NOVEL 3D MICROSCOPY TECHNIQUES AND IMAGE ANALYSIS ALGORITHMS TO STUDY CANCER-RELATED IN VITRO SPHEROID MODELS

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Recent progresses in 3D microscopy techniques revolutionise our way to study biological processes involved in cancer metastasis thanks to innovative imaging of biological models. *In vitro* spheroid model consists in an aggregate of one or several cell types labelled using fluorescent dyes or constructs and embedded in fibrillar type I collagen matrix [1]. Studying spheroid migration, invasion and proliferation properties involves the use of various microscopy techniques and the development of adapted processing, characterisation and quantification algorithms.

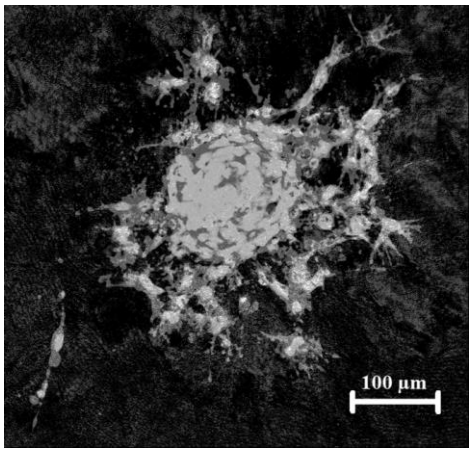

Figure 1: Z-projection of cathepsin B deficient CAF homospheroid in fibrillar collagen matrix imaged by Zeiss LSM 880.

In the present work, homo- or heterospheroids are imaged by laser confocal scanning microscopy while the surrounding collagen matrix is imaged by confocal reflectance microscopy or second-harmonic imaging microscopy. 3D spheroid images are either captured punctually or recorded over time to acquire time-lapses. The images are then pre-processed to increase the signal-to-noise ratio and segmented using local or global thresholding. Spheroids are divided into three parts: core, arms and invaded cells. 3D invasion profile is analysed by assessing spatial distribution around the spheroid core through distance, longitude and latitude parameters. 3D co-invasion is also considered in heterospheroids by comparing each cell position to identify invasion patterns between cell types. The matrix orientation and spatial distribution is evaluated as well from the grayscale images.

From these results, we show the influence of the collagen matrix initial architecture on the spheroid latitude invasion profile. We also highlight the difference of invasion profile and kinetics between different cell types in homo- and heterospheroids as well as cell-cell interactions during invasion process in the latter ones. Finally, cell-matrix interactions were studied by comparing matrix deformation and reorientation through time with spheroids composed of different cell type(s).

[1] S. Blacher; C. Erpicum, *et al*. “Cell Invasion in the Spheroid Sprouting Assay: A Spatial Organisation Analysis Adaptable to Cell Behaviour”, *PLoS ONE*, **9(5)**, e9719 (2014).