

# Scanning Tissue Samples using ZellSafe™ Tissue Chips for ChipCytometry Quick Guide

Below are the guidelines to describe how the x/y-coordinates of ZellSafe™ Tissue Chips are defined as well as how tissue samples are imaged.

## A. Adaption of Coordinates

1. Open the ScanApp.
2. Put the ZellSafe™ Tissue Chip with a tissue section onto the microscope.
3. Use the joystick to move the objective underneath the tissue section and adjust the focus manually.
4. In the upper right corner of the ScanApp display, the x/y-coordinates of the chip are shown (e.g. 26630.0µm x 12285.0µm ; X x Y, see Fig. 1).
5. Use the joystick to move to the edges of the tissue and record the new coordinates for X-start, X-end, Y-start, and Y-end, as shown in Fig. 2.

Fig. 1 | x/y-coordinates of the chip displayed within the ScanApp

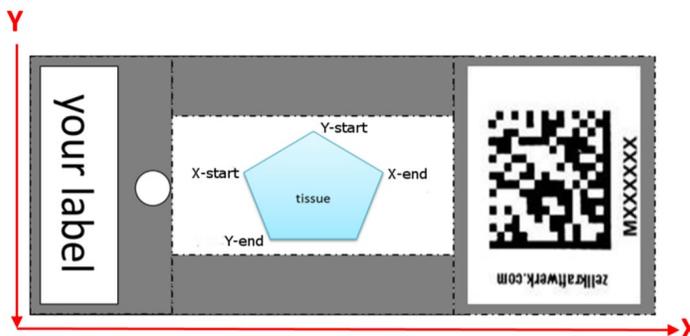
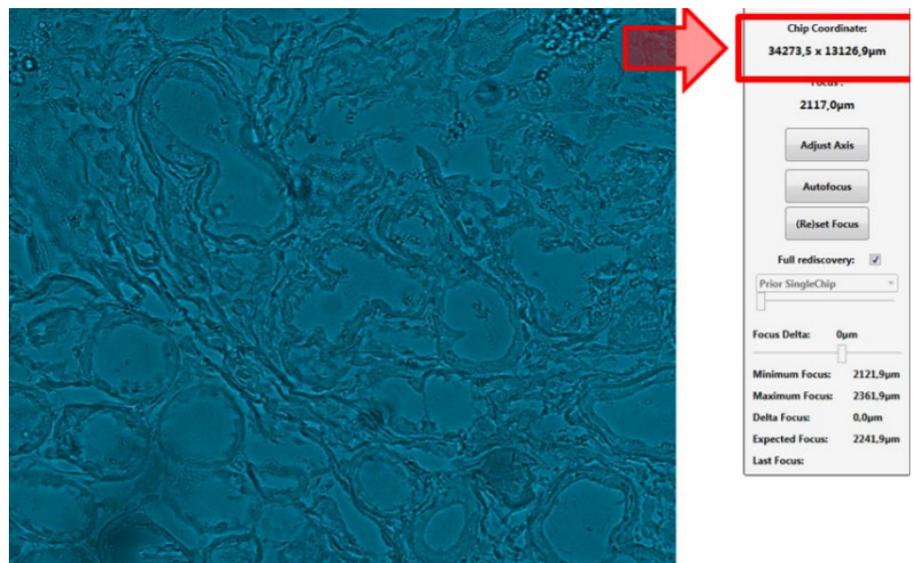


Fig. 2 | Example of x/y-coordinates of a tissue section

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## B. Change Coordinates

1. Open the ZKWApp.
2. Open the chip in the project manager.
3. Click on “Change Geometry” in the lower left corner and enter the new chip coordinates recorded earlier (Fig. 3).
4. Enable the checkbox “force coarse focus” to enhance focus quality in follow-up scans.

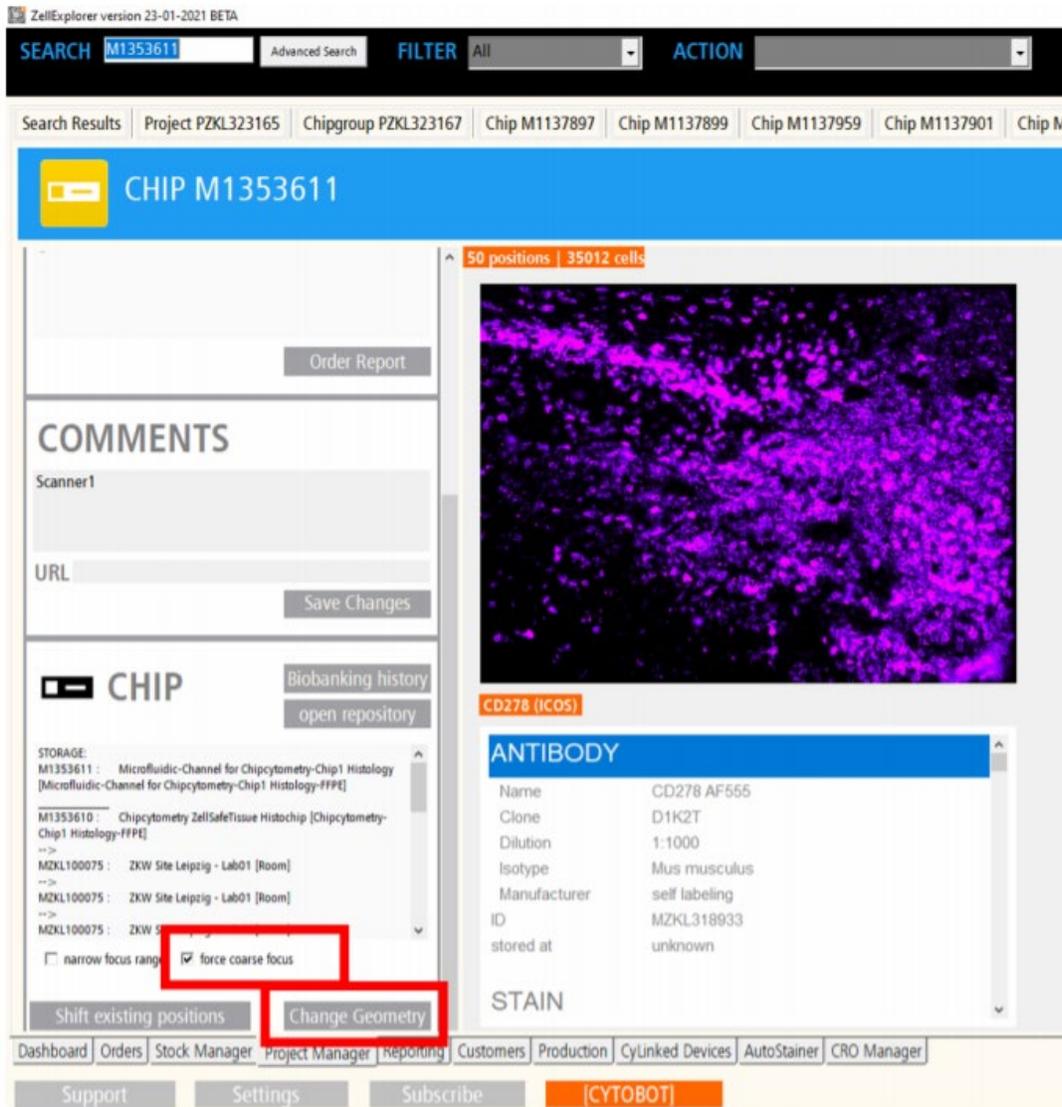


Fig. 3 | Locations in ZKWApp display to change the coordinates of tissue section

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## C. Initial Scan

1. Start with a background scan (BG) without bleaching. Any one of the five detection channels can be used as the recorded background for selection of the regions of interest rather than the background subtraction.
2. Omit positions that contain no tissue after the initial scan is complete:
  - i. Open the Datawizard
  - ii. Click “show overview”
  - iii. Hold Ctrl while clicking on any positions to exclude.
  - iv. Alternately, go to the “Action” dropdown menu, select “disable all positions” and then reactivate desired positions by holding Ctrl and clicking on those positions to include.
3. Make sure to check the initial background scan overview for folds, tears or detached areas in the tissue and exclude positions that contain any of these anomalies.
4. After the regions of interest have been selected, proceed with initial bleaching and background recording in all channels that will be used for the first staining.
5. Continue with staining or store according to the appropriate Quick Guide protocol.

## D. Staining

1. To confirm biomarker integrity, we recommend performing a tissue-specific biomarker fitness test (e.g., CD19 for B cell areas in spleen).
2. To identify regions of interest, use tissue-specific segmentation biomarkers, such as CD45 for immune populations, Ep-CAM for epithelial cells, etc. In case of uncertainty, consult an expert such as a pathologist.
3. Antibodies targeting surface and intracellular biomarkers must be diluted in appropriate buffers according to the optimal titration for that antibody and stained as outlined in the “Staining of FFPE and Ac/EtOH-Fixed Tissue Sections” Quick Guide. Use a total staining volume of 600  $\mu$ L. Please refer to our “Staining of Surface Markers”, “Staining of Intracellular Markers”, and “Staining of Intranuclear Markers” guides for more details.
4. Occasionally, antibody that is retained on the edges of the flow chamber might diffuse back into the channel during scanning. Rinse the chip with ZKW Wash Buffer during the scan to prevent back diffusion of antibody.
5. For staining of the nucleus, pipette 600  $\mu$ L of a DNA-binding dye (e.g., DAPI, Hoechst 33342, or propidium iodide) in the appropriate concentration to the chip and incubate 5 min at RT. Wash at least 2 min with ZKW wash buffer (~5 mL). Wait 2 min and repeat this washing prior to scanning. Scan with the appropriate filter set:
  - i. DAPI - FS395 and FS421
  - ii. Hoechst – FS395 and FS421
  - iii. Propidium iodide – FS560

Note: DAPI, Hoechst, and PI are not photobleachable.