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An Introduction to Practical Confocal Microscopy: The Ultimate Form of Biological Light Microscopy?

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INTRODUCTION

With the rise of the electron microscope in the late 1950s, the development of new techniques for using the light microscope (LM) in biology went into a period of relative decline for about 20 years. This ended in the late 1970s when the advantages that could be gained by applying video techniques to microscopy were highlighted by the work of Allen [1] and Inoué [2]. The essence of their contribution was point out:

a) That electronic imaging sensors are considerably more sensitive and linear in their response to light than is photographic film.

b) That the capabilities of the hardware then becoming available for digital image processing were sophisticated enough to substantially improve the visibility of features in electronically-recorded LM images.

As a result, microscopes with attached, digitally-processed video systems were then capable of displaying interpretable images of features that were either too low in intensity or too low in contrast to be seen otherwise.

KEY WORDS:

Confocal Microscopy, Light Microscopy

Although the spatial resolution of these electronically enhanced light microscopes inevitably remained far less than that of the electron microscope, they had the tremendous advantage that they could be used to study specimens that were alive or at least metabolically active. In addition, the ability to make visible features that were very low in contrast or intensity permitted the observer to detect the location and motion of structures that were considerably smaller than the limit of light optical resolution. A prime example of this type of application was the discovery that individual microtubules would move on a glass surface when provided with ATP. This observation led to the isolation of the motility protein Kinesin and its role in microtubule mobility [3].

During this same period other developments related to the specific staining of biological specimens also occurred. In immunology, the development of the techniques for producing monoclonal antibodies made it possible produce fluorescent probes with extremely high specificity [4]. More recently, the techniques of genetic engineering have made it possible to label specific nucleic acid sequences with fluorescent markers with the same specificity that the monoclonal antibody technique makes possible for labeling proteins. These and other techniques for labeling biological material with specific fluorescent stains are reviewed in [5].

As result of these developments, by the early 1980's, the sophisticated techniques of digital electronic imaging were being applied to the LM study of living specimens treated with fluorescent markers to make visible the location of specific proteins present in the plane of focus [6]. The sole remaining limitation was that, although biological specimens were three dimensional (3-D), the conventional LM imaged only a single 2-D plane. In addition, unless the specimen was first sliced into very thin sections,