Colocalization of cellular nanostructure using confocal fluorescence and partial wave spectroscopy

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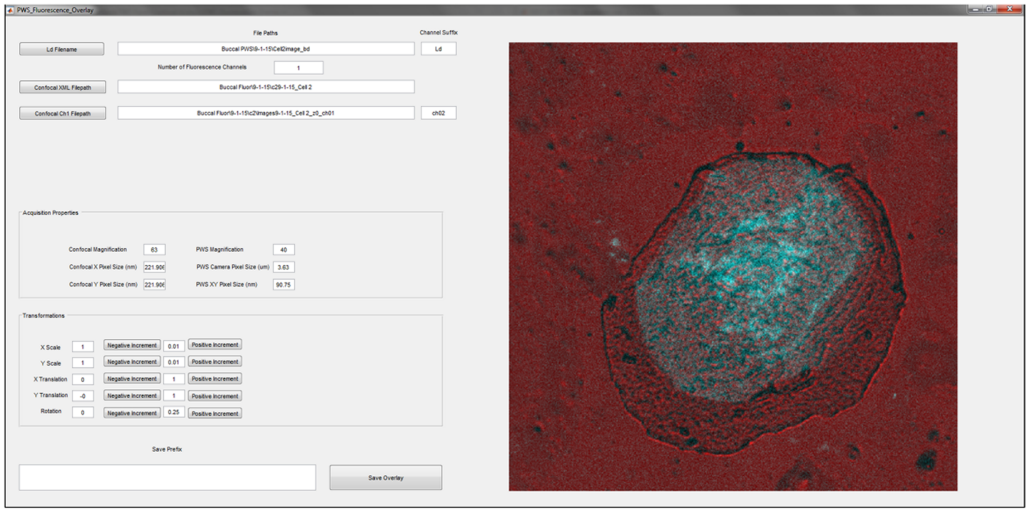
Supporting Information (Image Alignment and Overlay Image Processing):

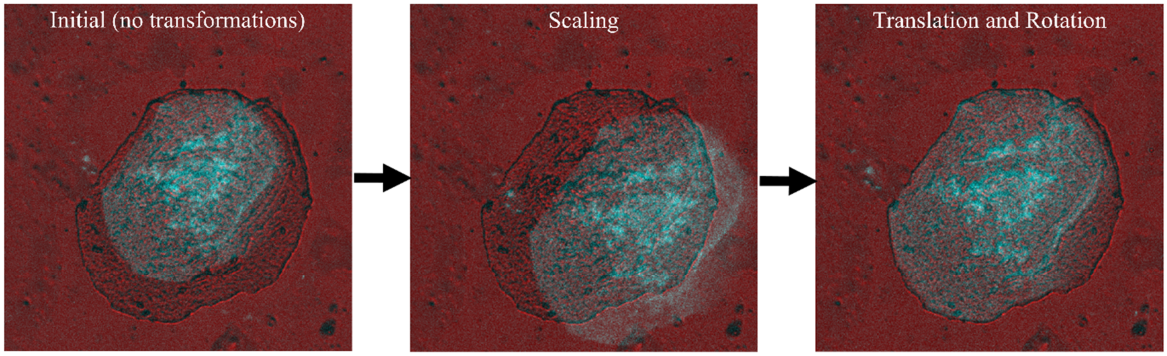
Several image alignment steps were undertaken following collection of the PWS and confocal data. Because different objective lenses and detectors were used for confocal and PWS acquisition, the image sizes differed in terms of field-of-view and pixels size. To correct for this a custom software interface was developed in MATLAB that allowed a user to overlay confocal image channels with PWS images from the same cell as illustrated in **Supplemental Figure 1**. When images were loaded, the program automatically rescales the confocal image to match the pixel size of the PWS image based on the known magnifications and pixel sizes of the acquired data. The user then has the option to manually adjust rotation and translation to align the confocal image with the PWS image. Supplemental Figure 1 illustrates this process. When the images are aligned the interface saves a transformation matrix that can be applied to all the confocal channels corresponding to that PWS measurement.

PWS images (Σ) are calculated from the spectral microscope images normalized by a glass reference spectrum to account for throughput of the optical path and the source spectrum. Σ was calculated at each pixel in the images as the standard deviation of the normalized spectrum. Images of Σ, such as those shown in column 2 of Figures 2 and 3, are displayed using the Jet colormap available in MATLAB using the scale limits shown by the colorbars and specified in the figure captions. To display the contrast provided by Σ within specific organelles as in column 4 of Figures 2 and 3, binary regions of interest defined by the fluorescence channels were segmented using the Mathematica implementations of Kapur's entropy method for the mitochondria and Otsu's thresholding algorithm for all other dyes. These regions of interest were then multiplied by the Σ image and assigned the color associated with the fluorescence channel used for the segmentation. Pixels outside the regions of interest segmented from the fluorescence channels in each sample were assigned to a third channel (red). All channels were then added together to yield an image showing the distribution of Σ color coded according to the organelles present at each pixel.

(a)



(b)



Supplemental Figure 1. (a) Screenshot of the software interface used to align PWS and confocal images. (b) Illustration of the process to align confocal (blue) and PWS (red) images. At the left, raw image data is shown with no transformations applied. Middle, the software interface automatically applies scaling based on the hardware parameters from each image making the effective pixel size in each image equivalent. Right, the user adjusts the translation and rotation transformations such that the two images align.