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

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Overview

Introduction

- Overview
- Motivation
- Existing Commercial Solutions
- Existing Freeware Solutions

Stitching with XuvTools

Examples

Technical Considerations
High resolution and large field of view

- image large structures as a whole
- maintain resolution to resolve details

[1] Csaba David, University Freiburg
High resolution and large field of view

- image large structures as a whole
- maintain resolution to resolve details
- maintain the morphology of the organism
- keep microscope usage at minimum
- lower stress on the probe, optimize usage of limited resources

[2] Peter Meister, FMI Basel
What is stitching?

Stitching is a special form of registration: intra-subject rigid registration. Generally, translation, rotation and scaling are assumed.

**Tile A:**
one or more channels imaged at one position

**Tile B:**
another position

**Overlay image:**
combination of tiles

channels are recorded interleaved
Commercial Solutions: Bundled with Hardware

<table>
<thead>
<tr>
<th>Name</th>
<th>Data type</th>
<th>Automation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeiss Mirax Micro</td>
<td>3D</td>
<td>full</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Data type</th>
<th>Automation</th>
<th>A priori info</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPFL MosaicJ for ImageJ</td>
<td>2D</td>
<td>semi</td>
<td>Manual pre-pos.</td>
</tr>
<tr>
<td>ASCR GlueMRC, LinkMRC</td>
<td>2D, 3D</td>
<td>semi</td>
<td>Reference points</td>
</tr>
<tr>
<td>Max Planck Fiji plugin</td>
<td>2D, 3D</td>
<td>full</td>
<td>Optional</td>
</tr>
<tr>
<td>XuvTools</td>
<td>3D</td>
<td>full</td>
<td>Optional</td>
</tr>
</tbody>
</table>

An in-depth comparison is available in our paper:
Freeware Solutions: ImageJ / Fiji

The Fiji ImageJ includes a free 2D/3D stitching plugin: http://pacific.mpi-cbg.de/wiki/index.php/Stitching_2D/3D, from Stephan Preibisch

- reads many file formats (LOCI)
- optimized for only one pair of images at a time
- no manual interactions or corrections possible
- does not support stage coordinates
- slower than XuvTools
Overview

Introduction

Stitching with XuvTools
- XuvTools Overview
- Stitching Modes
- XuvTools Usage
- Advanced Usage

Examples

Technical Considerations
XuvTools has been designed to work fully automatically, with little user interaction, and no microscope hardware requirements.

- confocal laser microscopy and 2-photon
- spinning disc
- electron microscopy
- optimized for thin structures: filaments
- manual mode and stage coordinates

Stitching Modes: Manual Stitching

Manual stitching is always available. The advantage is:

- no requirements towards microscope image acquisition.
- no overlap needed
- different stack sizes
- different magnifications
- different number of channels
- not good for 3D

Stitching Modes: Grid mode

Many microscopes allow for batch acquisition in a regular grid. For most common layouts, XuvTools provides a tool.

- images have to be acquired in a regular grid

Grid mode has many advantages:

- very fast stitching
- very memory-efficient stitching

[1] Csaba Dávid, University Freiburg
Stitching Modes: Stage Coordinates

Several vendors support writing stage coordinates with the image stacks:

- Metamorph STK
- planned: Zeiss LSM

Stage coordinates have all possible advantages:

- very fast stitching
- very memory-efficient stitching

[2] Peter Meister, FMI Basel
Calibration of the microscope stage is very important!

The stage coordinates and stack dimensions can in many cases be read from the image files.

If the microscope stage is wrongly calibrated, you need to correct for it in the XuvTools Layout Tool.
Stitching Modes: Fully automatic

There are two options for fully automatic stitching: Start from scratch, or refine the current layout. When using the first option, current tile positions are ignored.
Stitching Modes: Fully automatic

Fully automatic stitching is very powerful, and requires no stage coordinates or manual pre-alignment. It works with all microscopes.

- best results, easy
- requires good computer
- very memory intense
- slower than other modes
XuvTools Usage: A Sample Session

1. Add image stacks to the project
2. Generate thumbnails to validate the stitching
3. Set the performance slider according to your SNR. Higher Quality will take longer and require more memory.
XuvTools Usage: A Sample Session

1. To manually stitch a project:
   1. Add files to project
   2. Add files as new channel
   3. Set the Performance slider according to your SNR. Less memory used will make it faster.

4. Set the Similarity Threshold slider according to your SNR. Higher Similarity Threshold will take longer.

5. Start automatic stitching. You may want to grab a coffee now...

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XuvTools Usage: A Sample Session

6. This is very important:

6a. Set the Similarity Threshold to max

6b. Slowly decrease the Similarity Threshold. Always check tiles!

6c. If tiles ”clump”, go back

6d. Continue until complete stitching is achieved
XuvTools Usage: A Sample Session

7. Save the resulting stitched image
Advanced Usage

- If you have multiple channels stored as individual stacks, use 'Add channels' to select the next channel.

- If XuvTools should not work as expected, enable saving of logfiles in Advanced Parameters. Then send the logfile to development.
Live stitching session: Fully automatic mode,
Dataset: 'Flavio-brightborder/L21E13-16An1Sl2Sec1Im1.ims'

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- Quick Grid Example: stitching in seconds
- Huge Grid Example: the problem of background
- Fine Fibers Example: a difficult task
- Stage Coordinate Examples: fast and flexible
- Filament Tracer as a usage example

Technical Considerations
[4] Roland Nitschke, ZBSA Freiburg
[6] Susanne Theiss, University of Reading Whiteknights
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Grid Mode Stitching Examples
Fine Fibers Example
Stage Coordinates Examples
Filament Tracer Example

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Grid Mode Stitching Examples
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Filament Tracer Example

[3] Claudia Vittori, FMI Basel

stage coordinates

after stitching

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Bitplane Filament Tracer: neuron tracing on stitchings

MetaMorph stitching


our proposed stitching
Overview

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Technical Considerations
  ▶ Imaging Considerations
  ▶ System Requirements
  ▶ Specification
  ▶ Future Outlook
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
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

Supported input file formats are TIFF stacks, Zeiss LSM5, Bitplane Imaris IMS, Metamorph STK, BioRad PIC, Fluoview TIFF, PSIA TIFF, Nanoscope II/III, OME and RAW.
Outlook into upcoming features for XuvTools

XuvTools is under heavy development, many important features are just coming up!

- 2D stitching, and 3D projection
- File I/O based on LOCI, more output file formats (TIFF)
- Memory Management
- 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.

[5] Alida Filippi, University Freiburg

[1] Csaba David, University Freiburg
Summary and Remarks

- XuvTools is bleeding edge software, please be careful.
- Please check your results immediately after stitching.
- Please restart the stitcher after every stitching.
- Become an alpha-site, register to the newsletter.
- Last, not least, Similarity Threshold: always start high, then step to low.
Open Source - Do you want to join?

We are looking for developers and cooperation partners. You will get advanced support, a word in feature requests and access to beta versions.

Welcome are: money, programmers and power users for testers.

To keep the project alive, it is vital that you cite XuvTools and the corresponding paper. See http://www.xuvtools.org/ for a bibtex example.

**License:** You may use XuvTools free of charge. No fees, no hidden costs. But, **you have to cite the project.**
Acknowledgements

[1] Csaba Dávid and Jochen Staiger, Institute of Anatomy and Cell Biology, Albert-Ludwigs-University Freiburg, Germany

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[3] Flavio Donato, Dominique Spirig, Nadine Gogolla, Claudia Vittori and Ewa Bednarek from Group Pico Caroni, Friedrich Miescher Institute for Biomedical Research (Part of Novartis Research Foundation), Basel, Switzerland

[4] Roland Nitschke, Life Imaging Center in ZBSA, Albert-Ludwigs-University Freiburg, Germany

[5] Alida Filippi, Developmental Biology, Albert-Ludwigs-University Freiburg, Germany

[6] Susanne Theiss, University of Reading Whiteknights, UK
Acknowledgements of Publications:

Thank you for your attention!