A Note On Paraffin Waxes, Their Crystals, And Microtoming

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Paraffin wax is a mixture of (virtually) straight chain hydrocarbons. Note the word "mixture". Unless one goes to the enormous lengths of purifying or searching for a fine chemical supplier, the wax will *always* be a mixture. I used a "pure" wax once as a standard for gas chromatographic studies on paraffin waxes. There is a relationship between hydrocarbon chain length and melting point, but as the waxes are always mixtures, melting points are never exact, either in the compounding or the measuring.

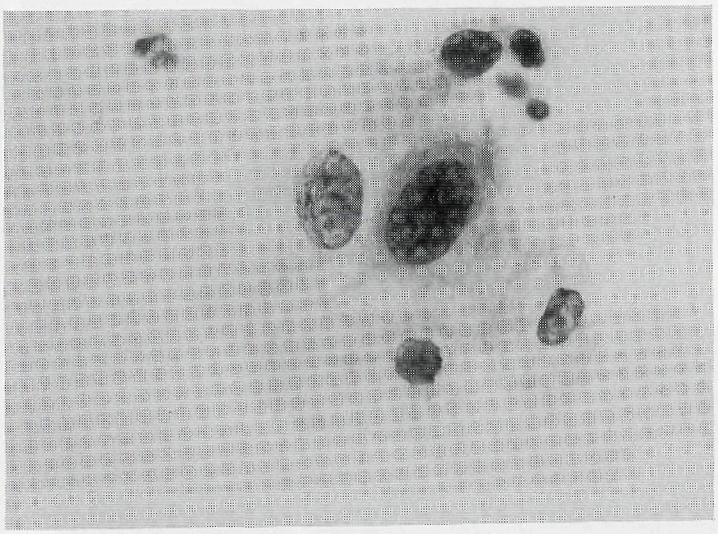
Perhaps more important than the melting point is the "plastic point", but that is virtually ignored by suppliers. The plastic point occurs about 10° C below the melting point, and its meaning should be fairly obvious - softening a piece of physiotherapy wax in your hands should explain all you need to know. The reason the plastic point is important is related to the sectioning properties of the wax, but we will come to that later.

Crystal size is important in the wax surrounding the tissue and in the tissue spaces, but not in the tissue *per se.* Molten wax infiltrates the specimen, and the size and shape of crystals will be influenced by the tissues as the molten wax solidifies (*i.e.*, crystallizes). So we cannot have "small crystals" infiltrating, although smaller crystals will result from soliditication in denser tissues.

Some of the theory behind this suggests that wax crystallizes first as flat "plates", the higher melting point hydrocarbons crystallizing first. The plate crystals pile up on one another as successively lower melting hydrocarbons crystallize. Distortion due to these dynamic events forces the edges or corners of the most well developed plates to curl and roll. Eventually, that gives rise to needle shaped crystals, which some workers consider most ideal for microtomy. All of this will be contingent upon the boundaries imposed upon the process by cell and tissue structures.

During microtomy, essentially two types of forces are exerted in the cutting process: flow shearing and point-to-point shearing. Flow shearing is the smoother of the two processes, and proceeds ahead of the edge of the blade.

CORRECTION: In our previous (April) issue, in the article "Histogram Stretching Or Histogram Equalization in Image Processing" by William R. Oliver, a printing error was made relating to Figures 4 and 5. With apologizes to Dr. Oliver and our readers, the correct figures are as follows:



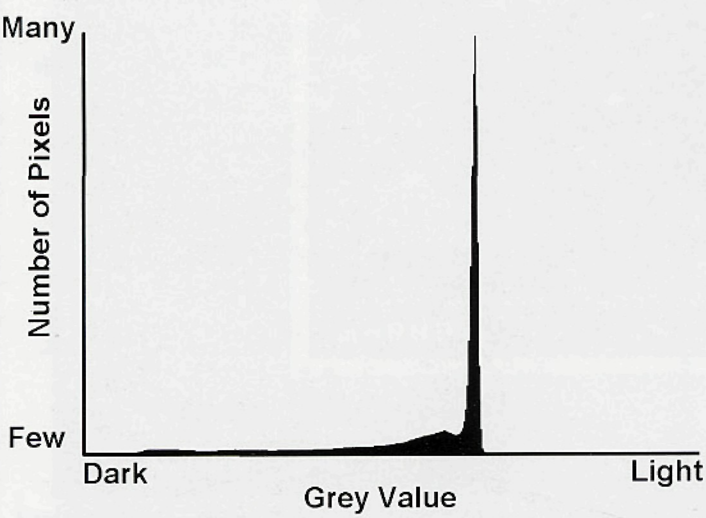
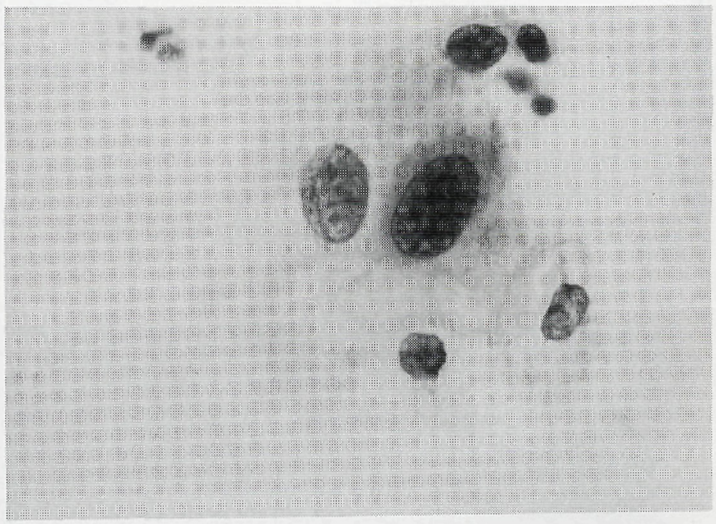


Figure 4: An image as digitized. Note that the lightest areas are still a little dark and the darkest areas are not completely black.



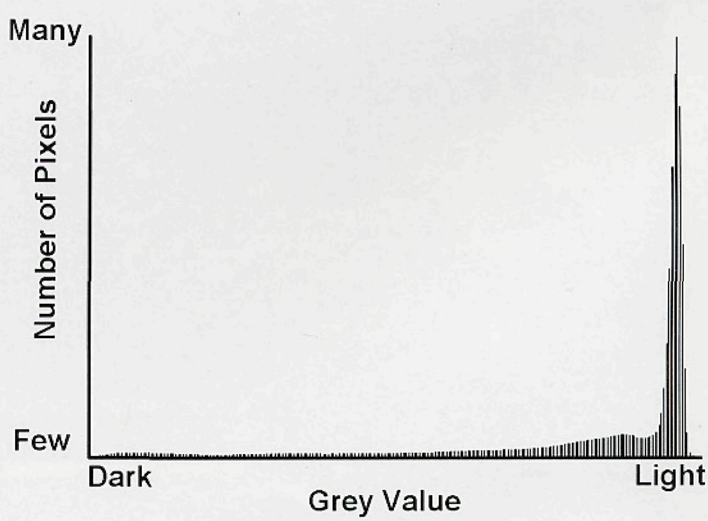


Figure 5: This is a simple histogram stretch. The brightest areas are now white and the darkest areas are now black. Everything in between is scaled. Notice the introduction of spaces between the columns in the histogram, as they are spread apart. Normal directly digitized images usually don't have gaps such as this. This kind of sawtooth pattern on a histogram is a hint that the image has been manipulated.

Point-to-point shearing has forces seeking the line of least resistance ahead of the blade, and these result in a section of uneven thickness - not that this would be noticed microscopically.

Imagine the difference between cutting through a jelly and cutting through a beef burger. Now the importance of the plastic point (as opposed to the melting point) can be seen. Additives to paraffin waxes are intended to minimize the point-to-point shearing and improve the plastic flow. The association between the words "plastic" and "polymers" should now be awakening. The additives to paraffin waxes are usually polymers (of known chain length, for they are synthesized exactly), with a major role in "harmonizing" the consistency of the paraffin waxes, in part at least by filling in between the wax crystals.

What about resin sections? This is a bit like cutting glass. Try to cut sections of glass, and there is no control over the direction of shearing. Resins are formulated to act as "liquid solids" - for that is what glass is - but with some control over cutting properties.

So, what is the point in all that? Purely academic, actually; I use plain paraffin wax with no additives in the belief that proper processing and a *sharp* blade are the central features of good microtomy. I have only ever come across one wax with significantly different crystalline structure to others and that is Ralwax, which can be helpful when cutting decalcified tissues, etc.

If anyone has access to a scanning EM and wants to see crystalline structures, simply fracture some wax, make a resin impression (via a silicone intermediary impression if necessary) coat and view it. Virtually the same thing can be done to paraffin sections (both top and bottom surfaces), but use 10% aqueous polyvinyl alcohol as the impression material, float cut sections onto the surface, cut out around sections, then remove wax by boiling.

Interesting crystals will be seen in the former, and familiar tissue structures in the latter. This will also give an impression of where the wax goes and does not go.

References:

Allison, R.T. 1978. The crystalline nature of histology waxes: a preliminary communication. Med. Lab. Sciences 35:355-363

Allison, R.T. 1979. The crystalline nature of histology waxes: the effects of microtomy on the microstructure of paraffin wax sections. Med. Lab. Sciences 36:359-372.

Culling, C.F.A. R.T. Allison & W.T. Barr. 1985. *Cellular Pathology Technique.* 4th ed. Butterworths, London. Chapter 4 pp58-59 and Chapter 5 pp91-98.

HEAVEN & HELL

Heaven is where:
the police are British,
the cooks are French,
the mechanics are German,
the lovers are Italian,
and all is organized by the Swiss

Hell is where:
the police are German,
the cooks are British,
the mechanics are French,
the lovers are Swiss
and all is organized by the Italians

Captured from Cyberspace by Larry Albright