

## Supplementary Information

### 1 **Confocal Raman Microscopy (microscope slides)**

2 Figure 1 shows Raman spectra of  $\beta$ -carotene loaded DSEs. The red trace shows  
3 the intensity profile of the carotene band from the Raman spectrum (shaded red)  
4 along the line (also shown in red) in the optical image. A unique olive oil band was  
5 identified at  $\sim 1650\text{ cm}^{-1}$ . The band at  $\sim 2900\text{ cm}^{-1}$  is due to overlapping  
6 contributions from various C-H vibrational modes of the emulsion components.  
7 The green and blue traces show the intensity profile of olive oil and CH bands  
8 respectively. The intensity profile correlates to the red line scan in the optical  
9 images shown in Figure 2, Figure 3, and Figure 4.

10 Figure 2 shows bright field reflectance optical images and Raman spectra  
11 corresponding to the line scanned region of DSE without  $\beta$ -carotene droplets in  
12 the optical image. Figure 3 and Figure 4 also shows the optical images and  
13 Raman spectra corresponding to the line scanned regions of DSEs with  $\beta$ -  
14 carotene incorporated in shell droplets and  $\beta$ -carotene incorporated in core  
15 droplets respectively.

16 As expected, there were no spikes in Raman intensity of  $\beta$ -carotene as DSE  
17 droplets without  $\beta$ -carotene were scanned because there was no  $\beta$ -carotene  
18 incorporated but changes in intensities of the CH band was observed (Figure 2b  
19 & d).

20 As DSE droplets with  $\beta$ -carotene-in-shell droplets were scanned (Figure 3a),  
21 increases or spikes in Raman intensity of  $\beta$ -carotene at the interfaces of DSE  
22 droplets were recorded and gradual decreases recorded as the scan continued  
23 across the core droplets (Figure 3b & d). The reverse case was observed with

24 DSE droplets with  $\beta$ -carotene-in-core droplets whereby spikes in Raman intensity  
25 of  $\beta$ -carotene (Figure 4b & d) were recorded while scanning through the core  
26 droplets (Figure 4a).

27 However, fixing the emulsions in agarose made it very difficult to isolate single  
28 droplets. The problem was compounded by the thickness of the sample as well,  
29 therefore it is likely that Raman scattering was being collected from more than  
30 one droplet at once. To overcome this challenge, the possibility of isolating a  
31 single droplet was explored with the use of a microfluidic channel.

32 Confocal Raman microscopy results from the DSEs fixed in agarose indicated  
33 that  $\beta$ -carotene incorporated in shell droplets of DSEs remained localised within  
34 the droplets but looking at the Raman spectra there appeared to be strong  $\beta$ -  
35 carotene signals in scanned areas corresponding to core safflower oil droplets  
36 and the inability to isolate droplets made it difficult to evaluate possible  $\beta$ -carotene  
37 migration.

### 38 **Confocal Raman Microscopy (Microfluidic channels)**

39 Microfluidic channels were used to overcome the challenge highlighted above.

40 Figure 5 and Figure 6 show confocal Raman images and Raman spectral plots  
41 of olive oil control emulsions, and DSEs with  $\beta$ -carotene-in-core respectively  
42 while, Figure 7 and Figure 8 shows that of trimyristin (processed above 56°C)  
43 control emulsions, and DSEs with  $\beta$ -carotene-in-core droplets respectively.

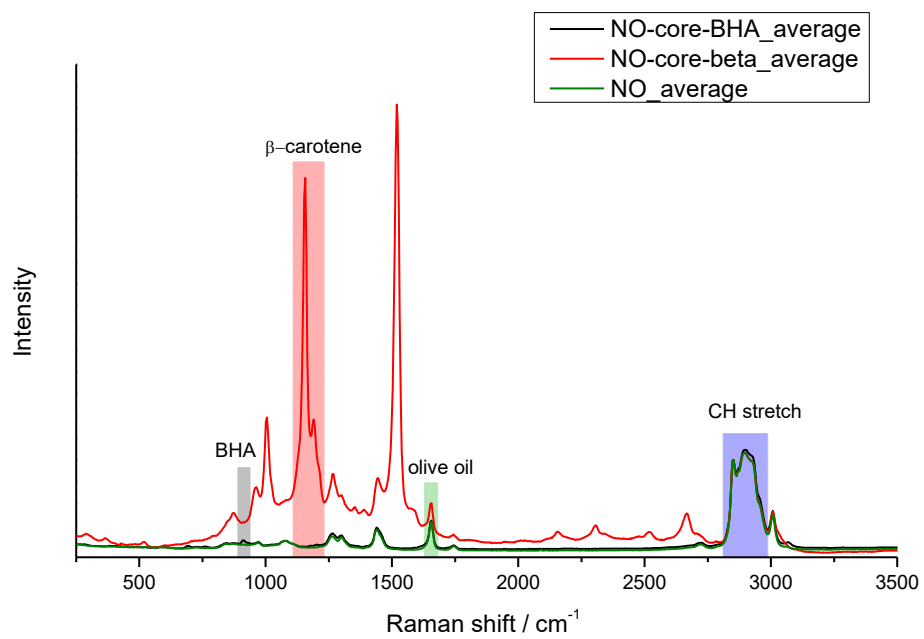
44 Raman intensity profile of olive control emulsions (Figure 5E) showed high  
45 intensities of  $\beta$ -carotene at line scan positions (~ 0-5, 20-30 & 40-50)  
46 corresponding to interfacial regions with olive oil shell droplets containing  $\beta$ -  
47 carotene while very low  $\beta$ -carotene intensities were obtained at line scan  
48 positions (10-20, 30-40) corresponding to core safflower oil droplets. The same  
49 trend was observed with trimyristin control emulsions (Figure 7E) which showed  
50 high intensities of  $\beta$ -carotene at line scan positions (~ 0-3, 17-21 & 36-40)  
51 corresponding to interfacial regions with trimyristin shell droplets containing  $\beta$ -  
52 carotene while very low  $\beta$ -carotene intensities were obtained at line scan  
53 positions (~ 3-16 & 23-35) corresponding to core safflower oil droplets.

54 Raman intensity profile across olive DSEs droplets with  $\beta$ -carotene-in-core  
55 safflower oil droplets (Figure 6E) showed high  $\beta$ -carotene and high CH band  
56 intensities at line scan positions (~ 2-14) corresponding to core safflower oil  
57 droplets. Low  $\beta$ -carotene and low CH band intensities were also observed at line  
58 scan positions (~ 0-1) corresponding to the interfaces of core safflower oil droplets  
59 composed of shell droplets without  $\beta$ -carotene.

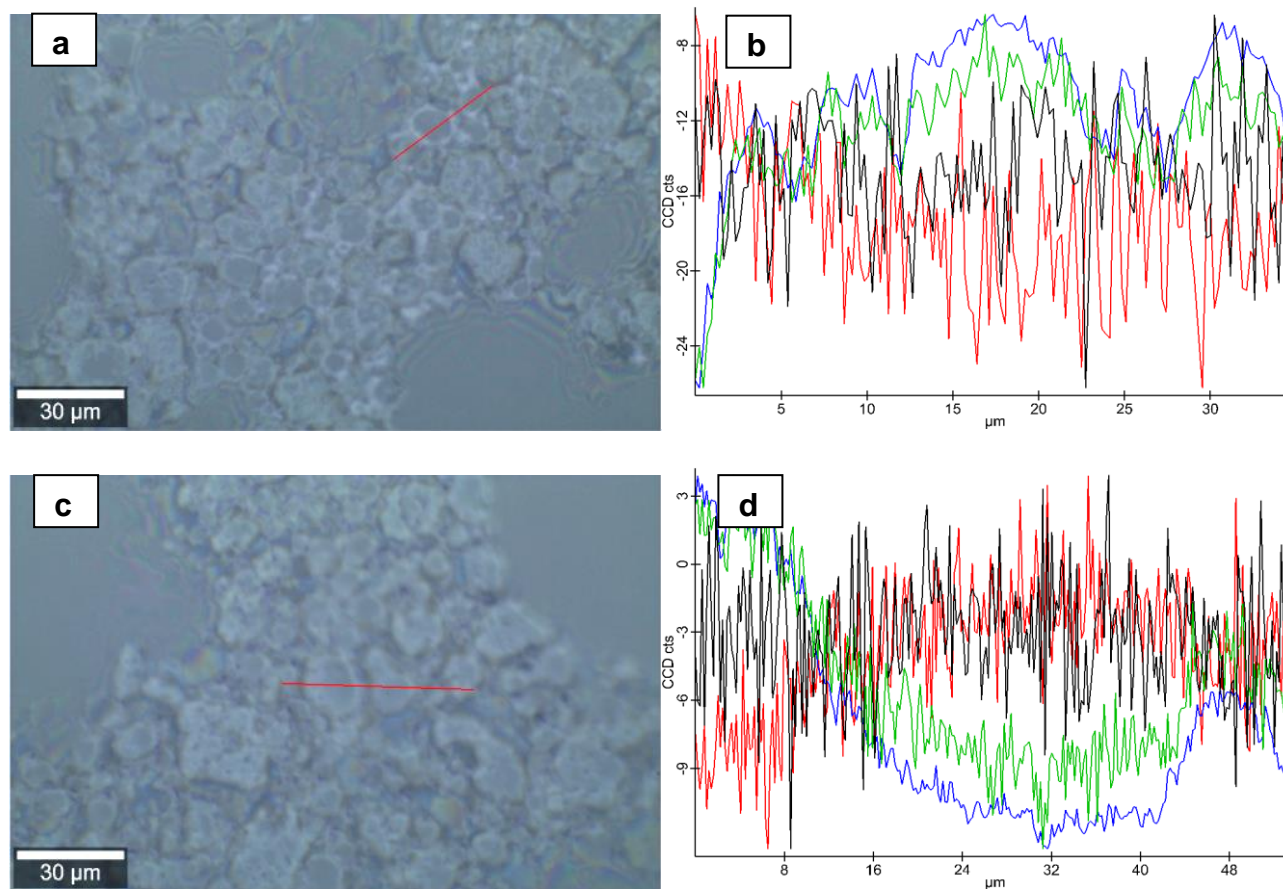
60

61 Raman intensity profile of trimyristin DSEs with  $\beta$ -carotene-in-core safflower oil  
62 droplets (Figure 8E) showed high  $\beta$ -carotene and high CH band intensities at line  
63 scan positions (~ 3-11 & 15-20) corresponding to core safflower oil droplets, and  
64 low  $\beta$ -carotene intensities were observed at line scan positions (~ 10-12)  
65 corresponding to the interfaces of core safflower oil droplets composed of shell  
66 droplets without  $\beta$ -carotene.

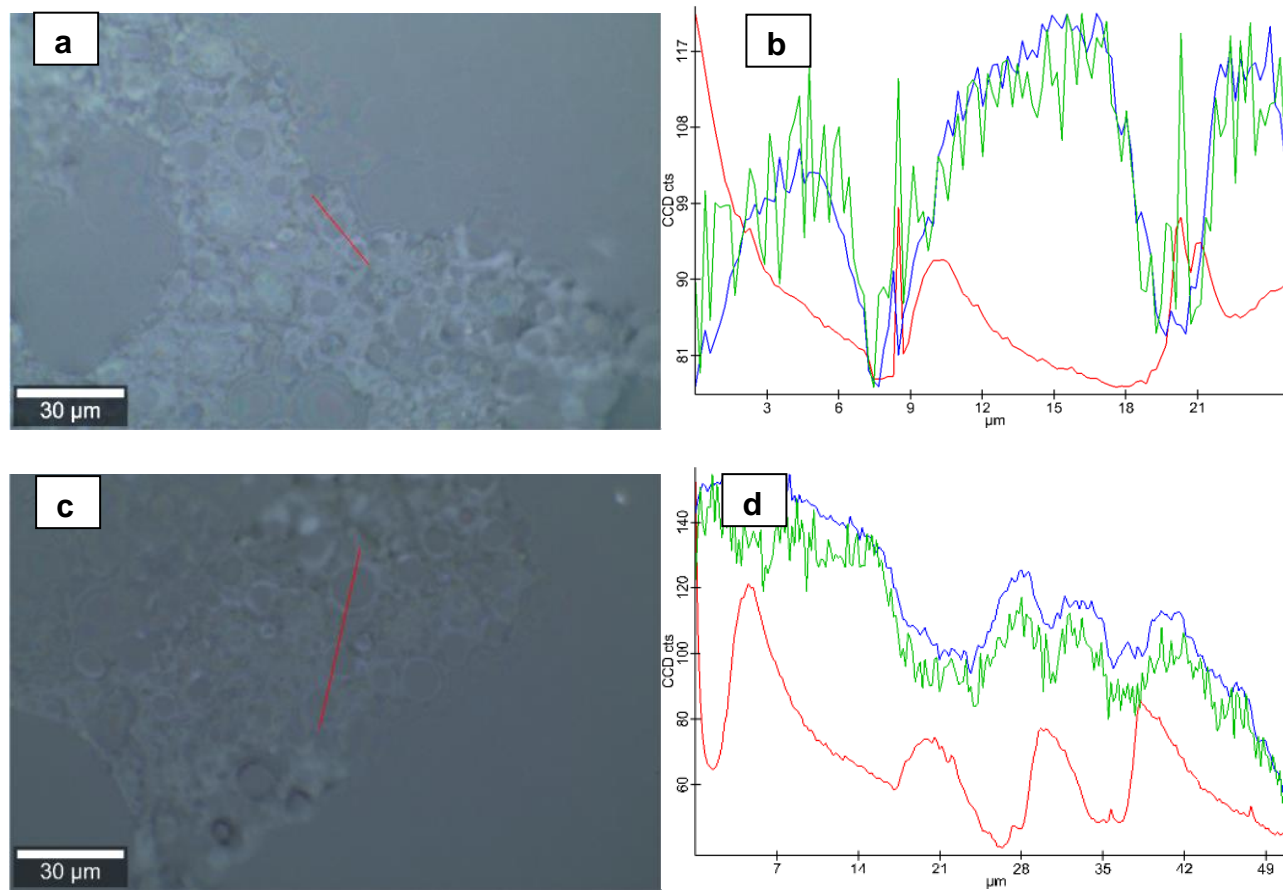
67 Figure 9 shows confocal Raman images of DSEs with  $\beta$ -carotene-in-core  
68 safflower oil droplets analysed at high power exposure (15 mW). The  $\beta$ -carotene  
69 channels indicate some photo-bleaching occurred while scanning at high power.



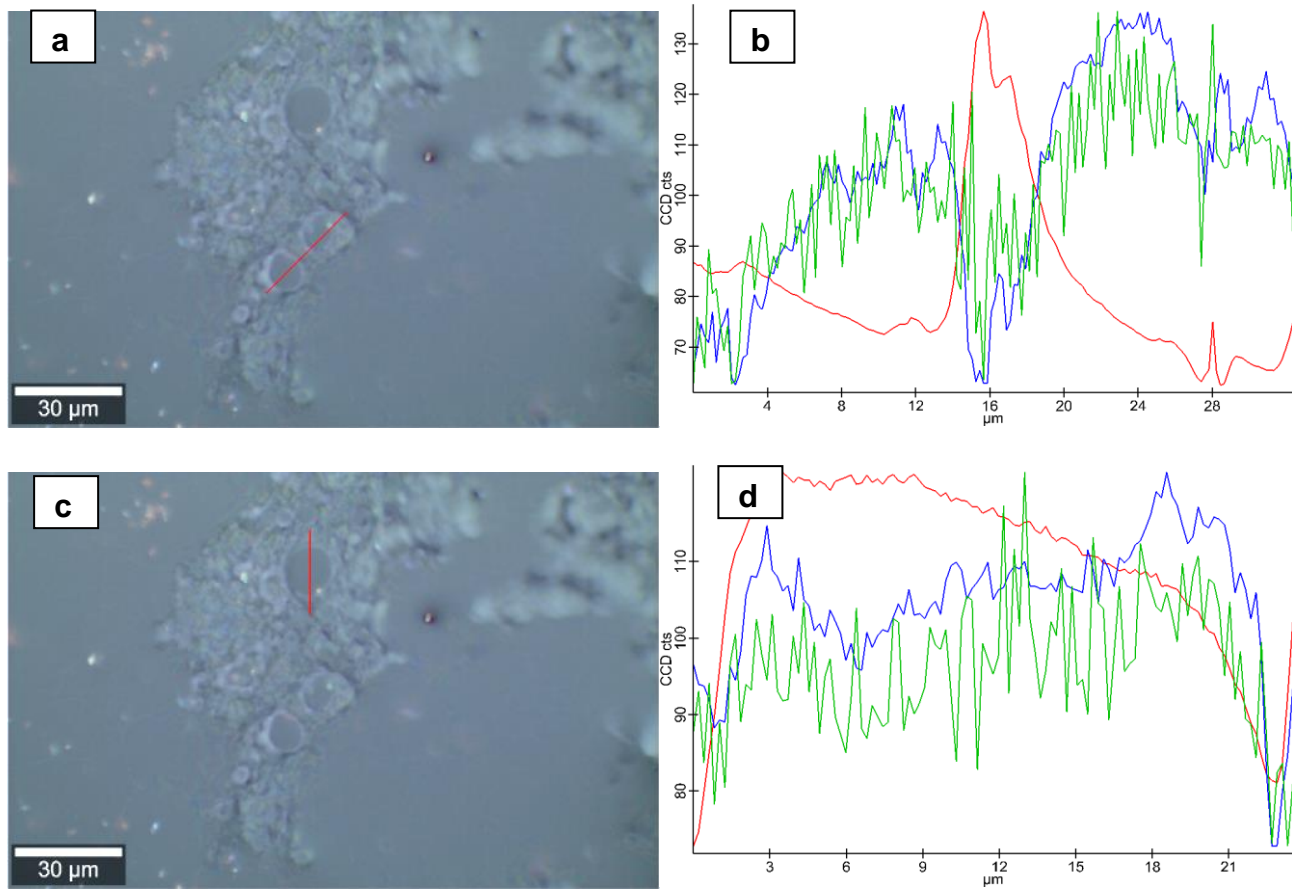
70 Figure 1: Raman spectra of DSEs with and without antioxidants (BHA & β-carotene)



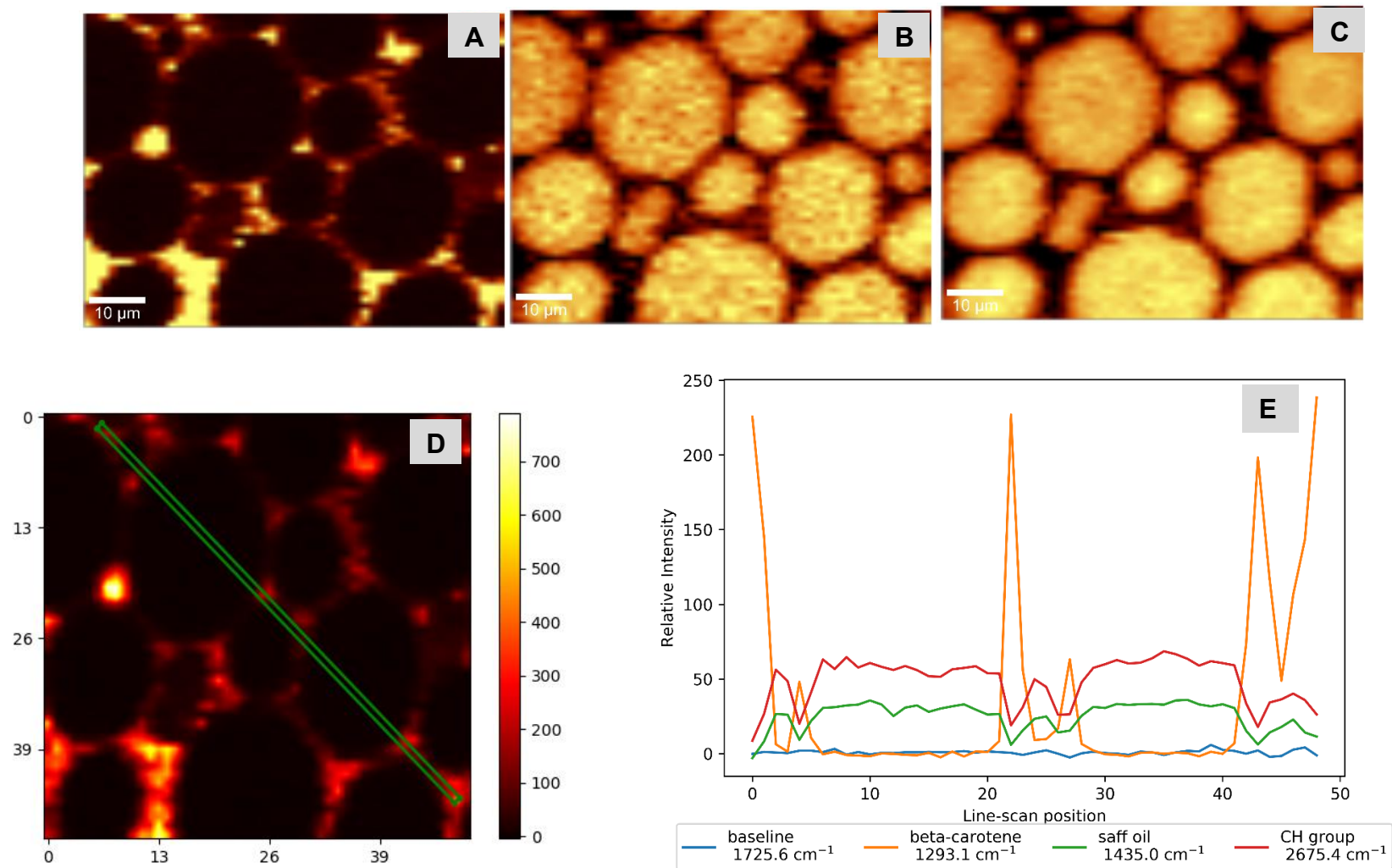
71 *Figure 2: Olive oil DSE (without B-carotene) with 150 points (top) & 265 points (bottom) (a & c) bright field reflectance mode images, (b & d)*  
 72 *peak integral distribution (red trace=  $\beta$ -carotene; blue trace= CH band; green trace= olive oil).*



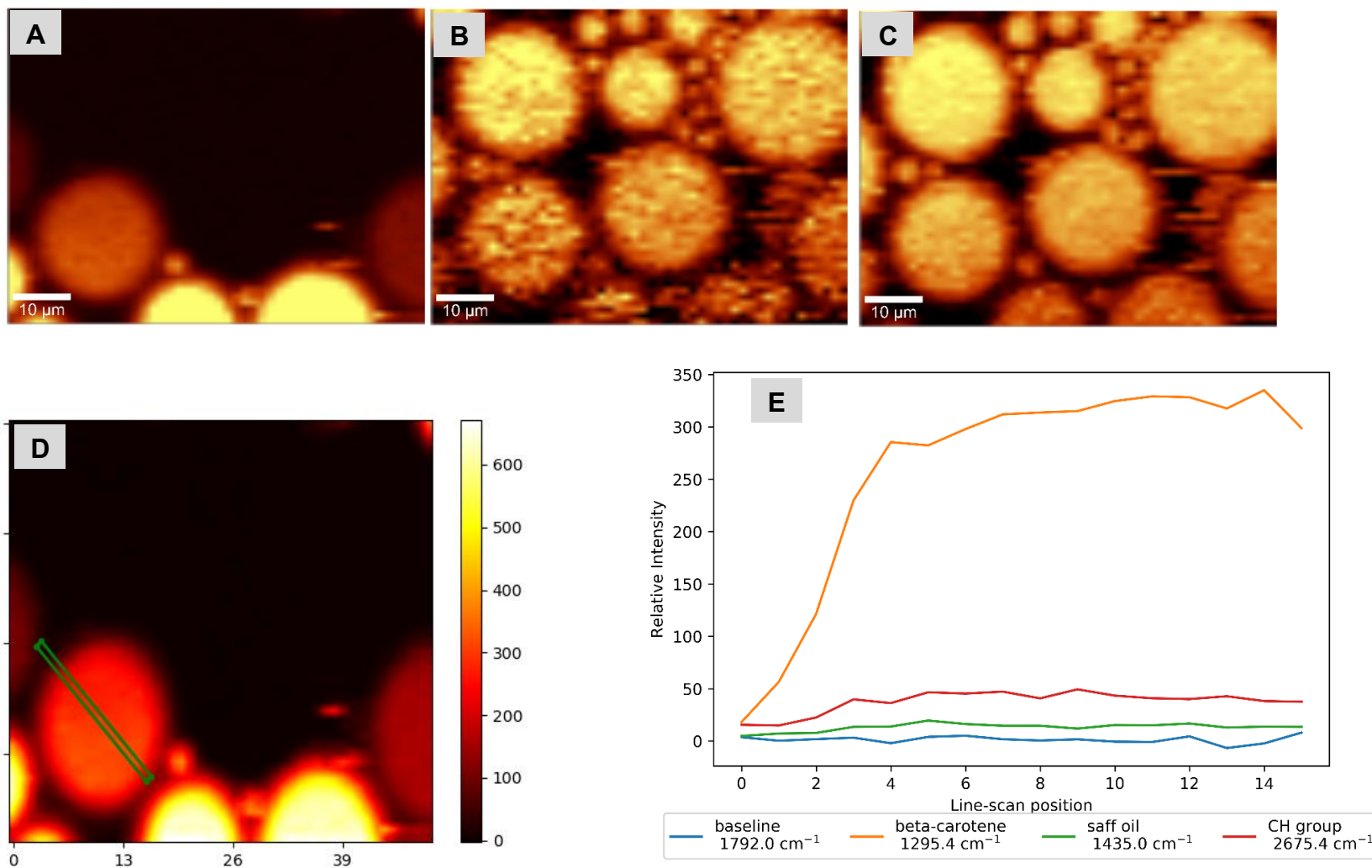
73 *Figure 3: Olive DSE (beta-in-shell) with 122 points (top); & 250 points (bottom). (a & c) bright field reflectance mode image, (b & d) peak*  
 74 *integral distribution (red trace=  $\beta$ -carotene; blue trace= CH band; green trace= olive oil).*



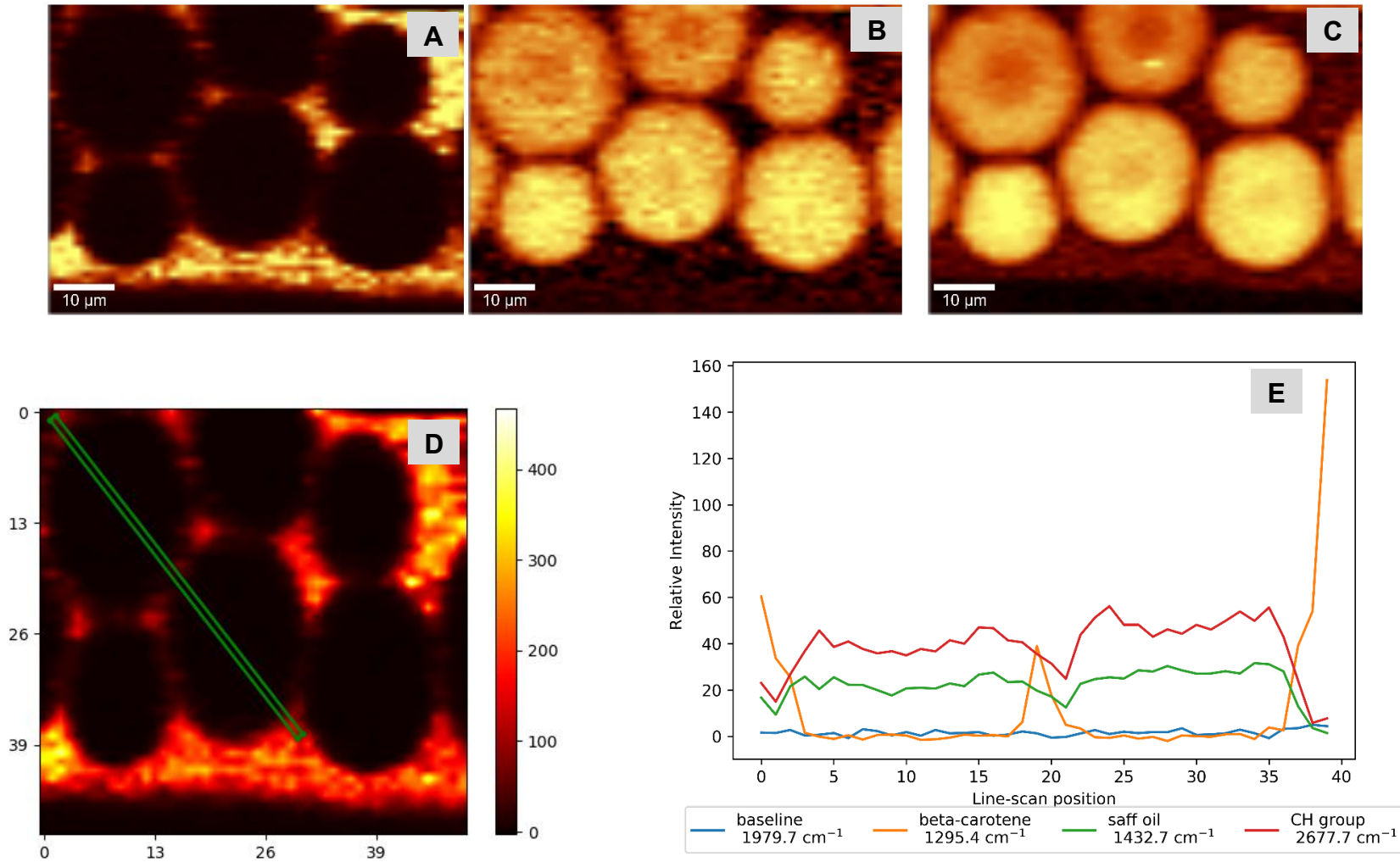
75 *Figure 4: Olive DSE (beta-in-core) with 158 points (top) & 115 points (bottom). (a & c) bright field reflectance mode image, (b & d) peak*  
 76 *integral distribution (red trace=  $\beta$ -carotene; blue trace= CH band; green trace= olive oil).*



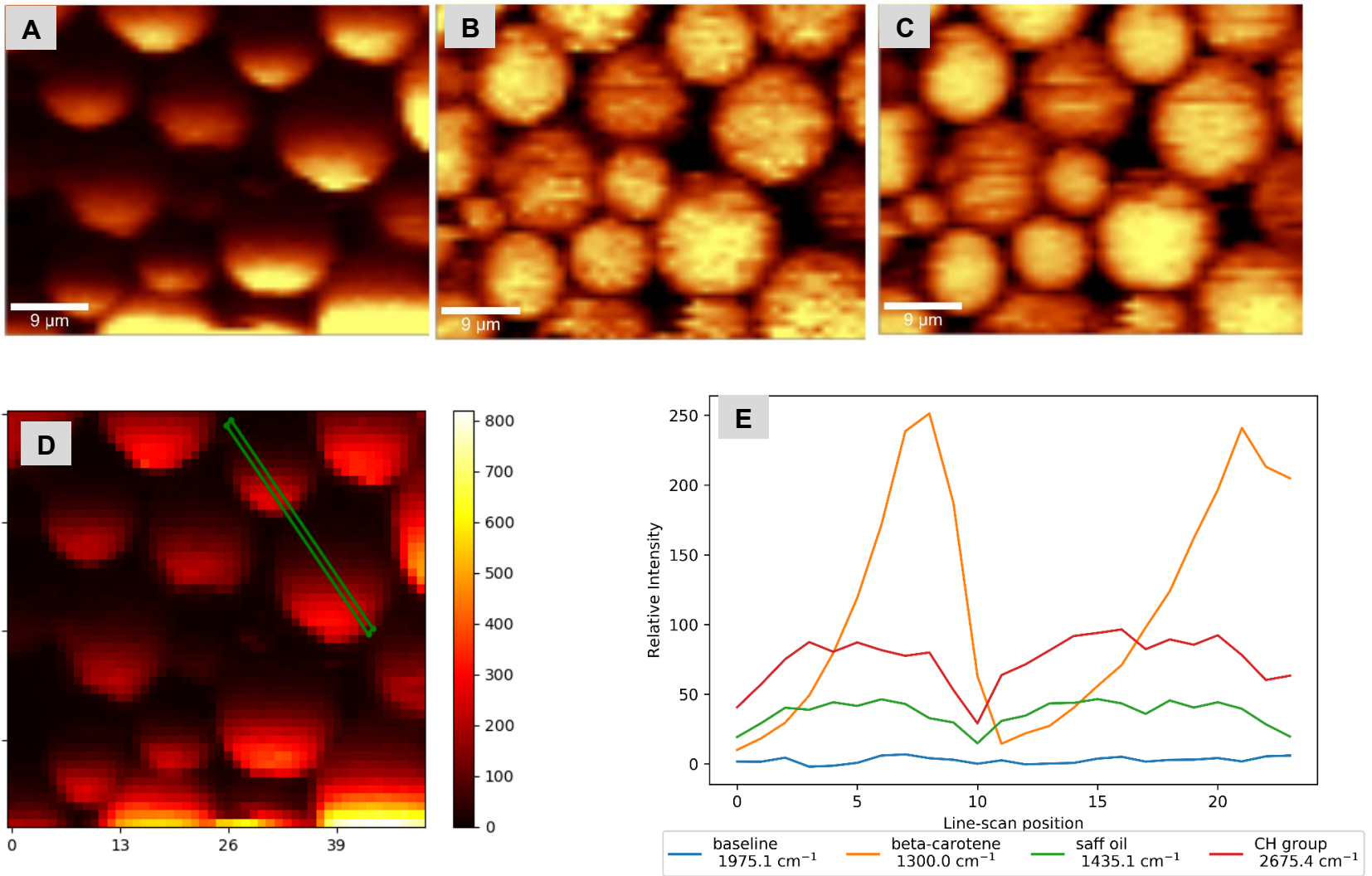
77 Figure 5: Confocal Raman microscopy images and intensity profile of Olive oil control emulsions (shell gently stirred-in) - A, B, & C=  $\beta$ -  
 78 carotene, safflower oil and CH band Raman channels respectively; D= Line scan location across droplets; E= Intensity profiles for selected  
 79 Raman channels across line scan shown in D.



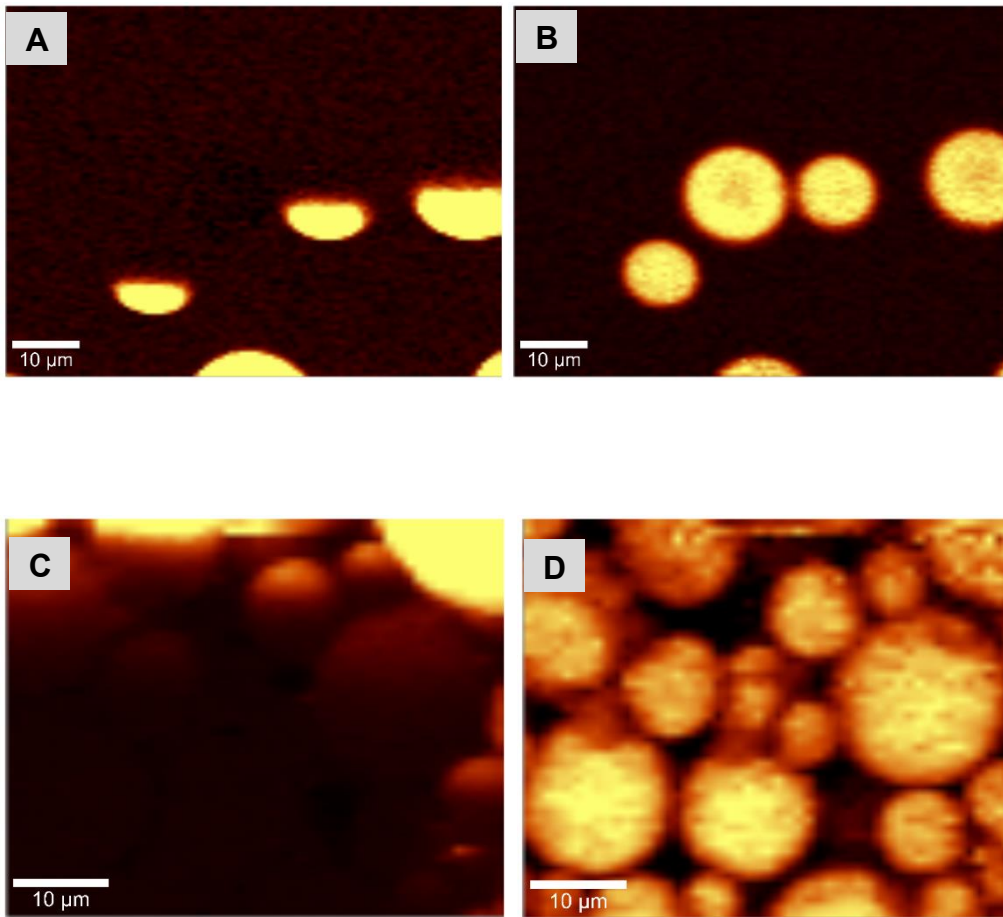
80 *Figure 6: Confocal Raman microscopy images and intensity profile of olive oil DSEs with  $\beta$ -carotene-in-core droplets- A, B, & C=  $\beta$ -carotene,*  
 81 *safflower oil and CH band Raman channels respectively; D= Line scan location across droplets; E= Intensity profiles for selected Raman*  
 82 *channels across line scan shown in D.*



83 *Figure 7: Confocal Raman microscopy images and intensity profile of trimyristin control emulsion (shell gently stirred-in) - A, B, & C=  $\beta$ -*  
 84 *carotene, safflower oil and CH band Raman channels respectively; D= Line scan location across droplets; E= Intensity profiles for selected*  
 85 *Raman channels across line scan shown in D.*



86 *Figure 8: Confocal Raman microscopy images and intensity profile of trimyristin DSEs with  $\beta$ -carotene-in-core droplets- A, B, & C=  $\beta$ -carotene,*  
 87 *safflower oil and CH band Raman channels respectively; D= Line scan location across droplets; E= Intensity profiles for selected Raman*  
 88 *channels across line scan shown in D.*



89 *Figure 9: Confocal Raman microscopy images of DSEs scanned at high power (15 mW)*  
90 *showing evidence of possible photo-bleaching of  $\beta$ -carotene. A & C=  $\beta$ -carotene Raman*  
91 *channels, B & D= safflower oil Raman channels.*