



Deep visual proteomics on mouse models of neurodegenerative disease

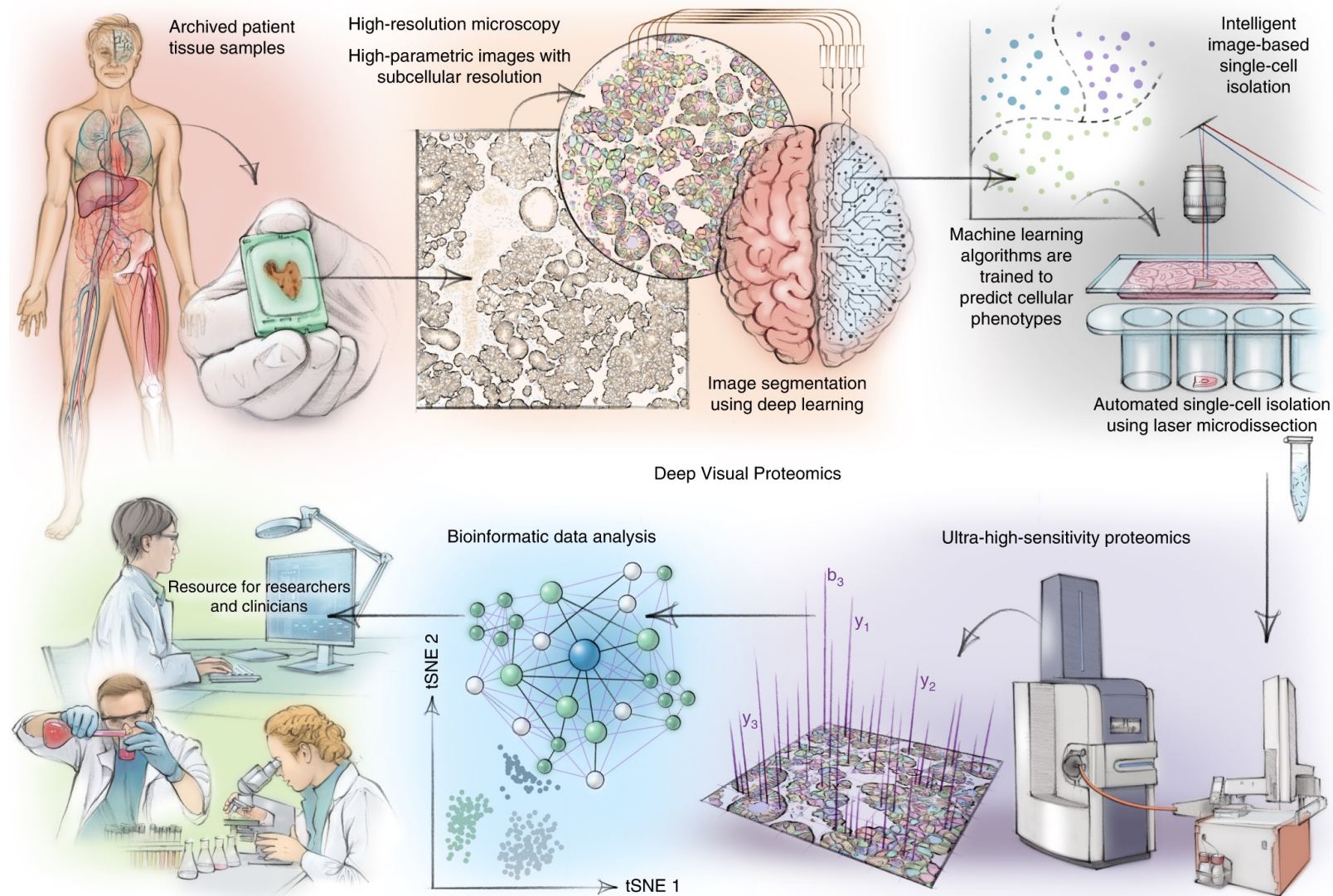
Nick Manfred | Abu-Remaileh Lab | GRIP | 09.16.2024

Overview: Project summary

CLN5 mutations are a risk factor for numerous neurodegenerative diseases, including Alzheimer's, Parkinson's, frontotemporal dementia, and Batten disease. My GRIP project investigated how CLN5 loss affects different types of cells in the brain at the level of the proteome (all the proteins made within that cell), including neurons, astrocytes, microglia, and oligodendrocytes. We hope to better understand how each of these cell types dynamically respond to the lysosomal stress imposed by this mutation, which will help us learn more about the progression of the disease and identify protective biological responses that may inform future therapies.

I was hosted by the Department of Proteomics and Signal Transduction at the Max Planck Institute of Biochemistry in Martinsried, Germany. Mentorship was provided by Prof. Matthias Mann, Edwin Rodriguez, and Marc Oeller, who taught me the pipeline for deep visual proteomics.

Overview: Deep visual proteomics pipeline



Step 1: Sample collection

ID	Sex	DOB	Genotype	Barcode	Age (mo.)
M0527	M	3/30/24	TH-/-; Cln5-/-	Barcode 0005020499	2
M0532	M	4/4/24	TH-/-; Cln5-/-	Barcode 0005020501	2
M0528	M	3/30/24	TH-/-; Cln5+/-	Barcode 0005020499	2
M0533	M	4/4/24	TH-/-; Cln5+/-	Barcode 0005020501	2
M0530	F	4/4/24	TH-/-; Cln5-/-	Barcode 0005020495	2
M0529	F	4/4/24	TH-/-; Cln5+/-	Barcode 0005020477	2
M0531	F	4/4/24	TH-/-; Cln5+/-	Barcode 0005020477	2
J0478	F	12/16/23	TH-/-; Cln5-/-	Barcode 0005020478	5.5
J0479	F	12/16/23	TH-/-; Cln5-/-	Barcode 0005020479	5.5
J0430	F	11/10/23	TH-/-; Cln5+/-	Barcode 0005020488	6.5
J0296	F	8/5/23	TH-/-; Cln5-/-	Breeding cage	10
J0485	M	12/29/23	TH-/-; Cln5+/-	Barcode 0005020479	5
J0483	M	12/29/23	TH-/-; Cln5+/-	Barcode 0005020478	5
J0399	F	10/12/23	TH-/-; Cln5-/-	Barcode 0005020482	8
M0460	F	11/10/23	TH-/-; CLN3-/-	Barcode 0004896152	6.5
M0454	M	11/10/23	TH-/-; CLN3-/-	Barcode 0004896151	6.5
M0456	F	11/10/23	TH-/-; CLN3+/-	Barcode 0004896152	6.5
J0011	M	9/1/22	TH+/-; GRN-/-	Barcode 0004720019	21
J0012	M	9/1/22	TH+/-; GRN-/-	Barcode 0004720019	21
J0013	M	9/1/22	TH+/-; GRN-/-	Barcode 0004720019	21
J0299	M	8/4/23	TH+/-; GRN-/-		10
M0557	F	5/8/24	TH+/-; Cln5+/-		1
J0270	M		TH-/-; Cln5+/-	Barcode 0005020491	
M0556	F	5/8/24	TH-/-; Cln5-/-		1

Key:

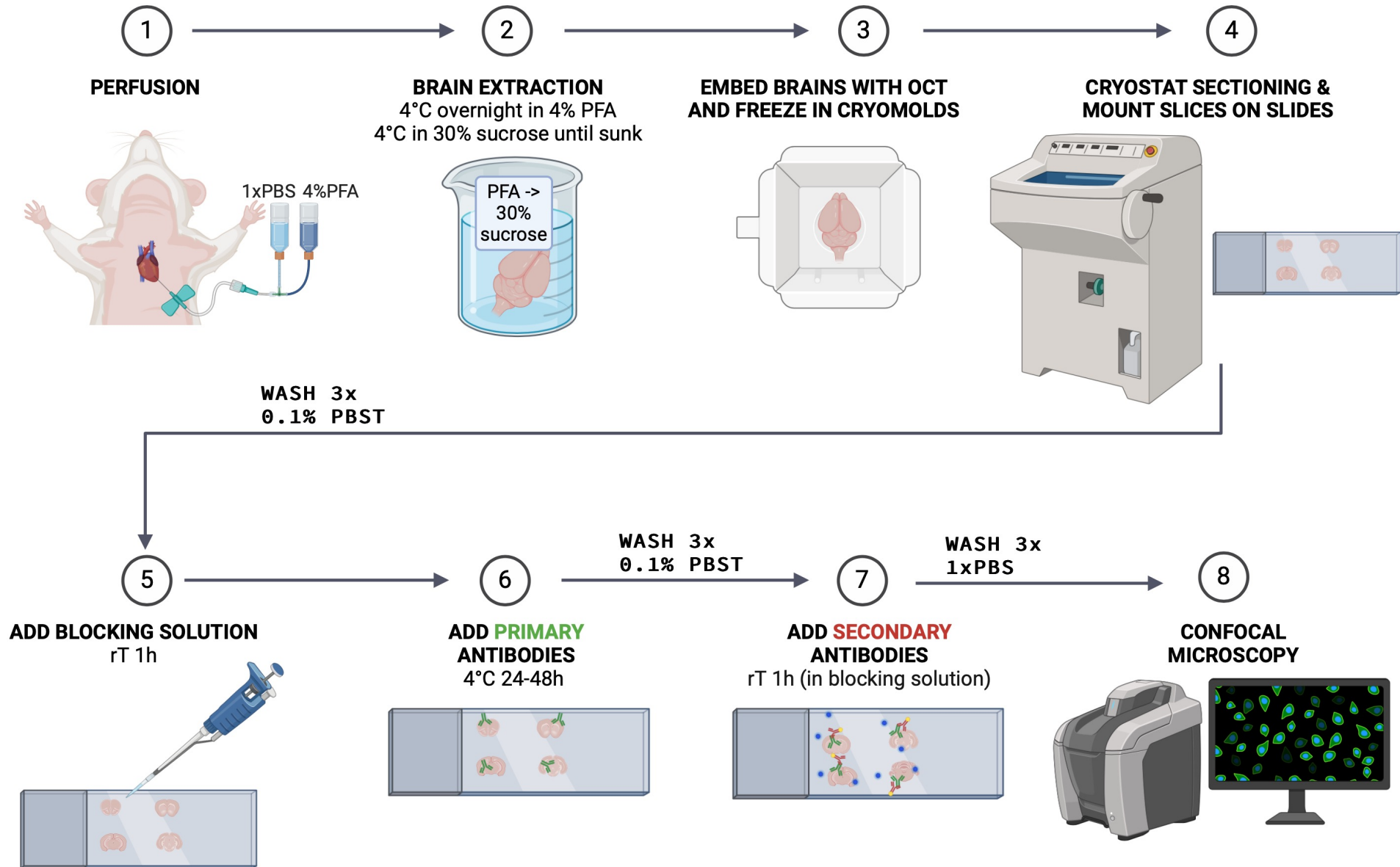
Male knockout

Male heterozygous

Female knockout

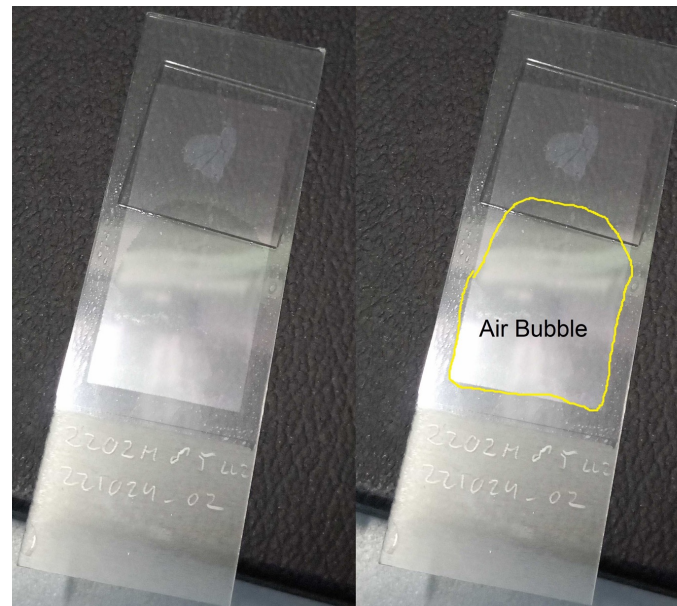
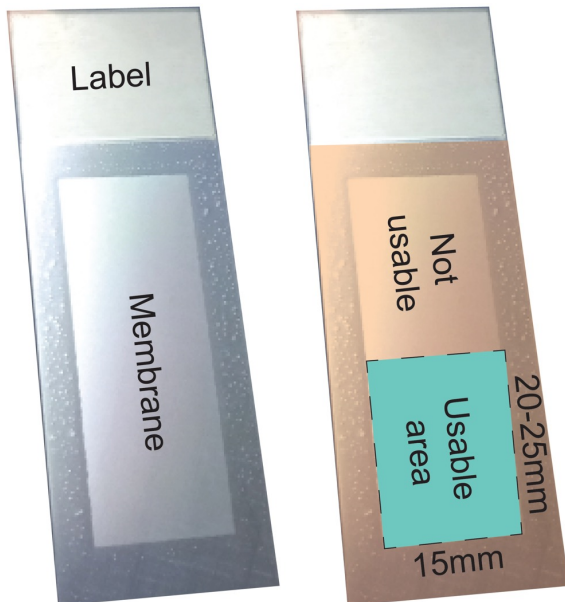
Female heterozygous

Step 1: Sample collection

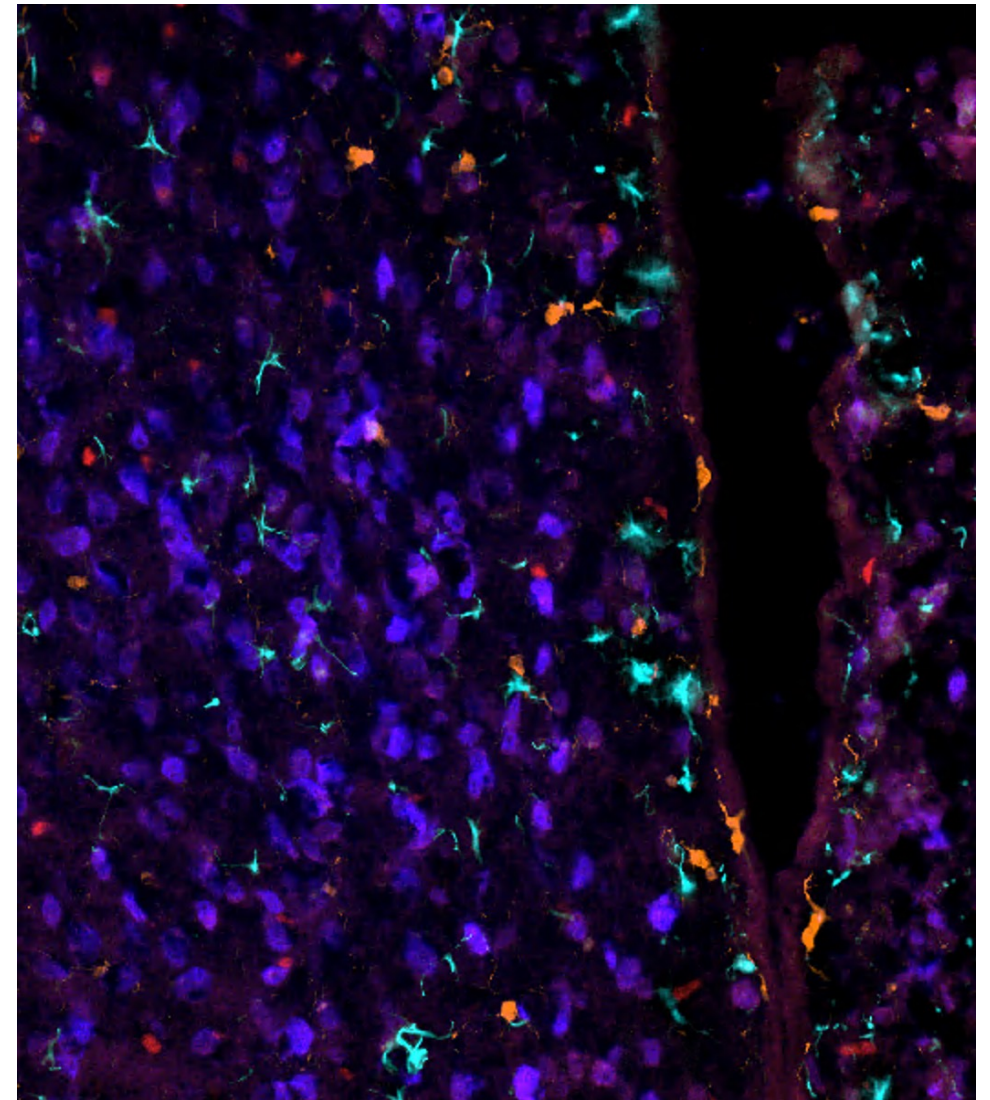
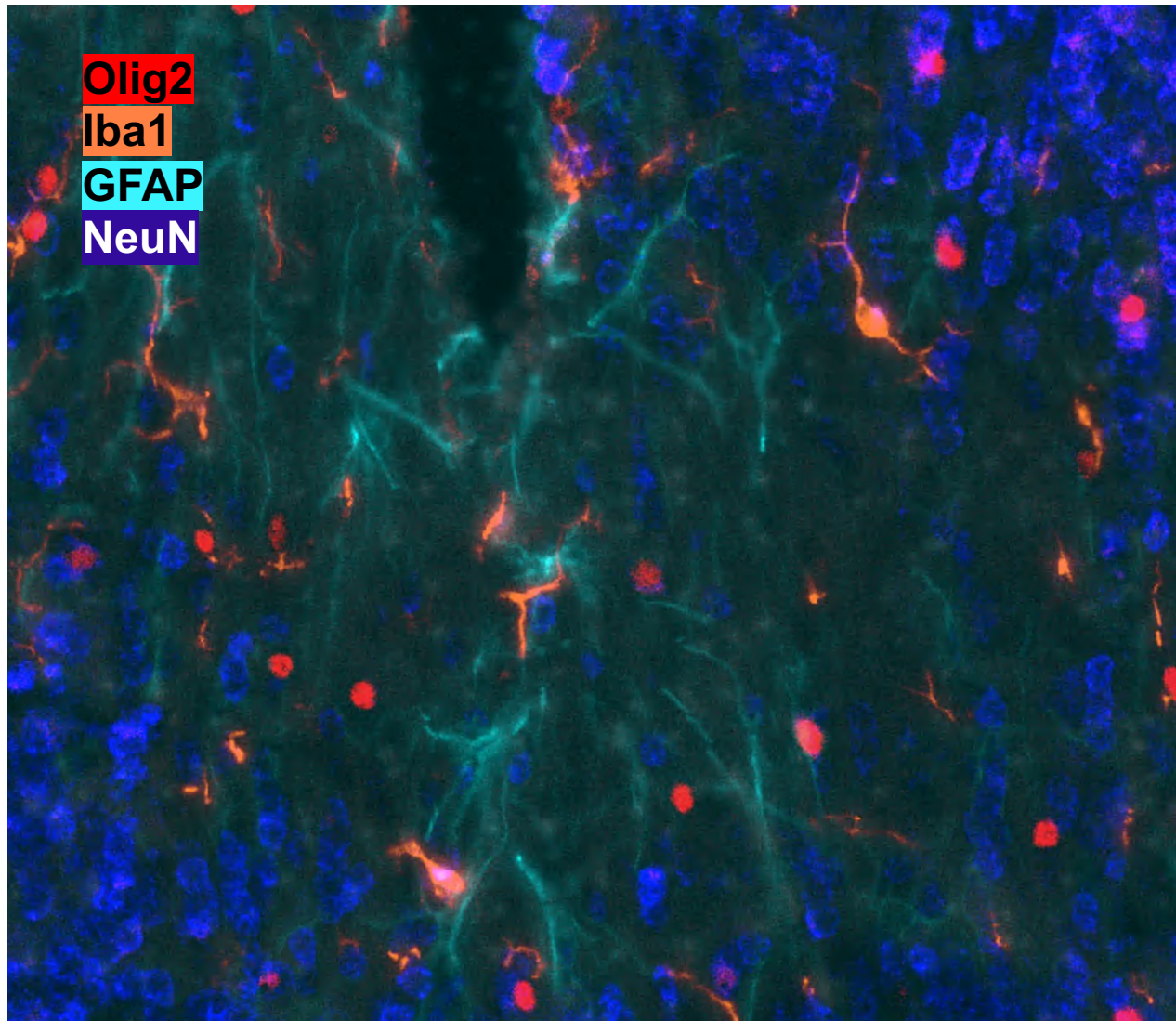


Notes: Sample collection

- *Requires special slides with adhesive membrane
- *Half of membrane area is usable (near label)
- *10 μ m coronal sections of dorsal hippocampus used
 - minimize section thickness (e.g. 5 μ m) to reduce staining overlap (less manual work later)
 - inverse relationship between section thickness and number of cells required for proteomics
 - keep section planes consistent across samples
- *Optimal antibody concentrations should be tested to achieve good staining
- *Use water-soluble mounting media to coverslip



Representative images (60x)



Step 2: Etch crosses in tissue for calibration

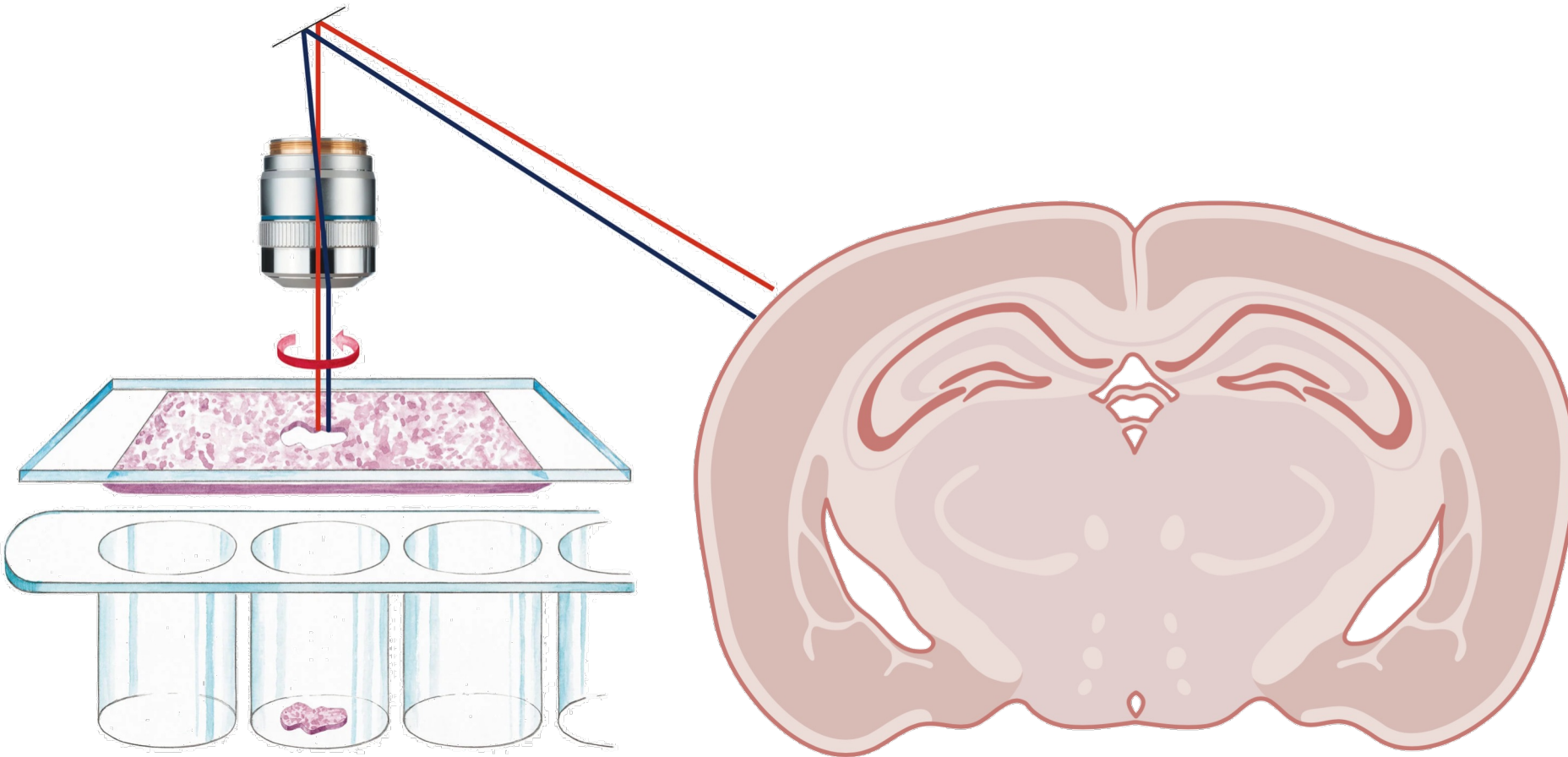


Image courtesy of Leica Microsystems

Step 2: Etch crosses in tissue for calibration

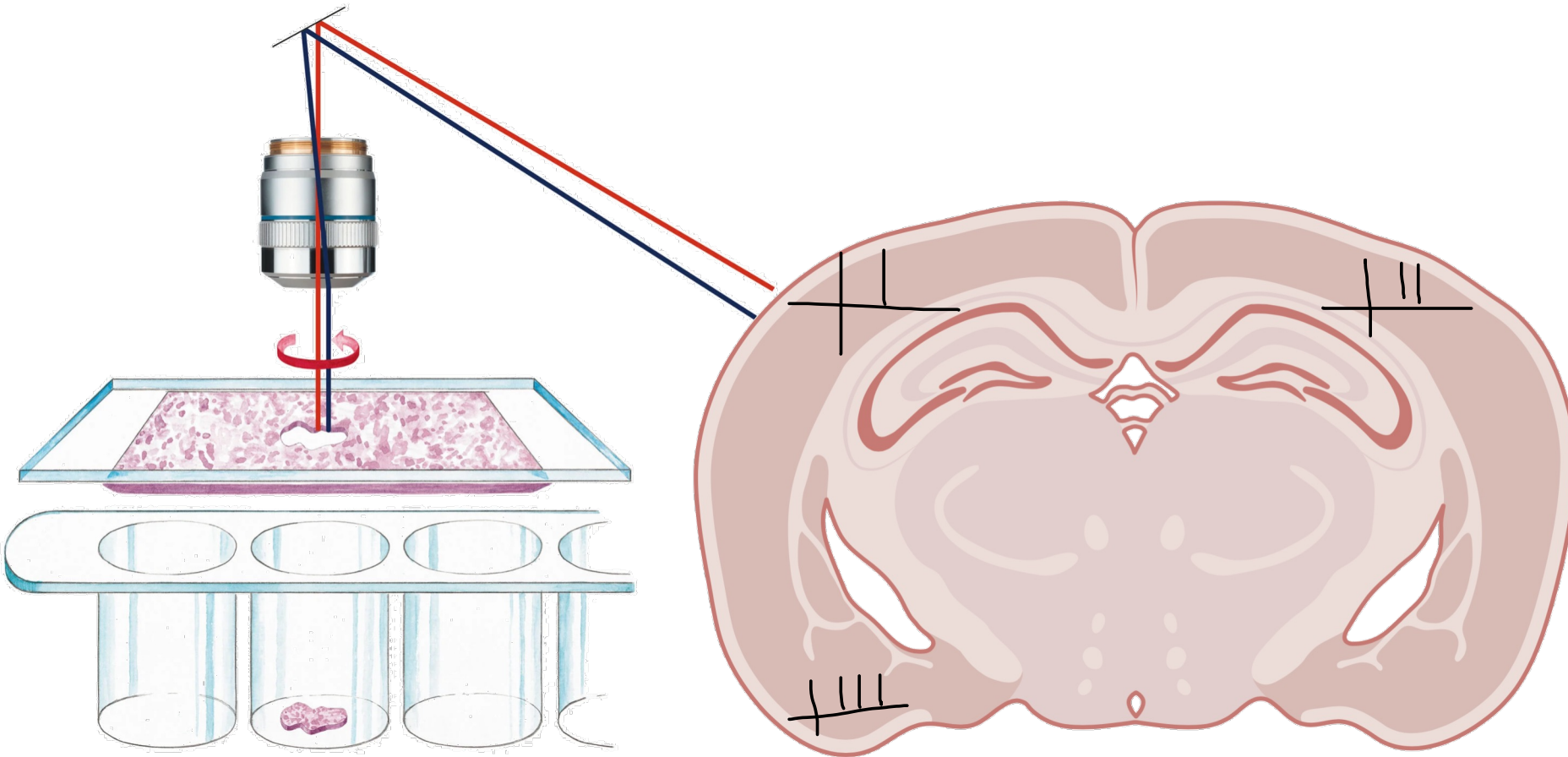


Image courtesy of Leica Microsystems

Step 2: Etch crosses in tissue for calibration

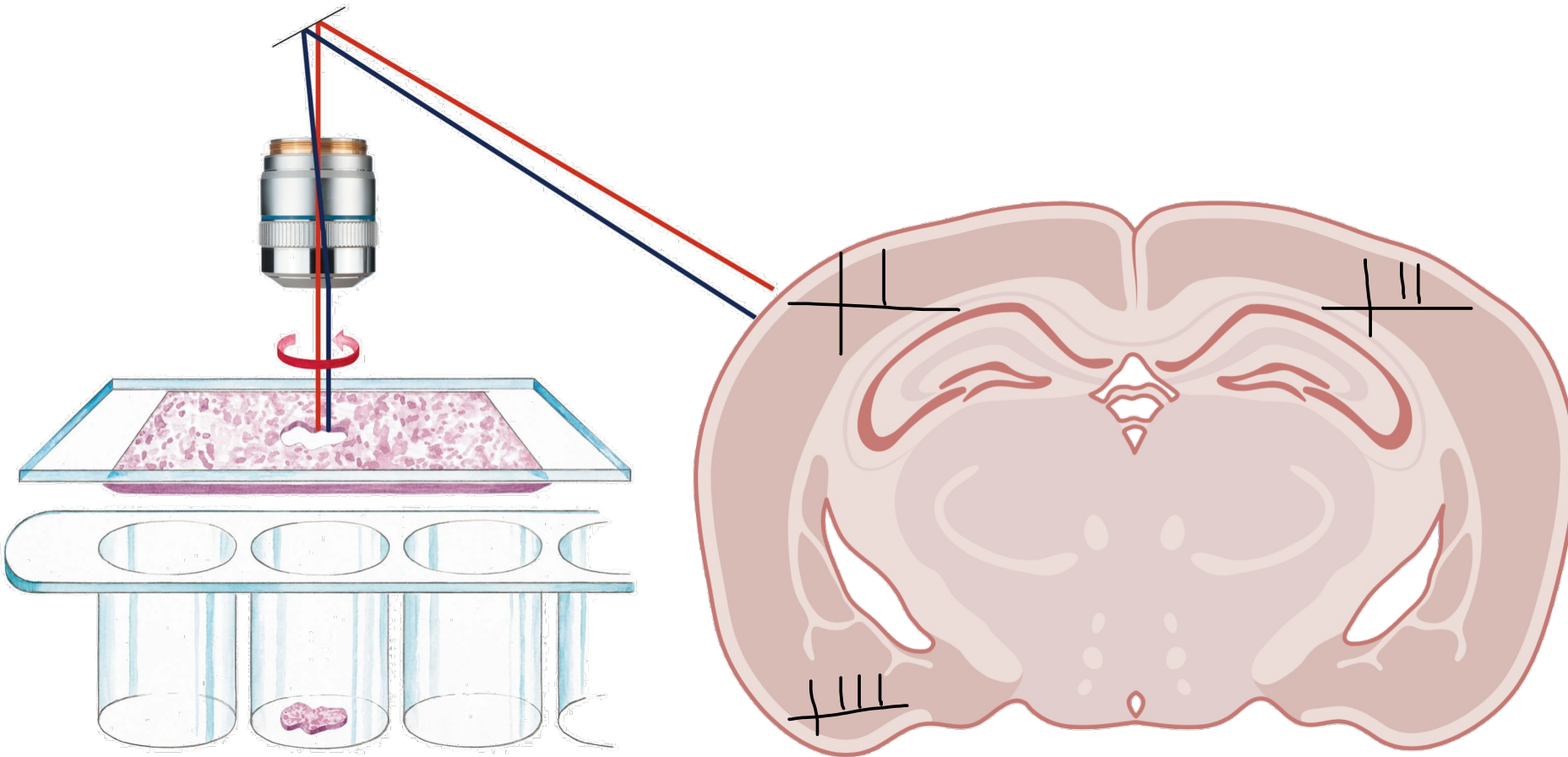
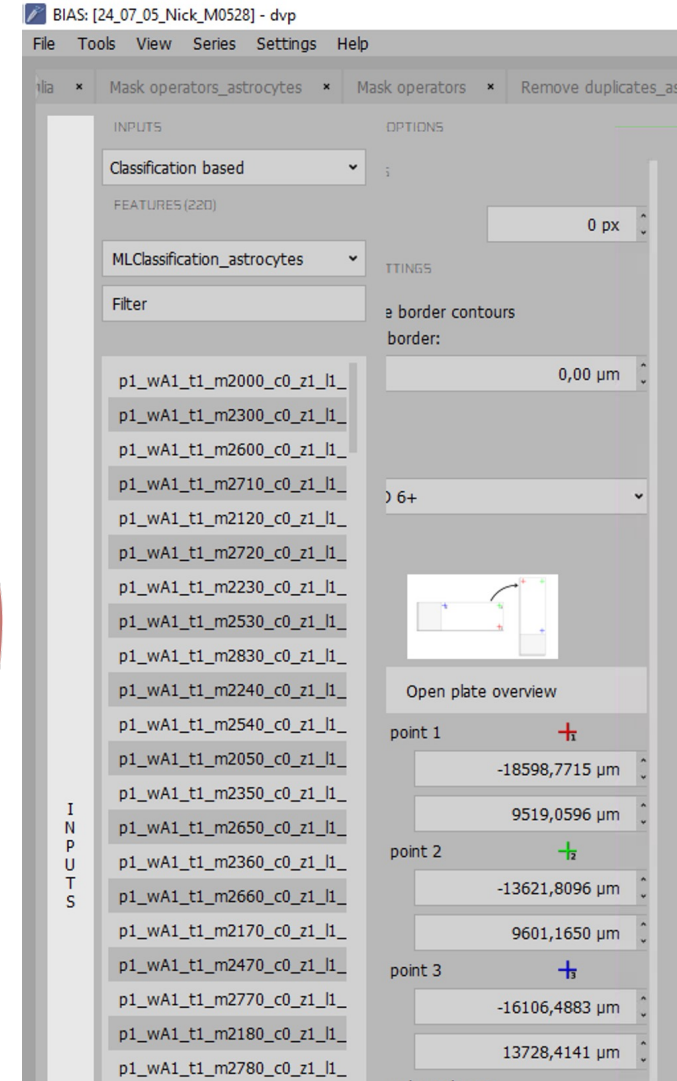


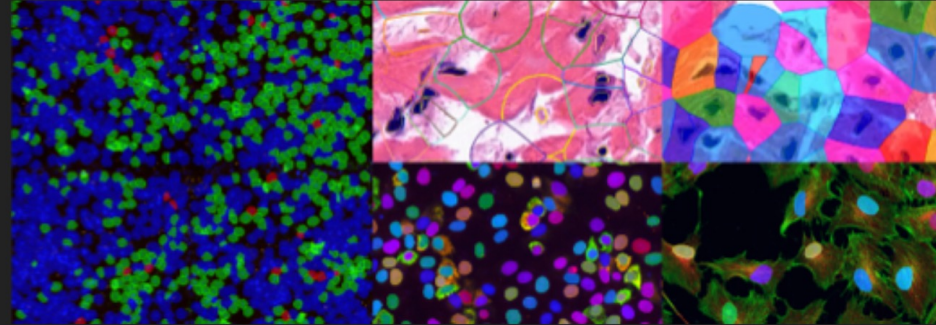
Image courtesy of Leica Microsystems



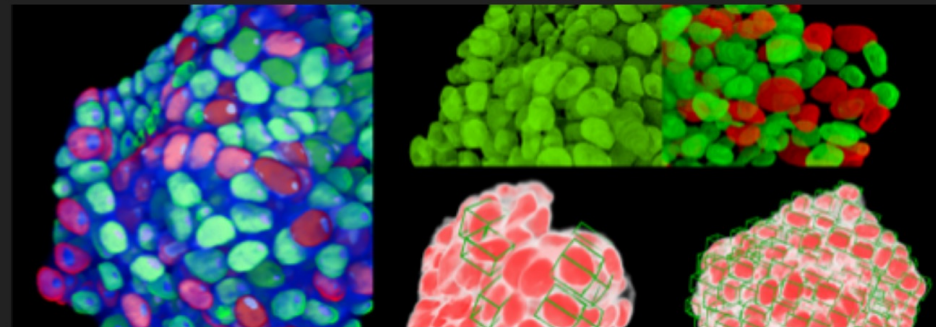
Step 3: Import images to BIAS



With the help of machine learning, **BIAS** can automatically find the most promising cell candidates.



The software provides the analytics of digital microscopy images for basic research in pathology, hematology, and biology.



BIAS also provides its full functionality for 3D data.

Images courtesy of Single-Cell Technologies®

Step 4: Obtain masks/contours for each cell type



Tabs:

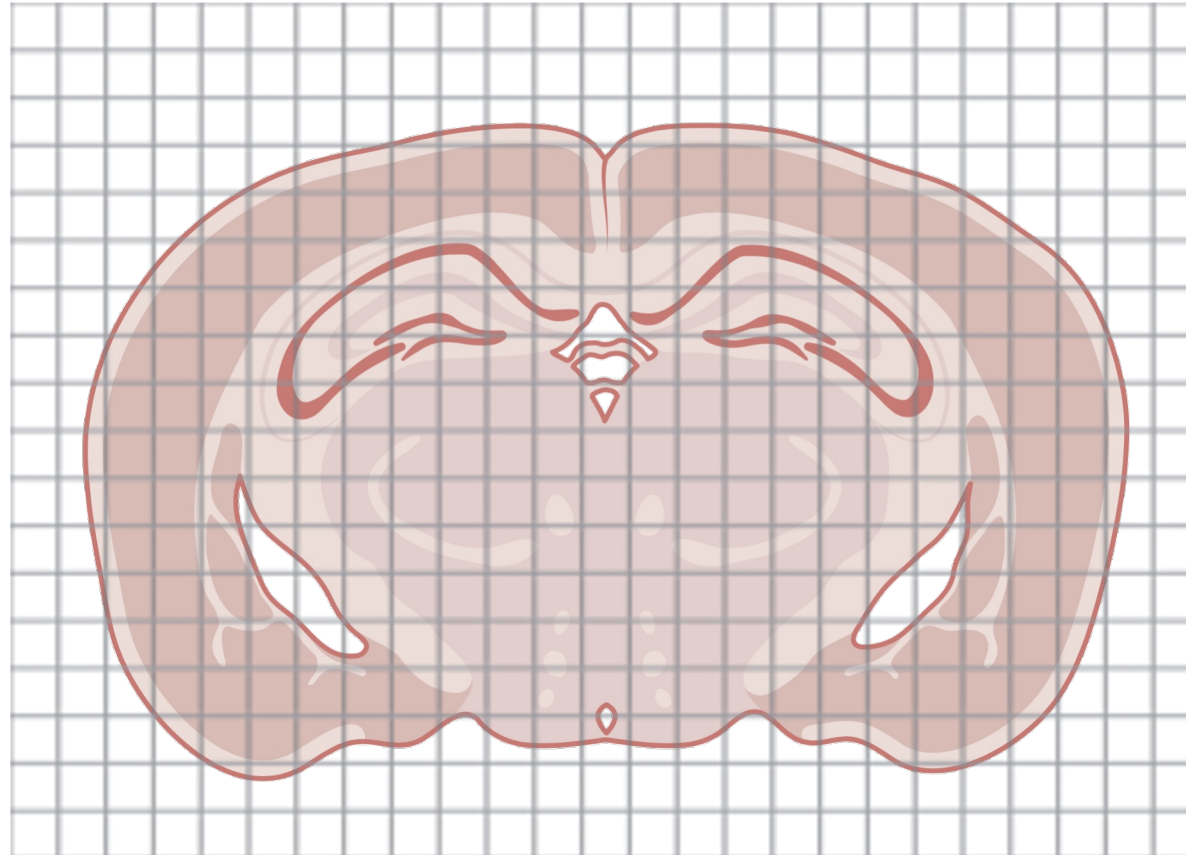
Selection -> Segmentation -> Remove duplicates -> Mask operators -> List creator -> Feature extraction -> ML classification -> Contour export

Step 4: Obtain masks/contours for each cell type



Tabs:

Selection -> Segmentation -> Remove duplicates -> Mask operators ->
List creator -> Feature extraction -> ML classification -> Contour export

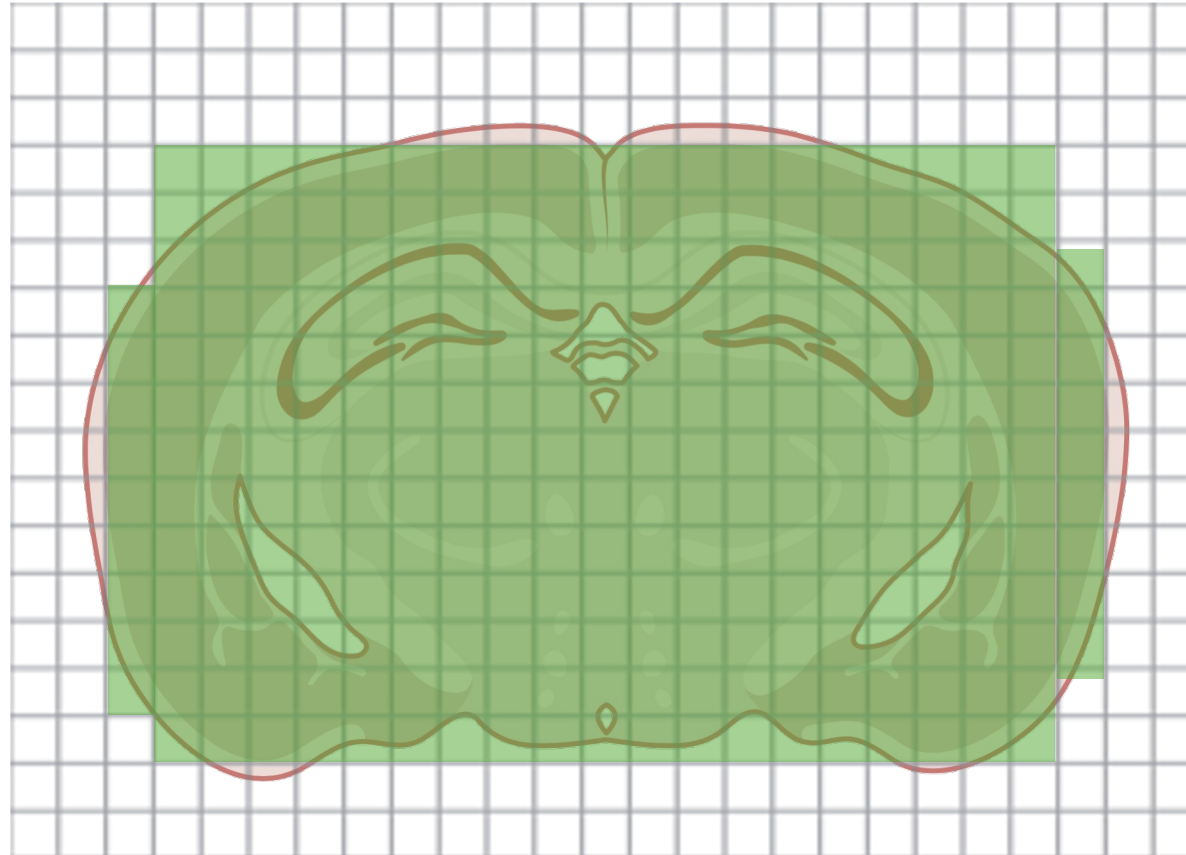


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Tabs:

Selection -> Segmentation -> Remove duplicates -> Mask operators ->
List creator -> Feature extraction -> ML classification -> Contour export

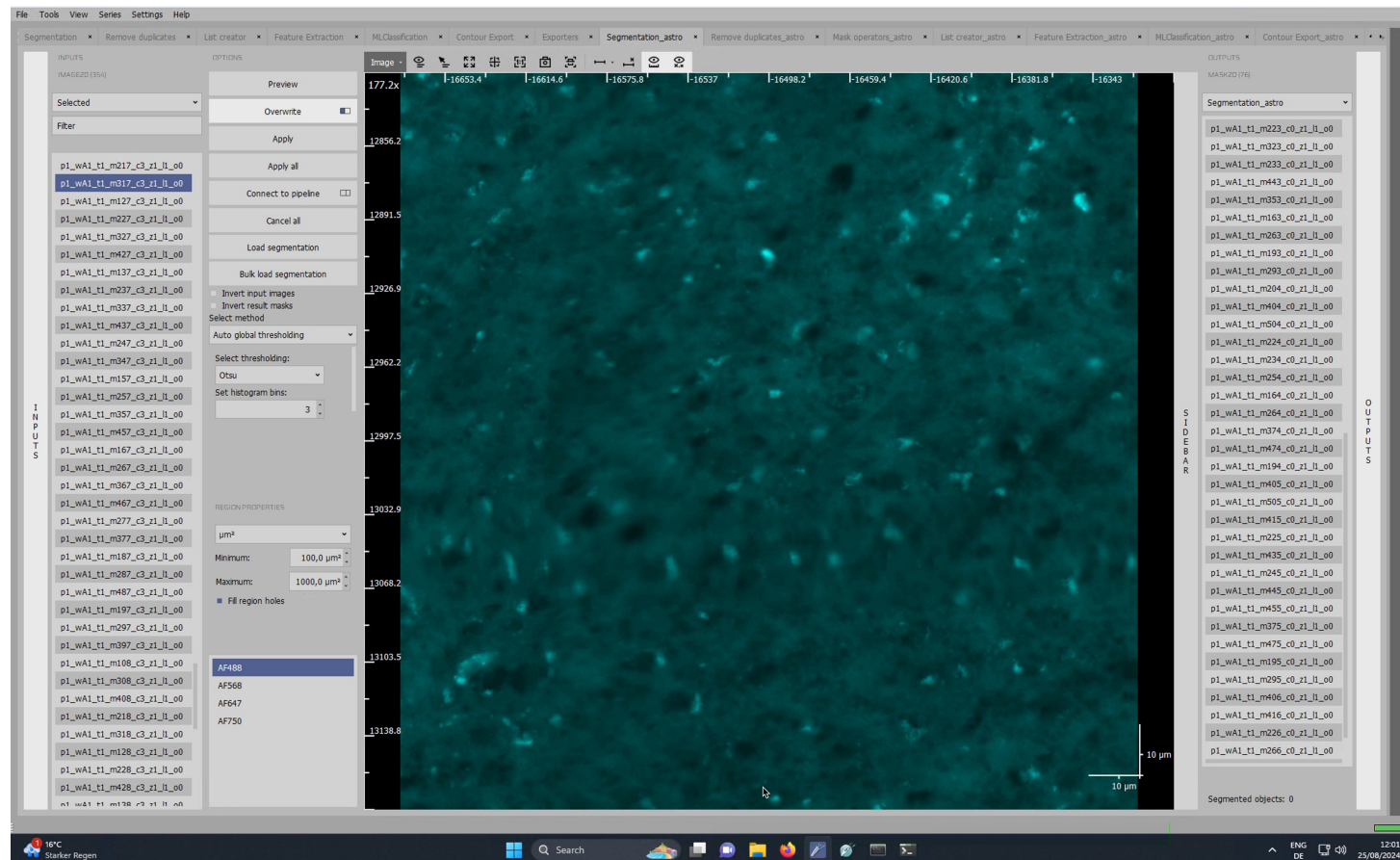


Step 4: Obtain masks/contours for each cell type



Tabs:

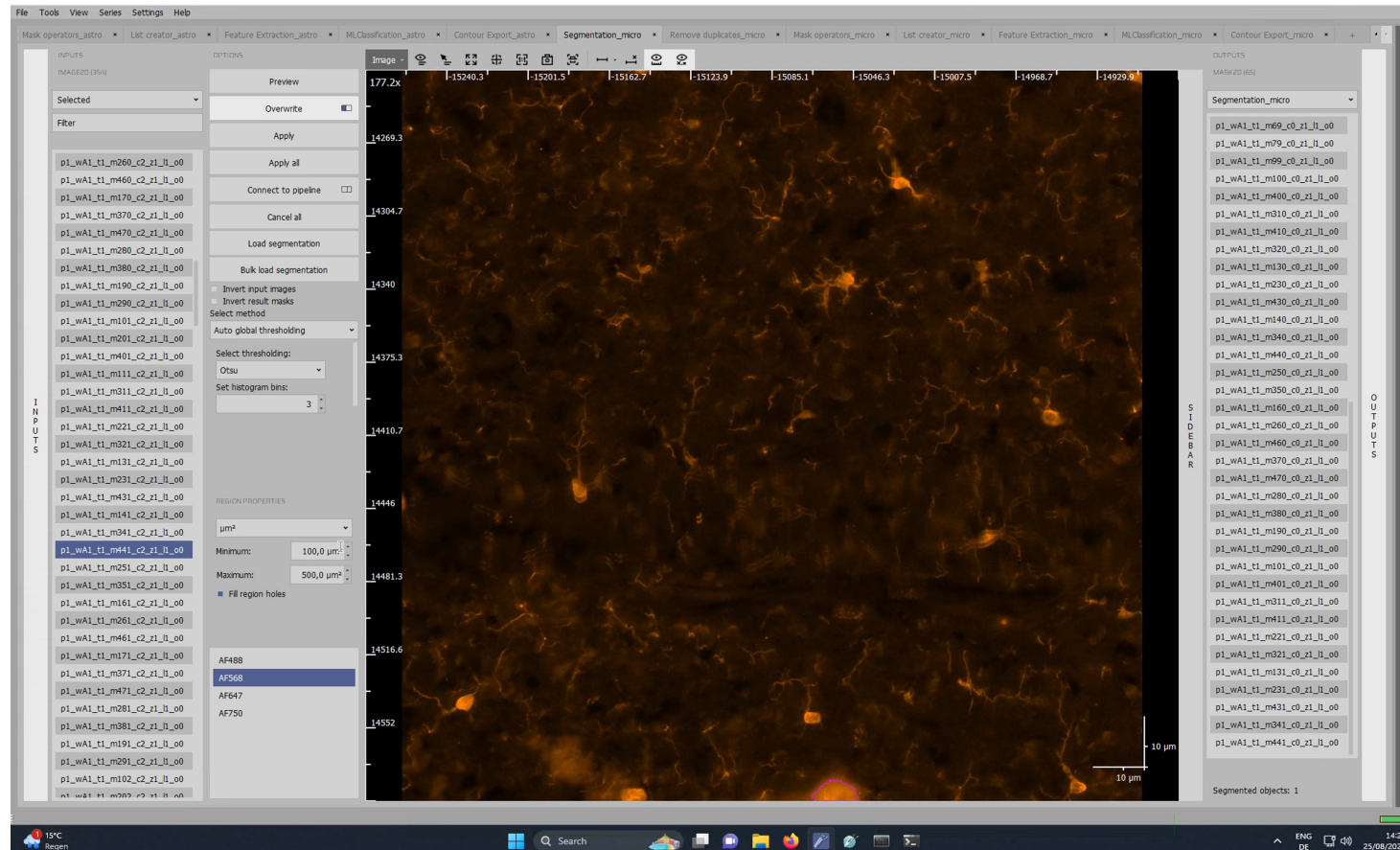
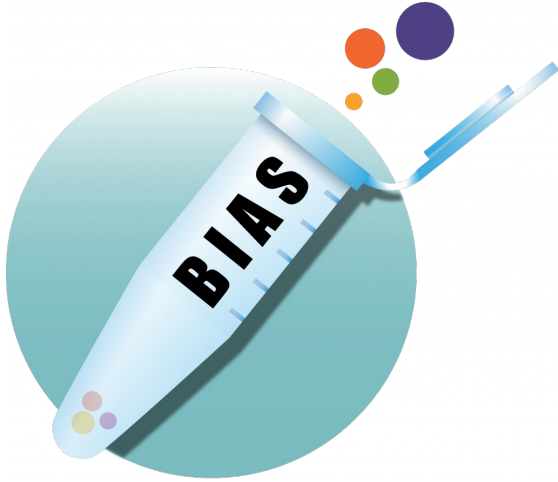
*Selection -> **Segmentation** -> Remove duplicates -> Mask operators -> List creator -> Feature extraction -> ML classification -> Contour export*



Step 4: Obtain masks/contours for each cell type

Tabs:

Selection -> Segmentation -> Remove duplicates -> Mask operators -> List creator -> Feature extraction -> ML classification -> Contour export

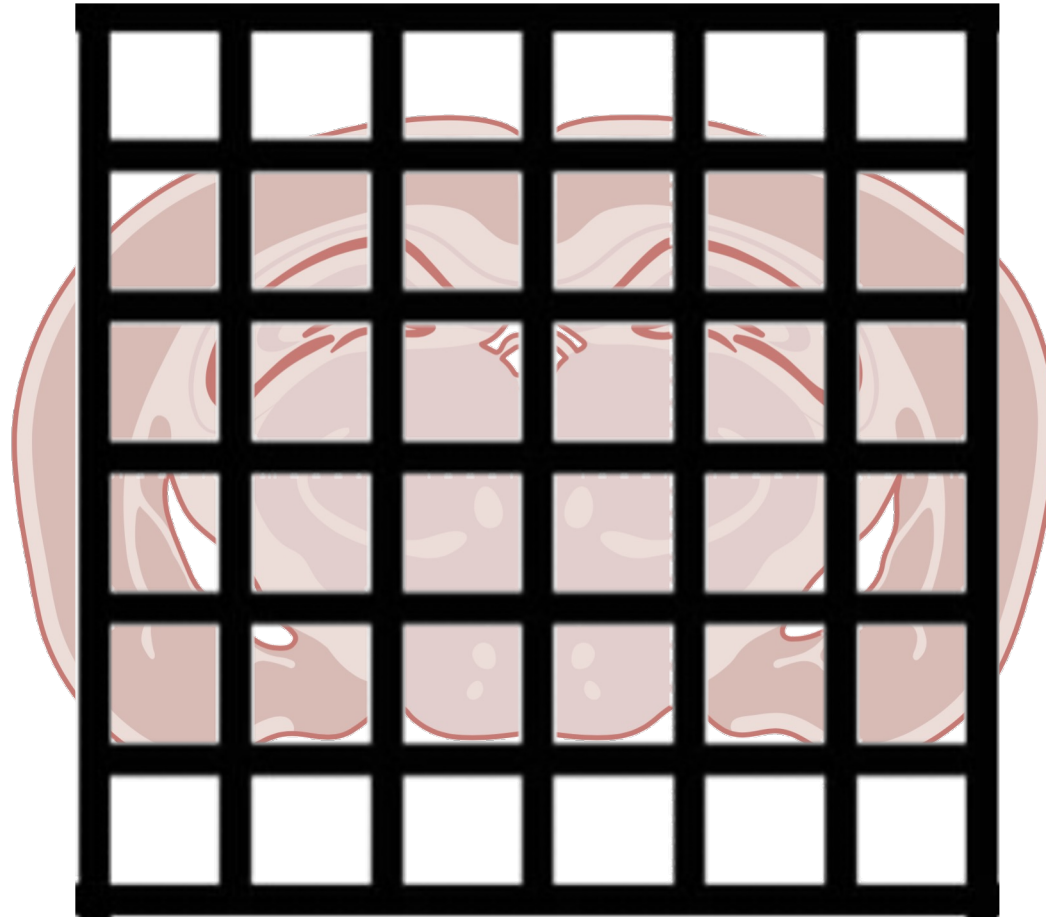


Step 4: Obtain masks/contours for each cell type



Tabs:

Selection -> Segmentation -> Remove duplicates -> Mask operators -> List creator -> Feature extraction -> ML classification -> Contour export

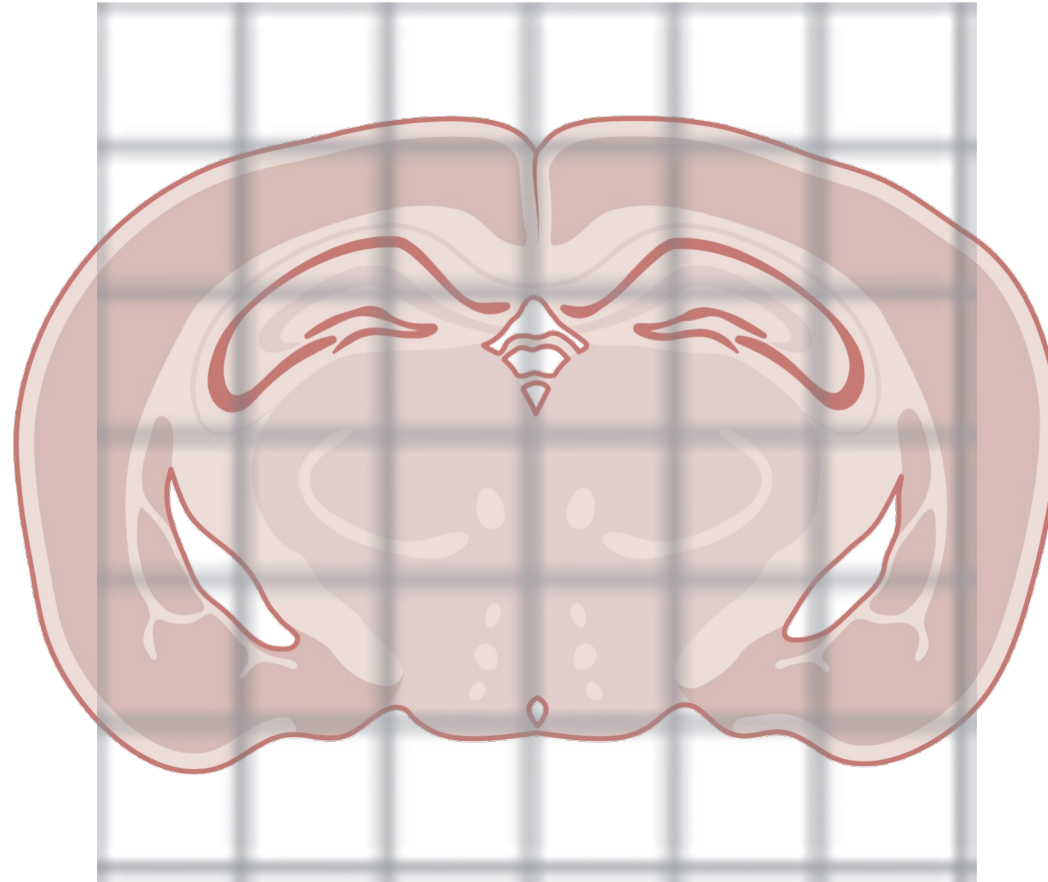


Step 4: Obtain masks/contours for each cell type



Tabs:

*Selection -> Segmentation -> **Remove duplicates** -> Mask operators -> List creator -> Feature extraction -> ML classification -> Contour export*



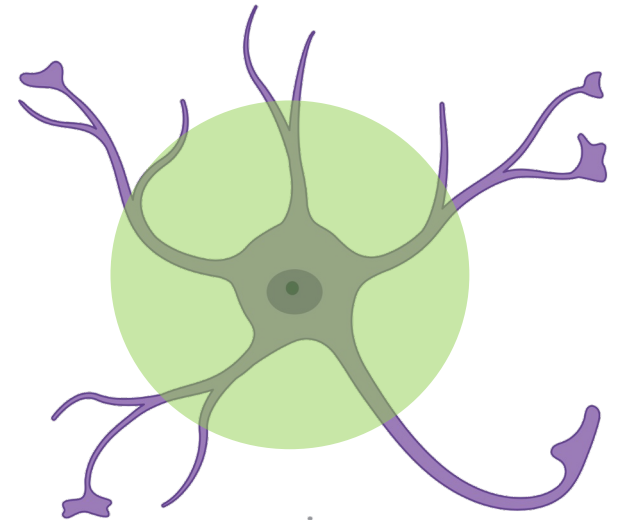
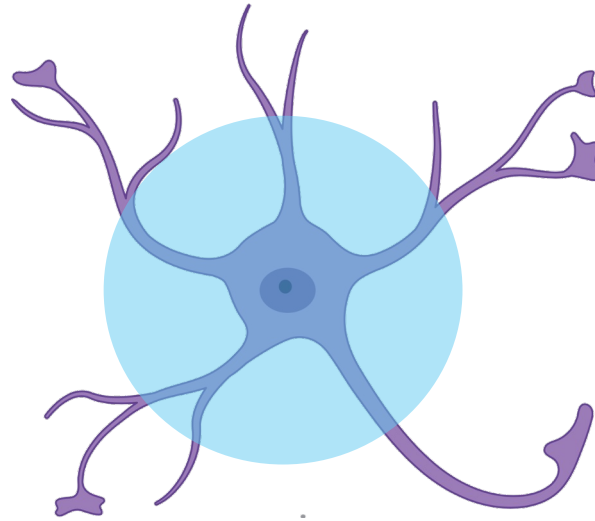
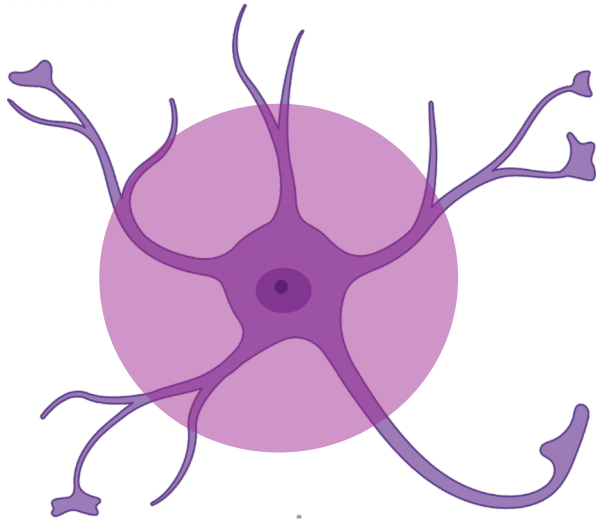
Step 4: Obtain masks/contours for each cell type



Tabs:

*Selection -> Segmentation -> Remove duplicates -> **Mask operators** ->
List creator -> Feature extraction -> ML classification -> Contour export*

Dilation



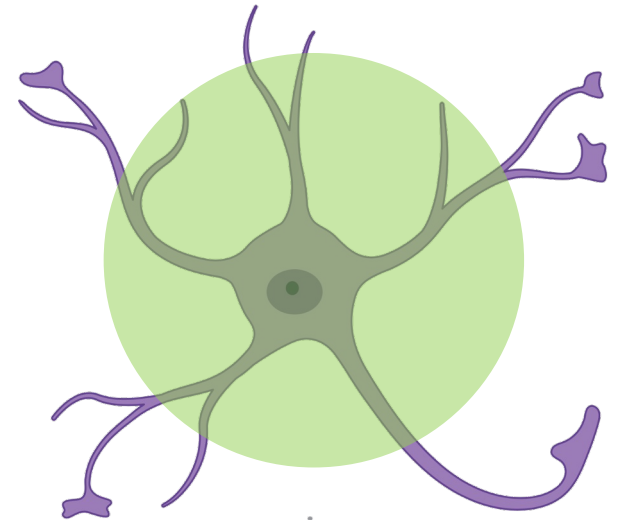
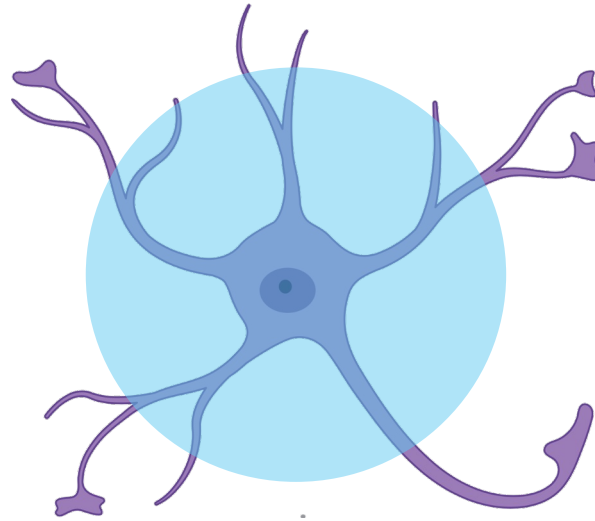
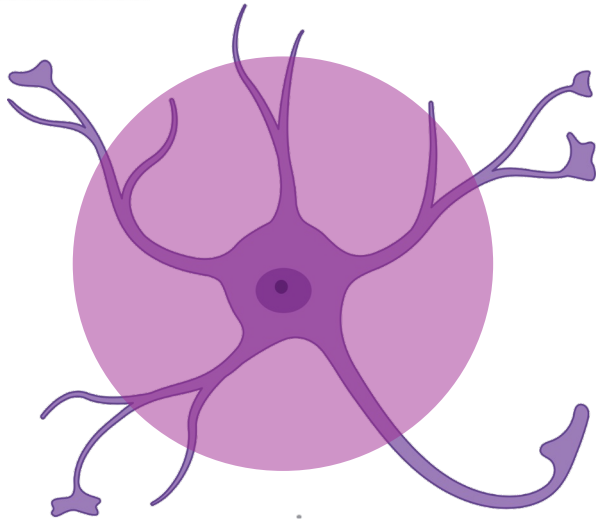
Step 4: Obtain masks/contours for each cell type



Tabs:

*Selection -> Segmentation -> Remove duplicates -> **Mask operators** ->
List creator -> Feature extraction -> ML classification -> Contour export*

Reduction

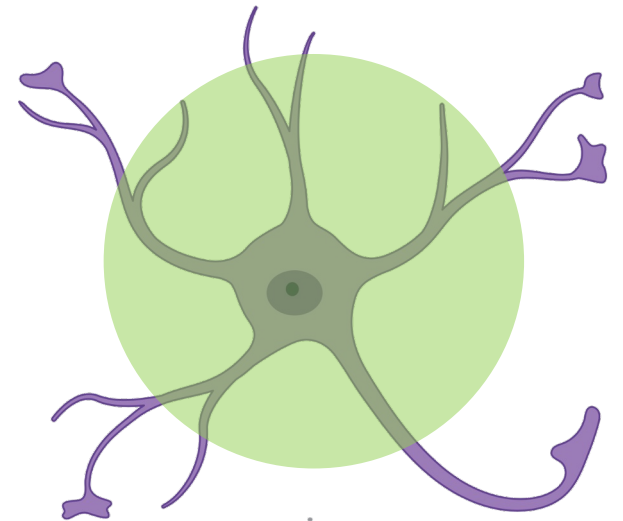
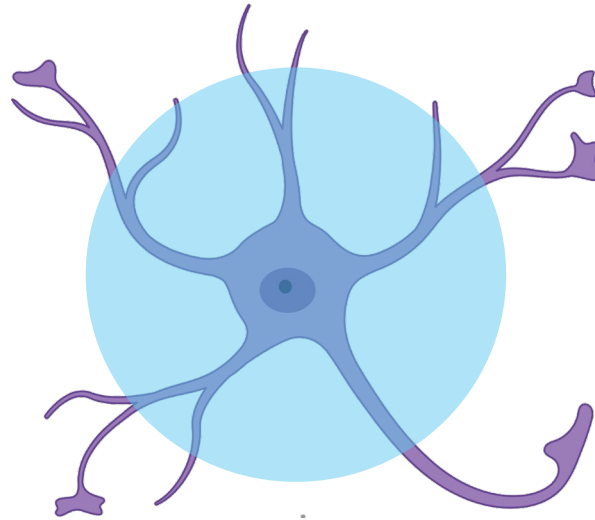
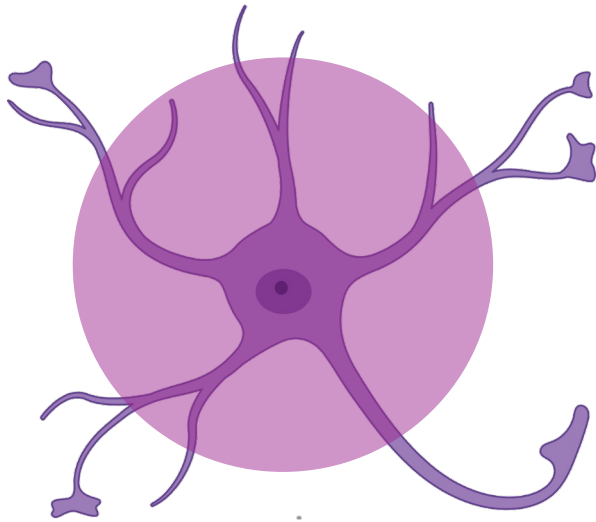


Step 4: Obtain masks/contours for each cell type



Tabs:

*Selection -> Segmentation -> Remove duplicates -> **Mask operators** -> List creator -> Feature extraction -> ML classification -> Contour export*



***Goal is to obtain shapes amenable to laser microdissection (large enough, blob-ish); “close shape” and “dilate” worked well throughout**

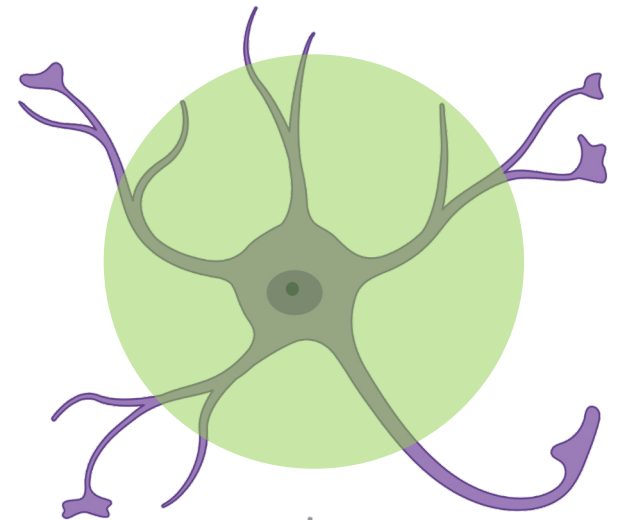
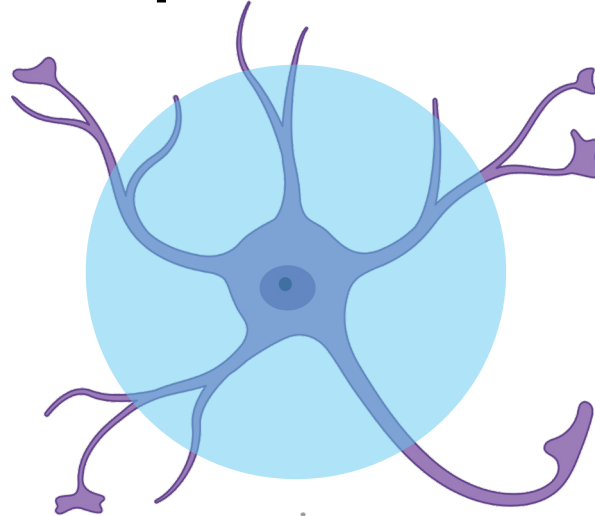
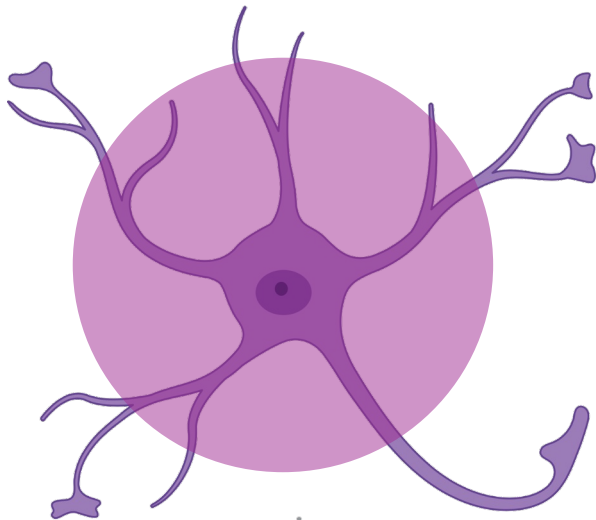
Step 4: Obtain masks/contours for each cell type



Tabs:

Selection -> Segmentation -> Remove duplicates -> Mask operators -> List creator -> Feature extraction -> ML classification -> Contour export

Select masks as input and the program outputs them as a list (required input format for feature extraction)



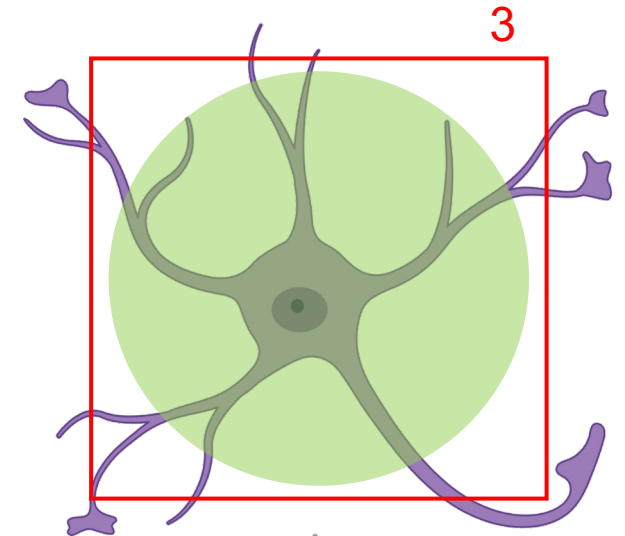
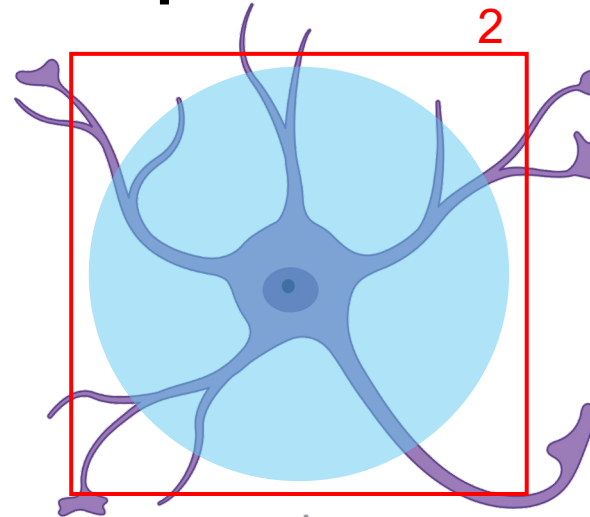
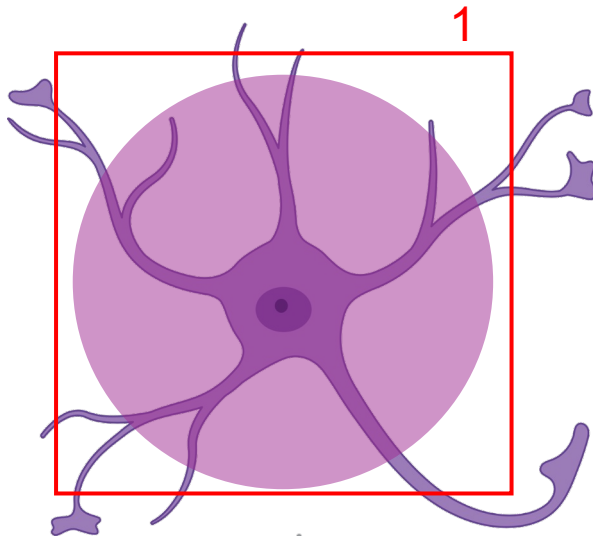
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Tabs:

*Selection -> Segmentation -> Remove duplicates -> Mask operators -> List creator -> **Feature extraction** -> ML classification -> Contour export*

Extracts dozens of differentiating features for each mask (e.g. eccentricity, area, length, width, intensity, etc.) to provide input for feature-based ML classification

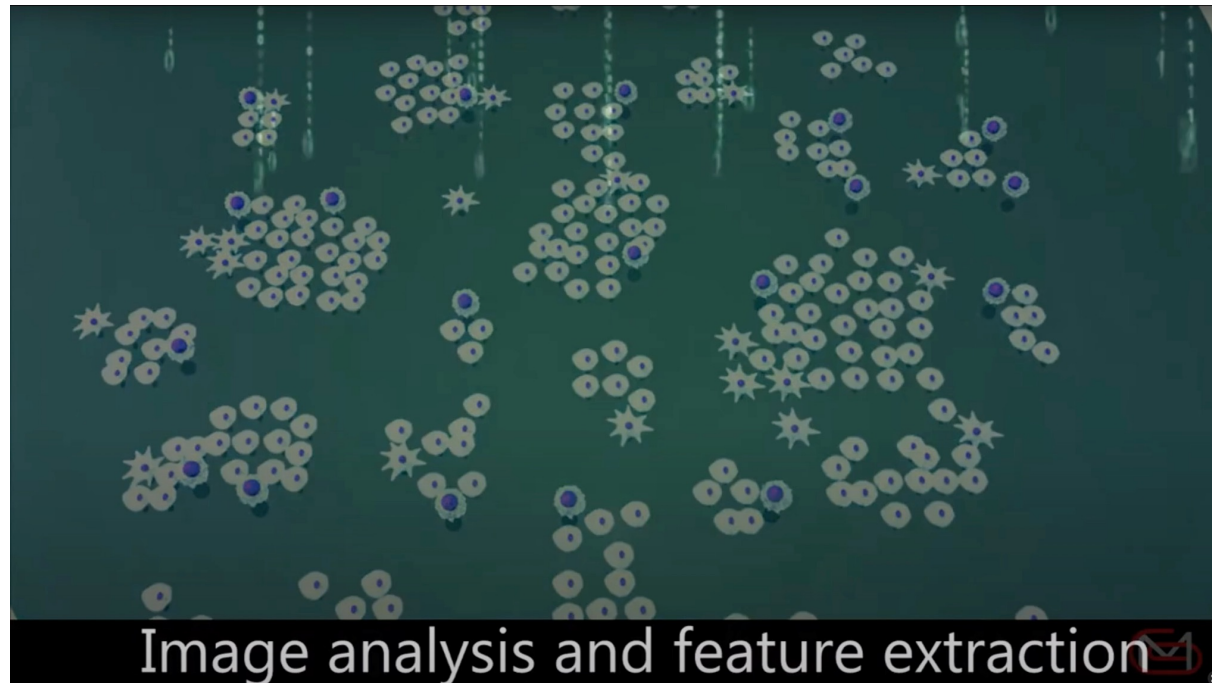
Step 4: Obtain masks/contours for each cell type



Tabs:

*Selection -> Segmentation -> Remove duplicates -> Mask operators ->
List creator -> Feature extraction -> **ML classification** -> Contour export*

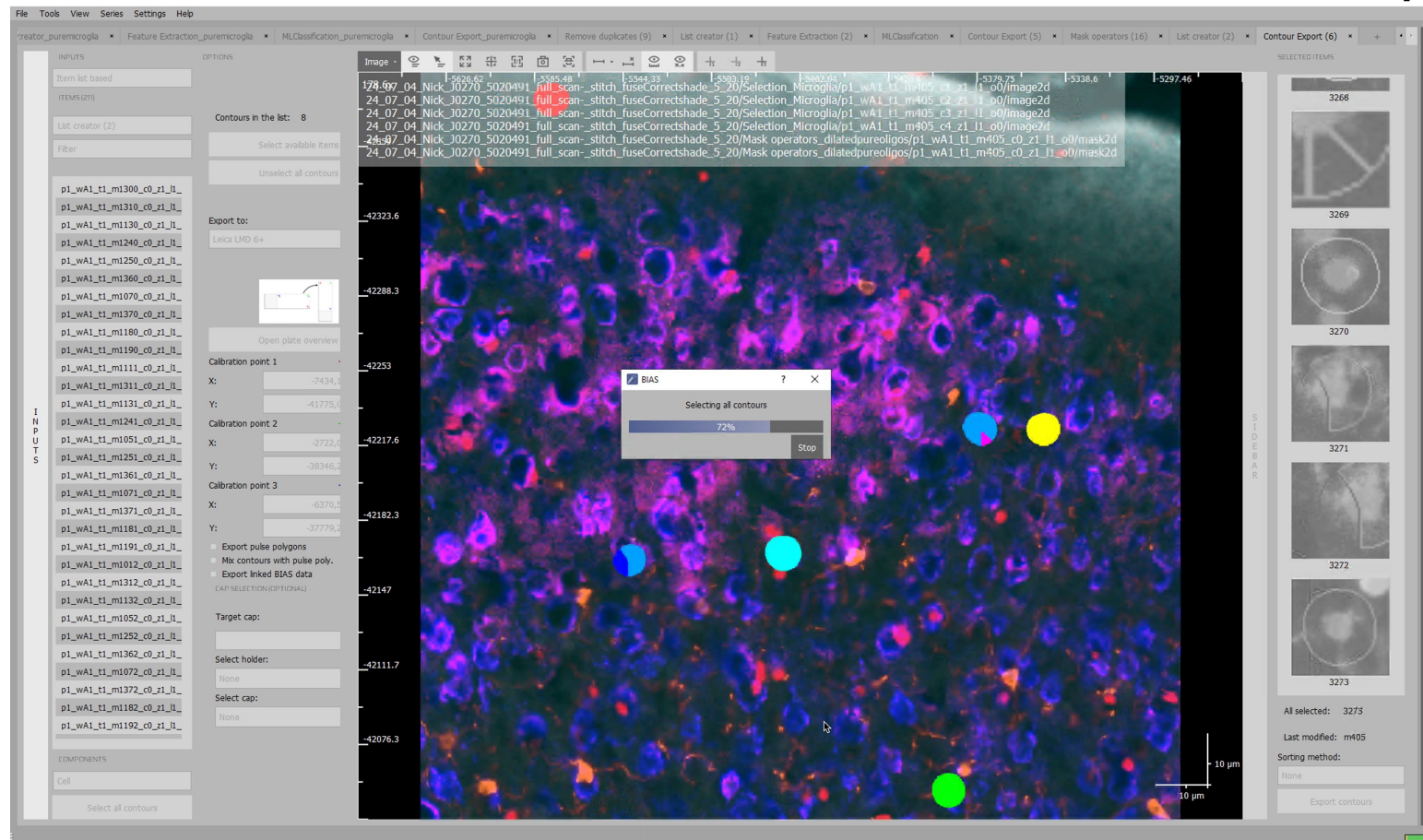
Create classes (i.e. real astrocytes, noise, unsure), train ML algorithm by manual selection of 10+ cells per class, and apply to all tiles



Step 4: Obtain masks/contours for each cell type

Tabs:

*Selection -> Segmentation -> Remove duplicates -> Mask operators -> List creator -> Feature extraction -> ML classification -> **Contour export***



Step 5: Laser microdissection (LMD)

**Masks superimposed on tissue and cut out with laser.
Specified wells aligned underneath each shape.**

Must first optimize for laser power, speed, and puncture settings.

Caveats: damaged membrane (liquid underneath) prevents shapes from falling into well (because they stay stuck)

Step 5: Laser microdissection (LMD)

remotedesktop.google.com/access/session/bc24efe0-7d9f-7043-15ac-a41ae1ed5405

Leica Laser Microdissection - [Live]

File View Camera Image Laser Auto Detection Options Help

Save Save +L Live Freeze MicCtrl Specimen Collector Focus Unload Laser

Select Draw Ellipse Rect PtoP Reuse Text Cut Text Detect Measure

Camera Settings

Exposure
☒ Automated
Exposure Time: Auto
Gain: 1.0

Camera:
Binning: Binning_Level_None
Color: White Balance
Shading correction: Shading Correction
☐ Switch Image

Laser Control

Revolver Position: 5
Magnification: 63x
Power: 52
Aperture: 52
Speed: 1
Midpulse Count: 50
Final Pulse: 1
Head Current: 60%
Pulse Frequency: 60%
Offset: 10
AF before cut: ☒ Field of View
Calibrate
Auto Calibrate
Factory Settings

Summary

Collected: ☒ Set 1 ☐ Set 2

No.	Line	C	D	E
1	Line			
2	Line			
3	Line			
4	Line			
5	Line			

619 objects, 116594 μm^2 total Area, 180 μm^2 average area
Cutting: 2h 25min 1sec estimated balance time 116594 / 355408 μm^2

Shape List

No.	Area (μm^2)	Mag	Cut
2213	139	63.00	G7
2214	199	63.00	G7
2215	74	63.00	G7
2216	181	63.00	G7
2217	74	63.00	G7
2218	125	63.00	G7
2219	111	63.00	G7
2220	201	63.00	G7
2221	308	63.00	G7

Total selected area (μm^2): 659951

Clear Clear All Summary

Leica MICROSYSTEMS Change GUI Color

Collection Device: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

Slide Holder

Stage Position Memory: 5 [4963 μm / 1687 μm] ☒ Restore Map

Add Clear All Clear Positions

Specimen Overview: Draw in OV area

Create Fast Overview ☒ Rectangle ☐ Ellipse

Inspect all collectors ☐ Autofocus

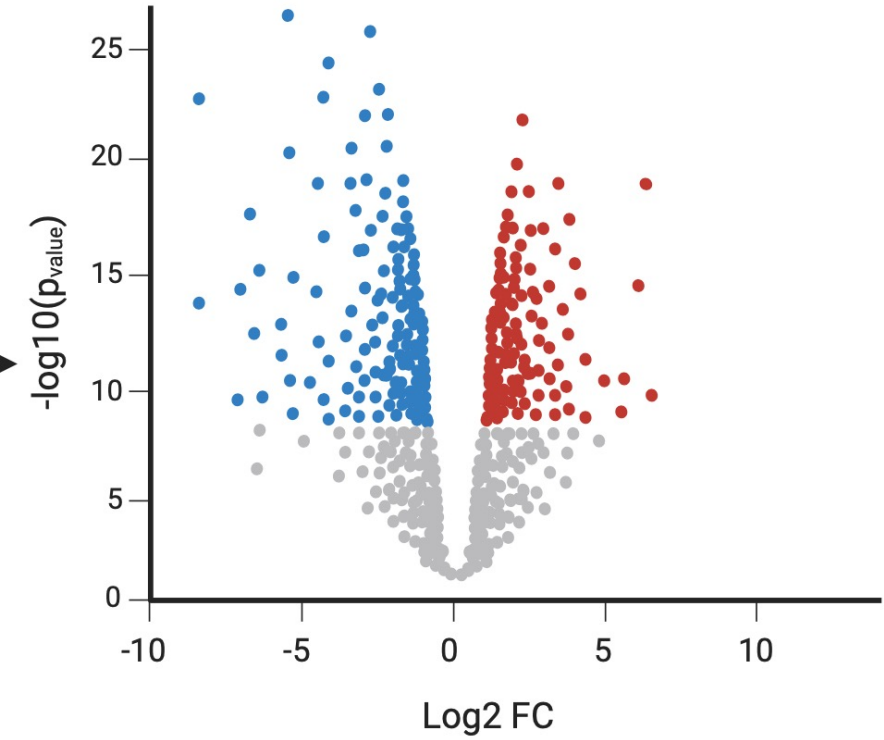
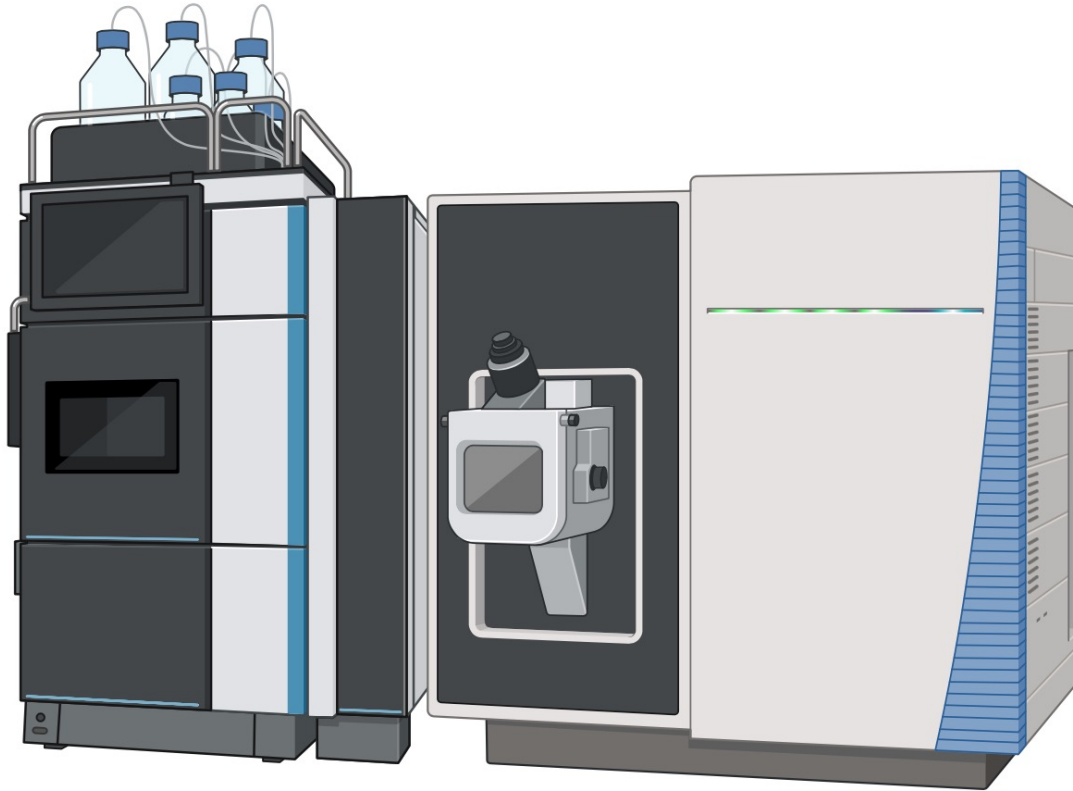
LEICA LMD7

HydraFourSlides

x-y-live: 1886 / 928 x-y-stage: 49786 μm / 15909 μm Magnification: 63x

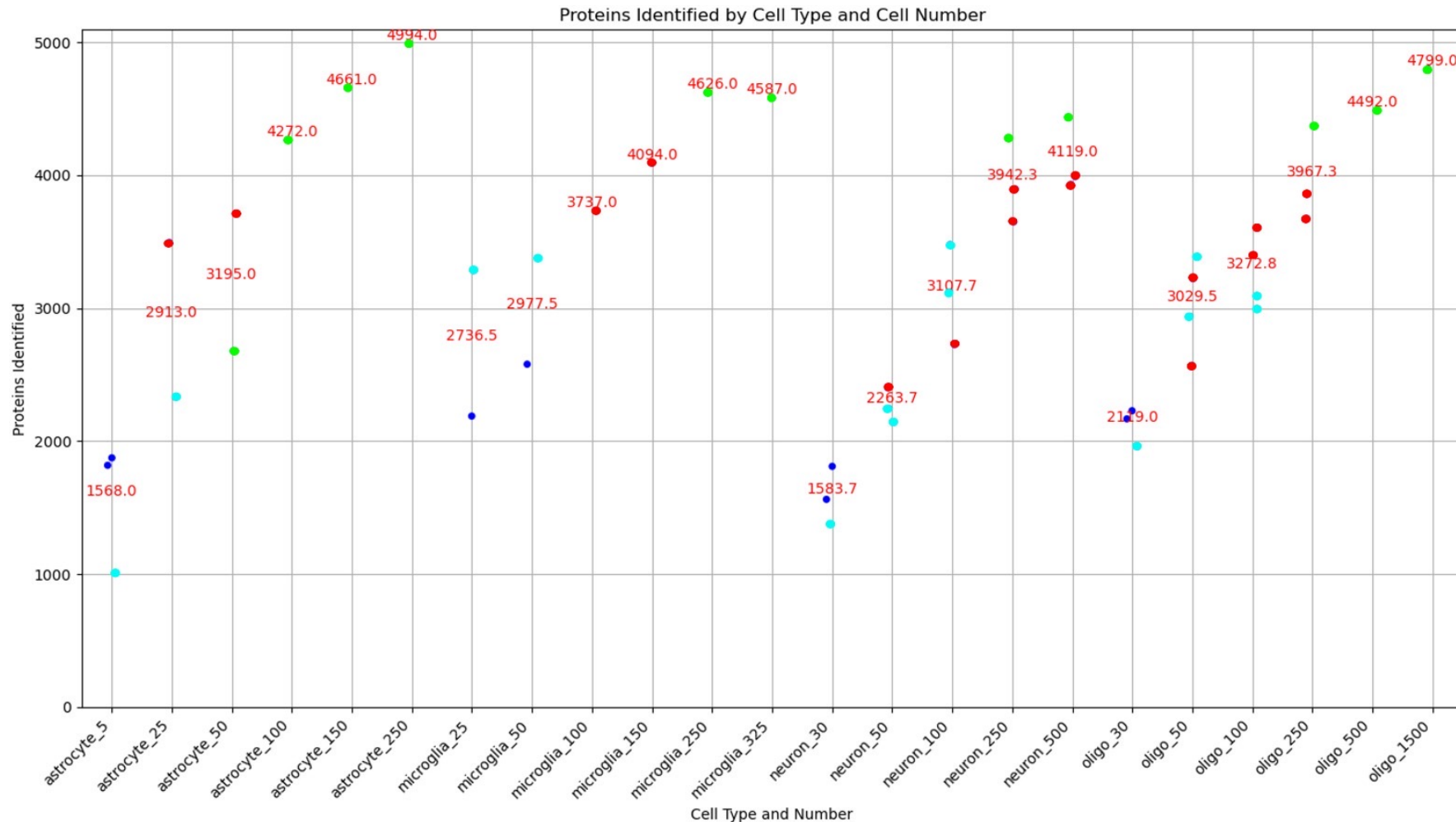
12:01 PM 7/26/2024

Step 6: Proteomics



Input Experiment:

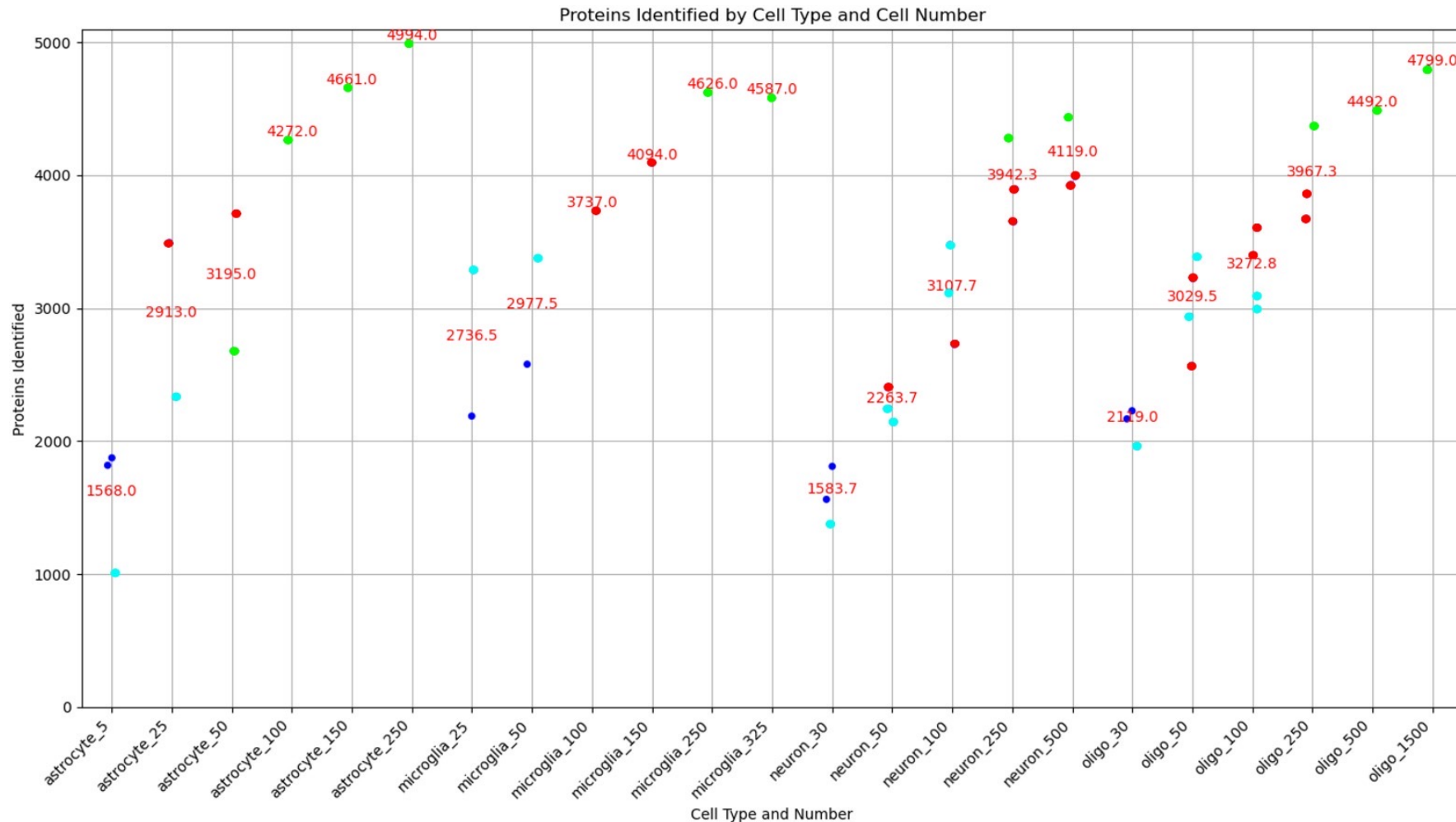
Determining number of cells needed to capture maximum number of proteins



Key:

- Method 1
- Method 2
- Method 3
- Method 4

Input Experiment: Determining number of cells needed to capture maximum number of proteins



Key:

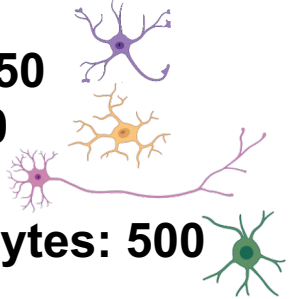
Method 1 (winner!)

Astrocytes: 250

Microglia: 250

Neurons: 250

Oligodendrocytes: 500



Next steps

IDX of all samples collected

Data analysis by cell type

Cross-validation by Xenium spatial transcriptomics

Future experiments on proteomic hits

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Andy Hims

Maryam Idris

Aleksandra Levina

Cindy Lin

Hunter Liu

Uche Medoh

Austin Murchinson

Shadler Nguyen

Kwamina Nyame

Eshaan Rawat

Samantha Scharenberg

George Walters-Marrah

Jian Xiong



Department of Proteomics and Signal Transduction, MPI of Biochemistry:

Matthias Mann | Marc Oeller | Edwin Rodriguez



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FÜR BIOCHEMIE

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MEDICINE

Neurosciences PhD Program

Stanford | The Europe Center

Freeman Spogli Institute and Stanford Global Studies

Stanford Club of Germany
Freunde der Stanford University e.V.



Final Thoughts



We were able to dissect out 4 major cell types from 3 mouse models of neurodegenerative disease. Over the summer, I established reliable methods for staining and conducting deep visual proteomics on murine brain tissue, which will help the scientific community conduct future experiments with other mouse models. We also now have highly accurate cell contour generating algorithms powered by ML, which will accelerate future studies, namely with neurons and oligodendrocytes.