

Application of pan-sharpening to SIMS imaging

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Higher resolution and increased intensity is always a goal for SIMS imaging experiments. One approach to achieving this goal might be to utilize complementary data sources that could be merged through the process of image fusion. The idea to incorporate the best aspects of two different image acquisition approaches to maximize information content. Here, we examine a subset of image fusion, pan-sharpening, that is utilized to combine relevant and redundant information from a pair of high resolution and low resolution images to create a hybrid image. To test applicability to SIMS imaging, two different scenarios are considered. First, a copper-mesh grid SIMS image is fused with a higher resolution SEM image to improve the intensity and contrast between gridlines and background. Secondly, an SIMS image obtained with an Ar_{4000}^+ cluster primary ion beam is fused with a higher resolution C_{60}^+ image to map specific lipid signals in a 3D depth profile of HeLa cells. Copyright © 2014 John Wiley & Sons, Ltd.

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Introduction

Image quality is important when examining SIMS images because the ability to convey the meaning of the data is often limited by a number of factors. These factors typically include primary ion beam focus, secondary ion intensity and mass resolution, among others. Certainly, physical improvements to SIMS instrumentation help to improve the images obtained. However, another route for improving the visual quality involves exploiting other data sources that are available during an SIMS experiment and implementing the mathematical approach of image fusion.

Image fusion is a technique that has already been applied to a number of disciplines, including medical imaging,^[1,2] satellite imagery,^[3] remote sensing^[4] and forensic science.^[5] The basis for image fusion is to merge relevant and redundant information from complementary image sources to create a hybrid image that better represents the scene than any of the stand-alone input images.^[6] While several subsets of image fusion techniques have been studied, here, we investigate pan-sharpening for SIMS experiments because it preserves color information,^[7] which is equivalent to chemical information in an SIMS image.

Pan-sharpening^[8] is performed using an adapted algorithm, described elsewhere.^[9] The low resolution, multispectral image source for pan-sharpening will be the SIMS image, where chemical information is encoded as color. For the higher resolution image source, there are two requirements, that the image contains more pixels (higher resolution) and that the data are scalar and represented in a single color channel. One possible source of high resolution data would be an SEM image, which would provide complementary information that is higher in intensity and pixels and contains topographic data.

Another source of high resolution data would be a second SIMS image from a different primary ion beam. When analyzing biological samples, such as cells, the distribution of lipid signals is an important focus of research. Lateral resolution up to 300 nm is achievable with a C_{60}^+ primary ion beam. However, spectra from a C_{60}^+ experiment lack adequate high mass signal at this resolution, which is crucial for identifying the exact nature of the lipid species. Instead, fragment ions, such as the phosphocholine

(PC) headgroup, are seen, which only characterize the general class of lipid. In order to obtain more molecular specificity, an Ar_{4000}^+ primary ion beam could be used for analysis because large gas cluster ion beams are capable of desorbing larger molecules.^[10] This type of analysis could provide significant molecular ion signal for lipids, such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). However, spatial resolution with an Ar_{4000}^+ primary ion beam is currently more than an order of a magnitude lower than that possible with C_{60}^+ . Thus, an image fusion experiment to combine the molecular specificity of an Ar_{4000}^+ experiment with the higher intensity and superior spatial resolution of a C_{60}^+ image could prove to be fruitful.

Experimental

Copper-mesh grid imaging

A 400-mesh-copper grid (Electron Microscopy Sciences, Ft. Washington, PA) was imaged using an SIMS instrument, described previously,^[11,12] equipped with a 40-keV C_{60}^+ primary ion beam (Ionoptika Ltd., Southampton, UK). The primary ion beam current was measured to be 54 pA and focused to a beam diameter of approximately 10 μm , determined by line scan from an SEM image. SEM images were acquired at 512 \times 512 pixels with a field of view of 480 μm . Without moving the sample, 256 \times 256 pixel SIMS images were acquired with the same 480 μm field of view. The primary ion beam fluence for the experiment was calculated to be 5.7×10^9 ions/cm².

HeLa cell imaging

HeLa cells were grown on the silicon substrates in a 6-well cell culture plate in media (Dulbecco's modified Eagle's medium, 1x,

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Corning Cellgro, Manassas, VA, USA) for 24 h in an incubator at 37 °C and 5% CO₂. The silicon substrates were sonicated in chloroform, Milli-Q water (Millipore Milli-Q System, Burlington, MA) and methanol, for 5 min each, then sterilized in 70% ethanol before submersion into the culture media. Once HeLa cells were grown to confluency on silicon, the samples were swiftly washed with 0.15 M ammonium formate three times and quickly dried with a stream of N₂ gas, followed by plunge freezing into liquid ethane and then transferred to a sample block precooled in liquid nitrogen. The frozen-hydrated samples were then transferred to the precooled sample stage (100 K) for SIMS analysis.

Dual beam depth profiling was performed using the *J105-3D Chemical Imager* (Ionoptika Ltd., UK). The sequential images were acquired by interleaving both 40 keV C₆₀⁺ and 20 keV Ar₄₀₀₀⁺ during the sample erosion process. A C₆₀⁺ primary ion beam was focused to 300 nm and used to do high resolution imaging over a field of view of 130 μm × 130 μm area with a resolution of 128 × 128 pixels. The primary ion dose was 3.2 × 10¹² ions/cm². For Ar cluster analysis, the primary ion beam spot size is ~5 μm, which was used to acquire the low resolution images overlapping with C₆₀⁺ images in a larger area of 260 μm × 260 μm with a resolution of 64 × 64 pixels. The primary ion dose was also 3.2 × 10¹² ions/cm². For overlapping and registering the pair of images, a 32 × 32 pixel window of the Ar₄₀₀₀⁺ was cropped to match the area analyzed by C₆₀⁺.

Image fusion process

Image fusion is performed using an adapted pan-sharpening algorithm, which has been applied to SIMS analysis and quantitatively evaluated using cross-correlation.^[9] For the copper grid experiment, an SEM image is used as the high resolution image source and an SIMS image as the low resolution image source. In the dual beam HeLa cell experiment, an image acquired using the C₆₀⁺ primary ion beam, mapping the PC headgroup signal, is first converted to grayscale values and then used as the high resolution, panchromatic image source. Then, an image acquired with Ar₄₀₀₀⁺, mapping a more specific chemical signal, is used as the low resolution image source.

Results and discussion

Applying image fusion to a set of SEM and SIMS images was first performed on a copper-mesh grid, shown in Fig. 1. The choice of a copper-mesh grid was made to establish the basis of applying image fusion to an SIMS experiment using a structured, well-known and reproducible system. The SEM image (512 × 512 pixels) is shown in Fig. 1a, and the corresponding SIMS image (256 × 256 pixels)

mapping the chemical signal for copper (m/z 62.9) in Fig. 1b. The SIMS image in Fig. 1b suffers from low resolution and pixelation because of low secondary ion intensity when compared with the SEM image in Fig. 1a. The gridlines appear broadened and blurred in the SIMS image, and noise signal exists in the areas between the gridlines. All of these factors combine to limit the visual quality of the image.

The result of fusing the SEM and SIMS image pair in Figs. 1a and b, respectively, is shown in Fig. 1c. The results demonstrate the effect of image fusion. Most noticeable is the lack of pixelation, as was observed in the SIMS image because the overall intensity of the fused image resembles that of the SEM image. This lack of pixelation leads to improved definition of the edges of gridlines as well as enhanced contrast between these gridlines and the silicon substrate. In addition, the gridlines incorporate topographical information that is contributed by the SEM image, such that the fused image represents more than just the spatial distribution of chemical signal.

Another example of improved visual quality due to image fusion could be found when exploring the features imprinted into the copper-mesh grid. In the SEM image, the letters 'P', 'Q' and 'R' are clearly identifiable. However, low secondary ion signal and decreased pixel resolution prevents the identification of the letters in the SIMS image. In fact, it would be difficult to claim that the features are letters without any prior knowledge of the system. After fusion, though, the letters are discernable, can be correctly identified and are matched up with chemical distribution information from the SIMS image. Cross-correlation values between the SIMS and fused images are calculated for each color band. Values range from -1, meaning, the two images are completely dissimilar, to +1, meaning, completely similar.^[8,13] The average for all three bands, 0.9775, quantitatively suggests the two images are strongly correlated.

While a copper-mesh grid provides a suitable starting point for applying image fusion to an SIMS experiment, a more complex system will be needed going forward. An important issue associated with image fusion and SIMS is what happens with a system containing more than one analyte of interest. Specifically, will image fusion incorrectly favor one species over another when two or more species are present? In addition, a protocol is needed to provide the color for overlapping signals.

To demonstrate image fusion with a different source of high resolution data, HeLa cells were imaged using both C₆₀⁺ and Ar₄₀₀₀⁺ primary ion beams. The top row of Fig. 2 shows selected layers of the 3D depth profile where m/z 184.1, corresponding to the PC headgroup, is imaged at a resolution of 128 × 128 pixels. The middle row is of the corresponding Ar₄₀₀₀⁺ images, mapping the sum of the DPPC M + H, M + Na⁺ and M + K⁺ peaks (m/z 734.4, 756.6 and 772.6, respectively). These images serve as the low resolution input for image fusion because the resolution is only

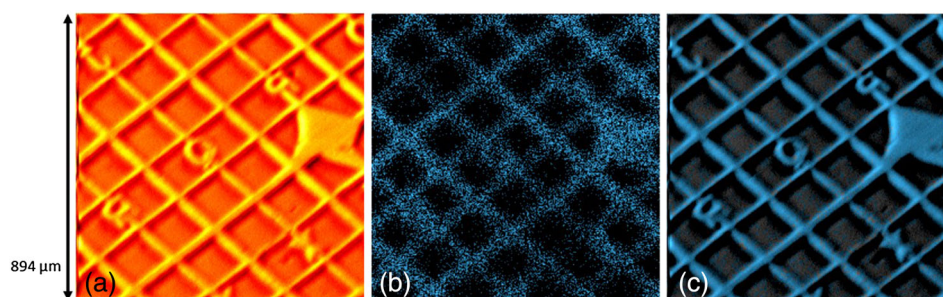


Figure 1. (Color online) SEM and SIMS fusion series. (a) SEM image of a 400-mesh-copper grid with a resolution of 512 × 512 pixels. (b) SIMS image of the same region, mapping copper (m/z 62.9) with a resolution of 256 × 256 pixels. (c) Result of fusing Figs 1a and 1b. Field of view for all images is 894 μm × 894 μm.

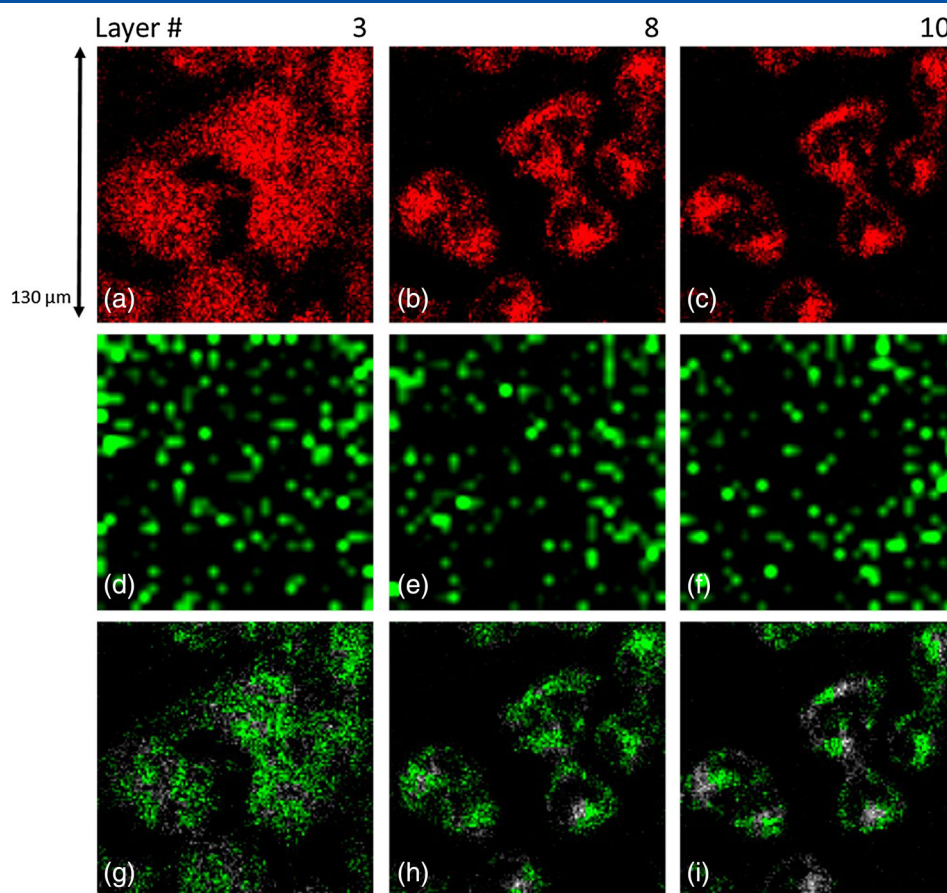


Figure 2. (Color online) Selected layers of a 3D depth profile of HeLa cells. (a–c) SIMS images of phosphocholine headgroup (m/z 184.1) using C_{60}^+ primary ion beam with a resolution of 128×128 pixels. (d–f) SIMS images of the 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine $M + H$, $M + Na^+$ and $M + K^+$ peaks using Ar_{4000}^+ primary ion beam with a resolution of 32×32 pixels. (g–i) Results of image fusion. Field of view for all images is $130 \mu m \times 130 \mu m$.

32×32 pixels. However, these Ar_{4000}^+ images provide more chemical specific information than the corresponding C_{60}^+ images, which only contain information about the class of the lipid species, mainly the exact identity of the lipid molecule.

Unfortunately, the DPPC images from the Ar_{4000}^+ primary ion source in Fig. 2 suffer from low secondary ion intensity and, because of experimental limitations, are only 32×32 pixels in size. Thus, the images are dark, pixelated and lack significant information. High intensity regions in these images could be argued to co-localize with the same features in the C_{60}^+ images, but no real conclusions could be drawn from these images alone. As these SIMS images lack the resolution to produce significant detail of the HeLa system, they do not deliver substantial information about the DPPC distribution throughout the cells.

After performing image fusion, the distribution of DPPC becomes clearer as the green molecular signal becomes localized with the red fragment signal in the C_{60}^+ images. Signal to noise has been drastically improved in the fused images, as seen by the lack of intensity in areas that do not correlate with PC signal from the C_{60}^+ images. For areas that do correlate, increased pixel intensity is observed. If there is DPPC signal from the Ar_{4000}^+ images, these pixels appear green in the resulting fused image, which improves the visual quality of the image. However, if there is no signal in the corresponding pixel, that pixel appears gray in the fused image. Gray pixels are the result of increasing the lightness of the pixel color when the original color is black. Thus, for this experiment, gray pixels represent the location of lipids that

contain the PC headgroup, but not DPPC, such as 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) or 1,2-didecanoyl-*sn*-glycero-3-phosphocholine (DDPC). The average cross-correlation values are 0.6932, 0.7526 and 0.7919 for layers 3, 8 and 10, respectively. These values are lower than previously observed in the copper grid fusion series, indicating less correlation between the C_{60}^+ SIMS and fused images. Gray pixels represent information not originally present in the C_{60}^+ SIMS image, and thus explain the lower cross-correlation values. Taking all of these factors into consideration, the resulting fused images are hybrid images combining high intensity, higher spatial resolution and chemical specificity that could not be expressed with any of the input images alone, which allows more information regarding the lipid identity and distribution to be conveyed.

Conclusion

SIMS images were fused with higher resolution image sources to produce hybrid images containing more information than any of the input images alone. SEM images provide intensity, resolution and topographical information that improve the visual quality of an SIMS image, seen with a copper-mesh grid. A C_{60}^+ primary ion beam was used to obtain a high resolution lipid SIMS image to fuse with high mass, molecular specific signal obtained using an Ar_{4000}^+ cluster primary ion beam. As a result, the output hybrid image displays improved visual

quality over the original SIMS image by combining multiple pieces of relevant and complementary information into a single image. This improved visual quality allows a reader to visualize the DPPC lipid distribution with more clarity and accuracy. Being able to visualize lipid specific signal to the degree as done with image fusion will be invaluable for imaging biological species with SIMS.

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