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## NEW IMAGE PRE-PROCESSING METHODS FOR TRACKING VARIOUS BIOLOGICAL OBJECTS MOVEMENT

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**Анотація.** Дослідження алгоритмів для відстеження динаміки руху різних біологічних об'єктів є актуальним. На вибір оптимальних методів і алгоритмів відстеження об'єктів, для конкретного завдання, сильно впливають особливості і характеристики цих об'єктів і умови їх візуалізації. Тому, щоб автоматизувати процеси адаптації алгоритмів розпізнавання-відстеження, в статті розглядаються кілька варіантів алгоритмів для стеження за об'єктами реалізованими в проєктах Labview. Специфіка цих об'єктів, умови їх візуалізації і параметри моделі сильно впливають на вибір методів і алгоритмів, які є оптимальними для конкретного завдання. Тому в цій статті для автоматизації процесів адаптаційних алгоритмів розпізнавання-відстеження запропоновано кілька алгоритмів попередньої обробки кадрів з використанням інструментів NI Labview і Vision Assistant. Попередня обробка включала вирівнювання загальної фонові яскравості зображення, усунення високочастотного шуму і різних артефактів (виділені області, проміжки, переломи) з вихідного зображення, контрастності, порогового значення, бінаризації і інших функціональних перетворень. Проєкти дозволяють швидко змінювати шаблони для навчання і перепідготовки системи. Вони адаптуються до швидкості об'єктів і статистичних характеристик шуму в зображеннях. У статті обговорюються нові методи попередньої обробки зображень для алгоритмів, які відстежують динаміку руху різних біологічних об'єктів. Будуть представлені і проаналізовані експерименти, проведені для тестування трекерів на реальних відеофайлах.

**Ключові слова:** класифікація, процес відстеження, безліч біологічних об'єктів, алгоритми попередньої обробки зображень, порівняння зображень.

**Аннотация.** Исследование алгоритмов для отслеживания динамики движения различных биологических объектов является актуальным. На выбор оптимальных методов и алгоритмов отслеживания объектов, для конкретной задачи, сильно влияют особенности и характеристики этих объектов и условия их визуализации. Поэтому, чтобы автоматизировать процессы адаптации алгоритмов распознавания-отслеживания, в статье рассматриваются несколько вариантов алгоритмов для слежения за объектами, реализованными в проектах Labview. Специфика этих объектов, условия их визуализации и параметры модели сильно влияют на выбор методов и алгоритмов, которые являются оптимальными для конкретной задачи. Поэтому в этой статье для автоматизации процессов адаптационных алгоритмов распознавания-отслеживания предложено несколько алгоритмов предварительной обработки кадров с использованием инструментов NI Labview и Vision Assistant. Предварительная обработка включала выравнивание общей фоновой яркости изображения, устранение высокочастотного шума и различных артефактов (выделенные области, промежутки, переломы) из исходного изображения, контрастности, порогового значения, бинаризации и других функциональных преобразований. Проекты позволяют быстро менять шаблоны для обучения и переподготовки системы. Они адаптируются к скорости объектов и статистическим характеристикам шума в изображениях. В статье обсуждаются новые методы предварительной обработки изображений для алгоритмов, отслеживающих динамику движения различных биологических объектов. Будут представлены и проанализованы эксперименты, проведенные для тестирования трекеров на реальных видеофайлах.

**Ключевые слова:** классификация, процесс отслеживания, множество биологических объектов, алгоритмы предварительной обработки изображений, сравнение изображений.

**Abstract.** The algorithms to tracking of movement dynamics of various biological objects now it is actually to studying. Features and characteristic of objects, conditions of their visualization strongly influence the choice of optimal methods and algorithms for a specific task it they tracking. Therefore, to automate the processes of adaptation of recognition-tracking algorithms, several Labview project trackers are considered in the article. Specificity of these objects, conditions of their visualization and model parameters strongly influence the choice of methods and algorithms, which are optimal for a specific task. Therefore, in this article, in order to automate the processes of adaptation algorithms of recognition - tracking, we suggest several frames pre-processing algorithms using NI Labview tools and Vision Assistant. Preprocessing included equalization of general background luminance of the image, elimination of high-frequency noise and different artifacts (highlighted areas, gaps, fractures, etc.) from the original image, contrasting, thresholding, binarization and other functional transformations. Projects allow changing templates for training and retraining the system quickly. They adapt to the speed of objects and statistical characteristics of noise in images. New pre-processing methods image for algorithms tracking of movement dynamics of various biological objects will be discussed. The experiments carried out to test the trackers on real video files will be presented and analyzed.

**Keywords:** classification, tracking process, pattern matching, plurality of biological objects, pre-processing algorithms

### 1. INTRODUCTION

The rapid development of researches in the field of andrology stimulates studying spermatogenesis process. In clinical practice, its evaluation is often based on the results of the patient's spermogram study. The main purpose of the clinical evaluation is to determine the quality of sperm cells in order to identify their potential ability to fertilize the egg and find possible causes of infertility for further development of its treatment method. To date, in clinical practice, there are two main parameters generally accepted as evaluation criteria of spermatogenesis: the percentage of motile sperm cells, and the domination of morphologically normal sperm. Both of these parameters are visually analyzed under the microscope and evaluated subjectively by a physician possibly leading to incorrect results and false diagnostic conclusion. Even if a highly qualified specialist evaluates ejaculate very carefully, the variation of quantitative and qualitative characteristics makes up not less than 10%, and highly increases in the case of defects overlay (at preparation, material sampling or evaluating). Therefore, to determine and correct the errors, both internal and external quality control should be held in every laboratory. A spermogram is a simple but important analysis, on which further activity of the urologist and andrologist is based. Sperm concentration and total sperm count, sperm motility, and morphology are the most important pa-

rameters of the spermogram [1]. Modern technologies of computer processing and image analysis [2-4] can improve the quantitative and qualitative indices of the patient's spermogram analysis. The main problem of tracking biological objects in video stream is automatic finding them in each separate frame. Usually tracking algorithms consist of such successive steps: selection and allocation of descriptors, their comparison and classification. At the comparison, a frame fragment, the descriptor of which is the most similar to the descriptor of the tracked object, is searched. When the corresponding fragment is found, then the found object is classified. When analyzing moving biological objects recorded by digital microscope using tracking algorithms, a large number of frames must be processed in the images stream. Counting the number of selected objects in each frame to determine the average of a standard set, usually 200 frames, and grouping and clustering by different parameters, including shape, speed, location, etc., are important subtasks [1]. And for each of these sub-tasks not only dedicated optimal image processing algorithms adapted to specific conditions, but the possibility to rearrange and integrate them easily into a single tool environment are required. There are well-established approaches to recognition of very noisy and correlated single objects [5, 6, 7, 8, 9] and sets of multiple objects [10, 11, 12], including moving ones [13], with simultaneous division into clusters. However, they are all very diverse and poorly integrated into single, flexible and configurable, and adaptive system or program. Therefore, when choosing a tool for research, we settled on Labview as the most powerful system-design platform and development environment for a visual programming language from National Instruments. [14].

Using Labview and its basic applications and modules enables relatively quick design of the required video analysis processing system of AVI files, which greatly simplifies the recognition and tracking of biological objects. We have developed a number of possible projects for detection and tracking of moving biological objects. NI Vision package is a powerful tool to work with images, supplemented by a set of NI IMAQ drivers and NI Vision Assistant module. Regardless of the software environment - Labview, Measurement studio, Visual Basic or Visual C ++, the package directly provides full control over all the types of analog and digital cameras and allows not to program on the register level. For the processing of static and animated images a basic NI Vision module is used. It contains a set of optimized functions to work with color, black-and-white or binary image, including filtering, statistical and geometric shape changes, pattern matching, and measuring picture parameters. The features of this package allow capturing geometric shape changes, compare the image with the standard, and change settings of the picture. The NI IMAQ video camera driver software is compatible with all National Instruments software, including NI DAQ. It enables easy integration of image processing to any of National Instruments' product. The main feature of the NI IMAQ is the extensive library of special functions. They include templates for cameras configuration, memory allocation functions, start-up initiations and the actual image acquisition both in continuous and single-image modes. A comfortable and functional addition to the NI Vision, greatly enhancing the user experience, is NI Vision Assistant. It enables to easily create own routines to capture, filter, process, analyze and edit the images, change settings of the cameras being used. These routines can be imported into Labview. Visualization and ease of use are the main advantage of such an approach, as the result of function application is visible at once [14].

Therefore, to automate the processes of adaptation of recognition-tracking algorithms, several Labview project trackers are considered in the article. New pre-processing algorithms, which improve the quality of real-time monitoring and tracking of multiple biological objects, are considered in the paper. Projects allow to change training templates and to retrain the system quickly. They adapt to the speed of objects and statistical characteristics of image noise. New features of image or image characteristics comparison, descriptors and pre-processing methods will be discussed. The experiments that were carried out to test the trackers on real video files will be presented and analyzed.

## **2. EXPERIMENTAL STUDIES OF IMAGE PRE-PROCESSING ALGORITHMS FOR EXTRACTION AND ANALYSIS OF MULTIPLE BIOLOGICAL OBJECTS**

There is a number of problems in visual tracking of biological objects that can degrade the quality of detection and tracking of objects. The lighting effects of objects in the frame, i.e. highlights and shadows, distort the actual results of detection and tracking of objects. When processing the image, a problem with strong texturing of the background may appear. Changes in the appearance of the object due to projection of 3D movements on the 2D plane (sequence of frames), may include geometric deformation of the tracked object (due to rotations around the axis, for example). For non-solid objects, the shape can be deformed for a long time. Sudden changes in subject's movement with increasing speed, which can cause the problem of finding and tracking of objects. When tracking an object in a liquid medium, the image may be blurred by this medium. Tracking such objects is difficult for visual tracking of the right path the object. An ideal algorithm of visual tracking of biological objects should overcome all of the problems described above. Therefore, one of the main objectives of research of tracking algorithm of moving biological objects is pre-processing of frames in the stream to improve the quality of their subsequent detection and tracking.

## 2.1 The algorithm of frames pre-processing, using morphological tool and local threshold, version 1

The algorithmic solutions consists of the three main stages:

- the research mode to find and select the toolkits for solving the task;
- expert processing, analysis and evaluation of results of image processing with the involvement of all available NI Vision Assistant means in interactive mode;
- automatic processing by fixed (pre-selected) sets of computational procedures, the use of which guarantees certain probability of correct processing results.

To eliminate the problems described above, one of pre-processing algorithms can be used (Fig.1). As shown in Figure 1a the original color image in addition to the objects of interest, has many false objects and complex background.

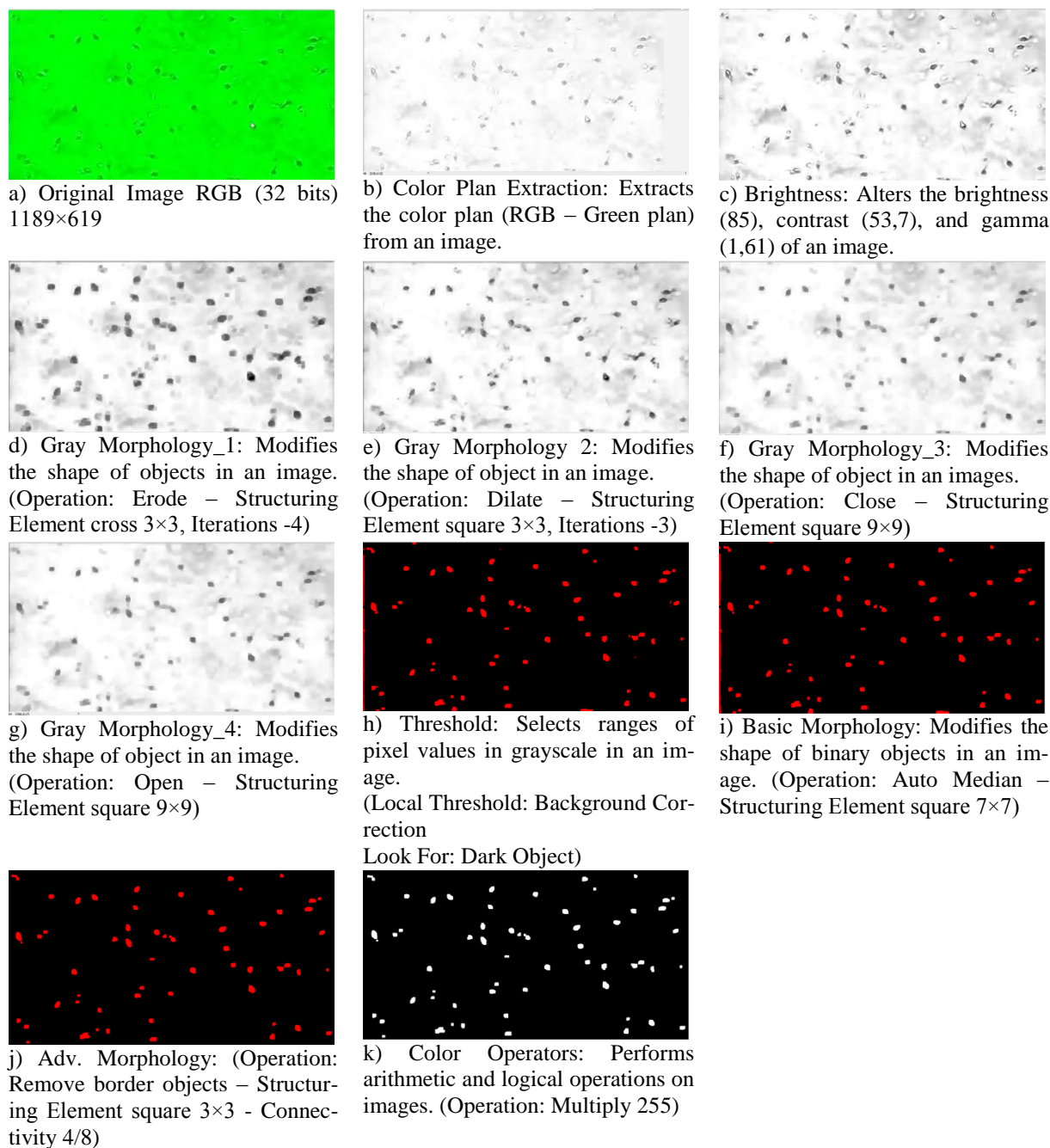


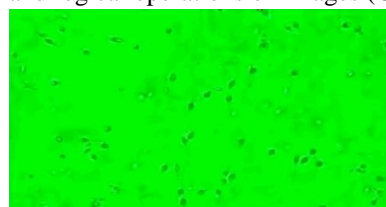
Figure 1 - The algorithm of frames pre-processing using a morphological tool and local threshold, version 1

Preprocessing includes equalization of common luminance of the image, elimination of the high frequency noise and various artifacts (highlighting of the separate regions, gaps, fractures, etc.) in the original image, contrast adjustments, threshold processing, binarization, and other functional transformations. Preprocessing by one

of the algorithms presented in Fig.1 includes a set of tools described below, which enable successive transformation of each image frame. To simplify processing by the next tools and operators, firstly one of RGB colors plan, for example, Green plan, is extracted from original Image RGB (32 bits) 1189×619 pixels (Fig.1, a, b). To improve the readability and processing quality of the image, Tool Brightness, which alters the brightness (85), contrast (53,7), and gamma correction (1,61) of an image (Fig.1, c) was used at the following stages. To improve the quality of extraction of biological objects, the morphological operators were used then to modify the shape of objects. At the first and second stages the Erosion - blur operations of the shape of object were used (Fig.1, d), then a part of it was removed (Fig.1, e). These operations have resulted in better finding of the object's shape. With the same purpose Close operation, which closes a space of area of the object's shape by a square  $9 \times 9$  structural elements (Fig.1, f)) and Open operation, which opens a space of area by a square  $9 \times 9$  structural elements during processing (Fig.1, g). To detect moving biological objects and ignore the background noise, Threshold tool and its local threshold function for searching dark objects were used. On this step the algorithm selects the ranges of pixel values of grayscale images with parameters (Local Threshold: Background Correction Look For: Dark Object) (Fig.1, h)). The resulting binary images were further transformed by means of the shape change operators of Basic Morphology tool and Remove border objects operator of Adv. Morphology tool. Basic Morphology modifies the shape of binary objects in an image with parameters (Operation: Auto Median – Structuring Element square  $7 \times 7$ ) (Fig.1, i)). Adv. Morphology performs high-level operations to the blobs in binary images (Operation: Remove border objects – Structuring Element square  $3 \times 3$  -Connectivity 4/8) (Fig.1, j)). For normalization across all levels of graduation image, Color Operators tool was used that performs arithmetic and logical operations on images (Operation: Multiply 255) (Fig.1, k)).

## 2.2 The algorithm of frames pre-processing using a morphological tool and local threshold, version 2

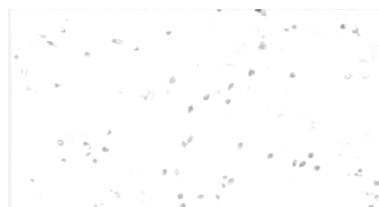
The second variant of the preprocessing algorithm is shown in (Fig.2). As can be seen from (Fig.1, a) the original color image, except of objects of interest, also has a lot of false objects and complex background. Pre-processing as well as version 1 includes equalization of the total background luminance of the image using Tool Brightness. The algorithm, in contrast to version 1, uses two operations Erode - Dilate by Tool Gray Morphology, the result of which is shown in Fig.2, d. These operations modify the shape of object in images. The background of the frame is ignored due to local threshold function (Threshold Type - Auto Threshold: Moment, Look For: Dark Object), see Fig.2. Further four Adv. Morphology operations are used in version 2, which perform high-level operations doing blobs in binary images (Fig.2, f). This Operations: Remove border objects (Structuring Element square  $3 \times 3$ , Connectivity 4/8, Iterations – 3); Filling holes; Convex hull; Removing small objects. For normalization across all levels of graduation image Color Operators tool is used, which performs arithmetic and logical operations on images (Operation: Multiply 255), (Fig.2, g).



a) Original Image RGB (32 bits) 960×512



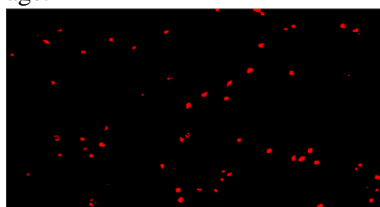
b) Tool Color Plane Extraction: Extracts the three-color planes (RGB, HSV, or HSL) from an image.



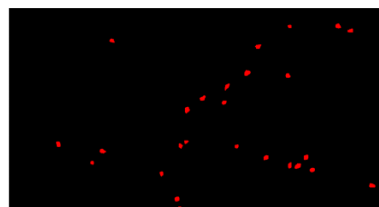
c) Brightness: Alters the brightness (85), contrast (53,7), and gamma (1,61) of an image.

d) Tool Gray Morphology 1-2: Modifies the shape of object in an image.

(Operation: Erode – Dilate; Structuring Element square  $3 \times 3$ ; Iterations -2)



e) Threshold: Selects ranges of pixel values in grayscale images. (Threshold Type – Auto Threshold: Moment, Look For: Dark Object)



f) Adv. Morphology 1-4: (Operation: Remove border objects – Structuring Element square  $3 \times 3$  - 4/8 Iterations-3; Fill holes; Convex hull; Remove small objects.)

Figure 2a, 2b, 2c, 2d, 2e, 2f - The algorithm of frames pre-processing using a tool of morphological and local threshold, version 2



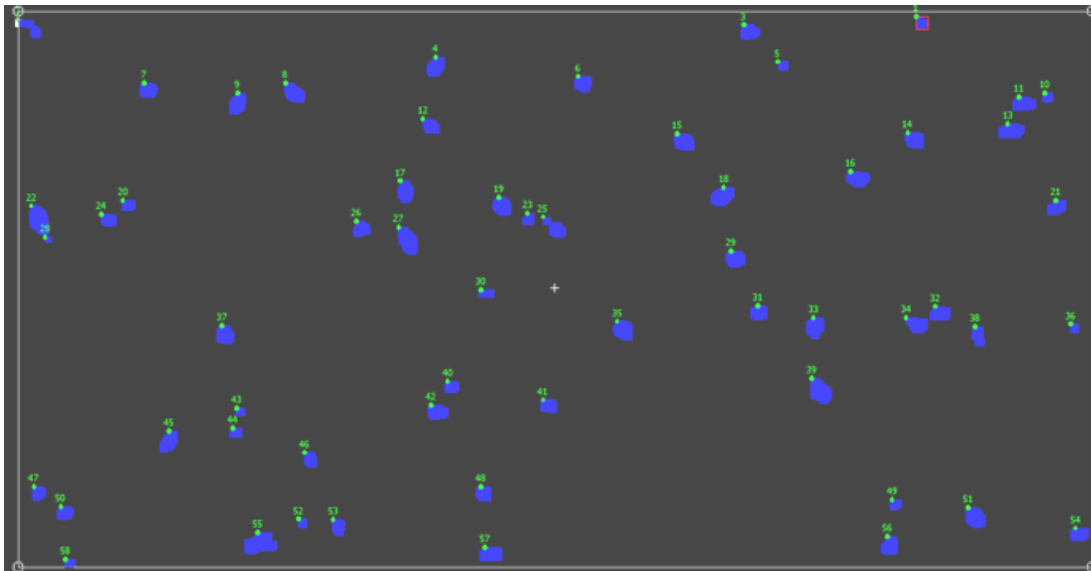
g) Color Operators: Performs arithmetic and logical operations on images. (Operation: Multiply 255)

Figure 2g - The algorithm of frames pre-processing using a tool of morphological and local threshold, version 2

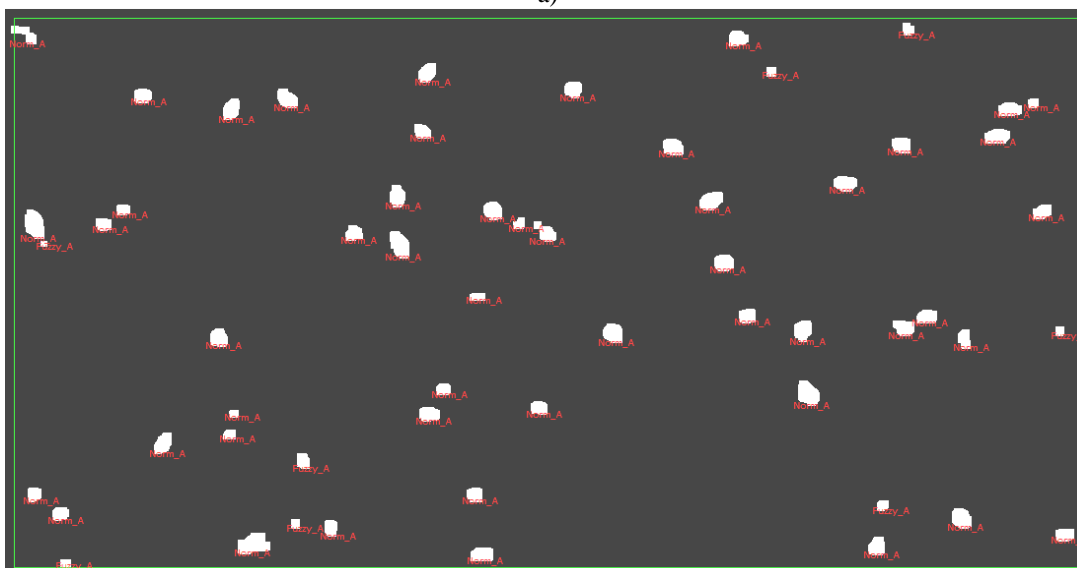
### 3. USING LABVIEW FOR REAL-TIME SIMULATION FOR CLASSIFICATION, TRACKING AND MATCHING WITH TEMPLATE OF MULTIPLE BIOLOGICAL OBJECTS

#### 3.1 The frames pre-processing algorithm using the classification of multiple biological objects

Fig.3 shows the result of frames pre-processing algorithm using the classification of multiple biological objects.



a)



b)

Figure 3 - The frames pre-processing algorithm using the classification operation of multiple biological objects

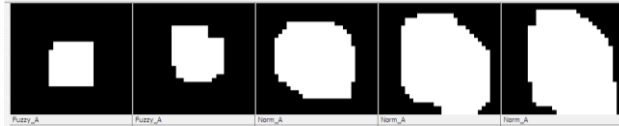
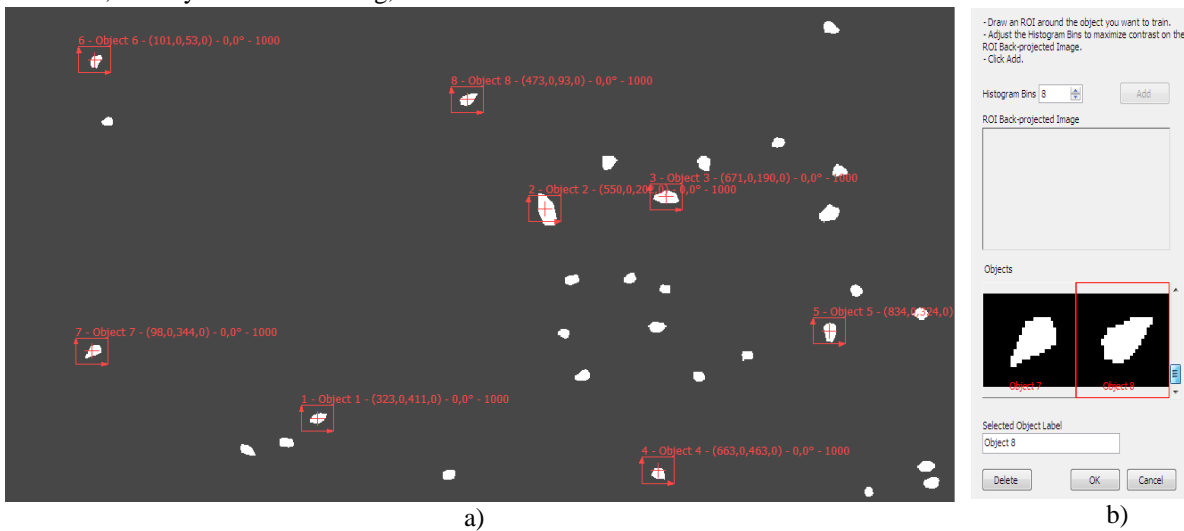


Figure 4 - Templates for two classes: Norm\_A and Fuzzy\_A

In NI Vision Assistant Interface a classification of 58 biological objects is made (Fig.3, a) on Norm\_A and Fuzzy\_A (Fig.3, b). Fig.4 shows the templates for two classes Norm\_A and Fuzzy\_A. Classification of particles is made by the method of Auto Threshold: Moments. Search of biological objects is done against a bright background with the Nearest Neighbor operation. The search method uses the metric - Sum. The result shows that the set of objects is divided by two classes with help of two types templates (Fig.4) - normal and fuzzy objects. And in the resulting window of image, every single object has its own index-number (Fig.3, a). A wide range of functional tools and qualitative detection of required fragments of objects images in the previous steps of the algorithm made it possible not only to classify biological objects more accurately, determine their parameters, shape, but also to evaluate their quantity. For comparison of the quality of the available in NI Vision Assistant tools we show in the next subsection the results obtained using Object tracking tool.

### 3.2 The frames pre-processing algorithm using tracking of multiple biological objects

Object tracking is a classical problem in computer vision and prerequisite solutions for many important tasks such as tracking of multiple biological objects. Such spermogram analysis is done on 200 frames. Both moving and slow-moving sperm is analyzed. Measuring sperm motility is determined by tracking operation with measuring the coordinates of sperm movement from frame to frame. Fig.5a, shows eight objects in the 106 frames with identification of their coordinates. Fig.5b shows templates for these objects. Tracking of biological objects is made from one frame to the next in a sequence of images. For tracking of biological objects Traditional Mean Shift algorithm with number of histogram bins - 8 and maximum iterations – 6 is used. The use of Object tracking tool makes it possible to determine the change of coordinates of these objects, to form and to process the coordinates with the ability to integrate to Excel. This enables clustering of multiple objects into groups with different moving speeds. This is an important parameter in the study of the sperm motility. Thus, we show that it is the use of NI Labview tools opens the possibility to build integrated environment still on stage of pre-selection of algorithms and designing of system, and makes it easy to go to the construction therein of ready-to-use projects. Some aspects of embedding the conveyor of preprocessing blocks on the basis of yet another final instrument, namely Pattern Matching, we will discuss in the next section.



Results ...	1	2	3	4	5	6	7	8
X Position	323,00000	550,00000	671,00000	663,00000	834,00000	101,00000	98,00000	473,00000
Y Position	411,00000	202,00000	190,00000	463,00000	324,00000	53,00000	344,00000	93,00000
Angle	0,000000	0,000000	0,000000	0,000000	0,000000	0,000000	0,000000	0,000000
Score	1000,00000	1000,00000	1000,00000	1000,00000	1000,00000	1000,00000	1000,00000	1000,00000

c)

Figure 5 – Eight objects in the 106 frames with the definition of their coordinates



### 3.3 The frames pre-processing algorithm using the Pattern Matching process for multiple biological objects

Let's consider the algorithm of frames pre-processing using the Pattern Matching process for multiple biological objects. In the Fig.6, the block diagram with graphical source code of the preprocessing algorithm is shown. And the Front Panel of the user interface built in NI Labview with controls and indicators is shown in Fig.7a. 200 frames of the investigated biological objects in \*.avi format are input from the Vision Acquisition source to the preprocessing algorithm, which is synthesized in the 1 Vision Assistant block. Vision Assistant 2 block uses the operation of Pattern Matching for multiple biological objects. The modules to count the number of objects in each frame, the sum total of all the frames and their average number are added to the project. The Fig.7a shows preprocessing by the algorithm of the biological objects and their number in each frame, the number of the frame processed, the total amount of the biological objects and their average numbers in frame. Fig.7b shows the results of processing of 200 frames using Pattern Matching process, and identification of 36 biological objects. Parameters for check and comparison of the template in the region of interest for multiple biological objects are shown.

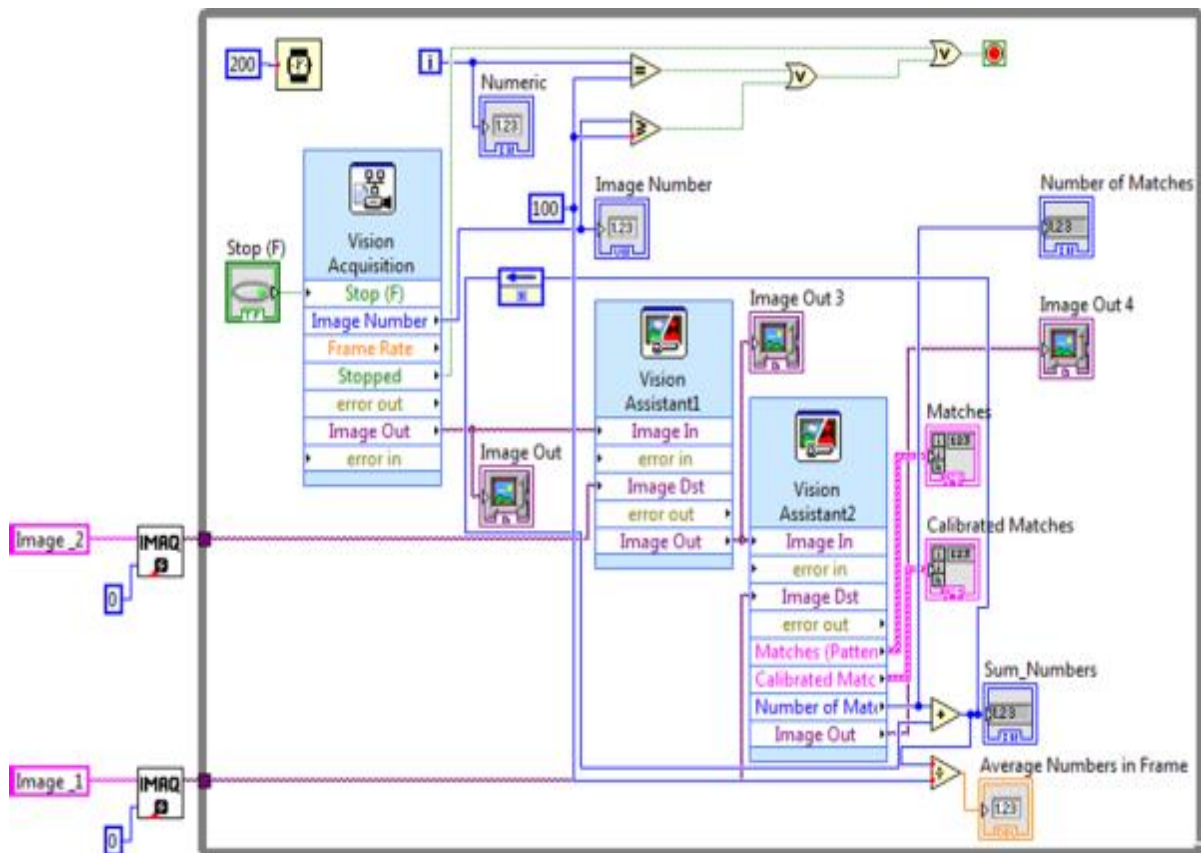


Figure 6 – The block diagram contains graphical source code of the preprocessing algorithm for detecting of biological objects

Similarly, to modules described in the sections 2-3, integral preprocessing modules are integrated into the finished project, or in their set, which sold a wide range of tasks and functional changes.

### 3.4 The frames pre-processing algorithm using the Geometric Matching process for multiple biological objects

The main indicators of sperm quality are the number of spermatozoons and their mobility. After preliminary image processing using NI Vision Assistant and Function Practical Analysis, we will count the number of spermatozoa and determine their basic parameters. Fig. 8 shows the input frame, the frame after preprocessing and the determination of mobility.

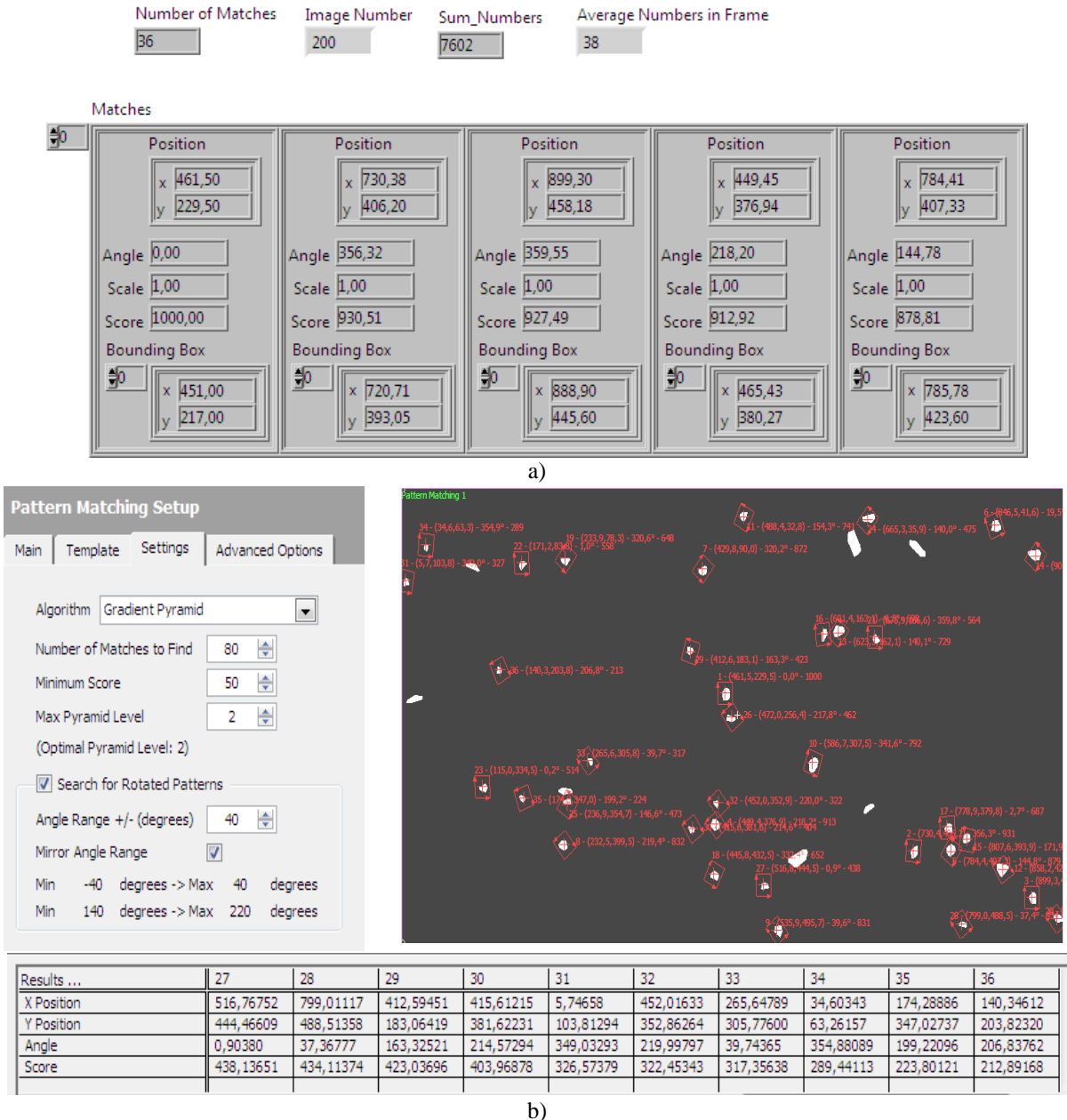
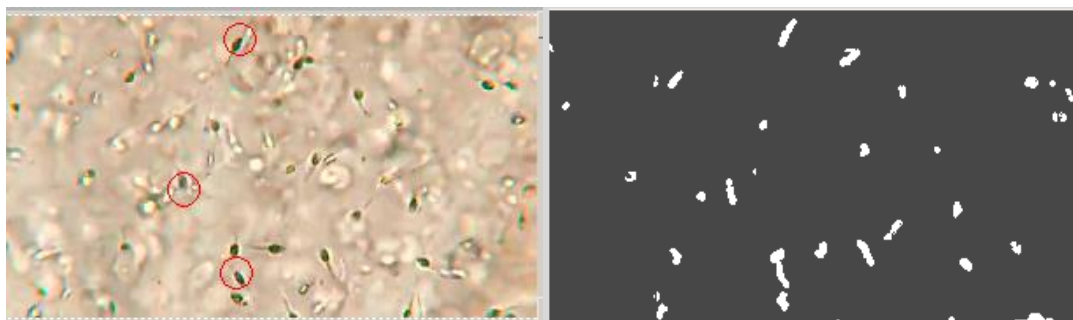


Figure 7 - The frames pre-processing algorithm using the Pattern Matching process for multiple biological objects. a) User interface built in NI Labview, the Front Panel with controls and indicators. b) Tool Pattern Matching: Checks the presence of a template in the entire or in a region of interest based on its intensity (Algorithm – Gradient Pyramid; Number of Matches to Find – 80; Minimum Score – 50; Max Pyramid Level – 2; Search for Rotated Patterns  $\pm 40$  degrees; Mirror Angle Range  $-40^\circ \div 40^\circ$ ,  $140^\circ \div 220^\circ$ )



a) Input frame with indicated best sperm b) Frame after mobility analysis  
Figure 8 - Simulation results of spermatozoon's analysis



In this example, the number of particles is 24, and the main parameters of the first five particles, namely the position and size, are shown in Table 1.

Table 1 – Parameters of the first 5 particles after analysis

Object #	Center of Mass X	Center of Mass Y	Bounding Rect Diagonal	Perimeter	Max Feret Diameter	Area	% Area/Image Area
1	529,57426	24,40594	16,97056	37,18959	14,42221	101	0,04384
2	430,05806	27,90323	22,80351	56,25136	19,31321	155	0,06727
3	120,67442	49,81395	12,80625	27,70276	10,19804	43	0,01866
4	220,13274	64,9469	18,02776	39,61967	15,52417	113	0,04905
5	618,75281	66,94382	15,81139	35,53274	13,60147	89	0,03863

To determine the motility of the spermatozoons, we perform a logical sum operation on the frames. As a result, during the motion of the sperms, their trajectories will be formed, according to which we can determine the number of progressively fast moving (class A), the number of progressively slow mobility (class B), the number of low active (class C) and fixed (class D).

#### 4.CONCLUSIONS

The tasks of real-time tracking of the dynamics of movement of various biological objects are researched in the work. Specificity of these objects, conditions of their visualization and model parameters strongly influence the choice of methods and algorithms, which are optimal for a specific task. Therefore, in this article, in order to automate the processes of adaptation algorithms of recognition - tracking, we suggest several frames pre-processing algorithms using NI Labview tools and Vision Assistant. Preprocessing included equalization of general background luminance of the image, elimination of high-frequency noise and different artifacts (highlighted areas, gaps, fractures, etc.) from the original image, contrasting, thresholding, binarization and other functional transformations. Several shortcomings have been identified by using the differential image shifted for one frame. Such a pre-processing operation is made to remove not moving objects or static elements in the image. For further reconstruction, the moving objects in the image are enhanced by sharpening and processed by algorithms. At that while preprocessing it can be seen that moving objects in the image are duplicated due to the difference of frames. Therefore, the counting results are inaccurate. Furthermore, both in the first and second versions of the algorithm preprocessing result in many fragments, which are not identified as biological objects. The paper shows the possibility of building frames preprocessing algorithms in the stream for a number of biological objects. Modern computer processing and image analysis techniques will improve the quantity and quality analysis of the patient's spermogram.

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## НОВІ МЕТОДИ ПОПЕРЕДНЬОЇ ОБРОБКИ ЗОБРАЖЕНЬ ДЛЯ ВІДСТЕЖЕННЯ ПЕРЕСУВАННЯ РІЗНИХ БІОЛОГІЧНИХ ОБ'ЄКТІВ

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