Broadband Coherent Anti-Stokes Raman Scattering highlights state of chromatin condensation in the high wavenumber.

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Key Words: Broadband Coherent Anti-Stokes Raman Scattering (BCARS), label-free imaging, HEK293 cell line, human fibroblasts, high wavenumber, chromatin.

Coherent Anti-Stokes Raman Scattering (CARS) microscopy has been extensively used to image prokaryotes, mammal and plant cells, human tissues, etc. This technique of vibrational microscopy permits to visualize biomolecules without external labelling [1]. Such technology is often used to analyse lipid content in cells due to the strong CARS signal of CH_2 bond at 2850 cm^{-1} [2]. Using this signature, many authors have been able to monitor cellular processes such as cell differentiation [3]. Nevertheless, only few studies take into account the position of cells in the cell cycle. Indeed, cells do not have the same intensity of vibrational signatures according to their position in the cell cycle. Until now, most of variations due to the cell cycle have been analysed mainly in the low-wavenumber spectral region (800-1800 cm^{-1}) [4].
The purpose of our work is to identify cells in different phases of the cell cycle by broadband CARS (BCARS) microscopy in the high-wavenumber region (2500-3200 cm^{-1}) with < 1 cm^{-1} spectral resolution. In this study, we considered the influence of cell fixation (operated here using paraformaldehyde (PFA)) on the vibrational signature, with living cells as the reference.
The HEK293 cells were blocked at the G1/S transition or at the beginning of mitosis (prophase) through thymidine or nocodazole treatments respectively. Then cells were analysed with BCARS around 2850 cm^{-1} (CH_2 stretching mode) and 2930 cm^{-1} (CH_3 stretching mode), corresponding to lipids and proteins respectively. The same study was conducted for cells labelled with DAPI, a fluorophore that stains DNA in the nucleus with a higher staining efficiency for heterochromatin. We found that CH_3 vibrational region allowed to visualise chromatin, mainly heterochromatin (DAPI staining and CH_3 signature overlapped). Therefore, CH_3 signature permits to visualise mainly heterochromatin through the signature of proteins that contribute to the high level of chromatin condensation. This analysis allows to discriminate cells in the different phases of the cell cycle.
Finally, the impact of PFA fixation was investigated with HEK293 cells and human fibroblasts in primary culture at the G1/S transition or in prophase. We show that the nuclear envelope is clearly highlighted in the case of living cells only. This may be correlated to the presence of heterochromatin.
In conclusion, BCARS microscopy in the high wavenumber permits to distinguish cells according to their position in the cell cycle. We could observe chromosomes during mitosis and the nucleus shape of living cells through the vibrational signature of proteins, like condensin complexes, that allow the hypercondensation of chromatin.

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