Diagnostic de bactéries individuelles par spectroscopie Raman assistée par holographie numérique

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Résumé

Timely microbiological results are essential to allow clinicians to optimize the prescribed treatment, ideally at the initial stage of the therapeutic process. Several approaches have been proposed to solve this issue and to provide the microbiological result in a few hours directly from the sample such as molecular biology. However fast and sensitive those methods are not based on single phenotypic information which presents several drawbacks and limitations. Optical methods have the advantage to allow single-cell sensitivity and to probe the phenotype of measured cells. Here a process and a prototype that allow automated single-bacteria phenotypic analysis are presented. This prototype is based on the use of Digital In-line Holography techniques combined with a specially designed Raman spectrometer using a dedicated device to capture bacteria. The localization of single-cell is finely determined by using holograms and a proper propagation kernel. Holographic images are also used to analyze bacteria in the sample to sort potential pathogens from flora dwelling species or other biological particles. This accurate localization enables the use of a small confocal volume adapted to the measurement of single-cell. Along with the confocal volume adaptation, every components of the spectrometer have been modified in order to optimize single-bacteria Raman measurements. This optimization allows for acquiring informative single-cell spectra using an integration time of 0.5s only. Identification results obtained with this prototype are presented based on a 65144 Raman spectra database acquired automatically on 48 bacteria strains belonging to 8 species.

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