application note

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Confocal Raman Microscopy - Imaging of Emulsions

Confocal Raman Microscopy provides the ability to non-invasively map chemical properties of a sample at the highest resolution and to acquire depth profiles. Using this technique, it is possible to image the distribution of different phases in multicomponent mixtures.

1. Three component mixture: Oil, Alkane, Water

In this experiment, a three component mixture of oil, an alkane, and water was investigated. Using the Spectral Imaging Mode of the alpha300 R, a spectrum at each pixel was acquired by performing a scan in the x-y-direction with a scan range of 50 µm x 50 µm and 120 x 120 pixels (= 14400 spectra). Integration time was only 50 ms. In order to optimize the signal to noise ratio, fit procedures can be performed by the integrated software tools. From a small region of interest, a basis spectrum is generated by averaging all included spectra. These basis spectra can be fitted to the measured spectra, resulting in an optimized image. Using this fit procedure, three images showing the distribution of the different phases were generated and combined into one color coded image (fig. 1). In order to obtain 3-D information, an x-z scan with a scan range of 80 µm x 25 µm on the white line was performed (Fig.2). In both images, the green area shows the alkane (green spectrum in fig. 3), the red areas correspond to the oil (red spectrum in fig. 3), and the blue regions (corresponding to the blue spectrum in fig. 3) represent the water.

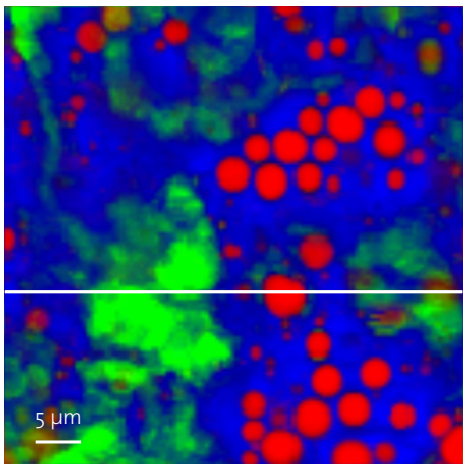

Fig. 1: Distribution of alkane (green), oil (red) and water (blue).

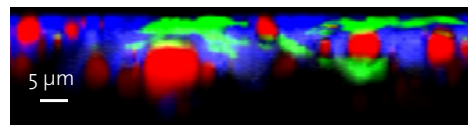

Fig. 2: Distribution of alkane (green), oil (red) and water (blue), depth scan.

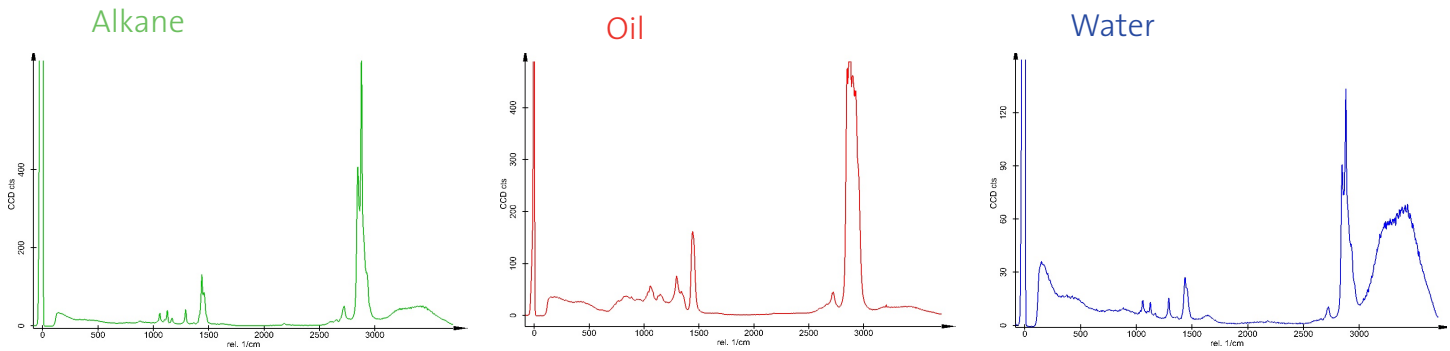

Fig. 3: Corresponding spectra of alkane (green), oil (red) and water (blue).

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2. Ointment

With the alpha300 R, it is also possible to show the distribution of multiple components in an ointment. For this experiment, a commercial ointment was deposited between two cover slips and imaged with an oil immersion objective (N.A.= 1.25). The scan range was 20 µm x 20 µm and 120 x 120 pixels (= 14400 spectra) with an integration time of only 100 ms per spectrum. With special software tools, the spectrum of the agent, Dexpanthenol in this case, was extracted from the measured spectra. In order to verify the generated spectrum, the pure substance was also analyzed (fig. 6). The image in fig. 4 was then created by the same fit procedures described in section 1. Fig. 5 shows a depth scan and fig. 7 shows the corresponding spectra in which the blue spectrum represents the Dexpanthenol (dissolved in water) and the red and green spectra correspond to the ointment bases.

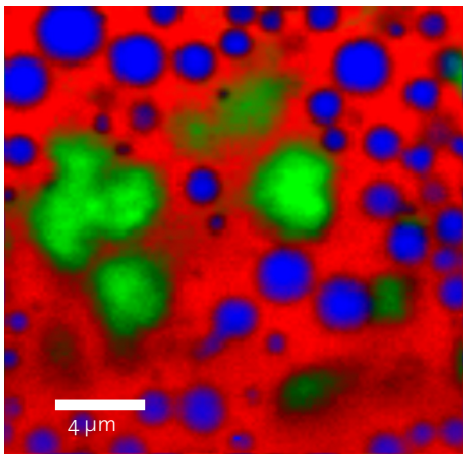

Fig. 4: Distribution of Dexpanthenol (dissolved in water) and ointment basis.

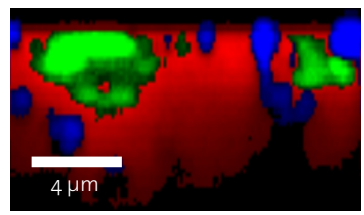

Fig. 5: Distribution of Dexpanthenol, depth scan.

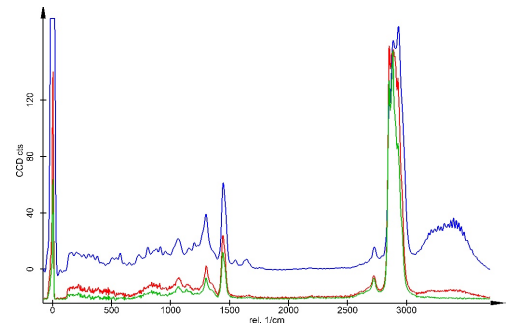

Fig. 7: Corresponding spectra of Dexpanthenol (blue) and ointment basis (green and red).

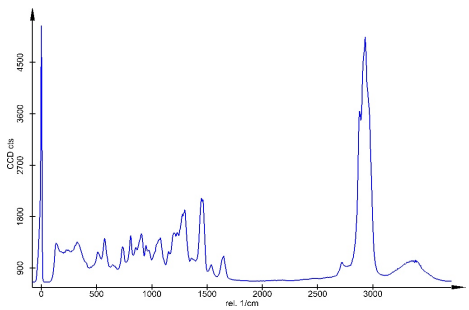

Fig. 6: Single spectrum of Dexpanthenol