Chapter 1 : Freezing Tissues for Cryosectioning

TISSUE FREEZING METHODS FOR CRYOSTAT SECTIONING Basic Tissue Freezing Methods. Preparing Tissue for Freezing. Then a quick overview of MHPL Cryostat sectioning Techniques.

They provide little useful information. For scientific or diagnostic purposes, tissue specimens must undergo substantial alteration in preparation for viewing under a microscope. There are four steps in tissue preparation. Fixation stabilizes and preserves the tissue. Embedding converts the tissue into a solid form which can be sliced "sectioned". Sectioning slicing provides the very thin specimens needed for microscopy. Staining provides visual contrast and may help identify specific tissue components. Most basic histology texts offer a minimal account of basic histological technique. Be aware that each step of tissue preparation introduces artifacts by altering or distorting the natural appearance of cells. Some artifacts are unavoidable. Fixation , by its very nature, kills cells and stabilizes dynamic cell processes. Enzyme activity is usually altered. Ions and small molecules are usually washed away. Some artifacts are intentional, most notably the colors added by staining. Still other artifacts are accidental. Cells may shrink or swell during fixation. Extracellular spaces may be distorted by compression or stretching. Ripples and wrinkles can be introduced during cutting and handling of sections. Unintended artifacts can be minimized by optimal procedures -- but optimal procedures are often impractical, especially with human specimens. Ideal tissue preparation preserves cells in a form that resembles the living state, but this ideal is seldom practical with clinical specimens. Often, especially in post mortem autopsy material, cells have been dead and deteriorating for several hours before fixation. Therefore, certain artifacts must be appreciated as part of the normal appearance of tissue specimens. The process of sectioning can introduce still other artifacts. The most common mode of routine tissue preparation involves fixation with buffered formaldehyde, embedding in paraffin, sectioning into slices about 5 micrometers in thickness, and staining with hematoxylin and eosin. Many of these involve sophisticated reagents based on the specificity of enzymes, immunological antibodies, or gene sequences to label and localize specific proteins or other molecules. Some textbooks present additional detail. This process is called fixation, and the resulting specimen is described as fixed. Boiling an egg and pickling a cucumber represent examples of fixation, in which heat or chemistry stabilizes the organic materials. A variety of chemicals can be used for fixing histological specimens. Routine fixation often uses a solution of formaldelhyde formalin to react with proteins and other organic molecules to stabilize cell structures. This solution is buffered and osmotically balanced to minimize shrinkage, swelling, and other collateral damage. Ideally, fixation should be accomplished extremely quickly to minimize post-mortem changes in cell structure. Since fixation rate is limited by diffusion, ideal tissue preservation requires that fixative be delivered as closely as possible to each cell. Rapid delivery of fixative can be accomplished either by perfusion or by immersion. Perfusion involves the delivery of fixative through the circulatory system of living tissue, by direct injection into a major artery. Such a procedure is commonly used with experimental animals but is obviously impractical for obtaining clinical specimens from patients. Successful fixation by immersion requires very small samples. However, surgical removal of very small tissue samples often entails incidental mechanical damage, especially with punch biopsies. These constraints on ideal fixation mean that tissue quality may vary across a specimen, with possible distortion near edges especially with needle or punch biopsies and with variation in fixation quality and attendant staining character in deeper areas into which fixative diffuses more slowly. An alternative to chemical fixation is freezing, followed by direct sectioning of the frozen specimen. Frozen sections are seldom as "pretty" as well-fixed specimens, but they do have certain advantages. Because frozen sections do not require hours for the normal schedule of fixation and embedding, they can provide immediate diagnostic information to a surgeon in the operating room. Frozen sections can also permit analysis of small diffusable molecules or of enzyme activity whose presence would be lost during chemical fixation. To embed a tissue sample, tissue water is replaced first by solvents such as alcohol and xylene and then with a liquid such as melted wax paraffin or epoxy solution which can be subsequently solidified by cooling or polymerization. Sectioning is the production of very thin slices from a tissue sample. A microtome may be as simple as razor blade, or it

may be a complex machine costing several tens of thousands of dollars for producing the ultrathin sections needed for electron microscopy. Most laboratory microtomes have the essential machinery of a baloney-slicer: For electron microscopy, sections are typically nanometers millimicrons in thickness. Sectioning necessarily reduces the specimen to a two-dimensional representation. Reconstructing the three-dimensional structure of the original sample requires either the "stacking" of multiple images from serial sections, or else judicious use of imagination 3-D visualization. A very small amount of three-dimensional information may be directly visualized under the microscope, by focussing up and down through the thickness of the specimen. For a further account of 3-D visualization, see here. Sectioning can certain introduce artifacts. Among the commonest artifacts, and most distracting for a beginner, are wrinkles. To appreciate why wrinkles form, imagine trying to lay a sheet of wet tissue paper representing the slice from the sample flat onto a table representing the microscope slide. Even with great care, wrinkles sometimes appear. Sometimes wrinkles are "forced" when the tissue section stretches unevenly around structures of differing consistencies. Another sectioning-related artifact is the disappearance of small structures which fall out of their proper place on the specimen, and the occasional reappearance of such structures at other inappropriate locations. This happens most often when the process of slicing separates a part which is attached only outside the plane of section, such as a hair shaft within a hair follicle. Except in the case of perfect lengthwise slices, the hair shaft will be cut into an oval slice that is not attached to the sides of the hair follicle and may therefore come out leaving the follicle apparently empty and then alight somewhere else as an odd oval structure anywhere on the slide. Yet other common artifacts are scratches and "chatter". Scratches are caused by flaws or dirt on the cutting edge, and appear as straight slashes or ragged tears across the specimen. The the process of slicing sometimes induces vibrations in the knife edge, which then cause variations in thickness ripples in the section. These appear as narrow parallel bands, usually evenly spaced, across a tissue specimen. They are often most evident in areas of smooth texture, such as the colloid in thyroid follicles. Even red blood cells, packed with hemoglobin, appear nearly colorless when unstained, unless packed into thick masses. Stains are used to confer contrast, to make tissue components visibly conspicuous. Certain special stains, which bind selectively to particular components, may also be used to identify those structures. But the essential function for staining is simply to make structures easier to see. NOTE that all stain color is artifactual and does not represent the natural color of the tissue. The same structures may have very different colors with different stains. You should generally use specific aspects of actual structure location, size, shape, texture to identify cells and tissues, rather than color. Color can offer additional information if used wisely, but is unreliable by itself. Hematoxylin is a basic stain with deep purple or blue color. Structures that are stained by basic stains are described as basophilic "base-loving". Eosin is an acidic stain with a red color. Structures stained by acid stains are described as acidophilic or eosinophilic and include collagen fibers, red blood cells, muscle filaments, mitochondria. Note that, basophilic cell structures are NOT necessarily acidic; they only happen to stain with basic stains. Likewise for acidophilic structures, which are NOT necessarily basic. Many tissue staining properties are determined by the complex chemistry of proteins and other macromolecules after interactions with fixatives and other processing agents, and defy simple analysis. Remember that nuclei are not really purple and collagen is not really pink. All such stain colors are artifacts, albeit intentional ones. Some cell structures do not stain well with aqueous dyes and so routinely appear clear. This is especially so for those which are hydrophobic, containing fat. Included in this category are adipocytes, myelin around axons, and cell membranes of the Golgi apparatus. Trichrome stain Trichrome uses three dyes hence the name , including one that is specific for the extracellular protein collagen. Depending on the particular stain combination, a trichrome stain may color collagen fibers sky-blue or bright green. The principle use for trichrome is to differentiate collagen from other eosinophilic structures, such as muscle fibers. Trichrome stains can be especially useful for highlighting an accumulation of scar tissue, as in glomerulosclerosis of the kidney see WebPath or cirrhosis of the liver. PAS Periodic acid Schiff is used for glycogen, glycoproteins such as mucus , and basement membranes which contain glycoprotein. Be aware that many other stain techniques exist, for special cases. Some of these are classical procedures can yield beautiful results but depend on mysterious art and alchemy for success. Other, more-modern techniques have been rationally

designed to exploit recent developments in molecular biology. In the "classic" category are a number of stains based on metal salts. A silver-based stain that demonstrates reticular fibers and basement membranes is especially useful for diagnosing certain pathologies of kidney glomeruli. A variety of silver stains have been very powerful for research into the central nervous tissue. Their only common feature is that silver grains form a dark precipitate on selected structures, with empirical variables determining which structures are visualized. Some cells have traditional names based on their demonstration with certain stains, such as the "argentaffin cells" cells with an affinity for silver and "chromaffin cells" cells with an affinity for chromium of the gastrointestinal tract. In the "modern" category are stains based on the application of particular molecules that can be selectively stained using radioactive labels, enzyme reactions or specific antigen binding.

Chapter 2 : The Art of Frozen Tissue Sectioning: Leica Biosystems

The Manual of Surgical pathology (2) in a section titled" Frozen Sections Are Not Permanent Sections" points to four reasons. These are: sampling error, ice crystal artifacts, lack of special studies, and lack of consultation.

Written by David Dawson Freezing dry food is a recent invention and many people discover its usefulness. What it means is that as you freeze dry your food all moisture and water content is removed from the food. This is different from traditional freezing. With this new method, the nutritional value of the food remains intact. This is exactly what you want when you go camping for days or are blocked from the world for a week or more during a disaster event. You need nutrition more than ever. Otherwise, the structure of the food changes drastically since it turns into hard crumbs. You will need warm water to un-freeze and un-dry the food so it becomes edible. There are a few steps to follow for proper freeze-drying, and we will discuss in this article. You should also know that foods with high content of water are the easiest to freeze-dry. Such foods are fruits and vegetables, e. Their structure remains intact, and only the water content is removed. Interesting enough you can freeze-dry other food like meat, pasta, oats, etc. Your imagination is your limit. When you get comfortable with the process, then you can move to other, more complicated, foods. You need to be aware about freeze-drying meat though. Meat must be freeze-dried right after cooking. This is when all its properties are intact and it will still be edible. If you freeze-dry raw meat, and then after months you decide to eat it and melt it, it will still be raw. And be sure that raw meat is hardly appetizing. Now, if dry freezing seems a bit too complicated, there is always the option of buying specially designed meal packages. We found some great options on Nomad Nutrition , an emergency food supplier for preppers all over the world. There are other ways to end up with freeze dried food. This is an easy way to have freeze dried food. During the first several hours the food will freeze. Then starts the sublimation process meaning, the drying process. This takes much longer â€" from one to several weeks. You can test if the food is ready, by taking a frozen piece from the freezer and let it melt. Another way to freeze dry food is to use dry ice CO2. This procedure is similar in concept to the above method. You can just use difference means to achieve what you want. Basically, dry ice lets all moisture evaporate from the food, leaving it freeze-dried. You will need a very large container, almost twice as the food you will freeze dry. Place the food in large plastic bags and seal them no dry ice should be able to penetrate the package. Cover the food entirely with the dry ice. Then place the container in the freezer. The container needs to have a few holes so that the gas and moisture can escape the container. This should happen with 24 hours so make sure to check regularly. A word of caution â€" when you do the above, always wear gloves and protective wear while you handle the dry ice. The best way is to keep it in plastic bags, but make sure to push the air out, since it contains moisture. This will speed up the spoiling of the food. You can use a vacuum packing machine if you want to be meticulous. First of all, freeze the food in the freezer a deep freezer would be even better and make sure that there is no other food inside â€" keep only the food you want to freeze-dry later. Let it stay for several hours. Avoid opening the freezer too often since that will slow the process, as ice crystals form very quickly when frozen air meats moist warm air. Set it to m Torr. The temperature must be 10o C 50o F. Again the process should take at a minimum 1 week, maximum a few more. It all depends on the parameters and how much moisture is in the food. When the sublimation is finished, place the food in storage bags and seal them well. Then comes the moment when we need to instruct you how to reconstitute your freeze dried food. This process is possibly the easiest of all. You need boiling water a cup or two and add it to the food. It will quickly start turning to its original fresh form, since it soaks with all the moisture you removed by following one of the above methods. A few more details and information about freeze dried food These details may be of interest to you. When you first freeze the food, it requires a temperature of about o C Then, in order to make the sublimation process happen, contrary to obvious logic, a warm temperature is needed â€" it gives energy so that this process can be started. The ice crystals need to evaporate, so a temperature of 35o C 95o F is needed for this. Also, while drying in the vacuum container, very low pressure is used. It is needed so that water almost immediately turns from one state frozen or liquid to another gas , and thus evaporates very quickly from the food. Without this process drying would take much

longer in normal conditions. Of course in the traditional method without using any fancy machines , freezing the food basically makes vacuum to be created around the water molecules. When the food is slightly warmed for drying these ice crystals quickly turn to vapor and leave the surface of the food. Another interesting fact is that water has only two conditions when put in a vacuum chamber or in any vacuum condition â€" it can be either gas or a solid. As the food is frozen vacuum created , and then moved to a slightly warmer condition, the frozen water can only evaporate as a gas. The thing is that even if the food is not kept in super low freezing temperatures, it still remains in sub-zero condition. That is the key to keeping it frozen, yet letting the water evaporate. This process is the so-called sublimation we mentioned above. The first stage is the freezing. Then the water transitions to the second vapor condition and begins evaporating. All the other methods require special chambers or dry ice in order for these two conditions to transition one from the other. As long as you know how water works, you should be able to control the process better. It is even believed that reconstituted freeze-dried food tastes and smells even better than traditionally dried food. The freeze drying process is the one that preserves all the qualities and nutrients in the food. Adding the boiling water only revives the food. The only nutrients that can be depleted somewhat are vitamins C and E, and folic acid. Freeze dried food can last for years. There are people and even markets that store food in this way so it can remain almost untouched by time and then be reconstituted when it needs to be eater or sold. This process is in fact very old and is being used by the Incas. They placed a piece of food during the night to freeze and then keep it in the hot sun for a whole day to dry completely. Freeze drying is also popular among hikers and campers since camping requires that you carry a lot of things in your backpack. Food can take a lot of space and make your backpack pretty heavy. If you add a few bottles of water and your main weight comes from food and water. So, substituting traditional food with freeze dried food is actually a great way to enjoy a week or so of trekking or camping without the burdens of heavy weight. It is also highly advisable that everyone has at least one-year of food stored for some future disasters or economic crises. Here comes the magic of freeze dried food. This makes it great for long-term storing away from moisture , since it takes very little space. If you want to know more about storing food, take a look at our article on emergency food storage. One such popular brand is Mountain House. It is particularly popular among tourists and campers, but if you need to store food you can buy in bulk from them. On the contrary, they have whole meals like lasagna, scrambled eggs, cottage cheese and almost everything you can think of. The truth is that almost any type of food can be freeze-dried. Some people even freeze dried ice cream. And to our surprise it worked. And finally, you can freeze dry meat. One very popular such food is beef jerky. You can also do the same with regular cooked beef. So, cook the meat, and then chop it to small pieces. Follow one of the above methods for freeze drying and you will end up with very small hard crumbs almost like bread crumbs.

Chapter 3 : Plant Microtechnique, a book from Oxford University Press

In order to visualize the detailed internal structure of a brain, e.g., histologically and/or microscopically, it's generally necessary to section the brain into very small slices. There are 3 major methods for this: 2 require freezing the brain--microtome and cryostat--and one does not, vibratome.

Concentration Time interval Fixation is best carried out close to neutral pH, in the range of Hypoxia of tissues lowers the pH, so there must be buffering capacity in the fixative to prevent excessive acidity. Acidity favors formation of formalin-heme pigment that appears as black, polarizable deposits in tissue. Common buffers include phosphate, bicarbonate, cacodylate, and veronal. Commercial formalin is buffered with phosphate at a pH of 7. Penetration of tissues depends upon the diffusability of each individual fixative, which is a constant. Formalin and alcohol penetrate the best, and glutaraldehyde the worst. Mercurials and others are somewhere in between. One way to get around this problem is sectioning the tissues thinly 2 to 3 mm. Penetration into a thin section will occur more rapidly than for a thick section. The volume of fixative is important. There should be a Obviously, we often get away with less than this, but may not get ideal fixation. One way to partially solve the problem is to change the fixative at intervals to avoid exhaustion of the fixative. Agitation of the specimen in the fixative will also enhance fixation. Hot formalin will fix tissues faster, and this is often the first step on an automated tissue processor. Concentration of fixative should be adjusted down to the lowest level possible, because you will expend less money for the fixative. Too high a concentration may adversely affect the tissues and produce artefact similar to excessive heat. Also very important is time interval from of removal of tissues to fixation. The faster you can get the tissue and fix it, the better. Artefact will be introduced by drying, so if tissue is left out, please keep it moist with saline. The longer you wait, the more cellular organelles will be lost and the more nuclear shrinkage and artefactual clumping will occur. Fixatives - general usage There are common usages for fixatives in the pathology laboratory based upon the nature of the fixatives, the type of tissue, and the histologic details to be demonstrated. Formalin is used for all routine surgical pathology and autopsy tissues when an H and E slide is to be produced. Formalin is the most forgiving of all fixatives when conditions are not ideal, and there is no tissue that it will harm significantly. Most clinicians and nurses can understand what formalin is and does and it smells bad enough that they are careful handling it. However, the mercury deposits must be removed dezenkerized before staining or black deposits will result in the sections. Glutaraldehyde is recommended for fixation of tissues for electron microscopy. The glutaraldehyde must be cold and buffered and not more than 3 months old. The tissue must be as fresh as possible and preferably sectioned within the glutaraldehyde at a thickness no more than 1 mm to enhance fixation. Alcohols, specifically ethanol, are used primarily for cytologic smears. Since smears are only a cell or so thick, there is no great problem from shrinkage, and since smears are not sectioned, there is no problem from induced brittleness. For fixing frozen sections, you can use just about anything--though methanol and ethanol are the best. Tissue Processing Once the tissue has been fixed, it must be processed into a form in which it can be made into thin microscopic sections. The usual way this is done is with paraffin. Tissues embedded in paraffin, which is similar in density to tissue, can be sectioned at anywhere from 3 to 10 microns, usually routinely. The technique of getting fixed tissue into paraffin is called tissue processing. The main steps in this process are dehydration and clearing. Wet fixed tissues in aqueous solutions cannot be directly infiltrated with paraffin. First, the water from the tissues must be removed by dehydration. Sometimes the first step is a mixture of formalin and alcohol. Other dehydrants can be used, but have major disadvantages. Acetone is very fast, but a fire hazard, so is safe only for small, hand-processed sets of tissues. Dioxane can be used without clearing, but has toxic fumes. The next step is called "clearing" and consists of removal of the dehydrant with a substance that will be miscible with the embedding medium paraffin. The commonest clearing agent is xylene. Toluene works well, and is more tolerant of small amounts of water left in the tissues, but is 3 times more expensive than xylene. Chloroform used to be used, but is a health hazard, and is slow. Methyl salicylate is rarely used because it is expensive, but it smells nice it is oil of wintergreen. There are newer clearing agents available for use. Many of them are based on limolene, a volatile oil found in citrus peels. Another uses

long chain aliphatic hydrocarbons Clearite. Although they represent less of a health hazard, they are less forgiving with poorly fixed, dehydrated, or sectioned tissues. Finally, the tissue is infiltrated with the embedding agent, almost always paraffin. Paraffins can be purchased that differ in melting point, for various hardnesses, depending upon the way the histotechnologist likes them and upon the climate warm vs. A product called paraplast contains added plasticizers that make the paraffin blocks easier for some technicians to cut. A vacuum can be applied inside the tissue processor to assist penetration of the embedding agent. The above processes are almost always automated for the large volumes of routine tissues processed. Automation consists of an instrument that moves the tissues around through the various agents on a preset time scale. The "technicon" tissue processor is one of the commonest and most reliable a mechanical processor with an electric motor that drives gears and cams , though no longer made. Tissues that come off the tissue processor are still in the cassettes and must be manually put into the blocks by a technician who must pick the tissues out of the cassette and pour molten paraffin over them. This "embedding" process is very important, because the tissues must be aligned, or oriented, properly in the block of paraffin. Alternatives to paraffin embedding include various plastics that allow thinner sections. Such plastics include methyl methacrylate, glycol methacrylate, araldite, and epon. Methyl methacrylate is very hard and therefore good for embedding undecalcified bone. Glycol methacrylate has the most widespread use since it is the easiest to work with. Araldite is about the same as methacrylate, but requires a more complex embedding process. Epon is routinely used for electron microscopy where very thin sections are required. Plastics require special reagents for deydration and clearing that are expensive. For this reason, and because few tissues are plastic embedded, the processing is usually done by hand. A special microtome is required for sectioning these blocks. Small blocks must be made, so the technique lends itself to small biopsies, such as bone marrow or liver. Sectioning Once the tissues have been embedded, they must be cut into sections that can be placed on a slide. This is done with a microtome. The microtome is nothing more than a knife with a mechanism for advancing a paraffin block standard distances across it. There are three important necessities for proper sectioning: MPEG movie [k] demonstrating sectioning technique with microtome. Knives are either of the standard thick metal variety or thin disposable variety like a disposable razor blade. The advantage of the disposable blade becomes apparent when sectioning a block in which is hidden a metal wire or suture. Plastic blocks methacrylate, araldite, or epon are sectioned with glass or diamond knives. A glass knife can section down to about 1 micron. Microtomes have a mechanism for advancing the block across the knife. Usually this distance can be set, for most paraffin embedded tissues at 6 to 8 microns. Sectioning tissues is a real art and takes much skill and practice. Histotechnologists are the artists of the laboratory. It is important to have a properly fixed and embedded block or much artefact can be introduced in the sectioning. Common artefacts include tearing, ripping, "venetian blinds", holes, folding, etc. Once sections are cut, they are floated on a warm water bath that helps remove wrinkles. Then they are picked up on a glass microscopic slide. Unstained section on glass slide. The glass slides are then placed in a warm oven for about 15 minutes to help the section adhere to the slide. If this heat might harm such things as antigens for immunostaining, then this step can be bypassed and glue-coated slides used instead to pick up the sections. Tray of unstained slides in drying oven. Frozen Sections At times during performance of surgical procedures, it is necessary to get a rapid diagnosis of a pathologic process. The surgeon may want to know if the margins of his resection for a malignant neoplasm are clear before closing, or an unexpected disease process may be found and require diagnosis to decide what to do next, or it may be necessary to determine if the appropriate tissue has been obtained for further workup of a disease process. This is accomplished through use of a frozen section. The piece s of tissue to be studied are snap frozen in a cold liquid or cold environment to Celsius. Freezing makes the tissue solid enough to section with a microtome. Frozen sections are performed with an instrument called a cryostat.

Chapter 4 : Freezing and refrigerated storage in fisheries - Freezers

This manual is a technical guide to methods in high pressure freezing and subsequent freeze-substitution fixation and embedding for high resolution electron microscopy and EM- immunocytochemistry.

Label base mold and partially fill the mold with frozen tissue matrix. Sacrifice animal by prescribed and approved euthanasia techniques. Remove desired tissues, trim and cut tissue no more than 5 mm thick. Place in pre-labeled base molds filled with frozen tissue matrix. Arrange tissue in the matrix near the bottom so tissue is easily exposed when sections are cut. Place a stainless steel beaker of 2-methylbutane in liquid nitrogen and allow to cool adequately. Place base mold with tissue into the beaker of cold 2-methylbutane and quickly immerse the block. If block is left in 2-methylbutane too long, the block may crack. Place the tissue block on the cryostat specimen disk. Adjust the positioning of the block to align the block with the knife blade. Cut tissue block until the desired tissue is exposed. Standard Immonuhistochemical Staining Procedure for Frozen Sections Please read entire procedure before staining sections. Perform all incubations in a humid chamber and do not allow sections to dry out. Isotype and system controls should also be run and must be matched to the isotype of each primary antibody to be tested. Label slides with a solvent resistant pen and demarcate the tissue if required. Rinse slides 3x in PBS, to remove the tissue-freezing matrix. Block endogenous peroxidase activity by incubating the slides in 0. Rinse slides 3x in PBS, 2 minutes each time. Alternatively, a buffered solution with a source of protein can be used as antibody diluent. Apply the diluted antibody to the tissue sections on the slide. Incubate for 1 hour at RT in a humidified chamber. Apply to the tissue sections on the slide and incubate for 30 minutes at RT. Apply the Streptravidin-Horseradish Peroxidase pre-diluted to the tissue sections on the slide and incubate for 30 minutes at RT. DAB is a suspect carcinogen. Wear gloves, lab coat and eye protection. Allow slides to incubate for 5 minutes or until the desired color intensity is reached. Wash 3X in water, 2 minutes each time.

Chapter 5 : How to Freeze Dry Food: Instructions And Best Methods

The preparation of cryosections does not involve the dehydration steps typical of other sectioning methods, and, furthermore, sectioning, labeling, and observation of specimens can usually be carried out in one day.

It requires experience, knowledge of clinical medicine and pathology, the capacity to make quick decisions under pressure, good judgment, an attitude that is conservative but not excessively so, and a keen awareness of the limitations of the method". Well, what are these limitations? I would like to discuss what I consider the limitations in frozen section. I will divide these into two categories. True limitations I consider insurmountable limitations by virtue of the technique and constraints placed on it by the urgency of the procedure. Avoidable limitations I consider handicaps that we are forced to deal with but can be minimized to a degree where our ability to provide the diagnostic information can potentially match that of permanent sections. In my experience the best ways to make a mistake are to be rushed or interrupted. When pushed to speed up a frozen section we should resist this pressure at all costs. If you are a well trained and skilled frozen sectionist, the time to cut and stain a slide will be only a few minutes. If you are the pathologist grossing the tissue this process can take from a few minutes to 10 minutes or more for a large complicated specimen requiring multicolor inking. For the pathologist reading the slide, this can vary from seconds to 10 or more minutes if you are searching for some minute clue, paging through books or consulting colleagues. We must be practical and consider our surgical colleagues, but on the other hand, if this frozen section was requested for the proper reason we are asked to make a decision that will alter the course of surgery. We owe it to the patient to try our best to provide a correct answer, even if it is delaying the case a few extra minutes. It is far less costly than a re-operation or providing a wrong answer. If we are swamped with a barrage of cases, complicated cases or numerous specimens, the best we can do is ask for help. If any of the multitudes of specimens is not going to influence a surgical decision, then these should be put to the end of the line or not done at all. Try not to cut corners on the grossing, preparation and reading of the slides. This is where your errors will be born. One thing experience teaches you is to recognize a situation beyond your ability. When faced with these cases I ask for whatever help is near. My advice is to tell your surgical colleagues all that you can be certain of and let them know they will have to be patient. Lets face it, in this day and age it would be malpractice try and sub classify lymphomas, sarcomas and all the things that mimic them without a few hardy trays full of immunoperoxidase stains and inspection of every twisted gene! Maybe the future will bring us more rapid studies that we can use at frozen section. As for now, this is a true limitation. Those of us in with large practices have the luxury of consultation with colleagues during working hours. The luxury of world expert consultation only an overnight express mail away is not an option. At the present telepathology systems are being used to provide remote diagnostic services to distant hospitals. What began slow limited technology is now developing into an efficient practical means of outside consultation. I predict in the future that we will see telepathology develop into a widely used tool providing immediate intra-operative pathology consultation. It is for this reason that ice floats. If it were not for this property the oceans would freeze over and we would not be here to think about it. Anyone who ever forgot a beer cooling in the freezer saw this principal in action. I believe the changes we see in tissues which are frozen are related to this expansion of water upon freezing. Like any artifacts we deal with in pathology, recognizing the artifact allows us to "read around them" so that we can make the correct interpretation. Below are phenomena which I believe are artifacts of freezing. Very edematous tissues freeze with an appearance similar to soap bubbles. The tissue in the right was never frozen and shows by comparison the edematous nature of the stroma. This is most evident in edematous tissues. The center picture shows the renal tubules being compressed by the clear ice crystals. From my observations this seems to relate to the type of tissue as well as the state of the tissue. I have noticed more of these crystals in damaged tissues. It makes sense that tissue which are damaged by cautery or ischemia would have loss of osmotic homeostasis an might therefore result in more "nuclear edema". It also seems that the more vesicular nuclei have greater tendency to show these ice crystals. I have made one very important observation: The thinner the tissue is cut the more these crystals appear as holes! The examples below clearly illustrate this

point. Lung Adenoarcinoma 6 Microns 3 Microns As I mentioned earlier some times even if our cryostat is set for 6 microns we will get "thin sections" which actually are much thinner. This will explain why sometimes these crystals are more numerous. Nuclear chromatin changes in frozen control. The chromatin is somewhat more condensed and hyper chromatic than the frozen section on the left which has been fixed rapidly. Notice the more vacuolated cytoplasm in the frozen sample, another very subtle example of freeze artifact. Frozen section Permanent section never frozen Avoidable limitations: A slide that can be fixed in a second or two in this time will show nuclear detail that rivals good cytology preparations. The exception to this will be the very vesicular nuclei which will retain its empty appearance which I consider a freezing artifact. See frozen section technique page 2 Sampling error - One of my favorite questions to my residents is" What is the most important thing we do in the frozen section room? It does not matter how perfect our slide turns out if we have sampled the wrong part of the tissue. I insist that the process of grossing in the frozen section room should be performed a meticulous systematic fashion. If I handle a breast biopsy or other solid tumor all external aspects should be observed and palpated carefully before inking. Then after inking, slicing or dissection is performed. The specimen is laid out in an orderly fashion. Then starting from the first slice, the tissue is examined with the eyes and palpated with the fingers slice by slice. Complex organs are examined by anatomic regions. One must be careful not to jump to the obvious nodule in one of the central sections because by doing this surely one day they will miss the 3 mm nodule at the edge. Another piece of advice to my residents is "When your looking at a specimen if you cannot smell the tissue you are not looking close enough. With a good gross examination the only sampling errors will be dictated by how many sections we have time to sample and cut. This greasy guy just does not freeze. But there are approaches to deal with him that can help us through most situations. I was not sure which category to place fat. By virtue of the laws of chemistry and physic this is an inaqueous material which does not freeze. But it our real purpose of freezing tissue is to harden it so that it may be cut thin. And in fact at very low temperatures this material does harden. BUt at that temperature the tissues we are interested will shatter. It is unquestionably our supreme nemesis in the cutting of frozen sections and will always be a handicap. Is the technique of frozen section to blame, or the inconsistent way it is taught? Can we blame a poorly stained slide on the stain or the person who stained it. Can we blame a wrinkles and shattered section on the technique or the technician? This brings us to the next avoidable limitation. There is inconsistency in who performs different parts of the task. Is the pathologist selecting the sections and dictating the approach to embedding? Is it a resident? I mentioned above that I believe the gross to be extremely important. Even in a small sample, failing to recognize a minute gross detail could create an error due to poor embedding. A large complex specimen needs to be systematically examined by a pathologist without question. Once embedded, the frozen should be cut and stained buy a properly trained individual. But again there is considerable variability in the training of these individuals. We may be asked to balance an icicle on the head of a pin. To accomplish this cryostats offer us a piece of steak on a tin plate and precede to squashes it with a hammer! Using conventional methods we may be asked to attempt to prepare precious minute tissues that may later disappear into a snowstorm. Up until now this is where the wizards had to resort to their magic. Through tedious and time consuming manipulations tissues are teased into various best attempts. The techniques I have offered in this web site will rapidly prepare blocks with a level of precision that surpasses paraffin embedding. I have resorted to using my limited artistic ability to demonstrate the level of facility and precision capable using these surprisingly simple techniques. Conclusion One must know what excellence looks like and sounds like in order to begin to approximate it. In all forms of art one can only achieve excellence if they are aware of all the ways to make mistakes and are aware when they are happening. I have also tried to provide the details necessary to distinguish problems as they were arising and solutions with which to approach them. These are the techniques and observations I have gained in my experience. I have no doubt that there are many experienced pathologists and histotechnologists who have a multitude of valuable observations and techniques which remain unshared. It is my hope that others will be stimulated by these writings to present their own unique methods. My intention in these writings is to offer a nidus of information for colleagues to add to or criticize.

Chapter 6 : Preparation and Staining of Frozen Tissue Sections | Protocols | BD Biosciences

Sectioning of Frozen Tissues Before cutting sections, allow the temperature of the block to equilibrate to the temperature of the cryostat (typically Â°C). Place the tissue block on the cryostat specimen disk.

History[edit] A diagram of a microtome drawn by Cummings in This allowed for the observation of samples using light microscopes in a transmission mode. One of the first devices for the preparation of such cuts was invented in by George Adams, Jr. The apparatus has enabled a precision in work by which I can achieve sections that by hand I cannot possibly create. Namely it has enabled the possibility of achieving unbroken sections of objects in the course of research. At the end of the s, the development of very thin and consistently thin samples by microtomy, together with the selective staining of important cell components or molecules allowed for the visualisation of microscope details. In most devices the cutting of the sample begins by moving the sample over the knife, where the advancement mechanism automatically moves forward such that the next cut for a chosen thickness can be made. The section thickness is controlled by an adjustment mechanism, allowing for precise control. The most common applications of microtomes are: From there the tissue can be mounted on a microscope slide, stained with appropriate aqueous dye s after prior removal of the paraffin, and examined using a light microscope. This technique is much faster than traditional histology 5 minutes vs 16 hours and is used in conjunction with medical procedures to achieve a quick diagnosis. Cryosections can also be used in immunohistochemistry as freezing tissue stops degradation of tissue faster than using a fixative and does not alter or mask its chemical composition as much. Sections are stained with an aqueous solution of an appropriate heavy metal salt and examined with a transmission electron microscope. This instrument is often called an ultramicrotome. The ultramicrotome is also used with its glass knife or an industrial grade diamond knife to cut survey sections prior to thin sectioning. These survey sections are generally 0. Thin sectioning for the TEM is often done with a gem quality diamond knife. Complementing traditional TEM techniques ultramicrotomes are increasingly found mounted inside an SEM chamber so the surface of the block face can be imaged and then removed with the microtome to uncover the next surface for imaging. These microtomes have heavier blades and cannot cut as thin as a regular microtome. For more detailed analysis of much smaller areas in a thin section, FTIR microscopy can be used for sample inspection. A recent development is the laser microtome , which cuts the target specimen with a femtosecond laser instead of a mechanical knife. This method is contact-free and does not require sample preparation techniques. The laser microtome has the ability to slice almost every tissue in its native state. Compresstome microtome[edit] A variation on the vibrating microtome is the Compresstome microtome, [12] which is designed and made by Precisionary Instruments. The device operates in the following way: The slight compression prevents shearing, uneven cutting, and vibration artifacts from forming. Note that the compression technology does not damage or affect the tissue being sectioned. There are several advantages of the Compresstome microtome: Modern sled microtomes have the sled placed upon a linear bearing, a design that allows the microtome to readily cut many coarse sections. Rotary microtome[edit] A rotary microtome of older construction This instrument is a common microtome design. This device operates with a staged rotary action such that the actual cutting is part of the rotary motion. In a rotary microtome, the knife is typically fixed in a horizontal position. Through the motion of the sample holder, the sample is cut by the knife position 1 to position 2, at which point the fresh section remains on the knife. At the highest point of the rotary motion, the sample holder is advanced by the same thickness as the section that is to be made, allowing the next section to be made. The flywheel in many microtomes can be operated by hand. This has the advantage that a clean cut can be made, as the relatively large mass of the flywheel prevents the sample from being stopped during the sample cut. The flywheel in newer models is often integrated inside the microtome casing. For hard materials, such as a sample embedded in a synthetic resin, this design of microtome can allow good "semi-thin" sections with a thickness of as low as 0. Cryomicrotome[edit] A cryomicrotome For the cutting of frozen samples, many rotary microtomes can be adapted to cut in a liquid-nitrogen chamber, in a so-called cryomicrotome setup. The reduced temperature allows the hardness of the sample to be increased, such as by undergoing a glass

transition, which allows the preparation of semi-thin samples. Ultramicrotome[edit] A ribbon of ultrathin sections prepared by room-temperature ultramicrotomy, floating on water in the boat of a diamond knife used to cut the sections. The knife blade is the edge at the upper end of the trough of water. An ultramicrotome is a main tool of ultramicrotomy. It allows the preparation of extremely thin sections, with the device functioning in the same manner as a rotational microtome, but with very tight tolerances on the mechanical construction. As a result of the careful mechanical construction, the linear thermal expansion of the mounting is used to provide very fine control of the thickness. Diamond knives preferably and glass knives are used with ultramicrotomes. To collect the sections, they are floated on top of a liquid as they are cut and are carefully picked up onto grids suitable for TEM specimen viewing. The thickness of the section can be estimated by the thin-film interference colors of reflected light that are seen as a result of the extremely low sample thickness. The vibrating microtome is usually used for difficult biological samples. The microtome of this type has a recessed rotating saw, which slices through the sample. Laser microtome A conceptual diagram of laser microtome operation The laser microtome is an instrument for contact-free slicing. Alternately this design of microtome can also be used for very hard materials, such as bones or teeth, as well as some ceramics. The device operates using a cutting action of an infrared laser. As the laser emits a radiation in the near infrared, in this wavelength regime the laser can interact with biological materials. Through the non-linear interaction of the optical penetration in the focal region a material separation in a process known as photo-disruption is introduced. By limiting the laser pulse durations to the femtoseconds range, the energy expended at the target region is precisely controlled, thereby limiting the interaction zone of the cut to under a micrometre. External to this zone the ultra-short beam application time introduces minimal to no thermal damage to the remainder of the sample. The laser radiation is directed onto a fast scanning mirror-based optical system, which allows three-dimensional positioning of the beam crossover, whilst allowing beam traversal to the desired region of interest. The combination of high power with a high raster rate allows the scanner to cut large areas of sample in a short time. In the laser microtome the laser-microdissection of internal areas in tissues, cellular structures, and other types of small features is also possible. Microtome knives[edit] A diamond knife blade used for cutting ultrathin sections typically 70 to nm for transmission electron microscopy. The selection of microtome knife blade profile depends upon the material and preparation of the samples, as well as the final sample requirements e. Knife design and cut types[edit] Profiles of microtome knives. Generally, knives are characterized by the profile of the knife blade, which falls under the categories of planar concave, wedge shaped or chisel shaped designs. Planar concave microtome knives are extremely sharp, but are also very delicate and are therefore only used with very soft samples. Finally, the chisel profile with its blunt edge, raises the stability of the knife, whilst requiring significantly more force to achieve the cut. For ultramicrotomes, glass and diamond knives are required, the cut breadth of the blade is therefore on the order of a few millimetres and is therefore significantly smaller than for classical microtome knives. Glass knives are usually manufactured by the fracture of glass bars using special "knife-maker" fracturing devices. Glass knives may be used for initial sample preparations even where diamond knives may be used for final sectioning. Glass knives usually have small troughs, made with plastic tape, which are filled with water to allow the sample to float for later collection. Sectioning[edit] Prior to cutting by microtome, biological materials are usually placed in a more rigid fixative, in a process known as embedding. This is achieved by the inflow of a liquid substance around the sample, such as paraffin wax or epoxy, which is placed in a mold and later hardened to produce a "block" which is readily cut. The declination is the angle of contact between the sample vertical and knife blade. If the knife is tilted, however, the relative motion of the knife is increasingly parallel to sample motion, allowing for a slicing action. This behaviour is very important for large or hard samples The inclination of the knife is the angle between the knife face and the sample. For an optimal result, this angle must be chosen appropriately. The optimal angle depends upon the knife geometry, the cut speed and many other parameters. If the angle is adjusted to zero, the knife cut can often become erratic, and a new location of the knife must be used to smooth this out. If the angle is too large, the sample can crumple and the knife can induce periodic thickness variations in the cut. By further increasing the angle such that it is too large one can damage the knife blade itself.

Chapter 7 : Histopathology: Difference between a Microtome and a Cryostat

The system provides distinct advantages over available methods for embedding tissue for frozen section, including speed, precision, reduced tissue wastage, ease of learning, and convenience. The speed in which the frozen sectioning is accomplished is improved on several levels.

Every 1 h 1 In both examples in Table 5, the freezer is correctly loaded since the product load matches the plant capacity in the weight of fish that can be frozen in 1 h. The above freezer would therefore be designed to hold 2 t of product A and when product B is frozen, only 1 t will be loaded and the product distributed to give uniform air flow. If however, 2 t of product B are loaded into the freezer at one time, the refrigeration plant will be overloaded. This is probably one of the most difficult aspects of freezer operation to explain clearly but in simple terms it means no matter how spacious your freezer and how much product can be loaded, you cannot freeze more fish than the refrigeration plant will allow. Good performance in batch air blast freezers is obtained by freezing the product in open trays without wrapping. Trays used in air blast freezers should transfer heat readily, be easily emptied and also be robust. Normally they are required to produce a pack that is of regular shape but when the product allows their use, trays with a taper on the sides of about one in eight can be emptied by applying a cold water spray on the underside for a few seconds and then giving a gentle tap on the edge. Trays used in this manner should never be filled above the tray edge or the product will be damaged during release. Cleaning and drying of trays before re-use is necessary to maintain a high standard of hygiene. Where the rate of production justifies the cost, an automatic tray washer may be installed. The reader will no doubt find other types of freezer available on the market which have not been mentioned. The design of many of these is based on combinations of two or more of the basic methods described. For instance, a variety of freezers make use of both contact and air blast freezing techniques. Other freezers may be identical in every respect with one of the methods described, but may use some other liquid, gas or contact method for heat transfer. These freezers will be seen to be similar to one of the types described and will therefore have the same advantages and disadvantages. Plate freezers do not have the versatility of air blast freezers and can only be used to freeze regularly shaped blocks and packages. Plate freezers can be arranged with the plates horizontal to form a series of shelves and, as the arrangement suggests, they are called horizontal plate freezers HPF Figure 2 1. When the plates are arranged in a vertical plane they form a series of bins and in this form they are called vertical plate freezers VPF Figure Modern plate freezers have their plates constructed from extruded sections of aluminium alloy arranged in such a manner as to allow the refrigerant to flow through the plate and thus provide heat transfer surfaces on both sides Figure Plate freezers are fitted with hydraulic systems which move the plates together and apart. The two main uses for this type of freezer are the freezing of prepacked cartons of fish and fish products for retail sale and the formation of homogeneous rectangular blocks of fish fillets, called laminated blocks, for the preparation of fish portions. The thickness of package or block frozen is 32 to mm and the freezer can readily adapt from the thicker to the thinner package provided the range required is made known to the supplier at the time of purchase. There is no direct contact between the fish and the freezer plates when freezing by this method since the fish is always packaged before freezing. If the operator is also careful not to spill water on the plates during loading and unloading, the freezer may be operated with only a light brush between each freeze to remove surface frost. The door may be left open overnight to allow the plates to defrost fully after being hosed down with warm water. A hot gas defrost arrangement is the quickest method to defrost an HPF, but even with this method, it may take 30 min or more. The defrosted plates must be completely free from frost or ice and dried before the freezer is used again. Horizontal plate freezers intended to be operated with a hot gas defrost are fitted with additional pipework which allow the cold refrigerant to be discharged from the bottom of the freezer as the defrost proceeds. Without this special pipework and operating valves, a hot defrost would clear the top plates only and leave the cold refrigerant in the plates at the lower levels. As in all hot gas defrost systems, the refrigeration system must have an adequate load to provide sufficient hot gas for an effective defrost. This system would therefore be better applied when there are two or more freezers operated from a common refrigeration system and each

freezer will then be defrosted in turn while the others are in operation. An HPF will only operate correctly if good contact is made on both the top and bottom surfaces of the pack or tray to be frozen. The faults shown in Figure 24 are some of those which make freezing times longer than necessary. If the product is frozen from one side only due to poor contact on the upper surface, the freezing time could be three or four times as long as the time achieved with good contact on upper and lower surfaces. The plates of the HFP are closed by means of a hydraulically operated piston to make contact with the upper surface of the product. The plate pressure applied to the product can easily be varied between 70 x mbar to suit the product and is increased by a factor of two as the fish expands during freezing. The main advantage of this type of freezer is that fish can be frozen in bulk without the requirement to package or arrange on trays. The plates form what is in effect a bin with an open top and fish are loaded directly into this space. This type of freezer is therefore particularly suitable for bulk freezing and it has also been extensively used for freezing whole fish at sea. The maximum size of block made by this method is usually 1 mm x mm. Other dimensions however, can be produced in which the thickness can vary from 25 to mm, but will depend on the fish to be frozen. The maximum weight and dimensions are also limited by the physical effort required from the operator to lift the block, and by the ease with which it can be handled so that damage to the fish is kept to a minimum. In most cases, fish can be loaded between the plates without wrappers and water need not be added either to strengthen the frozen block or improve the contact with the plates. With fatty fish such as herring, it has been found advantageous to use wrappers and add some water to fill the voids in the block. Fatty fish do not form blocks which are as firm and strong as blocks made from lean fish especially during seasons when the oil content of the fish is high. Water added helps to strengthen the block, protects the fish during subsequent handling and reduces the effects of dehydration and oxidation during cold storage. Well formed, rigid blocks are particularly important when freezing at sea. The product may be handled under particularly adverse operating conditions and poorly formed blocks, prone to breakage, would result in a high percentage of loose fish. Machine filleting or splitting of the fish for instance, may be difficult if fins and tails are broken. Wrappers have been used when freezing fatty fish in VPFs to protect the exposed fish on the outside of the block. A wrapper that has been found suitable for this purpose is a single layer paper bag, coated internally with polyethylene, and shaped to fit the space between the freezer plates. Wrappers made from polyethylene with a specially roughened outer surface to reduce slippage have also been used. Fish frozen in wrappers require a longer freezing time due to the insulating properties of the wrapping material. Some types of wrapper would have a considerable effect on freezing time but in sea trials the material described did not increase the freezing time by a significant amount. Vertical plate freezers are defrosted to release the blocks of fish after each freeze. Fish are in direct contact with the plates and the force required to release the blocks without a defrost could be excessive and result in plate damage. The defrost time need not exceed 3 or 4 min if a suitable supply of defrost gas or hot liquid is available. If a primary refrigerant is used in the plates, a hot gas defrost is generally used. Where there is a multiple installation, the freezers are defrosted in turn with the other units in operation providing the necessary refrigeration load for the compressor. When a secondary refrigerant is used, a reservoir of hot liquid has to be maintained and pumped through the plates to displace the cold liquid present. With this arrangement, it is possible to return the bulk of the cold liquid to the low temperature reservoir at the start of defrost, and also return the warm defrost liquid to the hot liquid reservoir for reheating at the start of the next freeze. This arrangement reduces the quantity of liquid interchanged at each defrost but provision must be made to maintain the liquid charges in both the cold and hot systems at the correct level. Defrost arrangements such as those described lead to more complicated and expensive refrigeration pipework. Attempts have been made to assist the release of the blocks by coating the plates with a low friction plastic material so that a defrost was unnecessary. Freezing times are longer due to the poor contact being made with the plates and because of the lower block density, more storage space is required for a given quantity of fish. The results of some tests that clearly show this difference in loading fish between warm plates and plates at refrigerated temperatures are given in Table 6. The first two results in the table were obtained when the fish were loaded between defrosted plates. The last results, which gave low density blocks and longer freezing times, were obtained when fish were loaded between cold plates.

Chapter 8 : Microtome - Wikipedia

STEEL PARTS SECTIONING GUIDELINES. Replacement of steel parts at factory seams and matching the replacement part configuration remain the preferred repair methods.

Facebook Twitter LinkedIn This article describes a system used for embedding of tissues for the preparation of frozen sections. This novel system uses simple techniques and apparatus to accomplish face-down embedding in freezing-temperature steel wells. The system is easy to learn and offers many advantages over conventional methods, including speed, high precision and predictability, and reduced tissue wastage. Show MoreShow Less Introduction As a practicing pathologist, I can think of no more intimidating task than preparing and interpreting the frozen section. This formidable task is a sharp taste of reality. This sense of wariness remains with a pathologist throughout his or her career. To provide the necessary answers to the questions put before us in the frozen section room, there are a number of steps that must be performed without flaw. These include gross examination and sampling of the tissue, accurate embedding of the tissue, cutting and staining high-quality slides, and finally interpreting the slide s. At each of these steps, a poor preparation can yield disastrous results. This article will concern itself with the process of embedding tissue for frozen section. Although embedding some cases is a simple process where orientation is not critical, there are many specimens where our ability to provide an answer relies on perfect orientation of the specimen within the block. In conventional cryostats, tissue is embedded for frozen section by placing it face up on a tissue holder and covered with an embedding medium. The tissue holder or "chuck" is then set upon a freezing temperature bar. A heat sink is applied to the top of the tissue at the proper moment to speed the freezing process and flatten the surface of the tissue to create a flat plane. There are many shortcomings to this system, which can lead to considerable frustration. The system works adequately in situations where a large volume of tissue is available and precise orientation is not an issue. A sizable sample can be embedded and simply trimmed to a level where the operator is satisfied with the tissue face. Unfortunately, current methods are inadequate in a significant percentage of cases where high precision and predictability of the prepared tissue face is essential. The result can be substandard preparations requiring significant trimming and attrition of tissue sample. Additional distortion can result from crushing of the tissue by the weighted heat extractor. This method of tissue embedding, at best, prepares rough approximation of what is possible with paraffin embedding. The problem is magnified when confronted with minute samples and thin cores. Further difficulties arise in situations where flimsy thin and difficult to handle samples need to be embedded in precise orientation. Standing these samples in a puddle of freezing embedding medium and teasing each into place while bent over the cryostat is an uncomfortable situation on many levels. Seasoned histotechnologists have devised a variety of techniques and makeshift apparatus in attempts to achieve optimum results. One such technique is to use pre-frozen chucks coated with a flattened layer of embedding medium. This affords the tissue a flat surface on which to stand rather than the irregular grids and furrows designed to grip the frozen tissue and medium that form the face of chucks. Others will flatten tissues against a frozen surface before embedding it face up on a chuck. Histotechnologists in the area of Mohs surgery will use a technique of incising the specimen in such a way as to relax the skin so that it can be more easily flattened. The frozen section process is very demanding by virtue of the ever-present pressure to provide a fast result. The stress is increased when multiple specimens are delivered by a number of surgeons simultaneously. Using the conventional system in modern cryostats it can take 90 s or more to freeze a block, during which we must monitor the freezing for the proper time to place the sole heat extractor over the freezing block. To speed the process some institutions have turned to alternate means of rapidly freezing tissues, such as immersing the tissue into liquid nitrogen or other super-cooled liquids. This rapid process affords less freezing artifact but is limited in precision. A handful of innovative minds have recognized the limitations and frustration with conventional methods and have marketed alternative means of embedding tissue for frozen section. These have included devices ranging from simple flattening devices to complex and elaborate instruments with the hope of providing the user with a more articulate means of achieving the proper orientation of tissues. So far, these products have not made their

way into the hearts of the major cryostat manufacturers. This article describes a system consisting of a simple apparatus and techniques used to embed tissue for frozen section. The system embeds tissue face down using freezing temperature wells machined into the surface of steel bars. Although similar to paraffin embedding, the system has a distinct advantage. This advantage is the physical property that causes tissue to stick to freezing steel. This property facilitates the setting of tissue into wells and is easier than standing tissue in solidifying paraffin. The advantages that will be outlined in this article include precision, predictability, speed, and reduced tissue wastage, ease of training, comfort, and convenience. The apparatus consists of a number of component parts described below. Embedding Well Bars Embedding is performed in wells machined into the face of one-inch thick stainless-steel bars Figure 1A. When cooled to cryostat temperature, these rather substantial bars provide a powerful heat sink for rapid freezing. Wells are machined with beveled walls, rounded edges, and polished surfaces for ease of release. In its current form, the author uses square wells with rounded corners of diameters 18, 24, and 30 mm across the base. Wells of varying sizes and depths can be made to fit different situations. Well bars are stored at the deepest convenient point in the cryostat where temperatures are lowest and brought to higher or more accessible location during the embedding process. In many cryostats a small shelf can be installed for this purpose. The width and depth of the channels allow for complete penetration of embedding medium when used cold and can therefore be stored at freezing temperatures to facilitate rapid freezing. The crossing grid pattern allows for extrusion of excess embedding medium so that chucks can be pressed flat to the well bar face. The chucks are made of stainless steel, maximizing their freezing power and durability and are stored in a bin at a convenient location low in the cryostat. Chucks freeze optimally when used cold but can also be used warm with use of the over-chuck freezing block described below. Stemmed chucks are most suitable for this process. The stem is the focal point to apply the necessary sharp tap resulting in an easy release of the block from the well. These chucks fit many of the major brand cryostats and can be used in most cryostats with use of an adaptor. Over-Chuck Freezing Blocks The over-chuck freezing blocks constructed of rectangular steel function as a heat extractor Figure 1C. They are designed to fit over the stem of the chuck. The freezing block also served as a dislodging tool. A light tap of the chuck stem cleaves the plane of adhesion holding the formed block to the well. These blocks also serve as a convenient flat freezing surface useful in plastering technique discussed below. Dispensing Slides The thin transparent dispensing slide is made of vinyl and serves to precisely orient the tissue into the desired position and as a means to accurately transfer tissue to the embedding well floor Figure 1D. The tissue is applied to the end of the transparent slide face down where it can be visualized from below and manipulated into position. The face that is visible will be laid onto the well floor and will ultimately be the embedded surface to be sectioned. The shelves are made in various sizes to accommodate most instruments. The shelf pictured accommodates three well bars capable of embedding 12 blocks. Well bars can be used on a convenient flat surface such as a brush holder in cryostats that are not compatible with an embedding shelf. Place the tissue to be embedded face down at the end of the slide Figure 2B. View the tissue through the transparent slide. Make any adjustments to the tissue to assure exact positioning of desired tissue face Figure 2C. Placing Tissue in the Embedding Well Tissue is pulled to slightly overlap the dispensing slide by about 1 mm. The overhanging edge of tissue is touched to the desired location in the well floor where it adheres to cold steel Figure 2D. The dispensing slide is pulled out from under the tissue, which falls and adheres to the well floor. Tissues requiring precise orientation can be manipulated into exact position as the dispensing slide is slowly pulled away. Filling the Well with Embedding Medium Fill the well with embedding medium to its maximum capacity so that a meniscus bulges above the level of the well bar surface Figure 2E. Any excess medium will be extruded through the channels in the chuck. This is a mandatory step when using a warm chuck or using the large 30 mm well. Optimal freezing conditions are as follows: Removal of the Block A sharp tap of the chuck stem with the over-chuck freezing block easily frees the prepared block Figure 2H and 2I. The width of the tip is chosen based on the tissue size and the well size in which the tissue is being placed. Looking through the slide offers a view of the tissue face that will be placed down on the well floor. While looking through the back of the slide, tissue can be adjusted into the precise orientation desired. This may be such that the epidermis or margin is visible or to place a tissue on edge. Then as the tissue leaves the slide, it is

guided to adhere in the desired position. A thin coat of embedding medium assures a clean easy release of the block and assures adhesion of tissue to the well floor. This layer of medium also provides a support for the tissue, aiding in manipulation on the dispensing slide and providing adhesion to the slide during transport. If excess embedding medium is used there will be a thicker layer of frozen medium than desired over the embedded tissue. This is an issue when extreme flatness is required such as when embedding multiple minute samples or needle biopsies flat. Figure 3 demonstrates an artistic example requiring extremely flat embedding. The samples in this case are colored sesame seeds. This could not have been accomplished without use of a very thin layer of embedding medium to adhere the sesame seeds to the well floor. If there is no risk of cross contamination, several samples of tissue can be placed on a single slide and pulled to the end for placement in one or more wells. For example in sampling a large tumor, four 1 cm squares can be placed in a 30 mm well. This allows a rather large amount to tissue to be sampled from a single sectioned block. If one were looking at separate specimens such as lymph nodes from four different sites; however, separate dispensing slides must be used to avoid cross contamination. Placing Tissue in the Well Orientation and location of the tissue in the well should be considered before placing the tissue. For example, if a 2 cm length of tissue is placed in a 30 mm well, it should be touched down beginning near the edge rather than in the middle.

Chapter 9 : SIU SOM Histology INTRO

Cryostat sectioning is a popular but labor-intensive method for preparing histological brain sections. We have developed a modification of the commercially available CryoJane tape collection method that significantly improves the ease of collection and the final quality of the tissue sections. The.

The pilot and feasibility program is intended to provide seed support for new and innovative research projects directed at basic, biochemical, translational, clinical, and community-based research questions that broadly pertain to diabetes, diabetes complications, and cardiometabolic disease. Pilot projects should be designed to generate sufficient data as the basis for further extramural funding e. In fact, in order to respond to NIH criteria for competitive renewal of the DRC, a key priority for your application should be to focus on subsequent extramural or R01 grant funding. Thus, your application will be reviewed with this in mind so please provide a short paragraph indicating how the award will facilitate R01 application development and the submission of a proposal. We will entertain 2 year projects if justification for the need is made in order to effectively develop an R Funding will be available to full time UAB faculty members who must meet one of the criteria listed below. Please note that applications from post-doctoral fellows will be considered if there is a clear plan and written documentation for transition to a faculty position at UAB before the initiation date of the pilot grant award. Established investigators with no previous work in diabetes who wish to apply their expertise to a problem in this area. Established investigators whose basic research project is related to the interplay between diabetes and other chronic diseases are encouraged to apply to the specific UCDC seed funding RFA www. Applications will be selected for funding based on merit using NIH study section criteria. Projects will be favored if consistent with the mission of the DRC http: Indeed, a cogent grant writing plan should be noted in the application and will be considered as a component of the priority score. More information can be found at the DRC website http: Application Procedure for Pilot Grant: The competitive process for these proposals is two-tiered: The project description should not exceed one page. The applications for first level review will be due Monday November 26, Please send these applications electronically to Ms. Jelisa Moseley jelisam uab. This application will use the PHS forms, an example of which can be found on our website http: The applications must include: If the budget allocations do not match the scope of the work, this will be considered as unfavorable in the review process. Include development plan for NIH grant application based on this work or other major national funding program. A list of three possible outside reviewers for your proposal. Applications for second level review will be due Friday February 15, Funding for those selected is anticipated to begin June 1, Acceptance of the pilot award will require the PI to adhere to a plan for submitting grants to extramural funding agencies, to present a research seminar at or near the time of study completion, and to report research progress to Dr. If you have questions concerning eligibility or the submission of these proposals, please contact Ms. Stuart Frank sjfrank uab.