

Study of the 3D repartition of vesicles normalized by the distance to the cell periphery

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We propose a tool to study the repartition of endocytic compartment organization in 3D fluorescence microscopy image. Although cell variation in size and shape usually prevents comparison and statistic generation about spatial organization, standardization can be obtained by normalizing the cells by micropatterning [1]. Another approach chooses to find a common frame to all cells, for example by fitting an ellipsoid to cellular compartment of reference (eg. Nuclei membrane in [2]). However, both approaches are not suitable for unconstrained cells with a wide range in shape and size such as lymphocytes. In order to study the endocytic compartments in activated T cells, we have created a 3D relative polar coordinate system oriented by the cell MTOC position and where the radius is normalized by the distance to the cell membrane (Figure 1). We have validated this approach to study cytokine containing vesicles repartition in T cells, where the common coordinate system origin is defined by the MTOC, automatically segmented by mathematical morphology. Cells were first semi-automatically segmented using AMIRA. Vesicles were then automatically segmented by 3D Gaussian fitting of regional maxima. The distance from the vesicles to the cell periphery was finally automatically computed as the distance between the center of gravity of the vesicles and the intersection point between the Vesicle-MTOC line and the cell surface. The accuracy of the distance computation had a maximal error equal to the size of a voxel in z (200 nanometers in our experiments), which is still within the deconvoluted 3D-widefield resolution (200 nm in xy). This framework can have a broad band of applications. For example we are also working on its application to loci detection and distance to the nuclear membrane in budding yeast.

[1] K. Schauer et al. "Probabilistic density maps to study global endomembrane organization," *Nat. Methods*, **7**, 560-566 (2010).

[2] AB. Berger et al. "High Resolution statistical mapping reveals gene territories in live yeast," *Nat. Methods*, **5**, 1031-1037 (2008).

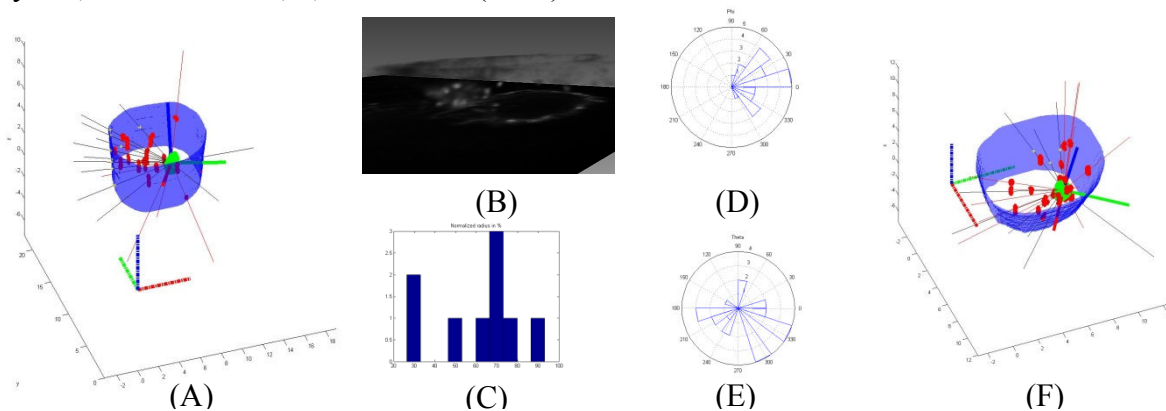


Figure 1: (A) and (F) Two segmented cells (transparent blue), MTOC (green) and vesicles (red). The yellow cross indicates the intersection point of the Vesicle-MTOC line on the cell surface. The image frame and the cell relative frame are shown. (B) Original cytokine-specific data set for cell (A); (C) Histogram of the repartition of the vesicles according to their normalized distance to the surface; (D) and (E) 3D normalized polar coordinates theta and phi.