

**INSTRUCTIONAL MANUAL  
CAT. 62800-10, 62800-20  
CryoJane Tape-Transfer System**



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## CryoJane Tape-Transfer System: Instructions

Required for installation are the following: Absolute alcohol, 1600W hair dryer, gauze, gloves

1: Remove release paper from ECU



2: Place ECU on the top left corner of the cryostat



3: Clean the chamber wall with absolute alcohol and gauze



4: Heat the left wall for Mech & Oil Bath placement



5: Place Oil Bath towards the rear of the left chamber wall



6: Route Oil Bath & Mech Cables underneath Mech



\*7: Place the Mech low in the chamber and near the front wall (placement near the freeze bar is ideal in most cryostats)



8: Route cables in door bar



9: Place the door bar with cables onto the front chamber ledge. If required, attach the Door Bar Support Strip underneath the door bar.



10: Adhere the cable holders along the top outer wall of the cryostat



11: ECU rear with cable and power connectors



12: Connect the Mech and oil bath cables to the ECU and the power cord to the ECU wall outlet. Turn on the ECU.



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13: Mech cable connected to ECU



14: External installation complete



15: Internal installation complete



## Freezing process

In the general freezing application, the tissue is typically frozen at about  $-30^{\circ}\text{C}$ . It is throughout this slow, gradual process that the ice crystals that form are usually large compared to cellular dimensions. It is important to note that these crystals can cause substantial damage to the tissue.

This device is designed to snap-freeze the tissue in approximately 8 - 10 seconds. The freezing agent can be liquid nitrogen, LN2 chilled isopentane, dry ice or a thermoelectric cooler (Peltier device). Tissues should be frozen at  $-60^{\circ}\text{C}$  or colder.

## CryoJane process

The CryoJane process is centered on three particular methodologies:

1. The capture of an undistorted, thin, snap-frozen section on a special cold adhesive tape.
2. The lamination of the captured section onto a cold glass microscope slide coated with an ultraviolet light curable pressure sensitive adhesive.
3. The curing of the adhesive on the slide with an 8 millisecond ultraviolet flash, and the subsequent removal of the adhesive tape, leaving a still frozen section firmly adhered to the microscope slide.

The CryoJane system has been built to welcome the application of these methodologies, and can be adapted to a variety of cryostat models.

## CryoJane components

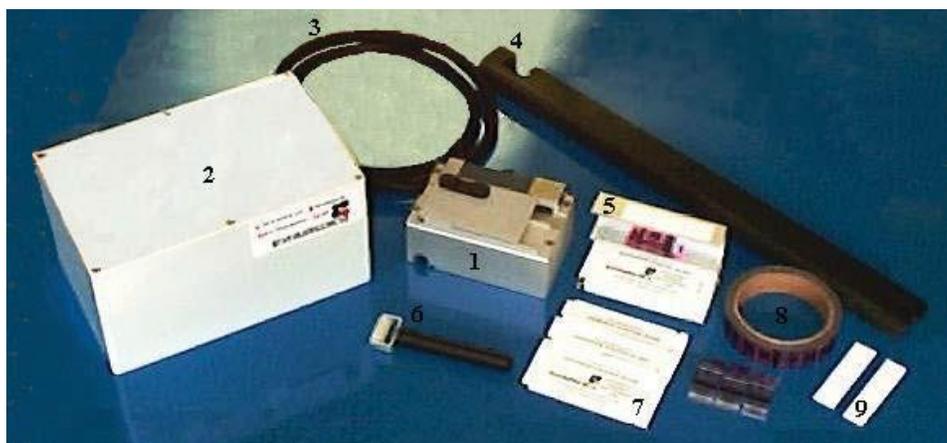


Figure 1

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1. Mechanism (Mech). See CRYOJANE MECH COMPONENTS for details.
2. Electronics Control Unit (ECU). See CRYOJANE ECU for details.
3. ½ inch diameter Mech Cable (connected to the Mech)
4. Door Channel
5. 3 Shelf Unit
6. Hand Roller
7. Adhesive Coated Slides
8. Tape Windows
9. Knife Facet Wipers
10. AC Power Cord (not shown)

## CryoJane ECU



Figure 2. CryoJane ECU (back)

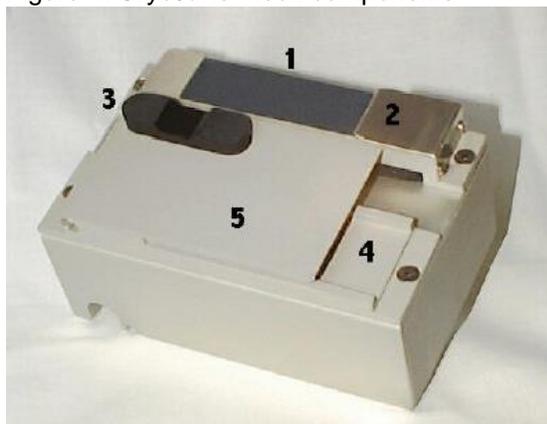


Figure 3. CryoJane ECU (front and right side)

1. ON/OFF switch
2. Pad LED
3. Power LED
4. Flash/Pad connector
5. Optional oil bath connector
6. Fuses
7. AC power cord connector

## CryoJane Mech components

Figure 4. CryoJane Mech components



1. Blue pad
2. Stainless steel spring clip
3. Black knob
4. Flash tray
5. Lid

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## Instructions for using the CryoJane

### Overview

In general applications involving sectioning, the user typically guides the cut section over the knife using materials such as a brush or an anti-roll mechanism. If a 2 to 4 micron thick section is desired, please note that even with the utmost attempt at precision and perfection, this step is rather difficult.

In order to mount the desired section, a room-temperature slide is touched to it. As soon as contact is made between the warm slide and the frozen section, the section will immediately melt, which will then convert the ice crystals back into water. Adhesion of the melted section to the slide is caused by surface tension. When the ice melts, water fills the spaces that were once filled with the ice crystals, rehydrating the nucleoplasm and cytoplasm, which causes erythrocytes to lyse and soluble substances to be displaced. Furthermore, flow of the melted solutions can distort and displace fine structure. At this point, the melted section is dried and surface tension forces continual distort, displace, and collapse tissue structure. Clinicians, pathologists, and other professionals alike have the skills necessary to detect substances that are normal and abnormal against this background of tarnished fine structure.

On the other hand, the CryoJane process is made to avoid air drying and melting. It utilizes a cold adhesive tape in order to provide support for and capture the section during the cutting step of this process. The frozen section on the tape is then transferred to a different cold adhesive-coated slide. Please note that in the mounting step, this section does not melt. The adhesive coating on the slide is then polymerized, and the tape is peeled off, which leaves the section permanently adhered to the slide will still frozen.

### Preparing for application

- Have a day's worth of adhesive coated slides and tape windows in the cryostat, already chilled and ready for use
- Cryostat temperature should be  $-25^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  (the model of your cryostat will determine the appropriate temperature)
- The Pad light (left LED) on the ECU should be lit green
- The right LED should display red if it is labeled as POWER, and green if it is labeled as OIL BATH

### Use

As soon as the tissue specimen has been frozen and then mounted in the microtome chuck, follow the steps below. Please refer to figures 5-22 on page 8.

1. If the Cryo-Vac-Away is installed, position the collection nozzle at the knife edge and step on the foot switch, immediately removing any debris during the trimming sequence. On the other hand, if the Cryo-Vac-Away is not installed, trim the block to the depth required in your application in order to get a full block face. Next, remove any visible debris from the knife. Be sure to set the micrometer to the appropriate section thickness and cut at least two to three sections in order to reassure a full cut section. See figure 5.
2. While holding a cool, pouched adhesive coated slide inside the cryostat chamber, remove the glass from the pouch. See figure 6.
3. With the frosted end held, quickly peel off the protective Mylar film, which covers the adhesive. Dispose of it. See figure 7.

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4. On the Blue Pad of the Mech, place the coated slide (adhesive-side-up) by sliding the frosted end of the slide underneath the stainless steel spring clip. See figure 8.
5. While inside the cryostat, pick up a pink tape and peel back the protective film that is covering the adhesive window. You may throw out the protective film once it is removed. See figure 9.
6. Hold the tape up by the narrow tab with the adhesive side towards the block face. Position the tape in front of the trimmed block so that the bottom edge of the exposed adhesive is aligned with the bottom edge of the block. Place the tape on the block face making sure that the bottom wider tab of the tape overhangs the knife. See figure 10.

**NOTE:** No part or section of the adhesive window should overhang the block face. To prevent the surface of the tissue from melting, do not touch the section of the tape that covers the block face.

7. Laminate the tape to the face of the block by moving the roller up and down, at least one to two times, and then left to right, being sure to cover the whole surface. This can be done with the use of the cold hand roller. For best adhesion results, apply light pressure. Once finished, place the hand roller back inside the cryostat, back to its original position. See figure 11.
8. Turn the flywheel slowly and evenly in order to cut a section. If the operator chooses to use a sharp, precise knife, then the use of a brush or anti-roll device is not needed, as the section captured on the tape will be flat, intact, and as thin as two microns. See figure 12.
9. Pick up the tape at either tab and take it to the slide on the blue pad. Make sure that the tape is kept down inside in the cryostat – this will avoid any melting that could occur. See figure 13.
10. Position the tape with the section side onto the adhesive coated slide. If referring to multiple sections, go to “Steps for multiple sections” in this manual. See figure 14.
11. Using the cold hand roller, laminate the tape to the slide. Then, evenly apply pressure to the section under the tape with the roller, at least two or three times. See figure 15.
12. Remove the slide from the blue pad by holding the frosted end, and take it to the flash tray. Carefully ease the slide in as far as it will go against the stop. Be sure to close the lid before actuating the flash. See figure 16.
13. Push and release the black knob to trigger the UV flash. The purpose of the UV is to cure the adhesive on the slide into a polymer. Doing so adheres the frozen section to the slide. See figure 17.
14. With the frosted end in hand, immediately remove the cured slide from the flash tray. See figure 18.

**NOTE:** To avoid the section melting, do not take the slide out of the cryostat during this time.

15. Making sure that the slide is completely, all the way inside the cryostat chamber, remove the pink tape. Doing so will reveal the frozen section on the slide. See figure 19.
16. In a diagonal, downward fashion, peel off the tape. This will minimize the tension on the section as well as insure a successful transfer. Be sure not to touch the section with your fingers as this could hinder results. Discard the tape after removing. See figure 20.

Once this step has been completed, the slide can now:

- Be fixed in the aqueous fixative
- Be freeze-dried in the cryostat before anhydrous fixation

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- Be melted or air-dried and used with the fixative of your choosing

**NOTE:** At this point the section should be immersed in the fixative inside the cryostat. When following through with fixation, room temperature should be maintained.

17. You can place multiple sections on the slide by placing the tapes perpendicular to the slide. When handling small tissue specimens, the tape can be cut into strips that are narrower. Follow steps 5-9.
18. Lift the lid of the mech and seat the slide on the flash tray in order to cure the adhesive on the slide. The tape tabs at this point will hang over the flash tray. See figure 21.
19. Close the lid of the cryostat and begin to push and release the black knob – doing so will trigger the UV flash. Next, lift the lid, remove the slide, and carefully peel each strip away as explained in steps 15 and 16. See figure 22.

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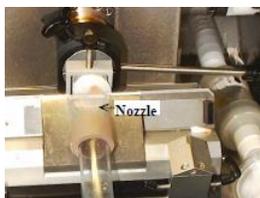


Figure 5. Block trimming and debris removal.



Figure 6. Adhesive coated slide in pouch.



Figure 7. Peeling mylar from adhesive coated slide.



Figure 8. Adhesive coated slide on blue pad.



Figure 9. Exposing the tape window.



Figure 10. Placing tape on block face.

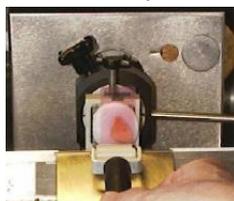


Figure 11. Laminating the tape to the block face.



Figure 12. Cutting the section.



Figure 13. Handling the cut section.



Figure 14. Positioning the tape on the slide.



Figure 15. Laminating the section to the slide.



Figure 16. Placing the slide into the flash tray.



Figure 17. Curing the adhesive on the slide.



Figure 18. Removing the cured slide.



Figure 19. Removing tape from cured slide.



Figure 20. Technique for removing tape from slide.



Figure 21. Processing multiple sections.

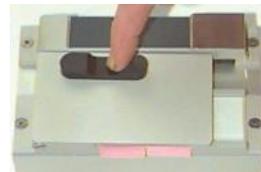


Figure 22. Curing multiple sections.

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## Summary of the frozen sectioning process

**NOTE:** Figures 23 – 27 can be found on page 10.

### Snap-freezing and embedding

A block holder is placed on the Gentle Jane snap-freezing device. CryoGel or other embedding material alike is dispensed onto the block holder and a tissue specimen is set on top. The chilled heat extractor is placed in its holder and then released. When the heat extractor comes in contact with the specimen, the tissue and the embedding medium is snap-frozen into a flat block. The plane of the flat block face serves to reduce the need to trim. When the shape of the specimen is vital, the tissue is snap-frozen using the CryoGel/Rubber Mold method. See figure 23.

### Cutting

After the block has been trimmed, a cold adhesive tape is adhered to the block face. This tape serves to support and capture the section while it is cut. This adhesion eliminates the need for an anti-roll device. See figure 24.

### Transfer to slide

A cold adhesive coated slide is placed on a temperature control pad. The adhesive tape is placed section-side-down on the adhesive-coated slide, and is laminated to the adhesive layer. This can be done with the use of a cold roller. See figure 25.

### Cutting the adhesive coating

A flash of UV light passes through the slide in order to polymerize the adhesive layer on the slide into a hard, solvent-resistant plastic, tightly adhering the section to the slide. See figure 26.

### Removal of tape

The tape is peeled away, which leaves the (still) frozen section tightly bonded to the plastic layer. See figure 27. The slide can now:

- Be fixed in the aqueous fixative
- Be freeze-dried in the cryostat before anhydrous fixation
- Be melted or air-dried and fixed with the fixative of appropriate for your application

## Special fixation protocols

### Aqueous Fixative

**NOTE:** This fixation should be used for all histology-related staining of CryoJane prepared sections

Please understand that this fixative melts and fixes the tissue simultaneously. Ice crystal artifacts (also referred to as “holes”) are masked. Nuclear morphology is generally successful. Some cell components, however, are lost and most of the red blood cells are lysed.

1. Preparation of aqueous buffer
  - Dissolve buffer-salt mix in 180ml of water
  - Store in refrigerator
  - Shelf life is 6 months
2. Preparation of aqueous fixative
  - Add 20ml of 25% glutaraldehyde to 30ml of aqueous buffer
  - Mix well

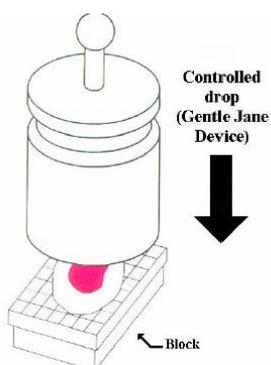
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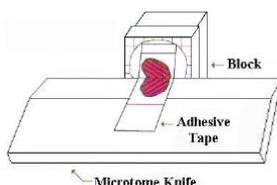
- Use at room temperature
- Discard after 1 week
- If using 50% glutaraldehyde, add 10ml of glutaraldehyde and 10 ml of water

### 3. Procedure

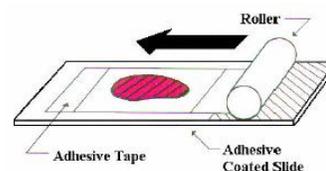
- After the adhesive coating has been polymerized, peel off the pink tape
- To avoid the chance of uncontrollable melting or drying of the section, immediately set the room temperature aqueous fixative into the cryostat and immerse the slide into the fixative. Dip 2-3 times.
- Maintaining room temperature, continue to fix for 15 to 30 seconds.
- Rinse the slide in water and continue with your application.



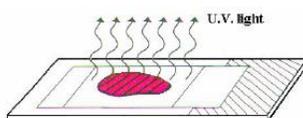
**FIGURE 23.** To minimize ice-crystal size, tissue is snap-frozen with a chilled heat extractor.



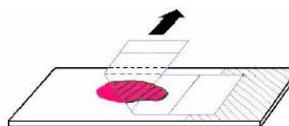
**FIGURE 24.** A cold adhesive tape is used to support the section as it is being cut.



**FIGURE 25.** The still frozen section, adhered to the tape is rolled onto the cold adhesive-coated slide.



**FIGURE 26.** An ultraviolet flash converts the adhesive coating into a hard solvent-resistant plastic.



**FIGURE 27.** The tape is removed. Several options are available

### Anhydrous fixation protocol

This process is to be followed after freeze substitution in a cold solvent (-30°C or colder). It aims to preserve the majority of cellular components and is recommended when using routine stains.

**NOTE:** Please note that this protocol is not recommended when ice crystals are large and widespread. This indicates poor freezing. In such a situation, the aqueous fixative protocol should be followed instead. An anhydrous fixative contains a maximum amount of 30% water.

- |                          |                                 |
|--------------------------|---------------------------------|
| 1. AN/DMF/Glutaraldehyde |                                 |
| Dimethylformamide (DMF)  | 35ml                            |
| Acetonitrile             | 35ml                            |
| 25% Glutaraldehyde       | 10ml (50% Glutaraldehyde – 5ml) |

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Add water to make up to	100ml
Wait at least one hour before use	
2. Formaldehyde-Ethanol	
Ethanol (100% alcohol)	86ml
Formaldehyde	14ml

Take the fixative to the cryostat and transfer the freeze-substituted slide directly from the acetone bath into the fixative. Continue the fixation process at room temperature for 15 to 30 seconds.

**NOTE:** The transfer process is made within the cryostat itself to avoid any condensation build-up on the cold slide, prior to fixation.

3. For sensitive antigens	
DMF/Glutaraldehyde	
Dimethylformamide	70ml
25% Glutaraldehyde	1ml
Add water to make up to	100ml

At this point, the freeze-substituted slide can be transferred from acetone to the fixative at +4°C for 30 seconds to 1 minute. This transfer process must be conducted within the cryostat. Once the fixation process has taken place, and before staining, rinse the slides in water.

## CryoJane Maintenance

### ECU

- Avoid placing anything on top of the ECU at all costs
- Any spills that could enter the ECU could seriously damage the ECU's electrical components
- Cleaning the ECU consists of wiping its surfaces with a dry rag. Alcohol wipes are also safe to use and acceptable.

**NOTE:** Any other solvents, especially acetone, will damage plastic surfaces of the ECU. Do not use them to clean the unit.

**NOTE:** Do not open the ECU. Only authorized personnel should open the unit.

### Mech

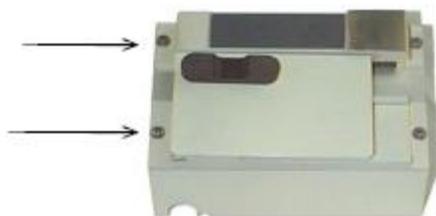
- Metal surfaces can be cleaned with alcohol or chlorine solutions
- When cleaning the flash tray, be sure to use alcohol to remove adhesive build-up. The flash tray should be cleaned periodically.
- Use extreme caution when cleaning the flash tray as the glass filter could easily be damaged
- Any liquid spills on the Mech should be cleaned immediately to avoid damage. As noted previously, any liquid spills could damage the electrical components within the unit.
- The Mech should not be immersed at any time, in solutions of any kind
- If removal of the Mech from the wall is needed for servicing or cleaning, unscrew the two screws shown below and then remove the Mech from the cryostat. Note that the mounting plate will stay intact with the wall of the cryostat.

**NOTE:** In order to avoid misplacement of any of the screws, screw them into the holes in the mounting plate that is on the wall.

- To reinstall the Mech, attach it to the mounting plate with the two screws.

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## Troubleshooting the Mech and ECU

PROBLEM	CAUSE	SOLUTION
The LED lights on the ECU are not on.	The ECU is not turned on.	Make sure that the ECU is connected to an electrical source properly. Make sure the ON/OFF switch is in the ON position.
	One or both fuses in the back of the ECU are blown.	Check the fuses on the back of the ECU and replace as needed. Only use 1AMP, SLOBLO, or Ceramic Core Wound fuses when replacing.
	The ECU is malfunctioning.	Do not open the ECU. Call Customer Service.
The left LED always displays a solid red light.	The ½ inch diameter Flash/Pad Power Cable is not properly connected.	Make sure that the Flash/Pad Power Cable is properly connected to the ECU and to the Blue Pad connector underneath the Mech.
	The temperature of the Blue Pad is too cold.	Increase the temperature of the cryostat.
	The ECU is malfunctioning.	Do not open the ECU. Call Customer Service.
The left LED always displays a flashing red light.	The temperature of the Blue Pad is too warm.	Lower the temperature of the cryostat.
	The ECU is malfunctioning.	Do not open the ECU. Call Customer Service.
The flash does not go on when triggered.	The ECU is not turned on.	Make sure the ECU is properly connected to an electrical source. Make sure the ON/OFF switch on the ECU is in the ON position.
	The ½ inch diameter Flash/Pad Power Cable is not properly connected.	Make sure the Flash/Pad Power Cable is properly connected to the ECU.

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	One or both fuses in the back of the ECU are blown.	Check the fuses on the back of the ECU and replace as needed. <b>NOTE:</b> If the flash unit is triggered more than 3 times on 1 minute, one or both fuses on the back of the ECU will blow out and will need to be replaced. This is a protective feature designed to prevent the chance of overheating. Only use 1AMP, SLOBLO, or Ceramic Wound fuses when replacing.
	The Flash triggering mechanism is jammed.	Check for ice build-up around the black knob on the Mech and remove if found. If by doing this does not solve the problem, call Customer Service.
	The flash unit is malfunctioning.	Do not open the ECU. Call Customer Service.
	The ECU is malfunctioning.	Do not open the ECU. Call Customer Service.

## CryoJane Process Troubleshooting

**NOTE:** The CryoJane process will not produce effective results if the cryostat and/or the microtome are malfunctioning. Please be aware that both units should be serviced periodically for optimal results.

PROBLEM	CAUSE	SOLUTION
The tape does not adhere to the block face.	The protective film covering the adhesive is not removed.	Peel away the protective cover film covering the adhesive window.
	The block is too cold or too warm.	Check the temperature of the cryostat and adjust if necessary. The temperature of the chamber should be set between -25°C to -27°C, depending on the model.
	There is moisture on the adhesive layer.	Use a fresh tape.
The tape does not capture the section.	The microtome is not advancing.	Check the microtome and adjust.
	The microtome is not advancing to the desired thickness.	Check the microtome and adjust. Cut two or three sections before applying the tape.

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	<p>The knife angle is too small or too large.</p> <p>The tape is defective.</p>	<p>Adjust the knife angle between 3 and 5 degrees.</p> <p>Use another tape.</p>
<p>The tape is cut during sectioning.</p>	<p>The block face is not flat.</p> <p>The adhesive on the tape overhangs the block and snags on the knife</p> <p>The knife and/or the block are not properly tightened.</p>	<p>Align and trim the block to obtain a flat surface.</p> <p>Position the tape on the block so that no adhesive is exposed to the knife when cutting the section.</p> <p>Check the knife and block for looseness and tighten all screws and clamps.</p>
<p>The captured section on the tape is not intact.</p>	<p>The knife is dull.</p> <p>There is dirt on the tape.</p> <p>Bubbles form while laminating the tape to the block face.</p> <p>There is vibration in the microtome.</p>	<p>If using a disposable blade, change the blade's position (the edge) or replace it. Sharpen the knife if it is of stainless steel or tungsten-carbide.</p> <p>Use a fresh, clean and cold tape.</p> <p>Adhere the tape to the block face while laminating it with the cold roller. Reapply the tape if bubbles still appear.</p> <p>Check the knife and block for looseness and tighten all screws and clamps.</p>
<p>The section on the tape does not transfer properly to the slide.</p>	<p>The mylar film on the slide has not been removed.</p> <p>The adhesive layer on the slide is not facing up.</p> <p>The slide is too cold or too warm.</p> <p>The tape is not being removed properly.</p> <p>The knife is dull.</p>	<p>Remove the mylar film.</p> <p>Position the slide on the blue pad so that the adhesive layer faces up.</p> <p>See the Mech and ECU Troubleshooting section for the left LED on the ECU always displaying either a solid red or flashing red light.</p> <p>Remove the tape carefully, peeling it back in a diagonal, downward direction to minimize tension.</p> <p>If using a disposable blade, shift to a different portion of the blade's edge or replace the blade. Sharpen the knife if it is</p>

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	A bubble may have formed when the tape was laminated to the slide.	of stainless steel or tungsten-carbide.  Apply the tape to the slide while laminating with the cold roller.
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## Troubleshooting Finished Slides

PROBLEM	CAUSE	SOLUTION
The polymerized adhesive picks up excessive red stain after H&E.	Eosin may be too acidic or too alcoholic. This may cause background setting.	Stain a shorter time, or use an aqueous eosin Y at pH 5.0 – 5.2.
There are chatter marks in the section.	There is looseness in the system.  The knife is dull.  Stop/start motion during cutting.	Check tightness of chuck. Clamp knife holder and/or blade holder vary tightly.  If using a disposable blade, shift to a different portion of the blade's edge or replace the blade. Sharpen the knife if it is of stainless steel or tungsten-carbide.  Cut slowly without stopping. If motor drive is used, lower the cutting speed.
There are tears in the section.	The knife has a nick or defect in the cutting edge.	If using a disposable blade, shift to a different portion of the blade's edge or replace the blade. Sharpen the knife if it is of stainless steel or tungsten-carbide.
Small clusters of cells are missing in the section.	Bubbles or debris was captured between the adhesive tape and the block face, or between the tape and the coated slide.  A hole in the block face may be due to groups of cells being plucked out during or prior to section.  Debris was caught between the tape and block, or between the tape and the coated slide.	To avoid bubbles, apply tape using the roller. If bubbles are visible under the tape on the block face, reapply the (clean) tape.  This may be a defect in the tissue block. Check the knife edge for dullness and correct.  Always remove trimming debris from the block face, the front and rear surfaces of the knife's cutting edge before sectioning.
The section did not transfer completely.		See the CryoJane Process Troubleshooting section.

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<p>There are holes in the nuclei and/or cytoplasm of the section.</p>	<p>Section may have melted and refrozen causing large ice crystals to grow.</p> <p>The initial freezing was poor, causing large ice crystals to grow. The section was taken deep in the tissue block where the freezing is the weakest.</p>	<p>Transfer the frozen block from the freezing device quickly to the cryostat to avoid thawing the tissue. Avoid finger contact with the central portion of the tape and/or slide Do not touch the block face with your fingers. DO not breathe directly onto the section. Keep tapes and slides inside the cryostat. The blue pad temperature may be too warm – see the Mech and ECU Troubleshooting section. All steps of the CryoJane process must be carried out deep inside the cryostat to avoid warm air contacting and melting the frozen section.</p> <p>Snap-freeze the tissue at -60°C or colder. Using the Power Jane or Stand Alone Gentle Jane or Dewar Jane snap-freezers with liquid nitrogen renders the best results for application. Snap-freeze the thinnest practical specimen. The sections located in the uppermost parts of the block will have the least amount of ice crystal artifacts. Use aqueous fixative. <b>NOTE:</b> You will receive optimal results in your application with freeze-substitution followed by anhydrous fixation only if the ice crystal size is minimal.</p>
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## Oil Bath Accessory Kit Components



Oil Bath  
Oil  
Oil brush  
Oil Bath power

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## Oil Bath Accessories Kit Operating Procedure

### Context

The vast majority of embedding media consist of water. The moment that a frozen tissue specimen is embedded in such media, and stored, the tissue as well as the medium will gradually, over time, dehydrate and degrade. The Oil Bath Accessory Kit allows the user to thoroughly cover the block face with the protective oil. The protective oil is generally maintained at  $-8^{\circ}\text{C}$  in a bath within the cryostat chamber. The tissue in the block will not melt if the surface is well coated. The oil has a freezing temperature of  $-10^{\circ}\text{C}$  and will freeze immediately onto the block face. If the frozen oil coating is stored in a freezer of  $-70^{\circ}\text{C}$ , tissue and dehydration can be avoided for up to one year. Furthermore, the oil can be used to repair a block that becomes damaged. By using the oil, holes or any other kinds of defects in the block face can be frozen over. The frozen oil consists of embedding medium which can be sectioned in similar fashions to those of other media.

### Preparation

- The temperature of the cryostat should be set between  $-25^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$
- Confirm that there is oil in the bath. If oil is added to the bath, wait approximately 1 to 2 hours for the oil to reach its set temperature of  $-8^{\circ}\text{C}$
- Make sure that the oil inside the bath is in a liquid state and not frozen
- The oil brush should be inside the oil bath
- The right LED on the ECU should be green

### Use

Tissue blocks can be stored in a freezer at  $-70^{\circ}\text{C}$  for up to 1 year if they are coated generously with the protective oil.

**NOTE:** During storage, the block cannot thaw nor can it refreeze during the time in which it is stored. If the block thaws or refreezes, please note that the tissue in the block will degrade.

### Storing a tissue block

To properly store a frozen tissue block, consider the following:

*For short-term storage in the cryostat*

- With the block in the cryostat, use the oil brush to cover the block face with the oil from the oil bath

*For long-term storage in a  $-70^{\circ}\text{C}$  Freezer*

- With the block in the cryostat use the oil brush to cover the entire block with the oil from the oil bath. Be sure to apply a generous amount of oil.

### Sectioning a stored tissue block

*For short-term storage*

- Trim away the frozen layer of oil in order to reveal the tissue and specimen
- Continue with usual procedure

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## Oil Bath Accessory Kit Troubleshooting Protocol

PROBLEM	CAUSE	SOLUTION
The oil inside the oil bath is frozen.	The ECU is not turned on.	Make sure that the ECU is properly connected. Make sure that the ON/OFF switch on the ECU is in the ON position.
	The oil bath power cord is not connected properly.	Make sure that the oil bath power cord is properly connected to the ECU as well as to the oil bath.
	One or both of the fuses on the back of the ECU are blown.	Check the fuses on the back of the ECU and replace as needed. Be sure to only use 1AMP, SLOBLO or Ceramic Core Wound fuses when replacing.
	The cryostat temperature is too cold.	Increase the temperature of the cryostat.
	The ECU is malfunctioning.	Do not open the ECU. Call Customer Service.
The oil bath LED always displays a solid red light.	The oil bath power cord is not connected properly.	Make sure that the oil bath power cord is properly connected to the ECU as well as to the oil bath.
	The temperature of the oil inside the oil bath is too cold.	Increase the temperature of the cryostat.
	The ECU is malfunctioning.	Do not open the ECU. Call Customer Service.
The oil bath LED always displays a flashing red light.	The temperature of the oil inside the oil bath is too warm.	Lower the temperature of the cryostat.
	The ECU is malfunctioning.	Do not open the ECU. Call Customer Service.

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