A dark field illumination probe linked to Raman spectroscopy for non-invasivety determination of ocular biomarkers

Appendix I

Impact

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Main objective, results and conclusions

The main objective of this dissertation was to develop a dark field probe for non-invasive determination of the molecular composition of the aqueous humor in the anterior eye chamber using Raman spectroscopy, without compromising the fragile posterior section of the eye by the strong excitation light necessary in this method. With the studies presented in this dissertation we demonstrated *in vitro* and *in vivo* in animal eyes a functional ophthalmic probe that enables to measure the concentrations of ocular drugs. The scientific and social impacts of this dissertation are highlighted in this section.

Scientific impact

The results published in peer-reviewed scientific journals and presented in (inter)national conferences contribute to researchers in the field of ophthalmology and physicists developing non-invasive diagnostic methods. Currently, clinical decision-making in ophthalmology mainly depends on the morphological screening by trained ophthalmologists. Molecular profiling could provide additional valuable information for a better understanding of the mechanics of ocular disease. Up to now however, real-time monitoring of disease progression is hampered by the fact that invasive biopsies are the only way to obtain this information. Thus, there is need for non-invasive and in-situ diagnostic methods. We propose Raman spectroscopy as a technique to be utilized for this cause. To do so, firstly spectra of target biomarkers need to be identified, as described in **Chapter 2.** This provides the spectra of two biomarkers related to four complex retinal diseases. Quantitative assessment of these molecules can be used as a diagnostic aid in patient care. Spectra and studies of another biomarker, sorbitol, that is generated in the pathway of diabetic cataract development in the lens, are presented in **Chapter 3**. This might help ophthalmologists to gain more insights into the mechanisms of early diabetic cataract development.

The feasibility of using Raman spectroscopy in drug delivery research in living animals is explored in **Chapters 4 to 4b**. The experiences and results gained from these studies will help researchers to obtain higher quality and more reliable data using spectroscopic methods in living animals. Not every preclinical research institute has the access or knowledge to acquire spectra in living animals. The original spectra released might be beneficial for them. The dataset can also be made part of a larger database with spectra for data mining to a comprehensive scientific output. Another potential use of the dataset is as a machine learning training dataset or other in other advanced data processing approaches to optimize further background subtraction modules, which might lead to a more universal and standardized data processing method of Raman spectra.

In current clinical practice, the strong excitation light needed in the Raman spectroscopy damages the fragile posterior section of the eye. Hence, we designed dark-field probe designs, which no direct illumination light reaching the retina. They are discussed in **Chapters 5 and 6**. Using these to obtain in-vivo ocular drug concentrations in

the animal eye will be of great help in evaluating the efficiency drug delivery into the anterior eye chamber when administrated topically. This might even be improved using methods like AADF presented in **Chapter 7**. The experimental settings and parameters proposed here may help physicists to establish such spectroscopic measurement systems for use in pre-clinical applications.

Social impact

Raman spectroscopy and mass spectroscopy are both very specific diagnostic techniques that provide the results in short time. That's why the Dutch minister of Public Health, Hugo de Jonge, recommended them as candidates for testing Covid-19 patients. However, only Raman spectroscopy is suitable for medical in vivo diagnostic applications. As the Raman effect is a very weak phenomenon, it is difficult to apply this technique directly in the vulnerable living eye.

Therefore, a safe way for utilizing Raman spectroscopy in ophthalmology will benefit a wider audience outside the scientific community in various forms. For clinicians and ophthalmologists, real-time molecular profiling will accelerate research procedures in clinical practices. For patients the burdens of biopsies will be significantly reduced by using non-invasive, *in situ* approaches like Raman spectroscopy. Animals might not necessarily be sacrificed to acquire drug data if these non-invasive molecular assessment methods become available. Hence, the usage of lab animals can be reduced. The economic benefit of reducing the number of lab animals will drive the pharmaceutical companies to broaden the usage of the Raman technique as an innovation in the industry. Furthermore, the granted patent might inspire and spread the application of the Raman technique in the medical device startups and companies. The biomarker measurement techniques by Raman spectroscopy and dark-field probes proposed in this thesis will be available and promoted to both scientific and industrial communities as commercial products and services.