Estimation of beta-carotene using calibrated reflection spectroscopy method: phantom study

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***Abstract*— In this work, we use compression and immersion optical clearing to enhance the accuracy of reflection spectroscopy to measure the concentration of beta-carotene inside biological phantom. The estimated results are in good agreement with exact value of beta-carotene.**

Keywords—beta-carotene; optical clearing; reflection spectroscopy.

Determining the amount of beta carotene in tissue as one of carotenoids plays an important role in human health. Recently, optical method such as Raman spectroscopy and reflection spectroscopy have been used to measure the amount of beta-carotene. The accuracy of these optical methods requires evaluation [1]. High pressure liquid chromatography (HPLC) is the main method for determining the amount of beta-carotene; beside its high cost, some amount of beta-carotene of the tissue will be lost after biopsy of tissue and will not give a precise comparison. Ermakov *et.al*. investigated the direct correlation of *in vivo* skin tissue carotenoid Raman measurements with subsequent chromatography derived carotenoid concentrations [2].

In this work, we report a low cost phantom study to validate results obtained by reflection spectroscopy. The phantom consists of two layers. We use mouse ear as upper layer (Fig. 1a). The lower layer includes agar, intralipid and different concertation of beta-carotene (0.2, 0.3, 0.4, 0.6 μM). We apply a tungsten halogen lamp (HL2000; Ocean Optics Inc., Dunedin, USA) with 20W power, and spectrometer (USB2000, Ocean Optics, Dunedin, FL, USA). An optical fiber probe (R200-7, Ocean Optics, Dunedin, FL, USA) is applied to collect data (Fig. 1b).

To accurately measure beta-carotene, we first reduce the scattering coefficient by immersion of mouse ear in Dimethylsulphoxide (DMSO) for 15 min, and then utilizing sample compression to reduce the role of blood absorption in the overall absorption spectrum of mouse ear and to make tissue somewhat thinner [3]. Consequently, the absorption spectrum of beta-carotene appears in absorbance spectrum (Fig. 1.c). A calibrated relation between estimated data and exact value of beta-carotene is applied to predict unknown values of the chromophore. For example, the exact value of 0.50 μM is predicted as 0.52 μM (error smaller than 5%), see Fig. 1d.

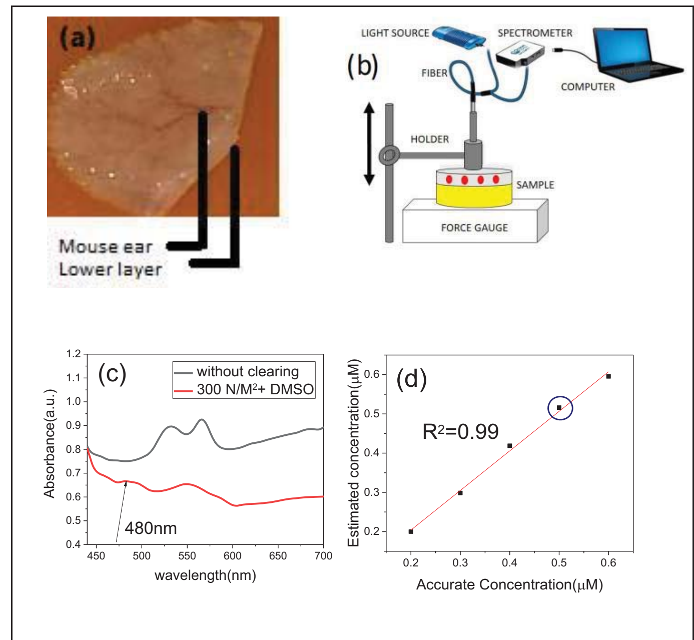

Fig. 1. A photo of phantom (a). A Schematic of reflectance spectroscopy (b) and, the influence of optical immersion (DMSO) and compresion (300 N/m^2) clearing on absorbance spectrum of phantom (c). The appeared peak under optical clearing (red graph), indicated by the black arrow at lambda=480 nm, shows the spectum of beta-carotene between 450 and 500 nm. The correlation between exact and estimated values of beta-carotene with corellation ratio of R^2 =0.99 is shown in the last graph (d).

This low cost method is a real time and a noninvasive that can be applied for measuring *in vivo* beta-carotene for nutrition and medicine researches.

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