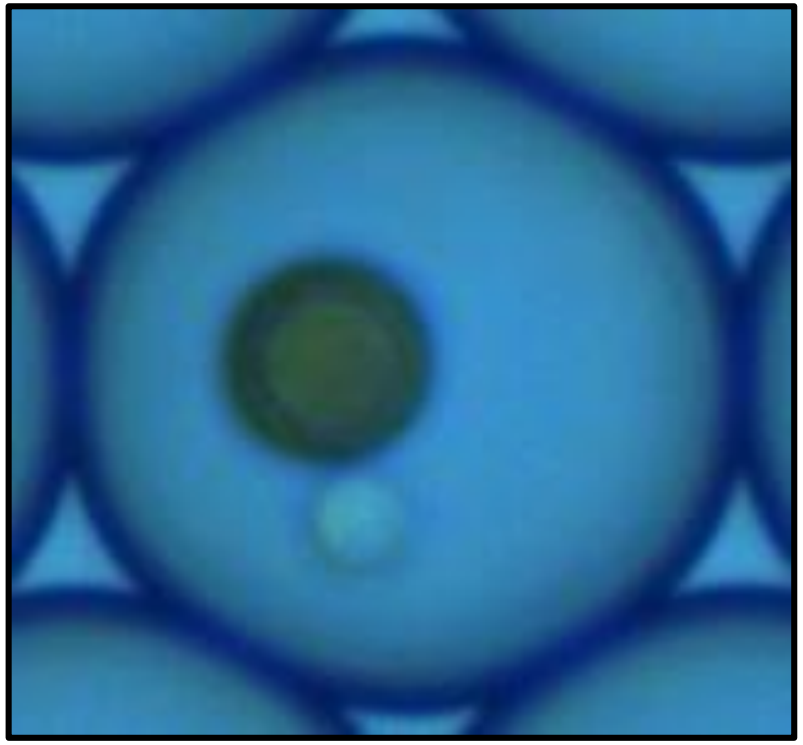
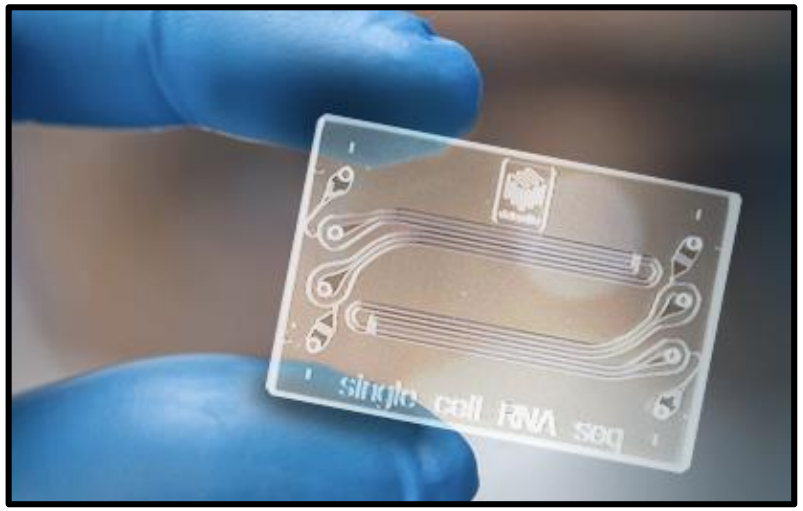




Microfluidics in biomedicine (single cell trapping) 3.3.2021

(Microfluidics and BioMEMS course)

Päivi Saavalainen, PhD, docent

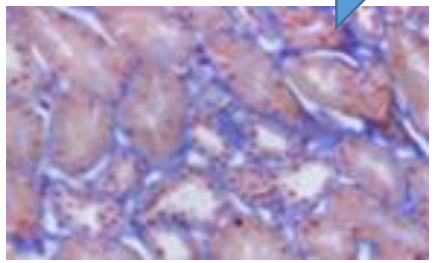
PI, Immunomics group, University of Helsinki

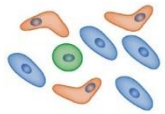
Single cell transcriptomics (scRNAseq)

Cells are not identical – Instead of **average** gene expression of millions of cells you now can measure it from **1000's of single cells individually**

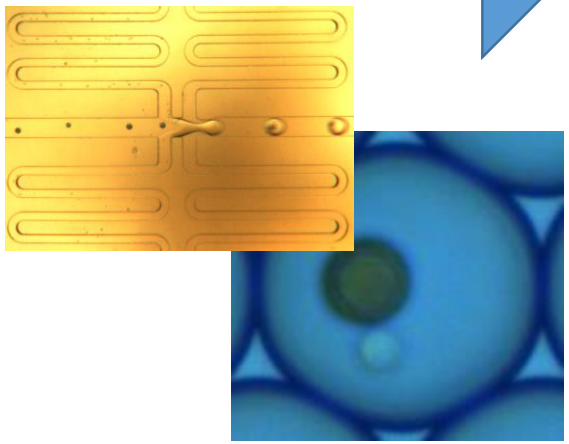
With single cell resolution:
-> Cell heterogeneity, rare cell types, cancer cells, drug responses, diagnostics, individualized therapies etc

CELL/TISSUE DISSOCIATION





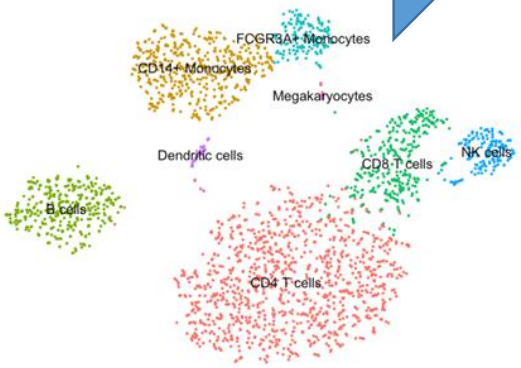
SINGLE CELL CAPTURE & TAGGING (BARCODING)



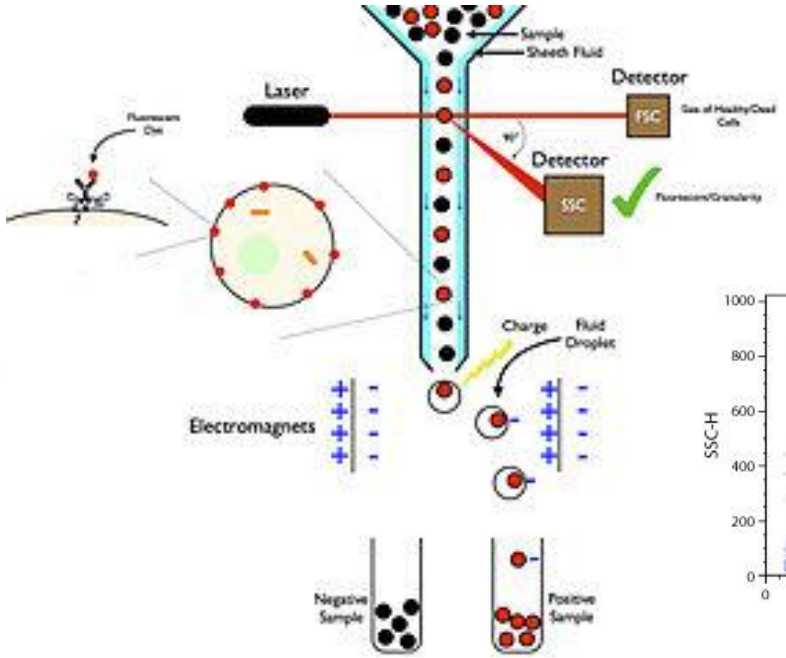
LIBRARY PREP & SEQUENCING

CellName	GTAAGC AGCGCG	CTTGGC AAGAAG	GCGAAT TCCCCT	GTAAGC AGTGGT	CGAGTC GGCCGT	GGTACT TGCTGG	GATTAC CGTGGG	ATTTAG GGAGCG	TCTACG AGTCAA
HBB	19480	18	2	243	4	0	0	0	36
HBA2	3163	4	0	33	1	0	0	0	4
HBA1	1248	3	0	19	0	0	0	0	3
MT-RNR2	481	350	205	1739	21	1523	457	285	841
BRSK1	200	0	0	1	0	0	0	0	0
UBB	119	14	0	5	0	0	0	0	1
RP11-138I1.4	98	12	0	0	0	0	0	0	0
GUK1	91	79	0	1	0	0	0	0	0
VTI1B	81	0	0	3	0	0	0	0	0

DATA ANALYSIS, CELL TYPE CLUSTERING



Traditional way to study and isolate single cells: Flow cytometry (FACS=fluorescense assisted cell sorting)



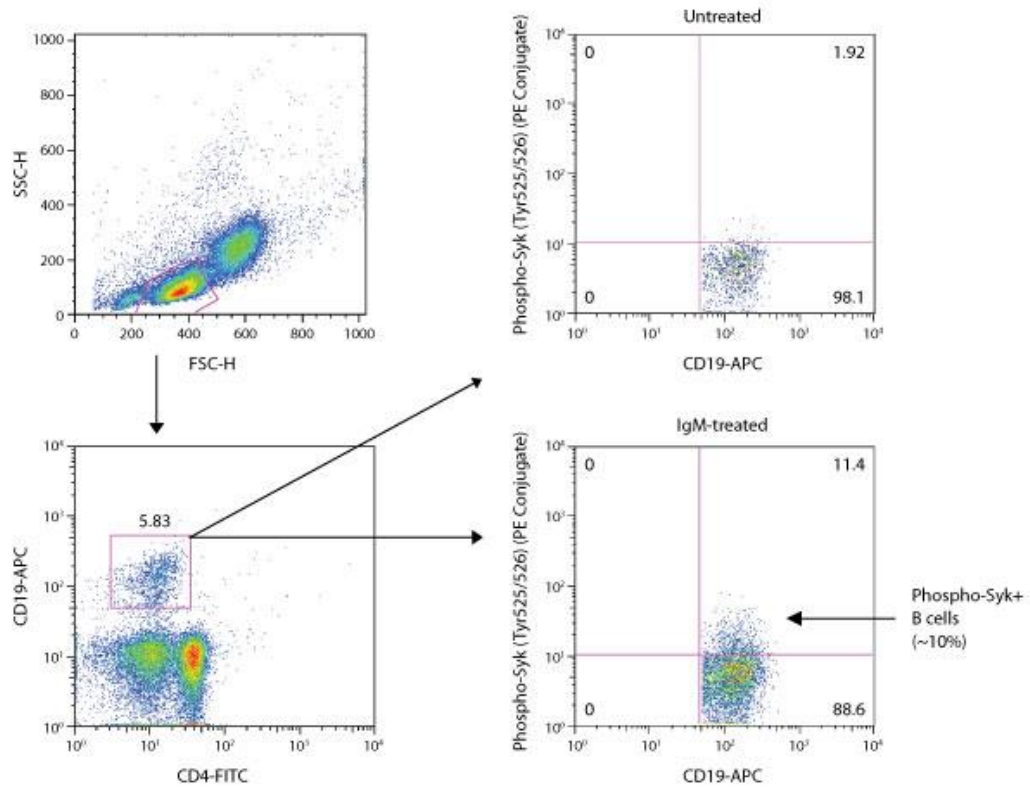


Table 1 Notable advancements in single-cell techniques

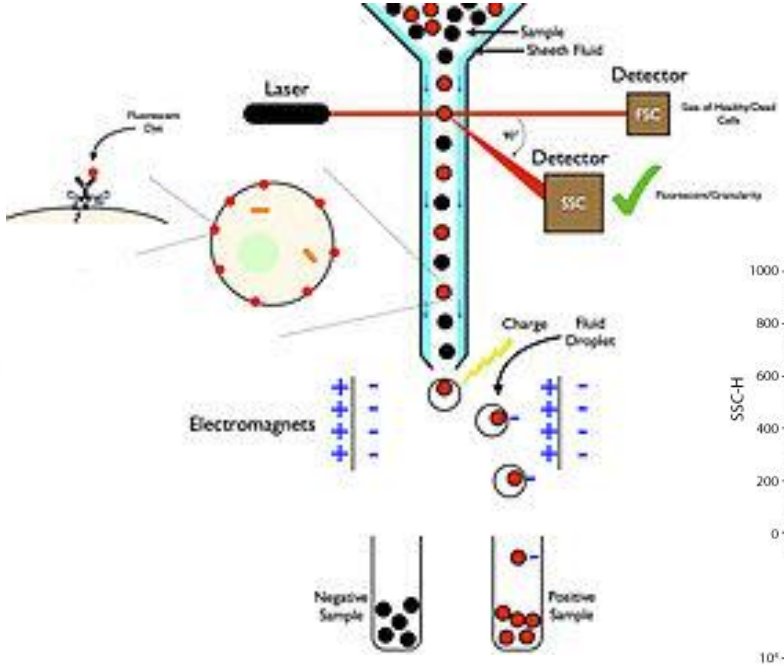
Year introduced	Notable technology advancements	Method cell range[a]
2009	Tang et al. [10]	1[b]
2011	STRT-seq [23]	< 100
2012	SMART-seq [24]	< 100
2012	CEL-Seq [25]	< 100
2013	Fluidigm C1 (IFC) [26]	< 800
2013	Smart-seq 2 [27]	< 1000
2014	MARS-seq [28]	10,000 s
2015	Drop-seq [29]	10,000 s
2015	inDrop [30]	10,000 s
2016	Chromium (10× Genomics) [31]	10,000 s
2017	ddSeq (Bio-Rad) [32]	10,000 s
2017	SPLiT-seq [33]	10,000 s
2017	Seq-well [34]	10,000 s

This is a non-comprehensive list of peer-reviewed studies that advanced single-cell isolation and preparation techniques

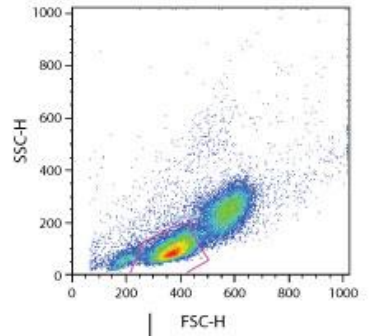
[a] The "range" lists the largest relative population that can or has been studied using this technique

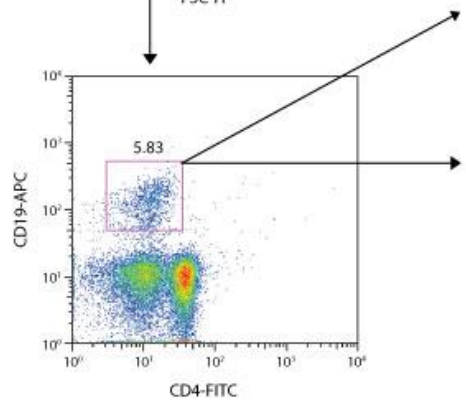
Ortega et al, Clin Trans Med 2017

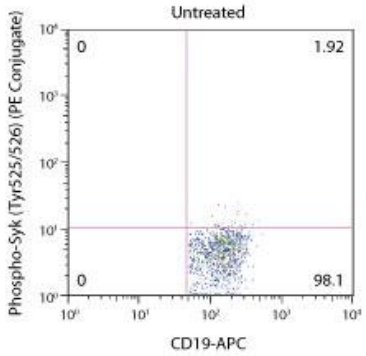
Traditional way to study and isolate single cells: Flow cytometry (FACS=fluorescense assisted cell sorting)

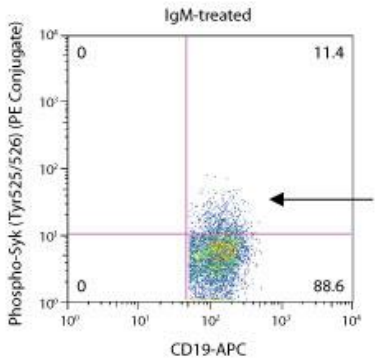


Sorting to tubes or 1 cell per Plate well



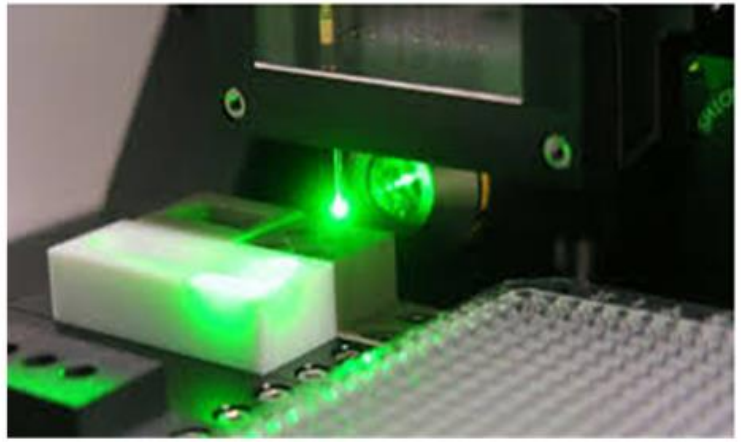


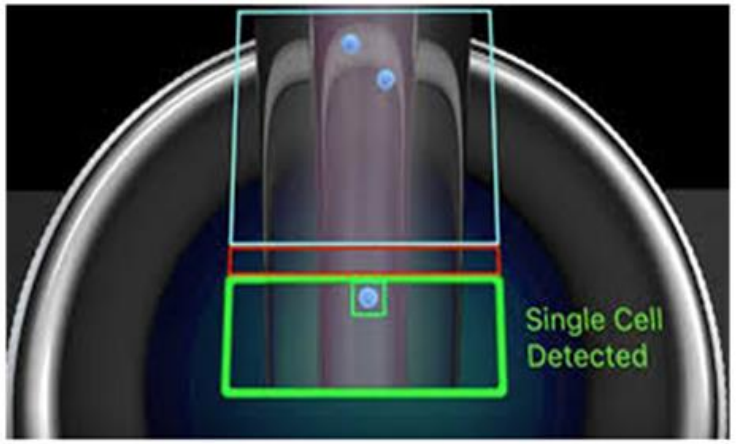


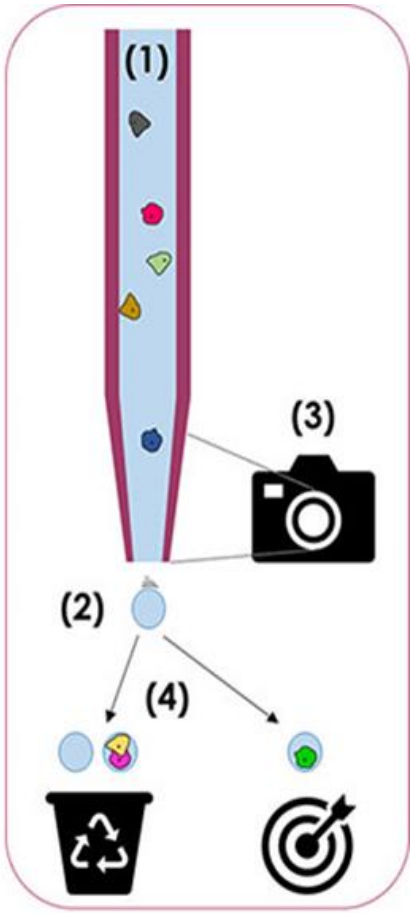


Phospho-Syk+ B cells (~10%)

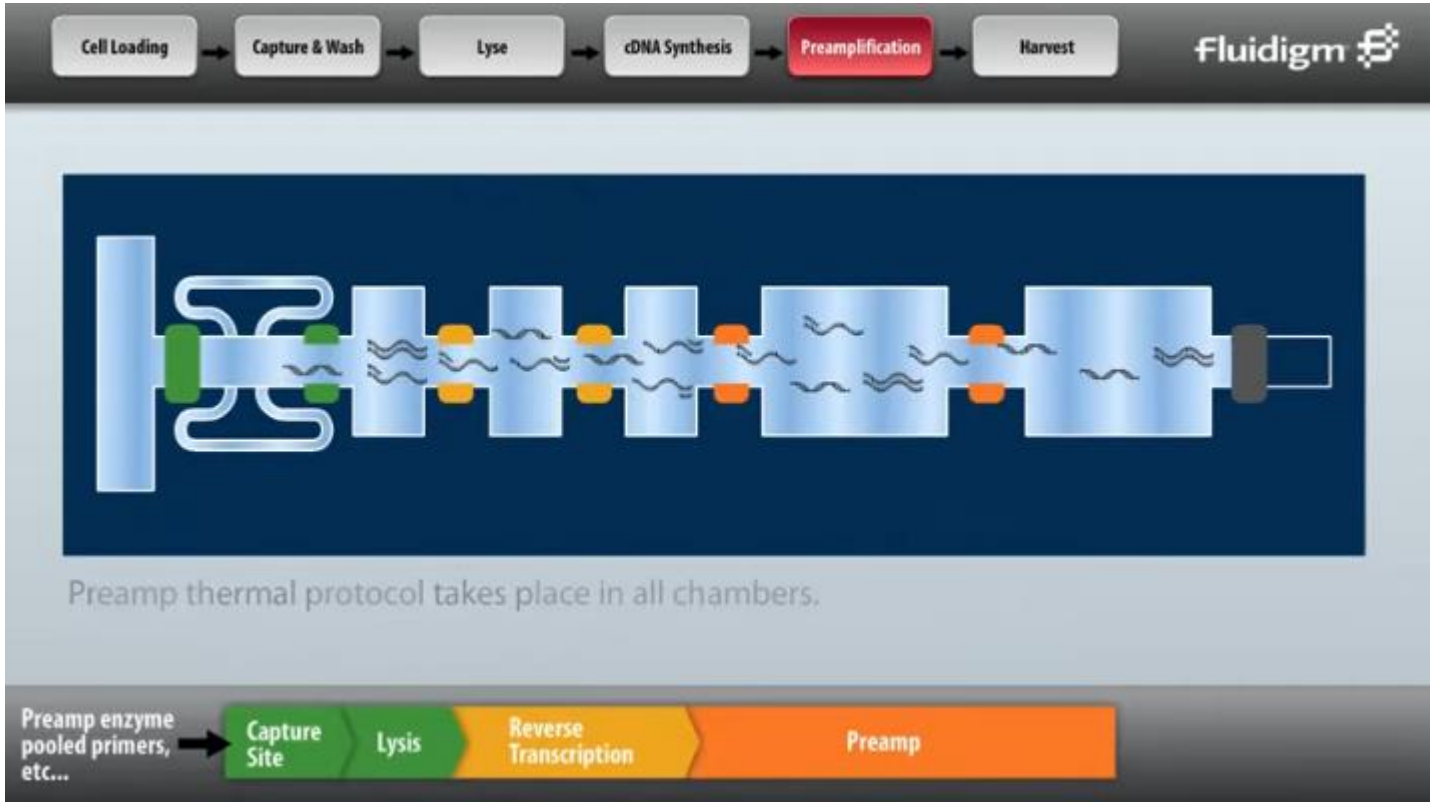
CellenONE single cell dispenser (at FIMM)







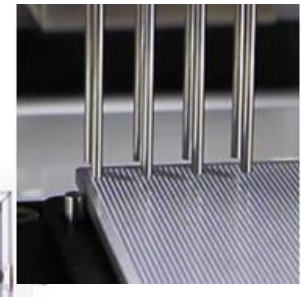
Non-Droplet systems: Fluidigm C1 (not very popular anymore)

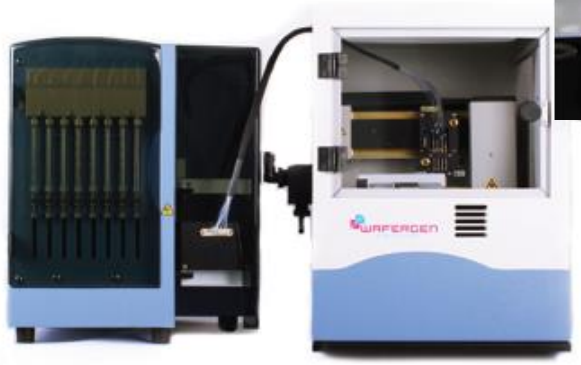


Non-Droplet systems:
Wafergen ICELL8 nanodispenser

SmartChip:
72x72=5184 wells

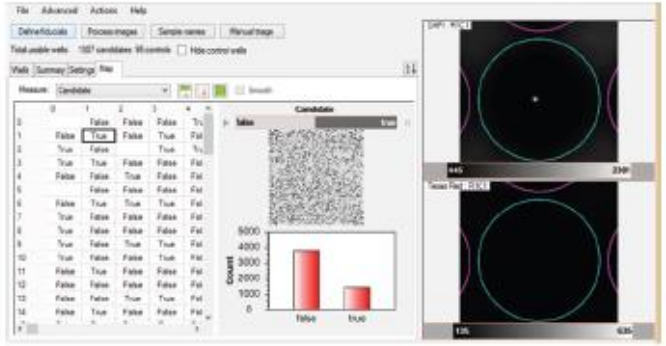
ICELL8 Chips and Reagents





MultiSample NanoDispenser

Imaging Station



CellSelect Software

Acquired by Takara

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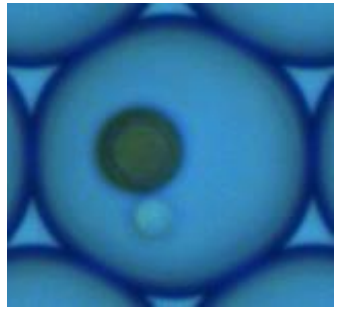
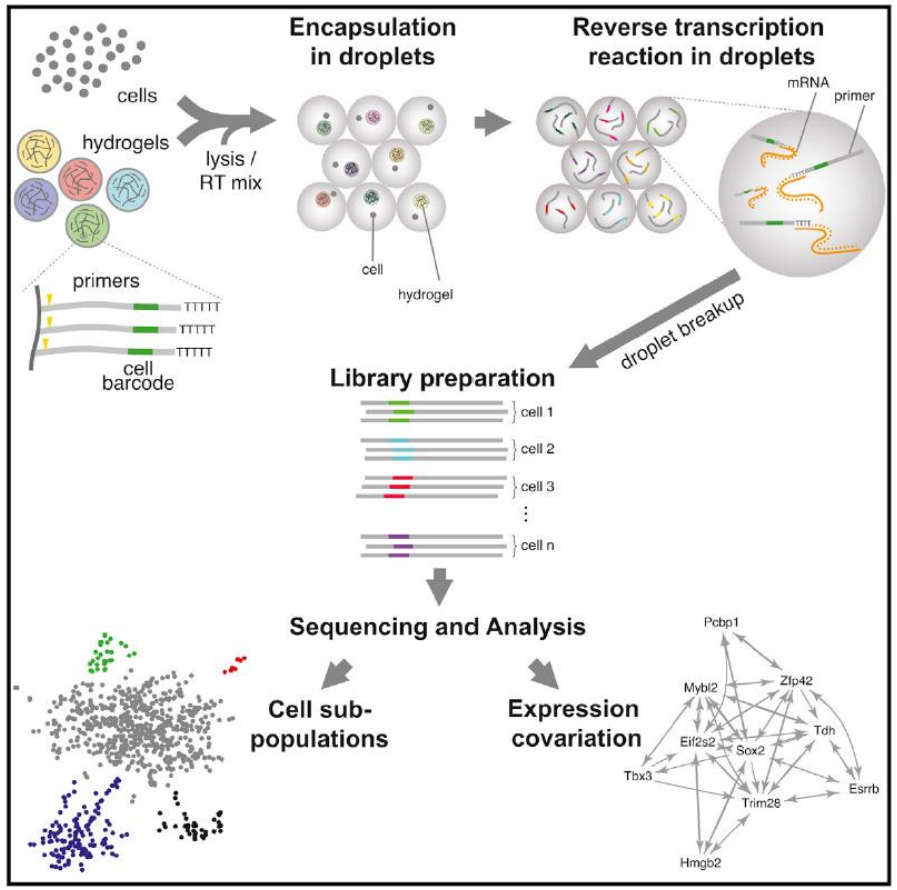


Ortega et al, Clin Trans Med 2017

New level of throughput: 2 droplet papers 2015 in Cell

Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells

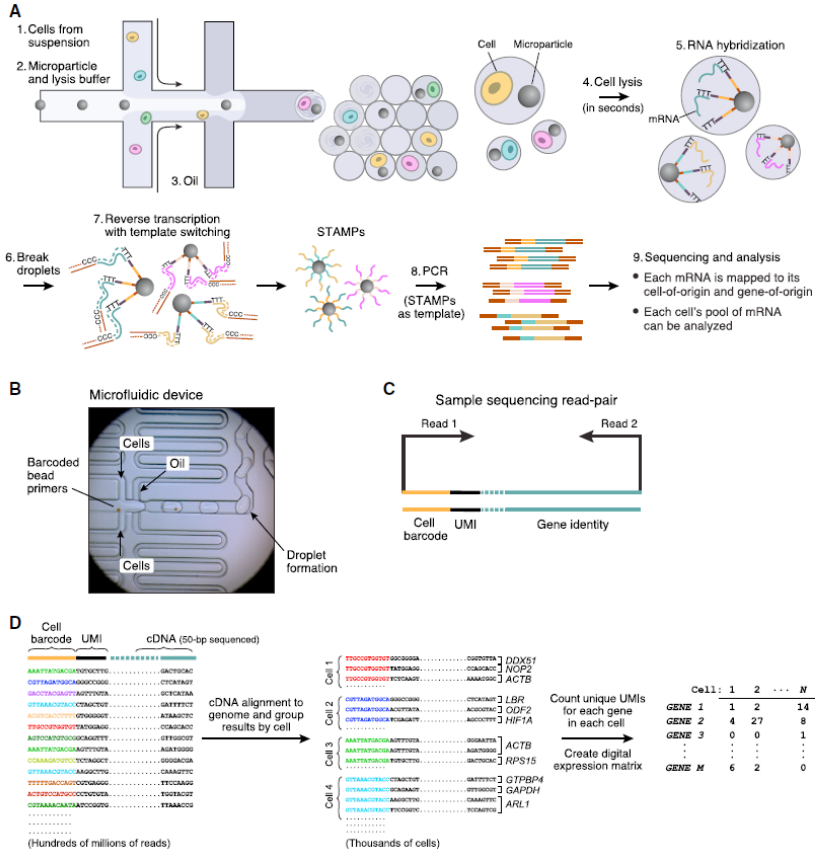
Allon M. Klein,[1,6] Linas Mazutis,[2,3,6] Ilke Akartuna,[2,6] Naren Tallapragada,[1] Adrian Veres,[1,4,5] Victor Li,[1] Leonid Peshkin,[1] David A. Weitz,[2,*] and Marc W. Kirschner[1,*]
[1]Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA



inDrop (indexing droplets)

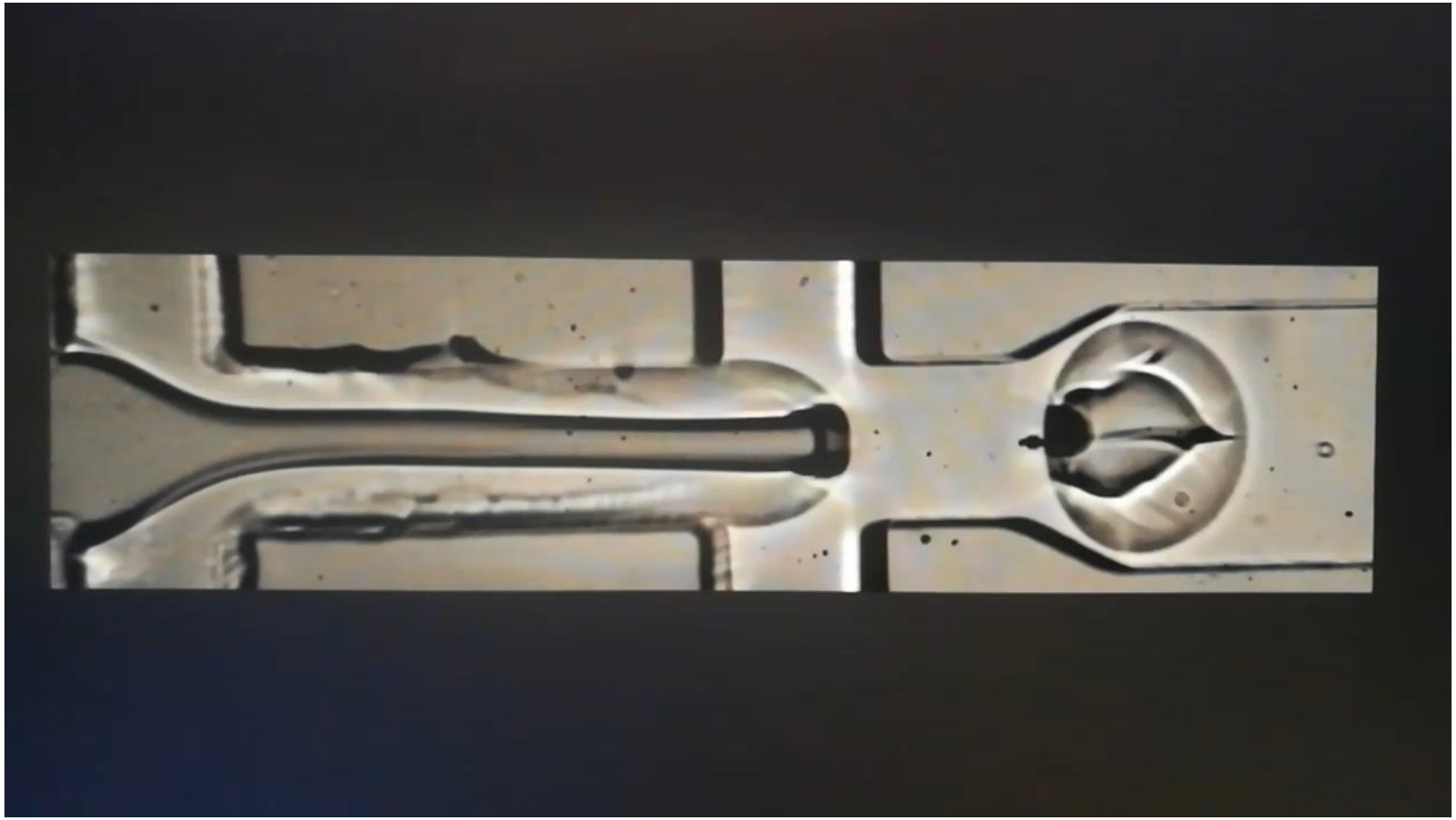
Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

Evan Z. Macosko,[1,2,3,*] Anindita Basu,[4,5] Rahul Satija,[4,6,7] James Nemesh,[1,2,3] Karthik Shekhar,[4] Melissa Goldman,[1,2] Itay Tirosh,[4] Allison R. Bialas,[8] Nolan Kamitaki,[1,2,3] Emily M. Martersteck,[9] John J. Trombetta,[4] David A. Weitz,[5,10] Joshua R. Sanes,[9] Alex K. Shalek,[4,11,12] Aviv Regev,[4,13,14] and Steven A. McCarroll[1,2,3,*]
[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA



Drop-Seq: 10.000cells, 12h, 6.5cents/cell
44,808 cells -> 39 cell populations in the retina

Droplet cell / bead capture video (DropSeq)



- http://mccarrolllab.org/wp-content/uploads/2015/05/Substack-4455-4504.gif

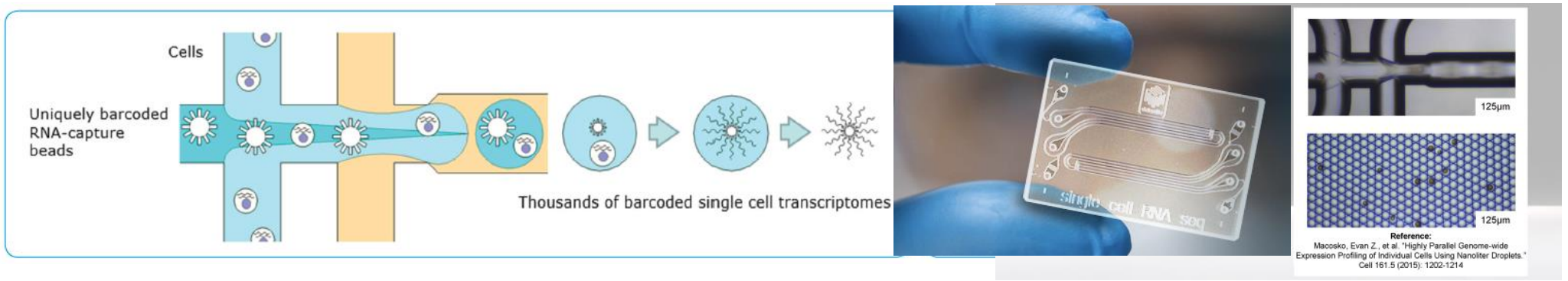
Commercial droplet based devices

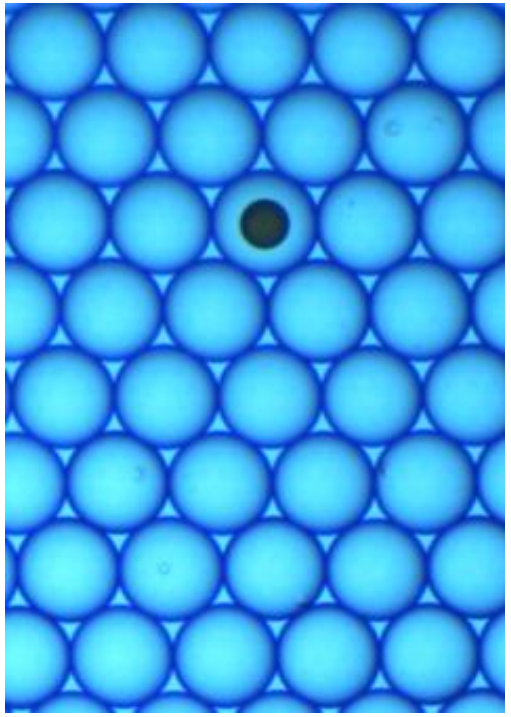
10x Genomics Chromium
120.000/50.000eur device

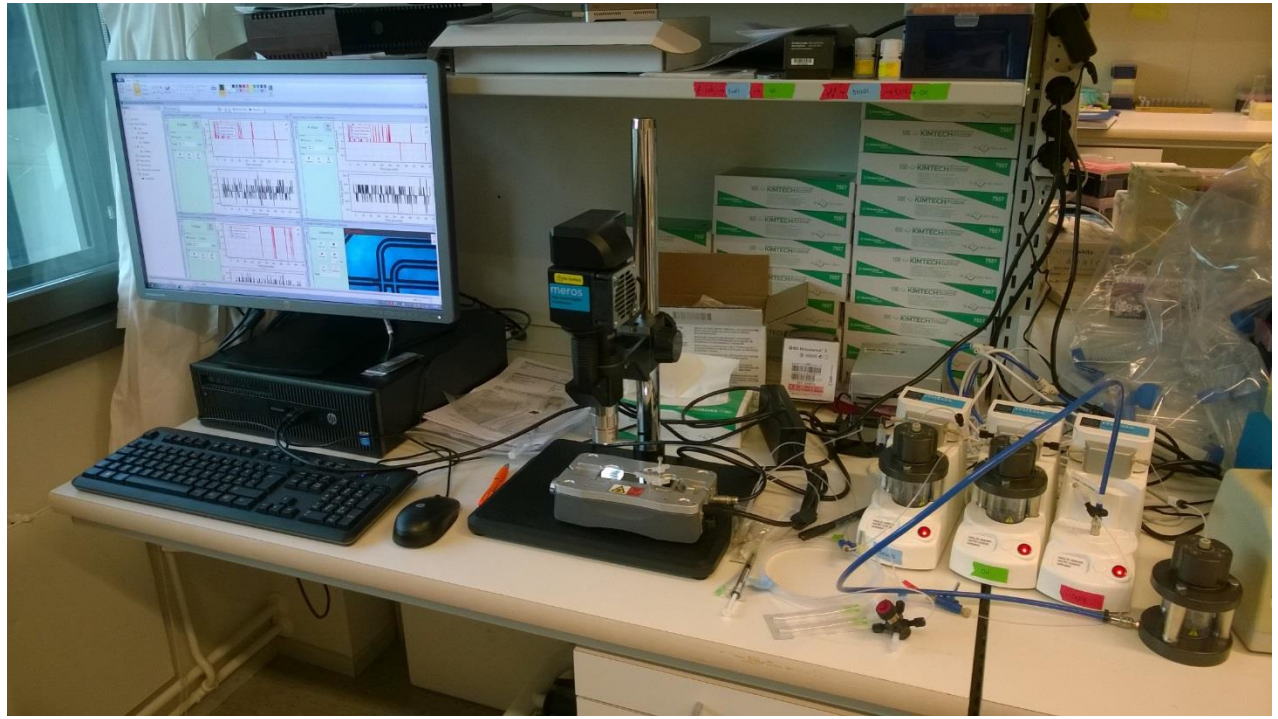
Bio-Rad Illumina ddSEQ
~50.000eur

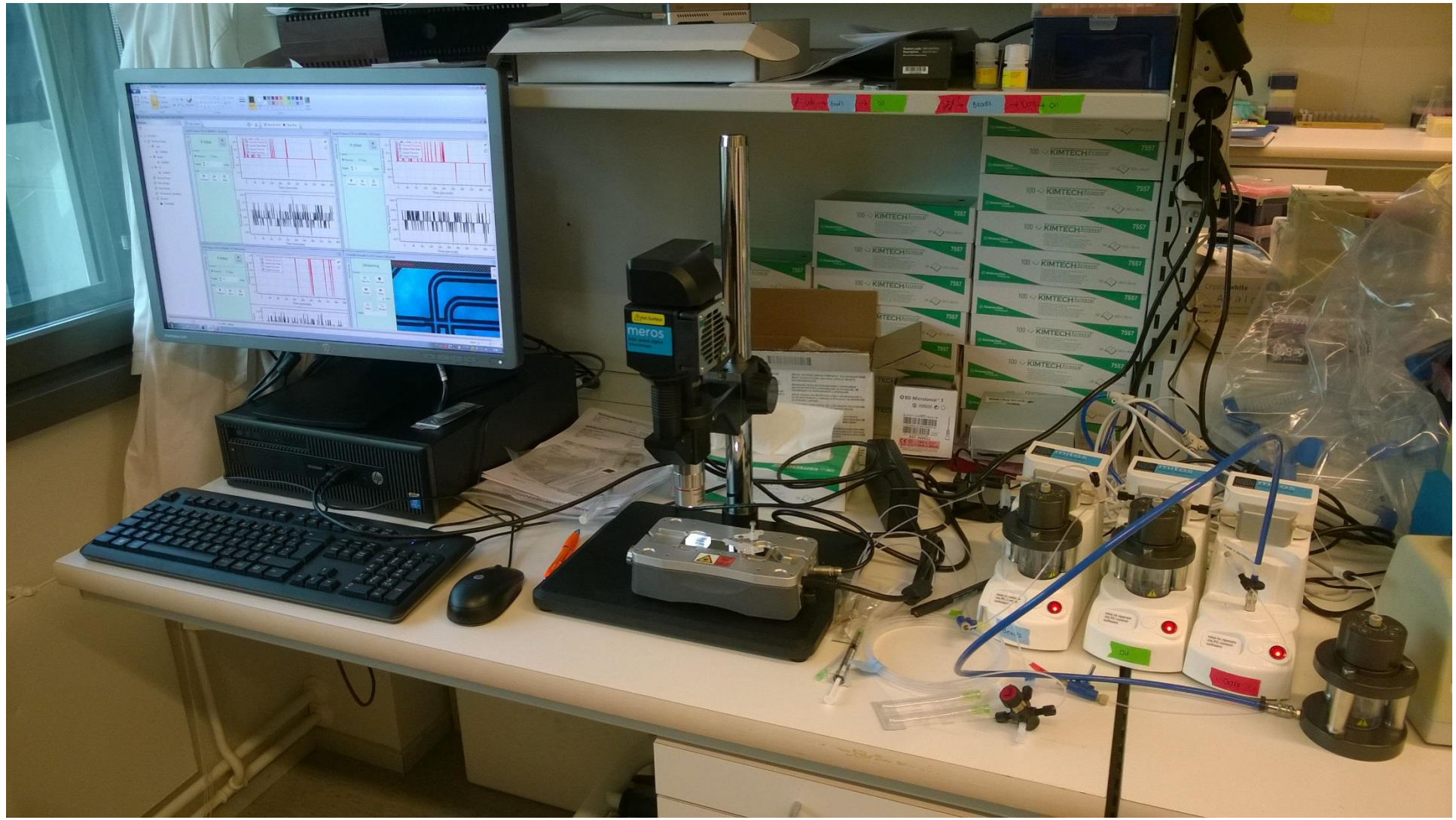
Dolomite Nadia (NEW)
~50.000eur

In our lab: the Harvard Drop-seq protocol & Dolomite Bio Single Cell RNAseq device

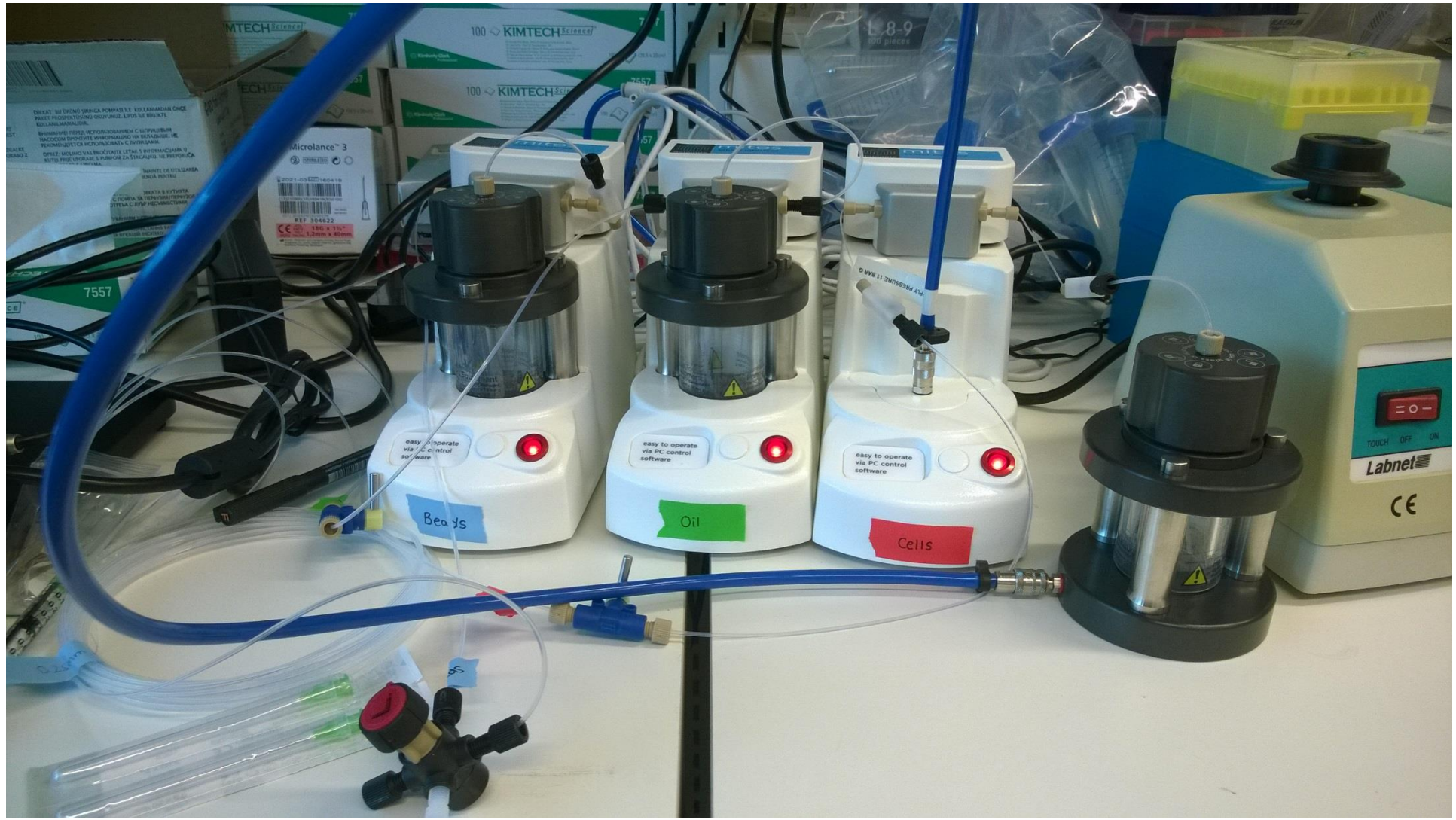


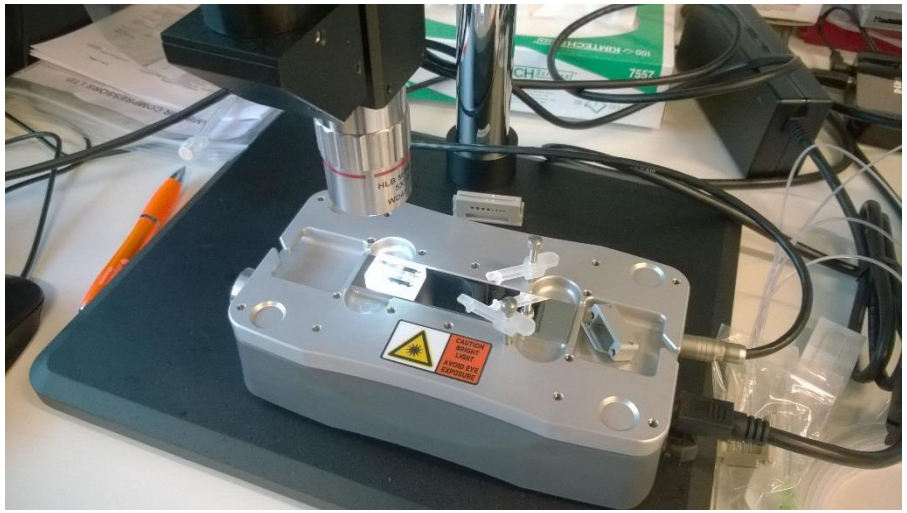


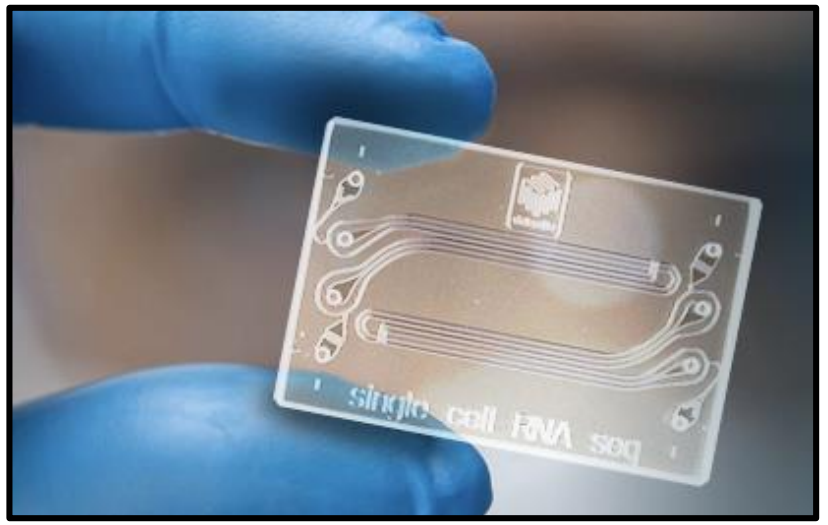


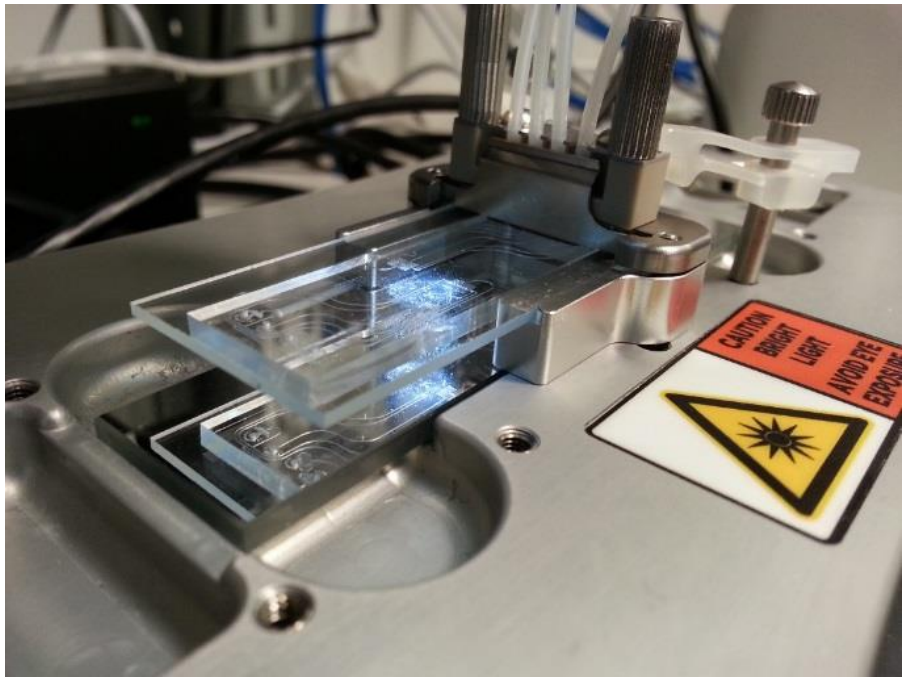

meros
KIMTECH
7557

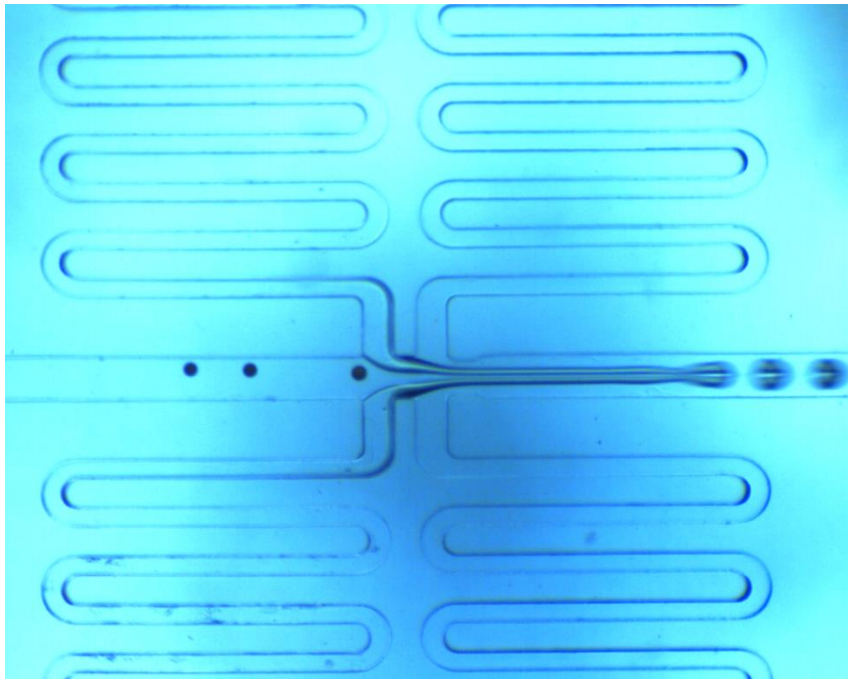

KIMTECH Science
7557
BD Microlance 3
Beads
Oil
Cells

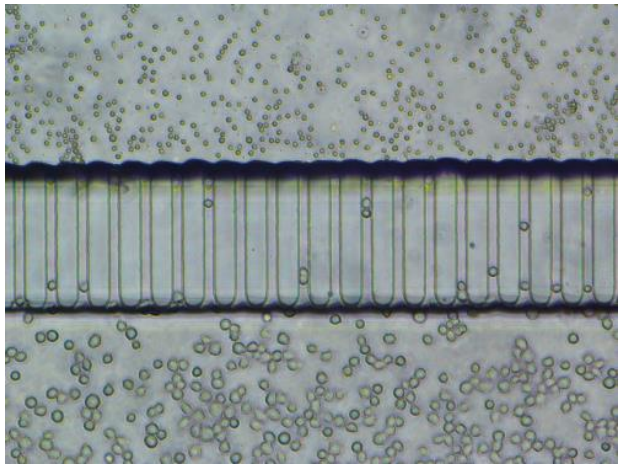

KIMTECH
Microlance™ 3
7557
easy to operate via PC control software
Beads
Oil
Cells
TOUCH OFF ON
Labnet
CE





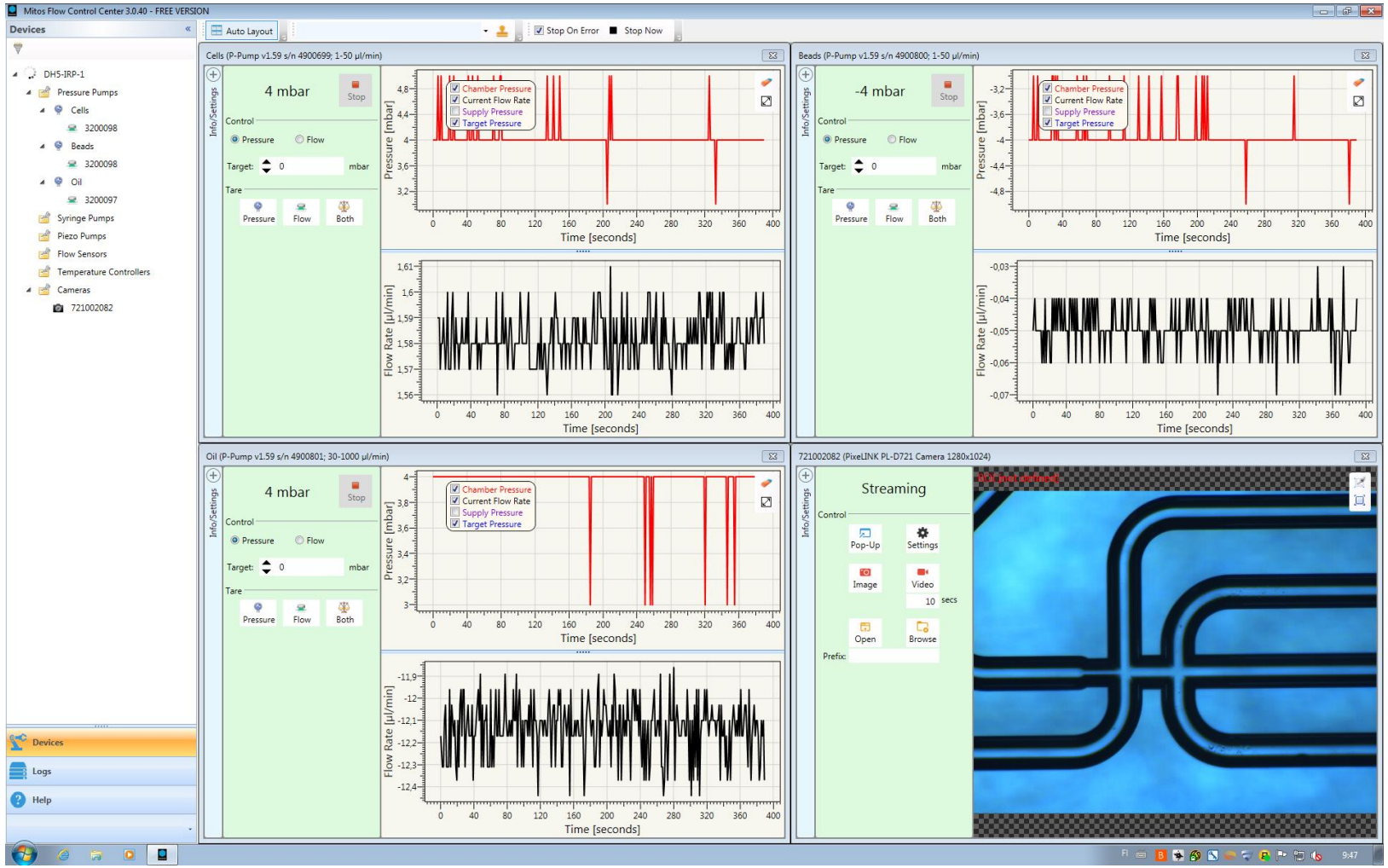


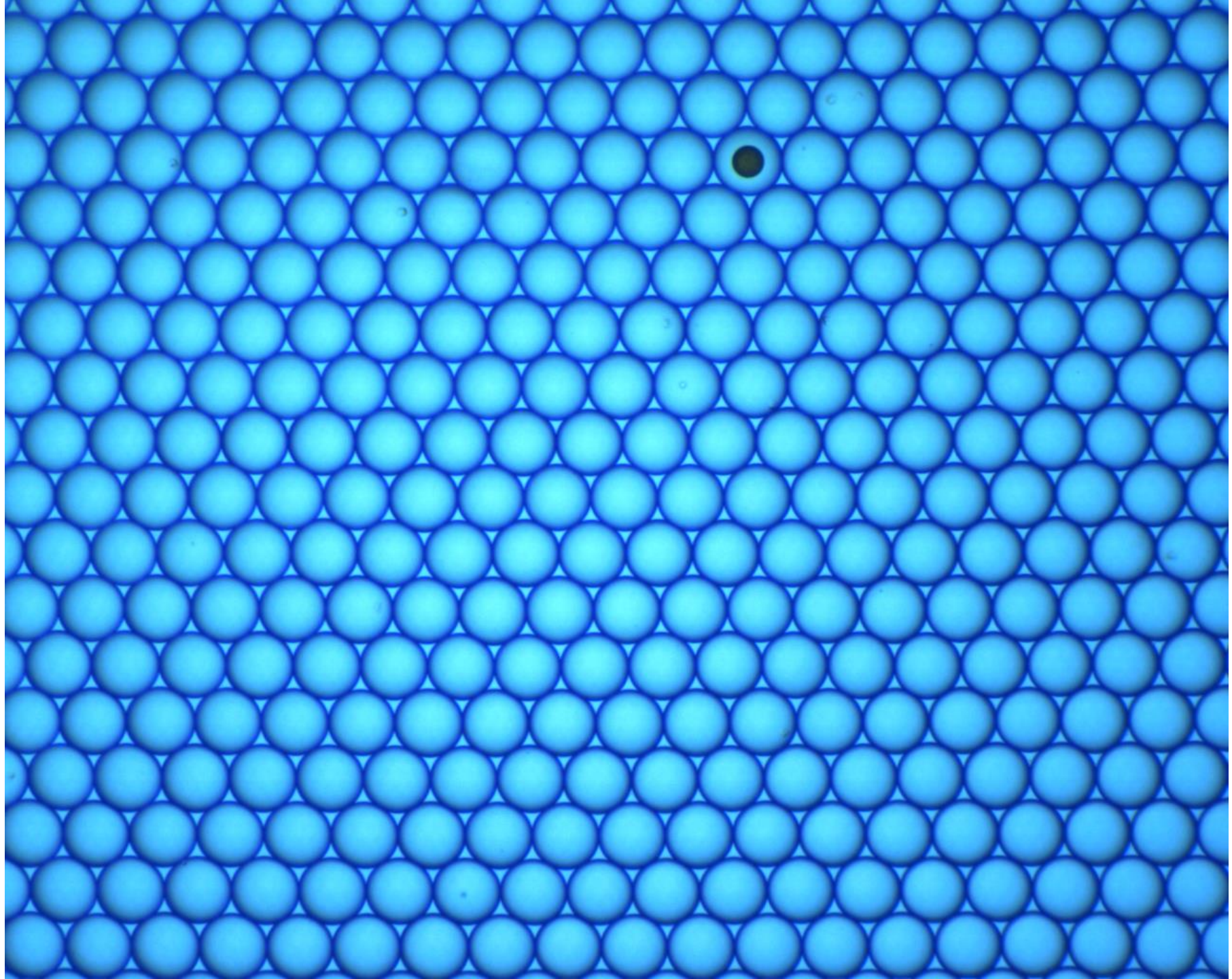




Cell migration/co-culture chip modified from Businaro et al, Lab Chip 2013

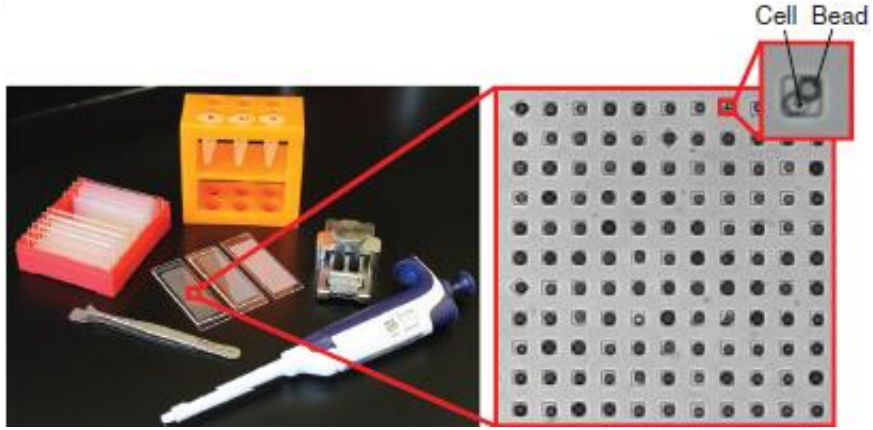
Droplet chips (Dolomite glass chip and in-house PDMS chip)


Mitos Flow Control Center 3.0.40 - FREE VERSION
Devices
Auto Layout
Stop On Error
Stop Now
DH5-IRP-1
Pressure Pumps
Cells
3200098
Beads
3200098
Oil
3200097
Syringe Pumps
Piezo Pumps
Flow Sensors
Temperature Controllers
Cameras
721002082
Cells (P-Pump v1.59 s/n 4900699; 1-50 µl/min)
4 mbar
Stop
Control
Pressure
Flow
Target: 0 mbar
Tare
Pressure
Flow
Both
Chamber Pressure
Current Flow Rate
Supply Pressure
Target Pressure
Pressure [mbar]
Flow Rate [µl/min]
Time [seconds]
Beads (P-Pump v1.59 s/n 4900800; 1-50 µl/min)
-4 mbar
Oil (P-Pump v1.59 s/n 4900801; 30-1000 µl/min)
4 mbar
721002082 (PixelLINK PL-D721 Camera 1280x1024)
Streaming
Pop-Up
Settings
Image
Video
10 secs
Open
Browse
Prefix:
Devices
Logs
Help

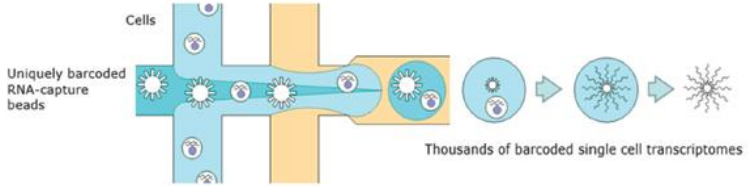


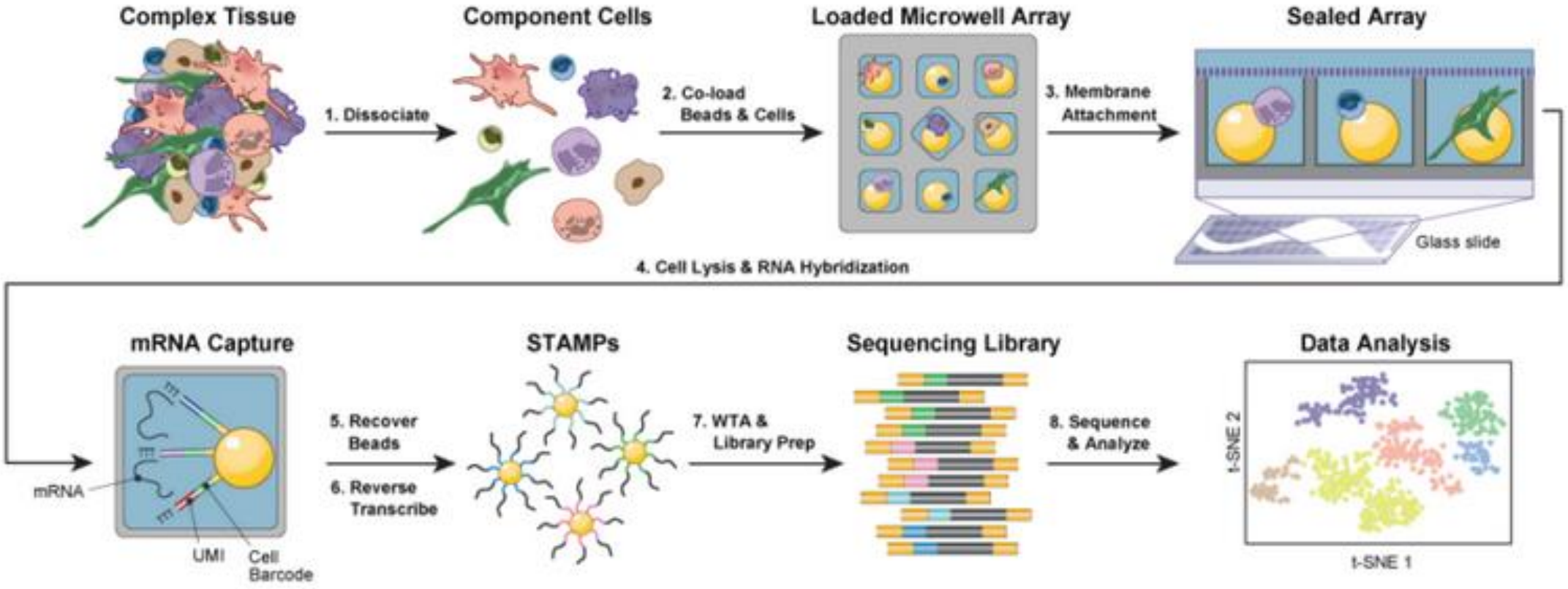
SeqWell - DropSeq chemistry but in microwells instead droplets:

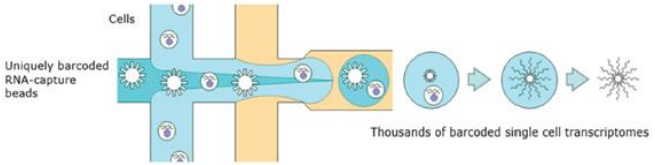
Portable, academic system
(MIT, Gierahn et al 2017)



Comparison to DropSeq:





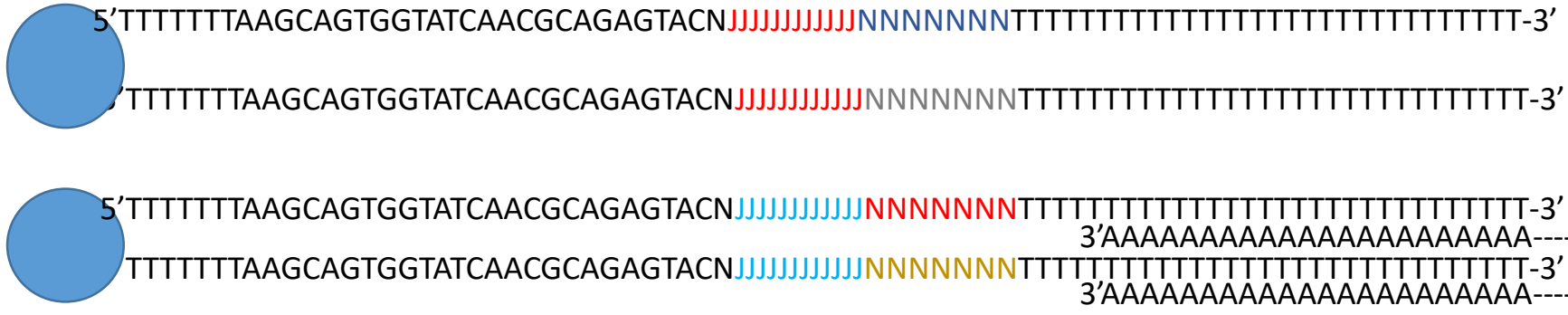


Within droplets
Or Array wells

12bp cell Barcode ~17 mio

8bp mol barcode (UMI)

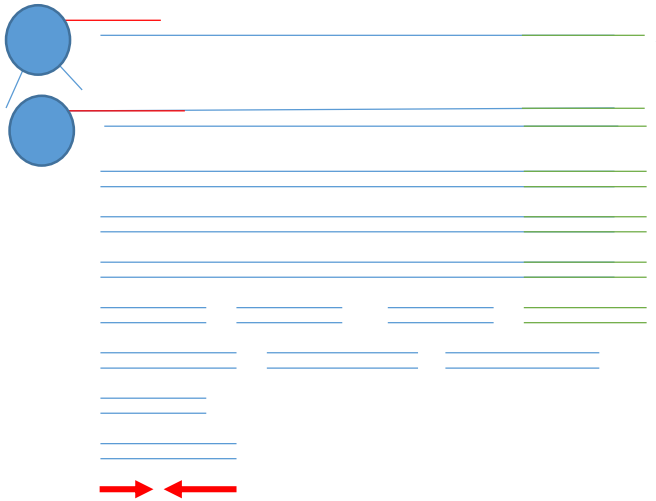
DropSeq: 180.000 cells + 180.000 beads
->5% captured + bead loss ~4000 cells
SeqWell: 10.000 cells + 110.000 beads -> 50% captured



polyA-RNA from the lysing cell

Collected droplets/emulsion
Library prep in bulk

Reverse transcription (42°C), with template switch oligo -> washes
ExoSap treatment (37°C) ->washes
Full lenght cDNA amplification PCR
AmpureXP purification of PCR product
Tagmentation (shortening+adapters) + PCR
AmpureXP purification
Illumina NextSeq/NovaSeq/MiSeq sequencing (20bp+55bp, from 3’ end)
Data analyses (QC filtering/trimming, demultiplexing, UMI counting, alignments to reference genome/transcriptome, cell type clustering, differential expression etc)



Template switching oligo

…gcatgacCCC (cDNA)
…cgtactg (RNA)
rGrGrGtgcatgctca (TSO)

…gcatgacCCC
…cgtactgGGGtgcatgctca

…gcatgacCCCacgtacgagt
…cgtactgGGGtgcatgctca

Single cell libraries -> Illumina sequencing

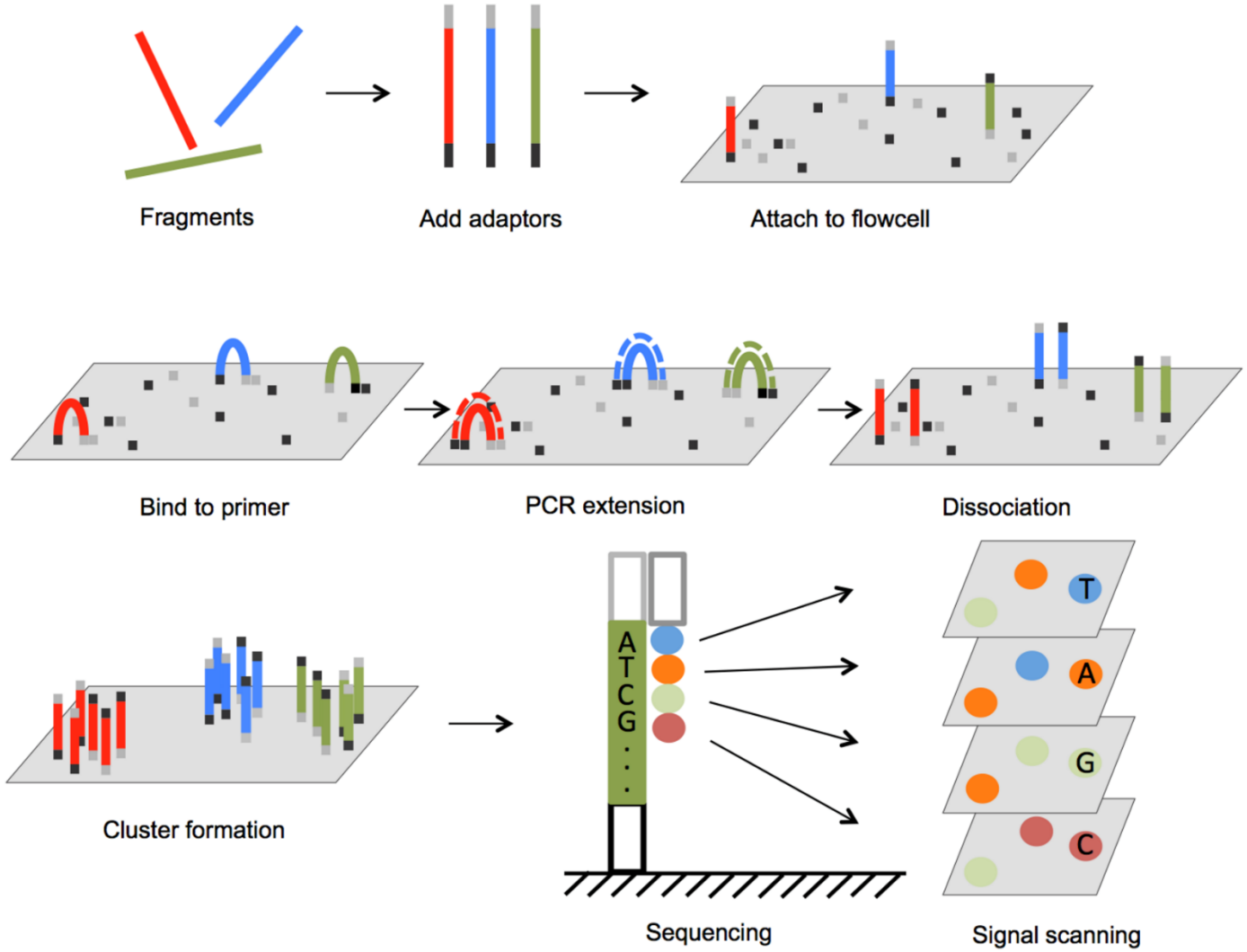
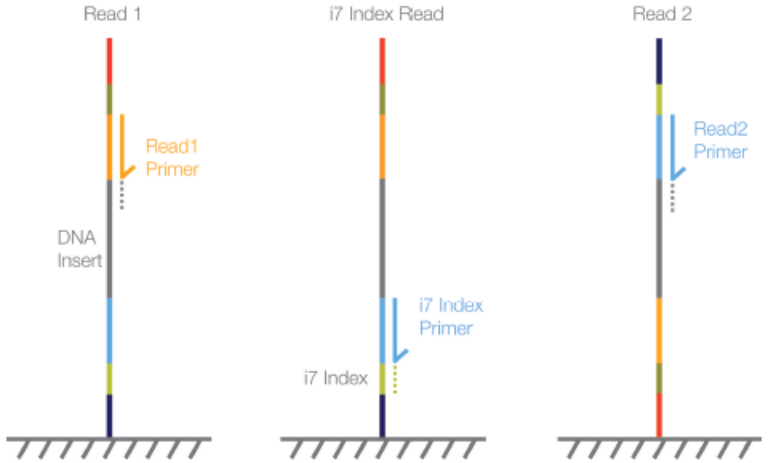


Figure 1 Single-Indexed Sequencing



1 **Read 1**–Read 1 follows the standard Read 1 sequencing protocol using SBS reagents. The Read 1 sequencing primer is annealed to the template strand during the cluster generation step.

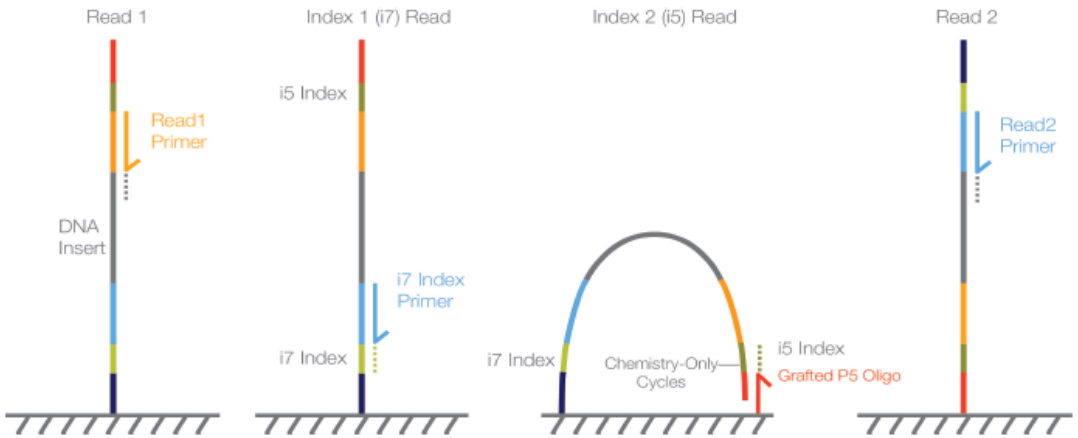
2 **Index Read preparation**–The Read 1 product is removed and the Index 1 (i7) sequencing primer is annealed to the same template strand, producing the Index 1 (i7) Read.

3 **Index 1 (i7) Read**–Following Index Read preparation, the Index 1 (i7) Read is performed. The read length depends on the system and run parameters.

4 **Read 2 resynthesis**–The Index Read product is removed and the original template strand is used to regenerate the complementary strand. Then, the original template strand is removed to allow hybridization of the Read 2 sequencing primer.

5 **Read 2**–Read 2 follows the standard paired-end sequencing protocol using SBS reagents.

Figure 2 Dual-Indexed Sequencing on a Paired-End Flow Cell (Workflow A) MiSeq



- **Workflow A**–The Index 2 Read is performed before Read 2 resynthesis, so the Index 2 (i5) adapter is sequenced on the forward strand.
- **Workflow B**–The Index 2 Read is performed after Read 2 resynthesis, which creates the reverse complement of the Index 2 (i5) index adapter sequence.

Table 1 Dual-Index Paired-End Sequencing Workflows

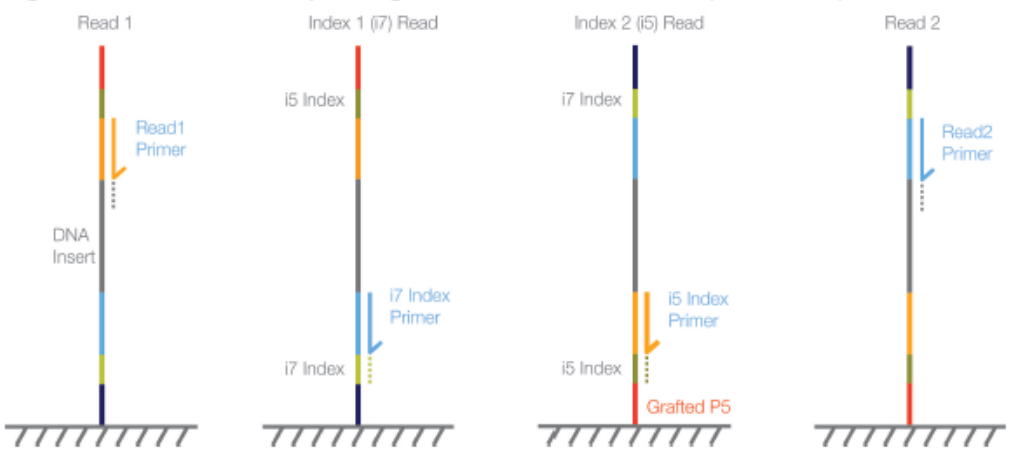
Workflow A	Workflow B
Read 1	Read 1
Index Read preparation	Index Read preparation
Index 1 Read	Index 1 Read
Index 2 Read	Read 2 resynthesis
Read 2 resynthesis	**Index 2 Read**
Read 2	Read 2 preparation
	Read 2

Dual-Indexed Workflow on a Paired-End Flow Cell

Dual-index sequencing on a paired-end flow cell follows 1 of 2 workflows, depending on the system:

- Workflow A is performed on the NovaSeq™, MiSeq®, HiSeq 2500, and HiSeq 2000.
- Workflow B is performed on the MiniSeq™, NextSeq®, HiSeq 4000, and HiSeq 3000.

Figure 3 Dual-Indexed Sequencing on a Paired-End Flow Cell (Workflow B) NextSeq



Drop-seq protocols, analysis programs, example data sets, discussion forum etc:

http://mccarrolllab.com/dropseq/

McCarroll Lab

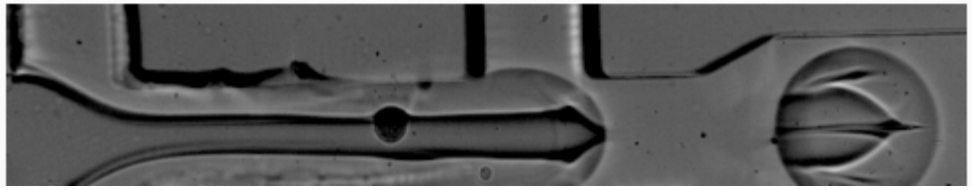
Welcome to Drop-seq!

Drop-seq is a technology that allows biologists to analyze genome-wide gene expression in thousands of individual cells in a single experiment. This work is described in Macosko et al., Cell, 2015. This site provides interested users with resources to implement Drop-seq in their own labs. We hope you do amazing things with Drop-seq. Tell us about it!

If you would like to be informed of new protocol optimizations, discussion forums, etc., please send an email to dropseq@gmail.com and we'll put you on the list.

Do you currently have Drop-seq working?

We are trying to gauge the level of adoption of the technology, and would very much appreciate hearing about your experiences. If you have Drop-seq working, please email dropseq@gmail.com with a picture of your first "Barnyard" plot and, if possible, the broad scientific direction/question you are hoping to address with the technology. We would really love to hear from all of you!



Drop-Seq Laboratory Protocol

version 3.1 (12/28/15)

Evan Macosko and Melissa Goldman

Steve McCarroll's lab, Harvard Medical School

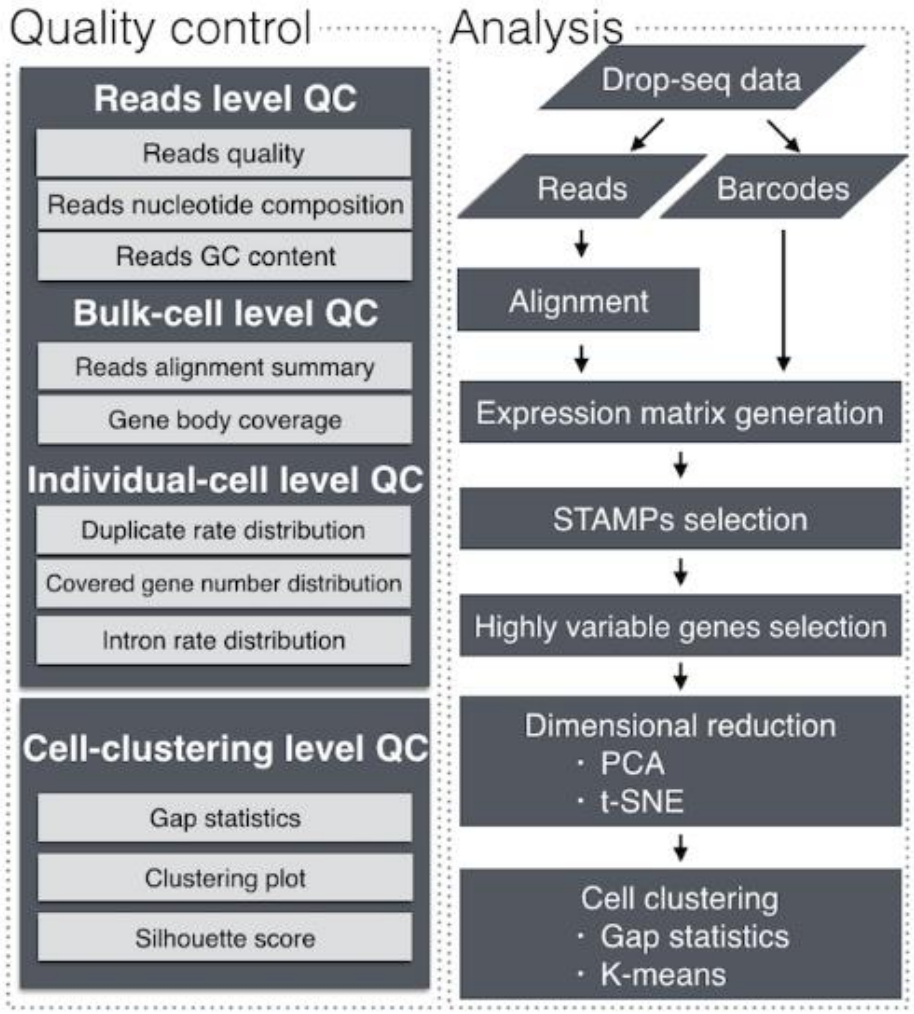
Bioinformatics of Drop-seq data

http://www.tongji.edu.cn/~zhanglab/Drseq/

Dr. SEQ

quality control and analysis for Drop-seq

Getting started | Overview | Manual | Output | Download | FAQ



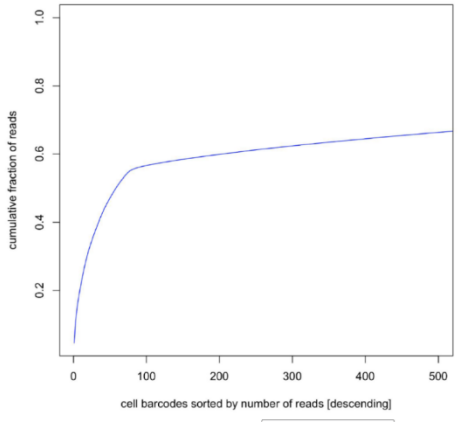
Drop-seq Core Computational Protocol

version 1.0.1 (6/11/15)

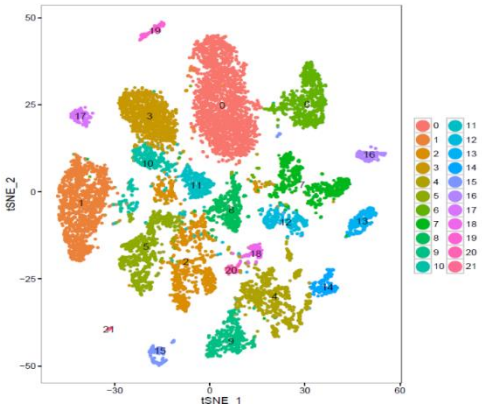
James Nemesh

Steve McCarroll's lab, Harvard Medical School

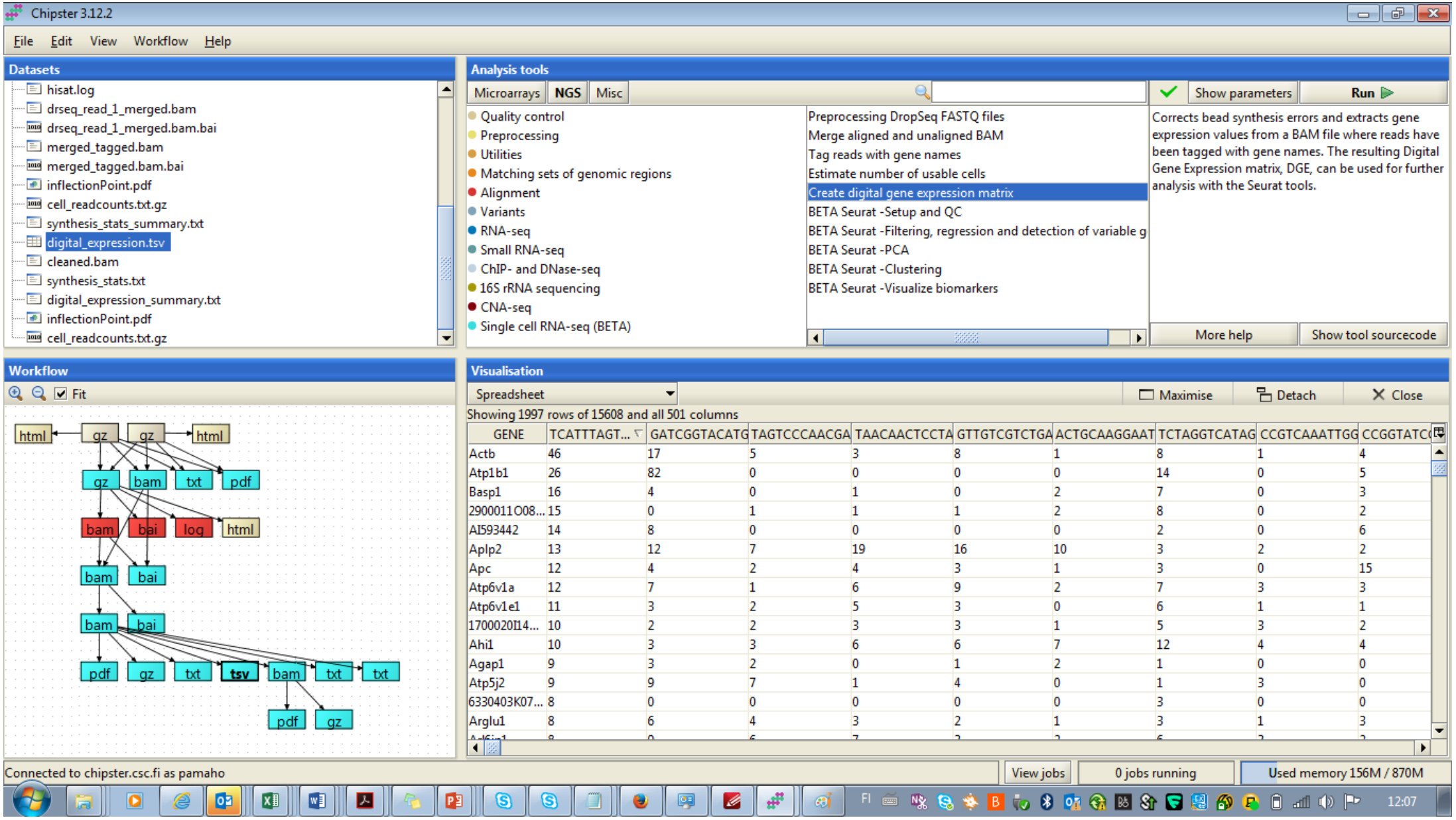
CellName	GTAAGC AGCGCG	CTTGGC AAGAAG	GCGAAT TCCCCT	GTAAGC AGTGGT	CGAGTC GGCCGT	GGTACT TGCTGG	GATTAC CGTGGG	ATTTAG GGAGCG	TCTACG AGTCAA
HBB	19480	18	2	243	4	0	0	0	36
HBA2	3163	4	0	33	1	0	0	0	4
HBA1	1248	3	0	19	0	0	0	0	3
MT-RNR2	481	350	205	1739	21	1523	457	285	841
BRSK1	200	0	0	1	0	0	0	0	0
UBB	119	14	0	5	0	0	0	0	1
RP11-138I1.4	98	12	0	0	0	0	0	0	0
GUK1	91	79	0	1	0	0	0	0	0
VTI1B	81	0	0	3	0	0	0	0	0



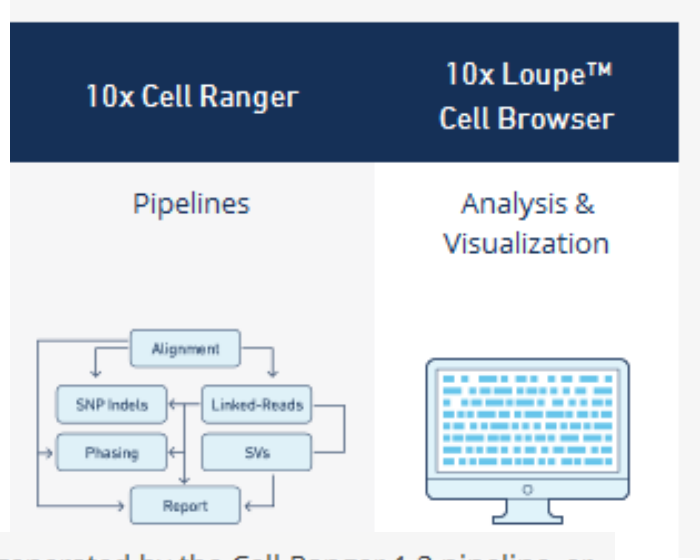
http://satijalab.org/seurat/



CSC/Chipster: Drop-seq pipeline + Seurat



10x Genomics Chromium (droplet based platform in FIMM) tools



Cell Ranger:

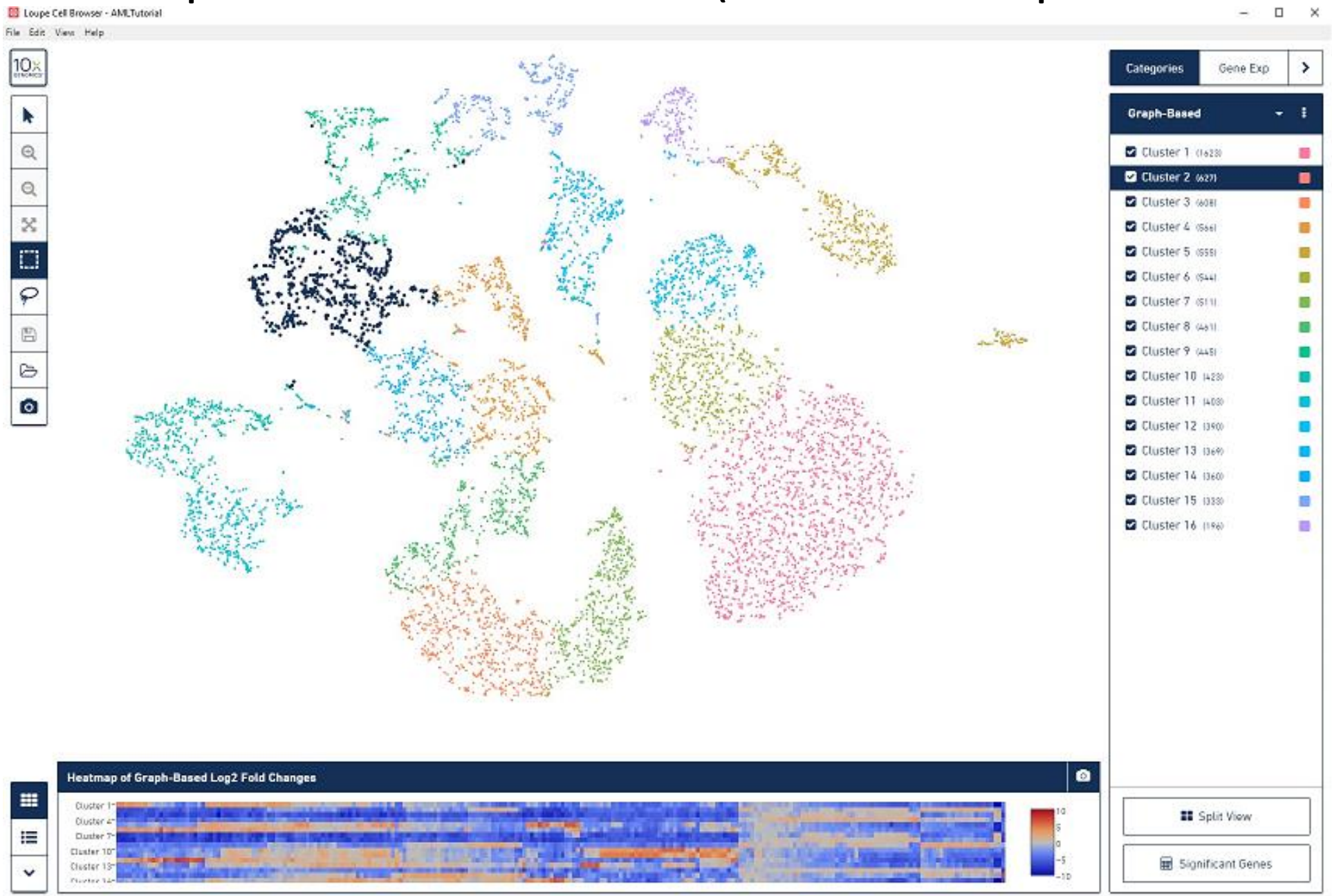
- cellranger mkfastq wraps Illumina's bcl2fastq to correctly demultiplex Chromium-prepared sequencing samples and to convert barcode and read data to FASTQ files.
- cellranger count takes FASTQ files from `cellranger mkfastq` and performs alignment, filtering, and UMI counting. It uses the Chromium cellular barcodes to generate gene-barcode matrices and perform clustering and gene expression analysis. `count` can take input from multiple sequencing runs on the same library.
- cellranger aggr aggregates outputs from multiple runs of `cellranger count`, normalizing those runs to the same sequencing depth and then recomputing the gene-barcode matrices and analysis on the combined data. `aggr` can be used to combine data from multiple samples into an experiment-wide gene-barcode matrix and analysis.
- cellranger reanalyze takes gene-barcode matrices produced by `cellranger count` or `cellranger aggr` and reruns the dimensionality reduction, clustering, and gene expression algorithms using tunable parameter settings.

Loupe Cell Browser:

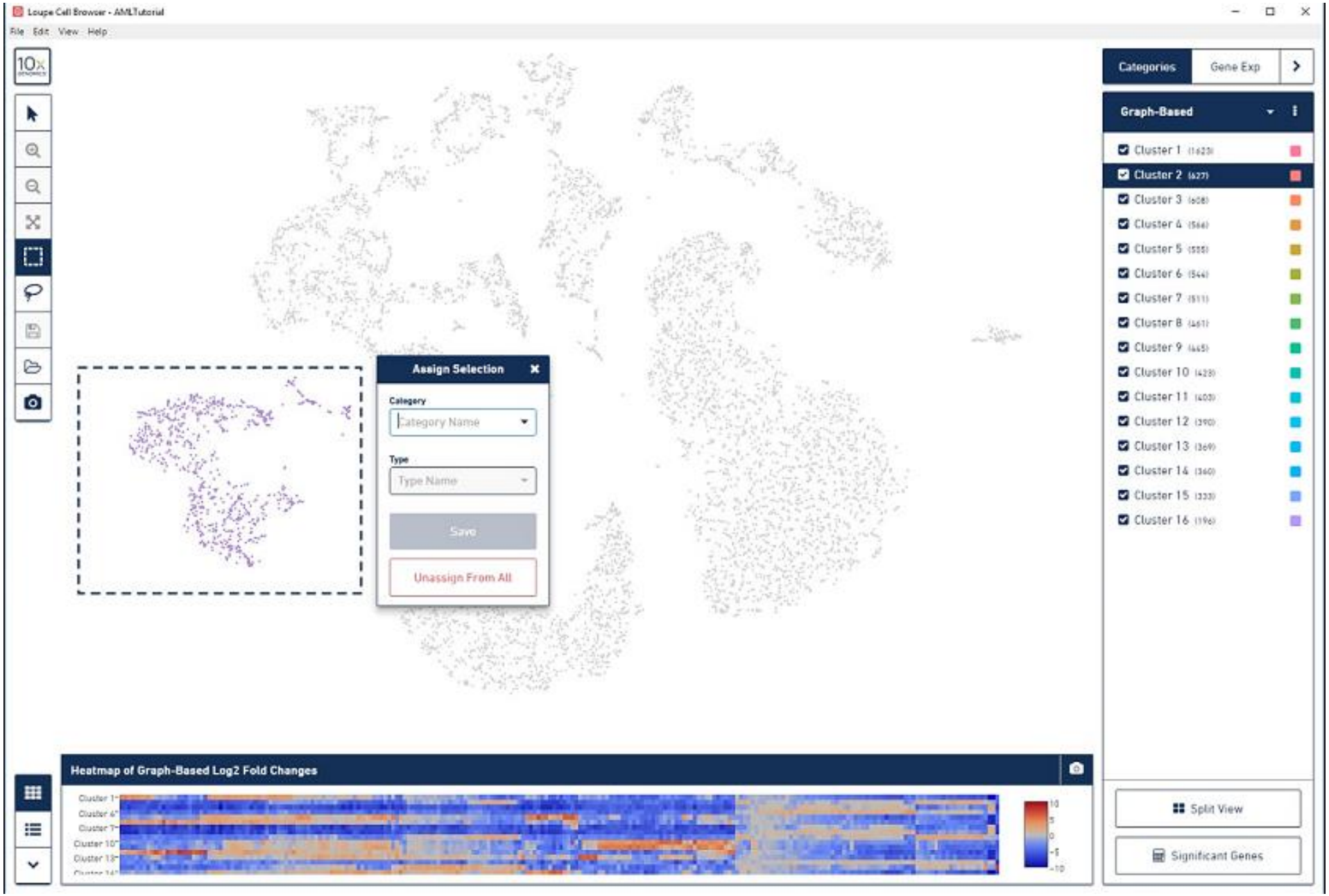
Loupe Cell Browser opens `.cloupe` files generated by the Cell Ranger 1.3 pipeline, or by `cellranger mkloupe` for older pipelines. Once your data is in Loupe Cell Browser, you can rapidly explore and gain insights from the data without writing a line of code:

- Finding Significant Genes
  Determine genes that uniquely characterize clusters at the press of a button.
- Identifying Cell Types
  Use gene lists and the gene expression view to locate different cell types and functional groups.
- Exploring Substructure
  Create custom clusters and use differential expression tools to identify even smaller subgroups in your data.
- Sharing Results
  Save genes of interest, export data tables, and capture screenshots of your single-cell data.

10x Loupe Cell Browser (also nice public data sets!)



10x Loupe Cell Browser



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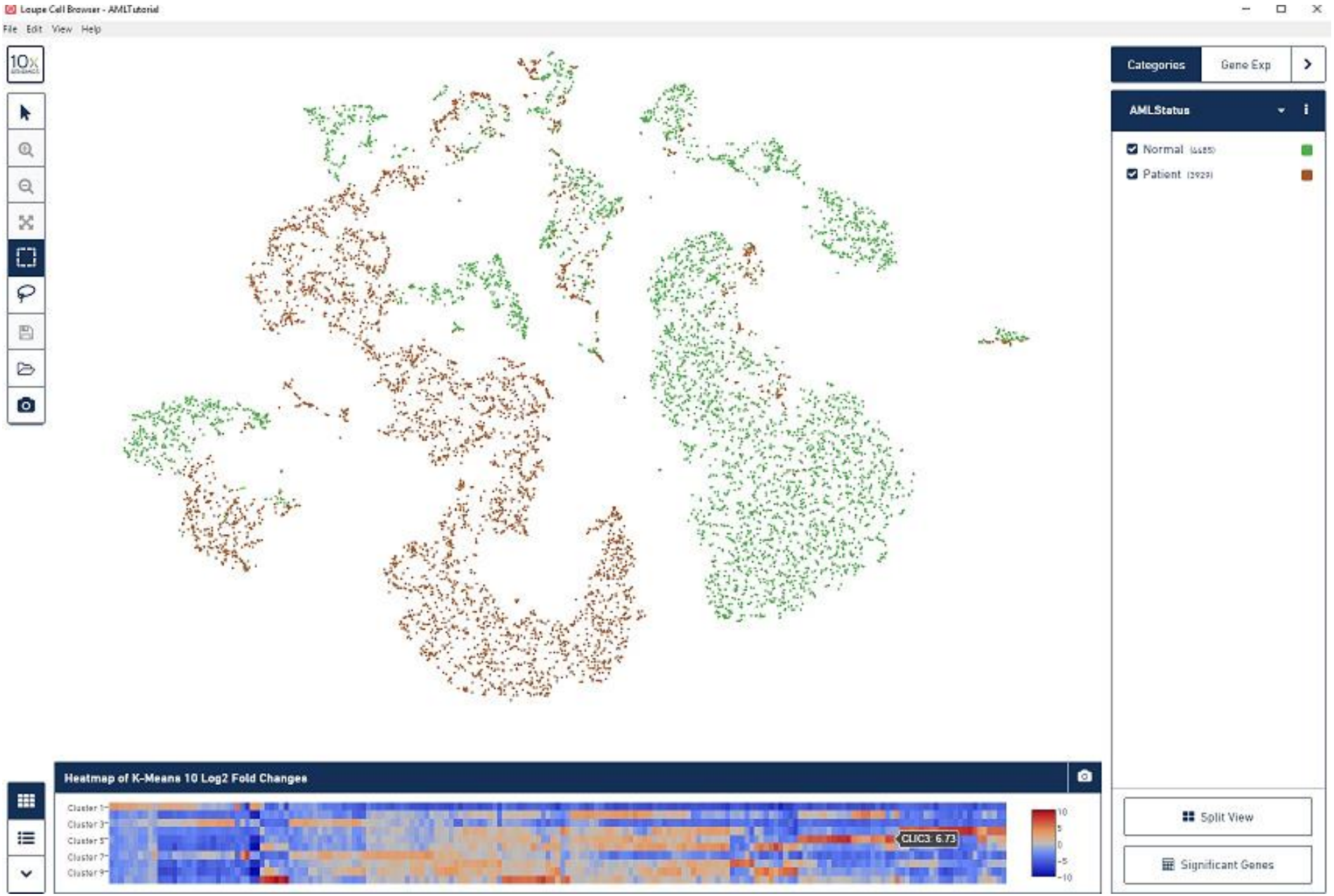
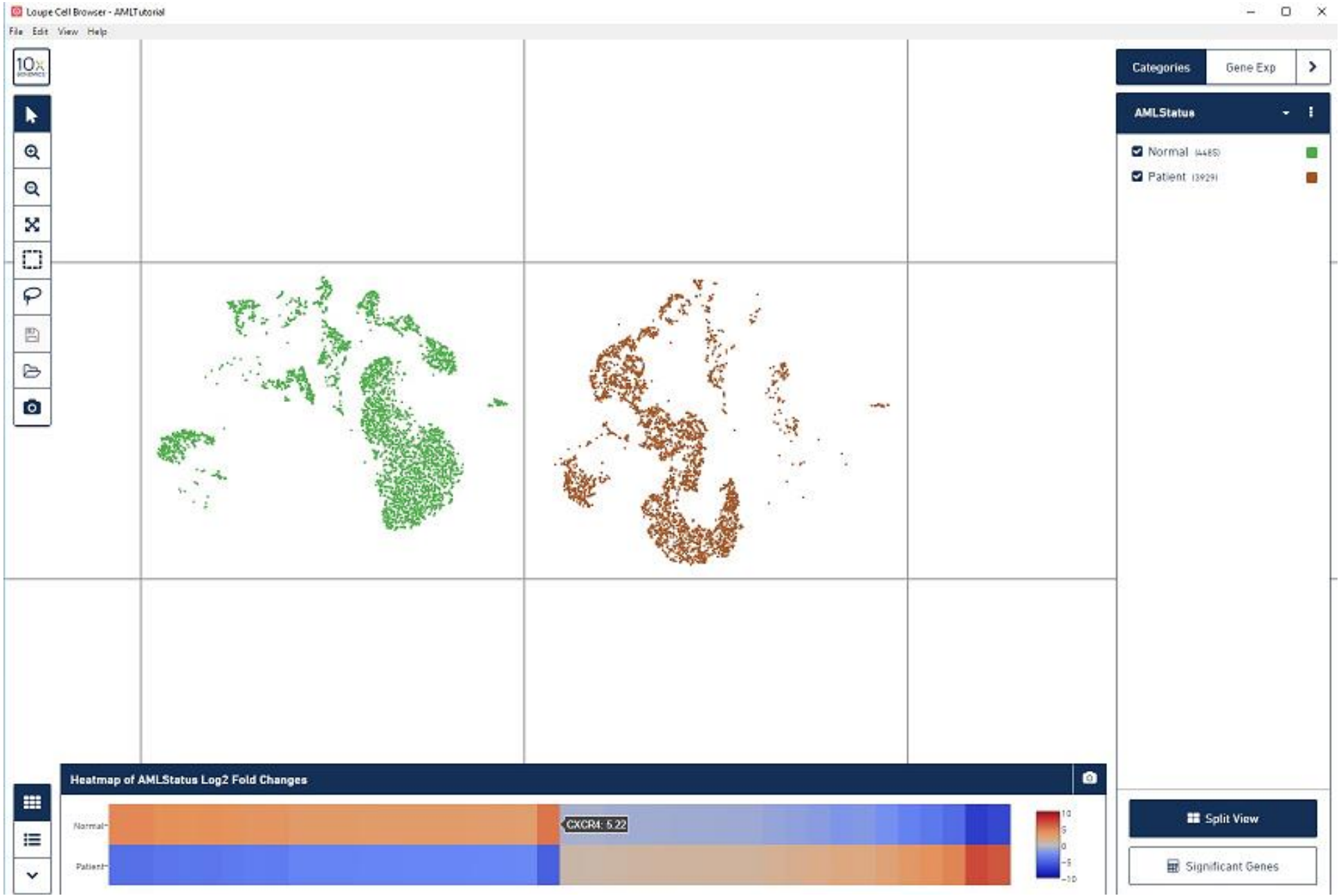
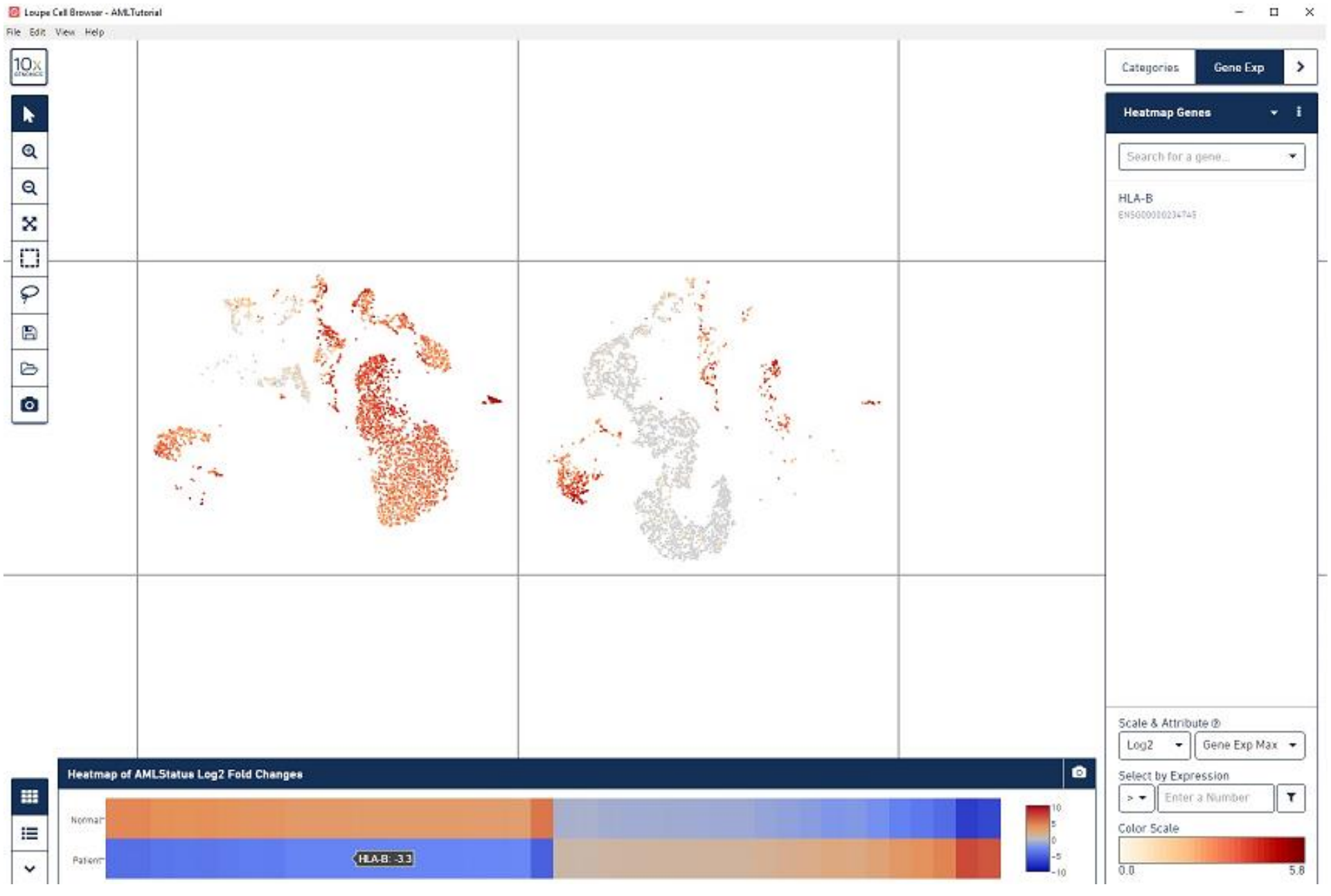


10x Loupe Cell Browser

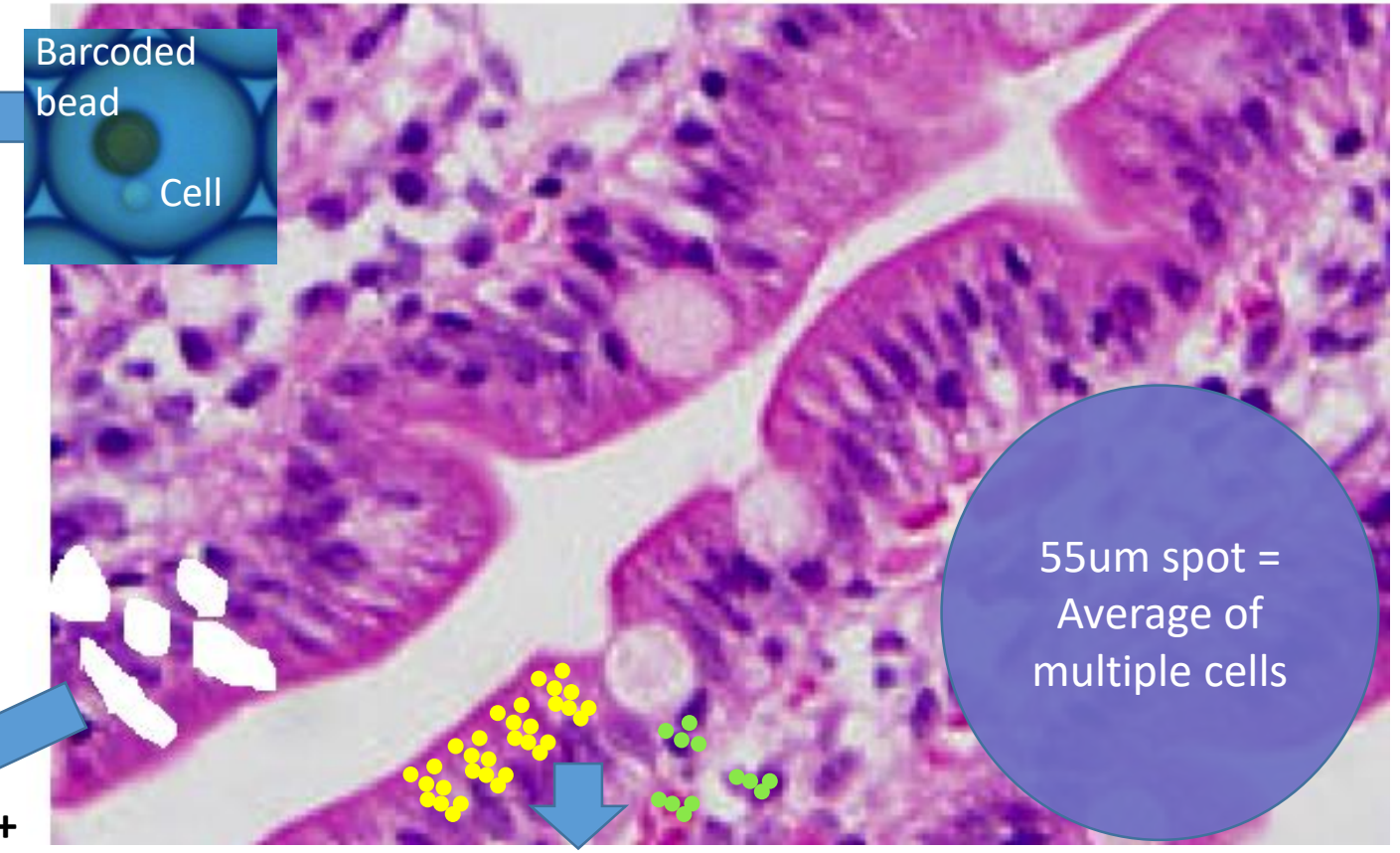


10x Loupe Cell Browser



Spatial single cell transcriptomics, directly from tissues -> original location of cells, cell-to-cell interactions…

Enzymatic/mechanic dissociation-> suspension-> barcode in droplets (e.g. 10x Genomics Chromium)



Laser microdissection + sequencing

Gene probing/in situ hybridization (e.g. Merfish, NanoString)

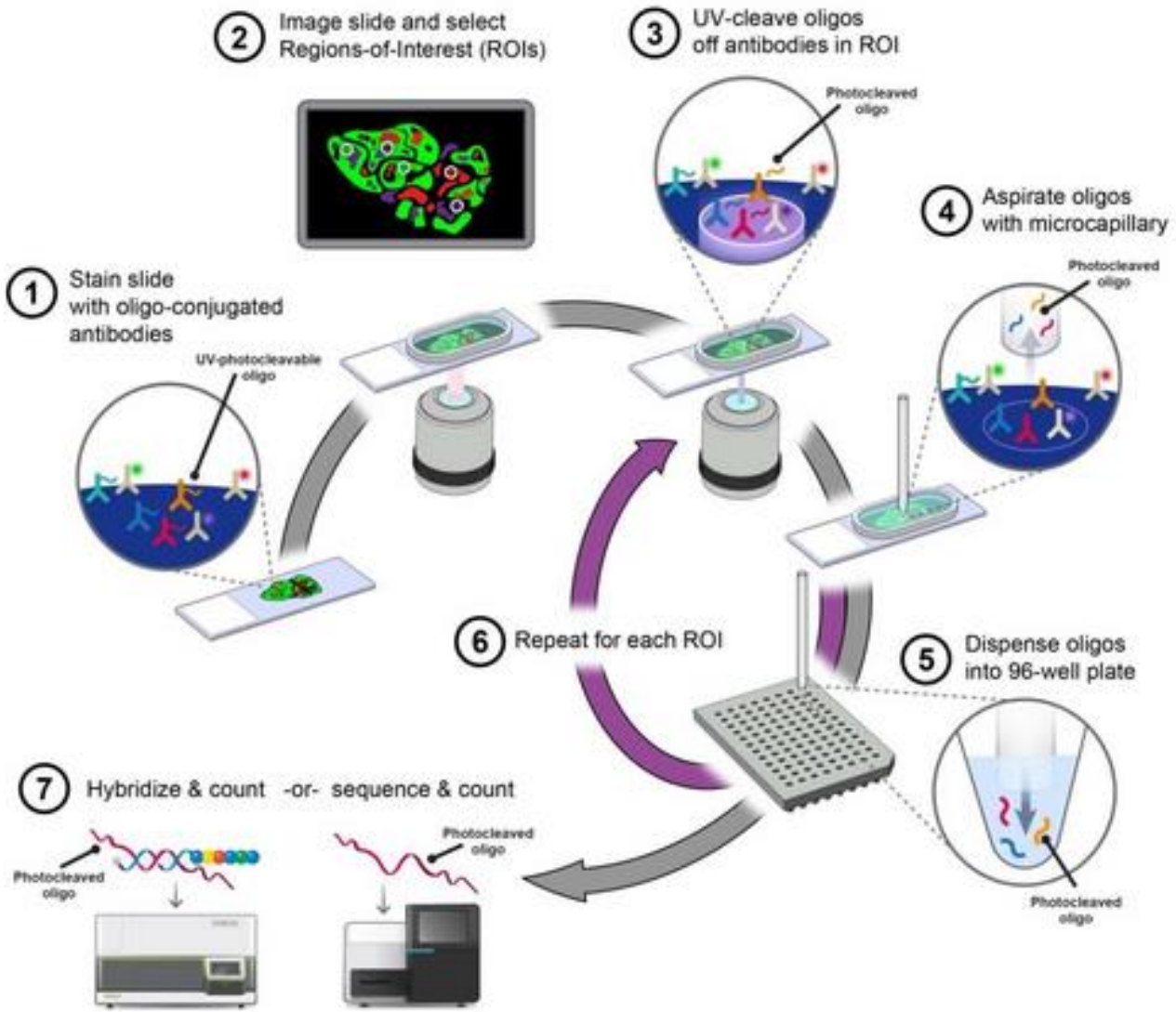
Spatial microarrayed barcode slides (e.g. Spatial Transcriptomics -> acquired by 10x Genomics -> Visium system

NanoString nCounter and GeoMx

GeoMx® Digital Spatial Profiler
Your GPS for Biology

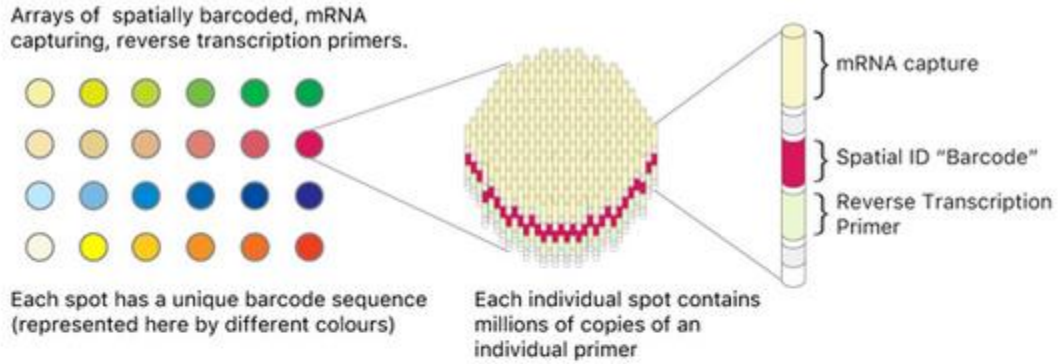
Morphological context and high-plex protein or RNA expression data – now possible from just one FFPE slide.

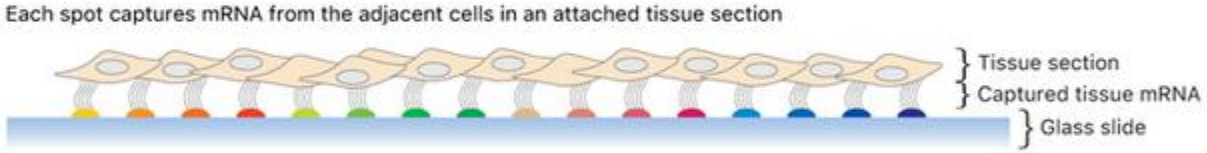
- Quantify up to 96 proteins and over 1000 RNA targets with spatial context
- Process 10-20 slides /day with readout on the **nCounter® System** or with NGS
- Preserve precious samples with non-destructive processing



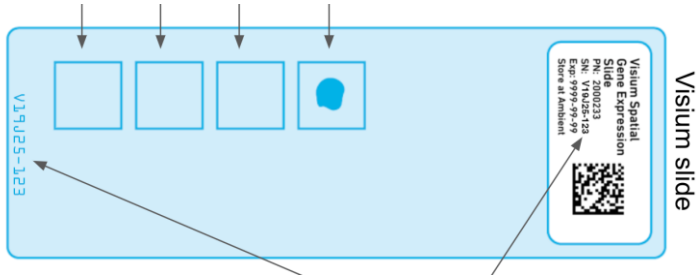
SPATiAL TRANSCRIPTOMICS

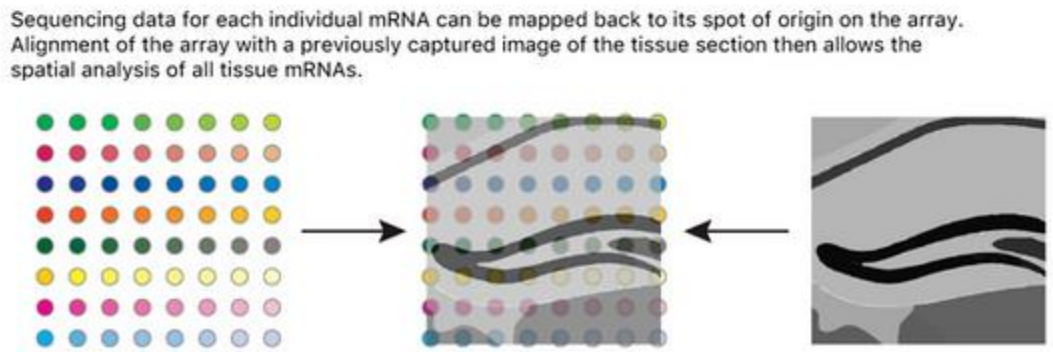
Spatial Transcriptomics
(Lundeberg, Frisen, Sweden)





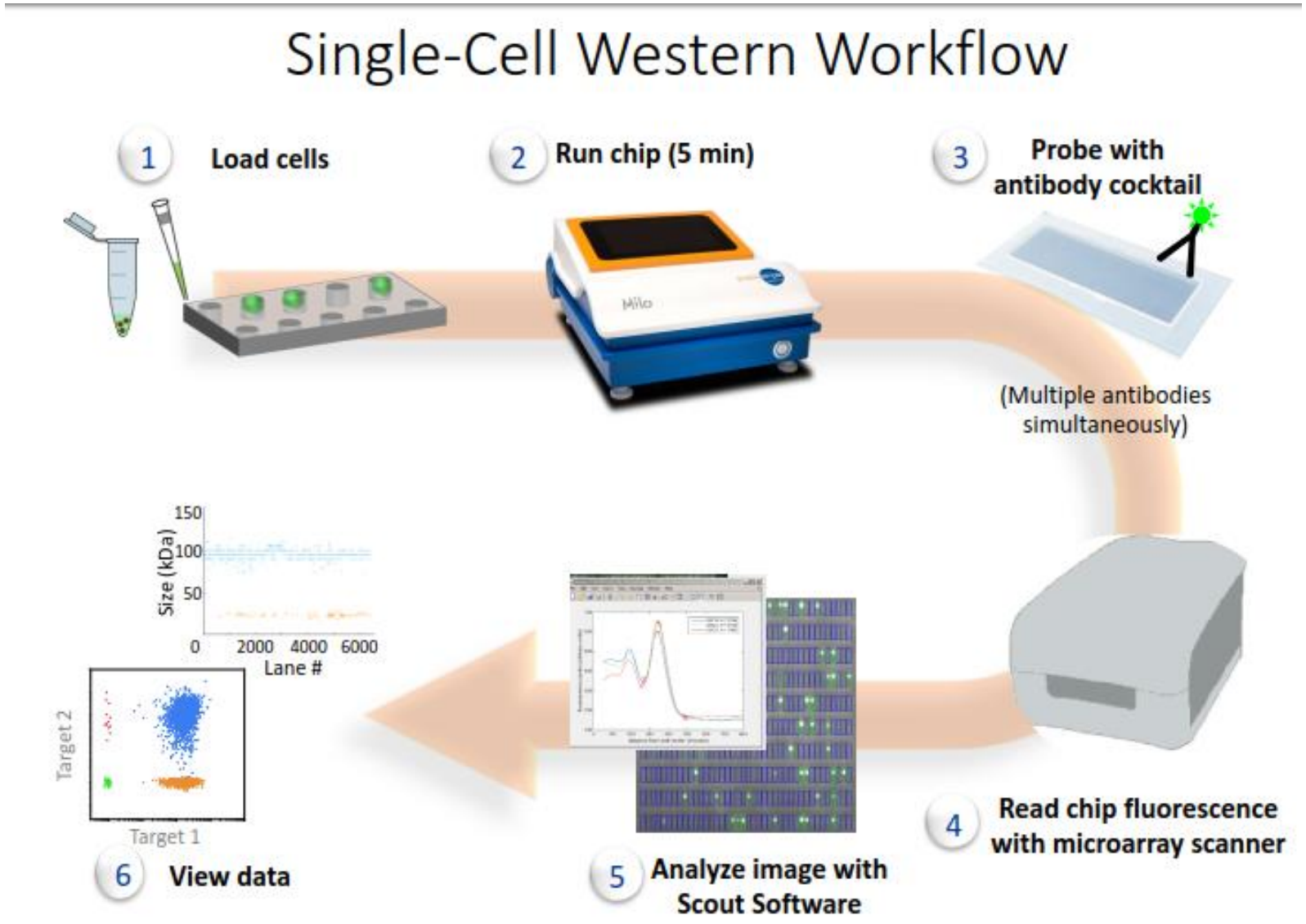
Acquired by 10x Genomics
-> Visium system
(at FIMM)
4 x 5000 spots
Diameter 55um each

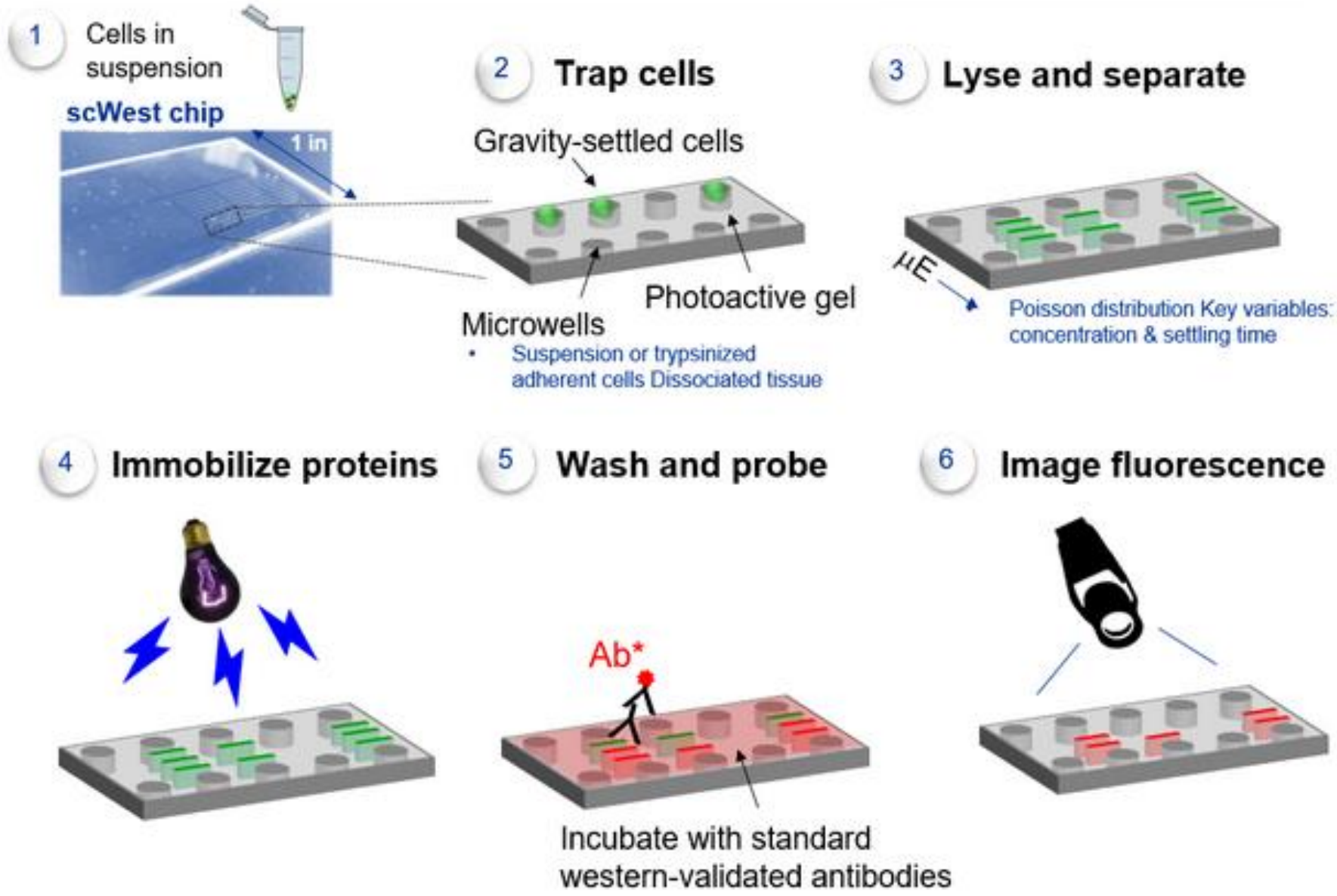




https://en.wikipedia.org/wiki/Spatial_transcriptomics

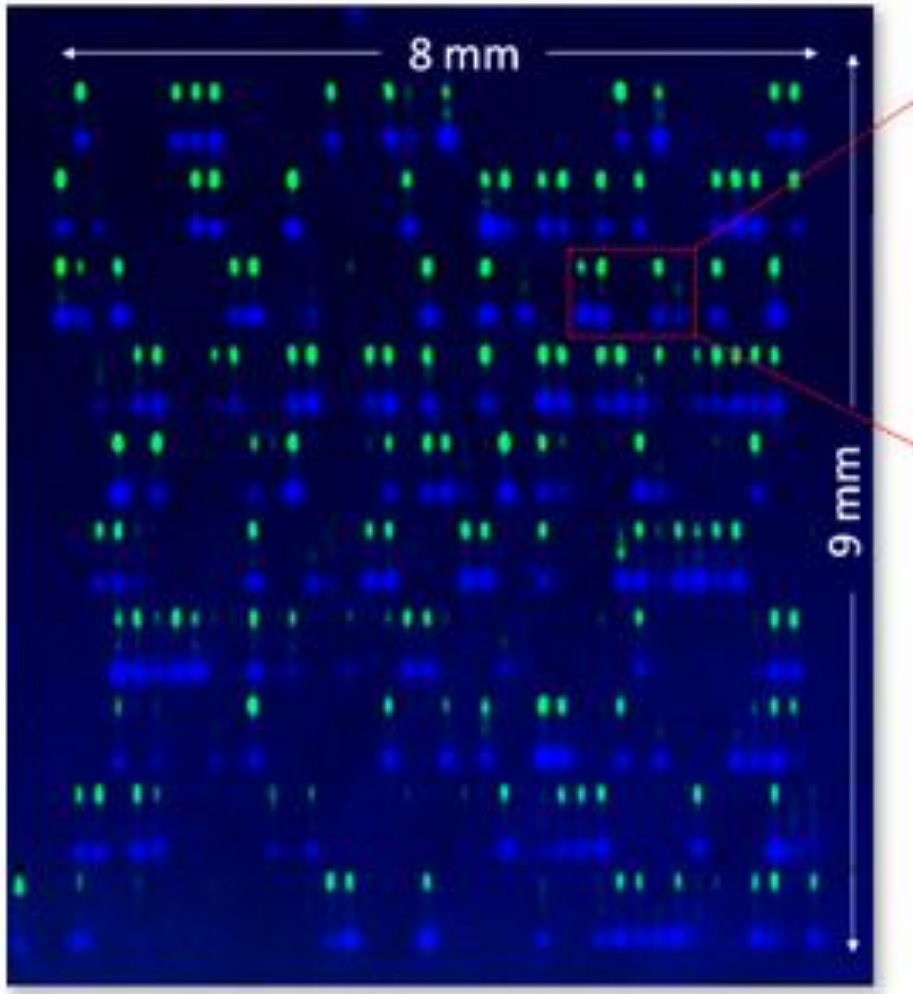
MILO single cell Western blotting



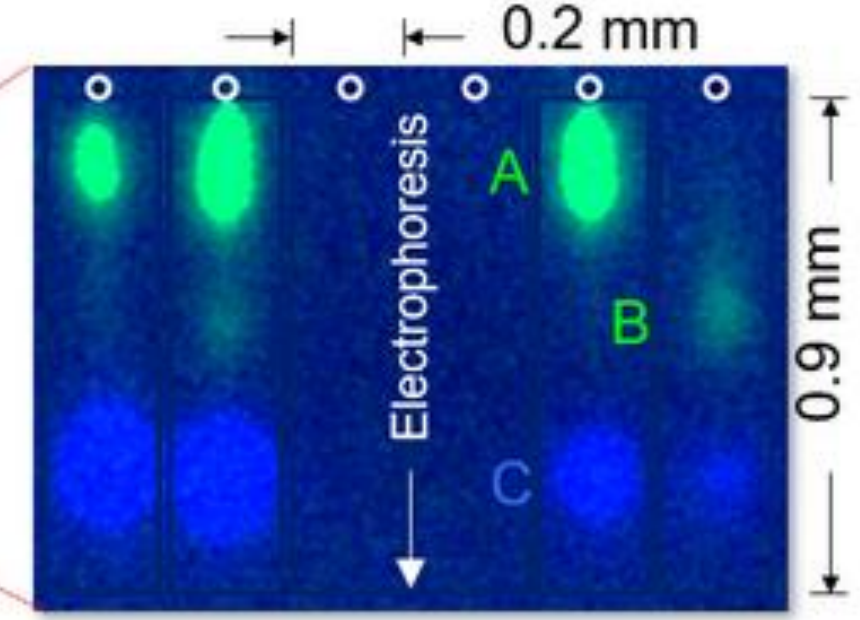

1
Cells in suspension
scWest chip
1 in
2
Trap cells
Gravity-settled cells
Photoactive gel
Microwells
Suspension or trypsinized adherent cells Dissociated tissue
3
Lyse and separate
μE
Poisson distribution Key variables: concentration & settling time
4
Immobilize proteins
5
Wash and probe
Ab*
Incubate with standard western-validated antibodies
6
Image fluorescence

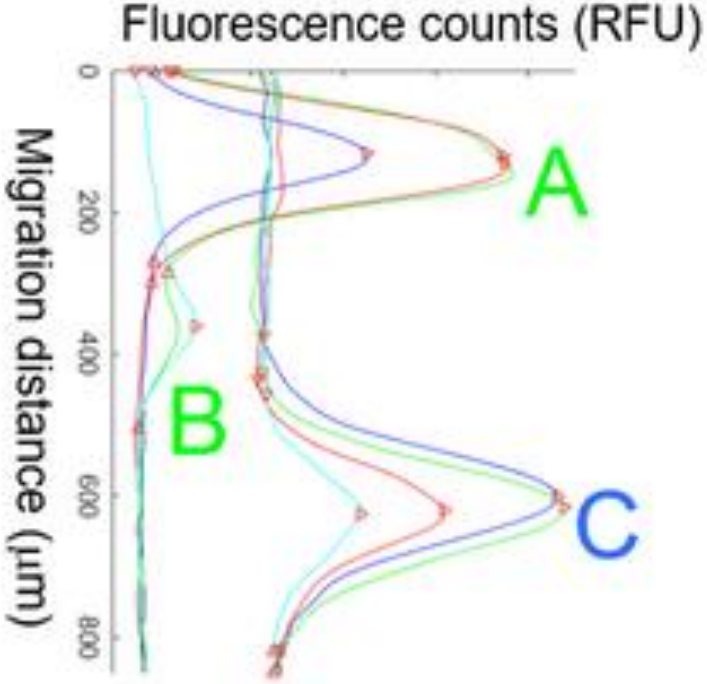
Slide scanning
(2 colours)
at FuGU Agilent
microarray
scanner

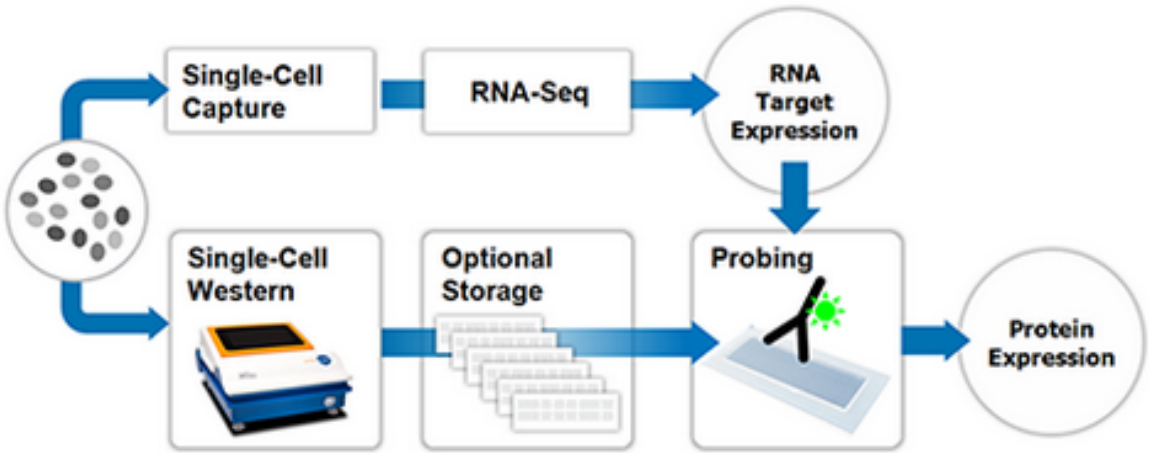
Scout software
for slide image
analyses
(free at
ProteinSimple
and also in UH
software
center)



1/16th of scWest chip







I need protein validation of my single-cell RNA-seq data

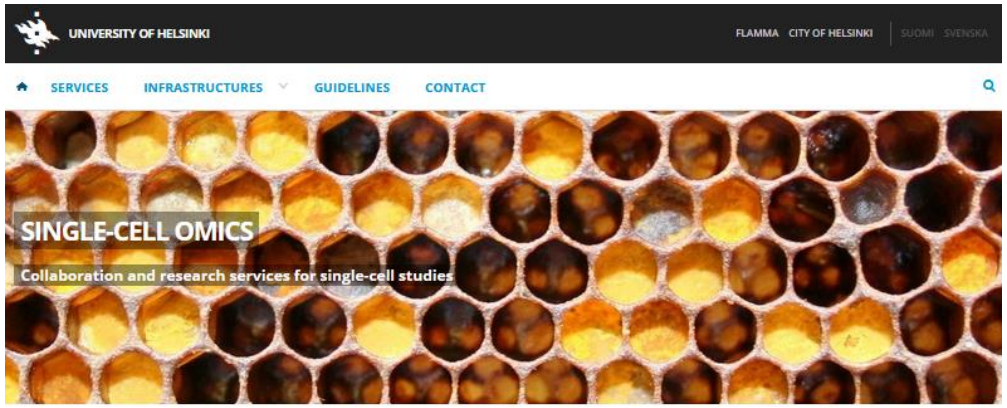
Use conventional western antibodies to validate your single-cell RNA-seq data with single-cell protein data. Plus, scWest chips can be archived for up to 9 months after you run them so you have plenty of time to get your sequencing results back before you have to probe for your targets of interest.

Learn More ›

Available single cell platforms in Helsinki area

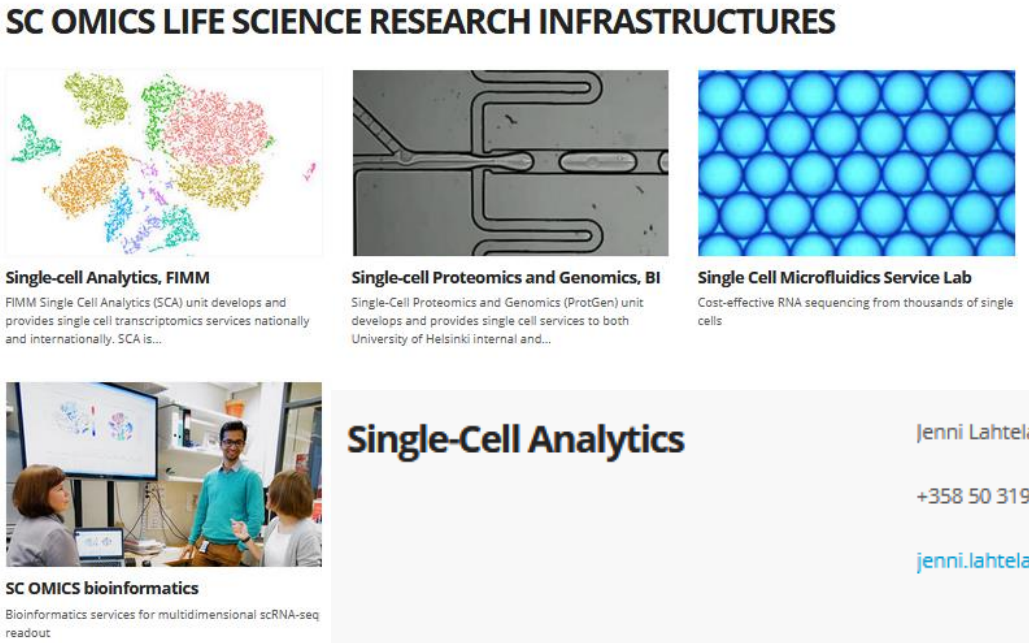
Single cell omics core facilities in Helsinki
https://www.helsinki.fi/en/infrastructures/single-cell-omics
FIMM: 10x Chromium, index sorting. CellenOne dispenser
Saavalainen/Functional Genomics Unit (FuGU):
Drop-seq, Seq-well, index sorting
Viikki: inDrops, Polaris, Helios CyTOF (mass cytometer)
FACS cores for single cell plate sorting

Other devices elsewhere:
Fluidigm C1: Anna Vähärautio/Biomedicum, Kristiina Iljin/VTT, Seppo Ylä-Herttuala/UEF
10x Chr**o**miums in Helsinki: FIMM, Viikki, Aaltonen and Alitalo labs



Single-Cell omics platform aims at bringing the latest single cell omics tools available for the research community. Services based on novel instruments are now available for single cell genomics and single cell proteomics to enable cutting-edge research in many areas of molecular biology and molecular medicine.

HiLIFE
HELSINKI INSTITUTE OF LIFE SCIENCE



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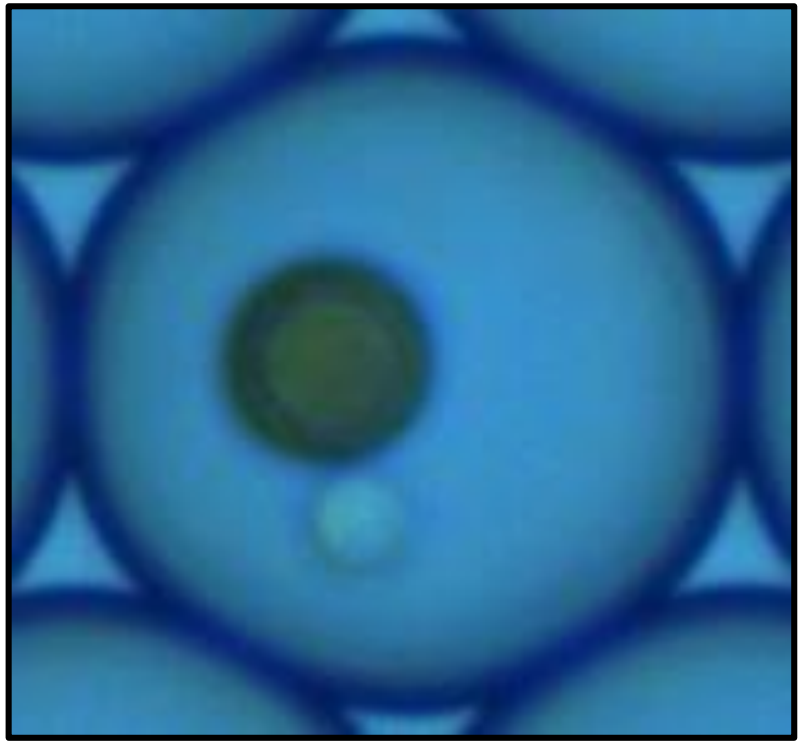

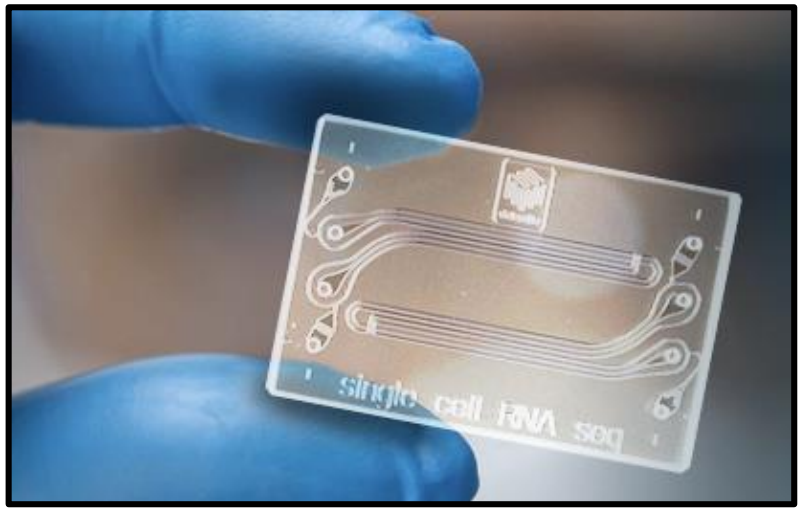

Thank you for your attention!