

Hyperspectral Stimulated Raman Scattering Microscopy for Monitoring Pharmacodynamics of Vancomycin in Biofilm

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Abstract

We report the development of a unique hyperspectral stimulated Raman scattering (SRS) microscopy technique which can be used for investigating pharmacodynamics of antibiotic in biofilms at the subcellular level. With the spectral focusing of 120 fs laser pulses, the hyperspectral SRS spectra can be achieved within seconds while retaining the full speed and image quality of narrowband SRS imaging. To better understand the antibiotic actions on biofilms, we apply hyperspectral SRS technique for direct visualization of the interplay between *Staphylococcus aureus* (*S. aureus*) biofilms and vancomycin coupled with alkyne tag. The conjugation of alkyne C≡C to vancomycin renders the antibiotic with a unique Raman peak at 2128 cm⁻¹ for highly specific SRS imaging of biofilms. Fig. 1 shows co-localized SRS images of vancomycin and biofilms. Whereby (a) and (b) are retrieved images of EPS and *S. aureus* from hyperspectral SRS image stacks. (c) is SRS image of biofilm incubated with alkyne-tagged vancomycin at 2120 cm⁻¹. The results show that vancomycin has preferentially bound to the *S. aureus* over extracellular polymeric substances (EPS). We further quantify their correlations by measuring the Mander's coefficient, a co-localization analysis that represents the proportion of alkyne pixels overlapping with the same registered pixels of each channel. There are more alkyne pixels that co-localizes with *S. aureus* pixels (0.731) than EPS pixels (0.358), which indicates that vancomycin retains its binding ability even in exposure to the inhibitory environments of the biofilms. This reveals that the ineffectiveness of vancomycin is not attributed to the loss of ability to bind the bacterial surface. The detailed pharmacodynamics of vancomycin in biofilms with different incubation times can also be observed directly by hyperspectral SRS imaging. This work opens a new avenue with SRS technique for characterizing the antibiotic behaviors in living organisms in a quantitative manner at the subcellular.

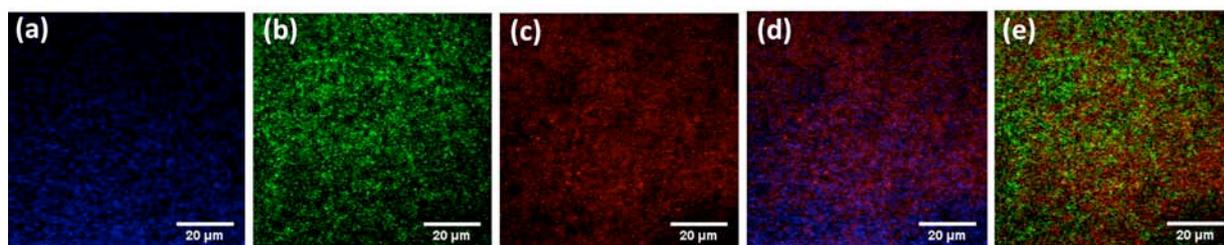


Fig. 1. Co-localized SRS images of vancomycin and biofilms. (a) and (b) are retrieved images of EPS and *S. aureus* from hyperspectral SRS image stacks. (c) SRS image of biofilm incubated with alkyne-tagged vancomycin at 2128 cm⁻¹. (d) is overlaid image of (a) and (c); (e) is overlaid image of (b) and (c).

References

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