Bioimage Informatics

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Course

Image Registration

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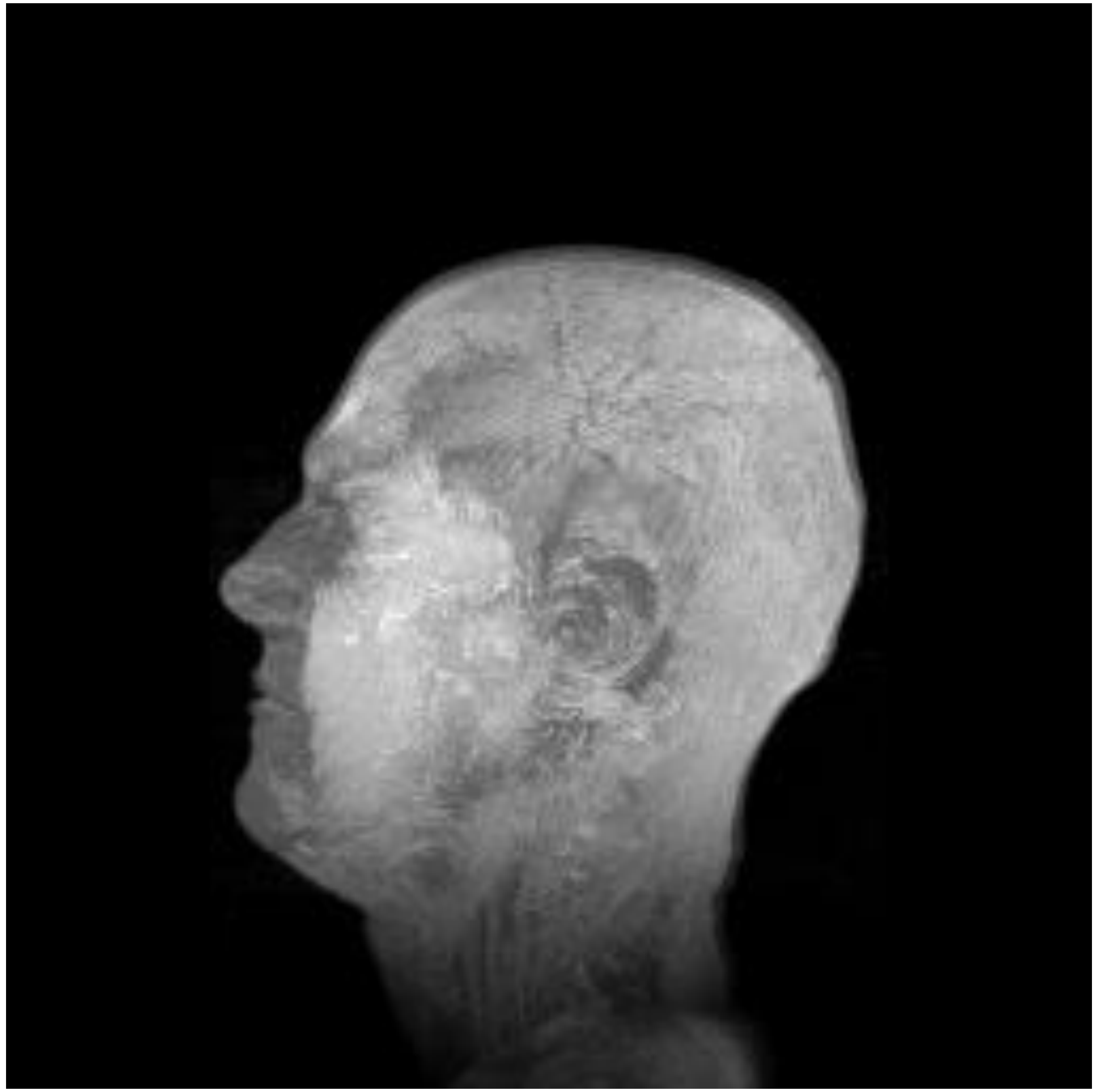
Image registration

- Overlay of two images taken from the same scene.
- Geometrical alignment

https://www.mathworks.com/discovery/image-registration.html

- Different viewpoints (multiview analysis)
- Different times (multitemporal analysis)
- Different sensors (multimodal analysis)
- Scene to model registration

Image Registration



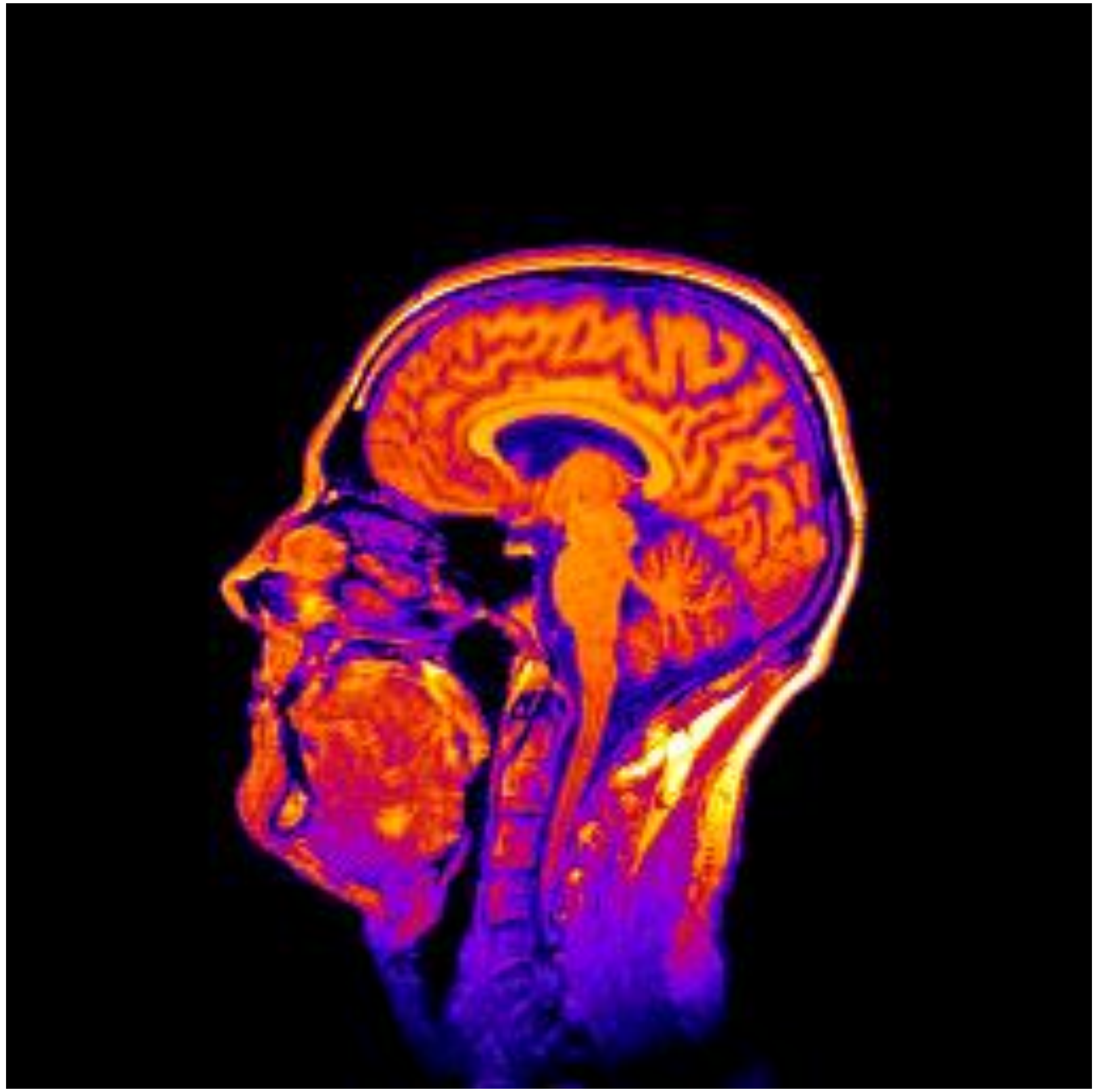


Image registration

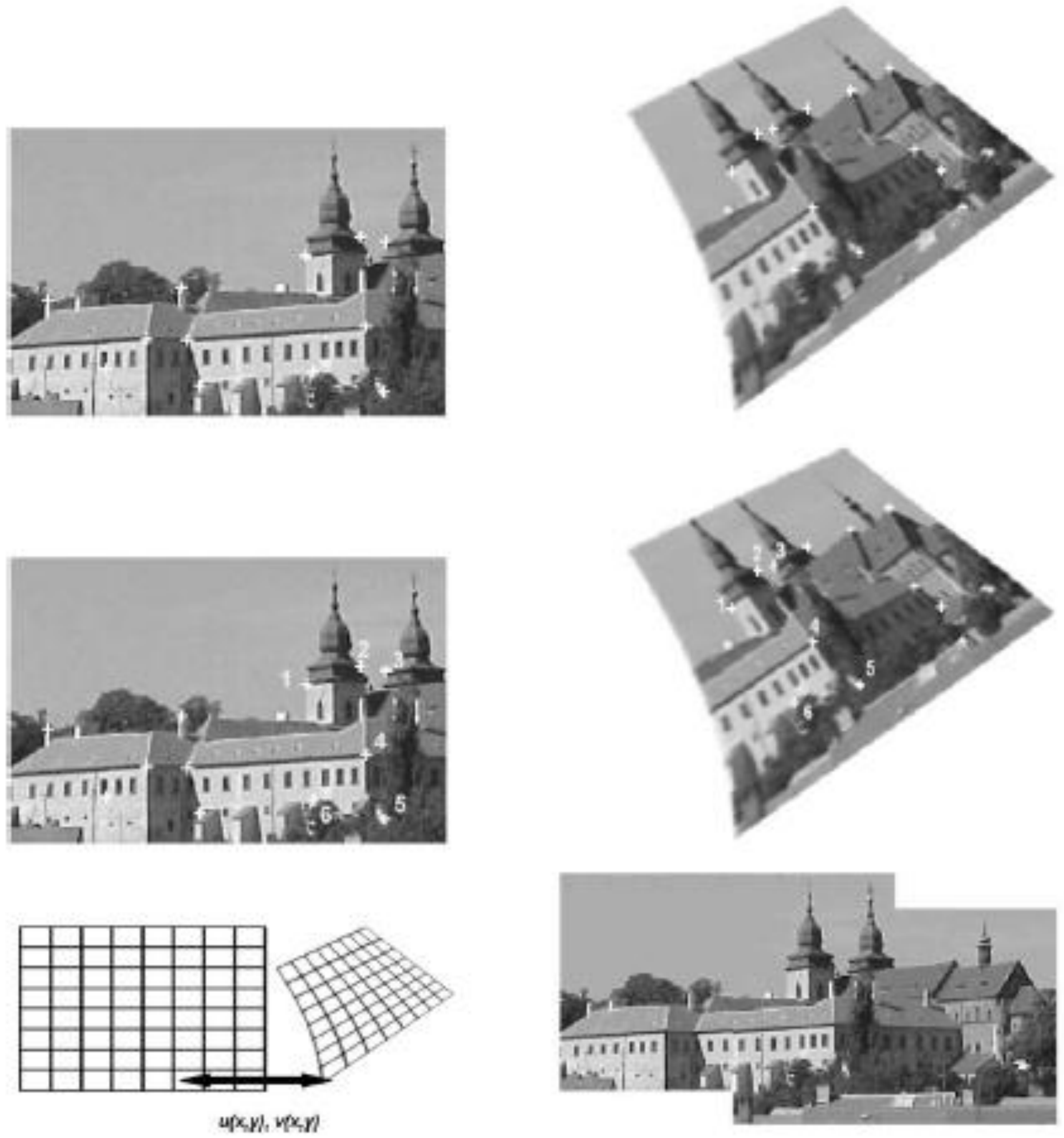


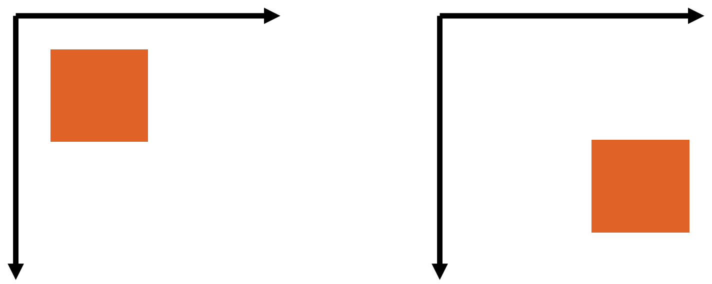
Image and Vision Computing 21 (2003) 977–1000

- Workflow
  - Feature detection
  - Feature matching
  - Transform model estimation
  - Image resampling and transformation

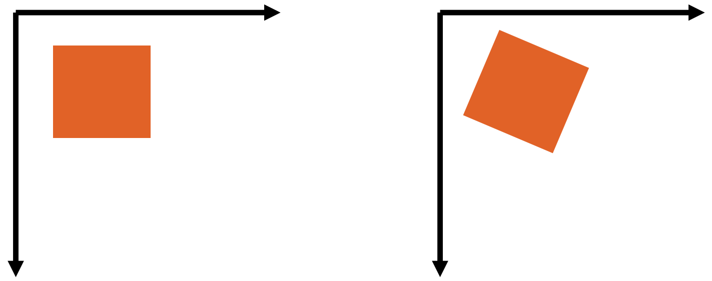
Tranformation

Geometric Operations

Translation: $\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} d_x \\ d_y \end{pmatrix} + \begin{pmatrix} x \\ y \end{pmatrix}$

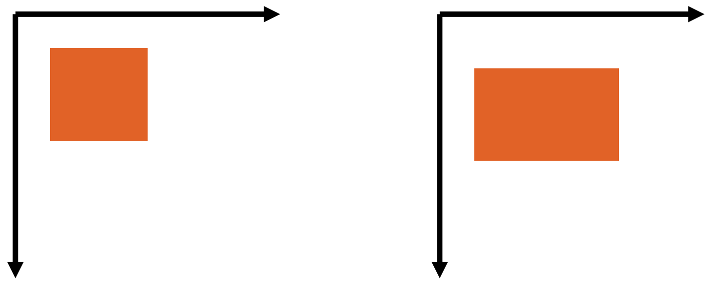


Rotation: $\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} \cos\alpha & -\sin\alpha \\ \sin\alpha & \cos\alpha \end{pmatrix} * \begin{pmatrix} x \\ y \end{pmatrix}$

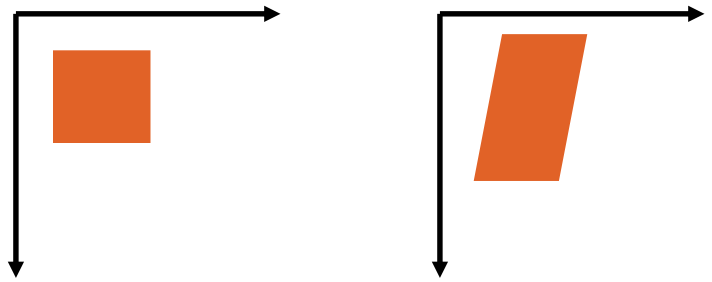


$$P(x, y) = P'(x', y')$$
$$x' = F(x)$$
$$y' = F(y)$$

Scaling: $\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} s_x & 0 \\ 0 & s_y \end{pmatrix} * \begin{pmatrix} x \\ y \end{pmatrix}$



Shearing: $\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} 1 & b_x \\ b_y & 1 \end{pmatrix} * \begin{pmatrix} x \\ y \end{pmatrix}$

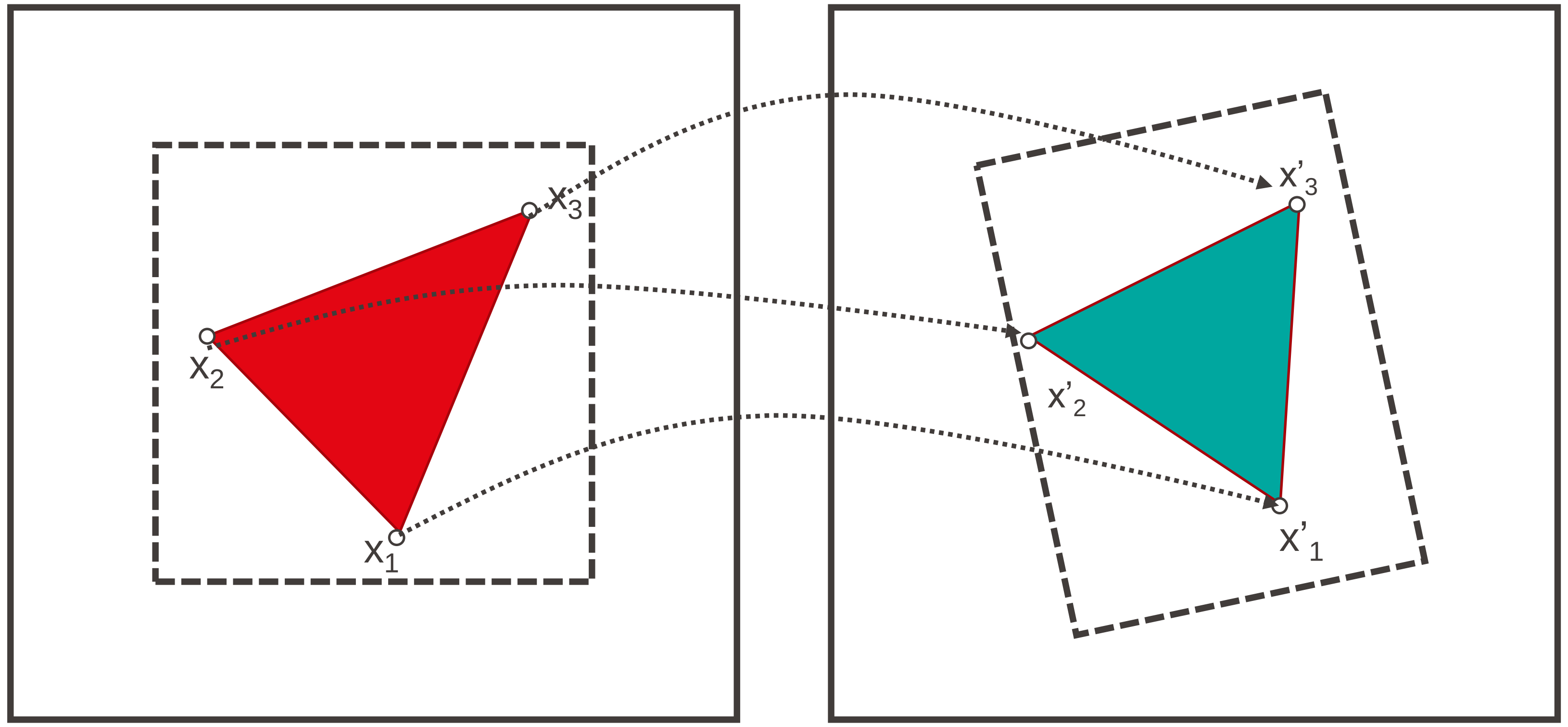


Affine Three point mapping

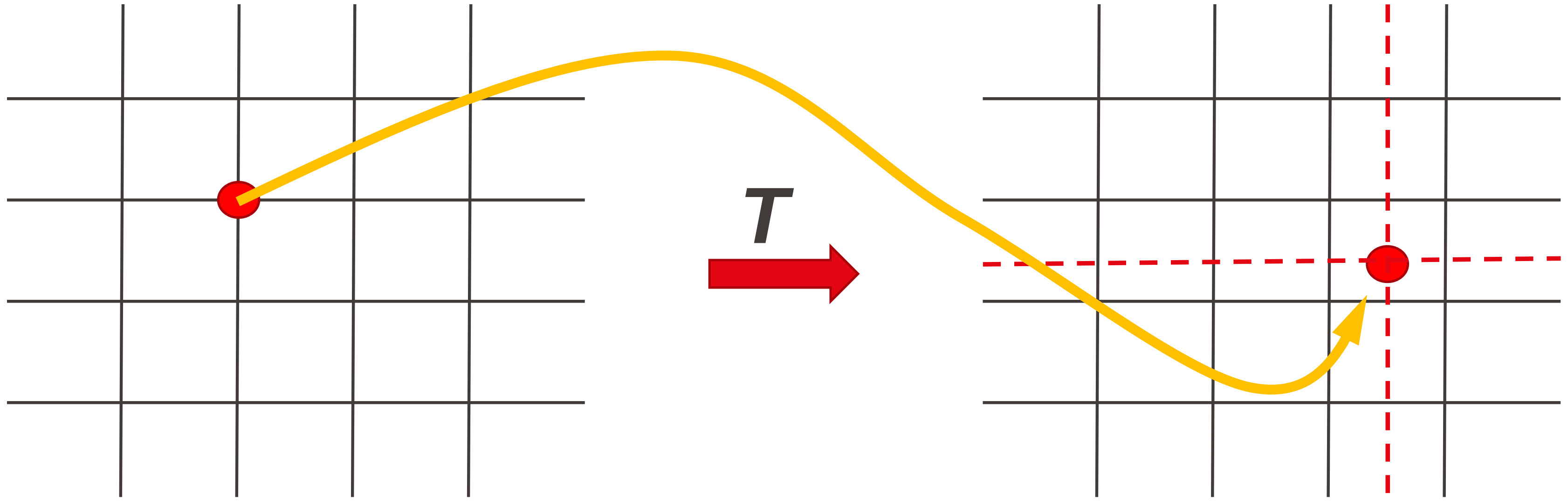
$$\begin{pmatrix} x' \\ y' \\ 1 \end{pmatrix} = \begin{pmatrix} x + d_x \\ y + d_y \\ 1 \end{pmatrix} = \begin{pmatrix} 1 & 0 & d_x \\ 0 & 1 & d_y \\ 0 & 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} x \\ y \\ 1 \end{pmatrix}$$

$$\begin{pmatrix} x' \\ y' \\ 1 \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ 0 & 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} x \\ y \\ 1 \end{pmatrix}$$

Transformation matrix T

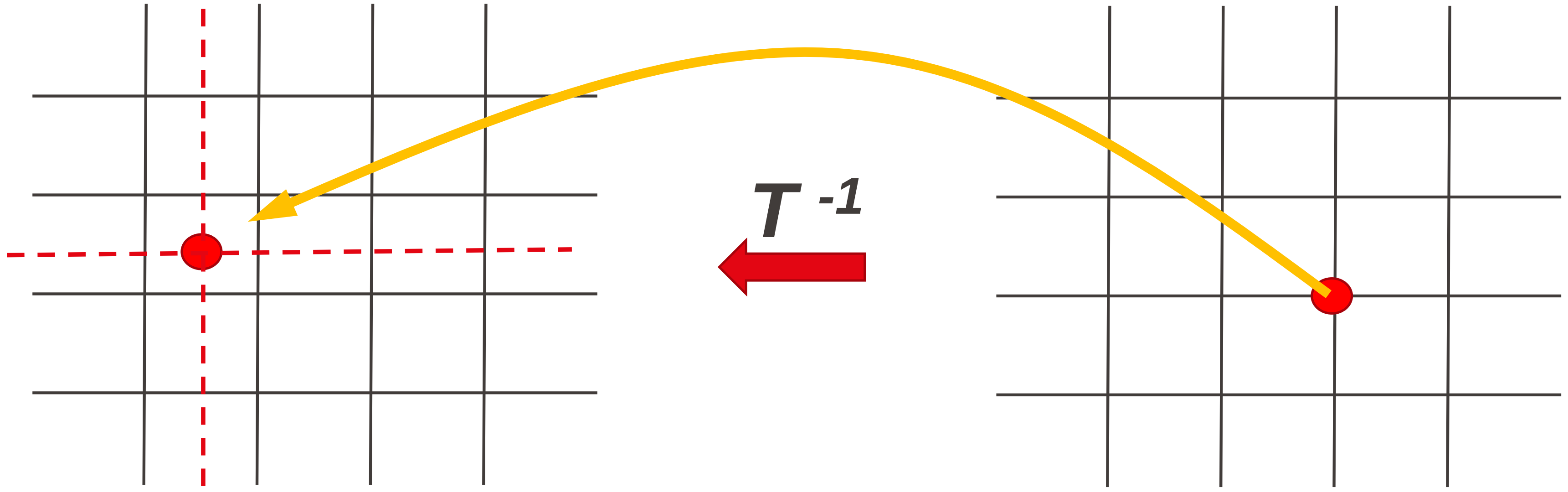


Resampling



Source-to-target mapping

source image target image

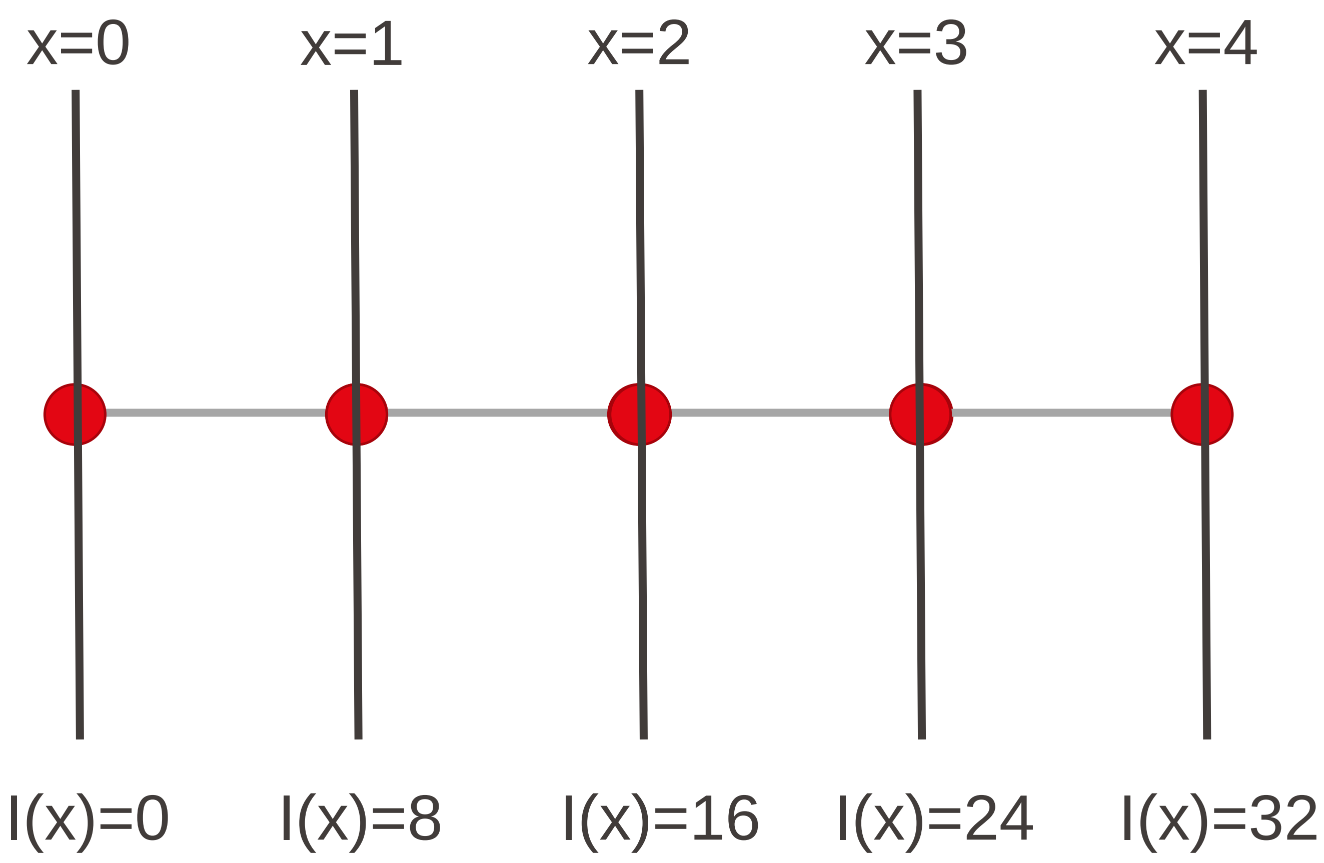


Target-to-source mapping

source image target image

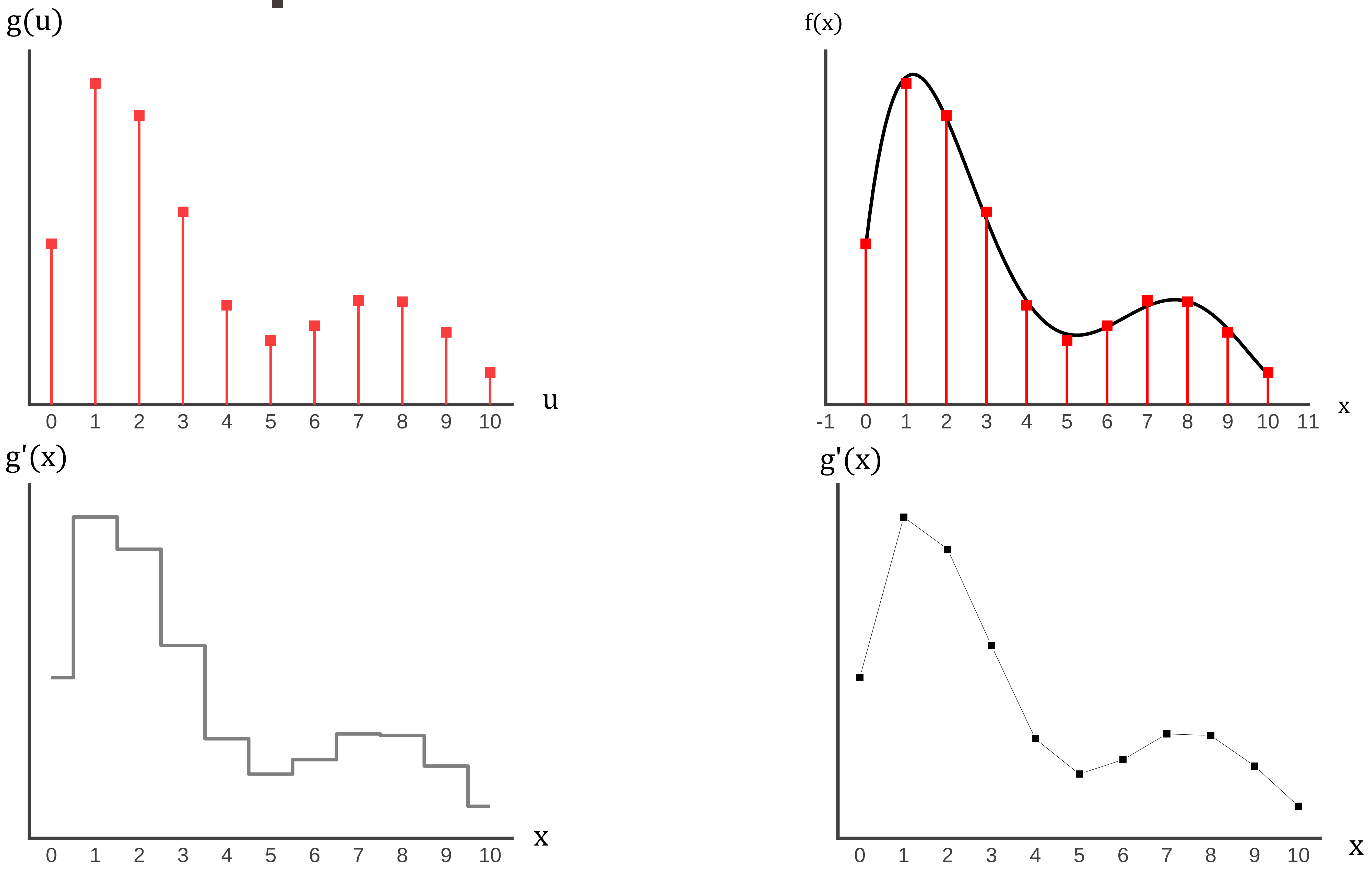
Interpolation

1D interpolation

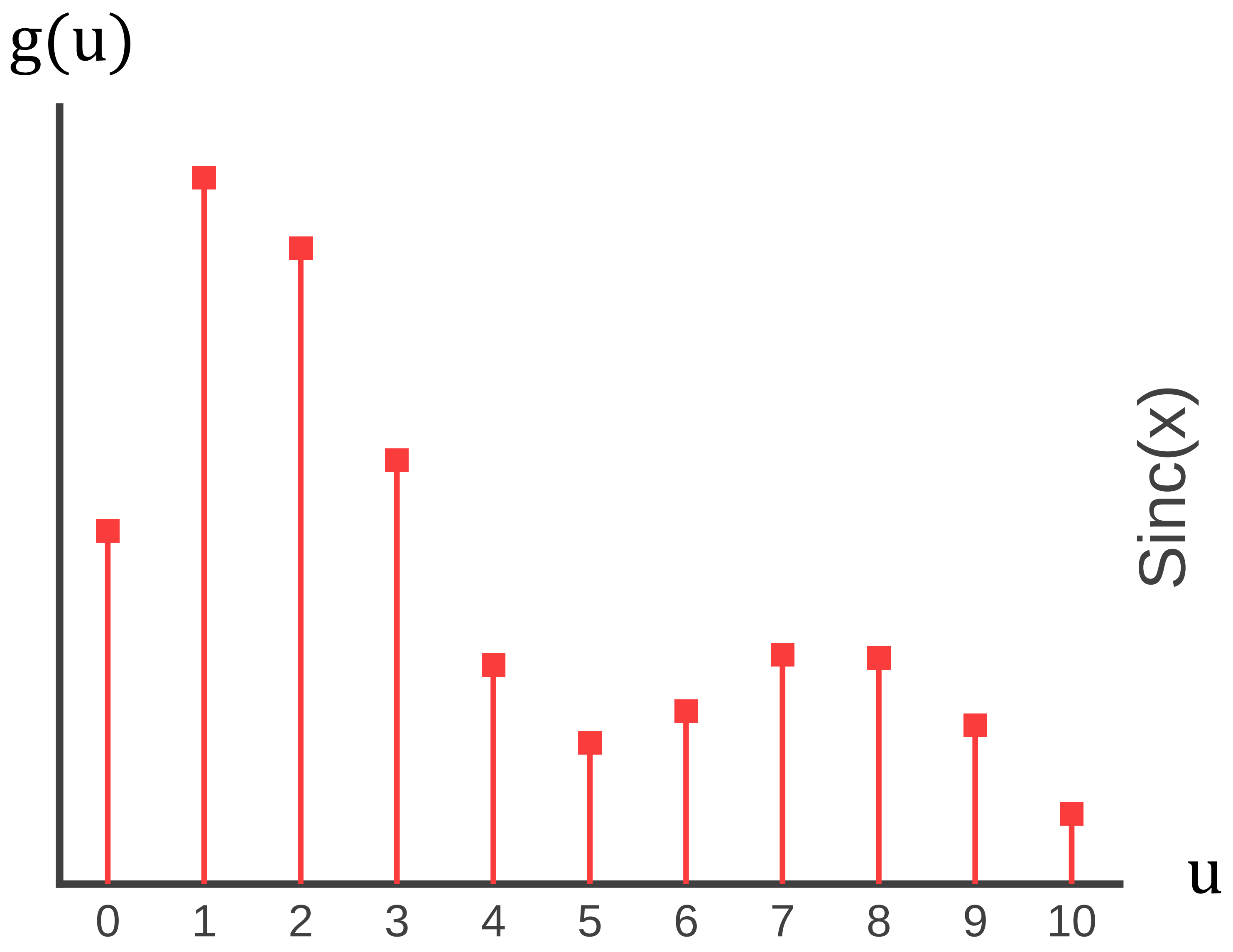


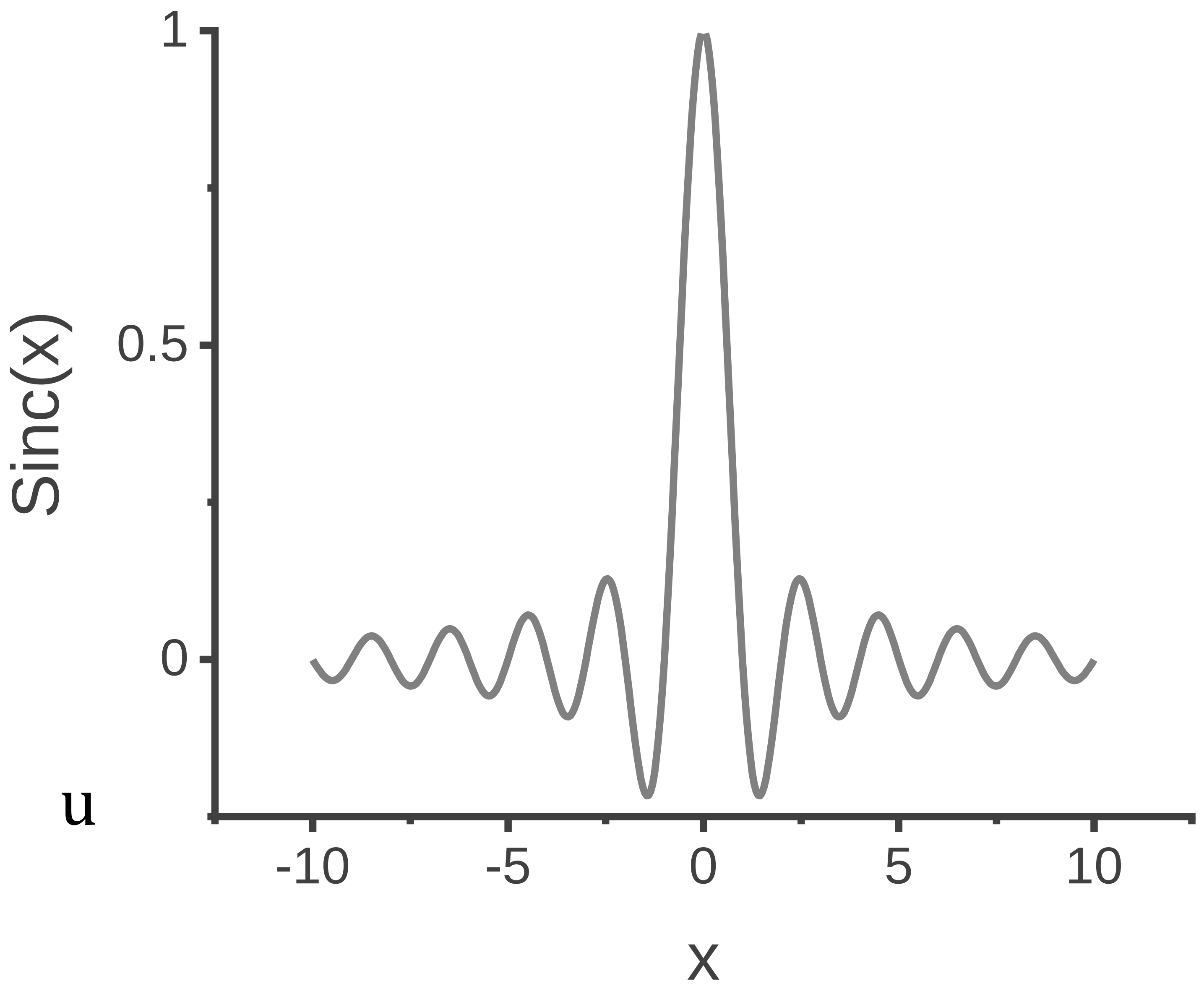
x	Nearest neighbour	Linear interpolation
0.25	0	2
0.5	0	4
0.75	8	6
1.25	8	10
1.75	16	14

1D Interpolation

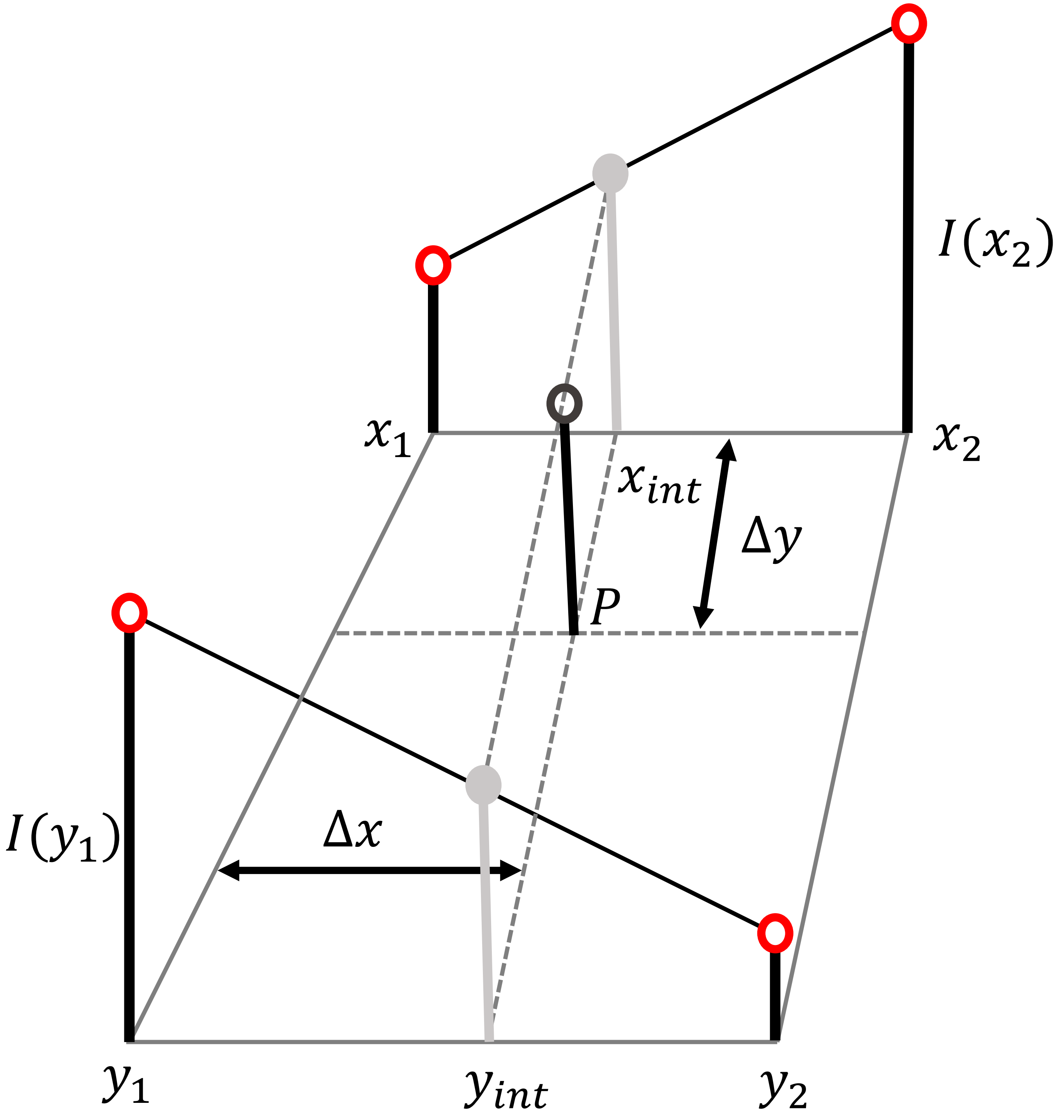


1D Interpolation





Bilinear interploation



$$I(x_{int}) = I(x_1) + (I(x_2) - I(x_1))\,\Delta x$$

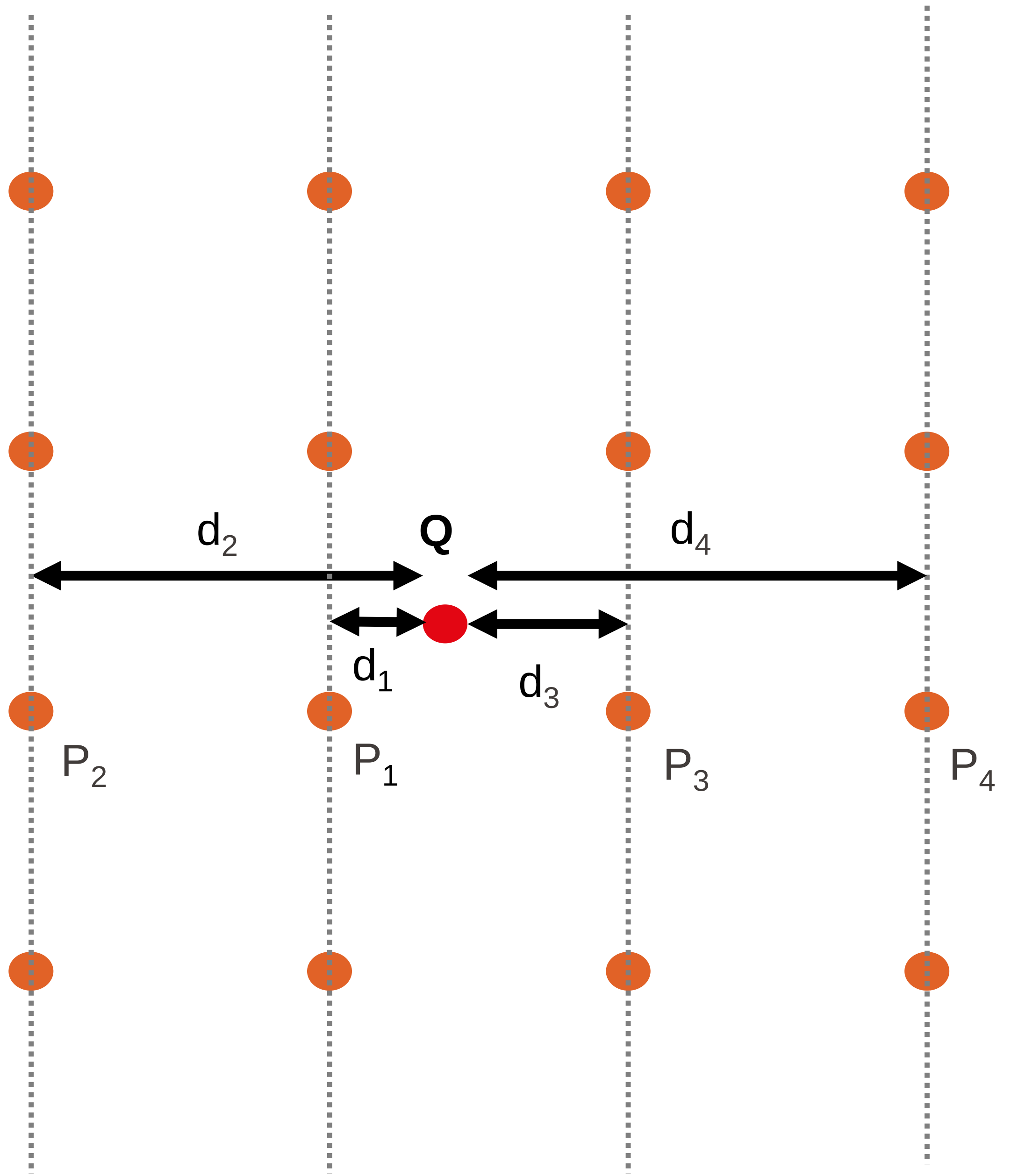
$$I(y_{int}) = I(y_1) + (I(y_2) - I(y_1))\,\Delta y$$

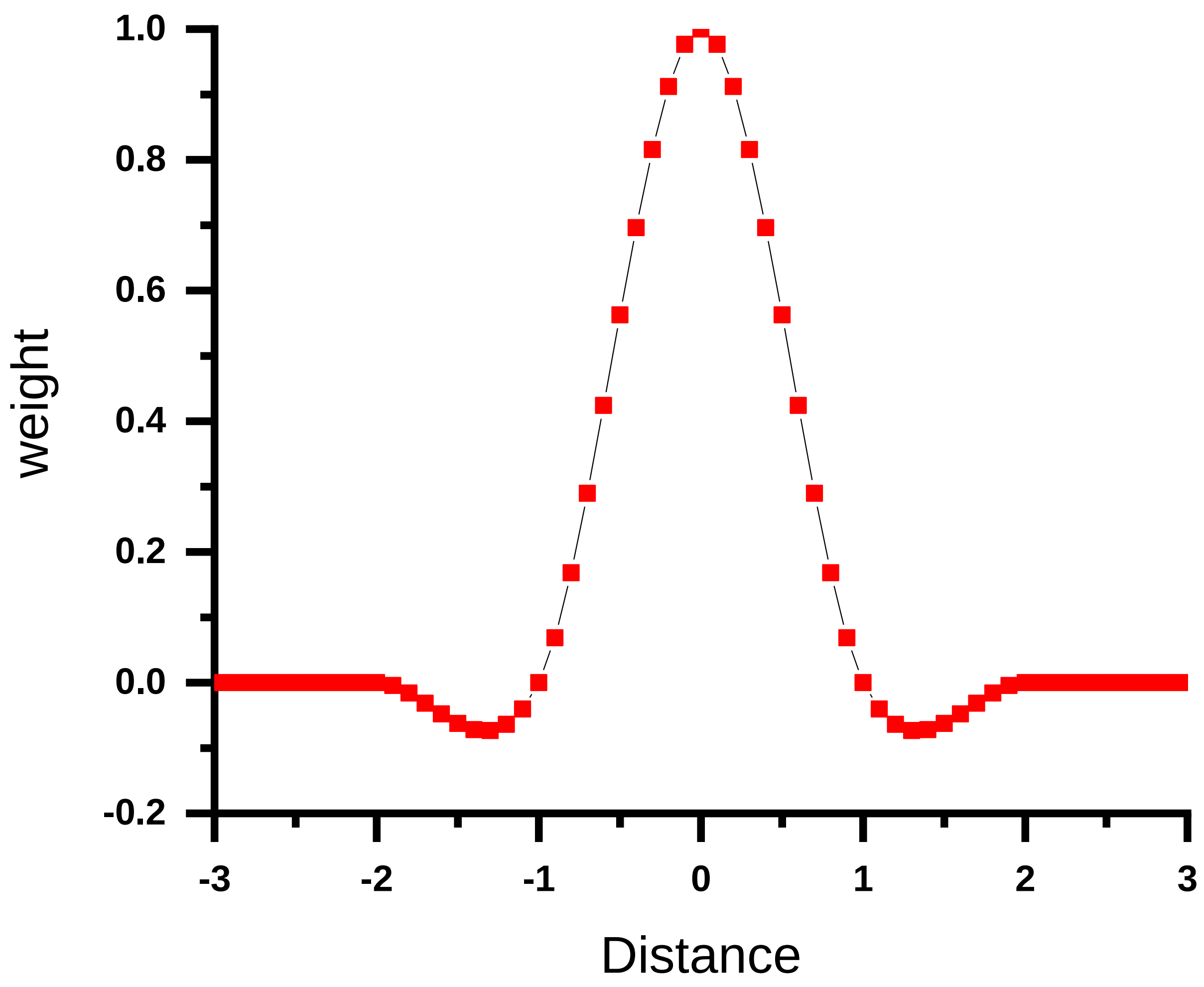
$$I(P) = I(x_{int}) + (I(y_{int}) - I(x_{int}))\,\Delta y$$

	x=0	x=1
y=0	0	16
y=1	32	64

	x=0	x=0.25	x=0.75	x=1
y=0	0	4	12	16
y=0.25	8	13	23	28
y=0.75	24	31	45	52
y=1	32	40	56	64

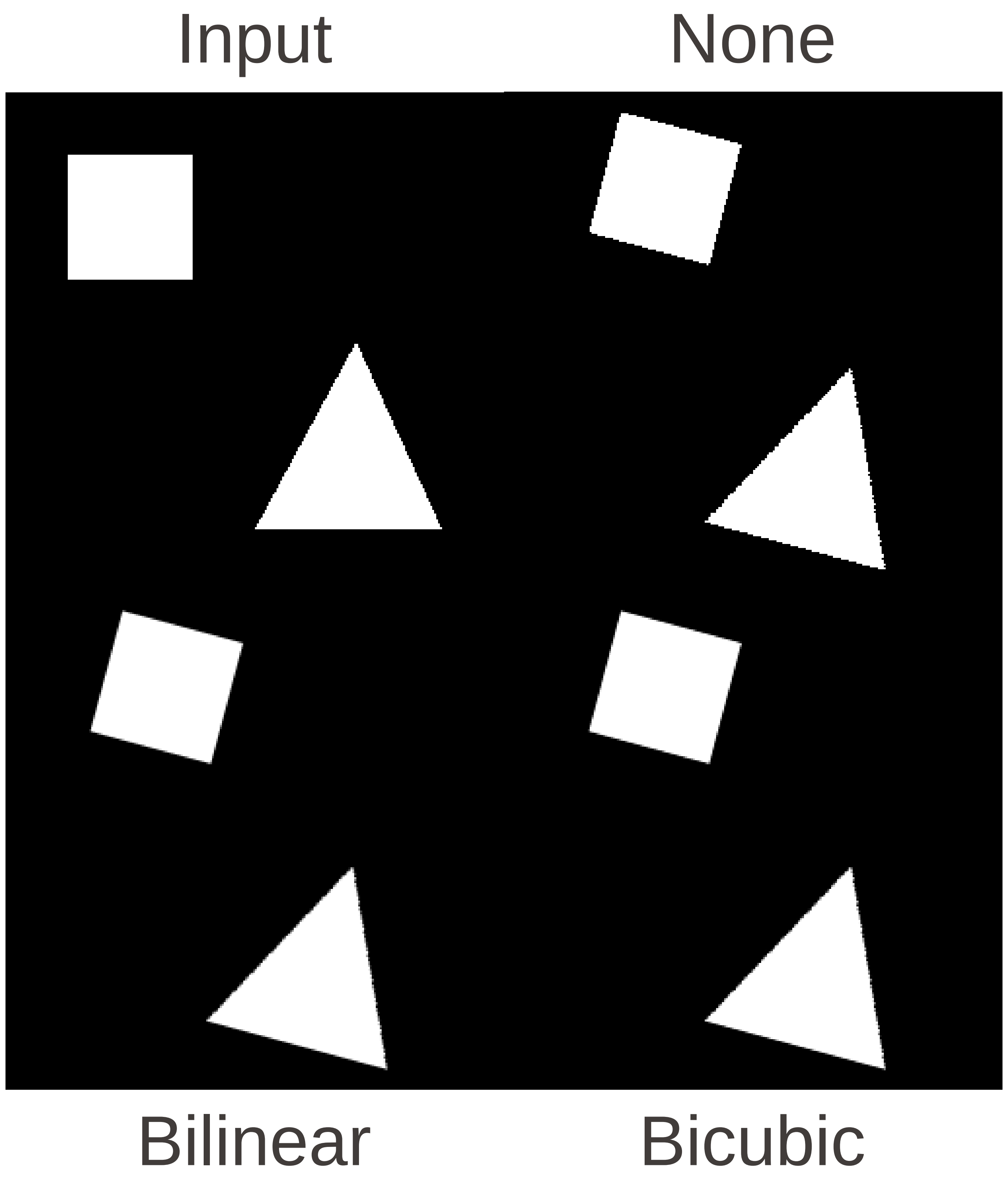
Cubic interpolation

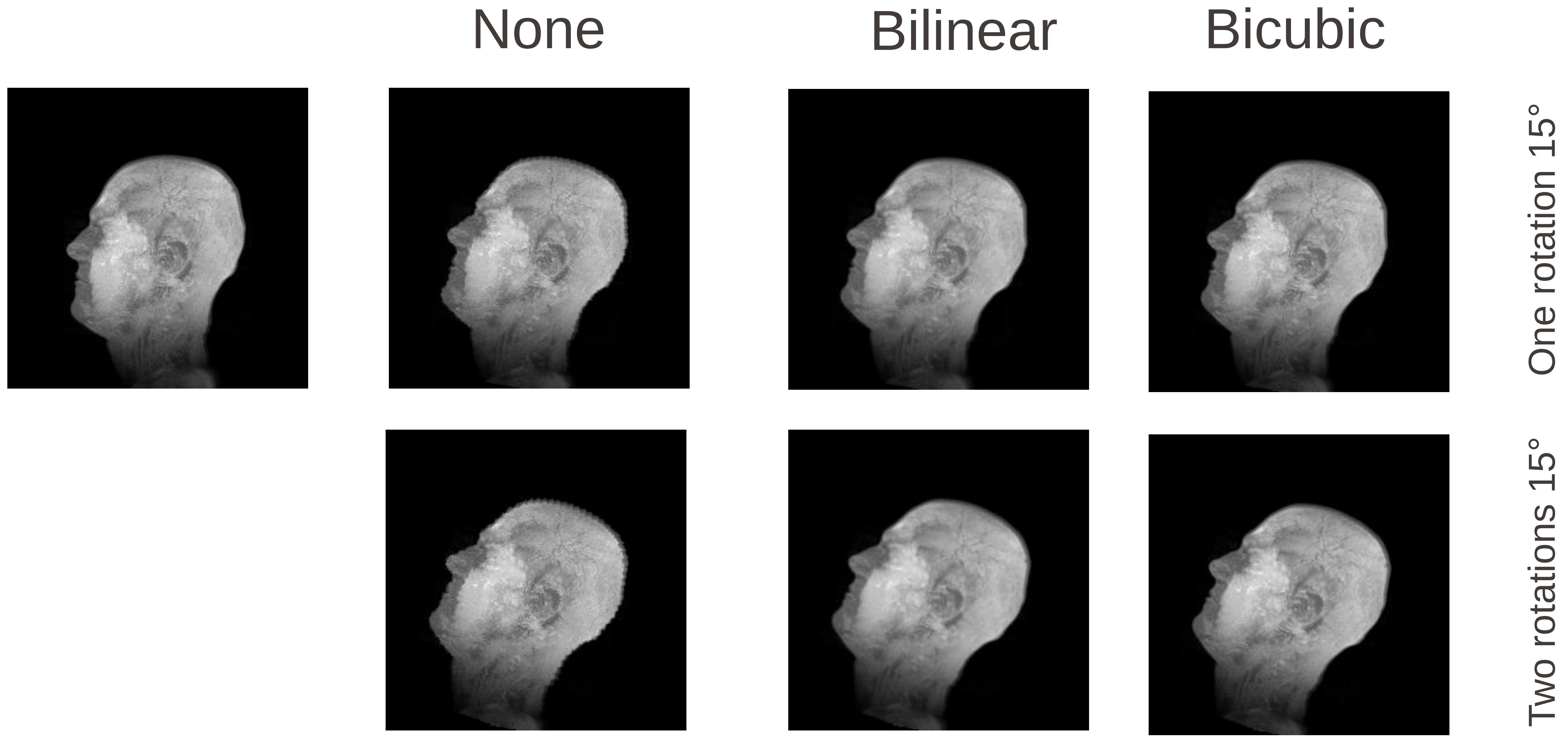




$$I_x(Q) = \sum_{4}^{n=1} I_n * w(d_n)$$

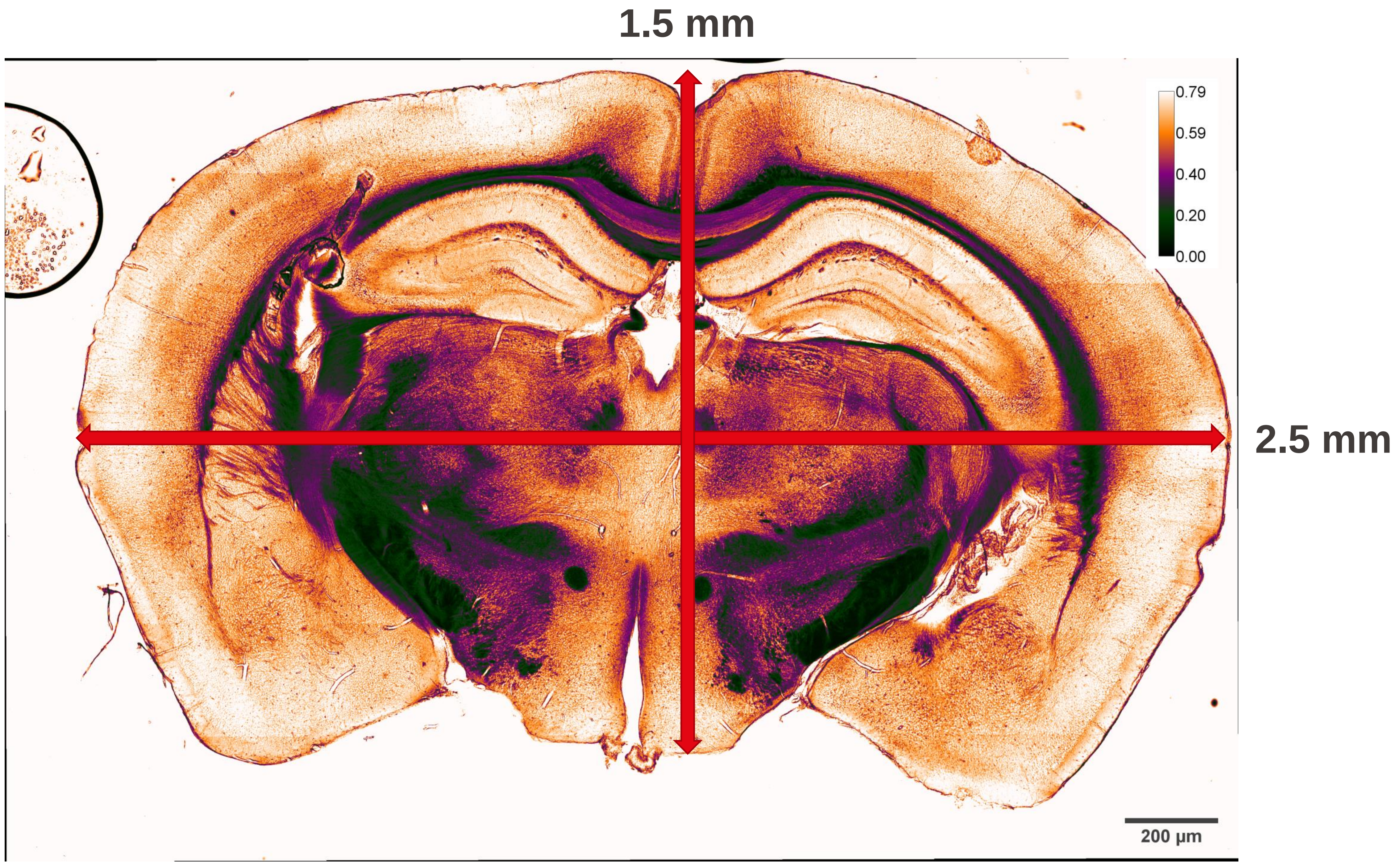
Interpolation



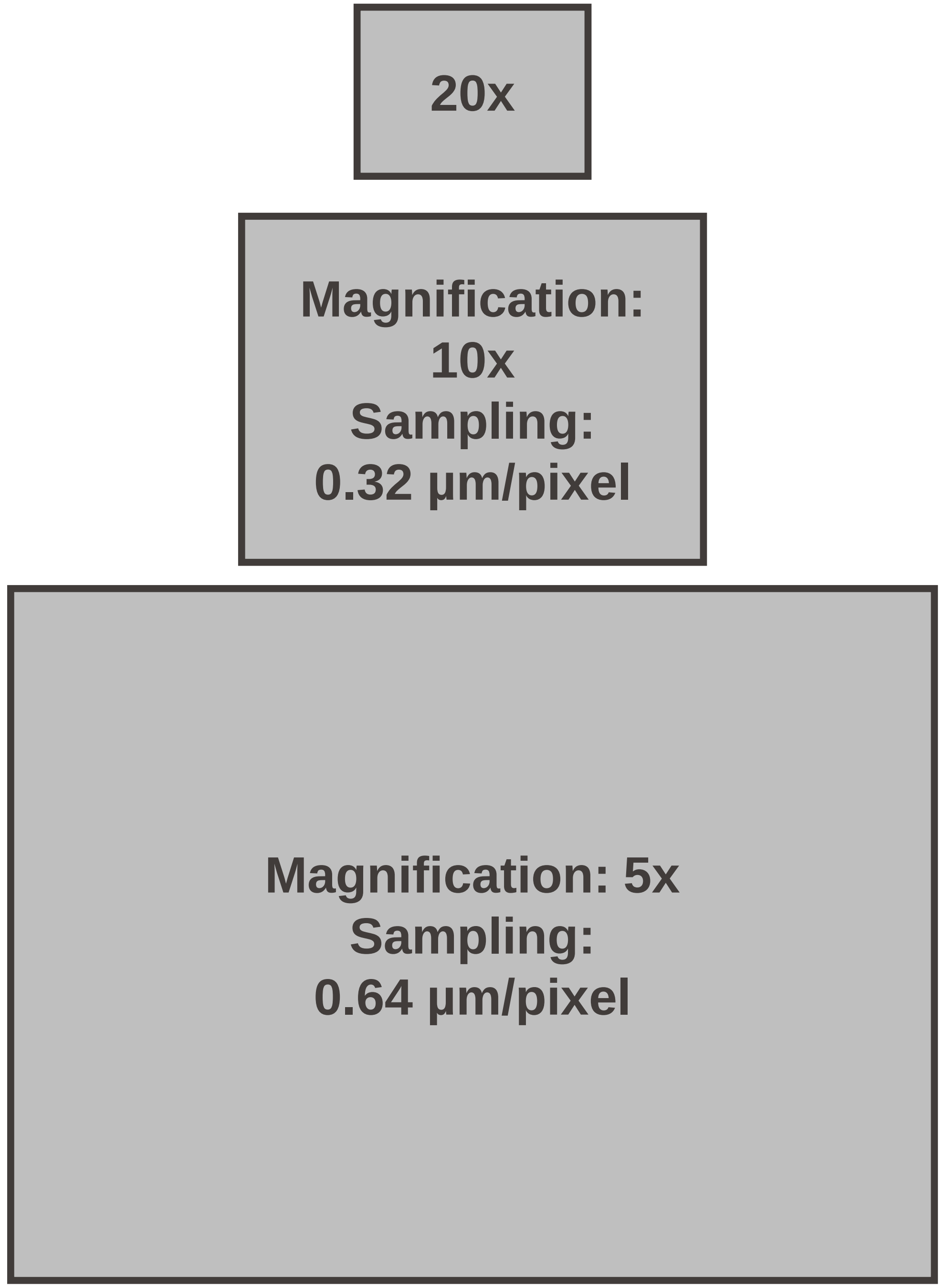

None
Bilinear
Bicubic
One rotation 15°
Two rotations 15°

Registration in microscopy

Motivation

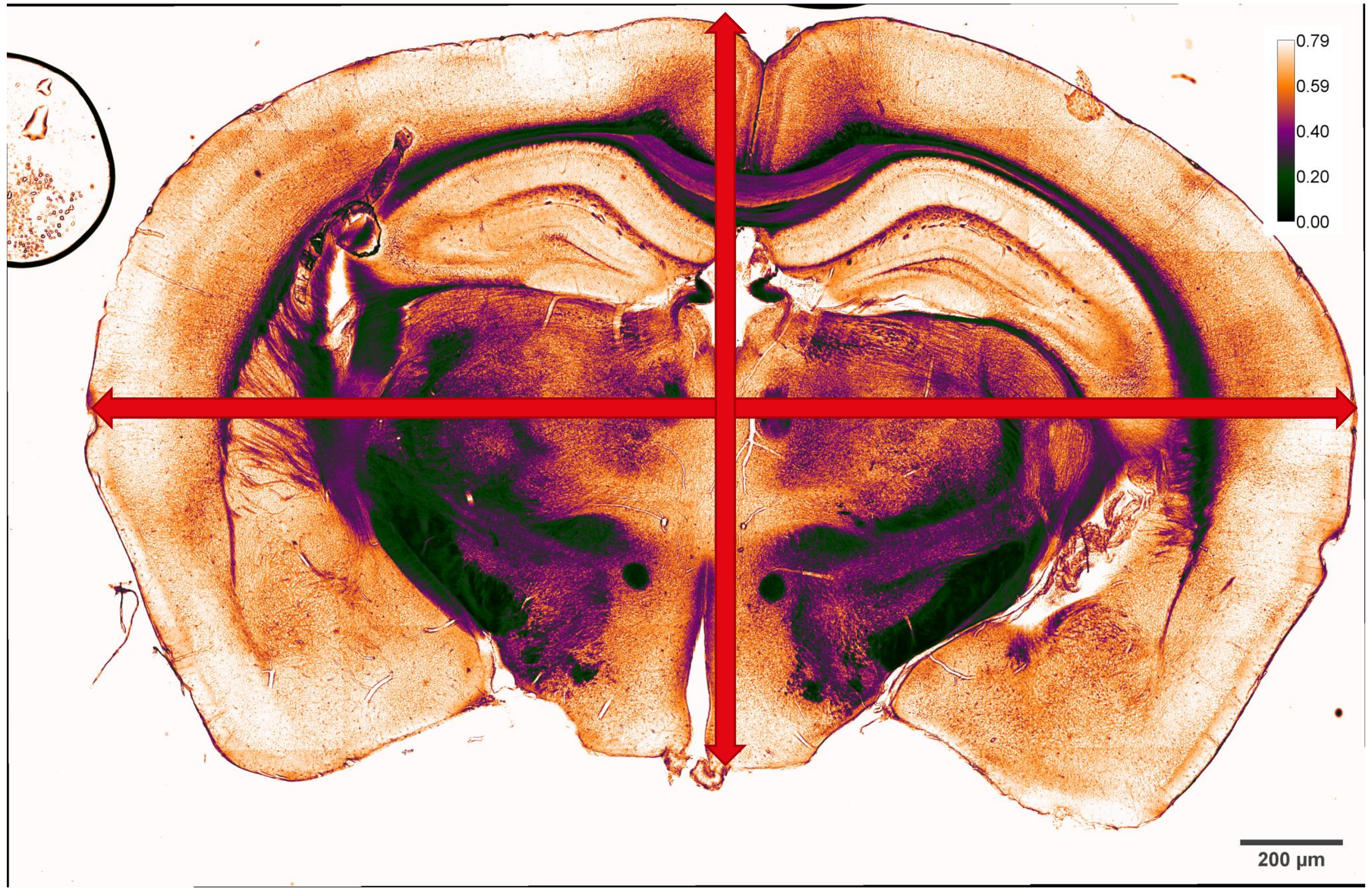


FOV sCMOS camera (color)



Motivation

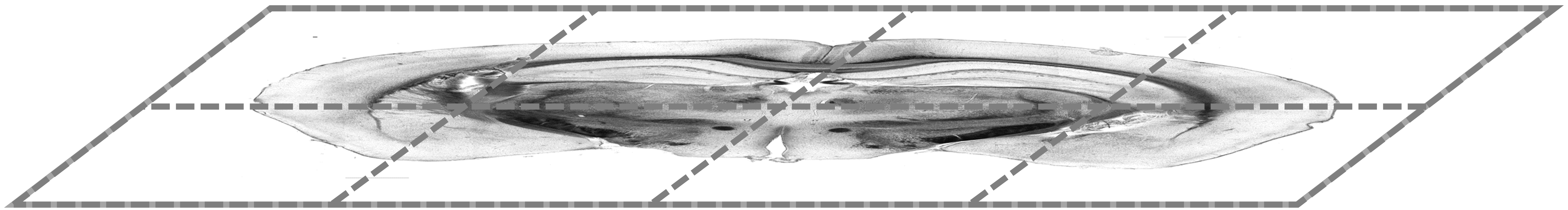
1.5 mm



2.5 mm

FOV sCMOS color	Tiles	N.A. resolution	rel. bright-ness
40x	224	0.95 0.29 µm	0.71
20x	56	0.8 0.34 µm	1.0
10x	8	0.4 0.69 µm	0.5
5x	4	0.95 2.1 µm	0.11

Tiled Imaging



Tiled Imaging

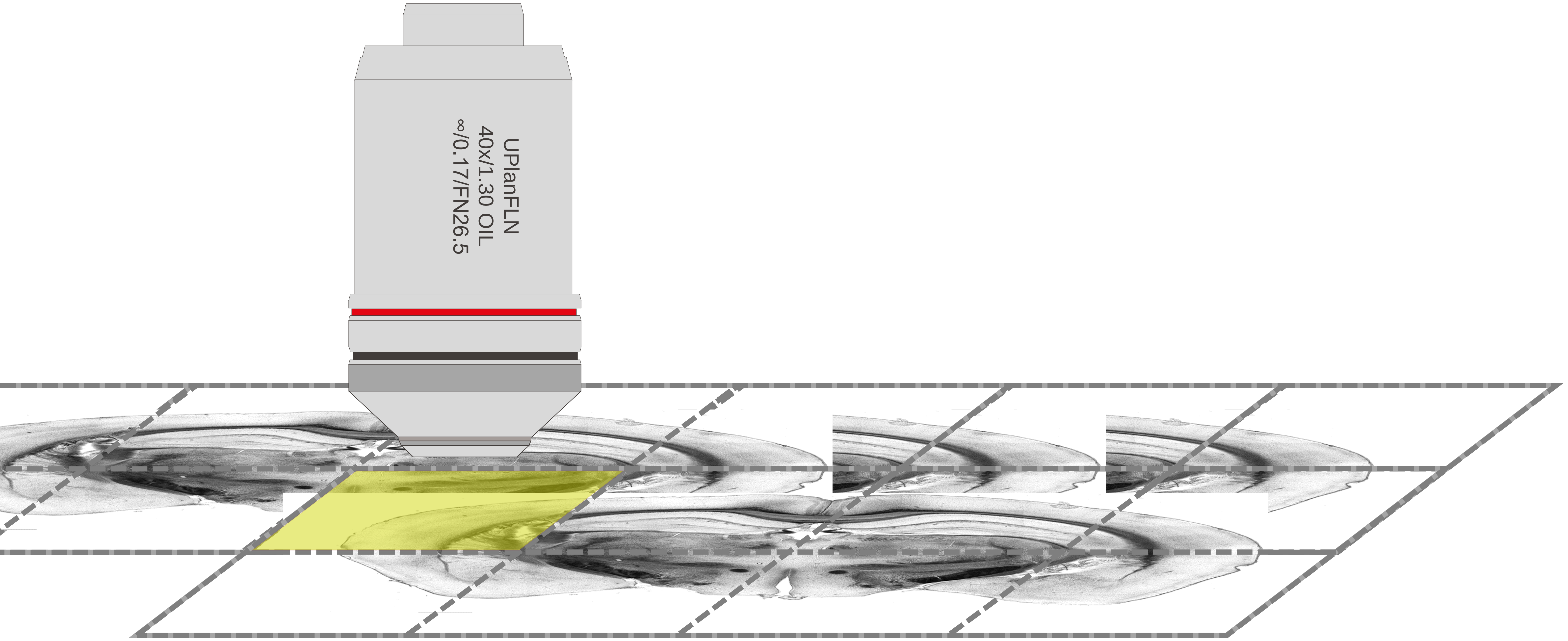
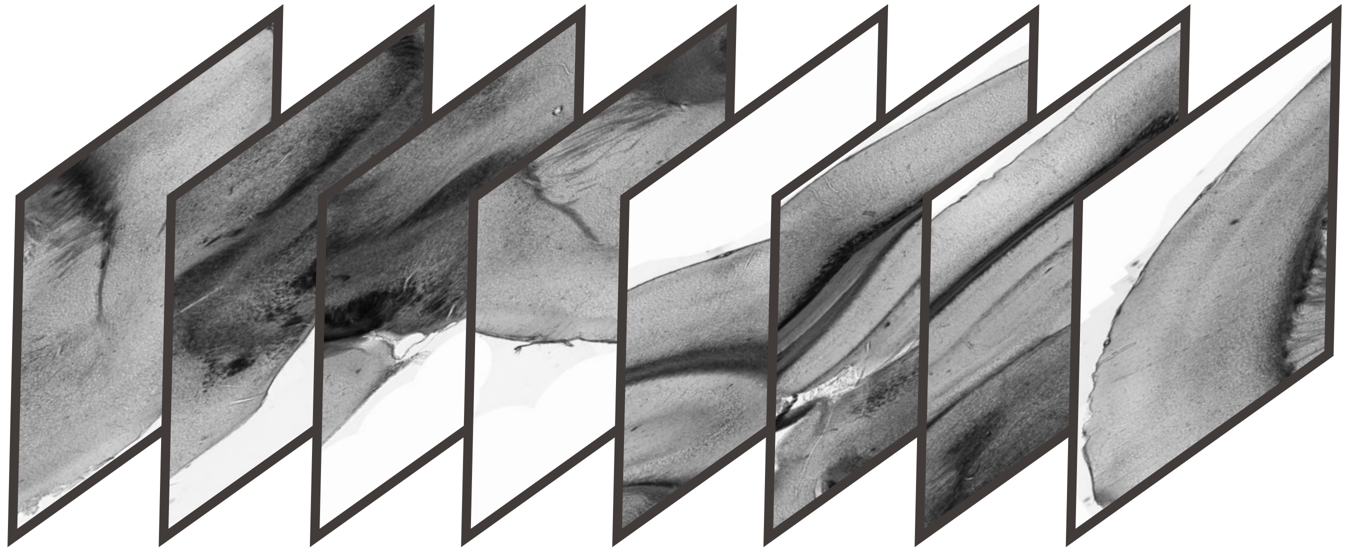


Image stack

Image Stack
2 x4



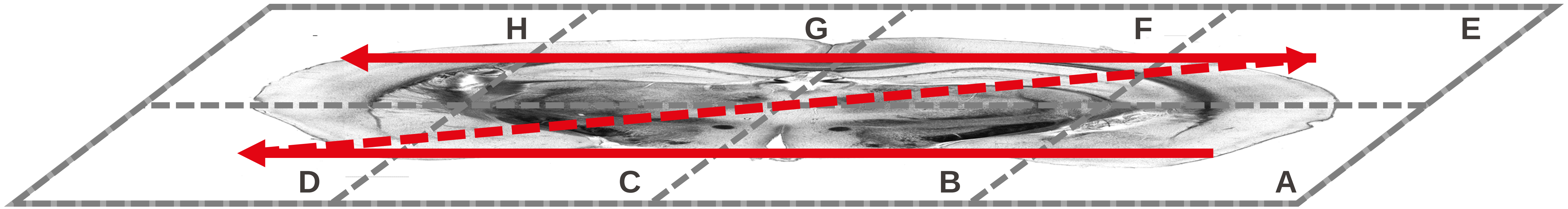
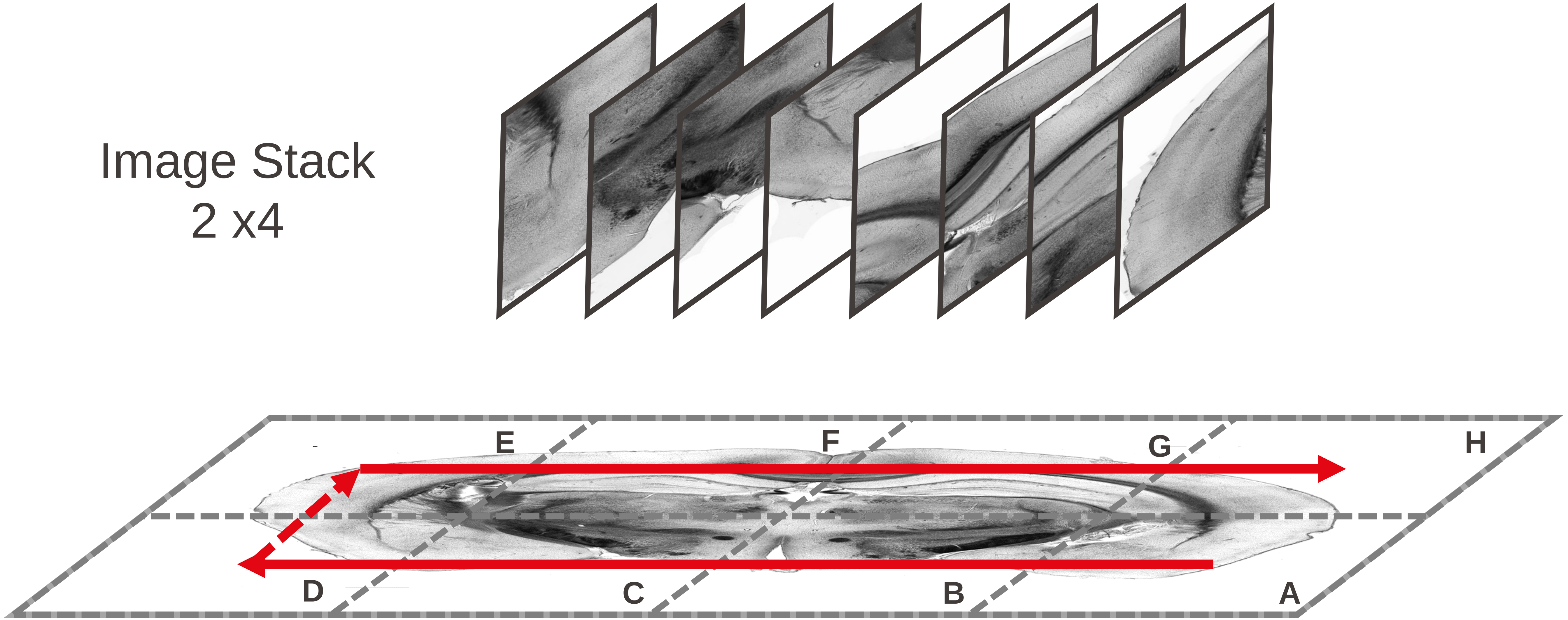
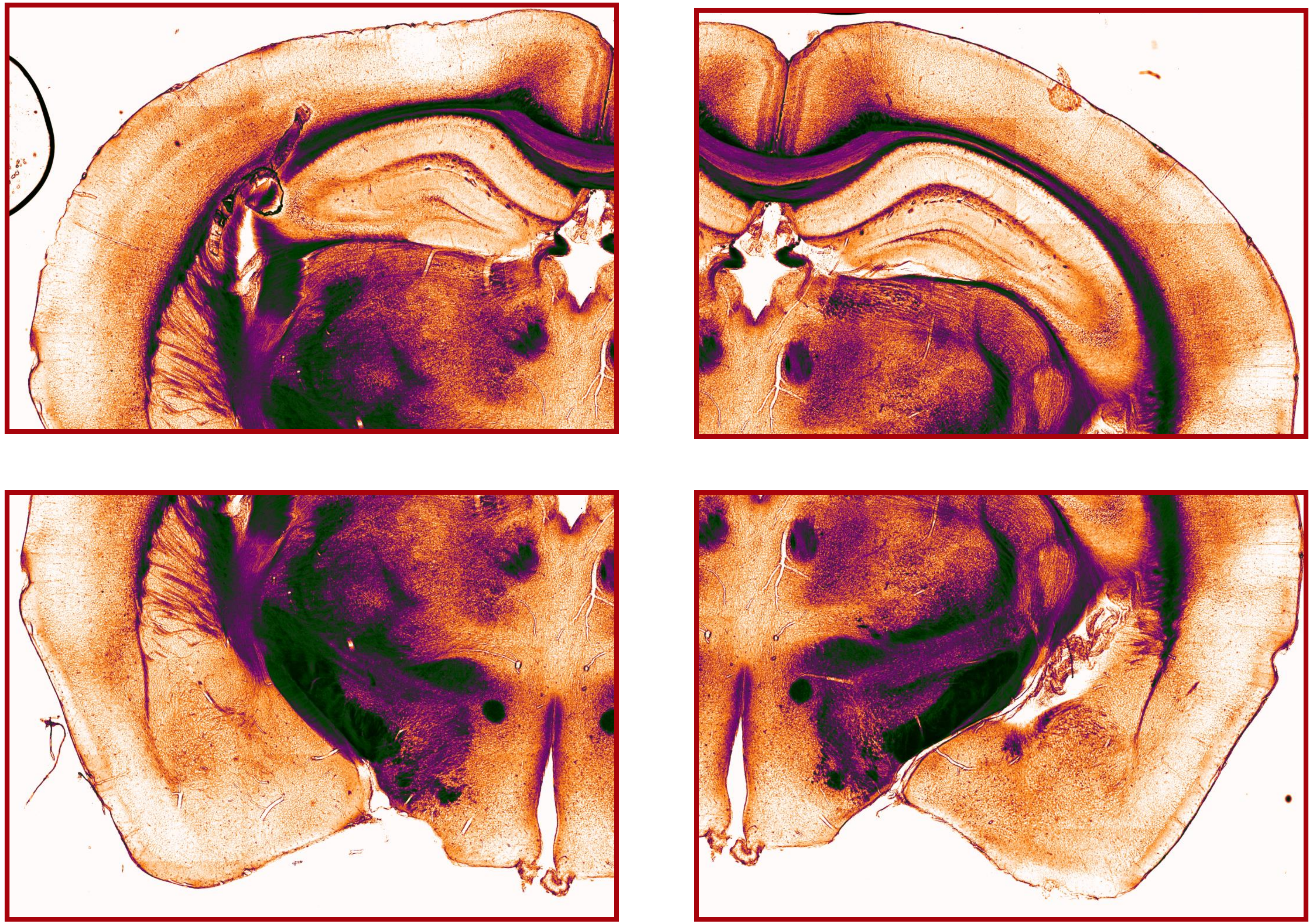


Image Stack

Image Stack
2 x4



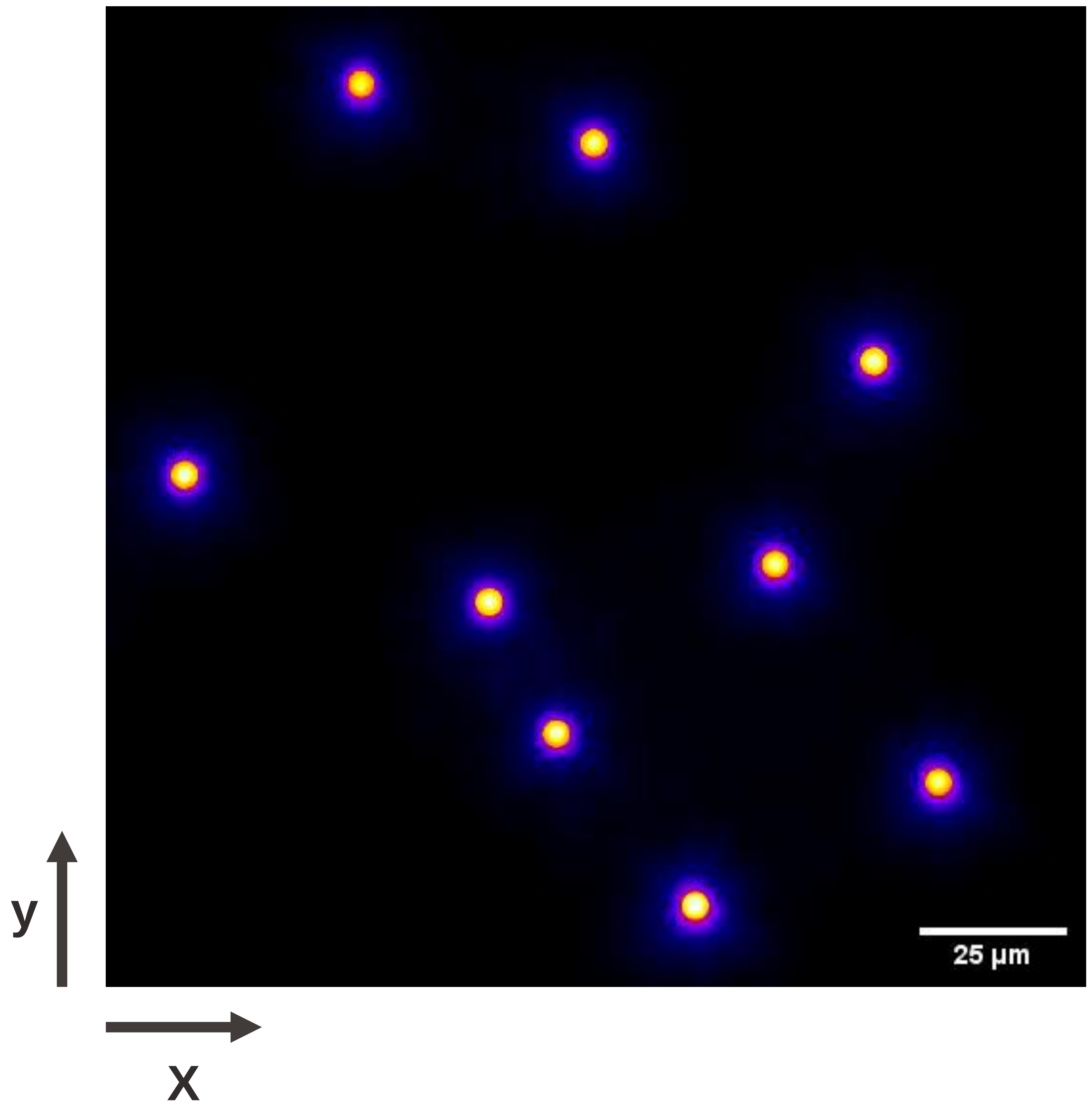
Alignment of individual tiles



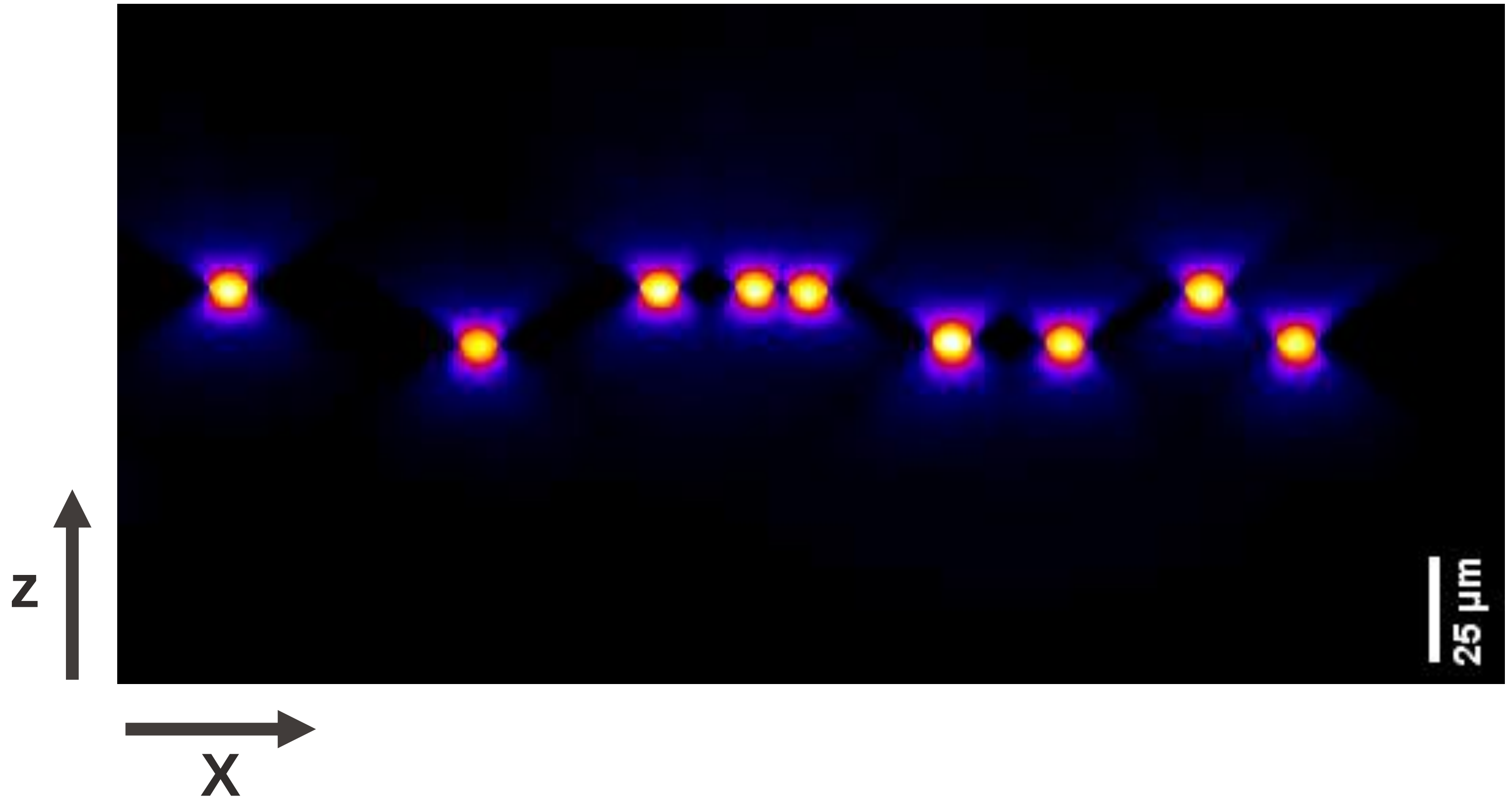
What speaks against a seamless acquisition strategy?

Temperature drift

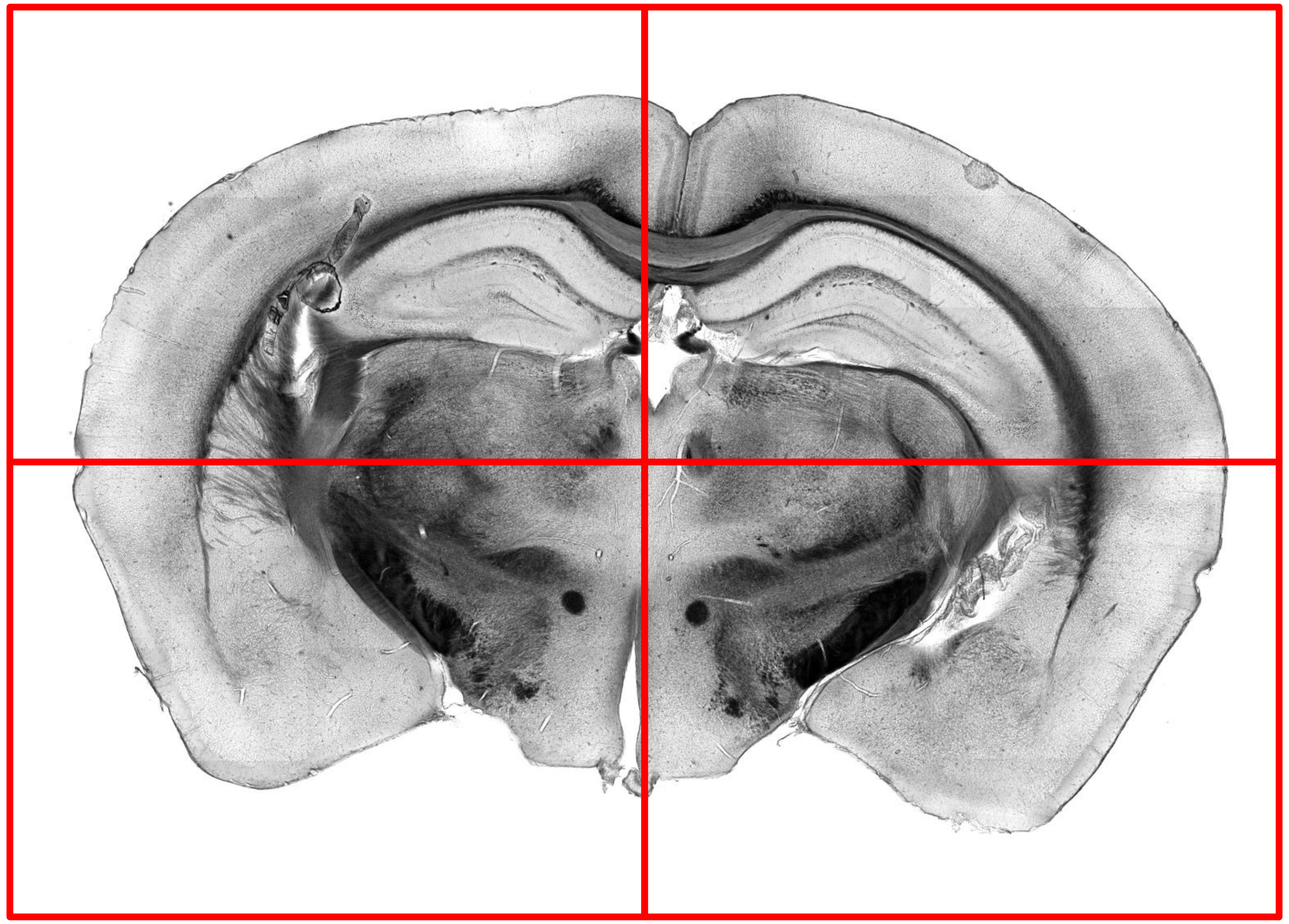
z-projection



y-projection



Alignment strategy

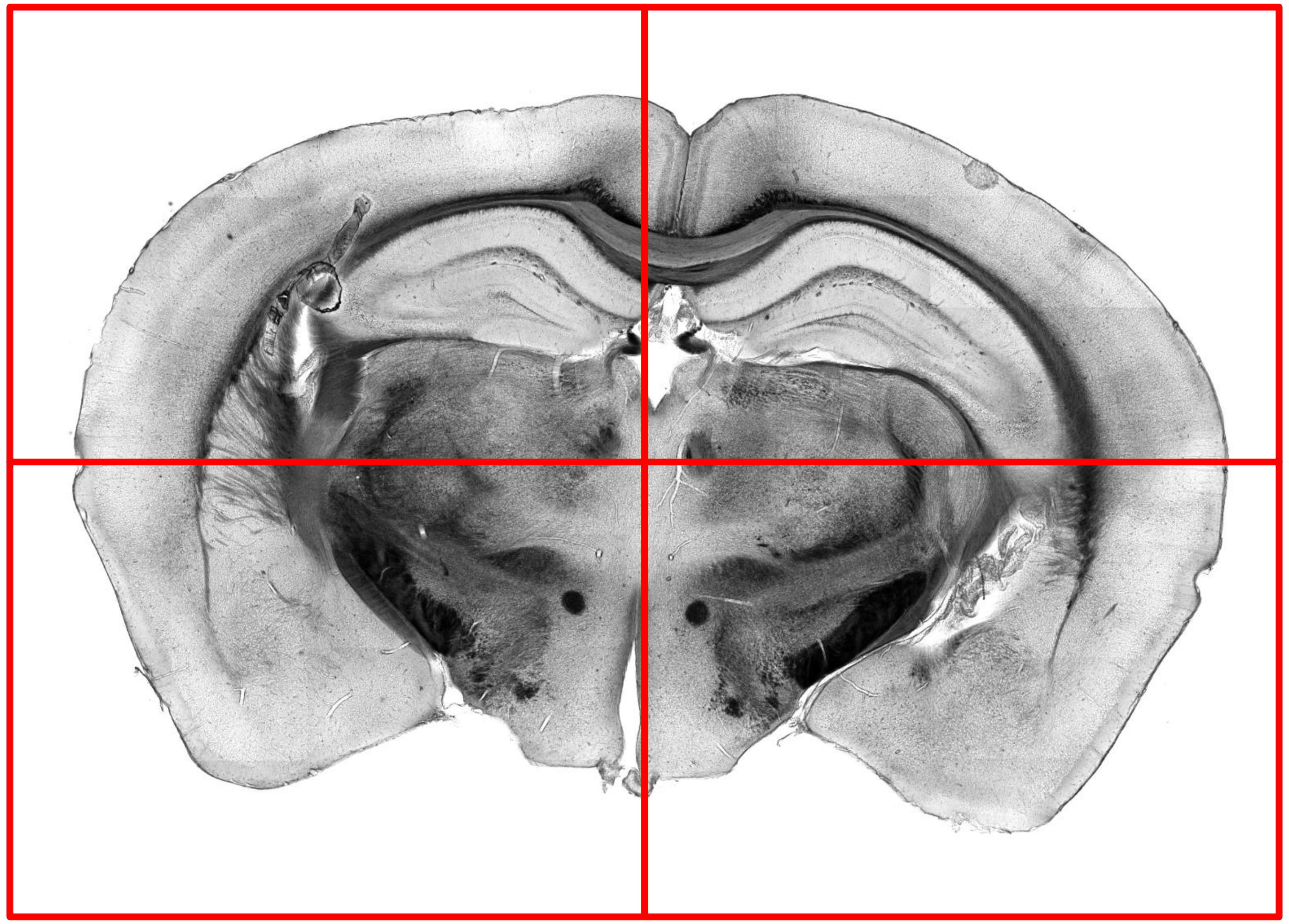


Alignment based on stage position (apriori knowledge)

- No post processing needed
- Limitation
  - stage precision
  - camera alignment
  - camera/sample orientation

In practice: used only for low resolution overview images.

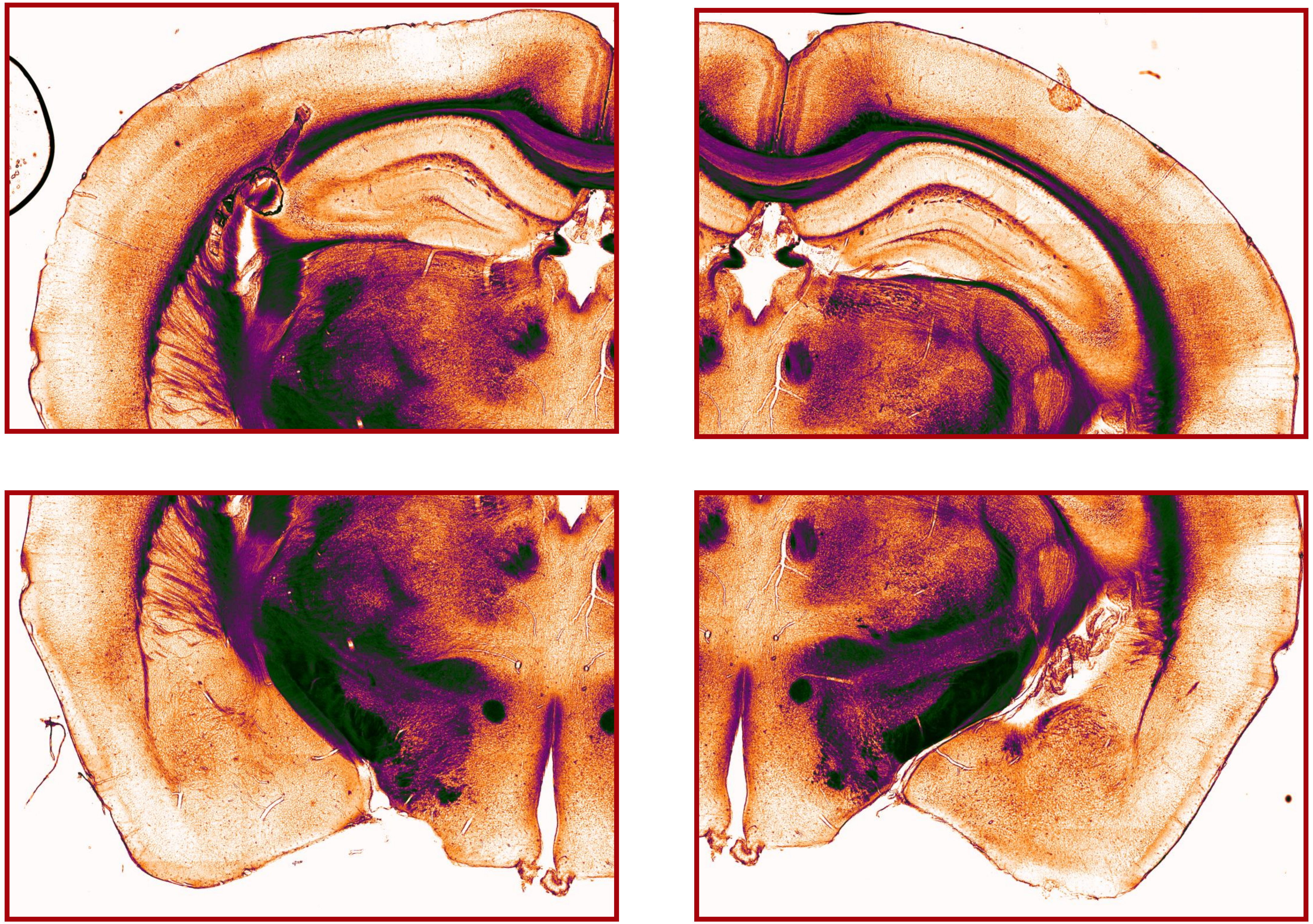
Alignment strategy



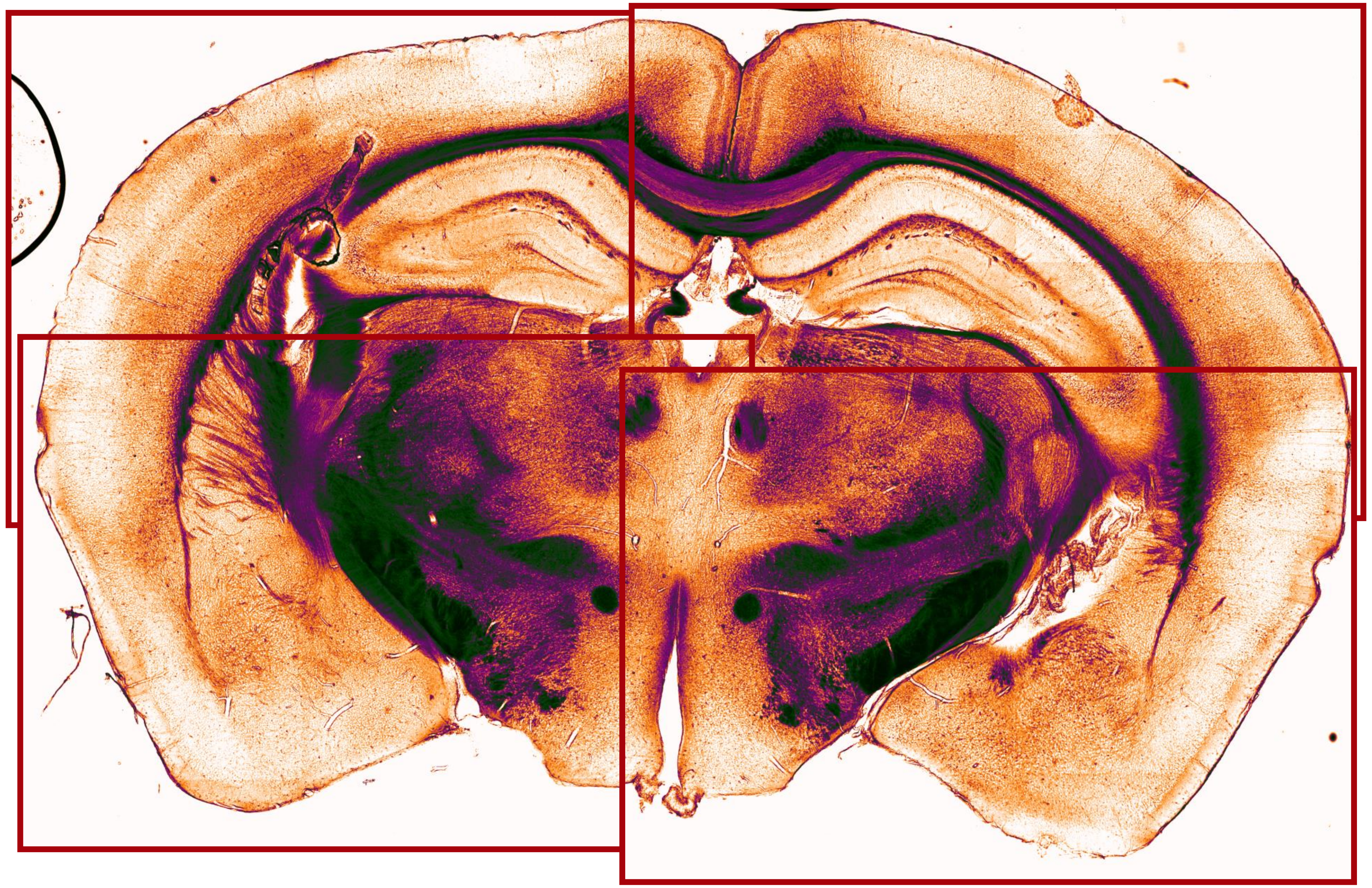
Alignment based on overlap

- Constant overlap/varying overlap
- Post processing needed
- High accuracy

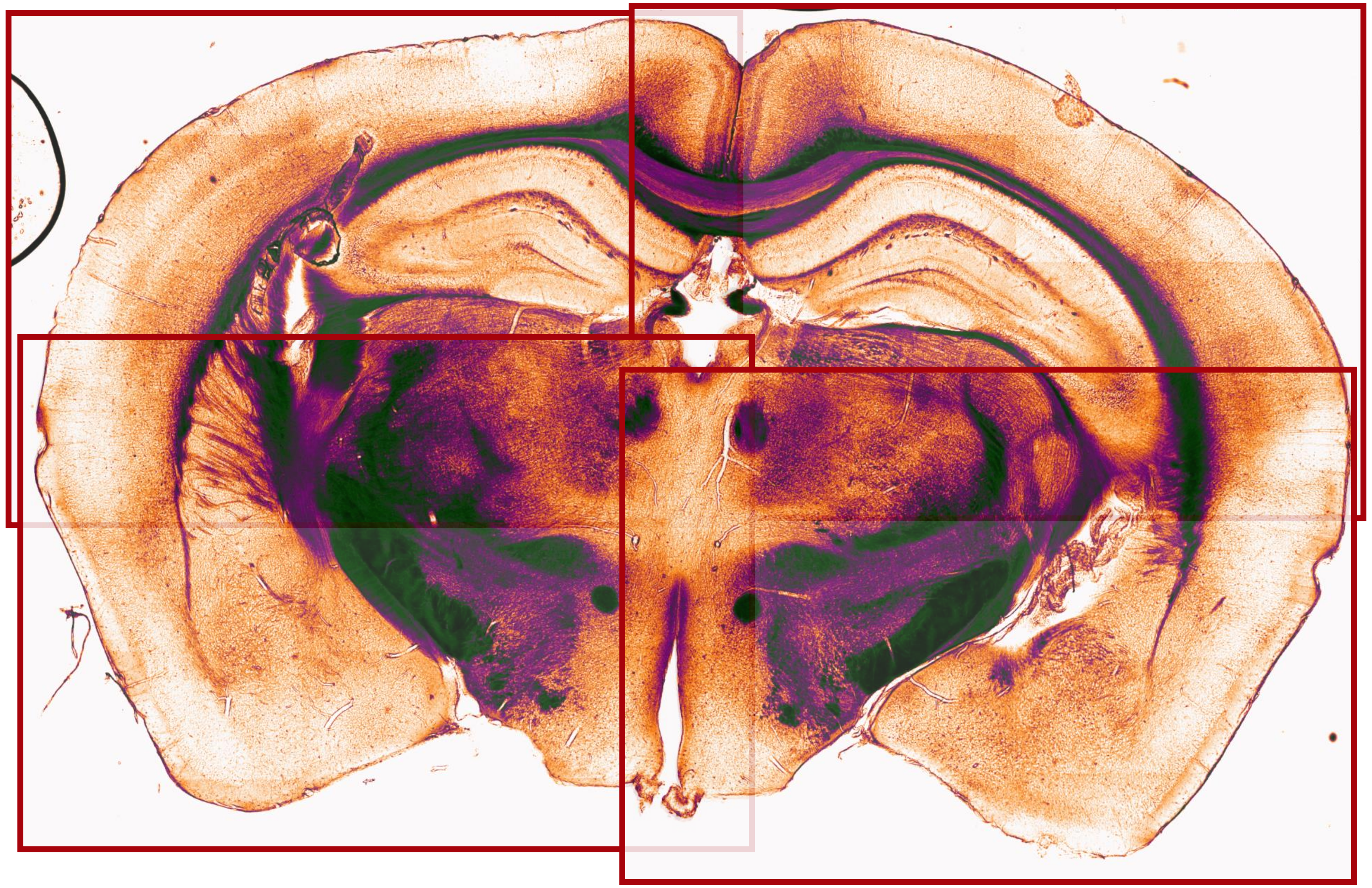
Alignment based on overlap



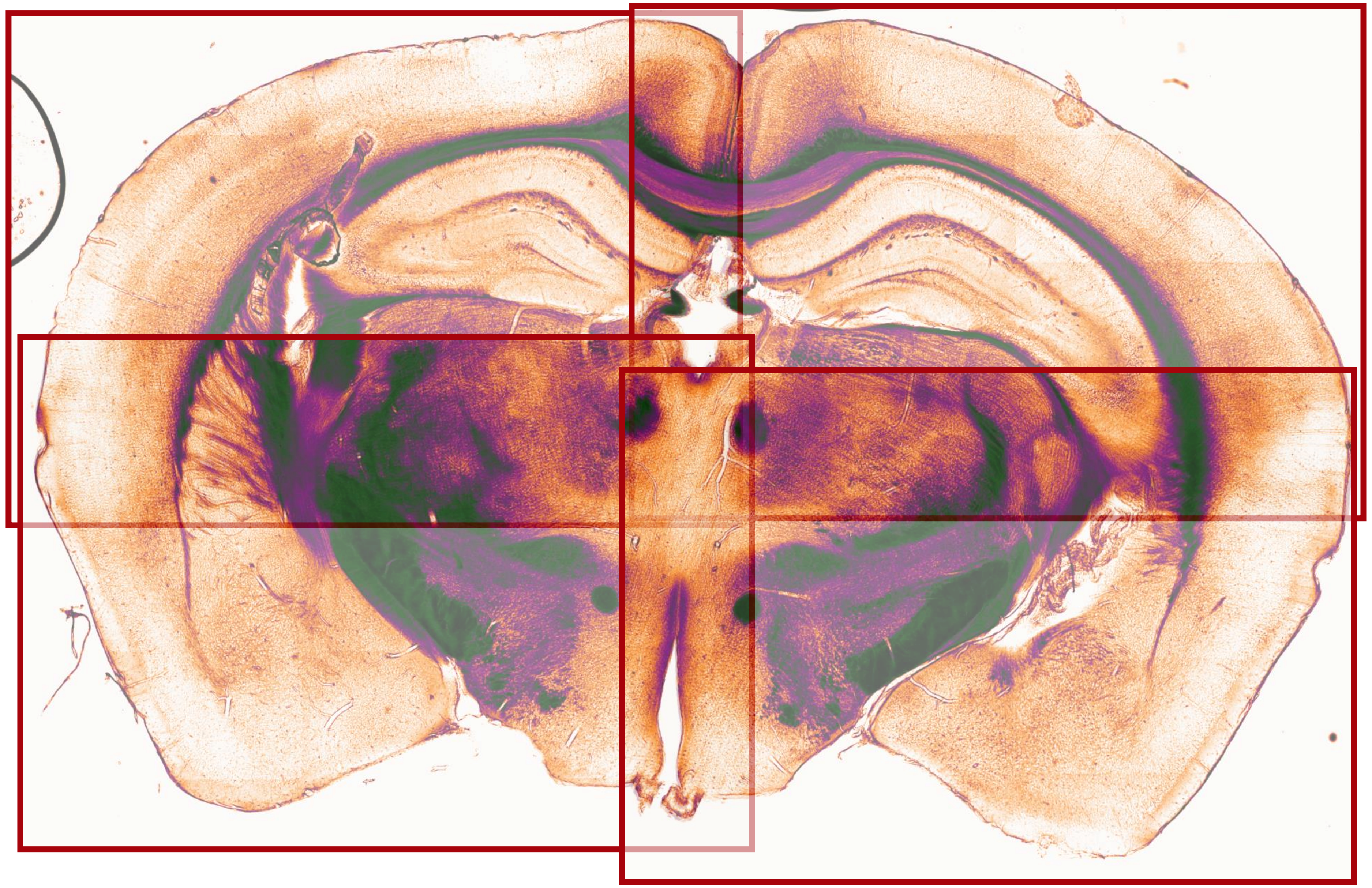
Alignment of individual tiles



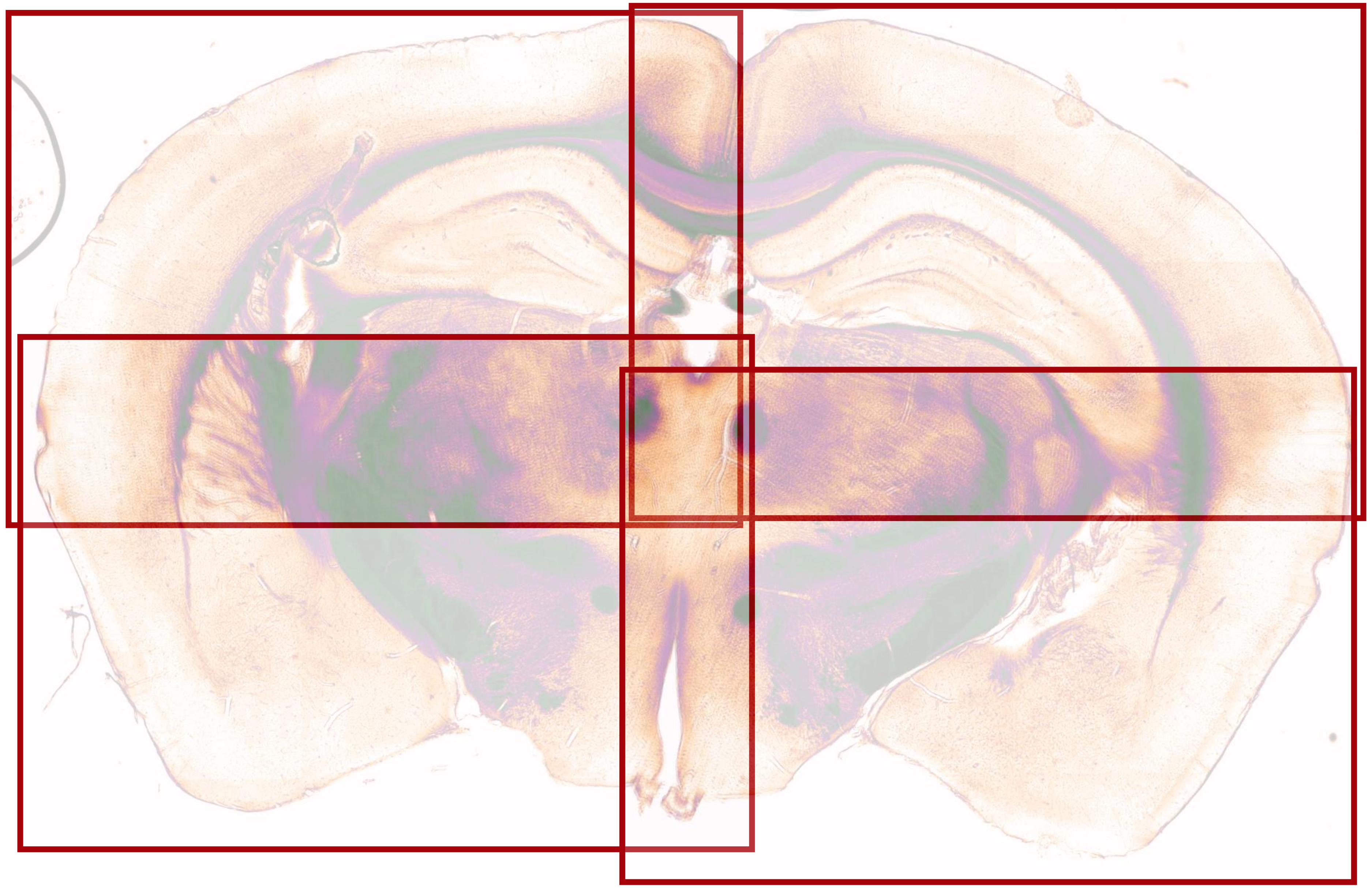
Alignment of individual tiles



Alignment of individual tiles

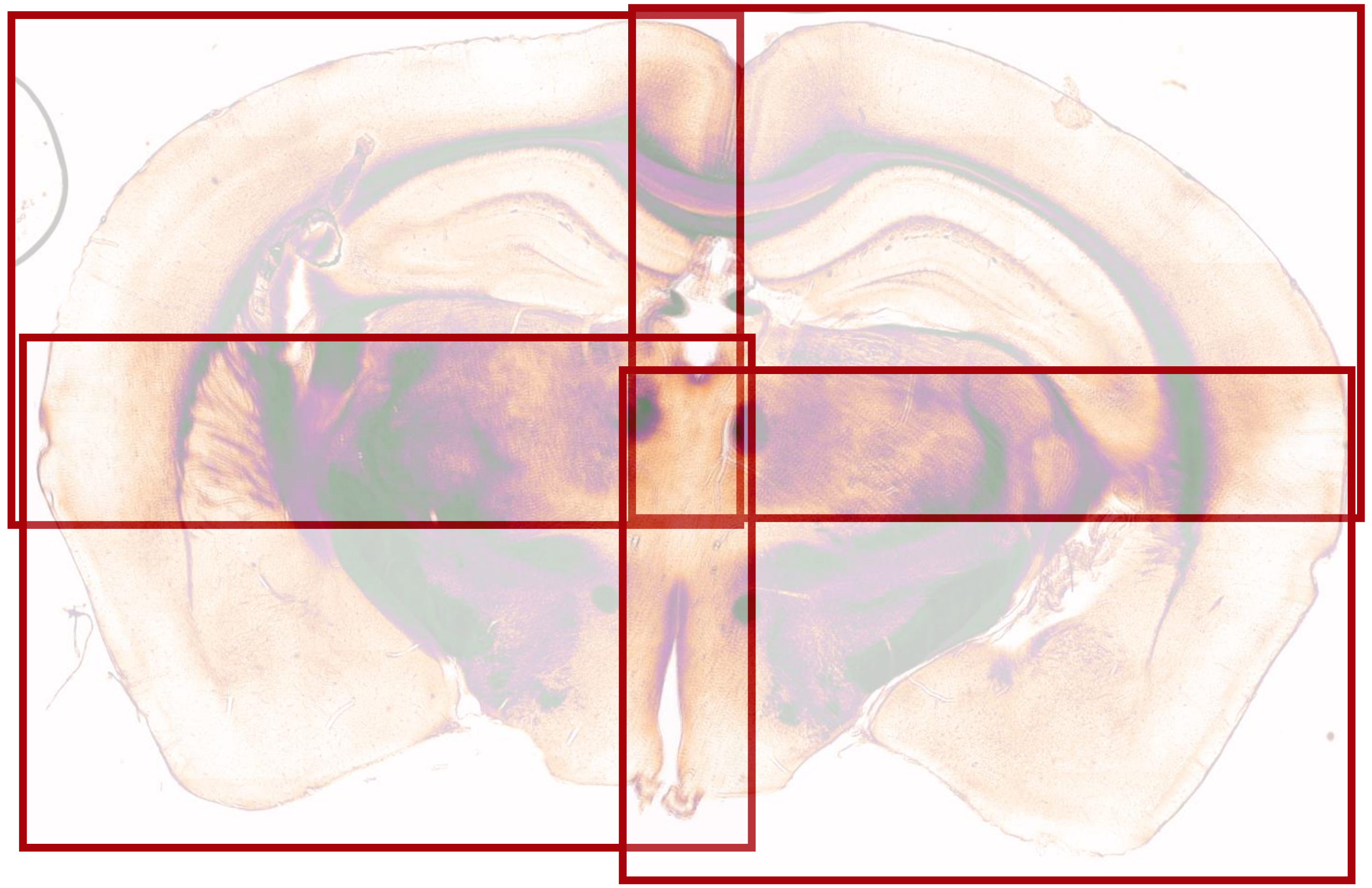


Alignment of individual tiles



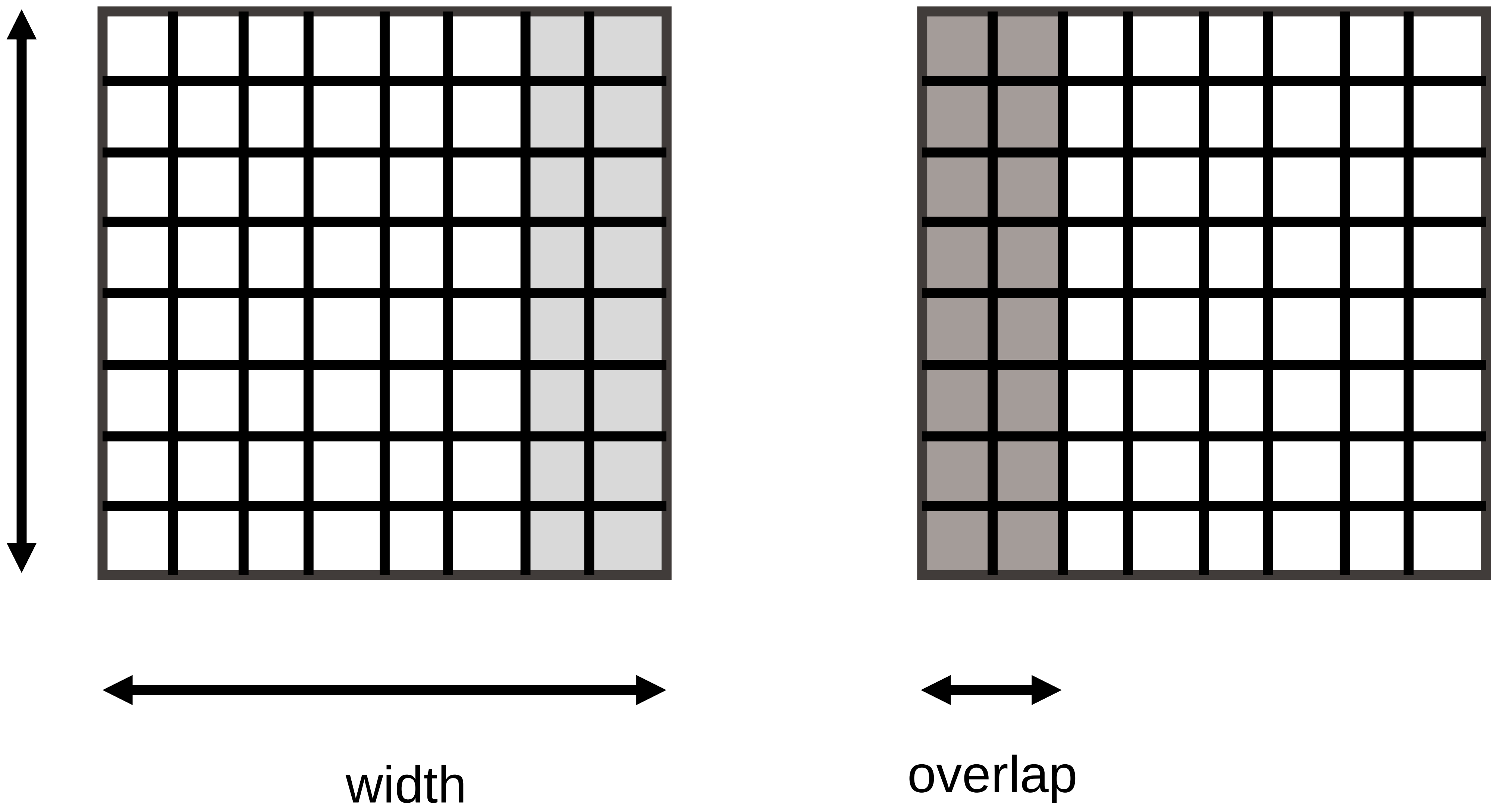
Stitching Tasks

- Define overlap region
- Align individual tiles
- Transformation
- Image fusion

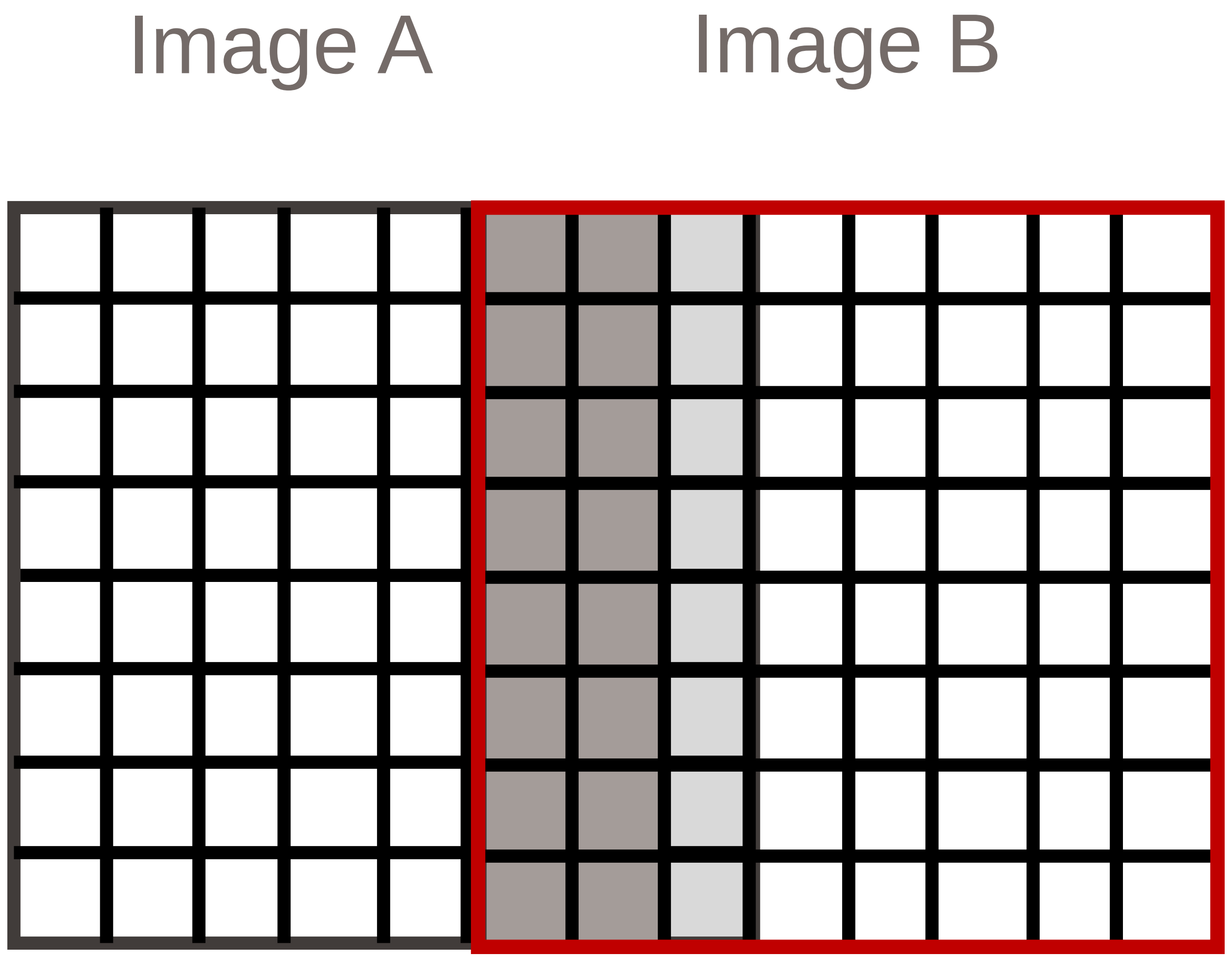


Tile alignment

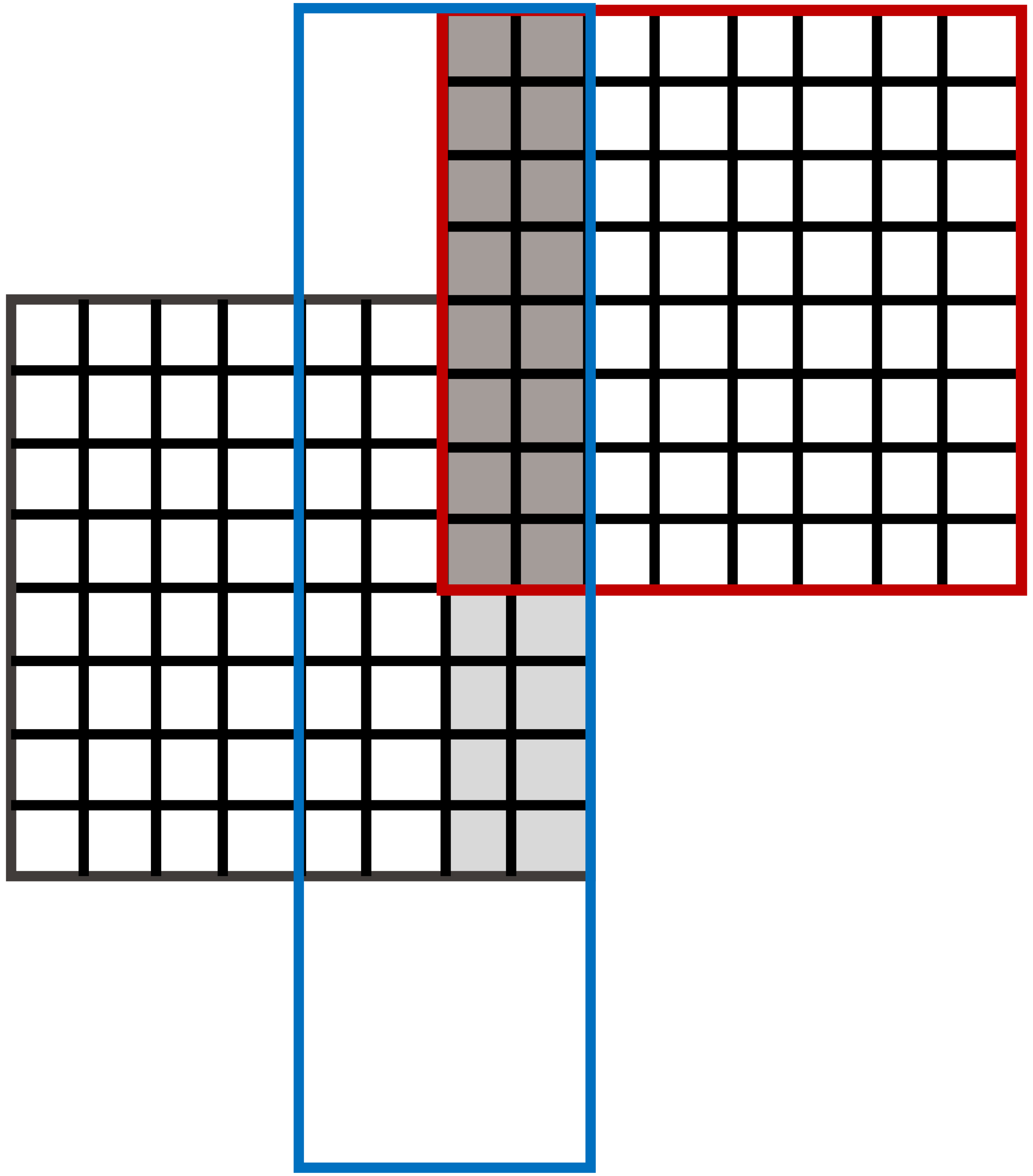
Intensity based alignment



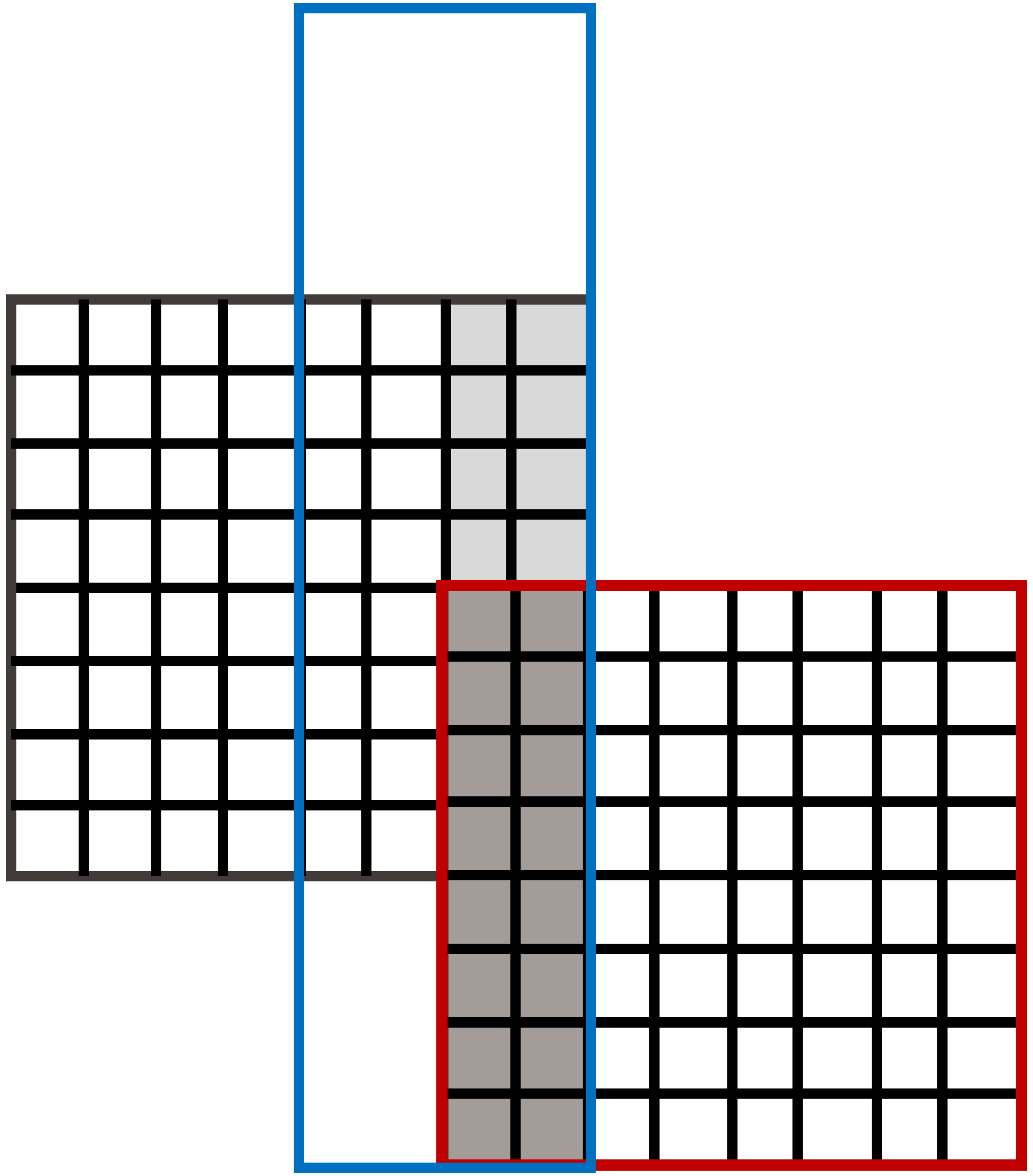
Intensity based alignment



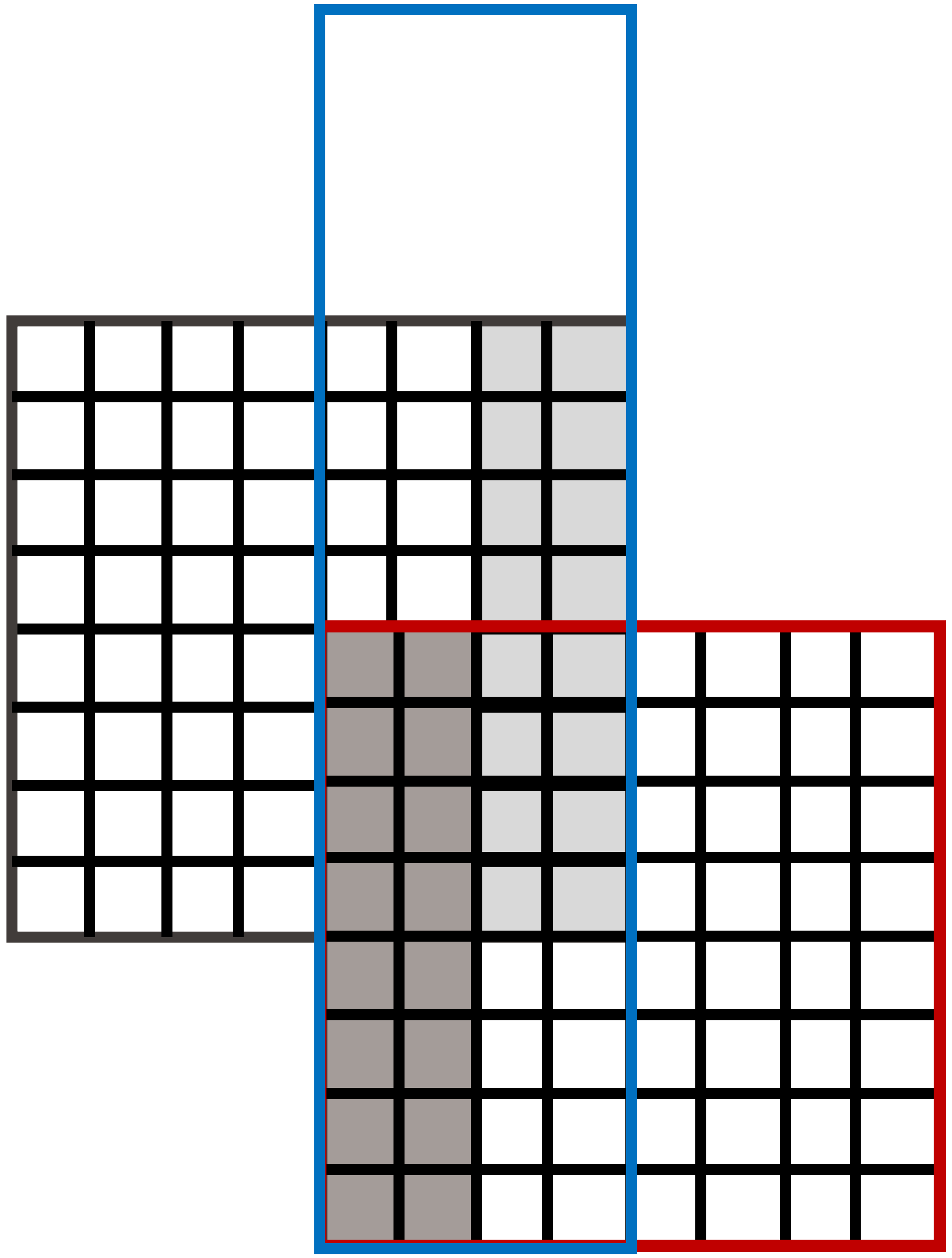
Intensity based alignment



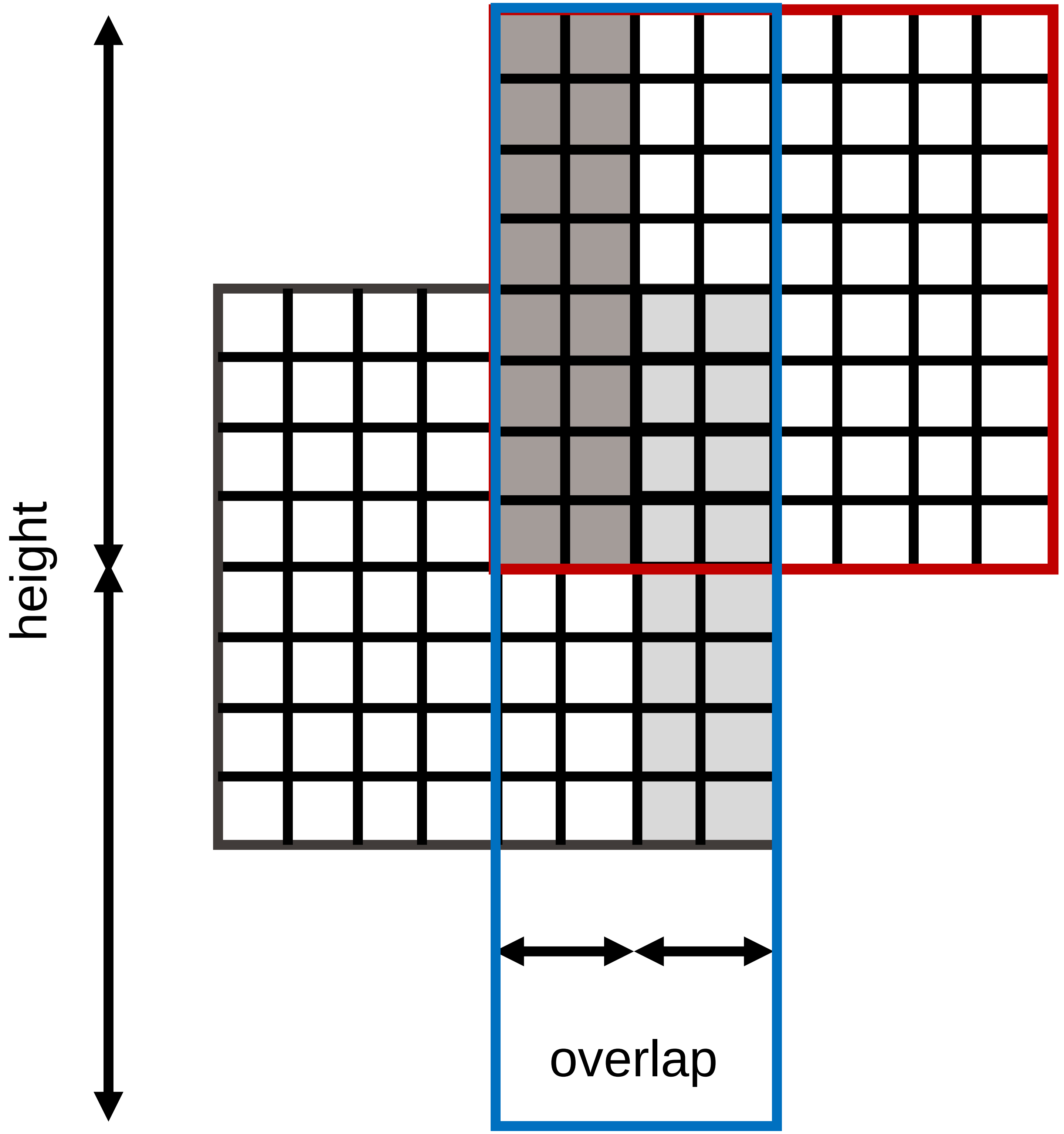
Intensity based alignment



Intensity based alignment



Intensity based alignment



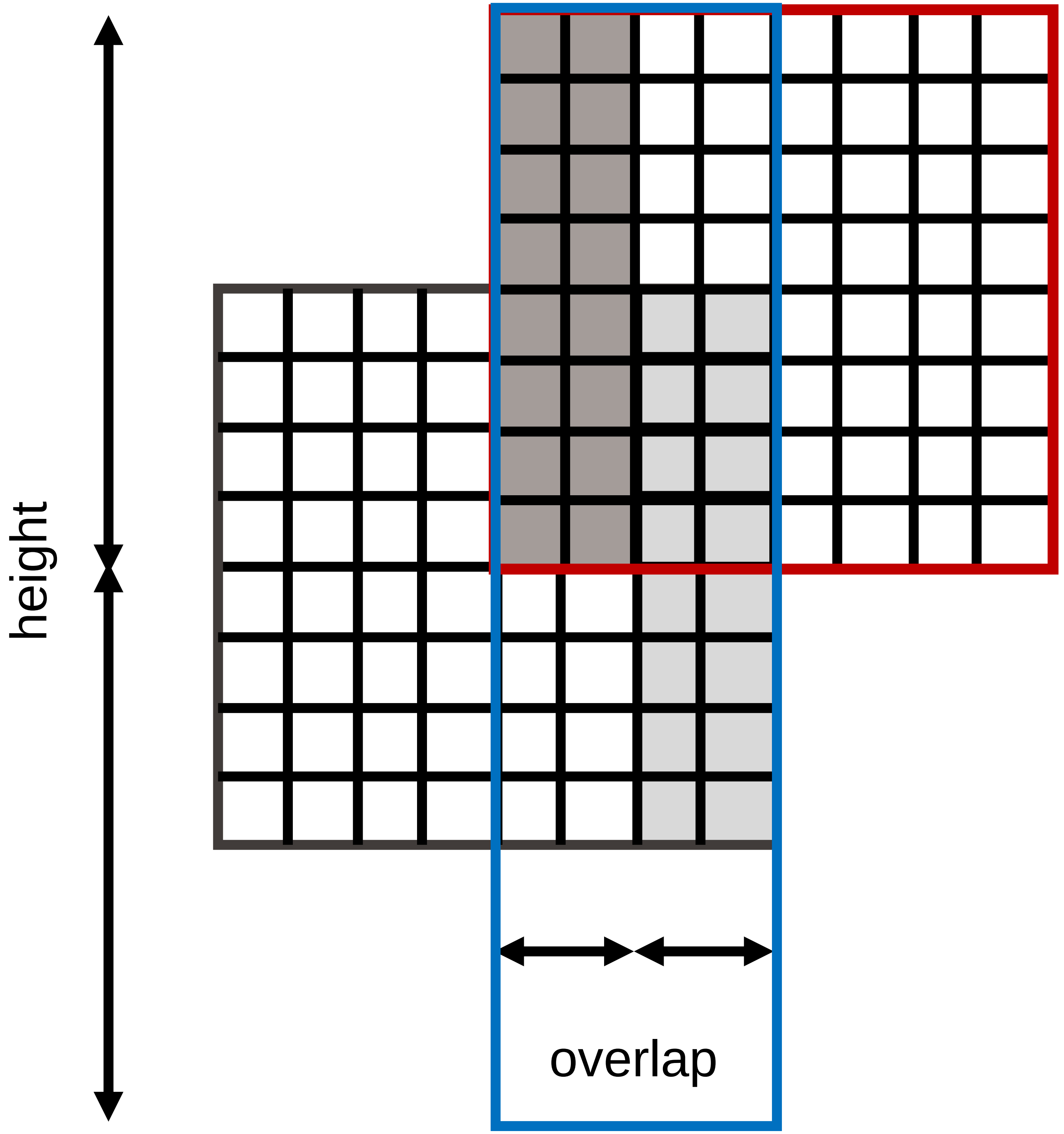
- Image similarity

$$D(u,v) = \sum_x \sum_y \left(T(x,y) - I(x-u, y-v)\right)^2$$

$$CC(u,v) = \frac{\sum_x \sum_y T(x,y) - \mu_T)(I(x-u, y-v) - \mu_I)}{\sqrt{\sum_x \sum_y (I(x-u, y-v) - \mu_I)^2 (T(x,y) - \mu_T)}}$$

Correlation theorem:
The FT of the correlation of two images is the product of the FT of one image and the complex conjugate of the FT of the other image.

Intensity based alignment



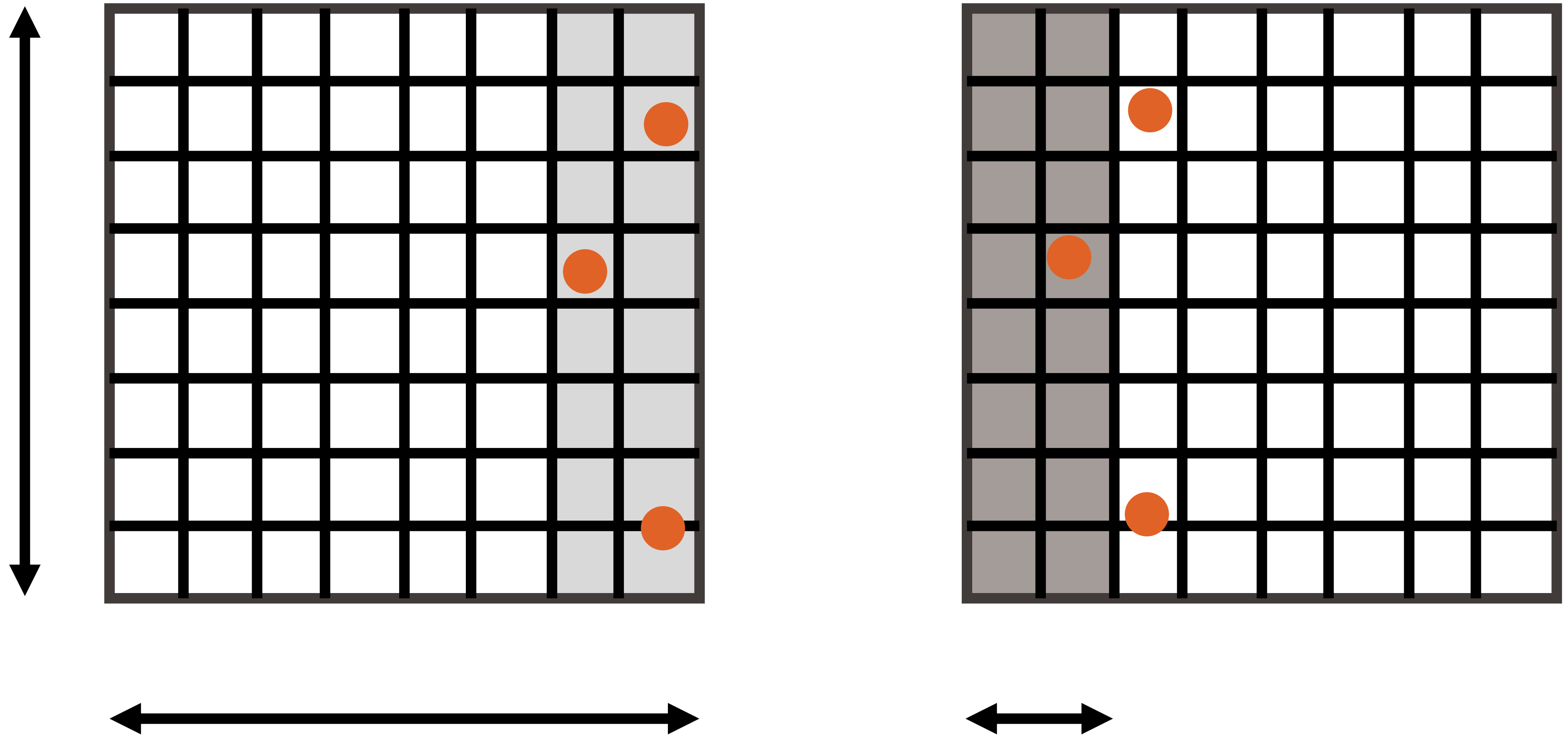
- Measure image similarity
- Works on any kind of dataset
- Rather slow

Image sensor: 2048 pixel *2048 pixel

Overlap: 10 %

Permutations 2*204*2*2048= $1.67*10^6$

Scale invariant feature transform



- Needs objects/features
- Fast

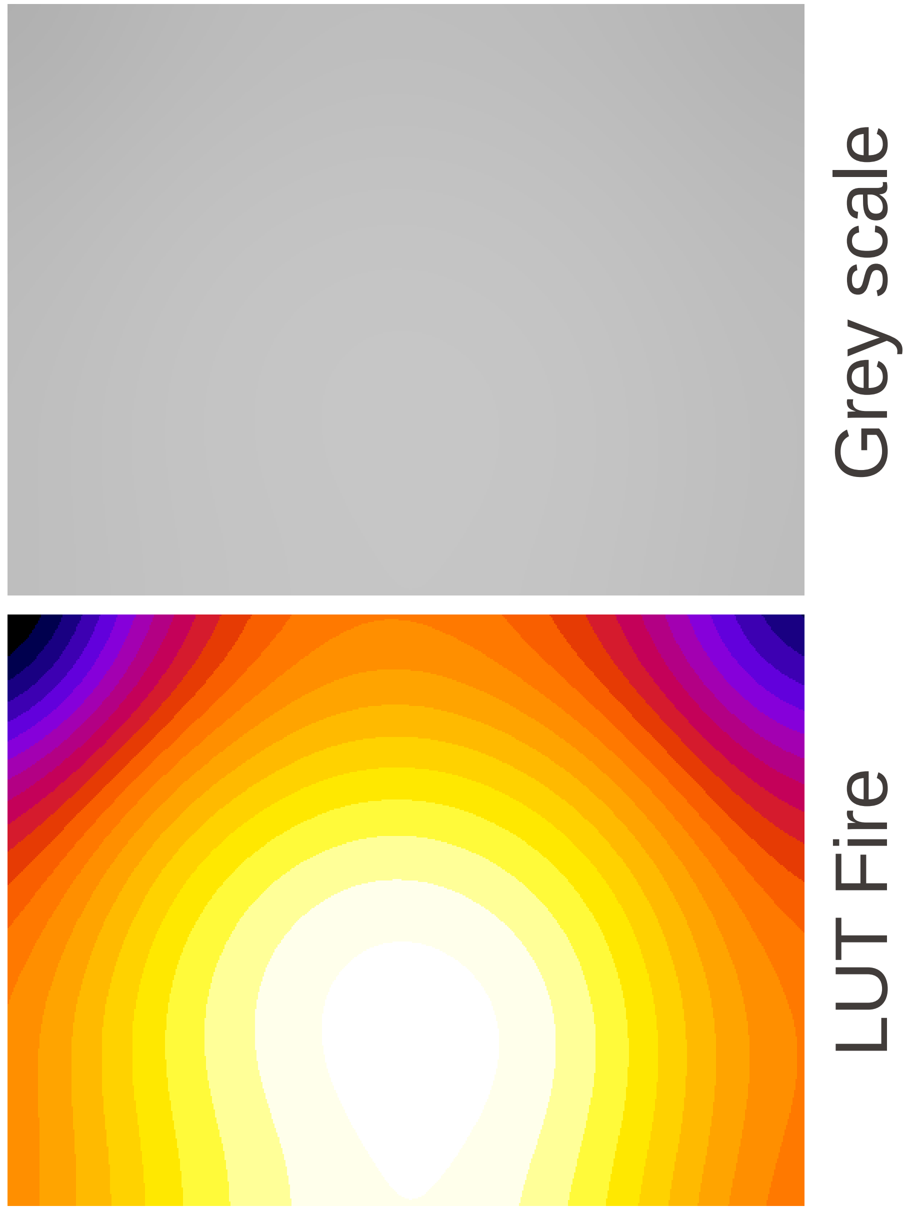
Practical aspects

Available software solutions

- Thévenaz, P., and Unser, M. (2007). User-friendly semiautomated assembly of accurate image mosaics in microscopy. Microsc. Res. Tech. *70*, 135–146.
- Emmenlauer, M., Ronneberger, O., Ponti, A., Schwarb, P., Griffa, A., Filippi, A., Nitschke, R., Driever, W., and Burkhardt, H. (2009). XuvTools: free, fast and reliable stitching of large 3D datasets. J. Microsc. *233*, 42–60.
- Preibisch, S., Saalfeld, S., and Tomancak, P. (2009). Globally optimal stitching of tiled 3D microscopic image acquisitions. Bioinformatics *25*, 1463–1465.
- Bria, A., and Iannello, G. (2012). TeraStitcher - A tool for fast automatic 3D-stitching of teravoxel-sized microscopy images. BMC Bioinformatics *13*, 316.
- Chalfoun, J., Majurski, M., Blattner, T., Bhadriraju, K., Keyrouz, W., Bajcsy, P., and Brady, M. (2017). MIST: Accurate and Scalable Microscopy Image Stitching Tool with Stage Modeling and Error Minimization. Sci. Rep. *7*, 4988.

Illumination artefacts

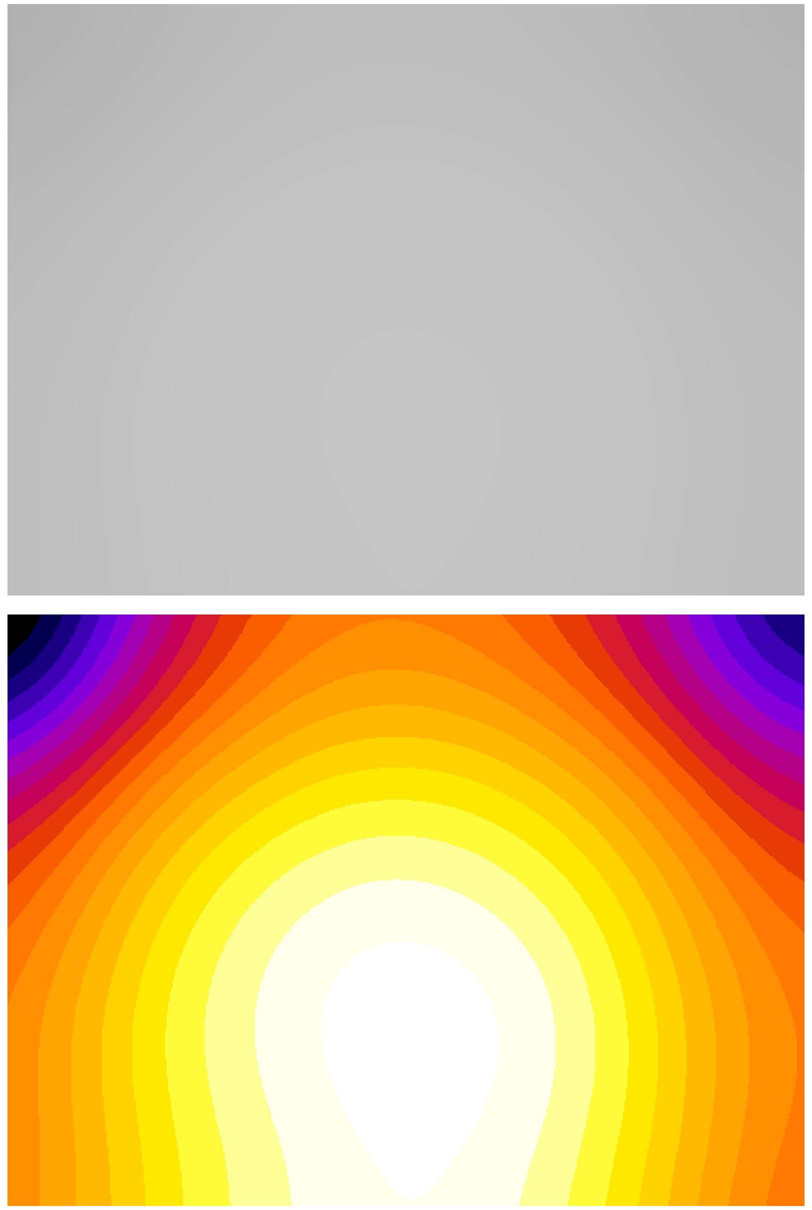
Flatfield image



Illumination artefacts

corrected image

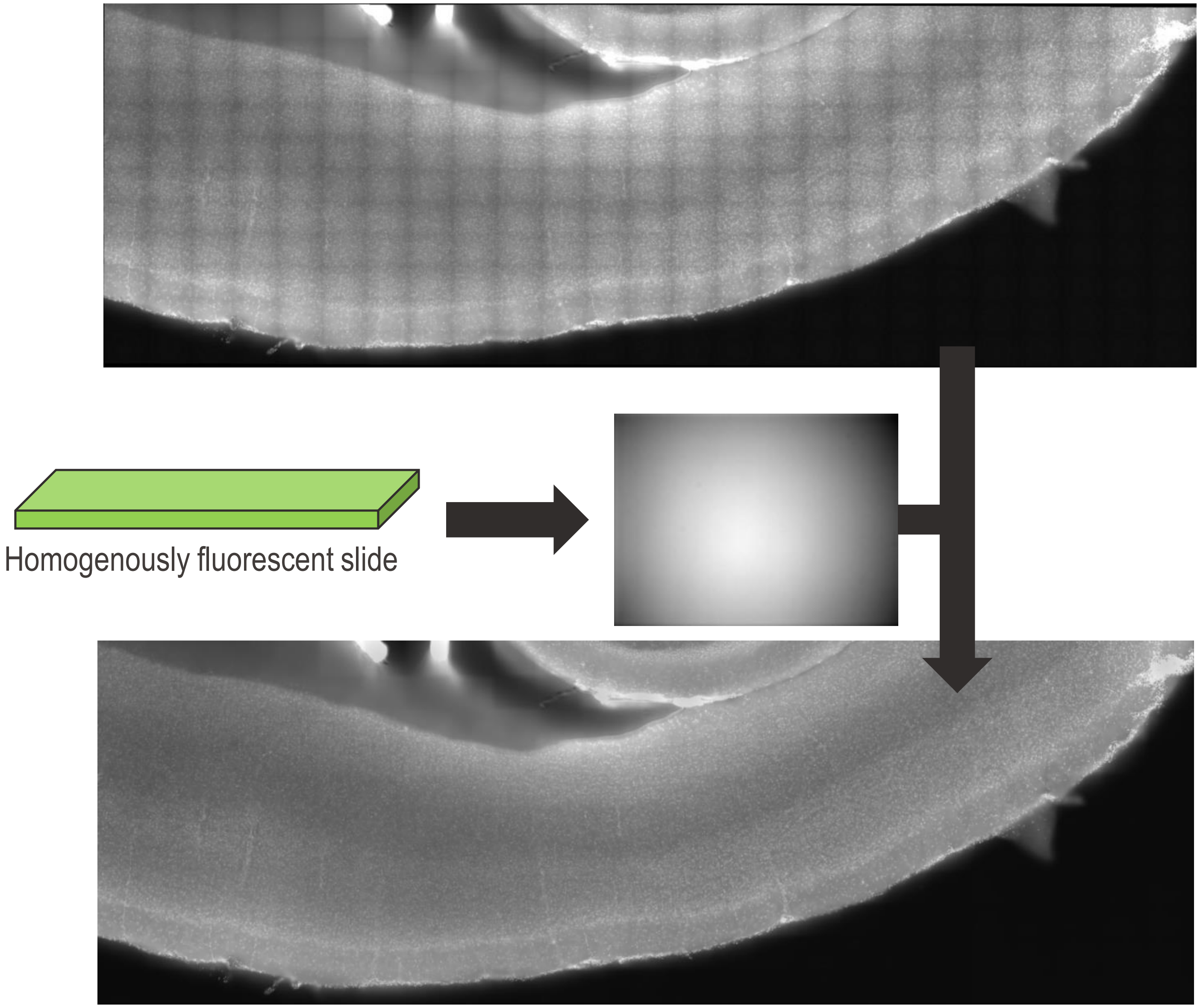
Flatfield image



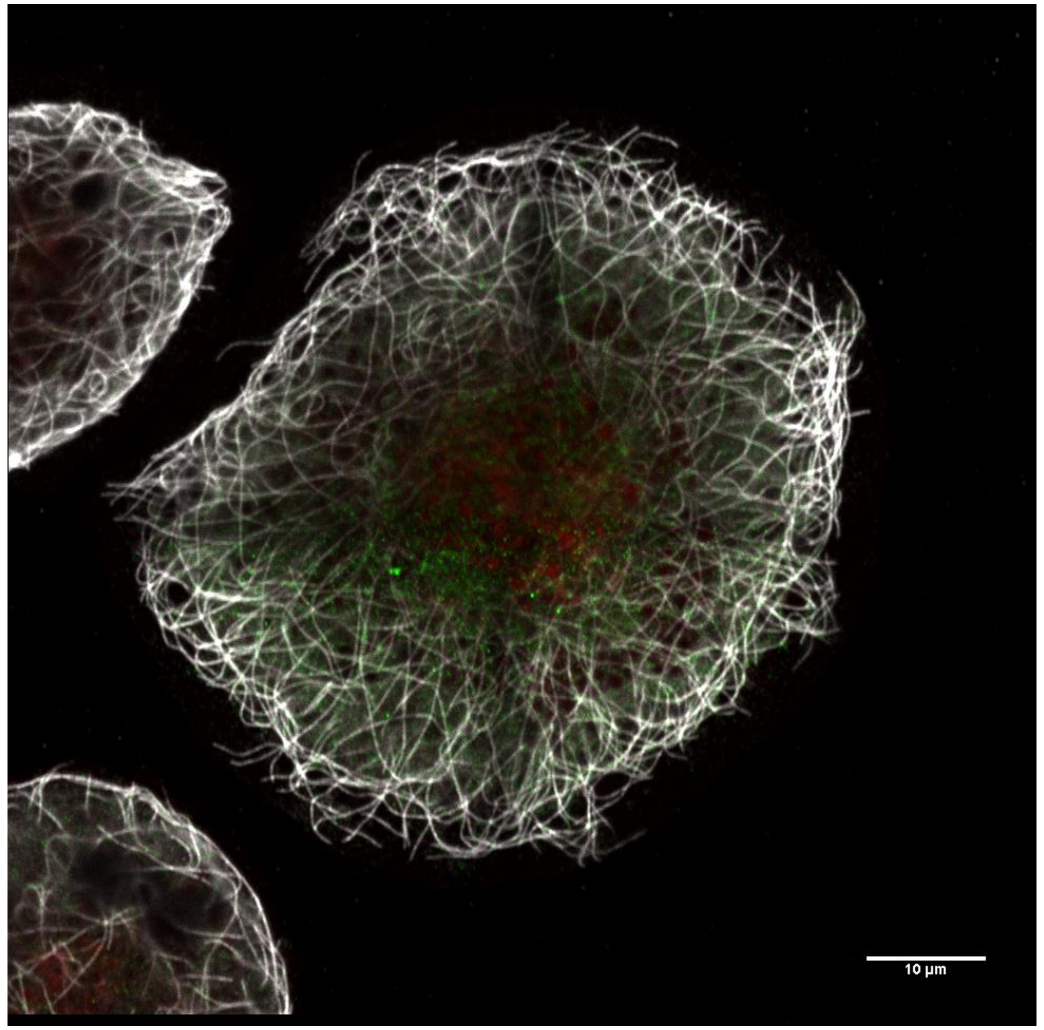
Grey scale

LUT Fire

Shading correction fluorescence

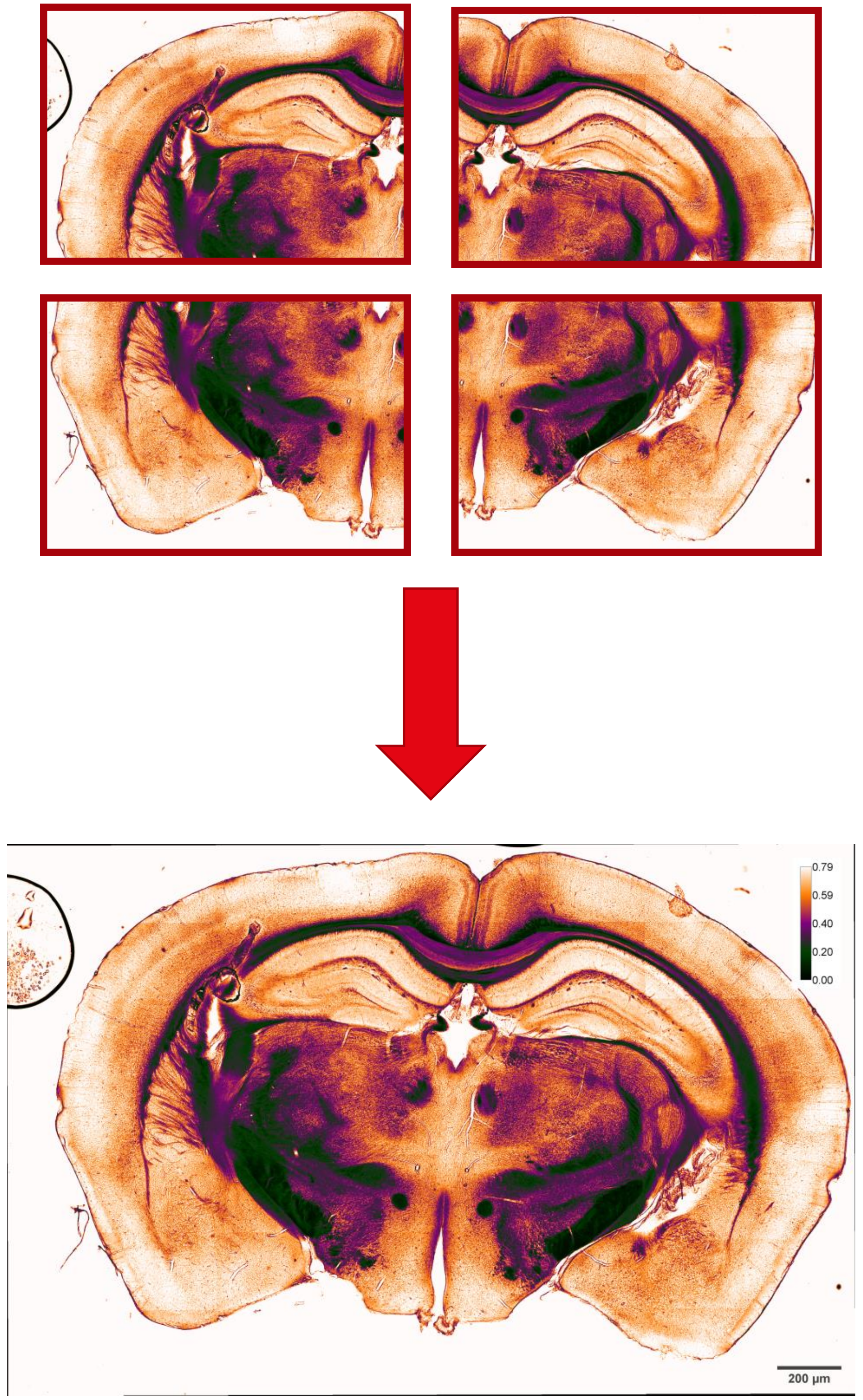


Bleaching



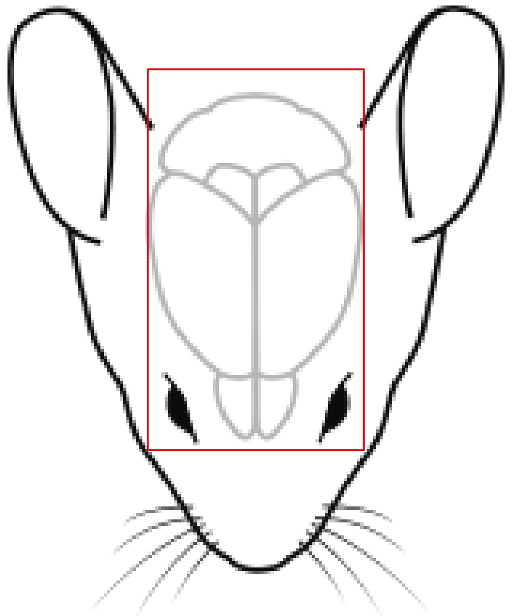
Summary

- Image Stitching
- Registration
  - Finding a transfer function between a source and a target image
    - Intensity based
    - Feature based
- Image Transformation
- Image Blending

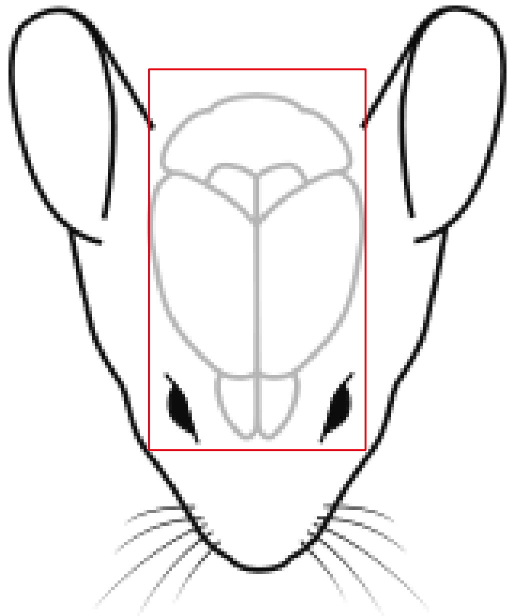


Scene to model registration

Brains can be extracted, fixed, cleared and fully imaged in 3D…

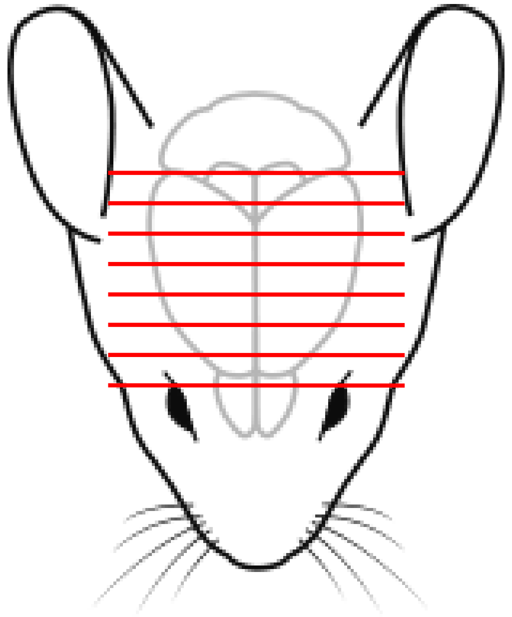


Imaging a fully cleared brain in 3D : it's not always the best choice



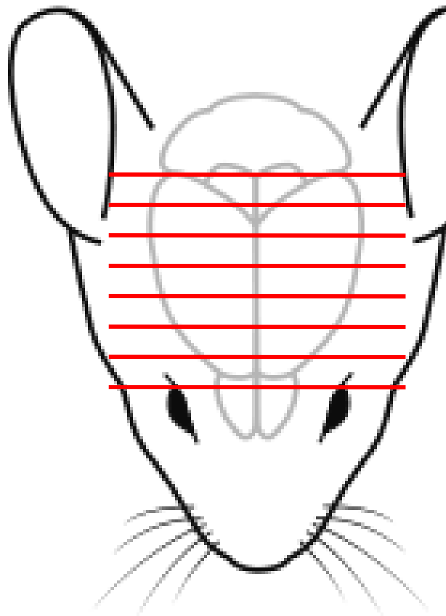
- Brains can be extracted, fixed, cleared and fully imaged in 3D
- It's not always the best solution:
  - long process
  - low multiplexing (antibody penetration issues)
  - immunostaining: long and costly

Imaging brain through thin serial sections brings more analysis flexibility

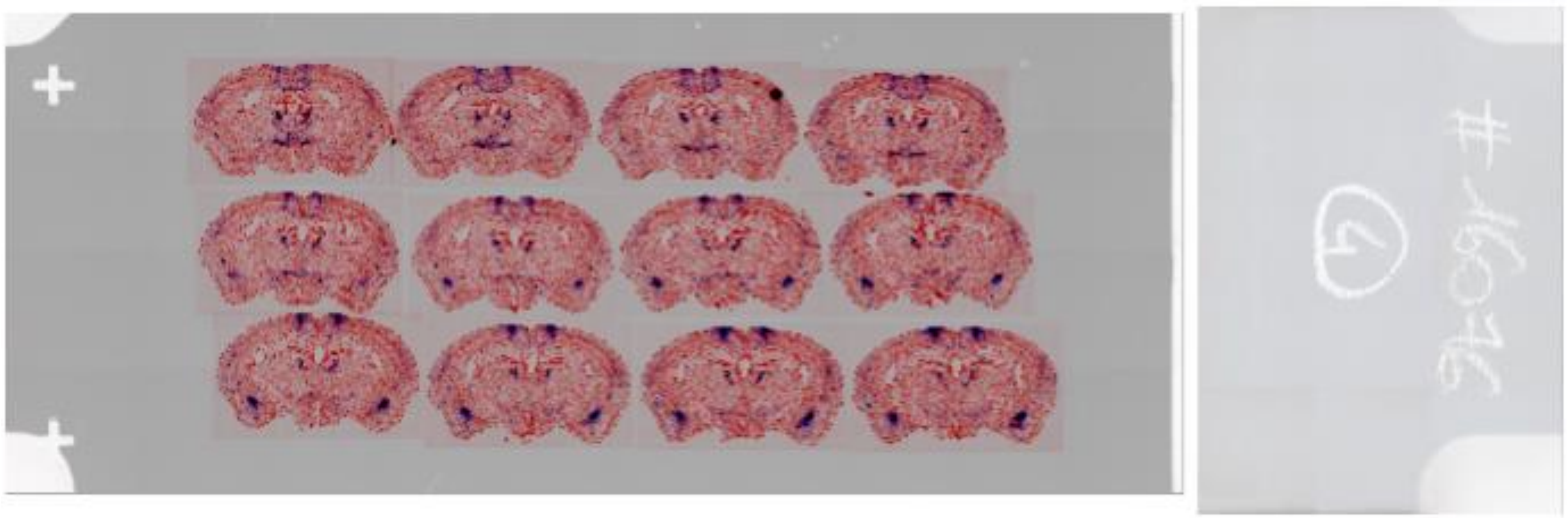


- Brains can be extracted, fixed, sliced

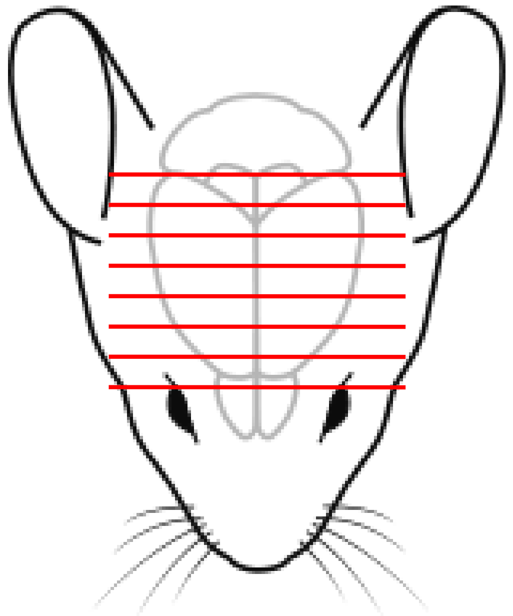
Imaging brain through thin serial sections brings more analysis flexibility



- Brains can be extracted, fixed, sliced, and imaged with a slide scanner



Imaging brain through thin serial sections brings more analysis flexibility



- Brains can be extracted, fixed, sliced, and imaged with a slide scanner
- High resolution 2D sections (~ 10 to 40 microns thick)
  - Image with slide scanner
  - very high xy resolution, low z resolution

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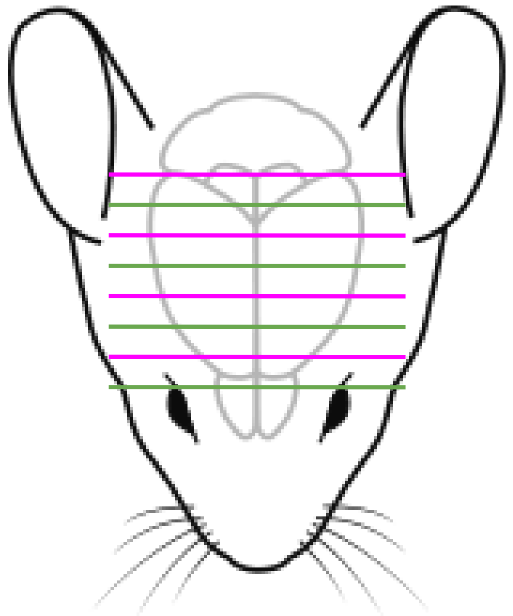
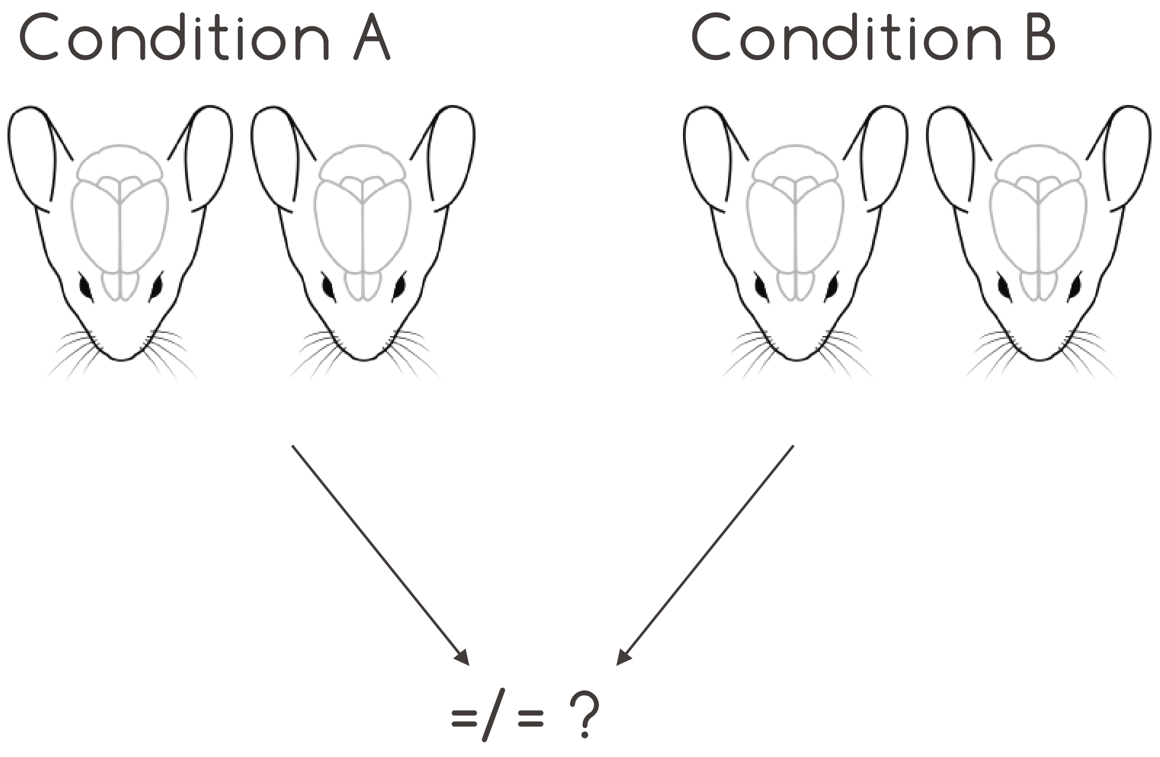
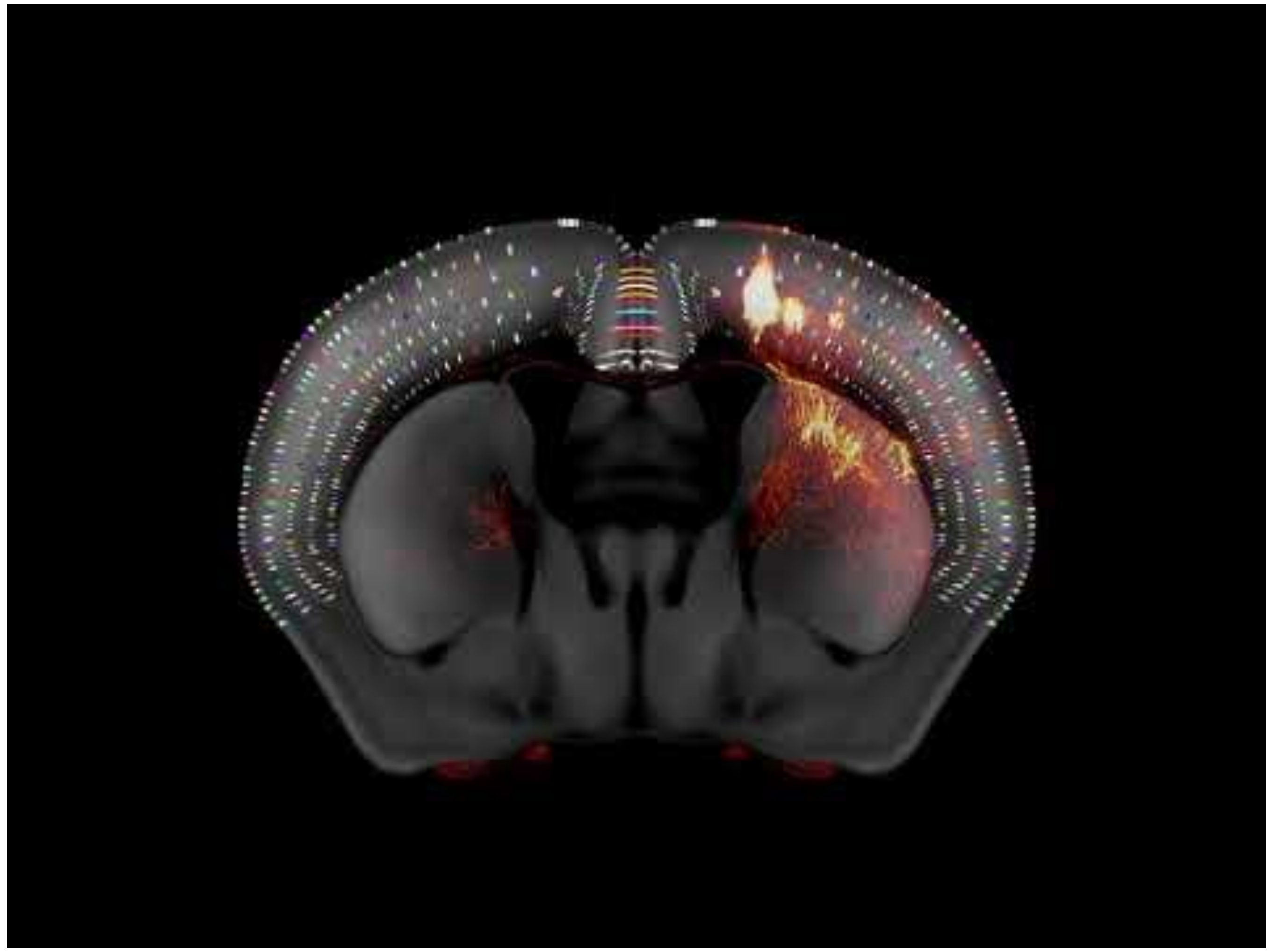


- Brains can be extracted, fixed, sliced, and imaged with a slide scanner
- High resolution 2D sections
  (~ 10 to 40 microns thick)
  - Image with slide scanner
  - very high xy resolution, low z resolution
- “Quasi 3d’ multiplexing:
  - Slice 1 : Antibody 1,2,3
  - Slice 2 : Antibody 4,5,6
  - Slice 3 : Spare
  - Slice 4 : Antibody 1,2,3
  - Slice 5 : Antibody 4,5,6
  - Slice 6 : Spare
  - etc.

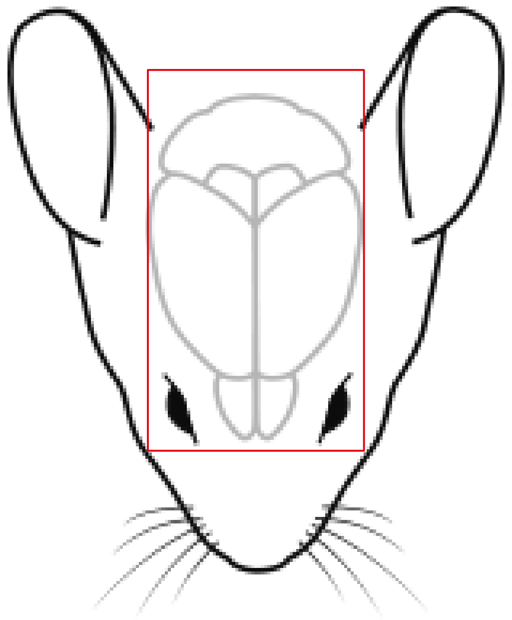
Aligning brain data to a common atlas allows for comparison between animals

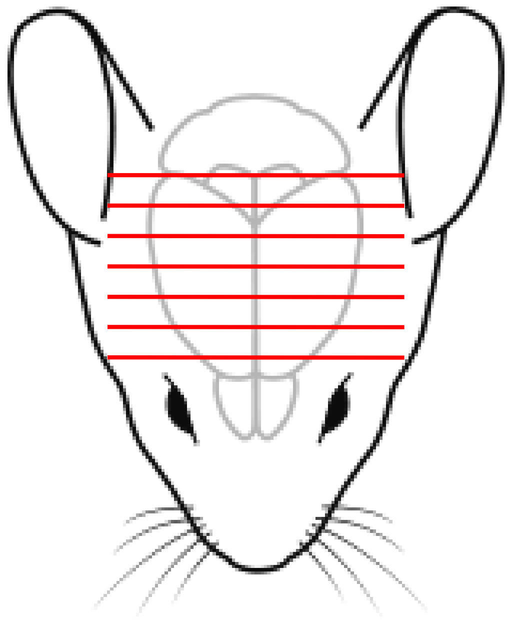




- How to compare animals between conditions ?
- Align animal brain data in a common reference
- Allen Brain Common Coordinates Framework v3 (and regions)

3D data and thin sections alignment do not pose the same challenge

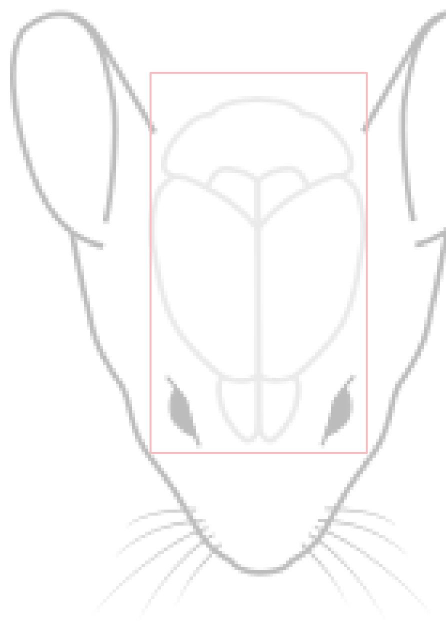




- Aligning 3D data to atlas: The overall brain structure is preserved

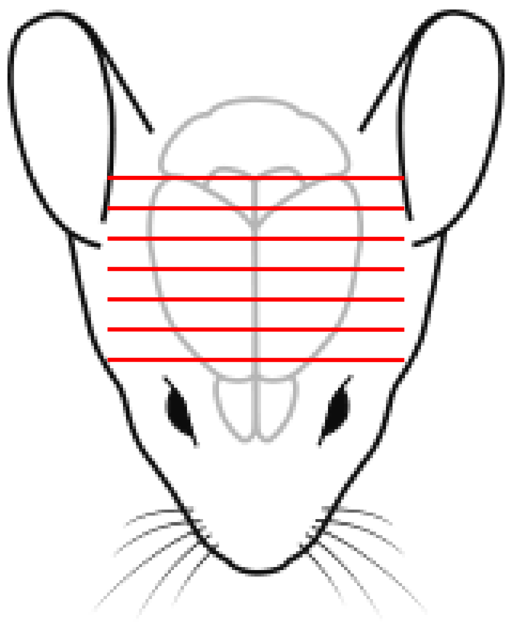
- Aligning sections to atlas: The brain is in many pieces…

3D data and thin sections alignment do not pose the same challenge



- Aligning 3D data to atlas: The overall brain structure is preserved

ABBA



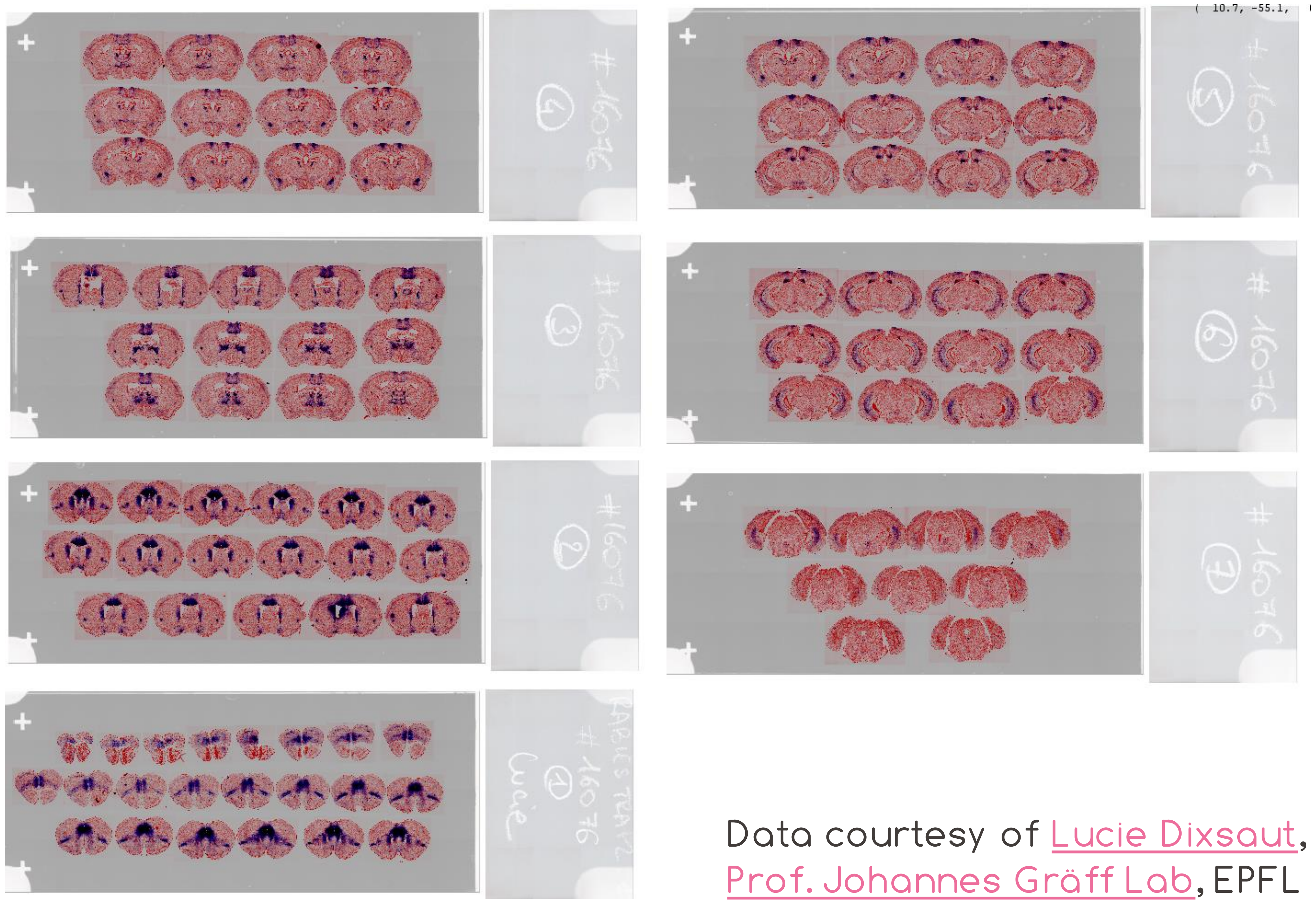
- Aligning sections to atlas: The brain is in many pieces…

Reconstructing a 3D mouse brain from microscopy images of serial sections: steps involved

- Stack sections along the slicing axis in 3D
- Move (translate, rotate) each section to match the atlas ‘in plane’
- Warp (with moderation) sections to match even better the atlas
- (Detect and classify cells of interest)
- (Position detected cells within the atlas)

Example dataset: 97 serial coronal sections (20 microns) of an adult mouse brain

Data available on Zenodo



Data courtesy of Lucie Dixsaut, Prof. Johannes Gräff Lab, EPFL

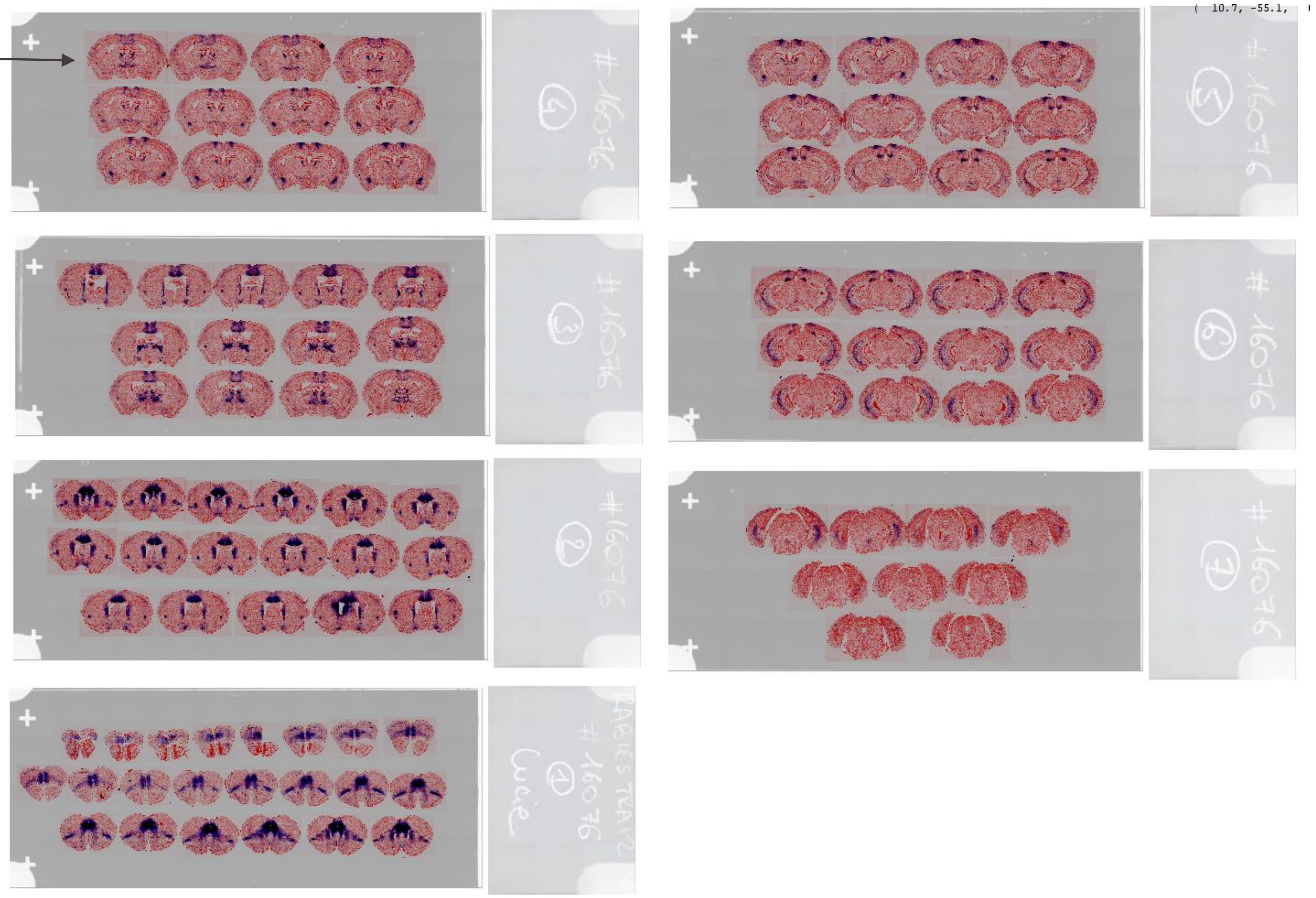
Reconstructing a 3D mouse brain from serial sections: challenges

Running an analysis pipeline *usually* involves **loading the entire dataset**:

Reconstructing a 3D mouse brain from serial sections: challenges

Dataset:

- Each section:
  - ~ 20 000 x 20 000 pixels
  - 3 channels
- Total uncompressed data: ~ 110 Gb



WSI = Whole Slide Images, and there are many!

Reconstructing a 3D mouse brain from serial sections: challenges

- Running an analysis pipeline *usually* involves **loading the entire dataset**:
  - the data can be on a network drive (100 Mb/s)
  - simply **opening** the full dataset of 110 Gb would take **15 minutes**.
  - Over Wifi ~ 2h30

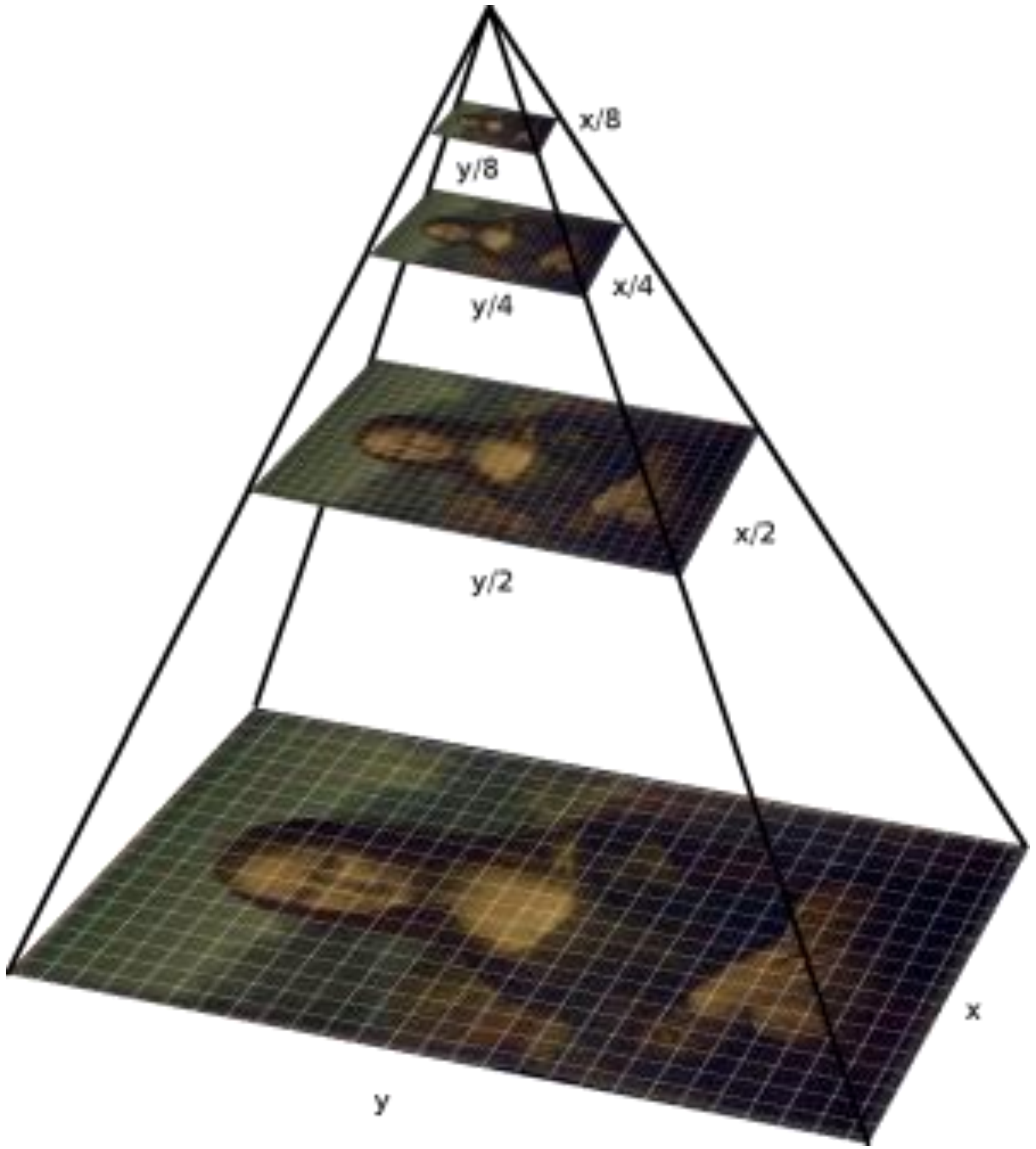
Whole Slide Images - Challenges

> 30’000 x 20’000 pixels ~ 2GB Uncompressed - **In a single plane for a single section !**

Visualizing the data is already a challenge

Whole Slide Images - Solution

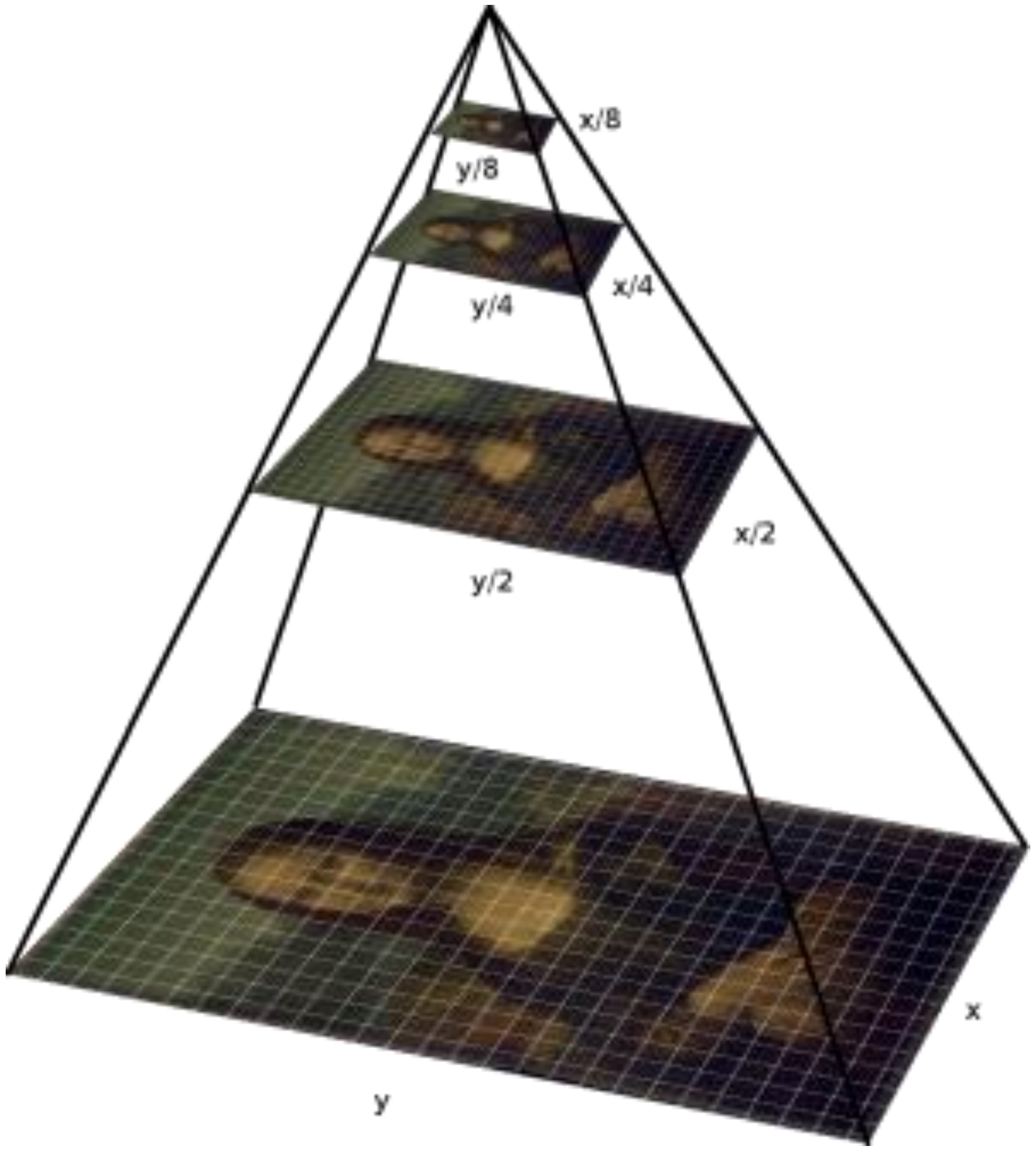
- > 30’000 x 20’000 pixels ~ 2GB Uncompressed
- **Google Maps- type approach**
- Load data on demand in blocks
- Use pyramidal representation



https://iipimage.sourceforge.io/documentation/images/

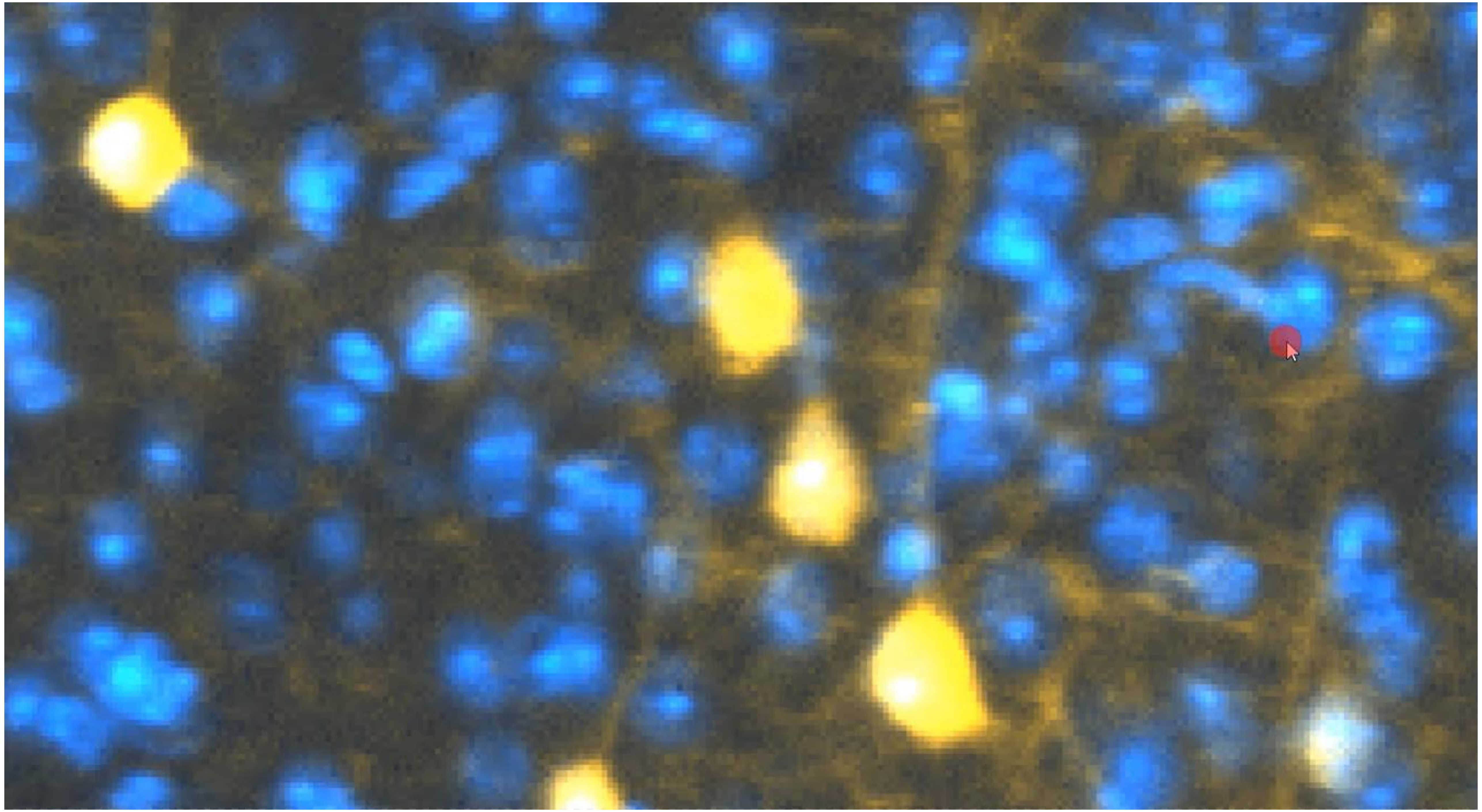
Whole Slide Images - Solution

- One can restrict data loading:
  - to the region required
  - at the resolution required
  - when required

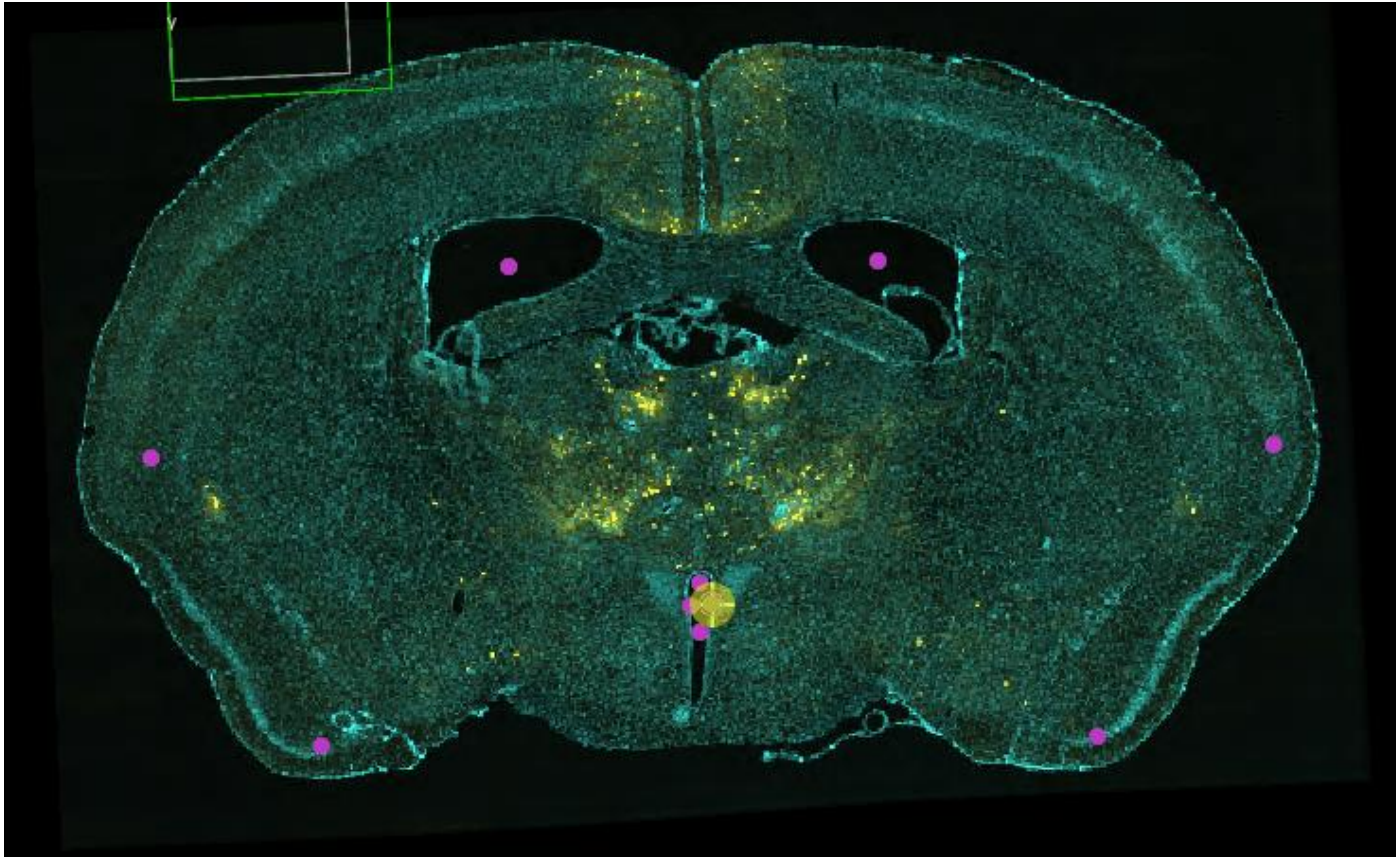


https://iipimage.sourceforge.io/documentation/images/

ABBA uses BigDataViewer for smart loading and display of big datasets



ABBA uses BigWarp to interactively display complex deformation of big datasets



ABBA speedrun

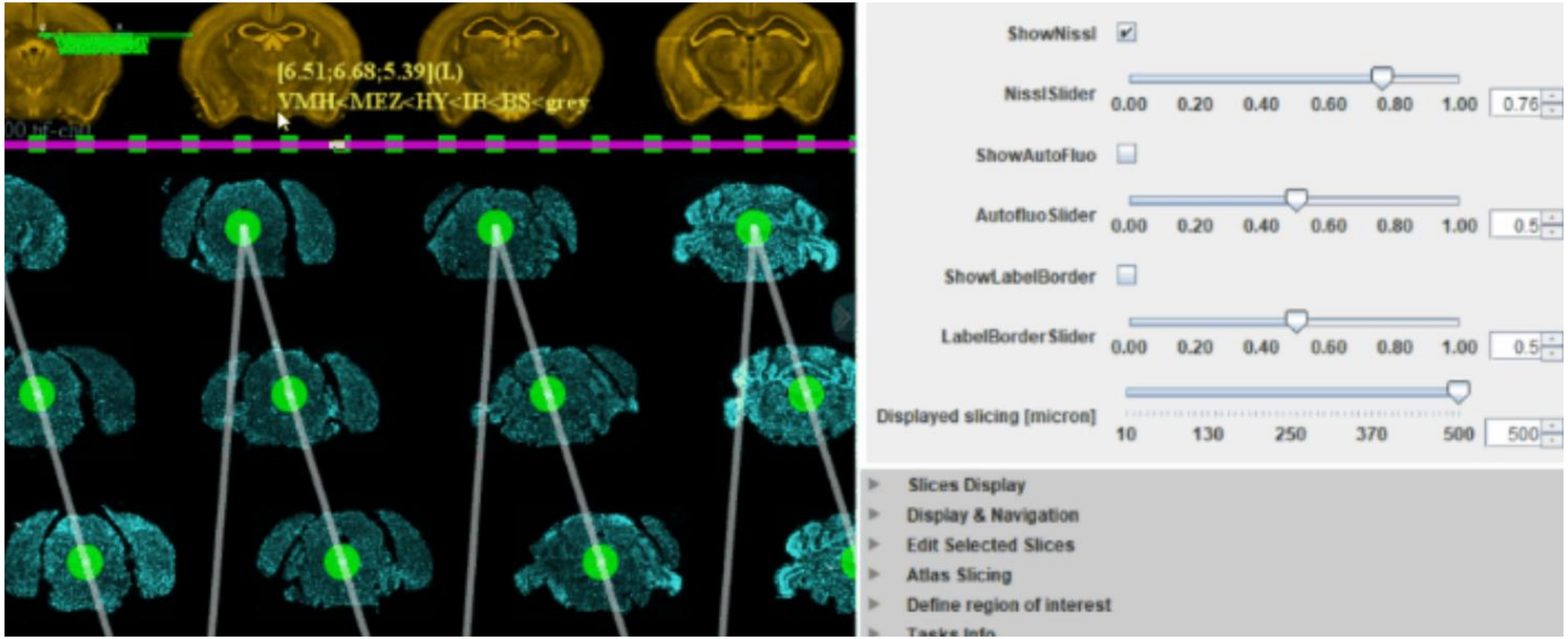
Antero-Posterior placement

In plane automated registration

In plane registration correction

Process sections in batch

For the initial placement of sections along the atlas, the position of many sections can be edited at once.



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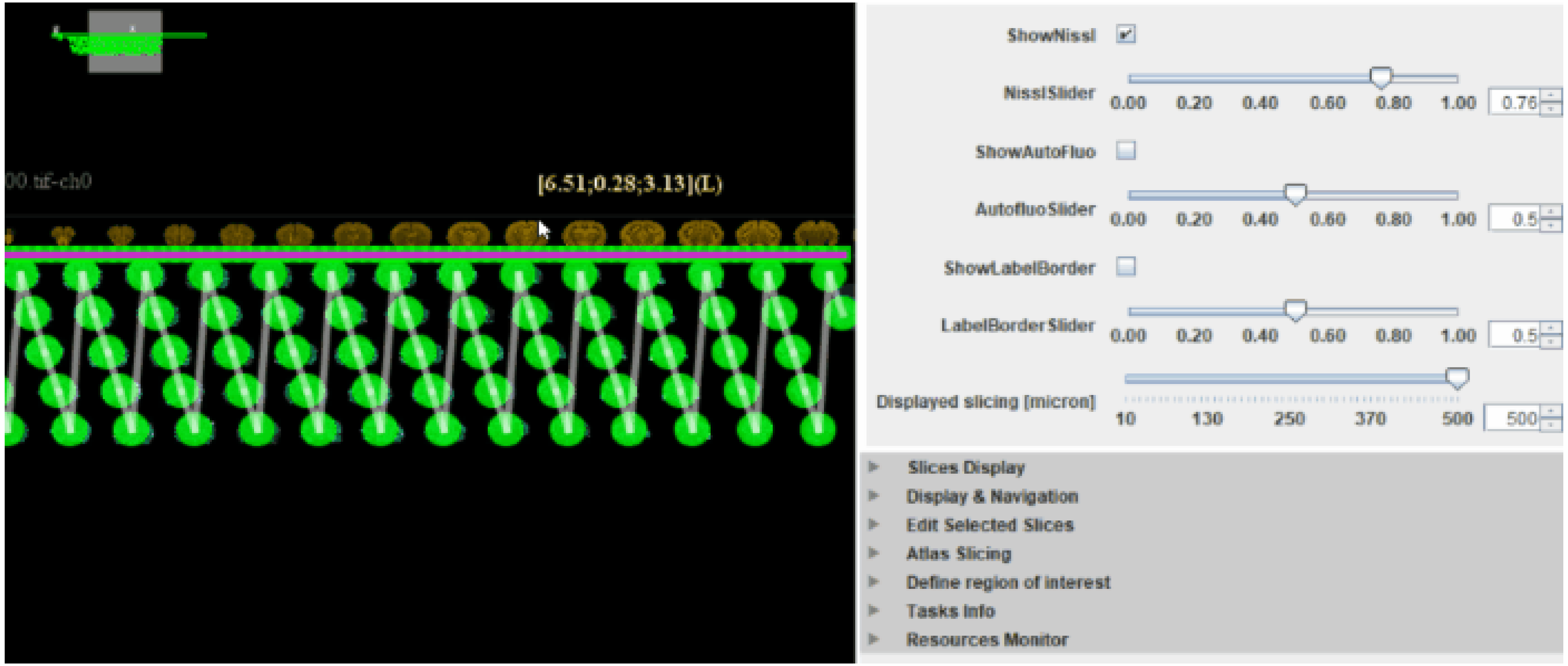

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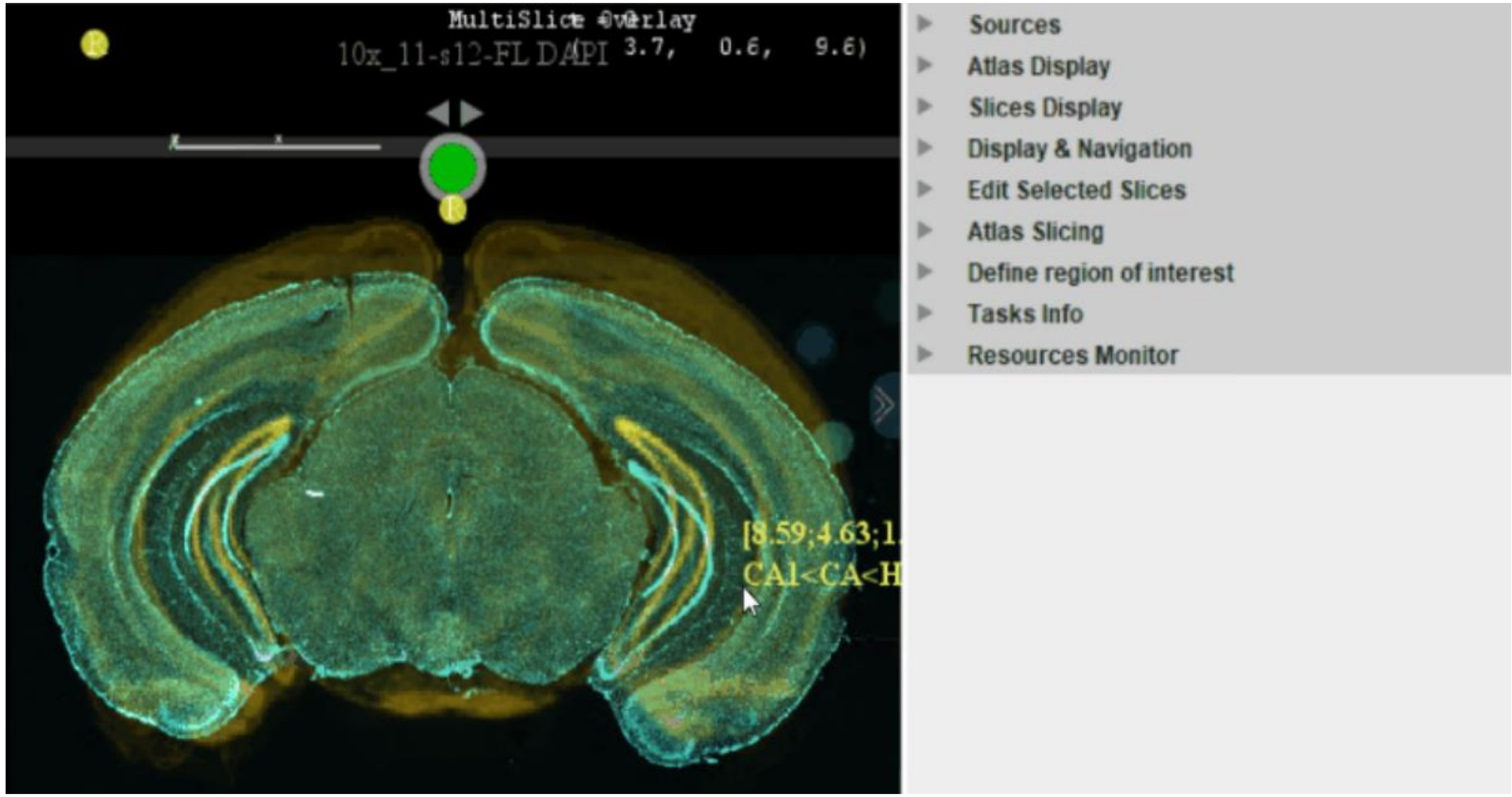
In-plane registration: ABBA uses a Elastix backend and provides automated affine or non-linear spline registration.



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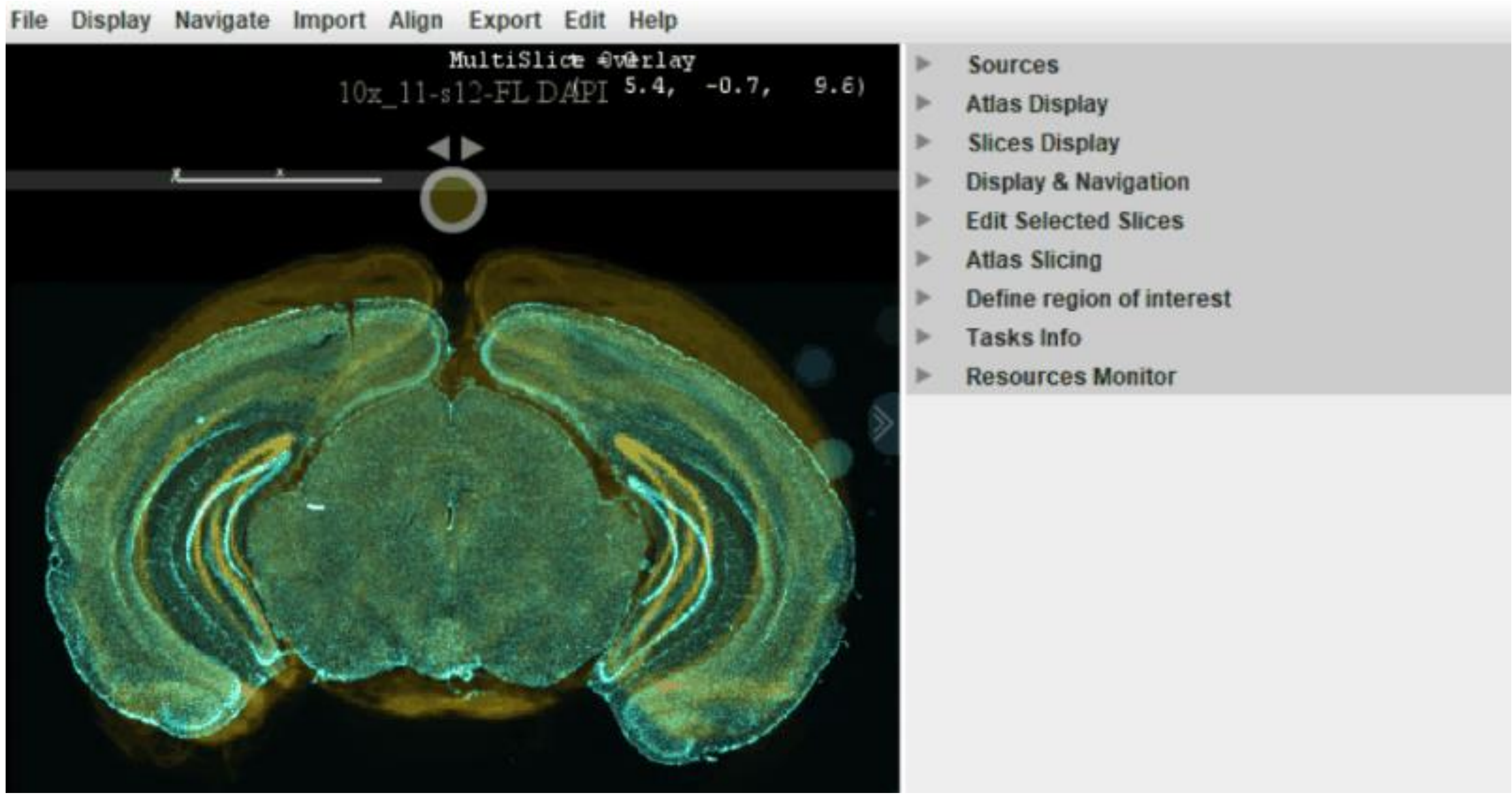

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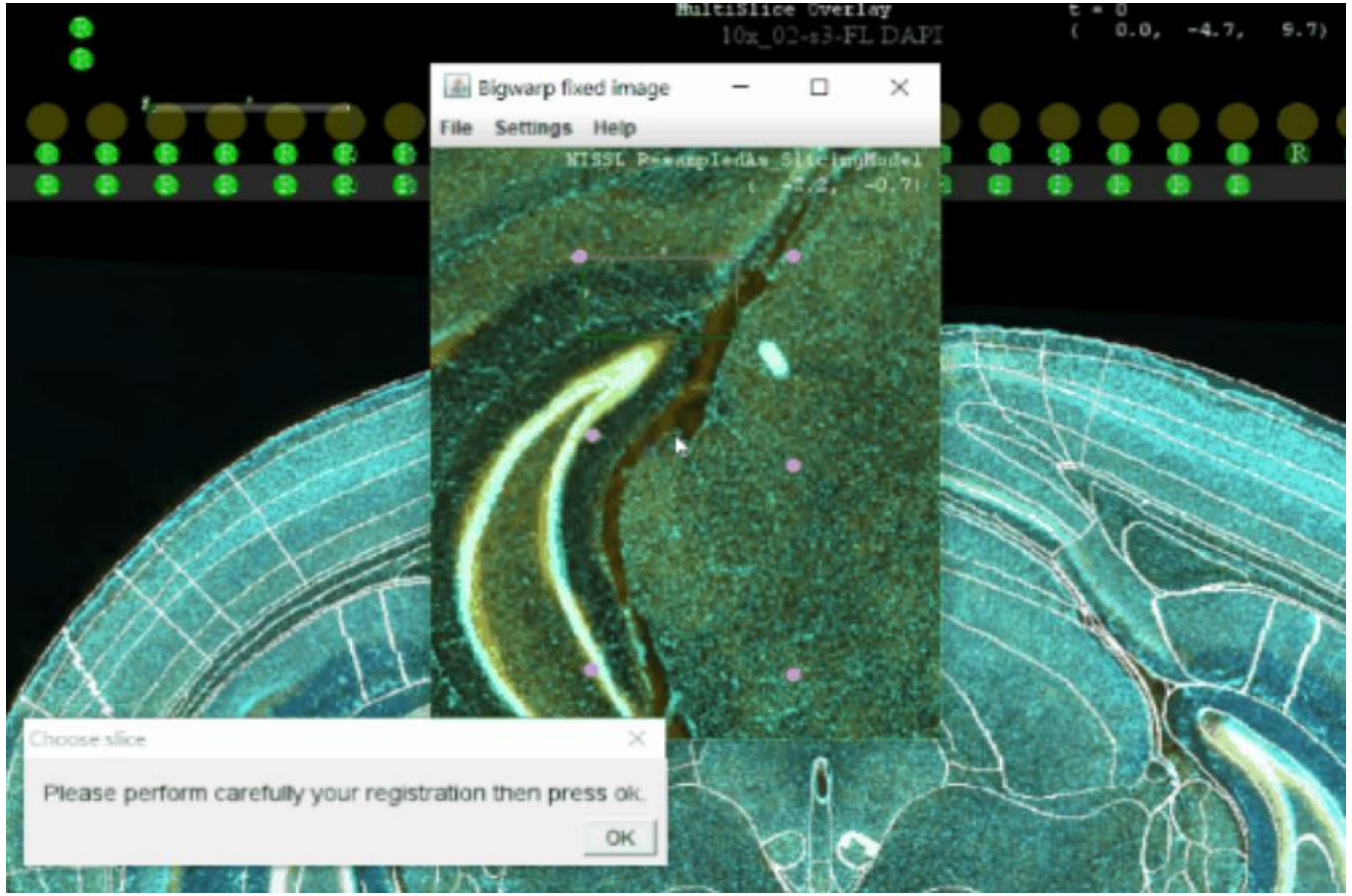

You can edit the result of an automated spline elastix registration with BigWarp.

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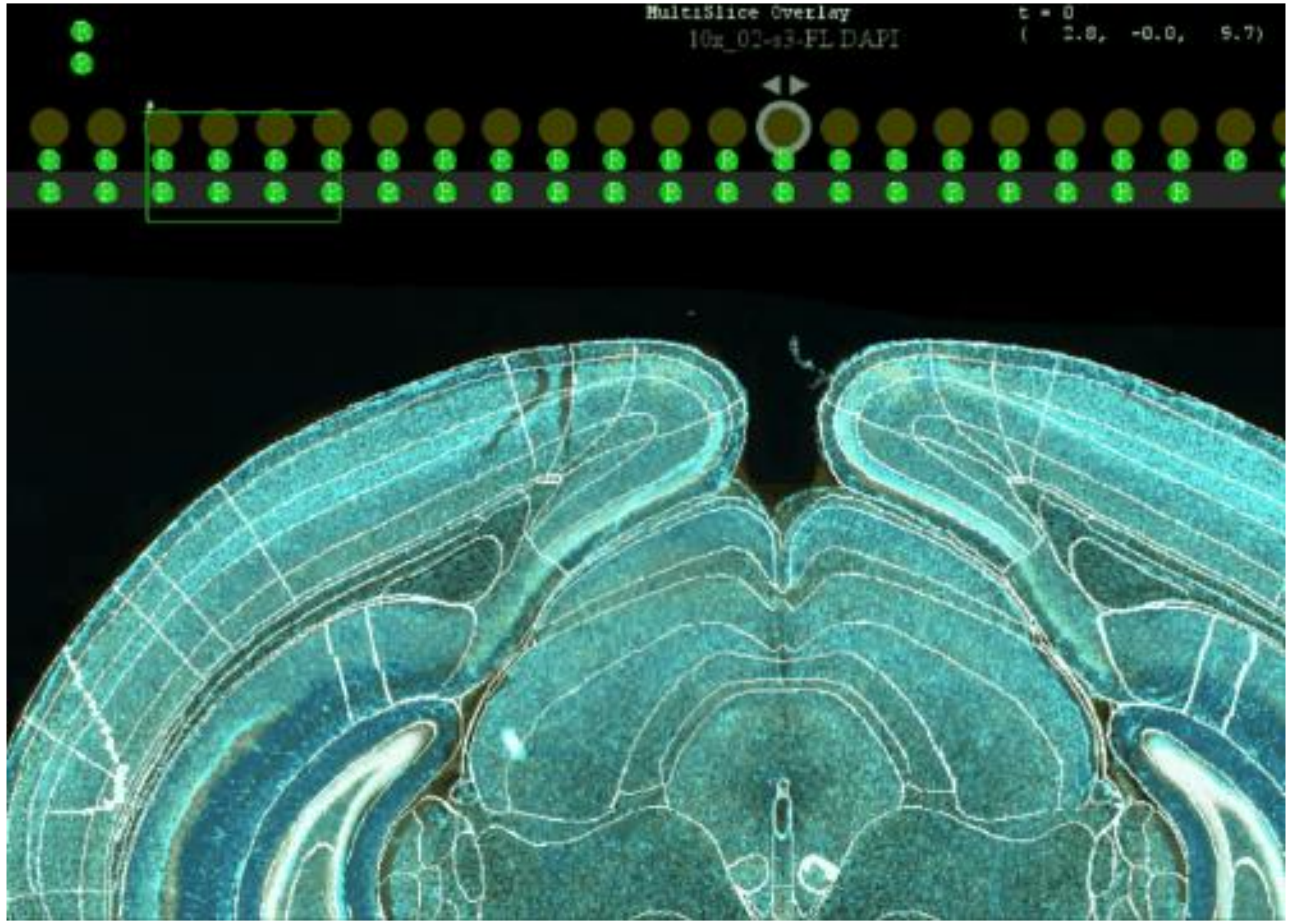

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ABBA speedrun

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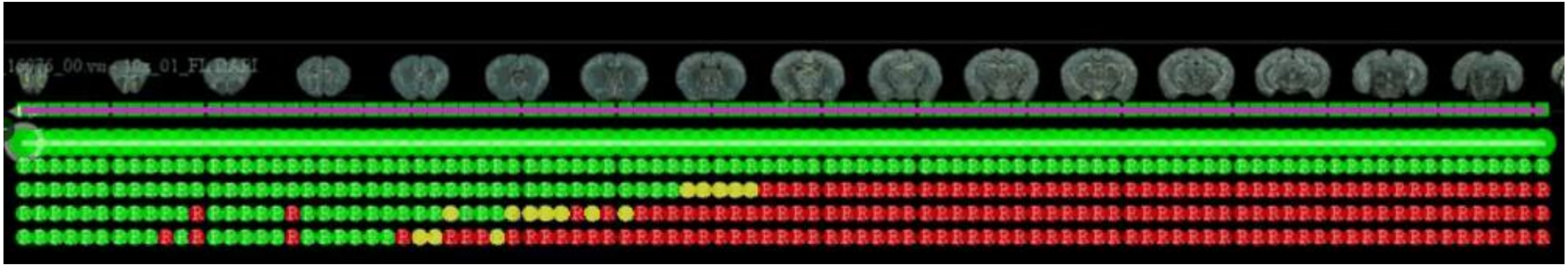
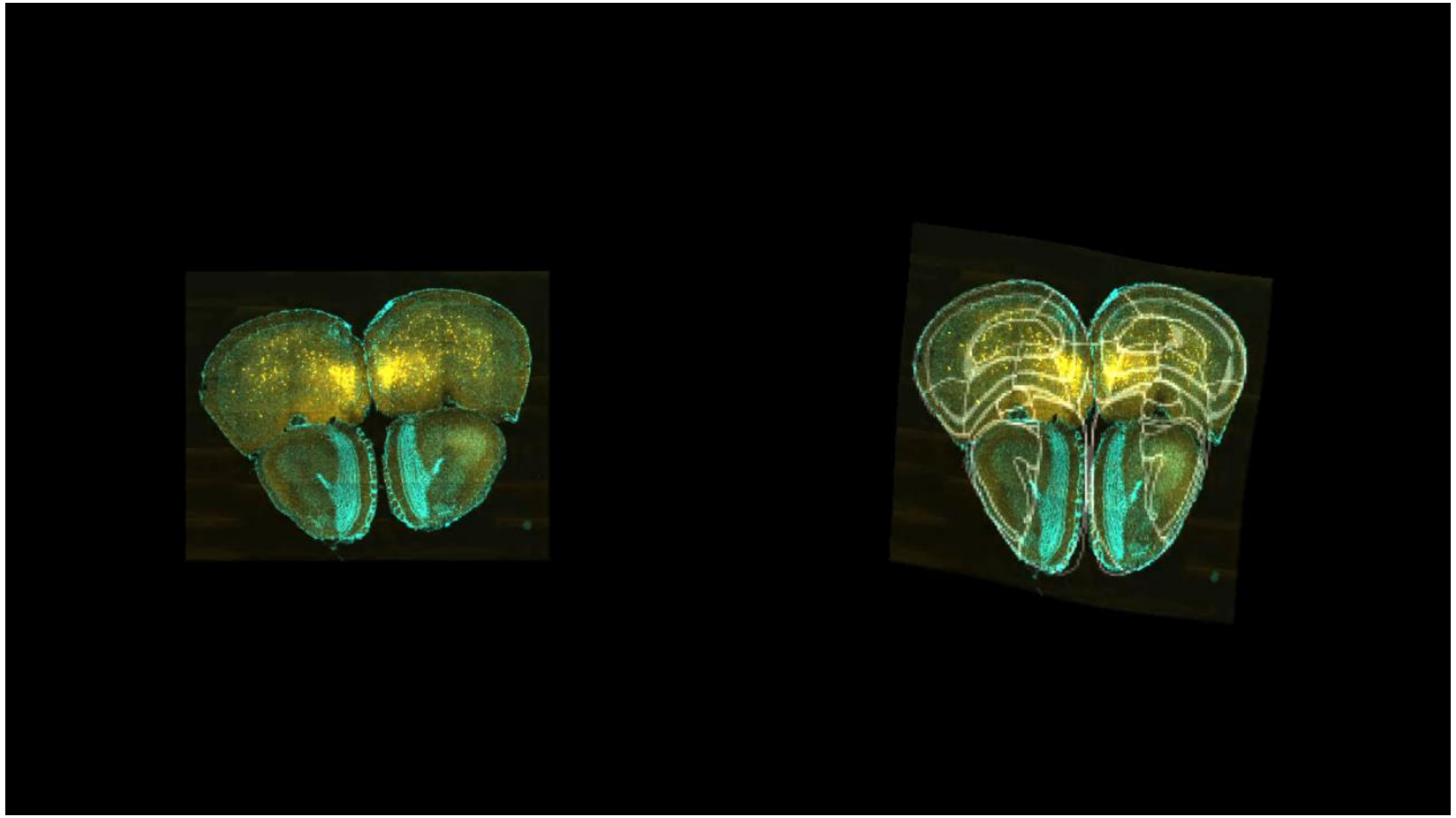
Process sections in batch

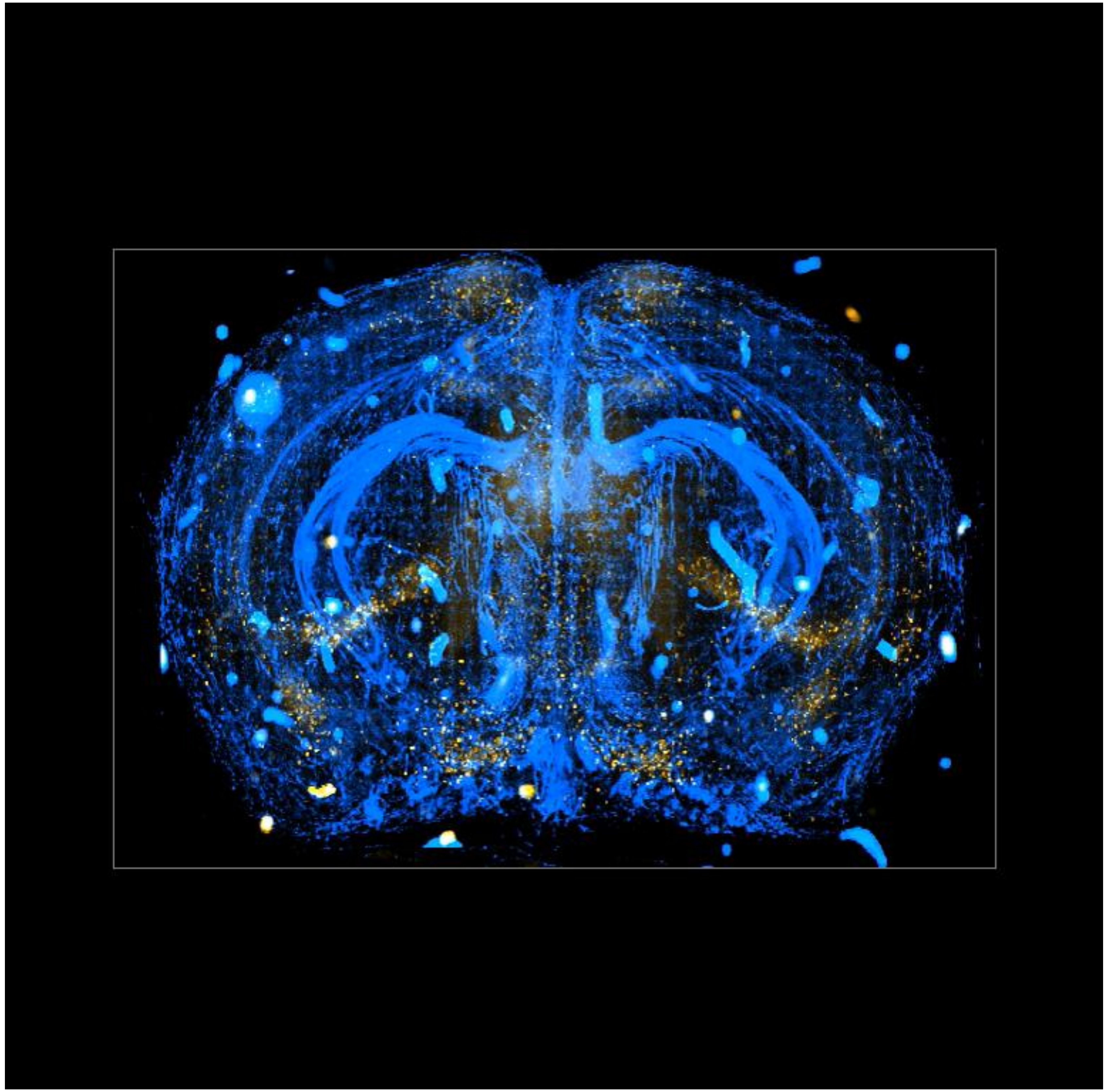
ABBA performs its tasks **asynchronously and in parallel**. (animation: 3 successive registrations performed on 90 slices: 1 affine then 2 splines, **total time ~ 15 min**/16 cores)



Visualizing aligned dataset in 2D

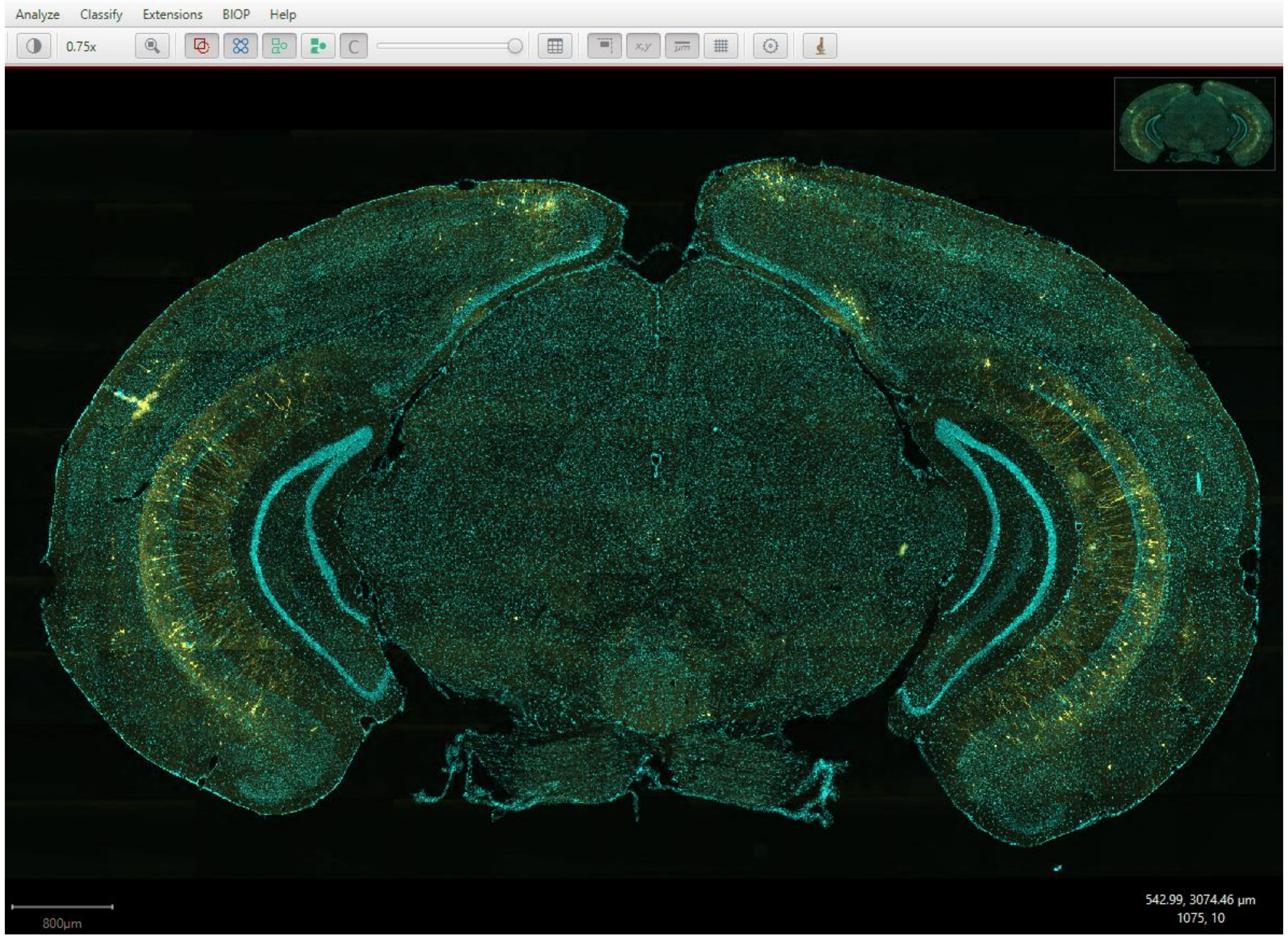


Visualizing aligned dataset in 3D



Fancy reconstruction with 3D script

ABBA interoperability
Importing results into QuPath



QuPath is the tool of choice for analyzing WSI dataset.

You can import the registered Regions in QuPath, and then **use your favorite scripts on a fully registered dataset**.