Topic
This technical note describes the hardware setup and software configuration necessary to achieve the best results with Volocity Acquisition automatic stitching. This functionality is available in the ‘Stitch’ tab of the acquisition setup dialog when a motorised XY stage is correctly configured.

Discussion
Volocity is able to stitch (produce montages of contiguous images) both 2D and 3D images.
In order to successfully stitch, three key criteria must be met by your imaging system.

1. Orientation
The orientation of images seen in the video preview must match the orientation of the image seen with your microscope’s ocular lenses. If this is not the case the orientation can be corrected by physically rotating the camera, or by using the rotation control to rotate the image.

2. Calibration
Ensure that the spatial calibration of the video preview is correct. Remember to check the calibration for every objective lens on your imaging system. We recommend the use of a stage graticule to accurately calibrate lenses.

Rotate either the camera, or the image, until the orientation of the image matches the orientation seen with ocular lenses.
3. Configuration

Select **Preferences...** from the **Volocity** (Macintosh) or **Edit** (Windows) menu. Select the **X-Y Stage** icon.

Ensure that the correct focus drive is selected in the dialog, and that the focus drive that you wish to use to store the Z position of acquisition points is selected (this may not be the same as the drive that you use for acquisition). There are also check boxes to **Invert X axis** and **Invert Y axis**. These options allow you to ensure that the origin of images matches the origin of the stage. If either of these options is incorrect, stitching will fail.

After setting preferences, you can check that they are correct by scanning an area of an informative sample such as a stage graticule.

Select the video preview. From the **Video** menu select **XY Stage**. From the **Stage** menu, select **Calibrate Stage**...

The stage will be moved through its full range of motion.

Locate the current field of view in the stage view and use the zoom tool to enlarge the XY stage view until individual fields can be clearly seen.
Use a ROI tool to select an area of three or four fields that are contiguous with the current field of view. Select **Scan Selected Area** from the Stage menu.

If the scanned fields are jumbled, the preferences have not been set correctly.

Re-open the Preferences dialog and select a new combination of stage preferences.
Your scanned area will be re-drawn using the new combination. Note: Scanned images are only roughly assembled and never look perfect. Use them only to ensure that tiles are not grossly jumbled. Once this is done, you can execute stitching acquisition protocols in which tiles are flawlessly aligned.
After stage editing preferences, tiles are no longer jumbled, although they are not perfectly aligned.