During the last decade, ultrasensitive microscopy has become one of the most important tools in biophysics. Using fluorescence excitation, sensitivities down to the single molecule detection limit can be achieved. While fluorescence microscopy is an extremely useful tool, it has the disadvantage that the sample needs to be labeled. Coherent Anti-Stokes Raman Scattering (CARS) microscopy as a non-linear optical technique suited for live cell microscopy provides an alternative approach in that contrast is generated on basis of vibrational spectra. Thus CARS microscopy provides contrast without the need for labeling. Applications of CARS microscopy suffer from non-resonant background which reduces the achievable sensitivity. For this reason, a number of different techniques have been developed recently, which reduce the non-resonant background. Here, we will present spectral focusing of the broadband laser pulses into narrow vibrational resonances [1] and a simultaneous dual-pump CARS microscope [2] as two ways to generate images with significantly improved sensitivity. We will discuss achievable sensitivities and show applications to imaging of live organisms.
