Chapter 17

Digital Photography in Biology Lab Teaching

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Introduction

If I had been able to use digital photography when I took ninth grade science I would have concentrated more on the science instead of getting depressed about being graphically challenged. My first homework assignment was to describe how a cow walked. (It was the traditional first assignment in my school, and I lived in a rural area.) We were supposed to make a diagram of which hoof went where, relative to the others, and when. (Hey, it's hard!) Even a slow-moving cow moves her feet pretty fast, and I had to keep track of all four at once. It took me a long time and I am still not sure I got it right.

Later, my teacher told us to draw some flowers on artist's paper with a pen. He was a talented illustrator, but I was not. "Just sketch them," he said, "It's easy." Well, maybe it was easy for him and for Leonardo da Vinci. He was trying to get us to notice the structures, and I do appreciate his methods; if you can draw it you have to observe it. I struggled; got a B and thought I was lucky, but also I began to think that biology was beyond my grasp. There must be many students with similar thoughts.

So, after many years I can finally complete my ninth grade science assignments and do them pretty well. Digital photography makes observing and recording flower structures and fast-moving cow's feet both easy, even fun. It is the nerd's answer to the artist.

Unlike most chapters in this volume, this one is not a single exercise that can be dropped neatly into your lab course. Instead, it is only a few tricks and hints to make digital photography easy and cheap. We will learn basic camera operation, then do close-ups, micrographs, and a movie, all with biology lab teaching in mind. The workshop is also just a snapshot in time; the date is 2001 in a rapidly evolving field.

Materials and Notes for the Instructor

Cameras

Here I present the three cameras I use as examples of what is available in mid-2001, and what is useful in teaching. A consumer digital camera I have found to be particularly useful is the Epson PhotoPC 650. It has a resolution of 1252 x 864 pixels, so it is a "one megapixel" consumer digital camera. (It can make a dot matrix image of one million dots.) The PhotoPC 650 has been

discontinued, but it might be available in clearance sales. I bought one in March 2000 for $250 US, and would buy lots more of them if I could. It got only mediocre reviews in the computer magazines in 1999 and was a commercial flop, but even so has some nice features (including being relatively rugged). It has a moderately wide-angle non-zoom lens equivalent to 38 mm focal length, if it were a 35-mm camera. It does automatic focusing down to about 6 inches. It takes 37-mm screw-thread filters (the size for video cameras); we use a yellow filter for fluorescent ethidium bromide DNA gels. It stores digital images on a removable 8 MB CompactFlash card. (A separate memory card reader came with the camera.) The camera has a flash, good to about 50 feet away, but it also has the desirable option of inactivating the flash. A feature we use a lot is its analog "video out" port which makes the camera into a continuous live video monitor using a TV or an AV computer (see below). It is also possible to play the contents of the camera's memory as video on a TV or an AV computer. The camera comes with the very useful PictureWorks/HotShots color graphics software for PC's and Macs, which can be used to manipulate pictures (crop, rotate, cut, paste, flip, enlarge, shrink, *etc.*). It can store pictures as an uncompressed TIFF file (maximum resolution, using all of the million pixels) or as three levels of JPEG compression (using less disk space but at progressively lower resolution).

We also use a Nikon Coolpix 880, which is a "3.3 megapixel" digital camera. It got rave reviews when new in late 2000, although it was discontinued in mid 2001 and has been replaced by the similar (and fortunately cheaper) Coolpix 775 and 885 cameras. The 880 costs $630 (US) new and then we had to buy $170 worth of accessories, including a CompactFlash card reader, an extra rechargeable battery, and a combination AC adapter and battery charger. It has a zoom lens equivalent to a 38-95 mm focal length zoom lens, if it were a 35-mm camera. It has no screw threads for filters, making me resort to unaesthetic "sticky tape mount" filters. It can make a short video, which would be perfect for the cow assignment mentioned above. It has a flash and the flash shut-off option, like the Epson. The Nikon has a bewildering array of features. The ones I like best are the high resolution allowing good quality blowups of small selections, very close automatic focusing (under 2 inches), multiple metering options including "scenes" programs which are setups for particular situations, and the video-out capability like the Epson. It comes with dedicated Nikon software for PC's and Macs, but unlike the Epson's software, the Nikon's software can be used only with Nikon cameras and it just displays the picture; no manipulation of pictures is possible, so separate graphics software (like Photoshop) must be purchased.

Our department purchased several COHU CCD cameras, model 4915-2010, several years ago. (This COHU or a similar model is still available through a web site given below.) The COHU is an analog video camera; it does no color photography, grayscale only, and has VGA resolution, 640 x 480 pixels, which is much less resolution that the two digital cameras above. It uses the freeware Scion/NIH Image for Macs and PC's, formerly NIH Image for Macs only. The COHU is the easiest camera for student use because it is easy to focus and compose on the computer monitor. This is a scientific camera, as opposed to a consumer camera, so it is expensive, over $800, and that does not include the lens. We added hundreds to the price by getting a good close-focusing lens. There is no flash. The COHU is not portable; it must be always connected to a power source because it has no battery and it must be connected to a computer because it cannot store images by itself. There is no shutter on the camera; it is either on or off and the software (Scion/NIH Image) provides the shutter. We use uncompressed TIFF images only. What makes the COHU distinctive and particularly desirable is its capability for "on-chip integration," which means it can make long-time exposures using an add-on image-capture or "frame-grabber" board in the computer. The time exposures are particularly useful for photographing dim images like micrographs of immunofluorescent (IF) cells. It is possible to photograph images that are too dim to see through the microscope eyepieces! The instructors in our Developmental Biology course and in our freshman labs decided to use lots of IF in their labs, so we recently made a big investment in COHU's and in Scion frame grabber boards for

Apple G4 computers (see "AV computers" below). The camera, software, and the required image capture boards are described on the Scion web site, *www.scioncorp.com*.

Computer requirements

USB computers

Until about 1998 the industry standard for image transfer from digital cameras, like the Epson or Nikon, to the computer was "serial transfer" to the serial connection port on a PC, or to the printer or modem port on a Mac. Serial transfer still works on the Epson camera but it is terribly slow for large images. A one-megapixel image, good enough to make a high quality 3 x 5-inch print, might take five minutes to download through a serial connection. That would really slow down a lab course. Within the last three years, the industry standard has switched from serial connections to the USB connection, which is at least ten times faster. We can download images from the camera through to a USB port on a computer, or better yet, we remove the camera's image storage card and put it into an external card reader (costing about $30 US), which is connected to a computer through a USB port. If you have a pre-USB computer, it might be possible to upgrade it to USB if your computer has a free PCI expansion slot. A card with two USB ports costs typically $40 (US). Upgrading to USB also requires updating the computer's operating system to Windows 98 (or later), or to Mac System 8.6 (or later). The camera and the card reader also have separate software. By the time this is published in 2002, the image transfer landscape will have probably changed once again, hopefully to the still faster Firewire (IEEE 1394) connection.

AV computers

The message here is: Do not throw away your old AV PC or Mac if you want to use COHU's. The AV (audio-visual) computers flourished for a short time in PC/Mac history, from about 1996 until 1999, but they are now obsolete. The AV computer has the capability of converting an analog image made by a TV camera or an analog CCD still camera (like the COHU) to the digital format the computer needs. Some computers have this feature on the motherboard (built-in) and others have an add-on board, called a "frame grabber" card, mentioned above. Now the best video cameras are 100% digital and communicate to computers through a digital Firewire connection, so the old analog-to-digital frame grabbers are really obsolete, unless you want to use your COHU's. Even the inexpensive Apple iMac has USB and Firewire connections, but no frame grabber. The new high-end PC's and Apple G4's have PCI slots which accept frame grabber cards but it seems a shame to me to spend much new money on old technology. Look at the Scion web site (see above) for COHU cameras and Scion frame grabbers, but be prepared for sticker shock. Nevertheless, we still need frame grabber function to use our considerable investment in old COHU cameras and to use digital still cameras in video mode (described a little above and more below). A low-cost option is to obtain a used AV Mac 7600 or 8600 (circa 1996) which have built in frame grabber function. (Mac 7600's can be bought on the used computer market for about $700 US.) Unfortunately, the frame grabber era did not overlap with the USB era, so we will either need separate computers for AV and USB functions or we will need to install a PCI USB card on the AV computer.

Storage cards

The Epson, Nikon, Kodak, and many other digital cameras use removable memory storage cards called CompactFlash cards, which are almost the industry standard, but not quite. Sony cameras store images on floppy disks or on their special MemorySticks, which work only on Sony cameras. Olympus digital cameras store images on still another type of storage card called SmartMedia.

Batteries and chargers

All of the cameras except the COHU have batteries that last a *short* time when the camera is on, so try to turn the camera off as much as possible. The little 1.8-inch LCD monitor on the camera drains batteries fast. Fresh batteries in the Epson will last about 20 minutes, and about 90 minutes in the Nikon. Then the batteries have to be replaced or recharged. We will use AC adapters instead of batteries when we can.

Software

For the COHU we need only Scion/NIH Image, which is free from the Scion web site. As mentioned above, for the other digital cameras we need image manipulation software, which comes with some but not all digital cameras.

File sizes and image compression

Digital images can take up lots of storage space. A typical "low resolution" image suitable for viewing on a monitor is 300 kilobytes (kb) in size. That means you can get only four of them on one high-density (1.4 MB) floppy disk. A typical "high resolution" image suitable for color printing at 3 x 5 inches is 1-2 MB. At its highest resolution, the Nikon 880 camera can make a good 8 x 10 inch print but the image size for that is 11 MB, which is even too large for the 8 MB CompactFlash card that came with the camera. To reduce the huge size of image files, the camera or computer usually compresses the image by the JPEG method. Most consumer digital cameras compress their images by default but the COHU does not. If we take pictures with the COHU or with the consumer digital cameras using freeze-frame of video output (see below) we also get uncompressed images called TIFF files (PC attachment .tif), in which each pixel is an independent morsel of information. In JPEG, pixels are grouped into clusters with a common average value instead. The degree of compression depends on the size of the cluster. JPEG images suffer some loss of resolution, evident on good printouts, but the compression is hardly noticeable on the average computer monitor. The HotShots or Photoshop software can compress images to different extents, controllable by the user. In my class, I encourage use of the computer monitor and discourage printing so we keep the image sizes small (under 300 KB).

Printing and Zip drives

We used to print out digital images in class but we spent entirely too much time waiting for the printer, so now we try to be "paperless." I encourage the students to buy 100 MB Zip disks to store their work. They can take their work home, edit it, put it into lab reports, and send the reports to me by e-mail. However, we must be sure the computers in class and in the student computer labs all have Zip drives. A new external Zip drive costs about $90 (US). A 100 MB disk costs about $7.50 (US) when bought in quantities of 100.

Clamps, stands, and mounts

To hold the cameras still for copy work or microscope work, we used ordinary chemistry lab clamps and stands in the workshop. This crude mounting method was only a temporary expedient to show that the method can work. I strongly recommend making or buying sturdier custom camera mounts. Whichever mounting method you use, you must be careful not to damage the cameras with the clamps

Microscopes

We can photograph anything we can see in the microscope. A trinocular head is not required.

Student Outline

Our workshop was a question and answer session followed by seven little projects. Following these directions, novices should start from project number one and do them in order. Those with experience can start in the middle, do them in any order, skip some, or spend all the time reading manuals.

1. Photographing large animals (people).
- Pick up the camera and turn it on. When the green light goes on it is ready but don't take a picture yet.
- Look at the LCD monitor on the camera while moving the camera around.
- Aim it at your buddy. Compose your picture on the LCD screen.
- Snap a picture by pushing the shutter release all the way down.
- Wait for about 10 seconds while the camera stores the image.
- Turn the camera off.

That is all you have to know to take pictures with a digital camera. But of course there are many fine points:

1) There are two LCD screens on the camera, a small one for information and a large one for the image. After you turn the camera on but before you take a picture, it will show what it sees on its bigger LCD monitor. We use that monitor to compose the picture.

2) After you push the shutter button, the camera makes a freeze-frame on the big monitor for about 2 seconds; the frozen image is the picture it just took. Then it stores the picture on its memory card. When the live action resumes on the monitor the camera is ready to take another picture. If you do not like what you saw in freeze-frame, you can snap another picture.

3) Unless you are connected to an AC adapter (to house current, 115 volts AC), the LCD monitor is eating batteries fast so turn the camera off if you do not need it.

4) The camera has autofocus and autoexposure, which occur when the shutter release is pushed halfway down.

5) Before taking the picture, you can choose the image quality or degree of compression. The image quality is given on the smaller LCD display as symbols (1-4 stars on the Epson) or words (fine, normal, *etc.,* on the Nikon). You push little buttons to change the image quality and several other adjustable parameters.

6) The smaller LCD screen also records the number of shots remaining on the storage card.

7) Quality and number of shots are related; lower quality (fewer pixels per picture) means more shots available. In the highest-quality mode, the Epson can store four uncompressed 2 MB TIFF images on its 8 MB card. It its highest-quality mode, the Nikon needs 11 MB of storage capacity per image, which is larger than the 8 MB card that came with the camera, so we had to purchase a larger card to take a picture at the highest quality. A 32 MB card cost about $60 US in mid-2001. Usually we use medium or low quality so can get more pictures on the storage card.

8) The camera has a playback feature to review the pictures it took on its monitor. (Better yet, you can review pictures on the computer as described below.) We do not have to save all the pictures. We usually take lots of pictures and toss most of them.

2. Looking at pictures and saving them (consumer cameras)

- Wait until you have accumulated several pictures in the camera's memory.
- Make sure the camera is off.
- Remove the memory card from the camera. There is a special button to release it.
- Put the memory card with your pictures on it in the card reader connected to a computer.
- An icon should appear on the computer screen; it is a folder of the card's contents.
- Double click on the folder icon; it should open up, displaying icons for each picture.
- Double click on a picture icon; it should open up displaying the picture. Look at all pictures.
- Decide which pictures you want to save and re-name them to identify them.
- Make a new folder on the hard drive for the pictures you will save.
- Drag re-named pictures to your folder on the hard drive.
- Erase the card by dragging all the pictures in it to the trash and emptying the trash.
- Drag the icon of the card to the trash.
- Now you can remove the empty memory card from the card reader.

Fine points:

1) Do not remove the memory card from the card reader until you have dragged its icon to the trash. If you forget, the card will get messed up and you will have to reformat it. The Nikon card icon will jump back out of the trash after you put it there, but it does not matter; remove the card from the reader anyway.

2) Graphics files need a graphics program. If you have no other graphics program, Netscape, Internet Explorer, and PictureViewer (in the QuickTime folder on all Apple computers) will open pictures to just view them but you will not be able to manipulate them. You will need a program like HotShots or Photoshop or NIH Image to manipulate the pictures.

3) Manipulate can mean to crop, annotate with arrows and text, *etc*. Students can make professional-looking annotated figures very easily, and that gives the graphically-challenged (like me) great confidence.

Figure 1. Fred Gurney, age 6. The picture was taken with the Nikon 880 and opened in Photoshop as a color image. A small selection of the original was copied and pasted into Scion/NIH Image, which opened it as a grayscale image.

4) Manipulation can also include erasing data, pasting in stuff that wasn't really there, etc. There are many opportunities for fraud in the manipulation of digital images.
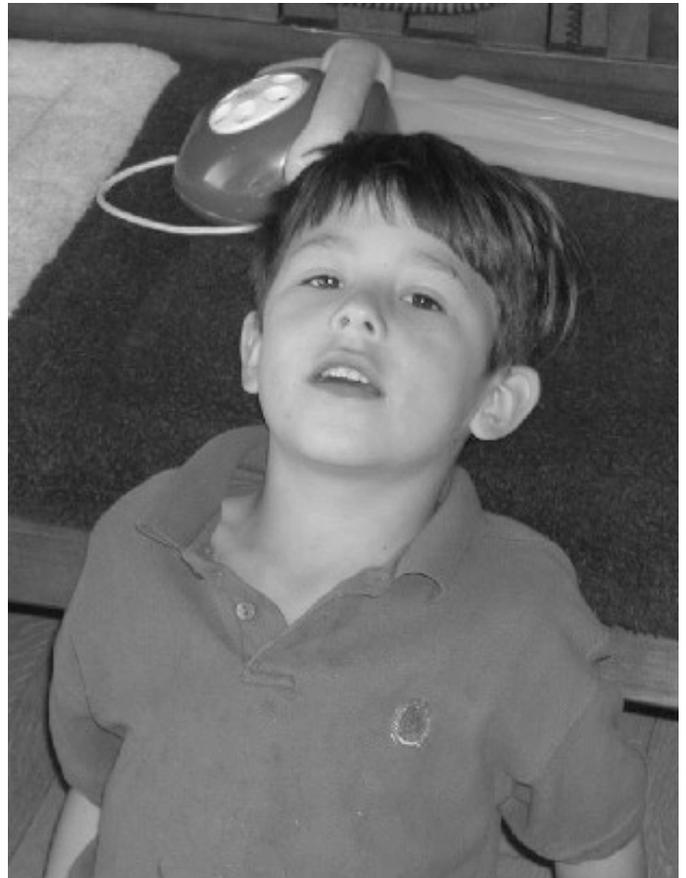
3. Close-ups and time delay (consumer cameras)

- Mount the camera on a copy stand (you could make a crude one out of chemistry stands and clamps).
- Find a small object to photograph.
- Arrange the clamps to hold the camera at the right distance from the object.
- Be sure the camera does not fall off and that the clamps do not damage the camera.
- Push little buttons on the camera to make a flower appear on the tiny screen (close-up mode).
- Push little buttons to make a stopwatch also appear (time delay).
- Push more little buttons to turn the flash off, lightning symbol with line through it.
- Compose the picture using the monitor.
- Push the shutter release and take your hands off to let the camera stop jiggling.
- It will take the picture 10 seconds after you push the release.

Fine points:

1) We turn the flash off for close-ups because the flash is arranged to illuminate things farther away. Instead of flash, we use available light and automatic time exposures but then we have to hold the camera still.

2) Close-ups are used to photograph small things like little flowers, insects, embryos, dissections, *etc*.

3) Most digital cameras have automatic exposure control and automatic focusing.

4) The default normal focusing range in Epson and Nikon cameras is about 18 inches to infinity but there is also an optional close-up range. The Epson's close-up range is 18 inches to 6 inches and the Nikon's close-up range is down to 1.5 inches, almost "macro-mode."

5) Our consumer cameras do not have cable releases to use with the time exposures we need with the flash off so there can be a terrible

Figure 2. Cheat grass seed head photographed with a handheld digital camera.

problem of camera shake making blurry images. We use the time delay feature to get around the shake problem.

4. **Photomicrographs (consumer cameras, color)**
- Turn the flash off.
- Using just your eyes on the microscope eyepieces, focus the microscope on the object.

- Focus at infinity. (For me, that means focusing with the distance, or upper part of my trifocals.)
- Then put the camera lens right where your eye was.
- Use a camera stand (or stands and clamps) to hold the camera in place.
- Adjust camera position to make the circle of light fill the little LCD monitor screen.
- Make sure illumination in the circle of light is uniform.
- Take a picture using the time delay method described above in the close-ups section.
- Using the same camera settings, photograph a hemacytometer to make a size scale.

Fine points:
1) We use chemistry clamps and stands to hold the camera as temporary expedients, just to show that it can work.
2) If you have a trinocular microscope, put an eyepiece in the third eye and use that eyepiece with the camera. You will still have to focus the microscope using one of your own eyes because the different eyepeices might not be parfocal.
3) If you have a hard time getting the circle of light to fill the field on the monitor, zoom the lens out, to longer focal lengths. Or, try using a wide-field eyepiece. The non-zoom Epson works best with a wide-field eyepiece. The zoom Nikon can work with a wide or narrow field eyepiece.
4) If the camera has the ability to fix its focus at infinity, use that; for instance, use the Nikon Landscape Scene, which means no flash plus focus fixed at infinity.
5) If you have shop talents, make a custom camera holder so you do not have to use the flimsy chemistry stands and clamps all the time. It would be lovely to have a mount with controls to make minor adjustments for positioning the camera precisely. Somebody might get rich making custom adjustable microscope mounts for consumer cameras.
6) I have used Leitz and Zeiss microscope mounts/adapters, each costing more than the camera itself and I have used PVC irrigation tubing, shims, rubber bands, duct tape, *etc.* There is no rule saying you must always use the most expensive equipment and that you can never be creative. However, the duct tape adhesive can be pretty destructive; watch out.
7) Download images from the CompactFlash card as before.

5) Photomicrographs with NIH Image (COHU or consumer cameras, grayscale)
- Position the camera to use the trinocular or third eye of the microscope. Make it secure.
- Run a cable from the video-out jack on the camera to the yellow RCA jack on the AV computer or to the Scion image capture board.
- The correct AV cable has a black end (camera) and a yellow end (computer).
- On the Epson, put slide at "view" on the back of the camera.
- Turn on the camera; green pilot light should go on.
- Launch NIH Image. From the Special menu, pull down "start capturing."
- You should get a live image of the object on the computer monitor.
- Adjust focus, stage, and light source to compose your picture on the screen.
- From Special menu, choose "stop capturing." You should get freeze-frame.
- From File menu, choose "Save as."
- Give the file a name and save it to your personal folder on the hard drive or your Zip disk.
- As above, photograph a hemacytometer under the same conditions to calibrate a size scale.

Fine points:

1) The big advantage of this method is the ability to compose the figure on the computer monitor instead of on the little LCD screen.

2) NIH Image stores images as uncompressed TIFF files.

3) NIH Image does grayscale only, not color, but most of what we do does not require color, and grayscale images are smaller.

4) The Nikon freeze-frame image has some annoying lettering which has to be edited out but the Epson and COHU freeze-frames are clean.
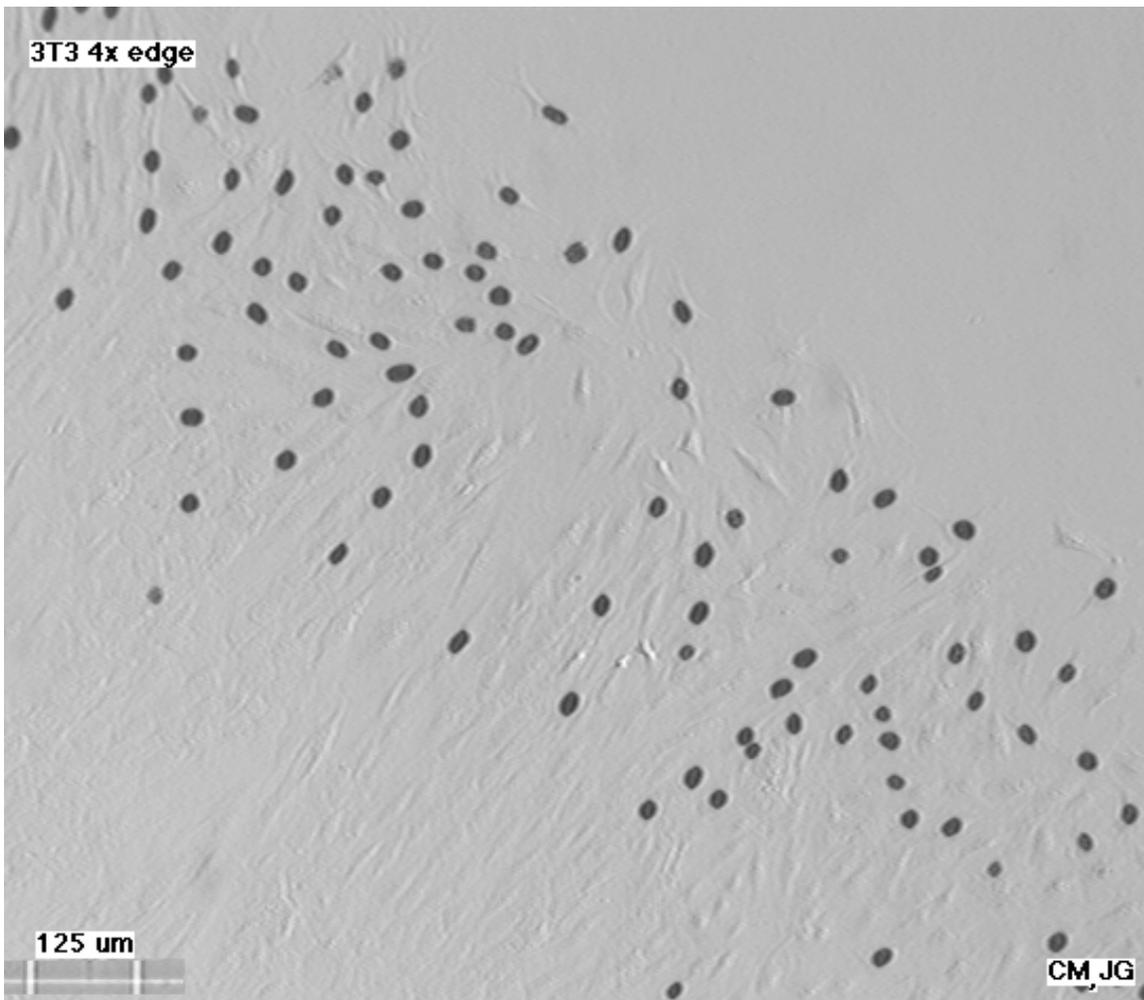

Figure 3. Autoradiograph of 3T3 cells labeled 6 h with tritiated thymidine. Made in Cell Biology lab, Autumn 1999, by two students. Used Leitz inverted microscope, 4x objective, COHU camera, and Scion/NIH Image. A little piece of a picture of a hemacytometer was pasted on to serve as a size scale. Black ovals are labeled nuclei; light gray blobs are unlabeled cells. The figure demonstrates contact inhibition of DNA synthesis because the sparser edge of the patch of cells was labeled but the dense middle (lower left) was not.

6. **Manipulating or editing a figure with NIH Image**
   - Open one of your color or grayscale images.
   - Select the box icon in the upper right of the artist's palette on the left of the screen.
   - Using the mouse, make a box around a small portion of the figure, selecting it.
   - There should be a dashed line around your selection.
   - Copy the box: "command-c" on Macs. ("Control-c" on PC's).
   - Close the image; don't save changes.
   - Open Scion/NIH Image (if you have not already) and get a new page to work on.
   - Paste in the selection you made: "command-v."
   - A grayscale version of the selection should appear on the new page.
   - Click the "A" icon; that means lettering.
   - With the mouse, click on the space below the figure to start lettering.
   - Type in a name for your selection.
   - Save the new figure in your collection, and give the figure a new name.

   Fine points:
   1) NIH Image is a paint program, so if you have done paint before (most high school kids have), this exercise will be ho-hum; if not, it will be a revelation. Skip this exercise if it is old news.
   2) Scion/NIH Image had an upper size limit to work with, 640 x 480 pixels. When asked for the size of page you want, type in 640 and 480. If your selection is bigger than 640 x 480, pasting will not work so start over with a smaller selection.
   3) Play with making lines and arrows and making text annotations on your micrograph. You can also rotate or flip the figure and there are many other tricks.
   4) There are unfortunately many opportunities for fraud using these tools; students might be tempted to erase data that "was not supposed to be there" and paste in data that "was supposed to be there."
   5) You can easily make a size scale for biometry. The x and y-axes of the figure are calibrated in units (pixels), so if you have a known size scale (a hemacytometer or the yardstick your buddy was holding in our exercise number one), you can calibrate distances in your figure by relating pixels to inches or micrometers. You could draw in a bar and letter it for a size scale.

7. **Time lapse movie made with Scion/NIH Image**
   To illustrate the method, we will photograph one of our favorite large animal subjects making some slow deliberate motions with her/his face and mouth.
   - Set up the Epson camera on a tripod aiming at a sitting person.
   - Run the video cable to AV computer.
   - Slide switch set to "view."
   - Turn on camera
   - Launch Scion/NIH Image
   - Under Special menu click "Start Capture"
   - Image should appear on computer monitor
   - Get the live image centered, make sure light and focus are OK
   - Draw the smallest possible box around the subject to make file size small
   - Under Stacks menu, click Make Movie...

- Dialog box appears
- Choose 20 frames
- Choose 1 second/frame
- Click Trigger and then First Frame
- Click OK
- Subject makes slow deliberate motions with face, nose, and tongue for 120 seconds.
- Type command-period to stop
- Save as "My Movie" (or something like that)

(A less personal but less interesting subject is to photograph the dial of an analog watch or clock.)

Play the movie by opening it and clicking the mouse. You can also go through the stack of images using the "<" and ">" keys or the page up or page down keys.

We use this method for making time exposures of slowly moving objects such as migrating tissue culture cells, or cultured chick embryo heart cells beating in culture. As an example of the kinds of things that are possible with time-lapse movies, students in my cell biology class made a movie of 3T3 cells migrating slowly on a Petri plate. Their time scale was one frame per hour. (In comparison, students who made a movie of beating chick embryo heart cells used a time scale of two frames per second.).

Acknowledgment