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Optical Diagnostics of Living Cells III

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ADVANCED TECHNIQUES IN ANALYTICAL CYTOLOGY

SESSION 1

Microscopy I

Introduction

The Advanced Techniques in Analytical Cytology IV meeting has been recognized by the International Society for Analytical Cytology (ISAC) as an ISAC-affiliated meeting. This is further evidence that SPIE BiOS meetings are now the place to learn about new techniques in analytical cytology. Many papers relevant to analytical cytology are spread throughout this volume and will appear in other BiOS 2000 proceedings. The Advanced Techniques in Analytical Cytology IV meeting contained many interesting papers on diverse subjects concerning new instrumentation and software, including standards, dyes, reagents, and applications.

The use of digital micromirror devices is a significant advance in microscopy, which includes, but is not limited to, making confocal microscopes with ultraviolet light excitation available at affordable prices. Other interesting microscope technology improvements were also described. Software advances to improve the classification and segmentation of nuclei were presented. The use of flow cytometry for drug discovery and development is a new and very interesting application. Dedicated instrumentation for achieving the discrimination of flow cytometry without the use of complex instrumentation can significantly expand the use of clinical measurements, such as CD4 quantitation in HIV patients. The dye studies included measurements of membrane fluidity and the use of lanthanide macrocyclic complexes without the need for time-gated instrumentation.

I have deliberately avoided mentioning any author by name because I believe that it would be inappropriate for me to steer the readers to any particular paper(s). I believe that all of the papers are informative and useful, and that each of the individual readers including myself, quite properly, has his or own interests.

I again wish to thank my cochairs, the other authors, the audience, and most of all you, the readers, for the success of our meetings.

Robert C. Leif

Improvements to Quantitative Microscopy Through the Use of Digital Micromirror Devices

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ABSTRACT

All of the different modes of microscopy deliver light in a controlled fashion to the object to be examined and collect as much of the light containing the desired information about the object as possible.

The system being presented replaces the simple circular or annular diaphragms of a conventional microscope with digital micromirror devices (DMDs, made by Texas Instruments) to enable digital light microscopy. The DMDs are placed in the optical path at positions corresponding to the field and aperture diaphragms of a conventional microscope. This allows for more precise and flexible control over the spatial location, amount, and angles of the illumination light, and the light to be collected.

Digital light microscopy enables the improvement of existing modes of microscopy, specifically for quantitative microscopy applications. Confocal microscopy has been performed, realizing improvements in resolution, flexibility, and cost. Three different combinations of image acquisition and post-processing algorithms have been used to generate confocal images, as well as a tomographic reconstruction image.

Keywords: confocal microscopy, quantitative microscopy, digital micromirror device, digital light microscopy

1. INTRODUCTION

Microscopy is used to produce magnified representations of dynamic and stationary objects. There are many different modes of microscopy such as brightfield microscopy, darkfield microscopy, phase contrast microscopy, fluorescence microscopy, reflected light microscopy, confocal microscopy, etc. All of these deliver illumination light in a controlled fashion to the object to be examined and collect as much of the information-bearing light from the object as possible. Normally this is accomplished using either Köhler illumination for transmission microscopy or Köhler epi-illumination for fluorescence microscopy. Both of these methods use appropriately placed diaphragms and lenses to control both aperture (illumination cone) and size of the illuminated area of the sample. In particular, for Köhler illumination, these diaphragms are placed in: 1) the conjugate image plane of the sample (to control the size and shape of the illumination spot) as a field diaphragm or pinhole, and in 2) a conjugate image plane of the light source (to control the angles of the light illuminating the sample) for the aperture diaphragm. Usually, these diaphragms are simple irises (as in brightfield, epi-illumination, and fluorescence microscopy), but sometimes they are more complex, consisting of cutout rings of different diameters (as in darkfield microscopy).

1.1. Conjugate image planes

The field diaphragm determines the area of the sample that is illuminated. This diaphragm is placed at a conjugate plane of the sample as projected through the condenser or objective (epi-illumination). The primary image plane of the microscope is an equivalent conjugate plane as projected through the objective lens. Thus, a location which transmits light in the field diaphragm plane illuminates a minimized (as demagnified by the lens set) spot in the sample. Likewise, each location in the primary image plane corresponds to a minimized spot in the sample. These three planes form the first key set of conjugate image planes. If only a single location is illuminated and detected, and if these spots in the sample are in the exact same location, then one has a confocal microscope. In fact, a confocal microscope usually has a single pinhole detector and a point source illumination spot.

The second key set of conjugate image planes includes the light source and the aperture diaphragm planes on the illumination and detection sides of the sample. Each spot on the light source corresponds to a spot in the aperture diaphragm planes. For

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a microscope using Köhler illumination, each spot in these planes also corresponds to an angle of light passing through the sample. Thus, in the illumination pathway of a bright field microscope, the aperture diaphragm controls the angles of light used to illuminate the sample. Likewise, on the image detection side of the microscope, the aperture diaphragm limits the angles of the light collected to form the image of the sample. For brightfield microscopy, these aperture diaphragms are simple fixed or adjustable circular irises. In darkfield microscopy, the illumination aperture will consist of a circular annulus limiting the illumination to a limited range of angles, while the detection aperture will usually accept a large range of detection light angles, but specifically not the angles used to illuminate the sample.

1.2. Microscopy using digital micromirror devices

The essence of the ideas being presented here is to replace these simple mechanical diaphragms with digital light processing units (digital micromirror devices[1], DMDs, made by Texas Instruments) or some other high contrast spatial light modulator. Placing a DMD at these locations allows for more sophisticated and precise control of the delivery of light to the sample and collection of light from the sample [2,3].

One of the most straightforward applications of using a DMD to perform dynamic illumination control is the creation of a confocal microscope[4, 5]. Illumination light is conducted from the light source, through an excitation light selection filter, reflected off a dichroic mirror, through a projection lens, onto a simple mirror, then onto the DMD. If one of the DMD micromirrors is ON, the light incident on it is reflected to the object lens. If the mirror is OFF, the light is conducted to a beam stop. The light conducted to the objective lens is transmitted and focused into a single location in the sample. Then, light generated by the sample (fluorescence) is collected by the objective lens, and transmitted to the primary image plane of the objective where the DMD is located. The DMD then reflects the light back to the simple mirror into the projection lens, through the dichroic mirror and an emission selection filter onto a light sensor that converts the light into an electronic signal for measurement. Each pixel in the primary image plane is mapped by the objective into a specific location in the sample. If the signal from the pixel which is mapped to the same location in the sample which is illuminated by the DMD, then information about that location in the sample is collected in exactly the same fashion as a regular confocal microscope.

1.3. Principal advantages of DMDs over mechanical diaphragms in microscopy

The advantages of DMDs over mechanical diaphragms are significant, particularly in quantitative microscopy:
Each micromirror is individually controlled through software. This allows one to reproduce illumination patterns precisely. The switching time of the micromirrors is under 20 μs. The fast switching time allows one to switch quickly between illumination patterns, and even entire modes of microscopy. Since there are no mechanical components to slide or rotate into the optical path, the possibility of disrupting the optical apparatus is eliminated. With diaphragms or annuli, one is limited to a small number of illumination patterns. The DMD can generate illumination patterns of essentially any shape and size to better match the sample being studied. The micromirrors can simulate 256 grey levels by switching ON and OFF many times within a single video frame.

2. RESULTS FROM DIGITAL LIGHT MICROSCOPY

Several image acquisition modes for digital light microscopy have been investigated. Semiconductor "targets" have been imaged under epi-illumination conditions. Biological samples have been imaged with transmission microscopy and fluorescence microscopy. Further, images have been collected using a single DMD in the field diaphragm position to control illumination pattern and size, and also with a DMD in the aperture diaphragm position to control illumination angles.

2.1. DMD placed in the field diaphragm plane

2.1.1. Demonstration of confocal condition

To demonstrate that digital light microscopy can be used to produce confocal images, a DMD was placed in the field diaphragm plane of a conventional microscope configured for epi-illumination. A plane mirror was used as the "sample". By turning a single DMD mirror ON, one spot on the plane mirror was illuminated. The mirror was scanned in the *z*-direction along the optical axis. At each position, an image of the illumination spot was collected.