Spectrozonal and multispectral raster lens-less microscopy of aquatic organisms: Towards new contact optical methods of environmental biotesting using standard model organisms in variable environments

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ABSTRACT

Biotoxicity testing using model aquatic organisms is crucial for assessing the impact of pollutants on ecosystems. Model organisms like *Hydra* provide valuable insights into the effects of contaminants on freshwater ecosystems. Monitoring of *Hydra's* response to toxins helps in understanding the potential risks posed by pollutants in aquatic habitats. The use of *Hydra* in ecotoxicology research allows for the assessment of water quality. These organisms serve as reliable indicators of environmental stressors due to their sensitivity to such substances for long-term monitoring of freshwater ecosystems in situ. This paper considers a novel technique for invertebrate hydrobiology and ecotoxicology based on the analog non-coherent spectrozonal raster lens-less microscopes with combined raster scan and cross-band LED shifting (developed by the author in early 2000th). Also, anaglyphic imaging (R+G or R+B channels) with a polarization system is proposed. Integral frequency characteristics and integral spatial characteristics can be used for the quality indication of the lens-less micrographs and provide optimality criteria of the images illustrating the structural effects of different agents on Hydra sp. at the tissue level. Time-lapse microphotographs of Hydra in different imaging regimes are provided in the text.

Keywords: Environmental tribology, surface qualimetry, sustainable development, lens-less microscopy, laser-assisted microroughness measurements, weathering, correlographic imaging, 2D FFT, 2D multi-angle laser scanning imaging

1. INTRODUCTION

1.1 Hydra as a model object

Representatives of the genus *Hydra*, family *Hydridae*, are a conventional "model object" in zoology and some other disciplines. As Galliot rightly notes[1], "the freshwater *Hydra* polyp emerged as a model system in 1741 when Abraham Trembley not only discovered its amazing regenerative potential, but also demonstrated that experimental manipulations pave the way to research in biology." Currently, *Hydra* is applied as a model object for the following tasks:

(1) As a model of biological development (both normal and pathological), histogenesis and cell differentiation[2], common to all *Cnidaria*[3] or all *Eumetazoa*[4]. This task also includes stem cell analysis during differentiation[5] and the abstract analysis of morphogenesis in mathematical biology[6]. In some cases, simple morphological features unique to a given taxon are modeled (hypostome, tentacles, foot[7]). In the more general and not taxonomically limited cases described above, the results are extrapolated to the level of general biological principles. For example, paradoxically, it is *Hydra* that is used to model diabetic microangiopathy: by thinning the membrane of its "leg" a reactive effect in the vessels to the introduction of the excess glucose is simulated[8]. Despite the fact that morphogenesis models of the *Hydra* leg (for example, the so-called "zipper model"[8,9]) are strongly different from the formal models of angiogenesis[10], all of them are based on the reaction-diffusion (activator-inhibitor) model[11,12].

(2) As a model of intercellular and intertissue interactions[13,14], a model of tissue regeneration common to all *Metazoa*[15]. This modality reflects not only the processes of tissue formation and cooperative histogenesis, but also autophagy[16]. In terms of regenerative biology, one cannot but agree with the old paper from Nature, which stated that: "hydra can regenerate and regulate. If the head of a hydra is removed a new head regenerate at the anterior cut surface. This regeneration does not require cell division, but stems from a reorganization of the tissues of the hydra"[17]. And further: "The implication is that a head acts as a source of some substance that actively inhibits head formation, and possibly also

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supplies “positional information” to cells within the hydra. When a head is removed the gradient of inhibitor relaxes and, it is postulated, when the concentration of inhibitor has fallen below a threshold value, which is a function of axial position, new head formation is initiated”[17].

(3) As a model of the aging processes, including replicative aging[18-20], as well as longevity, at least for the similar organisms[21].

(4) As a model of biochemical signaling, in particular those associated with reactive oxygen species[22], as well as a neurocytochemical model of the signal transduction[23], proving the commonality of the signal transduction mechanisms in hydras with vertebrates.

(5) As a good model for studying the biochemical basis of invertebrate sensing, including photoreception[24] and chemoreception (see below). The hydra opsins are shown to be related to the opsins of higher vertebrates and humans.

(6) As a model of the organism interaction with its environment during ontogenesis, both normally and under technogenic influences[25,26], including ecotoxicological aspects[27-32]. This allows *Hydra* to be used as a more available alternative to the higher animals for (eco)toxicological testing, although this approach often raises reasonable objections[33].

(7) As a model of interaction between an organism and its microbiome[34,35], as well as a model of interaction with different infectious and parasitic agents[36].

(8) As a molecular genetic model for various fields, including environmental genomics[37], applied eco-toxicogenomics[38], as well as molecular phylogenetics and evolutionary developmental biology[39].

(9) Finally, as a publicly accessible educational object for the school experiments and institute practices/workshops in biology and ecology[40,41].

Based on the foregoing, development and introduction of the microscopic methods and techniques that allow to observe in real time various aspects of *Hydra* behavior is required. However, for the studies of hydras, standard surface optical microscopy is usually used as 30-40 years ago[42-44] and even earlier (since the times of A. Trembley). Therefore, it is necessary to create and implement publicly available methods of hydra microscopy in situ for all the users who need continuous observation of the hydra response to various environmental factors for studying the problems of hydroecology, biotesting, and modeling the effects of various environmental factors on hydra as a model object. We propose and implement lensless microscopic technique for these purposes.

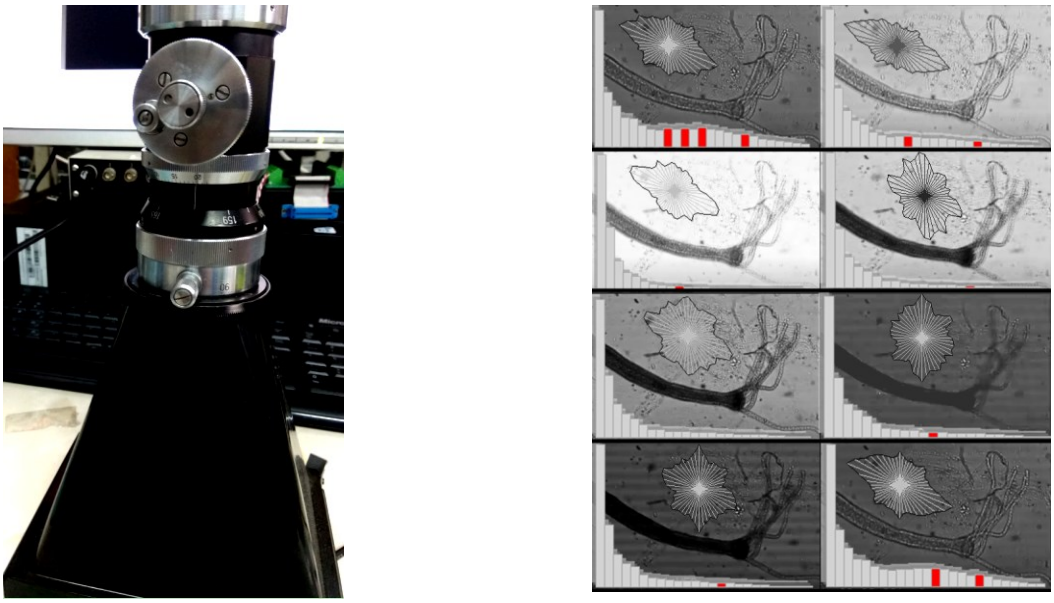
1.2 Criteria for selecting multiplexed lensless detection

What are the requirements for such a technique? A technique is needed that provides working at different wavelengths with contrast according to the criteria for distinguishing differently pigmented layers or tissues of *Hydra* as a polyp with the cell layers of different specialization. In addition, it is advisable to provide the possibility of phase and polarization control of the hydra image contrast (a transfer function of the detector). At the same time, this technique should not be extremely expensive and complicated (like hyperspectral microscopy or multilaser confocal scanning microscopy).

Obviously, it is necessary to ensure the possibility of simultaneous data acquisition from many hydras (in order to accumulate population statistics in synchronous experiments), but the field of view should not be too wide, like a binocular magnifying glass, since not the images of the entire population are needed, but the study of single hydras. That is, this instrumentation ideally should be so simple that it can simultaneously record tens or hundreds of hydras, which is impossible when using one expensive microscope. Consequently, we need a multi-channel but synchronously recording equipment with numerous detectors and microchambers for hydras (including those arranged in modules, where, in accordance with the experimental protocols, it is possible to change the temperature, ionic composition of the medium, toxicological contamination of the medium, pH, etc., up to microbiological and virological contamination). That is, we need a multichannel imaging system containing many data acquisition devices, in which it will be possible to connect various channels to the analytical devices without changing the parameters at the population level as a whole. It may also be useful to support a flow system (microfluidic or mesofluidic, as in the early organ-on-a-chip systems[45-51] and the ability to move the platform for analyzing orientation mechanisms[52].

2. MATERIALS AND METHODS

To implement the above principles, we have chosen a lensless microscope platform (one of the first designs developed by the author many years ago, initially analog with BNC connectors for output to a multi-channel ADC via a "quadroprocessor"/multiplexer), but subsequently digitized without changing the sensor, i.e. using a charge-coupled device. This type of lensless microscope was qualitatively different from the version with a CMOS sensor, controlled by a mobile phone processor[53,54], which made it possible not to strive for the highest resolution modern CMOS arrays (the last time developments in this area were carried out no later than 2011-2012[55-59]). This rather archaic handmade device is shown in Figure 1. By changing electrophysical parameters on the detector and the exposure time on a CCD sensor the photometric and densitometric characteristics of the frame (often displayed on vectorscopes or waveform monitors in IRE/voltage units) can be controlled. The frequency-contrast function of the sensor array differs depending on the spectral range/wavelength of the laser, therefore, by providing tuning along the wavelength or changing the range of the irradiation source or filter (for example, when rotating the interference filter), and using rastering when moving from one subband to another, one can provide simultaneous resolvometric and densitometric calibration and study of the detector signal and the object's own characteristics. As resolvometric (resolution measurement) indicators, one can use Integral Frequency Characteristics (IFC) and Integral Spatial Characteristics (ISC), calculated on the basis of 2D FFT[60] (this is a generally accepted practice from geography and hydrology to materials science and medical biophysics[61-70]). We carried out 2D FFT and real-time calculation of Integral Frequency Characteristics (IFCs) and Integral Spatial Characteristics (ISCs) using the QAVIS program (authors Goncharova A.A., Fischenko V.K., Pacific Oceanological Institute, Far East Branch of RAS, Vladivostok; https://oias.poi.dvo.ru/qavis/)[65-69]. The photograph of the lens-less instrument used in our experiments is provided on the Figure 1a.



(a) (b)

Figure 1. (a): A digitized lensless CCD microscope with a polarizer on the input optical path; (b): Automatic selection of the optimal configuration/operating mode of the installation for the analysis of hydra morphology using Integral Frequency Characteristics (IFC) and Integral Spatial Characteristics (ISC).

In this work, a fixed hydra specimen was studied, since 3D printing failed to provide a sufficient stability of lab-on-a-dish microfluidics to support *Hydra* in subchambers in an aquatic environment[70]. Subsequently, we could support aquatic organisms in commercially available transparent polymer (in particular, medical/diagnostic) fluidic chips on lensless microscopes.

3. RESULTS

We have tested various spectrozonal and channel-combined (including anaglyphic) versions of hydra imaging. The results obtained are publicly available at the videos listed in Table 1. The results of measuring the morphological

ordering of the test object (*Hydra*) and selecting the optimal mode for the detector response, in which this ordering is clearly visible, are shown in Figure 1b. In the top row there are manually selected optimal images on which it is clearly visible that ISC has a mono-axis orientation, and on the ISC histogram the program detects several well-defined bars that indicate the presence of statistically reproducible structuring/regularity of the object. The following images show the shift of the instrument from an optimal mode to a suboptimal one by changing the wavelength (spectrozonal range of the light source) and the projection angle of the LED beam with a known radiation pattern, as well as polarization on the polarizer which also affects focusing. As a result, multiple new maxima ("rays"/"poles") appear on the ISC, and the integral power of bars on the IFC drops dramatically, and the bars indicating regularity of the structure disappear or are reduced to one randomly preserved wandering peak. When returning to the measurement configuration close to the optimal one, the disappeared bars return, and the ISC again acquires a relatively sharp directionality and mono-axis orientation.

Table 1. Information on video files.

Images in different spectrozonal channels	Video
Spectrozonal lens-less imaging in B-channel.	https://youtu.be/Xt6PNtE3RqQ
Spectrozonal lens-less imaging in G-channel.	https://youtu.be/bJHDAueSDVs
Spectrozonal lens-less imaging in R-channel.	https://youtu.be/8C_eAp4ELvI
Anaglyphic lens-less imaging in G and B channels	https://youtu.be/Q4ywIdr1oaA
Anaglyphic lens-less imaging R and B channels	https://youtu.be/4N3hKh2q68w
Anaglyphic imaging (R+G channels) with polarization system POLMI A.	https://youtu.be/KNwswcuIkyE
Combined G+B channels with polarization system POLMI A.	https://youtu.be/UlOK9ljsvbw
Sequential lens-less imaging in R, G, B channels with polarization system POLMI A.	https://youtu.be/Xh9ytCf2UnI
Spectrozonal lens-less imaging in R, G, B-sequenced channels without accessories	https://youtu.be/oyTFIo4nPJ0

4. CONCLUSION

The image series in Figure 1b illustrate the changes in the quality of a lensless microscopic image with automatic changes in wavelength, contrast, light source brightness and line "duty factor" of the raster. In the short-wavelength range the resolution is shown to be better, but IFCs as well as ISCs directly depend not only on the wavelength, but also on the scattering and absorption of light when interacting with the microobject. Therefore, in some frames we can distinguish microanatomic features of *Hydra*, such as the tissue layers, while in the other we cannot. However, the results shown in Figure 1b indicate the possibility of objectively finding the optimal imaging parameters for registration of morphological features of *Hydra* by eliminating hardware artifacts or the intrinsic features of the detector transfer function in non-optimal modes. Due to the limited length of this paper, we cannot provide more than 30 pages of illustrations and data interpretation for different physiological states and morphological features of the test object *Hydra*. However, we look forward to submitting a full paper on this area of research in the coming years. Therefore, we avoid biomorphological and ecological specifics this short report.

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