Real-time mid-IR chemical imaging of dynamic processes: Proton-Deuteron exchange within a micro fluidic system using the Spero™ QCL based microscope.

There is a growing need to visualize the dynamic evolution of the mixing and reacting of chemicals at the microscale. Applications are diverse, ranging from monitoring the uptake of drugs in live cell experiments to controlling the syntheses of drugs in micro-flow channels. The new Spero microscope from Daylight Solutions is the first microscope of its kind to enable real-time monitoring of microscale flow chemistries over large areas at high spatial resolution and at video rates.

The Spero microscope employs tunable laser technology as its light source and works by shining this narrow band of infrared (IR) laser light onto the sample. The frequency of this light can be tuned across the fingerprint mid-infrared spectral range to correspond to molecular absorption peaks in the target compounds producing a functional group specific chemical contrast image at the detector. The user has full control of the excitation frequency of this illumination and the technique allows for simultaneous imaging of multiple chemistries within the same field-of-view at video frame rates. Additionally, complete infrared spectra can be acquired by tuning the laser source throughout the frequency range of interest

Other infrared techniques such as Fourier Transform Infrared (FTIR) microspectroscopy have been used to limited success in monitoring dynamic processes. However, these techniques are severely limited in dynamic response and spatial resolution. The Spero microscope is the only infrared imaging technique able to provide both *spatial and dynamic* chemical information in real time.

The Spero microscope is the first and only commercially available infrared microscope to take advantage of the quantum cascade laser (QCL) which has been commercialized over the past decade. The QCL source is 5 orders of magnitude brighter than a Globar source that is typically used in FTIR instruments and 3 orders of magnitude brighter than a synchrotron source. It offers higher sensitivity and the ability to use large format, uncooled microbolometer array detectors. These detectors provide mid-IR detection with pixel densities of 480 x 480, superior linear dynamic range, and with read-out rates of 30 fps. Furthermore, they are extremely reliable and require no cryogens or cool-down time.

Spero is uniquely suited to measurements where the spatial distribution of specific functional groups and chemical information is required from dynamic processes in real time.

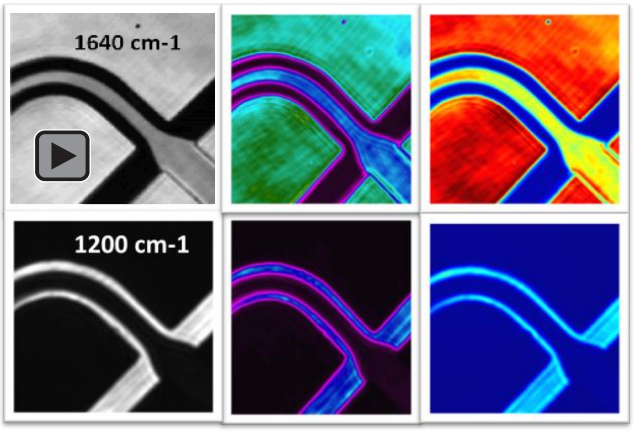

Figure 1: Discrete frequency images of H_2O and D_2O flows within a microfluidic channel at characteristic absorbances. Video recorded at 1640 cm^{-1} displays the transmission image where H_2O is black and D_2O is white. Click image to animate.

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Such applications include microfluidics and flow chemistry, reaction monitoring, diffusion studies, liquid-liquid interactions, melting, and crystallization as well as dissolution studies. Diverse applications including the observation of drug response in living cells and affinity studies can also be performed.

Visualizing Chemistry and flow within a Microfluidic System

Spero was employed to visualize the formation and distribution of the HOD species during deuteron/proton exchange between water and deuterium oxide streams within a microfluidic cell. The unique real-time imaging capabilities of Spero allow the observation of flow dynamics, diffusion, advection and reaction products at full 30 fps video with 1.4 µm per pixel resolution at user selected infrared frequencies. In addition, time histories of absorption for any pixel can be extracted from the video frames. And finally, full data cubes can be measured and individual spectra extracted for analysis.

Experimental

A standard configuration Spero microscope with 12.5X 0.7 NA objective having a field-of-view of 650µm x 650µm was employed for imaging. All data cubes were collected in transmission mode over the range of 1800 to 900 cm^{-1}. Video was recorded with discrete frequency illumination at 1640, 1442 and 1200 cm^{-1} corresponding to the approximate absorbance maxima of H-O-H, H-O-D and D-O-D respectively. Video was recorded in greyscale and various high contrast color palettes available within the ChemVision software. Hyperspectral image cubes were recorded at a resolution of 4 cm^{-1} over a spectral range of 1800 to 900 cm^{-1} resulting in an acquisition time of 5 minutes. A 3 mm thick CaF_2 window was used as a background reference for the images and data cube. A custom microfluidics cell with a "+" junction providing configurations for shield and laminar flow or up to three streams combining at right angles was used. The channels were approximately 100µm in width formed within a 20µm thick PTFE spacer between two CaF_2 windows.

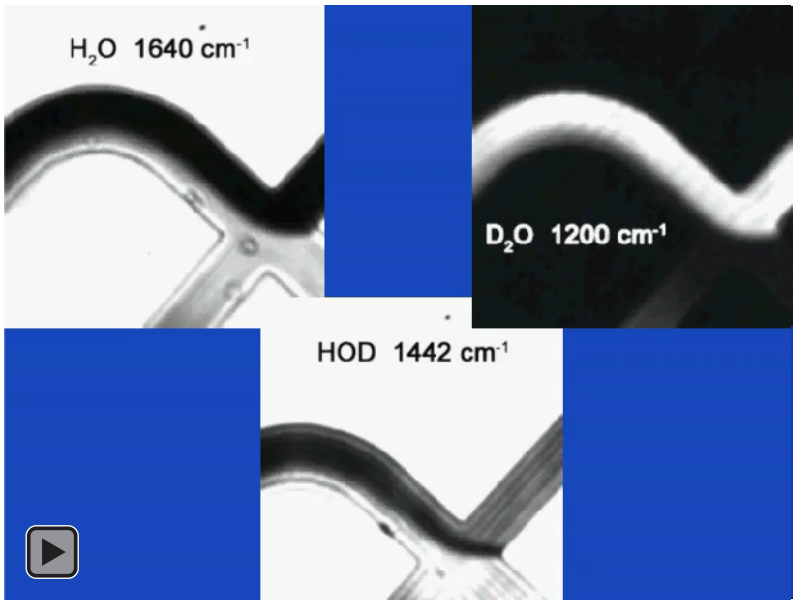

Figure 2: Discrete frequency video of H_2O and D_2O interface with proton deuteron exchange zone clearly observed at 1442 cm^{-1} Click image to play video

Flow was controlled by a syringe pump; (New Era, model NE-400) fitted with 5 mL plastic barrel syringes. Flow rate for the data cube and corresponding videos was approximately 20µL/hour as reported by the syringe pump. Deuterium Oxide, (Cambridge Isotope Laboratories Inc.) and deionized water were used at room temperature. The temperature of the cell was not controlled.

ChemVision™, Daylight Solutions software was used for instrument control, data cube and video acquisition. ImageLab (Epina GmbH) and MATLAB (MathWorks) were used in post processing.

Results and Discussion

Figure 1 shows the interaction of H_2O with D_2O at a flow rate of approximately 250µL/minute as the cell was initially primed. Images were acquired at frequencies corresponding to the H-OH and D-OD absorbance maximums. Each image depicts an area of 650µm x 650µm and consists of 230,400 individual pixels. Within the ChemVision software images can be acquired in greyscale or any number of color schemes to enhance contrast. Each pixel is a transmittance value ratioed to the previously measured background. In these images H_2O is entering the cell in the two channels at a right angle to the intruding D_2O stream. The image clearly shows the shape of the intrusion zone and the sharp delineation of the two liquids. The PTFE cell spacer has an absorbance band coinciding with the D_2O absorbance maximum at 1200 cm^{-1} and hence appears dark in the image.

The images in Figure 2 are still frames from video clips recorded at the H_2O, D_2O and HOD absorbance maxima with at a flow rate of 20µL/hour. At this flow rate a restriction

prevented the flow of water from the lower channel. Illumination at 1640 cm^{-1} shows a laminar flow at the interface of the H_2O and D_2O streams. Changing the frequency to the absorbance maximum of HOD at 1442 cm^{-1}, the area in which the proton/deuteron exchange is occurring and its distribution plume is clearly visible.

As in the previous figure, a video at each frequency can be played by using the mouse and left clicking within the image. To show the dynamic nature of the flow the pump was pulsed repeatedly perturbing the flow to depict motion.

The figures above were acquired with Spero's real-time chemical imaging mode. It's clear that spatial chemical information can be obtained by choosing the appropriate frequency for the functional group(s) of interest. The next step is to collect the complete hyperspectral image cube. In this mode, all of the IR frequencies are measured resulting in a 2D spatial image of the sample on the X-Y axes and IR absorbance on the Z axis. In this mode, *complete fully ratioed IR spectra can be extracted at any of the 230,400 pixels*.

linked to the cursor position. Left mouse clicking the image on the page will animate the cursor and display the linked spectra. The color pallet used is based on the recorded absorbance intensity and ranges from low (blue) to high (red) and corresponds to the HOD concentration. The plume of HOD is clearly visible as is the concentration gradient across the flow interface.

The spectra from a line of pixels through this interface were extracted and used to construct plots of normalized net area verses pixel number, shown in Figure 4. Here, integrated area normalized to unity from the characteristic absorbance bands for H_2O, D_2O and HOD are plotted verses pixel location. This plot shows the highest concentration of HOD corresponding as would be expected for a 1:1 reactant to product ratio, to the point at which the H_2O and D_2O integrated net absorbance intersects. With calibration this absorbance plot can be directly related to concentration.

Kinetic information can also be acquired from the experiment in several ways. Spectra extracted from the image cube can

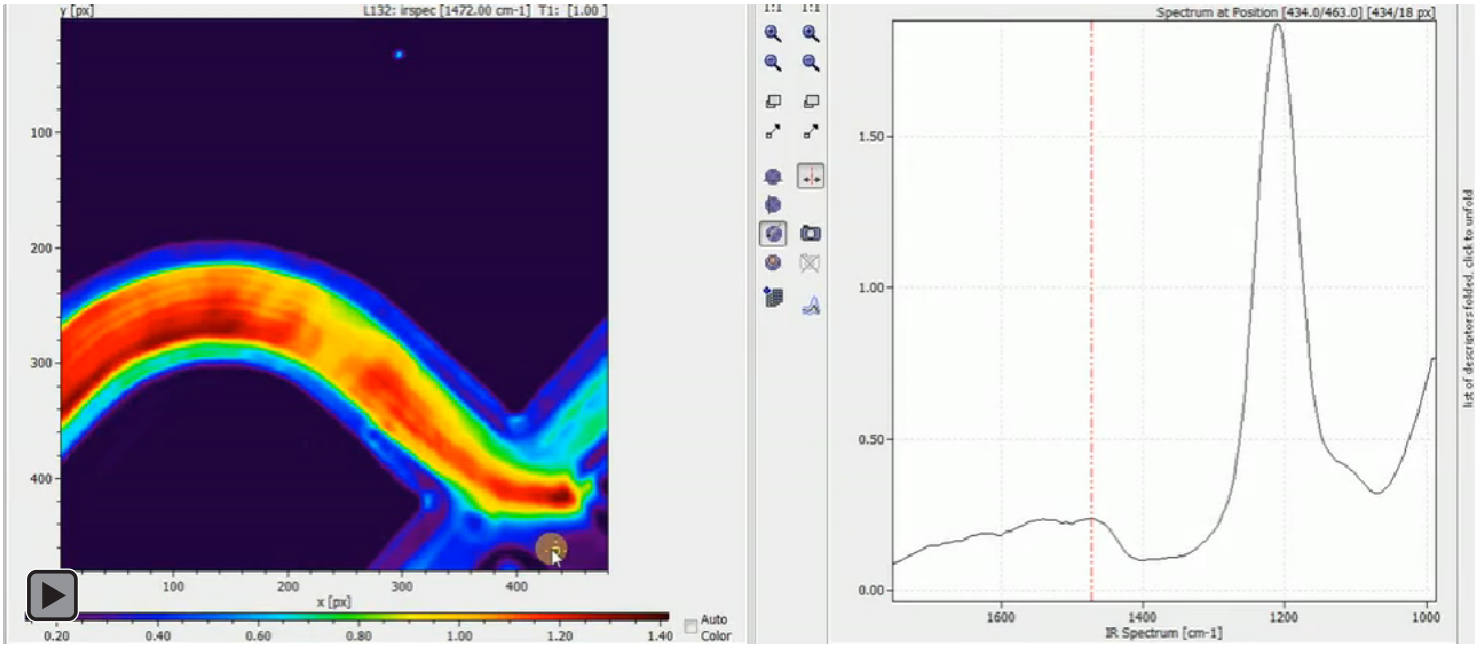

Figure 3: ImageLab display of the image cube of D_2O/H_2O flow showing absorbance image at 1442 cm-1 corresponding to HOD absorption. Red indicates high absorbance. Linked spectrum at right corresponds to cursor position. The figure can be animated by left clicking anywhere in the image.

Images can be displayed at any frequency using various contrast enhancements, such as univariate or bivariate peak height or area ratios, to sophisticated multivariate chemometric algorithms. Figure 3 shows the cell at the IR frequency corresponding to the HOD species, 1442 cm^{-1}. The spectrum in the panel on the right corresponds to the cursor location on the image to the left panel. This spectrum is

be related to time using a distance to time calculation if the flow rate is accurately known. Additionally, discrete frequency imaging can be employed as in Figure 2 where 30 fps video is recorded over the time period of the reaction. Each pixel in the video frame contains a fully ratioed absorbance (or transmittance) value which can be extracted and plotted vs time calculated from frame number.

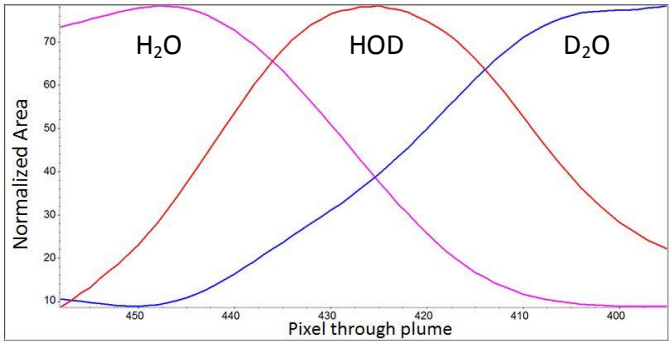

Figure 4: Histogram plot of normalized area vs. pixel for D_2O, H_2O and HOD

Spectral information in the image cube can be displayed in a variety of forms from simple waterfall plots of spectrum vs pixel position as in Figure 5 to complex 3D representations of components within the flow channel. Figure 6 shows the 3D representations of the concentration of H_2O (blue), HOD (red) and D_2O (green) within the fluidic channel.

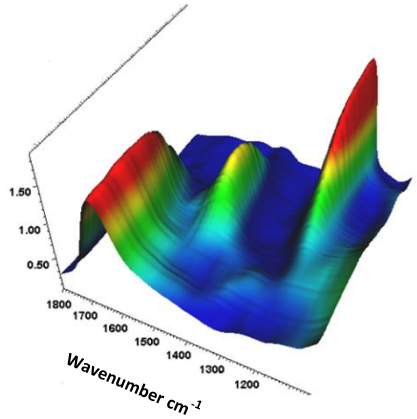

Figure 5: Waterfall plot of H_2O, D_2O and HOD spectra across the H_2O, D_2O interface.

Summary

Infrared spectroscopy is an invaluable tool in identifying, quantifying and trending reaction components over time. Spero expands upon this capability to simultaneously measure chemical content over large areas and to visualize their distribution and interaction in real time.

Spero provides the unique ability to visualize within microfluidic and flow chemistry experiments many parameters including:

- Spatial distribution of reactants, products and intermediates
- Flow dynamics
- Diffusion/partitioning
- Advection and transport
- Concentration
- Dissolution
- Kinetics

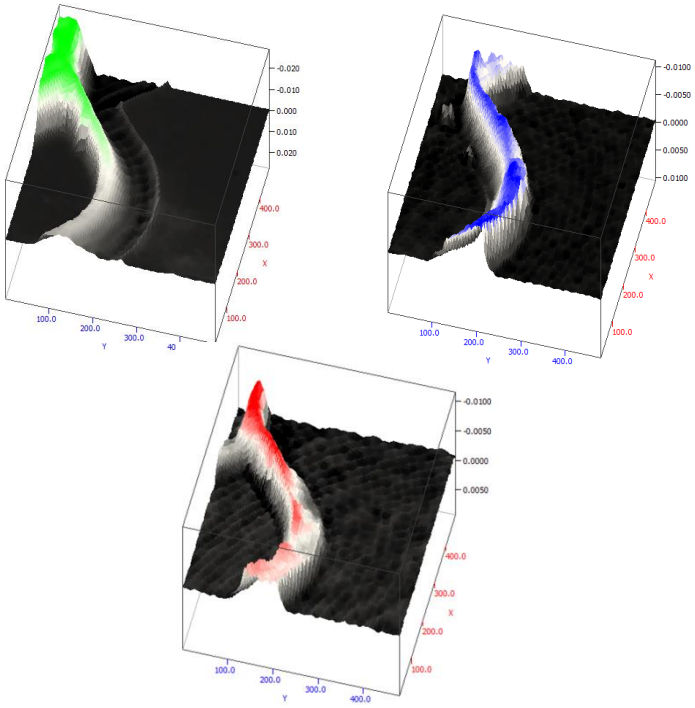

Figure 6: 3D representations based on the concentration of H_2O (blue), HOD (red) and D_2O (green) within the fluidic channel.

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