

Sample Carrier Application (i880/u880)

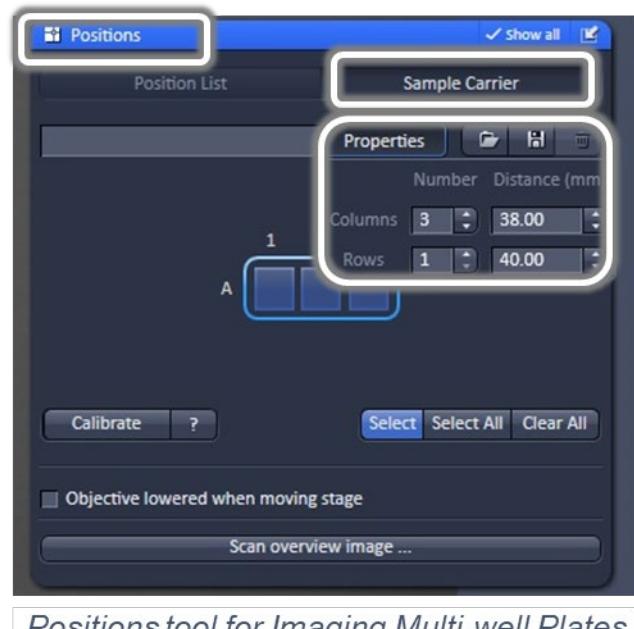
Tool Group Multidimensional Acquisition: Positions - The Sample Carrier application is used to image specimens (typically cells) which are grown in sample carriers like plates with a set number of wells.

Setup

1. Move to the center position of your well plate, and scan Live, while adjusting Gain and Offset settings (use Range Indicator) to optimize image quality.
2. Check *Positions* in the Multidimensional tool group, then open the Positions tab.
Click on the *Sample Carrier* tab.
3. Define the sample carrier by clicking on Properties. Set number of Columns and Rows of the sample carrier and the Distance in mm between the individual wells which is assumed to be equidistant within rows or columns:

E.g. For a 96-well plate, distance is ~ 9 mm; for a 6-well plate, distance is ~ 39.2 mm

4. To assign the actual position of the stage with a position in the sample carrier, move to the center well in the actual plate under the microscope then click *Calibrate*.
5. Click the field in the sample carrier representing this well of the plate (highlighted in blue, click *Select*. **Note that for an upright microscope (u880), the orientation of the actual plate is the reverse of the graphic displayed.**
6. Click on other wells desired for imaging, or click *Select All* to image all wells in the sample carrier. *Clear all* deselects all positions.
7. See instructions below if you want to use the **Autofocus** feature.
8. Click on *Start Experiment* to image selected wells.



Autofocus Option

1. After selecting the wells to image, open the *Focus Devices and Strategy* tab.
2. In *Autofocus Mode*, choose from either Reflection, Fluorescence, or Autofocus Map from the dropdown list.

For Autofocus Map, click on *Record Focus Map*. When setup is done, a *Focus Map* is created.

The system defines the auto-focus position for each of the positions in the sample carrier. This creates a Z-Map of the bottom layer of the sample carrier. This map is used to keep the sample in focus until another sample carrier is chosen or the carrier's orientation is changed.

- If Reflection (based on the reflection from the cover slip) is selected, the system determines the imaging configuration for the auto-focus. Can define an offset (see below)...
- If Fluorescence (based on fluorescence of sample) is selected, the auto focus looks for the brightest fluorescence signal using the present imaging configuration.



Tool for creating an Autofocus Map for multi-well plates

Offset: defines the distance between the actual position of the sample in Z and the position of the cover glass. It can be determined automatically:

- a. Focus onto the sample to be imaged then click *Find offset*.
- b. The value for the determined offset will then be written into the editing field.
- c. To test the performance of the Auto-Focus method (if Reflection is chosen, the value for the Offset will be taken into consideration), defocus the sample and click *Test*.
- d. The auto-focus procedure will be performed at the actual position and an image of the sample will be on the screen when completed.