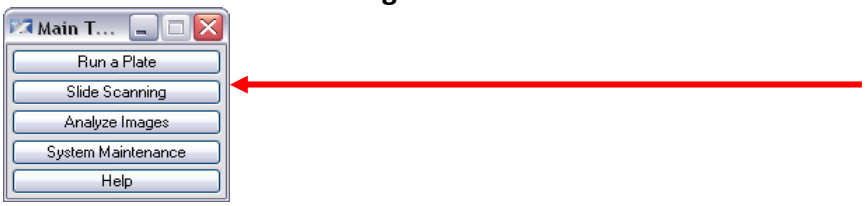
SLIDE SCANING QUICK START GUIDE FOR METAXPRESS 5.0

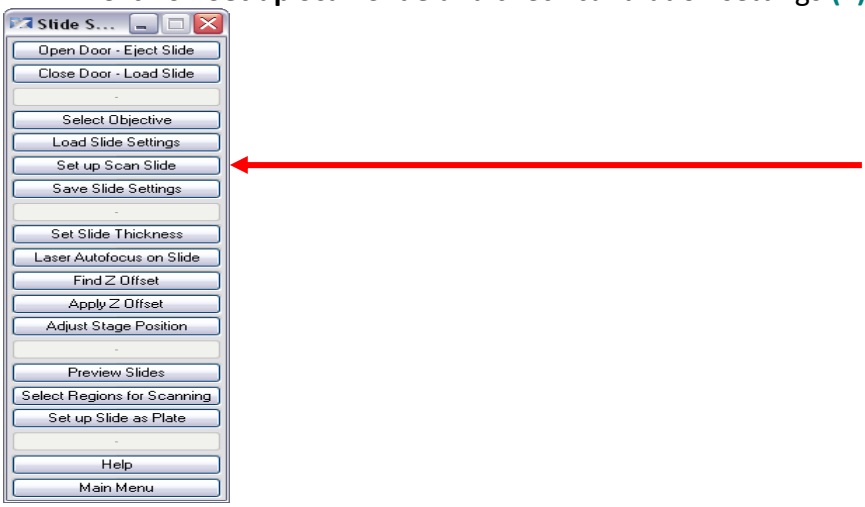
This guide will help you set-up Scan Slide. You will need IXMTaskbar v 5.1 or higher (available online on the Molecular Devices Knowledgebase)

The following are quick instructions to settings up Scan Slide. Please refer to **Sections A-D** below for more details.

1. Start MetaXpress and log in
2. Make sure the Main Taskbar is loaded (Journal → Taskbars → Load Taskbar …)
3. Click on **Slide Scanning**



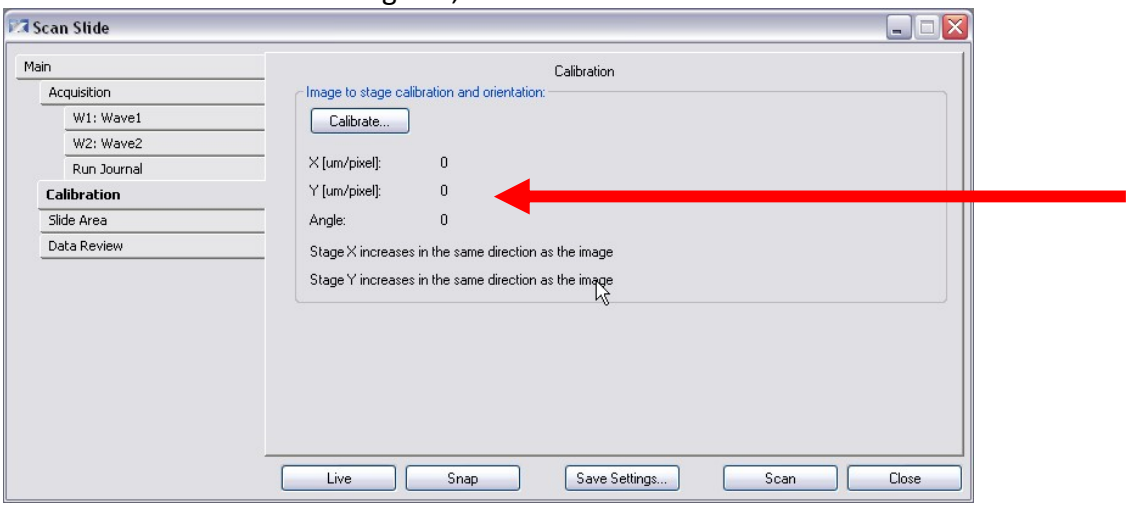
4. Click on **Set up Scan Slide** and check calibration settings **(*)**



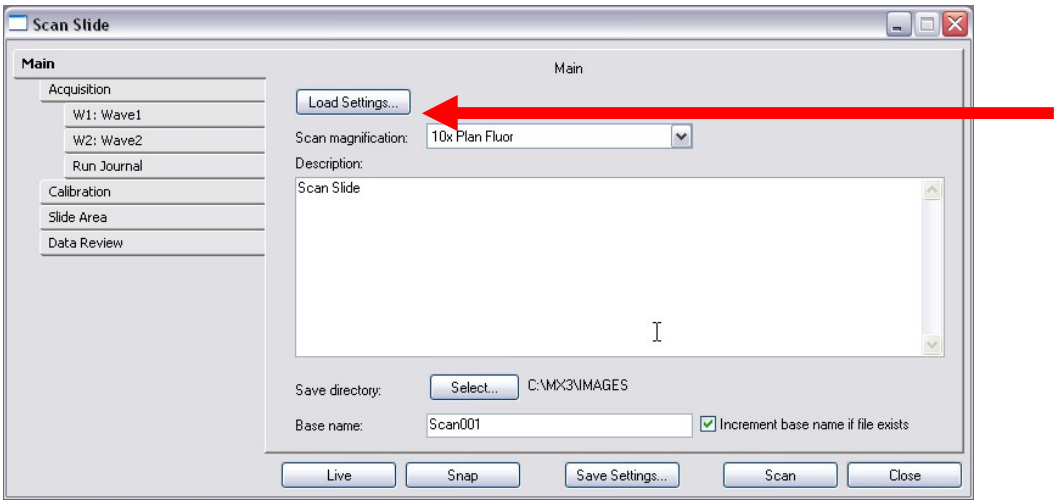
5. Create base settings for Preview Scan (preview settings) and Select Regions for Scanning (Acquire settings) if you do not have them already **(Section A)**
6. Click on **Preview Slides** to acquire slide at low magnification with one wavelength **(Section B)**
7. Click on **Select Regions for Scanning** to acquire slides at high magnification with all wavelengths **(Section C)**
8. Review images through Scan Slide dialog **(Section D)**

* Set-Up Scan Slide With Calibration Settings

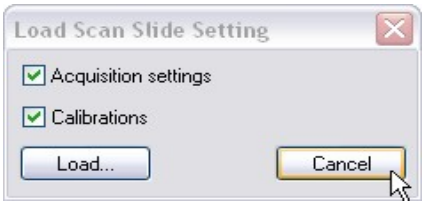
1. On the toolbar, click on Apps → Scan Slide . . .
2. In the Scan Slide dialog box, click on the **Calibration** tab



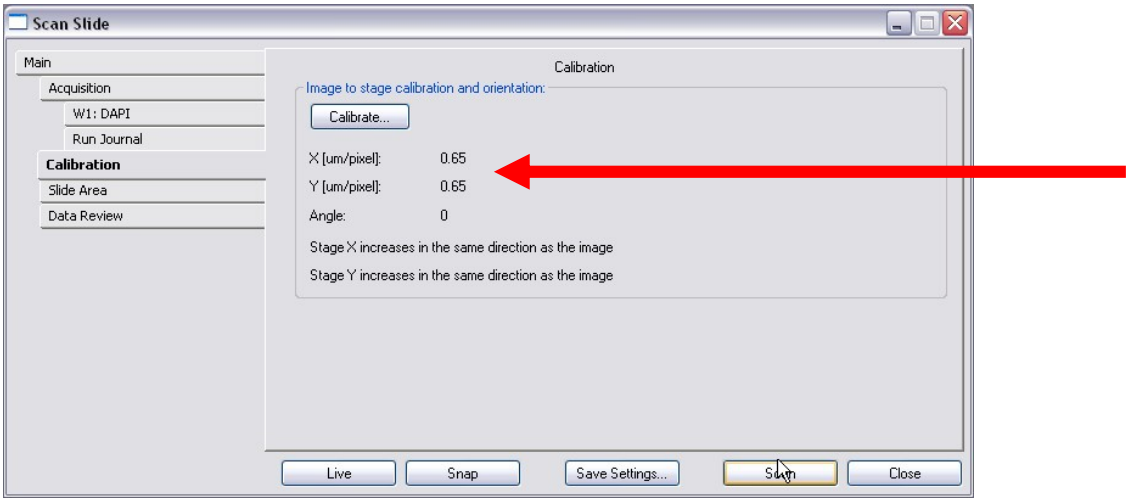
3. If there is a "0" next to X [um/pixel] and Y [um/pixel], do the following:
   a. Find the IXM_ScanSlide_Calibrations.scansetting file in C:\MX 5\Taskbars\Install\Files
   b. Copy the file into C:\MX 5\Scan Slide Setting
   c. Load this file into Scan Slide: Click on the **Main** tab. Click on **Load Settings**.



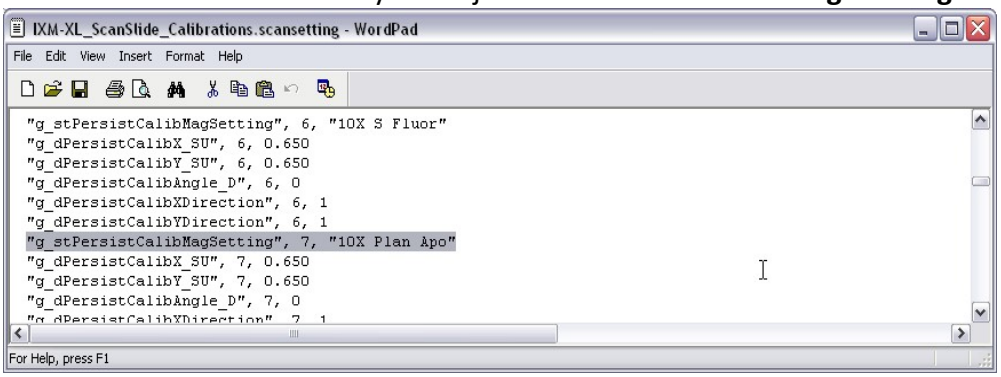
   d. Make sure there are check marks next to Acquisition settings and Calibrations



e. Click on **Load** and locate the IXM_ScanSlide_Calibrations.scansetting file
f. Click on the **Calibrate** tab. There should be numbers next to X and Y.

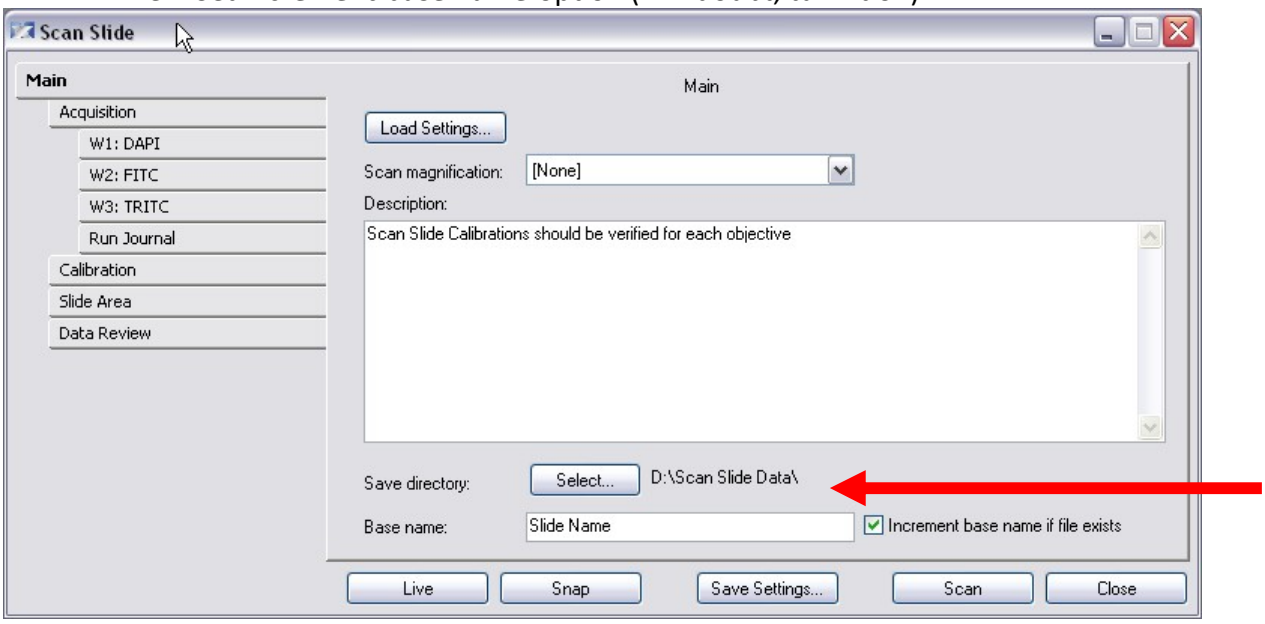


g. If no numbers are present, there may not be calibration settings for your objective or the objective name in the software (see the **Main** tab) does not match the calibration file. (Ex: 10x Plan Fluor is not the same as 10X Plan Fluor). You can either edit the name in the calibration file (opens in Wordpad) or edit the names of your objectives in **Devices → Configure Magnification**

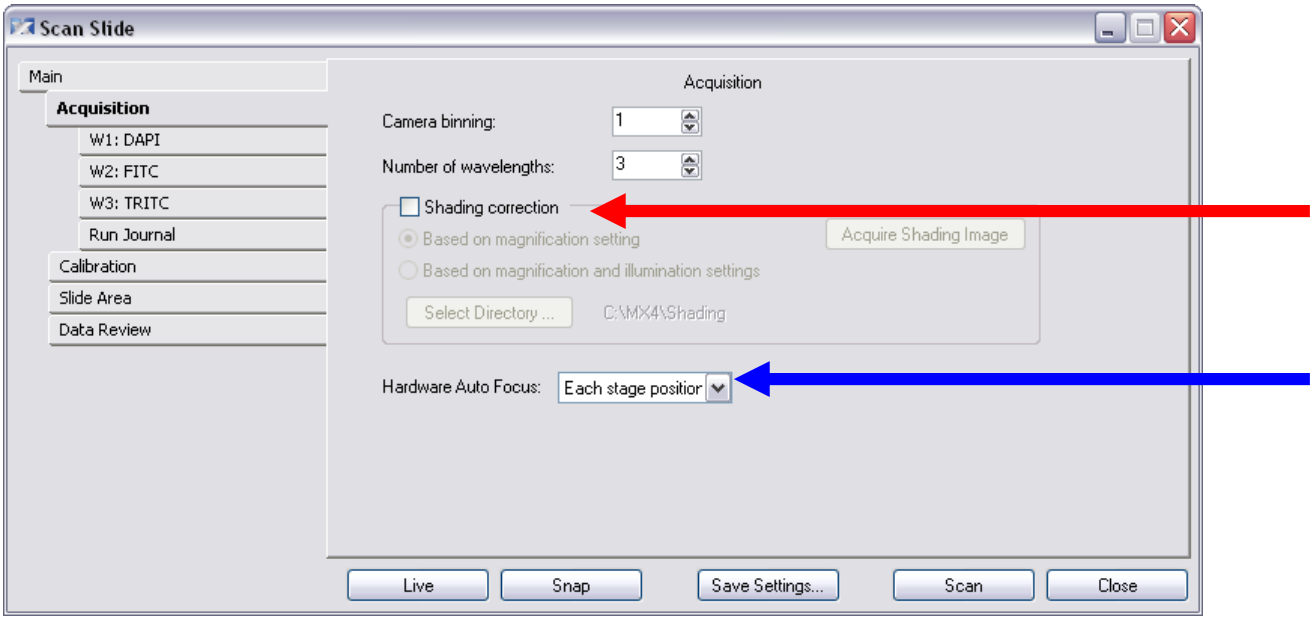


SECTION A: Slide Scan Dialog

1. Open Scan Slide (Apps → Scan Slide)
2. **Main tab:**
    a. Select an objective
    b. Load in any saved settings
    c. Enter a description
    d. Set ***Save Directory***
    e. Set ***Increment base name*** option (if in doubt, turn it on)

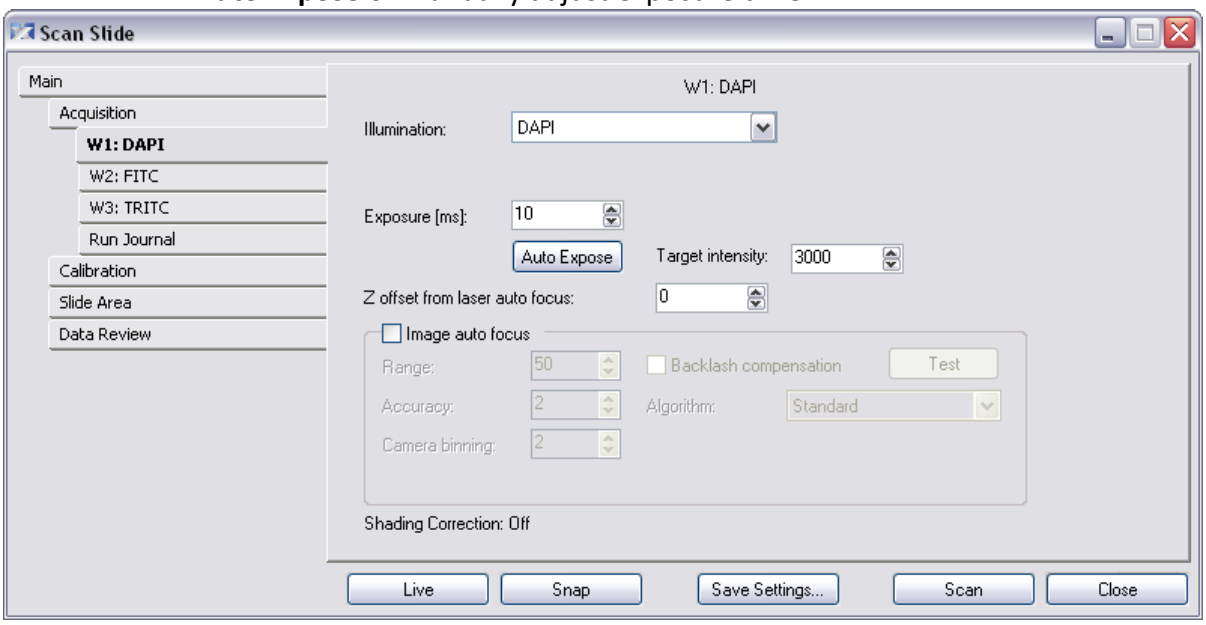


3. **Acquisition tab:**
    a. Set camera binning (1 or 2)
    b. Set number of wavelengths (3 for RGB color imaging)
    c. Set ***Laser Auto Focus*** to ***Each Stage Position*** if using settings for acquiring, set to ***Off*** if previewing slides
    d. If you want to apply shading correction, check the box and locate the directory. Shading correct should be ***based on magnification and illumination settings.***



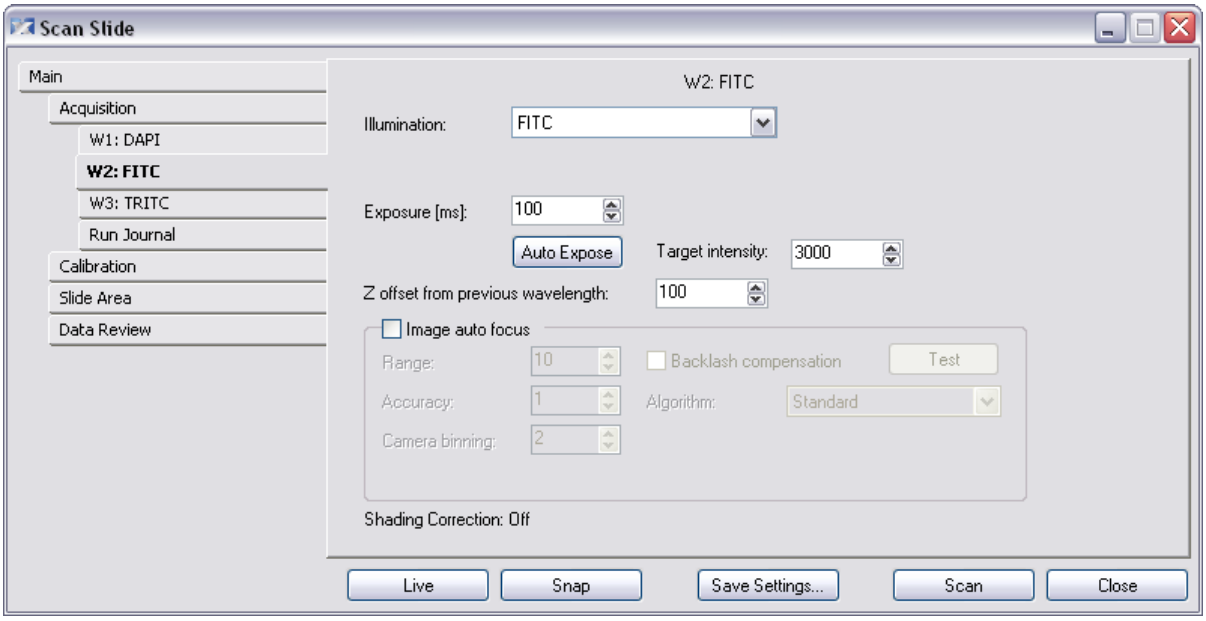
4. **W1 tab**:
    a. Select Illumination Setting
    b. Make sure you are in a suitable area of the slide:
        i. ***Recommended:*** Draw a region on the preview scan, and then click on ***Move to Region Center*** in the Data Review tab
        ii. ***Alternative:*** Use the ***Adjust Stage Position*** button on the taskbar and follow directions
    c. Click **Snap**.
    d. Click **Laser Autofocus on Slide** (take note of Z height in menu bar for checking offsets in later steps) on the taskbar to focus. If you have not previously defined your slide thickness in this section, you will be prompted to do so
    e. Make sure the image intensity is in a usable range (not saturating and not extremely dim). If necessary, press ***Auto Expose*** (make sure Target Intensity is set to 3000 for a standard IXM, 45000 for an IXM-XL) or manually adjust exposure time
    f. Determine offset from laser autofocus for W1 by clicking on **Find Z Offset** in taskbar and select ***Automatic: Image autofocus*** (take note of Z height and compare to Z height in step [d] above. If Z height does not reflect change, do step [i] below)
    g. If focus not optimal: click on **Laser Autofocus on Slide** and then click on **Find Z Offset** and select ***Interactive: Z-stack***
    h. Enter the resulting offset for W1 in Scan Slide.
    *i. Click on **Apply Z Offset** in the taskbar and enter the offset just calculated*

j. Click **Snap** again. Verify that the exposure time is suitable. If necessary, click **Auto Expose** or manually adjust exposure time

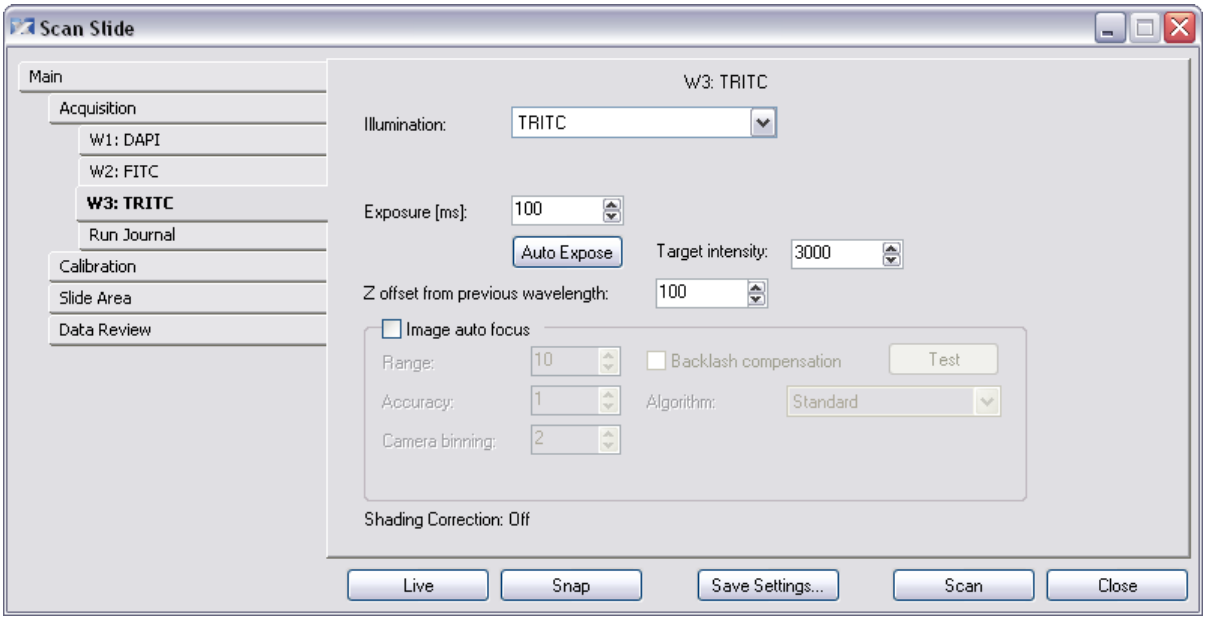


5. W2 tab:

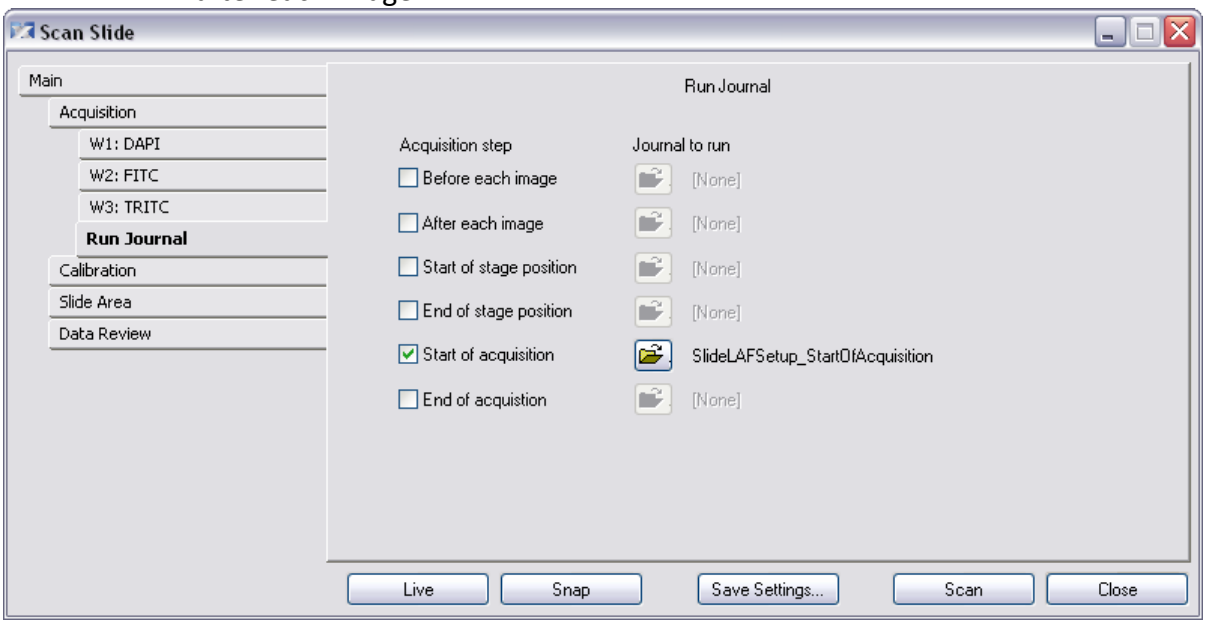
a. Select Illumination Setting. Click **Snap**
b. Make sure the image intensity is in a usable range
c. If you are not currently focused on W1, use **Laser Autofocus on Slide** and **Apply Z Offset**. Determine offset from W1 by clicking on ***Find Z Offset*** in taskbar and selecting the Automatic method.
d. If focus not optimal: repeat ***Find Z Offset*** and select Interactive to choose a plane from a Z-stack.
e. Enter the resulting offset for W2 in Scan Slide.
f. Click ***Snap*** again. Verify that the exposure time is suitable. If necessary, again press ***Auto Expose*** or manually adjust exposure time.



6. **W3 tab:**
    a. Select Illumination Setting. Click ***Snap***.
    b. Make sure the image intensity is in a usable range.
    c. If you are not currently focused on W2, use ***Laser Autofocus on Slide*** and ***Apply Z Offset***. Determine offset from W2 by clicking on ***Find Z Offset*** in taskbar and selecting the Automatic method.
    d. If focus not optimal: repeat ***Find Z Offset*** and select Interactive to choose a plane from a Z-stack.
    e. Enter the resulting offset for W3 in Scan Slide.
    f. Click ***Snap*** again. Verify that the exposure time is suitable. If necessary, again press ***Auto Expose*** or manually adjust exposure time.

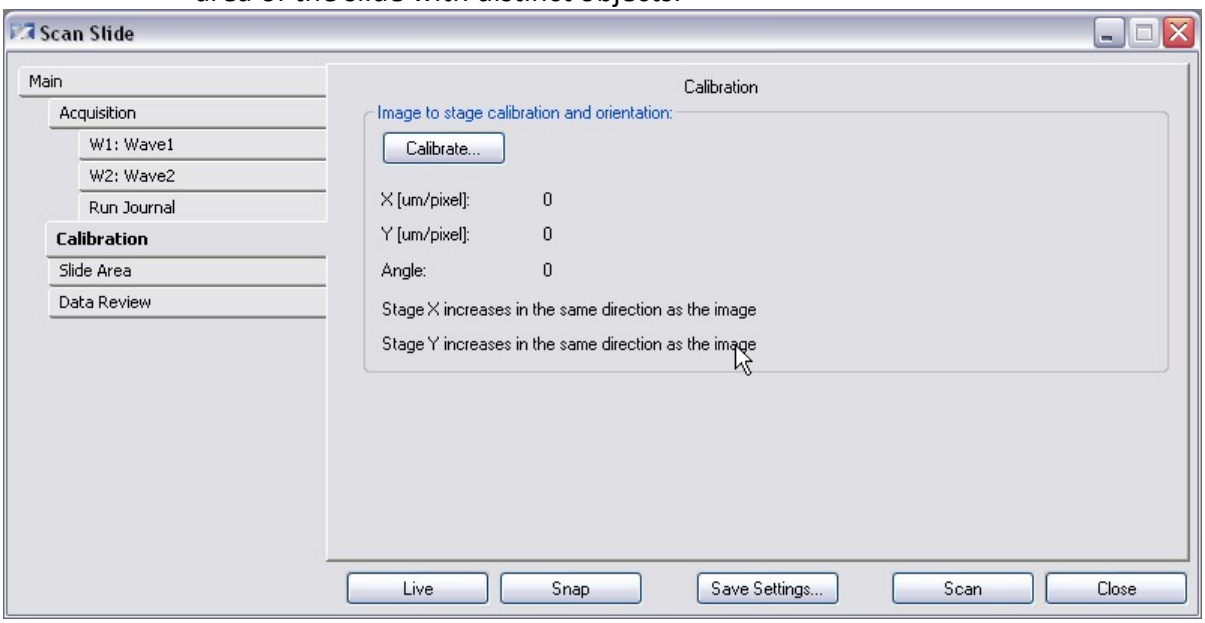


7. **Run Journal tab:**
    a. Make sure SlideLAFSetup_StartOfAcquisition is selected for the Start of Acquisition journal. Alternatively for a variable offset between the laser and W1, you can instead use the SlideLAFSetupWithAutoOffset_AfterEachImage.journal after each image.



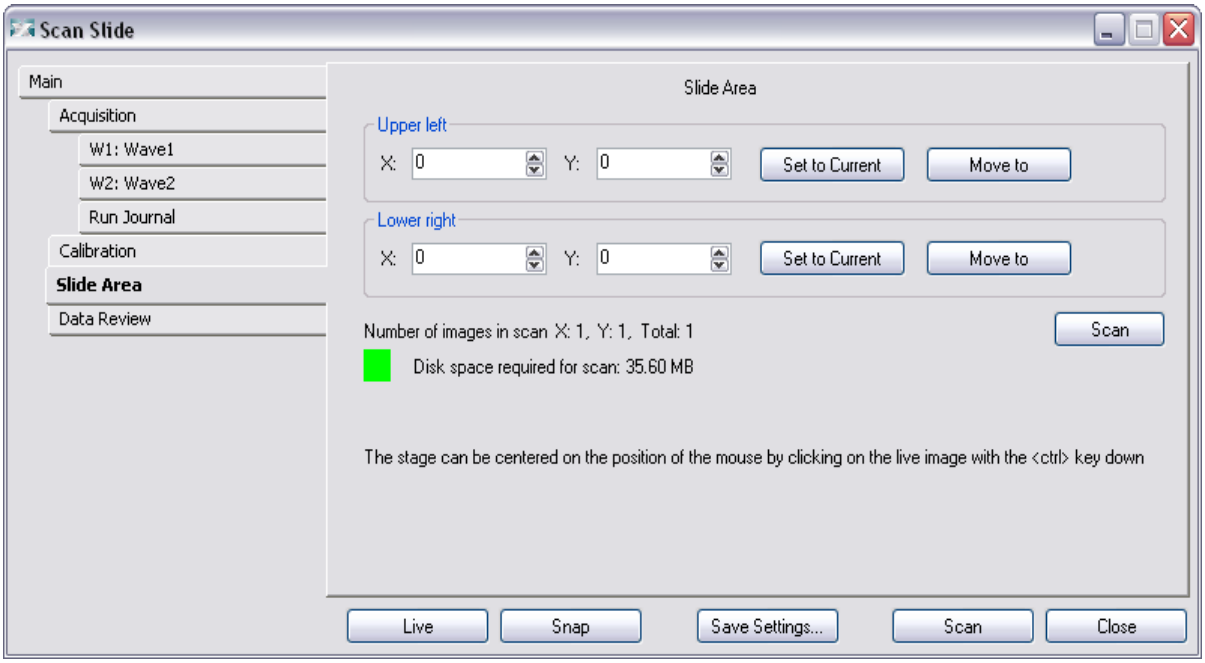
8. **Calibration tab:**

a. Make sure the X and Y calibration have been set (not 0). It is recommended to start with loading settings that already have the appropriate calibrations-see above.
b. If calibration settings are not available for your objective, set the binning to 1 and press Calibrate. Follow the steps to calibrate the stage. You should be in an area of the slide with distinct objects.



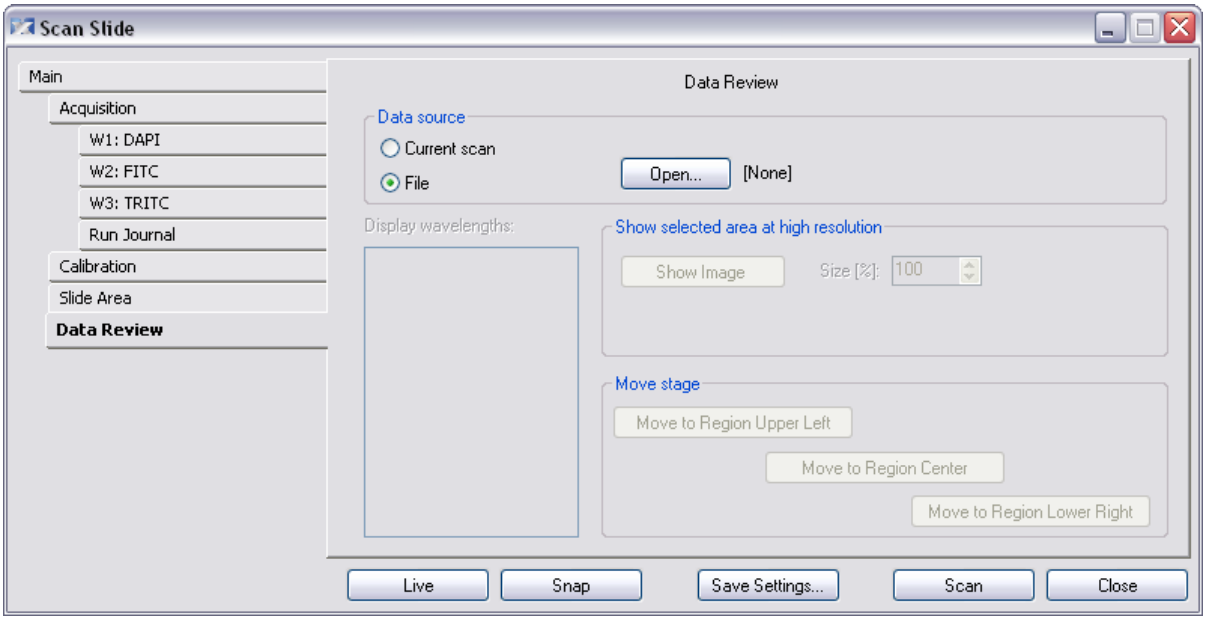
9. Slide Area tab:

a. If you are using the **Preview Slides** or **Select Regions for Scanning** tools, the slide area will automatically be set
b. If not, use the **Adjust Stage Position** tool and click on **Set to Current** for the Upper Left and Lower Right corners.



10. Data Review tab:

a. Nothing needs to be set here. This tab is used to view images after they are acquired.



SECTION B: Preview Slides (low-magnification whole-slide scanning to find objects of interest)

1. Click on **Preview Slides** on the taskbar. You do not need to have the slides loaded yet.
2. Select the slide positions you will be using.
3. Select the objective for the preview scan from the list (typically 2x or 4x). Click **Continue**.
4. Set the slide orientation / thickness.
5. You will be prompted to load in base settings for your preview scan.
6. Verify:
    a. Acquisition tab:
        i. Camera binning
        ii. Number of wavelengths (typically 1)
        iii. Laser autofocus settings (typically off)
    b. Wavelength tabs:
        i. Illumination settings
        ii. Exposure times
        iii. Z offsets
    c. Run Journal tab:
        i. SlideLAFSetup_StartOfAcquisition is selected for Start of Acquisition
    d. Calibration tab:
        i. Calibrations are present and correct for this objective. Settings with calibrations for most objectives are included in the taskbar (IXM_ScanSlide_Calibrations.scansetting OR IXM-XL_ScanSlide_Calibrations.scansetting, placed in the Scan Slide Setting folder).
7. Click **Close** the Scan Slide window.
8. You will be prompted to select a directory for image storage, then to enter a base name for the slide.
9. Now you will draw a region on the slide template image to select the area on the slide you want to cover. (This is why you don't load the slides yet). If no region is drawn, the entire slide will be imaged. This will usually include the extreme ends of the slide where it is sitting on the holder.

 **Region tools should be available in the toolbar. If they are not visible, right click on the toolbar, and select Region tools**

10. The door will now open. Place slide holder into system with A1 facing the labeled corner.
11. Click ***Continue*** for the system to proceed with scanning. Images will be saved as you have specified.

SECTION C: Select Regions for Scanning: high-mag scans

1. Make sure that the slides are loaded in the system with the same orientation and position used for the preview scan.
2. Select the slide positions you will be using.
3. Click **Yes** to let the system automatically name scans, **No** to manually name each region on each slide.
4. Click **Yes** to adjust wavelength settings separately for each region, **No** to use the same settings for all regions.
5. Select the magnification for high-mag scanning.
6. Set the slide orientation / slide thickness.
7. You will be prompted to browse and select the preview scan for the slide you are using.
8. Select one of the Scan (thumbnail) images (e.g. "DAPI Scan").
9. ***OPTIONAL***: When prompted, draw a region on one of the scan images for stitching and overlaying to review preview results. Click **Continue** and select a zoom percent.
10. The region you selected will be stitched for your review. If you have RGB images a color overlay will be created for your review.
11. When prompted, draw regions around areas of interest on the low mag Scan image. Press **Continue**.
12. You will be prompted to load in your settings for each slide. Select the appropriate settings. Exposure times and focus offsets should already have been optimized.
13. Select a directory for storing the data and a base name for the slide.
14. If you chose manual naming of scans, you will be prompted to enter a name for each region.
15. If you chose to adjust settings for each region, the Scan Slide window will open now and the system will go to the center of the region and autofocus on it. Verify wavelength settings and close the window.
16. The system will set up the scan for each region. Press **Continue** to automatically scan all regions.

SECTION D: Reviewing Scan Slide data

1. Go to **Scan Slide** and select the **Data Review** tab.
2. If the scan of interest is open, leave **Data source** set to **Current scan**. Otherwise, set **Data Source** to **File**.
3. Click on **Open** and browse to find the scan of interest.
4. Use the region tools to draw a region around an area to stitch on the preview images. Without any regions present the entire area will be stitched.
5. Click on **Show Image**. If this button is greyed out and a red warning is showing, the ***Size (%)*** setting needs to be reduced until the **Show Image** button is available and the warning disappears. This is because of a memory limitation on the computer being used.
6. The images will now be automatically stitched for each wavelength.
    7. Click on **Overlay Images** (Click on **Main Menu → Analyze Images → Overlay Images)** in taskbar to see the resulting color image.