

# Optical investigation of osteoarthritic human cartilage (ICRS grade) by confocal Raman spectroscopy: a pilot study

Raman Spectroscopy and Principal Component Analysis (PCA) were used to classify and assign a ICRS grade to osteoarthritic human cartilage

## Sample preparation

12 cartilage sections were collected from the knee of 3 patients. Four cartilage sections of International Cartilage Repair Society (ICRS) of ICRS grade I, II, and III were obtained. The assignment of ICRS grades were performed by two experienced orthopaedic surgeons. The cartilage samples were dissected perpendicular to the articular surface in a cubical shape with 3–4 mm side, fixed in formalin, and stored at 4 °C. For the analysis, samples were placed on a petri dish with the superficial layer of the cartilage facing the microscope objective. The petri dish was filled with PBS in order to prevent dehydration.

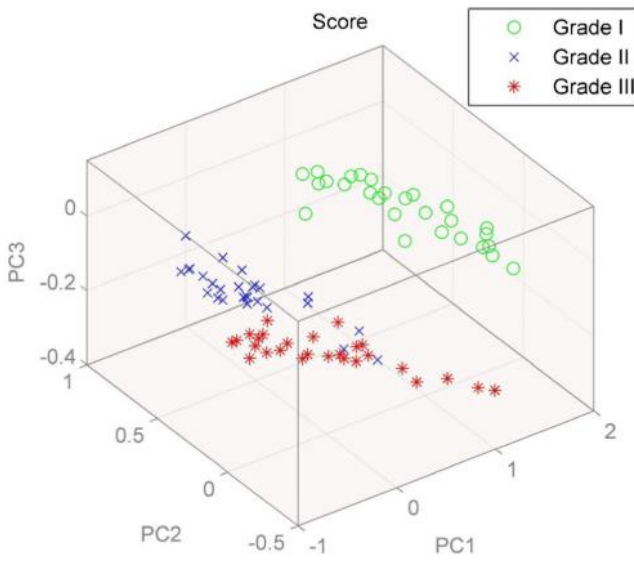
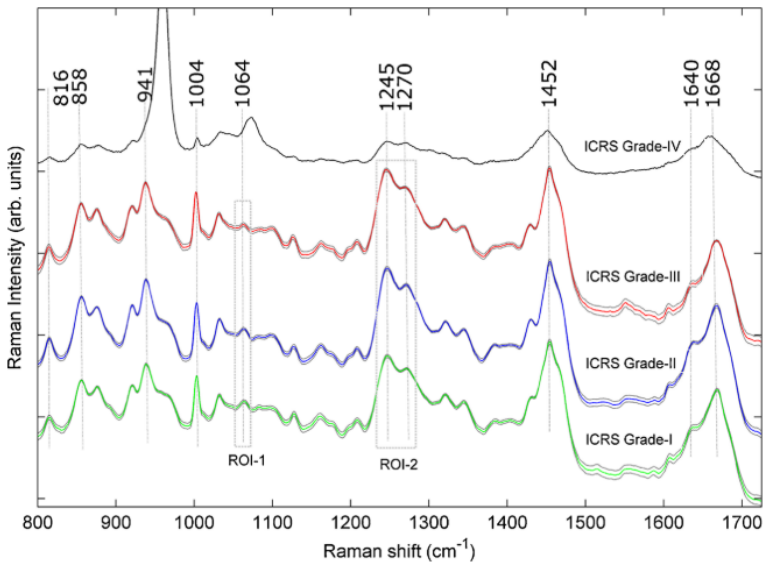
## Data acquisition conditions

Raman spectra were acquired using a LabRam HR800 HORIBA Jobin Yvon confocal Raman microscope with a spectral resolution of 2 cm<sup>-1</sup>. Spectral acquisitions were collected over the region 800-1725 cm<sup>-1</sup>, the fingerprint region of cartilage tissue. The acquisition time for each Raman spectrum was 20 s.

## Results

Mean normalized spectra is show in the figure, grouped by ICRS grade; PCA analysis was used to perform a dimensionality reduction and to allow clustering of the samples in the new space, the actual and predicted classification are shown in the following table, the overall predictive efficiency was about 85%.

Predicted classification			
	Grade I	Grade II	Grade III
Grade I	22	5	0
Grade II	4	23	0
Grade III	3	0	24



Mean (n=108 spectra) normalized Raman spectra obtained from ICRS grades I, II, III, and IV tissues (left). The solid lines indicate the average spectra while the shaded lines represent the standard error. ROI-1 and 2 represent regions of interest. Multivariate analysis-based PCA algorithm classifies different ICRS grades of osteoarthritis into separate clusters (grade I: green circle, grade II: blue cross, and grade III: red asterisk) (right)