Supplementary Materials

Experimental details

Detection system

The completed microfluidic device was placed onto a translation stage and appropriately aligned with a custom built fluorescence detection system. Briefly, the spectroscopic setup consists of 488 nm CW air-cooled argon ion (Omnichrome; Melles Griot, Cambridge, UK) and 632.8 nm HeNe lasers (Thorlabs, Ltd, UK). Beam steering Optics are used to bring the laser beams into an objective lens. A plano-convex lens (f = 300 mm, Thorlabs, Ltd, UK) is positioned at approximately 30 cm away from the objective lens and is used to focus the incident laser beam into a small spot at the back aperture of an objective lens. A custom-made dual-band dichroic mirror (z488/633rdc, Chroma Technology Corporation, USA), which is installed to permit simultaneous two-colour excitation by the 488 nm and 632.8 nm lasers, is used to reflect the laser beams into a 100× oil immersion objective lens (1.3 NA, f = 1.6 mm, Carl Zeiss Ltd, UK). The objective focuses the excitation light onto the microfluidic channels. Subsequently, fluorescence emission is collected with the same objective and transmitted through the same dichroic mirror. Any residual excitation light is removed using a custom-made dual-band emission filter (z488/635, Chroma Technology Corp, USA), positioned beneath the dichroic mirror. The fluorescence is then spectrally separated by another dichroic mirror (z630rdc, Chroma Technology Corp., USA). In the first channel (green detector), fluorescence reflected by the dichroic mirror is further filtered by an emission filter (hq540/40m, Chroma Technology Corp., USA) and focused by a plano-convex lens (f = 25.4 mm, Thorlabs, Ltd, UK) onto an avalanche photodiode (SPCM-AQR-141, EG&G Canada, Vaudreuil, Quebec, Canada). In the second channel (red detector), fluorescence passed through the dichroic mirror and is filtered by another emission filter (hq640lp, Chroma Technology Corp., USA) and focused by a plano-covex lens (f = 25.4 mm, Thorlabs, Ltd, UK) onto a second avalanche photodiode (SPCM-AQ-13, Perkin Elmer, Canada). The APD detectors operating in single-photon counting mode convert single photon events into TTL pulses (1 photon equals 300 ps 5 volt pulse). A digital counter (PCI 6601, National Instruments, UK) in combination with a programme written in LabView is used to control and perform data acquisition.