



SeBa2 Upright Digital Microscope Systems User's Manual



Version 4.0

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1. Introduction

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SYMBOL	DESCRIPTION
	STOP DAMAGE MAY OCCUR
	READ THE MANUAL BEFORE USE. UNSUITABLE OPERATION WOULD LEAD TO INJURY OR INSTRUMENT FAILURE
I	SWITCH IS ON
O	SWITCH IS OFF

2. Operation Notice



1. The microscope is a precision instrument, please operate carefully: avoid vibrating or jolting the microscope during operation.
2. Do not operate the microscope under conditions of direct sunlight, high temperature, high humidity, dust, or close to sources of vibration. Ensure the work surface is flat.
3. When lifting or carrying the microscope, use one hand to hold the arm of the microscope (1) and another hand to carry the front base (2).

****Note: The microscope will be damaged if the stage, focus knobs, or head are held when moving.***

4. The lamp may be hot when the microscope is on. Ensure there is enough space around the lamp housing for heat dissipation.
5. Ensure the microscope is properly grounded to avoid electric shock.
6. Ensure the power switch is in the “O” (off) position and wait until the lamp cools completely before replacing the bulb or fuse.
7. The input voltage is clearly marked on the back of the microscope. Ensure the power supply voltage is in this range.

Maintenance

1. Wipe the lens gently with a lens cloth. Carefully wipe off oil and fingerprints on the objective surfaces with a lens cloth moistened with a small Maintenance
2. Wipe the lens gently with a lens cloth. Carefully wipe off oil and fingerprints on the objective surfaces with a lens cloth moistened with a small amount of lens cleaning solution.
3. Lens cleaning solution is flammable. Turning on or off electronic devices (including the microscope) may produce a spark which could ignite the lens cleaning solution. Use these chemicals in a well-ventilated area.
4. Don't use organic solutions to wipe the surfaces of the other components. Please use a neutral detergent if necessary.
5. To clean the LCD display use a soft, dry, lint-free cloth to remove any dust from the screen, then clean the LCD display with a non-alcohol based cleaner made for touchscreen displays.
6. If the microscope is exposed to liquid during operation, power it off immediately and wipe it dry.
7. Never disassemble the microscope, the performance will be affected or the instrument will be damaged.
8. Cover the microscope with a dust cover when it is not in use.

3. Installation

Included Items

- SeBa Imaging System
- Power Supply
- SeBa2 USB Drive
- Dust Cover
- Immersion Oil
- Lens Paper
- Allen Wrench
- User Manual (On USB Drive)

Initial Installation

The SeBa2 Digital Imaging System is shipped with the objectives, condenser and stage pre-installed and aligned requiring only minimal assembly.

1. Remove the SeBa2 from its packaging and place it on a solid flat surface.
***NOTE: Keep all packaging**
2. Ensure power switch is in the "0" (OFF) position.
3. Plug power supply into the back of the microscope and plug cord into an outlet.

4. Instrument Components

Power On

1. Turn on tablet power switch which is located on the top left of the SeBa2 tablet.
2. Turn on microscope power switch which is located on the right side of the SeBa2 system.
3. The SeBa2 Touchscreen user interface will launch automatically after turning on the power.

On/Off Switch Tablet
(Small button on top left)



USB ports

On/Off Switch Microscope

USB Ports

1. Connect optional accessories into USB port(s) (mouse, keyboard, USB drive).

HDMI Port

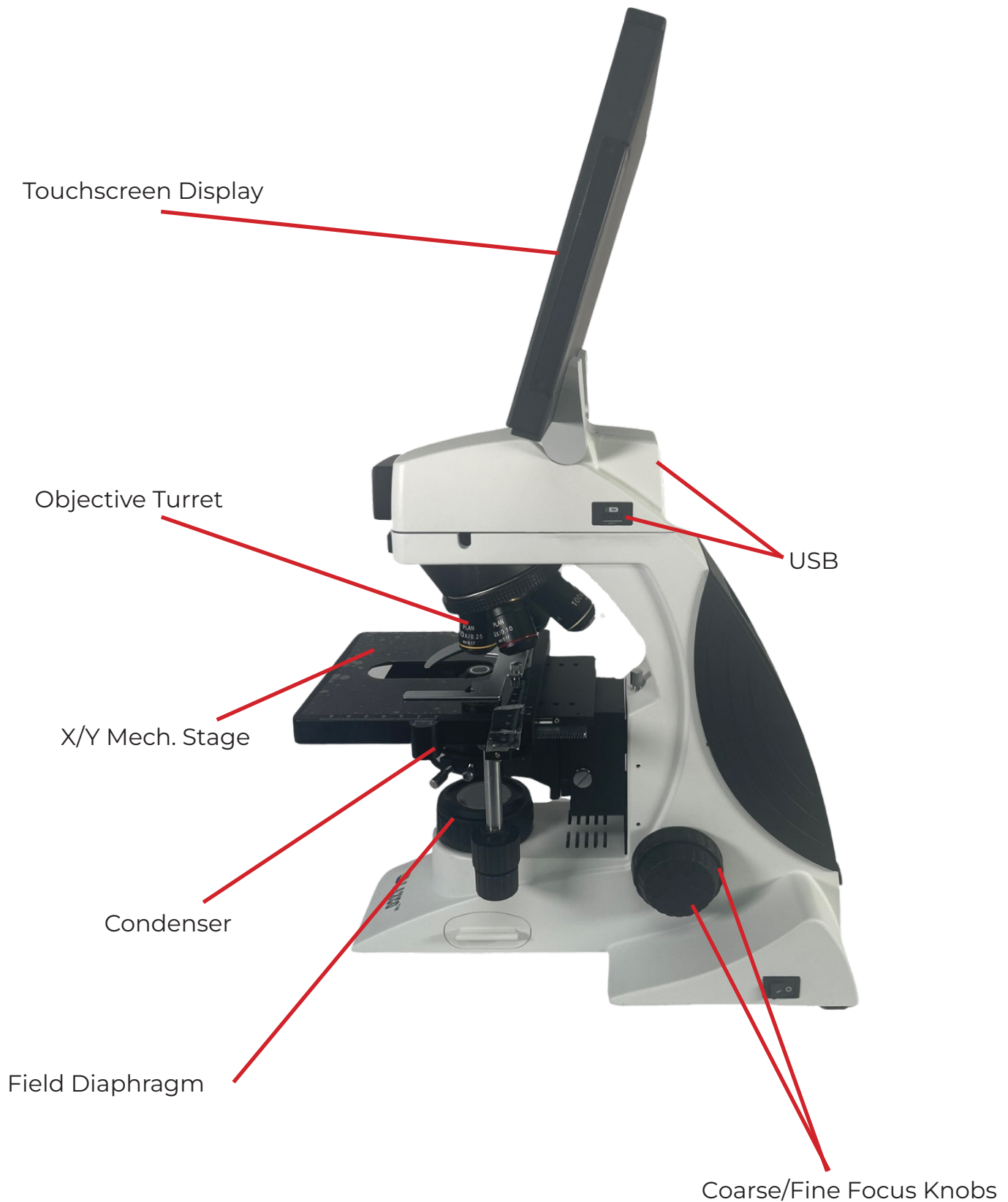
1. To display image on an external HDMI monitor/display, plug in HDMI cable to the port located on the back of the SeBa2 display and then plug HDMI cable into monitor.

****Note: HDMI cable not supplied with SeBa2 Digital Imaging System.***

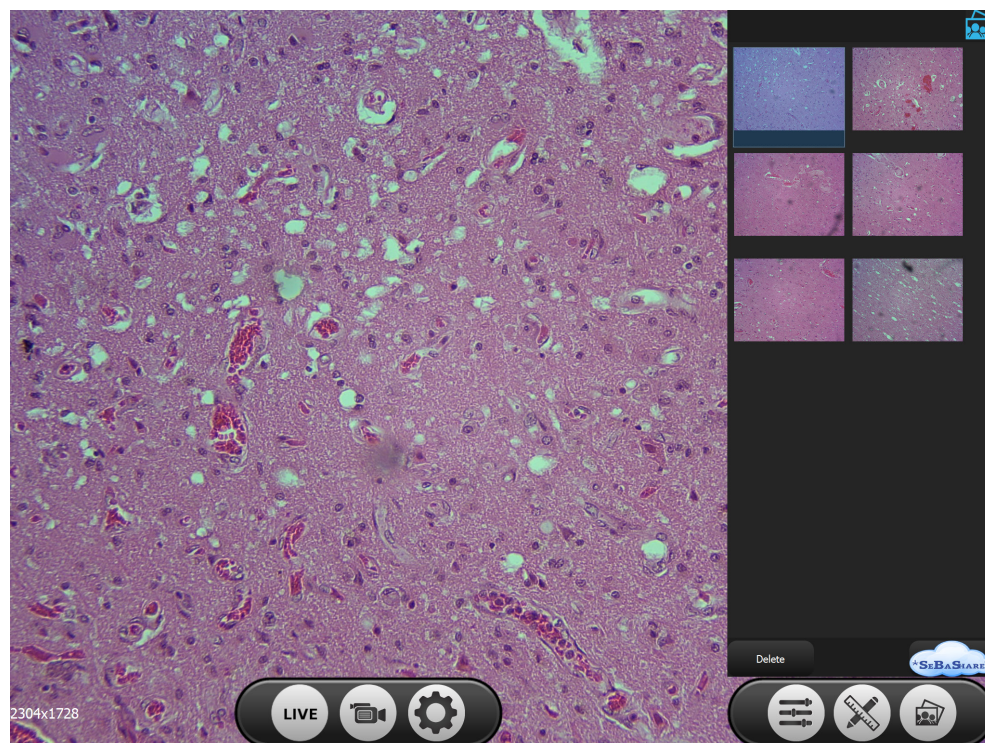
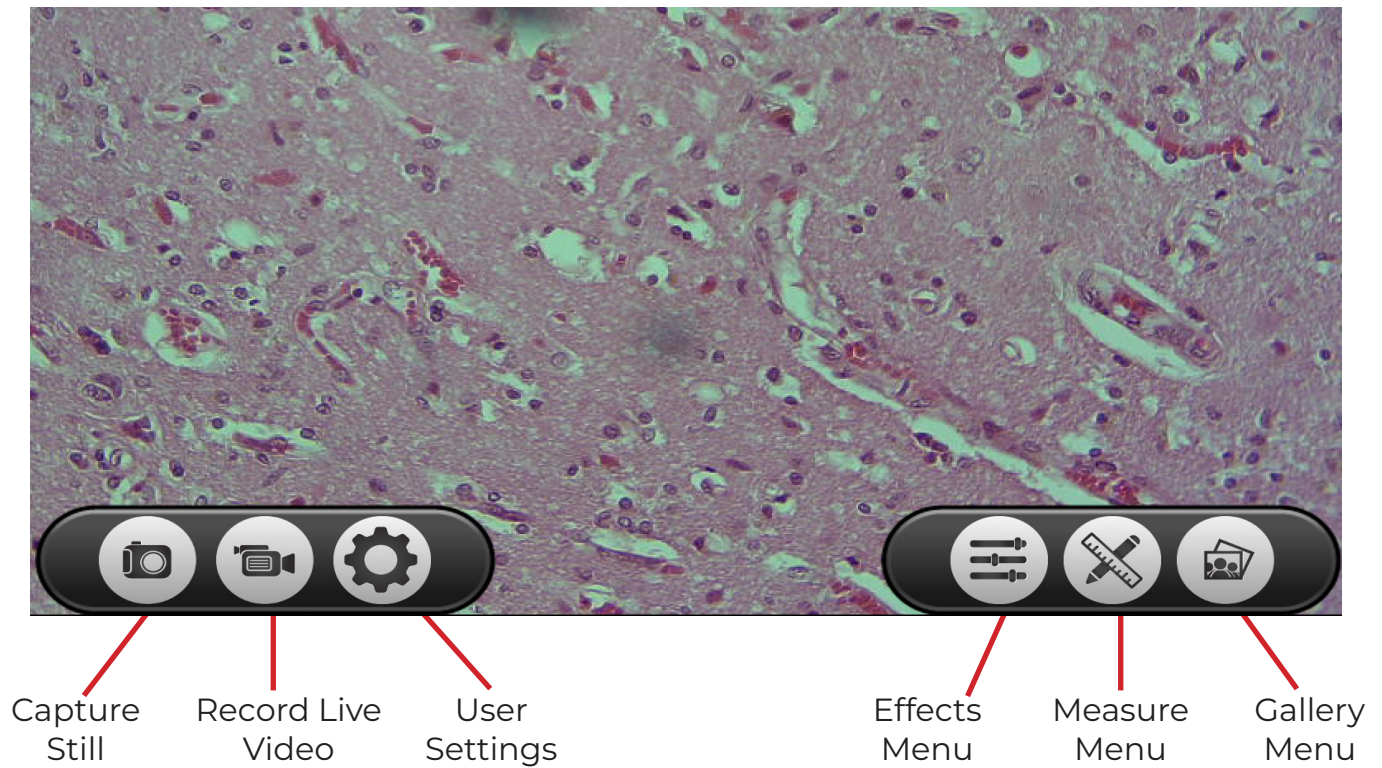


HDMI port

4. Instrument Components



5. SeBa2 Software Touchscreen Interface



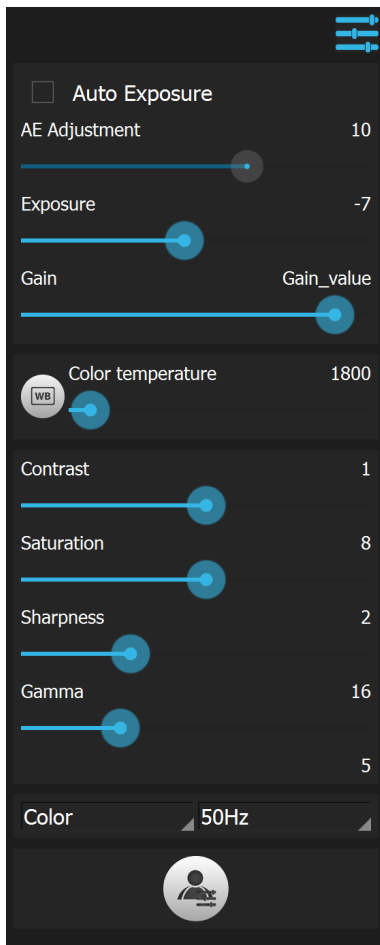
****Note: To return to full screen mode and “hide” the Effect, Measure or Playback menu simply touch the icon which corresponds to the open menu.***

5. SeBa2 Software Touchscreen Interface

Expanded Menu Details

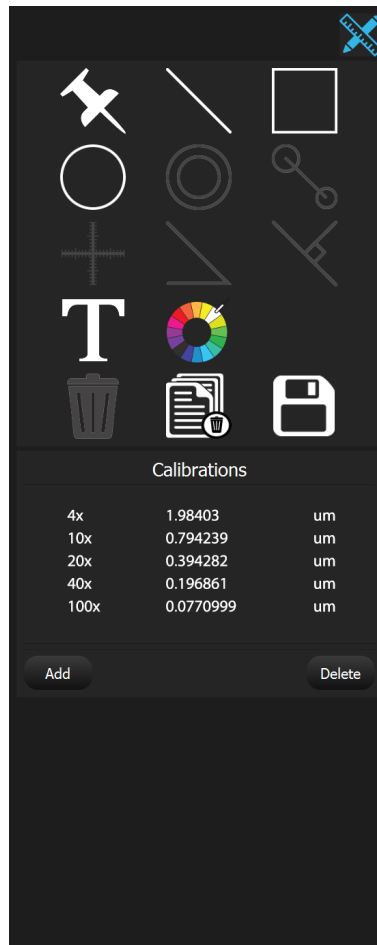


Effects Menu



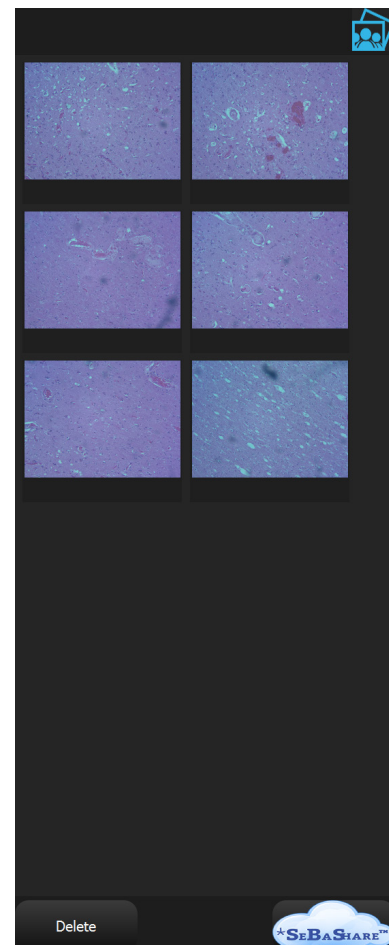
Adjust exposure, white balance, color, brightness, contrast of live image.

Measure Menu



Perform measurements on image.

Gallery Menu



Review captured images and upload to SeBaShare.

6. Operation

Basic Operation

The SeBa2 Digital Imaging System includes both manual and software based controls. Please refer to instrument components Section for the location of the controls referenced below. Software controls can be operated via touch or mouse.

1. Turn on the power to the SeBa2 Imaging System and allow the SeBa Software Interface to auto boot.
2. Place a microscope slide (coverslip up) into the slide insert on the microscope stage.
3. Select desired objective (It's recommended to start at a low magnification to initially focus on the slide then increase magnification).
4. Focus the sample on screen by using the coarse and fine focus knobs.
5. Adjust Illumination intensity to desired level.
6. Make any desired adjustments to the on-screen image as required. (white balance, exposure etc.)
7. Move the position of the sample by adjusting the x/y position of the stage to desired position.
8. To capture an image select the "capture still image" button.
9. To specify the location of capture images refer to the "Save Settings: Destination" section on page 14.

****Note: Destination should be specified prior to capturing an image.***

SeBaAccess Login

The SeBa2 Digital Imaging System includes access to a SeBaShare cloud account. To login to the SeBaAccess/SeBaShare account follow the steps below.

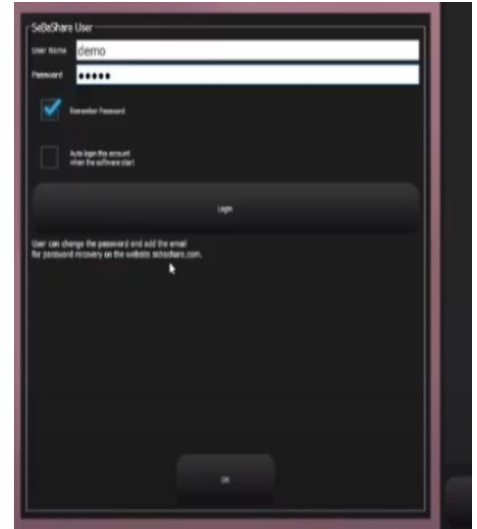
1. To connect to your SeBaShare account, select the SeBaSHARE Cloud Image in the lower right corner of the Image Capture window.

****Note: to upload images to the SeBaShare cloud the system must be connected to a WiFi network. Follow the steps in the "WiFi Network Connection" section.***


6. Operation

2. SeBaShare login screen opens
3. Enter USERNAME and PASSWORD.
4. Select LOGIN.

***Note: USERNAME and PASSWORD are assigned by Laxco.**



Capture Image

1. Click on the capture still image button  at the bottom of to capture and save a still image.

***Note: to specify the destination of saved images see the “Save Settings: Destination” section.**

Record Video

1. Click on the record video button  at the bottom of to record a video.

***Note: to specify the destination of saved images see the “Save Settings: Destination” section.**

2. When a video is being recorded a timer will be displayed on screen showing the length of the video and the record video

button  will change to a red stop button.

3. To stop the recording press the stop button.

Output to HDMI Display

1. To display image on an external HDMI monitor/display, plug in HDMI cable to the port located on the back of the SeBa2 display and then plug HDMI cable into monitor.

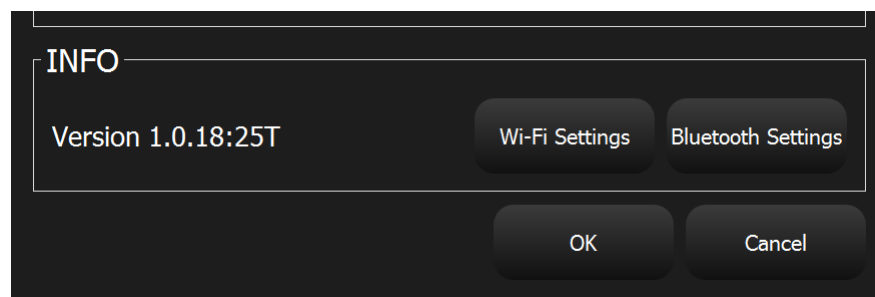
***Note: HDMI cable not supplied with SeBa2 Digital Imaging System.**

7. Advanced Software Settings and Controls

WiFi Network Connection

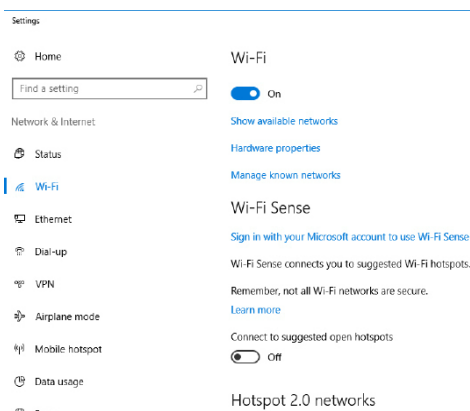
The SeBa2 can connect to a wireless network using the built in WiFi capabilities. This enables the use of the SeBaShare cloud for image storage and sharing capabilities. Refer to the SeBaShare section for additional details.

1. Click on the gear icon at the bottom of the screen to open the User Settings window. Under the “INFO” section click on the “WiFi Settings” button.

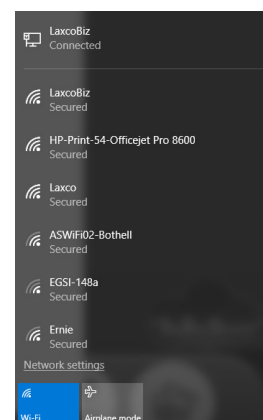


2. The Windows Setting window will open up on screen.
3. Under “WiFi” ensure that WiFi is on.
4. Click on “Show available networks” to open an new window in the bottom right corner of the screen listing the available WiFi networks.
5. Choose the appropriate network and enter password if required.
6. The SeBa2 is now connected to your WiFi network and can communicate with the SeBaShare Cloud.

2 -> 3 -> 4



5

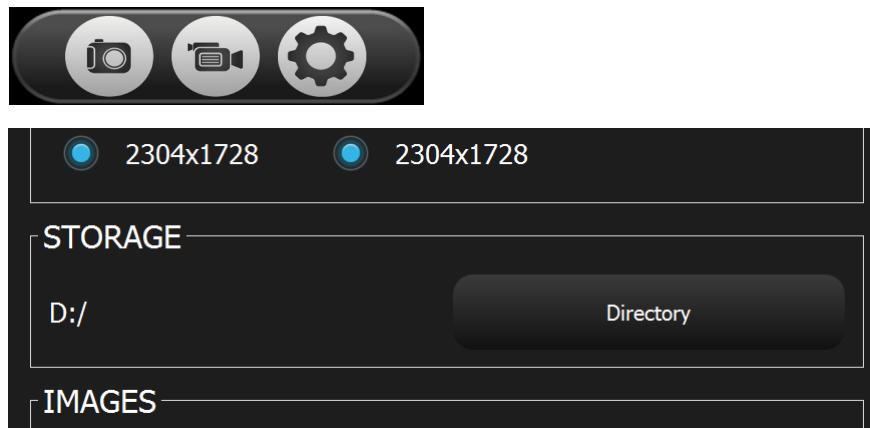


7. Advanced Software Settings and Controls

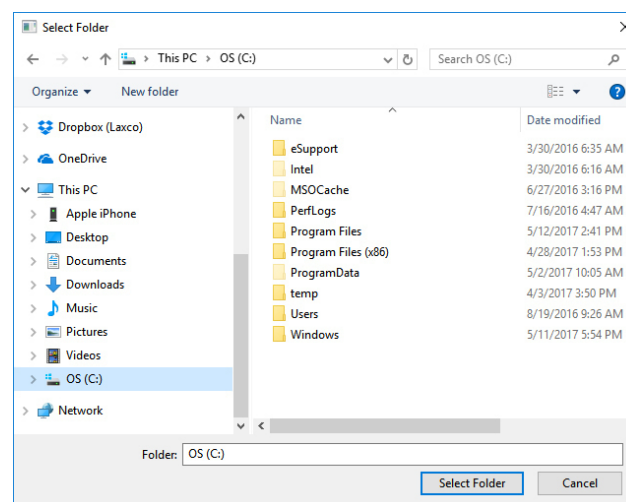
Save Settings: Destination

The SeBa2 can save images to the internal storage or a USB Drive. This setting must be made prior to saving an image to ensure the image is saved in the desired destination.

1. Click on the gear icon at the bottom of the screen to open the User Settings window. Under the “INFO” section click on the “Directory” button



2. The Windows “select Folder” window will open up on screen.
3. Browse to the appropriate folder or USB drive and choose “Select Folder”. This will now be the default location for all captured images.



4. To change the directory at any time, repeat the steps above.

7. Advanced Software Settings and Controls

Save/Preview Settings: Resolution and File Type

The SeBa2 can has separate resolution values for the Preview (live) image and save images. Captured images can be saved as .jpg, .png and .tiff. This must be set prior to capturing and image. For a full resolution, non-compressed image .tiff is the recommended file type.

1. Click on the gear icon at the bottom of the screen to open the User Settings window. Under the “Camera” section you will have access to the Preview/Capture Resolution and file type settings.



CAMERA

Preview Resolution	Capture Resolution	File Type
<input type="radio"/> 1024x768	<input type="radio"/> 1024x768	<input checked="" type="radio"/> jpg
<input type="radio"/> 1280x720	<input type="radio"/> 1280x720	<input type="radio"/> png
<input type="radio"/> 1600x1200	<input type="radio"/> 1600x1200	<input type="radio"/> tiff
<input type="radio"/> 1920x1080	<input type="radio"/> 1920x1080	
<input checked="" type="radio"/> 2304x1728	<input checked="" type="radio"/> 2304x1728	

STORAGE

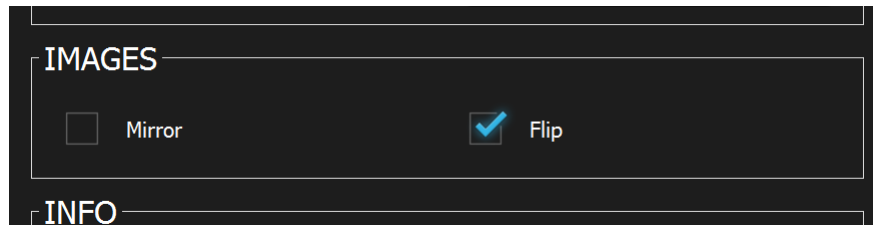
2. Choose the preferred resolution for Preview and Capture. These resolutions do not need to be the same.
3. Choose the file type for captured images.

7. Advanced Software Settings and Controls

Image Orientation

The Image Orientation is factory set and does not need to be changed unless you want the image displayed in an alternative orientation.

1. Click on the gear icon at the bottom of the screen to open the User Settings window. Under the “IMAGES” section you will have access to the “Mirror” and “Flip”.



2. Select the combination of Mirror and Flip until the image is displayed properly.

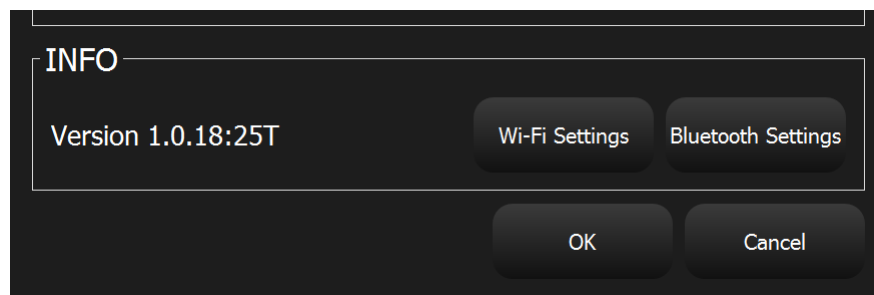
7. Advanced Software Settings and Controls

Bluetooth Settings

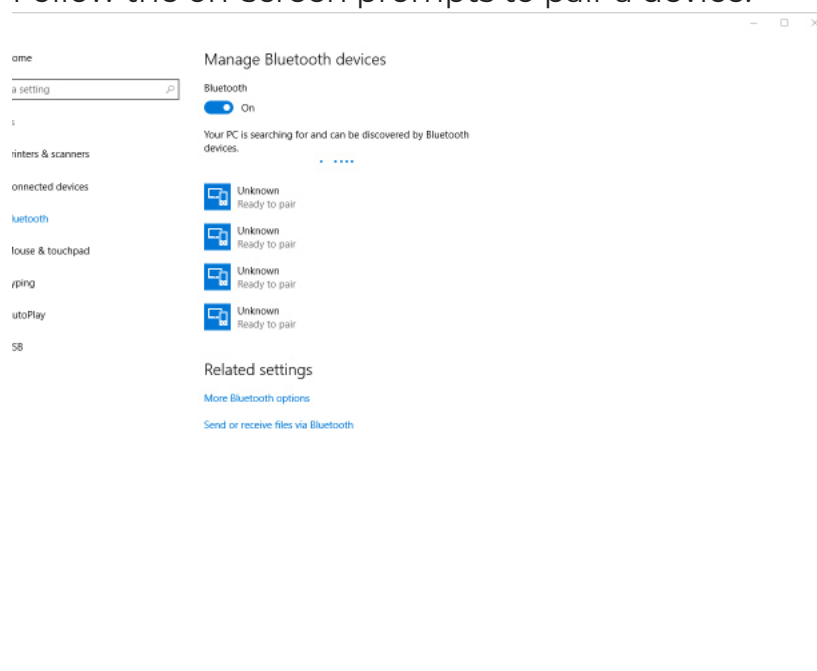
The SeBa2 can be connected to a Bluetooth device such as a keyboard or a mouse.

****Note: This does not permit saving images to a Bluetooth device.***

1. Click on the gear icon at the bottom of the screen to open the User Settings window. Under the “INFO” section you will have access to the “Bluetooth Settings”.



2. Clicking on “Bluetooth Settings” opens the Windows Bluetooth setting window. Follow the on-screen prompts to pair a device.



7. Advanced Software Settings and Controls

Camera Settings

1. Exposure

- Auto Exposure: Click box to enable Auto Exposure.
- AE Adjustment: When AE is active this slider will increase or decrease the AE target brightness.
- Exposure: When in manual exposure mode adjust slider until desired exposure is achieved.
- Gain: Increasing the Gain will increase the signal or brightness of the displayed image but will also magnify the camera noise. It is recommended only to use gain for low light situations when required.

2. White Balance and Color Temperature

- To perform a white balance of the camera, focus on a slide, move to a blank space on the slide (ideally on the coverslip), then click the “WB” button.
- To manually adjust the color temperature of the image adjust the slider until desired color temperature is achieved.
- Note: Color Temperature setting slider and White Balance function work independently. The Color Temperature slider will override a previously established White Balance.

3. Image Adjustment

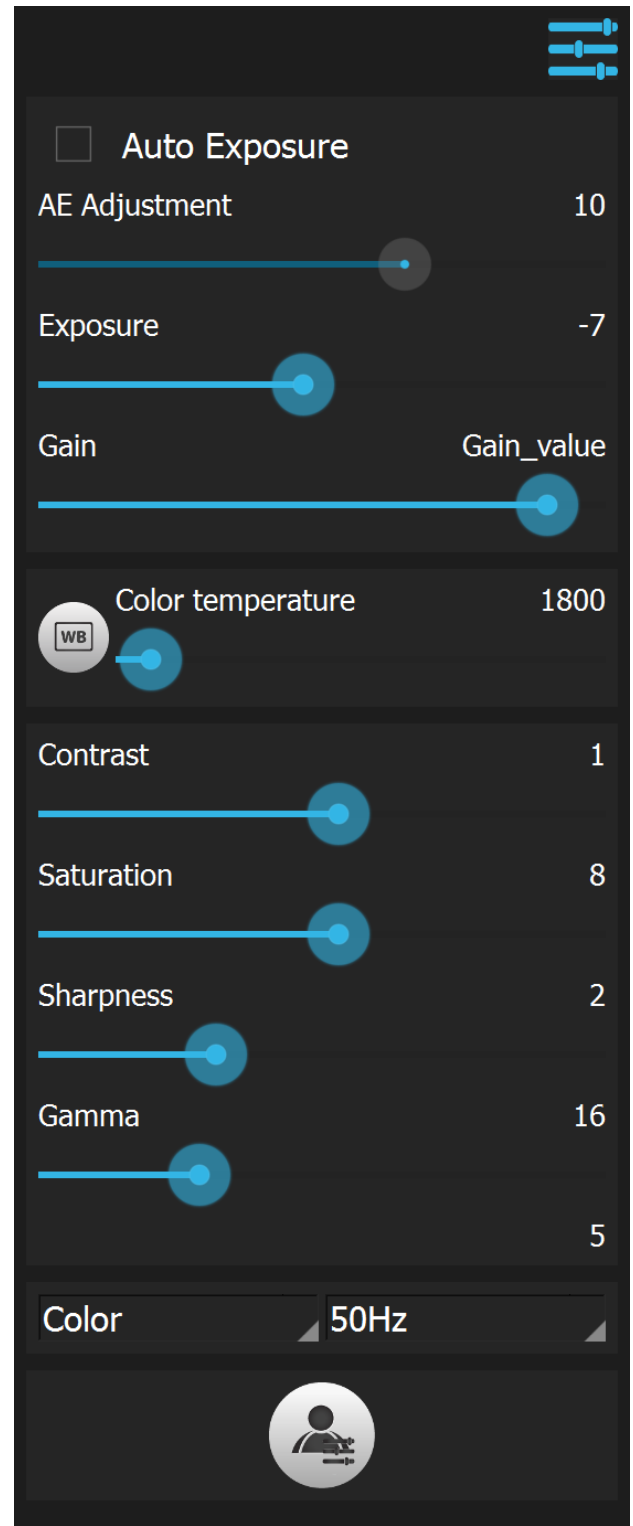
- Contrast: adjusts image contrast.
- Saturation: adjusts color saturation.
- Sharpness: adjusts image sharpness.
- Gamma: adjusts gamma of image.

4. Color Mode

- Allows you to change color mode between, color, monochrome and negative.

5. Power Frequency

- This can eliminate stroboscopic phenomenon but is rarely needed or used. Select between 50Hz, 60Hz or DC to eliminate stroboscopic effect.



7. Advanced Software Settings and Controls

Camera Settings (Cont.)

6. *Restore Defaults*

- To restore the settings in the Effects window to defaults click the “Restore Defaults” button.
- Note: This only applies to WB, Color Temperature, Contrast, Saturation, Sharpness, Gamma, Color Mode, and Power Frequency.

7. *User Presets*

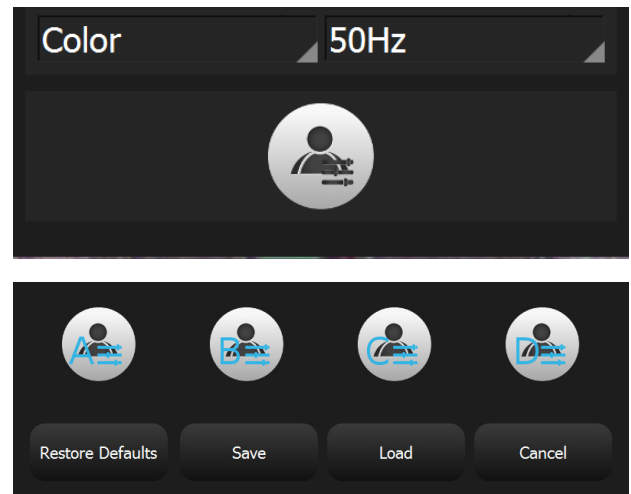
- Click on the User Preset button to open the user Preset Window.
- There are 4 available user presets; A, B, C, D.

8. *Save new Preset*

- To save a new preset, first make all of the necessary adjustments to the effects settings prior to opening this window.
- Click desired preset button, selected button will be highlighted.
- Click “save” to save current settings as selected preset.
- Note: This only applies to WB, Color Temperature, Contrast, Saturation, Sharpness, Gamma, Color Mode, and Power Frequency.

9. *Load Saved Preset*

- Click desired preset.
- Click Load.
- Setting for this Preset will be uploaded to the Effects settings.
- Note: This only applies to WB, Color Temperature, Contrast, Saturation, Sharpness, Gamma, Color Mode, and Power Frequency.



7. Advanced Software Settings and Controls

Measurements

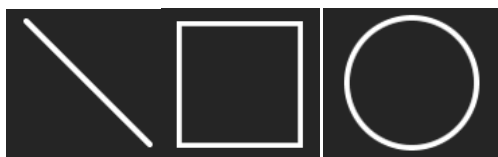
1. Calibration

The Seba2 will include basic calibrations prior to shipment. To add calibrations or re-calibrate the system follow the steps below.

***Note: Requires stage micrometer to complete process. Laxco PN: MP-SM100.**

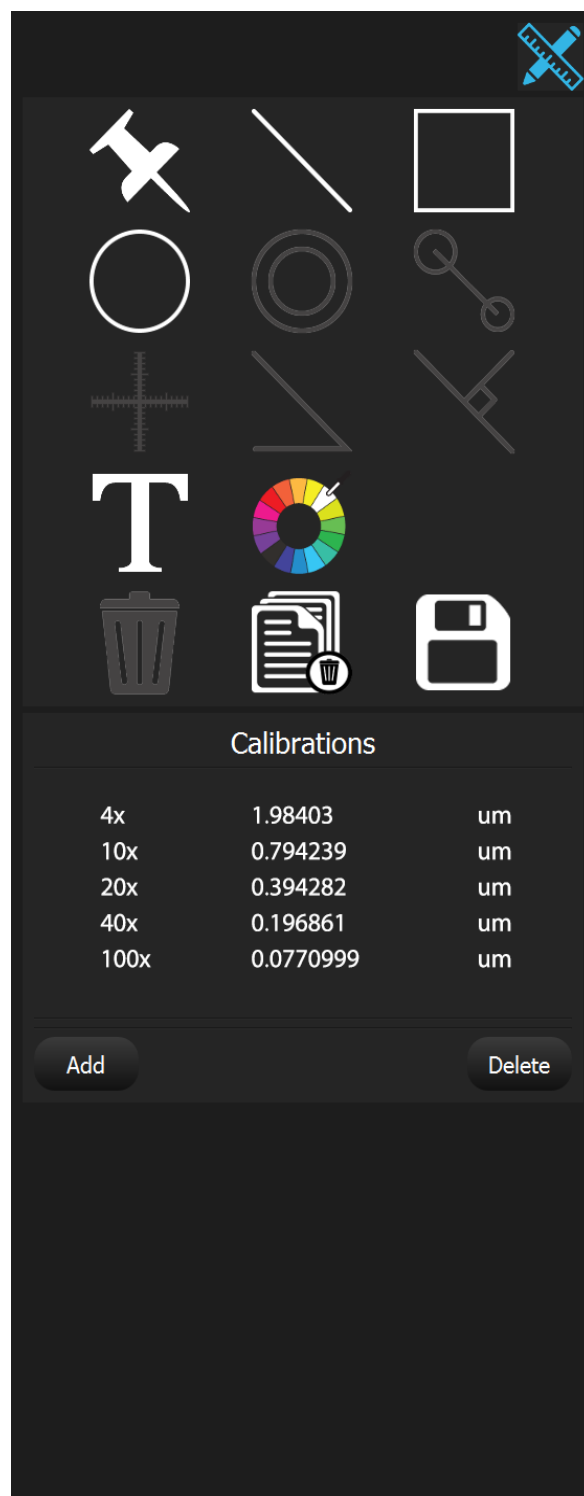
- Place stage micrometer on stage and focus on the linear scale using the objective you will be calibrating.
- Click “Add” under the Calibrations menu.
- Adjust the line on the image to match the length of the scale on the stage.
- Input the calibration name and length of the scale on the stage micrometer.
- Select the desired measurement units. (um, mm, inch etc.)
- Click “OK” to complete the calibration.
- The calibration should now be listed in the calibrations list.
- To delete a calibration, select the calibration in the list and click “Delete”.
- Calibration information can also be edited by double clicking on the calibration in the list.

2. Measurement Tools



- To perform a measurement (on live or captured image), first choose the magnification/calibration from the list of available calibrations.
- Select measurement type (Line, square/rectangle, or circle).

***Note grayed out measurements will be available in future versions of the SeBa2 software.**



7. Advanced Software Settings and Controls

Calibrated Measurement (Cont.)

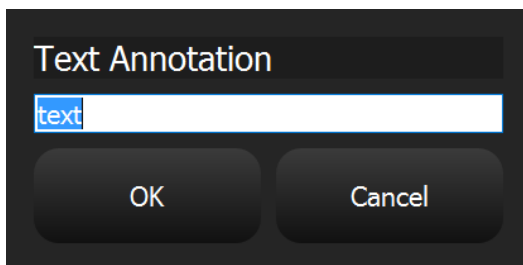
- The measurement will be placed in the upper left hand corner of the display.
- Move the measurement to desired location by clicking and holding the left mouse button or press and hold if using touchscreen.
- Adjust the size of the measurement by clicking and holding or pressing on the red square associated with the measurement and make necessary adjustments.

****Note: if the incorrect calibration was selected you can adjust all measurement on screen by clicking correct calibration and clicking anywhere on the image.***

3. Text Annotation



- Select the Text Annotation icon to open the Text Annotation dialog box.



- Enter annotation and click “OK”.
- Use the mouse or touchscreen to move the annotation to the desired location.

4. Point Counting



- Select the point counting icon to place a point in the upper left hand corner. This point will be numbered as “1”.
- Use the mouse or touchscreen to move the point to the desired location.
- Clicking the point counting icon again will add point numbered “2”. Add the desired number of points for your image.

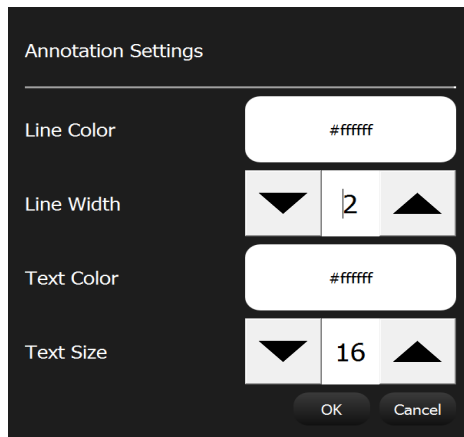
7. Advanced Software Settings and Controls

5. Measurement/Annotation Settings



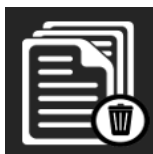
- Select the Settings icon to open up the Annotations Setting menu.

****Note: These settings will change all annotations and measurements.***



- Adjust Line Color, Line Width, Text Color and Text size and select "OK."
- Any current Annotations/Measurements will be updated to the new settings.

6. Delete All



- Select the Delete All icon to delete all annotations/measurements on the current image.

7. Save



- Selecting the "save" icon will save the current image with annotations/measurements at the current screen resolution.

****Note: The measurements/annotations are permanently "burned" into the image and cannot be removed.***

7. Advanced Software Settings and Controls

Gallery

1. View Captured Images

- Select the Gallery icon at the bottom right corner of the screen to open the Gallery menu.
- Images that are currently saved in the directory selected in “User Settings” (Pg.16) will be displayed in the Gallery menu.
- To open/view image double click/tap the image you want to view.
- To delete an image from the directory/gallery simply select an image and click the “Delete” button.

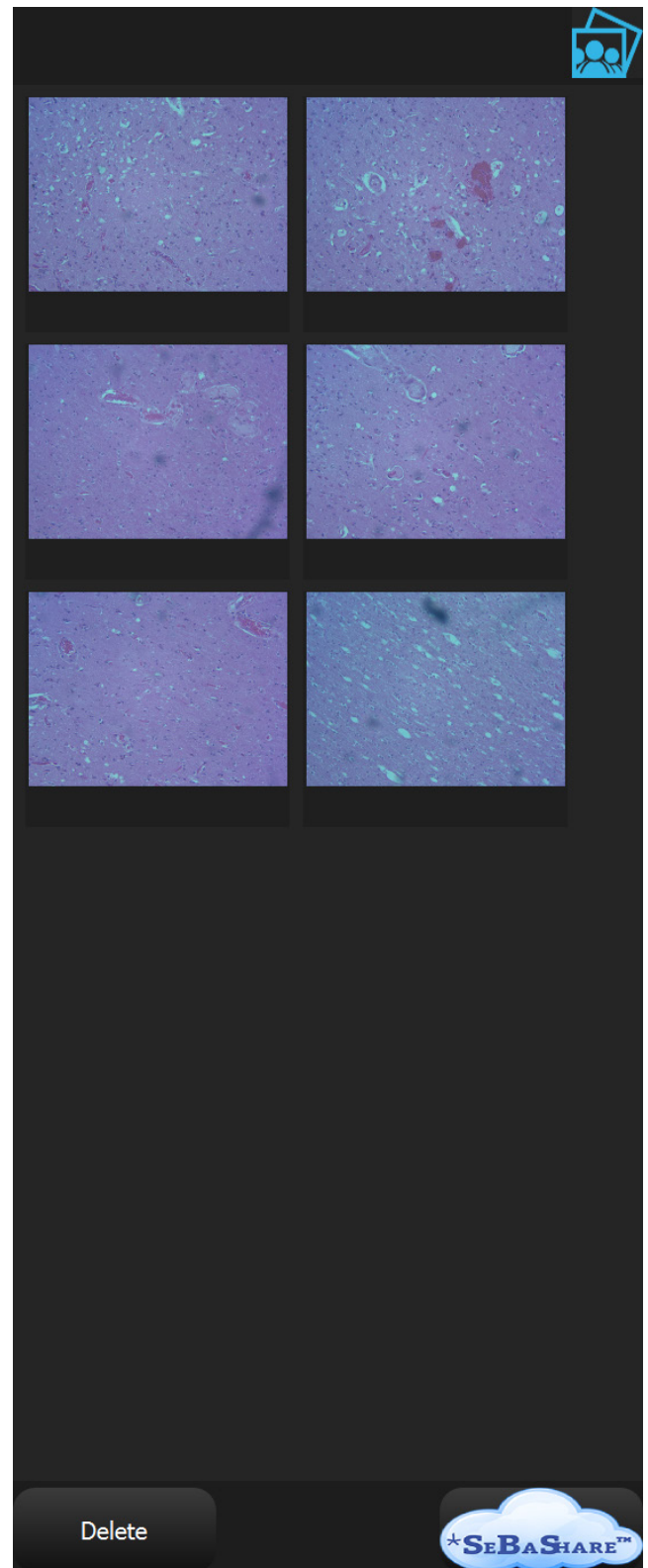
2. Upload to SeBaShare

- To choose a single image, tap the desired image in the gallery.
- To choose multiple images press/left mouse click and hold on the first image you want to select and drag your finger to highlight additional images.
- To upload the image(s) to SeBaShare, tap the SeBaShare cloud icon in the lower right hand corner. Follow prompts on screen to complete upload to desired folder.



- If the SeBaShare icon is grayed out you are not currently logged in to a SeBaShare account. To login, refer to “SeBa Access Login” steps.

***Note: To return to a live image when viewing a captured image or movie, press the “Live” icon on the lower menu.**



7. Advanced Software Settings and Controls

3. *Return to Live Image*



- When a captured image from the gallery is displayed the “capture” button changes to a “LIVE” button.
- Selecting the “LIVE” button return the display to the live image.

4. *SeBaShare Upload*



- To choose a single image, tap the desired image in the gallery.
- To choose multiple images press and hold on the first image you want to select and drag your finger to highlight additional images.
- To upload the image(s) to SeBaShare tap the SeBaShare cloud icon in the lower right hand corner. Follow prompts on screen to complete upload to desired folder.
- If the SeBaShare icon is grayed out you are not currently logged in to a SeBaShare account. To login, refer to “SeBa Access Login” steps.

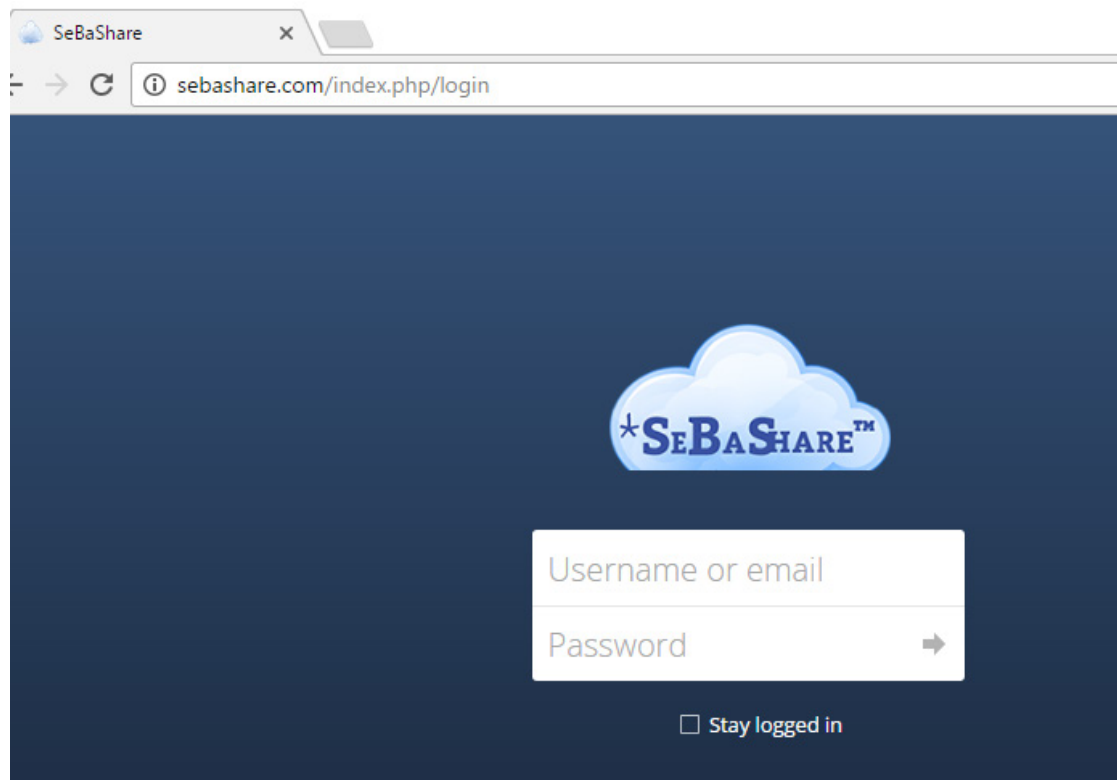
7. Advanced Software Settings and Controls

SeBaShare Website

The SeBa2 Digital Imaging System includes access to the SeBaShare Cloud for file storage and file sharing. To connect the SeBa2 system to your SeBaShare cloud refer to the “SeBaAccess Login” section.

1. *Initial Login/Account Setup*

- From a computer go to www.sebashare.com.
- Your initial username is located on a silver sticker located on your SeBaAccess card. The username is also your initial password to login to SeBaShare.
- Enter your Username and Password and click the arrow or press enter key to login.



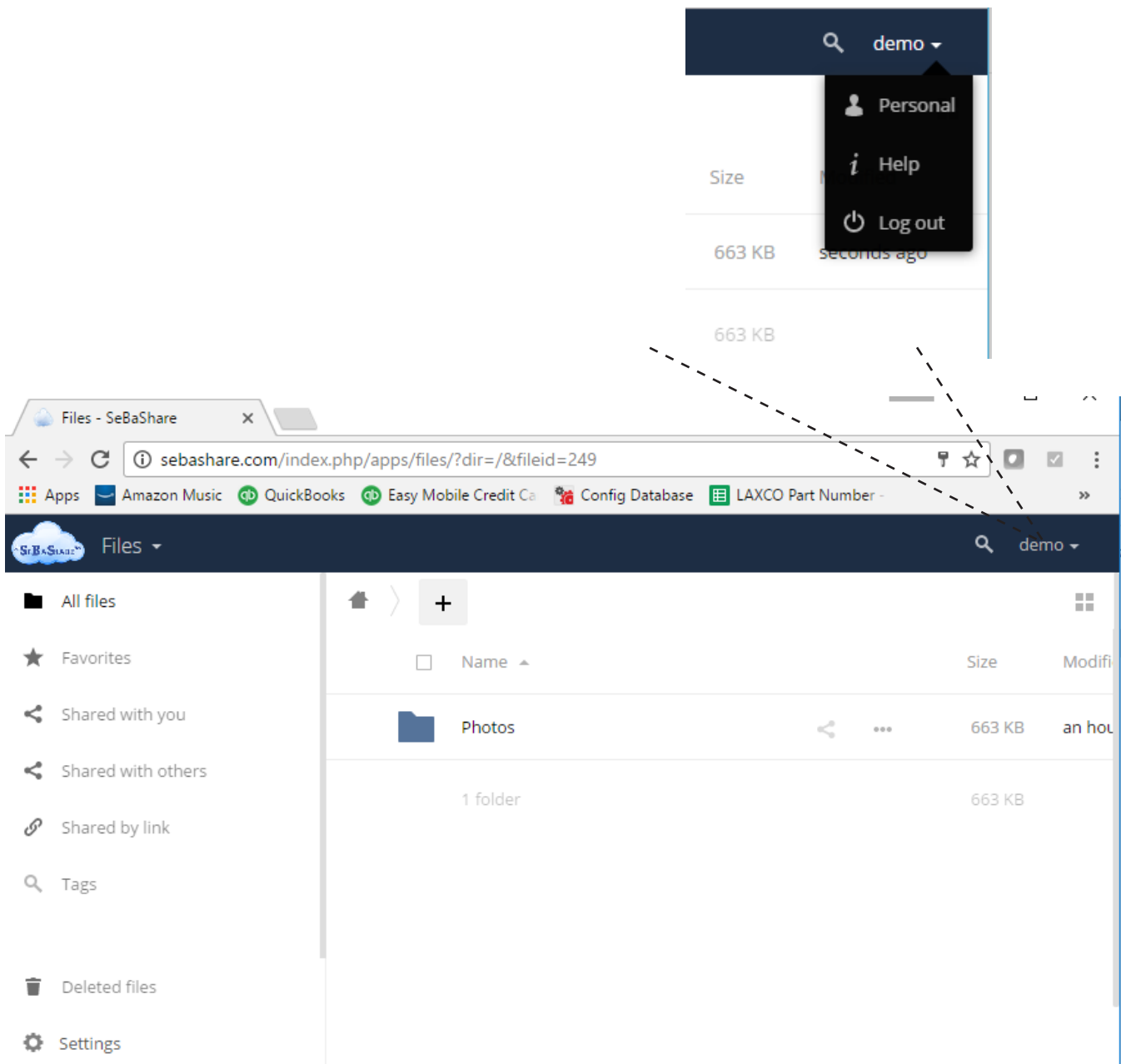
The screenshot shows a web browser window with the address bar displaying sebashare.com/index.php/login. The page has a dark blue background. In the center, there is a light blue cloud logo with the text "SEBAShare™" inside. Below the logo, there are two white input fields: "Username or email" and "Password". To the right of the "Password" field is a right-pointing arrow button. Below these fields is a checkbox labeled "Stay logged in".

7. Advanced Software Settings and Controls

2. Changing Username and Password

- To access your personal account settings click on the drop down menu in the upper right hand corner of the SeBaShare homepage and choose “Personal”.
- On the “Personal” page enter your email address. This can now be used as your new username for logging in to SeBaShare.
- Enter a new password and click “Change Password”.

****Note: For detail instructions on the use of SeBaShare click on the drop down menu in the upper right hand corner of the SeBaShare homepage and choose “Help”.***



8. Microscope Controls



Illumination Adjustment

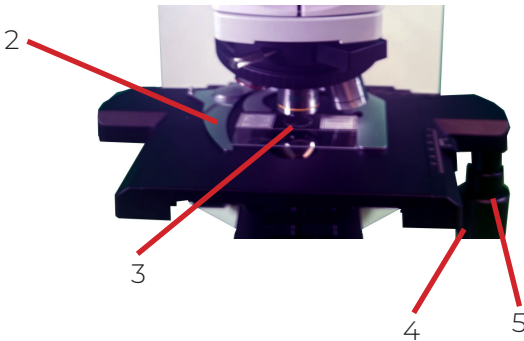
1. Turn on the microscope.
2. Adjust the light adjustment knob (1) to a desirable level . Rotate the light adjustment knob (1) toward the thick side of the indicator to increase brightness, and towards the thinner side of the indicator to reduce brightness.

Placing Slide on Stage

1. Open the stage clip (2) of the specimen holder.
2. Place the side with the specimen facing up; carefully release the stage clip (2) onto the slide (3).

****Note: Specimen holder can hold up to two slides.***

3. Rotate the X-axis knob (4) and Y-axis knob (5) to center the slide.



Focus Adjustment

1. Place slide on stage and rotate the 4X objective into the light path.
2. Loosen the stage height locking screw if necessary (6). Rotate the coarse focus knob (7) until the image is in focus and tighten the stage height lock (6).

****Note: The stage height lock screw can prevent the objective from touching the slide when focusing.***

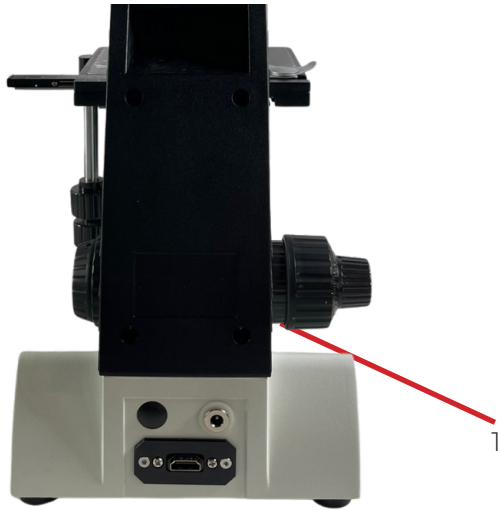
****Note: The stage height lock does not affect the fine focus knob.***

3. Adjust the fine focus knob (8) to improve the focus.

****Note: When observing with the 4X or 10X objective, open both the aperture diaphragm and field diaphragm to the maximum position and swing out the front condenser lens. See “Centering the Condenser” for condenser operations.***

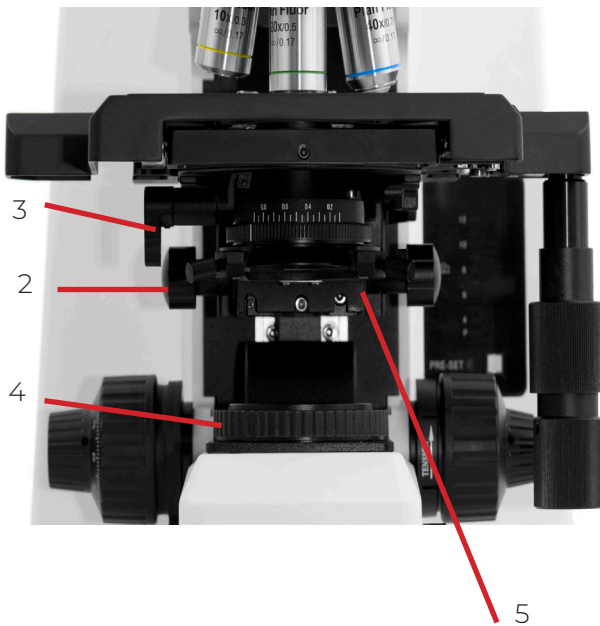


8. Microscope Controls



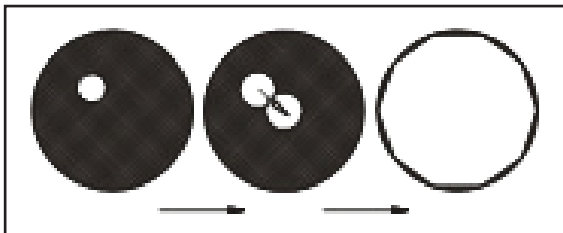
Focus Tension Adjustment

1. If the coarse focusing knob's tension is too high; or if the stage moves out of focus, adjust the focus tension (1) according to the indicator on the knob.

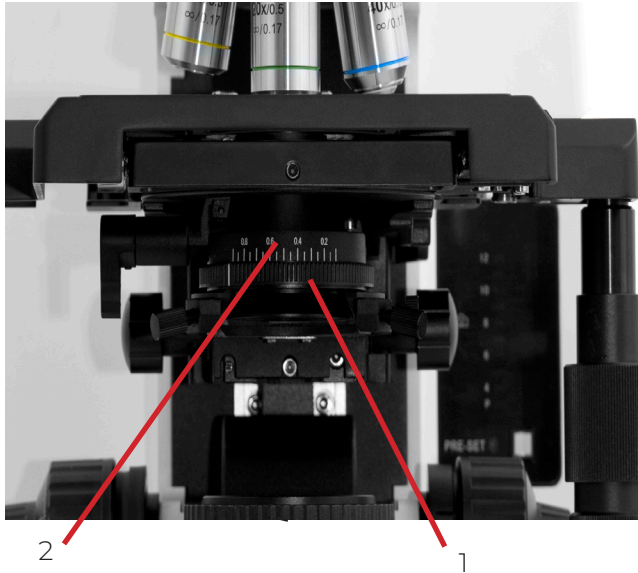


Centering the Condenser

1. Rotate the condenser knob (2) to raise it to the highest position.
2. Rotate the condenser swing-in lens (3) into light path.
****Note: Move the front lens of condenser into light path when the objective is 10X or above.***
3. Move the 10X objective into light path and focus the specimen.
4. Rotate the field diaphragm adjustment ring (4) to adjust the field diaphragm to the smallest position, the field diaphragm should be visible through the eyepieces .
5. Rotate the condenser adjustment knob to adjust the height of the condenser until the edges of the field diaphragm are in focus.
6. Adjust the condenser centering adjustment screws (5) to center the opening of the field diaphragm to the center of the field of view.
7. Open the field diaphragm gradually The image will not move away from the center if the condenser is centered properly.
8. The field diaphragm can be enlarged bigger than the field of view

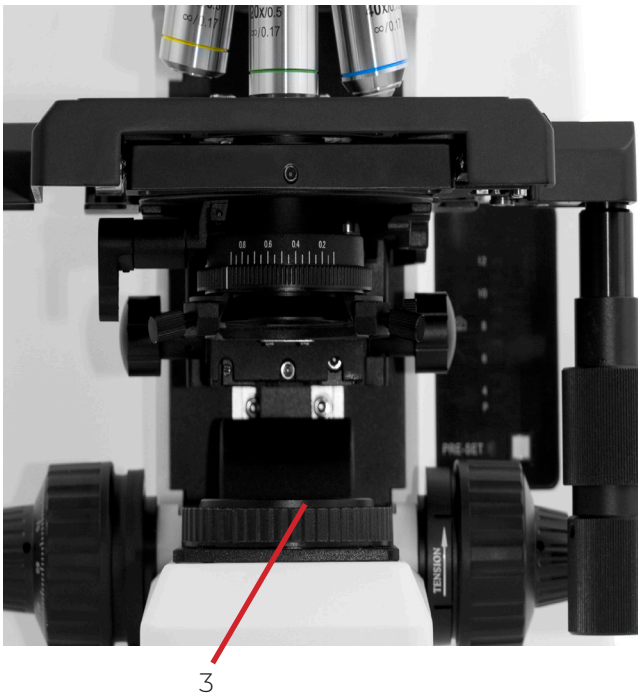


8. Microscope Controls



Adjusting the Aperture Diaphragm

1. The aperture diaphragm controls the numerical aperture (NA) of the illumination. A matching NA between the illumination and the objective will provide better resolution, contrast, and depth of field.
2. If your sample has low contrast, it is advised to adjust the condenser aperture diaphragm to be 70%-80% of the N.A. of objective. The eyepiece can be removed to observe this through the eyetube. Adjust the aperture diaphragm adjustment ring (1) until it matches the figure to the left.
3. Use of scale: set the scale of condenser N.A. to the 80% value of objective (2) N.A.
4. For example, use 40X objective (N.A. 0.65), set the scale of aperture diaphragm to $0.65 \times 0.8 = 0.52$.

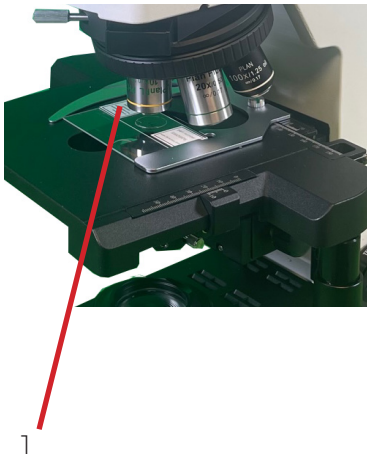


Adjusting the Field Diaphragm

1. By limiting the diameter of the beam entering the condenser, the field diaphragm (3) can prevent other light and strengthen the image contrast. When the image is just on the edge of the field of view, the objective can show the clearest image.

8. Microscope Controls

Using an Oil Objective (100X)



1. Use the 4X objective to focus the specimen.
2. Place a drop of oil (1) on the specimen
3. Rotate the nosepiece counterclockwise and rotate the oil objective (100X) to the light path.
4. Use the fine focusing knob to focus.
****Note: Make sure there are no air bubbles in the oil.***
5. After using, wipe the front lens with a tissue moistened with a small amount of alcohol or lens cleaner. Wipe the oil off of the specimen.
****Note: Avoid using another objective before removing the oil from the specimen.***

Assembly and Operation of Simple Polarization Components



1. Simple polarizing system includes polarizer (2) and 360° rotatable analyzer (3).
2. Remove the dust-cover if present from the filter slot (4) and insert the 360° rotatable analyzer (3) into the slot (4).
3. Place the polarizer (2) into the light well (5).
4. Rotate the 360° rotatable analyzer (2) to achieve polarizing observation.

9. Technical Specifications

Touchscreen	Microsoft Touchscreen Display
Camera	16MP Color CMOS Camera
Connectivity	WiFi Bluetooth 4 USB Ports HDMI
Seba Software	Capture Still images and video Upload images to SeBashare cloud Save to USB Drive Review, measure and annotate images
Optical System	Color corrected infinity optical system
Eyepiece	PL10X high eye-point plan eyepiece, field of view: 22mm.
Nosepiece	Reversed 4-position
Objective	Infinity plan achromatic objectives Infinity plan phase contrast objectives Infinity plan fluor objectives
Focus	Coaxial coarse & fine focusing system with limit-stoppper & tension adjustable. Travel range: 30mm. Height-adjustable bracket group stage. Fine focusing precision: 0.002mm
Stage	Double-layer mechanical moving stage. Area: 185×165mm. Moving range: 80x55mm. Accuracy: 0.1mm. Left or right hand position is optional.
Condenser	NA1.25 N.A condenser
Illumination System	100-240V wide range of voltage, transmission and reflection Koehler illuminator system, 3W LED

10. Customer and Technical Service

Returned Goods Policy for Repair or Replacement Parts

To return goods for repair or replacement, please contact Laxco Customer Service at 425-686-3081.

Please be prepared to supply the following information:

- Your name, return shipping address and telephone number
- Catalog/Model number of the item(s) you are returning
- Serial Numbers if applicable
- Description of the product's problem or reason for the return
- Date the item was purchased.

The Customer Service Representative will issue you a Return Materials Authorization (RMA) number. Please label the outside of your shipping container with this number.

Thank you for purchasing our products. If you are unable to complete the installation of your products, contact Laxco Inc. customer support or with suggestions or comments on this manual? We are always working to meet the needs of our customers, and we appreciate your suggestions.

Please e-mail us at info@laxcoinc.com, making sure to include the title of this manual and the pertinent page numbers.

For More Information on the SeBa2

Technical Service: 425-686-3081

Sales@laxcoinc.com

Website: www.laxcoinc.com

You Tube Link: <https://www.youtube.com/watch?v=hwo7XbuHPCY&t=99s>

11. Reference

KEY CONCEPTS

MAGNIFICATION

Magnification increases the apparent size of an object you are viewing through the microscope. Magnification by itself does not provide more information about an object unless there is also adequate resolution and contrast. The objective lens and oculars (eyepieces) determine magnification.

Magnification = eyepiece value X objective lens value.

RESOLUTION

Resolution is the ability to distinguish small objects that are close together. Resolution becomes more difficult as objects become smaller and closer together. At some point, the objects will “fuse” together and become indistinguishable. This point is the resolution limit of the microscope.

CONTRAST

Contrast of an object relative to the background is also necessary in order to resolve it. Without adequate contrast, it is impossible to distinguish an object from its background even when magnification and resolution are adequate.

Note: Resolution and Contrast are largely dependent upon specimen illumination. This involves using the illuminator (lamp), field iris, condenser iris, and objective lens. Optimal lighting for most specimens is achieved through Koehler illumination. Collector lens filters may also enhance image quality.

FIELD OF VIEW

The actual diameter of the observable field in the sample varies with the field number of the eyepiece and the magnification of the objective. It can be calculated by dividing the field number of the eyepiece by the magnification of the objective.

Example:

For an 18 mm field number eyepiece and a 10x objective, $FOV = 18 \text{ mm} / 10 = 1.8 \text{ mm}$ field of view.

12. Glossary of Microscopy Terms

A

Abbe Condenser: A simple condenser comprised of two lenses; corrects for chromatic aberration.

Aberration: Term used to describe any inaccuracy in focusing of light; derived from physical limitations of lenses and optics.

Aberration Chromatic: Inaccurate focusing of red, green and blue light.

Aberration Spherical: Inaccurate focusing of light due to curvature of lens surface.

Achromat: A classification of microscope objectives with simpler lenses corrected for chromatic aberration by bringing red and blue light to the same point of focus.

Aplanatic Achromatic: A term describing the level of optical correction; typically used for condensers. This term indicates that the optical device is corrected to produce a flat field (aplanatic) as well as to accurately focus red, green, and blue light (achromatic).

B

Beam Splitter: An optical device that divides the incoming light beam. The beam can be divided either in terms of wavelength (for example, reflecting shorter wavelengths but transmitting longer wavelengths) or partial reflection (for example, from a partially mirrored surface or at the angled surface of a prism).

C

C-mount: A type of camera adapter typically used to connect video cameras to a microscope.

CCD: Type of video camera using electronic chips as the detector. CCD stands for charged-coupled device. Light falling on the chip creates an electrical charge at a specific location.

Centering Telescope: A special eyepiece fitted with a focusing mechanism and a longer-than-usual focal length, used for observing the back focal plane of an objective. The centering telescope can be used for aligning components for phase contrast.

Concave: Referring to a lens surface; bowing inward.

Condenser: The optical component located nearest the specimen but between the specimen and the light source; responsible for the placement and angle of light approaching the specimen.

Contrast: Visibility of an object or feature against its background. Convex: Referring to the curve of a lens surface; bowing outward.

Coverslip Thickness: The thickness of a piece of glass used to protect and contain the sample on the slide. This value, usually 0.17 mm, is an optical requirement of the microscope, usually engraved on the barrel of the objective.

12. Glossary of Microscopy Terms

D

Depth of Field: The vertical distance in the sample through which features are simultaneously in focus. High numerical aperture objectives have a “shallow depth of field” and image only a very thin slice of information from the specimen.

Diffraction: Bending of light at the edges of features.

Dioptr Setting: The focus of one eyepiece with respect to the other to compensate for differences in focus between the microscopist’s eyes. A critical setting to avoid eyestrain and headaches.

E

Eyepiece: The optical component that provides the second step in magnification.

Eyepoint: The back focal plane of the eyepiece; the location at which the cornea of the eye is placed so that the information from the microscope can be imaged on the retina of your eye.

Eyepoint, High: A special optical design in which the eyepoint is raised about 18 mm above the top surface to accommodate people who wear eyeglasses.

F

Field Aperture: The opening controlling the diameter of the field to be illuminated. The opening is controlled by an iris called a field iris.

Field Curvature: One of the optical distortions. When the center of the image is in focus and the edges the field is said to “have curvature.” When the image is in focus from the center to the edges, the field is said to be “flat.” Plan objectives are corrected to produce flat fields.

Field Number: A number, usually engraved on an eyepiece, referring to the diameter of a baffle or raised ring inside the eyepiece. Determines the viewing field for the eyepiece. See also “Field of View.”

Field of View (FOV): The actual diameter of the observable field in the sample; varies with the field number of the eyepiece, magnification of the objective and other intervening optics.

Filter: A device that changes either the intensity or the wavelength of light interacting with it.

Flatfield: See “Field Curvature.”

Focal Length: Distance between the optical center of a lens and the point at which it will focus light coming from infinity.

Focal Plane: An imaginary, two-dimensional plane at right angles to the optic axis, comprised of an infinite number of focal points. Since an image can only be formed when light comes to a focus, the focal plane can be thought of as an imaginary “screen” on which the image is formed.

12. Glossary of Microscopy Terms

H

High Eyepoint: A design characteristic of eyepieces in which the back focal plane of the eyepiece is raised about 18 mm above the top of the eyepiece to accommodate microscopists who wear glasses.

I

Illumination, Axial: A contrast-enhancement technique for improving edge contrast. The condenser is closed most of the way, producing a highly coherent cylinder of light.

Illumination, Phase Contrast: A contrast-enhancement technique used to image phase objects. Using a specifically designed annulus or ring placed at the front focal plane of the condenser, the zero-order background light is carefully placed into a special optical device (phase plate), mounted in the back focal plane of the objective. The phase plate has two functions: it reduces the intensity of the background light to approximately 15% of its original value and decreases its phase by one quarter of a wavelength. The light passing through a well-behaved phase-altering specimen (such as a cell) slows down by a quarter wave on that interaction, then another quarter-wave as it passes through the thickest part of the phase plate. As a result, when it meets the background light at the primary imaging plane, it is a half-wave out of step. The resulting destructive interference enhances contrast, making the object more visible against the background. Phase images often suffer from bright haloes at the edge of fine detail, partially due to the background light scattering when it hits the edge of the phase plate.

Image: The focusing of light in an organized fashion to reproduce information collected from the object. The more accurately the light is focused, the more accurately the object is represented in the image.

Image Analysis: Any type of measurement performed on the image, ranging from particle sizing and counting to determinations of motility or field-specific parameters such as orientation.

Immersion Medium: The material used between the uppermost surface of the sample and the objective. The immersion medium could be air, water, immersion oil, etc.

In Phase: A relationship between waves of light. When waves are “in phase,” they must be coherent (come from the same source, have the same wavelength, travel in the same direction, at the same point in time, vibrating in the same plane) and will reach their peak and fall to their trough at the same time. They are, literally, in step with each other. Phase annuli must be centered to produce in-phase images.

12. Glossary of Microscopy Terms

Infinity Corrected Optics: A special optical design involving at least two lenses in which the object is placed at the focal plane of the first lens, causing the imaging rays to emerge parallel to the optic axis or some principal ray. Since the emerging rays never focus to make an image, they are said to be carrying that information to “infinity.” The second lens then picks up the information as sets of parallel rays, and brings them into focus at its back focal plane. In a microscope, the objective is the first lens, the eyepiece lens is the second.

Interpupillary Distance: The physical distance between centers of the pupils in the microscopist's eyes; usually measured in millimeters.

Iris, Condenser: Iris controlling the angle at which light emerges from the condenser and approaches the sample; located at the **front of the focal plane of the condenser.**

Iris, Field: Iris controlling the size of the illuminated field in the sample. Usually located around the light port. Reducing the size of the field iris is one technique for controlling haze and glare.

K

Koehler Illumination: An approach to microscope alignment that separates the illuminating set planes from an imaging set. A major goal of this approach is to illuminate evenly the back focal plane of the objective for maximum resolution and evenly illuminated background.

L

Light, Polarized: Light in which the waves vibrate in only one direction, perpendicular to the direction of travel.

Long Working Distance (LWD): A term used for specially designed objectives and condensers providing more clearance between the surface near the sample and the sample itself. Especially useful for tissue culture and microtitre work.

M

Magnification: A mathematical relationship between the size of an image and the size of the original object. If the image is larger than the object, the term used is “magnification”; if the image is smaller than the object, the term used is “minification.”

Micron: A unit of length measurement. A micron (or micrometer) is 10^{-6} meters (0.000001 meters) or about 1/25,000 of an inch.

Microscope, Compound: A microscope providing magnification in two stages, the first through the objective and the second through the eyepiece.

Microscope, Inverted: A standard microscope configuration in which the sample is viewed from below. Especially useful when working with very thick samples such as tissue cultures and microtitre plates.

12. Glossary of Microscopy Terms

Microscope, Stereo: A standard microscope configuration based on two independent imaging paths, separated by approximately 10-12 degrees, resulting in a stereoscopic image characterized by great three-dimensionality and great depth of field. Frequently uses lower power (0.5 to 300x total magnification). and for setting the limit of resolution for the entire system.

Microscope, Upright: A standard microscope configuration in which the sample sits face up on a stage, with the objectives mounted above it.

Microscopy: The art and science of making fine detail visible. The four major issues in microscopy today are magnification, resolution, contrast and measurement.

N

Neutral-Density Filter (ND): An amplitude object; an object that absorbs all colors of light equally.

Numerical Aperture (N.A.): A measure of information-collecting ability of a microscope optic. The numerical aperture is a product of the sine of half of the collecting angle and the refractive index of the immersion material. The greater the N.A., the better the resolving ability.

P

Phase: A property of light in which waves are “in step” with each other. See also “In phase.”

Phase Contrast: A contrast-enhancement technique that detects phase objects. It uses a special ring, placed in the condenser to control location of the undiffracted light, and a matching phase plate, placed in the back focal plane of the objective. A well-behaved phase sample will slow light by approximately one-quarter of a wavelength compared to the undiffracted background light. The phase plate is especially engineered to slow the diffracted light another quarter-wave. When the undiffracted light meets the diffracted light at the primary imaging plane to form the image, they will be out of step with each other by a half-wave, creating the condition of destructive interference and resulting in the darkening of the phase object and an improvement in contrast. Phase kits include a green filter (usually about 546 nm), which defines the wavelength for which the kit is optimized.

Photo Adapter: A special tube enabling cameras to be attached to the microscope.

Pixel: An electronic term used to describe the points of information used to map an image on a TV screen or computer monitor. Literally, a picture (“pix”) element (“el”). Each pixel carries at least the x,y location in the map and an intensity value (based on 256 gray levels).

Plan: An optical correction for objectives and condensers, indicating that the optical component has been corrected to produce a flat viewing field.

12. Glossary of Microscopy Terms

Primary Image: The first magnified image formed in the microscope.

Primary Image Plane: The location of the first magnified image formed by the objective.

R

Refraction: Bending of light as it passes, at an angle, across a boundary between materials of different refractive index; governed by Snell's Law.

Refractive Index (n or n_i): A number describing the relationship between the velocity of light in a material of interest compared to the velocity of light in a vacuum or air. The slower the velocity in the material, the higher the refractive index.

Resolution (R): The smallest distance by which two objects can be separated and still be imaged as two independent objects.

Reticle: A small disk with an engraved or photographic pattern such as a ruler or grid, placed in the eyepiece, in the primary image plane, so that the pattern will superimpose on the image of the specimen. Used for measurement (length, angle, counting, etc.).

S

Snell's Law: The law governing refraction, relating to angles of approach and exit as light passes, at an angle, from one material to a second material of different refractive index. Snell's Law states that light will bend toward the normal (an imaginary reference line drawn perpendicular to the surface at the point of entrance) as it passes from lower to higher refractive index

T

Trinocular Port: A special eyepiece, usually narrower in design than conventional eyepieces, used in the photo tube of the microscope to project a real image to the film plane or detector of a camera system.

Tube Length, Fixed: An optical design approach in which the object is placed at some distance in front of the objective, causing the image to focus at a specific distance behind the objective. Typical distances for the mechanical tube length in these systems are either 160 mm or 170 mm.

Tube Length, Mechanical: The distance from the objective shoulder to the seat of the eyepiece. When replacing objectives, the mechanical tube lengths must match.

Tube Length, Optical: The distance between the back focal plane of the objective and the primary image plane.

Tube Lens: In infinity corrected optics, a lens that works along with the objective to form the image at the primary image plane. See also "Telan lens."

W

Wavelength: The distance along a wave from peak to peak or trough to trough. In microscopy, wavelength is often correlated to the color and energy of light.

White light: Light containing all three primary colors: red + green + blue.



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