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Reconstruction of the Nanoscale Three-Dimensional Mass-Density Autocorrelation Function of Individual Cells

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Interrogating cellular nano-architecture may help reveal previously unknown structure-function relations. The three-dimensional mass-density distribution plays an important role in quantifying cellular structure. For instance, chromatin structure can be directly represented by the mass-density, as the individual molecules have similar mass and larger density generally indicates higher level of compaction. Chromatin compaction, in turn, is associated with gene transcription and is crucial in various aspects of fundamental biology, genetics, and oncology studies [1]. Moreover, it has been reported that a statistical representation of mass-density distribution by autocorrelation function (ACF) is crucial in stratifying risk of cancer in early stages [2]. Recent developments such as Partial Wave Spectroscopy [3] have gained success in cancer diagnosis by detecting three-dimensional sub-diffraction chromatin mass-density ACF alterations between malignant cells and healthy cells for various types of cancers.

While it has become routine to measure the 3D mass-density distribution of thin biological samples using high angle annular dark field (HAADF) electron tomography, such information for a complicated biological medium like a whole individual human cell has been hard to achieve. Technically, there are two conflicting aspects in obtaining such information: high resolution and large sample volume [4]: 1. The resolution of projection images is limited by sample thickness; 2. The voxel resolution of electron tomography is also limited by sample size, following the Crowther criterion; 3. The linear relationship between mass and HAADF signal may not hold for thick samples. To tackle the problem, we developed an experimental method combing HAADF and atomic force microcopy (AFM) and a novel de-convolution algorithm to reconstructed the 3D mass-density ACF for a real biological sample. As a demonstration of the method and the algorithm, we reconstructed the 3D mass-density ACF of a human cheek cell in the nucleus region.

First, we optimized the semi-collection angle of HAADF detector to maintain a linear relationship between grayscale image intensity and mass for thick polymer samples. Under the optimized conditions, we imaged a human buccal stratified squamous epithelial cell prepared by critical point drying at 10 nm pixel resolution (Fig.1 (a)). We then chose to use 5-micron polystyrene beads as external mass standard to calibrate the pixel-to-pixel mass projection of the cell nucleus region (Fig.1 (b)). A thickness map of higher resolution was measured using AFM of the same region (Fig.1 (c)). Density was calculated based on mass and thickness and the rotational averaged ACF of the density projection was analyzed (Fig.1 (d)). We find that the highest solid content is close to 60% for nucleus and 30% for cytoplasm. This is in good agreement with the reported value for the outmost layer of cheek cells [5]. Assuming the medium is statistically isotropic, we reconstructed the 3D ACF from the

2D ACF (Fig.1 (e)) of the density projection by a robust de-convolution algorithm using a "top-hat" function, while the accuracy of the algorithm was proved numerically.

In summary, we have demonstrated a method that is applicable to an entire human cheek cell, calculated the density, solid content, and shape of 3D ACF. The fact that these quantities are all in good accordance with the literature can be seen as promising for the proposed method. Future work will be aimed at applying this method to vast range of biological samples, and the mass-density distribution may provide valuable information to understand many cellular biological processes. In the presentation, we will focus on the technical details of experiments and math derivation of the reconstruction algorithm, as well as the possible directions of the applications of this method in biological and biomedical research.

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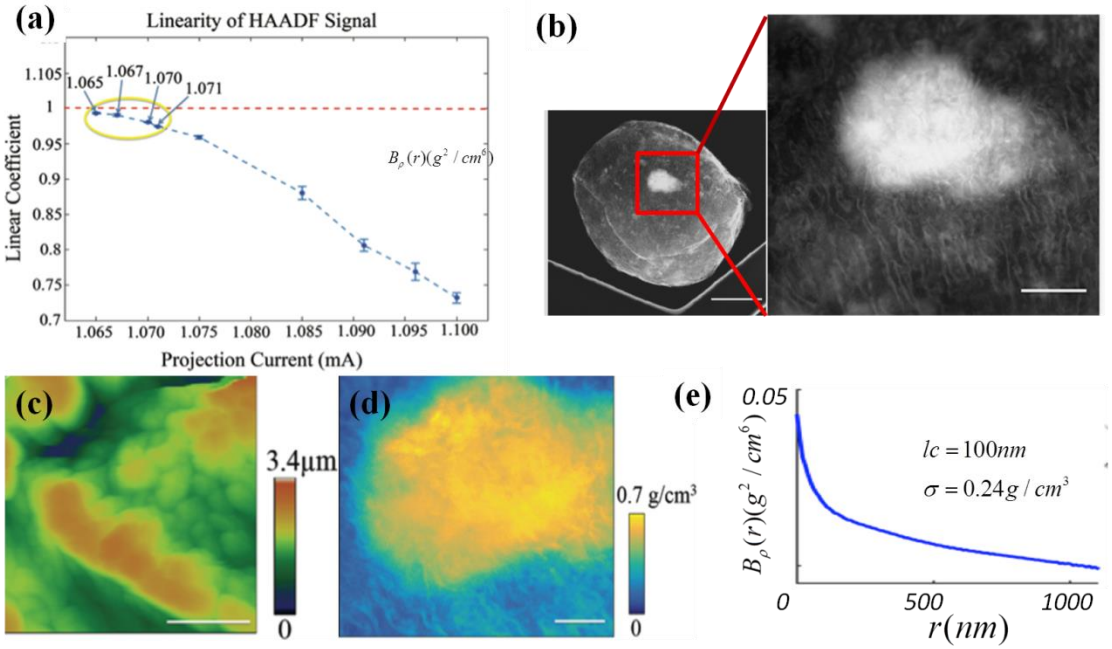

Figure 1. (a) The recovery of linear relation between HAADF signal and mass as collection angel increase. (b) HAADF image of a CPD cheek cell on carbon film at low (scale bar: 10μm) and high magnification(Scale bar: 3μm). (c) AFM image of nuclear region, scale bar: 2μm. (d) Density map of nuclear region, scale bar: 1μm. (e) 3D ACF of mass-density shown in (d), the correlation length (L_C) and disorder length ($\sigma \times n$) are listed in the graph.

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