characteristics to blood plasma, where the plasma proteins are synthesized by mesenchymal cells [30].

4. Conclusion

In this study, we have developed a new methodology for *in vivo* micro-vascular and micro-flow imaging by using two-photon excitation endogenous fluorescence in zebrafish. The 3D structures of fine microvascular networks in the brain were reconstructed based on the unique spectral characteristics of blood plasma TPEF signals. We demonstrated that imaging flow cytometry provides the quantitative counting of circulating blood cells, which could be used for *in vivo* monitoring the disease development and treatment, such as acute sterile inflammation. In addition, the calibrated counting of circulating leukocytes (neutrophils) in blood vessels of different flow velocity and size could potentially improve the accuracy of measurement of circulating cells in the whole circulation system. Finally, using a single wavelength excitation at 650 nm and spectroscopic detection, we achieved simultaneous four-color imaging of multiple blood cells and vascular structures. This imaging capability could potentially be used to address important biological topics such as the interactions between blood cells and vascular endothelial cells and red cells and monocytes/macrophages.

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