

# A MULTIPLEXED AND AUTOMATED IMAGING ASSAY OF CARDIAC MYOCYTE CONTRACTILITY

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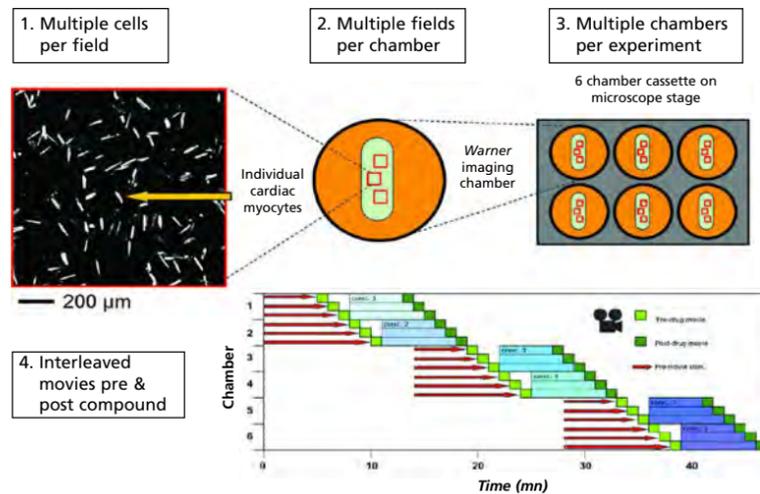
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## ABSTRACT

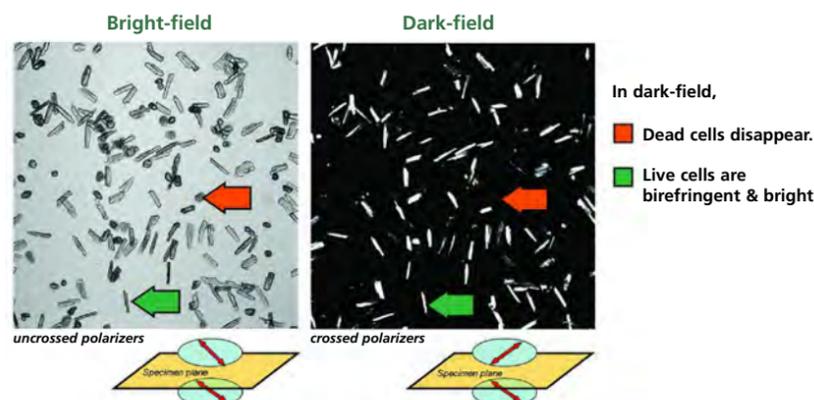
Cardiac myocyte contractility has traditionally been measured using single cell assays. In order to increase throughput and statistical power and facilitate quantitative measurement of the effects of small molecules on cardiomyocyte contractility, we have invented an automated imaging assay that achieves a throughput of more than a thousand cells per experimentalist-day. We took a parallel approach in which multiple cells are measured at once and multiple conditions explored within a computer-controlled experiment. Acutely-dissociated rat ventricular cardiomyocytes are maintained on the stage of an inverted microscope with environmental control in an array of multiple perfusion chambers with integrated electrodes for field stimulation. Imaging is accomplished using a low-magnification objective, and up to 30 contracting cells are imaged per field. Using a motorized stage, multiple fields are imaged per chamber. Custom control software interleaves movie acquisition before and after compound exposure. Movies are taken at high spatial and temporal resolution using a CCD camera and analyzed using custom segmentation and peak-detection algorithms. Distributions of contracted and resting cell lengths are quantified pre and post compound exposure, and dose-response curves computed based on changes in the distributions of contractile behavior. We have successfully used this platform to characterize compounds.

## STRATEGY

**A Parallel Approach** was developed to quickly measure contractility dose-responses in acutely dissociated rat cardiac myocytes.



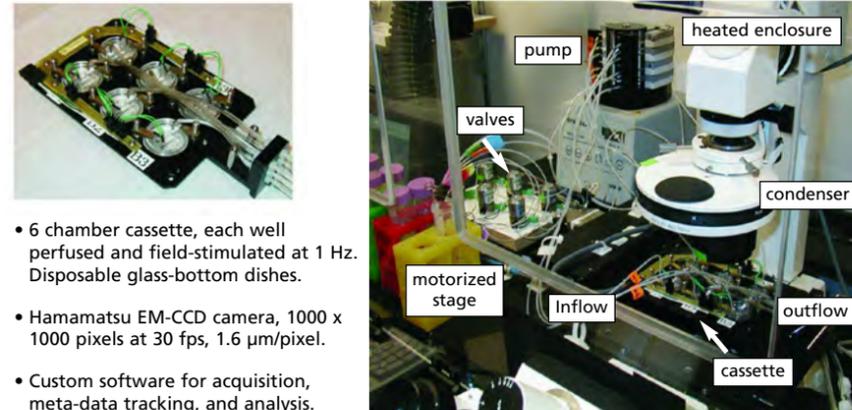
**Polarization Microscopy:** high-contrast without fluorescent labels



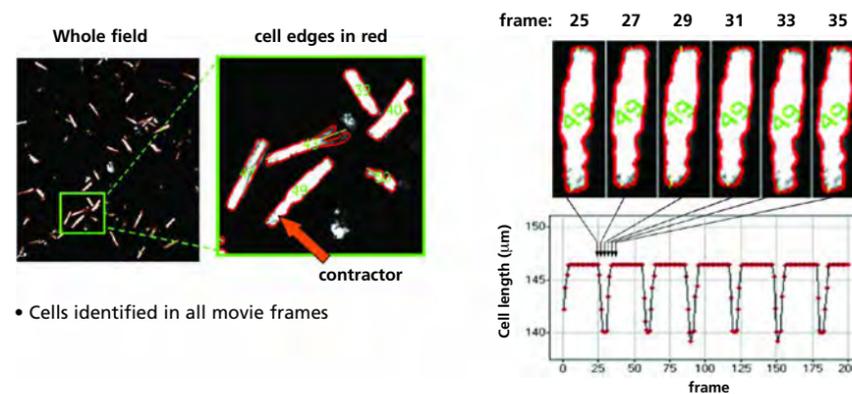
## AUTOMATED ASSAY STEPS

### Automated Microscopy

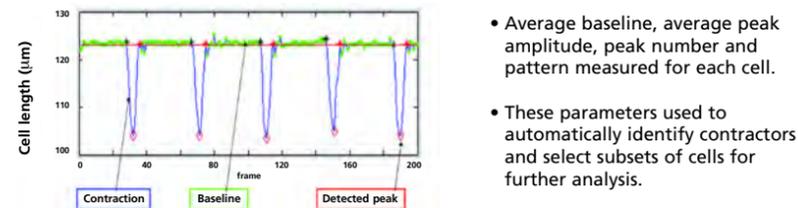
motorized microscope; computer-controlled fluidics, illumination, and field stimulation; environmental control and custom software.



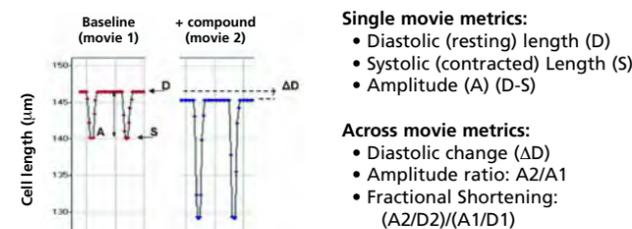
### Automated Cell Detection & Measurement



### Automated Trace Analysis

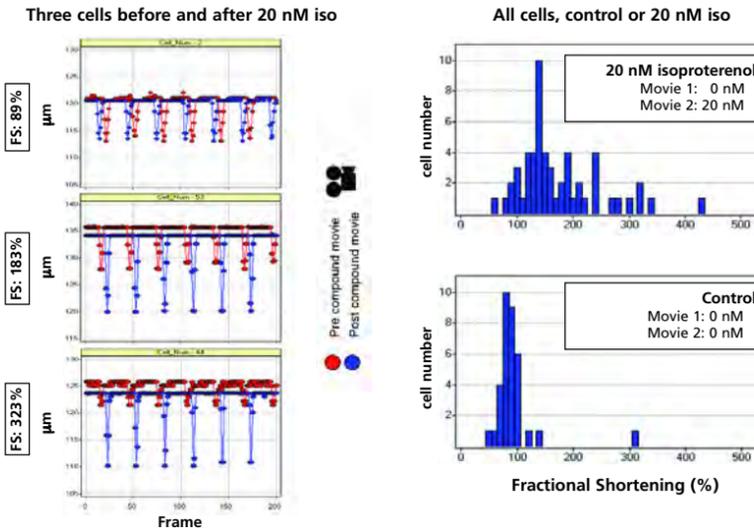


### Measuring Compound Effects

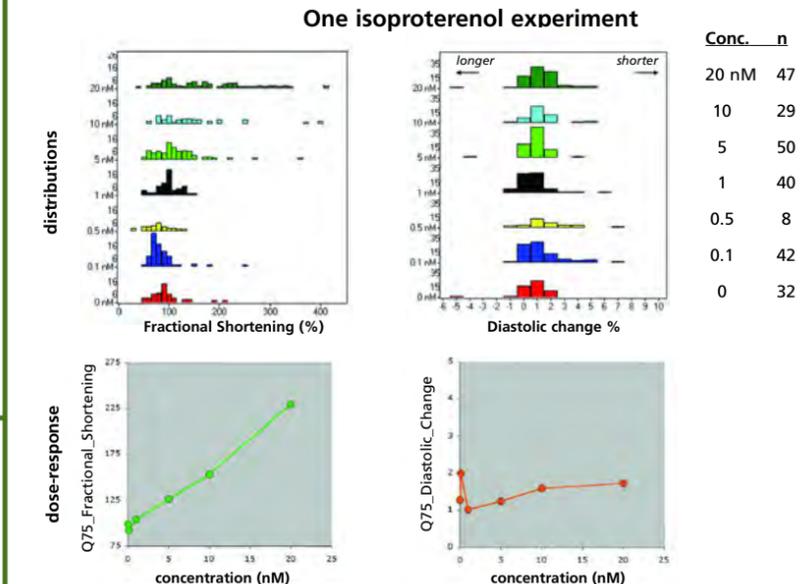


## RESULTS

### Contractility Responses are Broadly Distributed

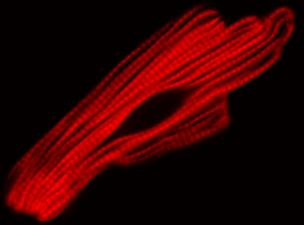
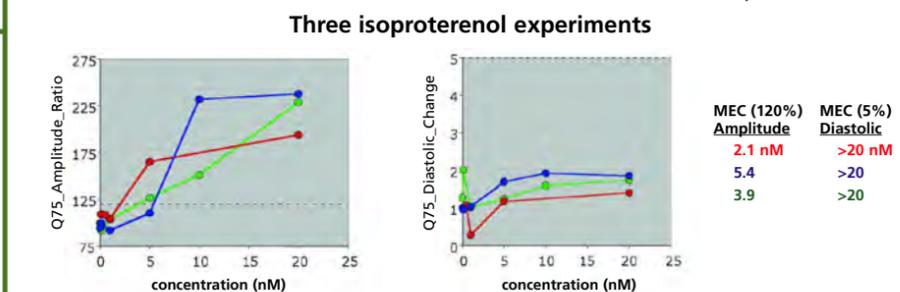


### Q75 Summarizes the Distribution Shift with Concentration



### Minimum Effective Concentration (MEC)

Concentration Where Curve Crosses a Threshold Describes a Dose Response



## CONCLUSIONS

- We have developed an automated assay platform that can measure contractility changes in hundreds of cardiac myocytes per hour.
- Six-point dose-responses for a compound can be acquired within an hour, with 10 – 80 cells per condition.
- Even with potent compounds such as isoproterenol, contractility responses (for all metrics) are broadly distributed.
- Response distributions shift with compound concentration, which is reflected by the Q75.
- MECs summarize dose-response curves.
- The platform may be adaptable to other assays, cell types, and imaging modalities.

## ACKNOWLEDGEMENTS

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