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CHARACTERISATION OF FLUORESCENT PENDANT DROPLETS

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Abstract. Laser induced fluorescence and amplification of fluorescence by laser pumped Rhodamine-6G solutions – having selected volumes – in water have been investigated. The results are comparatively presented for bulk and pendant droplets with emphasis on the amplification obtained in the second case. In the case of pendant droplets two different collection geometries of the fluorescence signal were used.

Key words: LIF, droplets, fluorescence amplification, Rh6G.

1. INTRODUCTION

Microfluidics is a research area that deals with generation and manipulation of small volumes of fluids. Systems based on small droplets are treated within the interdisciplinary field of microfluidics due to their potential applications in physics, biology [1], chemistry and pharmaceutical industry. Recent data on the use of the water solutions of medicines, laser irradiated are reported in [2–7]. In the last decades, in optics, an increased interest is oriented towards the microdroplets [8, 9] due to their spherical geometry with a near perfect surface induced by surface tension. Inside a sphere, an electromagnetic wave can suffer total internal reflection, taking place a confinement of light similar to that specific to an optical resonator. This phenomenon is known as "Wispering Galery Modes" (WGM) [8], [10–12].

A particular interest is devoted to the laser beams interaction with liquid microspheres, which can be resonant or unresonant [13]. The resonant interaction refers to the fact that the laser beam is partially or fully absorbed by the droplet's components and it is accompanied by phenomena such as laser induced fluorescence (LIF) [14], amplified spontaneous emission (ASE) [15], lasing emission [14, 15], or by photochemical induced modifications of the molecule's

structure [16]. We performed studies on resonant interaction with droplet containing different kinds of constituents: laser dyes for which lasing can be obtained [17], emulsions for which an enhancement of photoluminescence occurs or medicines whose molecular structures may be changed [16]. The unresonant effect takes place when the resonant effect is missing due to the lack of beam absorption by droplet's materials. The use of a very high energy laser beam properly tailored could be also responsible for the unresonant effects on droplets [13]. A combination for the resonant and unresonant effects on droplets obtained at incidence with a laser beam could produce both mechanical effects as well as light emission to the lasing limit.

Here, we report studies on the resonant interaction of a pulsed laser beam and pendant droplets containing solutions of Rhodamine 6G (Rh6G) in water.

2. MATERIALS AND METHODS

In our case, pendant droplets are constituted by a solution of Rhodamine 6G (Rh6G) in ultrapure water. Rh6G is member of xanthene derivatives family having a high fluorescence quantum yield, 0.95 at 25 ºC [18]. The Rh6G solution was prepared with ultra-pure water at several concentrations (10^{-5}M, 10^{-4}M, 10^{-3}M).

In Fig. 1, the experimental set-up used to expose the droplets to laser radiation is shown. The beads were generated using a computer controlled Droplet Generator System (MICROLAB 500 Dual Syringe Diluter, Hamilton). The droplet volume, used for these experiments, was 10 μl, corresponding to a diameter of 2.67 mm. The droplets were hanging on a hydrophobic, stainless steel capillary with the outer diameter 0.72 mm, so that the solution was not adhering to the inner or the outer surface of it. For droplet excitation, we used the second harmonic generation (SHG) from a pulsed Nd:YAG laser beam (Continuum, Surelite II) having available 532 nm wavelength, the pulse time width at half maximum 6 ns and the repetition rate 10 pps. The used energy per pulse was 2.2 mJ. The fluorescence signal was collected with a VIS optical fiber having the inner core of 400 μm. There were used two geometries for the collection of the fluorescence signal. In the first configuration, the optical fiber has been moved along a direction parallel to the optical axis from the input of the laser beam into the droplet to the exit of it from the droplet, with a step of 400 μm. In the second, measurements were performed by placing the optical fiber at different angles on the equatorial line of the droplet. The fluorescence signal was analyzed with an Acton Research Spectrograph coupled with an iCCD camera, controlled by computer. All the LIF spectra recorded were averaged for 10 pulses, for a better signal to noise ratio.

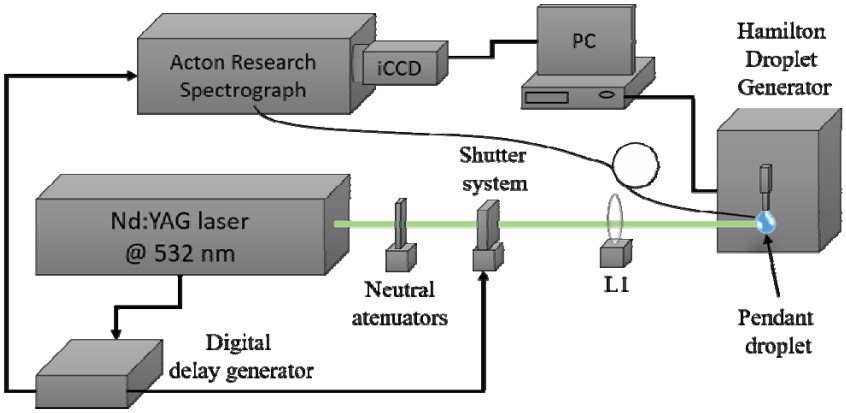

Fig. 1 – Experimental set-up used for irradiation of pendant droplets seeded with solutions of Rh6G. Evolution of the laser beam waist is represented in Fig. 2.

For comparison, LIF experiments were performed also on bulk samples, placed in a spectrophotometric cuvette, the liquid volume being 1 ml.

3. RESULTS AND DISCUSSIONS

The intensity and the width of the fluorescence bands depend on several parameters, such as the energy density introduced in the system, the solution concentration, the number of molecules excited by the laser beam and the temperature.

The laser beam is sent to the droplet through a lens (L1) with the focal length of 150 mm (Fig. 2a). For the same positions of the droplet and of the optical fiber, LIF signal was measured for several positions of the lens with respect to the droplet. The values of the fluorescence peak intensity as a function of distance between lens and droplet are shown in Fig. 2b. The intensity of the fluorescence is decreasing with the proximity to the focal point, since the power density is higher and localized on a small area and the number of the excited laser dye molecules decreases. Thereupon, in the focus point, the resonant effects are surpassed by the unresonant effects, and the droplet is destroyed [13]. After passing the focal point, the intensity is increasing, but it remains smaller than in the positions placed diametrically opposed.

For the next experiments, it was chosen a distance of 100 mm between the lens and the droplet. This corresponds to a beam waist of 2.5 mm. In such a way the beam is covering almost entirely the droplet that has the diameter of 2.67 mm. The droplet irradiated surface is a spherical cap with the area of 0.3 cm^2.

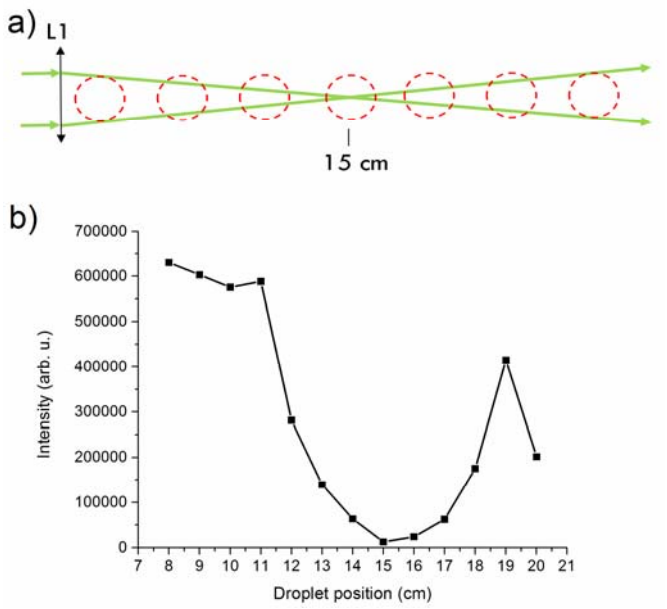

Fig. 2 – a) Cartoon depicting the evolution of the laser beam waist; b) intensity of laser induced fluorescence spectra of Rh6G 10^{-5}M solutions in ultrapure water taken in different focus positions.

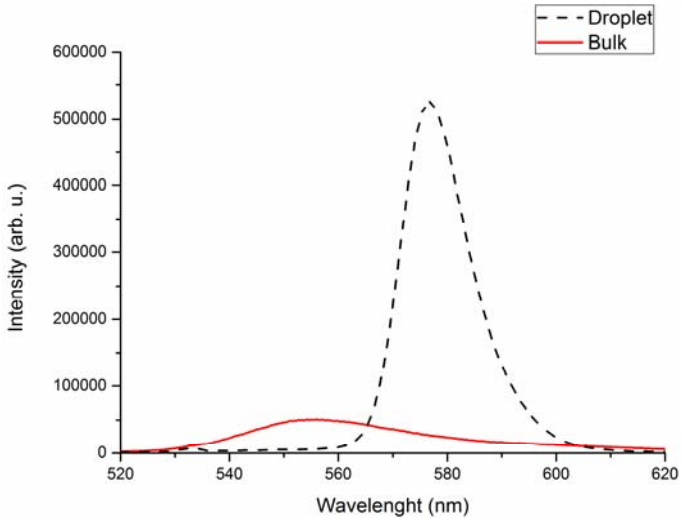

Fig. 3 – Comparison between fluorescence spectra emitted by a droplet and a bulk sample (placed in a spectrophotometric cuvette) containing Rh6G solution (10^{-5}M concentration).

In Fig. 3, a comparison is made between the fluorescence spectra measured for a solution of 10^{-5}M of Rh6G in ultrapure water in a bulk sample and in a pendant droplet. Both systems were irradiated with the same energy 2.2 mJ. Even if

the volume of the droplet is much lower than the volume of the cuvette, it is obvious that on the pendant droplets, the intensity of the fluorescence spectra is increasing with an order of magnitude with respect to bulk. Also the shape of the spectrum is changing and the maximum of the fluorescence band is moving to longer wavelengths. This is due to an amplification of the fluorescence signal inside the droplet. This effect is also reported in literature [12] where as active medium is placed in a microresonator with a spherical geometry. The sphericity of the droplet ensures the spherical resonant cavity, in which light could be confined by repeated total internal reflections at the surface of the droplet.

When the pumping beam is passing through the droplet, the intensity of the

$$I = I_0 e^{-\varepsilon l c}$$

beam is decreasing by the Beer-Lambert law, where I_0 and I are the intensity of the incident radiation and transmitted radiation, respectively, ε is attenuation coefficient, c is the concentration of the absorbent and l is the optical path length in the sample. The absorption coefficient (ε x c) depends on concentration and wavelength of the beam as presented in absorption spectra in Fig. 4. The absorbed energy contributes to excite the molecules of Rh6G from the ground singlet state to the first excited singlet state. From this state a significant number of the excited molecules could decay in the ground state through emission of fluorescence or other processes [19].

With respect to the Beer-Lambert law, after laser beam passes through the droplet, the intensity of the incident beam is attenuated N times, calculated in Table 1 for different concentrations.

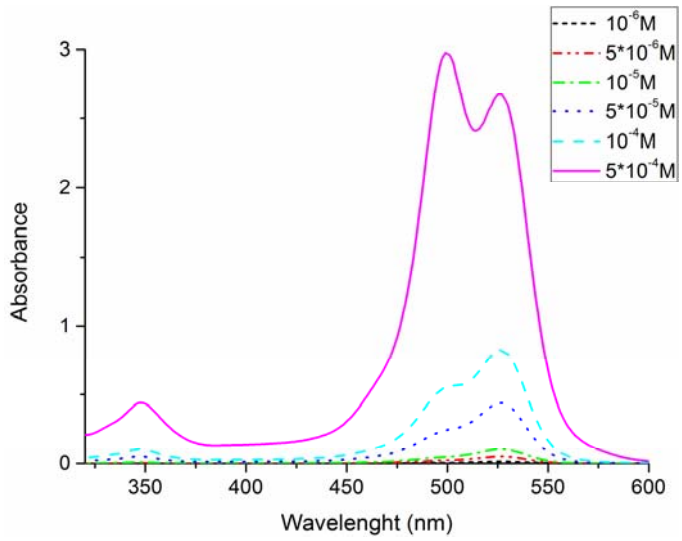

Fig. 4 – The absorption spectra of solutions of different concentrations of Rh6G in ultrapure water.

Table 1

Concentration [M]	Attenuation coefficient [$M^{-1}cm^{-1}$]	*d* (penetration depth) [mm]	*N* [%]
10^{-6}	126400	79.11392	96.76
$5 \cdot 10^{-6}$	93020	21.50075	88.60
10^{-5}	95620	10.45806	77.98
$5 \cdot 10^{-5}$	80308	2.49041	35.20
10^{-4}	75330	1.32749	14.10
$5 \cdot 10^{-4}$	49499	0.40405	0.16
10^{-3}	35868.6	0.2788	0.008

The radiations are emitted along a direction so that they will suffer a total reflection at the interface of water and air. The critical angle for total reflection at the interface water-air is 48.59°. This confined radiation could interact with other excited molecules and can give a stimulated emission. The emitted photons have the same frequency, phase and direction of propagation as the photons which produce them, and consequently we obtained the higher amplification of the signal compared to the bulk.

Studies were also made for concentrations of Rh6G in ultrapure water varied from 10^{-5} M to 10^{-3} M. LIF was measured in bulk, in a spectrophotometric cuvette (1ml of solution) and in pendant droplets. The LIF spectra measured for both samples are shown in Fig. 5a–b. For bulk, at the concentration of 10^{-5} M, it was obtained the typical fluorescence broadband of Rh6G. By increasing the concentration to 10^{-4} M the intensity of the fluorescence increases, due to the higher number of the molecules that are excited. For concentration greater than 10^{-4} M, the intensity of the fluorescence decreases, due to reabsorption processes and the formation of non-fluorescent dimmers [20]. With the concentration increase, the fluorescence bands are red shifted due to the reabsorption and consequently the reemission takes place at longer wavelengths [21].

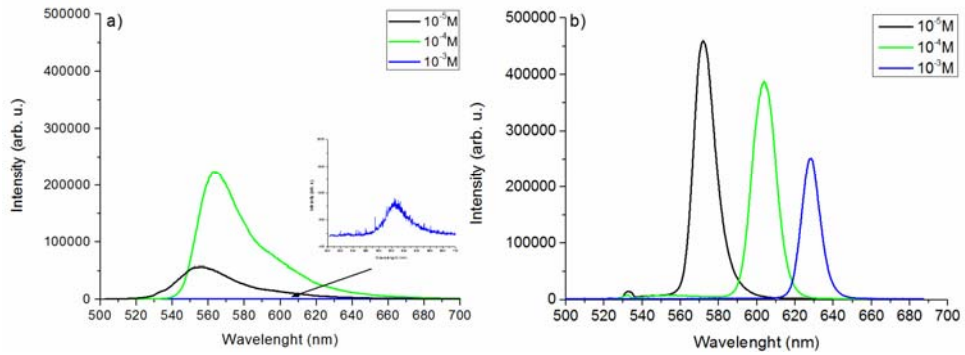

Fig. 5 – Comparison between fluorescence spectra of Rh6G solution emitted by droplet and bulk (spectrophotometric cuvette) at different concentrations.

By increasing the concentration of the droplet sample, the intensity of the fluorescence peak is slowly decreasing and the maximum is strongly shifted towards longer wavelengths.

Further, experiments were made for different signal collecting geometries. The position of the collecting optical fiber was changed along an axis which is parallel with the incident beam in the equatorial plane of the droplet, with a step of 400 μm. In Fig. 6, the emission spectra collected in three different positions (front, center and back as in Fig. 6a) are shown for a concentration of 10^{-5}M. In position 1, the spectrum obtained has three different peaks. The peak recorded at 532 nm, is the excitation laser beam, reflected on the droplet surface. The typical broad band of the fluorescence of Rh6G is obtained with the maximum intensity at 560 nm as it is also for the bulk sample case. The main difference between the fluorescence properties measured in droplet compared to bulk, is the rise of a third peak at 625 nm. This corresponds to the amplification of the fluorescence signal in droplet, which can be considered as a spherical resonant cavity [22]. When optical fiber moves towards the back of the droplet (positions 2 and 3), the reflection of the incident beam is no longer collected, the fluorescence intensity is drastically decreasing and only one peak is observed. The peak intensity of the emission with respect to the collecting position is presented in Fig. 6. The maximum intensity is near the position 1 and 3 and decreases significantly when the optical fiber reaches the center. The geometry of irradiation leaves a number of unexcited molecules that reabsorb the fluorescence, which leads to a lower intensity signal measured in position 2.

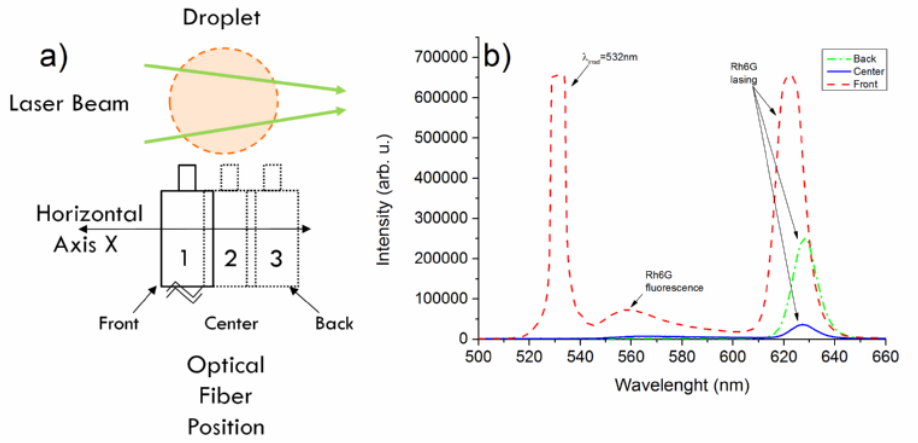

Fig. 6 – a) Cartoon depicting the optical fiber for measuring fluorescence at different positions on a droplet; b) laser induced fluorescence of Rh6G solution measured at different position on the equatorial plan of a droplet.

On the other hand, a red shift of the lasing peak measured from point 1 to point 3 occurs. This effect is characteristic for an increasing of concentration as it

was presented above. In this case, a concentration gradient is obtained in droplet due to irradiation. When the laser beam interacts with the first layers of the droplet, a part of the molecules are destroyed due to the high energy, which leads to a decreasing of concentration in this area, compared with the back of the droplet.

The LIF signal was measured also at different angles (Fig. 7a) around the droplet in the equatorial plane for droplet at 10^{-3} M concentration. It was observed that the intensity of the signal depends on the collecting angle and this can be seen in Fig. 7b.

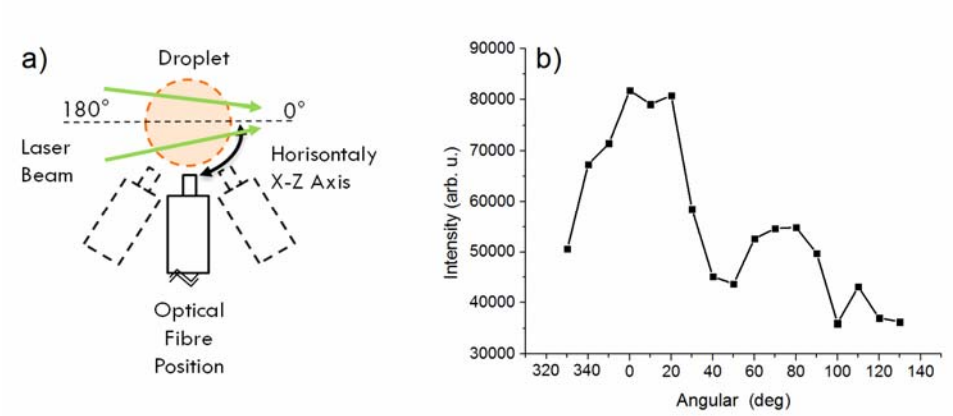

Fig. 7 – a) Cartoon depicting the experimental set-up for measuring fluorescence at different angles on a droplet; b) intensity of the signal measured at different angles.

The signal collection at 0° angle corresponds to the acquisition signal at the back face of the droplet on the direction of the propagation of the laser beam.

The acquisition at 180° is in the opposite position with respect to that at 0°. It must be kept in mind that the measurements are not possible at 180° due to the blocking of the pumping laser beam. The intensity of the amplification band showed a value higher near the 0° acquisition angle; then the intensity decreases according to a dumping pattern showing another maximum at around 90°.

4. CONCLUSION

The fluorescence of Rh6G solution has been investigated in pendant microdroplets. It was found that emission spectra for such systems has different characteristics compared with the ones measured for bulk. The main difference is the existence of a sharp peak at longer wavelengths and an amplification of intensity, even if the droplets contained a lower quantity of fluorophores. Also, the increasing of the concentration of Rh6G leads to a red shift and a slow intensity decreasing of the emission peak. In addition, the intensity and the position of the

laser induced fluorescence peak have a strong dependence on position of the droplet with respect to the focal lens and to the collection point.

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REFERENCES

1. A. Jonáš, M. Aas, Y. Karadag, S. Manioğlu, S. Anand, D. McGloin, H. Bayraktar, A. Kiraz, *In vitro and in vivo biolasing of fluorescent proteins suspended in liquid microdroplet cavities*, Lab. Chip, **14**, *16*, 3093 (2014).
2. T. Alexandru, A. Staicu, A. Pascu, E. Radu, A. Stoicu, V. Nastasa, A. Dinache, M. Boni, L. Amaral, M. L. Pascu, *Characterization of mixtures of compounds produced in chlorpromazine aqueous solutions by ultraviolet laser irradiation: their applications in antimicrobial assays*, J. Biomed. Opt., **20**, *5*, 051002 (2014).
3. M. C. Morán, T. Tozar, A. Simon, A. Dinache, A. Smarandache, I. R. Andrei, M. Boni, M. L. Pascu, F. Cirisano, M. Ferrari, *Toxicity study in blood and tumor cells of laser produced medicines for application in fabrics*, Colloids Surf. B. Biointerfaces (2015).
4. A. Simon, T. Alexandru, M. Boni, V. Damian, A. Stoicu, V. Dutschk, M. L. Pascu, *Interaction of solutions containing phenothiazines exposed to laser radiation with materials surfaces, in view of biomedical applications*, Int. J. Pharm., **475**, *1–2*, 270–281 (2014).
5. M. L. Pascu, B. Danko, A. Martins, N. Jedlinszki, T. Alexandru, V. Nastasa, M. Boni, A. Militaru, I. R. Andrei, A. Staicu, A. Hunyadi, S. Fanning, L. Amaral, *Exposure of Chlorpromazine to 266 nm Laser Beam Generates New Species with Antibacterial Properties: Contributions to Development of a New Process for Drug Discovery*, PLoS ONE, **8**, *2*, e55767 (2013).
6. M.-L. Pascu, V. Nastasa, A. Smarandache, A. Militaru, A. Martins, M. Viveiros, M. Boni, I. R. Andrei, A. Pascu, A. Staicu, J. Molnar, S. Fanning, L. Amaral, *Direct modification of bioactive phenothiazines by exposure to laser radiation*, Recent Patents Anti-Infect. Drug Disc., **6**, *2*, 147–157 (2011).
7. M. L. Pascu, A. Smarandache, M. Boni, J. Kristiansen, V. Nastasa, I. R. Andrei, *Spectral properties of some molecular solutions*, Romanian Rep. Phys., **63**, 1267–1284 (2011).
8. J. Ježek, Z. Pilát, O. Brzobohatý, A. Jonáš, M. Aas, A. Kiraz, P. Zemánek, *Liquid crystal emulsion micro-droplet WGM resonators*, 2014, Vol. 9441, 944108–944108–9.
9. A. Kiraz, M. Aas, A. Jonáš, *Optofluidic microlasers based on liquid droplet resonators for biophotonics*, SPIE Newsroom, Jan. 2014.
10. M. Tanyeri, R. Perron, I. M. Kennedy, *Lasing droplets in a microfabricated channel*, Opt. Lett., **32**, *17*, 2529 (2007).
11. M. Saito, H. Shimatani, H. Naruhashi, *Tunable whispering gallery mode emission from a microdroplet in elastomer*, Opt. Express, **16**, *16*, 11915 (2008).
12. J. Ward, O. Benson, *WGM microresonators: sensing, lasing and fundamental optics with microspheres*, Laser Photonics Rev., **5**, *4*, 553–570 (2011).
13. M. L. Pascu, G. V. Popescu, C. M. Ticos, I. R. Andrei, *Unresonant interaction of laser beams with microdroplets*, J. Eur. Opt. Soc. Rapid Publ., **7** (2012).
14. M. Boni, V. Nastasa, I. R. Andrei, A. Staicu, M. L. Pascu, *Enhanced fluorescence emitted by microdroplets containing organic dye emulsions*, Biomicrofluidics, **9**, *1*, 014126 (2015).

15. T. B. Rujoiu, A. Petris, V. I. Vlad, I. Rau, A.-M. Manea, F. Kajzar, Lasing in DNA-CTMA doped with Rhodamine 610 in butanol, Phys. Chem. Chem. Phys. PCCP, **17**, *19*, 13104–13111 (2015).
16. I. R. Andrei, T. Tozar, A. Dinache, M. Boni, V. Nastasa, M. L. Pascu, *Chlorpromazine transformation by exposure to ultraviolet laser beams in droplet and bulk*, Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci., **81**, 27–35, 2016.
17. M. Aas, A. Jonáš, A. Kiraz, O. Brzobohatý, J. Ježek, Z. Pilát, P. Zemánek, *Spectral tuning of lasing emission from optofluidic droplet microlasers using optical stretching*, Opt. Express, **21**, *18*, 21380 (2013).
18. R. F. Kubin, A. N. Fletcher, *Fluorescence quantum yields of some rhodamine dyes*, J. Lumin., **27**, *4*, 455–462 (1982).
19. J. R. Lakowicz, Ed., Principles of Fluorescence Spectroscopy, Boston, MA: Springer (US), 2006.
20. K. Cyprych, Z. Kopczyńska, F. Kajzar, I. Rau, J. Mysliwiec, *Tunable wavelength light emission and amplification in Rhodamine 6G aggregates*, Adv. Device Mater., **1**, *2*, 69–73, (2015).
21. R. Vogel, P. Meredith, M. Harvey, H. Rubinsztein-Dunlop, *Absorption and fluorescence spectroscopy of rhodamine 6G in titanium dioxide nanocomposites*, Spectrochim. Acta. A. Mol. Biomol. Spectrosc., **60**, *1–2*, 245–249 (2004).
22. M. Humar, I. Muševič, *Surfactant sensing based on whispering-gallery-mode lasing in liquid-crystal microdroplets*, Opt. Express, **19**, *21*, 19836 (2011).