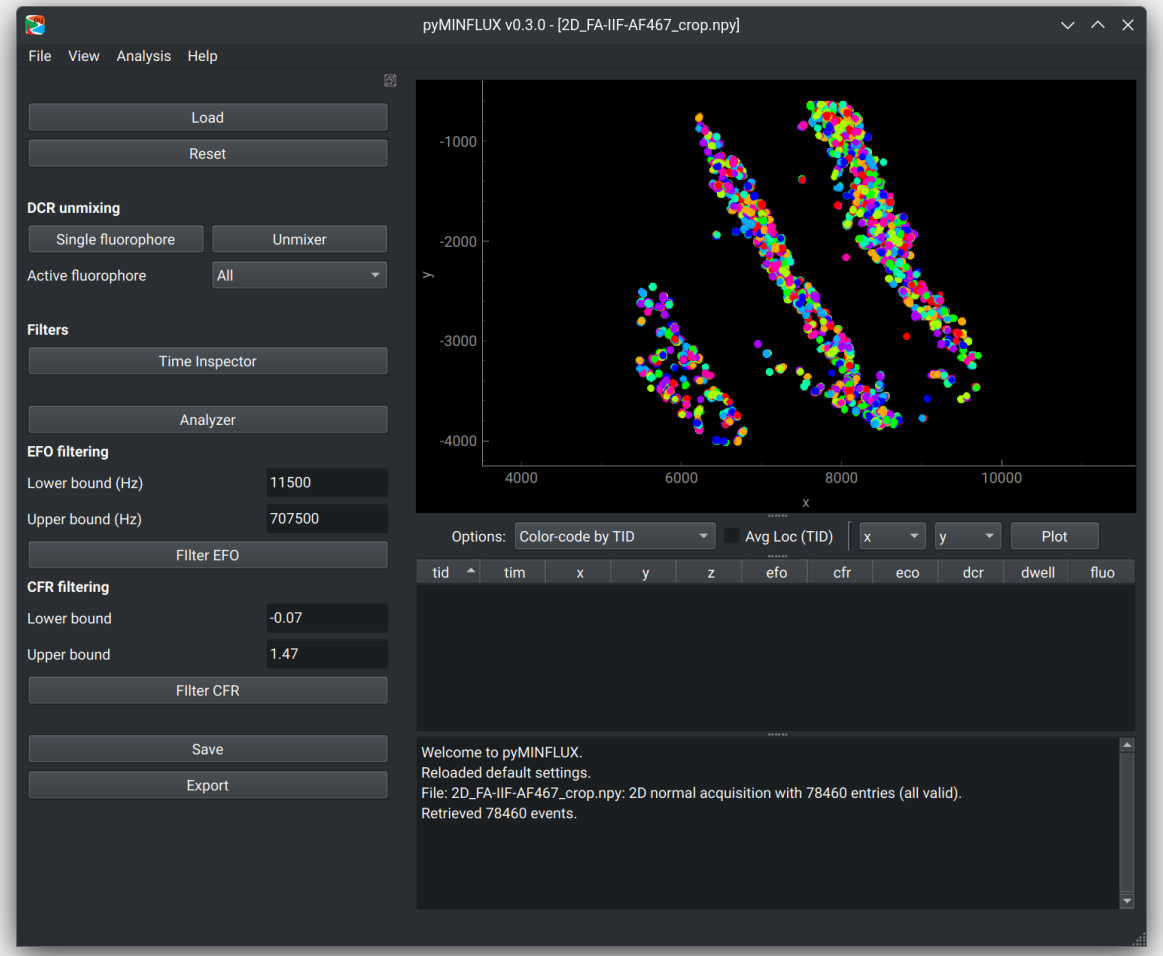
Collaborative expertise

Synergies between multi-disciplinary experts and end users in software development

Aaron Ponti



About me

Education

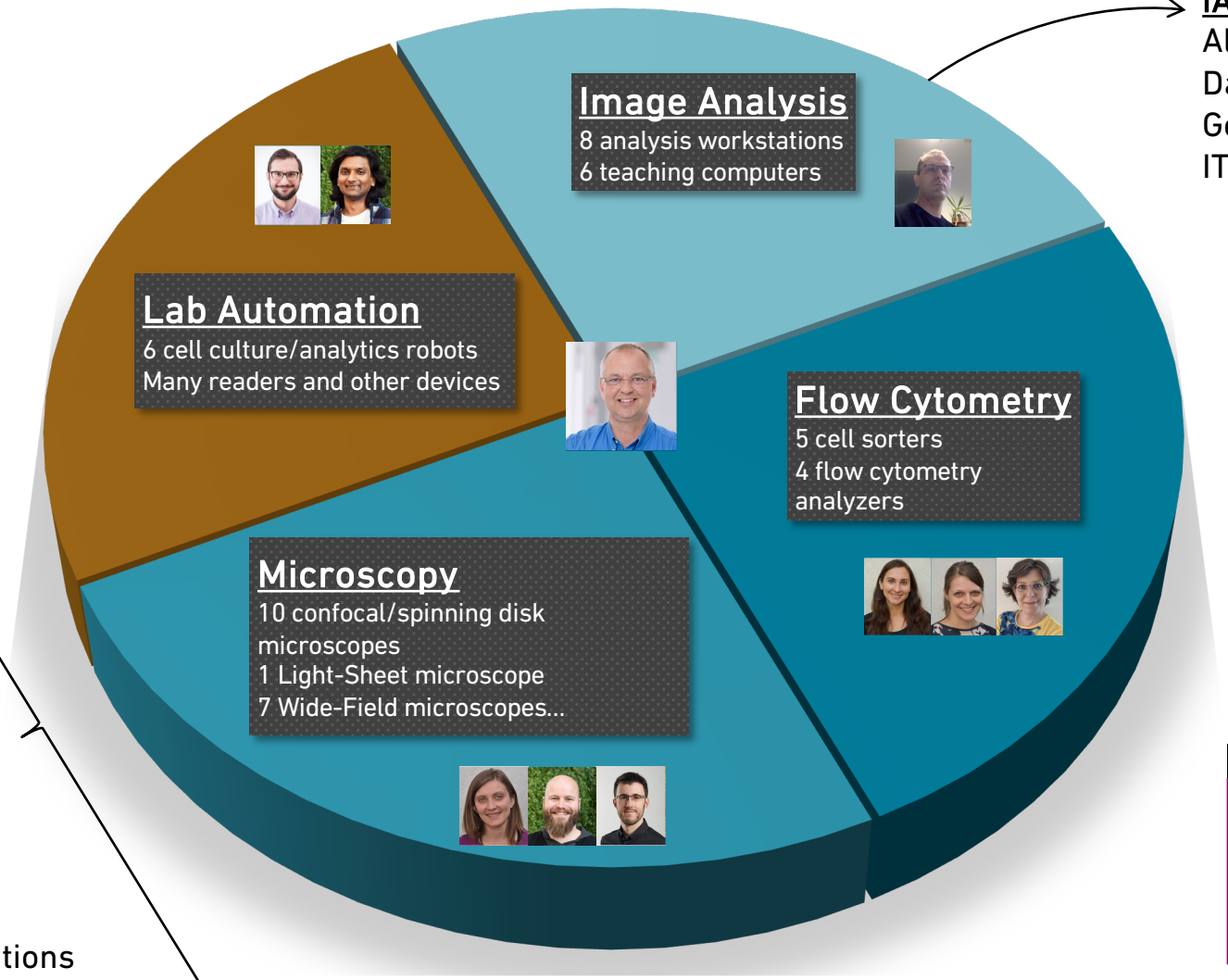
- M. Sc. Biotechnology
  D-BIOL ETH Zurich
- PhD Image Analysis
  D-MAVT ETH Zurich
- Post-doctoral fellow Image Analysis
  The Scripps Research Institute, San Diego, CA

Briefly
D-INFK ETH Zurich

As a grown-up

- Image analysis specialist
  Friedrich Miescher Institute :: Facility for Advanced Imaging and Microscopy
- Image Analysis Specialist & Software and Data Management Engineer
  D-BSSE ETH Zurich :: Single Cell Facility

About us



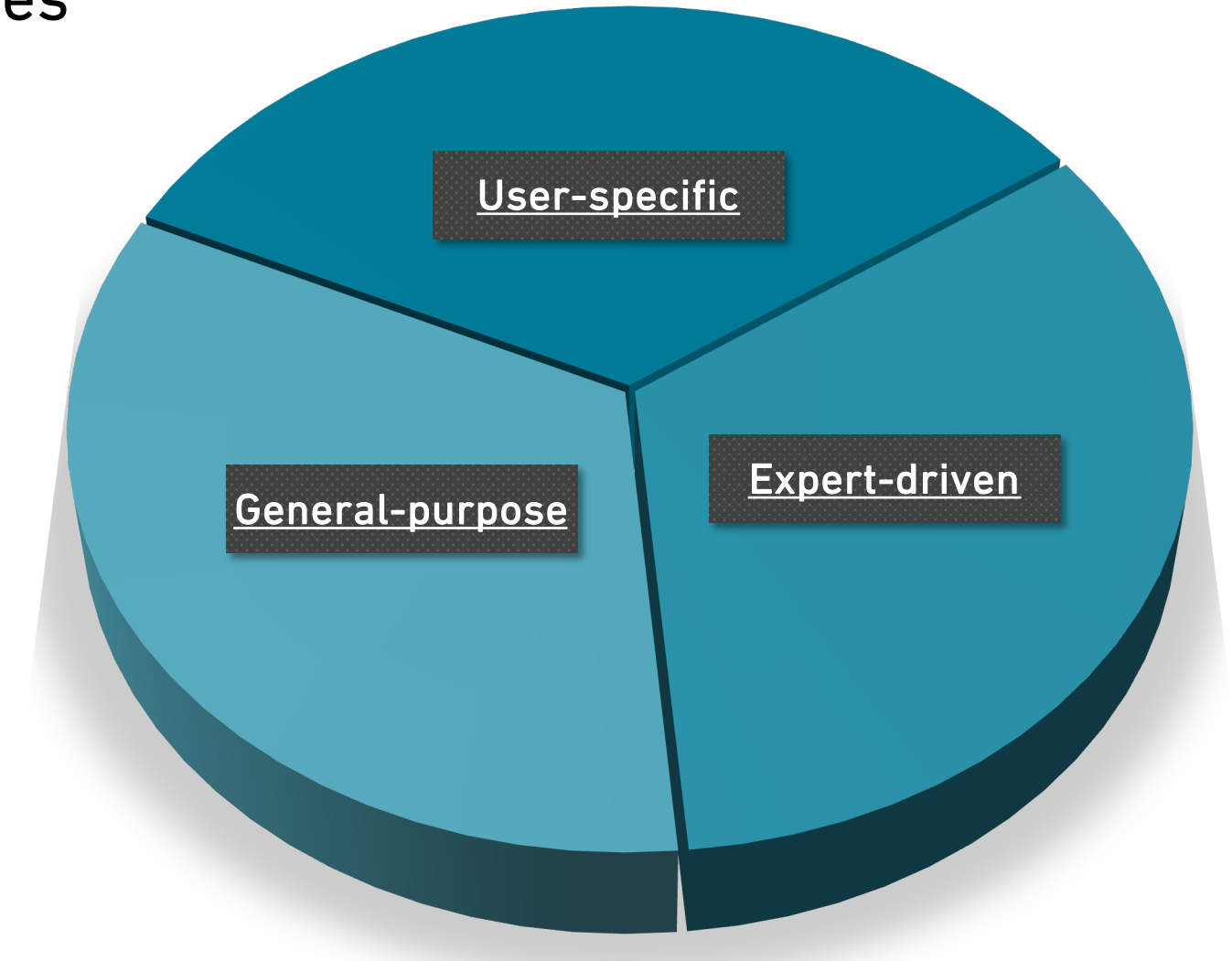
IA additional services
Algorithm development
Data management
Generic software engineering
IT support

General services
Maintenance of devices
User training and support
Teaching
Project discussions
Operator-assisted acquisitions

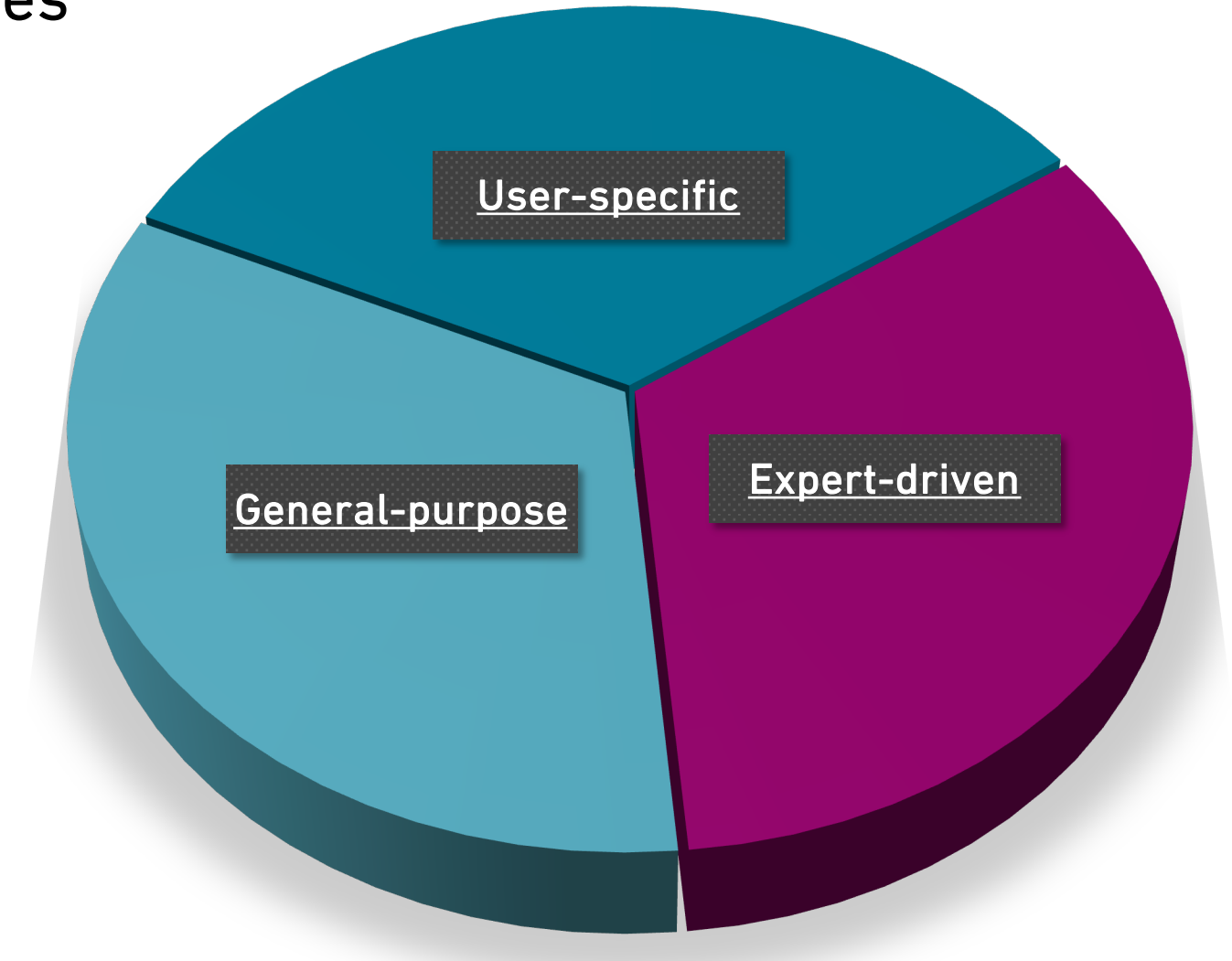
Department facilities

Single Cell Facility
Lab Automation Facility
Cleanroom Facility
Genomic Facility

IA project classes



IA project classes



Types of project

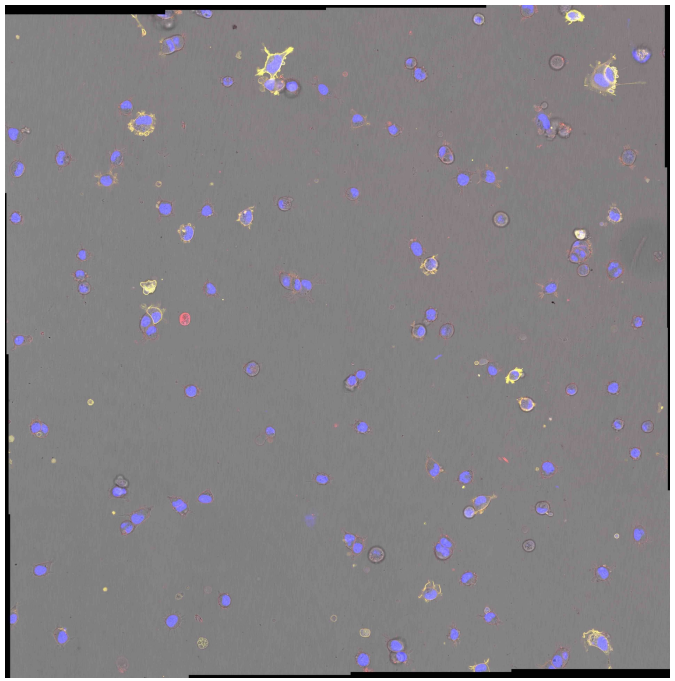
	User-specific projects	Expert-driven projects	General-purpose projects
Target audience	Single user	Several users	Many users
Specificity	Highly specialized	Specialized but scalable	Generalized
Team composition	Me (with user feedback)	Me and field experts (with user feedback)	Large and diverse team
Problem focus	Single, user-specific	Niche problems	Broad
Complexity	Varies	Complex	Moderate to complex
Scalability	Limited	Moderate	High
User input	Continuous	Initial and iterative (at release)	Initial and iterative (at release)
Project time	Short to moderate	Moderate to long	Long
Resource allocation	Moderate	High	Very high
Field knowledge	High (me)	Very high (experts)	Moderate
Code quality/testing	Minimal to moderate	High	Very high

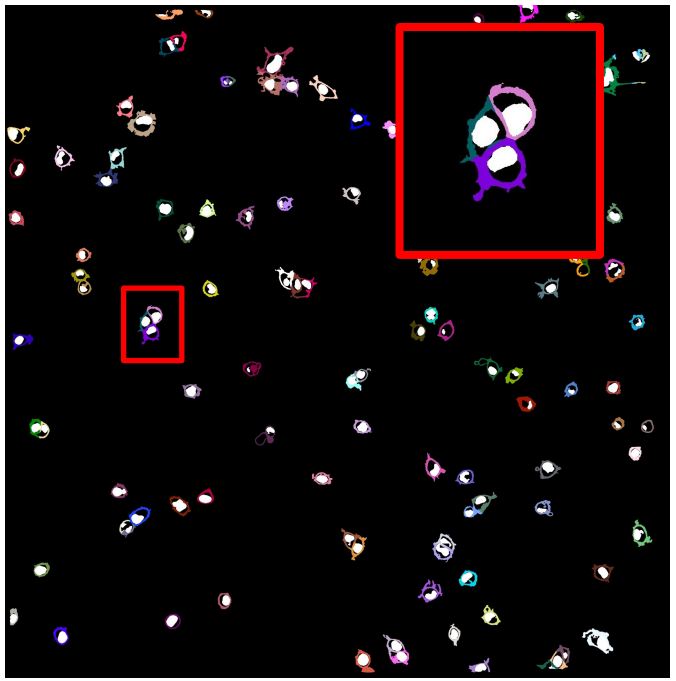
User-specific projects

	User-specific projects
Target audience	Single user
Specificity	Highly specialized
Team composition	Me (with user feedback)
Problem focus	Single, user-specific
Complexity	Varies
Scalability	Limited
User input	Continuous
Project time	Short to moderate
Resource allocation	Moderate
Field knowledge	High (me)
Code quality/testing	Minimal to moderate

User-specific projects :: Membrane localization study of a sensor

> *We designed a plasmid-based sensor consisting of two fusion-protein, that bind activated KRAS and can phosphorylate a synthetic transcription factor if it they dimerize. (...) As KRAS is a membrane protein and our sensor binds only to activated KRAS, membrane localization of our sensor-SYFP-fusion also tells us if KRAS is activated.*
> *(One of the) aim(s) of the study:* ***investigate differences in membrane localization of the sensor between mutant and wild-type KRAS****.*





	A	B	C	D
1	cell_id	membrane_signal	total_signal	ratio
2	62	5740.9854	6643.087	0.86420447
3	72	26428.516	33384.64	0.79163694
4	81	3419.3042	4384.9595	0.7797801
5	99	3454.4004	4615.084	0.7485022
6	100	5038.2393	5553.0483	0.90729254
7	102	4730.7227	5524.704	0.8562853
8	103	4360.502	4901.9644	0.88954175
9	104	67296.74	69806.89	0.96404153
10	107	3924.6875	5026.2783	0.7808337
11	108	3036.5955	4003.891	0.7584111
12	111	11538.633	16132.592	0.7152374
13	112	5822.574	6326.4824	0.92034936
14	125	5634.9688	6411.24	0.87892026
15	129	17054.633	21817.885	0.7816813
16	131	5955.9385	7285.884	0.81746274
17	134	3460.176	3846.4526	0.8995759
18	135	2996.8965	3478.8113	0.8614714
19	138	3821.9531	4512.3306	0.847002
20	139	5966.1807	8770.289	0.68027186
21	144	3959.9258	4624.1426	0.85635895

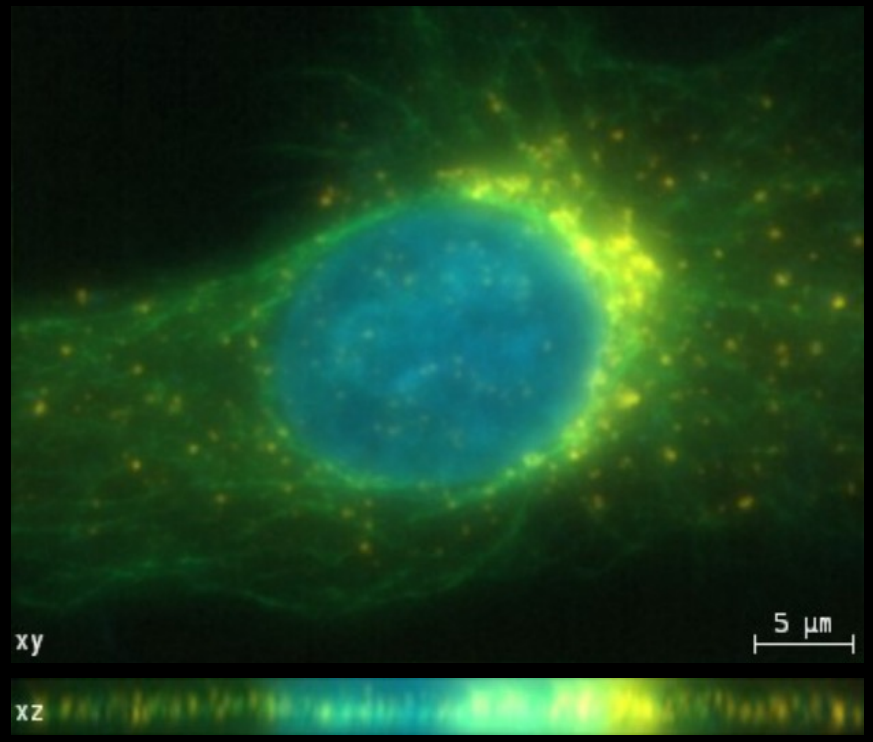
Stats

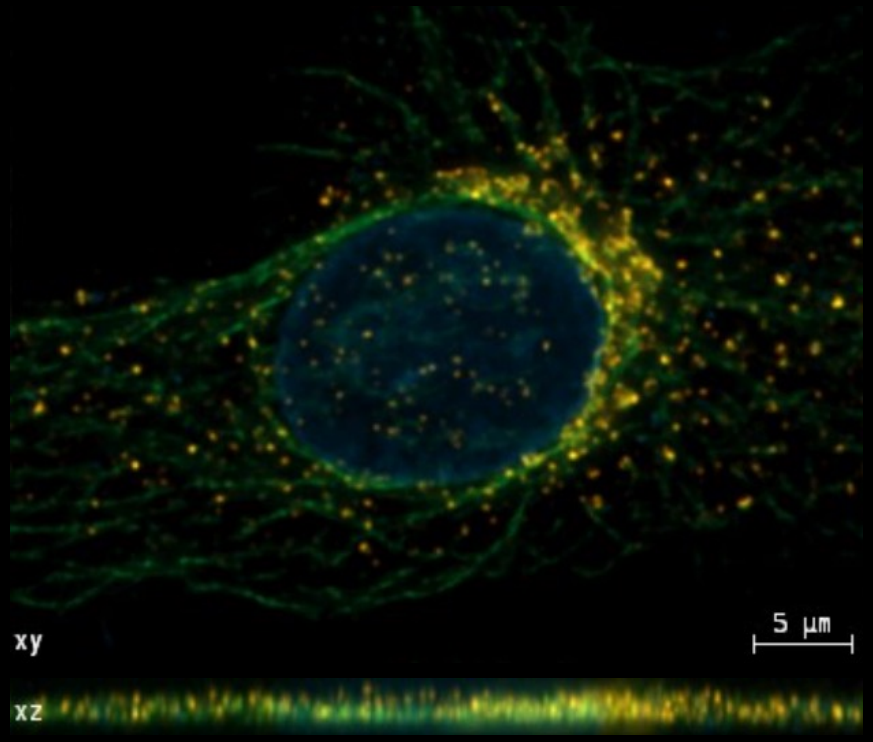
```
$ python analyze_membranes.py --folder E.GS3.56.1_reseeding --result result.csv --max-workers 8
```

General-purpose projects

	General-purpose projects
Target audience	Many users
Specificity	Generalized
Team composition	Large and diverse team
Problem focus	Broad
Complexity	Moderate to complex
Scalability	High
User input	Initial and iterative (at release)
Project time	Long
Resource allocation	Very high
Field knowledge	Moderate
Code quality/testing	Very high

General-purpose projects :: Huygens Remote Manager (HRM)

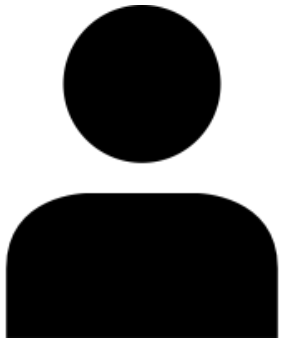




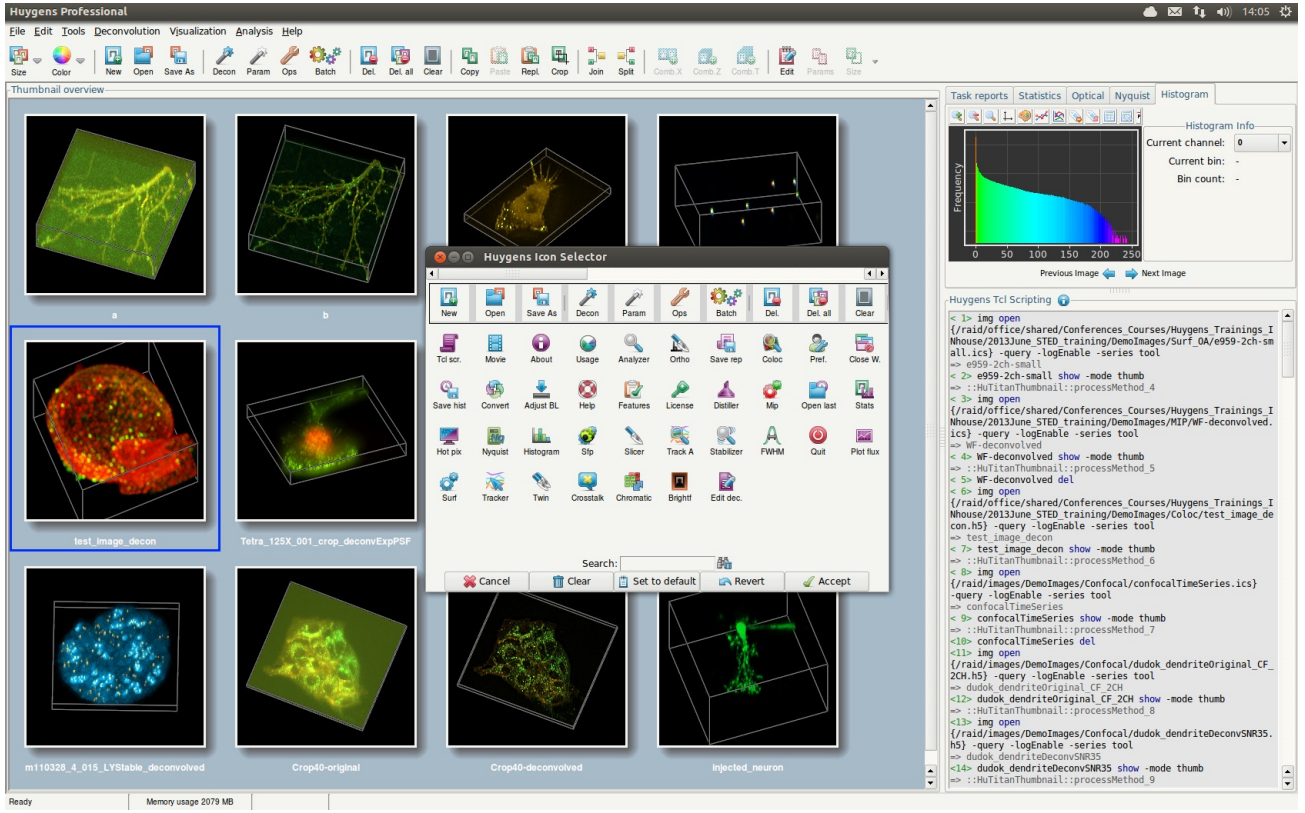
Deconvolution of an HeLa cell acquired on a widefield microscope.
Image courtesy Dr. Yury Belyaev. EMBL, Heidelberg, Germany.

General-purpose projects :: Huygens Remote Manager (HRM)

Scientific Volume Imaging
Deconvolution - Visualization - Analysis



Single-user desktop application



Huygens Professional

Ponti A., Sevilla Sanchez D., Ehrenfeuchter N., Belyaev Y. G.I.T. Imaging & Microscopy 2:22 – 24. 2015
Ponti A., Gulati A., Bäcker V. and Schwarb P. Imaging & Microscopy 9(2):57-58. 2007.

General-purpose projects :: Huygens Remote Manager (HRM)

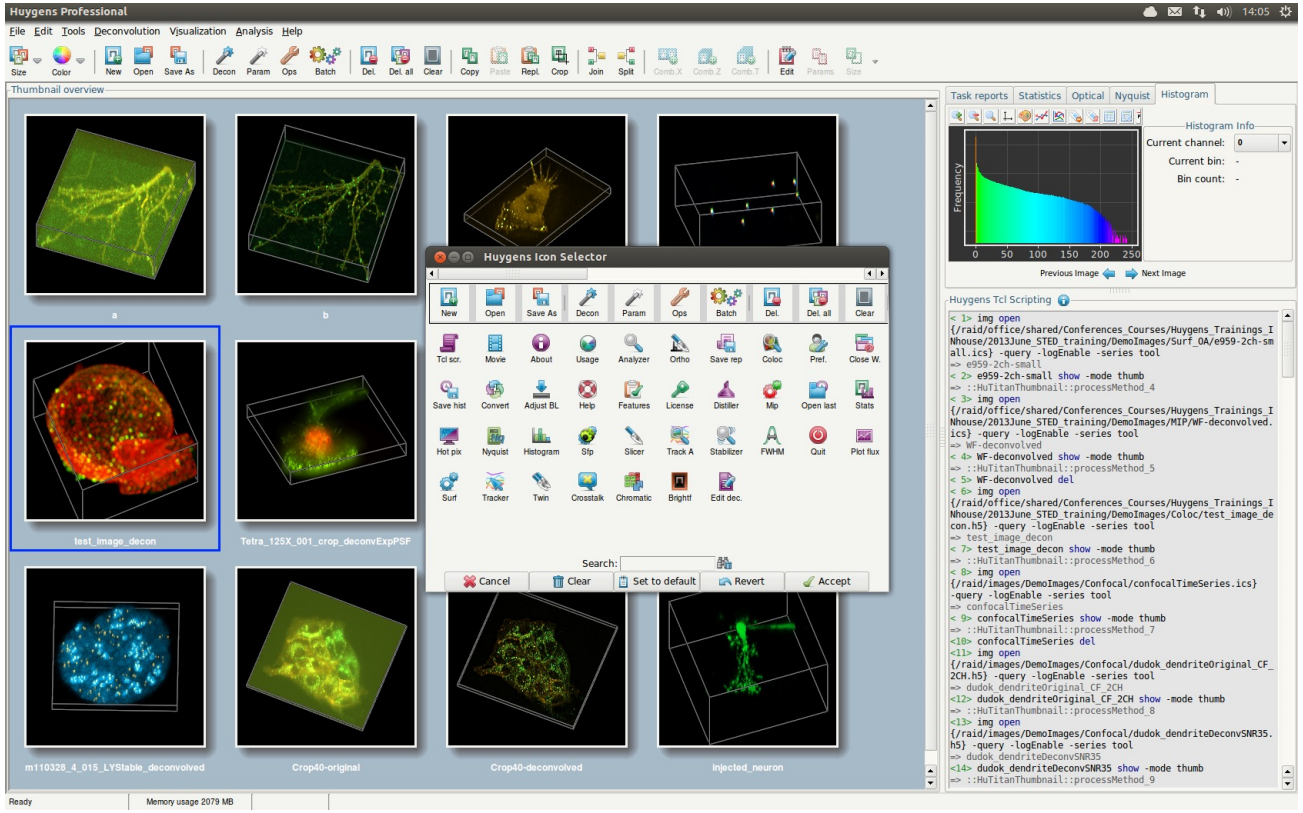
Our scope

Scientific facility with around
100 users

↕

1 workstation

Scientific Volume Imaging
Deconvolution - Visualization - Analysis



Huygens Professional

Ponti A., Sevilla Sanchez D., Ehrenfeuchter N., Belyaev Y. G.I.T. Imaging & Microscopy 2:22 – 24. 2015
Ponti A., Gulati A., Bäcker V. and Schwarb P. Imaging & Microscopy 9(2):57-58. 2007.

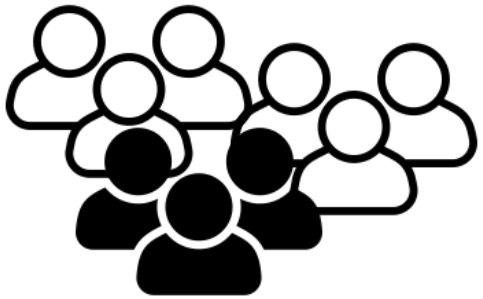
General-purpose projects :: Huygens Remote Manager (HRM)

Our scope

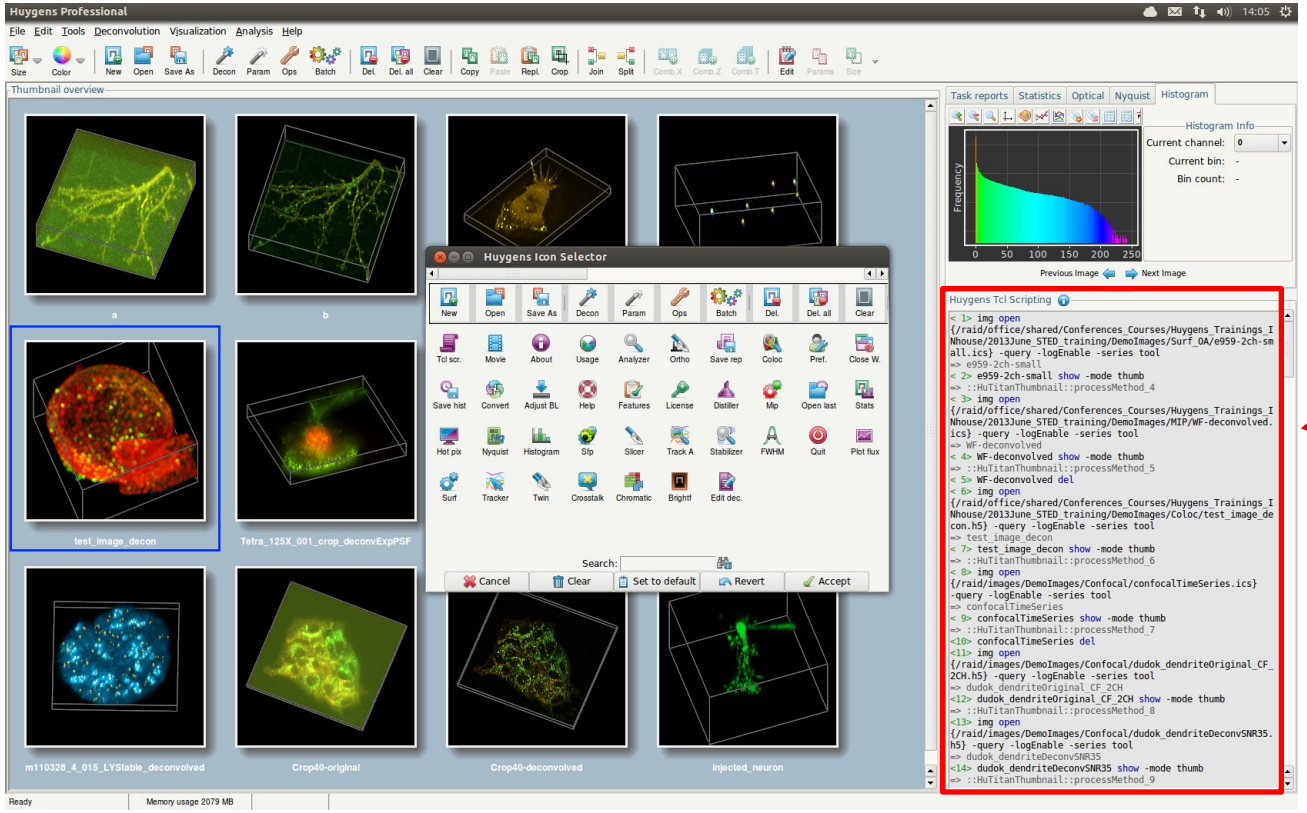
Scientific facility with around
100 users

↕

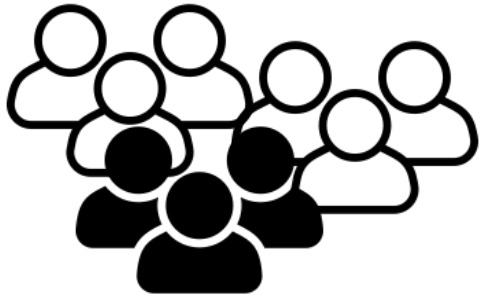
1 workstation



Scientific Volume Imaging
Deconvolution - Visualization - Analysis



Huygens Professional



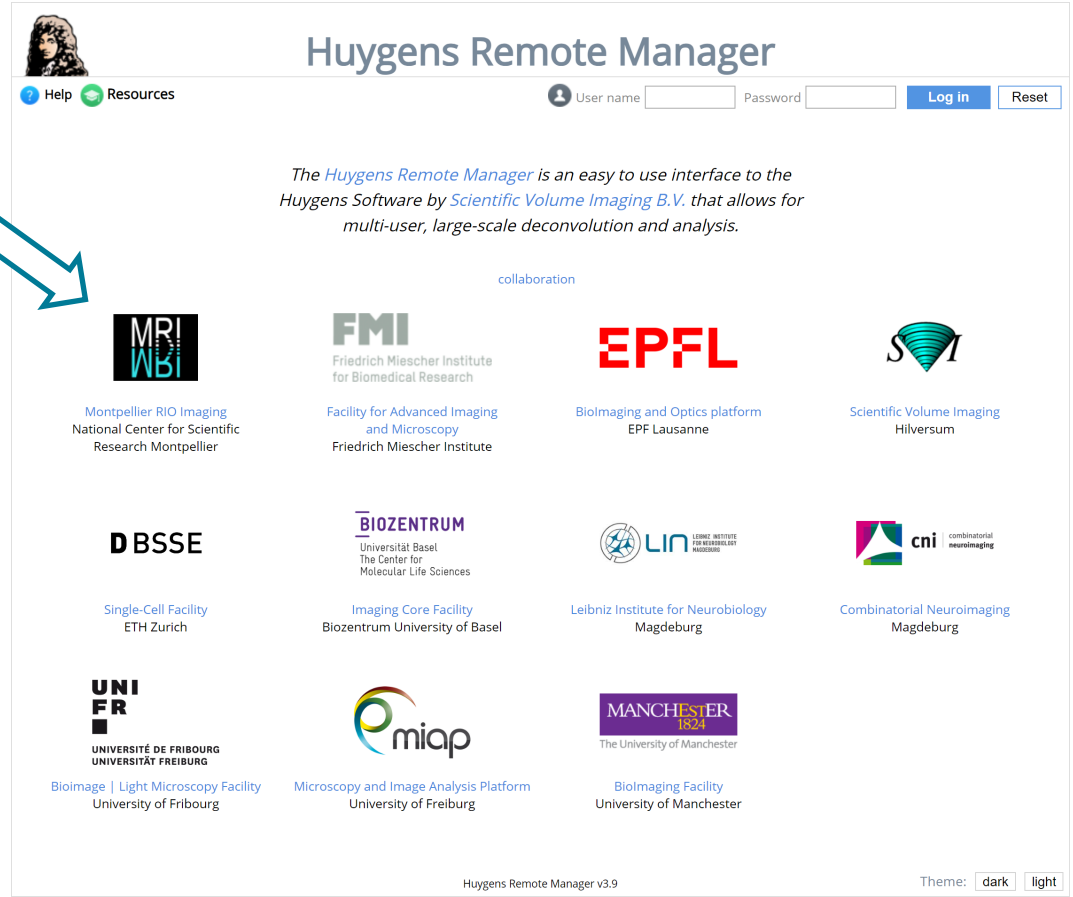
Fully scriptable

Huygens Core

Headless compute engine

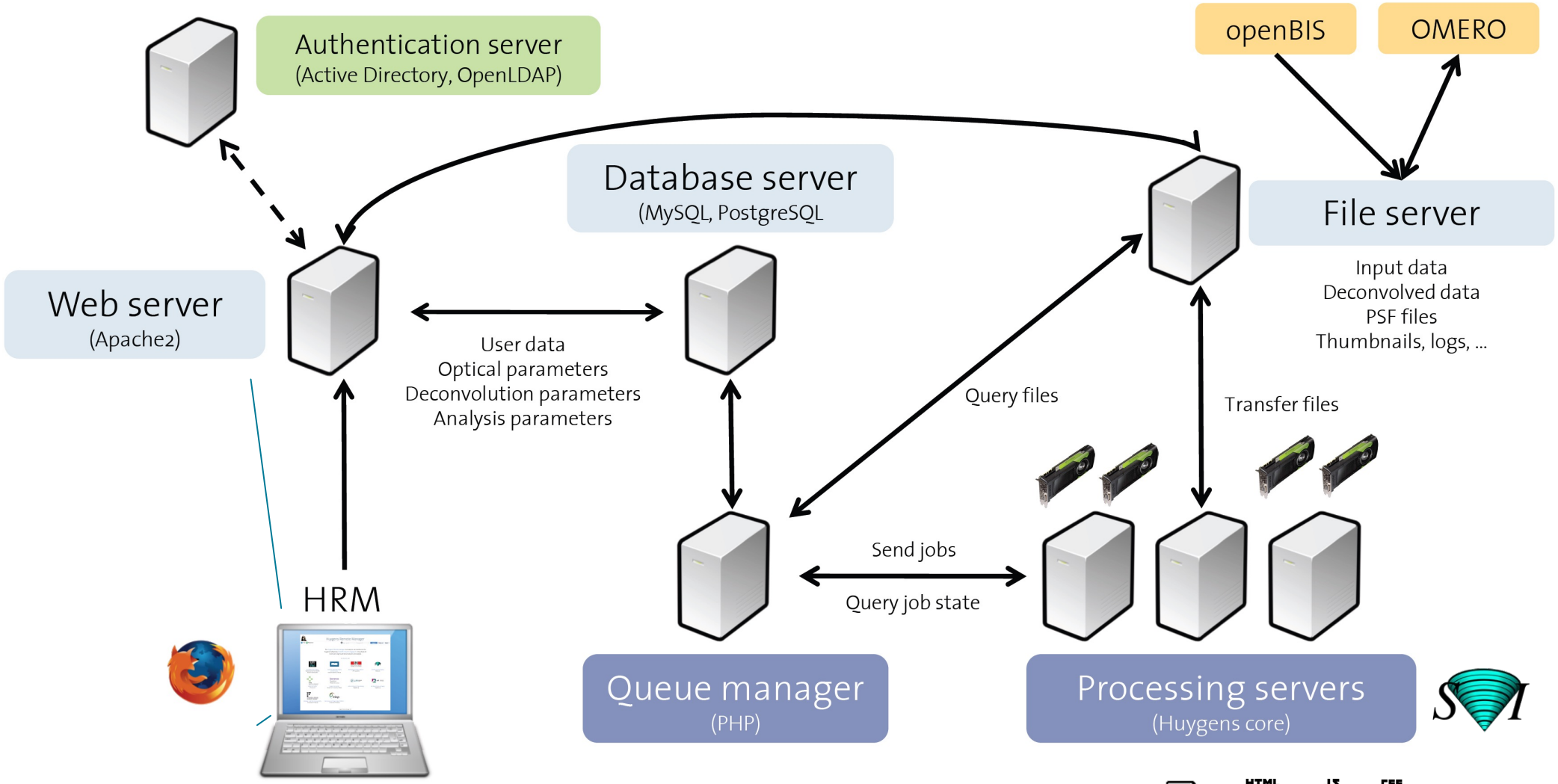
Ponti A., Sevilla Sanchez D., Ehrenfeuchter N., Belyaev Y. G.I.T. Imaging & Microscopy 2:22 – 24. 2015
Ponti A., Gulati A., Bäcker V. and Schwarb P. Imaging & Microscopy 9(2):57-58. 2007.

General-purpose projects :: Huygens Remote Manager (HRM)



https://github.com/aarpon/hrm/

General-purpose projects :: Huygens Remote Manager (HRM)



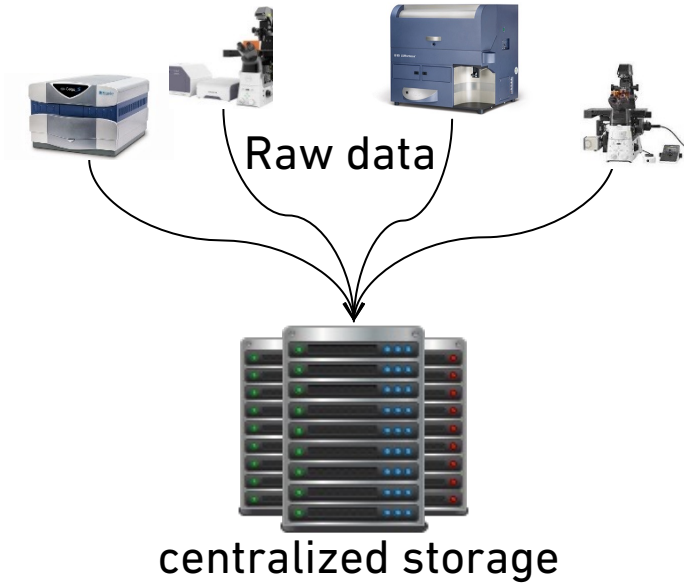
General-purpose projects :: openBIS Importer Toolset (oBIT)

~ 40x at SCF/LAF

Raw data

General-purpose projects :: openBIS Importer Toolset (oBIT)

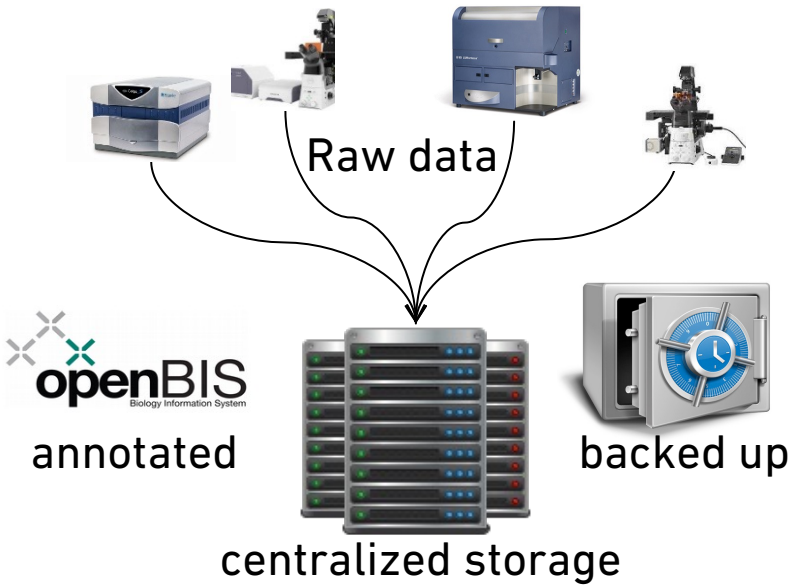
~ 40x at SCF/LAF



General-purpose projects :: openBIS Importer Toolset (oBIT)

~ 40x at SCF/LAF

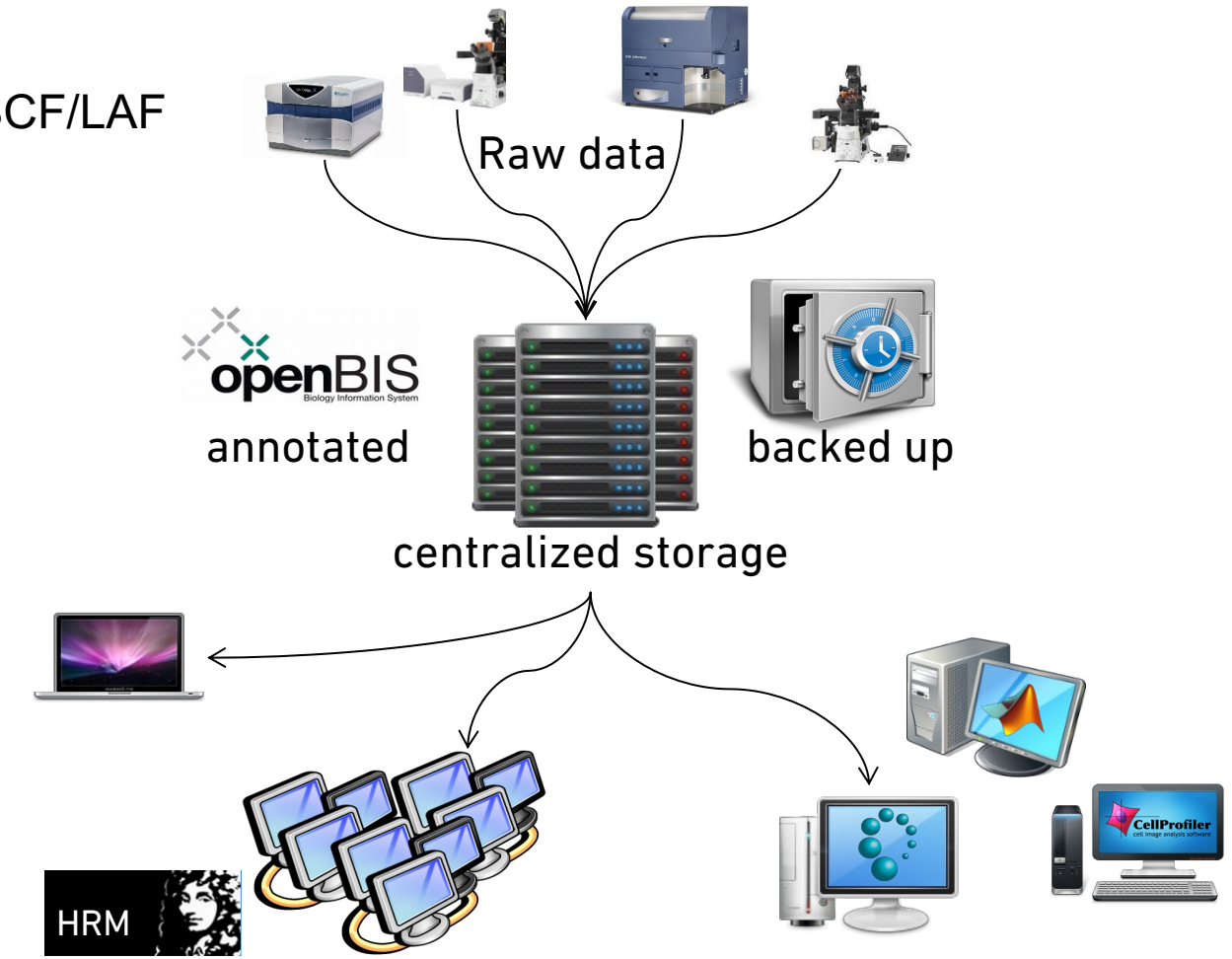
https://sis.id.ethz.ch/



General-purpose projects :: openBIS Importer Toolset (oBIT)

~ 40x at SCF/LAF

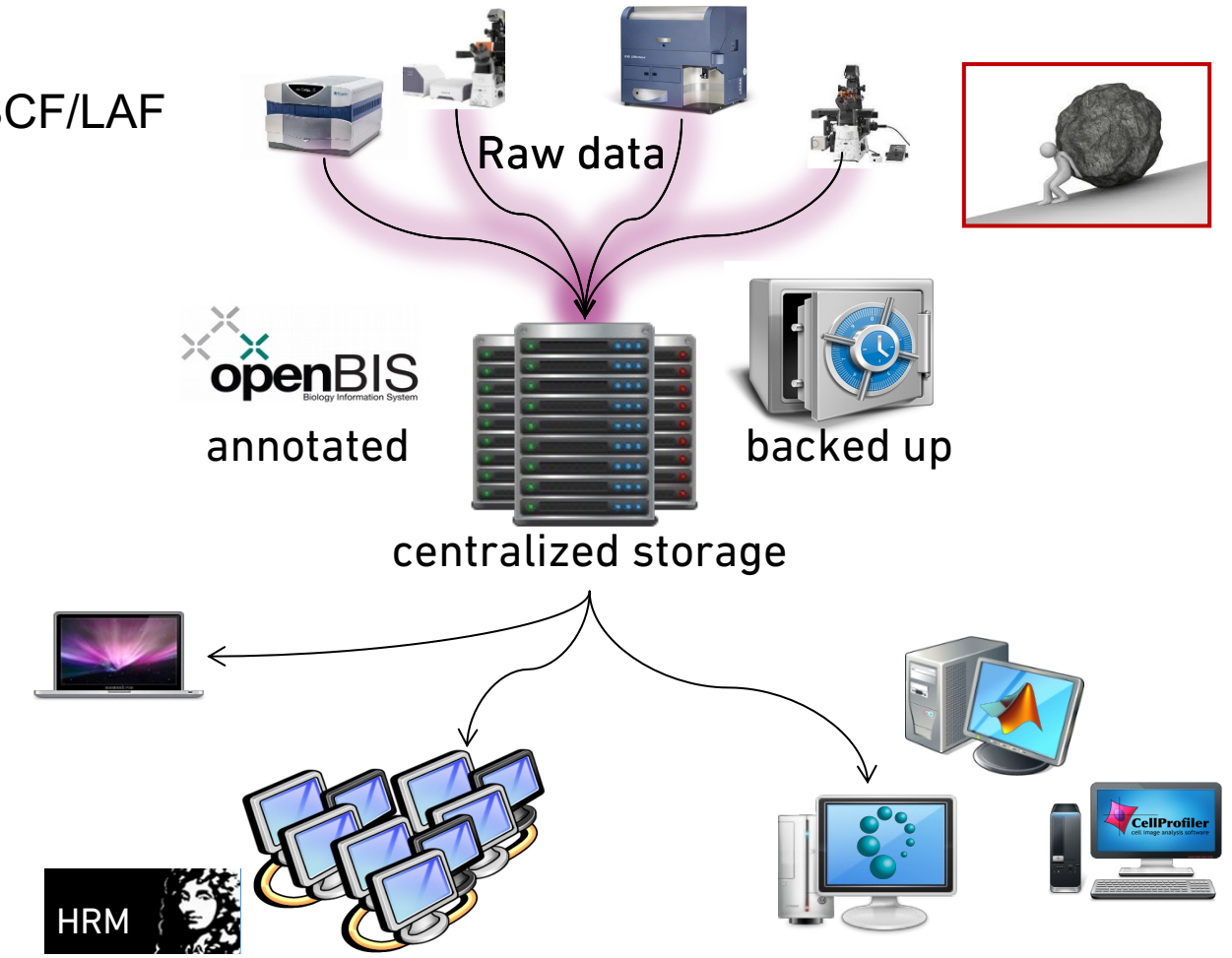
https://sis.id.ethz.ch/



General-purpose projects :: openBIS Importer Toolset (oBIT)

~ 40x at SCF/LAF

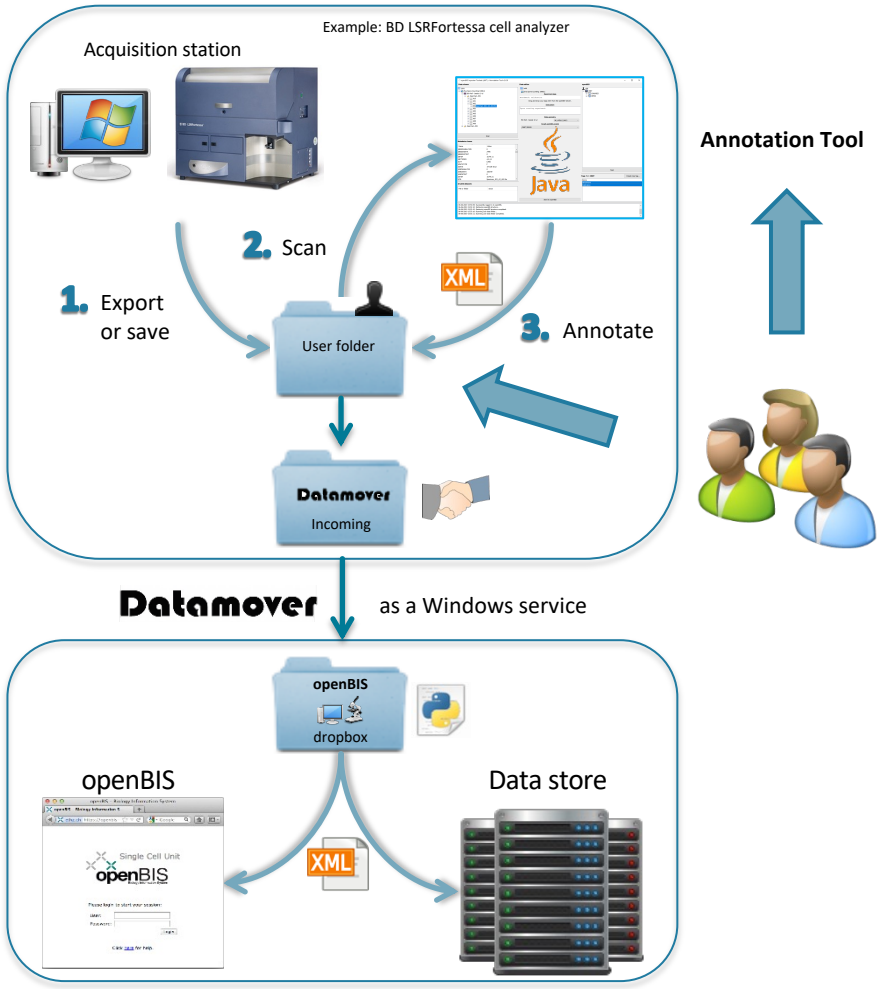
https://sis.id.ethz.ch/



General-purpose projects :: openBIS Importer Toolset (oBIT)

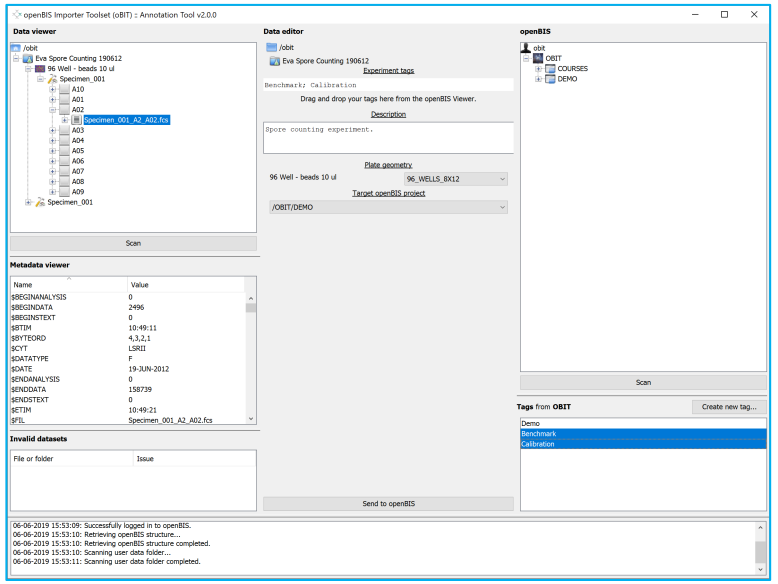
New openBIS core technologies

Microscopy
Flow Cytometry



Annotation Tool

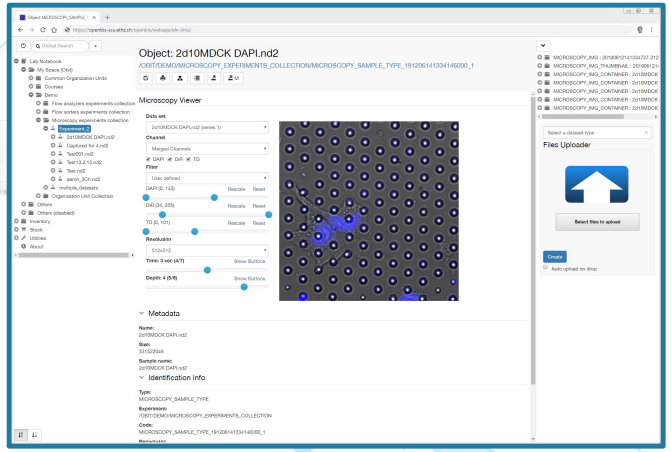
https://github.com/aarpon/obit

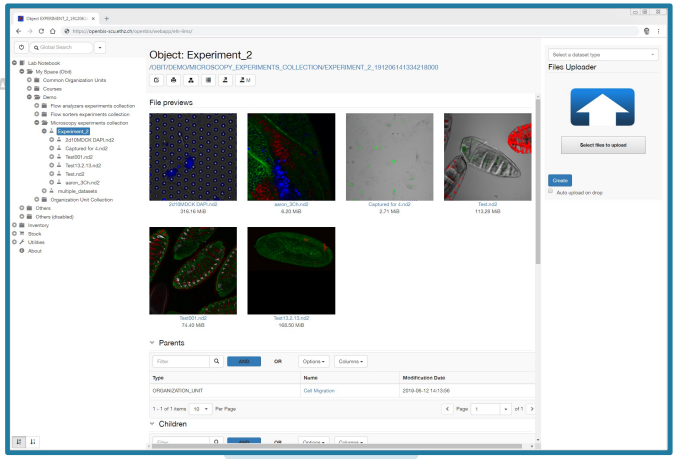


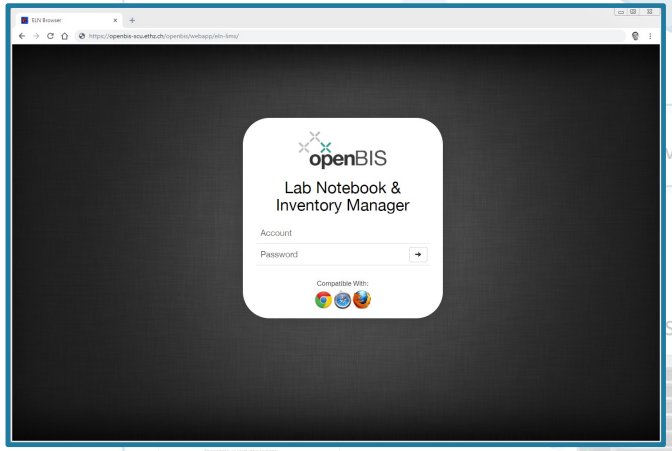
General-purpose projects :: openBIS Importer Toolset (oBIT)

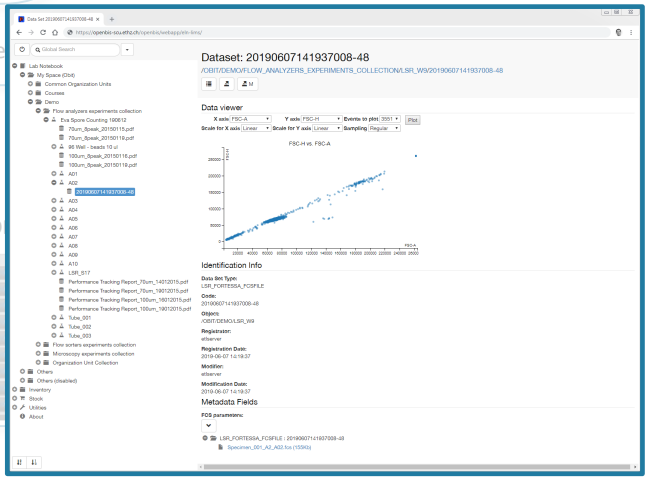
New openBIS core technologies

Microscopy
Flow Cytometry









Integration into ELN

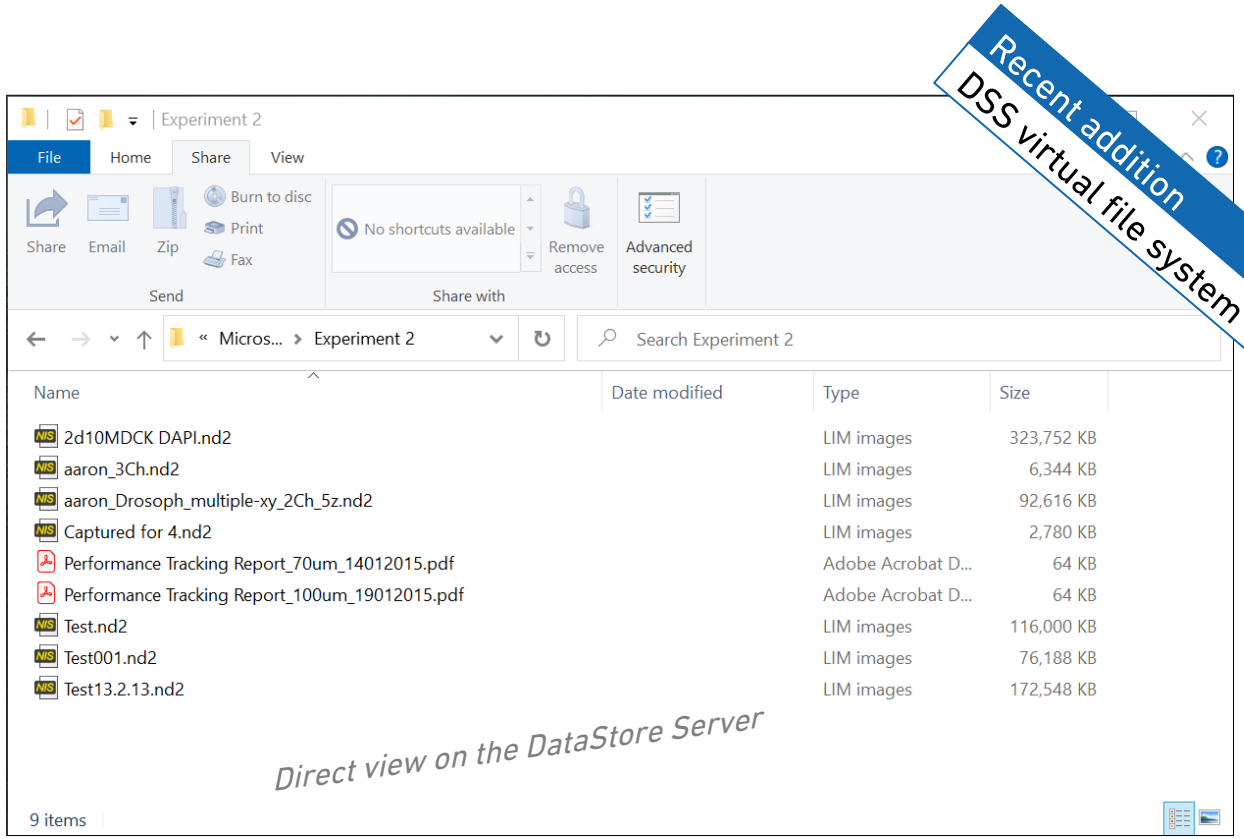
HTML

JS

CSS

Jython

General-purpose projects :: openBIS Importer Toolset (oBIT)



ETH zürich

Scientific IT Services

Expert-driven projects

	Expert-driven projects
Target audience	Several users
Specificity	Specialized but scalable
Team composition	Me and field experts (with user feedback)
Problem focus	Niche problems
Complexity	Complex
Scalability	Moderate
User input	Initial and iterative (at release)
Project time	Moderate to long
Resource allocation	High
Field knowledge	Very high (experts)
Code quality/testing	High

Expert-driven projects

	Expert-driven projects
Target audience	Several users
Specificity	Specialized but scalable
Team composition	Field experts (multi-user)
Problem focus	Domain problems
Complexity	Complex
Scalability	Moderate
User input	Initial and iterative (at release)
Project time	Moderate to long
Resource allocation	High
Field knowledge	Very high (experts)
Code quality/testing	High

Three example projects

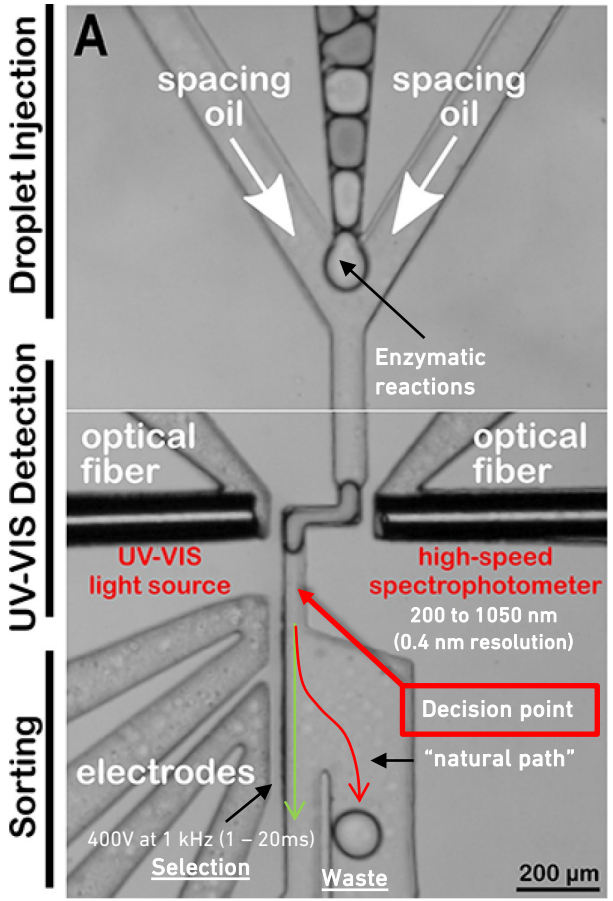
SpectraSorter
pyPOCQuant
pyMINFLUX

Expert-driven projects :: SpectraSorter

Duncombe T. A., Ponti A., Dittrich P. S. SoftwareX, Volume 19, 2022, 101160 DOI:10.1016/j.softx.2022.101160
Duncombe T. A., Ponti A., Seebeck F. P., Dittrich P. S. 2021. DOI: 10.1021/acs.analchem.1c02822

Goal

Design a **microfluidic platform** for the **high-throughput analysis** of (enzymatic) **reactions** inside small **droplets**.

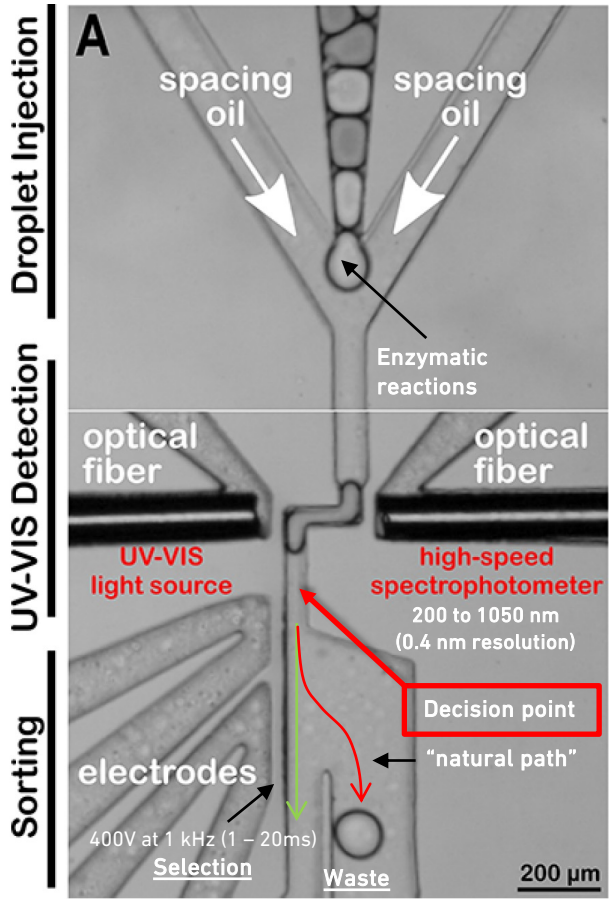


Expert: Todd Duncombe, Petra Dittrich Group, D-BSSE ETHZ

Expert-driven projects :: SpectraSorter

Duncombe T. A., Ponti A., Dittrich P. S. SoftwareX, Volume 19, 2022, 101160 DOI:10.1016/j.softx.2022.101160
Duncombe T. A., Ponti A., Seebeck F. P., Dittrich P. S. 2021. DOI: 10.1021/acs.analchem.1c02822

UVADS: UV–vis spectra-activated droplet sorter



High-throughput label-free chemical identification in droplets and **on-demand** collection (via electrodes)

In **droplet microfluidic platforms**, droplets containing cells or reagents flow though capillaries. Inside each droplet, a reaction takes place, and the output is measured *by some means*. These platforms aim at achieving high-throughput and/or massively parallel analytics.

Previous approaches were very low throughput (1-10 droplets / second) and used fluorescence microscopy to measure single-wavelength absorbance in the visible spectrum.

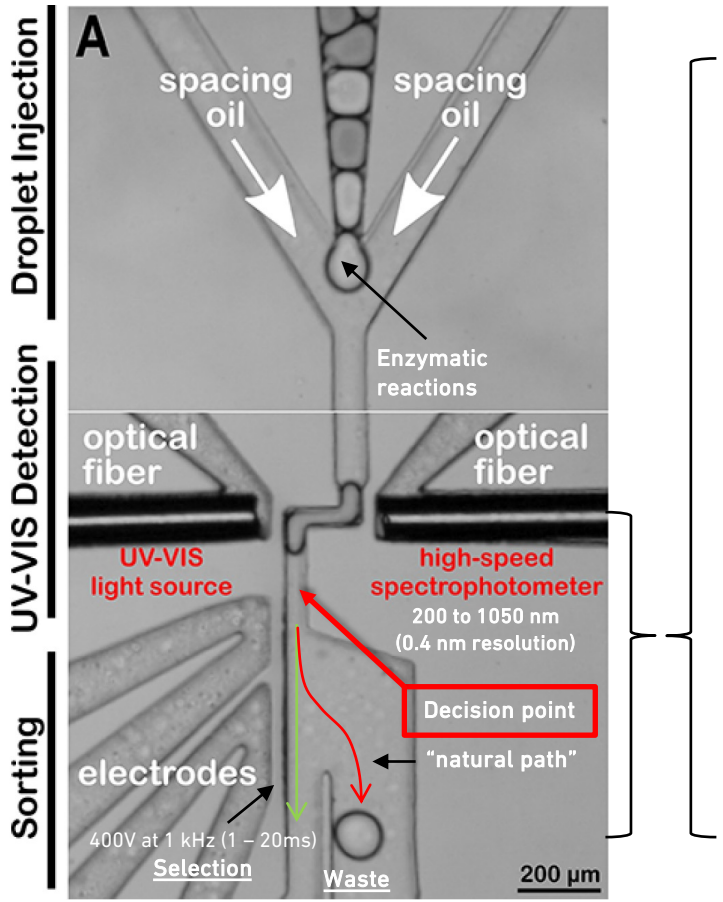
Recent **label-free UV–vis spectroscopy** interrogates **molecular structures** directly by **chemical absorbance** of incident light over a large spectrum (200 to 1050 nm)

Our **microfluidic platform** (**UVADS**) can perform screening assays of enzymatic activity in **droplets** of bacterial micro-colonies by directly measuring the conversion to product by **UV-vis spectroscopy** and **sort** selected droplets using **electrodes**.

Expert-driven projects :: SpectraSorter

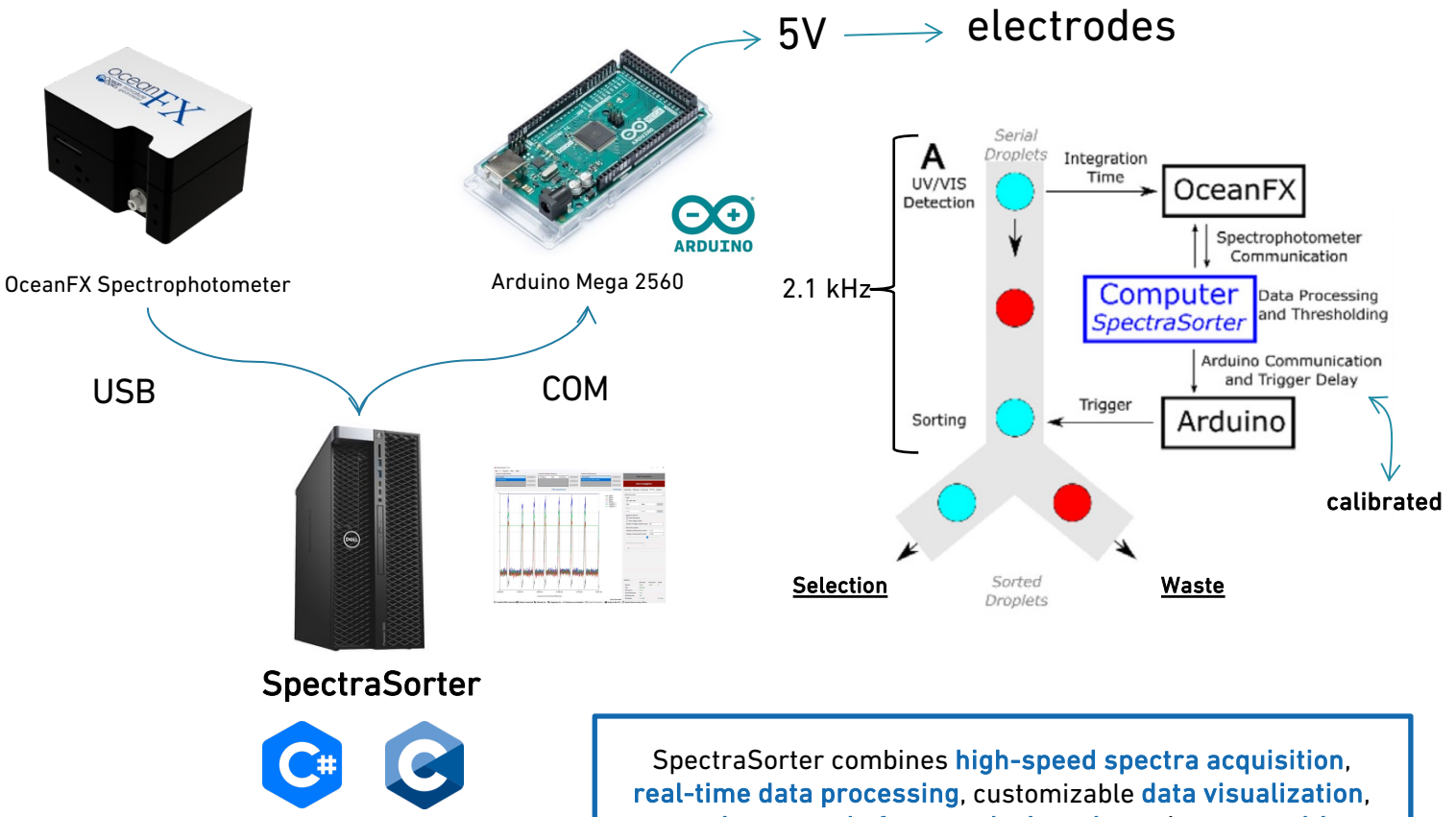
Duncombe T. A., Ponti A., Dittrich P. S. SoftwareX, Volume 19, 2022, 101160 DOI:10.1016/j.softx.2022.101160
Duncombe T. A., Ponti A., Seebeck F. P., Dittrich P. S. 2021. DOI: 10.1021/acs.analchem.1c02822

UVADS: UV–vis spectra-activated droplet sorter



High-throughput label-free chemical identification in droplets and on-demand collection (via electrodes)

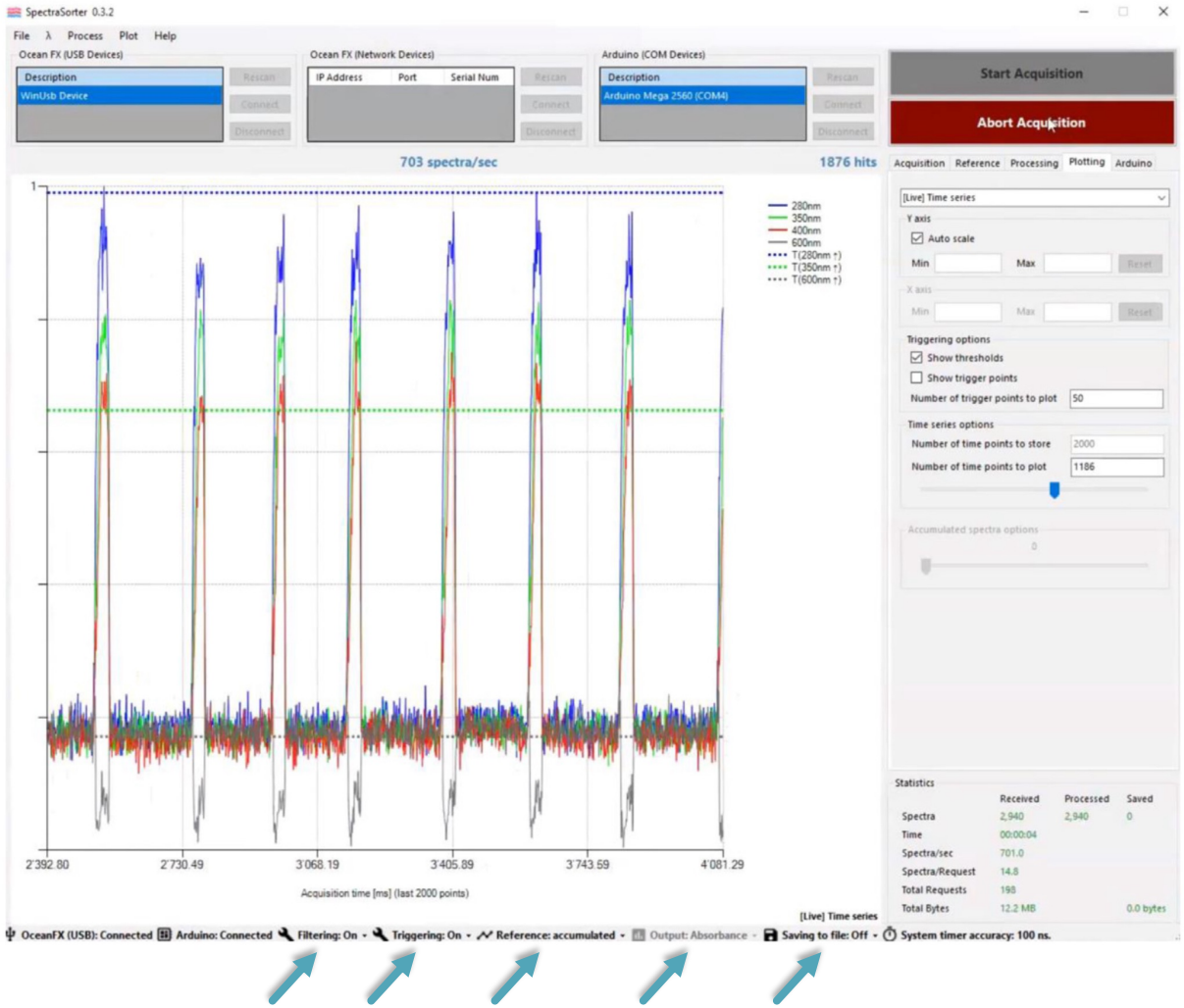
https://github.com/SpectraSorter/SpectraSorter



SpectraSorter combines high-speed spectra acquisition, real-time data processing, customizable data visualization, precise control of external triggering using any multi-spectral feature, configuration management, and more.

Expert-driven projects :: SpectraSorter

Duncombe T. A., Ponti A., Dittrich P. S. SoftwareX, Volume 19, 2022, 101160 DOI:10.1016/j.softx.2022.101160
Duncombe T. A., Ponti A., Seebeck F. P., Dittrich P. S. 2021. DOI: 10.1021/acs.analchem.1c02822

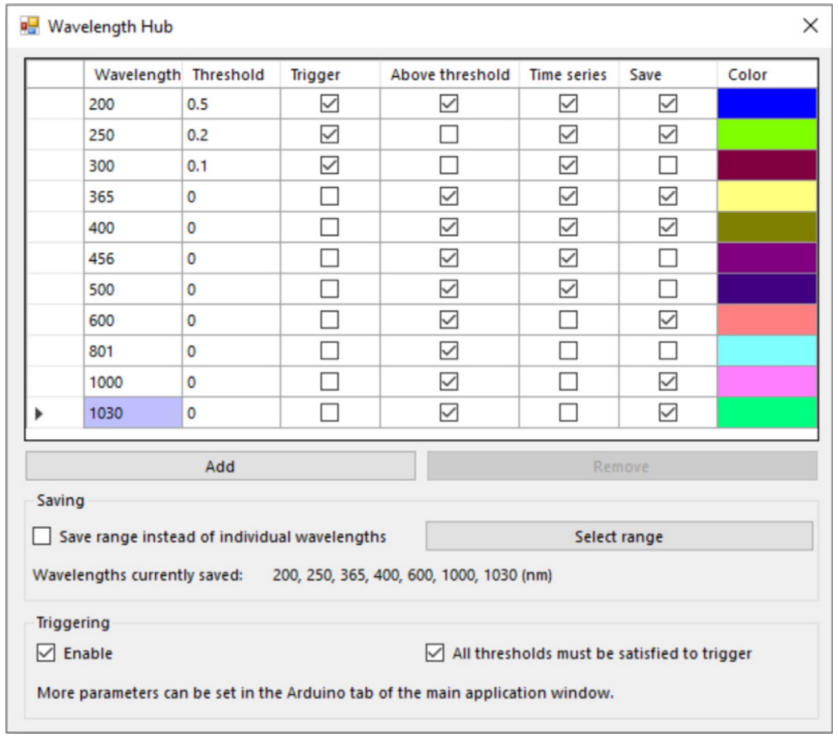


SpectraSorter runs four parallel queues:

- **Acquisition** queue: collects up to 4500 spectra/s from the spectrometer
- **Compute** queue: performs all operations to decide if an event should trigger the Arduino microcontroller
  (low-pass filtering, transformation for absorbance or transmission, dark- and reference-correction, testing against all user-defined thresholds, triggering via Arduino)
- **Plotting** queue: plots the last spectrum at very low rate (10 Hz) for visual feedback
- **Saving** queue: writes to file the processed wavelengths
  (either a selection, a range, or the full spectrum)

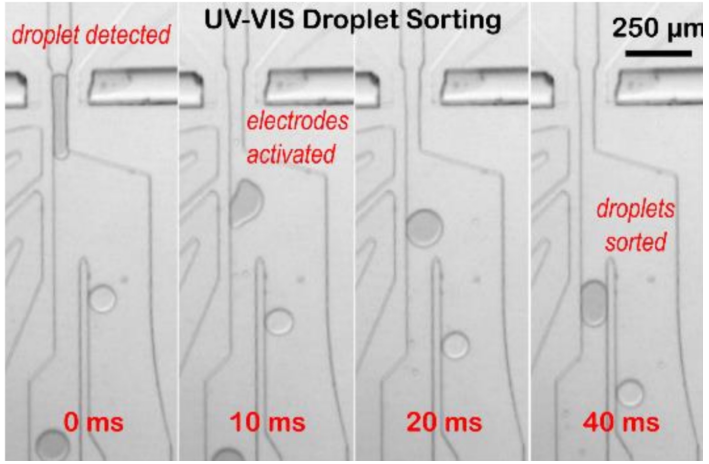
Expert-driven projects :: SpectraSorter

Duncombe T. A., Ponti A., Dittrich P. S. SoftwareX, Volume 19, 2022, 101160 DOI:10.1016/j.softx.2022.101160
Duncombe T. A., Ponti A., Seebeck F. P., Dittrich P. S. 2021. DOI: 10.1021/acs.analchem.1c02822



The **Wavelength Hub** allows selection of any number of wavelengths that:

- act as a **threshold** for a **triggering** event (and in which way)
- will be displayed in the plotter as **time series** and/or as **full spectra**
- will be **saved** to disk



Expert-driven projects :: SpectraSorter

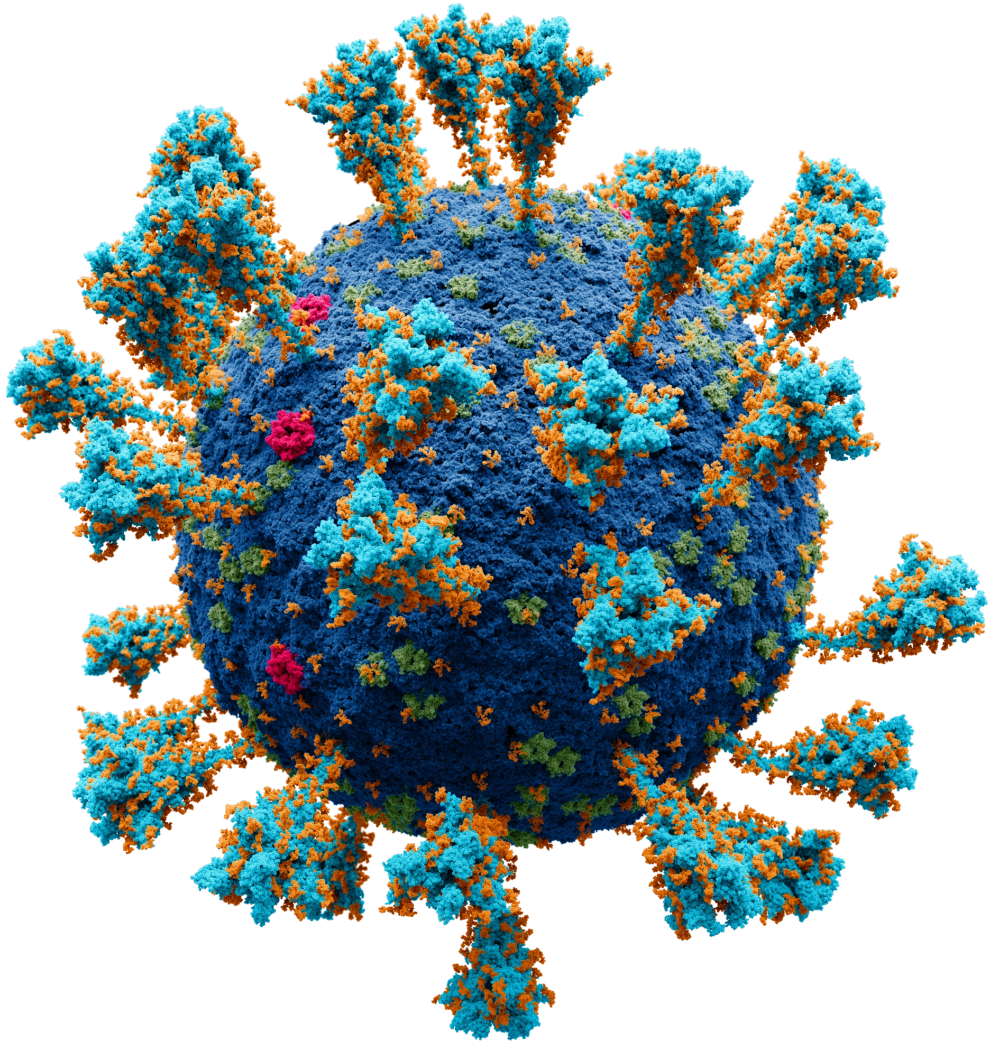
Expert contributions

Todd Duncombe: development of the UVADS methodology and the microfluidics chip
Aaron Ponti: software development

Users

Petra Dittrich group

Expert-driven projects :: pyPOCQuant



https://en.wikipedia.org/wiki/SARS-CoV-2

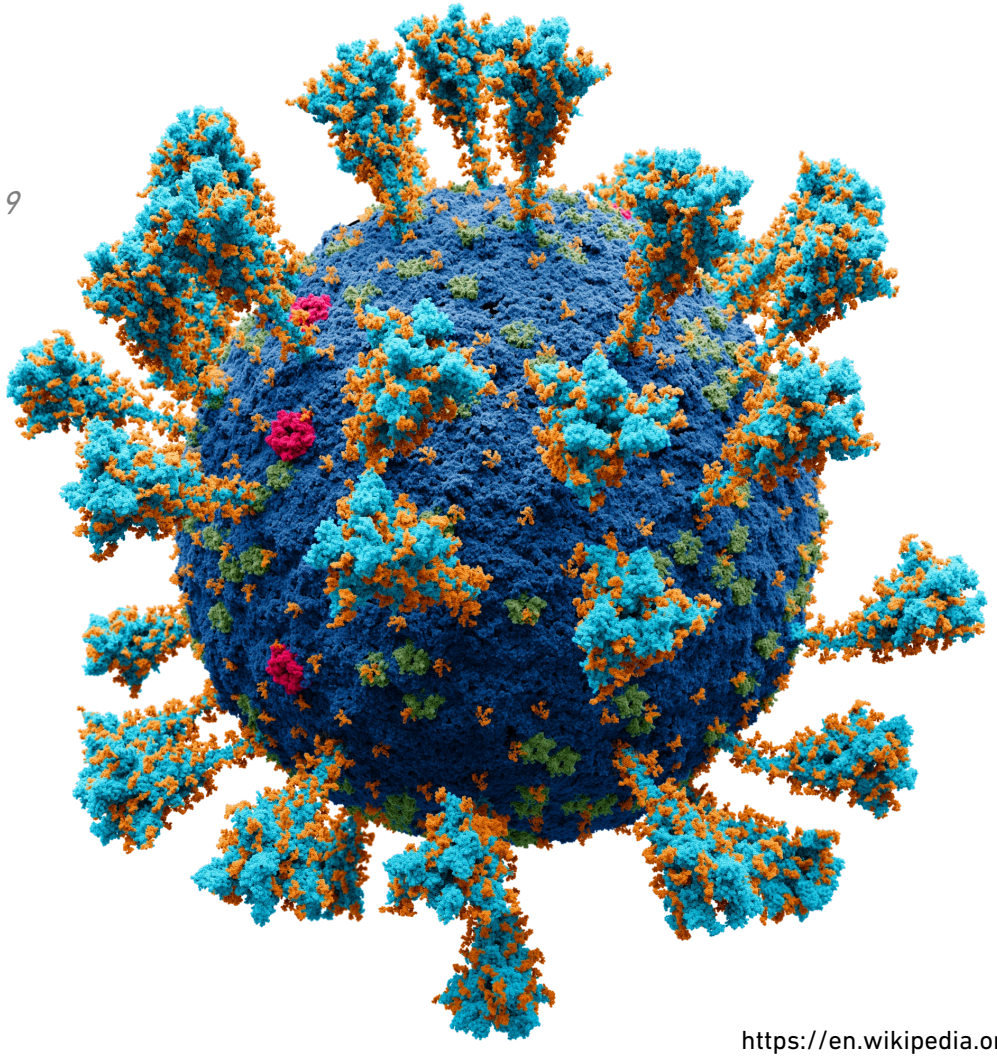
Expert-driven projects :: pyPOCQuant

On request by
Baselland Test Center (via Biolytix AG)

First wave of COVID-19
Spring 2020

Requirements

1. **Quantify** the levels of **IgG** and **IgM** antibodies in patients' blood samples **over time** to study the body's immune response to SARS-CoV-2
2. **Associate patient metadata** to each analysis and store the results in a database



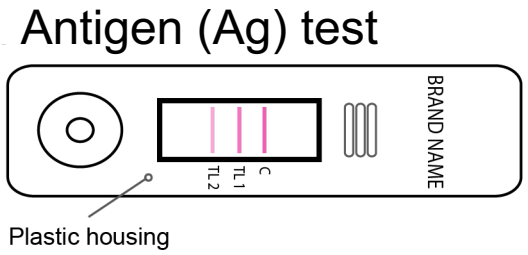
https://en.wikipedia.org/wiki/SARS-CoV-2

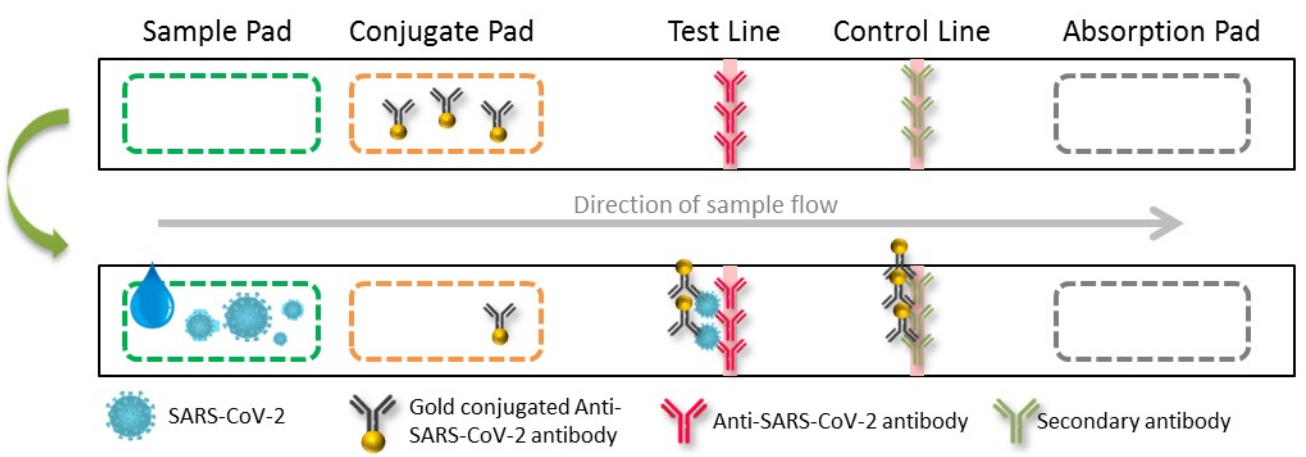
Experts:
Fabian Rudolf, Bundesamt für Gesundheit, CH
Andreas Cuny, Joerg Stelling group, D-BSSE ETHZ

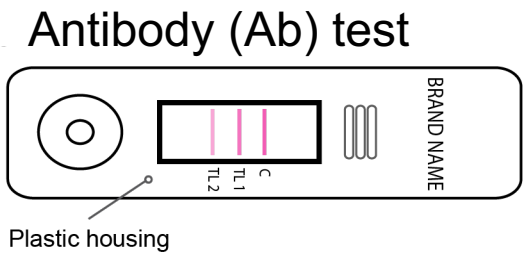
Expert-driven projects :: pyPOCQuant

Image source: https://www.acebiolab.com/EN/news/44

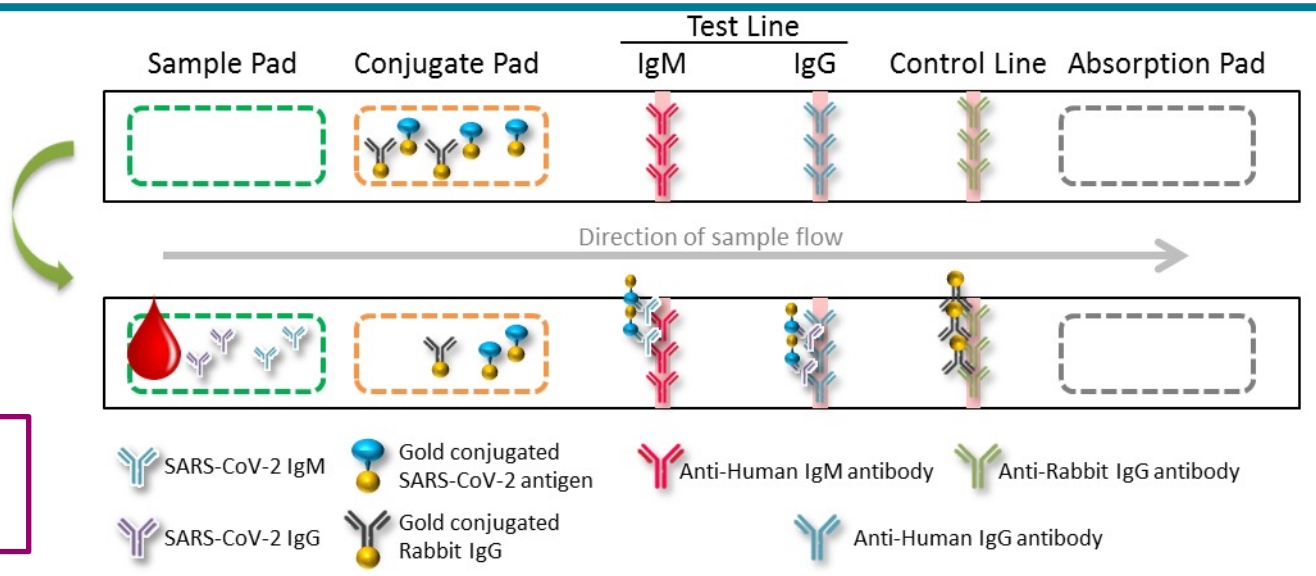
Lateral-flow point-of-care tests (POCTs)







Lateral flow Point-Of-Care Tests (POCTs) are a valuable tool for rapidly detecting pathogens and the associated immune response in humans and animals.



Expert-driven projects :: pyPOCQuant

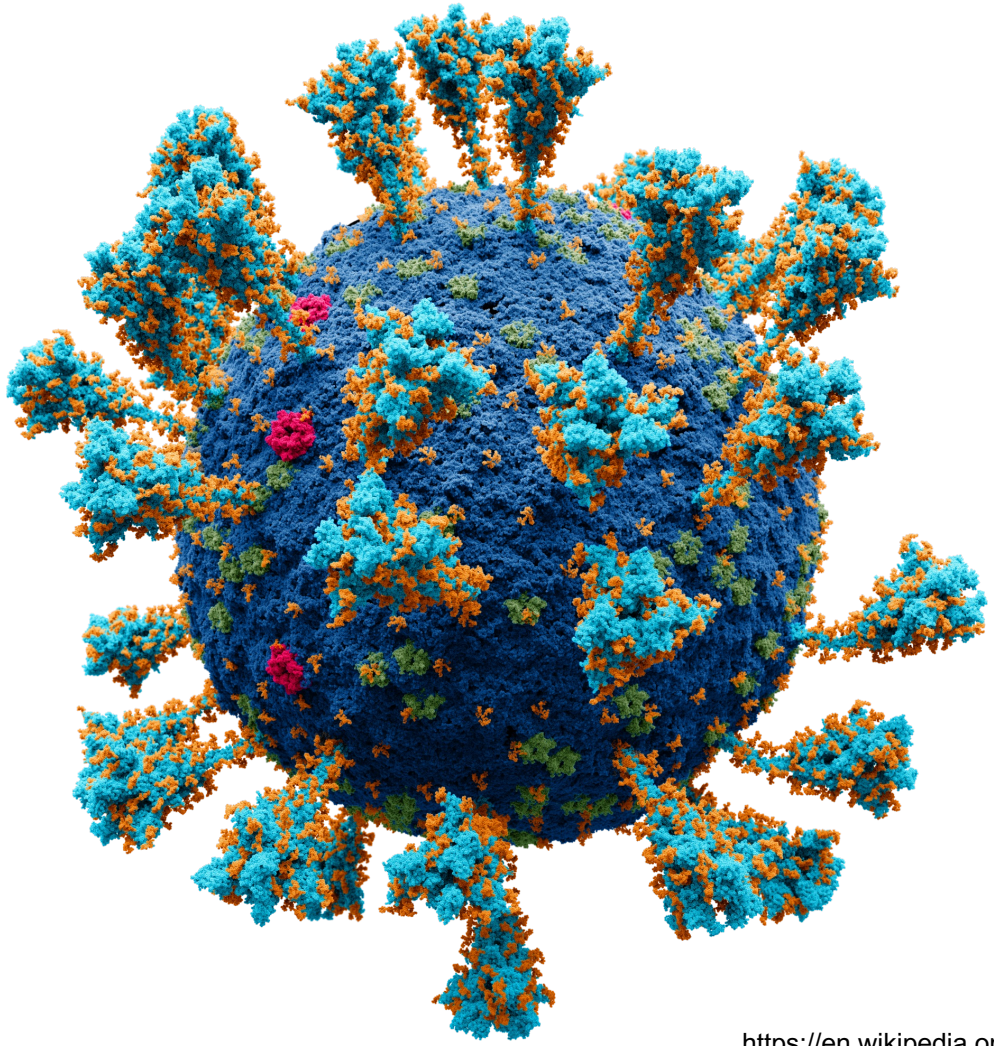
We don't want to waste any test!

Sudden, high demand for **robust** and **quantitative** analysis of POCTs from **different vendors** and from **large numbers** of images

Which ones are quantitative?

No freely available **pipelines** for robust, large-scale **batch processing**

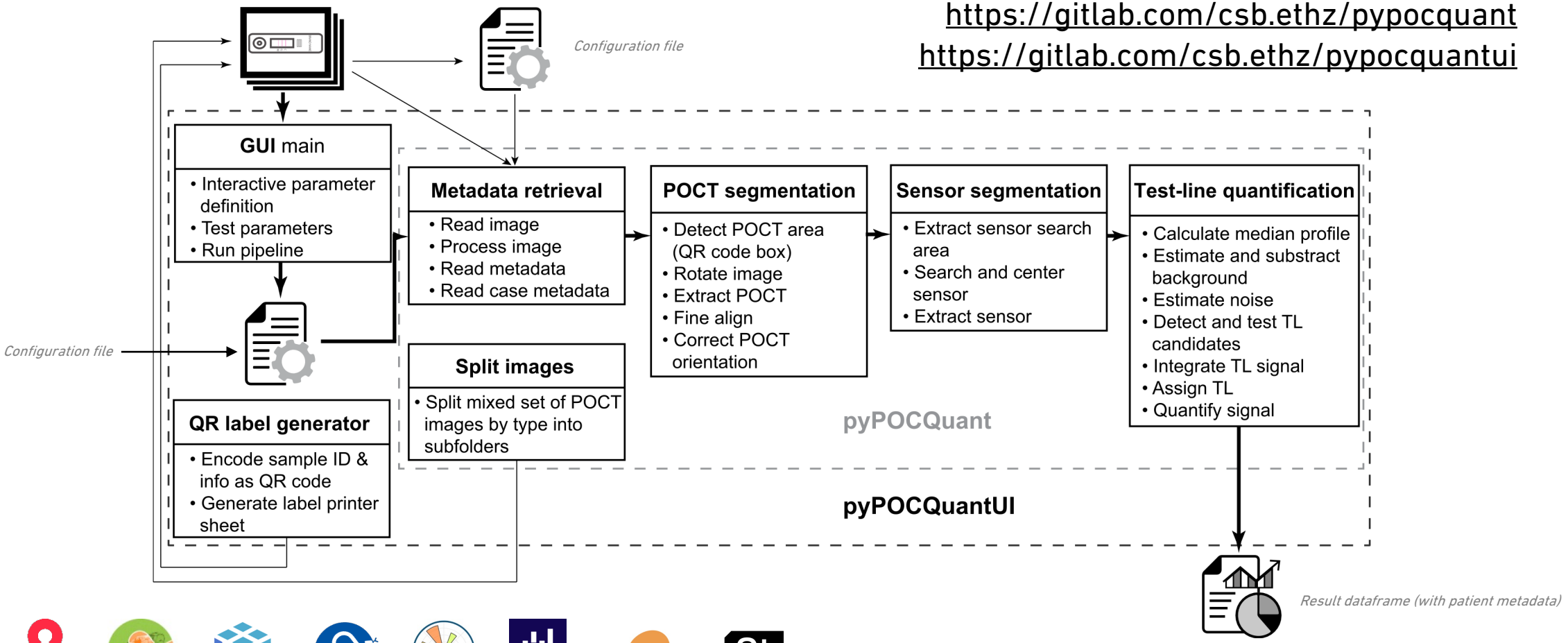
There were commercial readers, but they were slow, expensive, proprietary, and only compatible with certain types of tests.



https://en.wikipedia.org/wiki/SARS-CoV-2

Expert-driven projects :: pyPOCQuant

https://gitlab.com/csb.ethz/pypocquant
https://gitlab.com/csb.ethz/pypocquantui

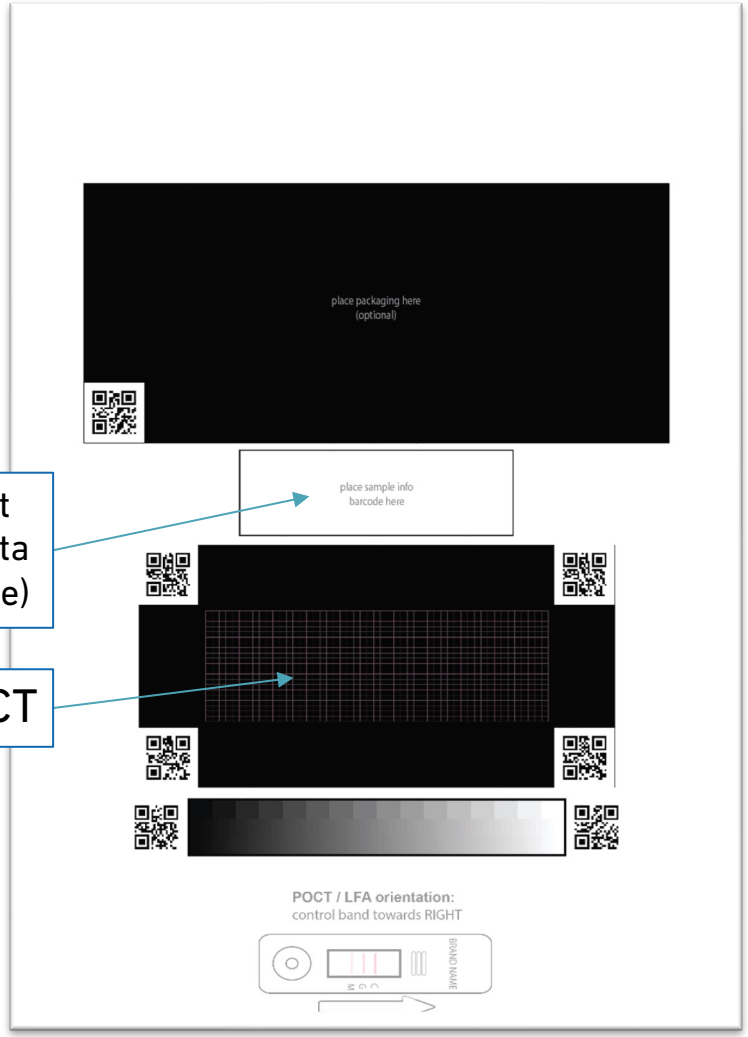


Expert-driven projects :: pyPOCQuant

- We needed to be able to analyse batches of **hundreds to thousands of tests** with very low failure rate
- However, several parameters strongly affect the robustness of our quantitative analysis:
  - **illumination variability** (given by acquisitions performed at different times of the day, weather conditions, shadows, reflections)
  - **scaling** and **orientation** of the images (due to freehand shots with cameras or smartphones)
  - **intensity corrections** by the camera (intensity stretching, white balancing, and compression) and its file formats (*e.g.*, RAW vs. JPG)
  - **different sizes**, **colors**, and **shapes** of POCTs from different vendors
  - **random positioning** and **orientation** of the POCT in the field of view
  - often **difficult localization** of the detector window

Expert-driven projects :: pyPOCQuant

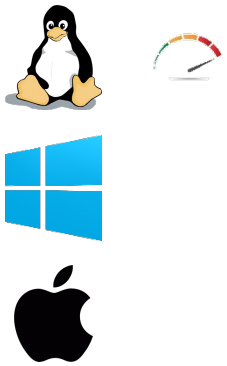
Slide courtesy: Andreas Cuny

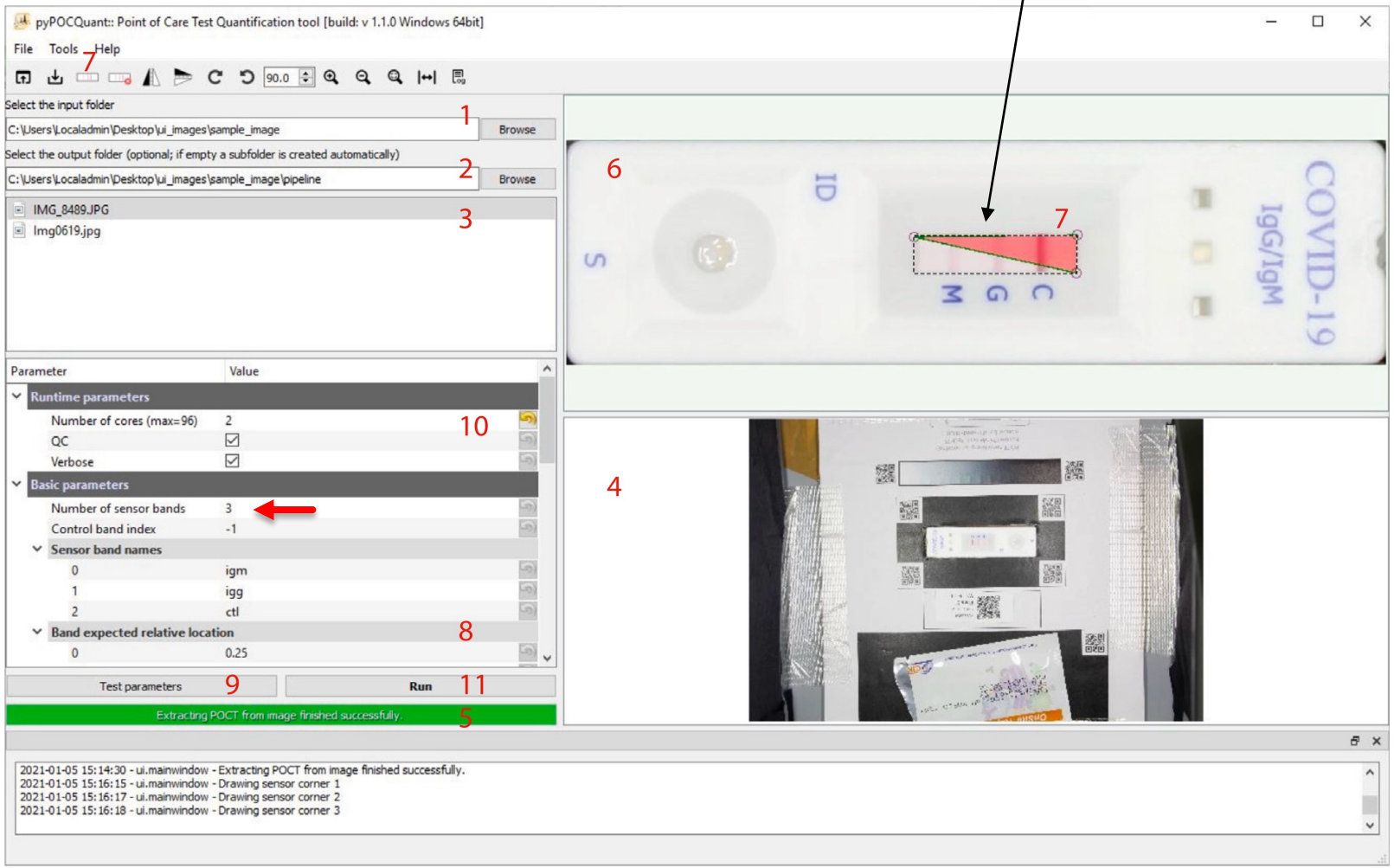


Work by Andreas Cuny

Expert-driven projects :: pyPOCQuant

User defines window and test line positions (and number)



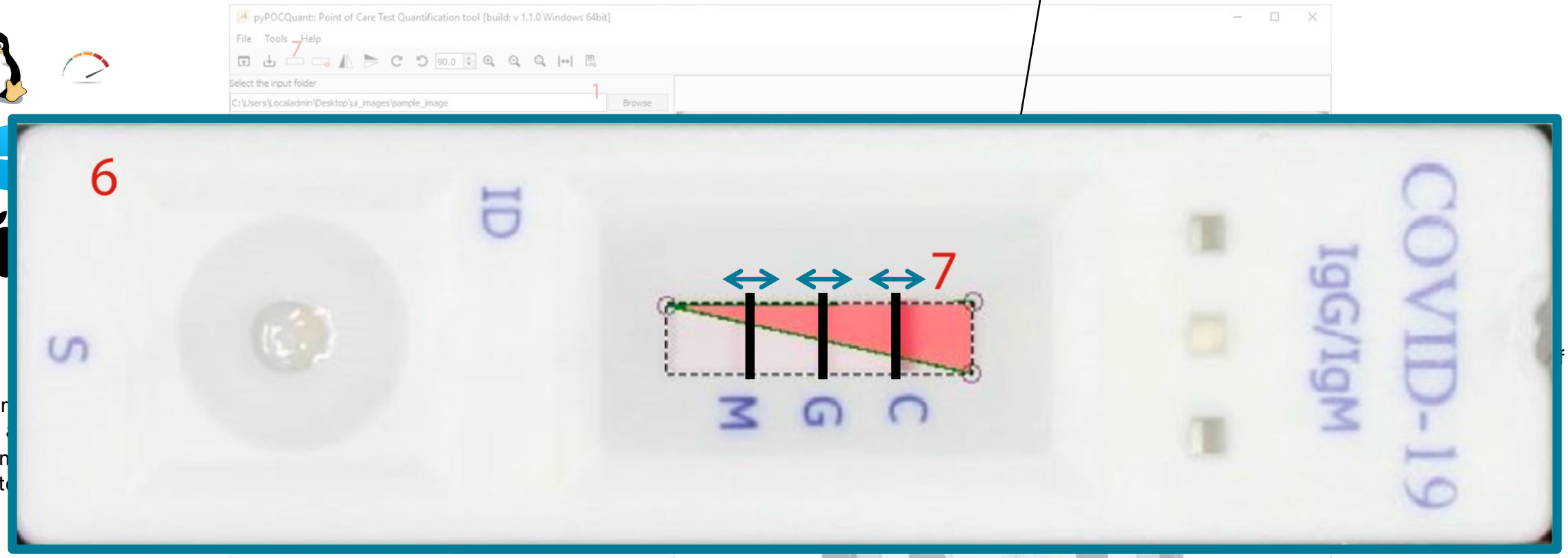


Interactively create and save a configuration for batch processing

Automatic extraction of POCT

Expert-driven projects :: pyPOCQuant

User defines window and test line positions (and number)



Inter
a
con
bat

Expert-driven projects :: pyPOCQuant

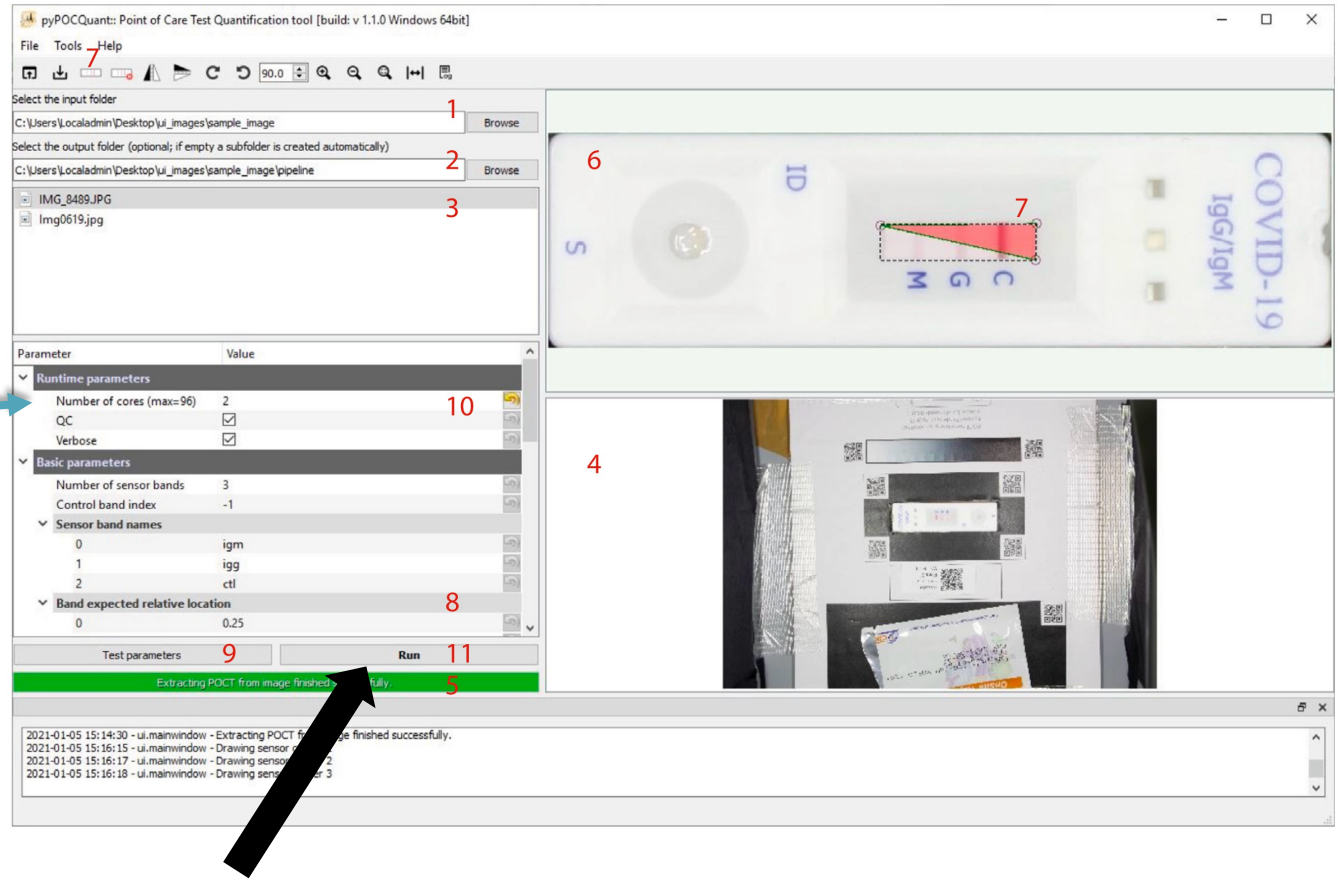
Run from console

```
python pyPOCQuant.py -f examples/images -o examples/images/results -s examples/config.conf -w 4
```

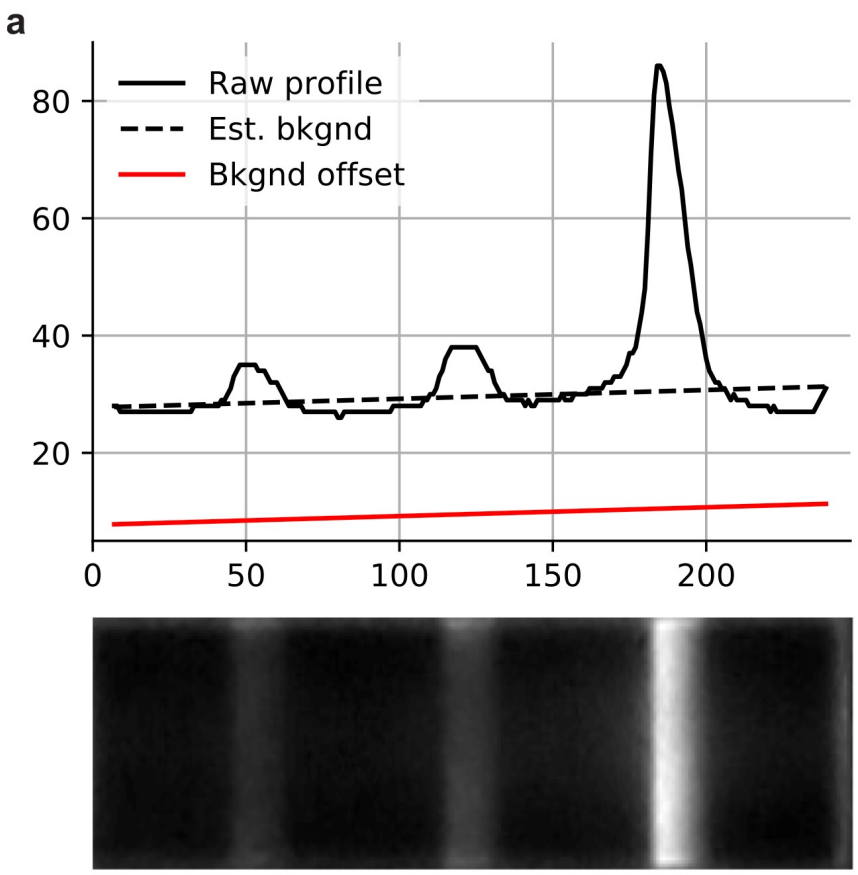
Run from scripts or notebooks

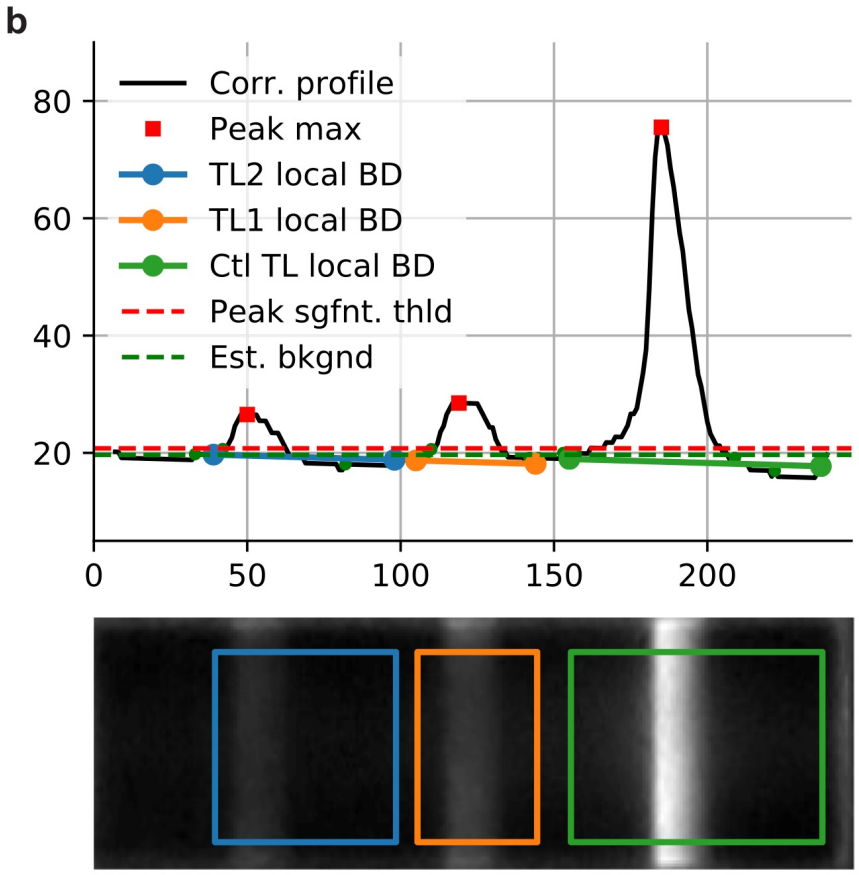
```
from pypocquant.lib.pipeline import run_pipeline
from pypocquant.lib.settings import default_settings

# Get the default settings
settings = default_settings()

# Change settings manually as needed
settings["sensor_band_names"] = ('igm', 'igg', 'ctl')

# Alternatively, load existing settings file
# from pypocquant.lib.settings import load_settings
# settings = load_settings('full/path/to/settings/file.conf')

# Set final argument
input_folder_path = 'full/path/to/input/folder'
results_folder_path = 'full/path/to/results/folder'
max_workers = 8

# Run the pipeline
run_pipeline(
input_folder_path,
results_folder_path,
**settings,
max_workers=max_workers
)
```

Run from user interface

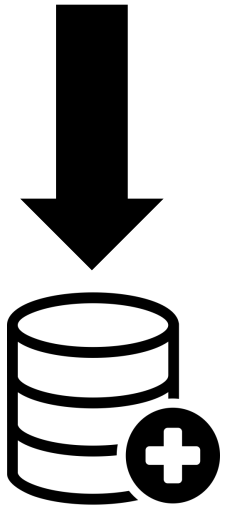


Expert-driven projects :: pyPOCQuant

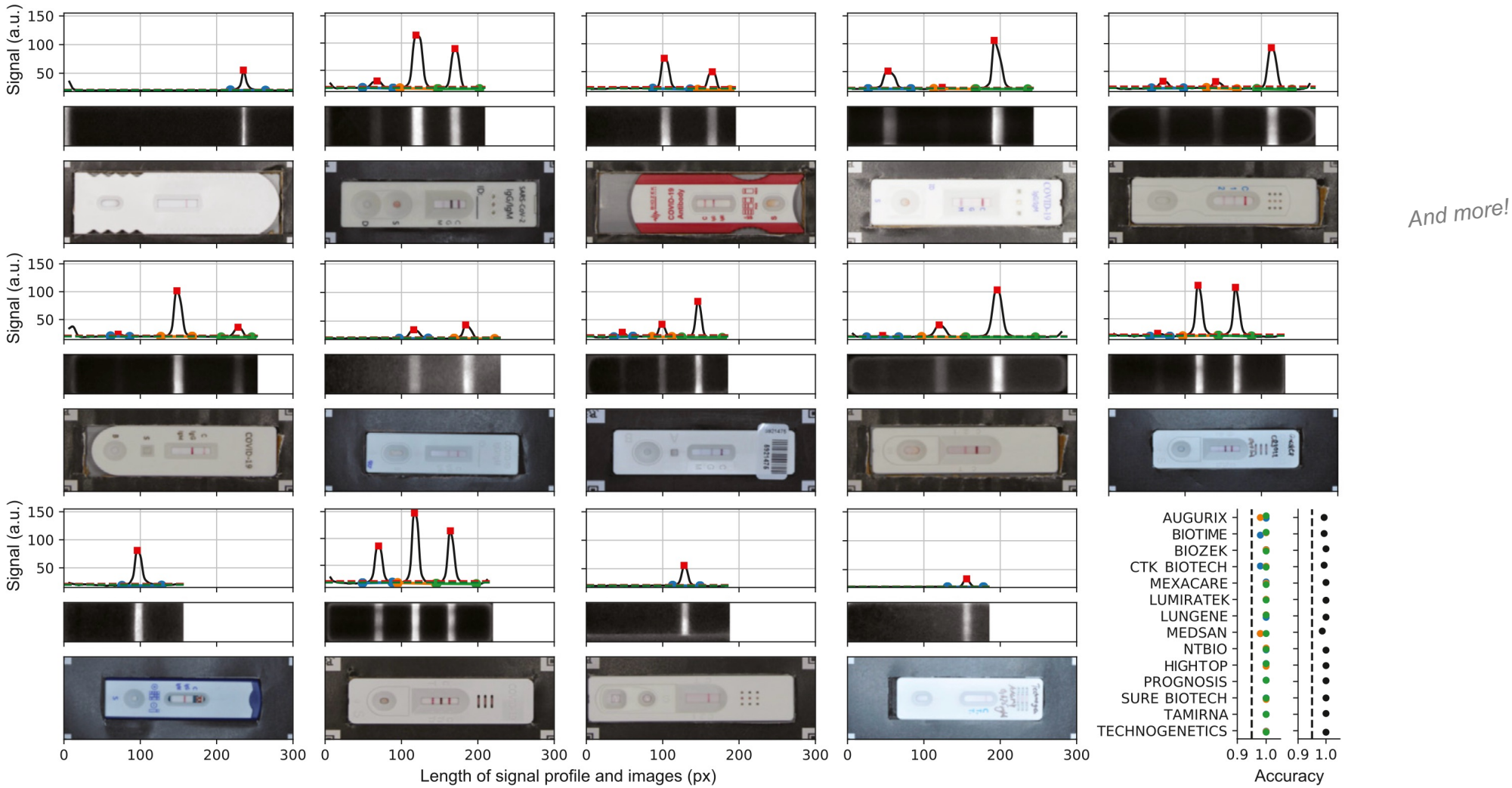




Analysis results
Patient metadata
Experiment metadata



Expert-driven projects :: pyPOCQuant



And more!

Expert-driven projects :: pyPOCQuant

Cuny A. P., Rudolf F., Ponti A. SoftwareX 15:2021, 100710. DOI: 10.1016/j.softx.2021.100710

Work by **Andreas Cuny**

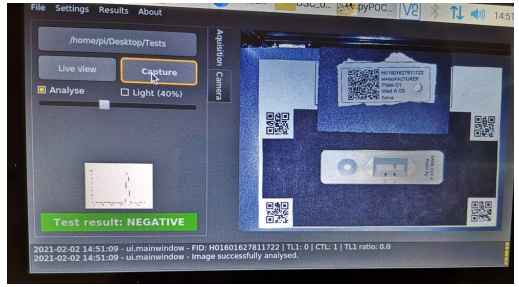
New hardware prototype (with touch screen and Raspberry Pi 4 Model B) for the Swiss Armed Forces

Expert contributions

Fabian Rudolf: development of the testing methodology, field work, and project management
Andreas Cuny: development of hardware components of pyPOCQuant, application development
Aaron Ponti: computer vision algorithm development, application development

Users

- Baselland Test Center (via Biolytix AG)
- Swiss Tropical and Health Institute (TPH), Basel
- Fachhochschule Nordwestschweiz (FHNW), Muttenz
- Swiss Armed Forces ← Large-scale testing before Rekrutenschule Summer 2020
- Canton Grisons and Swiss Federal Office of Public Health (Kantonaler Führungsstab Graubünden)
- Purdue University, Indiana, USA
- Test centers in Argentina and Greece



Adapted user interface (and template) for the new hardware

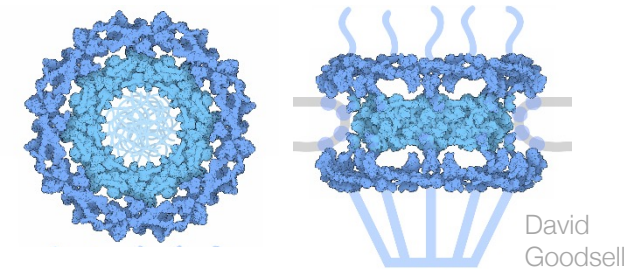
Expert-driven projects :: pyMINFLUX

Expert: Javier Casares Arias, Single Cell Facility, D-BSSE ETHZ

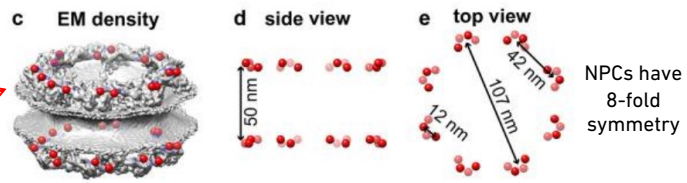
Slide courtesy: Javier Casares Arias

Expert-driven projects :: pyMINFLUX

QC sample: Nuclear pore complex imaging



Top view Side view

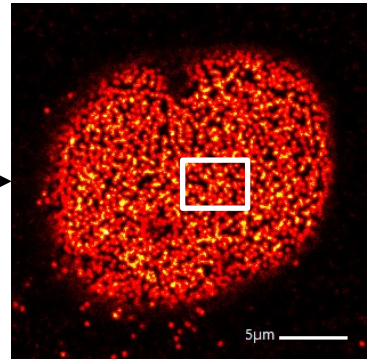


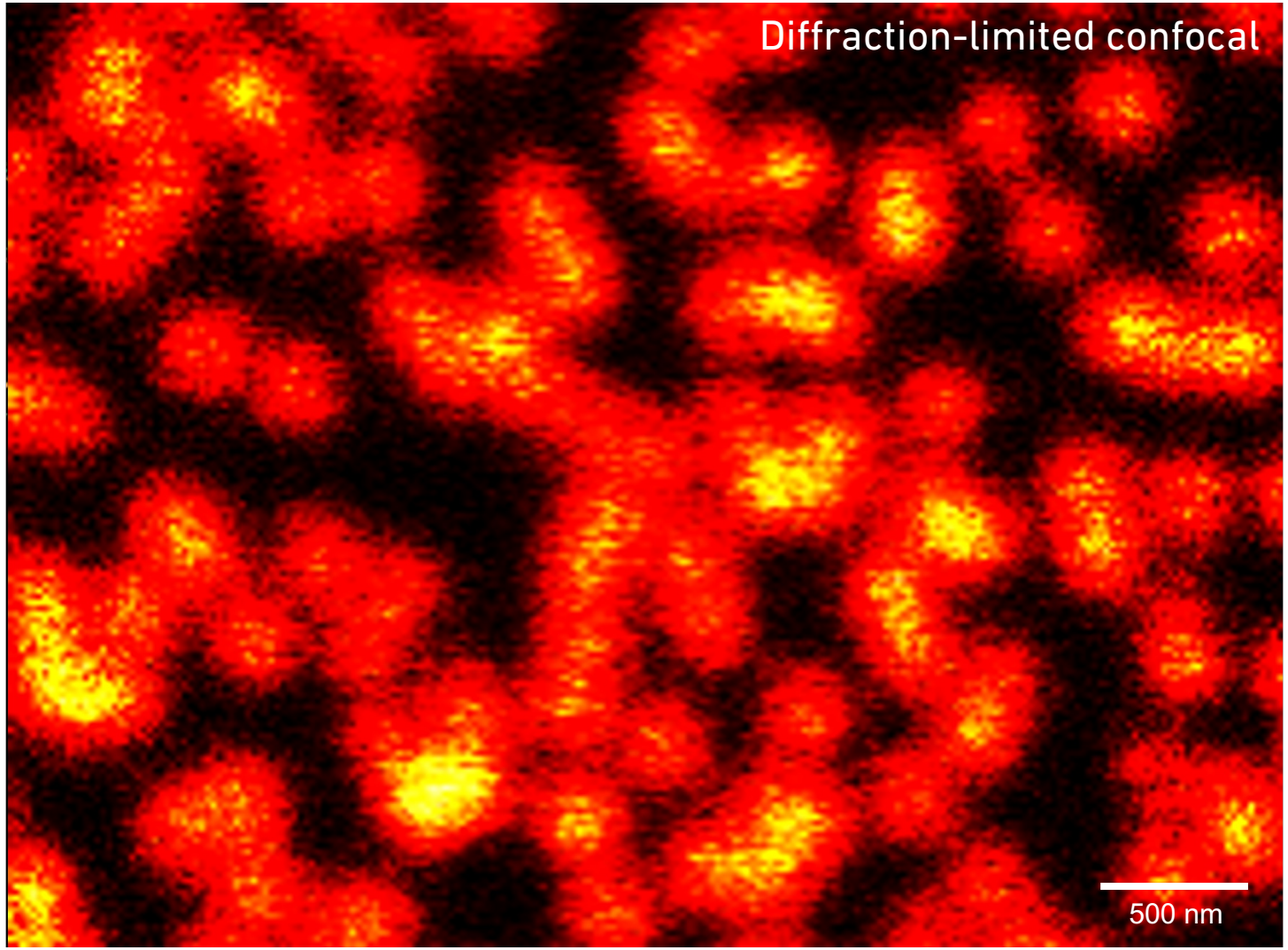
NPCs have 8-fold symmetry

Red C-termini of Nup96

doi: 10.1038/s41592-019-0574-9

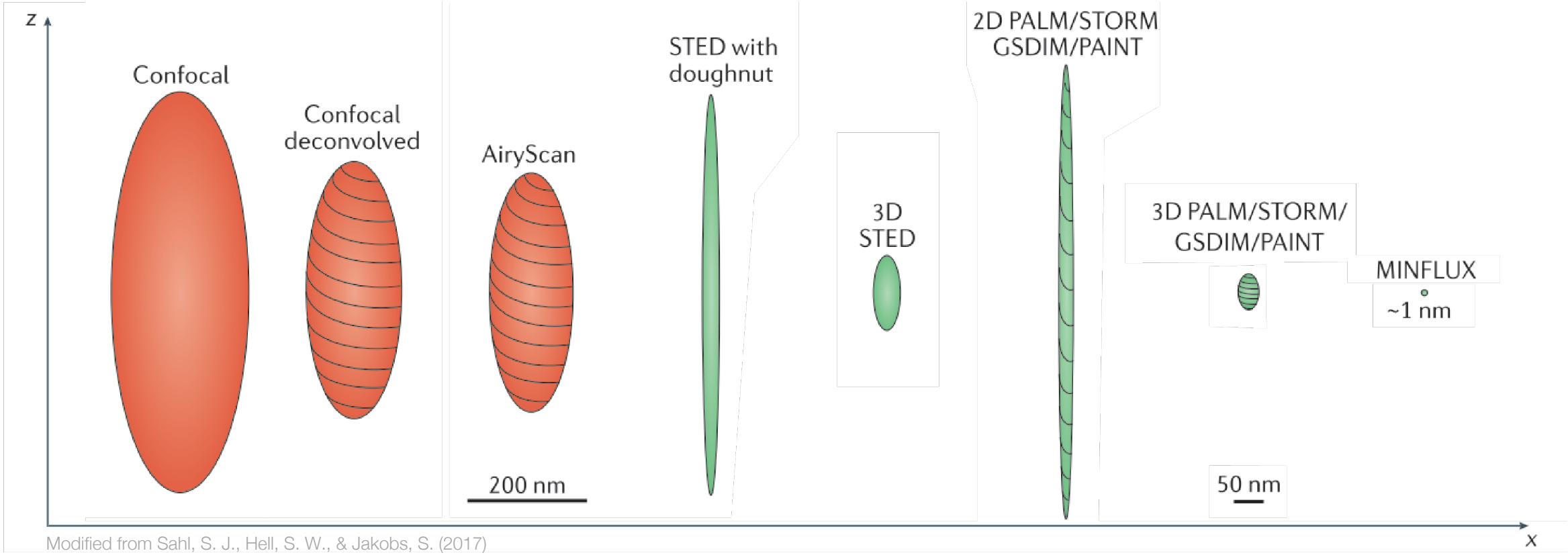
U2OS/Nup96-SNAP + BG-AF647





Slide courtesy: Javier Casares Arias

Expert-driven projects :: pyMINFLUX



Modified from Sahl, S. J., Hell, S. W., & Jakobs, S. (2017)

XY	200 nm	150 nm	120 nm	30 nm	50 nm	20 nm	20 nm	1 nm
Z	600 nm	400 nm	350 nm	600 nm	150 nm	600 nm	100 nm	1 nm

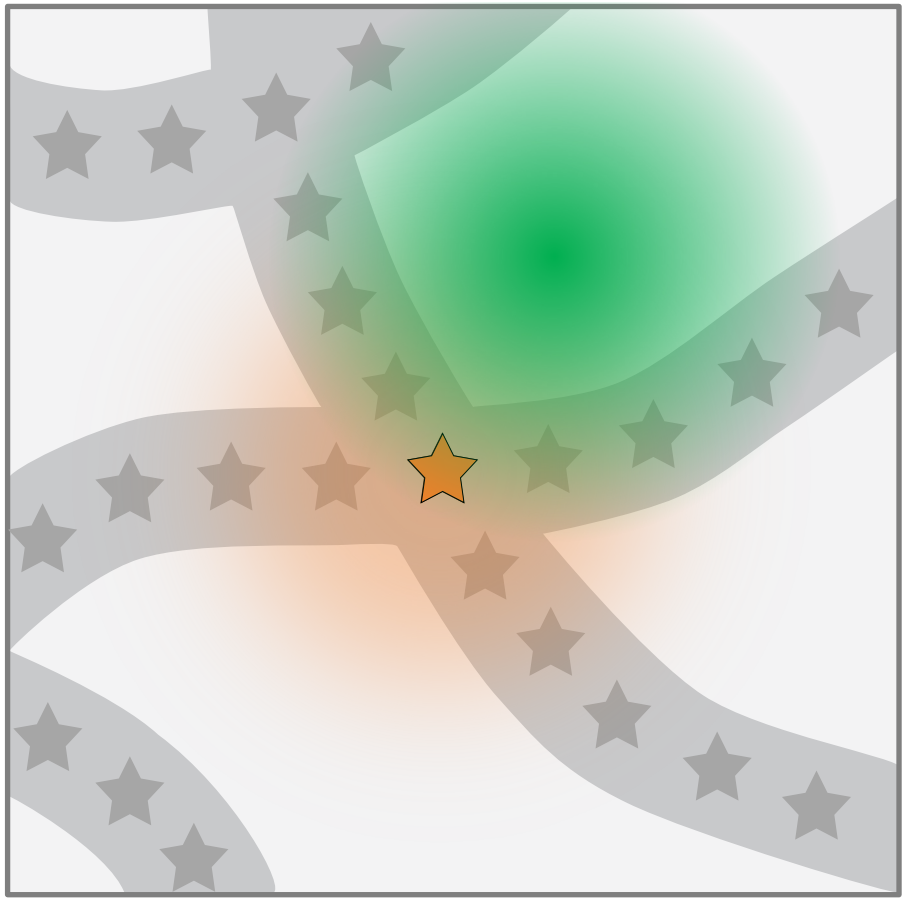
Expert-driven projects :: pyMINFLUX

When the minimum at the center of the **excitation donut** and the fluorophore overlap there is no excitation, and thus no emission

Beam position = Fluorophore position

Expert-driven projects :: pyMINFLUX

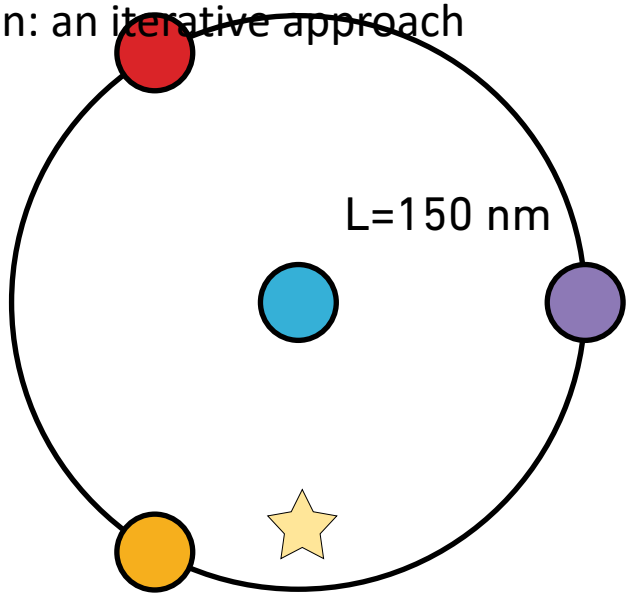
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

Expert-driven projects :: pyMINFLUX

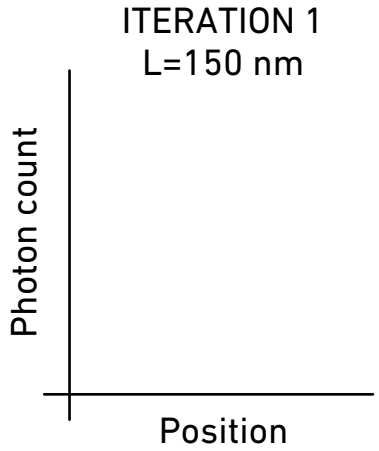
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

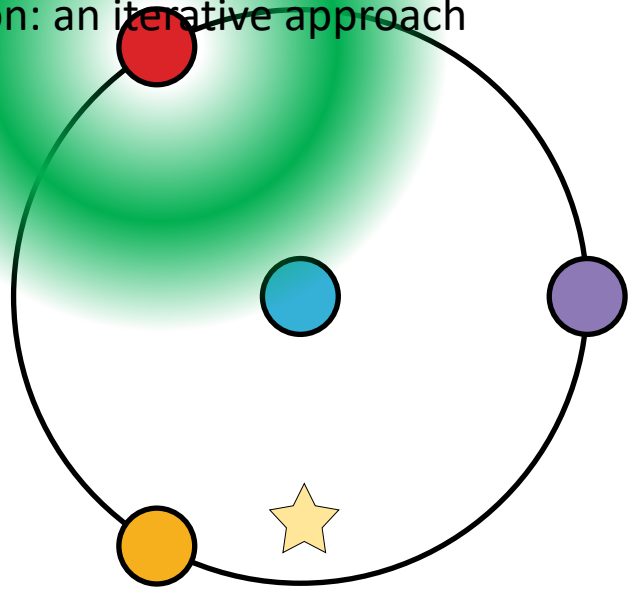
MINFLUX iteration:

1. Define scanning region according to previous estimation



Expert-driven projects :: pyMINFLUX

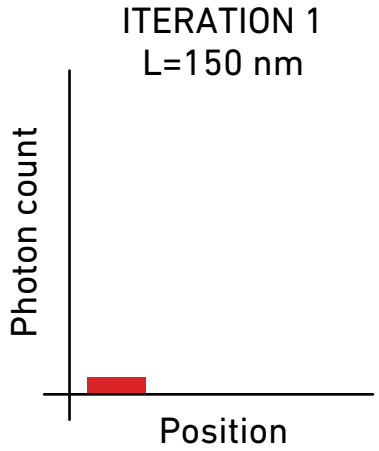
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

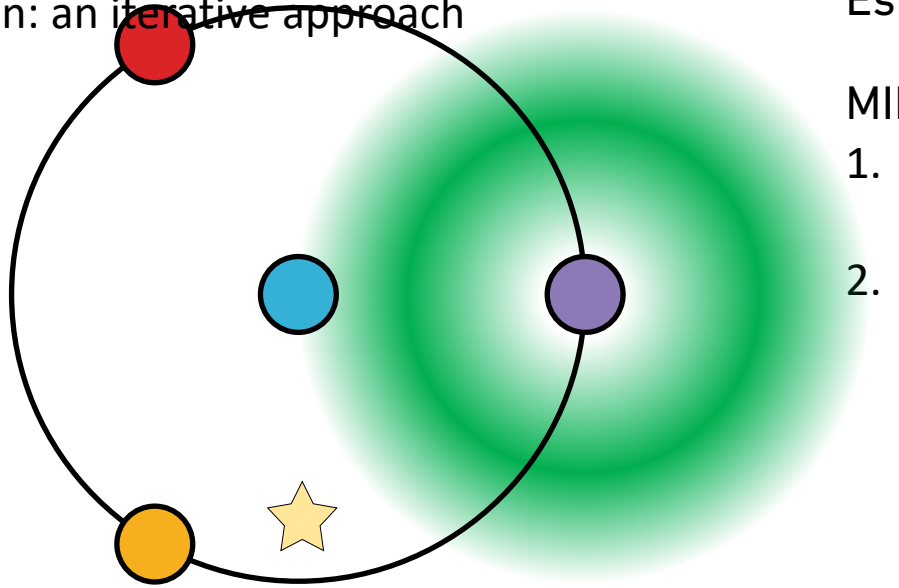
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern



Expert-driven projects :: pyMINFLUX

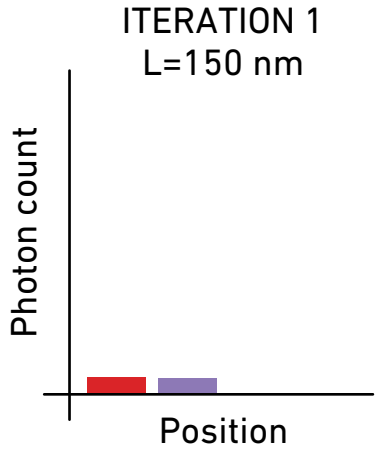
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

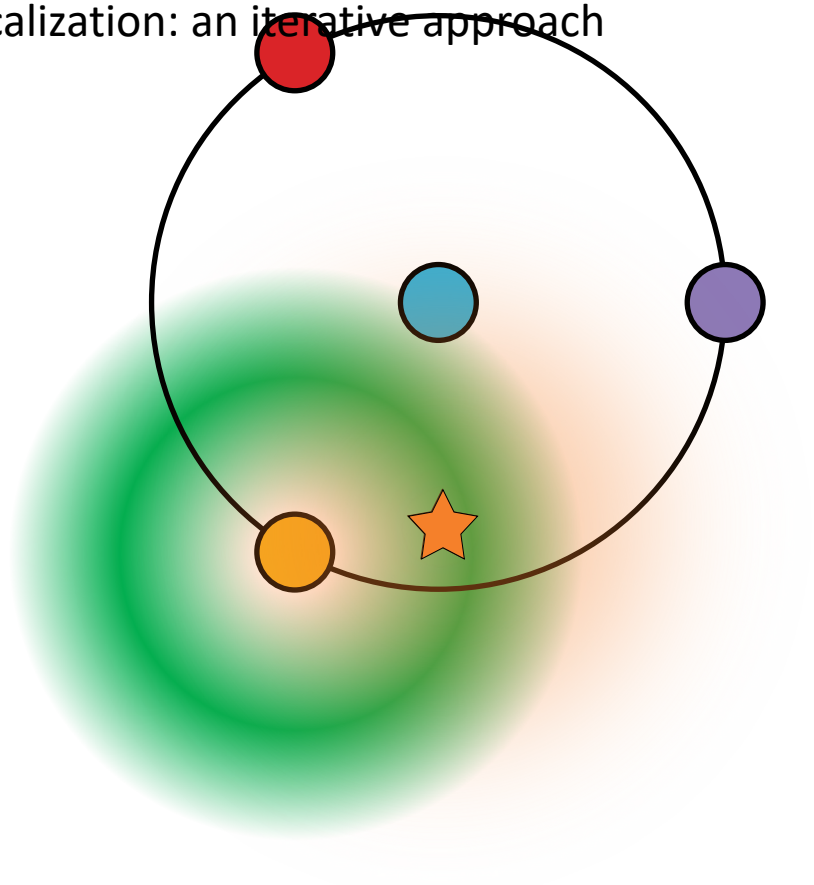
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern



Expert-driven projects :: pyMINFLUX

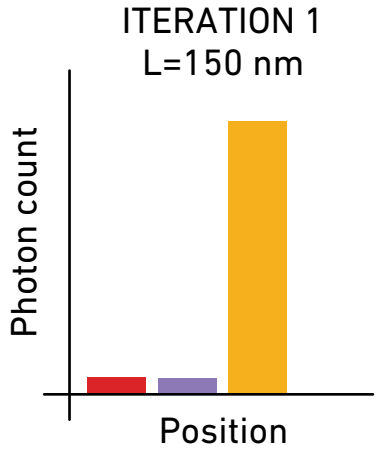
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

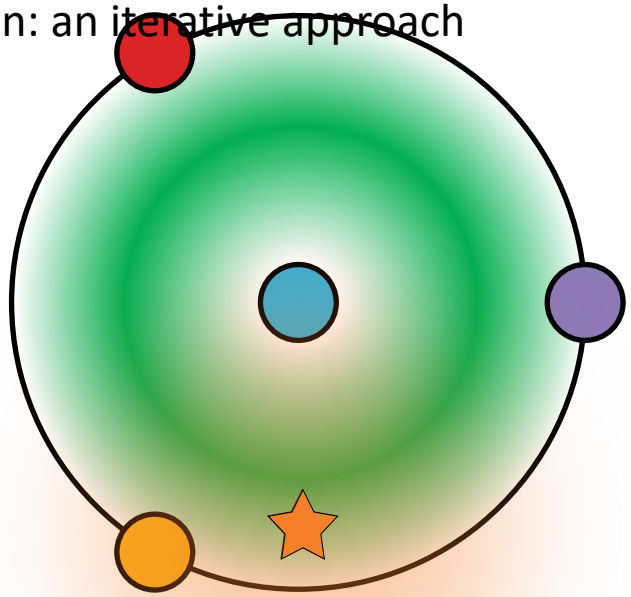
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern



Expert-driven projects :: pyMINFLUX

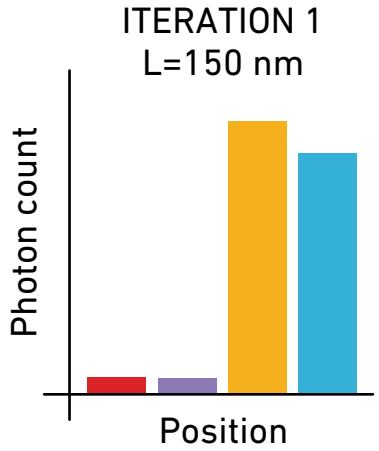
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

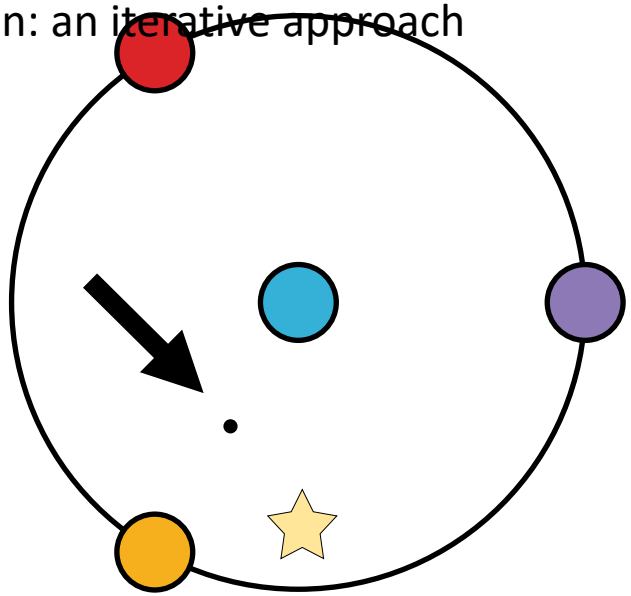
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern



Expert-driven projects :: pyMINFLUX

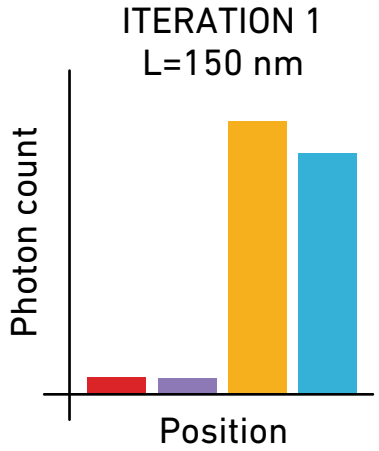
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

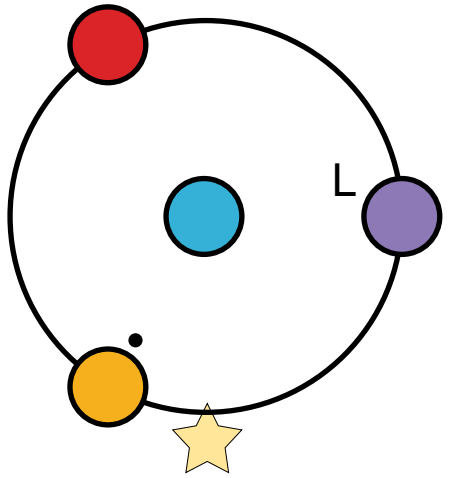
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation



Expert-driven projects :: pyMINFLUX

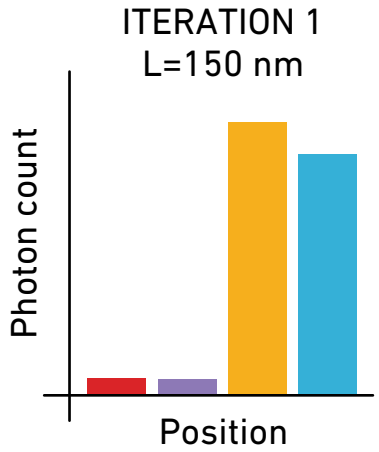
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

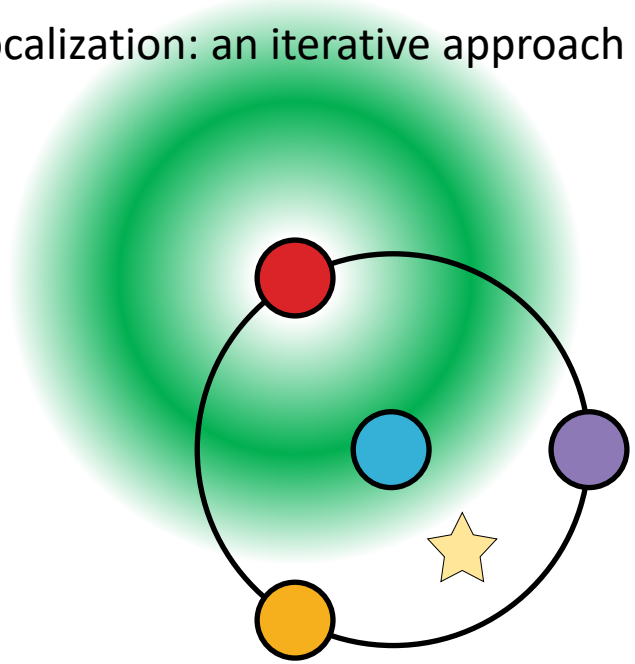
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

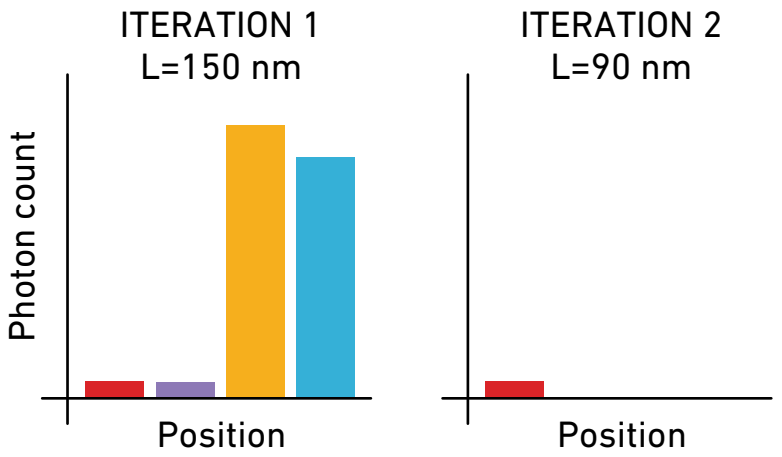
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

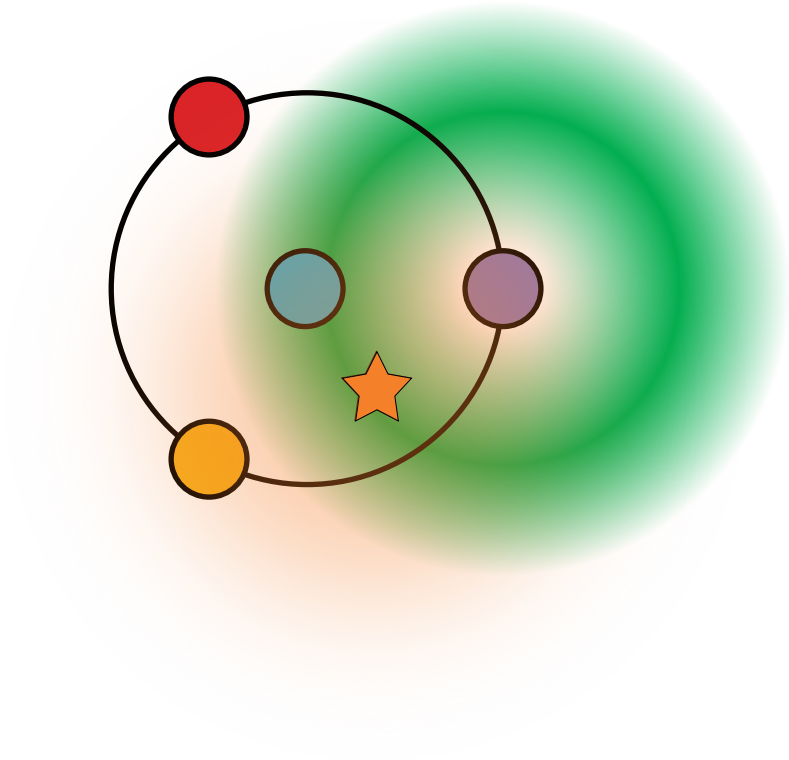
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

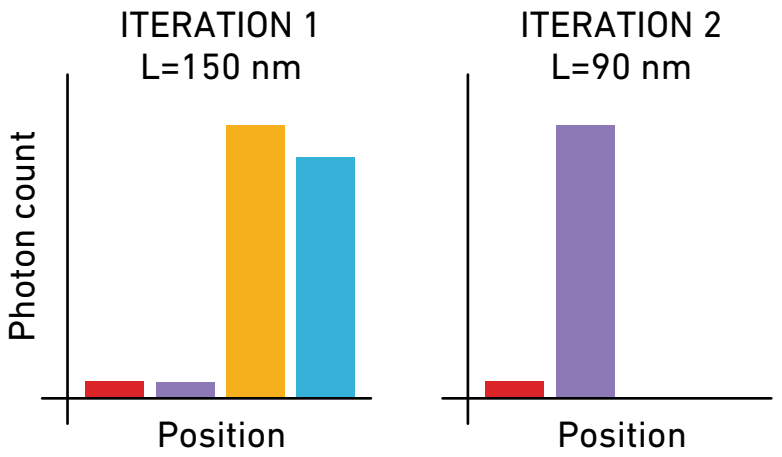
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

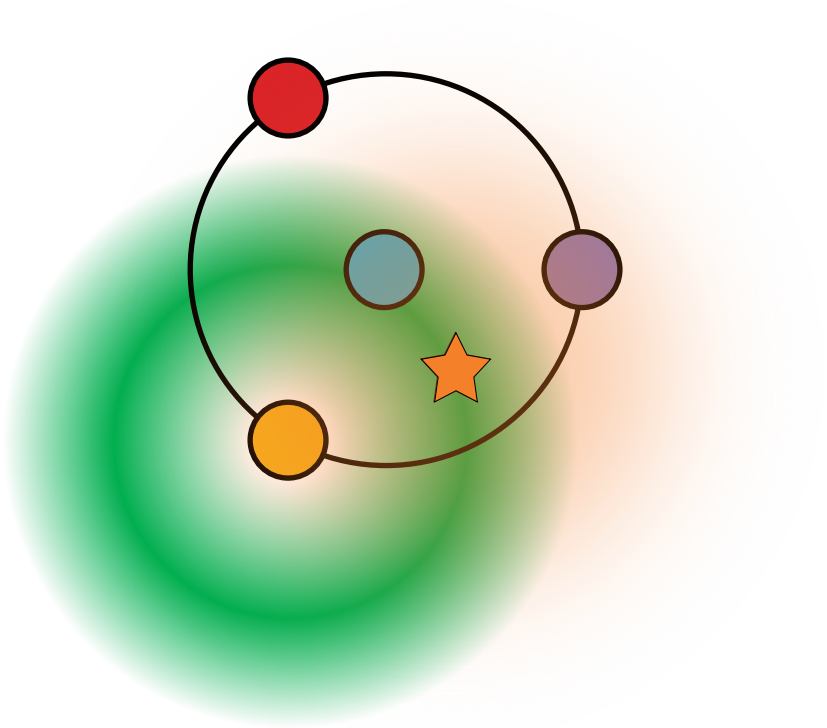
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

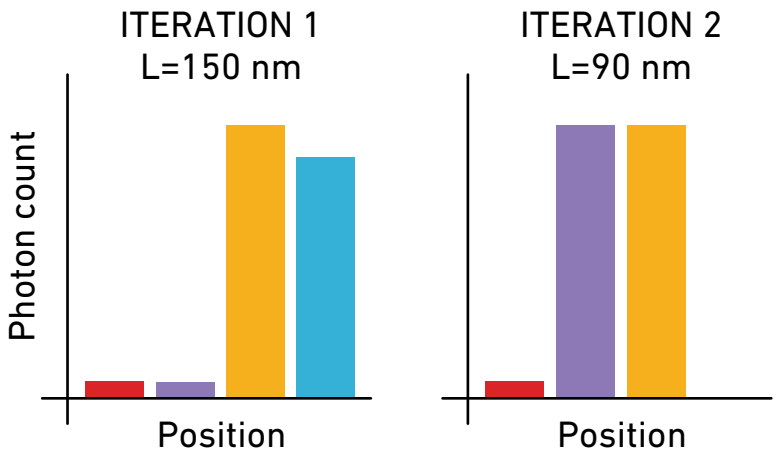
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

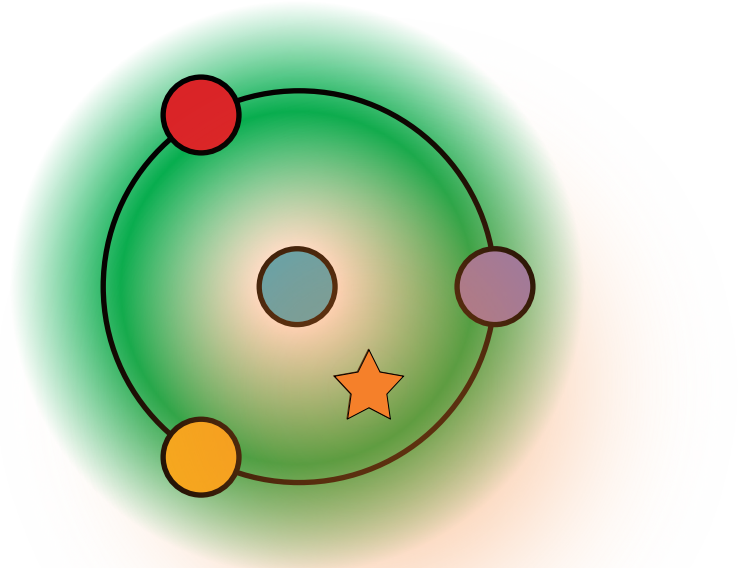
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

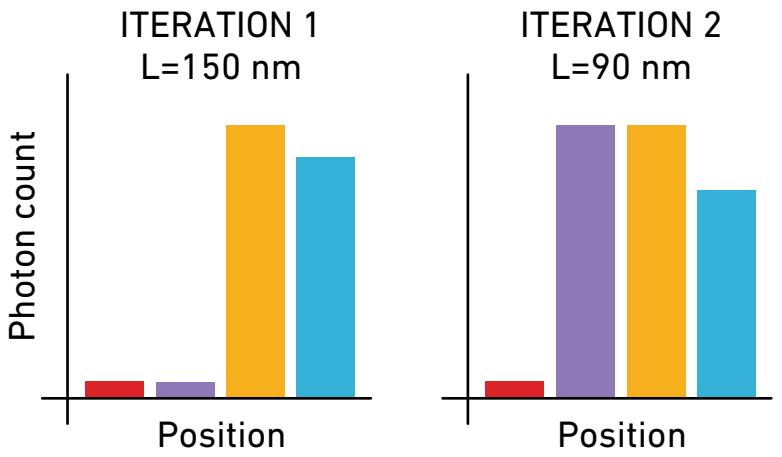
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

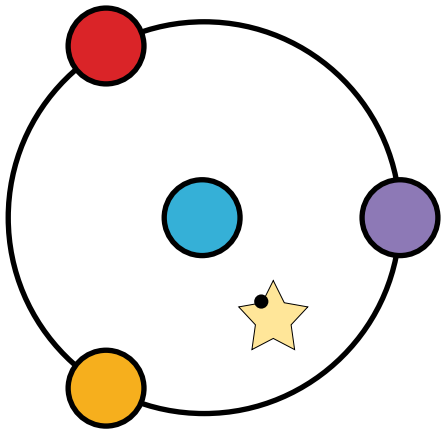
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

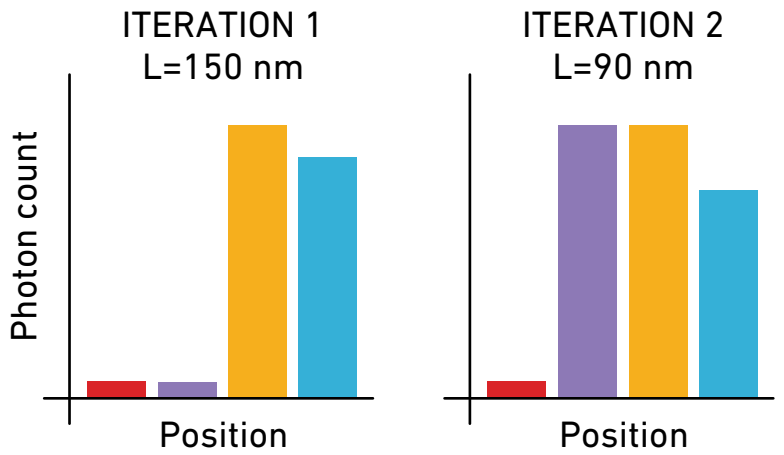
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

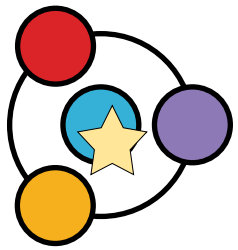
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

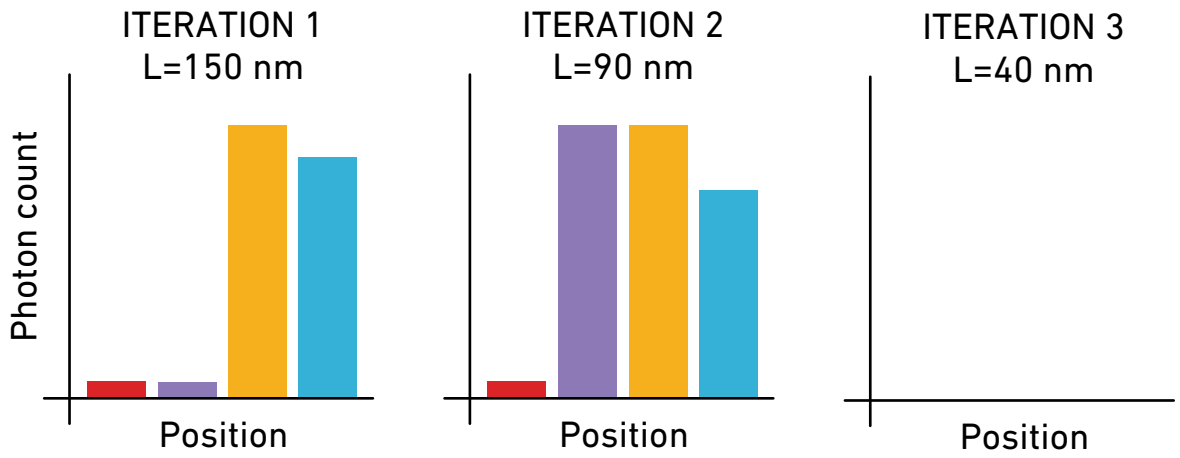
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

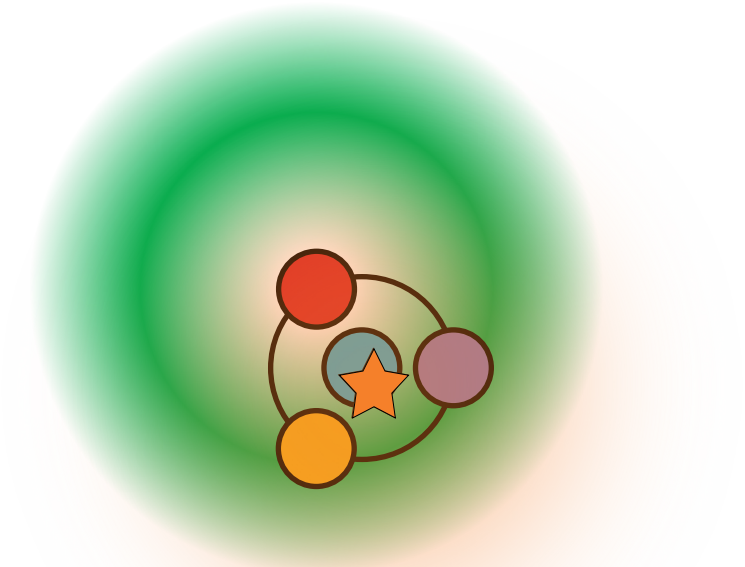
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

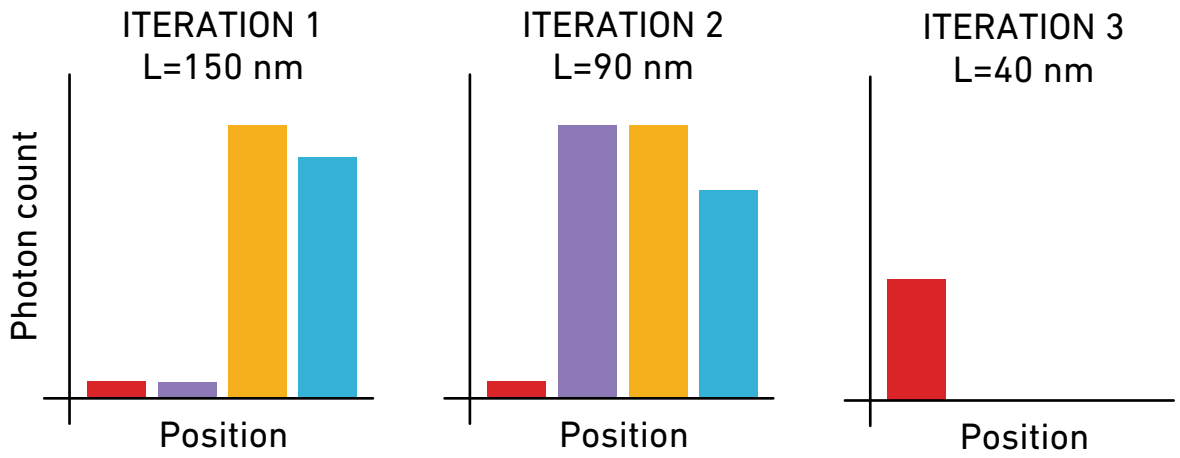
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

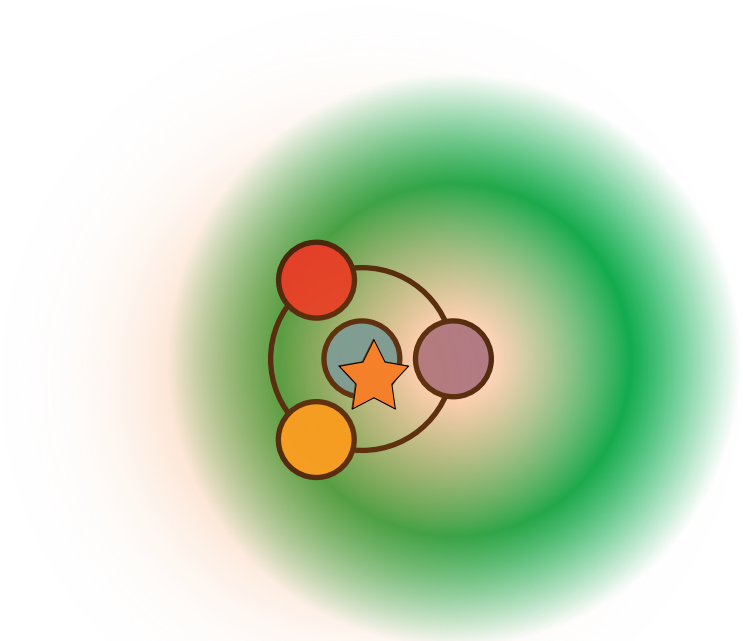
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

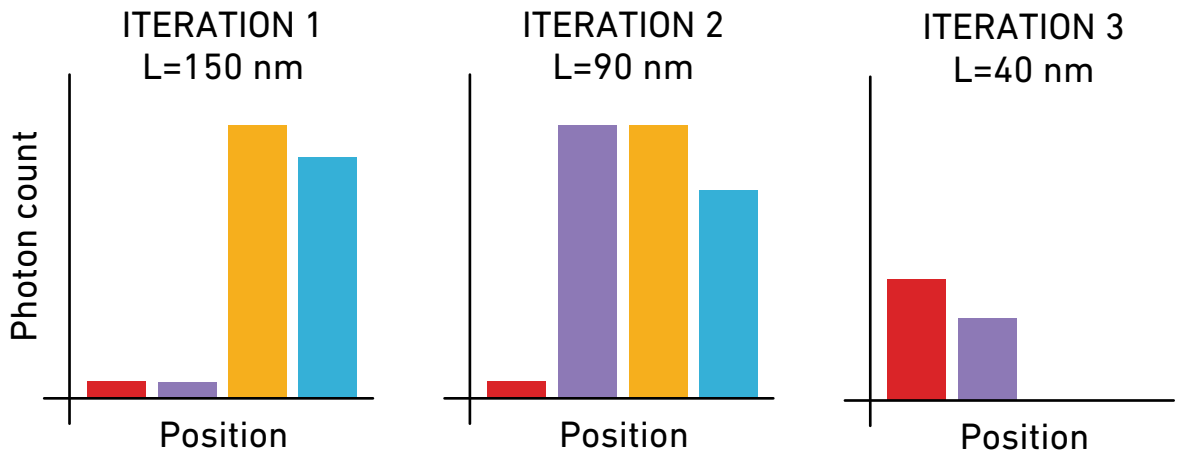
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

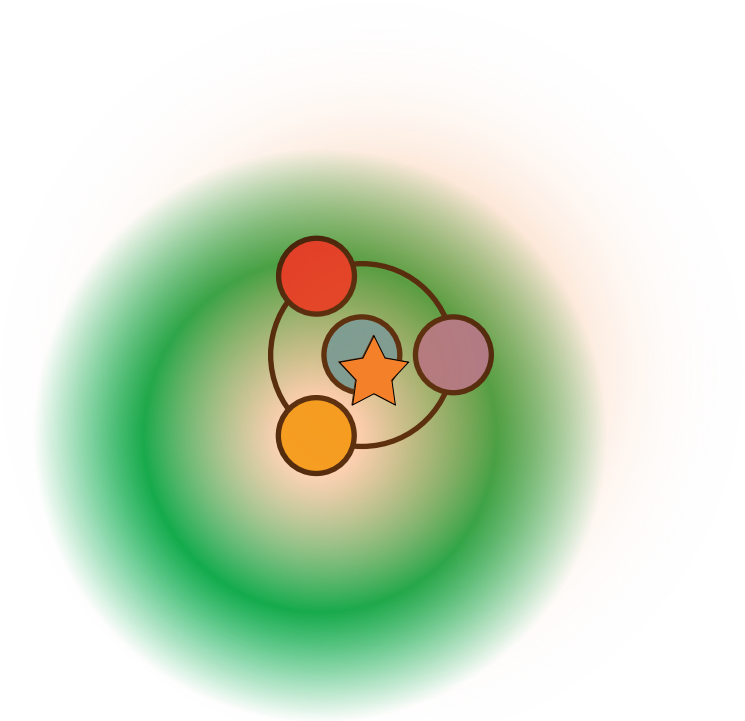
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

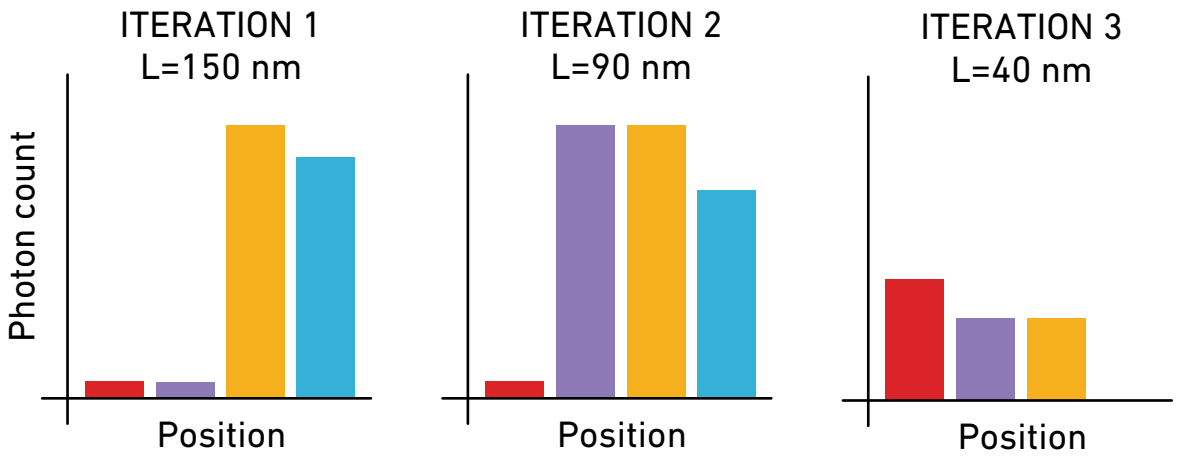
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

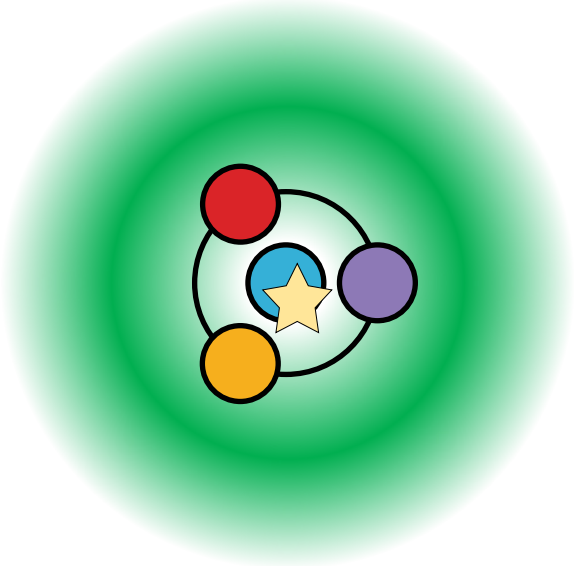
MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

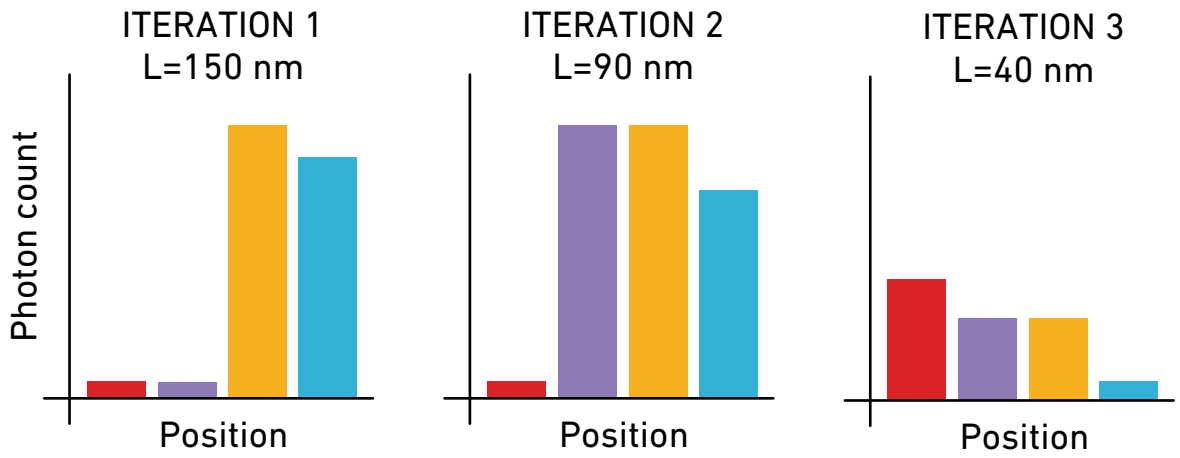
Localization: an iterative approach



Estimate fluorophore position with spot-shaped beam

MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

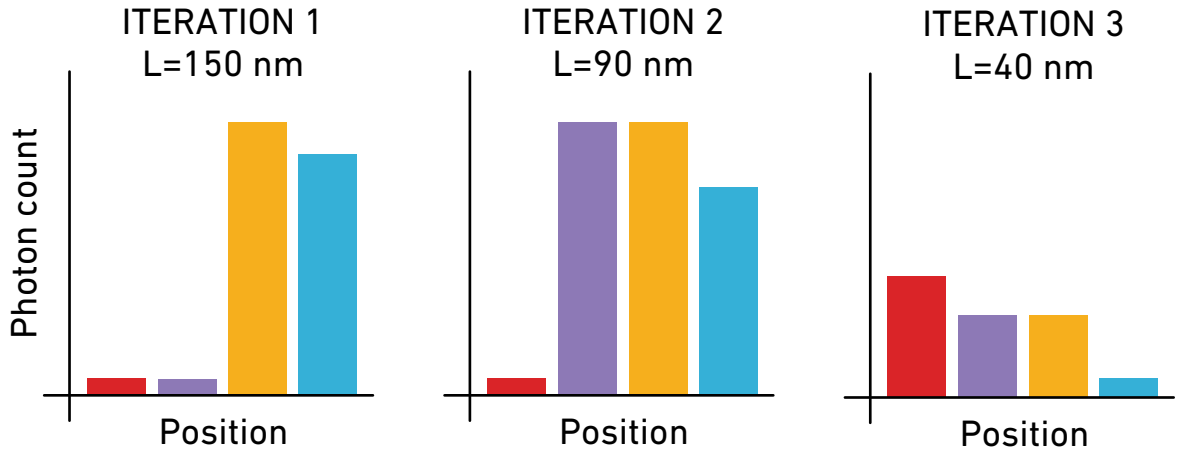
Localization: an iterative approach

FLUOROPHORE
LOCALIZATION

Estimate fluorophore position with spot-shaped beam

MINFLUX iteration:

1. Define scanning region according to previous estimation
2. Register emitted photons from every position in the pattern
3. Analyse photon counts and refine fluorophore position estimation
4. Decrease “L”



Expert-driven projects :: pyMINFLUX

Operation modalities

Localization

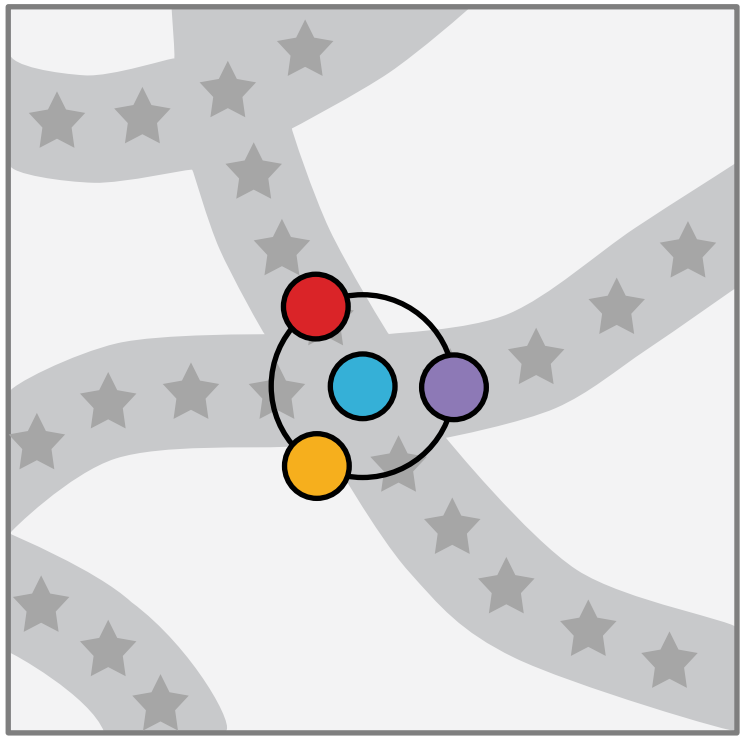
Important: only very few fluorophores must be emitting at any given time!

1. Fluorophores are localized 1 by 1

Expert-driven projects :: pyMINFLUX

Operation modalities

Localization

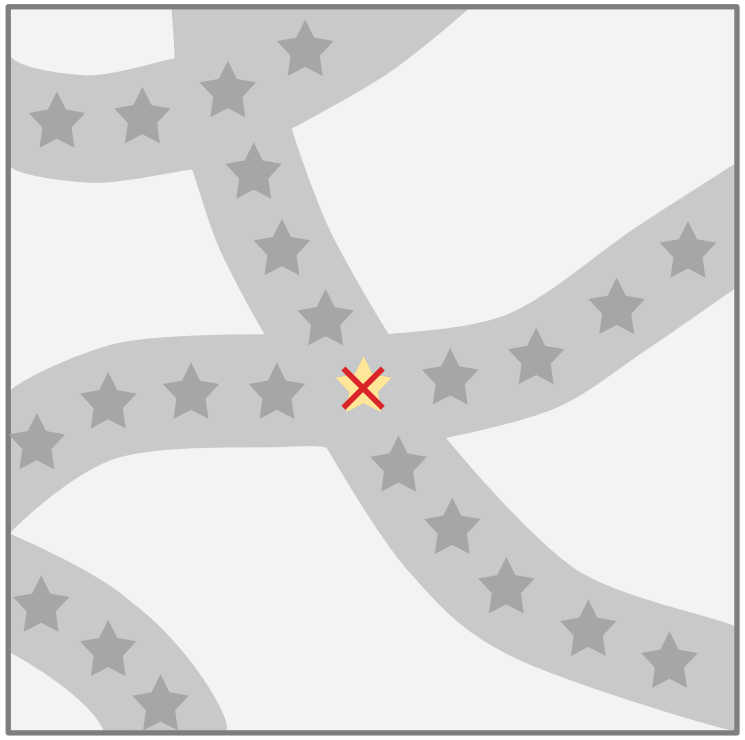


1. Fluorophores are localized 1 by 1

Expert-driven projects :: pyMINFLUX

Operation modalities

Localization

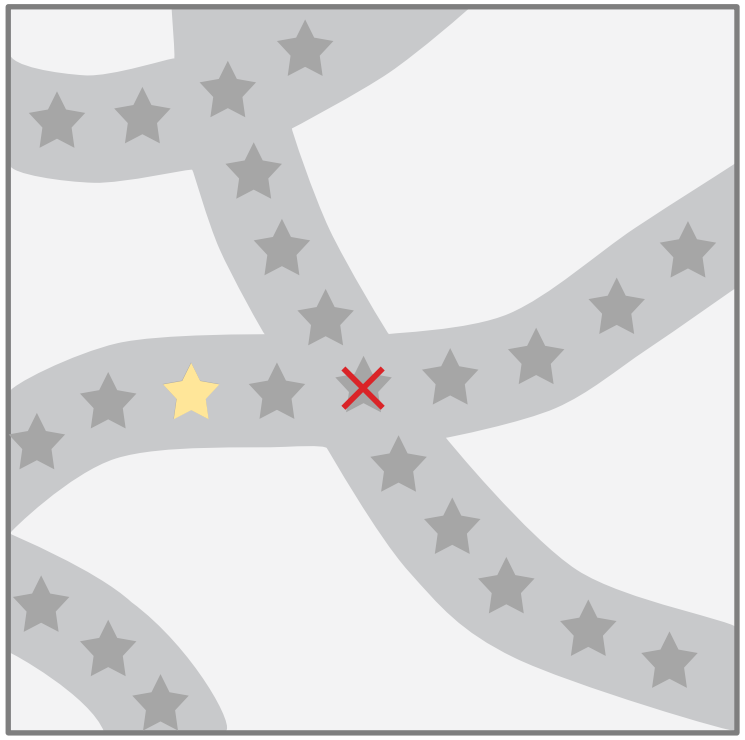


1. Fluorophores are localized 1 by 1

Expert-driven projects :: pyMINFLUX

Operation modalities

Localization

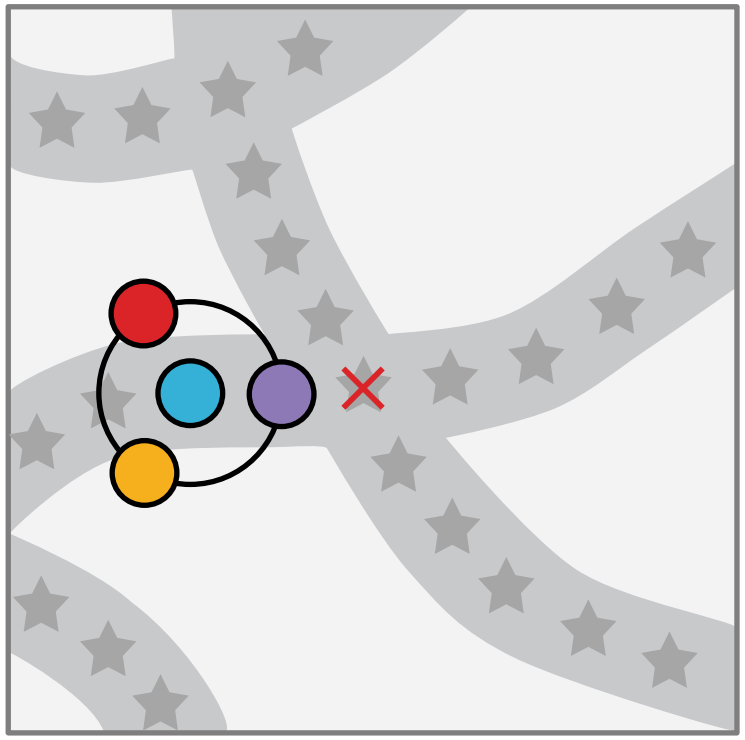


1. Fluorophores are localized 1 by 1

Expert-driven projects :: pyMINFLUX

Operation modalities

Localization

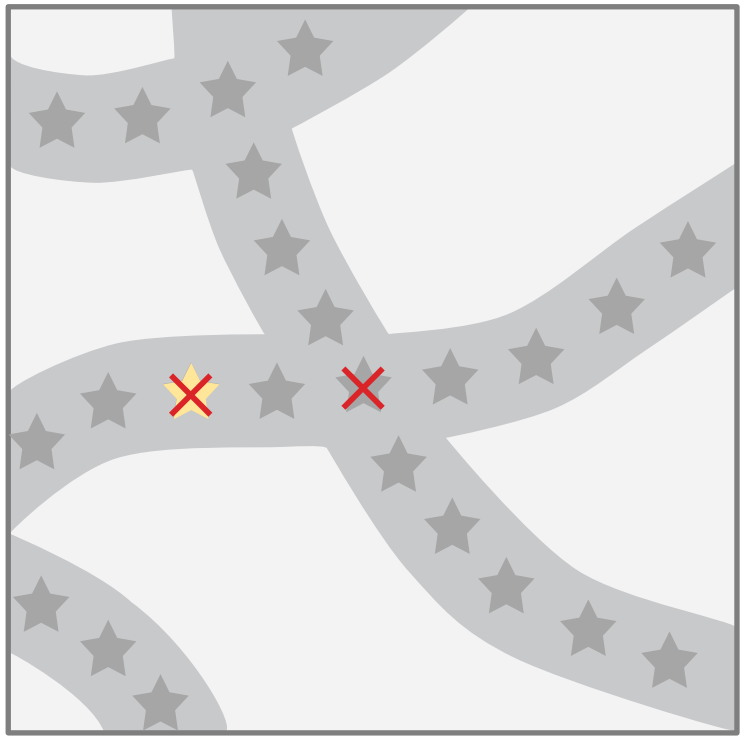


1. Fluorophores are localized 1 by 1

Expert-driven projects :: pyMINFLUX

Operation modalities

Localization

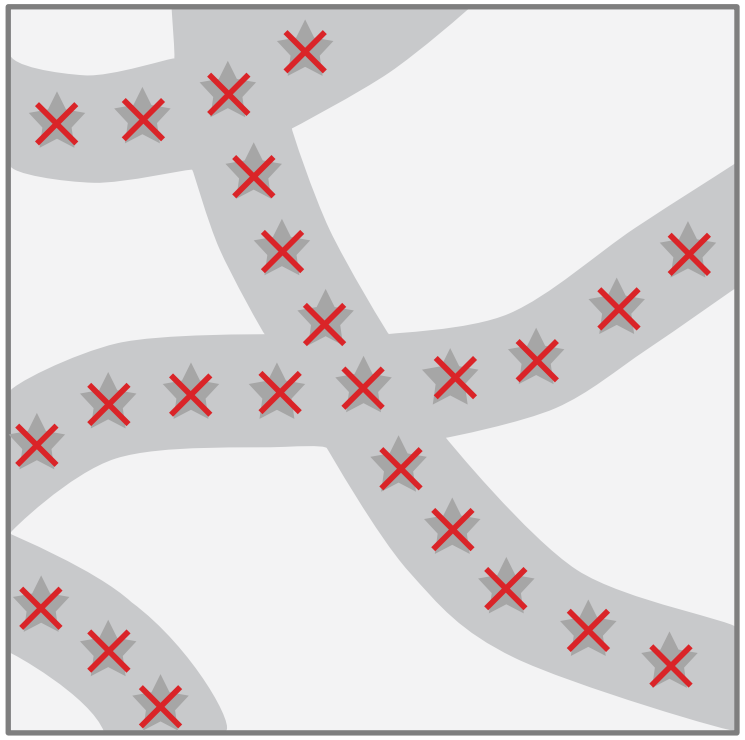


1. Fluorophores are localized 1 by 1

Expert-driven projects :: pyMINFLUX

Operation modalities

Localization

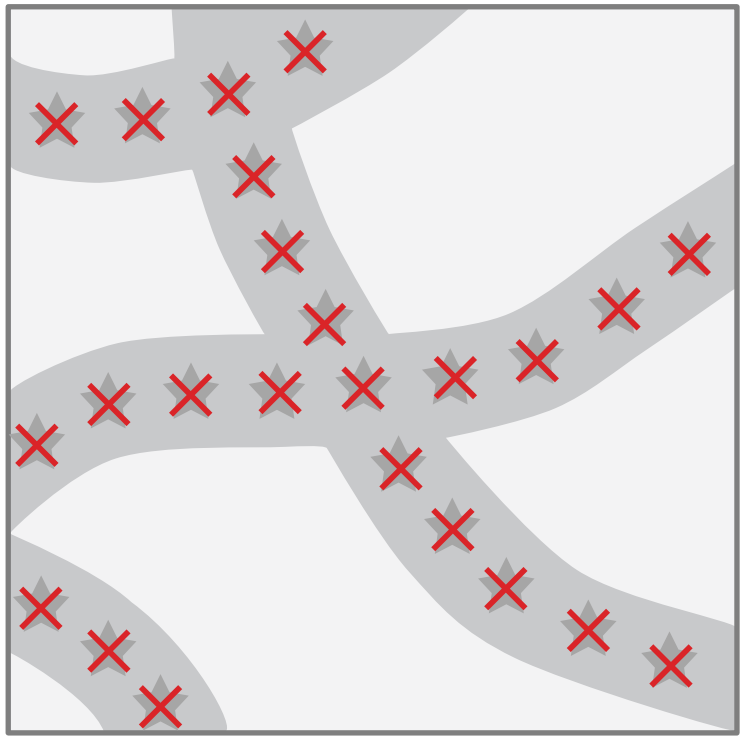


1. Fluorophores are localized 1 by 1

Expert-driven projects :: pyMINFLUX

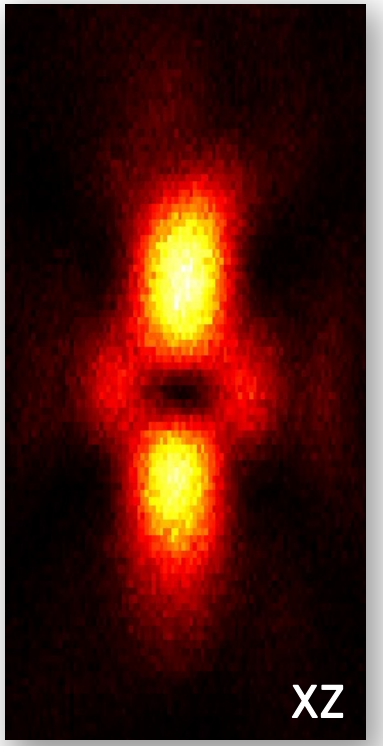
Operation modalities

Localization



3 nm - 2D

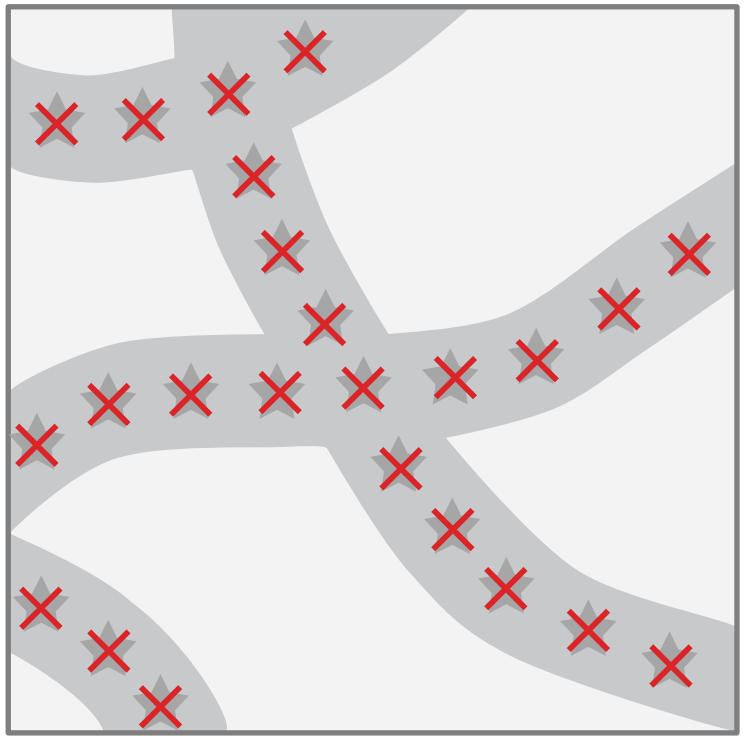
5 nm - 3D



Expert-driven projects :: pyMINFLUX

Operation modalities

Localization



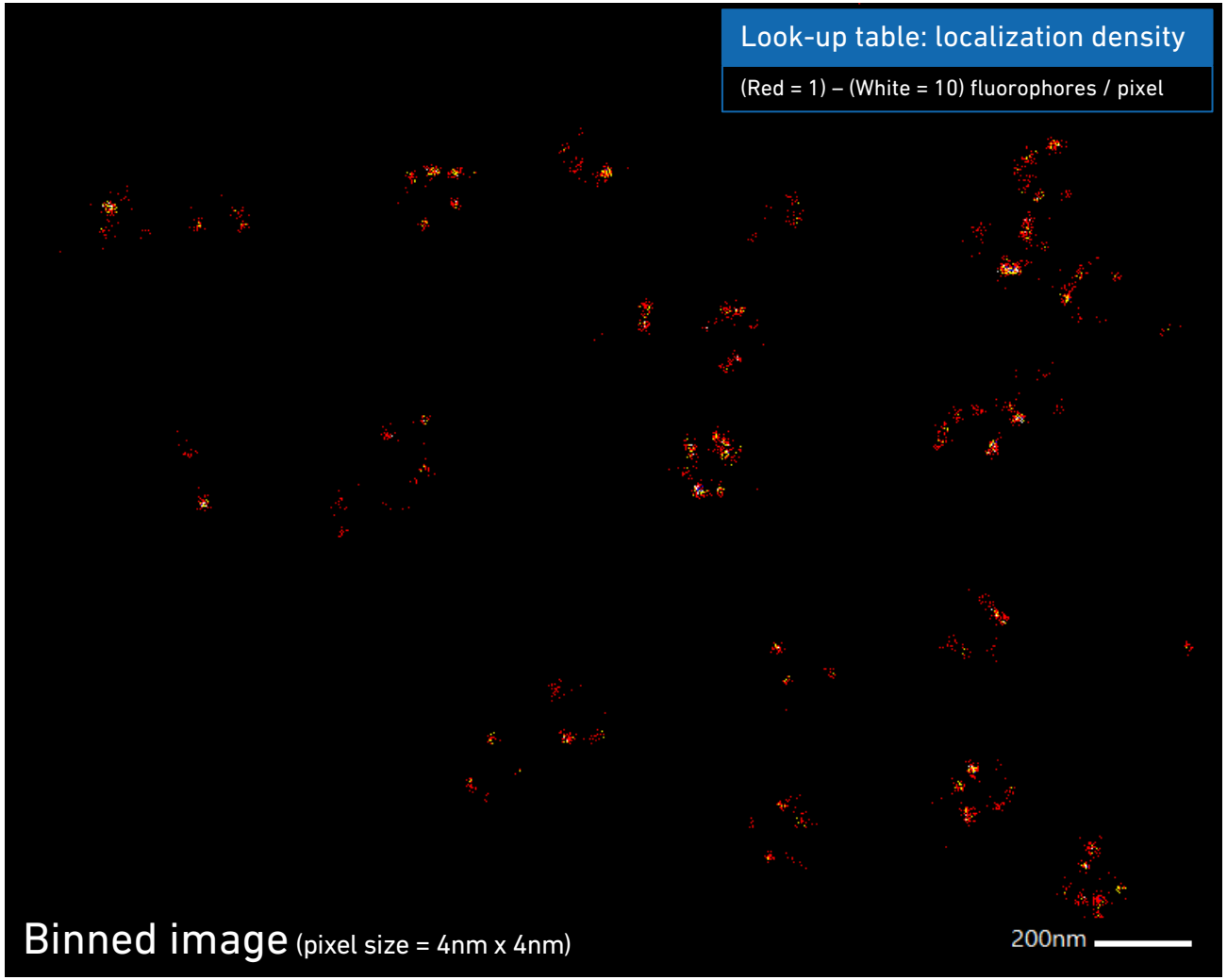
1. Fluorophores are localized 1 by 1
2. Coordinates turned into an image

Expert-driven projects :: pyMINFLUX

At this stage, we hadn't bough the microscope yet...

What we had:
- No localization data
- Low-res raster images

Testing phase



Expert-driven projects :: pyMINFLUX

At this stage, we ***still*** hadn't bough the microscope

What we had:
- Undocumented .npy files

```
python
>>> import numpy as np
>>>
>>> data = np.load("2D_FA-IIF-AF467.npy")
>>> data
array([[[(0,      88459982, [ 9.20032180e-06, -2.84379534e-06,  0.00000000e+00], [ 9.20032180e-06, -2.84379534e-06,  0.00000000e+00], 191,   0,  47754.77547755,
       nan, 0,  0.00000000e+00, 0.60512821, [2.880e-07, 2.880e-07, 2.880e-07], -2.72992425e-06, 9.32873000e-06,  0.00000000e+00,  0.00000000e+00, 0., -1.13871094e-07,
 -1.28408203e-07, 0., 1000.10001   ), (1,      89006469, [ 9.19750490e-06, -2.87824359e-06,  0.00000000e+00], [ 9.19750490e-06, -2.87824359e-06,  0.00000000e+00], 227
,  16,  75685.58806368,   5333.33333333, 0,  7.03125000e-02, 0.59030837, [2.880e-07, 2.880e-07, 2.880e-07], -2.84430902e-06, 9.20005812e-06,  0.00000000e+00,  0.00000
000e+00, 0., -3.39345703e-08, -2.55322266e-09, 0.,    0.        ), (2,      89268980, [ 9.19598929e-06, -2.84938952e-06,  0.00000000e+00], [ 9.19598929e-06, -2.84938952e-06,  0.00000000e+00], 113,  37,  56514.12853213,  18500.        , 0,  3.26324463e-01, 0.60504202, [1.512e-07, 1.512e-07, 1.512e-07], -2.84430902e-06, 9.20005812e-06, -3.39150003e-08, -2.55535439e-09, 0.,  2.88344971e-08, -1.51347656e-09, 0., 2500.31253907), (3,      89444092, [ 9.19754484e-06, -2.84625514e-06,  0.00000000e+00], [ 9.19754484e-06, -2.84625514e-06,  0.00000000e+00], 134,  15,  33508.37709427,   3750.        , 0,  1.11694336e-01, 0.57333333, [7.560e-08, 7.560e-08, 7.560e-08], -2.84430902e-06, 9.20005812e-06, -5.08727986e-09, -4.03714576e-09, 0.,  3.14115601e-09,  1.52385864e-09, 0., 3500.43755469), (4,      89794002, [ 9.19480421e-06, -2.84745348e-06,  0.00000000e+00], [ 9.19480421e-06, -2.84745348e-06,  0.00000000e+00], 163,  74,  32608.15203801,  14800.        , 0,  4.53918457e-01, 0.5398773 , [3.960e-08, 3.960e-08, 3.960e-08], -2.84430902e-06, 9.20005812e-06, -1.92328255e-09, -2.50337760e-09, 0., -1.22118530e-09, -2.75053711e-09, 0.,    0.        )], 0,  85, 2.24485005e+00,      85,  True, False, 255, 2),
       ([(0,      90231348, [           nan,            nan,            nan], [           nan,            nan,            nan],   0,   0,            nan,
       nan, 0, -3.05175781e-05,        nan, [2.880e-07, 2.880e-07, 2.880e-07], -2.84780267e-06, 9.19443312e-06,  0.00000000e+00,  0.00000000e+00, 0.,  0.00000000e+00,
  0.00000000e+00, 0., 1000.10001   ), (1,      90231482, [           nan,            nan,            nan], [           nan,            nan,            nan],   0
,   0,            nan,            nan, 0, -3.05175781e-05,        nan, [2.880e-07, 2.880e-07, 2.880e-07], -2.84780267e-06, 9.19443312e-06,  0.00000000e+00,  0.00000
000e+00, 0.,  0.00000000e+00,  0.00000000e+00, 0.,    0.        ), (2,      90231616, [           nan,            nan,            nan], [           nan,
   nan,            nan],   0,   0,            nan,            nan, 0, -3.05175781e-05,        nan, [1.512e-07, 1.512e-07, 1.512e-07], -2.84780267e-06, 9.19443312e-
06,  0.00000000e+00,  0.00000000e+00, 0.,  0.00000000e+00,  0.00000000e+00, 0., 2500.31253907), (3,      90231750, [ 9.19323572e-06, -2.84738854e-06,  0.00000000e+00]
, [ 9.19323572e-06, -2.84738854e-06,  0.00000000e+00], 118,  34,  39343.16912561,  11333.33333333, 0,  2.87414551e-01, 0.54615385, [7.560e-08, 7.560e-08, 7.560e-08],
 -2.84780267e-06, 9.19443312e-06,  0.00000000e+00,  0.00000000e+00,  0.00000000e+00, 0.,  4.14129639e-10, -1.19739990e-09, 0., 3500.43755469), (4,      90494261, [ 9.19419659e-06, -2.84644
64290e-06,  0.00000000e+00], [ 9.19419659e-06, -2.84644290e-06,  0.00000000e+00], 163,  81,  27173.46003167,  13500.        , 0,  4.96856689e-01, 0.52760736, [3.960
e-08, 3.960e-08, 3.960e-08], -2.84780267e-06, 9.19443312e-06,  4.16481912e-10, -1.18701399e-09, 0.,  2.74328613e-09,  9.50482178e-10, 0.,    0.        )], 0,  85, 2.26235652e+00,      85,  True, False, 255, 2),
       ([(0,      93567627, [ 6.49866944e-06, -3.91167479e-06,  0.00000000e+00], [ 6.49866944e-06, -3.91167479e-06,  0.00000000e+00], 165,   0,  82508.25082508,
       nan, 0,  0.00000000e+00, 0.67065868, [2.880e-07, 2.880e-07, 2.880e-07], -4.07989745e-06, 6.67882765e-06,  0.00000000e+00,  0.00000000e+00, 0.,  1.68222656e-07,
 -1.80158203e-07, 0., 1000.10001   ), (1,      93841025, [ 6.46988966e-06, -3.95146385e-06,  0.00000000e+00], [ 6.46988966e-06, -3.95146385e-06,  0.00000000e+00], 274
, 155, 274068.51712928, 155000.        , 0,  5.62896729e-01, 0.60583942, [2.880e-07, 2.880e-07, 2.880e-07], -3.91168455e-06, 6.49812452e-06,  0.00000000e+00,  0.00000
000e+00, 0., -3.97792969e-08, -2.82348633e-08, 0.,    0.        ), (2,      93928741, [ 6.45020677e-06, -3.98208635e-06,  0.00000000e+00], [ 6.45020677e-06, -3.98208635e-06,  0.00000000e+00], 280, 112, 280070.01750438, 112000.        , 0,  3.99902344e-01, 0.5795053 , [1.512e-07, 1.512e-07, 1.512e-07], -3.91168455e-06, 6.49812452e-06, -3.97631319e-08, -2.82287116e-08, 0.,  -3.06386719e-08, -1.96890381e-08, 0., 2500.31253907), (3,      94016457, [ 6.45068519e-06, -3.98938724e-06,  0.00000000e+00]
, [ 6.45068519e-06, -3.98938724e-06,  0.00000000e+00], 199,  87, 199049.76244061,  87000.        , 0,  4.35424805e-01, 0.60098522, [7.560e-08, 7.560e-08, 7.560e-08],
 -3.91168455e-06, 6.49812452e-06, -7.04052393e-08, -4.79192157e-08, 0., -7.29744873e-09,  4.79882812e-10, 0., 3500.43755469), (4,      94104175, [ 6.44684485e-06, -3.99075328e-06,  0.00000000e+00], [ 6.44684485e-06, -3.99075328e-06,  0.00000000e+00], 168, 105,  84021.00525131,  52500.        , 0,  6.24847412e-01, 0.64606742, [3.960
e-08, 3.960e-08, 3.960e-08], -3.91168455e-06, 6.49812452e-06, -7.76898370e-08, -4.74378589e-08, 0., -1.37889404e-09, -3.84180908e-09, 0., 5000.62507813)], 0,  87, 2.35260437e+00,      87,  True, False, 255, 2),
       ...,
       ([(0, 2883933185356, [           nan,            nan,            nan], [           nan,            nan,            nan],   0,   0,            nan,
       nan, 0, -3.05175781e-05,        nan, [2.880e-07, 2.880e-07, 2.880e-07], -4.57367997e-07, 3.65064404e-06,  0.00000000e+00,  0.00000000e+00, 0.,  0.00000000e+00,
  0.00000000e+00, 0., 1000.10001   ), (1, 2883933185490, [           nan,            nan,            nan], [           nan,            nan,            nan],   0
,   0,            nan,            nan, 0, -3.05175781e-05,        nan, [2.880e-07, 2.880e-07, 2.880e-07], -4.57367997e-07, 3.65064404e-06,  0.00000000e+00,  0.00000
```

```
[
    (
        "itr",
        [
            ("itr", "<i4"),
            ("tic", "<u8"),
            ("loc", "<f8", (3,)),
            ("lnc", "<f8", (3,)),
            ("eco", "<i4"),
            ("ecc", "<i4"),
            ("efo", "<f8"),
            ("efc", "<f8"),
            ("sta", "<i4"),
            ("cfr", "<f8"),
            ("dcr", "<f8"),
            ("ext", "<f8", (3,)),
            ("gvy", "<f8"),
            ("gvx", "<f8"),
            ("eoy", "<f8"),
            ("eox", "<f8"),
            ("dmz", "<f8"),
            ("lcy", "<f8"),
            ("lcx", "<f8"),
            ("lcz", "<f8"),
            ("fbg", "<f8"),
        ],
        (n_iters,),
    ),
    ("sqi", "<u4"),
    ("gri", "<u4"),
    ("tim", "<f8"),
    ("tid", "<i4"),
    ("vld", "?"),
    ("act", "?"),
    ("dos", "<i4"),
    ("sky", "<i4"),
]
```

iteration?

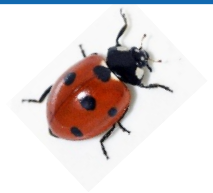
location?

timestamp?

trace id?

valid?

Testing phase



Slide courtesy: Javier Casares Arias

Expert-driven projects :: pyMINFLUX

Now, we bought the microscope

Abberior IMSPECTOR

Acquisition

Data export

Developed for STED

Pixel-based

MINFLUX data visualization requires "binning"

Licensed

Viewer version available

Windows-only

+

ParaView

3D rendering software

Built-in Imspector bridge

Tools + Plugins

Open-source

Cross-platform

Missing features

Click-and-inspect

Localization precision

Fluorophore unmixing

Filtering options

Metrics comparison

Not facility-friendly

Fully open raw data format

NumPy, MATLAB or JSON

Documentation from Abberior

Production

Expert-driven projects :: pyMINFLUX

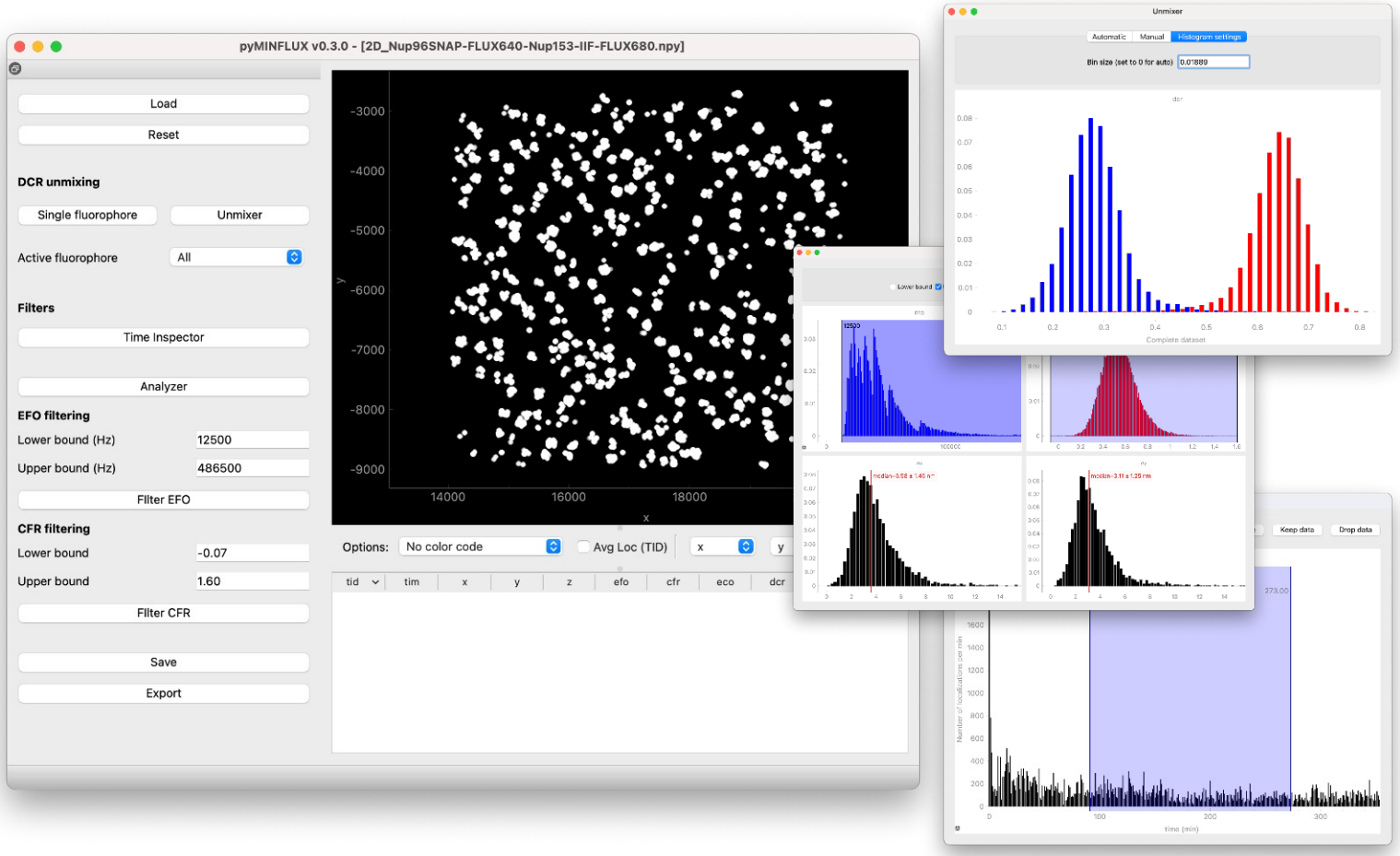
https://github.com/bsse-scf/pyMINFLUX

pyMINFLUX

Tailored for MINFLUX data

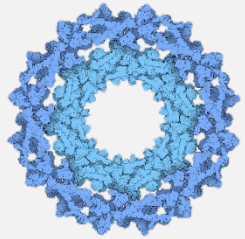
Open-access and cross-platform
- GUI-Independent API

Visualization, filtering and analysis tools
- Selection tool
- Data viewer
- Customizable scatter plot
- 2-Color MINFLUX unmixing
- Localization precision calculation
- Manual and automatic filtering
- Fourier-ring correlation analysis
- Integration with Paraview
- Custom .pmx format (metadata, processing parameters, …)

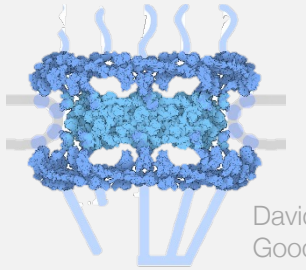


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



Top view



Side view

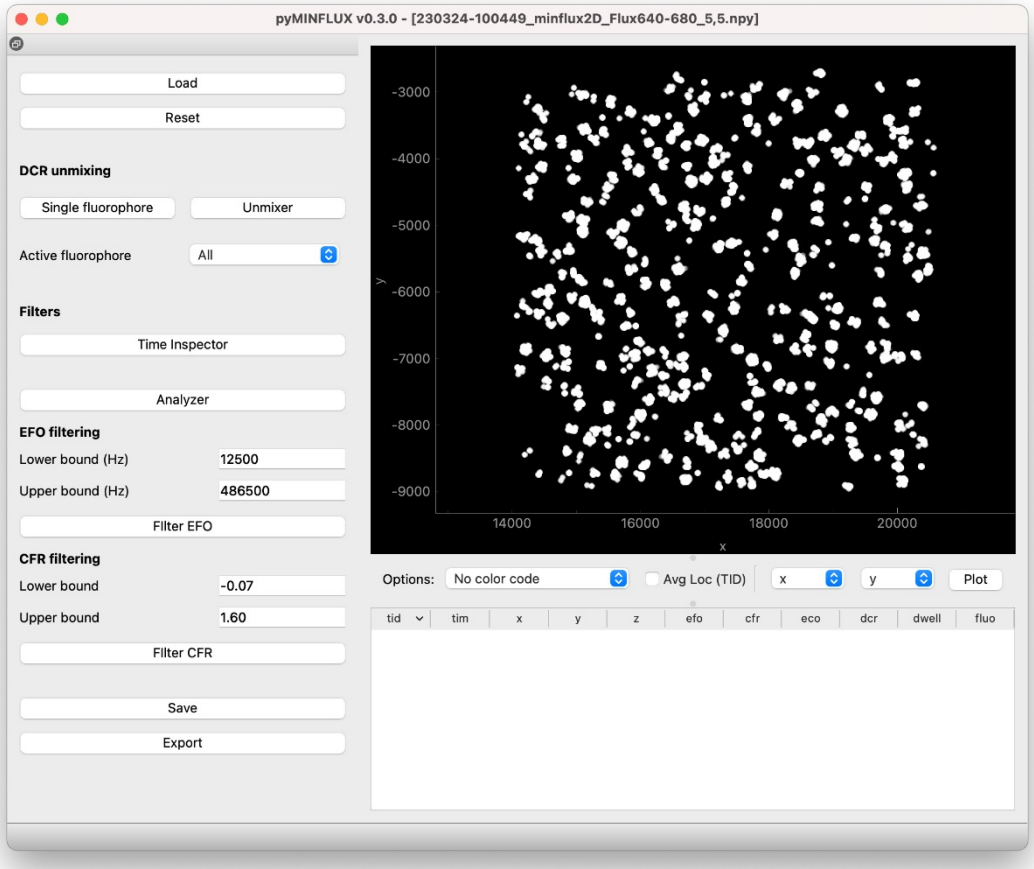
U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

pyMINFLUX workflow

Raw data import

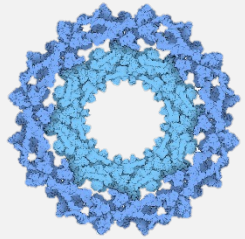
↓

Manual inspection

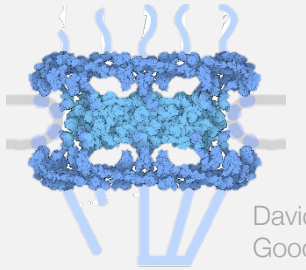


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



Top view



Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

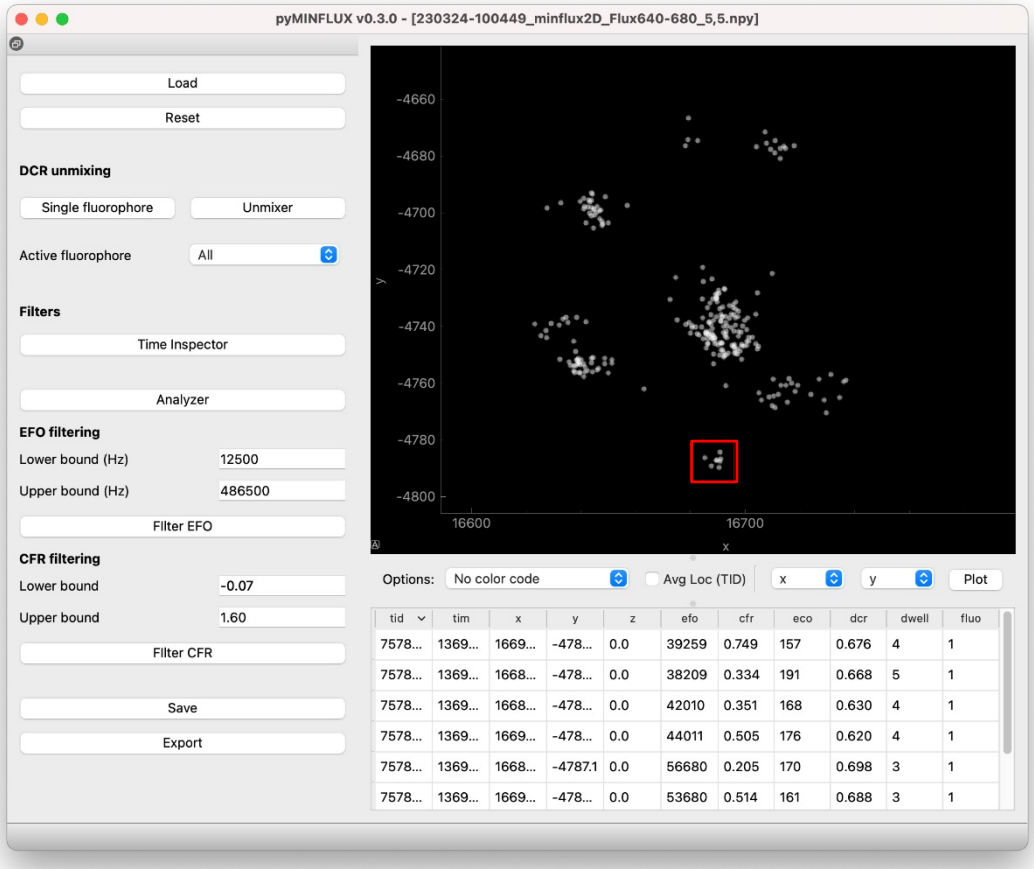
pyMINFLUX workflow

Raw data import

↓

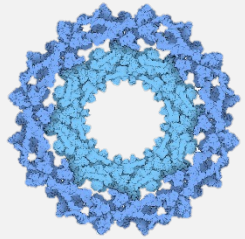
Manual inspection

Dataset navigation

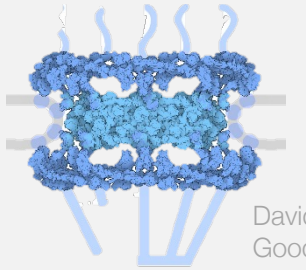


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



Top view



Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

pyMINFLUX workflow

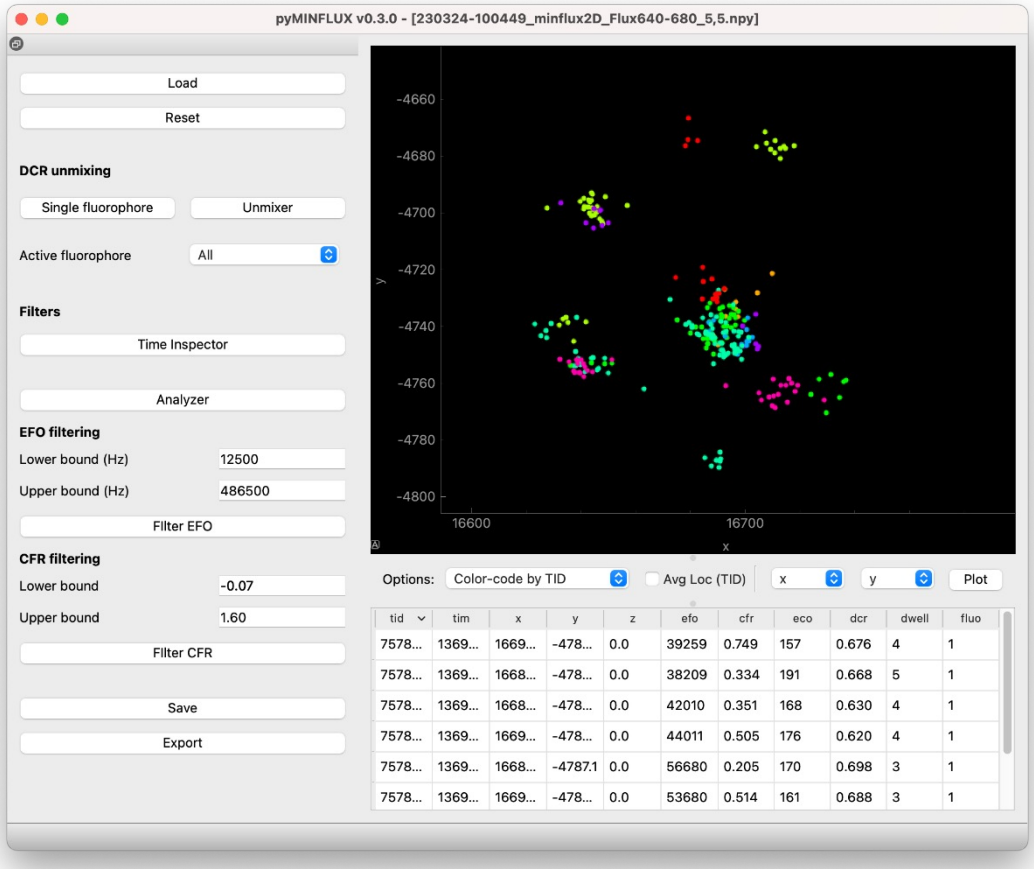
Raw data import

↓

Manual inspection

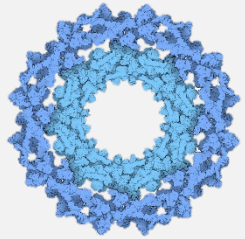
Dataset navigation

Trace coloring

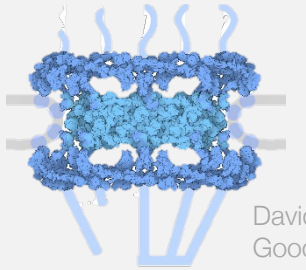


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



Top view



Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

pyMINFLUX workflow

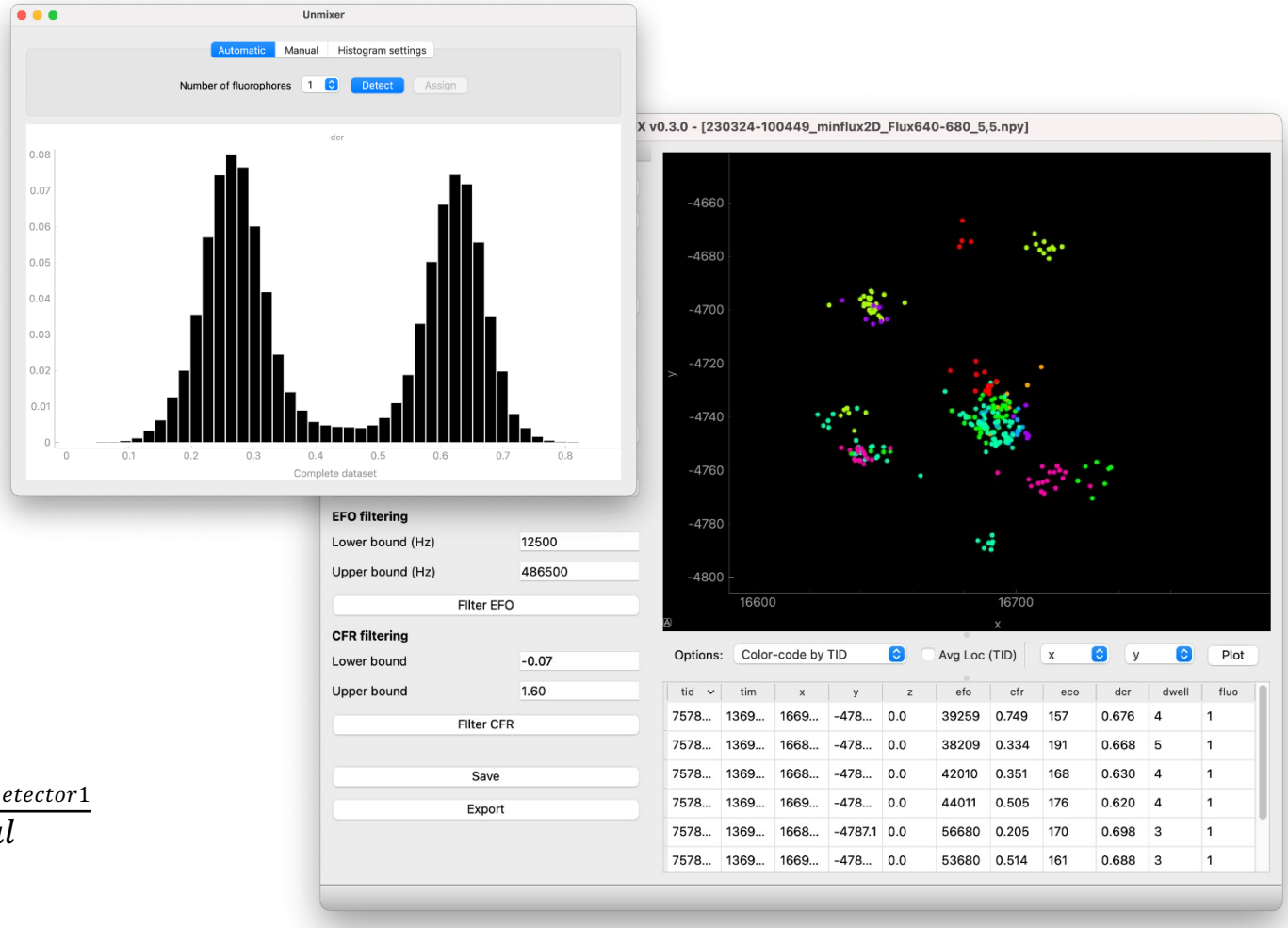
Raw data import

↓

Manual inspection

↓

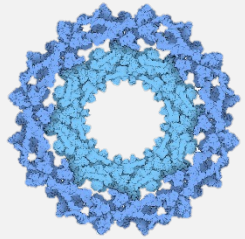
Color unmixing



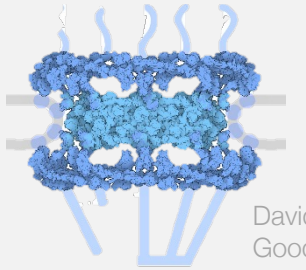
$$dcr = \frac{Photons_{Detector1}}{Total}$$

Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



Top view



Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

pyMINFLUX workflow

Raw data import

↓

Manual inspection

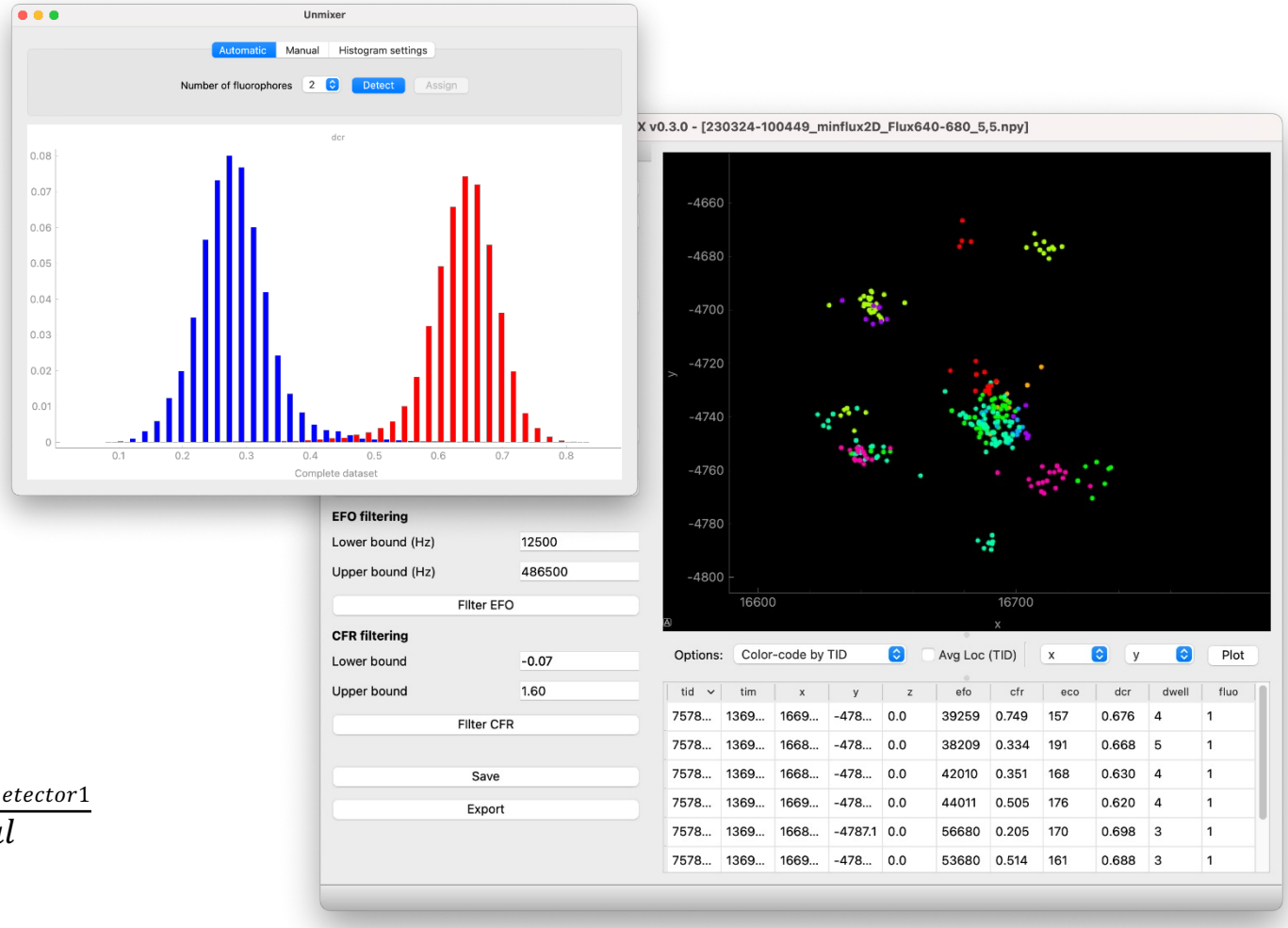
↓

Color unmixing

Automatic filtering

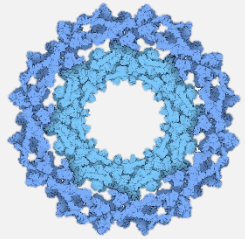
Gaussian mixture model

$$dcr = \frac{Photons_{Detector1}}{Total}$$

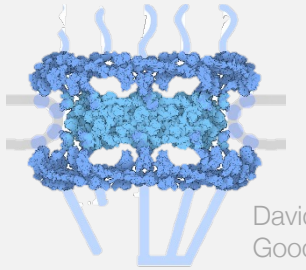


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



Top view

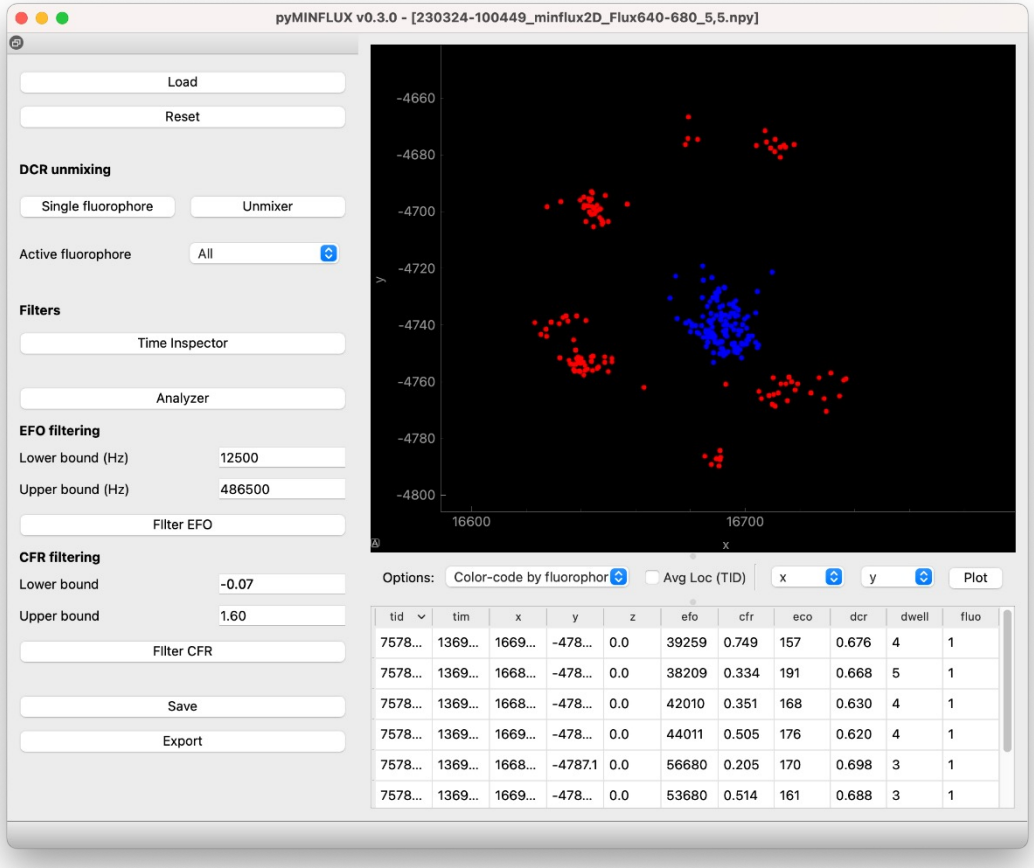


Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

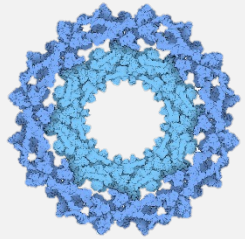
pyMINFLUX workflow

Raw data import
↓
Manual inspection
↓
Color unmixing

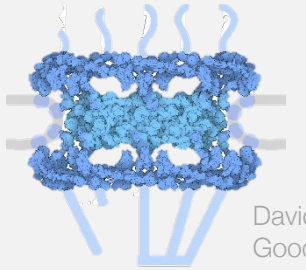


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



Top view



Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

pyMINFLUX workflow

Raw data import

↓

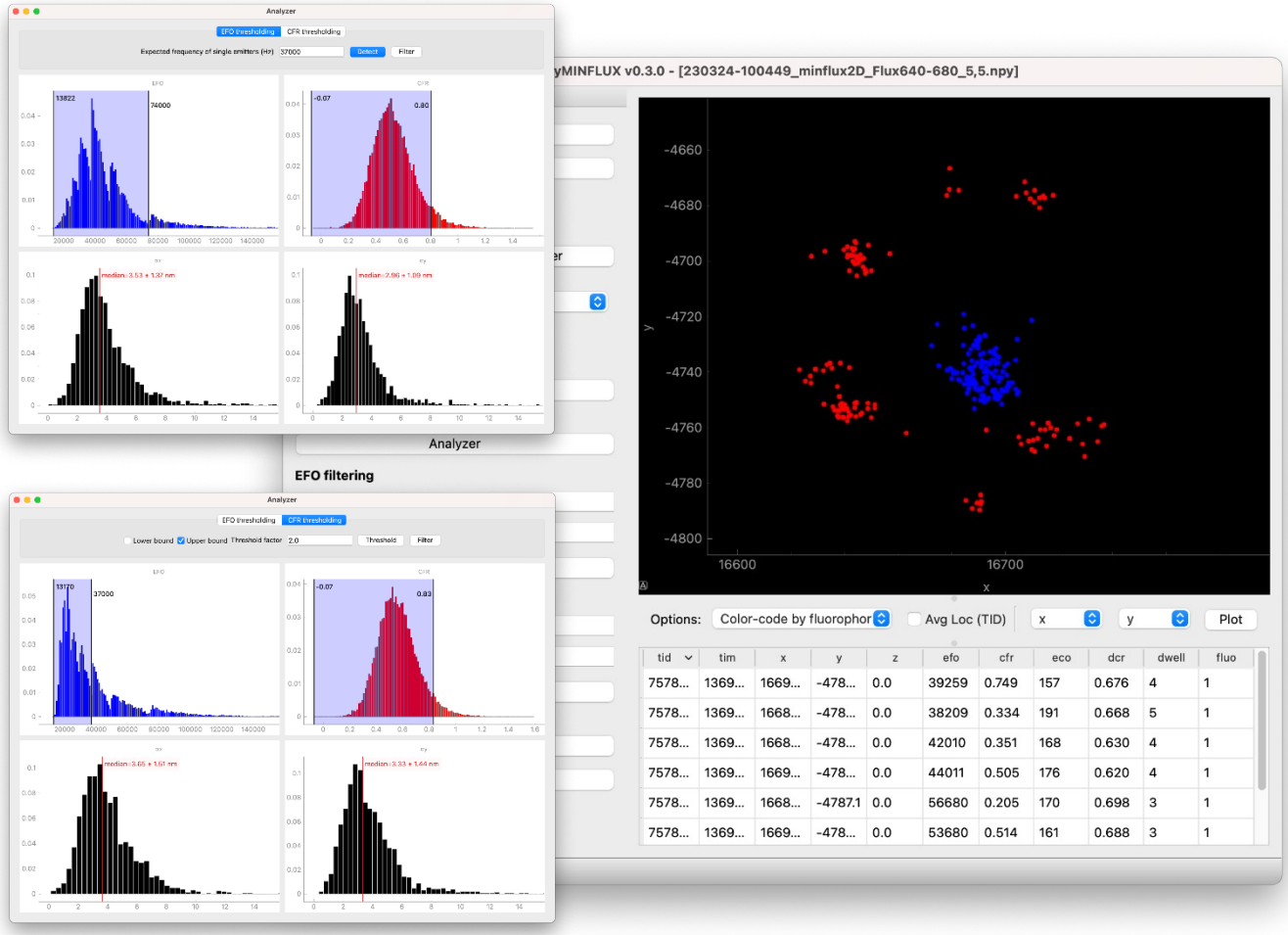
Manual inspection

↓

Color unmixing

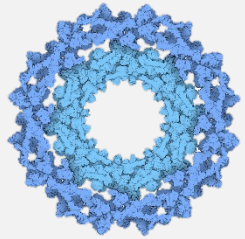
↓

Per-fluorophore filtering

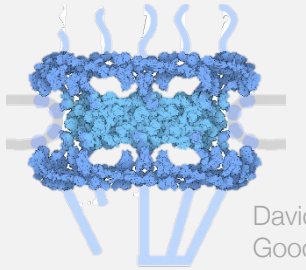


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



Top view



Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

pyMINFLUX workflow

Raw data import

↓

Manual inspection

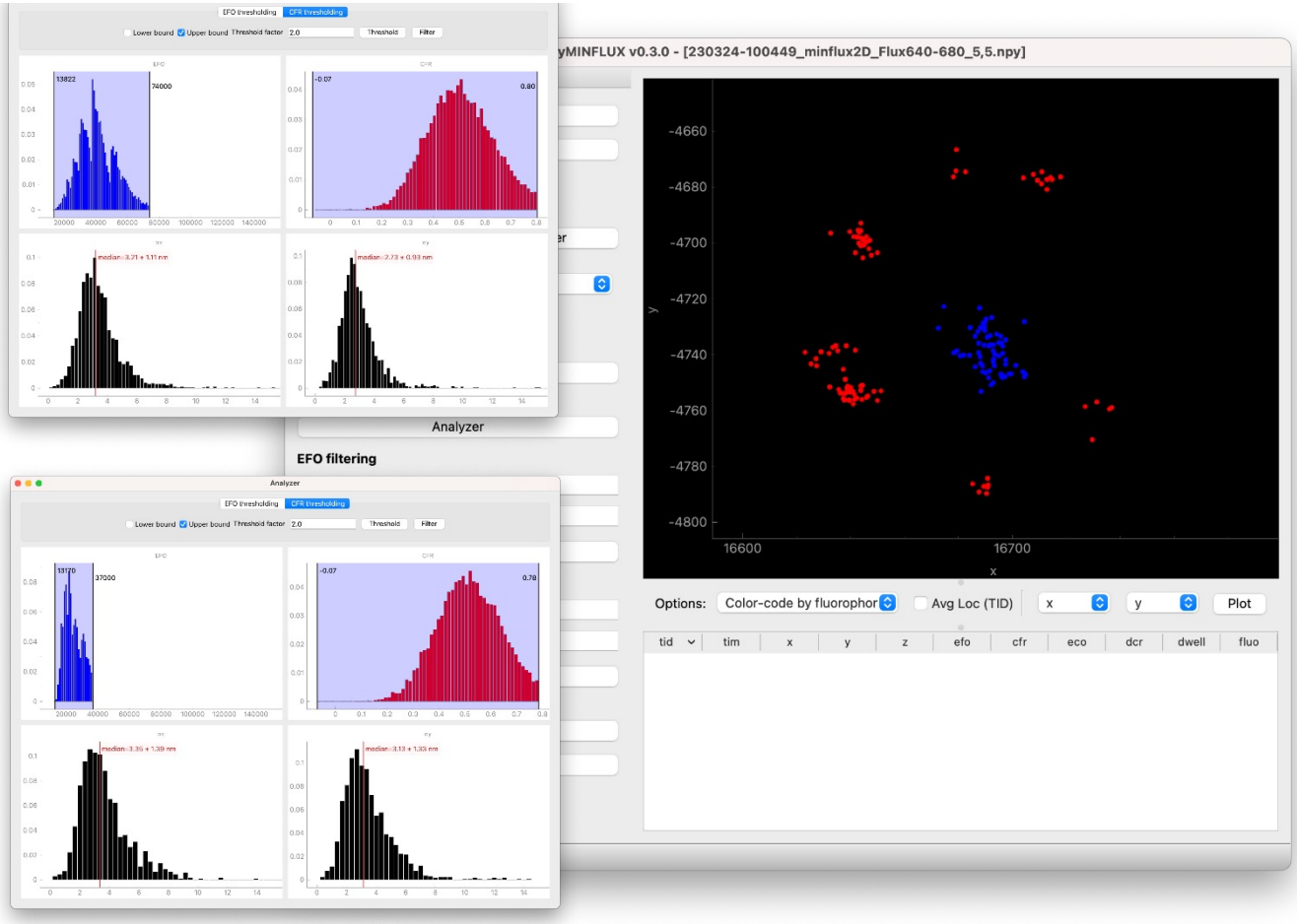
↓

Color unmixing

↓

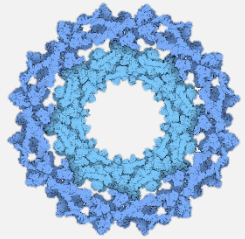
Per-fluorophore filtering

- Emission frequency
- Recentering estimator
- Localization precision

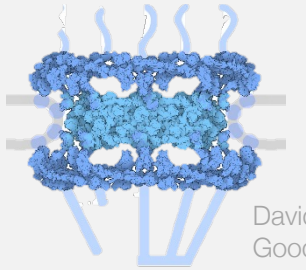


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



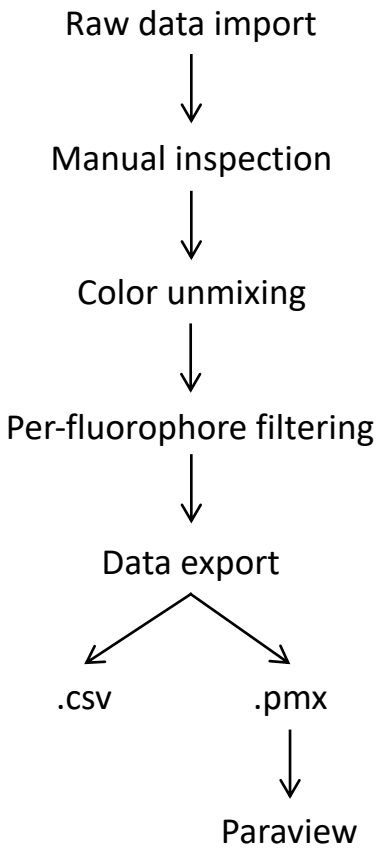
Top view

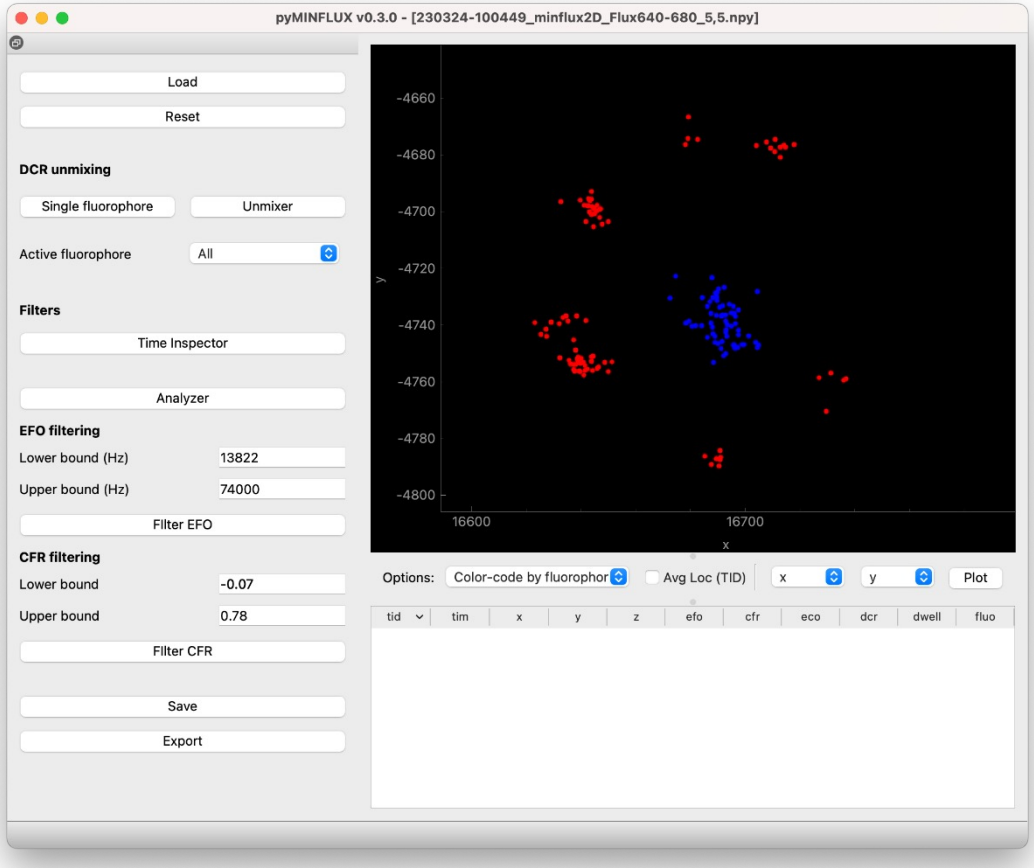


Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

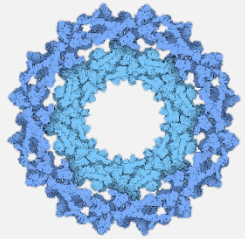
pyMINFLUX workflow



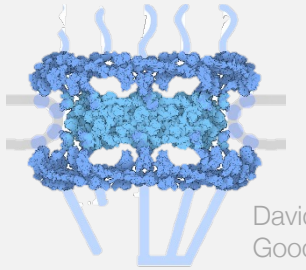


Expert-driven projects :: pyMINFLUX

QC sample
Nuclear pore complex



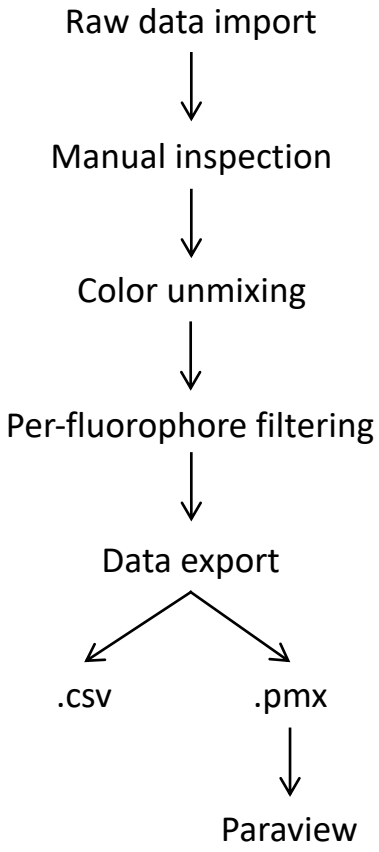
Top view

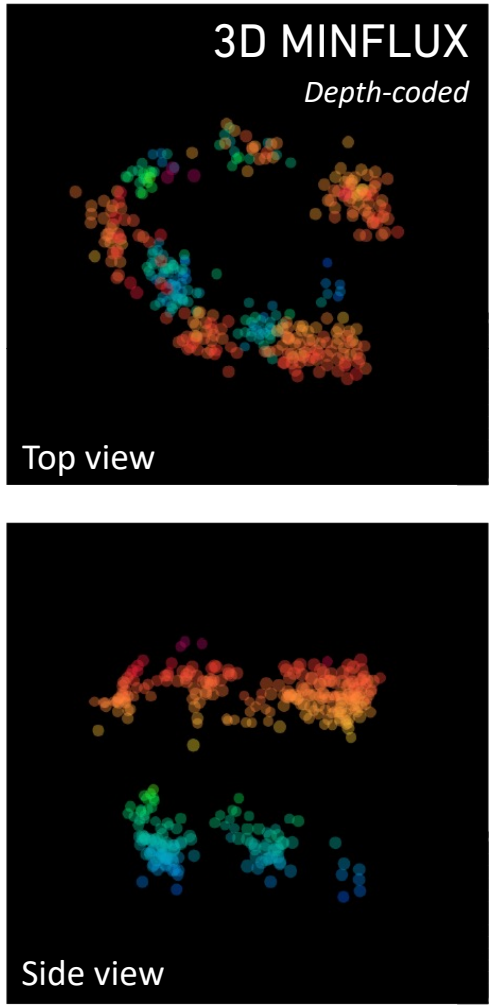


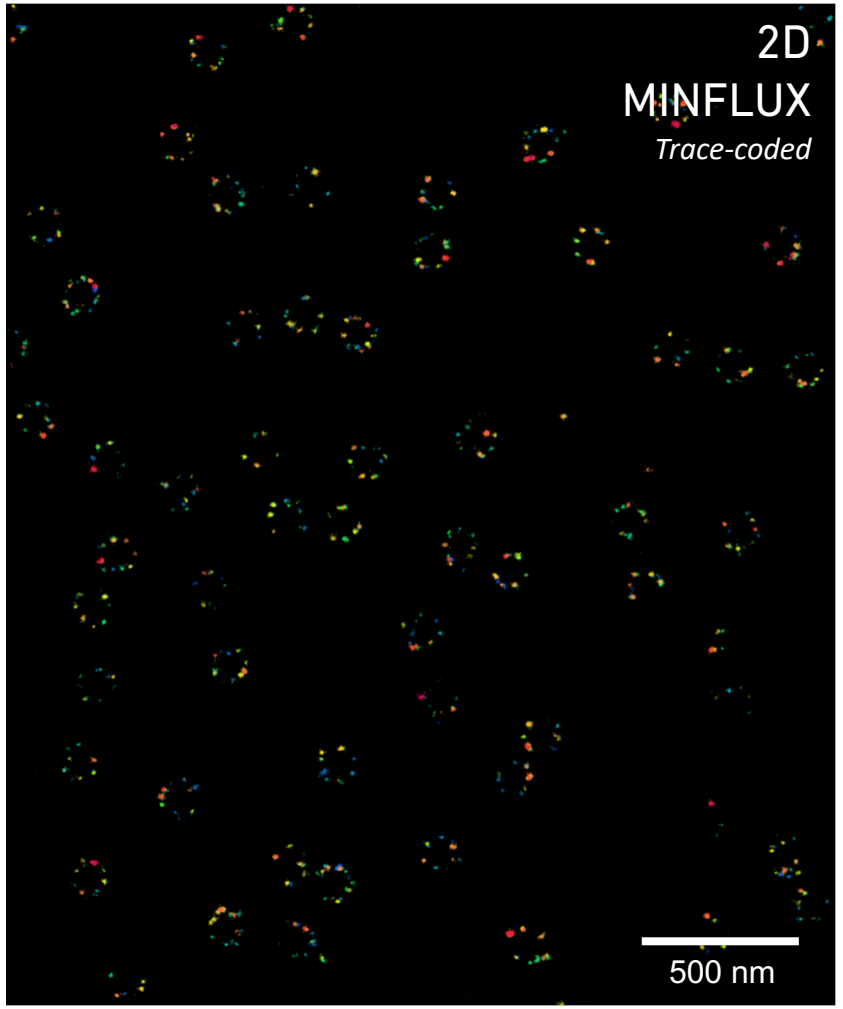
Side view

U2OS Nup96-SNAP cells
BG-Abberior Flux 640
Ms-Nup153 + 2^{ary} Flux 680
GLOX-MEA (10-15 mM)

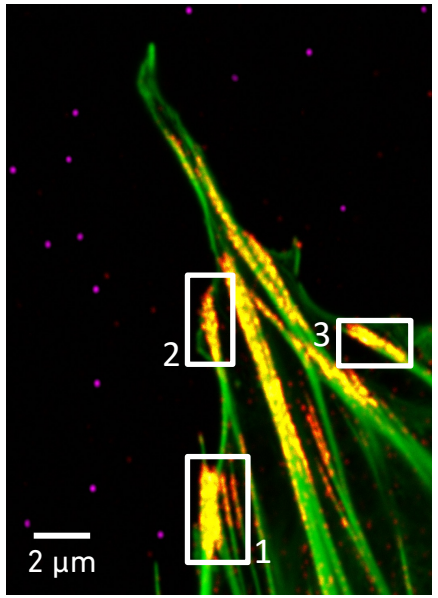
pyMINFLUX workflow







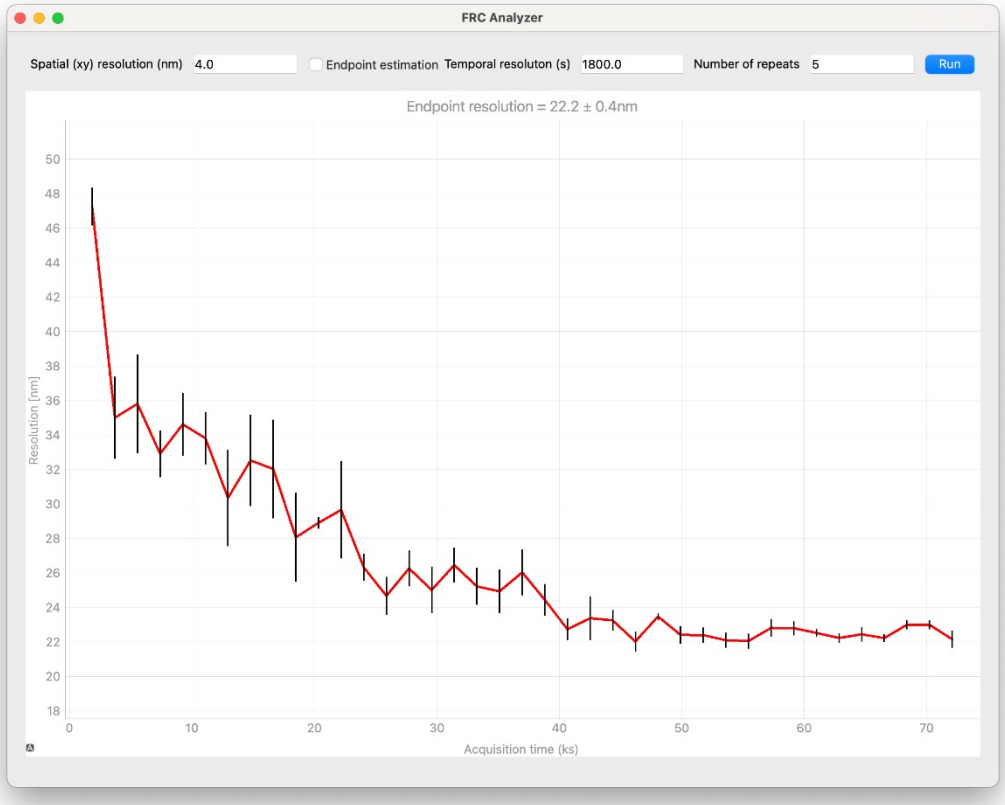
Expert-driven projects :: pyMINFLUX



MEFs
Phalloidin-AF488
Rat-Integrin + 2^{ary} AF568
Gold nanoparticles

Rb anti Paxillin
sdAb DNA-PAINT Atto 655

Fourier-ring correlation analysis (“endpoint indicator”)



Nico Strohmeyer, D-BSSE

Expert-driven projects :: pyMINFLUX

Expert contributions

Javier Casares Arias: MINFLUX specialist, designed a lot of controlled experiments to test filtering and analysis strategies
Aaron Ponti: development of pyMINFLUX

Users

- D-BSSE, Daniel Mueller Group (Michele Nava, Nico Strohmeyer, Matilde Lucioli, Krishna Kasuba)
- D-BSSE, Timm Schroeder Group (Germán Camargo)
- University of Basel, Thomas Ward Group (Michaela Slánská)
- University of Heidelberg (Charlotte Kaplan)

Summary

There are different classes of (image analysis) projects with different types of requirements and target audiences:

- **User-specific projects** usually have a small scope and are solved in a tight feedback loop with the end user.
- **General-purpose projects** target large audiences with less specific sets of functionality and often require larger development teams and better software engineering practices.
- **Expert-driven projects** require tight collaboration between experts with different sets of field knowledge with the goal of creating tools that appeal to reasonably large but niche audiences.

Acknowledgments

Thank you for your attention!

SCF Single Cell Facility

LAF Lab Automation Facility

Thomas Horn (head of SCF and LAF)

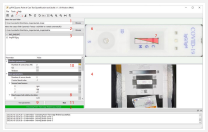
Microscopy
Erica Montani
Javier Casares Arias
Tom Lummen

Lab Automation
Sant Kumar
Daniel Gerngross

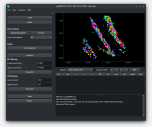
Flow Cytometry
Mariangela Di Tacchio
Aleksandra Gumienny
Chiara Cavallini

SpectraSorter


Todd Duncombe, D-BSSE

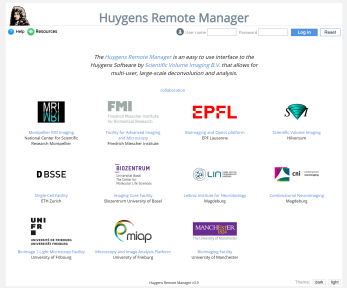
pyPOCQuant


Fabian Rudolf, D-BSSE and BAG
Andreas Cuny, D-BSSE

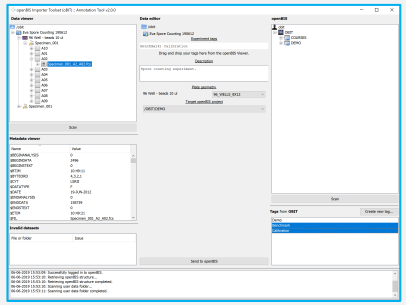
pyMINFLUX


Javier Casares Arias, D-BSSE

User-specific project
Gabriel Senn, D-BSSE

HRM


Volker Bäcker, Montpellier Rio Imaging
Daniel Sevilla, Scientific Volume Imaging
Niko Ehrenfeuchter, Biozentrum
Torsten Stöter, Leibniz Institute for Neurobiology
Felix Meyenhofer, University of Fribourg
Olivier Burri, EPFL
Egor Zindy University of Manchester.
Asheesh Gulati EPFL
Alessandra Griffa, EPFL
José Viña, Scientific Volume Imaging
Kevin Namink, Scientific Volume Imaging
Frederik Grüll Biozentrum

oBIT


Bernd Rinn, SIS
Chandrasekhar Ramakrishnan, SIS
Juan Fuentes Serna, SIS
Franz-Josef Elmer, SIS
Caterina Barillari, SIS
Piotr Kupczyk, SIS
Antti Luomi, SIS
Jakub Straszewski, SIS
Manuel Kohler, SIS
Vernon Bailey, ITSC
John Ryan, ITSC
Vincenzo Spanò, ITSC
Martin Fox, ITSC

D BSSE

All the users!