

# Cellular High Content Imaging in a Miniaturized Array Format



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## Brief overview

- **Miniaturized high content imaging assays in an array-based platform**
- **Cellular assays in 100 nL volumes**
- **Ultraminiaturized cell assays**
  - Reduce needs for cells and reagents
  - Enable broader use of rare / expensive cells and reagents

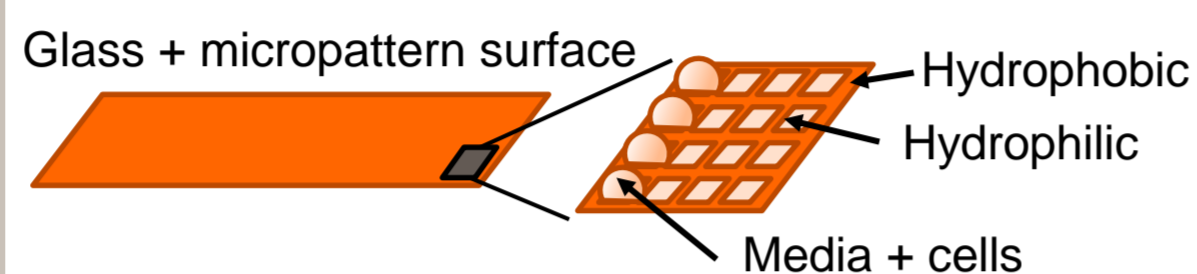
## Introduction

One strategy to enable the broader use of primary cells and stem cells in HTS and lead optimization is to ultraminiaturize the assays, and reduce the number of cells and reagent volumes needed per compound test. Here we present the development of cellular assays utilizing a micro-array device, based on a derivatized glass slide. This miniaturized format was used to develop high content imaging assays in 100 nL volumes. Significant savings can be realized in cell and reagent usage.

## Methods

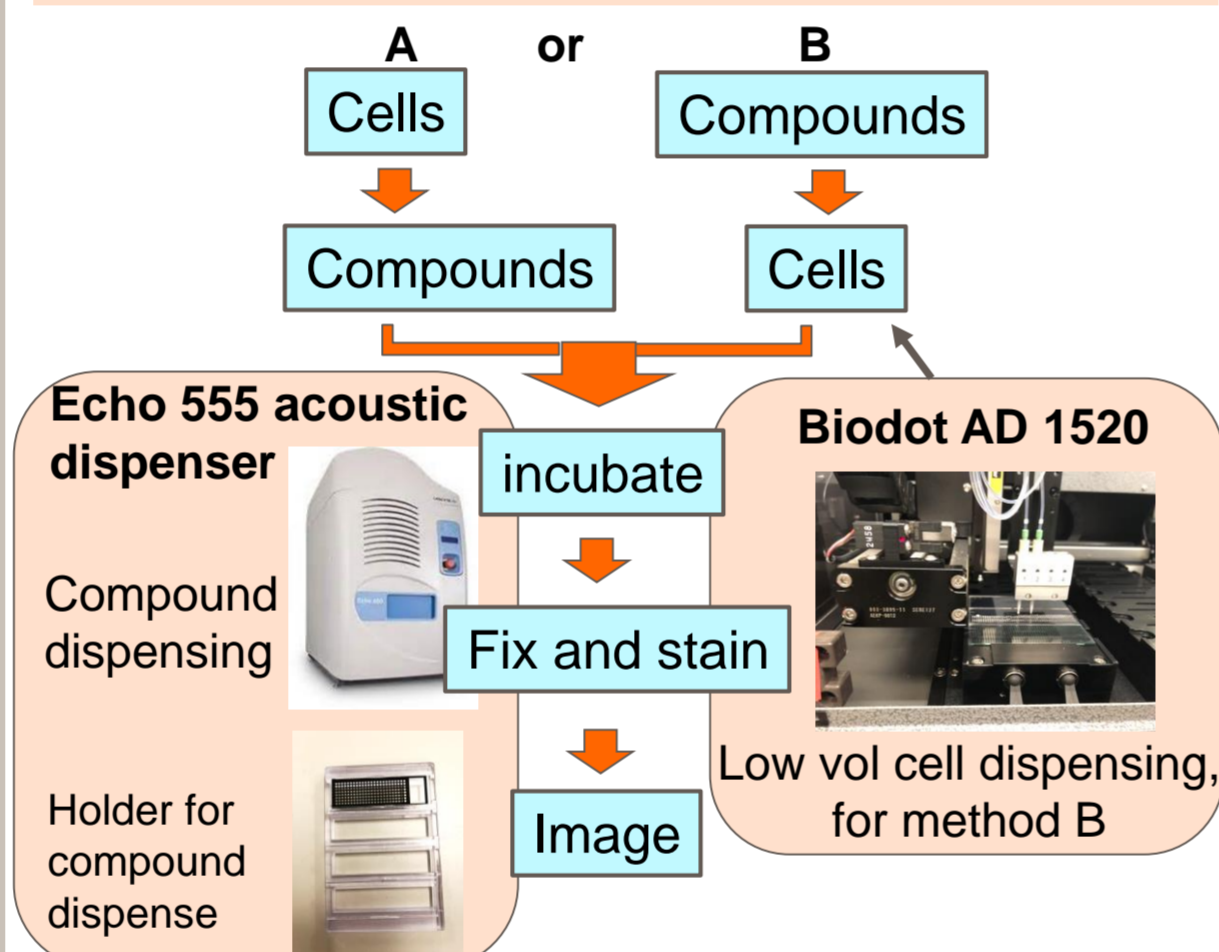
- All cell lines originally acquired from ATCC
- H2AX antibody from Cell Signaling
- Phalloidin and Hoechst stains from ThermoFisher
- Images captured with iPhone or GE InCell 2200 instrument
- Array slides from Aquarray ([www.Aquarray.com](http://www.Aquarray.com)) (see Popova AA et al., *Advanced Materials*, 2015, 27: 5217 for description and methods)
- Labcyte Echo instrument for compound dispensing
- Biodot AD1520 instrument for 100 nL cell dispensing
- Multidrop Combi dispenser used for 384 well cell dispensing
- Slide holder for fix and stain from ThermoFisher Scientific, for imaging from iBidi, for compound dispense, in-house 3D printing group.

## 1. Description of the device

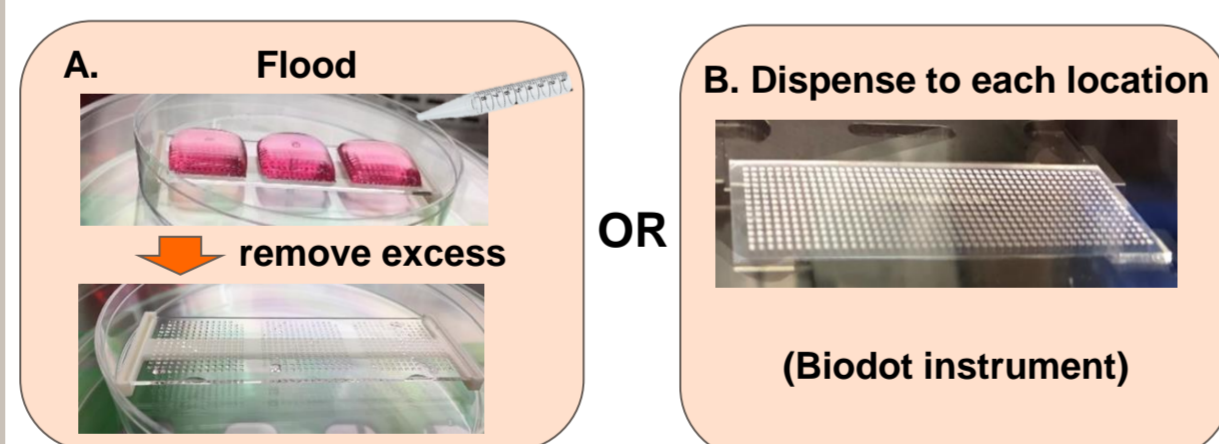


- Array: glass slide with a derivatized surface
- Alternating hydrophilic and hydrophobic regions
- Surface tension maintains discreet locations

## 2. High content imaging workflows



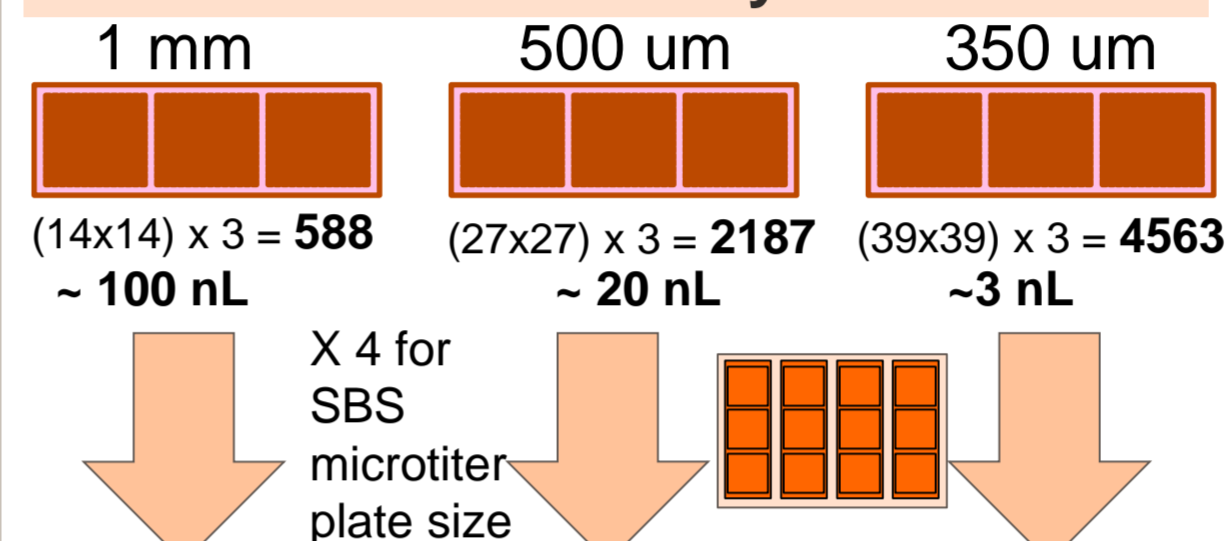
## 3. Two ways to add cells to the array



## 4. Fixing and staining for microscopy



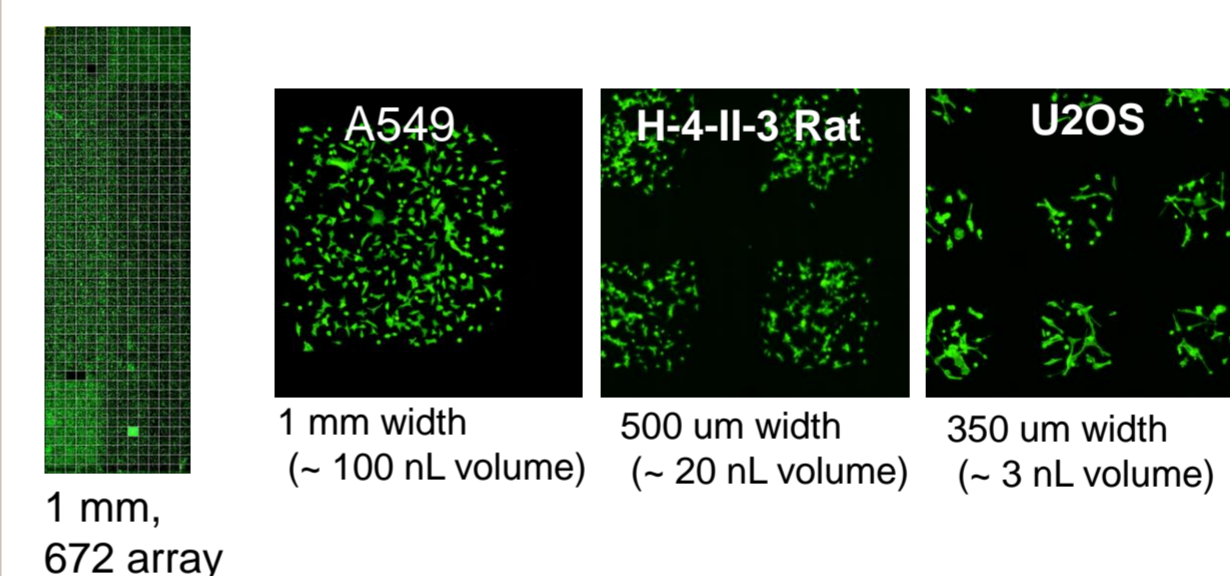
## 5. Different array sizes



Density for SBS microtiter plate sized footprint

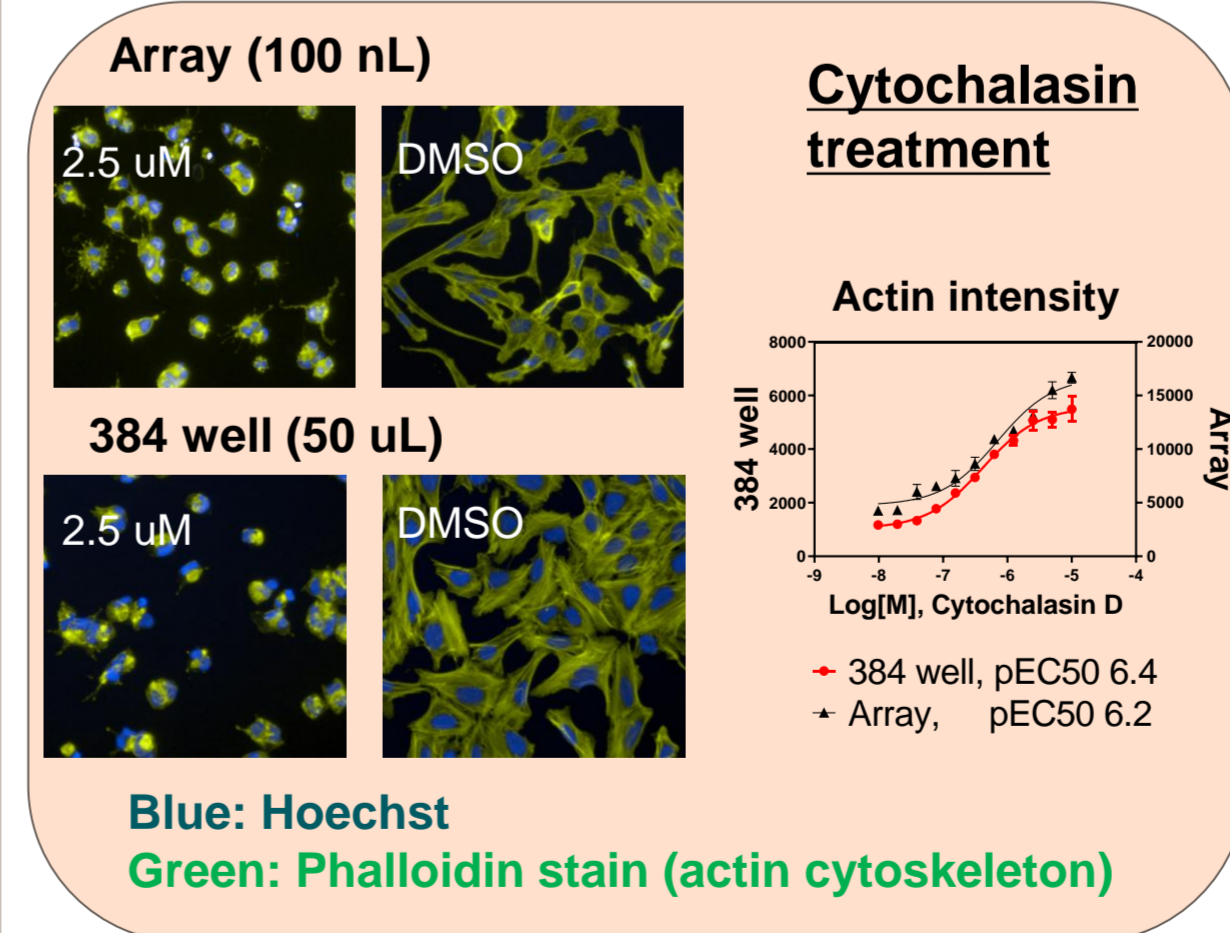
2352      8,748      18,252

## 6. Different cell lines on arrays



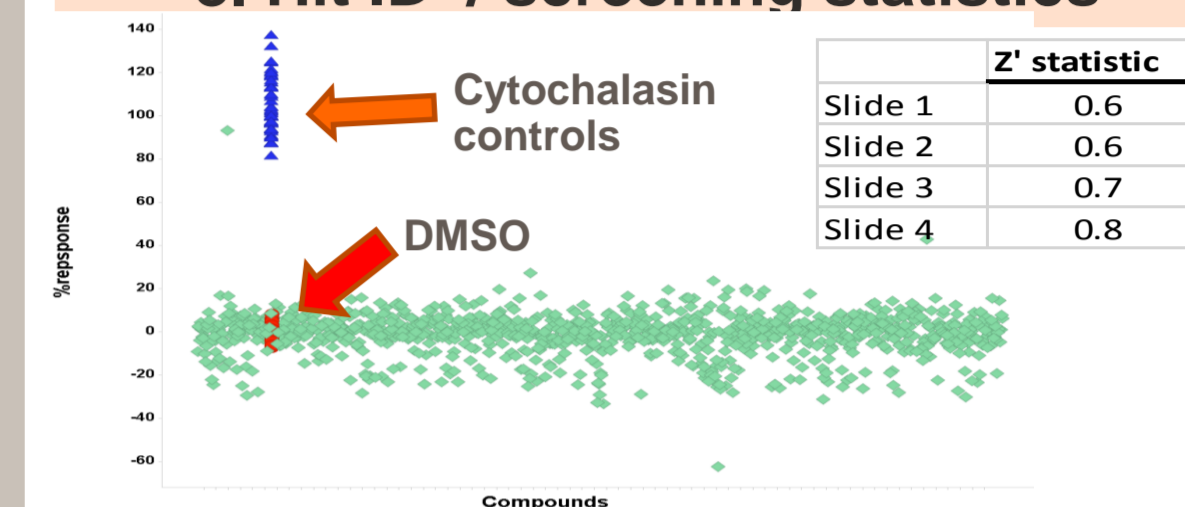
- All cell types tested attached and grew (o/n)

## 7. Pharmacology in 100 nL vs 50 uL



- Standard imaging stains + tool molecules
- Similar results, array 100 nL vs 384-well 50 uL

## 8. Hit ID / screening statistics



- Random 1000 cmpds, plus high/low controls
- Excellent screening statistics
- Z' values acceptable for hit identification

## Advantage over microtiter plates

- No plate washing / disposable tips
- Liquid exchange simplified

## Challenges

- Small volume compound dispensing
- Small volume cell dispensing
- Non-SBS standard formats
- Humidity control / evaporation

## Summary and Conclusions

- Assays miniaturized to 100 nL
- 5000 fold reduction in assay volume = reduced cell and reagent needs
- Pharmacology and Z' statistics comparable to 384 well
- Compatible with all adherent cell types tested
- Platform successful for both dose response and single concentration small molecule screening

## Acknowledgements:

Chris Watson (GSK) for initial array work, Bobby Silvers (GSK) Biodot instrument work, colleagues at KIT/Aquarray in Germany for advice and support, colleagues at Biodot for instrument usage and support, and colleagues at Labcyte for Echo instrument support.