Abstract

The thesis represents a journey towards the development of a new multimodal approach enabling construction of two dimensional elemental and molecular images relative to their location and distribution on the sample surface. By combining the advantages of various nuclear microprobe techniques and other imaging modalities we create an elemental and molecular visualisation tool for a better understanding of complex physiological processes in biological tissue.

The Particle Induced X-ray Emission - PIXE at Jozef Stefan Institute nuclear microprobe is the most frequently used analytical technique among others available at the installation, continuously used for the elemental mass quantification (Na to U) of different biological tissue samples. Quantification of elemental concentrations is achieved by simultaneous Elastic Backscattering Spectroscopy - EBS and Scanning Transmission Ion Microscopy - STIM. In order to extend the application to single cell organisms, an alternative quantification method within the sample approximation was developed, providing total elemental mass inventory of individual cells resulting in 1 pg sensitivity for selected elements. Human monocyte derived dendritic cells (MDDC) were used in the study to determine the total mass of ingested gold nanoparticles (GNP) as a prosperous drug delivery system. The same method was applied to determine the inventories of microparticles adsorbed on the lorica surface of the microzooplankton species *Tintinnopsis radix*, originating either from natural or anthropogenic sources.

To overcome the limitations of the μ-PIXE method imposed by providing solely the elemental distribution a Time-Of–Flight (TOF) analyzer was incrementally added to the microprobe measuring chamber for the furtherance of a novel high-energy technique known as MeV Secondary Ion Mass Spectrometry (MeV SIMS). The new setup broadens the capabilities of the nuclear microprobe to 2D molecular imaging and enables the distribution studies of various biomolecules in the tissue, spanning from small metabolites to complex proteins. The first step in developing a fully functioning imaging technique was the TOF mass analyser installation and its optimisation for mass spectrometry of desorbed sample fragments in the imaging mode. The MeV SIMS method was then successfully applied to study cocaine distribution in hair, metabolite and lipid distribution in various wheat genotypes and heterogeneous lipid composition of a small rat brain region.

The extent of the multimodality is shown by the MeV SIMS and PIXE dual imaging analysis, executed either simultaneously or sequentially on biological tissue sections. This is presented as one of the first known attempts of such MeV SIMS/PIXE imaging on biological tissue. In combination with other high-resolution imaging techniques a

correlation study was performed on a tea leaf cross-section to determine the elemental and molecular distribution in different leaf compartments of a control and Al-treated tea (*Camelia sinensis*) leaf.