Daphnia magna as biosensor for Ag-nanoparticles in water systems: Development of a computer vision system for the detection of behavioral changes

Jan Kunze*, Sarah Hartmann†, Klaudia Witte† and Klaus-Dieter Kuhnert*
*Institute of Real-time Learning Systems, Department of Electrical Engineering and Computer Science, University of Siegen.
Email of corresponding author: jan.kunze@uni-siegen.de
†Research Group of Ecology and Behavioural Biology, Institute of Biology,
Department of Chemistry and Biology, University of Siegen.

***Abstract*—We present a work-in-progress computer vision system for detecting behavioral changes of *Daphnia magna* under the influence of Silver-nanoparticles (Ag-NPs) in water systems. We invented a hardware set up that is able to track multiple Daphnia automatically in concordance with the OECD guideline for *Daphnia*. Our software design and first image results of the computer vision system are represented. We give an outlook on methods that we want to test and use in the future for the detection of behavioral changes.**

***Index Terms*—Assessment of organism behavior or behavior changes, organism tracking and movement analysis**

1. Introduction

Nowadays, manufactured nanomaterials (MNMs) are applied in a lot of technical applications [1]. After their use, those MNMs will enter through different pathways marine, freshwater or soil ecosystems [2]. The MNMs can be absorbed by zoo plankton filtering the water and enter the food chain this way. The effects for animals and humans are for the most part not known. Therefore there are several ongoing research programs investigating this matter [3] [4].

One water filtering organism is *Daphnia magna* [5] (Figure 1). *Daphnia* behaves very sensitive towards changes in environmental conditions or toxic substances [6] and are used as biosensors for testing the water quality. *Chevalier et al* [7] distinguish between two different use cases for tests with *Daphnia*: They are either used as an alarm system for ongoing water quality control [8] [9] [10] or in laboratory tests to determine the toxicity of substances [11].

Tests according to the OECD guidelines [11] mainly cover aspects like the LD_{50} of a substance where the number of dead or immobilized specimens after a certain time period is counted. However, systems like the *Daphtox II* from bbe Moldaenke [8] detect toxic substances by analyzing changed motion and behavior patterns of *Daphnia*, so called sub-lethal effects. These can appear at even low concentrations of toxins [7].

With the high interest in evaluating possible outcomes of MNMs exposure to the environment and lack of tools for the evaluation of presence and effects of MNMs, new techniques and methods in this area are needed. First studies [12] [13] showed, that mortality and behavior of *Daphnia* is influenced by MNMs, so that *Daphnia* can be used as a biosensor. In our tests we investigate the effect of Silver-nanoparticles (Ag-NPs) which are used in clothing, cosmetics or bandages, because of their antibacterial effect.

The tracking of multiple *Daphnia* with a computer vision system poses similar challenges in comparison to other animals like bees [14], flies [15] [16], mice and rats [17] [18], bats [19] or fish [20] [21].

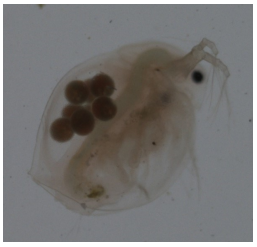


Figure 1. Adult *Daphnia magna*, size ca. 5mm, microscopic color image, recorded without contrast agent

Several studies already dealt with the evaluation of *Daphnia's* movements. *Dodson et al*. [22] surveyed the effect of food, light and container size on the swimming behavior. They analyzed their videos manually by measuring swimming distances with a ruler attached to a monitor.

Jeon et al. [23] used a grid counter device to quantify movement activity and created an index for changed behavior of *Daphnia* under the influence of copper [10]. *Horak et al.* [24] evaluated movement activity without tracking by subtracting consecutive images.

Lard et al. [25] invented a method where daphnids were marked with fluorescent colored quantum dots NPs to overcome the drawback of the specie's transparency. The use of quantum dot NPs did not have an influence on the behavior, the reproduction rate or the growth rate of *Daphnia*. *Lard's* approach was picked up by *Ekvall et al.* [26] and *Bianco et al.* [27], both using two cameras for 3D tracking. They were able to track the test specimen and improve the data quality in comparison to tracking without the quantum dot NP. The drawback of the quantum dot NP

method is that the particles have to be attached manually to the carapace which moults within two days. This prevents tracking the same specimens in long-term experiments.

Prez-Escudero et al. [28] developed a free software [29] for generic tracking of individuals in groups in 2D videos. They validated their result with mice, flies, fish and ants. While their software produces good tracking paths, the evaluation of videos is very time consuming and cannot be done in real-time or near-real time. This is problematic for large data sets. *Chevalier et al.* developed a *behavioral multi-cell exposure system* [7]. They tracked multiple cells containing several *Daphnia* at once with a single camera and detected changes in average speed under influence of different toxins [30]. They used the commercial software *ZebraLab* [31] for tracking and validated their results against the *Daphtox II* [8].

Noss et al. created a tracking system for multiple objects with two cameras and Matlab. This set-up was used for testing the influence of Titanium-dioxid-nanoparticles (TiO_2-NPs) on the swimming behavior of *Daphnia* [13]. They could detect changes in the swimming velocity under the influence of TiO_2-NPs. Their hardware set-up is similar to our approach. However, we want to find additional indicators or movement models for the detection of behavior changes.

With the need for detection and evaluation systems for effects of MNMs and the lack of a standard set up for performing behavioral tests with *Daphnia* outside the OECD mortality and immobility tests, our major goal is the invention of a system that uses *Daphnia* as a biosensor to test possible effects Ag-NPs. This way, we create an analytical tool for MNMs risk assessment. Our task is to invent a computer vision system that can (1) track several daphnids (2) automatically (3) in real-time and (4) detect changes in swimming behavior of *Daphnia* under the influence of Ag-NPs (5) according to OECD guidelines to design a set-up that could become a base model for a new guideline.

2. Materials and methods

We build the system from scratch to have influence on all parameters. One requirement for our system is the accordance to OECD guidelines [11] concerning tests with *Daphnia*. This permits the whole system to become an additional test tool for potential new guidelines for MNMs risk assessment using *Daphnia* as a biosensor. With this requirement, commercial systems like *Daphtox II* [8] that combine hardware and software can't be used, because hardware and software are not flexible enough to fulfill the needs of OECD guidelines. For more complex movement data we use two cameras. This supports our emphasis on finding useful indicators for detecting a change in behavioral pattern of *Daphnia magna*. With these requirements, we don't want to use commercial software like Zebralab [31] or EthoVision XT [32], which limit the possibilities to find indicators of behavior changes with new methods.

2.1. Experimental set-up

For recordings we use two monochrome Manta G-223B NIR from Allied Vision Tec [33] with a maximum frame rate of 53.7 fps, a resolution of two megapixel and a GigE Vision interface. The cameras support use of the *Robot Operating System* (ROS) [34] which is designed for the use in real-time applications. ROS is used as a middleware for the cameras, for data transport and storage. Cameras are synchronized with the help of a hardware trigger (AND-gate). C-mount lenses with a focal length of 50 mm and a minimum range of 20 mm were used with the cameras. Cameras are adjusted orthogonal to create a front and a top view of the test cell (see Figure 2).

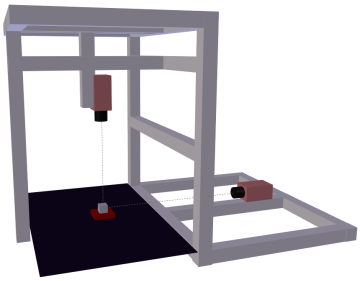


Figure 2. schematic with two cameras

For the recordings of image data a SSD hard drive (model Samsung 850 Evo) was necessary because of high data throughput. Normal HDDs with 7200rpm were too slow and lead either to huge data loss, if we decided to skip frames that could not be written immediately, or to a system crash when RAM and SWAP were filled.

Figure 3. Set-up, cover removed for better display

Illumination is a very important aspect for computer vision systems. It should lead to qualitative high image quality but also should not influence the behavior of test specimens. For experiments with *Daphnia* we use a CVI STAR BL-LED back light illumination with a frequency of 850 nm. NIR-Light in this spectrum can't be seen by *Daphnia* [35]. The set up is covered completely with molleton that absorbs any light from outside. The set up is placed in a laboratory room with controlled temperature and air humidity (see Figure 3).

2.2. Test execution

Tests are performed with 10 adult *Daphnia magna* of the same age (in days). As test cells we use cuboid cuvettes with the inside dimensions of 44 mm x 97 mm x 34.5 mm. Test cell size is in line with

OECD guidelines which demand for 2ml fluid per *Daphnia*. Tests are done for 96h. This duration extends the standard length of 48h that is used in the OECD guidelines. However, *Dabrunz et al.* [36] showed that a longer test duration leads to higher toxicity effects with TiO_2-NPs compared to the standard length of 48h. According to this, we extended our test time to 96h to trace long-term sublethal effects that alter the behavior of *Daphnia*. Tests are executed under total darkness (NIR-light is not recognized, see above) for the whole duration in concordance to the OECD guidelines.

A test series consists of 7 test cells including one control group. After the test cell is mounted onto the illumination there is a pause for 10 minutes until recordings are started. This way, *Daphnia* has time to return to its behavior pattern after experiencing vibrations through the manual mounting. Daphnids are recorded at eight recording times (0h, 3h, 6h, 12h, 24h, 48h, 72h, 96h) each cell for 2 minutes with the two synchronized cameras. Tests are conducted with different concentrations of Ag-NPs (NM-300k), with wastewater-borne MNMs and algae spiked with MNMs (see *Hartmann et al.* [37]).

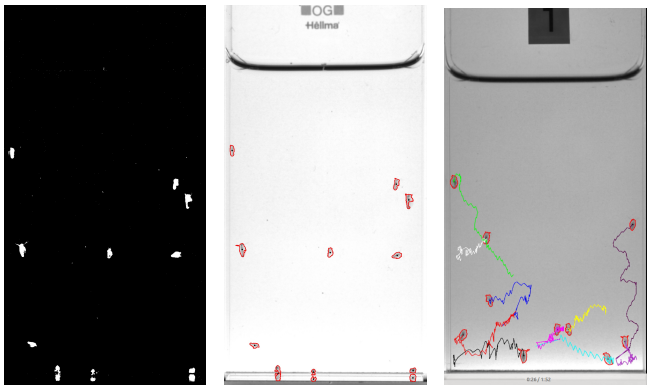
3. Tracking

Programming was done with the help of ROS [34] and OpenCV [38] in C++. The image processing for creating tracking paths includes three major steps:

In the first step, background segmentation is done using a mean filter. Here, the first 400 images are accumulated with a weight α. In doing so, moving objects won't be added to the background. This is tolerable because the *Daphnids* are usually highly active and are considered immobile if they are not moving within 15 seconds after gentle agitation of the test container [11]. This case automatically occurs when changing test vessels for recordings. After creating the background with this method (Fig. 4a), the creation of tracking paths starts.

In the second step, we detect the contours of specimens and calculate their mass centers (Fig. 4b) with the help of OpenCV. Too small contours are filtered out to reduce noise.

In the third step, these mass center points are used to determine tracking paths over time. At the start of the tracking a new track is created for every detected mass center. For each tracking path a Kalman filter is used to predict the position for the next frame. In the next frame all actual positions of detected mass centers are then globally compared with the predictions for each track using Global Nearest Neighbor (GNN) with the help of Munkres algorithm (also called Hungarian algorithm). The Kalman filter is then updated with the new found actual position and then creates a new prediction for the following frame. For the prediction in the Kalman filter four dynamic parameters are used, position and velocity. The adding of acceleration to use of 6 parameters let to worse predictions. The prediction model helps to cope with occlusion problems in 2D recordings which appear regularly in tests with this population-to-cell ratio. The resulting tracking paths of the *Daphnids* can be seen in Fig. 4c.



(a) Mean filter (b) Contour finding (c) Tracking paths

Figure 4. Steps for tracking, front view

A video with these images can be found at youtu.be/uYq%5fKEY9RJ4 [39]. Implementation of the tracking with 3D-coordinates is in progress at the moment.

One of our early goals, the detection of the heart rate, can not be accomplished with this set-up. The NIR-illumination makes it virtually impossible to detect the heart of *Daphnia*. This is caused by the *medical spectral window* for water and hemoglobin which encloses a range of 700nm to 900nm [40]. Most of the NIR-light is transmitted in that spectrum instead of being absorbed or reflected by water/hemoglobin which are two of the main components of the *Daphnia's* heart [41].

4. Detection of changes in swimming behavior

This section describes the goals of our project concerning the evaluation of our image data. The test series have recently finished, giving us a large data base for testing different methods to find indicators for changes in behavior and movement of *Daphnia*. This is work in progress which is in accordance to our time table. Results are planned to be presented at the beginning of 2017. We pursue three different approaches to find significant changes in behavior of *Daphnia*.

Our first approach is the statistical analysis of different indicators that are directly connected with the movement paths that are generated from the tracking of each *Daphnia* individually. We will test indicators like average speed [7] [8], speed distribution [8], swimming velocity [13], swimming height [8], turning based features [8] [42] and more. These can be used to evaluate statistically significant differences between *Daphnia* under the influence of Ag-NPs and the control group or a database created from control tests. These evaluations have the advantage that they are to some extend already validated through the daily use in commercial applications [8] regarding toxins and other studies in this field [13] regarding other MNMs.

We will secondly implement methods that don't rely on the tracking of individuals. The grid counter used by *Jeon et al.* [23] and *Jeong et al.* [10] counts the number of

detections in grid cells respectively the number of collisions with grid lines. This has low requirements on hardware but the resulting data set is sufficient enough to get evidences for behavior changes. The subtraction of images done by *Horak et al.* [24] is another interesting method. More complex crowd based methods that work for example with optical flow [43] [44] are considered, too.

Our third way is to describe the movement of the *Daphnia* in a physical model like the Random Walk or Active Brownian Particle theory. Both theories are already used in studies to describe the movement of *Daphnia* [45] [46]. It should be evaluated if those models are applicable for our real use case and if they can deliver an additional method for detecting behavioral changes of *Daphnia*.

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