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Fractal analysis limitations in digital analysis of Papanicolaou cytological images

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Abstract

In this paper there is shown, using a set of selected examples, estimation of the fractal dimension that can be used as an additional (not separated) tool for detection of abnormal cell nuclei. The perimeter P(R) is estimated using variable radiuses (R) and the fractal dimension is obtained. Fractal analysis of a boundary is not sufficient even for high resolution images apparently but extension of the number of parameters (cytoplasm and nuclei field) gives much better separation.

Keywords: fractals, image analysis, cytology, biomedicine, Pap smears.

Ograniczenia w analizie fraktalnej dla obrazów rozmazów Papanicolaou

Streszczenie

Geometria fraktalna pozwala na opis złożoności i zmienności obiektów biologicznych, w szczególności komórek. Metryki euklidesowe nie są wystarczające do klasyfikacji obiektów tego typu [4, 5]. W artykule pokazano wykorzystując zbiór wybranych przykładów, że estymacja wymiaru fraktalnego obwodu jądra komórkowego może być używana jako dodatkowy, ale nie jedyny wskaźnik służący do detekcji atypowych jąder komórek w analizie cytologii ginekologicznej. W typowej analizie cytologicznej rozmaz jest analizowany jest w powiększeniu 400x, dzięki czemu możliwa jest analiza nieregularności obwodu jądra komórkowego, które dla prawidłowych komórek powinno być gładkim okręgiem. Wybrane przypadki pokazano z zachowaniem skali na rys. 2. Założono wykorzystanie wymiaru fraktalnego dla obwodu, a do normalizacji obrazów wykorzystano średnicę Fereta. Obwód P(R) jest estymowany dla różnych promieni R, co pozwala na wyznaczenie wymiaru fraktalnego w oparciu o wykres Richardsona. Wymiar fraktalny nie może być brany jako jedyny wskaźnik atypii (rys. 4) gdyż niektóre jądra komórek mają ten sam wymiar fraktalny (to samo nachylenie), co komórki prawidłowe. Ocena atypii możliwa jest w przypadku zastosowania wymiaru fraktalnego, pola cytoplazmy i pola jądra komórkowego, co zwiększa różnicę między poszczególnymi przypadkami. Samo wykorzystanie obu pól nie gwarantuje dużej różnicy między tymi przypadkami. Założone aspekty analizy bazują części technik oceny wykonywanych przed osoby analizujące tego typu obrazy.

Słowa kluczowe: fraktale, analiza obrazów, cytologia, biomedycyna, rozmazy Papanicolaou.

1. Introduction

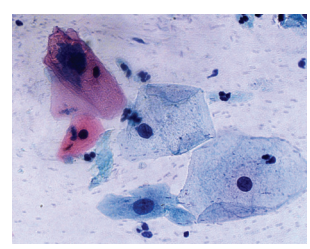
Fractal geometry is well suited to capture the complexity and variability of biological objects, especially cell nuclei. Euclidean geometric metrics are insufficient for biological object classification, so a suitable solution is to use mixed mode features, based also on fractal geometry [4, 5]. Fractal geometry [12, 14] may be used for synthetic generation of biological structures and deriving parameters like the fractal dimension in order to quantify the shapes of structures. There are several papers about fractal analysis in cytopathology of breast [11, 17], ovarian [10] and cervical cancer [4, 13]. It is connected with the fact that nucleus of the cell in pre carcinoma condition and carcinoma are very often irregulary shaped comparing with benign cells.

Objects may be characterized using fractal properties [1, 2, 3, 6], texture characteristics (lacunarity) and symmetry features [4]. The fractal dimension may be determinated by a box-counting algorithm [13]. It was observed that the fractal dimension of the nuclei increased as the degree of dysplasia increased [13] and statistically significant differences between benign and malignant cells were reported [15].

2. Cytoscreening

Cervical abnormalities are important especially for women of reproduction age. Cytoscreening is a standard test for early detection of precancerous conditions (squamous intraepithelial lesions), that may be effectively treated. The main point in work of cytoscreeners and pathologists is an identification of cervical intraepithelial lesions in a Papanicolaou screening test (Pap test) [9].

Overall, mortality from cervical cancer decreases continuously, but in many countries cancer of cervics uteri remains one of the main reasons for earlier mortality of women. It is estimated that cervical cancer affects 4000 women in Poland and is a reason for death in 2000 cases every year. According to the National Cancer Register 13 cases per 100 000 women are reported. Modern reports of cervical cytology are based on the Bethesda Classification System from 1998, revisited in 2001 [7].

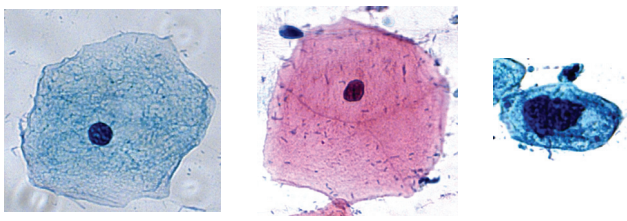


Rys. 1. Przykładowy obraz cytologiczny dla obiektywu 40x (powiększenie 400x)
Fig. 1. Example cytological image at 40x lens (magnification 400x)

Examination includes characteristic changes in cell nucleus morphology that include: size, pleomorphism, texture, staining intensity, enlarged nucleus area comparing with the cytoplasm area. The time between 8-10 minutes is typical for manual diagnosis (up to 300 movements of an optical microscope). In typical screening the smear is analyzed under the microscope with magnification up to 400x (objective 40x).

Cytologoical image analysis is very complex (Fig. 1) because there are many different phenomena [7] and image analysis should be based on multiple cells for classification purposes. Recognition of atypical cells is especially important for semi-automatic systems and detected cells are processed by the cytoscreener.

Selected examples are shown in the same scale. The colour of cytoplasm defines the age of a cell and it is a result of Pap processing (it is biochemical image segmentation). An example of the correct cell with a round cell nucleus is shown in Fig. 2 (left).



Rys. 2. Przykład prawidłowej komórki (z lewej), komórki uszkodzonej w trakcie przygotowania materiału (po środku), komórki z cechami atypii (z prawej)
Fig. 2. Example of the correct cell (left), correct cell mechanically disturbed in sample preparation (middle), cell with features of atypia (right)

Another problem in cytology is the representation of a specimen. Mechanical disturbance of the cell can influence the cell image. Fig.2 (middle) shows the correct cell that is disturbed by preparation (the cell nuclei is pressed).

An example of a cell with features of atypia is shown in Fig. 2 (right). The nucleus has many important differences like the very large size of cell nuclei in comparison to the cytoplasm and very rugged edges. Both parameters are important for cytoscreening, but precise diagnosis requires further histopathological examination that is necessary for confirmation or rejection of the disease suspicion (in some cases). The single cell analysis is not sufficient for detection of the disease.

The fractal dimension can be a valuable tool for detecting irregularities in atypical nuclei of the cervix uteri allowing the objective nuclear grading [13].

3. Estimation of fractal dimension

Computation of the fractal dimension is very important in image analysis applications. The most important technique is based on the analysis of the Richardson plot. Unfortunately, it is not possible to use this plot without additional analysis for the definitive assignment of the curve to the fractal or non-fractal. In [16] there are shown examples of non-fractal curves that may be interpreted as a fractal. In this paper it is not considered and the fractal dimension is estimated. The obtained value is useful for image analysis of the object independently of the fractal or non-fractal object origin.

The perimeter of cell nuclei is measured and the fractal dimension is estimated. Another approaches may be used for an object e.g. field dimensions. An image is acquired using AxioCamMRc5 camera with 2584x1936 resolution. This camera has a Bayer imaging sensor so theoretical optical resolution is reduced depending on the object. Very small details cannot be obtained at theoretical resolution in right way due to interpolation of the Bayer's filtered image. High contrast between cytoplasm (bright) and cell nuclei (very dark) improves optical resolution but it is dependent on the particular sample. Details that are less then 2x2 pixel size are at the edge of resolution practically.

Image segmentation between cytoplasm and cell nuclei is very hard to perform, due to the high variance of contrast even for a single sample. Moreover, there are cell nuclei artifacts that could be located at the edge of cell nuclei so simple segmentation techniques are not feasible. The segmentation is not possible without knowledge-based approaches. Manual segmentation of cell nuclei and cytoplasm is assumed in this paper. This is not convenient in massive image processing, but reduces the problem of validation of the automatic segmentation algorithm.

The processed image is converted to the binary one after the segmentation. The image is upsampled using the nearest neighbourhood algorithm three times and processed by the edge detection. The following formula is used for the edge detection:

$$E_{x,y} = \overline{I_{x,y}} \cap \left(I_{x-1,y} \cup I_{x+1,y} \cup I_{x,y-1} \cup I_{x,y+1} \right) \tag{1}$$

Such a detector returns 4-way neighbourhood edge. All points are collected in to the list of pixel coordinates and downsampled three times. The duplicated edge pixels are removed from the list finally. This technique is necessary if two areas are connected by the single pixel or line.

Feret diameter (F_{diam}) is necessary for normalization of the diameter. It is the largest distance between two edge points. The largest radius used in fractal measurements should be about three times smaller in comparison to the Feret diameter.

$$R_{\max typical} = F_{diam} / 3 \tag{2}$$

The perimeter $P(R)$ is estimated using variable radiuses (R) and the fractal dimension is obtained. Limitation of the maximal radius used in the estimation of the fractal dimension of the perimeter is necessary due to "fractal rabbits" phenomenon [8]. In particular examples the upper limit is additionally reduced to:

$$R_{\max} = F_{diam} / 7 \tag{3}$$

due to availability of “fractal rabbits” below (2) typical but empirical limit. The fractal dimension is obtained from the Richardson plot. Estimation of the single value corresponding to the fractal dimension is possible by linear regression fitting. The complexity of the object may change depending on the scale and line fitting approach may give strange results. The more convenient way is analysis of the fractal dimension for fixed ranges of scale or estimating them. The slope of line m in the Richardson plot (both axes are logarithmic) are used for estimation of the boundary fractal δ. The slope is measured for logarithm of the normalized perimeter length from 0.05 to 0.145 of the normalized range.

$$\delta = 1 + |m| \tag{4}$$

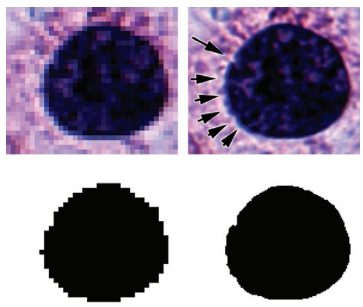
4. Boundary properties of cell nuclei

We selected only a small fraction of cells from a very large collection of Pap smear samples. Many separate cells are very similar, but from medical point of view the most interesting are extreme cases. Cancer cases are very rare due to early diagnosis and treatment of pre-carcinoma conditions.

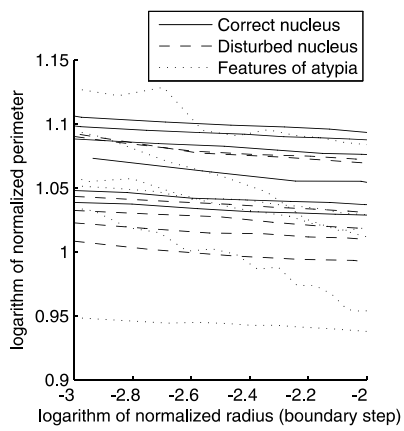
The high optical magnification and camera resolution is very important for detection of cells with features of atypia (Fig. 3). Image analysis should be performed at the highest possible resolution, which is technologically complicated (like one hour for a single slide and a few GB for uncompressed image at different focus settings).

Alone, fractal analysis of a boundary is not sufficient even for high resolution images. In Fig.4 there are shown the Richardson plots of selected samples. The fractal dimension for the correct and mechanically disturbed nucleus are similar (range 1.01-1.02) and it is corresponding to the knowledge of cytoscreeners. The fractal dimension of intraepithelial lesions features is much

dispersed. Some samples have the fractal dimension in the range of correct values. There are also large values of the fractal dimension (1.04-1.08) for visually rugged cell nuclei.

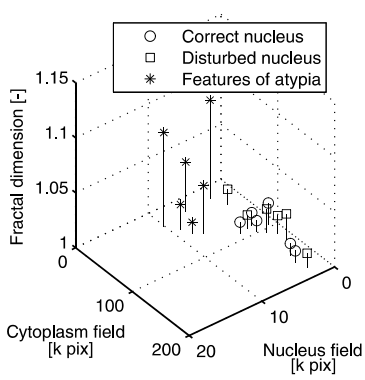


Rys. 3. Przykład atypowego jądra komórkowego dla powiększenia 100x (po lewej) i 400x (po prawej) oraz ich obrazy binarne (rozmiar znormalizowany)
Fig. 3. Example of the atypical cell nucleus at 100x (left) and 400x (right) magnifications (size normalized) and their binary images



Rys. 4. Wykres Richardsona dla wybranych próbek materiału
Fig. 4. Richardson plot for selected samples

Extension of the number of parameters (cytoplasm and nuclei field) gives much better separation of samples in comparison to the alone fractal dimension (Fig. 5). Both additional features are used by cytoscreeners.



Rys. 5. Rozkład próbek dla rozszerzonej analizy
Fig. 5. Distribution of samples in extended analysis

5. Conclusions

The single fractal dimension of nuclei boundary is not sufficient for complete analysis of cell abnormalities. Extension of the number of parameters for analysis gives better separation, which can be used for computer assisted analysis of cells. The fractal dimension of the analyzed samples is low valued. For such a class of fractal the theoretical range is between 1 and 2. The correct, but mechanically disturbed cell nuclei gives similar results to the good quality nuclei images.

Implementation of segmentation algorithms for separation of cell nuclei, cytoplasm and background allows processing a much larger set of examples, which will be considered in the further works. The fractal based analysis is a valuable tool for research and practical purposes in digital cytoscreening.

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6. References

[1] Ballerini L, Franzn L.: Fractal Analysis of Microscopic Images of Breast Tissue, WSEAS Trans. on Circuit, Vol.11, No.2 p.7, 2001. doi:10.1186/1471-2407-10-260.
[2] Bauer W., Mackenzie C.D.: Cancer Detection via Determination of Fractal Cell Dimension, 1999, http://cdsweb.cern.ch/record/376287.
[3] Bedin V., Adam R.L., de Sa B., Landman G., Metze K.: Fractal dimension of chromatin is an independent prognostic factor for survival in melanoma, BMC Cancer, 10:260, 2010, http://www.biomedcentral.com/1471-2407/10/260
[4] Blackledge J. Dubovitskiy D.: An Optical Machine Vision System for Applications in Cytopathology, ISAST Transactions on Computers and Intelligent Systems, Vol 2, Issue 1, pp 95-109, 2010.
[5] Dey P.: Fractal geometry: Basic principles and applications in pathology, Anal Quant Cytol Histol 2005, 27(5) pp 284–290, PMID: 16447821.
[6] Goutzanis L., Papadogeorgakis N., Pavlopoulos P.M., Katti K., Petsinis V., Plochoras I., Pantelidaki C., Kavantzas N., Patsouris E., Alexandridis C.: Nuclear fractal dimension as a prognostic factor in oral squamous cell carcinoma, Oral Oncology 44, pp. 345-353, 2008, doi: 10.1016/j.oraloncology.2007.04.005.
[7] IARC, Cytopathology of the uterine cervix – digital atlas, International Agency for Research on Cancer, http://screening.iarc.fr/atlascyto.php
[8] Kaye B.H.: A Random Walk Through Fractal Dimensions, VCH 1994.
[9] McKenna S.J.: Automated analysis of Papanicolaou smears. PhD Thesis, University of Dundee, 1994.
[10] Nielsen B., Albregtsen F., Danielsen H.E.: Fractal Analysis of Monolayer Cell Nuclei from Two Different Prognostic Classes of Early Ovarian Cancer, Fractals in Biology and Medicine, Mathematics and Biosciences in Interaction, 2005, Part 3, pp. 175-186, doi: 10.1007/3-7643-7412-8_16.
[11] Ohri S., Dey P., Nijhawan R.: Fractal dimension on aspiration cytology smears of breast and cervical lesions, Anal Quant Cytol Histol 2004, 26(2), pp 109–112, PMID: 15131899.
[12] Peitgen H.O., Jurgens H., Saupe D.: Fractal for the Classroms, Part 1: Introduction to Fractals and Chaos, Springer–Verlag, 1992.
[13] Sedivy R., Windischberger Ch., Svozil K., Moser E., Breitenecker G.: Fractal Analysis: An Objective Method for Identifying Atypical Nuclei in Dysplastic Lesions of the Cervix Uteri. Gynecologic Oncology 75, pp. 78-83, 1999, gyno.1999.5516.
[14] Seuront L.: Fractals and Multifractals in Ecology and Acquatic Science, CRC Press, 2010.
[15] Sharma N., Dey P.: Fractal dimension of cell clusters in effusion cytology, Diagnostic Cytopathology, Vol 38, Issue 12, pp 866–868, 2010, doi: 10.1002/dc.21299.
[16] Steven I.: Linear Richardson Plots from Non-Fractal Data Sets, Dutch Mathematical Geology, Vol. 25, No. 6, pp. 737-751, 1993. doi: 10.1007/BF00893176.
[17] Yokoyama T., Kawahar A., Kage M., Kojiro M., Takayasu H., Sato T.: Image analysis of irregularity of cluster shape in cytological diagnosis of breast tumors: Cluster analysis with 2D-fractal dimension, Diagn Cytopathol 2005, 33(2) pp 71–77, PMID: 16007648.

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