“Densely Multiplexed Fluorescence Imaging by Fourier Transform Flourometry”

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The goal of this K99/R00 proposal is to develop a new platform for highly multiplexed fluorescence microscopy, which could allow tens of thousands of biomarkers to be visualized in living cells. At present, multiple fluorescent markers are identified using differences in fluorescence emission wavelength. Here we propose to increase multiplexing density by simultaneously detecting different fluorescent markers with unique excitation, emission, and lifetime properties. The device that makes this possible is a novel Fourier transform fluorometer that is capable of measuring these fluorescence signatures in parallel. This technology has been pioneered by Dr. Leilei Peng, who is currently a Research Fellow at the Wellman Center for Photomedicine. Currently, the Fourier fluorometer is only capable of making single point measurements over a limited range of fluorescence wavelengths and lifetimes. For this proposal, Dr. Peng, will develop this technology further to dramatically expand the range of measurable wavelengths and lifetimes. Data acquisition speeds will also be increased to make this method compatible with microscopic imaging. Following these modifications, the fluorometer will be incorporated into a confocal microscopy imaging system and dense multiplexing will be tested and validated. The research will culminate with a demonstration of multiplexed microscopy in vitro. Guillermo J. Tearney, who runs a laboratory that develops microscopic optical methods for disease diagnosis, will mentor Dr. Peng and supervise development of the spectrometer and microscopy. Co-mentor Tayyaba Hasan is a renowned scientist in photochemistry and photobiology with specialties in photodynamic therapy and fluorescence imaging. Dr. Hasan will assist with the development and selection of fluorescent labels with distinct excitation, emission, and lifetime properties as well as functionalization of these labels for experiments in living cells. The opportunities provided by this award will not only allow Dr. Peng to develop this novel optical technique for biomedical imaging, but will also provide her with valuable mentorship, training, and resources to launch her career as an independent academic researcher.