

Zeiss Axiovert 135 Fluorescence Microscope Quick Guide / Operations Manual (v. 1.0 February '09)



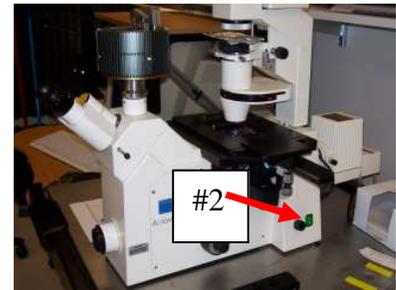
The Zeiss Axiovert 135 microscope is a manual stand with the ability to collect fluorescence and differential interference contrast (DIC) images with limited (single color) time lapse capability. It is equipped with a CCD camera run by MicroManager, and ImageJ-based, free software package. Examples of types of images that can be captured range from simple image capture (bright field or fluorescence), sequential capture of up to 4 fluorescent probes, and time lapse images of a single channel (fluorescence or DIC). The software provides user-friendly image capture with metadata annotation and a wide range of image processing capabilities.

Filters available on slider:

position	name	excitation	emission
1	DAPI	365-80	450/60
2	Blank	--	--
3	GFP/FITC	450-490	515-565
4	Red	546	590LP
5	Blank	--	--
6	DIC	--	polarizer

Turning on the System

- 1) Fire the arc lamp under the microscope table
(make sure nothing else is on)

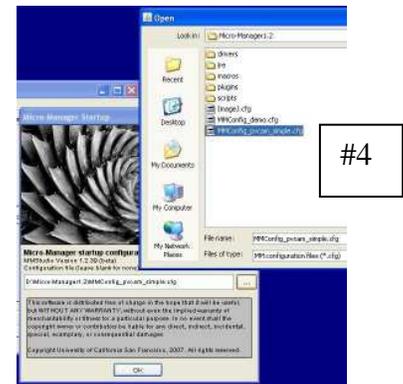


- 2) Turn on the brightfield bulb power switch on the microscope base (#2)



- 3) Turn on the camera next to the monitor(#3)

- 4) Log on and start the MicroManager software. Use the “. . .” button to change the configuration the PVCAM simple configuration.



- 5) Write down your start time on the log sheet

Shutting down the System

The arc lamp must be on for at least 20-30 min prior to turning it off, you must wait that long before turning it back on, too. Firing the arc greatly shortens the lamp life, so it's best to leave it running during the day when the schedule shows other users are coming—check the schedule, the last person MUST shut down the system.

If there is another user after you:

- 1) Clean any oil or water immersion objectives you used during your session.
- 2) Log off from your Windows session
- 3) Write down the time on the log sheet.

If you are the last person scheduled:

- 1) Clean any oil or water immersion objectives you used during your session.
- 2) Log off your Windows session, but leave the computer running (don't shutdown).
- 3) Turn off the camera (#3), brightfield power (#2), and the arc lamp (#1).
- 4) Cover the scope with the plastic cover. **THE LAMP HOUSING MAY BE HOT!** Don't cover that part.
- 5) Write down the time on the log sheet (how else will the next person know if system is cool enough to restart?)

System Anatomy

The Axiovert 135 is a manually operated microscope with automated digital image capture. The objectives, emission filter cubes, DIC prisms, and focus are all controlled manually. The software is only for image capture and processing, it does not run any aspect of the microscope.

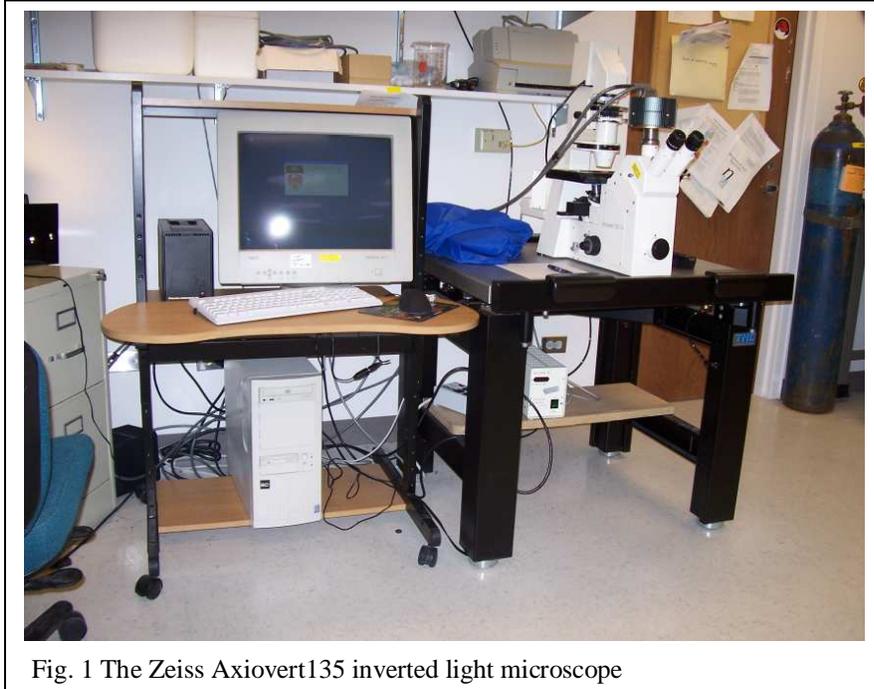


Fig. 1 The Zeiss Axiovert135 inverted light microscope

Before changing the magnification, lower the objectives by focusing the objective turret all the way down, so as not to disturb the sample or bash the objectives into the stage. There are three objectives available on this microscope: PlanNeoFluar 20x NA 0.5 dry, PlanNeoFluar 40x 1.30 oil, and PlanApoChromat 63x 1.40 oil. NA refers to Numerical Aperture, the resolving ability of the lens; bigger NA = higher resolution.

Brightfield and Fluorescence modes

There are six positions for fluorescence filter cubes in the slider below/behind the objectives (Fig. 2, “filter”). The filter position is changed by pushing the slider back and forth. The cube in the light path can be determined using the chart on the front of this manual. Since this is a manual microscope, the excitation/emission cubes must be changed by hand with the slider. Fluorescence uses excitation filters to excite your probe(s) with a narrow band of wavelengths, a dichroic mirror to reflect the excitation onto your sample while allowing the longer fluorescence to pass through, and a barrier (AKA emission) filter to block stray excitation light and limit the output color. All of the filter cubes on this microscope use

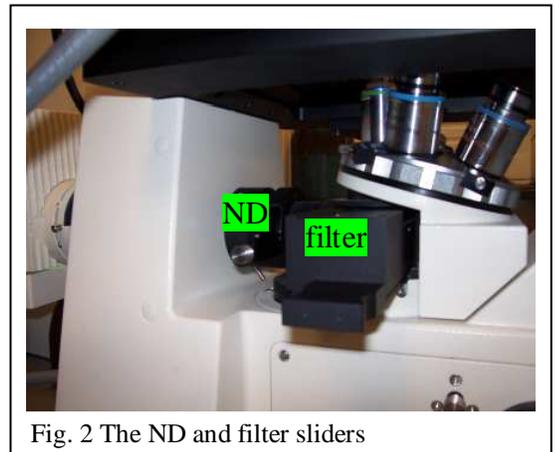


Fig. 2 The ND and filter sliders

dedicated excitation and band pass emission (barrier) filters that are coupled in the slider. This makes it easy to selectively excite most fluorophores properly and separate them from other fluorophores relatively well. Unfortunately, fluorophores with a large Stokes shift (like PI or Q-dots, which are excited by UV or near UV light, but emit in the green, red or far red range) may not work with this system.

A manual shutter slide is located behind the filter slider (Fig. 2, “ND”). There are three positions in this slider: all the way to the right blocks all light from the arclamp (for brightfield), the middle position is 50% of the arclamp output, and all the way to the left is 100% of the arclamp output. You should use as little light for live cell imaging as possible (both sliders pushed in) to forestall photodamage. Most imaging can be done with only the 50% slider pushed in (well suited for your eyes, too). You can use full light for dim samples, but be aware that you are probably getting high

autofluorescence and probes will bleach quickly. And remember to always check your controls with the same illumination intensities!

A brightfield lightsource is available (above the scope) and is controlled via the green switch (on/off) and knob (intensity up/down) on the back right of the microscope base (see above). There is a makeshift foil “shutter” to block brightfield light at the top of the condenser. It is sometimes easier to control the brightfield light by pulling this in and out than to turn it on and off with the switch.

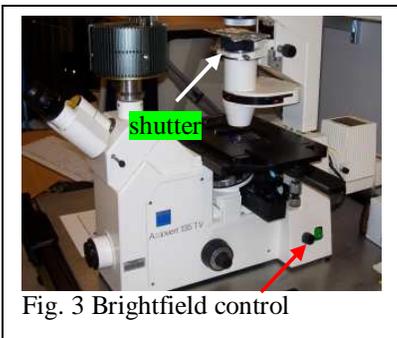
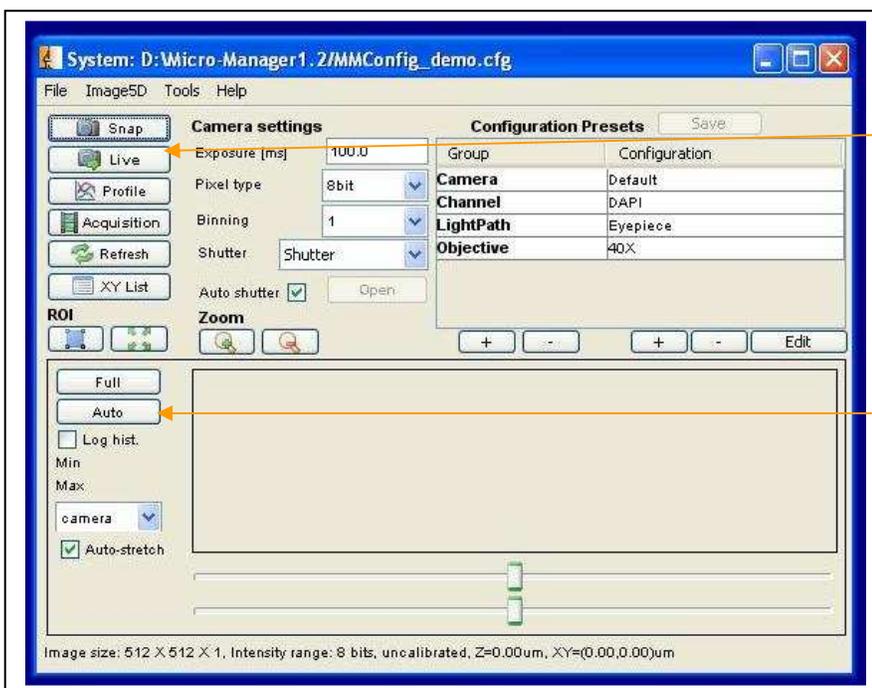


Fig. 3 Brightfield control

The MicroManager Software

All of the configuration of this microscope is manual, the software is only for image capture (i.e. is only aware of the camera). The software will not change the microscope settings, and it will not be aware of any changes that you have made to the microscope (or did not make to the microscope but should have, as the case may be). Once you have your sample in view with the magnification and color you want, and are ready to take an image, start the MicroManager software. MicroManager is built on top of ImageJ, the same free, open source software we recommend for image processing. When MicroManager opens, you will see the main MicroManager System window. To get a



live preview of your data, push in the slider on the lefthand side of the microscope, up near the oculars (it's labeled) and hit the “Live” button. If you don't see an image, make sure the ocular/camera slider on the side of the microscope is pushed in, then increase the exposure time. The default exposure time is 0.0 ms, which is obviously way too little. You can also use the “auto” button, which will autoscale the image display to make even low signals appear on the screen. The “Full” button returns the image display to the full range of whatever bit-depth you

select from the pulldown. A histogram representation of your image will appear in the large box at the bottom of the System window, to help you decide if your image is bright enough but not too bright.

To acquire an image press the “Snap” button. Then save the image using the “Save” or “Save As” button at the bottom of the Acquired image window. The default file format for saving single images is a .tif. Other file type options are available using the File → Save As feature from the main ImageJ toolbar menu. **If you want to keep a snapped image, you must save it right away**, otherwise it will be overwritten by live preview data or another snap image acquisition. If you want to change cubes between captures, you have to manually slide the filter slider between captures. If you want color channel images and DIC images, use the foil “shutter” to block the brightfield light when you are taking color images, it’s easier and more energy efficient than turning the bulb on and off with the power switch. The ROI button will allow you to capture only a portion of the image. Use the box tool from the main ImageJ toolbar to draw a box around a portion of your image, then hit the button with the blue box under ROI. Only that portion of the image will be captured, saving space in memory. To return to the full chip, hit the button with the four green arrows. If your image becomes too bright, too dim, or just plain funny looking after you draw the ROI, try going back to full chip, then make sure that the “auto-stretch” feature is turned off by unchecking the box. Hit the “auto” button to re-scale your image and then try drawing your ROI again. A feature for storing and re-visiting multiple ROIs is built-in to the software, but unfortunately since the software has no control of the microscope stage it is not possible to use this feature to collect multiple data points from a single dish/slide at this time.

Also, because the software cannot control the microscope’s filters or objective position, automated multi-channel (“Channel group”) or multi-z plane (“Slices (z)”) imaging is not possible. However, time lapse imaging of a single channel (one color or DIC) is possible. To collect a time series, hit the Acquisition button on the System window. You will get this Multi-dimensional Acquisition window. Once you have set your exposure time in the System window, use the “Frames (time)” box in the Multi-dimensional Acquisition window to set up your time lapse. Set the acquisition order to time-lapse, and press “Acquire!” to capture the images. Once the capture is finished, you can then save your images using either the “Save As” button from this window (if you want a series of .tif files in a folder created with the filename that you supply) or the File → Save As feature from the main ImageJ taskbar (if you want another filetype, such as a .tif stack .stk). If you want to write your data to disk as you go (nice for time lapses lasting several hours) you can set up a routine to write your images to disk as soon as they are captured. This could save you from data loss if the computer and/or microscope were to lose power at some point during your experiment.

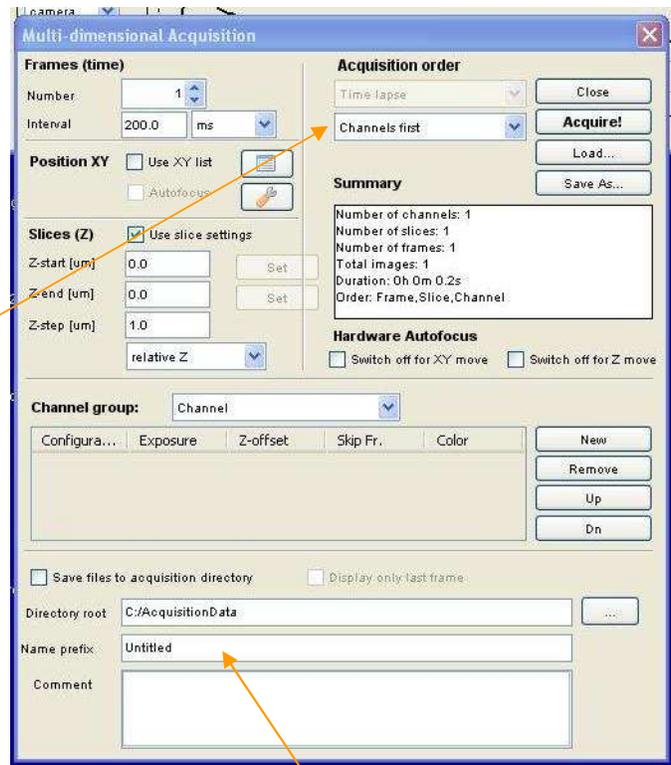
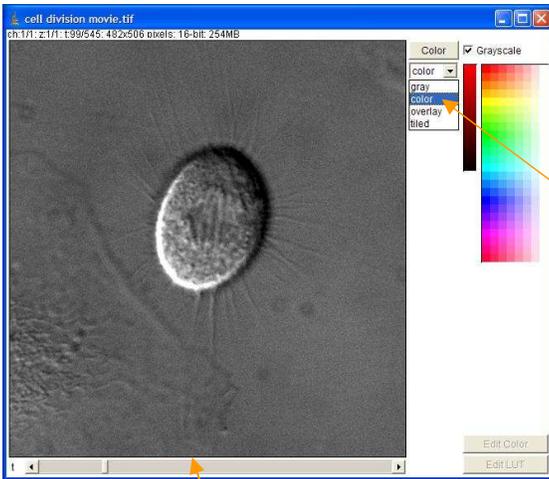


Image 5D Data Display

Images captured through the “Acquire!” button are displayed with ImageJ’s Image 5D plugin. This is a different way of displaying data from what most ImageJ users are used to, but it has some nice features. If you don’t like Image 5D or it does not meet your needs, your raw .tif images can be opened in ImageJ with the File → Import → Image Sequence function, which will display the standard ImageJ format. If you like Image 5D, it can be downloaded to your own computer and used to display .tif data from any microscope.



Basics of Image5D: Image5D is just a way to display .tif data, it is not a special image format. All .tif files acquired with the “Acquire!” button will be displayed with Image5D, but will save as regular .tif files or .stk tiff stacks. To add false color to a captured image, use the pull-down menu to choose the “color” option. Red will be assigned by default. To change this, hit the “Color” button on top of the pull-down menu and select a color from the menu that pops out of the right hand side. For timelapse images (and for z-stacks, if you open data from another microscope) a slider along the bottom of the image allows you to flip through your time points and/or z-planes. If you have volume over time, there will be two sliders, one for time and one for z. To save your data, you must first “convert” it to a stack. From the main ImageJ menu, choose Plugins → Image5D → Image5D to stack. A regular stack should appear (not in the Image5D-type window). This can then be saved as a .tif stack, .avi movie (Windows Media Player) or .mov movie (Mac Quicktime). Both movie types should allow you to set the playback speed (frames per second) and compression upon saving.

If you open a .tif stack with multiple colors or an RGB color image using Plugins → Image5D → RGB to Image5D, Image5D will at first only display one channel. You will be able to flip through the color channels with the large grey slider that appears under the pull-down menu. Choosing the “overlay” option from the pull-down menu gives a merged image of all the colors in the sample, with the option of toggling individual channels on and off with the checkboxes, and/or adjusting the color/brightness/contrast/etc. of a particular channel by selecting that channel (fill in the dot next to the name). Before saving, you must select a “conversion” method under Plugins → Image5D. If you choose Image 5D to RGB, and RGB image will be created as displayed, and then that image can be saved as a .tif or other file type with File → Save As or File → Save. These files will open in ImageJ, PowerPoint, Photoshop, etc. If you choose Save As Image5D, the file will be saved in the Image5D format and will only open with the Image5D plugin for ImageJ.

