## Scanning Transmission Helium Ion Microscopy- How Does It Compare to TEM?

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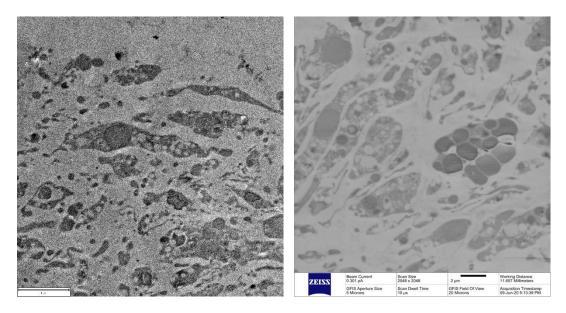
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Since its initial release a decade ago, the Helium Ion Microscope (HIM) has established itself as a tool of choice for many imaging as well as nanofabrication applications [1]. Throughout the past decade, technique developments such as the Secondary Ion Mass Spectrometer [2] have expanded the application space of the tool.

This work explores the HIM's analysis capabilities of unstained biological samples using a self-built dark field scanning transmission ion microscopy holder. For thin enough samples, such as thin sections of biological specimen on TEM grids, the high energy helium ions can penetrate through the sample. While the ion transverses through the thin foil, it undergoes collisions with the sample atoms and is deflected. The ion exits the sample at a deflection angle which is specimen thickness, ion energy as well as sample material dependent. The deflection angle can be determined using Monte Carlo simulations. The freeware program Stopping and Range of Ions in Matter was used in this work. This effect can be used to design a dark field scanning transmission ion microscopy holder (DF-STIM). The holder design is based on a previously reported experiment [3]. In principle, ions, which are deflected by a specific angle hit a metal conversion plate, which is mounted at a specified distance h below the sample. Here, the transmitted ions create a secondary electron signal which can be collected by the HIM's Everhart-Thonley Detector. Ions which are deflected less than the acceptance angle enter a hole in the holder which his located directly below the specimen. This hole acts as a Faraday cup. For this case, no secondary electron signal is created. For biological samples, areas with higher carbon density create signal while areas with lower carbon density create less signal and can this be distinguished in the DF STIM image.

The DF STIM holder is tested by imaging stained and unstained biological samples and the results are compared to TEM measurements (Figure 1).

The measurements show that the in house designed DF-STIM holder can be used in the HIM to record high signal to noise images of unstained biological samples, revealing nanosized internal features such as collagen fibres. This technique outperformed the TEM imaging capabilities for the unstained biological specimen used in the experiment [4].



**Figure 1.** Left: TEM micrograph of an unstained biological sample. Right: DF-STIM micrograph of the unstained biological sample.

## References:

- [1] FI Allen, Beilstein J Nanotechnol. **12** (2021), p. 633. doi: 10.3762/bjnano.12.52. PMID: 34285866; PMCID: PMC8261528.
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- [3] D Emmrich et al., Beilstein J Nanotechnol. **12** (2021) p. 222. doi: 10.3762/bjnano.12.18. PMID: 33728240; PMCID: PMC7934706.
- [4] Dr. Crystal Cooper is thanked for the many useful discussions and the sample preparation suggestions.