Comment on the influence of episcleral blood vessels in diffuse reflectance spectroscopy measurements of the bulbar conjunctiva

In response to: S.A. Lisenko, V.A.Firago, M.M. Kugeiko, and A.I. Kubarko. *J. Appl. Spectr.*, **83,** No. 4, 1-10. (2016).

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We read with interest, the recent article by Lisenko et al. [1] that described the use of diffuse-reflectance spectroscopy and a multi-variable spectral analysis model to estimate the concentrations of multiple chromophores in the human bulbar conjunctiva *in vivo*: namely oxyhemoglobin, deoxyhemoglobin, bilirubin within the vasculature, and melanin and neuroglobin within the scleral tissue. The authors employ inverse Monte Carlo modelling and measurement to adjust the concentrations of the chromophores assuming an additive-spectrum model to obtain a fit of the modelled spectra to experimentally recorded diffuse spectra. The authors claim that including the additional spectra of bilirubin and neuroglobin to the model provides an enhanced agreement between modelled and observed spectra. As highlighted in the paper however, inclusion of bilirubin and neuroglobin in the model has not been considered necessary in the well-established field of retinal oximetry, and neuroglobin has not previously

been observed outside of neutral tissue. By employing this model, the authors estimated bulbar conjunctival oxygenation to be in the range 74 – 91% *in vivo*.

We wish to highlight that whilst Lisenko et al. claim to have measured the spectral absorption of the bulbar conjunctival blood vessels, the absorption spectra of light transmitted through the sclera was probably also significantly influenced by the spectral absorption of episcleral blood vessels as discussed below.

Firstly, Lisenko et al. report an estimated bulbar conjunctival oxygenation of 74 - 91% in healthy humans at normoxia. It has been recently been shown, however that all bulbar conjunctival vessels normally exhibit high oxygenation (close to 100%) due to exposure to ambient air for imaging (i.e. when the eyelid is open). [2] This can be considered a "ground truth" for estimations of oxygenation in the bulbar conjunctiva, and this is contrary to the results reported by Lisenko et al.

Secondly, Lisenko et al. have employed a pseudo-annular, multi-spot illumination scheme to produce diffuse spectra free of surface reflections. Their rigorous Monte-Carlo simulation of blood vessels within the sclera shows that this light from a single spot on the sclera travels >1mm diffusely within the scleral tissue.[1] suggesting that the transmitted light spectrally interrogated a large volume of scleral tissue; this tissue volume will have contained multiple episcleral vessels that may have not been visible due to the highly scattering white scleral tissue, [3] but which could have contributed significantly to the absorption spectrum of diffuse light in scleral tissue. It has been shown that the oxygenation of episcleral vessels reflect the systematic blood supply of the body (i.e. ~97% for arterial blood and ~70% for venous blood), [2] so when averaged over a scleral region, recovered oxygenation would reflect a weighted average of both arterial and venous episcleral blood vessels. This fits well with the oxygenation range of 74 – 91% reported by Lisenko et al.

These two factors suggest that the spectral absorption measurements by Lisenko et al. are dominated by episcleral blood oxygenation rather than bulbar conjunctival blood oxygenation; this significantly alters their estimated blood oximetry and consequently impacts on the confidence in the concentrations of other parameters such as bilirubin and neuroglobin in their model. We would kindly recommend further consideration of this matter.

References:

[1] S.A. Lisenko, V.A.Firago, M.M. Kugeiko, and A.I. Kubarko. *J. Appl. Spectr.,* **83,** No. 4, 1-10. (2016).

[2] L.E.MacKenzie, T.R. Choudhary, A.I. McNaught, and A.R. Harvey. *Exp. Eye. Res.,* **149**, 48-58. (2016).

[3] P.A. Meyer. *Eye*, **2,** No. 5, 533-546. (1988).