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

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Chapter 9

Summary

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This thesis presents the use of Raman spectroscopy to investigate the ocular biomolecular status and introduces novel devices that allow high radiation energy to be delivered to the ocular system without damaging the vulnerable retinal tissue.

Chapter 1 introduces the background of this research. In current clinical practice, optical techniques are mainly used to provide anatomical, functional, and structural information. However, biochemical information is obtained by performing a biopsy of the tissue. This poses a risk to the integrity of the eye and cannot be performed on a regular base. We propose Raman spectrometry as a potential and powerful tool for a non-invasive and non-destructive investigation. The limitations of conventional Raman spectroscopy with direct illumination method in ophthalmic application are mainly safety concerns. In this thesis, biomedical applications of Raman spectroscopy are explored and a dark-field method in conjunction with confocal Raman spectroscopy (CRS) is proposed to avoid light damage of the retina.

In Chapter 2, characteristic Raman spectra of IL10 and ACE in their chemical form are reported. These could be used for future in vivo diagnosis applications for eye related disease.

Chapter 3 investigated the possibilities of using Raman spectroscopy for early diabetic cataract diagnosis. Previous research indicated that the sorbitol concentration in the lens was associated with the development of diabetic cataracts. However, non-invasive quantitative assessment is still a challenge for monitoring the sorbitol concentration in the lens. We obtained the sorbitol concentration in sorbitol induced cataracts mimicking diabetic cataracts lenses in vitro. The concentration of sorbitol in pig lens shows a good correlation to the degree of the lens cataractous via their Raman spectrum.

Chapter 4 presents an ocular drug delivery study using Raman spectroscopy to determine the ketorolac concentrations in vitro porcine eyes and in vivo rabbit eyes. The porcine eyes were enucleated and soaked in different concentrations of ketorolac whereafter Raman spectra were collected by two different setups. In the first setup we used a commercial objective lens as a focusing element, and in the second a doublet lens combined with a gonioscope lens. Twelve rabbits were treated with Acular™ for four weeks, while the concentrations of ketorolac from Acular™ in aqueous humor of the rabbits were measured by CRS. All these samples were also properly collected and tested in FDA “golden standard” high-performance liquid chromatography (HPLC). The comparison of Raman spectroscopy and HPLC showed good agreement in the ex vivo experiments, but lack of sensitivity in vivo measurement.

Chapter 4a shows the raw dataset of the Raman spectrum obtained from the in vivo experiments with the rabbit eyes presented in chapter 4. These raw datasets could provide experimental information for researchers who have no access to Raman spectra from the living rabbit eyes. The dataset might be processed by alternative data processing techniques for background suppression or characteristic peak identification of the biomarkers. The ketorolac treatments for the 12 rabbits are outlined in detail and raw Raman spectra data are presented accordingly.
In **Chapter 4b**, we describe the data processing procedure to minimize the hardware induced artifacts and fluorescence interference in the spectrum. A self-developed MatLab script for the automated correction of Raman spectra is presented. With the dataset in chapter 4a, the proposed data processing method demonstrated that it can be used for minimizing the hardware and fluorescence interference simultaneously.

**Chapter 5** proposed a novel device to acquire Raman spectra from the AC without jeopardizing the retina by high laser powers. In this study, the design of this dark-field device is presented. It uses a curved mirror system to guide the laser incident to the ocular system with a large angle of the optic axis of the eye. A three-dimensional computer model was built and evaluated by ray-tracing software Zemax to validate its optic performance. A prototype was manufactured and tested by AEM. The chemical compounds could be detected by the prototype in conjunction with CRS with acceptable SNR. However, the intensity of the Raman signal is still below our expectation. By means of ray tracing simulation, the alignment issue is the reason of low Raman signal collecting efficiency.

In **Chapter 6**, a further developed device without curved mirrors is proposed and validated in AEM. To validate, the prototype was tested by an ocular drug in AEM. The results indicated a 9 folds improvement in misalignment tolerance and a 36 folds improvement in the signal-to-noise ratio at the phenylephrine HCl 1002 cm\(^{-1}\) peak compared to a previous design. The prototype showed a good linear fit with the phenylephrine HCl concentration, with a R square of 0.996 and a sensitivity detection limit down to 0.05% phenylephrine HCl.

**Chapter 7** proposes an alternative approach to achieve a dark-field illumination method using a phase mask to generate an abruptly autofocusing beam in the anterior chamber. By modulating the phase of a circularly symmetric optical wavefront, the maximum intensity of the beams suddenly decreases by orders of magnitude right after the target point along the optic axis. In this simulation study, we demonstrate a setting that is capable of generating such a beam in the anterior chamber and create a dark-filed illumination to prevent excitation light to reach the retinal tissue directly. Results show that the efficiency of AADF is higher than the conventional focused Gaussian beam (FGB) method with center blocked. The peak value of the Raman signal intensity acquired using the AADF method is about 6 times larger than that of an FGB.

**Chapter 8** discusses the major findings of this thesis regarding to using Raman spectroscopy for biomedical applications in ophthalmology. The challenges and implications for future studies both discussed in a more general and broader perspective.

In the **appendix**, the scientific and social impacts of the research are elaborated. The scientific impacts mainly contribute to researchers in the field of ophthalmology and physicists developing non-invasive diagnostic methods. The social impacts are aiming to benefit a wider audience outside the scientific community in various forms like clinicians, ophthalmologists, and patients eventually. Pharmaceutical industry also can be a benefit from it by reducing the animal models in drug development.
In summary, Raman spectroscopy shows its unique potential for biomedical and pre-clinic applications in ophthalmology. The design of the eye probe makes one step closer to the clinic application and may serve as a groundwork for ocular biomarker non-invasive quantitative assessment for early diagnosis of ocular and systematic diseases.