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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Zhanna Kozyreva

PhD Program: Materials Science and Engineering

Title of Thesis: Fluorescent photoconvertible polymer microcapsules for cell labeling and tracking

Supervisor: Professor Gleb Sukhorukov

Name of the Reviewer: Dr Andrei Sapelkin

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**No conflicts of interest** **Date: 16-09-2024**

The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevance of the topic of dissertation work to its actual content
- The relevance of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications

The summary of issues to be addressed before/during the thesis defense

This project deals with development of photoconvertible microcapsules for the purpose of cell labelling and tracking. I found the overall project structure appropriate for the purpose with the thesis describing well the purpose, methodology and the key findings of this work. The structure of dissertation is very much appropriate and reflects well the work carried out under this project. The topic of the thesis is certainly relevant to the actual content of the dissertation also. The methods used to deliver the main goal are appropriate, well justified and the work is executed very well. The results reported in the dissertations can certainly be described as state-of-the-art and are competitive at the international level as also reflected by the results already published. There is clear relevance of the results reported in this work to the biomedical sciences and an excellent potential for practical applications.

Below are my comments on the content dissertation and suggestions for the changes (mostly minor) that should be made before this work is presented at the dissertation defense.

There are some very minor English language idiosyncrasies (ad a few typos) that I would like to see corrected, for example in the abstract, "Optical labeling of cells is usually performed via genetic modification for the following generation of photoconvertible proteins" should be changed to "Optical labelling of cells is usually performed via genetic modification to follow generation of photoconvertible proteins"; in page 7 "Moscow State University, Physical Department" has to be changed to "Moscow State University, Physics Department" etc. Please check again for English language throughout.

In the Abstract there are very good introductory remarks, problem statement and brief description of the methodology to address the problem. However, there is no information about the main findings: e.g. We found that …" or "we show that …". Abstract should essentially be a shortened version of the conclusions summarising briefly the main results of the work.

In page 63 the in Figure 2.21 it is nearly impossible to spot the difference between Cy5.5 and Cy3.5 dyes – please highlight the difference if the figure between the two dies. In the same page, the Cy7 is defined as "far-red channel" dye (835 nm). This should read "infrared channel" as 780nm-1mm is defined as infrared range (with 780-2500 nm typically referred as near-infrared range (NIR)).

Concluding remarks to the section on Cyanine dyes are very good (and welcome) in page 64 as they summarise the current understanding of the photoconversion mechanism. However, the following section on Xanthene dyes does not have the corresponding remarks that summarise understanding of photoconversion in that system, which begs the question of why they are even considered.

In general, I would suggest reviewing the structure of each section of the dissertation to make sure that each chapter, section, subsection etc. contain introductory remarks, concluding remarks summarising the main findings of the chapter/section/subsubsection and a sentence introducing the next part (e.g. "in the next section we will consider THESE, because of THIS and THIS" to maintain the logical flow across the thesis. For instance Section 2.3 ends abruptly in description of mechanism of photoconversion of various dyes, but there are no concluding remarks: what did we learn about the nature of various dye photoconversion and how is this relevant to the goals of this project?

I also found it odd that subsubsections (describing specific dyes) of subsection 2.3.2 are not enumerated, hence the corresponding parts do not appear in the Contents.

In page 77 there is generic statement "Nevertheless, such fluorescent markers have their disadvantages and limitations that should be taken into account, such as leakage, dilution with cell division, photobleaching, and cytotoxicity [21,22]. Also, staining of the nearby non-labeled cells is common [23], potentially distorting research results." This is far too generic and it is not clear why one should worry

about any of these and go onto development of alternatives. In other words, how these existing problems prevent further progress in relevant fields and what are the practical consequences of distorting the results (e.g. to patient health, hypothesis testing, development of new theories and/or methodologies etc.)?

In page 103 in third line the reference to the figure is missing – it appears as “Figure ??”.

In page 111 the units are missing in line 3 “± 0.5” – presumably microns? In the same line “which is almost two times less than that the initial capsules.” should be changed to “which is almost half the size of the initial capsules.”

In page 124 one of the dyes is abbreviated as PyB in the text, while it appears as PyrB in Figure 4.16.

In page 128 it is reported that “The increased signal after photoconversion appeared in the range of 505–540 nm for RhB and Rh6G excited by the laser with a 488 nm wavelength. At the same time, no such effect was observed for PyB.” It would be good to have some lines in the text to explain/hypothesise why no such effect was observed in PyB as it seems to contradict to the data in Fig 4.20j where there is an effect of increased intensity in 505-540 window.

In Fig 4.21 it would be useful to label the graphs with the names of the corresponding dyes for clarity.

In page 132 change “Also, after the following UV irradiation…” to “Also, after the UV irradiation…”.

In the concluding section (Chapter 7) it is stated that “The encapsulated dyes exhibited robust fluorescence after thermal treatment, except for TR, which did not withstand high-temperature treatment.” I think it is essential to add information on the temperature range as otherwise the reader has to search for the answers throughout the thesis.

In the same page (183) the statement that “RhB and Rh6G were found to be photostable and had remarkable photoconversion properties …” is far too generic: in what way are the found properties are remarkable – e.g. perhaps it would be important to mention at this point that photoconversion can be done with some colour/wavelength precision, enabling individual labelling of cells in large populations and/or multi-coding when more than one capsule is internalised by a cell, etc.

In the same page there is another very generic statement that “…detection parameters are more suitable for the Leica confocal microscope laser set. “. In what way are they “more suitable”? Please provide some details. Actually, I don’t think this is do with Leica system per in general, but perhaps the specific Leica system used in this work.

Provisional Recommendation
☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defense*
☒ *I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report*
☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense*