

**HSS Research Institute Histopathology Service
Shared Histopathology Resources**

Contact Information

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The **Histopathology Shared Equipment** consists of

1. Tissue Processor (room S-225)
2. Paraffin-embedding station (room S-225)
3. Two Microtomes (microtome-1 in room S-225, microtome-2 in room S-201)
4. Computer attached to a microscope and dissection scope (+camera) (room S-201)
5. Zeiss Axioscan 7 slide scanner (room S-223) [requires additional training and approval]
6. Osteomeasure (room S-223) [requires additional training and approval]
7. Zeiss Axio Observer Microscope (room S-223) [additional training and approval]

- Only **trained and authorized** individuals are allowed to use the shared equipment located on the 2nd floor of the S-building rooms S-225 and S-201 (microscope and microtome-2). Equipment in S-223 requires additional approval. Access to the equipment and rooms is provided to trained and approved users. Please, read and follow the guidelines.

- We reserve the right to revoke user privileges in cases where these guidelines, including proper equipment clean up, are not followed.

- Always wear appropriate personal protective devices such as goggles and lab coats when using the Histopathology setup. To conduct any bench work in the HSS Research Institute, users **must** have completed the mandatory EHS/WCM Lab Safety Training and annual refreshers. Additionally, to work alone laboratory areas, a valid C-14 certificate is required.

- To schedule **orientation and training**, please email to HistopathologyService@HSS.EDU For advice and questions regarding the **equipment usage**, please contact Samantha Lessard (lessards@hss.edu). For any other questions, please contact Miguel Otero (oterom@hss.edu), Kyung-Hyun Pak-Min (parkmink@hss.edu) or Erik Attia (attiae@hss.edu)

- Before using the equipment, please sign in using the online calendar assigned to each resource (**calendar access is provided to approved users**). Users must sign in a physical log to track equipment usage.

- When signing up for the Tissue Processor and the Embedding Station, please indicate the **number of samples** that you are going to process/embed, so that we know how often to change reagents. After processing, samples placed into the cassette bath of the paraffin-embedding station should be **embedded within 72 hours** to allow more users to process their samples.

- Pre-booked users have priority to use the equipment, but reservations will be cancelled if the machine is NOT in use within **1 hour** after the start of the reserved time. However, we ask the users to please not block microtomes by booking them for prolonged times in advance (e.g. 9 to 5, Monday to Friday) in order to allow all users timely access to the equipment.

- Users must clean up after using the equipment. Reagents, tools (e.g., blades) and any other material should be stored in the users' laboratory. Do **NOT store** any items in shared spaces.

a) Tissue Processor (room S-225):

- Sign up using the provided online calendar under “***Paraffin processor***”.
- We use **ONLY Clear-Rite and not Xylene** for the shared Tissue Processor. Never use Xylene when utilizing the shared Tissue Processor. If the reagents run low, please inform Samantha Lessard (LessardS@hss.edu).
- When signing up for the Tissue Processor, please make sure that you indicate the **number of samples** that you are going to process in the **physical log**, so that we know how often to change reagents.
- If necessary, refill the paraffin bucket if the paraffin is clean. If the paraffin is dirty, dispose of melted used paraffin and replace with fresh paraffin.
- The bucket should **not** remain in the paraffin after use. Users must remove, clean, and transfer the bucket to the Clear-Rite (#1).
- The Tissue Processor will be cleaned at least once a month, depending upon usage. Samantha Lessard (LessardS@hss.edu) will schedule the equipment clean up and maintenance and will contact users requesting help to clean up the Tissue Processor, when required.

- Reagent stations and position in carousel
 - 1) Clear-Rite
 - 2) Water
 - 3) 70% Alcohol (dehydrant or ethanol)
 - 4) 80% Alcohol (dehydrant or ethanol)
 - 5) 95% Alcohol (dehydrant or ethanol)
 - 6) 100% Alcohol (dehydrant or ethanol)
 - 7) 100% Alcohol (dehydrant or ethanol)
 - 8) 100% Alcohol (dehydrant or ethanol)
 - 9) Clear-Rite
 - 10) Clear-Rite
 - 11) Paraffin
 - 12) Paraffin

b) Embedding Station (room S-225):

- Sign up using the provided online calendar under “***Embedding station***”.
- When signing up for the Embedding Station, please make sure that you indicate the **number of samples** that you are going to process in the **physical log**. If the paraffin runs low, please inform Samantha Lessard (LessardS@hss.edu).
- After processing, samples placed into the cassette bath of the paraffin-embedding station should be **embedded within 72 hours** in order to allow more users to process their samples.
- After a completed run, open the processor, pick up the basket and drain the molten paraffin into the chamber.
- Transfer the basket (avoid dripping paraffin) into the cassette bath.
- Take one of the cassettes, place on work area, remove the lid and discard. Take mold from the mold warmer and place underneath the dispenser handle.
- Fill mold (~50-60%) with paraffin; use pre-warmed forceps to place sample into the mold (double-check orientation of the tissue).
- Place mold onto refrigeration spot while holding the tissue down, and allow for paraffin on the bottom to solidify. Place cassette on top of mold.
- Fill mold with paraffin and place cassette and mold at far end of cold plate for total solidification.
- **Clean up and Maintain** the Embedding Station after you finished:
 - a) Stack all cold molds and place into mold warmer
 - b) Use plastic scraper to combine liquid wax
 - c) Remove solid paraffin from work space (scrape into the trash).
 - d) Clean all forceps (use a gauze or paper towel).
 - e) If cold plate is off, dry with paper towels. If plate is on (cold), scrape wax
 - f) Clean wax from the collection tray (located underneath the wax dispenser)

c) Microtomes (room S-201 and S-225):

- Sign up using the provided online calendar under
 "Microtome-1" (S.225) or
 "Microtome-2" (S.201)

- Microtomes should be used within 1 hour of booking time. We ask that users to please not block microtomes by booking them for prolonged times: **maximum of 5 hours per day** to allow all users timely access to the equipment.

- Microtome blades must be provided by the user.

- Handle blades very carefully. Set up the microtome by (1) positioning sample first and (2) put in blade. Never put blade in before positioning the sample.

- Store blades in a covered container. **Never leave blades on countertops.** When leaving the microtome, make sure that the blade guard is in place.

- **Clean and Maintain the Microtome** thoroughly: microtome should be cleaned to remove tissue debris and wax
 - a) Clean microtome daily/after each use
 - b) Remove blade before cleaning
 - c) Remove excess wax/tissue using a dry paintbrush

- Clean work space after each use

- Empty and dry water bath after use



ZEISS Axioscan 7 Microscope Slide Scanner

The HSS Research Institute ZEISS Axioscan 7 Microscope Slide Scanner has a 100-slide capacity; 5x, 20x and 40x objectives; a circular Polarizer/Analyzer; and a 5 channel LED with 3 filter cubes for Fluorescence. The Scanner is accessible as a **user-driven shared equipment to trained and approved users from HSS-affiliated laboratories** (requires confirmation from Research Administration, and access to an HSS-provided server storage space), **or via the Histopathology Service on a fee-for-service basis**. To request training and user-driven access, please submit this form (only page 1) with all the required information to HistopathologyService@hss.edu and read the attached guidelines (pages 2 and 3 in this document).

User

Name:
Email:
Phone:
HSS-Affiliated Laboratory (PI name and email):

Scanning

Brightfield
Fluorescence

Non-human animal specimens (mark one): Yes No
IACUC #

Human specimens (mark one): Yes No
IRB #

Please remember: this scanner is for **research use**, human specimens should be **de-identified**, and **no PHI should be scanned**.

I confirm that **the scanned human specimens are de-identified and stripped of PHI:**

The laboratory has access to HSS-provided server storage space: Yes No

Signatures

User: printed name

Lab Director: printed name

User: signature and date

Lab Director: signature and date



Zeiss Axioscan 7 Microscope Slide Scanner Training Document

General Principles

- Turn off computer and scanner (front power button) at the end of the day or if you are the last scheduled user for the day.
- Do **NOT scan wet slides**.
 - Allow all slides to cure fully or are sealed and dried with nail polish before loading them into scanner.
- When logging off, never “Switch Profiles”; Always Shut down or Log Off

Turning Machine On

1. Turn computer on, log into your HSS account with NetID and Password
2. Turn Scanner On with front Power button
 - a. You should hear the slide tray move up and down, as well as the filter turret rotating through all positions
 - b. Wait approximately 2 mins for this to finish
3. Open Zen Blue 3.5
 - a. Click Zen Slide Scan (left button)
 - i. If this produces errors, you likely did not wait long enough after turning on the scanner; close and re-open software
 - b. Wait for Axioscan menu (top left) to finish initializing

Scanning a Pre-Configure Profile

Use this process if there is already a profile that will work for your slides

1. Load Slides
 - a. Press the Open/Close Door button
 - b. Gently open the slide trays
 - c. Use slide loader to load your slides and gently place them into the trays
 - i. The most common problem requiring a costly Zeiss service visit (cost >\$2000 per visit), is that you have not appropriately seated the slide holder into the tray, or you pushed the tray in too aggressively causing it to unseat inside the machine
 - d. Press the Open/Close Door button
2. Ensure that the **save location is appropriate** (in your save folder)!!
3. Choose the naming strategy you would like to use for the slides (default is Automatic)
 - a. Automatic
 - i. Select the appropriate naming convention from drop-down menu.



- b. Manual
 - i. Select all slides in menu
 - ii. Right click on one of them
 - iii. Select “Set all to manual naming”
(or do **one by one** with drop down menu)
 - c. Import
 - i. Open the Import Name Document (.csv) in Excel
 - ii. Type in all the information (name, profile, etc)
 - iii. Save document as .csv
 - iv. Import into Zen with import button
 4. Select Scan Profile
 - a. Using the check boxes (select all, unselect all etc.) select slides to which the scan profile will be applied.
 - b. Use either the top profile drop-down menu to select a profile for each slide
 - i. Or do one by one with the individual slide profile drop downs
 5. Click Preview Scan
 6. On first slide, use the gear button to select “Check and correct sample detection”
 - a. Adjust Scan area
 - i. There are many ways to do this but my preferred way is to use an auto detection profile and then right click on the detected area, split the area into regions I want to scan, then delete the areas I do not want to scan.
 - b. Adjust Coarse and Fine Focus Points
 - i. Add focus points with a right click
 7. Start Scan

Turning Machine Off

1. Close out of the Program
2. Shut Down Computer waiting for **ALL** software to close (never force stop a Zeiss Program)
3. Turn Off Scanner with front button.