This system can be further enhanced in two ways. Firstly, two–photon excitation with a Ti:Sapphire laser will improve the dynamic range for anisotropy imaging by an increase of the maximum anisotropy from 0.4 to 0.57. A tunable Ti:Sapphire will also allow two-photon excitation fingerprinting similar to what we demonstrated for one-photon excitation with the supercontinuum light source. Secondly, the EMCCD will be replaced by time-resolved detectors that will allow the measurement of individual photon arrival times. These technologies are already available and will enable extension to time-resolved spectropolarimetry [29,30].

The content rich images provided by HDIM and other new imaging techniques pose new challenges for data analysis and visualization. We proposed here types of representation to permit the intuitive visualization of data sets obtained by imaging spectropolarimetry. The most important applications that we foresee for hyper-dimensional imaging microscopy include (i) unmixing of multiple FRET-based biosensors [31], (ii) detection of interactions in multi-molecular complexes and (iii) enhanced contrast for tissue imaging. In our laboratories, HDIM will complement existing techniques that we are using for probing the molecular mechanisms underlying neurodegeneration [26] and cancer [32]. However, it is likely that HDIM will find numerous applications not only in biology and biophysics but also in material sciences and spectroscopy.

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