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TOF-SIMS IMAGING FOR COMBINATORIAL CHEMISTRY AND DRUG DISCOVERY RESEARCH

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1. Introduction

Mass spectrometric methods have become increasingly important to biotechnology and pharmacology because they provide a simple measure of molecular weight on sub-picomole quantities of material and because they may also provide ancillary structural information about unknown materials. Most measurements utilize either MALDI or electrospray ionization as tools for creating molecular ions. The SIMS approach, even with the advent of high performance time-of-flight machines, has not succeeded in making significant inroads into this vast research community. Typically, biomolecules experience fragmentation when bombarded by energetic particles. Surface contamination is also an annoying source of signal degradation.

Molecule-specific imaging is a clear distinguishing feature associated with TOF-SIMS measurements. With a Ga^+ ion probe from a liquid metal ion source, image resolution of less than 100 nm is now feasible. There are a number of research areas where imaging TOF-SIMS could provide breakthrough-type results and where the competing mass spectral approaches would not be appropriate. Here we examine the possible role of this technology in combinatorial chemistry experiments aimed at drug discovery, and speculate about applications in clinical chemistry involving efficacy trials on potential new drugs.

Combinatorial synthetic methods utilize a series of steps with multiple reagent choices for each step to create a repertoire of up to several million different compounds. The synthesis is usually carried out either on functionalized Si wafers or on 50-200 micron diameter polystyrene spheres. From a drug discovery point of view, the idea is to massively bioassay the entire library. The active compounds are then identified as leads after release from the bead followed by chemical assay. The chemical assay is now generally performed using MALDI or electrospray ionization,[1] or by using tagging strategies which yield molecules easily identified by a variety of mass spectral methods.[2] Since this strategy may require assay of thousands of samples, the conceptual power of combinatorial chemistry has yet to be realized on large libraries and current emphasis is directed toward studying libraries of several hundred members. The field is very active and many comprehensive reviews have recently been published.[3]

2. Combinatorial Chemistry Studies

Our idea is to array polystyrene spheres with biomolecules attached onto a device for keeping beads in a fixed position. If it were possible to chemically assay a single bead using SIMS, then it might be possible to determine the composition of many thousands of beads from this array with a single TOF-SIMS image.

Initial experiments focused on obtaining high quality TOF-SIMS spectra from molecules covalently attached to single beads using standard acid-sensitive linkers such as Sasrin©. As previously reported,[4] the presence of this linker essentially inhibits the formation of molecular ions, presumably because the energy produced in the desorption event breaks the weakest bonds first. To overcome this difficulty we found that exposure to trifluoroacetic acid (TFA) vapor for a few minutes releases the molecule from the surface but keeps it localized on the bead. Since our original report, image quality has increased dramatically. As shown in Figure 1a we have designed, using nanofabrication techniques, a means to position many beads in a single holder for imaging. In Figure 1b and 1c, we show the chemically resolved image of a single bead with a carboxylic acid derivative at m/z 214 exposed to TFA. Note that the molecule is completely localized on the bead and has not migrated to the surface.

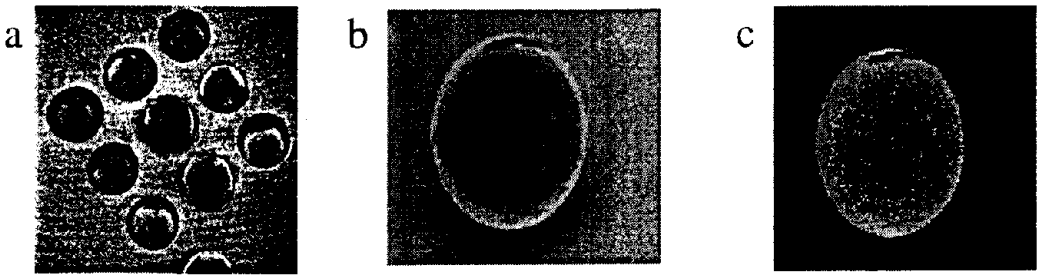

Figure 1(a) Total ion image of bead array. In this case, each bead is 200 microns in diameter. (b) Total ion image of a single 200 micron polystyrene sphere on a Si substrate. (c) Chemical image at m/z 214. the molecular ion for a carboxylic acid derivative that has been clipped from the bead after 30 min of exposure to TFA vapor. The spectrum contains 128x128 pixels with 10 Ga^+ ion pulses/pixel.

These images were obtained using a specially designed TOF-SIMS spectrometer[5] optimized to minimize field gradients from the extraction optics that are inherently set up across the bead. The extraction optics utilizes a large field of view (2.1 x 2.1 mm) with an extraction potential of 2500 V and an extraction gap of 10 mm. The Ga^+ ion source is from Ionoptika and yields a 150 nm probe size with a beam current of about 2 nA.

This very clean situation is not always observed, however, as shown in Figure 2, exposure to TFA vapor can cause some molecules to be washed off the bead onto the holder. In this case, the 60-micron bead was removed after the initial image in Figure 2a, leaving the halo-like residue associated with the Y_2+2 fragment ion of Val-Tyr-Val at m/z 281. Because clipping can produce artifacts like these, much more research is needed to find self-cleavable linking agents or alternative means of releasing target molecules from the bead without removal. In general, however, TOF-

SIMS imaging offers unique advantages over competing mass spectral methods since, as suggested by the image in Figure 1a, many beads can be assayed in a single measurement. Assaying speed is presently the major deterrent to fully exploiting the elegant concepts behind the combinatorial chemistry idea.[3]

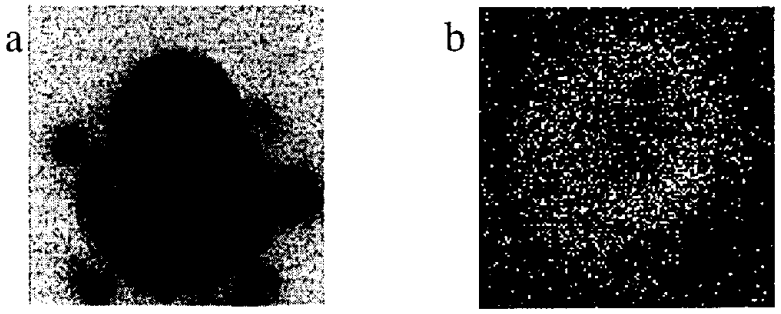

Figure 2(a) Total ion image of a 60 micron bead bound with Val-Tyr-Val. (b) 128x128 pixel image of m/z 281, 100 pulses per pixel.

3. Arraying of multiple samples for rapid assaying.

The ideas presented above for beads can be extended to include the assay of massive numbers of compounds distributed across a surface. To test this idea we have fabricated an array of pyramidal vials in Si which are 40 microns on each side and spaced by 40 microns. As shown below in Figure 3, an optical photograph of this device shows several domains with each containing 20x20 individual sample holders, and each domain measuring 1.6 mm per side.

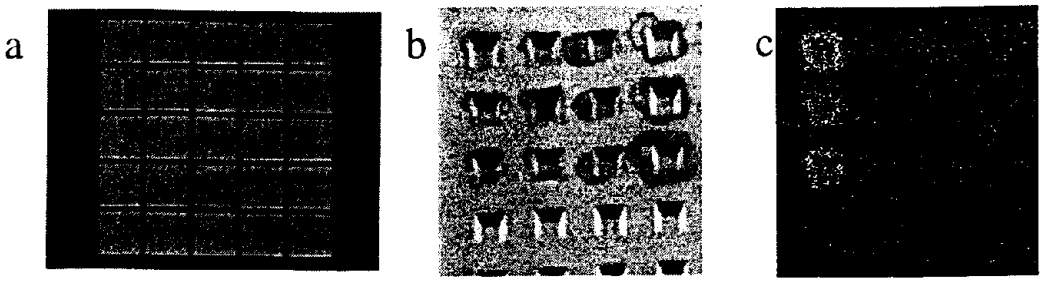

Figure 3(a) Optical photograph of vial array. (b) Total ion image of 16, 40-micron vials and (c) chemical image of m/z 229. See text for details.

A total ion SIMS image of 16 of these vials is illustrated in Figure 3b. Using a picospritzer device from General Valve, it is possible to fill individual vials with known quantities of different molecules. In Figure 3c, for example, we show the chemical image of m/z 229 associated with the molecular ion of Thionin. Each of the 3 vials has been filled with 10 pl of a 10^{-2} molar ethanol solution of Thionin, resulting in a total deposition of 100 femtomoles. The entire image was recorded with 256x256 pixels at 6 pulses per pixel. Considering the relative area of each vial, and a possible repetition rate of 10,000 pulses per second, each vial could be assayed in less than 1

sec. In the example shown, other molecules were deposited in adjacent vials. These data are similar to those shown in Figure 3c but are omitted because of space limitations and because the publishers do not allow the use of color overlays.

4. Summary

The above somewhat futuristic examples attempt to show how the marriage of nanofabrication, SIMS imaging and pharmacological applications can lead to new approaches for drug discovery. Of special interest is the possibility of preparing a diverse array of either beads or vials on a surface for rapid parallel assay. The biggest problem yet to overcome involves the need to desorb intact molecular ions with the Ga^+ ion probe. Although some classes of molecules, such as organic dyes, work quite well, other more fragile species such as carbohydrates, peptoids or other heterocyclic molecular libraries are still problematic. Many solutions to this problem present themselves for future research. For example, it is possible to synthesize libraries with embedded isotope labels which can be read much like supermarket bar codes.[6] These codes could easily be extracted from fragment ions and lead to a direct molecular identification. In general, the ability to apply SIMS imaging to such systems is quite unique and promises some major new applications in the pharmaceutical arena.

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