How to do film and digital imaging of microscopic preparations
Namboori B. Raju

Background

Fungal cells and nuclei are generally small and require microscopic observations at high magnifications. Their small size also mandates the use of fine-grain films or high-resolution digital cameras, so that enlarged images are not grainy. With the availability of fine-grain films in the last 30 to 40 years, 35 mm (24 × 34 mm) black and white negative films and color slide films have been widely used for photomicrography. They are more convenient and less expensive than the older, large format roll and plate films. Most research microscopes until 10 years ago are fitted with dedicated 35 mm film cameras, and many are still in use today. Since 1974, Raju has accumulated well over 10,000 film negatives -- mostly documenting Neurospora ascus development in wild type and in numerous mutant genotypes. The description that follows is based on his experience with films and his recent foray into digital imaging.

Procedure

***Film photomicrography*:** Films marketed for general photography are too grainy for microphotography. Over the years, I have used several fine-grain films that required specially formulated developers for obtaining high quality, continuous tone images (see Raju and Newmeyer 1977; Raju 1980, 1992 for photos). Unfortunately, several film-developer combinations have since been discontinued and new ones introduced. Thus, a particular film-developer combination that gave great results 10 or 20 years ago may not be relevant today, but the criteria for selecting a good film-developer combination will remain the same: thin emulsion, fine grain, and inherently high contrast but processed in a special developer to give continuous tone negatives. Such films are generally slow (<ISO 50), requiring relatively long exposures, but they are well-suited for bright-field images. Fluorescently stained cells and nuclei generally require long exposures, and fluorescence may fade during the exposures. Thus, faster films (ISO 200-400) are sometimes unavoidable. In the last 15 years, I have used Kodak TechnicalPan 2145 for bright-field photography and Ilford XP-2 for fluorescence photography.

Kodak Technical Pan 2145 is a very fine-grain, high-contrast film that is highly recommended for bright-field microphotography and other scientific applications. I have used it for photographing hematoxylin-stained nuclei and chromosomes in the developing asci. The film is rated at ISO 25 for general photography, but I have set the exposure index at 80 for photomicrography. The film is processed in HC 110 developer (dilution D, 1:40) at 20° C for 6-8 min for the desired contrast, density and sharpness. The same film and developer may be used for other applications by varying exposure, developer dilution, and development times (see Kodak publication # P255 and the instructions with the developer).

Ilford XP-2 is a high-speed (ISO 400) black and white film, but its processing is based on C41 color negative processing chemistry. I have used it mainly for photographing fluorescent images of acriflavin-stained nuclei and chromosomes (see Raju 1986 and *How to stain meiotic chromosomes using acriflavin*). The exposures are manually set at 30-60 sec, and the film is processed at a nearby Photo Lab (Longs, Walgreen or Walmart).

***Digital photomicrography*.** Almost all 10-20 year-old research microscopes can now be adapted for digital imaging, and I recommend such conversion as soon as possible. Black and White films for photomicrography are relatively expensive, and the Photo Labs that process and print them are becoming harder to find. Digital imaging is very convenient, quick, and inexpensive. In the last two years, I have adapted two research microscopes (Olympus BH2 and Nikon Microphot FX) for digital imaging using Nikon CoolPix 5000 and CoolPix 5400 digital cameras. Both are general-purpose 5 megapixel, consumer cameras. The present cost of adapting a digital camera (camera + adapters) varies from $1000 to $1200 per microscope. Microscope World (Encinitas, California, at www.microscopeworld.com) sells adapters for several Nikon, Sony, Olympus, and Canon cameras for $250-400 each.

Nikon Coolpix 5000 is attached to the video port of Nikon Microphot FX with three adapters: A Nikon C-mount adapter on the microscope's video port, a photo eyepiece-like lens adapter (MM-Cool from Microscope World), which connects to the C-mount adapter at one end, and to a Nikon camera adapter tube (UR-E6) at the other end. The UR-E6 in turn screws onto the outer mount of the camera lens. The same CoolPix 5000 camera setup can also be mounted on the C-mount adapter of Olympus BH-2 microscope. Nikon CoolPix 5400 is likewise adapted to the C-mount on either microscope with a MM3-45 (MM-3XS + M4537) adapter from Microscope World. Additional advice and appropriate adapters for several Nikon, Sony, Canon, and Olympus digital cameras may be obtained from the camera manufacturers/distributors or from Microscope World. Microscopes without the C-mount adapter may also be used by connecting the camera and the MM-Cool (or a similar adapter) directly to the phototube or to one of the binocular eyepiece ports with the supplied 23 mm sleeve.

The images of fluorescing nuclei in the developing asci and ascospores appear bright when observed through the microscope. However, the images are dim or hardly visible on the built-in small camera screen. Thus, it is necessary to frame and focus images of asci in bright field, and then switch the filter set and light path to GFP imaging. Another trick is to increase the ISO setting to 800, when the GFP images appear brighter for focusing, then change the ISO setting back to 100 for exposures. At ISO 100, the 5-megapixel sensors in both CoolPix cameras are capable of recording high definition images. For fluorescence imaging, typical exposure times are set manually at 4-8 seconds (F 5.4) with 10× objective, 2-4 seconds with 20× and 1-2 seconds with 40×. For such long exposures, the Noise Reduction feature of the camera is activated. For bright field imaging the cameras are set in Program mode; the built-in flash is, of course, disabled for both bright field and fluorescence imaging. Although both CoolPix 5000 and CoolPix 5400 have 5 megapixel sensors, I find that the fluorescent images appear brighter on CoolPix 5400 than on CoolPix 5000, perhaps because of the different lens adapters (MM-Cool vs. MM3-45).

References

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