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USE OF METHODS OF IMAGE PROCESSING AND ANALYSIS TO DETERMINE THE DISTRIBUTION CLASS OF CORTICAL GRANULES IN BOVINE OOCYTES

Summary

Image processing and analysis are one of the tools to achieve data coded in digital images. Development of these methods enables to gain more data coded in digital images, even those which are not visible to the human eyes. Therefore it is justified to create new computer systems appointed in functions and filters that support process of gaining new information coded in digital image. In this study system for classification of oocytes has been described. The cells are classified taking into account distribution of cortical granulae according to three-class scale. In addition, knowing the diameter of the follicle from which the oocyte was aspired and class of oocyte–cumulus complex, it is possible to determine developmental competence of oocyte.
***Key words**: image processing, image analysis, cortical granules, oocytes, bovine oocytes, COC*

WYKORZYSTANIE METOD PRZETWARZANIA I ANALIZY OBRAZU DO OKREŚLENIA KLASY ROZMIESZCZENIA ZIAREN KOROWYCH W OOCYTACH POCHODZENIA BYDLĘCEGO

Streszczenie

Przetwarzanie i analiza obrazu stanowią narzędzia do uzyskania danych zawartych w obrazach cyfrowych. Dzięki rozwojowi tych metod można uzyskać więcej informacji na temat danych zakodowanych w obrazach cyfrowych, nawet tych które nie są widoczne dla ludzkiego oka. Dlatego też uzasadnione jest tworzenie nowych systemów informatycznych wyposażonych w funkcje i filtry, które wspierają proces pozyskiwania informacji zakodowanych w obrazach cyfrowych . W pracy opisano system do klasyfikacji oocytów. Komórki są klasyfikowane pod względem rozmieszczenia ziaren korowych zgodnie z trójstopniową skalą. Ponadto, przy znajomości średnicy pęcherzyka, z którego został wyaspirowany oocyt i klasę kompleksu oocyt-kumulus, możliwe jest ustalenie w systemie kompetencji rozwojowej komórki jajowej.
***Słowa kluczowe**: przetwarzanie obrazu, analiza obrazu, ziarna korowe, oocyt, oocyt bydlęcy, KOK*

1. Introduction

Gaining data from digital images of microscopic samples is a step further in assess and classification process [1]. Analysis and processing of images allowing to obtain information encoded in graphical form requires the use of sophisticated and dedicated tools [2].

In agriculture engineering there is a strong trend to use image processing and analysis, therefore there is possibility to obtain information in quick and effective way [3-7]. Matlab Development Environment with Image Processing Tollbox [8] allow to create application *ComCell*, which aim was support and classification of cortical granulaes distribution (CG – *Cortical Granulae*) in bovine oocytes. *Comcell* was designed and created to support analysis and processing process of images from the stained preparation of the microscope. Created application allow classification of cortical granule distribution in bovine oocytes according to three-grade scale presented by Payton [9].

First class Payton's scale contains all oocytes where the equator are peripheral cortical distribution of grains with numerous clusters (Fig. 1).

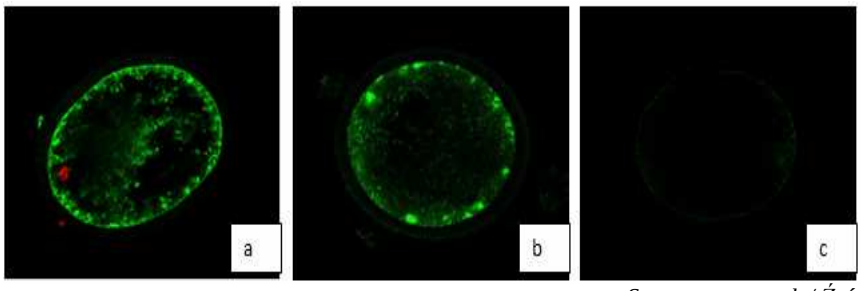

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Fig. 1. Payton's classification of cortical granulae distribution in bovine oocytes: a - oocyte where the equator is peripheral cortical distribution of grains with numerous clusters, b - oocyte, which is in a small number of particle aggregates, and their numerous cortical representation of individual units, c - cell, where the monolayer is without clear cortical particle aggregates
Rys. 1. Klasyfikacja rozmieszczenia ziaren korowych w oocytach bydła według Payton: a - oocyty, gdzie na równiku występuje peryferyczne rozmieszczenie ziaren korowych z licznymi ich skupiskami, b - oocyty, u których występuje niewielka liczba agregatów ziaren korowych natomiast obficie występują pojedyncze sztuki, c - komórki, gdzie występuje tylko monowarstwa ziaren korowych bez wyodrębnionych skupisk

In the second group are oocytes, which were observed in a small number of particle aggregates, and their numerous cortical representation of individual units. The third class are assigned to all the cells in which the monolayer were observed without clear cortical particle aggregates.

2. Characteristics of the application *ComCell*

The *ComCell* is an application created for the classification of digital images of microscopic preparations of bovine oocytes. Classification is made in the arrangement of cortical grains taking into account the size of the follicle, from which came the oocyte and oocyte complex class-cumulus (COC) [10]. Its aim is to support decisions regarding the accuracy of the oocyte and its competence development. The application is designed for personal computers equipped with the development environment Matlab, along with a package of Image Processing Toolbox. The application is created in C language dedicated to the environment Matlab using GUIDE (Guide User Interface Development Environment). The application was prepared using the built-development environment, based on requirements analysis conducted in accordance with the software engineering methodology based on UML 2.0 diagrams [11].

3. Functions of *ComCell*

Application functions as a GUI (Graphical User Interface), a system that facilitates the user the work in Matlab. The program is equipped with a main menu (Fig. 2), where under the button ’ComCell’ can save the currently analyzed and processed image and click 'About' where user can find information about the application.

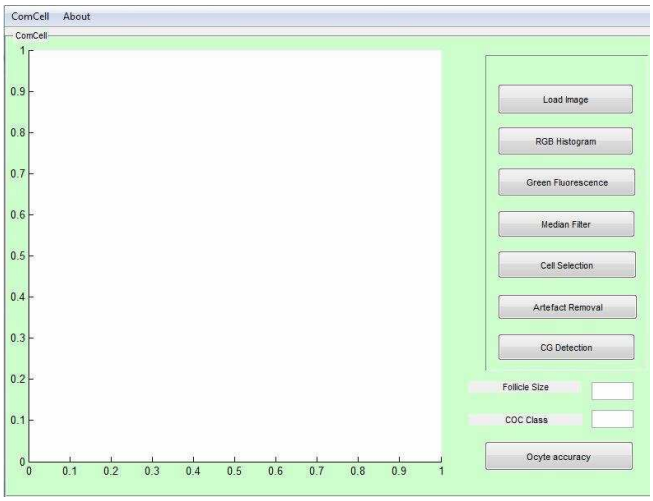

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Fig. 2. *ComCell* – main window
Rys. 2. ComCell – okno główne programu

The application has a context menu to work on the image (Fig. 3). In the context menu, user must first load the image, user can also display the RGB histogram that allows the analysis of individual color channels palette RGB [12] - use the 'RGB Histogram' button. Next step is to extract the green fluorescence, which represents cortical stained grains. Subsequently, apply a median filter to improve the visibility of the grain cortical [5]. In a further step, it is important to select an area on the image, in which the cell is located and removal the artifacts that could interfere with the assessment of cell.

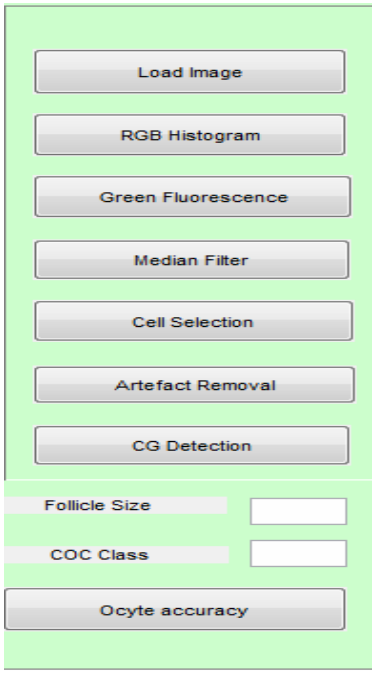

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Fig. 3. Context menu of *ComCell* application
Rys. 3. Menu kontekstowe program ComCell

The last step of image analysis is use the ‘CG Detection' button where information is obtained, to which class by Payton the oocyte belong (Fig. 4). Additionally there is possibility to check developmental competence, i.e. the capacity for fertilization and the development of the analyzed cells (Fig. 5).

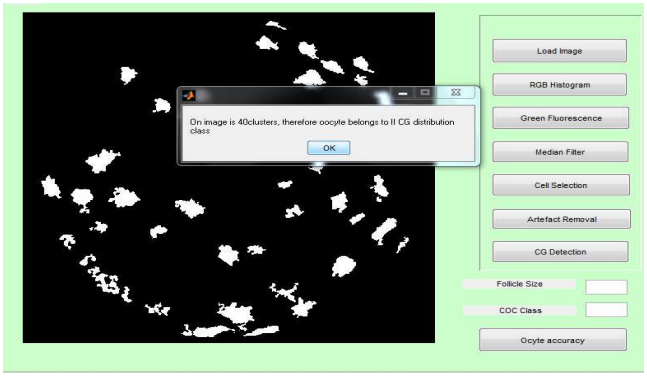

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Fig. 4. *ComCell* application output
Rys. 4. Wynik działania program ComCell

Knowing the class distribution of cortical grain, and taking into account the size of the follicle from which the oocyte was aspirated and COC class to which cell belonged, user can find out what quality will the embryo developed from a fertilized oocyte. This is possible only if user knows the parameters mentioned above for the individual cells analyzed. To check the quality of embryo user should in field 'Follicle Size' provide the diameter of the follicle in millimeters, from which it was obtained cell, and in filed ‘COC Class' must specify which class COC were among the analyzed cell.

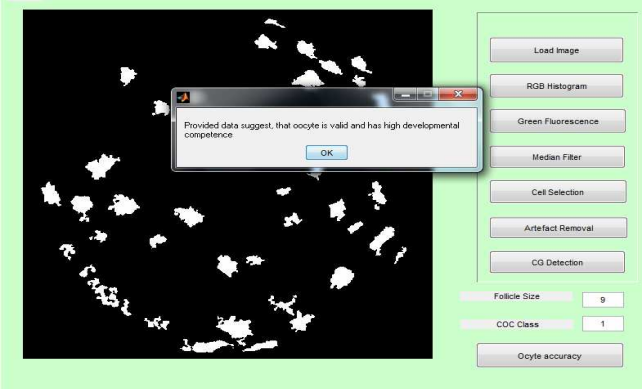

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Fig. 5. Checking the developmental competence of oocyte
Rys. 5. Sprawdzanie kompetencji rozwojowych oocytu

4. Functions used in application

At the built-in buttons in the application there was implemented a number of Matlab environment functions. The 'Load Image' allows the selection of the oocyte image it is based on the function uigetfile(), which opens a dialog box and lets user choose a photo file. There is here also a function imread(), which loads the main window, select the image file (Fig. 6).

The 'RGB Histogram' button has been coded with built-function imhist() to display the RGB histogram of the analyzed image (Fig. 7). This action is active at any time of image processing.

Cortical grain in the image are isolated using the 'Green Fluorescence' button (Fig. 8).

'Median Filter ' is a button that use buit-function medfilt2 function(). This function is applied to the image mask filter size [3 3] (Fig. 9).

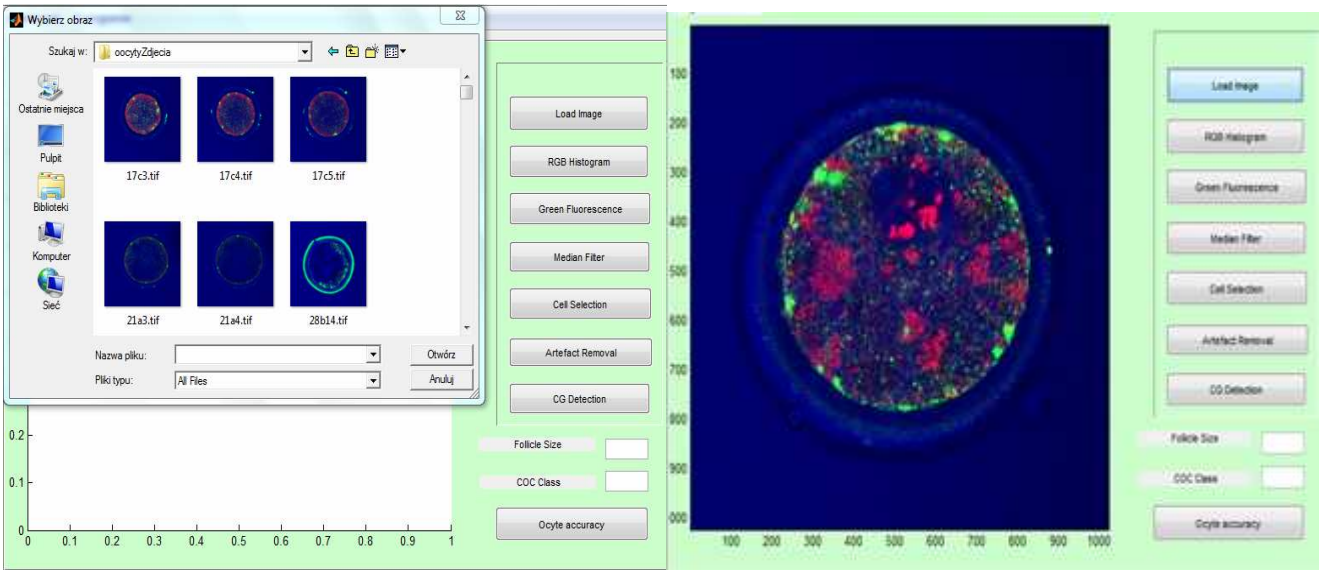

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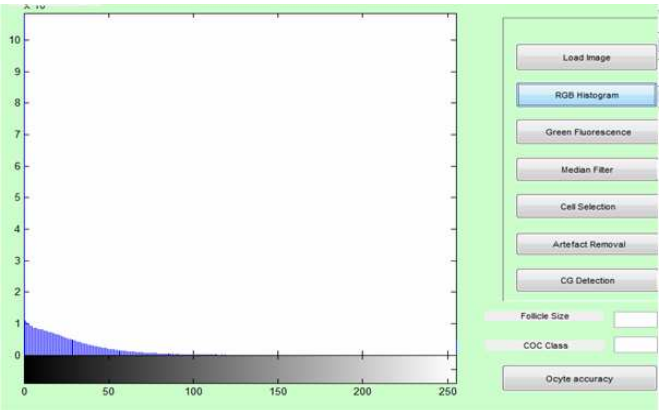
Fig. 6. Loading and displaying selected image in main field of *ComCell* application
Rys. 6. Ładowanie i wyświetlanie wybranego obrazu w oknie głównym program ComCell



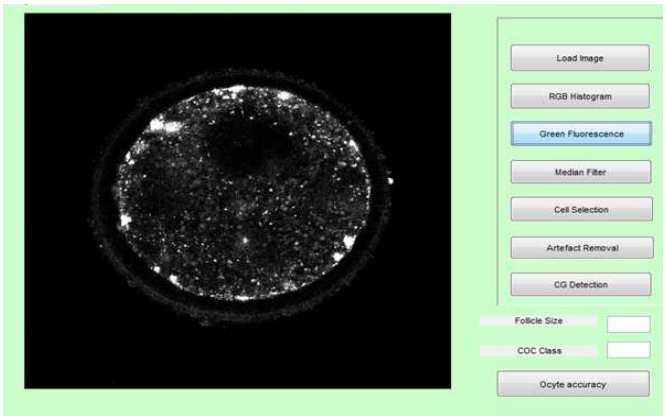


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Fig. 7. Displaying RGB histogram of analyzed image
Rys. 7. Wyświetlanie histogramu RGB analizowanego obrazu

Fig. 8. Isolation of cortical granules from image
Rys. 8. Wyodrębnianie na obrazie ziaren korowych

a) b)

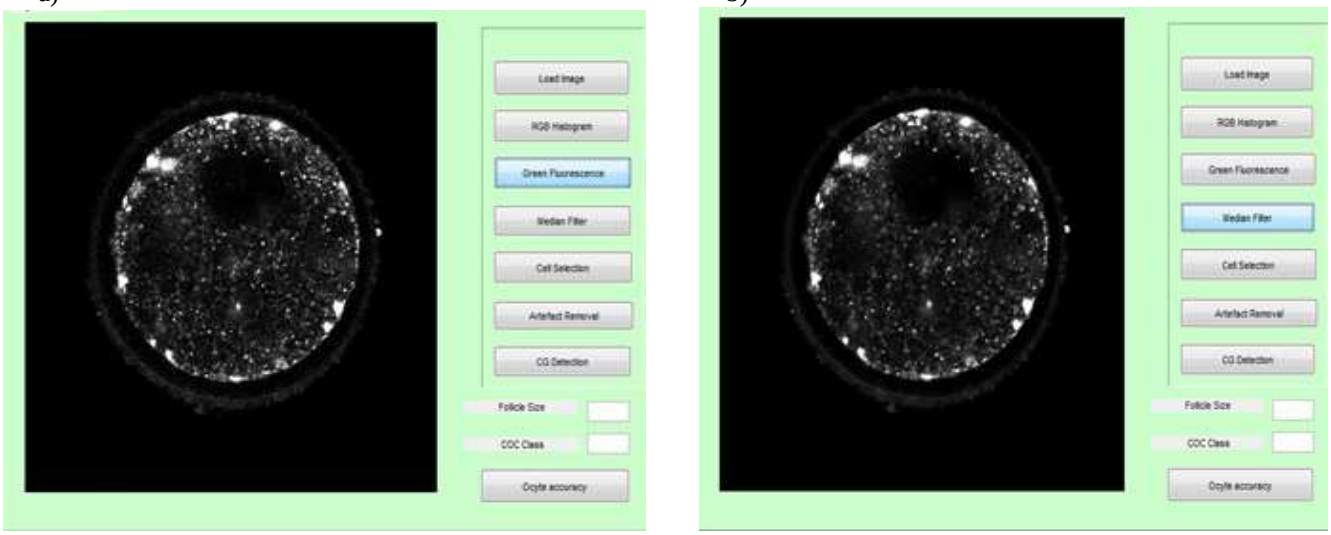

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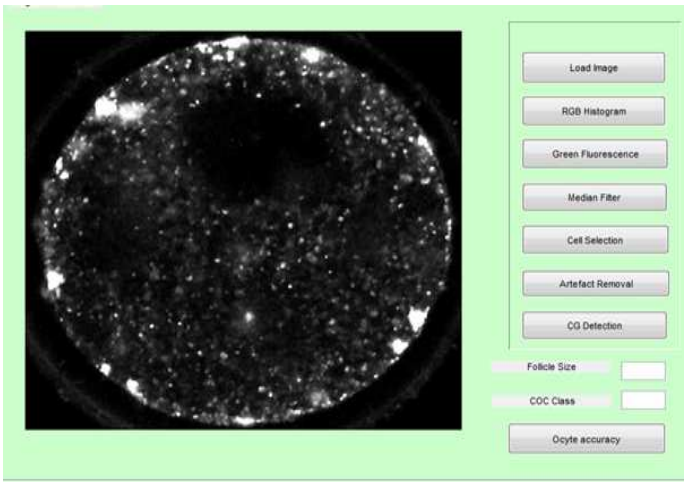
Fig. 9. Difference between image after (a) and before (b) applying mask filter [3 3] size
Rys. 9. Porównanie obrazu po (a) i przed (b) zastosowaniem filtra medianowego, wielkość maski [3 3]



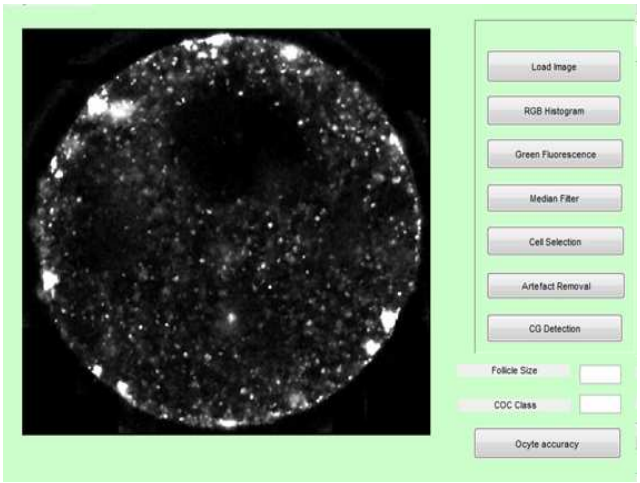


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Fig. 10. Excision with imcrop() function part of the image, where the cell is located
Rys. 10. Wycięcie przy pomocy funkcji imcrop() fragmentu obrazu, na którym znajduje się komórka

Fig. 11. The result of the imrect() function which aims to further removal of artifacts
Rys. 11. Wynik działania funkcji imrect(), która pozwala na usuwanie niepożądanych artefaktów z obrazu

Built-in Matlab environment imcrop() function allows excision of the whole picture this area, where there is a cell, in order to avoid reading errors associated with the occurrence of artifacts in the background of the image (Fig. 10).

Removing remaining artifacts from an image using the following imrect() function (Fig. 11).

5. Summation

Presented system *ComCell*, has been equipped with a number of functions for analyzing digital images. The generated application is dedicated to images of microscopic preparations of bovine oocytes and has been tested and used in the classification process of the distribution of cortical granulaes in these kind of cells.

6. Conclusions

1) *ComCell* application is useful tool supporting processing and analysis process of bovine oocytes.

2) Application can be used to assess the quality and quantity of images, and make decisions concerning the classification of the preparation in one of three classes of cortical granulae distribution in cells

3) Application *ComCell* allows user to obtain information about the developmental competence of the analyzed oocyte.

4) *ComCell* meets the requirements of design and creating software. The application is in accordance with the design goals and principles of software engineering.

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