This thesis focuses on characterizing the spectroscopic properties of carotenoids, the most frequently occurring biomolecules in living organisms, using Raman and fluorescence microscopy techniques. The aim is to determine whether carotenoids are the source of the observed green autofluorescence (GAF) in various organisms. The study analyzed pure carotenoids, including β-carotene and astaxanthin, and *in vivo* samples from microorganisms such as *Haematococcus pluvialis, Vischeria* sp., and *Rhodotorula glutinis*. Fluorescence spectra of β-carotene and astaxanthin exhibited peaks in the green region, with β-carotene ranging from 524 nm to 545 nm and astaxanthin redshifted to 556 nm to 574 nm. Both carotenoids displayed bathochromic shifts in solvents with higher refractive indices. Under blue excitation, the chemical maps of carotenoids in microorganisms showed colocalization with the green fluorescence maps at around 530 nm, supporting the hypothesis that carotenoids contribute to GAF. Additionally, the green fluorescence maxima in *H. pluvialis* and *Vischeria* sp. align with the values measured in carotenoid standards, astaxanthin, and β-carotene, respectively. However, the study cannot exclude the potential presence of other fluorescent compounds, such as metabolites or degradation products, colocalizing with carotenoids. Therefore, while evidence suggests carotenoids as a source of GAF, further experiments are needed to confirm their role definitively.