Nanoparticle-based 2D thermal bioimaging technologies (NanoTBTech)

The Horizon 2020 FET-OPEN-funded project NanoTBTech is leading the way to new thermal bioimaging technologies

Temperature measurements for biomedical technology have a two-pronged promise to fulfil, namely the detection and spatial mapping of temperature gradients for the better and earlier detection of diseases, and the real-time monitoring of hyperthermia treatments with the aim of making this a general non-invasive and innocuous treatment technique. To tackle those two complex challenges, the key technology need is non-contact thermometry achieved with a sub-micrometre resolution while providing high sensitivity thermal readout in a real-time mode. The NanoTBTech FET-OPEN project is leading the global effort to make these dreams come true.

The main pathways of the NanoTBTech approach are luminescent nanoparticles with high thermal sensitivity values, which are nontoxic, have long-circulating 'stealth', are functionalised, and are tumour-targeted. They are also none-invasive (e.g., NIR-to-NIR deep-tissue luminescence thermometry), and enable a real-time readout, both *in vitro* and *in vivo*.

Simple and compact prototypes coupling luminescent 2D time-resolved thermal imaging and optical microscopy imaging under NIR irradiation (or AC magnetic field) to monitor local hyperthermia in cells (CSIC) and to study *in vivo* time-gated and 2D hyperspectral magnetic- or optically-gated thermal transient thermometry in-depth tumoural models (Biospace Lab/FIBIRYCIS) are currently being developed.

Accurate temperature readouts

Temperature plays a central role in the myriad of biochemical reactions regulating life. For instance, the intracellular temperature depends on cellular activity, including cell division, gene expression, enzymatic reactions, and pathological states. Cells have developed thermoregulation mechanisms to neutralise large external temperature changes and to maintain homeostasis of the body temperature upon exposure to hot or cold, an intriguing and not yet fully understood mechanism. NanoTBTech is tackling the issue of accurate temperature readouts, both *in vivo* and *in vitro*.

Although currently less mature than other, clinically battle-hardened imaging techniques (e.g., magnetic resonance imaging (MRI) or positron emission tomography (PET), thermal imaging based on luminescence requiring neither long scanning times or post-processing analysis, providing a real-time readout far from the reach of all those competing techniques.

Achievements

During the last year (the project started in September 2018) NanoTBTech has reported several remarkable achievements, including:

- The design and synthesis of novel luminescent nanoparticles (*Opt. Commun*. 452, 342, 2019, Vinca) and heater-thermometer nanostructures (*J. Phys. Chem*. C 124, 8938, 2020, CNRS);
- The standardisation of light-to-heat conversion efficiency measurements (WPAS);
- The development of a general theoretical framework of single ion luminescent thermometers (UU); and
- The assessment of *in vitro* (CSIC/UAVR) and *in vivo* (FIBIRYCIS) temperature.

A general theoretical framework of single ion luminescent thermometers

A general theoretical framework of single ion luminescent thermometers was developed offering simple, user-friendly guidelines for both the choice of an appropriate emitter and the embedding host material for optimum temperature sensing (*Adv. Theory Simul*. 10.1002/adts.202000176 2020). The results show that the optimum performance (thermal response and thermal sensitivity) around temperature T_0 is realised for an energy gap ΔE_{21} between thermally coupled levels (two and one) between $2k_BT_0$ and $3.41k_BT_0$ (k_B is the Boltzmann constant). Analysis of the temperature-dependent excited state kinetics shows that host lattices in which ΔE_{21} can be bridged by one or two phonons are preferred over hosts in which higher-order phonon processes are required. Such a framework is relevant for both a fundamental understanding of luminescent thermometers but also the targeted design of novel and superior luminescent (nano)thermometers.

Accessing the *in vitro* and *in vivo* temperature

At *in vivo* level, the consortium reported a simple and straightforward analysis of emission bands of nanothermometers providing accurate and reliable thermal readouts (*Adv. Funct. Mater*. 2020, 10.1002/adfm.202002730). NanoTBTech researchers have found experimental evidence that the unique optical properties of living tissues may cause relevant spectral distortions in the recorded emission spectra from luminescent nanothermometers. The practical consequence of this is the traditional way of getting thermal readouts may lead to large thermal readout errors. The research team have also explored several routes such as the fluorescence lifetime or emission intensity as thermal indicators, in both *in vivo* measurements and *in silico* simulations. These new approaches are exploited using also super-bright luminescent nanothermometers. One example, with great potential for *in vivo* measurements, uses Ag_2S superdots fabricated by ultrafast photochemistry (*Nat. Commun*. 11, 2933, 2020) – see Fig. 1a. Thanks to their superior performance, it is now possible to measure the temperature of internal organs such as the liver (see: Fig. 1b).

For *in vitro* temperature readouts, the NanoTBTech consortium reported the development of a unique tool for real-time bidimensional intracellular temperature mapping (*Nano Lett*. 20, 6466, 2020). The publication describes innovative thermal probes consisting of lanthanide-bearing polymeric micelles and their application in the temperature mapping of breast metastatic adenocarcinoma cells. An adapted fluorescence microscope was developed which permitted the real-time recording of the emission of the thermal probes, which is subsequently converted to the intracellular temperature. This singular approach allowed the observation of a higher temperature for the nucleolus (signed with red dots in Fig. 1c) relative to the rest of the cell (blue dots in the Fig. 1c) and inhomogeneous intracellular temperature progressions (Fig. 1d, histograms obtained from the red and blue points indicated in panel c).

The challenge ahead: measuring the temperature in the presence of an external magnetic field

The NanoTBTech consortium will proceed with *in vitro* and *in vivo* studies by quantifying the temperature gradients induced by a magnetic field. Under external alternating magnetic irradiation, magnetic nanoparticles localised in a target organelle inside the cell or a living tissue induce a controlled and localised temperature increase, a decisive step forward towards the development of efficient novel magnetic hyperthermia cancer therapies. For *in vitro*, preliminary results demonstrated that temperature gradients are observed after exposure to the magnetic field. At present, experiments are underway to determine if these localised thermal gradients are enough to induce the cell apoptosis, achieving consequently the desirable therapeutic effect.

NanoTBTech's partners

CICECO – Aveiro Institute of Materials, Universidade de Aveiro (UAVR – Portugal) is co-ordinating the

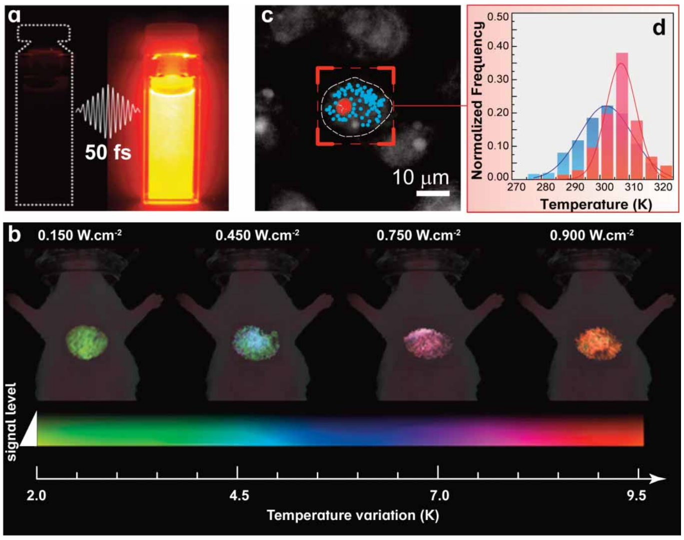

Fig. 1

project. Eight other partners participate in NanoTBTech:

- Fundacion para la Investigacion Biomedica del Hospital Universitario Ramon Y Cajal (FIBIRYCIS – Spain);
- Centre National de la Recherche Scientifique (CNRS – France);
- Agencia Estatal Consejo Superior de Investigaciones Cientificas (CSIC – Spain);
- Institut Za Nuklearne Nauke Vinca (Vinca – Serbia);
- Instytut Niskich Temperatur I Badan Strukturalnych Im. Wlodzimierza Trzebiatowskiego Polskiej Akademii Nauk (WPAS – Poland);
- Universiteit Utrecht (UU – The Netherlands);
- Nanoimmunotech Sl (NIT – Spain); and
- Biospace Lab SA (Biospace Lab – France).

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