

How to set up multiple Positions of a multi-well plate using “sample carrier” in Zen 2.6?

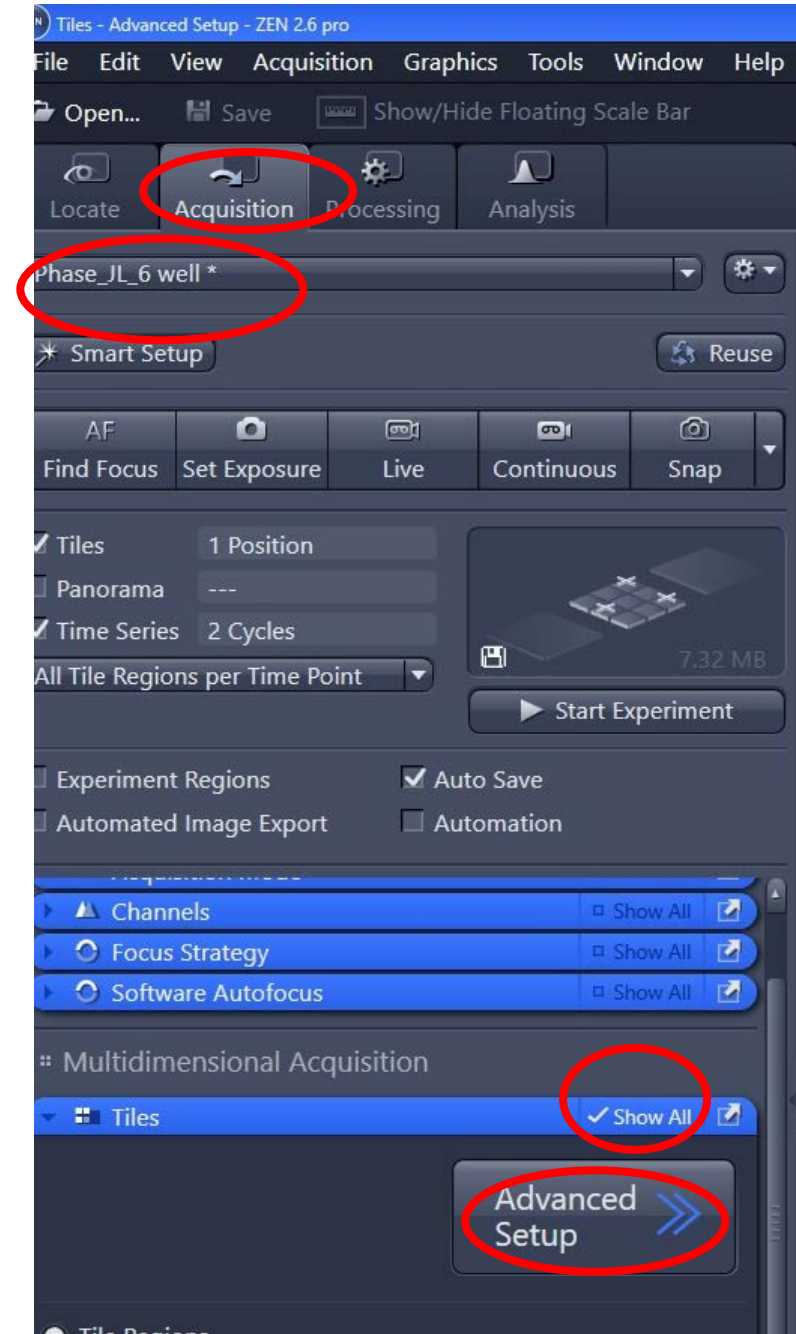
Goal: image multiple positions in a multi-well plate fashion. I.e. one position of each well out of a 6-well plate.

Logic (to use “Sample Carrier” to set up Positions of a multi-well plate)

1. First, calibrate the Carrier i.e 6-well plate
2. Then set up Positions by Carrier
3. After Positions setup, verify all Positions to update Z adjustment (focusing)

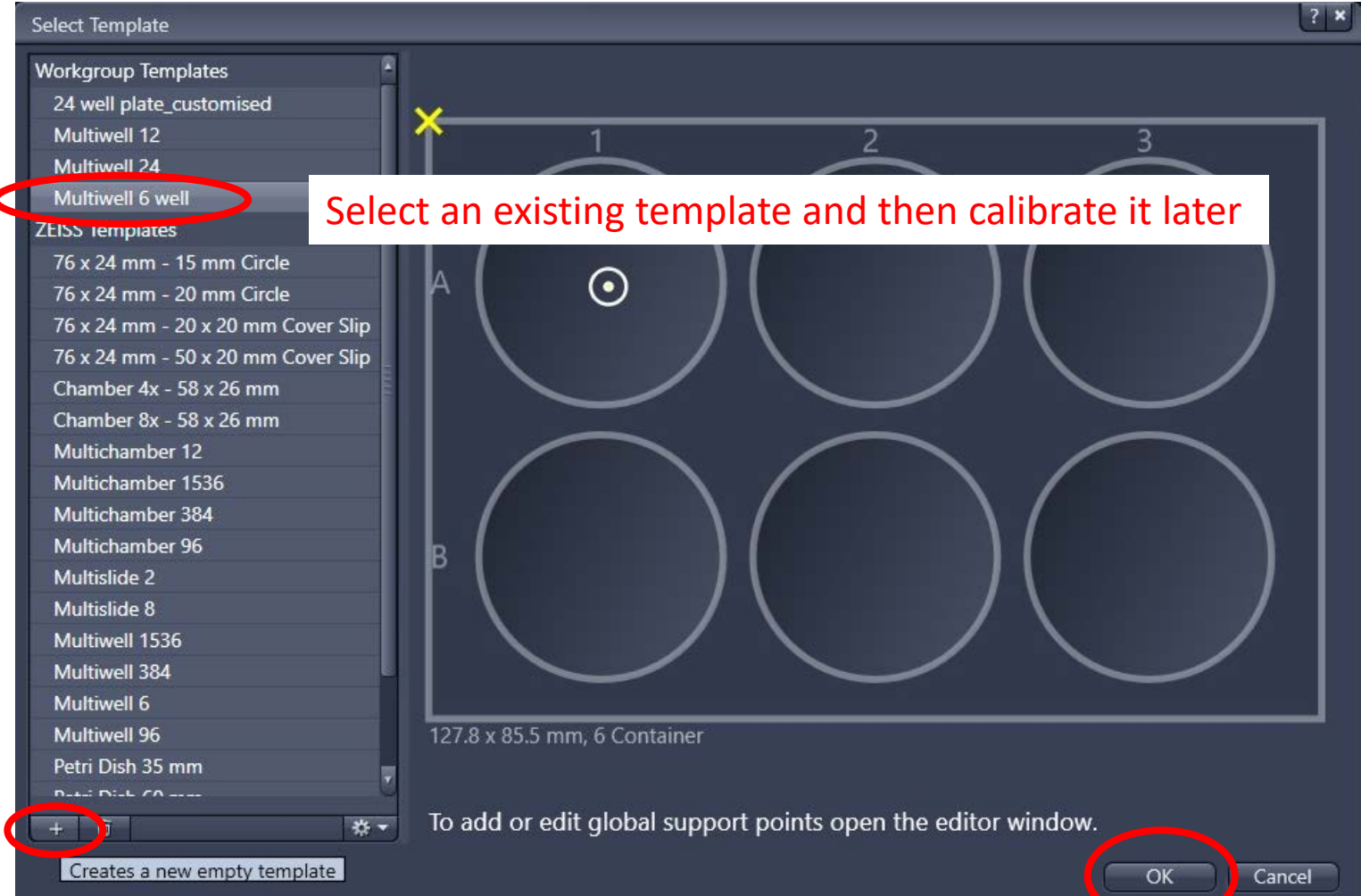
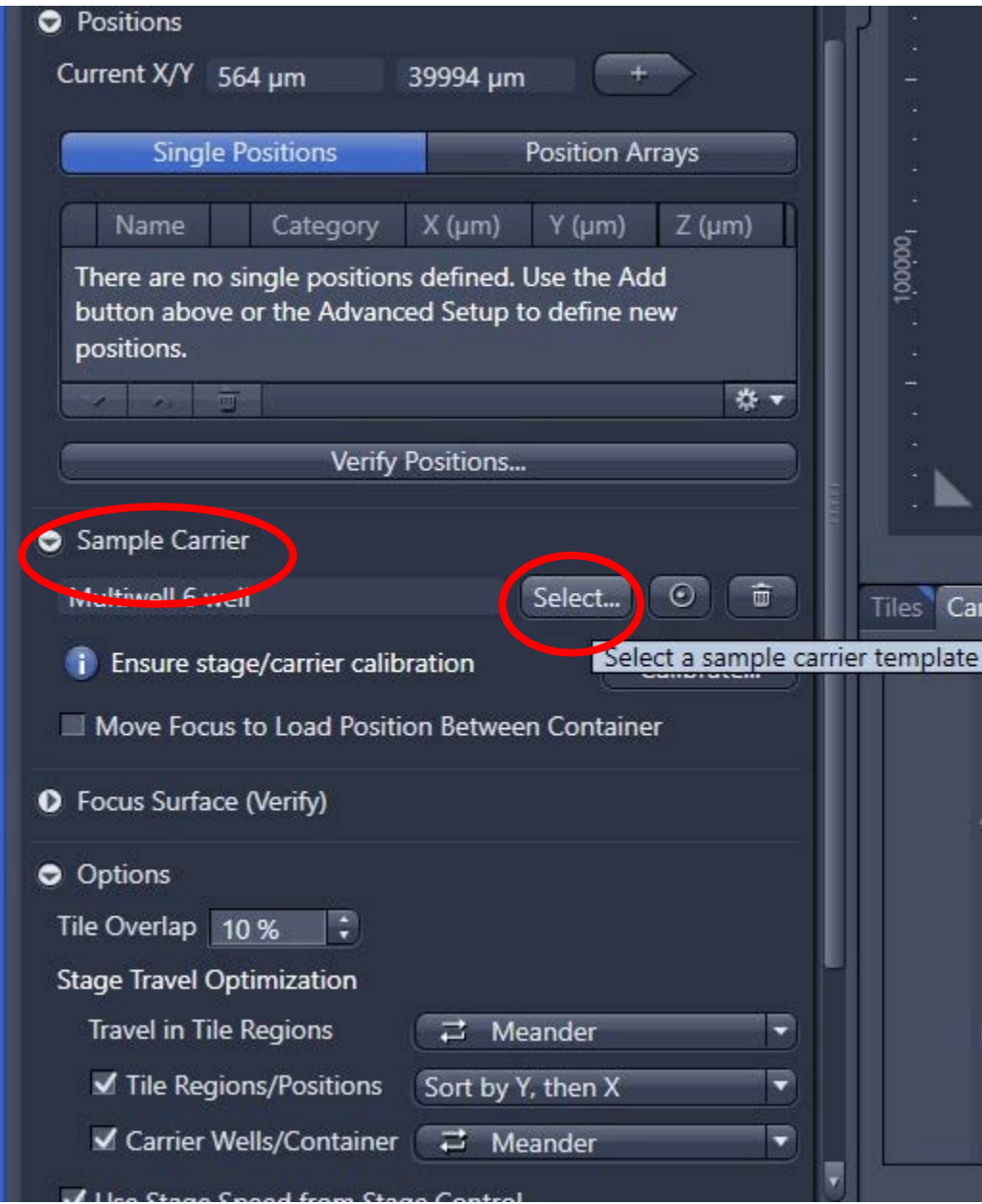
Notes:

1. Before you start Position imaging, you need to set up your channels as usual (get exposure times right for individual channels). Can start a new protocol or modify existing one (save as a new name then modify).
2. Don't forget to tick “Show All” for “Tiles”. Otherwise, “sample carrier” wont be visible.
3. Under “Tiles”, we need to go to “Advanced setup” to set up Positions by Carrier i.e. 6-well plate.



1. Set up Sample Carrier

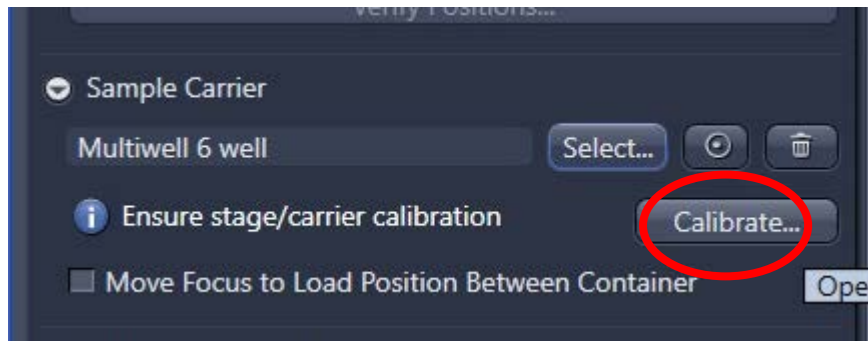
1.1 Choose a sample carrier template. Click "Sample Carrier" -> "Select" (to select a sample carrier template)



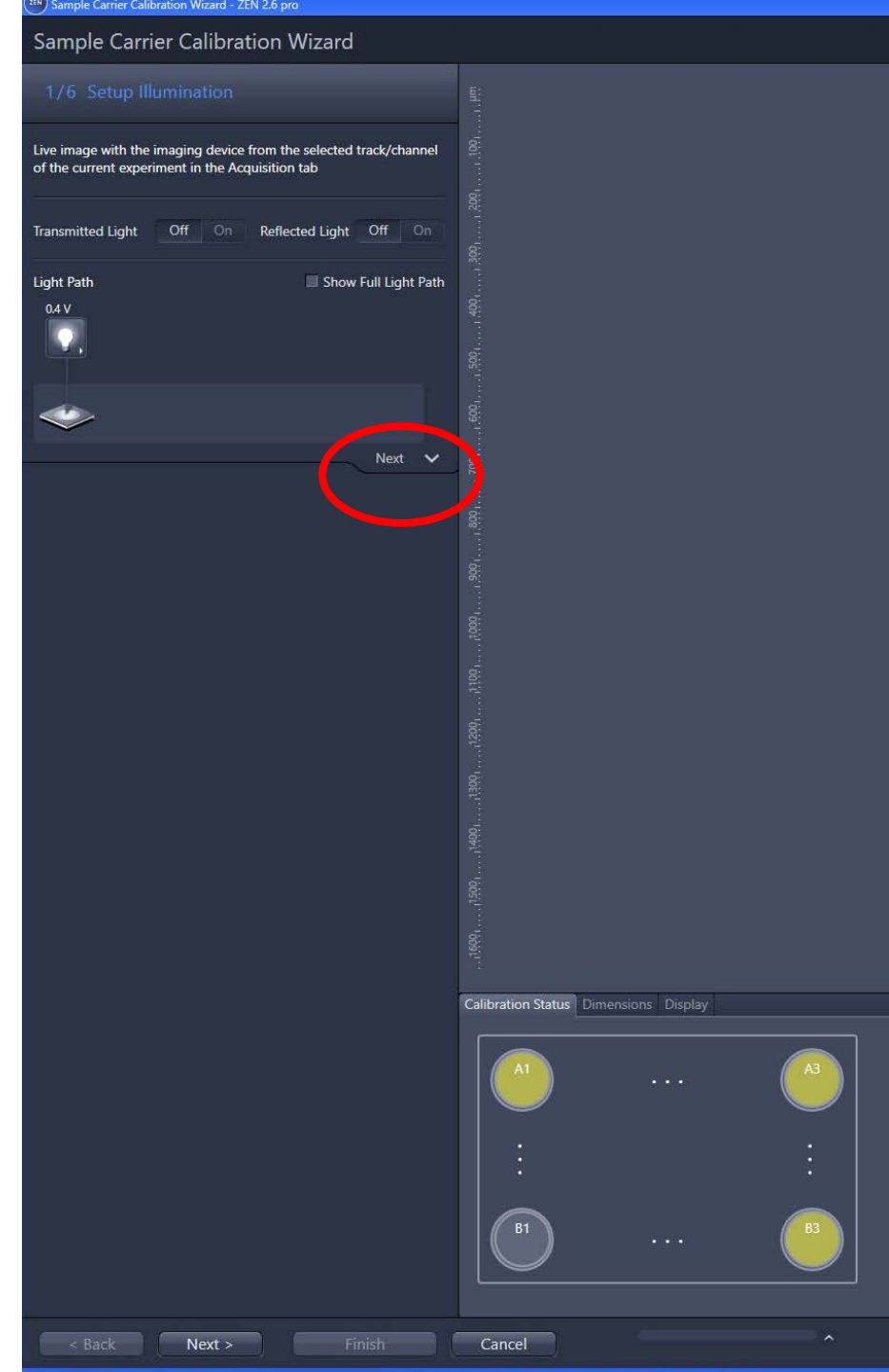
Or create a new empty template if you know have the dimension information about your sample carrier

1. Set up Sample Carrier

1.1. Click “Calibrate” to open up a wizard to calibrate the stage and sample carrier (here we use 6-well plate as an example)



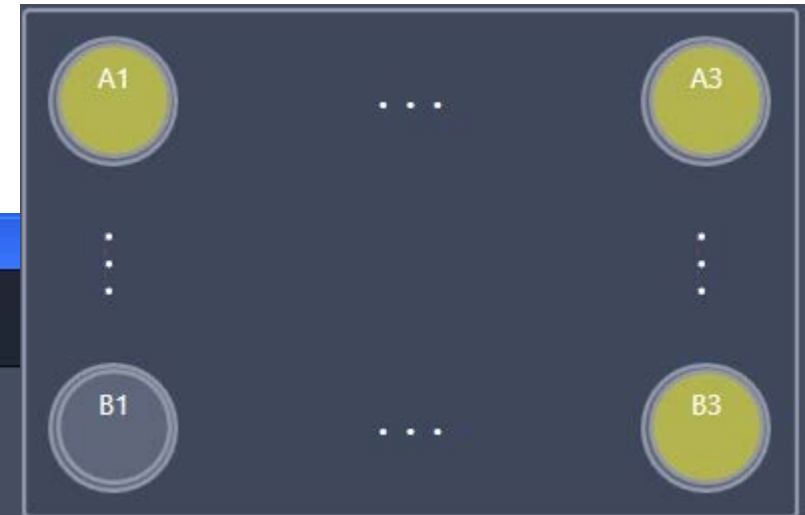
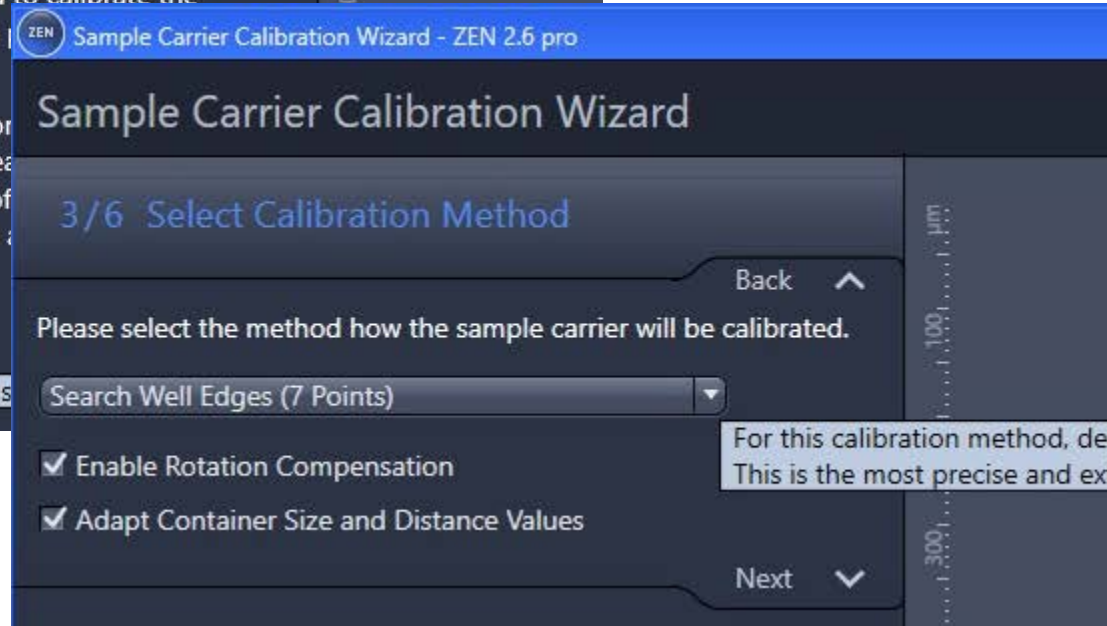
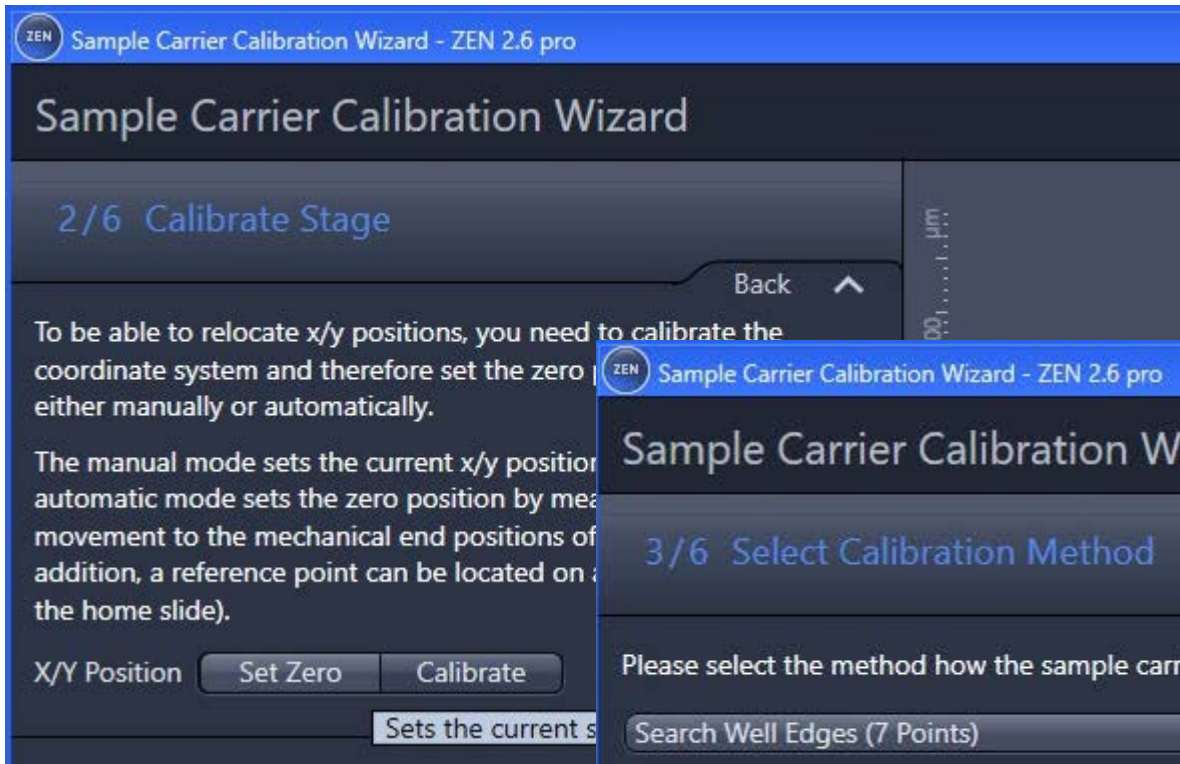
Then follow the wizard to go through 6-step calibration procedure



1. Set up Sample Carrier

1.2 Calibration: 2/6. I normally click “Set Zero” of the current location of the stage (it can be any where, i.e. where when the Well A1 is inside the current FOV)

1.3 Calibration: 3/6. Select calibration method. I usually use “Search Well Edges (7 points)”: Well A1 (3 points), Well A3 (3 points), Well B3 (1 points).



For this calibration method, dedicated edges of the three corner container have to be determined. This is the most precise and extensive method.

1. Set up Sample Carrier


1. 4 Calibration 4-6/6: Physically move the stage using the joystick to find those 7 well wall points and confirm.

Sample Carrier Calibration Wizard - ZEN 2.6 pro

Sample Carrier Calibration Wizard

4/6 Define Upper Left Well (A1)

Find the following positions in the upper left container.
Set the positions by clicking the corresponding buttons.



Reset Last
Reset All

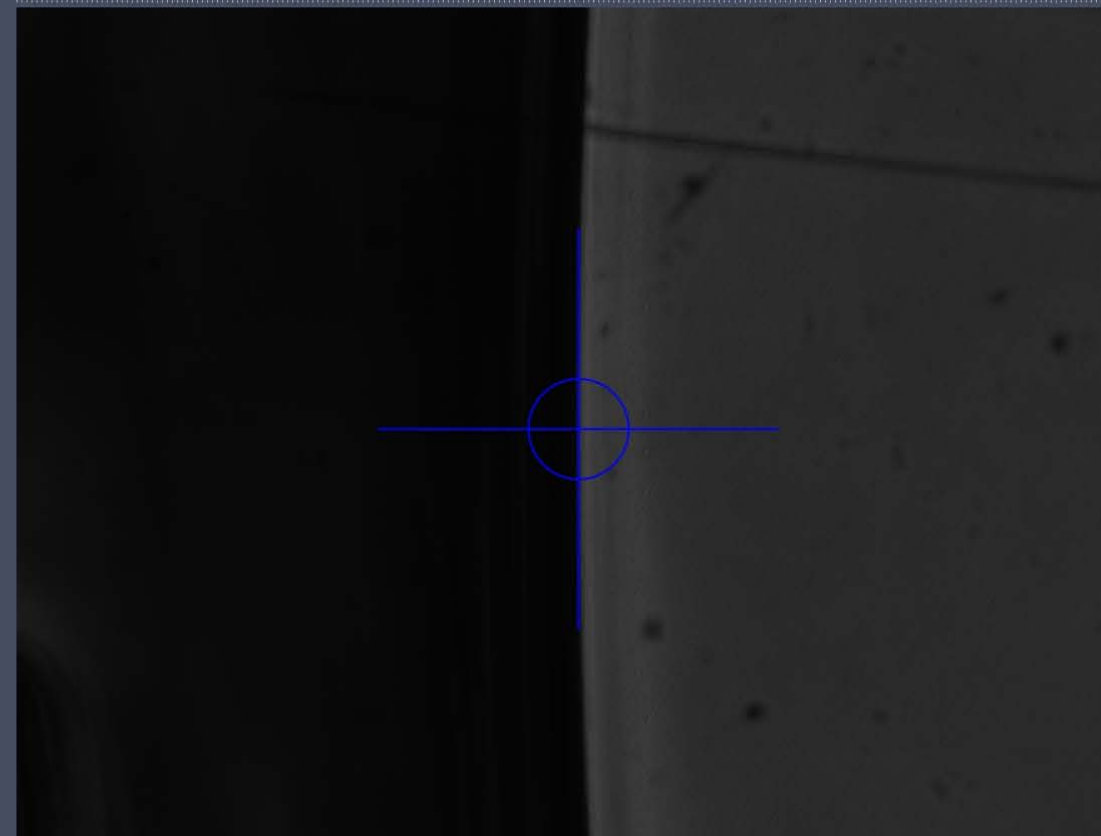
Determine Position: Right

Move Stage Automatically to each Required Position

| | | | | | |
|--------|---|----------------------|---|----------------------|-------------------------------------|
| Left | X | -17117 μm | Y | -691 μm | <input checked="" type="checkbox"/> |
| Top | X | 100 μm | Y | -18333 μm | <input checked="" type="checkbox"/> |
| Right | X | --- | Y | --- | <input type="checkbox"/> |
| Bottom | X | --- | Y | --- | <input type="checkbox"/> |

Back

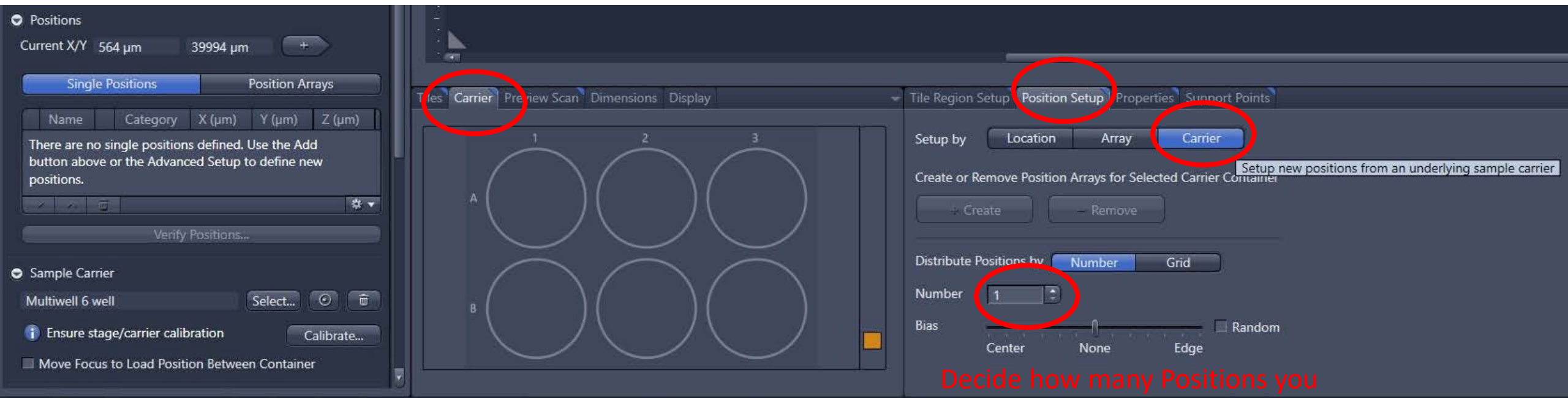
1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100 μm



Sets the current stage x,y position for the current container position to be determined.

1. Set up Sample Carrier

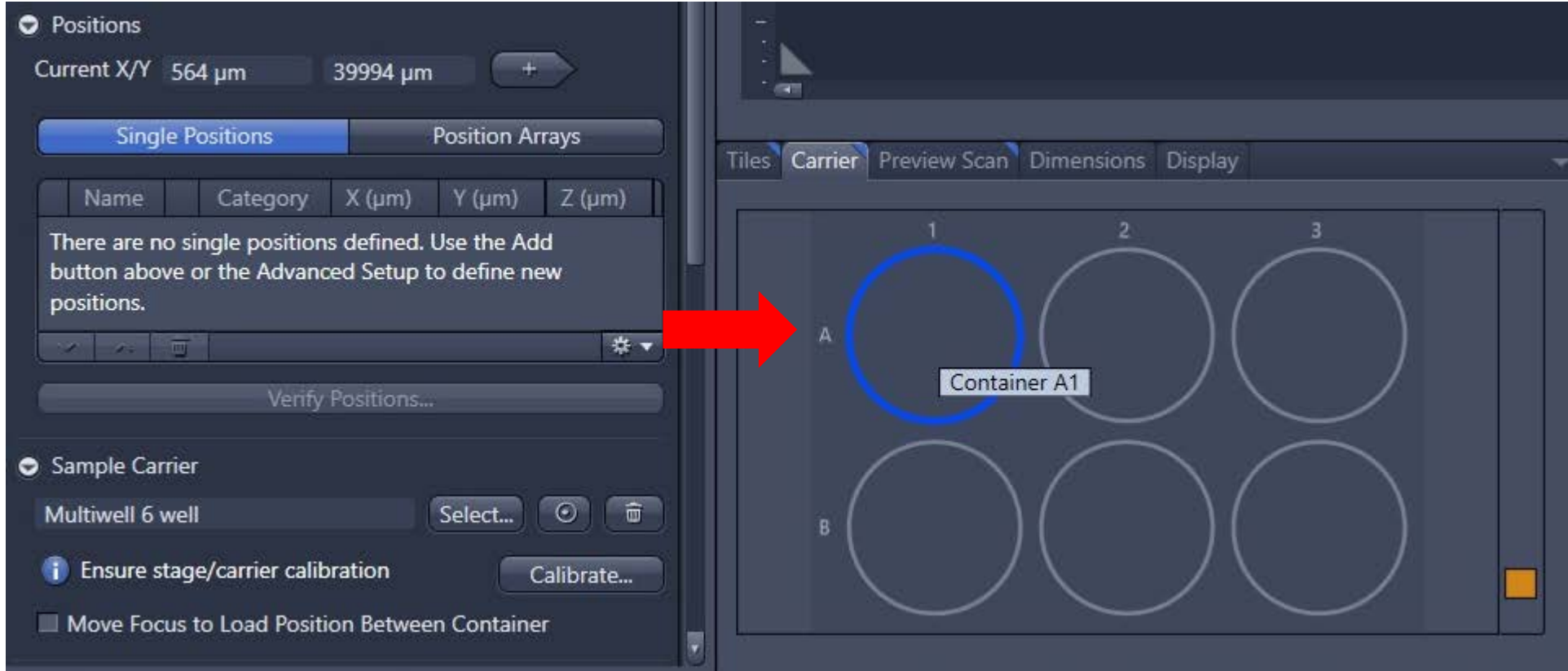
1.5. Now go back to “tiles”/”Advance setup”. Here, your 6-well plate (just calibrated) will pop up in “Carrier” Tab. Click “Position setup” tab



Decide how many Positions you like for each Well. Currently only 1 position per Well.

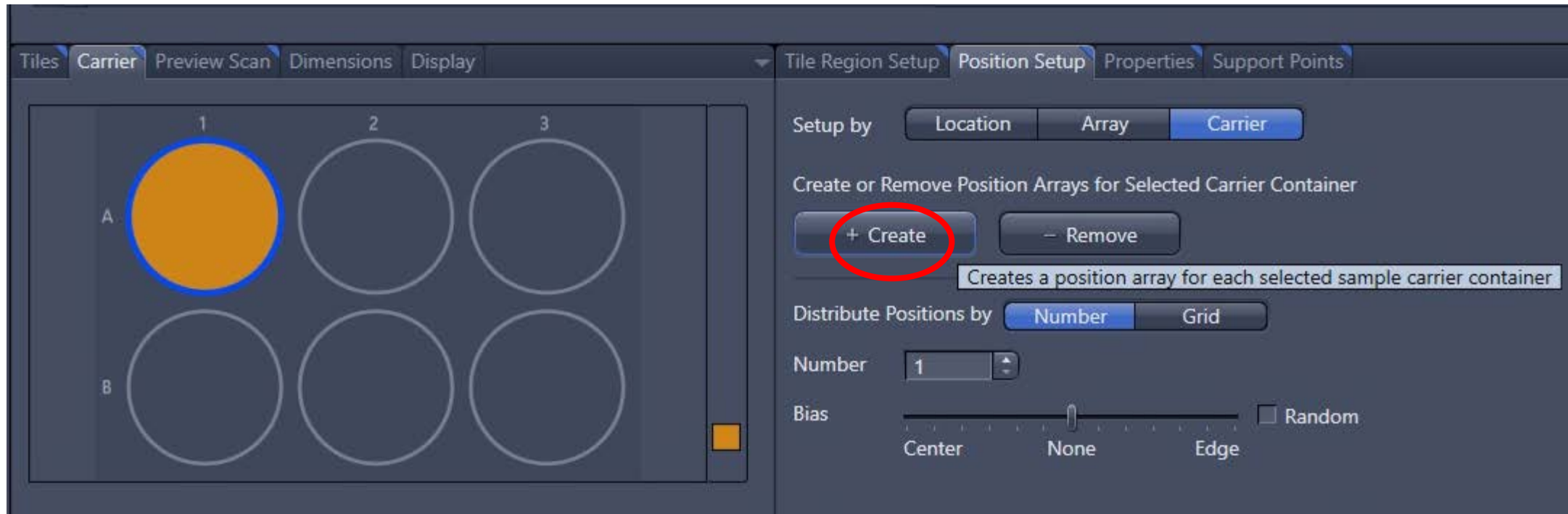
2. Position setup

2.1 double click the A1 well in the Carrier window to drive the stage to that well. You can physically move around A1 Well using the joystick and stop somewhere you can find interesting sample area



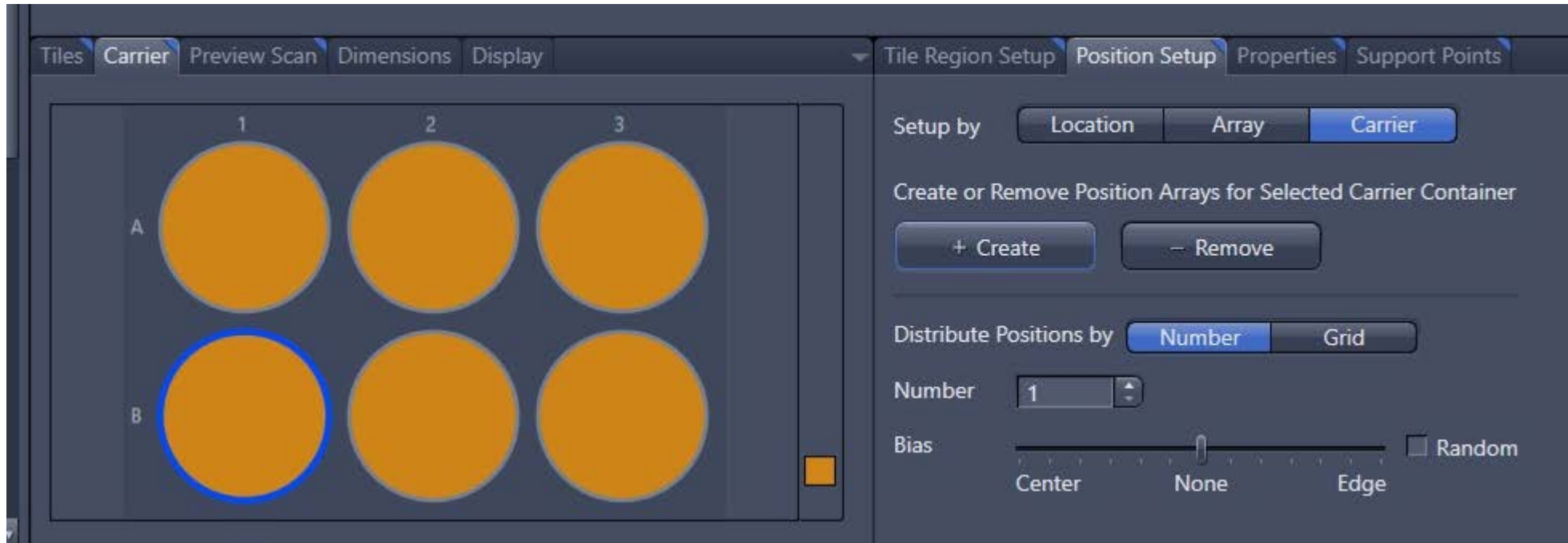
2. Position setup

2.2 then click on create and add the current position of this well (A1) as shown in this screenshot) as the 1st point. Once the Well is added to the Position, this well becomes yellow/orange color in “Carrier” window.



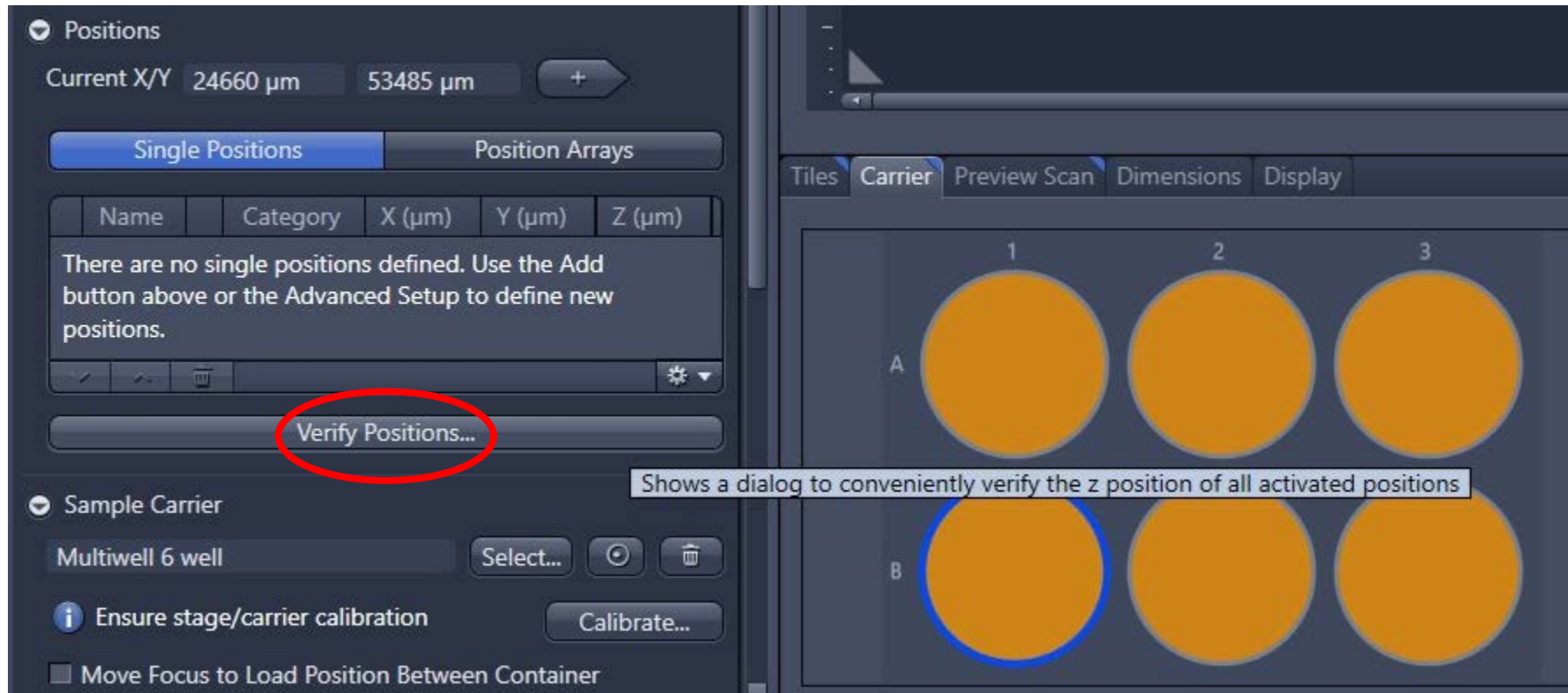
2. Position setup

2.3 same way to add all points from Wells A1 to B3. 6 Positions in this example (one out one Well)



3. Verify Positions

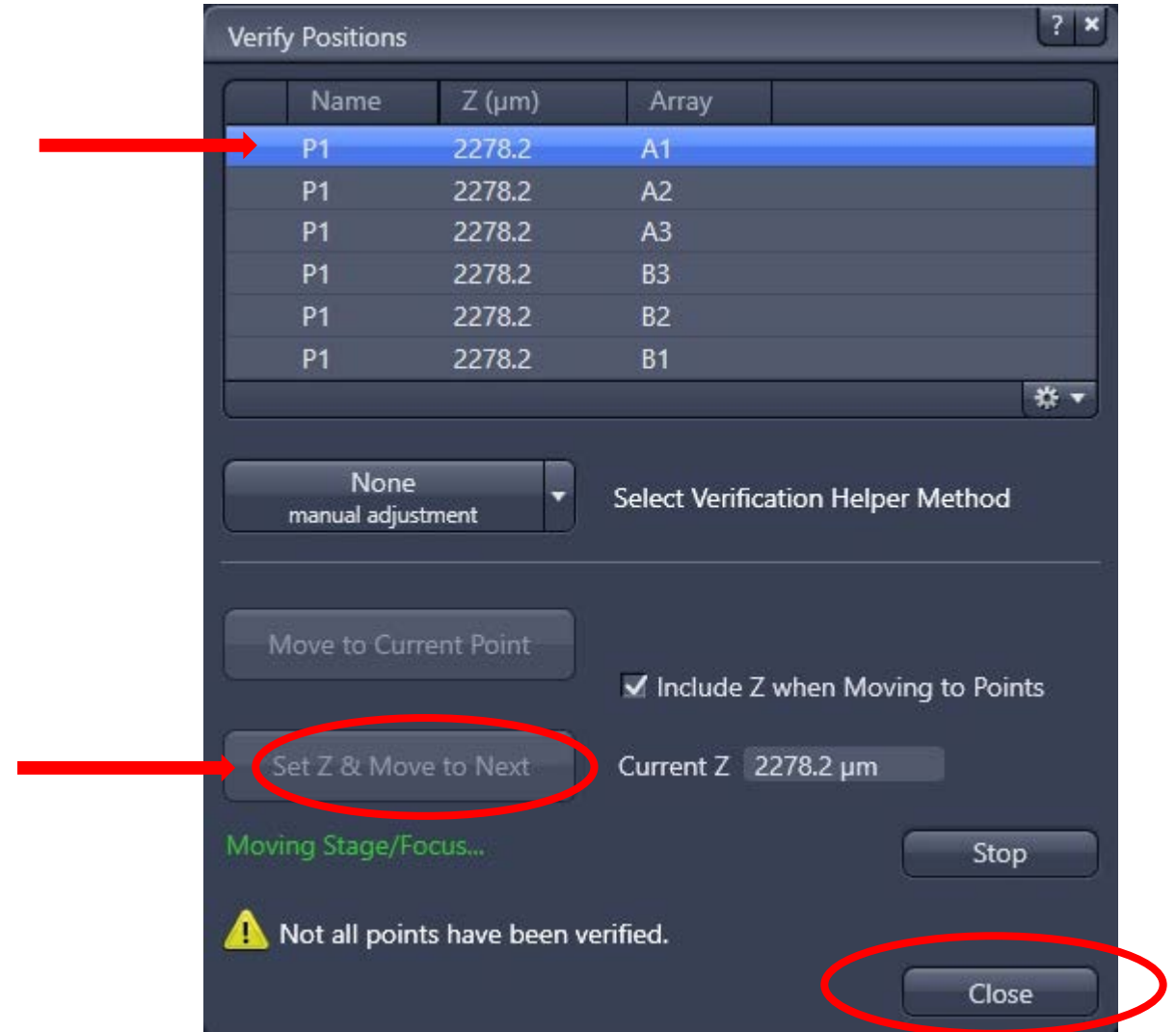
3.1 This is mainly to bring all selected positions in better focus and update Z position of all these Positions. Can run this 2-3 runs so focusing is good across the whole plate.



3. Verify Positions

3.2 Double click individual position i.e. P1 (from Well A1) to move the stage to that Position. Then focus the sample by physically adjusting the Focus Handle.

3.3 After Z position is adjusted, this “Set Z & Move to Next” will become activated. Click it to confirm the Z adjustment and move to next Position to continue verifying other Positions



3.4 After all positions verified, click “close” to close this window

4. Start Position experiment

Till now, all Positions have been set up using “Sample Carrier”. Finally, initialize “Position” experiment from here

