Volumetric structural mapping of API using polarized line-focus Raman microscopy

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Volumetric structural mapping of API using polarized line-focus Raman microscopy

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There has been a growing interest in analyzing biological samples using three-dimensional (3D) mapping. Traditional Raman based chemical mapping methods utitlise a laser point illumination mode that is known to result in several hours or even days for the collection of one volumetric Raman map at dimensions of 100x100x100 pixels. Volumetric Raman imaging can be used to reveal chemical responses via decomposition procedures such as non-negative least squares (NNLS), multivariate curve resolution (MCR) (see Fig. 1a) and have growing interest in biomedical applications during last years [1]. Herein, we developed an aberration corrected Raman setup capable to work in laser line illumination mode and simultaneously measure 256 spectra through the laser line (Fig. 1c). The key benefit of our setup is connected with special design of laser delivery optics. We used cost effective and powerful multimode laser (785nm, 0.5W) and transformed its inhomogeneous beam profile to the homogeneous diffraction limited line profile on the sample (Fig. 1d).
The fast mapping capabilities of our home-made setup becomes extremely important in applications that require kinetic related investigations using 3D chemical mapping. Hydrates have a pharmaceutical relevant in that they can influence stability, solubility and bioavailability of drug products. Here, we preset a single particle of nitrofurantoin monohydrate (NF MH II) where the dehydration mechanism is being investigated during thermally induced solid form transformations (Fig. 1c). Chemical mapping shows the inhomogeneous distribution of hydrated and dehydrated species of NF in particular regions in the single crystal of NF during dehydration. This is indicative of water leaving the single crystal in an isotropic manner (Fig. 1b). These species can be further decomposed by MCR as 3D separate maps of each crystal form (Fig. 1a). Our experiment requires collection time for one 3D Raman map to be less than 10 min per temperature point. This becomes possible with line-focus sample illumination with acquisition time of 0.3s per line (plus readout time of 0.3s), where total collection time per map (30x30x256 pixels) will be 9min.
Based on our previous study of NF MH crystals we concluded that the decomposition of NF crystal responses based on synchronous analysis of several polarized Raman channels could provide more detailed information of the crystal responses in Raman spectra. Therefore, we acquired the map of NF crystal at different laser polarization orientations (0(ZZ)0, 0(ZZ)90) (Fig. 1b). Obtained data were analyzed by MCR (Fig. 1a.) Herein, we developed a new technique of high-speed 3D structural sample mapping by polarized line-focus Raman microscopy.

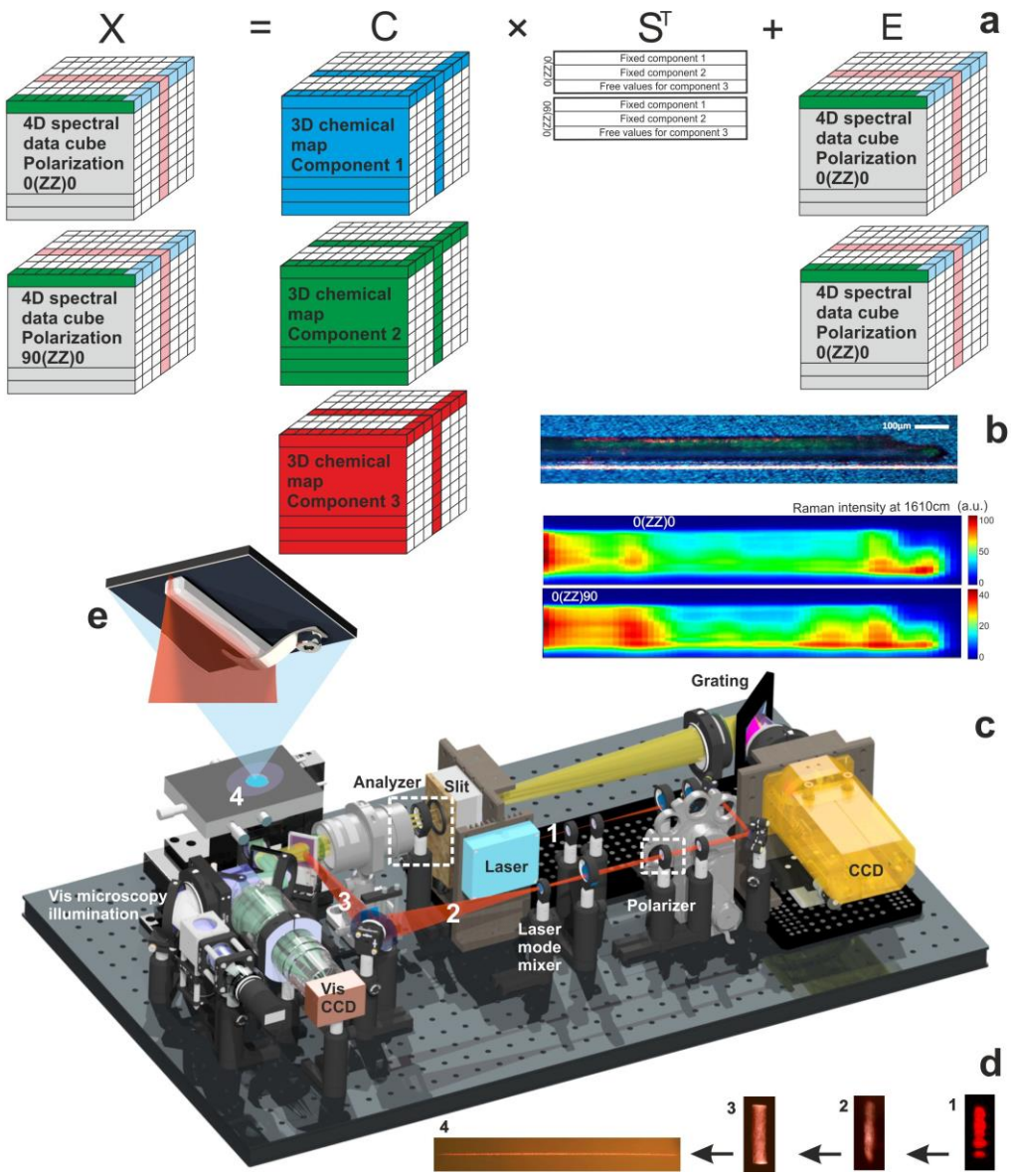

Figure 1. Volumetric chemical mapping of API. **a)** MCR algorithm for volumetric chemical mapping, **b)** line-focus Raman mapping of nitrofurantoin crystal, **c)** polarized lie-focus Raman setup, **d)** transformation of intensity distribution in laser beam profile, **e)** laser line focused on single crystal.

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REFERENCES

[1] C. Kallepitis, M. S. Bergholt, M. M. Mazo, V. Leonardo, S. C. Skaalure, S. A. Maynard, M. M. Stevens. Nature Communications. Vol. 8, 14843 (2017).
[2] P. Connelly, T, Vuong, M. Murcko. Nature Chemistry. 3, 692–695. (2011).

Volumetric Raman mapping of microcontainers for oral drug delivery

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Three-dimensional (3D) confocal Raman mapping is one of the most promising techniques to study the chemical composition of organic materials. However, it is limited in terms of applications due to 1) molecular fluorescence, 2) sample transparency at the laser excitation wavelength and 3) that it usually requires proper fit between the reflection indexes of the microscope objective immersion liquid, cover slip and sample. Herein, we focused on the last two problems related to polymer microcontainers (MC). MC are cylindrical microdevices with only the top side open, fabricated in the epoxy polymer SU-8 [1]. MC are filled with a polymer matrix (polyvinylpyrrolidinone, PVP) and a drug (i.e. ketoprofen) and used for oral drug delivery [2]. First, we verified that the Raman signal attenuation at the depth of 100μm (λ=785nm of laser excitation) was significant and consequently insufficient for mapping, as the total depth of MC was 365μm. The usual way of addressing this problem is to increase of the laser power and/or exposure time. However, this leads to an unrealistic total time of 3D map acquisition (>2 days) or, increasing the laser power simply overheats and burns the sample instead.

We solved these problems by placing MC inside a vial with a fused silica cover slip (Fig. 1a). For proper fitting of the reflection indexes of SU-8, PVP and cover slip we filled the vial with an agarose hydrogel (1%). The agarose hydrogel additionally works as an efficient heat transfer media. In order to reach an acceptable Raman signal at the bottom of the MC (365μm of signal attenuation), we increased laser power up to 70mW in the focal spot on the sample. Due to relatively high absorption of the laser excitation by the sample material, we placed the vial with MC on the surface of a Linkam stage and kept it at 8°C during the Raman mapping. 3D mapping of MC was done with a custom designed oil immersion objective having 50x magnification. The last objective lens was also made of fused silica to have an organized comparable reflection index through all layers in the measured system (Fig. 1a). Microscope objective parameters were optimized during the lens design process to maintain the diffraction limited point spread function (PSF) at the depth of 300μm in MC (Fig. 1b).

The above described method helped us to achieve high quality 3D Raman maps of MC (Fig. 1c). For this purpose, we measured Raman spectra of the pure chemical components presented in MC (Fig. 1c) and verified peak positions of each component with minimum peak overlapping. On Fig. 1d, we present 3D plots built on the peak intensity of amorphous ketoprofen (1000cm^{-1}), PVP (932.5cm^{-1}), SU-8 (809cm^{-1}) and the silicon substrate (520cm^{-1}). It is possible to conclude that i) amorphous ketoprofen was homogeneously distributed throughout the MC, ii) no signal from crystalline ketoprofen was recorded, iii) PVP was deposited on the top of MC walls and over the

surface of ketoprofen and iv) the silicon substrate response was recorded only at the bottom of the map. 3D map plotted based on the silicon peak demonstrates diffraction limited axial resolution of our self-made confocal Raman microscope.

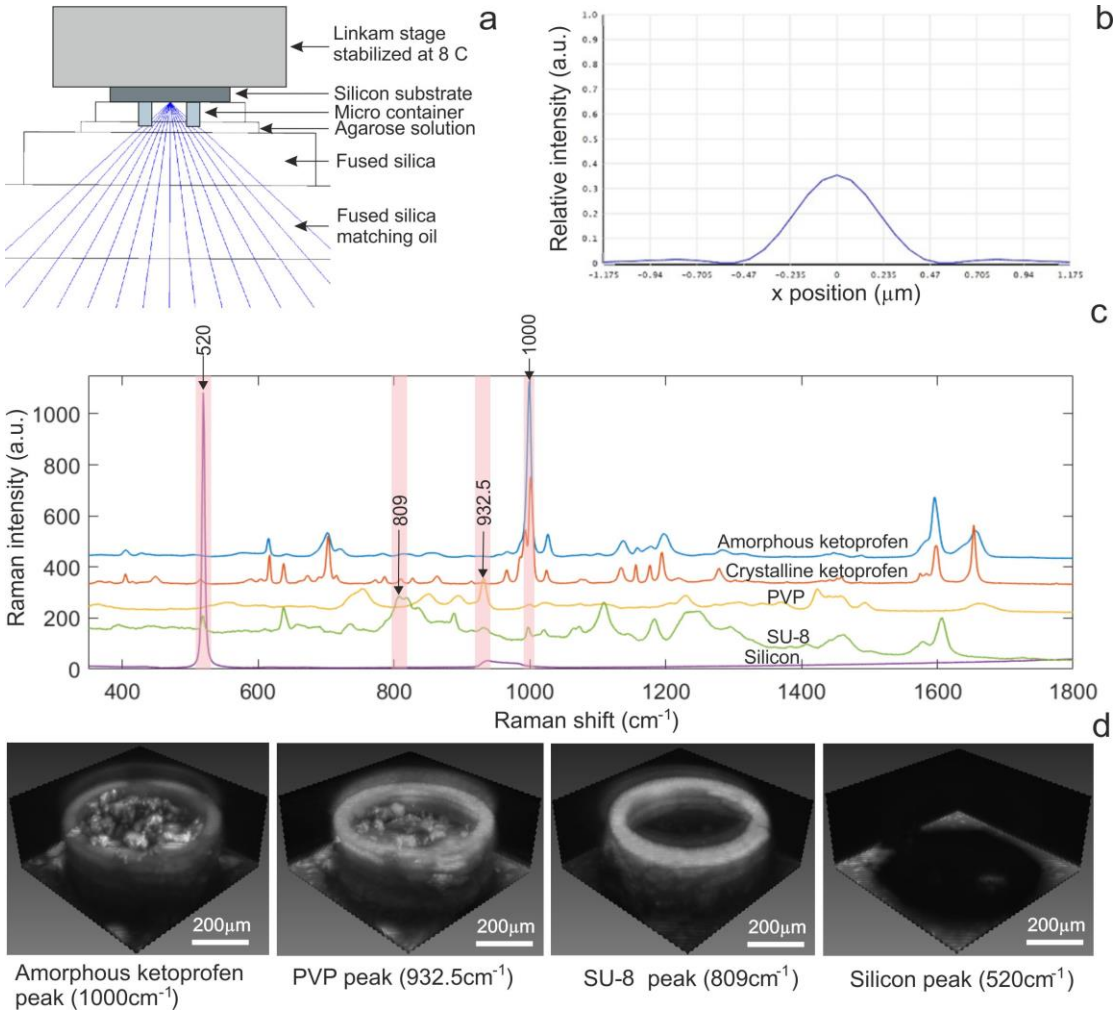

Figure 1. Volumetric Raman mapping. **a)** Reflection index matching geometry for 3D Raman mapping of MC, **b)** PSF of laser beam in the depth of 300 μm inside MC, **c)** Raman spectra of MC components, **d)** volumetric Raman maps of MC built based on peak intensity of chemical components.

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REFERENCES

[1] J. Nagstrup at al, Volume 88, Issue 8, 2342-2344, (2011).
[2] C. Mazzoni, F. Tentor at al, J. Control. Release. 268, 343–351, (2017).