

Microscopy based -omics

EMBL Rome



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Microscopy based -omics

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Talk and resources: https://github.com/alcrevenna/Fluigent_webinar

Twitter: @alvarocrevenna



Overview

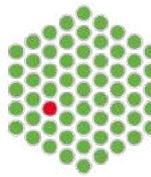
- Methods overview
- Choosing the right method
- Interfacing fluidics with a microscope
- Image analysis

Fluorescence microscopy based -omics

- Proteomics
- Transcriptomics
- Genomics
- Connectomics

Fluorescence microscopy based -omics

- Proteomics → Tissue profiling
- Transcriptomics → Spatial Transcriptomics
- Genomics → Chromatin conformation
- Connectomics → Neural connectivity (Neuroscience)



A good starting point



Review | Open Access |

DNA-Barcoded Fluorescence Microscopy for Spatial Omics

Florian Schueder, Eduard M. Unterauer, Mahipal Ganji, Ralf Jungmann

First published: 08 October 2020 | <https://doi.org/10.1002/pmic.201900368> | Citations: 1

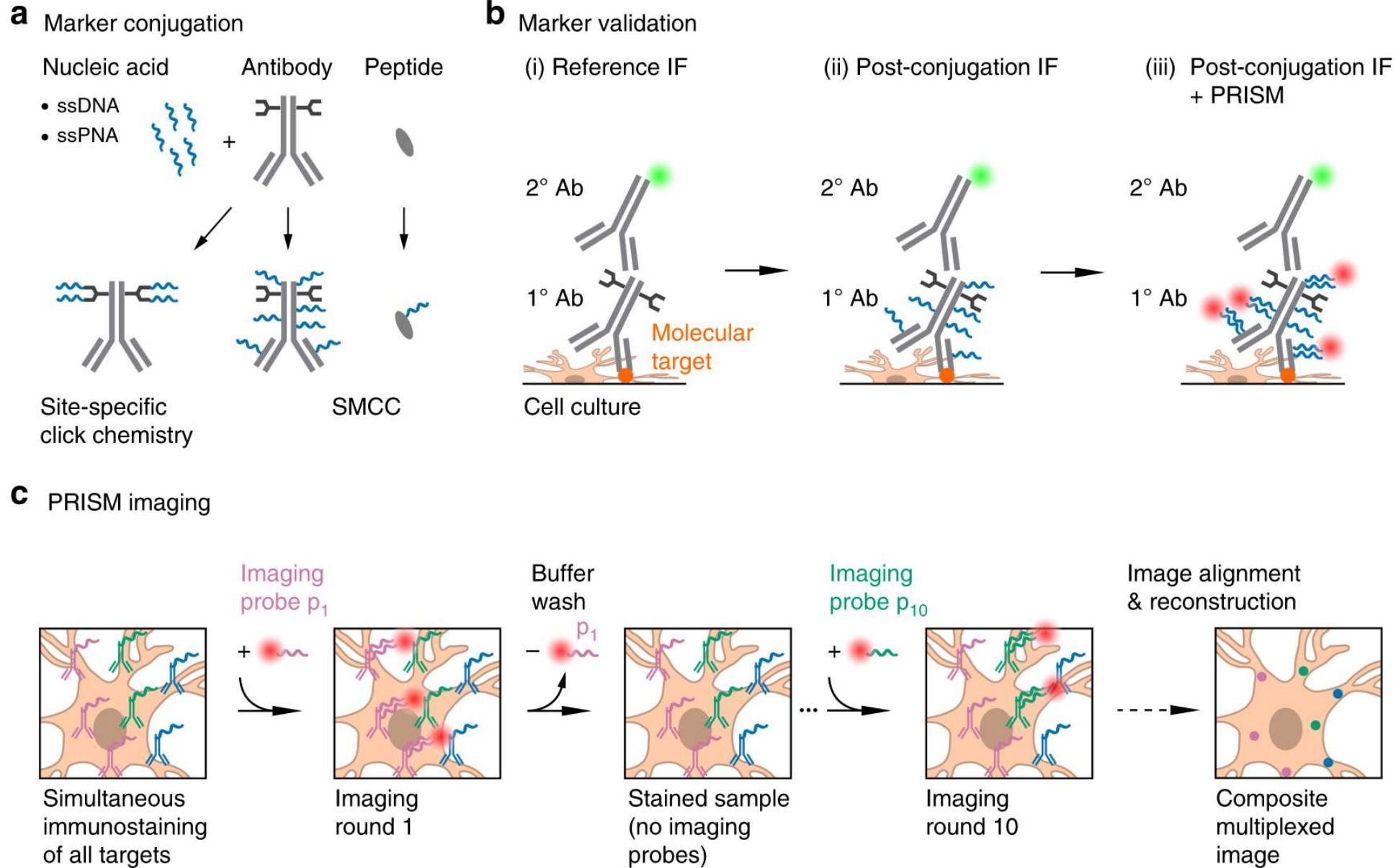
Fluorescence microscopy based -omics

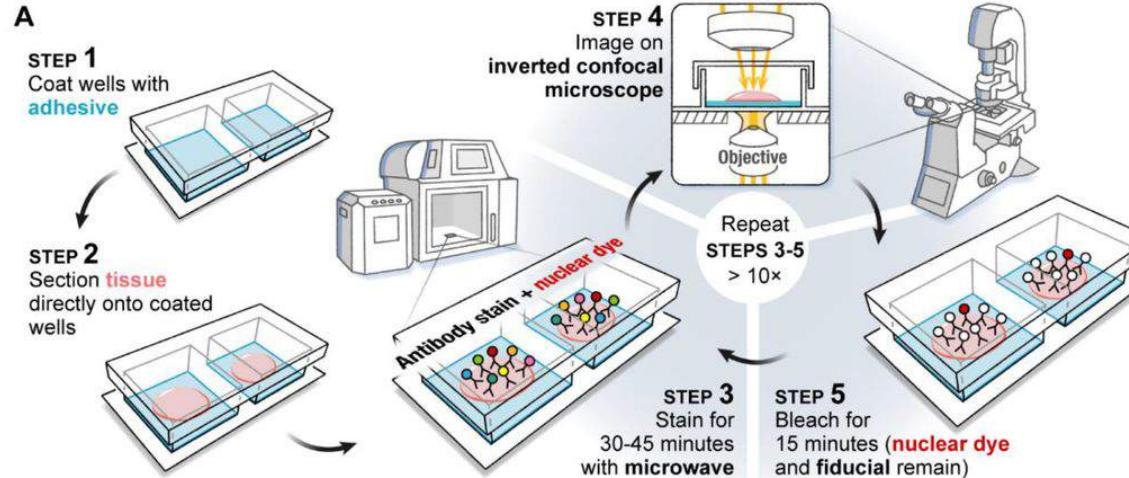
- Proteomics
- Transcriptomics
- Genomics
- Connectomics

Fluorescence microscopy based -omics

- Proteomics
 - Transcriptomics
 - Genomics
 - Connectomics
- Traditional IF is limited to 4-5 colors
--> How to scale up?

PRISM: Probe-based Imaging for Sequential Multiplexing





Benefits:
Simple cycles
Standard microscope

Limitations

Manual processing of samples for every cycle
Requires Fluorescent-conjugated primary Ab

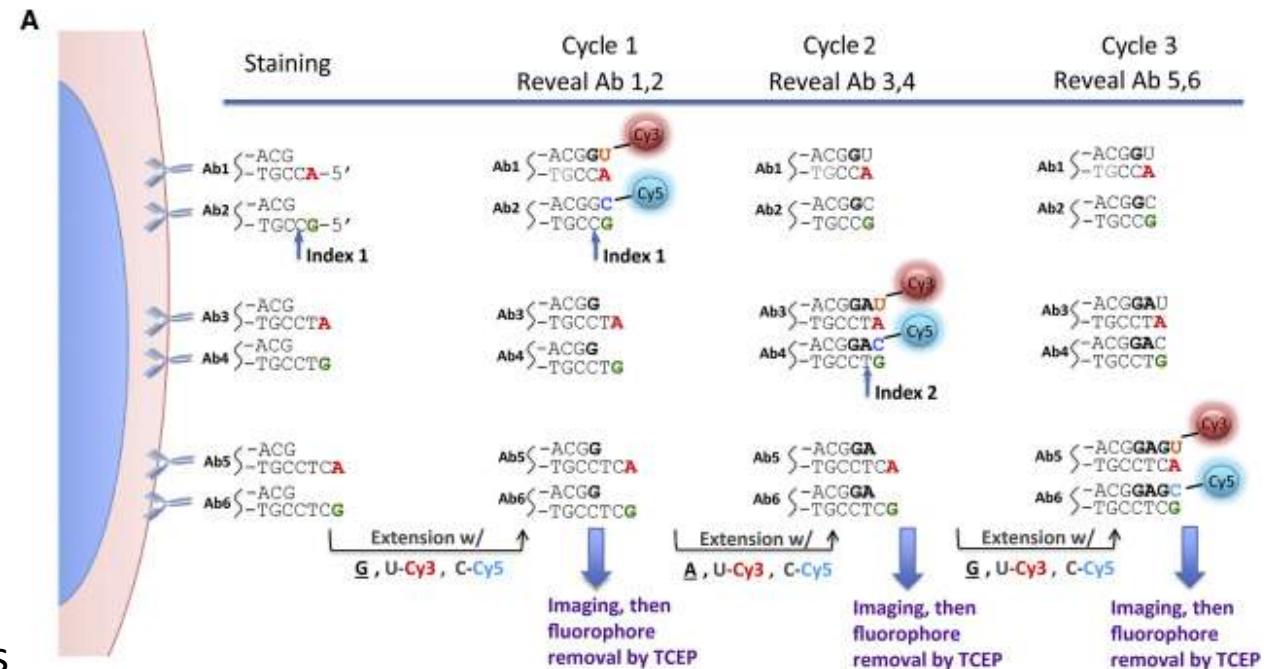
Radtke et al Biorxiv 2020

CODEX

Benefits:
Can be automated

Limitations:
Requires DNA-conjugated Ab
More complex cycles are needed

Goltsev et al Cell 2018
Commercialized by Akoya Biosciences

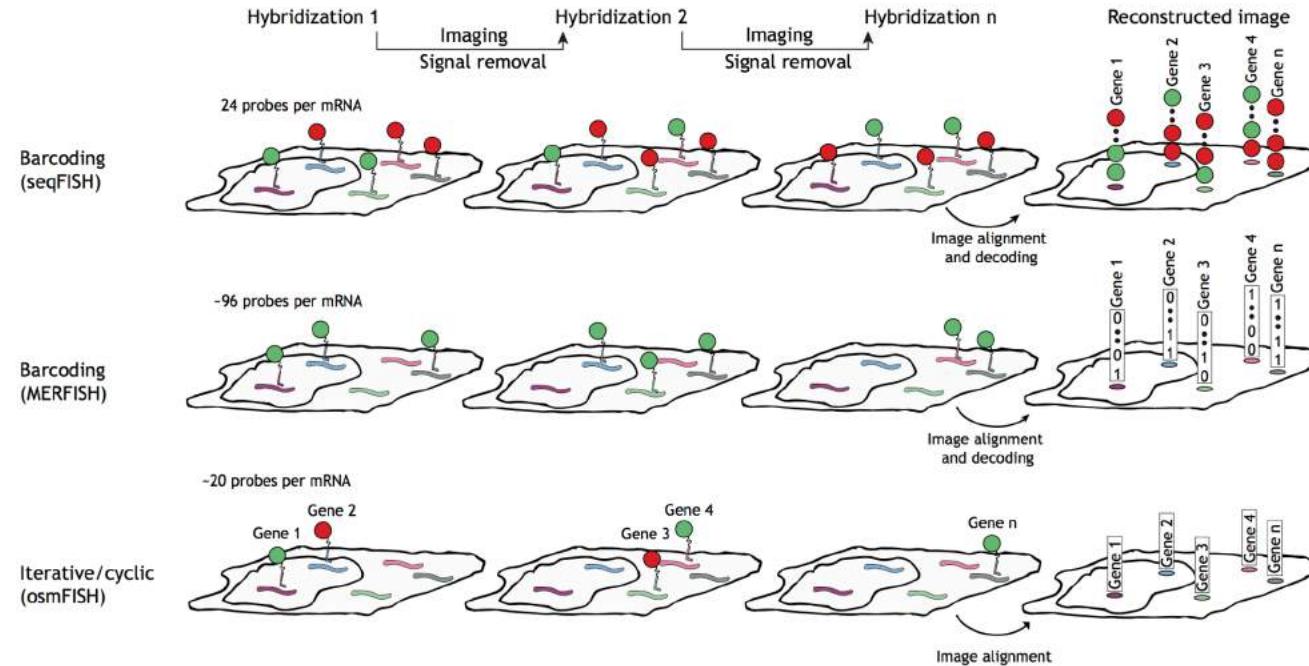


Fluorescence microscopy based -omics

- Proteomics
- Transcriptomics
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- Connectomics

Spatial transcriptomics methods

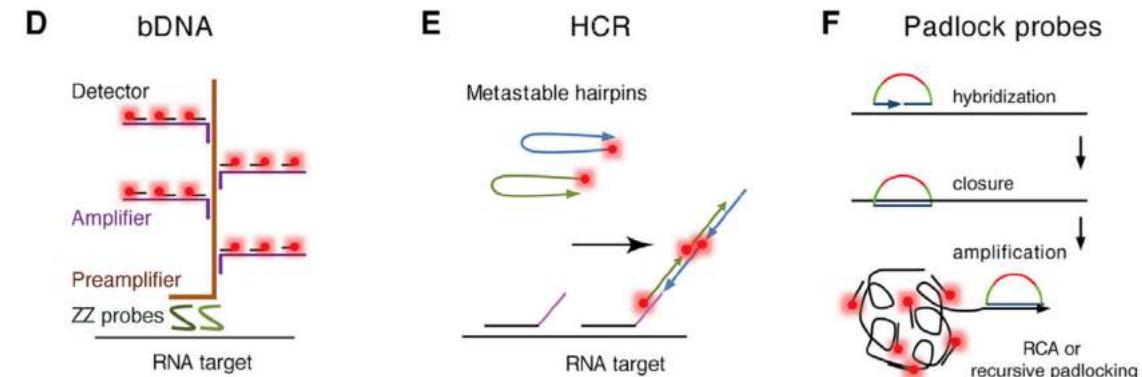
Multiplexing methods



seqFISH+ is scalable
Needs 24-50 unique primary sequences

Amplification methods

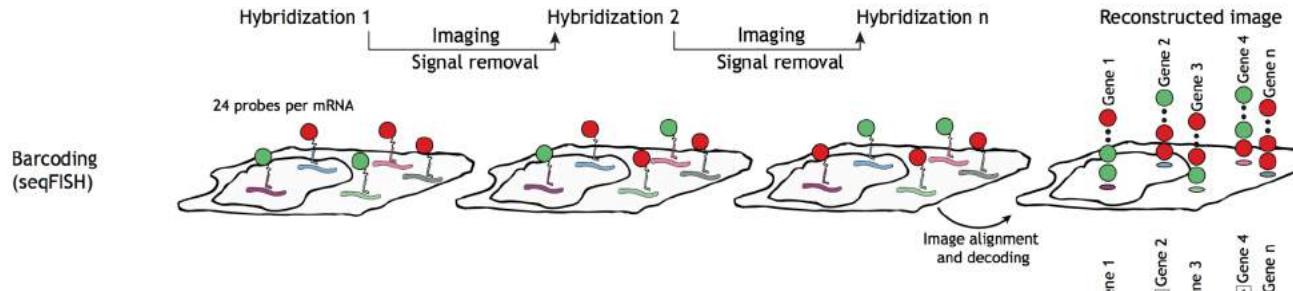
StarMap, SABER-FISH, Barista, etc.



Amplification methods need in theory 1 probe/RNA
(in practice they use 4-10)
It may be good for proteins with repeats or small RNAs

Spatial transcriptomics methods

Multiplexing methods

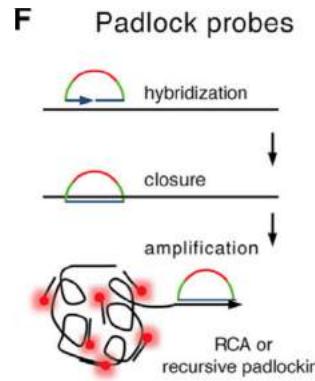


seqFISH+ is scalable

Needs 24-50 unique primary sequences

- Requires fluidics
- A single experiment can take 4-5 days
- Only 1 experiment can run on the scope
- 10,000 genes

Amplification methods

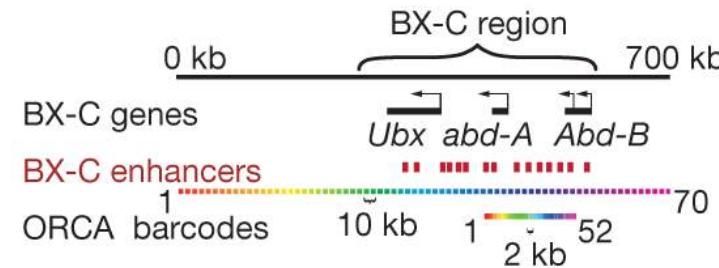
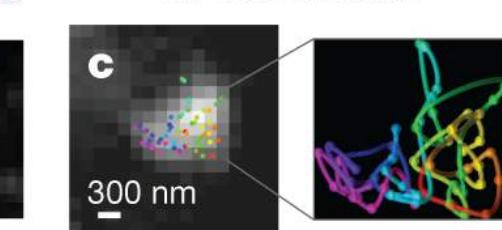
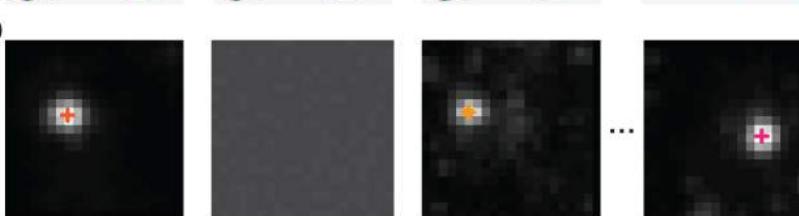
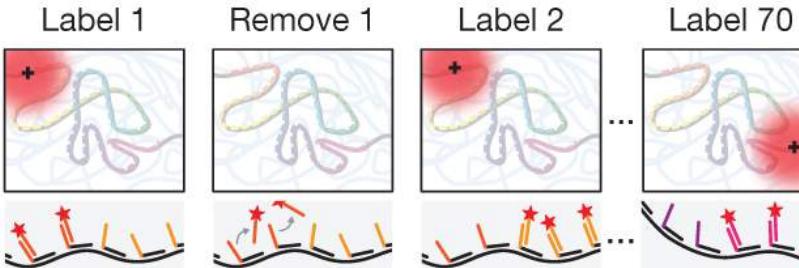
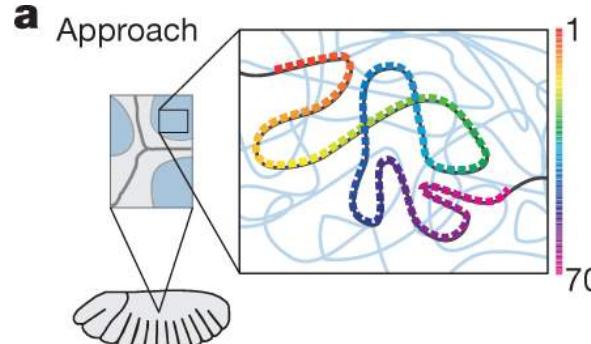


- 4-10 probes per RNA
- Does not require fluidics
- A single sample needs to be processed 7 times (ISS) or scales with RNA species (osmFISH, SABER-FISH)
- Multiple samples could be imaged in an automated manner using a slide scanner
- Max of about 200 genes

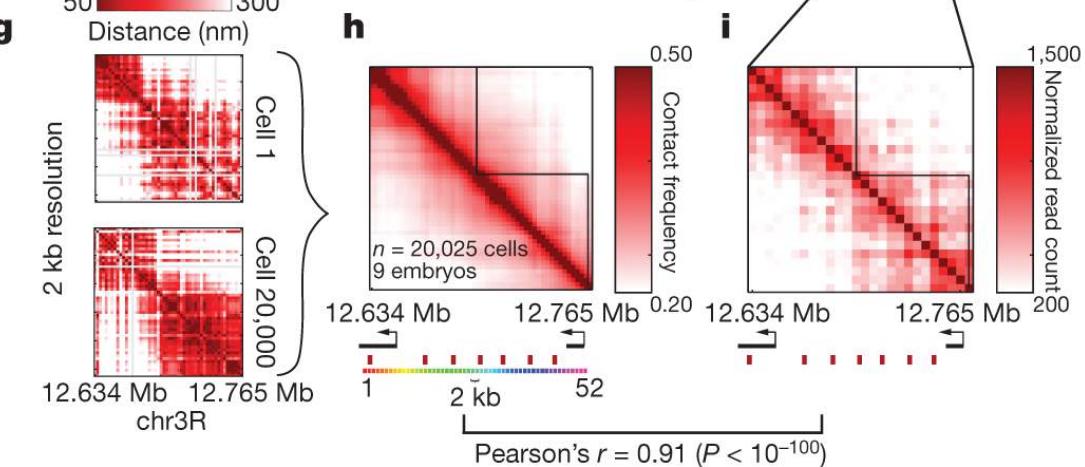
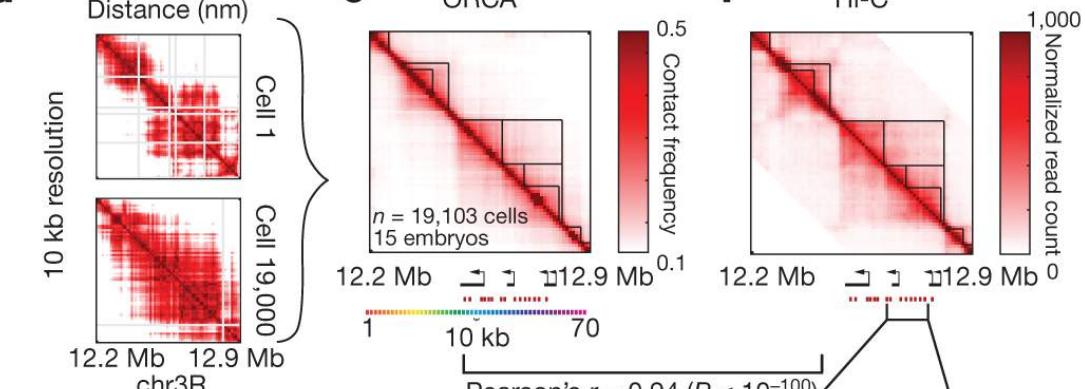
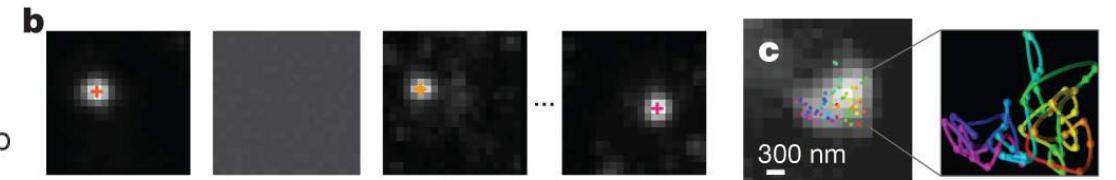
Fluorescence microscopy based -omics

- Proteomics
- Transcriptomics
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- Connectomics

Chromatin conformation



3D reconstruction

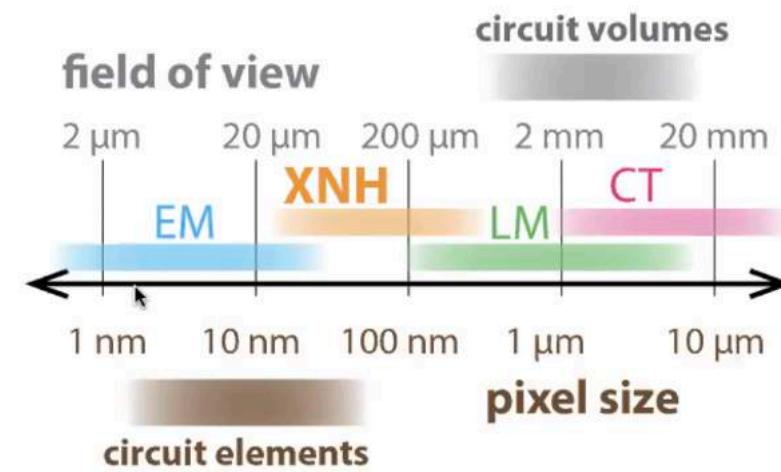
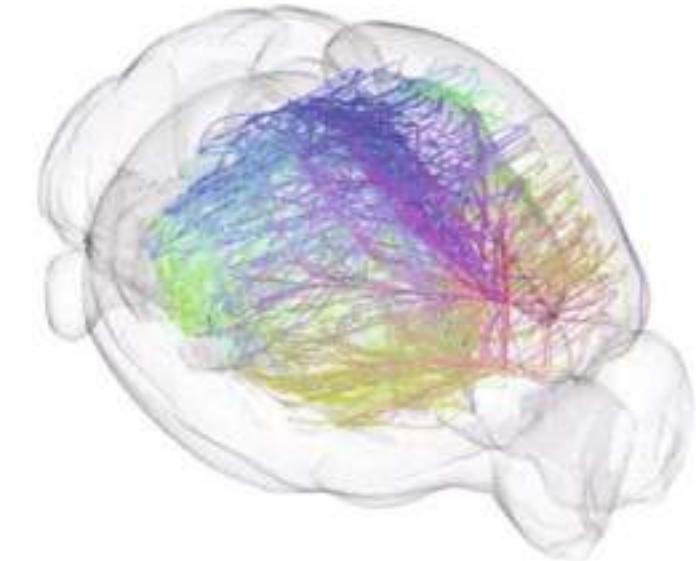
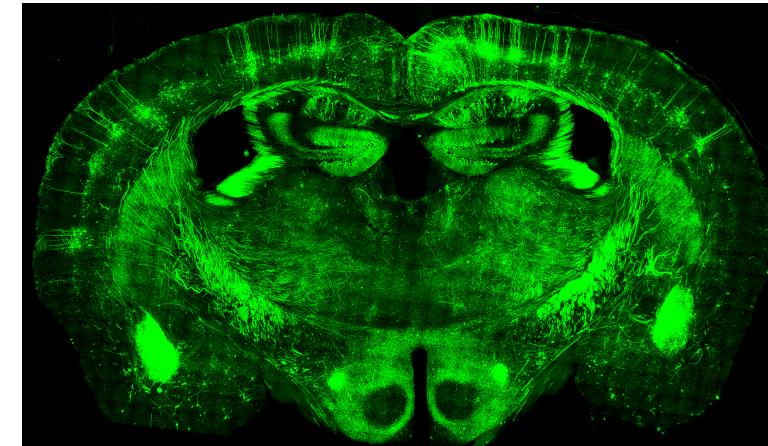
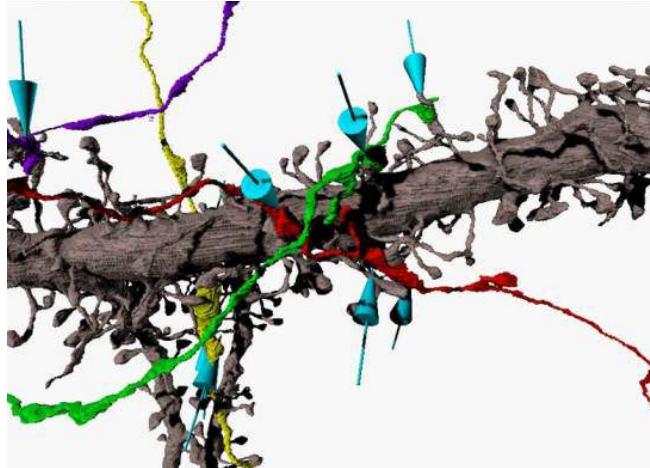


Similar principle for transcriptomics but probes target genomic traits

Fluorescence microscopy based -omics

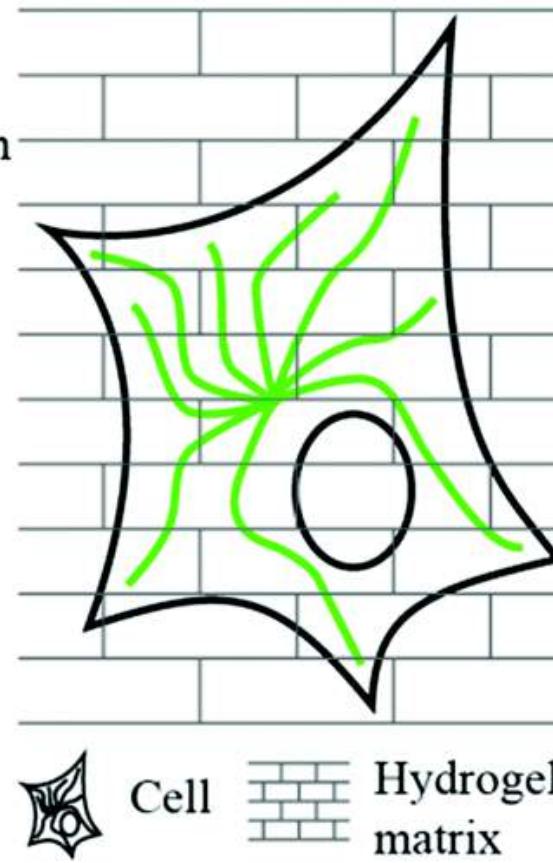
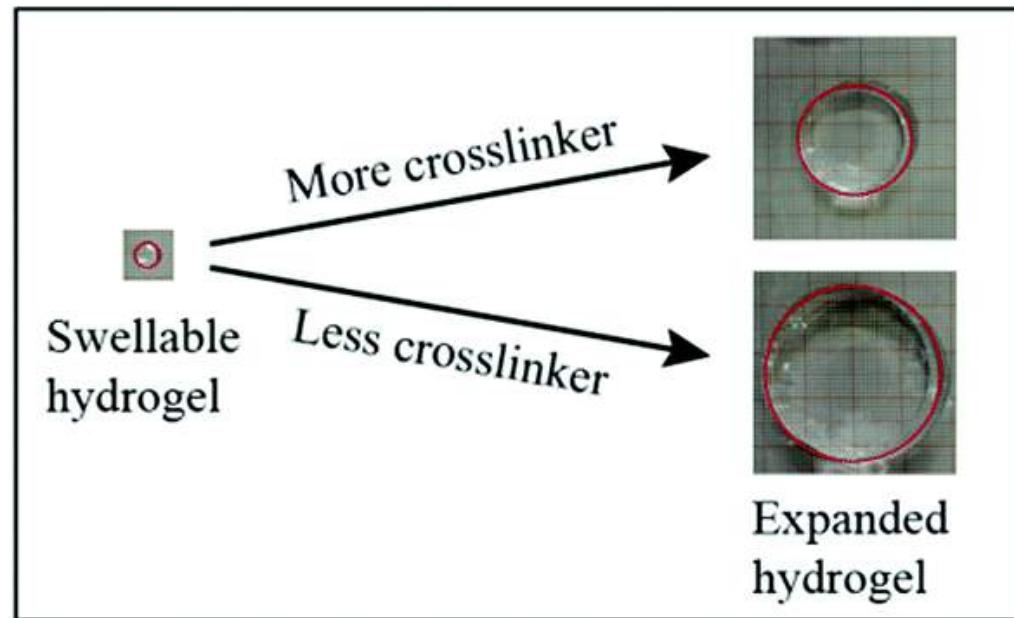
- Proteomics
- Transcriptomics
- Genomics
- Connectomics

Connectivity, a problem of scales



Connectomics

Idea 1: ExM + SIM



Combine:

SIM increase in resolution by a factor of 2

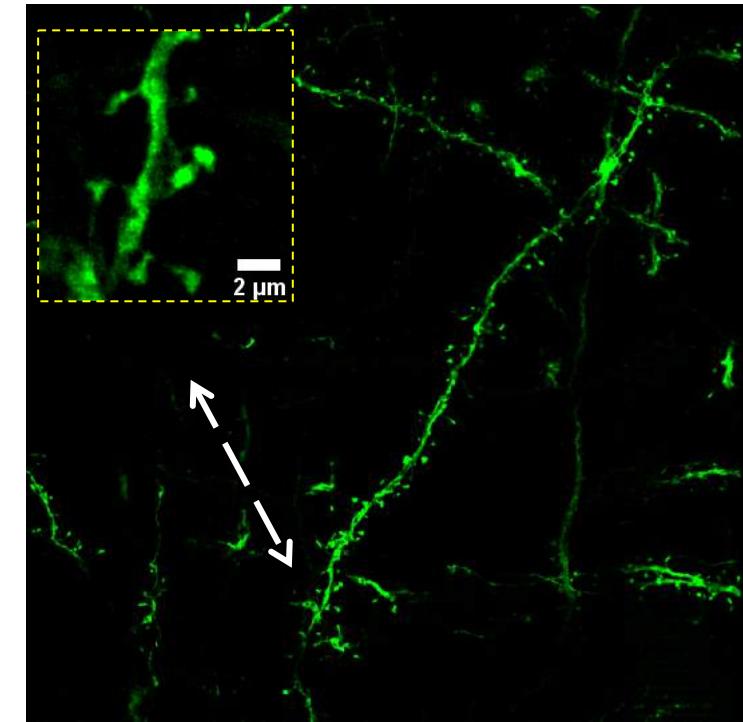
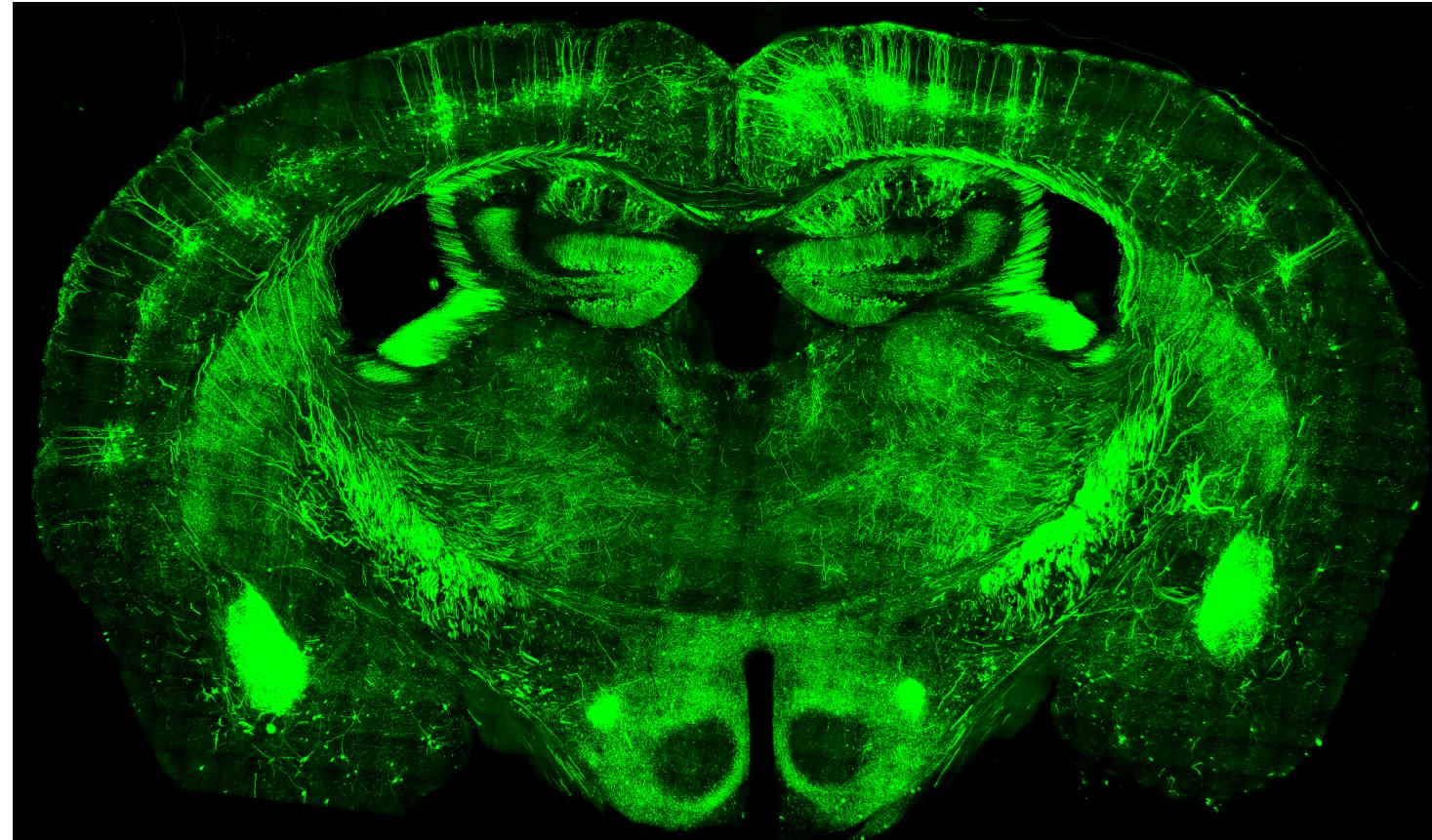
ExM

Increase in resolution by a factor of 4

= increase by a factor of 8!

Connectomics

Idea 1: ExM + SIM



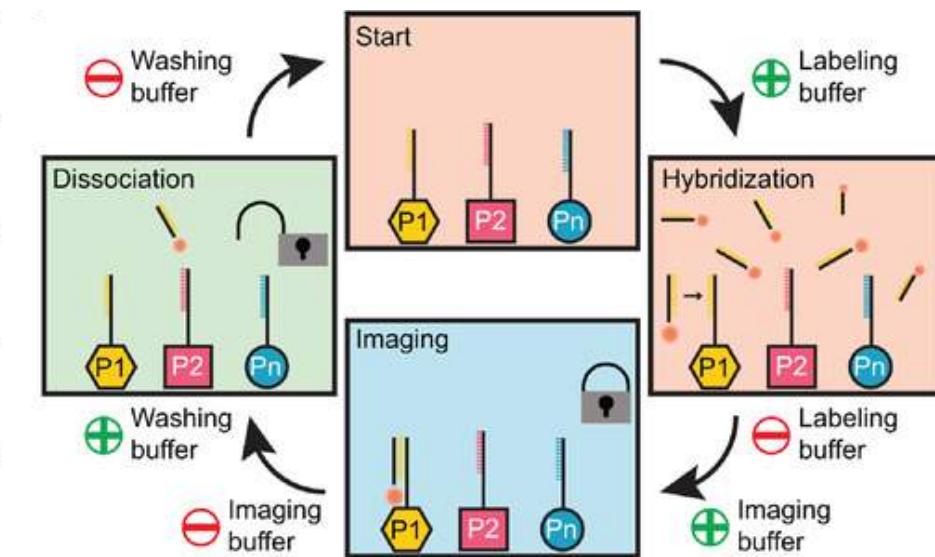
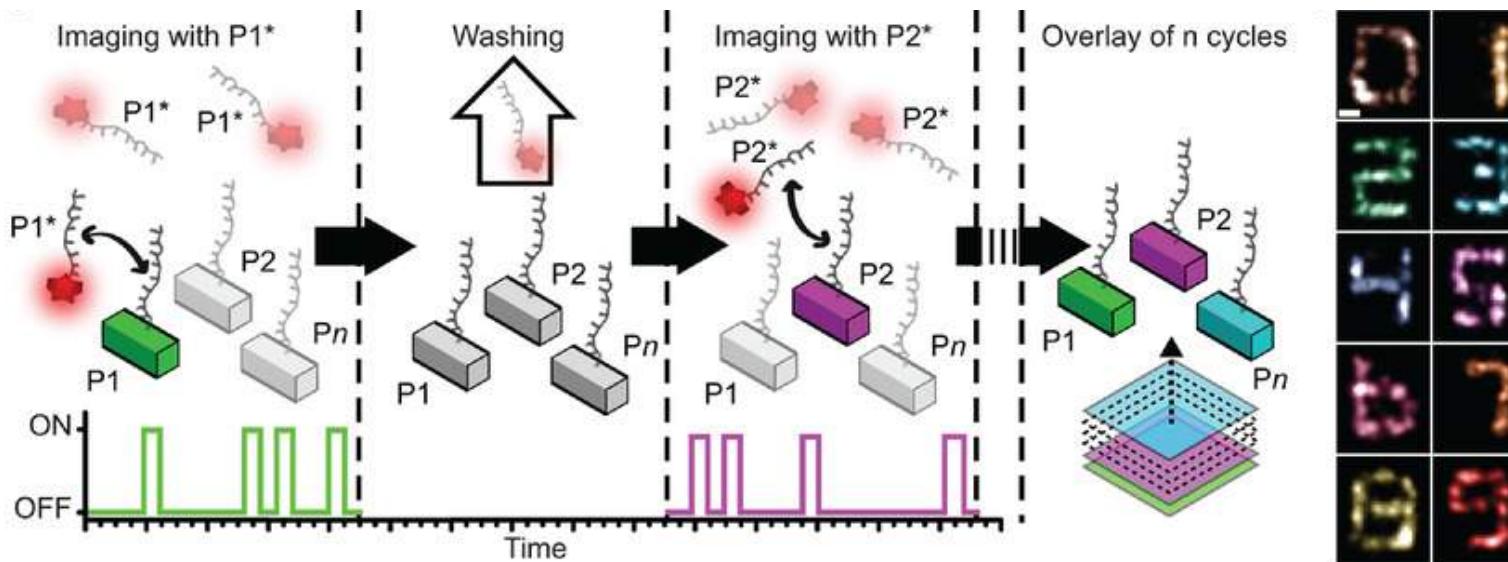
SIM + ExM

Thy1GFP
Mouse coronal section

Limitations: short working distance of high NA objectives, cannot image whole volume

Connectomics

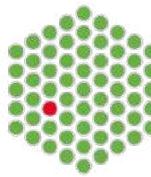
Idea 2: Light sheet + DNA-PAINT



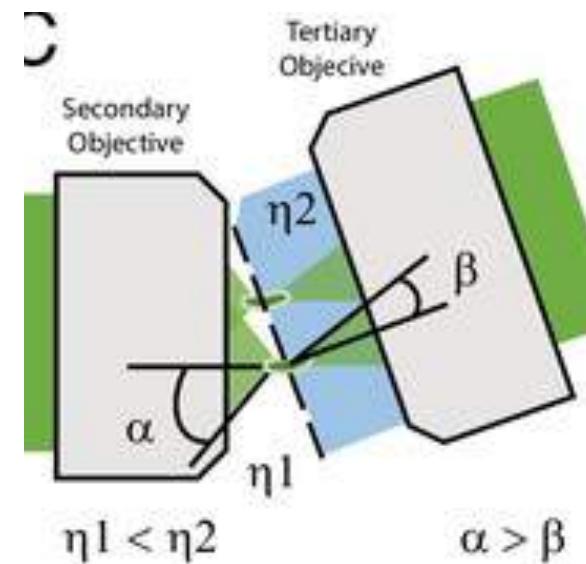
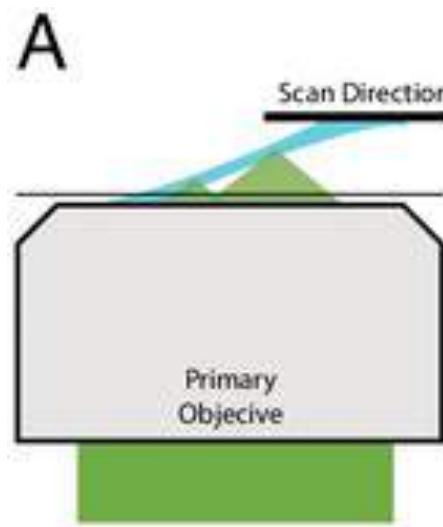
Limitations:

- conventional DNA-PAINT is rather slow (1-2 h for ~20nm resolution)
- Sectioning needed
- DNA-conjugated Ab required
- Small field of view (80x80 μm)

Schueder et al Proteomics 2020

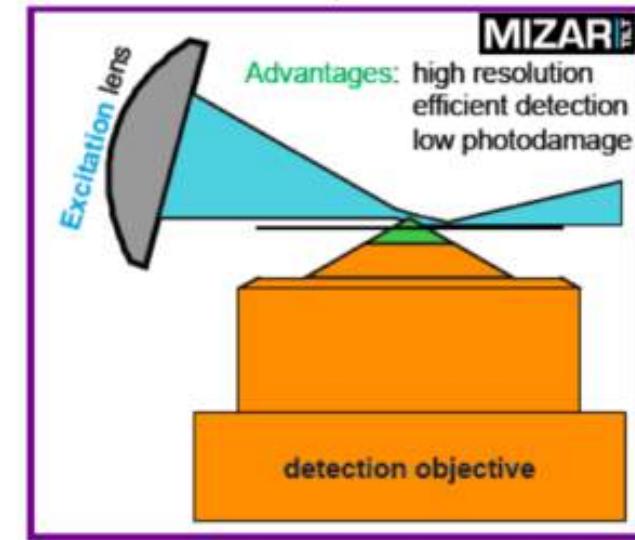


Open-top light sheet imaging at high resolution



Sapoznik et al eLife 2020

Will work for large tissue
Larger field of view
Fast scanning

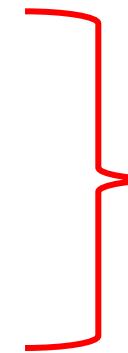


Fadero et al J Cell Biol 2018

Will work only for cells and small tissue/organisms
Large field of view
Fast scanning

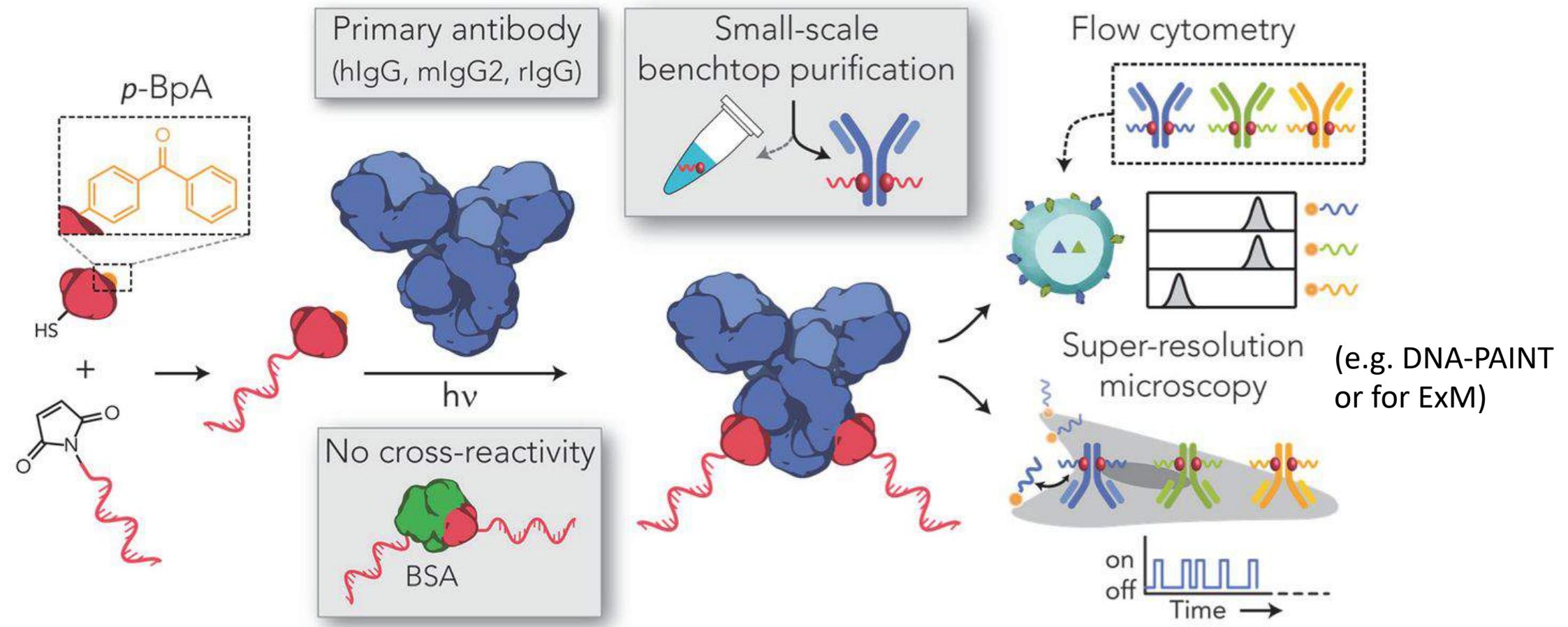
Fluorescence microscopy based -omics

- Tissue profiling – PRISM, CODEX
- Connectomics – DNA-PAINT, ExM
- Spatial Transcriptomics

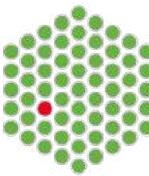


Need DNA-labeled Ab's

An efficient DNA-Antibody labelling pipeline



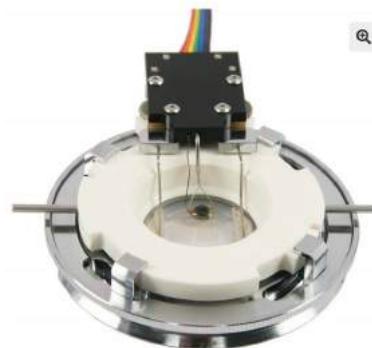
Interfacing a fluidic device with a microscope



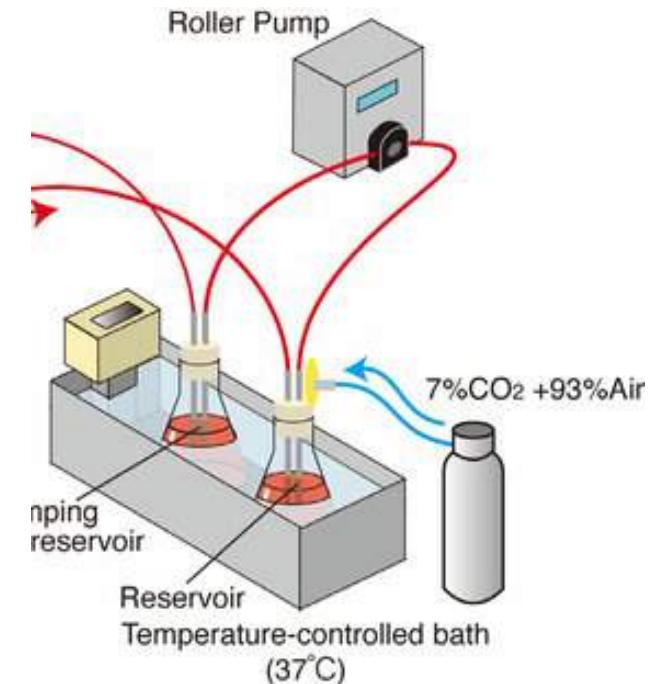
Microscope-based –omics need:



Microscope



A flow cell



A pump system and reservoirs

Microscopes tested

- Nikon TiE with a V3 X-light

spinning disk

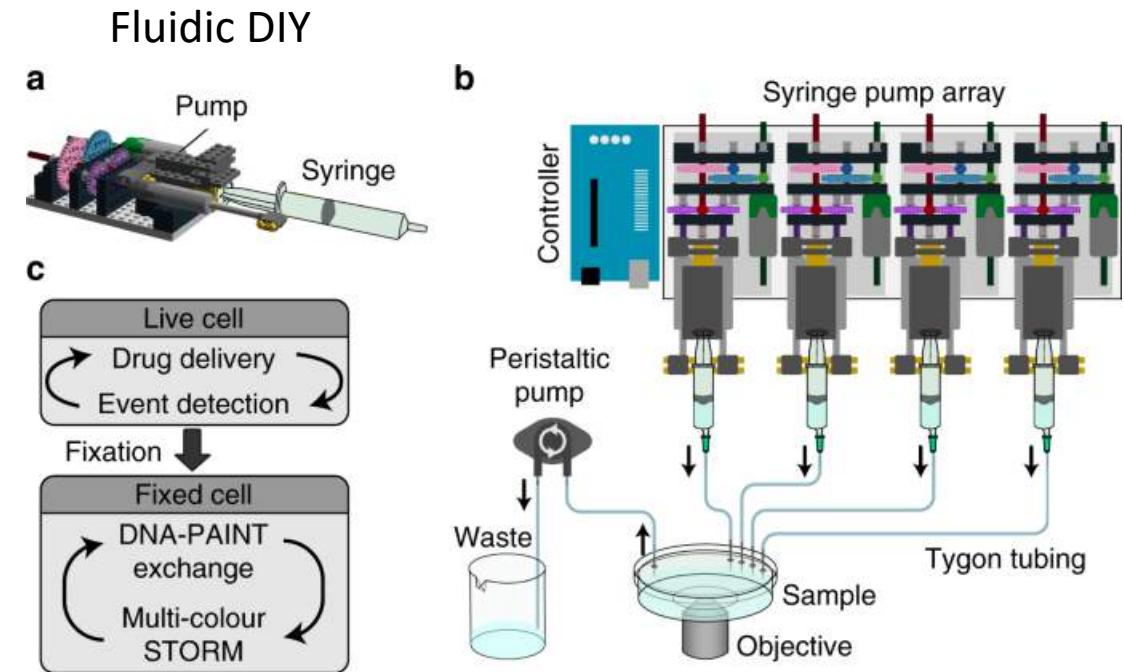
- Thunder Inverted WF Leica
- Elyra 7 Zeiss

Microscopes tested

- Nikon TiE with a V3 X-light spinning disk
 - → Simple
- Thunder Inverted WF Leica
 - → Simple
- Elyra 7 Zeiss
 - → Possible but cumbersome

Microscopes tested

- Nikon TiE with a V3 X-light spinning disk
- Thunder Inverted WF Leica
- Elyra 7 Zeiss



Almada et al Nat Commun 2019

Integrated fluidic unit "Aria" from Fluigent

Essential components

1. USB data card

Example:

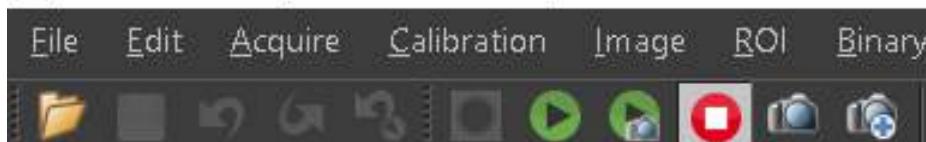


For Leica a trigger card
sold by Leica is needed

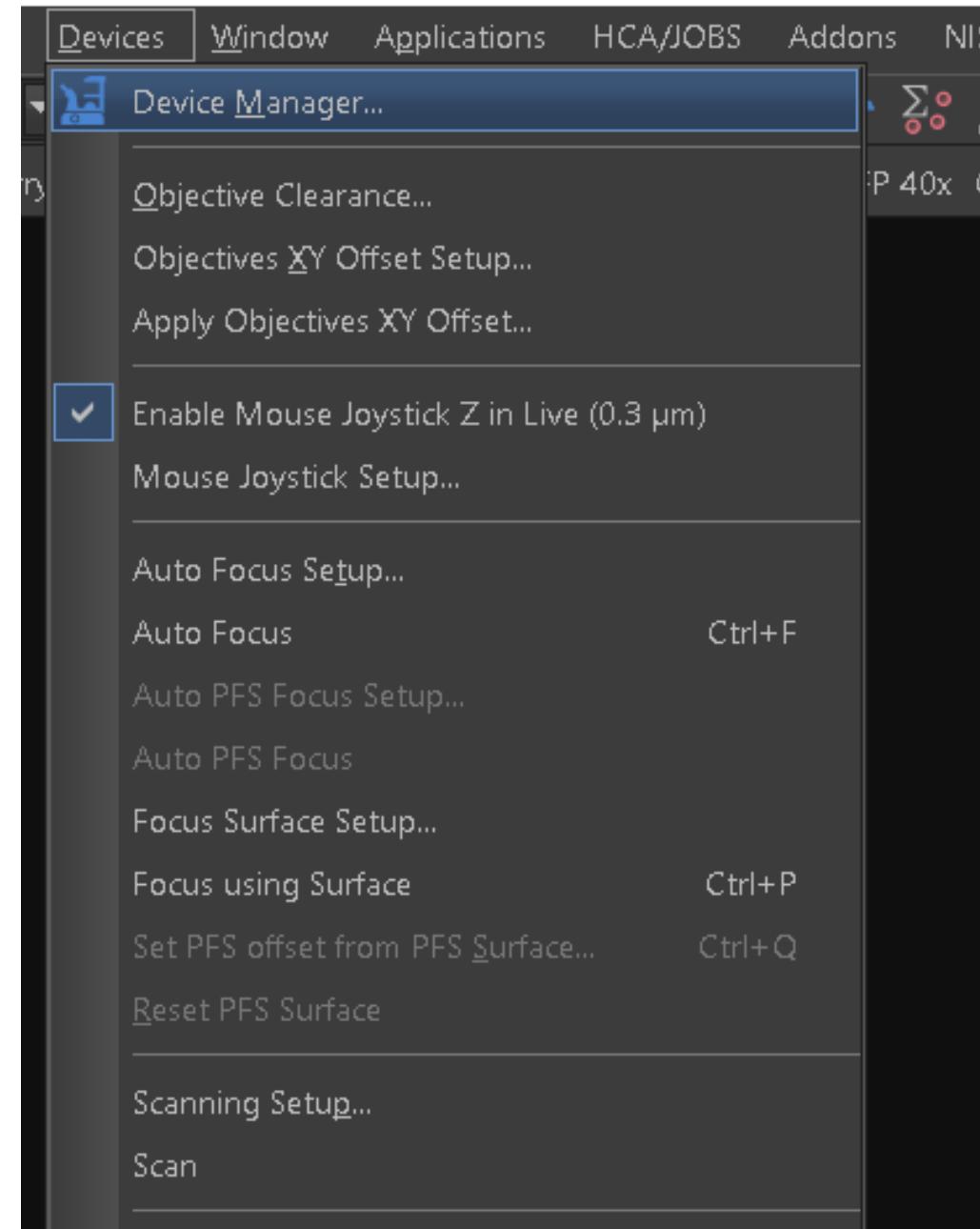
Works well for Nikon

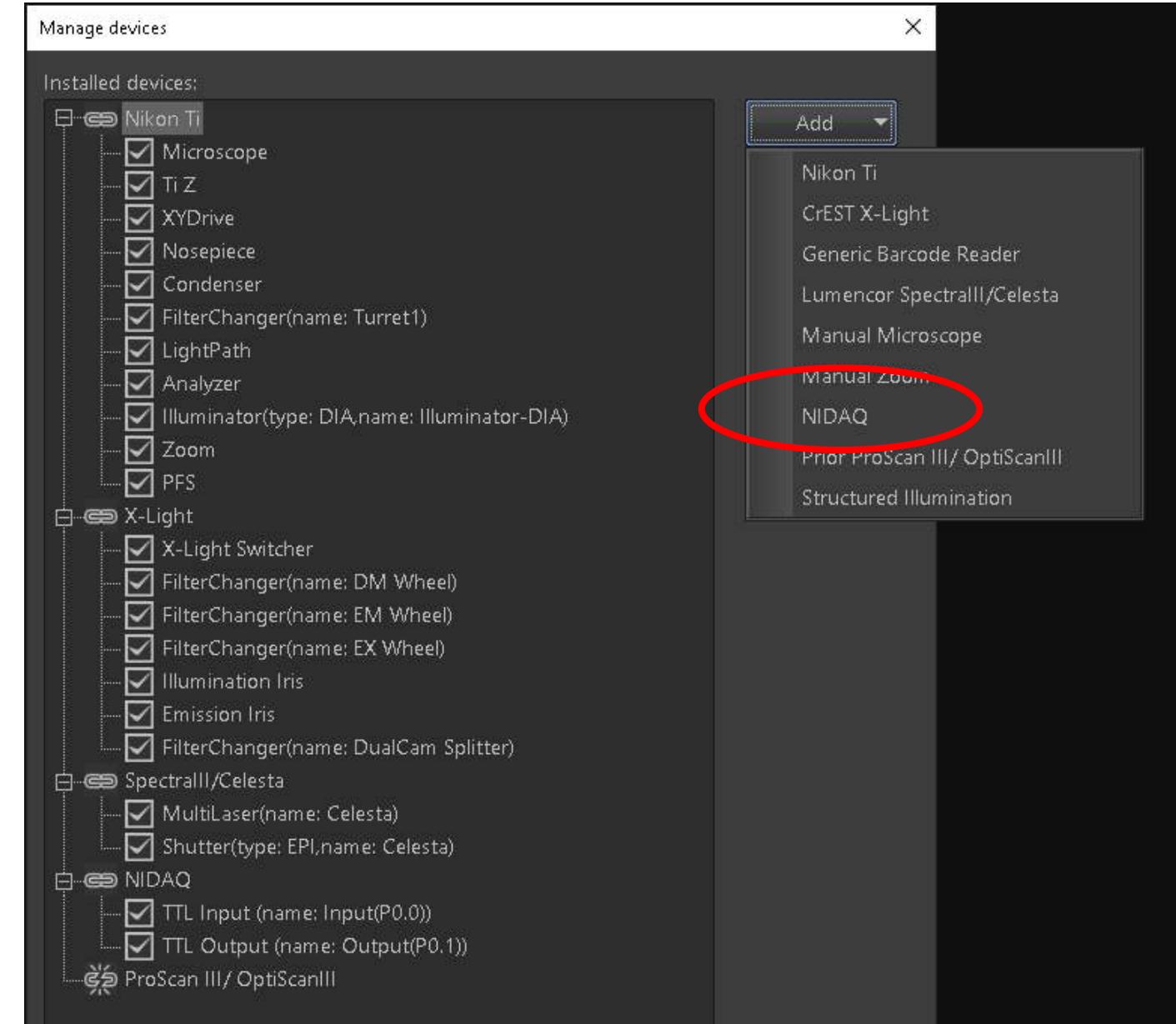
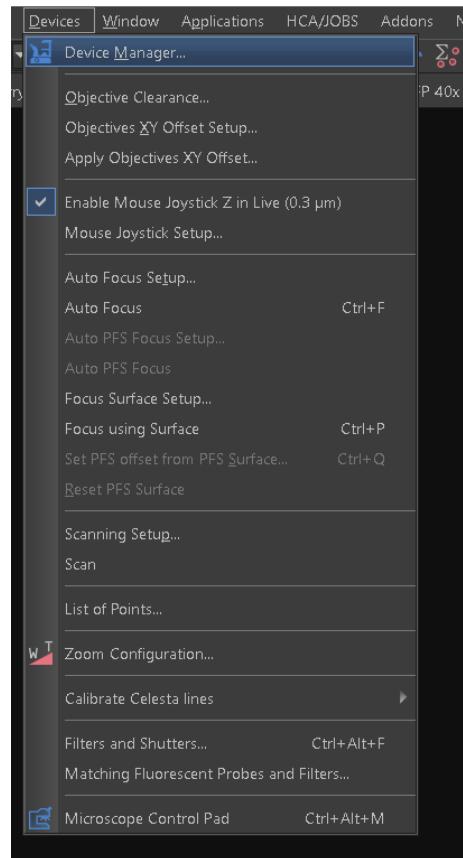
2. Cable (SKS Hirschmann BNC)





Open “Devices” on NIS-Elements AR





The screenshot shows the NI DAQ Configuration software interface. On the left, under 'Available devices', a list includes: TTL Input, TTL Output, Analog Input, Analog Output, Calibrated Analog Input, Calibrated Analog Output, RealTime TTL Input, RealTime Analog Input, Shutter, Piezo Z, Piezo XY, Piezo XYZ, Illumination Device, HW Box, Switcher, Filter Wheel, Exposure Signal TTL In, and Light. The 'TTL Input' item is highlighted with a blue selection bar. In the center, under 'Installed devices', two items are listed: 'TTL Input (name: Input(P0.0))' and 'TTL Output (name: Output(P0.1))'. Below these lists are two buttons: 'Add -->' and '-- Remove'. At the bottom of the main window, it says 'NIDAQ v19.6.0'. To the right, there's a 'Configure ...' button. At the very bottom, there are sections for 'Triggering' (listing 'Prime BSI' and 'USB-6501 (Dev2): Triggering not supported') and 'Connector' (listing 'USB-6501 (Dev2): Not Specified' and 'NI Simulated DAQ Device: /SimDev1/PFI0'). A vertical sidebar on the right lists various NI products with checkboxes, all of which are checked.

Experiment: ND Acquisition

 λ: Save to File

Path: X:\Silvia\Experiments\TdTom DiO\6487

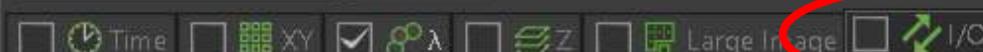
Browse...

Filename: 6487 4x002.nd2

Record Data...

 Custom Metadata

Order of Experiment ▾ Timing...



When Device Changes Then Do Action



Device Type

Name

Condition

Action

 TTL

Input(P0.0)

Falling

Start Experiment

On Experiment Event Set Device Output



Experiment Event

Device Type

Name

Output

Wait

 Phase 1 End

TTL

Output(P0.1)

... High 100.000 [...

Load ▾

Save ▾

Remove ▾

1 time loop



Run now

- Activate I/O tab
- Set Device and TTL input
 - → Action: Start Experiment
- Set End experiment TTL output
 - Wait after signal is sent



File Cameras Help

Setup



Hardware
Setup



Firmware
Update



Configure



Software
Updater

Components



DMI8



Condenser



Focusdrive



Nosepiece



IL-Turret



IL-Fast-Filter-
Wheels



Cameras



Ports



Function-Keys



Sequencer-
Advanced



SPECTRAX



XY-Stage

Subcomponents



System
Triggers



Laser Sources



Light Sources



TTL Signals



Analog
Signals



TTL Signals

Setup

▼ TTL Signals Configuration

Select Trigger Port	Name	Connect With	In/Out	Active	De
Slot1-I/O 1	Slot1-I/O 1	ITK Hydra Trigger 1	Input	High	
Slot1-I/O 2	Slot1-I/O 2	ITK Hydra Trigger 2	Output	High	
Slot1-I/O 3	Slot1-I/O 3	SpectraX BNC-B predefined	Output	High	
Slot1-I/O 4	Slot1-I/O 4	SpectraX BNC-G predefined	Output	High	
Slot1-I/O 5	Slot1-I/O 5	SpectraX BNC-R predefined	Output	High	
Slot1-I/O 6	Slot1-I/O 6	SpectraX BNC-T predefined	Output	High	
Slot1-I/O 7	Slot1-I/O 7	SpectraX BNC-C predefined	Output	High	
Slot1-I/O 8	Slot1-I/O 8	SpectraX BNC-V predefined	Output	High	
Slot2-I/O 1	TTL-OUT	Undefined	Output	High	
Slot2-I/O 2	TTL-IN	Undefined	Input	High	
Slot2-I/O 3	Slot2-I/O 3	Undefined	Input	High	
		Undefined	Input	High	

+ Add TTL-Trigger

► Reset triggers

Open projects

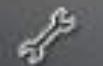
Acquisition

- Acquisition Mode: xyt

x y z t



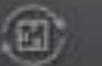
▼ Triggering

Trigger: TTL-IN Trigger linked to acquisition

Channels



Timelapse



Stage



Z pos.

Execution of trig. time dependent

 First cycle only Every cycle At cycles : 2,4,5 Every 1 th cycle Record trigger signals

▼ Triggering

Trigger: TTL-OUT Use in experiment

Number of pulses : 1 Duration (ms) : 100

 Trigger linked to acquisition

Channels



Timelapse



Stage



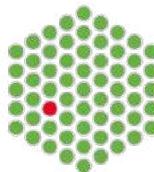
Z pos.

Execution of trig. time dependent

 First cycle only Every cycle At cycles : 2,4,5 Every 1 th cycle

Trigger

After acquisition

 Trigger independent of acquisition Record trigger signals

Microscopes tested

- Nikon TiE with a V3 X-light spinning disk • → Simple
- Thunder Inverted WF Leica • → Simple
- Elyra 7 Zeiss • → Possible but cumbersome

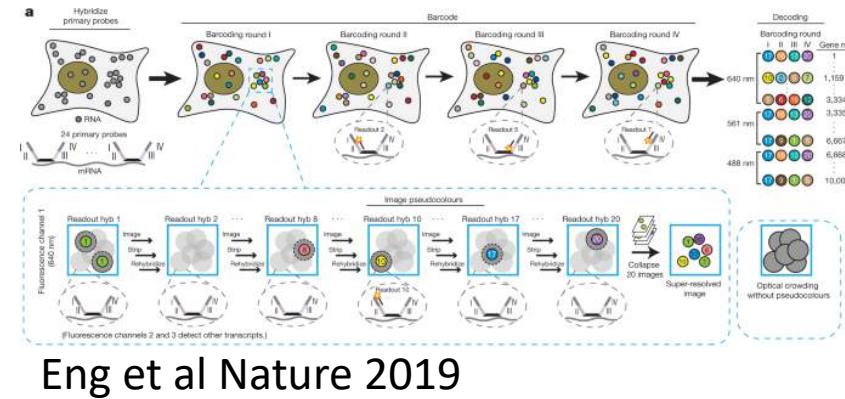
Solution:

Needs MyPic (visual basic macro) to control ZEN black
<https://git.embl.de/grp-ellenberg/mypic/-/wikis/home>

And then python custom code to interface fluidics with MyPic

Spatial transcriptomics – large scale

- Setting up seqFISH+



Benefits:

- Large number of genes (>10,000)

Limitations:

- High magnification and NA (60x) needed to resolve dots → slow for very large areas
- Long experiment (>several days on scope), requires 80 rounds

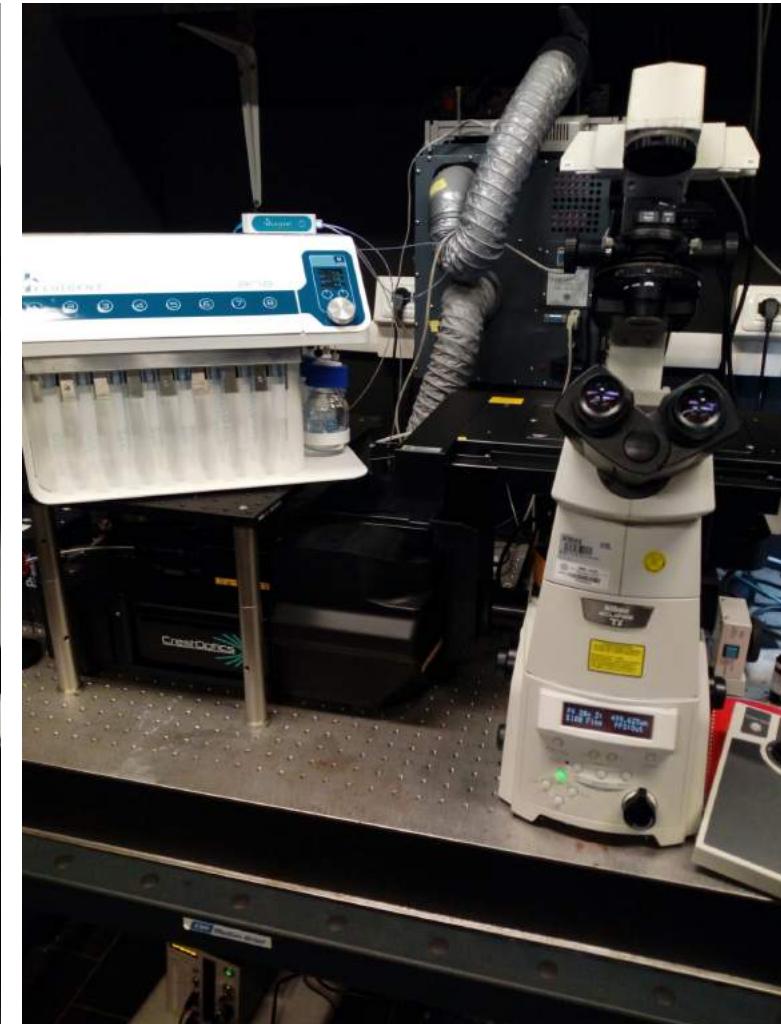
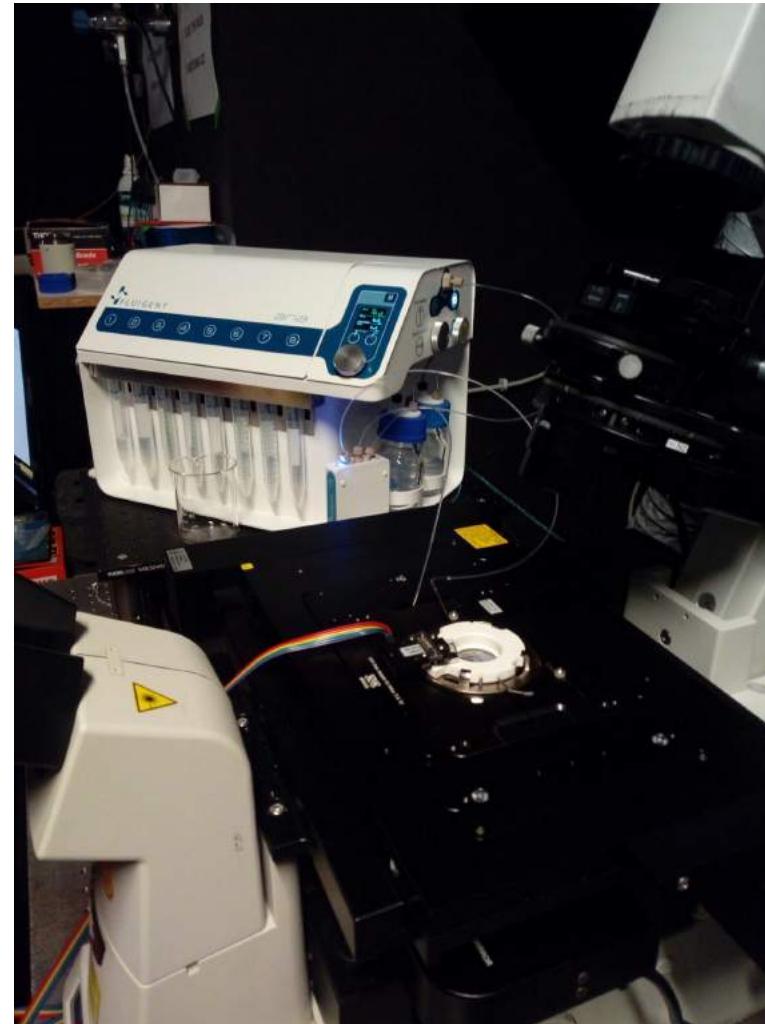
Spatial transcriptomics

- Setting up seqFISH+

How?

- Aria Integrated fluidic unit
- Nikon NIS – spinning disk

Limitation: manual change of ‘probes’
after couple of cycles



Spatial transcriptomics - routine

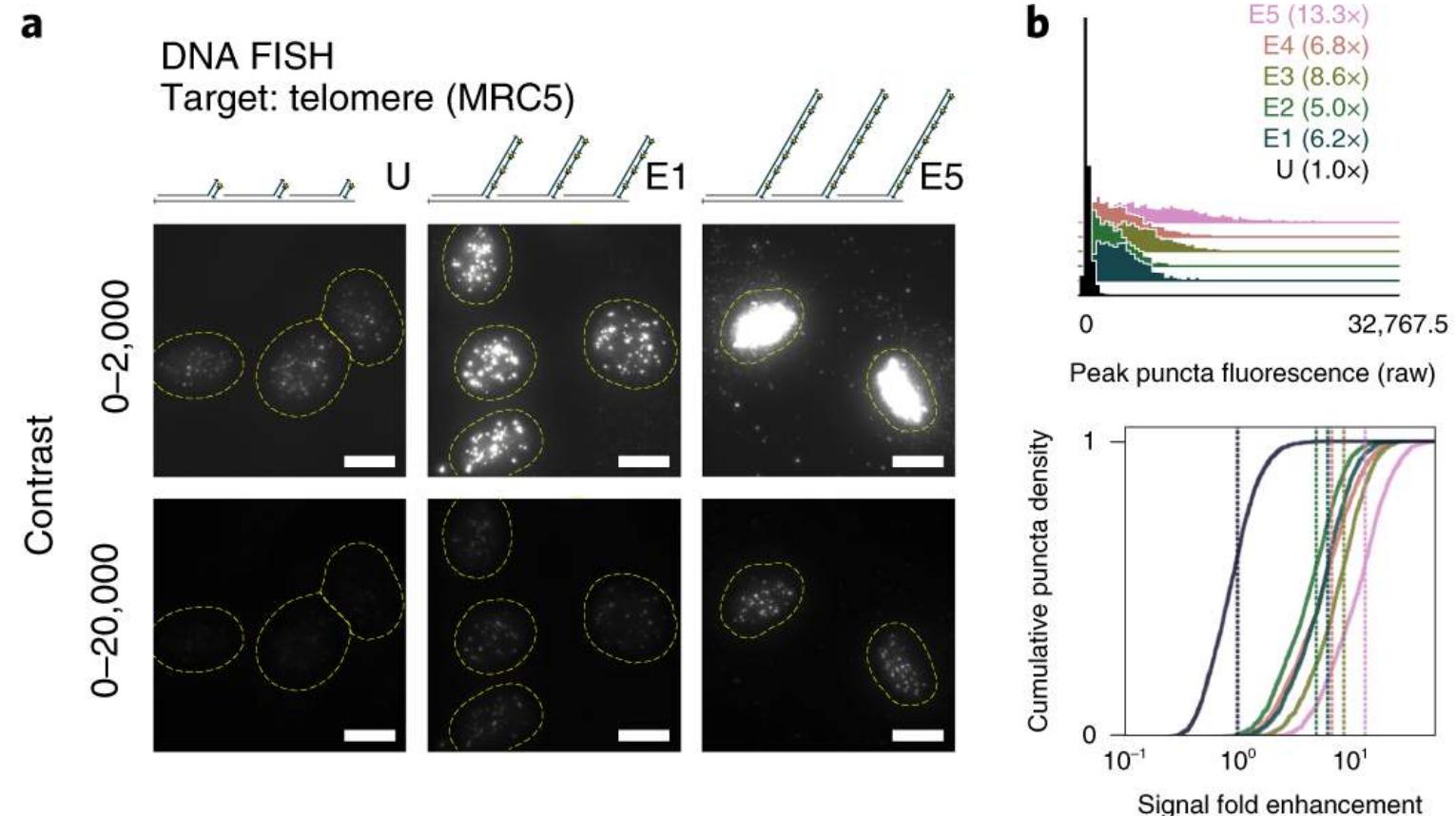
- Setting up SABER-FISH

Benefits:

- WF is ok to acquire signals
- Cost-effective
- Direct readout of signal

Limitations:

- Manual processing of samples between rounds
- <50 genes



Kishi JY, et al Nat Meth 2019

Spatial Transcriptomics

- Setting up SABER-FISH
- Trying out ISS (CARTANA kits)

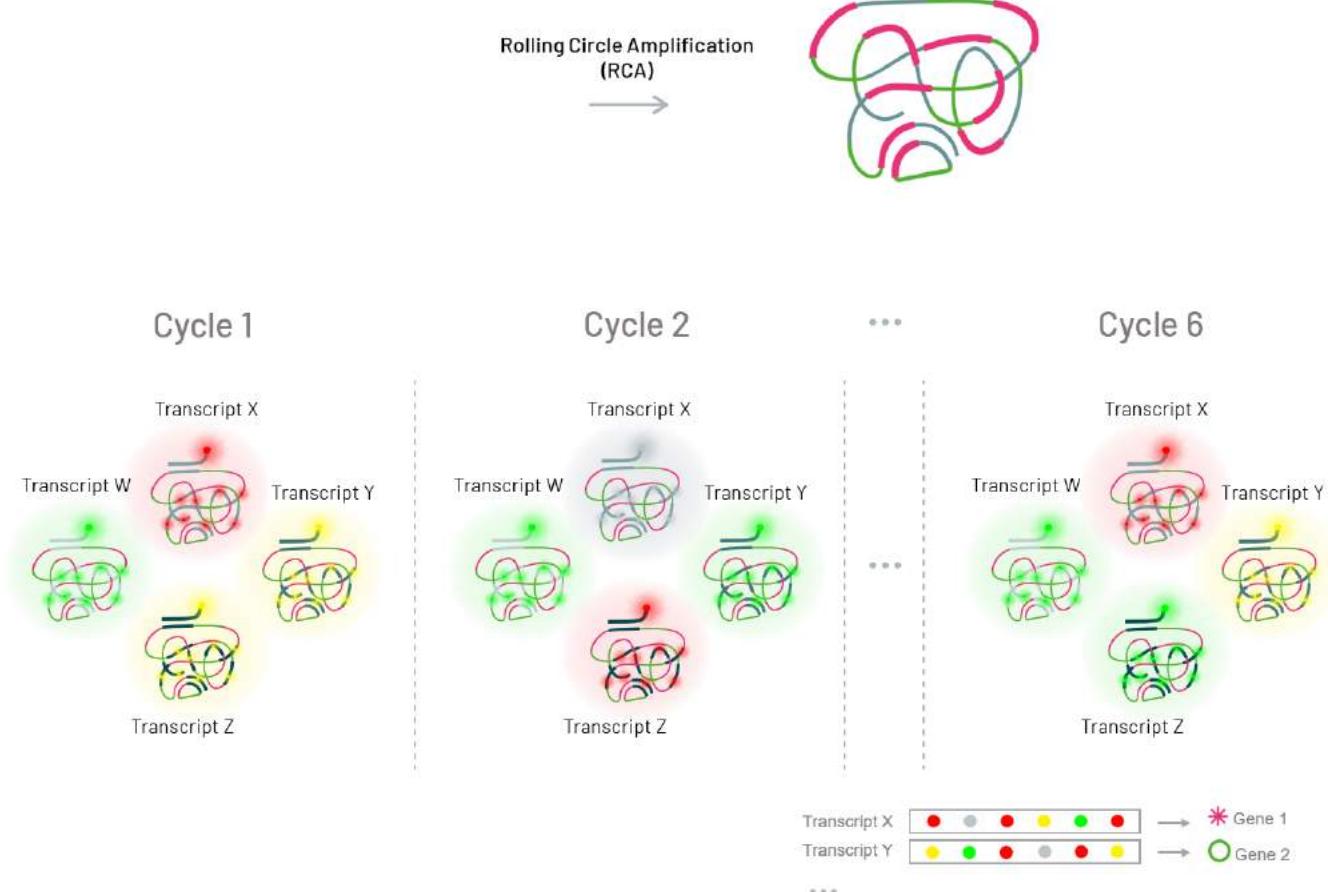
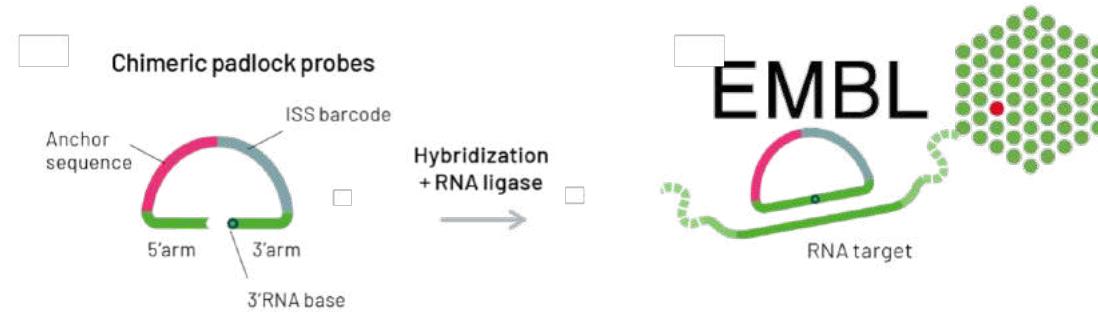
Benefits:

→ Large 'dots' do not need fancy microscopy (works great on the VS200 slide scanner)

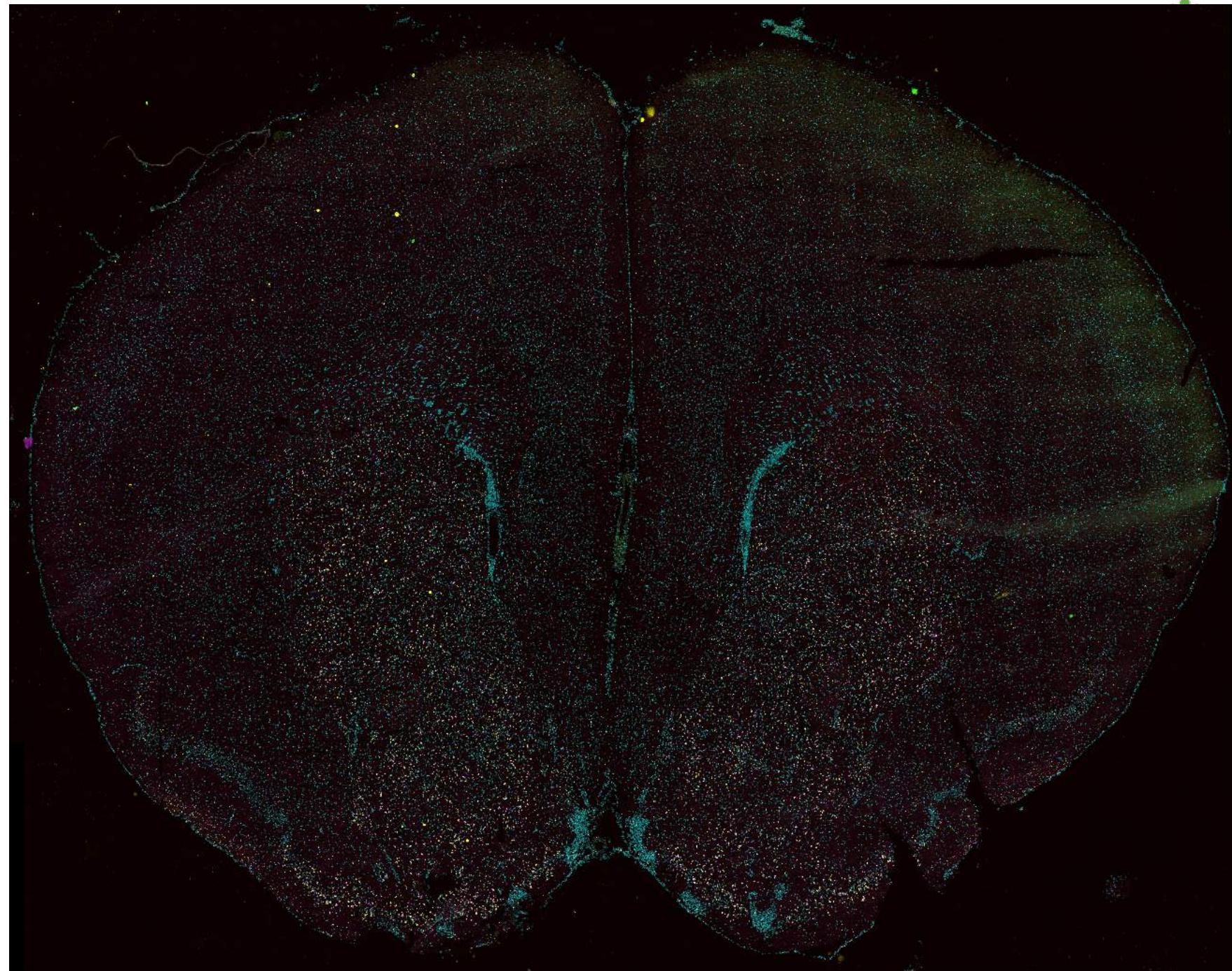
→ Only 6 rounds

Limitations

→ Manual processing of samples between rounds
→ ~200 genes max



CARTANA
Refence slide
Imaged with JOBs



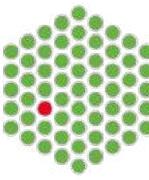
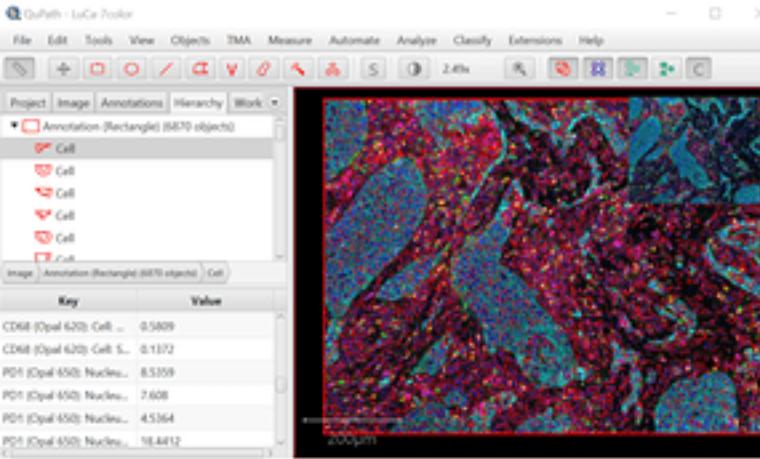


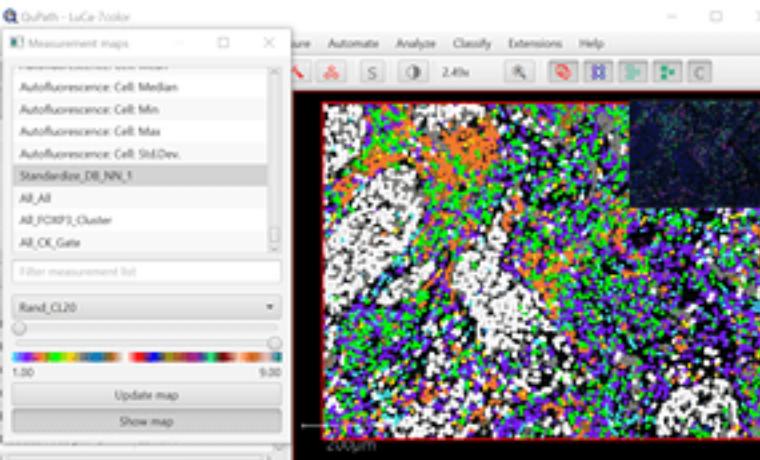
Image analysis

Spatial Proteomics Analysis pipelines

QuPath



Example:



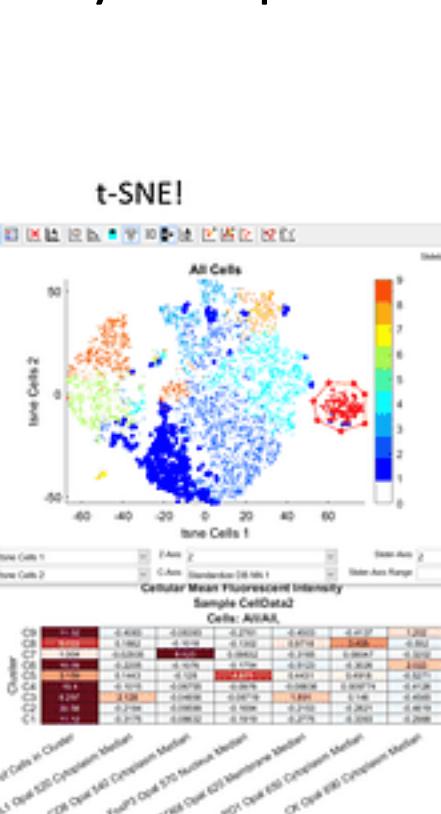
QuPath data.csv



CytoMAP results.csv

Import script.groovy

CytoMap



Heatmaps of clusters and gates!

Spatial Proteomics Analysis pipelines

Other examples:

New Results

Dec 2020

 [Comment on this paper](#)

Toward reproducible, scalable, and robust data analysis across multiplex tissue imaging platforms

 Erik Ames Burlingame,  Jennifer Eng,  Guillaume Thibault,  Koei Chin,  Joe W. Gray,
 Young Hwan Chang

doi: <https://doi.org/10.1101/2020.12.11.422048>

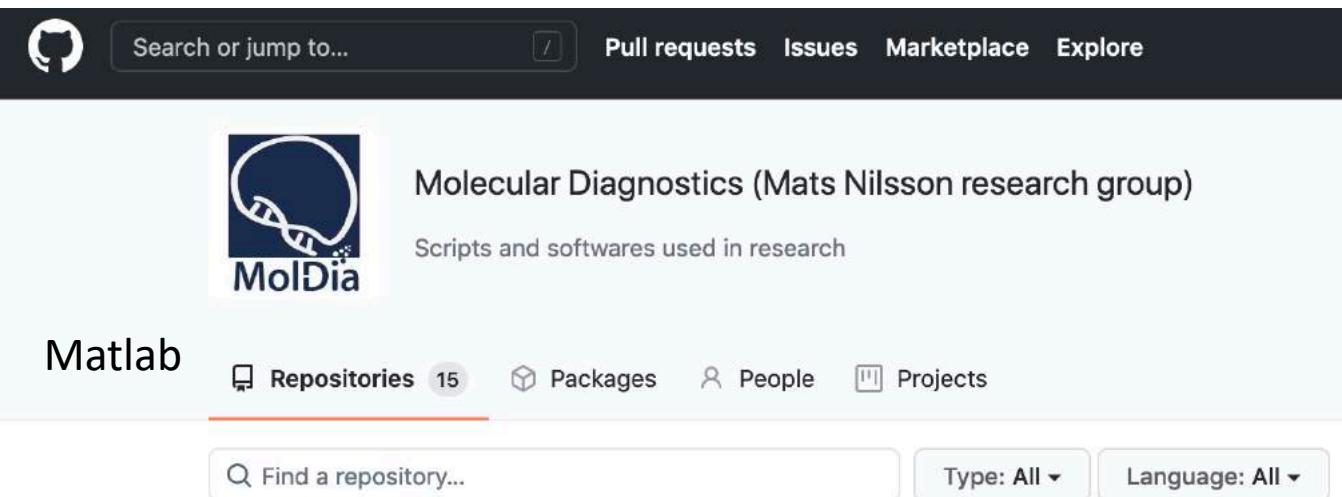
Sept 2020

cytominer: an R/Bioconductor package for visualisation of highly multiplexed imaging data

 Nils Eling,  Nicolas Damond,  Tobias Hoch,  Bernd Bodenmiller

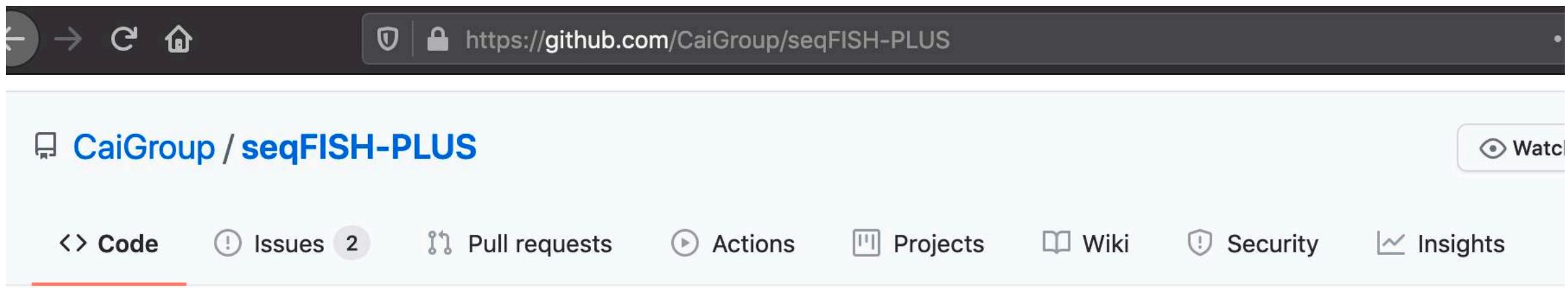
doi: <https://doi.org/10.1101/2020.09.08.287516>

Spatial transcriptomics image analysis



A screenshot of a GitHub repository page. The repository is named "Molecular Diagnostics (Mats Nilsson research group)" and is associated with "Scripts and softwares used in research". The page shows a "Matlab" section with 15 repositories. Navigation links include "Repositories 15", "Packages", "People", and "Projects". A search bar at the bottom allows users to "Find a repository..." and filters by "Type: All" and "Language: All".

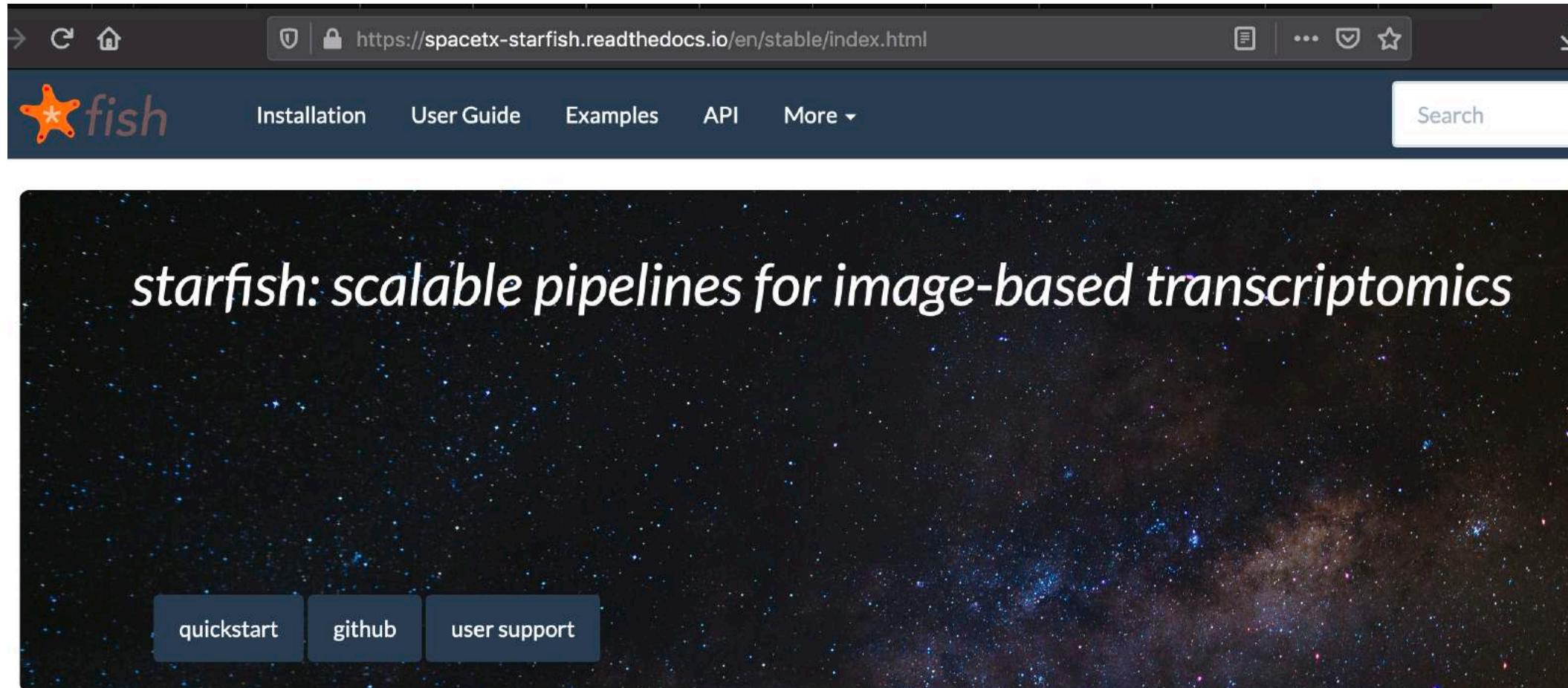
→ All home made
solutions and require
programming skills



A screenshot of a GitHub repository page for "CaiGroup / seqFISH-PLUS". The page includes a navigation bar with icons for back, forward, and home. The URL in the address bar is https://github.com/CaiGroup/seqFISH-PLUS. The main header shows the repository name "CaiGroup / seqFISH-PLUS" and a "Watch" button. Below the header are navigation links for "Code", "Issues 2", "Pull requests", "Actions", "Projects", "Wiki", "Security", and "Insights".

Matlab

Spatial transcriptomics image analysis



starfish: scalable pipelines for image-based transcriptomics

quickstart github user support

starfish is a Python library for processing images of image-based spatial transcriptomics. It lets you build scalable pipelines that localize and quantify RNA transcripts in image data generated by any

Features

Python-based

Spatial Analysis pipelines (after decoding)

Article | Published: 18 November 2019

Probabilistic cell typing enables fine mapping of closely related cell types *in situ*

Xiaoyan Qian, Kenneth D. Harris , Thomas Hauling, Dimitris Nicoloutsopoulos, Ana B. Muñoz-Manchado, Nathan Skene, Jens Hjerling-Leffler & Mats Nilsson 

Nature Methods 17, 101–106(2020) | [Cite this article](#)

 Giotto Home Documentation Installation Dataset Examples

Main page

Welcome

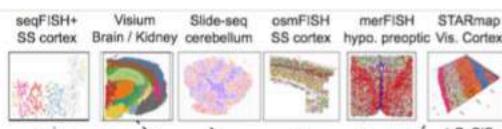
The Giotto package consists of two modules, Giotto Analyzer and Viewer, which provide tools to process, analyze and visualize single-cell spatial expression data. The underlying framework is generalizable to virtually all currently available spatial datasets. We recently demonstrated the general applicability on 10 different datasets created by 9 different state-of-the-art spatial technologies, including *in situ* hybridization (seqFISH+, merFISH, osmFISH), sequencing (Slide-seq, Visium, STARmap) and imaging-based multiplexing/proteomics (CyCIF, MIBI, CODEX). These technologies differ in terms of resolution (single cell vs multiple cells), spatial dimension (2D vs 3D), molecular modality (protein vs RNA), and throughput (number of cells and genes).

Reference:

Ruben Dries*, Qian Zhu* et al. Giotto, a toolbox for integrative analysis and visualization of spatial expression data. [Biorxiv doi.org/10.1101/701680v2](#).

What's new

Jun 13, 2020 - We recently broadcasted a Giotto tutorial on Zoom. Check out the slides in this [link](#). A recorded video session can be viewed at this [link](#).



Methodology article | [Open Access](#) | Published: 19 October 2020

Automated identification of the mouse brain's spatial compartments from *in situ* sequencing data

Gabriele Partel, [Markus M. Hilscher](#), [Giorgia Milli](#), [Leslie Solorzano](#), [Anna H. Klemm](#), [Mats Nilsson](#) & [Carolina Wählby](#) 

BMC Biology 18, Article number: 144 (2020) | [Cite this article](#)

236 Accesses | 17 Altmetric | [Metrics](#)

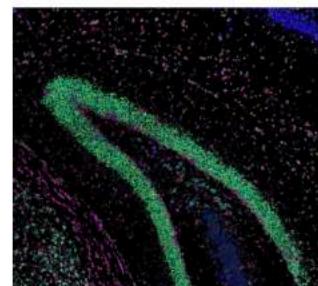
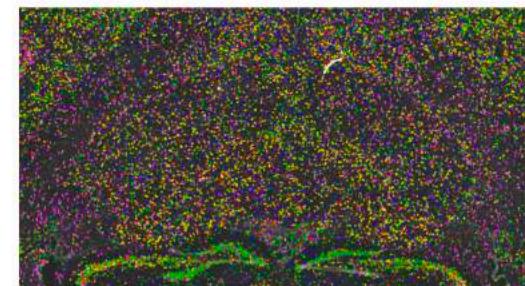
 **TissUUmaps**

HOME PROJECTS TUTORIALS PUBLICATIONS PE

Welcome to TissUUmaps

Projects

We develop computational methods to combine spatially resolved information on tissue morphology with *in situ* RNA sequencing and learning we can distinguish e.g. normal tissue from tumor tissue, and large-scale alignment of serial whole-slide images allows us to develop protein detection methods. We also develop tools for efficient visualization and data interaction, at multiple scales. In the long-term perspective will enable better diagnostics, prognostics, and treatment.



A unit's work



Emerald Perlas
Head of Histology
- Expansion microscopy
- Tissue clearing
- Sample prep for seqFISH and ISS



Cornelius Gross
Rahul Sureka (Postdoc)

Hiroki Asari

Santiago Rompani

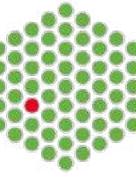
Neurobiology



Jamie Hackett

Matthieu Boulard

Epigenetics



Questions?