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## Presentation of Verified Algal Taxa as Reference Sources

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**PRESENTATION OF VERIFIED ALGAL TAXA AS  
REFERENCE SOURCES**

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AS REFERENCE SOURCES

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Information Management Completion Report

Project G-1549-32

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## ABSTRACT

### PRESENTATION OF VERIFIED ALGAL TAXA AS REFERENCE SOURCES

A data base of the algae of Arkansas ecoregions has been established to describe the numerous taxa that occur within the aquatic ecosystems included in these regions. The organisms were identified with the aid of diverse literature from throughout the world. These sources are written in multiple languages and the living organisms had to be compared with outline or silhouette drawings. These illustrations may include shading, but none present the true color of the organism but only the characteristics of the descriptive source. Primary characteristics used to identify algae is based upon pigmentation of the plastid and the number and position of the flagella as well as gross morphology.

The focus of this research project was to develop a photographic system which would permit the recording of living organisms with various forms of microscopy with correct color and with arrested flagellar movement. These parameters dictated the use of an electronic flash source with metering and control system after the light has passed through the microscope. Most commercially available systems use preset flash intensities and/or durations. These systems require the use of neutral density filters or other adaptations to attain specified magnifications-illumination method-flash settings.

The system for this project 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 diaphragms. The light is measured off-the-film so that the combined light from the microscope and flash are included. The flash is quenched and shutter closed based upon the combined reading. This system permits the operator to change magnification and/or illumination technique (brightfield, phase-contrast or Nomarski) without adjusting the flash or camera setting.

Richard L. Meyer

Completion Report to the U.S. Department of the Interior, Geological Survey, Reston, VA, June 1988.

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## INTRODUCTION

The only reference sources for the identification of freshwater algae with appropriately colored illustrations for the United States is by Wolle in 1887. Subsequent references have included line drawings. Some of these include illustrations include cytoplasmic detail while others are only silhouettes. Alternatively, the best illustrated taxonomic references, with 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, ie. plastids, stigma, nucleus, storage products, spores, etc.

Most photographic techniques are limited by the intensity of the light source and thus the duration of the exposure. More intense light sources tend to heat the organisms and modify or kill the cells. As newer methods of illumination were developed, i.e. phase-contrast, Nomarski differential contrast, and other systems, less light was available and exposures were longer. These exposure time prevented the capturing of flagellar movement. Most motile cells (vegetative, zoospores or gametes) are quite small. Also motile cells were very difficult to photograph because their movement is exaggerated by the magnification of the microscope.

The parameters necessary for the successful photomicrography of living algae include the true presentation of the plastid pig-

mentation, precise representation of cellular inclusions, and arresting the motion of the flagella as well as the intact cell or colony. These criteria could be met by the position of 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 had to be designed and tested. The required filmplane quenched flash control systems are not available commercially for microscopes. However, components are available via professional and advanced amateur cameras. The first short-term objective of this research project was to modify and adapt these components for attachment to various microscopes. Secondly, following modification and adaptation, was the testing of the developed unit on various microscopes and with different modes of illumination.

B. Related Research or Activities

During the testing phase of the research algae were collected from additional sampling sites and new taxa were added to our existing data base. Corollary to the equipment development was



the initiation of a research project on the genus Pleurotaenium (Desmidiaceae) and an ecological research program on a major drainage basin of a river system (Middlefork of the White River). These activities plus our ongoing phycology laboratory activities will provide opportunities to photographically record known as well as new taxa.

#### METHODS AND PROCEDURES

A careful analysis of the characteristics of several available amateur and professional 35mm camera systems was conducted. Criteria for selection of components to adapt and modify 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 disassembly 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 included compatibility with mounts of other photomicrographic and video equipment.

The equipment chosen for testing which met all of the above criteria was the Olympus OM 35mm camera components. Camera bodies from the OM-2 series or more recent models contain the off-the-film sensing circuitry. Several flash units are compatible with this body but the Olympus T Power Control 1 flash unit has an extend-

ible flash head (T 28). This head is detachable from the flash unit. Therefore, if the flash tube is damaged and requires replacing, only the head needs to be replaced. Also the control unit can operate on self-contained batteries, or with an external power source.

The alpha modification and adaption phase concentrated on the modification of the head to existing microscopes. Two primary sites for flash tube insertion are between the microscope lamp and collecting lens system, or between the field diaphragm of the illuminating system and the iris diaphragm of the condenser. The location near the microscope lamp may require a special adaptor/lens combination because of the extended lamp position. This adaptor would have to be tailored for the requirement of each microscope. The latter location 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 aperture 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 adaptation to various microscopes.

Beta refinements include improving the attachment mechanism so that the entire system can be conveniently transferred to other

microscopes. Bases for each type of microscope need to be developed to hold a standardized flash head. This latter development may require further modification of the flash head. 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 input from the microscope illumination and flash sources. Most of the units, therefore, lack the ability to readily measure various modes of illumination, ie. darkfield, brightfield, phase-contrast and differential interference contrast. Usually extensive experimentation is necessary and only fixed combinations of illumination intensity and flash intensity give satisfactory results.

The system developed uses an off-the-film metering circuitry, which senses the total light input to the film and quenches the flash. This system measures light from both the microscope lamp and the flash tube. It accomodates variations in modes of illumination as well as density of the specimen. These variations are controlled by setting the camera shutter speed to be of shorter duration than required for correct exposure by the microscope lamp only. Alternatively, the intensity of the microscope lamp can be reduced and the camera shutter setting constant. The supplemental 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 while sacrificing 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. Prior to the development of the flash, exposures were greater than one-fourth second at one thousand diameters magnification with brightfield illumination and several seconds with other types.

This new system, with further refinements, opens additional windows of studying and recording dynamic events in living organisms. These organisms can be recorded in correct color on high resolution films. A motor drive can be added so that sequential micrographs for time series analytical techniques can be applied. Increased medium viscosity methods are not required to arrest motion nor are temporarily non-destructive fixation techniques. With the flash, system specimens can be observed and studied at lower intensities/heat; thereby, reducing cellular damage.

In all, truer representations of living organisms can be developed as verified records to assist taxonomic studies, systematic analysis and ecological research. As the images are col-

lected and correlated with the existing data base new insights into adaptive mechanisms may emerge.

#### CONCLUSIONS

The utilization of off-the-shelf components to produce a autoregulated flash system adaptable to various microscopes and illumination systems has been constructed. The alpha testing of the systems indicates that it is functional and surpassed the goals of the initial phase of the research.

High resolution, color corrected images of motile organisms, zoospores or gametes have been produced using all illumination methods. The systems can now be used to develop an extended collection of images. Also newer film types should be tested.

To increase the flexibility and convenience of the system, further modification of the flash head and microscope adaptor are needed. Future modifications may result in adaptation to other types of instrumentation where a photographic record would be valuable.