Lightnovo

APPLICATION NOTE

Quantification of anti-cancer drug via SERS mapping

INTRODUCTION

Therapeutic drug monitoring (TDM) can improve clinical care when using drugs with pharmacokinetic variability and a narrow therapeutic window. Rapid, reliable, and easy-to-use detection methods are required in order to decrease the time of analysis and can also enable TDM in resource-limited settings or even at the bedside. Monitoring methotrexate (MTX), an anticancer drug, is critical since it is needed to follow the drug clearance rate and decide how to administer the rescue drug, leucovorin (LV), in order to avoid toxicity and even death. Quantification of anti-cancer drug via SERS mapping is challenging Raman microscopy applications that typically require research grade systems with deep cooling sensors.

TECHNOLOGY

Raman spectroscopy provides a unique opportunity to study the chemical composition of materials at the microscale.
Such capabilities come at the cost of extremely high requirements for instrumentation: lasers with stabilization of wavelength and power, low noise spectroscopic sensors, and a large clear aperture of spectrometer's optics. Therefore, demanding Raman spectroscopy and microscopy applications usually require high-end, bulky, and costly Raman instrumentation.
Lightnovo ApS found possible solutions to the most critical Raman miniaturization challenges: need for laser temperature and power stabilization, reduction of sensor dark noise, compensation on pixel-to-pixel quantum efficiency (QE) variation, laser optical isolation and achieving high spectral resolution.
As result a novel optics miniaturization strategy allows us to create **compact Raman spectrometers and microscopes** based on non-stabilized laser diodes, densely-packed optics, and non-cooled small pixel size sensors.
Lightnovo ApS proposed miniaturization concept based on real-time calibration of Raman shift and Raman intensity using an in-built reference channel that collects the Raman spectrum of polystyrene located in the spectrometer. We have demonstrated the miniaturization of the whole device dimensions down to several centimeters and achieved excellent sensitivity, low power consumption, perfect wavenumber and intensity calibration combined with high spectral resolution of around 7 cm^{-1} within the spectral range of 400-4000 cm^{-1}.

Details for miniRaman MRs patent

The optical design of our miniaturized Raman spectrometer allows confocal measurements because it utilizes a cross slit confocality concept1 as shown in Figure 1a, 1b. This feature helps to separate out of focus layers, which is beneficial for typical handheld Raman applications where the contribution from sample packaging or glass needs to be minimized. However, cross slit design also allows us to target confocal Raman microscopy applications when the device is additionally equipped with a three-dimensional motorized stage and a white light microscopy module (Figure 1c). The lateral resolution of our miniaturized Raman microscope was tested on polystyrene beads with a 1µm diameter (Figure 1d). Axial resolution was tested on the surface of a SERS substrate with BPE analyte at a concentration 100µM (Figure 1e). Cross sections in lateral and axial dimensions are represented in Figure 1f. They demonstrate a lateral resolution of around 1µm and an axial resolution of around 2µm, indicating a diffraction limited performance in both dimensions. To the best of our knowledge, the presented miniaturized Raman microscope is the smallest reported confocal Raman system that has been designed without compromising on basic performance.

This feature makes miniRaman technology an ideal solution for materials identification and quantitative measurements.

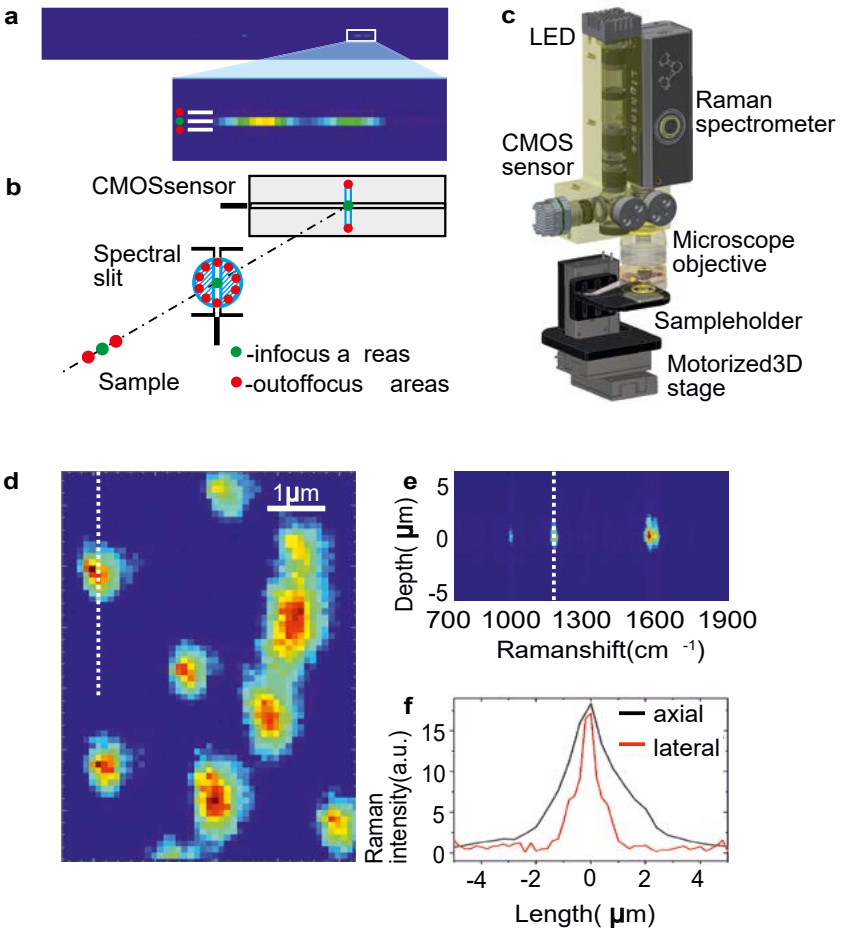


Figure 1. Miniaturized Raman system applied for biomedical Raman microscopy applications.

a) image from the CMOS sensor of the SERS signal of BPE; the zoomed region shows that the spectrum is compressed into one row on the sensor;

b) illustration of the cross slit design of miniaturized Raman spectrometer that is capable for confocal measurements;

c) optomechanical design of miniaturized Raman microscope based on miniaturized Raman spectrometer;

d) Raman microscopy image of polystyrene beads at the size of 1µm obtained with Zeiss objective 100x, NA=0.95, exposure time 0.2 sec per point;

e) depth scan by our miniaturized Raman microscope (equipped with Zeiss objective 100x, NA=0.95) through the surface of SERS substrate with BPE analyte at concentration 100µM;

f) axial (black curve) and lateral intensity distribution of Raman signal as a function of sample displacement (dotted white lines in Figure 2d, 2e indicate areas used for plotted axial and lateral intensity profiles); data demonstrate diffraction limited spatial resolution.

MATERIALS, SAMPLE PREPARATION AND MEASUREMENTS

MTX (98% purity) was initially dissolved in 50 µL of 1 M NaOH, and 2 mM stock solution was prepared in phosphate-buffered saline (PBS), pH 7.4, which was aliquoted and stored at −20 °C until further use. The MTX stock solutions were used to freshly prepare standards in PBS. The MTX standard solutions in PBS was mixed with methanol (MeOH) in various concentration of MTX: 0, 5, 10, 25, 50, 75 µM. Solvents, chemicals, and samples were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Fabrication of the AgNP SERS substrates and NPAS/SERS detection was described in our previous publication.

Microscopy test on PS beads. PS beads with size of 1µm in the form of aqueous suspension were purchased on Merck (MDL number: MFCD00243243). Suspension was deposited on polished stainless-steel surface for Raman microscopy mapping.

RESULTS

It has been shown that nanopillar-assisted separation (NPAS) method using SERS mapping by research grade Raman microscope with deep cooling EMCCD allows to measure MTX in PBS in the linear range of 5–150 µM with LoD = 5µM, LoQ = 25 µM[2]. Here, we also used NPAS method with SERS mapping of the SERS chip surface according to the methodology described in the original publication[2]. Typical SERS maps of SERS substrates measured by our miniaturized Raman microscope are shown in Figure 2a; total measurement time per chip was around 15 mins with exposure time of 0.1 sec per spectrum. In total, 24 SERS chips were used in this study following the NPAS procedure (Figure 2b, 2c). Calibration samples of MTX diluted in PBS were prepared in the range 0-75 µM. SERS spectra of MTX obtained after the averaging of SERS signals collected by mapping of the chip surface are shown in Figure 2d. Result of PLS calibration for MTX quantification is shown in Figure 2e, 2f demonstrating improved LoD = 3 µM, LoQ = 20 µM in comparison to previously reported data[2].

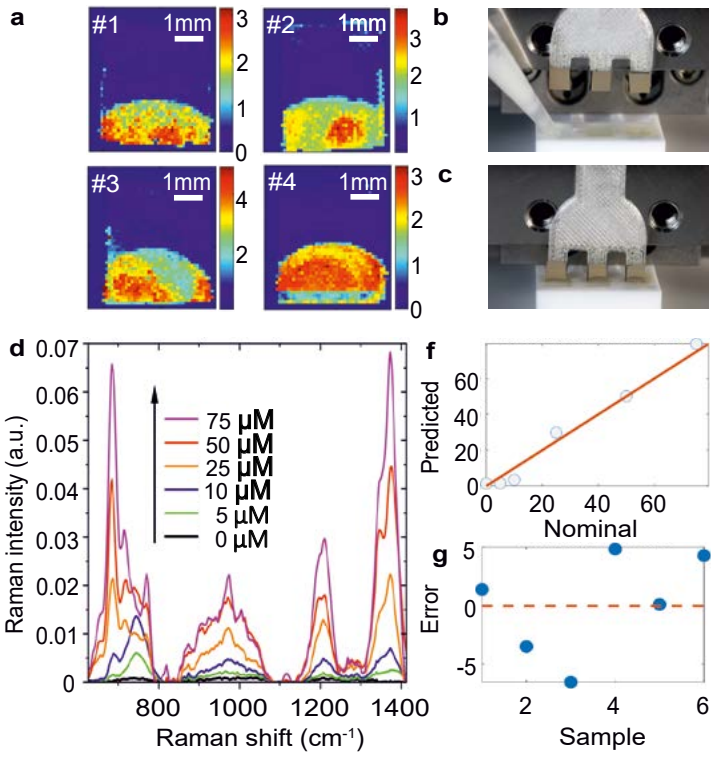


Figure 2. Miniaturized Raman system applied for biomedical Raman microscopy applications.

a) SERS maps of MTX deposited on silver coated NP SERS substrates at concentration of 25µM;

b), c) photographs demonstrating the process of analyte deposition;

d) SERS spectra of MTX at different concentrations (0 – 75 µM) obtained after the averaging of SERS signals collected by mapping of the SERS chip;

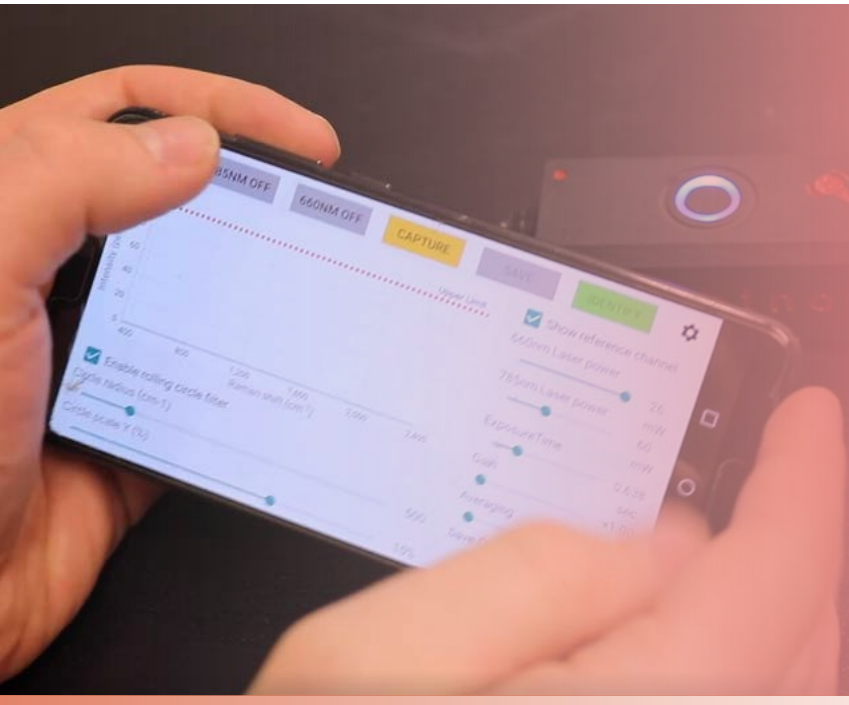
e), f) result of PLS calibration for MTX quantification.

CONCLUSION

Lightnovo ApS demonstrated technology for both miniaturizing and democratizing Raman spectrometers and microscopes, making Raman spectroscopy more accessible to researchers as well as consumers.
This case demonstrates that our miniaturized Raman microscope ideally suits for SERS mapping application and provides advantages in key Raman microscopy requirements such as LoD, LoQ, mapping speed and mapping resolution, system size and affordability.

LITERATURE

1 Slipets R, Ilchenko O, Mazzoni C, Tentor F, Nielsen LH, Boisen A. Volumetric Raman chemical imaging of drug delivery systems. *J Raman Spectrosc* 2020; **51**: 1153–1159.
2 Göksel Y, Zor K, Rindzevicius T, Thorhauge Als-Nielsen BE, Schmiegelow K, Boisen A. Quantification of Methotrexate in Human Serum Using Surface-Enhanced Raman Scattering—Toward Therapeutic Drug Monitoring. *ACS Sensors* 2021; **6**: 2664–2673.



Harness the power of Raman spectroscopy and make it widely accessible for the benefit of mankind.

- Lightnovo's mission

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