ICHF
Institute of Physical Chemistry PAS

the BioSpectroscopy group

SERS-based sensor for diagnosis of sexually transmitted diseases: a study of clinical samples

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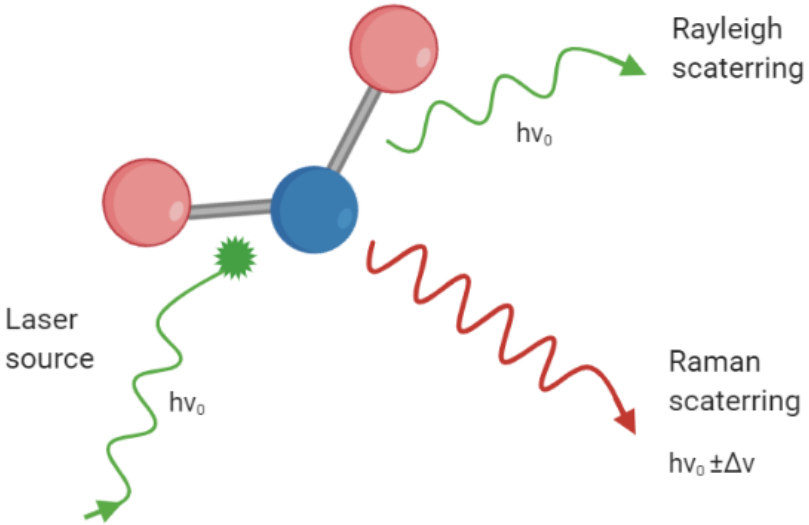
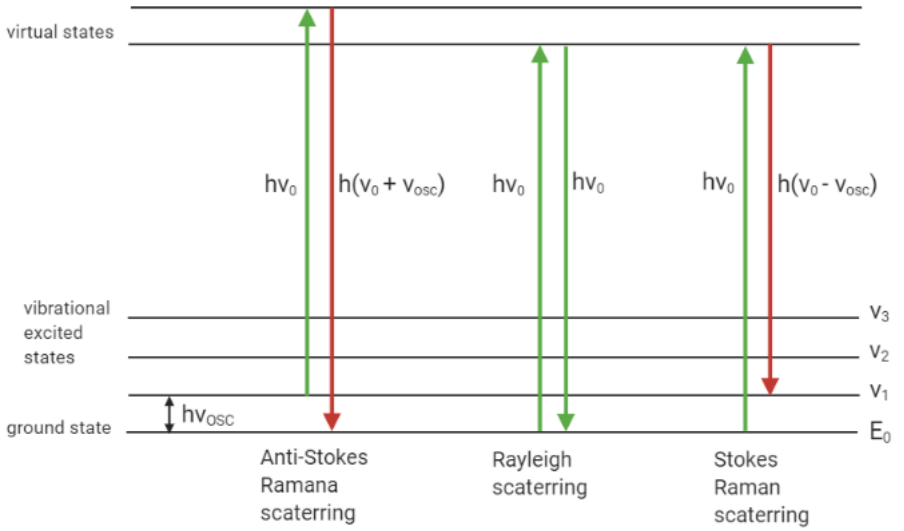
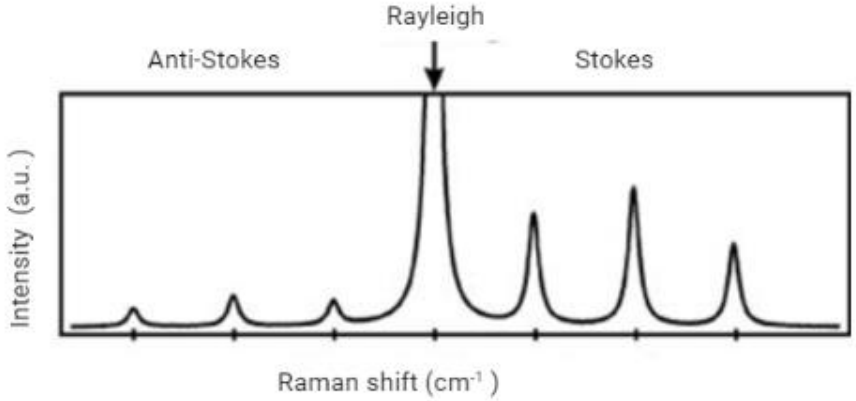
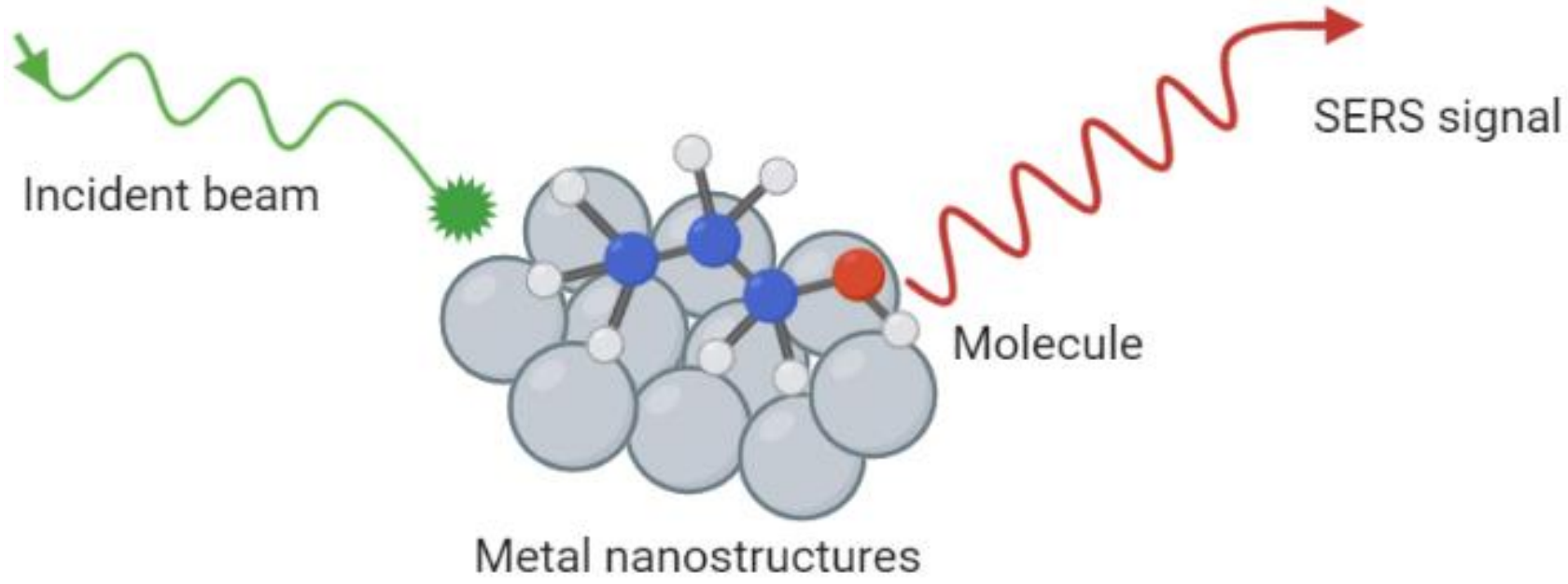
Raman Effect





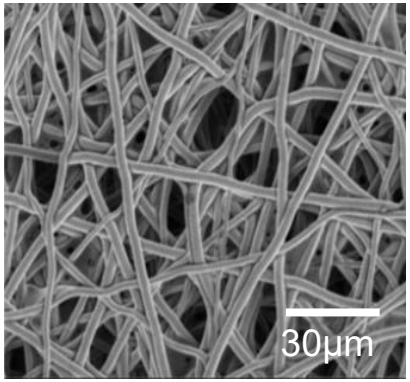


Surface-enhanced Raman Spectroscopy (SERS)

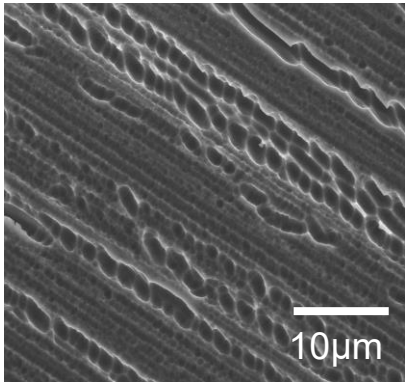


Created with BioRender.com

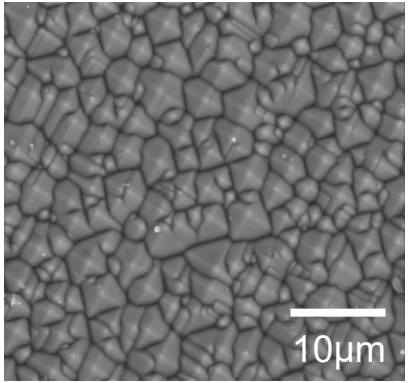
SERS substrates



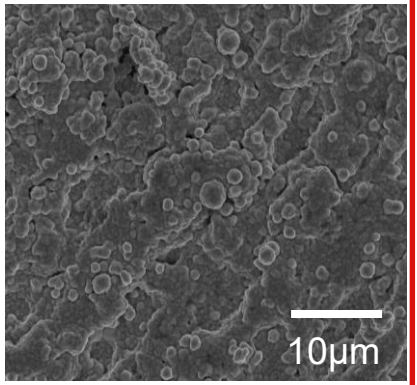
Polymer mat covered with 40 nm Au



Photovoltaic cells



Silicon after etching in KOH solution



Silicon after laser ablation covered with 100 nm Ag

Motivation

Sexually transmitted diseases (STDs):

- ✓ Caused by:

Bacteria (*Chlamydia trachomatis*, *Neisseria gonorrhoeae)*

Viruses (Herpes simplex virus - HSV, human papillomavirus - HPV)

Protozoa (*Trichomonas vaginalis*)

- ✓ It was estimated that each year around **214 million** people struggle with STD cause **only** by bacteria bakterie - *C. trachomatis* (127 millionów), *N. gonorrhoeae* (87 millionów). Since some infections have few, if any, recognizable symptoms, the presented data may be misleading.
- ✓ are one of ten **most popular** diseases in young adult males and women worldwide
- ✓ can lead to the numerous economic and **health consequences**, *e.g.*, infertility, adverse pregnancy outcomes, stillbirth, neonatal infections, ectopic pregnancy and pelvic inflammatory disease.
- ✓ Currently used methods (culture method, NAATs, microscopic examination) have many disadvantages

SERS + chemometric methods

Materials and methods

Clinical samples (male urethra swabs):

experimental group: samples from men diagnosed with chlamydiosis or gonorhea.

control group: samples from healthy volunteers.

Bacteria strains:

Neisseria gonorrhoeae ATCC 70825,

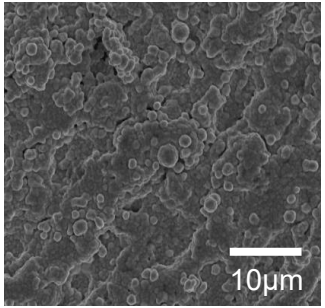
Neisseria meningitidis ATCC 13102,

Neisseria lactamica ATCC 23790,

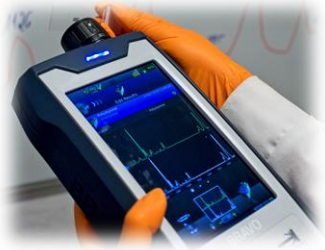
Neisseria sicca ATCC 9913

Cultured on BD chocolate agar (GC II Agar with IsoVitaleX for 48h in 37 °C, 5 % CO_2

SERS substrates: Silicon after laser ablation covered with 100 nm Ag



SERS: Bruker's Bravo spectrometer (Bruker MultiRAM sN:0006)



Chemometric methods:

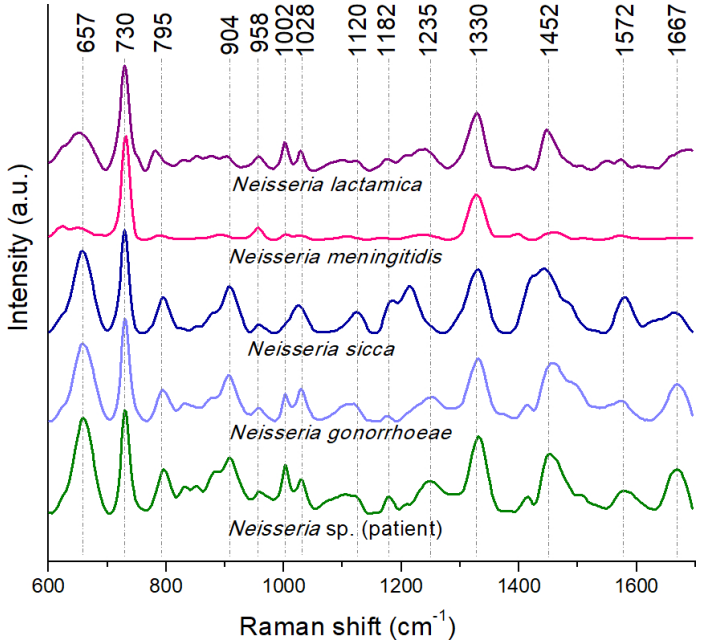
PCA Principal Component Analysis

PLS-DA Partial Least Square Discriminant Analysis

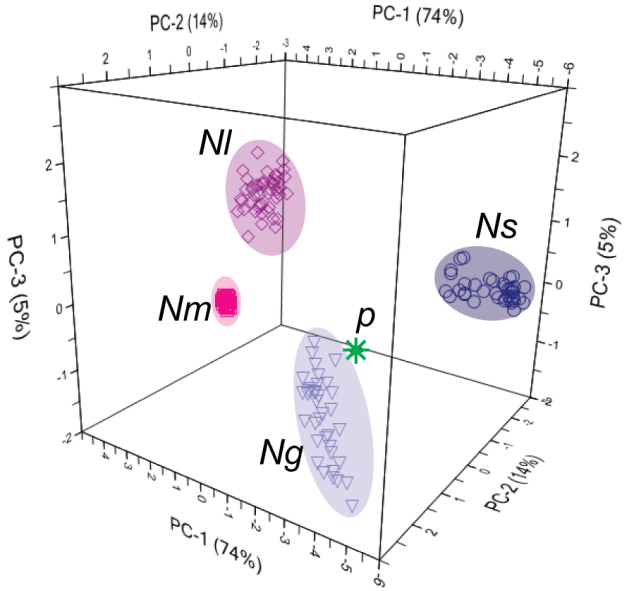
SIMCA Soft Independent Modelling of Class Analogies

Indirect approach

SERS



Principal Component Analysis (PCA)



Results were confirmed by

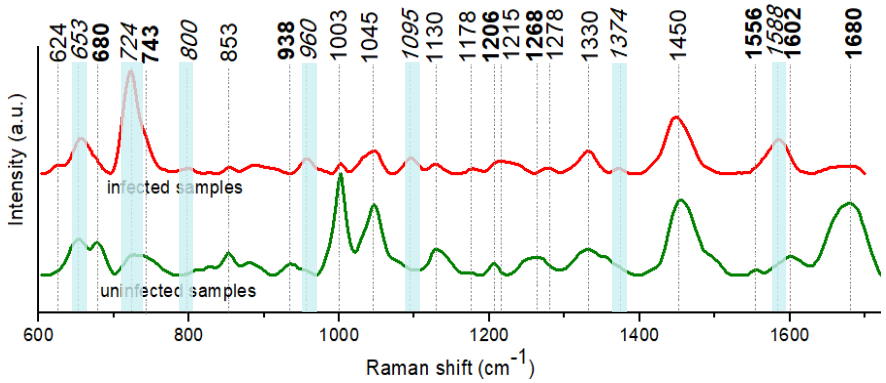
- Culture method (Chocolate Agar + PolyViteX vcAT3 agar (Biomerieux) 37°C, 5% CO_2
- Gram staining
- Oxidase test

Methods comparison

	SERS	Gram staining	Oxidase test	MALDI-TOF MS
Effect	Identification at species, and strain level	Cell wall type (Gram negative)	Biochemical reaction for the presence of cytochrome	Identification of bacteria at species level
Time of analysis	18-45 sec	10 min	2 min	1 min
Reagents	SERS active metal	Crystal violet, iodine solution, 95% ethanol, Safranin	Tetramethyl-*p*-phenylene-diamine hydrochloride	Ethanol, acetonitrile, trifluoroacetic acid, different matrices: α-cyano-4-hydroksycinnamic acid, sinapinic acid, ferulic acid
Visual assesment	No	Change in cell color to pinkish red	Change in cell color to dark-purple	No
Cost	low	low	low	high
Limitation	Reproducibility of SERS measurements	Colonies are usually difficult to interpret large number of steps and reagents	Can be performed only on fresh colonies (18-24h); Only platinum or inert transfer loops can be used.	high

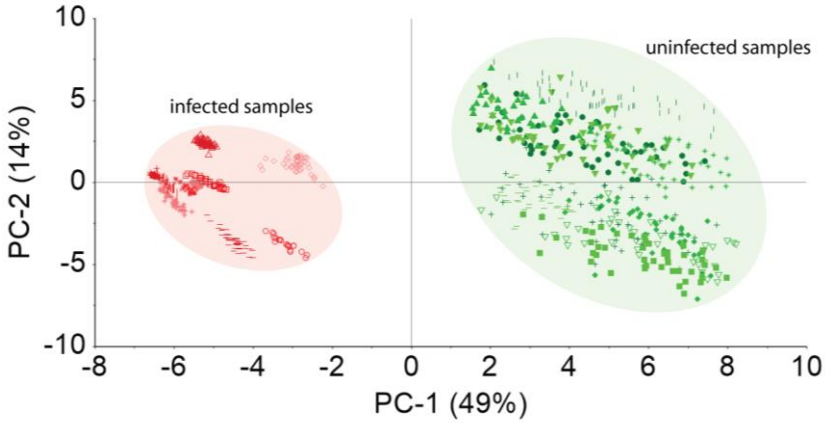
Direct approach

SERS

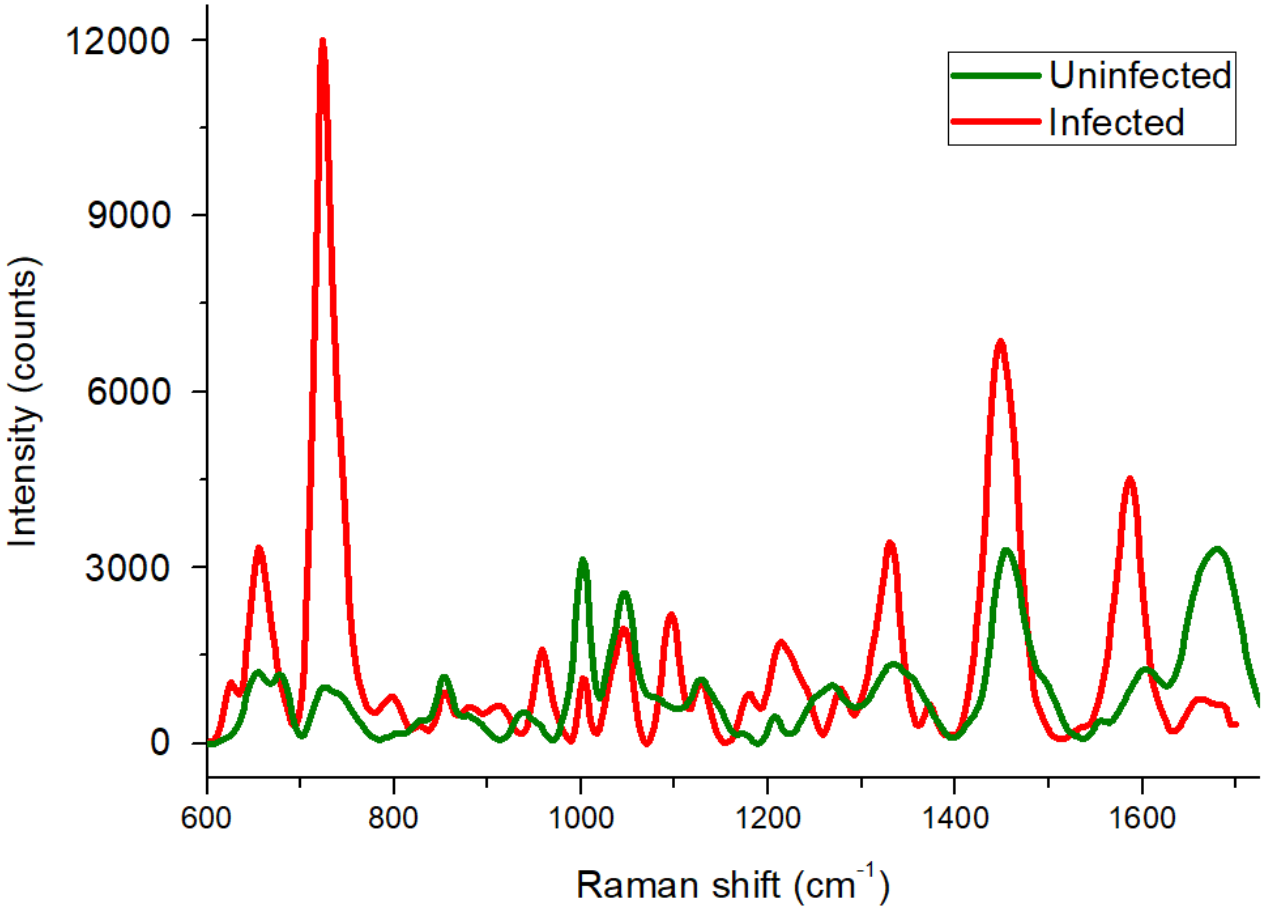


Raman shift (cm^{-1})	Tentative assignment
653	Guanine, D-glucose, lactose, O=C-N deformation in uric acid
853	C-C aliphatic stretching in tyrosine (proteins), (CNC)s stretching, CH_2 rocking
1003	Aromatic ring breathing in phenylalanine, C-N stretching in urea
1045	C-N symmetric stretching, Spermidine trihydrochloride
1450	CH_2, CH_3 bending in tryptophan, Albumin, Creatinine
1680	Cholesterol in lipids, Spermidine phosphate hexahydrate

PCA



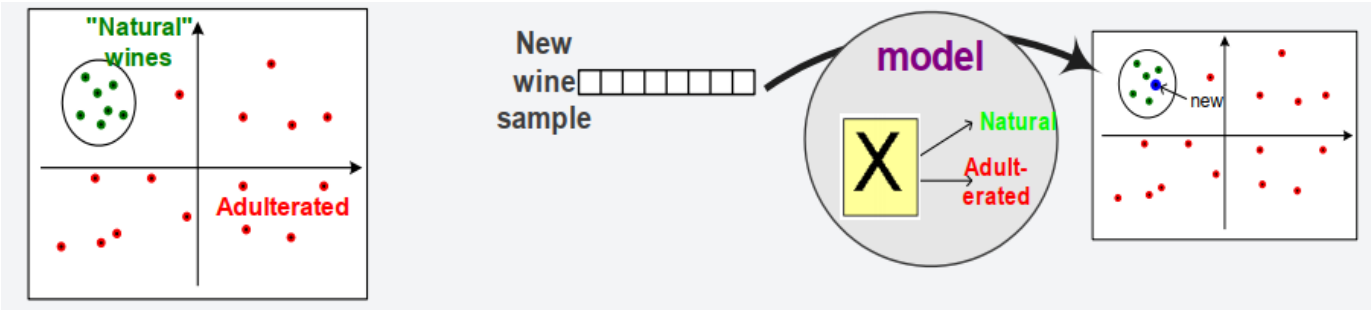
- Bands such as: **653, 724, 800, 960, 1095, 1374, 1585 cm^{-1}** on the spectrum of infected swabs are characteristics for bacteria cells
- PCA provides **binary differentation** between infected and uninfected samples
- Diet, age, healthy problem and other individual factors may influence the composition of tested sample - spreadness of the class
- It is estimated that in infected material there is about **10^6 cfu/ml** bacteria and it is sufficient for SERS detection



- Infected materials are characterized by a four times greater enhancement factor
- The contribution of chemical compunds in the spectrum is significantly lowered in favor of bacterial cells

Supervised methods

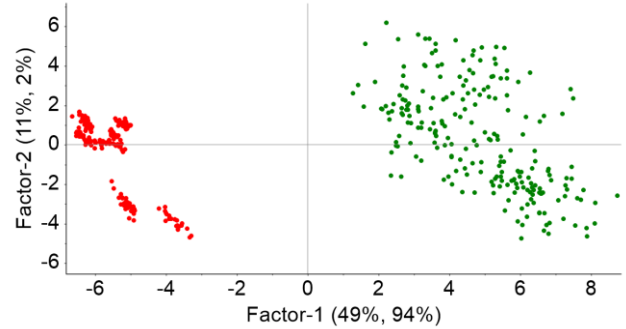
- **Calibration**: training set – 8 infected and 8 uninfected samples (for each sample 30 spectra were recorded) = total **480 spectra**
- **Validation**: test set – 2 infected and 2 uninfected samples (for each sample 30 spectra were recorded) = total **120 spectra**



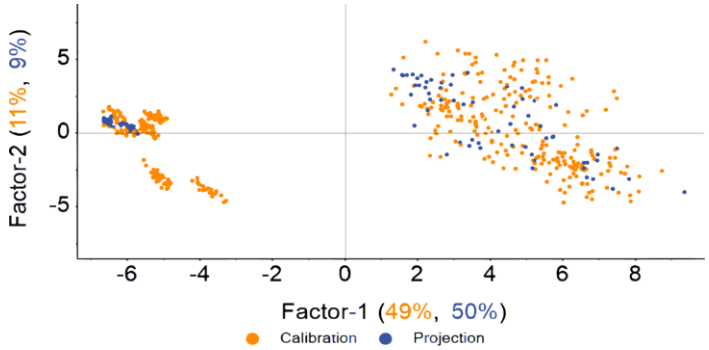
Source: Camo Analytics training materials

Partial Least Square Discriminant Analysis (PLS1-DA)

1) Preparation of calibration model



2) Projection of test samples onto calibartion model



3) Results of classification (selected spectra)

Samples	Class „Infected”		
	Predicted value	RSD	Reference value
Infe.1	1.0323	0.0430	1
Infe.2	1.0018	0.0419	1
Infe.3	1.0305	0.0429	1
Uninf.1	-0.1326	0.2077	0
Uninf.2	-0.0391	0.1609	0
Uninf.3	-0.1681	0.1517	0

- ✓ The first latent variable (LV1) explains 94% of the variance in the block Y with 49% of the spectral data (X matrix)
- ✓ the predictive values were always very close to 1 or 0
- ✓ The low values of standard deviation (0.04-0.31) ensure that the model is robust and provides correct classification
- ✓ Accuracy **100%**

Conslusions:

- ✓ For the first time,

we recorded spectra of urethra swabs taken from healthy volunteers and men infected with STDs,

we proposed band tentative assignments in SERS spectra of urethra swabs,

we recorded SERS spectra of pathogens leading to STDs,

- ✓ bacterial cells in infected samples give high contribution to its overall SERS pattern,
- ✓ PCA enables binary differentiation between infected and uninfected samples,
- ✓ The impressive predictive ability of created PLS1-DA or SIMCA model was obtained

PLS1-DA provides **100%** accuracy , **SIMCA** provides **89%** accuracy,

- ✓ Simplified and label-free procedure which does not involve a qualified staff and chemical reagents may facilitate a fast diagnosis od STDs,
- ✓ The integration of such a biosensing platform with a small, portable Raman spectrometer will develop the handheld point-of-care device

Acknowledgement

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Thank you for attention