# Micron-sized dielectric chips with gold nanostructures for intracellular label-free SERS

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*Label-free intracellular sensing using Raman spectroscopy has the potential to revolutionize the world of cell biology in a similar fashion as occurred with the invention of fluorescent labels. However, due to the weak intrinsic signals a method to enhance the Raman signal is required. By using colloidal gold and silver nanoparticles, several applications of highly sensitive and selective label-free single cell analysis have been demonstrated. On the other hand, Tip-Enhanced RS probes offer a fixed metal nano-pattern, but intracellular applications with these probes require an incision of the cell membrane during the measurement. We propose a new type of microchips, enabling reproducible and less invasive intracellular SERS measurements.*

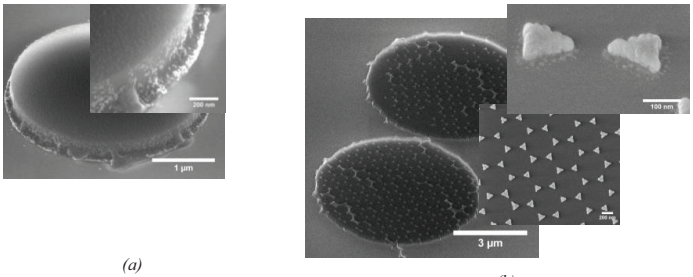
## Introduction

Raman spectroscopy, in which the characteristic vibrations of a molecule are probed, offers an interesting alternative to fluorescence-based techniques for monitoring intracellular processes and detecting intracellular molecules. While fluorescent labels offer the advantage of a good selectivity and strong intrinsic signals, fluorescence microscopy obviously requires the attachment of a label to the molecule of interest. This is undesirable in certain applications, as the label may influence the behaviour of the molecule, or labelling the molecule of interest may not be possible at all. Numerous applications of label-free Raman microscopy for cellular analysis have been demonstrated, like the discrimination of pathological from non-pathological cell types and the identification of specific cellular organelles[1]. However, spontaneous Raman scattering is a weak process and live-cell experiments require a limited laser power, short integration time and excitation wavelength in the near-infrared low-biodamage wavelength region. These limitations inhibit a fast and sensitive detection of molecules through Raman spectroscopy. Surface Enhanced Raman Spectroscopy (SERS) provides a possible solution to this issue. In SERS, a localized surface plasmon resonance is used to drastically amplify the Raman cross-section of molecules. As a result, the Raman scattering of molecules close to metal-nanostructures is increased by several orders of magnitude. As colloidal gold nanoparticles can be taken up by a variety of cell types, SERS is also of strong interest for intracellular experiments. Amongst others, gold nanoparticle based SERS is being used for measuring intracellular pH [2], enlightening the pathway of endocytosis [3] or monitoring drug delivery[4]. Despite these promising applications, the use of colloidal gold nanoparticles often leads to a poor reproducibility, due to a non-uniform distribution of the nanoparticles combined with their poor visibility under optical microscopes. Furthermore, it has been shown that these particles

tend to form clusters over time, strongly influencing the Raman enhancement factor [5]. This problem could be partially solved by using structures with a fixed and predefined metal configuration. This approach is being used in intracellular Tip-Enhanced Raman experiments [6], where a narrow gold- or silver- coated probe is pierced through the cell membrane. However this approach requires an incision of the cell membrane during measurement, which only allows short-term monitoring to limit cytotoxicity.
We propose a hybrid particle consisting of a planer silicon-nitride ($Si_3N_4$) chip decorated with a predefined gold nanopattern. As compared to colloidal nanoparticles and TERS, this approach does not suffer from particle aggregation while avoiding an incision of the cell membrane. In contrast to spherical microbeads[7], our planar structures offer an increased surface to volume ratio, which leads to a decreased consumption of cell-volume and possibly a more efficient cellular uptake. Furthermore these planar structures allow the use of top-down nano-antenna fabrication techniques. These features are an important step towards reproducible intracellular SERS experiments.

## Planar microchips for intracellular SERS

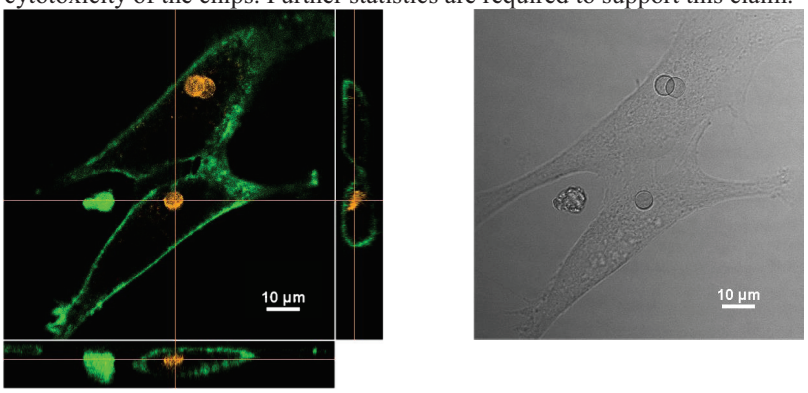
Silicon-nitride chips were fabricated by defining a large number of individual chips in a PECVD $Si_3N_4$ layer through UV-contact lithography and reactive ion etching. Next the chips were released form the underlying silicon substrate using a potassium-hydroxide wet etch. While etching, the sample was centrifuged in order to separate the detached chips. The potassium-hydroxide etchant was washed out with water in at least five centrifugation steps. Next, positively charged 10nm gold nanoparticles were adsorbed onto the negatively charged $Si_3N_4$ surface (figure 1a). In a second approach, the potential of using top-down lithography fabrication techniques was also demonstrated by fabricating nanotriangles through nanosphere lithography (figure 1b). Briefly, a monolayer of polystyrene beads was spincoated on the $Si_3N_4$ surface. This monolayer was used as a mask for metal deposition. After lifting off these beads, a repetitive pattern of nanotriangles remains on the chip. Choosing the appropriate size of polystyrene beads and metal deposition thickness allows tuning the resonant wavelength to match the Raman pump and stokes-shifted wavelengths. We used 500nm diameter polystyrene beads (Microparticles GmBH), on which a 50nm thick gold layer was deposited using electron-beam deposition. Subsequently individual microchips were patterned and released as described above.




*(a)* *(b)*

Fig. 1: SEM images of $Si_3N_4$ chips coated with colloidal gold nanoparticles adsorbed to the $Si_3N_4$ surface (a) and nanotriangles fabricated through nanosphere lithography.

Upon adding the microchips to a culture of fibroblast cells, the majority of these chips were incorporated by the cells after 24 hours. As shown in figure 2, the intracellular localisation of the microchips was confirmed by confocal fluorescence microscopy on live cells, were a fluorescently labelled $Si_3N_4$ (FITC, Sigma) chip is clearly surrounded by the green labelled cellular membrane (WGA- Alexa Fluor 488, Life Technologies). We frequently observed cell division of cells containing microchips, indicating a low cytotoxicity of the chips. Further statistics are required to support this claim.



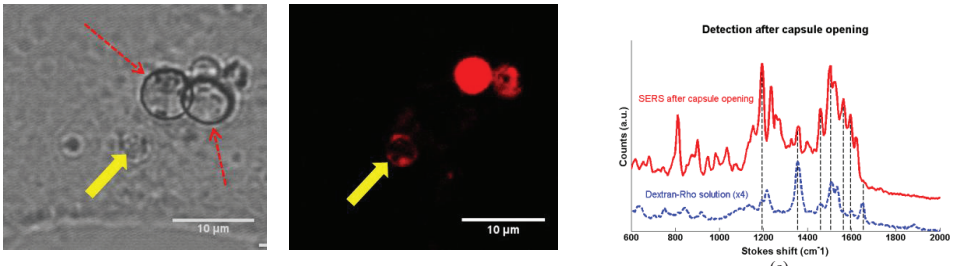

*(a)* *(b)*

Fig. 2: Confocal microscopy confirms the uptake of FITC-labelled (yellow) $Si_3N_4$ chips by human fibroblast cells, indicated by the WGA-labelled (green) membrane *(a)*. The corresponding transmission image is shown in *(b)*.

## Label-free monitoring of capsule-enabled release

The potential of our chips for in label-free drug delivery applications was demonstrated using correlative fluorescence and Raman microscopy for monitoring the intracellular delivery of foreign molecules. Polymer microcapsules were used for the delivery of these molecules to the cell, as reported earlier [8]. These microcapsules consist of a hollow core and a shell of alternating layers of oppositely charged polymers. Intermediate polymer layers contain gold nanoparticles in order to enable targeted release by laser-triggered heating. In this experiment, the microcapsules were loaded with a Rhodamine B isothiocyanate– Dextran dye (2kDa, 1mg/ml, Sigma), which shows strong fluorescence and a distinctive Raman spectrum. Both gold-nanoparticle decorated $Si_3N_4$ chips (figure 1a) and loaded microcapsules were inserted into a normal human dermal fibroblast cell culture. Electroporation was used to enhance the uptake efficiency of the capsules. After uptake, a cell containing both a capsule and a microchip was picked out. First, the cargo of the microcapsule was released by focusing a laser-beam on the capsule at a power density of approximately $30mW\mu m^{-2}$ (figure 3a-b). The release of the cargo is confirmed by a loss of fluorescence from the capsule core. Subsequently, SERS spectra were collected from the surface of a microchip in the same cell ($2mW\mu m^{-2}$, 0.2 sec integration time). In the collected SERS-spectra, specific Raman peaks originating from the released dextran-rhodamin were detected (figure 3c). Other present peaks can be attributed to the complex environment of the cell and the stabilizers used during gold nanoparticle fabrication. A commercial Raman microscope

(WITec Alpha300R) equipped with a 785nm diode laser, a 60x WI objective and a cooled CCD spectrograph was used for both opening the capsule and acquiring the SERS spectra. The investigated cells stayed alive during the experiment.




(a) (b) (c)

Fig. 3: Illustration of the label-free detection of dextran-rhodamin. $Si_3N_4$ chips and (a) Human fibroblast cell containing three dextran-rhodamin loaded capsules and two gold coated SiN chips (red dashed arrows).(b) Fluorscence signal after opening one capsule (yellow arrow) (c) SERS spectra measured on top of the SiN chips after capsule release (red) and reference spectrum of dextran-rhodamin (blue).

## Conclusion

We fabricated a new type of planar hybrid microstructures for intracellular SERS experiments. These are, to the best of our knowledge, the first structures which enable using top down nanoantenna fabrication techniques for intracellular sensing. We demonstrated this possibility with nanosphere lithography based patterns. Furthermore the potential of our structures in label-free drug-delivery applications was highlighted in a proof-of-concept experiment.

## Acknowledgements

Pieter Wuytens acknowledges the Research Foundation-Flanders (FWO) for a research grand and the Center for Nano-and Biophotonics (Universiteit Gent) for providing a platform for multidisciplinary research platform.

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