

Sampling Accessories for the High-Throughput Analysis of Combinatorial Libraries Using Spectral Imaging

Christopher M. Snively and Jochen Lauterbach

This article describes several special sampling accessories the authors designed that allow a variety of combinatorial systems to be analyzed.

The combinatorial approach has emerged in recent years as a method for rapidly and efficiently discovering new and novel compounds. This approach consists of two key steps: synthesizing a large number of potentially useful formulations (collectively referred to as a “library”), and the subsequent rapid testing of the library to determine the usefulness of each formulation to the specific application. Although this approach was originally developed for pharmaceuticals and small organic molecules (1), there has been much recent interest in extending it to the discovery of materials, particularly heterogeneous catalysts (2).

Numerous synthetic methodologies have been developed for rapidly and efficiently generating large combinatorial libraries. Once these libraries are generated, however, they must be characterized and tested for effectiveness using analytical techniques. Unfortunately, currently available analytical techniques are often incapable of keeping pace with the large numbers of compounds in the

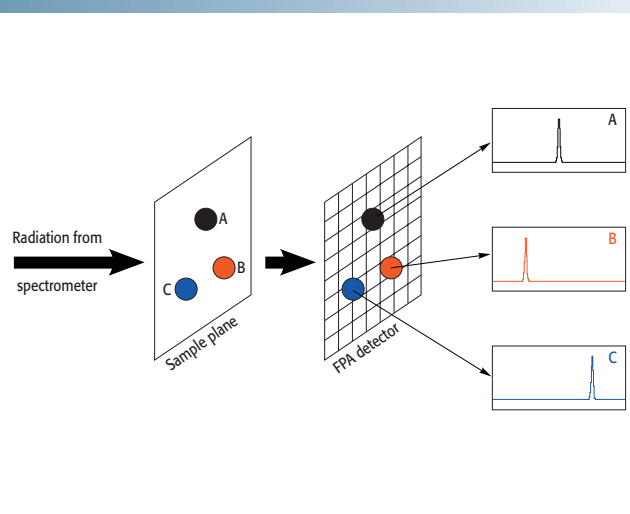
newly generated libraries. Because any process is only as fast as the slowest step, this creates a bottleneck that dramatically slows the entire combinatorial process. This bottleneck has created a serious demand for developing analytical techniques specifically designed for the high-throughput analysis of combinatorial libraries.

To date, three general types of approaches have been used for analyzing combinatorial libraries. The first approach is to simply implement currently available one-sample-at-a-time techniques, such as Fourier transform-infrared (FT-IR) spectroscopy (3), nuclear magnetic resonance (NMR) spectroscopy (4), mass spectrometry (MS) (5), and high-performance liquid chromatography (HPLC) (3, 4). Although this approach is the easiest and cheapest to implement with a small number of samples, it consumes too much time for libraries having hundreds to thousands of members because the screening time is directly proportional to the number of samples to be analyzed.

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Figure 1. Basic concept of the application of array detector-based analytical techniques to high-throughput screening. Information is collected from multiple samples simultaneously. In this scheme, the throughput is directly proportional to the number of samples in the field of view of the array detector. (Reprinted with permission from reference 13. Copyright 2000 American Chemical Society.)



The second approach is based on conventional serial techniques that have been modified using various automation approaches to decrease the screening time. Among these are scanning MS (6, 7) and resonance-enhanced multiphoton ionization (REMPI) (8). High-throughput scanning MS is a method of sequentially analyzing the gases from samples in a combinatorial library. Although this technique can analyze samples faster than previously possible, it has the key disadvantage of being able to analyze only one library element at a time. In REMPI, tunable lasers are used to generate photoions of reaction products, which are detected by an array of microelectrodes in close proximity to the samples. Although this technique is fairly straightforward to implement, a suitable laser frequency for each species of interest must be known and accessible to successfully apply it.

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The third approach involves the development of truly parallel screening techniques, which gather data simultaneously from all elements in a library. This category includes IR thermography and FT-IR imaging. Thermography detects the IR radiation emitted by objects and has been used to detect activity for exothermic reactions in combinatorial libraries in a truly parallel fashion (9, 10). However, thermal imaging does not possess the ability to chemically resolve product composition. Therefore, it is not clear whether the apparent activity is due to the desired reaction or unexpected side reactions. This issue is a key one when studying catalysts, and highlights the ne-

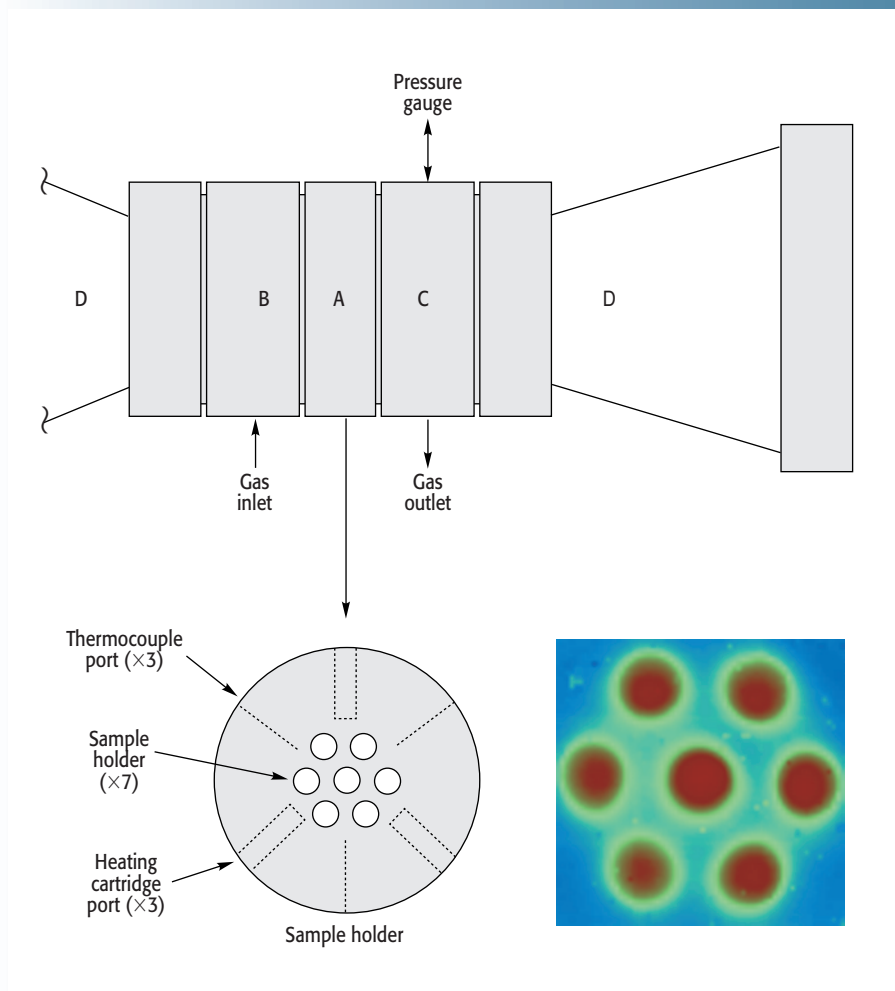


Figure 2. Diagram of reactor used for high-throughput testing of heterogeneous catalysts (left side omitted for clarity). The reactor is composed of (A) a central flange that holds seven samples, and contains heating cartridges and thermocouples for temperature control; (B) and (C) flanges that introduce reactant gases to the catalysts, remove product gases, and provide for pressure monitoring; and (D) conical flanges that keep the IR-transparent windows away from the hot central flange. A brightfield image through the reactor is also shown (bottom right).

cessity to gather chemically specific information from each library member.

During the past two years, we have demonstrated that FT-IR imaging can be successfully applied as a high-throughput analytical technique for a variety of combinatorial systems (11–13). FT-IR imaging is a technique that couples a focal plane array (FPA) detector with an FT-IR spectrometer to enable the simultaneous collection of spectral and spatial information (14). In previous FT-IR imaging studies, spectral information has been collected from a region of a sample to study its spatial heterogeneity. Conversely, the basic concept of the application of FT-IR imaging

to the analysis of combinatorial libraries (Figure 1) is to place several samples in the field of view of the instrument to analyze them simultaneously (15). This provides a multisample, multiplex advantage, in which chemically specific information is collected from multiple samples during a single experiment.

This additionally illustrates an important point for the development of future high-throughput analytical techniques. The development of truly scalable high-throughput analytical techniques is dependent on the development of suitable array detection schemes. This implementation is now apparent for light-based techniques with the advent of

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CCD and FPA detectors, but presents a serious challenge for techniques such as MS or NMR. The development of parallel detection schemes for these techniques is currently a topic of much research interest.

Sampling accessories. To successfully employ FT-IR imaging for the study of combinatorial systems, a completely new FT-IR imaging spectrometer was constructed. This was necessary because of the unique sample geometry requirements of the systems under study. This new FT-IR imaging spectrometer — which, for the first time, employed a rapid-scan spectrometer and refractive optical elements — allowed the imaging spectrometer to be built around the particular sampling accessory, allowing essentially any sample geometry to be studied (12). The necessity of this approach is realized when one tries to imagine analyzing samples inside of an ultrahigh-vacuum reactor at 500 °C on a microscope stage or inside of a small sample compartment.

Study of Adsorbed Molecules on Heterogeneous Catalysts

The activity of heterogeneous catalysts is highly dependent on the interaction of the reactants and products with the catalyst surface. Specifically, it is of interest to study the nature of the adsorbed species on the catalyst surface to better understand how the catalyst functions. This type of study has been performed on supported catalysts using transmission FT-IR spectroscopy, in which a powder composed of the catalyst supported on silica, alumina, or other inorganic support is pressed into a self-supported disk that is thin enough to allow for the transmission of IR light. This disk is held in a small reactor equipped with IR-transparent

windows to allow analysis via FT-IR. Using this approach, a wide variety of pressures (atmospheric to ultrahigh vacuum) and temperatures (cryogenic to several hundred kelvins) can be achieved.

To increase the throughput of this experiment, we designed a reactor that could hold seven supported catalysts. This reactor was constructed entirely of stainless steel ultrahigh-vacuum components. At the center of the reactor a flange held the supported catalysts and also provided temperature control through built-in heating cartridges and thermocouples. Additional flanges on either side of this central flange contained gas inlet and exhaust ports, a pressure gauge, and IR-transparent windows. A diagram of this reactor is shown in Figure 2. This setup not only allowed the study of seven supported catalysts simultaneously, but also had the advantage of

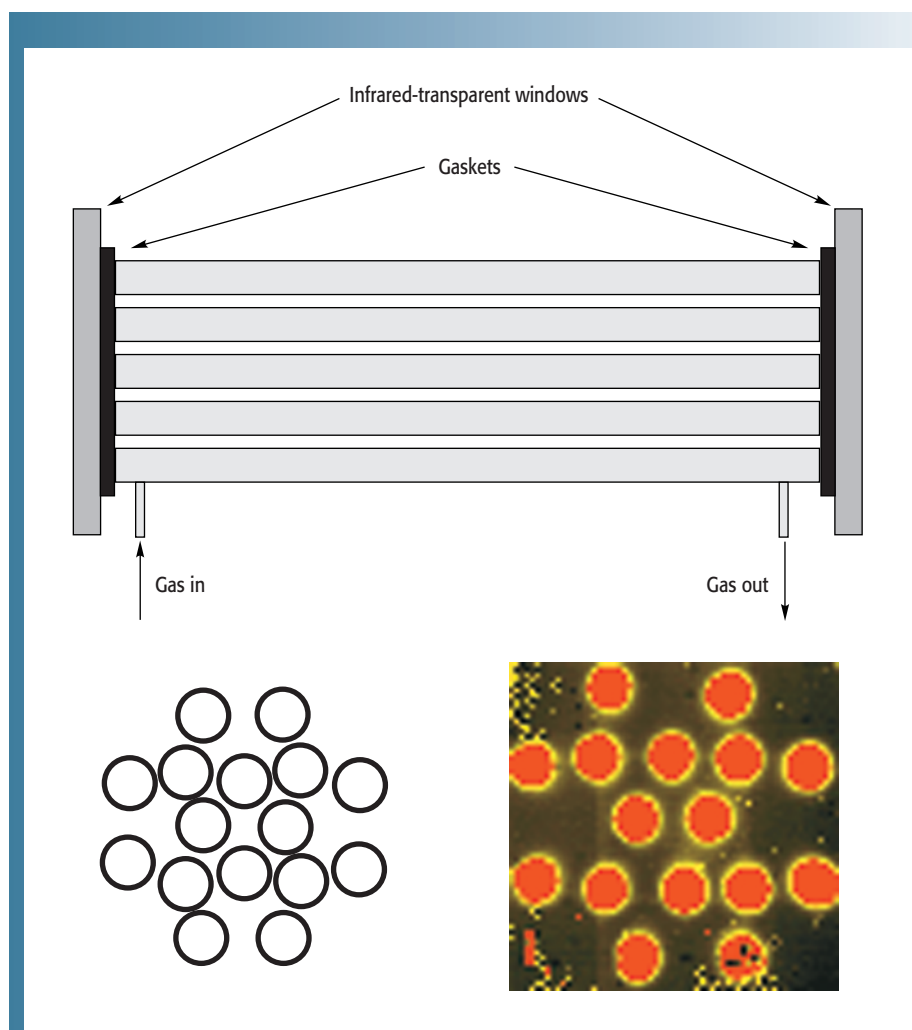


Figure 3. Diagram of gas-phase array used for sampling the gas-phase reaction products from 16 reactors simultaneously. Top: side view of the array, showing the arrangement of sample tubes, gaskets, and optical windows. The brackets holding the entire assembly together, as well as the optical mounts, are omitted for clarity. Bottom left: end-on view of the 16 sample tubes. Bottom right: brightfield image through the array.

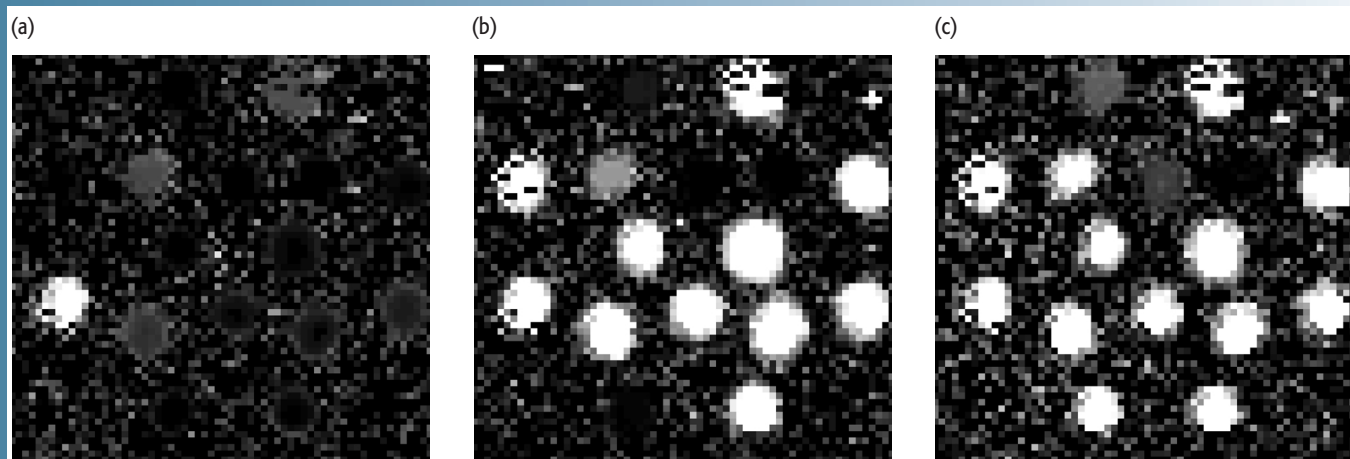


Figure 4. Spectral images of CO₂ concentration (2360 cm⁻¹) measured by FT-IR imaging at (a) 330 K, (b) 430 K, and (c) 530 K. The transition from a low reactivity state to a high reactivity state can be easily visualized for all elements of the array.

allowing the simultaneous pretreatment of all catalysts, which greatly improves the ability to compare results derived from different catalysts.

Monitoring of Gas-phase Reaction Products from Heterogeneously Catalyzed Reactions

To evaluate the performance of individual catalysts in a combinatorial library, it is desirable to directly determine their exit stream composition, for which IR gas-phase spectroscopy can be used. For this purpose, we developed a parallel array of gas-phase sampling cells for high-throughput analysis, composed of 16 stainless steel tubes, each with a separate gas inlet and outlet. The tubes were arranged in a pattern that afforded the tightest packing to minimize the size of the IR beam required to illuminate the array (Figure 3). (We have often been asked why a hexagonal packing arrangement was not used, but when you consider that each cell must be fitted with gas inlet and outlet tubes, the answer becomes obvious.) The ends of the tubes were sealed against IR-transparent windows using a gasket sheet consisting of polybutadiene rubber, fluoroelastomer (Viton), or expanded polytetrafluoroethylene (PTFE), depending on the specific chemical resistance required.

This gas-phase array was plumbed into series with the exhaust stream of a 16-element parallel reactor. To per-

form an experiment, reactant gases were flowed through the reactors, which contained the catalyst samples of interest heated to the desired temperature, and the product gases leaving the reactors were subsequently analyzed by collecting FT-IR images through the entire gas-phase array.

Thus, the product gas compositions from all 16 reactors were analyzed simultaneously. This technique can be used to monitor any gas that has an IR spectral absorption and is present in a concentration above the limit of detection of the specific setup. Our current setup is equipped with 15-cm-path-length tubes, which give a limit of detection (LOD) of approximately 1% for carbon dioxide for a single mirror scan (17-s collection time). As in standard FT-IR experiments, this LOD can be lowered by increasing either the pathlength or the data collection time.

To demonstrate the applicability of our setup to parallel reaction product analysis, we present here results for CO oxidation over an array of 16 transition metal-based supported catalyst samples at atmospheric pressure. Figure 4 shows three spectral images of the 2360 cm⁻¹ band (CO₂ concentration) for three representative catalyst temperatures. The transition from a low reactivity state to a high reactivity state can be easily visualized for all elements of the array. Typical spectra obtained in this manner are shown in Figure 5.

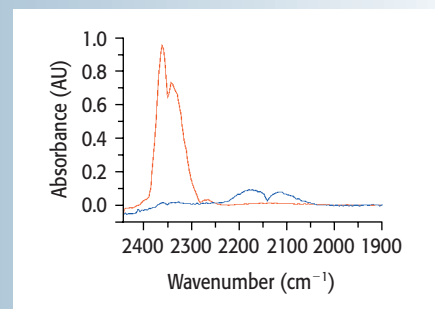


Figure 5. Representative IR spectra from active (red) and inactive (blue) catalysts.

With this approach, it now becomes possible to follow reactants and products in the exit stream in a quantitative manner and to therefore determine conversions and selectivities. Another key advantage of this setup is that it is independent of the specifics of the reactor, which allows this approach to be used with essentially any reactions, conditions, or chemistry, with only slight modifications in the experimental setup. We are currently developing a system that will allow the two setups described earlier to be combined into one, which will allow the correlation of the nature of the adsorbed species with the actual catalyst performance.

Identification of Supported Ligands and Monitoring of Reactions on Resin Beads

In classical combinatorial chemistry, for the generation of libraries of both small organic molecules and pharmaceuticals, the typical sample geometry is a ligand supported on small (10–1000 μm in diameter) polymer spheres. The desired reactions are performed on the attached ligands, and the reaction products re-

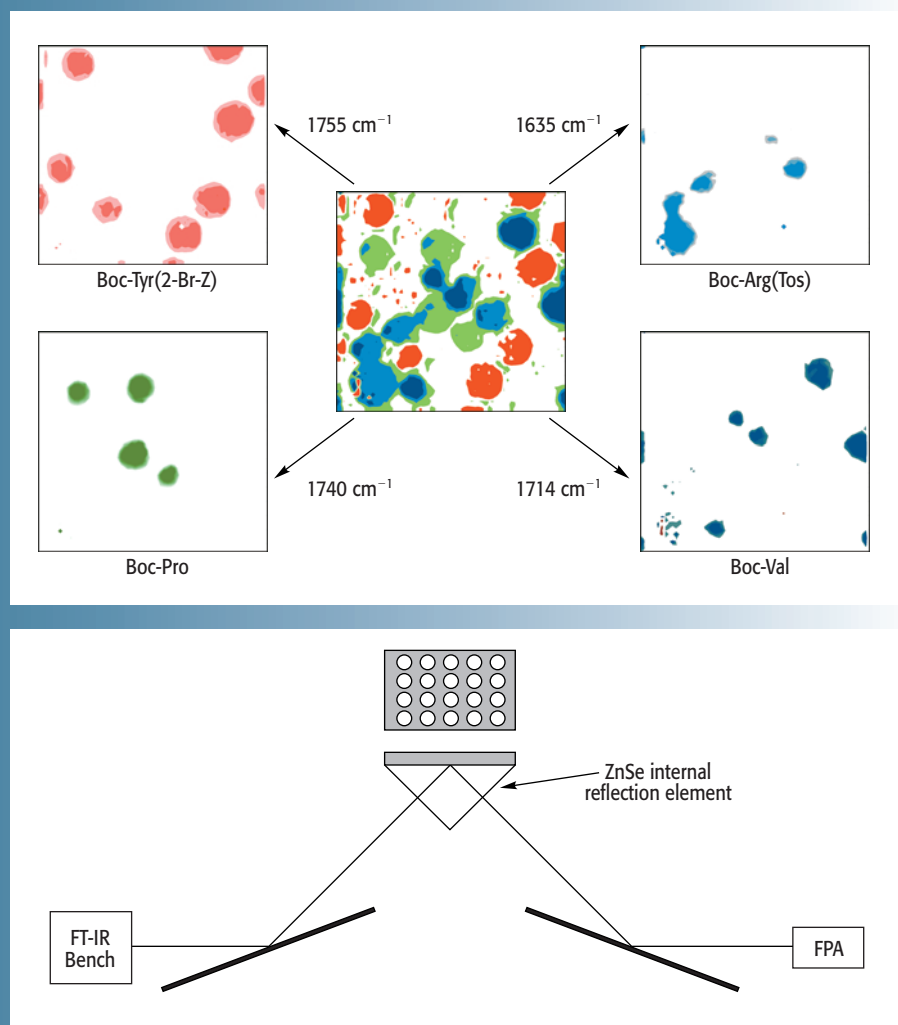


Figure 6 (left). Images of a mixture of beads with supported amino acid ligands. Images were generated by plotting bands specific to Boc-Pro (C=O stretch, 1740 cm⁻¹), Boc-Tyr(2-Br-Z) (C=O stretch, 1755 cm⁻¹), Boc-Arg(Tos) (C=N stretch, 1635 cm⁻¹), and Boc-Val (C=O stretch, 1714 cm⁻¹). The center image is a composite of all of the individual images, showing the location of all beads in the field of view. (Reprinted with permission from reference 13. Copyright 2000 American Chemical Society.)

Figure 7 (left). Diagram of the setup designed for the high-throughput analysis of liquid-phase combinatorial libraries. This setup consists of a 45° ZnSe prism internal reflection element and two mirrors to redirect the IR beam. The same optics that are used for transmission mode sampling are used here and are omitted for clarity. The top of the internal reflection element is outfitted with a PTFE gasket containing a series of holes, each of which holds several hundred microliters of each reaction mixture in the library.

main attached to the bead after the reaction is completed. This allows isolation of the reaction products by physically isolating the beads. These reaction products are then further reacted or chemically cleaved from the bead to isolate the desired compounds.

FT-IR spectroscopy and microspectroscopy techniques have been used for studying bead-supported ligands as methods that do not require cleaving of the ligands (5). We have developed FT-IR imaging techniques to study these beads in a high-throughput manner — both as a means of identifying the supported ligand, and as a method of studying the progress of reactions occurring at the ligand (13). For both of these types of studies, we have employed a simple parallel-plate geometry to contain the samples. This has consisted of either a standard fixed-pathlength demountable sample cell or

an adjustable-pathlength cell, both of which consist of two parallel IR-transparent windows held a fixed distance apart and sealed to retain the solvent.

For identification of resin-bound compounds from a split-and-pool synthesis, where beads carrying different ligands are mixed together, mixtures of beads were placed onto one window of the sample cell and swollen with methylene chloride. The solvent was allowed to partially evaporate, and the sample cell was assembled. Slight pressure was maintained to flatten the beads, which was necessary to obtain spectra free from saturation effects. The sample cell was placed in the field of view of the spectrometer, and images were collected. After this analysis, the beads could then be recovered without damage and submitted for further analysis. By selecting a spectral frequency that is unique for

each ligand and plotting the absorbance value for each pixel at that frequency, images were generated that revealed the location of each type of supported ligand, as shown in Figure 6. This type of analysis is not limited to ligands that have well-separated spectral bands, because more complex types of classification routines — such as principal component analysis — can also be coupled with this technique to identify ligands where the spectra of the individual ligands are highly convoluted.

Because of the dramatically decreased collection time of rapid-scan FT-IR imaging, it becomes possible to acquire in situ kinetic information from reactions occurring on the supported ligands. The ability to examine the progress of reactions in situ has the advantage that the reaction does not need to be perturbed to be analyzed, so it is no longer necessary to remove beads

from the reaction mixture and quench the reaction to monitor the reaction. This removes the need to repetitively manipulate and analyze single beads and also decreases the chances of introducing additional contaminants into the system. The beads are simply placed in the flow cell, the reaction is initiated by introducing the reactant solution, and data are collected (16).

Additionally, this approach can be extended to the study of liquid-phase combinatorial chemistry libraries. We are currently developing macroscopic ($\sim 2 \text{ cm} \times 2 \text{ cm}$) attenuated total reflection imaging capability, which allows the collection of IR spectral information from anything that comes in contact with the internal reflection element (Figure 7). Using this sampling geometry, it is possible to study liquid-phase combinatorial libraries, both for identification and monitoring of reaction kinetics.

Conclusions

We have demonstrated that FT-IR imaging has been successfully applied as a high-throughput analytical technique for studying several combinatorial systems. As with any specialized analytical technique, the successful application of FT-IR imaging to the study of combinatorial systems is highly dependent on the development of sampling accessories that allow spectral information to be collected from the specific sample geometry of interest. Further development of novel sampling accessories, as well as improvement to current accessories, will make this experimental methodology even more attractive to the study of larger and more diverse libraries.

Acknowledgments

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