AUTOMATIC SINGLE CELL RECOGNITION AND PLANNING FOR CORRELATIVE IMAGING

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ABSTRACT: The versatile functions of microscopic approaches developed allow cellular investigations to be conducted to acquire different spatial and temporal information. We have proposed automatic and correlative imaging of single cells, achieved by segmentation, imaging planning and correlation based on single cell images acquired by Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and X-ray Fluorescence Microscopy (XFM) (Figure 1a) [1][2]. A relatively large field of view (FOV) was first acquired upon which single cells were recognized given sufficient resolution and signal-to-noise level. An optimized geometric planning scheme that covers individual single cells with the increased magnification (reduced FOVs), as required by higher resolution imaging, was further developed [1].

A recent application is to quantify the uptake of antibiotics in single bacterial cells. Prepared on silicon membrane, bacterial cells (*Acinetobacter baumannii*) treated by iodine-labelled polymyxin were initially imaged by performing AFM measurement followed by elemental mapping with XFM imaging (Figure 1b). The acquired mapping data from the two sources were then registered, and used to determine the molarity and intracellular distribution maps for each single cell (Figure 1c). Normalized with the height data from AFM, intracellular distribution of polymyxin as represented iodine is determined to be uniformly distributed, contrary to the implication of "hot spots" based on mass concentration mappings only. The integration of microscopic approaches with the developed computational techniques offers a high resolution yet operationally efficient solution for correlative imaging of single cells.

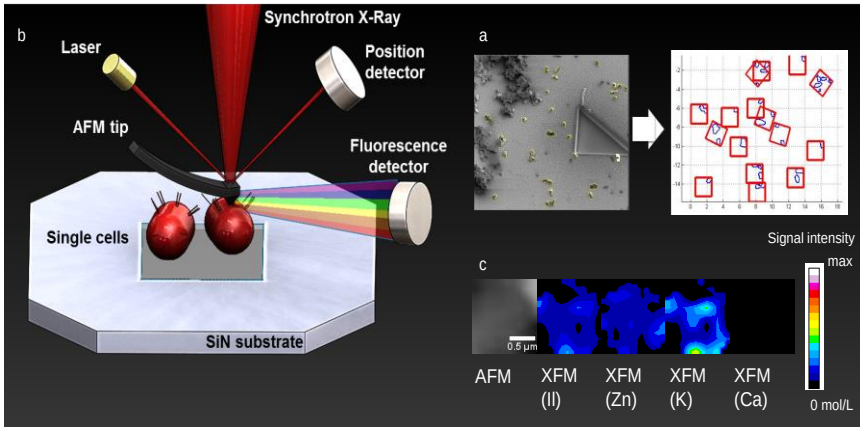

Figure 1. Schematics showing the experimental procedures initiated with (a) feature recognition and proposed planning scheme of target cells, followed by (b) XFM quantification and AFM topological measurement on single cells and (c) post-processing to derive the high resolution correlative images on one single bacterial cell (*Acinetobacter baumannii*).

REFERENCES

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