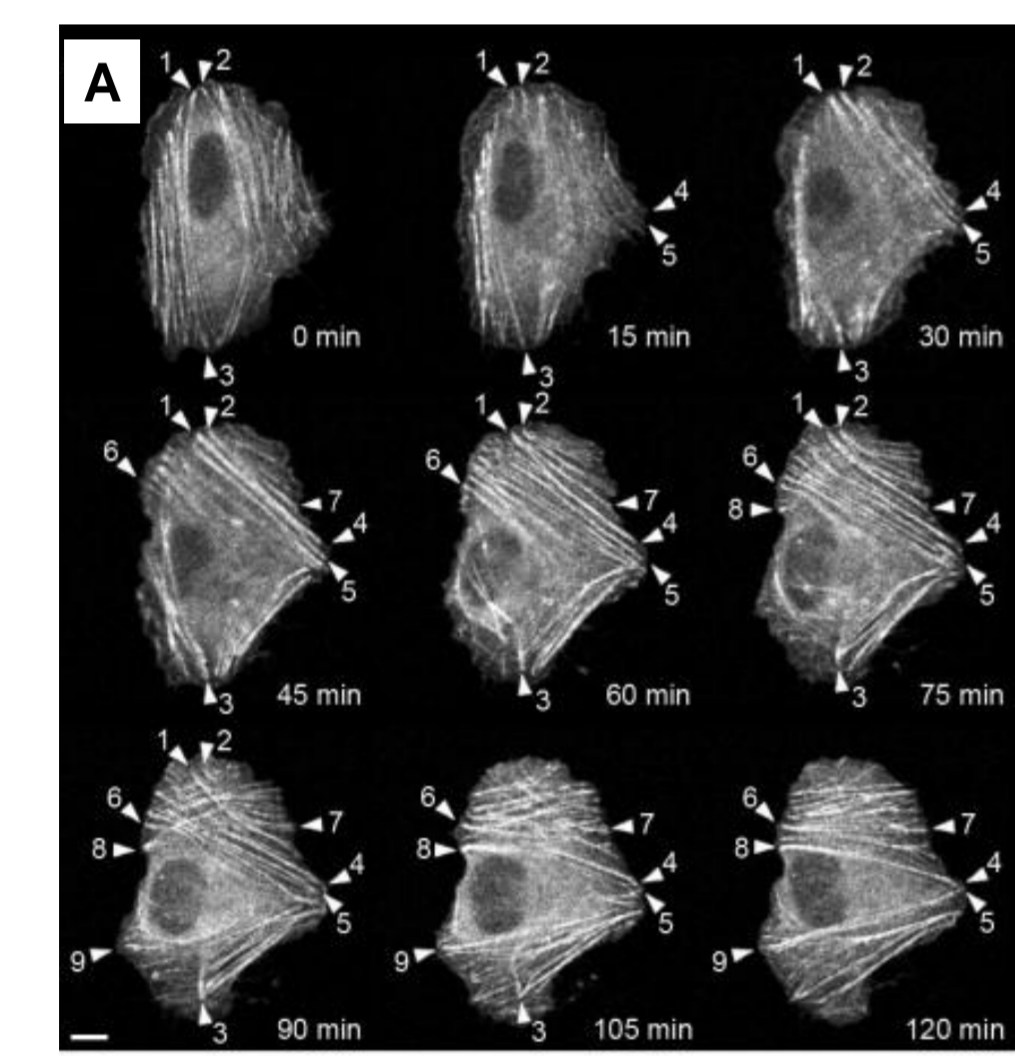
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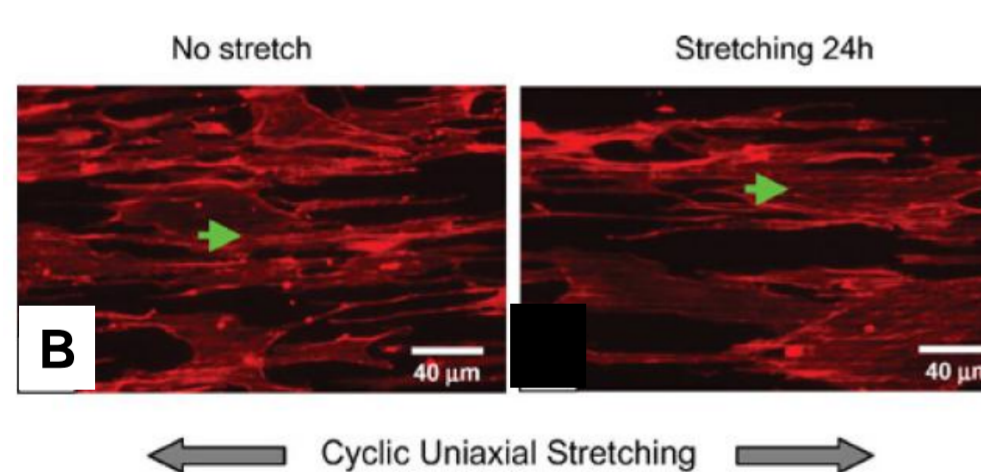
## Introduction

Cells are known to actively sense and respond to their (mechanical) environment. An important player in this respect is the actin cytoskeleton. Bundles of actin fibers - referred to as 'stress fibers' - actively respond to mechanical cues of the cellular microenvironment and as such are involved in cell traction forces, cell adhesion and migration.

The main trigger for stress fiber remodeling is a topic of debate. In a 2D environment, actin polymerization is deformation dependent. Under mechanical deformation, stress fibers form only in the direction of minimal substrate deformation and, as a consequence, cells exhibit a strain avoidance response when exposed to uniaxial cyclic strain<sup>1</sup>. However, contact guidance seems also to provide a competing stimulus for stress fiber remodeling<sup>2</sup> (Fig. 1).



**Figure 1.** A) Time-lapse images of U2OS cells expressing GFP-actin and subjected to 10% cyclic uniaxial stretch at 1 Hz. Stress fibers reorient perpendicular to the direction of the strain. Bar, 10  $\mu$ m (Lee *et al.*, *Biochem Biophys Res Commun*, 2010). B) The actin cytoskeleton of tendon fibroblasts (red) does not have a strain avoidance response when cell are cultured on micro grooved silicone surfaces and exposed to 8% cyclic uniaxial strain. (Wang *et al.*, *Ann Biomed Eng*, 2005)



## Aim of the project

The aim of this project is to dissect the impact of **strain** and **contact guidance** on stress fiber remodeling. We hypothesize that stress fiber alignment is driven by contact guidance, even under uniaxial cyclic strain.

## Approach

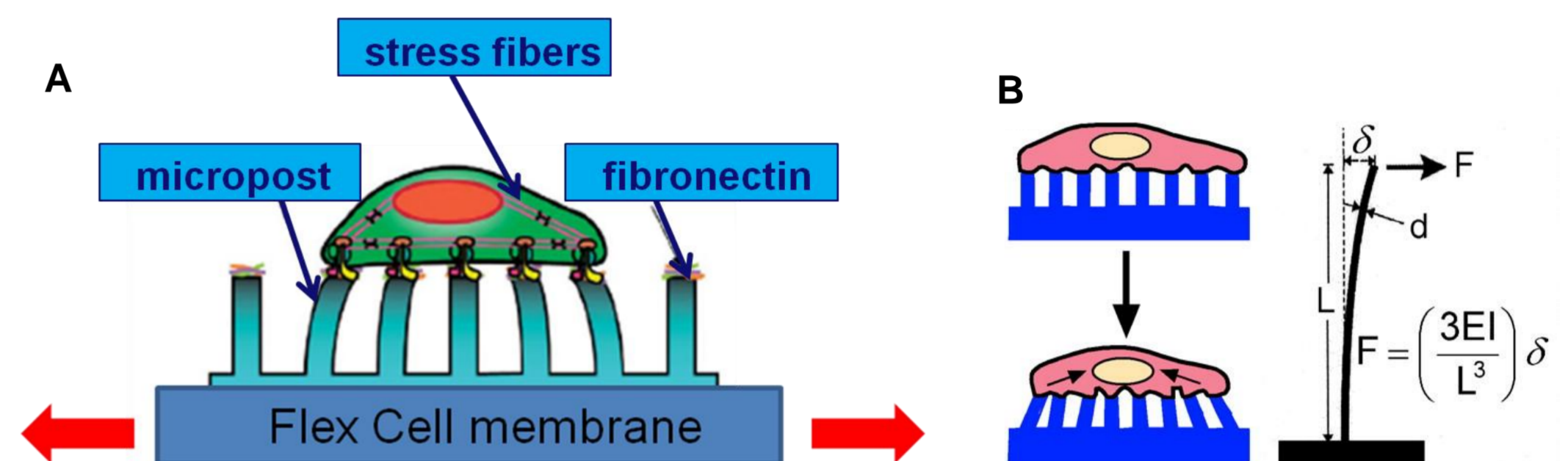
We will perform 2D live-cell imaging experiments using 3T3 cells under mechanical stimulation (**strain**) and topological affinity (**contact guidance**).

Stress fiber dynamics will be assessed from real-time single cell recordings of LifeAct-GFP transfected cells. This construct does not impair actin dynamics in the cells and offers an excellent signal-to-noise-ratio.

We are developing a model system of fibronectin coated micropost arrays mounted on a Flex Cell membrane, which provides:

### • Strain

The mechanical stimulation is provided by uniaxial cyclic strain applied to the Flex Cell membrane. By imaging the displacement of the micropost top, we are able to measure traction forces exerted by the cells on the microposts during uniaxial cyclic loading (Fig. 2).



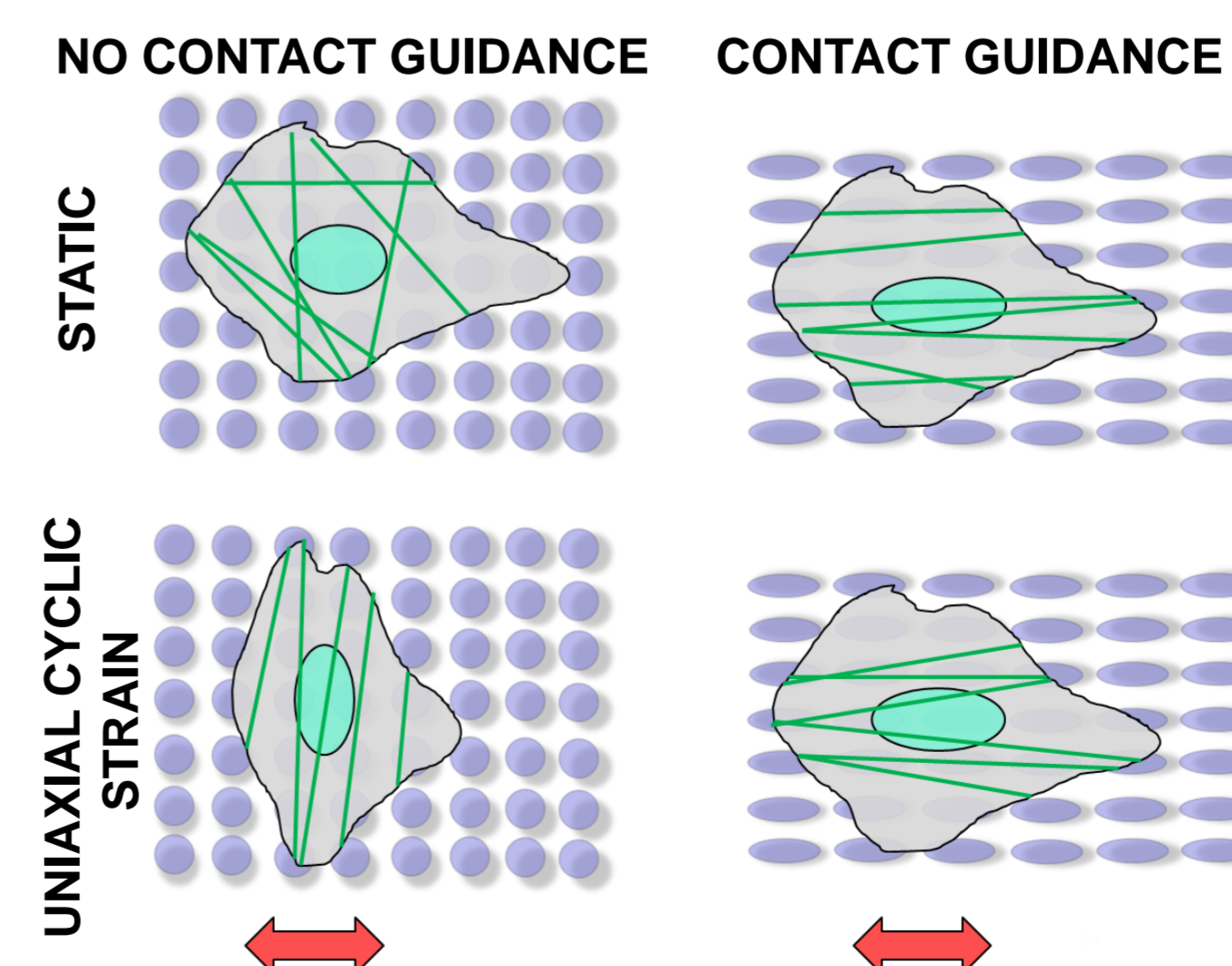
**Figure 2.** A) Schematic representation of our model system adapted from Sniadecki *et al.*, *Proc Nat Acad Sci*, 2007. Cells are seeded on an array of microposts coated with fluorescence-labeled fibronectin. The micropost array is glued to the Flex Cell membrane. The red arrows represent the direction of the uniaxial cyclic strain that can be applied. B) Cells exerting traction forces deflect the elastomeric posts, the traction forces can be calculated from the displacement of the micropost top. From Tan *et al.*, *Proc Nat Acad Sci*, 2002.

### • Contact guidance

The contact guidance cue is provided by the anisotropic geometry of the micropost cross-section (oval vs circular), while keeping the height constant

## Future work

As a first step, we will visualize actin dynamics for contact guidance and uniaxial cyclic strain (Fig. 3). At the same time we will measure forces exerted by these cells. By relating actin dynamics and forces we will unravel the dominant factor for stress fiber remodeling.



**Figure 3.** Top-view representation of the experiment. Cells are seeded on the micropost array (purple). Stress fiber (green) dynamics are followed under the combination of contact guidance and static vs uniaxial cyclic strain in order to detect which factor has more impact on stress fiber remodeling.