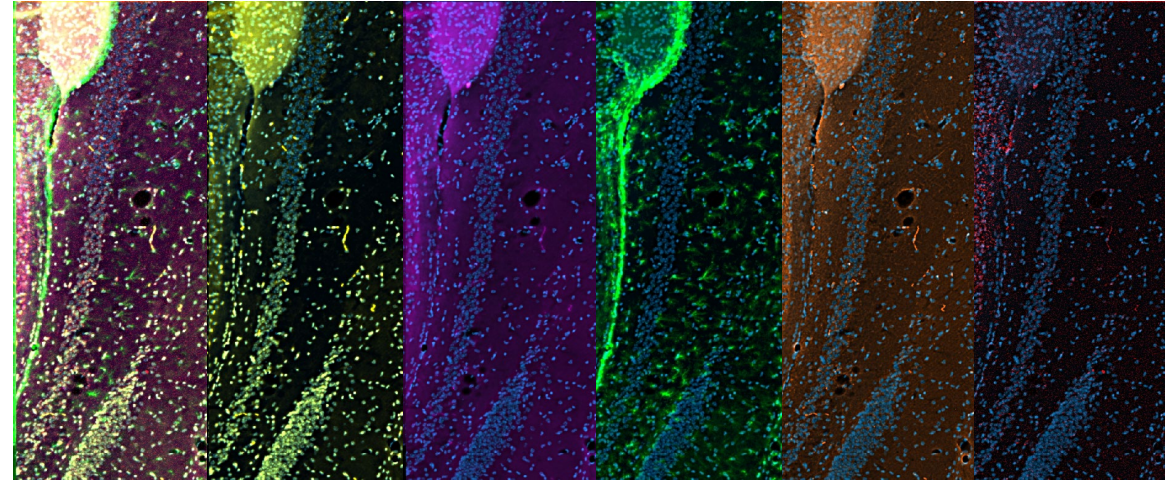
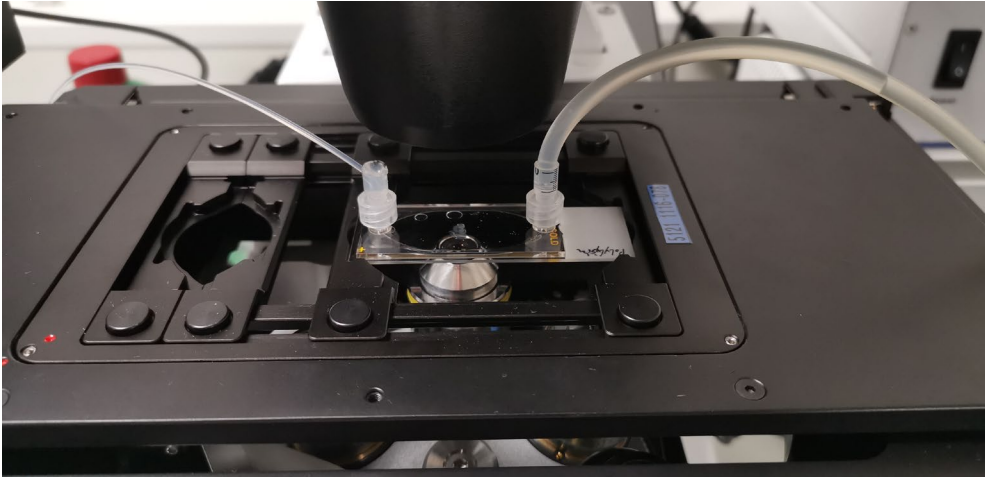
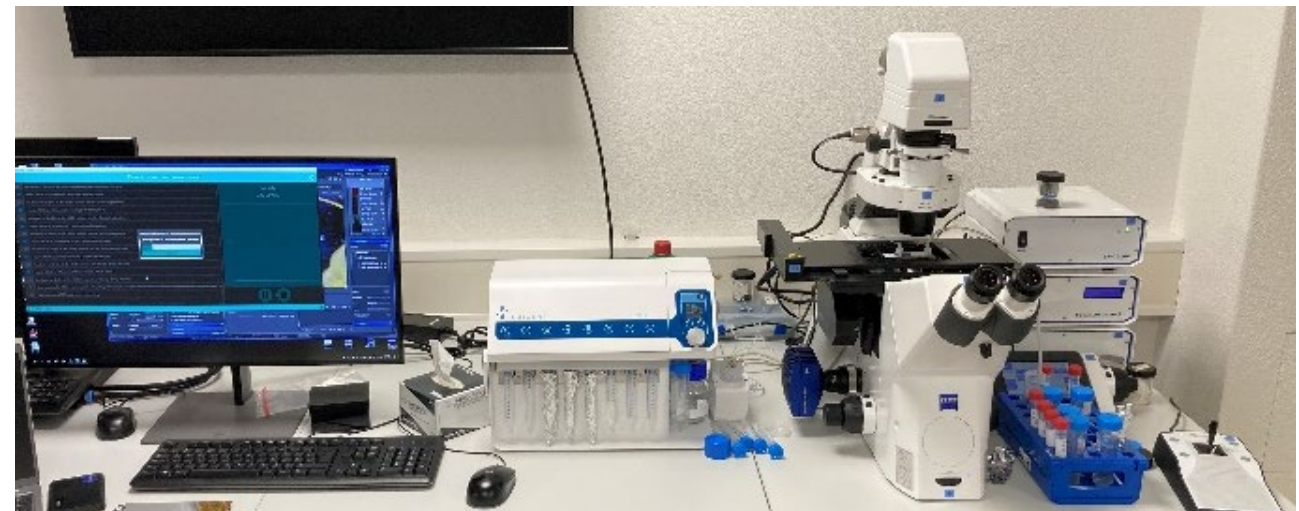


Microfluidics on Scope – Enabling modern applications like Spatialomics or SMLM



Philipp Seidel
Business Sector Life Sciences, ZEISS RMS

5 September 2024



01 Modern microfluidics with the Fluigent Aria

02 Better integration with ZEN software

03 Application examples

Fluigent Aria

Combined imaging with fluid delivery

Contamination?

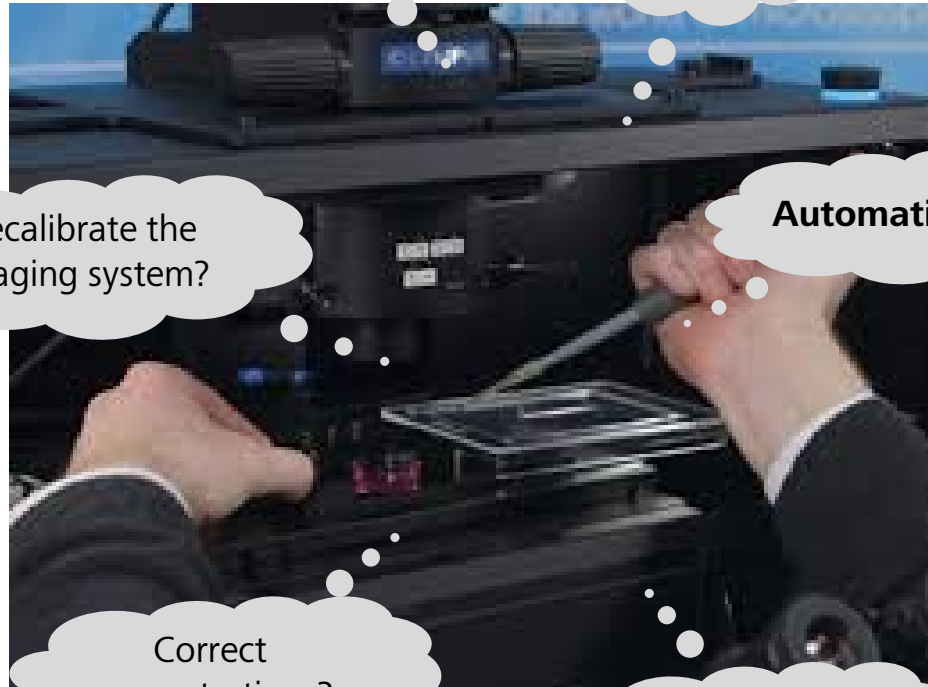
Spill over?

Recalibrate the
imaging system?

Automation?

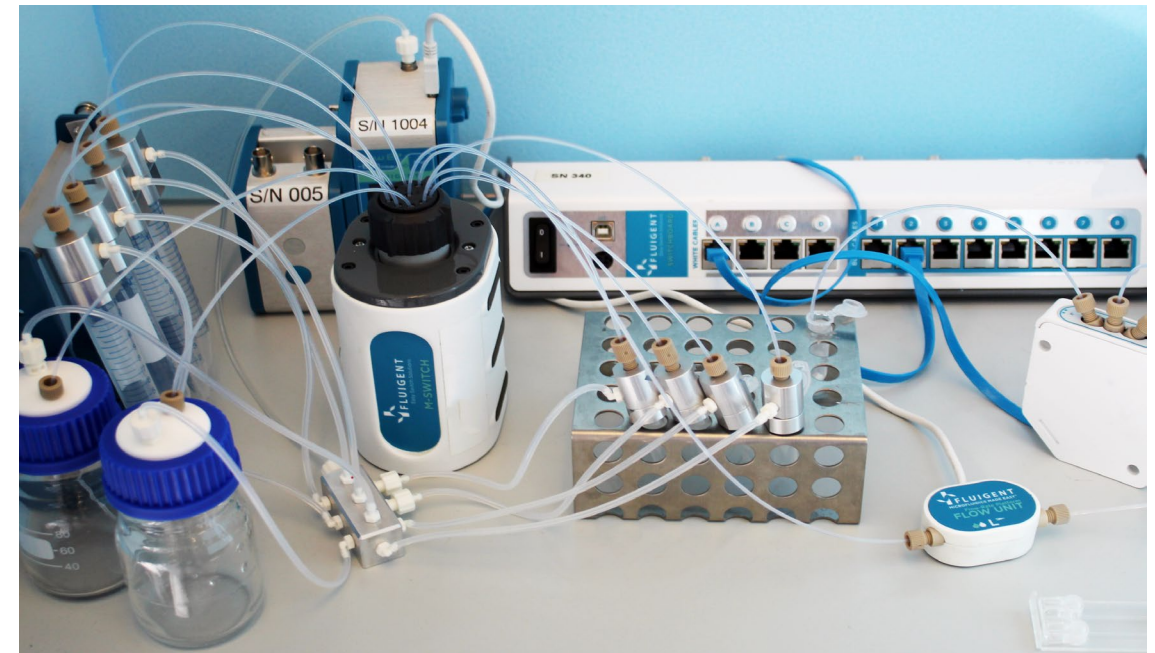
Correct
concentrations?

Proper Mixing?



Custom-built system

- Cost- and time-consuming implementation
- Detailed microfluidic expertise required
- Complex operation & custom coding



Aria instrument vs. custom-built microfluidic system

The Aria removes most technical overhead...



Fluigent ARIA

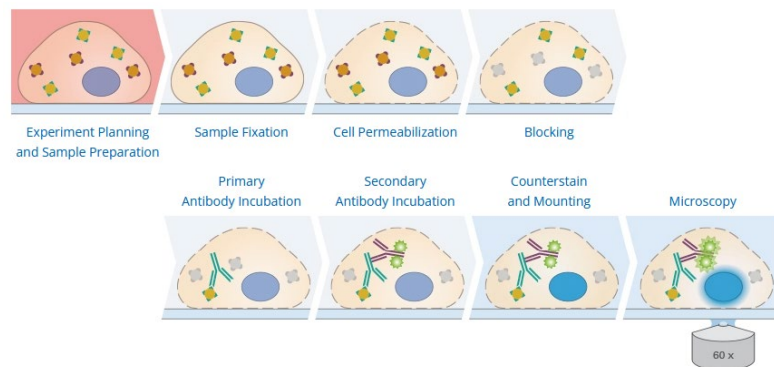
- All-in-one, affordable and plug & play
- No microfluidic expertise required
- Intuitive instrument operation



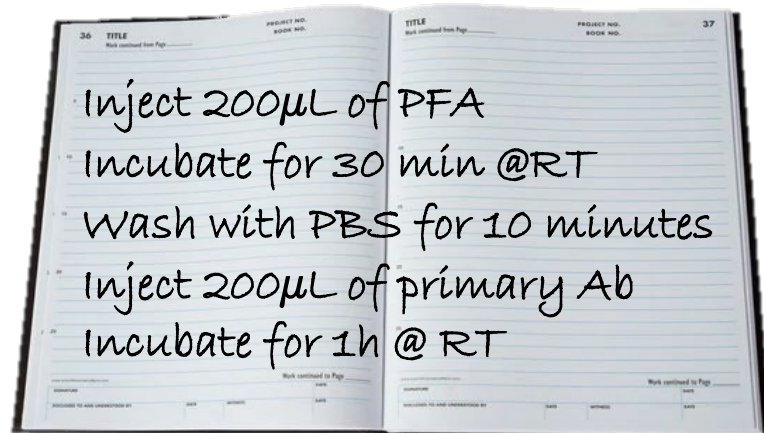
Intuitive operation

... with the Aria scheduling software

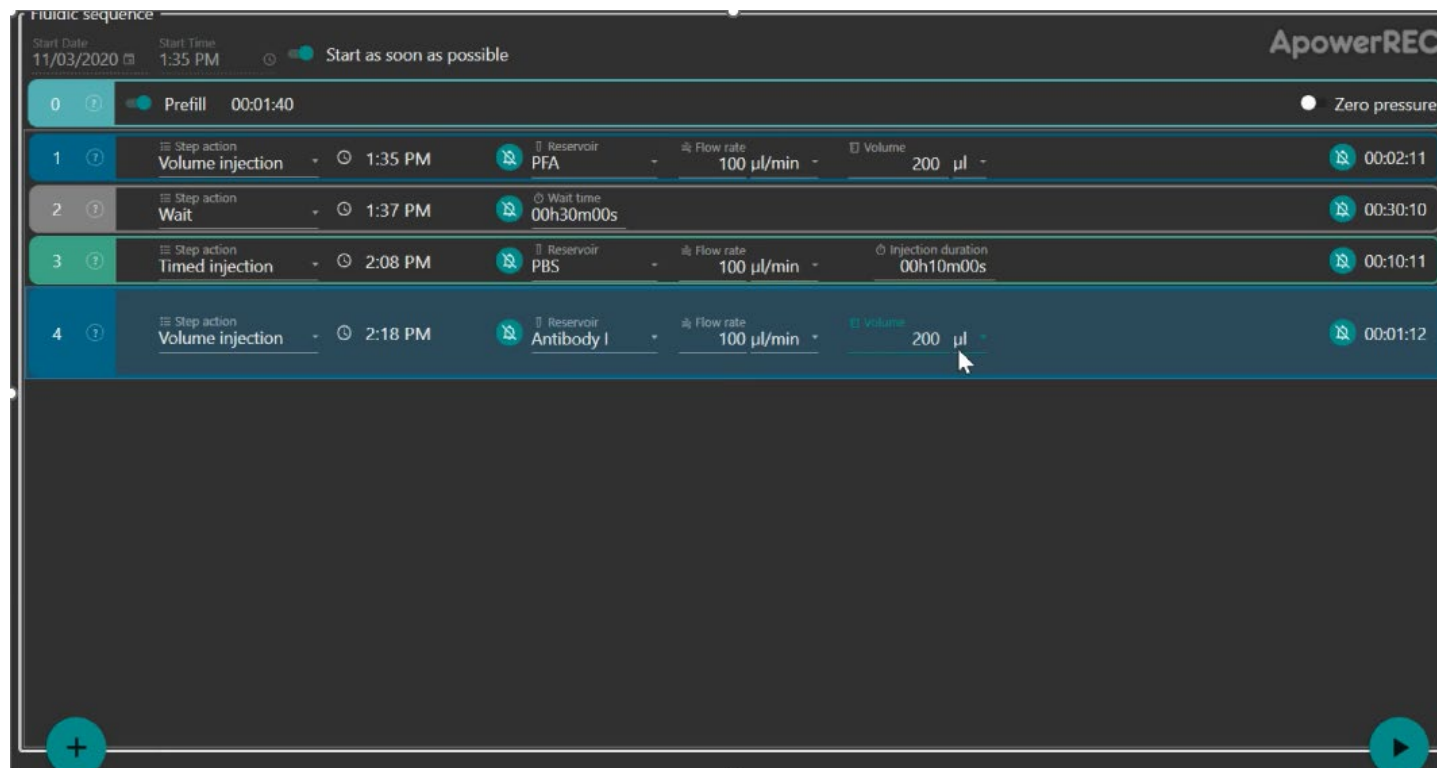
A typical Immunofluorescence workflow...



... and its staining protocol...



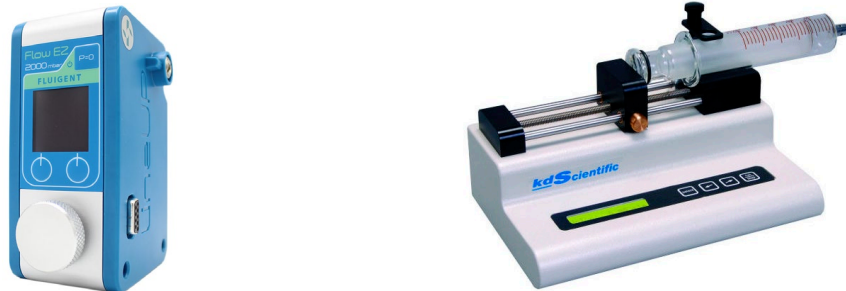
... translated into a valid Aria Sequence:



Step	Action	Time	Reservoir	Flow rate	Volume	Injection duration
0	Prefill	00:01:40				
1	Volume injection	1:35 PM	PFA	100 µl/min	200 µl	00:02:11
2	Wait	1:37 PM				00:30:10
3	Timed injection	2:08 PM	PBS	100 µl/min		00:10:11
4	Volume injection	2:18 PM	Antibody I	100 µl/min	200 µl	00:01:12

Precise fluid delivery

... with the best-in-class pressure-based flow control

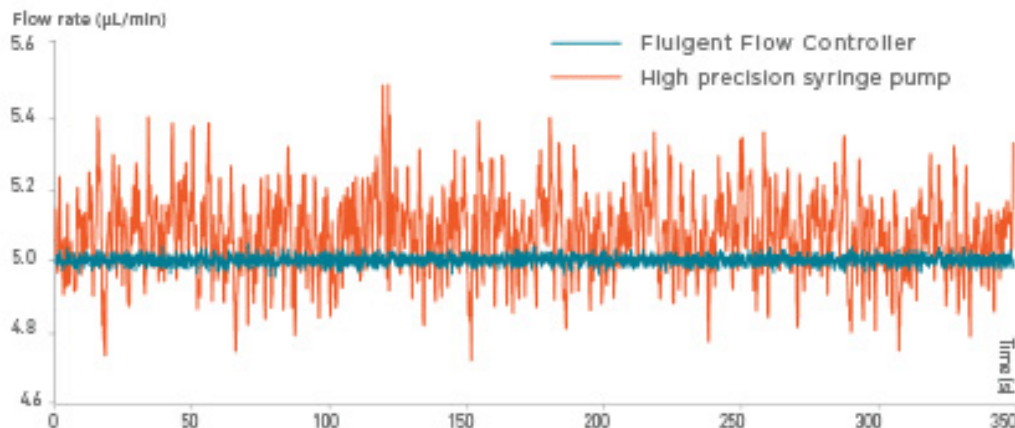


Fluigent flow control vs. High-precision Syringe Pump

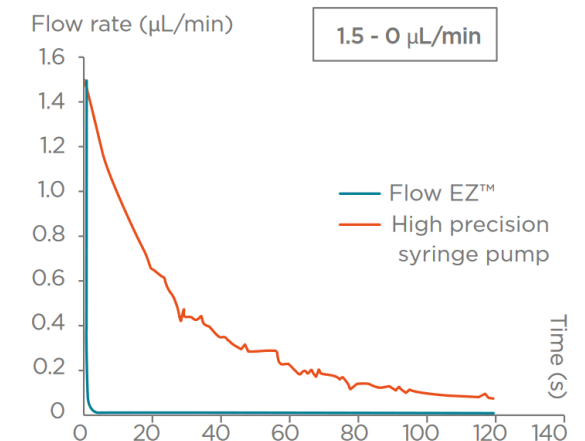
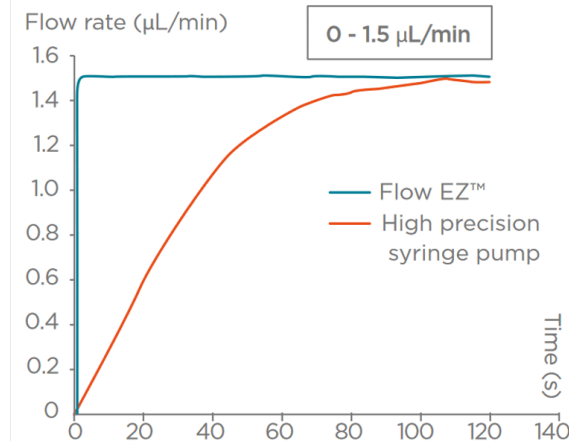
Smart fluidics handling

- Pre-stacking of sequential liquid in tubing minimizes dead volume and enable correctly timed fluid exchange
- Stop Flow functionality (no “backflow” during pause steps) enables reliable reagent incubation

Flow rate stability



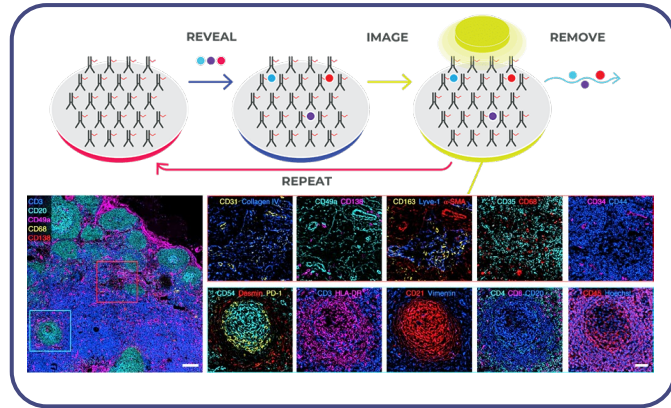
Fast flow rate changes



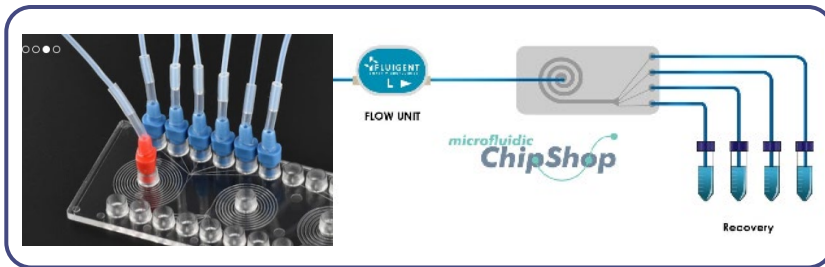
Open platform aspects

Versatility towards applications and protocols

Staining / Cyclic-Staining -> Spatialomics



Cell-Sorting / Separation



Imaging HW+SW

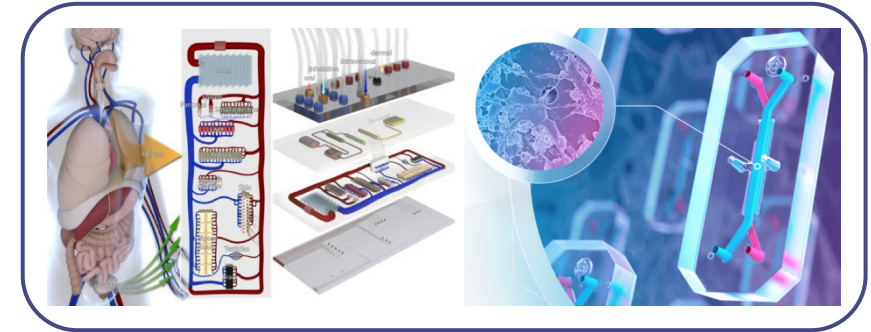


+
enabling technology
Microfluidics

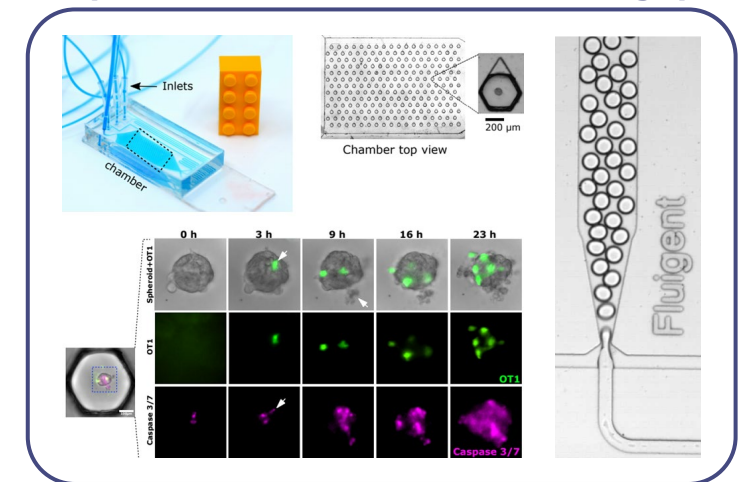
Live-Cell Imaging / Perfusion



MPS / Organ-on-a-Chip



Droplets -> Miniaturization / Throughput



Agenda



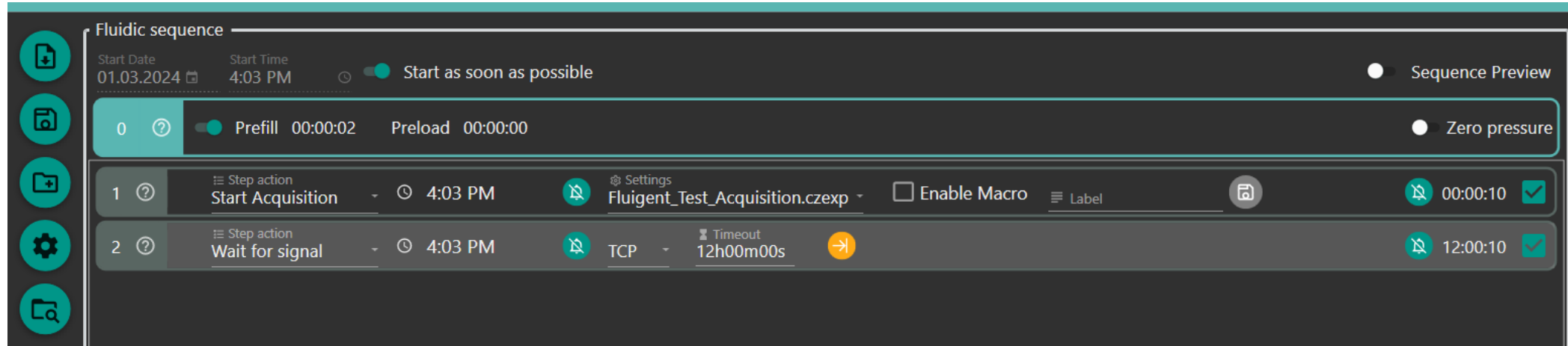
01 Modern microfluidics with the Fluigent Aria

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Combining fluidic and imaging workflows

The novel, robust and intuitive TCP-IP handshake ...



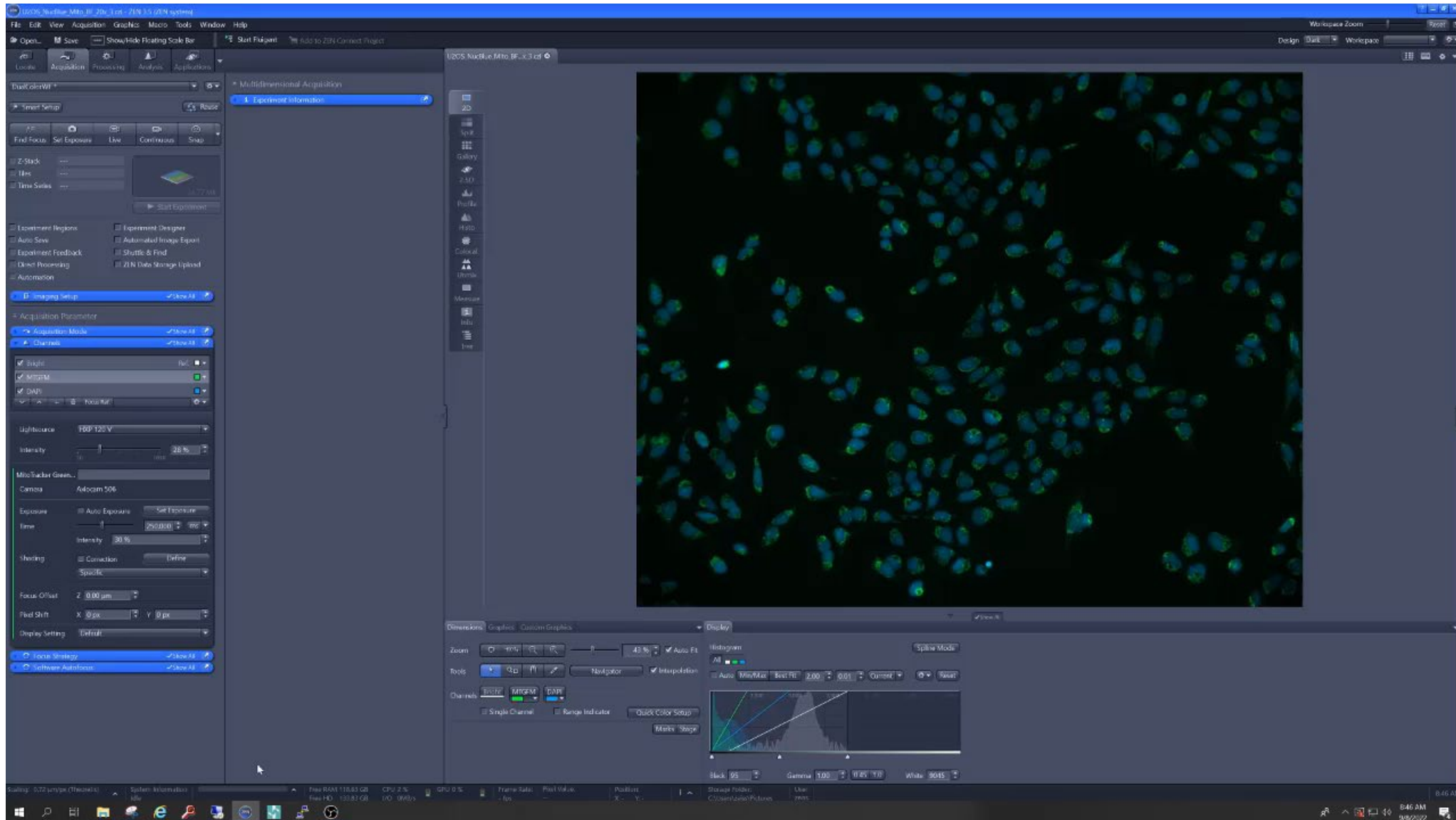
- schedules & initiates acquisitions



- provides available experimental settings
- provides feedback signal for correct synchronization

Combined fluidic and imaging workflows

... leads to intuitive and seamless workflows



Combined fluidic and imaging workflows

... leads to intuitive and seamless workflows



Fluidic delivery protocol

Fluidic sequence - DualcolorWorkflow7

Start Date
9/8/2022

Start Time
2:19 PM

Start as soon as possible

0

●

Prefill 00:00:00

Preload 00:00:56

●

Sequence Preview

●

Zero pressure

1	Step action	2:19 PM	Reservoir	MitoTrackRed	Flow rate	100 µl/min	Volume	100 µl	00:01:12
2	Volume injection	2:20 PM	Reservoir	Buffer	Flow rate	250 µl/min	Volume	500 µl	00:02:08
3	Acquisition	2:22 PM	Macro	FluigentExecute.czmac	Settings	DualColorWF.czexp			00:00:10
4	Wait for Signal	2:22 PM	TCP	Timeout	01h00m00s				01:00:00
5	Volume injection	3:22 PM	Reservoir	NucBlue	Flow rate	100 µl/min	Volume	100 µl	00:01:12
6	Volume injection	3:24 PM	Reservoir	Buffer	Flow rate	250 µl/min	Volume	500 µl	00:02:05
7	Acquisition	3:26 PM	Macro	FluigentExecute.czmac	Settings	DualColorWF.czexp			00:00:10

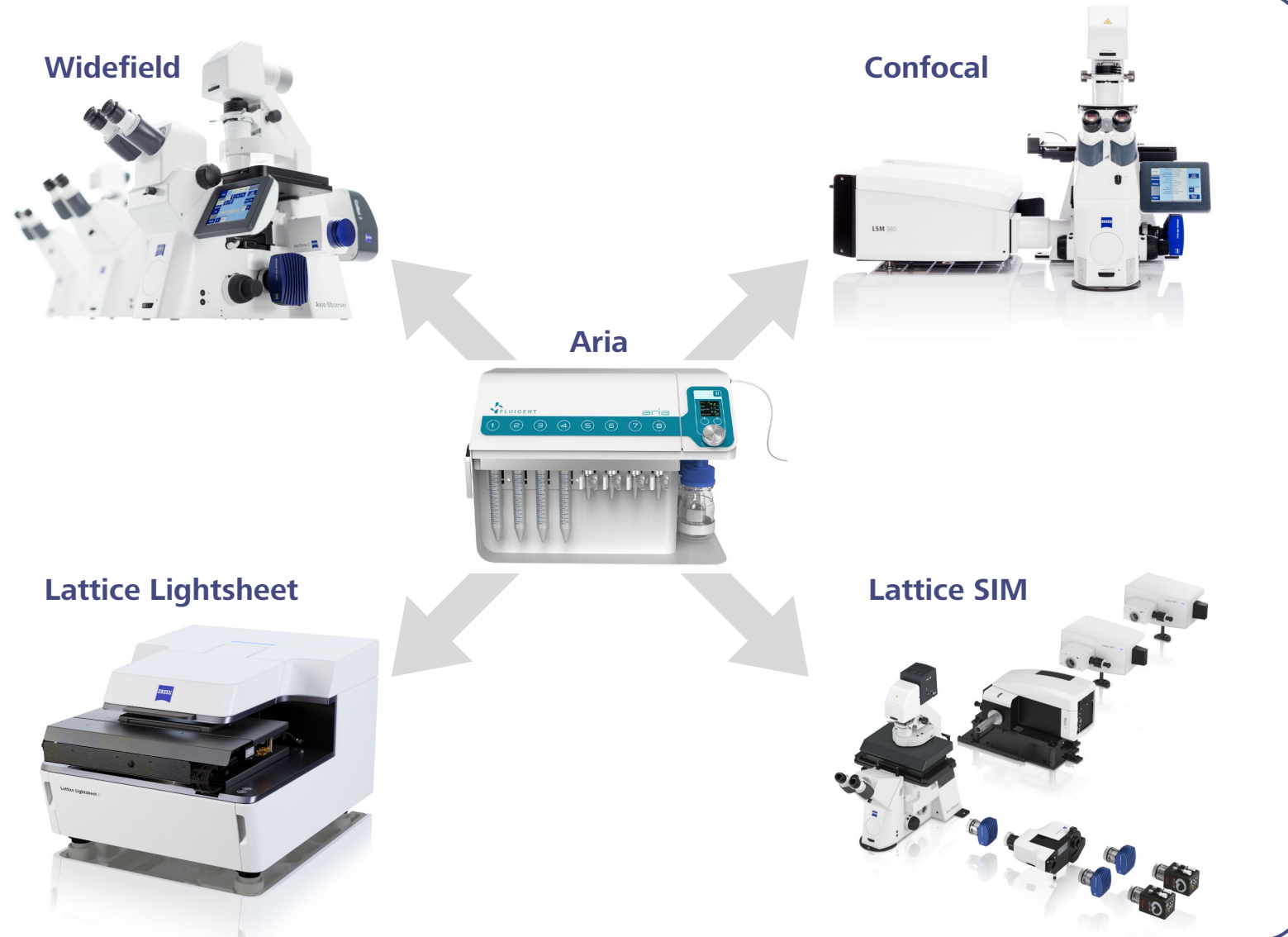
Minimum volume required

Reservoirs

MitoTrackRed	NucBlue
1	2
100 µl	100 µl
Reservoir 3	Reservoir 4
3	4
0 µl	0 µl
Reservoir 5	Reservoir 6
5	6
0 µl	0 µl
Reservoir 7	Reservoir 8
7	8
0 µl	0 µl
Buffer	Reservoir 10
9	10
1,241 µl	0 µl
Buffer	

Open platform aspects

Versatility towards imaging instruments



- With Aria, **users can choose an imaging instrument** according to their application and scientific goals
- **Other microfluidic systems** (e.g. Akoya Phenocycler-Fusion), often **hardwired to imaging instrument and application**

Agenda



01 Modern microfluidics with the Fluigent Aria

02 Better integration with ZEN software

03 Application examples

Application – Tissue Multiplexing

Introduction



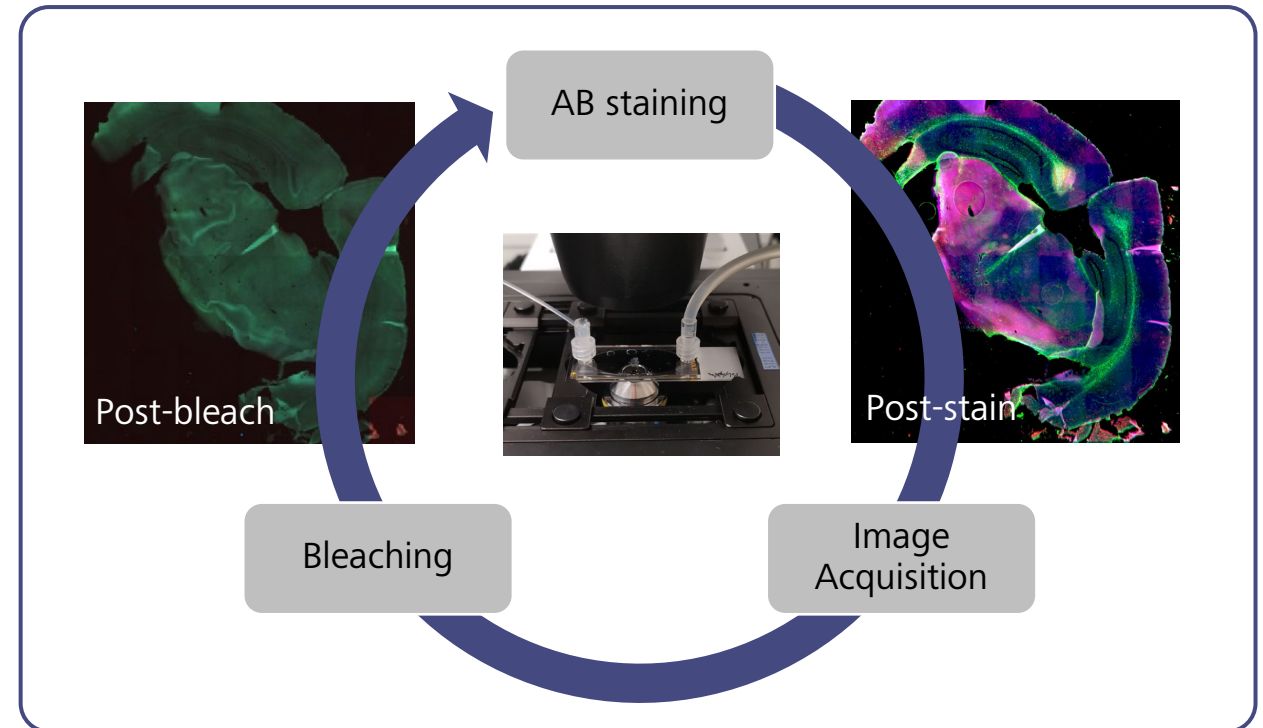
Dr. Wolf Heusermann



Dr. Julien Toquant

- Aim: **Proof-of-concept for tissue multiplexing** workflow employing the Fluigent Aria
- An **adapted IBEX protocol** was implemented (chemical bleaching)
- **Directly-labelled primary antibodies** were used for staining (kind gift from proteintech)
- Brain tissues were fixed to Ibidi “sticky-Slide Tissue” (80518)

In local collaboration with:



Radtko et al. IBEX: an iterative immunolabeling and chemical bleaching method for high-content imaging of diverse tissues. Nat Protoc. 2022

Application – Tissue Multiplexing

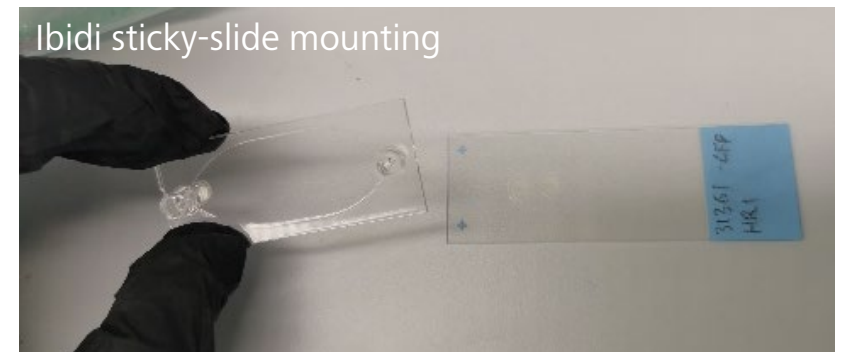
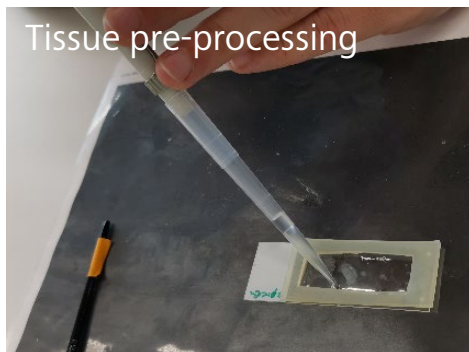
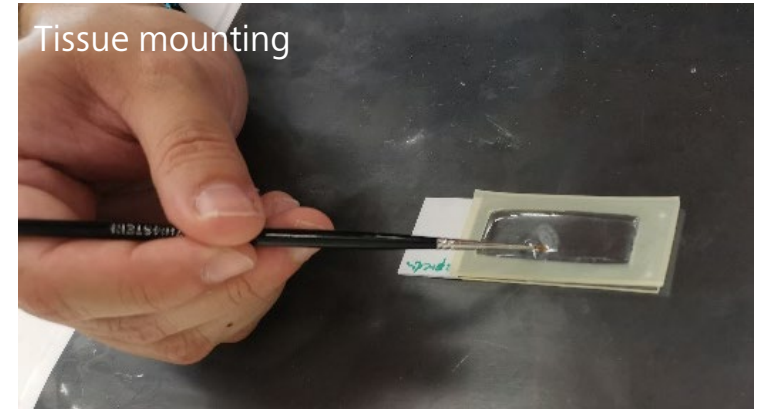
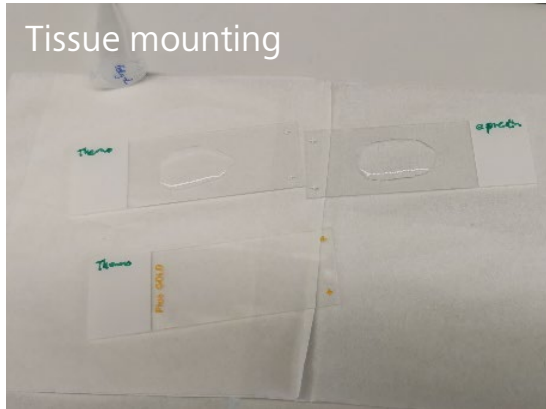
Sample and reagent preparation

Sample preparation

- **Poly-Lysin coating & tissue mounting** on standard glass object slides
- **Tissue pre-processing** (e.g. deparaffinization or antigen retrieval) is done before mounting the chamber
- **Ibidi sticky-Slide Tissue (80518) mounting**

Reagent preparation

- **Primary AB labelling** according to manufacturer's protocol (proteintech)
- **Buffer preparation for IBEX protocol**
(Washing buffer, Blocking buffer, Bleaching buffers)

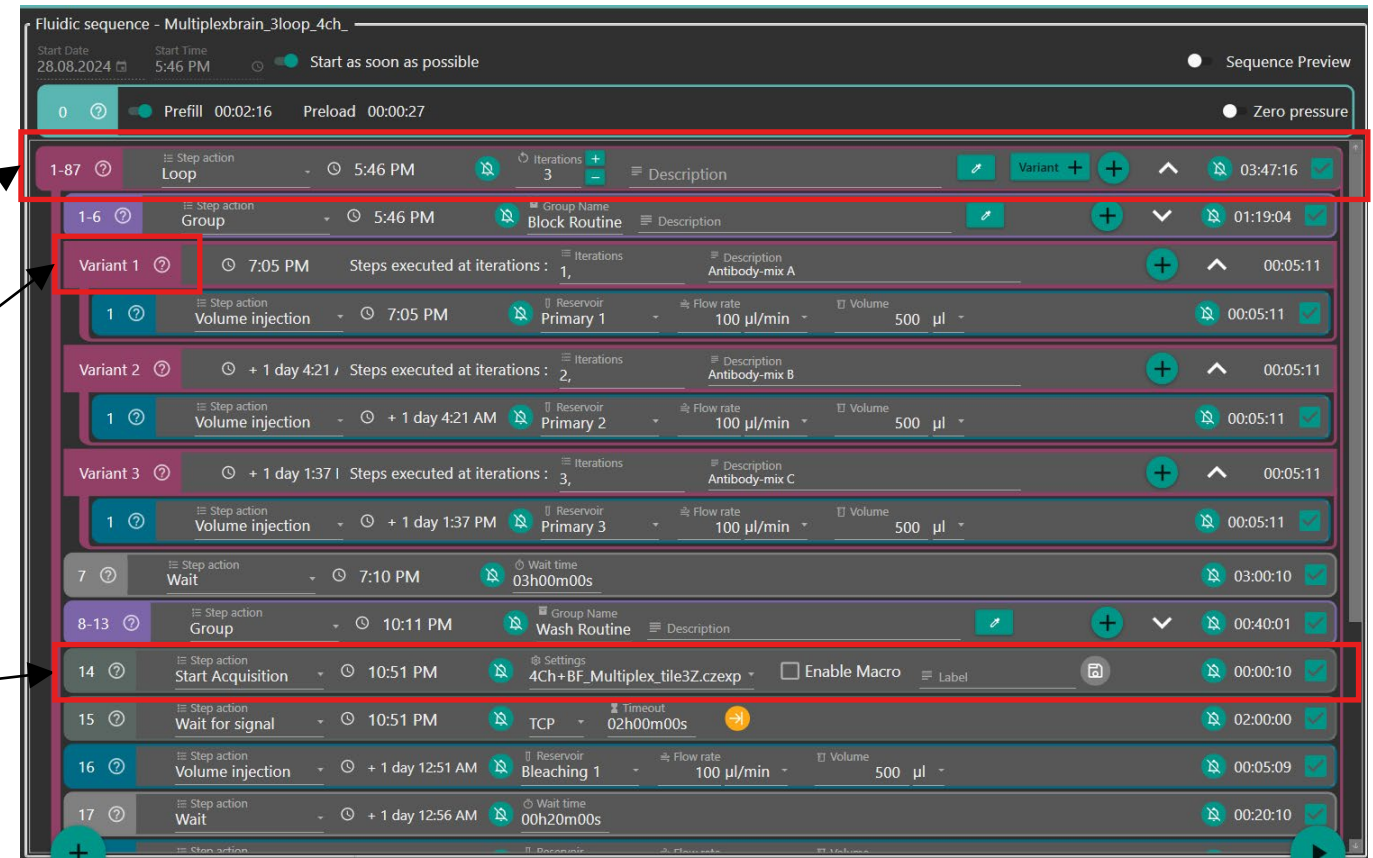


Application – Tissue Multiplexing

Workflow Aria Scheduler

Fluigent workflow...

- Controls reservoir, target sample, flow speed, flow volume and timing
- Overall **loop structure** to account for cyclic imaging runs
- Cyclic Ab staining is orchestrated via “**loop variants**” (here: change of reagent reservoir in each cycle)
- Zen acquisitions are toggled with pre-defined experiment



Fluidic sequence - Multiplexbrain_3loop_4ch_

Start Date: 28.08.2024, Start Time: 5:46 PM, Start as soon as possible

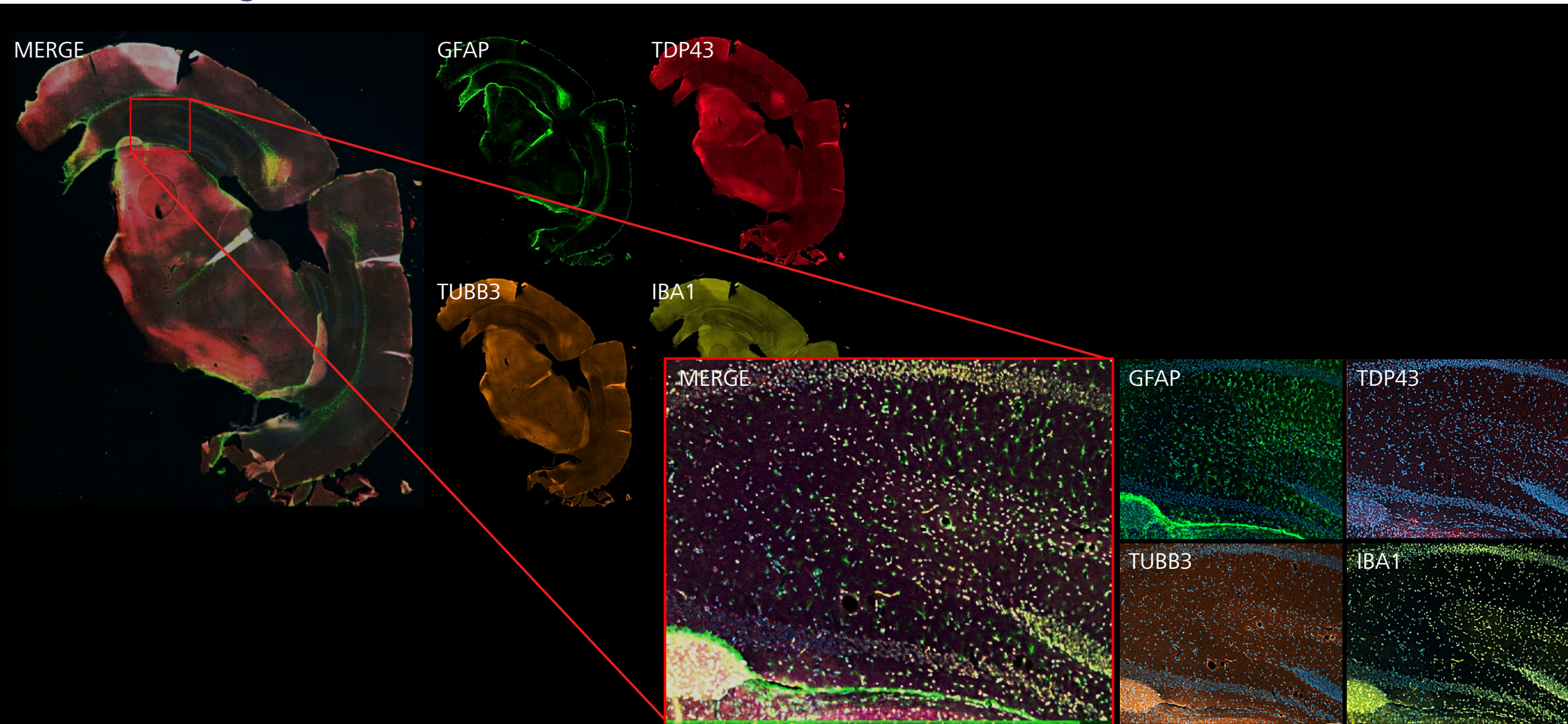
Sequence Preview

0, Prefill: 00:02:16, Preload: 00:00:27, Zero pressure

Step	Step action	Time	Iterations	Description	Variant	Time
1-87	Loop	5:46 PM	3			03:47:16
1-6	Group	5:46 PM		Block Routine		01:19:04
Variant 1		7:05 PM	Steps executed at iterations: 1,	Antibody-mix A		00:05:11
1	Volume injection	7:05 PM	Reservoir: Primary 1, Flow rate: 100 µl/min, Volume: 500 µl			00:05:11
Variant 2		+ 1 day 4:21	Steps executed at iterations: 2,	Antibody-mix B		00:05:11
1	Volume injection	+ 1 day 4:21 AM	Reservoir: Primary 2, Flow rate: 100 µl/min, Volume: 500 µl			00:05:11
Variant 3		+ 1 day 1:37	Steps executed at iterations: 3,	Antibody-mix C		00:05:11
1	Volume injection	+ 1 day 1:37 PM	Reservoir: Primary 3, Flow rate: 100 µl/min, Volume: 500 µl			00:05:11
7	Wait	7:10 PM	Wait time: 03h00m00s			03:00:10
8-13	Group	10:11 PM		Wash Routine		00:40:01
14	Start Acquisition	10:51 PM	Settings: 4Ch+BF_Multiplex_tile3Z.czexp, Enable Macro			00:00:10
15	Wait for signal	10:51 PM	TCP, Timeout: 02h00m00s			02:00:00
16	Volume injection	+ 1 day 12:51 AM	Reservoir: Bleaching 1, Flow rate: 100 µl/min, Volume: 500 µl			00:05:09
17	Wait	+ 1 day 12:56 AM	Wait time: 00h20m00s			00:20:10

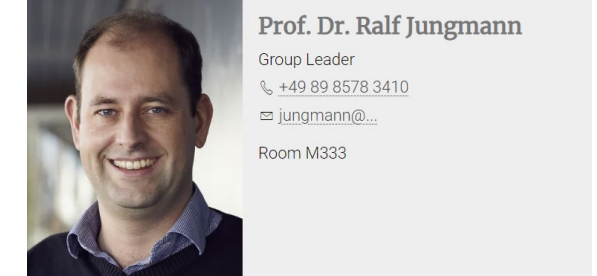
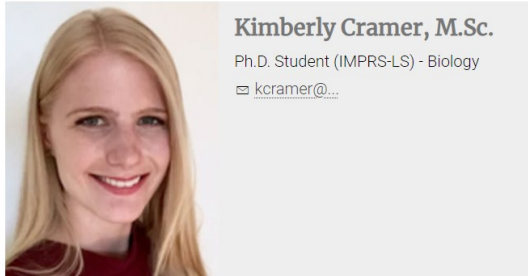
Application – Tissue Multiplexing

Result images

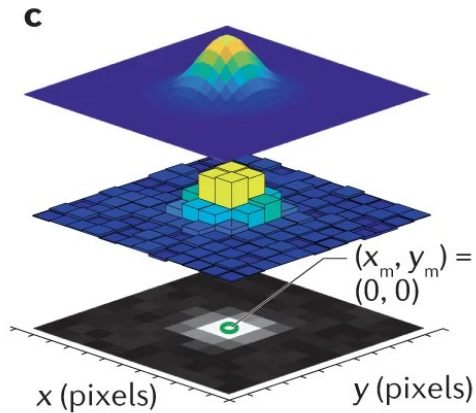


Application – DNA-PAINT

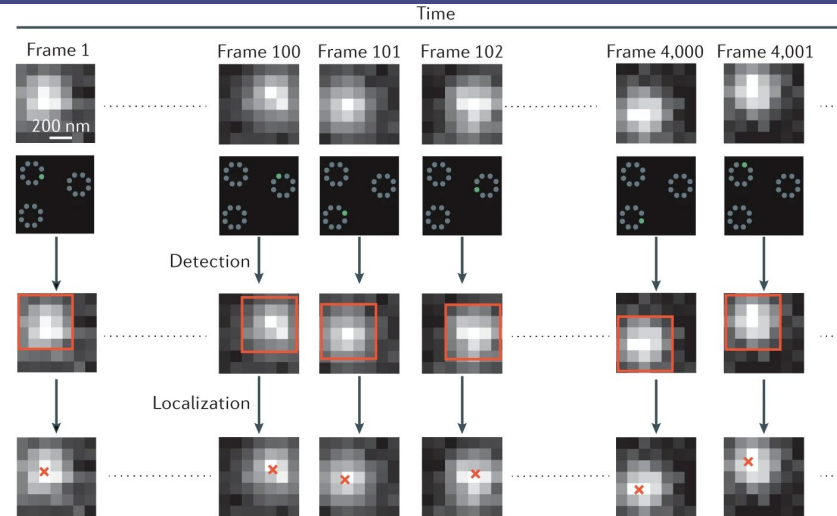
Introduction



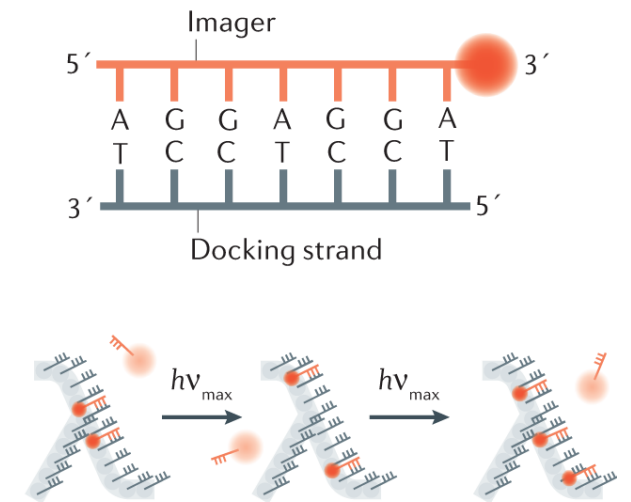
PSF to Localization



Detecting overlapping PSFs



Principle of DNA-PAINT

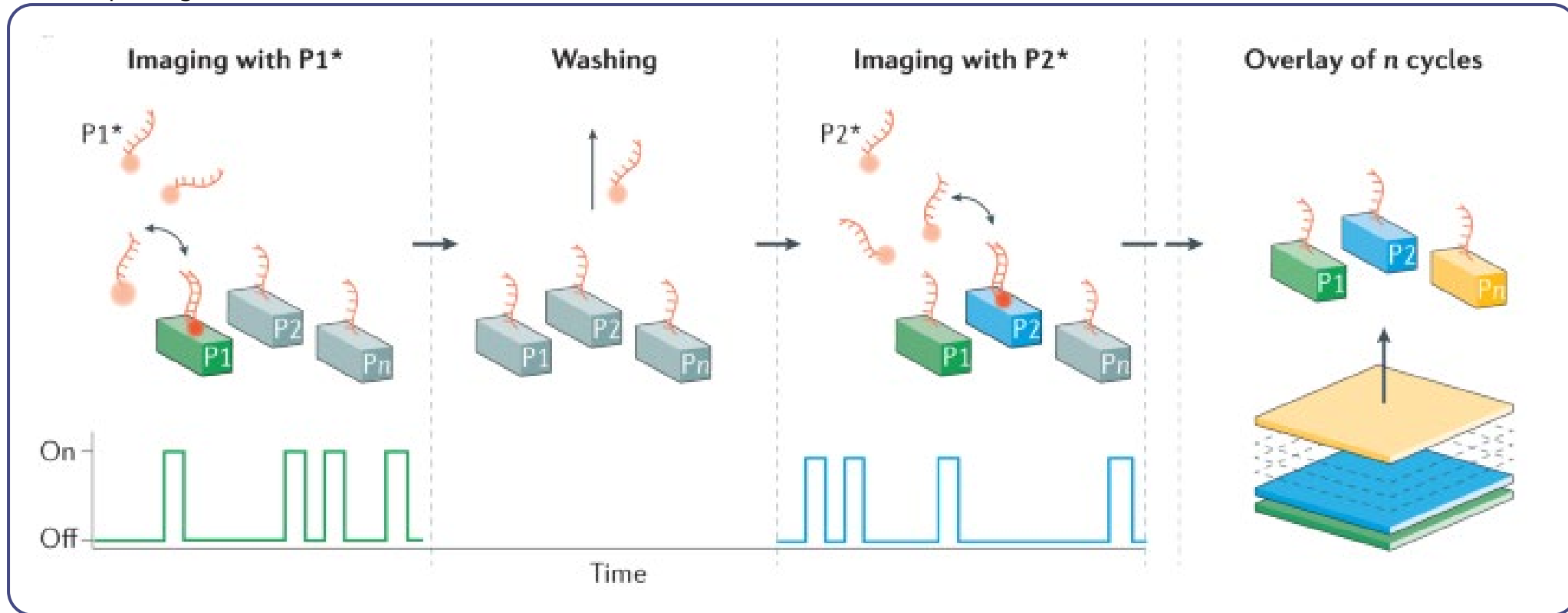


Lelek M et al. Single-molecule localization microscopy. Nat Rev Methods Primers. 2021

Application – DNA-PAINT

Exchange-PAINT benefits from Automation

“Multiplexing” with DNA-PAINT



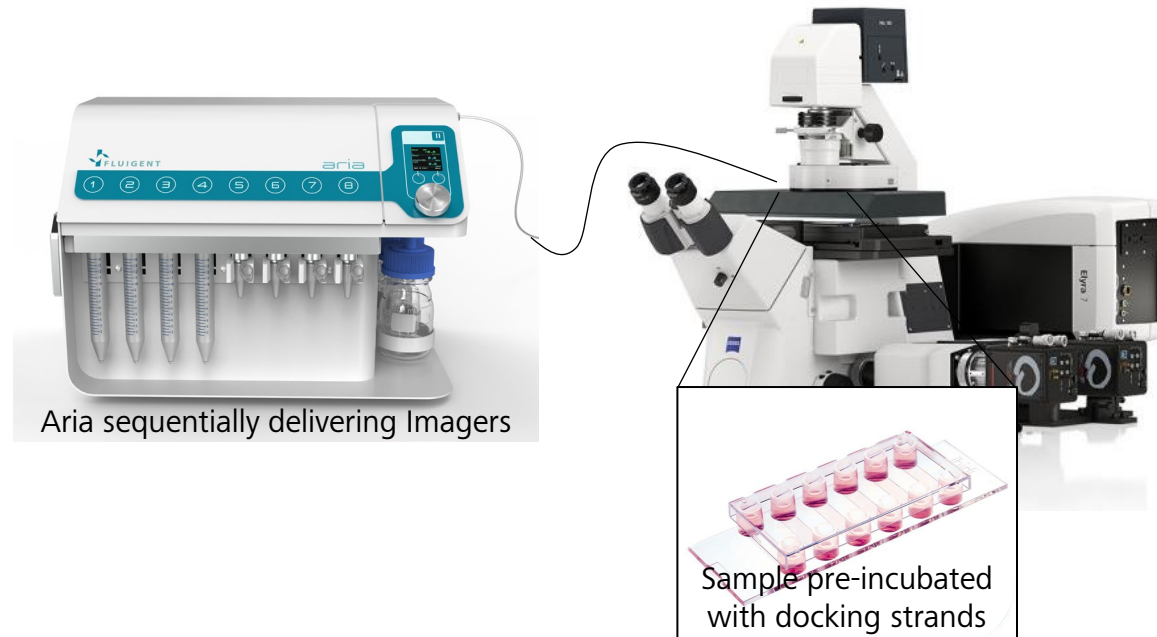
Lelek M et al. Single-molecule localization microscopy. Nat Rev Methods Primers. 2021

- Simultaneous detection of two or more targets is impossible with DNA-PAINT
- **But: Sequence specificity of imagers** allows for (theoretically unlimited) cyclic staining

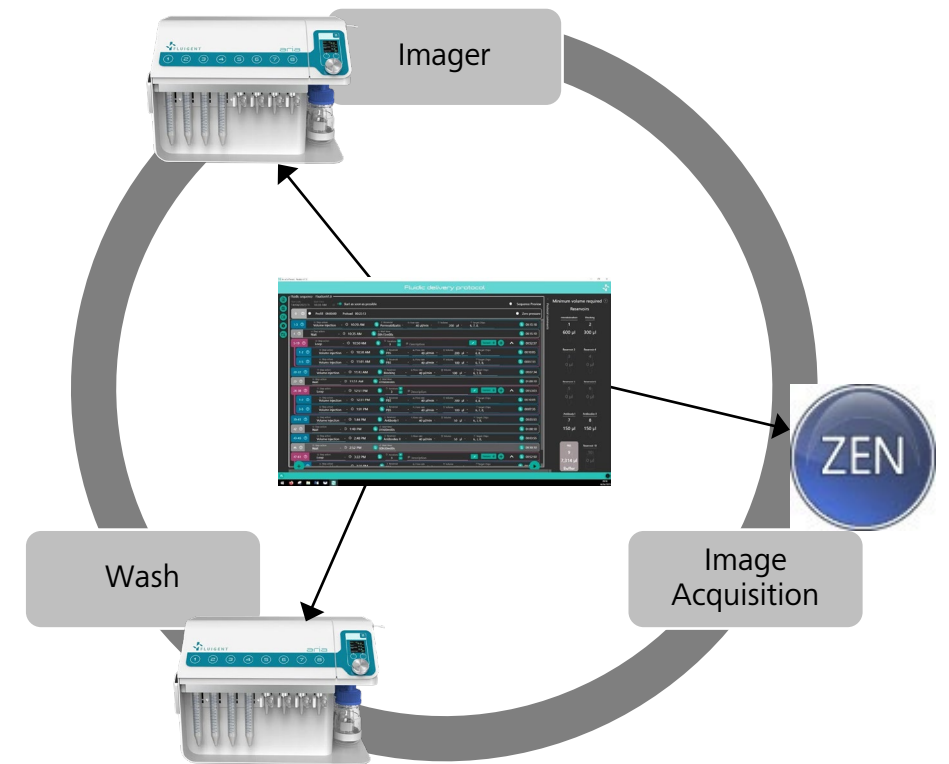
Application – DNA-PAINT

Experimental Setup

Hardware Setup



Workflow Setup



Application – DNA-PAINT

Final images – Exchange-PAINT

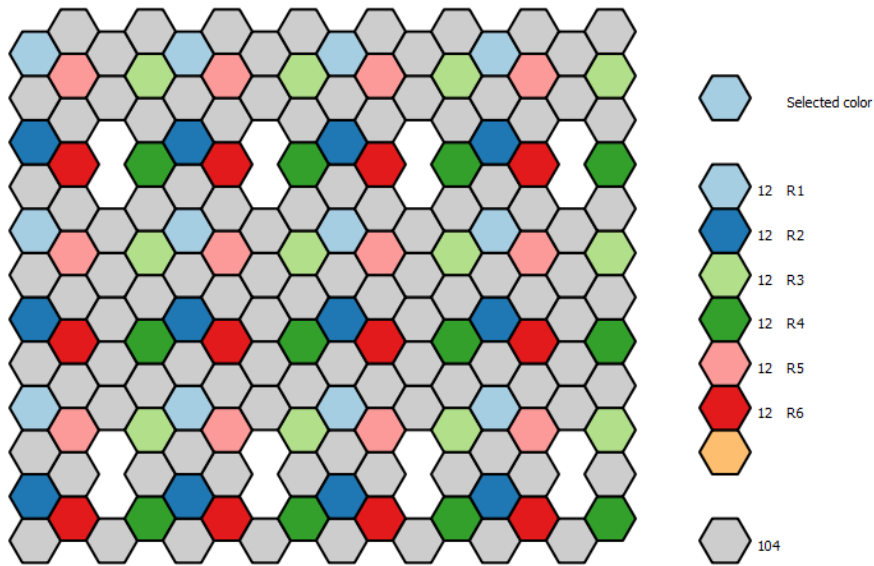


Figure 7: DNA origami pattern used in this experiment. The origami structure contains docker strands for the sequences R1 – R6 in a particular (4 x 3) shifted grid pattern. The sketch was created with the Picasso software (<https://github.com/jungmannlab/picasso>).

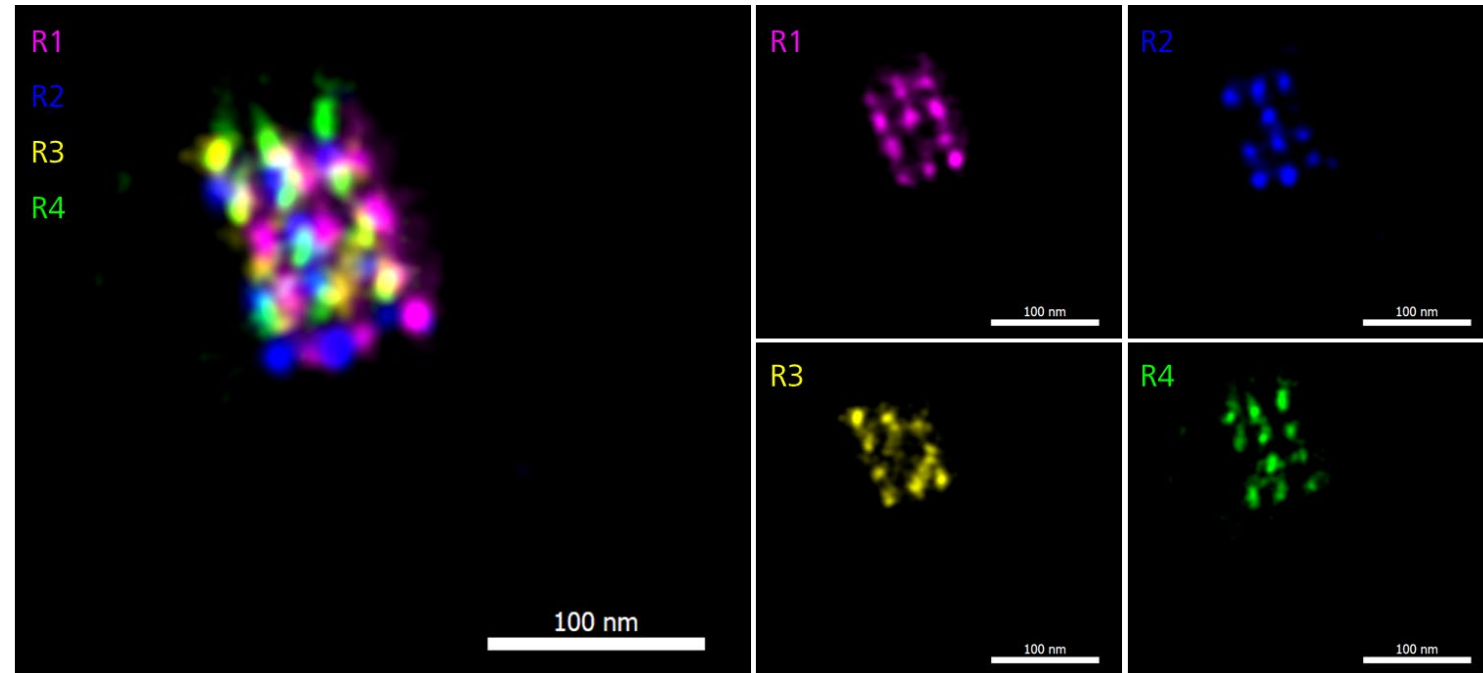
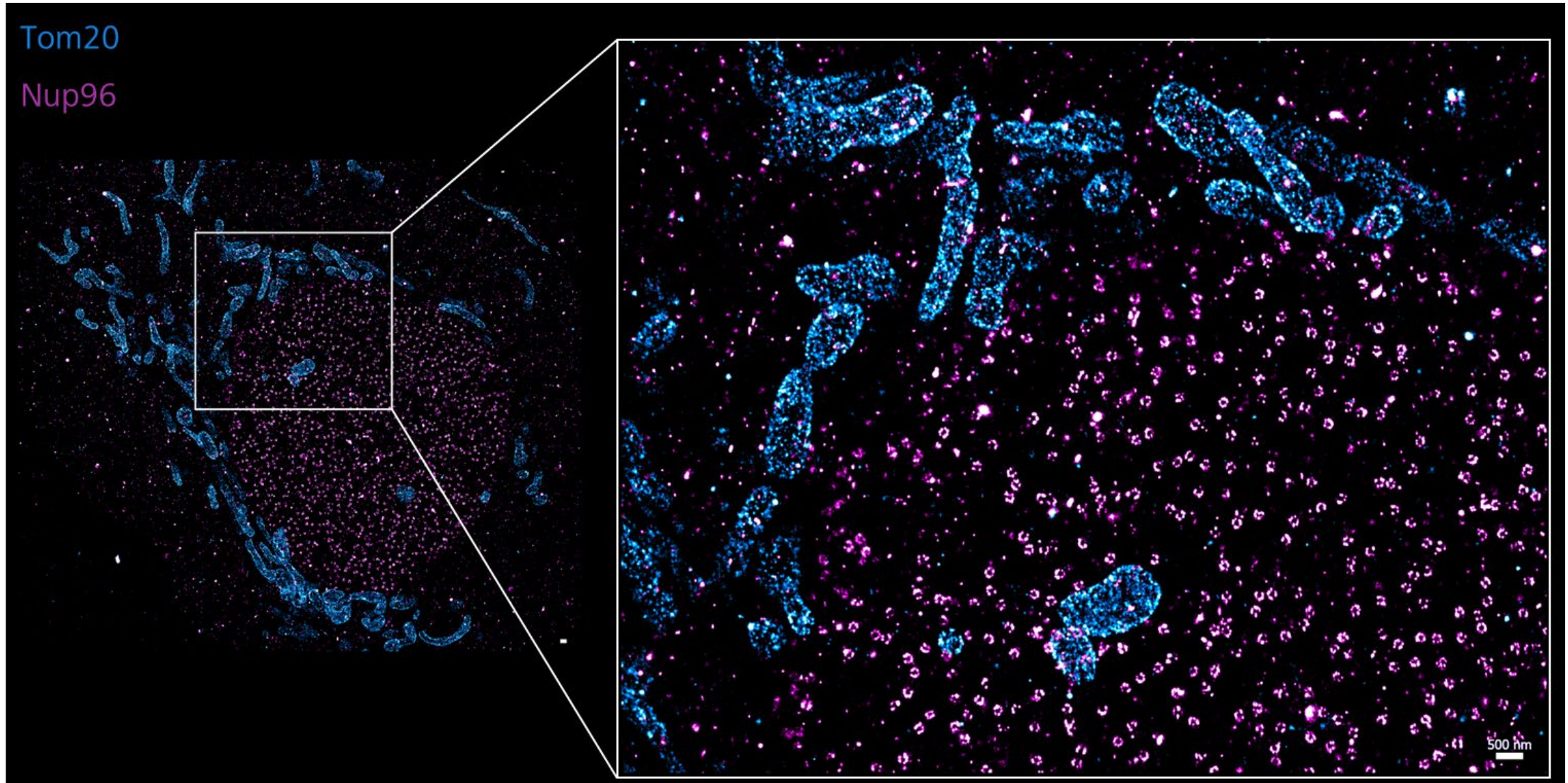


Figure 13: Final multi-channel super-resolved DNA-origami image. The 4x3 grid pattern (compare Figure 7) is visible for all four imagers. As expected from the origami layout, the grids are slightly shifted against each other. Screenshots were created with the Picasso software (<https://github.com/jungmannlab/picasso>).

Application – DNA-PAINT

Final images – Exchange-PAINT



Summary - Fluigent ARIA

- All-in-one, flexible, affordable and plug & play
- Intuitive instrument operation, no microfluidic expertise required
- Optimized integration with ZEN-based Zeiss instruments



Additional information...

- Customer Center in Oberkochen is now equipped with Aria and actively developing suitable demos
- A more detailed Sales Presentation / Quick Start Guide available on Widen, further collaterals (App Note, Info Flyer) are currently being prepared; more information available also on the [Fluigent webpage](#)
- If interested to purchase a microfluidic system together with Zeiss imaging instruments, please contact your local account manager, Debora.Olivier@zeiss.com (Product Management) or Philipp.Seidel@zeiss.com (Business Sector Life Sciences)
- Apart from the Aria, the “Developer Toolkit” for ZEN is needed for the “SW handshake”



Seeing beyond