Objective: Imaging mass spectrometry (IMS) application for the detection of human papilloma virus (HPV)-related proteins directly in the host specimen.

Introduction: HPV comprises a family of more than 130 types, with many types having preference to infect specific anatomical sites, causing lesions with distinctive clinical pathology. These include benign hyper-proliferative lesions such as cutaneous warts, and asymptomatic precursor lesions, that can in some instances progress to high-grade neoplasia and invasive cancer. Test systems are required to detect high-risk but also low-risk HPV subtypes with high specificity and sensitivity. We developed a proteomic-based IMS approach to investigate HPV molecular signals directly from host specimens.

Methods and Workflow: Formalin-fixed paraffin-embedded tissue sections from cervical cancer, genital and skin warts were prepared for IMS analysis according to an optimized standard protocol. Briefly, tissues were deparaffinized with xylene, rehydrated through graded ethanol and antigen retrieved in 10mM Tris buffer (pH 9) for 20 min at 95°C. Sections were sprayed with trypsin (0.025 µg/µl) using a T-FMS-Array (HTX Technologies, Chapel Hill, NC, USA) and incubated at 37°C for 2 hours. Alpha-cyano-4-hydroxycinnamic acid matrix solution (10mg/ml in 70%AcN/1%TFA) was deposited using the same sprayer device.

MS measurements for IMS were carried out using a rapifleX MALDI TissuetypeR (Bruker Daltonik GmbH, Bremen, Germany) at 50µm spatial resolution. Spectra were exported from HPV infected and non-infected regions and compared. SCI-Lab software (SCI-Labs, Bremen, Germany) was used to automatically find m/z values specifically co-localizing within the HPV infected annotated regions using a statistical Pearson correlation method with p<0.05. Protein identification was performed in-situ using an AutoflexSpeed MALDI-TOF/TOF (Bruker Daltonik) mass spectrometer.

Identification of HPV in Cutaneous Wart

Conclusions: Specific peptides related to individual HPV types were imaged and identified directly onto host specimens using IMS. Our results were in agreement with hybridization-based test systems used in routine diagnostics. Co-infections of a single wart with multiple HPV types were detected. The ability to rapidly identify signatures specific to microorganisms in tissue is a major advantage that greatly decrease both time and cost especially for the analysis of tissue with multiple simultaneous HPV infections.