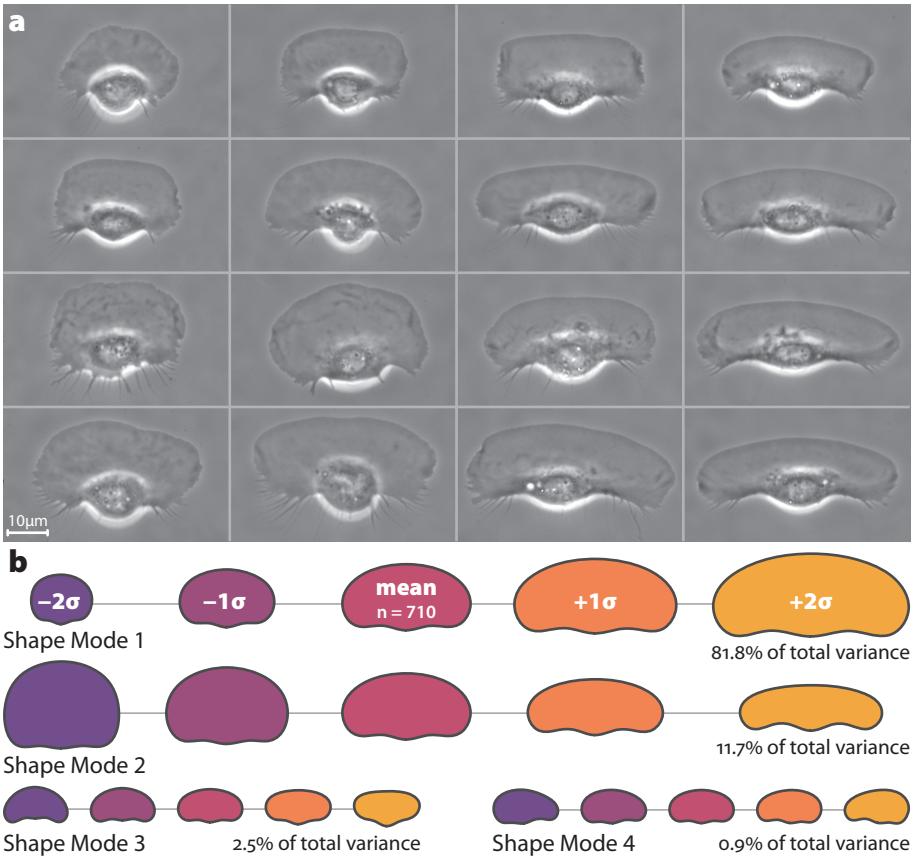
Image Cytometry Reveals Mechanisms of Shape Determination in Motile Cells

Zachary Pincus[1,2], Kinneret Keren[2], Greg M. Allen[2], Erin L. Barnhart[2], Alex Mogilner[3,4], Julie A. Theriot[2,5]

[1]*Yale University, Department of Molecular, Cellular, and Developmental Biology, New Haven, CT*
[2]*Stanford University School of Medicine, Department of Biochemistry, Stanford, CA*
[3]*University of California, Department of Neurobiology, Physiology and Behavior, Davis, CA*
[4]*University of California, Department of Mathematics, Davis, CA*
[5]*Stanford University School of Medicine, Department of Microbiology and Immunology, Stanford, CA*

The shape of motile cells is determined by many dynamic processes spanning several orders of magnitude in space and time, from local polymerization of actin monomers at sub-second time scales to global, cell-scale geometry that may persist for hours. Understanding the mechanism of shape determination in cells has proved to be extremely challenging due to the numerous components involved and the complexity of their interactions. We use novel measurement methods to capture information about the natural population variability of morphological and cytoskeletal phenotypes in a large population of motile fish epithelial keratocytes to reveal mechanisms of shape determination. We find that the cells inhabit a low-dimensional, highly correlated spectrum of possible functional states. We further link these measurements of morphology and protein localization directly and quantitatively to a physical model of shape generation. This simple model of actin network treadmilling in an inextensible membrane bag can recapitulate the observed phenotypic spectrum and predict both cell shape and speed. Our model provides a simple biochemical and biophysical basis for the observed morphology and behaviour of motile cells.

In addition to our biological findings, there are two major aspects of this work with implications for other cytometric applications: first, we demonstrate quantitative methods for measuring and describing the variability in morphology of natural populations - not just the average trends. Second, understanding this variability and its correlates led to a simple physical model that uses measures of morphology and actin network structure as inputs and outputs, providing an example of integrating image-based cytometry with molecular biophysics.



Keratocyte shapes are described by four primary shape modes.

(a) Phase-contrast images of different live keratocytes illustrate the natural shape variation in the population.

(b) The first four principal modes of keratocyte shape variation, as determined by principal components analysis of 710 aligned outlines of live keratocytes, are shown. These four modes — cell area, "D" vs. "canoe" shape, lamellipodial rear angle position, and left–right asymmetry — are highly reproducible; subsequent modes appear to be noise. For each mode, the mean cell shape is shown alongside reconstructions of shapes one and two standard deviations away from the mean in each direction along the given mode. The variation accounted for by each mode is indicated.