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AXON: An In-situ TEM Software Platform Streamlines Image Acquisition, Metadata Synchronization and Data Analysis, Enabling Deeper Understanding, and Improved Reproducibility of In-situ Experimental Results

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The adoption of in-situ transmission electron microscopy (TEM) studies, particularly those performed in a non-vacuum environment, has grown exponentially since early 2000s. Initially the greatest challenge posed by these types of experiments was how to safely introduce a liquid or gas environment into the TEM without risking the safety of the microscope. Now, users are wrestling with a new set of fundamental difficulties, one of which is complexity of accurately reporting and reproducing such experiments. A recurring refrain within the in-situ community is the need to develop minimum requirements for reporting experiment conditions and parameters to improve analysis and enable the reproducibility of results by other researchers [1]. The need to standardize reporting and enable other researchers to access metadata created during microscopy experiments was recently highlighted by Sarkans et al. as a general area for improvement within the wider biological imaging community [2]. In their review, Sarkans highlighted the FAIR principles, first proposed by Wilkinson in 2016, which identified that datasets need to meet the criteria of being (1) Findable (2) Accessible (3) Interoperable (4) Reusable [3]. In-situ TEM studies, due to both the size of the collected data and complexity is well suited to the application of FAIR principles and the community benefits if researchers can share and mine existing datasets to identify trends and behavior.

Protochips recently introduced AXON, a software solution designed to both address specific pain-points inherent to in-situ TEM studies such as stabilized tracking of dynamically moving systems, accurate dose quantification and management, and to consolidate experimental parameters and metadata across platforms. The AXON software platform consists of both a data-collection module (AXON Synchronicity) which connects, stabilizes, and integrates data streams from the microscope, detectors, and in-situ devices and a free-to-use post-processing analysis software (AXON Studio) that streamlines image sharing, analysis, and trend discovery. The hardware component, the AXON core, once installed on the microscope, enables the continuous collection of images and metadata produced by all three primary components during an in-situ experiment (TEM, detector, and the in-situ system) and automatically saves this data to the image. Once AXON is activated and connected to the detector or camera, any user-triggered changes, such as adjustments to beam conditions, detector acquisitions or in-situ stimuli is constantly recorded and integrated into the resulting data.

Because AXON has continuous access to the image and metadata streams it can apply computational and image analysis algorithms to calculate variables between images such as sample drift or changes in focus and apply the necessary corrections resulting in a high level of performance not previously possible. These calculated variables are a logged in real-time throughout the experiment and saved to their corresponding images. One of the most powerful AXON algorithms, developed to accommodate the inherently dynamic morphological changes that a sample undergoes during in-situ experiments, is "template morphing factor." This algorithm allows AXON to intelligently identify and quantify morphological changes that a sample undergoes in real time by continuously comparing the record of the sample image history to its most recently acquired image. When first activated, a reference template image is automatically generated by AXON, enabling the computation of comparative metadata on incoming images. This template is automatically refreshed after any operator-induced changes, or when the difference between the template and the current image exceeds a user-defined value. When the user initiates image changes by adjusting parameters like magnification, acquisition time, location, etc. AXON automatically refreshes the reference image. This morphed template feature is unique to AXON and is the foundation of how AXON can identify and react to a dynamically changing sample in real time. This feature is also what enables AXON to compute a value that Protochips terms the “match correlation.” At its most basic level, match correlation is a score of how dissimilar the sample currently looks to its most recent reference template. Like all metadata values collected or calculated by AXON, the match correlation value for a given image can be plotted in AXON Studio. This enables the user to scrub through many images and quickly identify trends or periods in which the sample undergoes morphological changes, such as in Figure 1.

From the AXON Studio interface, data sets or snippets of data sets consisting of images with their corresponding metadata can be easily saved as a new collection and shared among collaborators and colleagues allowing them to access the same complete data sets and interrogate the same data from their unique perspective. Thus, utilization of the AXON platform for in-situ experiments easily enables the application of FAIR principles to in-situ data management, and facilitates more robust analysis, data mining and review of in-situ experiments by outside researchers, increasing productivity and ultimately elevating the field of in-situ TEM.

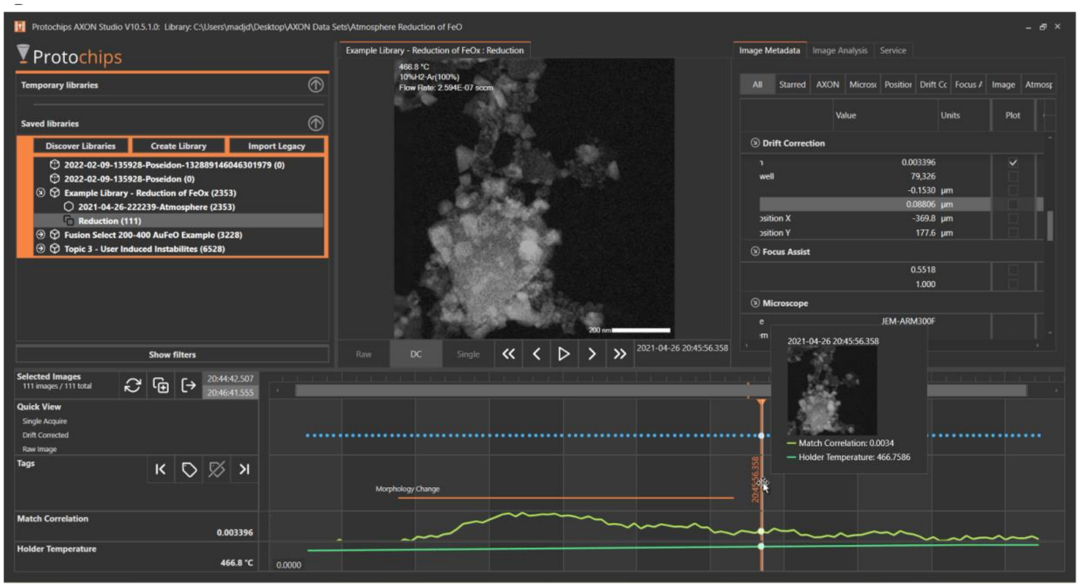

Figure 1. Example of the AXON User Interface for viewing image stacks and their associated metadata plotted over the course of a TEM experiment.

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