

Guide to High-Throughput Imaging

High-Throughput Imaging on Indigo

Automated high-throughput experiments can be setup on **Axio-Observer Indigo** to capture images in multiple positions/wells using fluorescence or brightfield. **MosaiX**, to capture large areas, can be combined with **Mark and Find**, to image entire wells, and further with **Timelapse**, to follow changes in these wells over time.

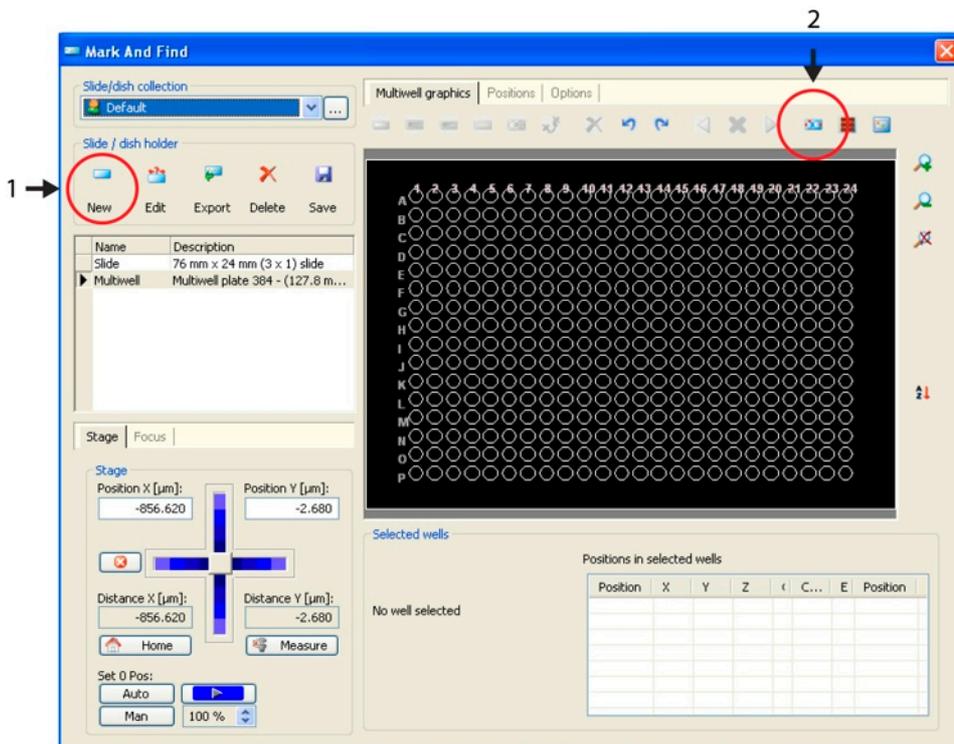
Setting Up a High-Throughput Experiment

Setting Up Channels

1. Setup the desired channels under **Multidimensional Acquisition** - see Axio Imager/Observer Guide.
2. Check the exposure time is suitable for all the wells in the experiment.
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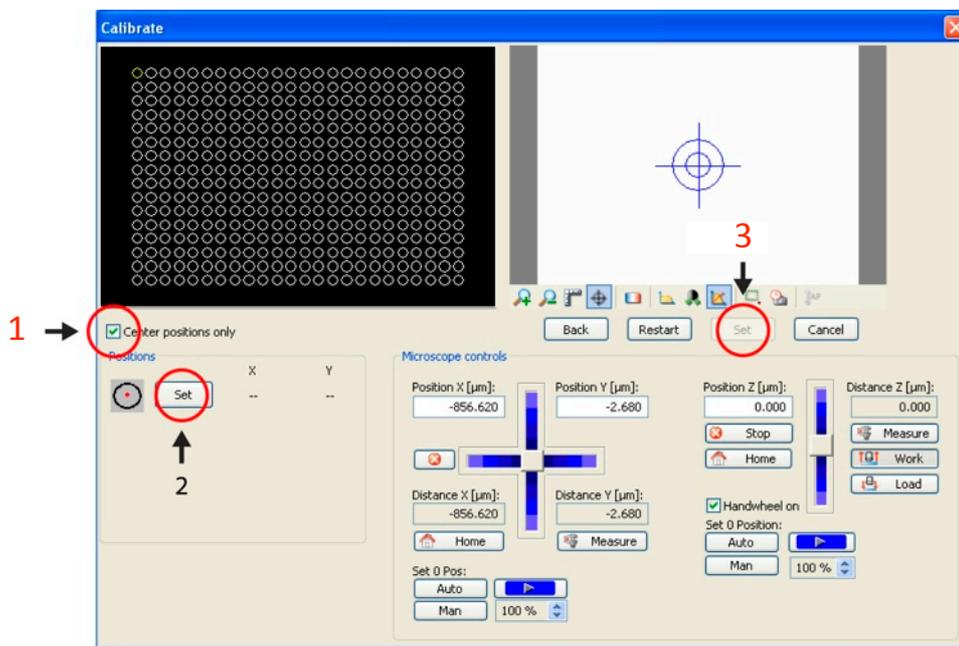
Setting Up Mark and Find

1. Click on the Mark and Find tab in Multidimensional Acquisition.
2. Tick on "positionlist" box at the top.
3. Click **MarkFind** – this will open the mark and find setup window.
4. In the Mark and Find setup window click **New**. (1)
 - In the window that opens select the type of plate you are using from the drop-down list and click "ok" - (in this case a 384 well plate is being used).
5. The outline of the plate will appear in the setup window.
6. Click on the "well calibration wizard" button at the top of the setup window. (2)
 - In the window that opens click "yes".

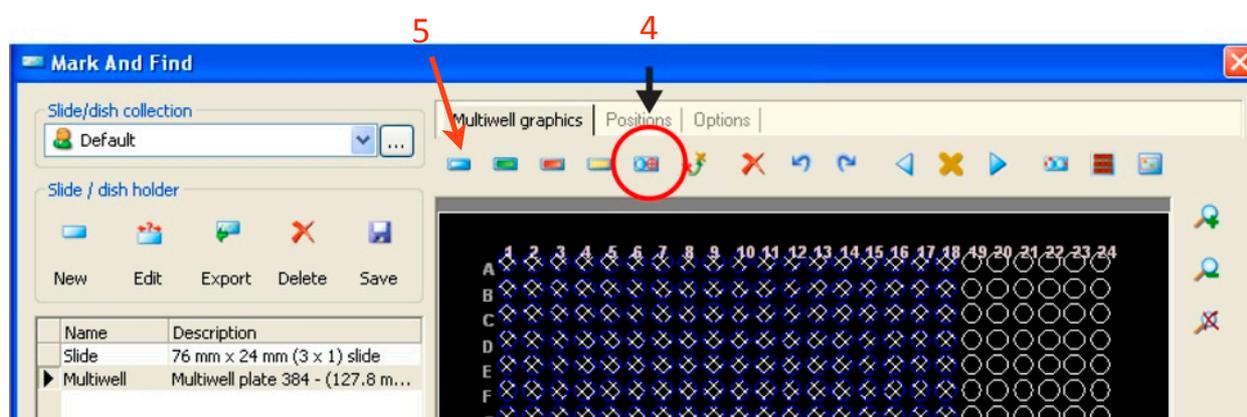


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7. In the “plate calibration” window that appears (as below) tick the **centre positions only** box. **(1)**
8. Move the stage so that you are in the centre of the top left well and click **set**. **(2)**
 - It is easiest to do this in **brightfield using the 2.5x objective**.
9. After pressing **set** the stage will automatically move to the top right well, adjust this so that it is in the centre and press **set** **(2)** again.
10. Repeat for the bottom right well.
11. The plate is now calibrated – click **set** in the top right. **(3)**



12. Now that the plate is configured you can double click any of the wells in the **Mark and Find** window and the stage will move to that well.
13. To create centre positions in the wells you are interested in click and drag a selection box over the desired wells then click the **generate centre positions for all wells** button **(4)**
 - Alternative you can add additional wells by holding the **Ctrl** button and clicking wells individually.



- *Instead of creating centre positions (for mosaix) it is also possible to double click a well, then move to regions of interest within that well and place a marker for imaging that particular spot - use the coloured buttons to do this. **(5)***

14. Once your positions are marked click **Save** and close the window
 - You are now ready take images of every marked positon (by clicking **start**)

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Setting Up Timelapse

Click on the timelapse tab in multidimensional acquisition and adjust how often you want images to be taken and the length of time you want the experiment to last for (e.g. every 15 minutes for 24 hours).

Make sure you give the microscope enough time to take an image of every position between timepoints.

Setting Up MosaiX

Use this option if you would like to take an image of the entire well (in combination with **marking centre positions in mark and find**).

1. Click on the **MosaiX** tab in Multidimensional Acquisition.
2. Tick on the “MosaiX” box at the top
3. Under *acquisition mode* choose **Center**. (1)
4. Under *region* choose the number of columns and rows needed to capture the full well.
 - For 96 well plates this is 2 x 3 using the 2.5x objective.
 - For 384 well plates this is 1 x 2 using the 2.5x objective.
5. Ensure the **overlap is set to 0%**.
 - This will make the full well visible without having to stitch the images together at the end of the experiment.



After the Experiment is Complete

1. Once the experiment is complete the image for each well/position will be visible in Axiovision in an experiment database.
 - Each image will be named according to the well it was taken from.
 - These files will be automatically saved in your “my pictures” folder.
2. Double click a thumbnail to see the image/movie at full resolution

