Raman mapping of erythrocytes to study the biochemical signature of ageing

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Red blood cell's (RBC) ageing is a physiological process, fundamental to ensure a proper blood homeostasis that, in vivo, balances the production of new cells and the removal of senescent erythrocytes. A detailed characterization at the cellular level of the progression of the ageing phenomenon can reveal biological, biophysical and biochemical fingerprints for diseases related to misbalances of the cell turnover and for blood pathologies. We exploited the imaging ability of Thermo Scientific Raman microscope to statistically analyse mean single cell spectra at different ageing times to highlight subtle spectral differences associated with conformational and biochemical modifications. Our results demonstrate a two steps ageing process characterized by a first phase in which proteins plays an important role, followed by a further cellular evolution driven by alterations in the membrane lipid contribution. Moreover, we analysed relevant spectral effects associated to reduction in haemoglobin oxygenation level and membrane fluidity induced by the ageing. The approach, which demonstrates to be robust and effective, allowing to classify the studied cells on the basis of their age and morphology, is particularly interesting and competitive, especially for bioanalytical and diagnostic applications where an automatic recognition, possibly based on intrinsic biomarker, would be preferable.

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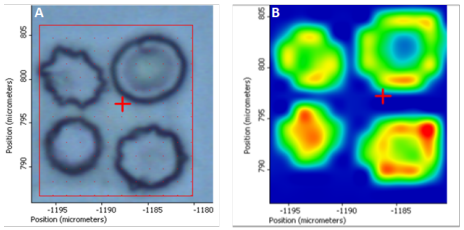

Figure 1. Optical (A) and spectral (B) maps collected with the Raman microscope on one of the smears. (A) map demonstrates the ability of the technique to distinguish between different RBC morphologies. At each point of the spectral map is associated a full range spectrum: (B) presents one of its possible visualization obtained by using 1585 cm^{-1} wavenumber as contrast parameter

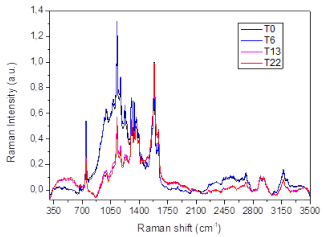

Figure 2. Mean Raman spectra for the 4 ageing times T0, T6, T13 and T22 as calculated by considering all the corresponding mean cell spectra..

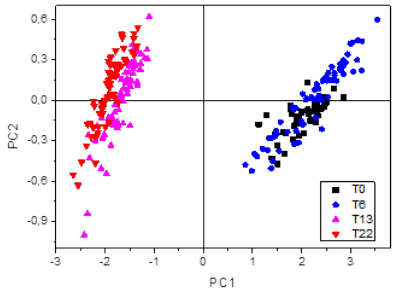

Figure 3. Score plot referring to the first two principal components PC1 and PC2 accounting for, respectively, 97.14% and 1.67% of the data variance