Short wavelength, low coherence, low noise lasers for improved resolution of submicron particles

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Background

A major barrier to single submicron particle analysis and sorting is the detection of small particles above the noise level of the cytometer system. Although cytometer "noise" is due to many factors, the laser source can be a significant contributor to the overall system noise. To improve analyses of submicron particles, particularly extracellular vesicles and viruses in the 50-500 nm size range, we sought to identify laser attributes that could improve signal-to-noise resolution for nanoparticle detection by flow cytometry.

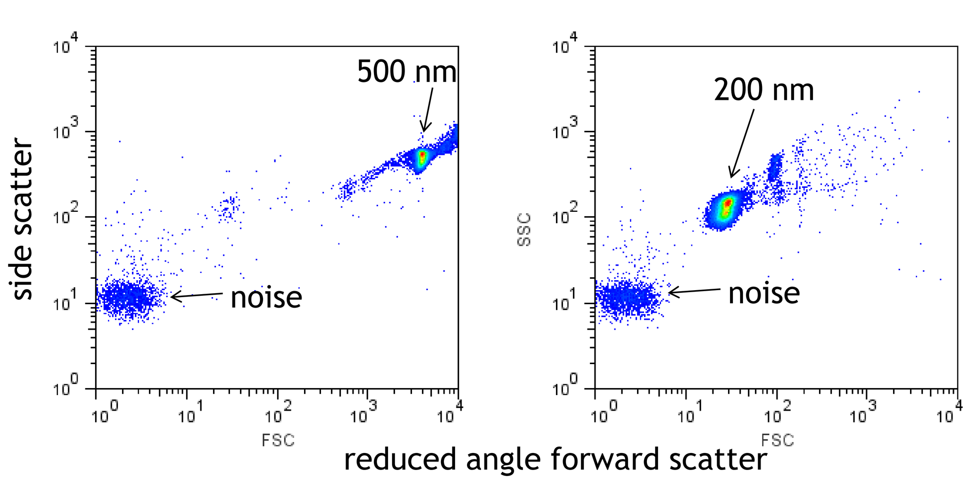
Methods

We employed laser sources with several unique attributes. First, the lasers tested in this study emitted at wavelengths shorter than the traditional 488 nm sources normally used for forward and side scatter measurement. Lower wavelength laser light would be theoretically expected to improve small particle resolution. Blue 450 nm, blue-violet 420 nm and violet 405 nm laser diode sources were compared to traditional blue-green 488 nm laser sources. Second, these laser sources used extremely low noise control electronics and power supplies to minimize laser noise to an RMS level of less than 0.05% for the 20 kHz to 20 MHz range. Third, these lasers emitted at lower coherence levels than most single wavelength lasers used in flow cytometry, with flat-top spectra. Active mode phase diversification of the laser emission produced lower levels of "speckle" than lasers with higher coherence levels. Laser speckle is a potential source of noise for flow cytometry. These modules also minimized laser speckle by other mechanisms, including internal combining of two beams with differing angles of polarization and generation of multimode beam geometries with sum frequency mixing of the resulting longitudinal modes. For the blue 450 nm module, the active mode phase diversification could be switched on and off, allowing comparison of small particle resolution for both operating modes.

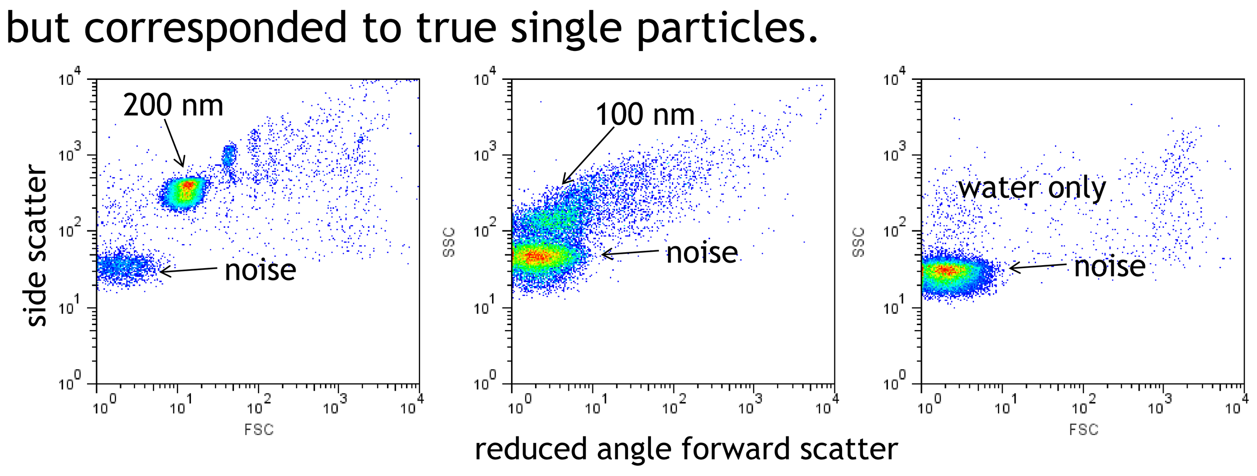
These specialized laser modules were compared to conventional laser diodes at the same wavelengths, and to a blue-green 488 nm also manufactured by PIC to the same low noise specifications as the shorter wavelength sources. All lasers were tested on a BD Influx cell sorter (BD Biosciences, San Jose, CA) equipped with small particle detection optics. Laser beam shapes, which did differ somewhat between lasers, were circularized using anamorphic prism pairs and measured using a beam profiling camera. Each laser installation was tested using 100 and 200 nm polystyrene or latex microparticles (Polysciences and Thermo Fisher Life Technologies).

Conventional laser systems

Using a "conventional" low noise MPBC Communications 488 nm fiber laser, installed on a BD Influx, 500 and 200 nanometer PS particles could be readily distinguished from background, on both the forward and side scatter axes .



In this configuration 100 nanometer particles can be distinguished from background using side scatter but not forward scatter. A low sample differential and inclusion of noise in the analysis indicated that this signal was not due to high-event coincidence, but corresponded to true single particles.



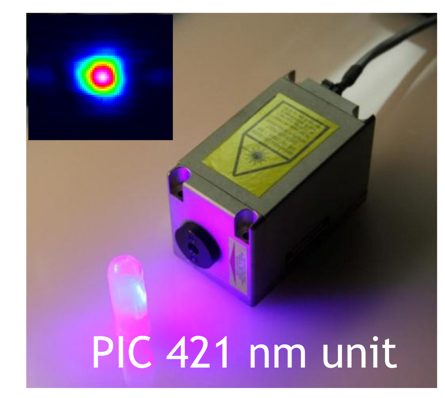
This resolution is equivalent to other Influx systems.

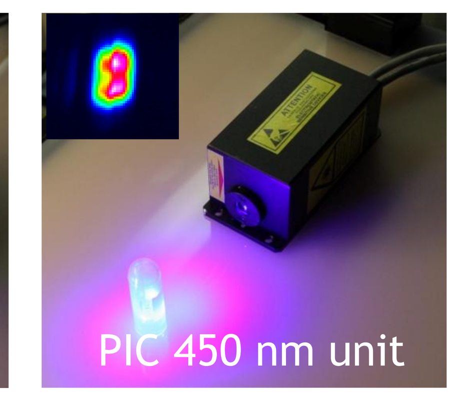
To explore whether improving the laser system could improve small particle resolution, a group of specially designed Pavilion Integration Corporation lasers with Nichia diodes were installed on the Influx in place of the conventional 488 nm source. These lasers (1) **emitted at shorter wavelengths than 488 nm**, (2) were equipped with **extremely low noise power supplies**, and (3) **designed with lower coherence, flat-top spectra and active mode phase diversification, to reduce laser "speckle"**. All of these characteristics could conceivably reduce laser noise and enhance submicron particle resolution.

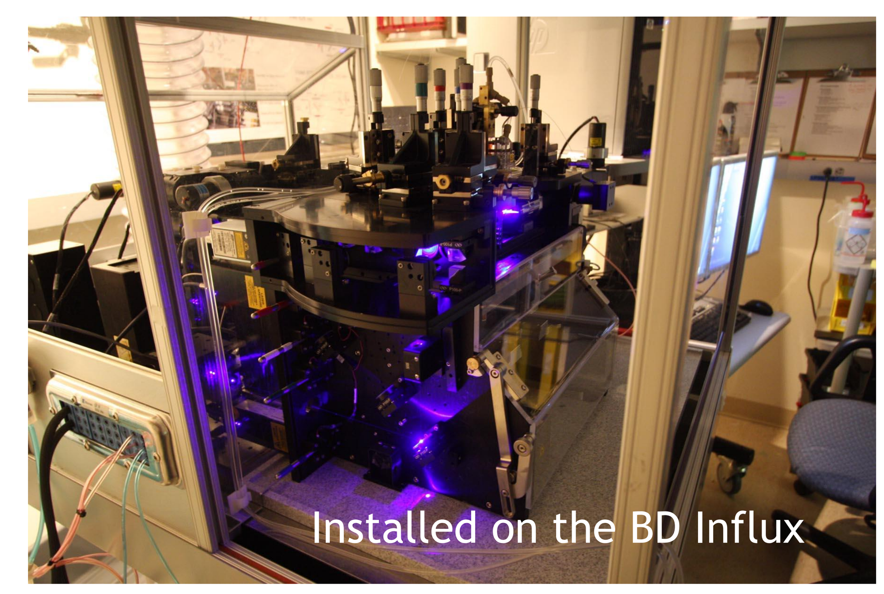
This work was supported by intramural research funds provided by the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services.

Results

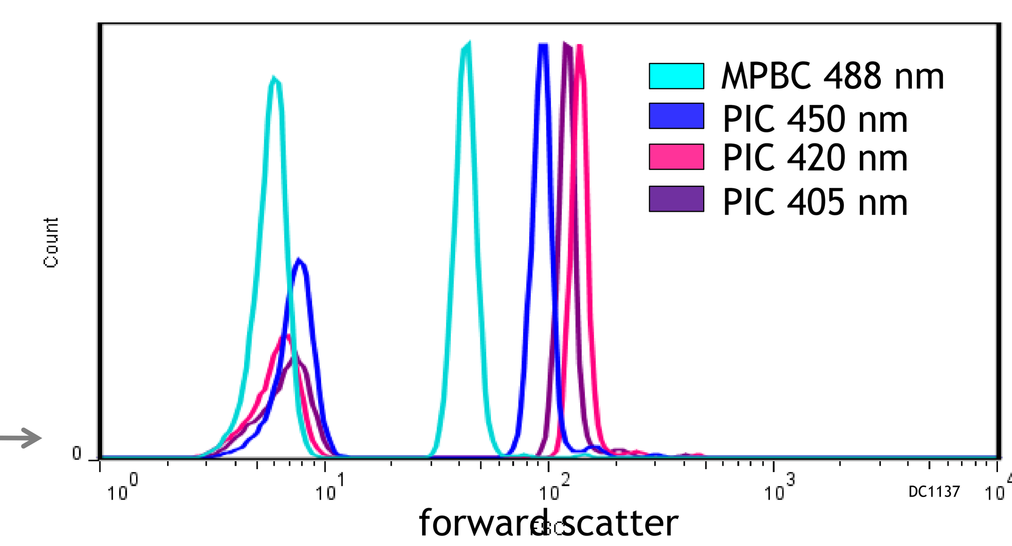
High power 405 nm, 420 nm, 450 nm and 488 nm laser modules were built by Pavilion Integration Corporation specifically for this study. These units were multimode, with beam shaping optics to render the final laser beam in a "blade" flat-top beam shape of roughly 0.7 mm x 2 mm in profile. This beam could be made nearly round (beam scans in insets) using the anamorphic prism beam shaping optics on the Influx. They were equipped with low electronic noise (WhisperIT) power supplies. All possessed low coherence relative to conventional laser sources, with active diversification of the wavelength to minimize laser "speckle". The blue 450 nm unit was configured to turn this active mode phase diversification on and off, so that its effect of laser noise and particle resolution could be observed.





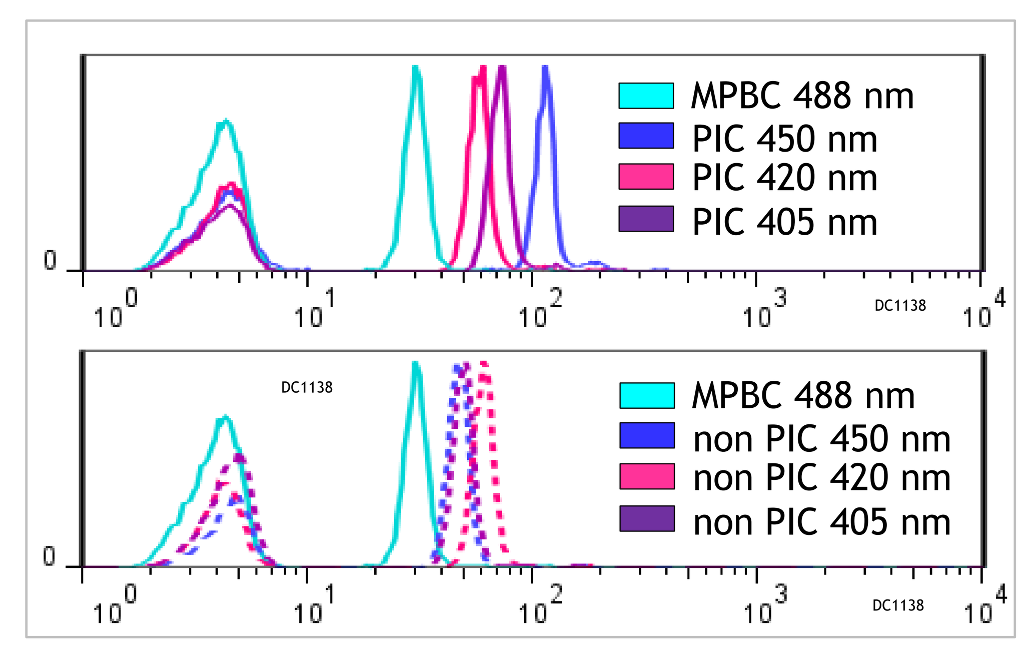


Left, Polyscience PS 200 nm particles analyzed on a BD Influx with 488nm, 405 nm, 420 nm and 450 nm laser all emitting at 100 mW. The shorter wavelengths show improved signal-to-noise ratio for forward scatter.

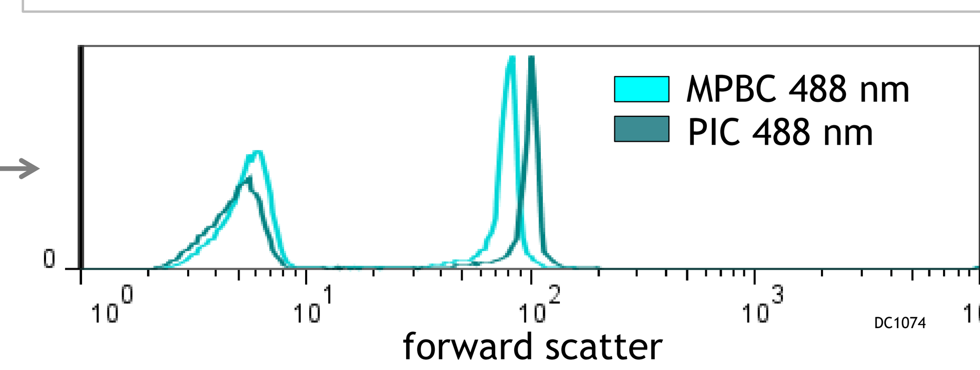


Left, PS 200 nm particles analyzed on a BD Influx with MPBC 488nm, PIC 405 nm, PIC420 nm and PIC 450 nm laser all emitting at 100 mW.

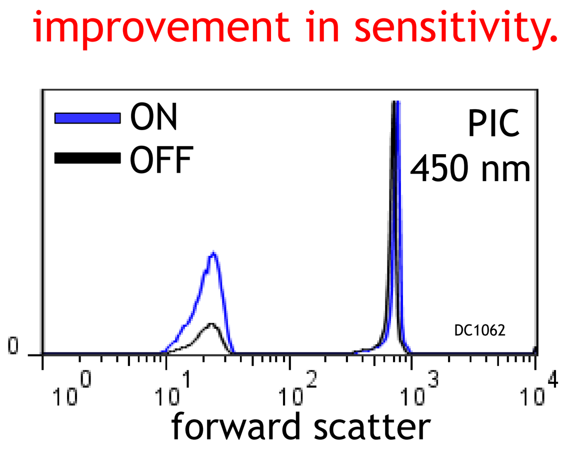
Left, The same experiment with conventional 405, 420 and 450 nm sources at 100 mW. The low noise, low coherence sources showed slightly better forward scatter resolution, although the precise mechanism is unclear.

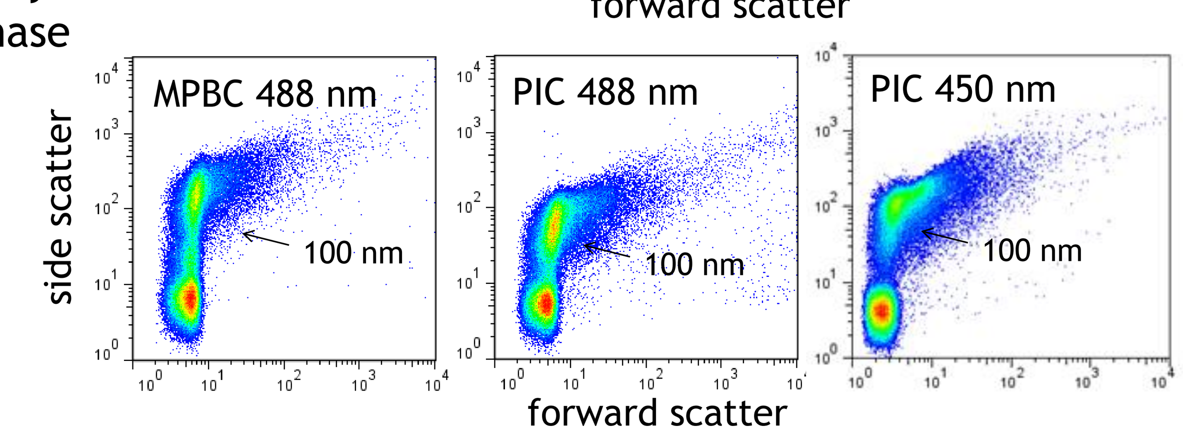


Left, PS 200 nm particles analyzed on a BD Influx with MPBC and PIC 488 nm laser sources. The PIC 488 nm source showed only a slight improvement, suggesting that laser attributes other than wavelength were less important.



Below, PS 200 nm particles analysis at 450 nm with active mode phase diversification ON or OFF. Background noise increased in the the ON mode but no improvement in sensitivity.





Above, Nano 100 nm latex particle analysis using PIC lasers (no side scatter filter). The decreased wavelength may be less critical for side scatter resolution of <200 nm objects.

While power supply noise and low coherence might reduce background noise, the **shorter wavelengths** of these units seemed to be the major factor in their improved resolution of forward scatter for submicron objects.