Quantification and detection of DNA in eukaryotic cells using Fourier transform infrared spectroscopy

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Fourier transform infrared spectroscopy has previously been highlighted as a potential method for the early detection of disease and elucidation of the biochemistry of disease progression. However, several non-Beer-Lambert type interactions between infrared light and biological specimens have hindered such applications. One such artefact, the 'Dark DNA' effect, deals with the apparent non-Beer-Lambert absorption of cellular DNA (Mohlenhoff *et al.*, 2005). This postulated effect dealt with the lack of DNA absorptions in the spectra of many cell and tissue samples and concluded that the high levels of compaction of some cellular DNA left it opaque and incapable of interacting with infrared light. Recently, the cause of the 'Dark DNA' effect was reconsidered when sharp, relatively intense DNA absorptions were seen in the spectra of hydrated cells. It was found that the conformation of DNA was causing the change in peak intensity rather than the level of compaction (Whelan *et al.*, 2011). DNA conformation, in turn, was found to be dependent on the amount of water in the sample with the dehydrated A- conformation of DNA detected as the water content was lowered. Identified B- and A-DNA specific absorptions have subsequently been used to quantify the percentage nucleic acid in both single and population spectra of hydrated and dehydrated avian erythrocytes. The percentage nucleic acid was successfully determined as 44.2±6.6% in nuclei (actual: 44.3%) and 12.8±4.3% in erythrocytes (actual: 12.5%; Whelan *et al.*, 2012). Moreover, by examining the changes to the infrared spectrum of the conformational transition from B-DNA to A-DNA, an observable difference in extinction coefficient, particularly for the DNA backbone moieties is observed, thus explaining the 'Dark DNA' effect completely and without any exception to Beer-Lambert's law. This research demonstrates that DNA absorption bands in cell and tissue spectra are directly relatable to the concentration of DNA. This is anticipated as enabling different levels of proliferation to be distinguished and, by extension, the detection of dysplastic and neoplastic cells. Further to demonstrating the quantitative and applied detection of DNA in cells, research conducted at the Australian Synchrotron has aimed to examine the spectra of single hydrated fibroblast cells at different stages in the cell cycle. The changes to the infrared spectrum as these hydrated cells move from mitosis through the G1, S, and G2 phases is easily elucidated using Principal Component Analysis. As well as several identified changes to lipid and carbohydrate composition, examination of the B-DNA backbone absorptions shows a clear decrease in DNA concentration before the cell enters S-phase and begins DNA synthesis.

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