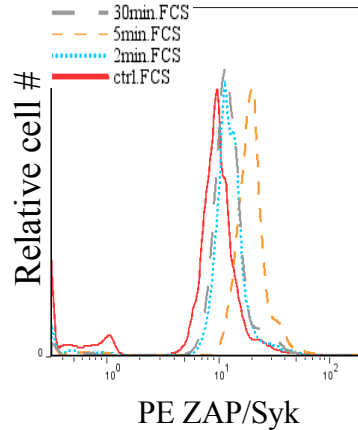
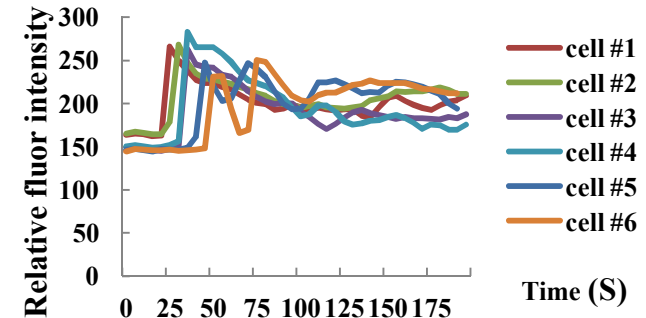
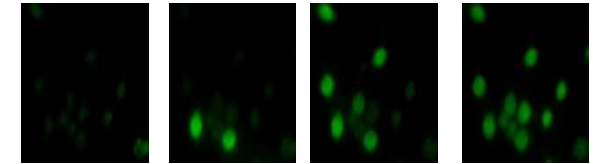


30 $\mu$ m (d), 200 $\mu$ m (w)

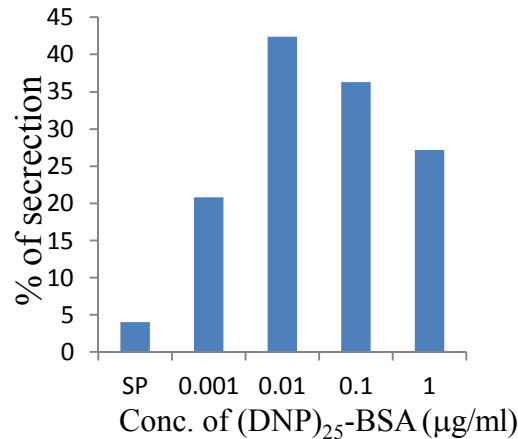
- < 1000 cells per assay
- Long term cell culture
- rapid fluid exchange allows fast kinetic measurement
- Multiplexed measurement (imaging and flow)
  - single cell resolution
  - self-contained and automated



phosphospecific  $\mu$ flow cytometry



Asynchronous changes in cellular  $Ca^{2+}$  in individual stimulated RBL-2H3 cells



$\beta$ -hexosaminidase release assay

## Abstract

As population-level assays do not reflect how individual cells respond to stimuli, we are developing an integrated microfluidic platform to systematically interrogate cellular signaling process on a single cell level with high-sensitivity and minimum reagent consumption. Our platform is comprised of a monolithic microfluidic chip and accessories such as electronic control valves, digital heaters and optical detectors. These components are assembled into a bench-top, semi-automated device that maintains cell viability and enables quantitative measurement of signaling events in single cells using high-resolution imaging and on-chip flow cytometry. The platform is being validated using rat basophilic leukemia cells (RBL-2H3), a mast cell model used to understand fundamental mechanisms that lead to allergic responses and asthma. Following on-chip cross-linking of the high-affinity receptor Fc $\epsilon$ RI for IgE by multivalent antigen, we examined early, intermediate, and late stages of signaling events including protein phosphorylation, calcium mobilization and the release of inflammatory mediators, demonstrating the ability of our platform to make quantitative measurements on a cell by cell basis from just a few hundred cells. The new platform enables analysis of the heterogeneity of cellular responses and makes it possible to analyze rare primary cells and tissue samples.