

INTRACELLULAR OXYGEN MEASUREMENT WITH SUBCELLULAR RESOLUTION USING WIDEFIELD FREQUENCY DOMAIN FLUORESCENCE LIFETIME IMAGING MICROSCOPY (FD-FLIM)

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Oxygen affects cellular responses in different aspects such as metabolic pathways and plasma membrane integrity. Therefore, accurate measurement of intracellular oxygen concentration is an essential task in biomedical research. Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) perchlorate (Ru(dpp)) has been widely used as a probe for luminescent detection and quantification of oxygen. The fluorescence intensity and lifetime of the dye ($\lambda_{\text{ex}} = 425\text{-}475\text{ nm}$, $\lambda_{\text{em}} = 575\text{-}625\text{ nm}$) are strongly reduced and shortened by the presence of molecular oxygen due to dynamic quenching. Unlike the intensity values can be affected by the fluorophore concentration, photo-bleaching, autofluorescence, and excitation light intensity, the fluorescence lifetime only depends on the presence of quenchers. As a result, the lifetime measurement is more accurate and straightforward when considering quantitative interpretation of the results. In this research, intracellular oxygen concentration with subcellular resolution is measured by a widefield frequency domain fluorescence imaging microscopy (FD-FLIM) system [1]. Here, the ruthenium complex (Ru(dpp)) doped solid silica nanoparticles (RuNPSiNPs) with diameters around 70 nm are used as sensors for oxygen measurement in living human lung adenocarcinoma epithelial cells (A549) [2]. Due to the

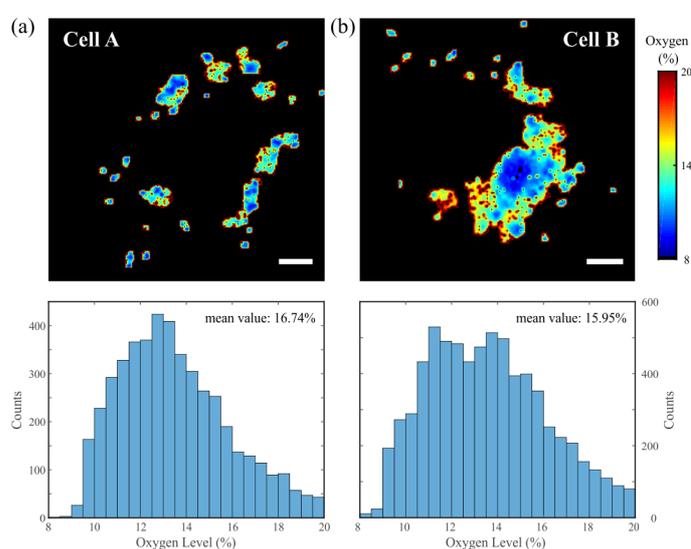


Figure 1: Contour plots (Top row) and histograms (Bottom row) of intracellular oxygen tensions in two different cells A (a) and B (b). Scale bar: 5 μm .

advantages provided by the widefield technique, the image acquisition speed of the system is faster and the exposure time can be reduced significantly resulting in less photo bleaching. Moreover, the measuring processes are less sensitive to surrounding environments. Therefore, it is suitable to be applied for time-lapsed observation to study transient phenomena. We believe the measurement technique developed in this research can be an efficient tool that greatly helps biomedical scientists to better understand intracellular oxygen distribution.

[1] H. M. Wu et al., "Widefield frequency domain fluorescence lifetime imaging microscopy (FD-FLIM) for accurate measurement of oxygen gradients within microfluidic devices," *Analyst*, **144**, 3494–3504 (2019).

[2] L. Ding et al., "Luminescent silica nanosensors for lifetime based imaging of intracellular oxygen with millisecond time resolution," *Anal. Chem.*, **91**, 15625-15633 (2019).