

Applications Note - Large format histology – Why thinner is better!

Dr Philip Bryant, Cardiff Metropolitan University, Cardiff, Wales, UK

Dr Neil Haine, CellPath Limited, Newtown, Wales, UK

[For the complete article, please refer to the Journal of Histotechnology](#)

The importance of large format histology as a method of assessing wide areas of tissue samples in cancer pathology is unparalleled. For a pathologist to be able to examine tumor extent, involvement of surgical resection margins and other prognostic parameters in a complete cross section of the tissue on a single slide is unrivalled in cancer diagnostics. However, there are several issues that need to be addressed before attempting to process large format, supra mega (SM) tissue samples in the laboratory. In order to achieve optimal high-quality tissue processing combined with a reduction in processing times, the ability to gross tissues consistently to 5mm thickness is crucial. However, due to the countless types and variable consistencies of tissues, this is not always possible in the grossing room. To overcome these challenges, it is often helpful to first gross the tissue into thicker slices before placing them into 100% reagent alcohol or ethanol for an hour in order to stabilize the tissue. This treatment not only firms the tissue to allow thinner slices to be taken but often reduces the need for reprocessing fatty or other problematic tissue. If tissues contain large amounts of fat, placing them into a fat solvent such as acetone is advantageous before attempting to gross the tissue into thinner 5mm slices. In addition, using isopropanol as the dehydrating agent during processing of fatty tissue is often recommended as it is a superior fat solvent compared to ethanol. However, if reagent alcohol is your preferred choice as a dehydrating agent, then extending processing times in that reagent would be required to ensure optimal processing. Since minimizing the thickness of slices is essential for quality processing of large tissue samples, commercial aids are available for the consistent grossing of tissue. Systems such as the ProCUT slicing devices (Milestone Medical) and the

TruSlice specimen cut up systems (CellPath) are not only convenient to use but also allow reliable production of thin slices of fresh and fixed tissues in the grossing room (Figures 1 & 2).

In large format histology, the choice of cassette plays a significant role in tissue processing and several types are available in the marketplace (Figure 3). Although the large format, SM cassettes have an external measurement of 75x52mm, they have an internal depth that varies between 6mm and 15mm depending on the cassette type (Figure 4). Open pore areas of the cassettes also play a central role in the processing of large slices of tissue and the latest hexagonal designs have an open pore area of 67% (Figure 5). These larger pore areas increase the flow of reagents, thereby improving diagnostic turnaround by substantially reducing the processing times. Cassettes with a reduced 6mm internal depth such as the SM mothership or SM slim cassette are available and help to ensure that tissues are grossed consistently to 5mm thickness. If 15mm deep SM cassettes are used, thin 5mm tissue slices may be separated by spacers in order to reduce the well depth of the cassette. As a result, tissues remain distortion-free and benefit from consistent, high-quality processing.

All large format cassette types are compatible with most modern automated tissue processors. All routine processors can be adapted for large format cassettes whether the tissue processing schedules employ reagent alcohol (ethanol) and xylene, isopropyl alcohol (with or without xylene) or xylene substitutes. Depending on the type of processor used, a maximum of 32 SM standard or 64 SM slim cassettes can be stacked or layered in the baskets and racks supplied. Processing times can be adjusted accordingly but will depend upon variables such as tissue type, the final thickness of the tissue slice, the style of cassette employed (standard, mothership or slim) and the method used in loading the cassettes into the processor (stacked or layered). Generally, processing times for tissues 5mm in thickness will be half that of

thick slices, with tissues taking roughly 18 hours to process. In practice, processing times of under 8 hours may be achieved for 5mm tissue samples such as prostate using microwave assisted processing.

Large format histology has proven to be cost effective and is able to meet the needs of the modern histology laboratory in the multidisciplinary approach to cancer diagnosis. If you are interested in introducing the large format system into your laboratory, please feel free to attend the Hands On Training Workshop #1 at New Orleans where you will have the opportunity to familiarize yourself with histology methods that utilize large format cassettes and associated consumables. Also, read the previous Fixation on Histology blog from July 2018 entitled 'Tumor sampling and large format histology – Why bigger is better!'

<https://www.fixationonhistology.com/home/tumor-sampling-and-large-format-histology-why-bigger-is-better>

Finally, look out for the article 'Application of large format tissue processing in the histology laboratory' that is destined for publication in the September issue of The Journal of Histotechnology.



Figure 1. The ProCUT grossing system from Milestone Medical showing the tissue on the base prior to grossing (left), application of a dome large enough to accommodate the tissue (center) and the tissue following grossing (right).

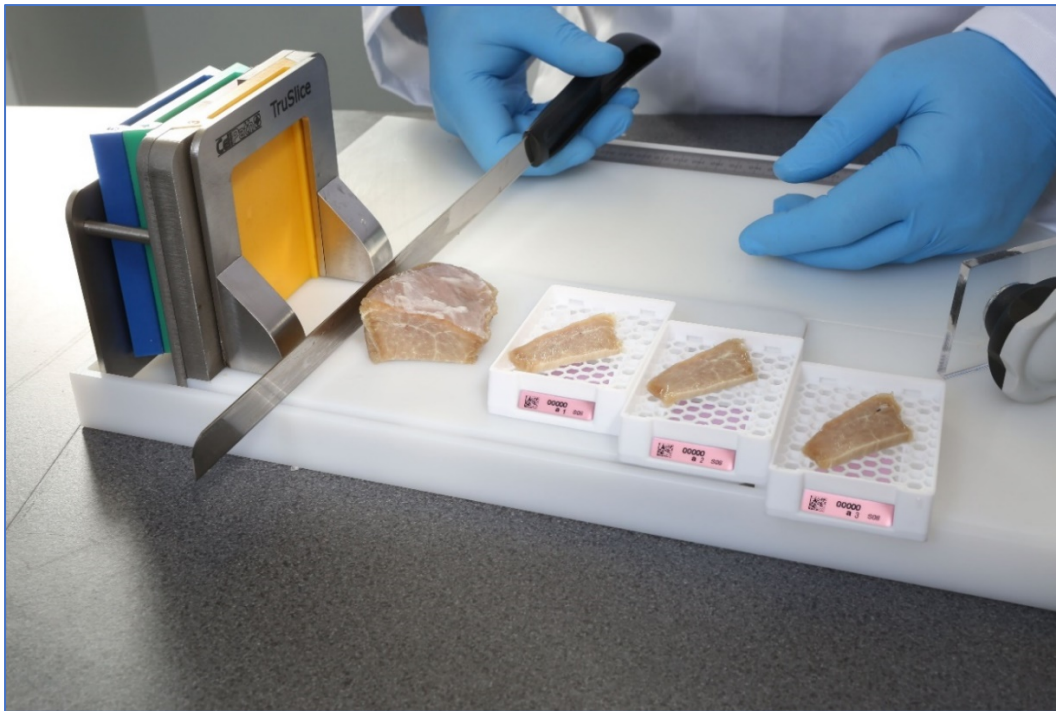


Figure 2. The TruSlice grossing system from CellPath shows a sharp blade used for slicing the tissue and SM mothership cassettes containing selected samples.



Figure 3. Large format cassettes showing the standard supra mega (SM) cassette (white), slim (blue) and mothership styles (yellow). SM mothership mold with SM mothership cassette (pink) are in the background.

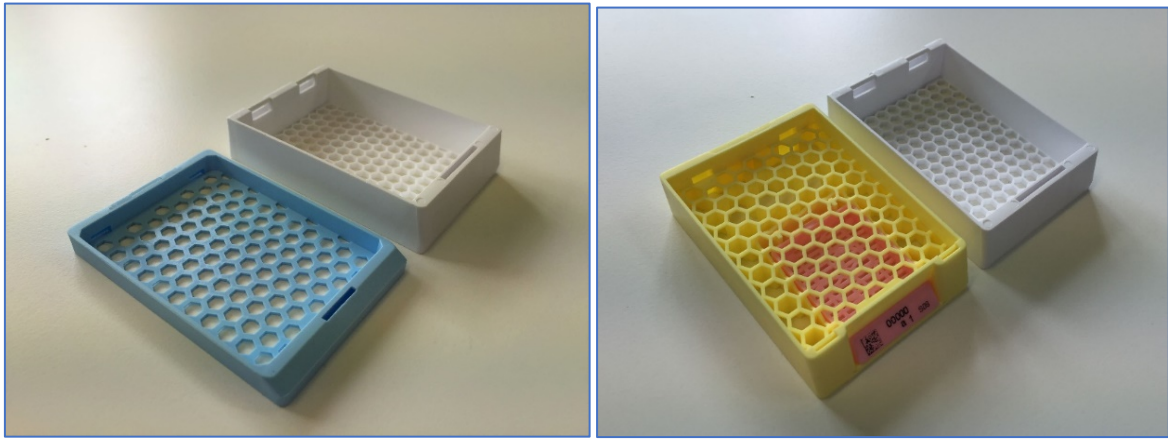


Figure 4. The image on the left shows the depth of the large format SM cassette (white) in relation to the reduced depth of the SM slim cassette (blue). The image on the right shows the SM mothership cassette (yellow) with the same external but shallower internal dimensions as the conventional large format cassette (white).

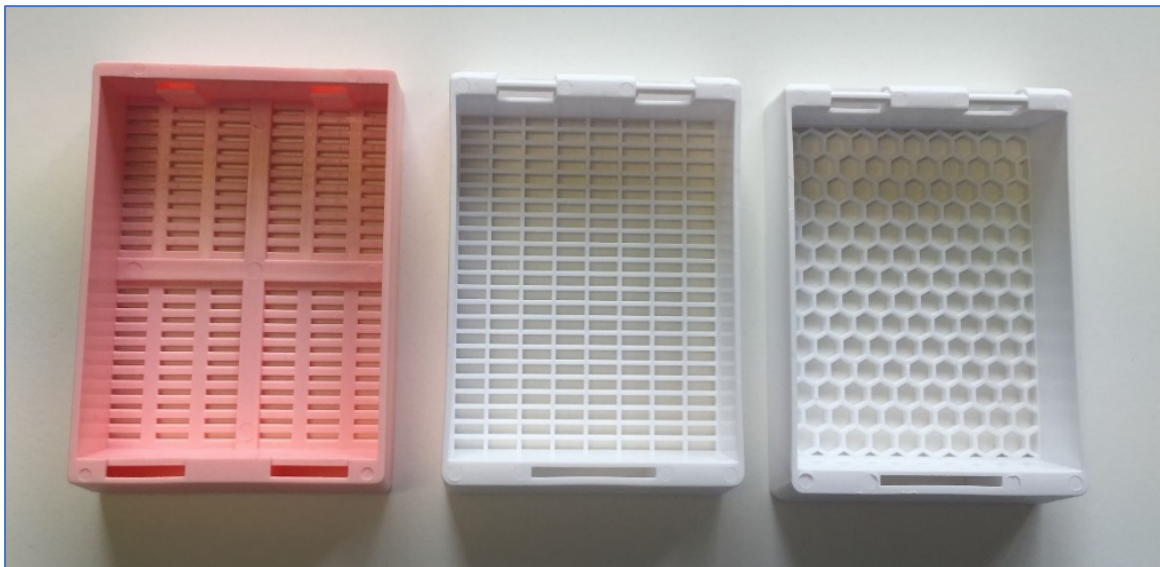


Figure 5. Large format cassette types showing (from left to right), the traditional (pink), the latest slotted (centre) and the hexagonal open pore area designs (white).