The zcorrectorgui

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An NIH National Center for Research Resources
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http://www.nb.engr.washington.edu
The Zcorrectorgui

- In ToF-SIMS depth profiles of 3D objects, the original Z-axis is inverted due to projection of 3D data onto 2D image plane
- Zcorrectorgui:
  - Matlab gui interface to correct 3D image stacks from Cell depth profiles
  - Corrects the Z axis of ToF-SIMS image depth profiles
  - Based off idea from Breitenstein et. al. (Angew. Chem. Int. Ed. 2007, 46, 5332 –5335)
- Requires Matlab (tested with Matlab 2006b)
  - Currently requires image processing toolbox
- Works with Iontof .bif and .bif6 files
- Can also load data from workspace (if formatted properly)
Why does the Z-axis need correcting?

- When depth profiling surfaces with topography the resulting images are 2D projects of the 3D surface
- This results in an inverted image matrix
3D object

Imaging with primary ions produces projection of 3D object into 2D image

Sputter beam etches away a layer of the surface

Cycle 1
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Cycle n
Why does the Z-axis need correcting?

The resulting image stack is inverted and the z=0 axis is incorrect.
Threshold the XZ or XY image cross-section of the total counts image

Use the thresholded image to find the contour line of the inverted profile. This line becomes the new Z=0 line.

Use the new Z=0 line to invert the image stack and get a corrected profile
Assumptions/Requirements

• Constant sputter rate (seems to be true for cells and polymers tested so far)

• Easy to find contrast between feature and background

• Minimal sputtering of substrate
Example

- ToF-SIMS image of NIH 3T3 cell
- Sputtered with C60+
- Imaged with Bi3+
- Known fragments of lipids and proteins were selected and exported in .bif images using Iontof measurement explorer.
- 56 .bif images were imported into zcorrectorgui
Zcorrectorgui main window

- Import Data From Directory
- Peak List
- Z corrected image XY
- Z corrected image YZ
- Z corrected image XZ
- Overlay Tools
- 3D Tools
- Create XY Corr Slice Movie
- Create XZ Corr Slice Movie
- Create YZ Corr Slice Movie

Dynamic graphs and visualizations are shown for thresholded total counts in XZ and YZ planes, with corresponding Z line diagrams.
Image stack correction

Original XZ image slice

Thresholded image

Profile line (new Z=0)

Corrected image slice

m/z = 56 (lipid signal)
Zcorrectorgui: 3D panel

Transparency Options
Be Patient This Works Very Slowly!

Update
ToF-SIMS AFM Comparison: PMMA bead on Si

AFM image before depth profile

SIMS Image from 3D profile

Top View

side View
ToF-SIMS AFM Comparison: NIH 3T3 cell

A) AFM

B) Corrected ToF-SIMS
Conclusions

- Matlab based program to correct the z-axis of ToF-SIMS depth profiles
- Shown to create accurate z-scale for beads and cells tested so far
- Available for download as part of the NB toolbox at: http://mvsa.nb.uw.edu/
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Why does the Z-axis need correcting?

- When depth profiling surfaces with topography the resulting images are 2D projects of the 3D surface
- This results in an inverted image matrix
When 3D objects are imaged by ToF-SIMS the resulting images are projections of the 3D data into a 2D image. A depth profile is then built from a stack of these 2D images.
Why does the Z-axis need correcting?

The resulting image stack is inverted and the z=0 axis is incorrect.

This process continues as you profile through a cell resulting in an inverted data matrix where data from the top of the 3D object is at the bottom of the data stack.
Threshold the XZ or XY image cross-section of the total counts image

Use the thresholded image to find the contour line of the inverted profile. This line becomes the new Z=0 line.

New Z=0 line

Use the new Z=0 line to invert the image stack and get a corrected profile

In order to correct this and display the data correctly, the correct Z axis must be determined. In the Zcorrectorgui, we use the total ion image to find the correct Z=0 line since in most cases the total ion image will show an inverted shape of the object being profiled.

To do this, first the total counts image is thresholded to find the shape of the object. Then the thresholded image is used to find the contour line of the object. The contour line is then set as the new Z=0 line and all pixels in the image are shifted by the appropriate factor to match the new Z=0.

This corrects the position of all pixels in the matrix and displays them in their true position.
Assumptions/Requirements

• **Constant sputter rate (seems to be true for cells and polymers tested so far)**

• **Easy to find contrast between feature and background**

• **Minimal sputtering of substrate**

For this correction to work, the sputter rate needs to be constant. This appears to be true for the systems we have tested.

If the sputter rate were not constant, the Z correction should still work, but would result in an extended Z axis in areas that sputtered slower than other areas.
Example

• **ToF-SIMS image of NIH 3T3 cell**
• **Sputtered with C60+**
• **Imaged with Bi3+**
• **Known fragments of lipids and proteins were selected and exported in .bif images using Iontof measurement explorer**
• **56 .bif images were imported into zcorrectorgui**
This slide shows a screen shot of the zcorrectorgui after the cell depth profile data has been imported and corrected.

The top left image shows the uncorrected data in the XY plane. The top right image shows the corrected data in the XY plane. The plots on the bottom of the window show the images from each step in the z correction process.
Image stack correction

This slide shows the individual images from the correction used for a given slice in the 3D data stack. The zcorrectorgui automatically calculates the corrected data for all slices in the 3D data stack.
This slide shows a screen shot of the 3D tools panel of the zcorrectorgui. On this panel any peak, or combination of peaks, in the data set can be displayed in 3D. The user can adjust the transparency of the 3D plot, selectively display selected slices of the stack, create and view isosurfaces, and overlay several isosurfaces. The user can also create a move of the 3D display as it rotates along any axis (X, Y, Z).
ToF-SIMS AFM Comparison: PMMA bead on Si

In order to check if the zcorrector is determining the correct z=0 line and accurately correcting the data, we did AFM on PMMA beads that had been melted onto Si wafer pieces. We then found the exact beads and depth profiled them and then corrected the depth profiles using the zcorrectorgui.

As seen in the figures the shape of the beads is accurately maintained in the corrected data.
An AFM ToF-SIMS depth profile comparison was then also done on an NIH 3T3 cell. As seen in the images above, excellent agreement is found between the AFM height image and the corrected ToF-SIMS depth profile.
Conclusions

• Matlab based program to correct the z-axis of ToF-SIMS depth profiles

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