Assessing Cellular Metabolism Using 2-Photon Imaging And Cancer Spheroids

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Introduction: Spheroids show great promise in being a better model for testing treatments for cancer *in vitro* when compared to monolayer cells. Single photon imaging of spheroids is limited by depth. Due to this reason, two photon imaging is necessary to obtain a full image of the spheroid. We developed a software that can evaluate the cellular metabolism of a spheroid by calculating the Redox Index (NADH divided by FAD). We tried to validate this software by treating the spheroids with an ATP antagonist.

Materials and Methods: Spheroids were made from the A431 skin cancer cell line in agarose coated 96-well plates and imaged using an Ultima IV 2-Photon Microscope (Bruker). The NADH autofluorescence intensities were imaged by exciting the spheroid at **760 nm**, while the FAD intensities were imaged at **880 nm.** A 3D stack of the entire spheroid was obtained. Spheroids were grown in the same manner but with different number of cells per well to ensure that there will be a difference in size. They were imaged using an Olympus IX71 inverted microscope with a 10x 0.3 N.A. objective and collected by a Scientific CMOS camera (ORCA-Flash4.0). Next, spheroids made from 1000 cells/well and 2000 cells/well were treated with Sodium Azide (NaN_3), which is an ATP antagonist. The concentration of NaN_3 ranged from 0.25 mM to 20 mM. A Live/Dead assay was applied to evaluate cell viability.

Results and Discussion: The software was able to calculate the redox ratio at each pixel and showcase that on a heatmap (Figure 1A). When treated with Sodium Azide, the spheroids showed a significant decrease in size (Figure 1B). Some spheroids treated with a higher concentration of Sodium Azide appeared to lose their shape after 46 hours and become a cluster of cells. The Live/Dead assay also showcased a difference between the control spheroid and the one treated with 20 mM Sodium Azide (Figure 1C). The control group had a higher number of live cells (indicated by the Green color) while the 20 mM group had a higher number of dead cells (indicated by the Red color).

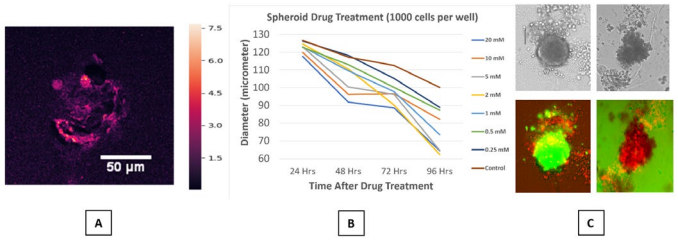

Figure 1. A) Redox Index map generated using the software. **B)** Graph comparing the size of spheroids treated with a different concentration of Sodium Aside. **C)** Live/Dead assay of the control group (Left) and the 20 mM group (Right)

Conclusions: We created a software that can analyze two photon images of a spheroid and generate a heat map of the redox index at each optical slice. Spheroids were also treated with an ATP antagonist and showed a decrease in size. Further analysis using a Live/Dead assay showed a higher number of live cells in the control group and higher number of dead cells in the 20 mM group. Future work includes looking at the redox index of spheroids treated with an ATP antagonist.

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References

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