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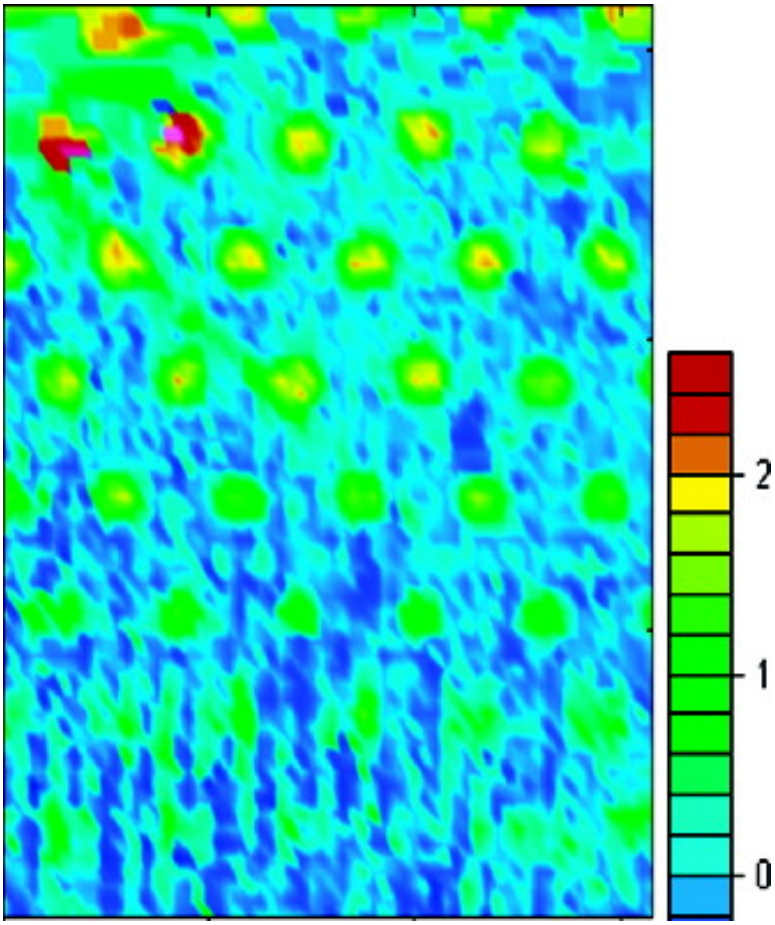

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Fourier Transform Infrared Imaging for High-Throughput Analysis of Pharmaceutical Formulations

K. L. Andrew Chan and Sergei G. Kazarian*

Department of Chemical Engineering, South Kensington Campus, Imperial College London, London SW7 2AZ, United Kingdom

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Fourier transform infrared (FTIR) spectroscopic imaging with infrared array detectors has recently emerged as a powerful materials characterization tool. We report a novel application of FTIR imaging for high-throughput analysis of materials under controlled environment. This approach combines the use of spectroscopic imaging with an attenuated total reflection (ATR)-IR cell, microdroplet sample deposition system, and a device that controls humidity inside the cell. By this approach, it was possible to obtain "chemical snapshots" from a spatially defined array of many different polymer/drug formulations (more than 100) under identical conditions. This method provides direct measurement of materials properties for high-throughput formulation design and optimization. Simultaneous response (water sorption, crystallization, etc.) of the array of formulations to the environmental parameters was studied. Implications of the presented approach range from studies of smart polymeric materials and sensors to screening of pharmaceuticals and biomaterials.

Introduction

High-throughput technology (HTT) analysis improves the efficiency of producing useful databases or libraries that could facilitate research and development.[1−3] The novelty of the HTT method extends further when the effects of different environmental factors to the library of samples are studied simultaneously. This significantly reduces the time, and hence the cost, of research that was economically not feasible to be carried out without HTT. Fourier transform infrared (FTIR) spectroscopy is one of the most powerful analytical methods available. The chemical specificity of FTIR spectroscopy allows different chemical components and morphologies to be distinguished on the basis of their absorbance in the mid-IR region. FTIR spectroscopy combined with the imaging capability of infrared array detectors provides a powerful tool to obtain both chemical and spatial information about the sample.[4] The focal plane array (FPA) infrared detector measures thousands of spectra simultaneously, which reduces the time required to acquire a FTIR image significantly, as compared to the traditional point-by-point mapping method.[5,6] Conventional FTIR microscopy was recently applied to study a continuous gradient combinatorial library for epoxy curing, but the spatial resolution of that approach was limited and the acquisition time was relatively long.[7] The advantage of FTIR imaging over conventional FTIR microscopy[8] has been recently utilized so that the spatial distribution of different components of a mixture can be obtained simultaneously in a single measurement for a dynamic rather than static system.[9−12] This is particularly important when the sample is subjected to a controlled environment, for example, controlled relative humidity. The potential of FTIR imaging to study heterogeneous materials was recently demonstrated.[5,6,13,14] One of the interesting applications of FTIR imaging involved the study of visualization of the patterned polymer film via imaging of organic solvent vapor sorption.[15] Unfortunately, these measurements were performed not in situ but after exposure to the solvent vapor. However, the ability of infrared array detectors to measure spectra from different locations in the sample in situ (when the sample is placed in a controlled environment cell) can also be utilized to obtain spectra of many different samples simultaneously. The most relevant studies by Snively et al.[16,17] have demonstrated the feasibility of FTIR imaging to study gaseous or solid-phase reactions in several transmission cells in parallel. That work had significant impact, and recently 49-channel parallel reactors combined with FPA were studied via FTIR imaging in reflection.[18] Snively and Lauterbach very recently presented a poster that demonstrates the feasibility of using ATR-IR imaging to measure six samples simultaneously.[19] Their approach, however, was not extended to enable the possibility of studying samples under a broad range of controlled humidities or applying HTT to pharmaceutical formulations. Study of the behavior of pharmaceutical formulations under different humidities is needed for the design of tablets for drug release and for finding ways to extend the shelf life of pharmaceuticals. Combining FTIR imaging with the controlled humidity cell provides an opportunity to study in situ water sorption into different domains of the sample and the behavior of the sample under a controlled environment. Recently we have applied FTIR imaging to study a formulation under controlled humidity.[20]

It is well-known that hydrophilic polymers in pharmaceutical formulations can absorb water vapor from the atmo-

* Corresponding author. E-mail s.kazarian@imperial.ac.uk.

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sphere. Sorption of water vapor may lead to undesirable effects on the molecular state of the drug (e.g., drug recrystallization) or potential polymorphic transitions of the drug. Thus, the effect of adsorbed water on the performance of pharmaceutical formulations in drug release and bioavailability may be significant. Furthermore, the adsorbed water may also influence the compaction properties in tablet preparation with an effect on particle morphology in the tablet, hence the considerable interest in studying water sorption in pharmaceutical formulations. Unfortunately, there is mainly an empirical approach in the preparation of polymer/drug formulations that is adopted by pharmaceutical companies. Studies of water sorption into polymers or pharmaceutical formulations usually involve one sample at a time and the sorption experiment is intrinsically slow as it depends on the diffusion mechanism.[21] Each of these experiments is time-consuming and thus slows progress in this area. There is an urgent need to develop faster screening methods for pharmaceutical samples subjected to a range of controlled relative humidities.[22,23] Fortunately, the opportunity exists, yet is unexplored, to apply spectroscopic imaging methods to study many samples at the same time in one such experiment. The spectroscopic imaging approach for fast simultaneous screening of many pharmaceutical samples under controlled environments has never been attempted before and represents the central basis of a research program designed to develop the new HTT approach for materials characterization under controlled environments.

Experimental Section

ATR-FTIR Imaging. The FTIR imaging system[24] consists of a step scan spectrometer (IFS 66/S, Bruker Optics) coupled with a macro chamber extension (IMAC) and a 64 × 64 FPA detector. Spectra were measured with 16 cm^{-1} spectral resolution and a spectral range of 3950−900 cm^{-1}. Twenty frames coaddition was used, and the total acquisition time was ca. 180 s. Images were acquired through an inverted ZnSe pyramid crystal (oil analyzer, Specac) that was originally designed for nonimaging purposes. The size of the FPA 64 × 64 detector is 3.8 × 3.8 mm^2. The size of the imaging area when the inverted pyramid from ZnSe was used is 3.8 × 5.3 mm^2 (due to the aspect ratio[24]).

Microdroplet Device. The microdroplet-on-demand device (AutoDrop, MicroDrop) consists of a dispensing control unit, two heated dispensing heads, an in-built computer, an *xyz* positioning system, and the *xyz* control unit. The sample can be heated to a maximum temperature of 160 °C to melt samples that are solid at room temperature. The dispensing control unit controls the droplet size and the number of droplets to be dispensed at each specific location. The possible droplet diameter ranges between ca. 30 and 70 μm. The dispenser was set to dispense 10 drops at each location to form samples on the ATR crystal with diameter of ca. 200−250 μm.

Sample Preparation. Poly(ethylene glycol) (PEG) (MW = 200) was purchased from Sigma and was used as received. Ibuprofen was supplied by Whitehall International. Nifedipine was supplied by Sigma. A solution of 30 wt % ibuprofen/PEG and 10 wt % nifedipine/PEG solution was made by heating the mixture to ca. 80 °C and mixing the two components in a beaker until all solid particles were dissolved. Micro droplet samples were dispensed directly onto the sampling surface of the ZnSe crystal. The pattern of sample deposition was programmed by using the macro software, and the deposition was carried out automatically with an *xyz* motorized robot arm.

Results and Discussion

FTIR imaging systems usually use an array detector with 64 × 64 elements, which allows one to simultaneously measure spectra from 4096 different locations in a sample (although arrays of different formats, for example, 32 × 32 or 128 × 128, have also became available). However, the FTIR imaging approach to study many samples in *transmission* will be challenging due to the difficulty of preparing many samples with precise and small thickness (since thick samples will absorb too much IR light). In addition, possible variations in the path length may affect quantitative data. Attenuated total reflection (ATR)-IR spectroscopy is a suitable approach that provides a highly reproducible small path length and requires minimal sample preparation. The latter is particularly important since a HTT method without sample preprocessing (which is required in transmission or reflection spectroscopic methods) will lead to substantial timesaving and will make the proposed HTT approach more efficient. Key ideas of our proposed methodology have been (i) a combination of macro ATR with FPA infrared detector for FTIR imaging,[10] (ii) the use of a microdrop system to deposit a microdroplet directly on the surface of the ATR crystal, and (iii) a combination of the macro ATR imaging accessory with a controlled humidity cell. This approach allowed us to image more than 100 samples simultaneously under identical conditions. A schematic diagram of the experimental procedure is shown in Figure 1. Dispenser head 1 was loaded with the sample containing the drug while dispenser head 2 was loaded with pure PEG. Droplets with different composition of drug (ibuprofen and nifidepine were used as model drugs) and polymer were prepared by dispensing a different number of drops from each dispenser head onto the same location.

Two arrays of sample have been produced using this micro-droplet device. The first array contains two different sets of samples. One set of the sample was deposited from dispenser head 2 and the other set was dispensed by head 1. The two sets of samples were dispensed adjacent to each other on the ZnSe crystal sampling surface. A visible image of part of the array is shown in Figure 2. The absorbance bands at 1060 cm^{-1}, $\nu(CH_3)$, and 1730 cm^{-1}, $\nu(C{=}O)$, have been used to characterize PEG and ibuprofen, respectively.[10] Images that represent the concentration distribution of PEG and ibuprofen have been generated by plotting the integral value of the corresponding absorption bands across the whole imaged area.

Figure 3a shows the distribution of PEG over the imaging area. Since all samples contain a high concentration of PEG, this image shows all the samples that have been measured. The samples were deposited in nine columns and 13 rows, which gave a total number of 117 samples being deposited

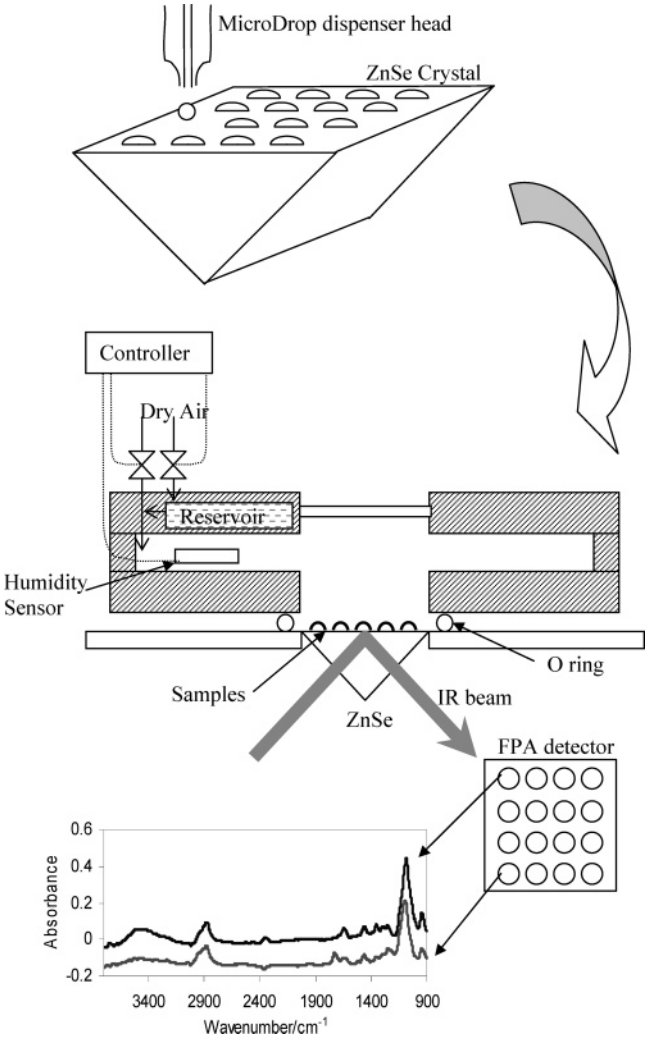

Figure 1. Schematic diagram showing the combination of microdroplet sample deposition with ATR-IR cell, controlled humidity chamber, and infrared array detector for simultaneous high-throughput analysis of many formulations under controlled environment.

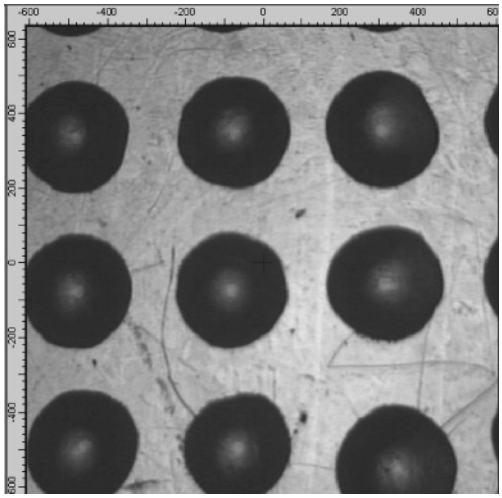

Figure 2. Visible image of part of the sample array deposited on the surface of ZnSe crystal. The scale shown is in micrometers.

for measurements. However, due to a number of bad pixels (bad detectors due to aging) at the top of the FPA, the top part of the image was not well resolved and the actual array size was ca. 9 × 11 samples; hence 99 samples were measured without compromising quality due to the bad pixels in the FPA. Nevertheless, the combination of the microdroplet device together with the relatively large field of view of the macro ATR-FTIR imaging system allows us to demonstrate for the first time that approximately a hundred samples can be analyzed simultaneously with an acquisition time of 180 s. In this particular experiment, each sample was measured by at least six detector pixels from which spectra can be extracted, giving the opportunity to reduce spectral noise by averaging the spectra. Figure 3b shows the distribution of ibuprofen over the imaged area, which can be used to identify the samples that contain ibuprofen. It has shown clearly that the samples on the top half of the image contain similar amounts of ibuprofen (dispensed by head 1), while the samples at the bottom half contain no ibuprofen (dispensed by head 2). This example demonstrates the possibility of studying many samples containing different components simultaneously.

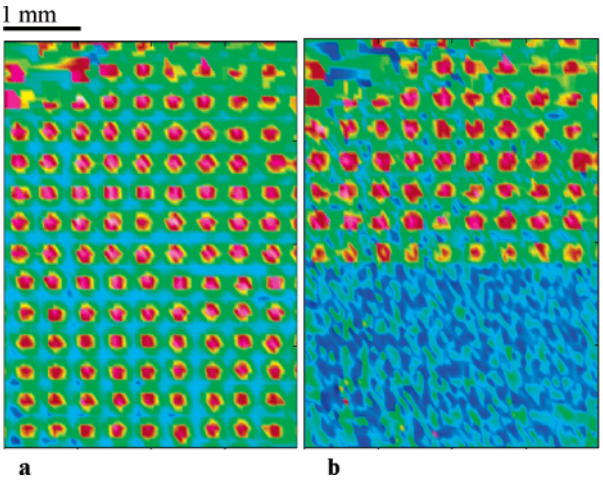

Figure 3. Macro-ATR images showing distribution of (a) PEG and (b) ibuprofen over the field of view (820 × 1140 μm^2). Red represents a high concentration while blue represents zero concentration.

The presented HTT approach can be applied to study a large variety of samples on the basis of the inherently rich information that is contained in the infrared spectrum. Thus, the relative concentrations of drug and polymer can be verified simply from the ratio of the corresponding absorbance bands against a calibration set. According to the Beer−Lambert law, the absorbance of a component in the sample is proportional to concentration of that component and the path length of IR light through the sample. A reproducible path length can be easily achieved by the ATR method; hence the concentration of ibuprofen in the sample can be quantitatively calibrated against set samples of known concentrations. The calibration relationship between the absorbance of ibuprofen, the ν(C═O) band in the ATR−IR spectra, and the known concentration of ibuprofen in PEG is shown in Figure 4. This relationship was used to validate concentrations of ibuprofen in the formulations prepared by droplet deposition. Spectral bands also convey information about the molecular state of drug in the polymer matrix and its possible alterations upon changing the environmental conditions.

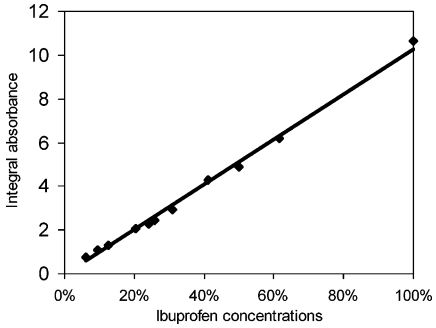

Figure 4. Relationship between the integral absorbance of ν(C=O) of ibuprofen as a function of ibuprofen concentration in PEG.

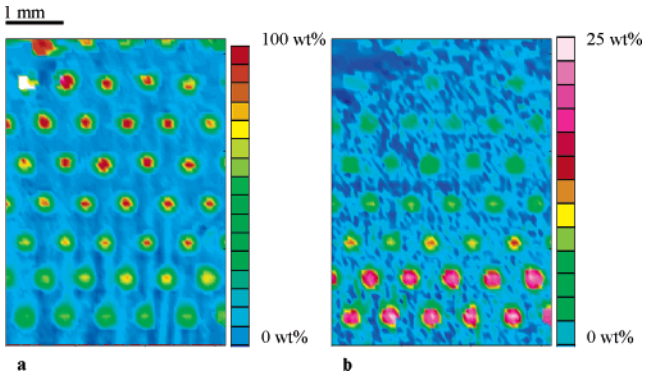

Figure 5. Distribution of (a) PEG and (b) ibuprofen in the same sample array. The color represents the concentration of PEG (a) and ibuprofen in PEG (b). The color scale was calibrated by use of the relationship presented in Figure 4.

Next, an array of the samples containing a range of different concentrations of ibuprofen has also been created. This sample array has been created by dispensing a different number of droplets from the two dispenser heads in each sample. The eight rows of samples have been deposited with each row containing different concentrations of ibuprofen. Figure 5 shows the concentration of ibuprofen at each sample. It is clear that the concentration of ibuprofen on the bottom of the image is the highest, and it gradually decreases toward the top of the image. Potentially, one could analyze samples with a greater variety of concentrations using this imaging approach, but as a demonstration of principle, only eight concentrations have been prepared in this study with each column having the same concentration of drug. The analysis of samples with the same concentration under identical environments can serve the purpose of testing reproducibility of the experiments, ensuring the reliability of the analysis. Following the deposition of this array, the sample was exposed to a controlled humidity as shown in Figure 1. Figure 6 shows the chemical images of the same sample array under 67% relative humidity for 30 min. The image that presents the distribution of water was generated by plotting the water absorption band due to OH bending at ca. 1640 cm^{-1} and has shown that water sorption is strong with samples that contain a lower concentration of ibuprofen.

The presented HTT approach also has tremendous potential for studying drug polymorphism. The polymorphic changes of PEG/nifedipine mixture[25] upon exposure to a controlled relative humidity have been studied with this HTT. The morphology of nifedipine is known to be affected by the surrounding environment, such as temperature and relative

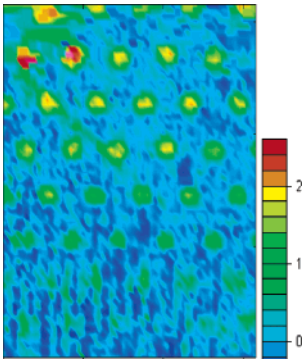

Figure 6. Relative amount of water in samples containing different concentrations of ibuprofen. The samples were exposed to 67% relative humidity. The color scale shows the integral absorbance value of water bending mode vibration.

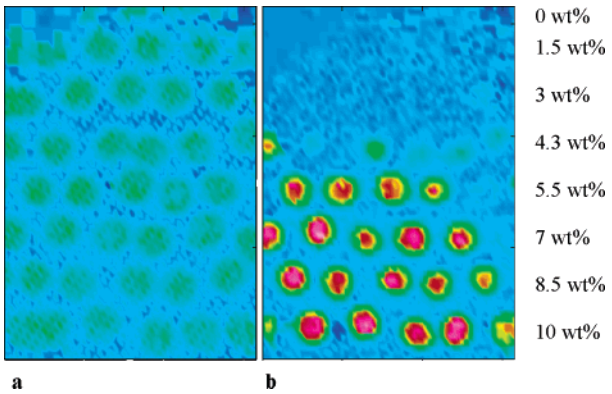

Figure 7. Distribution of (a) PEG and (b) crystalline nifedipine in the same sample array exposed to 95% RH. The concentrations of nifedipine in the formulation dispensed at each row are indicated on the right of the image.

humidity. We have recently[25] shown that PEG can prevent crystallization of nifedipine, in a dissolution test, when the drug-to-polymer ratio is low. The sample array was prepared in a similar manner as in the PEG/ibuprofen study. The result has shown that no crystallization of drug occurs at 85% RH. However, at 95% RH, the samples with drug concentration above 4−5 wt % crystallized (see Figure 7). It is also possible to use multivariate analysis to present these results quantitatively, but as it is outside the scope of this paper, it is left as future work.

Conclusions

In summary, by combining the microdroplet device with the FTIR imaging system, we have demonstrated for the first time that ca. 100 samples can be analyzed simultaneously. The number of samples could be further increased either by expanding the field of view with a larger ZnSe crystal and beam expander or by using a smaller droplet sizes. Both the sample deposition and spectroscopic imaging are fast. The proposed HTT methodology will allow fast screening of many different formulations under controlled environments for the first time. This allows us to rapidly and quantitatively identify specific formulations that exhibit drug recrystallization or polymorphism.[23] It will be possible to analyze relative concentrations of different polymorphs in many formulations. Chemically specific quantitative information (e.g., water sorption, ratio of polymorphs) will be obtained

from many samples at identical conditions. The data from the same experiment will provide information about the conditions at which a polymer is plasticized, forms a gel, or dissolves. Fast screening of many samples in a broad humidity range by this in situ spectroscopic imaging approach will allow us to identify comparative behavior of these formulations and obtain quantitative data with unprecedented speed. Furthermore, direct observation of the key events that often precede dissolution (water sorption, morphological changes, swelling, etc.) in many systematically different samples will allow, for the first time, direct comparison of the behavior of formulations to better understand the important processes relevant to drug release. This understanding is required for major advances in preparation and optimization of pharmaceutical formulations.[10,22]

The demonstrated HTT imaging approach will also impact many areas apart from the analysis of pharmaceutical formulations. For example, in catalysis the screening of the catalysts' efficiency can be achieved quickly via studying the spectroscopic response of numerous catalysts simultaneously subjected to a specific gas or gas mixture. The simultaneous response of many different modifications of smart polymeric materials to controlled humidity, temperature, or gas pressure can also be analyzed quickly by our approach. In fact, the demonstrated HTT methodology will be of great value in the area of ionic liquids research where the number of newly synthesized ionic liquids is growing rapidly. The success of the proposed HTT methodology may have significant impact for preparation of novel polymeric sensors[26] for environmental control; for example, micropatterned polymer-based electronic sensors were proposed to detect volatile organic compounds. The macro ATR imaging HTT approach could provide the means to facilitate such research with important implications for development of such sensors. One can also envisage that the outcome of the demonstrated approach may find applications in defense biotechnologies by screening the response of many different cells to the exposure of specific bioactive gases. Biomaterials[27,28] (e.g., in skin research[29]) are likely to benefit from this approach. The opportunity to combine the macro-ATR imaging approach with the use of bigger arrays, for example, 128 × 128 pixels, could increase a maximum number of samples further. Thus, it is believed that the imaging HTT approach under a controlled environment will spark the beginning of an exciting leap into new analytical capabilities for materials research.

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