PNAS
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Supplementary Information for

Imaging Lutein and Zeaxanthin in the Human Retina with Confocal Resonance Raman Microscopy

Binxing Li, Evan W. George, Gregory T. Rognon, Aruna Gorusupudi, Arunkumar Ranganathan, Fu-Yen Chang, Linjia Shi, Jeanne M. Frederick, Paul S. Bernstein

Paul S. Bernstein
Email: paul.bernstein@hsc.utah.edu

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Supplementary text
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www.pnas.org/cgi/doi/10.1073/pnas.1922793117

Supplementary Information Text

Supplemental Materials and Methods.

HPLC analysis. 10 µM of lutein and 10 µM of zeaxanthin methanol solutions were used to prepare the mixed carotenoid solutions, in which the percentage of lutein are 0%, 20%, 40%, 60%, 80%, and 100%, respectively. The final volume of each carotenoid preparation is 400 µL. 350 µL was added to a quartz cuvette for Raman measurement, and the left 50 µL was dried down by vacuum evaporation and dissolved in 1 mL of a reversed-phase solvent containing 80% methanol and 20% Methyl tert-butyl ether (MTBE) for HPLC analysis. Carotenoids were separated on an HPLC system (Thermo Scientific, Waltham, MA) equipped with an autosampler and photodiode array (PDA) detector, on a C30 column (YMC Europe GmbH, Germany, 250 x 4.6 mm i.d). The injection volume was 50 µL with a mobile phase flow rate of 1.0 mL/min. The gradient program consisted of methanol (A) and MTBE (B). The gradient included 95% A at 0 min, 70% A at 20 min, 40% A at 30 min, 5% A at 40 min, 95% A at 45 min, and hold at 95% until 50 min. The column was maintained at room temperature with a PDA detector operated at 450nm. Peaks were identified and confirmed using PDA spectra and by co-elution with authentic standards as necessary. Lutein and zeaxanthin were quantified according to the elution peak area. All the tests were repeated three times, and the data were plotted using Microsoft Excel software.

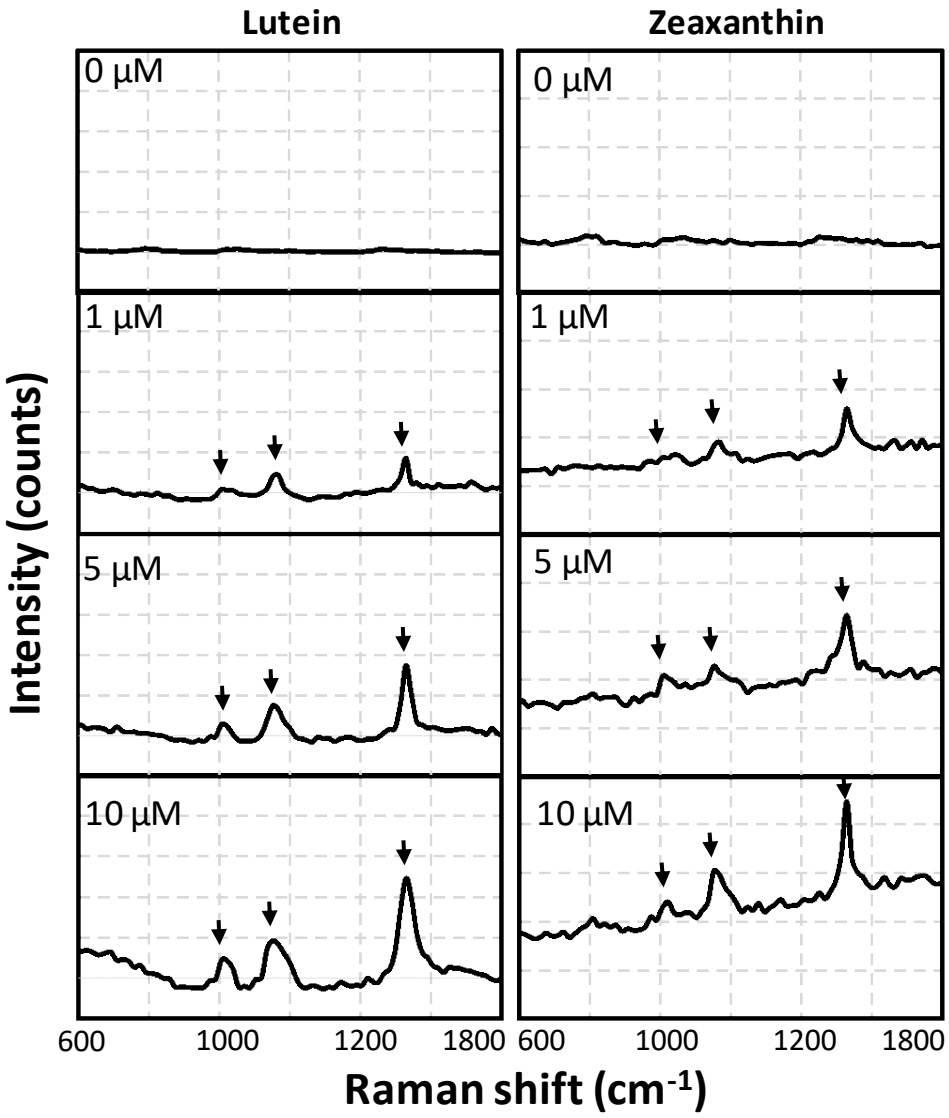

Fig. S1. Raman spectra of lutein and zeaxanthin dissolved in methanol at concentrations of 0 µM, 1 µM, 5 µM, and 10 µM. Arrowheads indicate the major peaks in the Raman spectra of lutein and zeaxanthin, and they are located at ~1500 cm^{-1} (v1), 1150 cm^{-1} (v2), and 1000 cm^{-1} (v3), respectively.

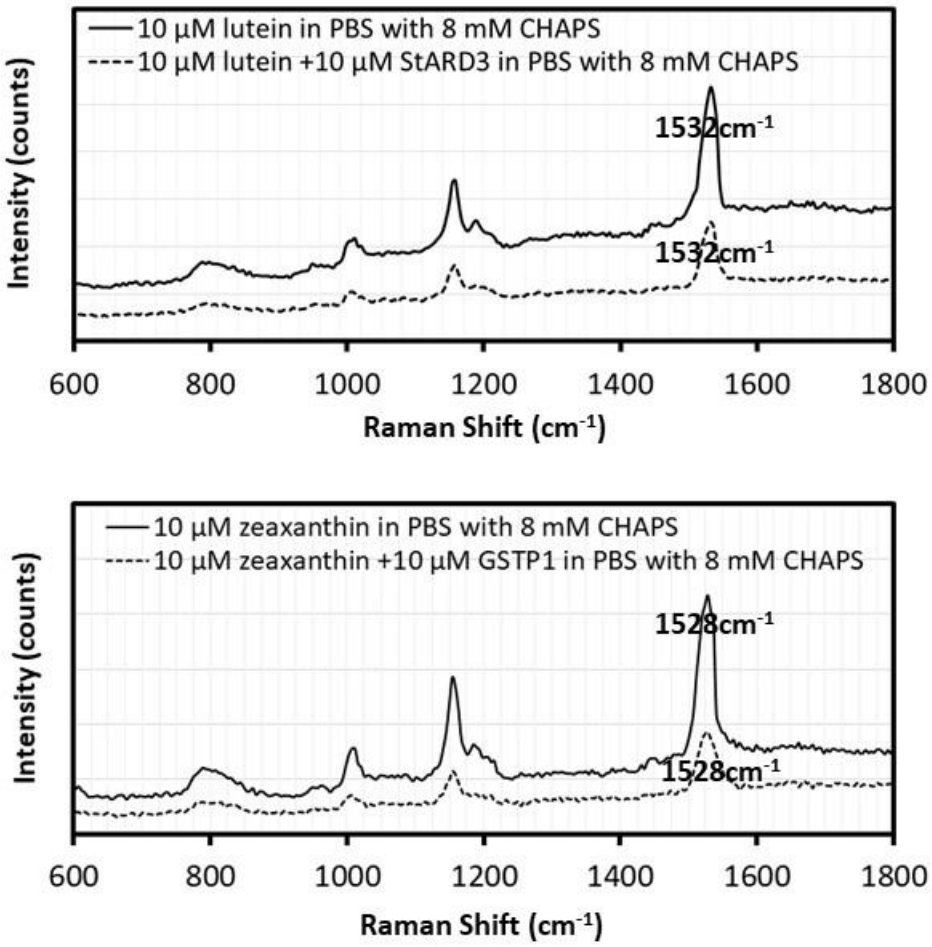

Fig. S2. Raman spectra of carotenoids free and bound to carotenoid-binding proteins in PBS buffer. No shift was observed between the v1 peaks of carotenoids and their binding proteins. (Top) Solid line, 10 µM lutein; dotted line, 10 µM lutein-StARD3 protein complex; (Bottom) Solid line, 10 µM zeaxanthin; dotted line, 10 µM zeaxanthin-GSTP1 protein complex. To solubilize lutein and zeaxanthin, 8 mM CHAPS detergent was added to the PBS buffer.

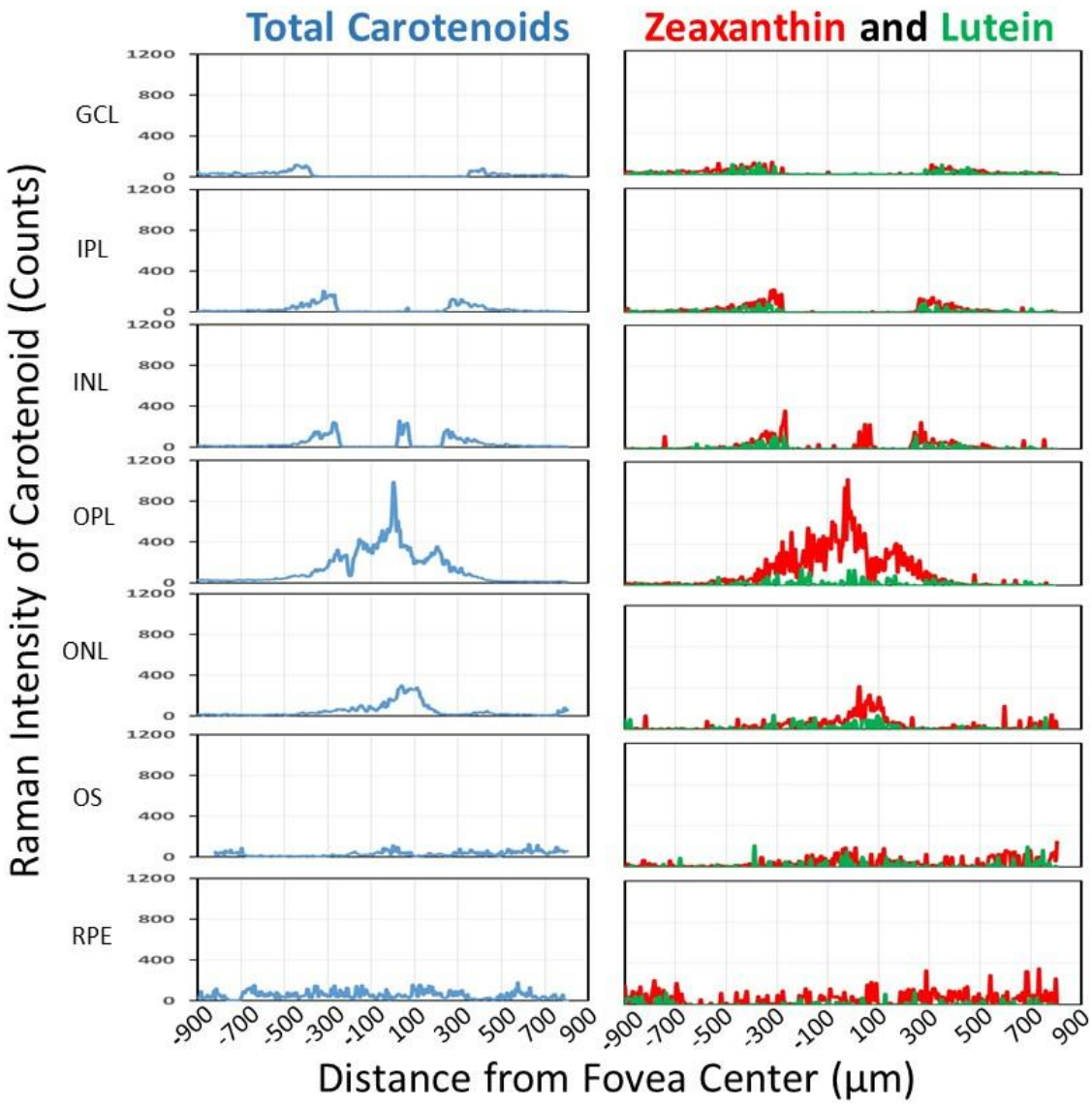

Fig. S3. Raman intensity profile in horizontal cuts at each retinal layer from the human retinal section shown in **Figure 6**. Blue, total carotenoids; green, lutein; red, zeaxanthin. A 3-pixel-wide horizontal line was drawn at the level of each retinal layer, and the average intensity of the v1 peak of the carotenoid at each level was plotted.

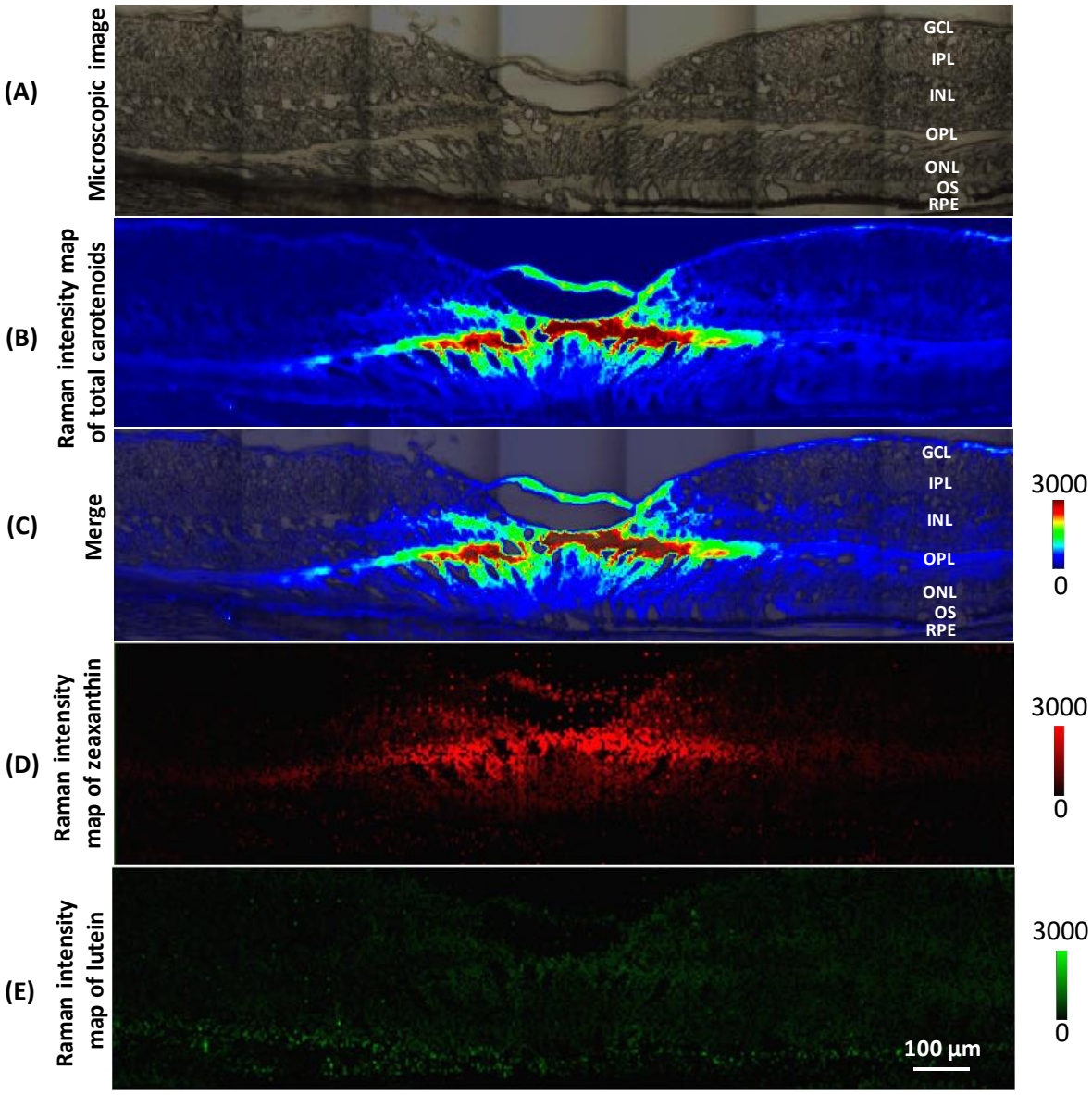

Fig. S4. Distribution of carotenoids in a human retinal section. (A) microscopic image of a foveal section from a healthy 81-y-o female donor; (B) an intensity map of total carotenoids created using Raman shifts ranged from 1500 cm^{-1} to 1550 cm^{-1}; (C) overlay of A and B; (D) intensity map of zeaxanthin generated using CLS fitting; (E) intensity map of lutein generated using CLS fitting.

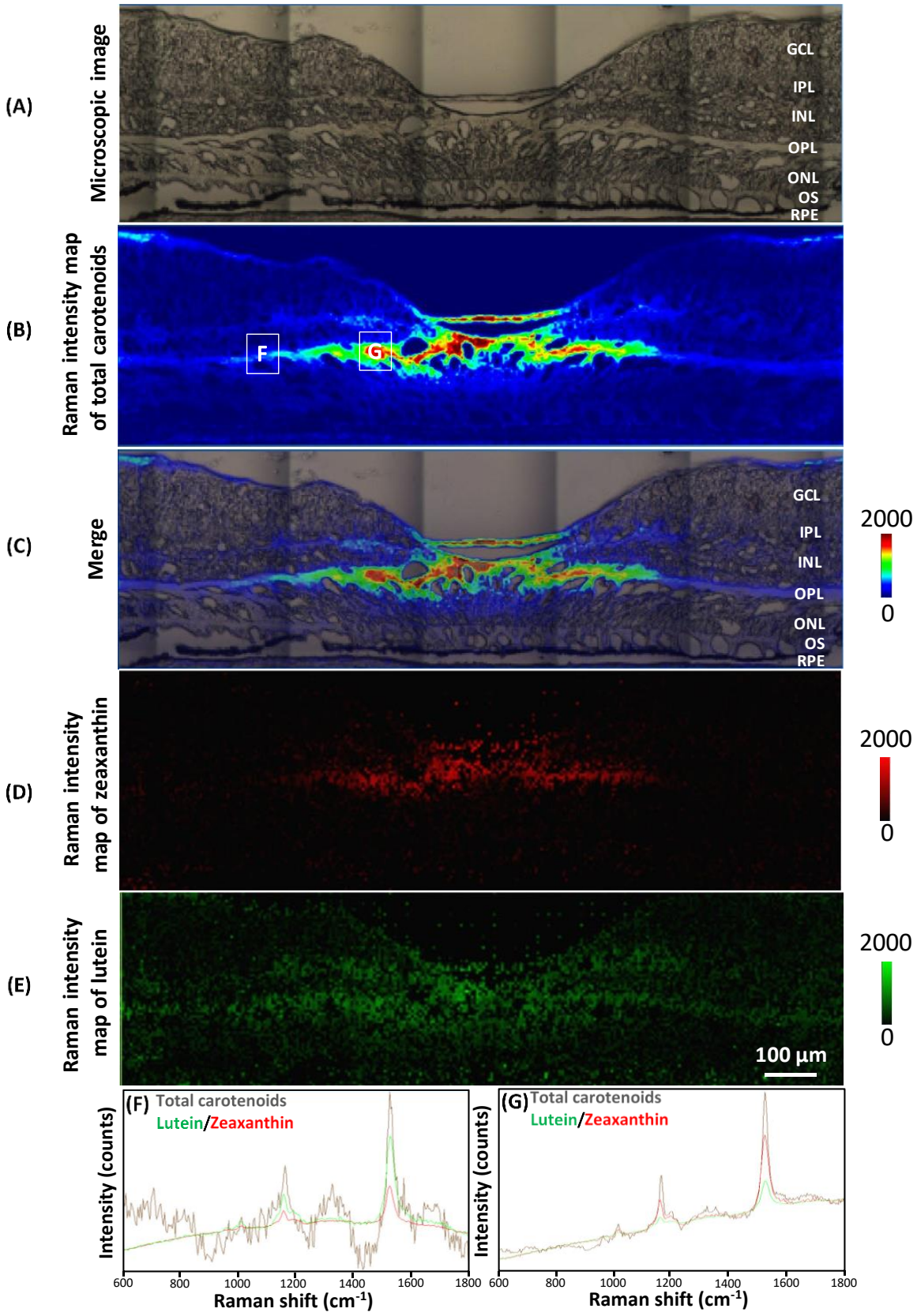
(A)
Microscopic image
GCL
IPL
INL
OPL
ONL
OS
RPE
(B)
Raman intensity map of total carotenoids
F
G
(C)
Merge
2000
0
(D)
Raman intensity map of zeaxanthin
2000
0
(E)
Raman intensity map of lutein
2000
0
100 μm
(F) Total carotenoids
Lutein/Zeaxanthin
Intensity (counts)
600 800 1000 1200 1400 1600 1800
Raman shift (cm-1)
(G) Total carotenoids
Lutein/Zeaxanthin
Intensity (counts)
600 800 1000 1200 1400 1600 1800
Raman shift (cm-1)

Fig. S5. Distribution of carotenoids in a human retinal section. (A) microscopic image of a parafoveal section from a healthy 81-y-o female donor about 40 μm away from the section in **Fig. S4**; (B) The intensity map of total carotenoids created using Raman shifts ranged from 1500 cm^{-1} to 1550 cm^{-1}; (C) overlay of A and B; (D) intensity map of zeaxanthin generated using CLS fitting; (E) intensity map of lutein generated using CLS fitting; (F,G) Raman spectra of two selected spots in (B), of which total carotenoid, lutein, and zeaxanthin are shown in gray, green, and red, respectively.