Fast Compressive Raman Imaging of Polymorph Molecules and Excipients in Pharmaceutical Tablets

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ABSTRACT: We implement a near infrared version (NIR) of compressive Raman imaging that incorporates a digital micro mirror device (DMD) and a single pixel detector for fast chemometric analysis and microscopic imaging. The NIR compressive Raman system is successfully used to detect and image active pharmaceutical ingredients exhibiting polymorphism within compact pharmaceutical tablets. We report the chemical imaging of a mixture of two clopidogrel polymorphs and three excipients in solid tablets with a pixel dwell time of 2.5 ms (0.5 ms per species). These results open the road to fast pharmaceutical tablet quality control imaging using compressive Raman technology.

Spontaneous Raman spectroscopy is a broadly recognized analytical technique spanning from research laboratories to industrial process lines[1]. In the pharmaceutical industry Raman spectroscopy has found interests such as the analysis and quantification of active pharmaceutical ingredients (API)[2] but also in the pharmaceutical monitoring processes and in-line quality control[3]. Among the plethora of pharmaceutical applications of Raman spectroscopy, the detection and quantification of polymorph molecules (same chemical composition but different conformation) play a central role, because polymorph API often exhibit very different pharmaceutical efficiencies[4]. This can be explained knowing that the physicochemical properties of polymorph molecules differ, such as their solubility, dissolution kinetic, thermodynamic stability some leading to the dramatic alteration of their bio-availability and then their pharmaceutical efficiency[5]. Because conventional analytical techniques to detect polymorph such as X-rays powder diffraction and solid state nuclear magnetic resonance require complicated sample preparation and may find difficulties when probing the variety of compounds found in pharmaceutical dosages, Raman spectroscopy appeared as a rapid non-destructive appealing alternative[6].

Ideally, Raman analysis should provide a spatially resolved maps for the API, including polymorphs, and the associated excipients necessary to ensure their bio-availability. However, Raman spectroscopy is often preferred over Raman imaging[7] because the acquisition of the complete Raman spectrum for each sample spatial pixel, coupled with the weak Raman scattering cross section and detector array noise, requires lengthy acquisition times and generates large data sets. Typically, spontaneous Raman imaging requires 0.1 s per pixel leading rapidly to an over-night acquisition time (> 25 hours) to image a mm^2 pharmaceutical tablet area with a spatial resolution of ~1micron[8].

Notably, acquiring the complete Raman spectra at each sample pixel is a very inefficient way to map the spatial distribution of known pharmaceutical compounds. A more efficient way takes advantage of the knowledge of the molecular compounds that are present in a sample[9]. This precisely what does the recently developed compressive Raman technology (CRT)[10, 11, 12, 13]. In CRT the measurement is directly designed to estimate the quantities of interest (*e.g.*, molecular concentrations), rather than deducing them from complete Raman spectra acquired at each pixel. Owing to the photon noise detection limit of single pixel detector and to the ability to retrieve the proportion estimation of chemical species with a low photons number, CRT was shown to perform Raman images ×10-×100 times faster than conventional EMCCD and CCD based Raman systems. Figure 1(a) shows a schematic view of a compressive Raman microscope[14] where a digital micro mirror device (DMD), acting as a fast programmable binary spectral filter, is inserted into a spectrometer to send the Raman photons onto a single pixel detector[10, 13]. Central to CRT are the design of the binary filters that are displayed sequentially to estimate the proportion of the chemical species, at each pixel (see Supp info paragraph 4 for a brief summary). The reported algorithms[10, 12] proceed in a similar way to design filters that maximize the precision of the chemical species proportions. This is done by the minimization of the trace of covariance[10], or the Cramer-Rao lower bound[12], matrices. The two approaches being equivalent when the number of binary filters equals the number of chemical species[12].

In here we follow the proportion estimation reported in[12] to map two polymorphs of clopidogrel, a commercial drug used to prevent platelet aggregation and to reduce heart disease risks and

strokes[15], and three excipients (starch, mannitol and PEG) found commonly in pharmaceutical tablets. We show that these chemical species can be successfully estimated at each micron size location on the tablet surface with a pixel integration time as low as 2.5 ms (0.5 ms per species). This allows to map 1 mm^2 area in 40 minutes with a spatial resolution of 1 μm.

EXPERIMENTAL SECTION

Compressive Raman Technology (CRT): The complete CRT setup is shown in supporting information figure S1. It uses a 785nm CW laser (IPS – 100mW) to minimize parasitic fluorescence with a laser line filter integrated to reduce the bandwidth down to 0.3 cm^{-1}, a two axis galvanometric scanner (Cambridge Technology) and a commercial microscope stand (Nikon Eclipse) equipped with a ×20, NA = 0.75 objective lens (Nikon CFI Apo Lambda). This objective focuses the 785 nm excitation light and collects the back emitted/reflected Raman photons. The latter are descanned and separated from the excitation beam by a dichroic mirror and sent to a custom build CRT spectrometer. At the spectrometer entrance a 785 nm notch filter (HSPF785.0 – Kaiser) reject all the remaining laser and Rayleigh light. The CRT spectrometer is composed of a high transmission (~85%) grating (1200 groove/mm) that dispersed the Raman photons towards the DMD (Vialux V-650L NIR) with optimized NIR reflection. The binary encoded spectral filter displayed on the DMD send the Raman photons towards a single photon avalanche photodiode (SPAD-ID Quantique-ID120) with a 75% quantum efficiency at 800nm. The galvo mirrors control and image acquisition is controlled by a custom LabView based software. This software controls also the DMD display via the Vialux interface. To end this paragraph, in our case, the spectral resolution is estimated to be $\delta\lambda \approx 12$ cm^{-1}, the spatial resolution 1 μm (x,y) and 10 μm (z axis).

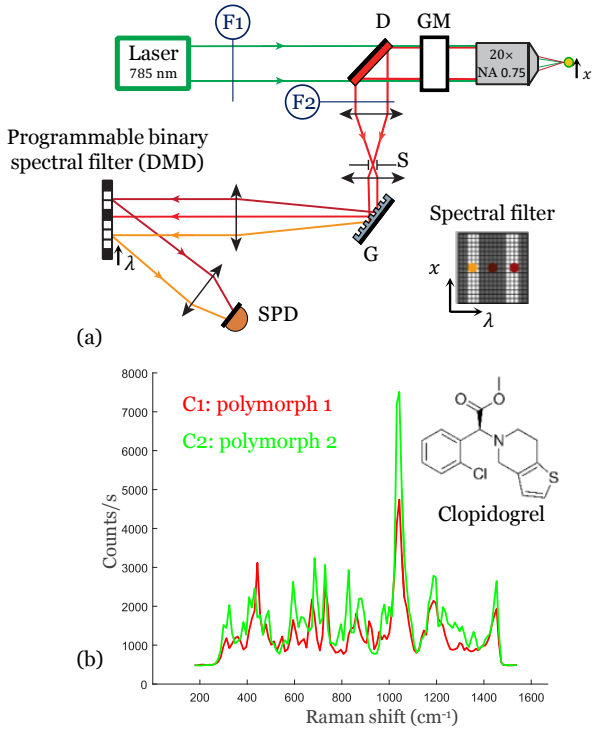

Figure 1. (a) Simplified sketch of a CRT setup (see supp info for a detailed setup description), in this work we developed a NIR CRT version with a laser emitting at 785 nm; D: dichroic mirror; GM: Galvanometric mirrors; S: confocal slit; G: amplitude grating (1200 lines/mm); DMD: digital micro mirror device; SPD: single pixel detector; F1: laser line filter, F2: notch filter. **(b)** Spontaneous Raman spectra of the two polymorph forms C1 (red) and C2 (green) of clopidogrel.

Polymorph CRT imaging: To illustrate the relevance of CRT to image APIs in tablets we choose two polymorph forms called C1 and C2. The spontaneous Raman spectra of C1 and C2 are shown in Figure 1(b). These known spectra are used to design the binary filters that minimize the variance of the estimated C1 and C2 proportions following[12, 13]. The designed binary filters are displayed in Figure 2 (a, b) for the two polymorph forms C1 and C2, respectively (Raman photons falling in the white stripes are kept while the photons falling in the grey stripes are rejected).

The CRT proportion estimate[12, 13] of C1 and C2 mixed in a test pharmaceutical tablet have been associated with an RGB filter and shown in Figure 2(c). When colored in red (C1) and green (C2) (Fig. 2(c)) and overlaid (Fig. 2(d)), they provided a colorful chemical image of the two polymorph distributions in the measured tablet. Figure 2(c) presents the proportion estimate of the C1 and C2 species. The background (in black) is also considered as a species and has its own binary filter (not shown). From the three species (C1, C2, background) proportions present at each pixel, a classification is carried out: on Fig. 2(d), at each pixel, a single color is displayed that corresponds to the dominant species. This classification is justified because the tablet has been produced from pure C1 and C2 compounds. Note that no further image processing (smoothing…) was performed to improve the image rendering.

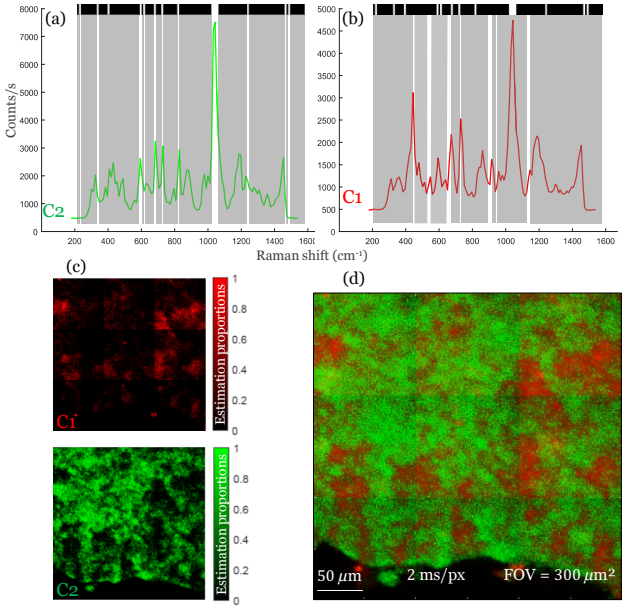

Figure 2. Designed binary filters (Raman photons falling in the white stripes are kept while the photons falling in the grey stripes are rejected) for clopidogrel polymorphs C1 **(a)** and C2 **(b)**. **(c)** Portion estimates for C1 and C2 as retrieved by the algorithm and colored in red (C1) and green (C2) using an RGB filter. (d) The overlaid of the two provides chemical imaging of the tablet (the image scan was performed near the tablet edge for clarity). Scale bar: 50

μm, pixel dwell time for each API image: 5 ms (10 ms in total), 300 × 300 μm (300 × 300 pixels)

Polymorph and excipient CRT imaging: Next we consider a powder mixture composed of the C1 and C2 clopidogrel polymorphs mixed with three excipients: starch (STA), mannitol (MAN) and polyethylene glycol (PEG). The powder chemical compounds were deposited on a CaF_2 substrate without further preparation and directly imaged with the compressive Raman microscope. Figure 3(a) shows the Raman spectra of the five considered species together with the numerically designed binary filters (top rows – white pixels correspond to selected wavenumbers whereas black pixels correspond to rejected wavenumbers). Figure 3(b) displays the estimated proportions results (that have been colored filtered) for each of the APIs and overlaid on the same image. All the APIs could be identified correctly and the proportion algorithms could retrieve the pure nature of the powder samples (each of the pixel is a pure chemical species). Note that at certain zones in the image, we see superposition of C1 and C2, which may not be fully identified spatially. We can assume that at these points a higher number of photon (and therefore a longer acquisition time) would be necessary in order to be able to perfectly discriminate between these two spectrally very close species. In supp info Fig. S2 we validate the CRT species identifications for 5 species by recording full Raman spectra. This validates the chemical species validated by CRT.

Complex pharmaceutical tablet imaging: As a final example we consider a compact tablet (1.5 cm diameter, ~2 mm thick) made of the two clopidogrel polymorphs and the three excipients in the relative proportion 10% C1, 40% C2, 40% STA, 5% MAN and 5% PEG. The five binary filters used for CRT imaging were the same as in figure 3 and demonstrate the ability of CRT to work with binary filters that have been designed once (Fig. 3(a)) and hold for further measurements. Figure 4 shows the estimation proportion of the three most concentrated APIs (C1, C2 and STA) over a region of interest of 300 × 300 μm located near the edge of the tablet. Note the chemical species MAN and PEG couldn't be found in this region. As in figure 2(d) and 3(b), no further image processing was performed to improve the image rendering.

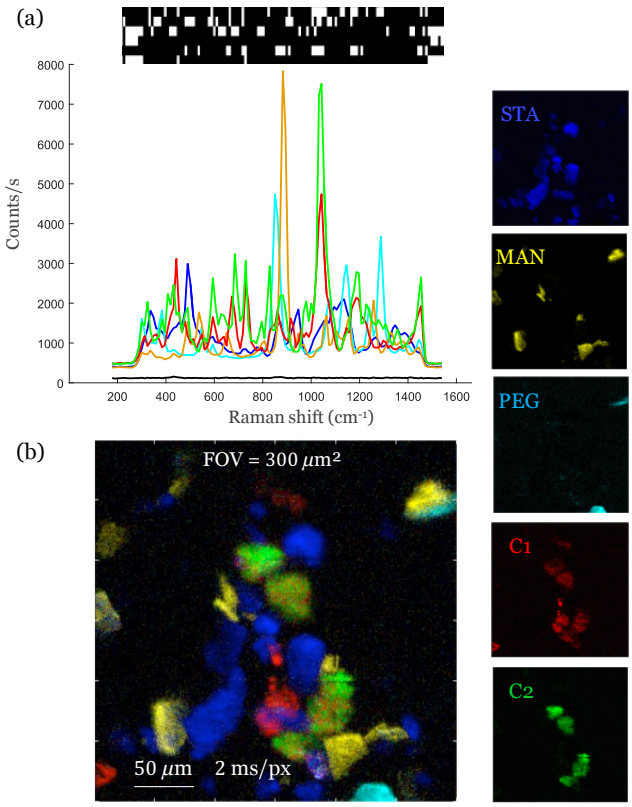

Figure 3. Powder samples: **(a)** Raman spectra of the five considered API, C1 and C2: clopidogrel polymorphs, STA: starch, MAN: mannitol, PEG: polyethylene glycol and the associated five binary filters. **(b)** Estimation proportions color filtered and overlaid on the same image. Scale bar 50 μm, pixel dwell time for each species image 2 ms, 300 × 300 μm (300 × 300 pixels).

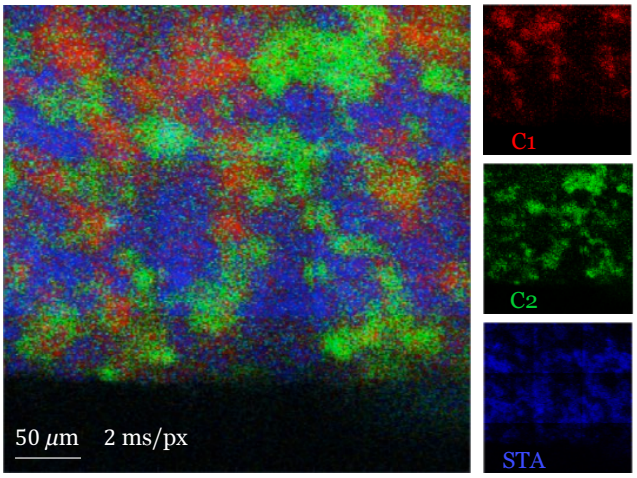

Figure 4: Complex pharmaceutical compact tablet composed of 10% C1, 40% C2, 40% STA, 5% MAN and 5% PEG as imaged by CRT. Only C1, C2 and STA could be retrieved in this region of interest located close to the tablet edge. Scale bar 50 μm, pixel dwell time for each species image 2 ms, 300 × 300 μm (300 × 300 pixels)

High speed pharmaceutical tablet CRT imaging: For this type of application where the compounds are *a priori* known, CRT is beneficial over conventional Raman because it uses

prior information on the chemical species Raman spectra from which the binary filters can be designed (Fig. 2(a) and 3(a)). To investigate the ability of our CRT setup to perform fast imaging of complex pharmaceutical tablets figure 5 compares the CRT image quality obtained with pixel dwell time ranging from 0.5 ms/pix to 10 ms/pix. The considered API sample and the region of interest is the same as in figure 4 (complex tablet). At 0.5 ms/pixel/species it is still possible to distinguish the three chemical species C1, C2 and STA. This probably sets the speed limit on this type of sample to access a decent signal-to-noise ratio for API detection. We noted that for increasing pixel dwell time, the error probability decreases and the estimator reveals the accurate chemical species at each pixel. As a consequence, with increasing pixel dwell time, there are more and more pixels that take the same color on macroscopic areas that correspond to a pure chemical compound.

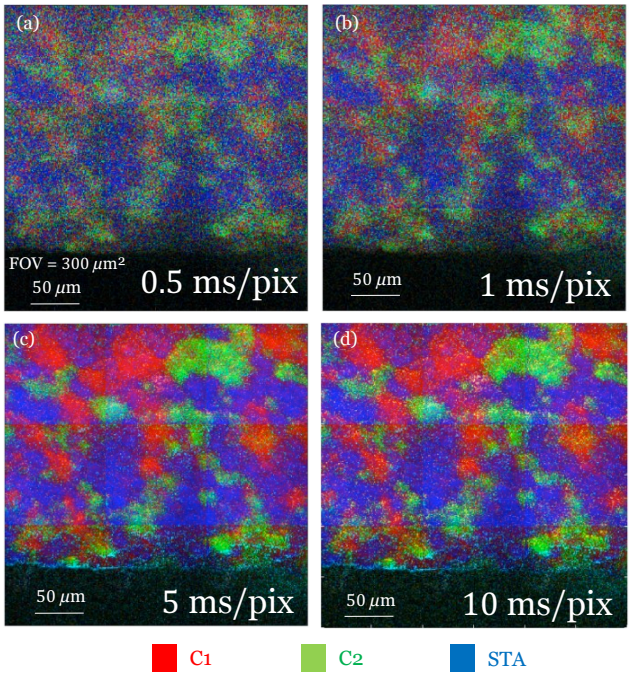

Figure 5. CRT imaging performed at different pixel dwell time. **(a)** 0.5 ms/pixel/species, **(b)** 1ms/pixel/species, **(c)** 5 ms/pixel/species and **(d)** 10 ms/pixel/species. C1 and C2: clopidogrel polymorphs, STA: starch. 300 × 300 μm (300 × 300 pixels).

CRT comparison with stimulated Raman imaging: As an interesting comparison in reference[16] we used stimulated Raman[17] to image in Epi collection the same tablet samples with a pixel dwell time of 400 μs per spectral point. Although a precise comparison between CRT and SRS is difficult we qualitatively estimate that the CRT image quality at 5 ms/pixel is similar to the SRS image quality at 0.4 ms/pixel. This make SRS superior by roughly a factor of 10 as compared to CRT for this type of compact pharmaceutical tablet samples; an advantage that comes at the price of a drastic increase in technological complexity for SRS (ultra-fast lasers, lock-in detection…)[16, 18]. Note that with improved Epi detection SRS can provide chemical images with a pixel dwell time as short as 100 ns[19].

DISCUSSION AND CONCLUSION

In this work, we have developed a NIR (785 nm) CRT using galvo scanners to quickly scan a focused laser beam across the sample. The CRT imaging system uses a DMD to display sequentially the optimally designed binary filters[12] for each scanned region of interest. This enables chemical proportion estimation of API across the imaging area with a transverse resolution of ~1 μm and a longitudinal resolution of ~5 μm. We have used this system to map APIs in compact pharmaceutical tablets among which two polymorphs show very similar Raman spectra (Fig 1(b)). We have successfully demonstrated the ability of CRT to estimate the chemical proportion for each polymorph and excipient at each pixel. This can be confirmed by performing a quick spectral scan using the DMD for each estimated pure chemical species (Supplementary figure S2). We have demonstrated that the imaging speed can be pushed up to 0.5 ms/pixel/species (2.5 ms in total for 5 species) (Fig. 5(a)) to map a mixture of complex APIs such as polymorphs and excipient in tablets. This leads to image a 300 × 300 μm region of interest in 45 s per species (~4.5 minutes for 5 chemical species. By stitching the field of view our current CRT system can image a 1mm^2 tablet area in 40 minutes with a resolution of 1 μm (Supplementary figure S3). These results suggest that CRT is a valuable alternative technology for pharmaceutical active ingredient mapping with potential application in fast in-line quality control

Supporting Information

The supplementary information includes:
Figure S1: detailed description of the CRT experimental setup
Figure S2: spectral validation of the estimated species
Figure S3: CRT image of 1mm^2 tablet sample area.

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Author Contributions

CG, CS and HR designed and built the experiment, CS and CG performed the measurements and the data analysis. The manuscript was written by CG and HR and approved by all authors.

The authors declare no conflict of interest

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