

## The Application Of Focused Ion Beam (FIB) On Biological Samples

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The focused ion beam (FIB) is a novel technique in electron microscopy that uses high-energy gallium ions to precisely and accurately sections or mill biological samples such as *Helicobacter pylori* cells, yeast cells and *Polyalthia* pollen spores irrespective of its place and location[1,2]. The step-by-step sectioning process will expose and reveal its internal structures, followed by observation with the FEG-SEM or conventional SEM for morphological comparison. The FIB also allowed a single cell to be sectioned at any desired position irrespective of its place and location. Some of the samples such as the pollen need no dehydration procedure.

Bacterial and yeast cells prepared on coverslips were fixed and dehydrated before coating them with osmium vapour while pollen spores were dusted to carbon stick stub and then osmium vapour coated. The bacterial and yeast cell samples for FIB need deposition with a layer of platinum (20 nm) to strength the cell structure. No deposition was required for the pollen spores. There was no collapse and destruction of cells when milled. The FIB-milled *H. pylori*(Fig 1-Fig 4), *C. albicans*(Fig 5 & 6) and pollen spores were then scanned with SEM and results obtained. Observation of FIB milled samples was also done at various angles. No much structural informations was obtained with the *H. pylori* milled samples. *C. albicans* milled samples showed many vesicles or inclusions bodies whereas the spore structure obtained after the spores were milled by FIB showed similarities in all three *Polyalthia* pollen spores(Fig 7 & 8). SEM observation of the spores also revealed three layer in the spore cut namely exine (outer layer), intine (central layer) and plasmalemma (inner layer). The result obtained showed that FIB could be used as an ultramicrotome in slicing the bacterial, yeast and pollen spores to observe the interior structure and for comparison purposes. In the case of the pollen spores, it also showed that external morphology but not internal structure of the pollen spores were important in species identification.

In conclusions, FIB can be used a micromaching tools for biological samples.

### References

- [1]. PR Munroe & MR Dickson, Proc. 10 EMSM (2001) 8 - 11.
- [2]. Y. Muranaka & Y. Hotta, Proc.10 EMSM (2001) 12 - 18.

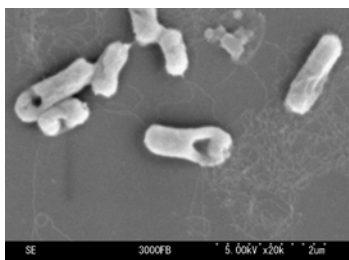


Fig 1: *H. pylori* before milling

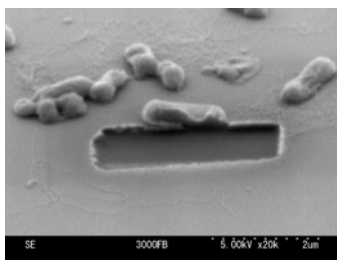


Fig 2: *H. pylori* after milling

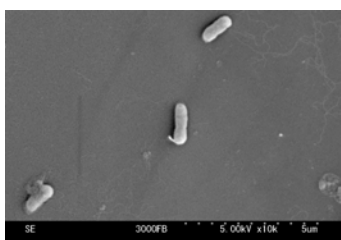


Fig 3: *H. pylori* before milling

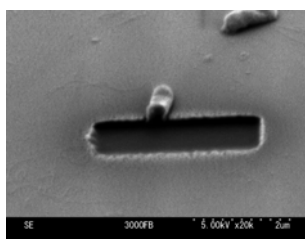


Fig 4: *H. pylori* after milling

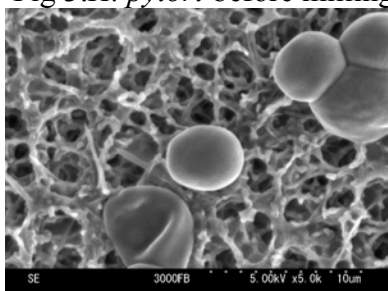


Fig 5: *C. albicans* before milling

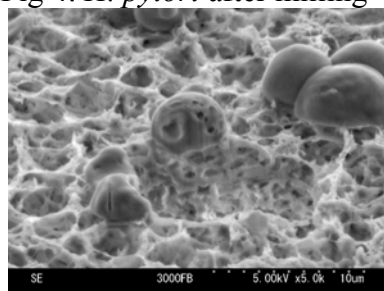


Fig 6: *C. albicans* after milling

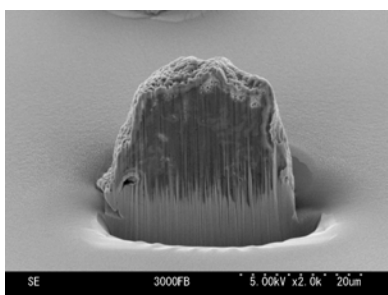


Fig 7: pollen spore after milling

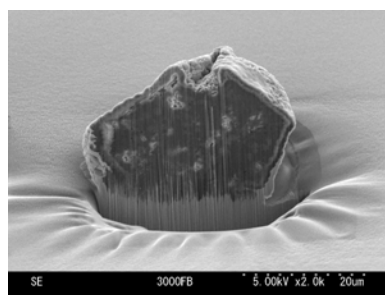


Fig 8: pollen spore after milling