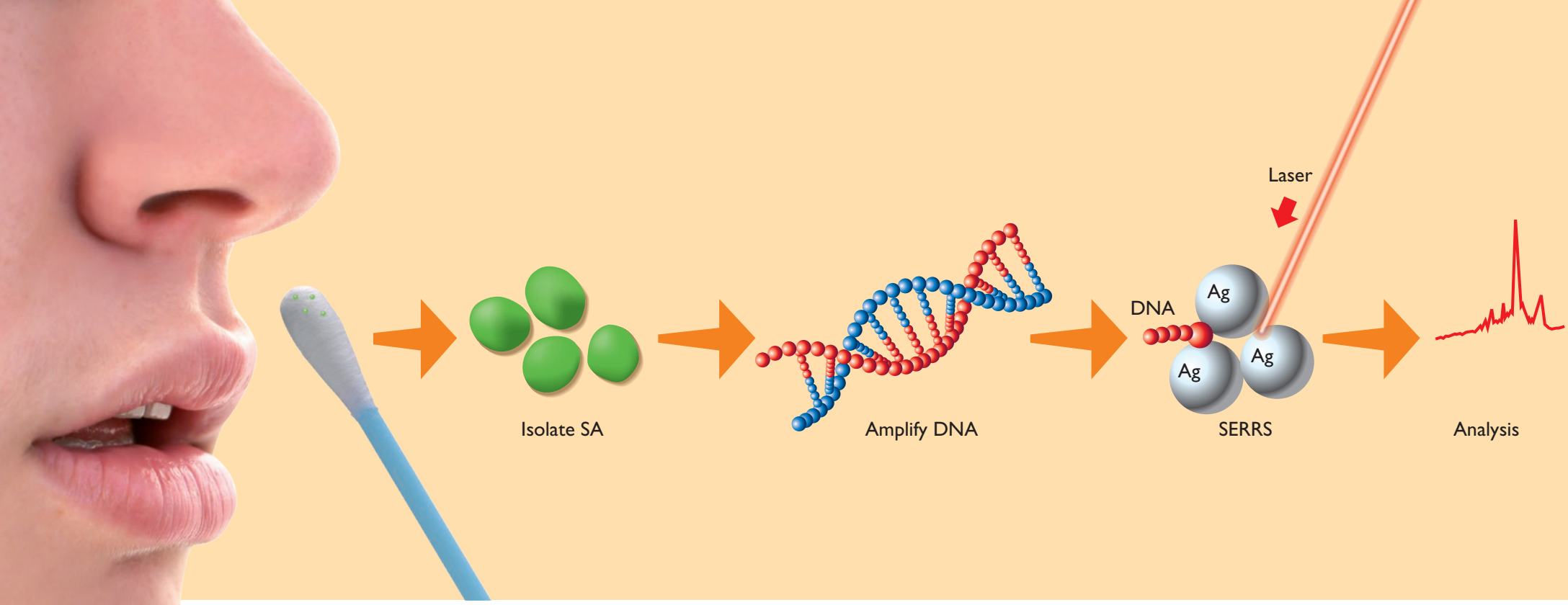


# Developing medical diagnostics methods

## using chemometric techniques

Measurements on biological samples are inherently complex because the measurement results can be disturbed by a large number of often unknown factors. For such complex measurements, the use of chemometric techniques for the design of the experiments and for the analysis of the data can prove indispensable. In this application note, we describe how our chemometric expertise was used in developing a diagnostic method for the characterization of infectious diseases.

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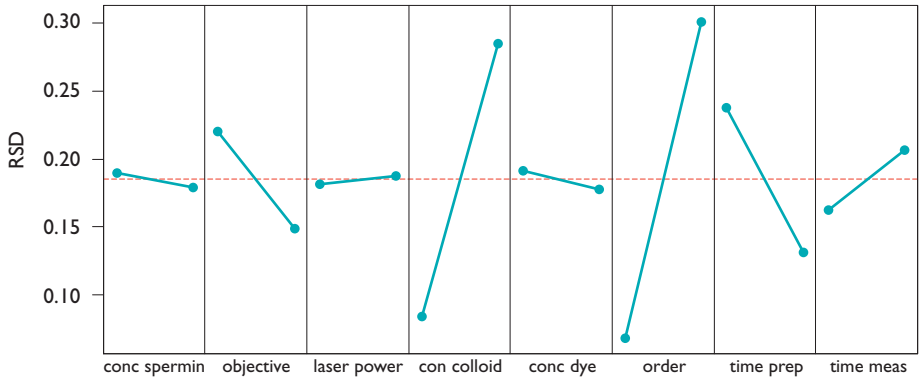



Fig. 1: Main effects plot of factors influencing SERRS measurement reproducibility.

**Chemometrics**

Chemometrics is a branch of chemistry that uses mathematical and statistical methods to design or select optimal measurement procedures and experiments and to provide maximum chemical information by analyzing chemical data. Chemometrics actually spans a wide range of different methods. There are techniques for collecting good data (optimization of experimental factors, design of experiments, calibration, signal processing) and techniques for getting information from these data (descriptive statistics, pattern recognition, classification methods, structure-property-relationship estimations). Chemometrics can hence assist in the experimental design, instrument response optimization, standardization and calibration as well as in the various steps involved in translating measurements (data) into useful information and knowledge.

## MRSA infections

In 2005 Methicillin-resistant Staphylococcus aureus (MRSA) infection was responsible for an estimated 94,000 life-threatening infections and 18,650 deaths in the United States. MRSA is by definition a strain of Staphylococcus aureus that is resistant to a large group of antibiotics. In the press MRSA is often referred to as a "superbug."

## Detection

The rapid detection and identification of the bacteria and their specific resistance against antibiotics is crucial for effective medical treatment. A recent Philips Research project aimed at measuring ten bacterial DNA strands simultaneously within seconds. The procedure is shown on the front page. A sample is taken, the bacteria are isolated, the bacterial DNA is amplified and labels are attached. Finally, the concentration of the labels and hence of the DNA strands is measured using a technique called SERRS (Surface Enhanced Resonance Raman spectroscopy).

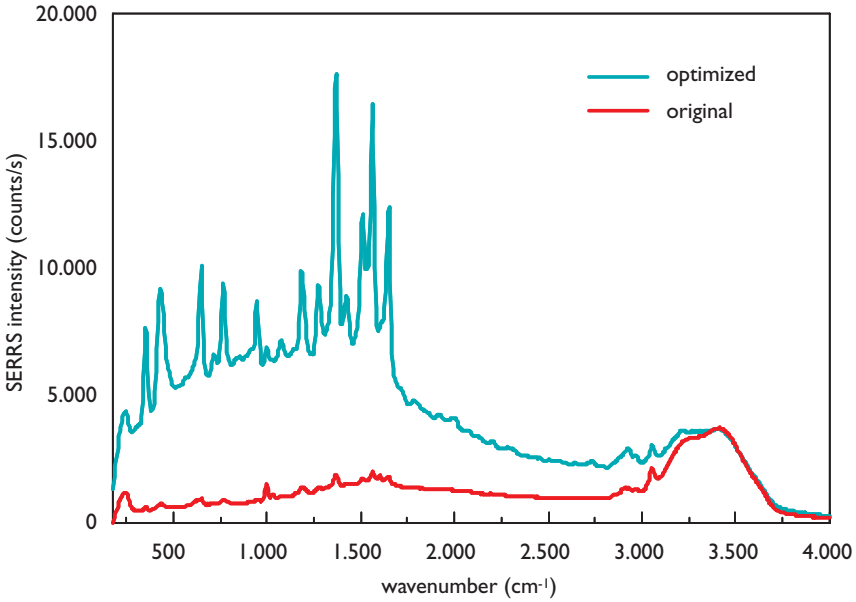
## SERRS

The molecular fingerprint information present in Raman spectra makes the technique both specific and quantitative. One drawback of Raman spectroscopy is its lack of sensitivity. The SERRS effect expands the applications of Raman to very low concentrations. In SERRS the low signals in Raman spectra are amplified by several orders of magnitude and hence the sensitivity is increased. This increase in signal is achieved by adsorbing the molecules onto rough metallic particles. However, these particles (typically silver or gold colloidal aggregates in the ten-nanometer range) tend to give variable signals, thereby making reproducibility of SERRS measurements a critical issue.

## Optimizing the SERRS signal

As noted above the reproducibility of SERRS measurements is a critical issue. Our aim was to find those variables that are chiefly responsible for variability in SERRS measurements. Design of Experiments (DoE) is a chemometric strategy for experimentation whereby all factors affecting a system or process are manipulated simultaneously according to rigorously formulated mathematical protocols. In DoE, the goal is to create representative and informative experiments. In the present case eight variables were selected. We used the relative standard deviation (RSD) as the response. This response was selected to achieve a small variation between repeats (small standard deviation) at high signal intensities (high average intensity).

In Figure 1, a main effects plot is shown. It is seen that two factors, the colloid concentration and the preparation order, have the largest effect on reproducibility whereas the effects of spermine and dye concentration are not significant. In a second experiment the optimum settings of these important factors were determined.



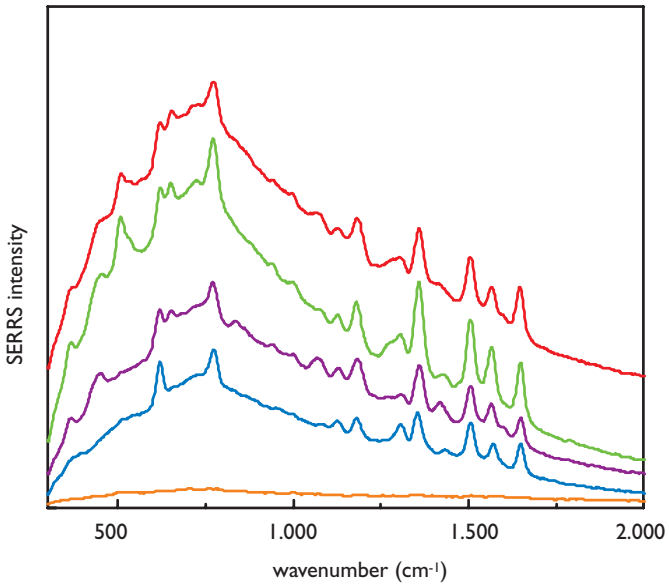




Fig. 2: Results of DoE experiment showing SERRS spectra before (original) and after (optimized) conducting the experiment.

The final results are shown in Figure 2. The investment of only a few days in planning and execution of the designed experiment, led to a reproducibility improvement of almost a factor of 10 (relative standard deviation now less than 5%) and an increase in signal of a factor of 20.

## Multivariate data analysis

In the MRSA problem, the information on the simultaneous presence of several DNA strands is hidden in the measured SERRS spectrum. The data measured are clearly multivariate and a robust data analysis method is required. More specifically, we need a method that is able to establish the presence of a specific DNA strand in the presence of several other DNA strands. We use the multivariate classification method PLS-DA (Partial Least Squares-Discriminant Analysis) to achieve just this.

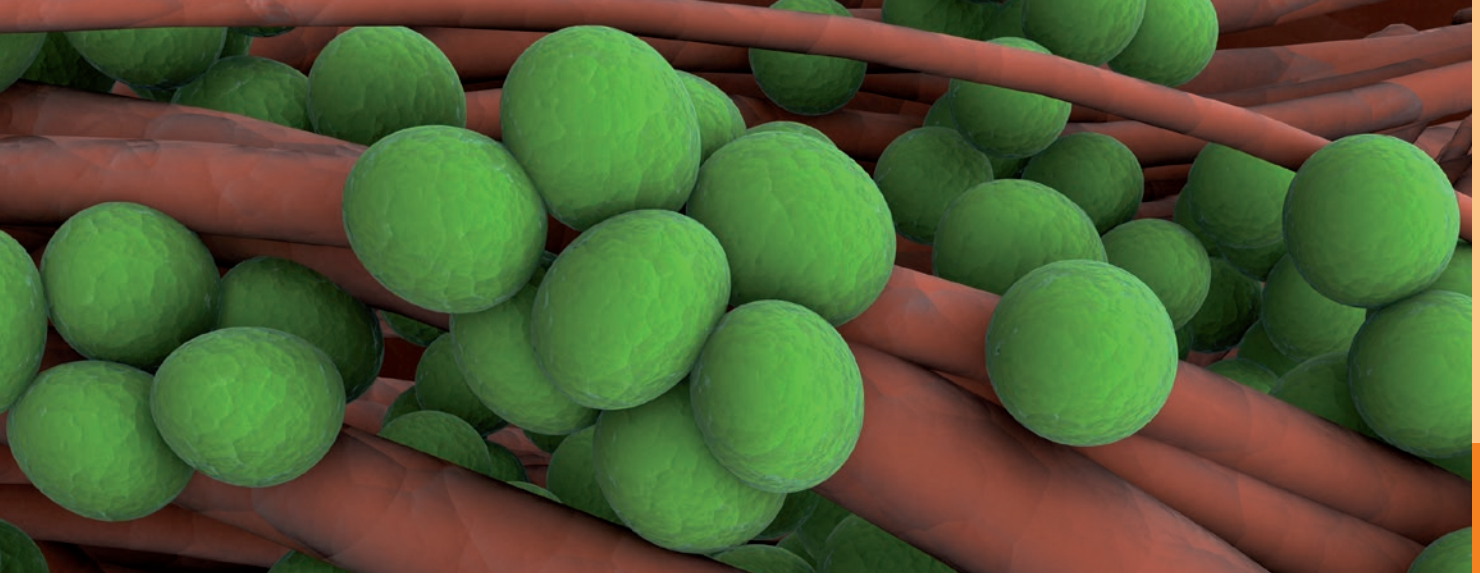
As with most multivariate methods, in PLS-DA, first a model is built using a training set of known samples. This model is then used to predict the presence of specific DNA strands in unknown samples. Fig. 3 illustrates the results of an experiment in which five DNA strands were classified simultaneously. In the experiment, mixtures of five different DNA strands were made using 500pM concentrations. In the prediction, all $2^5$=32 combinations were tested and predicted correctly.

## Conclusions

In the examples described above we have clearly shown the benefits of using chemometric techniques. With relatively little effort great improvements in the diagnostic methods were obtained.

| | A | B | C | D | E |
|---|---|---|---|---|---|
| # 3 | 1 | 1 | 1 | 1 | 1 |
| # 22 | 1 | 0 | 0 | 1 | 1 |
| # 2 | 0 | 0 | 1 | 1 | 1 |
| # 19 | 0 | 0 | 0 | 1 | 0 |
| # 20 | 0 | 0 | 0 | 0 | 0 |

Fig. 3: SERRS spectra of DNA strand mixtures (top) and corresponding compositions (bottom), (1=present, 0=not present). All 32 possible combinations were tested and predicted correctly.



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