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ESTIMATION OF THE Ki-67 PROLIFERATION MARKER EXPRESSION LEVEL WITH COMPUTER VISION TECHNOLOGY

Sergey A. Kotov[1], **Sergey A. Mozerov**[2,a], **Sergey O. Starkov**[2,b]
[1] *Model Spektr, OOO, Obninsk, Russian Federation, serj-kotoff@mail.ru*
[2] *Obninsk Institute for Nuclear Power Engineering, National Research Nuclear University MEPhI, Obninsk, Russian Federation*
[a] *mozerov@list.ru*, [b] *sergeystarkov56@mail.ru*

Abstract: lately, computer vision technologies have become common computer diagnostics tools. The study objective is the development of software tools to automatically detect cells with proliferation markers on medical images. We managed to qualitatively assess the number of cells with proliferation markers on a medical image sample. We closely cooperated with morphologists who identified the areas of interest on the image where the cells with proliferation markers were to be counted. Another enabler was extensive experience with developing computer vision systems for other applications. The medical images were tokenized by the morphologists. The images were slices where the proliferation marker cells were dyed. The organ under investigation was the tongue. We counted the immune-positive cells. It helped the morphologists to have some objective slice image metrics used to adjust the diagnosis and therapy plans.

Keywords: computer vision, Python, OpenCV, Ki-67 proliferation marker.

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ОПРЕДЕЛЕНИЕ УРОВНЯ ЭКСПРЕССИИ МАРКЕРА ПРОЛИФЕРАЦИИ Ki-67 С ПОМОЩЬЮ ТЕХНОЛОГИИ КОМПЬЮТЕРНОГО ЗРЕНИЯ

С. А. Котов[1], **С. А. Мозеров**[2,а], **С. О. Старков**[2,б]
[1] *ООО «Модель Спектр», г. Обнинск, Российская Федерация, serj-kotoff@mail.ru*
[2] *Обнинский институт атомной энергетики, Национальный исследовательский ядерный университет «МИФИ», г. Обнинск, Российская Федерация*
[а] *mozerov@list.ru*, [б] *sergeystarkov56@mail.ru*

Аннотация: в последнее время в качестве средств компьютерной диагностики широкую популярность приобретают элементы из области компьютерного зрения. Целью данной работы является разработка программных средств, позволяющих автоматически анализировать онкомаркированные клетки в образцах медицинских изображений. В процессе работы была решена такая задача, как выявление количества онкомаркированных клеток в образце медицинского изображения. Это стало возможно благодаря тесному взаимодействию с морфологами, обозначавшими интересующие их фрагменты снимка, где необходимо было произвести подсчет онкомаркированных клеток, и опыту программиста в решениях подобных задач других областей жизнедеятельности с помощью технологии компьютерного зрения. Медицинские изображения были размечены морфологами и представляли собой срез стекла, на котором были окрашены в определенный цвет онкомаркированные клетки специальным препаратом. Исследуемым органом стал язык. В результате был совершен подсчет иммунопозитивных клеток, что дает морфологам возможность наблюдать более объективную картину в изображении среза стекла образца, позволяющую скорректировать решение в отношении постановки диагноза и назначения необходимого лечения.

Ключевые слова: компьютерное зрение, Python, OpenCV, онкомаркер Ki-67.

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Introduction

Currently, there is a rapid growth in the development of computer-assisted diagnosis tools. Analytics itself has served medicine for more than 40 years, but only in the last decade its use in healthcare reached a new level: powerful tools, including those based on computer vision technology, machine learning, etc., emerged.

Computer vision is a technology for object detection and classification. As a field of science, it refers to the subject of image processing, and in its technical component it is used in video surveillance systems, industrial process control, object modeling, and recently such systems are very popular in the construction of augmented reality.

Software such systems are implemented using open-source computer vision algorithm libraries, such as Open computer vision (OpenCV) or Point Cloud Library (PCL).

The paper considers the possibilities of visual analysis of the marker, discusses the technical side of the implementation of determining its level of expression based on computer vision technology, and presents the main results of the analysis.

Ki-67 Proliferation Marker

The development of malignant tumors is based on cell proliferation, which leads to an increase in the number of atypical elements. To determine cell proliferation features of malignant tumors the most common marker of proliferation is the Ki-67 antigen, which is expressed practically in all phases of the mitotic cycle, except for the G_0 resting phase, and, accordingly, reflects the value of proliferative pool [1]. Ki-67 protein synthesis starts in the middle of the G_1 phase of mitosis and, gradually increasing, its level reaches a maximum in metaphase, then sharply decreasing in anaphase. Ki-67 antigen detected by appropriate monoclonal antibodies is a short-lived protein, it is destroyed within 1.5–2 hours. Therefore, antibodies to Ki-67 detect only dividing cells, because Ki-67 does not have enough time to accumulate and does not remain in resting cells.

The conventional mitotic activity estimation does not reflect the proliferative potential of the tumor, since mitosis itself takes several hours and preparation for it takes about 24 hours. A study of non-histone protein Ki-67 expressed in all cells coming out of G_0 and early G_1 phases of mitosis allows determining the latent proliferative potential of a tumor.

The proliferative activity directly correlates with the degree of histological malignancy, the degree of invasion, the presence of metastases. The physiological role of the Ki-67 antigen in cell life is still unclear. Nevertheless, its presence in all active phases of the mitotic cycle allows using this protein as a universal proliferation marker for the evaluation of malignant tumor growth activity. Tumor mass growth rate represents very important information for determining the tumor oncological status and aggressiveness. In such cases, its proliferative activity index is one of the decisive factors taken into account when choosing a treatment approach [2].

Artificial System for Assessing the Expression Level of the Ki-67 Marker

To build this artificial system for obtaining an objective assessment, it is necessary to solve the following problems:

1. Applying a mathematical method of selecting two types of spots: brown and blue, where cells are treated as spots of a certain color.
2. Calculation of the ratio of areas of spots of different colors in the image. A prerequisite is a classification concerning the ratio of the excess of one of the spot types.

Direct implementation of the system is performed programmatically in the Python programming language and with the help of the OpenCV computer vision library [3]. The structure of the software operations is shown in Fig. 1.

Let us consider the main steps of the proposed approach:

1. Stain highlighting.

After obtaining the primary image it is necessary to implement the procedure of spots extraction. For this purpose we use the Adaptive threshold from the OpenCV library [4]:

```
mask = cv2.adaptiveThreshold(cv2.cvtColor(img, cv2.COLOR_BGR2GRAY),255,\
```

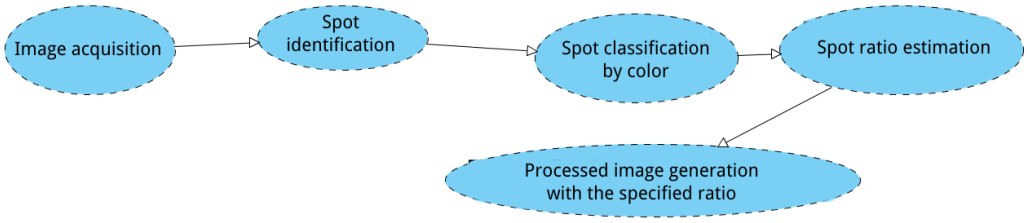


Figure 1. *Software flowchart*

cv2.ADAPTIVE_THRESH_GAUSSIAN_C, cv2.THRESH_BINARY,1931,3)

The next step is to clean the mask of too small pixel blobs. To do this, we blur the mask and apply the same adaptive threshold again. To compensate the small expansion of black spots during the cleaning procedure, the function of narrowing the black areas of the mask is applied. We also use the OpenCV library [5]:

```
mask = cv2.GaussianBlur(mask,(15,15),0)
mask = cv2.adaptiveThreshold(mask,255,\
cv2.ADAPTIVE_THRESH_GAUSSIAN_C, cv2.THRESH_BINARY,1931,3)\
kernel = np.ones((5,5),np.uint8)
mask = cv2.dilate(mask,kernel,iterations = 1)
return mask
```

A combination of these methods and functions results in a mask for cutting off the light background (highlighting spots):

```
mask = clear_mask(get_mask(img))
```

2. Classification of stains by color.

The next step is the operation of separation: which spots are brown and which are blue. To the brown pixels we will refer the pixels for which the following conditions are met:

1. Located inside the spot (mask == 0).
2. The intensity of blue is less than the intensity of red or both: blue is less than 120 and red less than 110, the intensity of green is less than the intensity of red.
3. To the blue pixels, we refer all the pixels of the spots that did not turn out to be brown.
4. Calculation of the percentage of stains.

The calculation of the number of brown and blue spots is based on the estimation of their total area. We find the ratios between the spots in percentages: ratios for both excess brown and excess blue spots, which differ only in what is in the denominator. But even though we count both images, the user sees one count: the minimum. The algorithm is as follows:

1. Find the ratio between them and the blue and brown cells.
2. Calculate which of the ratios is less.
3. A copy of the original image is created and a mask with selected spots on it is applied. A copy of the image is created, on which brown and blue pixels are applied.
4. The user is returned the percentage ratio and the processed final image.

Results

Based on the analysis of images, cells colored blue and brown were identified, their ratio was calculated, and the choice of processing one or more images was provided. When processing this program, the user receives a photo with selected cells and their percentage ratio. The graphical result is shown in Fig. 2.

Medical images of the Ki-67 tumor marker and the main results of the analysis – the excess of brown and blue cells obtained as a result of the program processing are presented in Table.

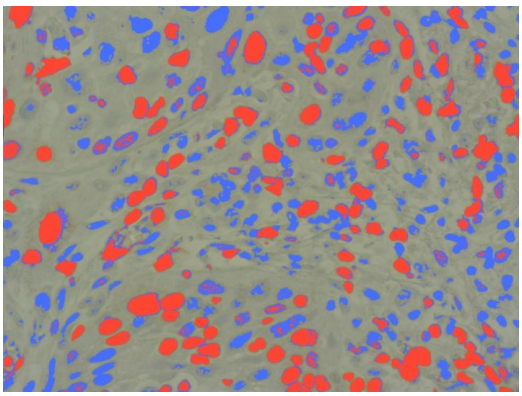
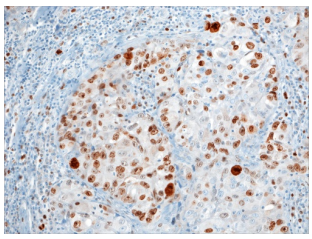
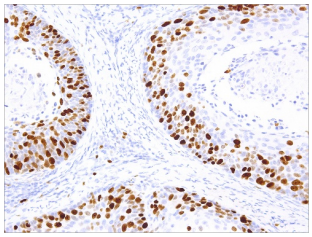
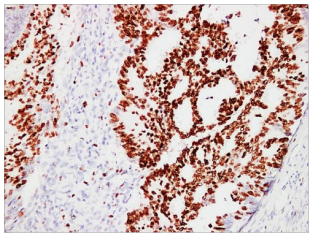
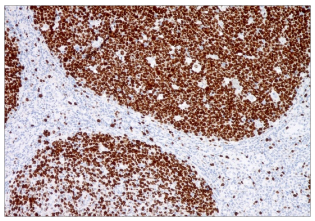
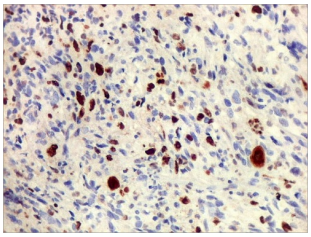


Figure 2. *An SW-generated image*

Table

# picture	Medical image	Excess
1		Brown cells 70 percent
2		Blue cells 30 percent
3		Brown cells 60 percent

# picture	Medical image	Excess
4		Brown cells 96 percent
5		Blue cells 39 percent

The results of the analysis are shown for the areas of direct interest to the physician, not for the entire medical image.

Conclusion

The first part of the software implementation solved the problem of detecting cells stained blue and brown (immunopositive cells) in photos and counting their ratio. Taking into account the fact that the proliferative activity of human tumor cells correlates with the degree of their histological and biological malignancy, the calculation of the proliferation index using computer vision significantly increases the objectivity of the data. Further development of the current software product can increase the color range of spots, expand the data set processed by the program, to refine the cell counting, because the error is 5 % so far.

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