

## Bacterial Monitoring of Drinking Water Sources Using Immunofluorescence technique, Image Processing Software and Web-based Data Visualisation

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**Abstract:** European organizations involved in updating water management regulations (WHO, OECD) insist in recent years on the need to improve methods for assessing and managing microbiological , physical and chemical safety of drinking water . Data obtained as a result of water quality monitoring should become a starting point for risk management actions.

The paper is mainly focused on presenting a highly efficient software application used to implement a more rapid microbiological method to detect pathogenic bacteria for human health, based on bacterial specific antibody-antigen interaction (Ag-Ab) , namely immunofluorescence technique , and microscopic digital image processing. Laboratory tests have proven that the proposed solution is reliable, stable and time-efficient for preventing microbiological contamination of drinking water.

This application software, the method and related instrumentation that the paper presents are parts of a demonstrative modular model which monitors water quality. The first module consists of instrumentation and software that serve a methodology applied to detect pathogenic bacteria in drinking water samples. The second module performs data transmission and storage in a relational database and enables real-time data visualization.

**Keywords:** water quality, digital image processing, digital filters, image segmentation.

### 1. INTRODUCTION

Water resources management is a global concern , not only in terms of their quantity, but also of their quality, as water is increasingly affected by various forms of pollution. Drinking water policy has been regulated by Directive 98/83 / EC as amended by the Commission Directive (EU) 2015/1787 on the quality of water intended for human consumption. The objective is to protect human health from the adverse effects of any contamination of water intended for human consumption. These regulations have been transposed into Romanian legislation by Law no. 458/2002, republished in 2011 and amended/supplemented by Ordinance No. 22/ 2017.

In recent years, automatic software processing of microscopic images of drinking water samples proves useful for water quality diagnosis. It can reliably detect the presence of bacteria and helps on their classification.

Operational monitoring of drinking water quality involves regular controlling of physical-chemical parameters (free residual chlorine, colour, turbidity, taste, odour, hydrogen ion concentration, conductivity, oxidisability, ammonium, nitrates, nitrite) and microbiological parameters (Colony count 22 °C and 37 °C, *Escherichia coli*, coli form bacteria, *Pseudomonas aeruginosa*, Enterococci).

Current methods for bacterial detection from drinking water are based on a bacterial concentration step by using a membrane filtration technique, followed by incubation on a selective bacterial growth medium. The colonies forming units (CFU) are then counted. These traditional microbiological methods are time consuming, the results being available after 48-72 hours. Advantages of an ideal early (on line) warning system over traditional detection systems based on bacterial growth would include a fast and specific bacterial detection to decrease the response time of action and the false-positive or negative results (Samendra et al., 2014).

The new method proposed to be used for the determination of accidental microbiological contamination of water with pathogenic bacteria is based on immunofluorescence detection using microscopy. The immunofluorescence technique uses antibody-antigen (Ag-Ab) interaction. The fluorescent dyes (fluorophores) have well-defined emission spectra which selectively identifies the analyzed bacteria.

The method that the paper describes uses software processing of digital microscopic images of drinking water samples that were treated using immunofluorescence technique.

The software application automatically creates a microbiological report on bacteria detection in the analyzed

water sample. The reports can be sent to dedicated web servers for storage and visualization on web pages.

## 2. RELATED WORKS

Paper (Posnicek et al., 2016) presents the development of a sensor system for a fast automated detection and quantification of *E. coli* bacteria in drinking water. *E. coli* bacteria in the water sample are captured on filter membrane. Reagents with the specific DNA probes are later added for fluorescence labeling. The digital images delivered by fluorescence microscopy are processed with a software applications for bacteria detection.

A method to analyze fluorescent microscopy images of *Pseudomonas aeruginosa* is presented in paper (Woźniak et al., 2018). Automated evaluation of fluorescence microscopy images by software processing is used to identify and count bacteria in dead and alive stadium.

Paper (Gross et al., 2010) presents an algorithms that implement a semi-automatic image segmentation using color and spatial relationships of user-selected foreground object pixels. Transmitted bright field and epifluorescence microcopies are used as sources for digital images.

The paper (Fernandez-Canque et al., 2008) presents a technique which is using together Machine Vision, Normarski Differential Interface Contrast (DIC), fluorescence microscopy, Fluorescein Isothiocyanate (FITC) and UV filters to detect the wall of cryptosporidium oocyst. Using a blue-fluorescent DNA, the sporozite nuclei are highlighted.

Paper (Gunda et al., 2019) presents a method for water quality monitoring using a deep convolutional neural networks (CNN) based on the fact that water specimens have a pink red color when *E. coli* is present. Paper (Chang et al., 2007) presents a method for image segmentation using voting, Voronoi tessellation, and level set methods. The method has been applied to a dataset produced from cell based fluorescence assays.

Paper (Du et al., 2010) presents and evaluates the performance of several algorithms for image segmentation, namely k-means clustering, Expectation Maximization (EM) algorithm, The Gaussian mixture model (GMM) and Otsu's method.

An extended review of image acquisition (including bright field and fluorescence) and image processing techniques for wastewater treatment is presented in paper (Shanono et al., 2018). Paper (Velusamy, V., et al., 2010) presents a comprehensive literature survey in the area of microbial pathogen detection, including bacteria detection conventional and standard methods such as culture and colony counting methods and immunology-based methods.

Paper (Sorea et al., 2016) presents the development of an electronic module that can act as a node in an Wireless Sensor Network (WSN). Paper (Stefan et al., 2018) presents the implementation of a WaterML Web services that offers controlled access to the resources of a heterogeneous SCADA monitoring and control structure for water monitoring.

## 3. OVERVIEW OF THE SOLUTION AND ITS NOVELTY

### 3.1 System architecture and novelty

The demonstrative modular model for bacterial monitoring of water sources contains two modules (See Figure 1).

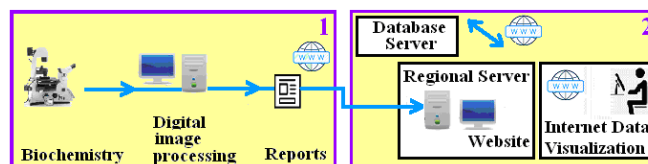


Fig. 1. The system architecture of demonstrative modular model for bacterial monitoring of water sources.

The first module consists of instrumentation and software application that serves a biochemical methodology (immunofluorescence technique) for bacteria detection. The software uses high-performance methods to automatically process microscopic images in order to create reports on detection the presence of pathogenic bacteria in drinking water. The second module performs reports transmission towards regional servers and data storage using a relational database located on a database server. The microbiological reports are visualized through the Internet by accessing the websites of regional water operators.

The paper presents several new scientific elements. Immunofluorescence technique is a new, alternative and faster detection method. It has a high specificity, reliability and accuracy for bacteria detection. It offers qualitative results regarding the type of target microorganisms and quantitative results regarding the number of target microorganisms. By the fact that the results are obtained much faster than traditional methods, it is suitable and efficient for bacteria detection when accidental microbiological contamination of drinking water sources occurs. The paper presents a particular case of image analysis and image processing software regarding an automatic procedure for detecting the presence of *Escherichia coli* in microscopic images. This procedure can be applied with a high degree of similarity for detection of other types of bacteria that may exist in drinking water. The software can be further extended with new modules for in depth water quality analysis without interfering with the existing ones. The software application running on regional server is a new tool for monitoring the biological decontamination at region level. In addition to these microbiological reports, the population has free access to view water quality reports regularly uploaded by regional water operators on their sites.

The modular demonstration module can be easily integrated into a superior water quality monitoring system that also targets physical and chemical parameter monitoring. The conceptual model describing the augmentation of the above demonstration model is presented in paper (Atanasescu et al., 2017).

### 3.2 The first module of the demonstrative modular model

The first module consists of instrumentation and software application that serves a biochemical methodology (immunofluorescence technique) for bacteria detection.

The modern methodology applied for the detection of pathogenic bacteria *Escherichia coli* in drinking water is based on antibody-antigen (Ag-Ab) interaction, whereby a particular antibody is bound to specific antigen. Bacterial growth of gram-negative bacteria strains *Escherichia coli* (ATCC 25922) was followed by bacterial labeling using Polyclonal antibody anti-E.coli tagged with FITC . The microscopic detection of fluorescent bacteria was performed using both Leica DMi8 inverted microscope at a 63X magnification and confocal microscope Leica TCS SPE DM6 at a 100x magnification. The samples are observed in both fluorescence and transmitted light, respectively.

The image observed in transmitted light is called "brightness image" while the image observed in fluorescence is called "fluorescence image". This pair of digital images of the same water sample, delivered by the microscopes, is processed by a dedicated software application in order to detect the presence of pathogenic bacteria. The software application automatically generates microbiological reports in form of files having .PDF format.

### 3.3 The second module of the demonstrative modular model

The microbiological reports are transmitted to regional servers and are assigned to a specific station where water samples is taken for bacterial monitoring. Every station is located on the Romanian territory. The microbiological reports identification items are stored on database server. A client-server software application running on regional server allows data visualizations using Internet browsers. Users must have proper credentials (User name /Password) for viewing the microbiological reports. People can freely access the water quality reports, which are periodically uploaded by regional water operators.

## 4. GREY IMAGE PROCESSING METHODS

### 4.1 Grey image histogram

Brightness image and fluorescence image delivered by microscopes are color images. Due to the fact that Polyclonal antibody anti-E.coli tagged with FITC is emitting in green light spectrum, the fluorescence image is converted to a grey image by extracting green color plane. Grey images contain a single color plane.

The image histogram is the graphical representation of pixel intensity distribution in an image. The histogram gives a simple yet useful information about image scaling within the whole range of grey levels. A good quality digital image should use the most of the available grey levels.

Mathematically, the grey image histogram is a function  $H$  defined on the discrete domain  $[0, \dots, 255]$  of the pixel values .

$$H(k) = n_k \quad (1)$$

where  $k$  is the grey level and  $n_k$  is the number of pixel with the value  $k$ . A histogram can have a linear or a logarithmic vertical axis (see Figure 2).

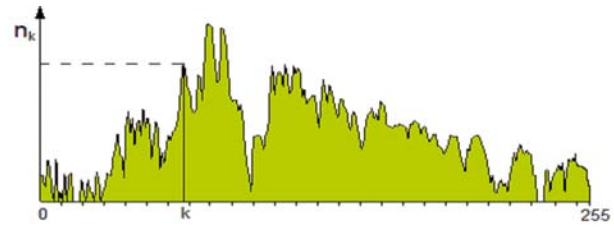


Fig. 2. Histogram of a grey image (linear vertical axis).

A grey image whose histogram has only two obvious peaks (a bimodal histogram) makes it easy to determine the objects contained in the image. The local minimum between the two peaks or the second peak may be used as the starting values for optimal detection of the objects in images . Otherwise, a further histogram processing is needed.

*Histogram equalization* improves grey image contrast. The existing frequencies in the grey image are uniformly distributed throughout the whole frequency range. For an  $M \times N$  grey image containing  $L$  levels of grey , histogram equalization is performed by applying the following formula:

$$h(v) = \text{round} \left( \frac{CDF(v) - CDF_{\min}}{M \times N - CDF_{\min}} * (L - 1) \right) \quad (2)$$

where  $CDF(v)$  is the cumulative distribution function of the image for  $v$  frequency (grey level) and  $CDF_{\min}$  is the minimum value of cumulative distribution function for the whole image (the value should be greater than zero).

*Linear filters* implement discrete two-dimensional convolution. Discrete convolution calculates a weighted average of pixels contained in a digital image  $F$  using a specified convolution kernel  $G$  ( an array). If image  $F$  has  $M \times N$  dimensions, then pixels of  $H$  image are obtained as follows:

$$H(i, j) = \sum_{m=0}^{M-1} \sum_{n=0}^{N-1} F(m, n) * G(i - m, j - n) \quad (3)$$

*Median Filter* eliminates the noise in the grey image and maintains the shape of compact areas. This filter uses a rectangular  $X \times Y$  window and implements the following algorithm:

- 1.The rectangular window is positioned in the upper left corner of the grey image being processed.
- 2.Pixels in the image covered by the rectangular windows are selected and sorted in ascending order of their values. The middle value is selected.
- 3.The value of the pixel located in the center of rectangular window is replaced in the original image by the value obtained in step 2. Steps 2, 3 are repeated until the window has passed the entire grey image through successive shifts.

### 4.2 Obtaining a binary image from grey image

A binary image has two components : background and foreground . The components can be swapped through an image reversal operation. Binary image analysis is usually done in the foreground. Elements to be analyzed are called

“generic objects” or blobs and represent distinct areas in the foreground.

Two approaches can be used in order to obtain a binary image from a grey image: *local threshold* and *global threshold*. An exhaustive mathematical review of different image threshold techniques is described in paper (Sezgin et al. 2004). Some aspects of obtaining a binary image by threshold techniques are presented as follows.

*Local threshold* takes into consideration a neighborhood around each pixel in a grey image. Calculation is carried out in a rectangular window which moves then over the entire grey image.

For a neighborhood of  $M \times N$  pixel in an image, where pixel having  $(i,j)$  coordinates has  $I(i,j)$  value, local mean is:

$$m(i, j) = \frac{1}{M * N} \sum_{i=0}^{M-1} \sum_{j=0}^{N-1} I(i, j) \quad (4)$$

and variance is

$$\sigma^2(i, j) = \frac{1}{M * N} \sum_{i=0}^{M-1} \sum_{j=0}^{N-1} [I(i, j) - m]^2 \quad (5)$$

Local threshold methods are as follows:

a) *Niblack algorithm*

$$\text{Threshold}(i, j) = m(i, j) + k * \sigma(i, j) \quad (6)$$

where  $k \in [0..1]$

If  $I(i,j) > \text{Threshold}(i,j)$

then  $I(i,j)$  belongs to the foreground

else  $I(i,j)$  is belongs to the background.

b) *Background correction*

The background3d-corrected image is computed as follows:

$$B(i, j) = I(i, j) - m(i, j) \quad (7)$$

in order to eliminate non-uniform lighting objects, then a global threshold is applied.

*Global threshold* enforces a unique threshold value over the entire grey image. The *manual choice* of a correct value for global threshold may be subjective and can be used merely for visual testing approaches.

*Automatic threshold methods* take into consideration an image with  $N$  grey levels ( $N=256$ ) where  $h(i)$  represents the number of pixels on grey level  $i$ . Some automatic threshold methods are presented as follows.

A) *Clustering*

The  $k$  value of the binary threshold is calculated so that the condition

$$\frac{\mu_1 + \mu_2}{2} = k \quad (8)$$

is true, where  $\mu_1$  is the arithmetic mean of the pixels values for values less than  $k$ , and  $\mu_2$  is the arithmetic mean of the pixels values for values greater than  $k$ .

B) *Entropy*

The probability of occurrence of grey level  $i$  is

$$p(i) = \frac{h(i)}{\sum_{i=0}^{N-1} h(i)} \quad (9)$$

For a threshold value  $k$ , entropy of the background is:

$$H_b = - \sum_{i=0}^k p(i) * \log_2 p(i) \quad (10)$$

and entropy of the foreground (white pixels) is:

$$H_w = - \sum_{i=k+1}^{N-1} p(i) * \log_2 p(i) \quad (11)$$

Optimal binary threshold  $k$  is the value that maximizes  $H_w + H_b$ .

C) *InterVariance (Otsu's method)*

Using the probability of occurrence of grey level described above, optimal binary threshold value  $k$  maximizes:

$$\sigma_b^2(k) = \omega_0(k) * \omega_1(k) * [\mu_0(k) - \mu_1(k)]^2 \quad (12)$$

where:

$$\omega_0(k) = \sum_{i=0}^{k-1} p(i) \quad (13)$$

$$\omega_1(k) = \sum_{i=k}^{N-1} p(i) \quad (14)$$

$$\mu_0(k) = \frac{\sum_{i=0}^{k-1} i * p(i)}{\omega_0(k)} \quad (15)$$

$$\mu_1(k) = \frac{\sum_{i=k}^{N-1} i * p(i)}{\omega_1(k)} \quad (16)$$

D) *Metric*

The optimal binary threshold value  $k$  is calculated so that the expression:

$$\sum_{i=0}^k h(i) * |(i - \mu_1)| + \sum_{i=k+1}^{N-1} h(i) * |(i - \mu_2)| \quad (17)$$

should be minimized, where  $\mu_1$  is the arithmetic mean of the value of all pixels having a value less than  $k$ , and  $\mu_2$  is the arithmetic mean of the value of all pixels having a value greater than  $k$ .

E) Moments

The binary threshold value is calculated so that the value of moments of the grey image are preserved in the binary image. Moment of k-order of an image is calculated as follows:

$$m_k = \frac{1}{n} \sum_{i=0}^{N-1} i^k * h(i) \tag{18}$$

5 ALGORITHMS FOR DETECTING BACTERIA USING DIGITAL IMAGES PROCESSING

5.1 Image processing workflow

The image processing workflow is presented in Figure 3.

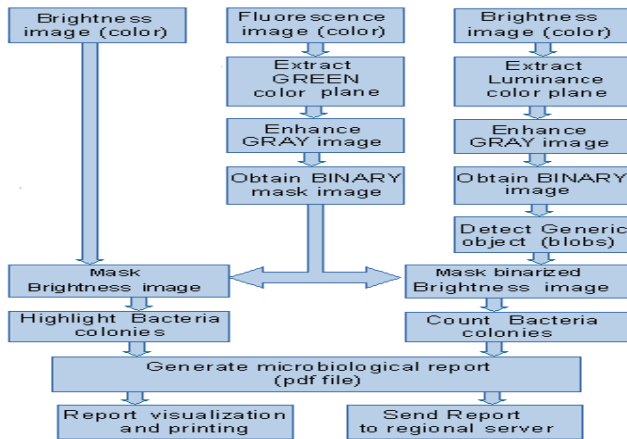


Fig. 3. Image processing workflow.

5.2 Fluorescence Image Processing

The processing pipeline is described as follows:

STEP 1: Load Fluorescence Image

The fluorescence image obtained as a result of immunofluorescence techniques is loaded from a graphical-type format file. The digital image contains three color planes. Image enhancement may be needed.

STEP 2: Green Plane extraction

According to the fluorescent image acquisition methodology, the Green plane will be extracted from the original image. The grey image obtained contains, besides useful information, many artifacts (levels of grey that do not belong to useful objects in the consequent binary image). These artifacts must be removed.

STEP 3: Histogram equalization

Histogram equalization is applied to the greyscale image obtained in STEP2. The contrast in digital image is increased.

STEP 4: Image threshold

The most important step is obtaining the binary image. The grey histogram indicates several local peaks (See Figure 4)

The first local peak corresponds to the grey levels in the image that does not contain useful information. The image threshold value was chosen as the value of the second local peak incremented by 1.

STEP 3 and 4 VARIANT: An alternative to STEP 3 and 4 is to use a local background correction filter for "light objects" identification. The filter dimensions were chosen as follows:

$$H = \text{Image height}, W = (\text{Image Width} / 4)$$



Fig. 4. Histogram of grey image.

STEP 5: Median Filter

The image still contains artifacts. The first step in artifacts rejection is applying a median filter.

STEP 6: Artifacts rejection

The artifact rejection continues with the removal of point-shaped objects, so called "small objects".

STEP 7: Fill Holes

Zero value pixels (if any) located inside the detected objects will have their value changed to 1.

STEP 8: Image Dilation

The image is dilated by 1 pixel to recover the scattered pixels from the outline of the useful objects obtained in STEP 4.

STEP 9: Count/save white objects

The image containing white foreground objects is stored in a graphical file named "fluorescence mask with blobs".

STEP 10: Outline contours of white objects

The contours of foreground objects are outlined. The image is stored in a file named "fluorescence mask with contours".

5.3 Brightness Image Processing

The brightness image of the water is processed using the fluorescence mask image in order to highlight the areas containing single bacteria or colonies of bacteria. The sum of these areas is used to calculate the density of bacteria presence in the whole image. Another important criterion is the number of isolated bacteria and bacteria colonies detected. That number can be used when different bacteria are present in the same water sample. Below there is a description of pipeline processing:

STEP 11: Load brightness image

The brightness image is uploaded from its graphical file.

STEP 12: Mask brightness image with "fluorescence mask with blobs" image

A logical AND operation between the brightness image and the "fluorescence mask with blobs" image is carried out. This new image shows only areas from brightness image that have

a correspondent object in “fluorescence mask with blobs” image.

*STEP 13: Superimpose “fluorescence mask with blobs” image over brightness image*

Objects detected in “fluorescence mask with blobs” image are superimposed over the brightness image. The selected areas are colored in green.

*STEP 14 Superimpose “fluorescence mask with contours” image over brightness image*

This is another way to highlight the colonies of bacteria which may exist in the brightness image. Contours contained in the “fluorescence mask with contours” image are displayed over the brightness image.

*STEP 15: Extract Luminance plane from brightness image*  
Conversion from RGB color space to luminance plane L

(a grey image) is performed with the following formula:

$$Y = 0.2126 * R + 0.7152 * G + 0.0722 * B \quad (19)$$

*STEP 16: Discrete convolution*

The filter used in this step uses a 9x9 kernel. All kernel's values are equal to -1, except for the value placed in the middle of the kernel which has value 100. As a result of applying the kernel dark grey pixels are highlighted.

*STEP 17: Auto threshold of grey image*

The grey image is converted to binary using an auto threshold METRIC criterion. The white areas in binary image correspond to single bacteria or colonies of bacteria .

#### 5.4 Bacteria counting using binary erosion

*STEP 18: Separate Objects*

If there are very thin connections (pixel size) between the objects, a separation of the connected objects is obtained through a binary erosion.

*STEP 19: Fill Holes*

Pixels having 0 value and located inside the detected objects will have their value changed to 1.

*STEP 20: Apply image mask and bacteria counting*

The binary image obtained at STEP 19 is masked with “fluorescence mask with blobs” image obtained at STEP 9. This is very important because it rejects miscellaneous artifacts that are not related to any bacteria.

#### 5.5. Bacteria counting using segmentation

A second approach to bacteria counting is using segmentation technique that partitions an image into segments (built around each generic white object) that are identified and labeled. Segmentation partitions the source image into regions centered on objects in the image that do not overlap and have a maximum area. The segmentation algorithm is described as follows:

a) the image obtained at STEP17 is partitioned into non-overlapping regions. Each region contains an unique white object

b) the border lines between adjacent regions are detected using a Canny filter

c) these lines are superimposed over the luminance plane of the brightness image

d) a background threshold method is applied followed by applying a binary image mask namely “fluorescence mask with blobs” obtained at STEP9.

The result is a binary image containing white bacteria colonies.

#### 5.6. Bacteria counting using watershed transform

A third approach to bacteria counting is using watershed transform. Watershed transform is used to automatically separate the touching white object of a binary image. It is using a distance map of a binary image that may be obtained as follows: each pixel of each white object of the binary image is assigned a numerical value from 0 to 255 as the smallest distance from the object border. A value greater than 255 it is replaced with value 255.

The algorithm used to count bacteria in brightness image using watershed transform is described below:

a) a grey image is obtained by selecting the luminance plane from the brightness image

b) a binary image is created applying a METRIC auto threshold on the grey image; the binary image is improved using binary morphology (artifacts rejection, fill holes)

c) a grey scale image is obtained from the binary image using distance map function

d) watershed transform using distance map is performed; separation lines between objects are obtained and objects are counted

e) separation lines are superimposed over the brightness image.

## 6. SOFTWARE IMPLEMENTATION

### 6.1. Software application for image processing

The software application for digital image processing is written in C++ using the Microsoft Visual C++ development environment , MFC paradigm and NI Vision software library. A screen capture of the application software is presented in Figure 5.

The user screen contains two windows. The left window is used to display images (colour, grey and binary) obtained through successive digital processing. It offers the possibility to scroll its content when the image is larger than the window. The position and area of every white object detected in fluorescence image are displayed into a list placed beneath the left window. The right window displays the compressed version of image being processed, the fluorescence image

histogram, the numerical characteristics of the histogram and two sliders to manually set the histogram threshold for testing purposes. There is also a predefined window for displaying a palette of software tools to draw a line, a rectangle and to show pixels position and value.

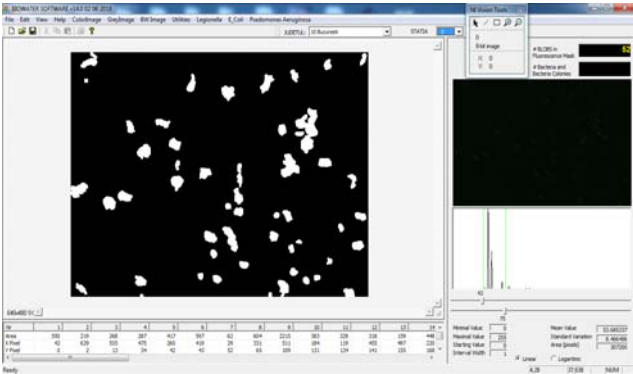


Fig. 5. Screen capture of software application.

Image processing functions are applied to color, grey and binary images and can be grouped into sequential processing units. Below are two examples of software functions:

```
void CBioWaterDoc::BW_SeparateObjects()
{
    int iResult=-1;
    int pKernel[9] = {1,1,1,1,1,1,1,1,1};
    StructuringElement structElem;
    structElem.matrixCols = 3; structElem.matrixRows = 3;
    structElem.hexa = FALSE; structElem.kernel = pKernel;
    iResult=imqSeparation(Image_1,Image_1,1,&structElem);
    if (iResult==0) {
        char* err = imqGetErrorText(imqGetLastError());
        MessageBox(NULL,err, "Error Separation", MB_OK);
        imqDispose(err);}
    UpdateAllViews(NULL);}}
```

```
void CBioWaterDoc::EColi_Count()
{
    //Bacteria counting
    numParticles_EC=-1;
    CString mszFileName;
    ColorPlaneExtraction(IMAQ_HSL ,3);//STEP 15
    Init_Convolution_Kernel(100.0,-1.0,9);//STEP 16
    Grey_Convolution(9);
    // Inverts the pixel intensities of the image.
    imqInverse(Image_8, Image_8, NULL);
```

```
imqAutoThreshold2(Image_1,Image_8,2,
    IMAQ_THRESH_METRIC, NULL);//STEP 17
    BW_SeparateObjects(); //STEP 18
    BW_FillHoles(); //STEP 19
    mszFileName.Format("%s\\Mask_EColi_BLOB.bmp"
    ,GetMaskDirectory());
    bSuccess=MaskInitialImage(Image_1,mszFileName,
    false,false); //STEP 20
    numParticles_EC =CountParticles();
```

The result of image processing functions is a microbiological reports generation having .PDF format. The microbiological reports are saved by default into a local database. The user can select any report from database for displaying it and/or sending it to the regional server.

### 6.2 Software for data transmission, storage and visualisation

The PDF file containing the microbiological report and its identification parameters are encapsulated into a POST request that send data to the regional server to which the station is assigned. Data integrity is ensured by transmission through the TCP / IP protocol.

The client-server paradigm is widely used for creating applications that handle a large amount of data having complex interaction relationships that require advanced processing algorithms.

A relational database is deployed and maintained on the database server. The database stores several predefined query and several tables as follows:

- identification items of microbiological reports sent by image processing application
- identification items of other water quality reports that are periodically uploaded by regional water operators
- identification items for stations, users and their credentials, regional water operators.

The client application connects to the database server based on credentials (user/ password) . Free access is also permitted for periodic water quality reports visualization. The client application can issue to the database server either a predefined database query or a user-defined database query using filters. The server processes the query and delivers the results. Data processed by database server are received and visualized on website dedicated pages through Client Interface which is an Internet browser.

The database server is deployed on a PC running LINUX Ubuntu Server 16.04.4 LTS and MySQL Server 5.7.22.

The client applications are deployed on several PC's running LINUX Ubuntu Server 16.04.4 LTS and Apache HTTP Server. In order to connect the web server (Apache HTTP Server) to the database server (MySQL), a software module

written PHP language is used. The PHP module is processed by database server and delivers the Html content to web server (client application).

7. EXPERIMENTAL RESULTS OF IMAGE PROCESSING

7.1. Brightness image

The brightness image used to test the presence of E.coli bacteria in water samples is shown in Figure 6. In order to increase the visibility of the results, a Region Of Interest (ROI) was selected from the initial image.

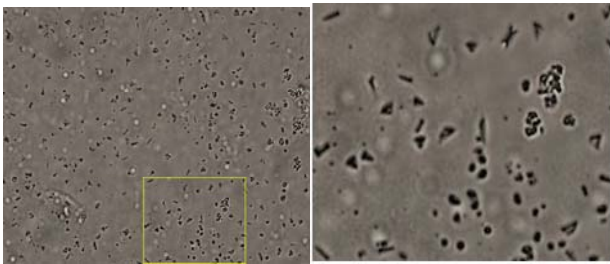


Fig. 6. Left-Brightness Image Right- Region Of Interest.

7.2. Enhancement of fluorescence image

Figure 7 below shows the ROI of the fluorescence image that is associated with the ROI of the brightness image. The histogram of the color image reveals the fact that pixels have low values. Image enhancement is required. The image is enhanced by multiplying all pixels values by 5. Values that exceed 255 are replaced with the value 255.

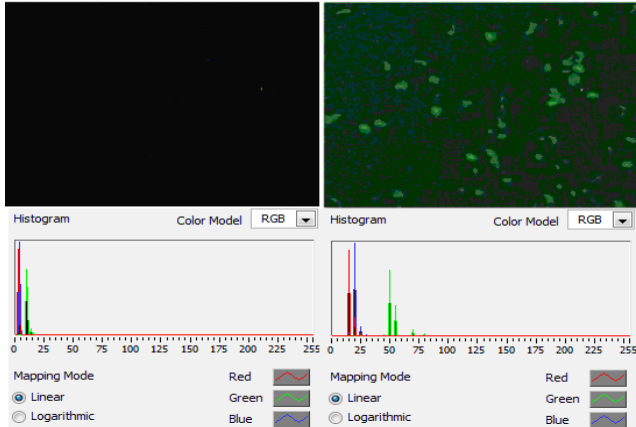


Fig. 7. Enhancement of Fluorescence Image.

7.3 Binary mask obtained from Fluorescence image

The binary masks are obtained as a result of the processing of the green plane of the fluorescence image. (See Figure 8).

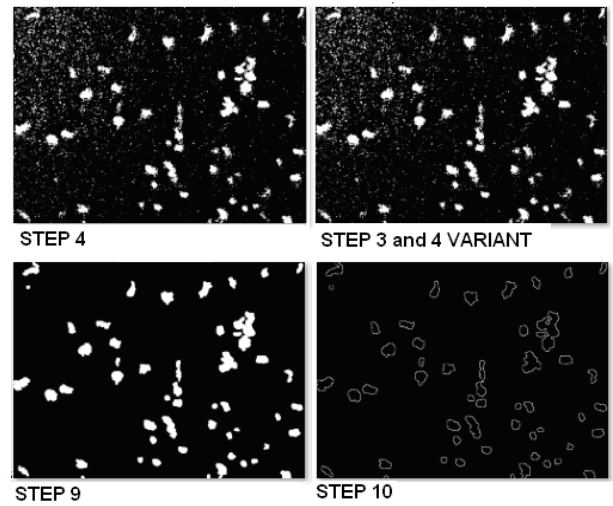
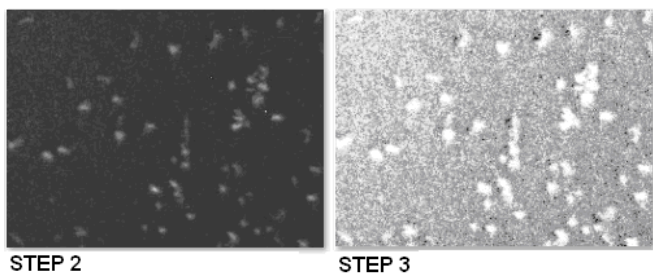


Fig. 8. Processing of the Fluorescence Image.

7.4 Brightness image processing

The results for bacteria highlight in brightness image are shown in Figure 9. Background is changed to white at STEP12 for better visualization.

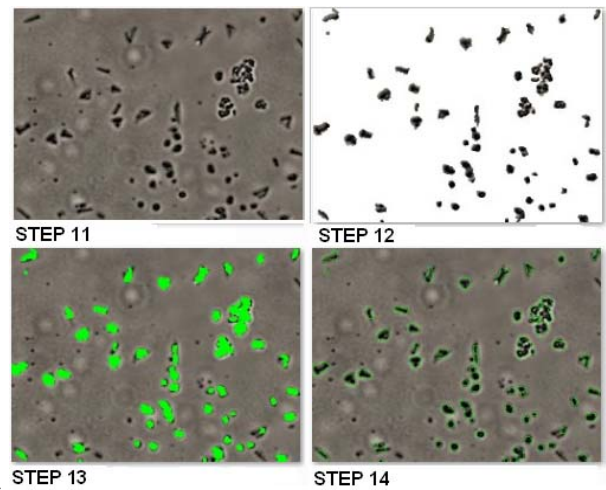


Fig. 9. Brightness Image containing E.coli bacteria.

The results of bacteria counting using binary erosion are shown in Figure 10.

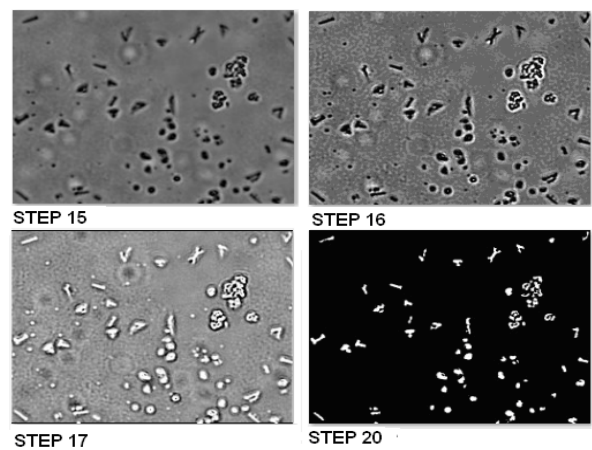


Fig. 10. E.coli bacteria counting using binary erosion.



The results of bacteria counting using watershed transform is depicted in Figure 11.

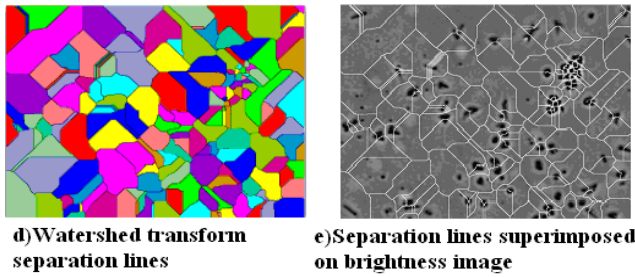


Fig. 11. E.coli bacteria counting using watershed transform.

The result of bacteria colonies counting using segmentation is depicted in Figure 12.

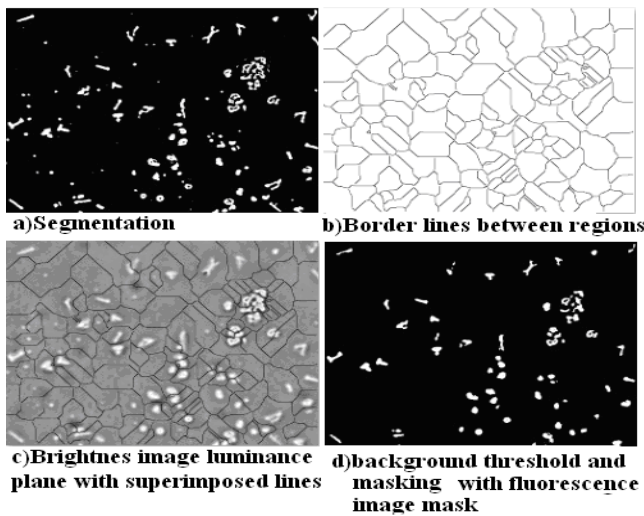


Fig.12. E.coli bacteria counting using segmentation

Fewer bacteria colonies are detected using segmentation method instead of binary erosion. The well known side effect of watershed transform is over-segmentation which artificially increases the number of detected bacteria colonies.

7.5 Microbiological report

The microbiologic report indicates the presence of E.coli bacteria as a result of image processing (See Figure 13). Bacterial identification and bacteria counting is carried out only for the selected ROI from the original image.

The report contains the brightness microscopic image having contours superimposed (the contours are obtained from “fluorescence mask with contours” image) and numerical information regarding both brightness image and fluorescence image.

Regarding to the brightness image, the image size and the cumulative number of isolated bacteria or colonies of bacteria are listed. As for the fluorescence image, the report contains the number of white blobs and their cumulative area, as well as the percent of white blobs in the whole image.

Computer operator can add observations, if needed.

In order to view on Internet a microbiological report of a water source located in a territorial station, the user must

firstly access the main page of the project website. Then the user selects the region (county) , the station and provide access credentials. Lastly, the user identifies the microbiological report using its type and date (See Fig. 14).

Detection Report nb. 7

County: Braila Station: 1 Date: 16-06-2018 Hour : 10:42:32

Detection of E.Coli

Image size: X=640 pixels Y=480 pixels Total Number of Pixels=307200

	Bacteria presence	Number of colonies in bright image	Number of areas in fluorescence image	Cumulative area in fluorescence image	Ratio of detected areas to the analyzed image
E. Coli	PREZENT	97	52	19986	6.506 %

OBSERVATIONS	
E. Coli	Bacteria presence single bacterium and bacteria colonies

Fig. 13. A microbiological report for E.coli detection



a)Map of Romania with a random station distribution



b)Web page to display the list of microbiological reports associated with a station

Raport masurare nr. 5

Stati: Braila Station: 1 Date: 29-06-2018 Ora: 07:52:11

Detectare prezenta E.Coli

Dimensiuni imagine: X=1920 pixeli Y=1420 pixeli Nr Total Pixeli=2726400

	Prezenta bacterii	Numarul colonilor in imaginea microscopica	Numarul zone in imaginea fluorescenta	Suprafata totala in imaginea fluorescenta	Procentajul suprafetei in imaginea fluorescenta
E. Coli	PREZENT	472	294	116611	4.383 %

OBSERVATII	
E. Coli	Bacterii izolate sau grupate in colonii

c)Viewing a microbiological report on a Web page

Fig. 14. Viewing microbiological report on the Website

## 8. CONCLUSIONS

The main goal of the research activities and experiments that the paper presents is to implement a rapid and direct method to evaluate the drinking water quality, especially for detection of pathogenic bacteria that may be present in drinking water samples.

Using automatic threshold segmentation of Green colour plane extracted from fluorescence image may produce artifacts. Several scenarios have been implemented for artifacts removal using proper digital filters and morphological operations. The binary image that is obtained is used to mask the original brightness image for artifacts removal and proper bacteria detection. A microbiological report is generated about presence/absence of E.coli bacteria in water samples.

The obtained results prove that image processing software together with immunological detection of antigenic structures associated with target microorganisms (immunofluorescence technique) can be used as a good diagnostic tools in rapid detection of target bacteria.

Future work will be focused on detecting different types of bacteria that may coexist in the same water sample.

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