A Fiber integrated optofluidic platform for sensitive microRNA FRET detection

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We demonstrated a fiber integrated optofluidic platform to perform miRNA detection with high sensitivity and specificity based on FRET principle. Recently miRNA has attracted extensive interest as a biomarker, providing important evidence for early disease diagnosis and post treatment [1]. Traditionally, numerous analytical approaches such as northern blotting [2], micro arrays [3], and real-time quantitative polymerase chain reaction [4] have been developed for miRNA detection. However, the existing methods have many technical challenges, for example, the concentration of miRNA in vivo is normally at low level, and the short sequence and easy degradation by RNAase makes it difficult for PCR. Therefore, we developed an innovative optofluidic platform that combines the advantage of droplet microfluidics and optofluidic technology, and provides a low-cost, compact and high specificity approach for identification and quantification of miRNA using FRET principle. Single-nucleotide differences within miRNA family can be distinguished by a novel Y-junction design of donor, receptor and miRNA sequence. We performed one step miRNA detection with simple device operation, and our platform achieved target miRNA identification at a low concentration and high specificity.

The device was composed of integrated optical fibers and a droplet generation structure, as shown in Figure 1. We employed a simple and robust insertion method of the excitation and detection fibers to achieve highly sensitive FRET detection. The droplet generation structure was used to encapsulate donor, receptor and miRNA sequence in pico-liter droplets. During the fast mixing of probes in droplets, the donor, receptor and miRNA formed a stable Y-junction complex and the FRET process occurs. When the droplets flow through the detection area, Cy3 linked fragment is excited by the fiber and transfers energy to Cy5 linked fragment in the Y-junction, and the emission was collected by the detection fiber. In order to ensure maximum collection of droplet fluorescence, we used multilayer photolithography to align the center of the flow channel, excitation fiber and collection fiber, and a two-layer PDMS device was replicated from the mold by soft-lithography. The integrated fiber delivered light to a dual wavelength PMT detection system. Time series data of dual channel was acquired for many droplets in Figure 2. According to ratio of fluorescent intensity, quantitative analysis of the miRNA in Y-junction complex system has a linear dynamic range from 5nM to 100nM, when dnor and receptor were kept at 100nM concentration. The detection limit for miRNA in our platform can reach as low as 5nM. By this novel Y-junction structure design, similar miRNAs within miRNA family can be distinguished.

In conclusion, we have successfully developed a fiber integrated optofluidic platform for miRNA FRET detection. The fiber integrated detection scheme avoided the bulk alignment and adjustment of the optical system, and achieved dual fluorescence simultaneous detection. Dynamic detection range for miRNA was from 5nM to 100nM, and the detection limit was 5nM. Using FRET principle, similar miRNAs can be distinguished by Y-junction structure design. Compared with conventional microplate reader, our platform could reduce the sample volume from 200ul to 500pl, which is favorable for low level miRNA samples in vivo. Moreover, reaction system was encapsulated in droplets, preventing the degradation of RNAase in the environment. Our platform proposed here offer a low-cost, robust, compact, high sensitivity and specificity method for identification and quantification of miRNA, and has potential for various application in biomedical research and clinical diagnosis.

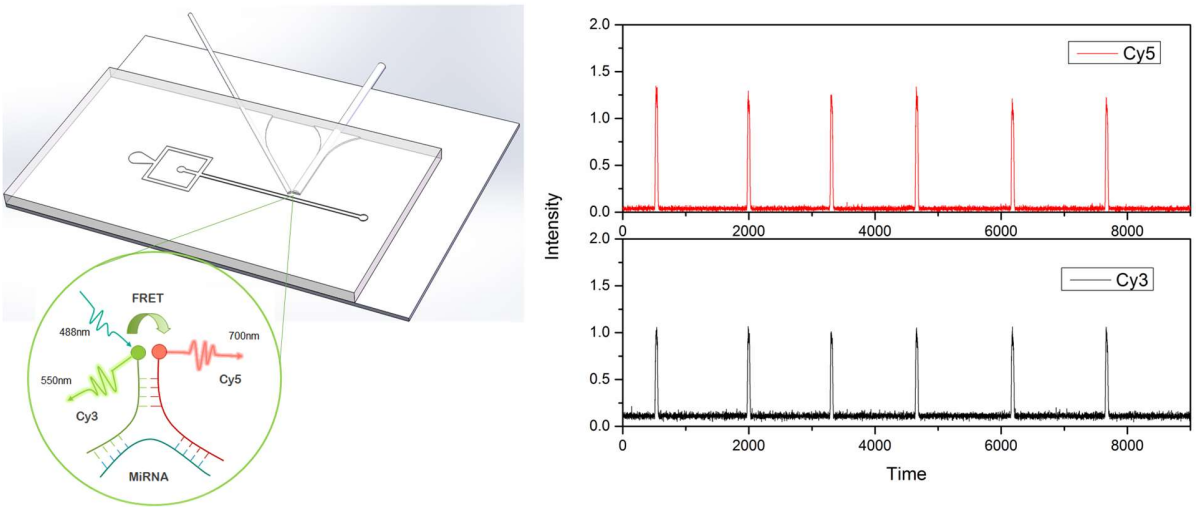

Fig.1 (a) Schematic design of a fiber integrated optofluidic platform for sensitive microRNA FRET detection;(b) Time series of dual wavelength signal for Cy3 and Cy5

Reference

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