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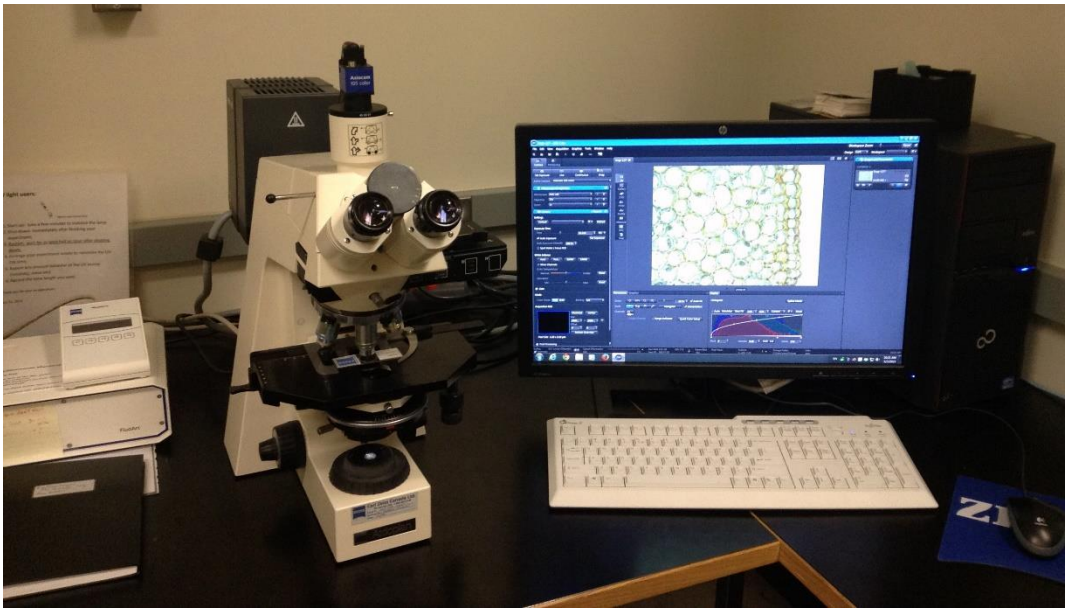
AxioPlan Fluorescence Microscope



Make/Model: Zeiss AxioPlan
Features & Applications: Microscope: Axioplan with 2.5X, 10X, 20X, 40X and 60X oil objective lens; DIC and phase contrast; 100W HBO lamp with Fluor control. Common UV filtering cubes; Digital CCD camera: AxioCam105 (Color, 5.0MP); Imaging software: ZEN. Conventional fluorescence microscopy for GFP-tagged or dye-stained cells or tissues. High quality image-acquiring and -editing system.

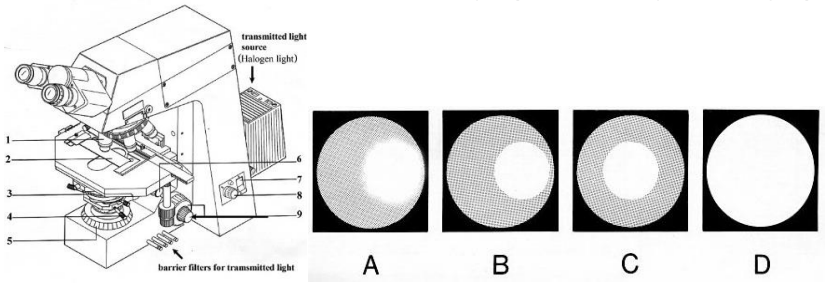
INSTRUCTION

Epifluorescence microscope is usually a compound microscope equipped with epifluorescent light path system, such as the Zeiss Axioplan in the following illustrations. The computer-assisted digital imaging operations greatly facilitate the users for acquiring images both in transmitted and fluorescent light condition.

1. Transmitted light (bright field, BF) microscopy

- Mount your specimen on a microscope slide and cover **with a coverslip**. For relatively permanent slides, or for oil immersion, seal the edges with nail polish.
- Place the slide on the stage, and secure**.**
- Turn on the transmitted light using the knob.
    - Note that the switch behind the knob should always be left with the top side pushed in. This is the main switch for the power supply to that light source.

- Make sure that there is light coming through the field diaphragm. Sometime this is left covered with a black cup, since transmitted light interferes with epifluorescence microscopy (discussed later).
- Adjust the distance between the ocular lenses so that you can see through both.
    - Like your shoe size, this will be a constant value. Most research microscopes have a scale that you can preset when using.
- Adjust the **focus**.
    - Using a low power objective (20x) focus on the edge of the slide – this will be at a slightly higher level than your specimens.
    - Some specimens are small and colorless, so it can be difficult to find the proper focal level. This strategy gets you close to the correct level very quickly. Continue to focus down using the fine focus.
- Objective lenses vary in two major ways: the **magnification** and the **numerical aperture**.
    - The numerical aperture (N.A.) is a measure of the light-gathering characteristics of the lens.
    - The highest NA currently available is 1.4 (in the 63x lens); other objectives have lower NA
    - The objective must be matched to a lens in the condenser, selected by rotating the ring under the stage.
    - Sometimes you need differential interference microscopy (DIC, discussed later) to increase the contrast of images.
- The **condenser** is a series of lenses below the stage (see the Figure at right). Checking by:

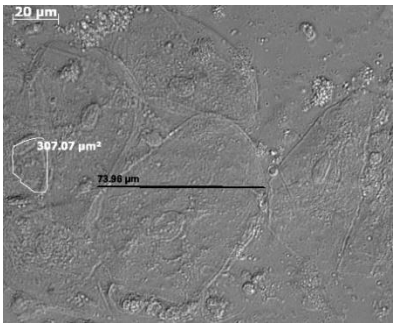


    - Close the field diaphragm by rotating the collar (#5).
    - Look through the oculars at your image.
    - The **condenser** can be out of adjustment in two ways: **focus** and **centering**.
    - Adjust the **focus** using the condenser (field diaphragm) focus knob (#6), so that the diaphragm edges are as crisp as possible, as shown in the figure below (**B**).
    - **Centre** the condenser using the two knobs (#4) so that the image resembles (**C**) in the figure below.
    - Open the **field diaphragm** (#5) until the edges are no longer visible (as in **D**).
    - This is called Köhler illumination, which is optimum for bright field transmitted light microscopy.
- The condenser focus should be checked each time you change objectives.
- The objectives on microscopes are parfocal: parfocal means that you can switch from one to another without major refocusing.
- Note that the 63x and 100x objectives are “oil immersion” lenses:
    - Oil immersion lenses require special immersion oil between the coverslip and objective for proper performance.
    - The oil assists in the objective gathering more light.
    - Oil immersion lenses have a black ring on them; dry lenses do not.
    - After using oil, both the objective and slide must be **cleaned** carefully.

    - Use special lens cleaner and lens paper.
    - Non-oil immersion lenses must be kept clean and dry.
    - When necessary, they are cleaned the same way as an oil immersion lens.
- Some objective lenses use water, or liquid growth medium, rather than oil, for immersion.
    - Water immersion lenses have a blue ring.
    - Growth medium should be rinsed off with distilled water, and then the objective should be blotted dry.

2. Differential interference contrast (DIC) microscopy

DIC microscopy uses polarized transmitted light to increase the contrast of images of unstained cells. This was developed by Nomarski and is also called Nomarski contrast. Briefly, the specimen is illuminated with plane polarized light, and after passing through the specimen and the objective, the light passes through a second polarizing filter that is at an angle to the first. The polarity of the light is changed as it passes through the specimen, leading to increased contrast and the appearance of a three-dimensional image. There are four parts to the optical system:

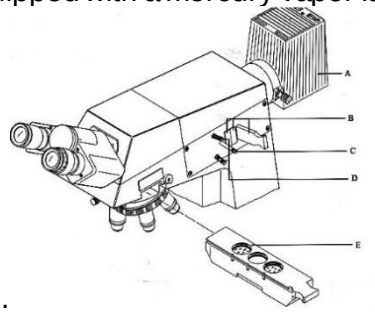


- The condenser lens must be appropriate to the objective – matched for numerical aperture N.A.
- The polarizer filter must be swung into the light path – this is below the condenser, but above the field diaphragm.
- The analyzer filter (see "slider for DIC"; just above the filter sets for epifluorescence) must be pushed into the light path.
- Sometimes, an additional small polarizing filter just above the objective must be adjusted for maximum contrast, although this may be incorporated into the analyzer filter. Either way, as this 'Wollaston" filter is rotated the image becomes more or less contrasted.

3. Epifluorescence microscopy

- **Fluorescent substances** (e.g. proteins or stains) absorb light of one colour (wavelength), and then release that energy as light of a different color (longer wavelength). e.g.
    - fluorescent substances that absorb blue light typically fluoresce (or emit) green light.
    - fluorescent substances that absorb green light typically fluoresce orange/red.

- The Zeiss Axioplan Epifluorescent microscope is equipped with a mercury vapor lamp light source **HBO-100**, as shown in figure at right (**A**).



- The light from the mercury vapor lamp goes through an aperture controlled by the slider **(B)** and one of a series of filter sets (excitation/emission cubes, or dichroic mirrors, **E**). The filter sets are (left to right)
    - I. G365/FT395/LP420.
    - II. 450-490/FT510/LP520.
    - III. BP546/FT580/LP590.
    - IV. Empty.
- To use **epifluorescence** microscopy, both the mercury light **Power Box** and bulb lifespan-monitoring control panel **FloArc** must be turned on.
    - Once turned on, the bulb should be left on for at least 20 minutes, so that it is completely warmed up.
    - Once turned off, it should be left off for at least an hour until it is completely cool again.
    - Mercury vapor bulbs are expensive and fragile, and this heating/cooling regime improves bulb performance and lifetime.
- Find your specimen using transmitted light, and then cover the transmitted light at the field diaphragm or turn the bulb off.
- Pull out the DIC slider: this absorbs a lot of light.
- Choose the correct filter set (**E**) for your fluorescent stain or protein.
- Pull back the slider covering to admit ultraviolet light (**C**).
- Note: Generally, it is easier to use epifluorescence in a darkened room.

*** Taking pictures with the digital camera**-- for detailed information, please see ZEN guide.