Versatility of Automated Tape-collecting Ultramicrotomy (ATUM) with the RMC Boeckeler ATUMtome

Introduction

Automated Tape-collecting Ultramicrotomy (ATUM) has developed into a unique and efficient approach for the non-destructive collection and collation of serial sections. This application is especially useful for efficient collection of large datasets and for 3D reconstruction at nano-scale spatial resolution.

Figure 1. RMC Boeckeler ATUMtome.

Instrumentation

The RMC Boeckeler ATUMtome showcases the capability for automated and unattended reliable collection of serial sections. It is ideal for large sample block face sectioning, 2mm x 3mm being a typical size, sometimes blocks with even greater surface areas may approached. The ATUMtome increases the rate and quality of acquisition of sections for both light and electron microscopy.

The ATUM tape collection attachment is combined with the RMC Boeckeler PowerTome PCZ (PT-PCZ) as a complete operating system. The computer-controlled instrumentation synchronizes the sectioning and section collection process and performs unattended to free the researcher to undertake other tasks.

Procedure

Various operational considerations can limit the use of the ATUM in its initial conceived operating mode; that is, collection of data sets from a large specimen block face area with many thousands of sections capable of being harvested. The more traditional approach to 'serial sectioning' using the much smaller block face sizes usually encountered in EM combined with the possible requirement for smaller data sets can present a challenge here. Researchers routinely needing fewer section numbers and with smaller regions of interest would find the ASH2 (Advanced Substrate Holder) more suitable in this case. With a small modification to technique, the versatility and flexibility of the ATUM can be used to great advantage.

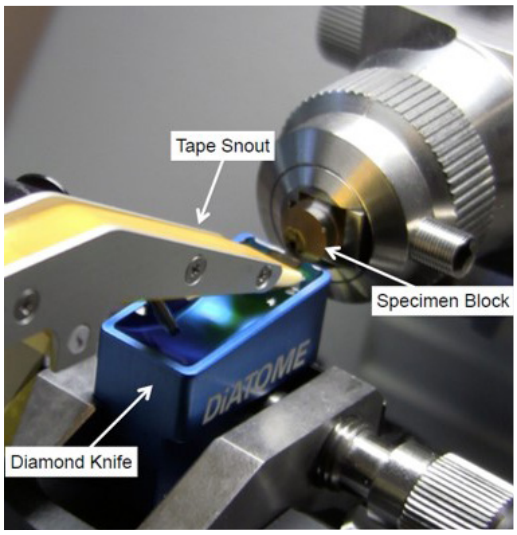

Figure 2.
Standard ATUM section collection setup, aka the 'Harvard' method.

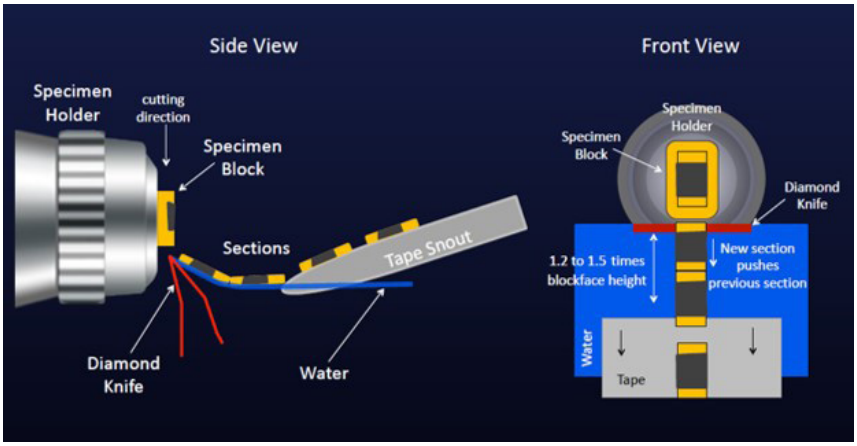

Figure 3.
Schematic diagram of standard ATUM collection. The block face needs to be of a minimum size to allow sections to progress toward the tape in an orderly and reliable fashion for unattended operation. Tape snout cannot be positioned too close to the knife edge.

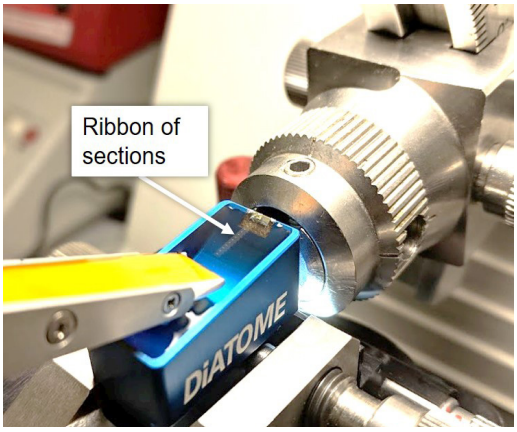

Figure 4.
Short ribbons of sections of 'traditional' size collected by the user. Note the snout head is moved back slightly, away from knife edge.

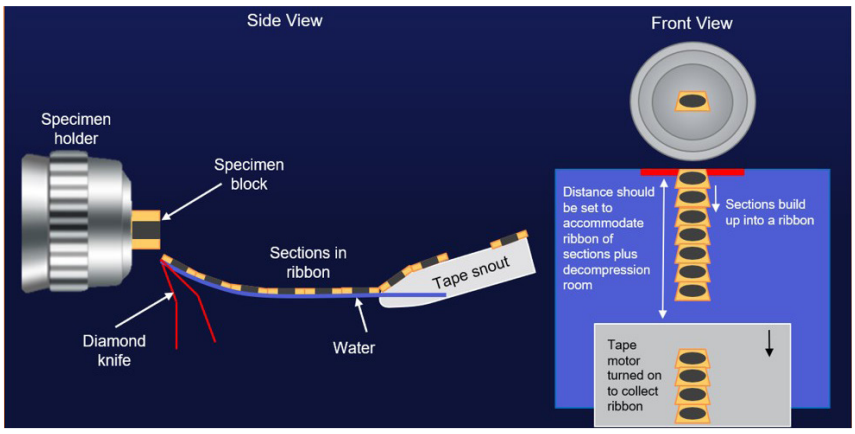

Figure 5.
Schematic diagram of ribbon collection.

Adaptation of the ATUM collection routine begins with dynamic user collection of ribbons of sections. After sectioning the sections are decompressed (e.g. with chloroform vapor). Ribbons are manipulated toward the tape snout head and the tape reels are manually started to collect the ribbon onto the Kapton tape. At this point, collection is visually confirmed, and the tape reels are stopped before repeating this process iteratively. Between the collection of each ribbon, while the tape and cutting are paused, it is advisable to use the 'Step Advance' function to back the specimen block away from the knife edge to avoid cutting a thicker initial section during the next sectioning cycle (due to thermal fluctuation/block 'creep', etc.). Once the required number of sections/ribbons are collected, the spools are wound to the take up the tape in the traditional ATUM method. Tape lengths can then be cut and mounted, in this case the tape was mounted onto 25mm or 50mm specimen stubs, but also be mounted onto 4-inch wafers or other imaging substrates.

Results

Advantages:
Quick and easy method for adaptable, user-controlled section collection. Glow discharge of tape is not required. Allows for the decompression/flattening of sections prior to collection. Uses smaller stubs and, thus, can utilize standard SEM chamber/imaging approaches (e.g. single beam SEM).

Figure 6.
Ribbon of sections after being decompressed and manipulated in the trough toward the tape edge in preparation for collection.

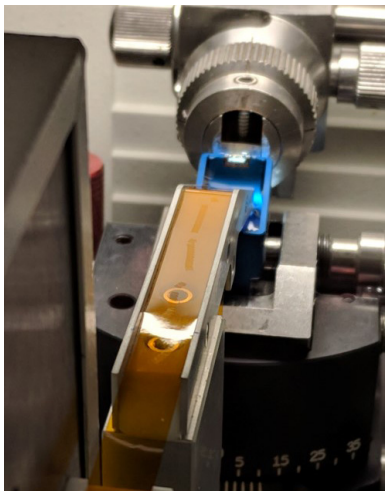

Figure 7.
Series of ribbons collected on tape.

Figure 8.
Strips of tape containing section ribbons is then mounted onto imaging substrates (e.g. 50mm SEM stubs).

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