

# 8

## Setting up an experiment

### 8.1

#### Placing the sample

- 1 Attach the sample to the sample holder.
- 2 Place the sample holder into the cuvette.

**NOTICE** Ensure that no medium gets into the microscope chamber.

### 8.2

#### Instrument Mode

In the **Measurement Wizard**, you can select the **Instrument Mode** for the ordered variant.

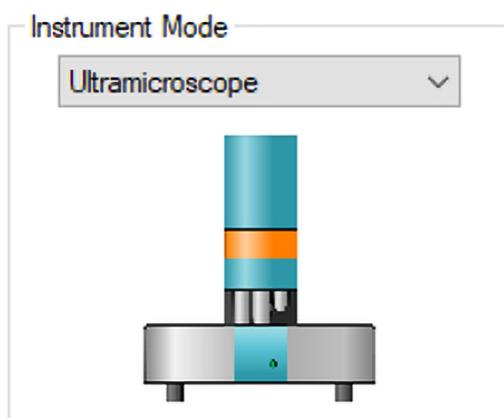


Figure 8.1: Instrument Mode list

All instrument modes are predefined by Miltenyi Biotec. If the settings need to be changed, contact Miltenyi Biotec Technical Support. See **Technical support on page 55**.

### 8.3

#### Measurement Mode

To acquire data, you must define the order of the devices to be used during acquisition. The user can freely define these devices by selecting them in the **Devices** lists or use predefined settings, called measurement modes. The available measurement modes can be selected in the **Measurement Mode** list.



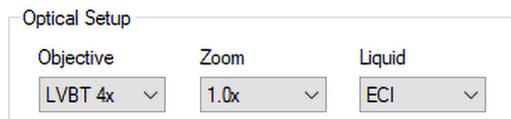
Figure 8.2: Measurement Mode list

All measurement modes are predefined by Miltenyi Biotec. If the settings need to be changed, contact Miltenyi Biotec Technical Support. See [Technical support on page 55](#).

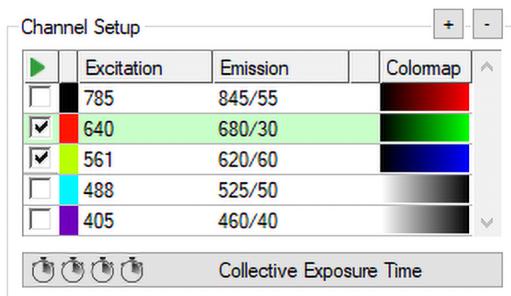
### 8.3.1 Aligning the horizontal focus on the sample

The alignment tool does not have exactly the same optical properties as a sample. Thus, the position of the horizontal focus must always be checked on the sample. This is also the case when changing objective lenses, as the FOV may differ slightly.

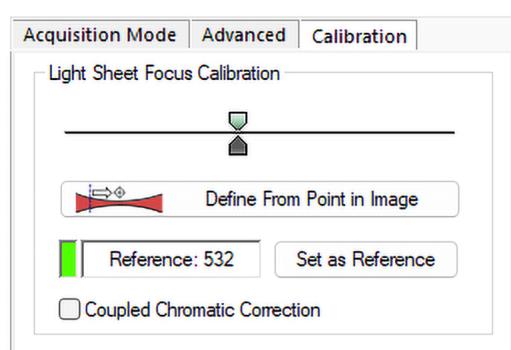
- Under **Optical Setup > Objective**, select the appropriate objective lens, the **Zoom** and the imaging solution used in the cuvette for **Liquid** in the **Settings 1** pane.



- Under **Channel Setup**, select the excitation and emission wavelengths for your sample.



- To set the current excitation wavelength as the new reference wavelength, click the **Set as Reference** button in the **Calibration** tab.



- Move the slider under **Laser Transmission Control** to set the laser power.

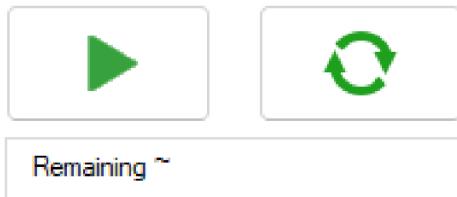
**NOTICE** Higher laser power bleaches the sample faster.

- Click **Light Sheet Selection > Select right light sheet**.





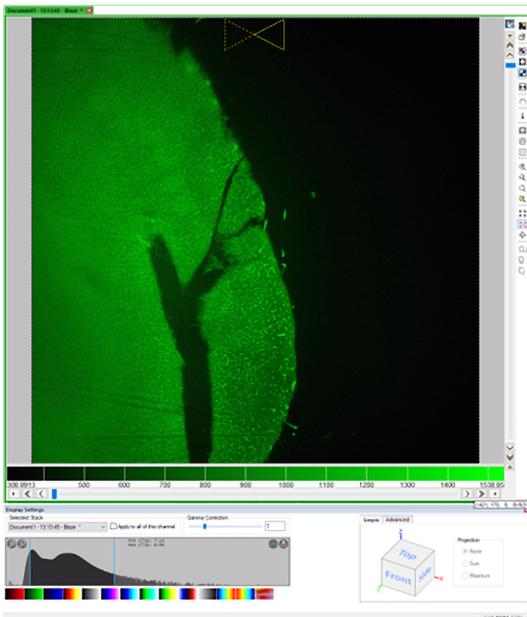
- 6 Click the **Start Live Preview** button in the **Measurement Wizard** to start the live preview.



- 7 Click and hold the **Objective Lens Focus > Move Detection Unit Down** button to move down the objective lens. The detection unit automatically stops.



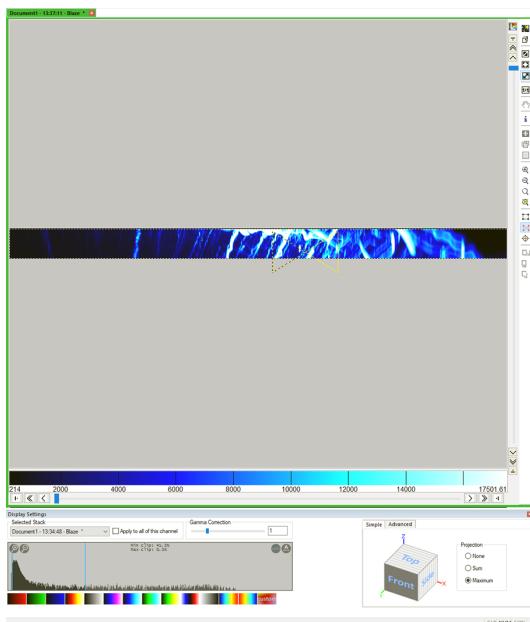
- 8 Use the jog wheel to move the sample inside the cuvette until the surface of the sample is visible in the FOV.
- 9 Focus on the sample.
- 10 Place the light sheet focus indicator on the sample, and check if it corresponds to the actual position of the light sheet focus. Move the top marker (**Global Offset**) of the **Light Sheet Focus Calibration** slider. Alternatively, click **Define From Point in Image** and place the small crosshair by clicking on the actual position of the light sheet focus.



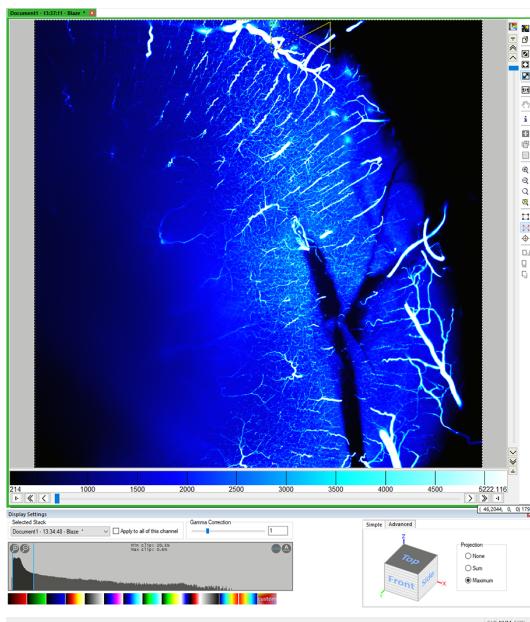
- 11 Move the light sheet focus indicator along the sample to check if the contrast is always best below.
- 12 Set up a z-stack of about 200 micrometers with two micrometers step size to confirm the calibration.
- 13 Select **Measurement Mode > Acquisition** and deactivate autosave.



- 14** Open **Display Settings** and perform a maximum projection of the front view. Check if the region with the best z-resolution corresponds to the indicated light sheet focus position. If necessary, click **Define From Point in Image** and place the small crosshair by clicking on the actual position of the light sheet focus.



- 15** Perform a maximum projection of the top view. Check if the region with the best contrast corresponds to the indicated light sheet focus position. If necessary, click **Define From Point in Image** and place the small crosshair by clicking on the actual position of the light sheet focus.



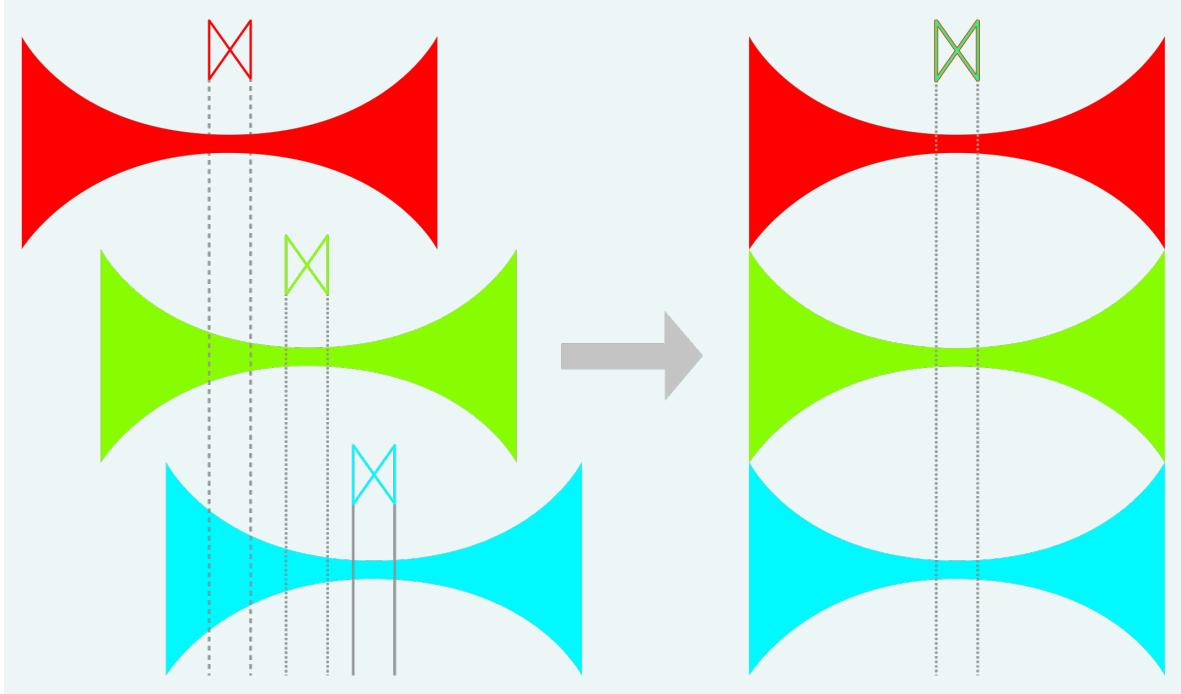
- 16** If the alignment is satisfactory, repeat the steps for the left light sheet.



- 17** Click **Save Settings** on the toolbar.

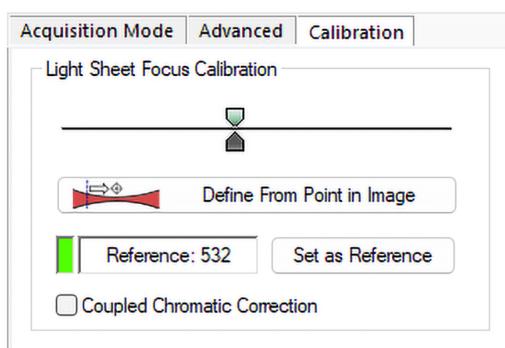
### 8.3.2 Correcting the chromatic offset of the horizontal focus

When using different wavelengths, the horizontal focus of each channel is in a different position compared to the reference wavelength defined in the **Calibration** tab. These chromatic offsets in the excitation path have to be corrected using a sample.



**Figure 8.3:** Chromatic offsets

- 1 Select the channel to be corrected.
- 2 Switch to the live preview, and focus on the sample.
- 3 Place the light sheet focus indicator on the sample, and check if it corresponds to the actual position of the light sheet focus. Move the bottom marker (**Chromatic Correction**) of the **Light Sheet Focus Calibration**. The marker changes color to the set color under **Channel Setup**. Alternatively, click **Define From Point in Image** and place the crosshair by clicking on the actual position of the light sheet focus.



- 4 Move the light sheet focus indicator along the sample to check if the contrast is always best below.
- 5 Optional: Capture a z-stack without autosave as described under **Aligning the horizontal focus on the sample on page 36**. If necessary, click **Define From Point in Image** and place the small crosshair by clicking on the actual position of the light sheet focus.
- 6 If **Coupled Chromatic Correction** is selected, the chromatic correction is applied symmetrically to the left and right light sheet. Otherwise, the chromatic correction has to be set individually for both light sheets.
- 7 Click **Save Settings** on the toolbar.



### 8.3.3

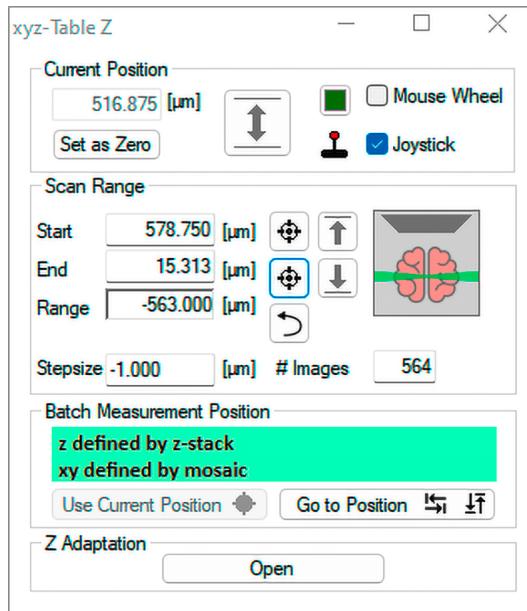
#### Acquiring an image



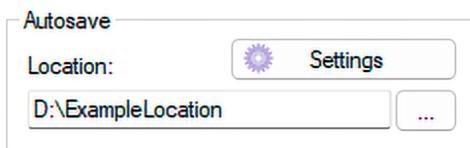
- 1 To start the live preview, click the **Start Live Preview** button in the **Measurement Wizard**.
- 2 Move in the z-direction to the start position.
- 3 Click **Current Position > Set as Zero** in the **Settings 2** pane.



- 4 Click the **Set current position as start** button under **Scan Range > Start**.
- 5 Move in the z-direction to the end position.
- 6 Click the **Set current position as end** button under **Scan Range > End**.
- 7 Define the step size, corresponding to the number of images, by entering it under **Scan Range > Stepsize**.



- 8 To stop the live preview, click the **Stop Live Preview** button. The laser is switched off.
- 9 Check **Settings** in the **Autosave** area of the **Experiment Manager**.



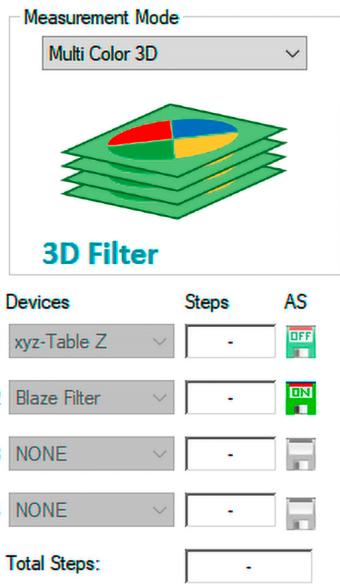
- 10 To start the acquisition, click the **Start Measurement** button.



### 8.3.4

### Acquiring a multicolor z-stack

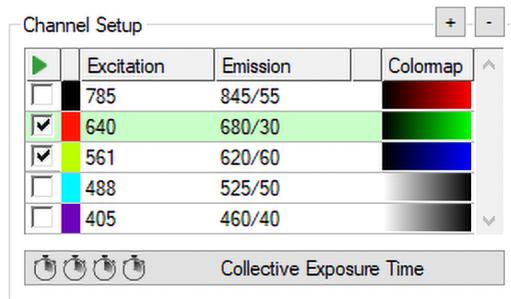
- In the **Measurement Wizard**, select **Measurement Mode > Multi Color 3D**.



- In the **Devices** list, select **xyz-Table Z** and **Blaze Filter**.



- Click the **Autosave** button at the end of the row under **AS**. The button turns green.
- Select the correct excitation and emission wavelengths for your sample under **Channel Setup** in the **Settings 1** pane. The selected checkboxes are used.



- Move the slider under **Laser Transmission Control** while the filter is highlighted to set the laser power for each individual filter.

**NOTICE** Higher laser power bleaches the sample faster.

- Select the shortest emission wavelength, and create a sharp picture.



- Correct the different focus lengths for different emission wavelengths using the **Chromatic Correction**, starting with the next longer wavelength.



- If all emission wavelengths have been corrected, click **Save Settings** on the toolbar.

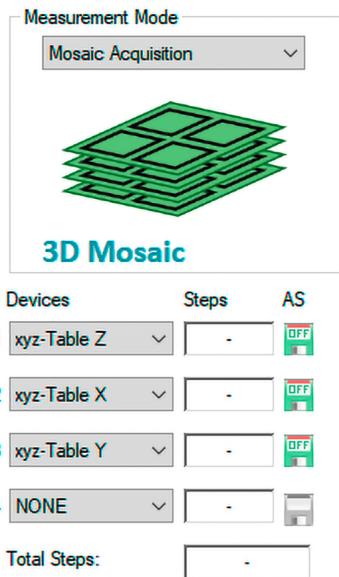


- To start the acquisition, proceed as described under **Acquiring an image on page 39**.

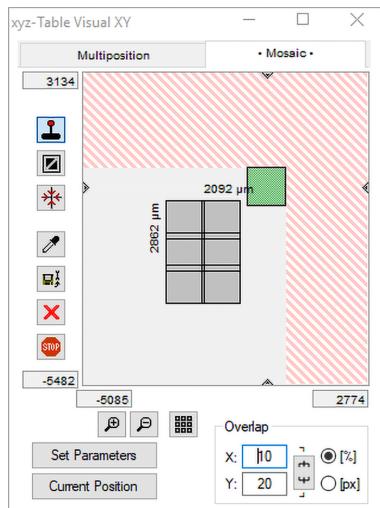
## 8.3.5

## Acquiring a 3D mosaic image

- 1 In the **Measurement Wizard**, select **Measurement Mode > 3D Mosaic**.



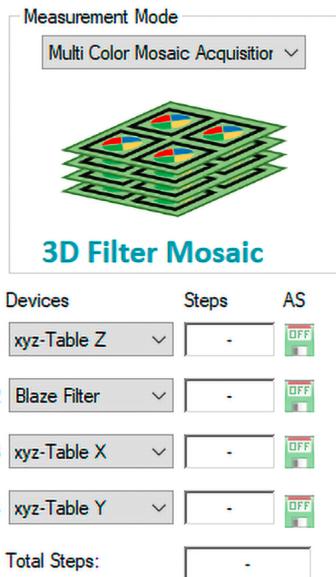
- 2 In the **xyz-Table Visual XY** view, click the **Mosaic** tab.



- 3 Delete all positions of this **Measurement Mode**.
- 4 Double-click the green tile representing the field of view. The tile turns gray.
- 5 Click and drag the black dots at the edges to create a mosaic.
- 6 To set an **Overlap**, enter a value and select percentage (%) or pixel (px).
- 7 Click the **Autosave** button at the end of the row under **AS**. The button turns green.
- 8 To start the acquisition, proceed as described under **Acquiring an image on page 39**.

### 8.3.6 Acquiring a multicolor mosaic image

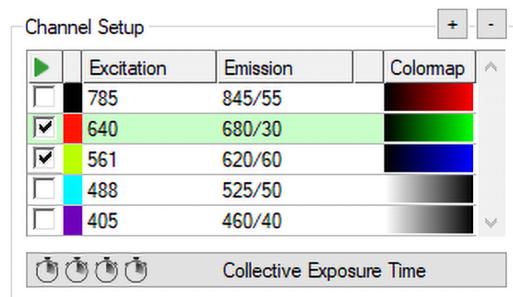
- 1 In the **Measurement Wizard**, select **Measurement Mode > Multi Color Mosaic Acquisition**.



- 2 In the **Devices** list, select **xyz-Table Z** and **Blaze Filter**.



- 3 Click the **Autosave** button at the end of the row under **AS**. The button turns green.
- 4 Select the correct excitation and emission wavelengths for your sample under **Channel Setup** in the **Settings 1** pane. The selected checkboxes are used.



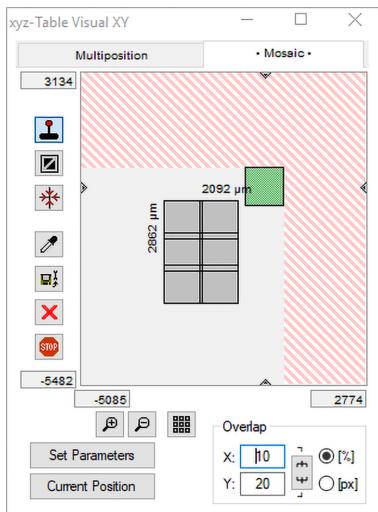
- 5 Move the slider under **Laser Transmission Control** while the filter is highlighted to set the laser power for each individual filter.

**NOTICE** Higher laser power bleaches the sample faster.

- 6 Select the shortest emission wavelength, and create a sharp picture.
- 7 Correct the different focus lengths for different emission wavelengths using the **Chromatic Correction**, starting with the next longer wavelength.
- 8 If all emission wavelengths have been corrected, click **Save Settings** on the toolbar.



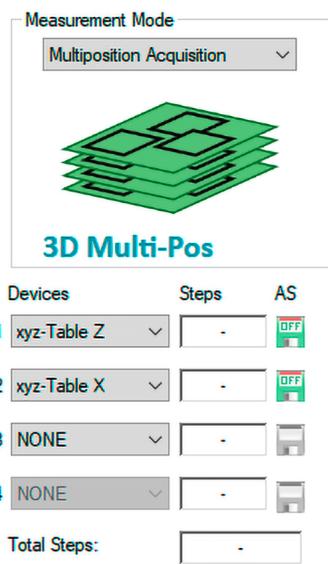
- 9 In the **xyz-Table Visual XY** view, click the **Mosaic** tab.



- 10 Double-click the green tile representing the field of view. The tile turns gray.  
 11 Click and drag the black dots at the edges to create a mosaic.  
 12 To set an **Overlap**, enter a value and select percentage (%) or pixel (px).  
 13 To start the acquisition, proceed as described under [Acquiring an image on page 39](#).

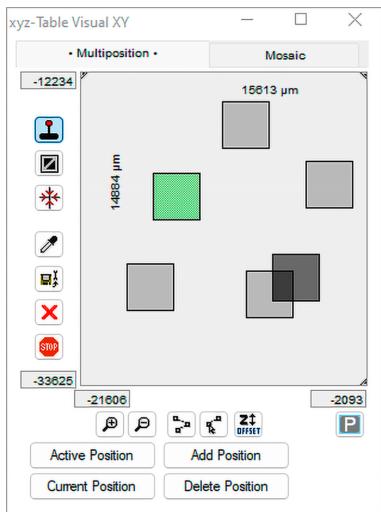
### 8.3.7 Acquiring a 3D multiposition dataset

- 1 In the **Measurement Wizard**, select **Measurement Mode > Multiposition Acquisition**.



- 2 In the **xyz-Table Visual XY** view, click the **Multiposition** tab.  
X 3 Delete all positions of this measurement mode.  
 4 Move the measurement positions to your region of interest.

- 5** Click **Add Position** for each position you want to add.



- 6** Click the **Autosave** button at the end of the row under **AS**. The button turns green.  
**7** To start the acquisition, proceed as described under **Acquiring an image on page 39**.

## 8.4

## Optimizing the measurement settings



- 1 Draw a rectangle over an illuminated and/or a dark region. If drawing is not possible, click the **ROI for Active Profile** button on the right in the stack window.



- 2 Click the **Auto Contrast** button to achieve a better contrast.
- 3 Click the **Rayleigh ROI** button to show the horizontal focus. Move the horizontal focus in the picture until you have the optimal contrast for your region of interest.
- 4 Select a suitable acquisition mode:
  - **Dynamic Focus**
  - **Fast Tiling Scan**
  - **LightSpeed Mode** (only available if ordered)
- 5 Optional: Move the slider under **Light Sheet Properties > Sheet NA** to reduce the numerical aperture of the light sheet. This creates a more homogeneous light sheet, but reduces the resolution in the z-plane.
- 6 Optional: Move the slider under **Light Sheet Properties > Sheet Width** to reduce the sheet width of the light sheet. This results in more light being focused on a smaller region of the sample.



The settings described may need to be adjusted when the zoom settings are changed.