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

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16.06.2011

Overview

Introduction

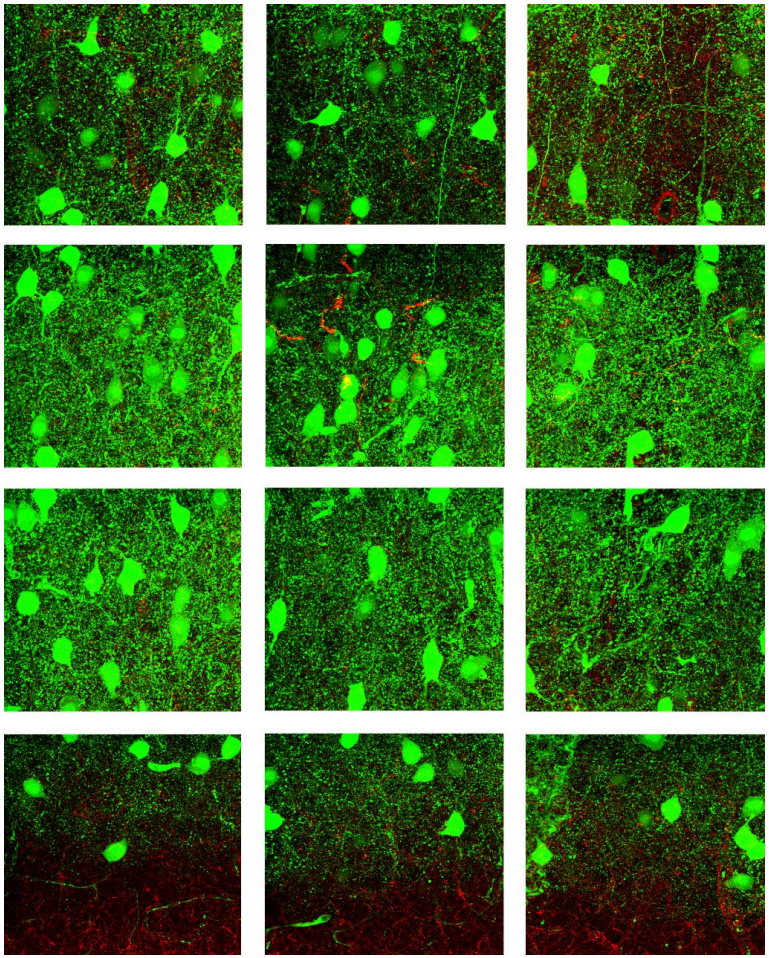
- Overview
- Motivation
- Existing Freeware Solutions

Stitching with XuvTools

Examples

Bio-Formats Integration

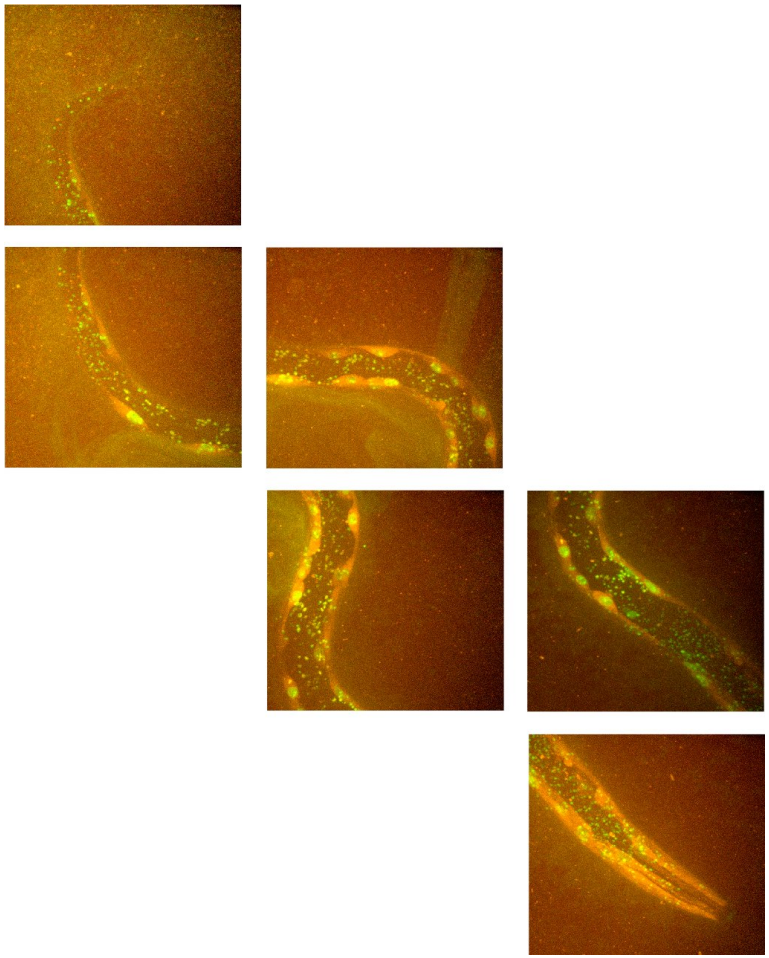
High resolution and large field of view



[1] Csaba David, University Freiburg

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

High resolution and large field of view

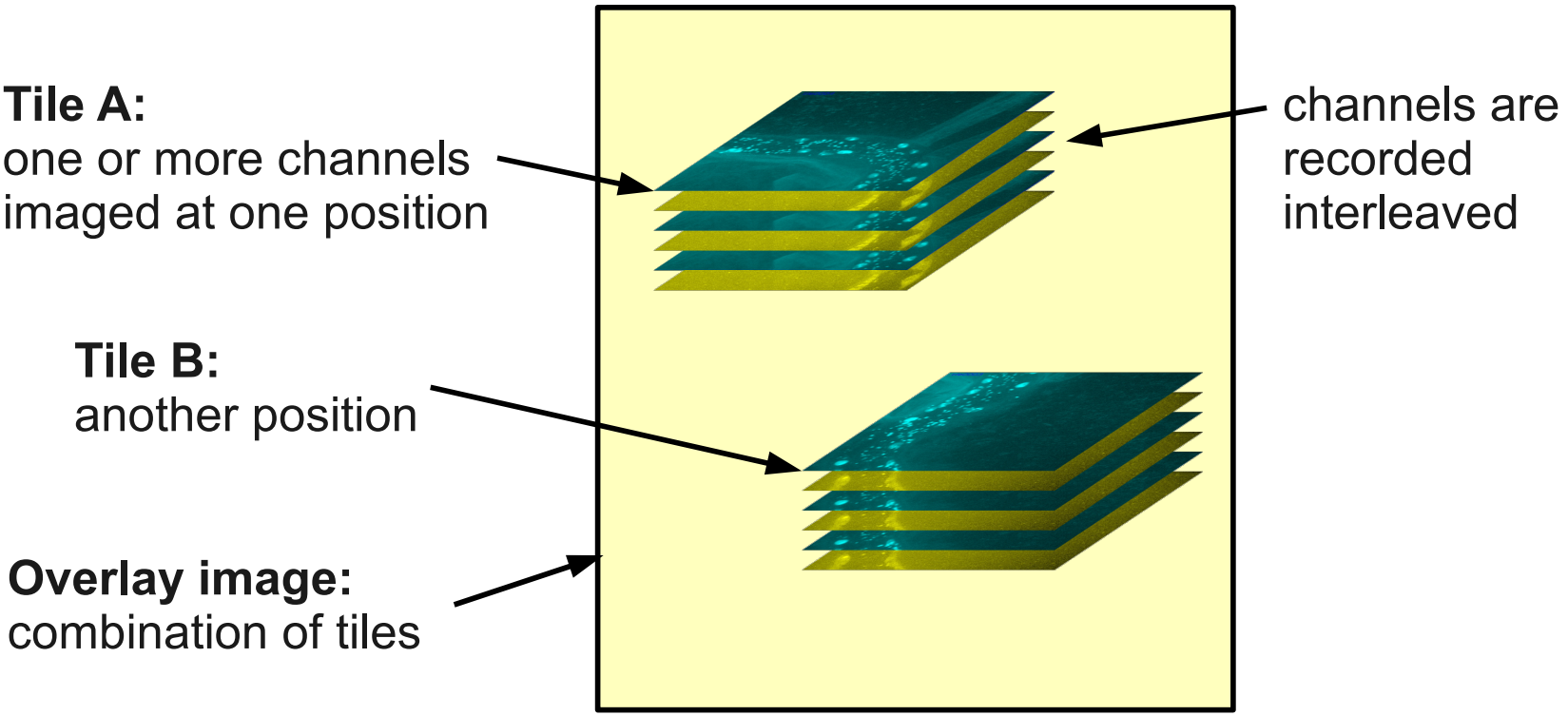


[2] Peter Meister, FMI Basel

- 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

What is stitching?

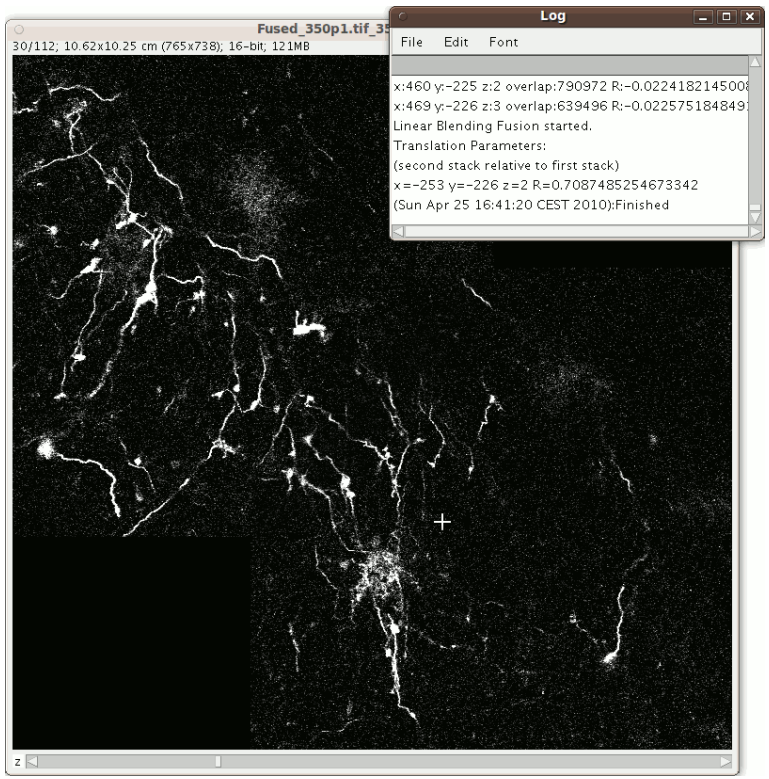
Stitching is a special form of registration: intra-subject rigid registration. Generally, allowed transformations are translation, rotation and scaling.



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)
- builds on the well-known ImageJ/Fiji
- similar algorithmic principle as XuvStitch
- no manual interactions or corrections possible(?)
- does not support stage coordinates(?)



Overview

Introduction

Stitching with XuvTools

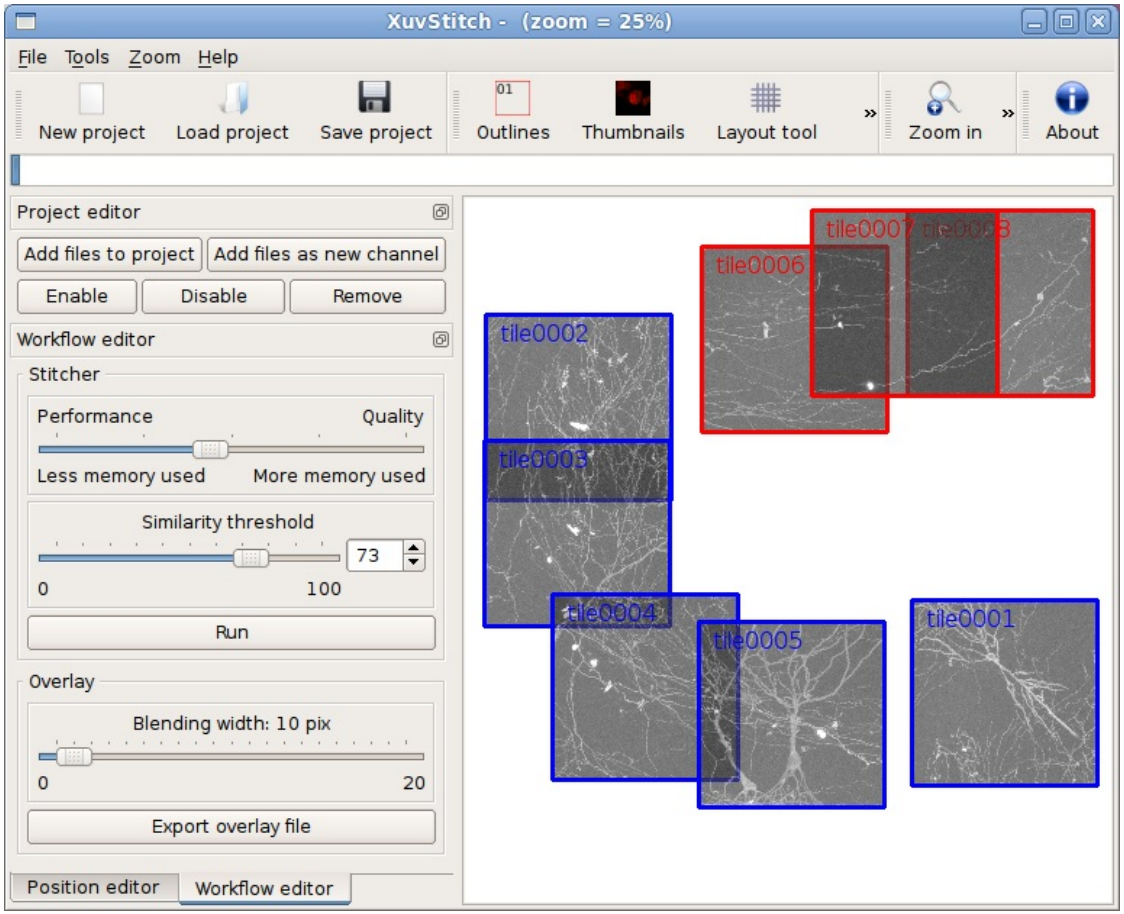
- XuvTools Overview
- Stitching Modes
- XuvTools Usage
- Advanced Usage

Examples

Bio-Formats Integration

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

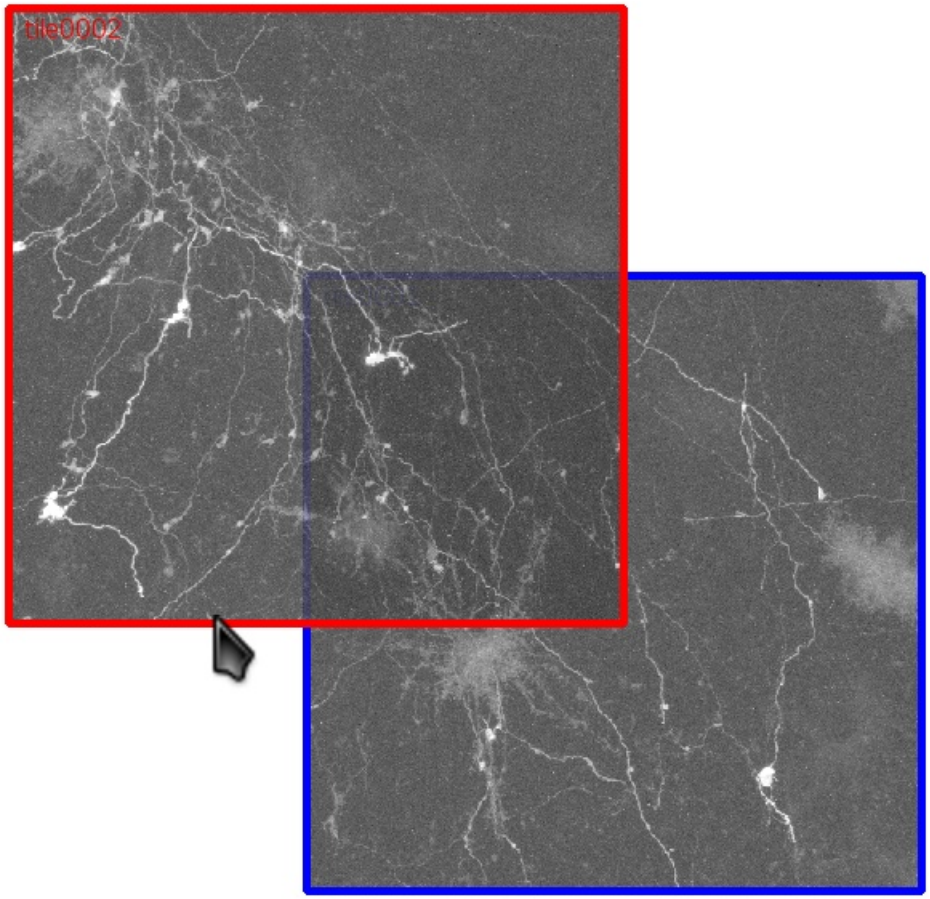


[3] Ewa Bednarek, FMI Basel

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



[3] Ewa Bednarek, FMI Basel

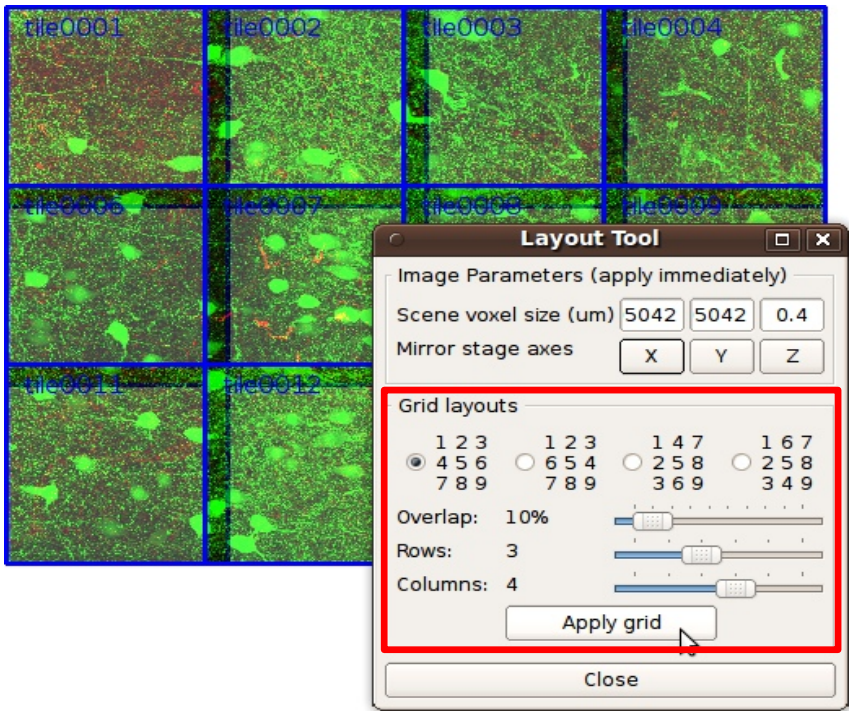
Stitching Modes: Grid mode

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

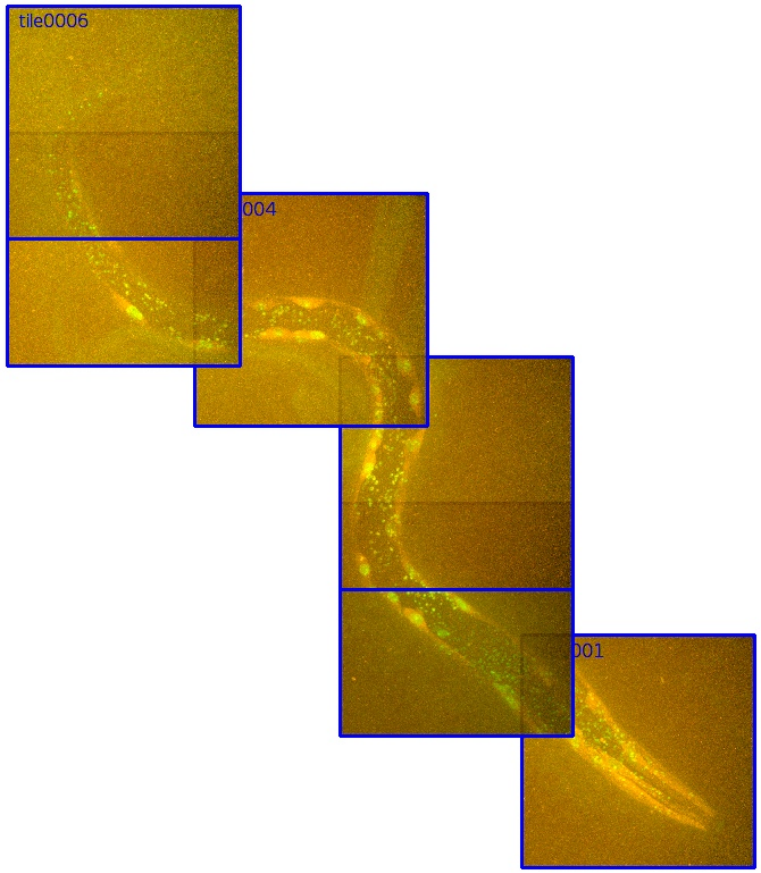
Stitching Modes: Stage Coordinates

Several vendors support writing stage coordinates with the image stacks

- many formats, thanks to OME LOCI metadata reader

Stage coordinates have all possible advantages:

- very fast stitching
- very memory-efficient stitching

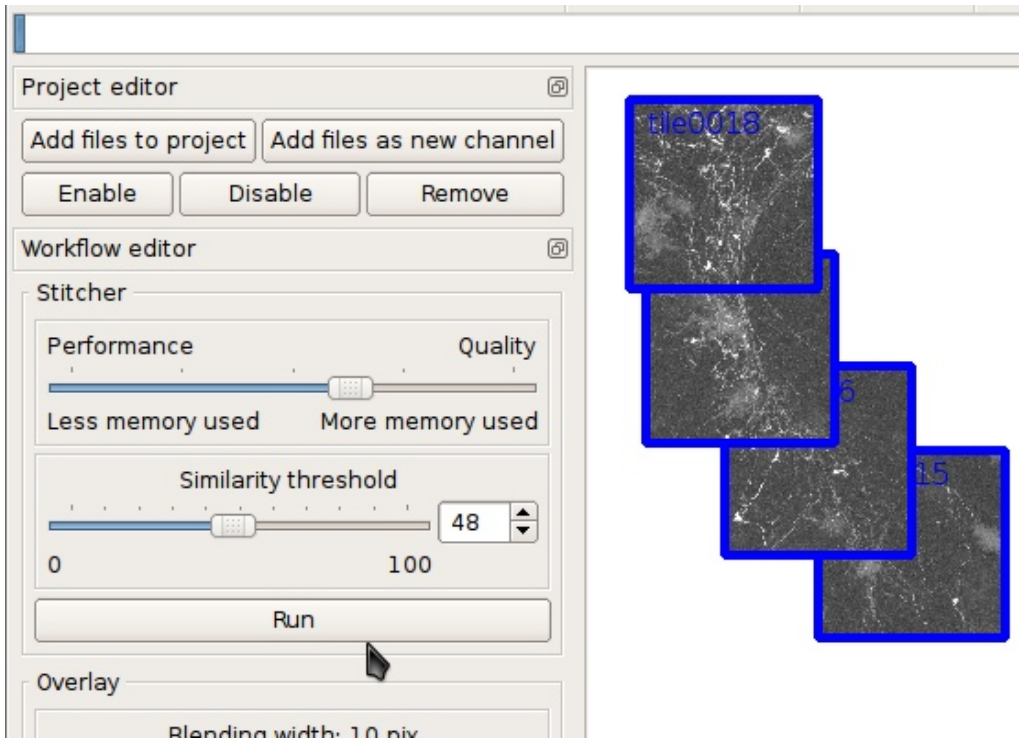


[2] Peter Meister, FMI Basel

Stitching Modes: Fully automatic

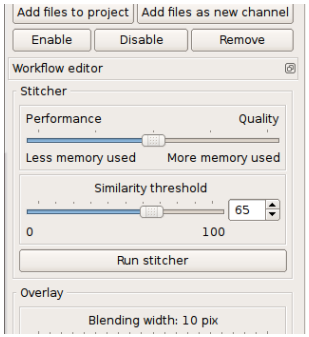
Fully automatic stitching is very powerful, and requires no stage coordinates or manual pre-alignment. It works with all microscopes.

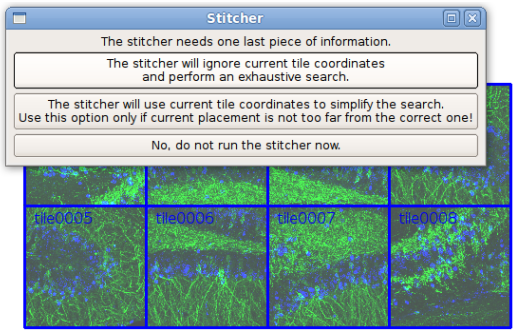
- best results, easy
- requires good computer
- very memory intense
- slower than other modes

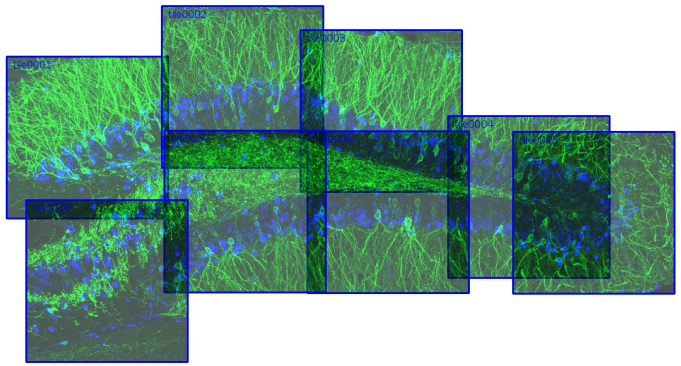


Live Stitching Session

Live stitching session: Fully automatic mode,
Dataset: 'Flavio-brightborder/L21E13-16An1Sl2Sec1Im1.ims'

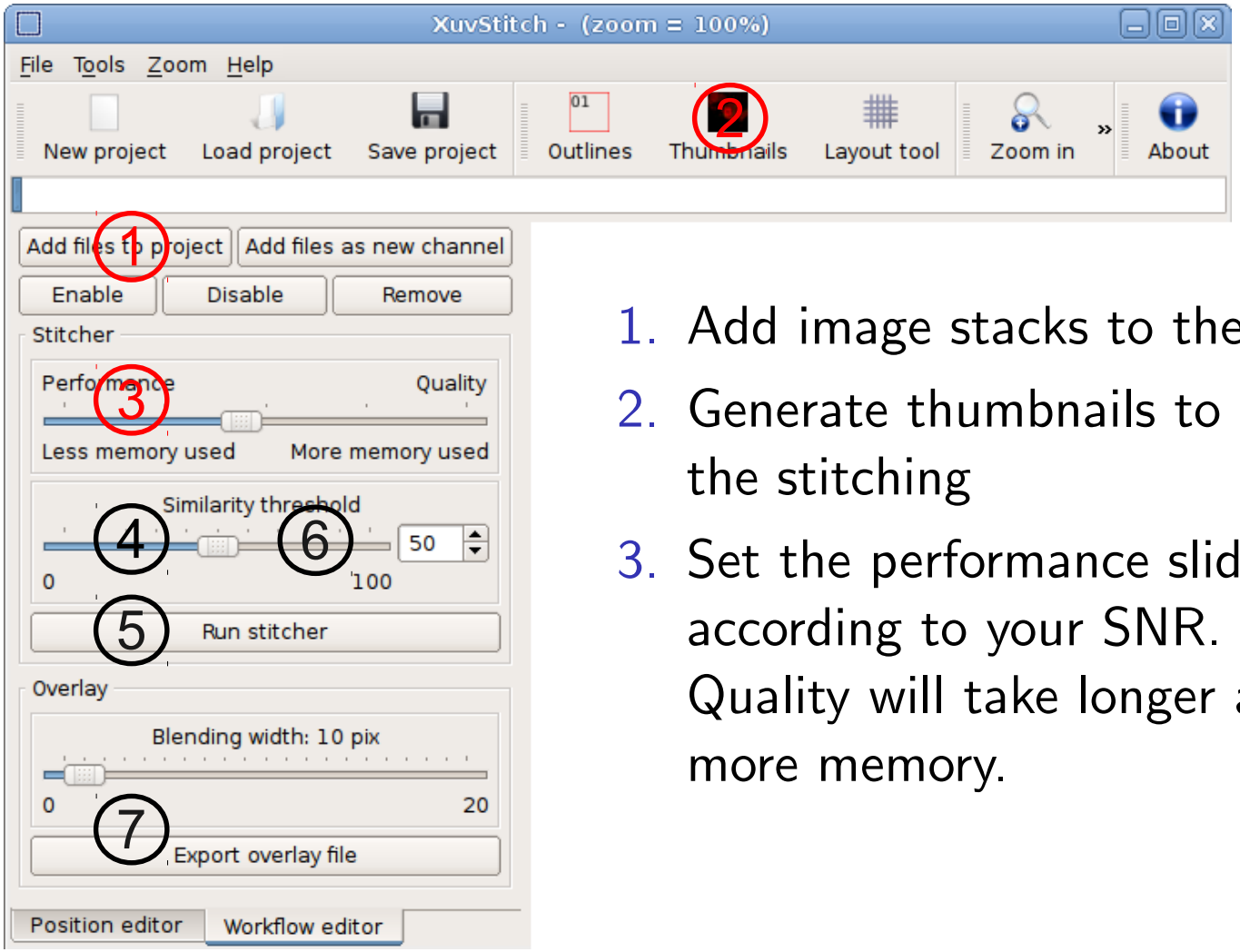






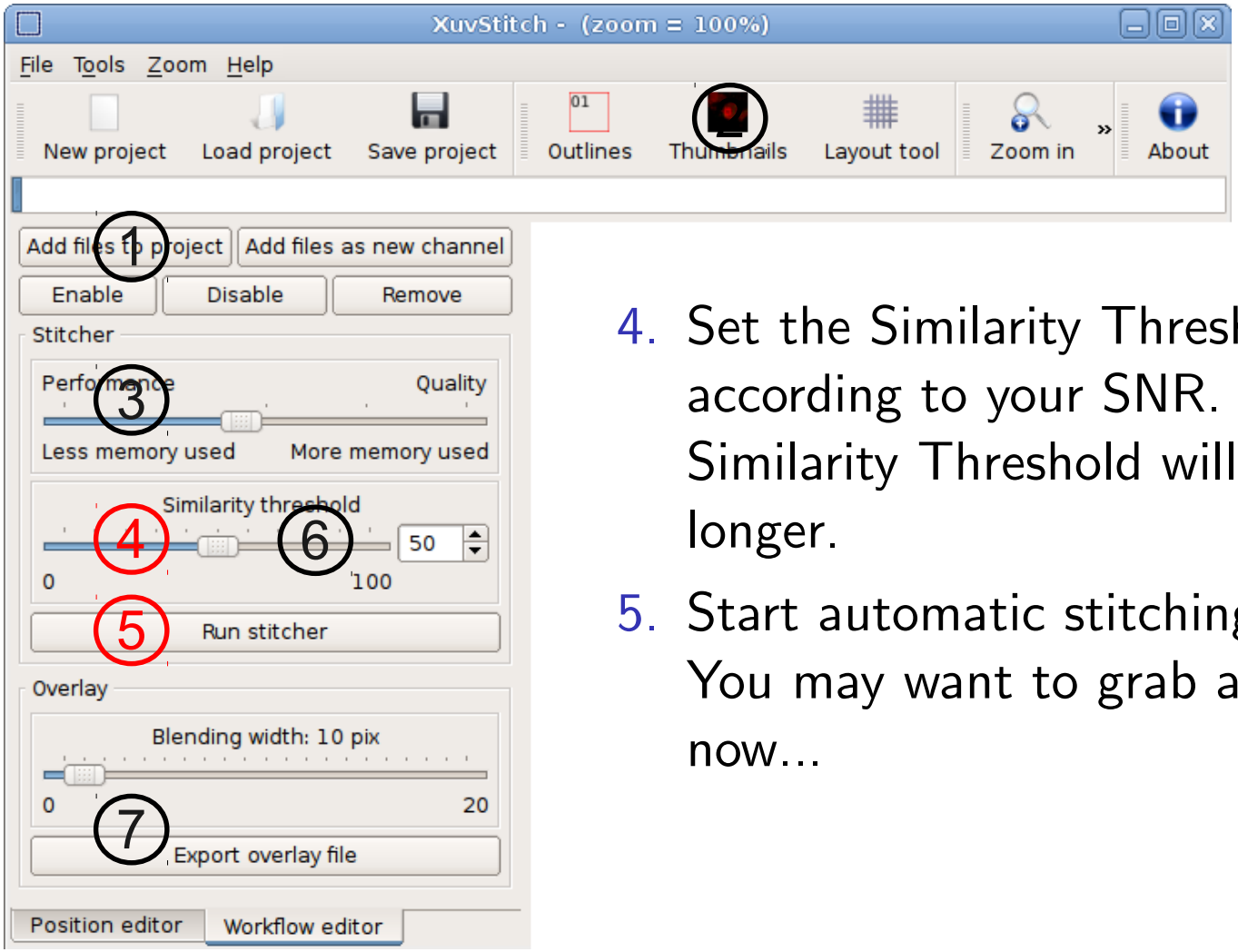
[3] Flavio Donato, FMI Basel

XuvTools Usage: A Sample Session



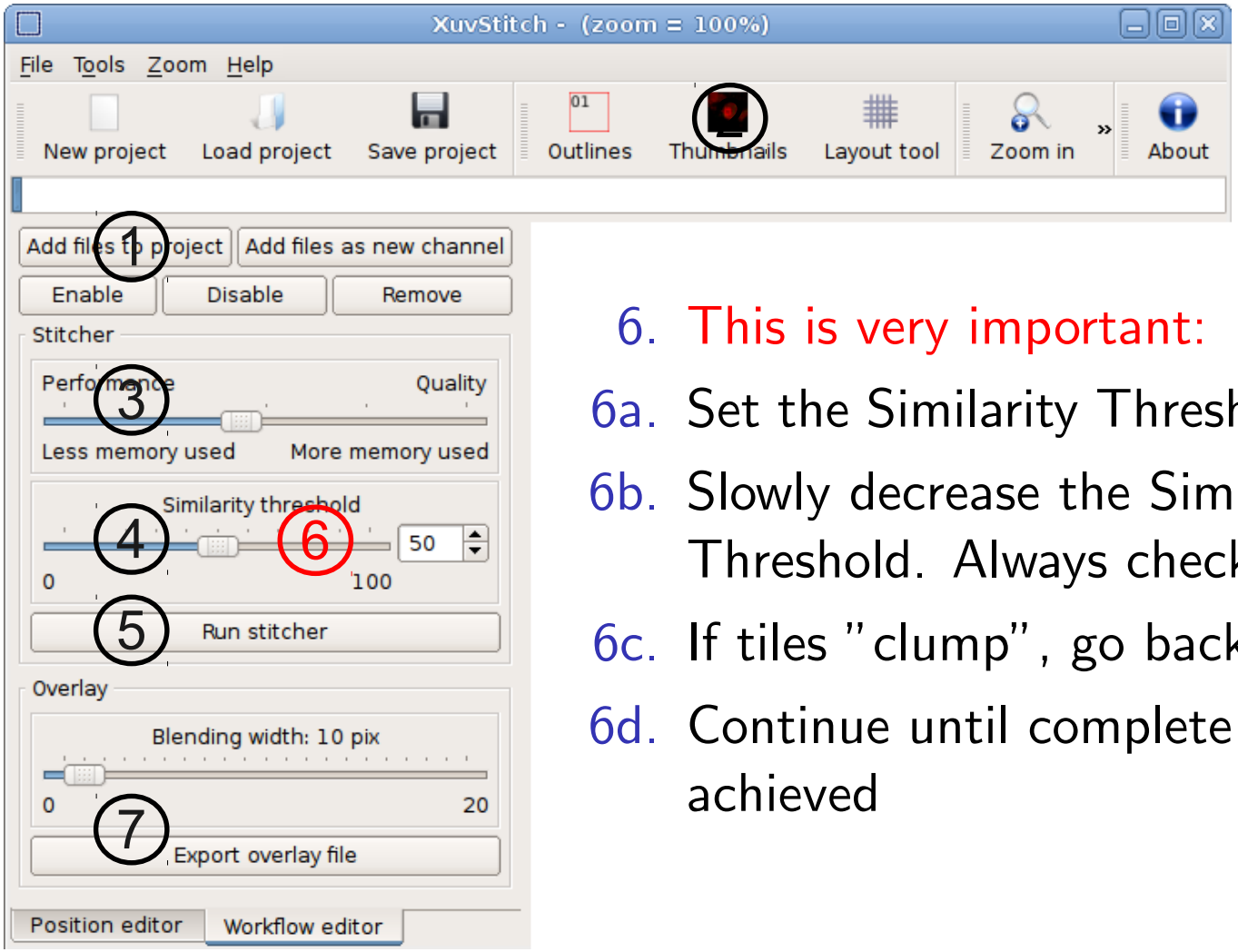
1. Add image stacks to the project
2. Generate thumbnails to validate the stitching
3. Set the performance slider according to your SNR. Higher Quality will take longer and require more memory.

XuvTools Usage: A Sample Session



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...

XuvTools Usage: A Sample Session



6. This is very important:

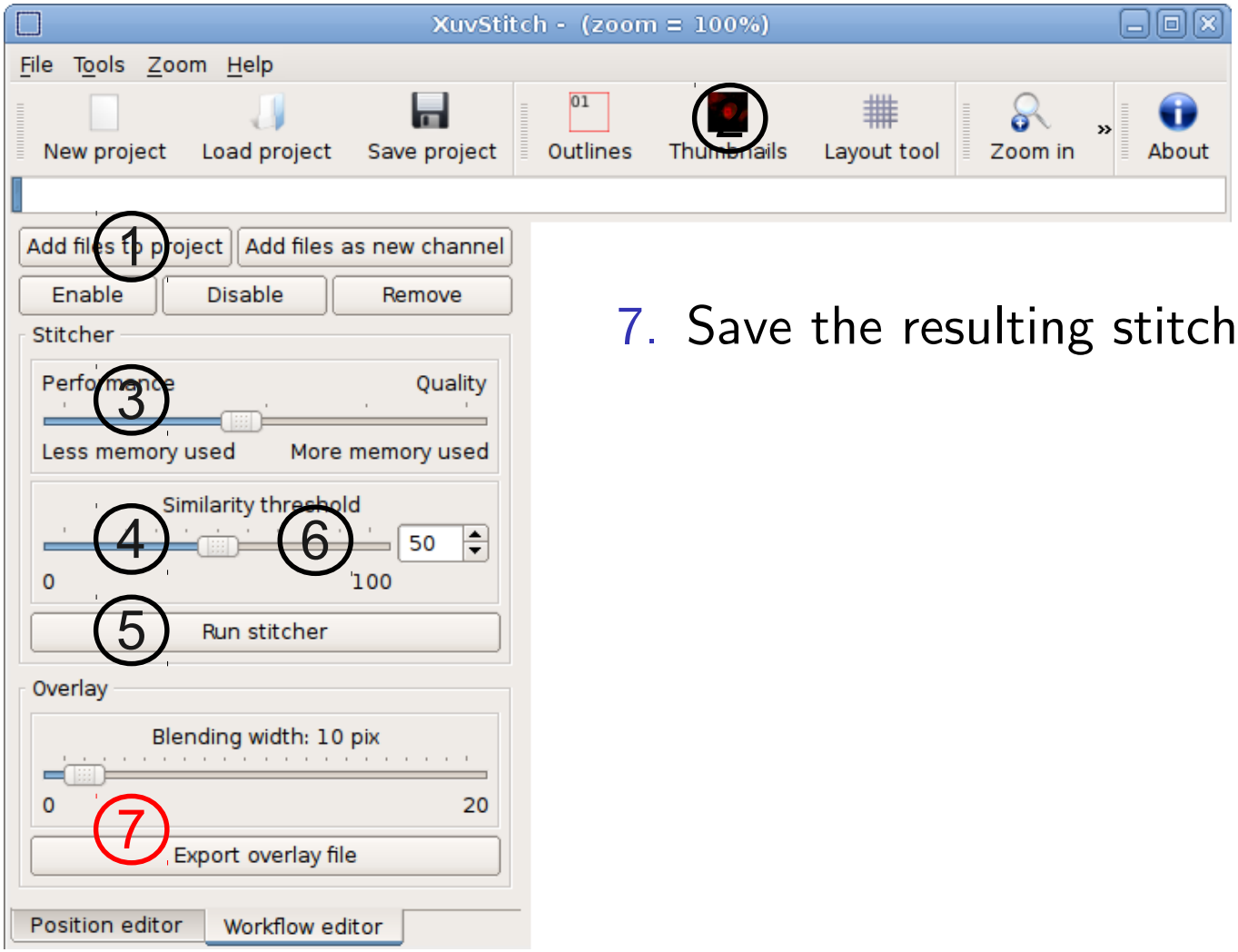
6a. Set the Similarity Threshold to max

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

Overview

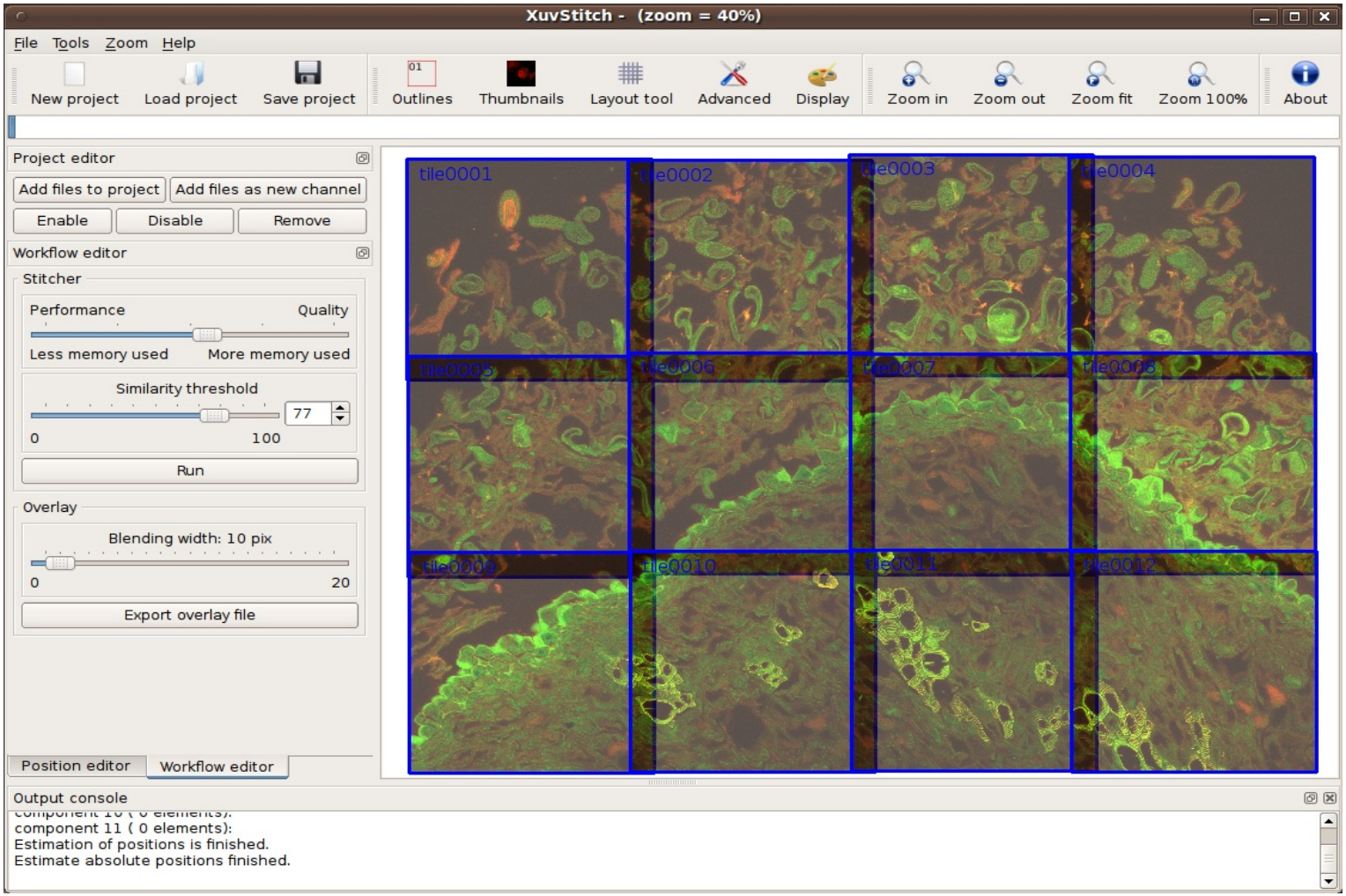
Introduction

Stitching with XuvTools

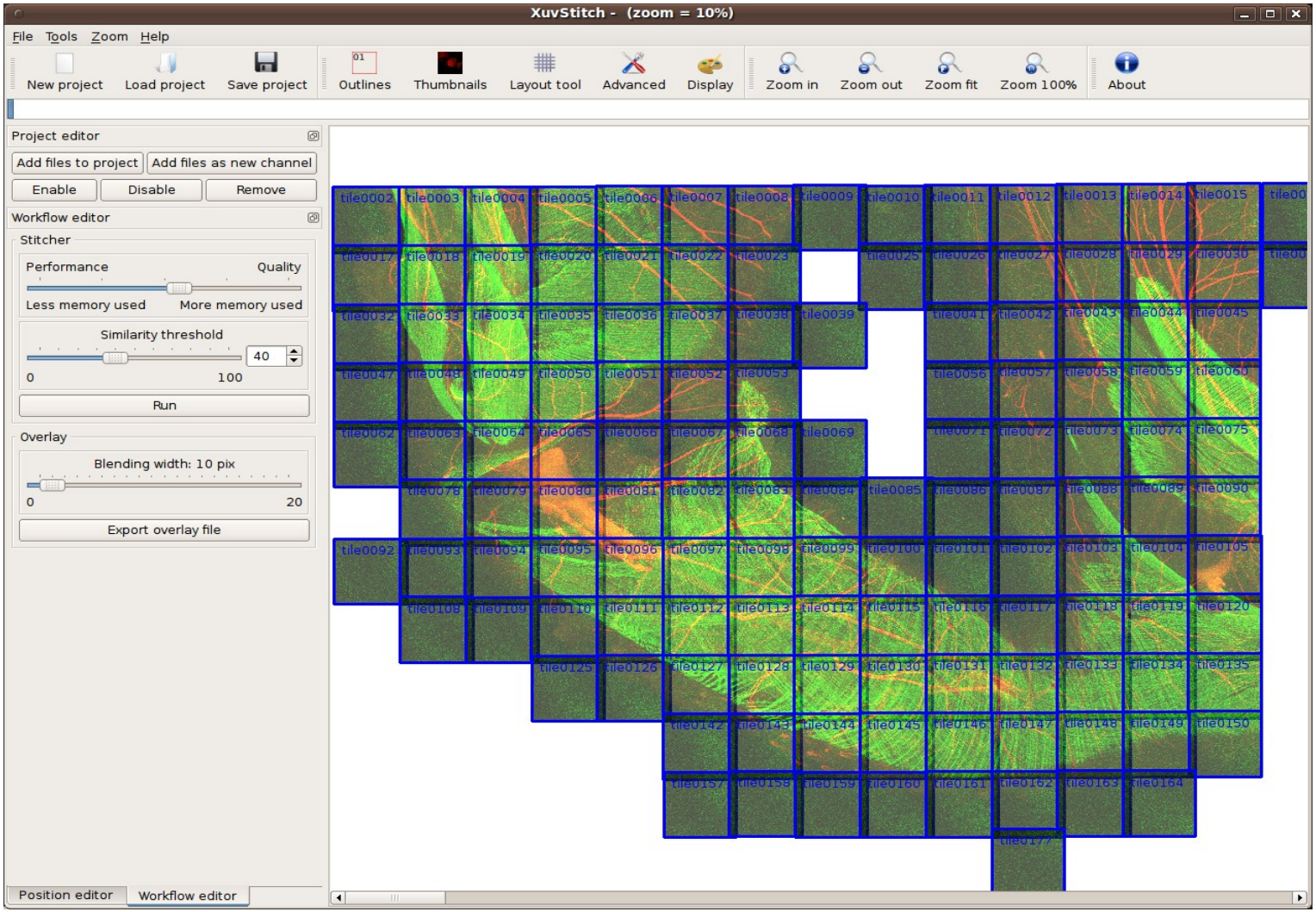
Examples

- 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

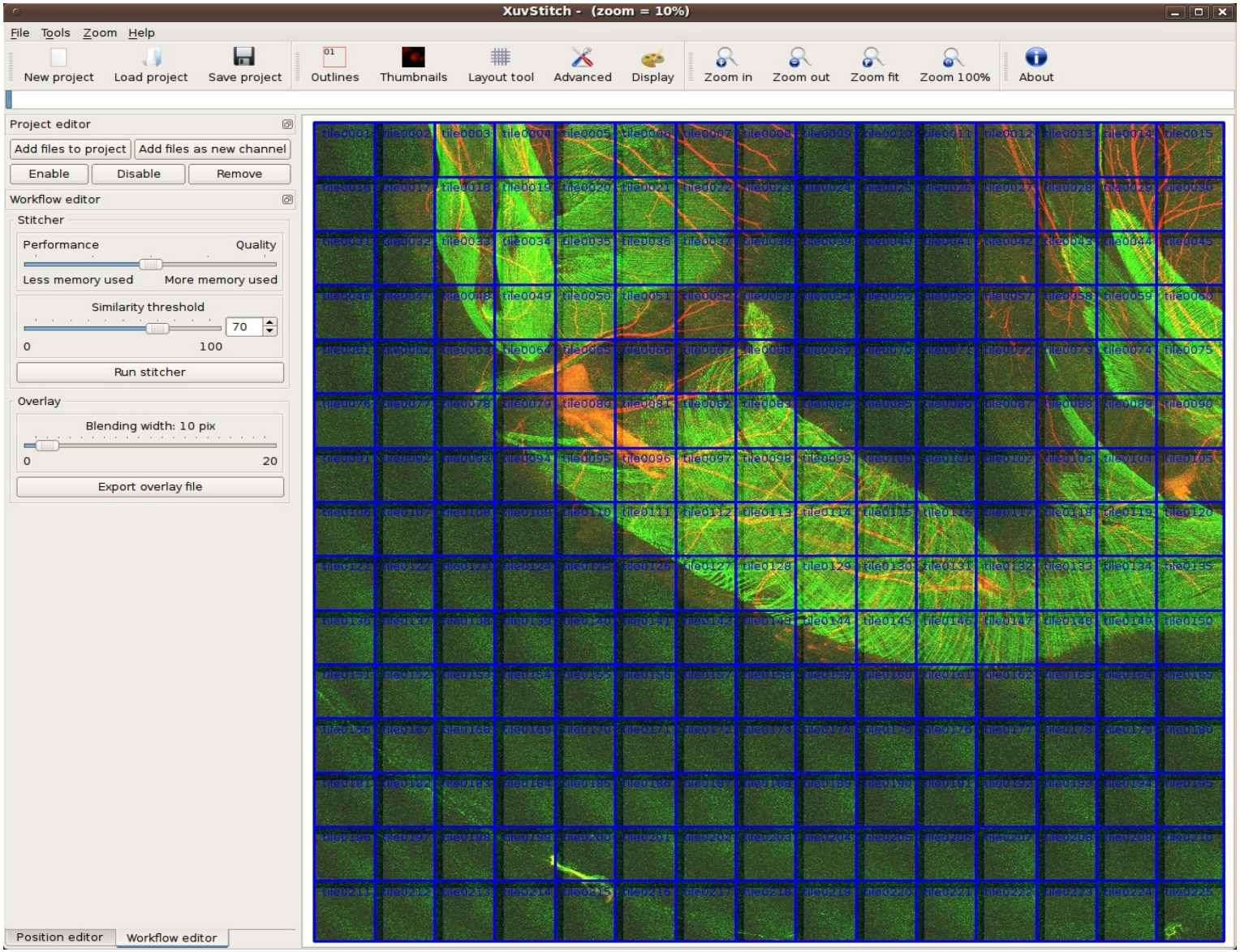
Bio-Formats Integration



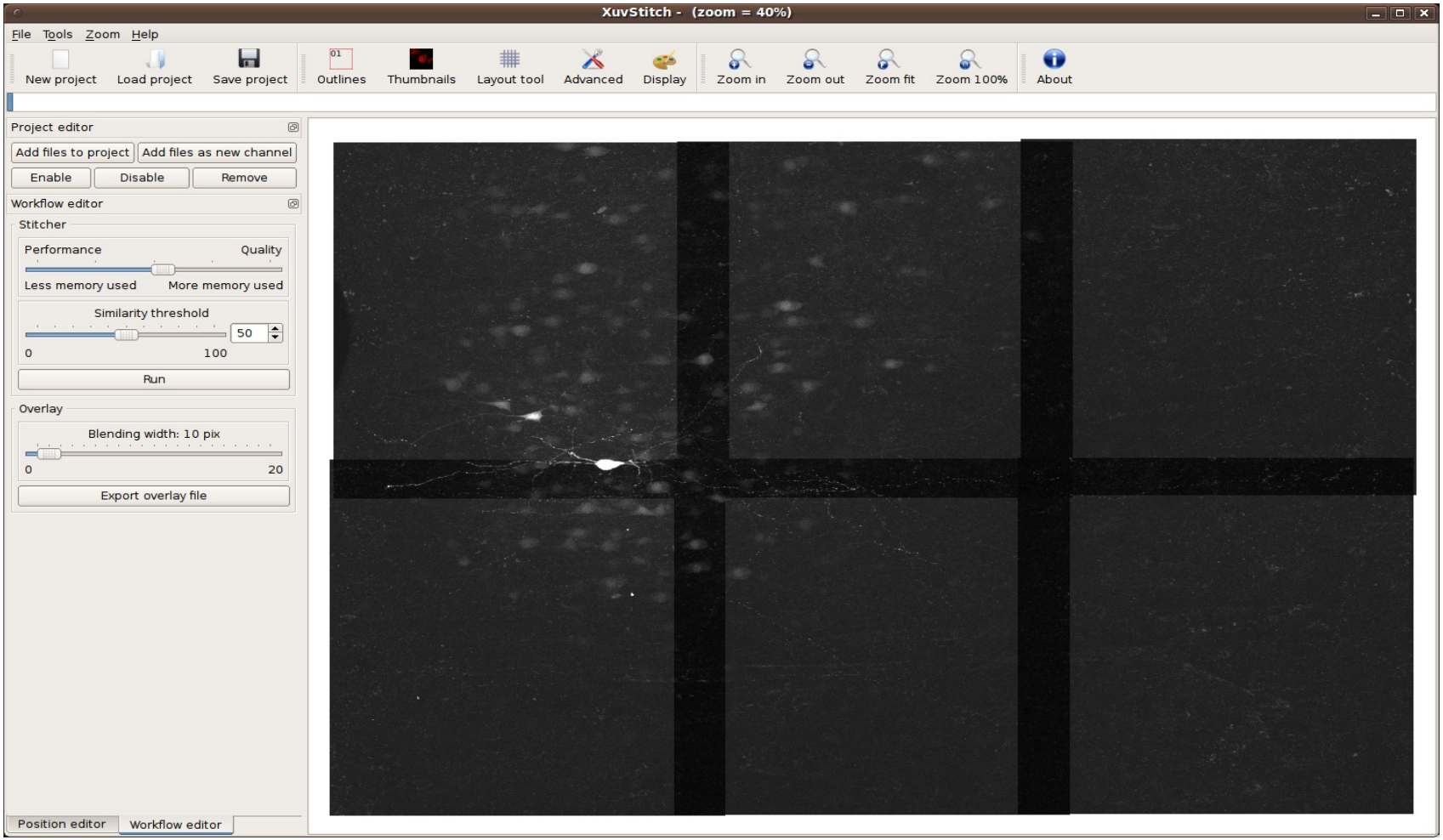
[4] Roland Nitschke, ZBSA Freiburg



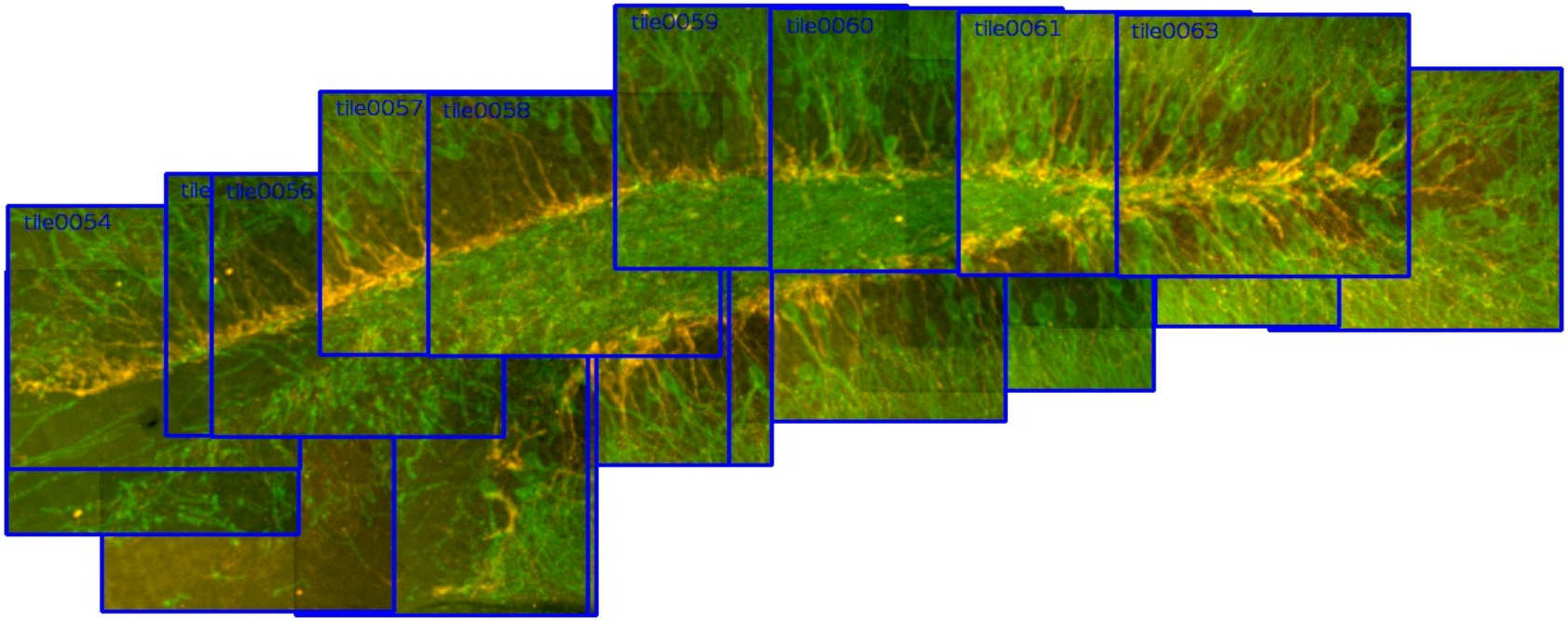
[6] Susanne Theiss, University of Reading Whiteknights



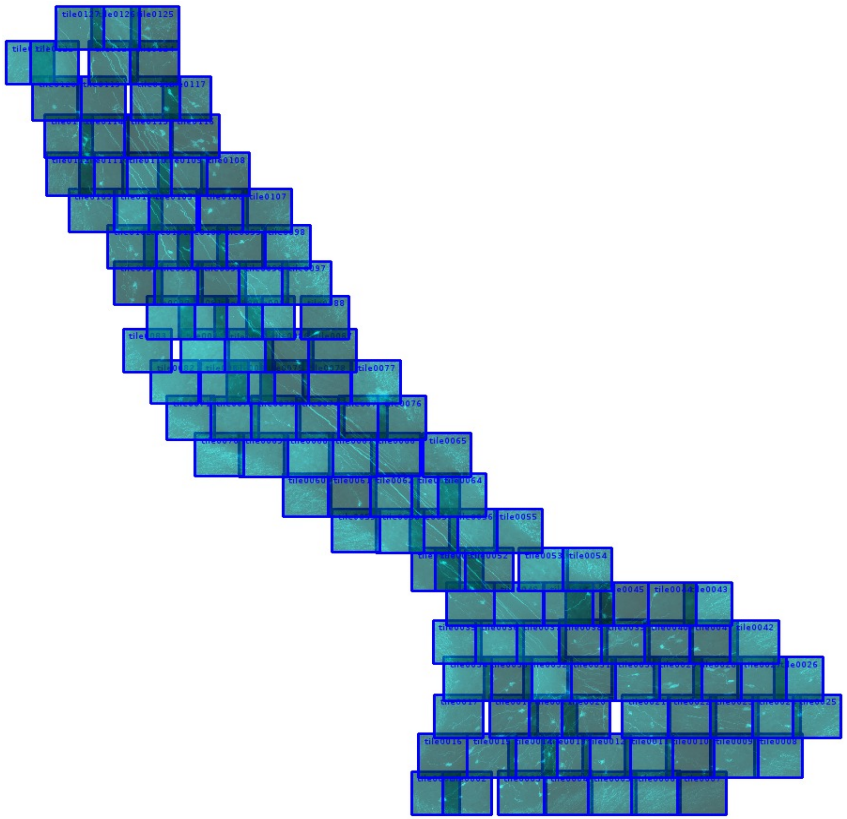
[6] Susanne Theiss, University of Reading Whiteknights



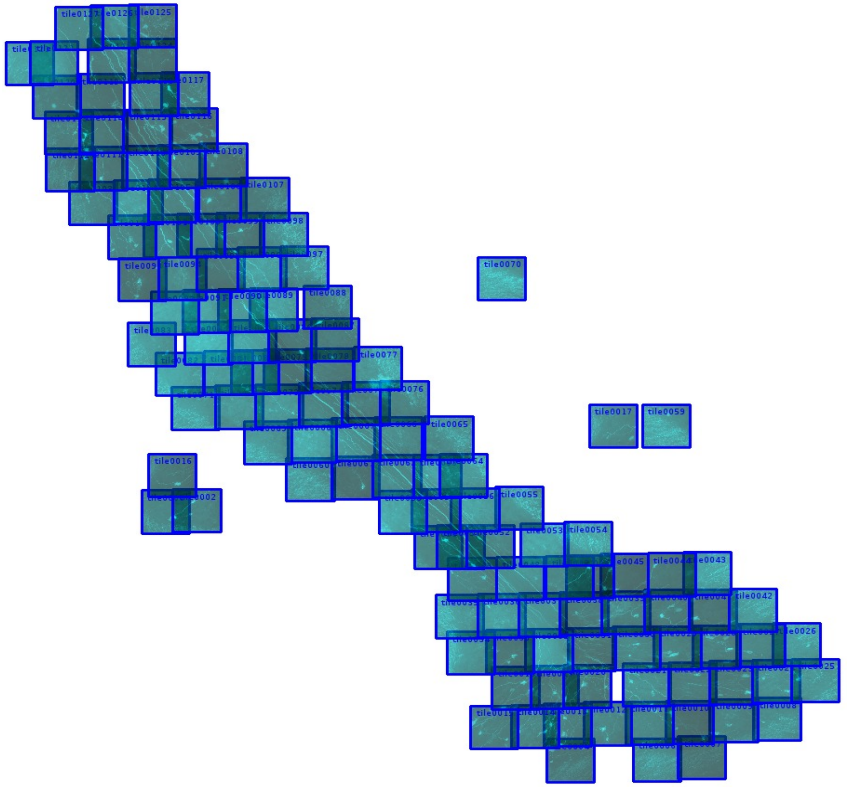
[1] Csaba Dávid, University Freiburg



[3] Dominique Spirig, FMI Basel



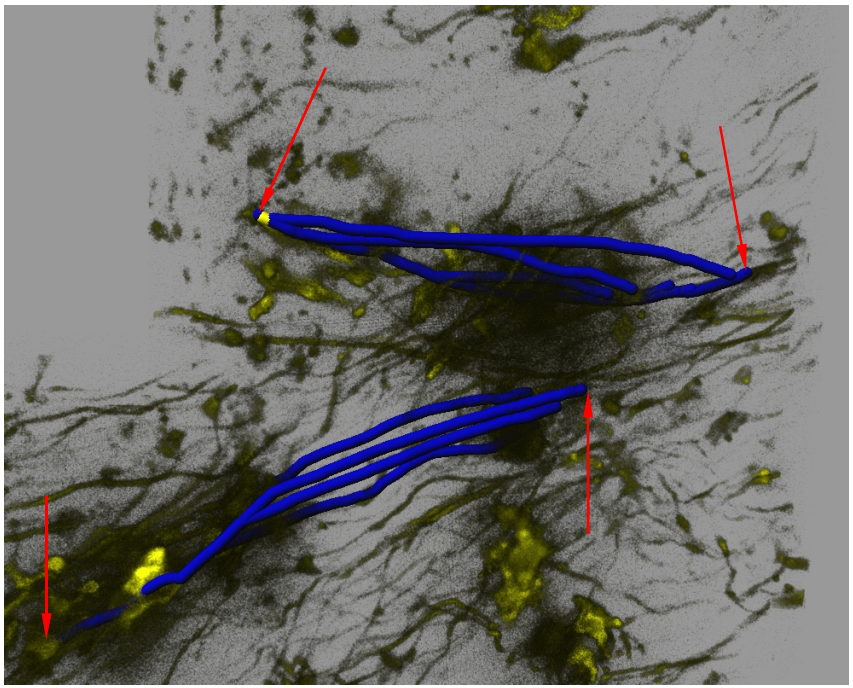
stage coordinates



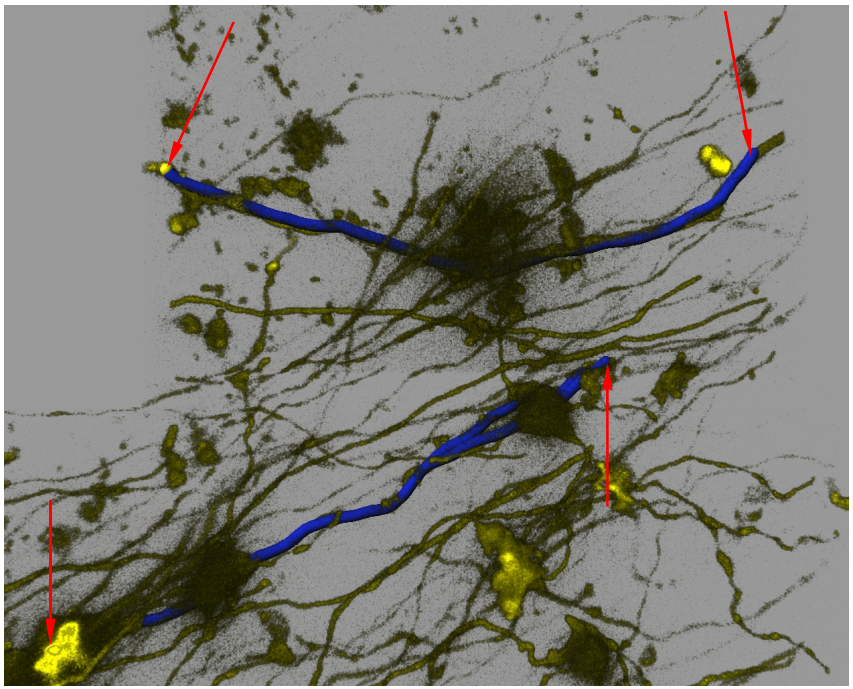
after stitching

[3] Claudia Vittori, FMI Basel

Bitplane Filament Tracer: neuron tracing on stitchings



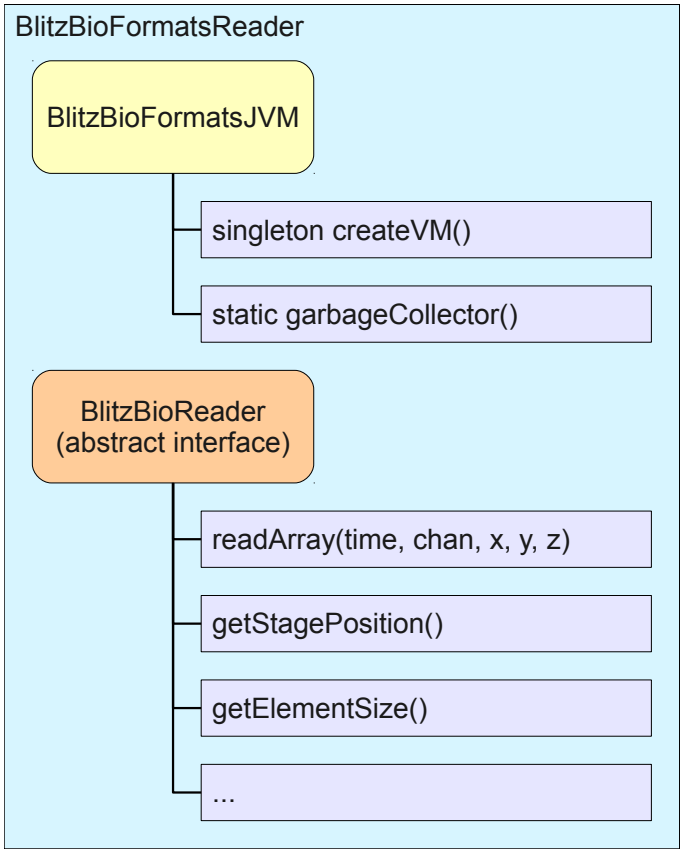
MetaMorph stitching



our proposed stitching

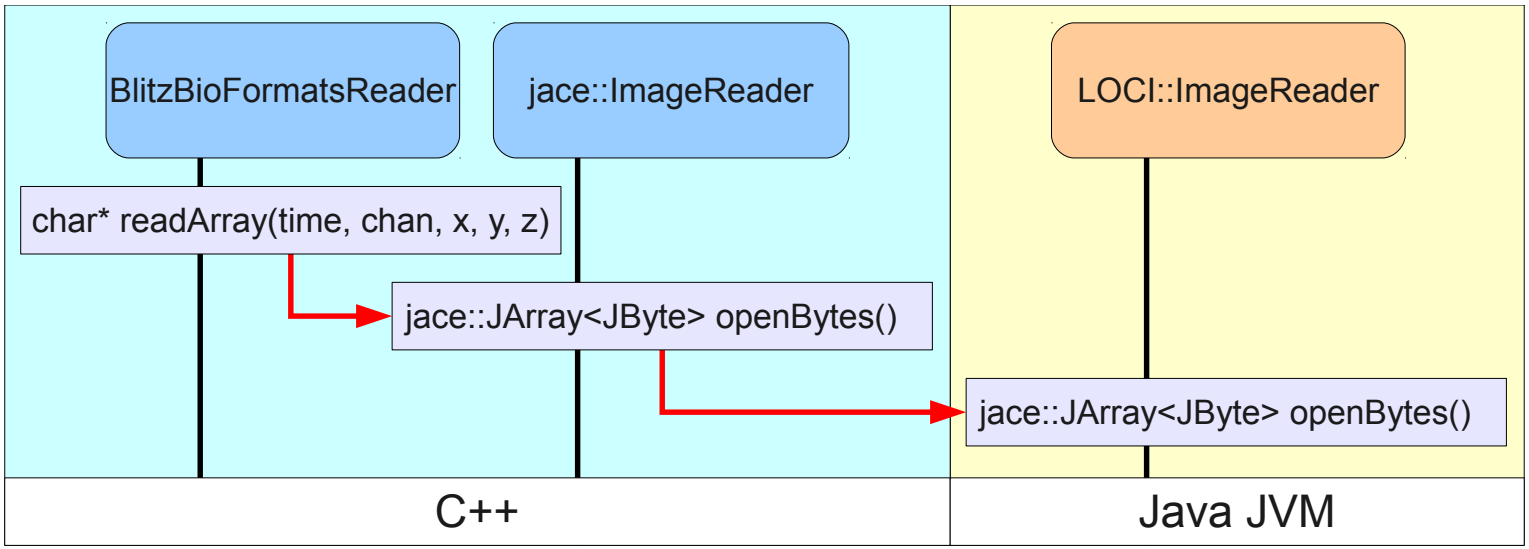
[3] Nadine Gogolla, FMI Basel

Integrating Java with C++ (Bio-Formats and XuvTools)



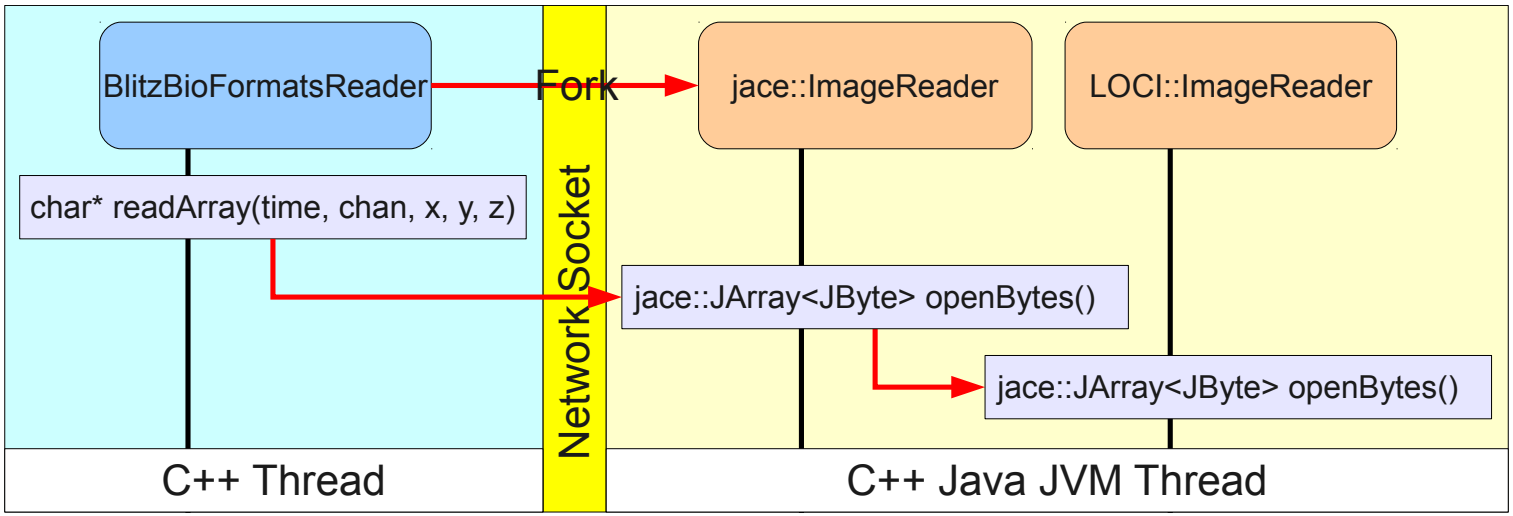

```
BlitzBioFormatsReader::
BlitzBioFormatsReader(std::string classPath,
                      size_t heapSize)
 : BlitzBioFormatsJVM()
{
  try {
    createVm(classPath, heapSize);

    // Assign the ImageReader
    OMEImgReader = new ImageReader();

    // ChannelFiller replaces indexed color data
    // with true color images on the fly
    OMEImgReader = new ChannelFiller(OMEImgReader);

    OMEImgReader->setMetadataFiltered(true);
    OMEImgReader->setMetadataCollected(true);
  } catch (jace::JNIException& e) {
  } catch (jace::proxy::java::lang::Exception& e) {
  } catch (std::exception& e) {
  }
}
```

Callstack of C++ to Java:



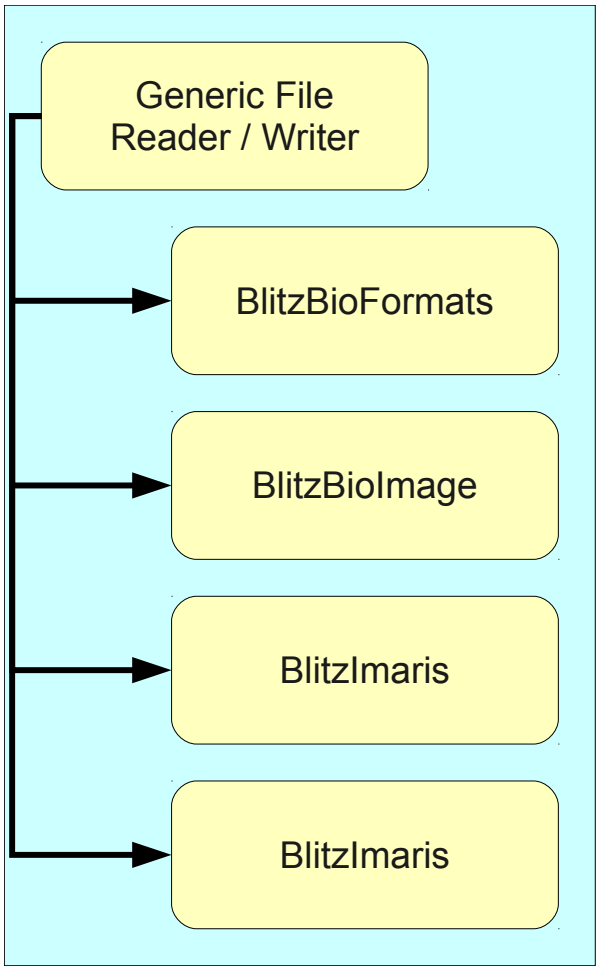
- C++ can call into Java, via jace wrapper classes
- However: calls into JVM are "relatively" slow
- Therefore: solve problems either in Java or C++, but avoid frequent switches (example memcopy).

Improved callstack of C++ to Java:



- Better place JVM singleton in a separate thread
- Recreating JVM allows changing of maximum java memory
- Create/Destroy JVM to free java memory

XuvTools file readers/writers



- BlitzBioFormats: OME Bio-Formats Java bindings
- BlitzBioImage: Dmitry Fedorov, University of California (http://dimin.net/software/)
- BlitzImaris: C++ HDF5 Bitplane Imaris Reader / Writer
- BlitzLSM: C libtiff Zeiss LSM Reader

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 (as 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: XuvTools is free and open source (GPL). No fees, no hidden costs. But, **please cite the project**.

Acknowledgements

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

[2] Peter Meister from Group Susan Gasser, and
[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:

FMI Basel, Aaron Ponti, Patrick Schwarb

University of Freiburg, Olaf Ronneberger

ZBSA Freiburg, Niko Ehrenfeuchter, Roland Nitschke

[2] Meister, P. et al., The spatial dynamics of tissue-specific promoters during C. elegans development. *Genes Dev 24 (8), 766 (2010).*

End

Thank you for your attention!