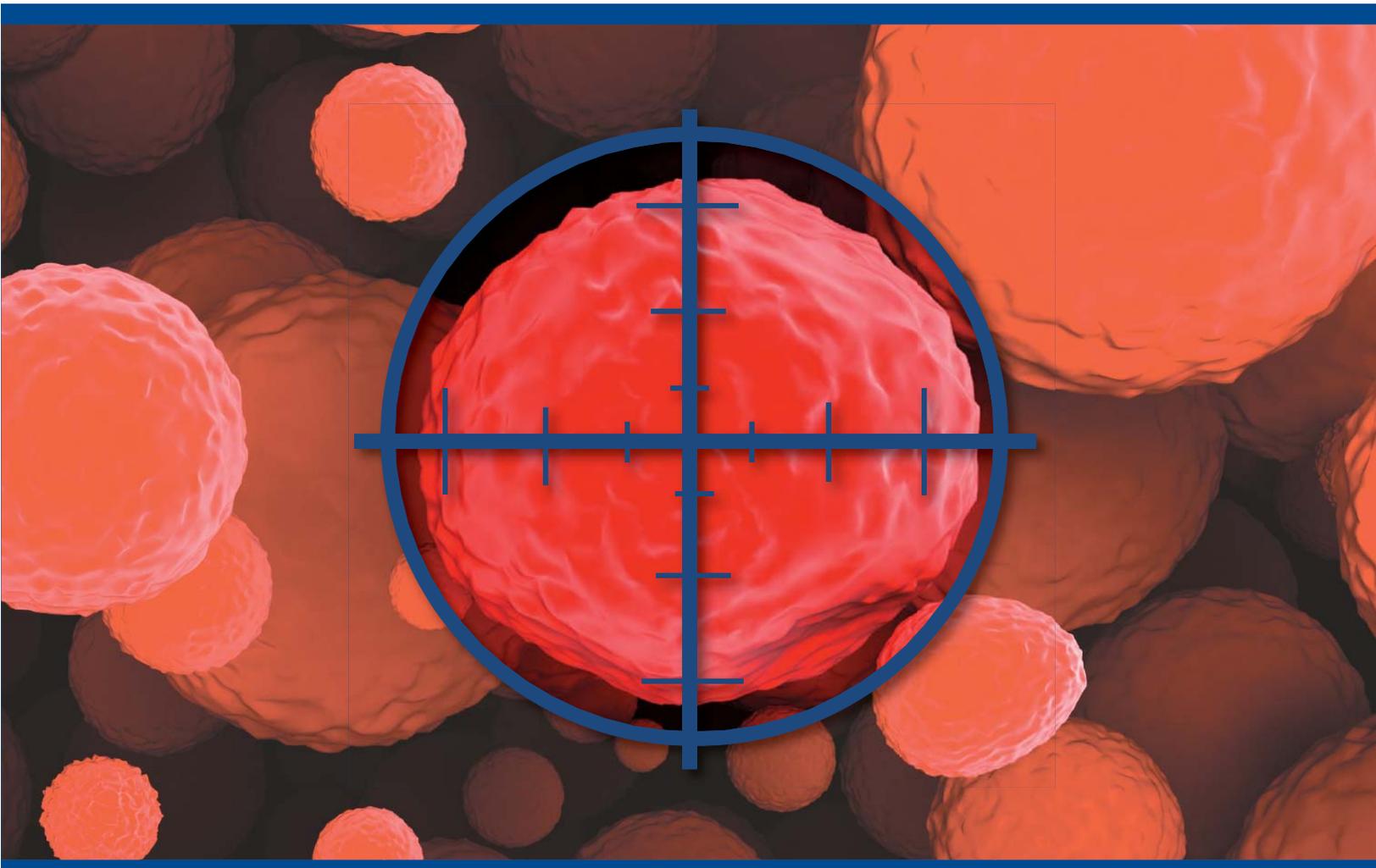


# Why Single Cells Matter

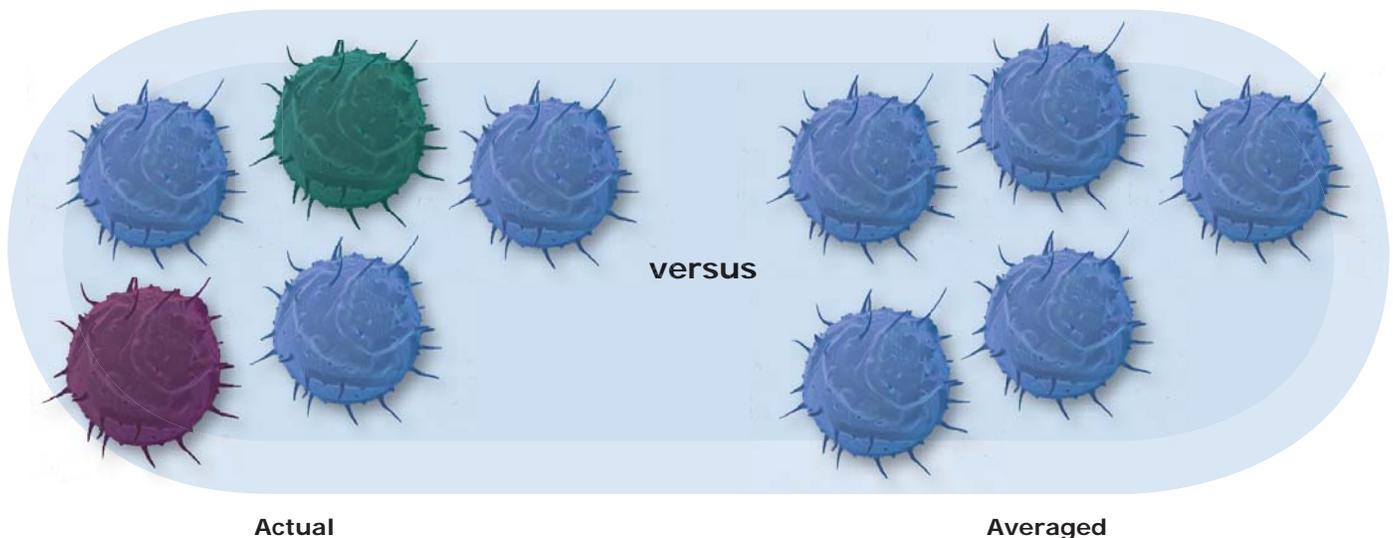


# A Whole New World: Expression Profiling of Single Cells

Within a population of seemingly identical cells, it is possible that variations in gene expression differ dramatically on a cell-to-cell level. These differences will be masked by the averaging effect of studying pooled samples. The solution is to examine multiple individual cells to identify those bearing unique transcriptomes.

*"There are very few people who pay attention to the advantages and importance of studying single cells," said Ron McKay, Chief of the Laboratory of Molecular Biology at the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, in an article entitled *A closer look at the single cell*, *Nature Reports stem cells*, May 7, 2009. "They talk as if they do. They use a FACS machine and act as if they have single-cell data. But they don't. They have data on a population, and that's a completely different thing."*

## Single Cell Studies Reveal Intracellular Differences



*Single cell gene expression analysis can reveal differences among cells even in cell populations that were thought to be homogeneous.*

## Who Wants to be Average?

- Measuring gene expression on a pooled population measures an average for all cells in the sample—not the variance among individuals.

## The Power of One

- The ability to profile individual cells enables not only the identification of previously unrecognized subpopulations, but also the dissection of regulatory networks. By looking at each individual cell instead of at the population average, correlations in expression between genes can be identified.

# The Fluidigm Solution for Single Cell Gene Expression

## Features

- **More Sensitive than Microarrays**  
Greater sensitivity and higher dynamic range than microarrays (6 logs for TaqMan® versus 2-3 logs for microarrays)
- **High Sample Throughput**  
Screen up to 96 single cells across 96 assays in one Dynamic Array™ IFC
- **Produce More Results Faster**  
Produce 9,216 data points in just four hours with one Dynamic Array IFC and one technician
- **Less Expensive than Next Generation Sequencing**  
Much less costly than next generation sequencing for follow-up gene discovery
- **Single Cell, Hundreds of Data Points**  
Use picograms of RNA from a single cell to screen against hundreds of genes
- **Assay Flexibility**  
Practice tried-and-true techniques such as TaqMan assays or other assays you already have in your lab
- **Real-Time or End-Point Runs**  
Real-time and end-point detection of PCR assays on Fluidigm IFCs
- **Productive Analysis Software**  
Powerful, easy-to-use software for gene expression



Fluidigm BioMark™ System for Genetic Analysis

## Best Solution for Focused Gene Expression Analysis

Fluidigm provides scientists with a practical means to rapidly achieve new insights from single cell analysis. The Fluidigm BioMark system and Dynamic Array IFCs enable hundreds of individual cells to be tested for the expression of hundreds of genes in a few hours. Fluidigm's open platform allows scientists to use off-the-shelf reagents and standard analysis tools. Fluidigm's Dynamic Array IFC provides an easy-to-use workflow and complete flexibility of assay configuration (just as with microwell plates, but without the tedious liquid transfer steps).

The BioMark system is ideally suited for focused gene expression analysis and hypothesis testing. Traditional microarrays or next generation sequencing platforms may be used initially to perform broad gene discovery research. Fluidigm's system, however, is far more sensitive than microarrays and far less expensive to use than next generation sequencing for follow-up gene discovery studies.

The BioMark system sets the new standard for high-throughput real-time qPCR assays, integrating thermal cycling and fluorescence detection on Digital Array™ IFCs and Dynamic Array IFCs. The BioMark system streamlines workflows for applications demanding sensitivity and dynamic range, at extremely high throughput.

# Stop Wasting Reagents

Only 240  $\mu\text{L}$  of master mix is required for 9,216 PCR reactions when using Fluidigm's technology. In comparison, 4.6 mL of master mix is required for the equivalent number of reactions in 384-well plates.



	384-well	96.96 Dynamic Array IFC	48.48 Dynamic Array IFC
Master Mix	46 mL	240 $\mu\text{L}$	480 $\mu\text{L}$
Primer-probe (20X)	4.6 mL	240 $\mu\text{L}$	480 $\mu\text{L}$
Plates	24	1	4
Time	8 days	4 hours	8 hours
Pipette steps	18,432	192	384

Comparison of materials and pipetting steps between conventional microplates and Dynamic Array IFCs; 96 samples against 96 assays.

## Ideal for Studies such as:

- Circulating tumor cells
- Stem cells
- Immunology
- Developmental Biology
- and many more

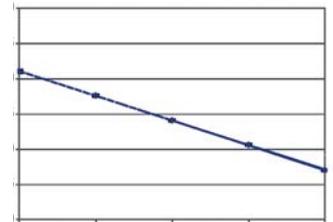


## Outstanding Data Quality

Linearity of six orders-of-magnitude or better

High intra- and interchip reproducibility

Lower rate of error due to fewer manual pipetting steps



## Workflow is Fast and Easy

The ease of use of the BioMark workflow makes it amenable to high-throughput studies. Generate results in four hours.

1

Obtain a single cell via FACS or other method of choice.

2

Extract mRNA from cell, reverse transcribe and specific target amplify (STA).

3

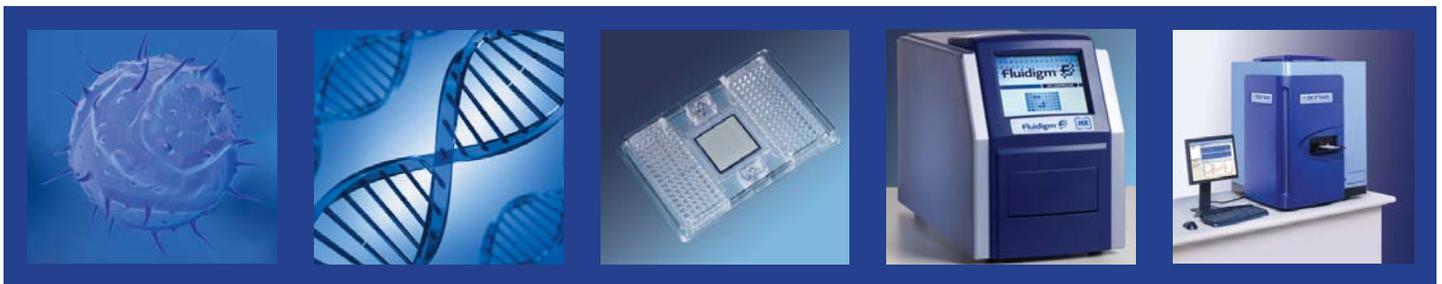
Pipette into 96.96 Dynamic Array IFC.

4

Load chip on the IFC Controller.

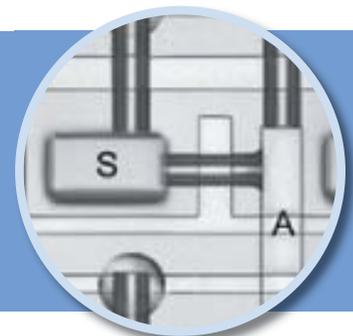
5

Run chip on BioMark System.



## The Power of Microfluidics

Using a Fluidigm Dynamic Array IFC, high-throughput multiplexing is easy. The microfluidic architecture does the work of combining samples and primer-probe sets into 9,216 simultaneous PCR reactions. That's 24-fold more data than is produced by a 384-well plate. This radical advance in experiment density is fully leveraged through a hardware/software system that automates setup and data analysis.



## Join the Single-Cell Analysis Movement

Many of the world's leading stem cell and developmental biologists are harnessing the power of Fluidigm technologies that enable single-cell level analysis of gene expression. With Fluidigm's BioMark™ system, researchers are beginning to dissect complex gene expression networks within individual embryos and to uncover the molecular basis of heterogeneity in the reprogramming process that generates induced pluripotent stem (iPS) cells, for example. This approach to gene expression analysis will undoubtedly unlock many biological discoveries.

Here's how some of Fluidigm's customers are using the BioMark System and Fluidigm Dynamic Array integrated fluidic circuits (IFCs) to study single cells:

Irving Weissman, M.D. Professor of Pathology, Director of the Institute of Stem Cell Biology and Regenerative Medicine, Stanford University, uses the BioMark System to perform single-cell gene expression to isolate homogeneous cell populations. His overall research pursuit is stem cell-based therapies for many chronic human diseases.

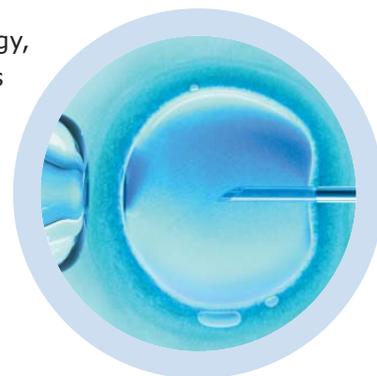
*"When we get to the point that we think we have a homogenous population, the real test is the fingerprinted gene expression,"* Dr. Weissman said. *"We do that as a matter of course now."*

Dr. Mikael Kubista, Head of the Gene Expression Laboratory at the Institute of Biotechnology and founder of the TATAA Biocenters, uses the BioMark system to study intracellular gene expression profiles of cancer stem cells.

*"This has not been possible before, because in science we had not been able to measure the differentiation on the cell level, at least not with high accuracy,"* Dr. Kubista said. *"Using Fluidigm's integrated fluidic circuit technology, measuring cell differentiation is becoming possible today. We are doing it on Fluidigm's BioMark system, using 50 genes per cell and using preamplification."*

Mylene Yao, M.D., and assistant professor at the Department of Obstetrics and Gynecology, Stanford University, uses the Fluidigm system to study gene expression in early embryos to improve human fertility treatments.

*Dr. Yao explained that single embryo data allowed her research team to identify genes that are consistently differentially regulated and to find rare outlier embryos expressing unique transcriptomes.*



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## PUBLICATIONS FROM FLUIDIGM STEM CELL AND SINGLE CELL BIOLOGY CUSTOMERS

- Ralston, A., Cox, B., Nishioka, N., Sasaki, H., Chea, E., Rugg-Gunn, P., Guo, G., Robson, P., Draper, J., Rossant, J., 2010. Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2. *The Company of Biologists Ltd. Dev.* 137:395-403.
- Yuan, P., Han, J., Guo, G., et al. 2009. Eset partners with Oct4 to restrict extra embryonic trophoblast lineage potential in embryonic stem cells. *Genes Dev.* 23:2507-2520.
- Nicholas, C.R., Haston, K.M., Grewall, A.K., Longacre, T.A., Reijo Pera, R.A., 2009. Transplantation directs oocyte maturation from embryonic stem cells and provides a therapeutic strategy for female infertility. *Hum. Mol. Genet.* Nov. 15, 18(22):4376-4389
- Leong, D., Hahn-Windgassen, A., Foygel, K., Jun, S., Behr, B., Yao, M. 2009. Morpholino-mediated gene knockdown in the early mouse embryo. *Nature Protocols* 110.
- Diehn, M., Cho, R.W., Lobo, N.A., et al. 2009. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* 458: 780-783.
- Foygel, K., Choi, B., Jun, S., et al. 2008. A novel and critical role for oct4 as a regulator of the maternal-embryonic transition. *PLoS ONE*.3(12): e4109.



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