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# Protecting the food supply chain from farm to fork: Utilizing SERS and portable Raman spectroscopy

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**Abstract** Ensuring food safety in various steps along the entire food value chain is crucial to prevent undesired and harmful substances entering the food humans consume. Pesticides and antifungal agents used during growth, processing or along the logistic chain from the field to the consumer can be toxic causing a range of symptoms from stomach pain to the death of the consumer even at trace levels of concentrations. To prevent dangerous additives and contaminants entering the food chain governmental restrictions on a large number of hazardous components have been put into place and are tightly monitored. For a large number of tests complex and sophisticated equipment is required along with time consuming sample preparation steps, not permitting instantaneous sampling of the specimen at the point of measurement or in a timely manner. In order to become a commercially applicable technique, the complexity of the sample preparation and the analysis routine needs to be simplified without losing performance in terms of identification and quantification of the dangerous contaminants. Raman spectroscopy is a technology to allow for quick and rigid analysis of materials without a large amount of sample preparation. The use of surface enhancement of the Raman signal by means of gold or silver nanoparticles would allow for taking this measurement to the field with high accuracy for even small concentrations. The high cost and poor reproducibility of commercially available substrates has so far limited the successful application of SERS measurements along the food value chain. The use of an affordable handheld Raman instrument with mass-producible SERS substrates will be

described in the frame of the contribution with respect to requirements imposed at various stages along the food value chain.

## 1 Introduction

It is well known that the toxic effect of consuming contaminated food producst can range from the unpleasant but mild digestive problems, up to and including death, depending on the toxin. In order to prevent dangerous substances to enter the food chain a large number of regulations have been introduced [1]. These regulations are overseen and enforced by numerous national and international agencies protecting consumers worldwide. Despite all these efforts, food contaminants can still end up in human or animal food causing significant health scares and economic consequences. The reliable and quick identification of even small amounts of food contaminants, down to trace levels, with high specificity and sensitivity is critical to ensuring the safety of the food chain and ultimately the quality of life for humans and animals alike. With a complex value chain and a high degree of specialization along the modern food value chain, plenty of possibilities arise for dangerous substances to enter the food chain either by accident or sometimes maliciously. The places of contamination vary from storage facilities of seeds, over fields plants grow on, respective upbringing facilities and slaughterhouses in case of livestock or basins for fish via the processing and logistics chain down to the storage and display in the store. Just as the place of contamination can be varied, so can the contamination itself be different. Additives like antibiotics to water or food fed to livestock or fish, fertilizers, fungicides, growth hormones and pesticides used on fields and in storage or even chemical contamination in processing plants can lead to contamination of food. In this paper, we describe how the novel combination of printed silver nanoparticle based SERS (Surface Enhanced Raman Spectroscopy) substrates and state of the art (portable) Raman instrumentation can be used to detect important contaminants such as melamine, fungicides and antibiotics at down to trace levels. The paper will address the design and manufacturing of the novel printed silver nanoparticle SERS substrates as well as present results of measurements of three typical contaminants. All measurements account for the need for quick and efficient point-of-need diagnostics

using portable setups to be used in the field or close to the production lines. The unique combination of silver SERS substrates with available Raster Orbital Scanning (ROS) schemes allows for significant increase in performance of the already powerful ROS setup. Employing silver-based substrates instead of gold increases the sensitivity for the above mentioned applications.

## 2 Raman Spectroscopy

Raman spectroscopy gives access to the chemical bonding structure of the compound to be identified by means of excitation of the substance using lasers and observing the relaxation of the excited species. The emitted radiation shows peaks that are characteristic for the emitting species like a fingerprint. Major drawback of Raman spectroscopy is the low cross-section for spontaneous Raman scattering, [2] making sound identification of the material of interest challenging, especially for low concentrations. To enhance the amount of scattered light, so called surface enhancement Raman scattering or SERS can be performed. The effect was discovered in 1977 when the compound of interest has been placed on a roughened noble metal surface [3–5]. Today, nanoparticles made from gold or silver are most often used as SERS materials. Sensitivity down to single molecule level has been achieved for specific cases. Amplification factors can range from single digits to several orders of magnitude [6, 7]. With recent advances in laser and detector technologies lowering the costs as well as dramatically reducing the size of the required instrumentation, Raman spectroscopy has become more accessible for use in applications that have traditionally be inaccessible.

## 3 Experimental Setup

All SERS substrates presented in this paper were mass-printed using the Ocean Optics printable SERS Substrate technology. A novel printing technique and production scheme of the nanoparticles allows a much increased shelf life of the silver SERS substrates by controlling the oxidization of the silver nanoparticles. Slowing down the oxidization of the silver particles allows producing and storing substrates in larger quantities compared to what has been possible up to this point, entering time

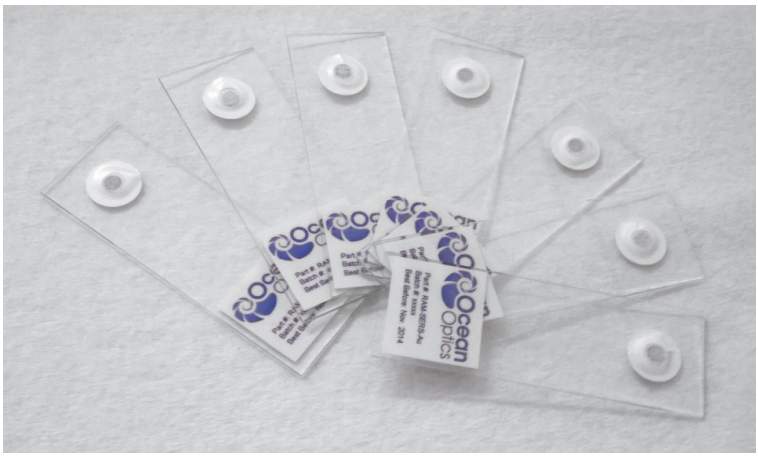


**Figure 21.1:** OceanOptics printed SERS substrates mounted on microscope slides.

frames that are up to this point only available for gold-based SERS substrates. The measurements have been carried out by drop-casting about 10µl of the analyte of interest, suspended in different solvents onto the printed SERS substrates mounted to conventional microscope slides as can be seen in Figure 21.1.

The samples have then been measured with either the OceanOptics IDRaman Reader using ROS or with a modular fiber coupled Raman system based on a QEPRO spectrometer configured for the use with a 785nm laser. The data was acquired using the OceanView spectroscopy software package. The setups are shown in Figure 21.2 and 21.3, respectively.

Using the IDRaman Reader or IDRaman Mini instruments with ROS schemes allow sweeping a tightly focused laser beam over the surface area to be interrogated, increasing the probability of hitting a SERS-active hotspot (localized region of Raman signal enhancement) on the SERS substrate. ROS results in a higher sensitivity compared to conventional Raman setups as well as a much reduced local heat deposition in the substrate, reducing thermal damage caused by the exposure of the sample to the power density of a tightly focused probing laser

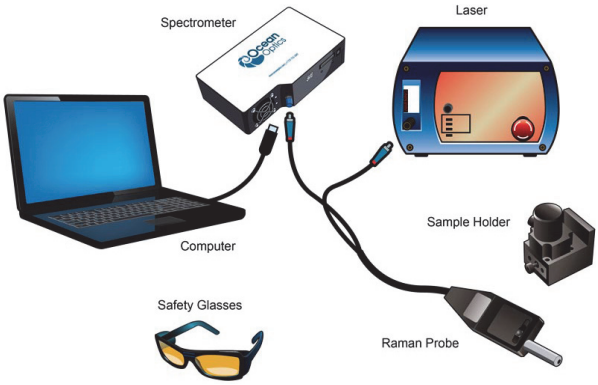


**Figure 21.2:** Modular fiber coupled Raman setup with OceanOptics QEPRO spectrometer, 785nm laser and fiber optic Raman probe.

beam. The raster orbital scanning scheme is schematically shown in Figure 21.4.

## 4 Melamine

Melamine has not traditionally been a substance that was tested for in the food industry. The Menu Food Pet Food recall of 2007 changed that when melamine had been added to pet food as non-protein-nitrogen to suggest a higher protein content than actually present. The short comings of not testing for melamine in food became even more obvious when in 2008 6 infants died as a consequence of being fed with melamine stretched milk powder. In 2010, the World Health Organisation (WHO) published the limits for a number of components in foodstuff in the codex alimentarius [8]. For melamine in foodstuff, a maximum value of 2,5mg/kg for general foodstuff and animal food and 1mg/kg for infant foods like milk powder may not be exceeded [9]. The detection at ppm level is thus needed.

Melamine can be detected using SERS at the required levels. The measurements shown have been acquired with a fiber coupled Ramam setup using an OceanOptics QEPRO spectrometer, a fiber coupled 785nm laser and a fiber optic Raman probe. The laser was set to yield


**Figure 21.3:** OceanOptics IDRaman Reader integrated Raman system with raster orbital scanning capability.

60mW of laser power at the sample. The spectrometer was set for an exposure time of 5s. Using the new mass printed silver-based SERS substrates, sub ppm levels of melamine have been detected successfully with good signal to noise ratio as is shown in Figure 21.5 or with greater detail in Figure 21.6.

To examine batch to batch reproducibility of the Ag-based substrates, several batches from different prints as well as from different nanoparticle synthesis have been tested at 10ppm level with the Ocean Optics 785nm-IDRaman Reader using raster orbital scanning. The power at the sample was set to 62mW and an exposure time of 1s has been used. As can be seen from Figure 21.7, the substrates are very reproducible.

## 5 Antibiotics

Enrofloxacin is a synthetic broadband antibacterial agent. It is effective against several bacteria including E. coli, Enterobacter, Salmonella and even methicillin-resistant strains of Staphylococcus . It has been used in poultry up to 2005 and is still used in aqua cultures to control bacteria levels. Enrofloxacin can cause bacteria to develop antibiotics resistant strains. Enrofloxacin is not labelled for use in food-producing animals for the United States of America and Canada according to the FAO [10]. According to the FAO, the quantification limit of the analytical meth-

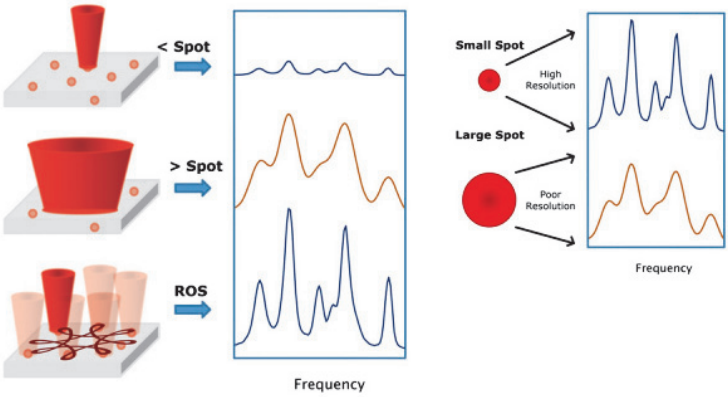


**Figure 21.4:** Raster orbital scanning schematically.

ods is 10 µg/kg for enrofloxacin in tissue and 5 µg/kg in milk. As a test for feasibility a rather high concentrated sample of enrofloxacin in water has been tested with the IDRaman Reader with raster orbital scanning. At long integration times of 40s the signature is clearly visible as is shown in Figure 21.8 , but a more efficient background subtraction mechanism is needed to reach the signal to noise ratio of the analytical limit. Nevertheless, the absolute count rates achieved with the silver substrates and the detector signal to noise are acceptable.

## 6 Fungicides

Two different fungicides have been studied in this work – malachite green and thiram. Malachite Green (MG) is an effective fungicide and is also used for parasite control. Malachite green is soluble in water as well as ethanol and attaches well to fat inside the body. MG has half-life times in the order of 45 days for salmon and carp and its use can thus be proven even months after fish have been exposed to MG. In several non-EU countries MG it is used in aquacultures to control fungi and parasites, while in the EU the use of MG for food producing animals is not allowed. The in consequence the European limit is 0 µg/kg for foodstuff. Typical detection limits of MG need to be off the order of

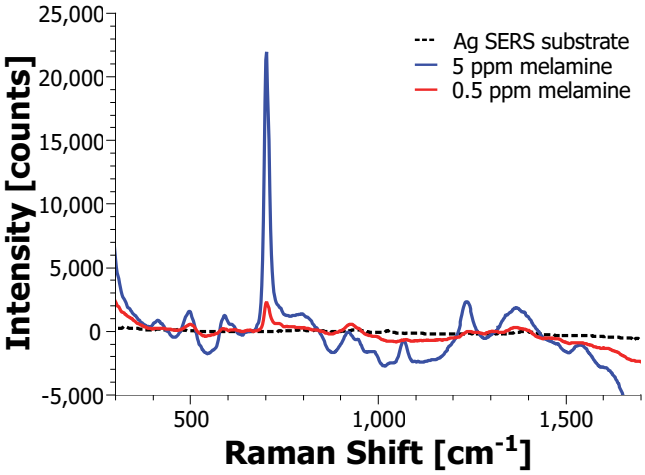


**Figure 21.5:** Melamine SERS spectra at concentrations of 0.5 and 5 ppm.

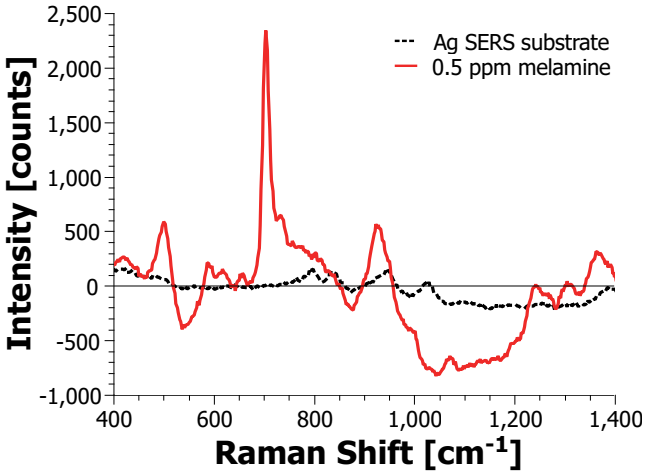


**Figure 21.6:** Close up of the Melamine SERS spectrum at a concentration of 0.5 ppm.

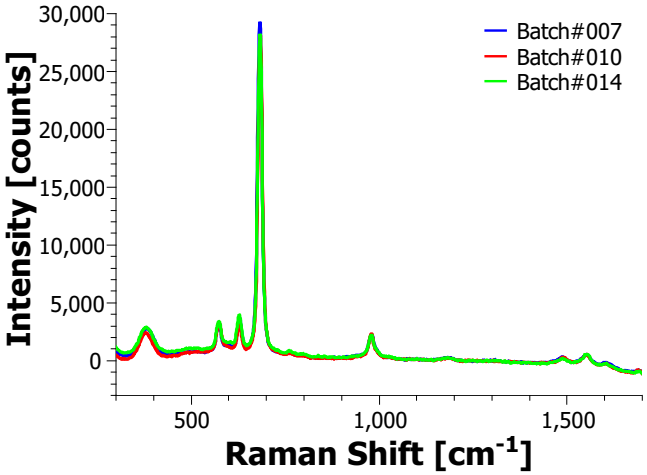


**Figure 21.7:** SERS spectra for different manufacturing batches at a melamine concentration of 10ppm, measured with IDRaman Reader 785nm and ROS enabled, $P_{785} = 62$ mW, $t_{exp} = 1$ s.

ppb to be acceptable. As with Enrofloxacin, the sensitivity of the silver-based substrates give promising result by almost reaching that value as is displayed in Figure 21.9.

In contrast to malachite green, thiram is a commonly used substance in agriculture. Thiram is used as a broadband fungicide, ectoparasiticide as well as animal repellant. Thiram attaches efficiently to soil particles and remained relatively immobile in clay or high organic matter soils. The half life time for thiram in soil is about 2 weeks. Thiram is nitrousamine precursor contaminating foodstuff. As in the case of melamine, adding Thriam to foodstuff suggests increased values for protein, when conducting traditional protein determination testing. While it is used for protecting young plants in the field and for impregnation of seeds and seedlings widely, its direct addition to meat is not allowed in Germany and the UK, but allowed in other EU countries as well as in the US. In these countries a limit of 200ppm may not be exceeded when added to fresh meat. [11] The SERS spectrum of Thiram solved in acetone is shown in Figure 21.10.

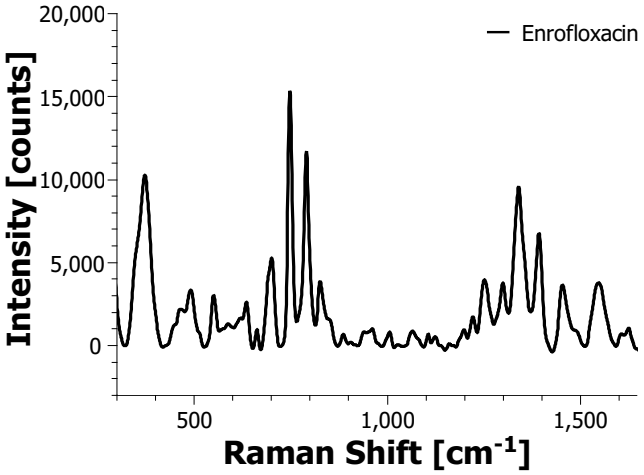


**Figure 21.8:** SERS spectrum of Enrofloxacin solved in water measured with the IDRaman Reader using ROS at a concentration of $10^{-4}$ M, $P_{785} = 62$ mW, $t_{exp} = 40$ s.

## 7 Summary

In this paper, a newly developed mass-printable silver nanoparticle based substrate for surface enhancement of Raman scattering has been introduced. It was demonstrated that the combination of these novel silver SERS substrates with Ocean Optics existing portable Raman instrumentation, ranging from modular fiber coupled systems to integrated Raman readers, is sensitive enough to meet the detection limits of for substances relevant to the food chain security. Results for melamine, antibiotics and antifungals have been shown in combination with the newly developed printed silver SERS substrates.

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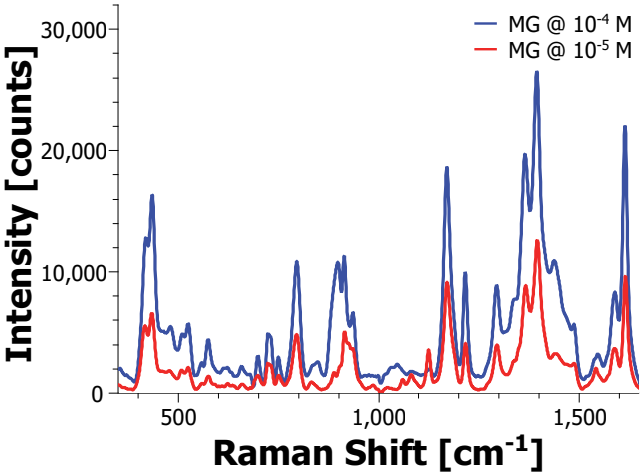


**Figure 21.9:** Malachite green solved in ethanol measured with the IDRaman Reader using ROS at concentrations of $10^{-4}$&$10^{-5}$ M, $P_{785} = 80$ mW, $t_{exp} = 10$ s.


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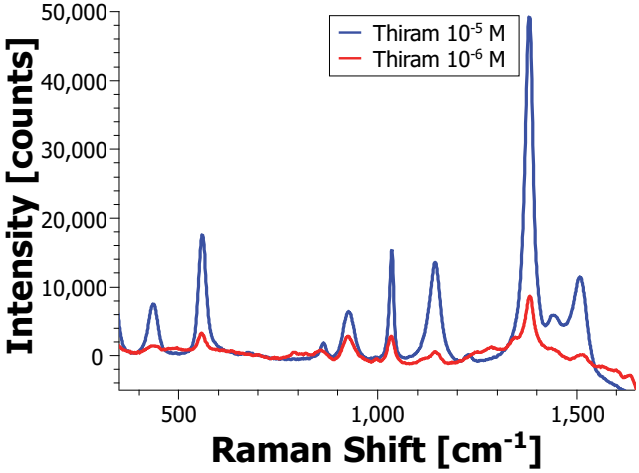


**Figure 21.10:** Thiram solved in acetone measured with the IDRaman Reader using ROS at concentrations of $10^{-5}$&$10^{-6}$ M, $P_{785} = 80$ mW, $t_{exp} = 20$ s.

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