Assessment of the Alzheimer Progression with Multiwavelength Stokes Polarimetry

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Abstract: Multiwavelength Stokes polarimetry imaging is used for screening fixed bulk mouse brain tissue blocks at different stages of Alzheimer’s disease, providing statistically significant difference sufficient for quantitative analysis of brain tissue. © 2021 The Authors.

1. Introduction

The accumulation of amyloid-beta (Aβ) plaques within the brain tissue is one of the major hallmarks of Alzheimer’s disease (AD). Currently, only histological analysis is able to provide definite diagnosis of AD with the help of immunohistochemistry (IHC) techniques which are used to label the Aβ plaques. The procedure of histological analysis is laborious and time-consuming; among other steps, it requires cutting the formalin-fixed paraffin-embedded (FFPE) tissue blocks and staining the obtained thin sections. In the previous studies, it has been demonstrated that polarimetry-based techniques have high potential to implement label-free, non-destructive express screening of brain tissues in order to facilitate the research of AD diagnostics and treatment [1,2]. In the current study, we performed the screening of bulk mouse brain tissue within the FFPE blocks without sectioning or staining the tissue. We used multiwavelength Stokes vector polarimetry and statistical analysis of the obtained polarization metrics of light backscattered from tissue blocks for distinguishing the tissues differently affected by β-amyloidosis.

2. Methods and Materials

The principal scheme of the multiwavelength Stokes polarimetric imaging setup developed in-house is shown in Fig. 1(a). The incident illumination was produced by the supercontinuum fiber laser (Leukos Ltd., France) and filtered by the high-speed acousto-optic tunable filter (Leukos Ltd., France) allowing to pick up a probing wavelength in the range of 450-650 nm. The linearly polarized light beam was altered into right-hand circularly polarized (RHCP) light by half- and quarter-wave plates. In the experiments, the RHCP light beam was focused on the surface of a sample at 55° angle. The sample was placed on the XY translation stage. Light backscattered from the sample was collected at a variable distance LSD away from the point of incidence [3] by a 20× objective lens at 30° angle. A 90:10 beam splitter directed 10% part of the beam to the CMOS camera which was used for control of focus on the sample’s surface; the rest of the beam was analyzed by the Stokes polarimeter (Thorlabs Ltd., USA).

The scanning of the samples was performed with 450, 550 or 650 nm incident wavelengths over 5 × 4 mm² area with a step of 20 µm (in Y) and 4 µm (in X) with the source-detector separation of 0, 50 and 100 µm. The total,
circular and linear degree of polarization (DoP) and full Stokes vector were measured at each scanning point. The spatial distributions of these metrics were analysed with statistical methods. The FFPE mouse brain tissue blocks investigated in the current study contained 24 brain hemispheres excised from sacrificed APP-transgenic mice (APPPS-21, APPtg) [4]. All protocols and procedures were performed in accordance with 2010/63/EU. The examined FFPE specimens were divided into two groups: less affected tissue (group I: 11 samples of animals 50-75 days old), more affected tissue (group II: 13 samples of animals 175-200 days old). Fig. 1(b,c) shows the histological images of brain tissue of animals of group I (b-1,b-2) and group II (c-1,c-2). Amyloid-beta (Aβ) plaques are observed as dark brown spots throughout the brain tissue due to IHC staining. The number, size and density of Aβ plaques is significantly higher in group II than in group I.

3. Results and Discussion

The results of polarimetric imaging have shown that the polarization metrics of backscattered light are significantly different for the samples from two groups. In particular, the DoP is found to be higher in most cases for the samples from group I than from group II at each incident wavelength and source-detector separation. Fig.2(a,b) shows an example of maps of DoP of two samples from group I (a) and group II (b) measured at 450 nm incident wavelength with different source-detector separations: L_SD = 0 µm (a-1, b-1), L_SD = 50 µm (a-2, b-2), L_SD = 100 µm (a-3, b-3).

It is observed in Fig.2(a,b) that tissue depolarization of the sample from group I was lower than that of group II. Additionally, due to the expansion of the sampling volume of the measurement resulting from the increase of L_SD, the DoP of both samples decreased when L_SD increased. Notably, the level of depolarization of the sample from group I measured at L_SD = 100 µm was equivalent to the depolarization of the sample from group II measured at L_SD = 0 µm. This indicates the enhancement of light scattering with the progression of the disease which has been also demonstrated previously in the alternative studies [5]. Thus, it is believed that the higher concentration of Aβ protein and its aggregations is responsible for the increase of scattering within the brain tissue. Fig.2(c) shows the comparison of the values of first statistical moment Z_1 (mean) of all measured parameters (total DoP, 3 components of Stokes vector, linear and circular DoP) for two groups, measured at 450 nm incident wavelength with L_SD = 100 µm. The Kruskal-Wallis test has shown that the differences in DoP, S_1, S_2, DoP linear and DoP circular between two groups are statistically significant.

The spectral dependencies of the values of linear and circular DoP measured with different L_SD are shown in Fig.3(a-c). Each data point represents a value of DoP averaged within the corresponding group; error bars represent standard deviation. P-values characterize the statistical significance of differences between the DoP values for two groups. The obtained data has shown that at L_SD = 50 µm, the differences between circular and linear DoP were significant at each incident wavelength. However, at L_SD = 0 µm, circular DoP exhibited more significant changes due to the tissue alterations if compared with linear DoP (especially at 450 nm incident wavelength), while at L_SD = 100 µm, linear DoP has shown more notable changes than DoP circular. Fig.3(d) shows Stokes vectors of light backscattered from the brain samples of two groups mapped on the Poincaré sphere (measurement at 650 nm incident wavelength with L_SD=50 µm). As one can see in Fig.3(d-2), the positions of Stokes vectors corresponding to two groups are different.
In the current study, multiwavelength Stokes vector polarimetry in backscattering configuration was used to examine FFPE specimens of mouse brain tissue divided into two groups by the severity of AD. The measurements were performed at different source-detector separations allowing variation between different sampling volumes. The results have shown that the polarization properties of backscattered light exhibit statistically significant differences for samples from different groups. The obtained results demonstrate that the considered polarization metrics, in particular, total linear and circular DoP and Stokes vector, could be used as sensitive markers of the presence of Aβ plaques. The proposed approach shows a potential to improve the existing practice of protein aggregate screening in AD diagnostics in terms of time and cost efficiency by using full FFPE blocks for the analysis without the need to slice and stain the tissue.

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6. References


