DEFORMABILITY CYTOMETRY: HIGH-THROUGHPUT, CONTINUOUS MEASUREMENT OF CELL MECHANICAL PROPERTIES IN EXTENSIONAL FLOW
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ABSTRACT
Cell state and function is often manifested in biochemical and biophysical markers. Traditionally, biochemical markers have been exploited for identification of phenotype while only recently have the mechanical or biophysical properties of cells been explored for the same purpose. Biophysical markers can be advantageous in that they do not require costly labeling or molecular analysis. However, current techniques to assay cell mechanical properties do not possess the throughput to be used clinically. Here, we present a high-throughput method which employs inertial microfluidic phenomena to measure whole-cell deformability and apply it to several pressing biomedical problems.

KEYWORDS: Cell mechanics, inertial microfluidics, label-free, intrinsic biomarkers, high-throughput measurements

INTRODUCTION
There is growing evidence that cell deformability (i.e. the ability to change shape under load) is a useful indicator of abnormal cytoskeletal changes and may provide a label-free biomarker for determining cell states or properties such as metastatic potential, cell cycle stage, degree of differentiation [1], and leukocyte activation. Clinically, a measure of metastatic potential could guide treatment decisions, or a measure of degree of differentiation could prevent the catastrophic consequences of transplantation of undifferentiated, tumorigenic stem cells in regenerative therapies. In order for deformability measurements to be clinically valuable, given the heterogeneity within a population of single cells, there exists a need for high-throughput automated assays of these mechanical properties. Here, we present a novel microfluidic device for the continuous deformation of cells in flow (~ 2000 cells/sec, throughputs comparable to traditional flow cytometry).

THEORY
A unique combination of inertial focusing and automated image analysis enables unprecedented mechanical measurements of cells. Previously, we characterized the important design criteria for high-throughput inertial focusing to align particles to precise positions in a flow [2]. Using these criteria we have devised and fabricated a channel where opposing streams of cells are precisely delivered to the center of an extensional flow where the cells undergo controlled deformation and are imaged with high-speed microscopy (Figure 1). This technique enables us to perform approximately 2000 deformations per second, >3 orders of magnitude over the current state-of-the-art methods for measuring mechanical properties of cells [3]. A custom MATLAB algorithm was written to track cells within the captured images and measure their initial diameter, circularity, and deformation. The code uses a polar mapping strategy to extract deformability information from images, similar to previous work. Here, we apply our system to effectively address two applications of extreme importance: measurement of metastatic potential of cancer cells and evaluating embryonic stem cell differentiation protocols for regenerative medicine.

Figure 1: Deformability cytometer operation. a. Complete device schematic (top view); b. Deformation and measurement region showing cell in lateral equilibrium position, Xeq; c. Example data from high-speed videos; d. Measurement definitions; e. Photograph of device with channel shaded for contrast.
EXPERIMENTAL
Microfluidic channels were fabricated using standard photolithographic methods. Briefly, polydimethylsiloxane (PDMS) was cast on a patterned photoresist mold then removed and bonded to glass with air plasma. Cell suspensions were loaded in syringes and pumped through PEEK tubing into the channel inlet. Cells are uniformly positioned in flow by inertial lift forces present at relatively high fluid velocities. They arrive at the center of an extensional flow and are stretched. No further external forces were applied. The cells were imaged with high speed microscopy before entering and while within the extensional flow region of the channel. Image files were processed to reveal single cell measurements of size and shape behavior.

We followed the example of Guck et al for demonstrating our ability to distinguish between populations of a normal cell line (MCF10A), a cancerous cell line (MCF7), and the same cancerous cell line modified to have increased motility or metastatic potential (modMCF7) [4]. We also compared mechanical measurements of self-renewing mouse embryonic stem cells (mESC) with mESC that were differentiated by two common protocols: adherent and embryoid body differentiation.

RESULTS AND DISCUSSION
In Figure 2 we show the measurements of deformability and circularity clearly distinguish normal cells from cancerous cells and malignant cells; the medians were determined by a nonparametric test to be statistically different with a confidence of P<0.01. This confirms trends observed by other techniques for measuring cell mechanical properties with greatly enhanced throughput, enabling adoption of the technique in settings where analysis of diverse cell populations is necessary.

![Deformability and circularity measurements](image)

**Figure 2:** High-throughput deformability measurements classify breast cancer cells by metastatic potential. Color density plots (red-to-blue indicates high-to-low density) of single cell deformability and circularity measurements of normal, benign, and enhanced motility metastatic cells. MCF10A (green) and MCF7 (magenta) centroids are indicated on subsequent plots for comparison.

We also compared the deformability of self-renewing mESC to mESC differentiated by adherent and embryoid body differentiation (Figure 3) and found that both methods resulted in an increase in cell stiffness. This statistically significant difference validates recent findings that differentiation state and associated cytoskeletal changes may manifest as a difference in cell mechanics. This distinction may find application in identifying failed-to-differentiate stem cells destined for implantation which could otherwise result in tumors in vivo.
We have also evaluated deformability of oil-in-water emulsions of known viscosities and elastomeric polymer micro-particles with known stiffness and determined a physical basis for our deformability measures connected to viscoelastic properties of cells. Further, we have chemically disrupted the individual cytoskeletal components of cells identifying their role in large scale, whole cell deformation. The dramatic increase in throughput provided by deformability cytometry will make it a useful tool for answering biophysical questions as well as enable the use of single-cell mechanical properties as a viable clinical biomarker for diagnostics, regenerative medicine, and cytoskeleton-acting drug discovery.

CONCLUSION
A cell's mechanical properties are often indicators of state or function. We have developed a high-throughput measurement of whole-cell mechanical stiffness using an inertial microfluidic system and have demonstrated this measurement's efficacy in distinguishing cell populations relevant to several clinical problems.

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REFERENCES

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