Tuning Luminescence Resonance Energy Transfer for Lifetime-Based Multiplexing Detection of Nucleic Acids

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Accurate and rapid detection of specific nucleic acids plays a critical role in disease diagnostics, especially in recent years as demands in public health soar during the pandemic. Polymerase chain reaction (PCR) is the gold standard due to its powerful capability of specifically amplifying the target sequence, offering ultimate sensitivity to detect even one single strand of nucleic acids in the sample. Nevertheless, a bottleneck for PCR detection lies in multiplexing to simultaneously identify different nucleic acids in the sample, which capability is limited by spectral crosstalk associated with conventional fluorescence color-based assays.

To overcome this limitation, we have developed a novel strategy for multiplexing PCR detection using the temporal dimension of luminescence lifetimes. It is based on competitive homogenous assays involving Cy5.5-labelled reporter oligonucleotides, which are complementary to the primers and a template sensitization oligonucleotide labelled with a europium complex (DTBTA-Eu), expect for a single-base mismatch introduced in the latter [1]. During PCR, the presence of any target sequence in the sample will consume its respective primers through the thermal cycles, so that the corresponding reporter oligonucleotide hybridize with nothing but the sensitization sequence at the end. This brings together the DTBTA-Eu and the Cy5.5 to close proximity, enabling luminescence resonance energy transfer (LRET) from the former to the latter. To enable multiplexed detection based on the lifetime, the template sensitization oligonucleotide is composed of sections that bind different reporter oligonucleotides, resulting in designated luminescence lifetime depending on the LRET distance between the dyes [2]. Moreover, the sample is processed using a microfluidic chip to generates tens of thousands of droplets [3], so that in each droplet only one type of reporter oligonucleotides will form double strands with the sensitization oligonucleotides to ensure one lifetime at a time. The droplets are then subject to lifetime measurement using a purpose-built time-resolved luminescence image cytometry. We are currently optimizing the annealing temperatures of the thermal cycles to minimize non-specific background, improving the precision in lifetime reading to increase multiplexing capacity, and exploring various samples to demonstrate our technique for practical disease diagnosis.

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