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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Julijana Cvjetinovic

PhD Program: Physics

Title of Thesis: Optical and mechanical properties of diatom algae and related materials

Supervisor: Professor Dmitry Gorin
Co-supervisor: Professor Alexander Korsunsky

Name of the Reviewer: Dr Andrei Sapelkin

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date: 08/09/2023**

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

The quality of thesis in my opinion is at appropriate level for the PhD and my assessment is further supported by the number and quality of the outputs (i.e. publications) produced by the candidate. The topic of the dissertation is certainly related to the thesis content with an impressive amount of material covered both in the literature review and in experiments. The content is undoubtedly scientifically significant at international level, delving into and delivering state of the art methods in characterization of diatom systems. I have little doubt that results of this work will find applications in several fields, most obviously in carbon capture and recycling, sensing applications and, perhaps, MEMS.

At the same time there is a number of structural and content changes that in my opinion are required to meet the standard of the PhD level dissertation. I outline these below together with the questions that I would like to address during the defense.

Abstract must contain a brief description of motivation for this work/problem statement, key methods/approaches used and summary of the outcomes. An Abstract is, essentially, a short version of the Conclusions and one expects to find in the Abstract a description of main findings: e.g. "Diatoms are a very interesting/important systems because …. However, our understanding of these systems is till incomplete, particularly in the area …" or "The following properties of these systems have not been studied/poorly understood/etc… There are following outstanding problems … In this project we used/develop the following methodology/approach/etc. to investigate/to deliver a paradigm shift etc … We found/discovered that …". The current version of the Abstract is far too generic and it is not clear to me from the Abstract what the motivation for this project was beyond nanoscale objects serving as sources of inspiration and that it is important to properly (what does that mean?) monitor diatoms to maximise their performance in CO2 capture (was that the main purpose of the project – if so, was the amount of CO2 captured by diatoms correlated with any other properties? What conclusions were made in that regard?). There are statements such as "Interrogating the structure and properties of diatoms down to nanometer scale leads to breakthrough advances reported here …" but no information is provided on what these advances are. What are the key findings of the project? Therefore, during the defence I would like to explore the motivation, methodology and the key outcomes of the project.

Much of the same arguments apply to the Introduction section. The purpose of Introduction is to provide background to the problems/challenges addressed in the project, current state of research and a clear picture of the challenges and approaches proposed to address them employed in this project. What I currently see in the introduction is that the motivation for the project is derived from:

1. Using diatoms for CO2 capture, hence their need to be grown in large quantities under optimised conditions. Correspondingly, I'll be looking to see analysis (e.g. original or in the Literature Review) of diatom performance in the context of current CO2 capture targets set by governments or international bodies. I'll also expect to find the methodology and experiments in the thesis that address that.
2. Diatoms provide high-performance optical and mechanical systems with potential use in new sensors and devices. Correspondingly, I would expect to see analysis of needs for such capabilities in the Literature Review, including explanation of why diatoms (as oppose, for example to man-made materials/systems) can provide a solution, followed by experimental evidence for their performance.
3. Modification of surfaces of diatoms to further improve their performance in sensing applications. Correspondingly, I would expect to see analysis of sensing applications that may require use of diatoms and justification that current man-made material/systems fall short of what is required. Again, I would expect to find evidence of such capabilities on the thesis.

I note that some of the statements as to the purpose of the project can be found in the Introduction, but they are currently rather generic and not supported at all by references. In other words, if there is a claim that there is a need for using diatoms for CO2 capture, there should be references to work that supports this statement. If such work is not present, then there should be some rudimentary analysis showing such a need. The statement says that "Hence, this thesis undertakes a comprehensive investigation of diatoms on three levels, encompassing the study of colonies in suspension, individual living diatoms, as well as purified frustules and diatomite powder, as schematically shown in Figure 1." At this point I would have expected the end of the Introduction. However, it then continues with rather generic convoluted text adding more objectives:

A. Fill the gap in understanding mechanical behaviour of diatom frustules.
B. Investigate the role of hierarchical structure in determining the optical properties of frustules.

These do not look to me like objectives in their own right, but rather form part of addressing 1-3 above. In other words. In order to achieve objective 2, one as to do A and B. However, currently Introduction suggests that there are distinct two sets of objectives – 1-3 and A-B – that are addressed in their own right. I would like this to be clarified in the Introduction.

I found that a large part of the Literature Review (LR) has been spent establishing the taxonomy of diatoms – I felt it may have been quite unnecessary: candidate should have explained instead what specific diatoms are subject of this project and how they fit into the overall diatom family, justifying/explaining the choice of the specific diatom types for addressing the project objectives. The purpose of the LR is to provide an overview of the current state of research in diatoms, building up to the key outstanding problems that will be addressed within the project, to provide a brief outline of the methods/approaches that will be used to address them and why. Some attempts to provide justification are made – a good example of that is in Fig 23 where potential optical applications of diatoms are summarised. However, it is not clear why one would want to utilise diatoms in contrast to, for example, man-made materials already capable of serving these applications. What advantages do diatoms provide? This style of writing is repeated throughout the LR where potential applications are proposed without demonstrating the actual needs for using diatoms. Sections 2.5 and 2.6 are examples of that style whereby a number of applications are proposed, but it is not clear why diatoms are a system of choice to address them. For instance, I would have expected to see some arguments involving the intricate hierarchical structure and morphology of diatom's silica structures that can be used to address a specific problem (or a class of problems) because the corresponding man-made systems are too inefficient/too complex (or impossible) to manufacture. E.g. why would one want to use diatom shell as MEMS device for high sensitivity microphones when modern MEMS research and industry can already provide very capable solutions? To conclude, a Literature Review must contain the summary of the review of published material, a problem (problems) statement and a brief description of the approach proposed to address the problem (problems, issues) – this is not currently present with the Literature ending abruptly with the description of parametrisation of eigenfrequencies. This is something that I would like to explore in some details during the defence.

Chapter 3 generally provides sufficiently detailed information to understand the experimental techniques, materials and methods used in this project. However, from section 3.8.1 it is not clear what specific in-house code or any other software were used for numerical simulations. There is no justification for truncation of the Fourier series to 97 harmonics. This section would also benefit from a schematic of the geometry of the calculations indicating the structure and the direction of EM light wave (e.g. how Z direction was aligned with the light propagation and sample geometry?).

In Chapter 4 a selection of existing methods for monitoring diatom grows conditions are introduced and their advantages and disadvantages are described. The candidate then proposes two new methods for monitoring: RSOM and Fluorescence (FL). However, it is not discussed these two methods address any shortcomings/disadvantages of the ones described earlier.

The results of the monitoring of a selection of diatom cultures by the RSOM and FL method are presented and compared to absorbance and radiant efficiency (how was this calculated?) using IVIS platform. However, from section 4.2 it is not clear how absorbance and fluorescence spectra were collected. Furthermore, it is said that the latter two were used to " … gain better understanding of state of diatoms during cultivation and their growth phase…" I found this statement rather generic to the point of being meaningless - what specific information did these two methods provide that RSOM and IVIS could not? Furthermore, it transpires further in page 130 that spectroscopic measurements where actually used to prove capabilities of IVIS and RSOM. This need clarification: was the purpose of this work to gain understanding or was it used as a benchmark to prove IVIS and RSOM? If both, then this should be stated at the start and use of absorbance and FL as benchmarks must be justified – e.g. are these methods more sensitive then IVIS/RSOM in monitoring diatom growth? If so, why are they more sensitive?

Also, I could not understand the difference between monitoring diatom growth (sections 4.2, 4.3) and monitoring diatom concentration (section 4.4) – are these not related (i.e. increase in concentration is due to growth)? What was the purpose of monitoring the concentration? I suspect the answer to the last question can be found in page 142 where it is stated that measurements of concentration can be used to evaluate ecological well-being and productivity of biomass. I would expect to find that statement at the beginning of the section to provide a rationale for concentration measurement using RSOM, which will then naturally address my the questions above. The same applies for section 4.4.2, where no rationale is given for using LED set up for concentration measurements. I guess that this is because LED set up is cheap and portable compared to RSOM – if so, this need to be stated at the start of the section. I also found it rather confusing that the data in this section are presented in way different to that of section 4.4.1, so direct comparison between concentration measurements using RSOM and LED set up is difficult. There is no comparison of RSOM and LED set up either in their performance as I would have expected, instead the latter is compared to absorbance and FL measurements – it is not clear to me why.

The purpose of section 4.5 is unclear – why division of diatoms needs to be monitored? What does one expects to learn from that?

In Chapter 5, section 5.1 one must provide rationale for using SEM, CLSM and FLIM for observing diatoms – i.e. what information each of these methods (and their combination) provide that enables better/faster understanding/classification of diatoms? The section must be concluded with a statement that "In this chapter we use SEM, CLSM and FLIM to monitor structure and morphology of diatoms". Furthermore, in the current version the Summary (section 5.5) is not informative and does not reflect the main findings of each method of visualisation of diatoms. In fact, what I would have expected to see is that SEM provides excellent capabilities to study diatoms down to nanoscale, but can only yield surface information on dead samples. CLSM can yield 3D visual information in live and/or dead samples while FLIM can target specific fluorophores, avoid influence of fluorophore concentration on the image contrast, improve penetration reach, potentially capable of assessing local pH, viscosity and concentration of chemical species of interest, etc. Also, there are no concluding remarks to any of the SEM, CLSM and FLIM sections. Those concluding remarks should then be summarised in the Summary.

In Chapter 6 there are issues similar to the ones I already described above: the purpose of SEM and AFM measurements and of Young's modulus is unclear form the outset as no rationale is provided at the start of the chapter. There are no concluding remarks to sections, hence it is difficult to understand what the key outcomes of the measurements are, there is no clear logical connections between the sections: why study of morphology is followed by AFM/AMAFM, followed by compliance analysis, followed by in-situ SEM indentation, followed by studies of vibration properties. How does all this fits into the coherent picture? Summary is again very much generic and doesn't provide a clear picture to what end all the above work has been carried out.

The introductory part of Chapter 7 is far too generic. It is not clear what changes to the wall geometry (e.g. numbers of layers, wall thickness, porosity, etc.) will be investigated. From section 7.1 I understood that the optical effects of two key factors were investigated: i) "physical dimension of the periodic structure" and ii) the "refractive index contrast". It is not clear to me from the description what is meant by i) – what is meant by that term (i.e. is it simply the size of a diatom, wall thickness, wall geometry, etc.)? This needs clarification. I also do not understand what exactly is meant by ii) – the contrast should be determined by the difference between the refractive index of silica and of air (for dried diatom shells, for example), but does ii) also include changes in the average refractive index of silica layers due to porosity? More details are required in this part to explain clearly what will be studied. In page 207 the structural parameters of the diatom structure are introduced, but without these parameters appearing in the corresponding schematic, it is difficult to tell what they correspond to. In page 209 the z-component of the Pointing vector is mentioned and diffraction in all channels at normal light incidence are discussed – again, a schematic of the setup would be essential here to understand the geometry of calculations. In the same page there appears a statement that "A dual-periodic photonic lattice due to diffraction increases the transmittance of light …" Again this statement is far too generic without specific parameters of the photonic lattice (e.g. its dimensions). I did not understand the significance of Figure 75 and the corresponding discussions in context of the results reported in Figure 74. I also couldn't understand what is meant by "For this purpose, we collected the absorbance spectra …" in page 210 as the purpose doesn't seem to be stated. In page 222 I do not understand the meaning of the sentence "The presence of these bright areas signifies the constructive interference of light waves and provides a foundation for further analysis of the underlying physical mechanisms governing interference.": it is quite clear what the physical mechanism of the interference is - it is the wave nature of light, so I suspect this sentence may be about something else. Again, the Summary of the section is rather generic – it is not clear what specifically has been learned for the simulations apart of the somewhat obvious result that interference on array of holes provides a certain intensity distribution. How is this distribution related to the geometry of the diatom? Does it help diatoms to optimise the use of available light? Is there a relationship between the whole size and density, and the layer spacing in diatoms?

In Chapter 8 it is not clear what rationale/motivation was for modification of diatoms with gold nanoparticles. It further transpires that the purpose was to use diatom/Au hybrid system for SERS measurements, however, it is not clear why such SERS system should be a good/viable alternative to already existing man-made SERS platforms. The summary of the section doesn't provide specific details on the levels of SERS enhancement.

I found Chapter 9 to be rather mixed as it provides some conclusions that are far too generic, while others are very specific (here we find out the level of SERS enhancement, for examples, that doesn't appear in the summary of the corresponding chapter), particularly considering the complexity and amount of research carried out. What one expects to see in the concluding section is a collection of clear statements (typically collected from the corresponding experimental chapter summaries) of the main findings and of their significance. How the results reported in each section changed our

understanding of diatoms? Why these results provide a significant contribution to the field? How they are likely to impact diatom research?

I should also mention some formatting problems. Having captions on pages separate to the actual figures is unacceptable and needs to be corrected. My general suggestion regarding the structure would be to check that Abstract is a short version of Conclusions and that Conclusions contain the brief summary found at the end of each experimental chapter (Chapters 4 through to 7). Summaries for each of the experimental chapters must contain information about specific findings and their significance in the context of the Literature Review and/or introduction to the section.

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*