

Economical digital photomicroscopy

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Abstract

Public domain software has been developed for remote exposure control of a consumer digital camera for use in photomicroscopy. A computer-controlled shutter release and remote control of various camera functions are essential to avoid mechanical disturbance of the microscope and slide during the imaging procedure. These features have previously been available only with expensive professional models. The digital images of diatom specimens obtained with this system captured the same detail as conventional photographs. The setup can therefore be used as a cost-effective tool for documenting taxonomic information of microscopically small organisms. In combination with the fast data transfer rates available via the Internet, microscopical studies can now be performed faster, more reliably, and more consistently.

Introduction

Digital imaging technology is increasingly used as a tool for documenting the morphologies of microscopic organisms such as diatoms (e.g. Stoermer, 1996; Joynt & Wolfe, 1999). In contrast to conventional photomicroscopy, digital imaging provides pictures instantaneously and no elaborate procedures or photographic equipment for producing prints are required. Electronic images can be easily transferred over the Internet. However, professional high-resolution digital cameras designed for microscopy, including remote control software, are still comparatively expensive, while the resolution of videocamera images is too low. On the other hand, the software that is

currently shipped with inexpensive high-resolution consumer cameras unfortunately does not typically provide remote computer control of the camera functions, requiring a manual shutter release for photomicroscopic applications. There is also an associated higher risk of accidental misalignment of the object during manual changing of the camera settings.

In this article we present a high-resolution digital imaging system that costs a fraction of the price of professional models and provides full remote control of numerous imaging options. This was accomplished by taking advantage of state-of-the-art megapixel digital cameras that are readily available on the consumer market. A public domain device driver for the camera was upgraded to allow remote control of all major camera functions through a graphical user interface (GUI), a feature otherwise only available with much more expensive professional models. The images were stored in a hierarchically and alphabetically organized website to accelerate their retrieval.

Material and Methods

We mounted a consumer digital camera (either a Nikon Coolpix 950 with 1600 x 1200 pixels or a Nikon Coolpix 990 with 2048 x 1536 pixels, both with 3x optical zoom) on a trinocular microscope (Olympus BX50). The 28 mm threaded camera lens (0.75 mm pitch) was connected to the phototube through a series of adaptors: a 28 mm to 37 mm step-up adapter (available as part number NKN-ADP from <http://www.ckcpower.com/adapters.htm>) and a 37 mm to 52 mm coupling ring attached to a universal LE (Lens-Eyepiece) Adapter (available from <http://www.stillwtr.com/leadapter>). To control the camera remotely by a computerized

interface, we linked it with a serial cable to a Pentium computer (48MB RAM, 3.1GB disk). We enhanced the public-domain camera driver program photopc (<http://www.average.org/digicam>); the modified version has been adapted to recognize the many specialized features available in the Nikon cameras, such as direct control of the optical and digital zoom. A simple GUI called xphoto, written in the platform-independent TCL language, was also developed for remote digital imaging. This GUI and the latest release of the specialized camera driver have been made freely available to the scientific community and can be downloaded from the web site <http://www.math.ualberta.ca/imaging>. Although these programs have been developed and tested under the public domain Linux operating system (and should work without modification on any UNIX workstation), they can be easily adapted to other computing environments. An executable version of the camera driver photopc for Microsoft operating systems has already been made available on the above web page.

High resolution images were taken with a 100x objective lens (oil immersion, N.A. = 1.35) and a 2.5x photoeyepiece, with transmitted light or differential interference contrast, and using the grayscale and infinity focus settings of the camera. The camera lens was set for telephoto. Focusing was controlled manually by adjusting the microscope stage until the sharpest image appeared on the 2.1" (1.8" for the Coolpix 990) LCD camera display (using a 2.5x digital zoom). To obtain maximum image resolution, the GUI automatically reverts to using a 1.0x digital zoom just prior to exposure. A screenshot of the GUI is shown in figure 1 and a complete list of remotely controlled camera options is presented in table 1. Diatom images were stored alphabetically and

hierarchically by using the public-domain WebMagick package (available from <http://www.simplesystems.org/webmagick>). For evaluating size differences, a uniform magnification was used for all diatom images. To portray absolute sizes, the images were superimposed on a stage micrometer micrograph with 10 μm divisions. We compared the digital images with conventional photographs of the same specimens taken with an Olympus C-35A 35mm film camera and exposure control unit.

Results

An untreated image, taken with the Coolpix 950 appears in figure 2a. Figure 2b shows the same image with the contrast enhanced by imaging processing software (figure 2b). Figure 2c shows the same object taken with the higher resolution camera Coolpix 990 in order to evaluate the increase in quality this camera affords compared with the Coolpix 950 (fig 2a, 2b). Figures 2e and 2f are enlargements of figures 2b and 2c, respectively. Additionally, we included a conventional photograph of the same object to demonstrate the differences between digital and traditional photography (figure 2d). Further examples of grayscale diatom images taken with the Coolpix 990 are depicted in figure 3. Hierarchically organized images of diatoms from several Canadian sites, which were made with the equipment described in this article and classified into genera and species, can be found at <http://www.math.ualberta.ca/~bowman/diatom>.

Images at the high resolution setting of the Coolpix 990 require 9.5 MB (uncompressed), but in the fine resolution mode, which uses JPEG compression, they typically occupied only about 500 kB. With a USB transfer speed of 250kB/s, it takes 38 seconds to download a high resolution image and 2 seconds for a compressed fine

resolution image. For light microscopy the difference between the high and fine resolution settings was found to be negligible. With the Coolpix 950, the image sizes were about 40% smaller, but due to the slower 10.5kB/s serial interface, the transfer times were 15 times longer.

Discussion

Upon comparing the conventional diatom photograph (figure 2d) with the megapixel digital images of the same specimen taken with the same microscopic system (figures 2b, c), one sees that the digital image depicts the same detail as the conventional photograph. There seems to be a better resolution in the central part of the *Cocconeis* sp. valve in the digital images. It is not clear to what extent this difference can be attributed to the imaging techniques since a slightly different focus in the conventional image might be the reason for less detail. We notice minor improvement in the photograph taken with the Coolpix 990 compared with the image taken using the lower-resolution Coolpix 950. The difference between the image resolution of the two digital cameras becomes evident at higher magnification of the respective images (figures 3 e,f). The quality of the resulting digital images also depends on factors like file format (e.g. JPEG compression can decrease image quality), image contrast, display pixel depth, postscript image density, printer resolution, and paper quality.

When compared to diatom micrographs in diatom floras the untreated images generally show less contrast and appear grayer. However, the contrast can be easily enhanced by image processing (figures 2a, b), a technique also applied to diatom

photographs in floras during print development.

Difficulties arise when imaging very small diatom specimen or diatoms with structures greater than 30 striae per 10 μm with the digital cameras, since the camera display is so small so that proper focusing is difficult. The resulting images are sometimes slightly out of focus and therefore may not offer the best resolution possible. This problem could be solved by increasing the magnification of the photoeyepiece or by connecting the camera to a television display, which would provide a larger image of the object than the LCD viewfinder. However, this issue perhaps applies only to objects that have structures close to the resolving power of a light microscope like diatoms and not to other larger microphotographic objects like chironomid head capsules or pollen.

In conclusion, the development of an affordable high-quality digital imaging system in combination with a freely accessible, easily searchable image database offers a new alternative for microscopical studies. In combination with the fast data transfer rates available via the Internet, microscopic studies can now be performed faster, more reliably and more consistently, with lower supplemental travel, literature acquisition, and photomicrographic production costs.

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References:

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Table 1: Examples of the digital imaging options that can be remotely controlled by the graphical user interface (xphoto) and the upgraded photopc driver.

options	choices
model	Coolpix 950 / Coolpix 990 / Coolpix 990–USB
resolution	1600 x 1200 (or 2048 x 1536), 1024 x 768, 640 x 480 (and 4 compression factors)
lens	telephoto, wide, fisheye
digital zoom	1.0x, 1.25x, 1.6x, 2.0x, 2.5x
image adjust	standard, contrast +/-, brightness +/-
white balance	auto, sunny, incandescent, fluorescent, flash, preset, cloudy
metering	center, spot, matrix
aperature	auto, high, medium, low
focus	macro, normal, infinity
flash	auto, force, off, anti–redeye, slow–synchronized
optical zoom	7.2 to 20.0 mm (8.2 to 23.4 mm for Coolpix 990)
exposure adjust	–2.0 to +2.0
shutter	exposure time in μ s (range for Coolpix 950 is 1/750–32 s)
count	exposure count (for interval photography; a %d in file name will be replaced by the frame number, e.g. image%d.jpg)
timer	seconds between snapshots
LCD	on / off
color	color / gray scale
keep	keep copy of image in camera (faster) instead of erasing it
transfer	automatically transfer each image to computer
autoview	automatically display each image
download card	transfer all images on flash card to computer
erase card	erase all images on flash card

Figures

Figure 1: Screenshot of the Graphical User Interface (GUI) xphoto for photopc. The main window of the program is on top; the lower window is the options menu.

Figure 2: Comparison of different photographic systems by imaging the same valve of *Cocconeis* sp. using identical microscopic equipment:

- a) Unprocessed image taken with the Coolpix 950.
- b) like a) with digitally enhanced contrast.
- c) Contrast-enhanced image taken with the Coolpix 990.
- d) Conventional photograph, no contrast enhancement.
- e) copy of b) enlarged.
- f) copy of c) enlarged.

Figure 3. Examples of fine-resolution digital diatom images (2048 x 1536) taken with the CoolPix 990 (see <http://www.math.ualberta.ca/~bowman/diatom/database/.JOPL>). a) *Navicula oblonga* Kützing. b) *Anomooneis sphaerophora* (Ehrenberg) Pfitzer. c) *Stephanodiscus niagarae* Ehrenberg. e) *Cyclotella bodanica* var. aff. *lemanica*. f) *Navicula cincta* (Ehrenberg) Ralfs. g) *Cocconeis* sp.





