Photo – Histometry A Modified Computer Assisted Morphometric Measurement Program

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Summary:

A promotion to a previous computer programmed technique "Photo-Micro Estimation Program" was carried out to compute the exact dimensions of cell and cell fraction in photographs of histological sections. "Visual Basic 6" was used as a language for building of the antecedent application forms. With the aid of a slide micrometer, pixels were substituted for micrometer (µm). The new procedure was termed "Photo-histometry program".

To test the suitability of this program, eight photographs of histological sections were selected randomly to be tested. Results were contrasted with those calculated by using ocular lens (calibrated by a slide micrometer).

Estimation of RBC diameter (well known, =7-8 µm) was the second step in assessing the adequateness of this new program.

Results revealed that this program was simple, fast, adequate and accurate. It was better than the calibrated ocular lens in being more precise (it enumerates up to the fractions of a micrometer)

Key words: *Histometry , Computer program*

Introduction:

Traditionally, histologists have relied on verbal descriptions of tissue structure and have seldom favored a mathematical approach. In recent years, however, measurement, of the microscopical images of tissues and cells, has been used increasingly both in research and in diagnostic work.

The term "Morphometry" is defined as a body of methods for obtaining numerical information about anatomical structure, macroscopic or microscopic, in terms of such quantities as volume, surface area, number of components, size of components [(1)] i.e. morphometry has a wide meaning of measurement of the forms of organisms. On the other hand, the term "Histometry" refers to measurement of tissues only [(Z)].

Histometry provides a sound basis for comparison of histological observation. It offers objectivity, increases precision compared with direct visual appraisal and makes statistical analysis easier. It improves assessment of certain histological change, which though may be recognizable by the eye, are accurately graded and their progression better appreciated by histometric quantization. Furthermore, histometry allows appreciation of three dimensional parameters such as volume, density or surface area per volume. Many of these parameters are directly applied to tissue function and correlation with physiological data can yield important information about tissue mechanisms (3).

Histometric quantitative analysis is now gaining popularity in determining the degree of malignancy [(4,5,6),] studying the effect of agfing on different tissues (7,8) and in judging the effect of treatment and exercise [(9)]. Moreover, it has a lot of applications in cytology (io)

Initially, most histometric methods entail the use of test grids, superimposed repeatedly and randomly on the structure in question. Estimates are based on probabilistic considerations and statistical analysis. Such quantitative histological studies, whether they entail counting cells or measuring areas of structures, can be extremely tedious and time consuming. However, the development of semiautomatic and automatic image analyzers has gone a long way towards solving these difficulties. The recent spectacular developments in objective analysis of cells, cell components and different parts of tissue sections are related to the developments in computer sciences that allowed for automation of many functions previously requiring manual calculation (3a)

Lastly, quantitative histometry using automation or semi-automation will remain the province of research workers rather than those in diagnostic departments for the foreseeable future. Because of marked shortage of instrumentation in Iraq and the great difficulties in achieving histometrical data in our research work, trials were done to modify a pre-existing computerized program (done by authors), looking forward to solve such local obstacle.

Materials And Methods:

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1. The original program "Photo-micro-estimation" [11] was designated to deal with photos transferred to the computer, where the intensity of the final reaction products, of some enzymes or stains, can be calculated. Visual Basic 6 was employed as the language for building this application. The minimum computer system requirements are IBM compatible computer with a Pentium processor, Windows 98 operating system, and at least 32 megabytes of RAM. Disk space necessary for instillation is around 15 megabytes depending on whether runtime V116 and ODBC files are already instilled. Further space is necessary to save the data files.
2. The modified program "Photo-histometry " was created to measure the diameter of each single layer or cell or even part of it, in pixels (computer terminology). The number of pixels was estimated versus micrometers, making use of a simple physiological apparatus (Neubauer haematocytometer counting chamber). So that:
At power (X10),1 pixel=1.25 pm (=0.00125 mm)
At power (X40), 1 pixel= 0.3125 µm (=0.0003125 mm)
3. Program testing and evaluation procedures: Two pathways of appraisal were followed:
A. Eight random histological sections were picked out to estimate the diameter of lining epithelium of jejunum (by two magnifications, X10 and X40), thickness of cornea (power X10), thickness of anterior epithelium of cornea (power X40), thickness of the iris (power X10), diameter of gall bladder mucosa (power X10), diameter of endometrial mucosa (by two magnifications X10 and X40). Calibrations were done using both the photo-histometry program and the ocular lens (calibrated by slide micrometer). Photo-histometry program was employed in calculation after photographing the histological section by a digital camera (resolution 640 x 480) and their delivery to the computer. All readings were standardized with identical resolution and magnification. While that, done by ocular lens, was enumerated directly from the same histological tissue sections. Twenty readings were obtained for each histological photograph or section respectively. Double-blind assessment was applied by both researchers (10 readings for each). Lastly, mean ± standard deviation was obtained.
B. A slide of fresh blood was prepared. A drop of normal saline was added to it (to maintain the size of RBC constant). Using the same digital camera, RBCs were photographed photographs were shifted to computer under identical conditions. The diameter, of 56 RBCs, was measured. Mean ± standard deviation was obtained.

Results:

During use, "Photo-histometry program" was very easy to apply, gives fast results and calculates dimensions up to the fractions of a micrometer.

Outcome, of testing, comparison and evaluation of this program, is detailed in table (1 and 2)

Table (1): Data of 8 items-counting, using both ocular lens and photo-histometry.

Tissue or cell	magnification	Diameter (µm)	
		Photo-histometry	Ocular lens
Epithelium of jejunum	X10	12.91 ± 0.9	10 ± 5.1
Epithelium of jejunum	X40	13.04 ± 1.3	14 ± 2.4
Cornea thickness	X10	104.16 ± 0.7	100 ± 5.9
Anterior epithelium of cornea	X40	14.29 ± 1.9	12 ± 2.2
Iris thickness	X10	71.34 ± 2.0	66 ± 3.1
Epithelium of gall bladder	X10	3.43 ± 1.6	4± 4.8
Epithelium of endometrium	X10	3.33 ± 1.3	4 ± 3.4
Epithelium of endometrium	X40	3.68 ± 0.9	4 ± 3.9

Data are expressed as mean of twenty readings ± standard deviation Table (2):

Disclosing measurement of RBC diameter by both methods.

RBC	Magnification	Diameter (pm)	
		Photo-histometry	Ocular lens
	X100	8.193 ± 1.1	6 ± 3.0

Data are listed as mean of fifty six readings ± standard deviation

Descusion:

All automatic systems aimed at a rapid automatic input of information from the specimen with minimum subjectivity and high speed handling of those data [12,13]. On the other hand, semi-automatic instruments accept a much lower rate of input and higher level of subjectivity, which, although much slower, allow a high degree of user control and involvement [14]. Our device belongs to the second category.

Both automatic and semiautomatic quantitative histometry techniques require highly sophisticated procedures and instruments and often applicable to a limited number of studies (6,13,14 . This program is pertinent nearly to all types of studies concerning quantitative histometry (distance measurement) in an accurate and easy way.

Studying tables (1 & 2) reveals that values measured by Photo-histometry have a very narrow standard deviation compared to the other method. This indicates a very narrow variability in reading dimensions eliminating the error caused by human factor which is clearly shown in the standard deviation obtained by the ocular lens method.

Comparing the two trials (calculation by ocular lens and photo-histometry), it had been found that photo-histometry program gave finer outcome (results were offered up to fractions of the micrometer), which couldn't be achieved by ocular lens. Moreover, photo-histometry program was easy to perform, reproducible, more sensitive and could be performed by a computer and digital camera only.

In conclusion, although the validity of this program has not yet been fully investigated, yet it is comparatively simple to perform and should find considerable application in the future.

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