# Monitoring tumor burden by multicolor in vivo flow cytometry

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## 1. Introduction

In vivo measurement of tumor burden, both in cancer research models and in patients, is an important parameter for the accurate assessment of disease progression and the response to therapeutic intervention [1]. Several in vivo imaging modalities have been utilized in the assessment of tumor burden, including functional magnetic resonance imaging, computer tomography and positron emission tomography [2, 3], fluorescence imaging [4, 5], intravital microscopy [6] and bioluminescence imaging [7]. More recently, the detection/quantification of circulating cancer cells has been explored as a method to evaluate tumor burden in the context of assessing disease stage, prognosis as well as monitoring disease progression following therapeutic intervention in cancer patients [8, 9]. Clinically, various ex vivo assays have been developed to detect cancer cells shed in circulation by primary tumors, including breast cancer, prostate cancer and small-cell lung cancer [10, 11].

In vivo flow cytometry has been developed as a method for real-time detection of circulating cancer cells injected into the circulation of experimental animals. The method does not require extraction of blood samples and is therefore well suited for long-term monitoring of circulating tumor cells. In this report, we report on the development of a multichannel in vivo flow cytometer to detect and quantify circulating cancer cells as a means of assessing the tumor burden in animal models.

## 2. Materials and methods

Development of a multichannel in vivo flow cytometer Multichannel in vivo flow cytometry combines the principles of confocal detection and flow cytometry in order to enable the real-time detection of fluorescently labeled cells circulating in a live animal. The system can be applied for the dynamic and simultaneous monitoring of multiple populations of circulating cells, which can be targeted and labeled with multiple fluorescent markers and probes. To accomplish this, light from up to three separate excitation lasers was focused by a cylindrical lens and then imaged across a blood vessel to form an excitation slit. Figure 1 illustrates the concept of the multichannel in vivo flow cytometer. When fluorescently labeled cells were flown through the excitation slit, the emitted fluorescence signal emitted was confocally detected by a photomultiplier tube in each of the detection channels of the system. The collected PMT signal was sent to an analog-to-digital converter to be digitized and then stored on a PC to be analyzed by Matlab software in order to identify cell peaks and extract quantitative information on the number of cells passing.

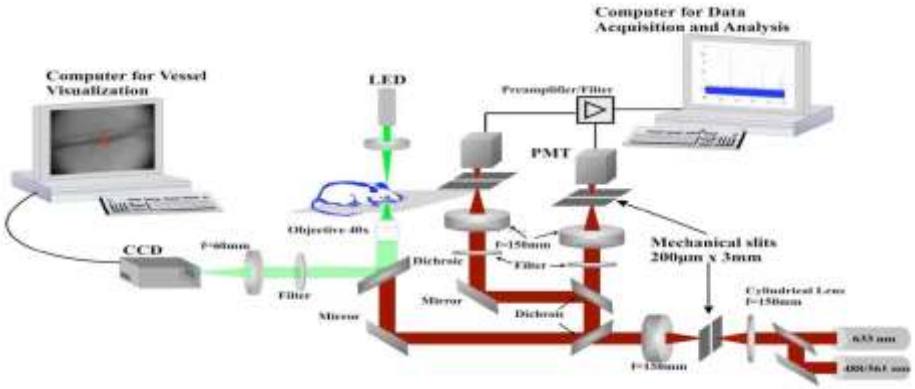


Figure 1. Schematic of the multichannel in vivo flow cytometer

The excitation lasers chosen were the 633 nm Helium-Neon laser, preferred due to its good penetrating capability through tissue and blood, as well as the 561 nm (Cobolt AB, Solna, Sweden) and 488 nm (Coherent Inc, Santa Clara, CA, USA) diode-pumped, solid-state lasers, chosen for their ability to excite fluorescence in a host of commonly used fluorochromes such as green fluorescent protein (GFP), fluorescein, and the yellow/red fluorescent protein variants (YFP/RFP). Arteries (with average diameter of 40-50 μm), rather than veins, were chosen for experiments because of faster blood flow and absence of cell-endothelium interactions.

*In vivo detection of circulating cancer cells*

Approx. 106 cells/ml from the MDA-MB-231 breast adenocarcinoma cell line were incubated with the Vybrant series of lipophilic fluorescent probes (each at a concentration of 5 µg/ml) and then separately injected through the tail vein of anesthetized male, 10-12 week old, CD1 mice in order to demonstrate the in vivo capabilities of the system. The anesthetized animals were placed on an imaging platform within 5 minutes after injection of the cells and an appropriate arteriole in the ear was chosen from which to obtain circulating cell count.

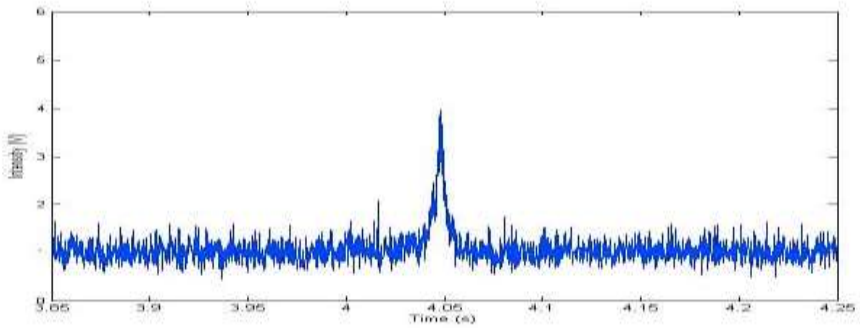


Figure 2. Breast adenocarcinoma cells labeled with the Vybrant DiD fluorescent probe and detected in vivo

## 3. Results and discussion

We have developed the multichannel in vivo flow cytometer for the dynamic monitoring of circulating cells and have demonstrated its capabilities in detecting and quantifying fluorescently labeled breast adenocarcinoma cells directly injected in the circulation of experimental animals. The system was designed and built with the ability to simultaneously assess the circulation kinetics of several distinct cell populations in a single animal. This will allow for a more efficient in vivo investigation of complex biological processes by enabling the simultaneous monitoring of the multiple cell populations that might be participating and interacting in such processes.

To better approximate the tumor environment in vivo, a mouse tumor model will be developed through the adoptive transfer of fluorescent protein expressing cancer cells in immune-compromised mice. Once the cells migrate to tumor growth areas and the tumors are established, the animals will be monitored long term using the multichannel in vivo flow cytometer in order to quantify the fluorescently labeled tumor-shed cells in circulation. Tumor burden will also be assessed via whole body reflectance imaging and the results will be compared to data on circulating cancer cells in order to validate the method for the in vivo assessment of tumor burden in animals. By quantifying circulating tumor cells, in cancer disease models that include a circulating cell component, the in vivo flow cytometer can be used to non- invasively track tumor burden and thus assess important cancer treatment parameters such as the tumor growth and the response to therapeutic intervention.

## 4. References

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