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First Report of *in vivo* Detection of Tumours
Final Version

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Abbreviations and Acronyms

Ag_2S	Silver sulfide
BW	Biological window
BL	Biospace Lab
CNRS	Centre National de la Recherche Scientifique
CSIC	Agencia Estatal Consejo Superior de Investigaciones Cientificas
FIBIRYCIS	Fundacion para la Investigacion Biomedica del Hospital Universitario Ramon Y Cajal
LNThs	Luminescent nanothermometers
PBS	Phosphate Buffered Saline
NIR	Near Infra-Red
NPs	Nanoparticles
UAVR	Universidade de Aveiro
WP	Work package(s)

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D6.4 First report of in vivo detection of tumours

Work package 6 (WP6) of the project is titled: "*Real time in vivo thermal images in BW-II and BW-III for tumour detection, tumour monitoring and magnetic/optical hyperthermia*". The report is mainly given by a lead beneficiary of the WP6: Fundacion para la Investigacion Biomedica del Hospital Universitario Ramon y Cajal (FIBIRYCIS); along with other partners:

- Centre National de la Recherche Scientifique (CNRS)
- Agencia Estatal Consejo Superior De Investigaciones Cientificas (CSIC)
- Biospace Lab (BL)

This report presents all the main results concerning the detection of tumours by means of luminescent thermometers. The impact of tumor development on the thermal relaxation dynamics could be cleared observed by using the model of the small animal imaging system proposed in the project

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1. Equipment, nanostructures and type of tumours utilized

Time dependence of intratumoral temperature was recorded by using the Ag_2S dots. A simple two-beam set-up was added to the small animal NIR fluorescence imaging system proposed in *Deliverable D6.3* - "Optical Set-Up for NIR small animal images" in order to measure the thermal relaxation dynamics of a tumor after moderate laser heating. The experimental procedure followed to evaluate the thermal relaxation dynamics of a tumor during its full development is schematically described in **Figure 1**. Briefly, the flank zones on the back right side of C57Bl/6 mice were intradermally inoculated with $1x10^6$ B16/melanoma tumor cells dispersed in phosphate buffered saline (PBS) solution to impel tumor growth. After cultivating the tumor cells into living mice, 50 µL of a 1.5 mg mL^{-1} dispersion of NIR-II Ag_2S LNThs in PBS were intradermally injected into flank region on the right and left side of the mice's back, associated respectively with the presence and absence of the tumor cells, in order to monitor the tumor developing by comparing the difference between the thermal relaxation time of a tumoral and healthy (control) tissue. Both the tumoral and the healthy tissues were subjected to an irradiation power density of 0.5 W cm^{-2} provided by an 810 nm laser (heating beam), where tissues' temperature exhibited an increasing trend with the time, warming up 5-7 °C after 2 min when the laser was finally switched off. Simultaneously with and after the application of the heating laser, a second 800 nm laser excitation (pump beam) was employed with a much low power density (0.03 W cm^{-2}) enough to generate luminescence from the NIR-Ag2S LNTHs (at about 1200 nm) without additional heating.

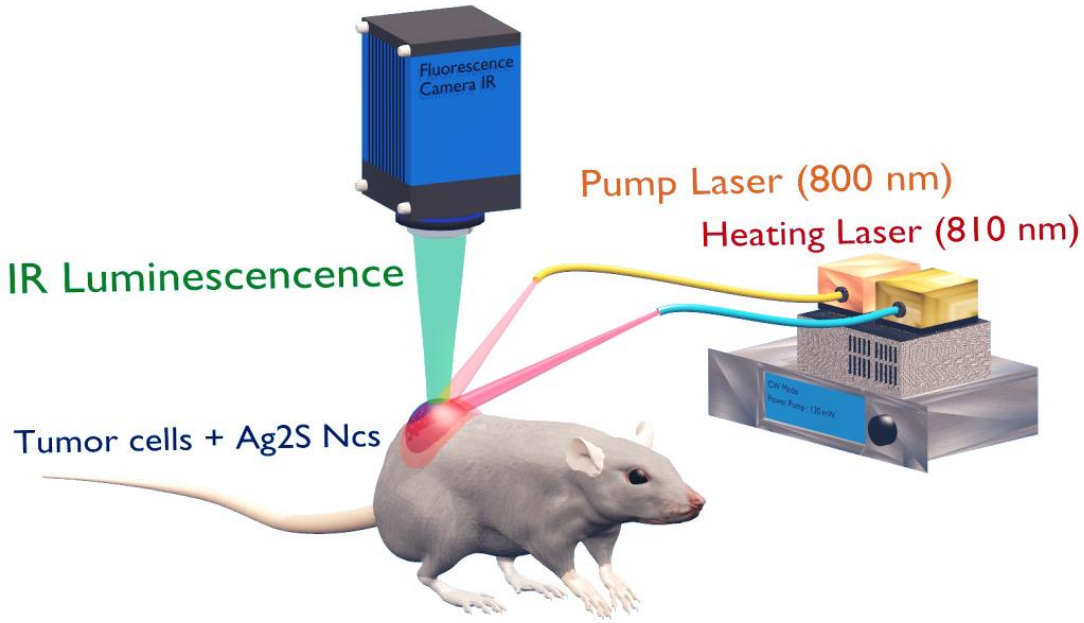

Figure 1. Small animal NIR fluorescence imaging system constituted by a simple two-beam set-up.

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2. Main results

The physical principle behind the detection of the tumor is found in the analysis of the time evolution of a tissue's temperature after being subjected to a heating pulse until it returns to its baseline value. This cooling profile, also known as thermal relaxation, can provide information about the health status of the tissue under study.

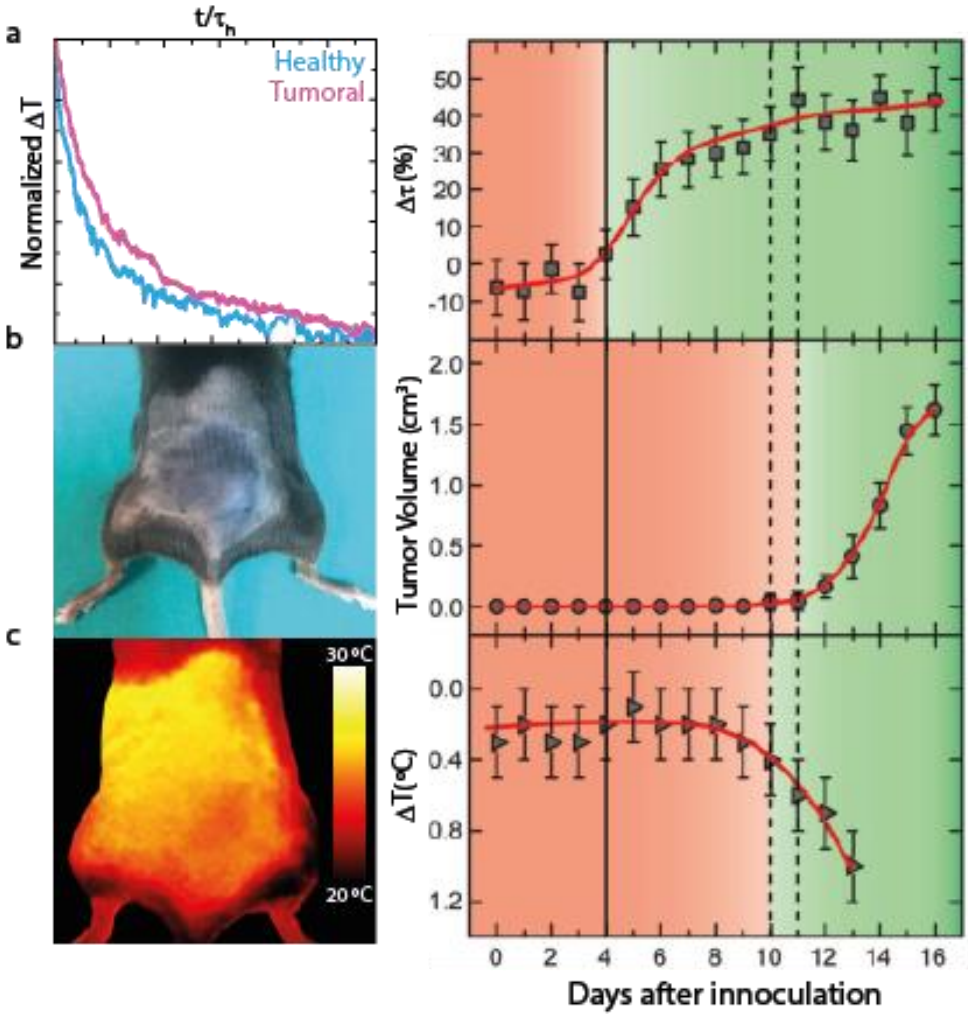

Figure 2. Experimental data values at different days after intradermally inoculation of B16/melanoma tumor. (a) Evolution of the relative change in the relaxation time. The left contains a graph of two representative relaxation curves obtained with healthy and tumoral tissues after 11 days of inoculation. (b) Tumor's size as measured by optical visualization. The left contains a representative optical figure of the mouse after 11 days of inoculation. (c) Temperature difference between tumoral and healthy tissues as measured with a thermographic infrared camera. The left contains a representative thermographic image of the surface of the mouse after 11 days of inoculation.

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Under our experimental conditions, after the heating laser was switched off, the NIR-II emission generated by the injected Ag2S nanothermometers gradually recovered, indicating the thermal relaxation of the tissue until it reached its basal temperature. For both tumoral and healthy tissues, we obtained the thermal relaxation curves by combining the thermal calibration of the Ag2S nanothermometers (as described in *Deliverable D6.1 – "First calibration datasheets for in vivo imaging"*) with the experimentally recorded intensity versus time curves. This allowed us to compare the characterizing cooling times of both tissues, which, in a first approximation, were given by:

$$\tau_{tumor} = \frac{\int \Delta T_{tumor}(t)dt}{\Delta T_{tumor}(t=0)}$$

$$\tau_{healthy} = \frac{\int \Delta T_{healthy}(t)dt}{\Delta T_{healthy}(t=0)}$$

If we define the relative change in the relaxation time as

$$\Delta\tau = \frac{\tau_{tumor} - \tau_{healthy}}{\tau_{healthy}}$$

Then we can easily see how Δτ is a highly sensitive parameter for monitoring tumor progression when compared to other techniques. In fact, the time evolution (measured since the day of tumor induction) of Δτ, of tumor size (Figure 2b) and of the surface temperature difference between tumoral and healthy tissues, as measured by infrared thermography (Figure 2c) demonstrate that. The experimental results shown in Figure 2a evidence that luminescence thermometry can detect the existence of a tumor only 5 days after the inoculation of cancer cells. This constitutes a great advance when compared with the traditional diagnosis based on optical inspection, which cannot achieve tumor detection until seven days later, i.e., eleven days after inoculation with tumoral cells (see Figure 2b). The same can be said if this technique is compared with infrared thermography, which, in turns, requires ten days after tumor induction to be able to detect its presence (Figure 2c). Taking into account that the characteristic tumor cycle time in our animal model is 14 days, it is safe to say that luminescence thermometry shortens the time required for tumor diagnosis by a remarkable amount of 60%.

According to literature, [1] the fundamental causes of this difference can be directly correlated to the changes in the blood perfusion that occur during tumor development due to the interplay between angiogenesis and necrosis.

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3. Conclusion and Perspectives

Though the demonstration of tumours detection was narrowed to a murine model of melanoma, it is safe to say that transient thermometry would also apply to any tumor whose development entailed a significant modification in blood perfusion (virtually all solid tumors), as long as it was accessible by a laser beam. As angiogenesis and necrosis are both common processes during tumor development, the technique here presented has the potential of being utilized in the further progress of the project.

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4. References

1. Santos, H. D. A. *et al.* In Vivo Early Tumor Detection and Diagnosis by Infrared Luminescence Transient Nanothermometry. *Adv. Funct. Mater.* **28**, 1803924 (2018).

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