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CEOS Environmental Science and Ecology

Research Article

Received Date: August 15, 2022
Accepted Date: September 15, 2022
Published Date: September 17, 2022

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Citation

Roshchina VV, Yashin VA, Kunyev AR, Podunay YA, Davidovich NA (2022) Fluorescent Analysis of Vitality and Stress State of Diatom Ulnaria Ulna Cells. CEOS Environ Sci Ecol 1(1):102

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Fluorescent Analysis of Vitality and Stress State of Diatom Ulnaria Ulna Cells

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[#]To memory of Dr .Valerii N. Karnaukhov

Abstract

Fluorescent analysis has been applied to the determination of vitality and stress state for living freshwater diatom *Ulnaria ulna* (Nitzsch) Compère (Bacillariophyta). In a population of the diatom cells it was possible to differ living, dead and stressory individuals by various luminescence microscopy including modifications such as laser-scanning confocal microscopy and microspectrofuorimeter. Their fluorescence spectra recorded by microspectrofluorimeter showed the chlorophyll maximum at 680 nm in living cells and at 520 nm in dead ones or in isolated frustules. Histochemical staining of the diatoms for the biogenic amines (dopamine, histamine) as stress indicators showed the presence of the compounds by fluorescent method at 460-470 nm in some cells.

Keywords: Biogenic Amines; Dopamine; Histamine; Laser-Scanning Confocal Microscopy; Luminescence Microscopy; Microspectrofluorometry

Introduction

In the studies of diatom algae populations, there is necessity to differ vital, dead and stressory cells. One of the approaches for similar studies may be fluorescent analysis their autofluorescence and emission after special histochemical staining. Among possible techniques are microspectrofluorimetry/microspectrophotometry and laser-scanning confocal microscopy which are still rarely used to study phytoplankton in the laboratory. In laboratory of Valery N. Karnaukhov, the first fluorescence spectra recorded with a microspectrofluorimeter patented in Germany, USA and Great Britain [1-3]. Apparatus was used in the study of intracellular metabolism in single cells and cell systems [4,5]. The technique applied in the studies of the dinoflagellate *Peridinium depressum* and the diatom Nitzschia longisissima [5, 6]. The attention was attracted to a fluorescence of marine diatoms as unique photosynthetic microorganisms that have a solid frustule.

Microspectrofluorimetry/microspectrophotometry and laser-scanning confocal microscopy were not used earlier for the analysis of freshwater diatom *Ulnaria ulna* (Nitzsch) Compère (Bacillariophyta) previously been assigned to the large and heterogeneous genus *Synedra* Ehrenber [7] and proposed to be useful for the laboratory cultivation and identification in the natural populations. The freshwater living diatoms visible in a light microscope used for the analysis of the development and reproduction [8, 9]. We aimed to apply microspectral analysis to this widely distributed and ecologically important diatom. Autofluorescence and fluorescence after the histochemical staining for stress biological amines dopamine and histamine – were analyzed.

Materials and Methods

Object and Cultivation

Samples were gathered in a small channel in Ghent (Belgium) in 2012. Single cells of an araphid diatom *Ulnaria ulna* (Nitzsch) Compère were isolated from the samples to start monoclonal cultures. During nine year, the last were maintained in a culture collection of the algae and microbiota laboratory, Karadag Scientific Station – Nature Reserve of the Russian Academy of Sciences [8, 9]. After that, clones 2.0419-A and 2.0903-A were cultivated in Pushchino laboratory of microspectral analysis of cell and cellular systems in Petri plates (3 cm in a diameter) in the medium which included 6.63, 6.51, 3.47 and 5 μg/l of K_2HPO_4, $CaCl_2$, NaCl, $MgCl_2$ accordantly and 2 μg/l of silicagel (*Merk*, Austria) as the source of silicon for the frustule formation. Frustules were isolated from organic materials by acid method as shown earlier [8,9].

Fluorescence Observation

The autofluorescence and fluorescence after the histochemical staining for biogenic amines was used as the test-reactions of the diatom cells on the object glasses (slides) as described earlier for unicellular probes [10,11]. All experiments were performed at room temperature 20-22 ^{O}C. The emission spectra were recorded, and images of living cells and their frustules were photographed by luminescent microscope Leica D 6000 B(Germany-USA-Austria), microspectrofluorimeter/microspectrophotometer MSF-15 (LOMO, Sankt-Petersburg) with photocamera *Levenhuk* M300 Base (USA). The images of living cells and separated volves were recorded and photographed by luminescence microscope Leica DM 6000 B (Germany-USA-Austria), microspectrofluorimeter MSF-15 (LOMO, Sankt-Petersburg) with photocamera *Levenhuk* M300 Base (USA) at ultra-violet light 360-380 nm excitation and laser scanning –confocal microscope *Leica TCS SP*-5 (Germany-Austria - USA), laser- 488 nm.

Fluorescent histochemical determination of biogenic amines (dopamine, histamine and serotonin) within cells , was carried out according to the methods primary described for animal cells and applied for plant cell as well [12,13]. Microspores were put on object glasses (slides) and moistened by drops of 1% aqueous solutions of 0.5 -1% solutions of glyoxylic acid for dopamine or o-phthalic aldehyde for histamine. After 10-20 minutes of staining with the reagent, samples were dried at 50-80°C during 5-10 min. Fluorescence reactions of forming products was studied under luminescence microscope *Leica* DM 6000 B or by camera *Levenhuk* M 300 (USA) at the excitation by light 360-380 nm. The fluorescence spectra recorded by microspectrofluorimeter MSF-15 (*LOMO*, Sankt-Petersburg).

Results and Discussion

To keep in mind that the algae have photosynthetic activity, we tried to study their autofluorescence related to chlorophyll using luminescence microscopy with modification such as laser-scanning confocal microscopy and microspectrofluometer.

Images In Laser-Scanning Confocal Microscopy

On Fig.1 single diatom *Ulnaria* cell is well seen in 488 nm laser of laser-scanning confocal microscopy as red-fluorescing structure unlike image in transmitted light.

Up to now a practical identification of diatoms at the species and generic levels is based on the morphology of the frustule valves [8,9]. During the vegetative phase of the life cycle, the cells of diatoms divide in two, and since they carry a protective silica frustules consisting of two flaps, one of which covers the other like a lid in a box ("box" model), each of the daughter cells gets one half. The second missing part is being completed. As a consequence, after division, one cell has the same size as the parent, and the second is slightly smaller. The cells are capable of both ordinary meiosis and sexual reproduction.

Autofluorescence has also shown the population of the *Ulnaria* cells in red and green channels of the confocal microscope (Fig.2). In red channels living cells emitted due to chlorophyll. If to see green channel only dead cells may fluoresce in green region. They have yellow-fluoresced structures which lack of chlorophyll.

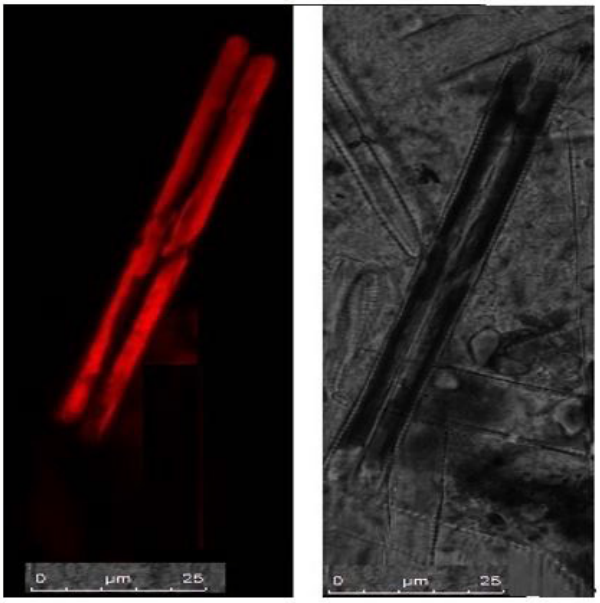

Figure 1: The single diatom *Ulnaria ulna* cell of laser-scanning confocal microscopy when excited by laser 488 nm (left) or in transmitted light (right)

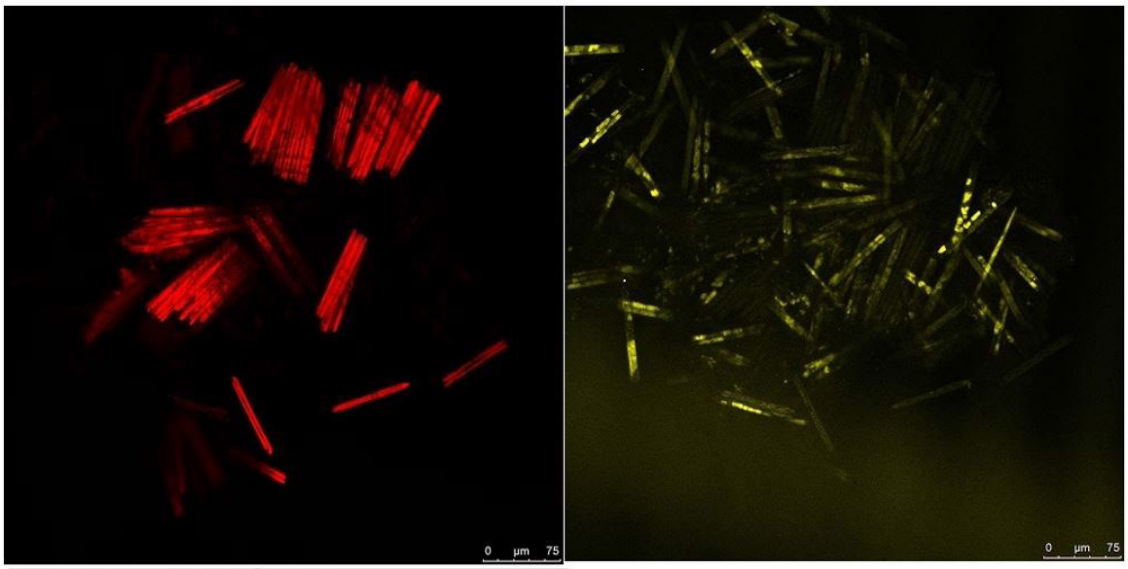

Figure 2: The fluorescing population of the *Ulna* cells in red (left) and green channels (right) of the confocal microscope. Laser 488 nm

Microspectrofluorimetry for A Determination of Living and Dead Cells

Living cells fluorescent in red (a) are fine seen in luminescence microscope (Fig. 3), and one can differ dead cells emitted in green (b) at 500-540 nm here. At UV excitation 360-380 nm fluorescence in blue was insignificant both in living and dead cells. Autofluorescence of alive cells has maximum at 680 nm which is peculiar to chlorophyll. Dead cells with destroyed chlorophyll fluoresce in green (520 nm). Within dead cells, there are greenish or yellow-fluoresced chloroplasts, with chlorophyll derivate. These effects make it possible to differentiate between living and dead cells. Frustules cleaned from organic material also demonstrated maximum at 520 nm in their fluorescence spectra. These structures seems to be a mixture of phenolic compounds and silicon.

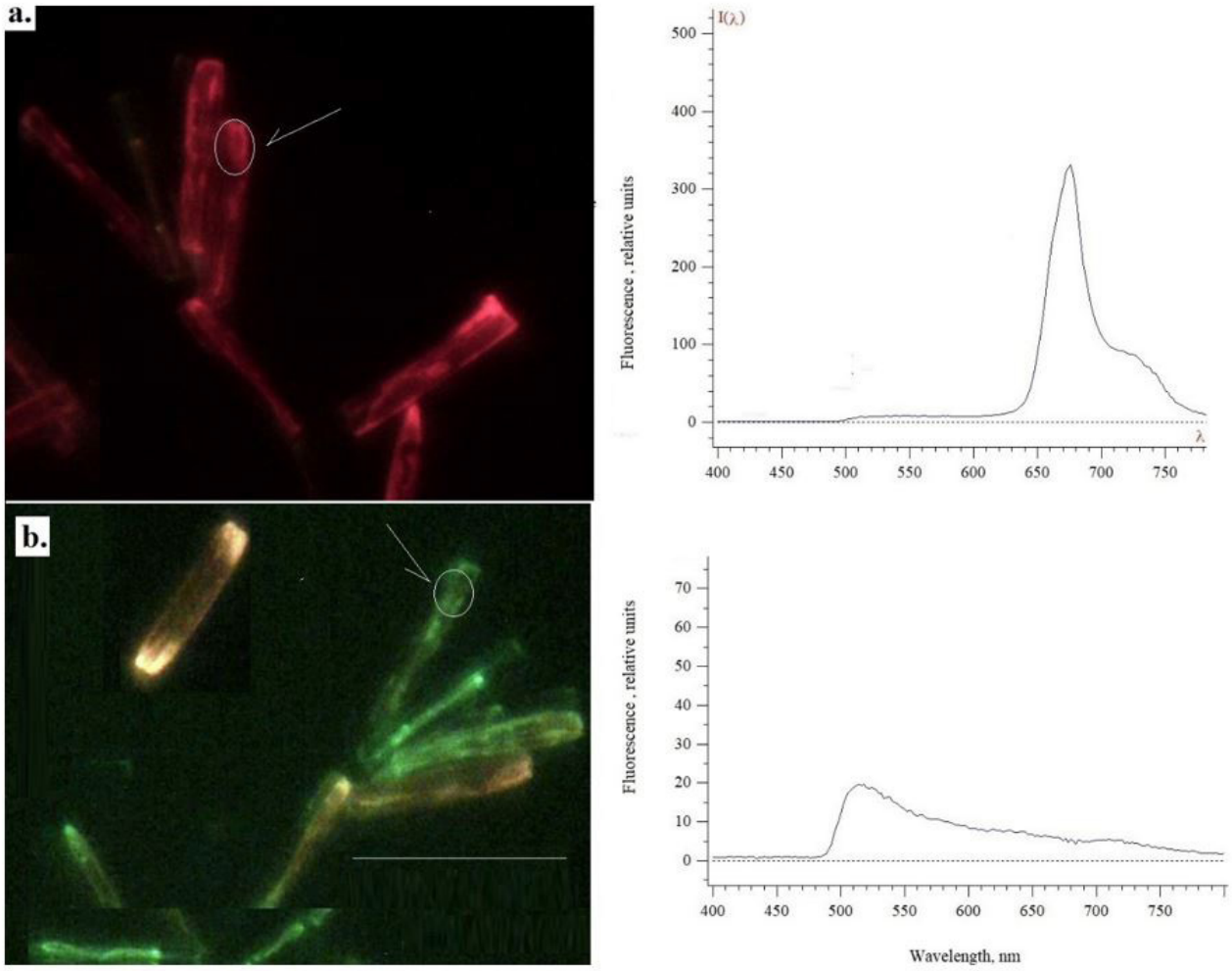

Figure 3: Microspectrofluorimetry of *Ulnaria* cells with images of fluorescence (left) and the fluorescence spectra (right). Excitation 430-450 nm. Arrow shows the part of single cell, from whom the spectrum has been recorded. a – red fluorescing cells ; b – green- fluorescing cells

As shown on Fig.3, living stick-looked cells fluorescing in red (a) are well seen in luminescence microscope, and one can differ dead cells emitted in green (b) here. At UV excitation 360-380 nm fluorescence in blue was insignificant both in living and dead cells. Autofluorescence of the diatoms clearly observed at the excitation 430-450 nm and has maximum 680 nm peculiar to chlorophyll. This effect permits to differ living cells from dead ones, which fluoresce in green. In second case, chlorophyll is destroyed, and the emission with maximum 520 nm belong to the frustules of diatoms. Individual isolated frustules also demonstrated maximum 520 nm in their fluorescence spectra. These structures consist from phenolic compounds in mixture with silicon.

Microspectrofluorimetry for Determination of Stress Biogenic Amines

Modern view to plant stressory mechanisms considers common agents in all kingdoms of living organisms – from microorganisms to plants and animals [12,13]. These are biogenic amines such as dopamine and histamine earlier found in plants [14].

By histochemical fluorescent method we could record also the presence of biogenic amines as stress indicator [11,12]. As seen in *Ulnaria* cells (Fig.4) dopamine and histamine are present in the individual cells that demonstrated the fluorescence at 460- 470 nm in the fluorescence spectra after the histochemical staining with the reagents glyoxylic acid for dopamine and ortho-phthalic aldehyde – for histamine [11,12]. The treatment completely masked weak chlorophyll emission at 680 nm. The fluorescence was observed mainly in blue spectral region. As a whole in this experiment, the fluorescence intensity higher, than in control before the staining. Amount of some stressory cells in the normal living population (percent per 100 cells) for dopamine was more than 10%, while smaller - for histamine. However, high concentration of histamine in single diatoms sometimes well seen basing on the intensity of blue emission. There is first approach to study biogenic amines in the diatoms by fluorimetry of individual cells. We suppose that will be following investigations of the stress amines in diatoms. A presence of the amines known as neurotransmitters in animals and also found in plants and microorganisms [14] is the field of great interest for the evolution of the irritability [13], beginning from ancient unicellular diatoms, where role of the compounds never studied. The luminescence microscopy permits to follow the investigations.

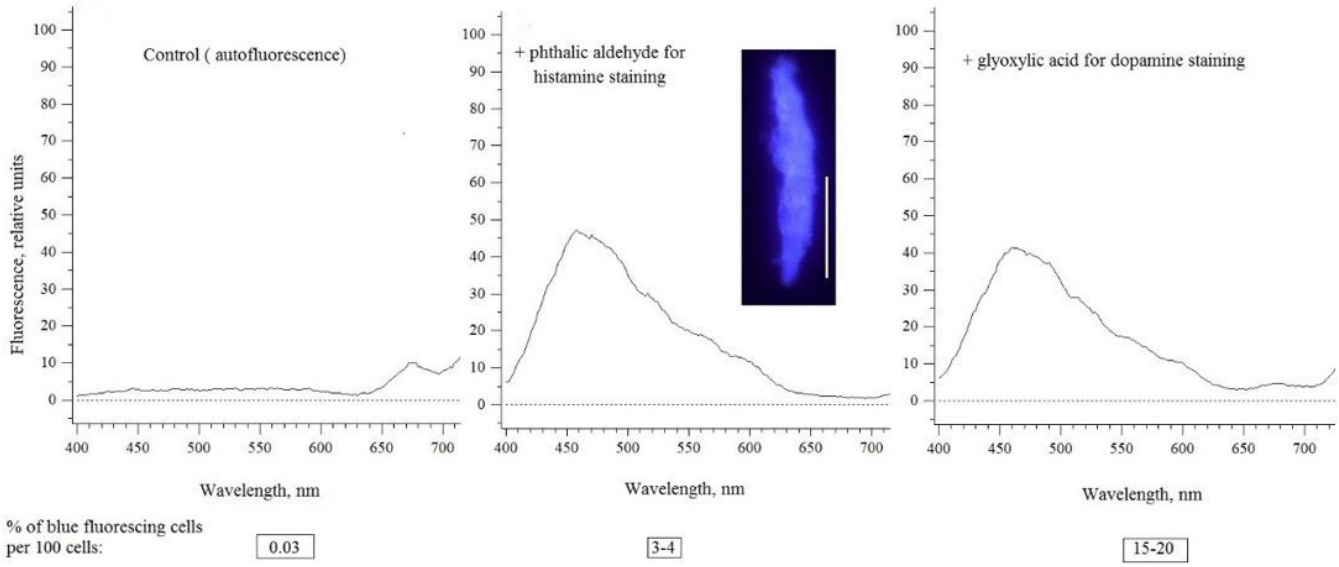

Figure 4: The fluorescence spectra of *Ulnaria* cells before (control) and after the staining for histamine and dopamine. Excitation 360-380 nm. Color image of blue fluorescence has shown in variant with histamine, bar = 40 μm. Under the spectra percent of blue fluorescing diatoms per 100 cells ±5 has been demonstrated

Conclusion

Our study shows that fluorescent methods may be applied in practical work with diatoms in environment if a laboratory has elementary luminescence microscope. Red emitted cells of *Ulnaria ulna* show that the object has chlorophyll and can live. Damaged or dead cells appear to observe in any cases due to weak reddish color or green color of their emission, relatively. If diatom losses chlorophyll only frustule may fluoresce. Population of diatoms may contain stressory cells fluorescing in blue after histochemical staining for dopamine and histamine.

Acknowledgment

We are thankful to the Optical Microscopy and Spectrophotometry core facilities, ICB RAS, Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences" for possibility to work with laser scanning confocal microscope.

Conflict of Interest

The Authors declare no conflict of Interest.

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