MetaXpress® 6 Software Guide

Setting Up Microscope Slides with Regions

Date Revised 07/14/15 Version B

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Chapter Purpose

The purpose of this chapter is to guide the user in setting up and acquiring microscope slide(s) with irregularly spaced features using the **IXM Taskbar.**

Microscope slides that have irregularly spaced features (i.e. tissues, TMA, etc.) are best acquired through this method.

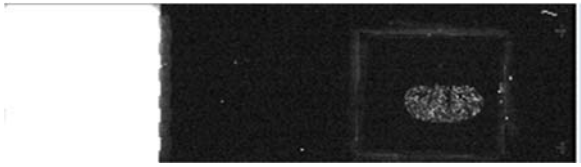
For regularly spaced features (chambers, multiple cover slips.), refer to the chapter on **Setting up Microscope Slides as a Micro-well Plate.**

Slide Scanning: Two Workflows

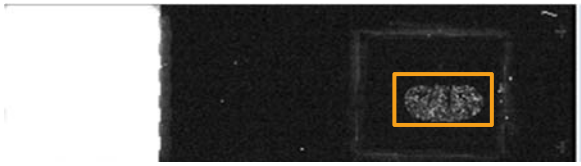
Slide Features Are Irregularly Spaced

(e.g. tissue slice or TMA)

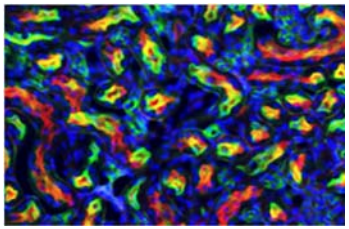
Perform Preview Scan
(low-magnification scan)



Create Scan Areas
(select regions for high-resolution scanning)

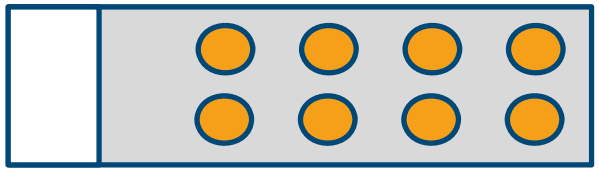


Acquire Slide

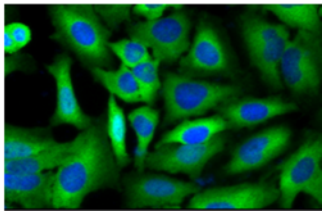


Slide Features Are Regularly Spaced

Set up Slide Dimensions
(create plate file)

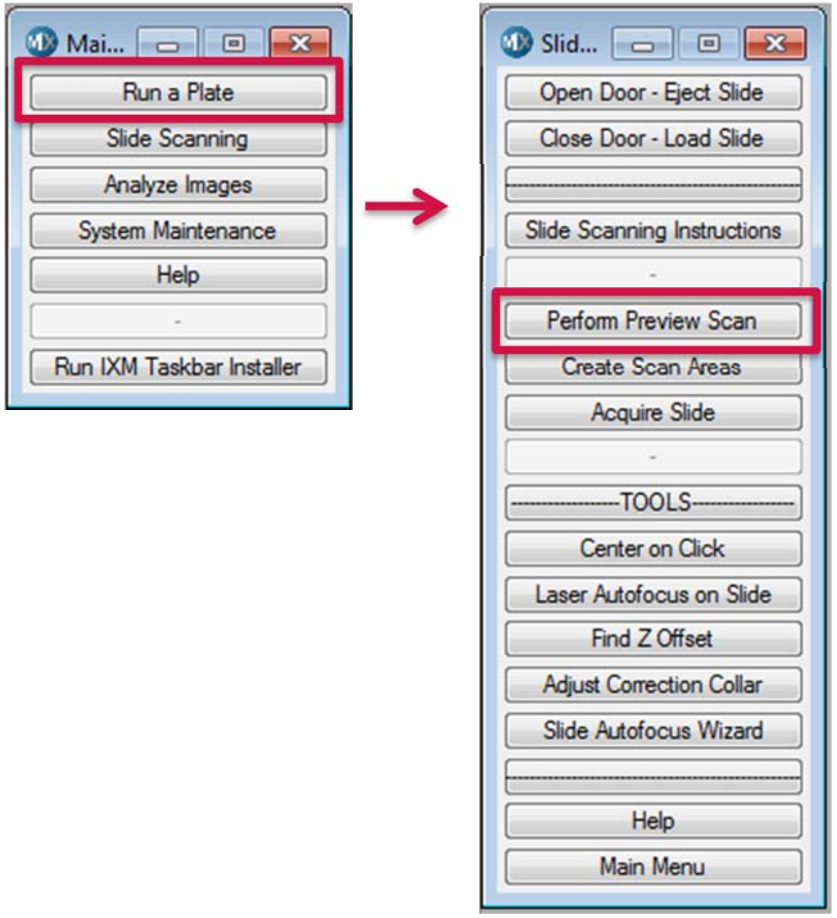


Acquire Plate



Setting Up Microscope Slides as a Plate

In preparation for microscope slide scanning, Molecular Devices recommends setting the correction collar of corresponding objectives according to the orientation of the slide as will be selected in this procedure. For example, if imaging through a standard microscope slide, set the correction collar to 1. If imaging through a standard coverslip, set to 0.17

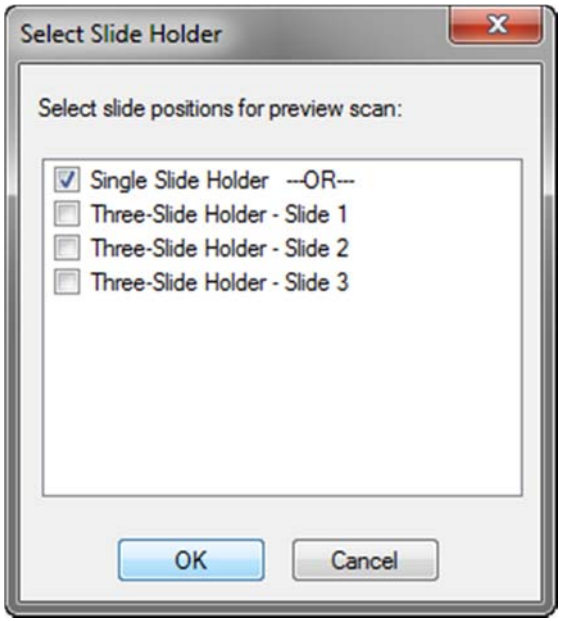


1. In the MetaXpress software, ensure that the **IXM Taskbar,** click on **Run a Plate**

2. Click on **Perform Preview Scan**

Setting Up Microscope Slides as a Plate

3. Select the appropriate **Slide Holder** type in the list.



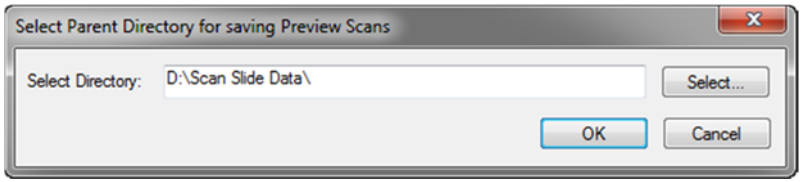
Single Slide Holder

3-Slide Holder

4. If using a 3-Slide holder, select all the positions that will have a slide.

5. Click **OK**

6. Select the directory to save the images from the preview scan and click **OK**. Ensure that the drive has enough space.



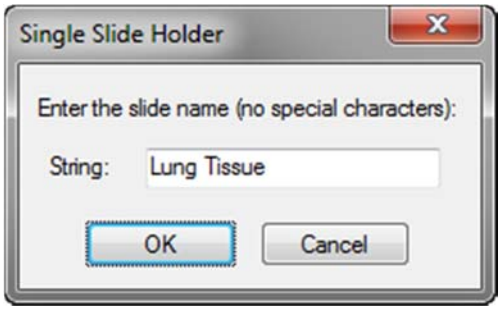
Setting Up Microscope Slides as a Plate

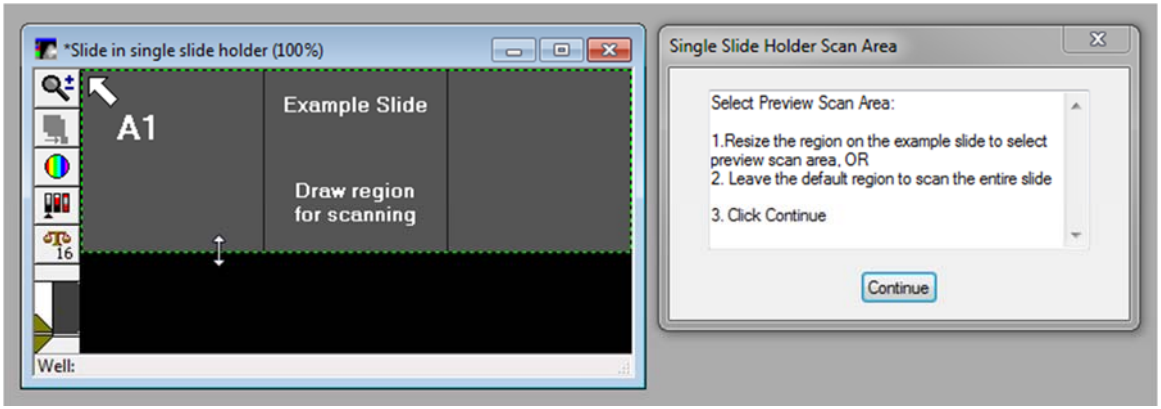
7. Enter a name for the slide and click **OK**

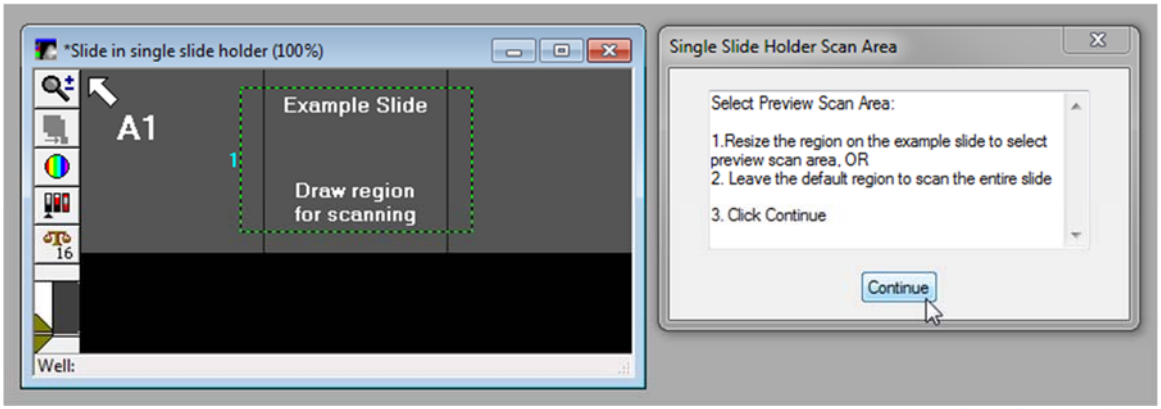
8. Select the preview area on the illustration of the slide by adjusting the region. Leave the region at the default position to acquire the full slide.

9. Click **Continue**

10. If using a 3-Slide holder, repeat for each slide



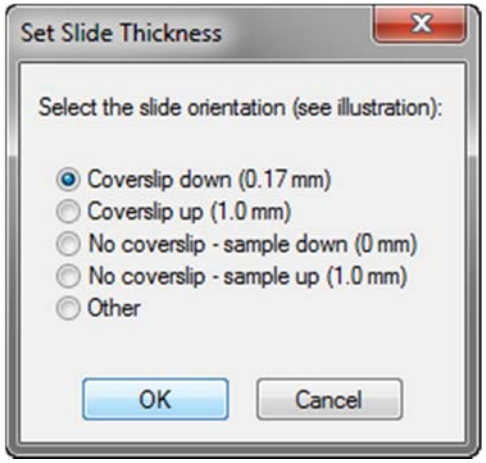


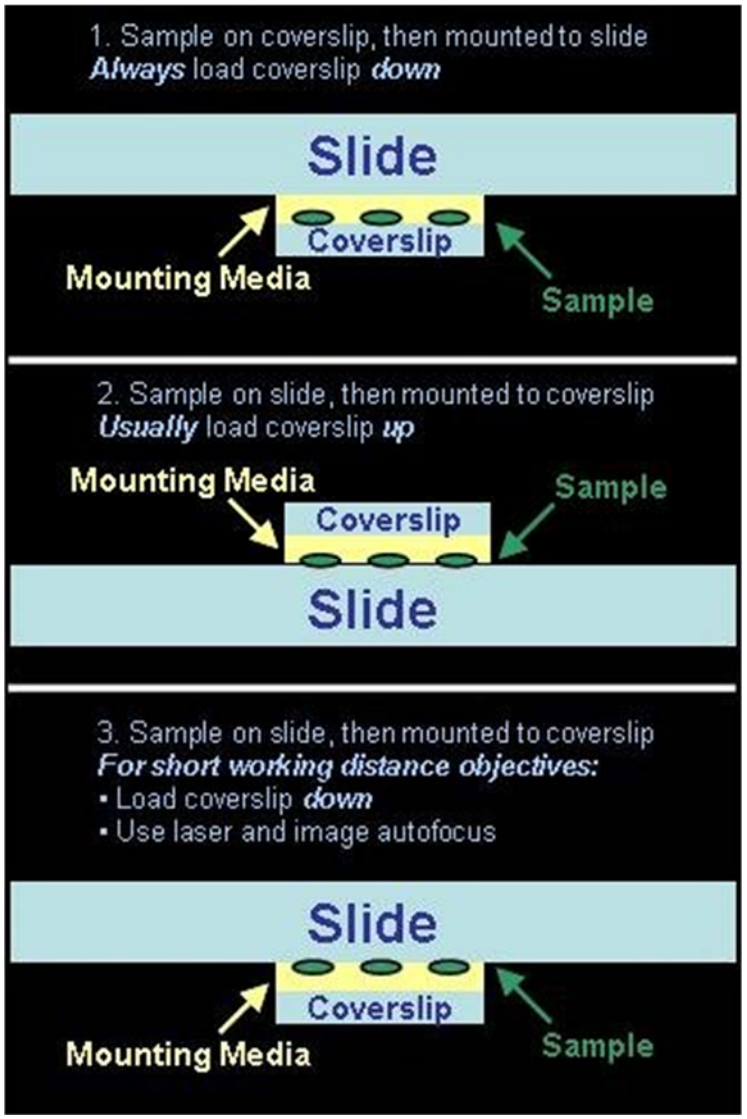


Setting Up Microscope Slides as a Plate

11. Select the appropriate **Slide Thickness** and click **OK**. Refer to the illustration for guidance.

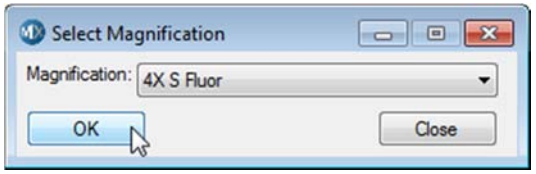
12. When prompted, load the microscope slide(s) in the slide holder into the system



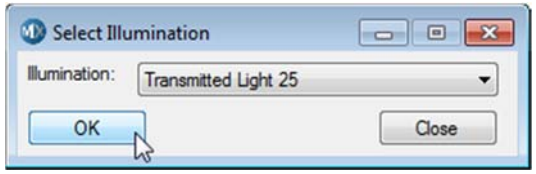


Setting Up Microscope Slides as a Plate

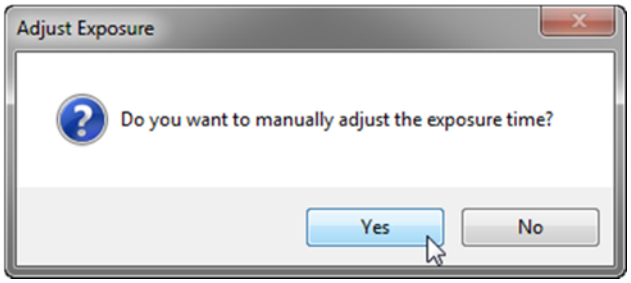
13. Select the desired (2x or 4x is recommended) magnification from the drop-down list and click **OK**



14. Select the desired illumination (brightfield or DAPI is recommended) from the drop-down menu and click **OK**



15. Review the image and select **Yes** to adjust exposure time manually, select **No** to continue

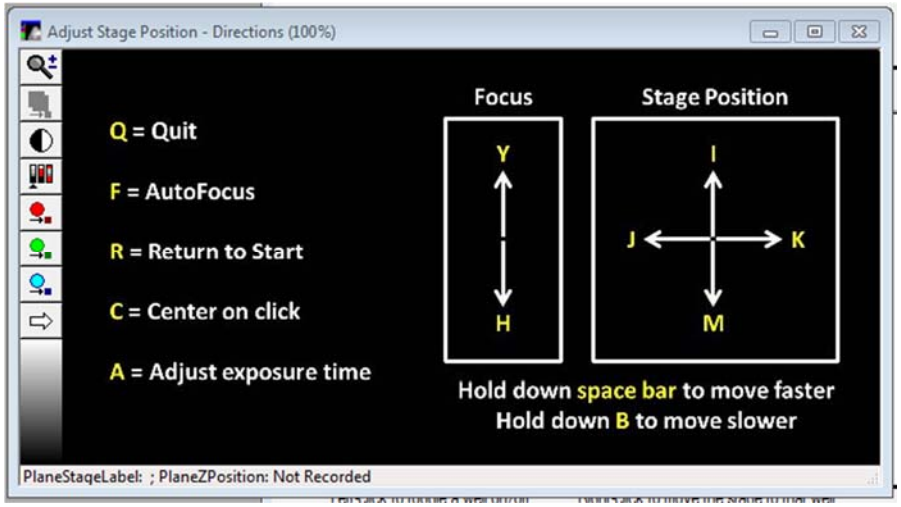


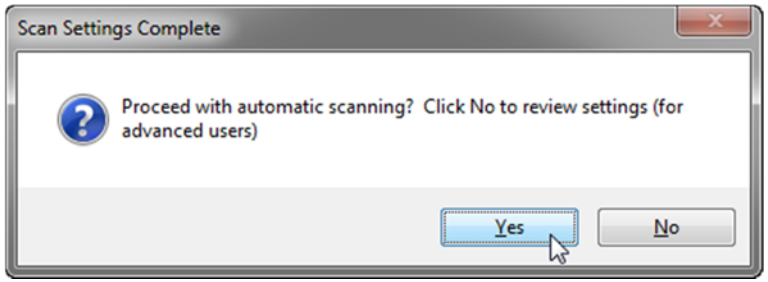
Setting Up Microscope Slides as a Plate

16. If **Yes** was selected, use the keyboard shortcuts as displayed in the illustration to adjust exposure and position

17. When done, press the **Q** button on the keyboard

18. Click **Yes** to continue with the scan automatically, click **No** to return to adjusting the settings

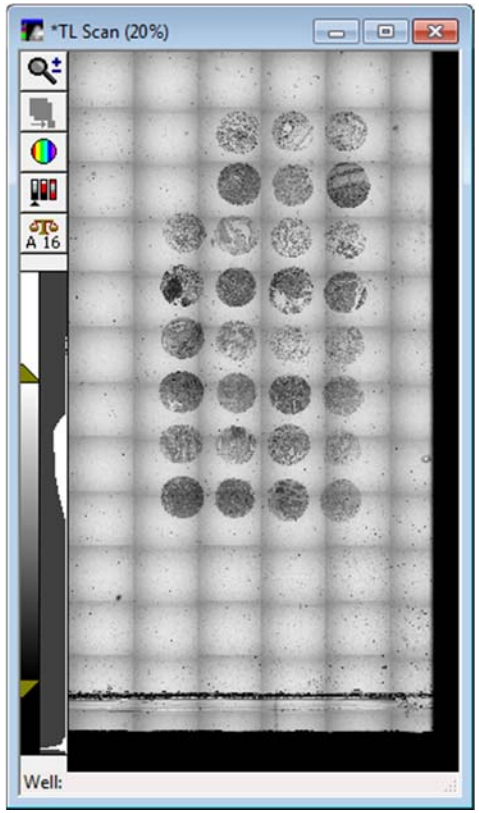


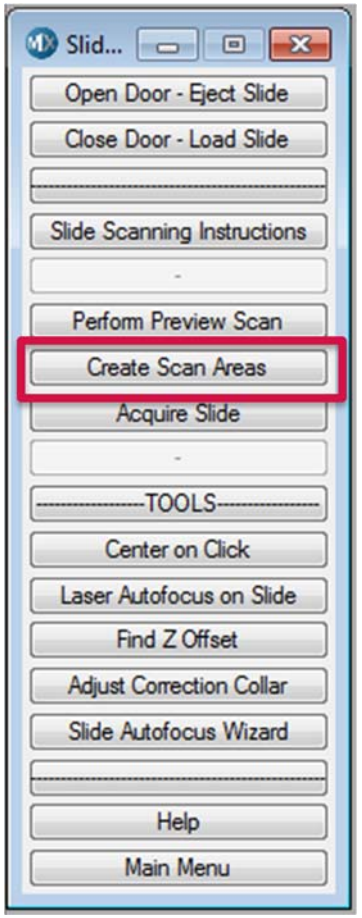


Setting Up Microscope Slides as a Plate

19. Once the preview scan has completed, proceed to defining the scan areas for higher magnification

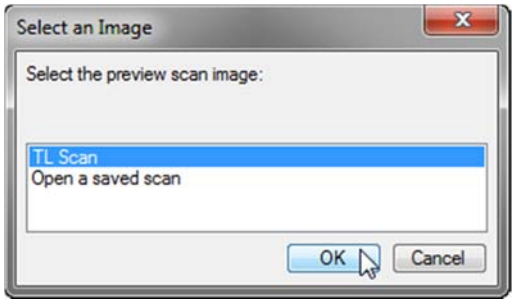
20. On the **IXM Toolbar**, click on **Slide Scanning > Create Scan Areas**



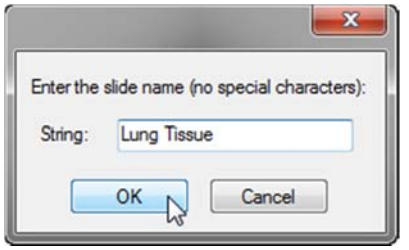


Setting Up Microscope Slides as a Plate

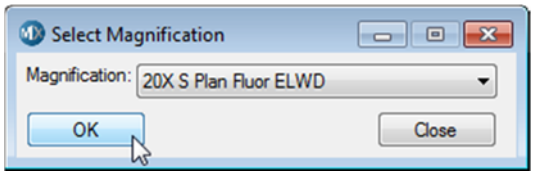
21. If the preview scan is already open (**Control > Acquire > Scan Slide),** select the name of the preview scan. Otherwise, select **Open a saved scan** and click **OK.** These files will have a .scan extension.



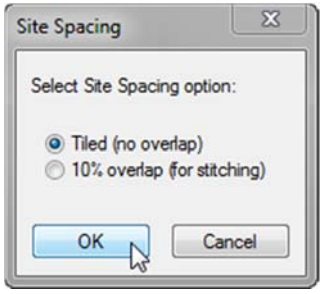
22. Enter and confirm the name of the scan. Click **OK**



23. Select the desire high magnification objective from the drop-down menu and click **OK**



24. Select the appropriate site spacing:
    - **Tiled** (no overlap): can be montaged
    - **10% Overlap**: overlap is required for stitching

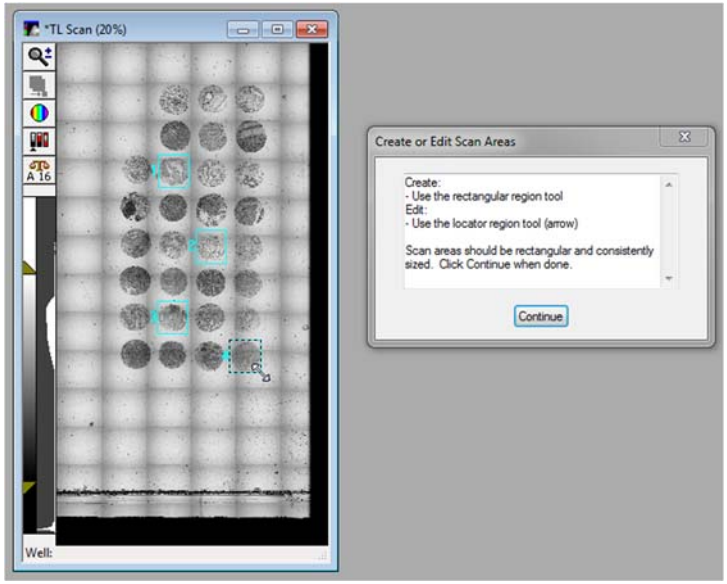


Setting Up Microscope Slides as a Plate

25. Draw **Regions** of interest (scan areas) or load saved regions onto the preview scan

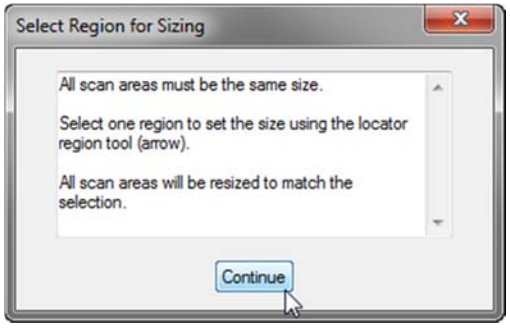
26. Each region represents one well (used as dimensions and location in **Review Plate Data**)

27. Regions should be rectangular Click **Continue** when you have completed drawing regions.



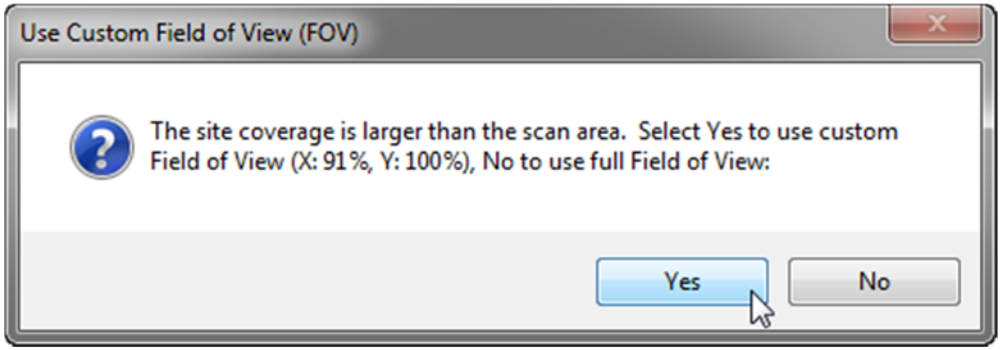
NOTE Use rectangle to create regions, arrows to resize/move.

28. All regions need to be the same size. Select a region (indicated by the active-clicking) that represents the desired size and click **Continue**

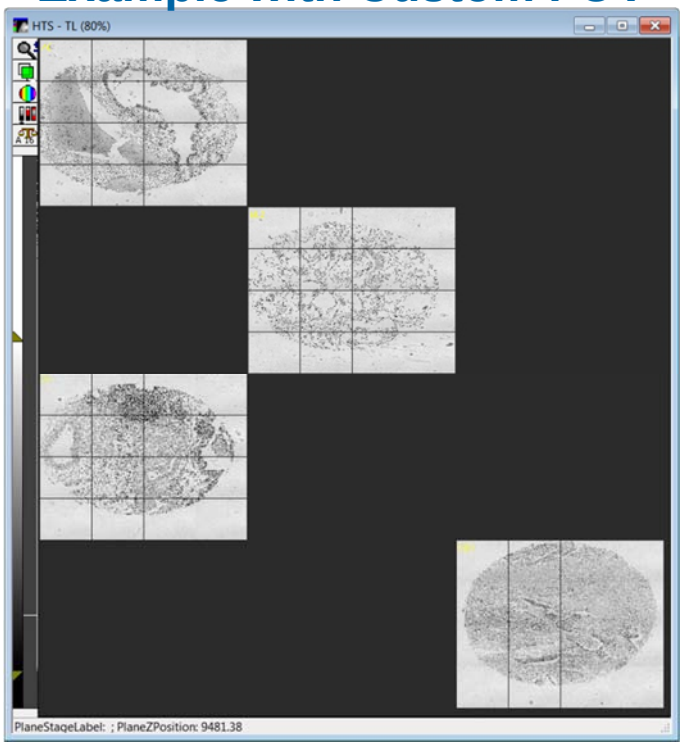


Setting Up Microscope Slides as a Plate

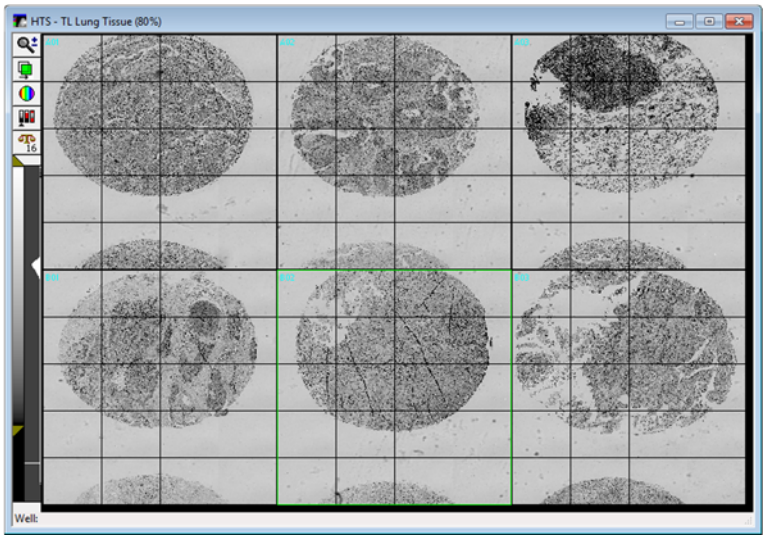
29. If the selected scan area does not correspond to a whole number of sites, select **Yes** to use a smaller field of view or **No** to image extra area



Example with Custom FOV



Example with Full FOV



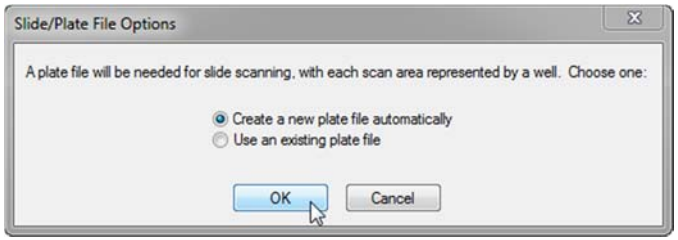
Setting Up Microscope Slides as a Plate

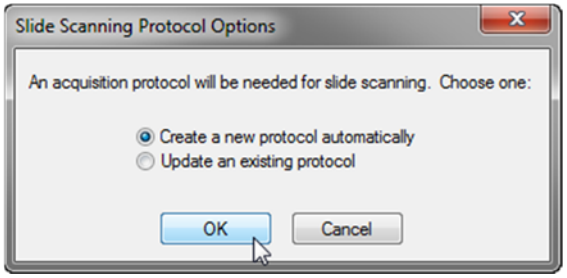
30. The number of images and other settings will now be calculated

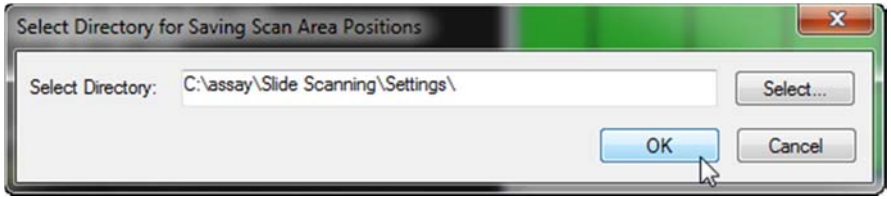
31. Select the appropriate **Plate File** option

32. Select the appropriate **Protocol** (acquisition settings) option

33. Select the directory of where to save

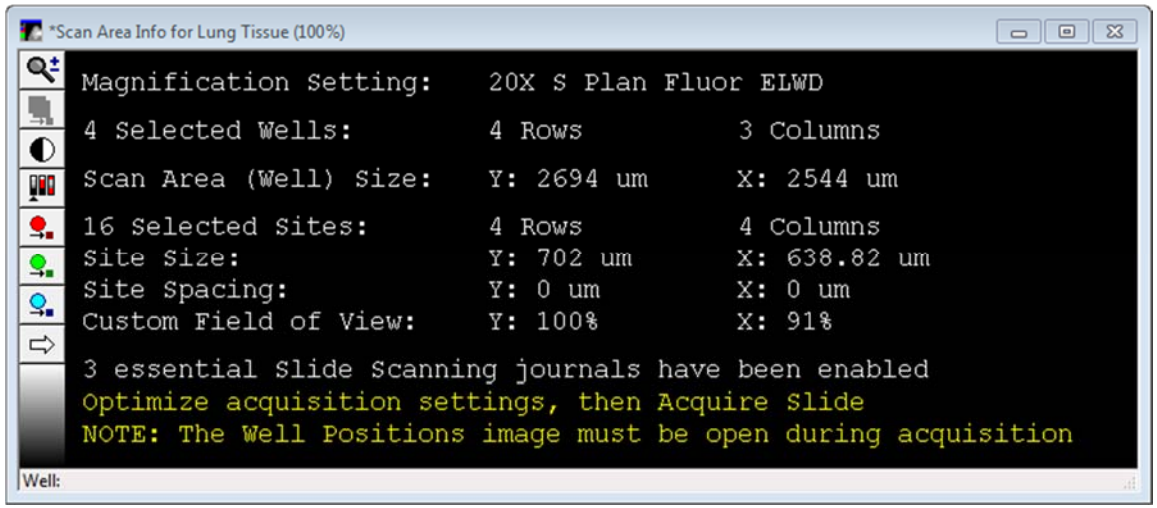




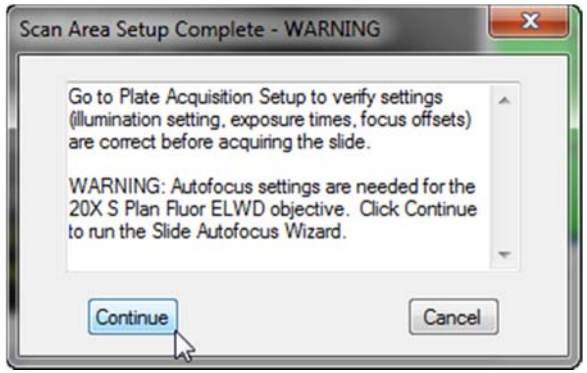


Setting Up Microscope Slides as a Plate

34. A summary of the scan area calculations is displayed

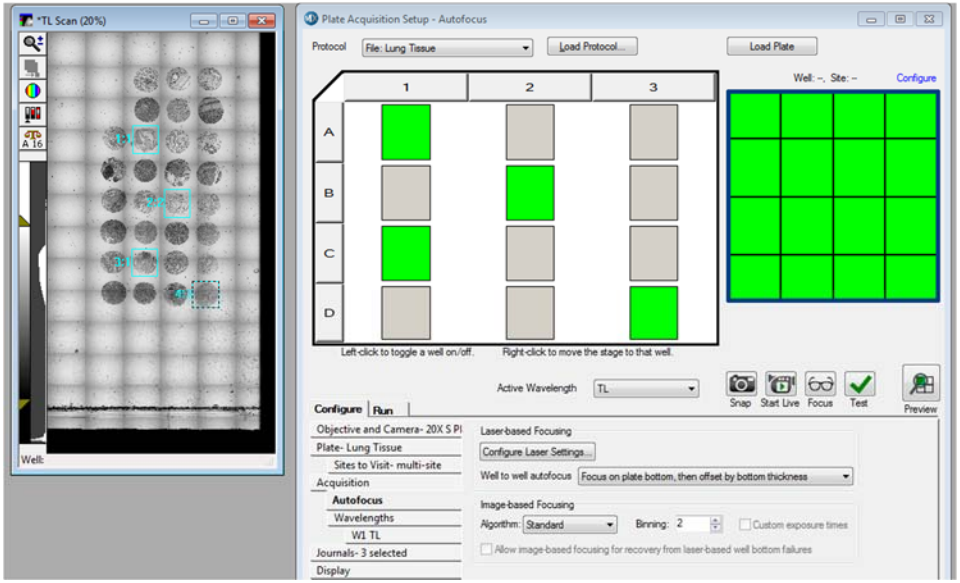


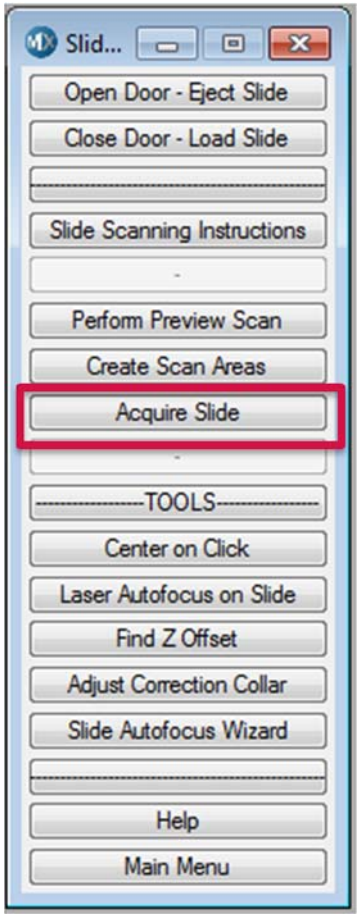
35. If laser autofocus settings are needed, click **Continue** to proceed through the **Slide Autofocus Wizard**



Setting Up Microscope Slides as a Plate

36. Review and optimize settings in **Plate Acquisition Setup** as you would normally for a micro-well plate including:
    - Description
    - Wavelengths
    - Exposure times
    - Z Offsets
    - Z-stack options





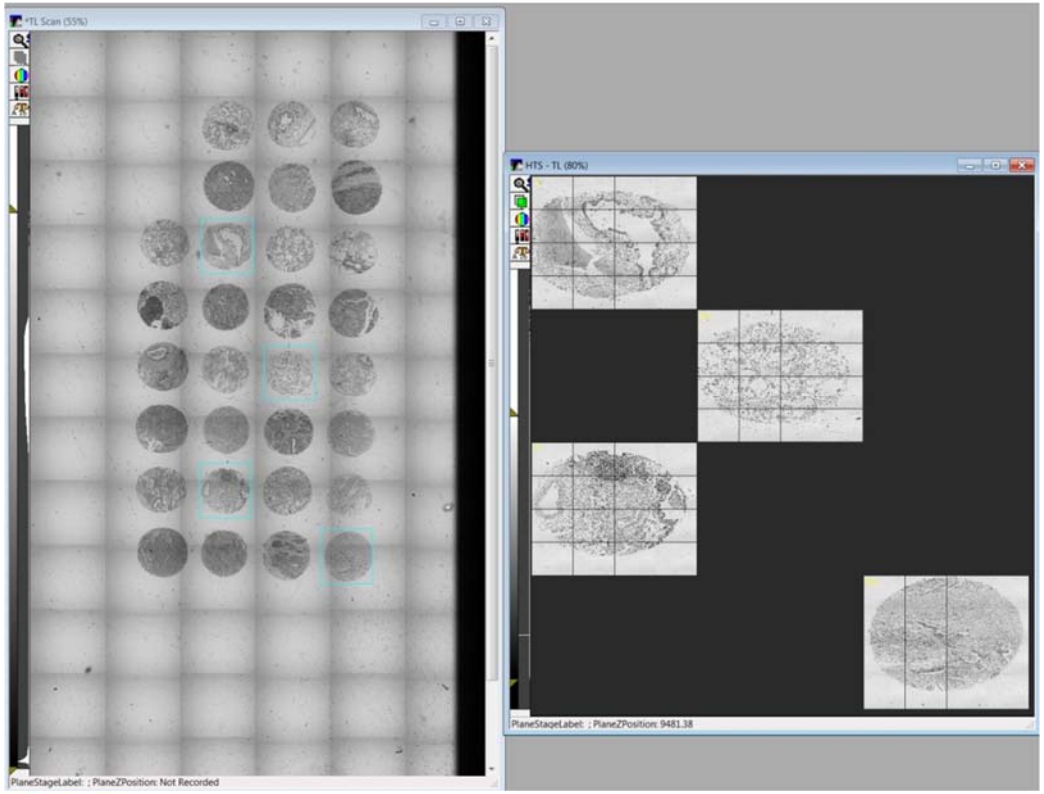
37. Acquire the slide by clicking on the **Acquire Plate** button in the **Plate Acquisition Setup** dialog or on the **IXM Taskbar**

Setting Up Microscope Slides as a Plate

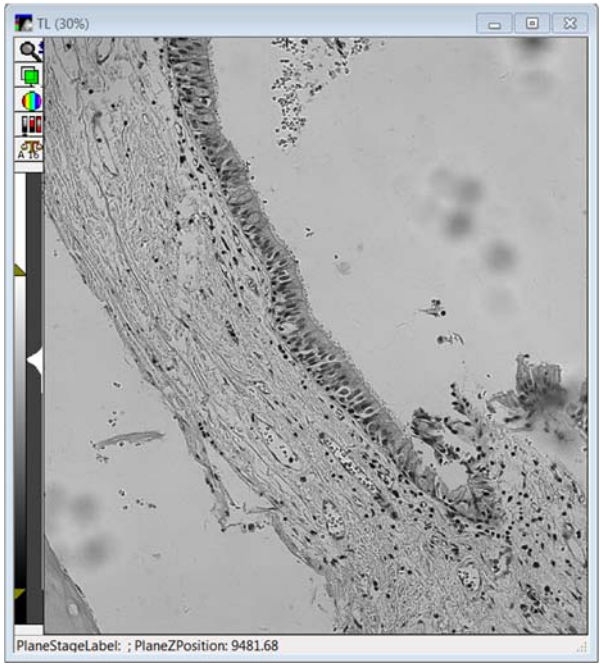
38. In **Review Plate Data**, each well corresponds to one scan area (region)

39. Well layout approximately corresponds to position in the preview scan

Preview Scan **HTS Montage**



Full-Resolution Image



Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments

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