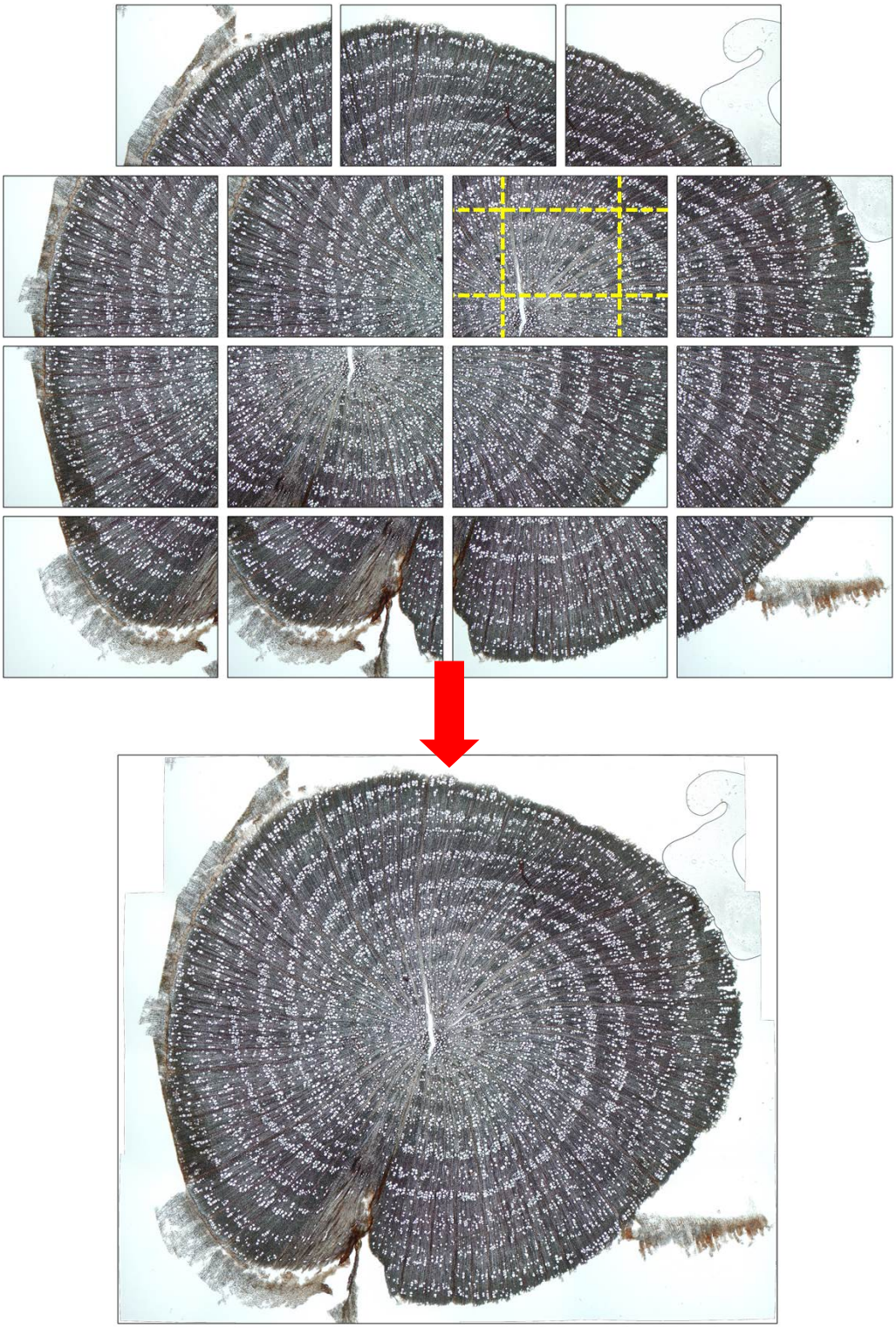
Stitching distortion-free mosaic images for QWA using PTGui

Georg von Arx



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A. Introduction and overview

PTGui (www.ptgui.com) is an affordable commercial stitching tool for creating panorama images of large scenes taken with a camera on a tripod. In this application it has to deal with camera rotation and distortions caused by lens systems.

However, with the appropriate settings and handling as described in this quick guide it is also a great tool for **creating mosaic images of microscopic anatomical samples**. Usually, the entire process is done **fully automatic**, and batch processing of created project files is available to enhance efficiency. Problematic samples can be **manually edited** if needed.

A great advantage of PTGui is the **correction of distortions** caused by some microscope and camera lenses (plan-type objectives are distortion-free!). Accordingly, one micrometer in the center of the mosaic image will be represented by the same number of pixels at the edges of the mosaic image. This is particularly valuable to avoid biases in quantitative image analysis.

I find PTGui very efficient and reliable compared to other stitching software I have evaluated, also for large mosaic images (composed of >100 individual images) A very similar tool in terms of efficiency, reliability and quality of output is Autopano (www.kolor.com). Please also note that using a **slide scanner** is nowadays the best choice, if available, because it directly and extremely fast produces an image of the entire anatomical sample in a very high quality.

B. Taking microscopic images

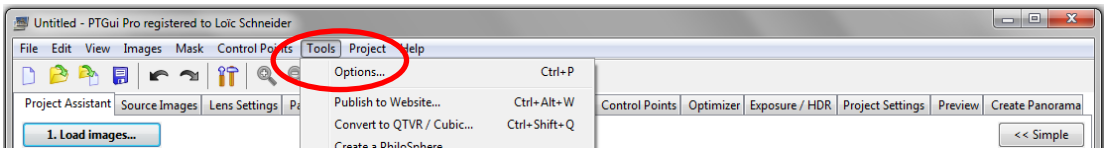
- Make sure individual images overlap by at least 15-25% in angiosperm and 30% in conifer samples
- Avoid rotation of the sample while shifting it on the light or object table. Use a X/Y-stage!
- Keep all microscope and camera settings constant for all your project.

C. INSTALLING PTGUI

1. Download full [licensed] version from: www.ptgui.com/download.html.
2. Run PTGui installer
3. Launch PTGui; you will be asked to provide the license information to activate the full version; exit PTGui when finished.
4. *Recommended:* download Smartblend-plugin [1] from: www.ptgui.com/plugins.html.
5. *Recommended:* unzip the Smartblend-plugin folder and move it inside the PTGui program folder.
6. You may want to create a desktop shortcut for PTGui and for the batch stitcher (RunStitcher.exe within PTGui program folder).

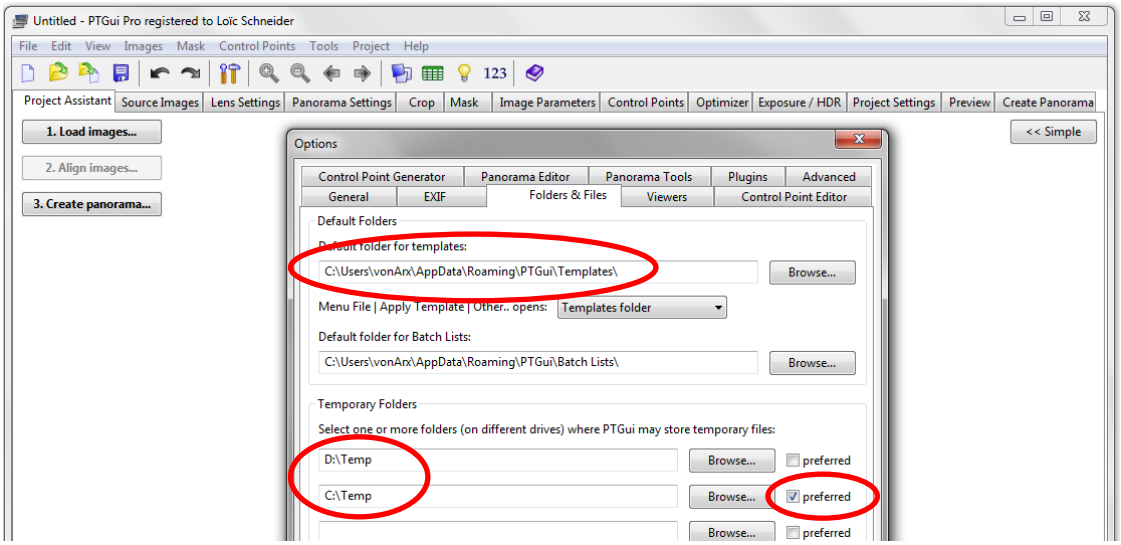
D. INITIAL SETUP

→ **Navigate to Tools > Options**



Folders & Files **tab:**

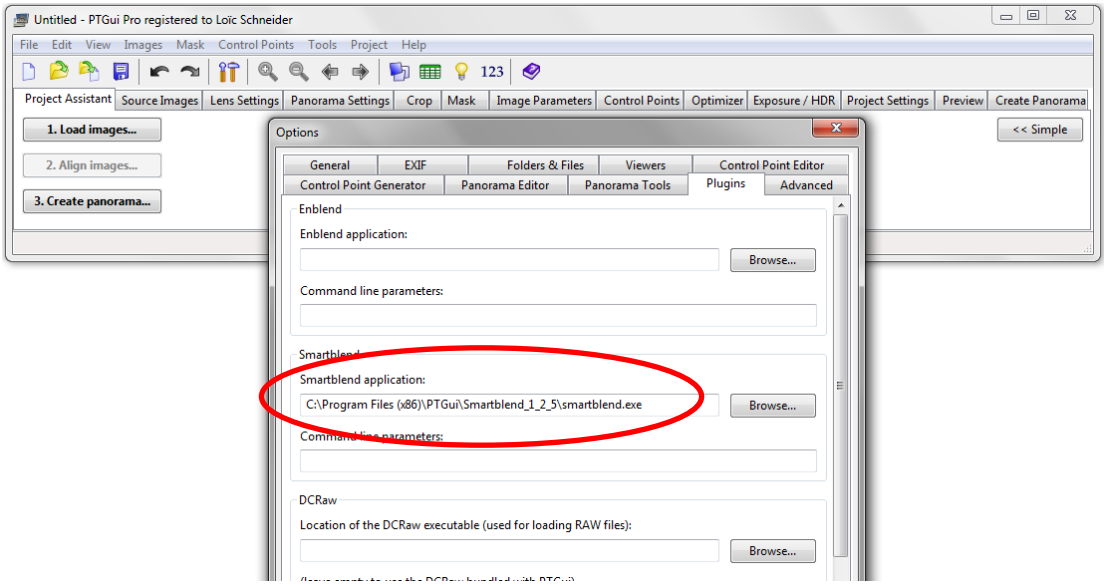
- Standard folder for templates: define a folder of choice
- Temporary folders: create one to several temporary folders that reside in a partition with plenty of free disk space and PTGui can use for memory-intense processes. Check this (these) folder(s) as preferred.



[1] Smartblend *evens out slight illumination heterogeneities between the stitched images; it also deals with slight parallax-issues when moving the sample around (parallax: shift of an object against the background that is caused by a change in the observer's position).*

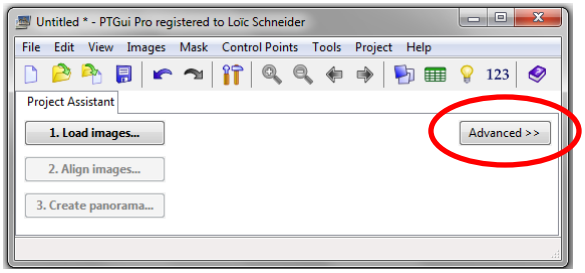
Plugins tab:

- *Recommended:* Smartblend: browse to the folder containing smartblend.exe and select it.

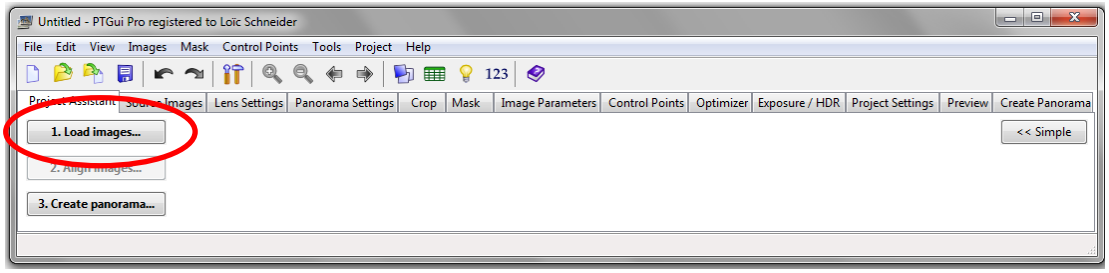


E. PREPARING AND EXECUTING STITCHING PROJECTS

1. Launch PTGui
2. *On first run only:* push the Advanced-button to show full program menu



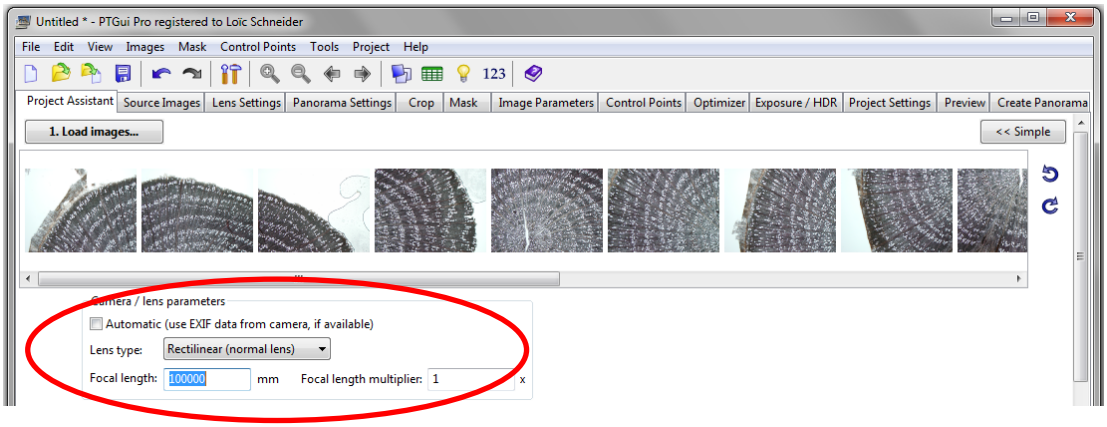
3. Load all images of the first stitching project (1. Load images... in Project Assistant tab)



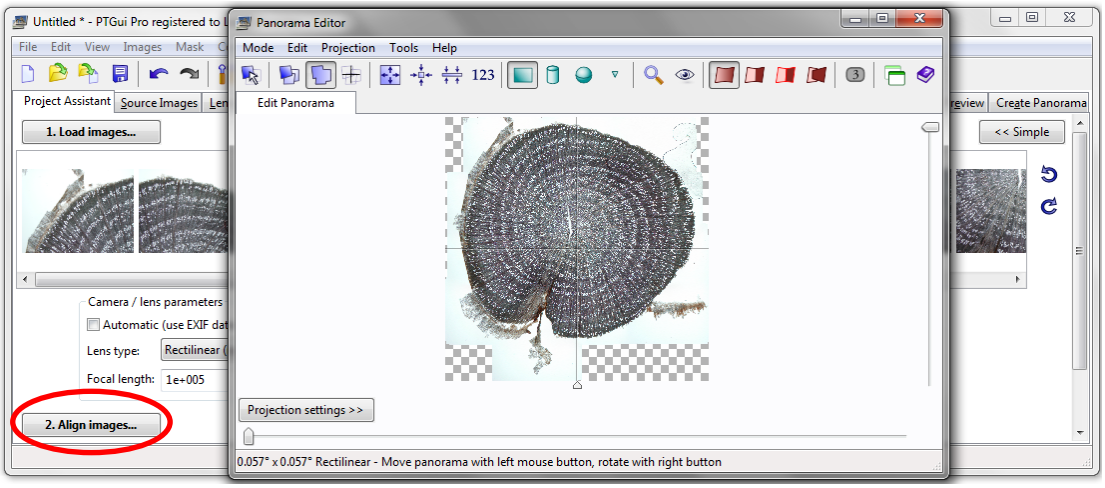
4. *On first run only:* Camera / lens data (EXIF)-window pops up; push Cancel [2]
   → *Option* Automatic (use EXIF data from camera, if available) *should be* ***unchecked****!*

[2] *The optics used in the microscope setup differs from the EXIF data (**EX**changeable **I**mage **F**ormat – extension containing the camera settings that were used to take the picture); it is misleading* PTGui*.*

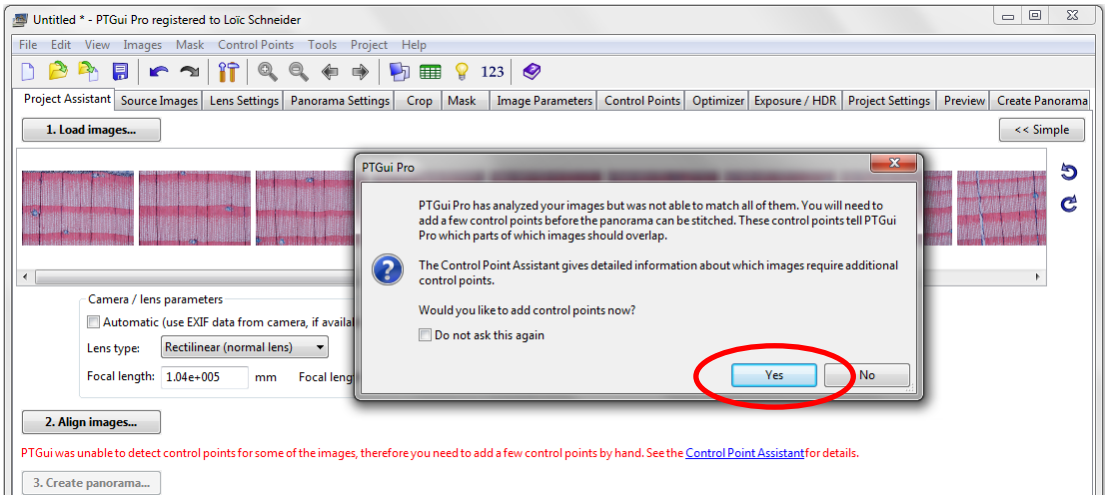
5. *On first run only:* Set Focal length to a large number such as “10,000” to “200,000” and Focal length multiplier to “1” [3]



6. Create a preview of the stitched image (2. Align images...)

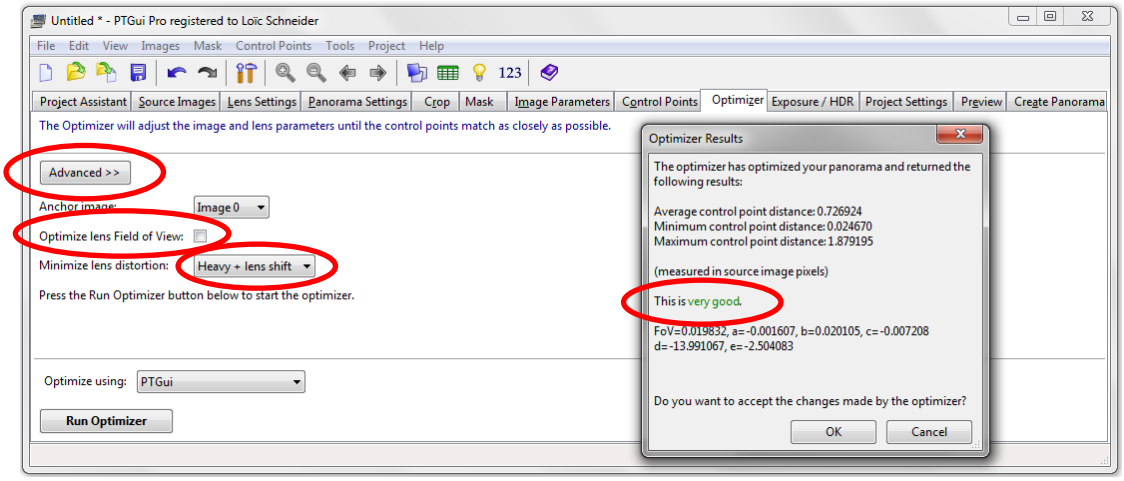


→ *See* G. CORRECTING REGISTRATION ERRORS *for trouble-shooting unsuccessful registration*

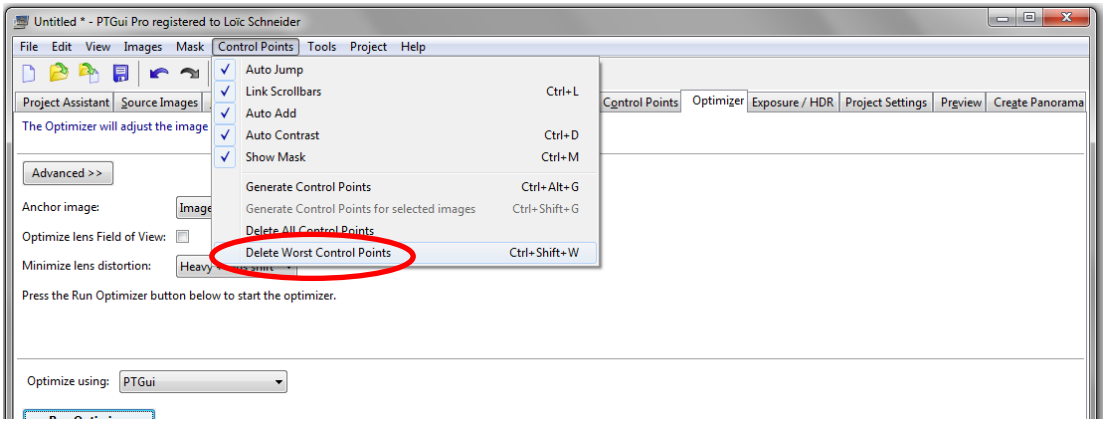


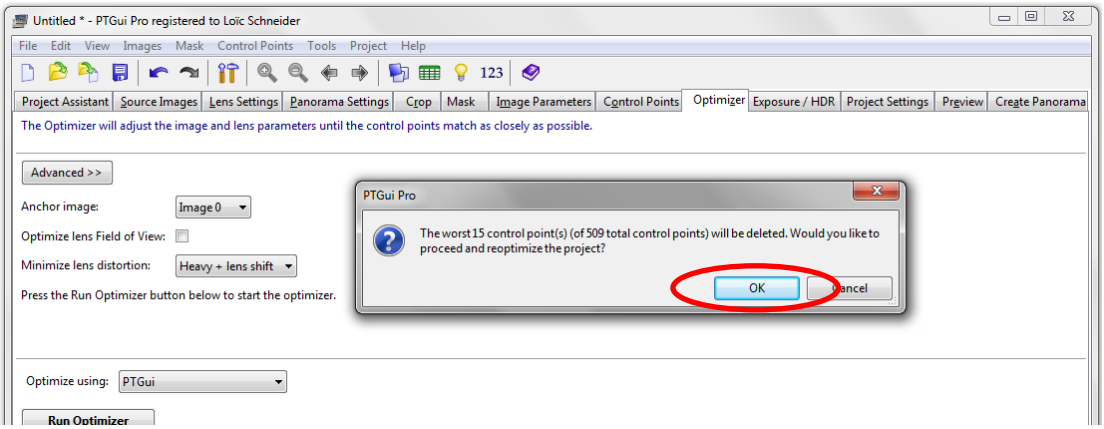
[3] PTGui *assumes that all images are taken from a fixed position and images were taken by rotating the camera relative to the captured object (e.g. as when using a tripod).. In the case of microscopic images, the sample is shifted under the microscope for each image, i.e. the camera is always perpendicular to the sample (“orthographic projection”). By setting the focal length to a large value, the rotation assumed by* PTGui *is reduced to <1°, which virtually eliminates any spatial contraction by* PTGui*, resulting in a ‘flat stitch’. See also: www.ptgui.com/support.html#5_5*

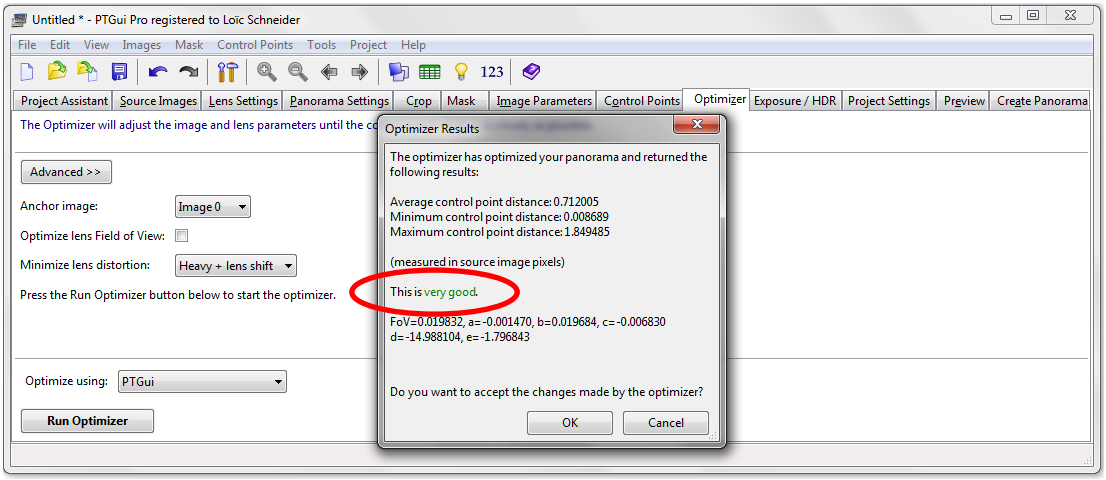
7. Go to the Optimizer tab and select Simple mode
   a) *On first run only:* Deselect Optimize lens field of view
   b) *On first run only:* Minimize lens distortion: select Heavy + lens shift
   c) Push Run Optimizer
   d) The Optimizer Results window pops up; hopefully it states a very good statistics; push OK



8. *Recommended:* improve the quality of the stitched mosaic image:
   a) In main menu, navigate to Control Points > Delete Worst Control Points
   b) A process summary window pops up; push OK.
   c) The Optimizer Results window pops up; hopefully it states a very good statistics; push OK
   d) *Optional:* Open the Control Point Table (Tools > Control Point Table) and delete Outliers if any (Distance much above other control point pairs); push Run Optimizer
   e) Cycle through a)-d) until you get satisfying statistics (e.g. Average: <0.8, Maximum: <2.0)

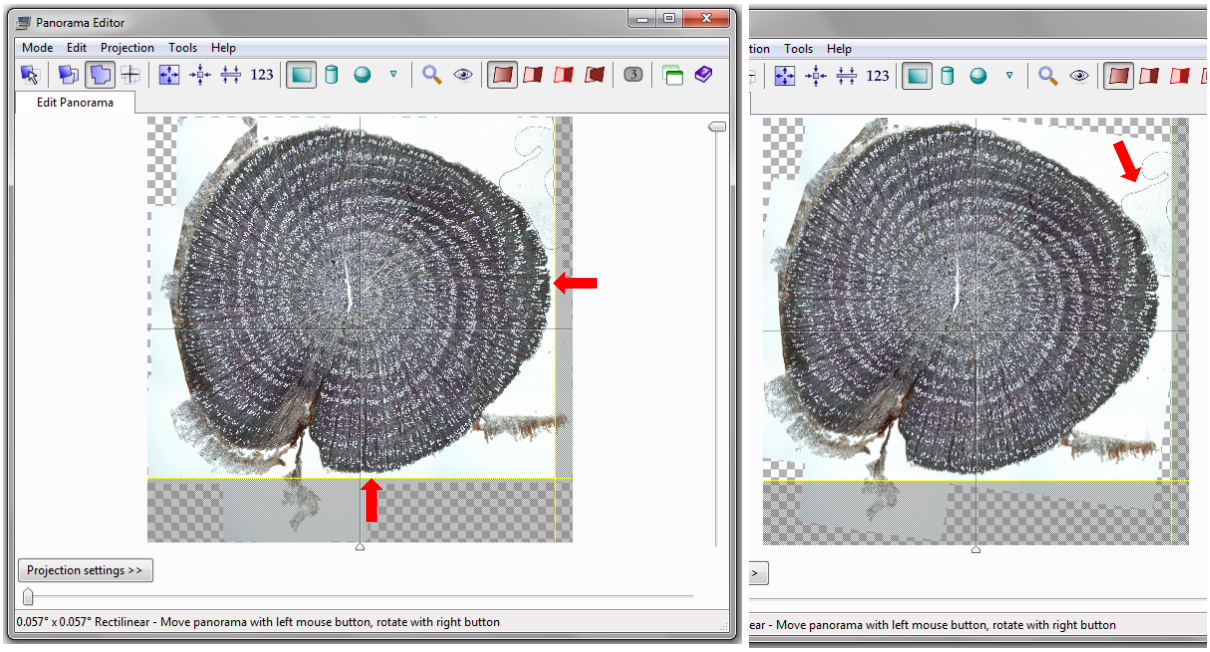






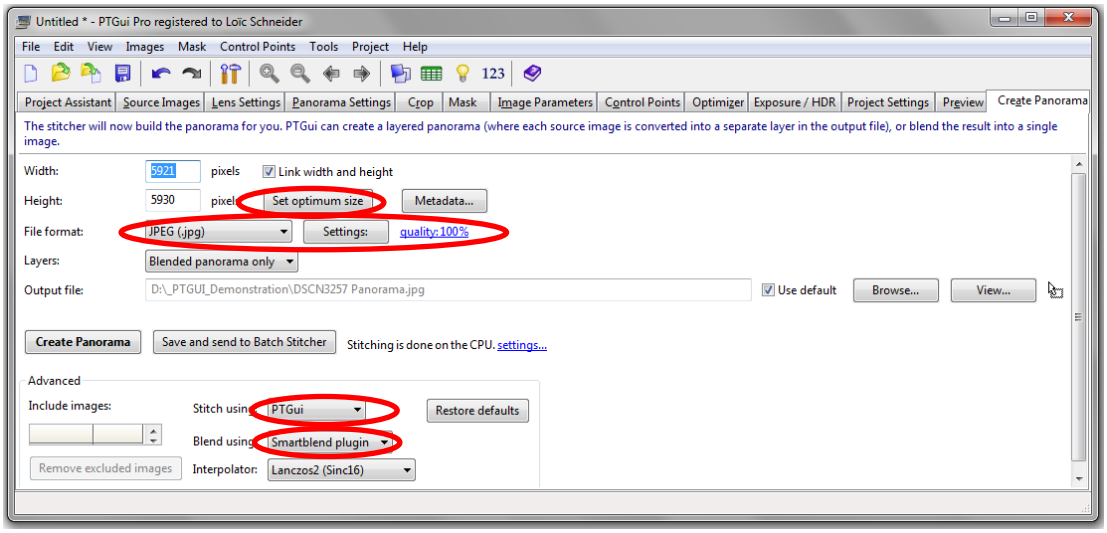
9. *Optional:* If you still are not satisfied with the results, follow the instructions in "G. CORRECTING REGISTRATION ERRORS".

10. *Optional:* crop/trim and rotate the mosaic image
    a) Select or open (CTRL + E) the Panorama Editor window
    b) Drag vertical and horizontal image edges with left mouse button; yellow lines show the applied trimming
    c) Hold down the right mouse button to rotate image



☞ *In linear anatomical samples (e.g. from an increment cores) you may want to rotate the image until the ring borders are aligned horizontally*

11. Go to the Create Panorama tab
    a) Push Set optimum size button to obtain mosaic image with same resolution/detail.
    b) *On first run only:* Choose an appropriate File Format and compression option in Settings (100% for JPG)
    c) *On first run only:* Blend using > Smartblend plugin
    d) *On first run only:* Output file > Use default



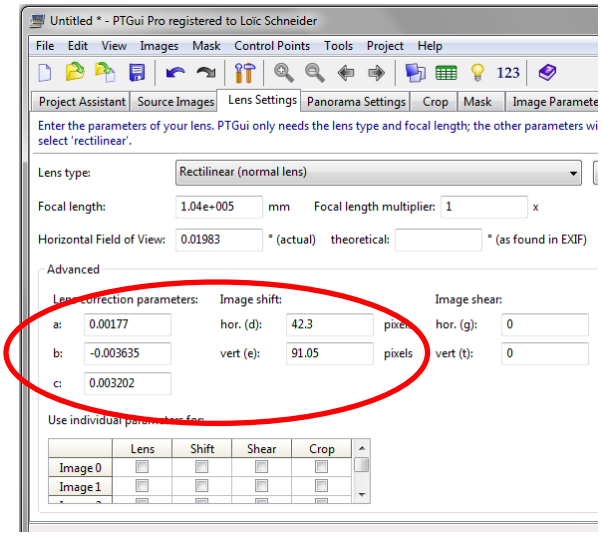
☞ *The maximum image dimension for JPG output is 25,000 pixels in any direction. Other file formats (e.g. TIFF) support larger mosaic images. For ROXAS analysis, the input image should not be larger than 32,768 pixels (corresponding to integer data type) in any dimension. Consider to create overlapping mosaic images to keep final image size within manageable limits.*

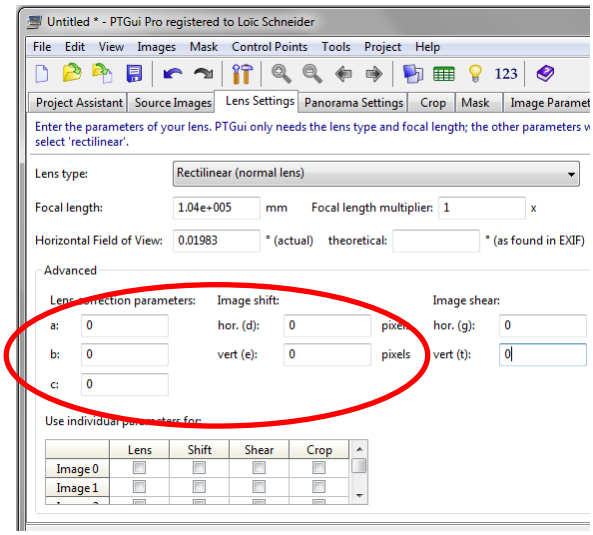
12. *On first run only:* In main menu select `File > Make Default`

    → *Most settings will be automatically applied to future projects as default values, i.e. the steps introduced by 'On first run only' are no more required.*

13. *Optional (only apply if using distortion-free plan-type lenses!):* remove residual tiny distortion (cf. step 5) by resetting in the `Lens Settings` tab the "a", "b", "c", "d" and "e" to "0".

    → *When asked, do not optimize the control points again!*



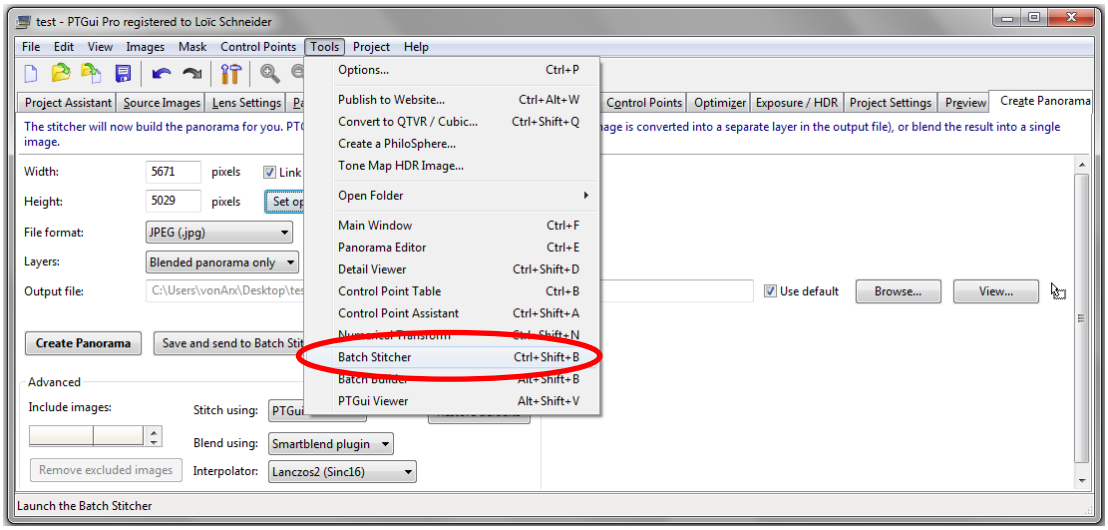


14. a) To stitch the image now:
    Go to the `Create Panorama` tab and push `Create Panorama`-button
    b) To stitch the image later, together with other projects (more efficient! cf. G.BATCH STITCHING MULTIPLE MOSAIC IMAGES):
    In main menu select `File > Save`; name the project

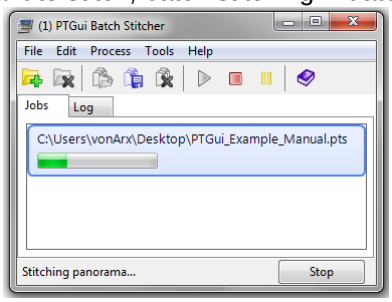
☞ *Saving the project even if stitched immediately (option (a)) is recommended because it allows later to check and reproduce the stitching settings*

F. BATCH STITCHING MULTIPLE MOSAIC IMAGES

1. Launch batch stitcher (`RunStitcher.exe`) or navigate in `PTGui` menu bar to `Tools` > `Batch Stitcher`



2. Add all projects (`*.pts`) you want to stitch; batch stitching initiates automatically



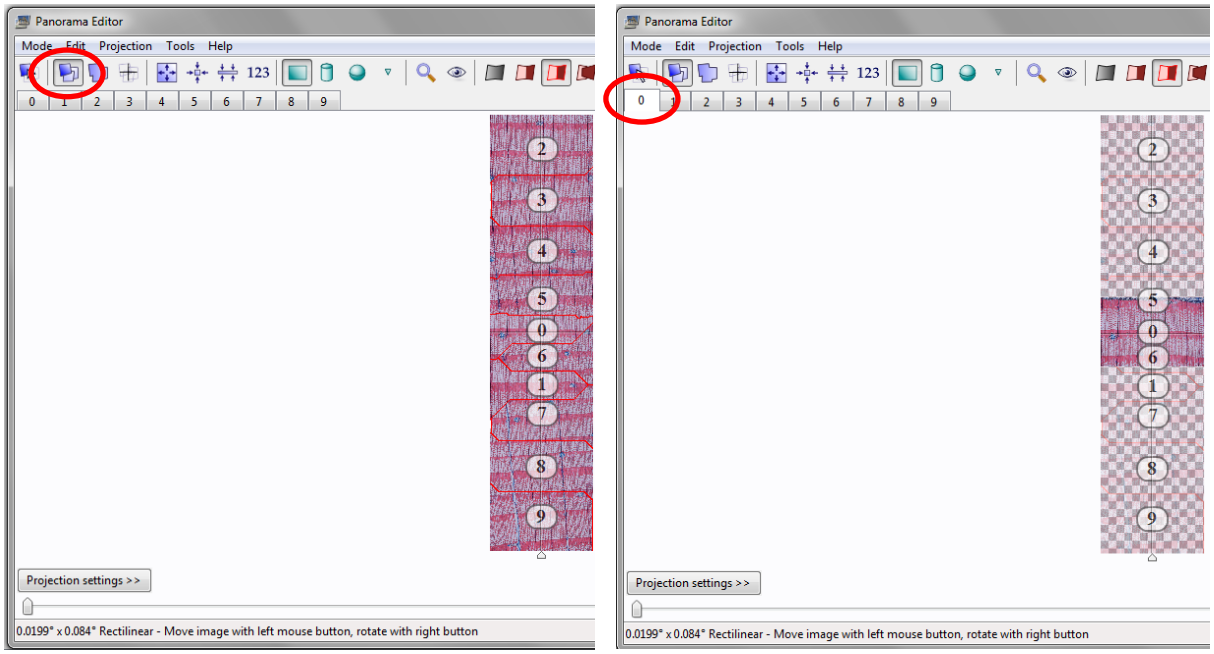
☞ *Don't stitch images if you intend to work on your computer at the same time, since stitching uses ample system resources!*

☞ *Stitched images are saved under the project name into the folder that contains the project*

G. CORRECTING REGISTRATION ERRORS

Option 1: manually position orphaned images:

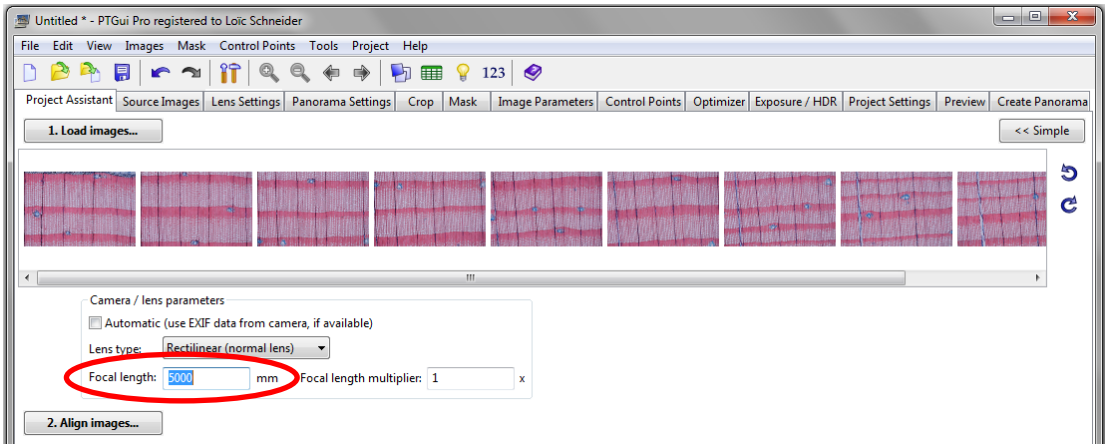
1. In the Panorama Editor window, select Mode > Edit individual images



2. Drag orphaned images to appropriate/corrected locations.
3. In the PTGui menu bar push Control Points > Generate Control Points for all images
4. Follow steps 7-14 in E. PREPARING AND EXECUTING STITCHING PROJECTS

Option 2: retry image alignment with changed field of view settings

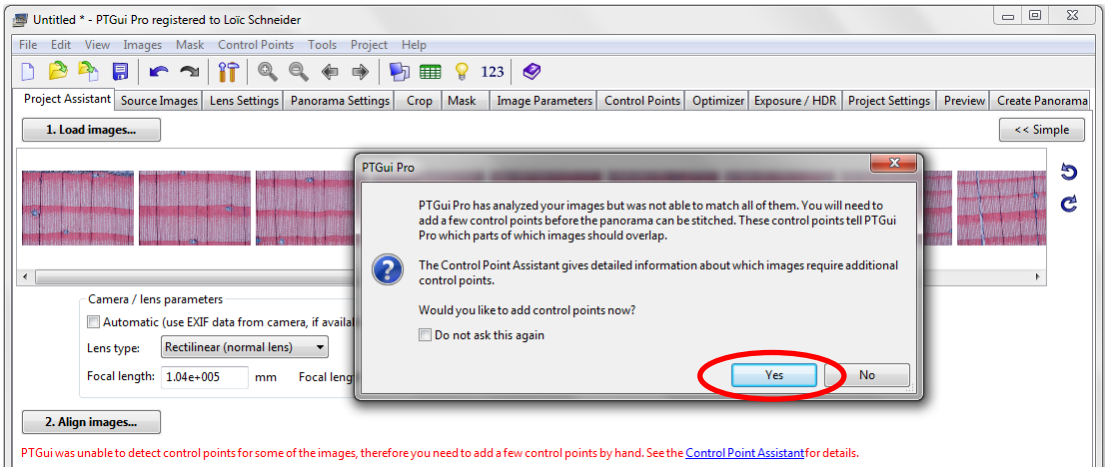
1. In the Project Assistant tab, Change the Focal length value to a smaller number (e.g. “1,000”, “5,000”; see step 5 in E. PREPARING AND EXECUTING STITCHING PROJECTS).



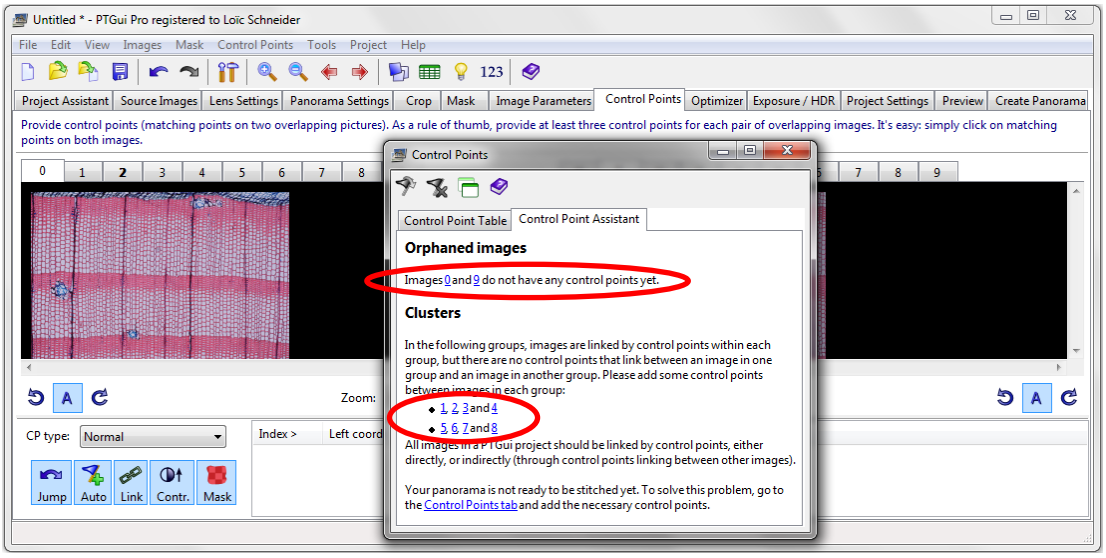
2. Follow steps 6-14 in E. PREPARING AND EXECUTING STITCHING PROJECTS

Option 3: manually add control points

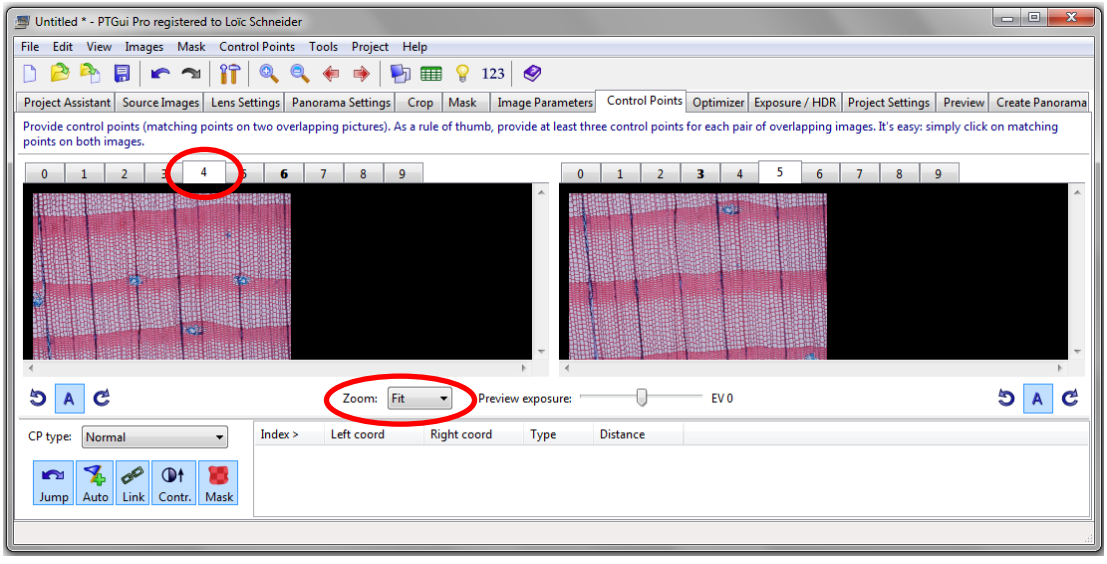
If PTGui was unable to find control points for all images, you may try to add them manually



In the example below images 0 and 9 are orphaned, while there are two clusters. Let's try to connect the clusters by linking image 4 and 5.



1. Select images 4 and 5 in the numbered tab and adjust the zoom level see a bit more detail



2. Position the images to see the overlapping regions (common structures). Then click on a conspicuous point in the left hand image and on the corresponding point in the right hand image.

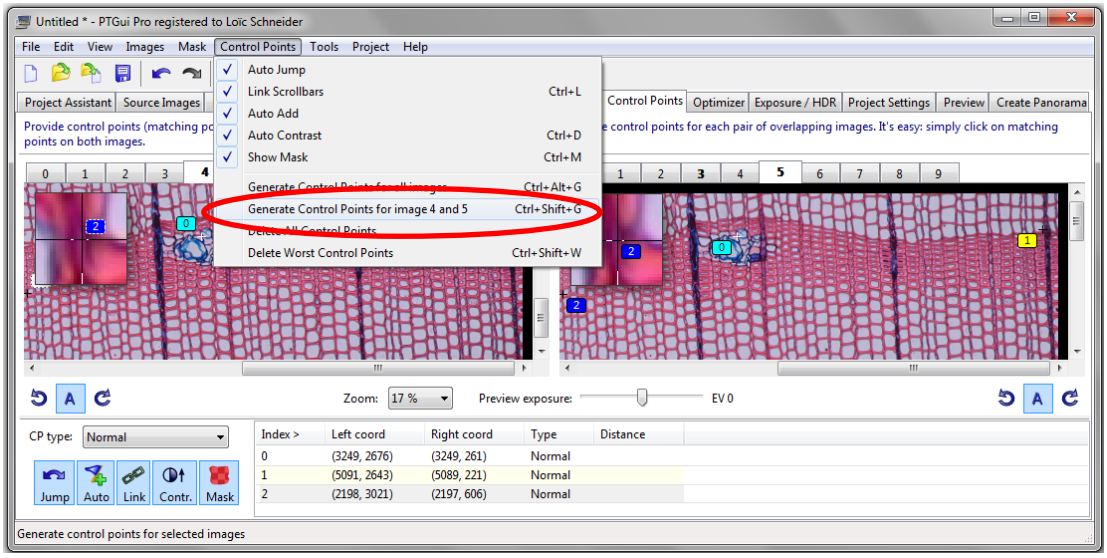
   → Use the arrow keys for fine tuning

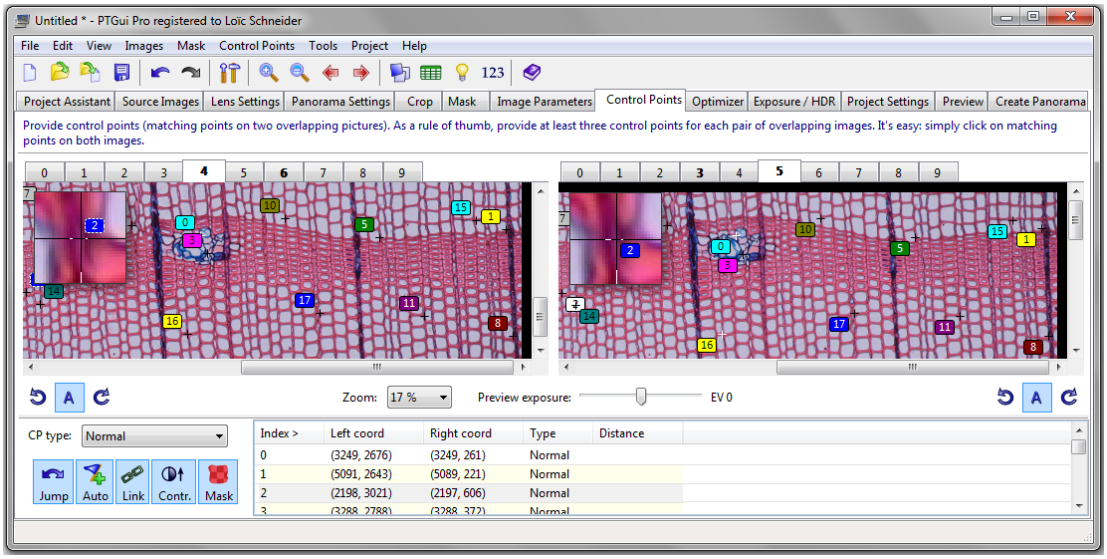
   → Try to well distributed the control points over the overlapping images

   → After adding two pairs of control points, the Auto Add feature will suggest the matching second point of a new pair of control points.

   → Read in the PTGui help files for more detailed information about manually adding control points

3. After adding at least 3 pairs of control points, navigate to main menu Control Points > Generate Control Points for image 4 and 5 to automatically add additional control points based on your manual selection.





4. Repeat steps 1-3 for all unconnected images and image clusters.
5. Follow steps 7-14 in E. PREPARING AND EXECUTING STITCHING PROJECTS

H. ELIMINATING DISTORTIONS IN SINGLE IMAGES

The following trick removes distortions from single images.

Note: You must have processed multi-image projects before applying the following procedure! In addition, the procedure has to be repeated for each optical setup.

1st time:

1. Open a project with multiple images
2. Go to the Lens Settings tab and jot down the values in Lens correction parameters for a-c.
3. In main menu select File > New
4. Load the image of a single-image sample **2×** (1. Load images...)
5. Go to the Lens Settings tab and paste the values for a-c. Set d and e to 0
6. Go to the Create Panorama tab and push Set optimum size button
7. In main menu select File > Save; name the project
8. Optional: you may stitch the image now
9. Navigate to the project file and copy it to your standard folder for templates (cf. D. INITIAL SETUP)
10. Rename the copy of the project file that from now on will serve as a template for stitching single images into something sensible, e.g. “Stitch_1_image.pts”

2nd and consecutive times:

1. Load the image of a single-image sample **2×** (1. Load images...)
2. Go to File > Apply Template and choose the previously created template (e.g. “Stitch_1_image)
3. Save the project or perform an instant stitching

I. DETERMINING THE SPATIAL RESOLUTION

For quantitative image analysis the spatial scale (pixels/unit) must be determined. While professional microscope-camera systems usually provide this information, there are two approaches for low-budget systems. Although approach 2 is more laborious, it will be more accurate and efficient in most cases.

Approach 1: Spatial reference in each (mosaic) image

1. Capture images of your sample with the desired zoom factor
2. Create the mosaic image (→ distortions removed)
3. Measure the extension (in pixels) of the spatial reference with a line measuring tool (e.g., using ImageJ) and calculate the pixels/unit conversion

Approach 2: Using focal length of camera and stage micrometer

1. Capture two images of stage micrometer (microscope slide with a scale etched on the surface), one with the lowest and one the highest camera zoom level

2. Stitch each of the single images to remove distortions (cf. H. ELIMINATING DISTORTIONS IN SINGLE IMAGES)
3. For each image, measure the distance (in pixels) of two distant divisions on the stage micrometer with a line measuring tool (e.g., using ImageJ).
4. For each image, divide the measured line length (in pixels) by the actual length (in the dimension of choice, e.g. microns) → pixel/unit
5. Repeat steps 3-4 at least 10× to get a robust statistical mean
6. For each image, get the focal length (a measure for the zoom level) from the EXIF file (*EX*changeable *I*mage *F*ormat – extension holding the camera settings that were used to take the picture; accessible by, e.g. IrfanView)
7. Use linear interpolation to calculate the pixel/unit-conversion for each intermediate focal length (zoom level).

J. IMAGE CROPPING

You may want to crop the output images. I recommend using IrfanView (www.irfanview.com), because it can handle very large images and will not compress the cropped image (alternatively: Photoshop, ImageJ, Image Pro Plus, etc.).

In IrfanView:
Make sure the plugins package is installed, then:

a) With left mouse, select crop area
b) Options > JPG Lossless Crop... (PlugIn)

☞ *Re-opening and overwriting the cropped image file (*File > Save as...*) will make the image much smaller without losing any information. It seems that the stitched images still contain the information from overlapping planes of the individual images.*

K. FINDING HELP

Check the help files and user-group (http://tech.groups.yahoo.com/group/PanoToolsNG) or the support/faq site (www.ptgui.com/support.html) for specific questions.

PTGui has a steep learning curve!