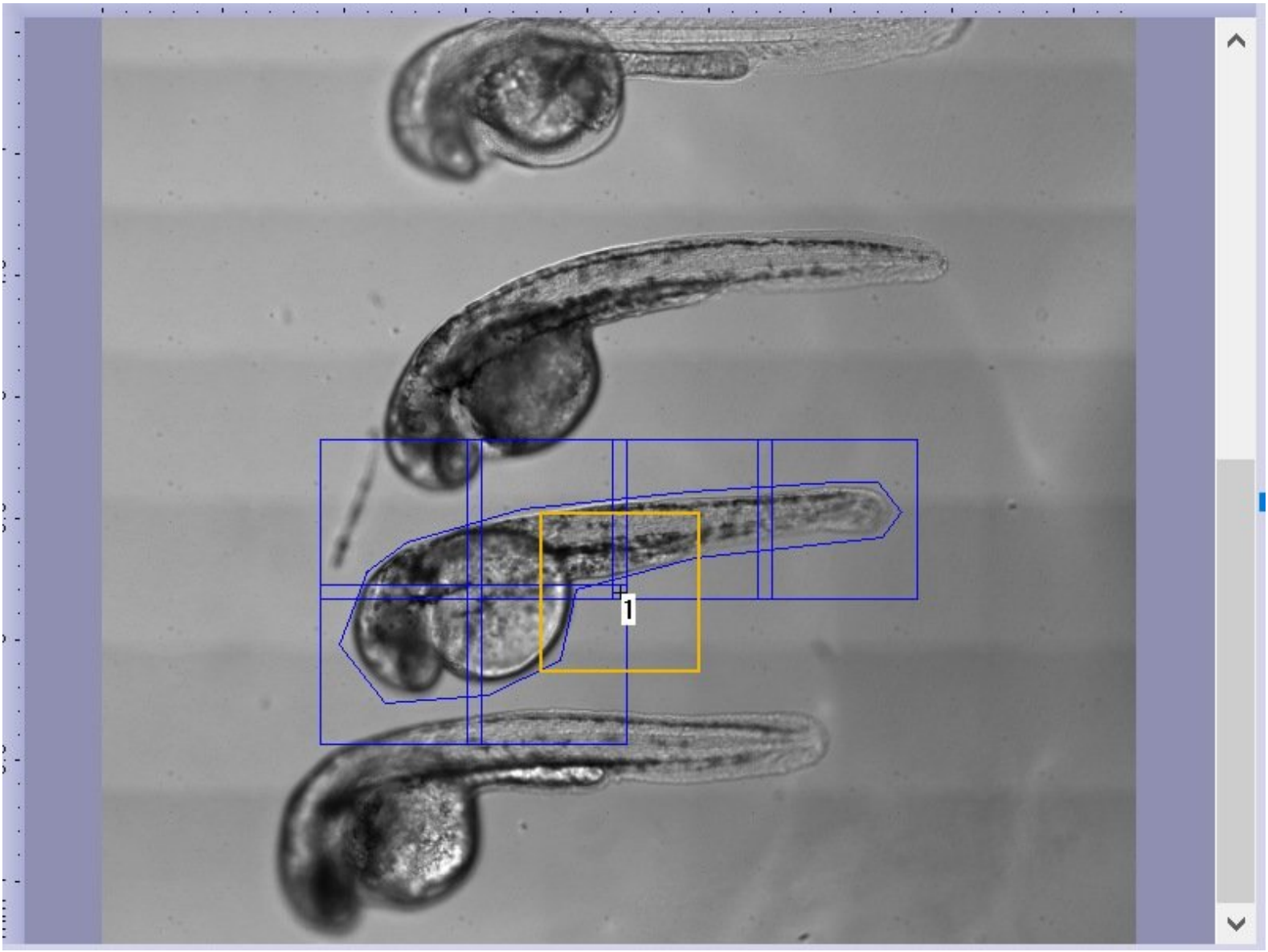
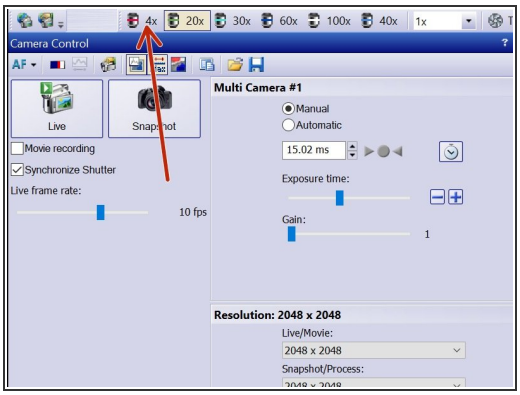
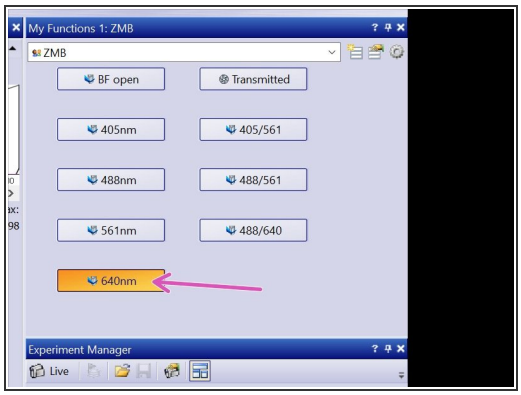
Olympus Spinning Disk - 3: Overviews + multi-positions + xyzt images

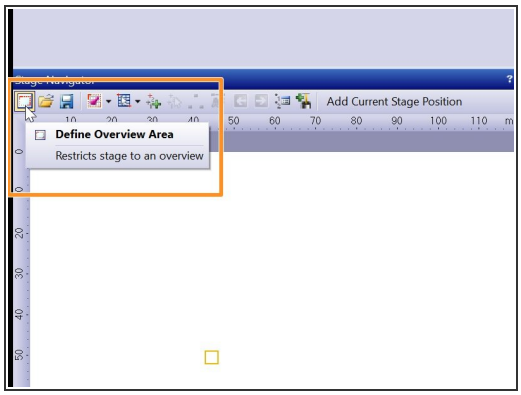
Written By: Joana Raquel Delgado Martins



Step 1 — Define an overview



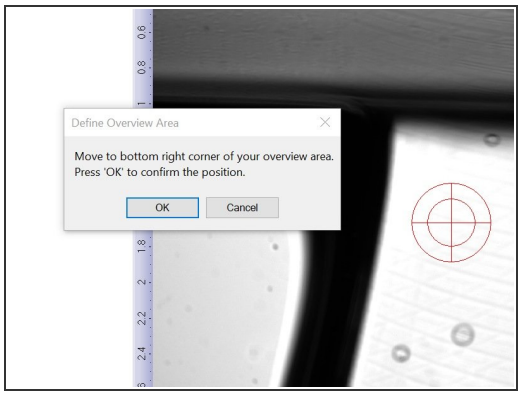


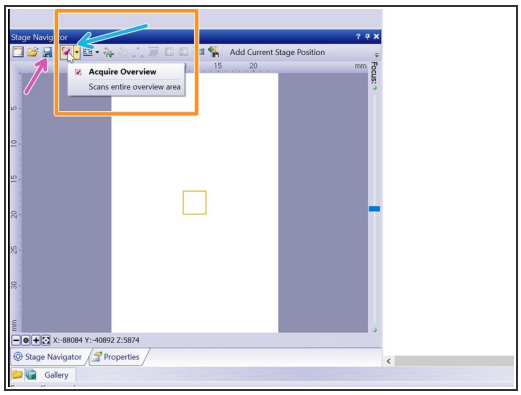


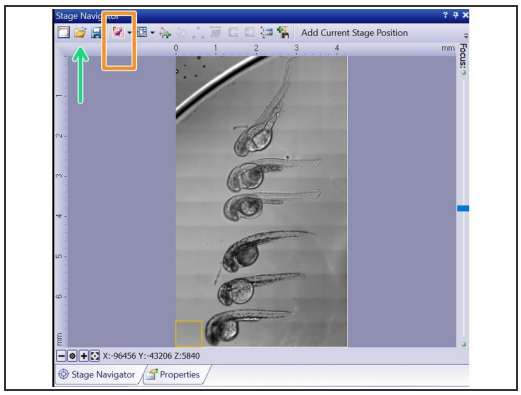
ⓘ When acquiring high resolution images, it can make sense to acquire an overview image of your sample at a lower magnification. This allows you to identify and navigate between your positions/regions of interest.

- Choose the 4x objective (or another low magnification objective).
- Choose the imaging setting you want to use for the overview (e.g BF, 640) and adjust it accordingly (laser intensity, exposure time) in the Live mode.
- You can define the overview area on the **Stage Navigator** window (bottom left).
- Click "Define Overview Area".

Step 2 — Define an overview





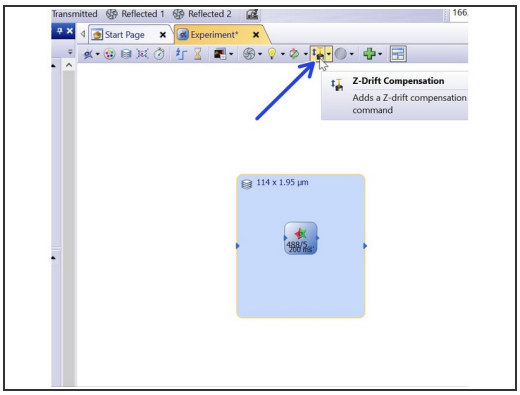


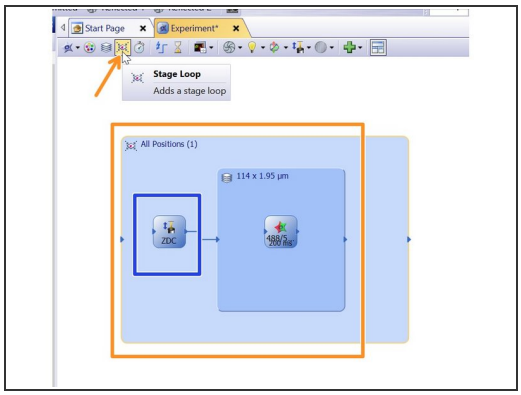
- Move to the top left and bottom right as indicated in the pop up messages.
- Click here to initiate the overview.
  - It is also possible to acquire a multichannel overview using the dropdown menu.

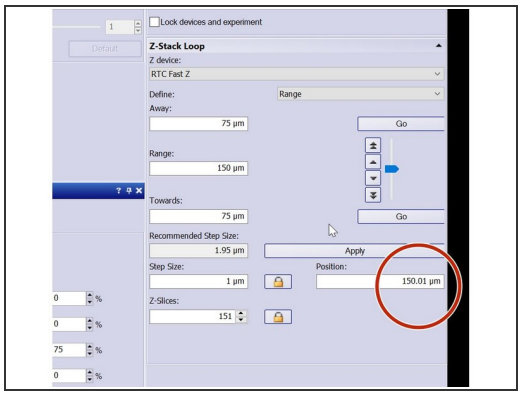
⚠ It won't be possible to add positions outside the overview.

- You have the option tosave your overview here.
- Here you can load the "Default Overview Area" or other previously saved overviews.

Step 3 — Define your imaging settings







- Select your objective of choice and define your imaging settings.

ⓘ If you need further information on how to set up your imaging please refer to the appropriate guides:

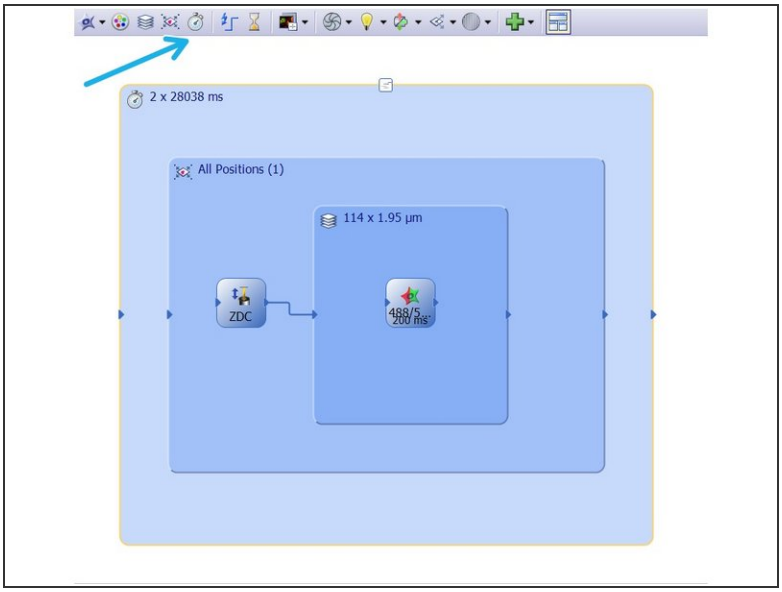
ⓘ Spinning disk mode: Multichannel + Z-stack acquisition

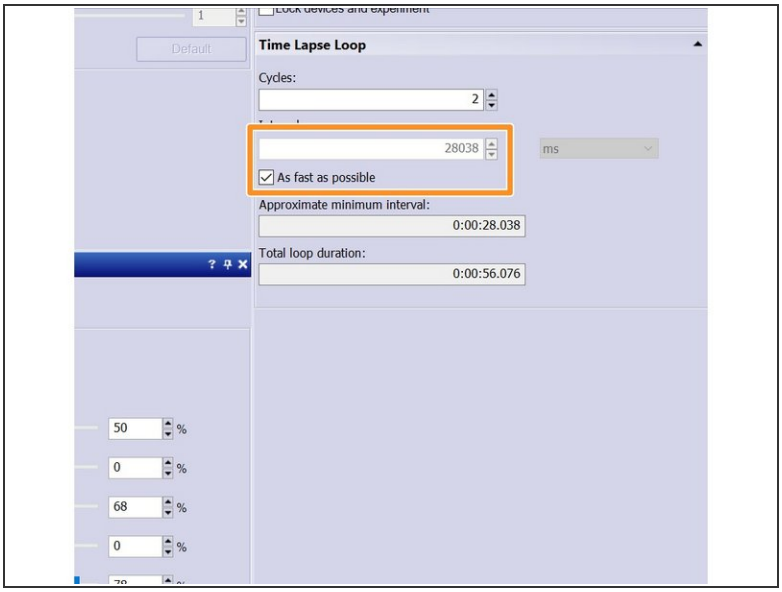
ⓘ Widefield mode

- Add the hardware autofocus through a **Z-Drift compensation** command before your acquisition sequence.
- Add a **Stage loop** to define the positions you would like to image.

⚠ While using RTC Fast for z-stacks please make sure it is in the appropriate position (middle=150um) before starting to define positions.

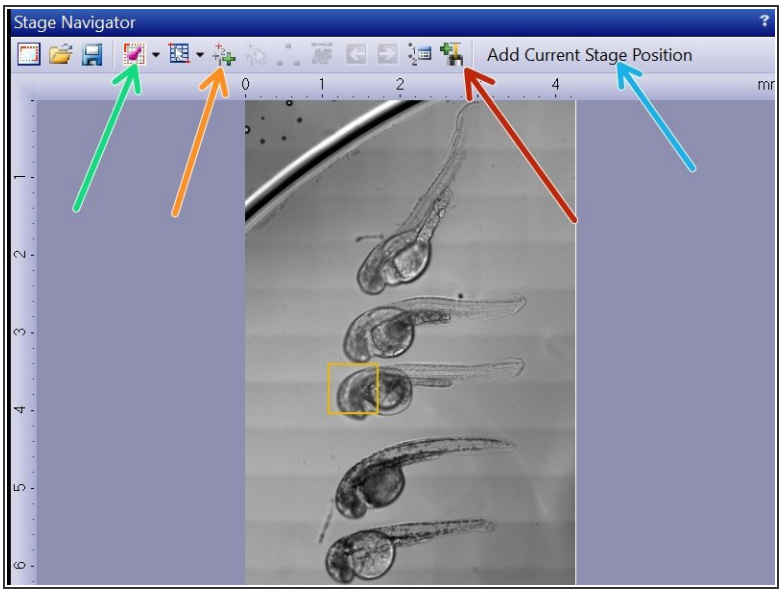
Step 4 — Defining your imaging settings - timeloop

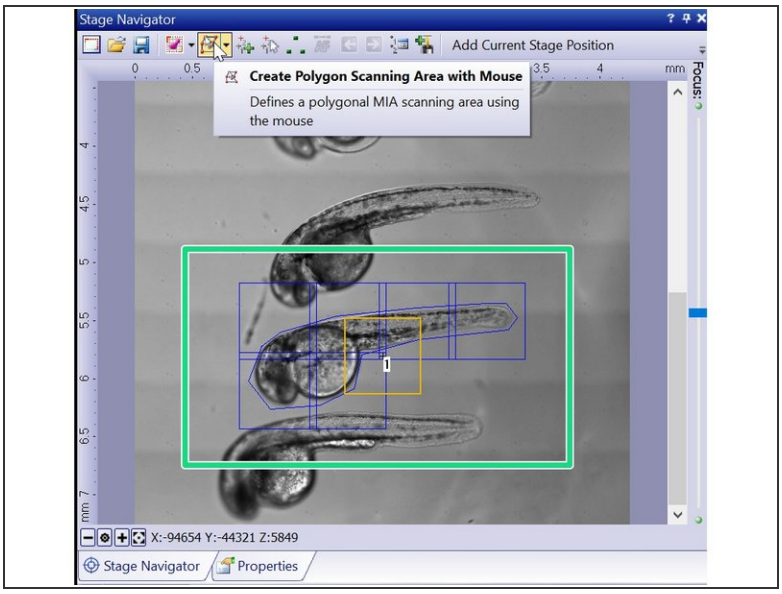




- Additionally you can also add a time lapse command if you wish to loop through all positions.
  - Here you can define the time interval
  - Or choose the minimal possible time interval by checking the "As fast as possible" box.

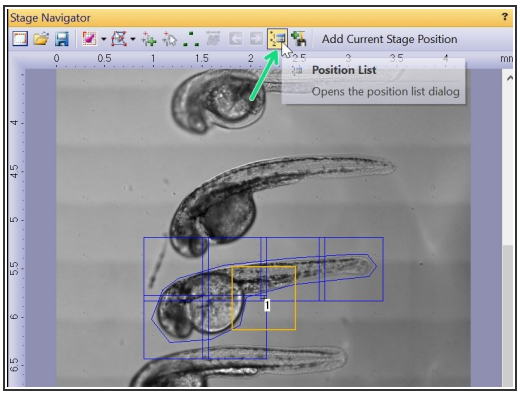
Step 5 — Adding positions

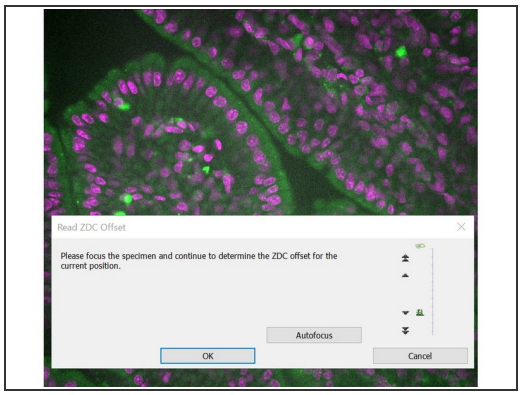


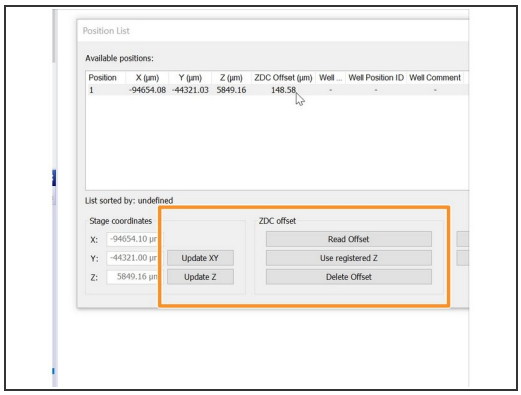


- Activate ZDC offset measurement when adding a new position.
- Go to your first position.
  - You can either add the current stage position here,
  - or add a select position directly in the overview.
  - To create a stitched image of a larger area than your field of view you can acquire and generate a MIA (Multiple Image Alignment).

Step 6 — Read position offset







- After adding a new position, focus your specimen.

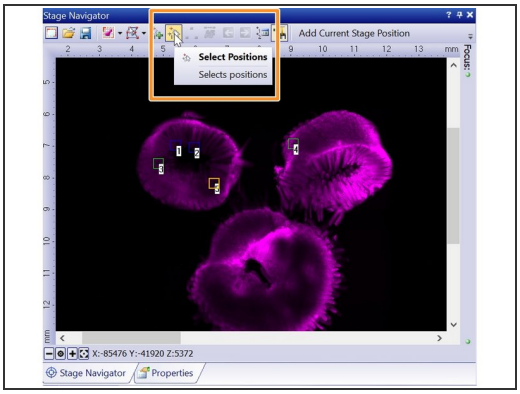
ⓘ In a MIA the central position will be used to assess the ZDC offset.

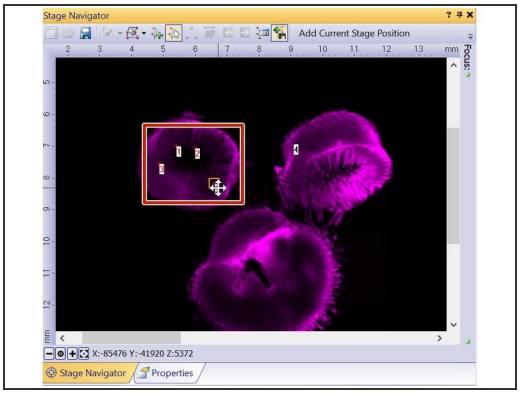
- Click OK once you are satisfied with your focus.

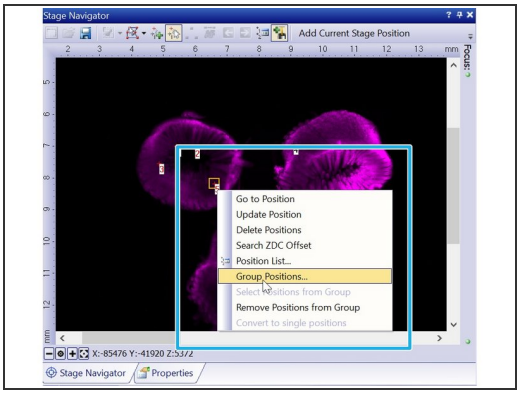
ⓘ You will hear a beep if the offset is successfully determined. If you hear 2 beeps, the measurement failed. Check for any air bubbles in immersion oil and repeat the measurement.

- You can check the coordinates of your positions in the Position List.
- Here you can also redefine xy or z coordinates as well as re define the ZDC offset.

Step 7 — Optional - Group positions





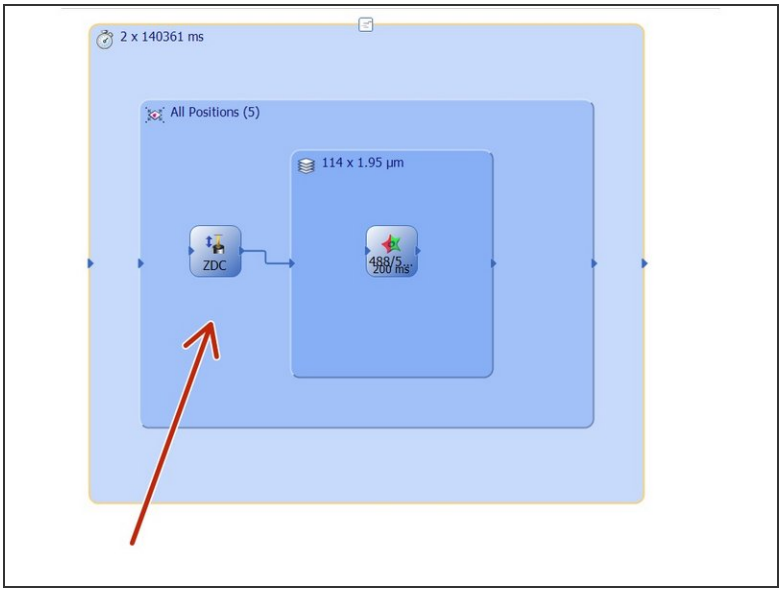


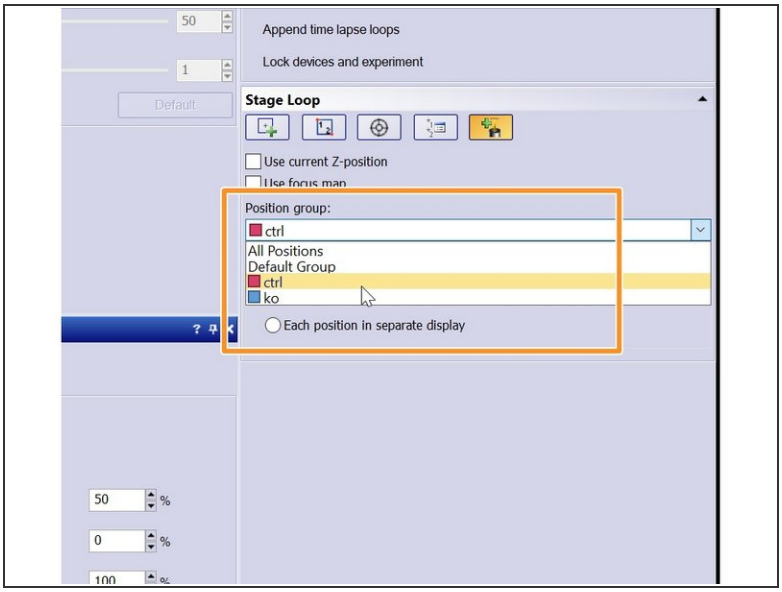
- Once you've added all your positions you can group them (e.g. according to the imaging protocol you'd like to run on each group).
- Use the arrow tool to select your positions (for multiple positions keep the shift key pressed while selecting the different positions)

ⓘ Selected positions will be formatted in red.

- Hover over the selected position and right click. Select **Group positions**.
- Name your new group.

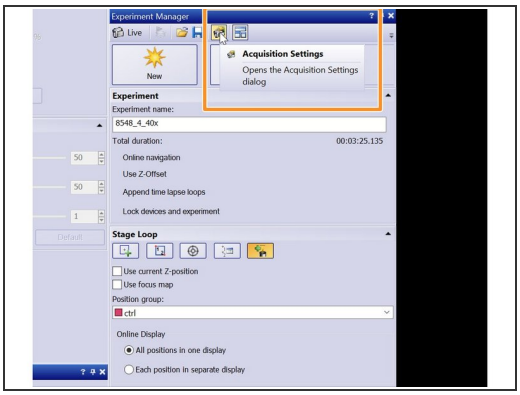
Step 8 — Optional - group positions

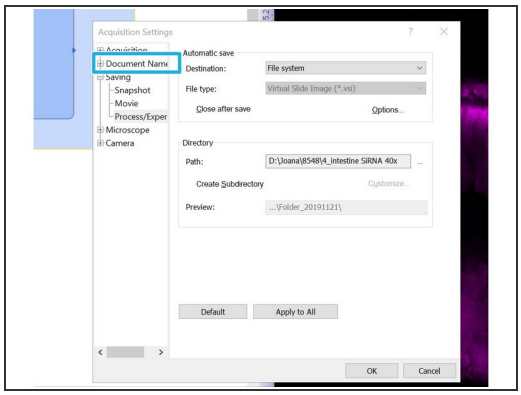


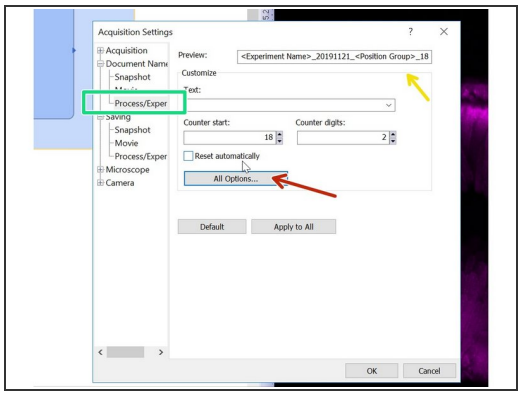


- Click on the stage loop box.
- You can now select (bottom right) if you want to image all your defined positions or just a restricted group .

Step 9 — Optional - document naming



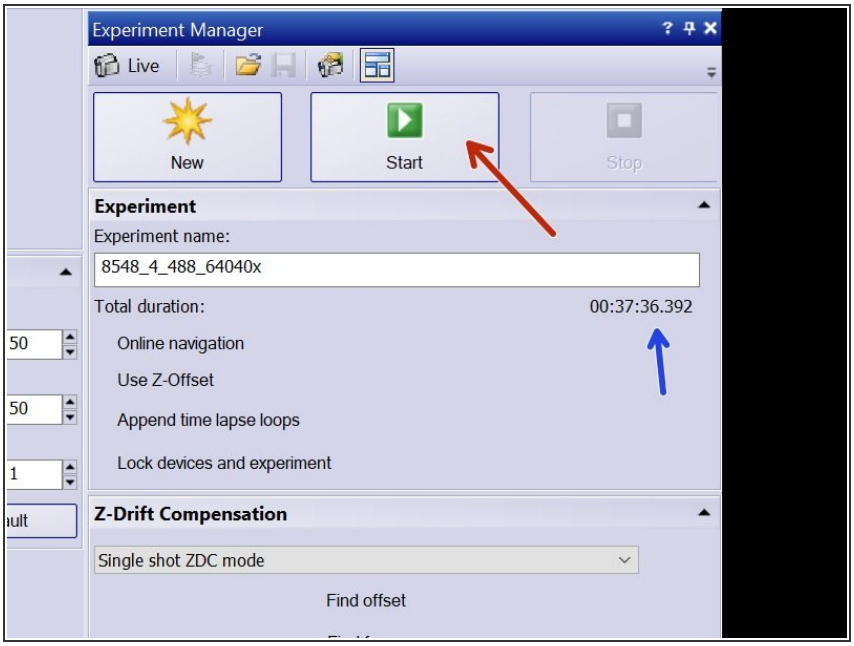




Under the **Experiment Manager** window you can redefine how you'd like to name your images.

- Open Acquisition settings.
- Chose "Document naming".
- Choose Process/Experiment.
- Here you can see how the naming is currently defined.
- Here you can redefine the document naming.

Step 10 — Start your experiment



- Here you can check what is the predicted total duration of your experiment.
- You can now start your experiment!

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