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Simplified, High-Throughput TOF-SIMS Analysis via HR^2 and Uniform Molecular Imaging of Rough Surfaces

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Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is becoming indispensable as a discovery tool in biological and complex materials research owing to the unique capacity for 2D and 3D imaging of molecular and elemental species at sub-micron spatial resolution, at high abundance sensitivity, and without the sample treatments required by e.g. MALDI or fluorescence microscopy. The technologies required for increased productivity and uniform imaging of rough surfaces are the focus of this discussion. Specifically, we will discuss the advanced HR^2 molecular imaging that is now the default mode of 2D molecular imaging because both high mass resolution and high lateral resolution are achieved simultaneously.

The ability to image samples having a large degree of surface topography is a highly desired quality in a TOF-SIMS instrument; imaging of rough, high relief surfaces is a particular strength for the PHI *nanoTOF* due to an inherently large angular acceptance and depth-of-field afforded by the *nanoTOF'*s TRIFT mass spectrometer. This imaging capability is demonstrated by the characterization of epicuticular wax components present at the surfaces of *Arabidopsis thaliana* organs, shown in Figure 1. The mass spectra associated with high resolution images of organs and single cells illustrate the capability to characterize biological chemistry at a spatial resolution of ≈ 0.3 μm without the artifacts associated with topography, i.e. reduced sensitivity, reduced mass range, and mass scale nonlinearity. Mass spectra in both the positive and negative secondary ion polarities reveal that the epicuticular surface of each *Arabidopsis thaliana* organ is comprised of distinct chemical components. Moreover, the mass spectra of specialized cells forming the stomata and trichomes reveal that, even for single cuticle cells, the wax composition is unique. The differences in wax chemistry on each of the interrogated organs and cells of *Arabidopsis thaliana* will be presented. This example demonstrates, in particular, the chemical identification and imaging of high relief surfaces while maintaining high sensitivity, uniform signal collection, low spectral background and high S/B, full mass range, and high mass scale linearity.

A new standard for TOF-SIMS analysis at high mass resolution and high lateral resolution simultaneously, i.e. high resolution squared (HR^2), has been introduced by PHI. The long-accepted model for TOF-SIMS analysis has been dogged by trade-offs in mass resolution, lateral resolution, sensitivity, and time of analysis. In order to achieve high sensitivity and short analysis times an analyst must often choose between the ability to identify chemical species at the same nominal mass, i.e. high mass resolution, and the ability to observe small chemical features, i.e. high lateral resolution. Several approaches have been utilized in the past to achieve respectable mass resolution together with useful lateral resolution, but these approaches typically result in a significant increase in analysis time, a detrimental reduction in sensitivity, a greatly limited mass range, or even a non-linear mass scale. None of the aforementioned limitations is commensurate with the discovery or problem-solving utilization of TOF-SIMS. The new HR^2 imaging mode, which boasts $m/\Delta m \approx 10{,}000$ mass resolution and $\Delta l \leq 500$ nm lateral resolution together, is accomplished at high analysis beam currents so that there is no sacrifice in acquisition speed or sensitivity. Further, the HR^2 imaging mode does not introduce either

limitations on the mass range or mass scale non-linearity; therefore, chemical and molecular identification is straight-forward. The advantages of HR^2 imaging will be demonstrated by failure analysis and molecular imaging of droplets on a surface as presented in Figure 2. In this example, a short, high sensitivity analysis allowed full molecular characterization and high contrast molecular imaging of the droplets and the surrounding surface.

Figure 1. Total ion images of *Arabidopsis thaliana* organs. (LEFT) Spores on a flower petal; marker = 10 µm. (CENTER) Stomata on the adaxial leaf surface; marker = 10 µm. (RIGHT) Trichome on the abaxial leaf surface; marker = 100 µm.

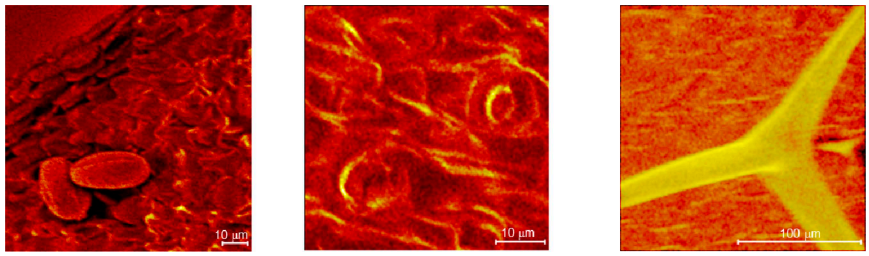
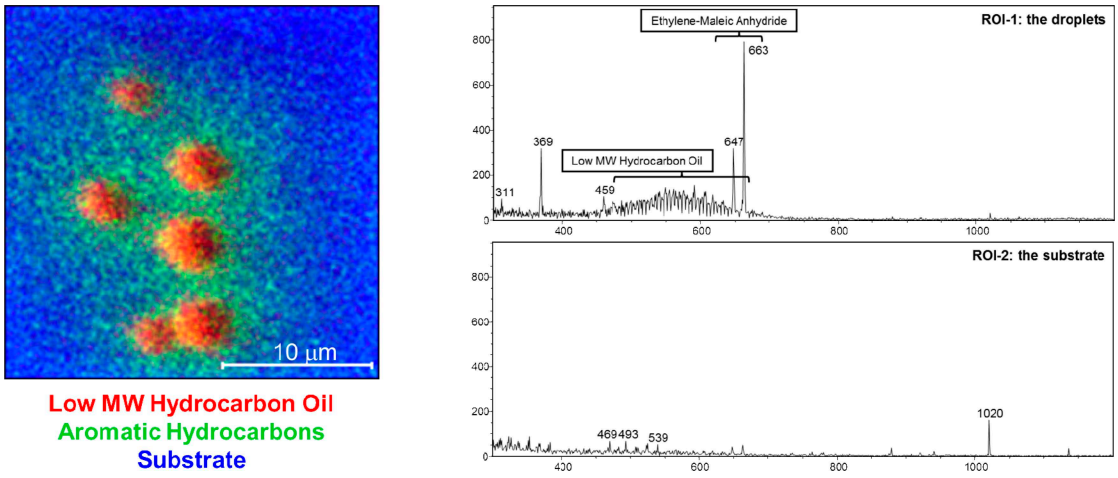


Figure 2. (LEFT; COLOR) False color overlay of molecular ion images revealing the organic droplets on an organic surface; marker = 10 µm. (RIGHT) Region-of-interest (ROI) mass spectra of the droplets, ROI-1, and of the surrounding surface, ROI-2, scaled to the same maximum intensity and with the molecular components identified.



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