Thesis title: Reloading the Confocal Data Matrix: Quantitative Characterization of Organic and Biological Assemblies by Optical Sectioning of Samples

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Entry Number: 2017BLZ8282

Abstract

The visualization of cells has significantly advanced since Robert Hooke's first observation of cork cells in the 1600s using a compound light microscope. This led to identification of cells as the basic structural and functional units of life. The mid-20th century saw the advent of fluorescence microscopy (1950s) and confocal microscopy (1970s), which enhanced imaging capabilities by allowing optical sectioning and improving the signal-to-noise ratio. Confocal microscopy, especially, has become a go-to technique for visualizing live cells in their native hydrated state due to its ability to generate three-dimensional data matrices through optical sectioning. However, more often than not the confocal microscopy has only been used as a 2D tool to increase signal:noise ratio in the images.These advancements in light microscopy have allowed researchers to delve deeper into cellular heterogeneity, recognizing that variations within cell populations are not merely noise but are critical for proper cellular function. This work utilizes confocal microscopy as a 3D tool to study, quantitatively characterize, various organic and biological assemblies leading to defining 3D innate morphological heterogeneity in cells at whole cell level and at the level of subcellular components with respect to their distribution within the cells' confinement.

The study begins with an examination of pseudopeptosome, pseudopeptide self-assemblies, in controlled chemical systems to minimize heterogeneity that might affect structural dynamics and then extends to studying morphological variations in RAW264.7 cells in different conditions.

The cells have been studied in their native condition and after synchronization at the G2/M phase. Synchronization reduces overall morphological heterogeneity, giving cells a common purpose of division, allowing for a focused study by minimizing variations in morphology of the whole cell and subcellular component distribution. Furthermore, the study investigates the effects of osmolarity on cellular morphology and organelle distribution. Despite extensive literature on osmolarity, a systematic study quantifying its impact on subcellular organelle distribution and

cellular pleomorphism has been lacking. As opposed to the popular belief that the hyperosmolarity increases pleomorphism and hypoosmolarity decrease it, with the help of quantitative image analysis of morphometric parameters,we report that both the conditions decrease morphological heterogeneity in the population. We further report the organelles in which the whole cell heterogeneity has been translated into. From our repertoire of 7 subcellular components, we report ER, mitochondria, and tubulin to be independent of whole-cell apico-basal heterogeneity of optical density while nuclear, plasma membrane, lysosomal, and actin fluorescence distributions are found to contribute to the apico-basal polarity of the whole cell. We report that the variation in trends of the parameters, related to extracellular osmolarity, observed at whole-cell level are translated into actin and tubulin parameter variations, while nucleus, mitochondria, and endoplasmic reticulum are independent of the whole cell morphology alterations. While doing so, we have also developed an image analysis algorithm utilizing 2D segmentation to analyze the single cells in 3D using confocal microscopy - a technique that allows us to analyze cellular states in their native hydrated state.