Inexpensive Digital Microscopy Workstations Engage Students in Integrative Biology

G. S. Withers[1,2*], and C. S. Wallace[1]

[1]Department of Biology, Whitman College, Walla Walla, WA 99362
[2]Center for Research on Occupational and Environmental Toxicology, Oregon Health Science University, Portland, OR 97239

Turning the pages of high impact journals in the life sciences reveals the extent to which many disciplines are converging on the technology of live cell imaging. Yet, due to the high cost of digital imaging equipment, few undergraduates have an opportunity to work with this powerful technology. We confronted this issue by designing a basic, inexpensive workstation that could be fitted readily to standard student microscopes, and by using this workstation to introduce interactive imaging exercises to all levels of our life sciences curriculum. By adapting a consumer-grade digital camera with live video to a student microscope and adding flat screen TV as a monitor, digital microscopy becomes a stimulus for student inquiry and discussion. With standard computers and minimal software, students can capture images or movies to document their observations in a digital lab notebook and perform analyses offline. In this context, familiar exercises like sea urchin fertilization, cytoplasmic streaming in Elodea or behavior of protists, can provide fresh data insights that engage student in critical analysis of the molecular regulation of life. Thus, a modest investment in technology generates an environment for active learning, pushes students to make cross-disciplinary connections and prepares undergraduates to work effectively with images as experimental data.

Keywords: digital microscopy; live cell imaging; undergraduate cell biology

1. Introduction

A major challenge in life sciences education is to organize training that will engage students as independent scientists from the outset [1]. This requires a shift in emphasis away from pedagogical methods that simply transfer essential information efficiently and crisply (e.g., lectures and "scripted" lab exercises) to exercises that are driven by student curiosity and allow students to learn principles by developing and testing their own hypotheses [2, 3]. One practical barrier to integrating classroom coverage of background material with fresh hands-on experience relevant to current science, however, is the high cost and complexity of the analytical tools that are framing the next generation of questions in life sciences research.

1.1 Imaging as a tool for integrative biology

Interest in imaging technology has converged to the point that researchers from many disciplines (e.g. biochemistry, genetics, cell biology and physiology) use living cells as test tubes to address fundamental questions [4]. This has promoted rapid, and mutually enhancing advances in cell culture, molecular labeling and digital microscopy. From the perspective of science education, the common focus on live cell biology is melting the borders between traditional academic disciplines as it raises a new set of "postgenomic" questions about how gene products assemble into life [5]. Our own professional interest in cell biology came from observing living cells in action and wondering how they did it. Thus, we began to revise our pedagogy by seeking ways to expose students to the phenomena that continue to

* Corresponding author: withergs@whitman.edu, Phone +1 5095275053

inspire our research imagination though the incorporation of digital microscopy and live cell imaging into our curriculum.

Our goal was to be able to put a digital imaging workstation in the hands of our students as early as their first introductory lab to help them develop an integrative and cross-disciplinary science perspective. In addition, we wanted to challenge them to begin building the skill sets they would need to use imaging effectively in upper level courses and ultimately to apply these tools to their independent senior thesis research. We approached this challenge by defining 4 objectives. The first objective was to design a digital microscopy workstation that would be affordable and accessible to undergraduate biology students. Second, we wanted to configure these microscopes to gather students into active discussion, rather than divide them individually as active observers and passive non-observers. Third, we wanted to design open-ended exercises that would expose students to living cellular physiology (e.g. cell motility, intracellular transport) and challenge them to account for their observations by applying the discipline of hypothesis testing while integrating the background information from the classroom side of the course (e.g. organization of the cytoskeleton, force generation by molecular motors). Fourth, we assigned projects that asked our students to analyze their images as primary data, and to deliver a product that reflected the critical standards they will encounter in the current literature (e.g. to generate publication quality figures). This digital microscopy initiative is an attempt to address the challenge put forth by the National Academy of Sciences to teach cell biology the way it is done by working scientists [1] and reflects the contemporary intellectual shift away from detailing molecular components toward understanding the emergent properties of life [6].

2. Critical elements of an inexpensive digital microscopy and imaging workstation

2.1 Identifying critical components of a research digital microscopy workstation

Surveying the facilities for digital microscopy in our research lab, we tried to identify the key components that make such a set up a powerful tool for discovery (Fig. 1a). We identified these capabilities as indispensable: 1) the ability to work in a digital medium, allowing interactive image processing and analysis (requiring a digital camera), 2) the ability to display live video on a screen, permitting discussion in real time as specimens are surveyed and images are acquired (requiring a color monitor to show the specimen as it is viewed in the microscope), and 3) the ability to rapidly analyze images and discipline visual impressions with quantitative measurements (requiring offline computer analysis). Figure 1b shows a simple student workstation that emulates these essential features. Note the parallels with the research workstation including (in addition to a microscope), a digital camera, monitor and computer for image analysis. Table 1 indicates how consumer grade equipment can substitute for much more expensive specialized components.

Fig. 1 A comparison between a) a research microscopy workstation and b) an inexpensive student workstation illustrates how the essential components of the research grade setup can be emulated to achieve a student grade digital microscopy workstation.

Table 1 Essential elements of a digital microscopy workstation for research and student training.

RESEARCH MICROSCOPE/DIGITAL IMAGING WORKSTATION	ADAPTATION TO STUDENT MICROSCOPE
DIGITAL CCD CAMERA	DIGITAL CAMERA (e.g. Nikon CoolPix)
DISPLAY	TRINITRON FLAT SCREEN TV
IMAGE ANALYSI S SOFTWARE	PHOTOSHOP ELEMENTS, NIH IMAGE

2.2 Essential components of a student digital microscopy workstation

We chose a Nikon Coolpix 4500 that produced 4.0 megapixel color still images and was able to acquire 35 sec movies with audio track for narration. This model is no longer commercially available, but there are a number of comparable alternatives, and with improved digital camera technology, cameras in a similar price range now have higher image resolution and longer movie recording capabilities. An additional advantage of building a system around a general purpose handheld camera is that it can be adapted flexibly to a variety of uses. In our biology department, for example, the cameras are also available for outdoor and macrophotography, such that students can use them to gather data, and record images for individual research and other class projects. In choosing a digital camera, we recommend that the camera 1) has manual as well as automatic exposure and flash settings, 2) has a live video out connector, 3) has a threaded filter ring that allows the camera to be attached to a microscope adaptor. The ability to control camera settings is particularly important in photomicroscopy, where automatic settings may not select the desired plane of focus, or exposure.

We used an aftermarket adaptor to connect the digital camera to our student microscopes and strongly suggest confirming that such an adapter is currently available for a candidate camera before configuring a system. Also, companies that manufacture such adapters (e.g. Qioptiq Imaging Solutions, www.qioptiqimaging.com) can be a valuable source of technical information because they must keep abreast of design and manufacturing changes as well as the variety of user applications. The simplest way to install a camera to a microscope (or wide variety of optical instruments) is to replace a standard diameter eyepiece with the adaptor, allowing the digital camera to slip into the ocular tube. This obviously prevents viewing through the eyepieces, however. For unobstructed viewing through the eyepieces in parallel with digital image recording, it is necessary to purchase a trinocular head for the microscope. Unfortunately, trinocular heads are not available for all commercially produced student microscopes, and this could be a limitation to working with already purchased microscopes.

Rather than purchasing a specialized high resolution imaging monitor, we opted to use 13" flat screen color TV (Sony Trinitron). There are two major advantages to having the monitor coupled to receive live feed from the camera. Most important, it allows microscopic images to be viewed in real time by a group, such that multiple active observers can share a single microscope. Also, in a large lab, such as our introductory biology class, an instructor can easily scan 12 monitors to draw attention to important phenomena or identify teams that need help. A second advantage of having a live monitor feed is that it makes focusing the microscope for the camera much easier for a beginner.

To allow for easy offline analysis of recently acquired images (and rotation of a large class onto a limited number of scopes), we added an extra memory card, so that some students can be working with their images offline while others are using the microscopy workstations. There are a number of options for computer digital analysis. We have used software that is freely available from the National Institute of Health (NIH Image, for Macintosh computers, http://rsb.info.nih.gov/nih-image/, Image J for PCs, http://rsb.info.nih.gov/ij/). These packages are ideal for quantifying data, such as determining size and scale. In addition, our institution purchased a site license for commercial software (e.g. Adobe Photoshop Elements) that was quite helpful in training students how to compose figures. The net cost of upgrading a student microscope to a digital workstation was under EUR 900 ($1200 USA), including the purchase of a trinocular head, adaptor, camera, monitor, memory cards and card reader (this cost excludes the microscope, offline computer and software site license).

3. Applications in teaching

3.1 Exercises to generate hypotheses about the molecular regulation of life

The ability to record living cells in motion can reveal key issues in current biology using the simplest of preparations. For example, three topics of active research in cell biology are cellular polarity, regulation of cell shape, and intracellular transport. Most undergraduates do not confront these important topics until upper level courses, if at all. Protists, such as the stentor (Fig. 2a) provide classic examples of cells as free-living organisms with complex behaviors. They are also excellent models of polarity (i.e. different regions of the cell are specialized for different functions) and they exhibit strikingly dynamic shifts in shape. Simply observing these organisms via short digitally recorded movies can prompt students to generate questions about how such complexity is regulated within a single cell. Elodea (fig. 2b) is a fresh water plant commonly used to demonstrate a form of intracellular transport known as cytoplasmic streaming. Using movies not only allows students to observe this phenomenon thoroughly, but also measure it quantitatively to distinguish active vs. passive transport. Because biological processes in living cells are a sequence of transient events, reviewing movies can help students to define these processes with greater precision, and provide the opportunity for an “instant replay” in the case where events occurred before they could be identified. For example, the initial events involved in the fertilization of sea urchin eggs are both rapid and subtle. Acquiring movies during fertilization enables students to go back and review the events rather than having to infer that these events occurred based on longer lasting cellular changes like the appearance of the fertilization membrane.

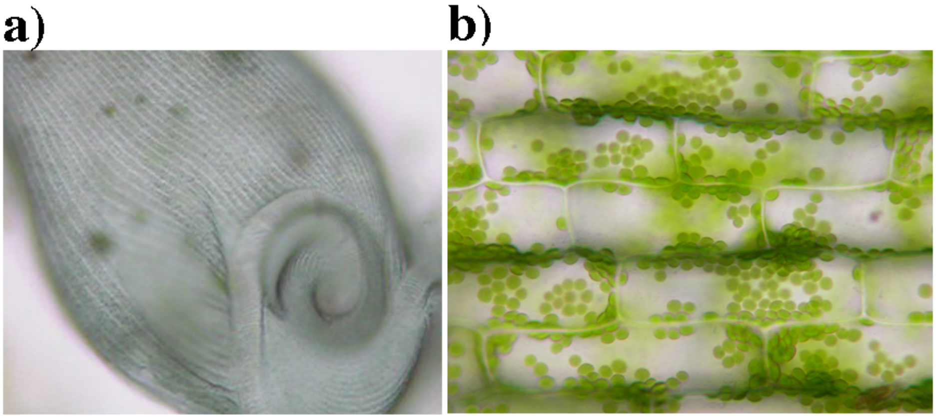

Fig. 2 High resolution images allow students to explore the microstructure of living organisms, stimulating questions about the underlying molecular machinery. Movies record a) morphological changes in the spiral shaped oral pore lined with cilia on the protist stentor; b) directional movement of pigmented chloroplasts in the aquatic plant Elodea reveals active intracellular transport. Examples of student-generated movies can be viewed on our website, address provided below.

3.2 Acquiring and analyzing digital images as primary data

Actively recording images of a particular phenomenon viewed through the microscope enhances a student’s critical perspective (i.e. by acquiring data with purpose as opposed to forming general impressions). This activity also generates a permanent record that can be analyzed and re-analyzed as hypotheses are tested and refined. Thus even the most familiar exercises can become freshly engaging when coupled with digital imaging. One simple experiment common to many introductory biology labs assesses how changes in osmolarity influence the organization of living plant cells. By comparing photographs of cells before and after hyper- or hypotonic conditions, students can quantify the extent to which the size of the cells change (fig. 3a). With quantitative measurements they can go on to determine the relationship between molarity and amount of change, as well as measuring the rate at which cells change.

Incorporating digital imaging into familiar exercises such as analysis of prepared slides (often regarded as dry, and passive observation assignments) can make these exercise more active and extend their lasting impact. We have used images to make digital atlases, or digital lab notebooks. These exercises help train students to use images as primary data, and enable analysis beyond the class period. For example, we have had students assemble a series of images to create an atlas of early sea star development (fig. 3b). One outcome is a concrete illustration of key concepts, e.g. that, although the number of cells increases substantially in early development, growth does not occur in an embryo until gastrulation begins). A second outcome is that students are prepared to record and measure anything that they can observe with their microscope. These kinds of projects can easily be extended to incorporate the images into lab reports, or for the images to be treated as figures for publication by including labels and writing a caption. Thus, by acquiring images, and assembling them into figures, students have an opportunity for critical review and quantitative analysis of data.

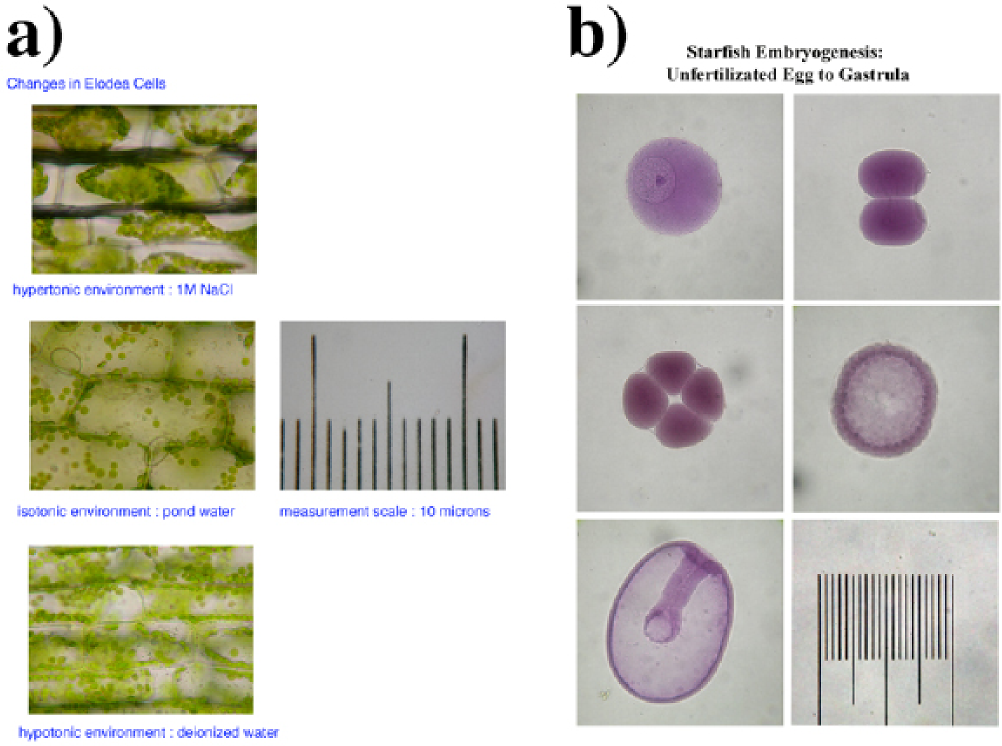

Fig. 3 Examples of student product. a) In our introductory biology laboratory, students analyzed the effects of changing osmolarity on the organization of living plant cells by generating a figure that allowed them to compare the morphology and size of individual cells in solutions of varied salinity; b) in an exercise studying development, students made an atlas identifying key stages of sea star embryogenesis. Both a and b are representative samples of original student work.

4. Assessment and Outcomes

4.1 Assessment of digital imaging exercises

We have used two avenues for evaluating the advantages of adding imaging exercises to our curriculum. The first is our subjective impression of an improved level of intellectual readiness and technical competence students are bringing to our upper level courses and to research internships within our lab. The students also report finding these exercises helpful. Using an anonymous survey, we asked students in a upper level course who had participated in imaging exercises to rate the value of the experience using a Lichert scale (i.e.,1 is least positive and 5 is the most positive) in response to the following two questions:

1. Did you find using the digital camera to acquire images from prepared slides useful? (Mean score: 4.73).

2. Would you continue to use the digital camera to acquire images and movies from living preparations? (Mean score: 4.64).

Student comments indicated that they preferred exploring microscopy in conjunction with the digital photography both because they thought it enhanced the process of learning and provided them a take home product they found more helpful than hand sketches they had done in the past. Students cited the ability to use the TV monitors as very helpful in allowing them work collaboratively with their partners as opposed to taking turns. Generating their own images allowed them to supplement textbook figures by creating their own illustrations that documented key phenomena in manner that made the most sense to them. Preparing a digital lab notebook extended their study time as they identified images and labeled key structure during figure composition, and enabled them to prepare for lab practical at home. Taken together, these comments suggest that students found themselves more actively and independently engaged in microscopy exercises that involved digital microscopy. We have found it beneficial for students to take away images rather than impressions for their lab projects.

4.2 Conclusions

Since its inception, the microscope has been a critical window to our understanding of the cell, and now, our understanding of the cell is advancing at an amazing rate through data gathered using digital microscopy and live cell imaging [4]. By building upon microscopes already present in many labs, the addition of simple digital imaging tools like those described here, allows students to participate in this microscopy revolution. From an educational standpoint, it transforms microscope use from a solitary exercise into an interactive learning experience. It also helps address the challenge of teaching biology as it is practiced, enabling students to generate and present images as data using state of the art technology, and in the process, enriching their understanding of cells as living machines.

Acknowledgements This work was supported by a grant from the W.M. Keck Foundation to Whitman College and by the National Science Foundation CAREER Award IBN-0135985 to GW. We also gratefully acknowledge technical assistance from Martha Holt and Heidi Geiser, the students in our classes at Whitman College from whose work we sampled to generate Figs 2 and 3, and Brad Changstrom for critical comments on the manuscript. Additional information can be found at our website at: http://www.whitman.edu/biology/dendrite/index.html

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