Polaris and Callisto: Advances in Cellular Biology

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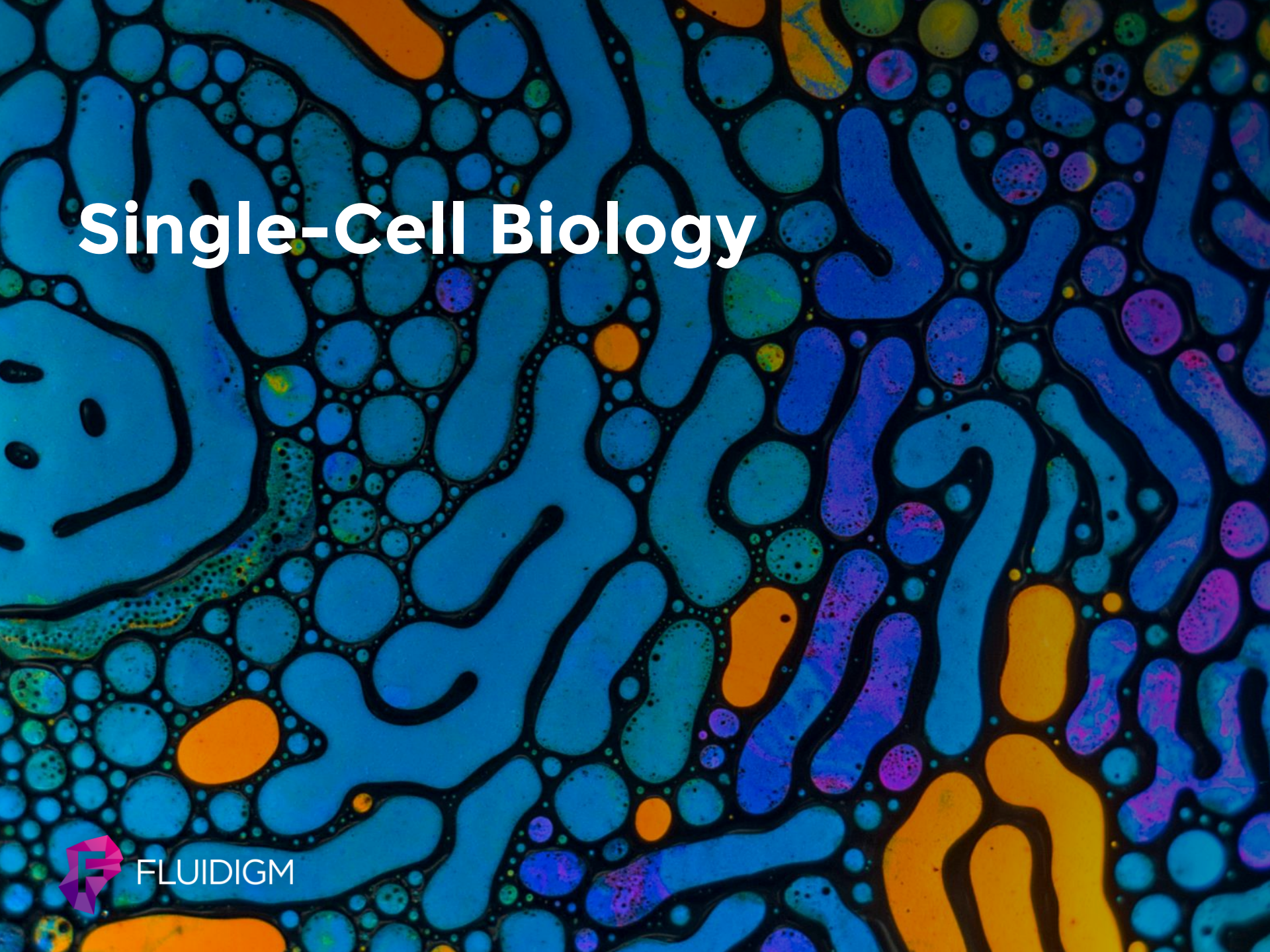
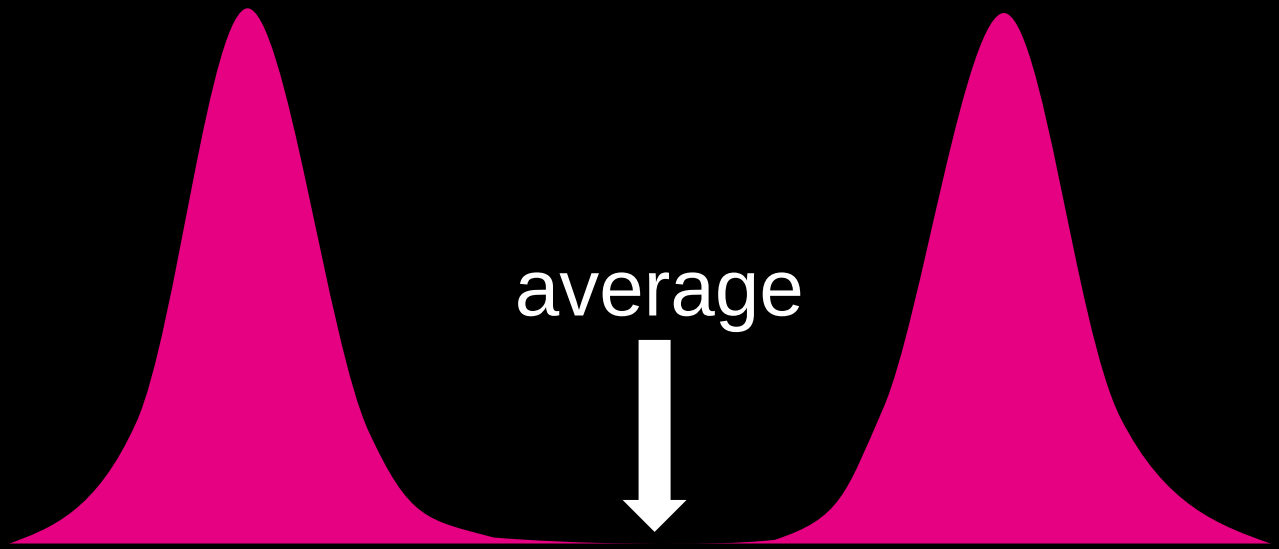
Senior Field Applications Specialist

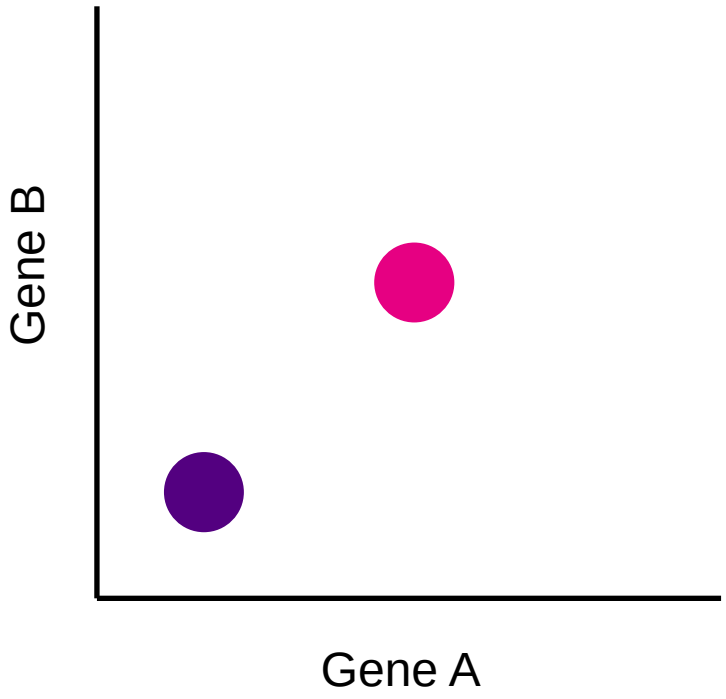
FLUIDIGM


Single-Cell Biology
FLUIDIGM

The population average is a lie

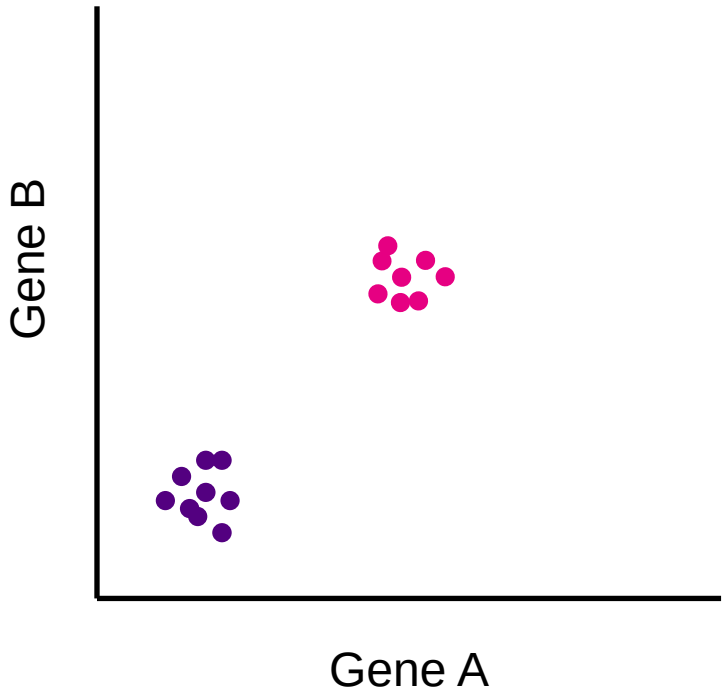


Bulk gene expression

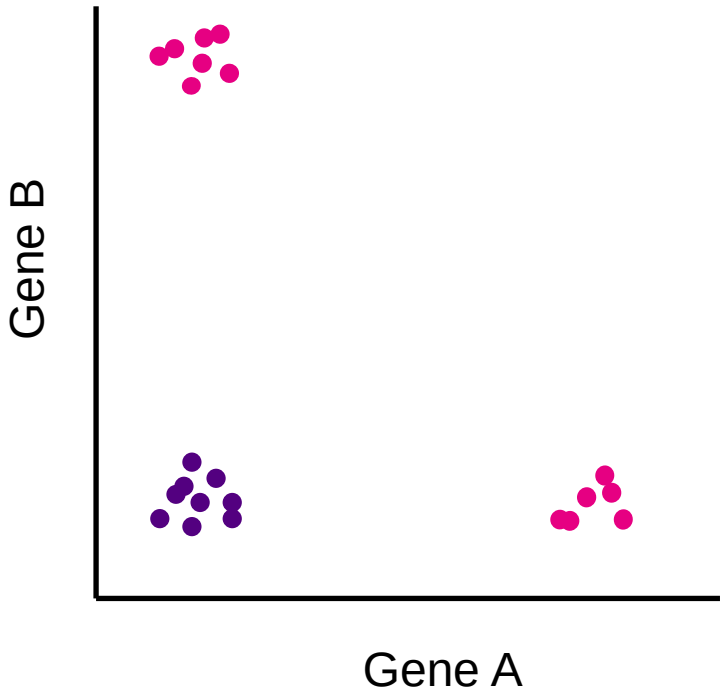


A, B fully correlated

Single-cell gene expression

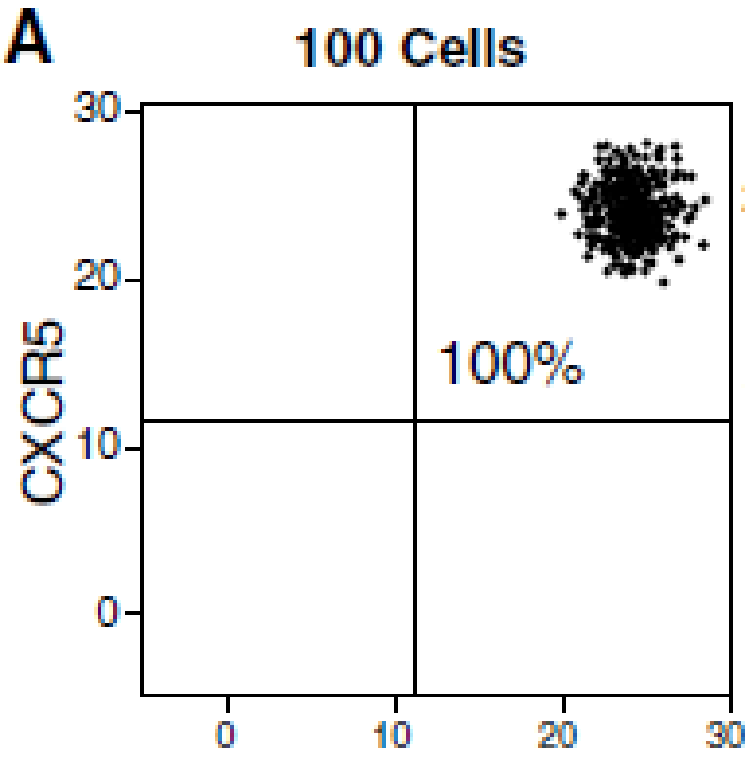


A, B fully correlated



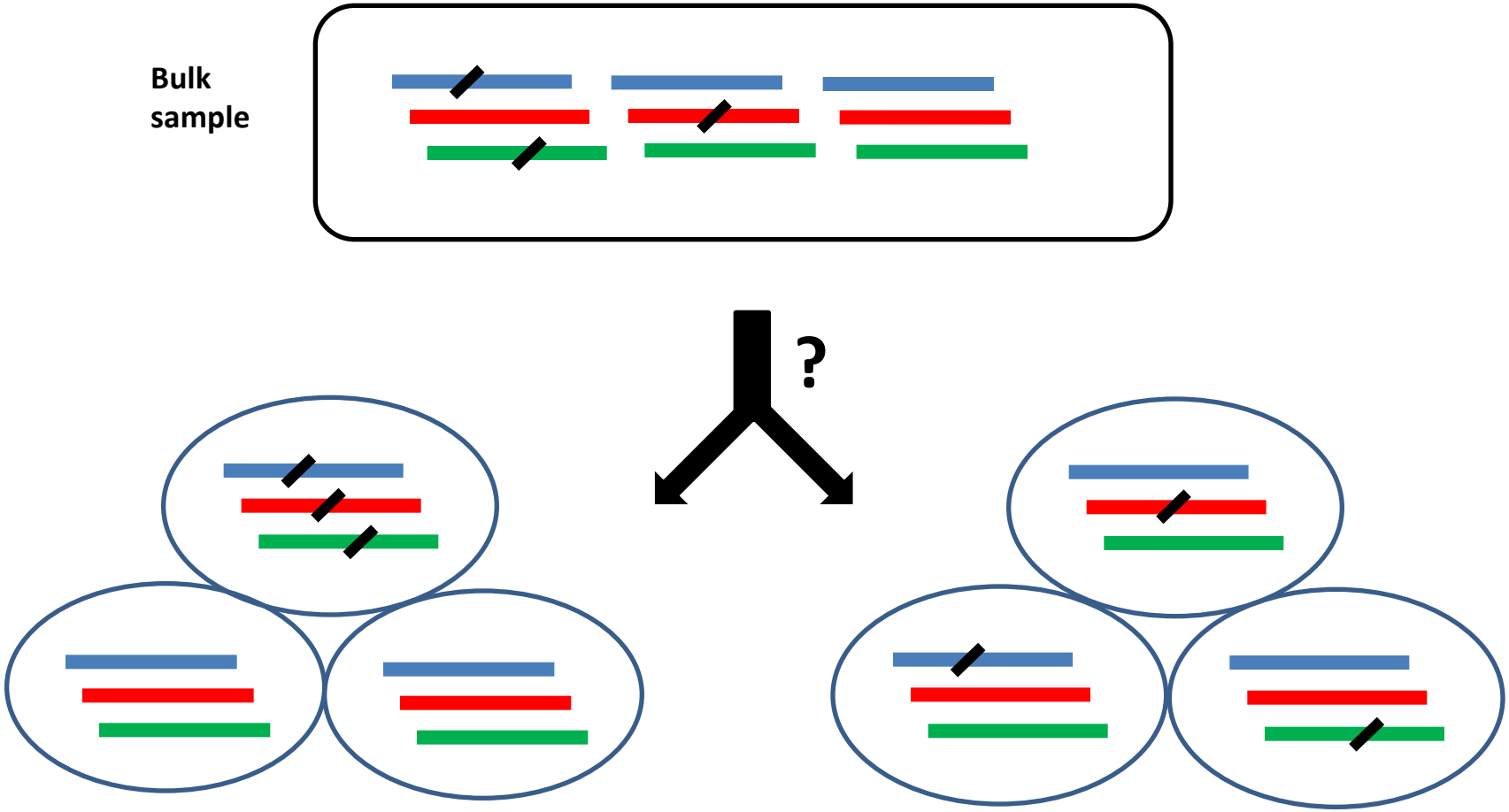
A, B ***anticorrelated***

Real world example: CD4+ T-cells



Dominguez et al. *J Immunological Methods* (2013)

The population average is a lie

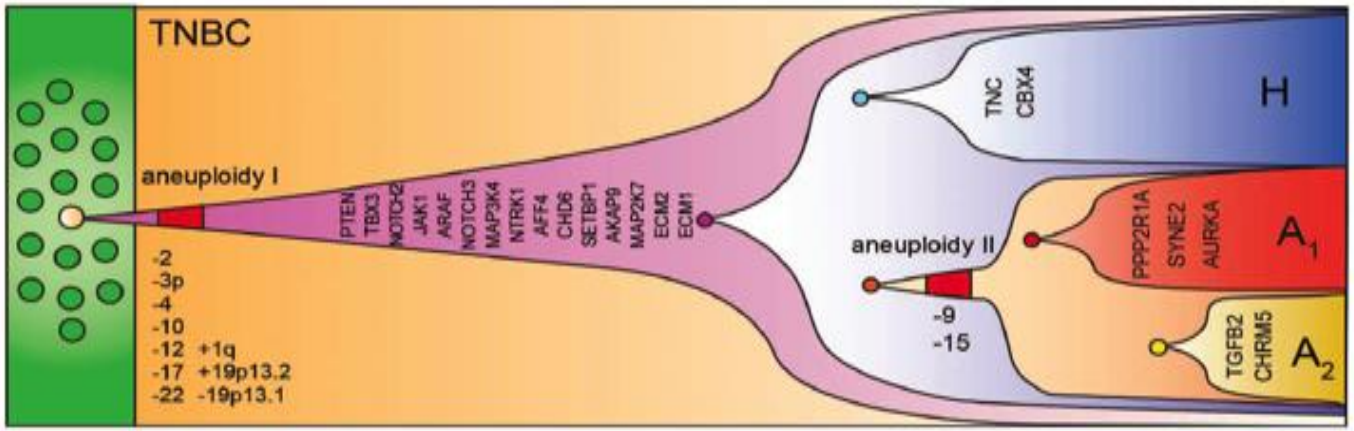


ARTICLE

doi:10.1038/nature13600

Clonal evolution in breast cancer revealed by single nucleus genome sequencing

Yong Wang[1], Jill Waters[1], Marco L. Leung[1,2], Anna Unruh[1], Whijae Roh[1], Xiuqing Shi[1], Ken Chen[3], Paul Scheet[2,4], Selina Vattathil[2,4], Han Liang[3], Asha Multani[1], Hong Zhang[5], Rui Zhao[6], Franziska Michor[6], Funda Meric-Bernstam[7] & Nicholas E. Navin[1,2,3]



Extended Data Figure 6 | Models of clonal evolution in breast cancer. **a**, Clonal evolution in the ERBC inferred from single cell exome and copy number data. **b**, Clonal evolution in the TNBC inferred from single cell exome and copy number data.

Wang et al. *Nature* (2014)

Polaris: Integrated Single-Cell Biology System

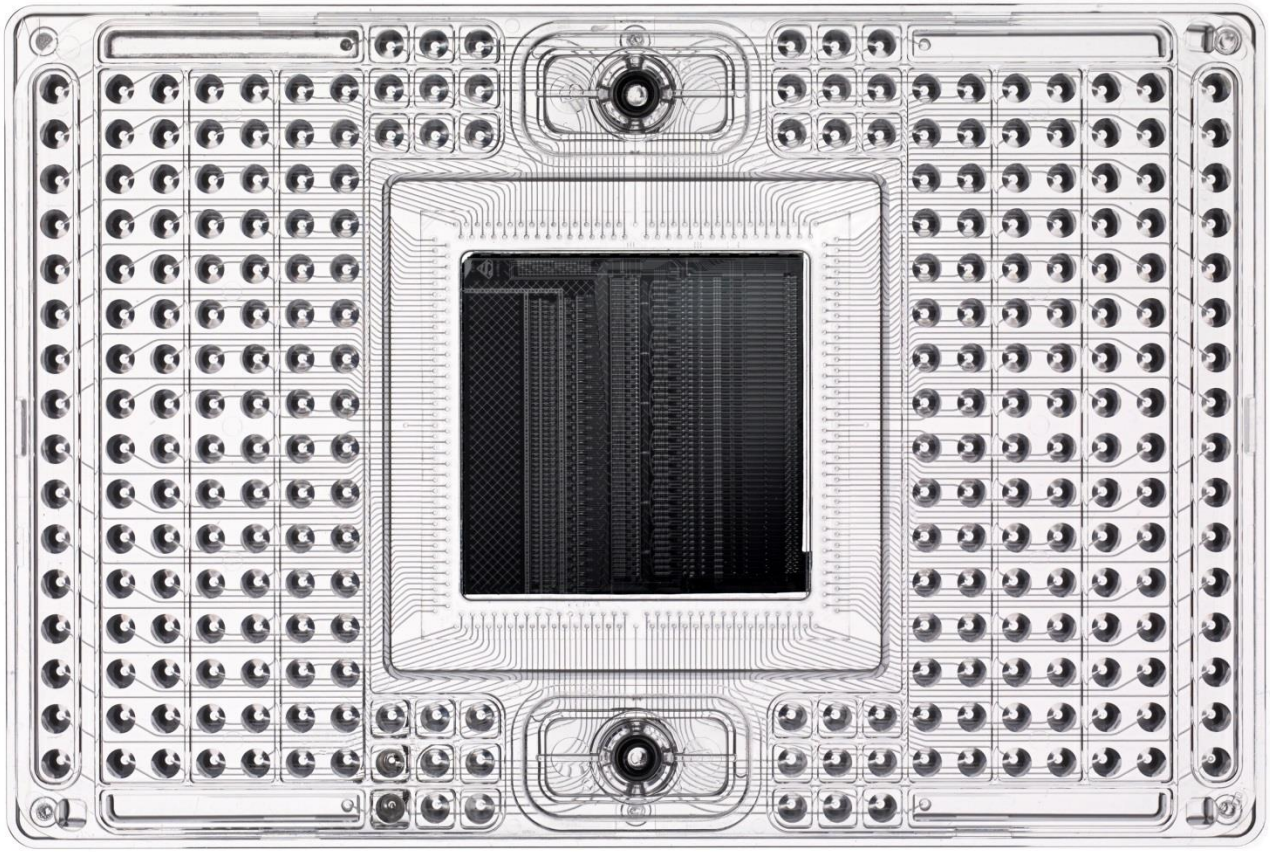
The ideal single-cell functional study

Select	Image	Simulate cell environment	Perturb	Measure
Obtain high purity of target cells	Correlate imaging data with expression	Re-create the cell's in vivo environment	Simulate normal and diseased states	Seamlessly determine expression levels

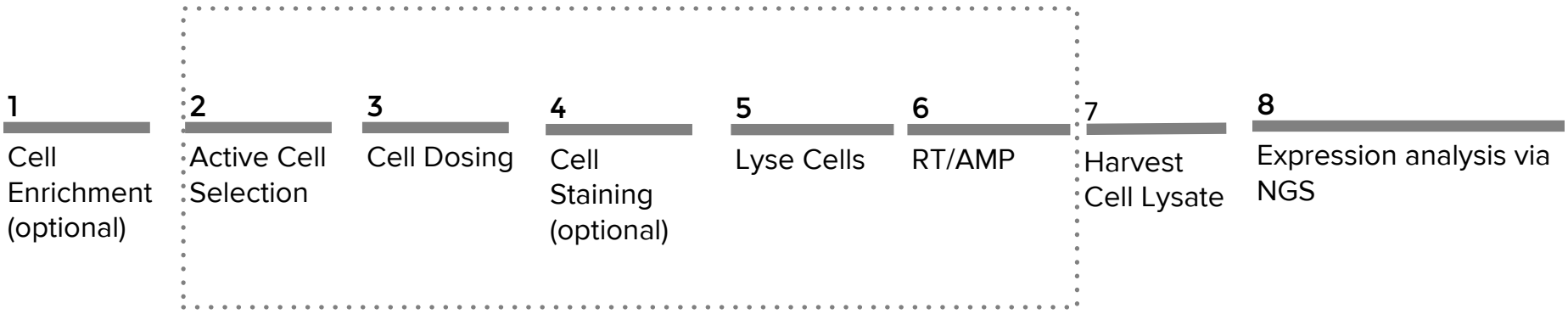
Polaris


Select an experiment to run.
Dose Response
6 Conditions/Concentrations
Sample A
Condition A
Condition B
Condition C
Condition D
Condition E
Condition F
Cell Population
FAM
VIC
1/30/2015 11:40:25 AM
10 hr 35 min
Time Course
6 Timepoints
Sample A
Condition A
Cell Population
FAM
1/30/2015 11:40:25 AM
10 hr 35 min
Culture Cells
Culture and feed with medium
Sample A
Feed
Cell Population
FAM
VIC
1/30/2015 11:40:25 AM
10 hr 35 min

Polaris IFC



The Polaris™ workflow

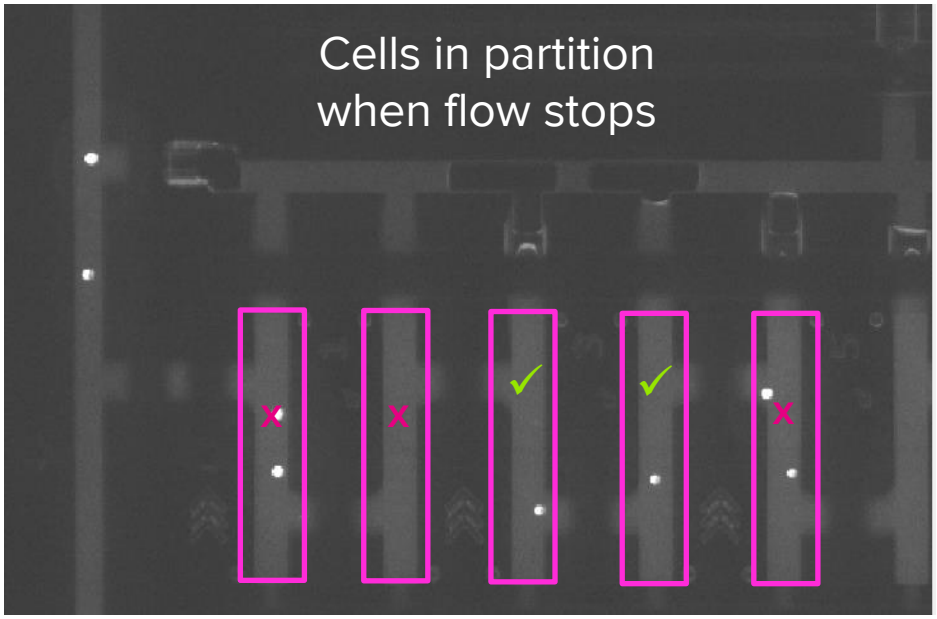


Dynamic selection of single cells

Select | Image | Simulate cell environment | Perturb | Measure

How it works:

- Cells are selected based on staining profile.
- Site detection algorithm run to qualify 48 cells of interest.
  - Stain intensity
  - Single vs. double vs. empty
- Only cells of interest are sent to unoccupied chambers for capture.



mRNA chemistry

Select | Image | Simulate cell environment | Perturb | Measure

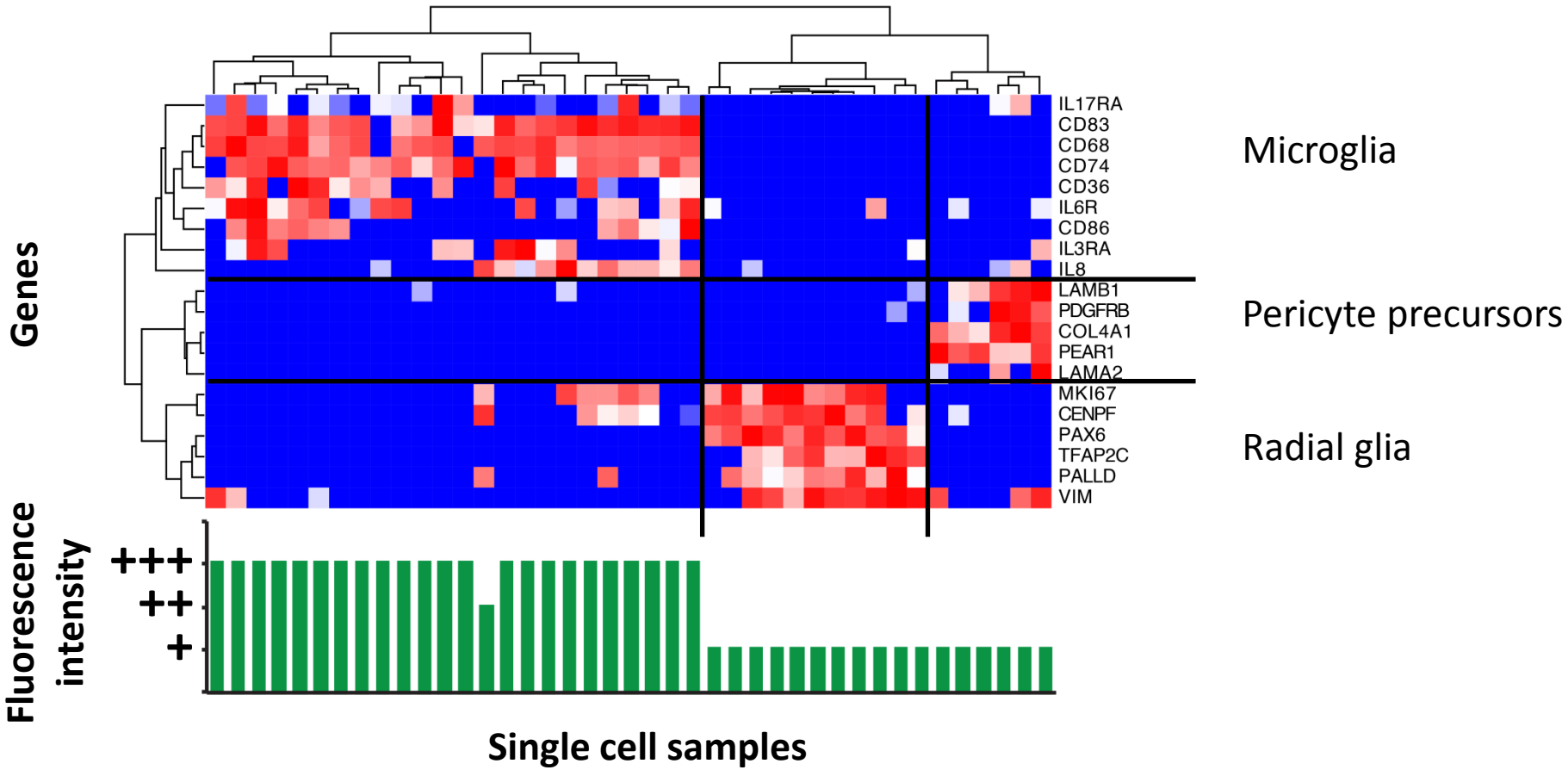
How it works:

- Same chip rxn architecture as first generation C1 IFCs
- Clontech® SMARTer® Kit
- Nextera® XT DNA Sample Preparation Kit
- Identical chemistry to current mRNA-Seq protocol

Why SMARTer chemistry?

- Read through alternative splice sites.
- Identify novel isoforms.
- Detect low-abundance transcripts.

UCSF collaboration: Primary human neurons on Polaris



Example applications on Polaris

Select different types of neurons; perturb a molecular pathway; measure the consequences in gene expression.

Observe the time-course of T cell response after HIV infection.

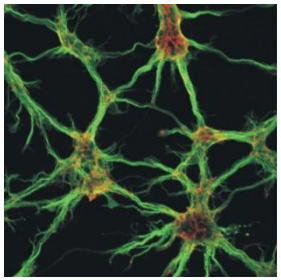
Explore the heterogeneity of cellular responses to inflammatory signals (e.g. dendritic cell response to LPS).

The Polaris System

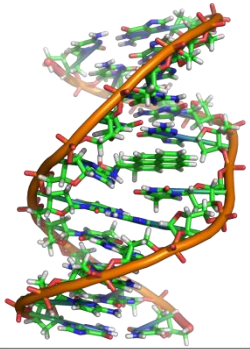
Seamlessly integrates cell biology with molecular analysis

- Actively **select** and isolate targeted cells.
- **Image** cells to ensure phenotype and cell viability.
- **Maintain** and feed single cells on IFC.
- **Perturb** cells with a wide range of factors, including RNAs, transcription factors, bacteria, small molecules and more.
- **Prepare** individual cells for mRNA sequencing.

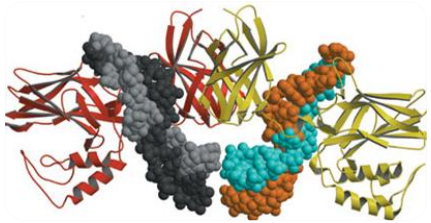
We are enabling comprehensive analysis at the single-cell level.



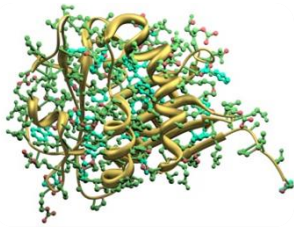
Cell



RNA & DNA

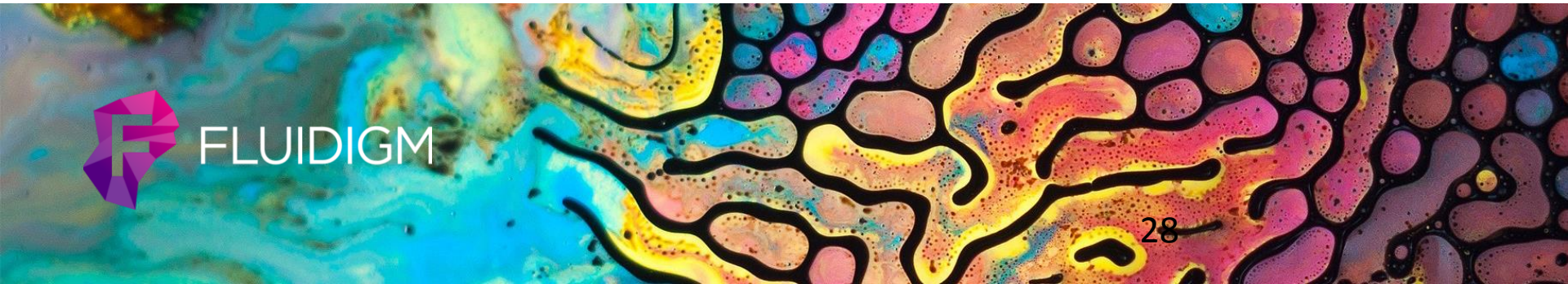


Gene regulation



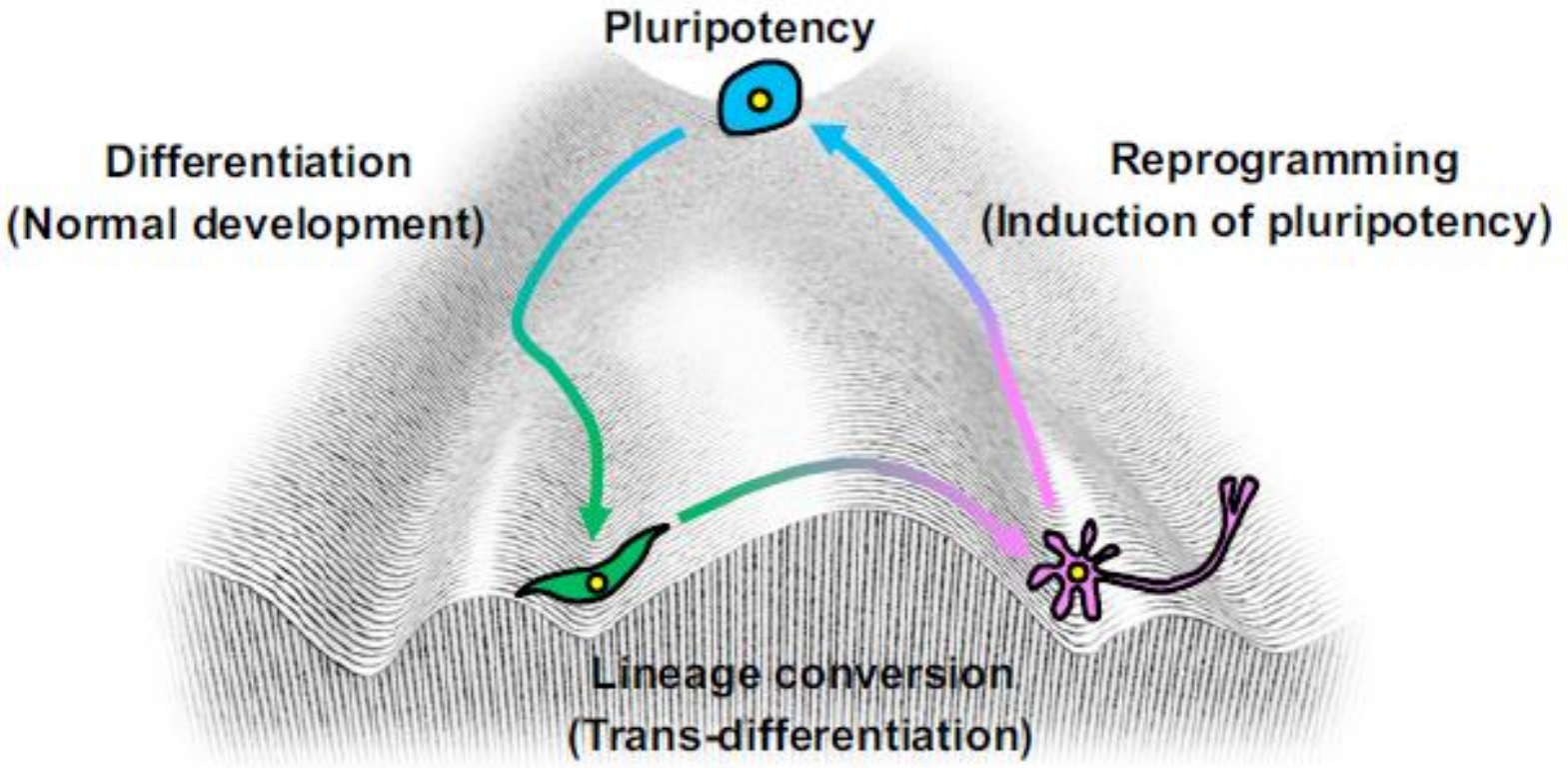
Protein

A Microfluidic Combinatorial Approach to Cell Differentiation and Reprogramming



Callisto™: Automated, combinatorial cell culture system

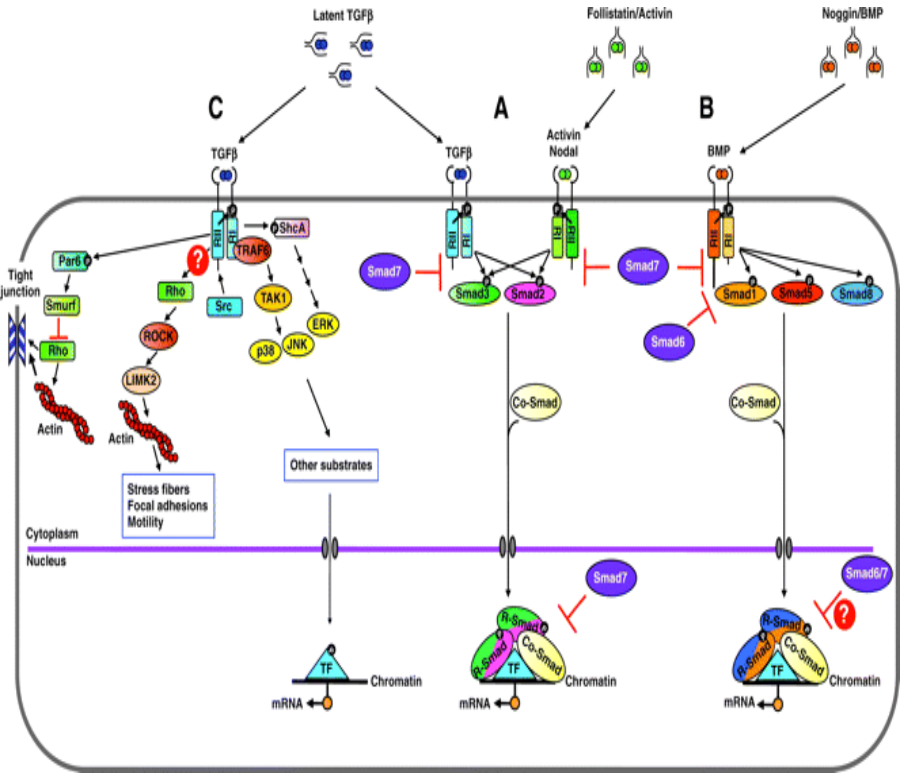
Cell Differentiation and Reprogramming



Cell fate changes on Waddington's epigenetic landscape.

Takahashi and Yamanaka, Development 2013, 140, 2457-2461.
Induced pluripotent stem cells in medicine and biology.

Many Factors Contribute to Cell Fates and Functions



Moustakas and Heldin , Development 2009;136:3699-3714. *The regulation of TGFβ signal transduction.*

Basic research

- Multiple factors contribute to every cellular process
- Cellular signaling is dynamic

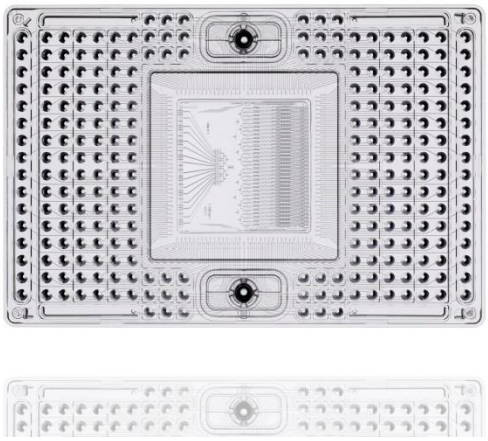
Cell-based therapy

- Efficiency and Safety
  - Chemically–defined conditions
  - Non-integrating methods

Combinatorial studies are important!

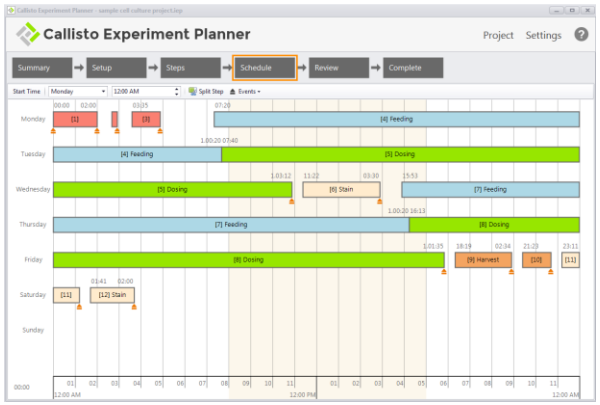
What is the Callisto™ System?

Callisto is an integrated microfluidic platform for automated cell culture and combinatorial dosing of cells



IFC

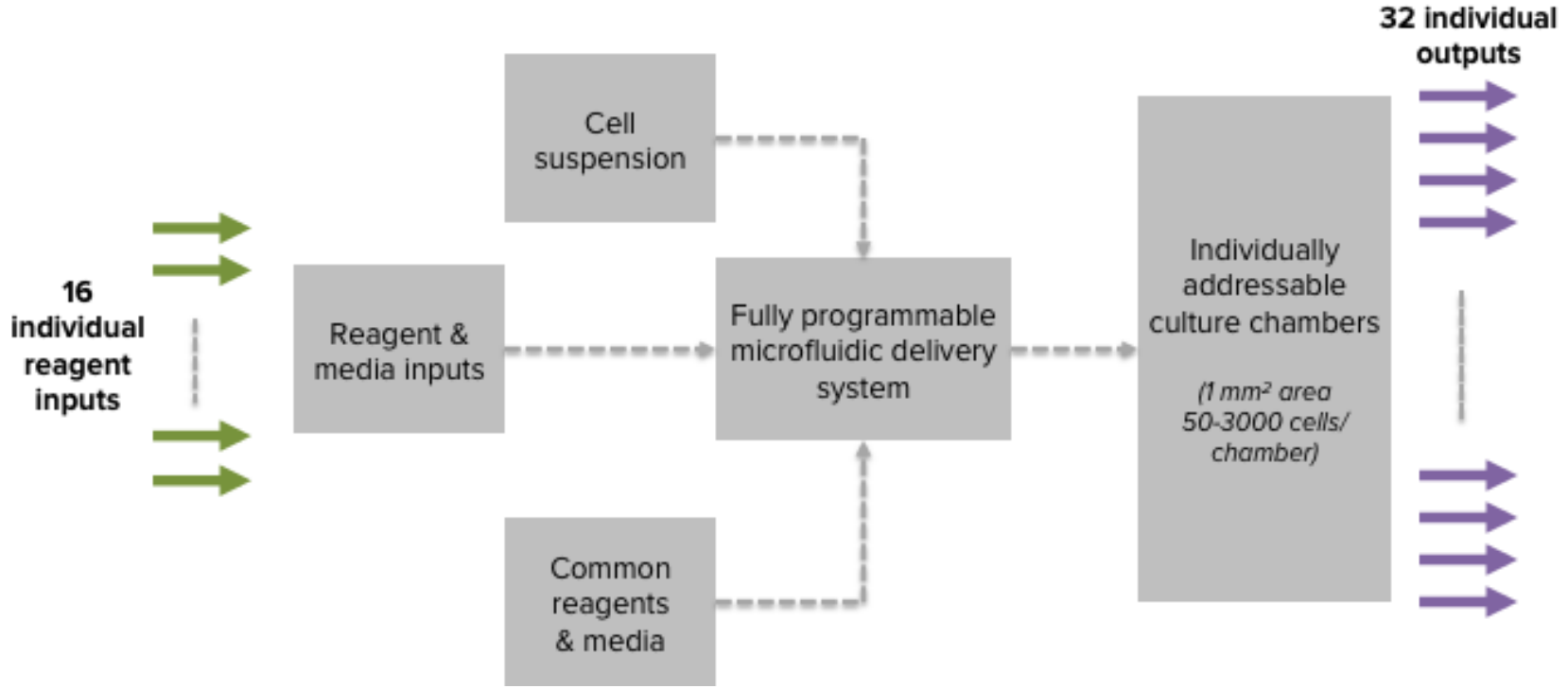
Instrument

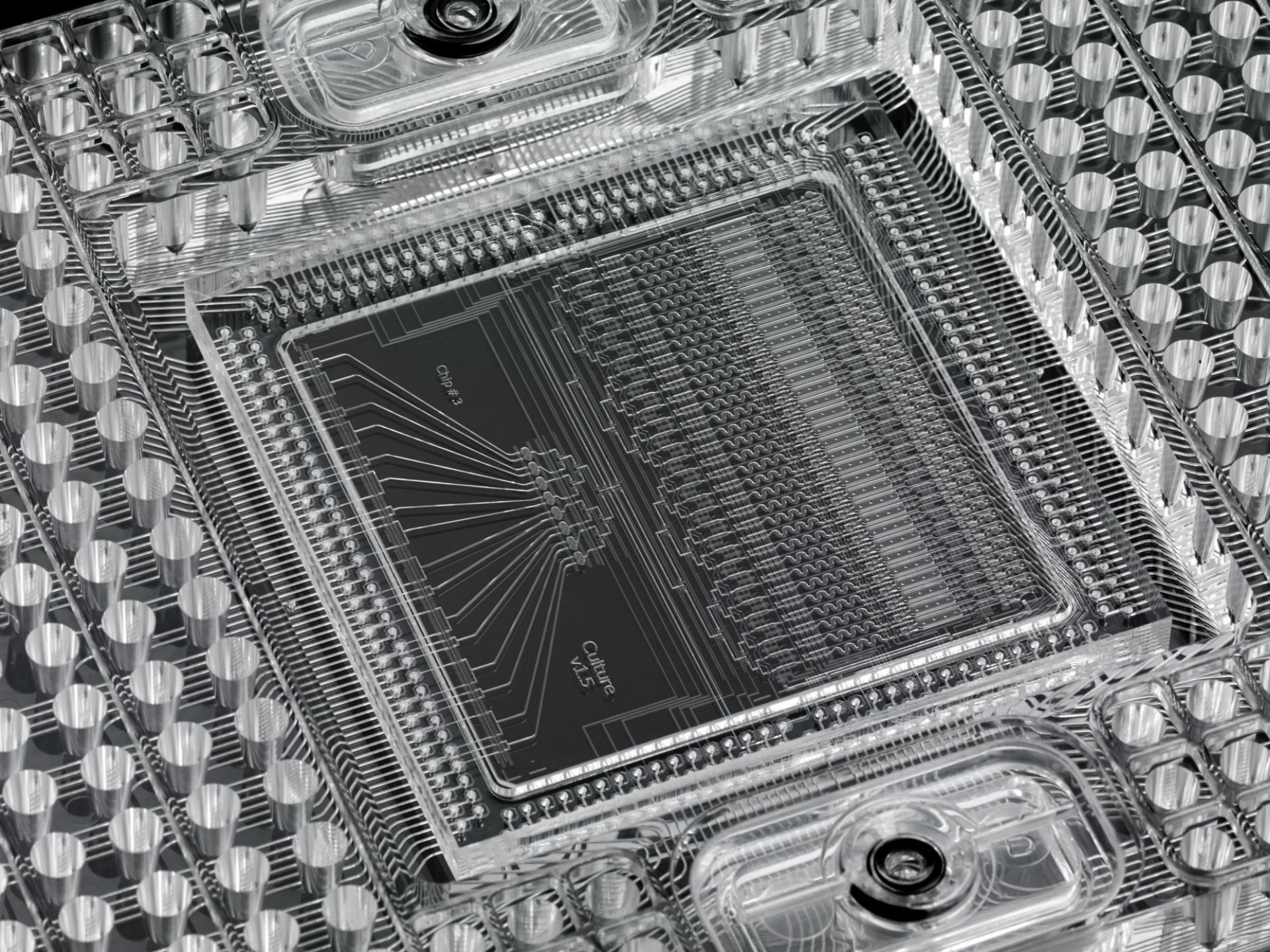


Software

What does it do?

- Enables combinatorial dosing of cells in 32 culture chambers with user- defined combinations of up to 16 separate reagents
- Supports long-term culture up to 3-4 weeks
- Compatible with downstream bulk or single-cell analysis
- Compatible with staining, imaging, gene expression and protein expression




Chip #3
Cultivate
v1.5

Unprecedented Capabilities

Each of 32 chambers can have different conditions

For example:

Chamber	Condition
1	A
2	B
3	C
4	A+B
5	A+C
6	B+C
7	A(t=0), B(t=2hr)
8	A(t=0), B(t=2hr), C(t=4hr)
9	…
⋮	⋮

A, B, C … H =

- Virus
- Proteins
- miRNA
- mRNA
- DNA
- Small molecules

Combinations can be programmatically varied over time

General Workflow

Pretreat IFC

Seed cells

Feed Cells

Dose

Dissociate

Lyse

Fix

Stain

Harvest

Cell Culture on Callisto

Single-Cell Genomics with the C1 System

Barcode

Bulk Gene Expression on Biomark HD

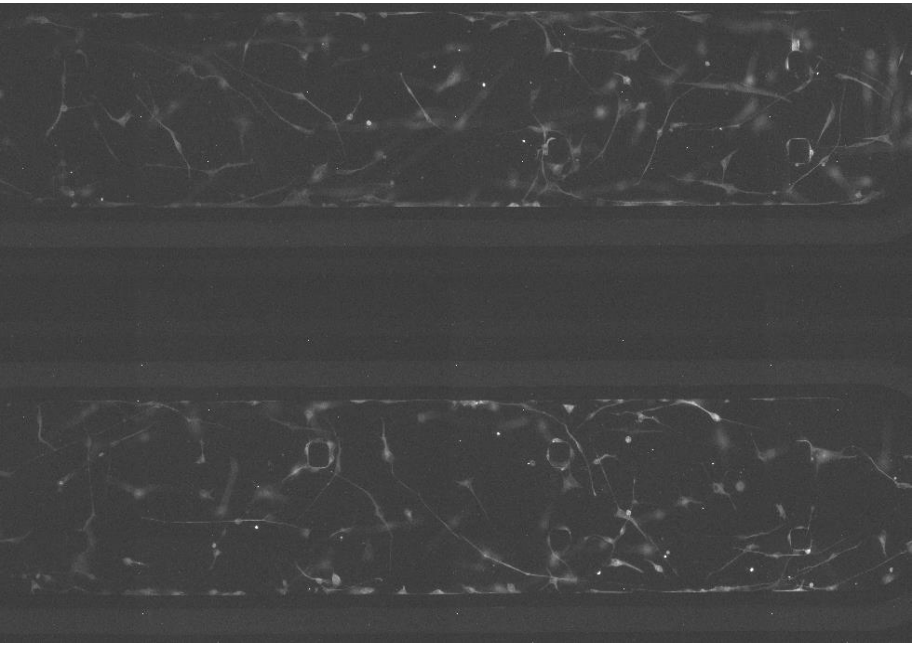
DNA or RNA analysis with Biomark or NGS

Single-Cell Protein Detection on CyTOF

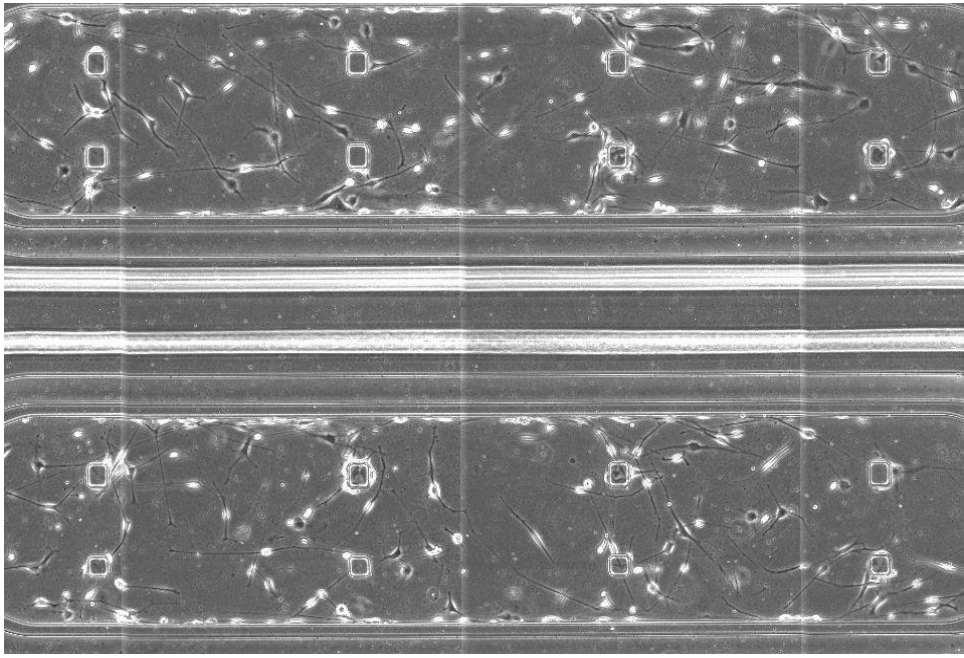
Imaging by Microscopy

Plate and Expand

Long-Term Cell Culture on IFC

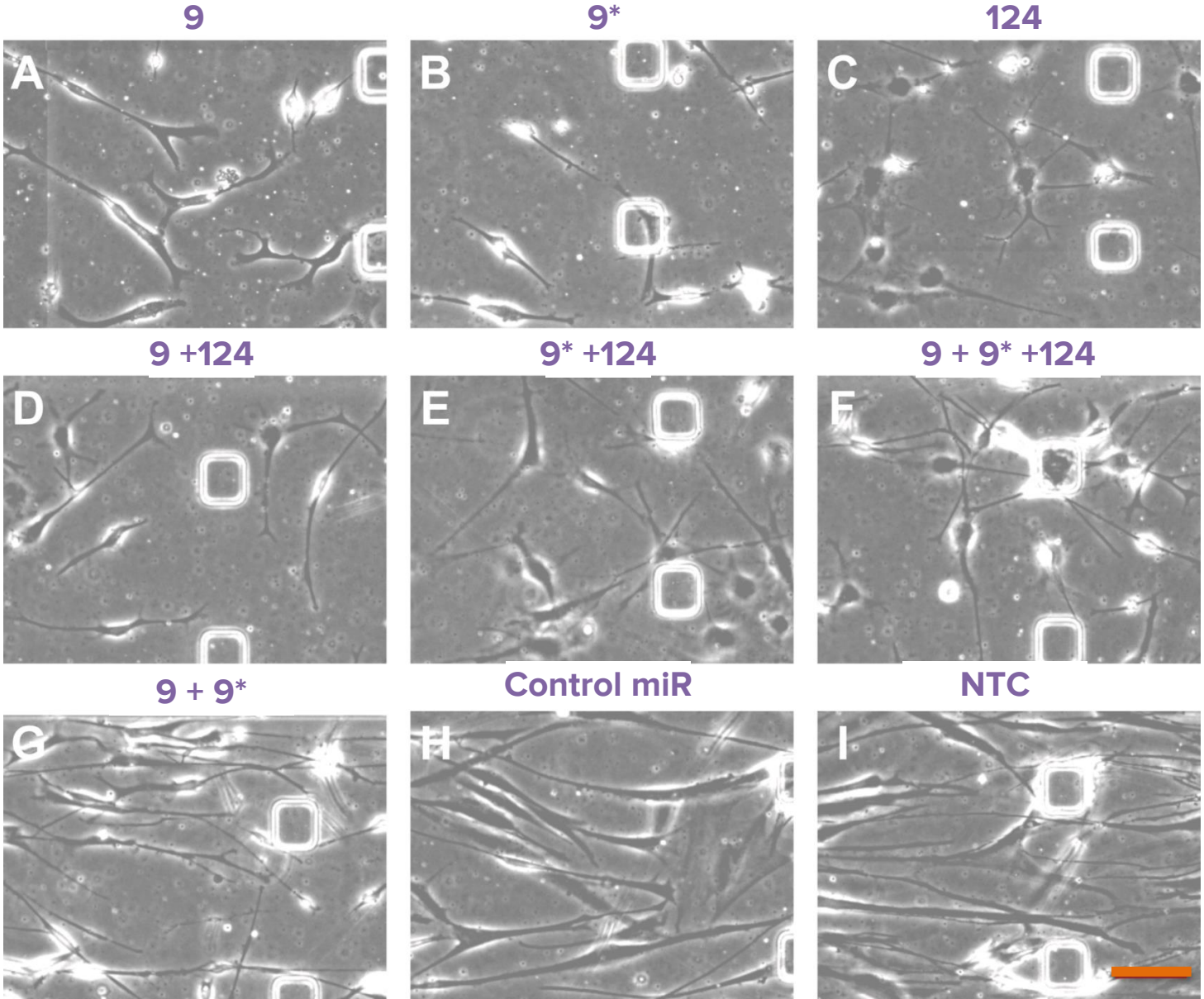


Fluorescence immunostaining



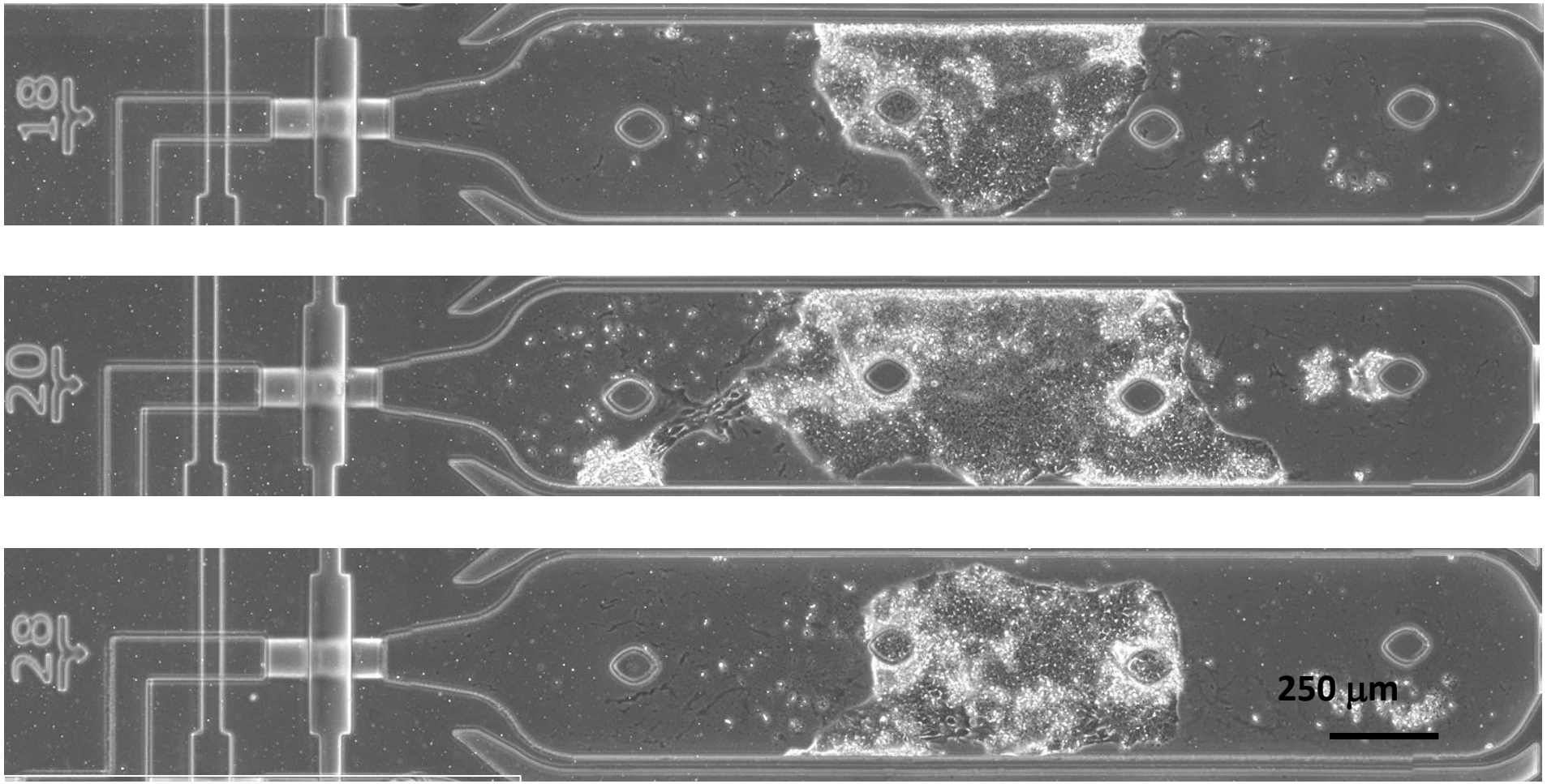
Phase Contrast

Transdifferentiation: Combinatorial Dosing of Three miRNAs Converts Fibroblasts to Neurons



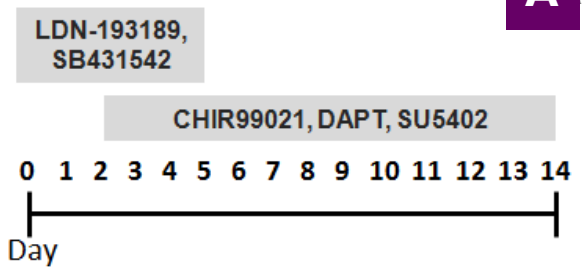
Human iPSC Growth and Live Staining

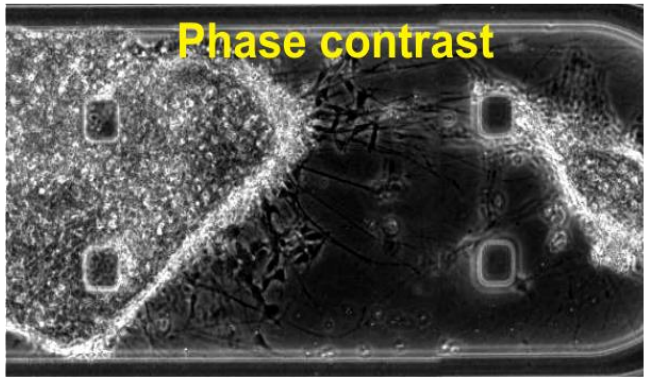
Phase contrast

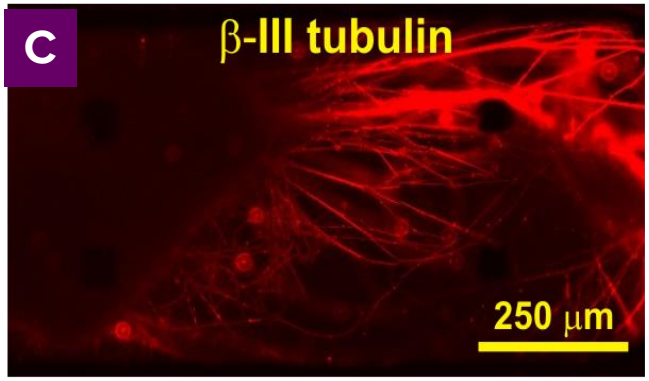


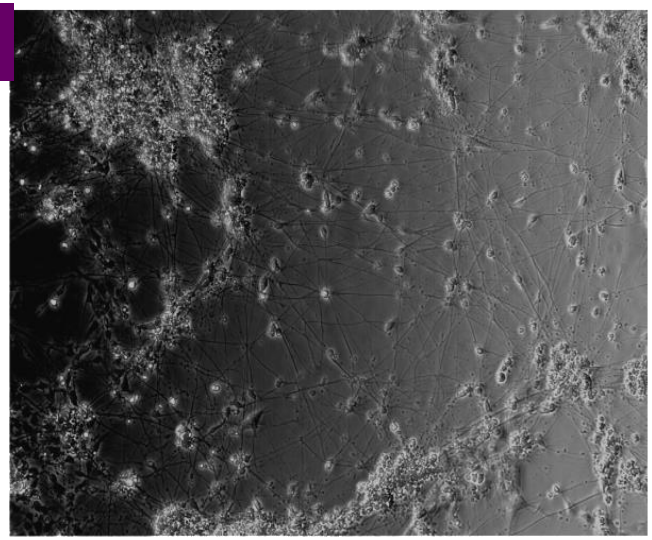
Day 5 on Matrigel in E8 media.

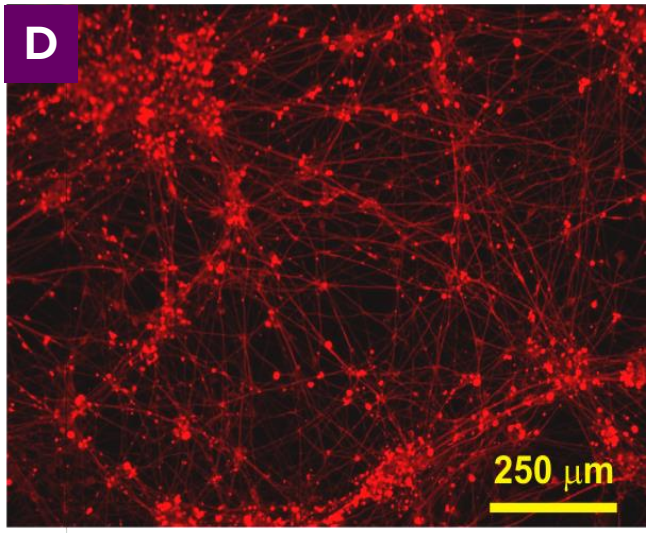
Neural differentiation: hiPSCs to Nociceptor Neurons





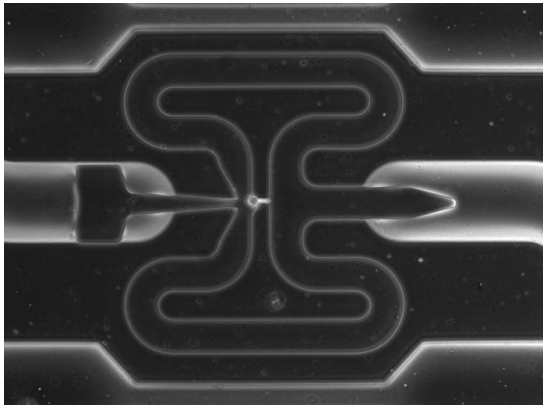






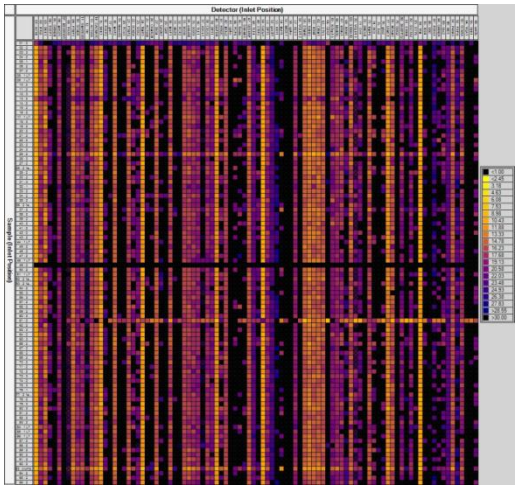
Coupling Cell Culture IFC with Fluidigm C1 Single-Cell Analysis Workflow

A



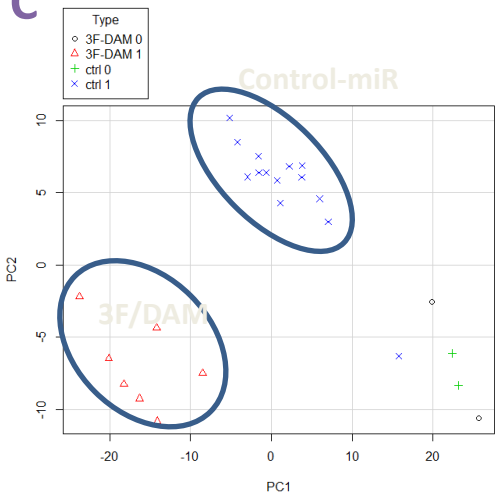
C_1^{TM} capture site

B



Heat map

C



PCA analysis

Conclusion

- We have demonstrated long-term culturing of human fibroblasts and iPSCs on chip and automated dosing of cells with combinations of miRNAs, mRNAs, and small molecules at predefined various times.

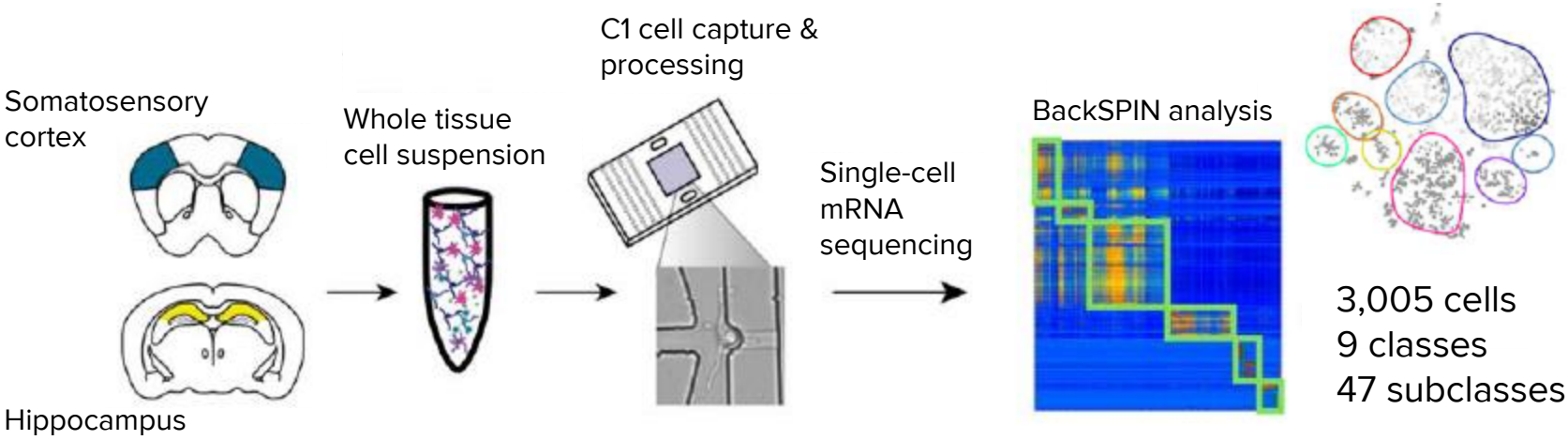
- Using combinatorial dosing, we were able to directly convert fibroblasts to neurons or differentiate hiPSCs to different lineages in chemically defined conditions on chip and in wells.

- Cells can be characterized by immunostaining or genomic analysis after exporting live single cells or cell lysate from the culture chip.

- The system may be used for studying and screening different conditions for cell proliferation, differentiation, and reprogramming.

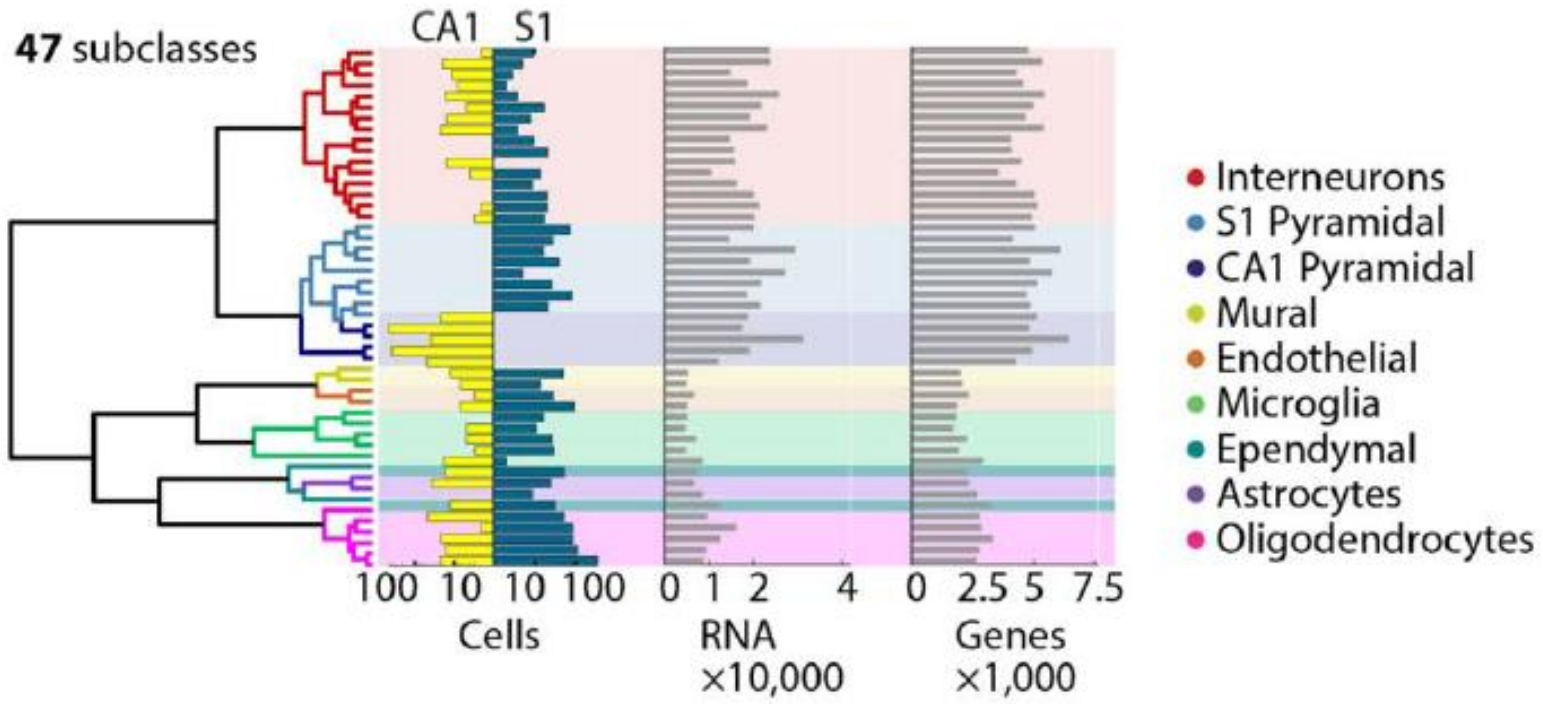
Increasing the throughput of the C1 system for single-cell mRNA sequencing

Large scale single-cell mRNA sequencing to classify cells



Modified from Zeisel et al. *Science*. (2015)

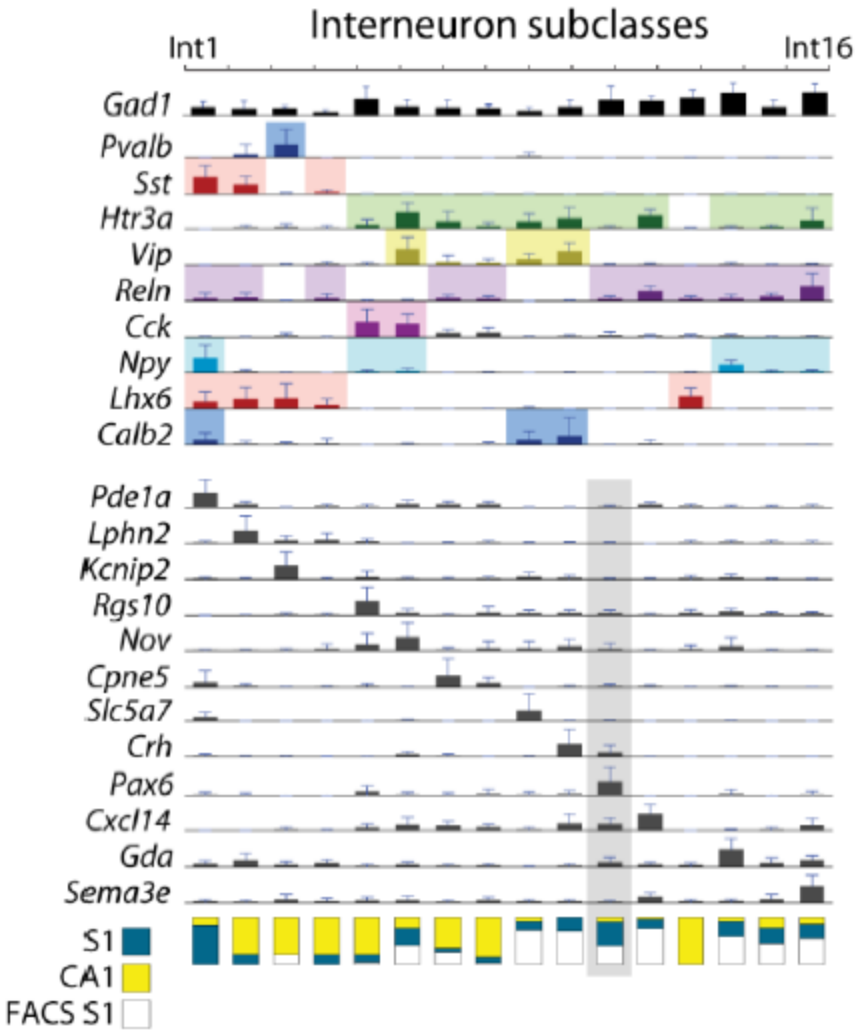
Cell types within subclasses can only be revealed by single-cell mRNA sequencing



Zeisel et al. *Science*. (2015)

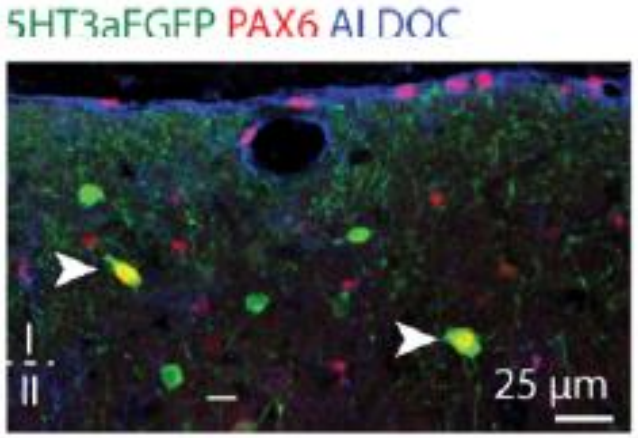
Single-cell resolution allows one to observe new cell types and molecularly distinct subclasses

Identification of interneuron classes



Zeisel et al. *Science.* (2015)

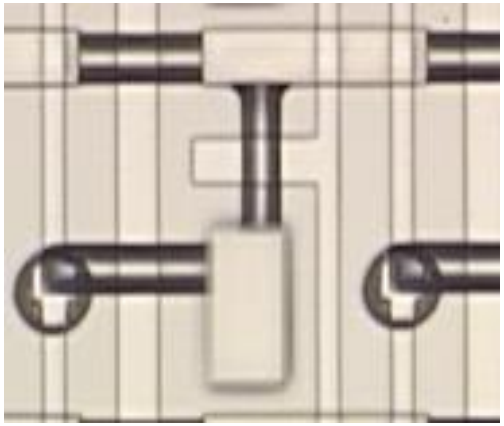
Interneurons residing in functionally distinct cortical structures are transcriptionally closely related.



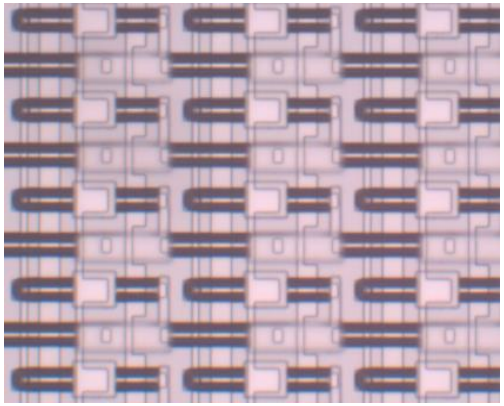
Immunohistochemistry demonstrating the existence and localization of novel PAX6+/5HT3aEGFP+ interneurons, Int11

Evolution of microfluidics

IFC for real-time PCR

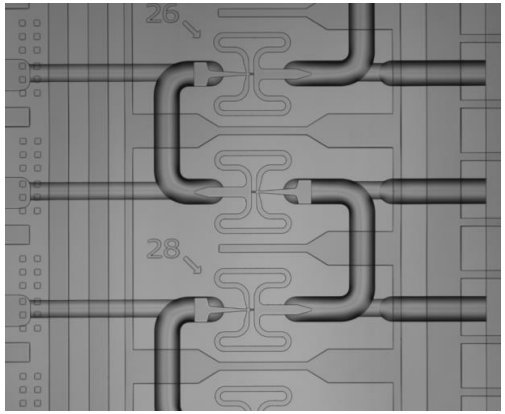


48.48 Dynamic Array

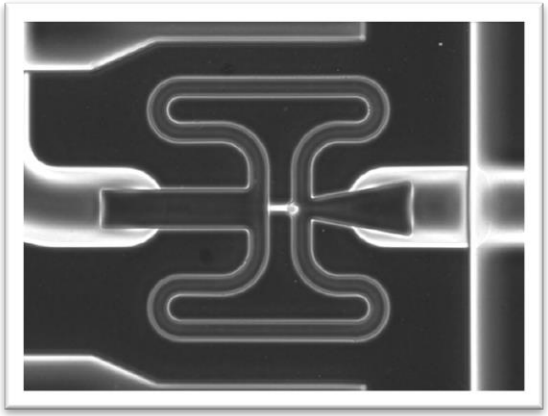


96.96 Dynamic Array

C1 (96) IFC

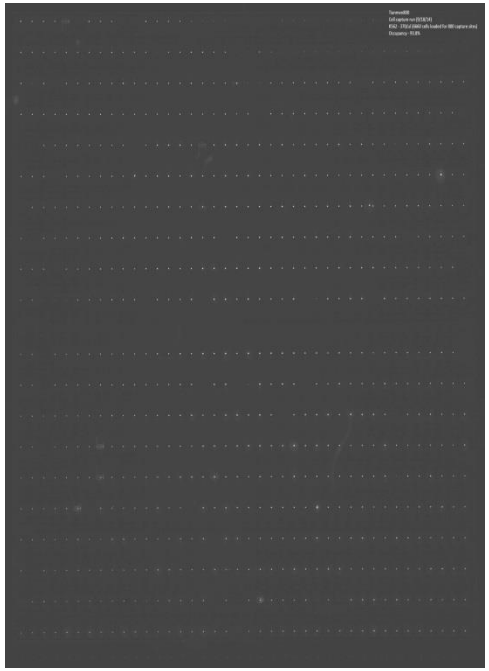


Capture sites 26, 27, and 28



Magnified capture site

C1 (800 cell) IFC



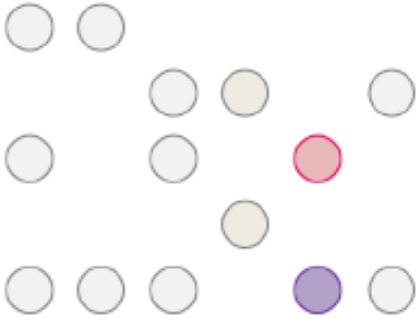
Eight hundred capture sites per IFC

Consider processing 4000 cells for mRNA sequencing

Ninety-six cell mRNA sequencing IFC	High-throughput mRNA sequencing IFC
• Forty-two C1 (96) mRNA sequencing IFCs	• Five C1 (800) mRNA sequencing IFCs
• Forty-two days of full-time cell-processing (with one C1 system)	• Two-and-a half days of full-time processing

The need for large number of cells

Rare cells are difficult to sample



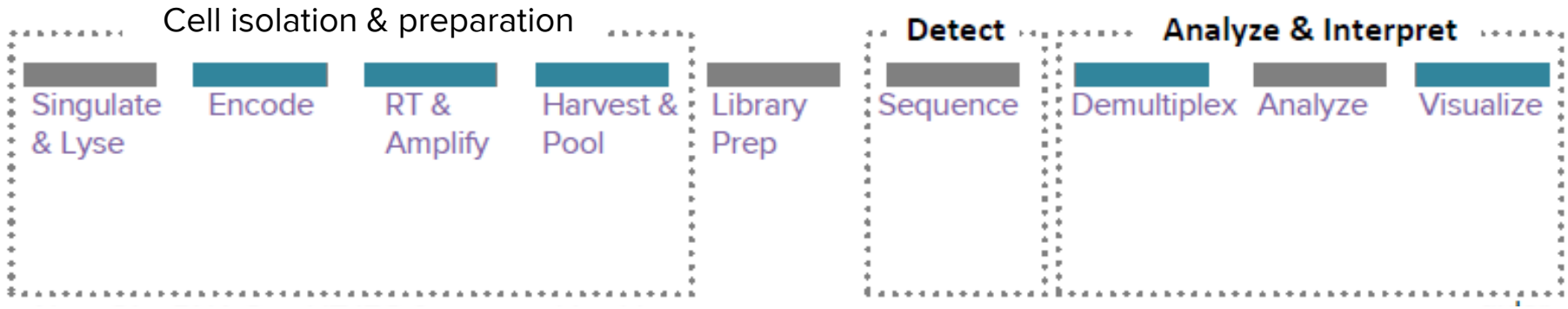
Stochaisticity creates noise, making it difficult to determine signatures

One thousand cells is the minimum number of cells required to provide the necessary power to robustly detect rare cell populations and states. (Jaitin et al. *Seminars in Immunology* (2015)).

High throughput mRNA sequencing C1 IFC

Product solution	Description
C1 mRNA sequencing high throughput IFC	800 capture sites per IFC
High throughput mRNA sequencing C1 module kits	To enable high density sample pooling
C1 system scripts	New scripts with new capture and thermal profiles. Enables two IFC processing per day
Demultiplexer software	On chip cell barcoding
Singular™ version 4.0 software	To allow for incorporation of barcodes and faster data processing
Flexible mRNA sequencing methods	Molecule 3' counting Template-switch Multiplexing

Workflow: high throughput mRNA sequencing IFC



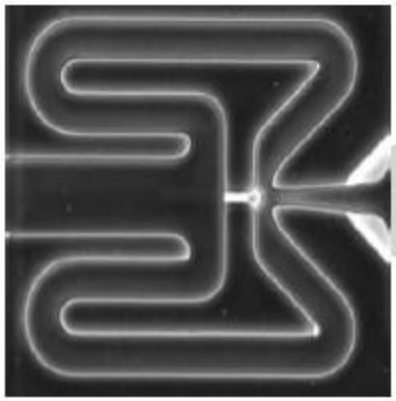
C1 system with high throughput IFC

Singular version 4.0

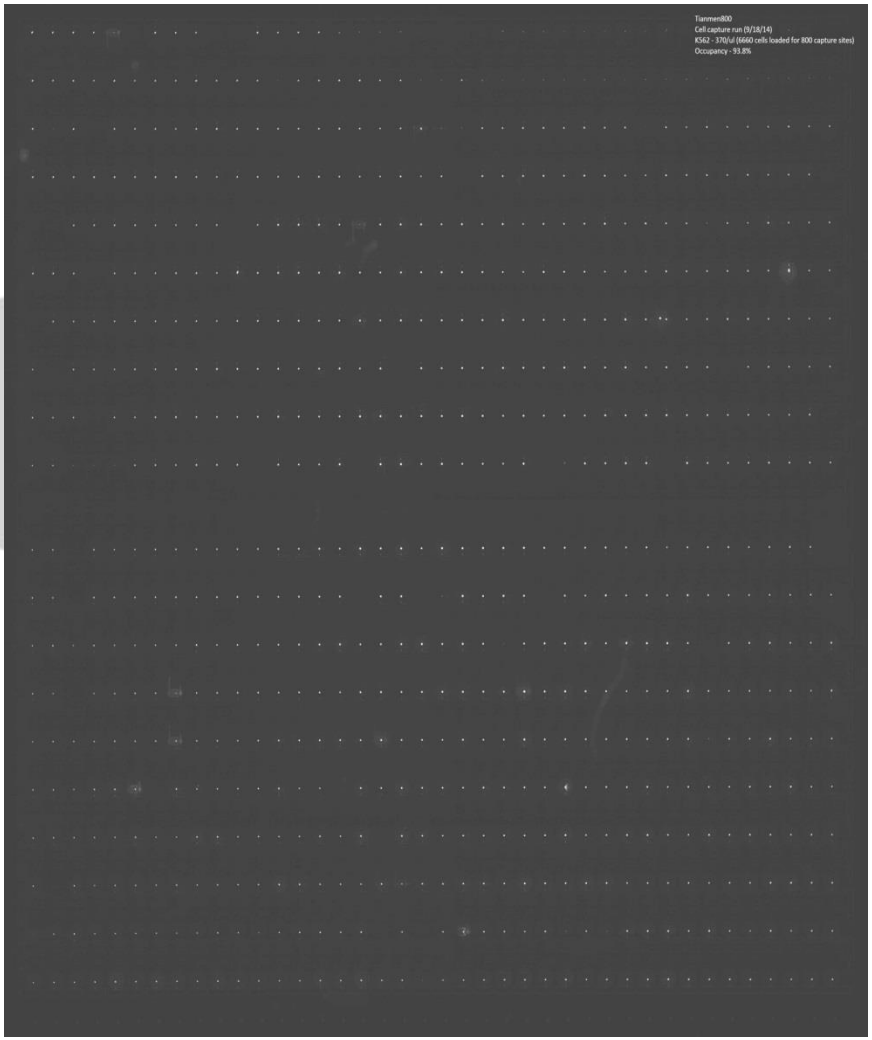
New workflow, chemistry and scripts allow researchers to process two high throughput IFCs per day.

Throughput becomes 1600 cells per day verses 96 cells per day.

Cell capture

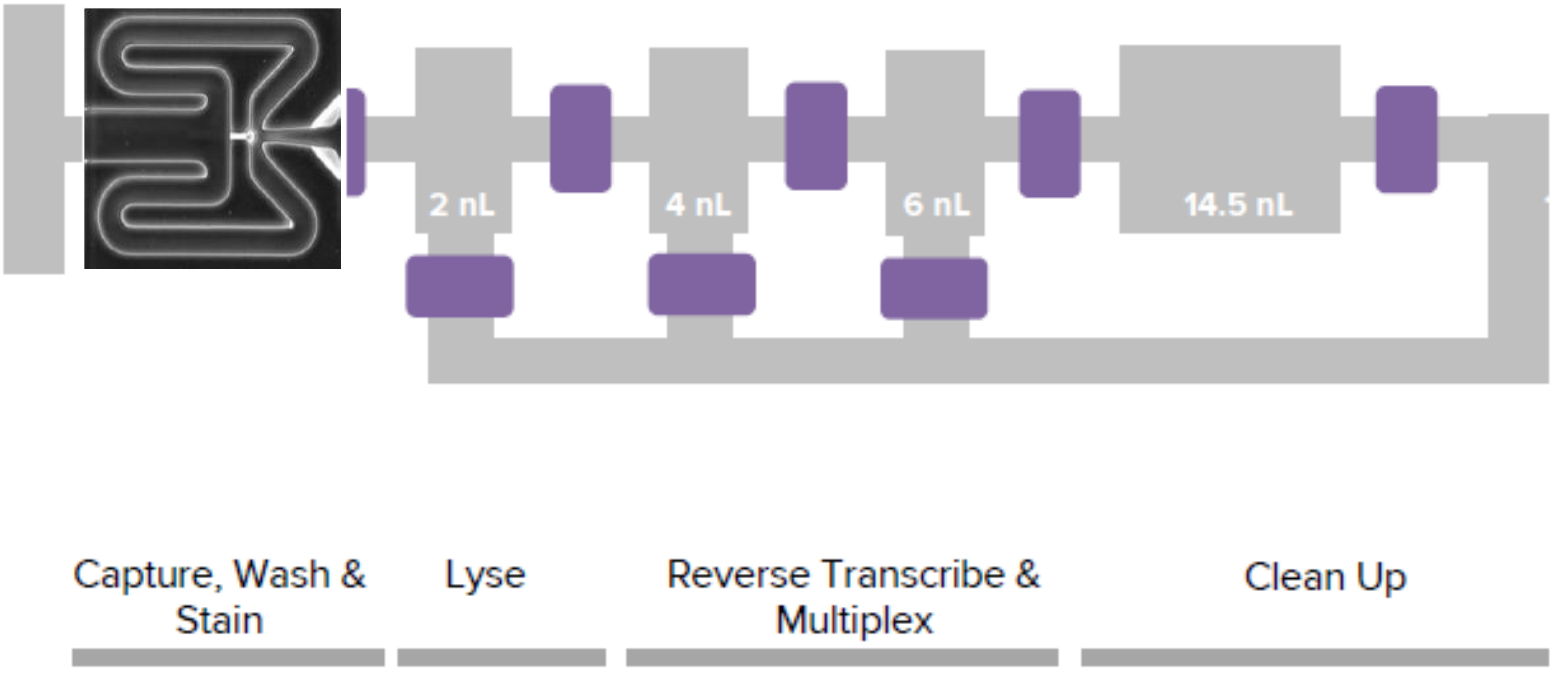


Redesigned capture site



Micrograph of over 700 captured cells using fluorescence

Cell processing



Simplify the complex quest to understand and apply biology.