Mass spectrometry methods of surface analysis and surface imaging help to unravel the molecular complexity of surfaces based on the ions desorbed from them. Most suitable for the formation of intact molecular ions from surfaces containing biological or synthetic macromolecules, in a wide range of polarities, is matrix assisted laser desorption ionization (MALDI). The surface is treated with a light-absorbing matrix and bombarded with a laser, and the desorbed ions are recorded in mass spectra that identify the molecules desorbed. If the surface is moved in two dimensions during MALDI, an optical image of the spatial distribution of a specific substance across this surface can be obtained. MALDI-MS surface analysis and imaging have been used to analyze the lipids desorbed from shed gecko lamellae and to identify and image the lipid residues left in gecko footprints. Since MALDI-MS can only probe the molecules in contact with the light-absorbing matrix, it is possible to characterize just the surface layer (~2 nm), without interference from lower layers, if the matrix is applied strictly to the surface. The validity of this approach has been confirmed by analyzing a surface on which different polymers were deposited sequentially; the latter method can also be used to probe segregation phenomena in multicomponent polymer films, as will be documented for a surface prepared by spin casting a blend of cyclic and linear polystyrenes.