AFMExplorer [version 0.22, 24-Mar-2008]

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Installation

By now you've gunzipped and untarred AFMExplorer-0.22.tar.gz in some appropriate place such as C:\Program Files\AFMExplorer-0.22\.

Notice the \bin subdirectory. You need to append a fully\qualified\path\to\bin\ to your global path, since it contains dynamically linked libraries AFMExplorer requires at runtime. E.g. most likely C:\Program Files\AFMExplorer-0.22\bin.

Execution

Now click on afm_explorer.exe.

The program will bring up four panes: one on the left, initially blank, but will eventually hold the images we want to look at; and three vertically stacked panes, from top-to-bottom: "Image Properties", "Image Processing Parameters", and "Component Data". Initially, "Image Properties" simply says, "No image loaded." – a state reflected in the status bar at the bottom that initially reads, "Ready to load image." (The status bar will always guide you to the next required step, or display the current processing while you wait.) Initially, "Image Processing Parameters" displays the default values it will use to filter the image prior to thinning its features to line components and then finding the backbones from connected components. (You may change these parameters at any time – more on this later.) Initially, "Component Data" displays an empty two-column list that will hold the ID and Length of the list of connected components it processes.

First, go to *File => Open*, and select a bitmap (.bmp) format image; or go to *File => Most Recently Opened*, and select a file from the list. The image will appear in the "Original Image" tab. Notice the image is displayed in *Fit to Window* mode. If you want to change the zoom settings, then go to *Zoom* and select the appropriate setting.

Next, go to *Run => Set Conversion Factor*. A dialog will prompt you to enter the number of nanometers (nm) that the longest dimension of your image represents. A default value is provided. If you provide a different value, then that value becomes the default for the duration of program execution. The next time you start the tool, the default will revert to its original value. Once you chose a value and press "OK", the conversion factor (nm/pixel) will display in the last line in the "Image Properties" panel.

Next, go to *Run => Process Image*. Notice the algorithm's progress is shown in the status bar. When the image is processed, two new tabs appear in the main image viewing panel: "Filtered Image" and "Backbone Image". Clicking on these will display the result of the algorithm. The connected component data now appears in the "Component Data" panel. The ID assigned to each connected component is given in the order of its discovery in a top-to-bottom, left-to-right ordering. Click on the columns to sort the list by that column's data. Click again to toggle the order from ascending to descending, etc.

From here you may save the filtered image (*File => Save Filtered Image*), save the backbone image (*File => Save Backbone Image*), and save the connected component data (in its current

sorted order) (*File => Save Connected Components*).

In any tab's image, click on a backbone component. It will turn red, and its entry in the "Component Data" panel will be highlighted. Alternatively, click on an entry in the "Component Data" panel to highlight its corresponding backbone component in the image.

If you want to adjust the image processing filtering parameters to perform better on your sample images, then go to *Run => Set Image Processing Parameters*. This will take you through a series of dialogs, prompting you for choices and values. These follow the following scheme:

First, choose a thresholding method: Fixed or Adaptive.

If you chose Fixed, then:

1. Select the pixel intensity level, above which the algorithm will eliminate it from further processing. These pixels are too bright.

2. Select the pixel intensity level, below which the algorithm will eliminate it from further processing. These pixels are too dim.

If you chose Adaptive, then:

1. Select the pixel intensity level, below which the algorithm will eliminate it from further processing. These pixels are too dim.

2. Chose whether you want to use a Mean or Gaussian adaptive thresholding method to evaluate pixel intensity values in the box (see next).

3. Select the dimension of the box representing the nearest neighbors to the pixel under consideration – at the center of the box. Thus, the dimension must be an odd value. The Mean method will decide whether to retain the pixel under consideration by computing if its intensity level is greater than the mean intensity level of all its neighbors. The Gaussian method does something similar, but imposes a Gaussian distribution on the intensity levels of the neighbors instead.

If this is the first time through parameter selection, then recommended values are provided. These will remain defaults until you change them, then the defaults will become the changed values. This holds over the duration of program execution. The next time you start the tool, the defaults will revert to their original values.

Notice that if your selections represent a shift away from the prior parameter values, then you will be prompted to reprocess the image if you like, since the currently displayed "Component Data" will not yet reflect processing of the image using these new parameters.

Also notice the new parameter values display in the "Image Processing Parameters" panel.

Known Bugs (for immediate fix or investigation)

1. The sliders in the image panel (irrespective of tab) misbehave, snapping back to their original position despite translating the figure in the window in the intended direction as far as the slider was placed. After trying about two dozen approaches to fix this, the reason for it escapes me. I will make this a high priority 0.22 release fix.

2. Reprocessing an image (at least via (re)setting the image processing parameters) does not refresh images in normal-size mode once components have been rendered upon them. One potential result of this is ghosted segments from prior processing using different image processing parameters. I will make this a high priority 0.22 release fix.

3. In fit-to-window mode, the segment rendering is poor, and even highlighted connected components appear degraded, despite their being clear in normal-size or zoom modes. I'm unclear as to the limitations imposed by compressed image renderings. If you want to see the segments, then view the image in normal size mode, or zoom in. This may not be fixable.

4. Mouse selection of connected components does not work in fit-to-window mode, for the obvious reason that the coordinates would have to undergo active transformation (each time the user resizes the window), and may suffer from cumulative rounding errors. This may not be fixable.

To Do (for version 0.30 — processing mode items)

1. Correct for dim streaks, often in the left-right proximity of bright spot artifacts.

2. See Known Bugs 1 and 2, above.

3. Allow batch processing of images, either on the command line or via menus, including outputting their segment (id, length) pairs.

4. Enable opening and processing of Nanoscope files, which use a higher precision (N-bit) embedded image than BMP or TIF (8-bit) images.

To Do (for version 1.00 — strategic items)

1. Model thermal drift to correct backbone length — much to be worked out here. One idea is to input an image series (e.g 20 images organized as follows: 10 pairs, each pair rendering a different molecule from the same species, the first and second of each pair separated by some known time interval, sufficiently large for thermal drift to reach equilibrium). This set of images can thus be used to calibrate thermal drift and the accuracy of measuring the backbones of a population of the same species of molecule (the variance should not exceed some stringent tolerance). Another idea entails using calibration molecules and a model of local distortions based on proximity and orientation of these to segments of the target object — an idea partially developed in separate notes.

2. Perform tip-deconvolution to correct backbone length — much to be worked out here.

3. To locate backbone breakages (restriction enzyme digestion sites): walk the backbone, examining change of intensity in the transverse and orthogonal directions.

Contact Information

Please contact Andrew Sundstrom regarding any questions or to report bugs.

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