Zeiss Axiovert 25 Microscope Operation

Introduction:

A microscope is an instrument that magnifies an image and allows visualization of greater detail than is possible with the unaided eye. Microscopes are usually classified according to their light source: visible light (LM-light microscope); polarization, phase contrast, interference, ultraviolet, and x-ray microscopes and electron beam (EM). [1]

Operate a microscope: (see figure on next page)

1. Turn the lamp on (14)
2. Place slide or tissue culture dishes/flasks on the tissue stage (3), make sure the part you want to view is over the hole in the center of the stage
3. View sample through the eyepiece (1)
4. Adjust the light intensity with illumination control (5)
5. Adjust the focus with focusing drive (22, 21), can use a smaller objective to focus first and then switch to the desired objective and do fine focus (21)
6. Move the sample to get area of interest on the sample
7. Adjust phase contrast filter to get increase contrast
8. Turn the camera switch to take an image on the computer (20)

Note: Always use day light filter (blue filter on top),

Operate the camera/computer:

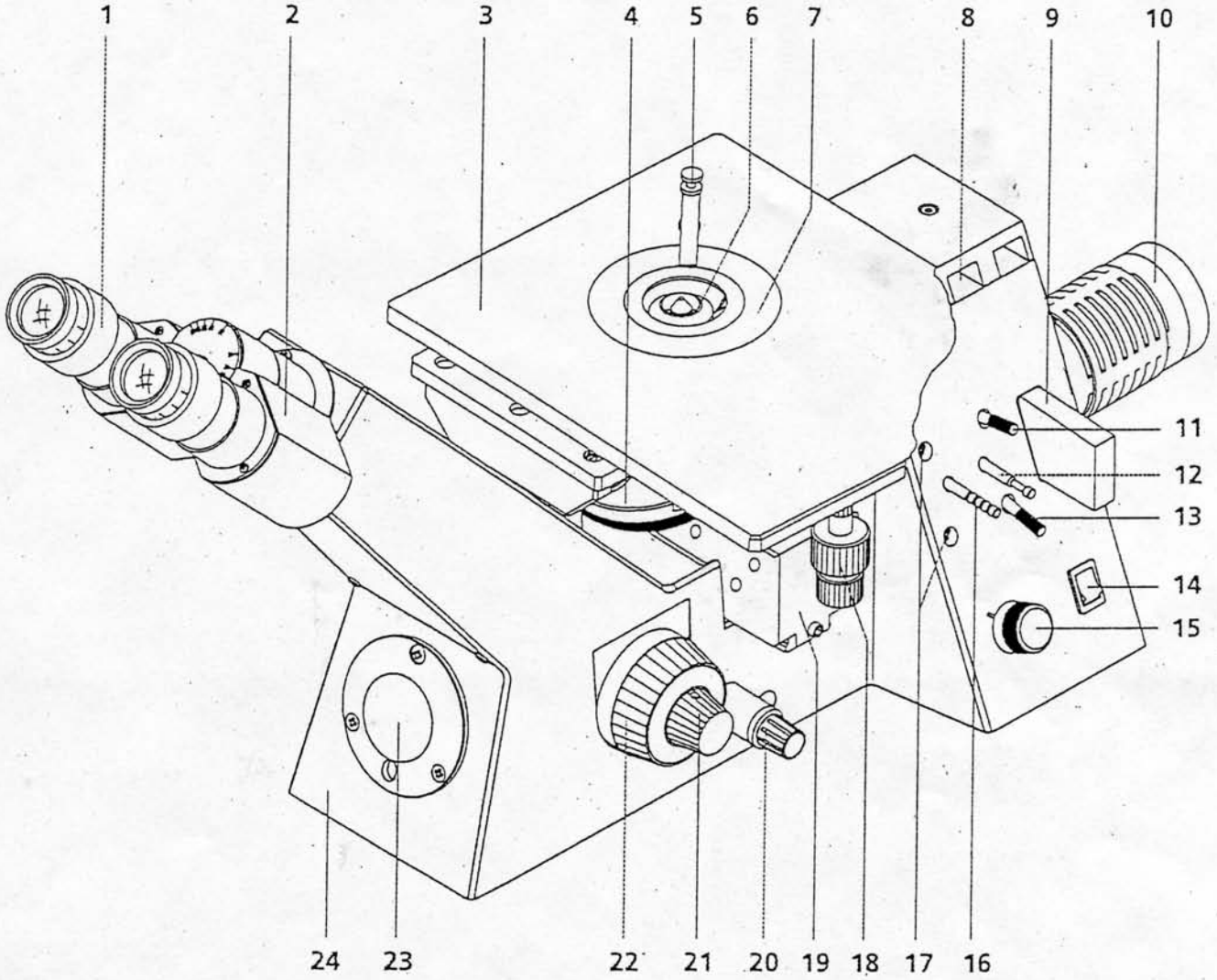
1. Open AxioVision on the computer
2. Turn on live view
3. Adjust the focus with the live image (focus between the eyepiece and cameras are sometime different). Note: focus bar can be used to assist focusing (right click the image ->focus bar)
4. Adjust the exposure time accordingly. Note: over/under exposure button can be used to assist with exposure (blue-white-red button on the lower left of the live image) Red indicate saturated spots (over exposed)
5. Adjust the color with white balance
6. Choose the correct objective to set the correct scale
7. Capture the image
8. Add scale bar
9. Save the image in Ziess format, and group images in an archive (This way all the attributes of the image are saved and can be changed later)
10. For experimental samples should take 1 to 2 image at 5x, 10x, 20x, and 32x objective
11. Optimize the image by using the image property window (right click on image -> properties)
12. If needed export the image to desired format (tiff, jpg)

Reference:

1. Laboratory Manual, The University of Vermont
2. Zeiss Axiovert 25 Microscope Manual

2 Operation

2.1 Control and function elements



1 Eyepiece
2 Binocular tube
3 Mechanical stage 30 x 30
4 Nosepiece (4x)
5 Stage clip
6 Objective
7 Glass stop
8 Compartment for DIC slider
9 Filter slider 2x
10 6V 25 W halogen lamp
11 Centering screw for aperture diaphragm
12 Pushrod for aperture diaphragm
13 Centering screw for aperture diaphragm
14 On/Off switch
15 Illumination control
16 Pushrod for luminous-field diaphragm
17 Centering screw for luminous-field
18 Coaxial drive for x-y stage movement
19 Reflector mount (3x)
20 Switch to change from observation to camera
21 Focusing drive (fine adjustment)
22 Focusing drive (coarse adjustment)
23 Front port camera/video
24 Microscope stand

1.2.2 Optical design

The state-of-the-art ICS (Infinity Color-corrected System) optics guarantee high optical performance for all methods (field number 23, tube factor 1x). Different combinations of objectives and eyepieces permit an optimum configuration for the intended application.

A universal port is available for documentation. Suitable adapter modules for photomicrography and video technology are offered.

The correct color temperature for color photography using artificial-light film is achieved automatically at the full lamp voltage.

A selector is used to set 100% of the light either for viewing or for documentation.

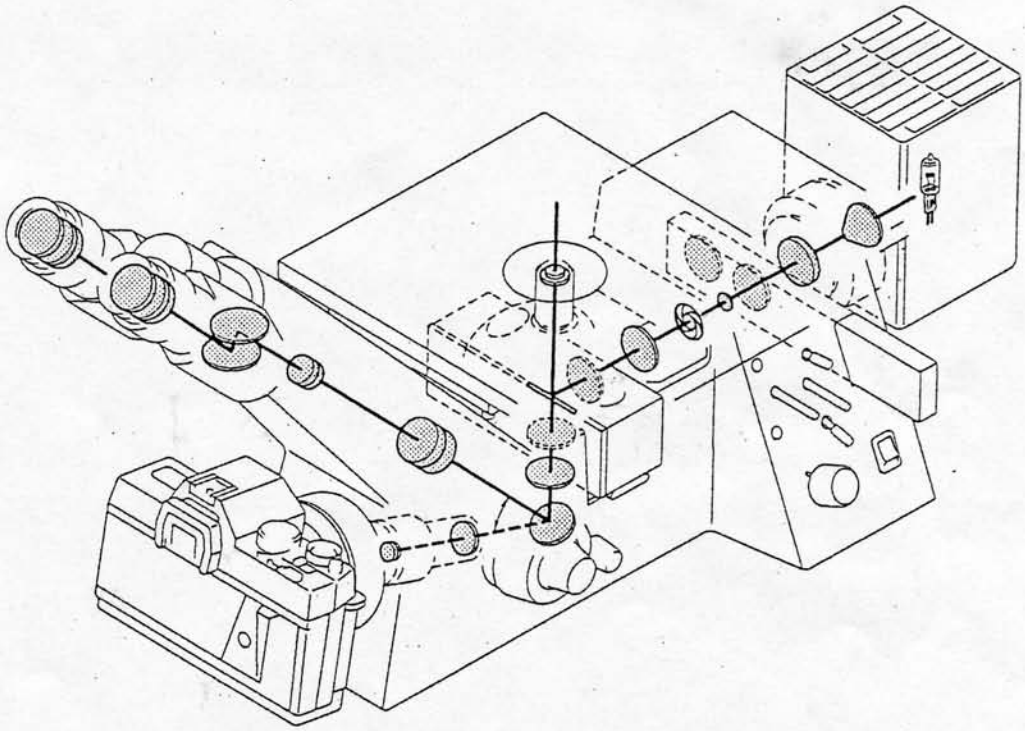
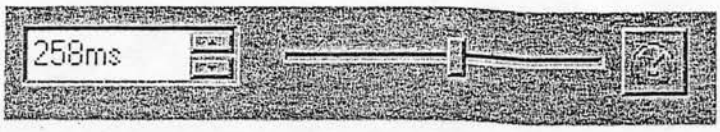


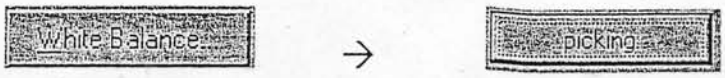
Fig 1-1 Optical design of Axiovert 25 CA

How do I grab a single image?

- Click on the Camera Properties icon  to switch to the camera control page.
- Make sure light is directed to the camera, and click the Live icon  .
- Click the Calculate icon  . The exposure will automatically adjust.
- Fine tune the exposure by adjusting the exposure slider or using the up and down arrows.



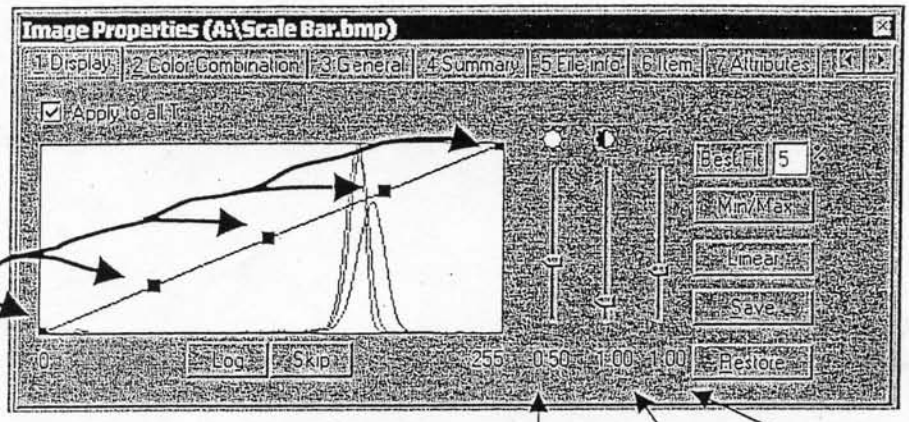
- In the case of a color camera click the White Balance button, which will change to picking… and click on an area that's white in the live image.



- Click the Snap icon  to capture the image.

How do I enhance captured images?

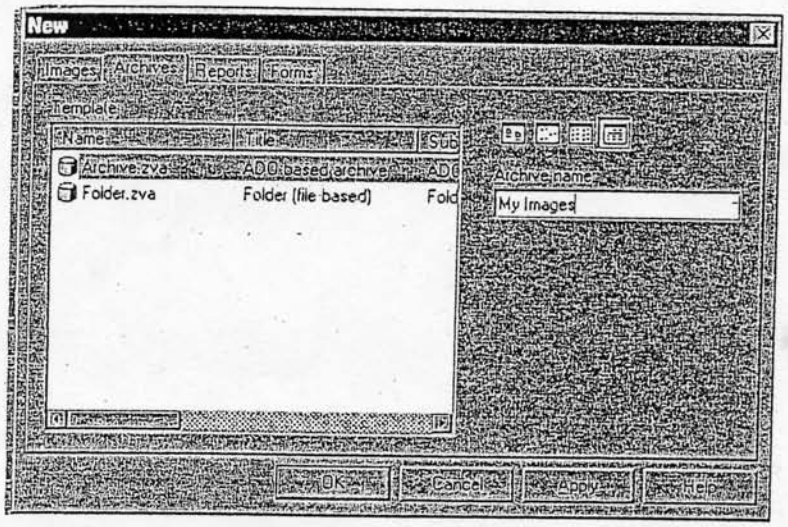
- Make sure your captured image is active and go to Veiw->Properties, or click on the Properties icon  .
- This will open the Image Properties window.



- Here you can move the slider for brightness, contrast and gamma up and down to adjust the image.
- You can also move the squares on the line provided.
- To find the best contrast and brightness automatically click the Min/Max button.
- To undo any changes you've made click on the Linear button.
- You can apply the same adjustments to multipe images by clicking on the Save button for your first image, and Restore for any subsequent images.
- If the image is saved in the archive you can always go back to the original data. When you export an image as a common file extension you need to apply the display adjustments (see pgs 15-16) you've made and cannot go back..

How do I use the archive?

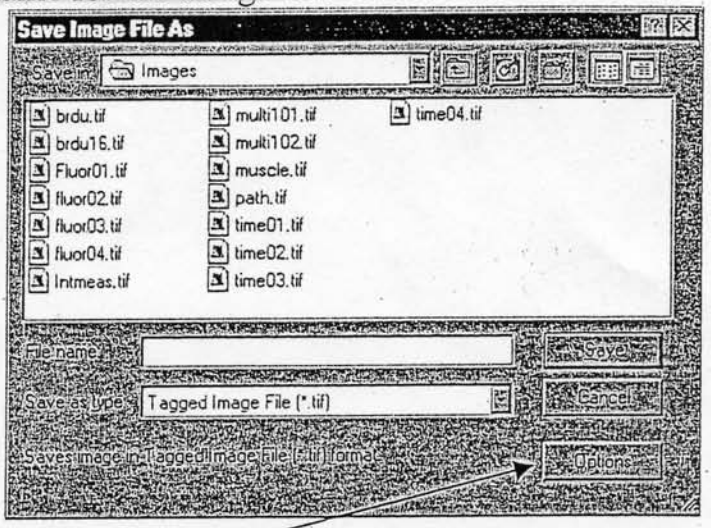
- To create a new archive go to the menu at the top and go to **File->New** and the New dialogue box will open.
- Go to the Archives tab.
- Enter a name for your archive in the space provided on the right and click OK.



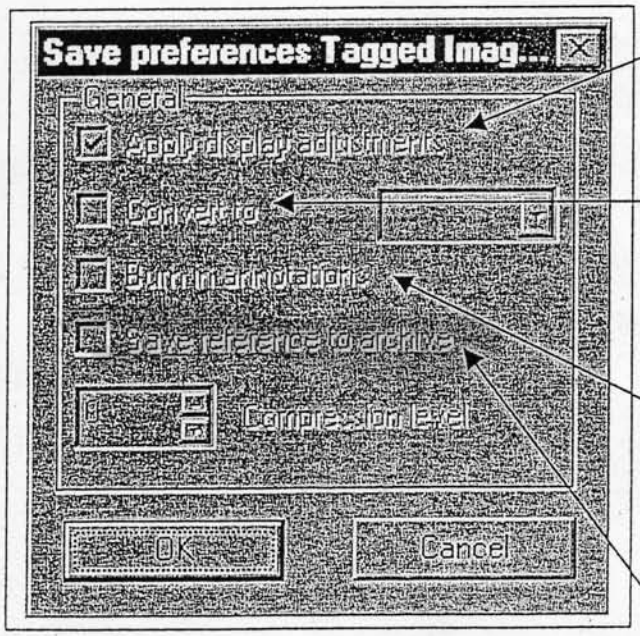
- To open an existing archive click on the Open Archive icon  .
- To add an image to the archive make sure the image you want to add is active and click the Add Active Image to Archive icon  .
- Add any information you would like to store with the image into the archive form and click OK.

How do I export images so they can be seen by other programs?

- For a single channel image you can go to **File->Save As** or click on the Save As icon  , which will bring up the Save As dialogue box.
- Enter a name for the image and select a file extension.



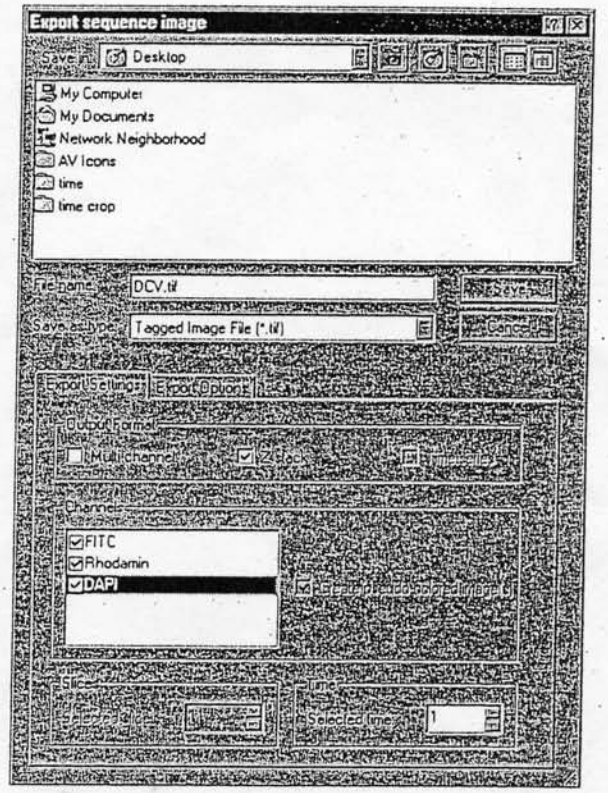
- Click on the Options button to open up more save options.



- To save changes made to contrast and brightness you must click the Apply display adjustments tick box..
- To convert a 12 or 14 bit image to 8 bits check the Convert to tick box. **This MUST be done to view an image in Photoshop or PowerPoint.**
- If you added annotations that you want to show up in the exported image you must check the Burn in annotations tick box.
- If you would like to save a reference to an AxioVision archive you must check here.

- Click OK in the Options window
- Select a destination folder and click Save in the Save As window.

- For a Multichannel, Z-Stack, or Timelapse image you must go to File->Export, which will open the Export dialogue box.



- Here you can select a destination folder, an image name and a file type.
- The Export Options tab provides the same information as the Options button described above.
- Under Output Format you can choose how you would like the image to be exported. If you only want to save certain channels, time points or z planes you do that here.
- Click Save.