

Molecule counting of organellar cargo

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With the advent of modern bio-imaging techniques and advanced computational power, quantitative biology has become a hot area of research. As more and more molecules are being administered to living cells for various cell biological experiments, it is important to understand how many molecules are uptaken and how are they processed compartment wise in various genetic backgrounds. Despite of development of several methodologies for absolute cargo quantification, exact quantification of cargo in subcellular compartments with spatiotemporal control has been a challenge. Here we describe a method to quantify amount of cargo DNA uptake by J774 cells in order to characterize the process of endosomal maturation. We use a special imaging technique called intracellular Single-molecule, High-Resolution Localization and Counting (iSHIRLoC) in conjugation with MATLAB and Python based image analysis to quantitate exact number of cargo molecules in early endosomes of J774 macrophage cells. This study sets up a general platform for quantitation of cargo in an intracellular compartment within a cell.

