Surface enhanced Raman spectroscopy: an alternative approach to detect Malaria strains (3D7 and CS2) spectra in different conditions

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Abstract

The Raman spectroscopy is the vibrational spectroscopy used to identify chemical components in the matter. The information of Raman spectra give a distinct fingerprint of the vibration mode of a particular molecule. In this study, the surface enhanced Raman spectroscopy is chosen as an alternative sensitive approach to detect Malaria stains. Malaria is one of global diseases currently threatening to the global health. Researchers around the world have put their great efforts to detect Malaria at an early stage. This challenge is, thus, the aim of this research. Raman spectrometers with 785nm (Peakseaker 785) and 1064nm (Rigaku handled Raman) laser wavelength are used to obtain the spectra of two Malaria strains 3D7 and CS2 cultured in human serum and cow serum. This spectra reveal different effects from these two kinds of stains in protein modifying the red blood cell membranes. Because the limitation of the portable Raman spectroscopy and a small concentration of molecule samples give obscure spectra, an enhancement method for the detection is inevitably required. Consequently, Onspec chip (NECTEC, Thailand), the surface enhanced Raman substrate fabricated in silver nanorod structure was applied. The results show that the surface enhanced Raman spectroscopy has a potential to identify the Raman peaks from the spectra of the infected red blood cell membrane.

Introduction & objective

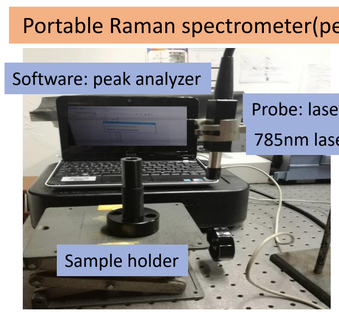
Plasmodium falciparum (or P. falciparum) is Malaria parasite specie. CS2 and 3D7 are 2 particular strains what chosen to be studied in this work. The two different strains can be differentiated by identify the knob association histidine-rich protein(KAHRP) created. The biologist wants to prove that 3D7 which's traditional strain and long time cultured in biology laboratory still create the protein on RBC or not? The study emphasized on the Raman spectra of 3D7 infected RBC in composition CS2 infected RBC and normal RBC by using Raman spectroscopy and surface-enhanced Raman substrate to augment signal.

Moreover, the serum that used to cultured the malaria parasites is also questioned to affect to parasite growing so the divergence of conditions will be obtained: infected RBC in cow serum(Albumax) and infected RBC in human serum.

Instruments & Method

Main instruments

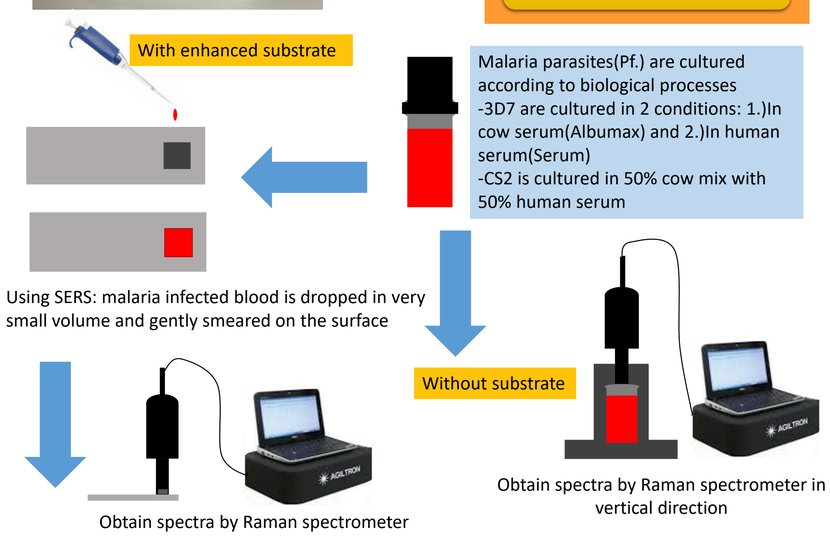
Portable Raman spectrometer(peakseeker785)



Rigaku Raman spectrometer

Surface enhanced Raman substrate

Experimental step



Conclusion & Future works

Although the full efficiency of surface-enhanced Raman substrate still can not be used from the Raman spectra what obtained refer to the composition in normal and infected RBC reasonably and the peak at Raman shift 1599 cm^{-1} can indicate the important protein that's focused in this work: the knob association histidine-rich protein(KAHRP). The intensity ratio from CS2 that cultured in 50% cow and human serum has the same value level as the intensity ratio from 3D7 cultured in human serum and from of event more from 3D7 cultured in cow serum.

However, the process used to make the thin film blood with suitable thickness on surface-enhanced Raman substrate has to be further investigated so on to obtaining the Raman spectra from Malaria infected blood, In addition, other study will be a part of the project "Optical technique used for Malaria detection" so other optical properties of composition in Malaria infected blood also what of interest.

See in website.

Acknowledgement

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Results

Power laser & Parasitemia level effect

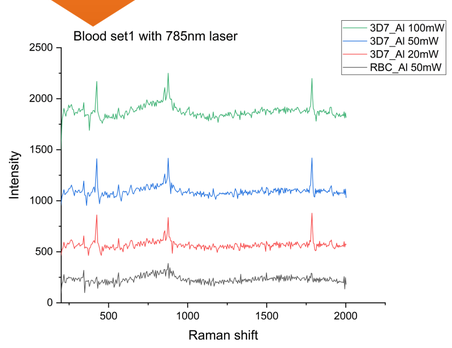


Figure 1: Raman spectra from blood set1 (parasitemia level 5%)with different power laser

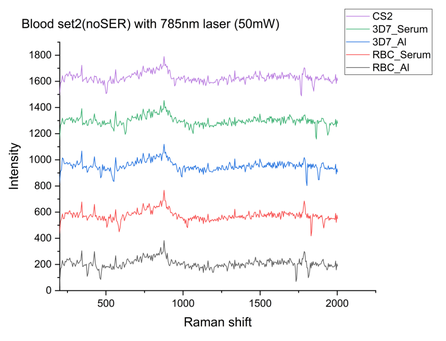


Figure 2: Raman spectra from blood set2 (parasitemia level 2%)

Raman spectra obtained from 20-100mW power laser are almost identical. The differentiation between RBC and infected RBC by 3D7 or CS2 depend on the number of parasites in blood(parasitemia level). Considering 5% and 2% of parasites, the changing in peak compositions of spectra are more obviously seen in 5% than 2%.

Table 1: Source of Raman peaks

Source	Raman shift
Thymine (ring breathing modes of the DNA/RNA bases)	436[9]
CCl_4	459[10]
S–S disulfide stretching band of Collagen ν(S–S) *gauche-gauche-gauche* (amino acid cysteine C-OH(505/508)	505[9]
S-S disulfide stretching in proteins(520)	528[1]
ν(S–S) *trans-gauche-trans* (amino acid cysteine)	540[9]
Cholesterol	548[9]
OH out-of-plane bending (free)(583,586)	585,586[9]
Proline, hydroxyproline, tyrosine, ν2PO2-stretch of nucleic acid	827[9]
Hydroxyproline	876[5]
(proline/glycogen), collagen(896)	893[9]
Proline, hydroxyproline, glycogen and lactic acid(918)	914[9]
ν3(CH3) of proteins (in RBC)	951[1]
Glycogen[7], Beta-carotene[8]	1029
ν4 Hb [ν(pyr half-ring)sym]	1376[4]
δCH2 of lipids	1468[9]
KAHRP, knob protein****	1599[1]
amino acids aspartic & glutamic acid(1712)	1722[1]
In-phase carbonyl C=O stretch(1776/1778)	1780[9]
bound diatomic heme ligands CO	1908,1913[6]
Lipid,ester group	1737[9]
C–O stretching mode	1119[9]

Source	Raman shift
Hemoglobin	378[3], 424[3], 567[3],673[3],745[4], 827[3], 1156[3],1222[3],1362[3], 1448[3], 1526[3], 1547[3], 1639[3],1653[3]
Hemozoin	342[3], 1003[3],1007[3],1625[3],1628[3]
Lipid in RBC	421[1], 724[1], 1087[4], 1439[4]

Strain conditions effect

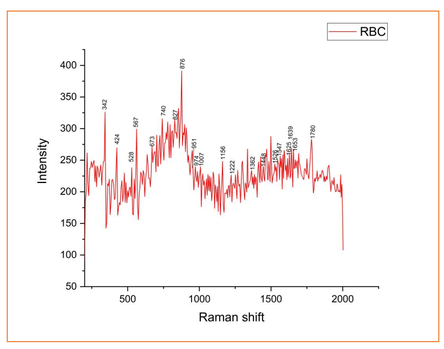


Figure 3: RBC Raman spectrum

Results from 785 nm power laser

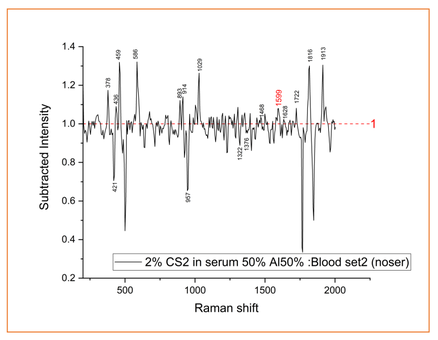


Figure 4: 2% CS2(in50% cow and 50% human serum) infected RBC subtracted spectrum

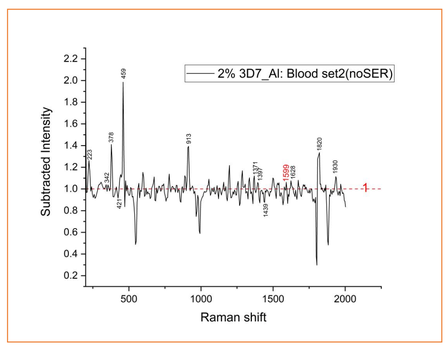


Figure 5: 2% 3D7(in cow serum) infected RBC subtracted spectrum

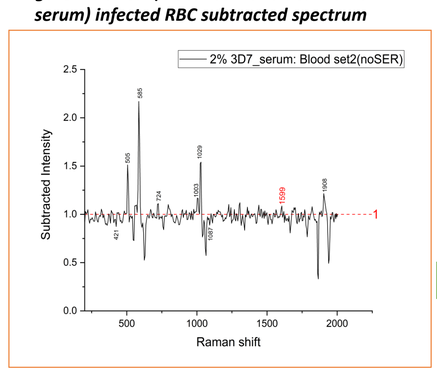


Figure 6: 2% 3D7(in human serum) infected RBC subtracted spectrum

With 785nm laser wavelength results, the crucial peak at 1599 cm^{-1} corresponds to KAHRP. The intensity ratio from 2% 3D7 in human serum is slightly different from the intensity ratio from 2% 3D7 in cow serum. However, from 1064nm laser , the peak from Raman shift at 1599 cm^{-1} of CS2 becomes more prominent than the one from 3D7 in cow and human serum.

Results from 1064 nm laser wavelength with SERS

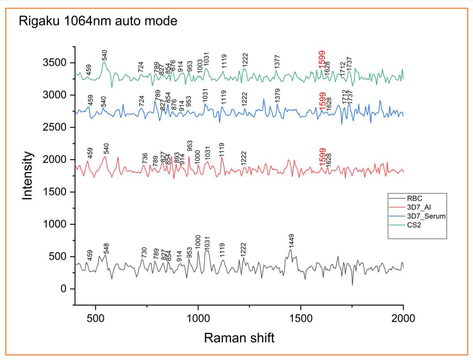
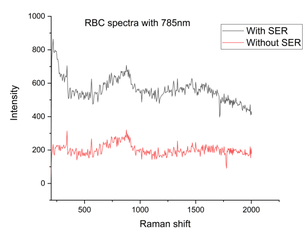
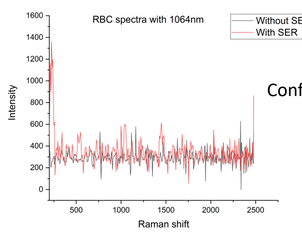


Figure 7: Blood set3(2% parasitemia level) spectra with 1064nm laser wavelength

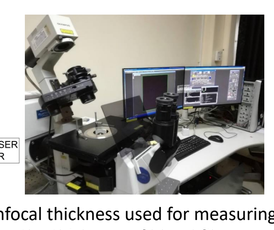
Surface enhanced Raman substrate effect



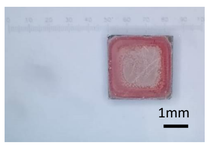
Graph 8: RBC spectra with 785 nm laser wavelength



Graph 9: RBC spectra with 1064 nm laser wavelength



Confocal thickness used for measuring the thickness of blood film



Blood on surface enhanced substrate

As shown in figure 8 and 9, it was found that the Raman peaks are barely enhanced by the SER. The Raman spectra at 785nm before and after using SER are almost unchanged. This suggests that the capability of SER cannot be fully exploited. What is thought to be the source of this in efficiency is the thickness of the blood film. The optical confocal technique given a thickness of 20 μm for blood film compare to only 0.2 μm of nanorod on the substrate[11].

References

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