REAL TIME ANALYSIS OF NUCLEAR FOCI AFTER DNA DAMAGE IN LIVING MULTI-CELLULAR TUMOUR SPHEROIDS BY LIGHT SHEET MICROSCOPY

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Light sheet microscopy (LSM), also known as Selective plane illumination microscopy (SPIM), is a useful technique for three-dimensional (3D) imaging of complex thick biological samples [1][2]. With a combined optical and chemical method based on an adaptive SPIM and a water-based clearing protocol, one can acquire high resolution in-depth images of optically cleared complex thick samples such as Multi-Cellular Tumour Spheroids (MCTS) [3]. Our team aims to study bystander effects that have been little investigated in human 3D models [4][5]. We would first like to study DNA damage in living MCTS, either by a radiation-induced or a drug-mediated procedure.

We chose to do so by creating drug-mediated DNA damage using either bleomycin or etoposide with HCT116 cell lines that over-expresses 53-binding protein-1 (53BP1) with red fluorophores for our experimentation. Apparition of foci after DNA damage with bleomycin deep inside spheroids was observed. Figure 1 (b-c) illustrates the significant increase in the number of foci with bleomycin. In order to quantify the evolution of in depth foci and over time, we compared the number of foci in three different conditions : living spheroids over time, fixed spheroids and fixed cleared spheroids. Imaging analysis was carried out with 3D Watershed in the open source image-processing Icy package and with Blob Finder in Arivis Vision 4D.

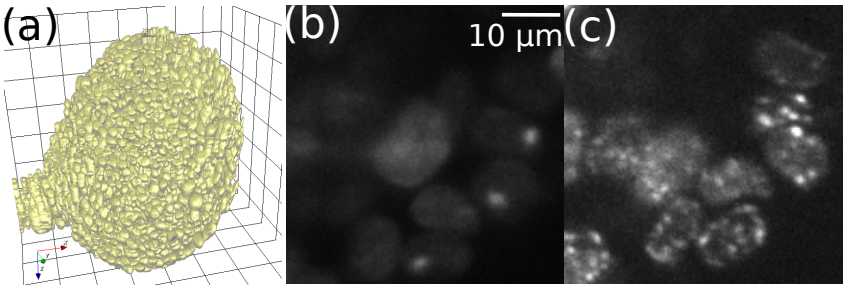

FIGURE 1 – (a) 3D segmented spheroid, (b) start point right after we take spheroid in bleomycin, (c) 3 hours after.

[1] Lorenzo. C, *et al*. Live cell division dynamics monitoring in 3D large spheroid tumor models using light sheet microscopy. Cell Div **6**, 22 (2011).

[2] Dodt. H.U., *et al*. Ultramicroscopy : three-dimensional visualization of neuronal networks in the whole mouse brain. Nat Methods **4**, 331-336 (2007).

[3] Masson. A, *et al*. High-resolution in-depth imaging of optically cleared thick samples using an adaptive SPIM. Scientific reports **5** (2015).

[4] Klammer. H, *et al*. Bystander effects as manifestation of intercellular communication of DNA damage and of the cellular oxidative status. Cancer letters **356**.1, 58-71 (2015).

[5] Lam. R. K. K., *et al*. Rescue effects : irradiated cells helped by unirradiated bystander cells. International journal of molecular sciences **16**.2, 2591-2609 (2015).