

SUPPLEMENTARY TEXT FOR

Correlative Imaging across Microscopy Platforms Using the Fast and Accurate Relocation of Microscopic Experimental Regions (FARMER) Method

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Full result of relocating MIN6 cells (complementing Fig. 5)

We used FARMER to relocate regions of interest (ROIs) that are groups of live cells in a glass-bottom dish, as described in the main text (Fig. 5). In the first imaging session, we imaged 5 uniquely looking ROIs and recorded their coordinates. In each of the two subsequent imaging sessions, we used FARMER to find and image all 5 ROIs (Fig. S1A). Between consecutive sessions, the dish was taken off the stage and remounted with deliberate rotation. The time taken to find the coordinates of the cells after each remounting was about 3 minutes. Note that each field of view contained many cells even though only a few of them were fluorescently labeled (Fig. S1A).

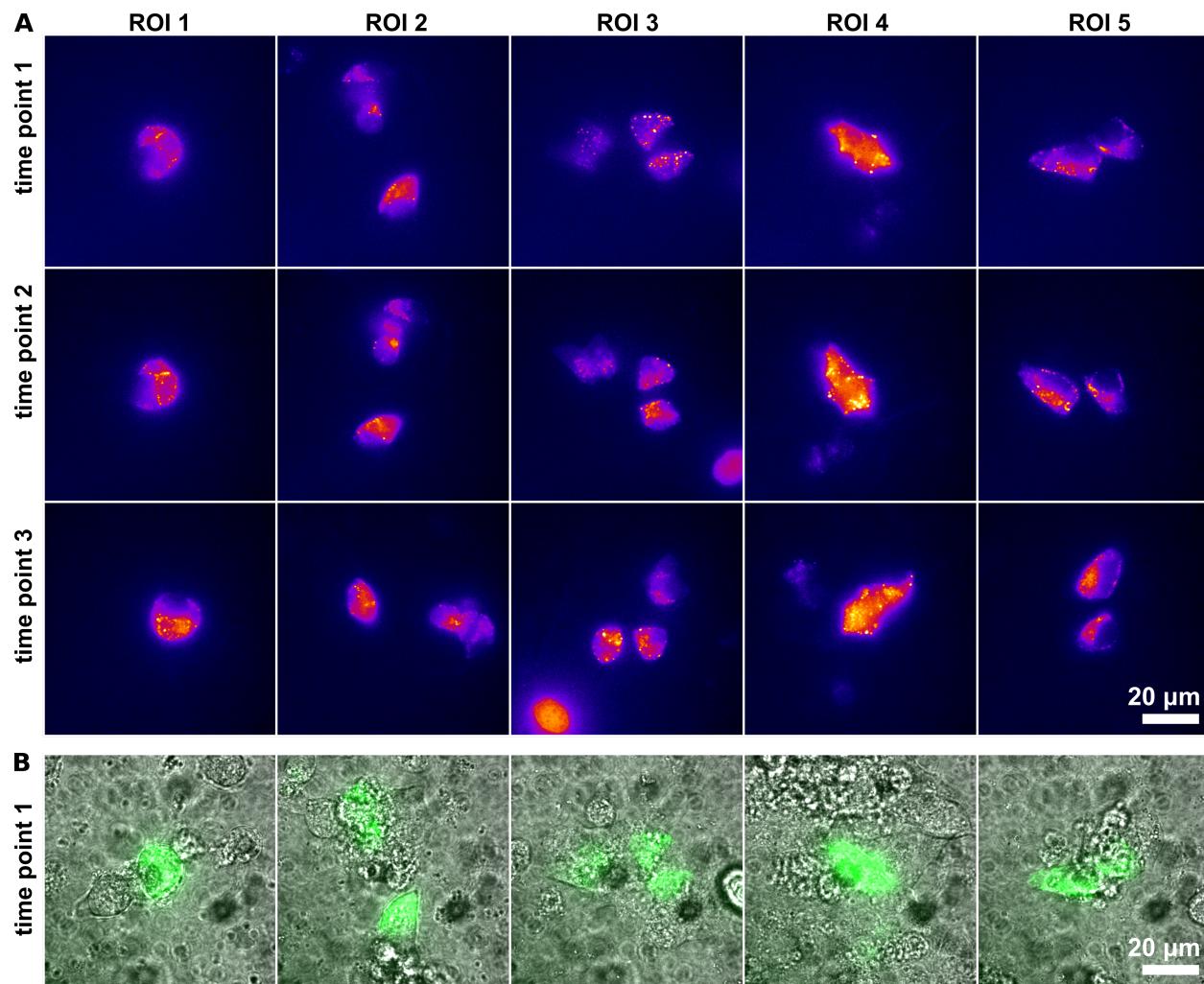


FIG. S1. A) Multiple regions of interest (ROIs) of MIN6 cells at different time points, imaged with spinning-disk confocal fluorescence microscopy using a 100x objective. Between consecutive time points, the dish containing the cells were taken off the microscope and then remounted with intentional rotation. The lookup table is ImageJ Fire. B) Overlay of brightfield and fluorescence images corresponding to time point 1 in A. The lookup tables of the brightfield and fluorescence channels are gray and green, respectively.

Errors due to deviations from the FARMER implementation

We evaluated the errors that would occur when FARMER were implemented with intentional deviations, using the results of relocating MIN6 cells (Fig. 5, S1) as an example.

Firstly, if we had used the features made by the permanent markers without adjusting the focus (Fig. 5C), the calculated coordinates would not have deviated much (0-4 μm in any direction) (Fig. S2A).

Secondly, without the scaling/shearing operator, we would have had small deviation in the xy-plane (0-4 μm), but no deviation in focus: the maximum difference 0.06 μm is smaller than the stage resolution 0.1 μm (Fig. S2B). The non-zero deviations in the xy-plane indicate the need for scaling/shearing if better accuracy is needed, even though they are equivalent to about 5% of the field of view and would not have caused a problem in locating the cells. Another advantage of scaling/shearing is that arbitrary units can be used; for example, the recorded numbers on the optical microscope used to image the nanoparticles were in micrometers (μm), while they were in millimeters (mm) on the SEM.

Finally, the algorithm is set up for a right-handed coordinate system, while not all systems are set up as such in practice. We simulated a left-handed coordinate system on the microscope by flipping the signs of the z-coordinates, and found enormous errors in focus ($\sim 14000 \mu\text{m}$), while errors in the xy-plane were practically zero (Fig S2C). If the coordinate system in the 1st session was right-handed, while that in the next session was left-handed, the errors were also similar, $\sim 14000 \mu\text{m}$ in z-direction, and $<1 \mu\text{m}$ in the xy-plane.

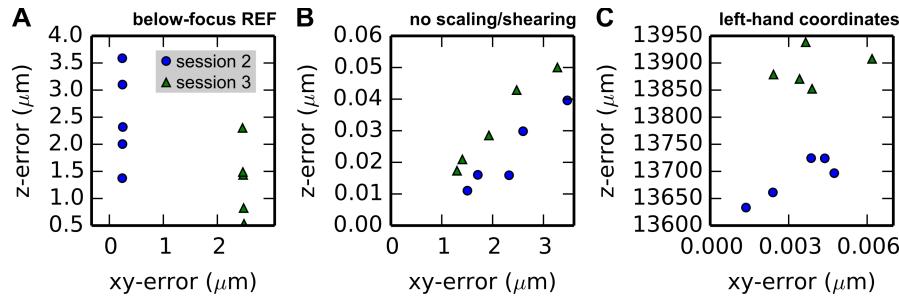


FIG. S2. Errors (distances from the centers of the fields of view) from relocation experiments done with MIN6 cells (Fig. 5, S1) with intentional deviations in the implementation of FARMER. (A) Plot showing errors when reference points (REF) are on a focal plane below the regions of interest (ROIs) and above the objective (Fig. 5C). (B) Plot showing errors when no scaling/shearing operator was used. (C) Plot showing errors when the coordinate system of the microscope stage was left-handed, and was a mirror image of the system used in the calculations. Note that all values are absolute values.

Aligning images from different microscopes (complementing Fig. 6)

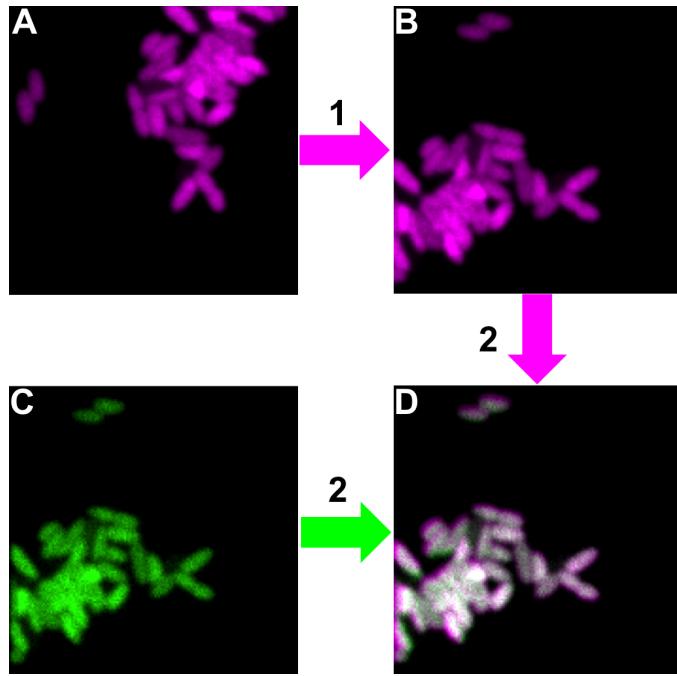


FIG. S3. Alignment of MFM and confocal images (Fig. 6). (A) The confocal image from Fig. 6C is (1) rotated 90° counterclockwise and flipped vertically to produce the image in (B). (C) The MFM image from Fig. 6B. (D) The MFM image in C is overlaid with the transformed image in B (2) to produce the overlay image. The resultant (overlaid) image is white showing the perfect alignment of the green and magenta channels clearly indicating that the bacterium image is the same shape and size after accounting for specimen orientation. The images in Fig. 6 were resized to account for the differences in magnification between the microscopes. Image rotations and flips occur since the number of reflections (mirrors in the emission light path which result in flips in the image) and camera orientation (rotation) are not the same between the two setups.

TABLE S1. Compilation of stages used in this work.

Figure number	Stage
1	3, 4 Ludl 99S108-O2-LE
2	5 Prior ProScan II H117P1I4
3	6 Prior ProScan H117E1N5/F
4	6 Ludl 99S106-N2K-LE2
5	7 Built-in stage of Horiba LabRAM HR Evolution
6	7 Built-in stage of FEI Nova NanoSEM 230

Algorithm source code in Python 2.7.6, with numpy 1.8.0

Usage:

- (0) Install Python and numpy. Choose (or make) a folder and put all the files mentioned below in that folder.
- (1) Copy Relocation.py into the working folder.
- (2) Make Original.txt, which contains the list of coordinates of REFs and ROIs (space delimited, one point per row), from the design or an empirical search
- (3) Make REFIndex.txt, which contains the indices of REFs in Original.txt (counting starts at 0, in 1 line). For example, if REFs are the 1st, 5th, and 11th points, REFIndex.txt's content should be “0 4 10” (no quotation marks).
- (4) At the beginning of the imaging session, find the coordinates of the REFs after sample mounting. Save in Input.txt (space delimited, one point per row).

- (5) Run the Python code. For example, in the terminal (or command line), change the directory to the one in (0), then type “python Relocation.py” (no quotation marks).
- (6) Make sure Output.txt is made. This file contains the coordinates of the ROIs on the stage (space delimited, one point per row).
- (7) Use the stage controlling software to read the coordinates, or manually input the coordinates.