Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Three-Dimensional Molecular Imaging of p-Cresol in a Micro-Capillary Cell using Near-Infrared Raman Microprobe Chemical Tomography

Katsuhiro Ajito [a] & Masao Morita [a]

[a] NTT Basic Research Laboratories 3-1, Morinosato-Wakamiya, Atsugi, Kanagawa, 243-01, Japan

Version of record first published: 04 Oct 2006

To cite this article: Katsuhiro Ajito & Masao Morita (1998): Three-Dimensional Molecular Imaging of p-Cresol in a Micro- Capillary Cell using Near-Infrared Raman Microprobe Chemical Tomography, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 314:1, 191-196

To link to this article: http://dx.doi.org/10.1080/10587259808042477

Mol. Cryst. Liq. Cryst., 1998, Vol. 314, pp. 191–196
Reprints available directly from the publisher
Photocopying permitted by license only

Three-Dimensional Molecular Imaging of *p*-Cresol in a Micro-Capillary Cell using Near-Infrared Raman Microprobe Chemical Tomography

KATSUHIRO AJITO and MASAO MORITA
NTT Basic Research Laboratories
3-1, Morinosato-Wakamiya, Atsugi, Kanagawa 243-01, Japan

A three-dimensional (3-D) near-infrared (NIR) Raman microprobe chemical tomography (Raman-CT) system was developed by combining a highly sensitive NIR Raman microprobe spectrometer and a two-slit confocal arrangement. The system provided a very clear 3-D NIR Raman-CT image of *p*-cresol (a model compound for tyrosine) in a 20-μm square micro-capillary cell. The image obtained using NIR laser light contained much less fluorescence interference than those obtained with visible laser light and this indicates that the system has sufficient sensitivity and spatial resolution to identify the distribution of organic or biological molecules in micro-scale samples.

Keywords: near-infrared; three dimensional; Raman CT imaging; *p*-cresol; tyrosine

INTRODUCTION

Two-dimensional (2-D) Raman microprobe imaging using visible laser light has been utilized for various types of micro-scale samples such as oxides[1] and organic materials[2] to investigate their chemical distribution. Recently, this technique has been combined with confocal microscopy[3] to generate three-dimensional (3-D) images[4]. This technique, however, is not suitable for application to the study of organic or biological micro-scale samples due to the fluorescence interference with the Raman spectrum. An effective way to resolve this problem is to use near-infrared (NIR) laser light in the 700 to 1300-nm range as the excitation light source for the Raman measurement. This is because low-energy NIR laser light eliminates fluorescence background signals[5]. We have developed an NIR Raman microprobe chemical

tomography (Raman-CT) system by combining a highly sensitive NIR Raman microprobe spectrometer and a two-slit confocal arrangement. One of the slits is perpendicular in relation to the other and their use makes it easier to obtain the optical alignment needed for confocal Raman measurements than with a pinhole. The 3-D Raman-CT image of *p*-cresol (a model compound for tyrosine) in a micro-capillary provided sufficient sensitivity and spatial resolution for the molecular distribution to be identified and there was much less fluorescence interference than when using visible laser light.

EXPERIMENT

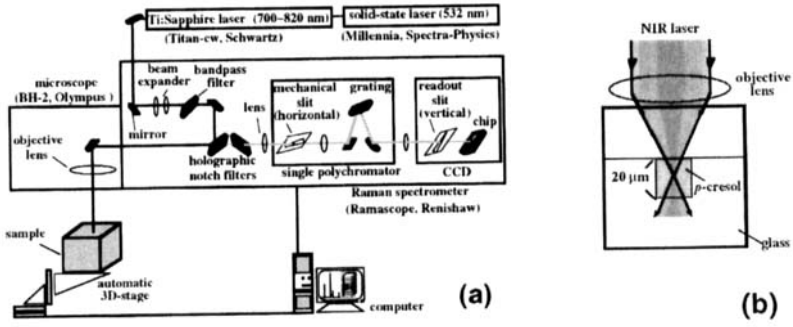

FIGURE 1 (a) The 3-D NIR Raman-CT imaging system. (b) Schematic of the sample and the optical arrangement.

Figure 1(a) shows the Raman-CT system which consists of an NIR Raman microprobe spectrometer, a 3-D stage for the sample, and a computer system. The spectrometer is a specially modified Raman microprobe spectrometer (Ramascope, Renishaw Ltd.) for NIR light. The 780-nm line of a Ti:Sapphire ring laser in the TEM_{00} mode at an output power of 1 W is used as the excitation light source. The expanded beam is reflected by the front holographic notch filter (HNF) and then focused onto the sample using an objective lens. The same objective lens used to focus the laser is used to collect the scattered light. After passing through two HNFs, which remove the Rayleigh scattering component, the collected the light is focused onto the entrance slit of a single polychromator. The confocal arrangement is achieved by using two slits: the very narrow entrance slit of the polychromator and the

very narrow readout window in the CCD detector. The sample is placed on a 3-D piezo transfer stage with a spatial resolution of 10 nm. A Windows-type personal computer system is used to control the NIR Raman microprobe spectrometer and the 3-D stage and to store acquired data. The 2-D and 3-D images are rendered with Slicer software (Fortner Research LLC).

Figure 1(b) shows a schematic of a micro-capillary (20 μm × 20 μm) cell, which we formed in a quartz glass plate by dry etching and then covered with another quartz plate. *p*-cresol (reagent grade) was melted in a hot water bath and poured into the micro-capillary. NIR laser light was focused onto this sample by using a specially adjusted objective lens with high magnification (M = ×50) and a large numerical aperture (NA = 0.55). The laser spot was approximately 2 μm in diameter at a power of about 100 mW.

RESULTS and DISCUSSION

A. 2-D NIR Raman-CT measurement

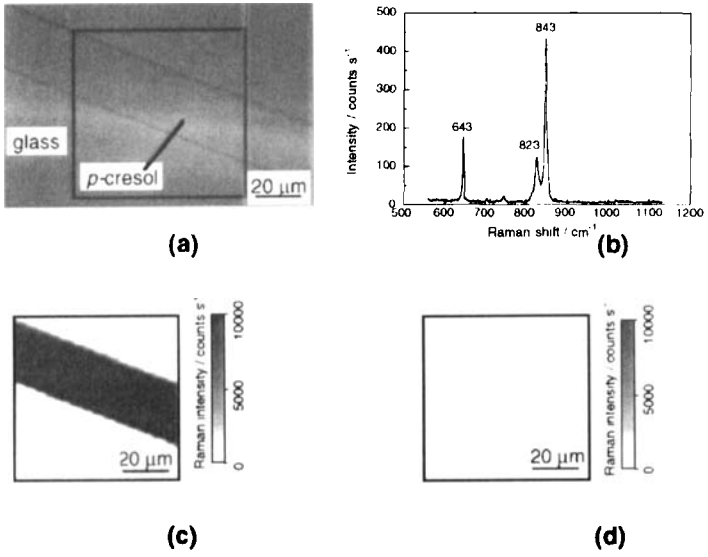

FIGURE 2 (a) An optical micrograph of a micro-capillary filled with liquid *p*-cresol. (b) A typical Raman spectrum of *p*-cresol in the micro-capillary. (c) A 2-D Raman-CT image of *p*-cresol at the peak of 843 cm^{-1} and (d) a background image measured at 900 cm^{-1} of the area in (a) enclosed in the square.

Figure 2(a) shows an optical micrograph of a micro-capillary cell filled with liquid *p*-cresol. The square micro-capillary 20 μm in width was lying in the quartz cell. The *p*-cresol in the micro-capillary was very uniform and contained no bubbles. Figure 2(b) shows a typical Raman spectrum of *p*-cresol obtained near the center of the micro-capillary. The spectral resolution of this mode is about 2 cm^{-1}. The doublet peaks of *p*-cresol at 823 cm^{-1} and 843 cm^{-1} are well separated and very clearly observed. These peaks have been observed for a large number of para-substituted benzenes [6]. The intensity of the background signals is very low in this spectrum. This indicates that the NIR laser light removes fluorescence background signals, which are mainly caused by reagent impurities and/or contamination during sample preparation, and is much better than visible laser light for Raman microprobe measurements. The signal-to-noise ratio determined as the ratio of the peak height to the standard deviation of the baseline noise in the spectrum reached over 80 for the 843 cm^{-1} band of *p*-cresol at 0.3 s, which was sufficient to identify the molecular species.

To obtain a 2-D Raman-CT image, the sample was moved a given distance on the plane and another Raman spectrum acquired. After this point-by-point measurement, the Raman intensity at each point was obtained as the sum of the data within a given spectral range. Figures 2(c) and (d) respectively show the 2-D Raman-CT image of the *p*-cresol band at 843 cm^{-1} and the background at 900 cm^{-1} taken in a square (72 μm × 72 μm) region, which corresponds to the area enclosed by the square in Fig 2(a). The total exposure time in this area was about 6 min. The distance between each point was 2 μm and the spectral range for the sum of the data in each band was 10 cm^{-1}. The intensity of the background image in Fig. 2(d) is very low and uniform. On the other hand the molecular distribution of *p*-cresol is clearly observed in Fig. 2(c). The darker region in Fig. 2(c) corresponds to the *p*-cresol area in the micro-capillary and the brighter regions to glass. No Raman peak was detected for glass at 0.3 s in the spectrum range shown in Fig. 2(b). The darker region is about 1 μm wider than the original shown in Fig. 2(a). This difference is related to the size of the laser spot and the distance between each acquisition point.

B. 3-D NIR Raman-CT measurement

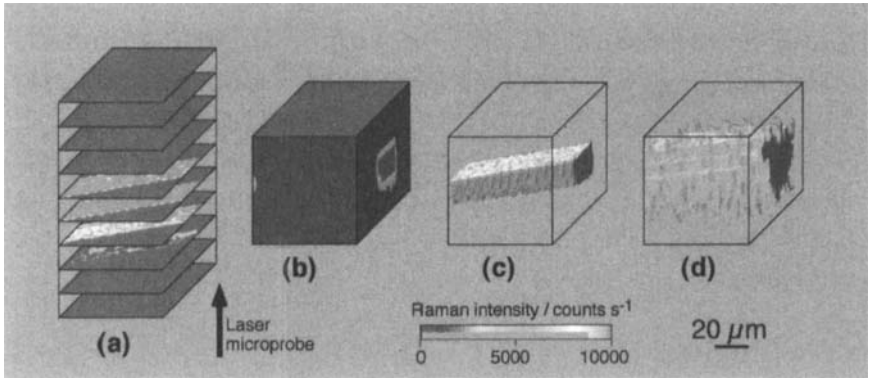

FIGURE 3 (a) Sequentially measured 2-D Raman-CT images of the *p*-cresol in the micro-capillary measured using the confocal arrangement with the two slits. (b) A 3-D Raman-CT image reconstructed from the 2-D images in (a). (c) A transparent representation of (b). (d) A transparent representation measured without the second slit. (See Color Plate I).

Figure 3(a) shows sequential Raman-CT images of the *p*-cresol in the micro-capillary. The transfer stage was sequentially moved after measuring each image plane. These image planes were measured under the same conditions as the image shown in Fig. 2(c). The distance between each plane was 8 μm. The NIR laser beam was focused from the bottom of the sample. The Raman intensity is very low near the top and bottom of these images and the shape of the *p*-cresol was detected near their center. This shows that the two-slit confocal arrangement shown in Fig. 1(a) provides sufficient depth resolution for sample observation

Figure 3(b) shows a 3-D representation reconstructed from the sequential 2-D Raman-CT images in Fig. 3(a). The data were interpolated linearly between each image. The cube was 72 μm × 72 μm × 72 μm. The cross-sectional image of the micro-capillary located on the right side of the cube shows the square shape distribution of *p*-cresol near the cube's center. Figure 3(c) shows a transparent representation above an intensity of 7000 counts s^{-1} of the image in Fig. 3(b). The *p*-cresol distribution is a horizontal square pillar and the surface of each side is very flat. This is in good agreement with the shape of the original micro-capillary and indicates that our Raman-CT system has sufficient resolution and sensitivity to allow us to observe the 3-D molecular distribution of micro-scale samples. The two-slit confocal arrangement

provides a focal depth of about 5 μm. The first slit is a 35 μm mechanical incident slit and the second is a readout slit 130 μm wide, which corresponds to six readout pixels in the opposite direction of the spectral dispersion on the CCD detector. Figure 3(d) is a 3-D representation of the same sample measured with the second slit at a width of 780 μm, which corresponds to the slit being removed. In this image the shape of the *p*-cresol is very different from the original; without the second slit the vertical spatial resolution is very poor.

CONCLUSION

We developed an NIR Raman microprobe chemical tomography system by combining a highly sensitive NIR Raman microprobe spectrometer and a two-slit confocal arrangement in order to obtain the molecular distribution in a micro-scale sample. The 3-D Raman-CT image of *p*-cresol in a micro-capillary cell obtained using NIR laser light shows much less fluorescence interference than when using visible laser light and provides sufficient resolution and sensitivity to identify this molecule in the sample. We believe that this technique will be a viable tool for the 3-D direct molecular imaging of heterogeneous organic and biological micro-scale materials and possibly for 3-D one-line diagnostics.

Acknowledgments

The authors would like to thank Dr. Y. Iwasaki for helpful discussions and Dr. N. Matsumoto for his encouragement.

References

[1.] K. Ajito, J. P. H. Sukamto, L. A. Nagahara, K. Hashimoto, and A. Fujishima, *J. Electroanal. Chem* **386**, 229 (1995).
[2.] M. D. Schaeberle, V. F. Kalasinsky, J. L. Luke, E. N. Lewis, I. W. Levin, and P. J. Treado, *Anal Chem.* **68**, 1829 (1996).
[3.] K. P. J. Williams, G. D. Pitt, D. N. Batchelder, and B. J. Kip, *Appl. Spectrosc.* **48**, 232 (1994).
[4.] C. J. H. Brenan and I. W. Hunter, *Anal. Chem.* **69**, 45 (1997).
[5.] J. M. Williamson, R. J. Bowling, and R. L. McCreery, *Appl. Spectrosc.* **43**, 372 (1989).
[6.] M. N. Siamwiza, R. C. Lord, M. C. Chen, T. Takamatsu, I. Harada, H. Matsuura, and T. Shimanouchi, *Biochemistry* **14**, 4870 (1975).