XuvTools: Fast and reliable stitching of large 3D datasets

M. Emmenlauer[1], O. Ronneberger[1], H. Burkhardt[1],
A. Ponti[2], P. Schwarb[2], R. Nitschke[3], and W. Driever[3]

1)Albert-Ludwigs-Universität Freiburg, Institut für Informatik
Lehrstuhl für Mustererkennung und Bildverarbeitung
Georges-Köhler-Allee 52
D-79110 Freiburg
{emmenlau,ronneber,burkhardt}@informatik.uni-freiburg.de

2)Facility for Advanced Imaging and Microscopy,
Friedrich Miescher Institute (FMI),
Maulbeerstrasse 66,
CH 4058, Basel, Switzerland
{aaron.ponti, patrick.schwarb}@fmi.ch

3)Albert-Ludwigs-Universität Freiburg, Department of Biology I
Developmental Biology Unit
Hauptstrasse 1
D-79104 Freiburg
{roland.nitschke, driever}@biologie.uni-freiburg.de

► Motivation

Ongoing biomedical research often requires imaging of large and thick specimen. Such specimens do not fit into the field of view (FOV) of a standard confocal microscope. Moreover the variations in the fluorescence intensities (due to absorption effects) can not be resolved with a single recording. To overcome these limitations a recombination of multiple recordings can be done. While software for such a recombination exists, we have often found it to be limited in applicability. Furthermore recent objectives provide a distortion-free image even at the border of the field of view (FOV). Such datasets allow the use of new fast, efficient and robust techniques.

► Approach

Stitching:
Large scale imaging needs to be done at arbitrary positions in the sample to keep recording time and exposure of the probe at a minimum. With most stitching software, these arbitrary positions are especially hard to stitch, as there is no information to which stacks will correlate. We use a full 3D brute force technique to overcome this limitation, while special care is taken to achieve good runtime performance.

We use a modified phase-only correlation based on the normalized Fourier spectrum, that allows us to determine pairwise best displacements while being invariant to linear gray value changes. Normalization of the Fourier amplitude is done with the square root, which has shown to be very robust against noise.
The precise pairwise displacement is determined using normalized cross-correlation, to attain a quantitative measure of similarity. Correlation windows are extracted at interest points which are selected based on image contrast. From pairwise displacements we find the globally optimal positions of image stacks using singular value decomposition.
To maintain speed, a multi-scaling approach and a heuristic for choosing likely correlation candidates have been developed.

Bleaching correction:
From the overlapping region, incident image bleaching can be estimated. We have found bleaching to be modelled by a linear process, where the parameters can be gained from the level-wise gray value cooccurrence matrix. Distribution from the overlap into the probe is modelled by a normal distribution where the variance resembles the likeliness of the fluorophore to bleach. This parameter has to be gained from the user by visual inspection.

► Results

We have applied our software toolset to various bio-medical datasets ranging from zebrafish to mouse braincell slices to trichome datasets to single human braincells. With the before mentioned heuristic, very large datasets of up to 68 stacks recorded at arbitrary positions could be stitched in less than 6 minutes on a state of the art single core computer, given a sufficient amount of RAM. For smaller datasets, stitching takes on average less time than needed to load and store the data, typically below three minutes.

► Acknowledgements

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► References, Availability

The presented software toolset "XuvTools" is freely available as open source from the software website at

http://lmb.informatik.uni-freiburg.de/lmbsoft/xuvtools/

Future enhancements and updates will be made available at this website as well. We have released XuvTools under the Gnu Public License (GPL) v2, so it can be used and extended by as many people as possible. Commercial licensing is available upon request, please contact Mario Emmenlauer (emmenlau@informatik.uni-freiburg.de).

To the software, we have a corresponding publication "XuvTools: Free, Fast and Reliable Stitching of Large 3D Datasets". In it we describe all used methods, the application of XuvStitch to a broad range of datasets as well as a comparison with other commercially and freely available tools. The paper has been accepted by the Journal of Microscopy, and is currently pending publication.

[1] M. Emmenlauer, O. Ronneberger, A. Ponti, P. Schwarb, A. Griffa, A. Filippi, R. Nitschke, W. Driever, and H. Burkhardt. XuvTools: Free, Fast and Reliable Stitching of Large 3D Datasets. To be published in the *Journal of Microscopy*, 2008. URL http://lmb.informatik.uni-freiburg.de/people/ronneber/

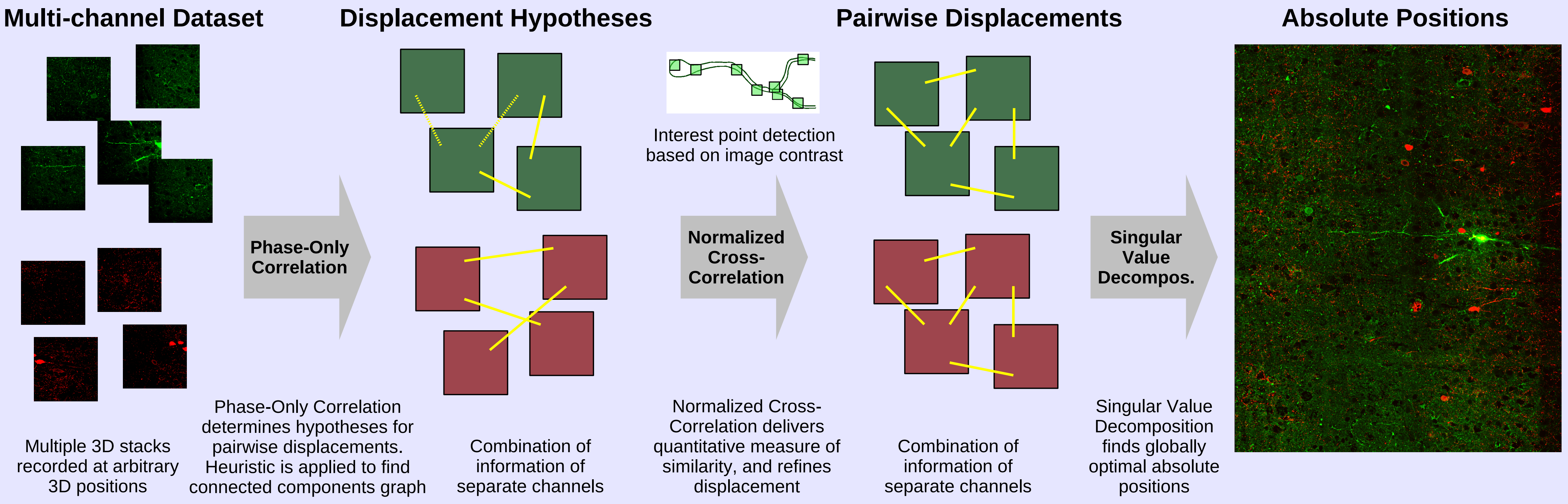
Benchmarks

All benchmarks were performed on a single core of a 2.6 Ghz Xeon computer with 32GB Ram. Results are comparable to those on a Core 2 Duo personal computer with 16GB of Ram.

For the large datasets, a large fraction of the time is needed for loading and storing the image data. Shown are times for stitching, without bleaching correction. All sizes are in voxels.

Dataset	Size	Stacks	Time
Mouse, cereb. sec.	9300x4200x77	68	6m 10s
Zebrafish	970x970x480	4	30s
Mouse brain	5000x6300x128	20x2	2m 33s
Single neuron cell	2800x2000x80	6	13s

Stitching of large 3D Datasets


Multi-channel Dataset

Displacement Hypotheses

Pairwise Displacements

Absolute Positions

Phase-Only Correlation

Interest point detection based on image contrast

Normalized Cross-Correlation

Singular Value Decompos.

Multiple 3D stacks recorded at arbitrary 3D positions

Phase-Only Correlation determines hypotheses for pairwise displacements. Heuristic is applied to find connected components graph

Combination of information of separate channels

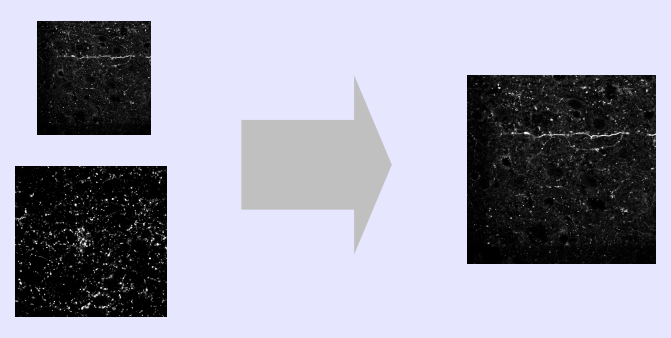
Normalized Cross-Correlation delivers quantitative measure of similarity, and refines displacement

Combination of information of separate channels

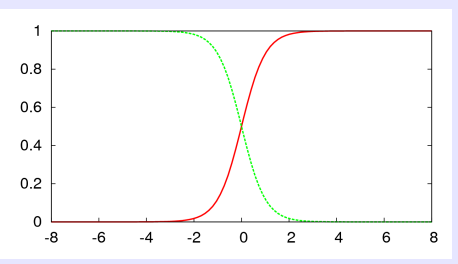
Singular Value Decomposition finds globally optimal absolute positions

Additional Steps (as required)

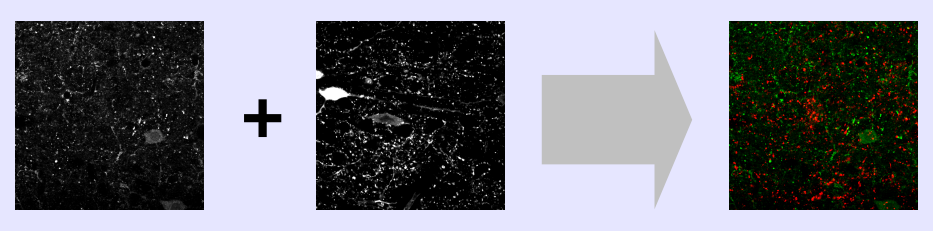
Scaling to finest element size, using trilinear interpolation



Blending images into each other using a sigmoid function



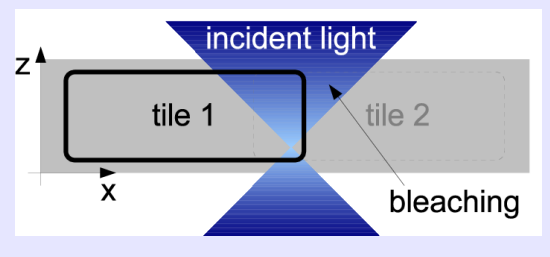
Combining multiple channels into one overlay image



Bleaching Correction

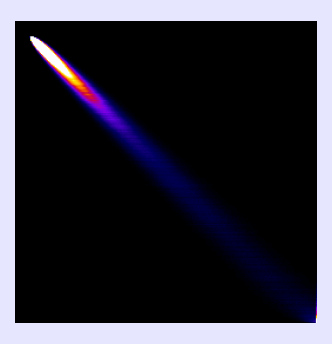
Level-wise estimation of linear parameters

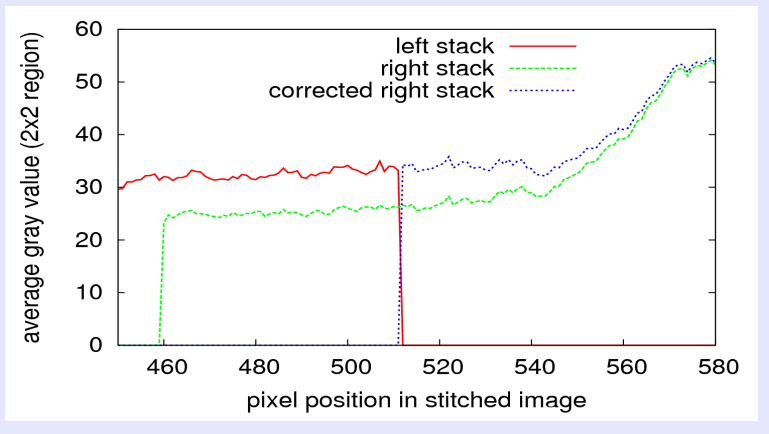
Incident image bleaching can be estimated from the overlapping parts of two images, where gray values from before and after a recording are available.



Incident light above and below the focal plane can cause bleaching even outside of the currently recorded tile. Stray light has a similar effect.

The gray value cooccurrence matrix supplies information on the linear parameters of the bleaching function, as well as on its standard deviation.



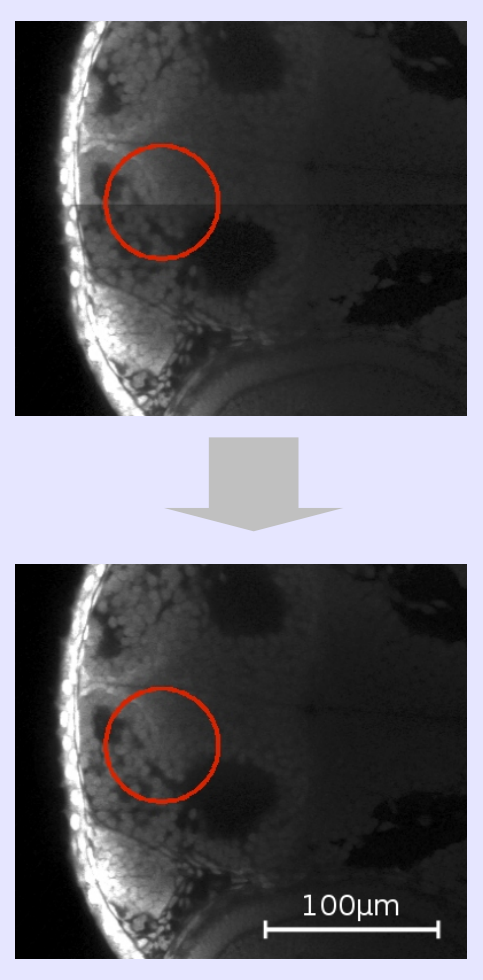


Bleaching visualized:
Average gray values of two overlapping images.
Red the first recording, green the second. Visible is the drop in intensity in the overlapping part (green curve below red curve).

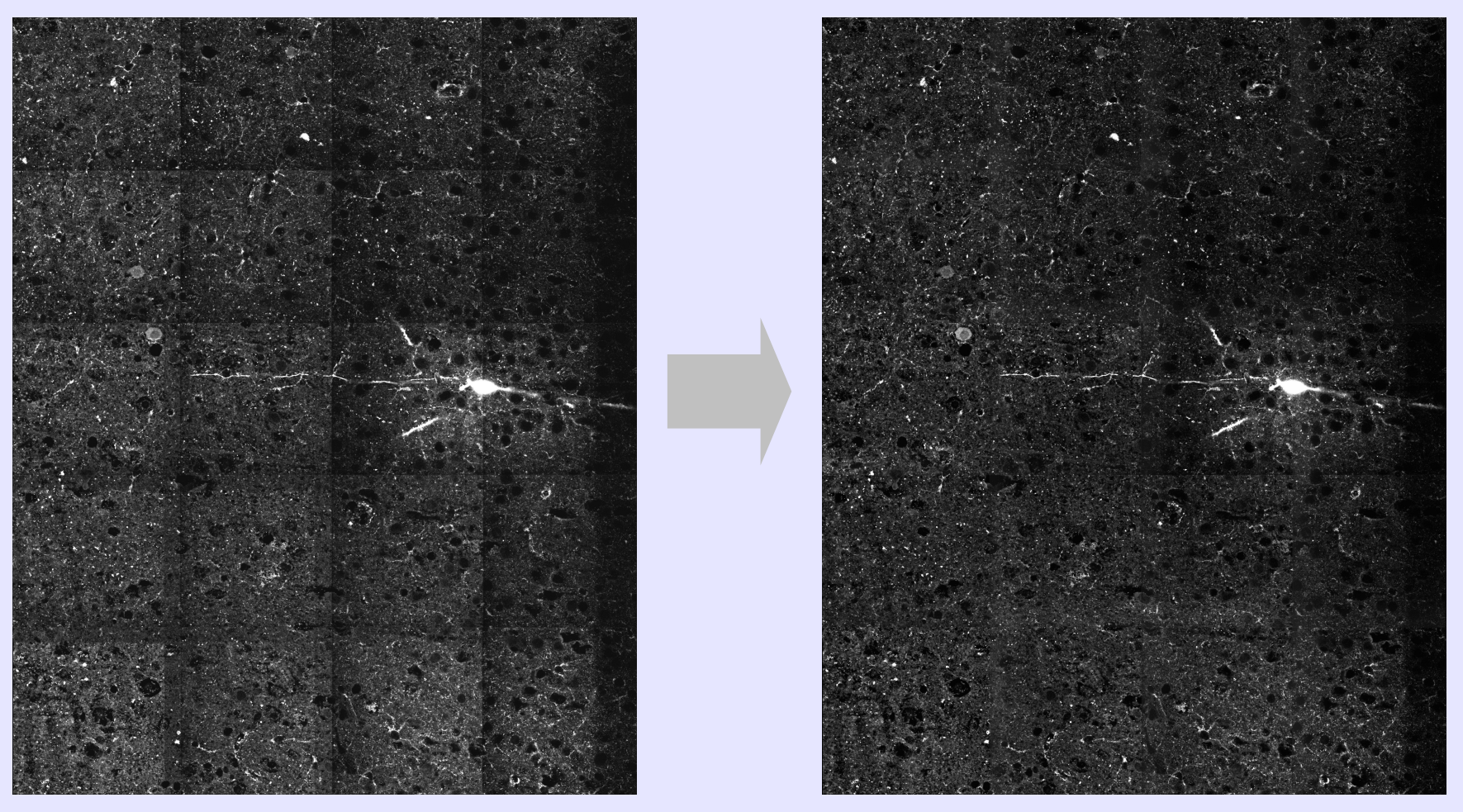
The blue curve depicts the estimated original intensities.

Corrected sample Datasets

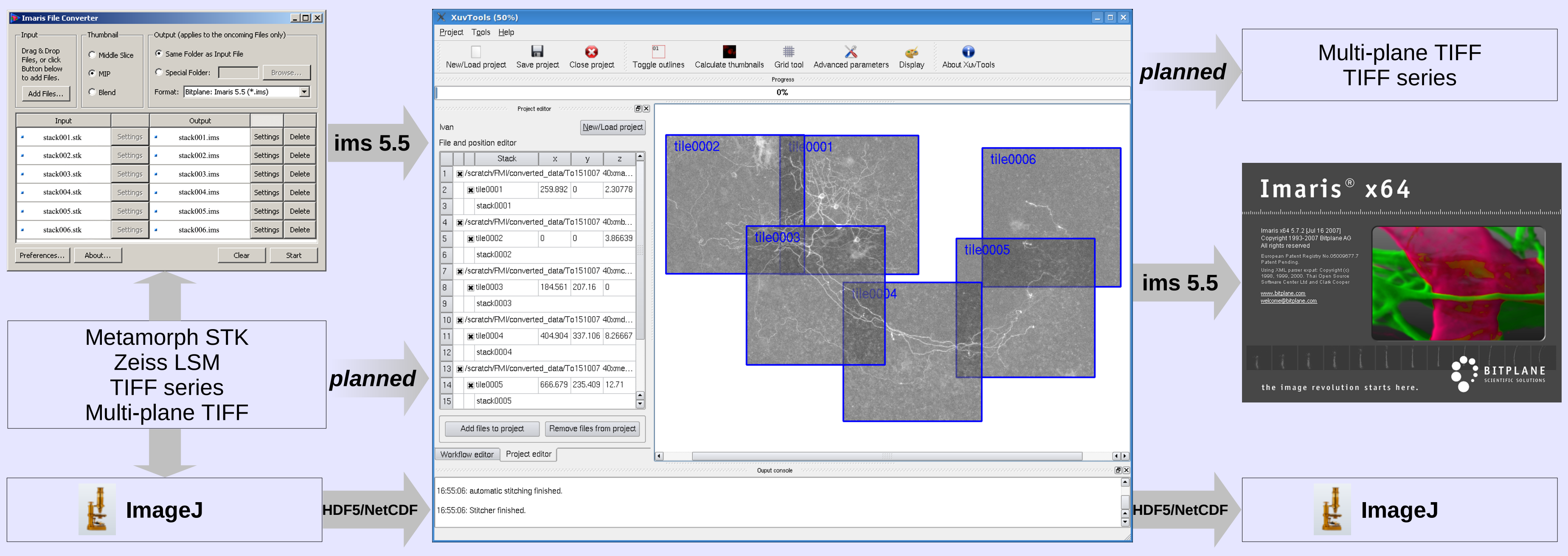
Zebrafish
Part of two overlapping Stacks, good correction.



Pyramidal neuron, in primary somatosensory cortex of the rat
Image consisting of 20 stacks (one channel of two).
Bleaching is reduced, but still visible.



Workflow with XuvTools



16:55:06: automatic stitching finished.
16:55:06: Stitcher finished.

The XuvTools natively read and write generic HDF5 and NetCDF and Bitplane's Imaris 5.5 file format. Through conversion, either in ImageJ (to NetCDF or HDF5) or with the Imaris File Converter (to ims 5.5), all major microscopy formats can be indirectly input into XuvTools thus making our tools widely applicable for the microscopy community.

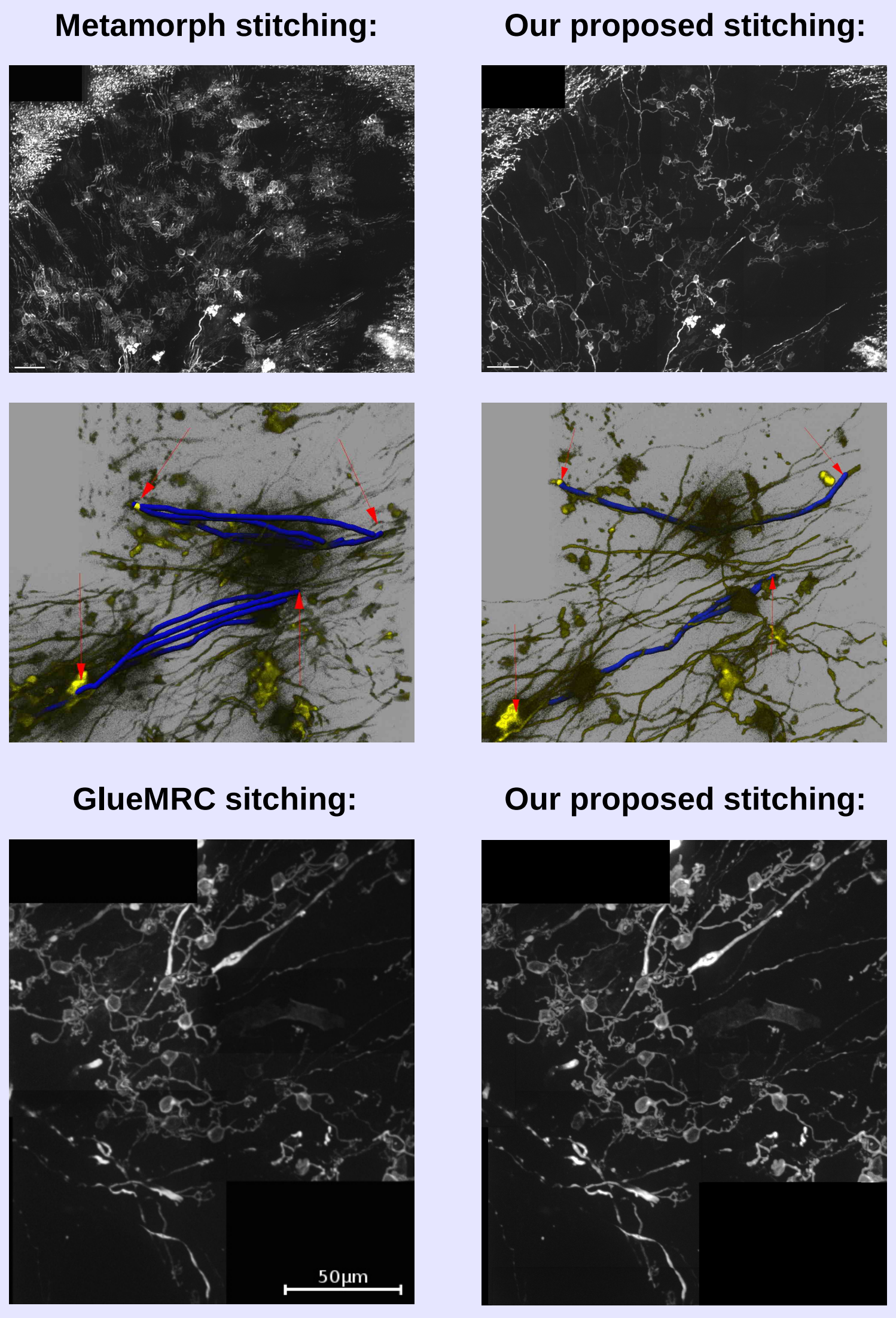
Currently, the best workflow uses the Imaris file format both as input and output format. Indeed, the conversion to NetCDF and HDF5 in ImageJ is somewhat limited, whereas the Imaris File Converter performs a better job. Moreover, saving the result of the stitching as an Imaris file allows one to exploit the "huge datasets" capabilities of Imaris to smoothly visualize datasets that can easily grow to tens of GB in size.

Support for **direct** reading of some of the major microscopy formats, Metamorph's 'stk' and Zeiss' 'lsm' among others, is planned.

Comparison with other Software

Available Stitching software for our Comparison
We have compared XuvTools with several common stitching tools (see [1]). However, of the available tools, only Molecular Devices Metamorph, Zeiss Axiovision and GlueMRC of the Academy of Sciences of the Czech Republic where able to perform stitching of 3D datasets.

Here we provide an exemplary comparison with Metamorph and GlueMRC, for details please refer to [1].


Metamorph stitching:

Our proposed stitching:

GlueMRC sitching:

Our proposed stitching:

Maximum Intensity Projection (MIP):
Metamorph (left image) performs plane-by-plane stitching independently, without Z-correction. While the stitching of each individual plane is usually good, artifacts can appear between planes. In the MIP, these artifacts are visible as multiplicities of the same structure.

Filament Tracing:
We have employed filament tracing on the result of Metamorph stitching (left image) and of our proposed stitching. The artifacts introduced from the pseudo-3D-stitching in Metamorph can lead to multiply detected filaments which are not there in the original image stack.

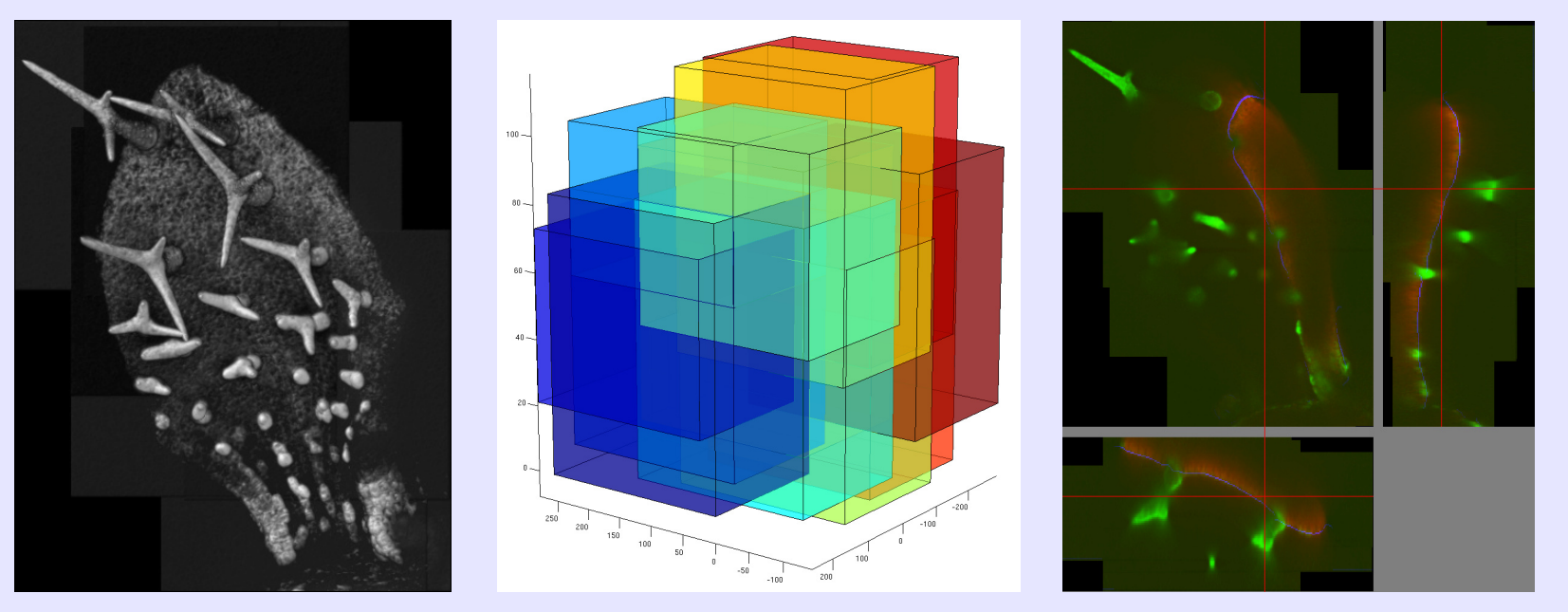
GlueMRC semi-manual stitching:
GlueMRC (left image) is a freely available stitching software which performs full 3D stitching.
The results are generally very good. For every two image stacks, the user has to select two corresponding structures which will then be correlated (thus semi-manual stitching).

The XuvTools stitching (right image) is identical to the GlueMRC one, but was achieved without user interaction in a fraction of the time.

Additional Datasets

Arabidopsis Trichome Dataset

Image consisting of 12 stacks, recorded at arbitrary 3D positions.

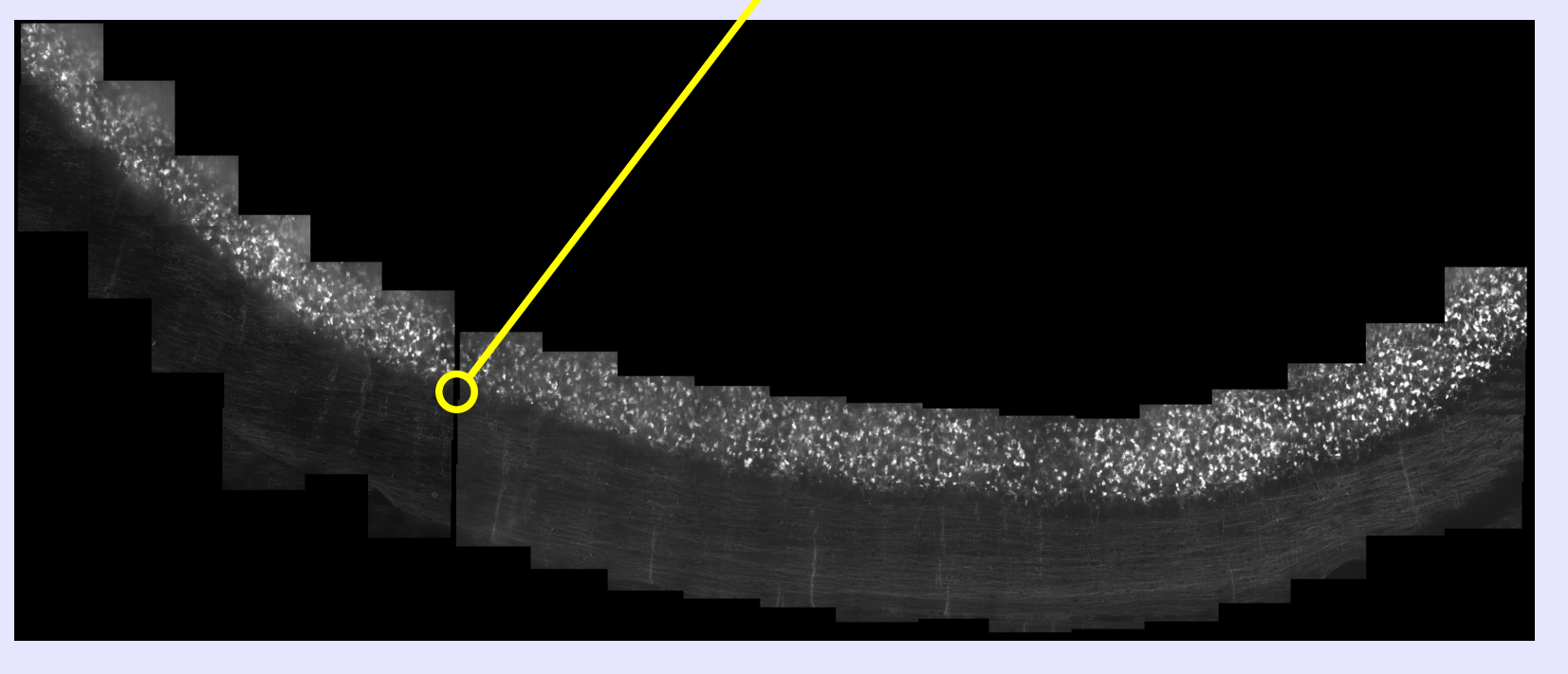

Shaded volume rendering

Estimated stack positions in 3D

Orthoview projection

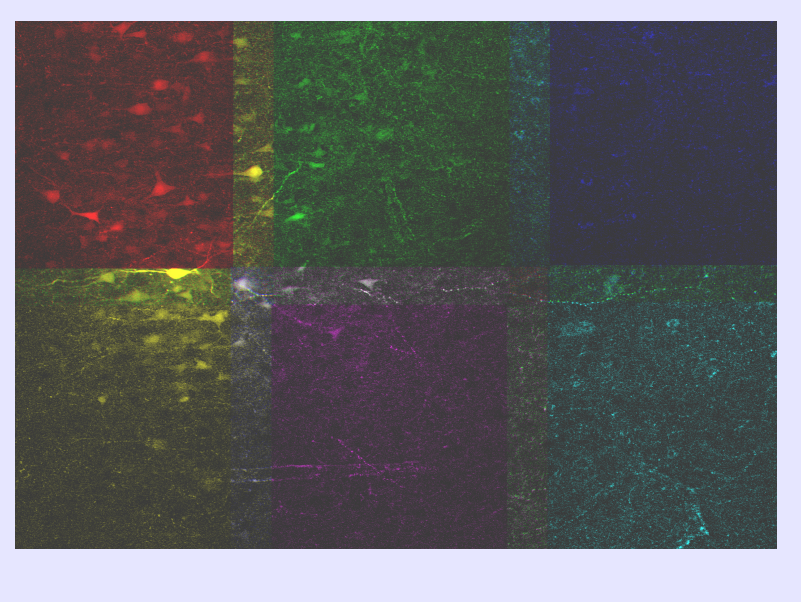
Membrane-GFP transgenic mouse line

Cerebellar section.
Stitching sectioned in 20 + 43 stacks, due to too small overlap of less than 16 pixels (see border).



Rat neuron cell

Color overlay shows good matching of the fine dendrites.



Human brain cell

From the original 12 stacks, only the 7 containing dendrites could be matched.