Changes to the Conformation and Concentration of DNA Detected Using Fourier Transform Infrared Spectroscopy.

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The ability to accurately detect DNA both quantitatively and qualitatively inside cells using Fourier transform infrared (FTIR) spectroscopy has been disputed [1]. Recently, we have demonstrated that the variability of DNA absorptions is in fact due to the dehydrated nature of most biological samples prepared for FTIR spectroscopic measurement [2]. In the dehydrated state DNA in cells and nuclei assumes an A-DNA conformation instead of the native B-DNA form and, in this conformation, the molar extinction coefficients of the antisymmetric and symmetric phosphate absorptions change significantly. This leads to unexpectedly low DNA absorption intensities that nonetheless are Beer-Lambert in nature. The Beer-Lambert nature of these absorptions was demonstrated by infrared spectroscopy of avian erythrocytes and extracted nuclei in conjunction with Partial Least Squares regression analysis to quantify cellular DNA [3]. The DNA content of extracted erythrocyte nuclei was successfully estimated as 44.2±6.6% (actual: 44.3%) and of intact erythrocytes as 12.8±4.3% (actual: 12.5%). DNA absorptions of spectra of hydrated single cells throughout interphase were also used to investigate the quantitative and qualitative biochemical changes involved in the G_1, S and G_2 phases of the cell cycle [4]. Using Principal Component Analysis cells only two hours apart within the same cell phase were successfully clustered. The Loadings Plots elucidated changes in the lipid, protein and DNA concentration as well as conformational changes to protein and lipid ordering.

The detection of the B to A conformational transition of the cellular DNA in eukaryotes has also been used to demonstrate that *in situ* A-DNA can be rehydrated to the native B-DNA state. Furthermore, the same B to A to B transition of cellular DNA that was previously observed in eukaryotes has also now been observed in several species of desiccation resistant prokaryotes that are viable after rehydration. This highlights the biological significance of a previously unreported *en masse* transition of cellular DNA into the A-form in response to desiccation stress and demonstrates that FTIR spectroscopy is an important non-invasive technique for monitoring DNA polymorphism as well as concentration in the cells.

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