Chemical Imaging with 100nm Spatial Resolution: Combining High Resolution Flurosecence Microscopy and Ion Mobility Mass Spectrometry

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Overall research goals: Our research goals were to combine, in a single instrument, high spatial resolution optical microscopy with the chemical specificity and conformational selectivity of ion mobility mass spectrometry. We apply this technique to thin films of molecular semiconductor materials in order to probe the conformer distribution within the film and relate this distribution to the luminescence properties.

Significant achievements in 2009-2010: In our initial design the microscope objective and the sample scanner were placed in vacuum (see Figure 1 below and the diagram of Figure 2). We designed and constructed a chamber to replace the existing source chamber such that the microscope chamber could be inserted and removed without disrupting the alignment of the microscope or ion optics. Ions were formed by focusing the desorption laser (frequency-tripled YAG laser 355 nm, 100 nJ/pulse 7 ns
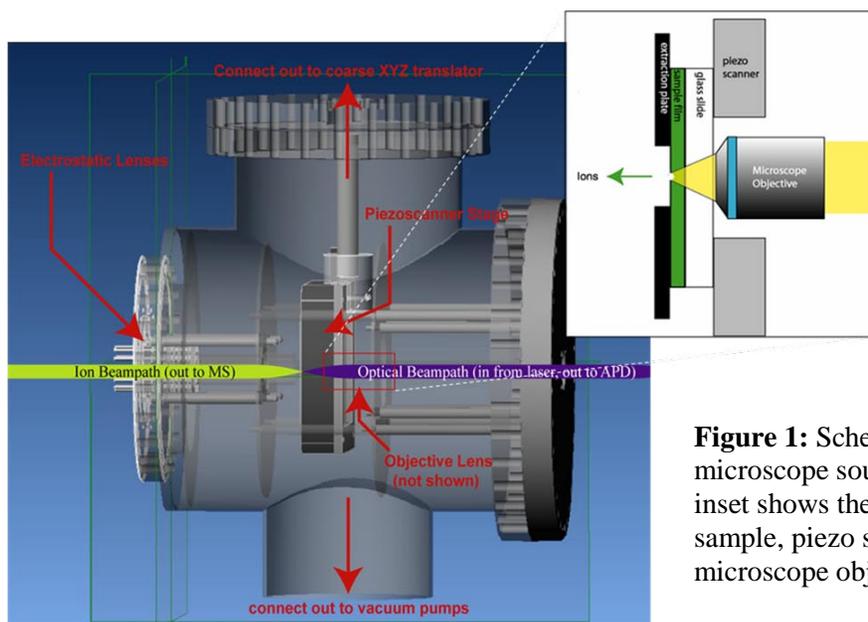

Figure 1: Schematic of the microscope source chamber. The inset shows the relative position of the sample, piezo scanner, and microscope objective.

pulse width) using the same microscope objective as used in optical microscopy. Positive ions were extracted by applying a voltage (of order 100 V) to the extraction plate as seen in Fig. 2b. Positive ions were then collected and guide using the ion optics of the mass spectrometer. While our design worked well-enough for mass spectrometry, our signals were too low to perform the ion mobility measurements
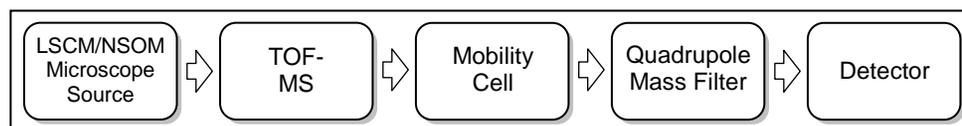

Figure 2. Block diagram of instrument.

important to our overall goals. Our attempts to improve the signal-to-noise ratio of this initial version centered on the addition of an ion funnel to enhance our ion collection efficiency. During the design phase of these improvements we realized that a new prototype instrument from Waters Inc. included the

high ion collection efficiency we required. The Bowers lab helped develop the ion mobility part of this instrument and had one available in the lab. We were able to modify the existing LSCM source such that it could be coupled to the ion port of the Waters machine. A schematic of this coupling (as well as photos of its implementation) is shown in Figure 3.
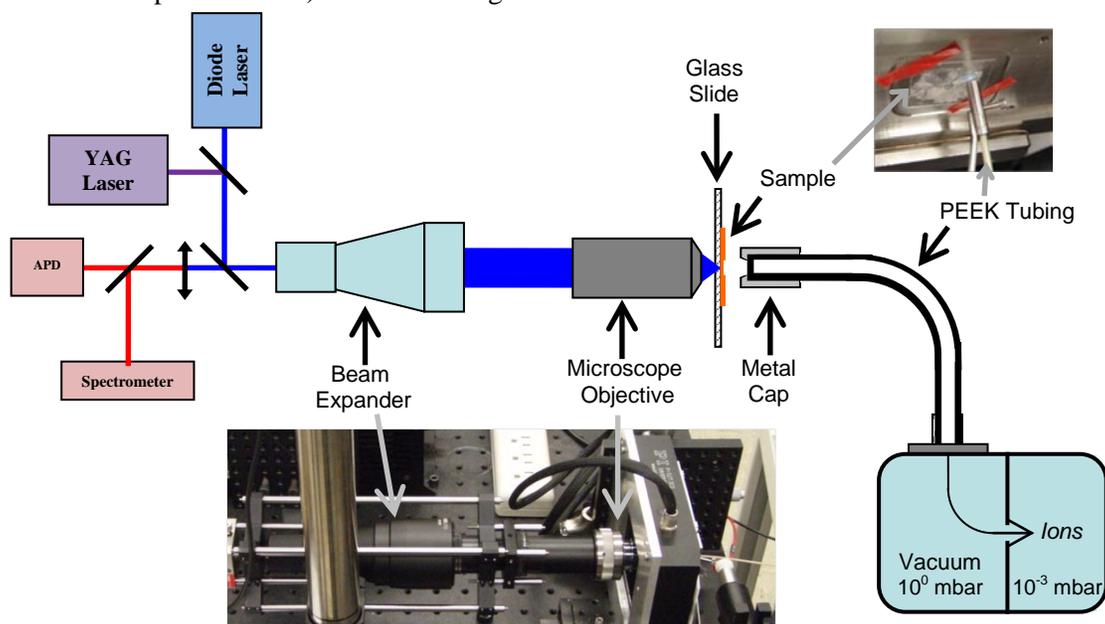

Figure 3. Schematic and photos of the LSCM source. The scanning stage is visible in the bottom photo, but is omitted in the schematic. The upper photo shows the sample, metal cap and the beginning of the PEEK tubing.

The new LSCM source was mated to a prototype of the Synapt HDMS system from Waters (as depicted in Fig. 3). For the development of this new source, the instrument operates as a normal quadrupole TOF. The Synapt prototype was chosen as a platform around which to build the new LSCM source for two reasons: i) the instrument is very sensitive, that is, nearly all ions that enter the instrument are transmitted and detected; ii) the instrument is designed such that all ions that arrive at the detector are recorded and stored by the software. These features are critical because as the amount of sample analyzed decreases as the spatial resolution of the microscope increases. The number of ions ablated decreases accordingly and maximum detection efficiency is required. The Waters instrument also has the capability to perform traveling wave ion mobility spectroscopy (T-Wave) experiments. In the T-wave experiment absolute collision cross sections cannot be directly measured, but the presence of multiple molecular conformations can be detected and a distribution of shapes is obtained.

Ions are produced by laser ablation using the third harmonic (355 nm) of a diode pumped ND:YAG laser (Lumanova Flare; Model 200–100; Hannover, Germany) focused through the microscope objective to a single spot on the sample surface. The laser is operated at the maximum repetition rate, 334 Hz, and pulse energies of ~100 µJ. With each laser pulse that hits the sample a plume of ions is generated near the surface. The glass slide and matrix surface are grounded and the ions are pulled towards a metal cap, which is biased to -1.3 kV ($\mathcal{V}$ = -1.3 kV). In our initial experiments the metal cap is placed approximately 1 mm (d = 1 mm) from the sample surface and the orifice (1.0 mm diameter) of the metal cap is centered over the laser spot. As the ions near the metal cap, the pressure gradient drives the gas flow into the vacuum, pulls the ions through a 0.76 mm diameter PEEK tube (polyetheretherketone, Sigma, St. Louis, MO). The PEEK tubing is 30.5 cm long and is attached to the entry port of the modified q-TOF with a custom cone designed to accept the tube. The mechanism of ion transmission into the instrument is purely due to the pressure gradient between the atmospheric pressure in the lab and the first stage of vacuum pumping in the SYNAPT machine (10^{-3} mbar). Once the ions enter the vacuum, they are collected and transmitted through the instrument as they would with the original source in place.

An example of the performance of our new LSCM source is shown in Figure 4. The sample in this example is a thin film of 2,5 dihydroxybenzoic acid (DHB) approximately 200 nm thick as measured by AFM. DHB was chosen since it is readily used as the matrix for matrix-assisted lased desorption ionization (MALDI). The film was prepared via drop casting ~50 μL of a 100 mg/ml DHB solution in methanol onto a glass coverslip (20mm x 20mm x 17μm).
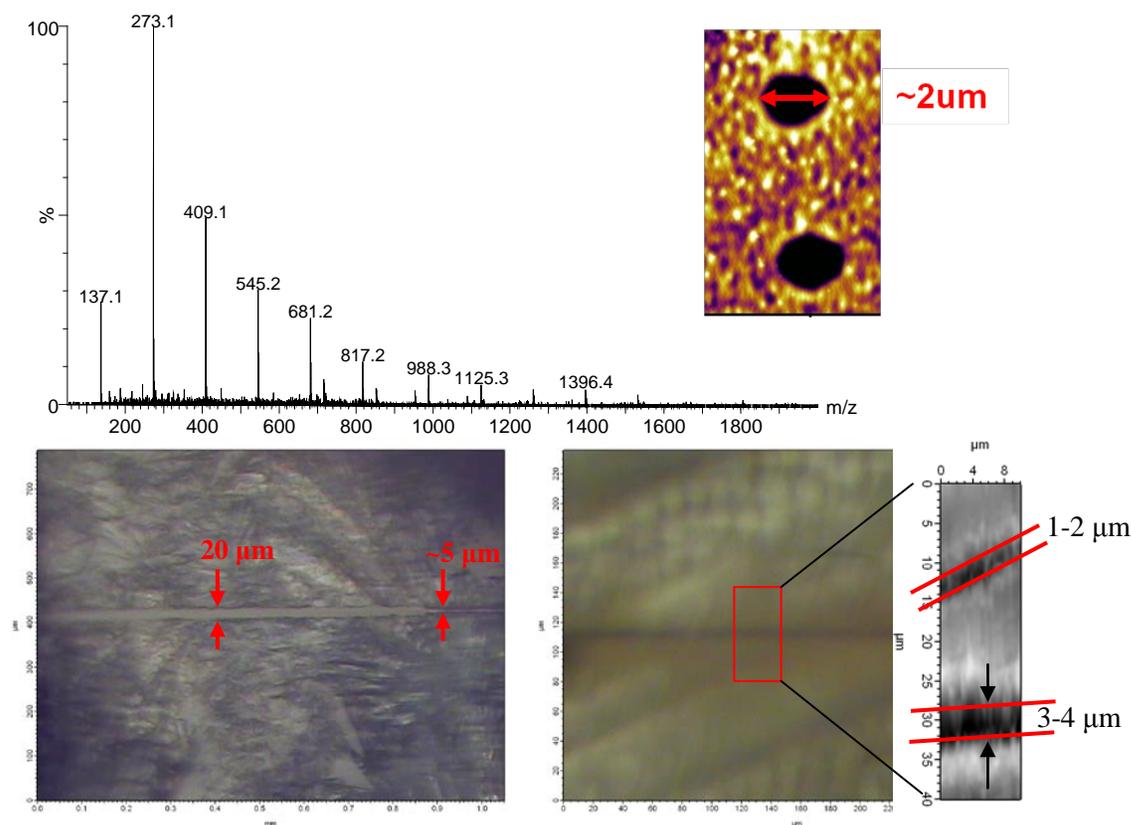

Figure 4. Mass spectrum of DHB matrix ablated using the LSCM source from a 2 μm spot. The peaks and the signal analysis are presented in the text. The upper inset is an AFM image of two such spots. The diameter is approximately 2 μm and the depth of the hole shows that all of the DHB has been removed.   The micrographs below the spectrum shows a line of ablation (acquired while scanning). The change from a 20 μm to 5 μm thickness represents a change to a higher magnification objective. The lower inset shows the thickness of 2 lines of ablation with thickness of the order of 1 μm.

The DHB-coated glass slide was placed onto a custom built frame and attached to the scanning stage described earlier. The slide is held in place or rastered in front of the microscope objective as the mass spectra are recorded. A mass spectrum recorded from a 2 μm spot of DHB is shown in Figure 11. The well known 137 m/z and 273 m/z peaks correspond to the $[DHB + H–H_2O]^+$ and the $[2DHB – OH – H_2O]^+$ ion respectively. The successive m/z peaks at 409 m/z, 545 m/z, 681 m/z, etc., are all clusters of DHB matrix. For example the 409 m/z peak is $[3DHB – 3H_2O + H]^+$ and each larger cluster is the addition of 136 amu ($[DBH – H_2O]$). The mass spectrum in Fig. 4 is created by the summation of individual spectra recorded every 90 μs for 10 s. At a repetition rate of 334 Hz, approximately one ion is detected for every 7.5 laser pulses that hit the matrix surface. This certainly represents a low estimate for the ions generated per laser pulse as the matrix surface is completely ablated well before all 334 pulses hit the slide each second as observed by AFM. The S/N ratio of the spectrum of Fig. 4 is more than 50 times better than observed in our vacuum version.

We have demonstrated the capability of the microscope and the ion mobility mass spectrometer on a thin films of oligo-p-phenylene vinylene (OPV) molecules, a molecular semiconductor used as the active layer in organic light-emitting diodes. A high resolution fluorescence image of an OPV film is presented in Figure 5 along with the arrival time distribution of the conformers in the film taken using ion mobility mass spectrometry.
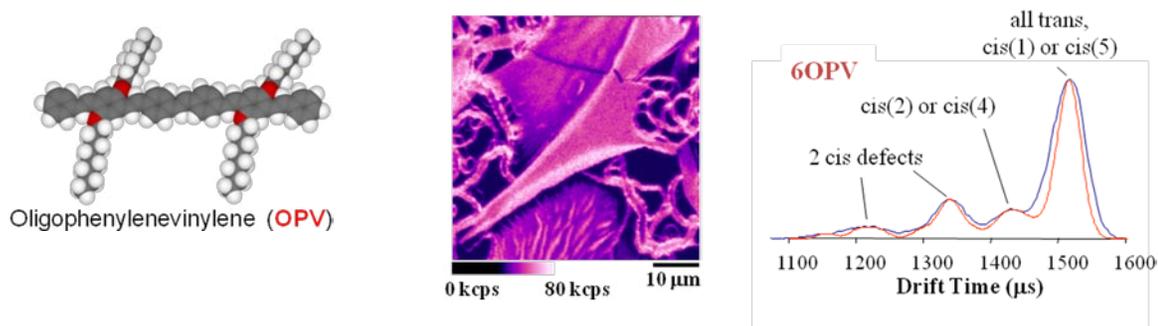
We have also used the microscope to image annealed thin films of 4-((2′,5′-dioctyloxy-4′-styryl)styryl)styryl stilbenylmethane (6OPV). [25] LSCM fluorescence images of 6OPV films are shown in Figures 6a and 6b. The films were thermally annealed at 140 °C after casting from solution. The films contain intricate polycrystalline structures resembling frost on a windowpane. Two major crystalline domains are evident in Figs. 6a and 6b. Fig. 6a shows an example of sheet-like crystal formations that occur in the annealed 6OPV films.

Figure 6: LSCM fluorescence micrographs of two annealed 6OPV films prepared at the same time. Film **a)** 75 x 50 micron image shows mostly sheet-like structures, while film **b)** 20 x 20 micron image contains mostly spaghetti features.

References:
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