Name:	Olympus BX53F Fluorescent Microscope Procedure	**Created:**	September 23, 2025
Number:	Histo-6	**Revised:**	
Category:	Instrument Operation	**Author(s):**	Katie Tooley

1.0 Purpose

The purpose of this SOP is to describe the proper procedure of use of the Olympus BX53F fluorescent microscope located in the Missouri S&T histology core, Bertelsmeyer 220.

2.0 Policy

2.1 The use of all histology equipment in the histology core lab at Missouri S&T is currently managed by the Center for Biomedical Research (CBR) staff.

2.2 All personnel working in the histology core are required to complete general laboratory safety and BSL-2 training through EHS.

- 2.2.1 More training may be required in the future. Please check with CBR staff before beginning work to ensure all required training is complete.

2.3 Eating or drinking is not permitted in the lab.

2.4 PPE is required for all users. This includes, at minimum, gloves and a lab coat. A mask and/or goggles should also be worn if working with noxious chemicals (i.e. xylene) or chemicals with the potential to splash.

2.5 Bertelsmeyer 220 is a shared lab space, therefore all users must be familiar with the supplies and equipment available to them before keycard access to the lab will be granted.

2.6 All samples should be labeled with your name, date, and sample identification. **Any samples not labeled will be thrown out.**

2.7 Each user is required to sign in and out of each equipment logbook while working in the histology core.

- 2.7.1 Please also schedule all equipment use through the online Outlook calendar for the Histology Users – MST Outlook group.
  - 2.7.1.1 Please view the Outlook calendar instructions pdf sent with the Outlook group invite for further information.

2.8 Each person working in the lab is responsible for cleaning work surfaces, such as benches, and any used equipment before leaving.

- 2.8.1 Cleaning tasks must be documented daily on the provided checklist.

2.9 Each person leaving the lab, including temporary visitors, is required to wash their hands before leaving.

2.10 No user fee is currently being charged, however, a list of supplies to be provided by users is outlined in the Supplies section below.

2.11 The operator is responsible for carefully following all steps outlined in this SOP, performing the required cleaning after each use, and immediately reporting any equipment malfunctions, damage, or safety concerns to the lab supervisor.

2.12 Please contact Anna Chernatynskaya or Katie Tooley if you have any

questions.

3.0 Microscope Procedure

3.1 All slides should be coverslipped before viewing them on the microscope.

3.2 Sign in to the logbook located in the drawer underneath the embedder.

3.3 Remove the dust cover and make sure the stage of the microscope is fully lowered before placing the slide in the clamp. Additionally, ensure the objective is set at the lowest setting.

3.4 Flip the switch located at the back of the left side of the microscope to turn it on.

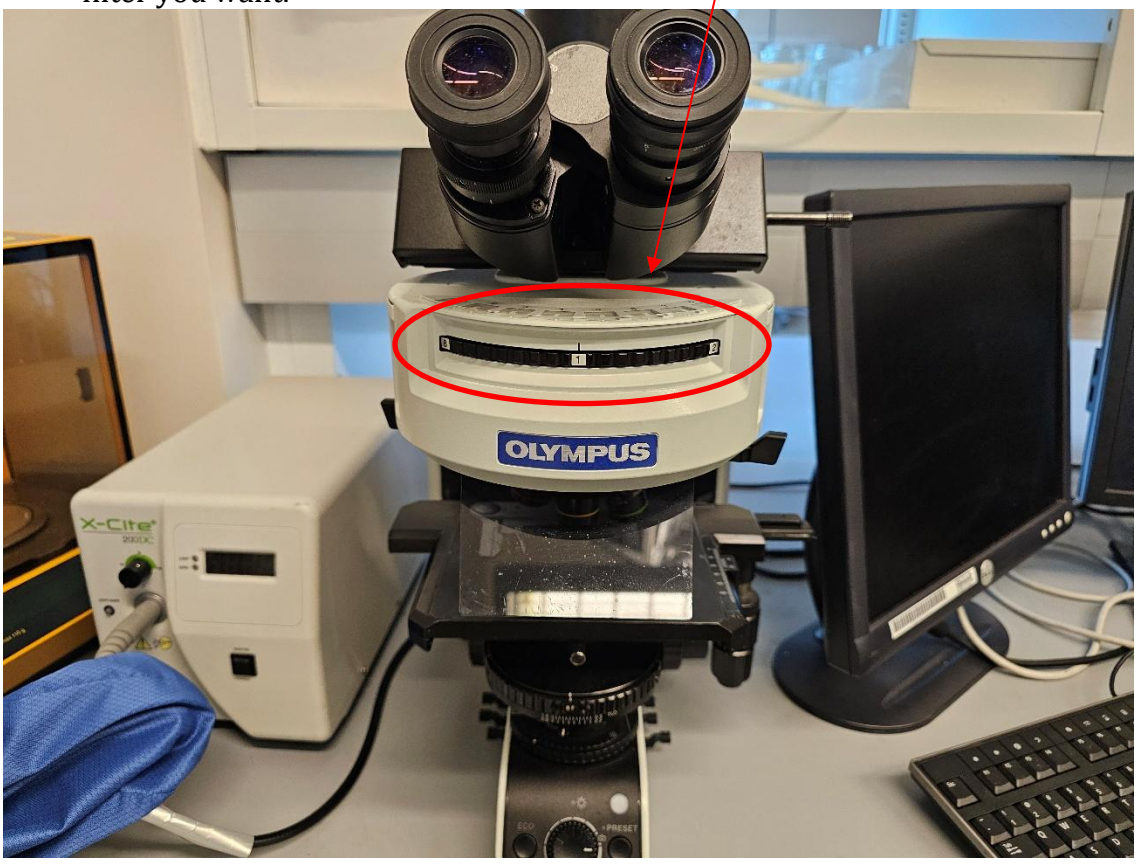
3.5 Adjust the Y-axis using the Y-axis knob attached to the underside of the stage.

3.6 Once you have located your desired sample section, use the coarse and fine adjustment knobs located on either side of the microscope underneath the stage to focus the image.

3.6.1 You can also adjust other minor settings like the light path and diopter. See the microscope manual for more information.

3.7 After focusing, you can then move on to higher objectives and refocus if needed.

3.8 If you need fluorescence imaging, rotate the disc below the eyepieces to select the filter you want.



3.8.1 Three fluorescence filters are available: blue (setting 2, used for DAPI), green (setting 3, used for FITC), and orange (setting 4, used for TRITC).

3.8.2 Turn on the UV lamp to view the fluorescence using the switch on the front of the lamp.

3.8.2.1 Do NOT leave the UV lamp on if you are not viewing the fluorescence or capturing an image. This will bleach your sample.

3.8.2.2 Remember to turn off the UV lamp when finished.

3.9 Adjust the brightness if needed using the brightness adjustment knob at the bottom.

3.10 If you want to capture an image on the computer, continue on to section 4.

3.11 If you are done viewing your sample, turn off the microscope, lower the stage, switch to the lowest objective, remove your sample, and gently wipe the eyepieces and the stage with a kimwipe.

3.12 Replace the dust cover and sign out of the logbook.

4.0 Capturing Images

4.1 Turn the computer on and login to the CBTRR group profile using the password "CBTRR". This is also located on a sticky note on the side on the computer tower.

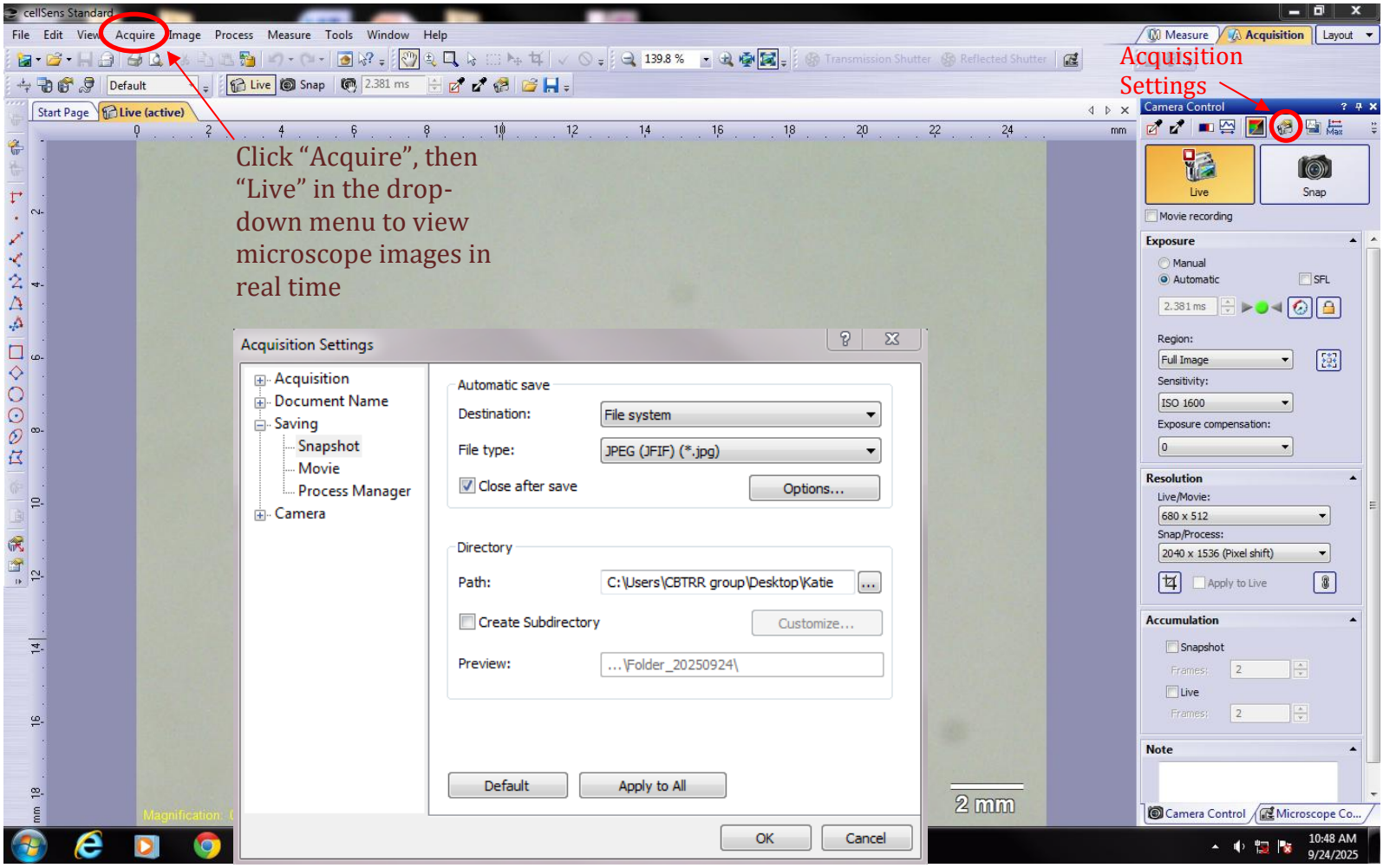
4.2 If you have not already done so, create a folder on the desktop with your name to store your captured images. **Do NOT save your images individually to the desktop.**

4.3 Open CellSens from the desktop.

4.4 Click "Acquire" on the top toolbar, then click "Live" from the dropdown menu. This will show you a live image of the microscope.

4.4.1 If the microscope is already turned on and focused, you should see the same image in CellSens. If not, please follow the steps listed in section 3.

4.5 In the Camera toolbar on the right side of the screen, select "Acquisition Settings"

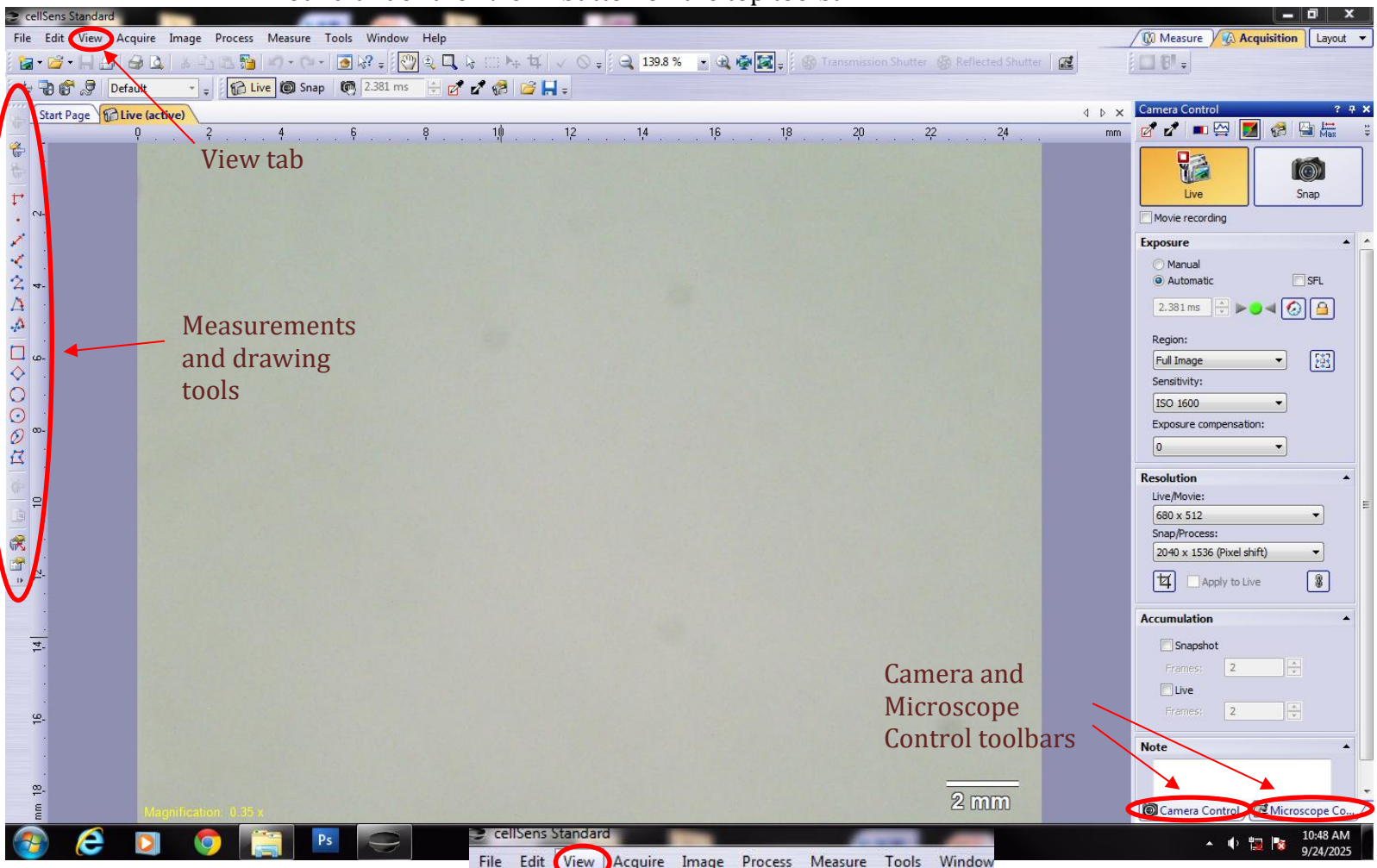


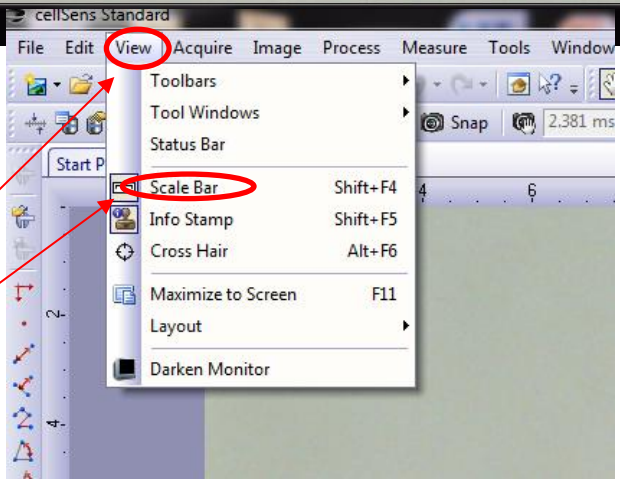
near the top. Choose the folder you want to store your images in. You can also change the desired format here. You will need to check this each time you open the program.

4.6 Various settings like exposure time and brightness can also be adjusted through the computer using the Camera and Microscope control windows. See the CellSens manual for more details.

4.7 You can also add different measurements and illustrations to the image using the toolbar on the left side.

4.8 You may additionally want to add or remove a scale to your image. This can be found under the "View" button on the top toolbar.





Click "View", then click "Scale Bar" to insert or remove a scale bar in the lower righthand corner of the image

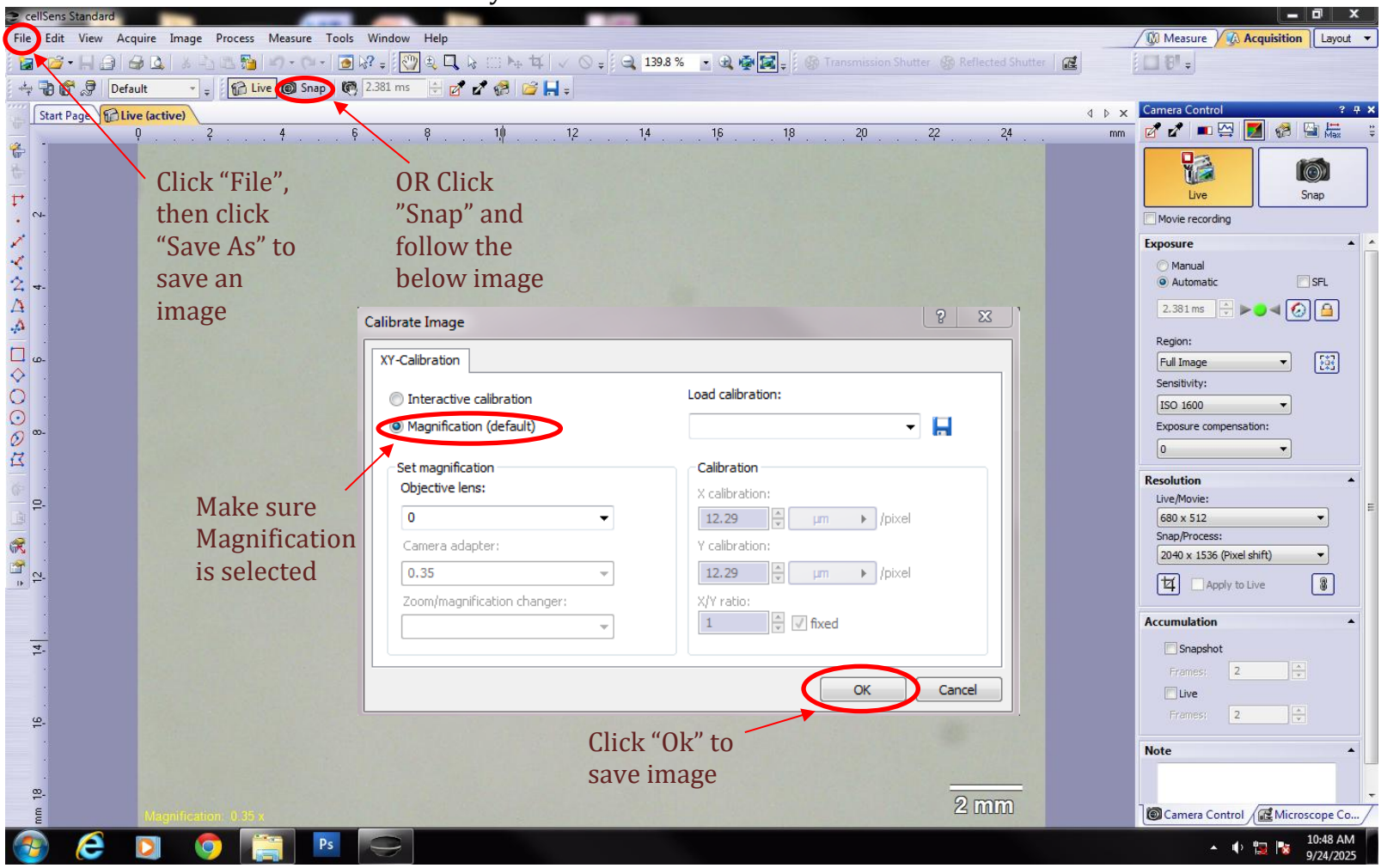
4.8.1 Make sure you select what objective you're using on the toolbar at the top. Your scale will be incorrect if you don't do this.

4.9 After finalizing adjustments, you can capture an image one of two ways:

4.9.1 The first option is to select "File" at the top lefthand corner, select "Save As" in the drop-down menu, and select the folder you want to save the image in. You can also change file type and file name before saving.

4.9.1.1 NOTE: TIF file should be selected if you want to retain the true dimensions of the sample for future measurements and adjustments. JPEG file can be selected if you only want to save that specific image and will not want to take further measurements. JPEG files are also higher quality, so they should be chosen only for your final product.

4.9.2 The second option is to select "Snap" in the top toolbar. The calibration screen will pop up. Make sure automatic calibration is selected and click okay. The image will be saved in the chosen format to the folder and format you selected in section 4.5.



4.10 Once you have finished capturing the images you want, close CellSens and shut down the computer. Then follow the steps listed in sections 3.10 and 3.11.

5.0 Supplies provided by users:

5.1 **Blades for microtome and cryostat**: Epredia HP35 Ultra microtome blades (please stick with this exact product, our microtome is set up for easy change-outs of these specific blades) - $289.09 for a pack of 50, Catalog #31-537-35, https://www.fishersci.com/shop/products/thermo-scientific-ultra-disposable-microtome-blades-2/3153735?searchHijack=true&searchTerm=thermo-scientific-ultra-disposable-microtome-blades-2&searchType=Rapid&matchedCatNo=3153735

5.2 **Camel hair brushes, small** (links include what we use, but feel free to shop around, must be camel hair) - $70.75 for a pack of 12, Catalog #1910, https://www.fishersci.com/shop/products/cryotome-cryostat-accessories-camel-hair-brush/1910#?keyword=1910%20brush

5.3 **Camel hair brushes, large** - $26.28 each, Catalog #03-661, https://www.fishersci.com/shop/products/fisherbrand-long-handled-camel-s-hair-brush/03661#?keyword=03661

5.4 **1 gallon of Fisher histological grade ethanol** (only needed if you'll be processing more than 50 samples) - $113.10, Catalog #A405F-1GAL, https://www.fishersci.com/shop/products/ethanol-anhydrous-histological-fisher-chemical-3/A405F1GAL?searchHijack=true&searchTerm=ethanol-anhydrous-histological-fisher-chemical-3&searchType=Rapid&matchedCatNo=A405F1GAL

5.5 **Glass microscope slides** (charged slides are best for tissue retention during staining) – Fisherbrand Superfrost Plus Microscope Slides, $47.66 for pack of 144 slides, Catalog # 22-034979, https://www.fishersci.com/shop/products/fisherbrand-superfrost-plus-stain-slides/22034979?searchHijack=true&searchTerm=fisherbrand-superfrost-plus-stain-slides&searchType=Rapid&matchedCatNo=22034979

5.6 **Glass cover slips** (personal preference, but this is what we use) - Epredia Signature Series Cover Glass, $83.55 for a pack of 10 boxes, Catalog #22-050-232, https://www.fishersci.com/shop/products/signature-series-cover-glass-24-x-50mm/22050232#?keyword=22050232

5.7 **Microscope slide box** (feel free to shop around, item linked is an example) – Fisherbrand Microscope Slide Box, 100 slots, $9.58, Catalog #03-446, https://www.fishersci.com/shop/products/fisherbrand-microscope-slide-boxes-numbered-slots-3/03446#?keyword=03-446

6.0 References

6.1 Evident Scientific Manuals (search BX53) https://evidentscientific.com/en/downloads/manuals

6.2 CellSens Standard Manual (found on microscope computer desktop)

6.3 Missouri S&T EHS Laboratory Safety Training https://ehs.mst.edu/trainingindex/

SOP REVISION HISTORY

VERSION #	APPROVED	DETAILS
1	9/9/25	Created
2		