

Supplementary protocol 2

Application 3: Automated serial-section TEM

A detailed step-by-step video tutorial from SerialEM screen grabs is provided as Supplementary Movie M2.

For each cell of interest on the initial section, we create a map at a moderate magnification (1000-2000 \times , depending on what is the lowest non-LM mag of the microscope) with a sufficiently large field of view for realignment. We then select the Navigator items of all interesting cells we like to propagate to the next section, in this case the “target” section, for acquisition.

The py-EM script *duplicate_items* then duplicates these map items into a new registration. This can be done multiple times, when trying to populate multiple serial sections at once. In order to register to the target section, we select a few easily recognizable cells on both the initial and on the target section and use these features as *Registration Points*. Taking overview montages at low magnification (\sim 300 \times) can help finding cells back and speeds up the process. We then apply the resulting transformation to all duplicated map items. These items are now placed approximately at the correct position on the target section. The few microns of shift that usually occur can be corrected using the Realign To Item procedure when the cellular features provide sufficient visual similarities between adjacent sections.

The acquisition of new maps on the target section is done by running a SerialEM script which also labels the Navigator entries such that they match the source Navigator labels. This keeps the indexing of the cells consistent to avoid confusing the many navigator items (cell43_s1, cell43_s2, etc.).

The entire relocation procedure is then repeated for each transition to a neighboring section. We usually start from a single section where we identify the target cells and then follow them section-by-section in both directions.

The script is available at <https://serialscripts.nexperion.net/script/43>.

For acquiring tomograms using the fast scripts, the parameters such as tilt range, angular sampling, target defocus and storage directory are provided in the master script. The user can also specify, whether a separate map or additional tomogram at lower magnification should be recorded directly after the first. The naming of the files is automatically done by the script such that they match the corresponding navigator label.

The script for fast tomography is available at <https://serialscripts.nexperion.net/script/42>.