

Large Format Tissue Processing



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Foreword

We, at Northwest Pathology, have been utilizing whole mount technology for almost a decade now. Its utility in our lab has continued to grow since its adoption. We routinely utilize whole mounts for prostates, melanoma, breast lumpectomies, and even some brain autopsies. Having the ability to visualize the complete margins negates the need to mentally reconstruct the tissue and permits the use of zero magnification photographs. This is an extremely useful tool in our laboratory. It allows us to demonstrate tumor features and growth patterns, all in relation to the margins.

Pathologists, surgeons, and oncologists who attend our tumor boards find them extremely useful. Interest has been increasing rapidly nationwide and if you are not already doing whole mounts, you are behind the curve.

I would encourage every lab to consider bringing in this technology.

– Jeremy Johnston, Laboratory Manager

The team at Northwest Pathology, Washington, USA, has been providing specialty Anatomic Pathology services for over 25 years. Their primary focus has been on Anatomic Pathology and they have numerous areas of subspecialty, including Women's Health and Neuropathology. Northwest Pathology has recently broadened their scope and brought on a state of the art Clinical Pathology laboratory, Northwest Laboratory, to round out their service offering. Jeremy Johnston oversees a multi-disciplinary team in the Anatomic Pathology lab.

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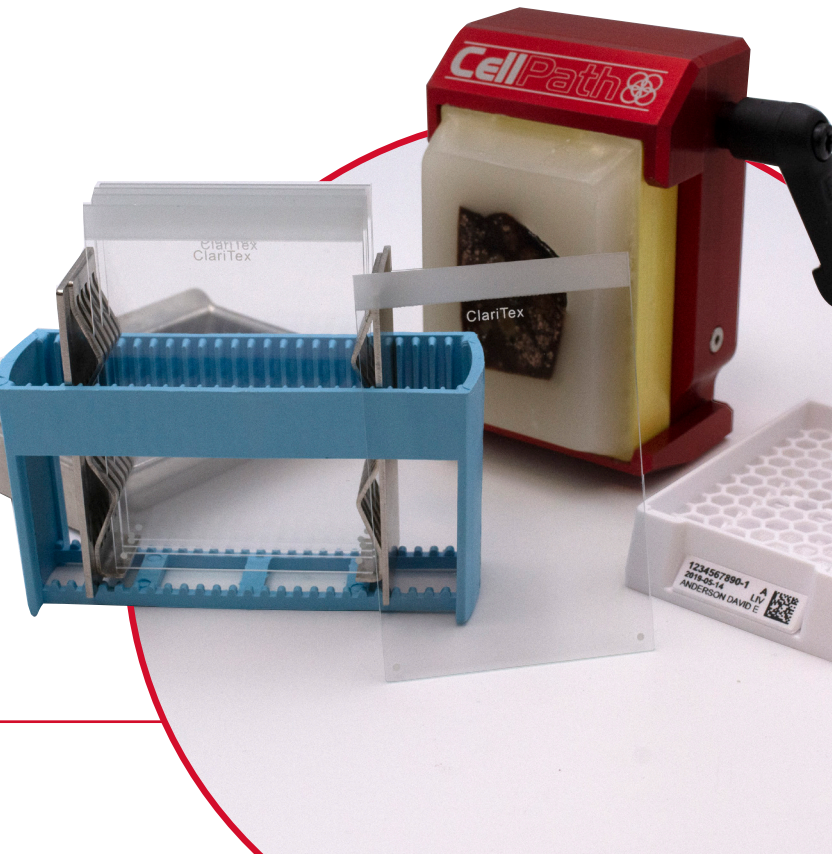


1.

What is large format tissue processing?

Large format tissue processing is a technique which can be introduced into histology laboratories to allow them to process bigger pieces of tissue using the same instruments as standard tissue processing.

Large format tissue processing is also known as Supra Mega or, depending on tissue type, whole mount histology. To accommodate larger sections of tissue, larger consumables are used. This means more tissue, and therefore more information, can be seen on each slide.



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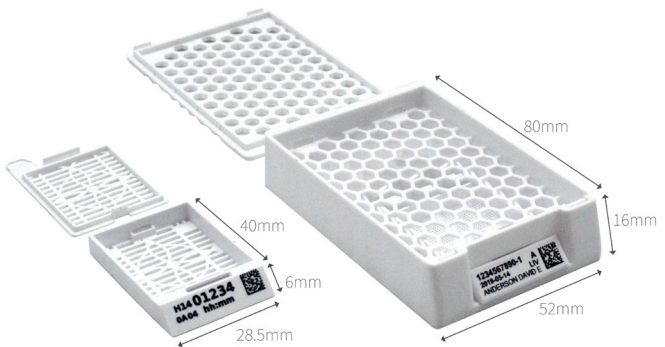


2. How is large format tissue processing different to standard tissue processing?

In large format histology, the consumables are bigger- this includes slides, coverslips, cassettes and base molds.

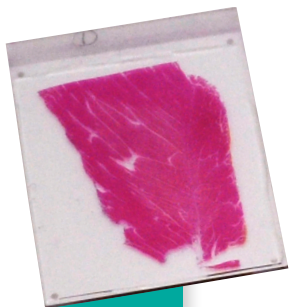
For example, standard slides measure 25mm by 75mm whereas large format slides measure 50mm by 75mm.

Supa Mega cassettes are also bigger than the standard sized alternative. Standard cassettes are typically 28.5x40mm whereas large format cassettes are 52x80mm. A range of depths of cassettes are available which means thicker pieces of tissue can be processed if desired.



3. What is unique about this method?

With large format histology, pathologists can see much more tissue at once. Instead of receiving one specimen with sections split between several slides, they can have all the information from each section of the specimen on one slide. The larger slides allow better examination of section margins and mean there is less need for mental reassembly.



Supa Mega Slide



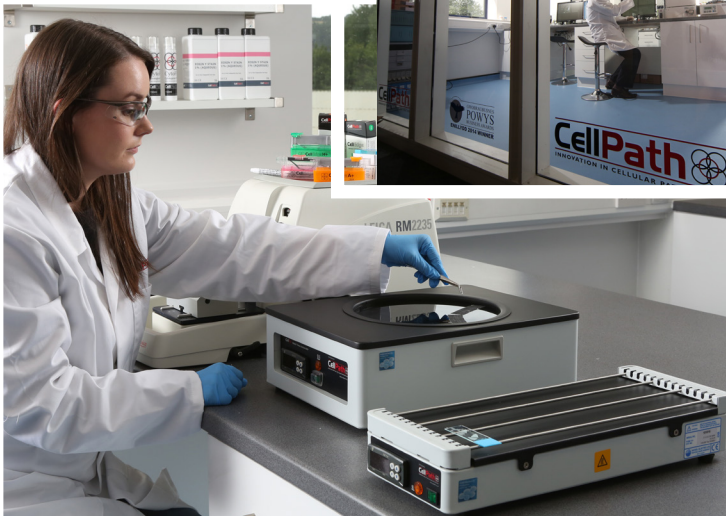
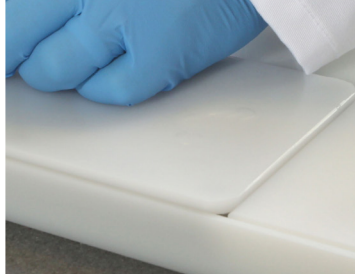
Standard Size Slide

4. What stays the same when using large format tissue processing?

Overall, the steps required for tissue processing are exactly the same for large format processing as standard format processing. Though processing time increases, minimal adjustment to existing equipment is required. Large format tissue processing can be integrated easily alongside existing processes.

Once processed, the analysis of slides also remains similar with the advantage of less need for manual reassembly of small pieces of tissue.





The method is widely used throughout the UK and Europe with a concentration of CellPath Supra Mega equipment users in Scandinavia.

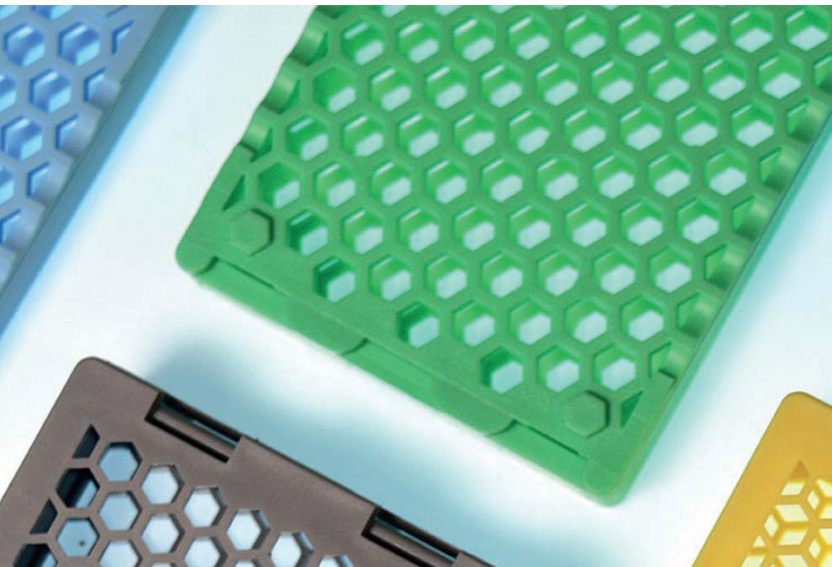
5. Who is using large format tissue processing?

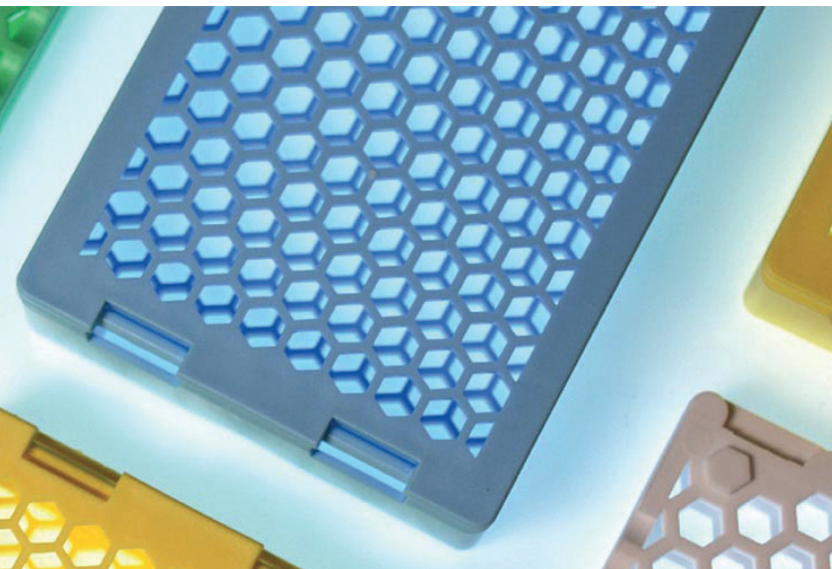
Globally, large format histology is used in a variety of labs and can be used to process a wide range of tissues. Large format processing can be used with many tissues including bone, bowel, brain, breast, kidney, liver, lung, pancreas, prostate, skin and thyroid. This method can also be used in veterinary labs where tissues such as vocal cords and jaws have been studied.



6. Why should I use large format tissue processing?

Large format histology can save labs both time and money, as well as having a positive impact on patient diagnosis. Studies have shown all three of these factors can be positively influenced by adopting large format histology when appropriate.





7. How will using large format histology save me time?

Time can be saved throughout tissue processing and analysis by using large format histology. Grossing can be quicker because, once tissue is accurately and thinly sliced, it can be placed straight into large format cassettes without the further dissection normally required to fit tissue pieces into smaller cassettes.

Pathologists can also save time as they have less need to mentally reassemble tissues pieces as more information is presented on one slide. They also have fewer slides to switch between. Productivity of the laboratory is increased overall due to the reduced number of blocks and slides which must be prepared and processed.



8. How will it save money?

Several studies have been conducted to explore the potential cost savings of large format histology over standard size processing. For example, Ibarra (2012) reported savings of \$26 per case when large format techniques were correctly implemented. Though these savings are small at first, they can accumulate quickly due to the broad range of tissue types suited to large format processing.

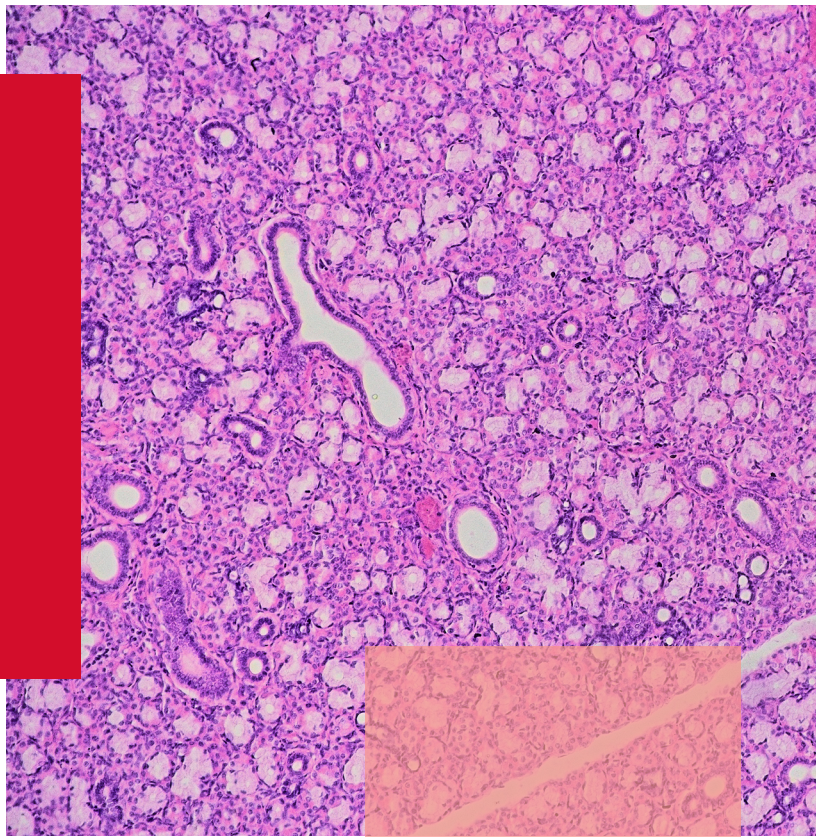
Of course, it is very difficult to put a monetary value on the improved patient outcomes experienced when using a large format system efficiently.



9. How will it affect patient diagnosis?

Positively! Studies show for multiple cancer types that there are clinical benefits from the use of large format histology. The ability for pathologists to see large sections all at once means they do not have to conduct the large amount of mental jigsaw puzzling previously required.

It can be easier to see the tumor profile on a large format slide which may lead to the discovery of otherwise unknown but significant characteristics. Ibarra (2012) found that large format histology provided further diagnostic advantages as large slides make it easier for pathologists to be confident that a tissue edge represents the margin and is not an artifact created by tissue sectioning. Foster et al (2012) identified findings with a significant impact on diagnosis, which were only visible when using large format histology, in over a quarter of the 656 cases they studied.



10. How will using large format histology affect pathologists?

Pathologists regularly identify growing workload as one of their biggest challenges.

Kamel (2011) explored the pressures facing the pathology industry and identified growing necessity to cut turnaround time as another of the key challenges to be addressed.

By switching to a method of slide analysis which allows pathologists to view large format slides, they can effectively study four slides at once. This helps to save time and reduce workload, as well as minimizing sources of mental fatigue and strain which can lead to diagnostic error.

11. How will introducing large format histology affect the staff in the lab?



Some training may be required to get the greatest potential out of large format techniques as many labs decide to introduce Supra Mega processing alongside standard size processing.

Ultimately, the tissue requires the same treatment, just on a larger scale.

12. When should I use large format tissue processing?

Large format histology can be used with a wide range of tissues including bone, bowel, brain, breast, kidney, liver, lung, pancreas, prostate, skin and thyroid. It is most commonly used with prostate, bowel and breast tissue. For some organs, such as the prostate as shown, this method even allows a whole organ section to be studied on one slide. This allows for better understanding of the tumor characteristics.

Large format histology is particularly useful for big specimens as more of the sample can be seen on one slide and there are fewer margins for the pathologist to piece together.

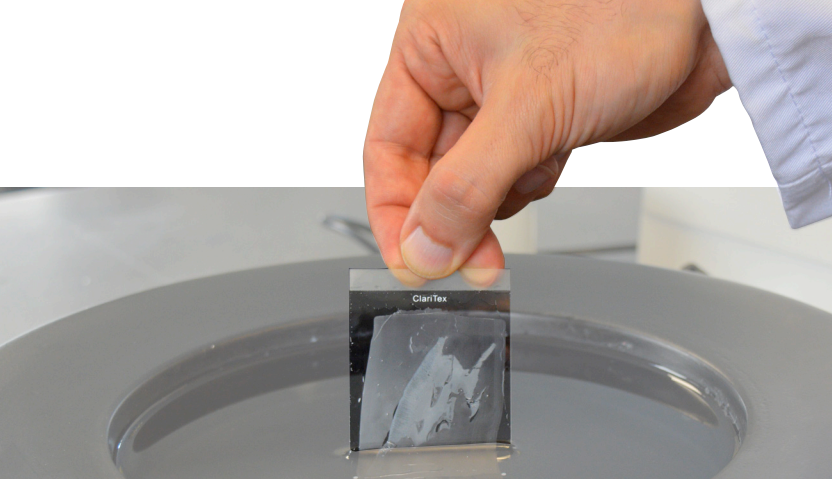


13. When should I not use large format tissue processing?

There are some types of tissue for which large format histology would not bring any additional benefit. Needle core biopsies, for example, should be processed using specialist micro consumables such as CellSafe+ capsules or the Lumea BxChip™. Within cytology, there would be no additional benefit to using a large format system.

It is also unlikely that large format techniques would ever be used in combination with frozen sections due to the urgent time constraints of this method.



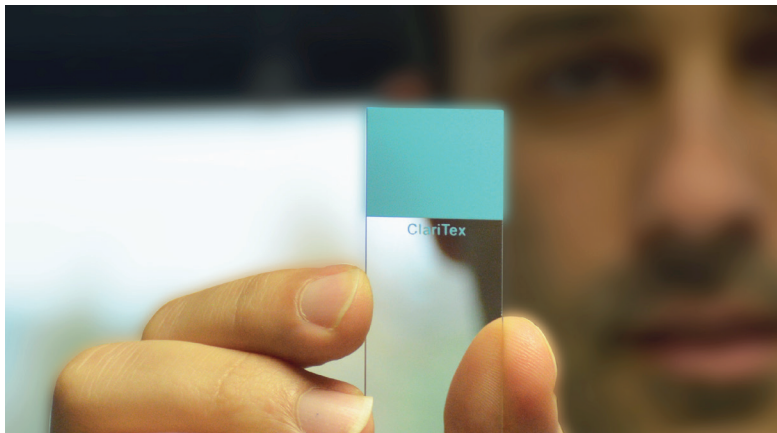


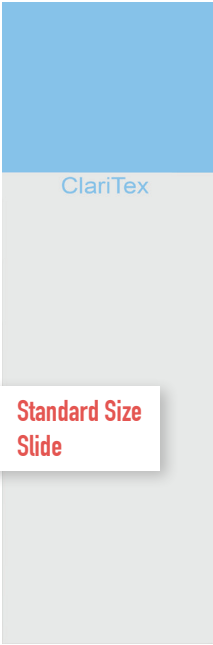
14. When will it be compatible with my current equipment?

For many standard laboratory practises the equipment you already have will be ready to work with large format processing techniques straight away. There is no need to change your processing or staining machines and only the simple adaptation of adding a clamp to fit Supa Mega cassettes means your current microtome can also be used.

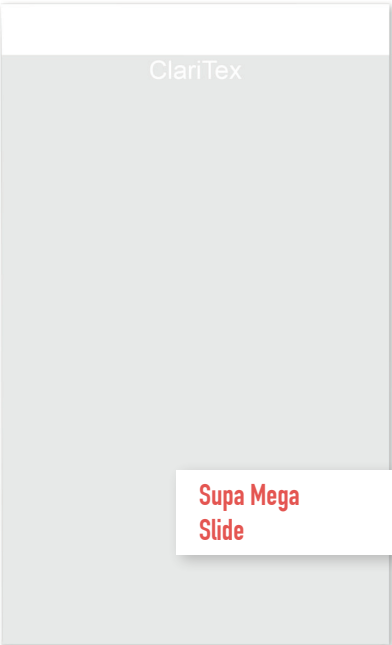
15. When will it not be compatible with my current equipment?

Large format histology typically integrates very well with standard laboratory practice. You may find you need to adapt your identification methods- see Question 24- and your current block and slide storage trays and cabinets may also need to be upgraded to maximise your use of space.

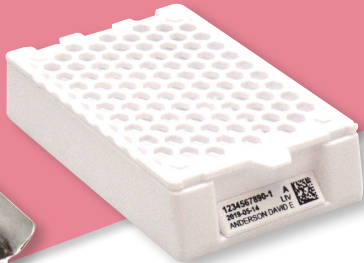
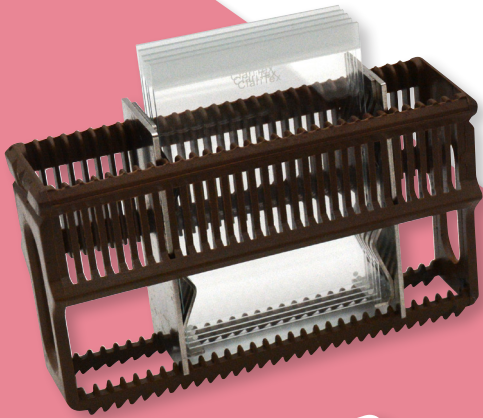




**Standard Size
Slide**



**Supa Mega
Slide**



16. What do I need to buy new to use large format histology?

To get started, a lab needs to purchase large format slides, coverslips, cassettes, base molds, slide rack adaptors and a universal microtome clamp. Be sure to consider compatibility between your equipment when choosing a microtome clamp. For example, the Universal Supa Mega Cassette Clamp produced by CellPath Ltd. can be attached to most popular brands of microtome. You may also need to consider your transport and storage systems, but some standard systems can be adapted to suit. Bryant and Haine (2017) estimate the cost of initial set up to be \$1,500.



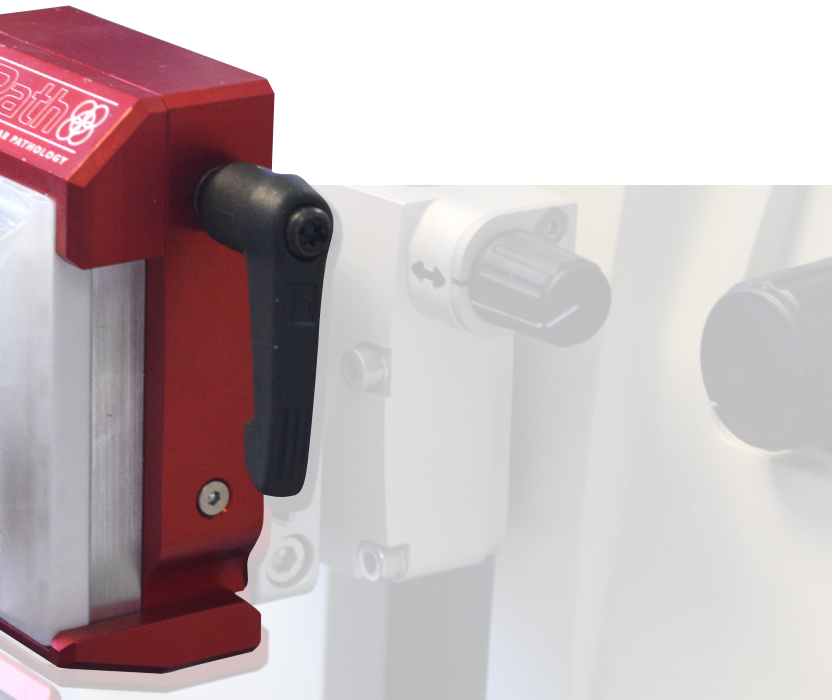
17. What can I adapt in my lab?

Ingenious technicians and pathologists are always discovering new ways to adapt their existing equipment to accommodate new technologies and extend the life of any expensive purchases. Laboratory equipment manufacturers, like CellPath Ltd who



design and manufacture consumables at their premises in mid-Wales, have also developed some methods which allow the range of functions of large equipment to be extended.

These developments include slide rack adapters which can be inserted into the slide racks used in staining machines and allow large format slides to be stained as easily as standard slides. Another useful invention is a universal microtome clamp which fits all depths of large format cassettes. These clamps can be fitted to most brands of microtome eliminating the need for the additional expense of purchasing an extra microtome.



18. How do I gross tissue in a large format system?

Grossing for large format tissue processing requires similar techniques to standard tissue processing. The benefit of large format techniques is that, by adopting methods such as 'bread loafing,' it may be possible to contain much more information about the tumor in a single tissue section. Thicker slices of tissue can also be processed in some large format cassettes if desired. For example, CellPath Supra Mega cassettes have a maximum depth of 16mm.

Care should be taken when combining large format processing with xylene free methods if thicker tissue sections have been cut. Tissue is more prone to excessive swelling with xylene free methods and labs will be keen to avoid imprinting from the cassette affecting the tissue.

As a rule, tissue should still be grossed accurately to 5mm which fits well inside slimline Supra Mega cassettes and Mothership cassettes. If deeper cassettes are used, spacer blocks can be added to allow thin specimens to remain flat without "pringling" during processing. Grossing aids, such as CellPath's Truslice, are available to ensure consistent slices are cut- just as important when using deeper cassettes.



19. How do I process tissue in large format cassettes?

Tissue processing can still be conducted in exactly the same way when using Supa Mega cassettes. Large format cassettes are placed into baskets of an automated processor- naturally, as they are bigger, fewer cassettes will fit at once. You will need to adjust the timings on your processor to accommodate the larger pieces of tissue and processing will take longer than for standard sized cassettes.



Average processing times for large format cassettes are around 20 hours, with thicker tissue sections taking longer to process. Consistent grossing to 5mm ensures consistent processing time. It is possible to complete processing in as little as 7 hours with microwave assistance. Examples of typical protocols can be seen in the appendices of this book.

20. How do I embed tissue in a large format system?

Embedding and casting of tissue follows the same protocol as when using standard sized consumables. Some people may find it easier to manipulate and orientate larger specimens.

Base molds exist in a variety of shapes, depths and materials to suit the needs of each lab. Some may prefer to use shallower base molds which cast quicker. An average deep mold will take approximately 60 minutes to cast with a shallow mold taking around 20 minutes.

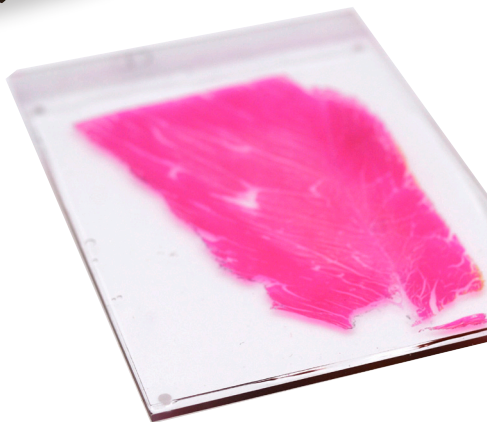
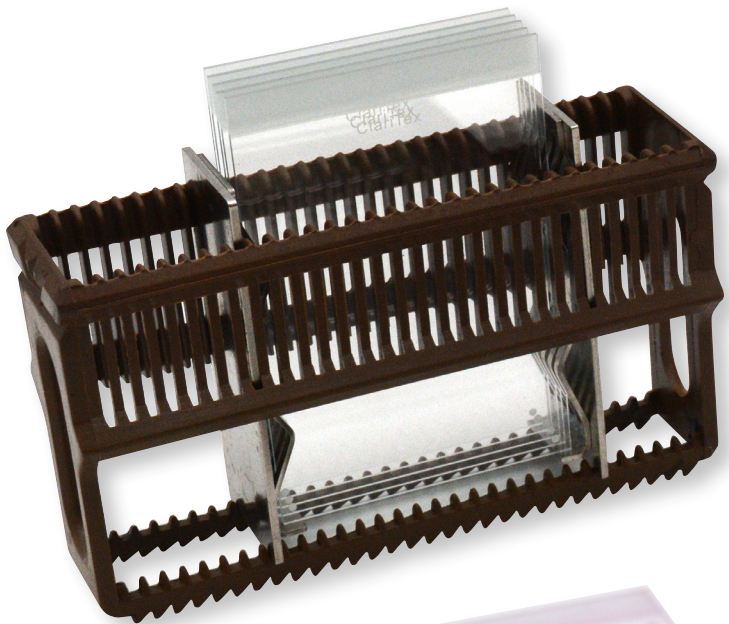
21. How do I section Supa Mega blocks?

Once a suitable large format clamp has been attached to your microtome you will find sectioning follows the same process as in a standard format system. High quality sections can be cut using either sliding, sledge or rotary microtomes from a wide range of manufacturers. Adaptations are available to ensure all depths of Supa Mega cassettes can be used. For example, if you have chosen a slim cassette you may need to use a spacer block to hold the cassette in place during sectioning.

Leica 2200 series microtome users may find increased likelihood of collisions between the base of the clamp and the knife holder if they use the Leica microtome clamp. Removing the plastic cover of the knife holder and orientating the block to the zero position will help to minimise these. Using a 15mm deep base mold with slim or mothership cassettes will also help. Alternative clamps, such as the CellPath Universal Microtome Clamp have been specifically designed to eliminate these issues.

Standard format sectioning typically requires the blade to be moved between three positions to ensure the most use is had from the blade. Large format cassettes allow the blade to remain in the same position throughout.





22. How do I stain the sections?

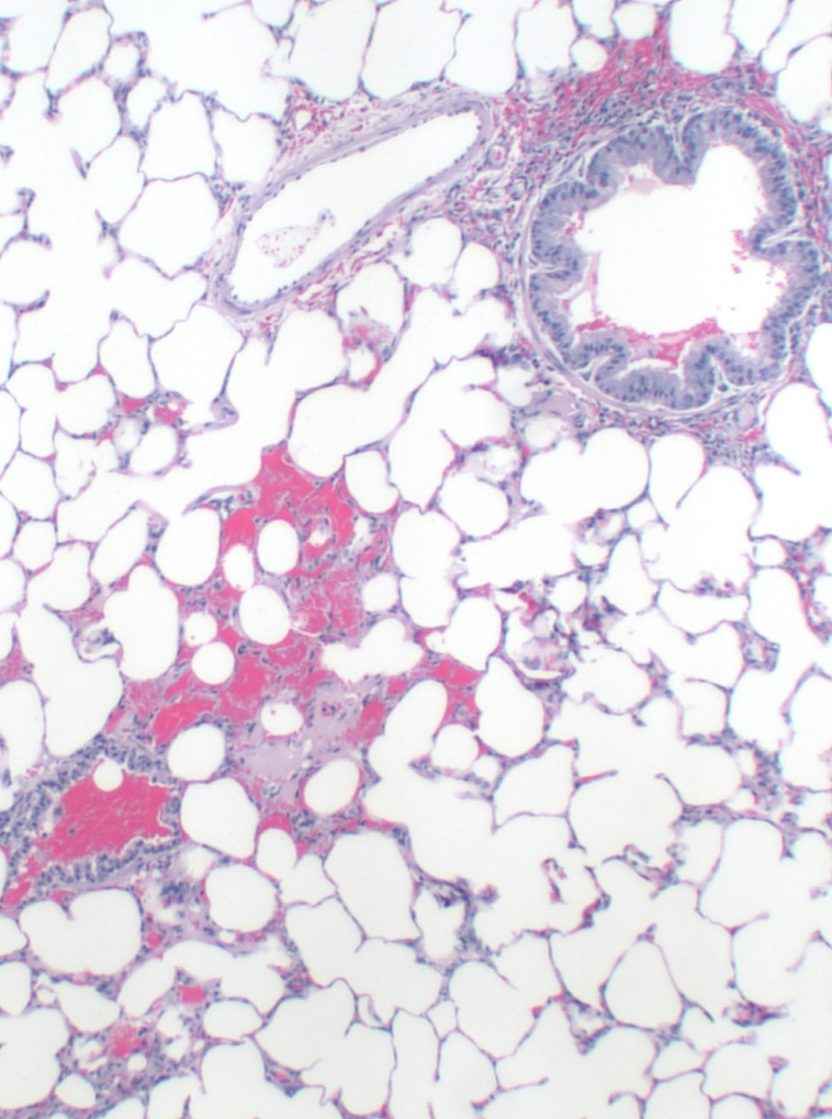
Both automated and manual slide staining protocols can be applied to Supa Mega slides bearing large sections of tissue. The only obstacle most labs encounter is fitting their large slides into their slide racks. Fortunately, slide rack adapters ensure an easy solution to this problem.

The adapters fit into the slide racks of all the most common automated stainers and allow five large slides to be stained at once. Manual stainers typically hold slides horizontally, meaning the greater width of Supa Mega slides causes no problems with manual staining provided each bath is filled deep enough to cover the slide.



23. How do I analyze the stained slides?

With ease! Large format slides can be studied on a standard microscope platform. Pathologists should expect to see more information on each slide so it is to be expected that analysis may take longer per slide. However, with reduced need to switch between slides and reconstruct a mental jigsaw, time savings can be expected overall.



24. How do I identify slides and cassettes?

Printed identification of large format consumables will require adjustment to your current practices. Printing on equipment, rather than handwriting, ensures samples are clearly identified throughout. The College of American Pathologists produced guidelines in 2015 which state that laboratories should ensure that all blocks and slides are clearly labelled using two patient identifiers. To comply with most Laboratory Information Management Systems (LIMS), laboratories must ensure their samples can be traced electronically. These factors have led labs to increase their use of printed identification.

Though there are currently no printers for large format cassettes, you can still use printed methods of identification by utilizing a standard cassette with a Mothership cassette. The standard sized identifying cassette is printed as normal on your standard printer and then fitted securely into a large format Mothership cassette containing tissue for processing. CellPath holds the patent for Supa Mega Mothership cassettes which they design and produce. They are available in a range of colors to suit your needs.



To identify large format slides, a slide label can be printed and applied. There are several options for slide labels, including long thin labels which will make best use of the available space on a large format slide. Alternatively, some users may choose to use standard labels and trim them to better fit a large format slide whereas others use them as they are.

Of course, all Supa Mega consumables are suitable for manual hand-written labelling if required.

25. How do I store Supa Mega samples?

You may find you are able to adapt your current system to accommodate larger block and slide storage. For example, trays which typically hold 9 standard width slides can be used to hold 4 double width large format slides.

Storage solutions specifically designed for larger slides and blocks are also available and allow convenient long- and short-term storage of essential materials. These solutions include cardboard boxes and durable metal cabinets with dimensions specific to Supa Mega block proportions. Slide transport trays and storage boxes are also available.





26. How is it designed?

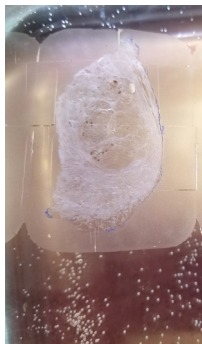
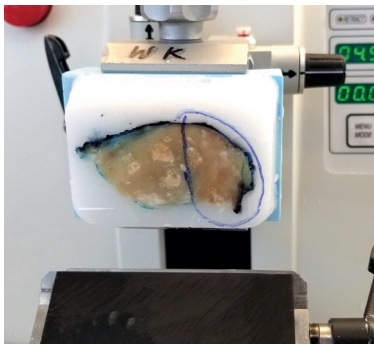
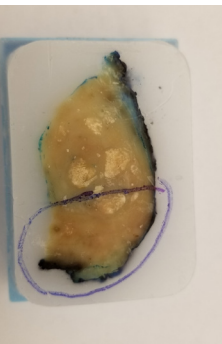
Large format consumables are designed with all the same criteria in mind as standard consumable equipment. This includes maximizing flow of reagents through cassettes and ensuring precision and clarity of slides. Some of the design challenges are exaggerated with larger format consumables.

For example, in order to maintain the necessary rigidity across a larger surface area, manufacturers may trial several different pore shapes and sizes to strike a compromise between structure and reagent flow. Other pieces may be designed with cost savings in mind. For example, by designing adapters which allow labs to continue using their existing slide racks or microtomes CellPath shows awareness of the needs of lab equipment to be as multifunctional as possible.

27. How is large format equipment made?

All manufacturers will have different techniques for ensuring their ranges are produced to the highest possible standards. With a variety of materials used to make pathology consumables, some manufacturers will produce their consumables on site through facilities such as injection molding and others may outsource production processes.

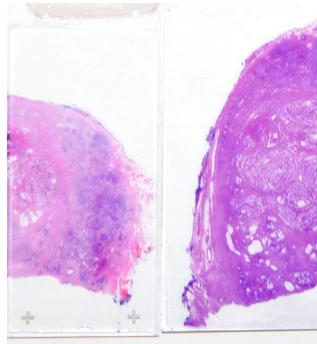
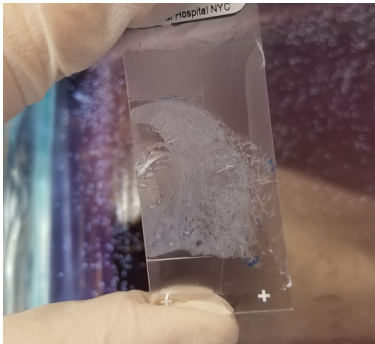
At CellPath Ltd, dedicated research teams regularly develop and produce new products for use in large format histology. As holders of ISO 13485:2016 certification we are able to produce high quality pathology consumables in-house at our manufacturing facility in mid Wales, UK.



28. Can I use large format processing with IHC?

At time of writing, there are no platforms for immunohistochemical (IHC) staining which accommodate larger than standard sections and it remains more common to use standard sized equipment for IHC. However, some laboratories are already using novel techniques to employ a combination of Supra Mega processing with IHC.

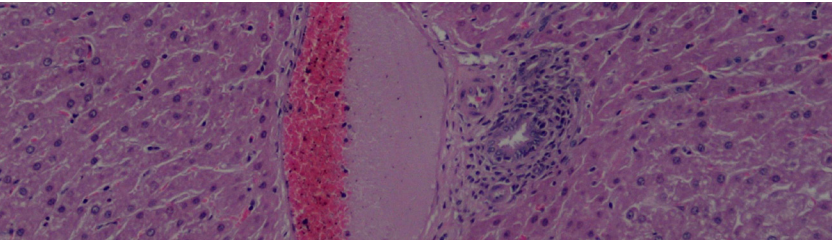
A laboratory may choose to use large format techniques to prepare a specimen for presentation to a pathologist. If the pathologist then requires IHC to be used on a region of interest the lab can adopt an alternative technique to continue using automated IHC. One such technique, as described by Bryant et al (2019), requires the large format section to be floated onto the water bath and the region of interest separated with forceps from the large format section. This segment would then be mounted onto a standard positively charged slide in order to proceed with automated IHC.

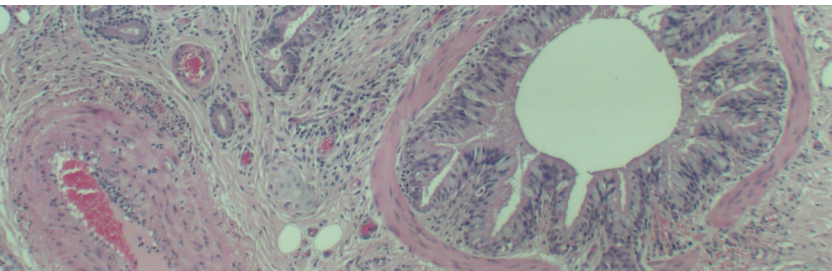


29. How does large format histology integrate with digital pathology?

Digital pathology is on the rise with many benefits already identified including the ability for remote slide analysis by geographically distant pathologists. The principles of large format histology are well supported by the benefits of digital pathology. Not only are more pathologists able to see the tissue, there will also be more tissue to see.

Though slide scanning technology is at different rates of development in different countries, many platforms are already available for scanning larger slides as well as standard sized.





30. What is the future of large format histology?

As large format processing techniques continue to gain popularity around the world, laboratory staff and pathologists are likely to see more developments in the field as manufacturers strive to make it easier to integrate large format histology into standard laboratory practice.

Already, many of the challenges of integration have been met and it is likely that equipment manufacturers will be keen to ensure large format techniques can be adapted to any new technologies as they emerge.

References

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Kamel HM. Trends and challenges in pathology practice: choices and necessities. *Sultan Qaboos University Medical Journal*. 2011 Feb;11(1):38.

Appendices

Conventional processing

	Tissue 5mm; Cassettes stacked	Tissue 8-10mm; Cassettes random
Reagent	Timing (Hours)	Timing (Hours)
Alcohol 70%	1.00	2.00
Alcohol 100%	n/a	n/a
Alcohol 100%	1.30	2.00
Alcohol 100%	1.30	3.00
Alcohol 100%	1.30	3.00
Alcohol 100%	1.30	3.00
Alcohol 100%	1.30	3.00
Alcohol 100%	1.30	3.00
Xylene	1.30	3.00
Xylene	1.30	3.00
Xylene	1.30	3.00
Paraffin Wax 60°C	1.00	3.00
Paraffin Wax 60°C	1.00	3.00
Paraffin Wax 60°C	1.30	3.00
Paraffin Wax 60°C	1.30	3.00
Total Time	18.00	37.00

Fatty tissue- Tissue 8-10mm

Pre-treatment of fatty tissue.

Ensure that all fatty tissue is well fixed in formalin.

Fatty tissue may require extended protocols if ethanol is used as dehydrant - isopropanol is a more superior fat solvent.

Acetone, alcoholic formalin or proprietary solutions such as Pen-fix (which contains formalin, ethanol, methanol and isopropanol) may also be used as substitutes to pre-treat fatty tissue.

Reagent	Timing (Hours)
60% Isopropanol	3.00
70% Isopropanol	3.00
80% Isopropanol	3.00
95% Isopropanol	3.00
95% Isopropanol	3.00
100% Isopropanol	3.00
100% Isopropanol	3.00
Xylene	1.50
Xylene	1.00
Paraffin Wax 65°C	1.50
Paraffin Wax 65°C	2.00
Paraffin Wax 65°C	3.00
Total Time	30.00

Bone- Tissue 8-10mm

Reagent	Timing (Hours)
60% Isopropanol	4.00
70% Isopropanol	4.00
80% Isopropanol	4.00
95% Isopropanol	4.00
95% Isopropanol	4.00
100% Isopropanol	4.00
100% Isopropanol	4.00
Xylene	4.00
Xylene	4.00
Paraffin Wax 65°C	4.00
Paraffin Wax 65°C	4.00
Paraffin Wax 65°C	4.00
Total Time	48.00

Xylene free- Tissue 8-10mm

Reagent	Timing (Minutes)	Temperature (°C)	Pressure/Vacuum (mBar)
Formalin	5	55	Ambient
85% Ethanol	60	55	Ambient
85% Ethanol	95	60	Ambient
80/20 Ethanol/IPA	90	60	Ambient
95% Isopropanol	130	60	Ambient
100% Isopropanol	60	60	Ambient
100% Isopropanol	130	60	Ambient
Xylene	230	60	Ambient
Paraffin Wax	180	85	Vacuum
Paraffin Wax	130	85	Vacuum
Paraffin Wax	120	65	Vacuum
Total Time	20 Hours, 30 Minutes		

Microwave assisted- Tissue 5-8mm

Program Method	Timing (Minutes)	Temperature (°C)	Pressure/Vacuum (mBar)
Fixation	20	40	n/a
Fixation	40	40	n/a
Fixation	20	50	n/a
Fixation	40	50	n/a
Ethanol	30	50	n/a
Ethanol	100	50	n/a
Isopropanol	30	55	n/a
Isopropanol	70	55	n/a
Isopropanol 2	30	60	n/a
Isopropanol 2	70	60	n/a
Isopropanol 2	20	68	n/a
Isopropanol 2	60	68	n/a
Vaporization	1.50	n/a	600
Wax Impregnation	0.50	70	995
Wax Impregnation	30	70	500
Wax Impregnation	20	70	400
Wax Impregnation	20	70	300
Wax Impregnation	20	70	200
Wax Impregnation	250	65	150
Wax Impregnation	59.5	65	800
Total Time	15 Hours, 31 Minutes, 30 Seconds		

Microwave assisted- rapid prostate, tissue 5mm

Reagent	Timing (Minutes)	Temperature (°C)	Pressure/Vacuum (mBar)
Formalin	15	50	
Formalin	15	50	
Flushing	1		
Rinsing 1	2		
Rinsing 2	2		
Ethanol	15	65	
Ethanol	30	65	
Isopropanol	15	68	
Isopropanol	45	68	
Vaporization	1		600
Paraffin	30	70	995
Paraffin	15	70	500
Paraffin	15	70	400
Paraffin	6	70	300
Paraffin	6	70	200
Paraffin	6	70	150
Paraffin	210	65	150
Total Time	7 Hours, 9 Minutes		

Supa Mega Product Range

Product	Part Number	Pack Size
<i>Supa Mega Hex Cassette (White, Green, Blue, Pink, Yellow, Red)</i>	EAG-01XX-02A	100
<i>Supa Mega Slim Cassette (White, Green, Blue, Pink, Yellow, Red)</i>	EAN-01XX-02A	100
<i>Supa Mega Mothership Cassette (White, Green, Blue, Pink, Yellow, Red)</i>	EAO-01XX-02A	100
<i>Supa Mega Base Mold 60 x 45 x 15mm</i>	GBC-6014-05A	5
<i>Supa Mega Base Mold 36 x 36 x 15mm</i>	GBC-3614-05A	5
<i>Supa Mega Slim Base Mold 60 x 45 x 8mm</i>	GBC-6014-05B	5
<i>Supa Mega Mothership Base Mold 60 x 45 x 8mm</i>	GBC-6014-05C	5
<i>Supa Mega Slim Microtome Chuck Spacer Block</i>	JFA-0100-00A	1
<i>Supa Mega Slim Microtome Chuck Spacer (Vice Clamps)</i>	JFA-0100-00B	1
<i>Supa Mega Universal Cassette Clamp</i>	JFB-0100-00A	1
<i>Claritex Supa Mega Plain Slide</i>	MAC-1400-02A	100
<i>Claritex Supa Mega Twinfrost Slide</i>	MAD-1400-02A	100
<i>Histobond+ Supa Mega Slides</i>	MAD-1402-09A	50
<i>Claritex Supa Mega Coverslips No. 1.5, 50 x 64mm</i>	SAF-5064-02A	100
<i>Claritex Supa Mega Coverslips No. 1.0, 50 x 64mm</i>	SAG-5064-02A	100
<i>Slide Rack Adapters, Stainless Steel</i>	RMC-2000-63A	2
<i>BlocFile 1 SM Cassette/ Slide box (Assembled/Flatpack)</i>	WCB-1100-00A/08F	1/25
<i>BlocStor 3 SM Cassette/ Slide box (Assembled/Flatpack)</i>	WCB-0500-01A/08F	10/25
<i>OmniStor 4 Standard (Assembled/Flatpack)</i>	WEA-0700-01A/08F	10/25
<i>FiloSlide 100 Box (for Supa Mega Slides)</i>	WEQ-0906-00A	1
<i>SlideRite Supa Mega Slide- Cardboard Mailer</i>	WEX-0500-01A	10
<i>OmniStor Supa Mega Cabinet</i>	WEA-130X-00X	1
<i>OmniStor Base Plinth (Wheeled)</i>	WEA-0601-00A	1
<i>OmniStor Base Plinth (Static)</i>	WEA-0501-00A	1



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