

Electron cryo-tomography for measurements of macromolecular changes induced by test compound within a cell

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Introduction

Structures obtained by NMR, X-ray diffraction and single particle cryo-electron microscopy lack the cellular context. In the cell, quite often chemicals that are supposed to bind a specific target show lower affinity and are not delivered to the target or they interact with unpredicted cellular components with possible toxic side effects.

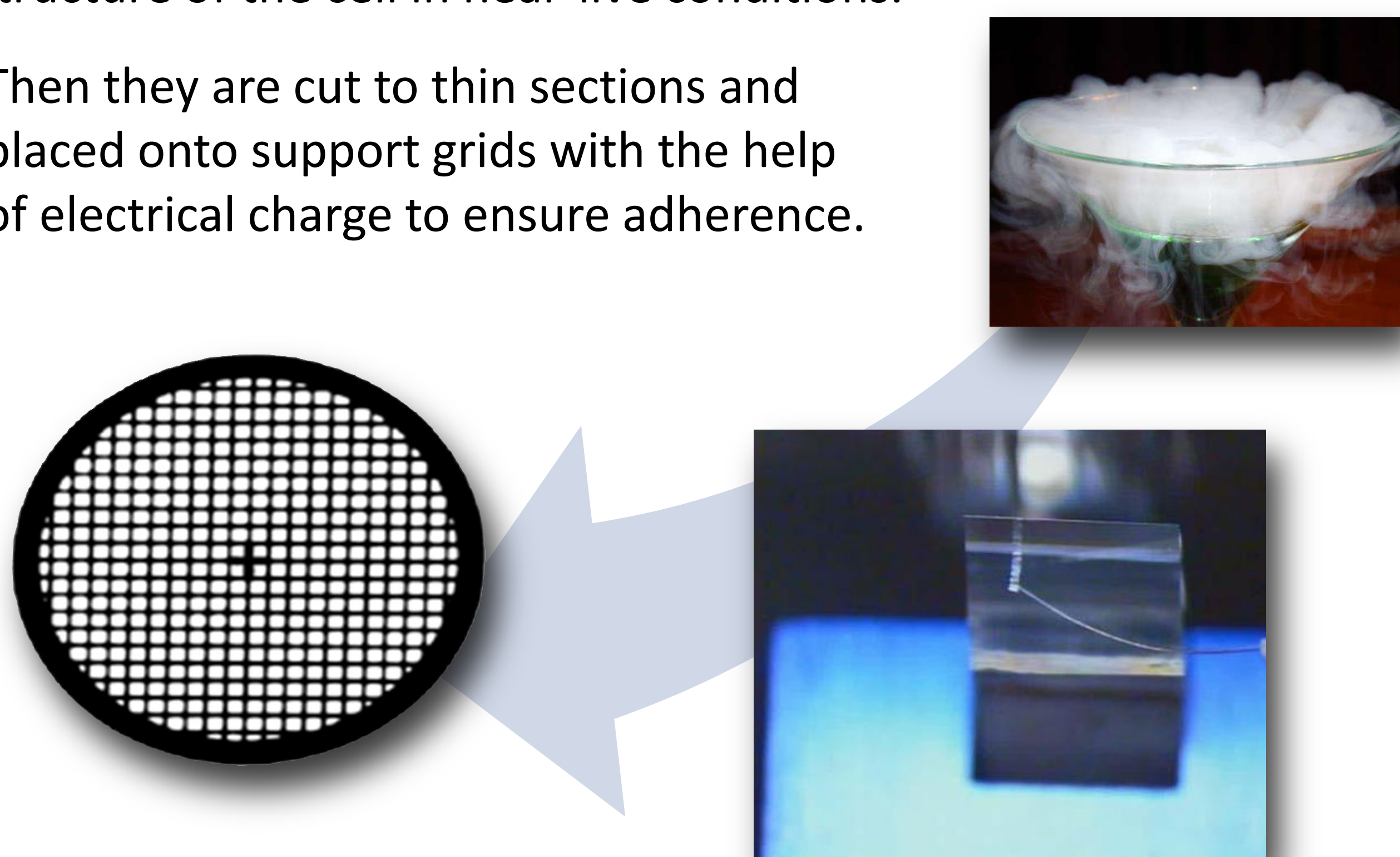
Cryo-electron tomography is a new emerging tool that allows us to visualize macromolecular complexes within high pressure frozen cells (1) With novel methods for generating vitreous cryo-sections and iterative reconstruction techniques, developed within the project, we will push forward the resolution to the point of identifying structural changes induced by drugs on macromolecular complexes in a native cellular context (2).

The implementation of this technology will bring additional dimension to our understanding of the toxic effect of chemicals in the cell.

Step 1: Sample Preparation

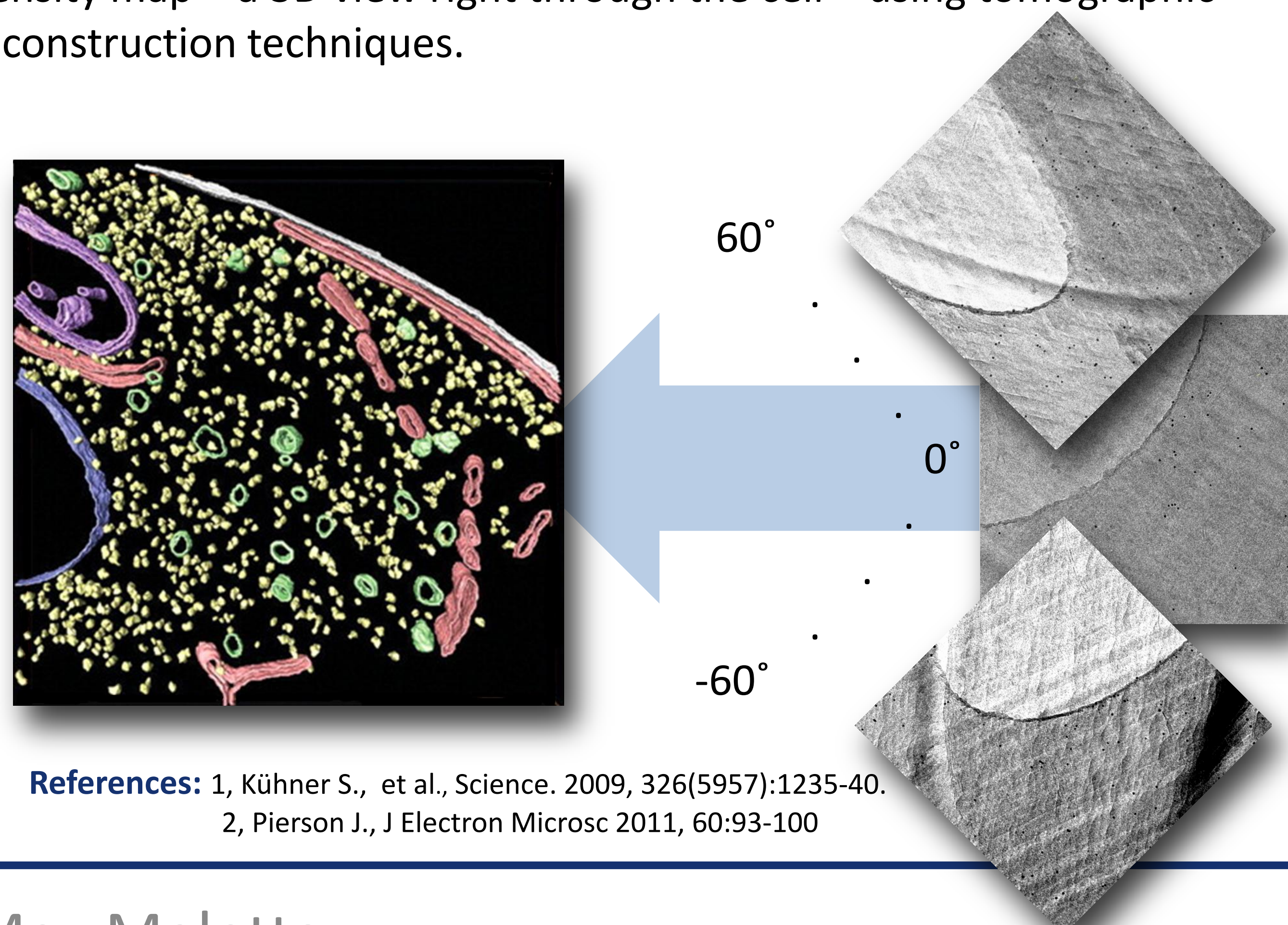
The specimen of interest is high-pressure frozen, preserving the ultra-structure of the cell in near-live conditions.

Then they are cut to thin sections and placed onto support grids with the help of electrical charge to ensure adherence.



Step 3: Tomographic reconstruction

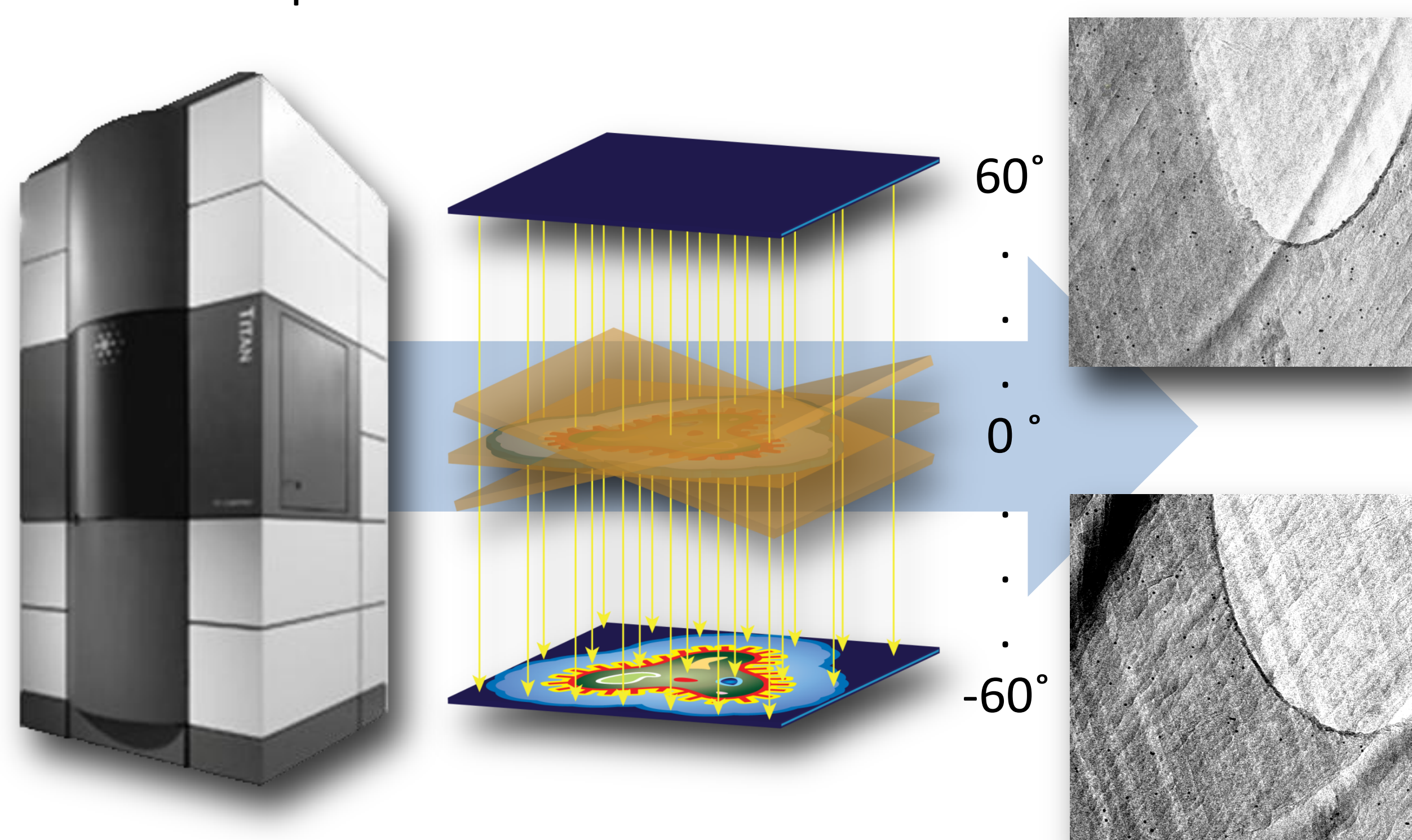
The 2D images from tilt-series are computationally combined into a 3D density map – a 3D view right through the cell – using tomographic reconstruction techniques.



References: 1, Kühner S., et al., Science. 2009, 326(5957):1235-40.
2, Pierson J., J Electron Microsc 2011, 60:93-100

Step 2: Image Acquisition

A set of low-dose images of the samples from multiple angles – a tilt series – is acquired using state-of-art electron microscopes.



Step 4: Analysis

Using sub-tomogram averaging, template matching, and cross-correlation scores, an average structure of a protein is obtained from the 3D density map.

By extracting and spatially localizing multiple complexes in the cell, a “cellular macromolecular atlas” can be built.

