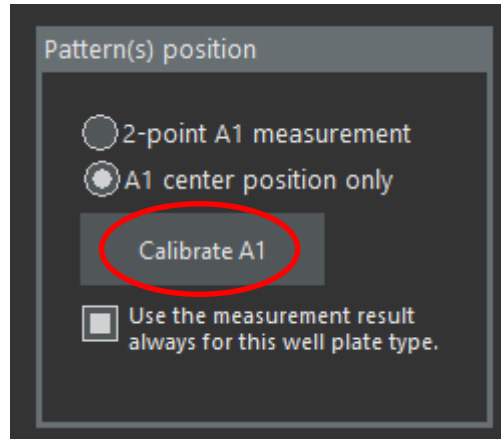


1. Go to “Edit” and select any well type
2. Click “New” and rename it as “Flexible multiple ROI”
3. Set the conditions as in the screenshot below. “Spacing” is not important and “Well width/Height” should be set between 333um (40x Objectives) and 1300um (10x Objectives). Standard value of 1000um will always work.
4. Set also the Well bottom properties. For robustness, if you are working with a slide, unclick “Use always...” and set the thickness to 170um and Refr.index to 1.59

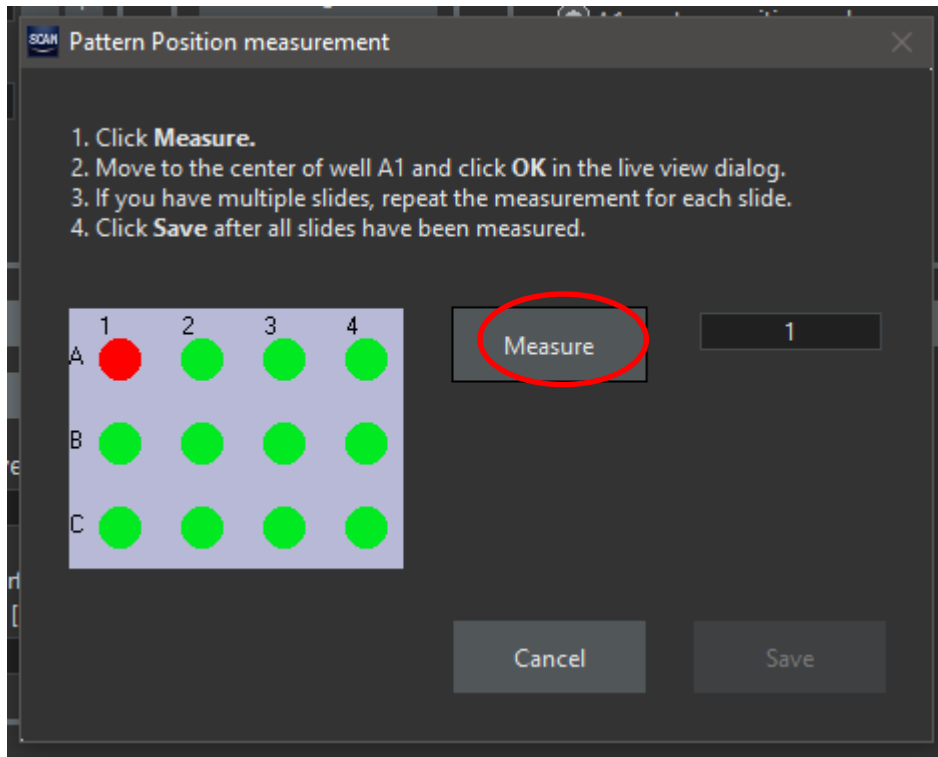
The screenshot shows the configuration interface for a well plate type named "Flexible multiple ROI". The interface is divided into several panels:

- Type:** Flexible multiple ROI (with a green status indicator "A1 position calibrated").
- Pattern:**
 - Columns: 1
 - Rows: 1
 - Repetition: 1
- Spacing:**
 - Column [μm]: 9000
 - Row [μm]: 9000
- Well geometry:**
 - Shape: Rectangle
 - Well width [μm]: 1000
 - Height [μm]: 1000
- Pattern(s) position:**
 - 2-point A1 measurement (unselected)
 - A1 center position only (selected)
 - Calibrate A1 (button)
 - Use the measurement result always for this well plate type. (checkbox, unselected)
- Well bottom:**
 - Plate bottom properties:**
 - Thickness [μm]: 170
 - Refractive index: 1.59
 - Lower surface variation [μm]: +/- 250
 - Offset and position calibration:**
 - z-offset [μm]: 107
 - Calibrate Z (button)
 - Lower surface position [μm]: 2440
 - Use always for this well plate type. (checkbox, unselected)

5. Click calibrate A1



6. Click measure

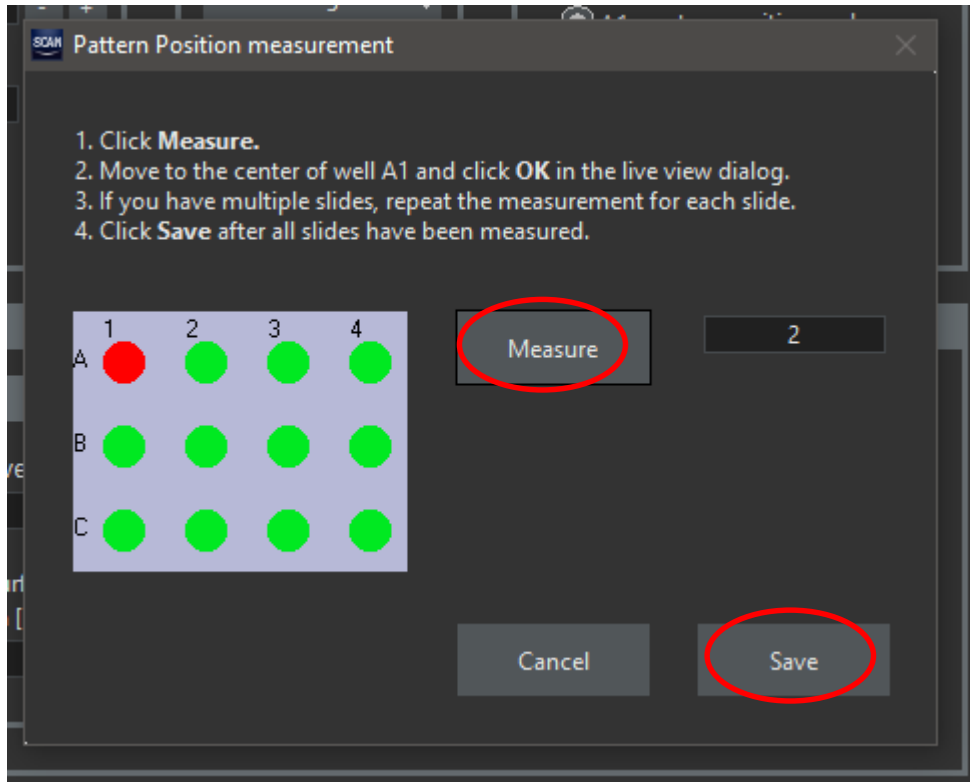


7. Move with the stage to the first ROI (FOV) you want to image and click OK

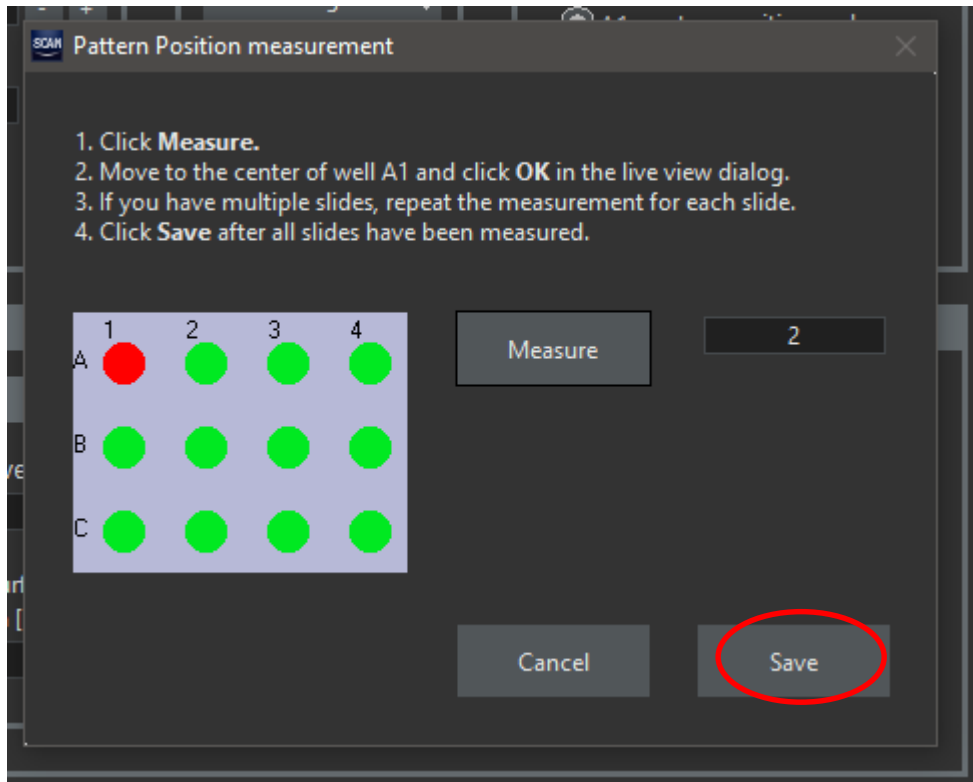
The screenshot displays a microscope software interface with a central live view of a sample. The interface includes several control panels:

- Left Panel:** A vertical scale from 0 to 4096. Below it, a histogram shows the intensity distribution. A "Clipping" control is set to 0%.
- Central Live View:** Shows a grayscale image of a sample with a bright, irregular boundary. The image is framed by a grid pattern.
- Bottom Center:** A status bar displays "2048x2048 0.25X 116 (1049,2036)".
- Right Panel:** Contains several control sections:
 - Z focus / XY navigation:** Includes "Coarse" and "Fine" focus controls with "+" and "-" buttons, a "Step size" of 3.00 μm , and a "Current position" of 5650.92 μm .
 - Camera:** "Exposure time [ms]" is set to 50, with "Auto" selected. "Binning" is set to 1x1.
 - Microscope:** "Filter cube" is set to DA/FI/C3/C5-SE. "Objective" is set to UPLSAPO 2 40x.
 - Defined Color Channels:** Set to FITC.
 - Illumination:** Set to Fluorescence at 100%.
 - LED excitation:** Set to Cyan (470).
 - Emission filter:** Set to 515/30 (FITC).
- Bottom Right:** "Cancel" and "OK" buttons. The "OK" button is circled in red.

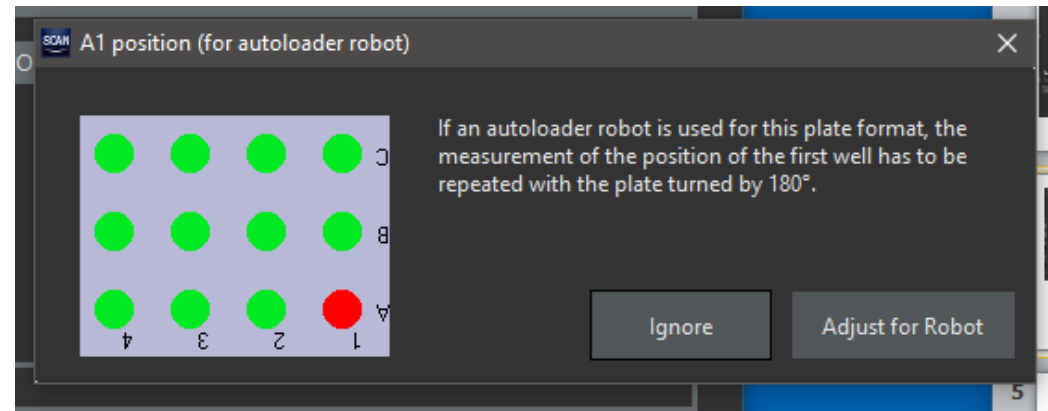
8. Click Measure again to select the second ROI you want to image. Perform this as many times as ROIs you want to measure.



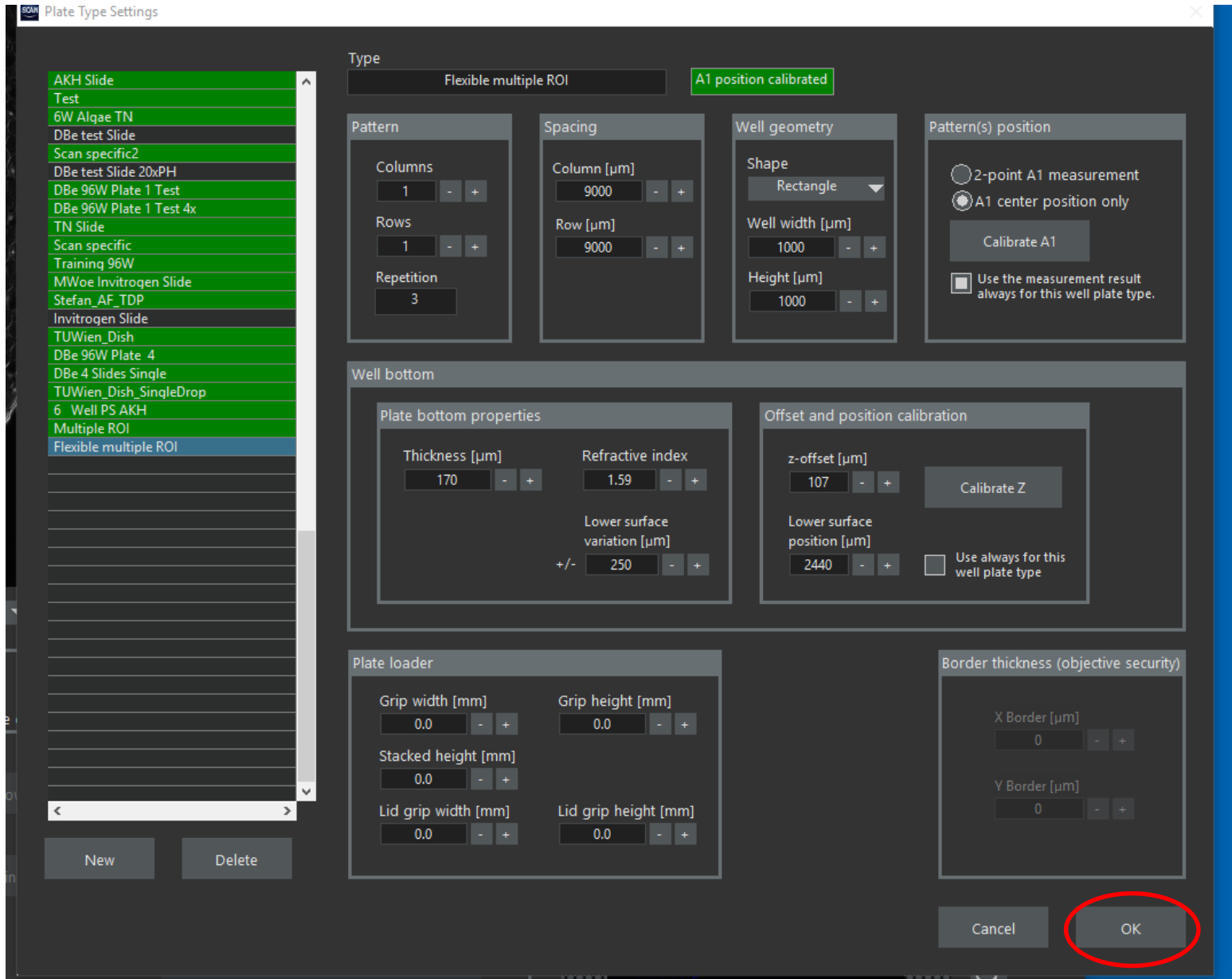
9. Once you have selected all the ROIs you want to measure, click Save



10. Click Ignore, if you are not using a Robot



11. Click OK



12. Check the ROI pattern you have generated. You can Zoom it in for deeper look

The screenshot displays the 'Edit Scan' software interface. The main window is titled 'Edit Scan' and has tabs for 'Plate Manager', 'SW-Autofocus', and 'Acquisition'. The 'Well pattern' section is active, showing a large gray area with a red oval highlighting a specific well. Below this area, there are instructions: 'Left click to toggle wells on/off' and 'Mouse-drag (+ Ctrl) to select (deselect) wells'. There are also 'scan' and 'skip' buttons. The 'Well pattern' configuration window is highlighted with a red border and shows three blue squares labeled '1', '2', and '3' arranged in a diagonal pattern. Below the squares, there are instructions: 'Left click to toggle wells on/off' and 'Mouse-drag (+ Ctrl) to select (deselect) wells'. There are also 'scan' and 'skip' buttons. The main window also shows various settings: 'Meander wells' (checked), 'Continuous ZDC' (checked), 'Wells selected' (3), 'Well width [μm]' (1000), 'Well height [μm]' (1000), 'Plate' (AKH_6Well_CountCells), 'Type' (Flexible multiple ROI), and 'Calibration status' (A1 position calibrated). The 'Acquisition order' section shows 'Single' and 'Center' options. The 'Field of view [μm x μm]' is 333 x 333, and 'Total positions' is 1. At the bottom, there is a 'Scan Setup Status' bar with the message 'Info: This scan will result in 9 images acquired.' and 'Cancel' and 'OK' buttons.

Well pattern

Left click to toggle wells on/off
Mouse-drag (+ Ctrl) to select (deselect) wells

scan skip

Well pattern

Left click to toggle wells on/off
Mouse-drag (+ Ctrl) to select (deselect) wells

scan skip

Meander wells Wells selected 3

Continuous ZDC Well width [μm] 1000

Well height [μm] 1000

Plate
AKH_6Well_CountCells Edit selection table

Type
Flexible multiple ROI Edit plate types

Calibration status: A1 position calibrated

Acquisition order
Single Center Show order

Field of view [μm x μm]
333 x 333

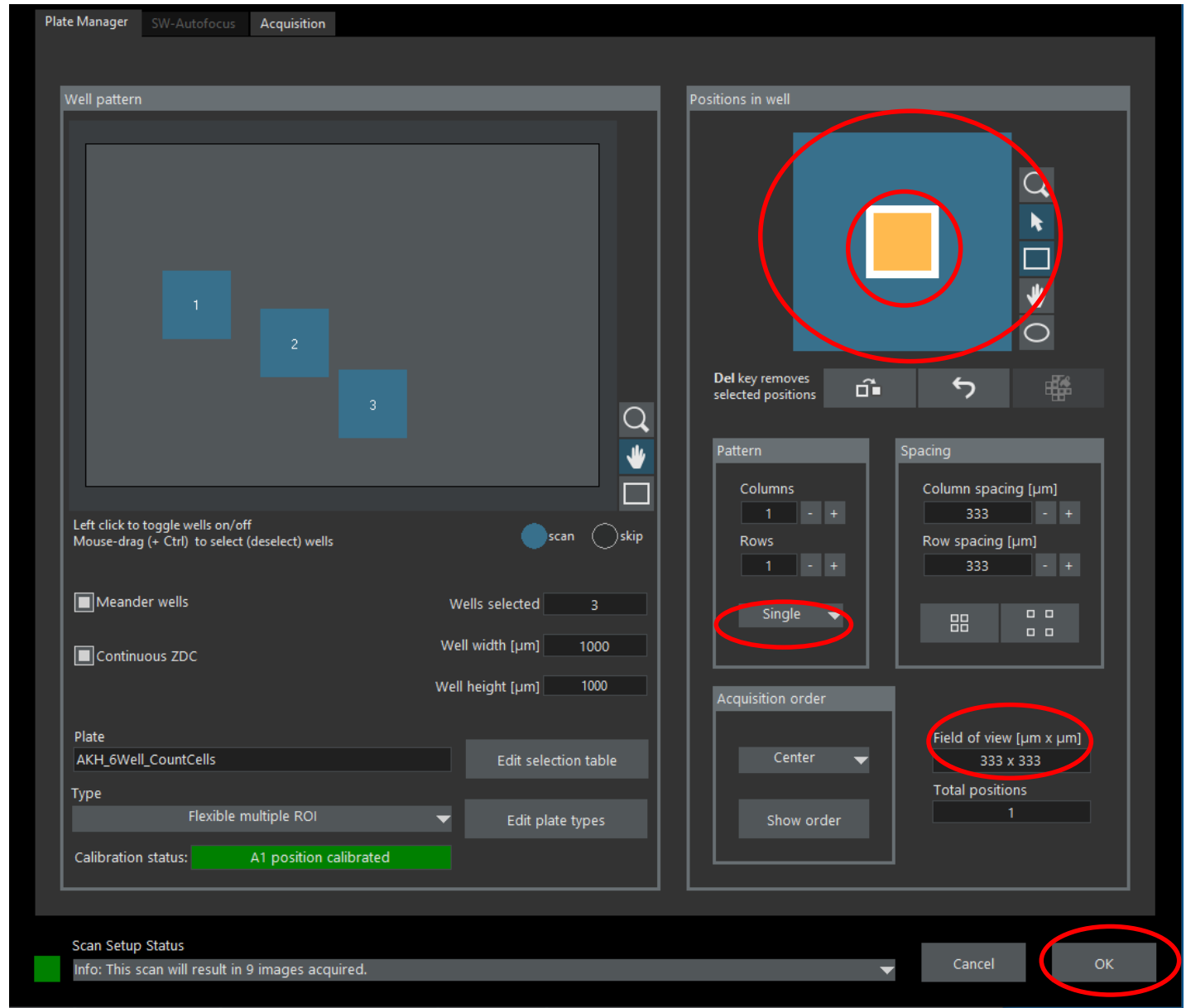
Total positions
1

Scan Setup Status
Info: This scan will result in 9 images acquired. Cancel OK

13. You can check the FOV with respect to the ROI in this Window. There can be a mismatch depending on the settings of the well width, set on step 3 before.

14. Normally, you only image a Single point but you can increase this number if necessary, depending on the real ratio of the ROI and the FOV

15. Click OK



15. Now you can start the scan and check the different ROIs.

The screenshot displays a software interface for well plate scanning. At the top, three windows show microscope images of wells, labeled "Well No. 1", "Well No. 2", and "Well No. 3". Each window shows a 240x240 pixel image at 1X magnification. The central image shows a circular well with a bright ring of cells. Below the images is a "Plate" control panel. On the left, a vertical color scale ranges from 0 (orange) to 0 (green). The main area of the panel is a grid where three wells are highlighted in green and numbered 1, 2, and 3. To the right of the grid are three dropdown menus: "Object type" (Main), "Measurement type" (Counts), and "Gate" (All). Below these is a "Reference Gate" dropdown (All). At the bottom of the panel are buttons for "Well Results", "Descriptions >>", "Restore", and "Close". A legend at the bottom left indicates "Dynamic" and "Absolute" modes, and a color key for "Selected" (green) and "Skip" (grey). A note states: "Left click to toggle wells on/off" and "Mouse-drag (+ Ctrl) to select (deselect) wells".