Two-photon flow cytometer with non-diffracting beams

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In vivo flow cytometry is used for medical diagnosis and the quantifying of circulating cells. This technique uses laser light for excitation to detect the fluorescent or acoustic signals after light is absorbed by molecules within the cell. A challenge in this approach is the limited detection depth due to the scattering of light by living tissue. Two-photon excitation based flow cytometers use infrared light and have longer detection depth, but limited sample volume. In this project a light-sheet microscope was made to increase the sample volume. In conventional flow cytometer Gaussian beams are commonly used, but it has limited sample volume as focused Gaussian beams diverge quickly after focus. So a self-healing and non-diffracting beam named Bessel beam is used to generate a light-sheet. Bessel beams have concentric rings that create background noise, but can be reduced through the use of two-photon excitation. This can be further improved by using Airy beams which have been shown to produce a three-fold increase in detection depth when compared to Bessel beams. In this study a scanning two-photon Airy beam light-sheet is implemented for greater resolution, sample size, and detection depth of microfluidic channels, therefore future in vivo flow cytometry.

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