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APPLICATION OF INFORMATION TECHNOLOGIES FOR VISUAL EVALUATION OF LEMNA MINOR BIOASSAYS

Oto Haffner, Erik Kučera, Štefan Kozák, Barbora Urminská

Abstract:

This article deals with the problem of automatic evaluation of experiments with Lemna minor. One of the convent methods for evaluating experiments with Lemna minor is number counting of new grown leaves of the Lemna minor. This article deals with the possibility of implementing algorithms of recognition and evaluation of Lemna minor leaves using visual system implemented in the single-board computers. Result of this paper is theoretical proposal of system for automated counting of Lemna minor leaves number.

Keywords:

ecotoxicity testing, image processing, single-board, Microsoft Azure, visual system, segmentation, IDS uEye XS, Raspberry Pi 2, machine vision

ACM Computing Classification System:

Computer vision, Visual inspection, Image segmentation, Cloud based storage

Introduction

The possibilities for monitoring the environment are in continuous progress and it is necessary to develop more advanced wastewater treatment methods and better monitoring of toxicant contents in treated water. Because of these facts, ecotoxicological tests are considered to be relevant approach for evaluation of impact of treated wastewater to ecosystems. No wastewater treatment plant removes pollution perfectly, it just lowers the pollution to an acceptable level. It is very important to judge whether the level of ecotoxicity of treated water is low enough and what will be the effect on the water organisms [8,10].

One of the plants which are used to measure ecotoxicity is Lemna minor. During the ecotoxicity tests the inhibition or biostimulation of plant growth caused by tested samples is watched for 7 days. The aim of the test is the quantification of substance effects on vegetable growth using number of leaves and biomass. Average increase of number of leaves in reference sample must be 7 times for the legitimacy of test. Also the pH of water must not change more than 1.5 unit and the level of EC50 for standard substance – potassium chloride KCl must be between 5.5 to 10 g L^{-1} [7].

The classical biotests utilizing duckweed are based on dry plant biomass comparisons between the control and experimental samples. These are invasive methods and do not allow the continuation of the experiments, since the plants are harvested for biomass measurements. Another method is based on manual counting of the number of thalli (‘‘leaves’’), regardless of their size. A more reliable and more accurate method of defining the growth of biomass is comparison of the area of thalli in the control and experimental samples.

This is a non-invasive method allowing the continuation of the experiment. Computer image analysis methods provide objective support for data collection. [11].

Big scientific institutions which deal with topic of wastewater management have devices with partially automated process of evaluation of Lemna minor experiments. Among these devices there are leaf area meters and chlorophyll fluorescence imaging instruments. However, these devices are expensive and not available for smaller institutions or scientific teams. Therefore the number of leaves at the end of the exposure period has to be counted manually by a laboratory worker. This is a very time consuming procedure and can be loaded by human mistake. Moreover, the unhealthy leafs can vary also in size and color – dead tissues have white color (necrosis), yellow color of leafs is caused by chlorosis – but these parameters cannot be quantified by human eye and are often overlooked. The majority of bioassays are based in a visual observation approach, where the observer evaluates symptoms of the toxic impact of pollution on bioindicators.

Machine vision has become a key technology in the area of manufacturing and quality control due to the increasing quality demands of manufacturers and customers. Machine vision utilizes industrial image processing through the use of cameras mounted over production lines and cells in order to visually inspect products, read or direct products and guide robots in real time without operator intervention. Machine vision can be used not only in industrial environment but also in various laboratories to support evaluation or analysis tasks, which would be difficult or time consuming if they were done by humans [11].

Using the digital images for the evaluation of the test would allow to measure the total leaf area and simultaneously to determine an average color of the plant. This method is therefore more accurate and much less time consuming compared to the manual counting of leaves.

Our study aimed to proposed a low-cost approach to objective and quantified biomonitoring based on computer image analysis, which can be used by a researcher without the need to purchase expensive specialized hardware. The aim of this study is to propose basic solutions for bioassay analysis using computer vision (standard digital camera) and framework software, which can be used in the future used for test populations of the widespread freshwater macrophyte Lemna minor L., treated within a toxicity bioassay of non-ionic detergents and compare the conventionally method (manual by research worker) and our new proposed. The function proposed algorithm was verified on set of lemna minor images.

1 Lemna minor

Lemna minor or lesser duckweed (Angiospermae, Lemnaceae) is a freshwater plant occurring in most countries of the world, especially in the lowlands and foothill areas with stagnant and slow-flowing waters. Body of the plant is made up of relatively long root and oval shaped leaves of 2 to 5 mm which float on the water surface. Plant usually creates the colonies of two to five leaves. A single lesser duckweed plant can reproduce itself about every 3 days under ideal conditions in nutrient-rich waters [9]. Bioassay on Lemna minor is quite common and easy to perform. The endpoint of the test is growth inhibition after 7 days exposure to tested samples and the aim is to quantify the effects of substances on vegetation growth.

2 Computer vision methods and algorithms

Computer vision is a field that includes methods for acquiring, processing, analysing, and understanding images. Computer vision applications range from tasks such as industrial machine vision systems to research into artificial intelligence and computers or robots that can comprehend the world around them. [2]

Computer vision covers the core technology of automated image analysis which is used in many fields. Machine vision usually refers to a process of combining automated image analysis with other methods and technologies to provide automated inspection and robot guidance in industrial applications. [1]

Thresholding

Thresholding is one of the most used method for image segmentation. Let us consider image as function f. Thresholding is then transformation of input image to output image g using

$$g(x,y) = \begin{cases} 1 & for\ f(x,y) \geq T \\ 0 & for\ f(x,y) < T \end{cases} \tag{1}$$

There are some methods for automatic setting of the threshold.

Shape modelling

This method uses two degree function for histogram approximation. Between this function and histogram is minimized sum of squares. The solution is found using iteration [6]

$$T_{opt} = \min \left[\sum_{g=0}^{T} [b_1(T) - g]^2 + \sum_{g=T+1}^{L} [b_2(T) - g]^2 \right] \tag{2}$$

$$b_1(T) = m_0(T)/P(T) \tag{3}$$

$$b_2(T) = m_p(T)/[1 - P(T)] \tag{4}$$

Local thresholding

In some cases, global thresholding give us unsatisfied results. Advantage of local thresholding is respect of different light conditions in different parts of image.

The threshold is set for each pixel based on its surrounding. The imaginal mask is moved over the whole image. [5]

Binary mathematical morphology

Next morphological transformation will be expressed using Minkowski formalism. [4]

Dilation

$$X \oplus B = \{p \in \varepsilon^2 : x + b, x \in X, b \epsilon B\} \tag{5}$$

Erosion

$$X \ominus B = \{p \in \varepsilon^2 : p + b \epsilon X\ for\ each\ b \epsilon B\} \tag{6}$$

Opening and closing

Using combination of this two projection, we get morphological operation opening and closing. [5]

Opening is defined as dilation after erosion.

$$X \circ B = (X \ominus B) \oplus B \tag{7}$$

Closing is defined as erosion after dilation.

$$X \bullet B = (X \oplus B) \ominus B \tag{8}$$

RGB color model

The RGB color model (
Fig.1) is an additive color model in which red, green and blue light are added together in various ways to reproduce a broad array of colors. The name of the model comes from the initials of the three additive primary colors, red, green and blue. A color in the RGB color model is described by indicating how much of each of the red, green, and blue is included. The color is expressed as an RGB triplet (r,g,b), each component of which can vary from zero to a defined maximum value. If all the components are at zero the result is black; if all are at maximum, the result is the brightest representable white. [3]

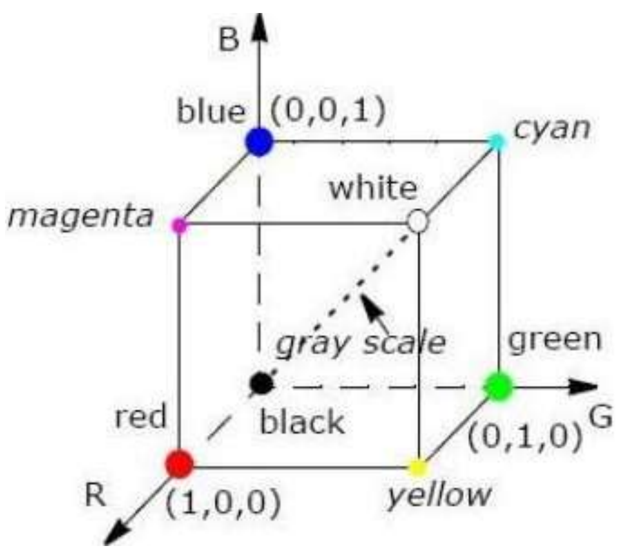

Fig.1. Representation of RGB color model

HSL/HSV color space

HSL and HSV color models (
Fig.2) are the two most common cylindrical-coordinate representations of points in an RGB color model. The two representations rearrange the geometry of RGB in an attempt to be more intuitive and perceptually relevant than the cartesian (cube) representation. Developed in the 1970s for computer graphics applications, HSL and HSV are used today in color pickers, in image editing software, and less commonly in image analysis and computer vision. HSL and HSV are the two most common cylindrical-coordinate representations of points in an RGB color model. The two representations rearrange the geometry of RGB in an attempt to be more intuitive and perceptually relevant than the cartesian (cube) representation. Developed in the 1970s for computer graphics applications, HSL and HSV are used today in color pickers, in image editing software, and less commonly in image analysis and computer vision. [6]

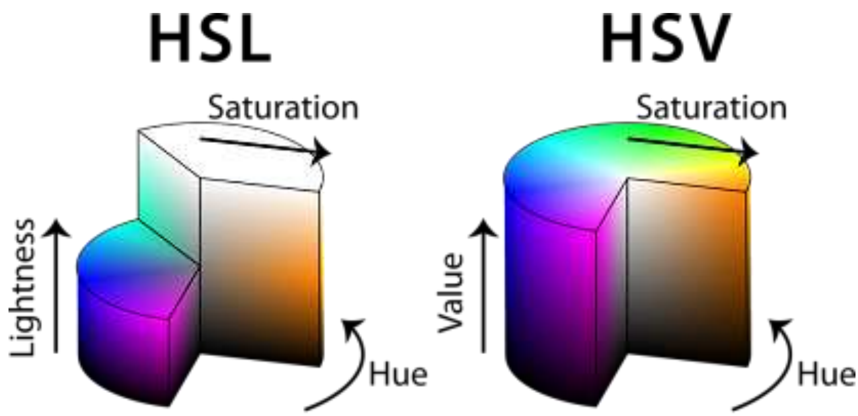

Fig.2. Representation of HSL/HSV color model

3 Proposal of methodology for experiment evaluation

The main motivation of computer vision Lemna minor evaluation is to reduce the mistakes caused by human. In some specific cases (e.g. small laboratories) the counting process of growth leaves is done by laboratory workers eyes. This can cause careless mistakes and is time-consuming.

Lemna minor specifications

The leaves are shown in Fig.3. In experiment, there are two containers. The first container is referral with clean water and the second is the same sample of contamined water with Lemna minor. In this experiment, the growing of number of leaves and the color of leaves is important.

Proposal of the method

For proposing of methodology, we will work with Lemna minor in laboratory environment.

Fig.3. Lemna minor laboratory experiment

Leaves segmentation

The first step is to segment the leaves in the image picture. The aim is to get only pixels in image which represent the area and shape of examined leaves (Fig. 4 left).

For segmentation can be used HSL color segmentation. This method is based on known color of object. The object can have various saturation of color e.g. Fig. 8 right, where the leaves are not so saturated and are faded.

In HSL color segmentation are very important set parameters for Hue, Saturation and Luminance. Not suitable parameters can cause unwanted results. In Fig.5 we can see segmented reflections in canister. To solve this problem by setting different values for HSL parameters can cause losing of information about leaves which have same HSL values as reflections. This problem will be discussed in further pats of paper.

In Fig.6 the results of wrong set Luminance parameter can be seen. The right parameters of HSL must be set experimentally and they also depend on lighting of the scene.

Counting of area

The better indicator than number of leaves is the area of leaves. Number of leaves are used because it is easy to count the number of leaves by laboratory workers. However, the leaves do not have the same size and this can cause some errors. The better way is compute the area of leaves. This task is very difficult for human laboratory worker, however for computer vision it is a very simple task. After watershed segmentation, the number of pixels are counted. The area of one pixel depends on used optics and camera sensor size. It is expected, that the level of water and leaves are still in the same distance from the camera. In this case, the simple measurement can be used to find out the real size of one pixel in image.

Leaves tint

One of the experiment indicator is color of leaves. In Fig.3 are shown two canisters. On the left side, there is non-toxic environment. On the right side, there is toxic environment. We can see, that color of leaves in toxic environment is desaturated and slightly yellowish. The exact color tint of leaves can be easily measured by meaning all HSL values of each pixel. For measuring the right color tint, it is necessary to have stable lighting environment.

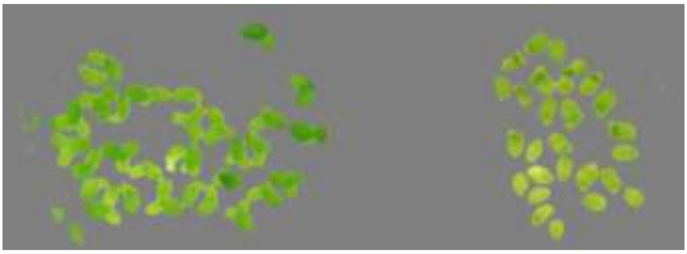

Fig.4. Lemna minor segmentation

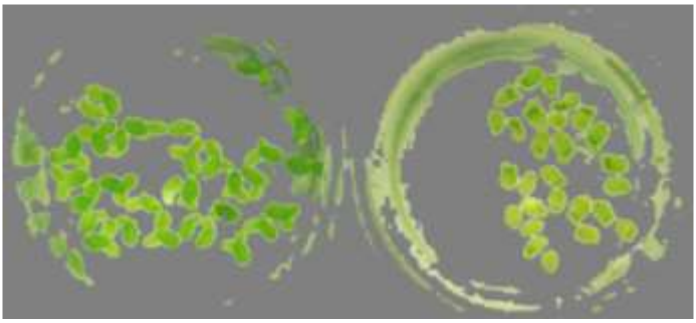

Fig.5. Lemna minor reflection segmentation

Fig.6. Lemna minor wrong set Luminance segmentation

Algorithm

In Fig.7 we can see the scheme of whole algorithm for experiment evaluation.

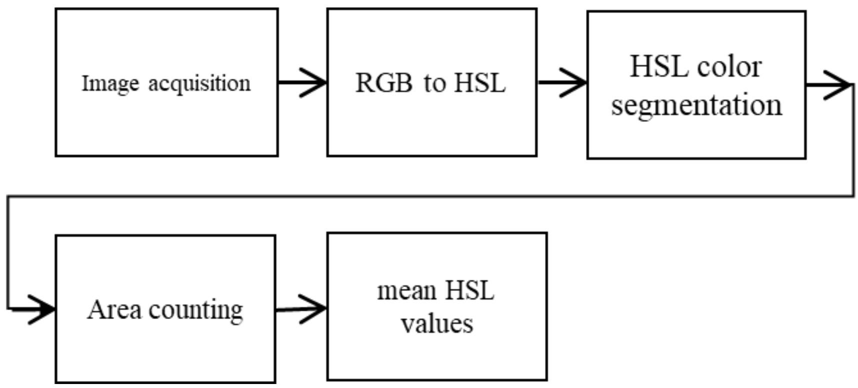

Fig.7. Lemna minor surface evaluate algorithm

4 Proposal of visual system experiment

Leaves tint

As we mentioned in section IV, there are a few problems in process of segmentation. The biggest problem are reflections in glass containers (Fig.8 left). These reflections cause mistaken segmentation, which cannot be removed by setting ideal HSL values. The possible solution is using not glass container but matt black. Container with mat black finishing would be ideal to suppress the reflections. Another solution can be using a region of interest in image. This can be used only when the position of camera and the container is still the same.

In Fig.9 we can see effects of water tint itself. Water from toxic environment can have color tint. This cause also error during segmentation. Container with mat black finishing would be ideal to suppress this error.

Luminance

In Fig.6 we showed the effect of non-uniform lightning to the segmentation. In case, that some parts of leaves are lighted non-uniform can cause error in segmentation. However,this can cause also errors in computation of color tint. This problem can be solved by proper lighting design.

In Fig.9. Water tint we can see effects of water tint itself. Water from toxic environment can have color tint. This cause also error during segmentation. These errors can be also removed with container with mat black finishing.

Fig.8. Reflections

Fig.9. Water tint

Lightning of laboratory experiment

The lightning of the scene can be done in more ways. In

Fig.10 we can see the circle shape of lightning. Light with circle shape supplies more diffused light with direction from the camera lens. This configuration gives bright image field.

In Fig.11 there is a scheme with dome shape lightning. This configuration gives ale bright image field same as circle shape lightning. However, it gives much better diffused light. Adding of light beams inside of canonical surface makes shadowless light. The surface of *Lemna minor* leaves can be rough, so shadowless light can improve quality of scene image.

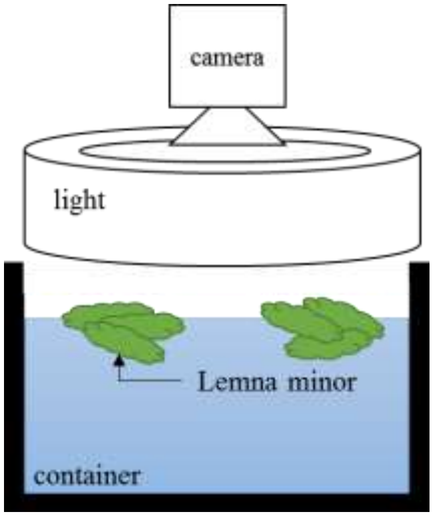

Fig.10. Circle lightning of laboratory experiment

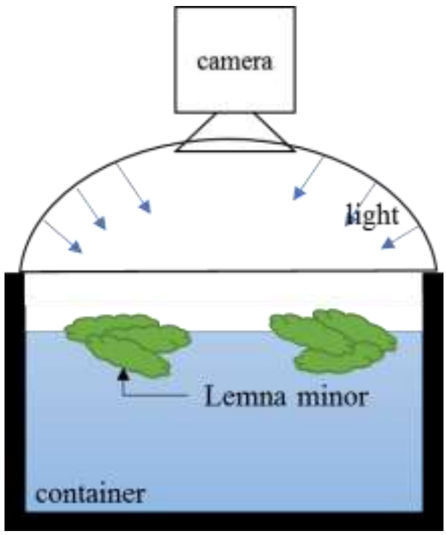

Fig.11. Dome shape lightning of laboratory experiment

5 Implementation

Proposed algorithm and visual system experiment must be implemented. The implementation scheme is shown in Fig.12

The whole system consists of single-board computer, visual system (camera) and cloud service. Camera is connected to the module for communication. The acquisitioned images are transformed from RGB color space to HSL. After transformation, the HSL color segmentation is done. Result of color regimentation is watershed segmented. After this, three computing tasks are done. To the .txt file is written the number of leaves, area of leaves and mean HSL value. To the .txt field it is also written value from pH probe. This .txt file is processed to be suitable for cloud communication module. This module sends the data to web service (database) to be accessible from any place or to be processed and evaluate in future.

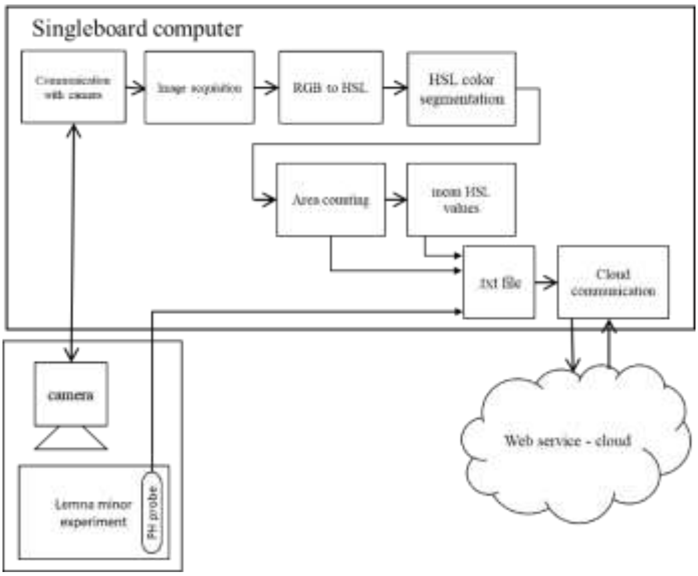

Fig.112. Dome shape lightning of laboratory experiment

Conclusion

In our work, we bring theoretical background about Lemna minor toxicity experiments. Based on this, the computer vison algorithms are presented, which can be used for morphometric measurements of plant surface. This approach can be new standard for a more objective and quantified assessment of the negative influence of toxins on bioindicators. Automation can increase the speed and accuracy of the tests evaluation. The next part of the work presents theoretical purposing of Lemna minor experiment examination and evaluation method.

Big scientific institutions which deal with topic of wastewater management have devices with partially automated process of evaluation of Lemna minor experiments. However, these devices are expensive and not available for smaller institutions or scientific teams. Our proposed algorithm would remove these deficiencies, because it uses low cost devices, can be implemented on any image processing framework (e.g. Matlab, NI Vision Assistant etc.) and will be easy to use. Using the area of leaves can be more precise than the number of leaves. Number of leaves does not take into account the fact, that the leaves can vary in size.

The future task of our work will be the implementation and evaluation of our algorithm comparing the conventionally method (manual by research worker) and our new proposed and its implementation to single-board computer.

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References

[1] E. Davies. *Computer and machine vision: theory, algorithms, practicalities.* 4th ed. Amsterdam : Academic Press, 2012. s. 871.

[2] O. Haffner. *Edge detection in image.* Bachelor thesis. Bratislava : FEI STU, 2011. s. 52 (in Slovak).

[3] R. Gonzales and R. Woods. *Digital Image Processing.* 3rd. Upper Saddle River : Pearson, 2008. s. 976.

[4] M. Klíma, M. Bernas, J. Hozman and P. Dvořák. *Image information processing*. Praha: Vydavatelství ČVUT, 1999. 177s (in Czech).

[5] E. Šikudová, et al. *Objects detection and recognition.* Praha : Wikina, 2011(in Slovak).

[6] M. Šonka,V. Hlaváč and R. Boyle. *Image processing, analysis, and machine vision.* Toronto : Thomson, 2008. s. 829.

[7] ISO 20079:2005. Water quality - Determination of the toxic effect of water constituents and wastewater on duckweed (Lemna minor) - Duckweed growth inhibition test.

[8] Mendonça, E., Picado, A.,Paixão, S., Silva, L., Barbora, M. and Cunha, M. A. (2011). The role of ecotoxicological evaluation in changing the environmental paradigm of wastewater treatment management. The 6th Dubrovnik Conference on Sustainable Development of Energy, Water and Environment Systems (SDEWES'2011). DOI: 10.13140/2.1.2168.3205.

[9] Free Floating Plants: Lemna minor L., lesser duckweed; Lemna trisulca L., startduckweed or ivy duckweed. Department of Ecology: State of Washington [online]. 2012. http://www.ecy.wa.gov/programs/wq/plants/plantid2/descriptions/lemmin.html

[10] Bundschuh, M. (2014). The Challenge: Chemical and ecotoxicological characterization of wastewater treatment plant effluents. Environmental Toxicology and Chemistry. Vol. 33, Issue 11, p. 2407.

[11] Mazur, R., Szoszkiewicz, K., Lewicki, P. and Bedla, D. (2016). The use of computer image analysis in a Lemna minor L. bioassay. Hydrobiologia.

Ing. Oto Haffner, PhD.
Faculty of Electrical Engineering and Information Technology, Slovak University
of Technology in Bratislava, Bratislava, Slovakia
oto.haffner@stuba.sk

Ing. Erik Kučera, PhD.
Faculty of Electrical Engineering and Information Technology, Slovak University of Technology in Bratislava, Bratislava, Slovakia

Prof. Ing. Štefan Kozák, PhD.
Faculty of Informatics, Pan-European university, Bratislava, Slovakia

Ing. Barbora Urminská
Faculty of Chemical and Food Technology, Slovak University
of Technology in Bratislava, Bratislava, Slovakia