



Fund “Nauka” Project № 16007 Resume – Competition-Based Session 2016:

“Three-dimensional visualization of glial cells and tumor cells in colorectal cancer“

Project leader: Prof. Nikola Yordanov Kolev, MD, PhD, DSc

Project Goals: Three dimensional visualization of enteric neuronal and processes glial cells in colorectal carcinoma and in normal colorectal mucosa.

Necessary equipment: Inverted microscope and software modules, which will be used with our multichannel fluorescence microscopic system. This will allow better visualization of 3D objects in the glial cells.

Expected impact: Proving the existence of glial and neuronal processes in colorectal cancer. The project will assist the PhD of Dr. Alexander Zlatarov from the Department of General and Operative Surgery, University Hospital “St. Marina” – Varna, and the scientific work of young scientists (Dr. Pavel Pavlov, Dr. Nedyalka Zgurova – Departament of Clinical Pathology, University Hospital “St. Marina” – Varna). A paper in an international journal with an impact factor is intended.

Achieved results:

One of the original tissue clearing methods, called CUBIC, was modified by a change in the protocol. This method was chosen for tissue clearing because it uses only incubations into chemical compounds, without the need to apply additional expensive equipment such as platinum electrodes, which are not available in standard histological laboratories. The aim was to optimize the possibility of 3D visualization of human colon tissue without the need for a confocal microscope. Optimization included modifications of various steps that allowed better results. By applying the optimizations, a robust signal for β 3-tubulin was detected in the lamina propria of the normal intestinal wall as well as in the tumor. Only by using standard epifluorescence, a good signal was achieved at a depth of 300-500 μ m tissue slice thickness, as well as at 3-4 mm tissue slice thickness. Fluorescent images were transformed into 3D reconstruction using easily accessible programs such as *Fiji* and *ilastik*. Innervations in human colon material and adjacent colorectal cancer were first visualized using classical immunofluorescence. Nerve structures were stained in both the normal colonic wall and the tumor, neuroendocrine cells in normal crypts, and tumor cells and for further study and better 3D reconstruction, the slice thickness was increased above

50 μm , resulting in reduced image quality with standard methods. As a solution, the CUBIC tissue cleaning method was modified and applied, and thus tissue slices up to 100 μm thick were successfully processed. $\beta 3$ -tubulin gives a robust staining after the CUBIC treatment and might serve as a control when investigating human colon wall and tumor using other antibodies for labeling.