# Nuclear morphometric application in the quantitative description of breast lesions

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## Abstract

Morphometry is the quantitative description of geometric features of structures such as tissues, cells, nuclei, or nucleoli. One of the most important functions of morphometry in pathology is the study of nuclear morphometry in differentiating benign lesions from malignant lesions based on their nuclear parameters. Morphometric techniques are fairly simple and inexpensive, but time-consuming in routine applications. In this review, an attempt is made to examine the significance of nuclear morphometry in the quantitative evaluation of breast lesions, and different techniques applied by various researchers.

**Keywords:** Morphometry, Malignant lesions, Breast lesions.

## INTRODUCTION

### MORPHOMETRY

**Background:** Morphometric analysis started as early as 1925 by Jacobi who found that the volume of a normal cell doubles before cell division. Heiberg and Kemp, in 1929 were the first to substantiate the subjective impression that cancer nuclei are larger than those of normal cells. In the 1950s and 1960s an increased interest amongst anatomists and biologists gave a strong impetus to morphological and stereological analysis in biomedicine. In the late 1970s and early 1980s the application of morphometric analysis to pathologically changed tissues became increasingly popular and widely applied, particularly in cancer [1].

**Morphometry includes**

1. Stereotactic techniques estimating fraction of different tissue components, inner and outer surface density, as well as shape and volume by means of a test system of lower dimension ( i.e. point or line grids) than the structure itself, and
2. Measurement of geometric features of structures in a two-dimensional microscopical image which is also called planimetry [2].

Recently, morphometric assessments were improved by advanced computer-assisted image analysis system where the microscopic image is recorded by a video camera and displayed on a computer screen which makes it possible to trace the outlines of nuclei on the screen and then compute nuclear areas as well as nuclear shape using dedicated software that are able to produce quantitative data in the form of cytogram and histograms [3].

## METHODOLOGY

Different authors have applied different methods to perform the computer assisted nuclear morphometric study which are currently in practice.

Tan *et al* [4] have used paraffin embedded tissues fixed in 10% buffered formalin and sectioned at 4µ thickness and stained with Haematoxylin and Eosin. Nuclear morphometry was carried out using The Kontron Electronik imaging system, comprising a light microscope with a camera linked to a computer.

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KS400 Release 2.0 software was used. Frozen sections were excluded due to collapse of nuclei. One hundred fifty ductal epithelial nuclei were randomly selected from lesional areas at a magnification of X 40. The images were then digitized and the nuclei outlined using a mouse attached to the computer. Some of the nuclear parameters they studied were nuclear area, nuclear perimeter, feret circle, a shape factor, is calculated using the formula, $4\pi$ area/ perimeter$^2$ and feret ratio, a ratio of minimum to maximum feret diameter. Wolberg et al [5] selected fine needle aspiration samples fixed in 95% ethanol and stained with Haematoxylin and Eosin. For computer analysis, the operator used a microscope with a x2.5 ocular and a x63 objective to visually select a field which seemed most atypical and avoided areas where there were distorted and overlapped nuclei. A 640 x 400 pixel digital image of this field was produced by a video camera on the microscope and a frame grabber card in a computer. A mouse button was used to outline each cell nucleus on the computer monitor. Since data storage accommodated only a single image, analysis was performed on 10 to 20 nuclei per patient. Beginning with this user-defined approximate border, a deformable spline technique precisely located the actual nuclear border. Nuclear features studied were:.

a. Radius was computed by averaging the length of radial line segments from the center of the nuclear mass to each of the points of the nuclear border.
b. Perimeter was measured as the distance around the nuclear border.
c. Area was measured by counting the number of pixels in the interior of the nuclear border and adding one-half of the pixels on the perimeter.
d. Perimeter and area were combined to give a measure of the compactness of the cell nuclei using the following formula: perimeter2/area.
e. Smoothness was quantified by measuring the difference between the length of each radius and the mean length of adjacent radii.
f. Concavity was determined by measuring the size of any indentations in the nuclear border.
g. Concave points counted the number of points on the nuclear border that lie on an indentation.
h. Symmetry was measured by finding the relative difference in length between line segments perpendicular to and on either side of the major axis.
i. Fractal dimension was approximated using the "coastline approximation" described by Mandelbrot that measured nuclear border irregularity.
j. Texture was measured by finding the variance of the gray scale intensities in the component pixels.

Teague M et al [6] conducted morphometric analysis of 56 FNAC samples stained with Haematoxylin and Eosin and fixed with 95% alcohol using image analysis system called Xyct developed at the University of Wincosin. For each case, a single image projected through a x 63 objective was generated using a color video camera and captured by a Computer Eyes color framegrabber board (Digital Vision, Inc., Dedham). The image chosen was representative of the most atypical- appearing nuclei on the slide. They manually traced the individual outlines of 10-20 nuclei within the video-captured image to provide a representative sample. But the software used was capable of storing data from only one high power field per case, which limits the number of nuclei that can be analyzed. Nuclear size, shape, and texture were represented by ten computer-generated nuclear characteristics, each of which had a corresponding mean value, worst value, and standard error. From these 30 nuclear characteristics, 3 (worst area, mean texture, and worst smoothness) were used to classify each case as either benign or malignant. Marciniak A et al [7] conducted morphometric examinations of cell nuclei on the cytological material obtained by fine needle biopsy. Biopsy without aspiration was performed under the guidance of ultrasonography with a needle of 0.5 mm. Smears from the material were fixed in spray fixative (Cellfix of Shandon company) and stained with Haematoxylin and Eosin. The time between preparation of smears and their preserving in fixative never exceeded three seconds. All cancers were histologically confirmed and all patients with benign disease were either biopsied or followed for a year. The image for digital analysis was generated by a color video camera mounted atop a microscope. The slides were projected into the camera with 10 and 160× objective and a 2.5× ocular. One image was generated for enlargement 100× and nine for enlargement 400×. First the background elimination by thresholding hue component was applied, then the actual segmentation was done with region growing technique. Morphometric measurements characterizing the shape and size have been mainly used for feature extraction. The extracted features are: size, circularity, perimeter, compactness, lengths of axis of ellipse circumscribing the nuclei, ellipticity and eccentricity of ellipse circumscribing the nuclei.

**Few of the softwares that are used for image analysis are**

Image J software: "Image J" is a freely available java-based public-domain image processing and analysis program developed at the National Institutes of Health (NIH) [8]. Wayne Rasband is the core author of Image J who is a Special Volunteer at the National Institute of Mental Health, Bethesda, Maryland, USA; after developing the Macintosh-based NIH Image for 10 years, he started afresh with Image J using the Java programming language. To run Image J, a given system needs only the operating system-specific Java runtime environment. Java runtime environments (JRE) are freely available, either from Sun or bundled with platform-specific installations of Image J (rsb.info.nih.gov/ij). With JRE available for most operating systems, Image J is platform-independent, running on Macintosh, Windows and Linux [9].

Image J's plugin architecture and built in development environment has made it a popular platform for teaching image processing [10]. The source code for Image J is freely available [11].

1. Image pro-express version 4.5 developed by Cybernetics Inc. USA.
2. Prodit 3.1, Promis Inc, Almere, The Netherlands.

Several authors have done studies on the role of nuclear morphometry in breast lesions which has been well documented in literature, carried out both on fine needle aspirates samples as well as tissue sections and found to be quite objective in differentiating the benign and malignant lesions.

Pienta KJ, Coffey DS [12] in a retrospective analysis of 60 patients quantified the changes in nuclear morphology using the DynaCell Analysis System in a blinded fashion. They found that the nuclear area increases from an average of 25µ$^2$ in normal patients to 59µ$^2$ in patients with metastatic disease and suggested that the metastatic potential correlates with increased nuclear area. Dey P, Ghoshal S, Pattari SK [13] selected 24 histologically proven infiltrating ductal carcinomas of the breast and 10 benign breast lesions (fibroadenoma) to correlate visual cytologic grade with automated nuclear morphometry of carcinoma of the breast. MNA, standard deviation of nuclear area, nuclear diameter, convex area, convex perimeter and perimeter were significantly increased from benign versus grade 1 carcinomas and grade 1 versus grade 2 and 3 carcinomas. However, there was no significant difference in grade 2 versus grade 3 carcinomas. Rajesh L, Dey P, Joshi K [14] conducted computerized morphometric analysis in histologically confirmed breast cancer in 79 patients (19 cases of ILC and 30 cases of IDC, 20 cases of benign lesions) to analyze the role of automated image morphometry in distinguishing infiltrating lobular carcinoma (ILC) of the breast from benign, borderline and infiltrating ductal carcinoma (IDC). They observed that all the nuclear morphometric features of ILC were much

lower than those of IDC and borderline lesions, whereas nuclear morphometric data on ILC were only marginally more than those on benign cases. ANOVA showed that mophometric data were significant (P < .05) in all the variables between ILC and IDC. However, there was no significant difference between ILC, and borderline and benign cases. Rezanko T, Pehlivan F, Evcim G, Sirkeci G [15] retrospectively evaluated 45 breast equivocal FNA cytology with histologic confirmation using computerized image software to obtain MND, MNP and MNA. Morphometric values were compared with histopathogical diagnosis. In the benign group, MND was 9. 9$\mu$; MNP was 29.26$\mu$ and MNA was 68.19 $\mu^2$. In the malignant group, however MND was 10$\mu$; MNP was 30.28 $\mu$ and MNA was 70.03 $\mu^2$. No statistical differences in mean nuclear diameter, perimeter and area were found between malignant and benign groups with nonparametric tests. Nuclear morphometric analysis based on image analysis did not help to distinguish the borderline lesion. Elzagheid A, Collan Y [16] studied the potential of nuclear morphometry in supporting the interpretation of fine needle aspiration biopsy samples of the breast to outline the nuclei of breast epithelial cells in breast cancer, fibroadenoma and fibrocystic disease using image analysis. They found that the MNA of cell groups of malignant samples varied from 42 to 125$\mu^2$, in fibroadenomas from 30 to 50$\mu^2$ and in fibrocystic disease from 26 to 57$\mu^2$. The MNA of free cells varied as follows: cancer, 66-181$\mu^2$; fibroadenoma, 33-70$\mu^2$; fibrocystic disease, 35-60$\mu^2$. They suggest if the mean nuclear area of cell groups is < 42$\mu^2$, the lesion is probably benign; if > 57$\mu^2$, malignancy should be considered. The differential diagnosis between carcinoma and fibroadenoma could be based on free cells: mean area of free cell nuclei ≤65$\mu^2$ suggested a benign lesion, and of ≥71$\mu^2$ suggested a malignant lesion. Abdalla F, Boder J, Markus R, Buhmeida A, Collan Y [17] retrospectively studied 132 breast cancer samples using computerized nuclear morphometry to determine the role of nuclear morphometry in the evaluation of breast cancer prognosis and the relation of morphometry with clinicopathological features. Nuclear morphometric values were higher in premenopausal, large tumor ( p=≤0.03), higher histological grade (p=≤0.0001), advanced stages (p=≤0.04), infiltrating ductal carcinoma and lymph node positive tumors (p=≤0.001). The Univariate analysis and survival analysis indicated that short survival time was associated with high nuclear morphometric values. Survival among patients with MNA <71 $\mu^2$ was significantly better than among patients with MNA >71 $\mu^2$.

## CONCLUSION

Nuclear morphometry is an efficient and successful tool in distinguishing benign and malignant lesions. When faced with an inconclusive diagnosis of aspirates of breast masses, image analysis can help in the further classification of such lesions providing a more appropriate triage for surgical biopsy.

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