





# Visual proteomics using whole-lamella 2D template matching

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## Abstract

Localization and characterization of biomolecules inside a cell is the fundamental quest of all biological imaging. Fluorescence microscopy can localize biomolecules inside whole cells and tissues, but its ability to count biomolecules and accuracy of the spatial coordinates is limited by the wavelength of visible light. Cryo-electron microscopy on the other hand provides highly accurate position and orientation information of biomolecules but is often limited to small fields of view inside a cell, providing only limited biological context. In this study we use a data-acquisition scheme called “Fast Observation of Whole Lamella” (FOWL) to collect cryo-electron microscopy data over thin central sections, representing roughly 1% of the total cellular volume, of neutrophil-like mouse cells. We use 2D-template matching to determine localization and orientation of the large subunit of the ribosome in these cross-sections. We furthermore use 2D-template matching to test for complex formation with the small ribosomal subunit and used relative orientations of ribosome to assign ribosomes to polysomes. Overall these results provide a “map” of translational activity in cross-sections of mammalian cells. We envision that using these high-throughput cryo-EM data collection and 2D template matching approaches will advance visual proteomics to complement other single-cell “omics” techniques, such as flow-cytometry and single-cell sequencing.

## Introduction

Understanding of cellular processes requires knowledge of the amount, location, and interaction of biomolecules inside the cell. Techniques that measure this can broadly be divided into label, and label-free techniques. In label-techniques a probe is physically attached to a molecule of interest that is able to produce a high signal-to-noise signal, such as a fluorescent probe. In label-free techniques the physical properties of molecules themselves are used for detection. An example for this is proteomics using mass-spectrometry. Broadly the advantage of label-free techniques is that they can provide information over thousands of molecules, while label-techniques offer higher fidelity information for a few molecules. Especially spatial information can most of the time only be achieved using label-techniques.

Cryo-electron microscopy has the potential to directly measure the arrangement of atoms that compose biomolecules inside of cells, thereby allowing label-free detection with high spatial accuracy. This has been called “Visual proteomics”. The predominant technique at the moment is cryo-electron tomography. However, the throughput of these methods is low due to highly complex sample preparation, data acquisition, and data processing. We have shown that molecules can be identified by their structural “fingerprint” in single projection using “2D template-matching”.

Here we apply 2D-template matching to neutrophil-like murine cells. By employing a new data-acquisition scheme we obtained coverage of the whole “lamella” covering roughly 2% of the cellular volume.

## Materials and Methods

### Grid preparation

ER-HoxA9 cells were maintained in RPMI supplemented with 10% FBS, penicillin/streptomycin, SCF, and estrogen [1] at 37°C and 5% CO<sub>2</sub>. 120h prior to grid freezing cells were washed twice in PBS and cultured in the same medium, except without estrogen. Differentiation was verified by staining with Hoechst-dye and inspection of nuclear morphology. Cells were then counted and diluted to  $1 \times 10^6$  cells/ml. Grids (either 200 mesh copper grids, with a silicone-oxide and 2  $\mu$ m holes with a 2  $\mu$ m spacing or 200 mesh gold grids with a thin gold film and 2  $\mu$ m holes in 2  $\mu$ m spacing) were glow-discharged from both sides using a ... for ... . 3.5  $\mu$ l of cells suspension was added to grids on the thin-film side and grids were automatically blotted from the back-side using a GP2 cryoplugger (Leica) for ... s and rapidly plunged into liquid ethane at -185°C.

### FIB-milling

Grids were loaded into a Aquilos 2 FIB/SEM microscope with a stage cooled to -190°C. Grids were sputter-coated with platinum for 15s at 45 mA and then coated with a layer of platinum-precursor by opening the GIS-valve for 45s. An

overview of the grid was created by montaging SEM images and isolated cells at the center of gridsquares were selected for FB-milling. Lamella were generated automatically using the AutoTEM software, resulting in 6-8  $\mu\text{m}$  wide lamella with 150-200  $\mu\text{m}$  thickness as determined by FIB-imaging of the lamella edge.

## Data collection

Grids were loaded into a Krios Titam TEM operated at 300 keV. The microscope was setup with a cross-grating grid on the stage by setting the beam-diameter to 900 nm, resulting in the beam being completely visible in the camera. To establish fringe-free conditions, the “Fine eucentric” procedure of serialEM was used to move a square of the cross-grating grid to the eucentric position of the microscope. The effective defocus was then set to 2  $\mu\text{m}$ , using the “autofocus” routine of serialEM. The objective focus of the microscope was changed until no fringes were visible. The stage was then moved in Z until images had a apparent defocus of 2  $\mu\text{m}$ . The difference in stage Z-position between the eucentric and fringe-free conditions was calculated and noted to move other areas into fringe-free condition.

Low magnification montages were used to find lamella and lamella that were sufficiently thin and free of contamination were selected for automated data collection.

## Data pre-processing

## Template matching

## Data analysis

## Introduction

## Introduction

## Figures

## References

1. **Inhibition of Dihydroorotate Dehydrogenase Overcomes Differentiation Blockade in Acute Myeloid Leukemia** David B Sykes, Youmna S Kfoury, François E Mercier, Mathias J Wawer, Jason M Law, Mark K Haynes, Timothy A Lewis, Amir Schajnovitz, Esha Jain, Dongjun Lee, ... David T Scadden  
*Cell* (2016-09) <https://doi.org/f3r5jr> DOI: 10.1016/j.cell.2016.08.057 · PMID: 27641501 · PMCID: PMC7360335



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