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MODELLING THE FUTURE

LIVE CELL *IN VITRO* CANCER RESEARCH USING A FEW-CYCLE LASER

NANOTECHNOLOGIES
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ABSTRACT
The Synchronous Red Green Blue – Fluorescence lifetime imaging microscope (SyncRGB-FLIM) [1], an extension of the conventional multiphoton MP-FLIM method, deploys a sub-10 fs ultra-broadband (>400 nm bandwidth) laser capable of simultaneous excitation of spectrally well-separated dyes or autofluorescence molecules. The d-scan technique is used for pulse control and measurement at the focus, to maintain the few-cycle laser pulse throughout the microscope with subsequent delivery of the optimized pulse at the sample plane [2]. We use SyncRGB-FLIM to study the metabolic activity of cells in 2D and 3D models of lung cancer.

RESULTS
By quantifying the fluorescence lifetime properties of endogenous metabolite NAD(P)H we are able to map the cellular bioenergetics [3], upon anticancer treatment with the drug paclitaxel (PTX).

DISCUSSION
Cancer cells tend to be more glycolytic, characterized by the presence of unbound NAD(P)H (short lifetimes). During treatment with PTX, cells shift to an oxidative phosphorylation state, leading to an increased presence of bound NAD(P)H (long lifetimes). This is observed in monolayers of lung cancer cells H460 (Fig. 1) increasing the average lifetime (tm) by 45% and decreasing the ratio of contributions of short lifetimes (a1)/long lifetimes (a2) by 43% (Fig. 2). Towards testing the method in 3D cancer models we used H460 spheroids. PTX cause the disruption of the spheroids (Fig.3) increasing the tm by 16%.
The sub-10fs laser was efficient at generating autofluorescence signal, at the focus of the objective, in 2D and 3D cell models, without causing photo induced damage to this samples.

Label-free metabolic imaging during PTX treatment on 2D model of H460 cells

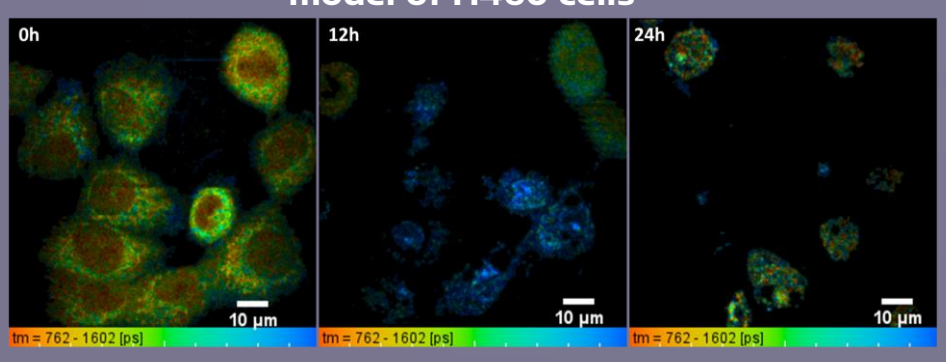

Fig.1: FLIM images of H460 cells incubated with PTX for 0h, 12h and 24h.

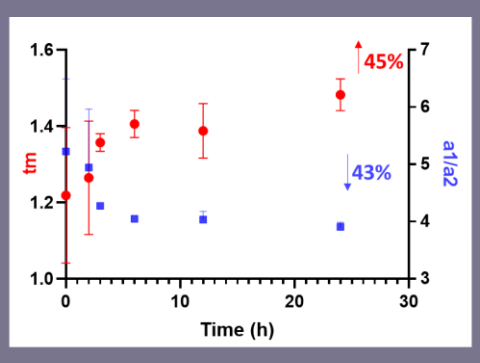

Fig.2: The variance of tm and a1/a2 ratio on cells during treatment.

Label-free metabolic imaging during PTX treatment on 3D spheroid model of H460 cells

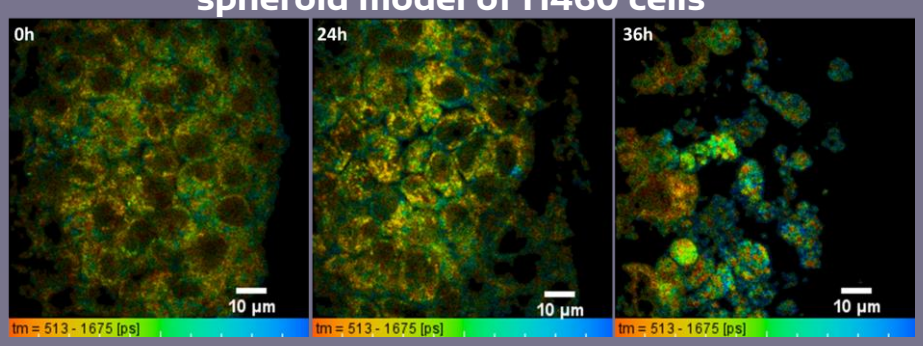

Fig.3: FLIM images of H460 spheroids incubated with PTX for 0h, 24h and 36h.

CONCLUSIONS AND OUTLOOK
A label-free novel bioimaging technique, capable of tracking the efficiency of cancer treatments is presented.
This demonstration paves the way for this imaging technique to contribute to the pharmacological development of drugs tested in latest 3D cellular disease models.

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