

# Dorsal Root Ganglia Neurons Morphometry Analysing Method Optimization

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Oxaliplatin (OHP) is an anticancer drug known for its peripheral nervous system toxicity. Indeed, it can be considered as a robust model of neuronopathy; therefore, it is the ideal setting in which the issue of tailoring and ameliorating the morphometrical analysis of dorsal root ganglia (DRG) neuron can be addressed. Usually, DRG morphometry relies on manual measurements acquired by a trained operator; even so, an improvement of the standard morphometry protocol could allow to rule out potential subjective measurement bias and, eventually, it could be the basis for an automated or semi-automated measurement development.

Analyses were performed on DRG from rats treated with OHP (3mg/kg, i.v., twice a week for 4 weeks), and compared to control (CTRL) animals. Acquisitions of light micrographs of methylene-blue stained DRG sections were obtained, after sacrifice, both at the end of the treatment and after 6 weeks of follow up. Measurements were performed by two blinded operators. Manual tracing of neuronal soma, nuclei, and nucleoli outlines, along with the calculation of corresponding areas, were carried out using *ImageJ* software. We followed a specific algorithm to save and label the outlines each examiner traced, facilitating the subsequent validation of measurement accuracy and the verification of the correct identification of the targets of interest (i.e., soma, nucleus, nucleolus).

A statistically significant decrease has been identified by both examiners in all components in rats treated with OHP at the end of the treatment, compared to CTRL animals. At the end of the 6-week follow up, due to a partial recovery, the reduction was statistically significant only in the nucleolar area. Both examiners achieved the same level of statistical significance in their assessments for all parameters at all time points.

As there was minimal and non-significant variation between the examiners, we can suggest that our algorithm is adequately reliable. Therefore, the proposed algorithm enabling a persistent segmentation of soma, nucleus, and nucleolus areas will be subsequently used as the ideal starting point for the implementation of automated or semi-automated measurement methods, relying on machine learning algorithms.