



## High Power 380 nm UV Laser for Flow Cytometry Applications

### Introduction

Stem cell research is fueling an increased demand in UV excitation in flow cytometry using dyes like Hoechst 33342 for Hoechst side population studies. So far instrument designs are based on large and inefficient water-cooled Argon/Krypton ion lasers or quasi-CW DPSS lasers, which has limited the use of UV based flow cytometers due to their size and cost. We will discuss experiments using Hoechst 33342 and DCV and compare 355 nm vs 380 nm laser excitation, using a high power 380 nm laser diode.

### UV Laser Technology

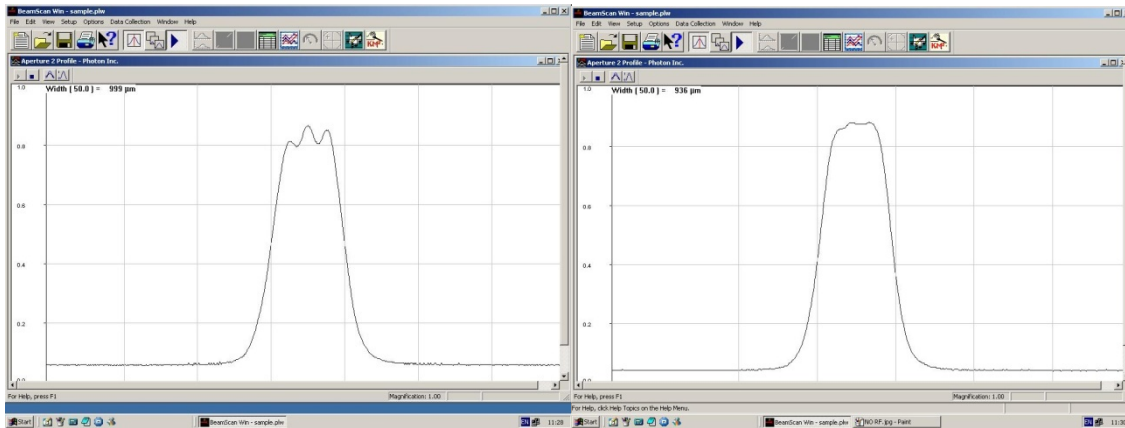
In the past the only viable UV laser sources for flow cytometry applications were water-cooled Argon/Krypton ion lasers. Such lasers are large in size and very inefficient and make the instrument design and operation cumbersome. A first improvement came several years ago with the availability of quasi-CW DPSS lasers. These modelocked lasers produce a train of ps pulses at very high repetition rates, which allows the conversion of the IR laser light into 355 nm UV light due to the high peak power of these ultrashort pulses, but appears to the fluorescence detection as a quasi-continuous-wave excitation. Such lasers can produce a couple of 100 mW of output power at 355 nm, but are still relatively large in size (about 20,000 cm<sup>3</sup>, laser head and power supply combined) and inefficient (electrical utility requirement < 750 W). Due to the still complex cavity design these quasi-CW DPSS lasers have enabled only a slight cost-of-ownership reduction compared to ion lasers. As such the use of UV lasers in flow cytometers is still limited to high end instruments.

The promise of smaller and more efficient UV lasers came with the availability of 380 nm GaN based laser diodes. 380 nm compared to 355 nm is not ideally matched to the fluorescence dye absorption (the excitation cross section of UV dyes for 380 nm is typically only half the excitation cross section at 355 nm) combined with only low output power levels of 20 mW so far has limited the use of these lasers in biotechnology applications. Now higher power levels on the order of 100 mW are available from GaN diodes from broad emitter diode structures. Of course a laser beam directly from a broad emitter diode is not directly usable. In the following section we will describe how to stabilize the beam from such a broad emitter diode and turn it into a useful laser beam for applications like flow cytometry.



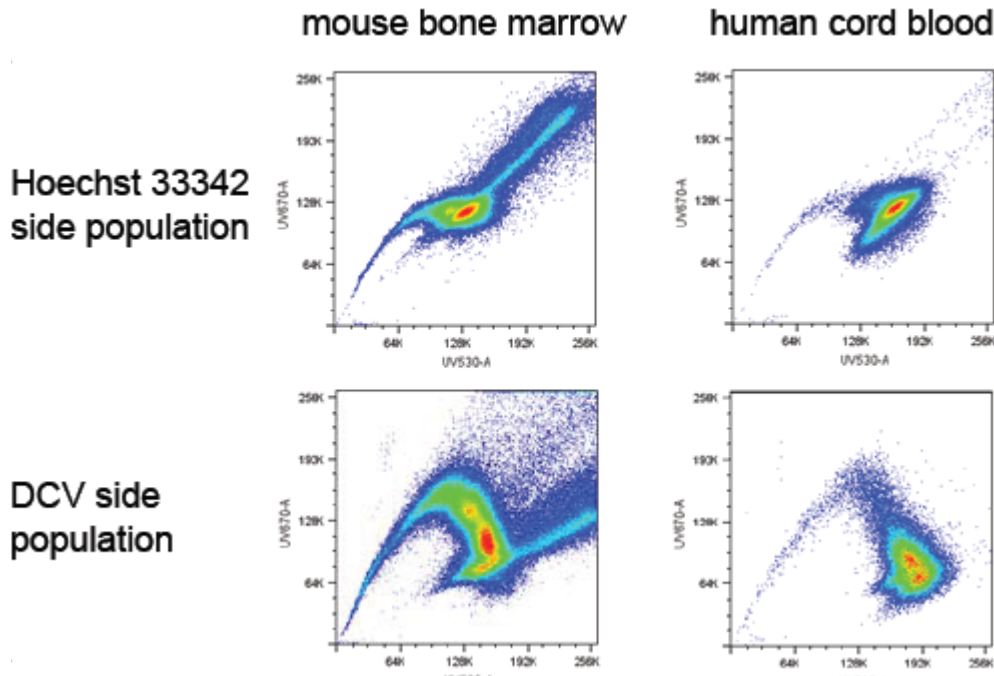
Laser diodes exhibit longitudinal mode hops that result in power instabilities and noise. By just stabilizing the temperature such mode hops can never be completely avoided. Eventually the diode will age and mode hops will occur due to a change in diode current to compensate for an output power degradation. PIC's WhisperIT® technology has been proven to eliminate the mode structure of laser diodes and therefore guaranteeing a low noise operation under all operating conditions and over the life of the laser.

In the case of broad area diodes operation on several transversal modes causes instabilities and large intensity variations of the beam intensity profile. Using the same technology PIC can stabilize and smooth out the transversal intensity profile of broad area diodes as demonstrated by the following tests using a 250 mW broad area 405 nm laser diode. The left intensity profile was taken without PIC's WhisperIT® technology and shows intensity modulation across the laser beam. Using WhisperIT® technology the intensity modulation disappears and the laser intensity profile is converted to a flat-top shaped beam.



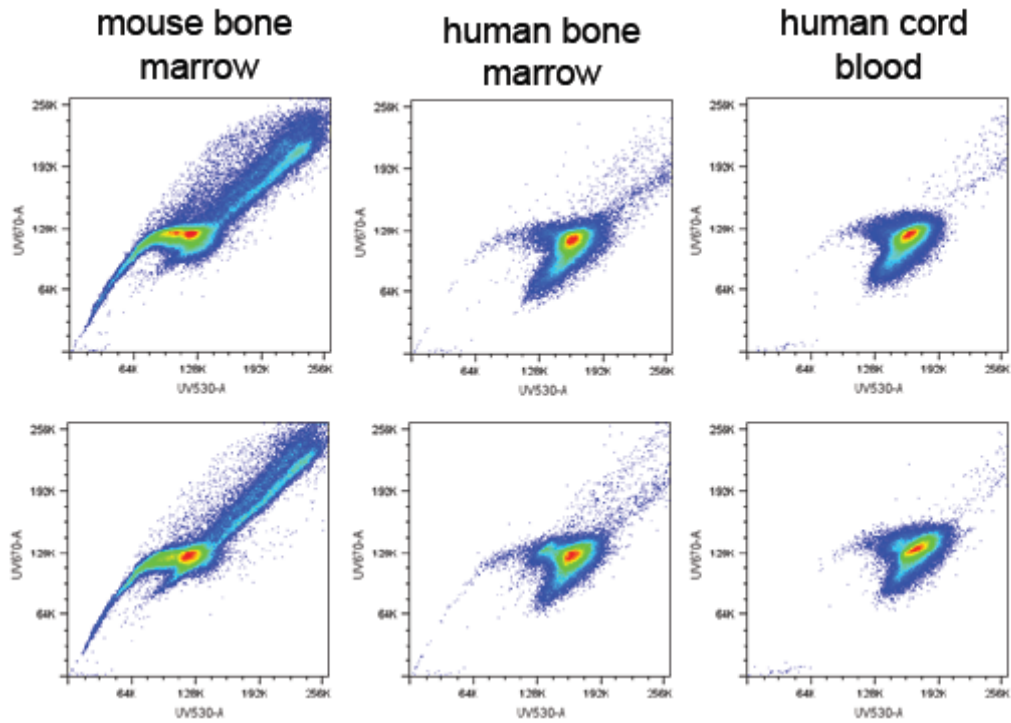
## Applications in Flow Cytometry

The data presented in the following section have been achieved by Bill Telford, NIH using the 380 nm laser described above at 100 mW with a 3:1 aspect ratio in commercially available flow cytometers with virtually no modification. We would like to thank Bill Telford for performing the tests and making these data available to us.



The measurements above were taken with a Whisper380 laser using Hoechst 33342 and DCV fluorescence dyes for mouse bone marrow and human cord blood samples. The experiments below are comparing the excitation of different cell cultures with 2 different laser types. The top row was obtained with a 355 nm modelocked laser at 20 mW, the bottom row used the Whisper380 laser at 100 mW.

These experiments show clearly that even so 380 nm is not perfectly matched to the dye absorption of some dyes, the higher power available now from broad area diodes at 380 nm compensates for that. 380 nm can now be used to excite different UV dyes and delivers comparable results to bigger, less efficient and more costly 355 nm DPSS lasers.



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