Visual proteomics using whole-lamella 2D template matching

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Abstract

During hematopoiesis all cells of the blood derive from a common set of stem and progenitor cells in the bone marrow. How is the differentiation of these morphologically and functionally diverse cells orchestrated and how is differentiation outcome is encoded in progenitor cells? To provide answers to this question we are using a "visual proteomics" approach where we determine cellular state by quantifying the localization, orientation, and functional state of biomolecules in cells by label-free cryo-electron microscopy (cryo-EM) imaging. Our lab has developed an algorithm called 2D template matching, where all possible projections of a reference structure are compared with cryo-EM images of cells to derive the location and orientation of all instances of a biomolecule. We used this approach to find large ribosomal subunits in cryo-EM images of differentiating hematopoietic precursor cells. A new data-acquisition scheme called "Fast Observation of Whole Lamella" (FOWL) was employed to find ribosomes in complete cellular cross-sections. We find highly heterogeneous distribution of ribosomes in differentiated cells and are working on optimizing data-collection and data-processing to achieve the throughput that allows imaging the equivalent of multiple cellular volumes. We envision that using these high-throughput visual proteomics approaches can become a complimentary technique to other single-cell techniques, such as flow-cytometry and single-cell sequencing.

Introduction

References