PROJECT SUMMARY

The goal of this project is to develop a device to acquire a white blood cell count (WBC) non-invasively in real-time, *in vivo*. Clinically, white blood cell counts are required to diagnose and monitor infections and inflammation related to diseases, as well as disease-related leukopenia and neutropenia. In many cases, frequent monitoring of the WBC would be beneficial, especially for the early detection of sepsis, a blood infection, in newborns, and monitoring WBC levels in cancer patients or immune-compromised patients to help direct treatment. However, obtaining a diagnostic WBC currently requires blood withdrawal and laboratory analysis of the sample, limiting the frequency of WBCs which can be acquired from a patient. Additionally, blood withdrawal can be very difficult, if not dangerous, in certain patient populations. For example, in patients with small blood volume, such as newborns and premature infants, withdrawing adequate and frequent blood samples often results in the need for blood transfusions. Blood withdrawal is dangerous in immune-compromised patients, such as AIDS, chemotherapy, or transplant patients, because skin penetration carries an inherent risk for infection. The proposed research aims to develop a two-photon *in vivo* flow cytometer (TIFC) that continuously and non-invasively monitors the WBC without the need for blood withdrawal.

White blood cells contain tryptophan, an amino acid in proteins, and can be identified by their tryptophan fluorescence, whereas red blood cells do not exhibit tryptophan-based fluorescence. The TIFC will use this endogenous tryptophan fluorescence to identify white blood cells in circulation. This will allow the identification and quantification of cells by their endogenous fluorescence without the need for labeling with external fluorophores. Using the process of two-photon absorption, the TIFC will excite tryptophan using visible light at 590nm, rather than using the ultraviolet radiation required for one-photon excitation. The longer wavelength visible light will reduce light scattering in the tissue, allowing targeting of dermal vasculature, as well as avoid tissue photo-damage caused by UV exposure.

The proposed research consists of three aims. The first aim is to build the two-photon *in vivo* flow cytometer for the excitation and detection of tryptophan fluorescence. For verification purposes, the instrument will have the capability to perform both one-photon and two-photon fluorescence detection. The second aim is to test the efficiency of the instrument. Since existing commercial flow cytometers do not have the capability to detect tryptophan autofluorescence, we will use micro-fluidic flow channels, developed by Dr. Vacanti's lab at MGH, with the TIFC as an *ex vivo* test of the system. A known number of white blood cells will be injected through the micro-fluidic channel, and the number of cells will be quantified by two-photon tryptophan fluorescence. The third aim is to count the number of white blood cells circulating through a mouse's ear based on their tryptophan fluorescence and to confirm the accuracy of these counts. Accomplishing these three goals will show feasibility that the TIFC can be used to non-invasively quantify circulating white blood cells.