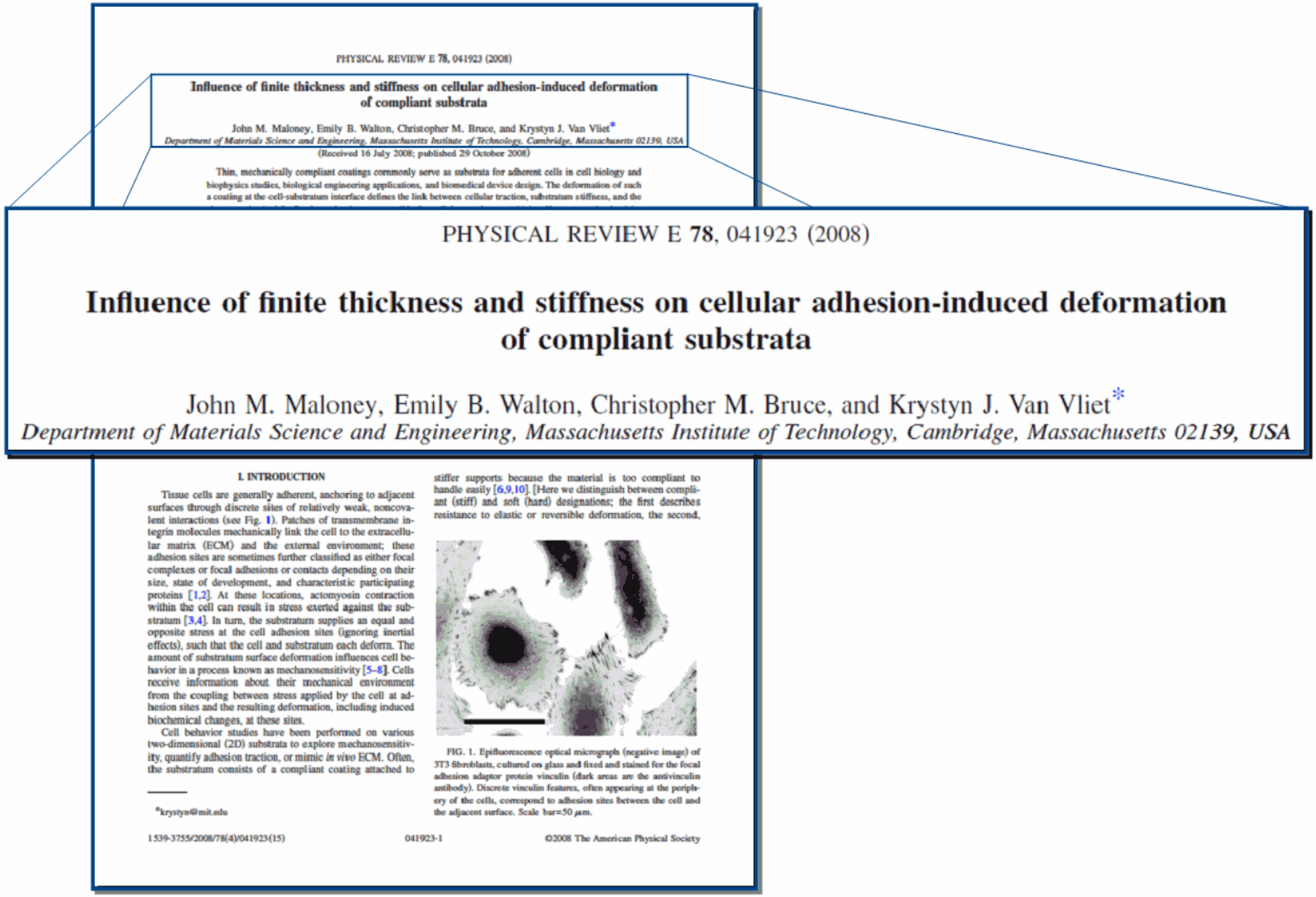

PHYSICAL REVIEW E 78, 041923 (2008)

Influence of finite thickness and stiffness on cellular adhesion-induced deformation of compliant substrata

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(Received 16 July 2008; published 29 October 2008)

Thin, mechanically compliant coatings commonly serve as substrata for adherent cells in cell biology and biophysics studies, biological engineering applications, and biomedical device design. The deformation of such a coating at the cell-substratum interface defines the link between cellular traction, substratum stiffness, and the

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I. INTRODUCTION

Tissue cells are generally adherent, anchoring to adjacent surfaces through discrete sites of relatively weak, noncovalent interactions (see Fig. 1). Patches of transmembrane integrin molecules mechanically link the cell to the extracellular matrix (ECM) and the external environment; these adhesion sites are sometimes further classified as either focal complexes or focal adhesions or contacts depending on their size, state of development, and characteristic participating proteins [1,2]. At these locations, actomyosin contraction within the cell can result in stress exerted against the substratum [3,4]. In turn, the substratum supplies an equal and opposite stress at the cell adhesion sites (ignoring inertial effects), such that the cell and substratum each deform. The amount of substratum surface deformation influences cell behavior in a process known as mechanosensitivity [5–8]. Cells receive information about their mechanical environment from the coupling between stress applied by the cell at adhesion sites and the resulting deformation, including induced biochemical changes, at these sites.

Cell behavior studies have been performed on various two-dimensional (2D) substrata to explore mechanosensitivity, quantify adhesion traction, or mimic *in vivo* ECM. Often, the substratum consists of a compliant coating attached to stiffer supports because the material is too compliant to handle easily [6,9,10]. [Here we distinguish between compliant (stiff) and soft (hard) designations; the first describes resistance to elastic or reversible deformation, the second,

FIG. 1. Epifluorescence optical micrograph (negative image) of 3T3 fibroblasts, cultured on glass and fixed and stained for the focal adhesion adapter protein vinculin (dark areas are the antivinculin antibody). Discrete vinculin features, often appearing at the periphery of the cells, correspond to adhesion sites between the cell and the adjacent surface. Scale bar=50 μm.

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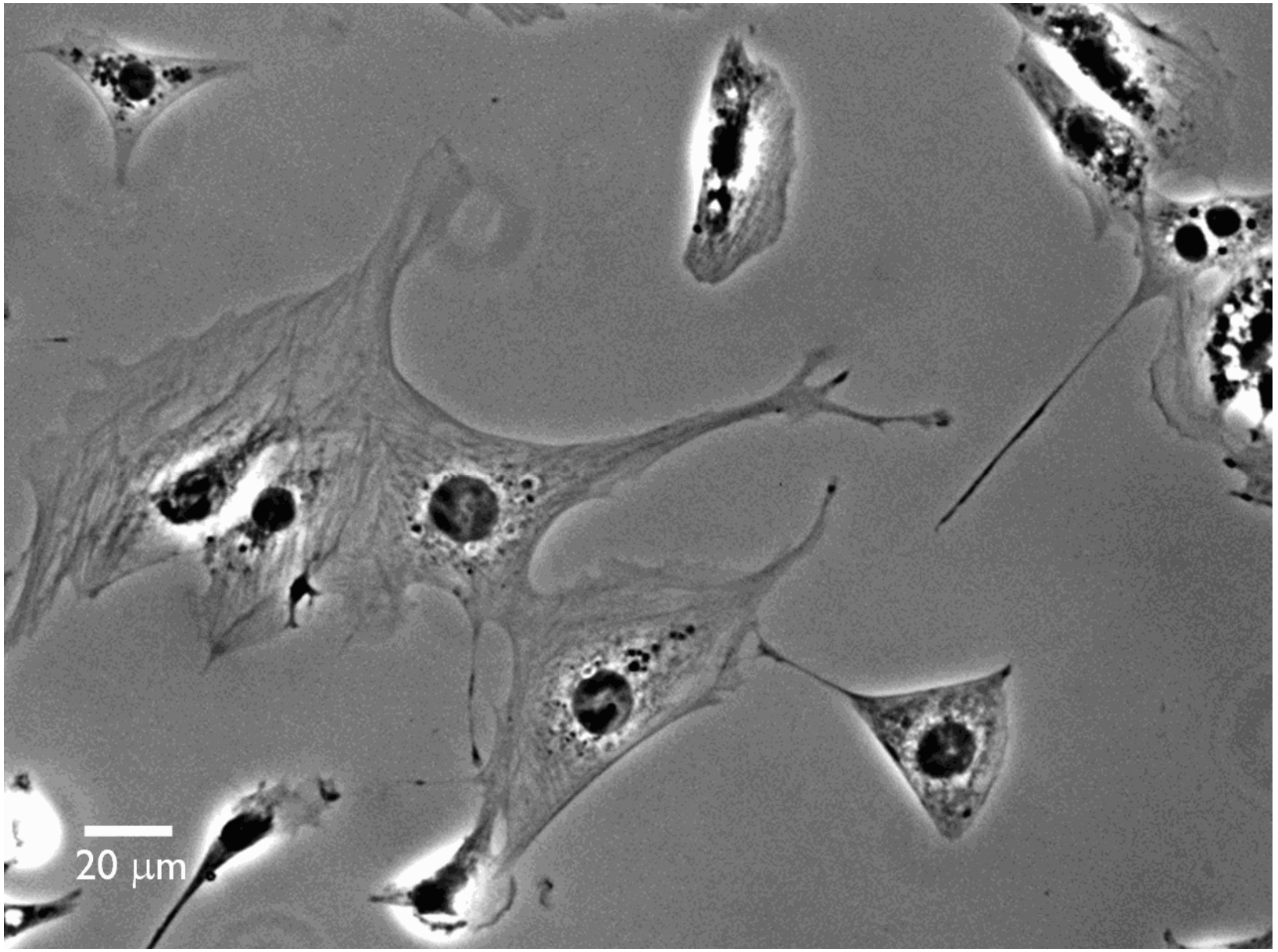
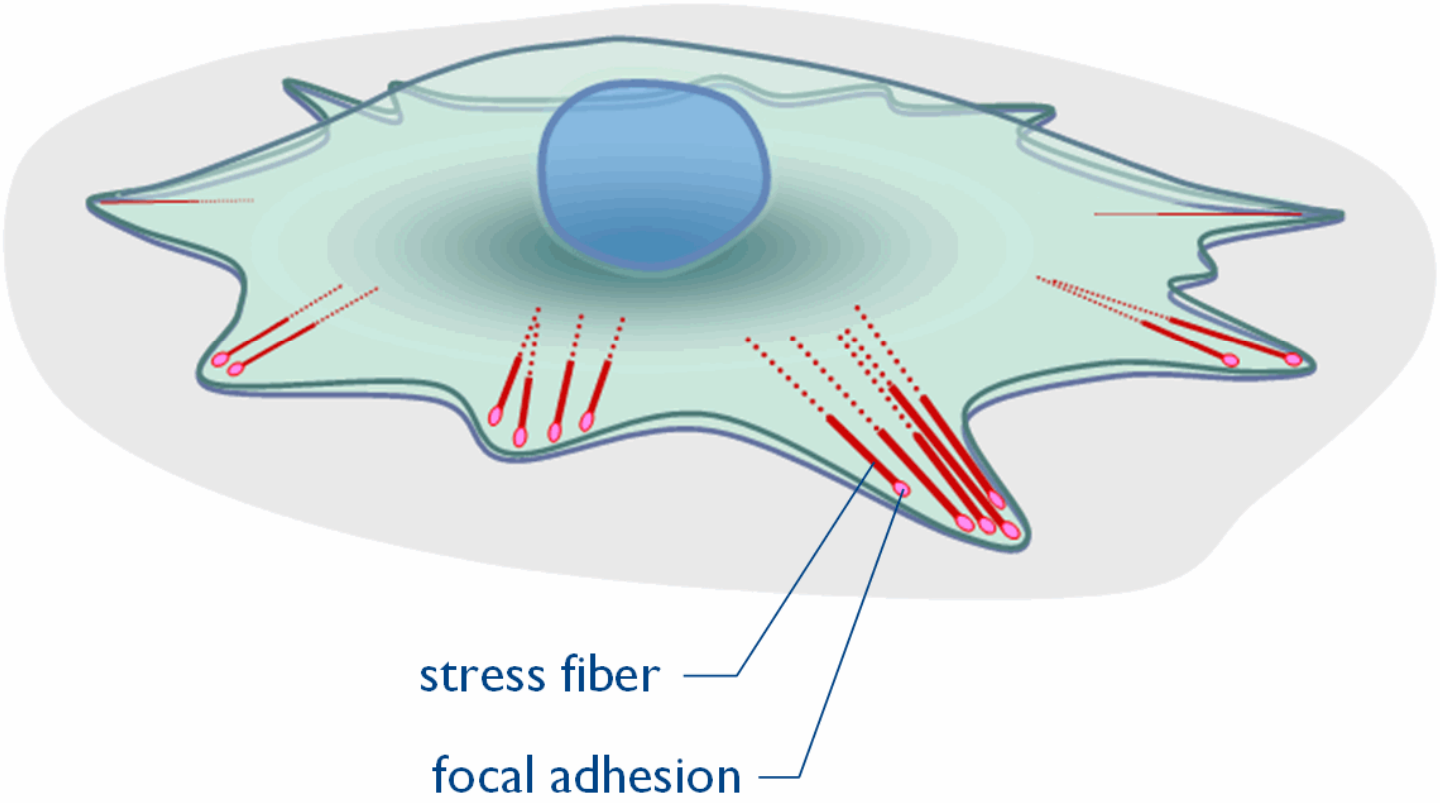
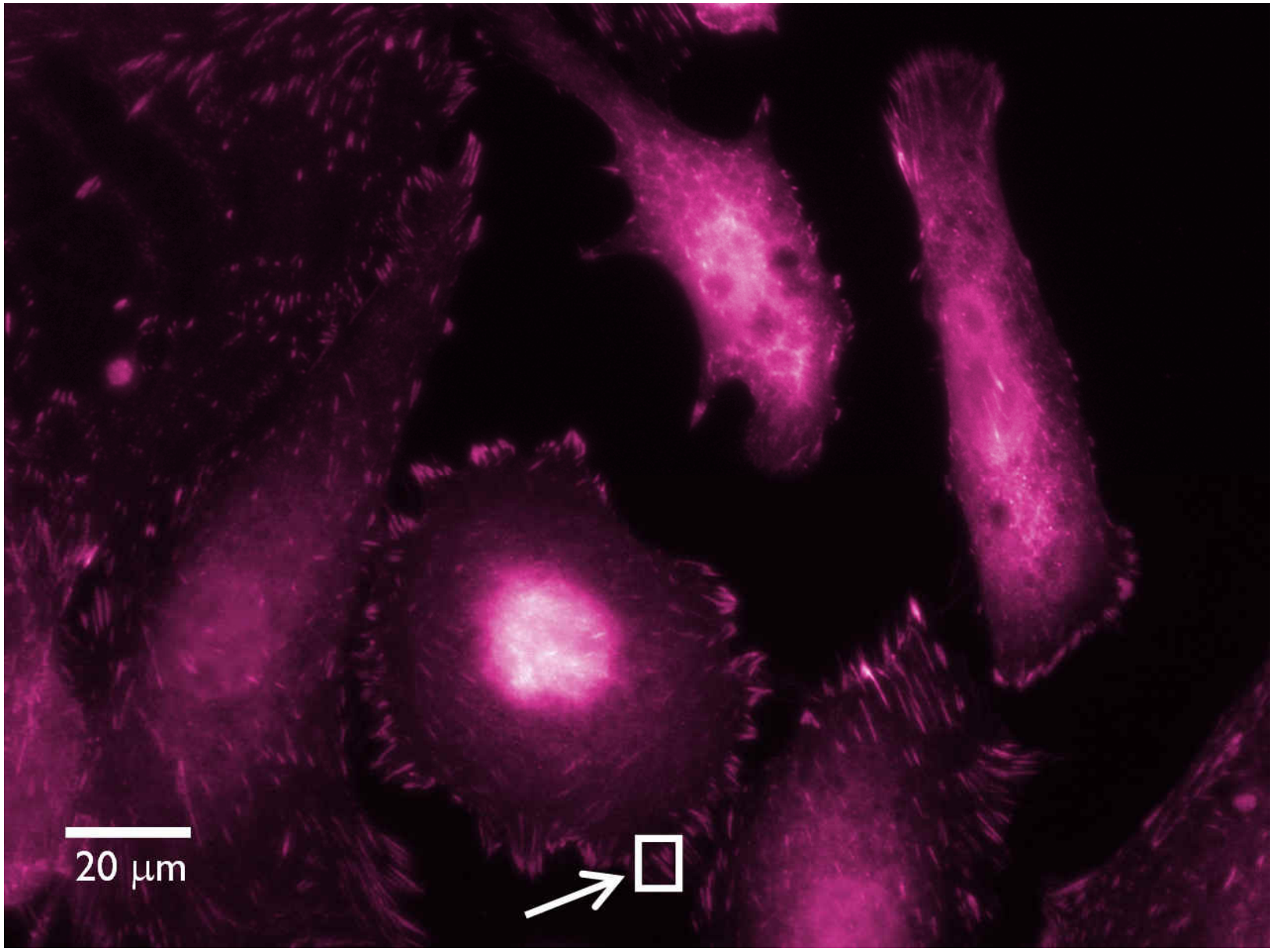
How deep can tissue cells feel? That is, how far into their surroundings can adherent cells sense a change in stiffness? I and my colleagues Emily Walton and Christopher Bruce, and our advisor, Prof. Krystyn Van Vliet, recently studied this question to learn about cells' mechanosensory abilities and the design of synthetic substrates for in vitro cell study.



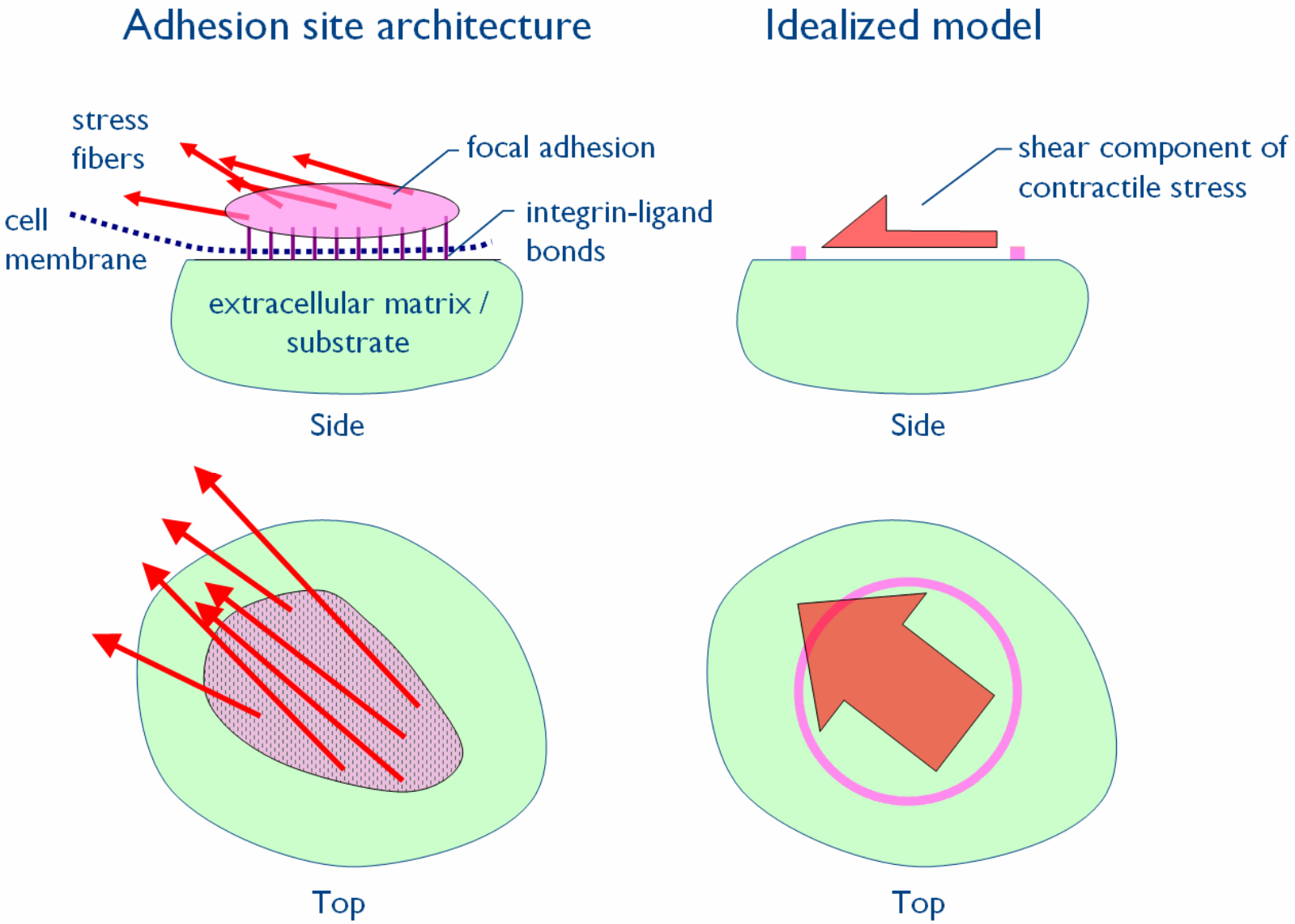
Many tissue cells are adherent, like these individual fibroblasts stretched out and growing on a flat polystyrene surface, or substrate. But cells aren't uniformly sticky.



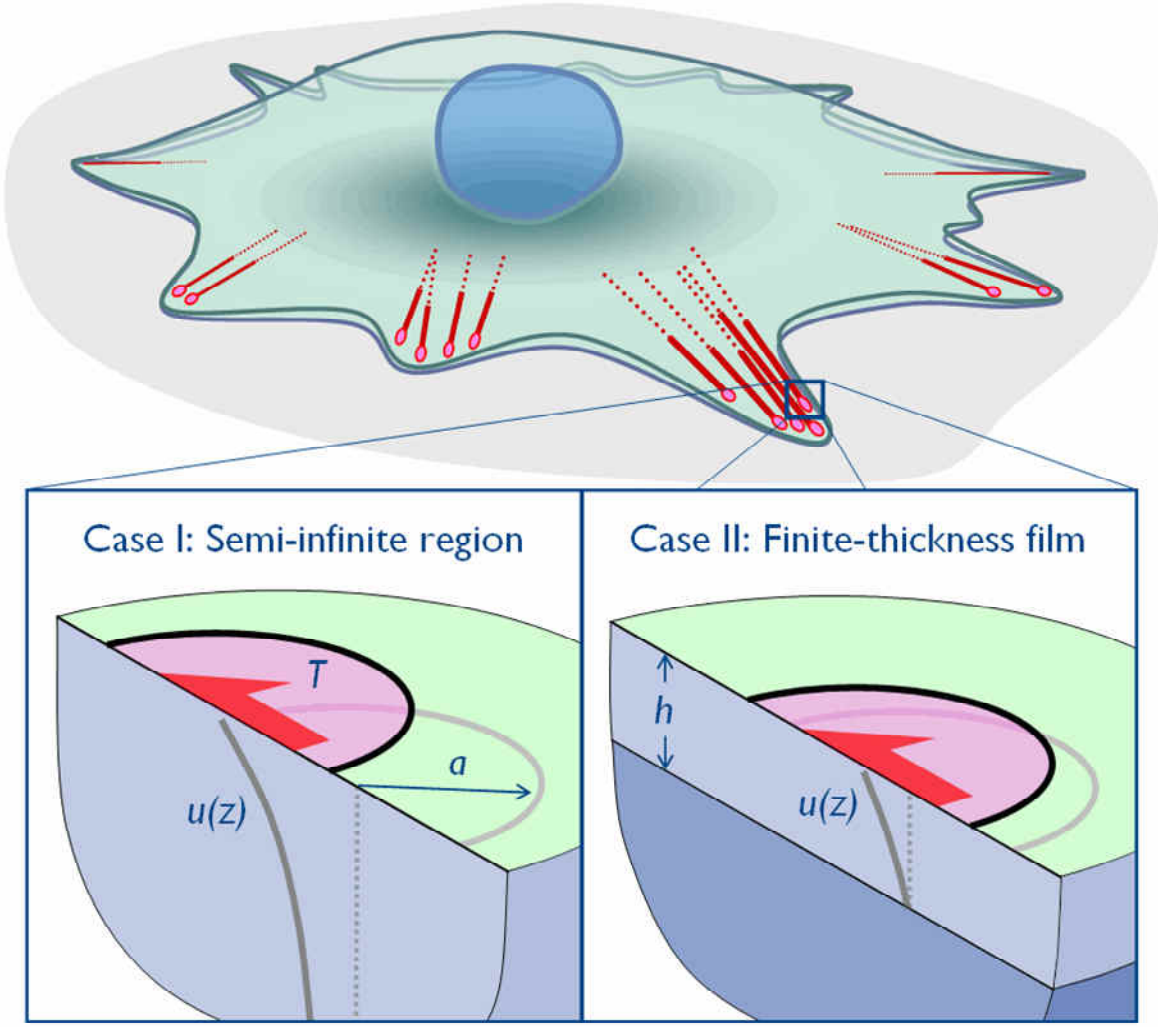
Rather, they attach to their surroundings at discrete, dynamic patches called “focal adhesions.” Tugging on these adhesion sites are cytoskeletal stress fibers of filamentous actin that hold the cell in a state of mechanical tension, the substrate underneath in compression.



We can identify adhesion sites, which measure one to several microns large, and are often located at the periphery of cells, by fluorescently staining for one of the participatory proteins—in this case, vinculin.



At focal adhesions, the cell pulls at the adjacent substrate via transmembrane integrin molecules and senses the resulting deformation as a measure of the substrate's compliance. We idealize these regions as circular areas where a uniform shear stress acts on the surface.



Let's consider two possible substrate configurations: on the left, a compliant semi-infinite region; on the right, the same material in the form of a film atop an underlying, perfectly rigid base. This second configuration is common in laboratory experiments, for example when compliant hydrogels, used to mimic soft tissue, are supported by glass or plastic. In both configurations, the traction at the adhesion site deforms the surface. But for sufficiently thin films, the deformation is attenuated by the presence of the rigid base, which the cell begins to feel. The minimum thickness to avoid interference from the rigid base has been described as a "critical thickness."

Definition of "critical thickness"	Effect of increasing substrate stiffness?
Depth of an arbitrary strain (e.g., 0.1%)	Decreases
Film thickness that maintains an arbitrary surface deformation	Increases
Film thickness that attenuates surface deformation by some percentage (e.g., 10%)	Independent

But how exactly should we define this critical thickness? We found that multiple definitions were possible and in use. For example, the depth where one would find an arbitrarily small strain (say, a tenth of a percent) could be said to be a critical depth; inserting a rigid underlying base at this depth would have little effect on surface movement. Or, we might define a critical film thickness as one that maintains a certain surface deformation and thus sends a certain cue to adherent cells. A third definition might be the film thickness that attenuates surface deformation by a certain fraction, perhaps a tenth. These values change in different ways in response to changes in substrate stiffness. Stiffer substrates reduce the depth of strain propagation, but they require thicker films to obtain the same deformation. And the film thickness that cuts surface deformation by a certain fraction doesn't depend on film stiffness at all. To see why this is true, let's look closer at how these deformations are calculated.

Green's tensor

force vector

displacement vector

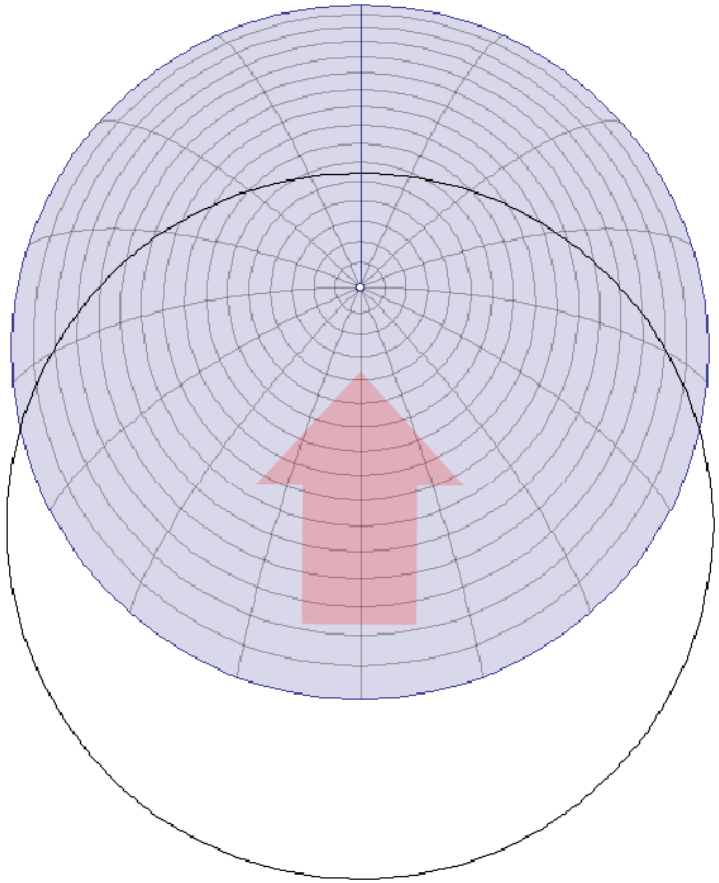
$$\vec{u} = \mathbf{G}\vec{F} = \int_S \mathbf{G}\vec{T}\, dA$$

$$G = \frac{1}{2\pi}\int_0^\infty \begin{pmatrix} -\Phi_1 J_0 + \frac{x^2-y^2}{x^2+y^2}\Phi_2 J_2 & \frac{2xy}{x^2+y^2}\Phi_2 J_2 & \frac{x}{\sqrt{x^2+y^2}}\Phi_{13} J_1 \\ \frac{2xy}{x^2+y^2}\Phi_2 J_2 & -\Phi_1 J_0 - \frac{x^2-y^2}{x^2+y^2}\Phi_2 J_2 & \frac{x}{\sqrt{x^2+y^2}}\Phi_{13} J_1 \\ -\frac{x}{\sqrt{x^2+y^2}}\Phi_{31} J_1 & -\frac{y}{\sqrt{x^2+y^2}}\Phi_{31} J_1 & -\Phi_{33} J_0 \end{pmatrix} d\rho$$

Bessel function

$$\frac{6+4\nu^2-11\nu-2e^{2h\rho}[4\nu^3-20\nu^2+h\rho(1-\nu)+24\nu-(r\rho)^2(3-\nu)-9]-e^{4(h\rho)}[8\nu^3-20\nu^2+21\nu-2(h\rho)^2(1-\nu)+2h\rho(1-\nu)-8]}{(1+e^{2h\rho})[3-4\nu+e^{4h\rho}(3-4\nu)+2e^{2h\rho}(2h^2\rho^2+8\nu^2-12\nu+5)]}$$

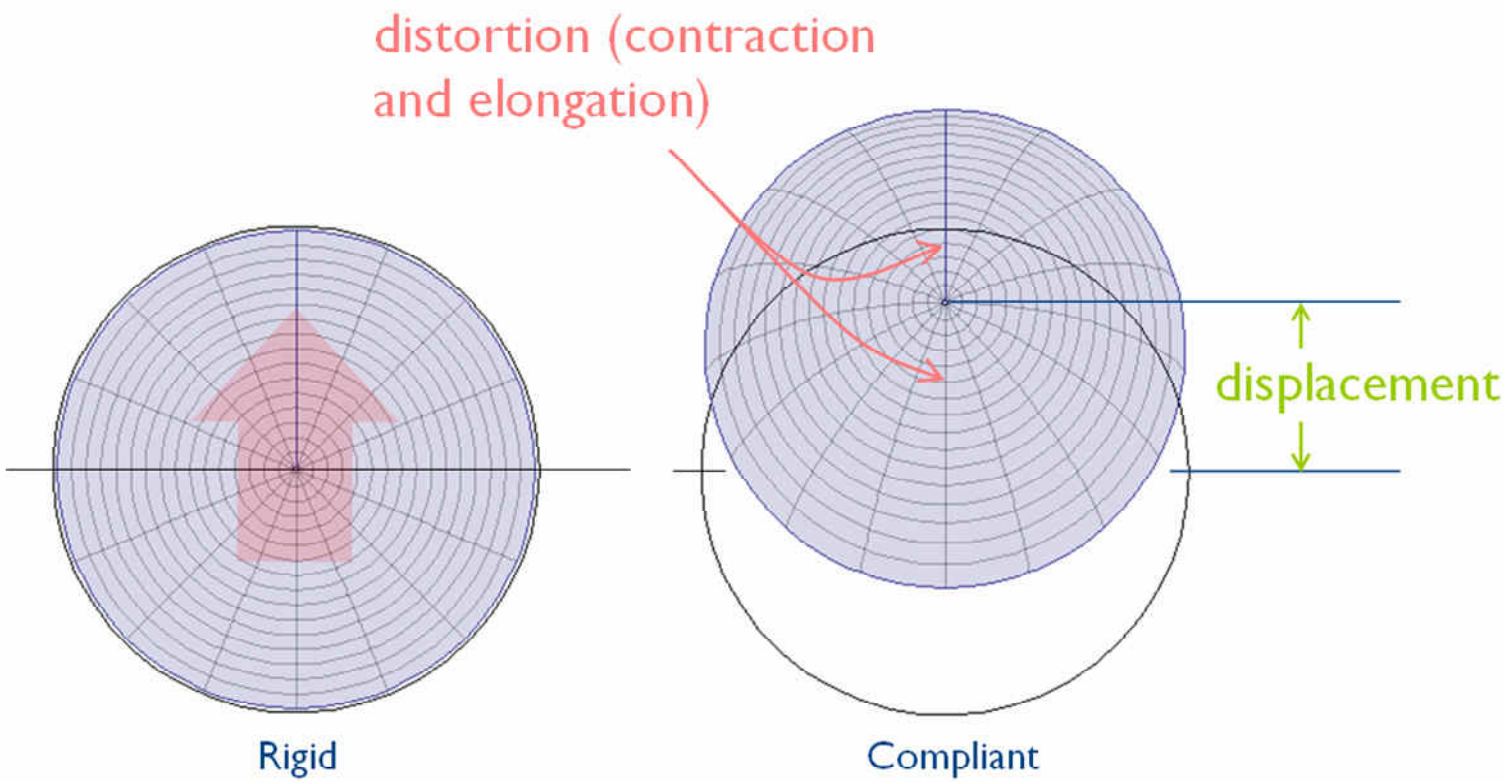
We use the Green's tensor, which couples an applied force to the resulting displacement. In the case of surface deformation of a finite-thickness film, the Green's tensor incorporates several Bessel functions and intricate unique characteristic terms best manipulated with a computer. Because we're interested in the response from a shear stress, we integrate the Green's tensor over the circular adhesion site area.



Compliant

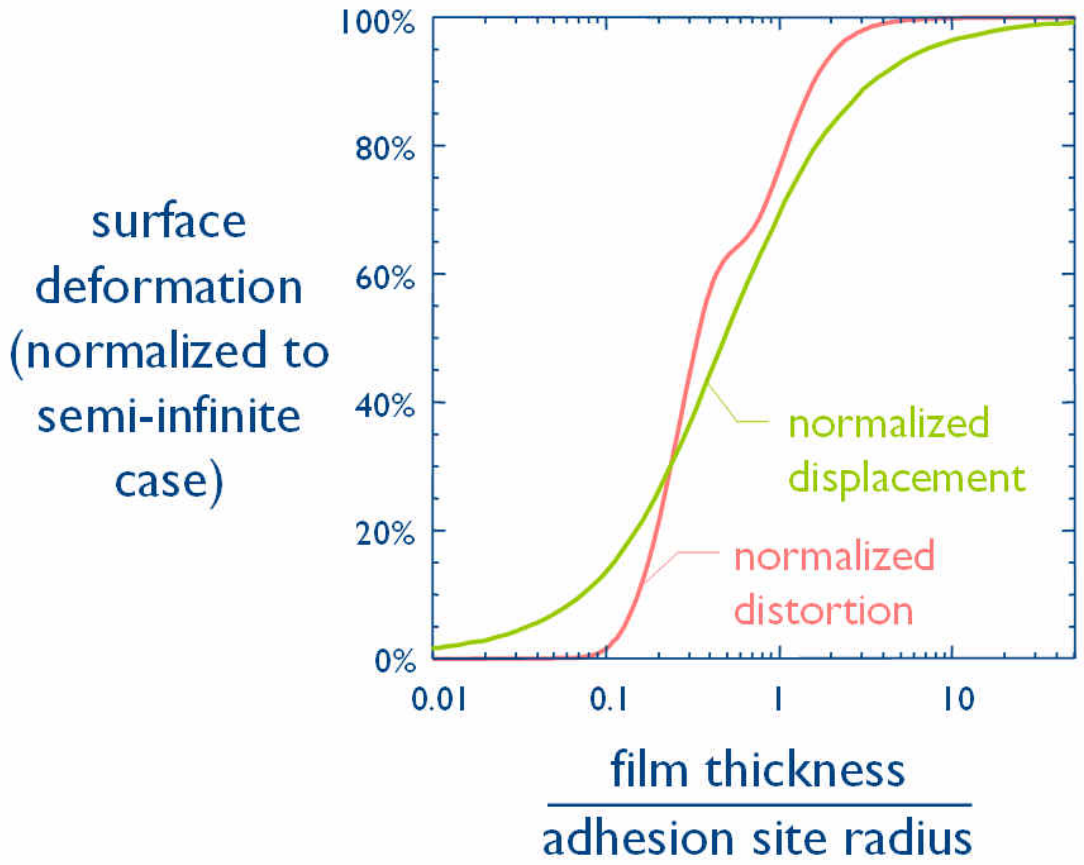
Rigid

Numerical calculations let us predict the deformation of an individual circular area, representing the adhesion site, for substrates of any stiffness. Not surprisingly, the site is motionless for very stiff substrates; after all, a cell can hardly deform glass, whose stiffness is 70 GPa, or polystyrene, about 1 GPa. But a cell can more easily deform hydrogels, whose stiffness can be in the kilopascals, as compliant as cells themselves, and a thousand times more compliant than soft rubber. Let's look more closely at this deformation.

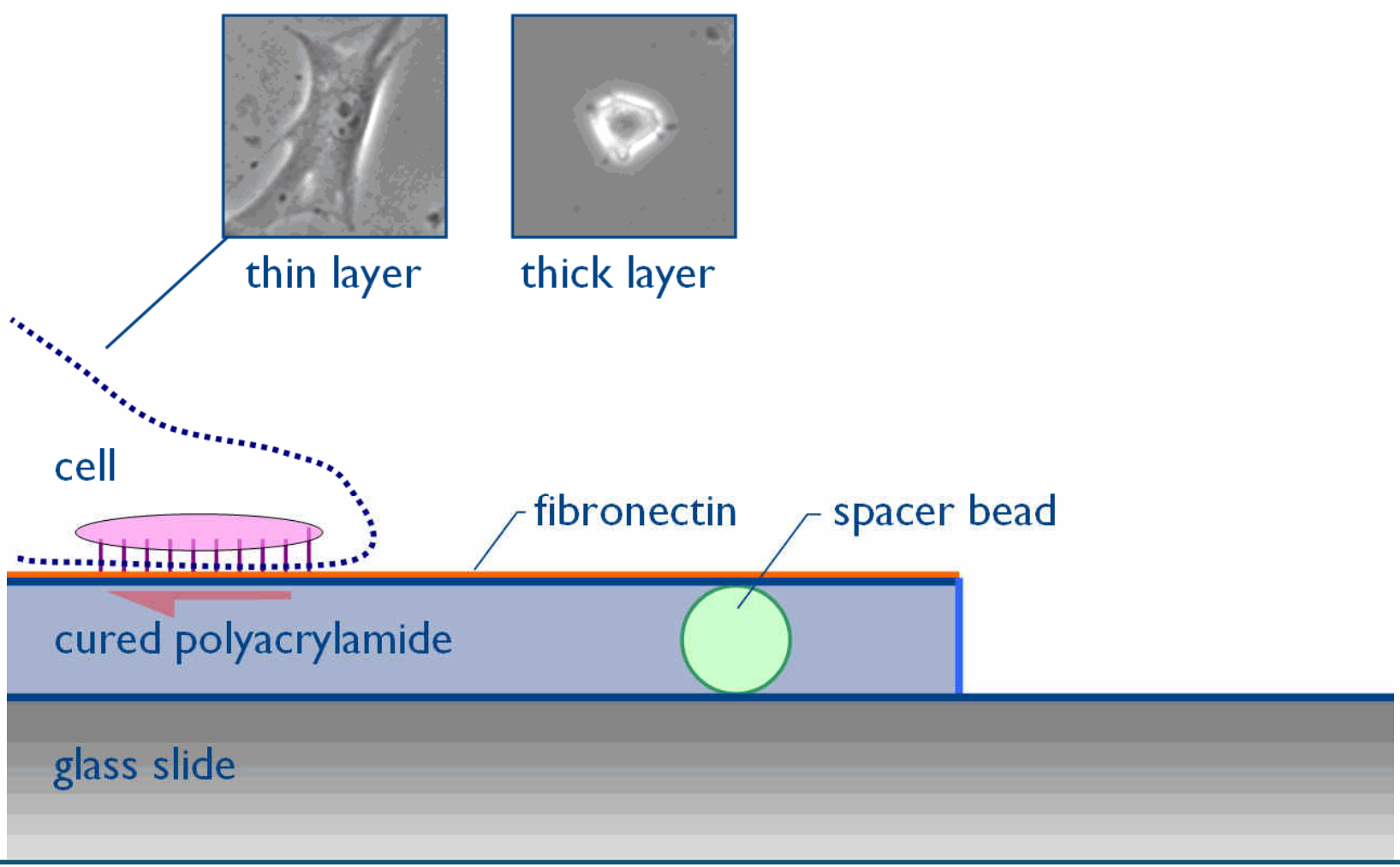


$$\text{deformation} \sim f\left(\frac{\text{film thickness}}{\text{adhesion site radius}}\right)$$

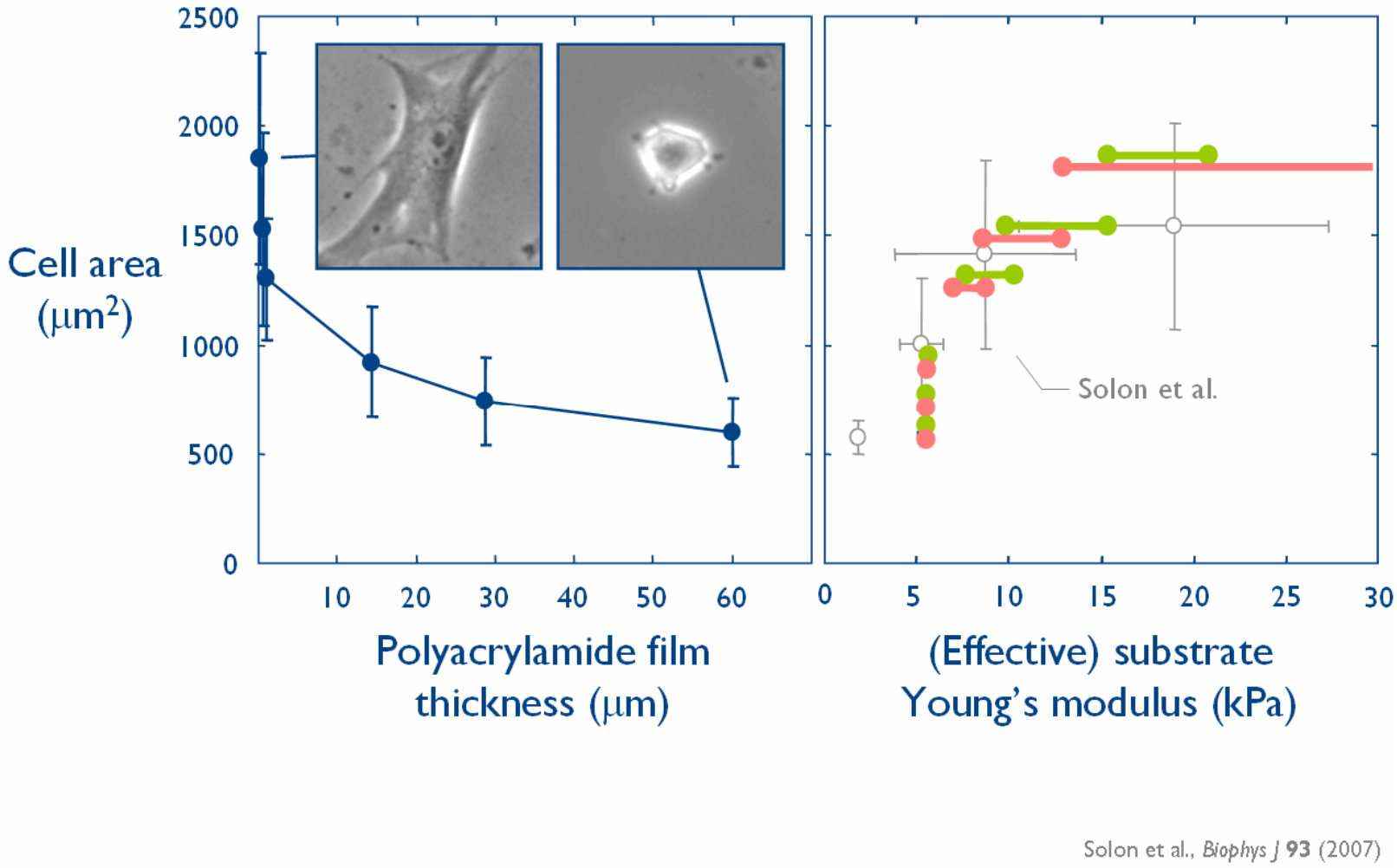
The most obvious way to characterize surface motion is by displacement of the center of the circular area. But a more subtle mode is the distortion: the compression of the leading edge and elongation of the trailing edge. These two modes turn out to be attenuated in slightly different ways. We bother to distinguish deformation modes because it's not yet known exactly how cells sense the mechanical properties of their environment. For all modes, however, a dimensionless number, the ratio of the film thickness to adhesion site radius, emerges in the equations. The appearance of this ratio alerted us to a deep connection between critical thickness and focal adhesion size.



Let's consider two extremes in the value of this ratio. For the case of very thin films atop an underlying rigid base, all deformation is reduced to zero, as we'd expect. Conversely, when the film is very thick, the circular area displaces and distorts as if the cell were on a semi-infinite region; that is, as if no base existed. We numerically calculated the curves connecting these two extremes. Deformation is predicted to be affected (for example, reduced by a tenth) when the compliant film thickness (the vertical distance to the rigid base) is about the same as focal adhesion size. We also predict that reductions in film thickness will be detected by the cell as increases in effective stiffness; after all, the only mechanical input to the cell is surface deformation, triggered by its own contraction. Note that material properties don't appear here; that's why we say that this measure of critical thickness is independent of substrate stiffness.



We experimentally tested these predictions by seeding fibroblasts on compliant polyacrylamide gels of different thickness and using their spread area as an indication of mechanosensitive response. Coauthor Chris Bruce prepared acrylamide solutions containing polystyrene spacer beads with diameters ranging from sub-micron to tens of microns. He compressed the films with a glass slide while they cured so that the bead size would determine the film thickness, and adsorbed a layer of fibronectin to promote cell adhesion. We indented very thick films with atomic force microscopy to determine the bulk gel stiffness, which was about 6 kPa for the recipe Chris used. Cells seeded atop the films were more spread out on thinner films, which corresponds to how the same cells behave on stiffer materials.



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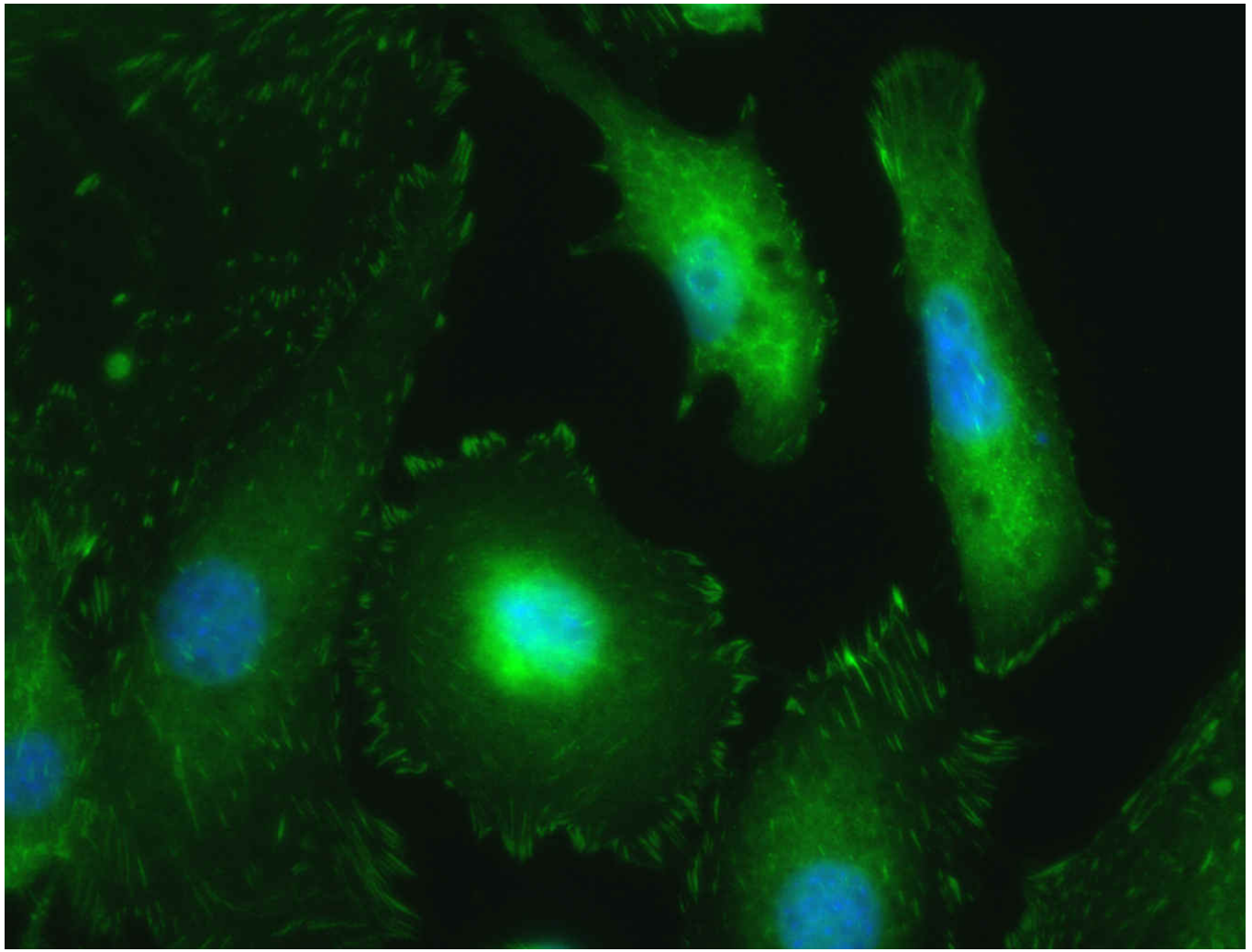
The quantified spread cell area shows that cell morphology changes considerably when the films are on the order of one to ten microns thick. We interpret this data as the cells' sensing a larger effective stiffness when film thickness is reduced to the size of focal adhesions. Other groups have quantified how fibroblasts behave on thick polyacrylamide gels of various stiffness. When we calculate an effective stiffness by dividing the bulk stiffness of our gels by the numerical attenuation factor described earlier, we find that our data, replotted, corresponds well to these literature reports and thus confirms our predictions.

Julia Woolf (http://www.juliawoolf.com)

In the old story of the princess and the pea, a mystery woman's royal identity is proven by her delicate reaction to a single pea hidden under a pile of mattresses.



Individual adherent tissue cells also prove remarkably sensitive, responding strongly to changes in stiffness one to ten microns beneath the surface upon which they attach. Our studies of cell-induced deformation are intended to support and advance both broad investigations of cell mechanosensitivity and, in a practical context, guidelines for synthetic substrate design. We're now looking for similar two-dimensional *in vivo* tissue configurations (such as the so-called basement membrane of blood vessels) so that we might apply our model to processes occurring in our bodies right now.

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Funding: Arnold and Mabel Beckman Foundation, National Science Foundation, National Defense Science and Engineering Program

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