Identification of some of biologically active polyamines using *Identity* Raman Plate Reader.

**INTRODUCTION**

Polyamines are well known to play a significant role in cell functioning [1-6]. All known cellular organisms possess polyamines such as spermine, putrescine, and others, often in significant (up to 10mM), but always tightly regulated, concentrations [7]. Although the precise role of polyamines in cellular processes is not always understood, some important areas of polyamines' involvement are known. These include regulation of levels of *c-fos*, *c-jun* and other transcriptional factors [1] and participation in early signal transduction and cell proliferation [2], control of growth arrest and apoptosis [3], sequence-specific binding to DNA and regulation of chromatin acetylation [4, 5]. The latter makes polyamines important in epigenetics and specifically, in cancer development and prevention [6]. Bioactive polyamines do not possess any chromophore moieties, thus making their non-invasive label-free *in situ* monitoring difficult. On the other hand, polyamines possess distinct Raman characteristics in the 500-3500 cm\(^{-1}\) spectral region [8], which makes detecting them feasible by this technique. The use of Surface Enhanced Raman Spectroscopy (SERS) may additionally increase the sensitivity of polyamine detection below the level of physiologically relevant concentrations. Digilab, Inc. has recently introduced *Identity* (Figure 1), an innovative microplate reader based upon the power of Raman spectroscopy, which is suitable for many applications, including polyamine analysis [9,10]. This Application Note addresses the issues of identification and quantitation of different polyamines 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° backscatter configuration by a spectrometer with a Peltier-cooled CCD array detector capable of <10 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. Silver colloids were made as described by Lee and Meisel [11] using solutions of silver nitrate (Sigma S6506) and sodium citrate (Sigma C8532). The colloid was diluted 8-fold with 0.1M NaCl (Sigma) before adding 1:1 to sample serially diluted with the same 0.1M NaCl to wells of the glass bottomed 384 well plate (Greiner 781892). Colloid was added last, immediately before scanning with Identity. All other chemicals were from Sigma. All solutions were made using Nano-Pure water. SERS spectra were analyzed by using Panorama 3 software (LabCognition).

SERS Spectra of Selected Polyamines

![Figure 2. SERS spectra of the five polyamines at 10mM concentrations in 0.1M NaCl. Commercial sources of Polyamines: Spermine x4HCl (Sigma S1141); Spermidine base (Sigma S0266); Putrescine x2HCl (Sigma P5780); Ethanolamine base (Sigma E0135); Ethylenediamine base (Sigma E1649). Identity model RMI-53200-1 was used, total collection time 5 min with 10 co-additions.](image)

CONCLUSIONS

1. Polyamines demonstrate strong SERS signals within the areas of 400-1800 cm\(^{-1}\), 2800-3000 cm\(^{-1}\) and 3200-3400 cm\(^{-1}\) (Figure 2). Although the pattern depends on the concentration of the polyamine (see, for example, Figure 3E), it remains quite characteristic for each type of the polyamine and therefore could be used for the polyamine identification in a complex mixture.

2. Identity Raman Plate Reader can be successfully used for monitoring biologically active polyamines (spermine, spermidine, putrescine) in aqueous solutions both by traditional Raman technology, and SERS method. Using SERS method helps extending the limit of detectable polyamine concentration below 10ppb when strong peaks around 2800-3000cm\(^{-1}\) are used for comparison (Figure 3A-3C).

3. Straight concentration dependence of SERS signal was confirmed for the 1300-1500 cm\(^{-1}\) peaks of spermine within the concentration range 0.3-30mM (figure not shown).
Figure 3. SERS spectra of the five chosen polyamines at different concentrations (0.01-100 μg/ml) in 100mM NaCl. Spermine (A); Spermidine (B); Putrescine (C); Ethanolamine (D); Ethylenediamine (E). Identity model RMI-53200-1, total collection time 5 min with 10 co-additions.
REFERENCES


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