# Paneling and Stitching – Collecting, stitching and special considerations

## Collecting Panels:

1. **Consider binning your images** – If you will be collecting a large number of panels, using 2x2 or 3x3 binning during acquisition is an effective way of reducing the file size of a stitched image.
2. **Use the correct overlap value during collection** – If panels will be deconvolved, you must ensure that the overlap value listed in the Panels tab of the Experiment Designer is at least 2x the border roll-off of the image. Follow the steps below to check what the border roll-off value is:
    a. Select the correct image size, bin value and objective in the Resolve3D window.
    b. In the Resolve3D window, select **File > Snapshot**.
    c. Activate one channel and select **Do It**. (Note: it is not necessary to have a sample on the scope or the correct channels selected for this portion.)
    d. In the main *softWoRx* window, select **Process > Deconvolve**.
    e. Drag the window ID number for the snapshot into the Input field in the Deconvolution Window.
    f. In the Deconvolution Window, select the **More Options** button.
    g. In the More Options Window look for the field labeled **Border Roll-off**. Note the value of the border roll-off and double it for the **Overlap** value during panel acquisition.
3. **Flat-Field Calibration** - Flat Field Calibration compensates for variations in sensitivity of the camera detector as well as any obstructions or distortions in the light path. It will make it so that illumination is even across the field of view, which reduces the appearance of dark edges around individual images in a larger stitched image. For specifics on how to do this, refer to the "Creating Calibration Files" document.
4. **Deconvolve your images** (if you would like to) and crop border roll-off.

## Stitching Panels:

1. **Quick Projection** – If you have collected several Z sections, but a 2D view is all you need from your stitched images, consider projecting your images (**View > Quick Projection**) by Max Intensity before stitching. This will greatly reduce both the file size of your stitched image and the processing time required to stitch.
2. **Rotation** – For most systems there is a slight rotation adjustment required during the stitching process. This adjustment is made in the **More Options…** menu of the stitching tool. Normally, the "correct" rotation lies between -1 (clockwise) and +1 (counterclockwise) degree. Finding the proper rotation is a matter of trial and error.
3. **Reduction Factor** – A parameter located in the Stitching tool, adding a reduction factor (>1) is a way of reducing the file size of a stitched image post-acquisition.

## Special Considerations:

1. **Paneling over time** - When you collect paneled data, each panel is saved as a time point. When you collect a panel image for a *single* time point, the stitching

tool can easily read this information in order to produce a properly stitched image for that time point. However, when paneled data is collected over time, both the panels and time points are saved as time points and the tool has no way to distinguish between last image of time point 1 and the first image of time point 2.

To get an image of this kind to stitch correctly, you first need to manually edit the image header. An example follows. This example assumes that the paneled image has 1 Z section, 5 columns and 2 rows of images (for a total of 10 images in each panel) and 15 time points. This would result in a raw image with one Z plane and 150 time points. (NOTE: If your image contains several Z sections, Panels AND Time points, you will have to Quick Project the image before proceeding.)

a. Edit the Image's Header:
    i. Save a back-up of the file that you would like to stitch.
    ii. Close the image that you need to edit (the Edit Header tool cannot write to an open file).
    iii. In the main softWoRx window, select **Edit > Header**.
    iv. In the Edit Header window, select the **File** button and navigate to your image.
    v. When your image is loaded properly, look at the field labeled **Z-Sections, Wavelengths, Time-Points**.
    vi. The example file would read: 1 1 150
    vii. Change that field to: **10 1 15**. (Please make sure that you have a single space in between the numbers. The middle value should already match the number of wavelengths for your image.)
    viii. Select **Save Changes** and **Done**.
    ix. Open the file. You would now see 10 Z-Sections and 15 Time Points.
b. Stitch the File:
    i. In the main softWoRx window, select **View > Stitch**.
    ii. Load your file into this window.
    iii. Select **More Options…**
    iv. Click on the dropdown next to **Stitch Type** and select **Along Z**.
    v. Select the **Close** button.
    vi. Select **Do It** in the Stitch Window

2. **Adding an AF event before each panel** – Adding an AF event before each panel is not something that it achievable through the Design/Run Experiment GUI but is possible with some custom macro editing. The easiest way to do this is to set up as much of your experiment as possible through the Design/Run Experiment GUI and then just add whatever lines are necessary to customize it.
    a. Create a "Dummy" time lapse to set the AF command syntax:
        i. Use the Design/Run Experiment GUI to design a time lapse that contains an AF event with the same parameters that you would like to use during your paneling experiment.
        ii. Select **File > Save** and then **File > Edit** in the Design/Run Experiment window. This opens the Macro Editor window. (NOTE: If you need more of an explanation of what each macro

command does, you will find a list of all the valid commands on the right hand side of the macro editor window. If you highlight any of these commands, you will see a brief description of what it does and its correct syntax at the top of the macro editor window.)

iii. Scroll through the macro until you find the Autofocus command, highlight the entire command line (it should look like this: `AUTOFOCUS 2,0.386,6.184,0,0.000`, although the numerical values will most likely be different) and select **Edit > Copy**.

b. Set up the Paneling Experiment:
   i. Fill out the Sectioning, Channels and Panels tab as you normally would.
   ii. Select **File > Save** to save the macro and **File > Edit** to open the macro for editing.
   iii. Find the `NEXTPANEL` command and select **Edit > Paste** to insert the Autofocus command from the "dummy" macro on a line below the NEXTPANEL command
   iv. Select **File > Save** to save the changes to the macro (you will probably see a warning telling you that the macro has been changed, you can ignore this) and then close the macro editor window.
   v. In the Design/Run Experiment window, select the Run experiment tab, type in an Image filename and press the green play button.

3. **Stitching and viewing very large data sets** – It is possible to collect more panels than your workstation is able to display. If this happens and you can not use any of the techniques previously mentioned to reduce the file size (i.e. binning and/or using a reduction factor), it is possible to change the memory parameters in softWoRx to accommodate a large dataset.
   a. In the main softWoRx window, select **Utilities > User Parameters**.
   b. Increase the **Working Unit** until it is large enough to allow the system to display one section (number of rows*columns) of the stitched image.
      i. The Working Unit may not exceed the Working Set.
      ii. The Working Set should be at least 10Mb smaller than the Shared Memory Total.
      iii. The Shared Memory Total should not be larger than 95% of the available workstation RAM.

      NOTE: In the User Parameter window, selecting any of the parameters (Shared Memory Total Size, Working Set or Working Unit) will open a help window with more information about each of the parameters.
   c. If adjusting the user parameters still does not allow you to view your stitched image, you have a dataset that is too large to be displayed. In this case you will have to either add a reduction factor during stitching or acquire the panels again using 2x2 or 3x3 binning.
   d. To restore the default memory parameters, enter 850, 750 and 350 for the Shared Memory Total Size, Working Set and Working Unit values, respectively.