Ultrathin Section Thickness and Interference Color

Introduction

Students of ultramicrotomy are usually introduced to the well-known “Peachey Chart”. This chart shows the relationship between section thickness and their observed interference colors while floating in the knife trough and illuminated by the diffuse white light of the ultramicrotome (Peachey, 1958). Since his original article, a chart of interference colors versus section thickness was made by one of the earlier manufacturers of ultramicrotomes, Ivan Sorvall, Inc. and this chart was copied by other commercial firms and became very commonplace in electron microscopy laboratories. However, this chart was an artist’s rendition of the original data by Peachey, and does not accurately portray the colors seen with modern ultramicrotomes’ improved lighting and designs. Therefore, RMC Boeckeler undertook a simple study to display the interference colors, observed and photographed with a typical biological specimen embedded in a commonly used epoxy resin.

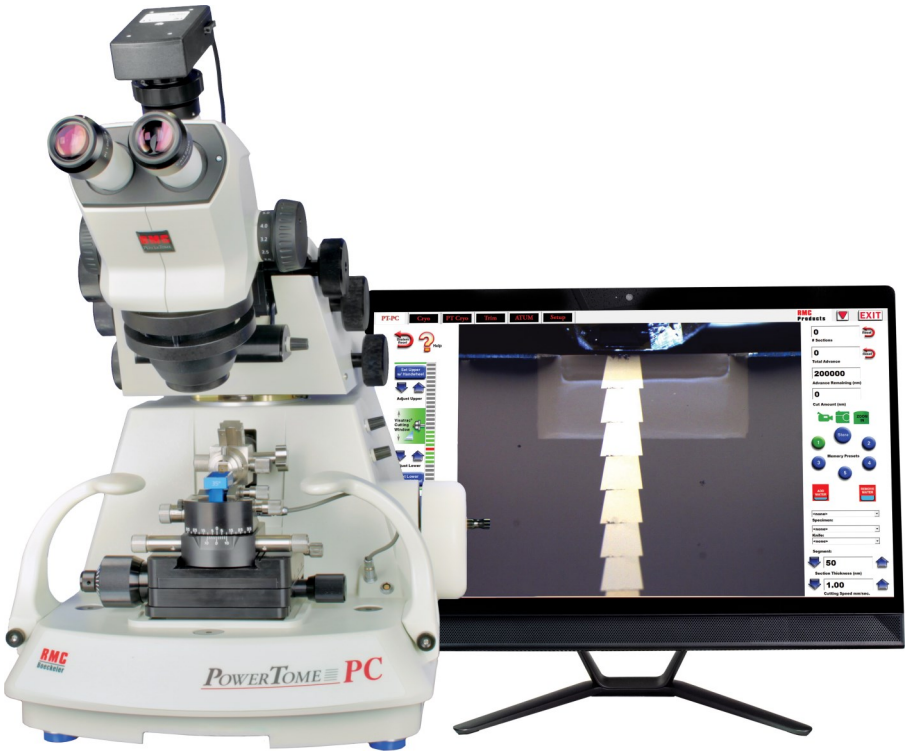

Figure 1.
The RMC Boeckeler PowerTome PCZ ultramicrotome.

Instrumentation

The RMC Boeckeler PowerTome PCZ was used for this study. It is equipped with a mechanical advance system which has a precision digital linear scale for highly reproducible cutting arm advances. This advanced model of ultramicrotome also features a touchscreen PC and trinocular stereomicroscope with high definition camera for capturing pictures and videos of the sections as they are sectioned. This imaging system also has a measurement tool which was useful in measuring section dimensions and areas for comparison to those of the sample block face for estimation of sectioning compression.

Procedure

A mouse spleen was fixed in 4 % formaldehyde and 1 % glutaraldehyde and was post-embedded in Embed 812 resin from Electron Microscopy Sciences (EMS, Hatfield, PA, USA). This is a commonly used EPON 812 replacement resin and is representative of the epoxy resin mixes used for biological specimens in many laboratories. A hard mix was used, per manufacturer instructions. A production PT PCZ ultramicrotome was used for sectioning. The mechanical advance of the cutting arm was verified by an independent method, using a Mitutoyo micrometer (Model # ID-C112E) which can read linear distance down to 1µm increments. Advances of the sectioning arm by 10 µm were made with the instrument controller and these distances compared to the readings of the Mitutoyo micrometer throughout the entire 200 µm advance range of the cutting arm. The two readings were in agreement to within 1 % variance. The mechanical advance of the cutting arm is implemented with a stepping motor and feedback control from a digital linear advance system, thus the smaller advances for ultrathin sectioning are expected to be similarly accurate. For example summed multiple advances of 100 nm steps result in the same reading on the Mitutoyo micrometer as larger advances of 1 µm and 10 µm.

Sections were cut in the range of 30 nm to 400 nm and photographed as they were sectioned and also after “stretching” (decompression) by $CHCl_3$ vapor. The camera settings for gain and exposure were set such that the resulting images closely matched the color and brightness observed directly through the stereomicroscope. Section images were arranged using the image processing software ImageJ (NIH, USA) to produce Figure 2.

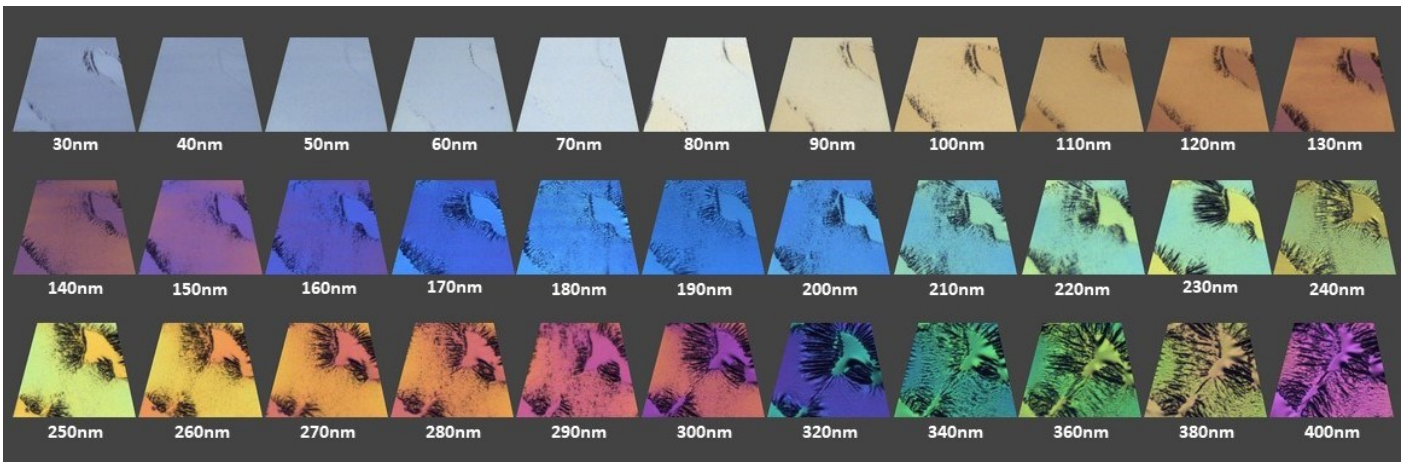

Figure 2.
Sections ranging from 30 nm to 400 nm following ultramicrotomy and decompression. The clear areas in the upper right and lower left are lumina without tissue.

Results

The stable and precision advance system of the RMC Boeckeler PT PCZ was optimal for sectioning a typical epoxy resin embedded specimen to record the interference colors of the sections. Subtle variations in the gold colors of sections between 70 nm and 100 nm thick were easy to distinguish. A bright blue color was seen around 170nm, much thinner than the 190 nm to 240 nm originally reported by Peachey on an older instrument.

Figure 2 displays sections "stretched" by $CHCl_3$, given this is a standard technique in many laboratories. Others often use a device with a heated filament passed over the sections to obtain a similar decompression of the section. Decompression of sections is beneficial in ameliorating effects of sections stretching and moving within the beam of the transmission electron microscope (TEM). We found that section lengths were compressed approximately 10 % as compared to the sample blockface. This was reduced to appoximately 8 % compression following stretching by $CHCl_3$. Consequently, actual thickness of the sections may be equivalently decreased. Figure 2 was constructed from actual sections created by an instrument confirmed to be advancing accurately, producing a more accurate and updated reference for ultramicrotomists, and therefore more useful than previous charts produced by artists and distributed commercially. This chart also covers a greater thickness range, demonstrating how some interference colors repeat as section thickness increases.

References

Peachey, L.D. (1958). J. Biophys. Biochem. Cytol. 4, 233.

Sjöstrand, F.S. (1967). Electron Microscopy of Cells and Tissues. 281-287.

RMC Boeckeler

4650 S. Butterfield Drive
Tucson, Arizona 85714, U.S.A.
T +1 520-745-0001
F +1 520-745-0004
E info@boeckeler.com
www.boeckeler.com