

Supplementary information for ROOT-ExM: Super-Resolution Imaging of Proteins in *Arabidopsis* Roots by Expansion Microscopy

Yutaro Shimizu^{1, #}, Guillaume Maucort^{2, #}, Mónica Fernández-Monreal², Florentin Dumas¹, Emmanuelle M. Bayer¹, Yohann Boutté^{1, *} and Magali S. Grison^{1, #, *}

¹Laboratoire de Biogenèse Membranaire, Université de Bordeaux, UMR5200 CNRS, Villenave d'Ornon, France

²Université de Bordeaux, CNRS, INSERM, Bordeaux Imaging Center, US4, UAR 3420, Bordeaux, France

*For correspondence: yohann.boutte@u-bordeaux.fr; magali.grison@u-bordeaux.fr

[#]Contributed equally to this work

1. Install "Miniforge" (once for the first time)
<https://github.com/conda-forge/miniforge>
2. Download "expansion_analysis_protocol" (→)
https://src.koda.cnrs.fr/bic-tai/expansion_analysis_protocol
3. Open Miniforge Prompt
4. Navigate to the repository folder
5. Create the environment
6. Activate the environment
7. Launch "jupyter notebook"
8. Open "notebooks" → "expansion_analysis.ipynb"
9. Run the cells
10. Load your images
11. Indicate a tentative expansion factor

First a simple overlay image (without registration or transformation) of the pre- and post-expansion images will be shown. The pre- and post-expansion images are displayed in magenta and green, respectively, in the overlay.

The similarity transformation will be applied to correct global translation, rotation, and isotropic scaling differences. The process can also calculate the isotropic expansion factor.

The calculated expansion factor based on the number of pixels

Following the global alignment, the non-linear B-spline transformation will be applied to capture local deformations.

Feature points will be sampled from the binary foreground to calculate RMS errors.

The pipeline will generate the globally aligned image, non-linearly deformed image, RMS displacement CSV file, and visualization plots of deformation magnitude.

Figure S1. Image analysis workflow