Emerging Techniques in Modern Microscopies

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DNA microspectroscopic infrared (IR) signatures using Fourier Transform-IR microscope ARO objective

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The infrared absorption profile and marker bands of dry samples of DNA and DNA-protein complexes obtained with an IR microspectroscope using a diamond attenuated total reflection (ATR) objective has recently been reported as a contribution to support forthcoming interpretation of FT-IR signatures of chromatin [1]. However, the examination of samples on gold-covered slides with the "all reflecting objective" (ARO) has also been recommended for FT-IR analysis to avoid direct contact with the preparations [2]. In the present study we investigated the adequacy of using an ARO objective for analysis of DNA dry samples.

Calf thymus and salmon testis double-stranded DNA samples (Sigma, St. Louis, USA) differing in base composition [3,4] were examined with the Illuminat IR II™ microspectrometer (Smith Detection, Danbury, USA) equipped with a liquid-cooled mercury-cadmium-telluride detector and Grams/AI 8.0 spectroscopy software (Thermo Electron Co., Waltham, USA), Olympus microscope and ARO objective (magnification, 15 x). The performance validation of the equipment used a low signal-to-noise ratio (7929:1). The spectral absorption signatures were obtained at wavenumbers between 4000 cm^{-1} and 650 cm^{-1}, with a spectral resolution of 4 cm^{-1}. Ten spectral profiles were obtained for each sample. Each spectral profile was subjected to baseline correction and normalization; an average profile was then calculated by the Grams software. Peak fitting and "estimate" procedures were applied to absorption band peaks especially assigned to DNA PO_2^- symmetric (v_s) and antisymmetric (v_{as}) stretchings.

Similar to findings obtained with the ATR objective, those obtained with the ARO objective permitted to clearly identify the DNA PO_2^- (v_s) and (v_{as}) stretchings and that the intensity of the PO_2^- v_s was greater than that corresponding to PO_2^- v_{as} for both DNA types. As regards the ratio PO_2^- v_{as}/ PO_2^- v_s, it was practically the same for the salmon DNA analyzed with both ARO (after peak fitting) and ATR objectives, but differed for the calf DNA because the intensity of the PO_2^- v_{as} of this DNA was not much different from that of the PO_2^- v_s when estimated with the ARO objective (Table 1). In contrast with the results obtained with the ATR objective, the most prominent band peak obtained after using the ARO objective was found at the ~3420-3410 cm^{-1} range for both DNA types (hydrogen bonds?). The peak assigned to adenine (1660 cm^{-1}) was more prominent for the salmon DNA in comparison with the calf DNA when the ARO objective was used. In contrast, the peak at 1400 cm^{-1}, assigned to nitrogen bases [1], that was the highest one in the salmon DNA profile when using the ATR objective became less evident when using the ARO objective.

Despite the differences on the FT-IR profiles of the DNA samples when comparing results obtained with the ARO and ATR objectives, the discrimination of important vibrational characteristics of the DNA could be made with the ARO objective. Selection of the microscope objective to be used in FT-IR DNA comparative studies will thus depend on specific vibrational groups of interest.

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DNA samples	ARO objective (after peak fitting)		ATR [1] objective	
	v_{as}/ v_s ratio	Wn (cm^{-1})	v_{as}/ v_s ratio	Wn (cm^{-1})
Calf thymus[a]	0.92	1215/1085	0.67	1220/1079
Salmon testis[b]	0.58	1262/1085	0.54	1220/1079

[a]Plurirmodal base composition [3]; [b]AT-biased DNA [4]; Wn, wavenumbers at which absorbances were obtained for the calculation of the ratio

Table 1. The PO_2^- v_{as}/ v_s ratio for calf thymus and salmon testis double-stranded DNA