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Ga^+ Ions and Xe^+ Plasma: Complementary FIBs for Resin-Embedded Life Science Sample Analyses.

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As researchers seek to understand high-level processes within living organisms, finer detail in three-dimensions of cell cultures and tissue needs to be observed using electron microscopy. Over the last several years, the Ga^+ focused ion beam (FIB) has been implemented for cross-sectioning resin-embedded samples to get an accurate representation of life science samples.[1-2] The Ga^+ FIB is capable of milling much finer slices ($\geq$ 5 nm) of material than traditional sectioning methods. [3] When coupled with automated software packages, such as FEI's Auto Slice and View™ and Amira™ or Avizo™, complete 3D-reconstructions of specimens are possible.[1-2] One limitation of the Ga^+ FIB is the moderate cross-section size prepared in a reasonable amount of time. The Xe^+ inductively-coupled plasma FIB is able to produce much larger cross-sections in faster time, while only sacrificing minimal prepared-surface quality. In this study, zebrafish specimens, prepared via osmium tetroxide staining and high pressure freezing followed by flat-embedding in epon resin, were used for evaluating and comparing the cross-sectioning capabilities of the Helios NanoLab™ Ga^+- and Vion™ Xe+-based FIBs, and subsequent imaging, with respect to life science samples. Zebrafish (Danio rerio) are often used in biological studies due to their fast growth and regenerative abilities. The biological parallels with humans and the inexpensive cultivation of zebrafish make them attractive model organisms for large-scale studies.[4]

Figure 1a illustrates the increased speed and viewable area of cross-sectioning with the Xe^+ FIB. Both cross-sections were prepared in roughly the same amount of time. A large final polishing current using Xe^+ (15 nA compared to 0.79 nA with Ga^+) was used resulting in minor curtaining. High-resolution backscattered electron (BSE) imaging of the embedded tissue was possible without any further clean-up using the Ga+ FIB, as shown in Figure 1b.

Figure 2 shows a direct comparison of large cross-sections prepared with both FIBs. All ROIs were prepared in roughly the same time and only varied in beam current used. Sections prepared using the Ga+ FIB (Figure 2b) show a large damage area in the vicinity due to taking single "snapshots" for pattern positioning. The damage produced surrounding the sections prepared using the Xe^+ FIB (Figure 2a) consisted of a larger halo, but was not as locally damaging to the resin surface. However, when using the highest beam currents, i.e. 1.3 and 0.47 μA, shrinkage of the resin was observed, creating a distorted cross-section.

While the Helios' Ga^+ FIB has been established as a tool for analyses of resin-embedded life science samples, [1-2] the Vion Xe^+ ICP-FIB has been shown as complementary instrumentation. The fast speed of cross-section preparation combined with a more than adequate final polish allow large areas of tissue and cell cultures to be exposed and subsequently imaged using the highest resolution Elstar™ SEM column available on the Helios. Final clean-up of the large cross-section can be easily performed using the Ga^+-based FIB column of the Helios if needed.

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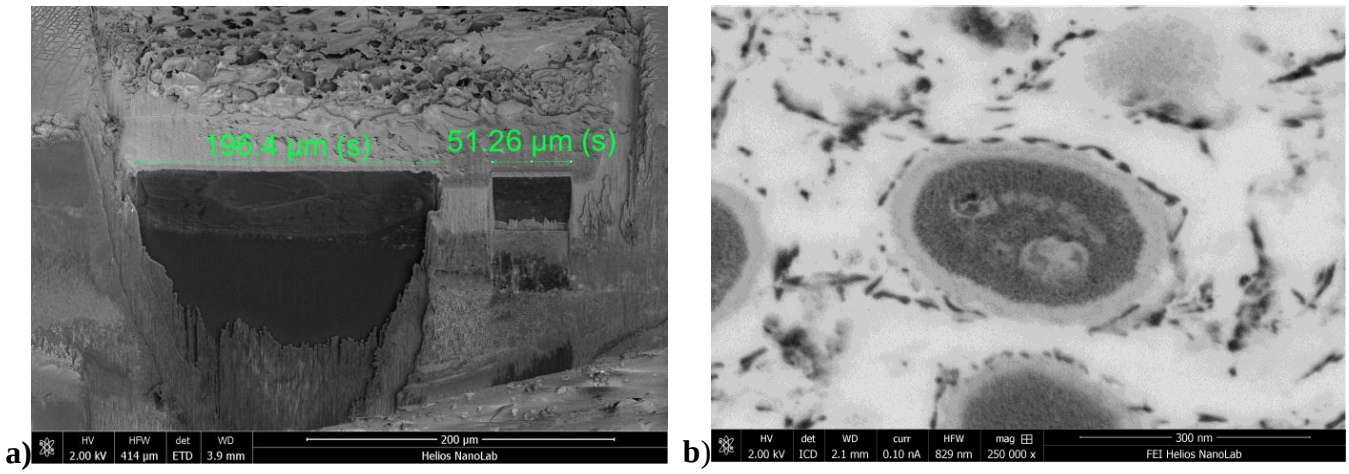

Figure 1. a) Zebrafish cross-sections prepared using Vion Xe^+ ICP-FIB (left) and the Helios NanoLab DualBeam utilizing Ga^+(right). Both cross-sections of equal final quality were prepared in roughly the same amount of time. **b)** The final 15 nA polish using Xe^+ creates high quality cross-sections with few curtains for high-resolution BSE imaging in the Helios. (contrast inverted)

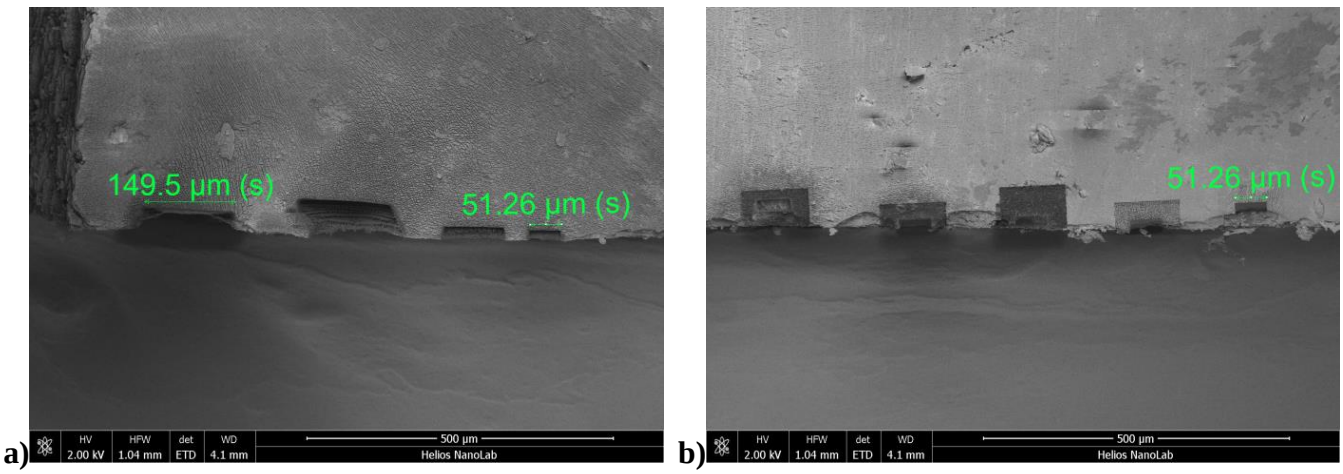

Figure 2. Series of cross-sections prepared in epon resin for comparison using **a)** the Vion Xe^+ ICP-FIB with beam currents of 1.3 µA, 0.47 µA, 180 nA, and 59 nA (left-to-right, respectively) and **b)** the Helios NanoLab DualBeam using Ga^+ with currents of 65 nA, 47 nA, 21 nA, 9.3 nA, and 2.5 nA (left-to-right, respectively). All cross-sections were prepared in roughly 5 minutes using the respective beam current.

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