2900/3220 cm^{-1} CARS signal ratios. An overlay map of these two ratios is presented in Fig. 5(d). This figure contains considerably more structural contrast than that found in the single CARS frequency image (Fig. 5(a)). Furthermore, it can be seen that the two signal ratios vary across the fiber, and thus represent distinct measures of structure in these fibers. In particular, it is observed that the relative strength of O-H stretch signal diminishes markedly at the sharp bend. This indicates a local disruption in inter-chain hydrogen bonding that may be due to stress/strain-induced structural changes [56]. We clearly identify gross positionally-varying changes in the CARS spectra in these samples. However, as shown by Zimmerly *et al.* [51], the birefringent nature of cotton fibers necessitates full polarization control and analysis in ascribing local spectral changes to structural orientations. Thus, the combination of polarization-resolution and CARS spectral imaging in the CH and OH regions will prove a powerful tool for future studies structured biopolymer assemblies.

4. Summary

We have identified a strong CARS response in common carbohydrate polymers such as starch and cellulose, and have shown that, together with the commonly-measured C-H stretch (2750 cm^{-1}–2970 cm^{-1}), the O-H stretch region (3000 cm^{-1}–3400 cm^{-1}) is an important source of structural-information and image contrast in these materials. We showed that our experimental scheme comprising of a single femtosecond laser-source and associated supercontinuum Stokes source allows for rapid scanning of the CARS spectrum and for simple simultaneous SHG and CARS imaging. Together, these complimentary nonlinear imaging modalities provide both information on local bond density and structural orientation. Their joint use shows considerable potential for the study of condensed carbohydrate polymer systems. The addition of concurrently-obtained TPEF signal is easily implemented, but as the samples under consideration for this article show only small levels of endogenous fluorescence, these images have not been included. We are currently expanding this research to the study of glycogen in live cells and tissues, and to the elucidation of starch degradation and retrogradation process during heat-moisture swelling. It is also of interest to investigate the apparent anti-correlation between the CARS and SHG signals in striated starch grains, and the disruption in hydrogen-bonding that occurs at kinks/bends in cellulose fibers.

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