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A new technique for measuring oxygen concentration in cells providing metabolic "fingerprints" of cancer cells

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In the field of biology it is a general consensus that cancerous cells often use other metabolic pathways, than corresponding healthy cells, and thereby consume less oxygen. If it is possible to measure the oxygen levels of cells by fluorescence microscopy this could be exploited as a future tool in clinical cancer diagnosis. However, measuring oxygen concentration of live cells is not totally straight forward. Traditional fluorophores have too short excited state lifetimes (nanoseconds) to be significantly influenced by molecular oxygen collisions, typically taking place in the microsecond time range.

TRAST for O2 monitoring

A new technique called Transient State Imaging (TRAST) is set to change the way oxygen concentration of cells is measured, by taking advantage of transitions to and from the dark lowest triplet state (T1) of fluorophores (1). T1 is a photo-induced, long-lived non-fluorescent state, found in essentially all fluorophore molecules. Combining fluorescence microsocpy with a modulated laser source, and systematically varying the modulation characteristcs, it is possible to extract kinetic information about the T1 state. The T1 state lifetime is proportional to the oxygen concentration. Imaging how the fluorescence intensity varies with the laser modulation can then provide direct information about the local oxygen concentration around the fluorophores. TRAST combines high detection sensitivity (by the fluorescence readout) with high environmental sensitivity (from the long lifetimes of the dark states analysed), and is feasible with a broad range of fluorophores. Even weakly emitting auto-fluorescent compounds can be used (2,3).

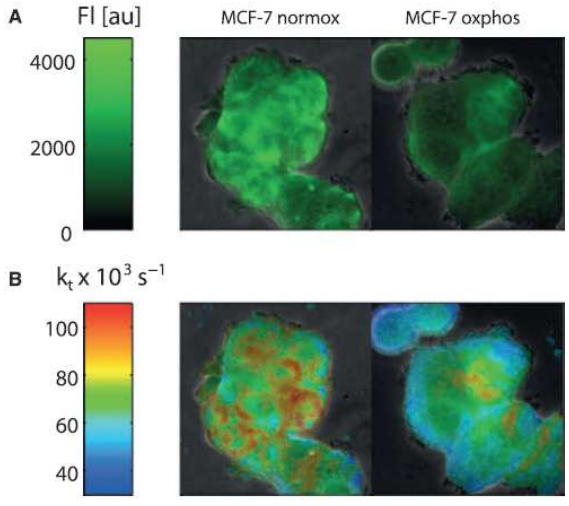

Figure 1: (A) Fluorescence intensity images of cells undergoing a cancer cell specific metabolism (left) and a normal cellular metabolism (right). (B) Corresponding TRAST images, showing the T1 decay rate in the cells. The decay rates are lower in cells with normal metabolism, indicating lower local oxygen concentrations, and thus higher oxygen consumption

Cancer-specific cellular metabolism

Using the high triplet yield dye Eosin Y, TRAST imaging was applied to cells from different breast cancer and fibroblast cell lines (1), Cells from the breast cancer cell line MCF-7 were cultured in different media, driving the cells either into a cancer cell specific metabolism, or into a metabolism typical for normal cells. The differently cultured cells were imaged with TRAST to check for differences in their oxygen consumption rates. Figure 1 illustrates these results and shows that the MCF-7 cells undergoing normal cellular metabolism consume more oxygen than the cells undergoing a cancer cell specific metabolism.

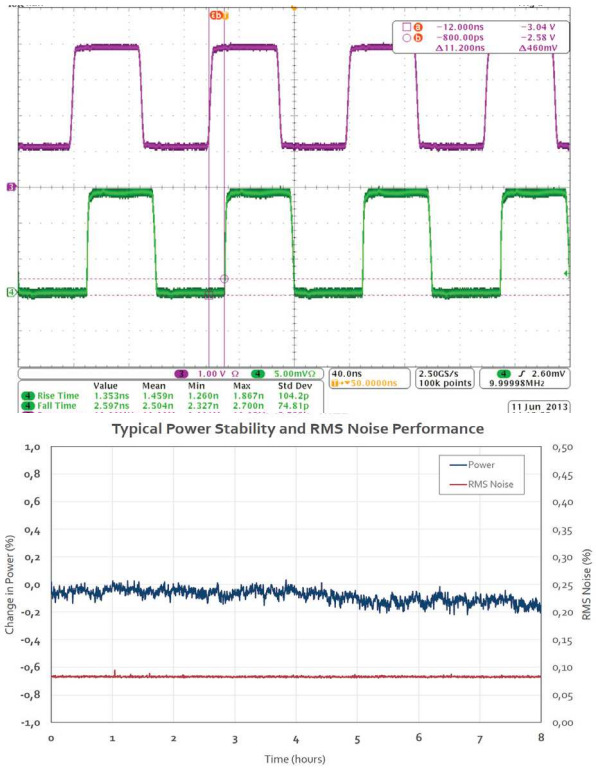

Figure 2: Typical modulation waveforms (A) and noise performance (B) of lasers from the Cobolt 06-01 Series

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Lasers for TRAST and fluorescence microscopy

The TRAST technique is a promising way to measure oxygen concentration in living cells. Various diseased cells show altered metabolism and oxygen consumption. TRAST can thereby offer a means to distinguish normal cells from diseased cells, such as cancer cells, or infected cells. Compact and cost effective lasers can be used. This naturally simplifies and reduces the cost of instruments for future use in e.g. detection and analyses of cancers. Lasers for TRAST need to be reliable and robust however, with excellent performance characteristics in terms of power and TEMoo beam stability and quality. It is clearly also an advantage if the lasers can be directly modulated. All of these specifications can be met in the Cobolt 06-01 Series of plug and play modulatable cw lasers, offering a full spectrum with powers up to 250 mW, as well as the Cobolt Modulated DPSS Lasers Series with integrated AOM.

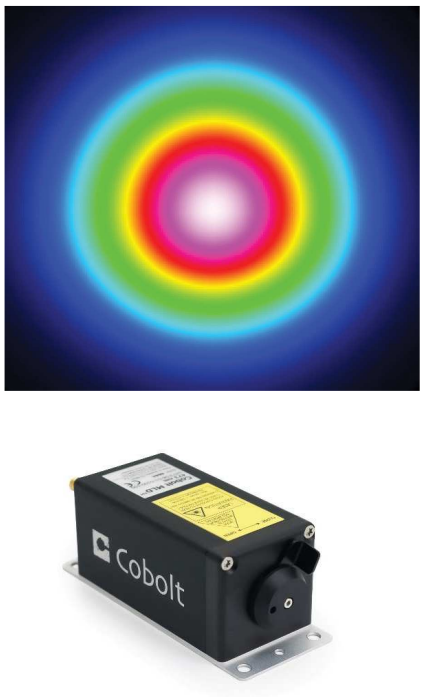

Figure 3: Typical beam profile of the Cobolt 06-MLD laser (top), Cobolt 06-MLD laser (bottom)

Conclusions

The TRAST technique has been demonstrated to be a feasible method for monitoring oxygen concentration in living cells. In turn, this can be used as a precursor to detecting cancerous cells in new sensitive ways, via their distinctive metabolic features/fingerprints. A wide variety of Cobolt lasers are ideally suited to such inspiring applications.

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References:

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(3) Tornmalm J, Widengren J "Label-free monitoring of ambient oxygenation and redox conditions using the photodynamics of flavin compounds and transient state (TRAST) spectroscopy", Methods, in press 2018