Nanostructured optical components in biosensors

NANOINNOVATION 2016

Luigi Pierno 21/09/2016

LEONARDO

LAND & NAVAL DEFENCE ELECTRONICS

EDA NANOCAP – EDA BIOTYPE

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‘Novel NANOstructured optical Components for CBRN detection and high performAnce oPto-Microwave Links’
‘BIOsensors for point detection based on nanostructured opTical components for quick deploYment in an overall CBRN EuroPEan operational capability.’

Detection of:

- ⇨ Sarin (GB), chemical weapon (list 1), accidentally produced by pesticide industrial plants, high potential of threat to human health
- ⇨ TNT, low vapor concentration at ambient in air, need of trace level detection in near- real time with low false alarm ratio
- ⇨ Bacillus Anthracis,in spores collected from aerosol, low lethal concentraion

‘Grant: European Defence Agency (EDA)

jip cbrn

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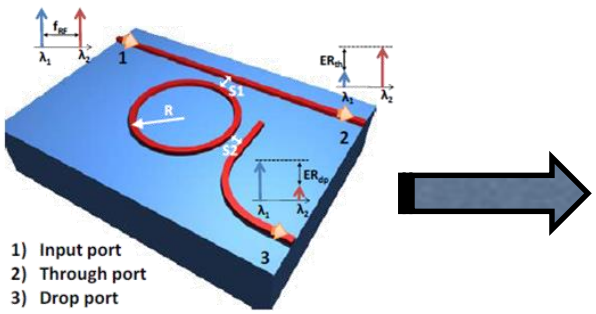
Technological platform:

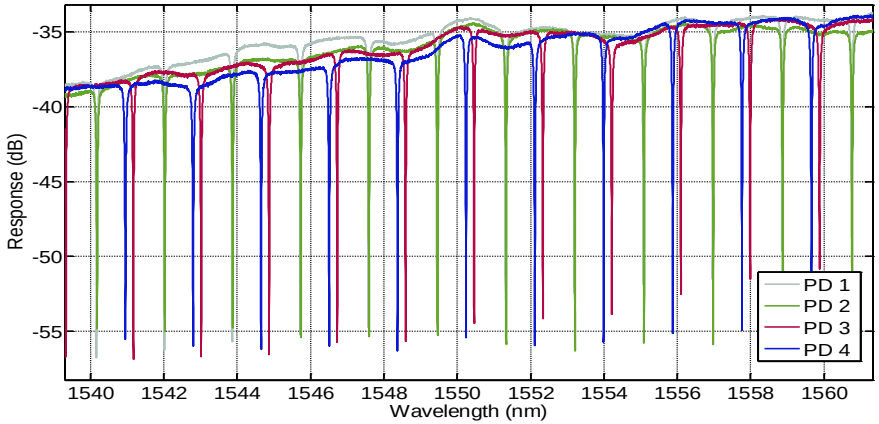
1-Nanofabricated Photonic Integrated Circuit PIC technology also for CMOS photonics

2- Chemical surface nano-functionalisation for bio - sensing of chemical agents and explosives in air and biological agents in aerosol.

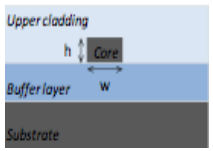
Photonic Integrated Circuits (PICs) for the sensitive detection of chemical agents in air

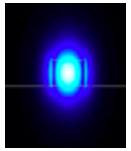
- Ring coupled to waveguide ⇨ transfer function exhibiting a stop band response at the resonance frequency

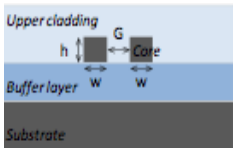


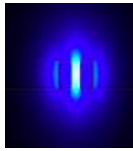


- Light propagation in waveguide by total internal reflection
- I/O realized by coupling optical fibers
- Compactness, high operating bandwidth ⇨ solution for highly demanding aplications in severe conditions
- Immune to electromagnetic interferences









Capability of PIC for monitoring interactions between molecules

- ✓**Tight confinement of light in integrated structures**
  ⇨**increased light-matter interaction to be used for high sensitivity**
- ✓**Resonant effect in high Q structures**
  ⇨ **Increase sensitivity of integrated structures**

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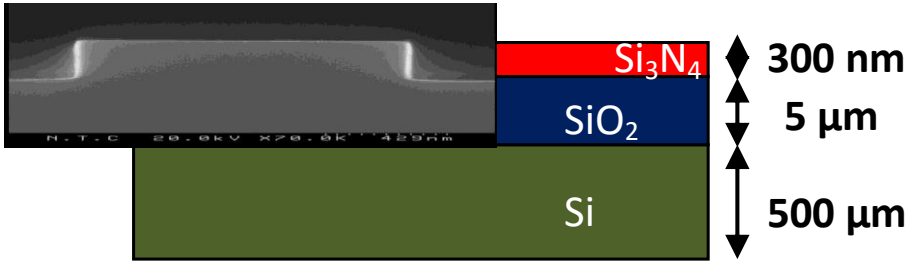
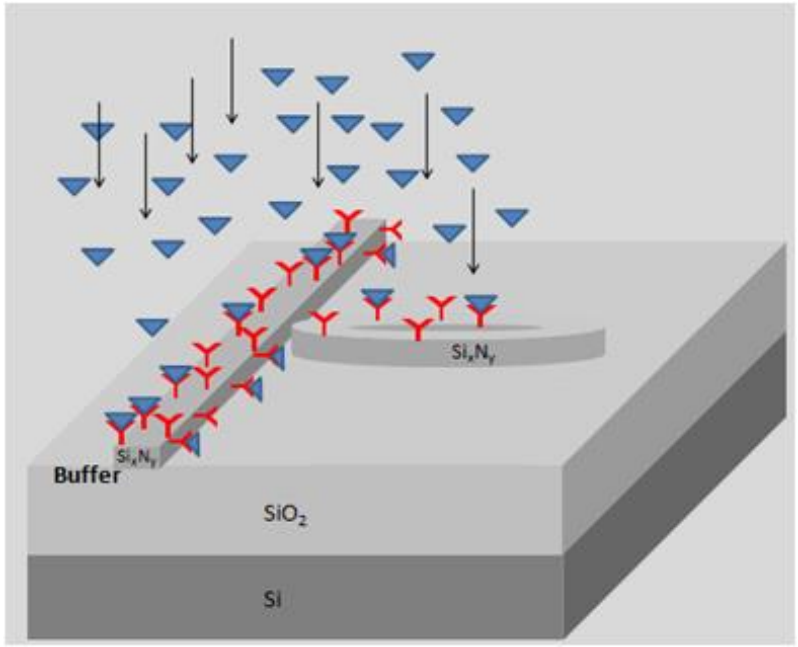
Features of the photonic nanostructured resonator @ 1550nm

- Dimensions: The waveguide is a Si3N4 channel with a cross section of 1x0.3µm. Proteins can be selectively attached only to the surface of the waveguide.





- The sensing area is the one formed by a R=100µm ring made with the photonic waveguide. Therefore the extension of the sensitive area is smaller than in SPR.
- Higher sensitivities to the Refractive Index are expected in the photonic waveguides surface.

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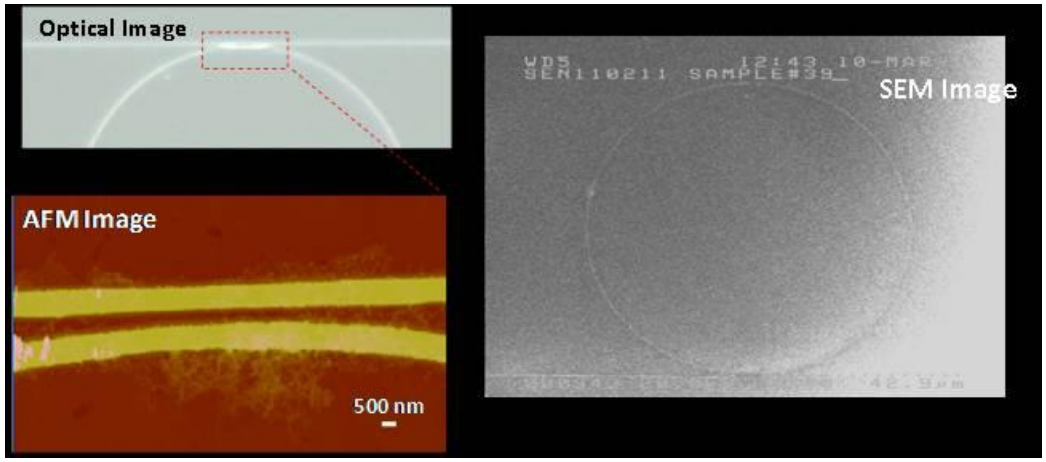
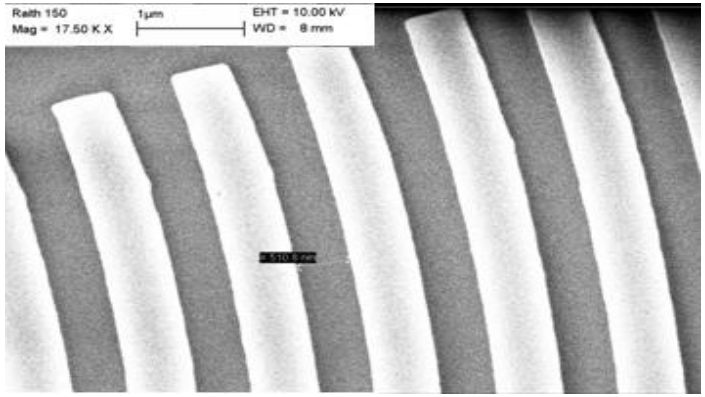
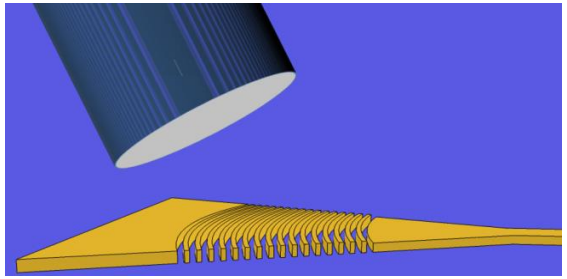
NANO optical structures of the photonic nanostructured resonator @ 1550nm



- Gap in the range of 500nm.
- Low tolerance on the surface → Electron Beam Lithography manufacture





- Fiber optic coupling gratings

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The use of Odorant Binding Proteins (OBPs) for highly specific recognition of targets

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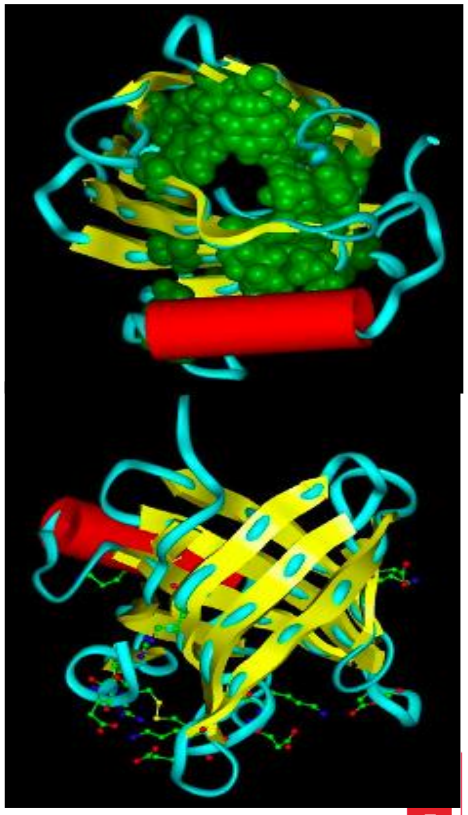
- Concept not new
  - ✓molecular recognition of ligand/receptor to analyte is observed in immunological systems of living animals
  - ✓working principle of immunoassays

- OBP = Part of the sense of smell
  - ✓come from the snout of cows (b-OBP) or pigs (p-OBP)
  - ✓optimized by random mutation + high-throughput screening
  - ✓heat-resistant (> 100°C) due to rigid β-barrel
  - ✓works in solution or gas phase



Technology for the detection of CWA and explosives in air

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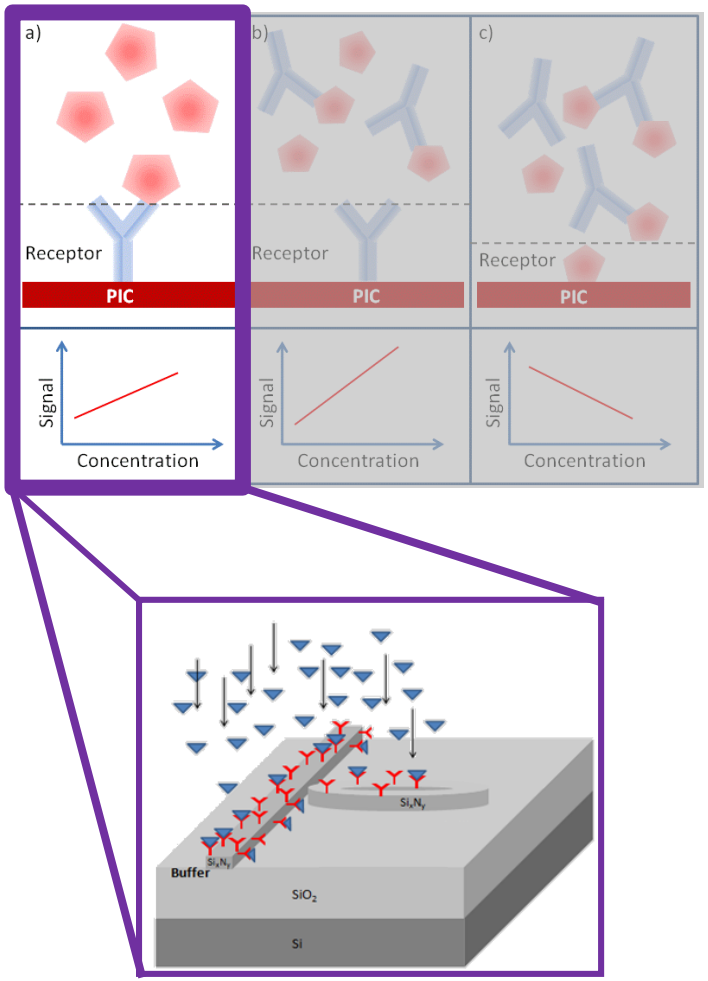


Direct non-competitive detection format

Selective trap + High sensitivity of photonic signal to state of ligands and binding of analytes

⇩

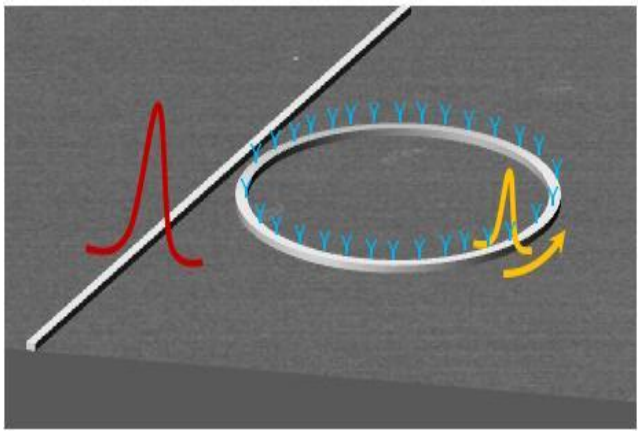
Modification of ring resonator Refractive Index ⟺ Production of a propagation wave

⇩

Wavelength shift

≅

measured phenomenon



! Specific recognition leading to lower false alarm ratio !

Immobilizing mutant bovin-OBPs on PICs

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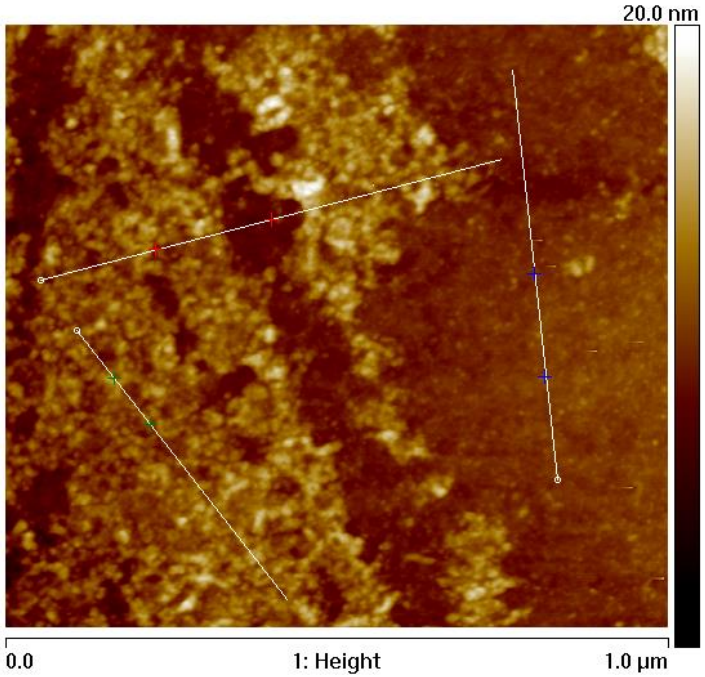
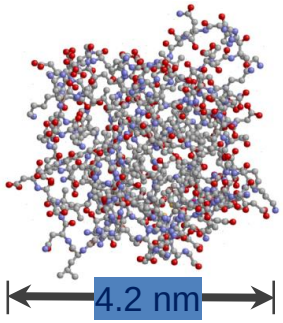
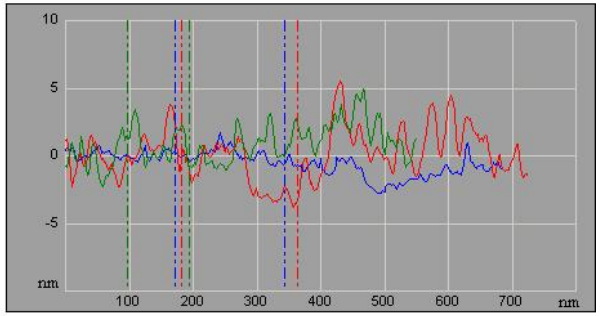
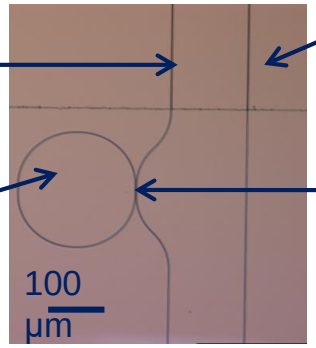






Dense mat of aggregates z = 5nm
(93% coverage)

Dense mat of aggregates z = 10nm + smaller particles



Dense mat of aggregates z = 2nm

Dense mat of aggregates z = 4nm + biger particles (96% coverage)
Same on resonator ring

AFM microscope topography

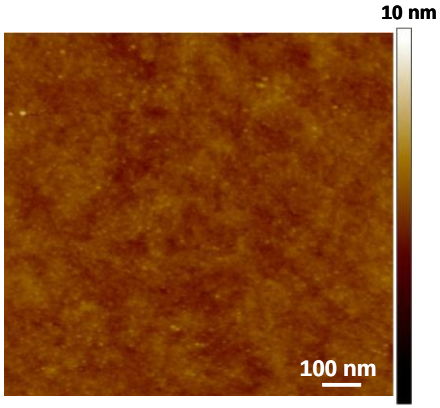
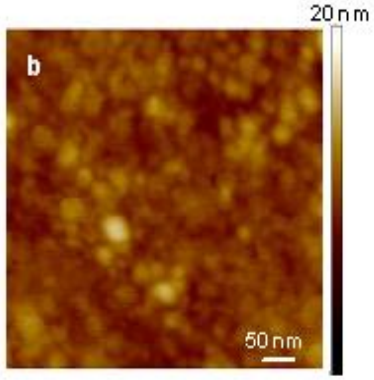


Figure 10. Surface image of a S_3N_4 wafer.



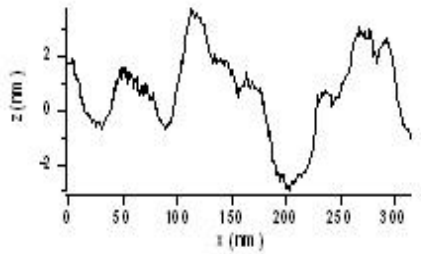
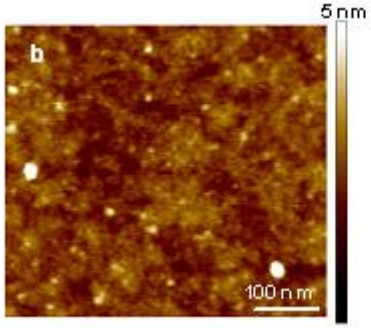


Figure 12. Topography images and section graphs of the chip with proteins for TNT performed on the resonator

The AFM images show a homogeneous and continuous film of TNT sensitive proteins in the detection area. Proteins are present both on ring resonator and waveguides. The coverage ratio of the resonator is of 94%.



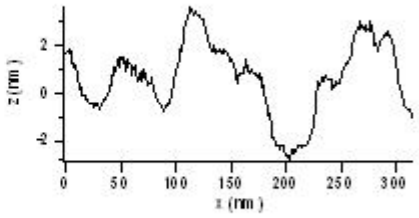


Figure 13. Topography images and section graphs of the chip with proteins for DMMP performed on the resonator

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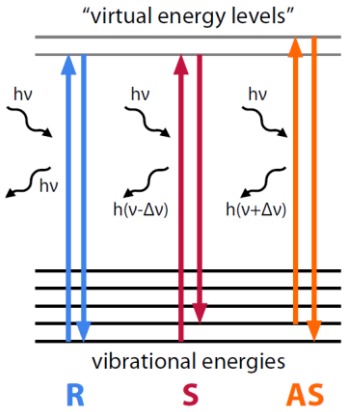
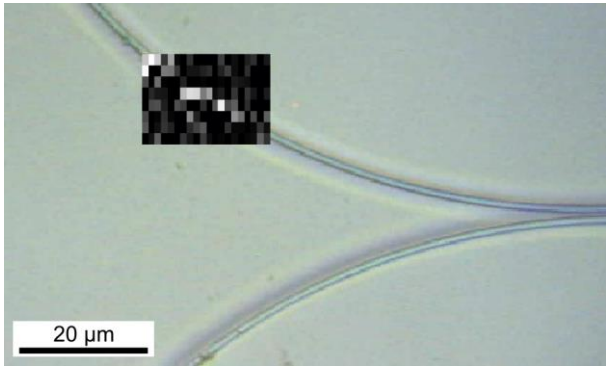
Raman spectroscopic characterization of chips



Scheme of the energy levels and transitions involved in Raman spectroscopy: Rayleigh (R), Stokes (S) and anti-Stokes (AS) scattering.



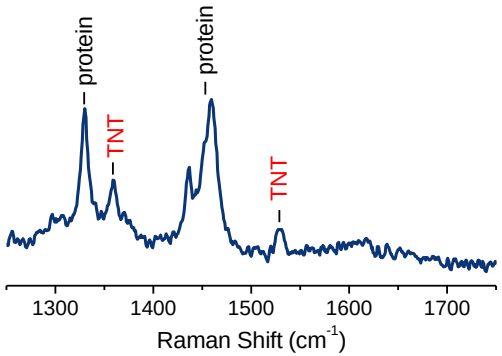


Figure 18. Raman mapping measurement (1.5 µm resolution) carried out on a functionalized ring resonator for TNT detection (left). The gray level indicates the intensity of TNT signals. Corresponding Raman spectrum with visible TNT and protein peaks (right).

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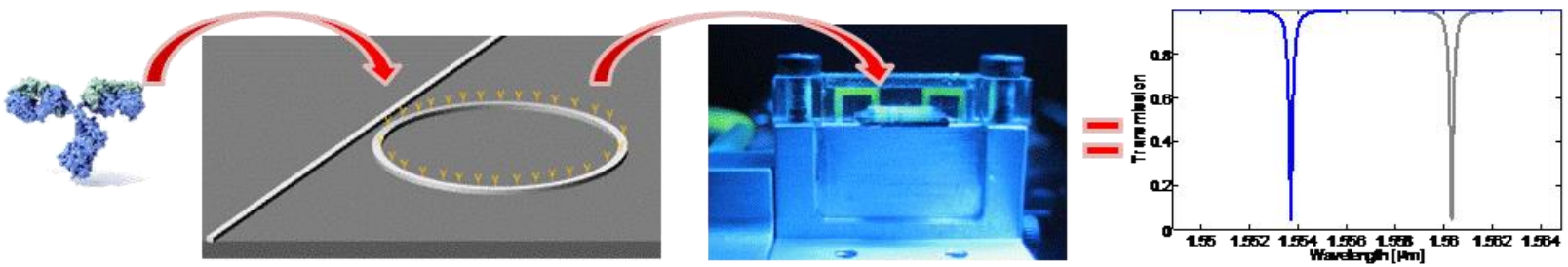
Testing Nanocap

Biophotonic sensor for the sensitive and specific detection in air of DMMP, a simulant of Sarin nerve agent

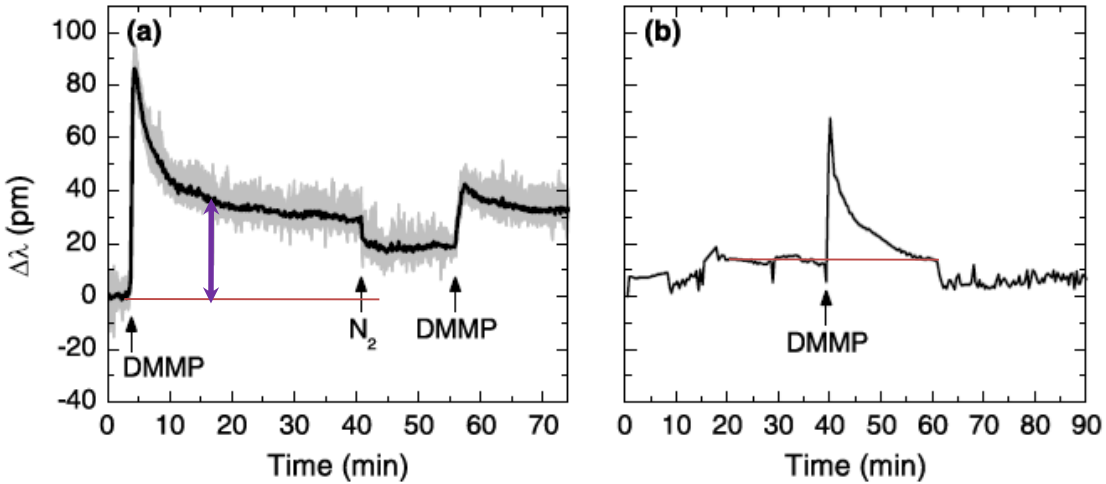
Receptor + PIC + Sampling System = Detector



20 ppb of DMMP in N_2

Proteins

⇨35-40 pm residual shift



No proteins

⇨ no residual shift

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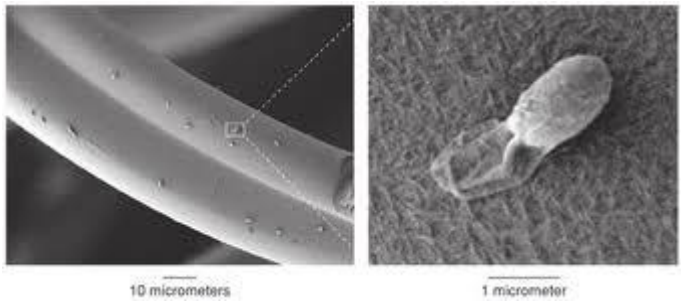
EDA - RAMBO
Rapid Air-particle Monitoring against BiOlogical threats

An eventual terroristic release of Biological Agent would be detected and identified through the syntoms diffusion statistics in the victim population (some days delay).
A **'detect to warn' paradigm** requires novel sensing architectures.
Stand off detection is the most promising but reliability and sensitivity are still to come…
A **first alarm point sensor network** able to detect in less than **5 minutes** a release of spores of microrganisms i.e. *Bacillus Anthracis* with concentration down to **100 CFU/l** of air is proposed.
With a high **reliability** the **impact** of agent on the population is measured **during the release** through sensor network and an immediate response of the emergency will be set up.



'Grant: European Defence Agency (EDA)

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RAMBO concept

2 stages *sensing operations, for (DIM) detection, identification and monitoring of biological threat/pathogenic agents in air. The RAMBO system includes all schemes:*

•Stage one: fast 5 minutes continuous detection and monitoring sensing and pre-alarm based on ***SERS optical biological detection unit****;*

• Stage two: alarm based on ***real time PCR*** *that allows in 45 minutes for a high reliability identification of the microorganism that allows to start a proper response by the first responders*

The operation mode and connection between stages is highly automated due to a ***microfluidic-based platform****.*

The SERS technique can be useful for identifying simultaneously multiple agents by increasing the ***spectrum data base of fingerprints*** *and improving the spectrum processing capabilities.*

The processing and analysis unit enables to record the signals related to the event of detection of the threat while the microfluidic reservoir enables the storage of the sample for a custody chain of sample proof .

RAMBO :

- investigates the use of Phagi as **stable,** receptors for **in vivo** .They will have a **natural** high affinity to bacteria.
- exploit the **selectivity intrinsical of phagy +SERS;** test with **interferents** bacteria for low false alarm
- **detecting and classifying** bio-material, by analysing its convoluted vibrational **SERS** spectra;

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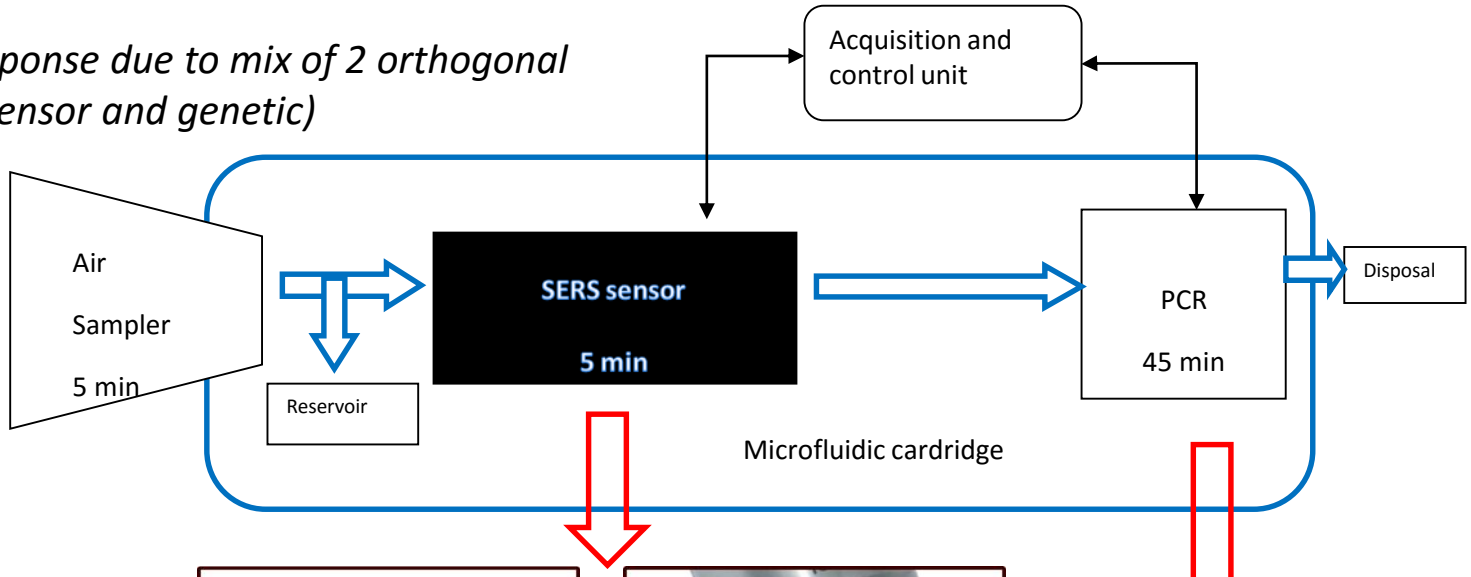
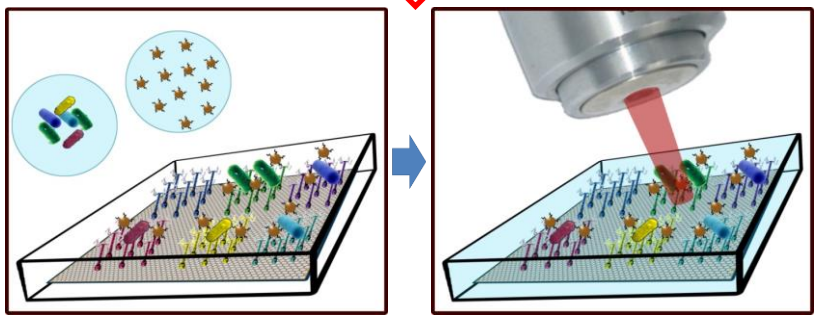
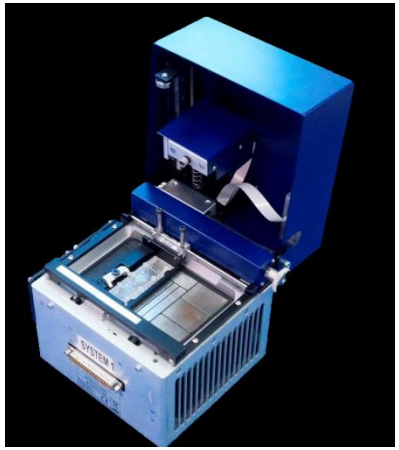
Sensor architecture

Reliability *of response due to mix of 2 orthogonal techniques (Biosensor and genetic)*



Custody of proof
microfluidic reservoir enables the storage of the sample





Micro-Raman spectroscopy is a well known fast and sensitive tool for the detection, classification, and identification of biological microorganisms.
- *no sample preparation,*
- ***highly specific*** *and can provide a chemical fingerprint of several samples.*

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EDA - RAMBO
Rapid Air-particle Monitoring against BiOlogical threats

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Technological platform:

1- Nanostructured GaN substrates for Surface Enhanced Raman Spectroscopy

2-Chemical surface nano-functionalisation for bio-sensing of biological agents in aerosol.

'Grant: European Defence Agency (EDA)

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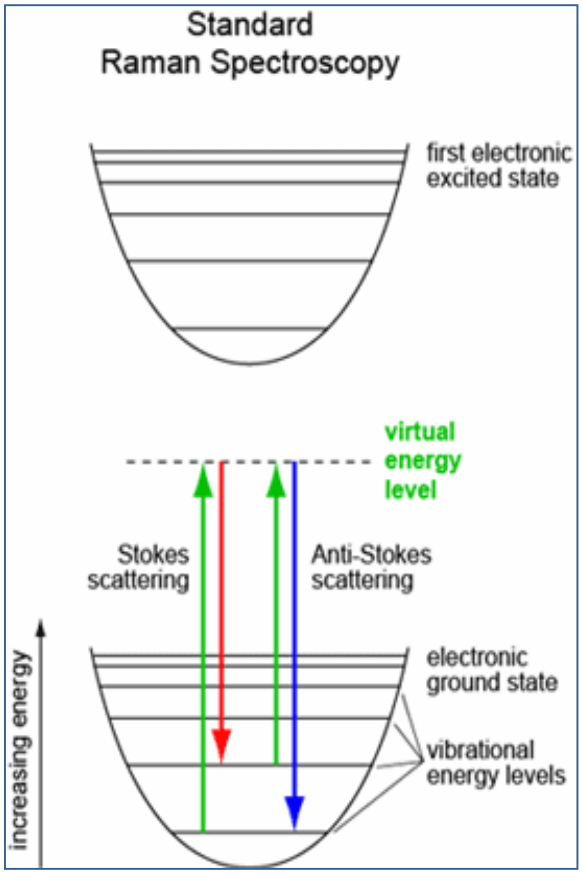
Raman spectroscopy

Raman spectroscopy, based on molecular vibrations/inelastic light scattering, has become a versatile and important tool to study the complex and **heterogeneous biomaterials**, including **bacteria.**

The Raman spectrum, that give information on molecular structure and interactions and intracellular effects, is obtained by measuring the intensity of the scattered light as a function of the frequency difference.

Among the many advantages that this technique offers, as well as the non invasiveness and the **non destructivity**, there is the possibility to **study the biological samples** in their **physiological environmental** because of the low Raman scattering cross-section of **water**.



BUT

the Raman effect is relatively weak and only 1 in 10^8 incident photon are inelastically scattered

SO

the application of the technique suffers of this intrinsic weakness

the intensity can be increased by several orders of magnitude if the sample is adsorbed on the surface of metal **nano-particles**.

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Raman Spectroscopy on Bacillus spores

The Raman spectroscopy can take advantage of the following peculiarities:

- Raman bands are generally many times narrower than most fluorescence bands, minimizing the potential overlap of contributions from different sources in a given spectral region.
- The best excitation wavelength for Raman is not strongly dependent on the adsorbed molecule, allowing the use of a single excitation source for multiple species.
- The Raman spectroscopy is not subject to photo-bleaching, so allowing to average the signals over long periods, in order to decrease the limit of detection.

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Surface Enhanced Raman Scattering (SERS)

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SERS is a powerful spectroscopic technique to detect analytes at very low concentrations (the signal can be **enhanced** by factors of **10^6 to 10^{11}** due to **nanoparticles** as Au/Ag)

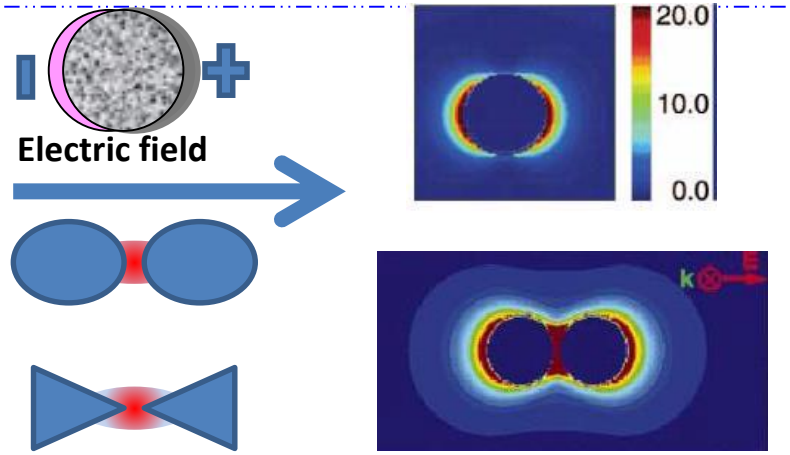
Although the mechanism of SERS is not completely clear it is proposed that SERS enhancement originates mainly from the **excited surface plasmon** of the metal

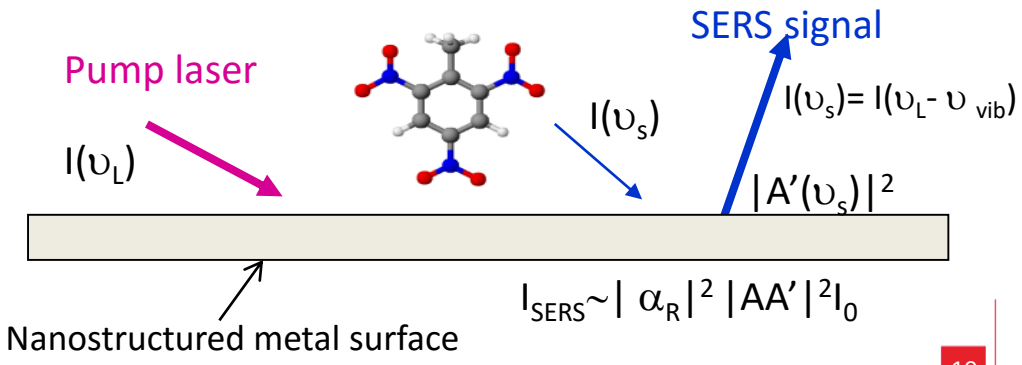
1) Electromagnetic field theory

Based on a change in electric field of the incident laser light close on a discontinuous surface leading to an interaction between the analyte and the surface plasmons of the discontinuous metallic surface

2) Chemical field theory

Based on the chemical bonds that can be produced by the transfer of electron density between the analyte and the surface or by the formation of strong covalent bonds with the metal. This enhancement is generally less than 100





SERS technique on Bacillus spores

The SERS technique offers three major advantages over standard Raman spectroscopy

- Very high sensitivity (a typical Raman scattering intensity increase of 10^3-10^6 can be expected). As a result of enhanced Raman cross-sections vibrational signatures may be obtained with greatly **reduced data accumulation times and incident laser powers** leading to the rapid observation of Raman spectra at the single cell level and the development of low cost portable Raman instrumentation.
- $EF = I_{SERS}N_{NR}/(I_{NR}N_{SERS})$

where N_{SERS} and N_{NR} denote the number of molecules adsorbed on the SERS probe within the laser spot area and the number of molecules probed by RAMAN

- **High selectivity**: owing to the metal surface proximity dependence of the SERS enhancement mechanisms, only cellular surface constituents **within few tens of nanometers** can be expected to contribute to SERS spectra. The resulting molecular selectivity in the SERS spectra of bacteria, as compared to the corresponding bulk Raman vibrational signatures, may be a useful feature for the development of both analytical and structural probes of these microorganisms.
- Because the **fluorescence** of molecules adsorbed **on metal surfaces become quenched**, the fluorescent background that appear in SERS spectra loses its intensity, and so the Raman band that was hidden by the fluorescence become easier to observe.

… and one *major critical issue*:

- reproducibility and reliability of the vibrational signatures owing to the very sensitive dependence of the enhancement phenomenon on the microscopic morphology and **stability** of the SERS active substrate.

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SERS technique: Direct SERS Sensing

- The **functionalized** SERS surface will capture selectively the target bio-molecules, sorting out different species into separated spots or strain dependent arrays.
- After a suitable time for the bio-molecules to get stuck to the surface, the cell will be **rinsed out** in order to dispose of any substance apart from the captured organisms. The surface array is then analyzed by the Raman probe which scans the sample and provides a map of positive or negative response for the target species.
- During the measure, a laser beam of suitable wavelength and power is focused into a spot volume on the sample surface and the inelastically scattered radiation is collected by means of a properly designed optical system. For each spot a Raman spectrum is produced and classified by means of chemometric techniques.

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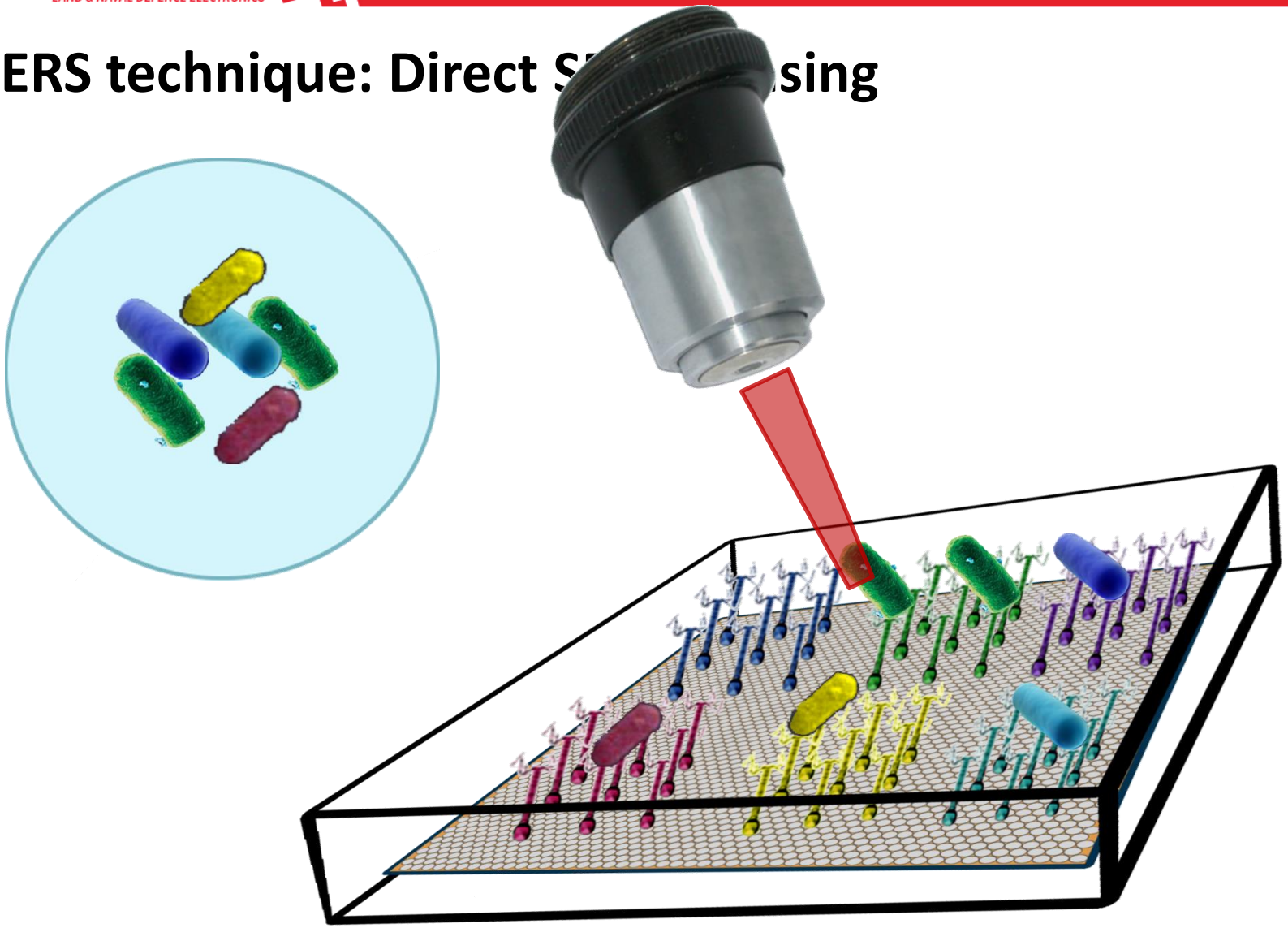
SERS technique: Direct S sing



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SERS technique:Extrinsic Raman Labelling

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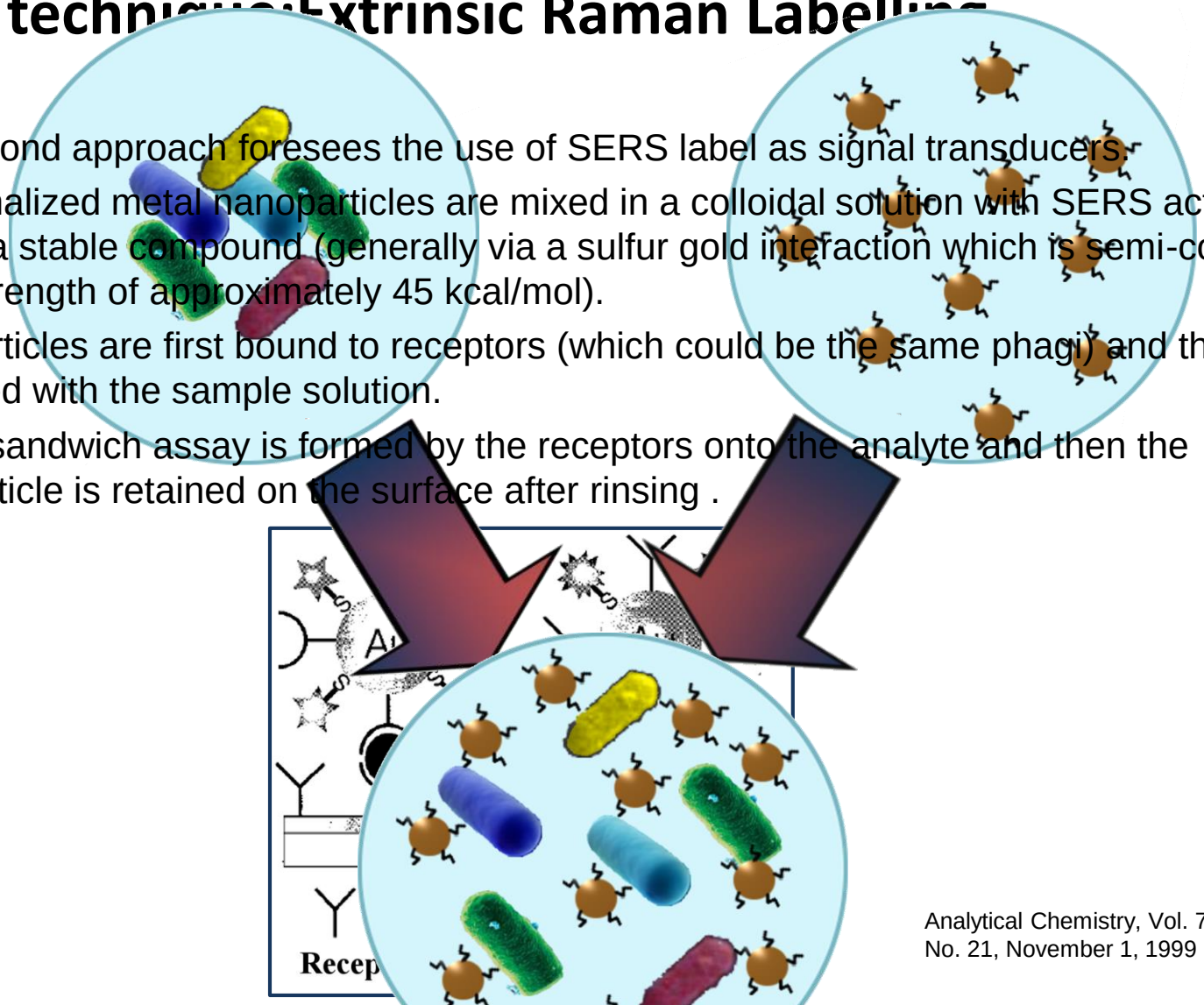
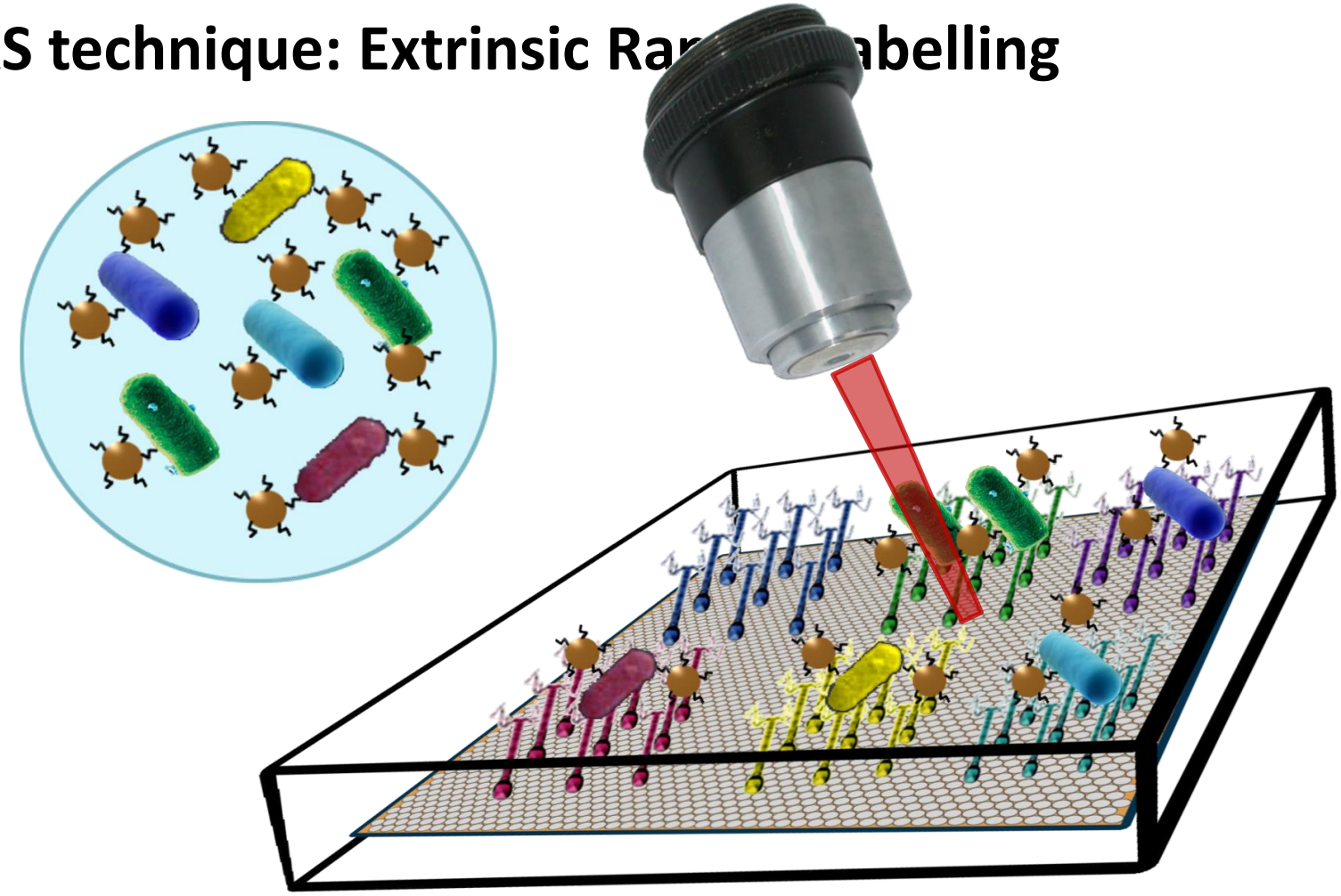
- This second approach foresees the use of SERS label as signal transducers.
- Functionalized metal nanoparticles are mixed in a colloidal solution with SERS active Labels to form a stable compound (generally via a sulfur gold interaction which is semi-covalent and has a strength of approximately 45 kcal/mol).
- Nanoparticles are first bound to receptors (which could be the same phagi) and then incubated with the sample solution.
- Then a sandwich assay is formed by the receptors onto the analyte and then the nanoparticle is retained on the surface after rinsing .



Analytical Chemistry, Vol. 71, No. 21, November 1, 1999

- The final detection proceeds as in sing method.

SERS technique: Extrinsic Ra abelling



In order to achieve an even better spectrum the extrinsic Raman Labeling [9] of the nanospheres are considered, it will be based on the functionalization of the labeled nanospheres dispersed in the liquid with Gamma Bacteriophages receptors.

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Selection criteria of the SERS substrates

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High Ehnhancement Factor EF in the range 10^4 – 10^7 depending on the type of molecules and platform configuration.;
→nanopillar-based substrates from SILMECO (Silicium based) and UNIPRESS(GaN based)

- are **stable** over long time in tha air and water - I think that it excludes only silver based SERS substrates (like Q-SERS or Ag based SILMECO platform). →gold or gold/silver alloy

- can be electrografted

- Can be reused

- uniform EF on the whole platform

Nanopillar fabrication on GaAs for SERS [1]

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Surface Enhanced Raman Scattering (SERS)-active surface based on photo-etched (KOH) and 90um Au-coated GaN. The highest enhancement factor (EF) in SERS and high reproducibility of spectrawere obtained from surfaces covered with bunched nanopillars which were produced by relatively long defect-selective photo-etching.

EF of the order of 2 10^6 for p-mercaptobenzoic acid (PMBA). Same EF wrt conventional RAMAN Ag surfaces but stability in RAMAN spectrum more than 3 months.

Cleaning procedure available (H2O2/NH3)→ reusability for SERS experiments

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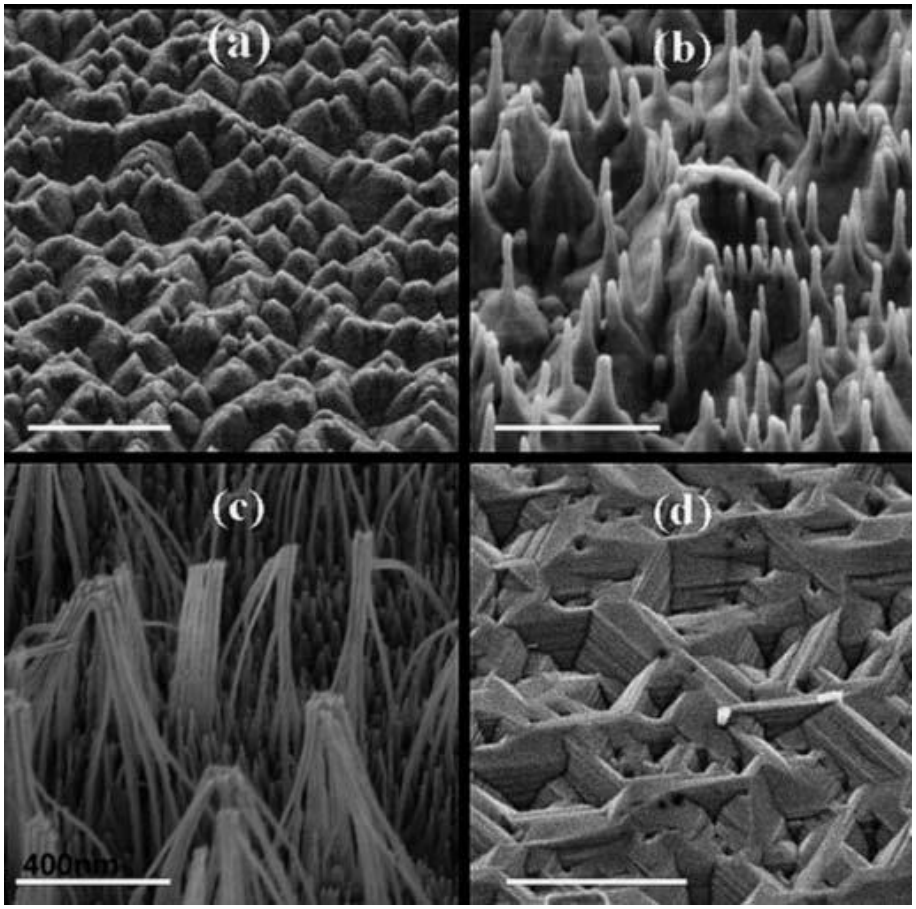

SEM images of GaN samples after photo-etching for: 5 minutes (a), 10 minutes (b), 15 minutes (c) and 3 minutes followed by subsequent etching in hot KOH solution (d). The bars represent 1 um.

Dealloying of sputtered Ag Au thin film [2]

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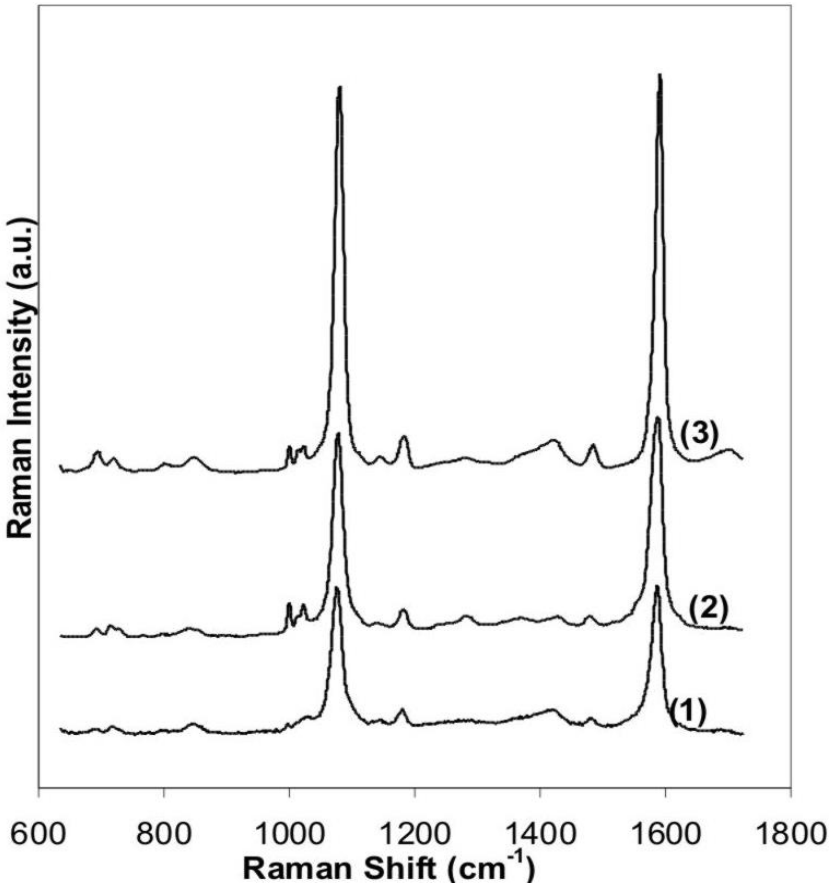
SERS substrates with thin film gold-rich Au57Ag43 alloy (70/30 wt. %), it is possible to preserve about 19 at. % of silver in the layer, even after etching in nitric acid for up to 24 h.

A large EF >10^ 7 proven on PMBA molecules attached to such porous Au-Ag metal layer is obtained due to the presence of a high percentage of Ag.

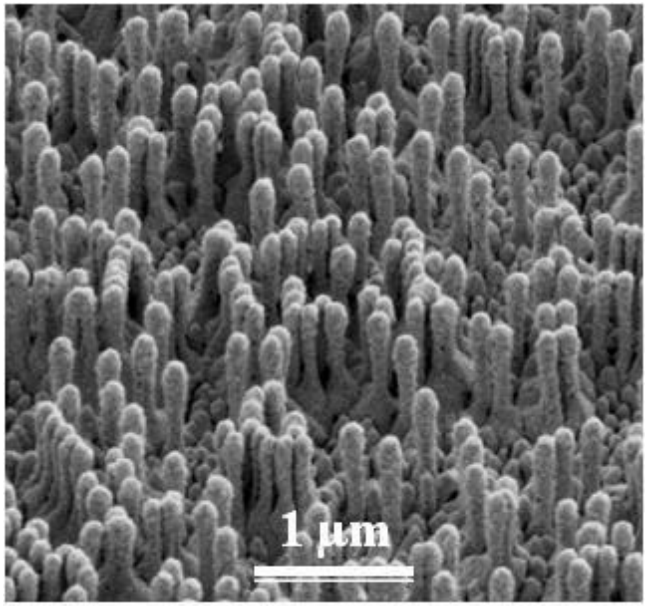


SERS spectra of *p*-MBA on GaN-based platforms covered by pure gold (1) and Au-Ag alloy (2,3). Samples 1 and 2 not etched, sample 3 de-alloyed for 24 hours
(reprinted from ref. 2).

Dealloying not feasible onto Si based substrates

GaN Based substrates features

1. Standard size of SERS substrates: 5 x 5 x 0.4 mm
2. Surface morphology: individual pillars or bunches of pillars;
3. Nobel metal coating: gold or Au/Ag = 70/30 wt% alloy. De-alloying for increased nano-scale roughness for 3 hours in Nitric Acid;
5. Analysis of analytes at low concentration in a liquid or solid deposits;
6. Long time stability of delivered SERS platforms (up to 90 days).



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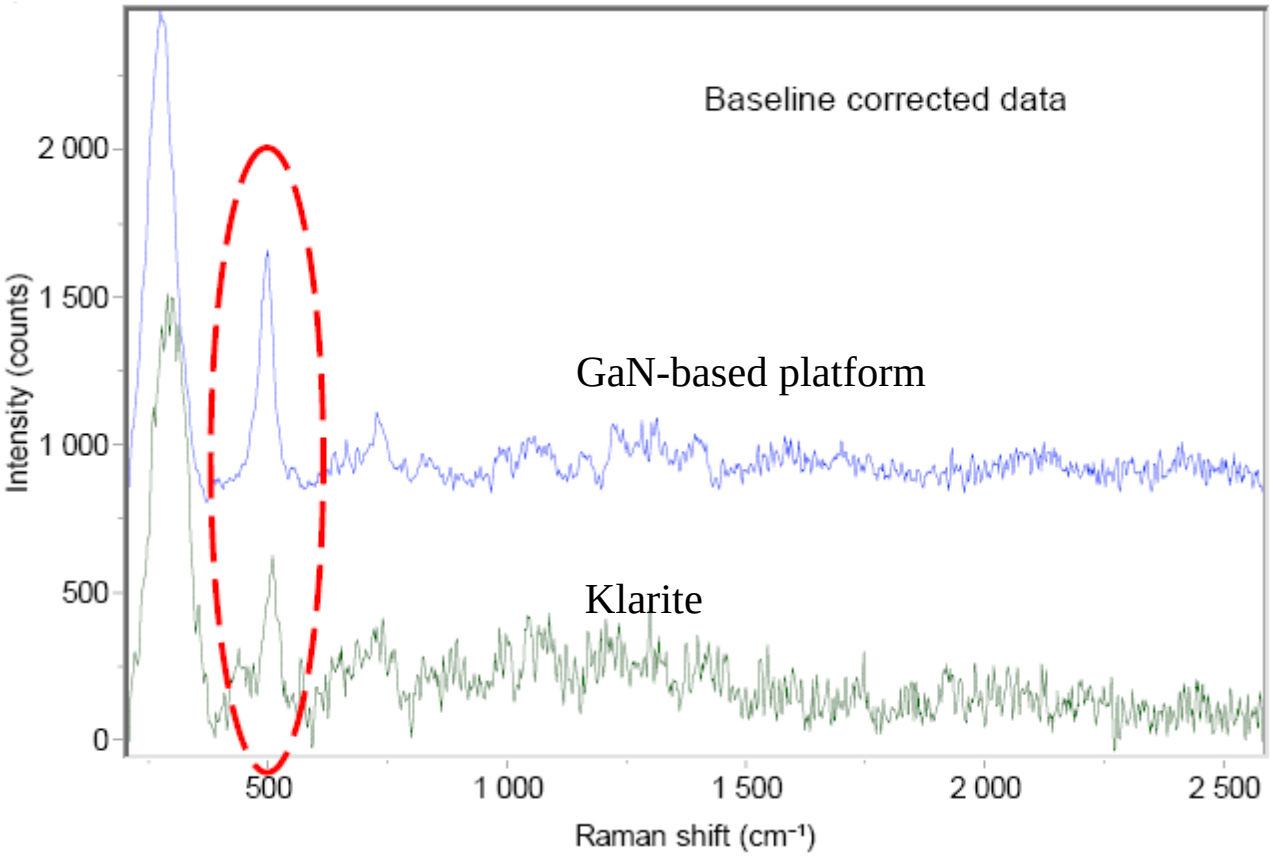
DTT SERS detection comparison: GaN-based platform vs commercial platform

(By courtesy of Dr C. David, Horiba Jobin-Yvon, France)



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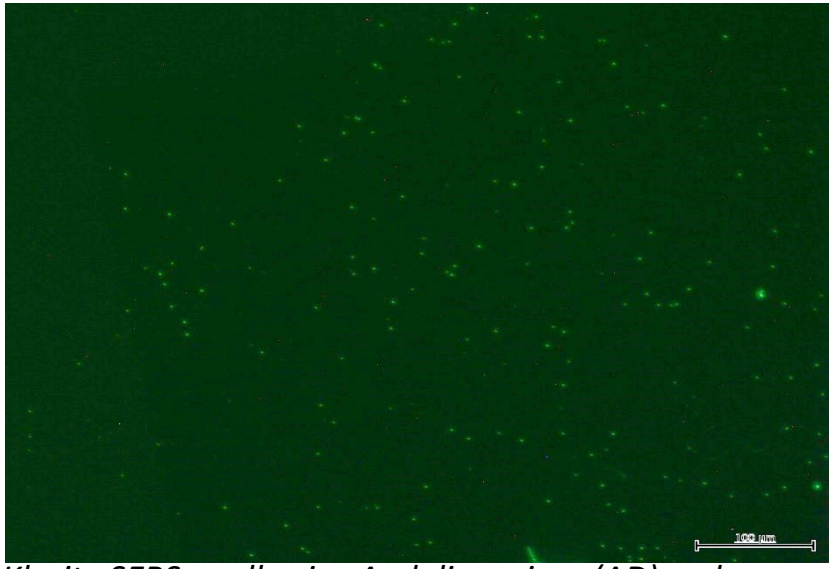
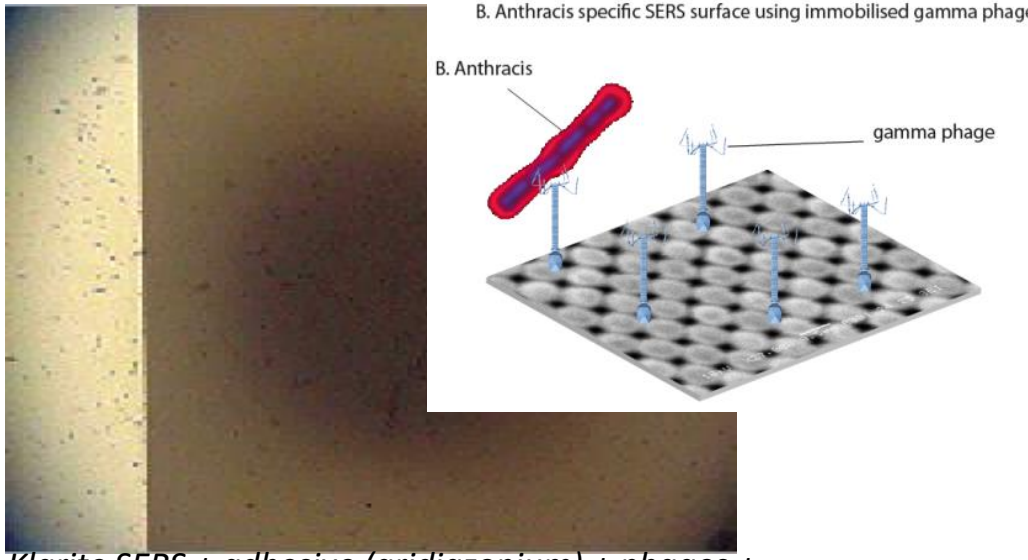
SERS functionalised substrates



Klarite SERS + adhesive Aryl diazonium (AD) + phages + FITC

Concentration: ***10 Phages in 100um sq diameter***



Klarite SERS + adhesive (aridiazonium) + phages + captured BT

- *Grafted phages can be randomly oriented on the surface*
- *They are supposed to create an irreversible binding to the Bacillus (a Phagolithyc cycle)*
- *Range of binding to be verified*

Phages based bio *sensor:*

- *High specificity, high avidity of Phages →sensitivity*
- *Label free*
- *Non competitive assay*
- *High resistance to the envrionment (WRT to antibodies)*
- *simple and* ***low no. of chemical reagents***

For Spores a different capturing technique will be implemented based on immobilisation of ***Peptides on the SERS surface***

Seq	SLLPGLPGGGC. βAla-βAla -βAla K.K.K

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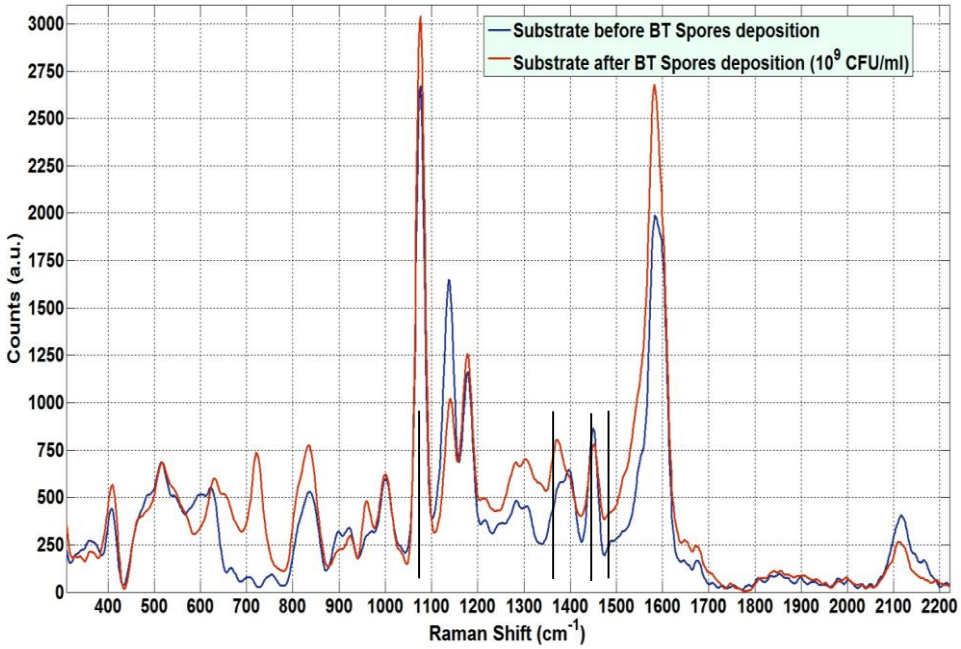
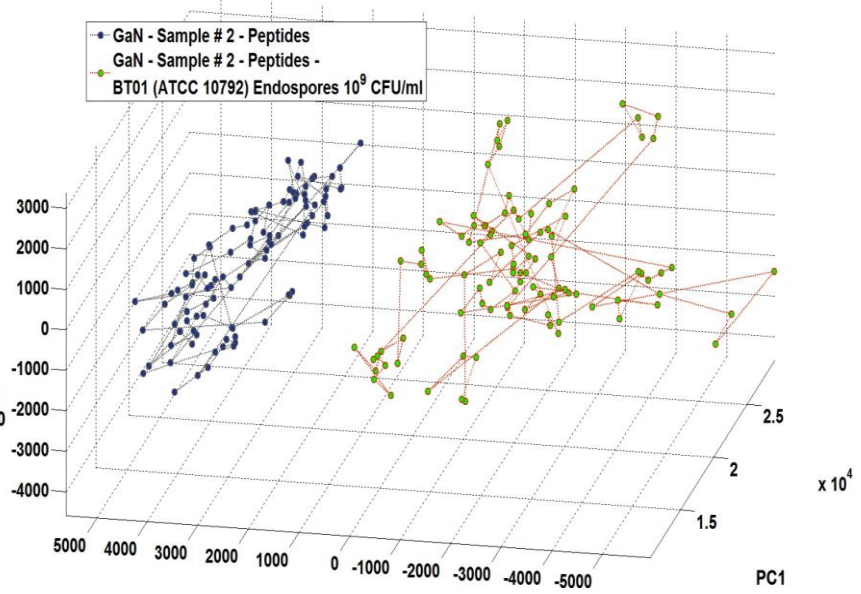
Raman spectra (average comparison) and PCA analysis of BT endospores on GaN/Au-Ag substrates functionalized with peptides

20 μl of endospores suspension (10^9 CFU/mL) was dropped. After waiting 45 minutes, in order to assure the interaction with peptides, the sample was rinsed with MilliQ water and finally measured





The main fingerprint peak at 720 740 cm^-1 is the glyconilic ring in the peptitoglicane molecule in the BT spore membrane

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NANOCAP

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