# New features in Look@NanoSIMS:

## isotopic reconstruction in 3D and correlation with ultra-high resolution electron microscopy

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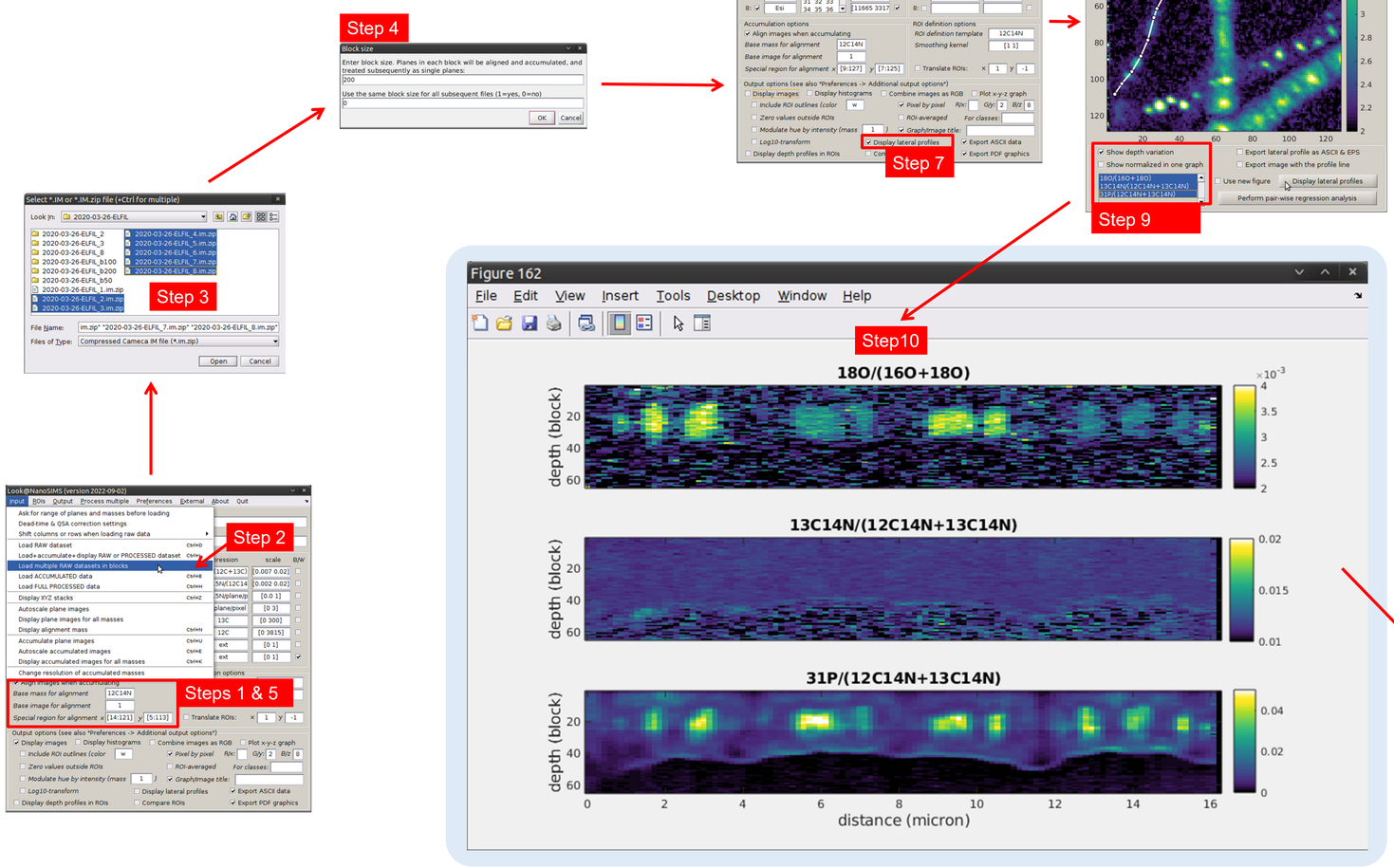
## 3D variation of the isotopic composition within microbial cells

### Analysis:

**Measure secondary ions until sample completely eroded**

- Typically: a chain analysis with 1000 planes per file
- recommended: keep dwell-time at 1000 µs/px to minimize effects of image drift that cannot be corrected for by data processing
- variable dwell-time can be accounted for later




### Data processing in Look@NanoSIMS:

1. Define how the planes will be aligned and accumulated in blocks.
2. Load multiple raw datasets in blocks.
3. Select input datasets (hold Ctrl to select multiple input files).
4. Define number of planes per block. It can vary among input datasets, but it can also be set as constant for all datasets.
5. Align and accumulate all planes.
6. Define expressions for ion count ratios.
7. Select “Display lateral profiles”.
8. Select ion count ratio from the top pop-up menu, define a lateral profile as a **polyline**, and specify the profile thickness.
9. Select “Show depth variation”, select multiple ion count ratios in the bottom list, and click on “Display lateral profiles”.
10. Inspect the data.
11. At the end, save the full processed dataset in a mat file. This will eliminate the need to perform steps 1-5 if you want to analyze the same data again.

## Correlation with ultra-high resolution electron microscopy images

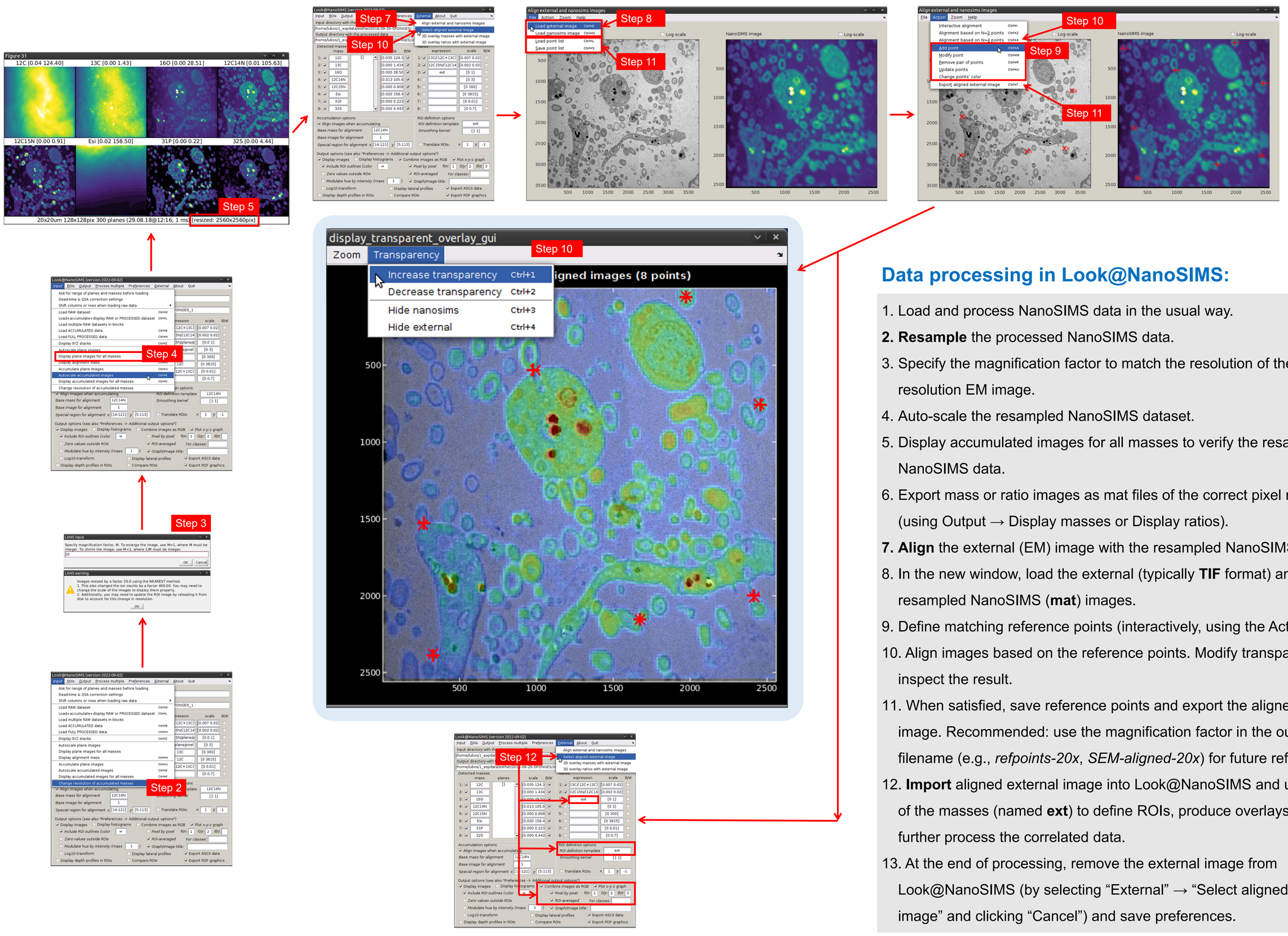
### Analysis:

**Acquire ultra-high resolution image of the sample**

- For example, using electron microscopy (TEM or SEM).
- Typically: imaged area 20−30 µm, pixel resolution 3000−4000 pixels.

**Measure secondary ions from (roughly) the same area**

- Typically: pixel resolution of 256 or 512 pixels (i.e., roughly 10-fold lower)
- Issue: the two types of images are **distorted** relative to each other (due to image drift during lengthy NanoSIMS measurements)




### Data processing in Look@NanoSIMS:

1. Load and process NanoSIMS data in the usual way.
2. **Resample** the processed NanoSIMS data.
3. Specify the magnification factor to match the resolution of the ultra-high resolution EM image.
4. Auto-scale the resampled NanoSIMS dataset.
5. Display accumulated images for all masses to verify the resampled NanoSIMS data.
6. Export mass or ratio images as mat files of the correct pixel resolution (using Output → Display masses or Display ratios).
7. **Align** the external (EM) image with the resampled NanoSIMS image.
8. In the new window, load the external (typically **TIF** format) and resampled NanoSIMS (**mat**) images.
9. Define matching reference points (interactively, using the Action menu).
10. Align images based on the reference points. Modify transparency to inspect the result.
11. When satisfied, save reference points and export the aligned external image. Recommended: use the magnification factor in the output filename (e.g., *refpoints-20x*, *SEM-aligned-20x*) for future reference.
12. **Import** aligned external image into Look@NanoSIMS and use it as one of the masses (named **ext**) to define ROIs, produce overlays, etc., to further process the correlated data.
13. At the end of processing, remove the external image from Look@NanoSIMS (by selecting “External” → “Select aligned external image” and clicking “Cancel”) and save preferences.