ScopePlot: Development of a single molecule fluorescent *in situ* hybridisation (smFISH) pipeline for the detection of host-pathogen interactions

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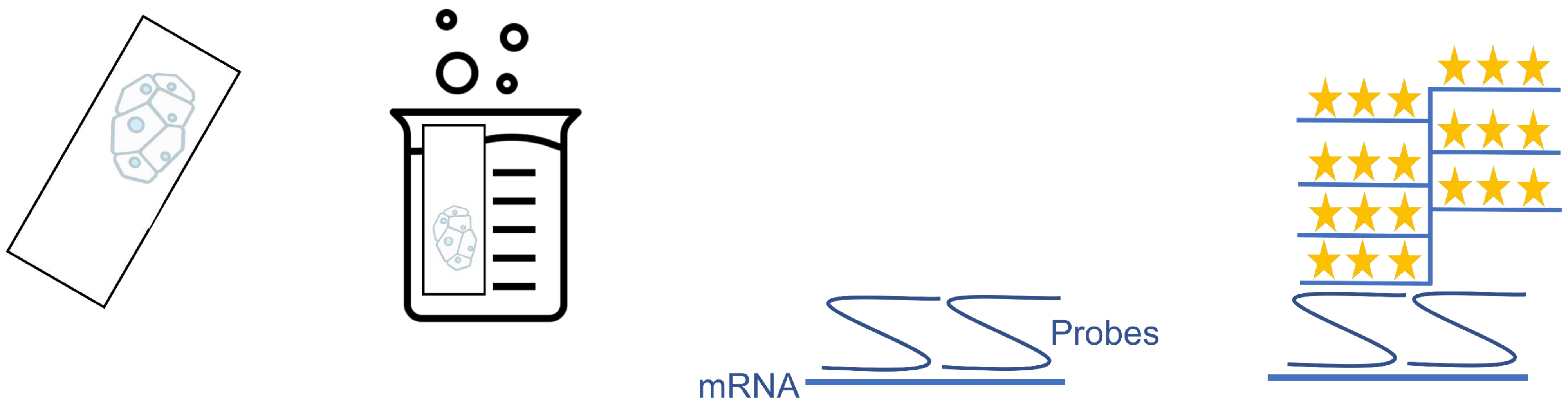
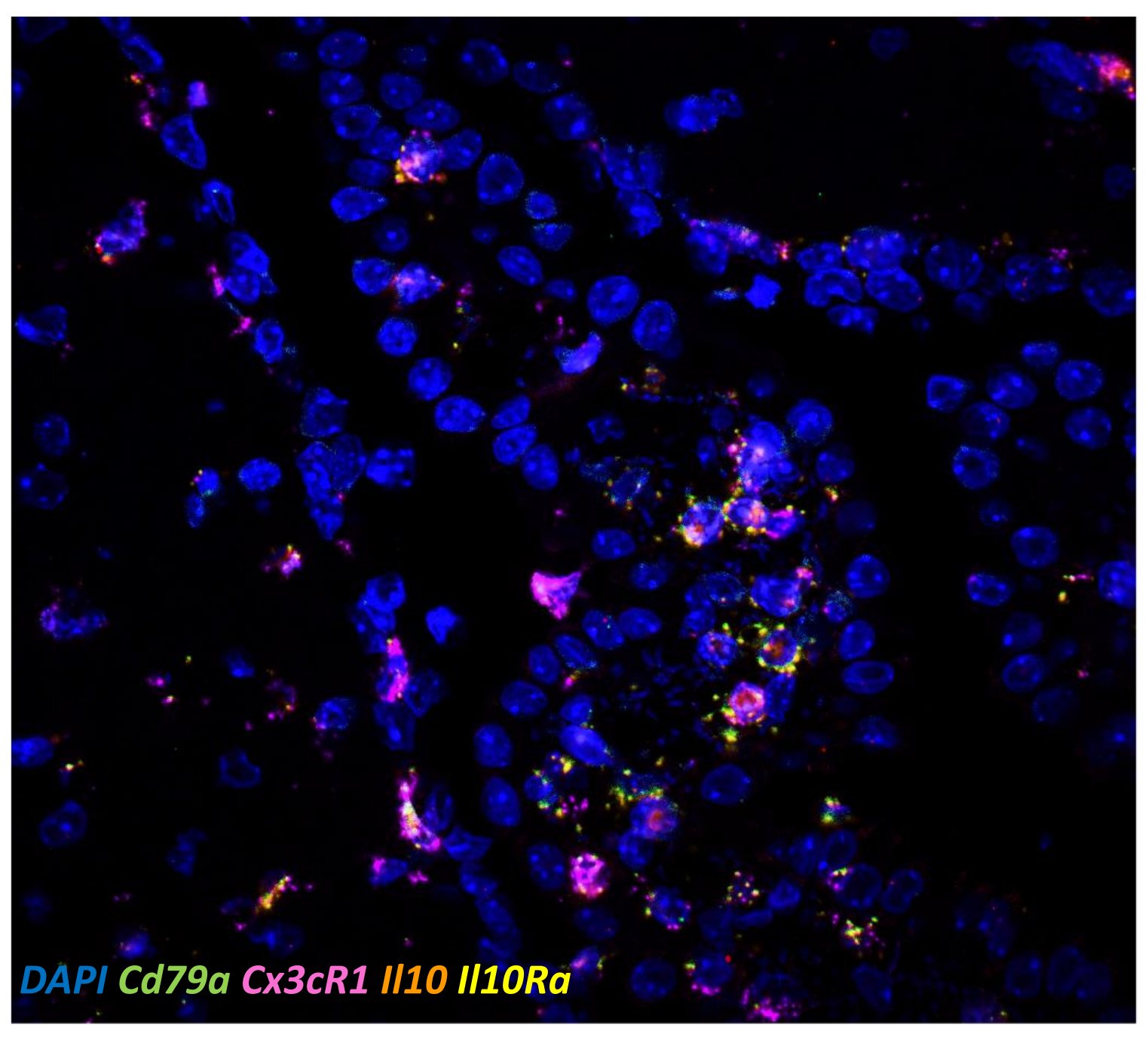
Context: We have previously predicted an interaction between brain-dwelling *Cd79a*⁺ B cells and *Cx3cr1*⁺ microglia, mediated by IL-10 signalling, that we validated using smFISH. However, a **significant hurdle in this process that had to be overcome was the accurate and unbiased data processing** of the outputs generated with standard imaging processing softwares such as QuPath.

Research Aim: To overcome this challenge, we developed **ScopePlot**: a rapid, automated, and user-friendly open-source workflow for the analysis of smFISH.

1 RNAScope Data Acquisition (3 days)





FFPE tissue section

Deparaffinisation
Rehydration
Antigen retrieval

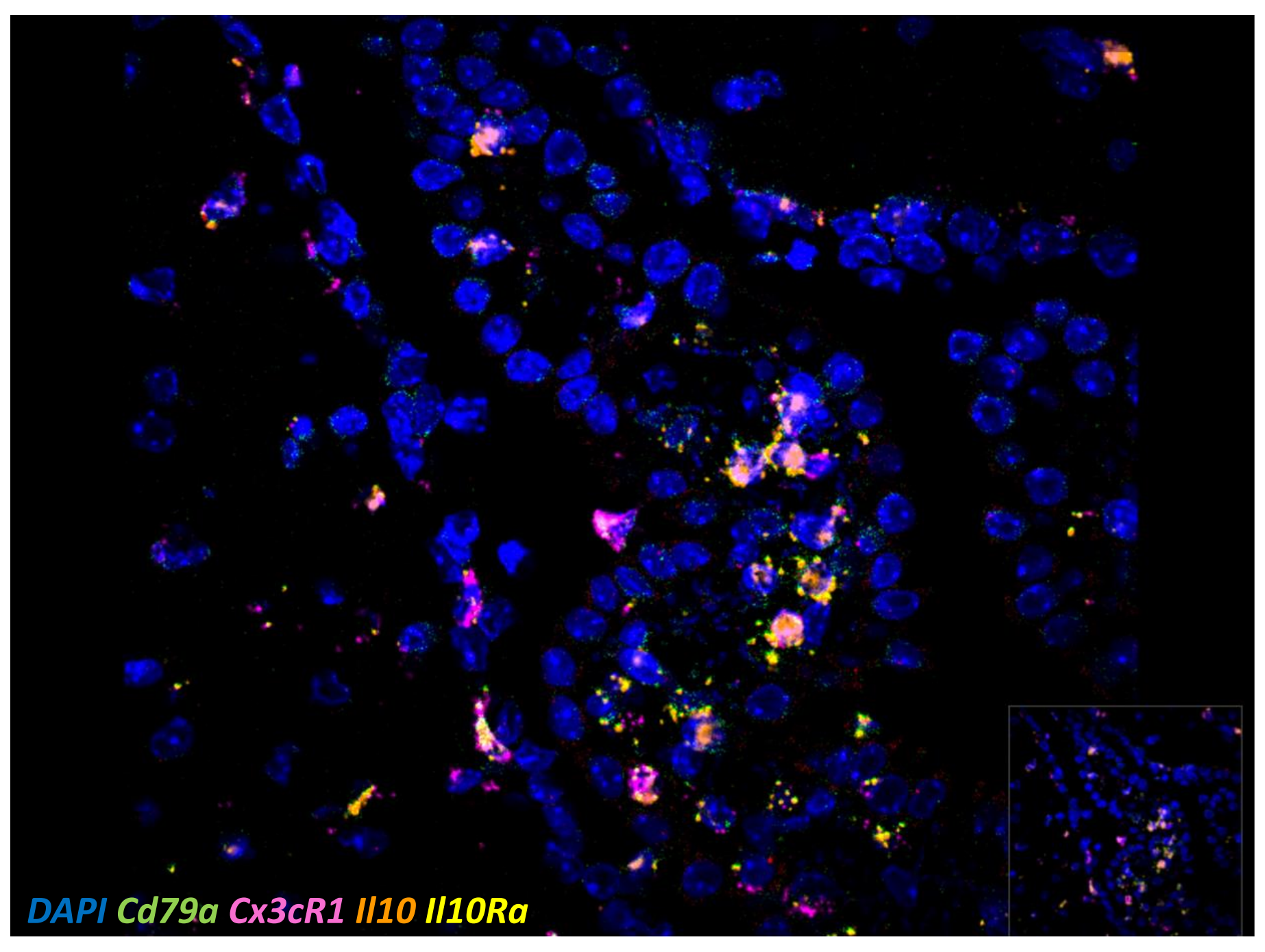
Probe hybridisation

Signal amplification

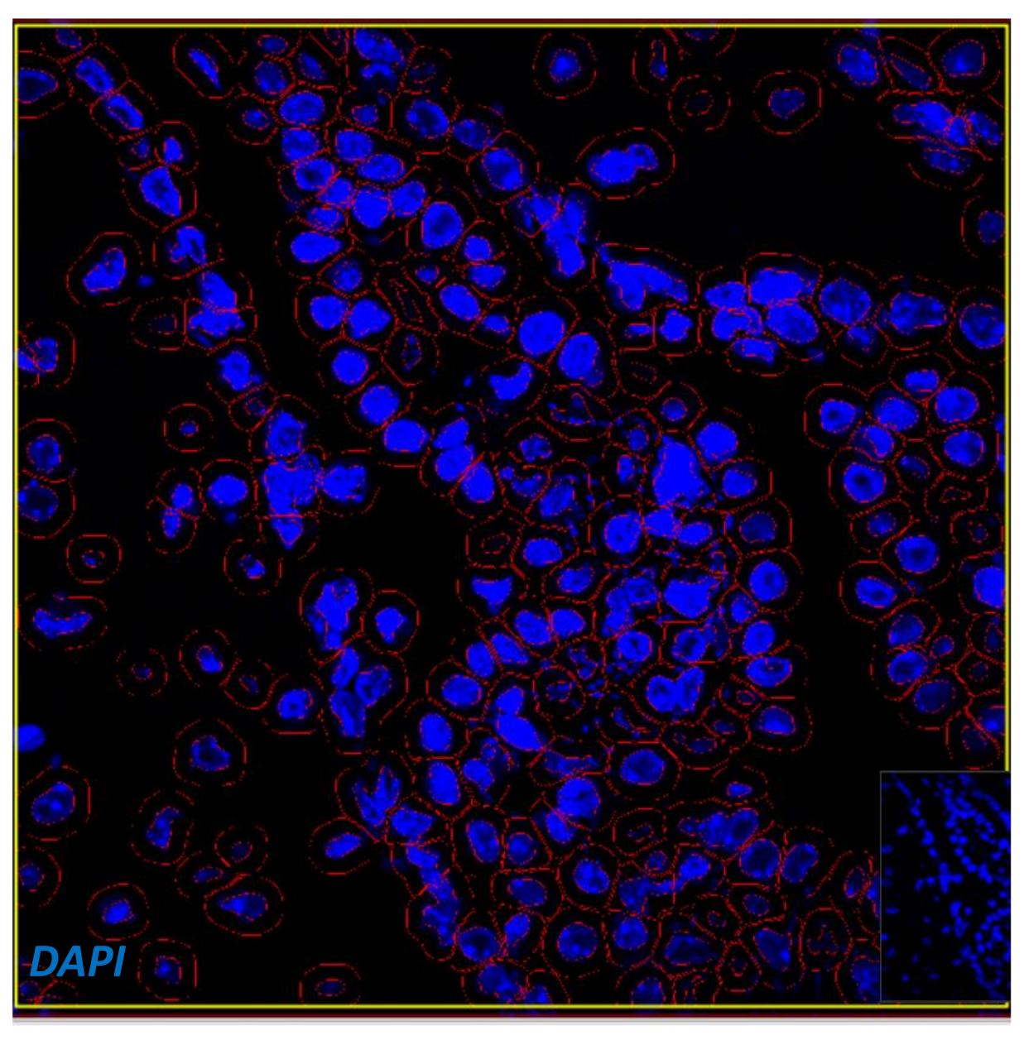
Imaging

Day 1 → Day 2 → Day 3

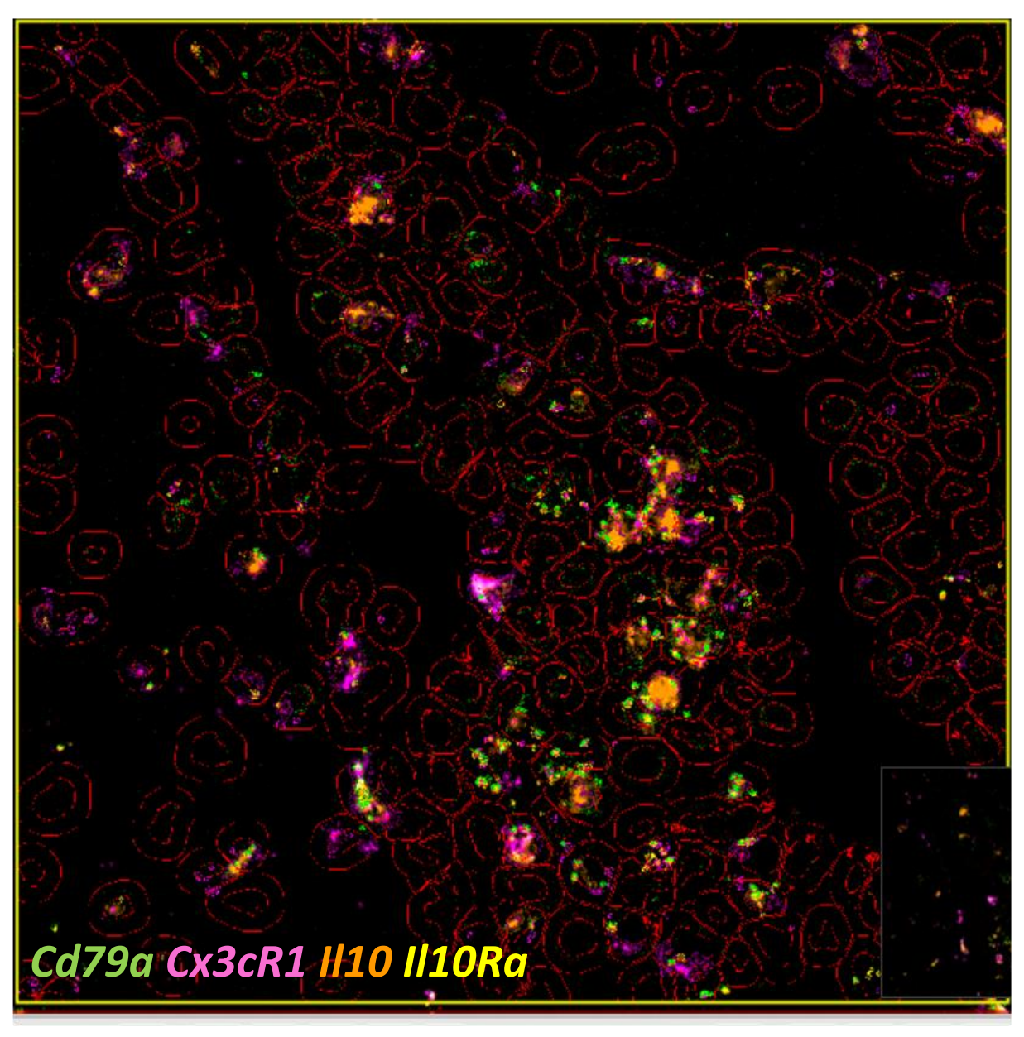
2 Quantification of cells and transcriptional spots using QuPath (<2 hours)



Remove background fluorescence



Detect cells



Detect subcellular spots

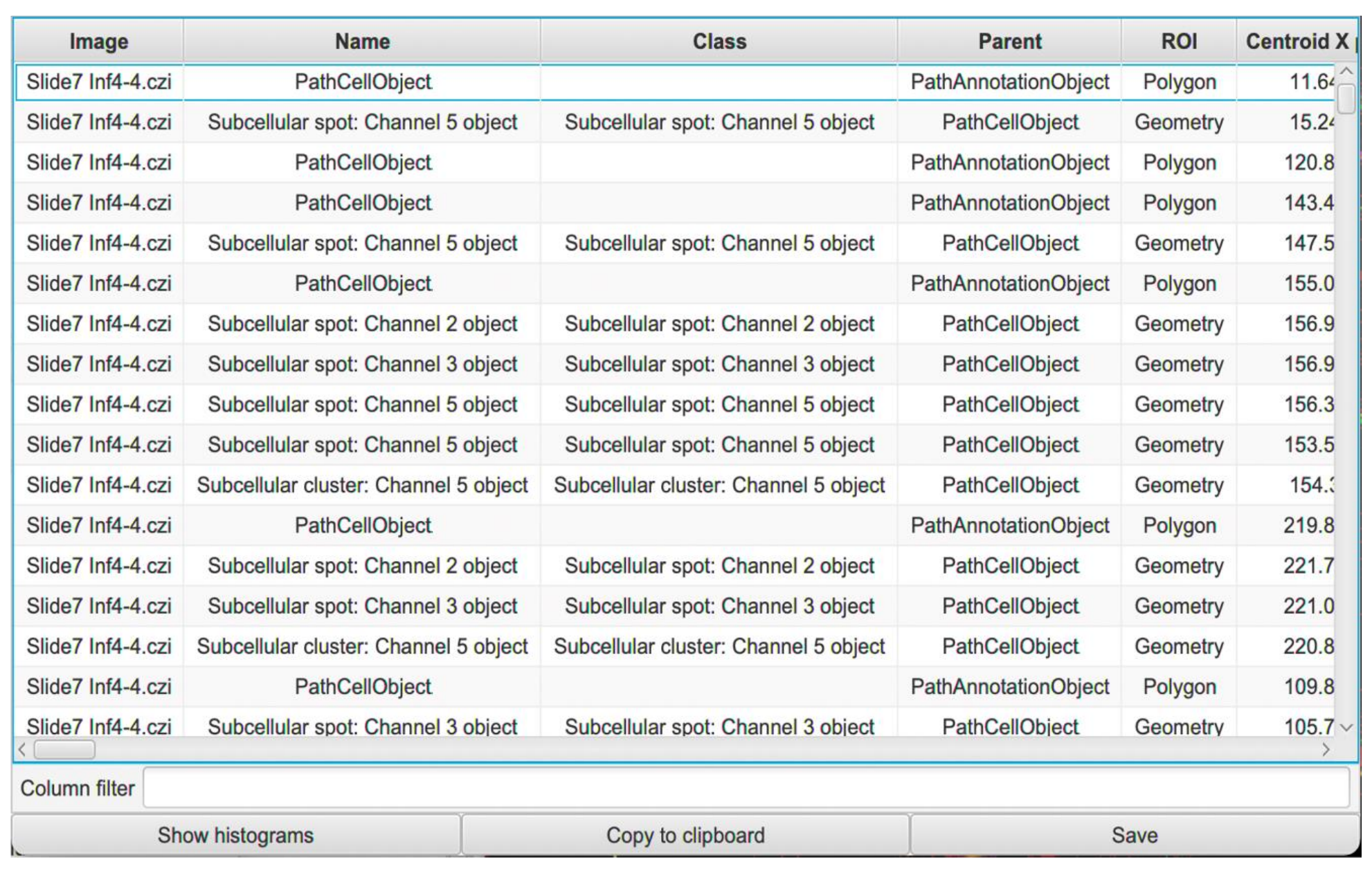
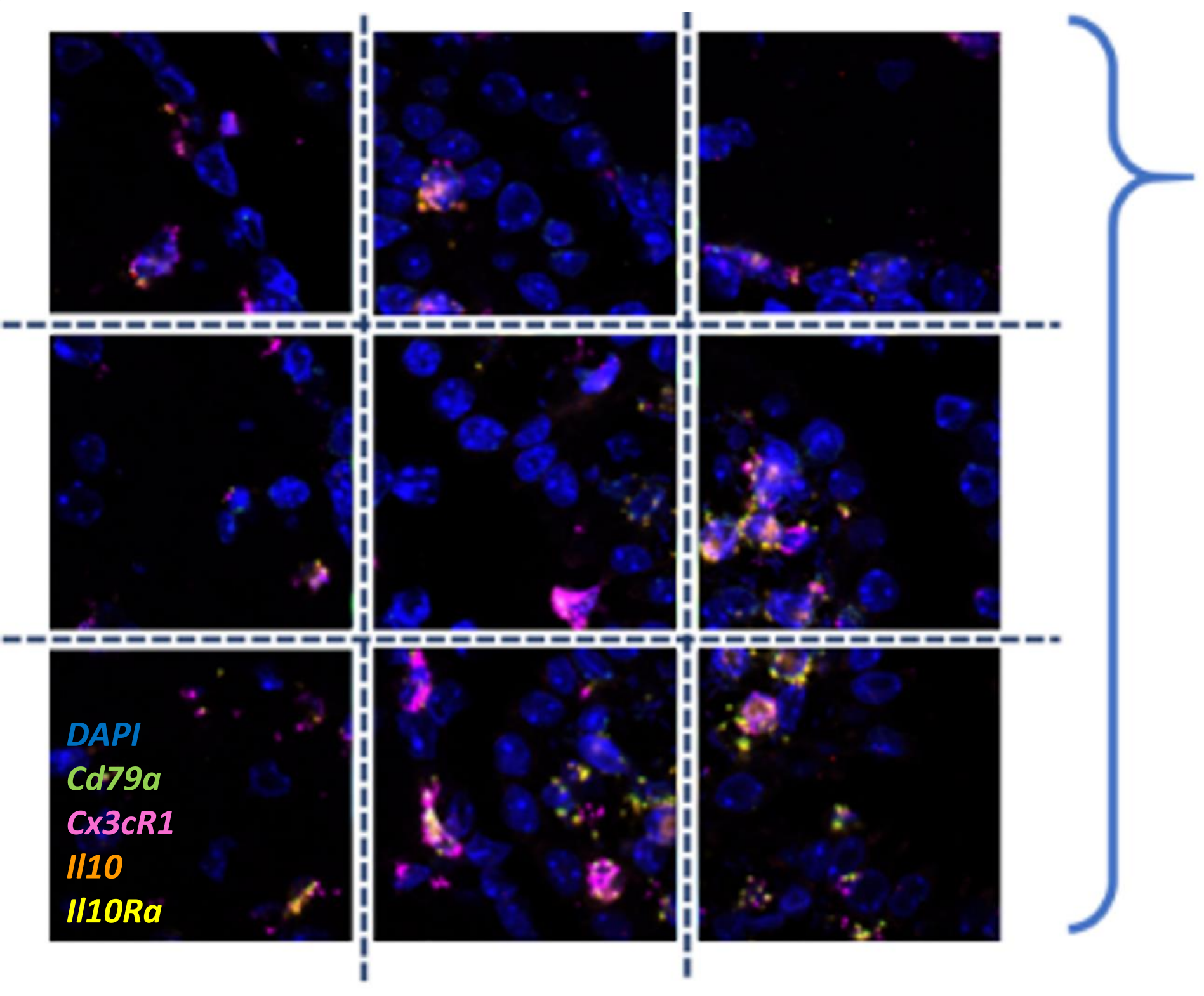


Image	Name	Class	Parent	ROI	Centroid X
Slide7 Inf4-4.czi	PathCellObject		PathAnnotationObject	Polygon	11.6
Slide7 Inf4-4.czi	Subcellular spot: Channel 5 object	Subcellular spot: Channel 5 object	PathCellObject	Geometry	15.2
Slide7 Inf4-4.czi	PathCellObject		PathAnnotationObject	Polygon	120.8
Slide7 Inf4-4.czi	PathCellObject		PathAnnotationObject	Polygon	143.4
Slide7 Inf4-4.czi	Subcellular spot: Channel 5 object	Subcellular spot: Channel 5 object	PathCellObject	Geometry	147.5
Slide7 Inf4-4.czi	PathCellObject		PathAnnotationObject	Polygon	155.0
Slide7 Inf4-4.czi	Subcellular spot: Channel 2 object	Subcellular spot: Channel 2 object	PathCellObject	Geometry	156.9
Slide7 Inf4-4.czi	Subcellular spot: Channel 3 object	Subcellular spot: Channel 3 object	PathCellObject	Geometry	156.9
Slide7 Inf4-4.czi	Subcellular spot: Channel 5 object	Subcellular spot: Channel 5 object	PathCellObject	Geometry	156.3
Slide7 Inf4-4.czi	Subcellular spot: Channel 5 object	Subcellular spot: Channel 5 object	PathCellObject	Geometry	153.5
Slide7 Inf4-4.czi	Subcellular cluster: Channel 5 object	Subcellular cluster: Channel 5 object	PathCellObject	Geometry	154.
Slide7 Inf4-4.czi	PathCellObject		PathAnnotationObject	Polygon	219.8
Slide7 Inf4-4.czi	Subcellular spot: Channel 2 object	Subcellular spot: Channel 2 object	PathCellObject	Geometry	221.7
Slide7 Inf4-4.czi	Subcellular spot: Channel 3 object	Subcellular spot: Channel 3 object	PathCellObject	Geometry	221.0
Slide7 Inf4-4.czi	Subcellular cluster: Channel 5 object	Subcellular cluster: Channel 5 object	PathCellObject	Geometry	220.8
Slide7 Inf4-4.czi	PathCellObject		PathAnnotationObject	Polygon	109.8
Slide7 Inf4-4.czi	Subcellular spot: Channel 3 object	Subcellular spot: Channel 3 object	PathCellObject	Geometry	105.7

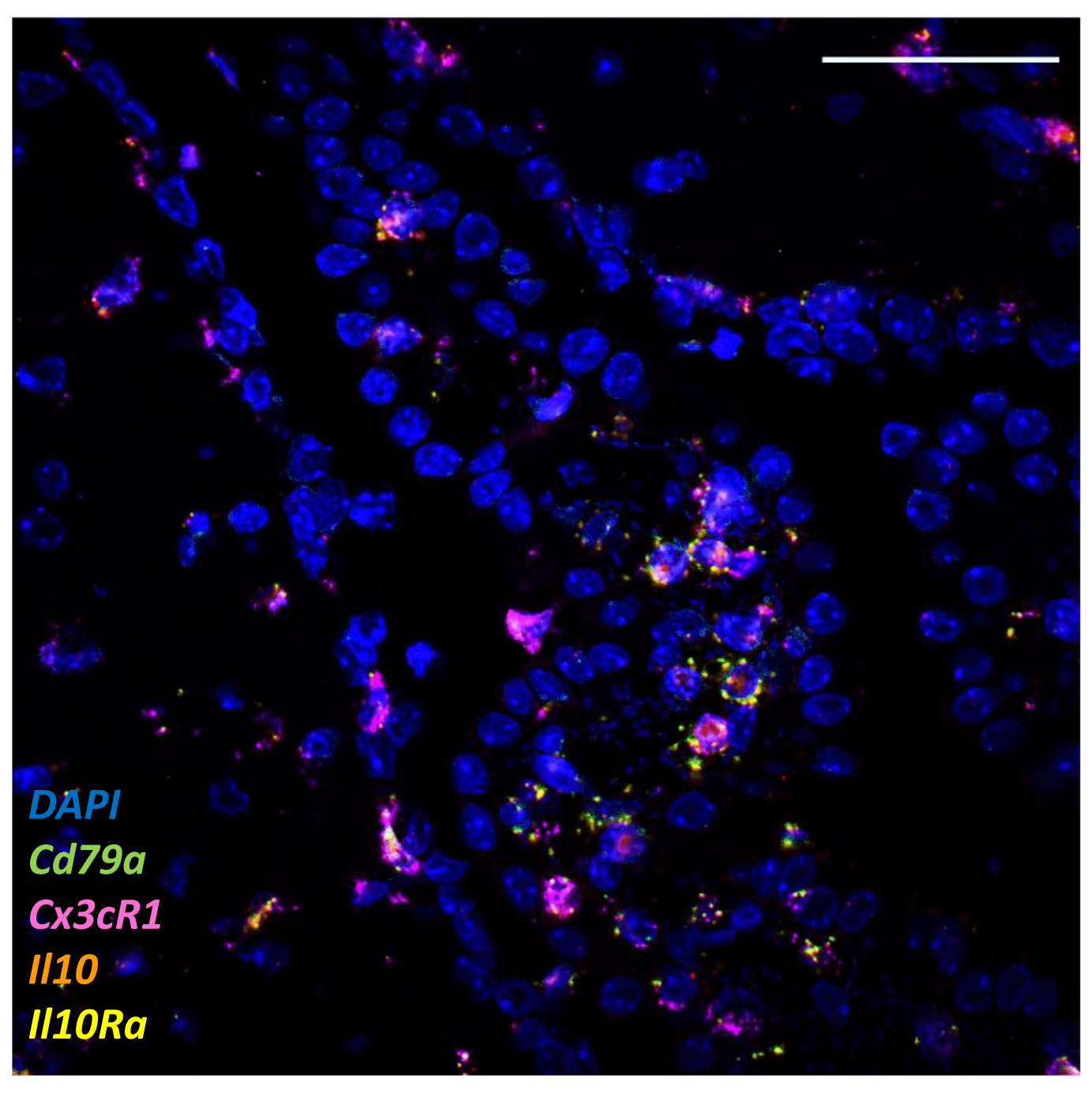
Export dataset using QuPath

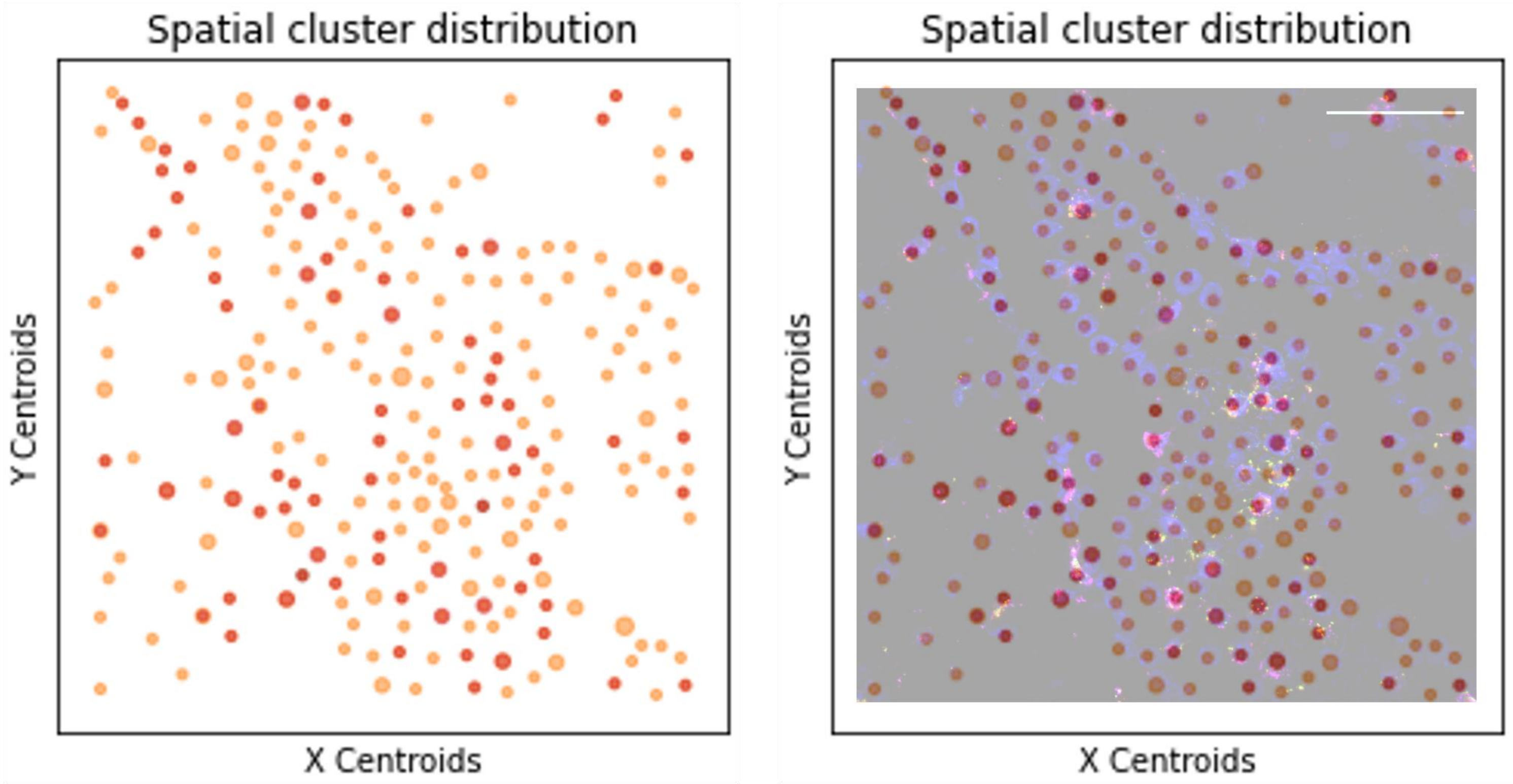
Scripted for automated batch processing

3 Rapid data processing of cellular information with ScopePlot software (<15 mins)



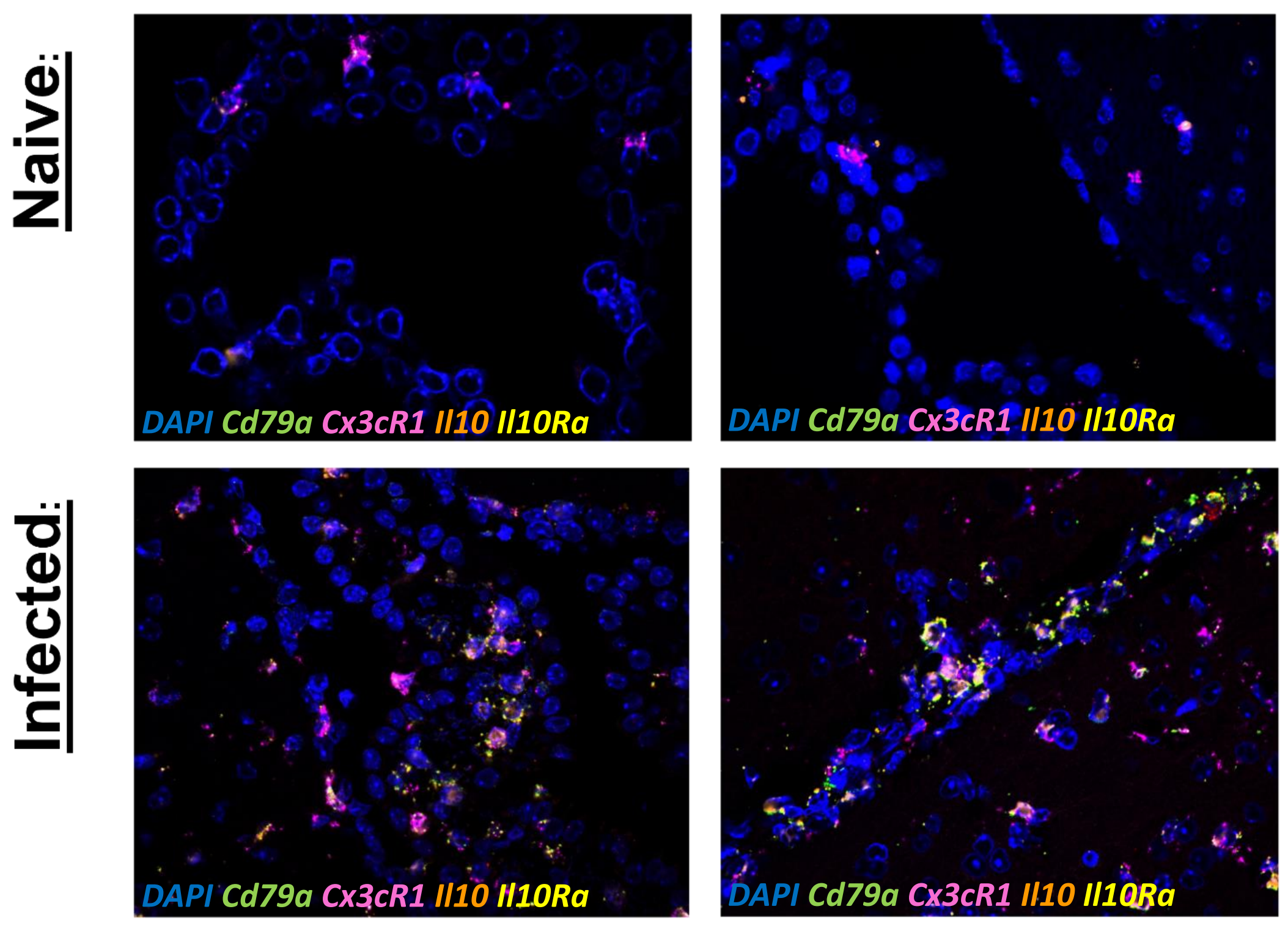
Multi-image dataset concatenation



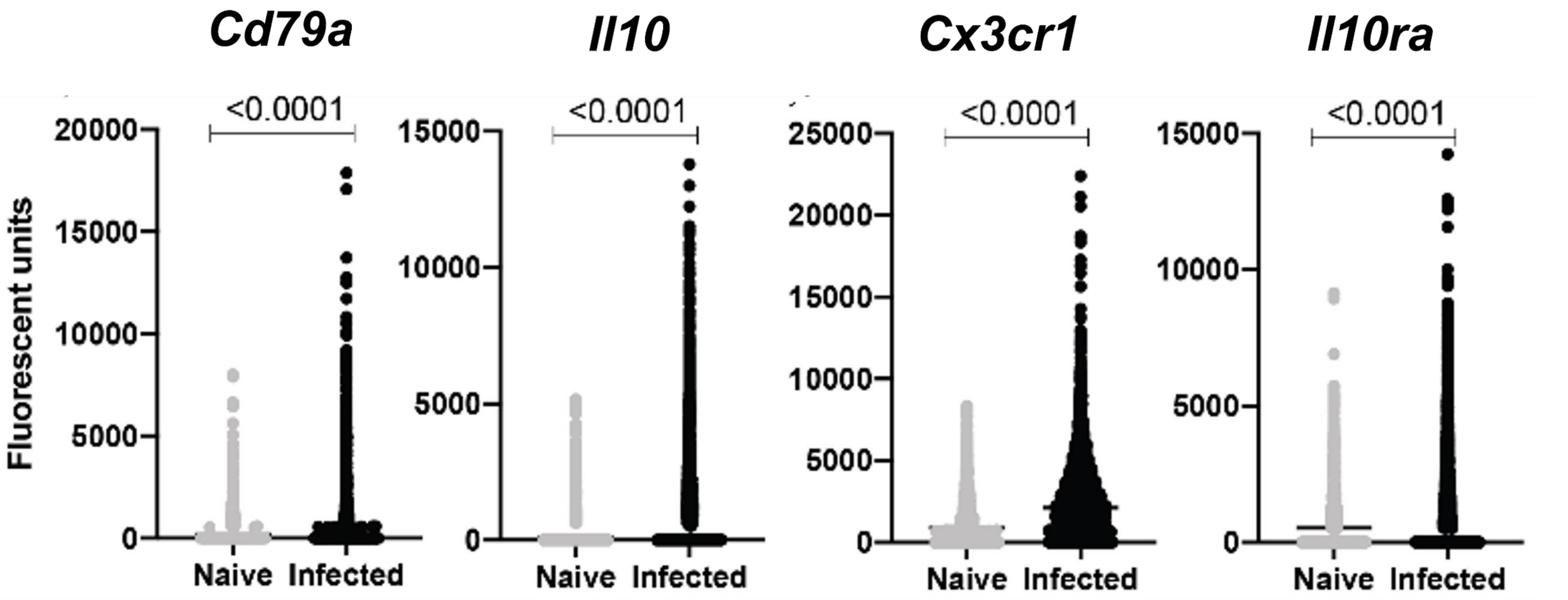


Partition cells by user-selected RNA target and plot distribution

4 Analysis and visualisation of subcellular transcript expression using ScopePlot and Prism (<15 mins)



Multi-dataset analysis in series



Streamlined visualisation of multi-target, multi-dataset transcript expression

We have generated a new pipeline for the **analysis of RNAscope image data** and tested this pipeline on a validated dataset. The pipeline is capable **of quantifying cellular and sub-cellular data** in RNAScope images and can **partition data automatically** for rapid, target-focused analysis.

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