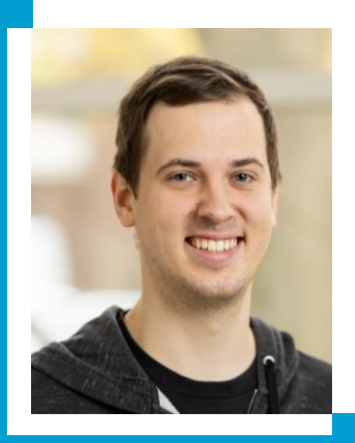# Introducing FISCAS – a software tool for compiling single cell MSI data
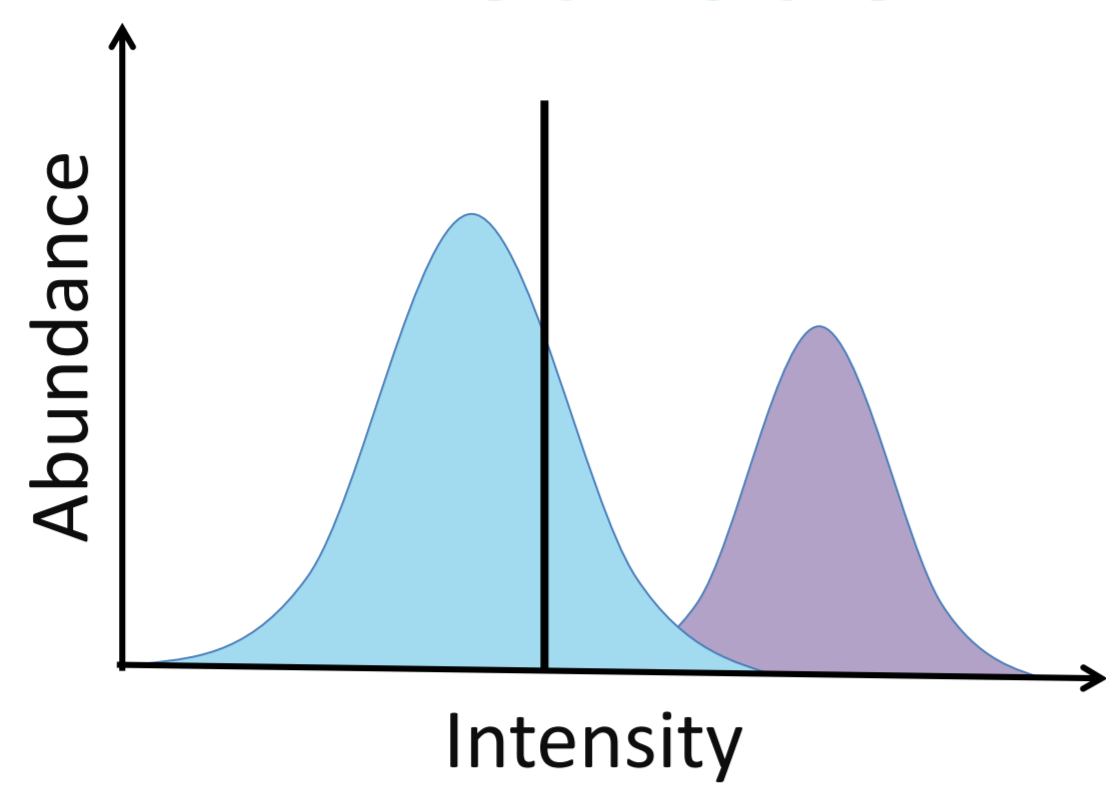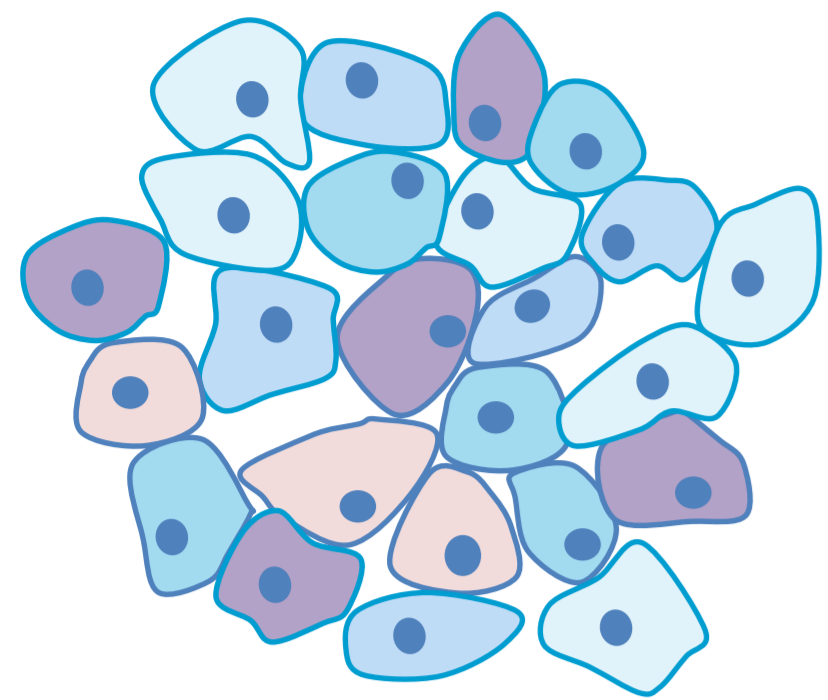
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1: Institute of Hygiene, University of Münster
2: Imaging Network, University of Münster
3: Bruker Daltonics GmbH & Co. KG, Bremen
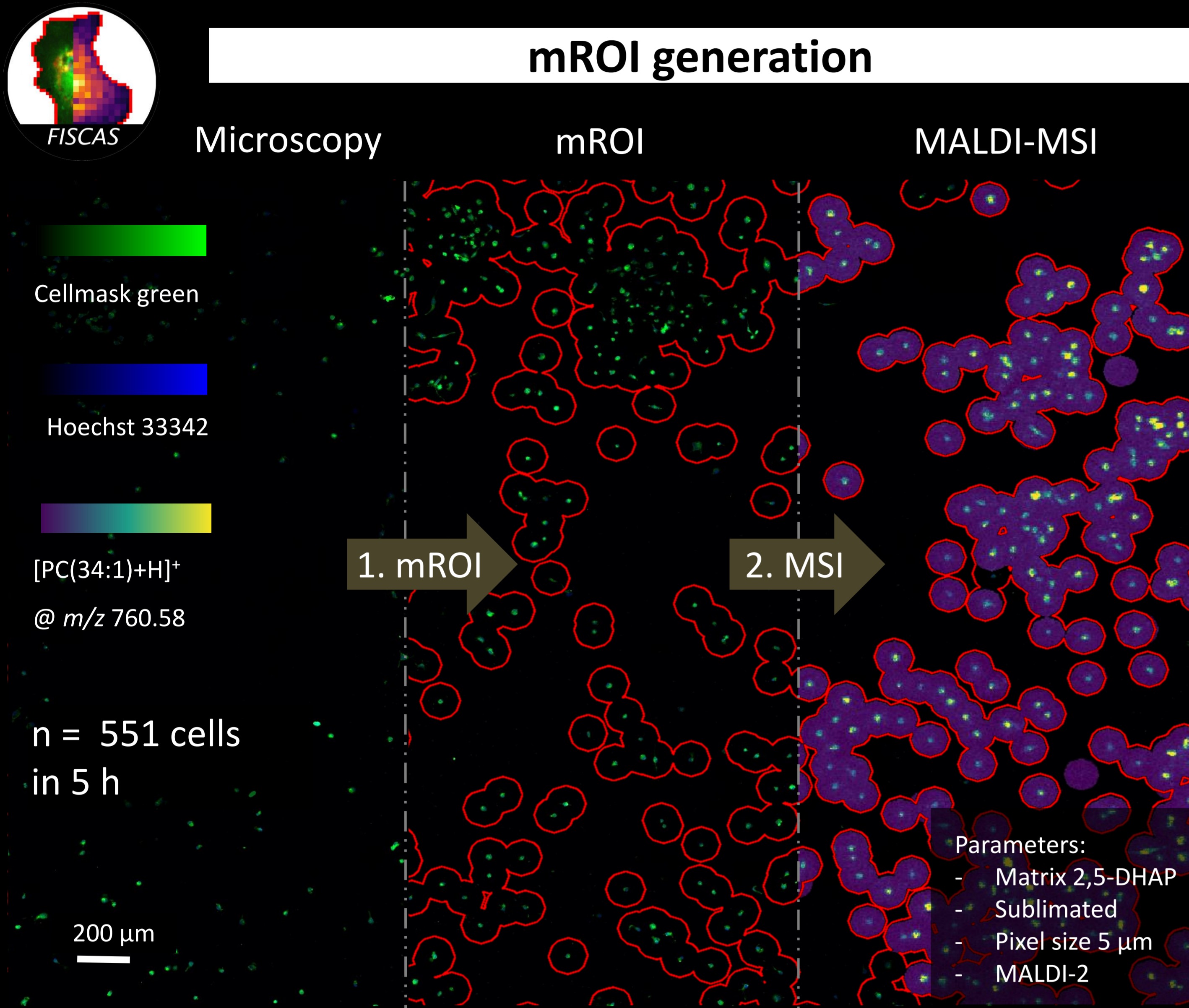
Universität Münster

Motivation

Why single-cell with MALDI-MSI?
- Cell populations are heterogeneous
- Bulk analysis methods are not capable of representing distributions

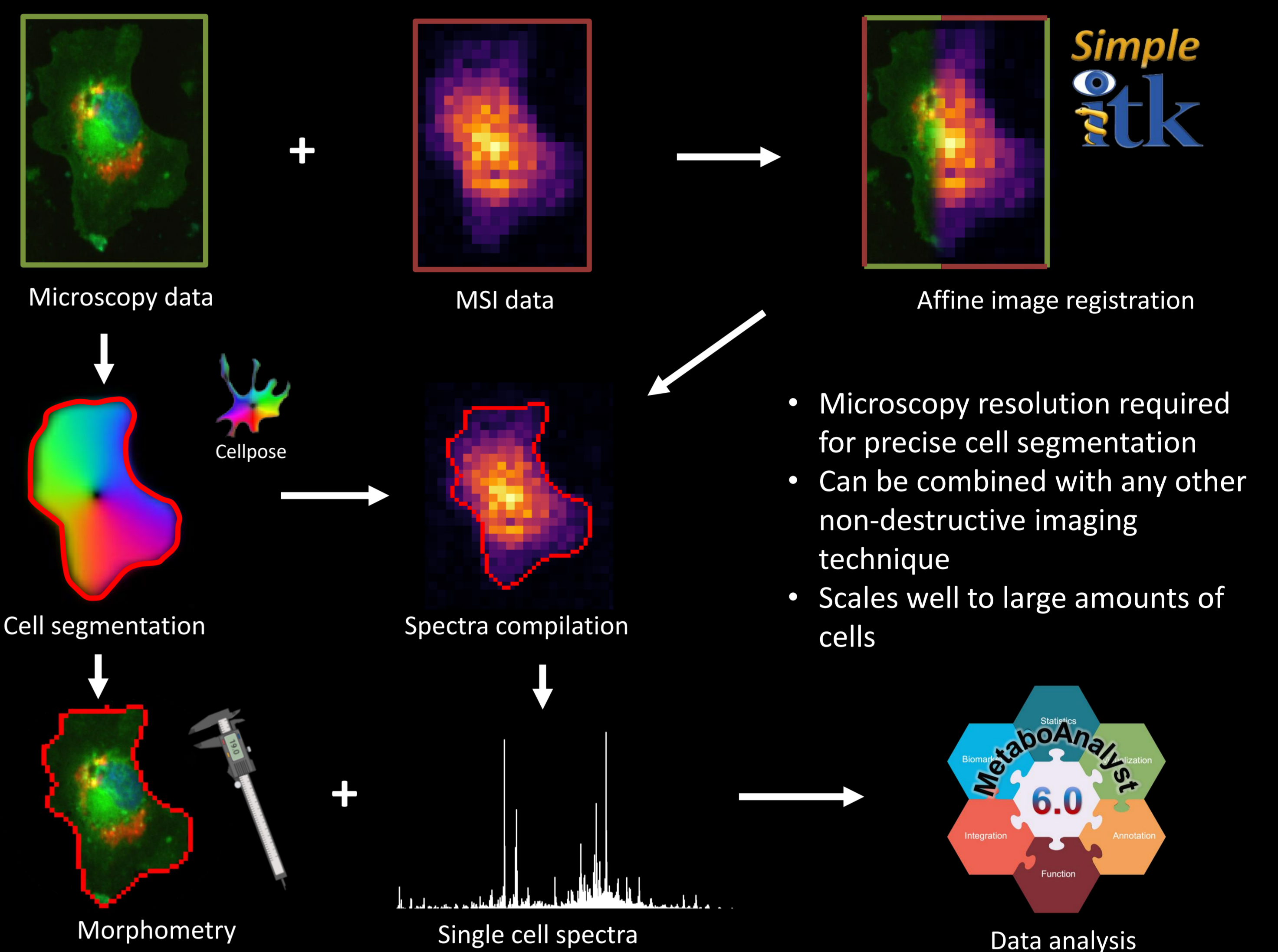Abundance / Intensity

FISCAS

Fluorescence Integrated Single Cell Analysis Script
- Tool to generate single cell data based on MSI and fluorescence images
- Saves measurement and work time by generation of measurement Regions of Interest (mROI) and compiling single cell spectra in an automated fashion
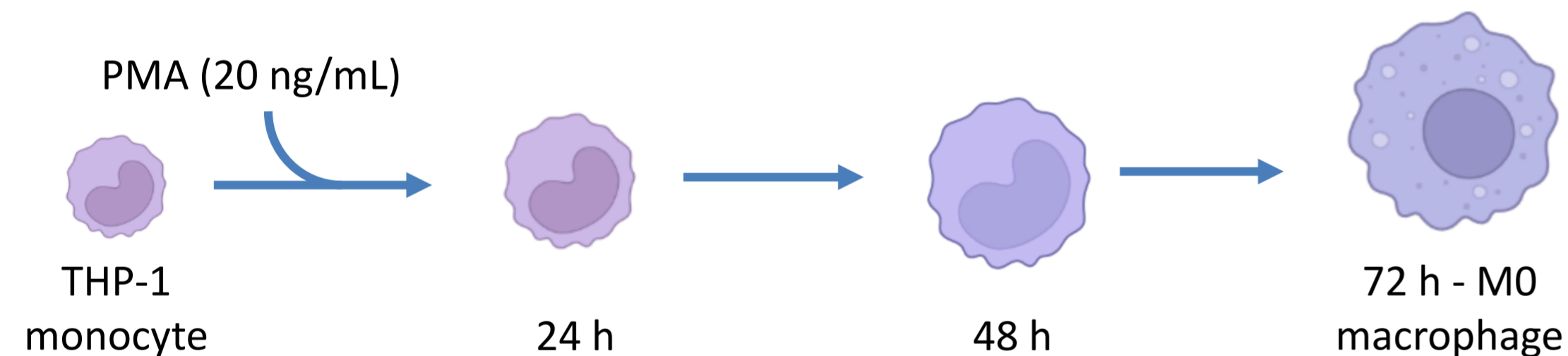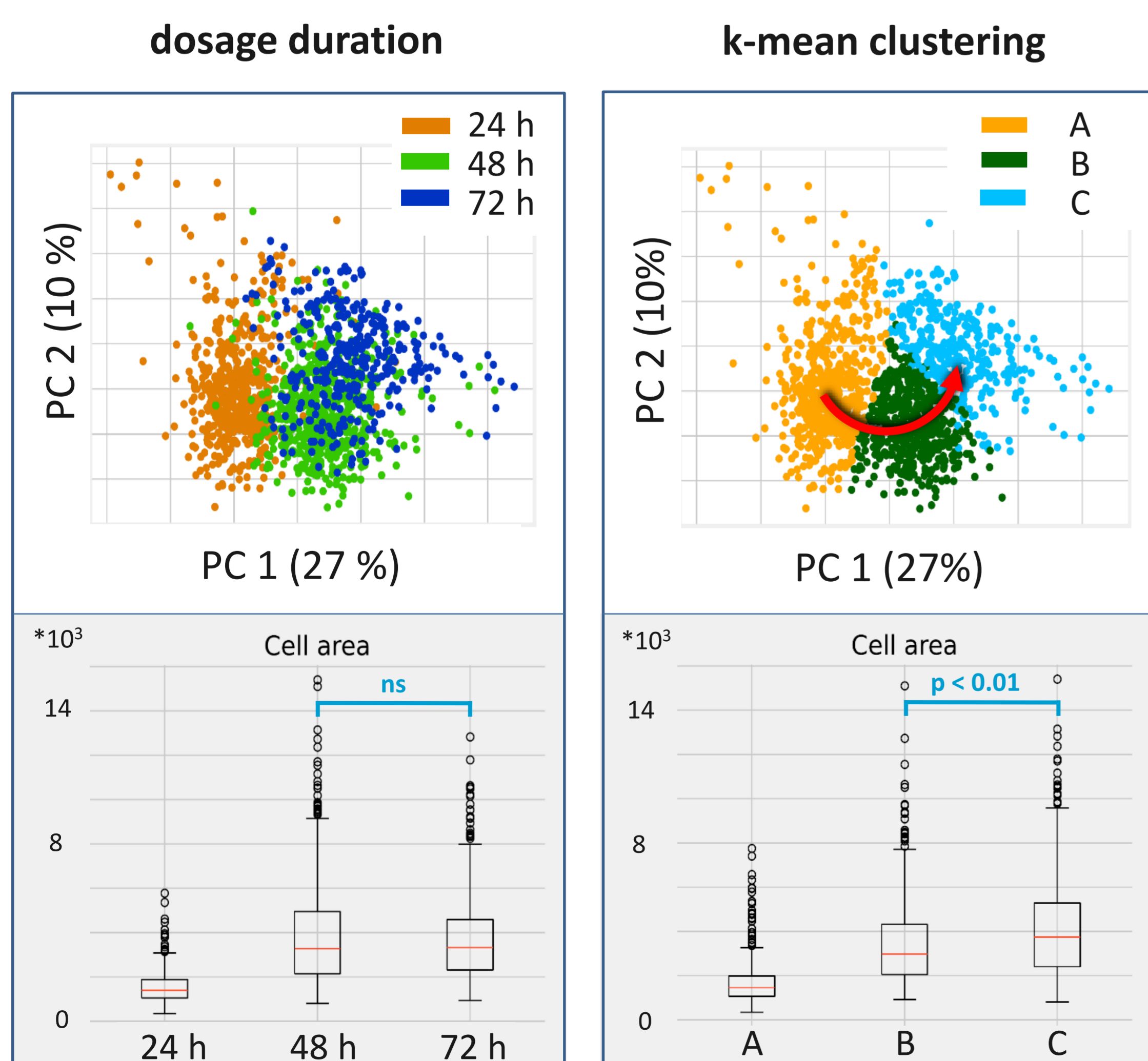
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mROI generation

FISCAS

Microscopy | mROI | MALDI-MSI

Cellmask green

Hoechst 33342

[PC(34:1)+H]$^+$
@ m/z 760.58

n = 551 cells in 5 h

200 µm

1. mROI → 2. MSI

Parameters:
- Matrix 2,5-DHAP
- Sublimated
- Pixel size 5 µm
- MALDI-2

Single cell spectra compilation

Microscopy data + MSI data → Affine image registration (Simple ITK)

Cell segmentation (Cellpose) → Spectra compilation

- Microscopy resolution required for precise cell segmentation
- Can be combined with any other non-destructive imaging technique
- Scales well to large amounts of cells

Morphometry + Single cell spectra → Data analysis (MetaboAnalyst 6.0)

Proof-of-Principle Study

PMA (20 ng/mL)

THP-1 monocyte → 24 h → 48 h → 72 h - M0 macrophage

- Apply FISCAS workflow to THP-1 cells during differentiation
- Sampling points after 24 h, 48 h, 72 h
- Individual cells differentiate at different rates
- Cell size (marker for differentiation) correlates better with a clustering based on MSI data then with the time of dosage

dosage duration

PC 2 (10 %) / PC 1 (27 %)

24 h / 48 h / 72 h

k-mean clustering

PC 2 (10%) / PC 1 (27%)

A / B / C

*10^3 Cell area — ns
14 / 8 / 0
24 h / 48 h / 72 h

*10^3 Cell area — p < 0.01
14 / 8 / 0
A / B / C

Current Development - GUI

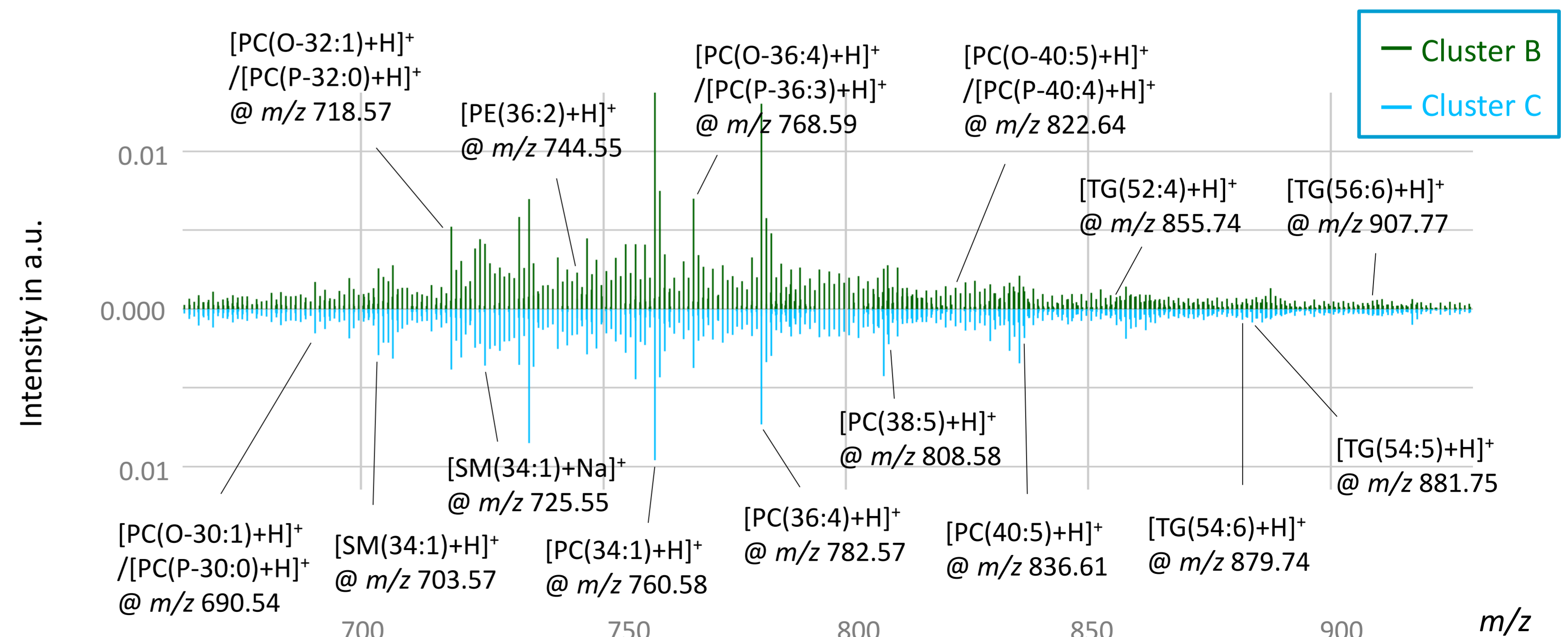Load Data → Registration → Segmentation → Spectra Generation

GUI for easy access
- Guided workflow
- Import/Export of external data at every step for easy integration into other pipelines
- Supports a broad range of input data
- State of the art algorithms
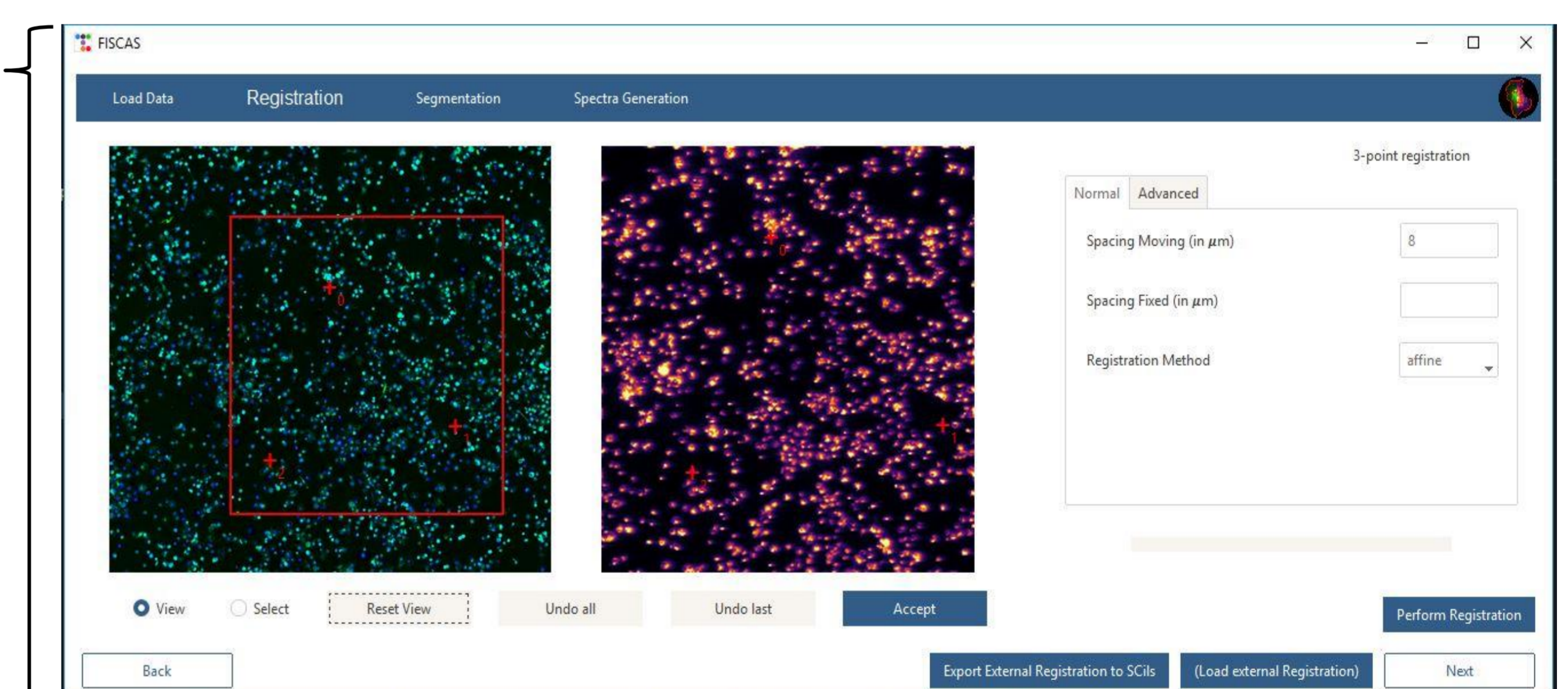
View of the registration window to co-register both imaging modalities using SimpleITK

[PC(O-32:1)+H]$^+$ /[PC(P-32:0)+H]$^+$ @ m/z 718.57

[PE(36:2)+H]$^+$ @ m/z 744.55

[PC(O-36:4)+H]$^+$ /[PC(P-36:3)+H]$^+$ @ m/z 768.59

[PC(O-40:5)+H]$^+$ /[PC(P-40:4)+H]$^+$ @ m/z 822.64

[TG(52:4)+H]$^+$ @ m/z 855.74

[TG(56:6)+H]$^+$ @ m/z 907.77

[PC(O-30:1)+H]$^+$ /[PC(P-30:0)+H]$^+$ @ m/z 690.54

[SM(34:1)+H]$^+$ @ m/z 703.57

[SM(34:1)+Na]$^+$ @ m/z 725.55

[PC(34:1)+H]$^+$ @ m/z 760.58

[PC(36:4)+H]$^+$ @ m/z 782.57

[PC(38:5)+H]$^+$ @ m/z 808.58

[PC(40:5)+H]$^+$ @ m/z 836.61

[TG(54:6)+H]$^+$ @ m/z 879.74

[TG(54:5)+H]$^+$ @ m/z 881.75

Intensity in a.u. / m/z

Cluster B
Cluster C