Simultaneous Raman-Fluorescence Flow Cytometry

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We demonstrate simultaneous Raman-fluorescence flow cytometry. The flow cytometer consists of a femtosecond Ti:sapphire laser coupled with a homemade pulse pair generator and microfluidic chip. The pulse pair generator rapidly scans optical delay to accomplish interferometer-based Fourier-transform coherent anti-Stokes Raman scattering (FT-CARS)[1,2] and Fourier-transform two-photon excitation (FT-TPE)[3,4] measurements. Sample cells are suspended in buffer solution and pumped through the microfluidic chip, where acoustic focusing is used to pass them singly through the measurement position. Using this scheme, we measured metabolic response of the microalga *Haematococcus pluvialis* to four different photo-stress conditions using Raman scattering from its secondary metabolite astaxanthin and autofluorescence from its chlorophyll and chlorophyll catabolites at a throughput of 10 events/s.

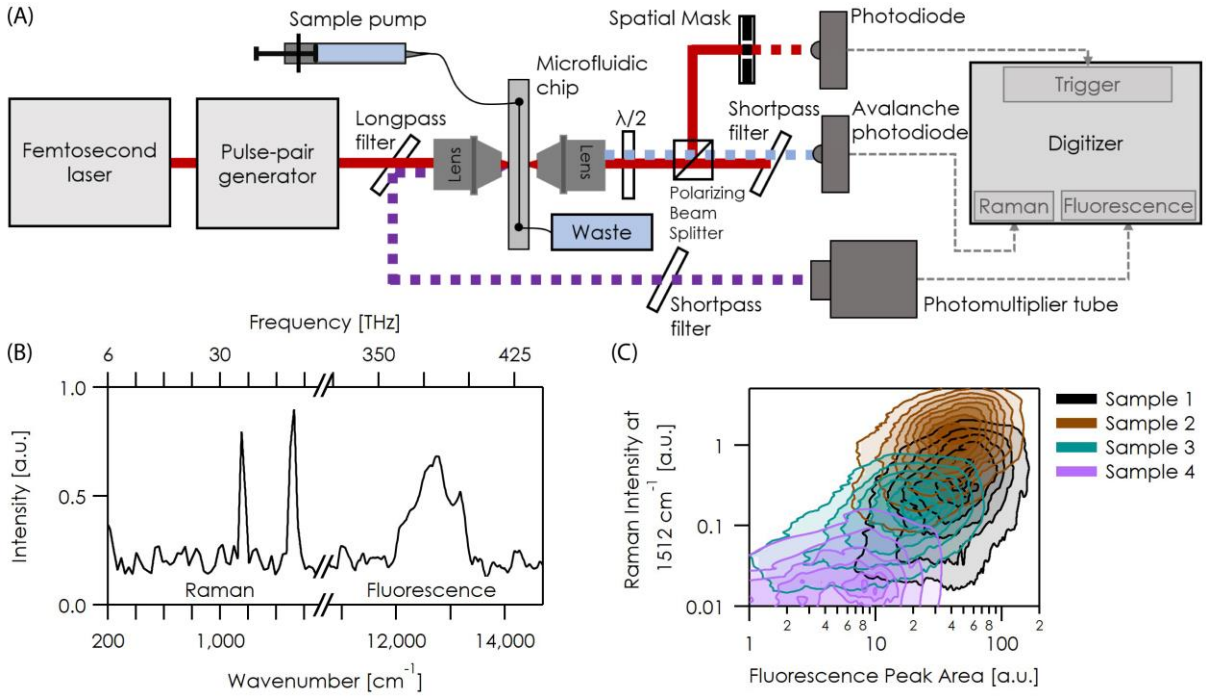

Figure: Simultaneous Raman-fluorescence flow cytometry. (a) Schematic of the flow cytometer showing trigger and FT-CARS signals detected in the forward scattering regime, and FT-TPE signal detected in the backward-scattering regime. (b) Raman and fluorescence excitation peaks from a single *H. pluvialis* cell at a flow rate of ~2 cm/s. Characteristic Raman peaks of astaxanthin are visible at 1052 and 1512 cm^{-1}. The fluorescence peak is typical of chlorophyll autofluorescence excitation under the laser's power spectrum. (c) Astaxanthin vs autofluorescence from *H. pluvialis* samples cultured under different photo-stress conditions. N=2000 for samples 1, 2, and 3. N=1392 for sample 4.

References

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