APPLICATIONS OF IMAGE ANALYSIS IN HEMATOLOGICAL CYTOLOGY

ESSAY

Submitted For Partial Fulfillment Of Master Degree

Of Clinical Pathology

BY

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(M.B;B.Ch.,1988)

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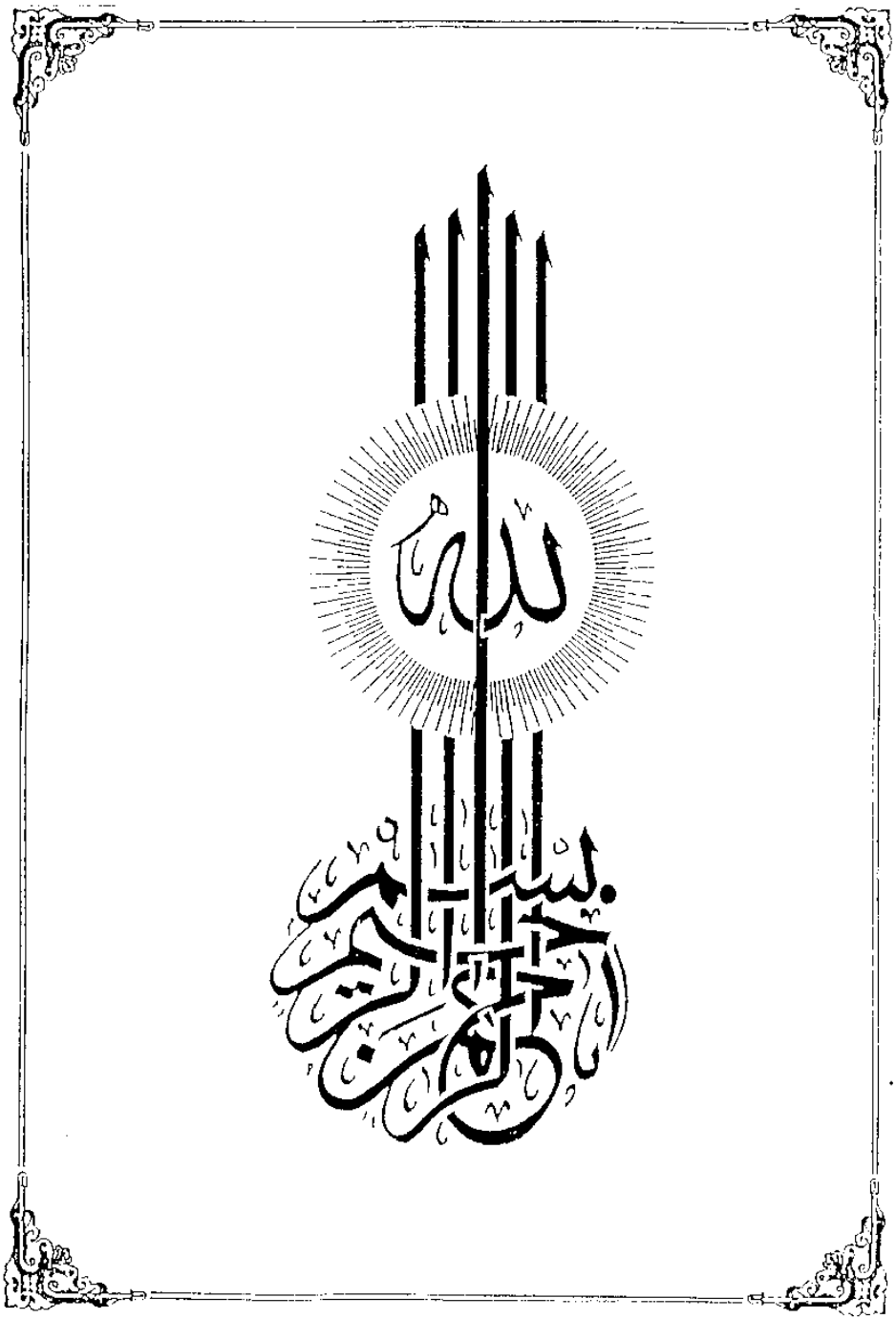
AIN-SHAMS UNIVERSITY

1995

بسم الله الرحمن الرحيم

﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَآ إِلاَّ
مَا عَلَّمْتَنَا
إِنَّـــكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ ﴾

[سورة البقرة : آية ٣٢]



ACKNOWLEDGMENT

I am greatly indebted to ***Dr.Salwa Mohamed Abu El-Hana****, assistant professor of clinical pathology, Ain-Shams University. I would like to express my sincere gratitude to her, for her encouragement and generous support.*

I am express my deep thanks and gratitude to ***Dr. Hala Mahmoud Hamdy Abaza****, assistant professor of hematology, Ain-Shams University, for her advice, guidance and constructive supervision throughout this work.*

My hurtful thanks to ***Dr. Hanaa Mohamed El-Sayed Afifi****, lecturer of clinical pathology, Ain-Shams University, for her kind support and trustful help throughout this work.*

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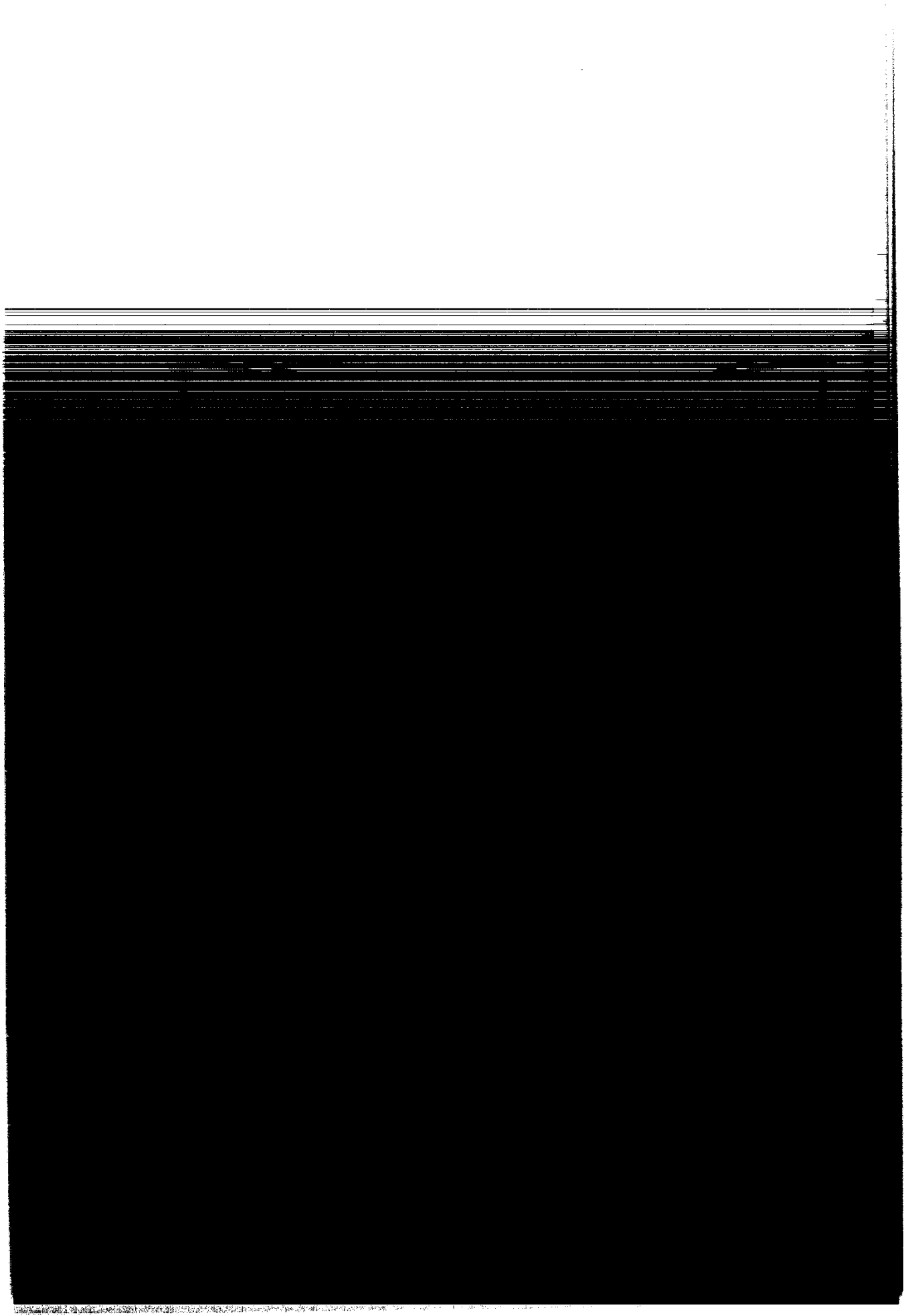


INTRODUCTION AND AIM OF THE WORK

Image analysis (IA) is a technology that has undergone rapid development since the 1960. It has increasing numbers of medical applications, including radiological imaging and microscopy **(Auer et al., 1989).**

Image cytometry includes automated or semiautomated computer-based methods in which image information is digitized, captured, stored and subjected to quantitation of image features. This process might also be referred to as "image cytometry", "cytophotometry", "static cytometry", and "microspectrophotometry " **(Weid et al., 1989).**

Image analysis, when combined with video microscopy, can measure features of biological interest in cells and tissues, especially when probes and special stains are employed to label specific cellular components. So, the microscope-based image analysis is a powerful analytical tool capable of multiparametric cellular measurements that are potentially useful in research and diagnosis **(Suit and Baior, 1990).**

With image analysis, one can analyze a great variety of tissue and cell preparations, including touch imprints, cytospins, cytological smears and tissue sections. Moreover, a vast array of

measurements can be performed on paraffin sections of tissues or destained cytological preparations **(Dawson et al., 1990).**

Routine clinical applications of image analysis will include: analysis and measurement of DNA contents, karyotyping, detection of nuclear antigens and oncogene expression **(Bacus et al., 1989).** Meanwhile, new developments in image analysis will make this technology increasingly attractive and useful for clinical laboratory. Commercial instruments are now available, and the price of such integrated imaging system is highly competitive with flow cytometry **(Weinberg, 1993).**

AIM OF THE WORK:

The aim of this essay is to review the basic technology of image analysis and its clinical applications in hematological cytology in comparison to flow cytometry.

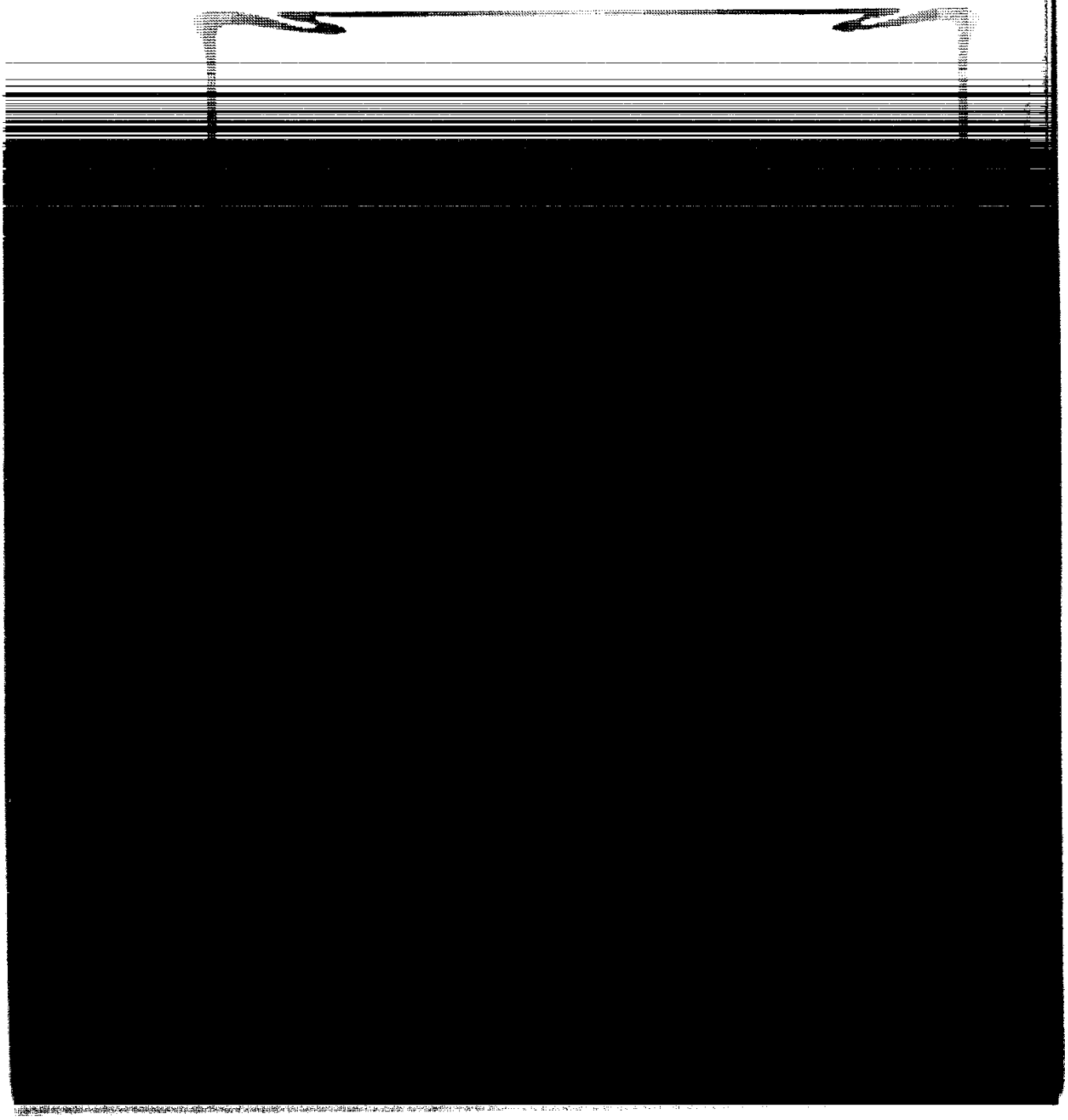


IMAGE ANALYSIS TECHNOLOGY

Image analysis is considered to be a technique of analytical cytology, yet this technique has common scientific roots dating back to the first attempts at measuring cells and cell components with a high degree of resolution **(Bacus et al., 1988).** It is a technology that has undergone rapid development since 1960, at first mainly stimulated by the needs of the aerospace and defense industries. Image techniques have found widespread applications in industry, primarily in the field of robotic vision and automated inspection. There after, increasing numbers of medical applications including radiological imaging and microscopy are documented **(Auer et al., 1989).**

HISTORICAL ASPECTS:

A few critical developments stand out as common landmarks in the history of this technique. The first attempt at measuring cells, by the French investigator "Donne", was published in 1845. In 1890, Miescher identified DNA (as thymo-nucleic acid), through his discovery of the staining reaction specific for this acid, now known as the Feulgen stain. This was followed by recognition that DNA was the carrier of the genetic properties of the cells by (**Avery et al., 1944).** Overlapping to some extent with these developments was the pioneer work on fluorescence of nucleic acids and proteins in cells, carried out by **Caspersson** and his students in the 1930.

Culminating with the observation on differences in nucleic acid content between benign and malignant cells published in 1942, the most important recent development in this technique was the development of small, powerful computers **(Coon and Weinstein, 1993).**

COMPONENTS OF IMAGE ANALYSIS SYSTEMS:

The image analysis (IA) systems are usually constructed from the following hardware components **(Weinberg , 1993)** **(Figure I)**:

1. A device to capture the image, usually a video camera.

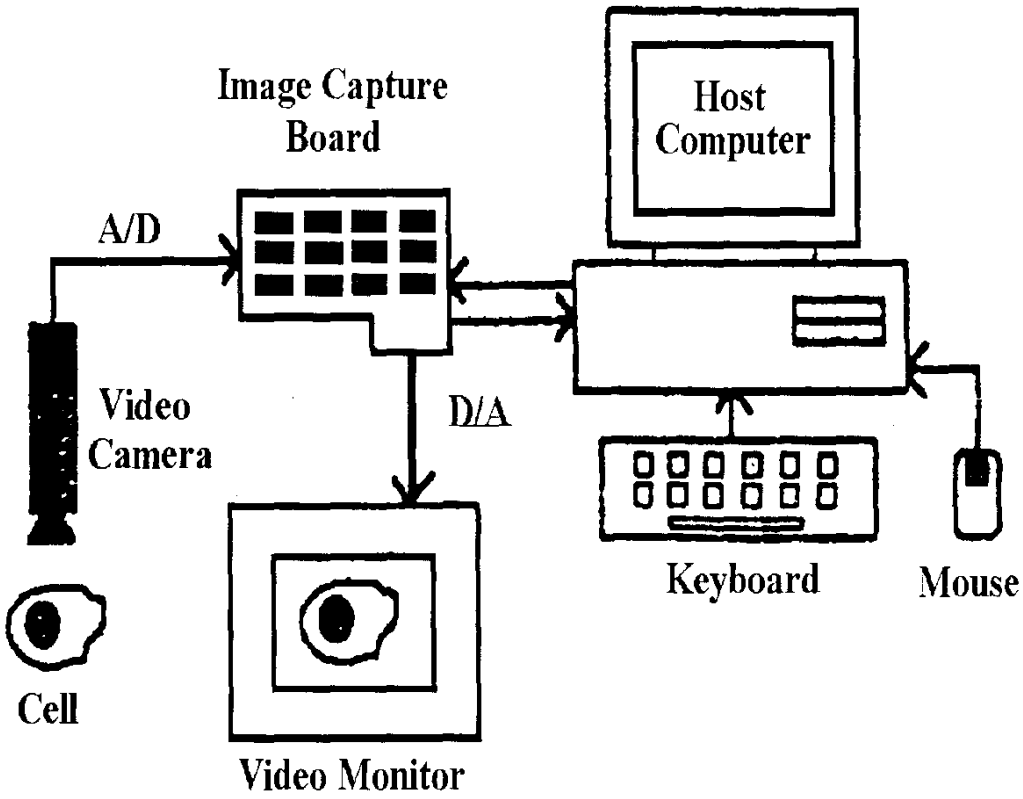
2. An image capture board which samples the continuos (analog) video signal, digitizes the signal and directs the numerical values to specific memory locations.

3. A computer which typically houses the image capture board and is used to perform a variety of software driven operations on the digital image data, as well as, to provide an interface for the user.

4. A video monitor, which displays the live or stored "captured " image.

5. A storage media, typically magnetic disc or tap.

6. A user interface, such as a key board, moise or light pen.



(Fig. Ib): Schematic diagram of a typical image analysis configuration

Perhaps, the most important component of the image system is the software, which greatly influences the ease of use of the system and types of measurements that can be made. Some systems also include extensive software facilities for handling and analyzing data, such as data bases and statistics packages **(Weinberg, 1993).**

Image analysis, includes automated or semiautomated computer-based methods in which image information is digitized, stored and subjected to quatitation of image features. This process might also be referred to as "quantitative digital imaging", "image cytometry", "static cytometry", "cytophotometry", and "micro-spectrophotometry" **(Weid et al., 1989).**

PRINCIPLES OF IMAGE ANALYSIS:

In this imaging systems, the video signal from the camera (or another device) is transferred to an image capture board, which samples and digitizes the analog signal. The digital value of the signal sample is proportional to the amplitude of the video signal and,thus, is related to the light intensity of a given portion of the image. The choice of digits depends on the computer capabilities and computer language used. The process of converting electrical signals into numerical values was very slow, it took well over an hour to scan and digitize a single cell. The digital numbers are then stored in a video memory **(Weinberg, 1990).**

Analog-to-digital (A/D) converters are used to distinguish 256 distinct levels of light intensity , or "gray levels" , ranging from pure black to pure white **(Marchevsky et al., 1987).**

Essential for certain types of measurements of biological interest, including nuclear DNA content, is the conversion of light intensity values to optical density. This is because the relationship between incident light (I_0), transmitted light (I_1) and optical density (OD) is, figure (II):

$$* \quad \frac{I_1}{I_0} = T \qquad\qquad OD = - \text{Log} \{ T \}$$

Measurements of cell components are based on computer generated histograms of optical density, taking into account the Beer-Lambert low. So according to this low, the concentration of a substance at the point being measured is linearly proportional to the optical density. When properly calibrated, optical density measurements at specific wave lengths of light can provide quantitation of staining reactions specific for cellular features. For example, the value of the total optical density of a cell nucleus stained by the Feulgen reaction (which is specific for DNA) provides a measure of the nuclear DNA content. The Feulgen-stained nuclei can be visualized on a television screen and selected

for analysis. The visualized nuclei can be either accepted or rejected when they appear to either accepted or rejected when they appear to be damaged **(Wied et al.,1989).**

Another important feature of digital conversion is that the continuos image is broken up into discrete picture elements, or "pixels" or "discrete square unit", with each pixel assigned a specific gray level. The most common format used in imaging today digitizes frames with dimensions on the order of 512 x 512 pixels. This can be attributed, in large part, to the low cost of video equipment compatible with 500-line television standard. In contrast, the human retina is able to resolve the equivalent of approximately 10,000 lines in a visual field **(Crissman et al.,1991).**

PHOTOMETRIC PROPERTIES:

Another important attribute of digital imaging system is its photometric properties. This is determined by the signal to noise ratio of the camera electronics, and by the number of gray levels that the analog signal from the camera is converted to by the digitize. Most systems represent pixel density information as 2, or 256, gray levels. In contrast, the human visual systems is only able to discriminate about 64 non-adjacent gray levels in an image. The 256-gray level standard has been adapted in large part, because most digital computers process information in 8 bit units termed " bytes" **(Marchevsky et al., 1987).**