FIB-SEM methods for biological applications

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IN²UB Meeting

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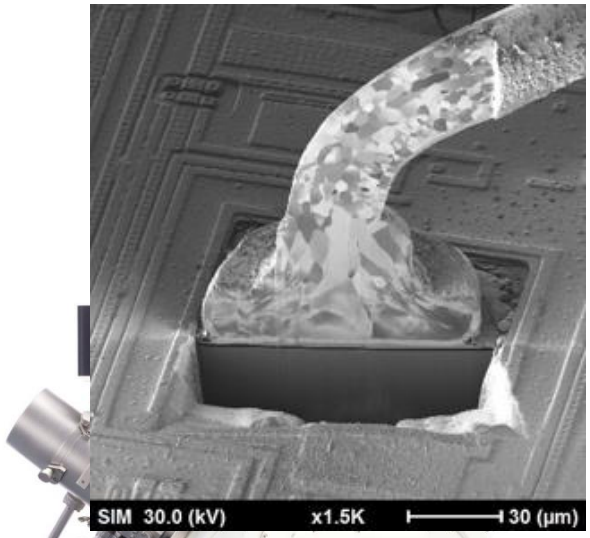
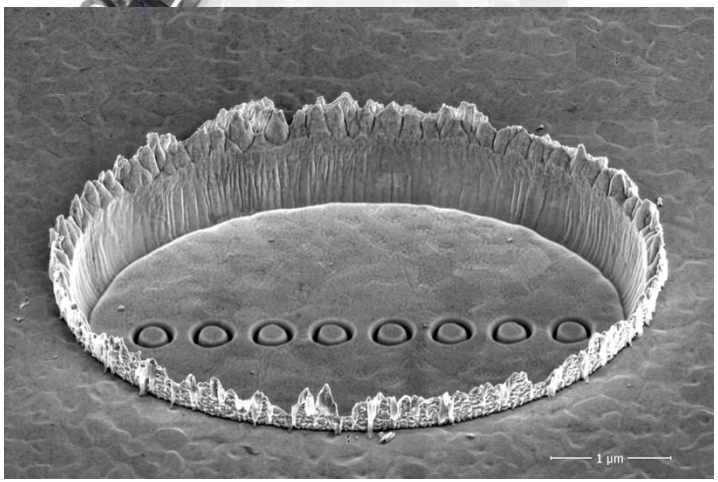
FIB-SEM INTRODUCTION

FIB and FIB-SEM Systems

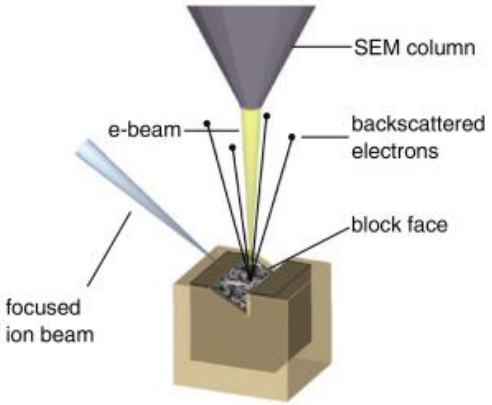
- The Focused Ion Beam (FIB) column is an optical instrument which focuses and scans an accelerated ion beam on a sample in a vacuum chamber.
- The most commercially available FIB systems are using gallium (Ga) ions.
- Such a column can be used for two main purposes:
  - To form scanning ion images by collecting the secondary electrons (SE) generated by the interaction of the incident ions and the sample surface.
  - To locally sputter the material surface to directly fabricate arbitrary nanostructures.
- FIB, previously restricted to the materials sciences and semiconductor fields, is becoming a **powerful tool for ultrastructural imaging of biological samples**.

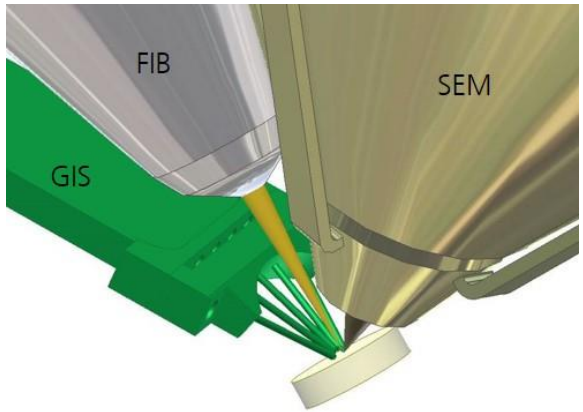




Fundamentals of FIB-SEM technologies

- Commercial dual-beam platforms incorporate both a FIB column and a SEM column in a single system.
- The typical dual-beam column configuration is a vertical electron column with a tilted ion column. In dual-beam platforms, the tilt angle of the FIB column can vary from manufacturer to the model, but usually it is between 52° and 55° tilt to the vertical.
- Both **SEM and FIB can be used to acquire high-resolution images** by collecting the secondary electrons (SE) that are emitted from the interactions between the beam and the surface atoms, although backscattered electrons (BSE) and/or secondary ions (SI) can contribute to form images.



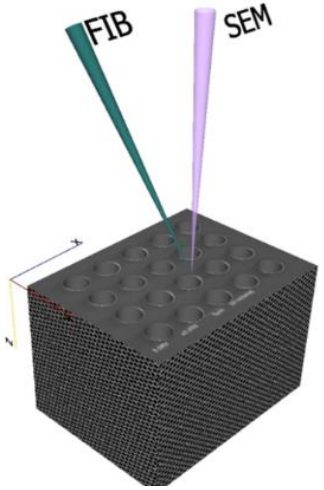


Basic applications of FIB

Ion milling

Ion milling, as the fundamental application of FIB systems, is a continuous sputtering process that occurs during ion beam exposure on the sample. Milling is actually an atomic collision process that ends up in the removal of the material from the ion.

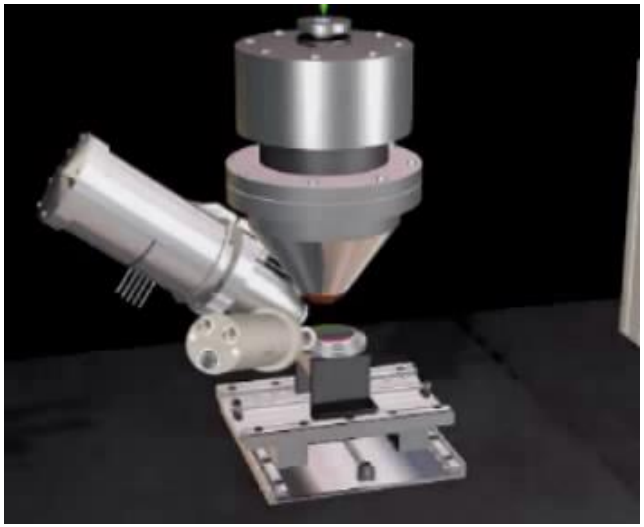
Ion milling can be used to create both simple structures, such as lines, rectangles, or circles in the material or big trenches in order to observe inner features of the sample.



Deposition

Deposition is the second most powerful feature of FIB technologies, as the ion or electron beams can be used in a deposition system, allowing the addition of material instead of removing the material.

Deposit materials are often supplied by an internal gas delivery system that locally exposes a chemical compound close to the surface impact point via gas injection systems (GIS) that can be incorporated into dual-beam platforms. The chemical gas compound is usually in the precursor form and consists of organometallic molecules.

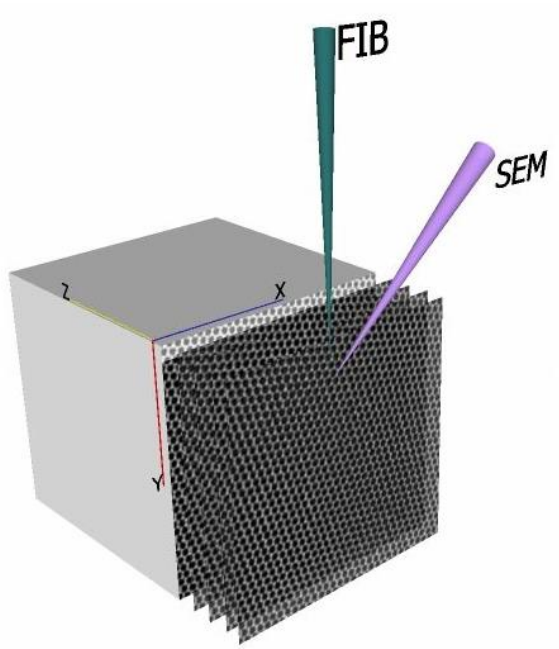


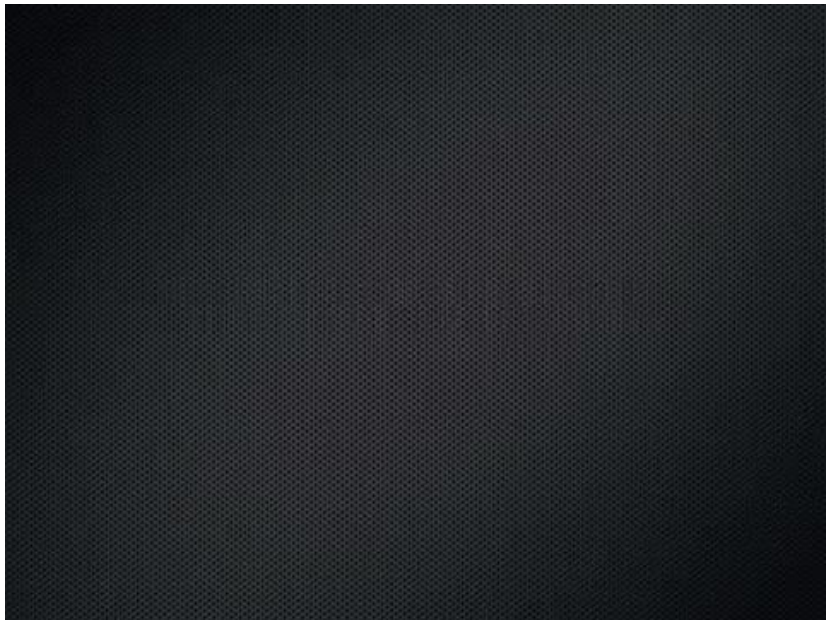
FIB-BASED PROCESSES

Serial slicing and imaging

Dual-beam platforms provide the use of electron and ion beams simultaneously, which opens a way to perform cross sectioning by means of sequential ion milling and monitoring and/or acquiring images of the corresponding cross section of the specimen at the same time using the electron beam.

A **rapid monitoring of inner structures** of several materials, obtaining information of the features down to a few nanometers is possible with serial slicing and imaging techniques.

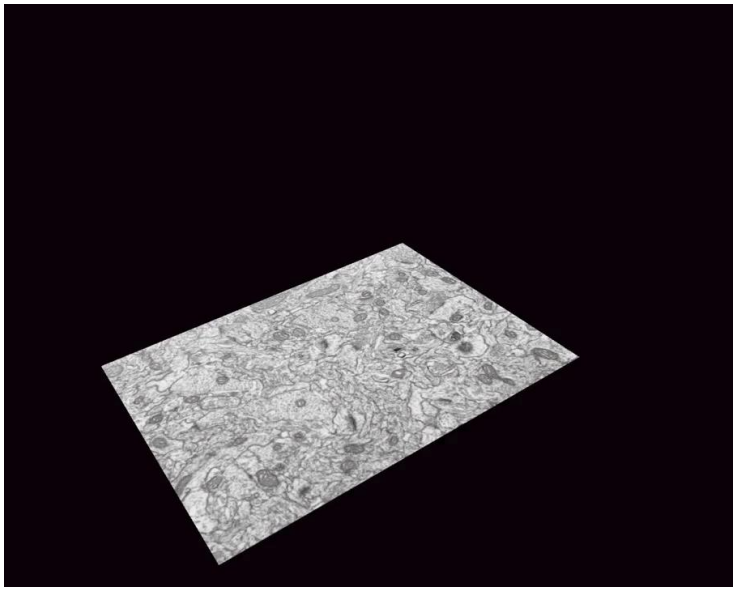




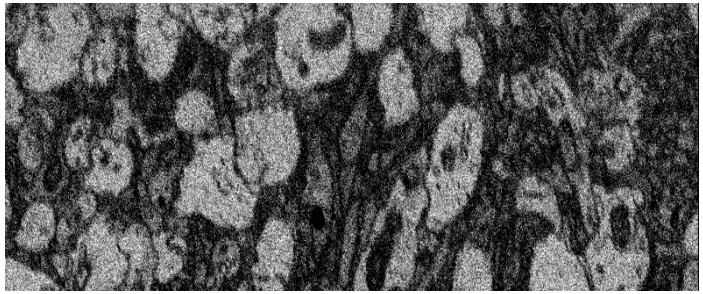
3D characterization and FIB tomography

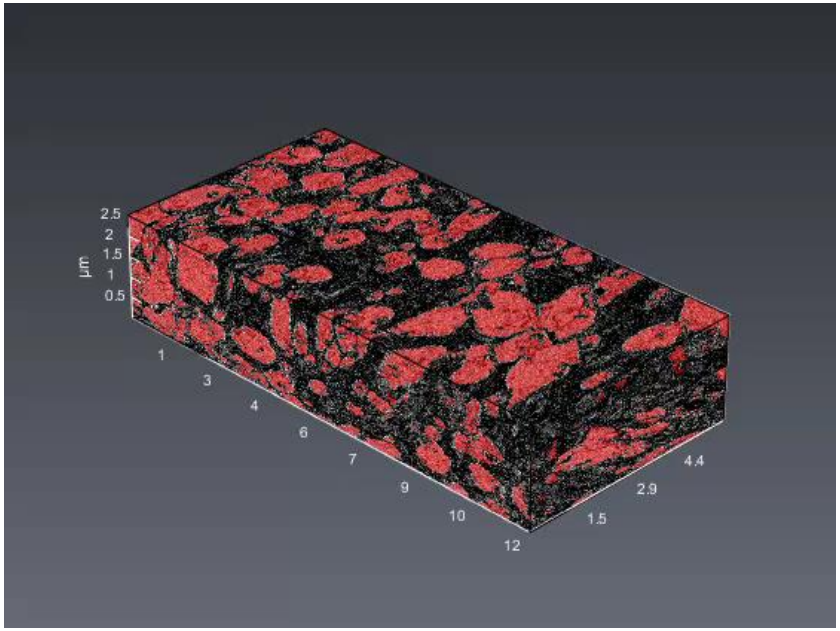
One step further of serial slicing and imaging application is the **3D FIB tomography**, which is based on the principle that continuous 2D data are collected from the surface of the bulk material by serial-sectioning and are stacked together to form reconstructed data, giving information in 3D.

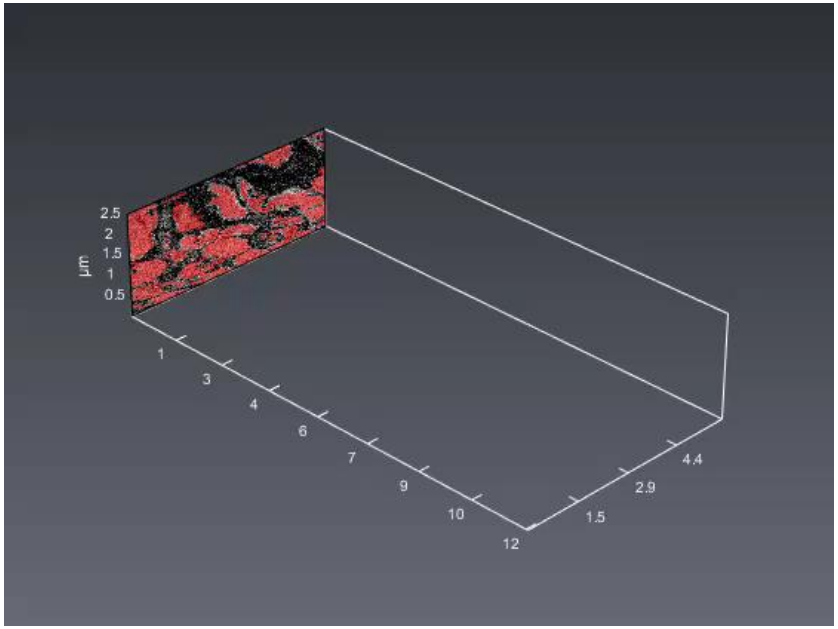
FIB-SEM enables three-dimensional information methodologies, especially for quantitative characterization of materials (the number of features per unit volume, feature connectivity, real feature shapes and sizes, and spatial distribution information).



Applications in UB





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