

**Referee's report on PhD Thesis of Mgr. Ing. Daniel Hadraba
titled
"Computational Collagen structures from cell culture to intact tendon"**

Daniel Hadraba has chosen a topical field for his study. The subject of his thesis is aimed at the use of label-free microscopy methods for the investigation of type I collagen structure and its multi-hierarchical function in connective tissue.

The thesis is organized into seven chapters, beginning from Chapt. 1 **Introduction** that is a well written review of the current state of research, based on a high number of fresh references (the total number of references used in this Thesis is 213). The Introduction leads to a conclusion that the mechanism of collagen aging is still not fully understood, thus introducing the rationale of the presented Ph.D. project. In this chapter, relative pros and cons of various sources of image contrast enabling the visualization of collagen structures and tissues are presented in brief, but concise way, indicating that the author is aware of a real applicability of various imaging techniques in particular type of assays.

Next there are five chapters, Chapt. 2 to 6, focused on particular research problems included to this Thesis. Each of them is opened by its specific Introduction, followed by Methods, Results and Discussion. I found this approach to be very efficient and clear way how to present all the achievements of this Thesis. These five Chapters are organized in a logical order, going from studies on cell cultures to the assays on tissues of increasing complexity. Moreover, these Chapters share a common methodological aspect focused of SHG techniques that combine the advantage of optical tomography performed without a confocal pinhole with the of label-free alternative to the immunofluorescence detection of type I collagen.

The final Chapter 7, General Conclusions, is a concise summary of achieved results. Here, I am pleased to emphasize that the author provided robust evidence concerning the clear advantage of SHG techniques over standard immunofluorescence microscopy of collagen structures under a variety of experimental conditions.

A few specific comments on the text are presented below, sorted according to the page numbers, not according to their relative importance:

- p. IV π defined as irrational mathematical constant. There are also other irrational mathematical constant, like, e.g., Golden ratio. Ludolph's number seems to be a more appropriate term. Anyhow, this definition is clearly unnecessary.
- p. IV Here E is defined as electromagnetic field, which is obviously less proper than the correct definition used in p. 13 (i.e., the strength of the optical vector field).
- p. 18 Microscopy of VICs culture

- p. 30, 31 “Sections visualization using fluorescence and SHG microscopy”. Here, a diagram defining the polarization angles (orientation of the polarization plane) would be helpful.
- p. 38 Whole the chapter 4 is rather sketchy and thus difficult to read. In particular, it is nearly impossible for a reader to follow whether or not the presented formulae are adequate for the characterization of examined object properties. It would be helpful to include an Appendix with details of this formulae derivation.
- p. 49 It is not completely clear how one can find in Fig. 5.2 (7 days) the parameters schematically shown in the diagram of Fig. 1.
- p. 51 Why the wavelengths displayed in the “illustrative comparison of amplitude/ wavelength relationship with age” are markedly different from the wavelengths shown in other figures of this Chapter. Moreover, the difference between the two figures at the right side of Fig. 5.3 is not explained in the legend to this Figure.

General comments:

- References to particular Figures would make the Discussion easier to follow.
- I missed an explicit link to be presented between Chapters 2 to 6 and the list of published papers.
- A personal contribution of the candidate to the published papers should be clearly specified during his thesis defence.

Conclusions:

Despite some criticism raised above, the main results presented in this thesis can be referred as new and valuable. The thesis of Mgr. Ing. Daniel Hadraba meets the requirements of Ph.D. program, thus **the author can be given the desired degree.**

Prague, April 20, 2017

Prof. RNDr. Jaromír Plášek, CSc.