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# Imaging of Biomacromolecules in Mass Spectrometry Using Timepix Detectors

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The Timepix (TPX) is a micropixelated imaging detector capable of recording both the arrival time and position of individual ions. In this study, we have integrated TPX detectors to three different mass spectrometers (MS) for the spatially resolved detection and structural analysis of macromolecular assemblies (MMAs). First, a dual microchannel plate (MCP) stack-TPX quad detection assembly has been coupled to a nano-electrospray ionization (nanoESI)-orthogonal time-of-flight (TOF)-MS for the analysis of multiply charged non-covalent protein complexes of molecular weight in excess of 800 kDa. Using this experimental setup, we demonstrate the ability of the TPX to unambiguously detect and image individual macromolecular ion events, providing the first report of single-ion imaging of protein complexes. The single ion imaging capability has been further exploited to gain a better understanding on the effect of ion and voltage parameters on the MCP response for the detection of a broad mass range of 192 to 800,000 Da. Moreover, we have used both the impact position and arrival time information of the ions at the detector to visualize the effects of various ion optical parameters on the flight path of ions. This led to the identification of the origin of an unexpected TOF signal that could easily be mistaken as a fragment of the protein complex as the secondary electron signal arising from ion-surface collisions inside the TOF housing. The TPX detector used for this work is limited by a moderate time resolution (20 ns here, at best 10 ns) and single-stop detection for each pixel that can bias the detection of ions with a low TOF at high count rates. Our second work has been benefited from the implementation of the next generation Timepix3 (TPX3) detector that offers 1.56 ns time resolution, per-pixel multi-hit functionality and kHz readout rates. In this experimental set up, a TPX3CAM (optically coupled to MCP via a fast scintillator) has been added to a MALDI (matrix-assisted laser desorption/ionization)-linear TOF MS, which allowed the detection and ion imaging of singly and doubly charged intact protein ions of mass to charge ( $m/z$ ) ratio up to 1,150,000 Da. We also demonstrate the spatial and temporal separation of metastable neutrals produced in MALDI MS, and the effect of the matrix structure and laser power on the metastable decay rate for various proteins. Additionally, TPX and TPX3 assemblies used in the first two experimental studies have been added to an in-house developed nanoESI-Orbitrap-linear/orthogonal-TOF MS platform. This innovative imaging approach targets the structural determination of MMAs by analyzing the relative positions of the fragment ions produced from the precursor MMA ion via ultraviolet photo dissociation (UVPD).

**Primary author:** Ms MATHEW, Anjusha (Maastricht MultiModal Molecular Imaging (M4I) Institute)

**Co-authors:** Dr D. KEELOR, Joel (Amsterdam Scientific Instruments (ASI)); Dr G. M. ANTHONY, Ian (Maastricht MultiModal Molecular Imaging (M4I) Institute); Dr LONG, Jingming (Amsterdam Scientific Instruments (ASI)); Dr PRANGSMA, Jord (Amsterdam Scientific Instruments (ASI)); Prof. J. R. HECK, Albert (Biomolecular Mass Spectrometry and Proteomics); Dr PAPANASTASIOU, Dimitris (Fasmatech Science and Technology); Prof. A. MAKAROV, Alexander (Thermo Fisher Scientific (Bremen)); Dr R. ELLIS, Shane (Molecular Horizons and School of Chemistry and Molecular Bioscience); Prof. M.A. HEEREN, Ron (Maastricht MultiModal Molecular Imaging (M4I) Institute)

**Presenter:** Ms MATHEW, Anjusha (Maastricht MultiModal Molecular Imaging (M4I) Institute)

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