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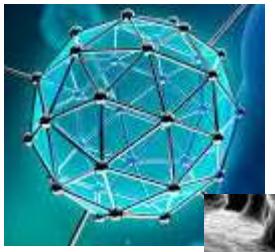
The Prospects of Aquaphotomics in Biomedical Science and Engineering

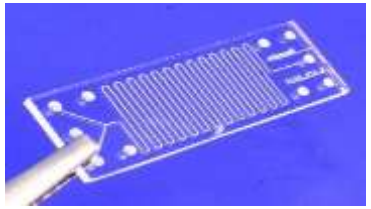
What Aquaphotomics can do?

1) Characterization of biomaterials (nanomaterials)



H_2O

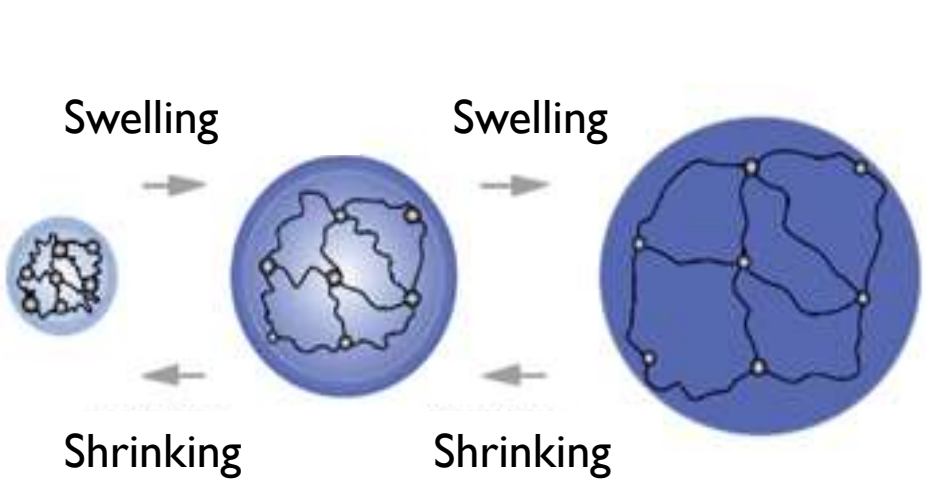
2) Biomedical measurements and monitoring



3) Early diagnosis and screening

Biomaterials Characterization – Soft Contact Lenses

- State of Water in Soft Contact Lenses
- Detection of Protein Deposits



www.iopinc.com/wp-content/gallery/osmed-hydrogel/

Hydrogels

Hydrogels

Wound healing

Transdermal drug delivery

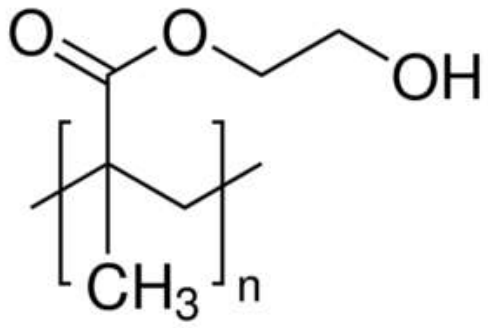
Tissue engineering

Drug delivery

Contact lenses &
Ocular drug delivery

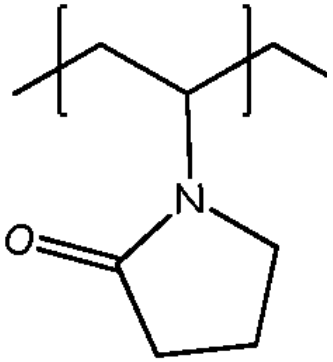
Biomaterials Characterization – Soft Contact Lenses

- State of Water in Soft Contact Lenses (silicone and non-silicone: pHEMA)



pHEMA

Add other monomers to increase water content



vynil pyrrolidone

- Functionality depends strongly on the water content and their ability of water-transport and oxygen transmissibility characteristics

❖State of Water in Soft Contact Lenses (pHEMA based)

Water is crucial, because oxygen is crucial

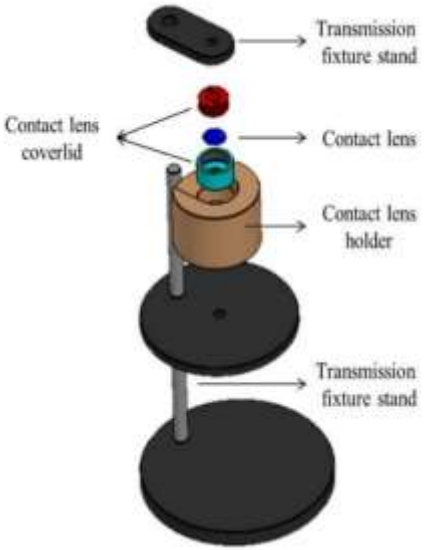
Free water
freezable

Bound water (tightly &loosely)
non-freezable and freezable

= still poorly understood

❖State of Water in Soft Contact Lenses (pHEMA)

	Low H_2O	Medium H_2O
Material	**VSO38**	**VSO50**
British Approved Name	pHEMA Filcon I I	Co-polymer of HEMA & VP Filcon II I
Water content	38.1% ±2	48%±2
Average measured Dk (Fatt)	9	15



Minispectrometer Hamamatsu, Transmittance mode

(900nm to 1700nm) - real time

(3 exp. x 12 lenses x 5 consecutive replicas)

Savitzky-Golay smoothing filter (25pt)

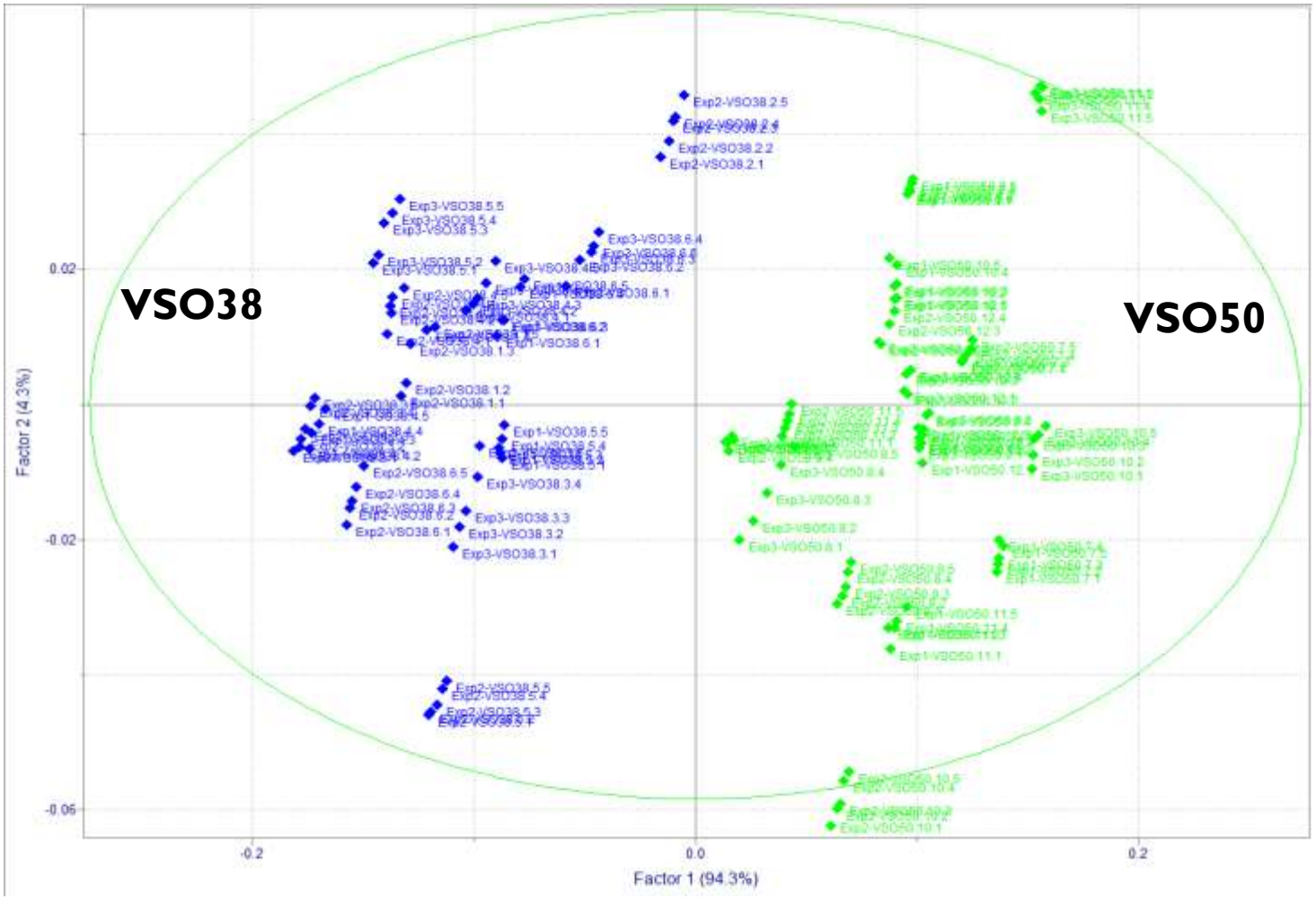
Mean centring and SNV

Principal component analysis

Aquagrams

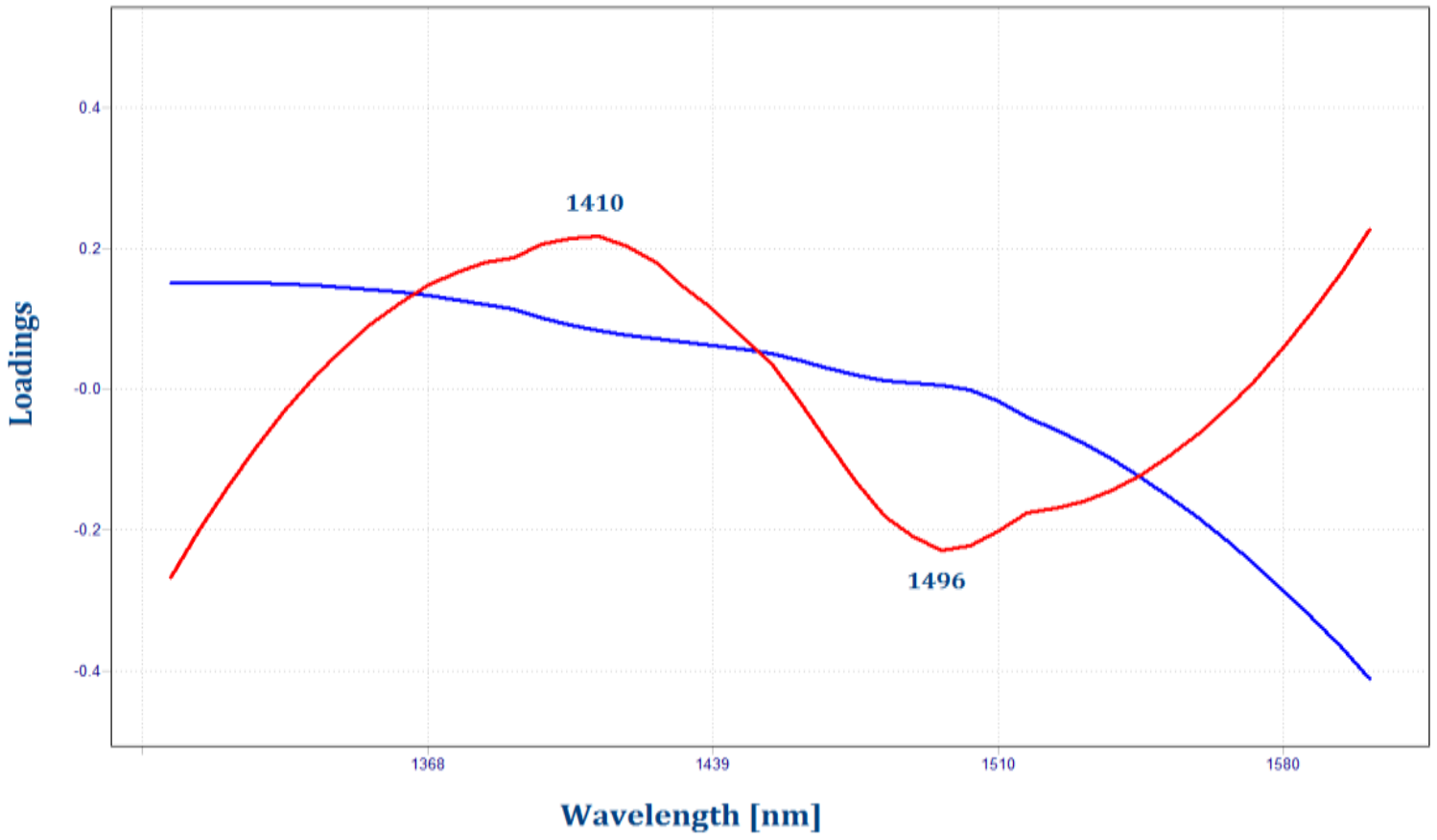
Biomaterials Characterization – Soft Contact Lenses

❖State of Water in Soft Contact Lenses (pHEMA)



Biomaterials Characterization – Soft Contact Lenses

❖State of Water in Soft Contact Lenses (pHEMA)



❖State of Water in Soft Contact Lenses (pHEMA)

Tightly bound

Free

Loosely bound

❖State of Water in Soft Contact Lenses (pHEMA)

- ✓ In material with higher water content more free water and loosely bound water exists in addition to tightly bound water
- ✓ Faster changes in the water state of VSO50 material, which could be due to higher content of unbound water that can more easily be extracted or escape as vapour
- ✓ The resulting state of water in VSO38 pHEMA Filcon 1 material is due to the hydroxyl groups (-CH2CH2OH) of pHEMA
- ✓ In VSO50 co-polymer of HEMA & VP Filcon II 1 the higher water content is achieved due to the presence of amide moiety (pyrrolidone group -NCOCH2-), which is very polar and two molecules of water can become hydrogen-bonded to it
- ✓ However, amide group does not bind water as strongly as hydroxyl group and that is why VSO50 contact lenses contain more loosely bound and free water when compared to VSO38.

Biomaterials Characterization – Soft Contact Lenses

❖ Detection of protein deposits on spoiled contact lenses

Surface deposits are composed of components normally present in the tear film and are primarily made of proteins, while lipids and carbohydrates are present in smaller amounts

The purpose of this exploratory study was to discriminate between the groups of spoiled (used, worn) and unspoiled (not used, new) soft contact lenses in hydrated state, using NIR spectroscopy and Aquaphotomics

* Promotes microbial cell attachment
* Increases risks of inflammatory complications

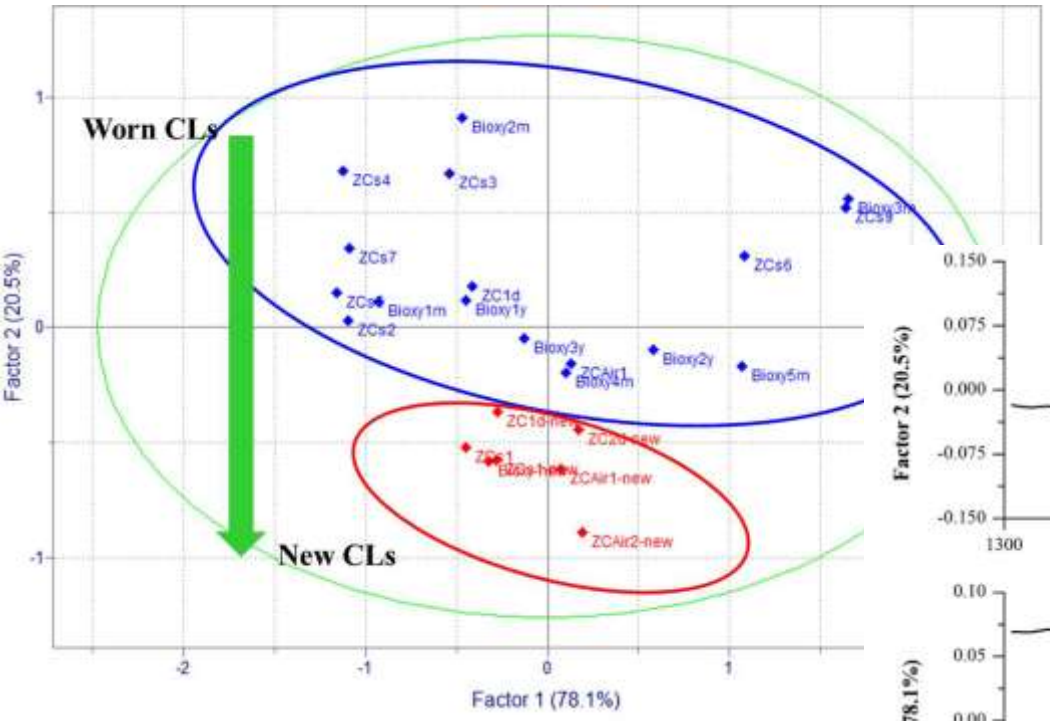
Biomaterials Characterization – Soft Contact Lenses

❖ Detection of protein deposits on spoiled contact lenses

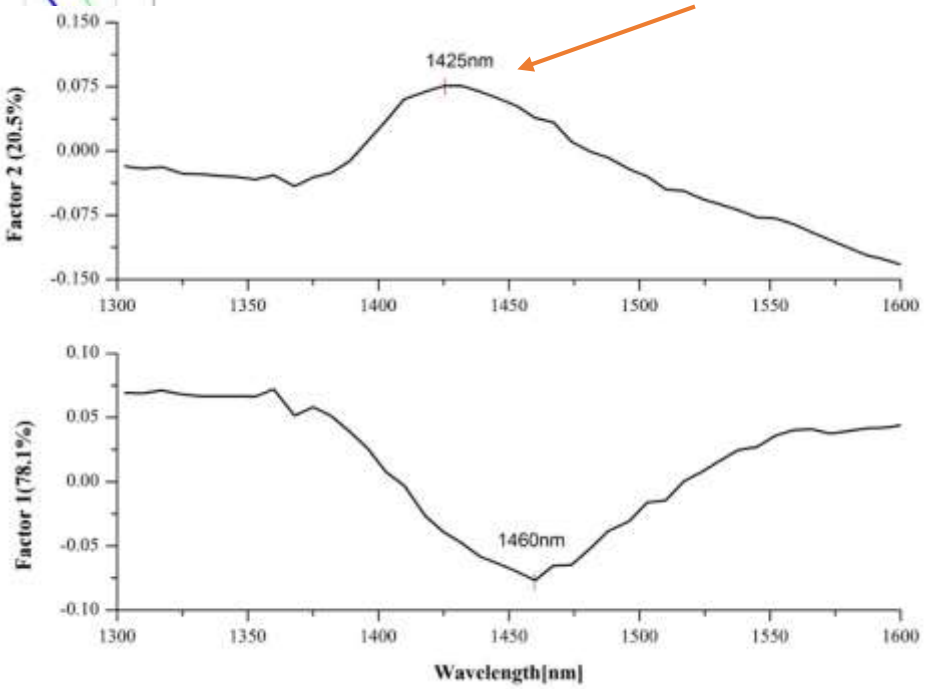
Manufacturer	Products	Material	Polymer type	No. of worn contact lenses	No. of new contact lenses
Barnaux Healthcare	Eyeye I Maxx	Etafilcon A	Silicon hydrogel	3	I
	Eyeye Bioxy	Hioxifilcon A	GMA-HEMA copolymer	5	0
	Zeiss Contact Day I Easy Wear	Methafilcon A	HEMA-MAA copolymer	I	2
Zeiss	Zeiss Contact Day 30 air	Aerofilcon A	Silicon hydrogel	I	2
	Zeiss Contact Day 30 compatic	Vitafilcon A	Sulfobetain and amino acids	9	2
			Total	19	7

Biomaterials Characterization – Soft Contact Lenses

❖ Detection of protein deposits on spoiled contact lenses

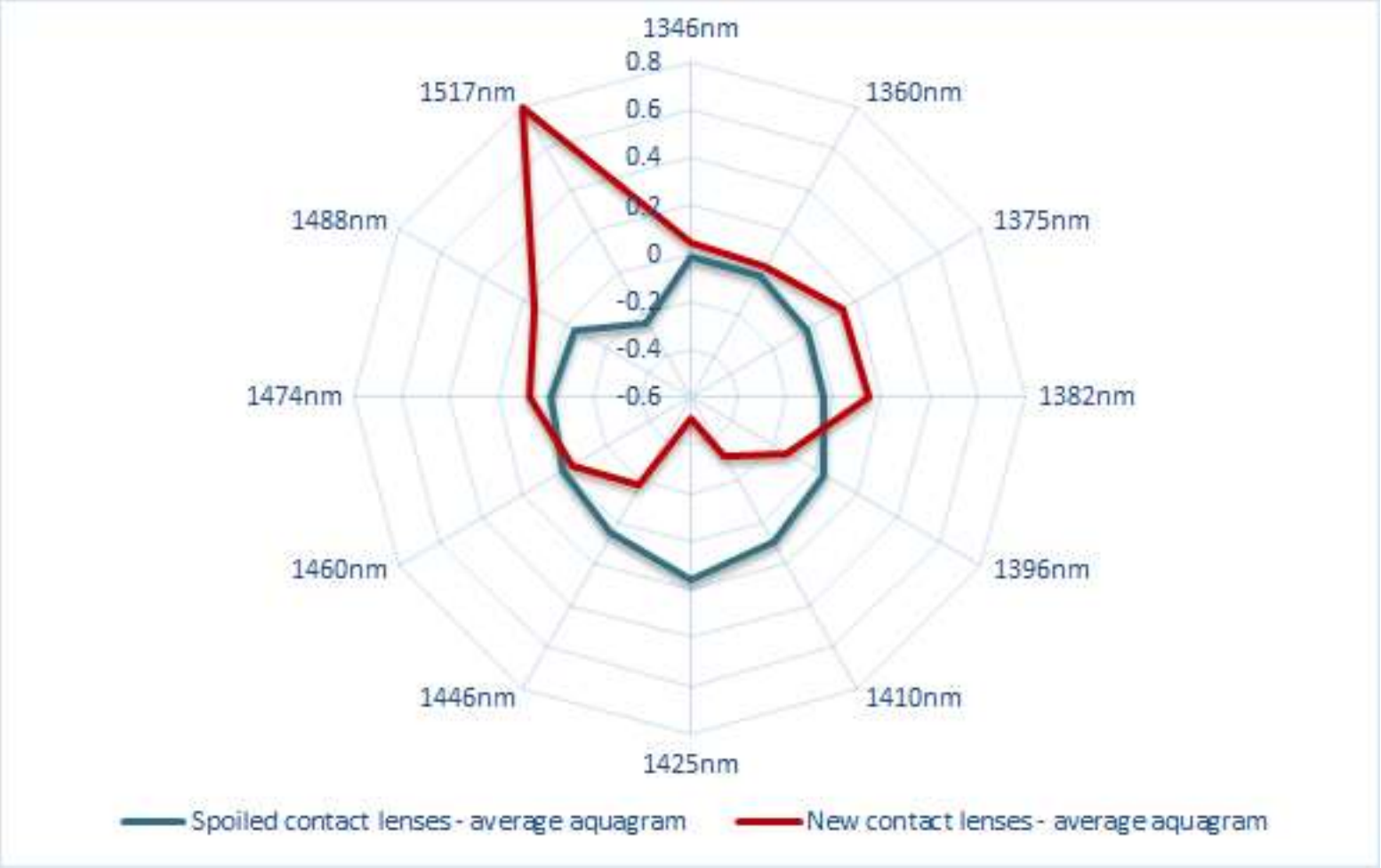


Water-mirror



Biomaterials Characterization – Soft Contact Lenses

❖ Detection of protein deposits on spoiled contact lenses



Biomaterials Characterization – Soft Contact Lenses

- Better understanding of water states in soft contact lenses and better understanding of the process of spoliation
- Non-invasive rapid characterization in conditions similar to biological (hydrated)
- **Future directions:**

  Better understanding of protein adsorption: time resolved spectroscopy

Šakota Rosić, J., Munćan, J., Mileusnić, I., Kosić, B., & Matija, L. (2016). Detection of protein deposits using NIR spectroscopy. *Soft Materials*, *14*(4), 264-271.

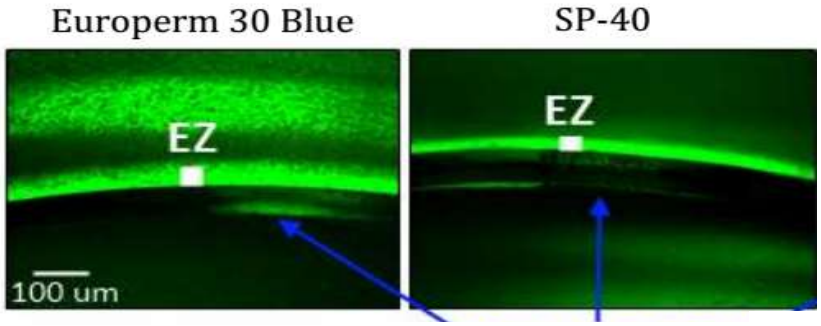
Munćan, J., Mileusnić, I., Šakota Rosić, J., Vasić-Milovanović, A., & Matija, L. (2016). Water Properties of Soft Contact Lenses: A Comparative Near-Infrared Study of Two Hydrogel Materials. *International Journal of Polymer Science*, *2016*.

Biomaterials Characterization – Soft Contact Lenses

❖ Future directions:

Utilization of water properties: water exclusion zone

Better material designs

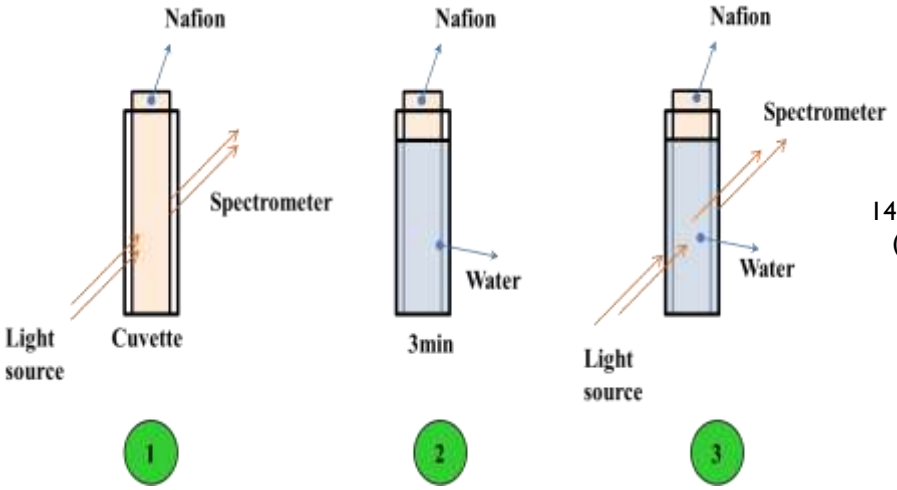


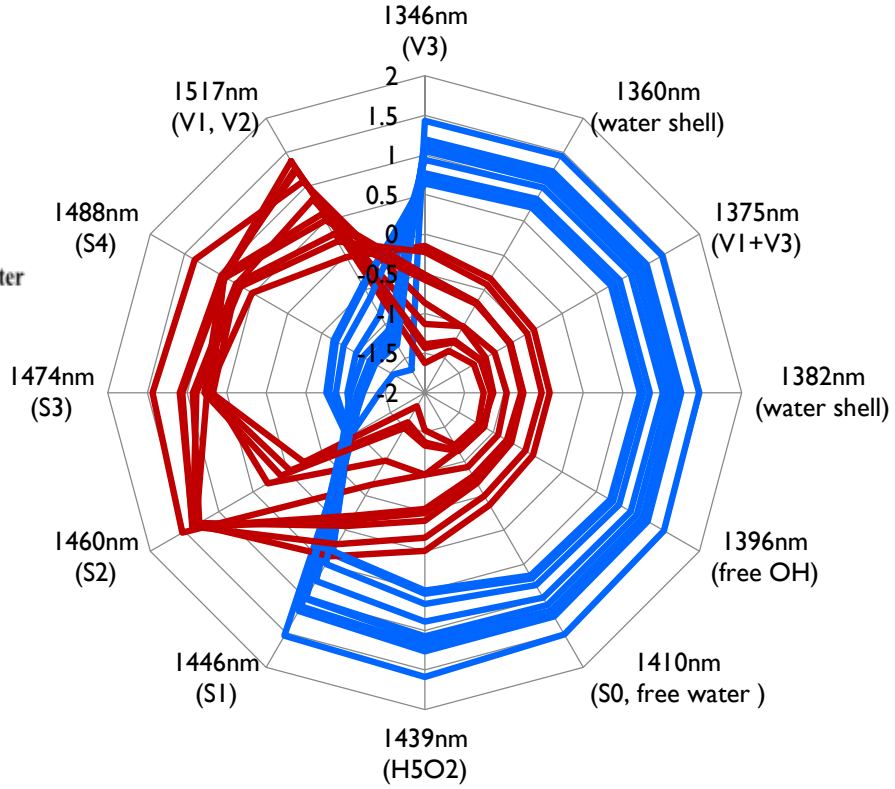
Contact lenses

Z.Golubovic, Investigation of interaction of deionized water with hydrophilic and hydrophobic materials, biomolecules, and hydrogenized carbon nanomaterials, PhD thesis, University of Belgrade, 2012

Biomaterials Characterization – Soft Contact Lenses

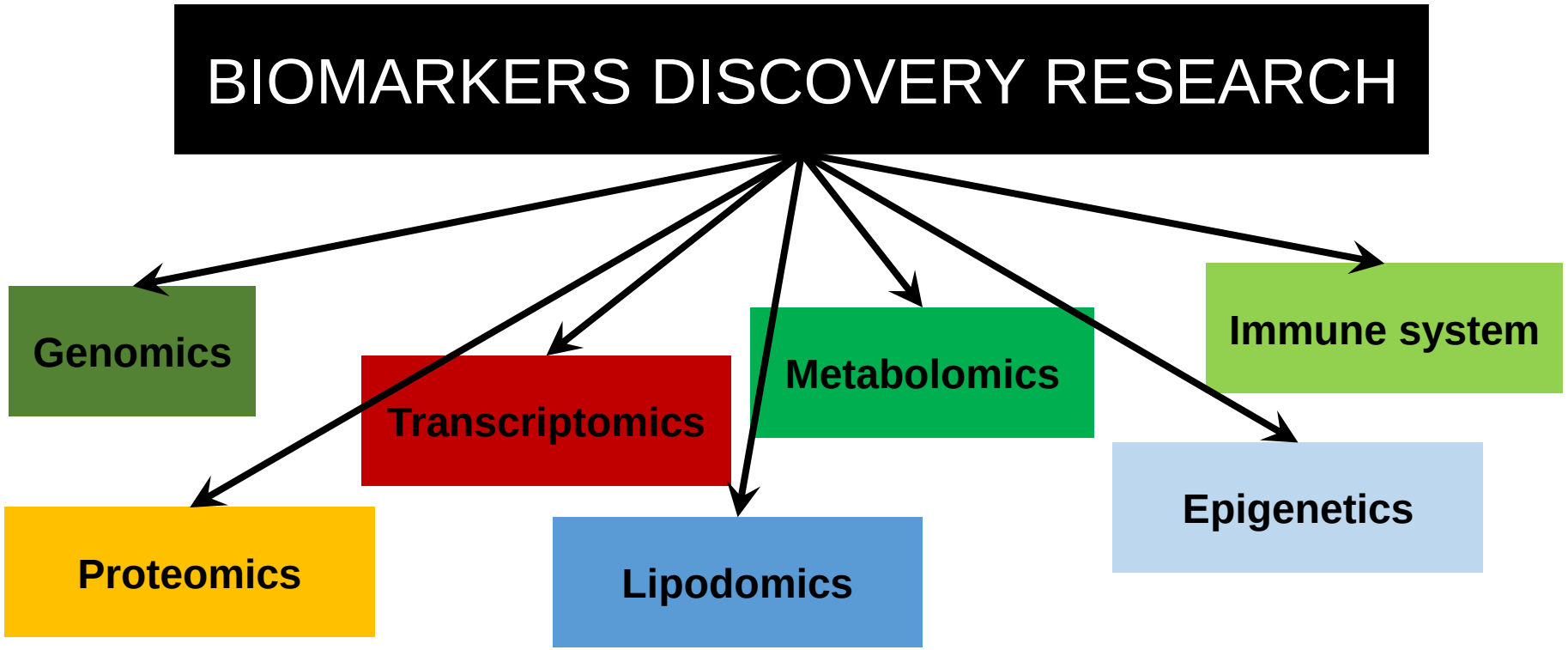
- Near Infrared Spectroscopic Study of Water in Contact with a Hydrophilic Polymer Nafion (EZ water) - unpublished





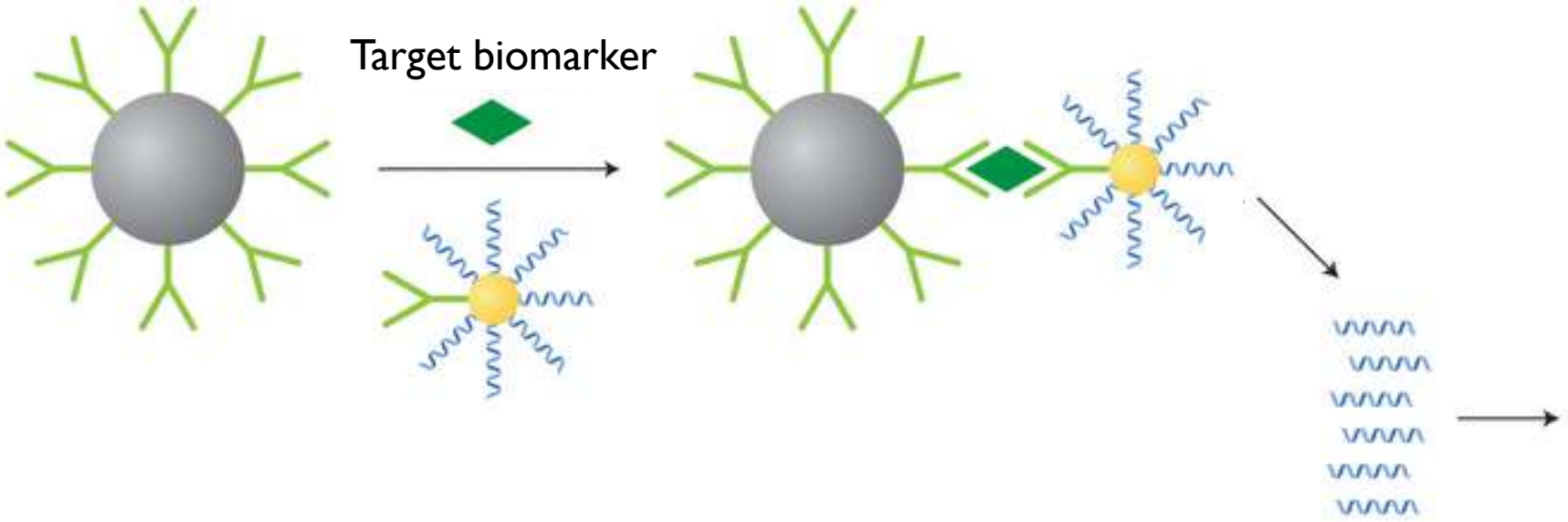
Current methodology in medicine: **REDUCTIONISM**

Disease diagnostics: **BIOMARKERS** (single)



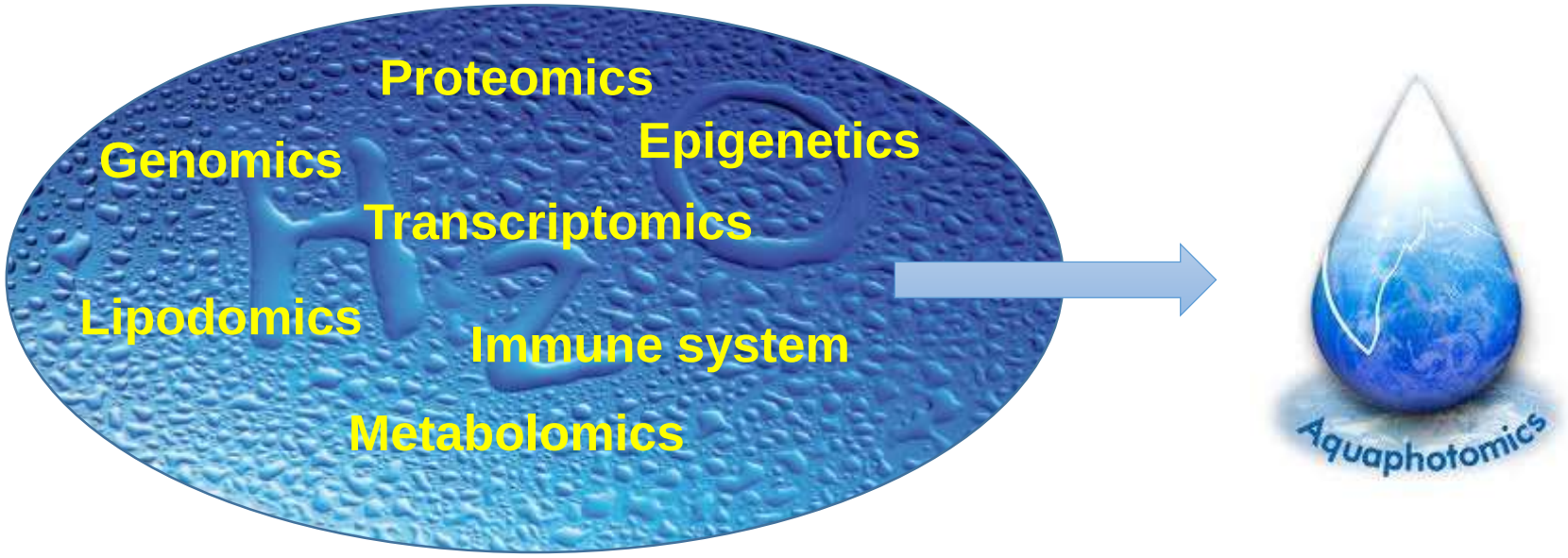
Current methodology in medicine: **REDUCTIONISM**

Disease diagnostics: **BIOMARKERS** (single, low concentration)

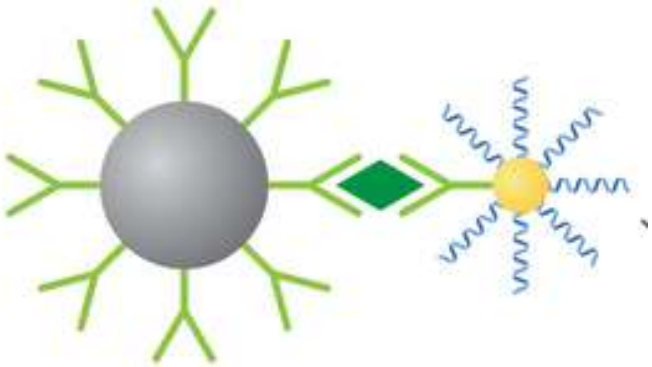


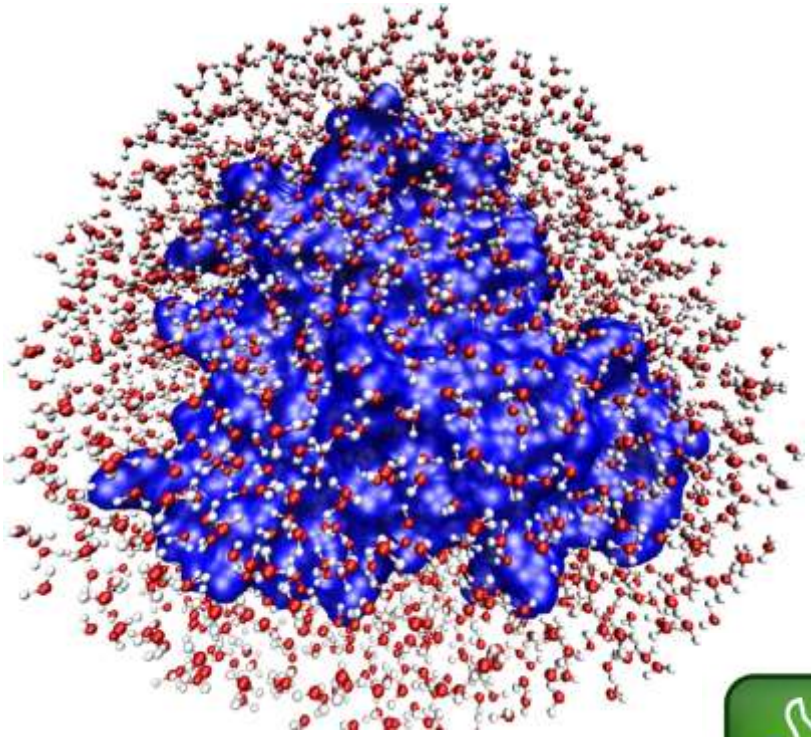
Current trends: nanotechnology, signal amplification

Future methodology in medicine: **Measure cumulative effect**



Future methodology in medicine: **Use natural amplification**





- **Ongoing studies:**

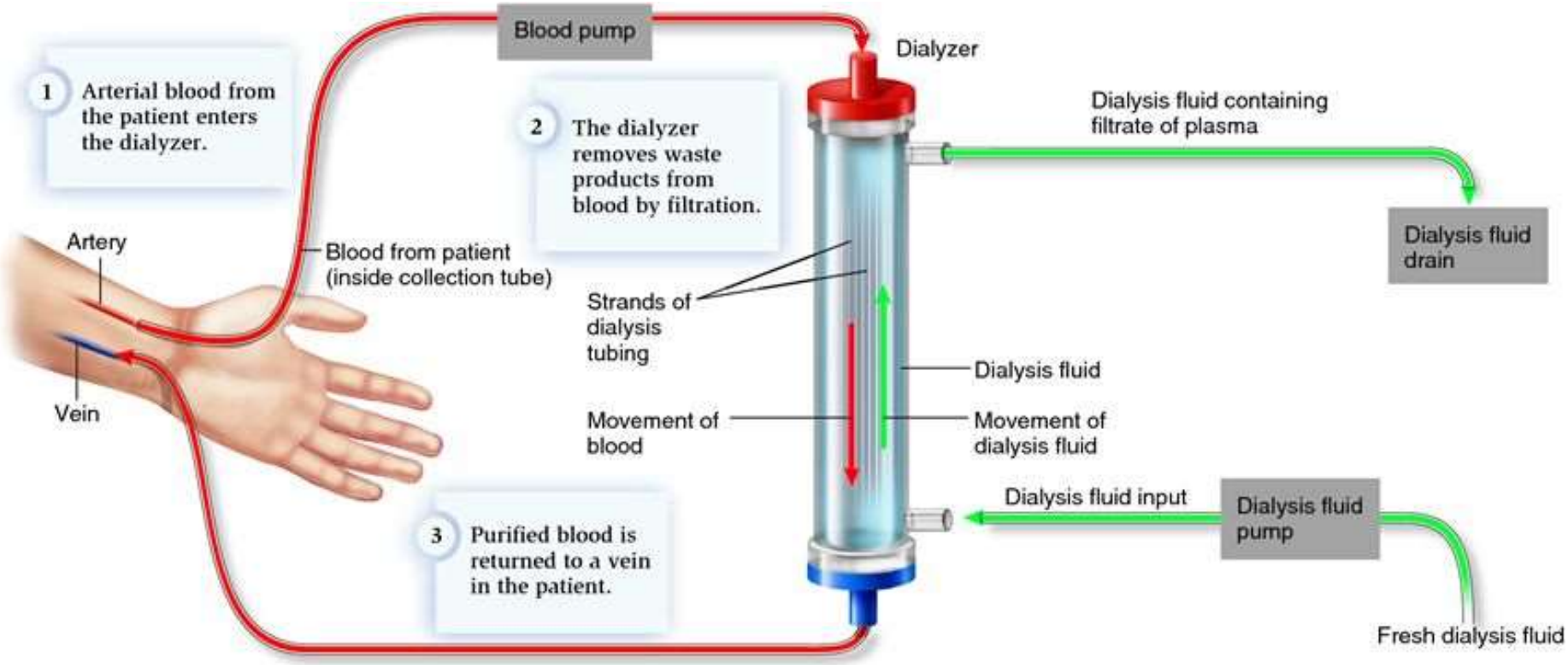
  Optical Monitoring of Spent Dialysate

  Early screening of colorectal cancer

- **Objective:** Translate the results to human medicine

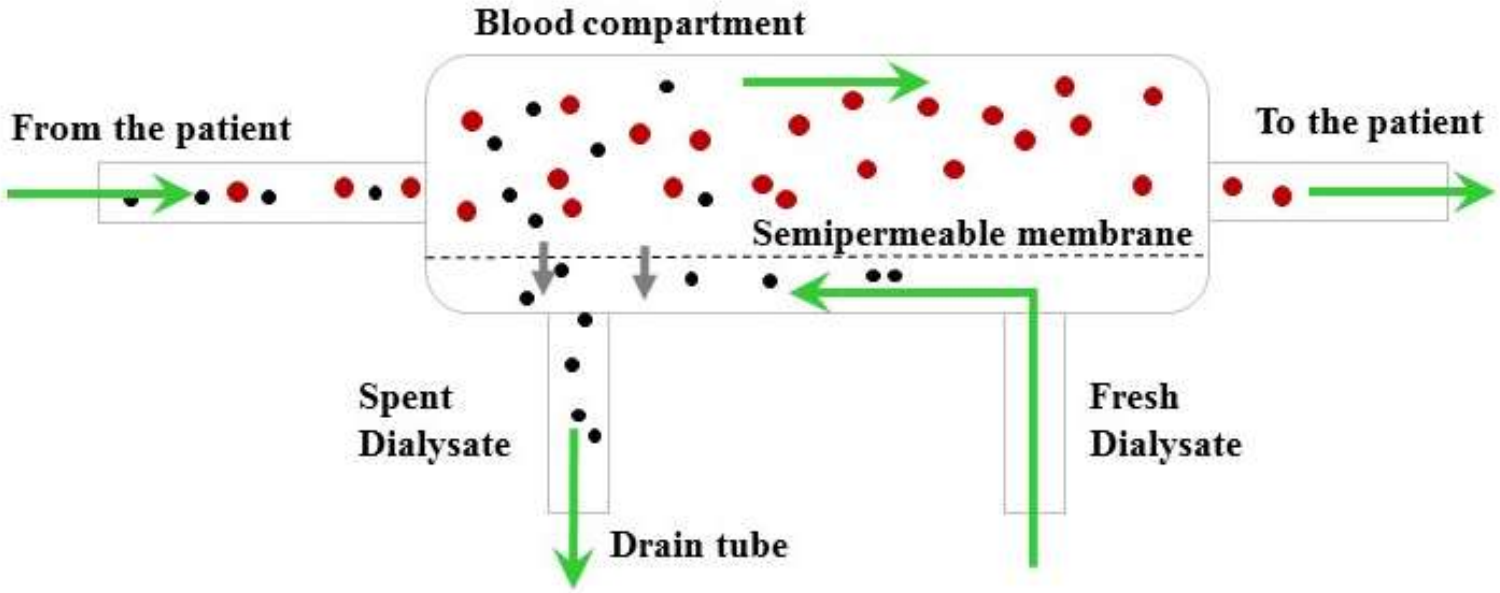
Optical Monitoring of Spent Dialysate

❖ Dialysis is a process of blood filtration



http://siamhealth.net/public_html/Disease/renal/hemodialysis.html

Optical Monitoring of Spent Dialysate



NIRS & Aquaphotomics

- Current methodology: blood sampling, UV spectrophotometry
- Problems: monitoring small concentrations of UV absorbing solutes
- Consequences: poor efficacy, inflexible therapy, undertreatment & overtreatment

Optical Monitoring of Spent Dialysate

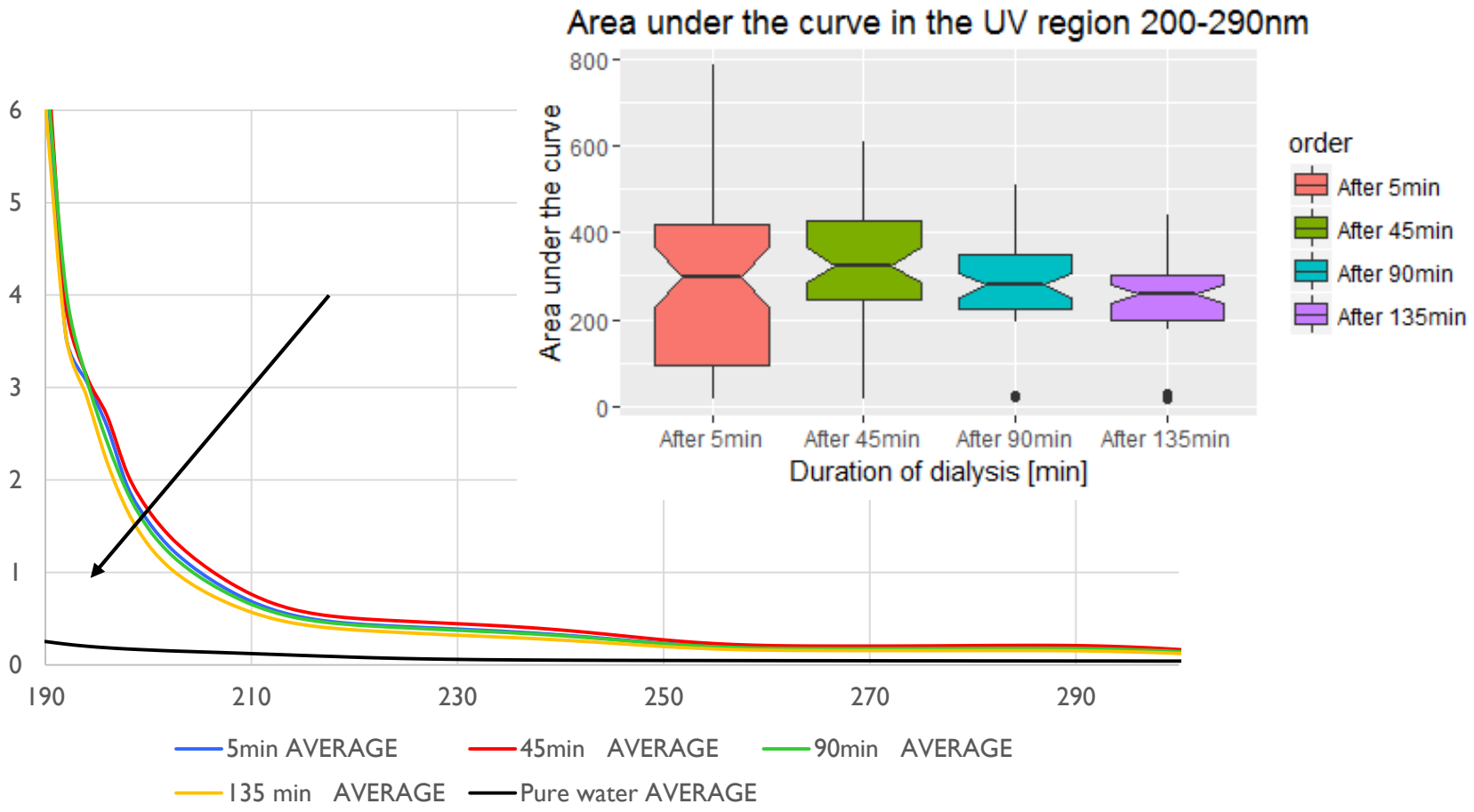
- The samples of spent dialysate were taken from the drain tube of the dialysis machine after 5, 45, 90 and 135min of each dialysis session.
- The samples were sent to the laboratory and the spectral acquisition was performed using double beam spectrophotometer Lambda 950 (Perkin Elmer Italy), in the range 190nm-1700nm with the resolution of 2nm.
- A quartz liquid sample cell (1 mm path length).
- For all 18 dialysis sessions, 4 samples were collected at planned times (50ml) and recorded three times giving in total 216 spectra.

Table 1. Dialyzers data		HdF100s	F70s	170H
Clearances [ml/min] Qd=500ml/min	Urea	271-354	190-245	196-321
	Creatinins	252-329	177-220	186-281
	Phosphates	240-315	174-216	180-266
	Vitamin B12	190-258	127-145	137-178
Blood flow [ml/min]		300-600	200-500	250-500
Dialysate flow [ml/min]		1000	1000	800
Effective surface [m²]		2.3	1.6	1.7

1p 6p 3p

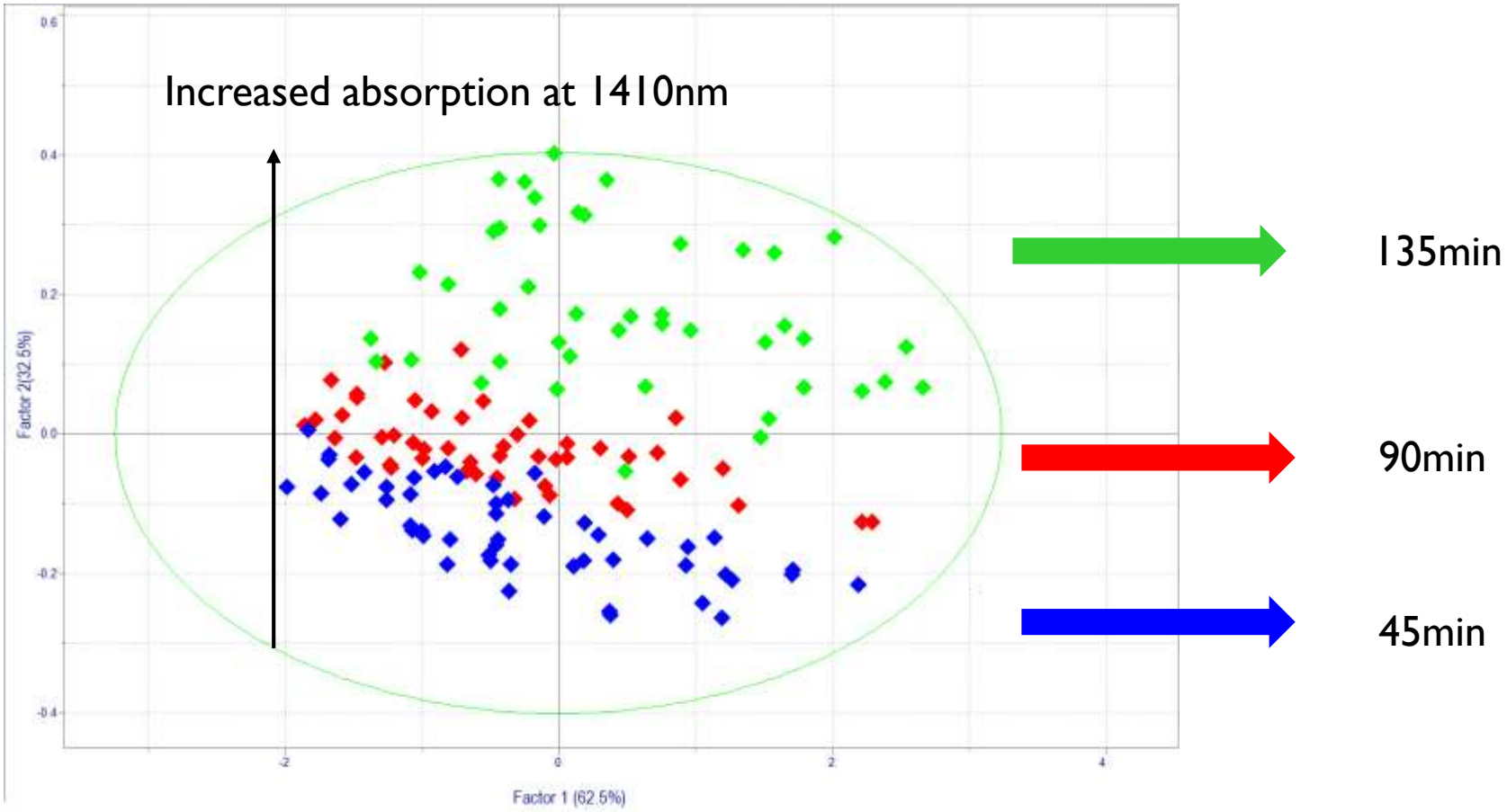
Optical Monitoring of Spent Dialysate

❖ UV region: confirmation of filtration process



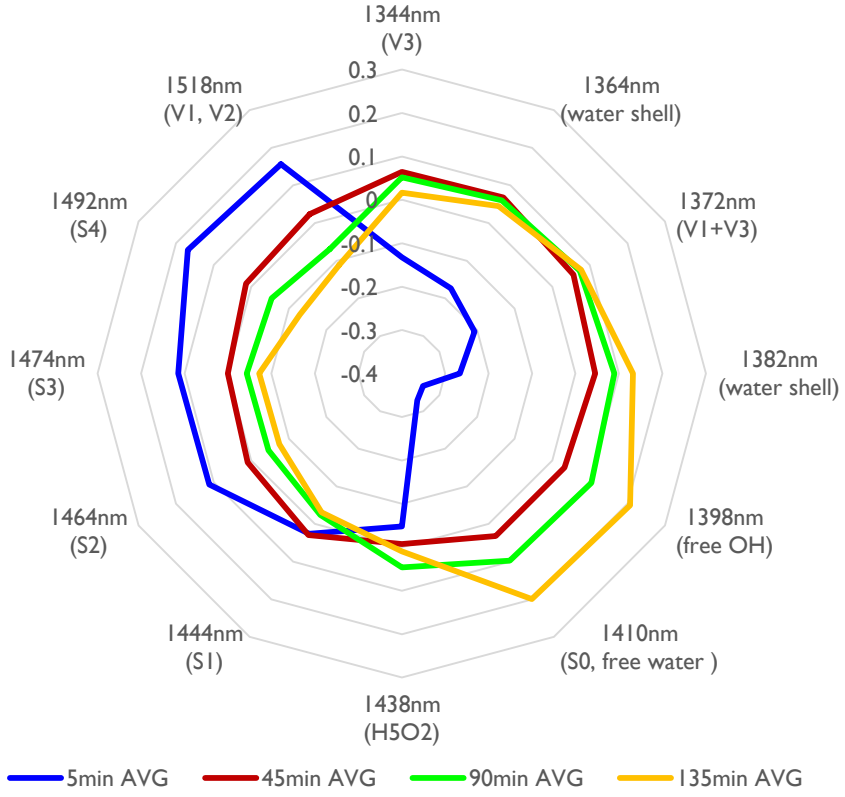
Optical Monitoring of Spent Dialysate

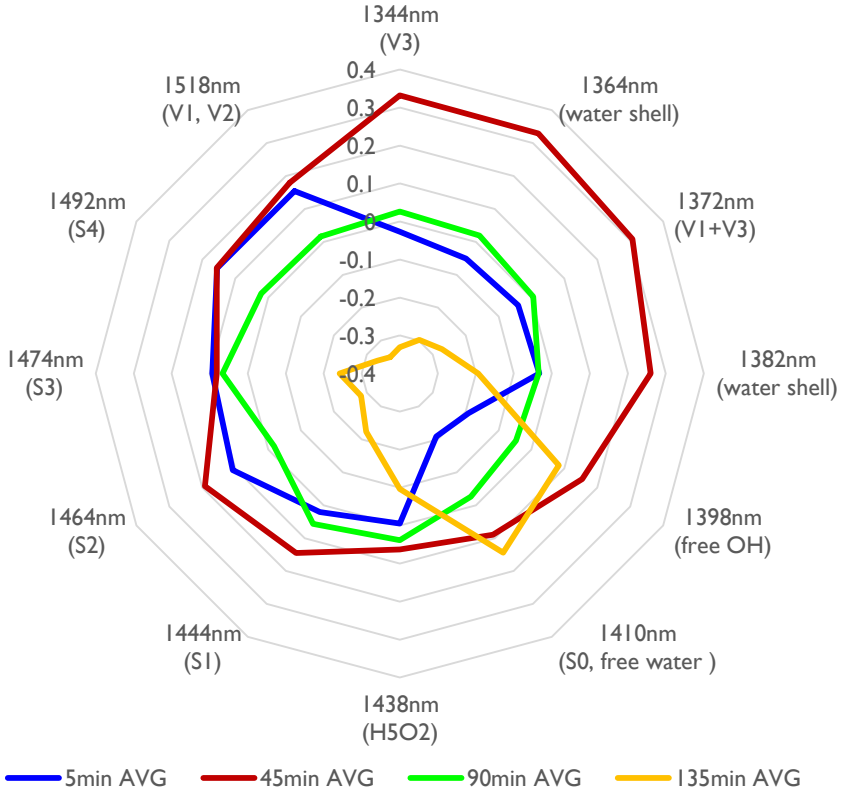
- NIR region: confirmation of filtration process – PCA analysis



Optical Monitoring of Spent Dialysate

❖ NIR region: confirmation of filtration process





Optical Monitoring of Spent Dialysate

- NIR region: proof of concept for the 1300nm-1600nm

- Future directions:

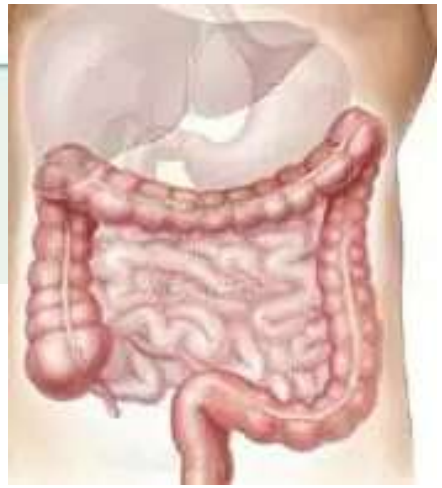
1) Increase the number of time points – time resolved spectroscopy
2) Tests for urea, creatinine, potassium, phosphate, glucose in spent dialysate – correlation

Early screening of colorectal cancer

- Third most common cancer in the world
- 1.4 million cases diagnosed (2012) → 2.4 million cases in 2035
- 95% of all colorectal cancers are adenocarcinomas
- Most people with early colon cancer don't have symptoms

Reduce your risk of colorectal cancer.

1. Get screened regularly.



- Current state of the art: FOCB and FIT
- Samples of stool are checked for blood (…only 5%)
- Sigmoidoscopy, Colonoscopy, Double contrast barium enema: CT colonography

Early screening of colorectal cancer

- Aquaphotomics → noninvasive or minimally invasive screening test
- 70 patients: 33 with benign colon lesions and 37 with confirmed malignant lesions (adenocarcinoma) after the surgery
- The samples of urine and serum acquired on the morning before the surgery

- Methods:
- FTIR, Frontier 400, Perkin Elmer, ATR mode, resolution 4cm^{-1}, 650-4000cm^{-1}
- Vis NIR, Lambda 450, Perkin Elmer, transmittance mode, resolution 1nm, 400-1700nm

Early screening of colorectal cancer

❖ Data analysis:

Pretreatment: Smoothing (…pt), SNV, 1st Derivative, 2nd Derivative

Classification: PLS-DA

Orthogonal signal correction

❖ Accuracy of classification: confusion matrix

$$\text{Specificity} = \frac{TN}{TN+FP} \qquad \text{Sensitivity} = \frac{TP}{TP+FN}$$

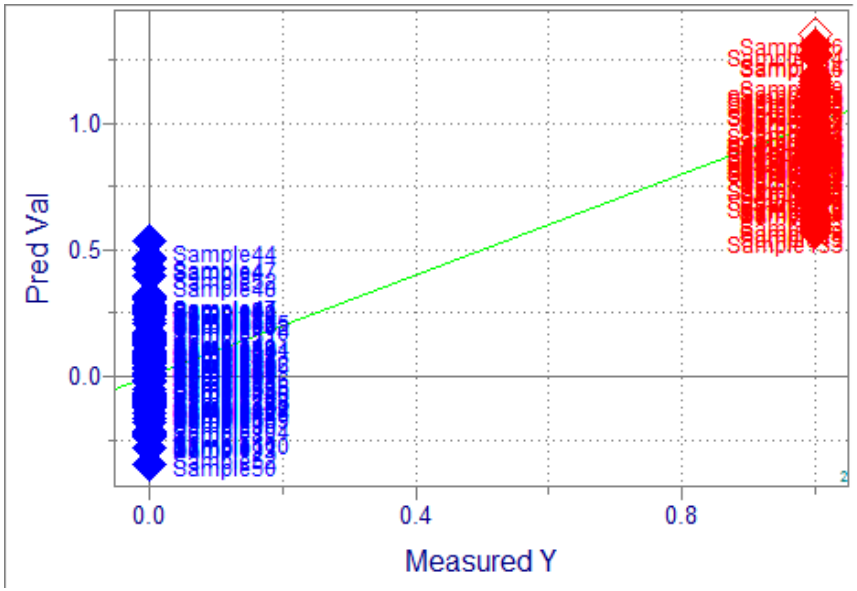
$$\text{Accuracy: } \frac{TP+TN}{TP+TN+FP+FN}$$

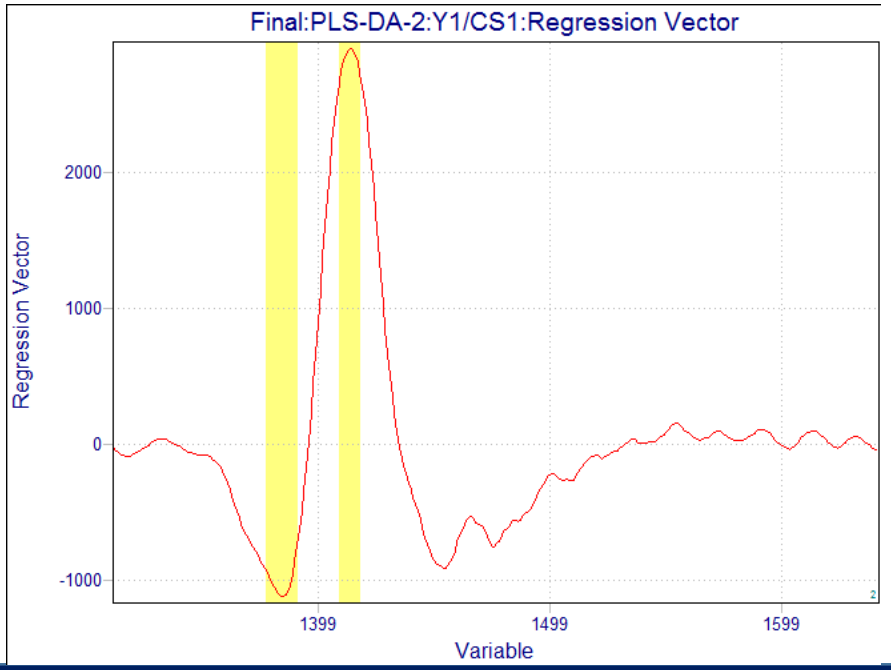
TP, TN, FP and FN denote numbers of true positives, true negatives, false positives and false negatives

Early screening of colorectal cancer

❖ Serum

- ❖ Partial least squares discriminant analysis (PLS-DA) using the 1300–1600 nm interval of NIR spectra with mean-centering, smoothing (21 pts), 2nd derivative (25pts), orthogonal signal correction (with one component), and leave-3-out cross-validation; 86.63% of samples were classified correctly
- ❖ Regression vector of PLS-DA revealed strong peaks at C4: 1380–1388 nm and C5:1398–1418 nm.



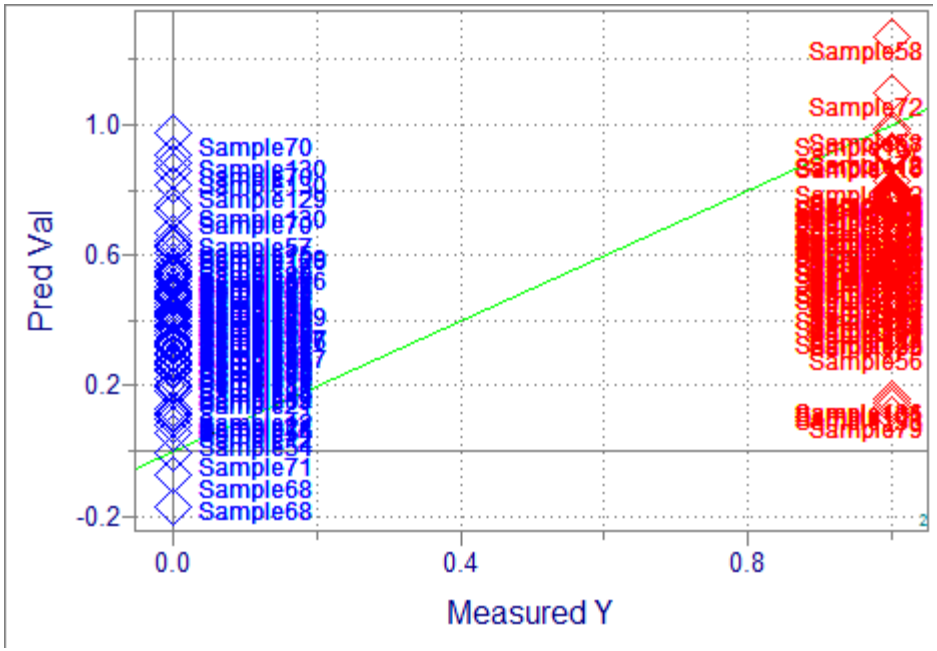


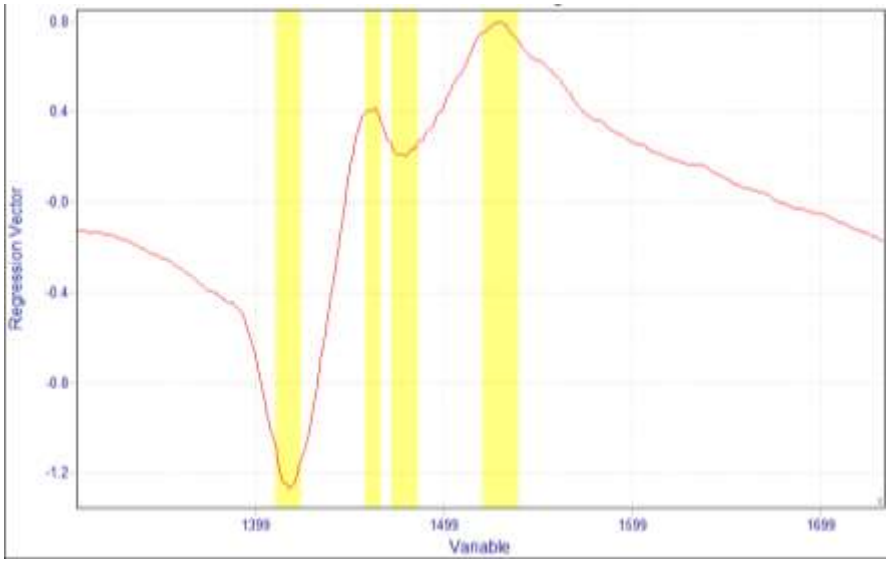
Early screening of colorectal cancer

❖ Urine

- ❖ Partial least squares discriminant analysis (PLS-DA) using the 1300–1600 nm interval of NIR spectra with mean-centering, smoothing (35 pts), SNV, orthogonal signal correction (with one component), and leave-3-out cross-validation; 70.44% of samples were classified correctly

- ❖ Regression vector of PLS-DA revealed peaks at C5:1398–1418 nm, C8:1448-1454nm, C10: 1472-1482nm,C12: 1506-1516nm





Early screening of colorectal cancer

- Serum — Specificity = 80.77%, Sensitivity = 91.49%, Accuracy = 86.63%
- Urine — Specificity = 73.17%, Sensitivity = 67.53%, Accuracy = 70.44%

- More than 30 biomarkers in blood/serum: Shah, Reena, et al. "Biomarkers for early detection of colorectal cancer and polyps: systematic review." *Cancer Epidemiology Biomarkers & Prevention*(2014): cebp-0412.
- Almost 30 biomarkers in urine: Nguyen, Minhhuyen T., and David S. Weinberg. "Biomarkers in Colorectal Cancer Screening." *Journal of the National Comprehensive Cancer Network*14.8 (2016): 1033-1040.

- Future directions….explaining why…
- Building a cancer database

And for the conclusion….

Aquaphotomics for smart diagnostics…

Thank you very much for your attention ☺

Acknowledgments:
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Authors are grateful to the patients and the staff of the Department of Nephrology, Clinical Centre Zemun, and First Surgical Clinic, Clinical Centre Belgrade, Serbia for their help and collaboration.

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