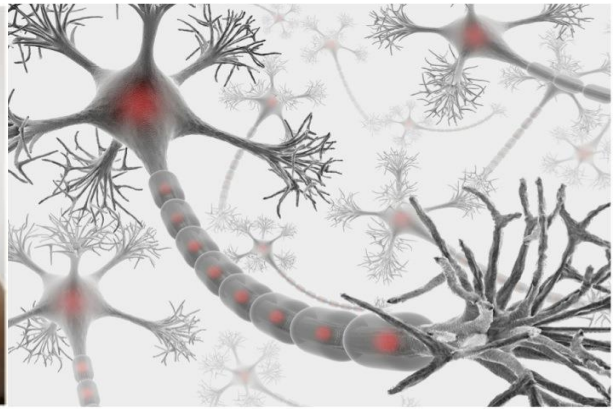
Molecular Imaging of the retina by Raman spectroscopy for retina and brain diseases





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Outline

- Molecular imaging of the Central Nervous System (CNS)
- Laser based molecular imaging: Raman Spectroscopy
- Preclinical development
- Prototype development
- Clinical validation
- Next steps and conclusions

Molecular imaging for developing biomarkers for neurological and retina diseases

- The CNS is very difficult to access in vivo ➔ imaging technologies offers the best opportunities to interrogate the brain
- Identifying biomarkers of disease progression and response to therapy is critical in order to develop new therapies for brain diseases
- Molecular imaging as a big promise: accessing the brain functioning by imaging + providing molecular details
  - MRS: few metabolites and requires big voxels
  - PET: few radioligands, complex development, expensive
  - NIR: limited to cortex surface
- The retina allows direct analysis by laser technologies and reflects many changes common to CNS damage
  - Optic Neuropathies: MS, AION, Glaucoma, other
  - Neurodgenerative diseases: AD, PD, FTD, Heredoataxias, ALS, etc
  - CVD

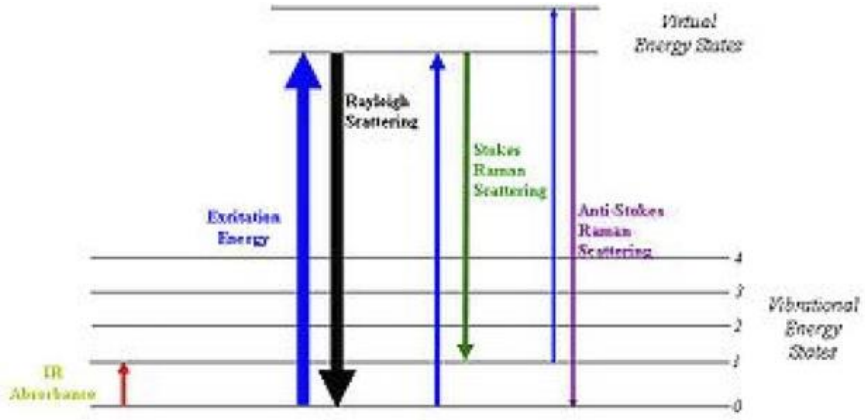
Laser based molecular imaging: Raman Spectroscopy (RS)

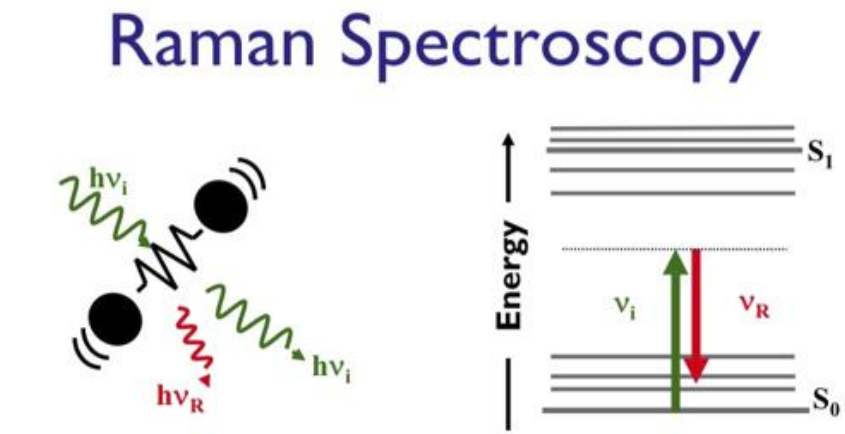
Advantages

- RS is present in almost all molecules ➔ chemical library of the retina
- RS profile is based in the chemical structure (bonds)
- RS use near-infrared laser (safe for human use)
- Confocal imaging allows defining RS from a neuronal layer (e.g. RGC)
- Linear relationship between RS signal and molecular levels

Limitations

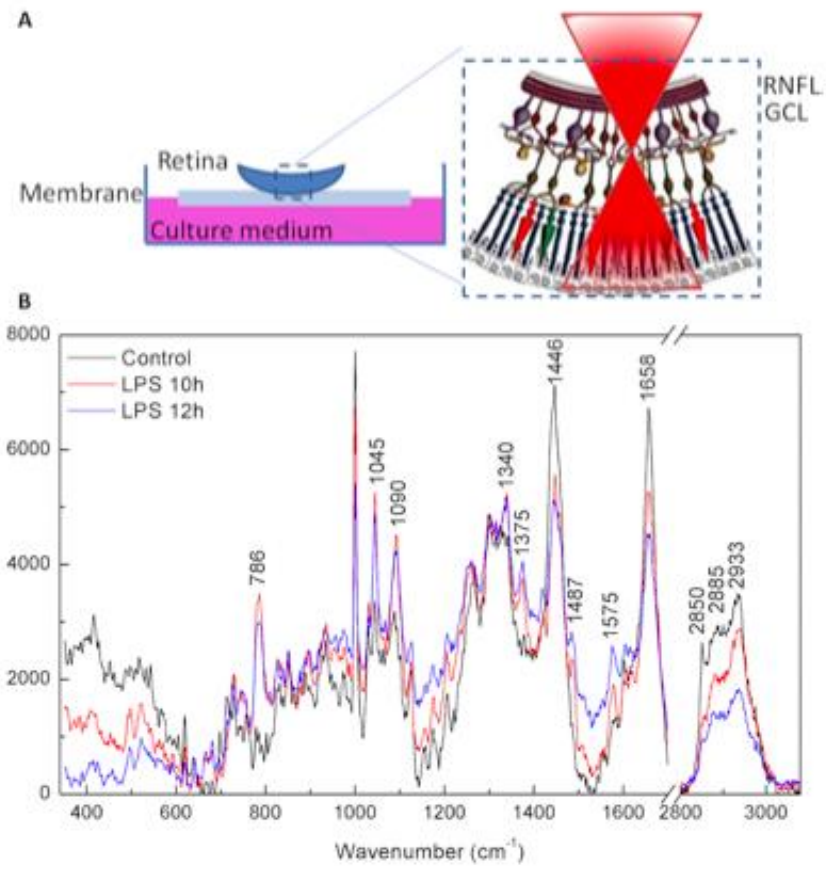
- low energy / low signal to noise ration (requires sensitive methods and long acquisition time)
- Macromolecules (proteins) produce low and complex signal





Preclinical studies: Raman spectroscopy of retina cultures

- Raman spectroscopy from retina tissue was tested in mice retina cultures and imaged with the Raman Invia microscope with confocal point in the Retinal Ganglion Cell (RGC) layer
- Inflammation was induced in retina cultures with Lypopolysaccharide (LPS)
- Spectra from healthy and inflamed retina were analyzed using bioinformatic tools, RS database and custom retina RS library



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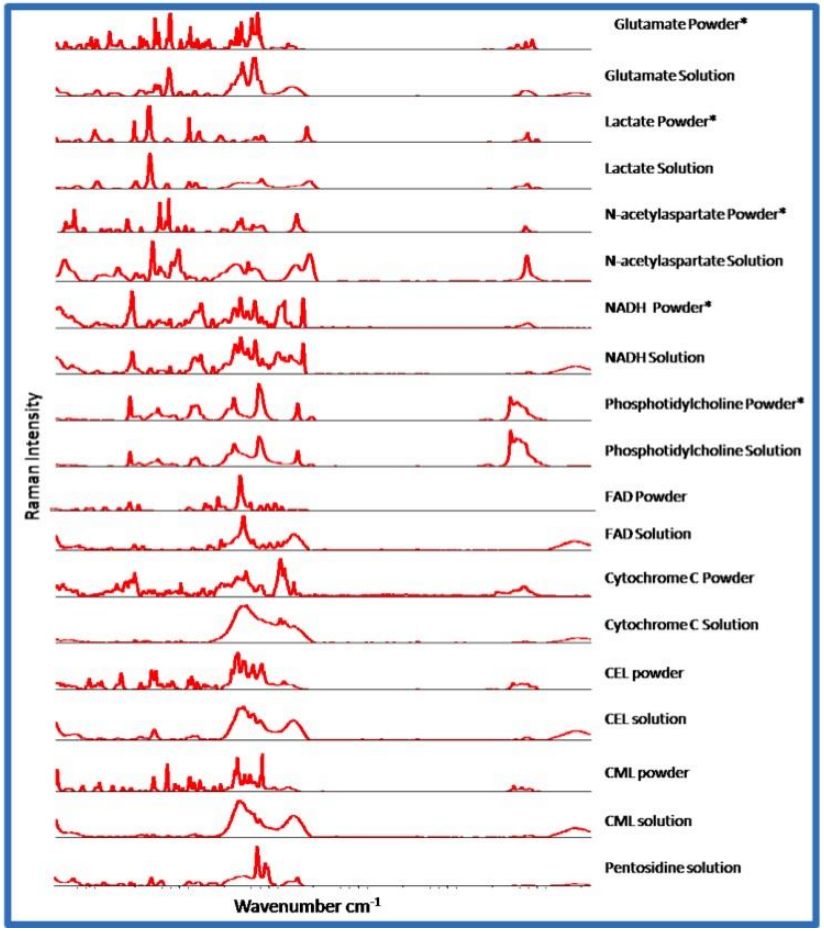
Custom RS library of candidate molecules

- Excitotoxicity: L-Glutamic Acid
- Axon: N-Acetyl-L-Aspartic Acid
- Mitochondria: Cytochrome C, L-(+)-Lactic Acid, NADH, FAD
- Lipids: L-α-Phosphotidylcholine
- Advance Glycosilation End-product (AGE): N-carboxymethyl lysine (CML), N-carboxyethyl lysine (CEL), Pentosidine



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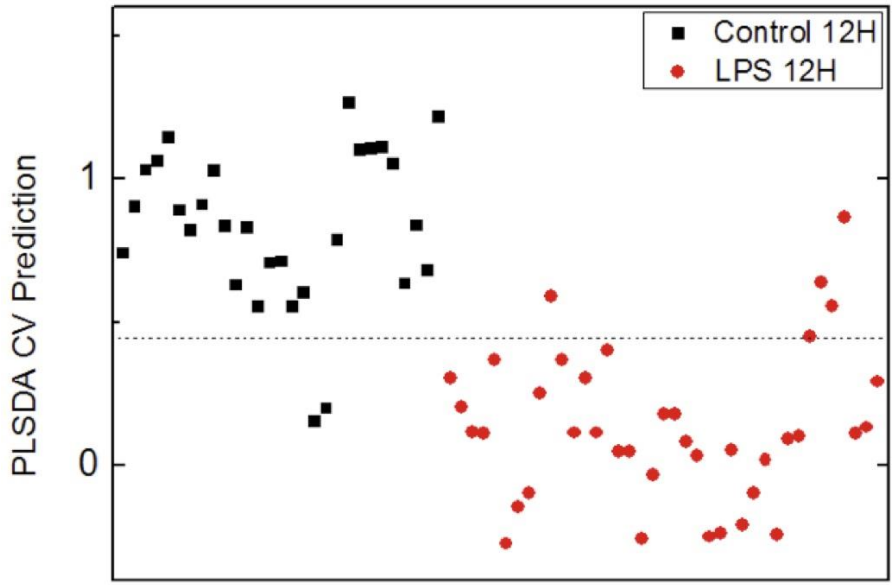
RS discriminate between healthy and inflamed retina and identify dynamic changes in key molecular process

- Raman spectroscopy discriminate between healthy and inflamed retina using bioinformatic tools (PLS-DA)
- Dynamic changes of RS combined with RS databases revealed molecular process triggered by inflammation

⑩ ↑ Pro-inflammatory mediators: Lipo-oxigenease, iNOS, TNFa

⑩ ↓ energy-metabolism: Cytochrome C, Phenylalanine, NAD/NADH

⑩ ↓ membranes and second messenger: Phosphatidyl-choline



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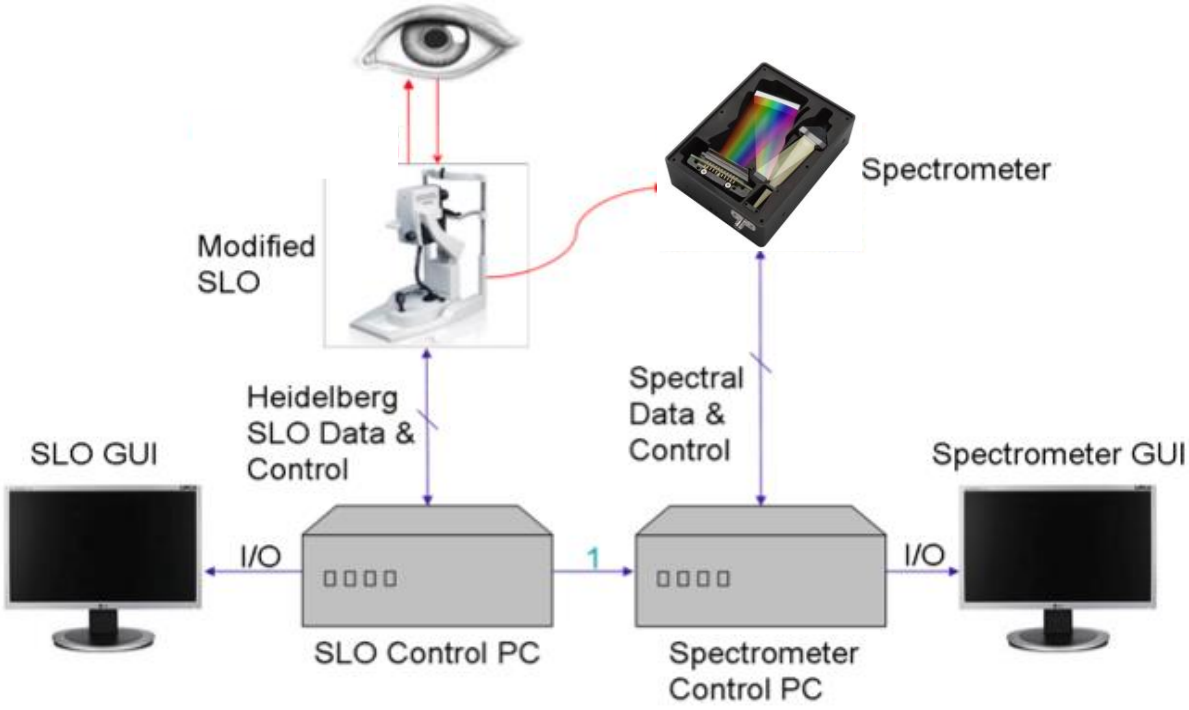
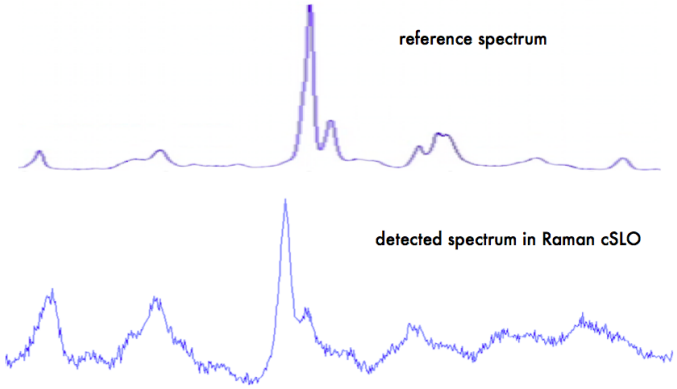
Raman Spectroscopy prototype for human use

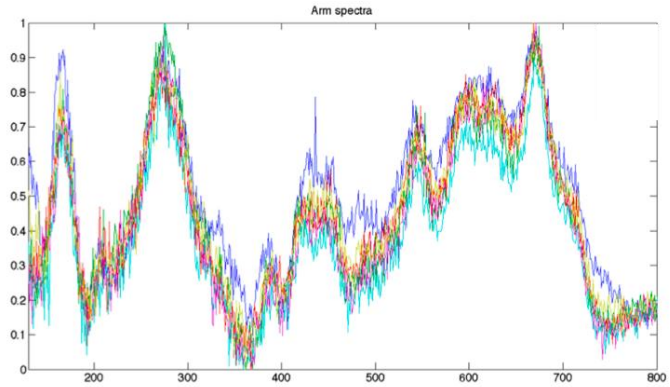
- A Raman spectrophotometer has been built on top a Scanning Laser Ophtalmoscope (SLO) from Heidelberg Engineering
- The device has been approved for human use by the Spanish Drug Agency (October 2014)



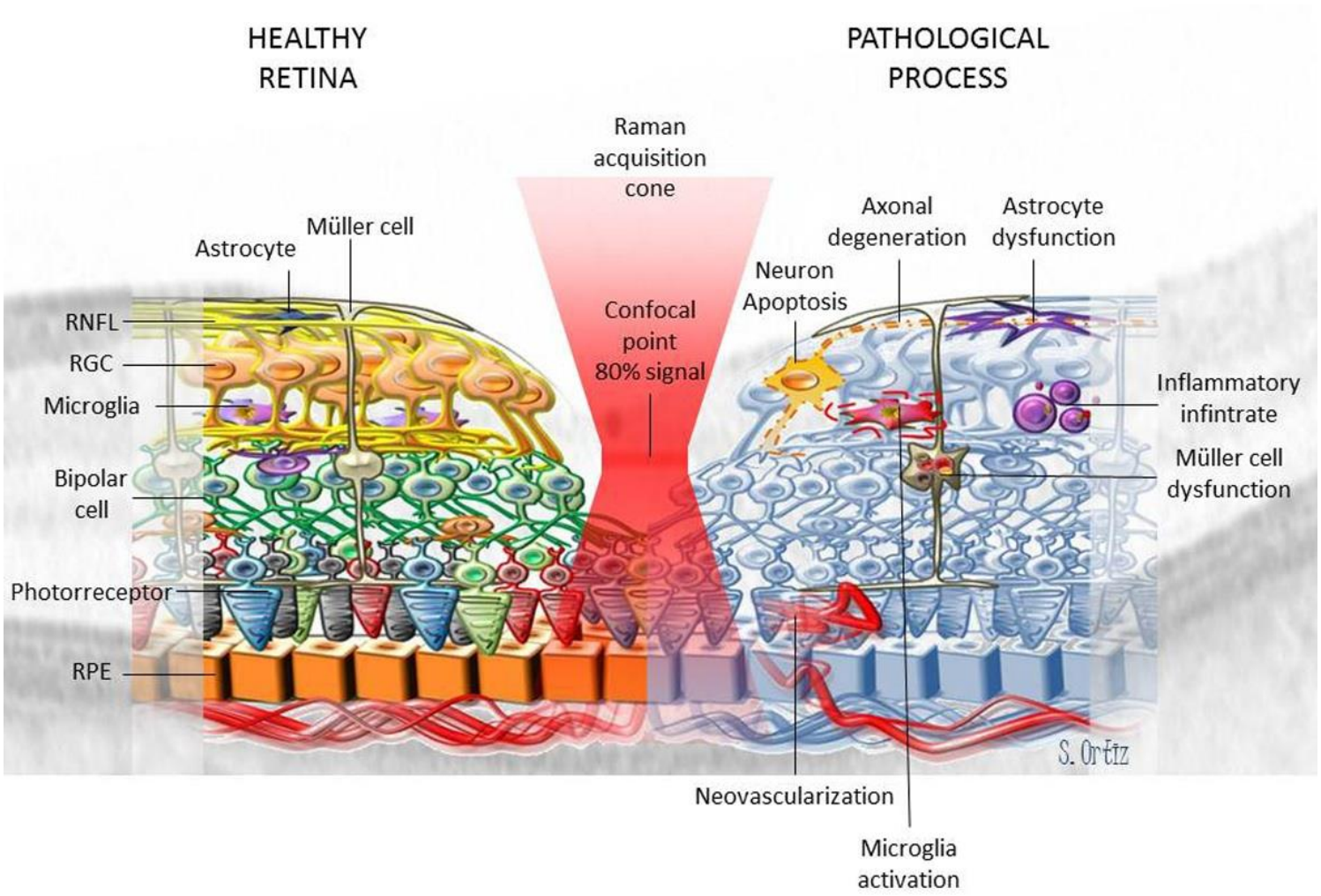
Molecular imaging of the retina by Raman spectroscopy

- The Raman spectrophotometer prototype has been optimized for obtaining a high-quality signal
  - Detection of reference compound (e.g. Ethanol)
  - 99% reproducibility intra-individual and >90% reproducibility between subjects





Molecular imaging of retina diseases: biological changes



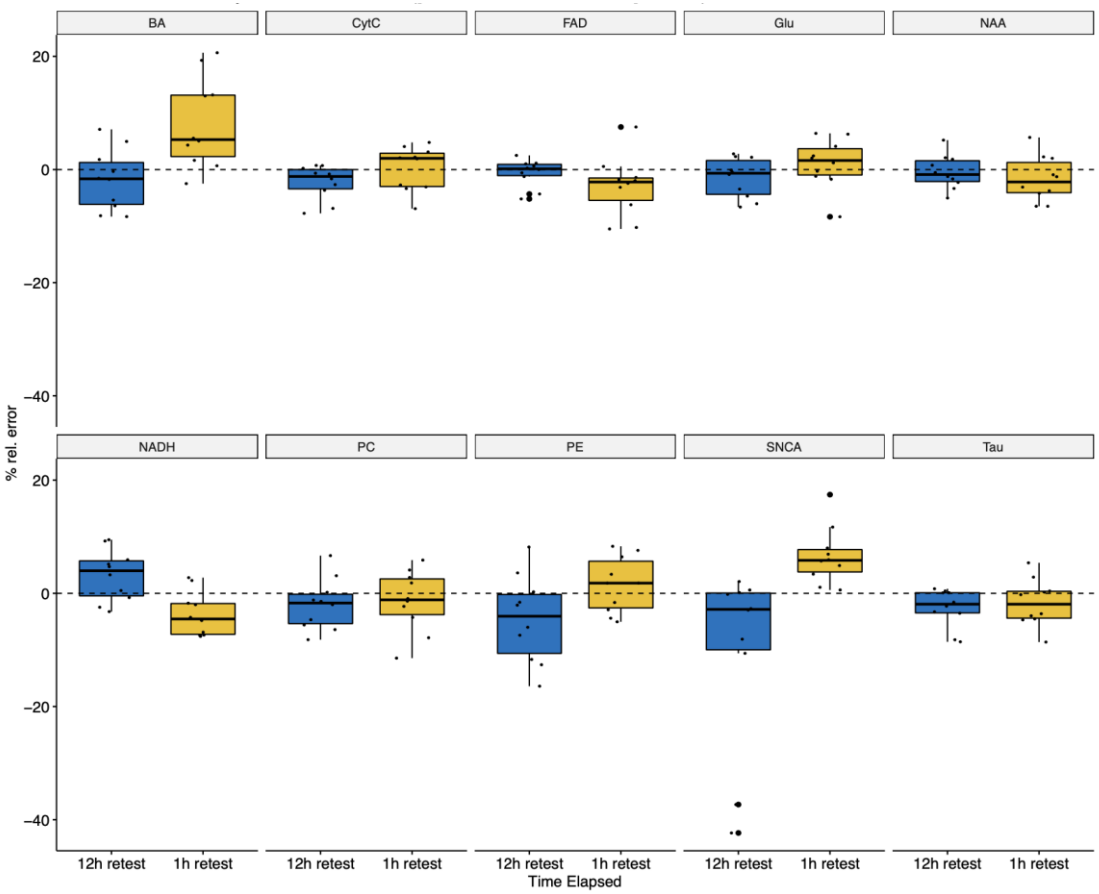
Artwork: Santiago Ortiz-Perez

Metabolites (candidate approach)

- N-Acetyl-Aspartate (NAA): axonal integrity
- Nicotinamide adenine dinucleotide (NADH): axonal energy
- Flavin adenine dinucleotide (FAD): axonal energy
- Cytohrome c (CytC): apoptosis
- Glutamate+Glutamic acid (Glu): excitotoxicity
- Tau: neurodegeneration and axonal biology
- Alpha-Synuclein (SNCA): neurodegeneration and synapsis biology
- Beta-Amyloid (BA): neurodegeneration
- Phosphatidil-Choline (PC): second messenger, membrane integrity, myelination
- Phosphatidil-Ethanolamine (PE): second messenger, membrane integrity

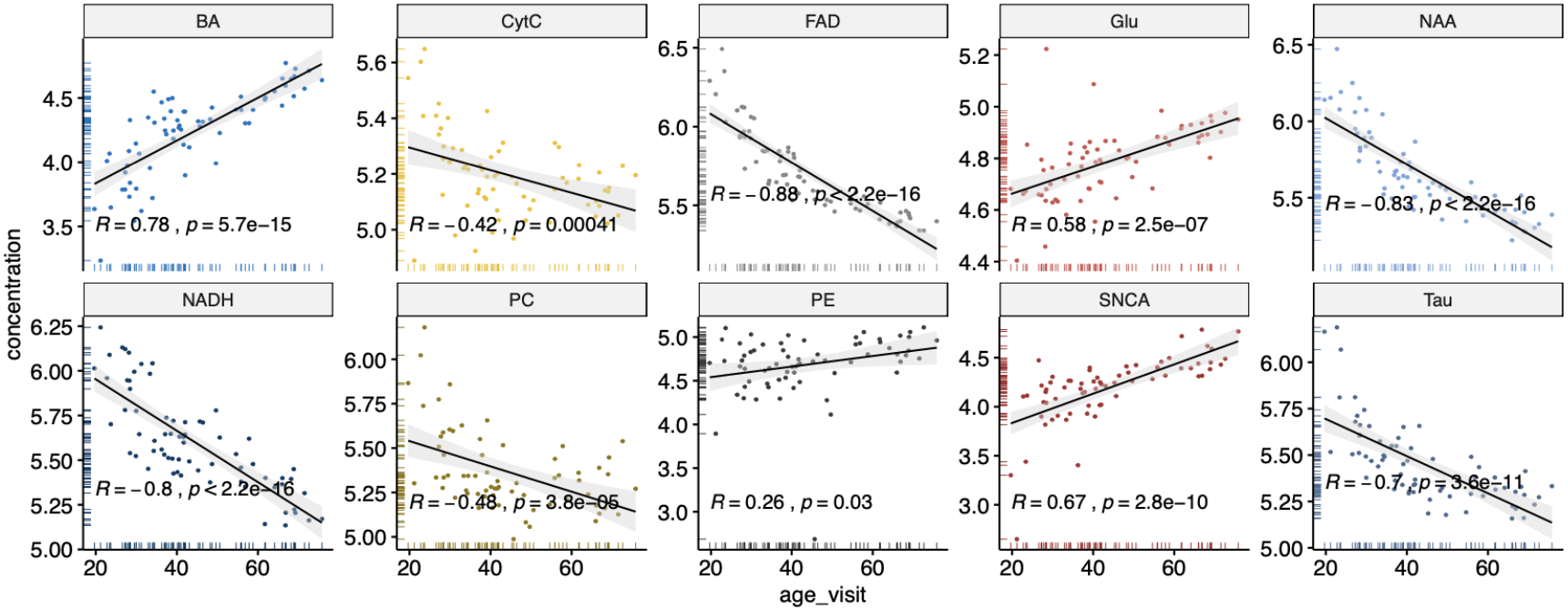
Results: test-retest 10 metabolites by RS

- rescanned a subgroup of 6 HCs and 5 MS cases at 1 and 12h
- low error in the 1h retest: less than 5% (except for Abeta and SNCA)
- low error in the 12h retest: less than 5% (except PE and SNCA)

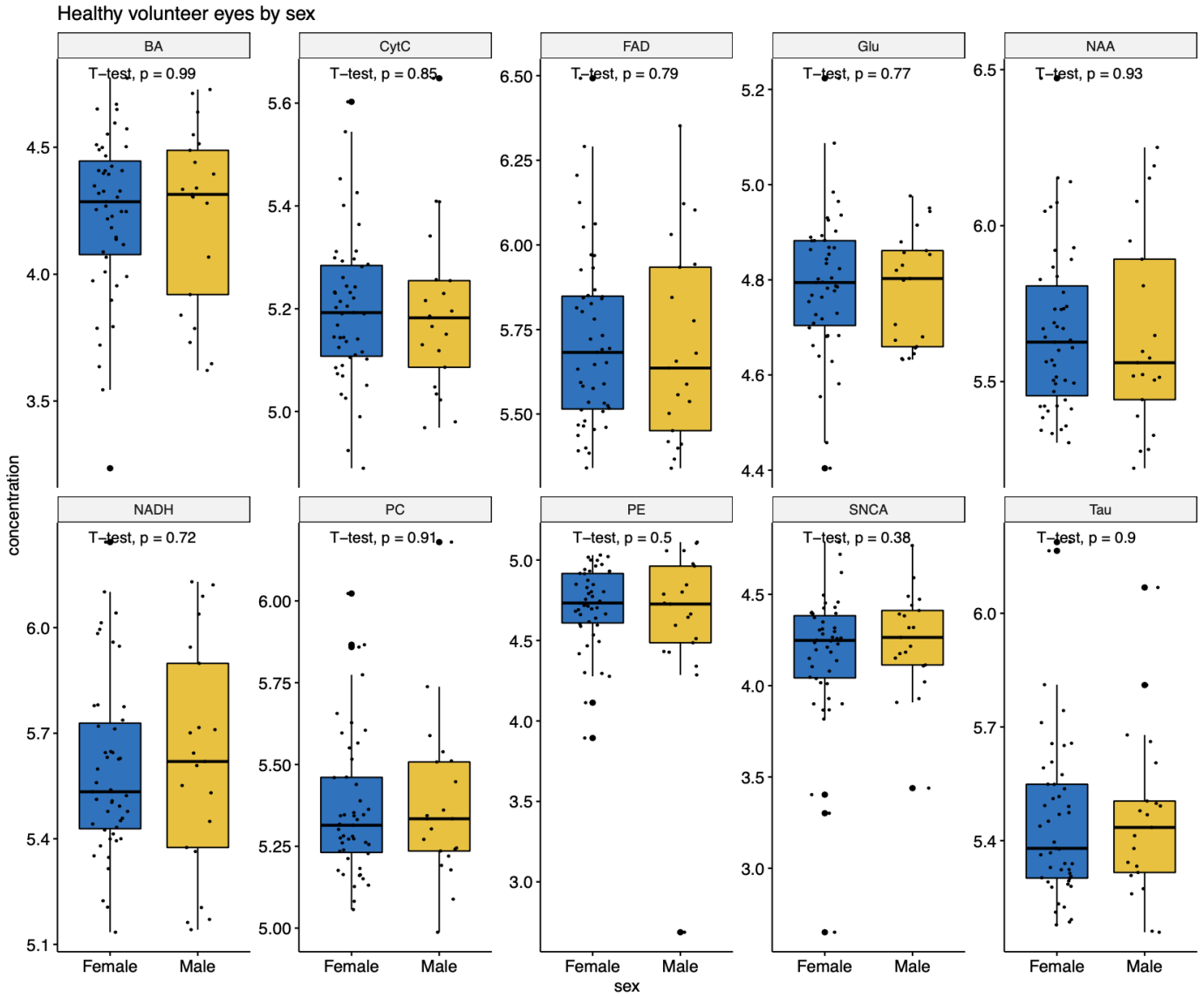


Molecule	1h retest	12h retest
Abeta	8.583 (7.373)	4.582 (2.999)
CytC	3.425 (1.528)	2.573 (2.728)
FAD	4.581 (3.750)	1.690 (1.775)
Glu	3.392 (2.738)	2.956 (2.273)
NAA	3.622 (2.079)	2.397 (1.646)
NADH	4.713 (2.442)	4.470 (3.110)
PC	4.277 (3.307)	3.941 (2.733)
PE	4.325 (2.460)	6.982 (5.324)
SNCA	6.568 (5.018)	10.700 (15.774)
Tau	3.451 (2.662)	2.881 (3.150)

significant correlation of metabolite levels with age

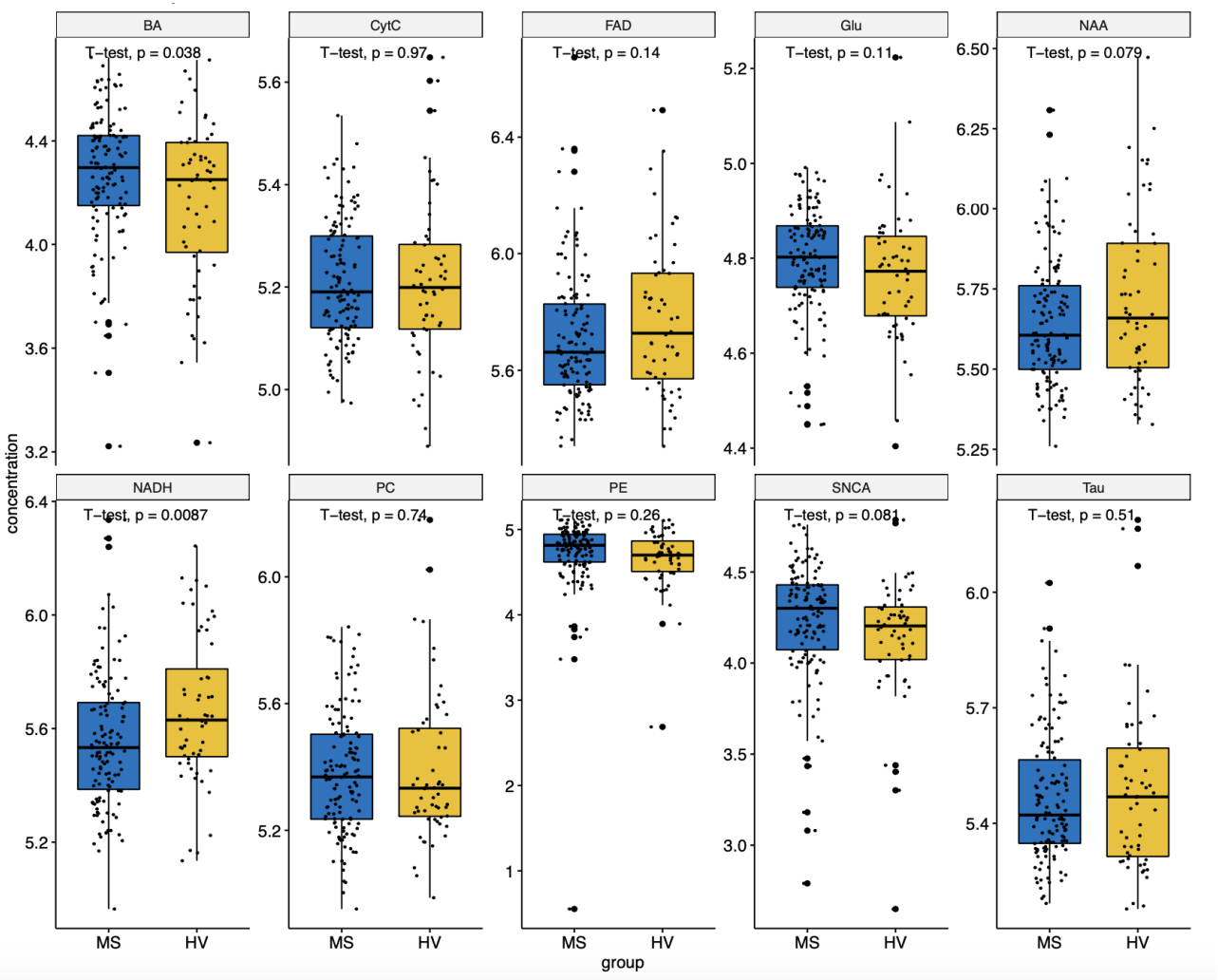


No significant sex effects



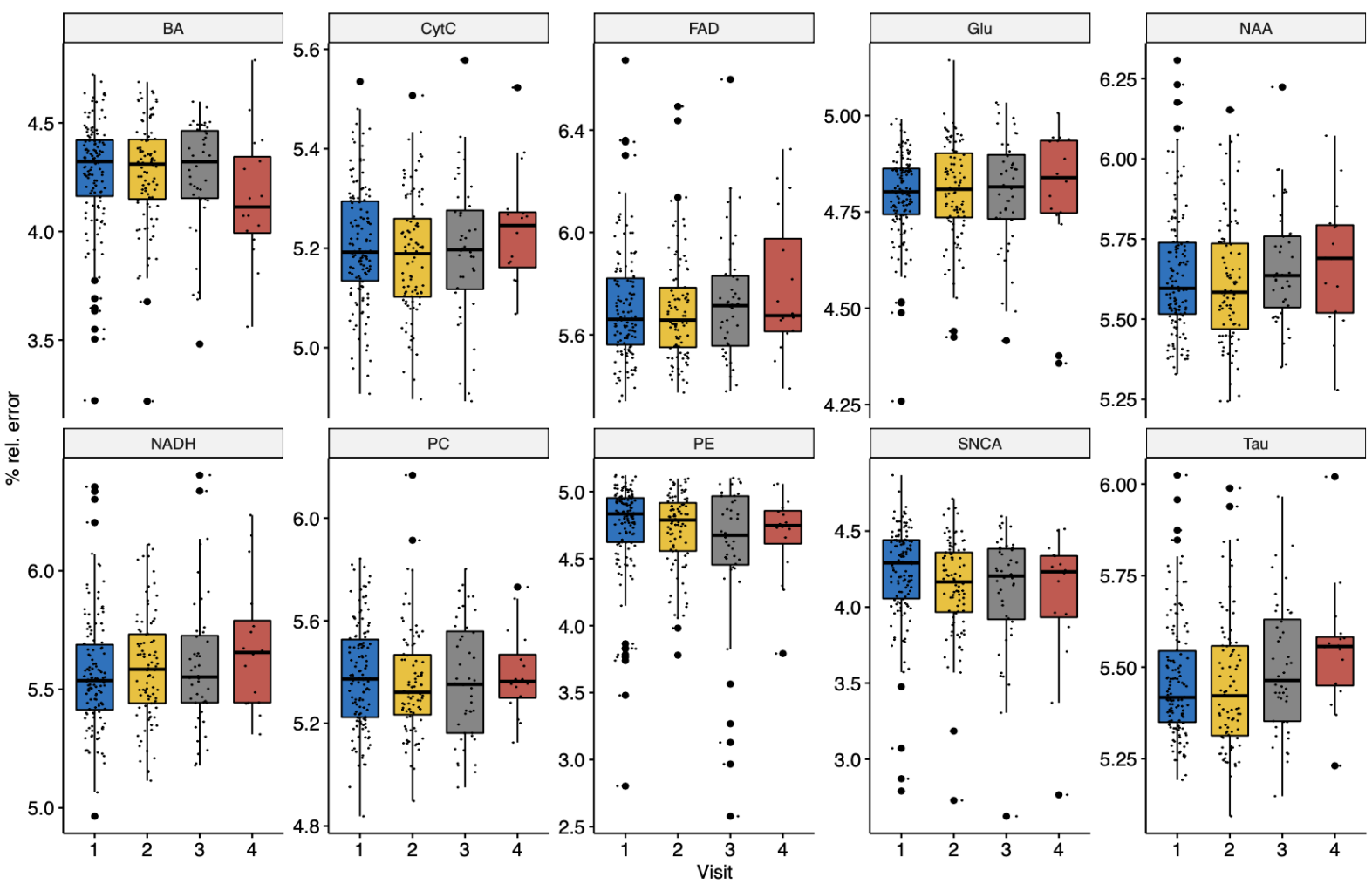
differences MS vs controls crossectional

There was a significant decrease in NADH and a trend towards a decrease in NAA in MS patients, as well as an increase in Abeta compared to healthy controls.



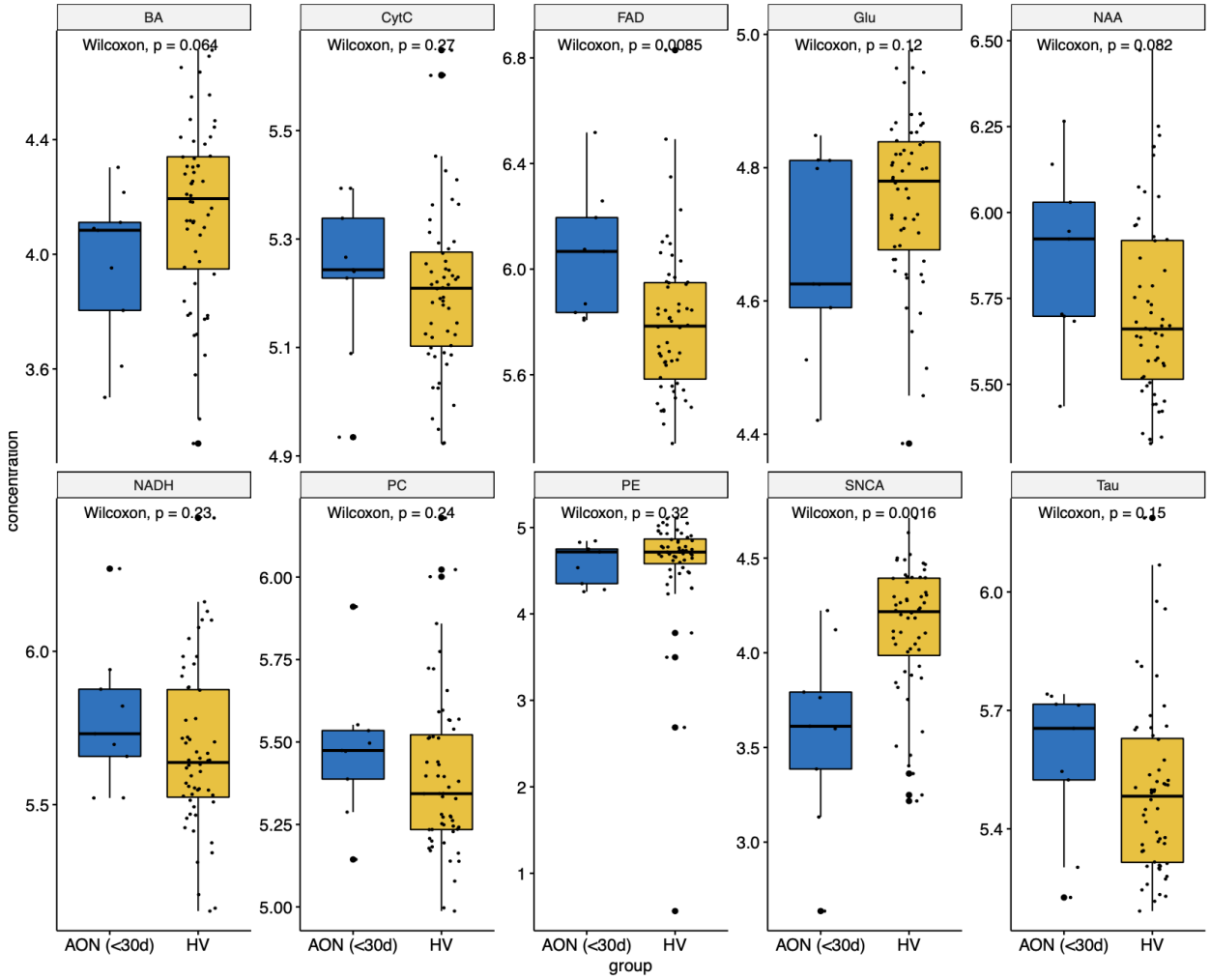
Longitudinal changes in MS patients

NADH and FAD increased overtime during follow-up, whereas Abeta diminished



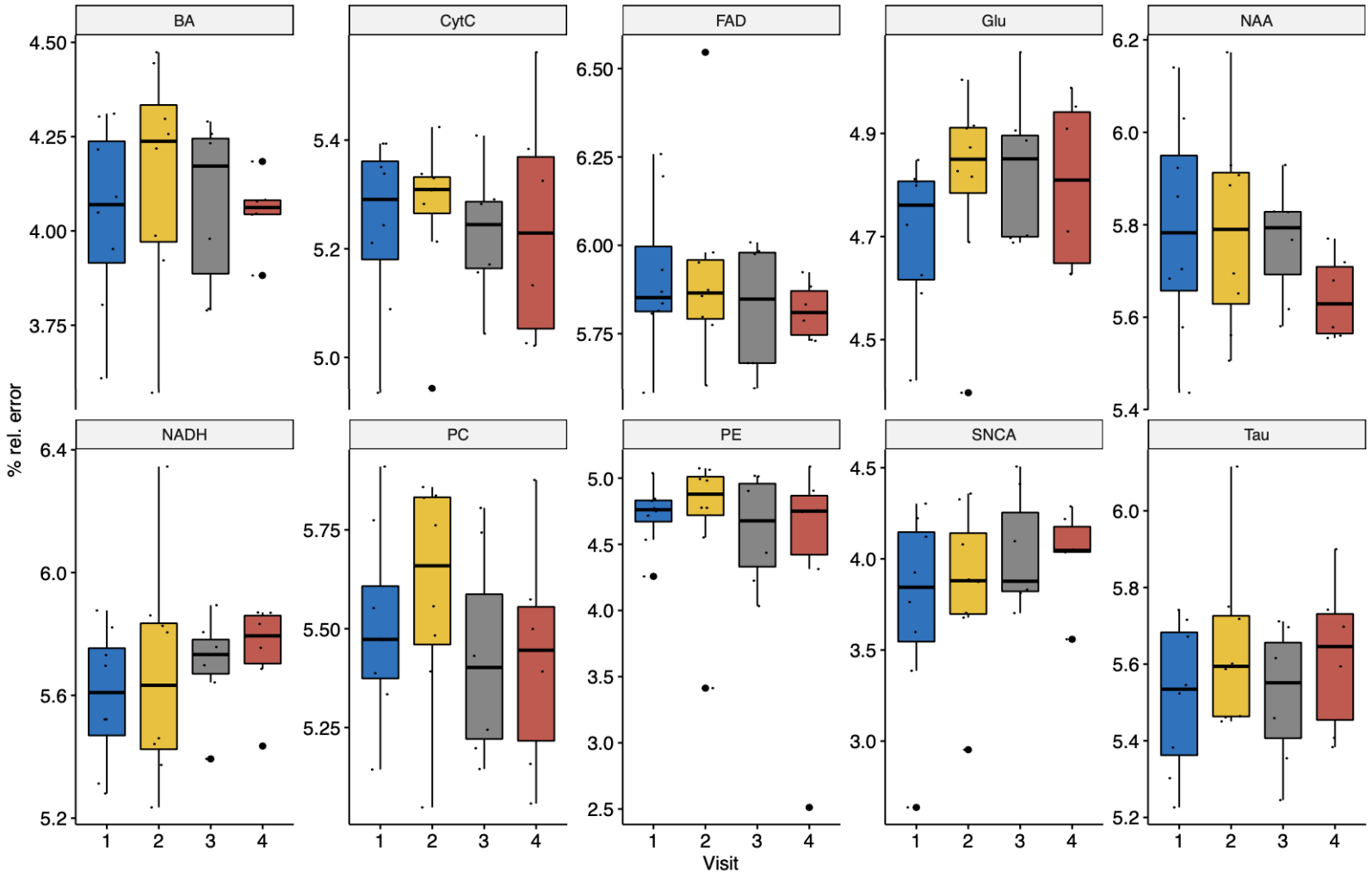
Metabolite changes during acute Optic Neuritis

Significant increase in FAD and a decrease in SNCA in the affected retina



Longitudinal changes in AON

Glutamate levels increased in the affected eyes after a 6-month follow-up



Conclusions

- Raman spectroscopy allows to perform molecular imaging of human retina in vivo
- Metabolites significantly change with age => adjusting for this factor
- MS: changes in metabolites related with energy supply, axonal maintenance and synaptic maintenance
- AON: changes in metabolites related with energy supply, excitotoxicity and synaptic maintenance

- Next steps
  - Developing prognostic and predictive biomarkers
  - Monitoring molecular changes at early and late stages
  - Expand metabolite analysis