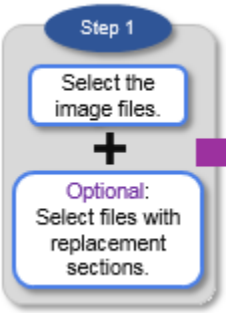
BRAINMAKER: Using the workflow

To open the workflow, go to *Registration>BrainMaker Workflow*.

In the workflow, you can see the list of steps, and below the list, the settings specific to the step selected.

To return to a previous step, click the name of the step in the list.

STEP 1: SELECT THE SLIDE IMAGE FILES



Before you start

- Note that you can't use image stacks.
- Use the **Biolucida Converter** to convert large images in non-virtual formats (e.g., *.tif, *.jpeg, *.bmp).
- Do not perform this operation over a LAN.

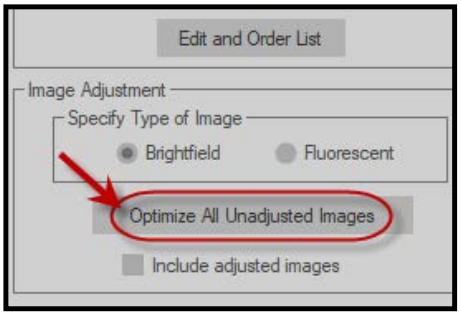
Procedure

1. Click the *Add slide(s) button* to open the *Image Open* window.
2. Select all the image files necessary for the reconstruction (hold down SHIFT or CTRL to select multiple files).

   The order of the slide image files in the list is important because it dictates the order of the images in the reconstruction.

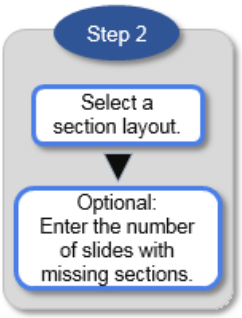
   If you have files with re-imaged data because of missing sections in your original images, add them to the end of the list (See Working with missing sections in the user guide).

3. Optional: To re-order the files or delete files, click the *Edit and order list* button.
4. Optional: To optimize image intensity, click the *Optimize all unadjusted images* button in the panel.



You can return to this step later from step 2 or step 3 if you need to make changes.

STEP 2: SPECIFY THE SECTION LAYOUT



The layout you specify will be used across the slides you selected.

The default is *Sequential/Variable Number Of Sections Per Slide/No Missing Section*.

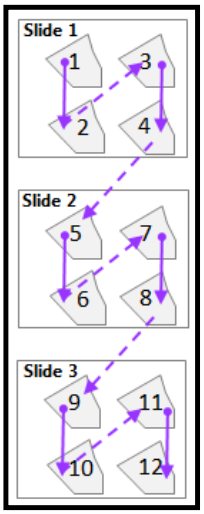
To see the layout options, check *Show advanced ordering options*. If there are unusable/missing sections in your original slides, enter the number of slides containing replacement sections in this step.

Sequential

All sections are ordered sequentially within in the first slide, then within the second slide, and so on.

- If the number of sections varies depending on the slide, select *Variable*.
- If the number of sections per slide is constant, enter the number of sections in the *Sections per slide is* field.

Example: Vertical sequential with 3 slides and 4 sections per slide ->

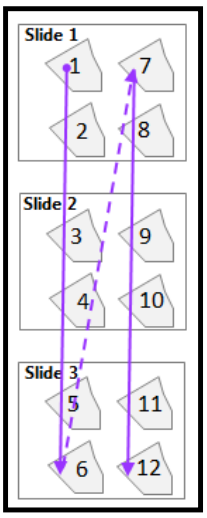


Staggered

Sections are ordered sequentially horizontally or vertically across slides. All slides must contain the same number of sections.

Enter the number of sections for each slide in the *Sections per slide is* field.

Example: Vertical staggering with 3 slides and 4 sections per slide ->



Slides for missing sections

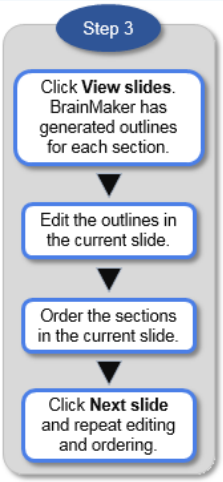
Enter the number of slides containing replacement sections. You need to return to step 1 to add these slides.

STEP 3: EDIT AND ORDER THE SECTIONS

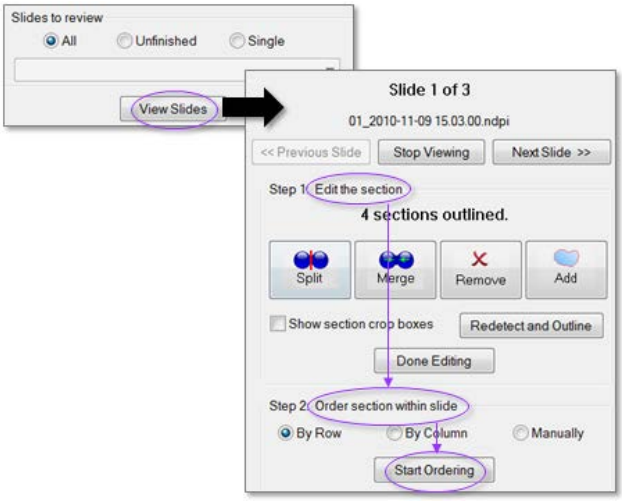
Edit and order the tissue sections that will be used in the reconstruction.

- For detailed instructions on editing, see Edit Section Outlines in the user guide.
- To take missing sections into account in the ordering, see Working With Missing Sections in the user guide for the complete procedure throughout the workflow.

You can interrupt the reviewing process and return to it later without losing your work. When you start the workflow again, BrainMaker applies the edits and ordering from the previous session.

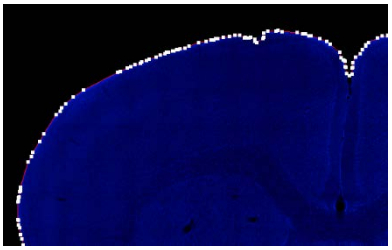


When you click *View Slides*, each slide is displayed with red section outlines generated by *BrainMaker*.



Editing & ordering: Process overview

1. Under *Slides To Review*, select *All*.
2. Click *View Slides*. The first slide is displayed with the section outlines.
3. Click the outline you want to edit to select it. The selected outline is represented by a series of white points ->



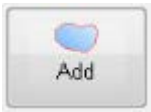
4. To edit the outline, use the tools provided in the workflow panel. You can also refine the outlines manually. See Edit Section Outlines in the user guide for detailed instructions.
5. Under *Order Section Within Slide*, select a method then click *Start Ordering*.
6. If you selected *Manually*, click inside an outline to number it.
7. Click *Next Slide* and repeat steps 3-5 until all slides are edited and ordered.
8. Click *Next Step*.

Slides to review (settings)

- ALL: BrainMaker displays all the slides you selected.
- UNFINISHED: BrainMaker only displays the slides that have not been ordered (either because you haven't performed this step yet, or because you already performed this step but skipped ordering for some slides).
- SINGLE: BrainMaker displays the slide selected in the drop-down menu.

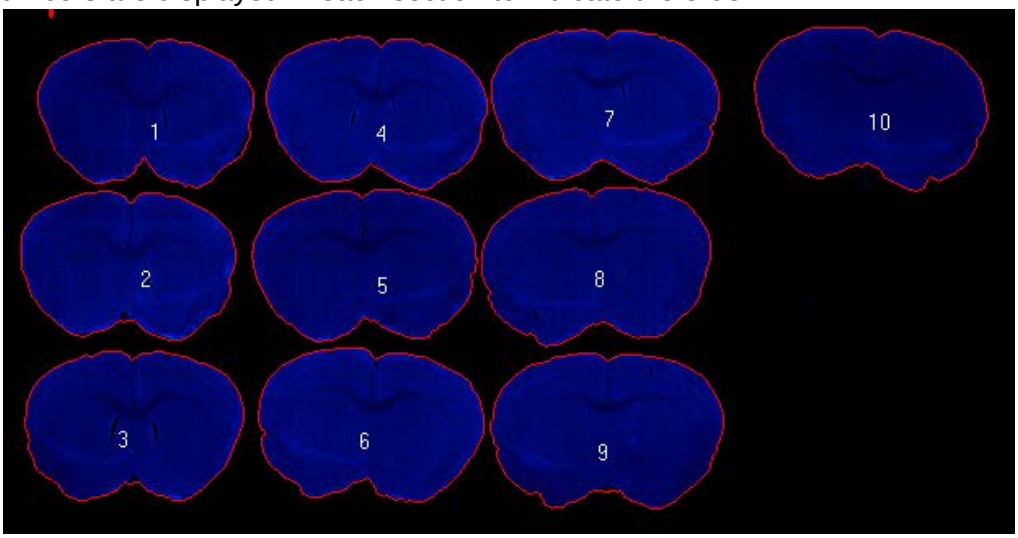
Edit the section (settings)

	Click a contour to select it then click the button.
	Click a contour. Hold down the CTRL key and click a second contour.

	Click *Merge*.
	Use this function to create a placeholder for a missing tissue section (see Working with missing sections in user guide) OR to draw a contour manually for a section that isn't delineated (see DRAWING AN OUTLINE MANUALLY in Edit section outlines in user guide).
	Use this function to refine the contours or to revert to the original contours generated by the program. When you change the settings, these settings persist the next time you use this button. Click the *Use Defaults* button to revert to the original settings. You can also refine the contours manually.

Order the sections within the slides (settings)

- BY ROW (BY COLUMN): Sections are automatically ordered and numbered by row (column).
- MANUALLY: Click inside each section contour in the order to be used for the reconstruction. Numbers are displayed in each section to indicate the order.



If you used BY ROW or BY COLUMN, you can edit the ordering manually:

- Right-click to undo the last section ordered.
- Once a section is no longer numbered, you can click it to number it.

NOTE: If you edit the slide after ordering, the ordering is lost.

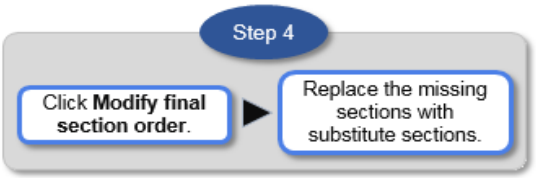
About the outlines generated by *BrainMaker*

BrainMaker first detects connected regions bounded by large image gradients (edges); these regions are interpreted as individual sections. After detection, the program generates an outline for each individual section detected.

Where two sections overlap, *BrainMaker* automatically identifies “pinch points” to split the outline into two separate outlines. Pinch points are defined as being close to each other in 3D space but distant from each other along the outline path.

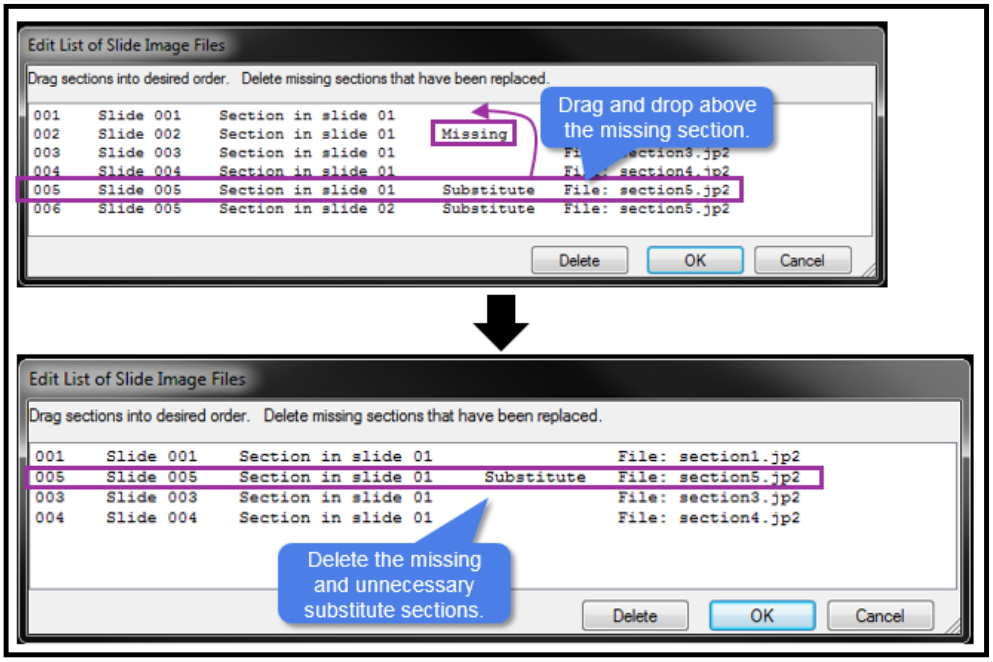
STEP 4: FINALIZE SECTION ORDER

If there are no missing sections, you can skip this step.



To include the missing sections:

1. Click *Modify Final Section Order*.
2. Select the replacement section in the list (labeled SUBSTITUTE) and drag it over the section to be replaced (labeled MISSING).
3. Highlight the MISSING section you are replacing and click the *Delete* button.



STEP 5: ALIGN THE SECTIONS

In this step, *BrainMaker* automatically aligns the sections previously delineated with the contours. During the alignment process, *BrainMaker* has the ability to correct for flipped or damaged sections. You can review and correct the alignment in step 6.

1. *BrainMaker* automatically identifies the image type as BRIGHTFIELD or FLUORESCENT.
2. If the type is BRIGHTFIELD but you only need to use one color, select FLUORESCENT then select a CHANNEL FOR ALIGNMENT from the drop-down menu.
3. Select one of the three methods (described in the workflow panel).
4. Click the *Perform Section Alignment* button.

Accuracy & processing time	Method	Description	Rotation	Flipping	Interior image features
− ↓ +	Align using centers	Aligns the centers of each section			
	Align using shape	Aligns sections based on the section's contour shape	X	X	
	Align using shape and image features	Aligns sections based on the section's image content	X	X	X

About the alignment process

BrainMaker uses multiple image registration algorithms.

Image registration identifies a geometric transform that maps points in one image onto corresponding points in another. The registration process adjusts transform parameters to optimize a metric that compares the image intensities at the corresponding points.

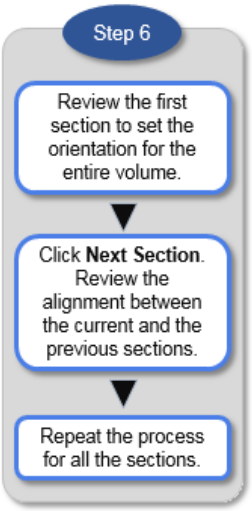
The transforms and comparison metrics vary depending on the alignment method you select.

STEP 6: REVIEW THE ALIGNMENT

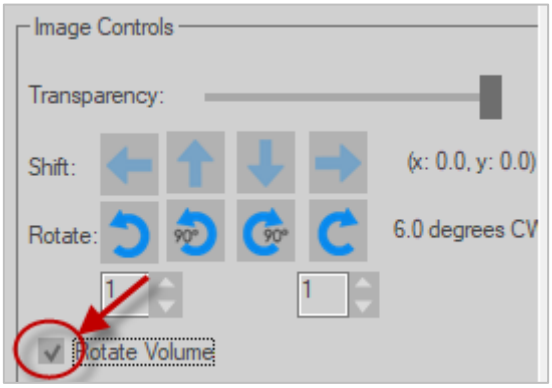
In this step, only the first section is displayed initially so that you can adjust the rotation and set the orientation for the entire volume.

- To rotate a section, use the *Image Controls* buttons in the workflow. From your keyboard, you can also use the "." (period) key to rotate right and the "," (comma) key to rotate left.
- To help with the adjustments, use the grid (check the SHOW GRID box in the workflow panel). You can adjust the cell size with the corresponding slider.

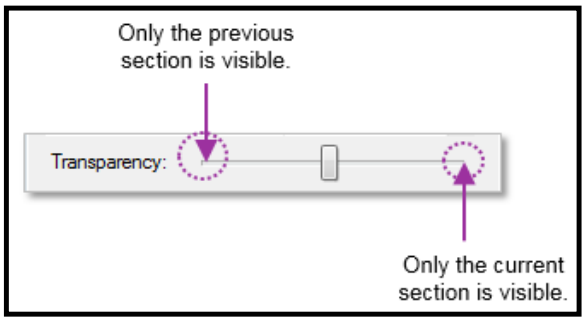
Once you've reviewed the orientation based on the first section, you can adjust the alignment of the sections, two sections at a time. When two sections are displayed, they both appear as semi-transparent. You can move the current section to align it with the previous section.



NOTE: If you can't determine the tissue orientation from small sections cut early in the block, use Rotate volume to rotate all the sections at once from any section.

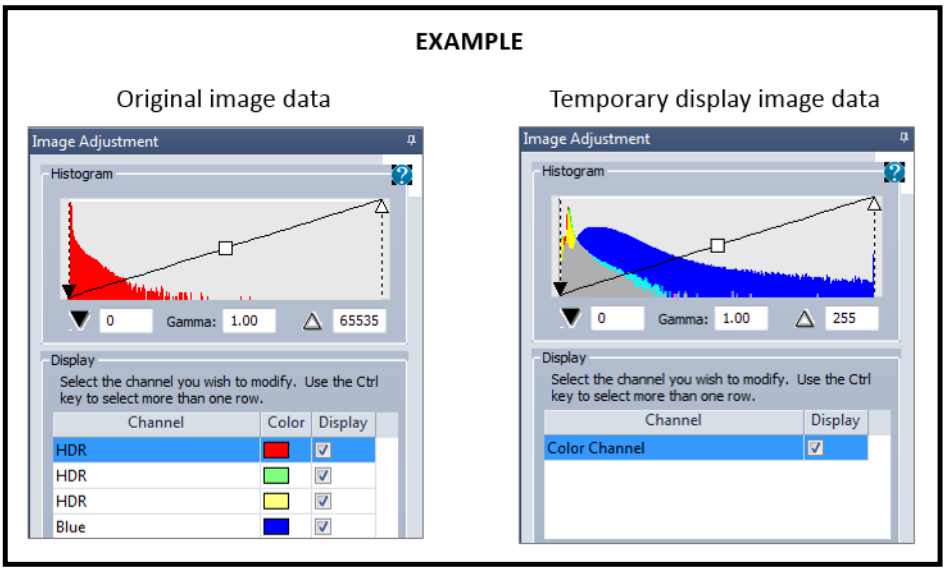


- To move the current section, use the arrow buttons in the workflow panel OR the arrow keys on your keyboard, OR drag the section.
- To adjust the transparency, use the TRANSPARENCY slider in the workflow panel OR the "+" and "-" keys on your keyboard.
- To show only the previous section, move the slider all the way to the left; to show the only current section, move the slider all the way to the right.

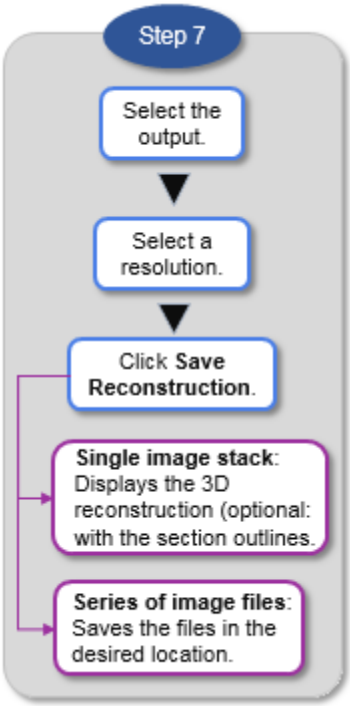


- To refine a manual adjustment (rotation or translation), click the REFINE button.

NOTE: You may notice that the image information in IMAGE ADJUSTMENT doesn't reflect the original image data. This is only temporary and designed to make the alignment process more efficient. BrainMaker will use the original image data for the reconstruction.

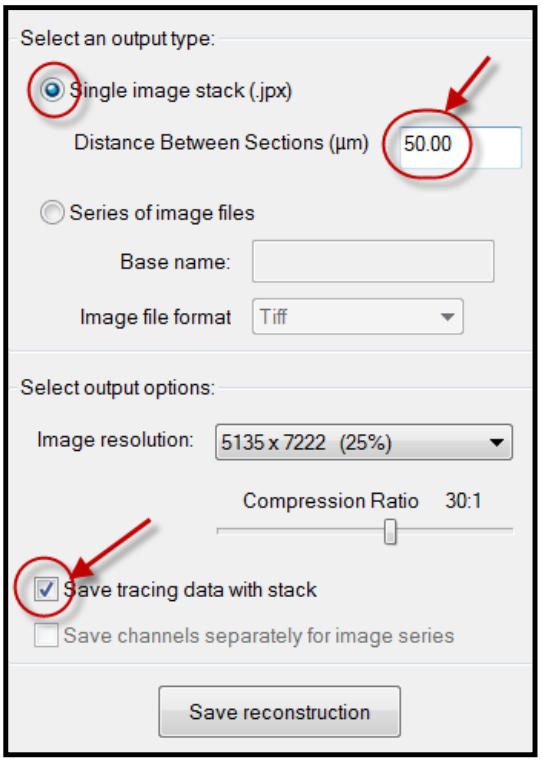


STEP 7: SAVE THE RECONSTRUCTION



BrainMaker produces a full-resolution 3D image stack from aligned sections, or saves aligned sections as a series of images.

Single image stack



The stack is saved as an MBF JPX file which preserves the intensity resolution.

1. Verify the DISTANCE BETWEEN SECTIONS.
2. Select an IMAGE RESOLUTION.

To save the section contours with the stack, check the *Save Tracing Data With Stack* box. You'll be able to use the Serial Section Manager in *BrainMaker*, *Neurolucida*, or *Stereo Investigator*.

The 3D reconstruction is displayed after clicking SAVE RECONSTRUCTION. See 3D Visualization for details on the 3D window.

Series of image files

1. Choose a BASE NAME.
2. Select an IMAGE FILE FORMAT.
    - MBF JPEG2000: Preserves all the channels.
    - TIFF or JPEG: Saves the image using a 24-bit format (8 bits per channel)
3. Select an IMAGE RESOLUTION. When you select *Enable X And Y Scaling*, resolution is automatically set to 100%.
4. Optional: For a multichannel image, check the *Save Channels Separately For Image Series*.
5. Optional: If your images have no scaling or if *BrainMaker* can't read the scaling, check *Enable X And Y Scaling* and enter the values manually.

NOTE: The files are saved in the folder you designated upon clicking *Save Reconstruction*; there is no reconstruction displayed.

About image resolution

BrainMaker determines a maximum resolution for each image based on the original image resolution and the alignments.

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