Acquire image

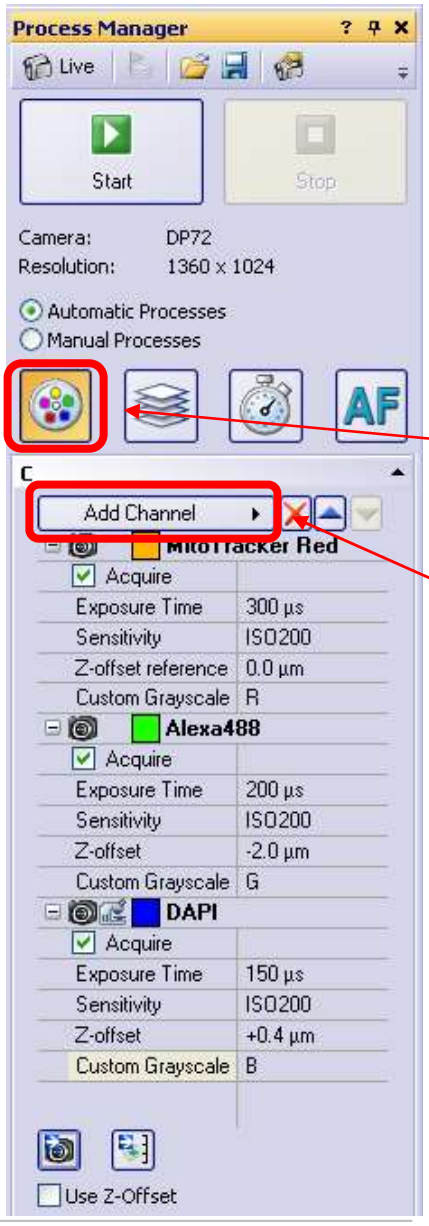
① Acquire multicolor image

1. Carry out all the microscope settings. In the **[Microscope Control]** tool window, click the desired objective's button. Click the observation method with the excitation that has the longest wave length (e.g., " MitoTrackerRed ") in the **[Observations Methods]** toolbar.

2. Go to the **[Camera Control]** tool window.

   - If you are working with a color camera click the Toggle **[RGB / Grayscale Mode]**  button and change to the gray-value mode.
   - Set the image resolution for the acquisition. Reduce the image resolution in the live mode to fasten live fps.

3. Switch to the live mode and bringing the sample into focus. Should the exposure time become longer than 300 ms, reduce the exposure time by increasing the sensitivity resp. the gain.



   - If you are using DP72 **[Custom Grayscale]** is effective to minimize the exp time as well.
   - In the camera's black & white mode you can reduce the diffused light by clicking the **[Online-Deblur]** button in the toolbar at the top of the tool window.

4. Go to the **[Process Manager]** tool window, located on the right-hand side of the user interface. Select the **[Automatic Processes]** option. Click the **[Multicolor]** button to the appeared clicked status, to make the **[C]** group appear.

5. Click the **[Add Channel]** button. Select the first color channel (e.g., " MitoTrackerRed "). Select the other channels (e.g., "Alexa488" and "DAPI") in the same manner.

6. Click in the first color channel.

   - Camera: The camera icon means that this channel will really be acquired. Click in this field to have this color channel not acquired during the acquisition process, resp. to have it again also acquired. In this way you can quickly remove a channel from a process, or

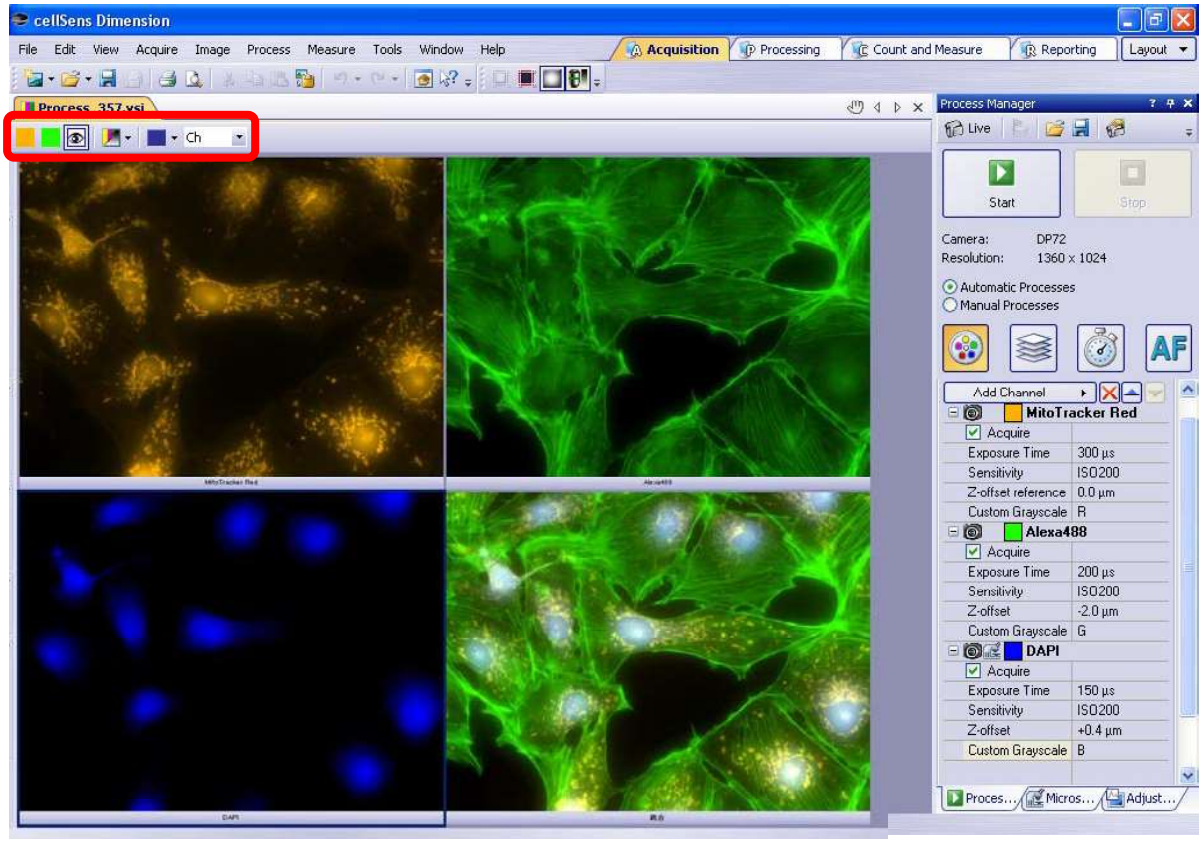
add one to it, without having to change the basic process.

- Microscope: The microscope icon means that this channel's observation method is currently set on your microscope.
- Fluorescence color: The color field shows the current fluorescence color.
- Additional properties of the color channels: Click the **[+]** sign in front of a channel to view additional channel properties. These include the exposure time and the sensitivity resp. gain of the acquisition.

7. Click in the fist color channel. The channel has now been activated. Switch to the live mode. Select manual exposure time in the **[Camera Control]** tool window. Optimize the exposure time and the sensitivity resp. gain, for this channel. Click the **[Read Settings]**  button, located under the color channels, in the **[Process Manager]** tool window. The exposure time and the sensitivity resp. gain, will be adopted in this channel. Activate each of the other channels, optimize the two parameters and adopt them in the channels. Finish the live mode.

8. Click the **[Start Process]** button in the **[Process Manager]** tool window. The multi-channel acquisition will start immediately. The image window will be divided into the individual channels so that you can directly observe the individual images.

9. The **[Start]** button will then change itself into the **[Pause]** button and the **[Stop]** button will become active. That's how you'll recognize that the acquisition is running. You can make a pause in the acquisition process, or cancel it completely. In the status bar, located at the bottom left of your user interface, you will find a progress bar, the number of images already acquired, and their total (e.g., 1/3).

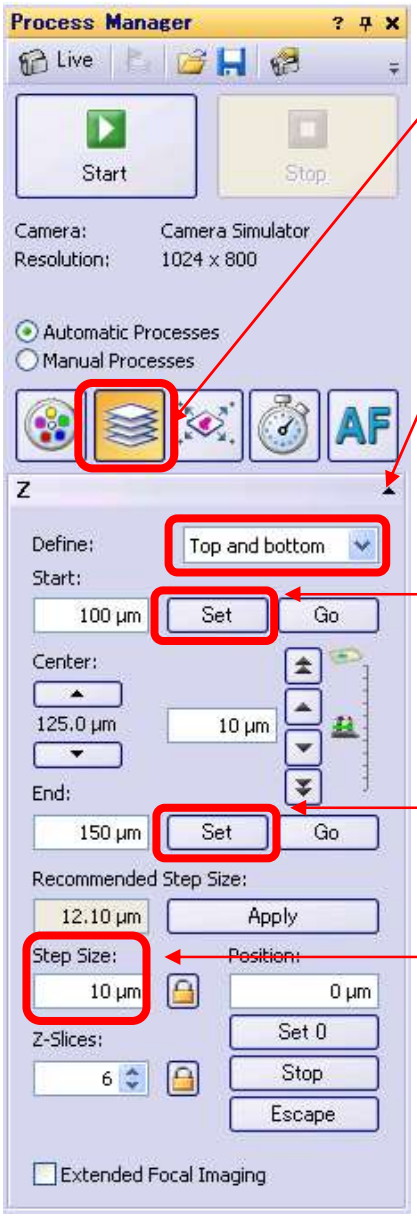


10. The acquisition has been completed when you can again see the [Start] button in the [Process Manager] tool window. The navigation bar will be displayed at the top of the image window. It contains a button for each channel, to enable you to display or hide that channel  . The eye icon indicates that the channel is currently visible. Click these buttons one at a time and take a look at each of the channels individually.

② Acquire Z-series image

1. In the [Microscope Control] tool window, click the button corresponding to the objective and observation method you've set. Go to the [Camera Control] tool window. Switch to the live mode. Optimize the exposure time. The software makes sure that the exposure time is kept constant during the Z-stack acquisition.

2. Go to the [Process Manager] tool window. Select the [Automatic Processes] option. Click the Z-stack button to the appeared clicked status, to make the [Z] group appear.



3. Select the [Top and Bottom] entry in the [Define] list. While observing the live-image, move your microscope focus upwards, to the Z-position at which the lowest-lying sample structures are sharply focused. Click the top [Set] button. The current Z-position will be adopted in the [Start] field.

4. Move your microscope focus downwards, to the Z-position at which the highest-lying sample structures are sharply focused. Click the bottom [Set] button. The current Z-position will be adopted in the [End] field.

5. In the [Step Size] field, enter the distance between two frames in the Z-stack. If you are using high NA objective you can minimize this distance. [Recommended Step Size] shows the automatically calculated according to the objective's NA. Click the [Apply] button, located next to the [Recommended Step Size] field, and transfer the necessary distance in the [Step Size] field. The number of frames in the stack will be automatically calculated on the basis of the Start and End values, and the Z-spacing. Make sure that the [Lock Parameter]  button next to the field isn't in the clicked status.

6. Finish the live mode and click the [Start] button.

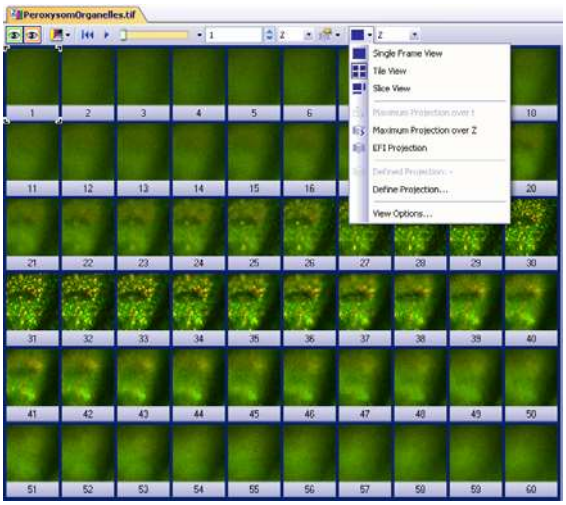
The acquisition of the Z-stack will start immediately. In the image window you will see the last frame that has been acquired. You can follow the changing of the focus directly. The **[Start]** button will then change itself into the **[Pause]** button and the **[Stop]** button will become active. That's how you'll recognize that the acquisition is running. You can make a pause in the acquisition process, or cancel it completely. In the status bar, located at the bottom left of your user interface, you will find a progress bar, the number of frames already acquired, and their total (e.g., 3/7).

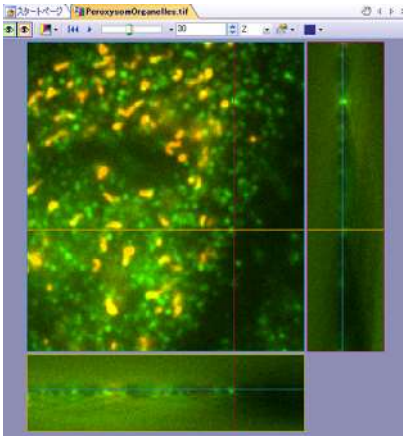
7. The first Z-frame will be displayed in the image window. The navigation bar will be displayed at the top of the image window. Move the slider slowly to the right, and go through the sample by changing the focus. Take a look at the Z-stack's Z-frames.

8. Click the little arrow next to the **[Tile View]** button in the navigation bar, then you can select below types of image display.

   - Single Frame View: By default you will find yourself in the single view. In the single frame view, only one image will be shown in the image window.

   - Tile View: Use the tile view to attain an overview of all of the individual images that make up a multi-dimensional image. In this view, you can also select individual images.

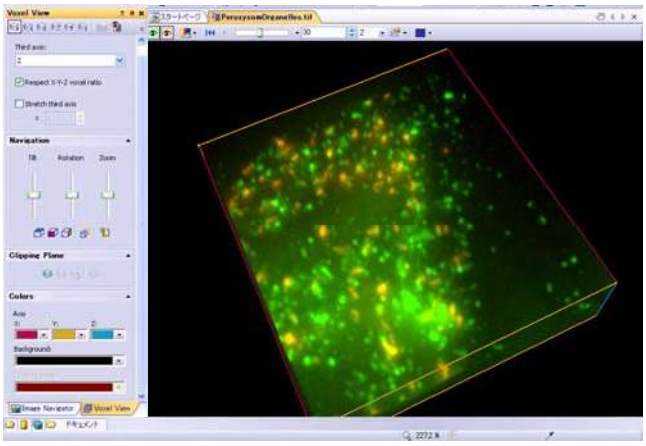




   - Slice View: The Slice View image window view is only available for image series. An image series can be e.g., a time stack or a Z-stack. Use this view to look at cross sections of an image series. In it, the cross sections lie perpendicular to the X, Y and Z-direction (t-direction).

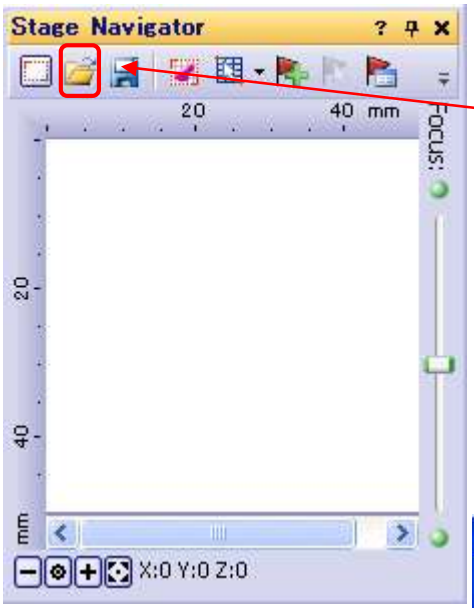
   - Voxel View: (3D deconvolution option is necessary)： By using the Voxel View, you can display an image series spatially. Voxel is a made-up word and stands for volume element. A voxel is the

smallest part of a three-dimensional image. It is the result of a pixel to which a height is assigned. With a Z-stack, this height is equivalent to the Z-distance between 2 frames. One can also describe a voxel as a 3D-pixel. The intensity in a voxel is constant.



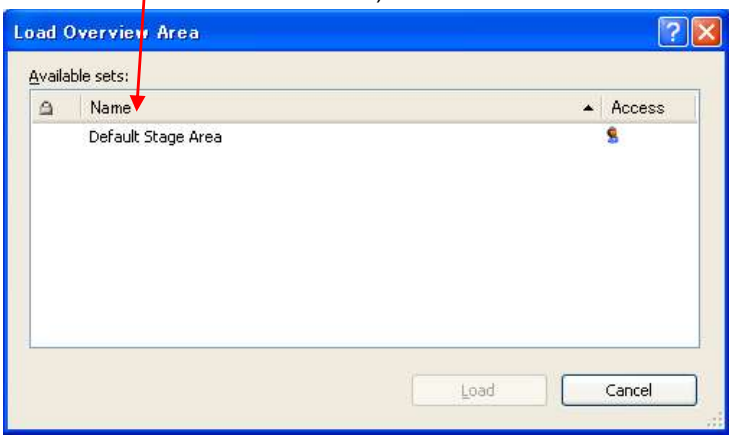
③ Preparation for MIA image acquisition (Stage navigator setting)

1. Carry out all of the necessary microscope settings. Set the objective with the smallest magnification. In the **[Microscope Control]** tool window, click the button corresponding to the objective you've set. Go to the **[Camera Control]** tool window. Switch to the live mode. Optimize the exposure time. The software makes sure that the exposure time is kept constant during the overview acquisition. Bring the image into focus.

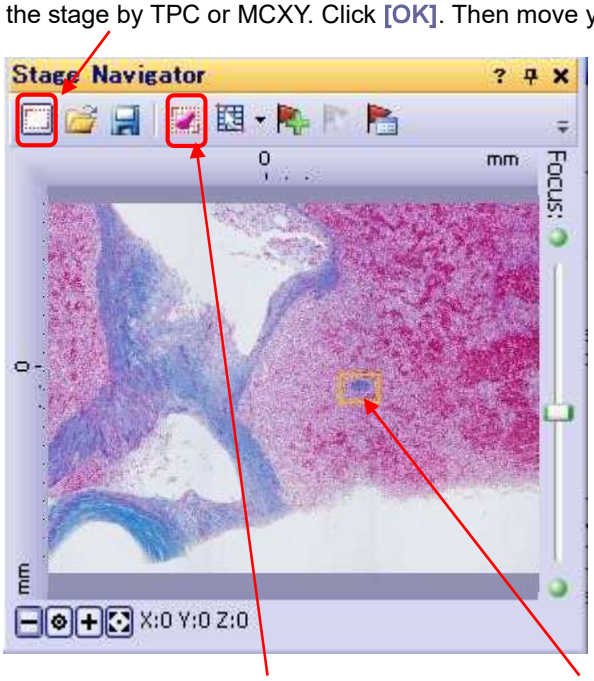


2. Go to the **[Stage Navigator]** tool window, located at the bottom left of the user interface. Click the **[Load Overview Area]** button, at the top of the tool window.

3. Select the **[Default Stage Area]** entry, and then click **[OK]**. The white field in the **[Stage Navigator]** tool window shows the complete stage area, as it has been defined by stage limits, during the calibration. (In the process, the overview area defined in the calibration will be overwritten.)



4. Click the **[Define Overview Area]** button, at the top of the tool window. The live-image will appear. You'll receive the request to move your stage to the top left vertex of the complete sample, so move the stage by TPC or MCXY. Click **[OK]**. Then move your stage to the bottom right vertex. Click **[OK]**.



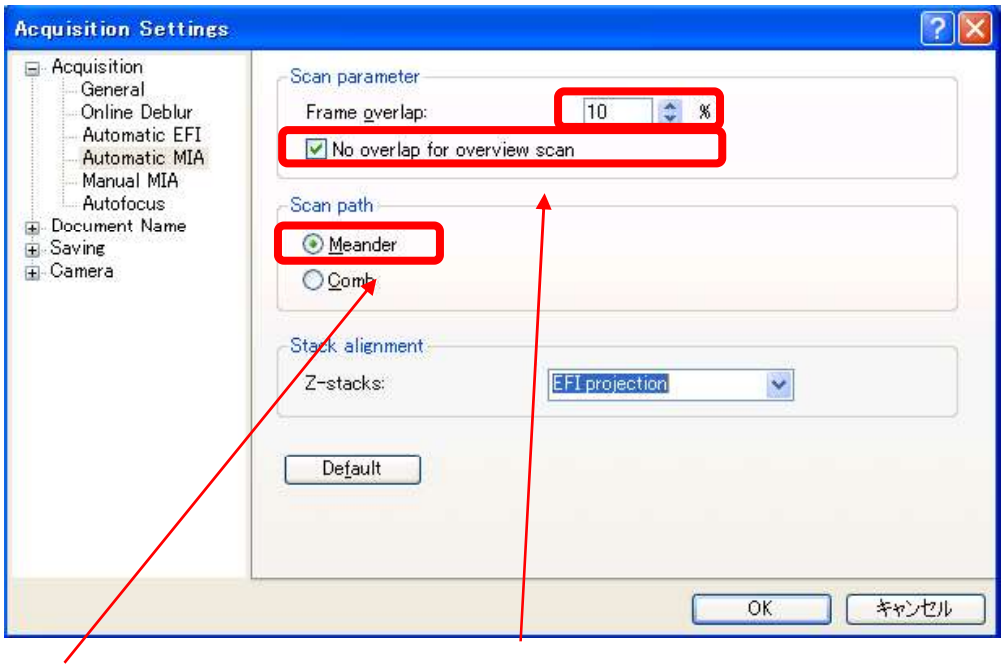
5. Click the **[Acquire Overview]** button, at the top of the tool window. The overview acquisition begins immediately. (Next to the progress bar located at the bottom left of your user interface, you'll find the Cancel button, use it when you want to cancel the overview acquisition.) The individual images will be acquired and placed side by side without being calculated against each other. In the image window, you can watch how the overview image grows. The small rectangle in the **[Stage Navigator]** tool window shows you the current stage position. The size of the rectangle depends on the objective magnification used.

6. Grasp the tool window by its header, and release it from its docked position. Enlarge the tool window. Take a look at the overview image. At the bottom of the **[Stage Navigator]** tool window you'll find a small button 

, with which you can change the overview image's zoom factor. Click the **[Zoom In]** button, or alternatively, use your mouse wheel, to zoom into the image. You can grasp the overview image with your left mouse button depressed, and move it. Take a closer look at some of the more significant positions on the sample. The **[Center current frame]** button shows the area around the current stage position. Click the **[Zoom]** to **[Fit]** button when you want to see the complete overview image again.

④ Acquire MIA image

1. Go to the **[Process Manager]** tool window, located on the right-hand side of the user interface. Then click the **[Acquisition Settings]** button in the toolbar at the top of the tool window. Open the **[Acquisition]** > **[Automatic MIA]** entry in the tree view. In the Frame overlap list, a value of 10% is in many cases sufficient. It can make sense to use a higher value when you scan a sample with little structure.

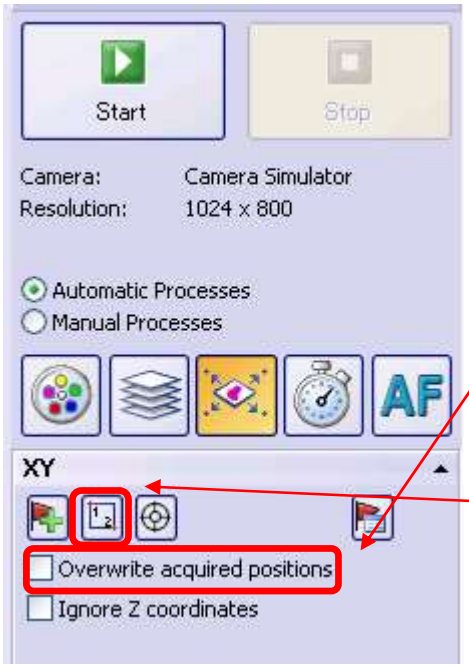


Make sure that the **[No overlap for overview scan]** check box has been selected. Select the **[Meander]** option in the **[Scan path]** group. A stage path in the meander form is shorter than one in the comb form, which results in a shorter acquisition time during the process. (The comb form stage path does offer the advantage of a higher exactness; this however, is irrelevant for MIA acquisitions). Click **[OK]**.

2. Select the objective and go to the **[Camera Control]** tool window. Switch to the live mode. If you want to scan a large area of the sample, the composite image can become very large. To reduce the file size, you can reduce the camera's resolution. In the **[Snap/Process]** list, located in the **[Resolution]** group, select the resolution for the individual images.

   Optimize the exposure time. The software makes sure that the exposure time is kept constant during the MIA acquisition. Bring the image into focus. Finish the live mode.

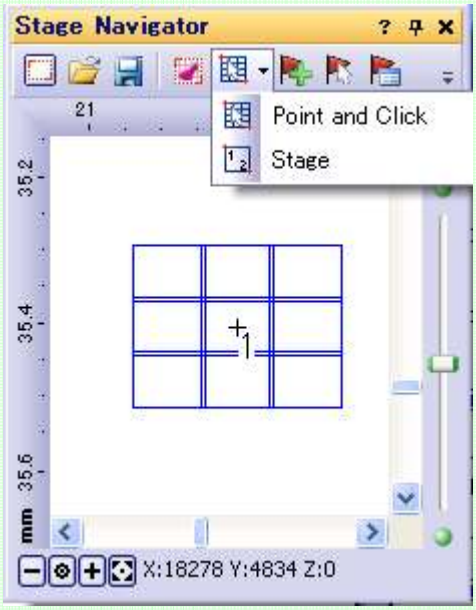
3. Go to the [Process Manager] tool window. Select the **[Automatic Processes]** option. Click the **[XY-Positions MIA]** button to the appeared clicked status, to make the **[XY]** group appear.



4. You can define the scan area within the overview area, that you determined. To begin with, select the **[Overwrite acquired positions]** check box. By doing this, you make sure that scan areas that have already been acquired, won't be scanned again, when you define a new scan area. (Otherwise, the software makes a note of all of the previous scan areas and XY-positions, then moves over them one after the other, during the MIA acquisition.) Click the **[Define MIA Scanning Area with Stage]** button, located in the **[XY]** group.

5. The live-image will appear. You will receive a message. Use the joystick to move the stage to the top left vertex of the area that you want to scan. Click **[OK]**. Then move your stage to the bottom right vertex. Click **[OK]**. Select the **[Ignore Z coordinates]** check box. Then the current focus position will be used. (Otherwise, the Z-position from the definition of the scan area is used.)

MIA POINT!



If you have acquired an overview image of your sample, you can define scan area more easily!!

You can set a rectangular area on your sample, for the acquisition of a stitched image. Click ▼ next to **[Create MIA Scanning Area]** and select **[Point & Click]** button. You can now use your mouse, with depressed left button, to define the MIA scan area, on the image area in the **[Stage Navigator]** tool window. For this definition, you won't need to move your stage. The scan area will be displayed with blue grid and you can immediately know how many frames are necessary to acquire the MIA.

6. To execute autofocus during the processes, bring **[Software Autofocus]** button, located next to the **[XY Positions/MIA]** button to clicked status, to have the Autofocus group displayed. In the Software Autofocus group, select the **[Multiposition / MIA]** check box, then select the **[Once at position]** or **[Every MIA frame]**option. (When you do this, the starting value of the autofocus depends on whether the Ignore Z coordinates check box has been selected, or not.)

   - **[Once at position]**: execute autofocus at the first frame of a MIA
   - **[Every MIA frame]**: execute autofocus with every MIA frame

7. Click the **[Start Process]** button. The MIA acquisition will start immediately. The individual images are acquired, then immediately assembled. In the image window, you can watch how the stitched image grows. The **[Start]** button will then change itself into the **[Pause]** button and the **[Stop]** button will become active.