

ScanScope FL Scanning Guide

Before you start: Check your slides on your microscope to ensure the staining worked. It can save time to identify a small area of tissue with good staining and features of interest to use for setting scan parameters.

Starting the system:

1. Turn on the system in the following order:



1. FL light source

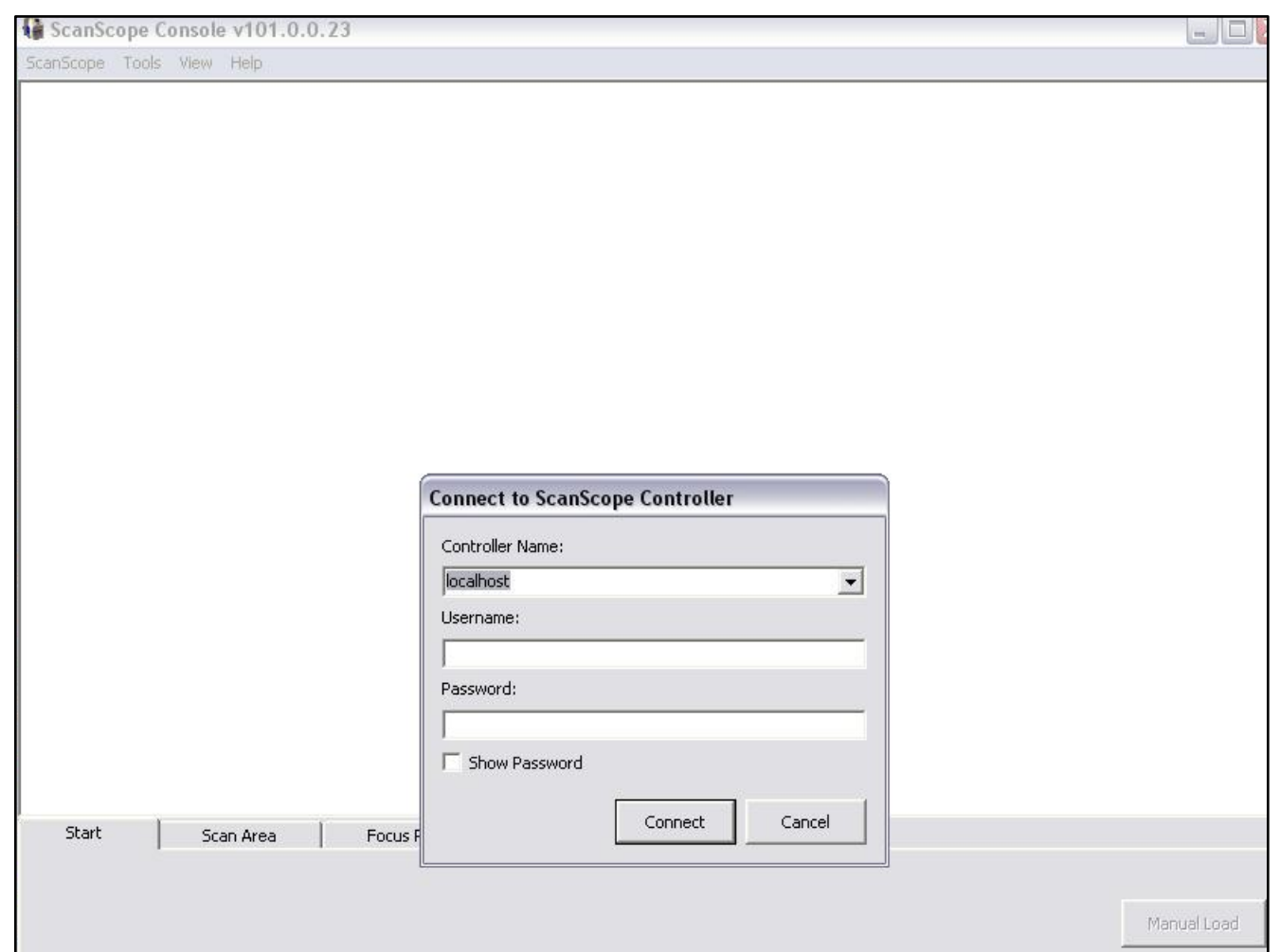
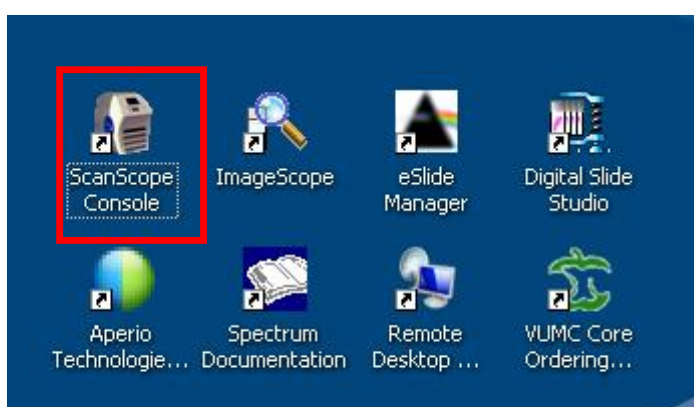


2. ScanScope



3. PC

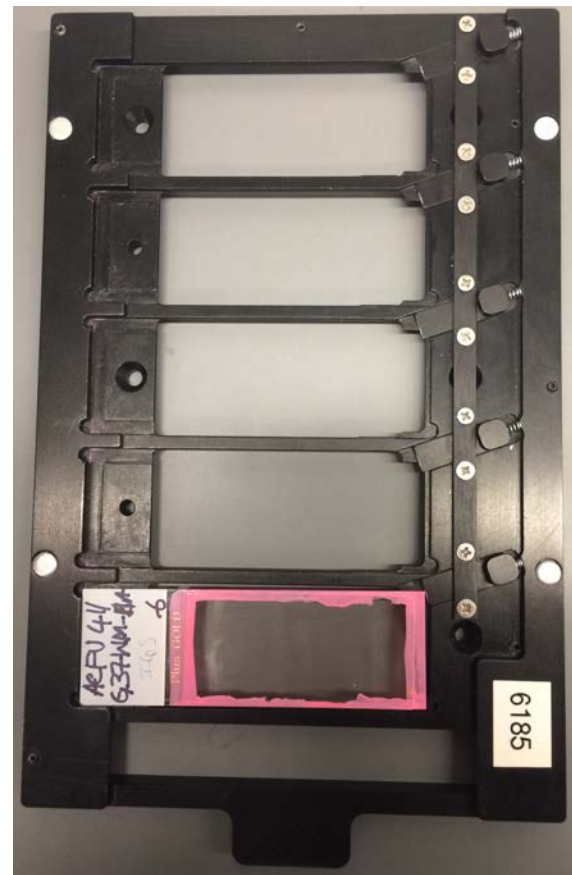
2. If you are the first user of the day, login to PC using password: **Sc@nscope**
3. Wait for ScanScope LCD to read: **ScanScope Ready**
4. Start the ScanScope Console program by double clicking the icon on the desktop and logging in with your username and password.



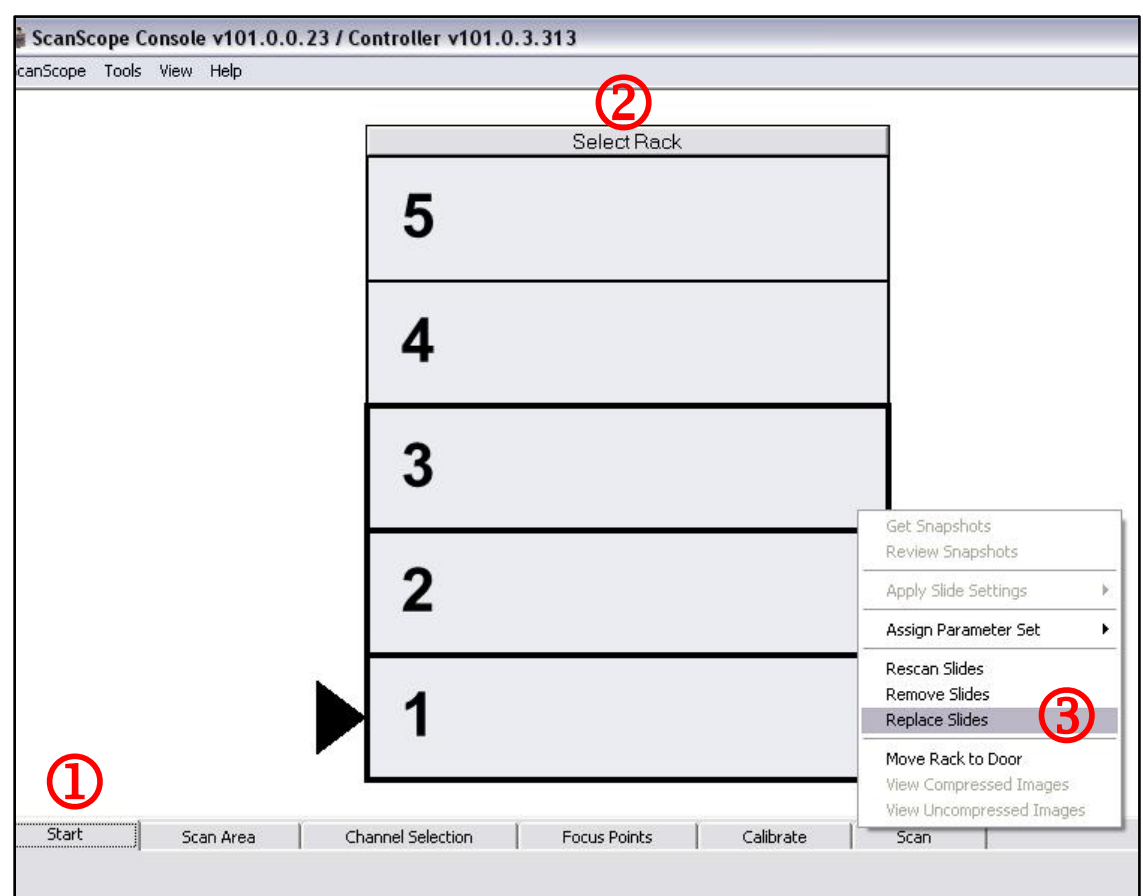
I. Loading slides

Slides must be completely sealed, dry, free of any mounting medium, and polished before loading into ScanScope.

1. Carefully remove slide tray from scanner.
2. Load slides into slide tray from bottom to top, with the coverslip facing up and the label oriented to the left.
2. Re-insert slide tray into scanner, ensuring it is seated properly.

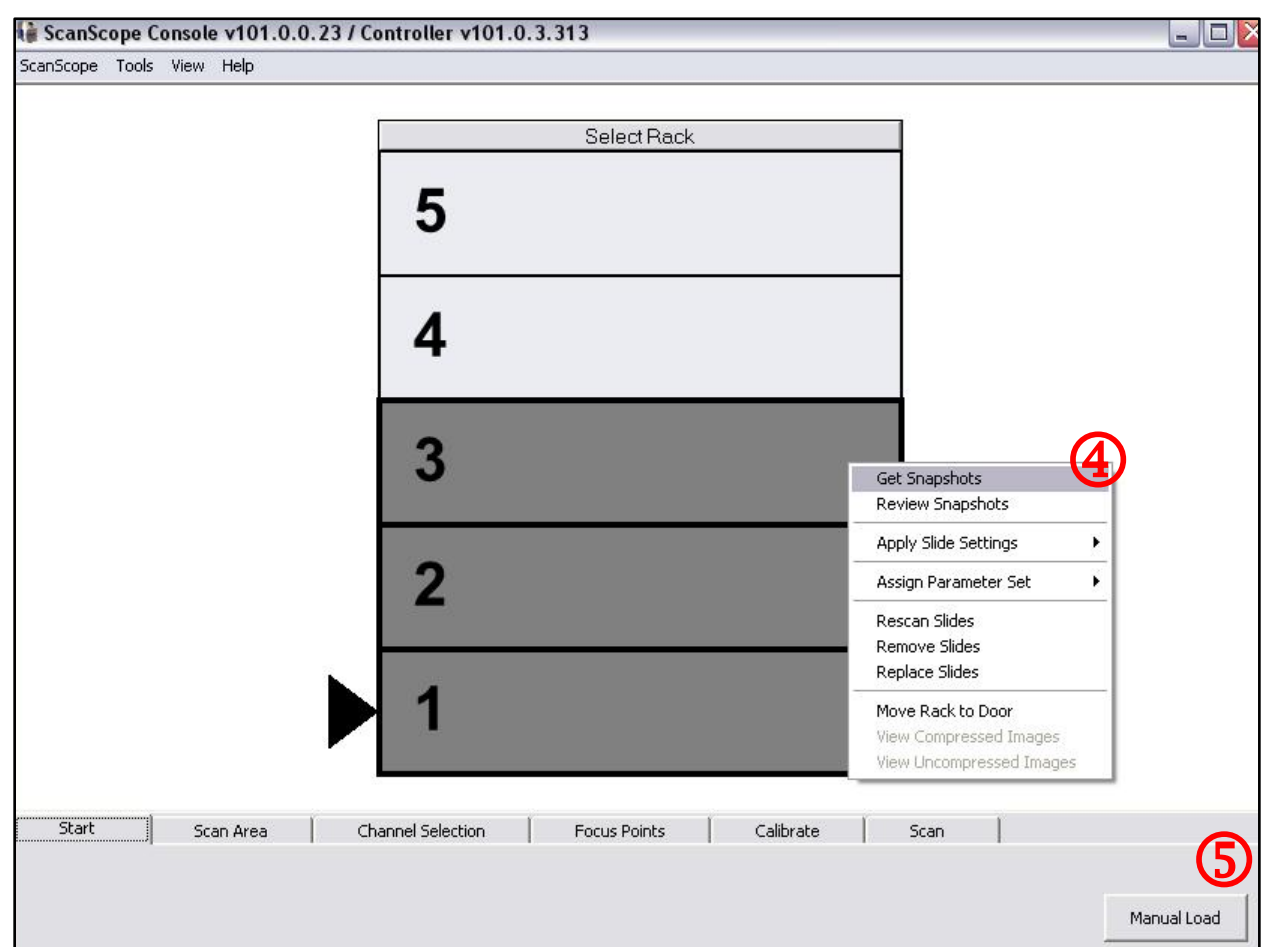


4. In ScanScope Console program, go to **Start tab** ①. Highlight slides by clicking on position 1. While holding the Ctrl key, click on all positions containing a slide or click **Select Rack** ② to highlight every position. Positions containing slides should be highlighted with a **bold** box.



5. Right click on rack diagram and select **Replace Slides** ③.

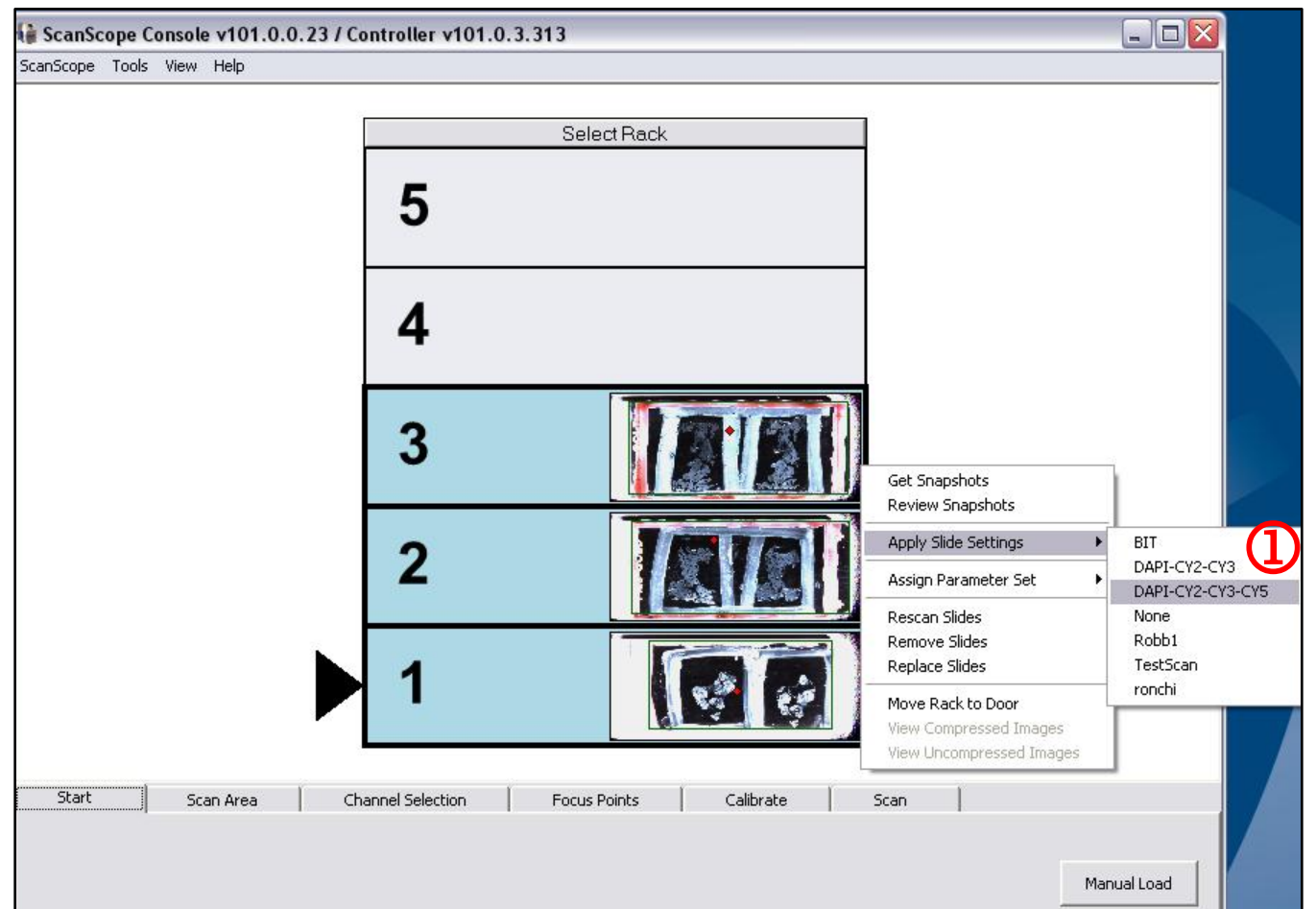
6. Right click on rack diagram and select **Get Snapshots** ④. The scanner will load an image of each slide.



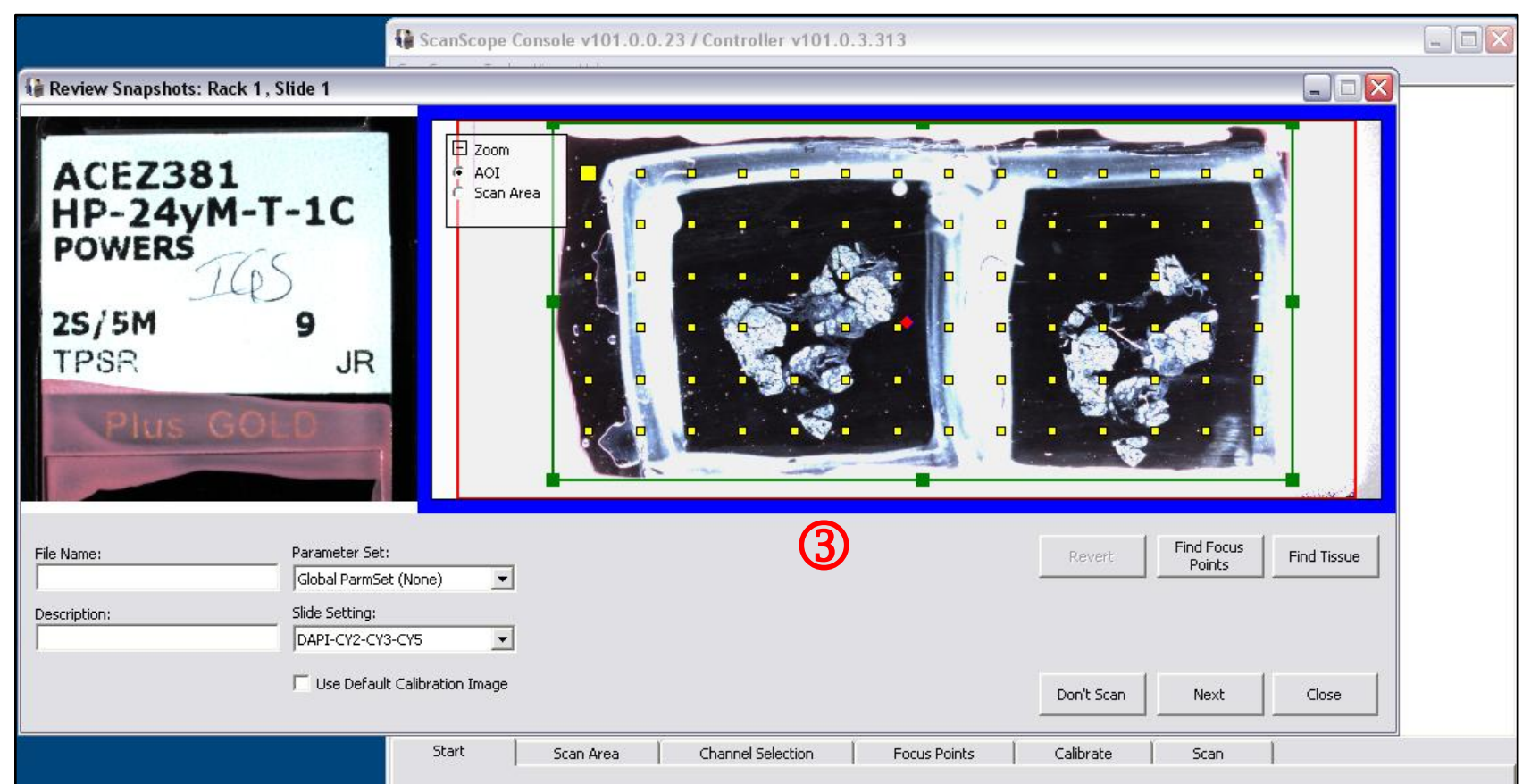
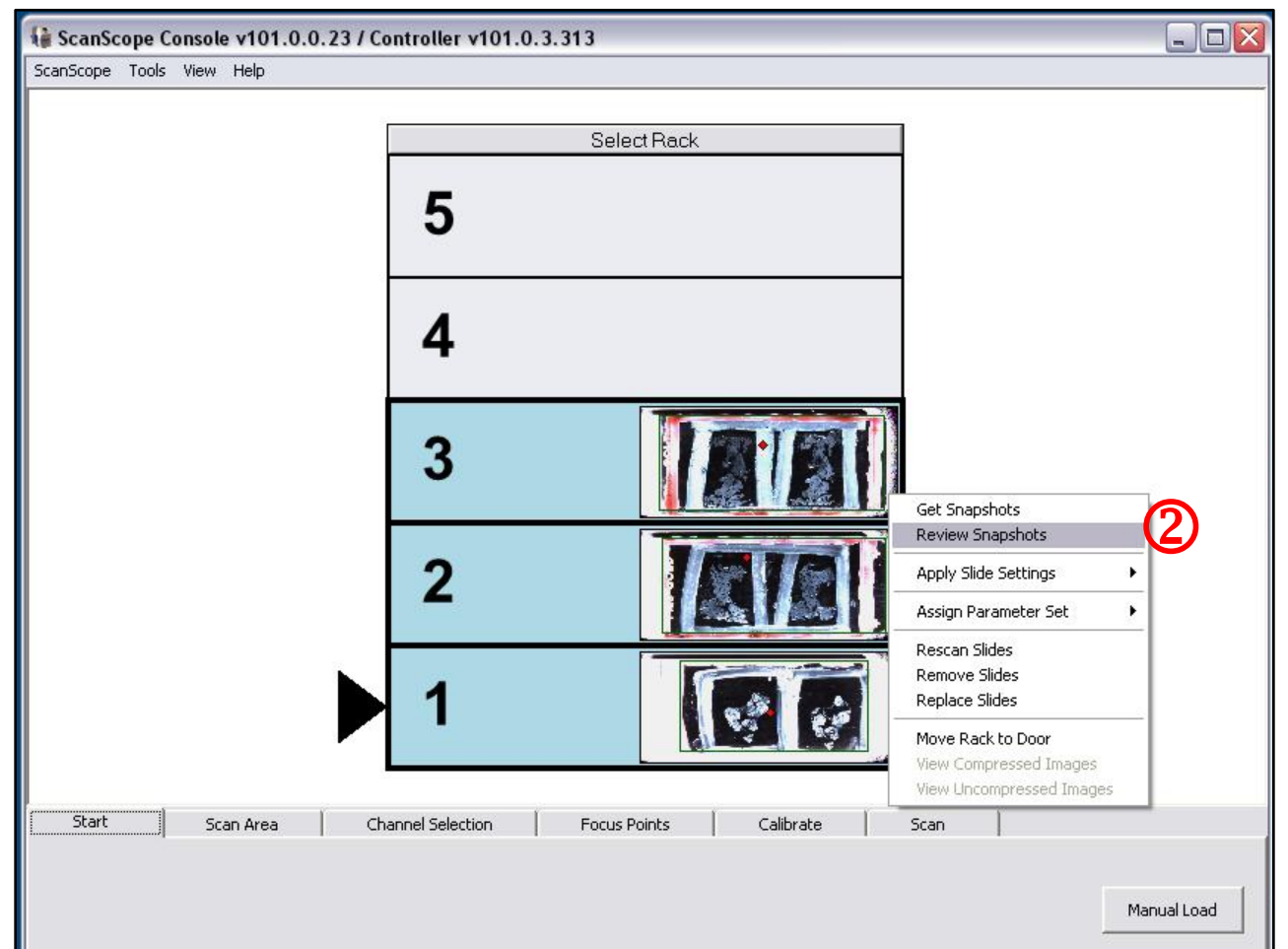
7. Review slide images. If dark field images of slides show smears or debris, click on **Manual Load** ⑤. The stage will move to the front of the scanner; remove rack, clean slides, and re-insert rack. Repeat **Get Snapshots**.

II. Setting Up Scan Parameters and Reviewing Snapshots

1. Right click on slide in position 1, choose **Apply Slide Settings** and select the applicable fluorophore combination saved in the system ①.

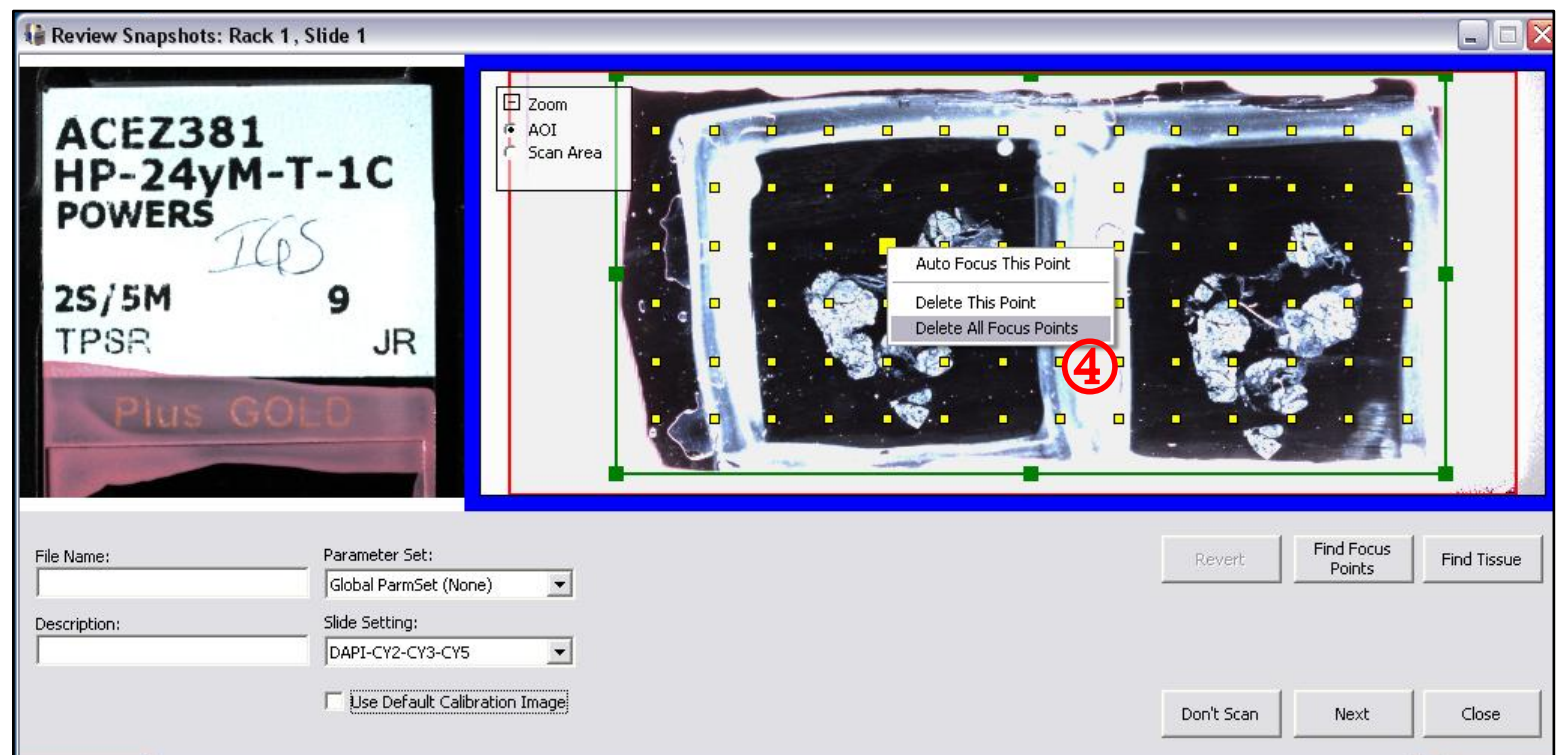


2. Right click on slide in position 1 and choose **Review Snapshots** ②. A window will open in which the entire slide is visible ③.



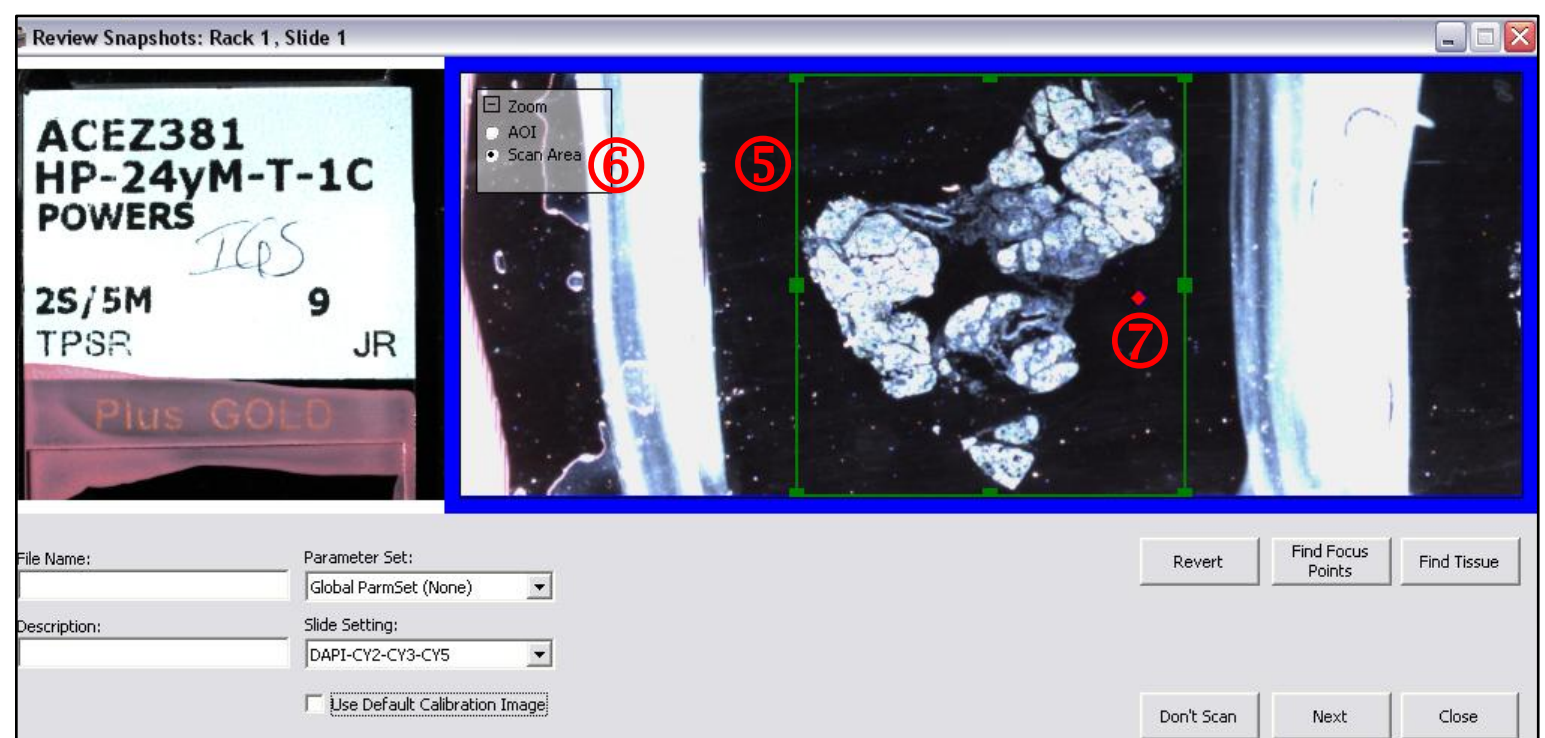
II. *Setting Up Scan Parameters and Reviewing Snapshots*

3. Right click anywhere within green box and select **Delete All Focus Points** ④.



4. Resize green box ⑤ to set scan area to one tissue section. Select "Scan Area" in Zoom box ⑥ to zoom into scan area or "AOI" to see entire slide.

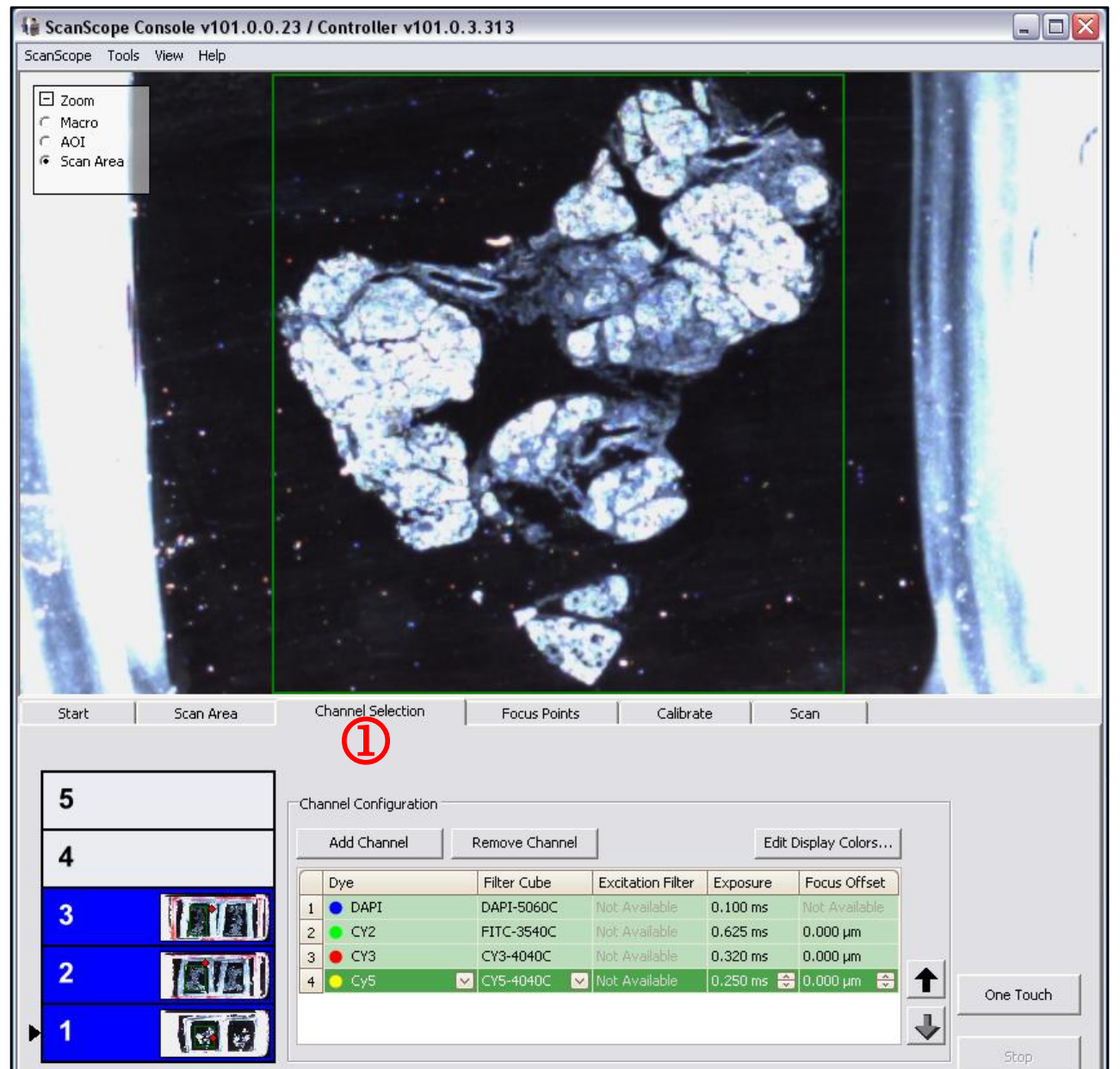
5. Click and drag red diamond ⑦ to the cleanest, blackest spot on slide (either inside or outside scan area) to set calibration point. It is crucial that this point not be located on tissue to achieve proper shading correction.



6. Close Review Snapshots window.

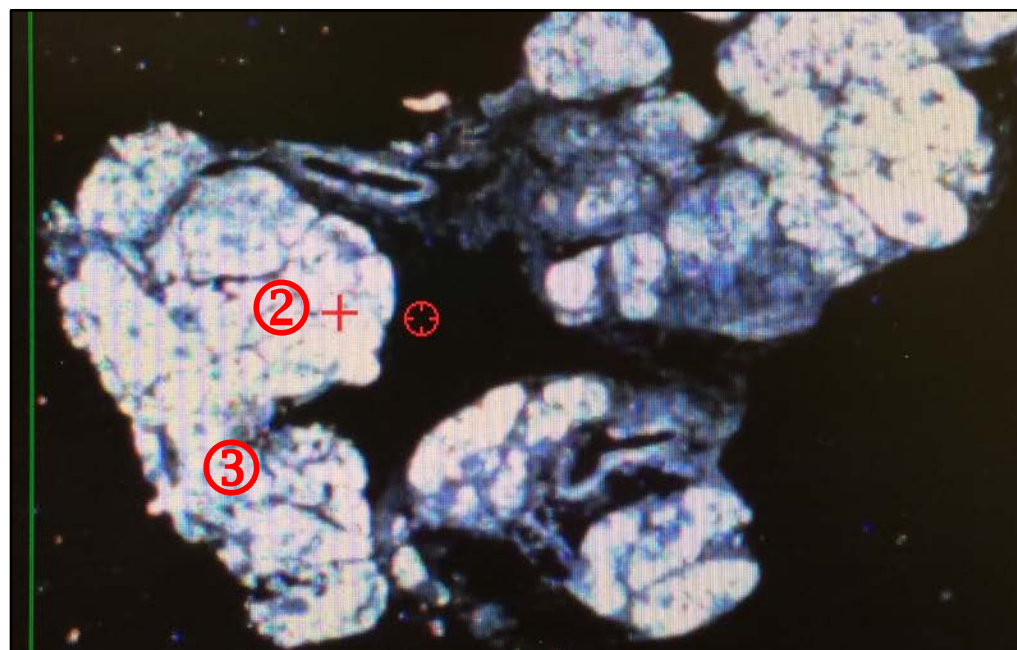
III. Adjusting Exposures

1. Select **Channel Selection** tab ①.

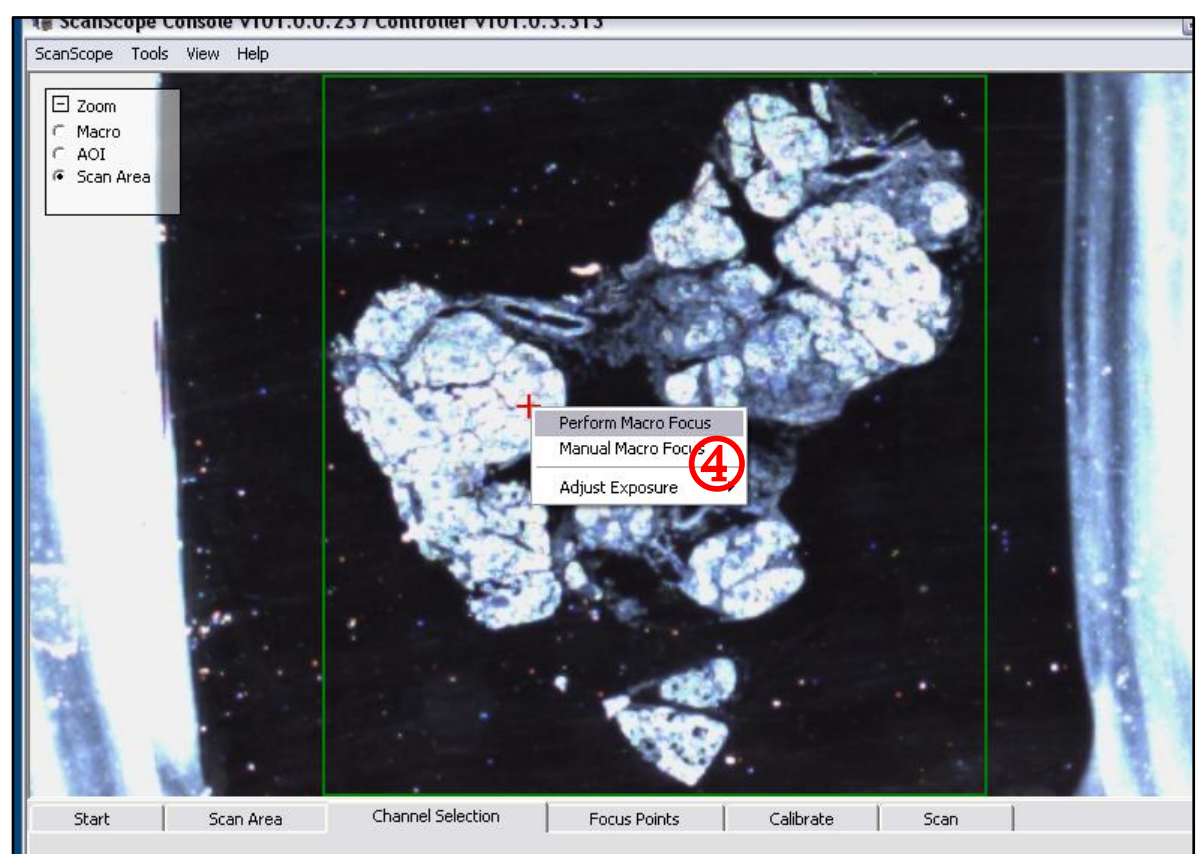


2. While hovering over image, cursor will change to red crosshair ②. Click on an area of the section to insert a red cross ③. This cross can be moved by clicking on another area of the tissue.

Note: Select an area with good staining and features of interest to set optimal exposures for each channel. It can be helpful to have an area chosen beforehand using a microscope.



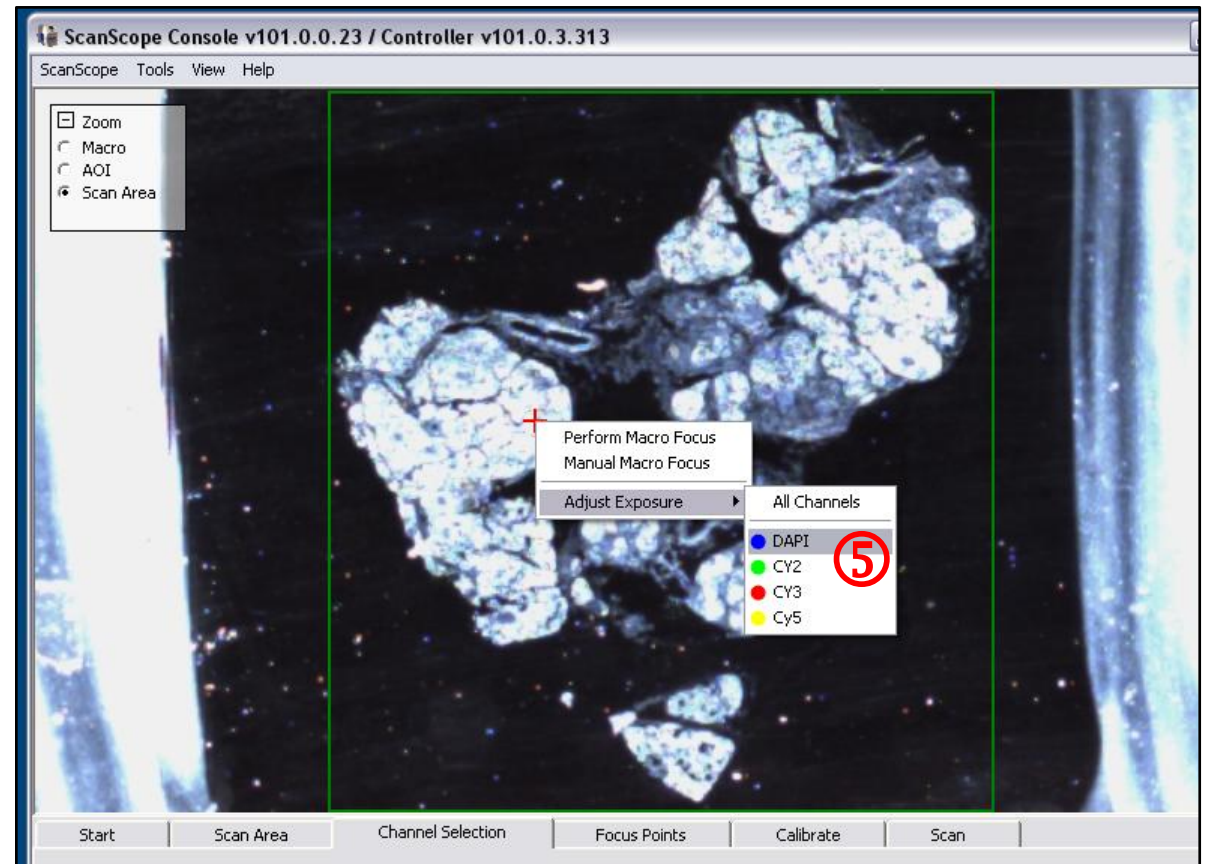
3. Align crosshair directly over cross, right click, and select **Perform Macro Focus** ④.



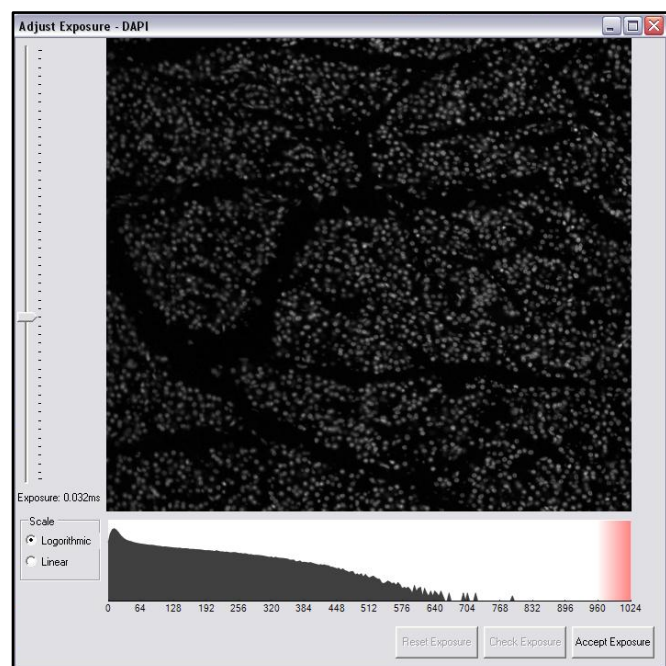
III. Adjusting Exposures

- Align crosshair directly over cross, right click, and select **Adjust Exposure** then **DAPI** ⑤.

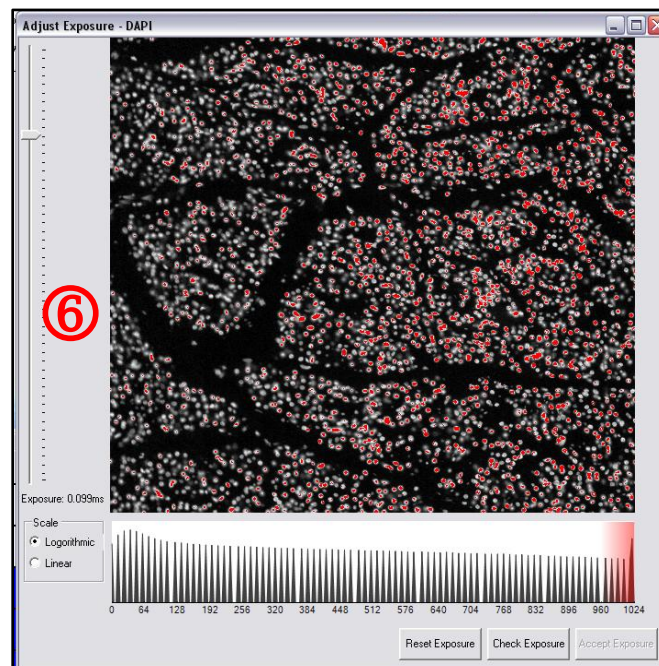
Note: If resulting DAPI image is blurry, follow MANUAL FOCUS instructions on page 8, then return to Channel Selection tab to readjust exposures.



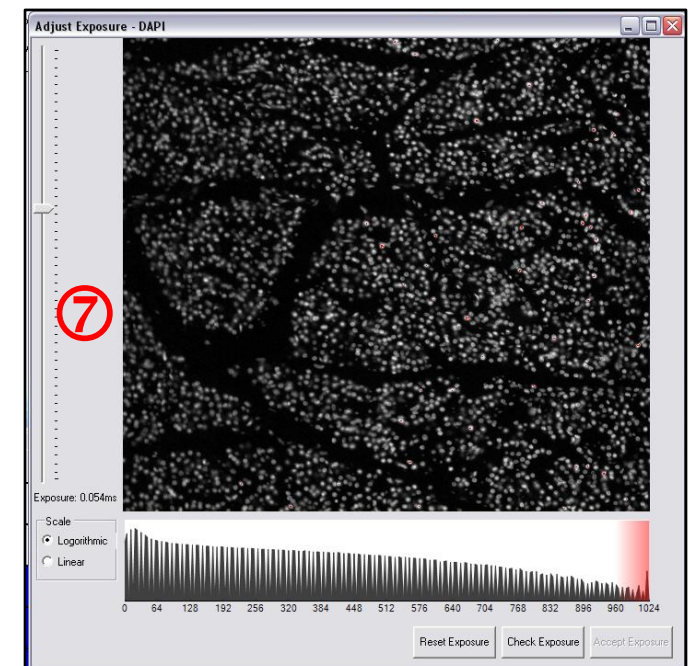
- In the **Adjust Exposure** window that appears, optimize exposure time using the slider ⑥. Raise exposure time until image is saturated (shown by red pixels), then lower it until most red pixels disappear. Click **Check Exposure** ⑦.



Too low



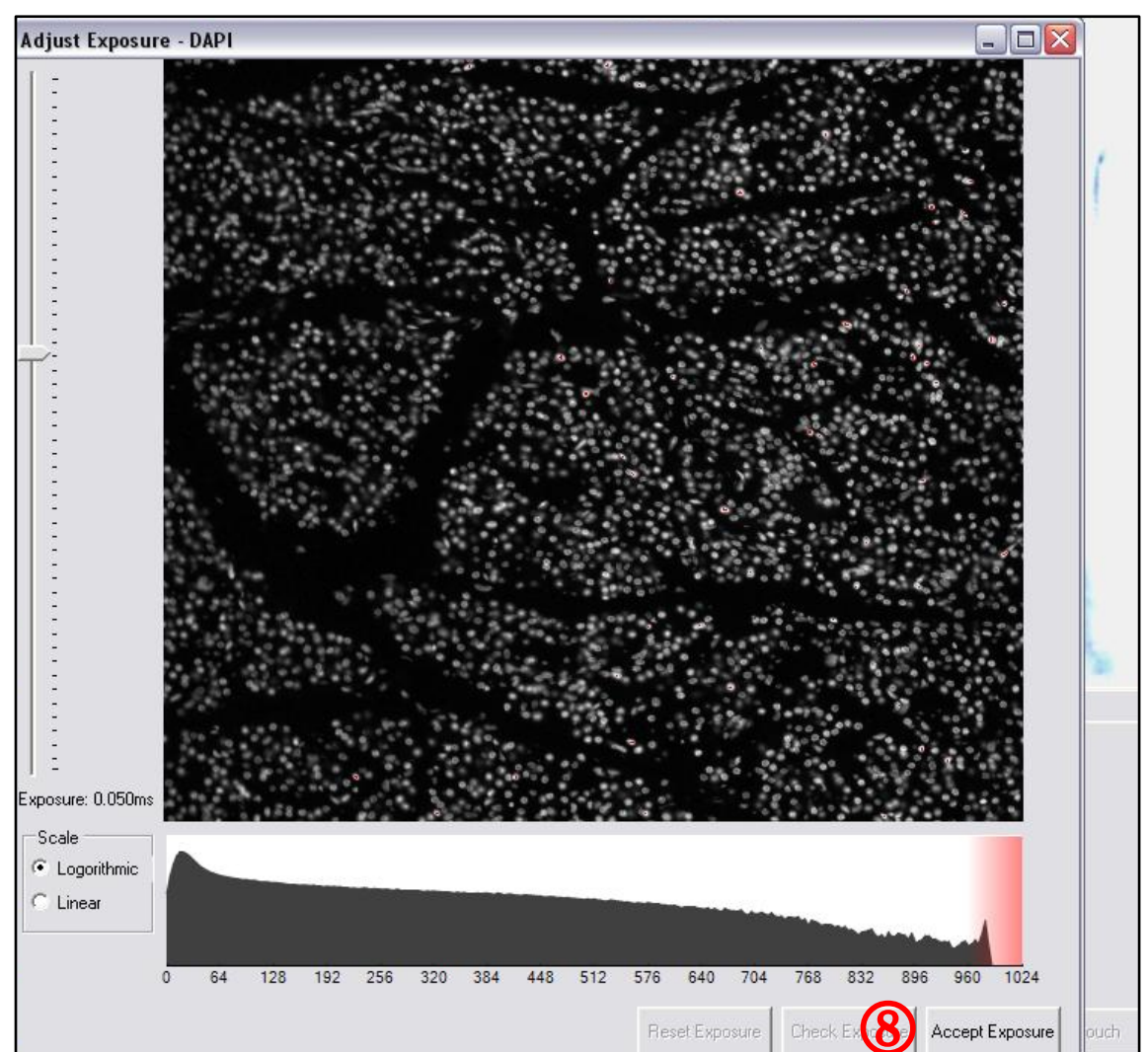
Too high



Optimal exposure time

- If exposure time is satisfactory, click **Accept Exposure** ⑧.

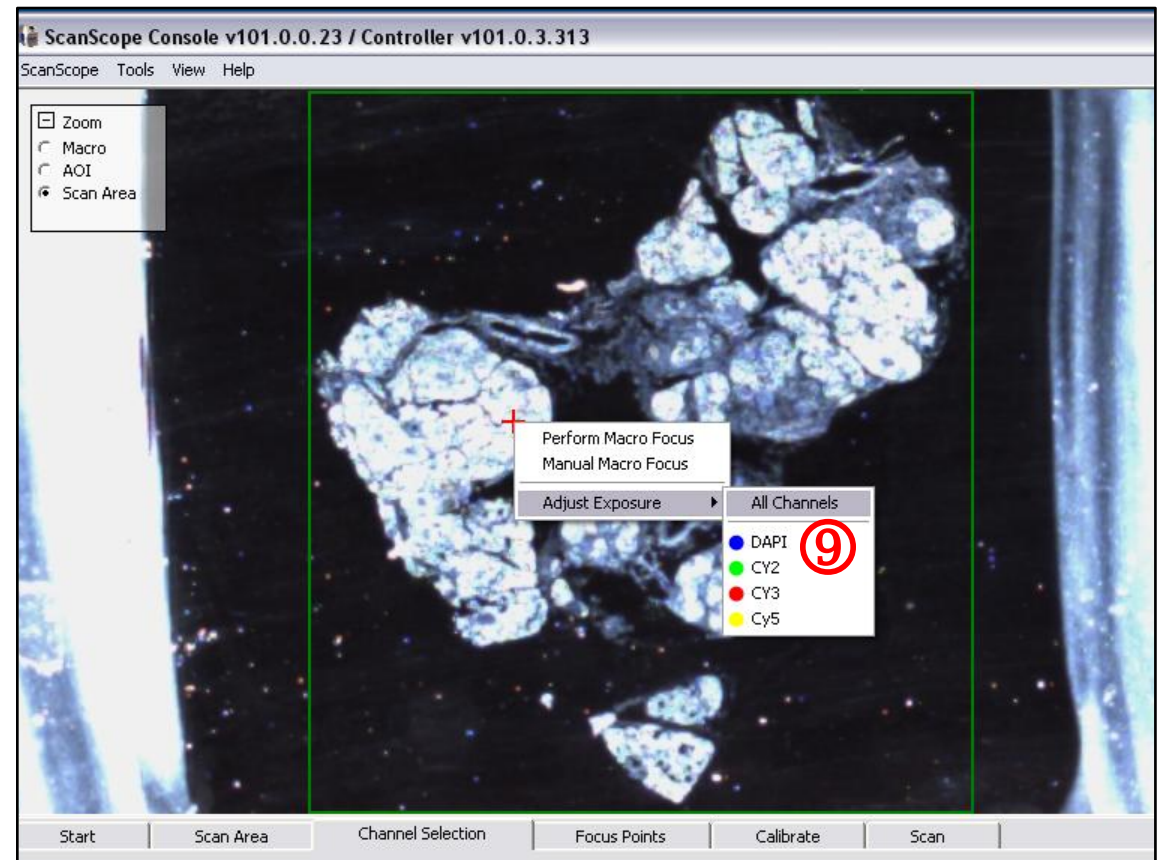
Note: Every time an exposure time is adjusted, Check Exposure must be clicked, followed by Accept Exposure.



III. Adjusting Exposures

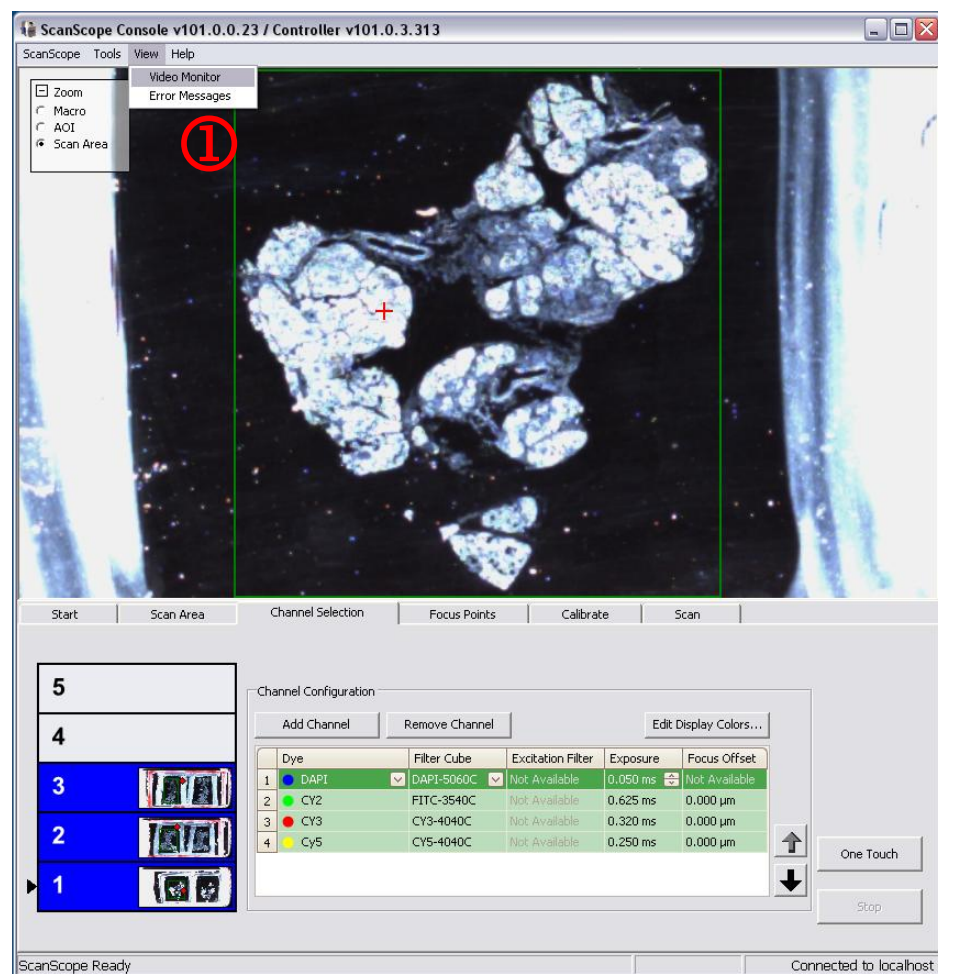
7. Repeat **Adjust Exposure** for each remaining channel by selecting **All Channels** ⑨ or by selecting each channel individually.

Note: When adjusting exposures for all channels, the DAPI channel will need to be reset.

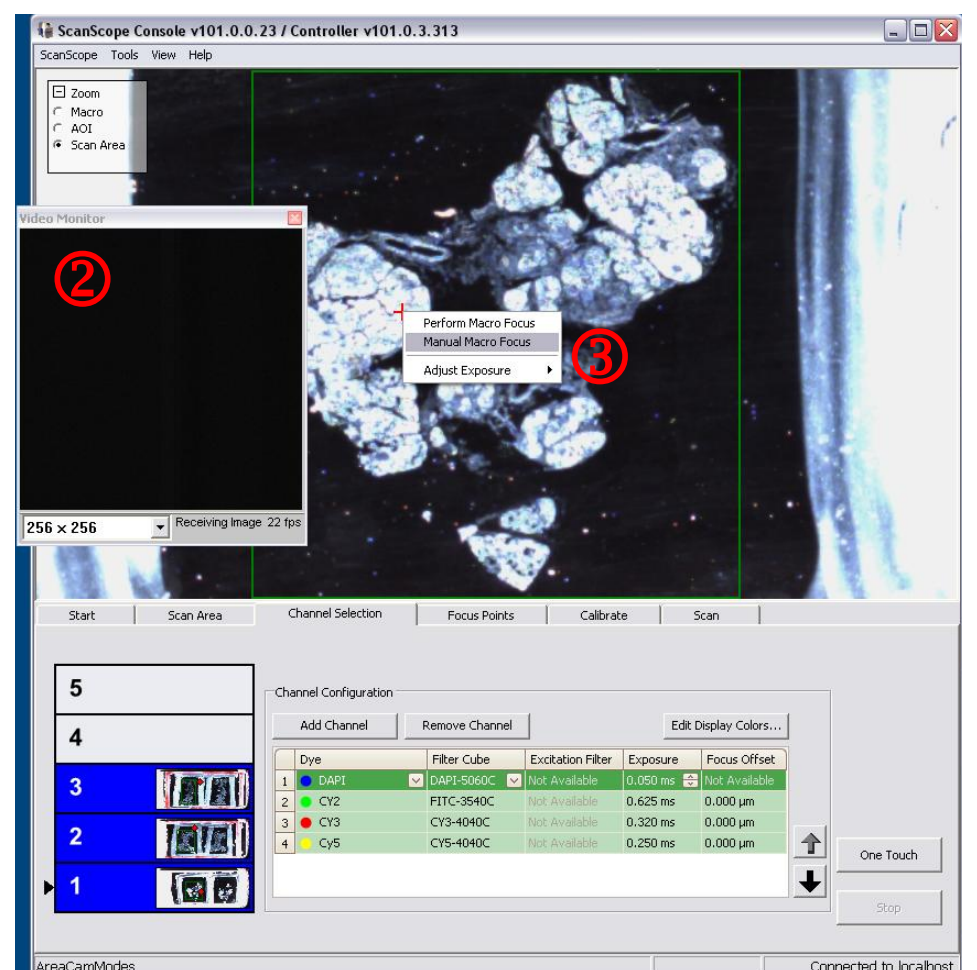


IV. Setting Up Manual Focus

1. Select **Video Monitor** ① from the **View** dropdown menu. This will open a Video Monitor window ②.

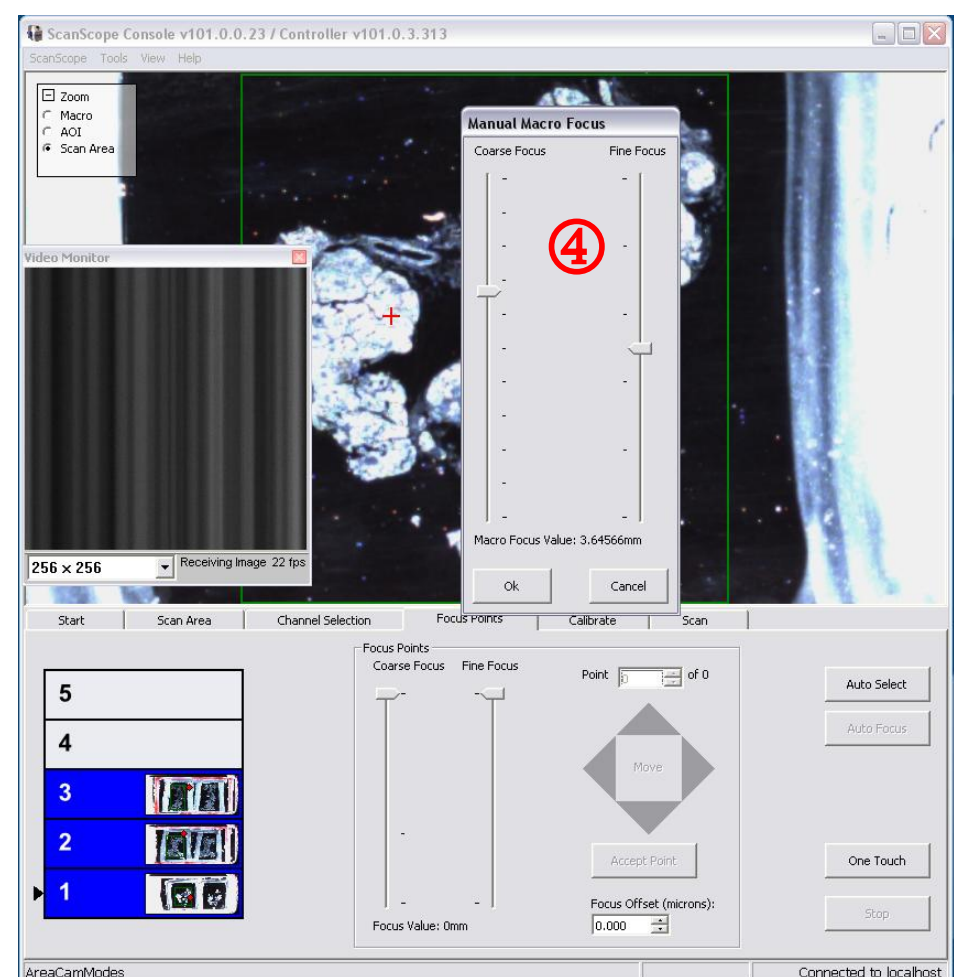


2. Align crosshair over red cross on tissue section, right click, and select **Manual Macro Focus** ③.



3. In the Manual Macro Focus window that opens ④, use the coarse and fine focus sliders to set the best focus/highest definition of the grey bars in the video feed.

4. Select OK in the Manual Macro Focus window. Close both this window and the Video Monitor window.



V. Acquiring Default Calibration and Testing Exposure Settings

1. To test exposure settings, go to **Scan Area** ① tab and resize the green box to a small area around the red cross.

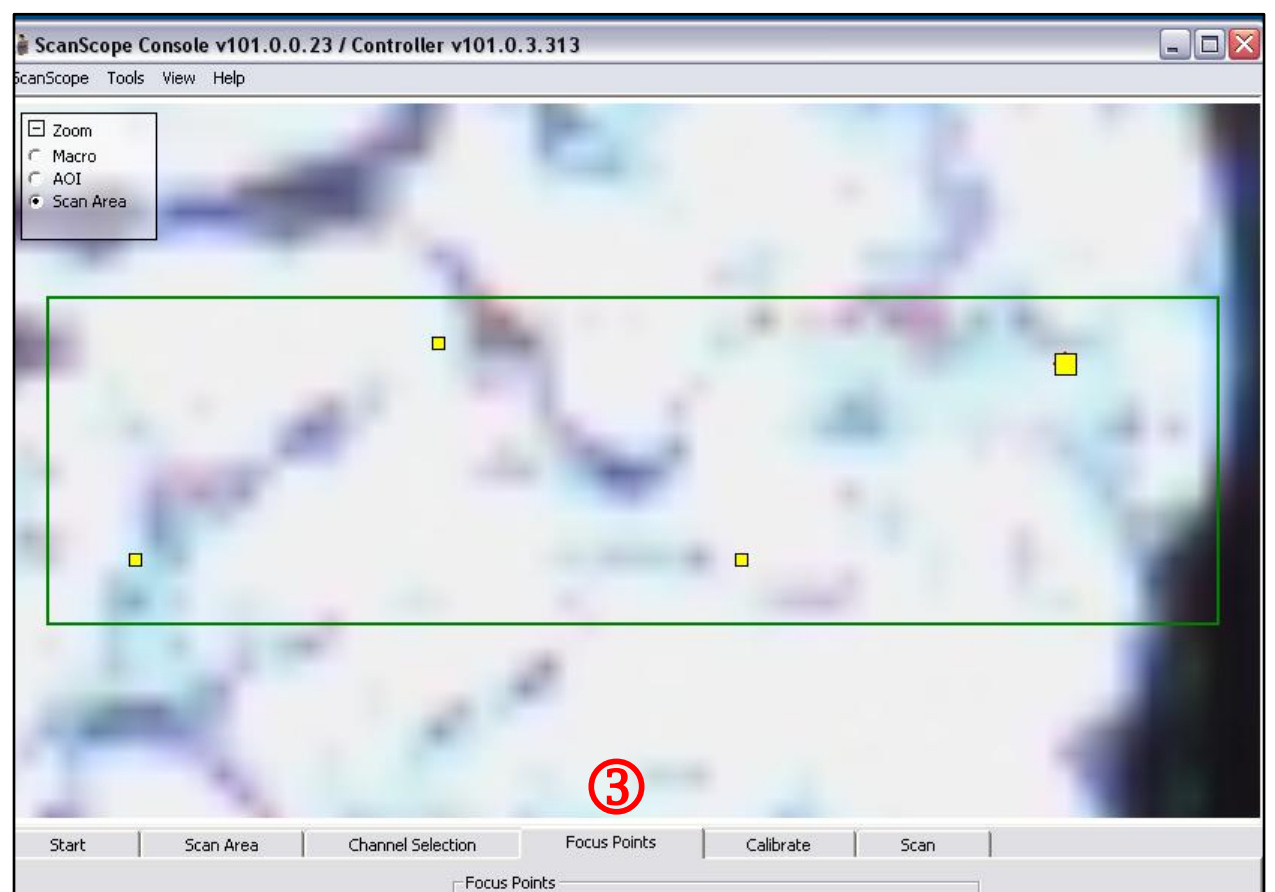
Note: A short, wide area works best to generate at least 3 stripes in the scan. This will allow you to see at least two seams in the image.

2. Click **Scan Area** ② in **Zoom box** to zoom into scan area.

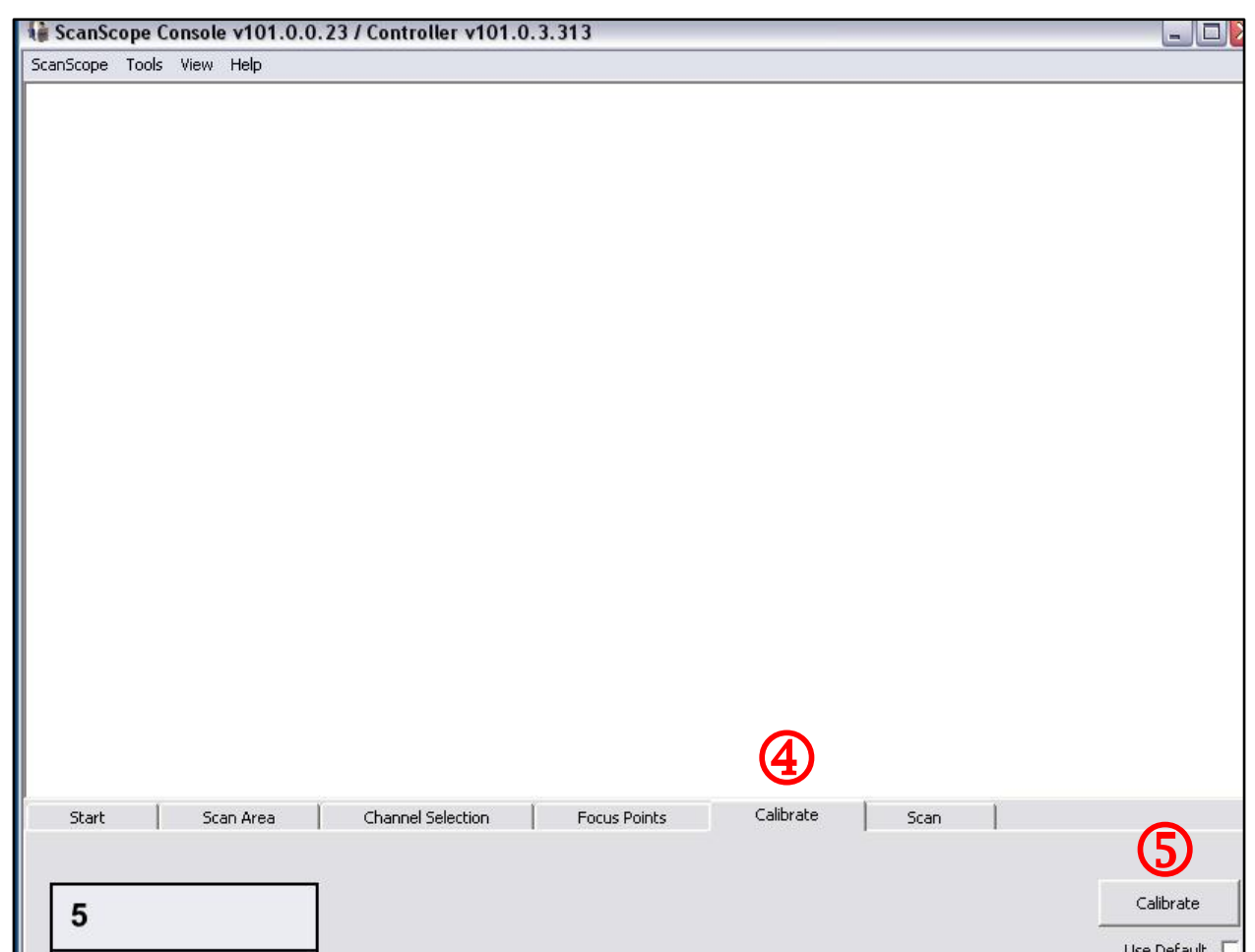


3. Go to **Focus Points** tab ③ and add 4 focus points to scan area by double clicking. A yellow square will appear at focus point, which can be moved around the scan area by clicking and dragging.

Note: While placing focus points alternate top and bottom positions within the scan area (as pictured).



4. Go to **Calibrate** tab ④ and click the **Calibrate** button ⑤. The program will automatically move back to the Focus Points tab.

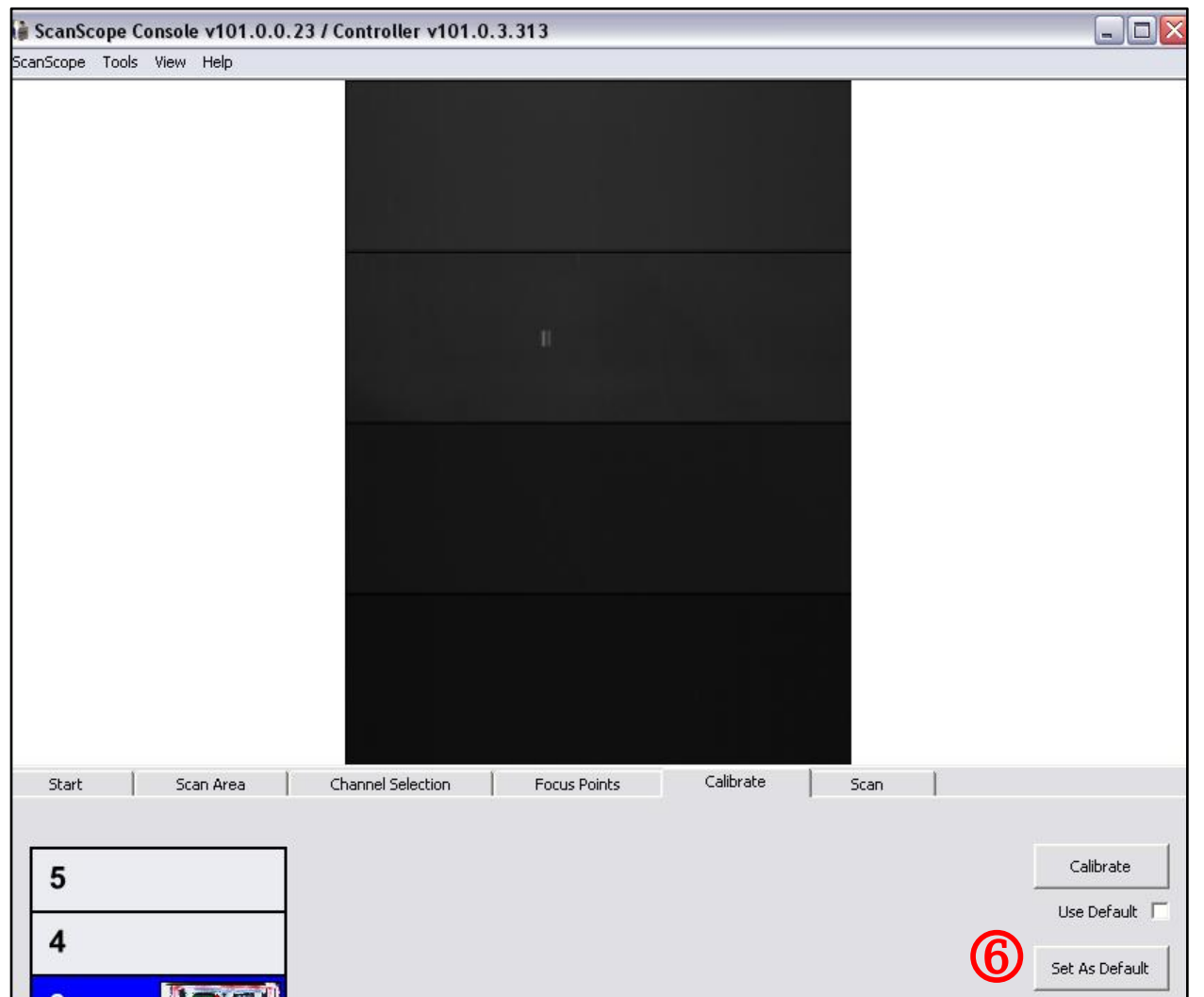


V. *Acquiring Default Calibration and Testing Exposure Settings*

5. Focus points will turn green as the scanner focuses.

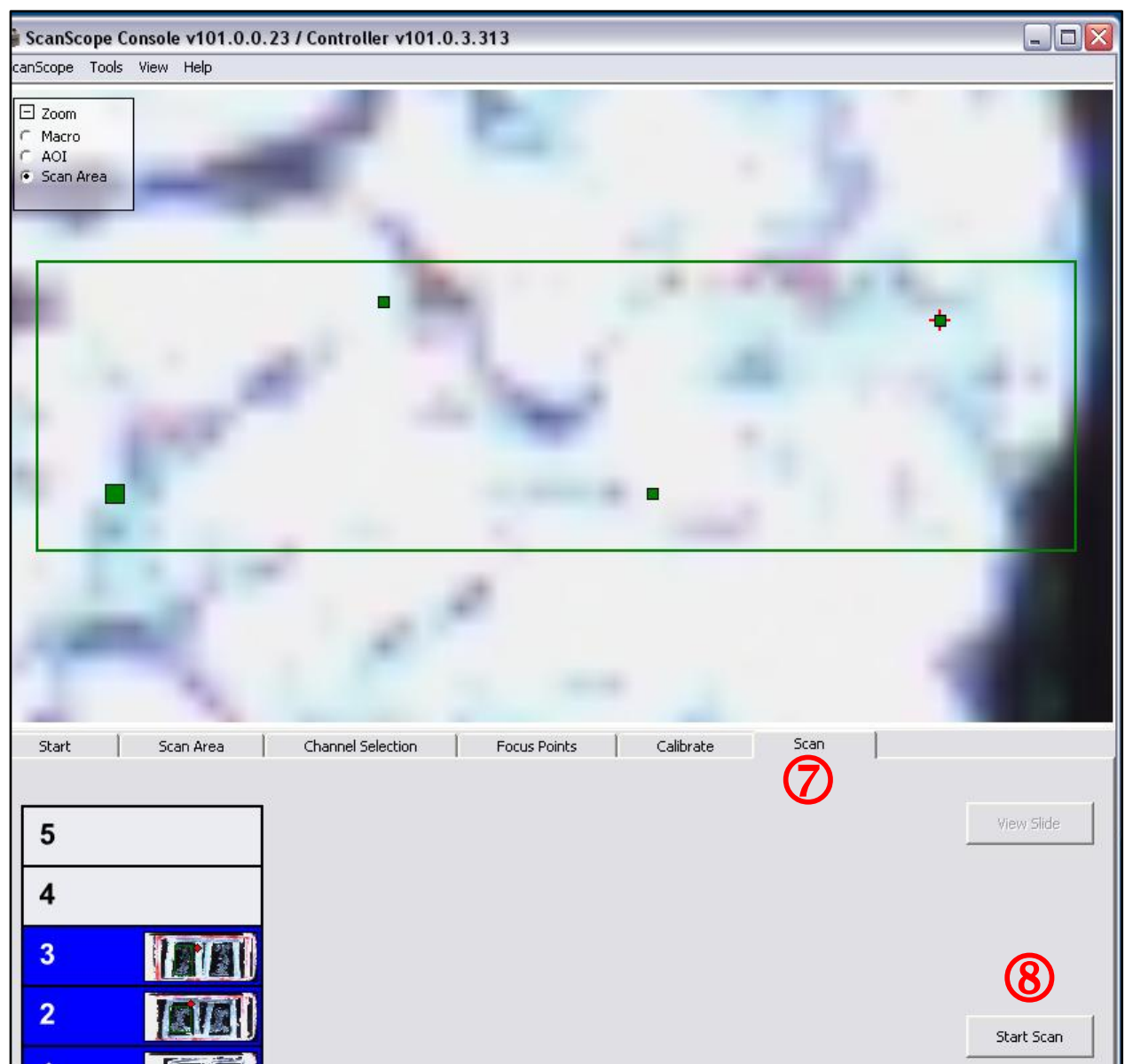


6. With calibration complete, the program will move back to the Calibrate tab. If no large smears, or numerous bright pixels are visible in the resulting image (see example), select **Set As Default** ⑥.



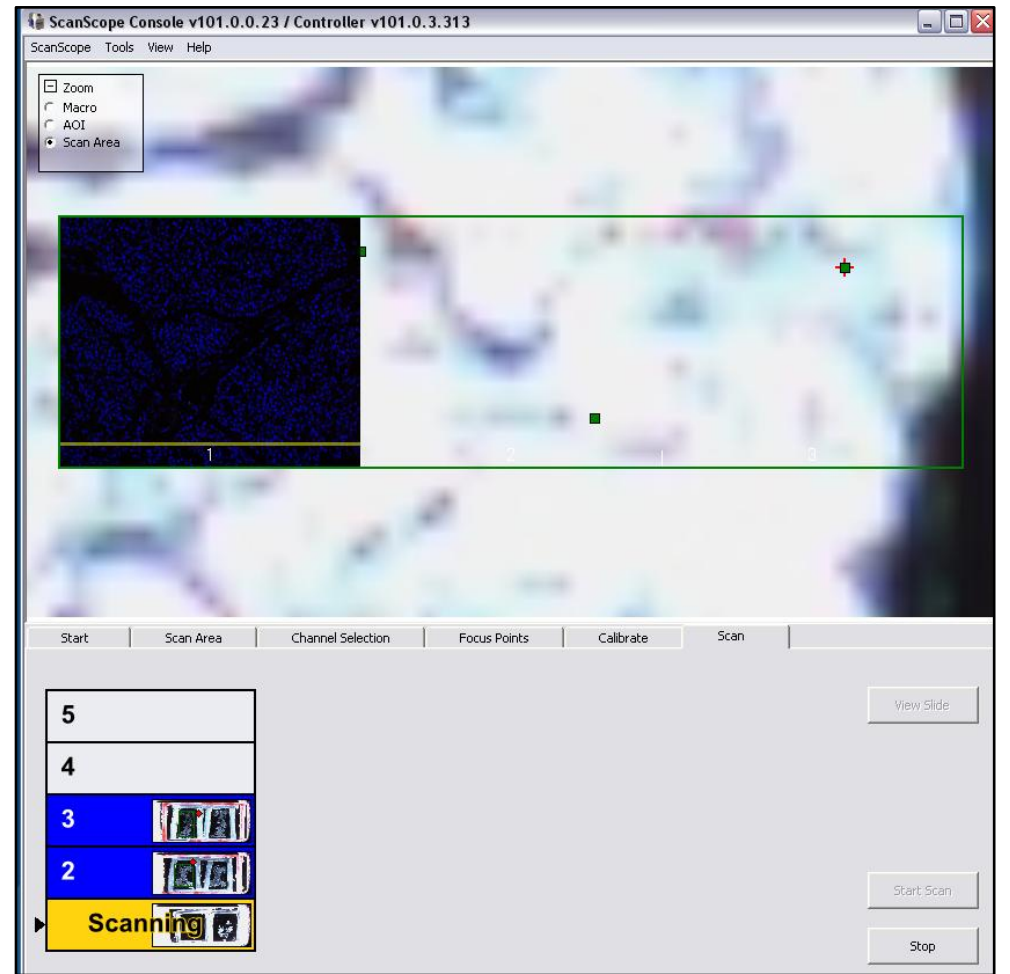
7. If **Calibration** is not satisfactory, return to **Scan Area** tab, reposition red diamond, and acquire a new Calibration.

8. Go to **Scan** tab ⑦ and select **Start Scan** ⑧.

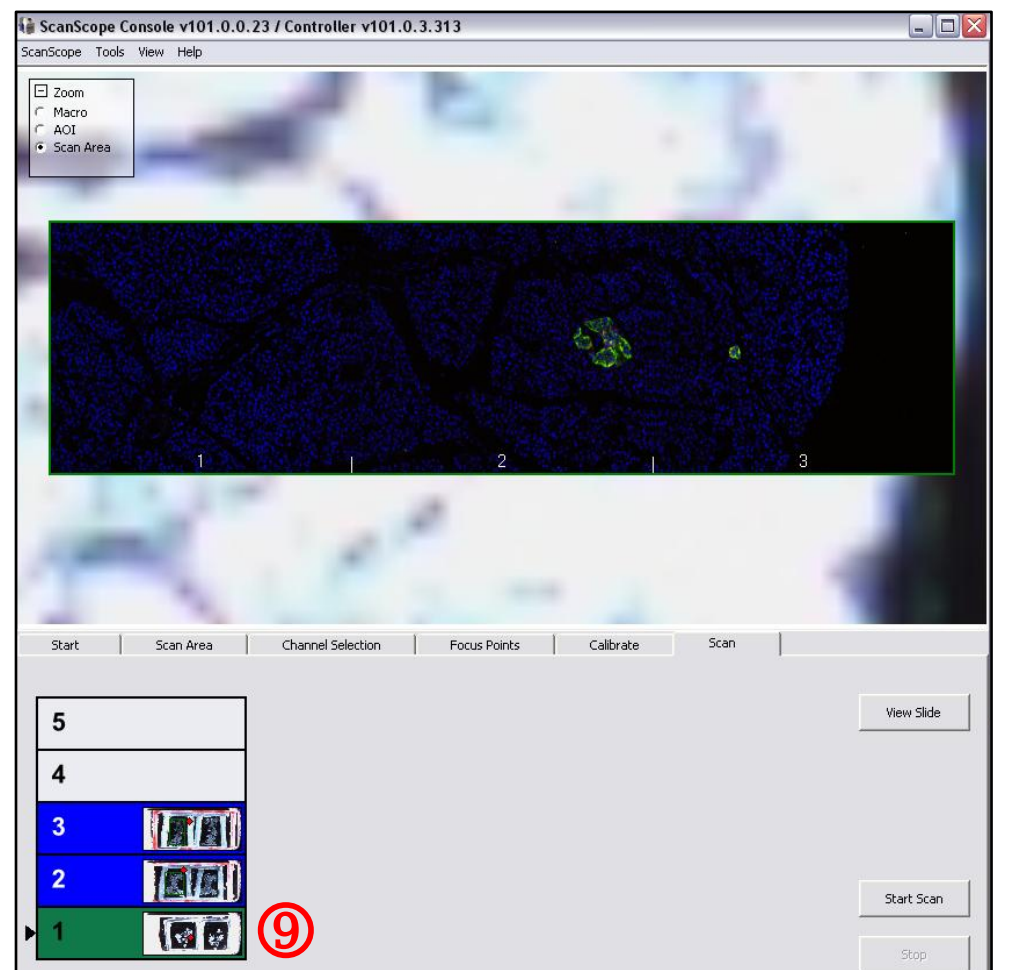


V. *Acquiring Default Calibration and Testing Exposure Settings*

9. Scan area will be imaged one channel at a time and will be displayed on screen.

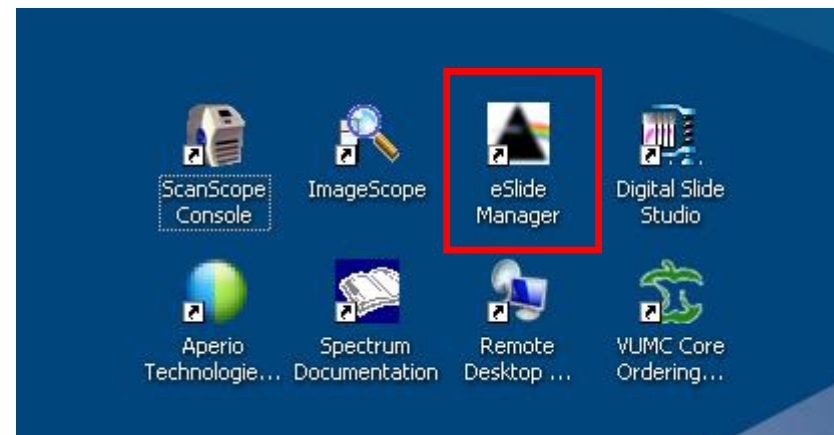


10. Slide will turn from yellow to green to indicate slide was successfully scanned ⑨.

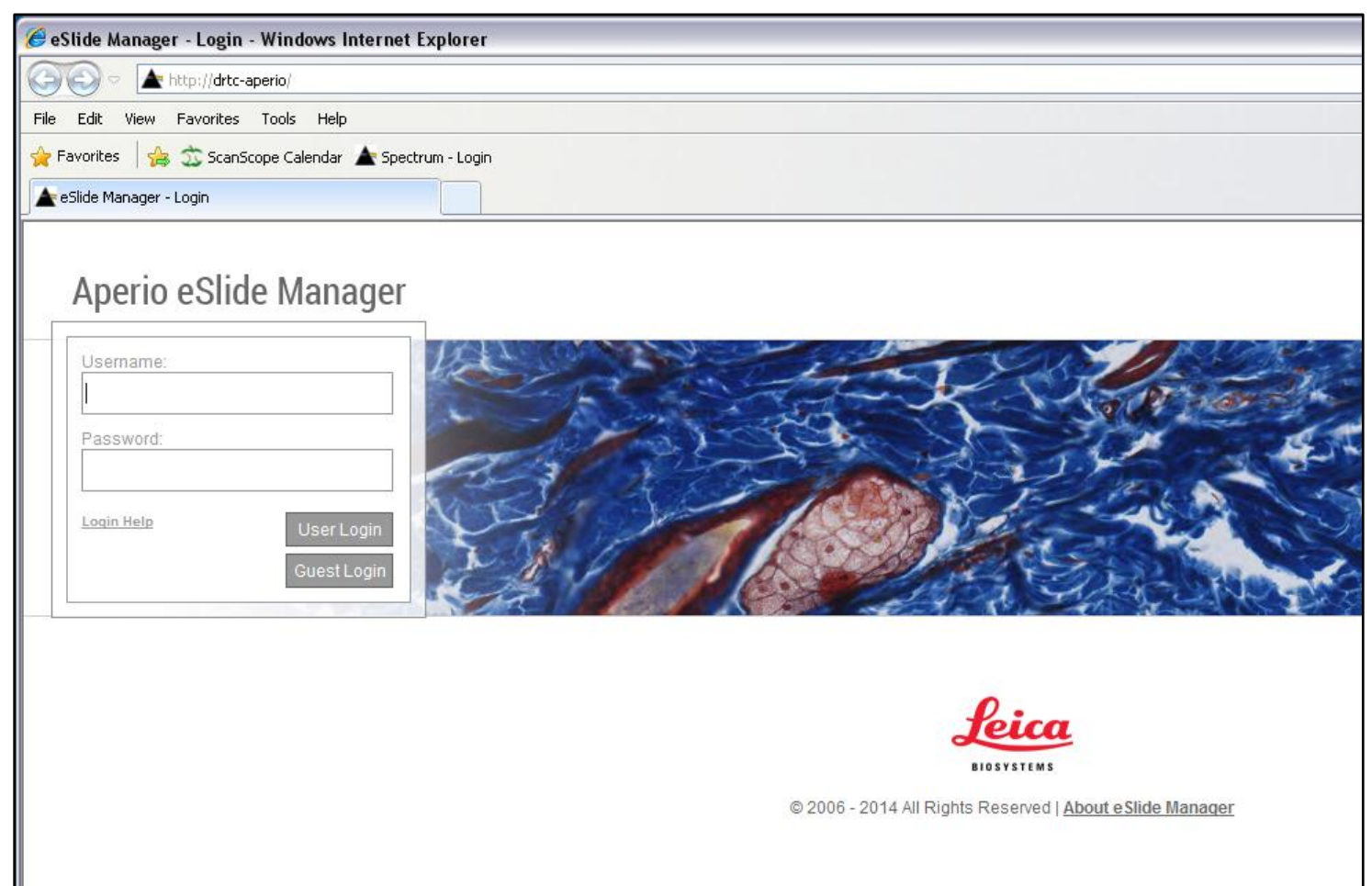


VI. Reviewing Test Pre-scan/Managing Final Images

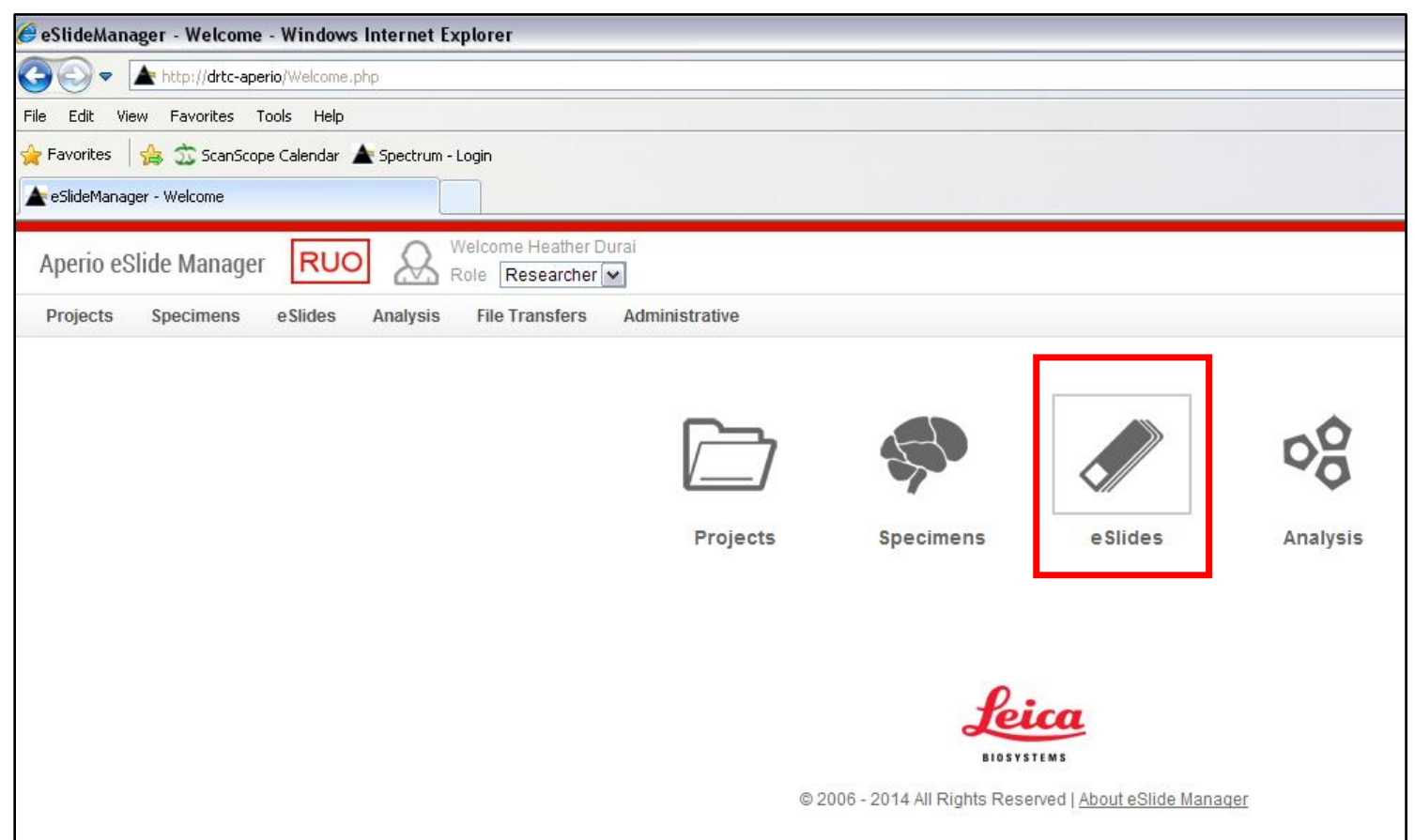
1. To view scanned images, open the **eSlide Manager** program from the desktop.



2. Log in to eSlide Manager with your username and password.

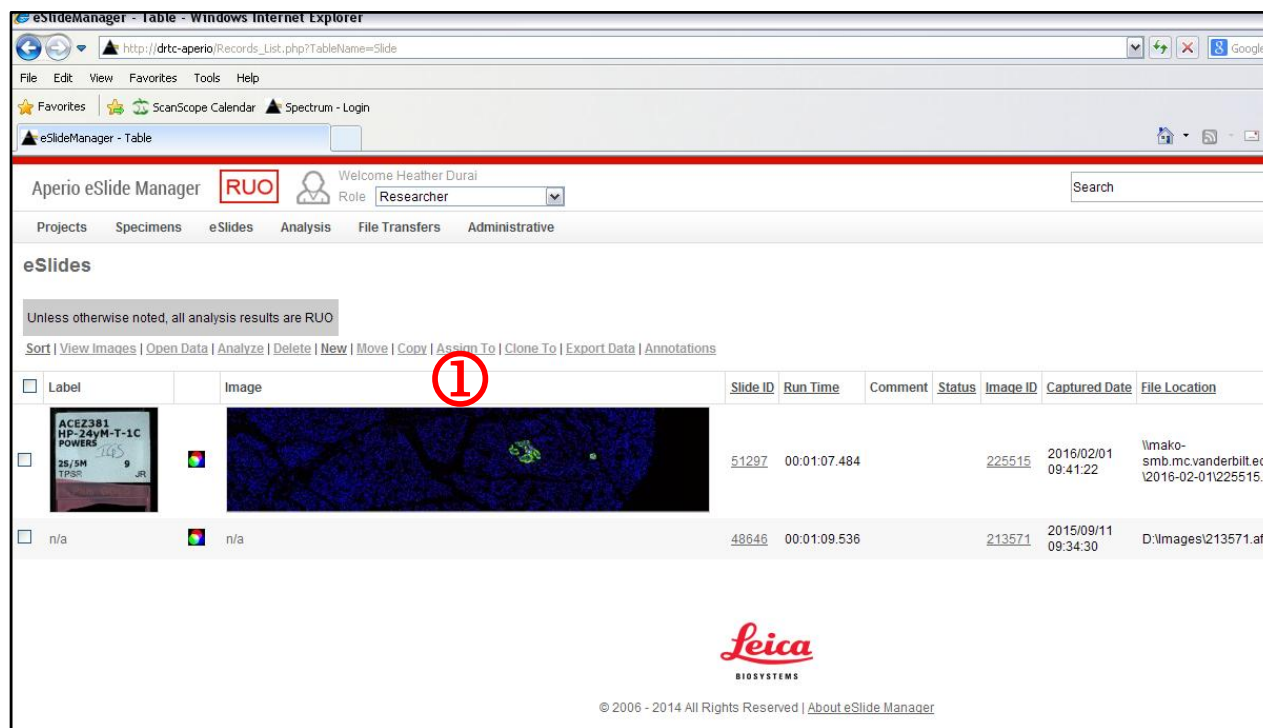


3. Select **eSlides** icon.

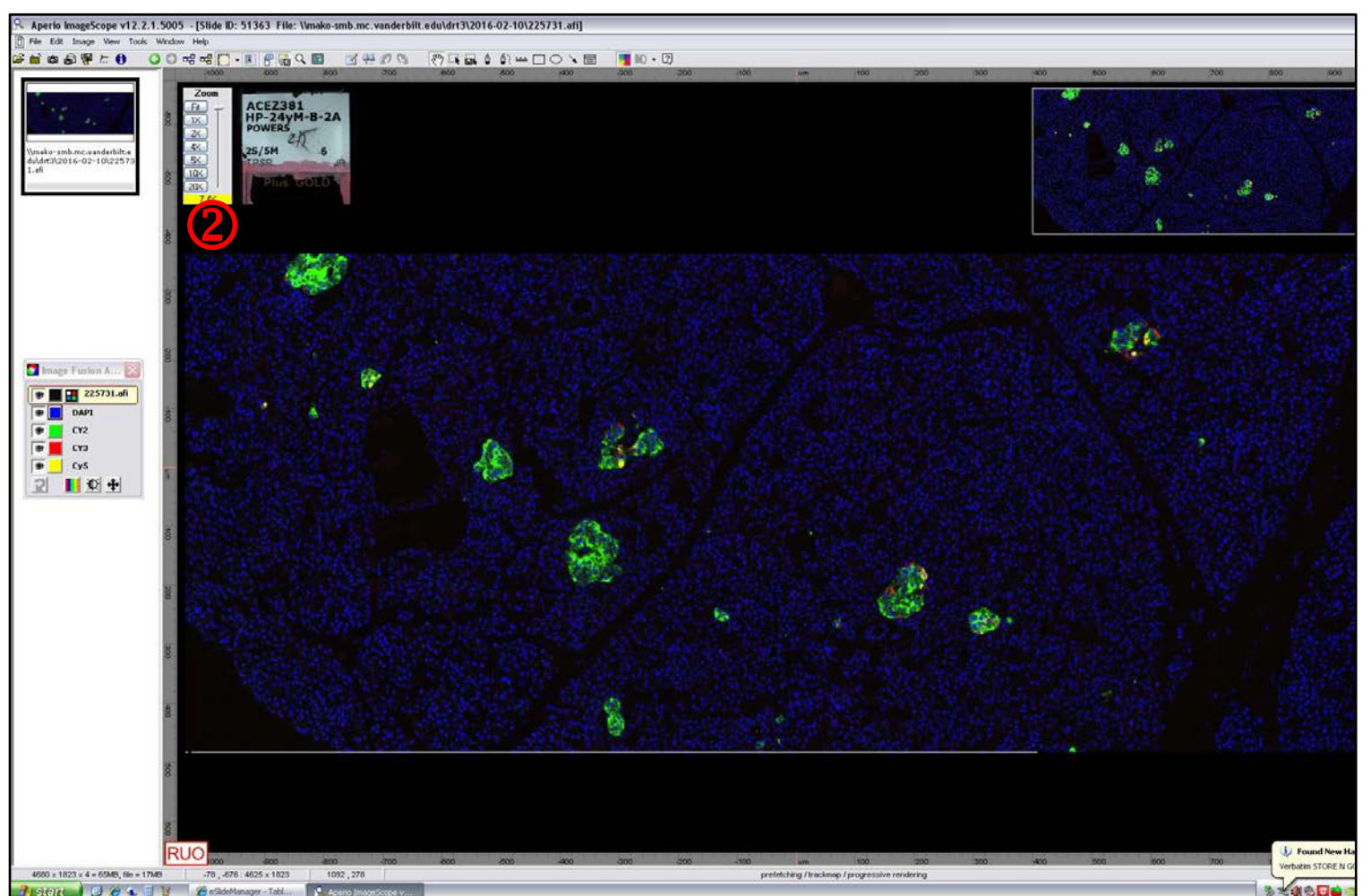


VI. Reviewing Test Pre-scan/Managing Final Images

4. Double click on the thumbnail image of your test scan area ①.

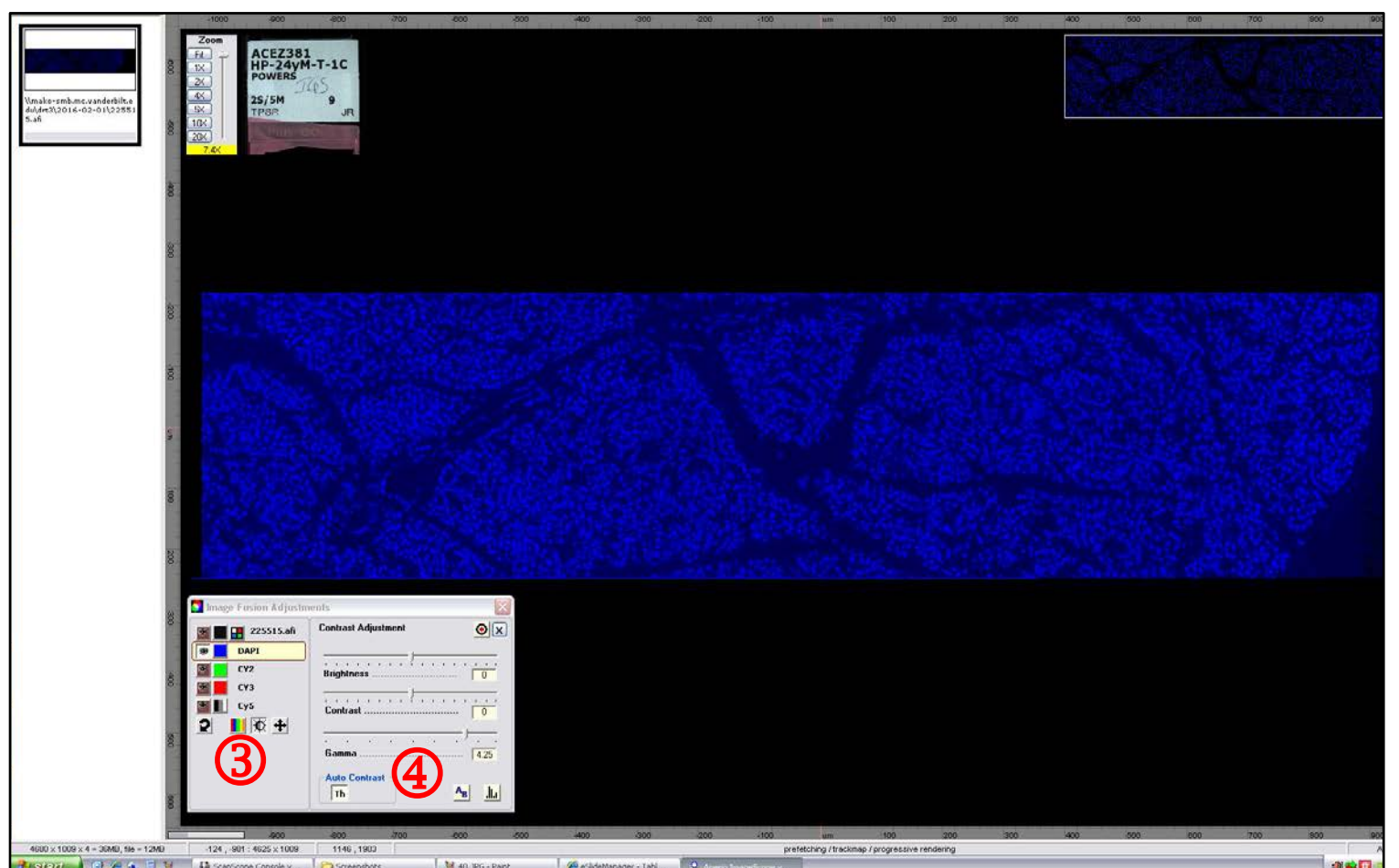


5. A window in the **Image Scope** program will open, displaying your image. You can zoom into an area within the image by double clicking on the area, or by selecting the magnification in the **Zoom** window ②.



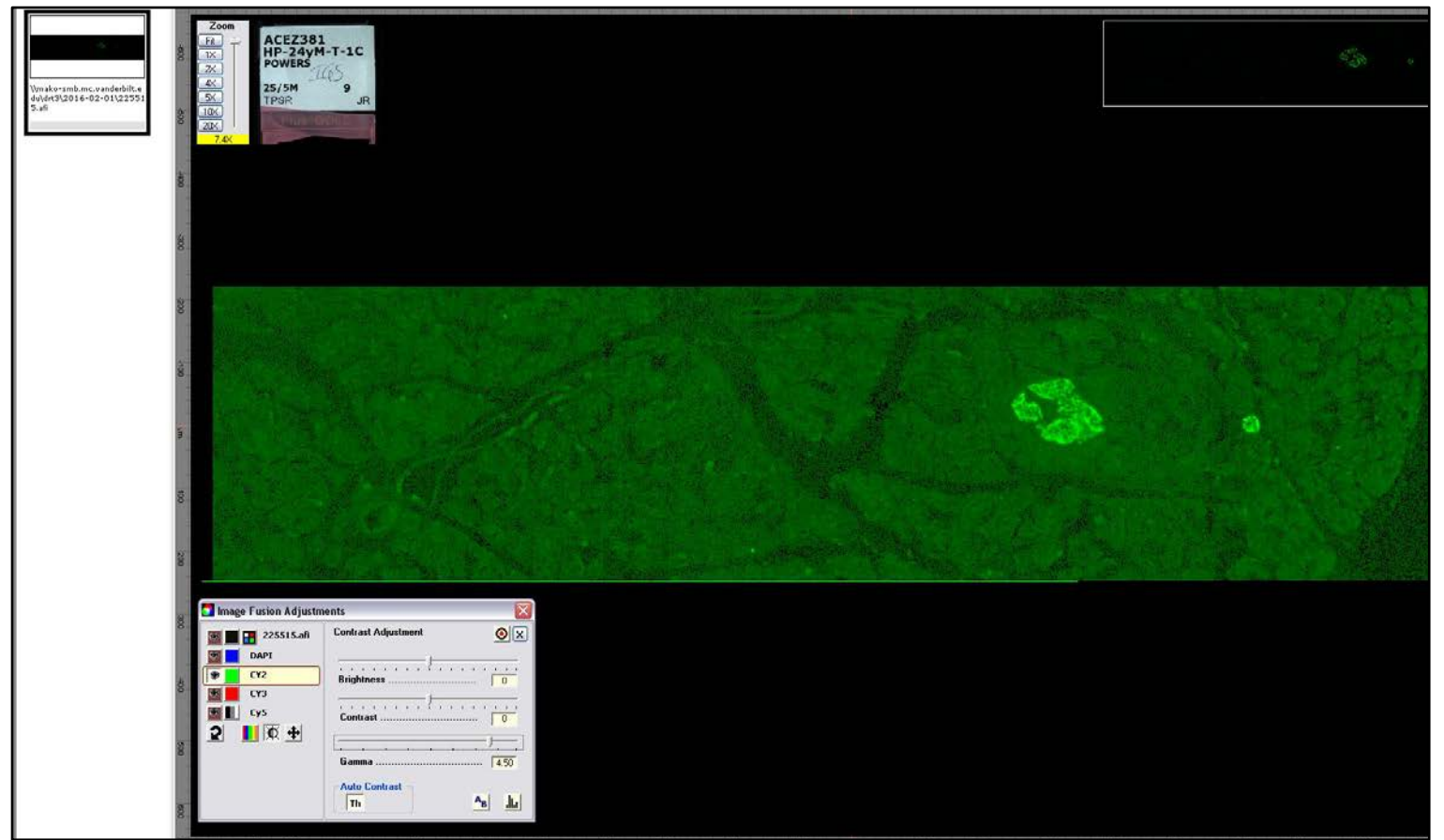
DAPI:

6. Check the quality of each channel by turning off all other channels using the eye icons in the **Image Fusion Adjustments** window ③. You can adjust the **Gamma slider** ④ to view seams between scanned stripes. If images show significant seams in each channel, acquire a new **Calibration**

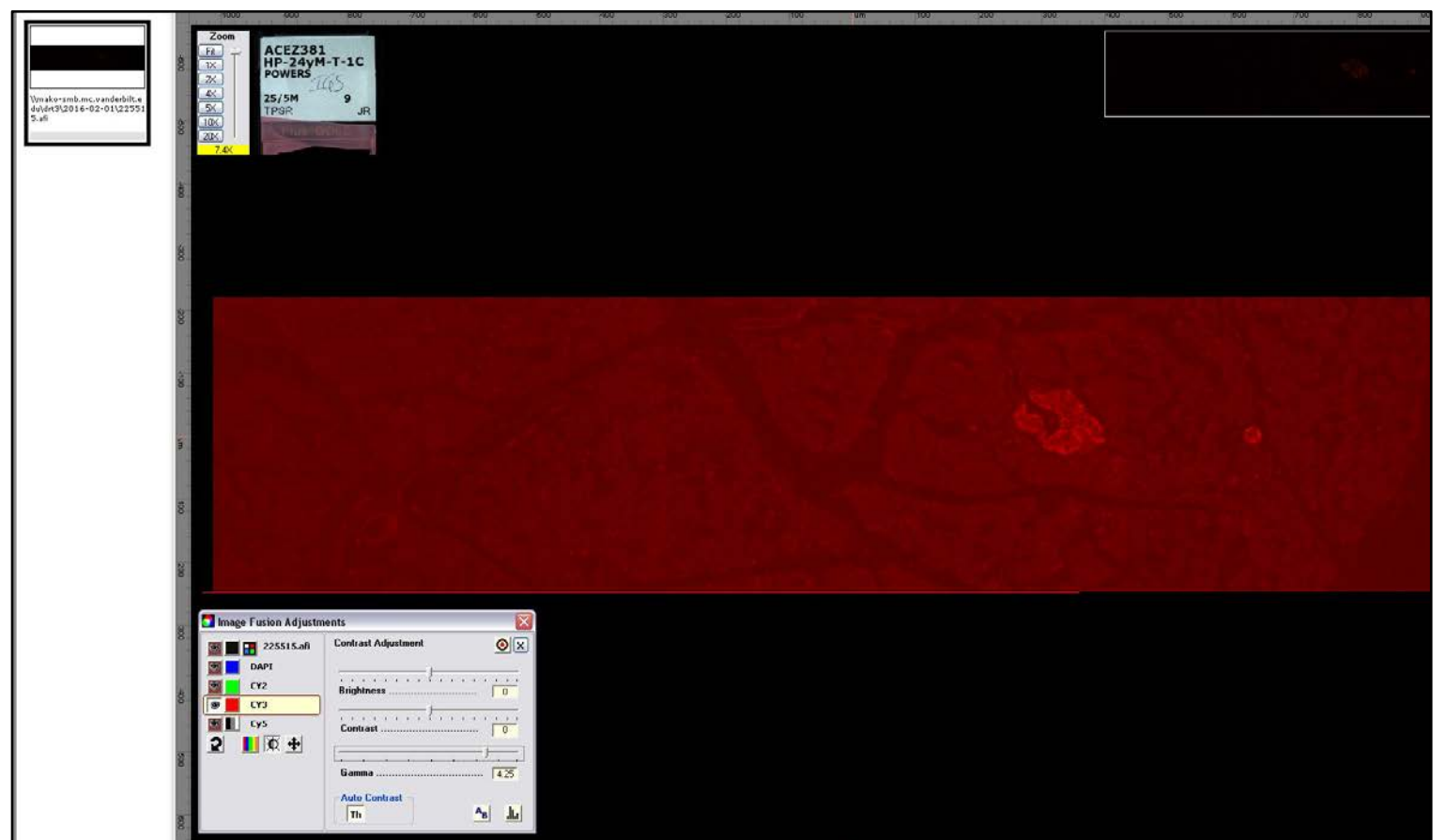


VI. Reviewing Test Pre-scan/Managing Final Images

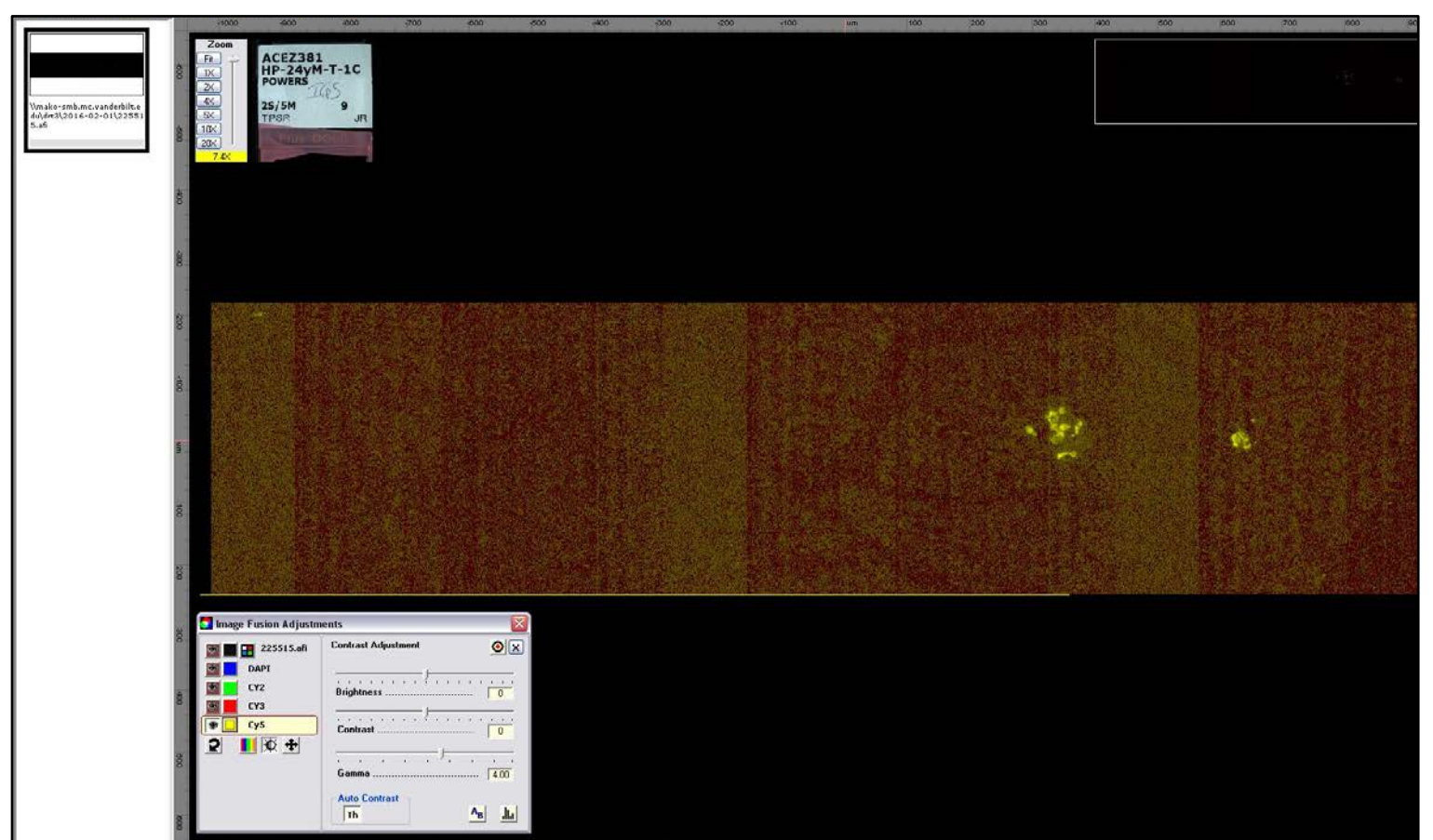
Cy2



Cy3



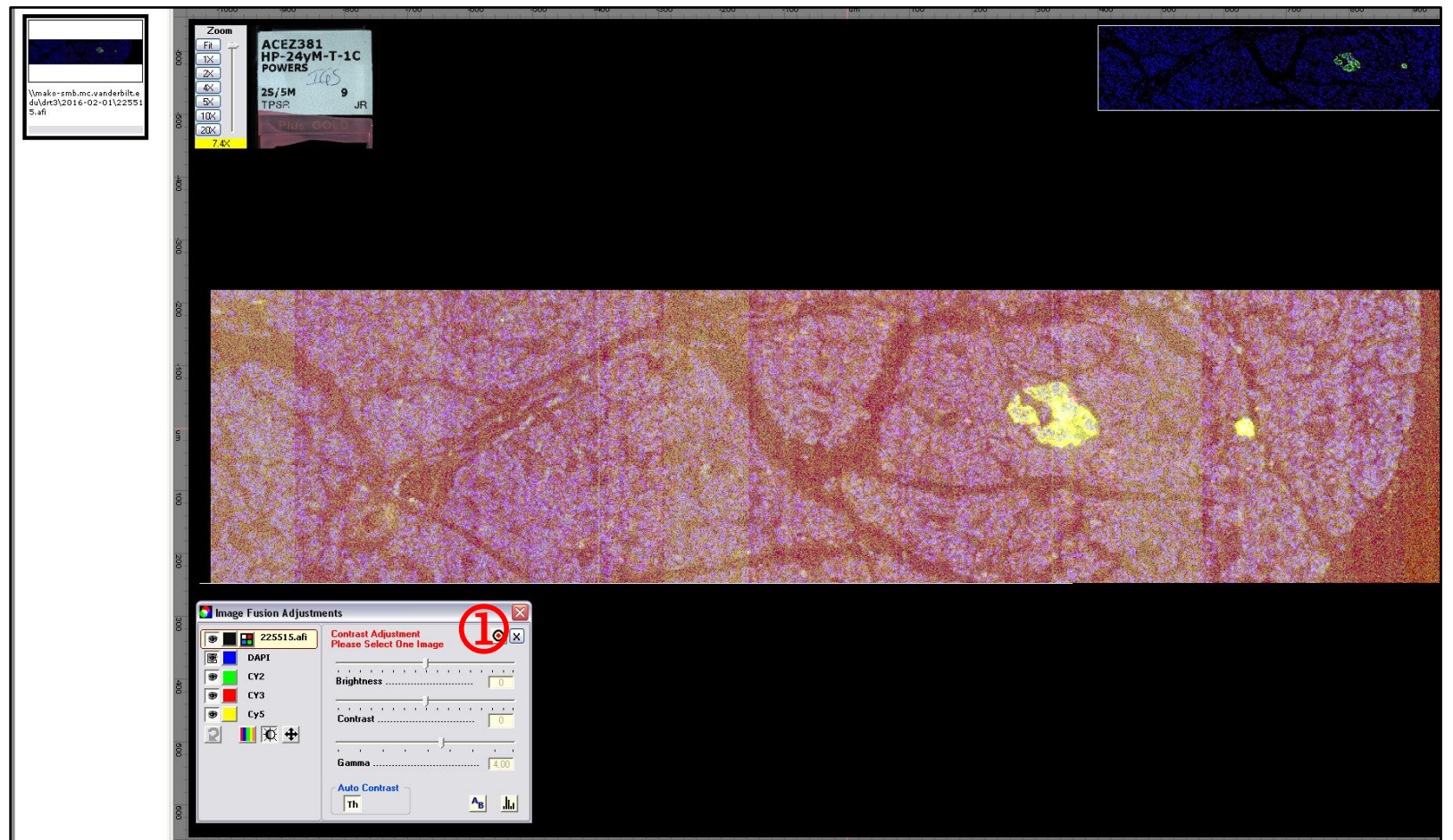
Cy5



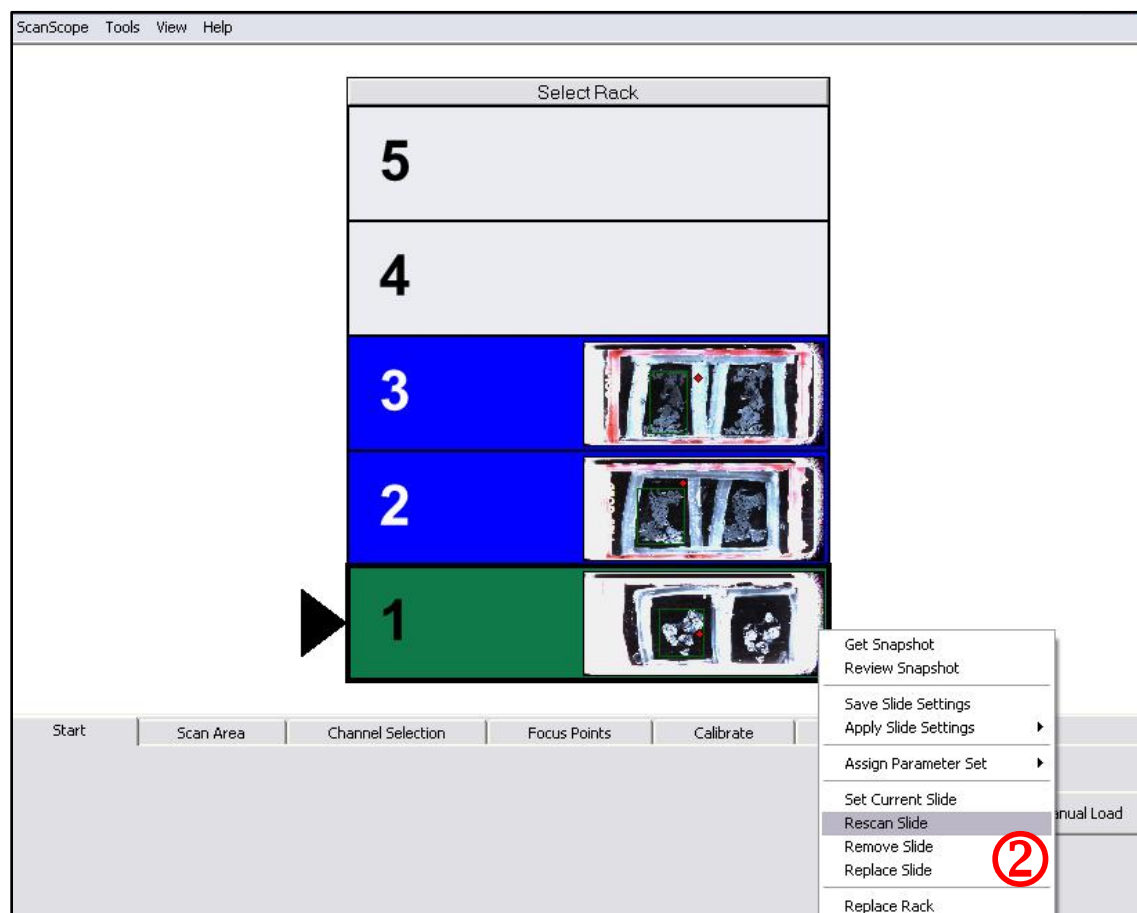
Note: seams and noise are often visible when turning Gamma up in Cy5 channel.

VI. Reviewing Test Pre-scan/Managing Final Images

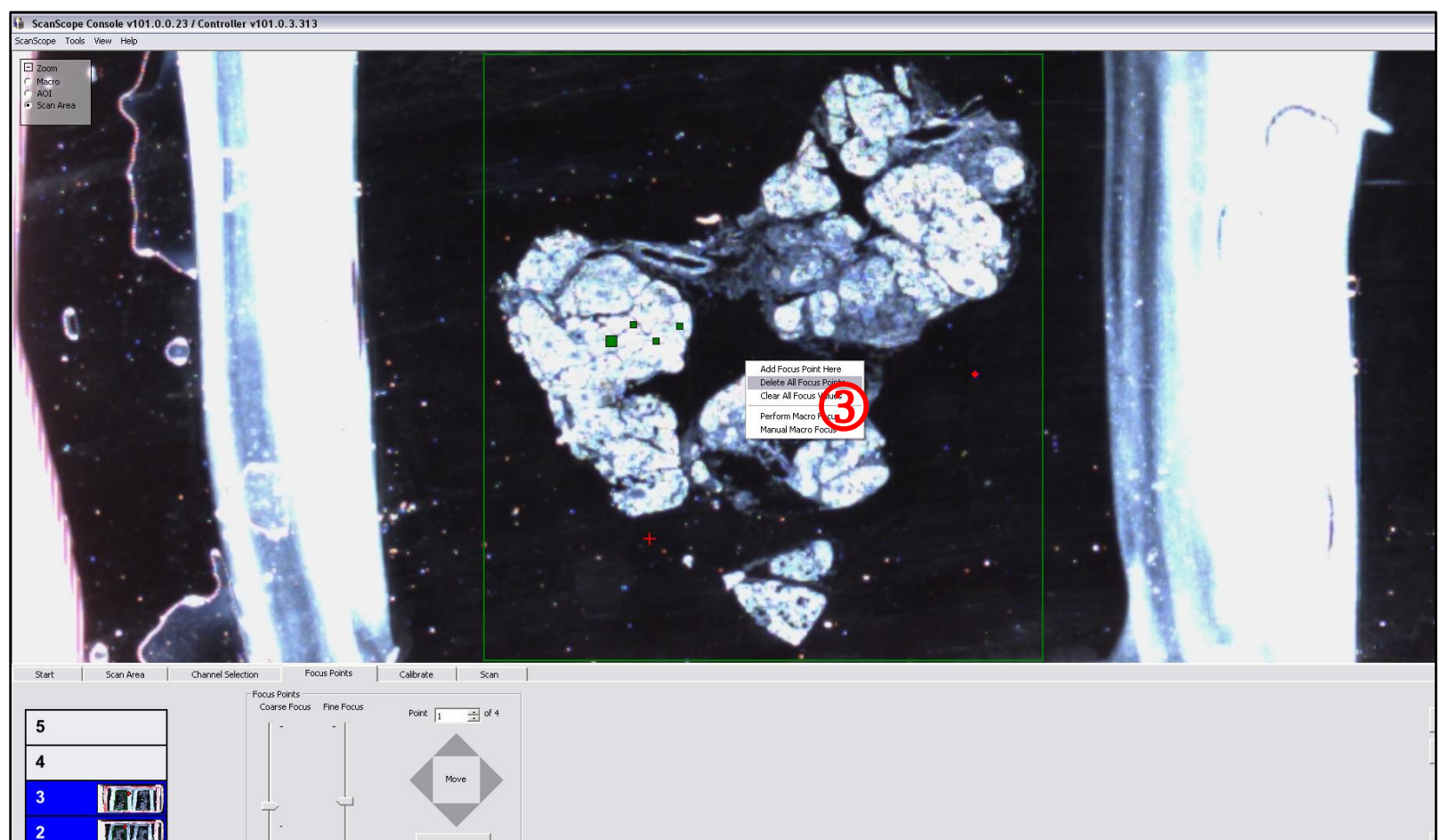
7. Revert image back to original settings by clicking the bullseye ① in the Image Fusion Adjustments window.



8. Return to the **ScanScope Console** program, right click on slide 1 and select **Rescan Slide** ②.

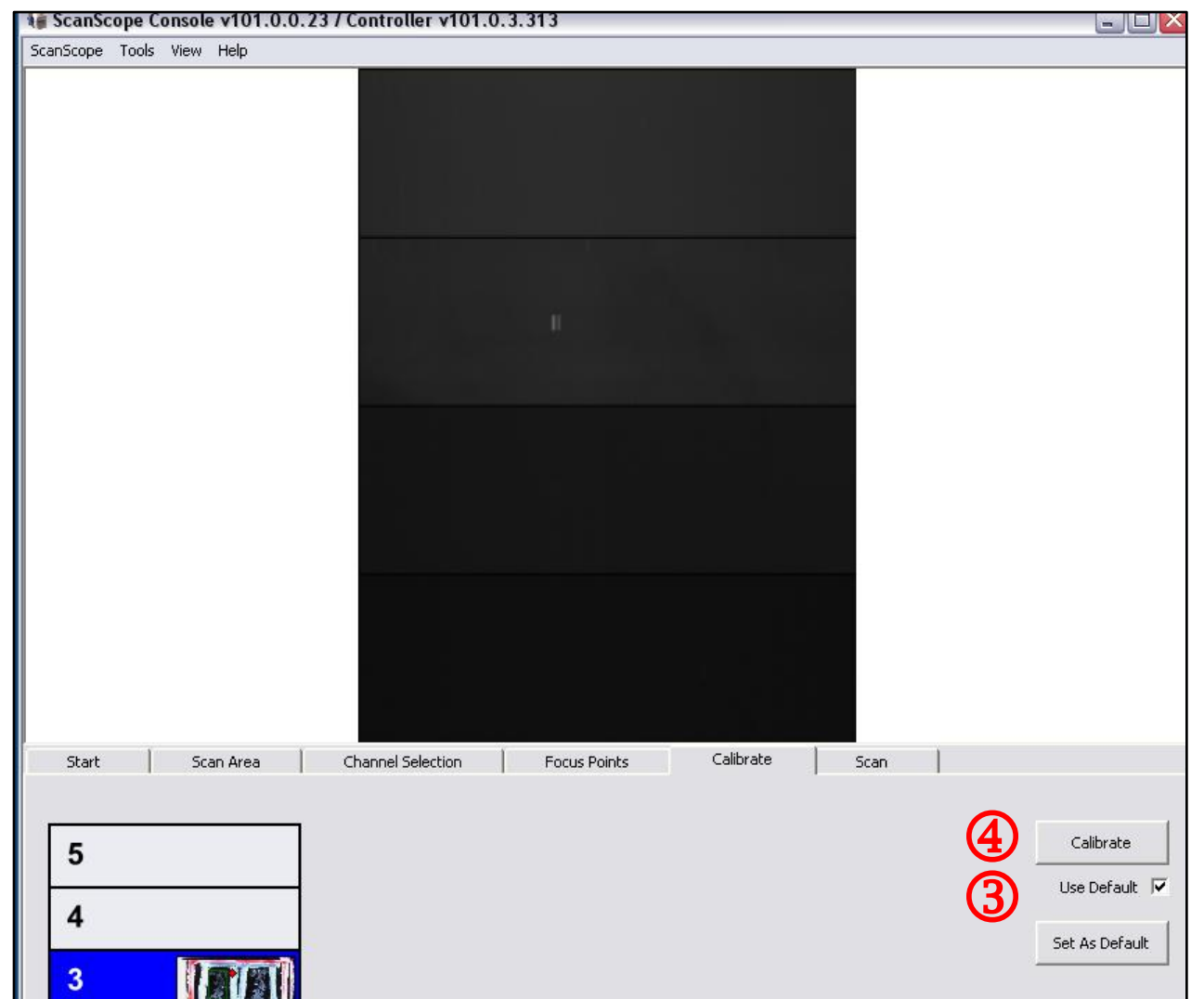


9. In the **Focus Points** tab, right click over tissue and select **Delete All Focus Points** ③. Re-size the scan area by clicking and dragging the edges of the green box to contain the entire section. Then, add new focus points throughout entire section by double clicking on tissue.



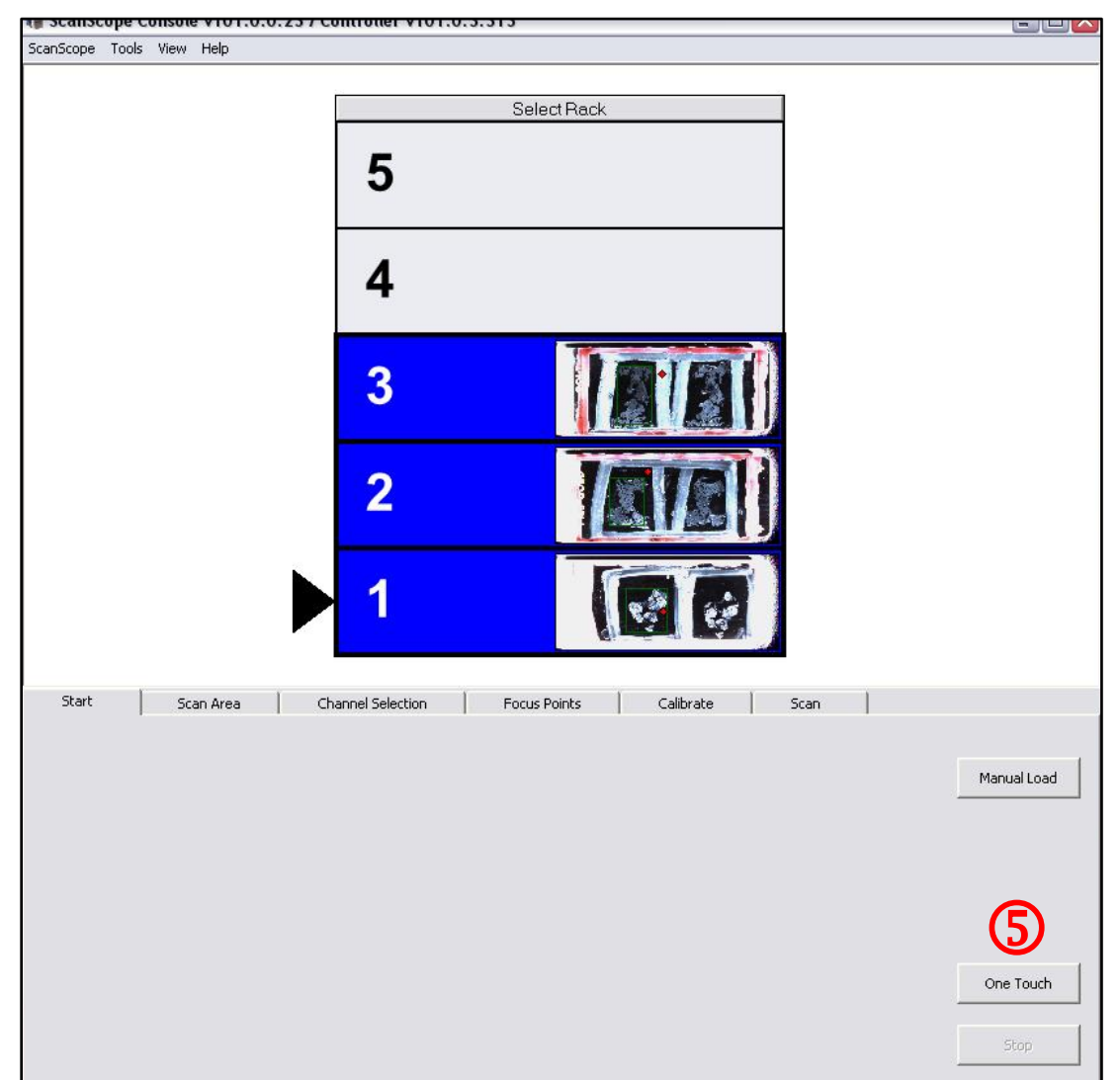
VI. Finalizing Slide Set-up and Scanning

1. Move to **Calibrate** tab and select **Use Default** box ③ (provided that calibration settings were set as default for test scan). Then click **Calibrate** button ④.



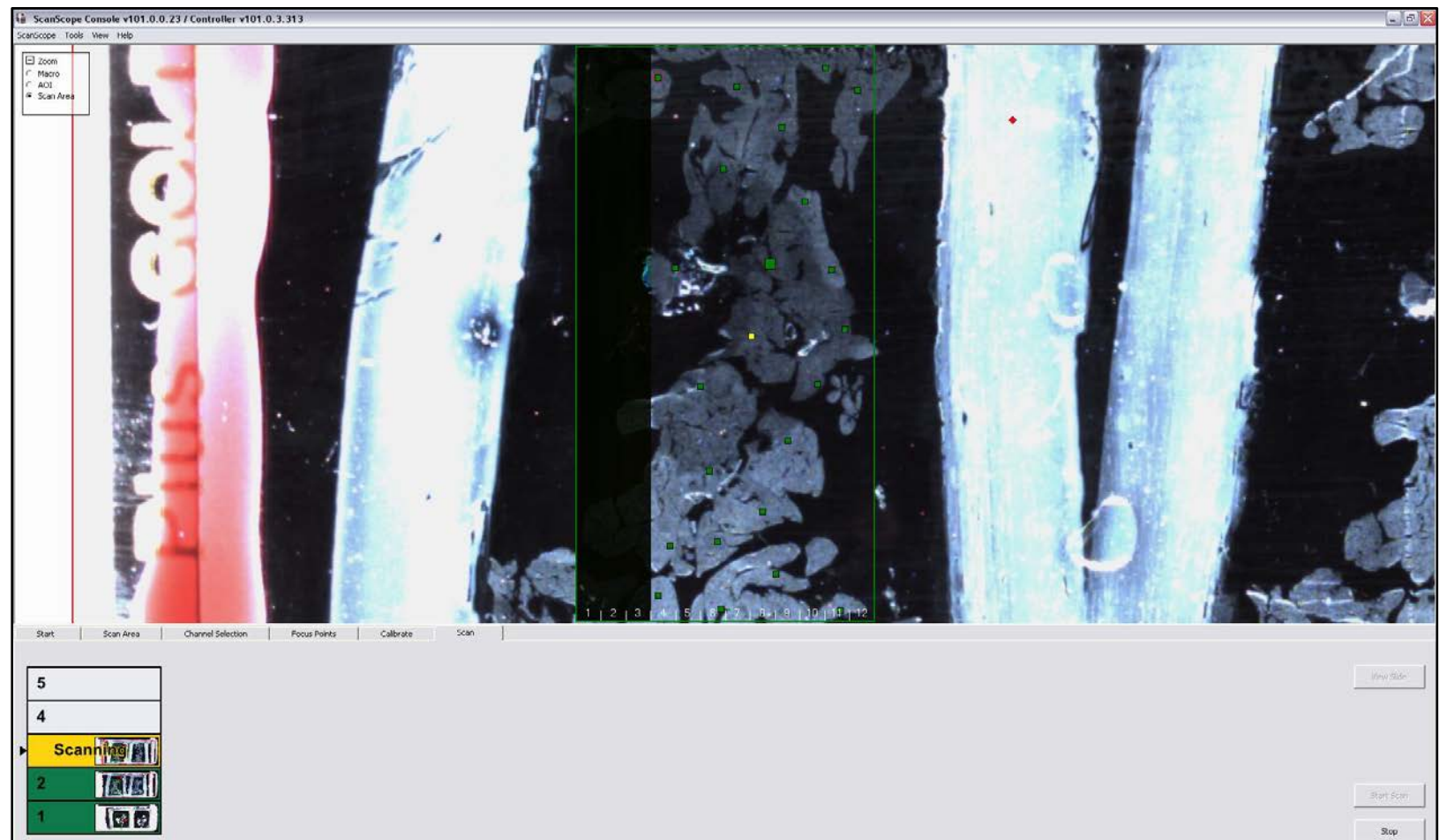
2. Move to **Start** tab and **Set Current Slide** to the next slide position.
3. Repeat steps 1-7 in the **Section IV. Adjusting Exposure**.
4. Move to **Calibrate** tab and select **Use Default** box ③ and click **Calibrate** button ④ as shown above.
5. Repeat steps 2-4 for remaining slides.

6. With scan areas, exposures, focus points, and calibration set for each slide, select **One Touch** ⑤.

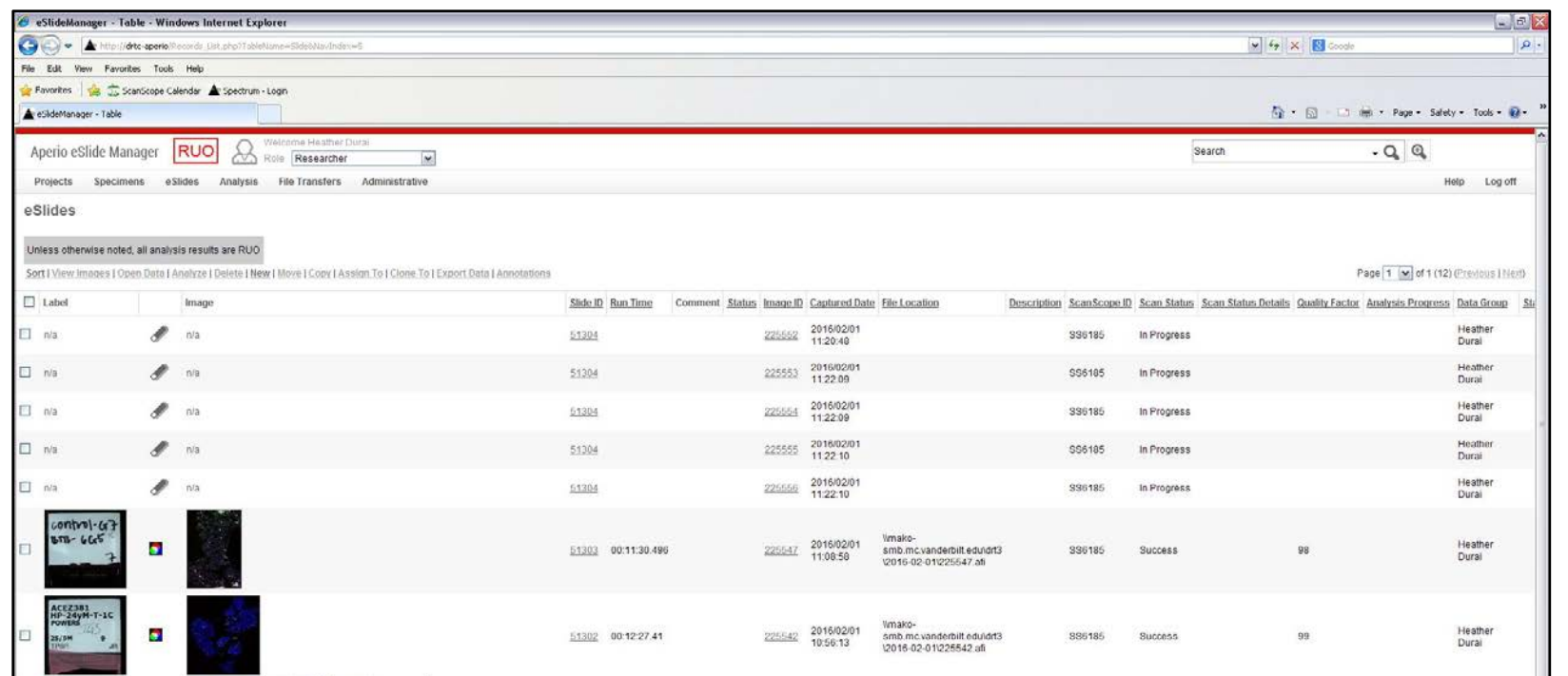


VI. Finalizing Slide Set-up and Scanning

7. Scanner will display a yellow slide with **Scanning** message for the slide currently being scanned. Successfully scanned slides will be displayed in green.



8. Scanned images and imaging progress can be managed in **eSlide Manager**.



9. When scanning is completed, move to **Scan** tab, right click, and select **Remove Slides** ①. This leaves the rack empty for the next user.

10. Remove slides from scanner and carefully replace slide tray into scanner. Leave system **ON** if there is another user signed up on the CORES calendar. If you are the last user of the day, turn system off in the **SAME ORDER** it was turned on.

