Micro-Organisms Detection in Drinking Water Using Image Processing

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Abstract— **The work presented in this paper uses a novel approach into the detection and identification of micro-organism oocysts, based on Machine Vision technology applied to drinking water. Our new concept of detection uses image processing which allows detailed inspection of parasite morphology to nanometer dimensions making the detection more reliable than existing manual methods. Combining Normarski Differential Interface Contras (DIC) and florescence microscopy using FITC and UV filters the system provides a reliable detection of micro-organisms with a considerable reduction in time, cost and subjectivity over the current labour intensive time consuming manual method.**

I. INTRODUCTION

It is evident that hundreds of micro-organism could be concentrated in a single drop of a particular water sample. Micro-organism in drinking water has been widely recognized as a serious cause of concern, with a very large number of waterborne infections caused by its oocysts. The problem is increased because a small dose of some micro-organism can produce an outbreak for some micro-organisms conventional water treatment process, including chemical disinfection, cannot guarantee to remove or destroy them completely. Regulatory bodies from all over the world acknowledge the continuous monitoring of water sources for micro-organism as imperative. Many requirements, rules and regulations are in place to attempt to address the control of micro-organism which threatens the safety of drinking water. The current European Union drinking water directive [1] requires that the drinking water is free from micro-organisms to a certain level that does not constitute a potential danger to human health. In order to assure the implementation of these regulations some tasks need to be covered as they are briefly presented in the block diagram presented in figure 1.

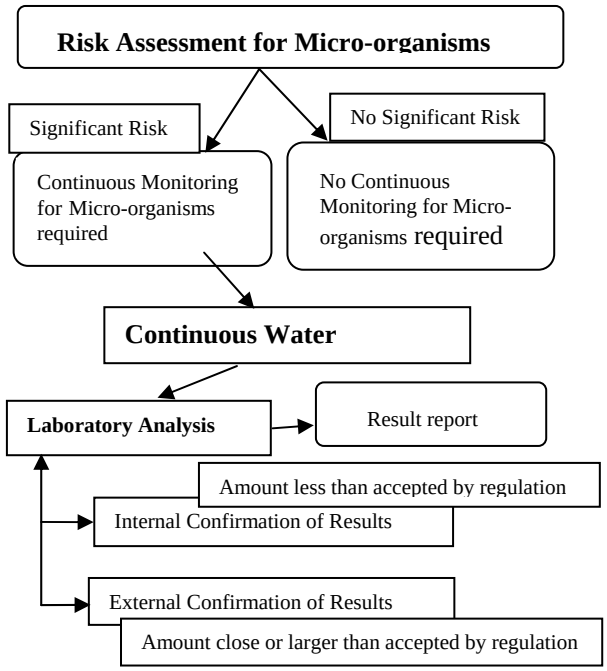

Fig.1. Operating Protocol for the monitoring of Micro-organisms

The Machine Vision System developed by Glasgow Caledonian University in cooperation with the Scottish Parasite Diagnostic Laboratory and the Technical University of Cluj Napoca has been designed and implemented to simplify and improve the detection and analysis procedures required by these directives.

II. MACHINE VISION APPLICATIONS

The evaluation and impact of new technologies in machine vision [2] has opened up new possibilities in application to medical and biological engineering. Table I compiles published papers in machine vision over a period of 4 years [3] in various journals.

The spread of image processing's applications in the different fields indicate only 4.2% dedicated to medical and biological engineering. The main reason for this low number of applications is the fact that the living cells' size and shape usually change and even their colour vary sometimes during their life cycle. Also in terms of automatic classification the

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amount of artificial intelligence embedded in these applications is relative complex and high. The application of image processing to detection of Micro-organisms in water present the problems associated with the physical variance of living cells and requires the development of specialized algorithms.

TABLE I
MACHINE VISION APPLICATION STATISTICS

Field	Number of Papers	Percentage
General algorithms or technologies	172	48.2%
Industrial related application	93	26.0%
Health,Microorganism, Living cell	15	4.2%
Agriculture, Food Industry	51	14.3%
Flight Control, Car Control, Automatic Tracking, Traffic Safety	18	5.0%
Textile, Leather, Jewellery	8	2.3%

III. MICRO-ORGANISMS IN WATER

Micro-organisms exiting in water have tremendously varied forms and sizes. They can be classified in groups according to their size: There is a Ciliate group which is relative large, over 100 um, and then the Flagellates generally less than 100 um followed by the Desmids and Diatoms which can be less than 60 um. Also there are some Algae group with dimension from about 20 to 80 um. According to the type of micro-organism found in drinking water it is possible to find: *Worms* The eggs and larvae of various intestinal worms found in man and warm-blooded animals pollute the water at times. They do not generally cause widespread infection for several reasons: they are relatively few in number and are so large they can be filtered out of water with comparative ease. The typical size of parasitic worms or helmiths, such as flukes, tapeworms, hookworms, ascris, pin worms, trichina worms, and filaria worms is 30-50 microns in diameter. *Protozoa.* Many live as parasites in the bodies of men and animals. N*ematodes* have long, cylindrical bodies which have no internal segments Nematodes can be a problem in drinking water because they impart objectionable tastes and odours to water. *Viruses*. The smallest of the infectious micro-organisms is that group of parasitic forms known as viruses. Viruses are capable of causing disease in both plants and animals. Viruses can pass through porcelain filters that are capable of screening out bacteria. Viruses, such as those producing infectious hepatitis, poliomyelitis, meningitis, and gastroenteritis, can be waterborne. Drinking water contaminated with any of these viruses is hazardous. Where these organisms are pathogenic or disease-producing, they may make water unsafe to drink.

IV. MATERIAL AND METHOD

Large micro-organism can be filtered with relatively easy; other can be made non-disease producing by chemical treatment. The main problem of drinking water is the need to detect and quantify smaller micro-organisms that are not easily eliminated due to their size. Manual existing methods and our system address this problem.

Detection of micro-organism oocysts using Normarski Differential Interference Contrast (DIC) microscopy is a tedious and time-consuming procedure required to assess micro-organism presence. The current method consists on manual microscopic examination of the recovered deposit for the detection and enumeration of Micro-organism oocysts. Following the laboratory preparation, each slide well must be scanned in a systematic fashion, ensuring that no field of view is missed and no duplicate counting occurs.

This method is very unreliable due to the fact that under DIC microscopy the contrast between the object of interest and the background is minimal; therefore objects are very difficult to distinguish by a human operator. Also under DIC microscopy it is impossible to see the nucleus inside sporozoites; hence sporozoites detection is based only on the shape deformation of the of the external oocyst wall – a very subjective process.

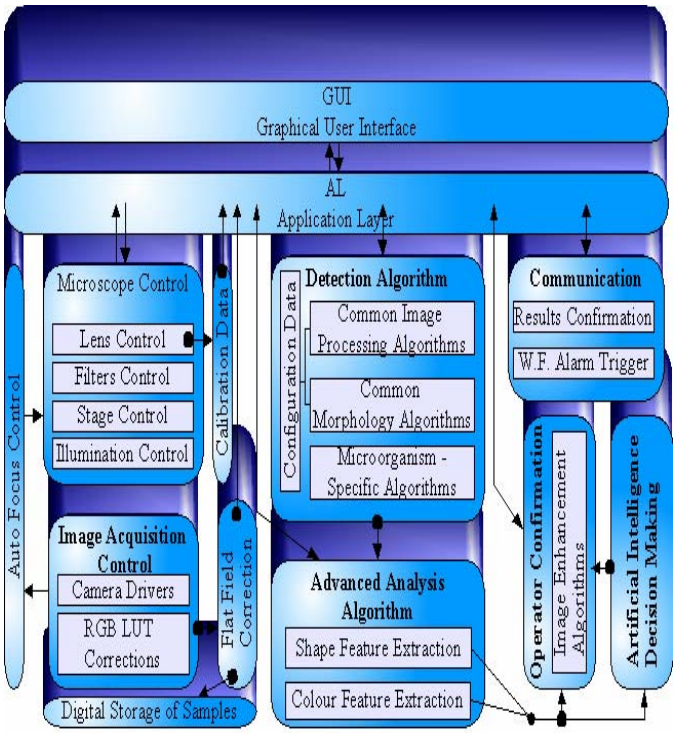

Fig. 2. Machine Vision Software Architecture

The architecture of our novel system is presented in figure 2. Our unexplored system uses the concept of making micro-organism detection under DIC microscopy more reliable using Machine Vision Technology.

The system can be tailored to detect and quantify different types of micro-organisms. Algorithms can be developed for the different micro-organism that present significant treat to health in drinking water.

To illustrate the proposed system the detection of Cryptosporidium is presented. Cryptosporidium is one micro-organism that has presented a large amount of concern in the last decade with numerous outbreaks [4][5]. Due to its size Cryptosporidium is difficult to filter and cannot be destroyed easily by chemical disinfestations. The method illustrate here for Cryptosporidium can be used to detect other Micro-organisms of similar size and different morphology.

V. RESULTS

The Cryptosporidium oocyst structure is shown in figure 3. The three microscopy techniques are commonly used: DIC microscopy, Fluorescent using FITC filters and Fluoresce

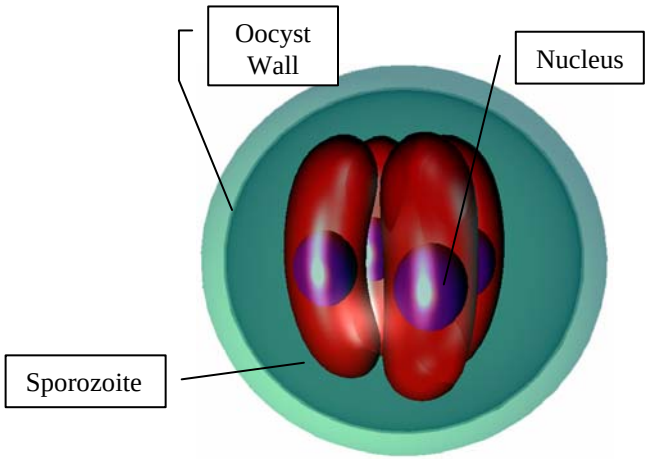

Fig. 3: Computer generated model of Cryptosporidium

using UV filters [6][7].

A series of images from a test slide containing Cryptosporidium, viewed under DIC microscopy with a 200x objective magnification is shown in figure 4.

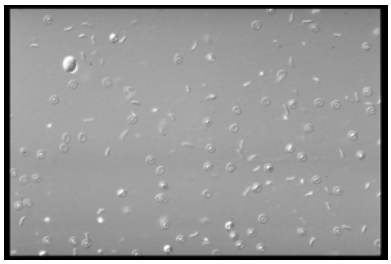

Fig.4.Test slide under DIC microscopy, 200x total magnification.

It is needed to isolate objects of interest from the background. A LUT transform proved to be completely inefficient in improving the separation. The use of special digital filter developed by us provides a significant improvement, as shown in figure 5.

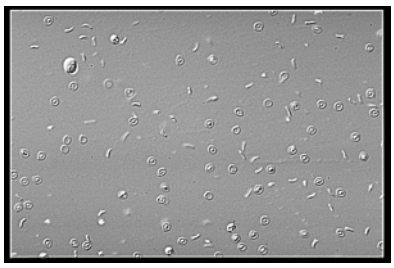

Fig. 5: Details Highlighted

The separation between the background and the objects of interest is defined to a certain degree that threshold algorithm can be performed as seen in figure 6.

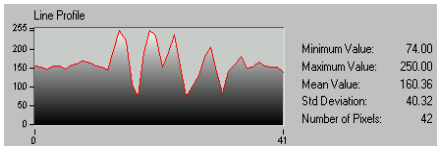

Fig. 6: Cryptosporidium profile after image processing

A threshold is performed, in two phases, one for the lighter component, and one for the darker component, then the results are combined using an OR function shown in figure 7.

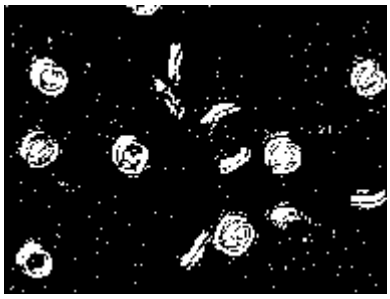

Fig. 7: Threshold images combined

It can be noted that the resulting image present prominent binary noise and the objects of interest are incomplete. All objects truncated by the acquisition process, namely objects which are partially in the image and partially outside the image boundaries are eliminated.

The binary noise present is efficiently eliminated using our developed algorithm [7]

The result is presented in figure 8. It can be noted that, unlike other binary noise reduction algorithms, the objects of interest are unaffected.

Objects too small to be Cryptosporidium oocysts are

Fig. 8: Binary noise clean-up

eliminated.

Then a distance function is applied, and Danielsson [8] circle detection algorithm is used for Cryptosporidium detection. The result is presented in figure 9.

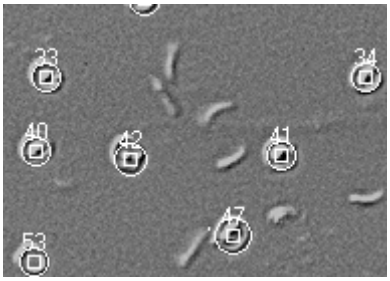

Fig.9: Detection first stage

The presented algorithm is able to detect presumptive Cryptosporidium oocyst on a slide viewed under DIC microscopy. However, from our laboratory test results, the detection rate was 93.75%. Although this is not a disappointing recognition rate, the target detection rate in order for a Machine Vision approach to be used as a standard operating environment must be 100%.

Identification of Cryptosporidium oocysts using exclusively DIC microscopy was considered unreliable therefore not recommended for Cryptosporidium recognition.

Although using DIC microscopy used with image processing does not provide a 100% detection of cryptosporidium, it can be used as a first stage of the new system proposed to provide a reliable detection which combining it with fluorescent microscopy. The new vision system proposed has been designed to implement the requirement of “Standard Operating Protocol for the Monitoring of Cryptosporidium Oocysts in Treated Water Supply" (SOP)[9].

One aim is to include as much as possible the human expertise accumulated at Scottish Parasite Diagnostic Laboratory in the identification of micro-organism to provide a reliable machine vision solution. The proposed system is able to incorporate current knowledge in the field and also be open for future development. Our aim is to match exactly in software the procedure followed by a human operator under current SOP.

The current process of Cryptosporidium identification is based on a series of Colour Feature Extraction and Shape Feature Extraction under FITC and DAPI, at 1000x total magnification. Therefore for each object to be analysed two images are acquired and processed. This is a time consuming process, during which the sample has to be exposed to UV light. Our solution is based on our algorithm that quickly detects Potential Cryptosporidium Oocysts. The second stage or Advanced Analysis of our system uses detection of FITC stained Cryptosporidium with Epi-illumination fluorescence microscopy, it is considered the best method available, and it is also employed in the current Standard Operating Protocol. It provides higher contrast between the objects of interest and background, but samples have to be chemically processed prior to examination.

An image of a slide containing FITC stained Cryptosporidium acquired using Epi-illumination fluorescence microscopy is presented in figure 10.

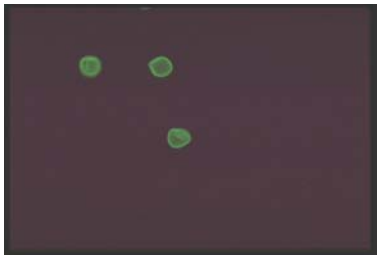

Fig.10: FITC stained Cryptosporidium under Epi-illumination fluorescence microscopy

VI. DISCUSSION

The excellent contrast between the objects of interest and background provided by this method make thresholding and binarisation possible using a simple clustering, with little noise presented in the binary image. Noise clean-up is performed, followed by objects reconstruction. Then Danielsson Circle detection is employed to perform potential oocyst detection, figure 11. Danielsson circle detection algorithm is able to detect circular objects touching each-other. The circles with corresponding Cryptosporidium size are defined as suspicious objects detected and their position is recorded for further analysis. In the image processing for this Micro-organism our system have the advantage over human operator in determining accurately small dimensions and morphology using separation of red and green plane form a colour frame.

In our laboratory test results, detection using this algorithm was 100% successful.

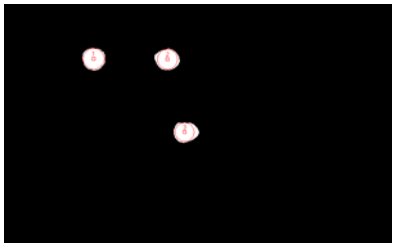

Fig. 11: Detected Cryptosporidium

The Advanced Analysis algorithm is a feature extraction algorithm, extracting relevant information under FITC and DAPI filters at 1000x total magnification, for each potential Cryptosporidium oocyst tagged by the Detection Algorithm. These parameters are stored together with the images acquired for each potential Cryptosporidium oocyst for each mode.

After the completion of the Advanced Analysis the system goes into the next stage – Artificial Intelligence Decision Making. This stage is completely transparent to the operator, and uses a fuzzy logic inference engine to mimic human knowledge based decision making. The classification is based on the features extracted by the Advanced Analysis Algorithm and a customisable rule base.

The reliability of the system is dependent on the quality of the acquired images. Therefore algorithms have been developed to assure the proper quality: Auto-focus and Flat Field Correction.

VII. CONCLUSIONS

The Machine Vision approach presented in this paper can perform automated analysis to determine whether or not Micro-organism oocysts are present in treated water supply. The system can reliable determine the presence of micro-organisms and enable the sample to be accurately and efficiently reviewed by an operator if required.

The novel approach of detection proposed here allows a reliable detection of waterborne micro-organism with substantial reduction in process time and cost than the current methods in use and permit the assessment of large quantity of water quality.

The implemented algorithms accommodate feature such as micro-organism size, shape, nucleons number, DAPI internal staining and typical or atypical FITC staining. The application example presented in this paper provides a reliable detection of FITC stained Cryptosporidium with Epi-illumination fluorescence microscopy.

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