Neuroanatomy, 2003, Volume 2, Pages 6-8.

Technical Note

Published online 24 February, 2003 © neuroanatomy.org

A simple low-cost method for two dimensional microscopic measuring and stepping on the microscopic plate

Esat Adiguzel (1)
S. Ender Duzcan (2)
Ilgaz Akdogan (1)
A. Cevik Tufan (1)

(1) Pamukkale University, School of Medicine, Department of Anatomy, Denizli, Turkey.
(2) Pamukkale University, School of Medicine, Department of Pathology, Denizli, Turkey.

Correspondence Address
Assist. Prof. Dr. Esat Adiguzel
Pamukkale Universitesi Tip Fakultesi
Anatomi A.D. Dekanlik, Kinikli 20020 Denizli, TURKEY
Telephone: (90) 258 213 31 05
Fax: (90) 258 213 28 74
e-mail:adiguzel@pamukkale.edu.tr

Received 18 November 2002; accepted 18 February 2003

ABSTRACT

In this study, a simple low cost method to be used in morphometric studies on microscopic anatomical structures is described. Increasing need for stereological methods depend on laboratories equipped with specially designed devices to do this type of studies. However, high-technical automated and/or computerized systems increase the cost of these studies there by limiting them to a small number of institutions. Here we suggest a simple two dimensional measurement technique that can be adopted to any laboratory.

Key words: microscopy, measurement, stereology, stepping, thoma glass.

Introduction

In edition to its importance in the analysis of gross anatomical structures, morphometric methods have recently been implicated in the assessment of cellular structures and dimensions in microscopic studies [1-3]. Among the implication areas of morphometry in these microscopic studies are the assessment of cell number, volume, surface area and dimensional measurements. The main goal of these studies has believed to be to obtain results that are dependable, unbiased, and cross-comparable with results from other studies in this field [4]. Recent development and establishment of computerized and/or automated systems for these measurements are very high-cost dependent methods for morphometric studies. In addition to its high-cost, often times the computerized and/or automated systems require technical knowledge and support from the manufacturers. Thus, the usage of high-technology based morphometric techniques in experimental basic science studies is limited to a small number of institutions [5]. Most researchers desire cheaper and simpler, yet still efficient, dependable, and unbiased techniques to work with [6-8].

In this study, we suggest and describe a simple, low-cost, efficient, dependable, and unbiased microscopic morphometric measurement method that utilises a light microscope connected to a calibrated simple screen or even a simple drawing tube without the need for an extra, automated measurement device in between them. Through this method a microscopic image can be analyzed in a 2-D way for random sampling of areas of interest, distance measurements, surface area measurements, etc.

Material and Method

In this study a light microscope (Nicon Eclipse E 600) attached to a monitor through a CVC camera (Figure 1), a Thoma Glass, and transparencies were used to work on a 2-D scale for the measurement of distances, and/or areas of interest on a given image.

CALIBRATION OF THE SCREEN

The goal was to calibrate the monitor screen according to a given Thoma Glass scale on the microscope stage for a given magnification. This allowed us to work on a magnified image on the screen with a known, 2D scale for the measurement of distances and areas. The scale on the Thoma Glass was magnified with either 10, 40 or 100X objective (Figure 2A) and the images on the monitor screen for each magnification was transferred onto an individual transparency (Figure 2B). Any scale with a known unit distance can be used for this calibration purposes. However, the advantage of the use of a Thoma scale is the fact that this scale has already been divided into calculated unit square areas (0,0025 mm^2 in our study), which allows faster calculation of any given area on a given image. Furthermore, on the magnified scales transferred on transparencies each unit square is big enough for further equal square subdivisions to increase the sensibility of the scale for smaller, or shorter area or distance calculations, respectively.

MICROSCOPIC MEASUREMENTS

Calibration of the measurement-transparencies according to the Thoma Glass scale was repeated everytime the microscope was turned on. Each selected tissue section

 ISSN 1303-1775 (electronic) 1303-1783 (printed)

Table 1 | A summary for the advantages and disadvanteges of the technique.

ADVANTAGES
- Does not require any additional attachments on microscope, which makes it easier to work with microscope.
- Does not require any equation to calculate the magnification factor etc., since the measurements are performed on a grid scale magnified with the same objective as the image of interest.
- It is cheap, and does not require a high-technical qualification.
DISADVANTAGES
- Requires more time, thus relatively slows down the research.
- Requires special care for the stepping on the stage of microscope.
- Requires calibration of the grid scales on transparencies each time the microscope turned on.

Figure 1 | The microscope system used in this study. A light microscope (Nicon Eclipse E 600) is attached to a monitor through a CVC camera.

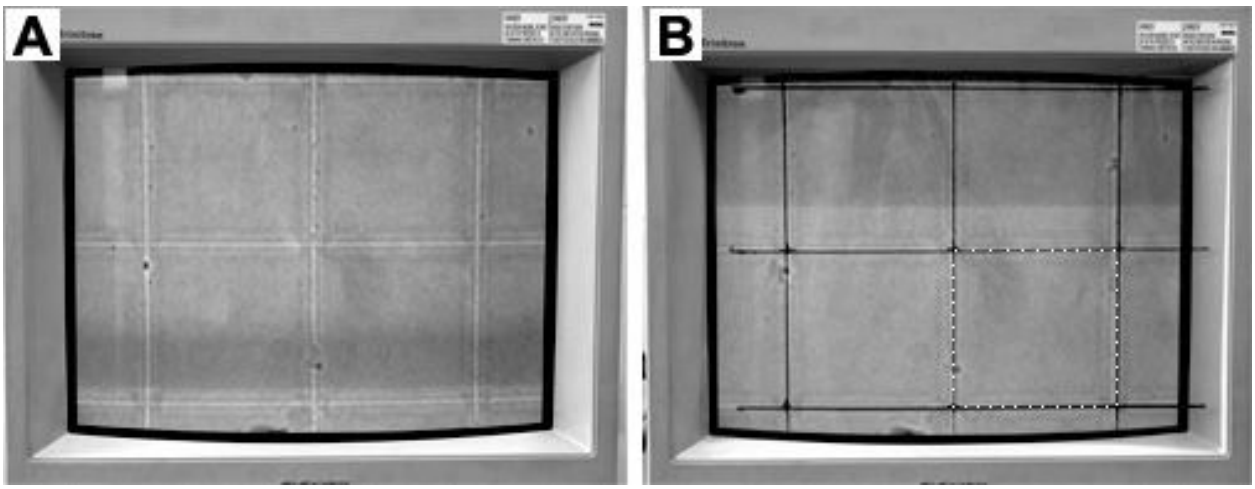

Figure 2 | Calibration of the screen according to the Thoma Glass scale. A) The scale on the Thoma Glass was magnified with either 10, 40 or 100X objective and visualised on the screen. This image was taken with 100X objective. B) The image of the Thoma Glass scale on the monitor screen for each magnification was transferred on to an individual transparency. Unite square area shown with dashed lines equals to 0,0025 mm^2 in our study.

was visualised under a given magnification and the obtained image was transferred onto a screen. The suitable transparency calibrated for that given magnification was then superimposed onto that image and the distance in-between two selected points was measured in terms of μm through that superimposed scale (Figure 3A).

TWO DIMENSIONAL (X-Y) STEPPING ON THE STAGE OF MICROSCOPE

Two dimensional (x-y) stepping is specially important for the calculation of planar section areas of anatomical structures or areas of interest. This study suggests a technique, which does not require the use of a computerised or automated calculation system, yet still can be precise and effective in this type of calculations. The distance in between the reference points was measured as discussed above for both x and y axes, and the area of interest was estimated through these two dimensions (Figure 3A and B).

THE ADVANTAGES AND DISADVANTAGES OF THE TECHNIQUE

This technique can easily be used in two dimensional measurements and the advantages and the disadvantages are given in Table 1.

Conclusion

Morphometric studies on microscopic anatomical structures may be performed using high-technical automated and/or computerised systems. However, the cost of these techniques makes them limited to a small number of

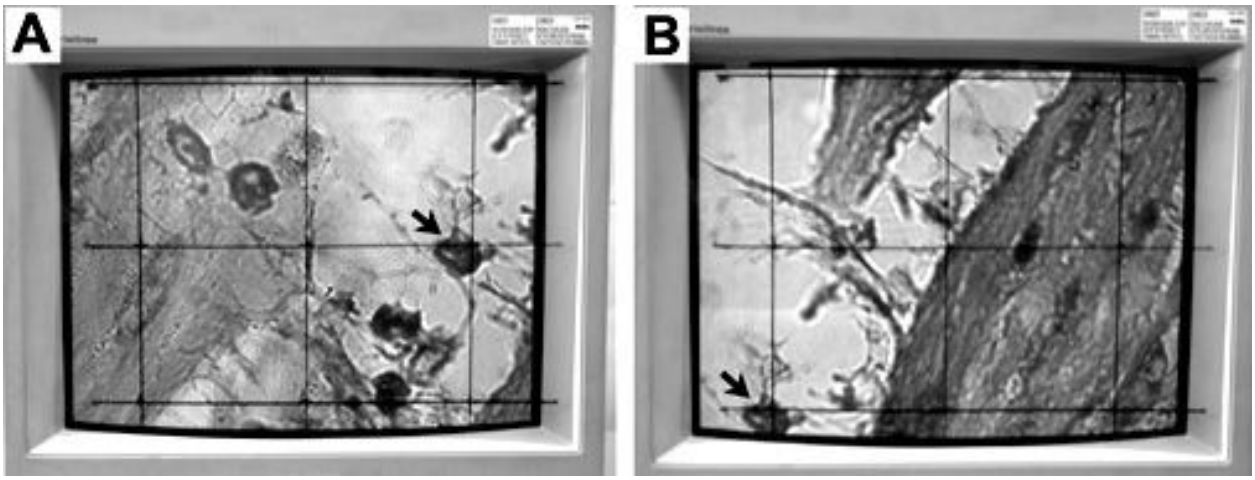

Figure 3 | Two dimensional (x-y) stepping on the stage of microscope. The reference point shown with a black arrow in panel A is moved 50 μm in y axis and 100 μm in x axis as shown in panel B.

institutions or laboratories. The technique suggested in this study allows every researcher to perform two dimensional morphometric studies using a simple and low cost, yet effective and accurate method. We believe that, this technique may help improve morphometric studies especially in newly founded laboratories.

References

[1] Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the disector. J. Microscopy. 1984 (134) 127-136.

[2] West MJ. New stereological methods for counting neurons. Neurobiol. Aging. 1993 (14) 275-285.

[3] Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS. 1988 (96) 379-394.

[4] West MJ. How to count. Neurobiol. Aging. 1996 (17) 503.

[5] Gundersen HJ. Stereology of arbitrary particles, A review of unbiased number size estimators and the presentation of some new ones, in memory of William R. Thompson. J. Microscopy. 1985 (143) 3-45.

[6] Korkmaz A, Ciftci N, Bosnak M, Agar E. A simplified application of systematic field sampling and low-cost video recording set-up for viewing disector pairs-exemplified in rat cochlear nucleus. J. Microscopy. 2000 (200) 269-276.

[7] Korkmaz A, Tumkaya L. Estimation of the section thickness and optical disector height with a simple calibration method. J. Microscopy. 1997 (187) 104-109.

[8] Kaplan S, Canan S, Aslan H, Ünal B, Sahin B. A simple technique to measure the movements of the microscope stage along the x and y axes for stereological methods. J. Microscopy 2001 (203) 321-325.

N