

## Detection of DNA using *Identity* Raman Plate Reader.

### INTRODUCTION



Figure 1. Identity Raman Plate Reader

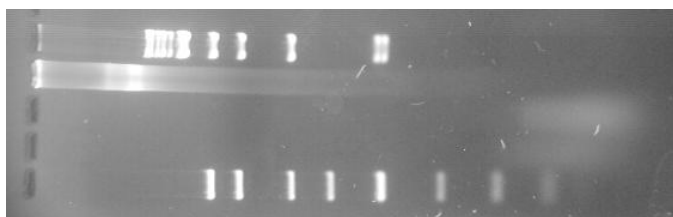
Nucleic acids play exclusive role in many vital cellular processes. Non-invasive localization, detection and quantitation of different nucleic acids is important for many practical applications, including cell sorting. Although nucleic acids can be easily non-invasively detected in solutions due to their strong UV and fluorescence properties, these methods are insensitive to the nucleic acid length and discrimination between RNA and DNA just based on their optical spectra is often difficult, specially inside the cells. It has been shown that Raman spectroscopy (both in traditional and SERS

modes) has capacity to discriminate between DNA molecules of different conformations and sizes [1, 2]. Use of Raman spectroscopy for cell sorting purpose was suggested recently [3]. Based on the internal cellular concentration of nucleic acids (calculated values are about 1.4% for DNA and 1-4% for RNA), both RNA, and DNA should be detectable by traditional Raman spectroscopy for sorting purpose. SERS mode may additionally allow for discrimination between DNA (and, probably, RNA) by size in solutions at significantly lower concentrations (down below  $10^{-5}\%$ ), suitable for analytical and molecular biological purposes [1, 2]. This Application Note addresses the issues of identification and quantitation of DNA in solutions by traditional Raman, and SERS techniques using Digilab's Identity Raman Plate Reader.

### PROCEDURE

1. All measurements were performed with a Digilab Identity Raman plate reader (Figure 1). The system is configurable with either a 532 nm (Model #RMI-53200-1) or a 785 nm (Model #RMI-78500-1) laser. Raman scattering is collected in an  $180^\circ$  backscatter configuration by a spectrometer with a Peltier-cooled CCD array detector capable of  $<10 \text{ cm}^{-1}$  spectral resolution. The Identity supports standard clear, flat bottom 96 and 384 well microtiter plates, as well as custom plate formats. The laser is focused through the bottom of the plate into the well for the analysis of liquids. Sensitivity of the reader is below 1ppm in regular mode (with iso-propanol as a standard) and below 0.2ppb for silver colloid-based SERS method (with melamine as a standard).
2. Commercial sources of DNA used in the study (the size was estimated on the basis of gel-electrophoretic separation in agarose gel, Figure 2):
  - DNA1 (*Sigma* D3159), 20-50 bp oligonucleotides, free acid;
  - DNA2 (*Amresco* 0644), 20-50 bp oligonucleotides, Na-salt;
  - DNA3 (*Sigma* D1626), highly polymeric ( $>10 \text{ kB}$ ), Na-salt.DNA was dissolved in 0.1M NaCl in Nano-pure  $\text{H}_2\text{O}$  to make final concentration 1mg/ml.

## APPLICATION NOTE

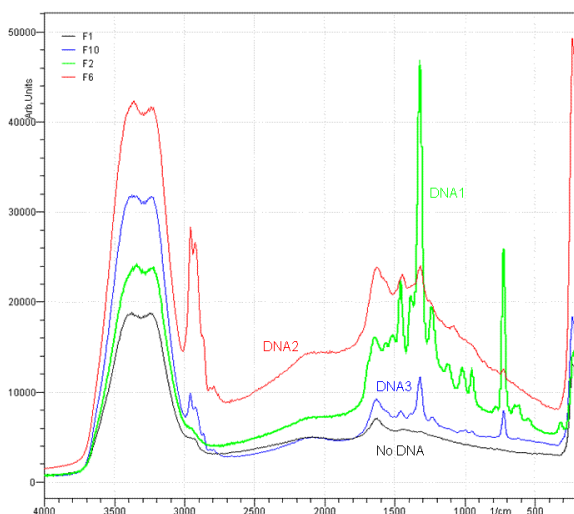


Ladder 1  
DNA3  
DNA2  
DNA1  
Ladder 2

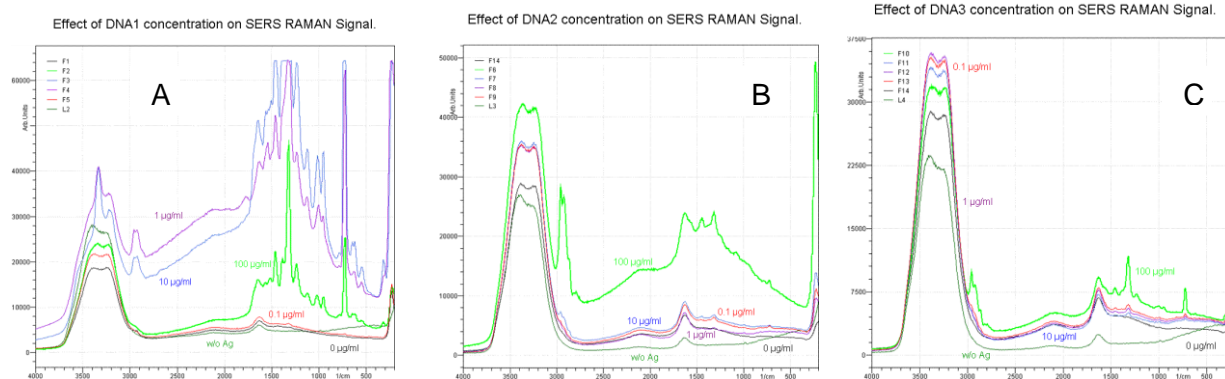
**Figure 2.** Gel-electrophoretic separation of the DNA samples in 2% agarose. Ladder 1: 1kB DNA Ladder (New England Biolabs); Ladder 2: exACTGene Low Range DNA Ladder (Fisher)

- Silver colloids were made as described by Lee and Meisel [4] using solutions of silver nitrate (*Sigma S6506*) and sodium citrate (*Sigma C8532*). The colloid was diluted 8-fold with 0.1M NaCl (*Sigma*) and added 1:1 immediately before plate reading to the DNA sample in the wells of the glass bottomed 384 well plate (*Greiner 781892*).
- Both traditional Raman and SERS spectra were analyzed by using Panorama 3 software (*LabCognition*).

SERS Spectra of DNA (0.1 mg/ml each) from Different Vendors.



**Figure 3.** SERS spectra of the three DNA samples (in 0.1M NaCl). Identity model RMI-53200-1 was used, total collection time 5 min with 10 co-additions.



**Figure 4.** Effect of concentration of DNA samples used in this study (from 0.1 to 100  $\mu\text{g/ml}$ ) on SERS spectra. DNA samples and measurements were as stated above in the legend to Figure 3.

## CONCLUSIONS

1. DNA molecules demonstrate strong SERS signals within the areas of 600-1800  $\text{cm}^{-1}$  and 2800-3000  $\text{cm}^{-1}$  with oligonucleotides in general showing a stronger signal, than the highly-polymeric DNA molecules (Figure 3).
2. Identity Raman Plate Reader can be successfully used for monitoring DNA in aqueous solutions both by traditional Raman technology, and SERS method. Using SERS method may extend the limit of detectable DNA concentration to lower than 0.1  $\mu\text{g}/\text{ml}$  when strong peaks around 250, 750 or 1300  $\text{cm}^{-1}$  are used for comparison (Figure 4).
3. Some SERS pattern differences between oligonucleotides and long DNA molecules were observed and may reflect conformational differences between the molecules.
4. DNA in the form of a free acid showed stronger SERS signal, compared to a Na-salt form at the same conditions (Figure 4).

## REFERENCES

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2. Igor Zlatkin, Sarah Mendelowitz, David Drapcho "Analysis of DNA-polyamine interactions using SERS method in Identity Raman Microplate Reader". XXII International Conference on Raman Spectroscopy, Boston, MA, USA, poster 099 (2010) (request a copy).
3. J. W. Chan, D. K. Lieu, T. R. Huser, R. A. Li "Label-free separation of human embryonic stem cells (hESCs) and their cardiac derivatives using Raman spectroscopy" LLNL-JRNL-406938 (2008).
4. P. C. Lee and D. Meisel, "Adsorption and Surface-Enhanced Raman of Dyes on Silver and Gold Sols" *J. Phys. Chem.* 86 3391-3395 (1982).
5. D. L. Drapcho, I. Zlatkin, F. Inscore, C. Shende, A. Sengupta, H. Huang, and S. Farquharson, "High-Throughput Trace Analysis Using SERS-Active Microtiter Plates with a Raman Plate Reader" *Spectroscopy* 25 Suppl. 42-50 (2010) (request a copy).

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