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PHYSICS AND ASTRONOMY
NOVEL-RESULT

Xanthene-stained nanoparticles for phosphorescence anisotropy measurements

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Abstract

For the measurement of flow-induced microrotations in flows utilizing the depolarization of phosphorescence anisotropy, suitable luminophores are crucial. The present work examines dyes of the xanthene family, namely Rhodamine B, Eosin Y and Erythrosine B. Both in solution and incorporated in particles, the dyes are examined regarding their luminescent lifetimes and their quantum yield. In an oxygen-rich environment at room temperature, all dyes exhibit lifetimes in the sub-microsecond range and a low intensity signal, making them suitable for sensing fast rotations with sensitive acquisition systems.

Keywords: fluid dynamics; flow diagnostics; luminescence anisotropy; vorticity; phosphorescence

Introduction

In the field of Particle Imaging Velocimetry (PIV), stained Rhodamine B particles are commonly used (Raffel et al., 2018). Recently, a method for the measurement of flow-induced rotations of nanoparticles has been proposed (Schmidt, 2021). For the novel measurement method to be easily applied, it would be convenient to have phosphorescent particles that could be used in an existing PIV setup with only minor modifications. Rhodamine B (RB) and other species of the xanthene group like Eosin Y (EY) and Erythrosine B (EB) exhibit phosphorescence at different levels of efficiency (Benkovics et al., 2017; Bowers & Porter, 1967; Eads et al., 1984; Parker & Hatchard, 1961). In the case of EY and EB, the heavy ions bromine and iodine enable a higher phosphorescent quantum yield compared to RB. Also, xanthene dyes can form supramolecular complexes with cyclodextrins (Hartmann et al., 1996), potentially altering their luminescent properties. Khurana et al. incorporated RB in Sulfobutylether-β-Cyclodextrin (SbCD) and observed a reduced photodegradation of the dye (Khurana et al., 2018). In combination with the molecular rotor 9-(2-carboxy-2-cyanovinyl)julolidine (CCVJ), cyclodextrins have been successfully applied to alter the fluorescence lifetime (Schmidt et al., 2017; 2021). Thus, the potential influence of these nanocavities is to be investigated.

Objective

The optical properties of the three dyes in question were previously investigated under special laboratory conditions, such as in an ethanol solution, in the absence of oxygen, and at temperatures well below ambient. For experiments in fluid dynamics, the dye may have to perform in solutions with oxygen present and at ambient temperature. In this case, which reflects the many experimental conditions in fluid mechanics, the phosphorescence lifetime and a sufficient intensity signal are essential. To a lesser degree, the fundamental anisotropy of the dyes is of interest. Lifetime and anisotropy are to be examined for both the dye in solution and embedded in particles. In addition, the influence of a supramolecular structure with SbCD on the optical properties is investigated.

Methods

Solutions of the dyes, as well as stained nanoparticles, are analyzed. For the solution, Rhodamine B (Sigma-Aldrich, R6626, CAS: 81-88-9), Eosin Y (Sigma-Aldrich, E4382, CAS: 17372-87-1), Erythrosine B (Sigma-Aldrich, 198269, CAS: 16423-68-0) and SbCD (MedChem Express, CAS 182410-00-0) were used without further purification. Polystyrene nanoparticles were purchased from micromod Partikeltechnologie GmbH (Rostock, Germany), stained with RB (article 30-00-102, 250 *nm*) and custom made EY (article 14-00-102, 100 *nm*). In addition, EY stained PMMA nanoparticles were produced in-house according to Angelis et al. (2014) in variation EY4 with a diameter of 300 *nm*. The solutions have a concentration of 10 $\mu mol L^{-1}$ of dye and 2 $mmol L^{-1}$ SbCD. The suspensions have a particle concentration of 0.1 $mg\ mL^{-1}$. A schematic of the setup is presented in figure 1. A New Wave Research Solo PIV 120 Nd: YAG laser (532 *nm*, 100 *mJ*, 5 *ns*) is used for excitation of the samples. The laser beam is expanded into a sheet, and polarization is achieved by a Glan - Taylor Polarizer (Thorlabs, SM1PM10). On the emission side, the signal is filtered with a Schott OG550 longpass filter. A Cairn Research OptoSplit II Bypass is mounted onto an intensified and gated camera (PCO dicam pro). For the lifetime measurements, the OptoSplit is set to bypass mode for lifetime measurements. A Stanford DG535 delay generator is used for timing the acquisitions.

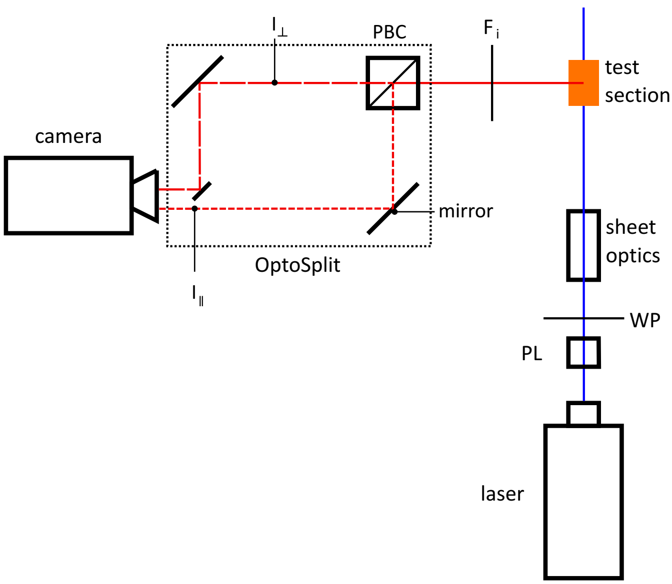

Figure 1. Experimental setup in polarization mode. The light's polarization is defined by the polarizer (PL). A $\lambda/2$ waveplate (WP) can be inserted and alters the polarization direction by 90°. A laser sheet optic is used to create a light sheet in the test section. The camera is equipped with a series of filters (F_i) to attenuate scattered laser light and other fluorescence from unwanted sources. The OptoSplit is operated with a linear polarizing beamsplitter cube (PBC), dividing the signal into perpendicular and parallel signals ($I_{\parallel}$, $I_{\perp}$). These signals are projected onto separate regions of the camera chip. The PBC is removed for lifetime measurements in bypass mode

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Results

The results of the lifetime measurements are best approximated with a two-exponential decay model. The results for the solutions without SbCD can be found in table 1. The entries with N_2 were flushed with nitrogen for 30 minutes before measurement. In contrast to Khurana et al. (2018) measurements with added SbCD are not presented but resulted in no change in lifetimes.

For the particles the results can be found in table 2 and figure 2. Note that the normalized intensity values cannot be directly be compared to table 1.

Table 1. Fits of the lifetime analysis for solutions. Intensity values are normalized to the smallest value.

	$I_1[a.u.]$	$I_2[a.u.]$	$\tau_1[ns]$	$\tau_2[ns]$
RB + N_2	699	372	129	580
RB	276	229	94	441
EY + N_2	118	82	114	553
EY	78	89	87	421
EB + N_2	18	11	125	734
EB	10	9	134	862

Table 2. Fits of the lifetime analysis for nanoparticles. Intensity values are normalized to the smallest value.

	$I_1[a.u.]$	$I_2[a.u.]$	$\tau_1[ns]$	$\tau_2[ns]$
micromod RB + N_2	47	36	108	533
micromod RB	40	37	100	492
micromod EY + N_2	7	7	125	630
micromod EY	7	7	112	545
EY4 + N_2	3	2	143	692
EY4	3	2	126	553

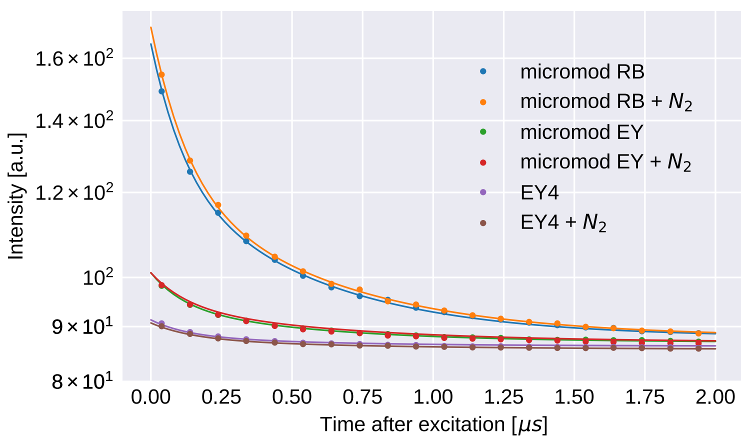

Figure 2. Semi-logarithmic plot of particle emission lifetime (dots) and the respective fits (lines)

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For the anisotropy r, one exemplary result is presented. It is computed from the two polarized signals as $r = (I_{\parallel} - I_{\perp})(I_{\parallel} + 2I_{\perp})^{-1}$. Further anisotropy measurements were not pursued due to the insufficiently short lifetimes for the considered fluid dynamical experiments. The spatially resolved, uncorrected anisotropy of EY4 can be found in figure 3.

Discussions

The results show a successful measurement of the long-lived luminescence. The two lifetimes are thought to be the delayed fluorescence and the phosphorescence. Overall, the intensity signals and the quantum yield are low with respect to the acquisition system, with multiple integrations on the sensor necessary for a sufficient signal-to-noise ratio. The presence of oxygen leads to a quenching of luminescence. In contrast, the application of SbCD does not result in a measurable change in the optical properties, indicating that no complex with the dye has been formed and quenching persists. In all cases, the emission lifetimes are in the sub-microsecond range. The low quantum yield of EB is remarkable, even though it also exhibits the longest lifetimes.

Conclusions

The lifetimes of the dyes remain below 1 μs. The sensitivity of the method relies on this lifetime being comparable to or longer than the inverse of the flow-induced microrotation rate (vorticity), which results in measurable vorticities of magnitude $1 \times 10^{6}\, s$ to $1 \times 10^{8}\, s$. For slower flow regimes, the dyes are not

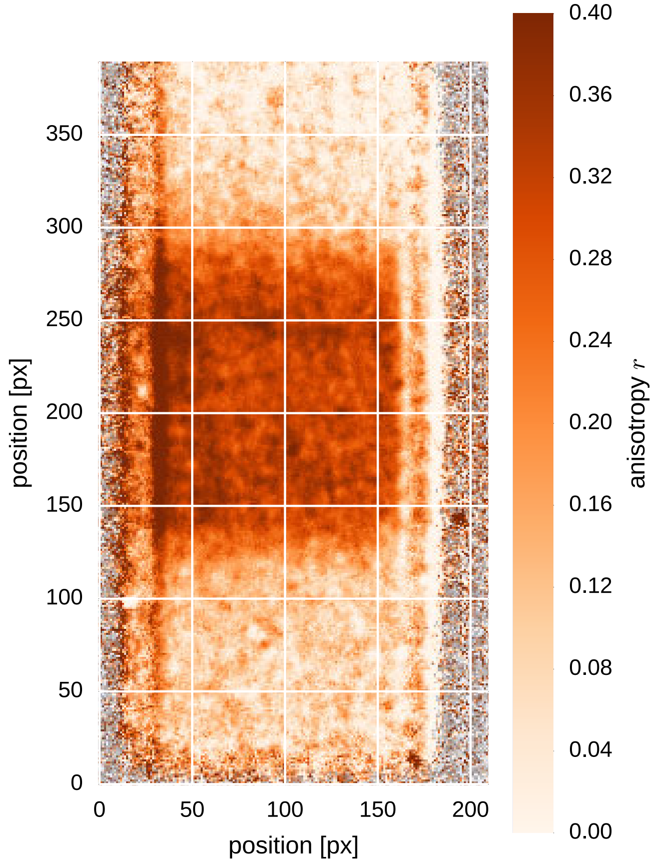

Figure 3. Spatially resolved anisotropy of EY4 in a 3.5 mL cuvette

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suitable. Even in cuvettes flushed with nitrogen, the lifetimes and quantum yields only increase slightly and stay well below the values for ideal lab conditions. Furthermore, the formation of a supra-molecule does not appear to influence the optical properties, indicating a continued interaction of the dye with its environment.

Acknowledgments. The authors thank Julian Schwarz for the conduction of lifetime experiments during his Bachelor's thesis.

Author Contributions. MJS and TR designed the study and wrote the manuscript. MJS performed the experiments and analyzed the data.

Data Availability Statement. The data are provided upon request.

Conflict of Interest. Markus J. Schmidt and Thomas Rösgen have no conflicts of interest to declare.

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Peer Reviews

Reviewing editor: Prof. Stefano Camera[1,2]
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[2]University of the Western Cape, Physics & Astronomy, Bellville, South Africa, 7535

This article has been accepted because it is deemed to be scientifically sound, has the correct controls, has appropriate methodology and is statistically valid, and has been sent for additional statistical evaluation and met required revisions.

doi:10.1017/exp.2021.12.pr1

Review 1: Xanthene-Stained Nanoparticles for Phosphorescence Anisotropy Measurements

Reviewer: Dr. Francesco Di Stasio

Date of review: 15 April 2021

Conflict of interest statement. The reviewer declares none

Comments to the Author: The authors present photoluminescene lifetime and anisotropy measurements of various commercially available chromophores and emissive nanoparticles. The results are very incremental but fit the scope of the journal.

Additional comments:

- The author should include the results with SbCD even if the cyclodextrin does not impact the photoluminescence properties

- The author refers to the PLQY as "low" throughout the text but they do not provide a number. Please, quantify the PLQY.

Score Card

Presentation

3.0 /5

Is the article written in clear and proper English? (30%)	3/5
Is the data presented in the most useful manner? (40%)	3/5
Does the paper cite relevant and related articles appropriately? (30%)	3/5

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Does the title suitably represent the article? (25%)	3/5
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Analysis

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Does the discussion adequately interpret the results presented? (40%)	3/5
Is the conclusion consistent with the results and discussion? (40%)	3/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	3/5

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Review 2: Xanthene-Stained Nanoparticles for Phosphorescence Anisotropy Measurements

Reviewer: Di Peng

Shanghai Jiao Tong University, School of Mechanical Engineering

Date of review: 20 May 2021

Conflict of interest statement. Reviewer declares none.

Comments to the Author: This article describes a useful work. It aims to extend application areas of flow-induced rotations' measurement. Please consider the following suggestions.

1. The authors need to add the detailed setup for lifetime measurement. As far as I know, using a PCO camera can't get a two-exponential lifetime result.

2. "The results show a successful measurement of the long-lived luminescence, the delayed fluorescence, and the phosphorescence." Is there any delayed fluorescence in those dyes? Please provide more explanations.

3. The author need explain the anisotropy's meaning in figure 3.

Score Card

Presentation

3.6 /5

Is the article written in clear and proper English? (30%)	4/5
Is the data presented in the most useful manner? (40%)	3/5
Does the paper cite relevant and related articles appropriately? (30%)	4/5

Context

4.0 /5

Does the title suitably represent the article? (25%)	4/5
Does the abstract correctly embody the content of the article? (25%)	4/5
Does the introduction give appropriate context? (25%)	4/5
Is the objective of the experiment clearly defined? (25%)	4/5

Analysis

3.4 /5

Does the discussion adequately interpret the results presented? (40%)	3/5
Is the conclusion consistent with the results and discussion? (40%)	4/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	3/5