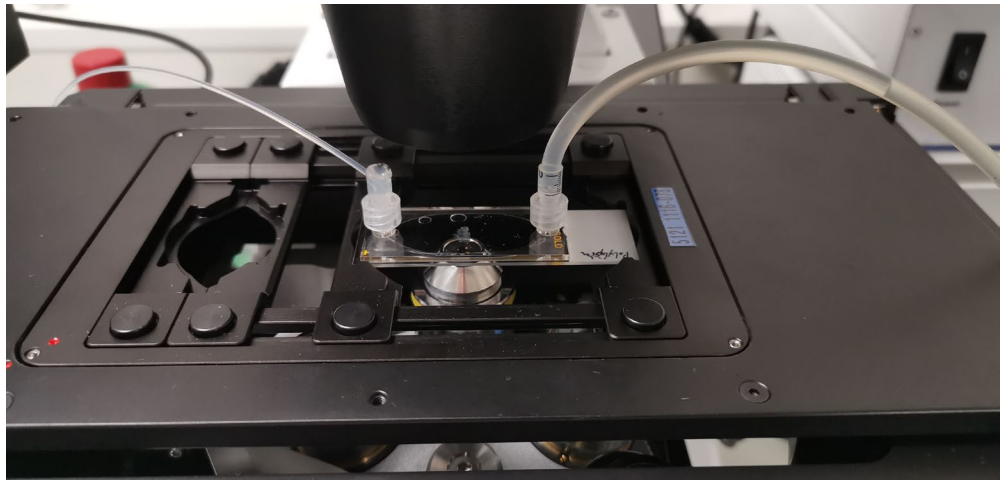
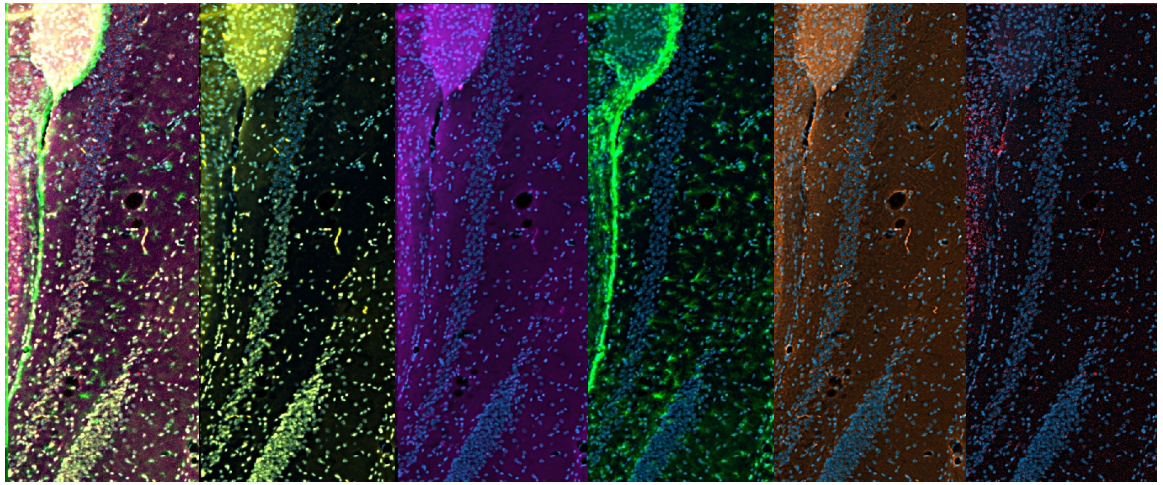
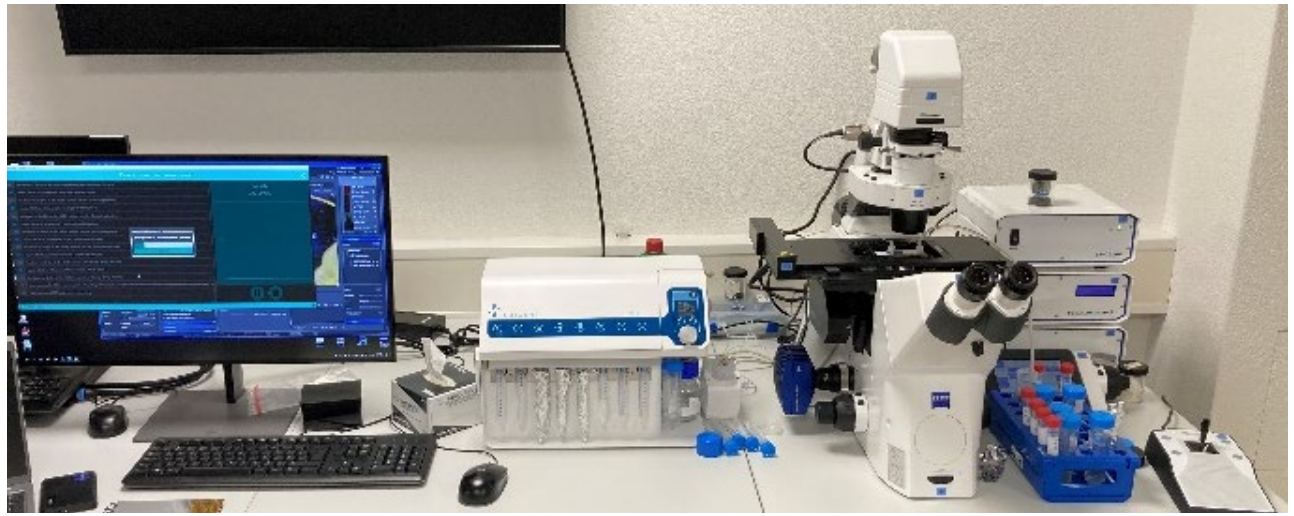
Microfluidics on Scope –
Enabling modern applications like Spatialomics or SMLM







Philipp Seidel
Business Sector Life Sciences, ZEISS RMS

5 September 2024

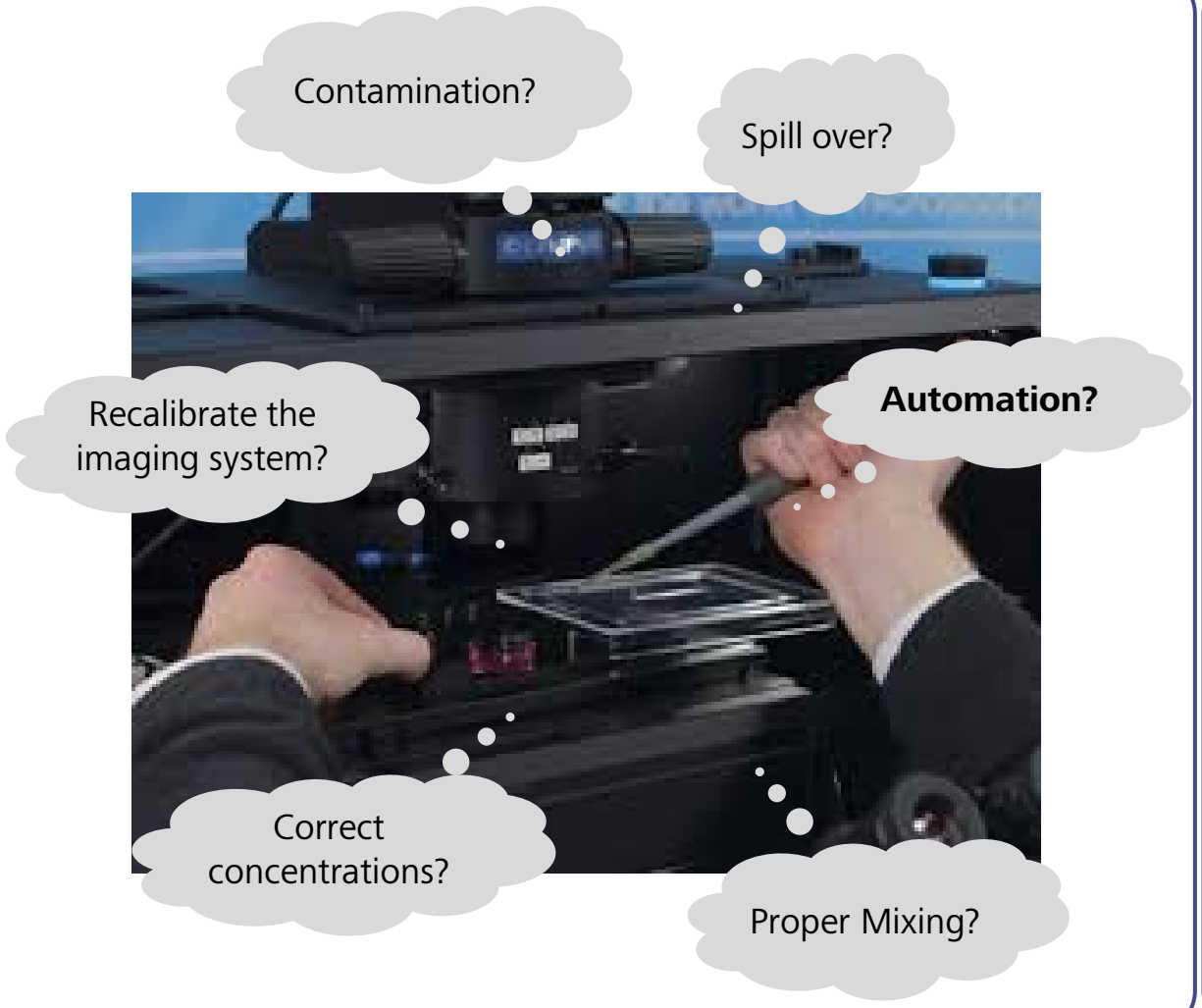
Agenda

01 Modern microfluidics with the Fluigent Aria

02 Better integration with ZEN software

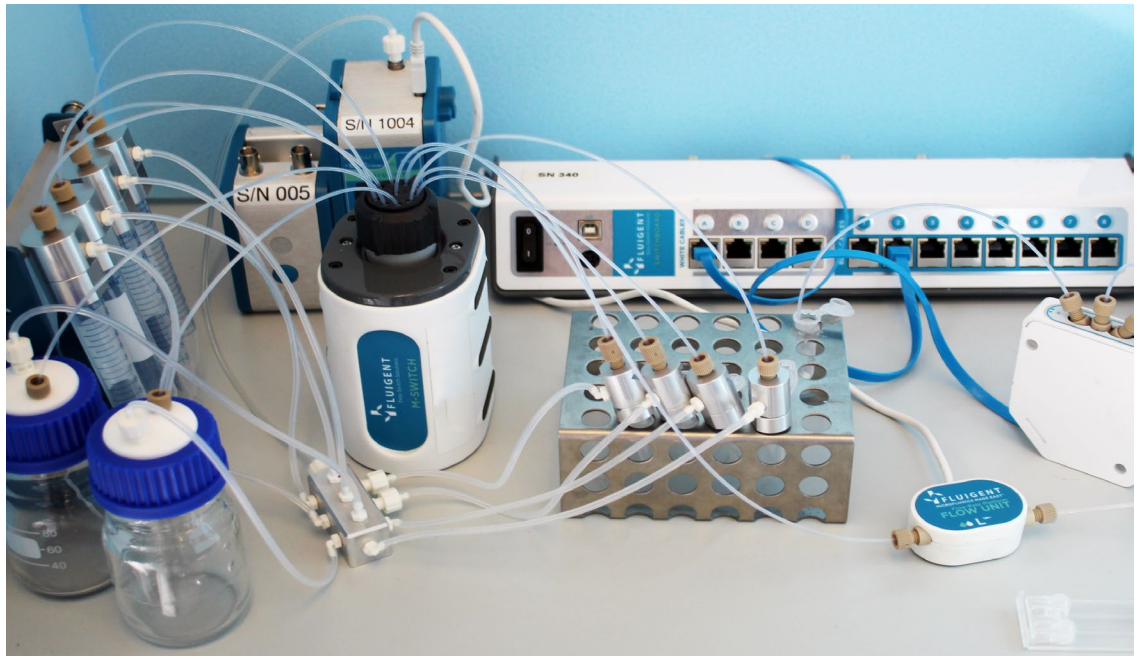
03 Application examples

Fluigent Aria
Combined imaging with fluid delivery



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…

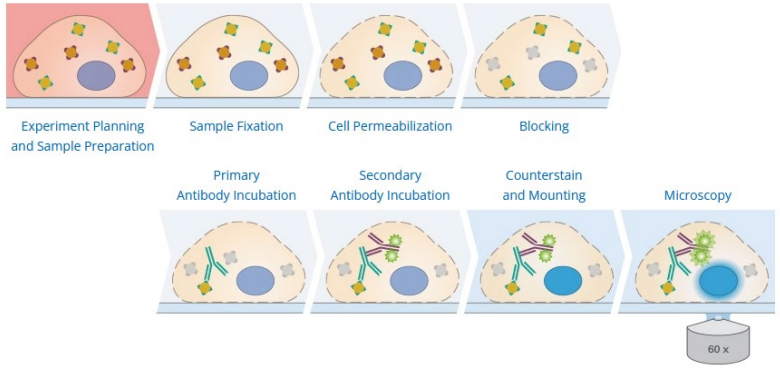
Fluigent ARIA

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

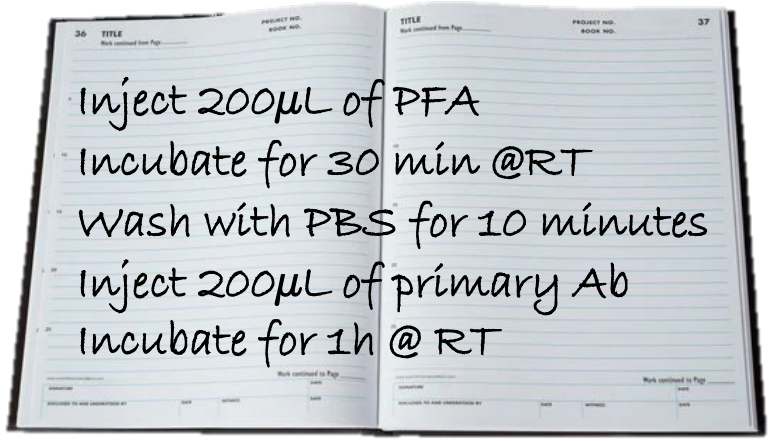
Intuitive operation

… with the Aria scheduling software

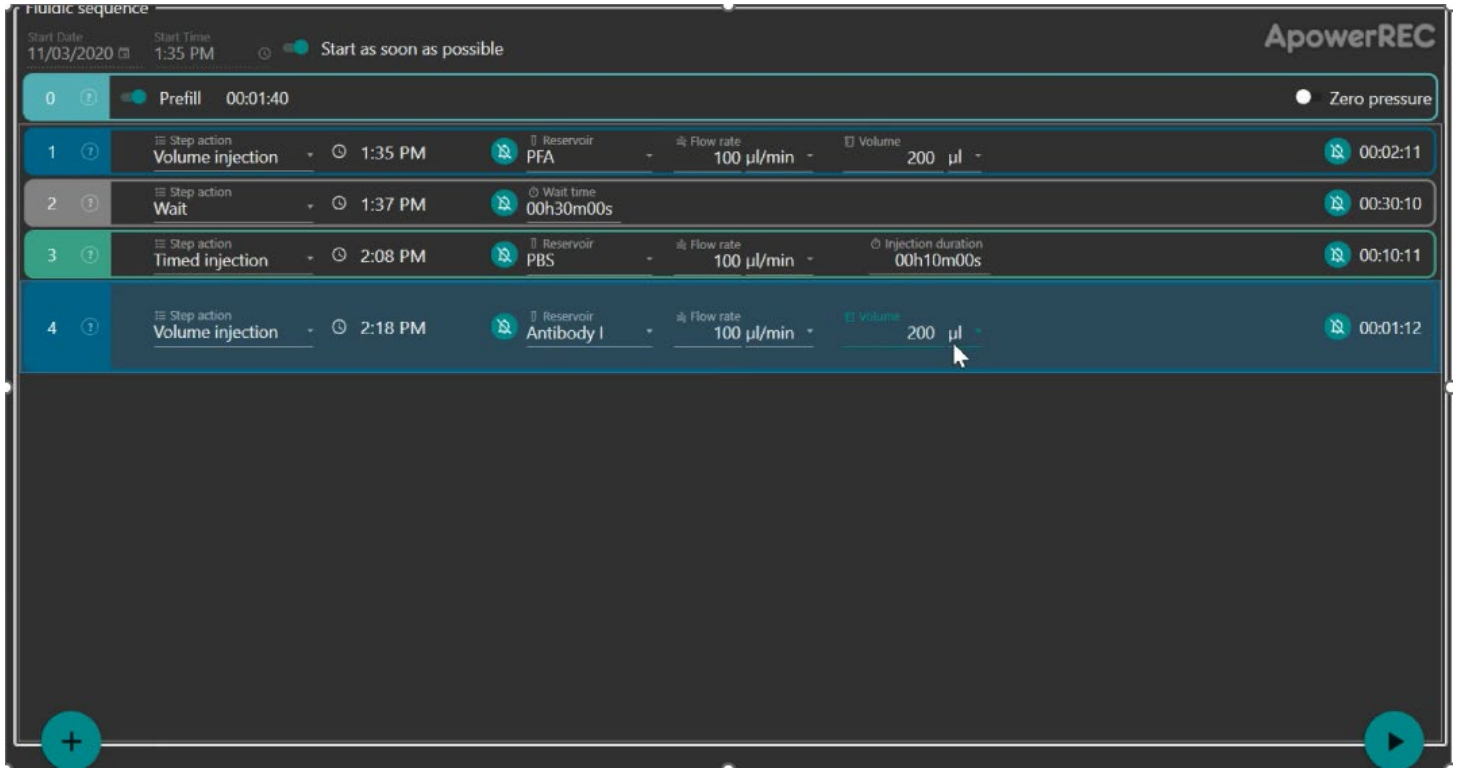
A typical Immunofluorescence workflow…



… and its staining protocol…



… translated into a valid Aria Sequence:



Precise fluid delivery

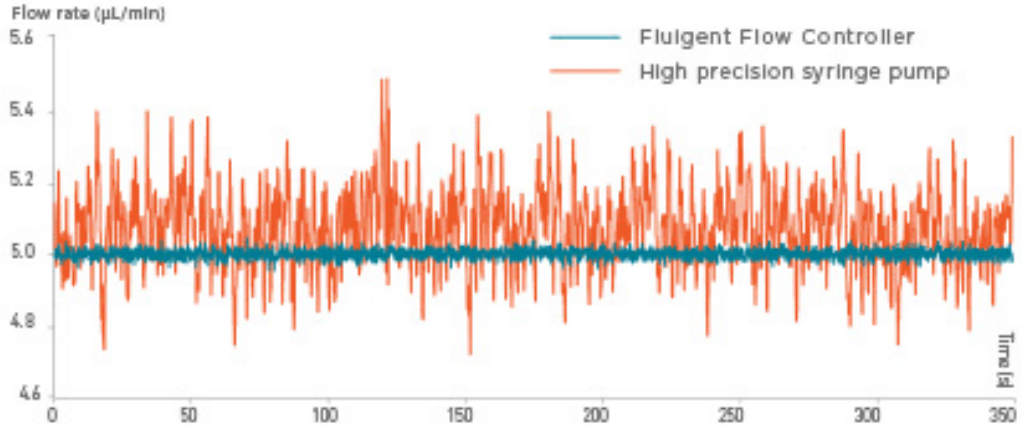
… with the best-in-class pressure-based flow control

Fluigent flow control vs. **High-precision Syringe Pump**

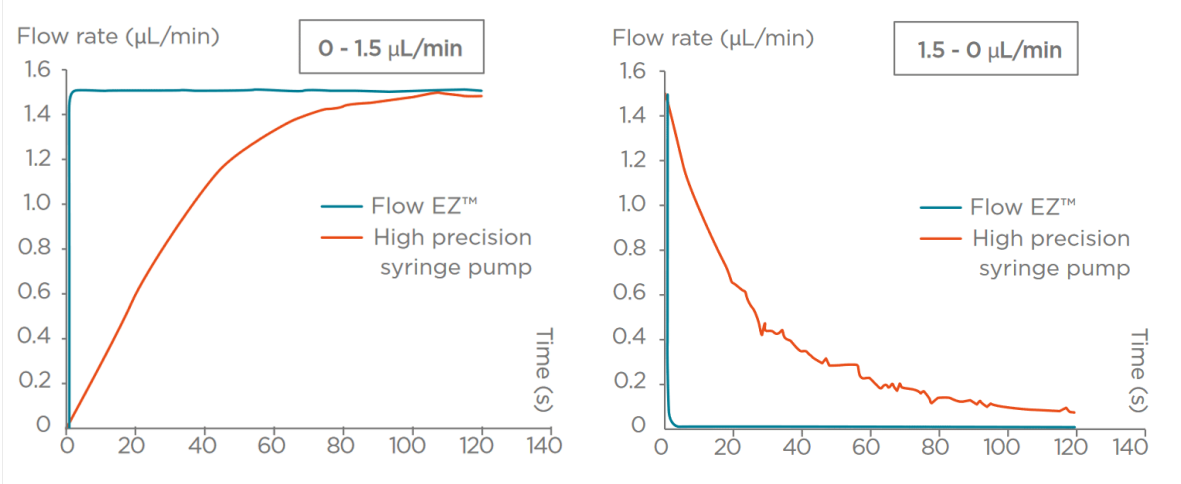
Smart fluidics handling

- 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



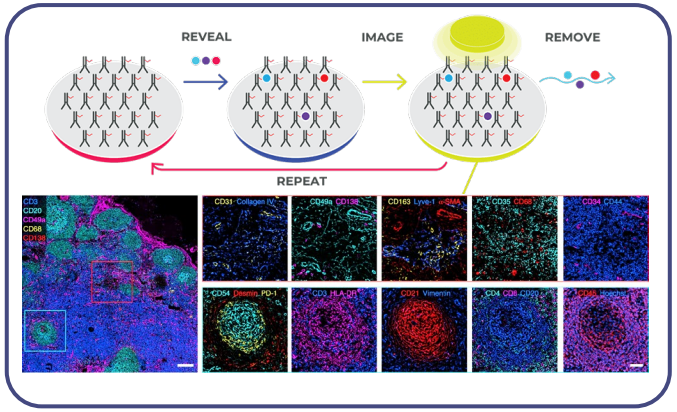
Open platform aspects

Versatility towards applications and protocols

Imaging HW+SW

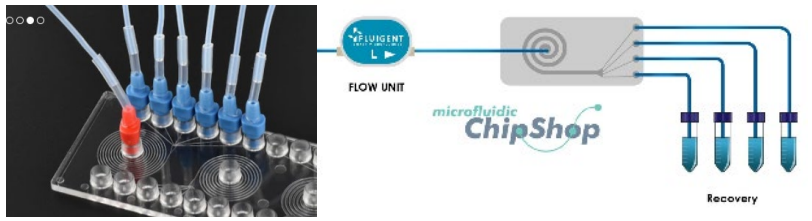
+
enabling technology
Microfluidics

Staining / Cyclic-Staining -> Spatialomics



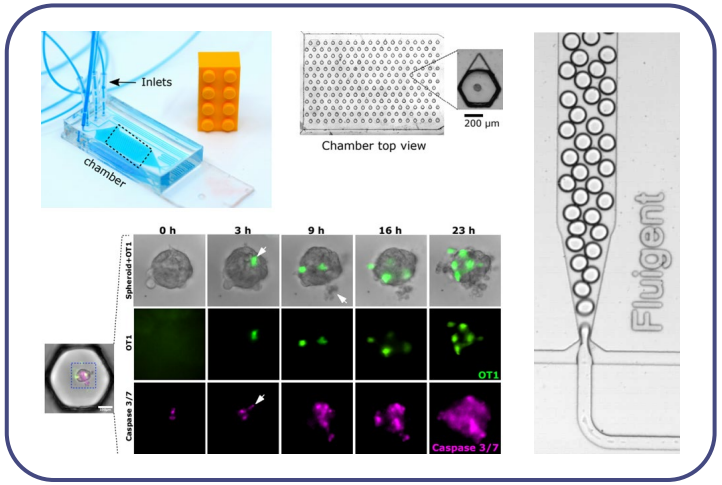
MPS / Organ-on-a-Chip

Cell-Sorting / Separation



Live-Cell Imaging / Perfusion

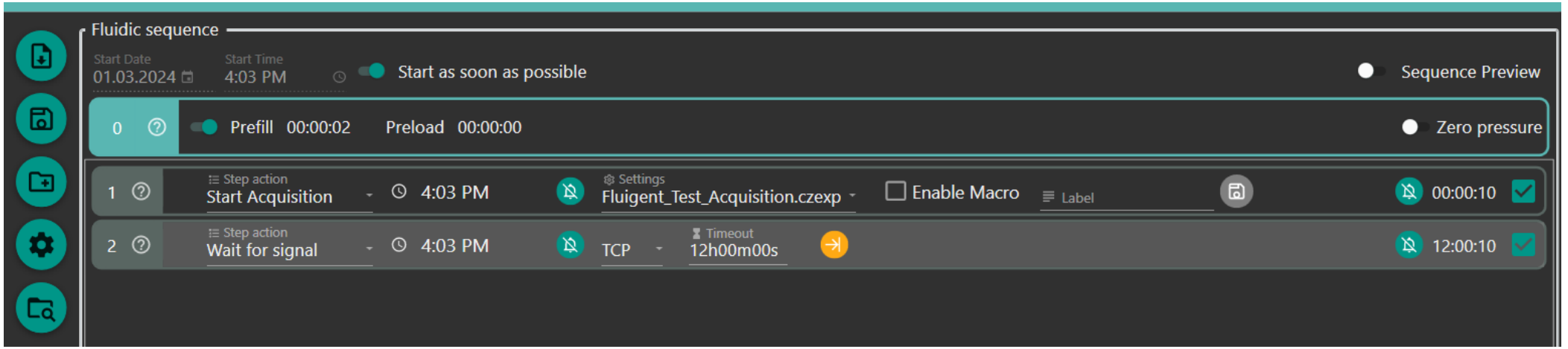
Droplets –> Miniaturization / Throughput



Agenda

Combining fluidic and imaging workflows

The novel, robust and intuitive TCP-IP handshake …



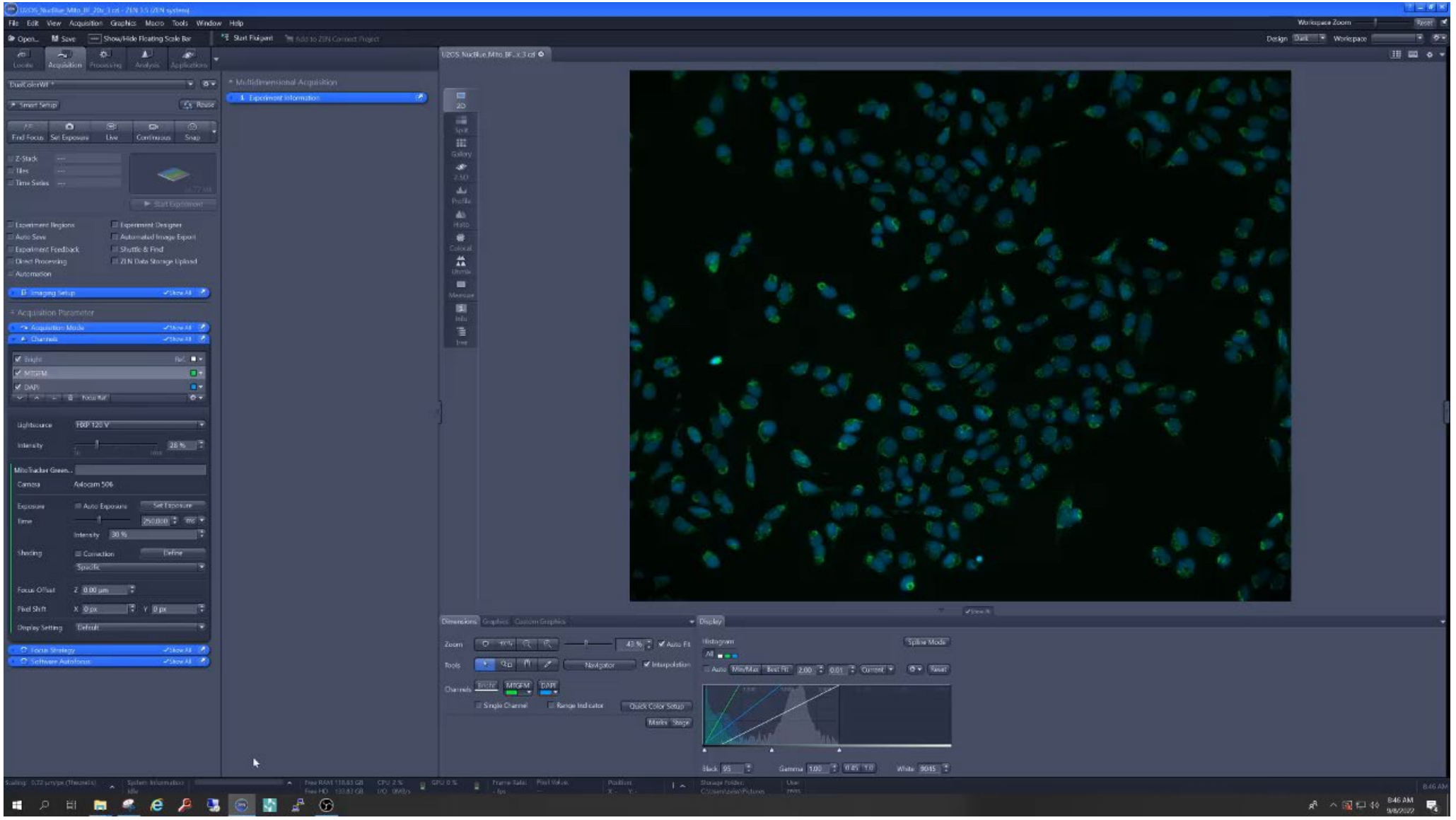
- schedules & initiates acquisitions

ZEN

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

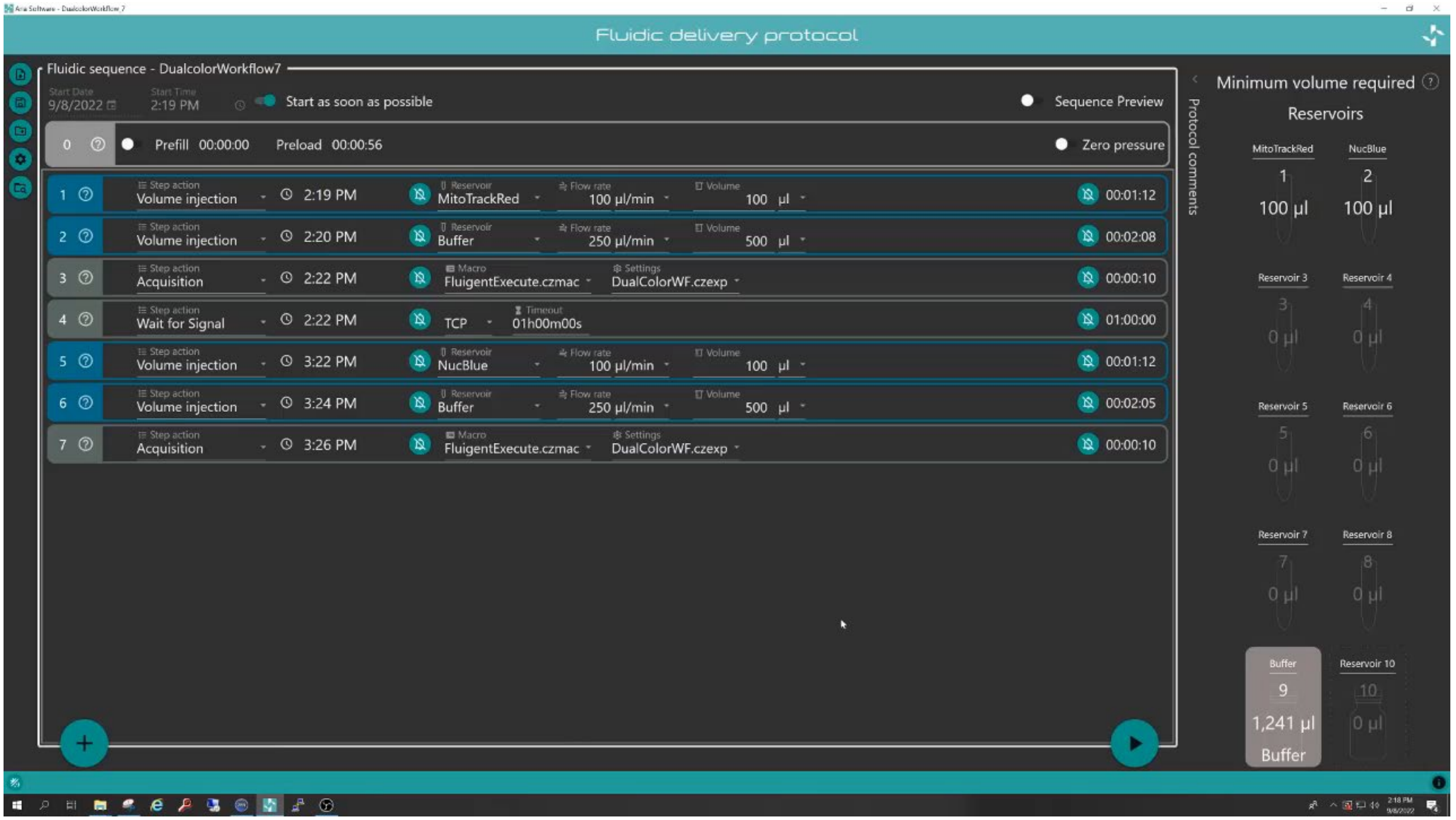
Combined fluidic and imaging workflows

… leads to intuitive and seamless workflows



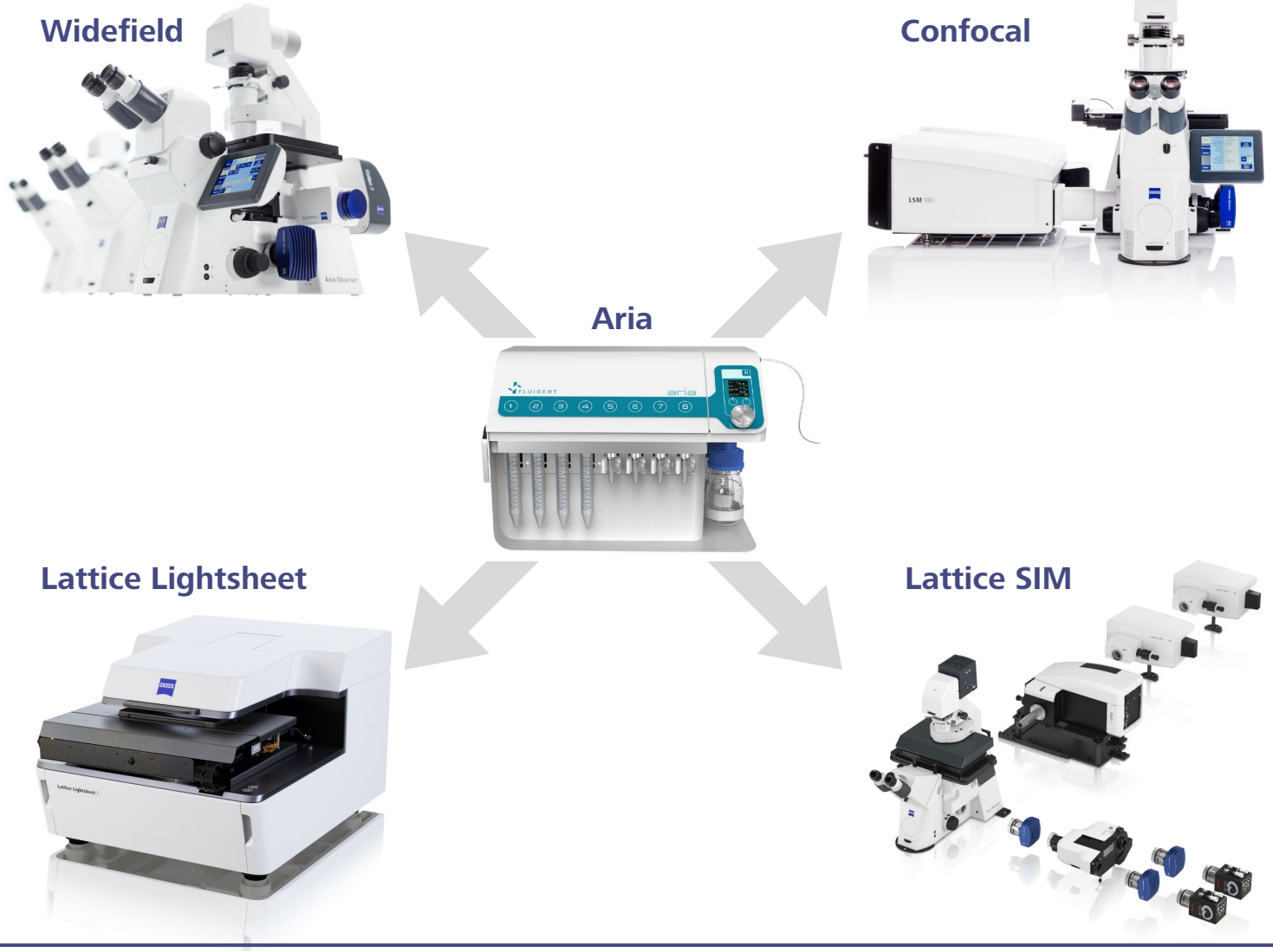
Combined fluidic and imaging workflows

… leads to intuitive and seamless workflows



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

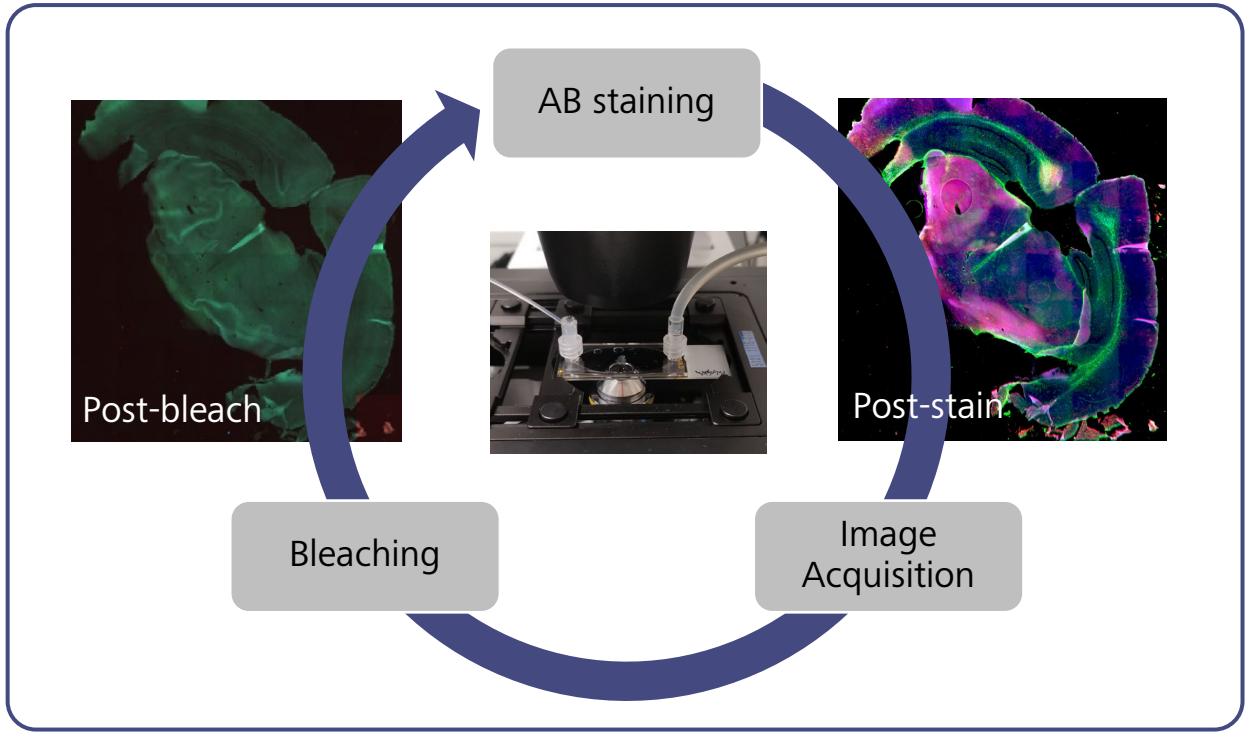
Application – Tissue Multiplexing
Introduction

Dr. Wolf Heusermann

Dr. Julien Toquant

In local collaboration with:

- 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)



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

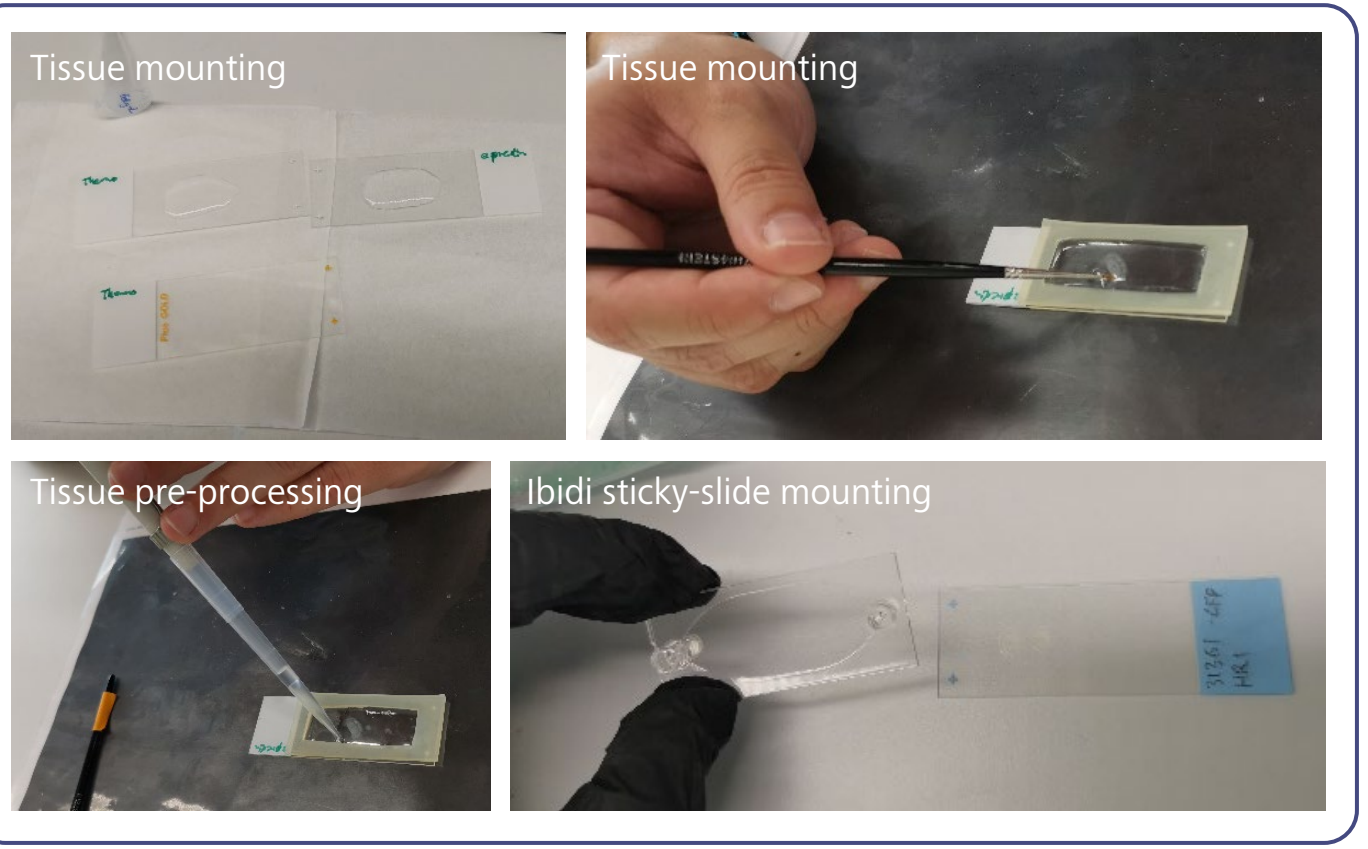
Application – Tissue Multiplexing
Sample and reagent preparation

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

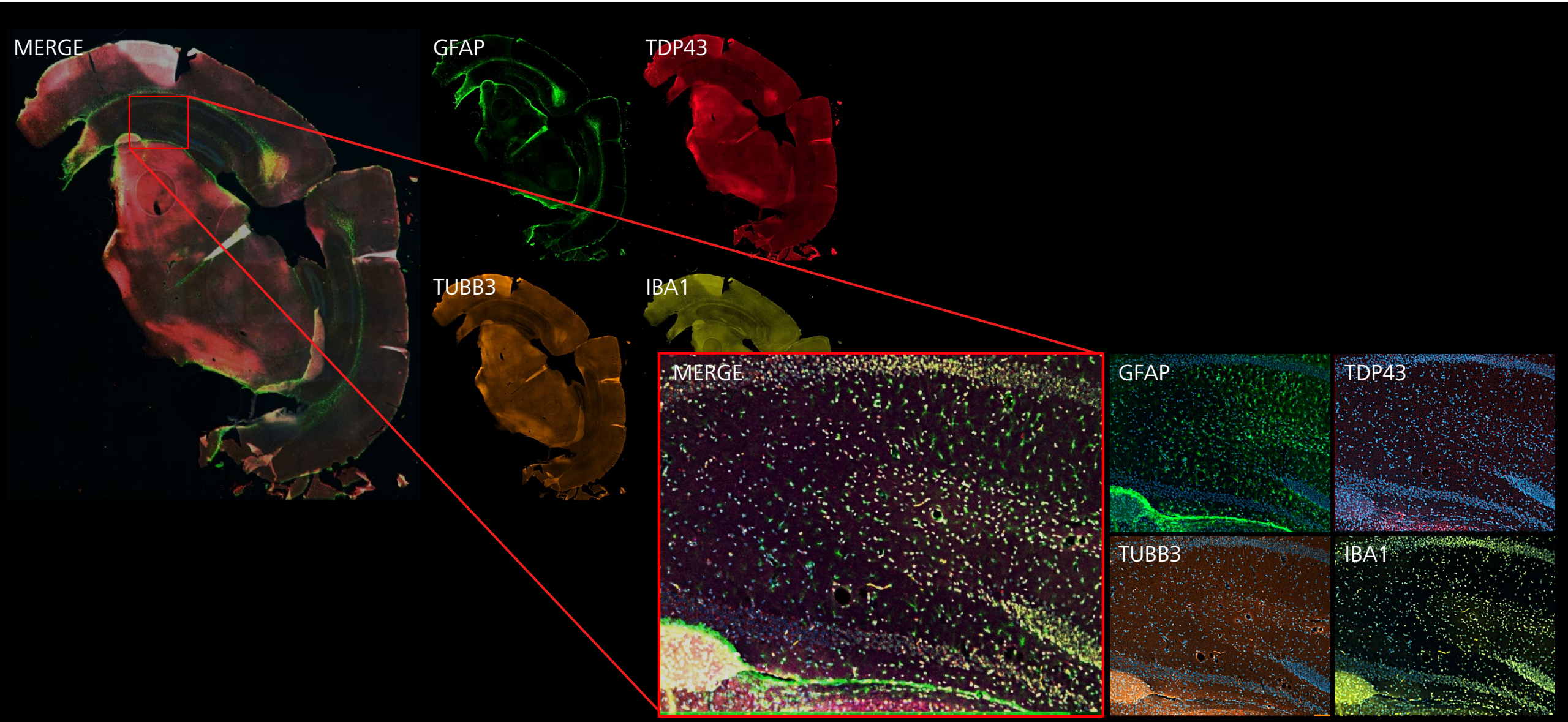
Workflow Aria Scheduler

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

Application – Tissue Multiplexing

Result images



Application – DNA-PAINT
Introduction

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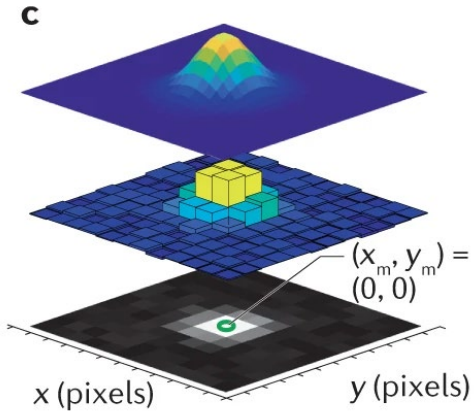
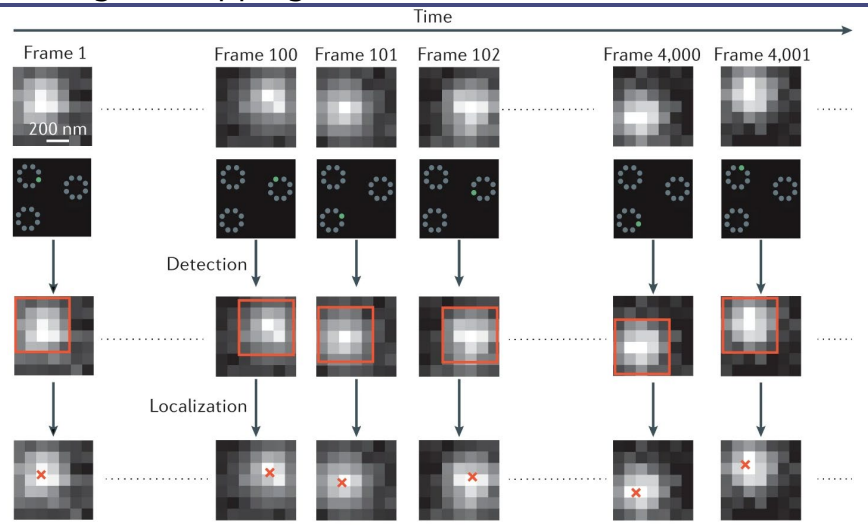
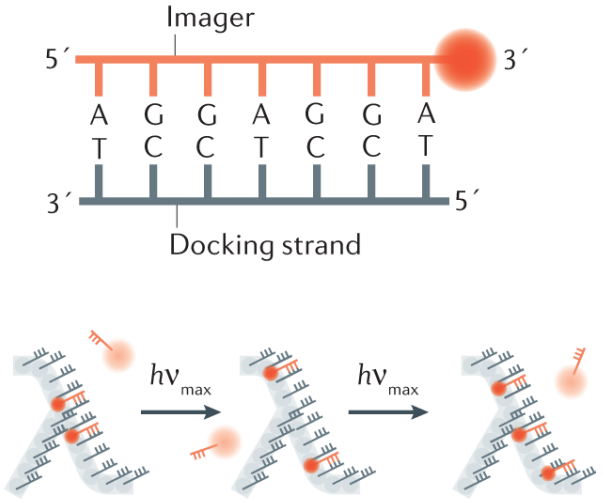
PSF to Localization



Detecting overlapping PSFs



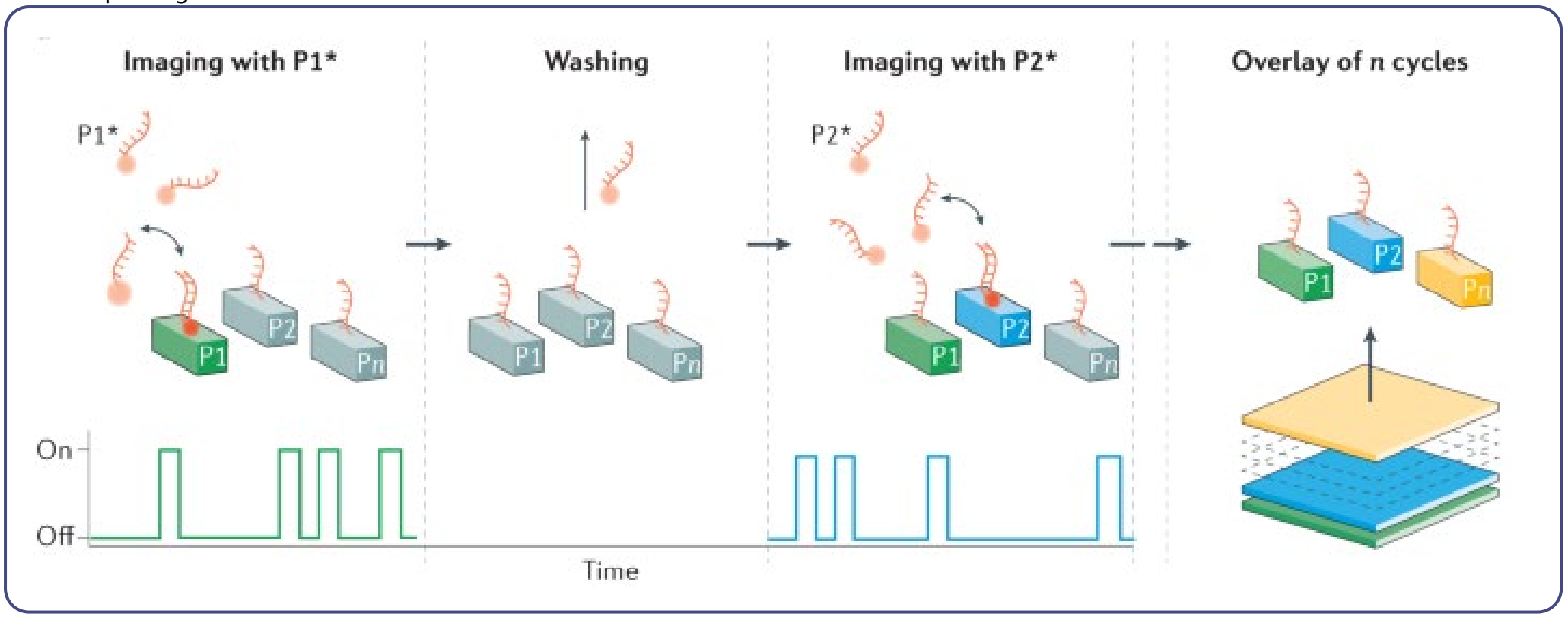
Principle of DNA-PAINT



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

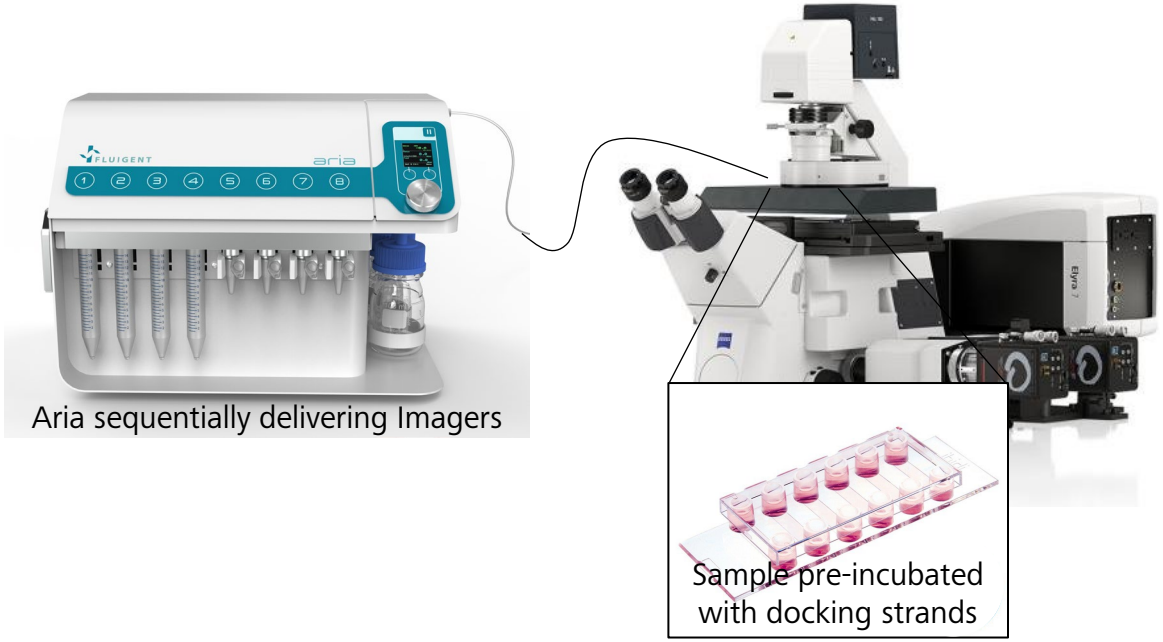


Lelek M et al. Single-molecule localization microscopy. Nat Rev Methods Primers. 2021

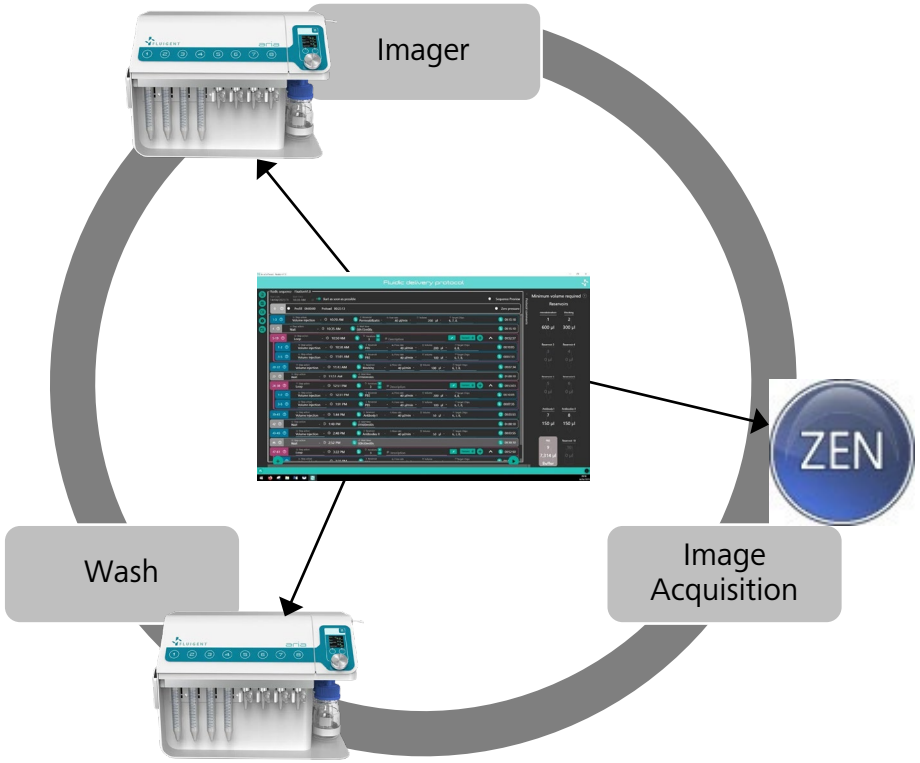
- Simultaneous detection of two or more targets is impossible with DNA-PAINT
- **But: Sequence specificity of imagers** allows for (theoretically unlimited) cyclic staining

Application – DNA-PAINT
Experimental Setup

Hardware Setup



Workflow Setup



Application – DNA-PAINT
Final images – Exchange-PAINT

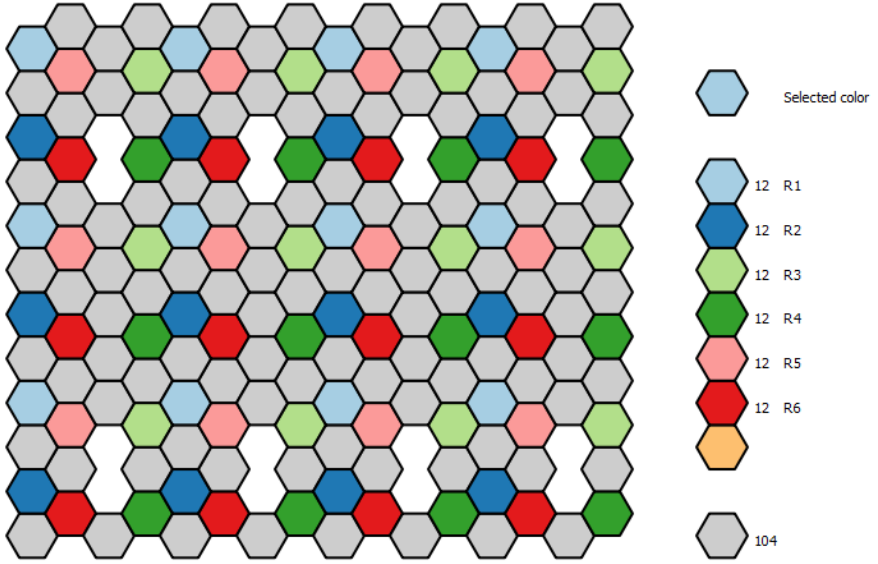


Figure 7: DNA origami pattern used in this experiment. The origami structure contains docker strands for the sequences R1 – R6 in a particular (4 x 3) shifted grid pattern. The sketch was created with the Picasso software (https://github.com/jungmannlab/picasso).

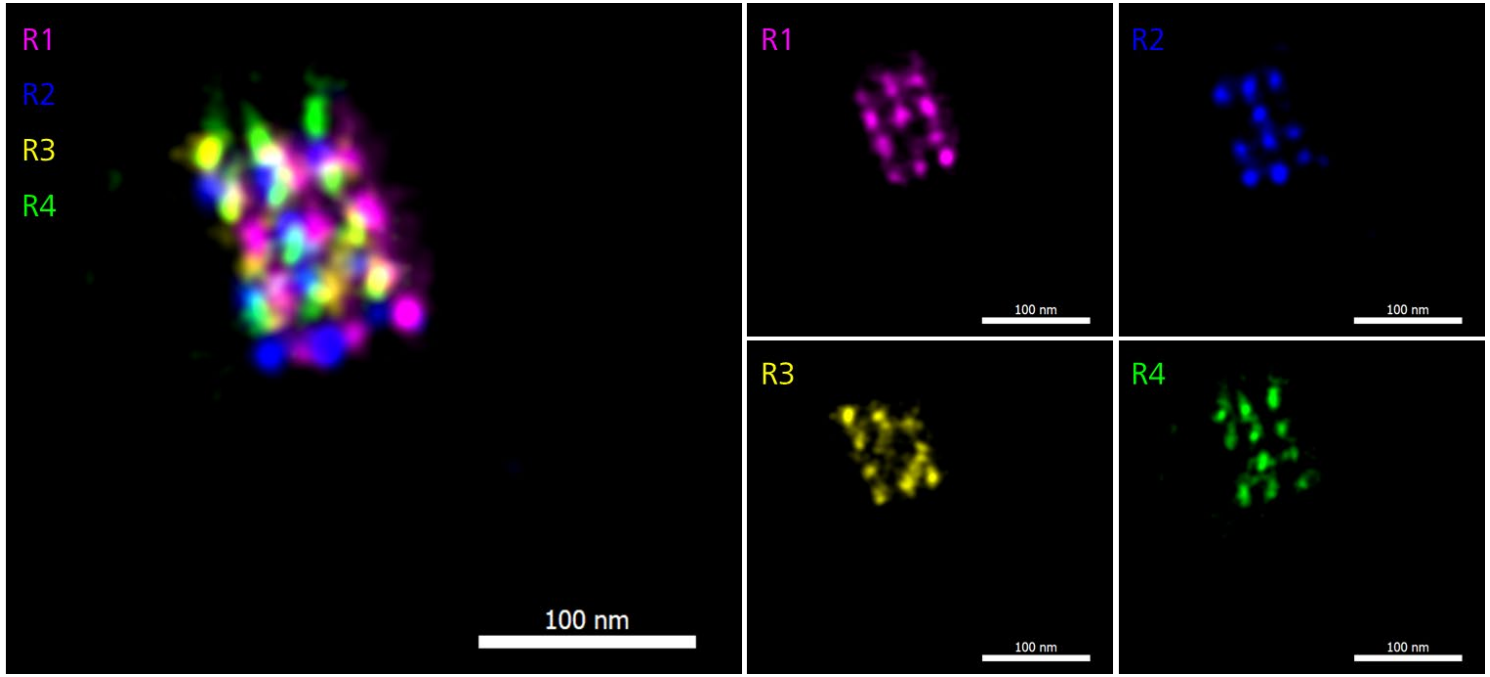
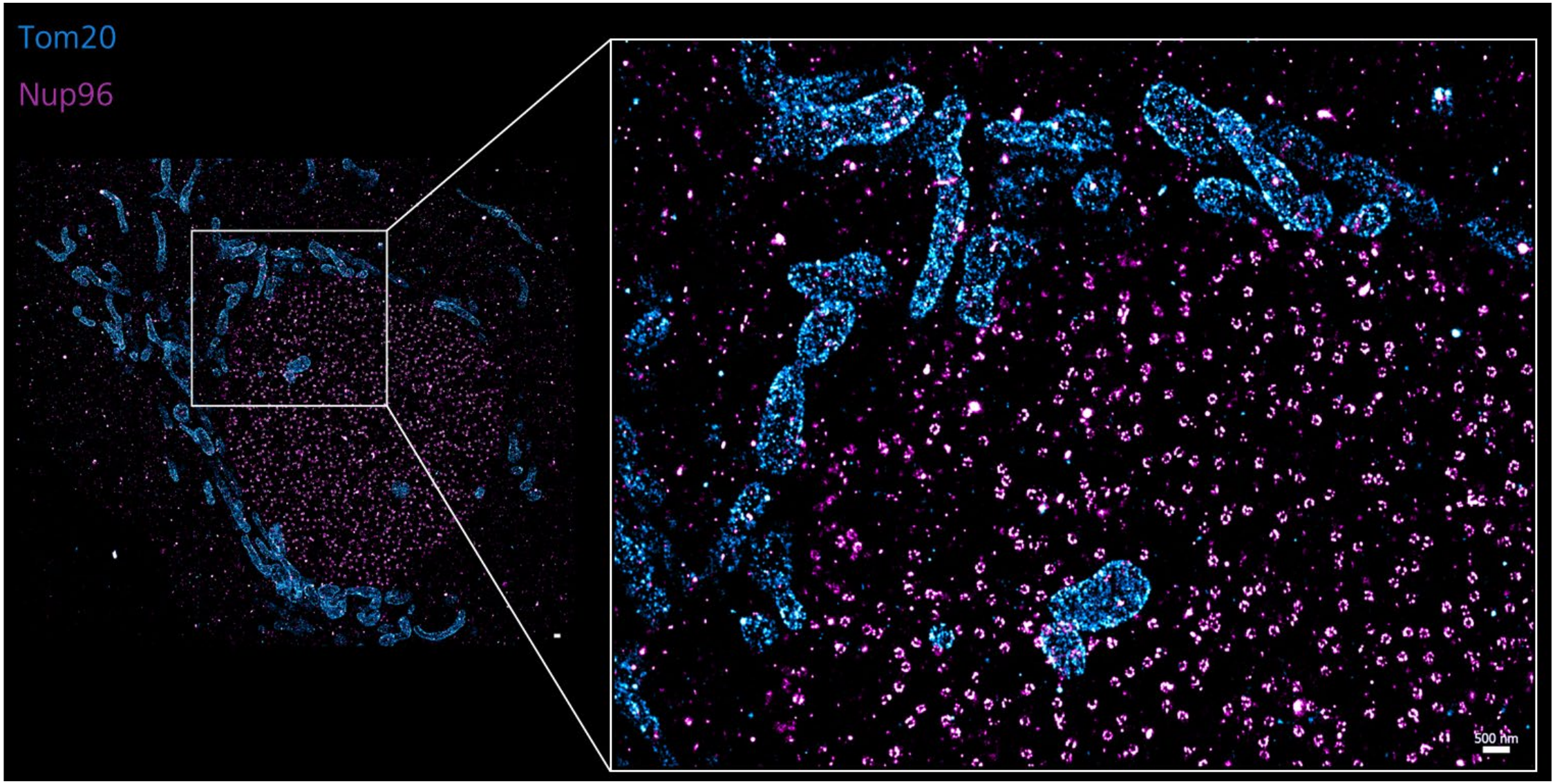


Figure 13: Final multi-channel super-resolved DNA-origami image. The 4x3 grid pattern (compare Figure 7) is visible for all four imagers. As expected from the origami layout, the grids are slightly shifted against each other. Screenshots were created with the Picasso software (https://github.com/jungmannlab/picasso).

Application – DNA-PAINT

Final images – Exchange-PAINT



Take home messages

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