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The Scanning Mass Spectrometry Probe: A Scanning Probe Electrospray Ion Source for Imaging Mass Spectrometry of Submerged Interfaces and Transient Events in Solution

Peter A. Kottke[1], **F. Levent Degertekin**[1,2], and **Andrei G. Fedorov**[1,2]

[1]G.W.W. School of Mechanical Engineering Georgia Institute of Technology, Atlanta, GA

[2]Petit Institute for Bioengineering and Bioscience Georgia Institute of Technology, Atlanta, GA

Abstract

The scanning mass spectrometry (SMS) probe is new electrospray ion source. Motivated by the need for untargeted chemical imaging of dynamic events in solution, we have exploited an approach to electrospray ionization (ESI) that allows continuous sampling from a highly localized volume (~picoliters) in a liquid environment, softly ionizes molecules in the sample to render them amenable for mass spectrometric analysis, and sends the ions to the mass spectrometer. The key underlying concepts for our approach are

1) Treating the electrospray capillary inlet as a chemical scanning probe, and

2) Locating the electrospray point as close as possible to the sampling point, thus providing the shortest response time possible.

This approach enables chemical monitoring or imaging of submerged interfaces, providing access to details of spatial heterogeneity and temporal changes within liquid samples. It also permits direct access to liquid/ liquid interfaces for ESI-MS analysis. In this Letter we report the first demonstrations of these capabilities of the SMS probe, and describe some of the probe's basic characteristics.

Chemical imaging with micrometric resolution of dynamic biochemical interfaces, especially interfaces immersed in liquid, is an area of considerable interest. Cell biologists, for instance, are excited about the ability to use such techniques to elucidate details of complex cellular processes.1 Proteomics studies increasingly find that data including correlation in time and space of protein presence is essential to biological understanding. Currently, all non-spectroscopic chemical imaging techniques are inherently limited in their discovery potential by the requirement that target analytes be identified ahead of time. Spectroscopic methods of chemical imaging, on the other hand, can provide information about multiple and possibly unanticipated analytes. Among spectroscopic methods, mass spectrometry holds the greatest promise for biochemical imaging. Mass spectrometry (MS) is a powerful analysis method because it allows identification of multiple chemicals simultaneously, displaying considerable chemical specificity and sensitivity.[2] In particular, with the advent of soft ionization methods, MS has become unparalleled in its ability to identify large biomolecules. In this Letter we report the first demonstrations of an MS-based technology that provides a means for obtaining the spatial or temporal distribution of chemicals in solution, and hence dramatically expands that applicability of imaging mass spectrometry (IMS).

The scanning mass spectrometry (SMS) probe is an electrospray ion source that helps fill a void in currently used imaging mass spectrometry technologies (e.g., MALDI,[3] MALDESI,4 LAESI,[5] SIMS, [6,7] DESI,[8,9] and PESI10) in that it allows for continuous imaging of transient events at submerged interfaces in solution.[11] This is accomplished by direct sampling of the

liquid sample through a capillary without active pumping. The current state of the art for electrospray based IMS is the liquid micro-junction surface sampling probe (LMJ-SSP) developed by van Berkel and collaborators. 12–14 The LMJ-SSP samples through a 127 μm (or greater) diameter capillary that is typically 8 cm in length, with resulting relatively large spatial and temporal resolutions.15 Application of the LMJ-SSP involves active pumping of solvent to exposed surfaces, creation of a wetted sample area, and then withdrawal of liquid from the wetted spot using aspiration. Another ESI-MS capillary based scanning probe was developed by Modestov et al., for imaging of electrochemically heterogeneous surfaces and transient electrochemical events.16 Their probe, called the scanning capillary microscopy mass spectrometer (SCM-MS) has two coaxial capillaries, like the LMJ-SSP, and uses forced flow of solution to the sample site in the outer annulus. Instead of using a liquid microjunction and assist gas flow induced aspiration to drive liquid sampling flow, the SCM-MS encloses the entire sample in a pressure chamber to allow a forced pressure difference to drive flow through the 25 μm ID sampling capillary with a temporal response of ~ 1 min.

To improve spatial resolution one must reduce the sampling capillary radius, while enhancing temporal resolution requires a decrease in both capillary radius and capillary length. The theoretical limit for spatial and temporal resolution would be realized by an ion source in which the electrospray event occurs at the sampling point, i.e., capillary length is zero. [17–20] The SMS probe (Fig 1) is a first attempt to approach this theoretical ideal by sampling through a capillary of small length and radius. The flow through the SMS probe is driven by electro-hydrodynamic phenomena supplemented, if necessary, by a slight vacuum at the spray location in a manner first demonstrated by Felten et al.[21] Thus the probe passively samples directly from liquid at ambient pressures. This enables two important modes of operation:

1) *Scanning Probe Imaging:* The sample is moved under the probe and mass spectra are then correlated with probe position; and,

2) *Transient Monitoring:* The probe is positioned in the desired location and spectra are collected as a function of time.

In this work we demonstrate the application of the SMS probe in both operational modes.

EXPERIMENTAL SECTION

Reagents

Solvents used were water (Ricca, deionized reagent grade, >18 MW/cm) and cyclohexane (JT Baker min 99.0%). Dissolved or mixed into the solvents were D(+)-Glucose Monohydrate (Fluka >99.5% HPLC), Sodium Formate (Sigma Aldrich 99+% ACS reagent), Formic Acid (Fluka ~98%), Trihexyl(tetradecyl)phosphonium bis(trifluoromethanesulfonyl) (Strem min 97%), Adenosine 5'-triphosphate magnesium salt from bacterial source (Sigma 96% Purity HPLC), Hexokinase, Type III: from Bakers Yeast (Sigma 51 u/mg solid) and ammonium bicarbonate (Fluka >99.5% Ultra).

Apparatus

The probe is connected to a Brüker Daltonics MicrOTOF mass spectrometer from which the original ESI ion source has been removed. A schematic of the SMS probe is provided as Supplementary Information, as are details of its operation. We have used fused silica sampling capillaries ranging from 10 μm inner diameter to 50 μm inner diameter and from 8 mm to 3 cm in length. All of the capillaries have a tapered outer diameter at the spray end. Some of the capillaries were purchased (New Objective Silica Tips, which have tapered inner diameters) while others we fabricated in-house using meniscus etching (these do not have tapered inner diameters).[22]

Transient Reaction Monitoring

A capillary with a 20 μm inner diameter and a length of 1.5 cm was used in the SMS probe to demonstrate the ability of the probe to monitor transient chemical reactions. Two solutions were prepared: one of 1 mM adenosine 5'-triphosphate magnesium salt (ATPMg), 1 mM D(+) Glucose (Glucose) and 50 mM ammonium bicarbonate in DI water, and one of 1mg/mg Hexokinase and 50 mM ammonium bicarbonate in DI water. A 100 μL drop of the ATPMg/ Glucose solution was deposited on a grounded, clean aluminum SEM stub that served as the sample stage. Positive mode ESI operation of the probe was initiated as described in the Supplementary Information. Once a stable mass spectrum with peaks indicating the presence of glucose and ATP (m/z = 198.1 and 582.9, respectively) was established, 20 μL of the Hexokinase solution was added by pipette to the sample droplet.

Interface Scan

A capillary with a 50 μm inner diameter tapering to 8 μm at the tip, and with a length of 2 cm was used in the SMS probe to demonstrate the ability of the probe to image a liquid/liquid interface. Two solutions were prepared: one of 500 mg/mL sodium formate and 0.1% formic acid in DI water, and one of 10 μM Trihexyl(tetradecyl)phosphonium bis (trifluoromethanesulfonyl) in cyclohexane. Trihexyl(tetradecyl)phosphonium bis (trifluoromethanesulfonyl) is an ionic liquid (IL) which raises the conductivity of cyclohexane to enable ESI.[23] A 30 μL drop of the aqueous solution was placed inside a polycarbonate tube (inner diameter 3.175 mm) with one open end affixed to and sealed by an aluminum SEM stub which was electrically grounded and attached to the X-Y-z stage. A 20 μL drop of the cyclohexane/IL solution was placed atop the aqueous solution, and a meniscus between the two immiscible liquids was clearly visible through the transparent polycarbonate. To initiate electrospray in positive ESI mode the procedure to start probe operation as described in the Supplementary Information was performed with one exception: the spray chamber was initially maintained at a slightly positive pressure with respect to the surroundings. This enabled moving the stage so that the probe tip was in the aqueous solution prior to initiating spray: the probe tip was approximately 0.5 mm below the water/hexane interface when the pressure in the spray chamber was reduced and electrospray commenced. Once a stable mass spectrum with peaks indicating the presence of sodium formate clusters (e.g., m/z = 498.9, 566.9, 634.9, 702.9, etc.) was observed, the stage was moved down at 1 mm/s to bring the tip almost instantaneously across the meniscus and into the hexane. Once it was clear that the probe was electrospraying hexane (m/z peaks at 483.5 and 1247 corresponding to the IL cation and to an adduct of the IL with one IL cation, respectively) the stage motion was repeated in reverse, moving the probe tip back across the hexane/ water interface at 1 mm/s into the water.

Imaging

In general, IMS requires software that takes two sets of data and combines them to make an image. The first data set contains peak intensities at multiple mass-to-charge ratios as a function of time. The second data set is probe position as a function of time. The two are combined to produce a data set consisting of peak intensity for mass-to-charge ratios as a function of probe position, and the images are graphical representations of subsets of this data. We have not yet implemented a software system for IMS with the SMS probe. Instead, the “image” of the interface is a 1-D filled contour plot indicating a single base peak intensity (m/z = 498.9) as a function of time juxtaposed with a graphical representation of the position of the probe tip.

Temporal Response Characterization

The temporal response of the SMS probe coupled to the TOF mass spectrometer was characterized as described in the supplementary information.

RESULTS AND DISCUSSION

Imaging mass spectrometry requires the ability to ionize a sample from a well defined small volume. Furthermore, practical implementation requires the ability to operate (scan) at a reasonable speed. Therefore it is clear that spatial and temporal resolution are key characteristics of any ion source developed for IMS. Our goal has been to develop an ion source that can be applied to IMS within liquid samples. The key motivation is the desire to obtain transient biochemical images of chemical events originating from live samples. There are numerous other potential uses, especially involving bio-active surfaces. The experiments performed for this work are meant to demonstrate that the SMS probe has the fundamental characteristics necessary for application to transient biochemical imaging.

To demonstrate the temporal response we used the SMS probe to monitor chemistry during an enzyme catalyzed reaction as described in the Experimental Methods section. Hexokinase catalyzes the transfer of a phosphate group from ATP to glucose and has been immobilized in ultra-microelectrode based biosensors used to measure transient concentrations of ATP.[24–26] We demonstrate the ability of the SMS probe to monitor transient concentrations of the reactants and products after addition of hexokinase to a solution of glucose and ATP (Figure 2). The kinetics of this reaction depend upon the concentration of the reactants, products (ADP and glucose-6-phosphate) and the concentration of the enzyme[27] and thus by varying the initial concentrations the rate of reaction was matched to the temporal response of the probe.

Characterizing the ability of the probe to image spatial heterogeneity of chemical species in solution is ideally performed on a system with well defined concentration differences that do not vary with time. Such a system is difficult to create in a single liquid system due to the effects of diffusion; however, it can be realized at a liquid/liquid interface. We used a water/hexane interface to demonstrate spatial imaging with the SMS probe (Figure 3). The resulting "image" is based on the intensity of a single peak (m/z=498/5) chosen from the series of peaks present due to sodium formate clusters in water.

It seems self evident that the size of the capillary inlet is the key determinant of spatial resolution. We have used a variety of capillary geometries with the SMS probe, and have found, for capillary lengths of 0.8 cm to 3 cm, that the capillary inner diameter also determines the temporal resolution. We characterized the temporal response of two capillaries of different geometries using a batch addition to approximate a step change in input conditions (see Supplementary Information). For a 1.5 cm long capillary with a 20 μm inner diameter tapering to 10 μm at the tip the response time is ~2 seconds. For a 1.5 cm long capillary with a 50 μm inner diameter tapering to 8 μm at the tip the response time is ~20 seconds. Thus, it is clear that a reduced diameter provides for improved spatial and temporal resolution. In addition to determining the fastest changes in chemistry that the probe can monitor, the temporal resolution, combined with the spatial resolution, limits the possible scan speed, i.e. $\text{scan speed}_{\max} = \frac{\text{spatial reolution}}{\text{temporal resolution}}$. Using the inner diameter of the sampling capillary to define the spatial resolution, the maximum scan speed would be 10 μm/s and 2.5 μm/s for the 20 μm and 50 μm inner diameter capillaries, respectively. Therefore smaller diameter capillaries yield not only improved spatial resolution and temporal resolution, but also higher scan speeds.

Both of the fused silica capillaries used for the temporal response characterization experiment are commercially available. Fused silica capillaries that can be easily incorporated into the current SMS probe design can be made using meniscus etching[22] of capillaries with smaller inner diameters (e.g., 10 μm, 5 μm and 2 μm). Although we have successfully used such in-house fabricated capillaries with a 10 μm inner diameter in the SMS probe, the performance

was erratic, and we have had no success in attempts with smaller inner diameters. The problems with smaller diameter capillaries seem to arise from clogging, inability to clear bubbles from the capillary, and difficulty initiating or maintaining sufficient flow.

To assist in developing successful probes with smaller sampling radii and hence improved spatial and temporal resolution, we have developed an order-of-magnitude scaling model that predicts the conditions necessary for successful electrospray from a capillary with the inlet immersed in a liquid at ambient pressure (see Supplementary Information). The model indicates that two competing requirements limit the possible capillary geometries, described by sampling path radius and length. The need to focus the electric field sufficiently to achieve stable electrospray without dielectric breakdown of air imposes a minimum on capillary length for a given radius. With liquid flow driven by an effective pressure difference across the capillary, a maximum capillary length for a given radius is determined by the need to be able to supply liquid at a sufficient flow rate to maintain the electrospray. These two competing requirements define an envelope of geometries for which stable electrospray is expected to be achievable for a given capillary material, sample solution, and applied pressure difference, i.e., spray side vacuum. They also define a minimum sampling capillary radius (~1–10 μm for water with conductivity of 1 mS/cm in a fused silica capillary and with applied pressure difference of ~10 kPa). It is notable that this minimum corresponds well with the capillary geometries which presented difficulties for probe operation as described above.

We have demonstrated that the SMS probe is capable of responding to changes occurring on a timescale of seconds, and can be repeatably used for spatial imaging with a 20 μm inner diameter capillary. In addition to the improved temporal and spatial resolution offered by our probe, which are not trivial advances, the key difference between our work and that of previous researchers is the ability to passively sample from a well defined microenvironment. It is this important difference, which comes both *with* and *as a result of* a reduction in sampling capillary dimensions, that makes the possibility IMS of dynamic submerged interfaces a reality. The challenge of application to living biological systems will require strategies for dealing with unseparated complex mixtures with usually high salt content, and implementation of a system to accurately control probe-tip/ sample relative position. The technology also will benefit from continued size reduction and concomitant resolution enhancement, and from implementation of strategies for extraction of quantitative information from imaging data sets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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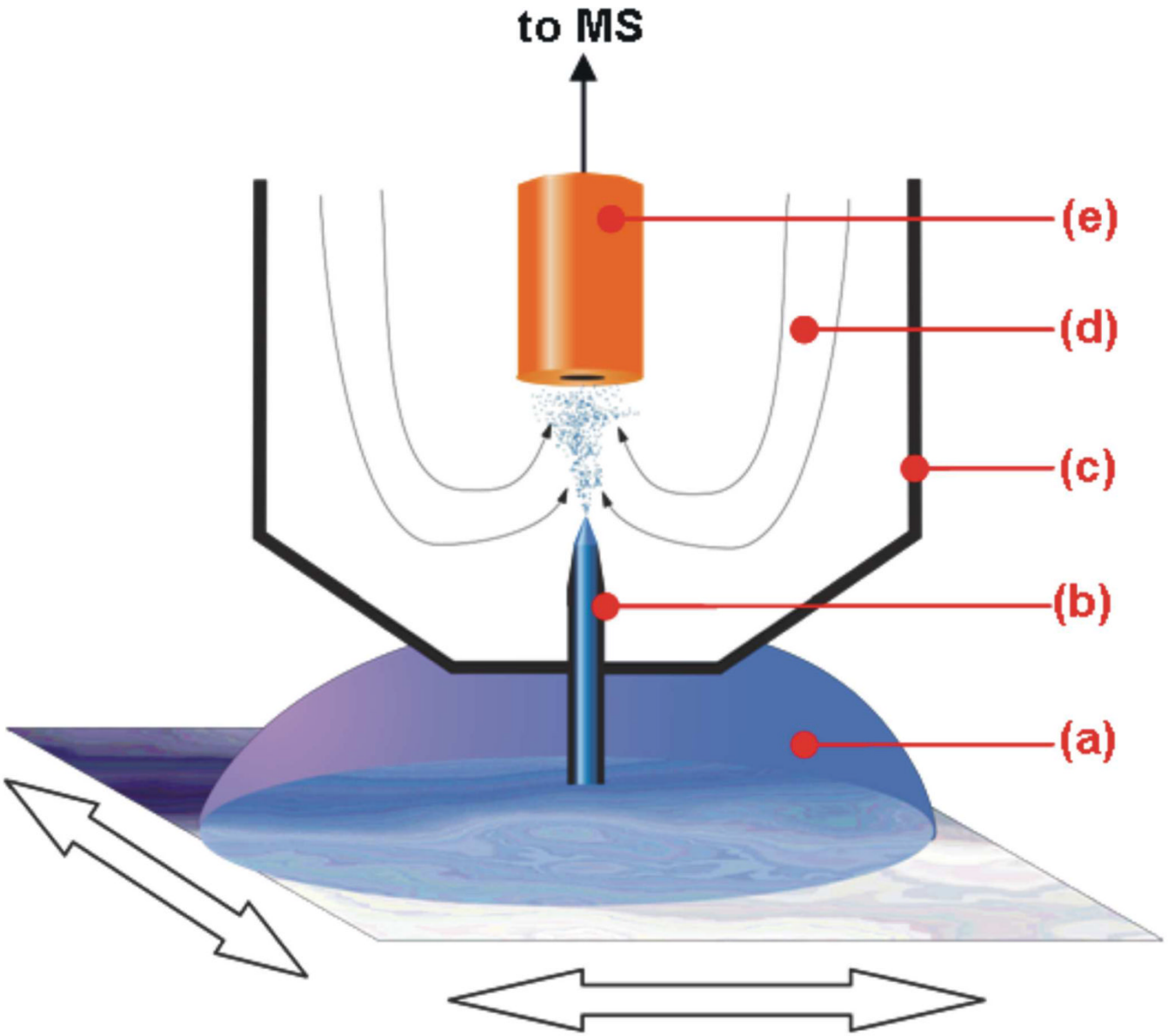

Figure 1.
Conceptual schematic of the scanning mass spectrometry probe (SMS probe) an electrospray ion source for imaging mass spectrometry of submerged surfaces and transient events in solution. Key elements are (a) Sample – can be dynamic and spatially heterogeneous, (b) Sampling capillary – key determinant of temporal and spatial resolution, (c) Barrier – separates liquid sample from spray chamber, (d) Spray atmosphere – can include assist flow, drying gas, and variable pressure control, and (e) counter electrode/ ion transport tube.

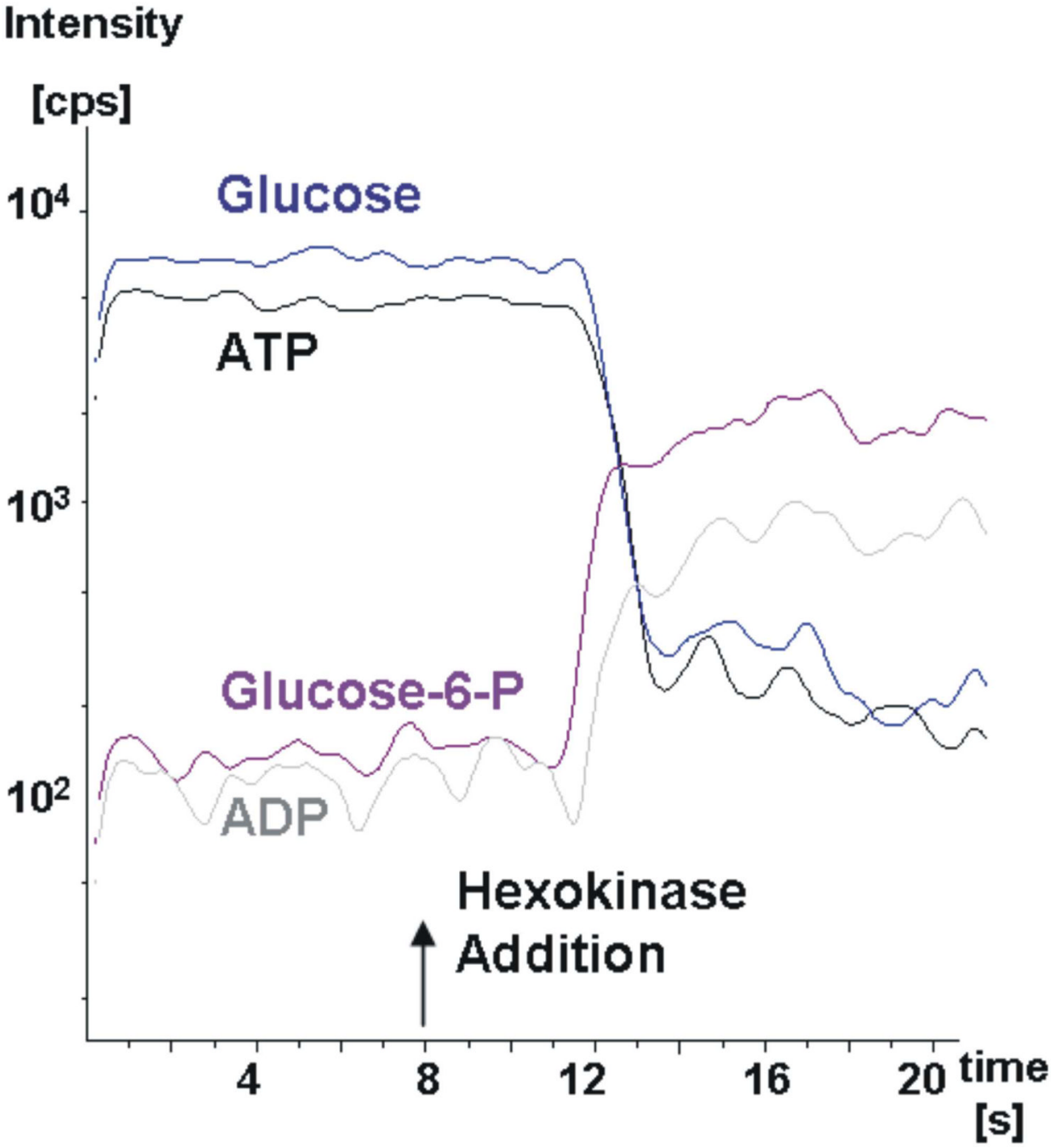

Figure 2.
Application of the SMS probe for real time monitoring of a transient enzyme catalyzed reaction. Yeast hexokinase, added at time 8 s to a solution of 1mM ATPMg, 1 mM glucose, and 50 mM ammonium bicarbonate in DI water catalyzes the transfer of a phosphate group from ATP to glucose, yielding glucose-6-phosphate and ADP. The reaction kinetics are visible to a temporal resolution of ~ 2 seconds.

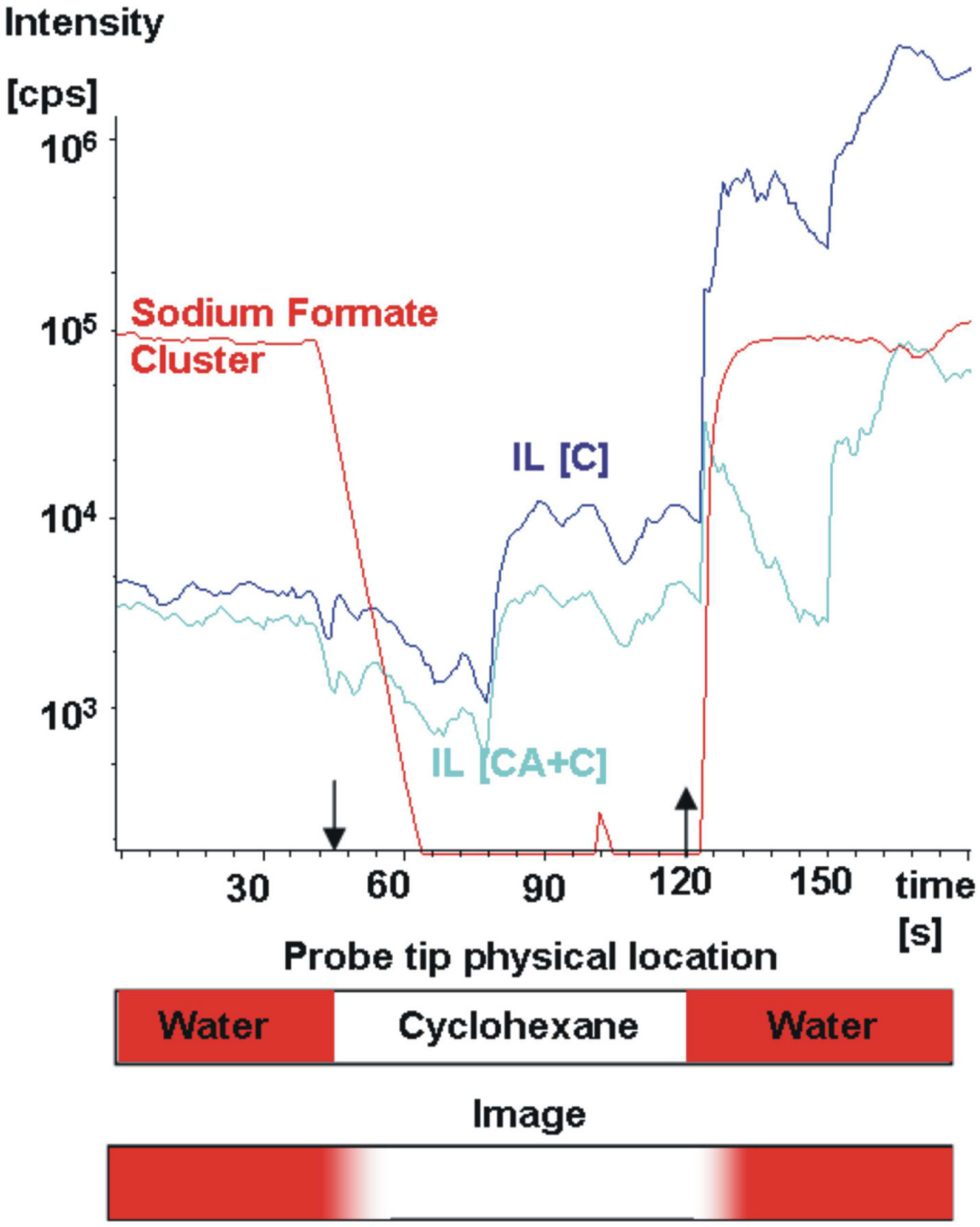

Figure 3.

Application of the SMS probe to image a spatially heterogeneous liquid sample of two immiscible liquids, water and cyclohexane. Initially the probe tip is in a solution of 0.1% formic acid and 500 mg/mL sodium formate in water. At time = 45 seconds the stage is moved at 1 mm/s for 1 second to cause the tip to traverse across the meniscus between the aqueous solution and a solution of 50 μM ionic liquid in cyclohexane. At time = 120 seconds the stage motion is repeated in reverse, moving the tip back into the aqueous solution.