

# INCREASING THE NUMBER OF DETECTABLE PHOTONS DURING WHOLE ANIMAL IMAGING: FLUORESCENCE BY RADIATIVE EXCITATION EMISSION OF QUANTUM DOTS BY BIOLUMINESCENT BACTERIA

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Genetically targeted luminescence is a powerful technology enabling a wide variety of biological processes to be visualized non-invasively within intact living organisms, and especially in small animal models such as rodents. Consequently, bioluminescence has been applied to studies on infection, tumor development, and calcium signaling. However, many useful bioluminescent probes, (e.g. aequorin and bacterial/firefly luciferases) emit light between 400 – 650 nm. Inasmuch as this overlaps strongly with the major absorption spectra of mammalian tissues constituents including oxyhemoglobin, de-oxyhemoglobin and melanin the detection of luminescent light in vivo can be challenging due to light scatter and photon absorption. This has provoked efforts to improve the sensitivity of whole animal bioluminescence imaging techniques by red-shifting emitted light to a range that is less affected by these problems [1, 2]. Along these same lines previous studies have reported chemiluminescence resonance energy transfer (CRET) using luminol-H<sub>2</sub>O<sub>2</sub> as a donor and fluorescent quantum dots (QDs) as an acceptor [3, 4]; wherein conditional upon the donor and acceptor molecules being in close proximity (1 – 10 nm) non-radiative (dipole-dipole) energy transfer occurred ensuring a red-shift in the detected wavelength [4]. Here we present a singular exception to CRET we term Fluorescence by Radiative Excitation Emission (FREE). We demonstrate in vitro and in vivo that QDs can be efficiently excited by blue-green photons emitted from bioluminescent bacteria expressing conventional bacterial luciferase (lux operon). We show that this phenomenon does not involve, and is distinct from, resonance energy transfer because it occurs over long distances ( $\mu\text{m}$  – cm) indicating that the donor and acceptor molecules do not need to be in molecular proximity. Further, the detection of red wavelength shifted fluorescent emission from QDs is entirely dependent upon the presence of bioluminescent bacteria. Finally, we demonstrate the utility of FREE for bioluminescent *E. coli* and Qtracker 705 *in vitro* inasmuch as it yields a red-wavelength shift that definitively and greatly improves the sensitivity of bioluminescence detection in vivo.

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