Panoptes: A 12 Parallel Microscope System for HCA

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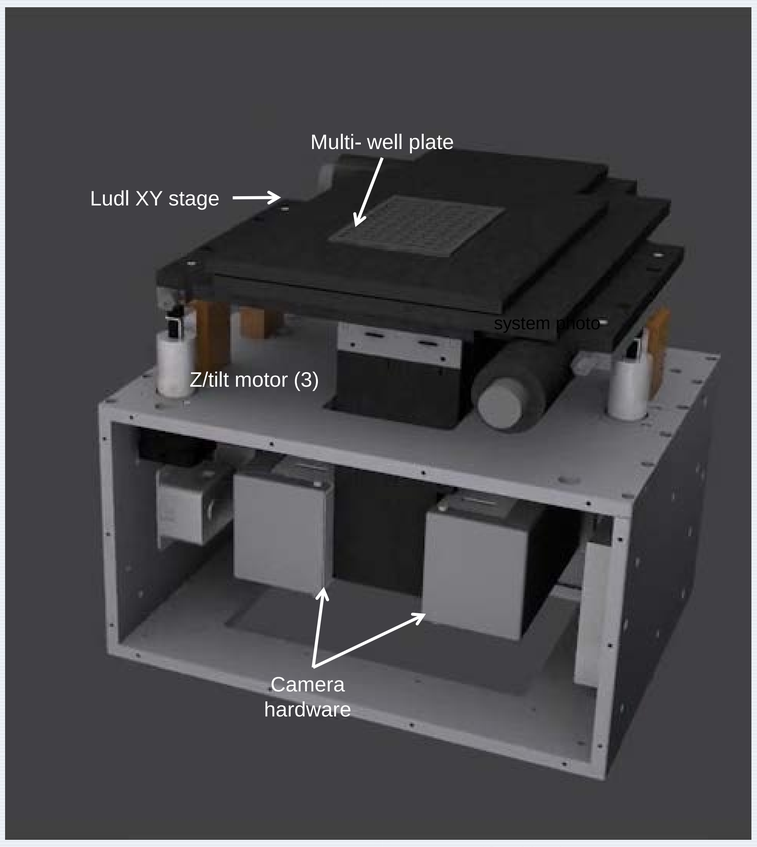
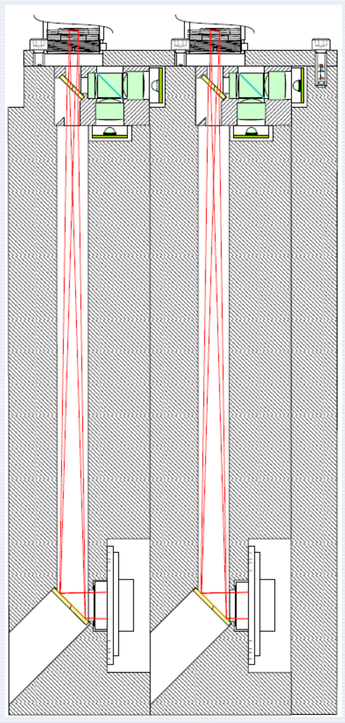
Panoptes: A Solution for Mechanobiology

Mechanical biology is poorly served with high throughput technologies. We have addressed the bottleneck at *imaging*, recognizing that a solution there could then be applied to any other technologies for multiwell plates. Our solution, Panoptes, is an imaging-based system applicable widely, to studies including rheological properties of biofluids, biofilms and biomaterials, cell mechanics, and drug carrier transport. The design goals challenge us to place an array of 12 cameras so that we can obtain the full 96 wells in 8 steps of data acquisition. The system is based on the DragonFly 2 camera from Point Grey Inc; it achieves ~55 fps at VGA resolution in a small package, suitable for our dynamics. The lateral arrangement tiles the cameras 6 x 2.. A typical experiment acquires data from four offsets (C) within each well, with about 1 minute of video collected at each position. With the 8 well-to-well translations, data can be collected in a 96 well plate in under one hour. Each camera feeds an independent computer for image acquisition and data analysis(D). Note that one hour of video in 12 channels is ~ 1 TB! The analysis of the experiments *must* be pipelined (E). The payoff is remarkable—ninety six experiments, from data collection through analysis, in ~ 3 hours, **all unattended.** Any plate, 1-1536 wells, as well as custom-designed microfluidic systems, can be used on Panoptes.

Twelve imaging channels (2 x epi-fluorescence) & independent, electronic auto-focus





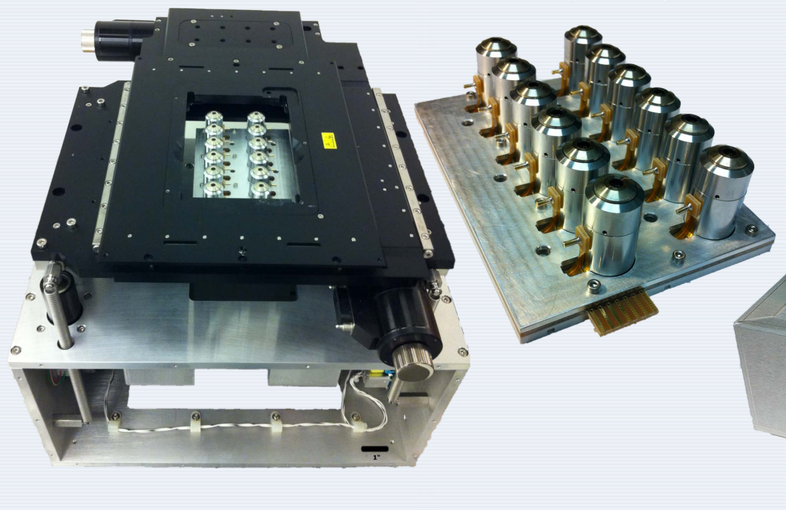
Electrically focusable objective for auto z-focus with no moving parts

liquid lens (Varioptic)

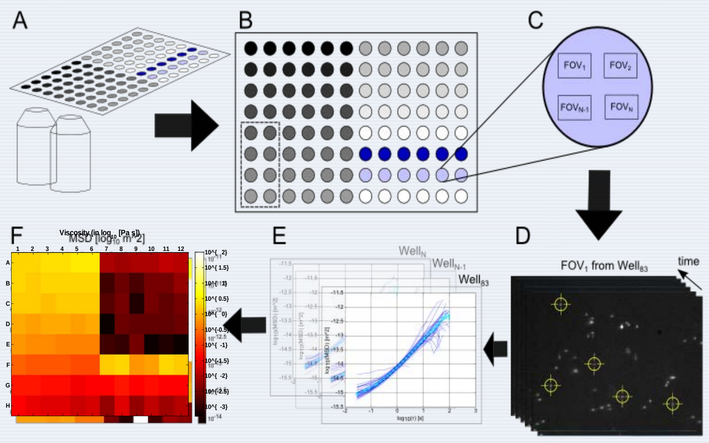
40x objective

Blue and amber LEDs for fluorescence channels: Red or YG beads, FITC, E-GFP, rhodamine, texas red

40x objective array



Experiment Workflow

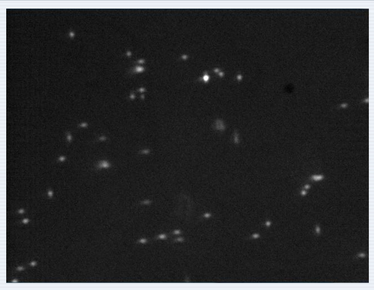


System Features

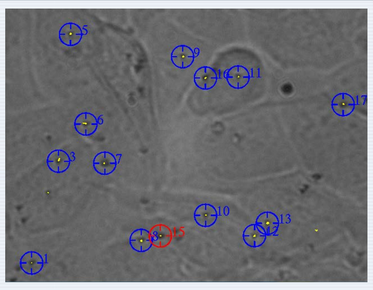
- Independent channel control
- Images 200 nm – 2 µm beads
- 50 fps for 1µm beads
- Simultaneous autofocus and image acquisition on twelve video channels with no added mechanical noise.
- Microcontroller based control system for hardware that launches events with optimal synchronization (LEWOS).
- Automated data-analysis pipeline

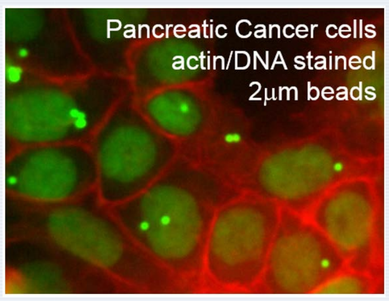
Cancer Cell Mechanics: data collection, representation, filtering and analysis in high throughput system

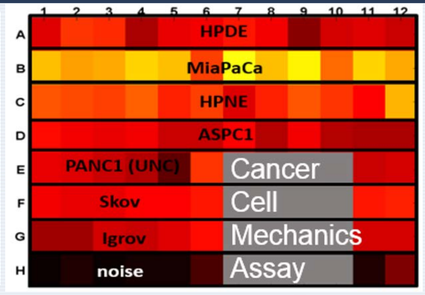
Understanding how cell behavior and function relate to physical structure and properties is a major goal of current cell mechanics studies. Elucidating this relationship is especially important in the study of cancer biology where the Mechano-phenotype of cells is seen as an informative diagnostic of cell metastatic potential. We show that the thermal diffusion of micron-sized beads connected to integrin surface receptors via fibronectin can distinguish ovarian and melanoma cancers of varying metastatic potentials. With sampling sizes in the thousands, we report elastic moduli differences between cancer cell types. Our results support the inverse relationship between mechanical stiffness and invasion behavior, and ultimately, demonstrate the value of our high throughput instrument and passive rheology assay as a screening tool for studying relevant signaling pathways involved in cancer cell mechanics.



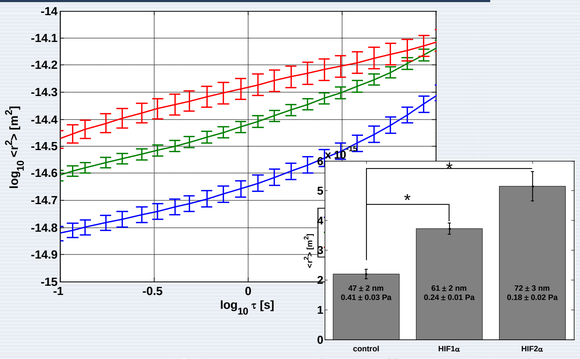
2 um fluorescent beads coated with fibronectin target attachment to integrins. Integrins receptors connect directly to the cytoskeleton of the cell allowing mechanical properties of the cells to be determined from the thermal motion of the bound beads.







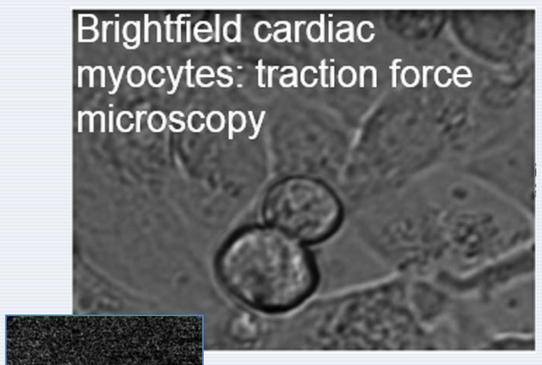
The 96-well plate layout of an experiment can be represented by a heat map of the apparent viscosity, here, at a 10 second time scale. Heat maps provide a way to visually check well-to-well consistency and differences.

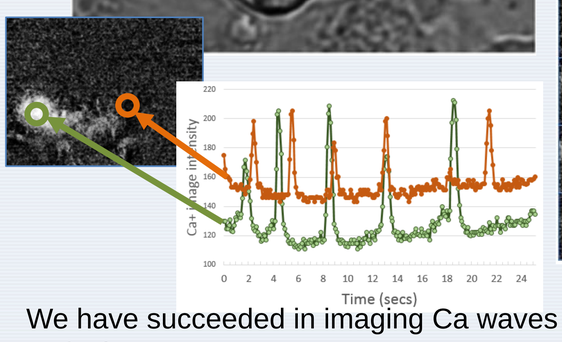


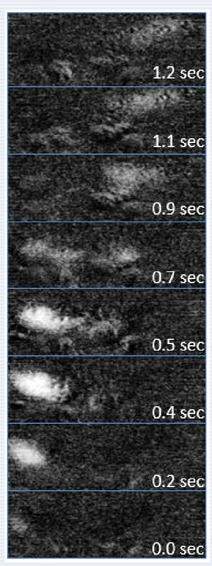
Average MSD curves for cell type. Each MSD curve above is a collection of 100s (melanoma) of beads. The bar plots represent average MSD at the 1-second time lag, the maximum timescale in which Brownian motion is the primary contribution to the motion of the bead. Error bars = SEM. * P < 0.0001.

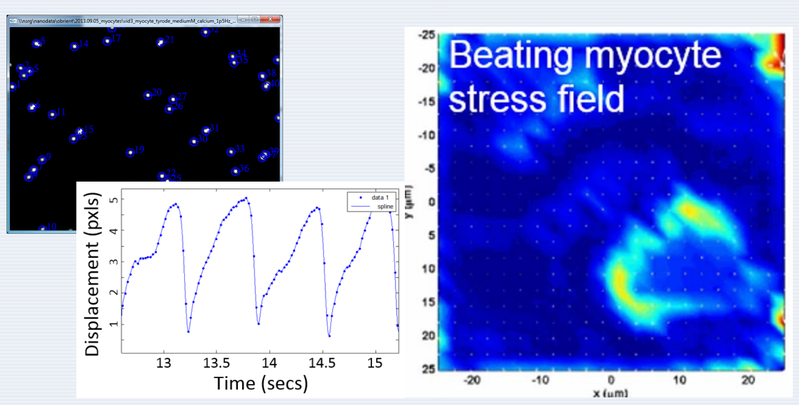
Cardiac Myocyte Functional Assay: Automated Traction Force Mechanics

While efficient methods to generate human cardiomyocytes from iPSCs have been developed, the field is hampered by the lack of robust methodologies to promote functional maturation of human cardiomyocytes to levels found in adult heart. There are a large number of soluble and extracellular matrix factors as well as cell mixture combinations that can potentially influence both cardiac differentiation and functional maturation. Thus we are looking for new ways to rapidly assess myocyte contractile function in a high throughput screening assay in the presence of various growth factors, small molecule compounds and non-cardiomyocytes to engineer accelerated cardiac maturation. Further, the field of Cardiac Toxicology will also benefit from a force assay of myocyte function.









Traction force microscopy requires good quality fluorescent bead images at appropriate frame rates. We have demonstrated tracking of labeled beads at 40fps, 5 msec exposure times. We are working with the group of Prof. Jeff Fredberg at Harvard in incorporating their TFM code into the pipeline for the Panoptes instrument.

We have succeeded in imaging Ca waves in the Panoptes optical system.

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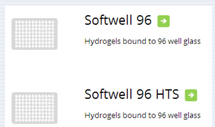
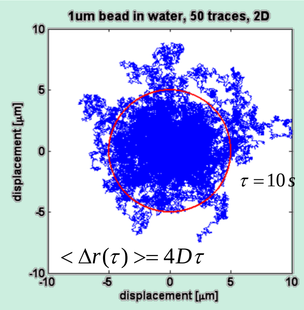
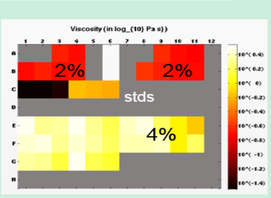


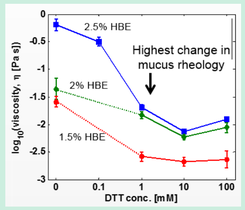
Other Applications: Mucus Rheology, Biofilms

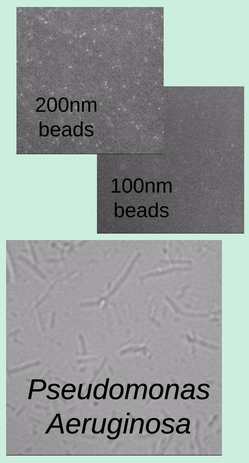
Microbead Rheology



$$< \Delta r(\tau)^2 >= \frac{2kT\tau}{3\pi a \eta}$$







Light collection and resolution (in quantifying positions of labeled virus-sized particles ~100nm) challenge us for the mucus and biofilm projects (S9 Lai and C10 Ribbeck projects), where viruses and drug delivery vector transport through biofilms is of central importance. Measuring 100nm particles or labeled endogenous vesicles is of great interest for cancer cell mechanics (C1 Blobe and S7 Kim) and the cell mechanics of motility (S5 Campbell) and cell polarity (S4 Guilak). We also want to measure cell features (organelles/nucleus) and beads at the same time for understanding cell state and for locating beads with respect to cell /nuclear boundaries.

Collaborators: *David Hill (UNC), Nenad Bursac (Duke), Gerry Blobe (Duke), Mythreye Karthekian (USC), Katerina Ribbeck (MIT), Jeffrey Fredberg (Harvard)*

This work was supported by the National Institutes of Health, including grants 5-P41-RR02025-21 and P41-EB002025.

Project Director: *Rich Superfine[1]*

October 2013