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Highly parallelized detection of single fluorescent molecules: simulation and experiment¹ BRIAN K. CANFIELD, JASON K. KING, WILLIAM N. ROBINSON, WILLIAM H. HOFMEISTER, LLOYD M. DAVIS, Center for Laser Applications, University of Tennessee Space Institute — We are developing an ultrasensitive, fluorescence-based detection system in highly parallel microchannels. Multichannel microfluidic devices have been fabricated by direct femtosecond laser machining of fused silica substrates. We approach single-molecule detection sensitivity by introducing dilute aqueous solutions ($\sim pM$) of fluorescently labeled molecules into the microchannels. In a custom-built, wide-field microscope, a line-generating red diode laser provides narrow epi-illumination across a 500 μ m field of view. Fluorescence is detected with an electron-multiplying CCD camera allowing readout rates of several kHz. Rapid initial assessment is performed through digital filtering derived from simulations based on experimental parameters. Good agreement has been shown between simulation and experimental data. Fluorescence correlation spectroscopy then provides more detailed analysis of each separate channel. Following optimization, microfluidic devices could easily be mass-produced in low-cost polymers using imprint lithography.

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Brian K. Canfield Center for Laser Applications, University of Tennessee Space Institute

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