Receding meniscus induced docking of yeast cells for quantitative single-cell analysis

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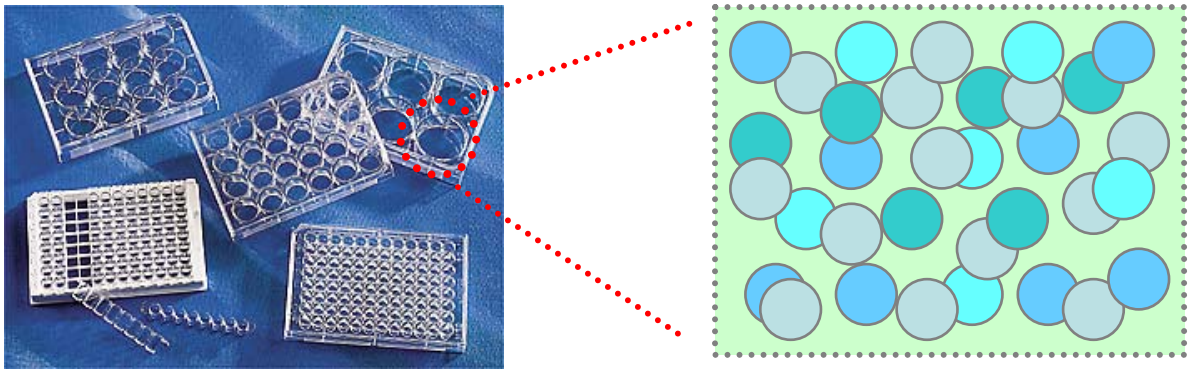
Single-cell analysis

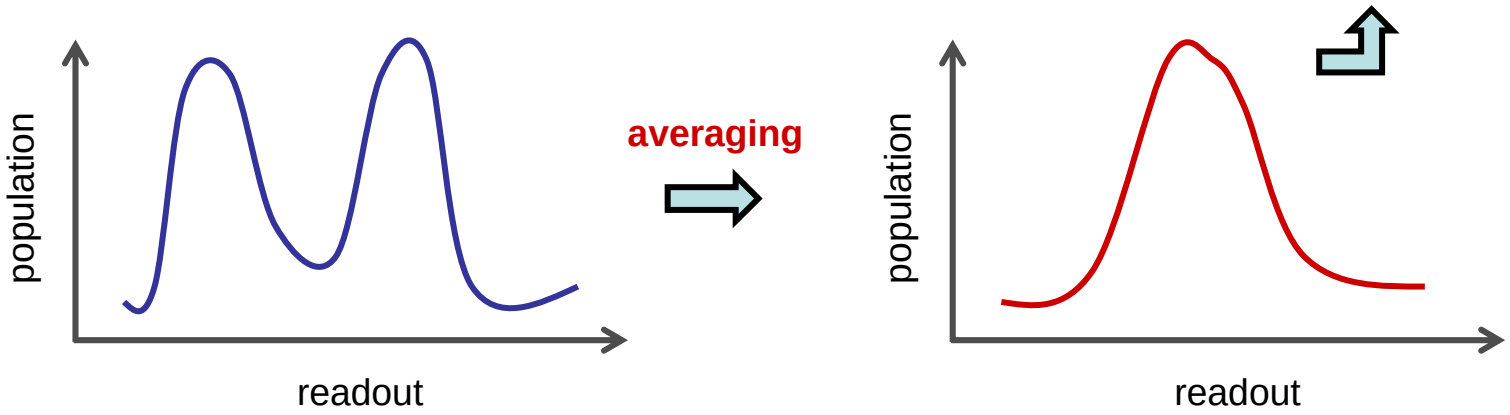
Bulk population based analysis



well plate

heterogeneous response

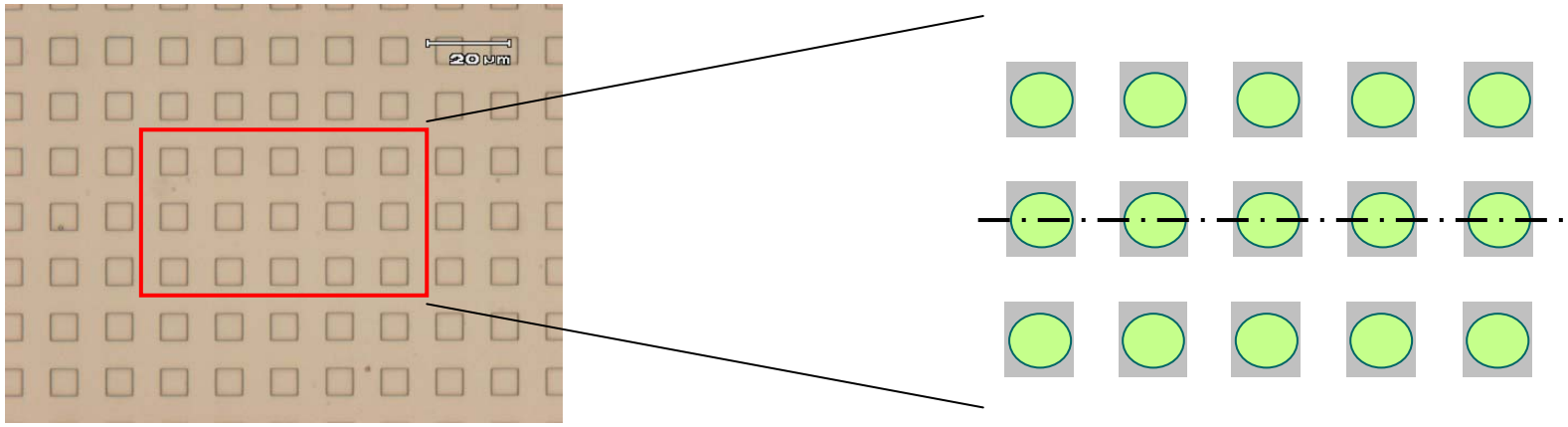
misconception from bulk population



Ensemble averaging problem !

Why single-cell analysis?

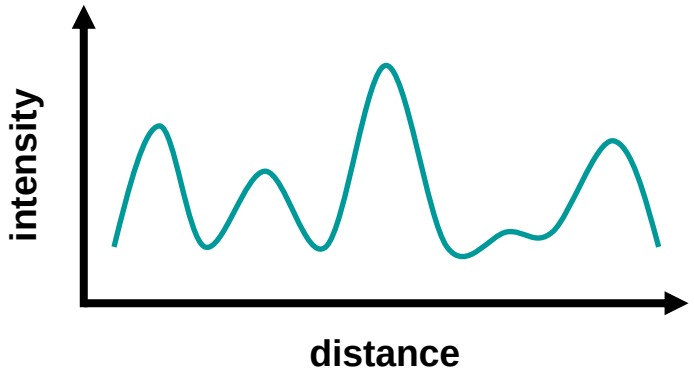
- **At single-cell level**



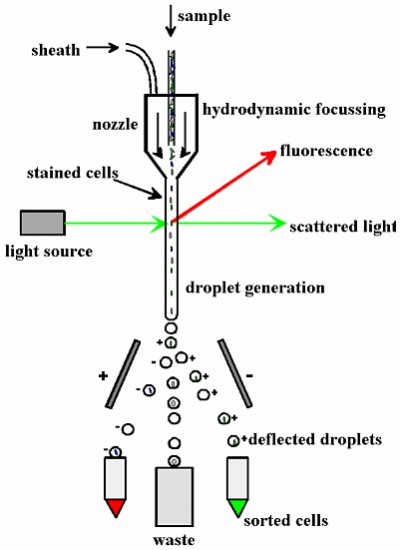
microwells

heterogeneous intensity

- Stochastic expression of cell
- Observing Single-cell information

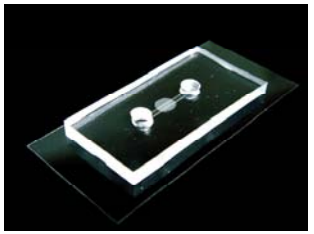


Cell chip (single-cell array)



Flow Cytometry (FACS)

- cannot interrogate individual cells repeatedly
- cannot capture cell images with high resolution
- cannot observe the spatial localization of fluorescence

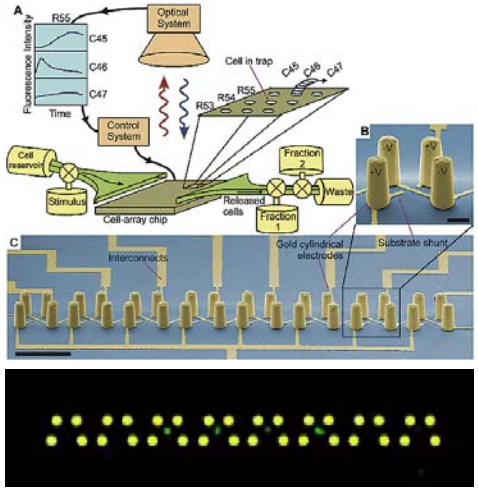


Automated microscope combined with cell chip

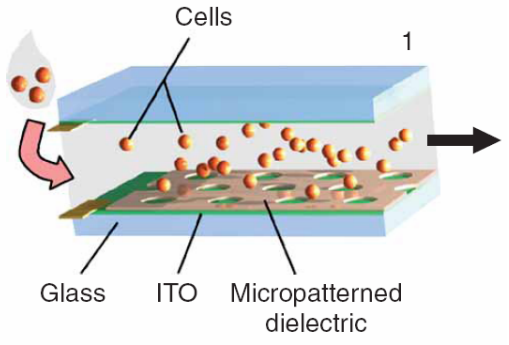
- can take hundreds of images of single cells at different time points
- can provide quantitative insight into cellular behavior
- can be used to determine heterogeneity among a population of single cells

High-throughput and high-content single-cell analysis

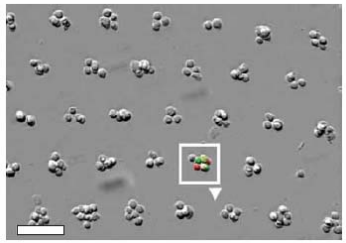
Single-cell docking



Voldman et.al., ***Anal. Chem.*** (2002)



Dielectrophoresis

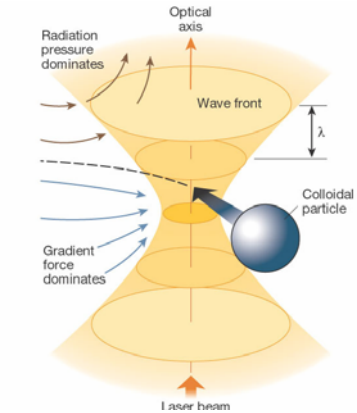


Bhatia et.al., ***Nat. Methods*** (2005)

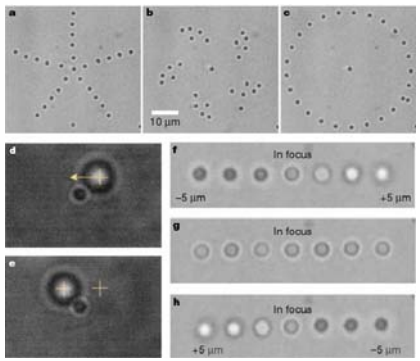
Folch et.al., ***Lab. Chip*** (2005)

Sedimentation

and so on ..

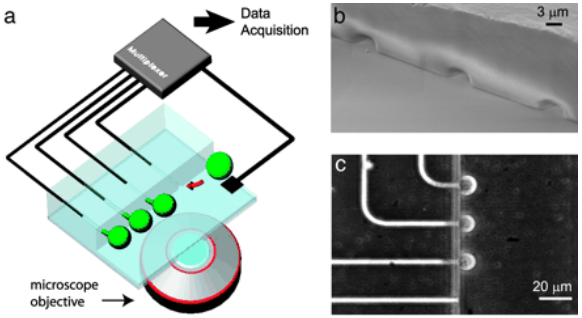


Optical tweezers



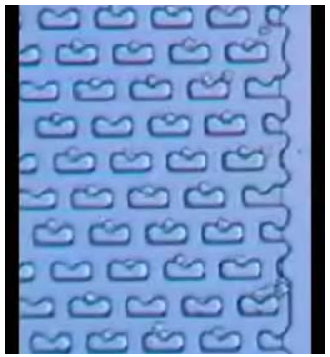
Grier, ***Nature*** (review) (2003)

Microfluidic patch clamp



Luke Lee et.al., ***PNAS*** (2005)

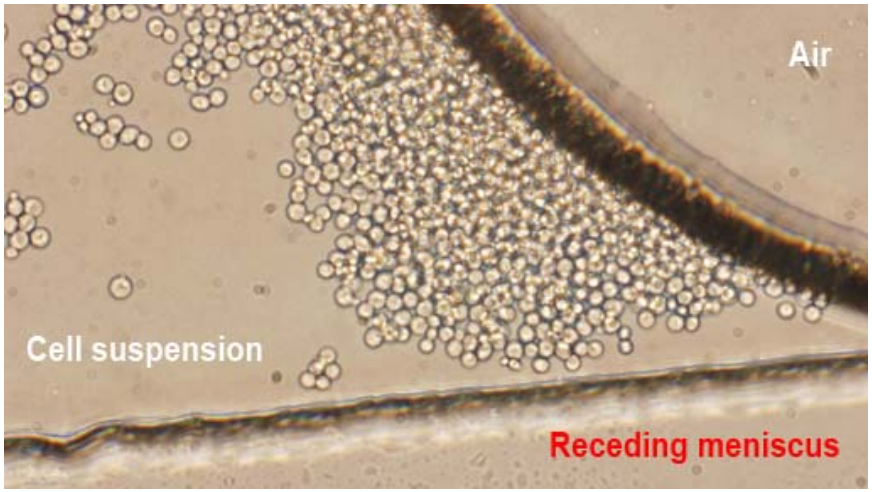
Physical structure

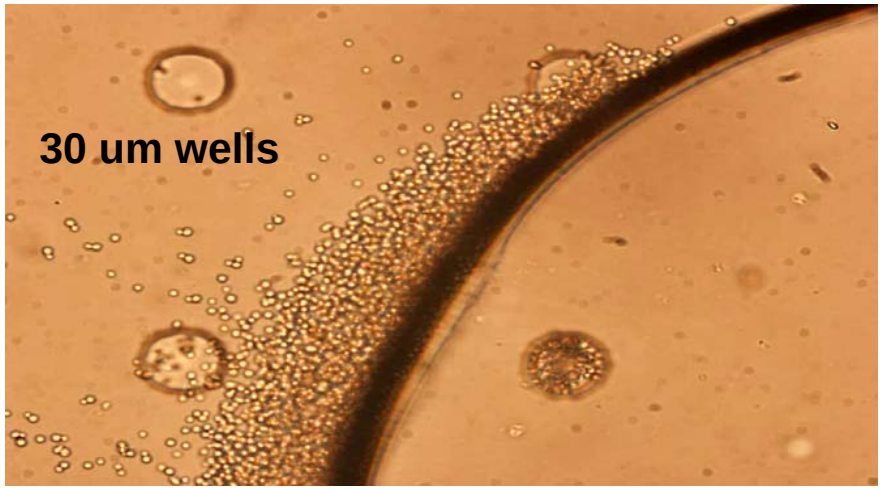


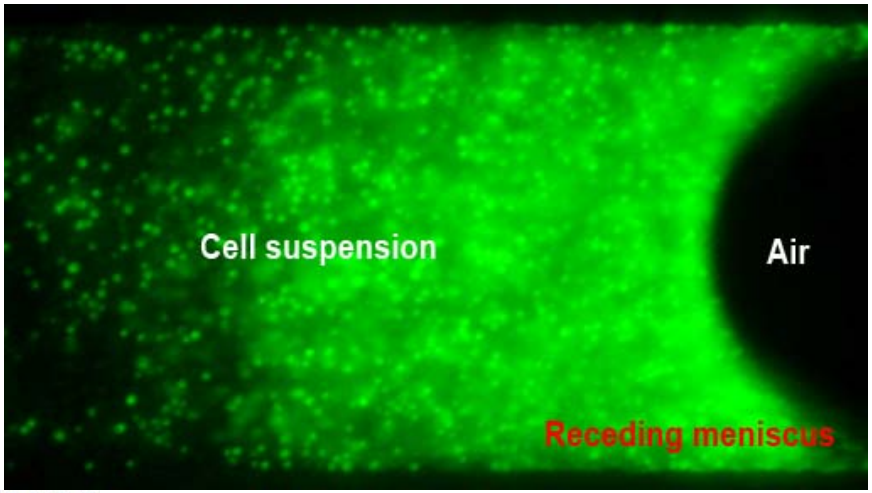
Luke Lee et.al., ***Lab. Chip*** (2006)

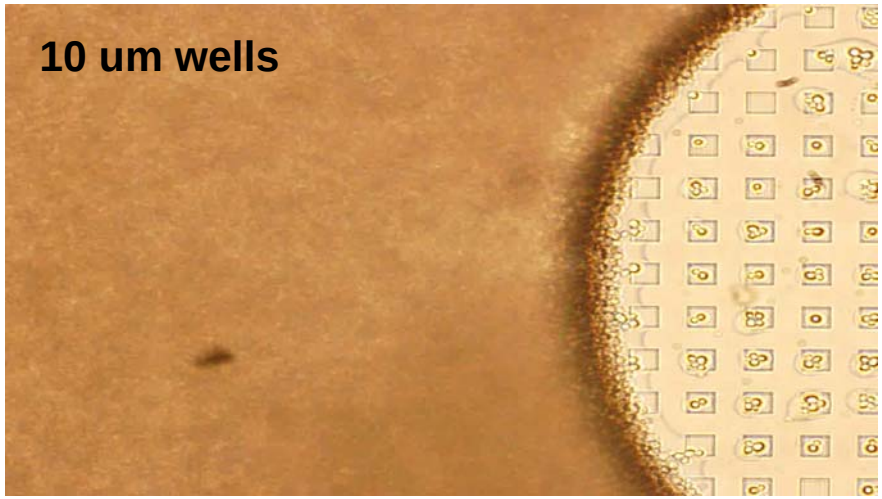
Our approach

- **Receding meniscus induced cell docking (Lab Chip, 6, 988, 2006)**

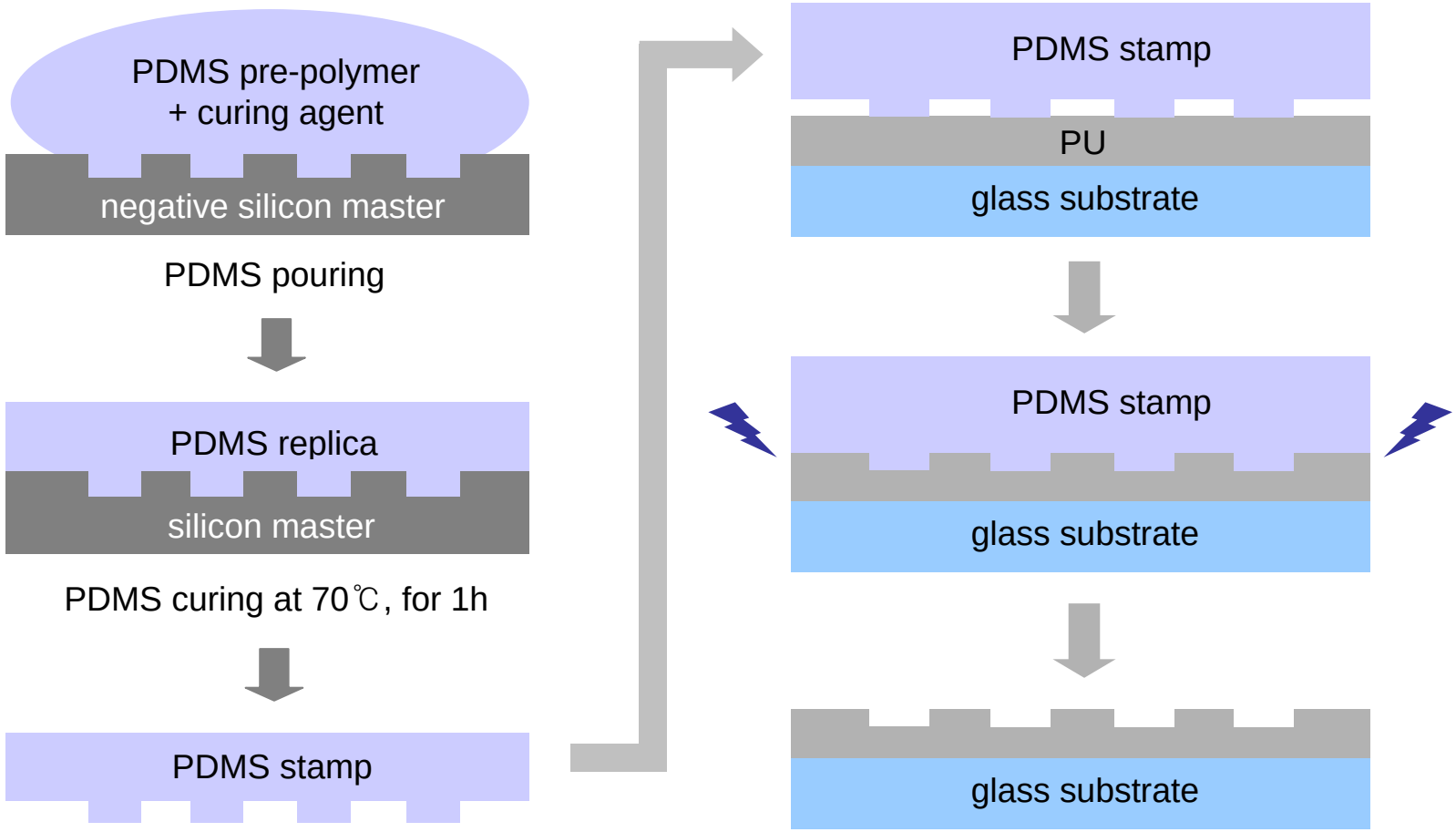






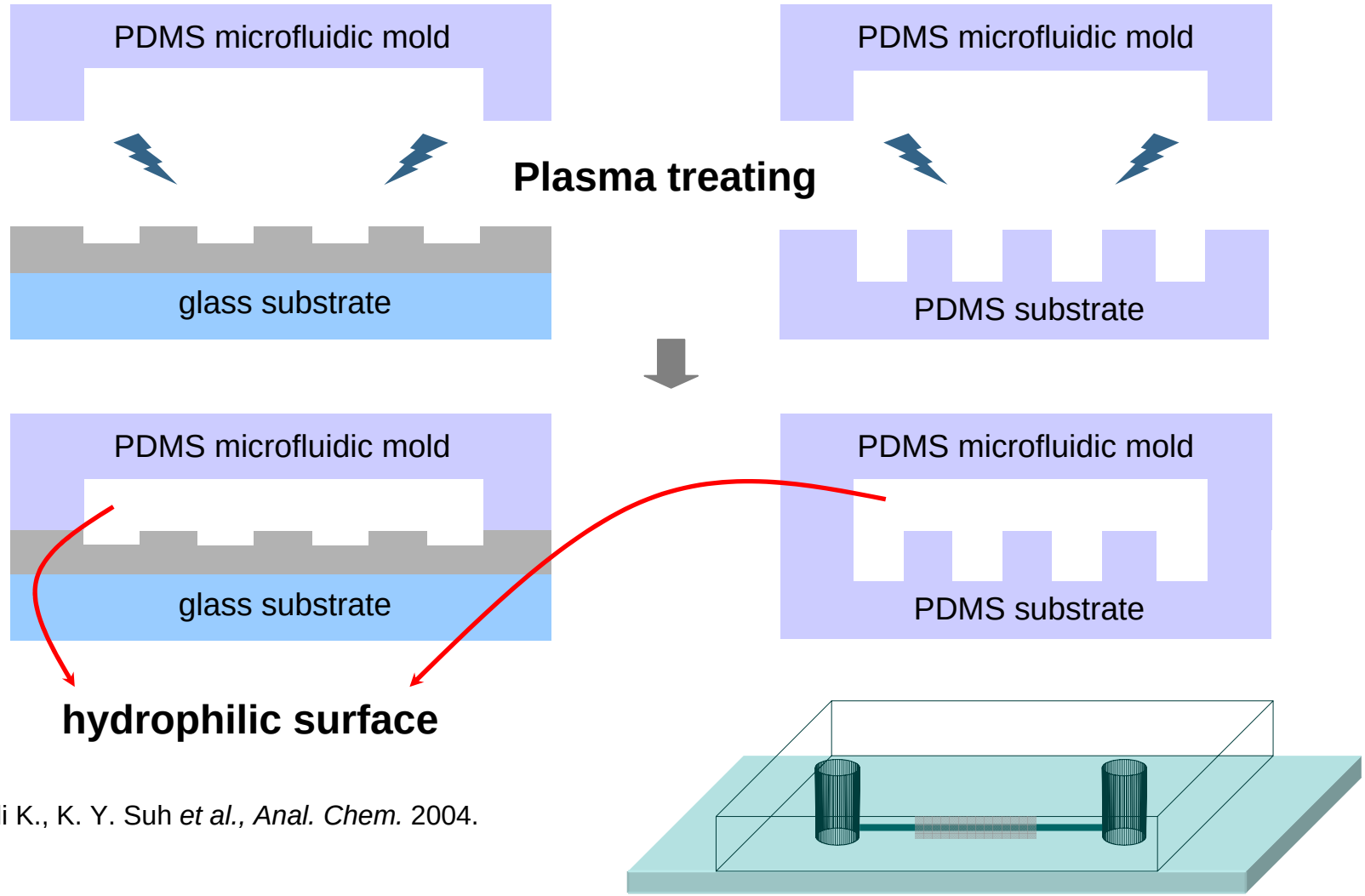


Microwell Patterning



K. Y. Suh *et al., Adv. Mater.* 2001.

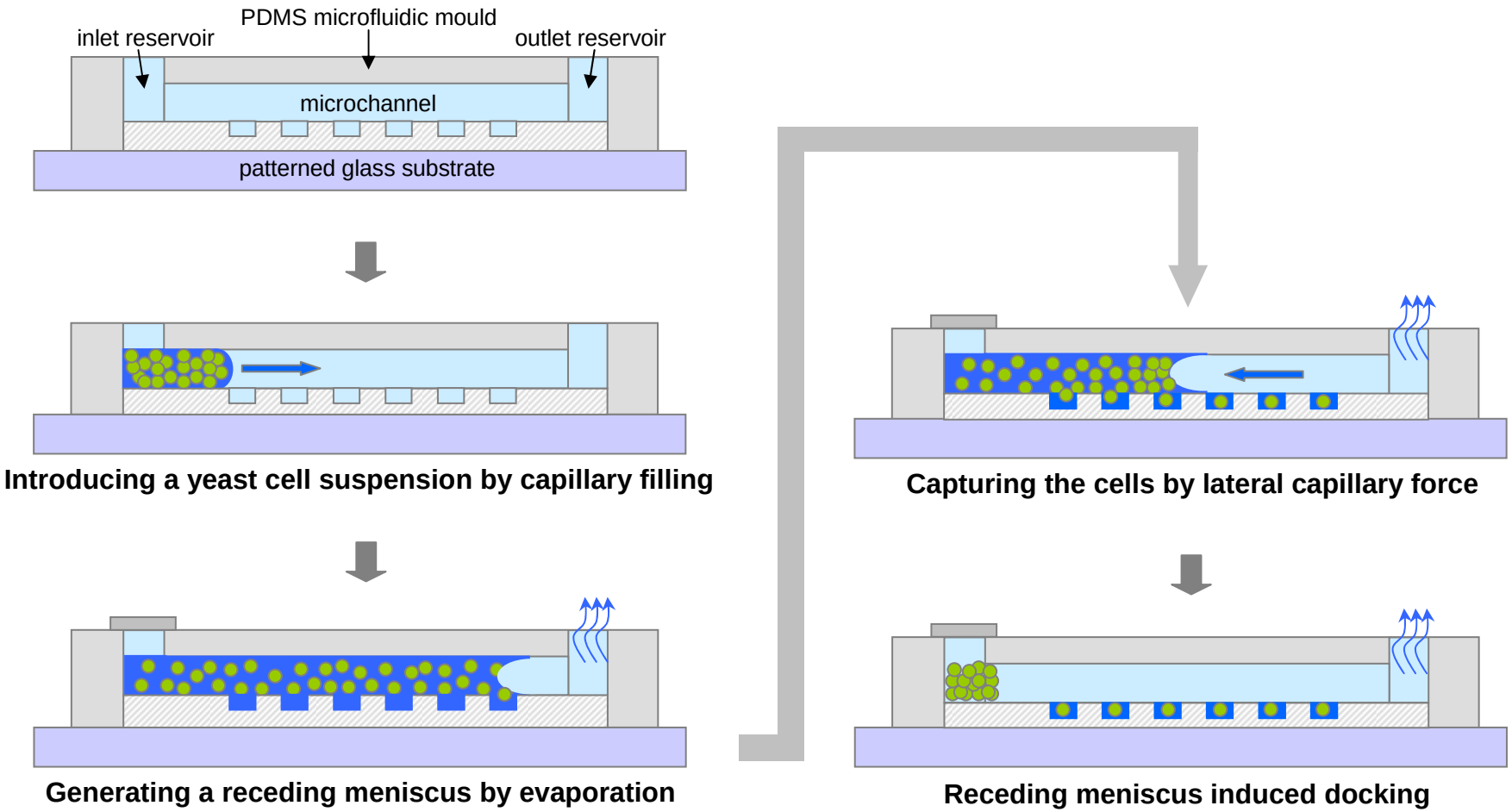
Patterned Microfluidic Channel



Ali K., K. Y. Suh *et al., Anal. Chem.* 2004.

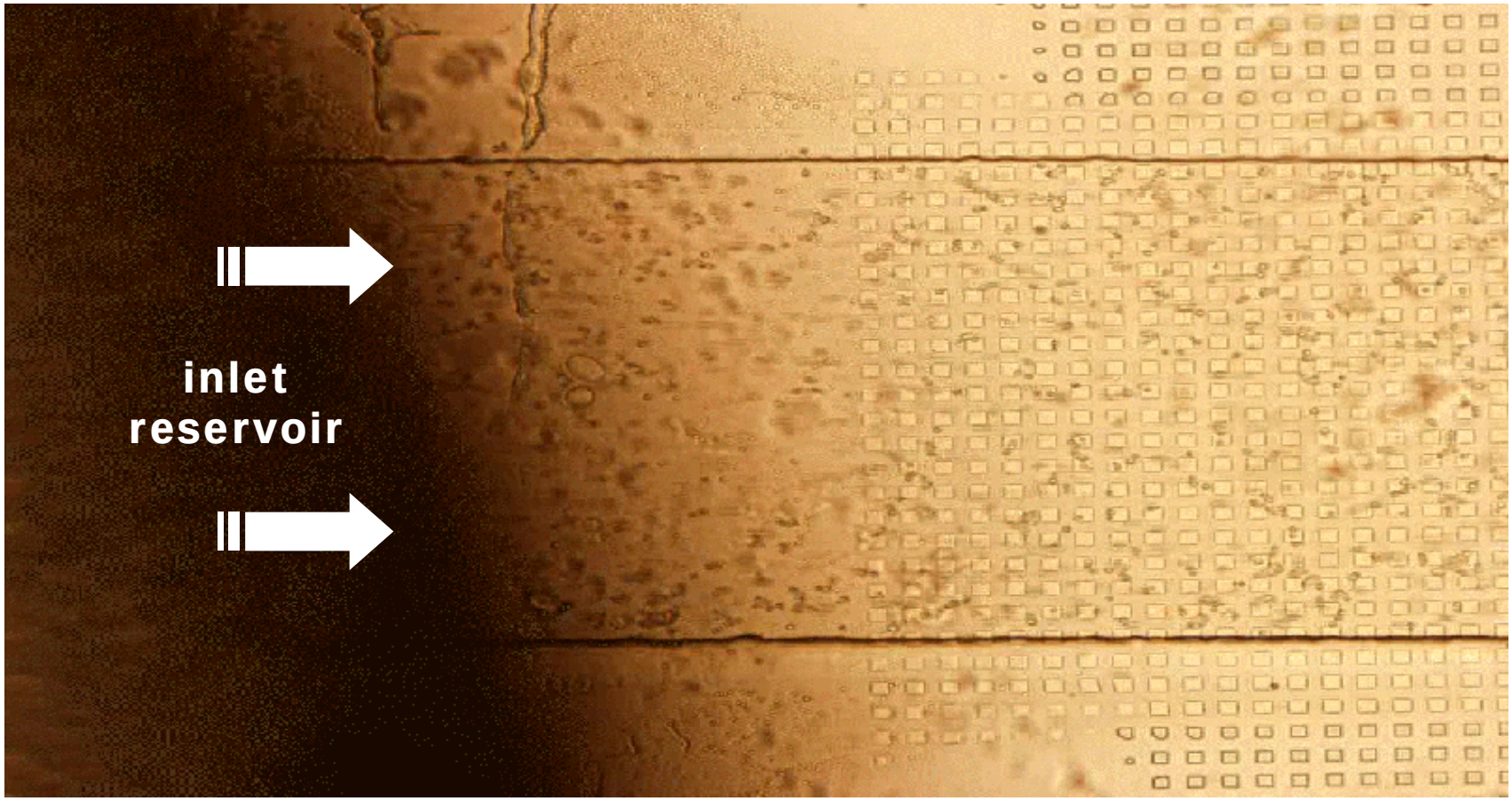
Receding meniscus induced docking

- **Schematic diagram**



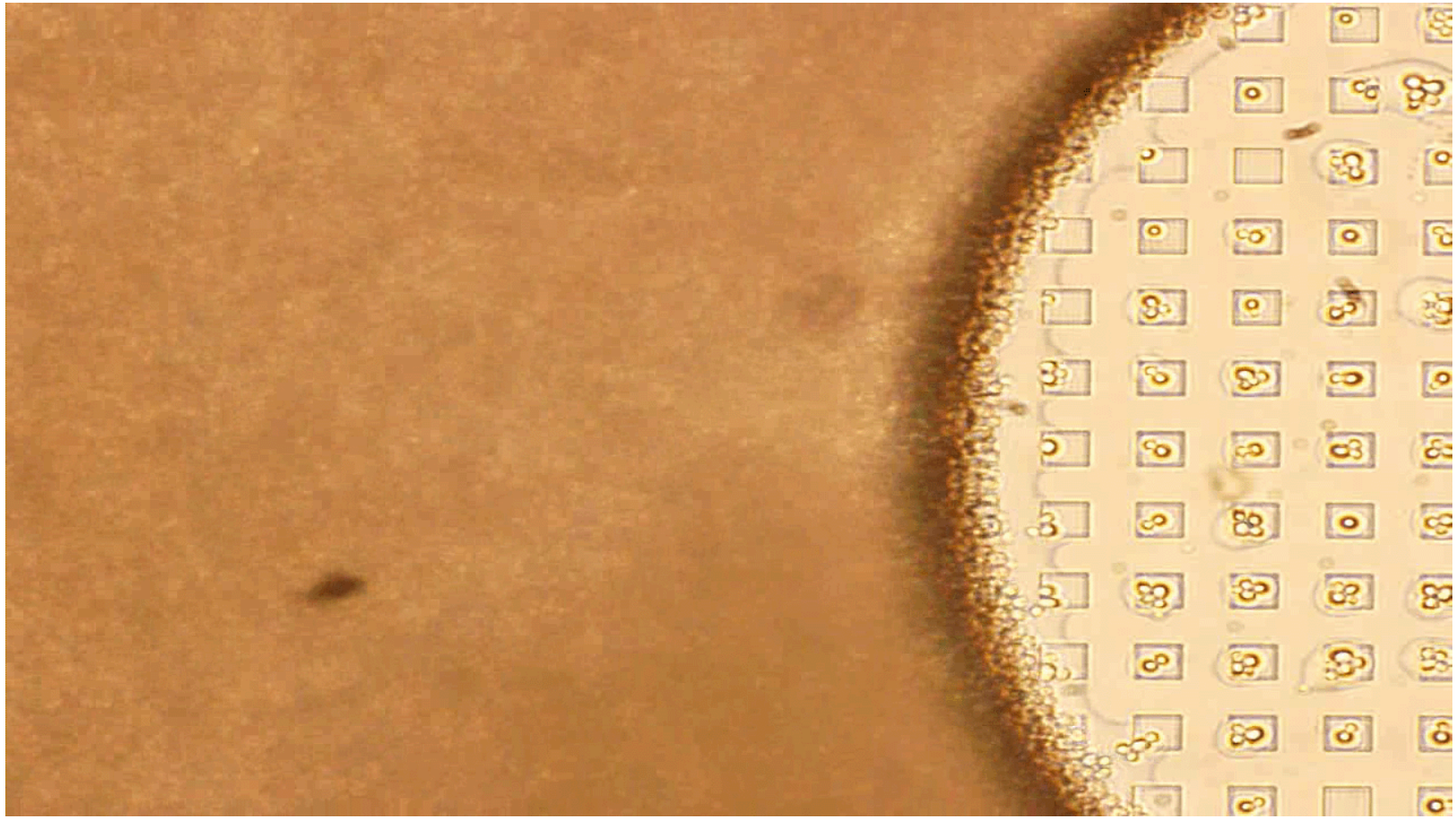
Receding meniscus induced docking

- **Surface tension driven capillary flow**

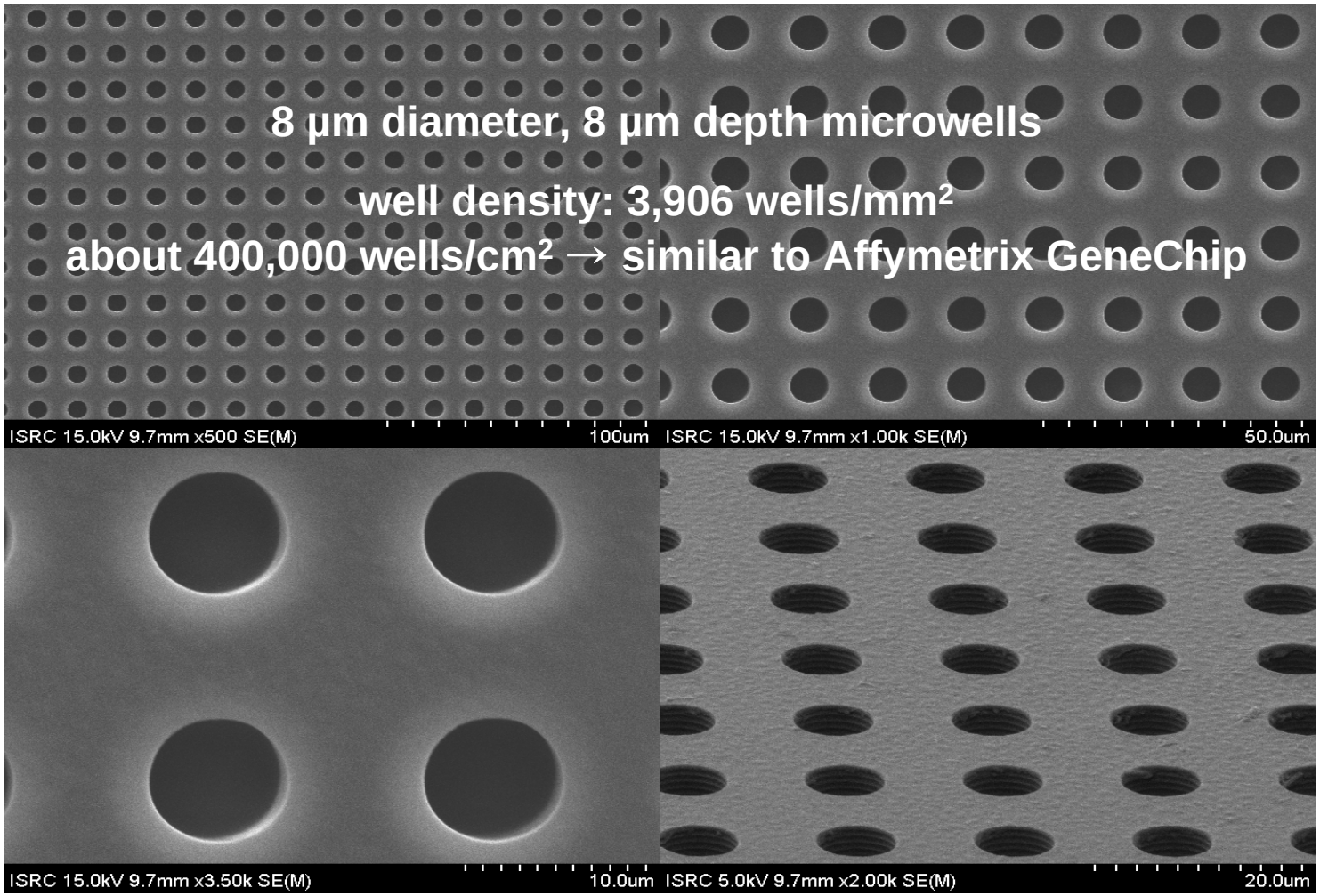


Receding meniscus induced docking

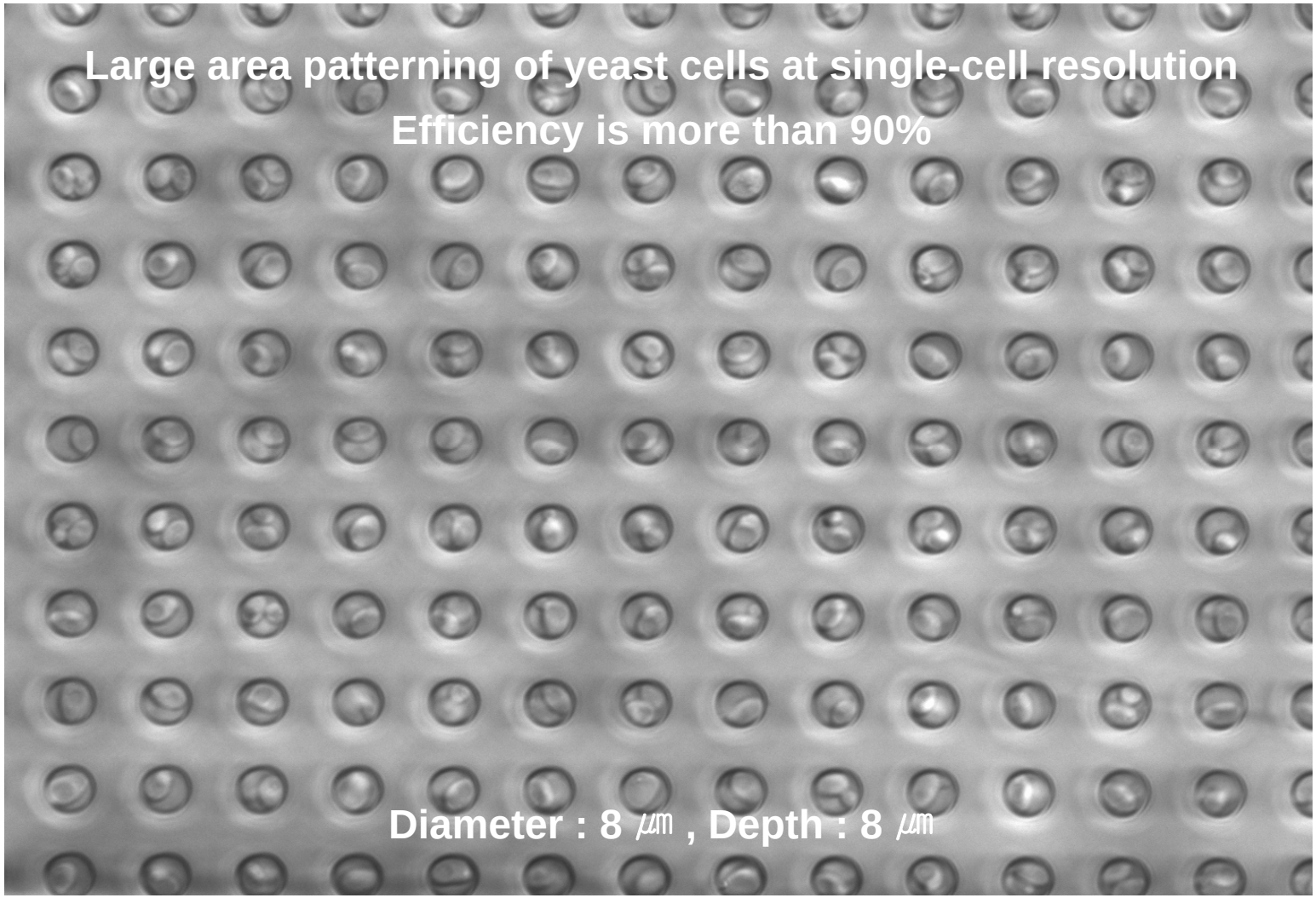
- **10 μm width, 1 μm depth microwells**



High-throughput single-cell analysis

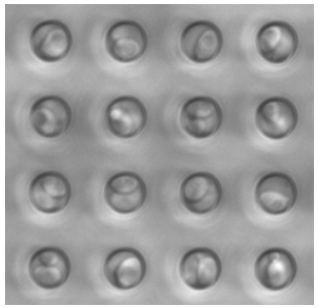


High-throughput single-cell analysis



Quantitative single-cell analysis

Experiment Flow



Single-cell docking

Automated microscope

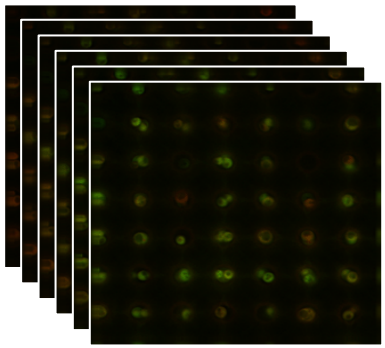


Image acquisition

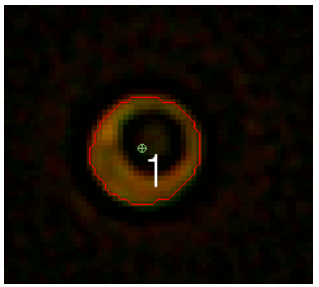
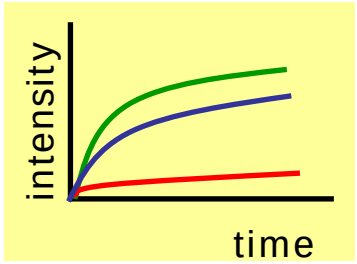


Image analysis

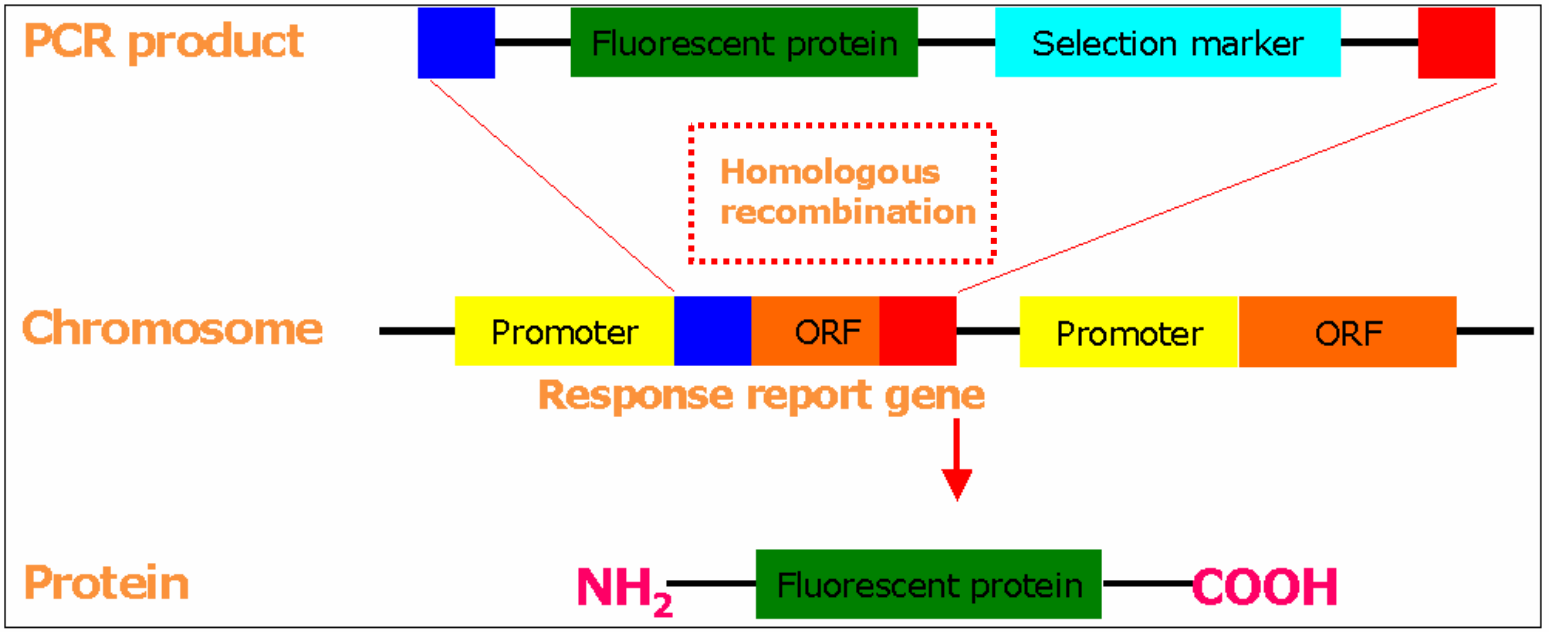
Data handling



Data analysis and modeling

Quantitative single-cell analysis

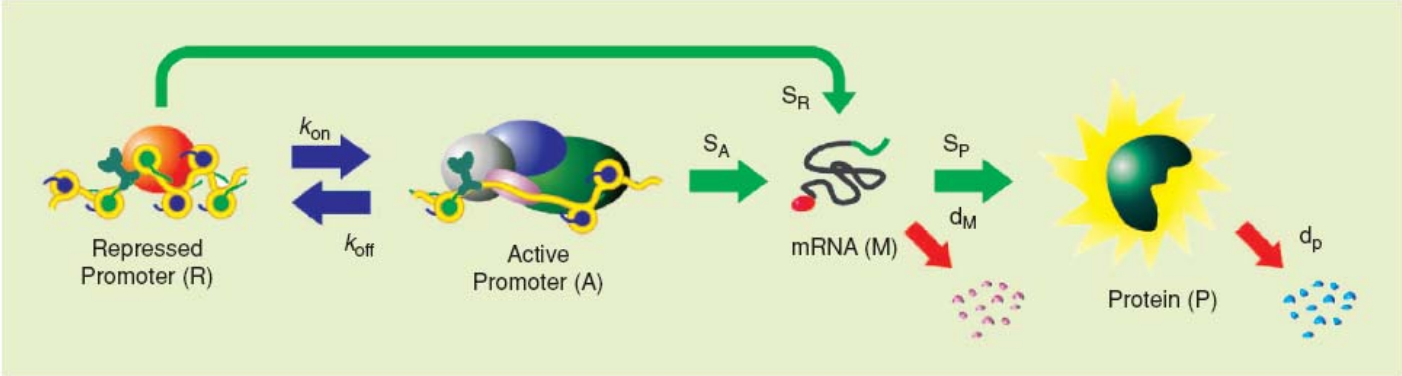
Yeast strain construction



	Response gene	Fluorescent protein
Mating	Fus1	EGFP
High osmolarity	Gpd1	Tdimer2

Quantitative single-cell analysis

Yeast strain construction



signal → **DNA** → **mRNA** → **protein**

transmission transcription translation

SH129 (P_{Fus1}-EGFP, P_{Gpd1}-Tdimer2)

alpha factor → Fus1 promoter activation → EGFP mRNA synthesis → EGFP

signal transmission transcription translation

KCl → Gpd1 promoter activation → Tdimer2 mRNA synthesis → Tdimer2

signal transmission transcription translation

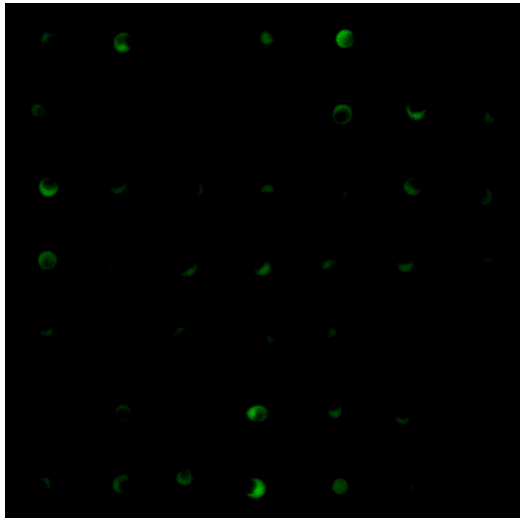
Quantitative single-cell analysis

Automated time-lapse fluorescent imaging

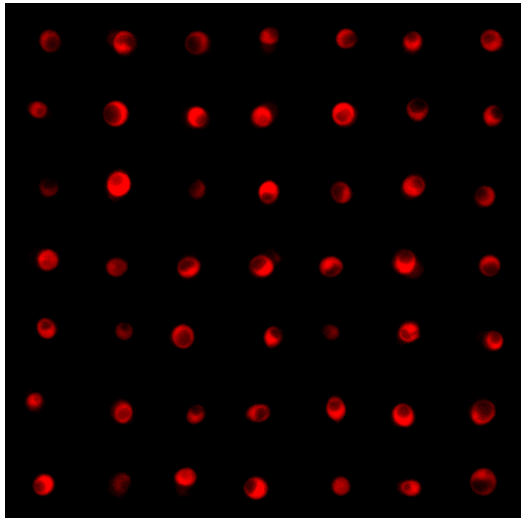
Live cell imaging

AppliedPrecision®

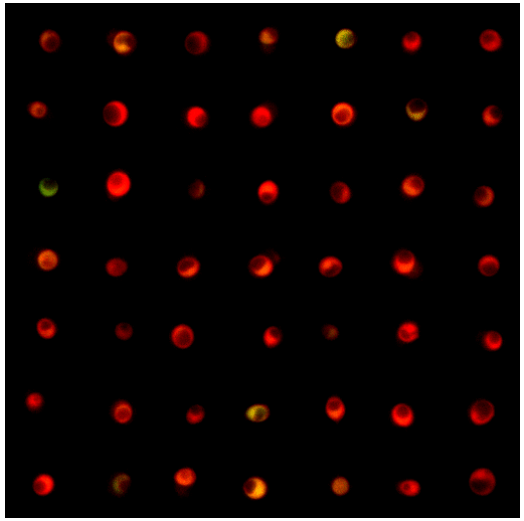
DeltaVision RT



EGFP (green)



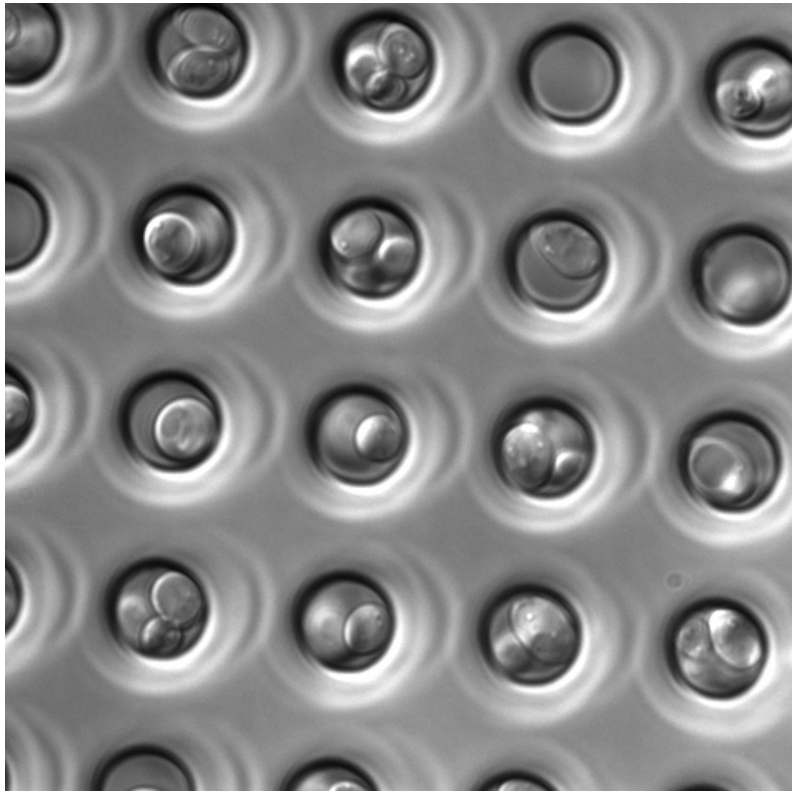
Tdimer2 (red)

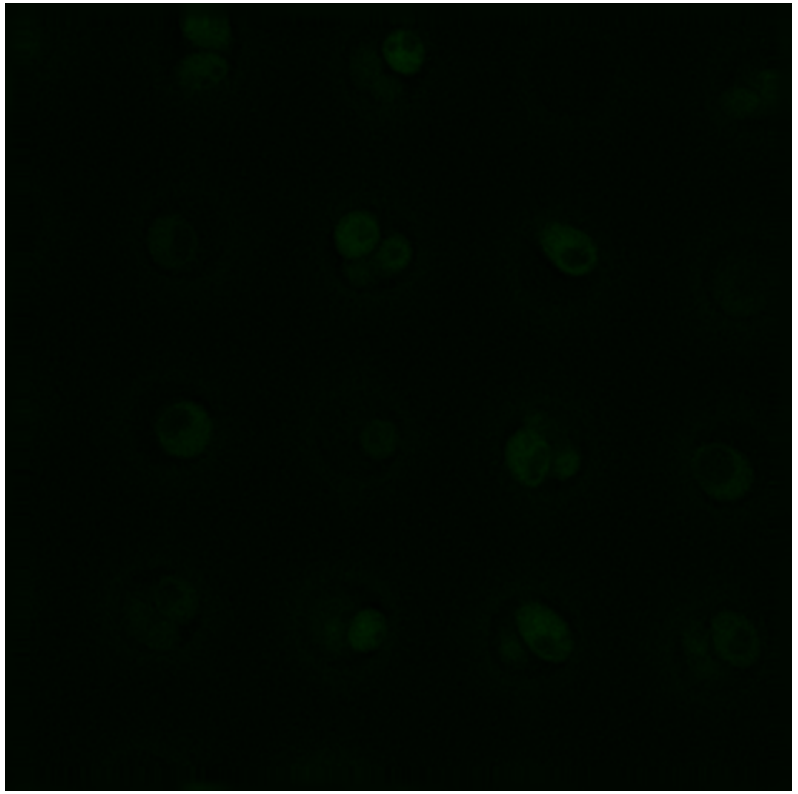


Merged

High-throughput single-cell analysis

- **Automated microscope**

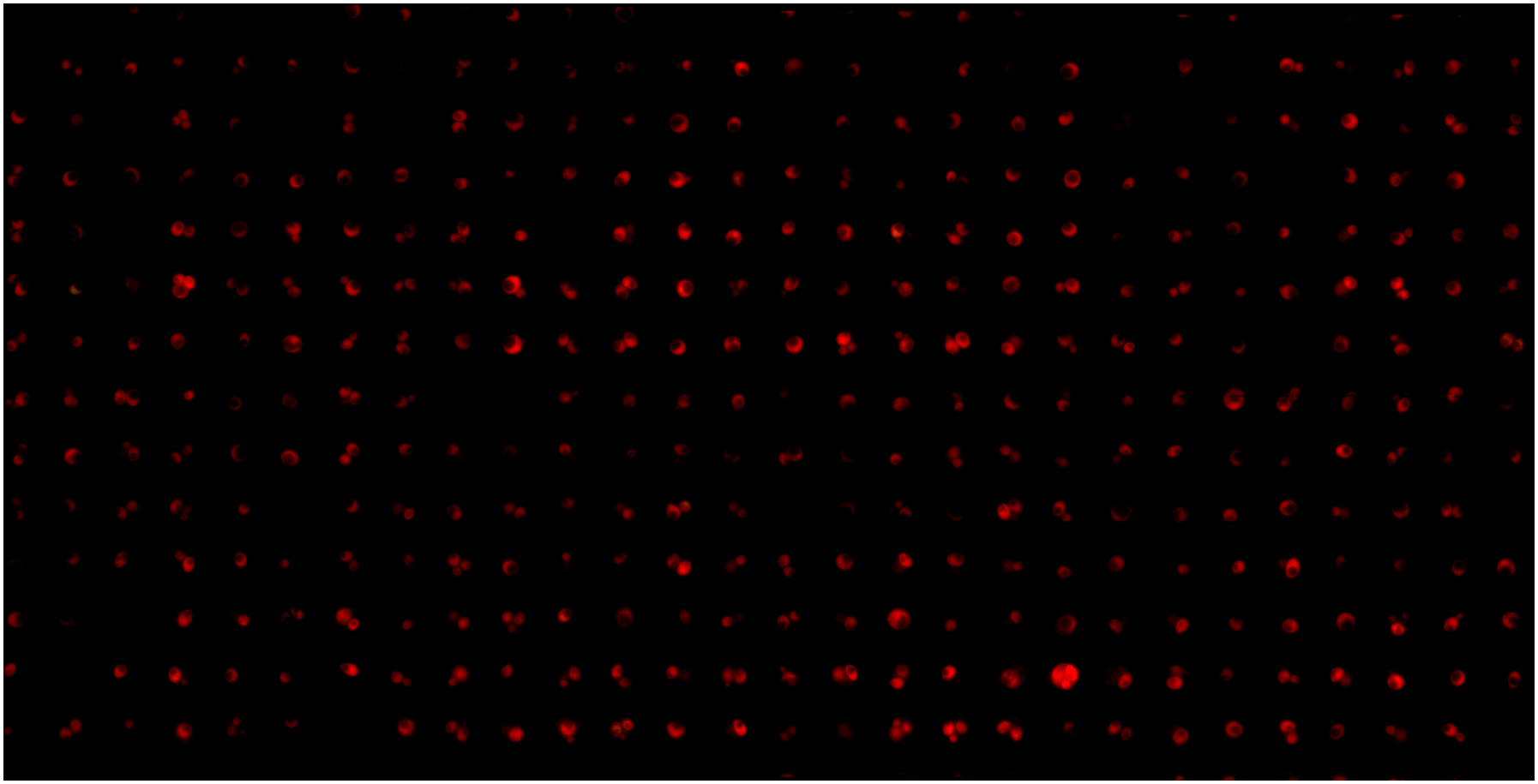




Time-lapse image at a point (120 min)

High-throughput single-cell analysis

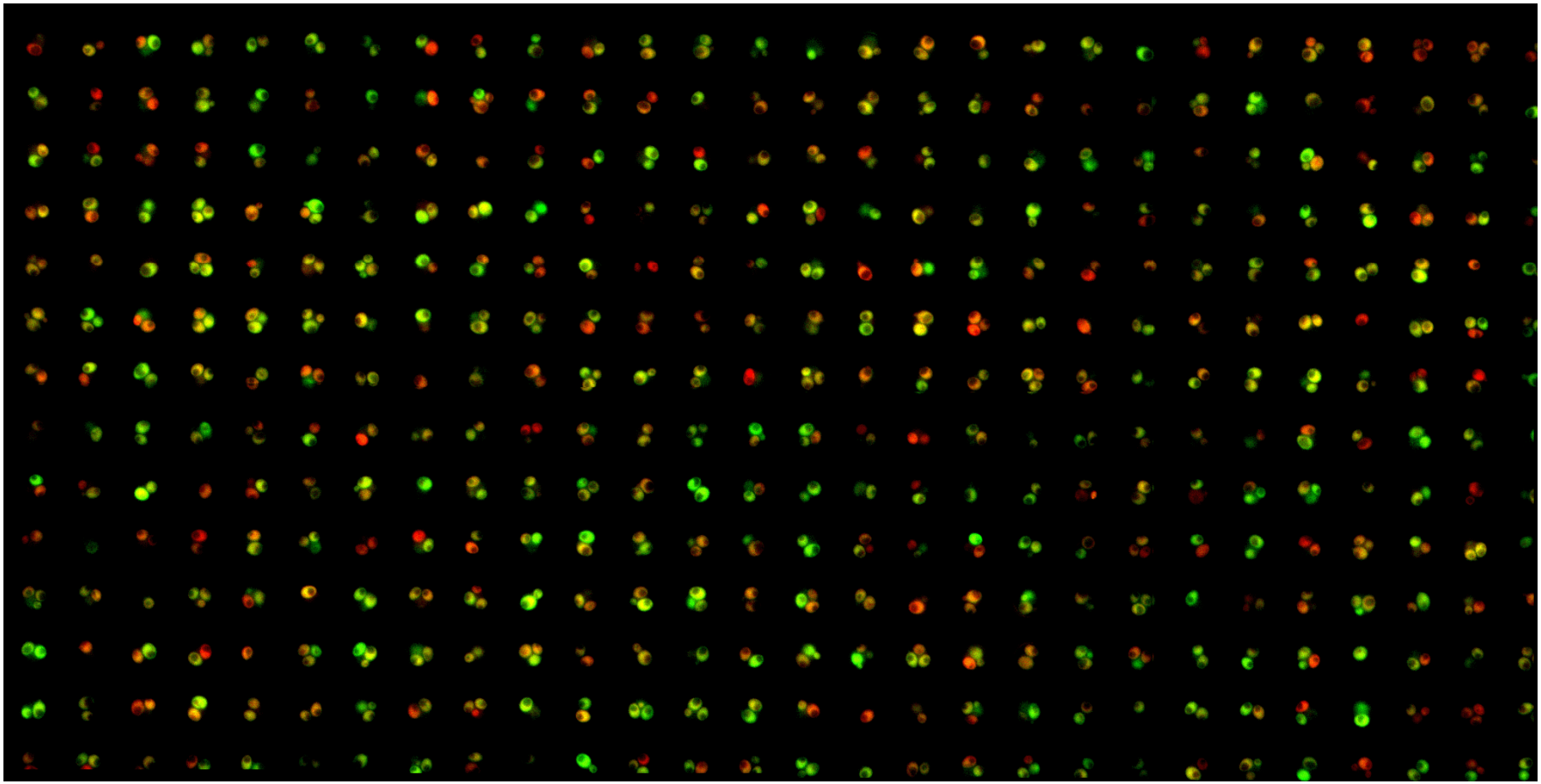
Mating signal response (alpha factor)



GFP is a reporter of mating signal response (120 min)

High-throughput single-cell analysis

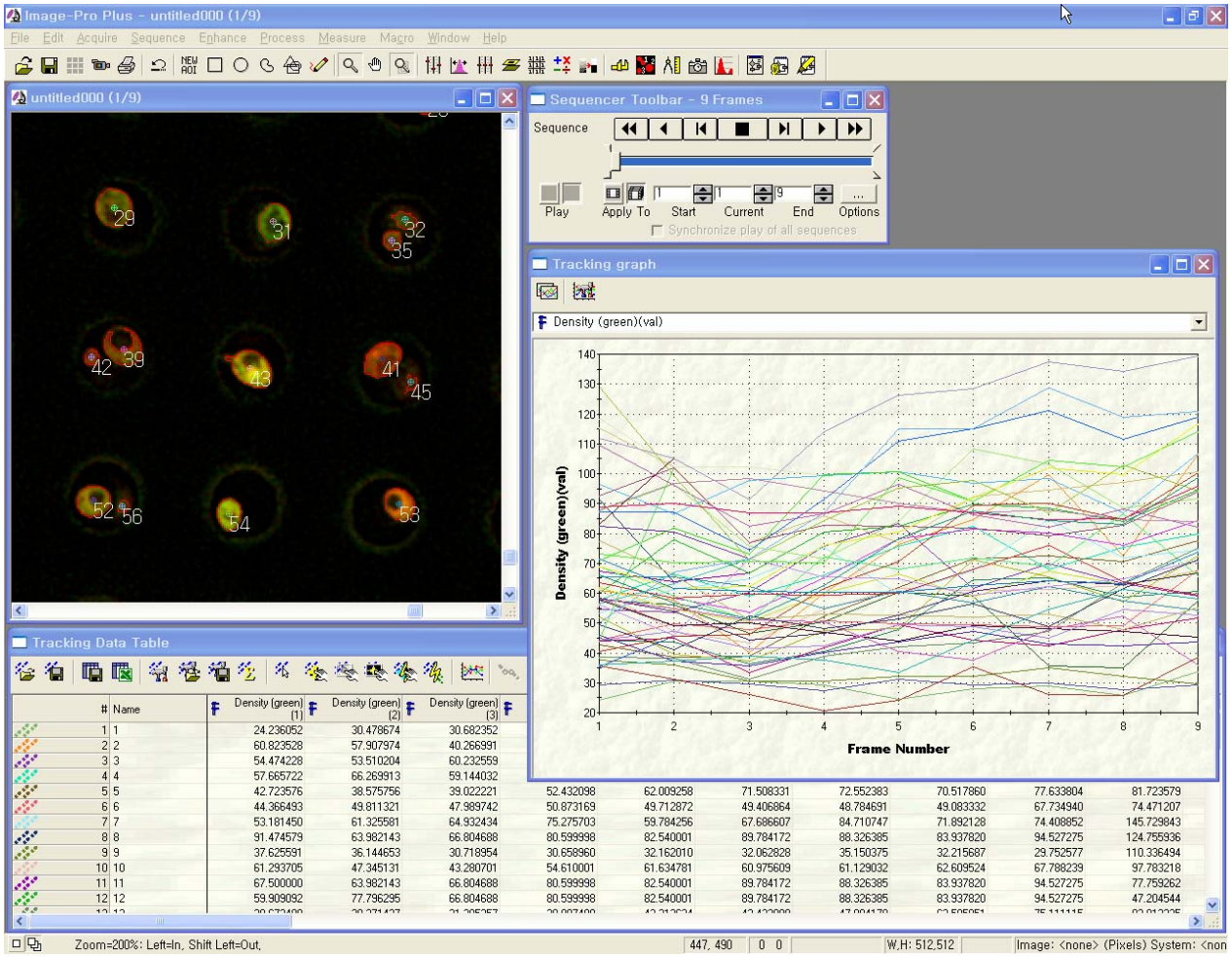
Salt-stress signal response (KCl)



RFP is a reporter of salt-stress signal response (120 min)

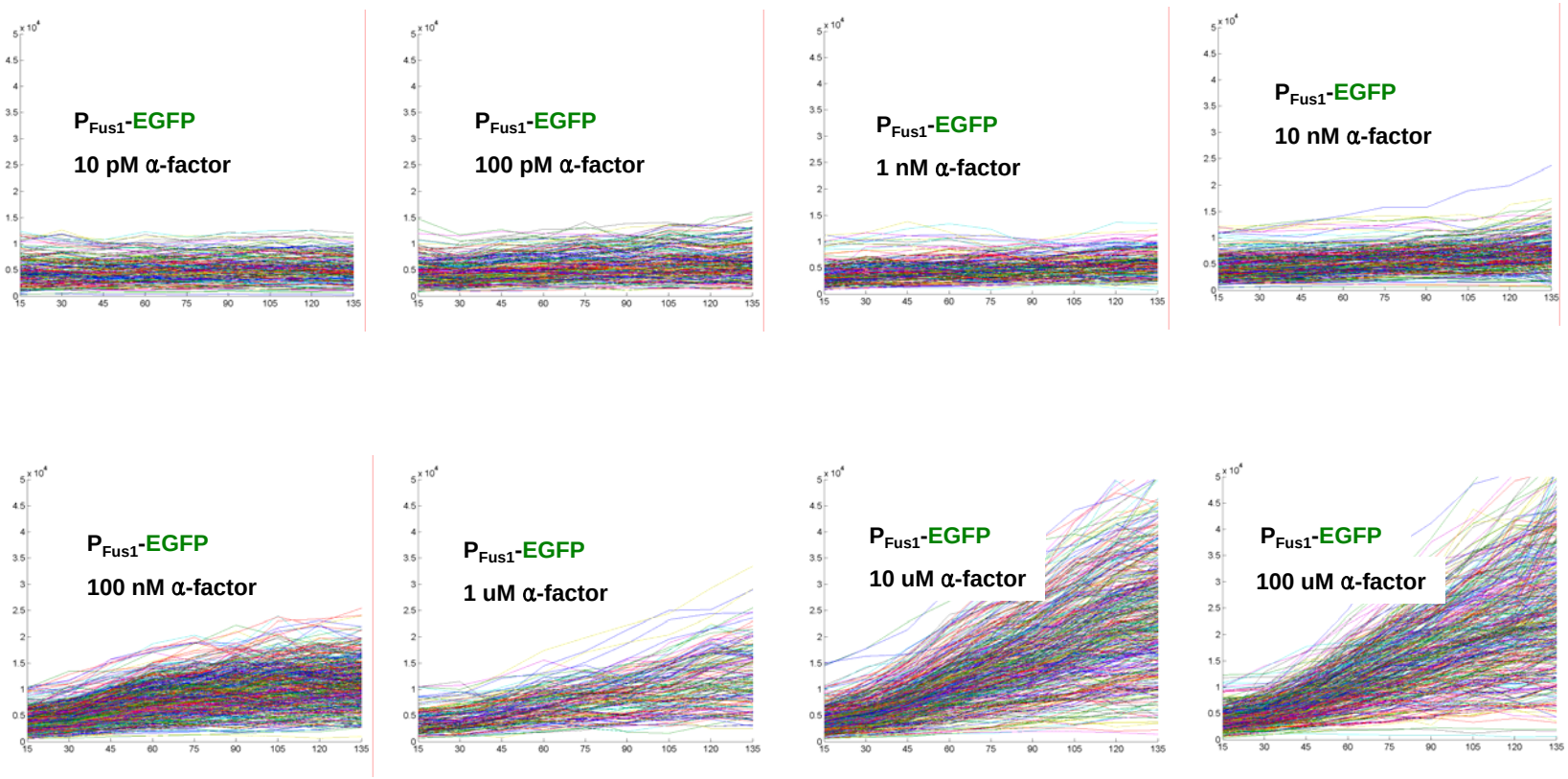
Quantitative single-cell analysis

Image analysis – ImagePro™ (Media Cybernetics, Inc.)

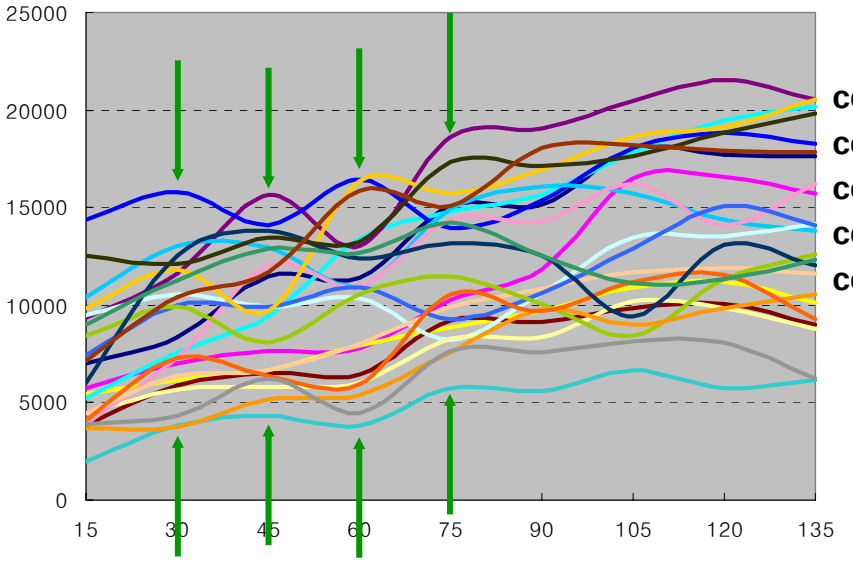


Quantitative single-cell analysis

Image analysis (SH129)



Stochastic dynamics

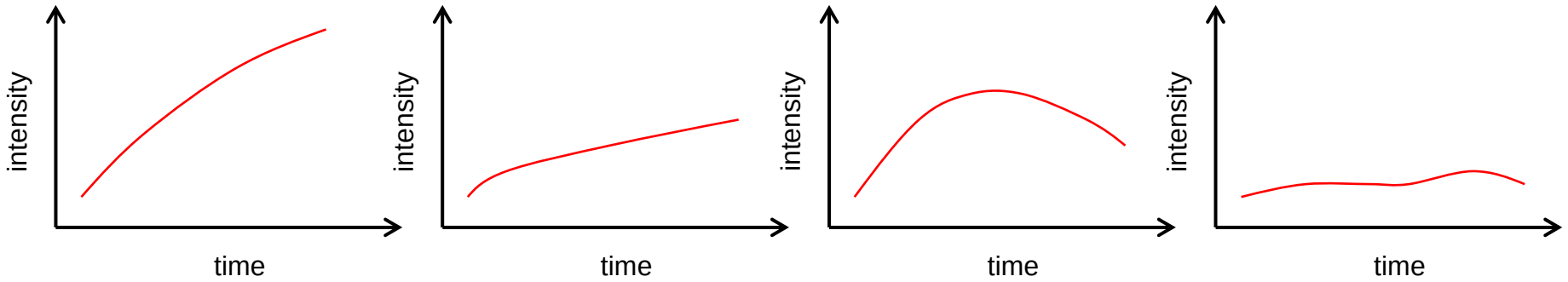


cell 1 dynamics
cell 2 dynamics
cell 3 dynamics
cell 4 dynamics
cell 5 dynamics

⋮

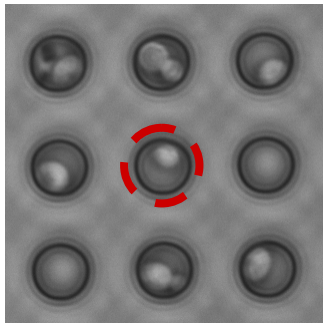
“stochastic dynamics” (pattern analysis)

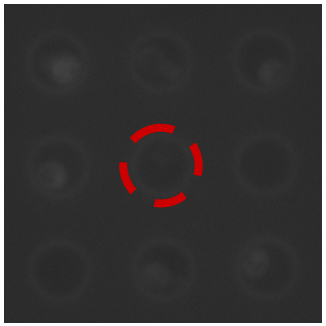
“noise” ≈ Std. dev. / Avg. (point analysis)

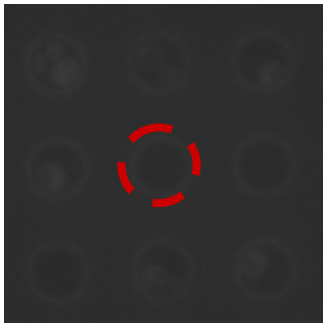


Stochastic dynamics

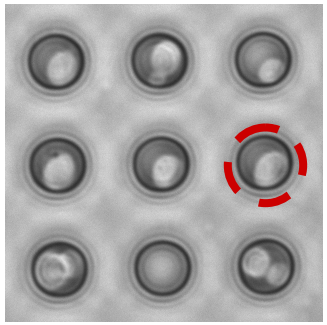
Single-cell recovery (SH129)

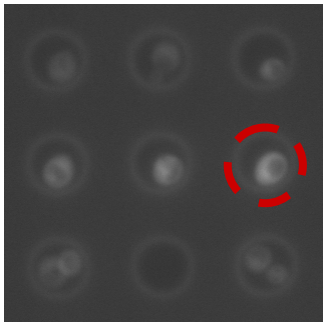


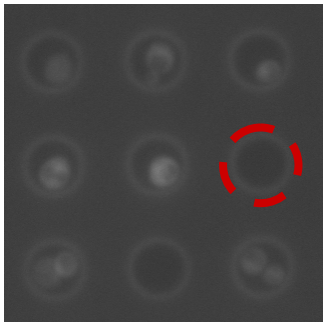




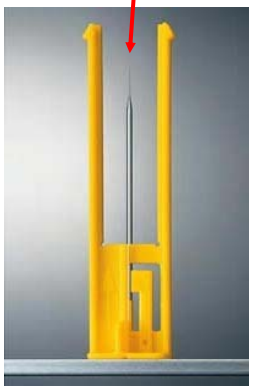
weak







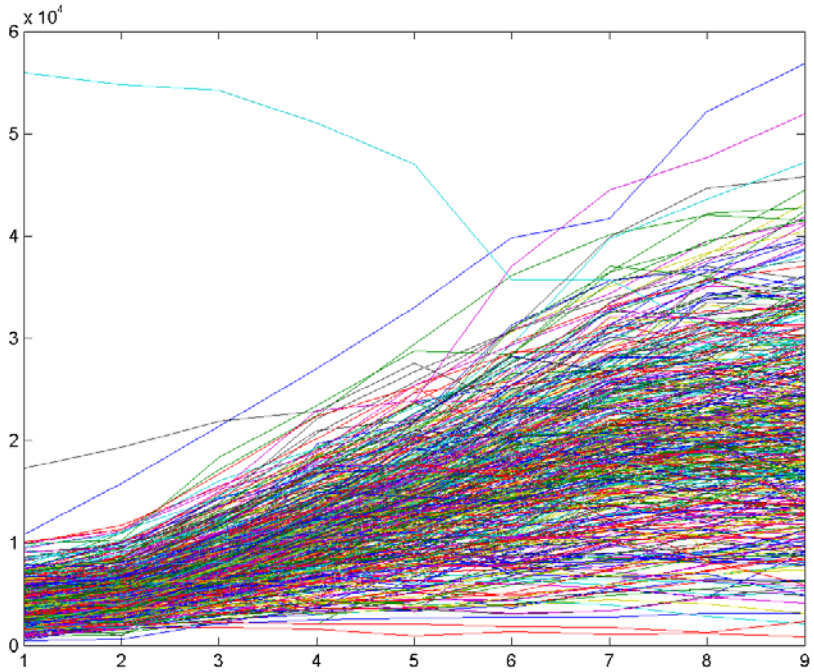
strong



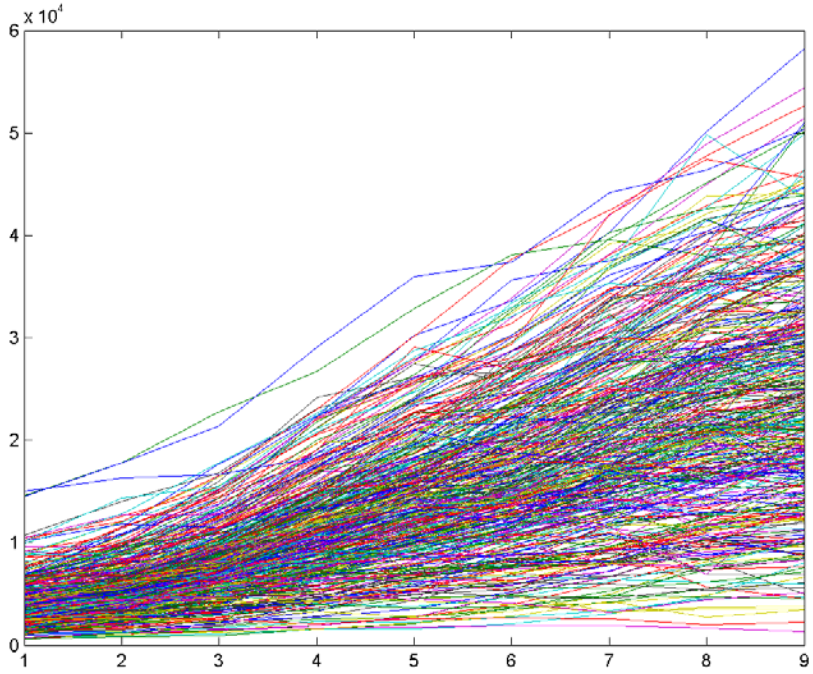
capillary tube

Stochastic dynamics

Single-cell recovery (SH129)



weak



strong

- Same stochastic behavior was observed
- Survival strategy of cells: flexible response to sharp changes in environments

Summary

- A receding meniscus induced cell docking scheme was developed.
- Cell response at single cell level was analyzed with statistical significance
- Stochastic cell response was observed at different conc. of signaling molecules
- Stochastic cell response is intrinsically programmed to survive various changes in cell environments.

Acknowledgements

- Micro Thermal System Research Center of Seoul National University
- Minuta Technology
- Ministry of Science and Technology through the Bio Tool R&D Project for Cell Research

Thank you for your attention!