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Information Management Completion Report

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ABSTRACT

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The focus of this research project was to continue the development of a photographic system which would record living organisms using various forms of light microscopy with correct color and with arrested movement. These demands dictate the use of an electronic flash source with metering and control system located in a position following the passage of the light through the optical train. The system developed uses off-the-shelf components with a modified flashtube holder which positions the tube in the axis of the light beam between the field and iris diaphragm. The light is measured off-the-film so that light from the microscope and flash are combined. The flash is quenched and shutter closed based upon the combined reading.

The second phase of the research project concentrated on (1) improving the adaptability of the equipment to various microscopes, (2) testing the system under various lighting conditions, and (3) producing high quality micrographs of selected verified taxa.

The micrographs will be incorporated into an existing ecological data base of the algae of Arkansas. Many of the organisms are ubiquitous. Therefore, micrographs are useful for comparison with other sources as well as serving as a verified reference resources of Arkansas taxa.

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INTRODUCTION

The only reference source for the identification of freshwater algae with appropriately colored illustrations for the United States is by Wolle in 1887. Most of the subsequent references have included line drawings. Some of these include illustrations with cytoplasmic detail, while others are only silhouettes. Certain texts and articles may include monochromatic micrographs of fixed, dried, cleared, or treated organisms. The major taxonomic references containing stippled drawings are in Russian, German and Polish. Although stippled drawings may present a three-dimensional view of the organisms, they lack the true color and shadings of the cytoplasmic inclusions, i.e. plastids, stigma, nucleus, storage products, spores, etc.

Most photographic techniques are limited by the intensity of the light source and therefore require extended exposure times. Intense light sources tend to modify or kill the cells due to heating or photodegradation. Newer methods of microscopy, i.e. phase-contrast, Nomarski differential contrast, and other systems, pass less light to the film plane and exposure time increased. These extended exposure times prevent capture of gliding activity and flagellar movement. Most motile cells (vegetative, zoospores or gametes) are quite small and erratic in their rapid movement. Also, motile cells are very difficult to photograph because their movement is exaggerated by the magnification of the microscope.

The requirements necessary for the successful photomicrography of living algae include true presentation of the plastid pigmentation, precise representation of cellular inclusions, and arresting the motion of the flagella as well as the intact cell or colony. These criteria can be met by positioning an electronic flash tube in the axis of the microscope light beam. Systems developed earlier by Leitz for their Ortholux microscope have successfully demonstrated that the flash could be centered in the beam between the field and iris diaphragm. However, this system did not include automatic quenching of the flash output.

A. Purpose and Objectives.

The long-term objective of the research is to develop a verified photographic record of algae from ecoregions, communities and subcommunities of algae within Arkansas. In order to accomplish this objective, special equipment was designed and tested. The required film plane quenched flash control systems are not available commercially for microscopes. However, components are available via professional and advanced amateur cameras. The first phase of this research project was to modify and adapt these components for attachment to various microscopes. The objectives of the second phase included (1) the improved adaptability of the unit to fit various microscopes, (2) testing the developed system under various lighting conditions, and (3) producing high quality micrographs of selected algal taxa.

B. Related Research and Activities

Algae were collected from additional sampling sites and new taxa were added to our existing data base. The developed unit was applied to develop a photographic record of the midwestern species, varieties and forms of the genus Pleurotaenium (Desmidiaceae). In addition, selected taxa of coccoid green algae, cyanophytes, as well as flagellaged and rhizopodial chrysophytes were photographed. These new images are included within an existing collection and are cross-referenced with a taxanomic data base.

New films have become available during the research period. Minimal information for microscopic applications was available. Of particular interest was the new Kodak Ektar 25 color print film. This film has excellent color balance (daylight) with extremely fine grain structure. The images can be enlarged up to 35 times with acceptable results as either color or black/white prints. The application of high resolution color print film is very desirable for developing a reference source which can be readily copied, annotated and distributed.

Other tested films include Kodak Ektachrome HC 100 and similar Fuji products. These films produce positive images for projection. Films of higher speed tend to produce lower resolution, but may be desirable for fluorescent plus flash montague images.

METHODS AND PROCEDURES

Careful analysis of the characteristics of several available amateur and professional 35mm camera systems were conducted. Criteria for selection of components to be adapted and modified were developed. These included: 1) off-the-film or equivalent flash controlling circuitry, 2) flash units which can be extended from the camera body, 3) dissectable flash unit which will permit disessembly and modification, 4) dampened shutter closure to minimize camera movement and disturbance of the specimens, 5) available microscope adapters, and 6) matching photo eyepieces for varying focal lengths of microscope objectives. An additional desirable characteristic includes compatability with mounts of other photomicrographic and video equipment.

The equipment chosen for modification, adaptation and testing which met all of the above criteria were Olympus OM 35mm camera components. Camera bodies from the OM-2 series or more recent models contain of-the-film sensing circuitry. Several flash units are compatable with this body, but the Olympus T Power Control 1 flash unit has a detachable flash head (T 28). This control unit can operate on self-contained batteries or with an external power source.

The modification and adaption phase concentrated on the modification of the flash head to fit existing microscopes. The flash tube located between the field diaphragm and condensor has given satisfactory results and permits the flash unit to be transferred

to various microscopes without special adaptation.

The flash head was modified by introducing an axially located 2 cm aperature in the head body. Non-essential components were removed from the head and the power/control cable was moved from the posterior surface to a lateral position. These modifications retained the parabolic reflector, but permitted the microscope lamp beam to pass through the housing and simplified further adaption to various microscopes. A simple holder for the modified head is under development. With further experimentation, it may be possible to develop a universal adaptor with an adjustable, self-centering clamping mechanism.

PRINCIPAL FINDINGS AND SIGNIFICANCE

Available microflash units for microscopes lack off-the-film circuitry to control the combined light imput from the microscope illumination and flash sources. Most of the units, therefore, lack the ability to readily measure various modes of illumination, i.e. darkfield, brightfield, phase-contrast and differential interference contrast. Therefore, extensive experimentation is necessary to develop fixed combinations of illumination intensity and flash intensity/duration to produce satisfactory results.

The developed system described here uses an off-the-film metering circuitry, which senses the total light input to the film and quenches the flash (cf. Meyer, 1989). This system measures light from all sources, both the the microscope lamp and the flash

tube. It accomodates variations in modes of illumination as well as density of the specimen. The combined exposure is accomplished by setting the camera shutter speed for a shorter duration than required for correct exposure by the microscope lamp only. The microscope illumination can be reduced in intensity as well as from a reflected or fluorescent source. The additional light is from the flash source. The duration of this constant intensity source is monitored and controlled by the camera. The flash duration is controllable from 1/40,000 to 1/10,000 sec.

The constructed system has been tested on microscopes containing brightfield, darkfield, phase-contrast and differential interference contrast illumination. The intense illumination from the flash unit permits the use of films with greater resolution but of lower film speed. Thus, higher quality images are recorded.

This system effectively stops organism and organelle motion. Flagellar action is arrested at all levels of magnification and with various illumination systems. With the flash system, specimens can be observed and studied at lower intensities/heat; thereby, reducing cellular damage and without the introduction of destructive or non-destructive adjuncts.

The system was used to examine twenty (20) species, varieties and forms of the genus Pleurotaenium. This taxon was photographed at various levels of magnification, 5 to 1,250x, with brightfield, phase-contrast and differential interference microscopy. Similiar techniques were used with representatives of the coccoid greens,

euglenoids, desmids, bluegreen algae and several motile and amoeboid chrysophytes.

Most of the micrographs were recorded on low speed, high resolution positive color films. These provide high quality projection images. Detailed analysis may be achieved by viewing through a dissecting microscope. If daylight corrected films are used, the resultant colors closely matched those of the specimen. However, some daylight films may be weak in the yellow range of the spectrum while others emphasize the blues. For example, Kodak Ektachrome HC 100 provides high speed, moderate resolution, but enhanced blues with increased contrast. These characteristics may be valuable when photographing fluorescent images. The use of color correcting filters may significantly reduce film speed to that of higher resolution color balanced films.

The availablity of high resolution, but low speed, color print film has added a new dimension to the research. Kodak's Ektar 25 (ISO 25/15^{o}) produces fine grain color prints with excellent color balance. The negatives can be enlarged up to 35x for either color or black/white prints. This new film and color prints are ideally adapted to the development of a verified reference source of algae. The prints are easier to reproduce, do not require projection equipment and can be annotated to highlight specific characteristics. Also, monochromatic prints can be incorporated into scientific publications. Therefore, color prints and positive images will be produced as a component of the reference resources.

CONCLUSIONS

The utilization of off-the-shelf components to produce a auto-regulated flash system adaptable to various microscopes and illumination systems has been successful. The system is functional and has surpassed the goals of the initial phase of the research.

High resolution, color correct images of motile organisms, zoospores or gametes have been produced using all illumination methods. The system is now being used to develop an extended collection of images. Also, newer film types have been tested and the addition of high resolution color print film has added a new dimension to the development of the reference resource.

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