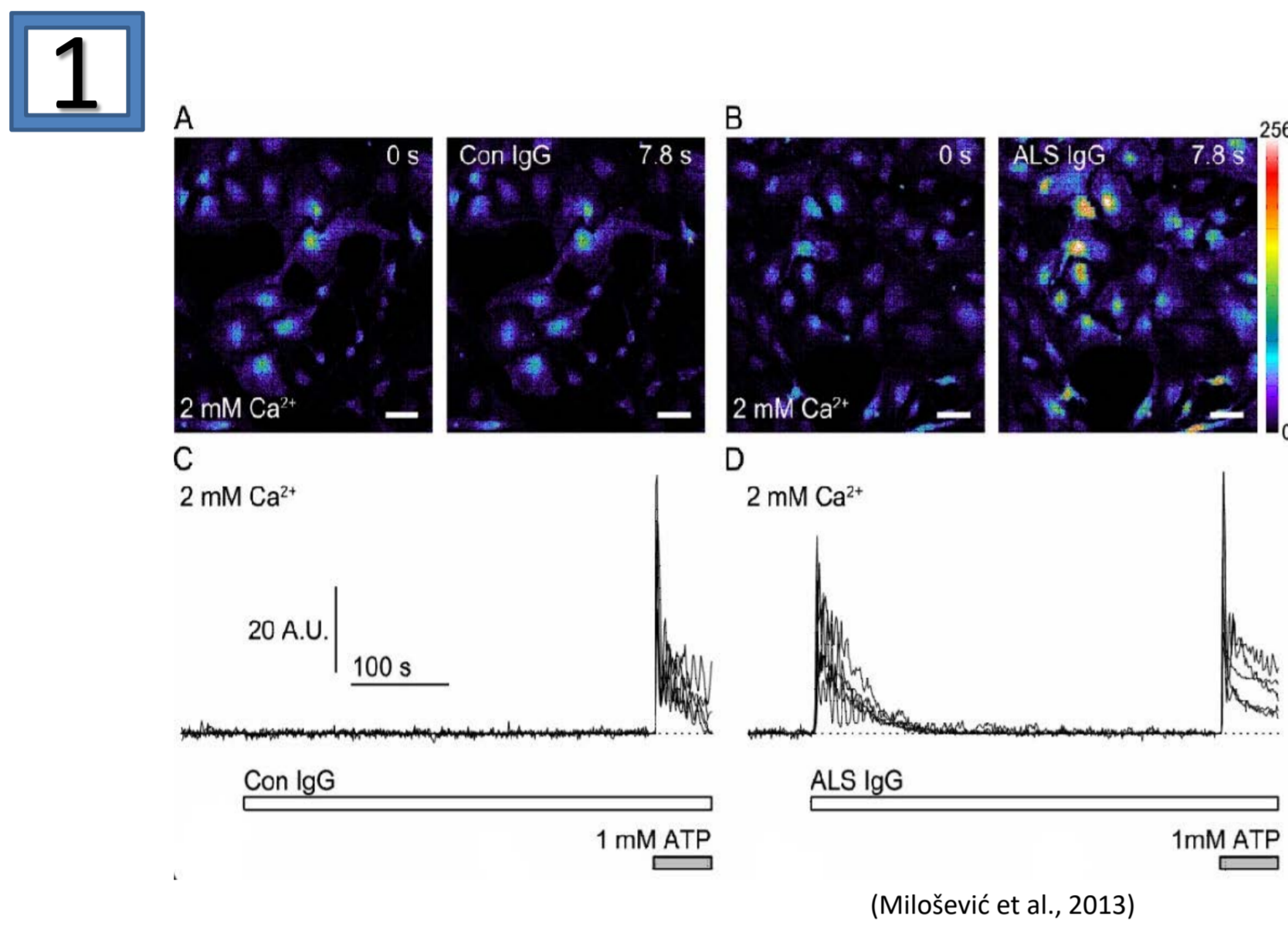


# The road to NIMOCHIP™ project: past present and future

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**Background:** Early experiments with ALS IgGs of Stanley Appel and Jozsef Engelhardt (e.g. PNAS 1991 88:647). Our studies with ALS IgGs on diverse physiological phenomena *in vitro*: a) rise in frequency of postsynaptic currents (Andjus et al., 1996,1997); b) intracellular calcium mobilization in response to ALS IgGs on neurons and glia (Milošević et al.,2013); c) acute free radical release in a microglial cell line (Milošević et al., 2017); and d) increase in the mobility of acidic vesicles in primary cortical astrocytes (Stenovec et al., 2011).



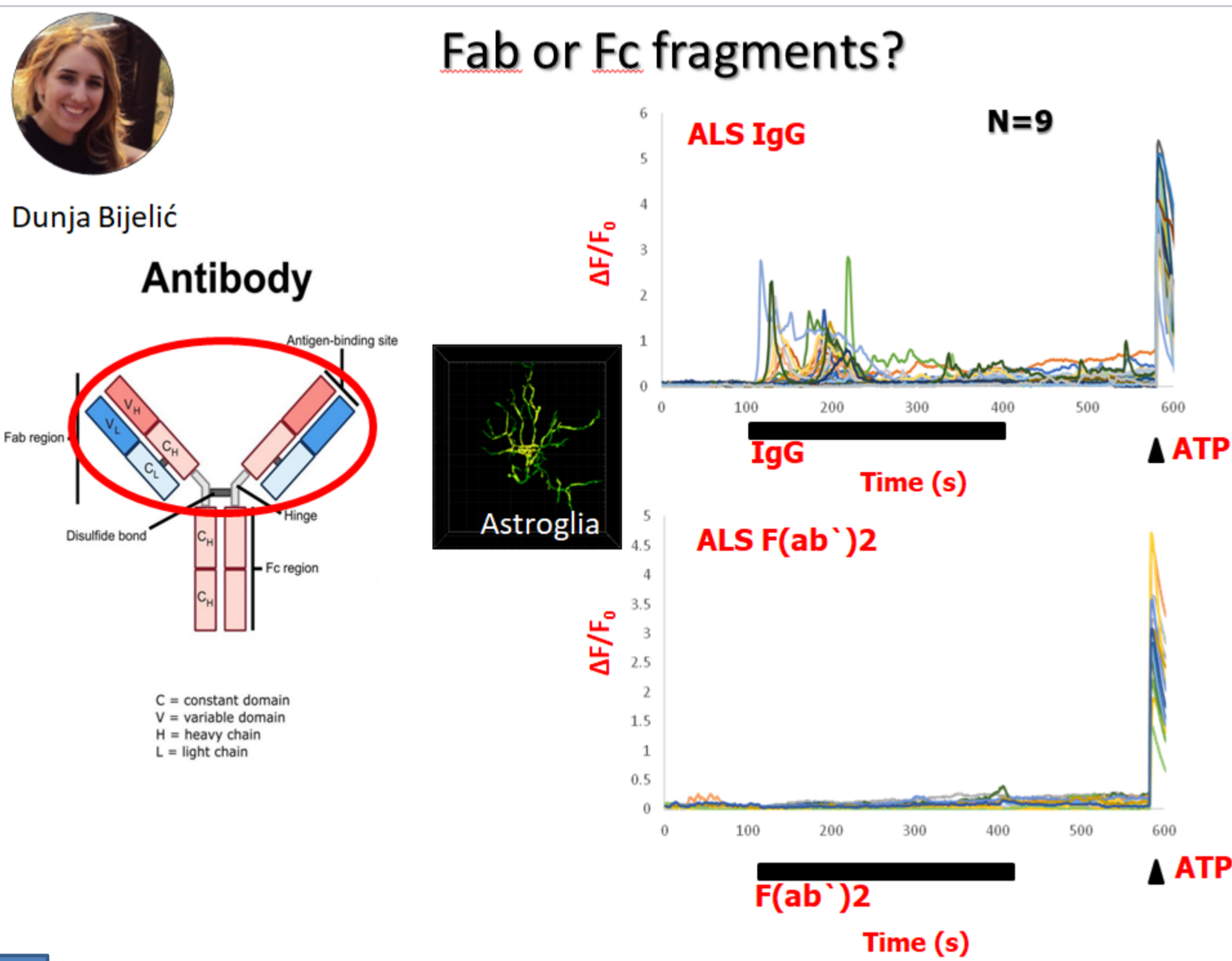
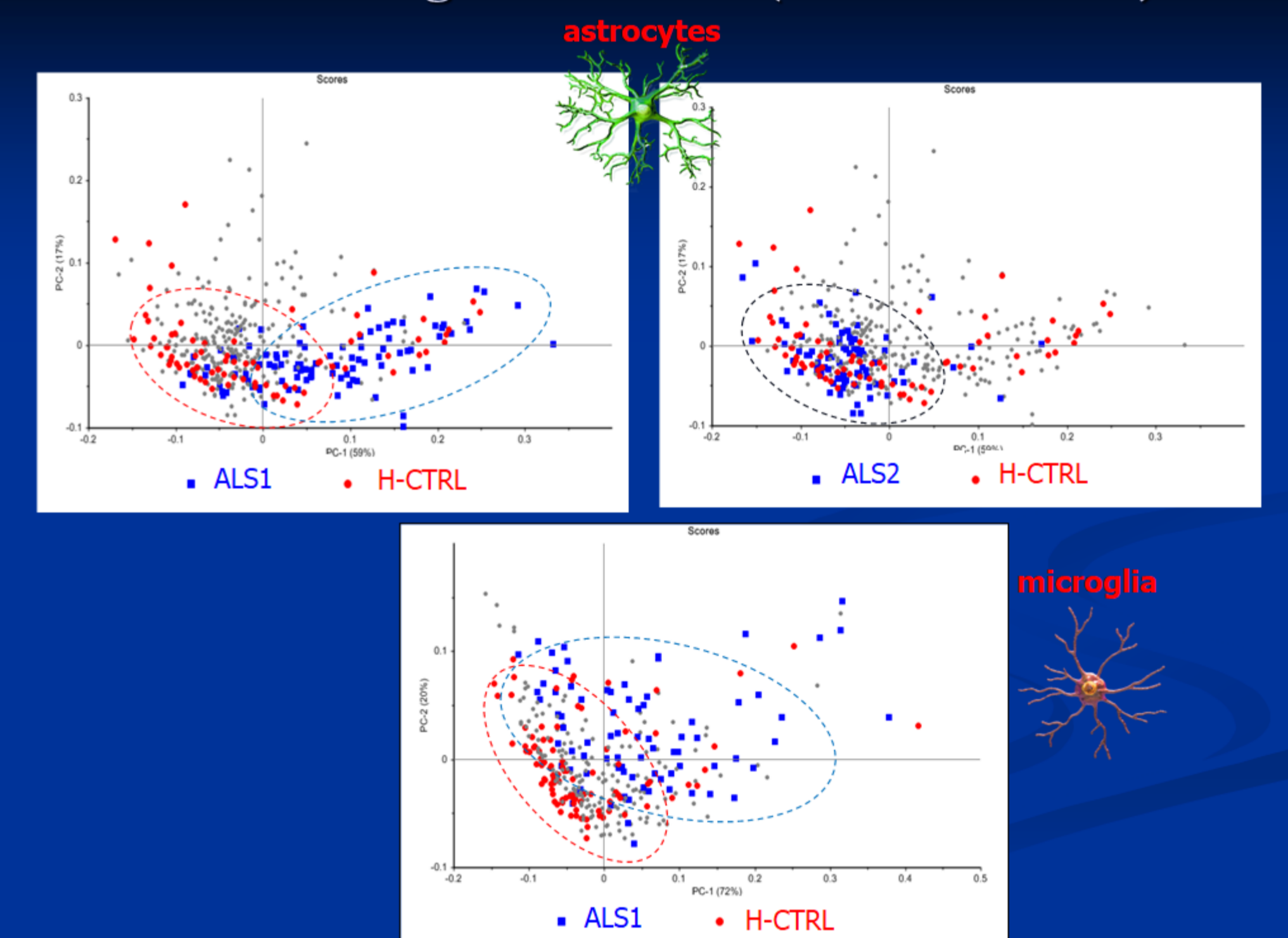
**(1) Method & Experiment:** Ca<sup>2+</sup> imaging of the IgG effect on rat astrocytes *in vitro*.  
**Finding:** The effect is diminished or absent after cutting the IgG Fc fragment.

**(2) Method & Experiment:** FTIR using ALBA synchrotron light source of freeze dried astrocytes and microglia *in vitro* after IgG treatment.

**Finding:** Principal Component Analysis reveals that 20 min – IgG treatment induces changes in glial lipids compared to control (healthy) IgG. Effect is not seen in all ALS patient samples. Changes are also recorded in bands from proteins and nucleic acids (latter only in astrocytes) – *not shown*.



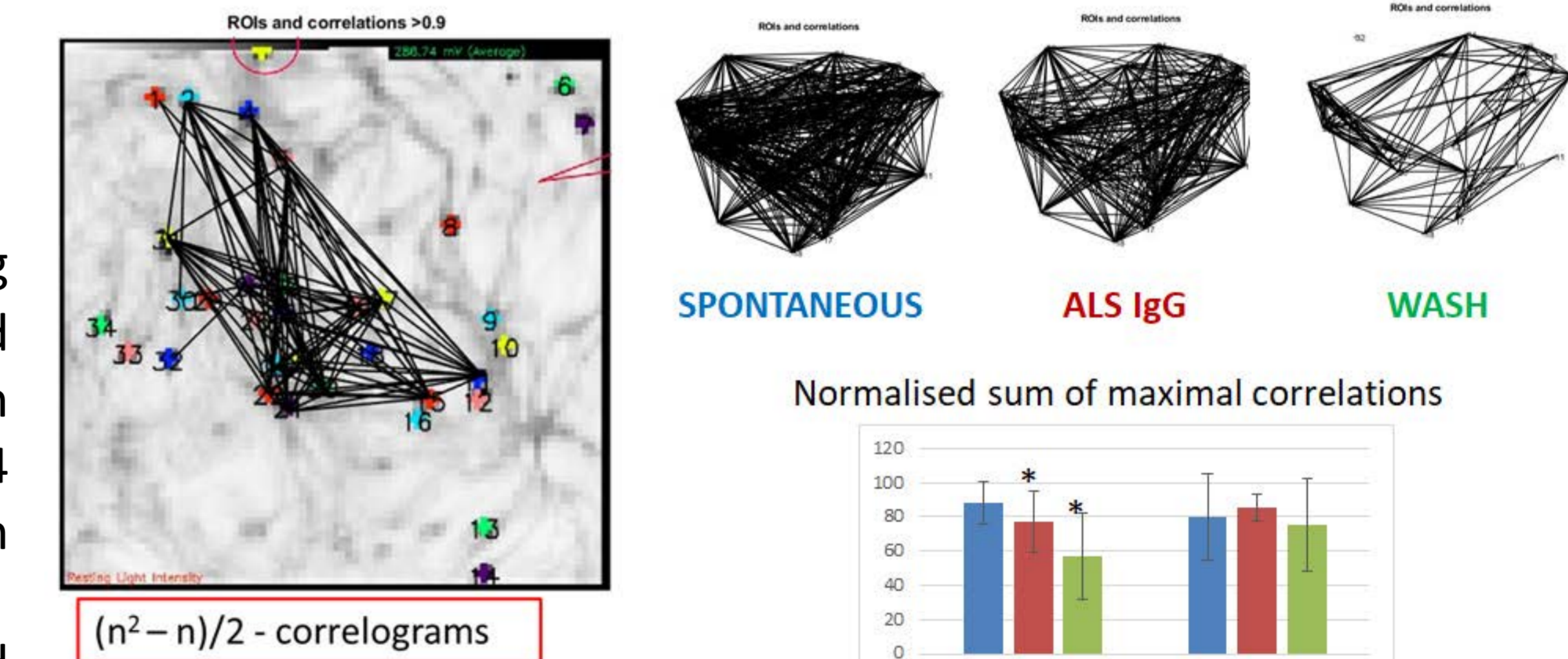
20 min in IgGs – LIPIDS (3100 – 2800 cm<sup>-1</sup>)



**(3) Method & Experiment:** Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) of fixed astrocytes after IgG treatment (20min).

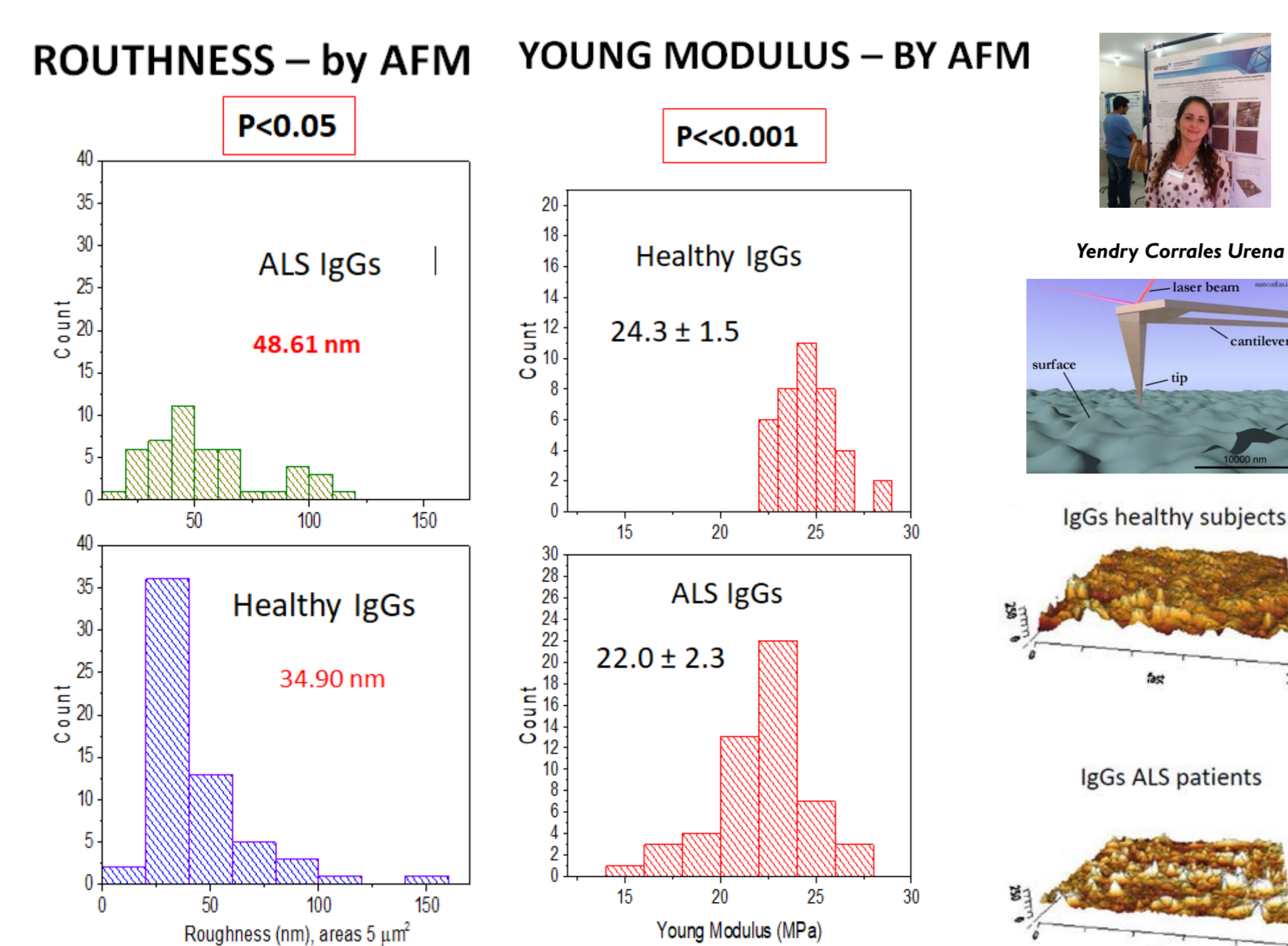
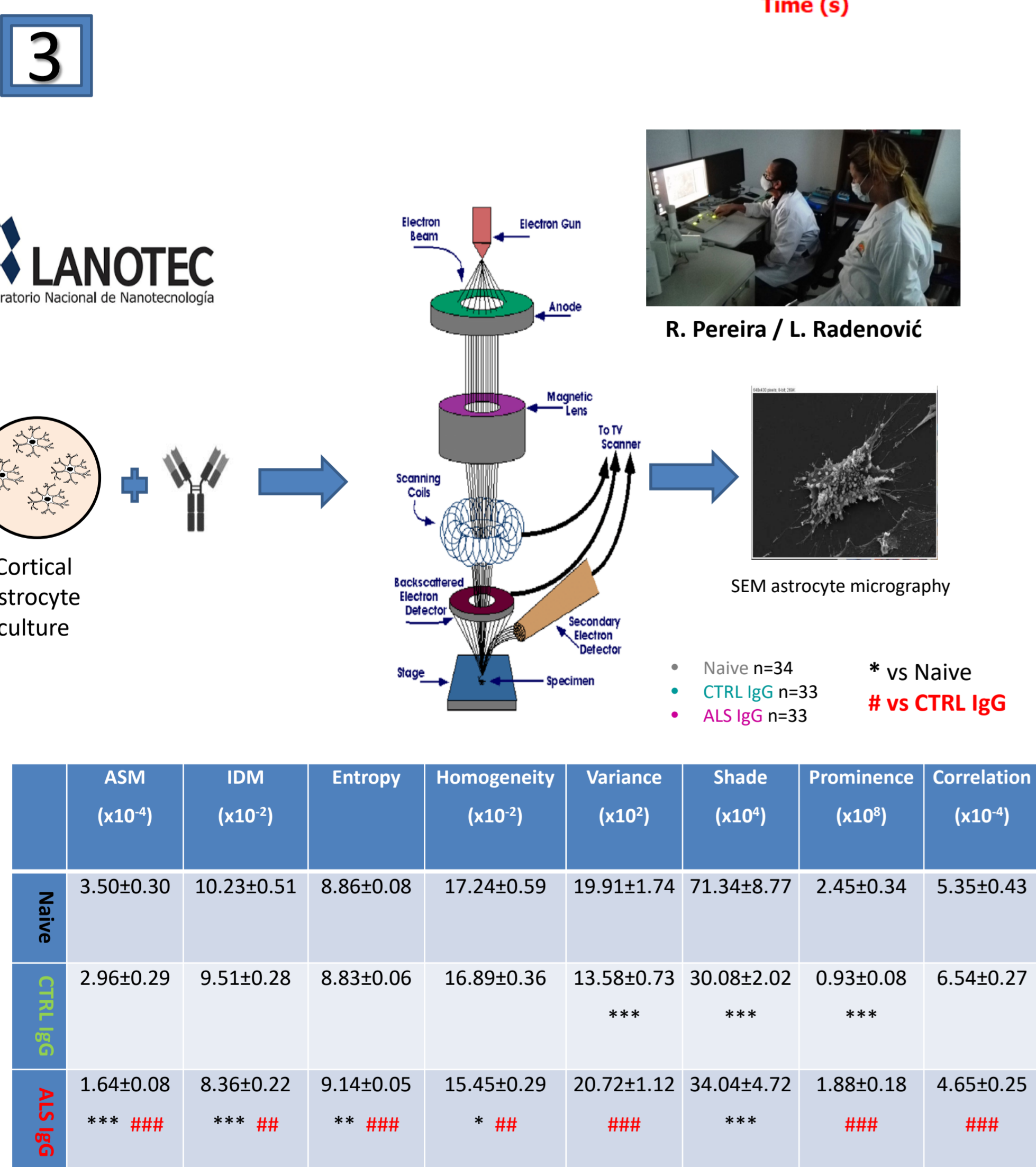
**Finding:** SEM - Texture analysis: grey-level co-occurrence matrix reveals significant difference from the control IgG in all parameters except Shade. AFM – ALS IgG induces roughness and „softens“ the astrocyte membrane.

**(4) Fluorescence recordings and analysis of IgG-induced Ca<sup>2+</sup> transients in hippocampal neurons *in vitro*.**



**(4) Method & Experiment:** Ca<sup>2+</sup> imaging on rat hippocampal neurons *in vitro* and analysis of correlation of activity with correlograms (each line in panel 4 connects a pair of neurons with correlation coeff>0.9)

**Finding:** ALS IgGs diminish correlation and synchronization of spontaneous activity that continues even after IgG wash.



**(5) NIMOCHIP™ device:** A microfluidic Lab-on-a-chip that uses time series fluorescence recordings of ALS IgG-induced reactions as a physiological biomarker and by means of machine learning is trained for precise personalized diagnostics.

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