Fast, reliable and automatic 3D alignment of confocal image stacks: Additional Material

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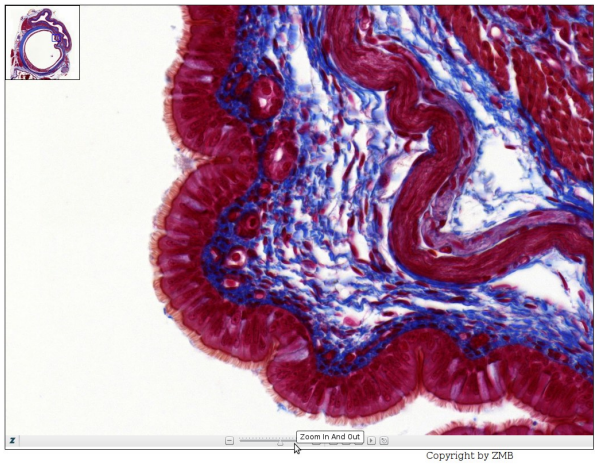
Commercial Solutions: Bundled with Hardware

Name	Data type	Automation
Zeiss Mirax Micro	3D	full



Solutions are geared towards automation. Alignment quality ranges from poor to sufficient, i.e. mostly dependent on the microscope stage. Few user interaction possible. Requires custom hardware.

Commercial Solutions: Standalone Software

Name	Data type	Automation	A priori info
Microsoft Powerpoint	2D	none	None
Adobe Photoshop	2D	full	None
Imagic ImageAccess	2D	semi	Reference points
MediaCy. Image-Pro	2D	semi	Defined grid
CarlZeiss AxioVision	2D, 3D	semi	Scanning stage
Mol.Dev. Metamorph	2D, 3D	semi	Scanning stage

All tested commercial standalone software packages provide poor automation, and have limited 3D support. Require a priori information to work. Often limited to grid-like structures.

Freeware Solutions

Name	Data type	Automation	A priori info
EPFL MosaicJ for ImageJ	2D	semi	Manual pre-pos.
ASCR GlueMRC, LinkMRC	2D, 3D	semi	Reference points
Max Planck Fiji plugin	2D, 3D	full	Optional
XuvTools	3D	full	Optional

An in-depth comparison is available in our paper:
Emmenlauer et al, XuvTools: free, fast and reliable stitching of large 3D datasets. *Journal of Microscopy*, 233(1):4260, 2009

Overview

Imaging Considerations

If you meet the following imaging requirements, you are likely to be successful with stitching!

- Neighboring images need 5% to 10% overlap
- In the overlapping region, there needs to be structure (neuron, cells, beads, etc). Any structure is good
- Always use zoom=1.0 (no zoom), else there are border artifacts (barrel distortions) that make stitching difficult
- Record at least 4 slices in Z, for every X/Y stack
- No rotation allowed

Imaging Considerations

If you meet the following imaging requirements, you are likely to be successful with stitching!

- No need to use a regular grid when imaging. Feel free to use the joystick for positioning.

- Multiple channels are good, and improve stitching quality. The more, the better.
- Structure is sufficient in one of the channels for every pair of tiles.
- Sensor dynamic range of 8, 12, or 16 bit are all supported. The more bit, the better.

System Requirements for running XuvTools

XuvTools works well on Microsoft Windows XP 64bit or newer, Apple Leopard or newer and on Ubuntu Linux Hardy or newer.

The optimal system should have:

- Lots of RAM (16GB or more recommended)
- Hard disk space (128GB or more recommended)
- Intel Core2 or Xeon CPU (or compatible)
- Fast hard disks (RAID recommended)

It is useful to have Bitplane Imaris or another 3D microscopy image visualization software available, that allows for large 3D data.

XuvTools Technical Specification

Written in C++ with gcc-4.4 or Visual Studio w/ Intel Compiler.
Very generic framework, designed for 64bit from ground up.

No limitation on:

- Number of tiles
- Number of channels
- Size of the data set
- Overlapping tiles

Other Specifics:

- Different resolutions can be mixed
- Reads stage coordinates
- Manual pre-alignment

With XuvTools-1.8.0-beta12, several file reader interfaces exist. Most relevant are Bio-Image (C, Dmitri Fedorov, many tiff-based file formats), and Bio-Formats (Java, OME LOCI, almost all biological image formats).

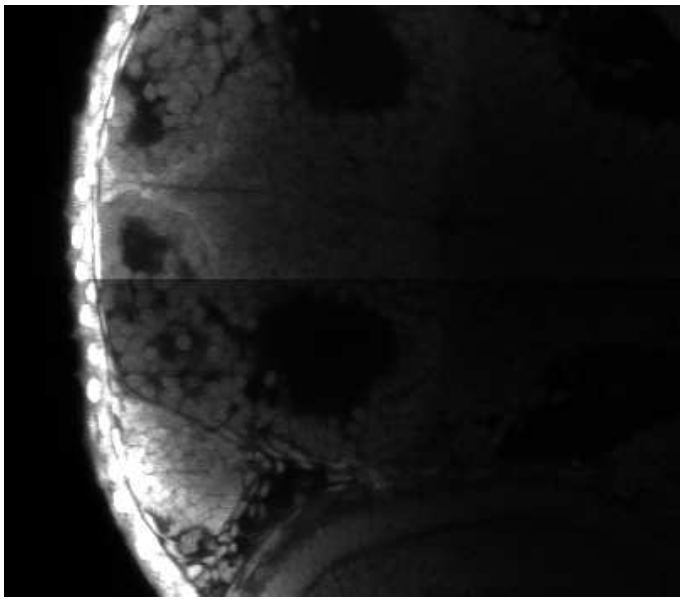
Outlook into upcoming features for XuvTools

XuvTools is under heavy development, many important features are just coming up! Note: No fixed release cycles: new features released as they are ready, and if you find a bug, it can be fixed ASAP.

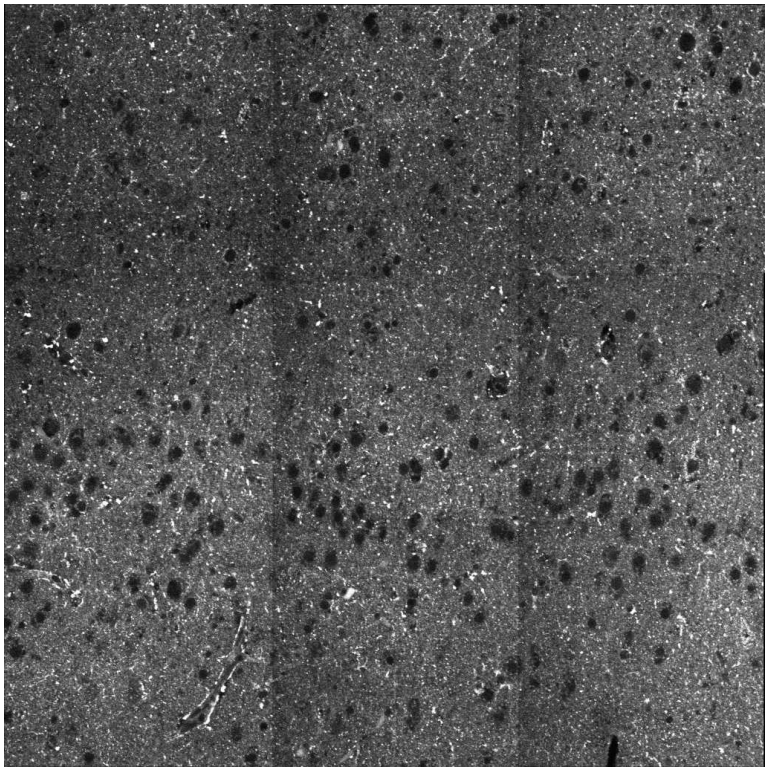
- 2D stitching, and 3D projection
- Memory Management
- Saving of large overlays
- Displacement editor
- Batch stitching support
- Bleaching correction

Bleaching correction: Without correction

Laser in can permanently damage fluorophore, thereby bleaching the sample. Affects surrounding area of image stacks.



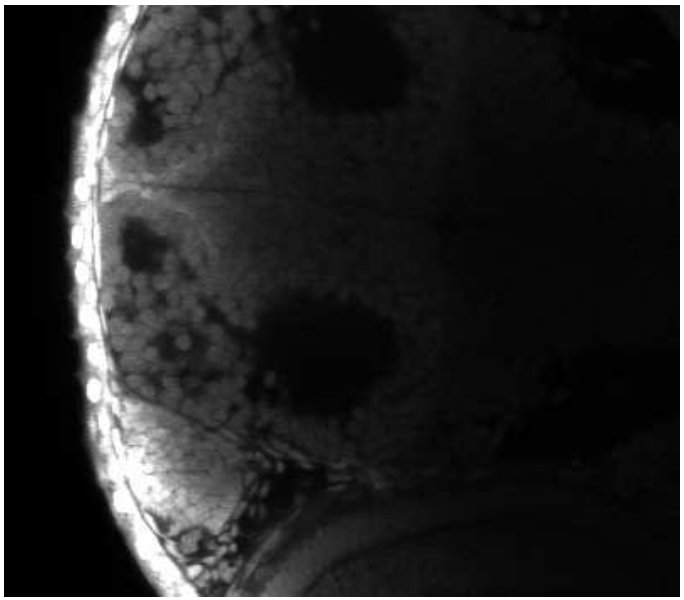
[5] Alida Filippi, University Freiburg



[1] Csaba David, University Freiburg

Bleaching correction: With correction

Laser in can permanently damage fluorophore, thereby bleaching the sample. Affects surrounding area of image stacks as well.



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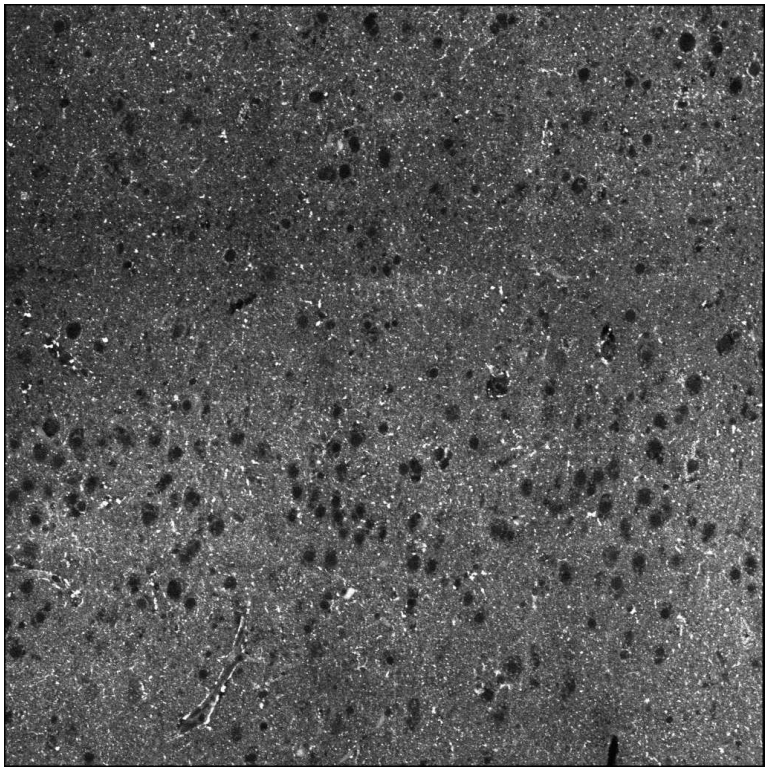

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