



prokarymetrics Documentation

Release 0.4.0

Shareef M. Dabdoub

June 18, 2012

CONTENTS

1	Introduction	3
1.1	Software Requirements	3
1.2	Running ProkaryMetrics	3
1.3	User Interface	4
1.4	Updating ProkaryMetrics	5
2	Data	7
2.1	Opening Files	7
2.2	Projects	8
2.3	Exporting	8
3	Data Interaction	9
3.1	Exploring the Rendered Image Data	9
3.2	Image Sets as Layers	13
3.3	Marking and Recording Bacteria	14
3.4	Capturing Screenshots	18
3.5	Keyboard Commands Reference	18
4	Analytics	19
4.1	Data Display Panel	19
4.2	Volume Estimation	19
4.3	Bacteria Orientation	22
4.4	Community Distance Information	23
5	Indices and tables	25

Release 0.4

Date June 20, 2012

ProkaryMetrics is a software package providing tools to analyze microbial biofilms through the 3D reconstruction of fluorescent microscopy image data and the manual annotation of bacteria. ProkaryMetrics allows full 3D interaction with such data sets and provides users with visual and quantitative analysis tools to gather quantitative measurements on individuals as well as the biofilm as a whole.

Contents:

INTRODUCTION

ProkaryMetrics is a software package designed to provide:

- 3D reconstruction and visualization of 8/16-bit B&W fluorescent microscopy z-stacks
- 3D interaction with the reconstructed data
- manually-assisted annotation of the individuals in microbial biofilms
- visual and quantitative analysis tools to gather quantitative measurements on individuals as well as the biofilm as a whole

1.1 Software Requirements

The software is written in Python (2.7) and requires the following libraries:

- VTK (the Visualization Toolkit). At least version 5.6.
- wxPython (windowing toolkit). At least version 2.8.10.
- PIL (the Python Imaging Library). At least version 1.1.7.

The simplest means to getting the appropriate Python environment with the appropriate libraries is to install the Enthought Python Distribution (free for Academic users) which can be found at:

<http://enthought.com/products/epd.php>

ProkaryMetrics requires at least version 7.0 to run due to the need for Python 2.7.

In the near future, I hope to provide a self-contained app package for Mac/Win that will not require any installations.

1.2 Running ProkaryMetrics

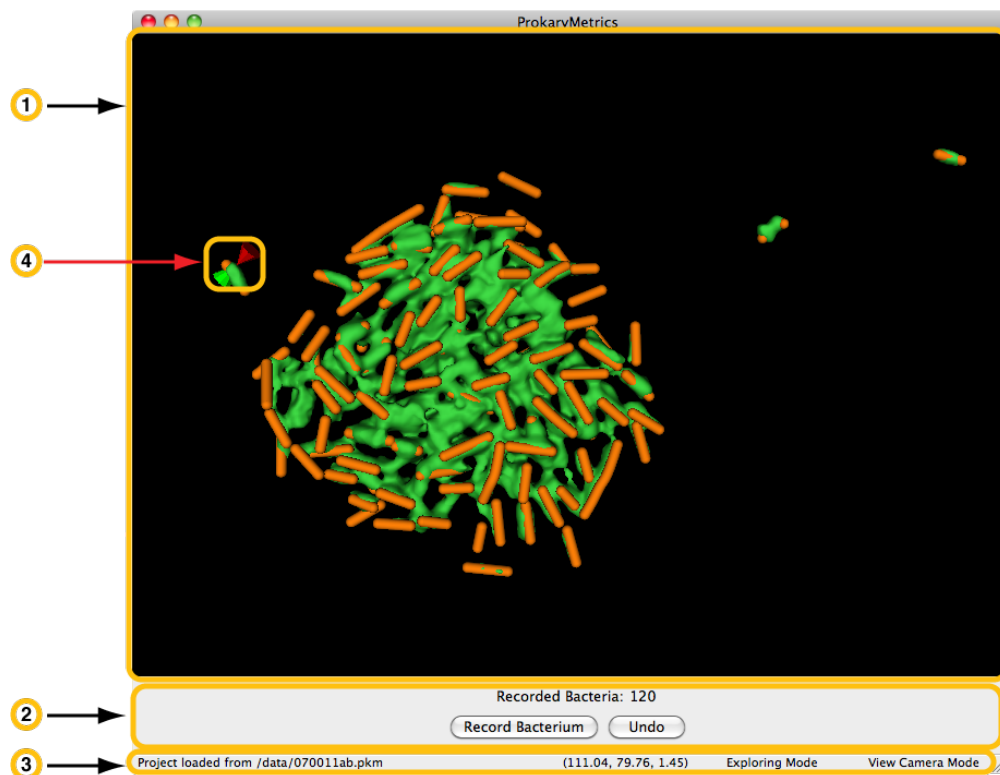
Once you have the appropriate Python environment and libraries installed, use a console to navigate to the ProkaryMetrics folder contained with this file in the downloaded archive and type:

```
$> python pkMetrics.py
```

This command will launch the program, and everything else is done through interacting with the GUI.

1.3 User Interface

Below is a screenshot of the basic user interface for ProkaryMetrics. The major interface elements are highlighted, as well as the important data kept track of at all times.



1. **Visualization Window** - This is the main focus of ProkaryMetrics; providing the user with 3D visualization of their reconstructed data, and allowing mouse-interaction for 3D data manipulation and exploration.
2. **Actions Panel** - This section of the program window provides the count of bacteria the user has marked, and presents the option to either save a set of marked points as a bacterium, or undo the addition of the last recorded bacterium.
3. **Status Bar** - The status bar is divided into four sections. From left to right: the most recent status message (seen here, the successful loading of a saved project), the current mouse pointer location in terms of X, Y, and Z within the rendered data, the mouse click mode (Exploring allows the user to examine the data by rotating, zooming, etc..., the other option Recording allows the user to annotate bacteria), and the camera mode (this feature is still in testing).
4. **Mouse Cursor** - The red and green cones represent the location of the mouse, with the clicking tip between the points of the cones. The two-cones cursor attaches itself to the nearest surface of the reconstructed data, allowing the user to trace the surface and thus permit accurate marking of bacteria.

Here the cursor is tracing the surface of a section of the image data (green) representing a bacterium. The orange data is a user-recorded marking of the bacterium from the image data.

1.4 Updating ProkaryMetrics

The latest version of the software is always available through the main website:

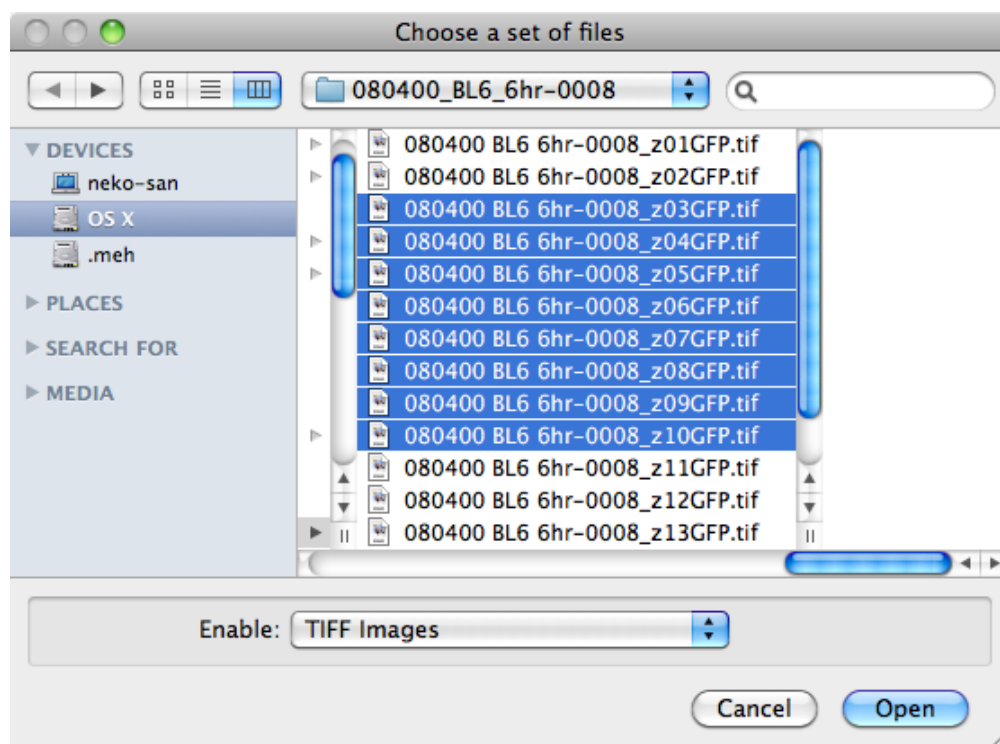
<http://www.justicelab.org/pkm>

DATA

ProkaryMetrics is designed to reconstruct volumetric image data by combining sets of 2D images known as z-stacks that represent optical slices of a physical data sample. Currently the default image format used by ProkaryMetrics is the Tagged Image File Format (TIFF), but the software also supports JPEG images.

2.1 Opening Files

Image data is added to the current rendering window through the File >> Open Image File(s)... menu item. Since a z-stack is expected, the file selection dialog will accept multiple file selection.



Clicking the “Open” button will cause ProkaryMetrics to attempt to load the specified image files, and render a 3D surface representation of the fluorescent pixels (see the User Interface subsection in the Introduction section). Currently, ProkaryMetrics has a default pixel-brightness level it uses when performing the 3D

reconstruction, which will not be appropriate for all image data. Particularly, 8-bit image data which only has pixel values between 0 and 127 will not be properly reconstructed initially. If the reconstructed data does not look right, or does not appear at all, you can modify the pixel value used to perform the reconstruction. This and other user-modifiable settings are described in detail in the Data Interaction section. Eventually the initial value will be intelligently chosen using the content of the image data itself, but users may still want to tweak the setting.

2.2 Projects

Once you have loaded some image data and maybe marked some bacteria, you will want to save your work. ProkaryMetrics supports saving and loading of project files. A project consists of the current state of the program including all loaded image files, any recorded bacteria, and any modified settings. Both saving and loading projects can be done through the File menu.

Note that ProkaryMetrics does not store a copy of the loaded image data, it merely stores the location of each loaded image file. This means when you load a previously saved project file ProkaryMetrics will look for the image data in the location it was when you saved the project. If the images have been moved or deleted it will pop up a message telling you it cannot locate them, and will give you the option to select the folder they have been moved to. If it can find all the images in the new location, it will load them and continue loading the rest of the project information.

2.3 Exporting

Given that one of the main goals of ProkaryMetrics is to provide quantitative and statistical information about microbial communities, it is often useful to use that data in other software for additional analysis or display. To this end, ProkaryMetrics provides the facility to export certain data. Currently, this includes: bacteria and lengths. Both options are available through the File >> Export menu.

2.3.1 Bacteria

Exporting bacteria will result in a file that lists the marked end and interior points for each bacterium. Each line corresponds to an individual bacterium, and each line is a comma-separated list of 3-tuples that indicate the X, Y, and Z coordinates of each marker that makes up the recorded bacterium.

```
1.23, 3.44, 0.65, 18.36, 4.40, 2.21
```

This example shows a line of 6 numbers separated by commas. As mentioned above, the line is logically grouped into 3-tuples indicating axis values. This line has 6 values, and so indicates two markers representing a single bacterium.

2.3.2 Lengths

The export lengths option creates a single-column CSV file with a header line of “len”. Each line in the file gives the length of a single bacterium in micrometers.

DATA INTERACTION

The main purpose behind 3D rendering of microbial communities is to be able to interact with the data (rotating, zooming, cropping, etc...) in order to gain an understanding of your data that may not be possible through viewing 2D slices. This section describes the facilities ProkaryMetrics provides to allow you to explore your data.

3.1 Exploring the Rendered Image Data

The mouse is the main means of interacting with rendered image data. The few keyboard commands available will be discussed as appropriate in this section. A list of all the keyboard commands is at the end of this section with a short description of each key's purpose.

3.1.1 Rotating Image Data

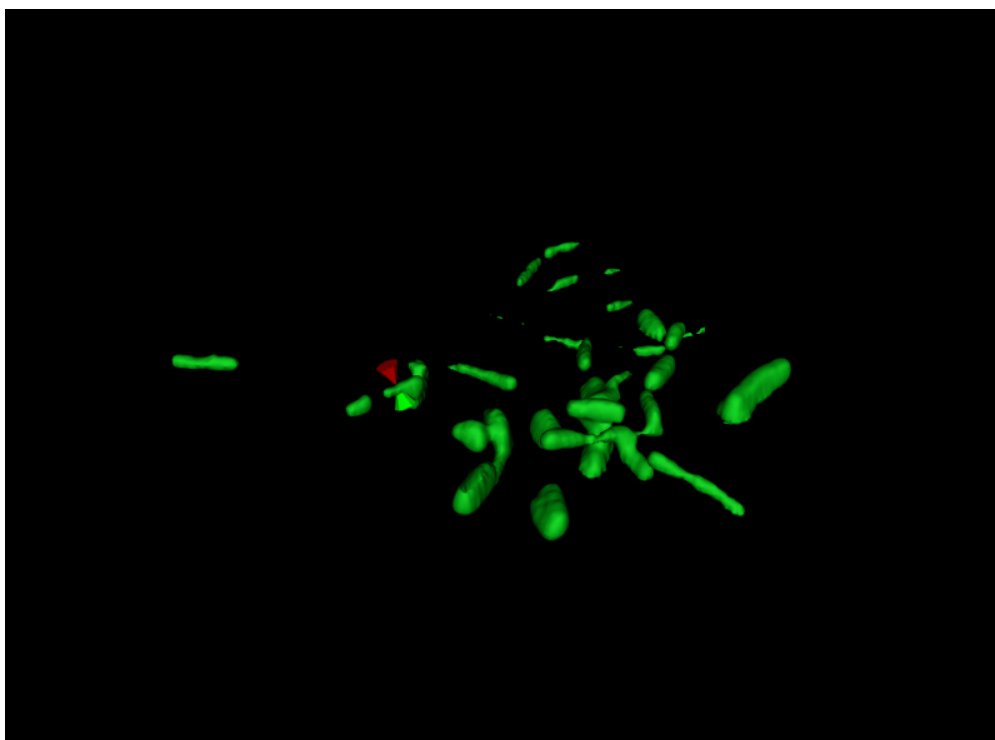
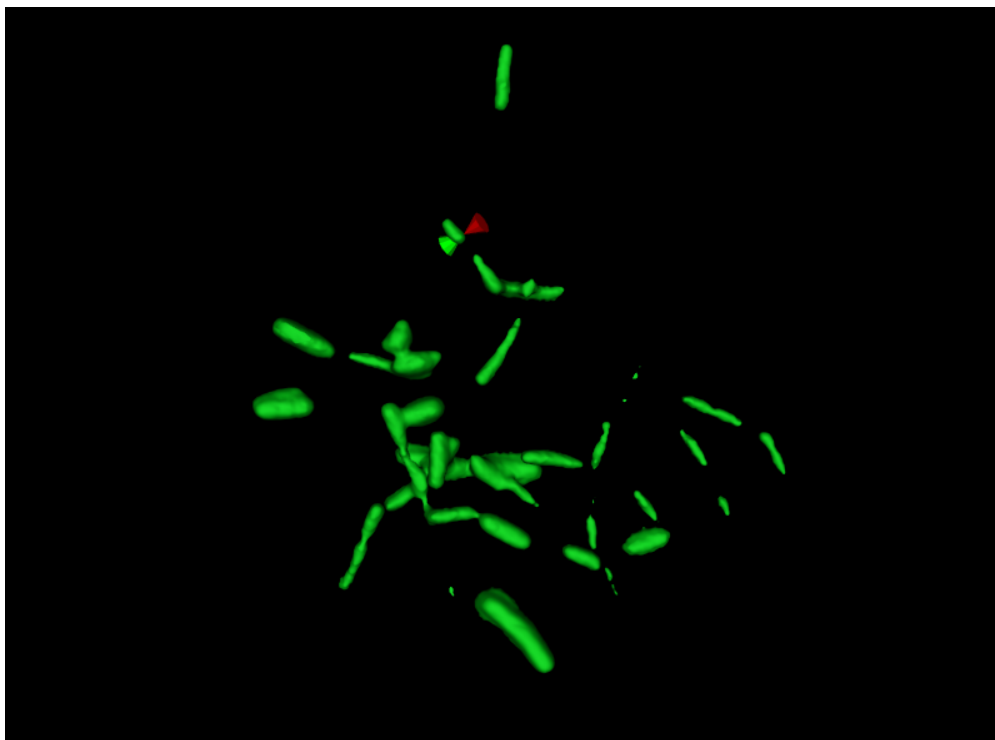
Imagine the the rendered image data is contained within a transparent hamster ball floating in space. If you grab the hamster ball, you can rotate it in any direction and the image data will rotate along with it. In ProkaryMetrics, you can use the mouse to rotate the hamster ball. Begin by left-clicking (and holding the mouse button down) anywhere within the Visualization Window. While holding the button, move the mouse and any data in the window will rotate in the direction you are moving the mouse. The first example below shows an initial rendering of a sample image set with the cursor near one bacterium.

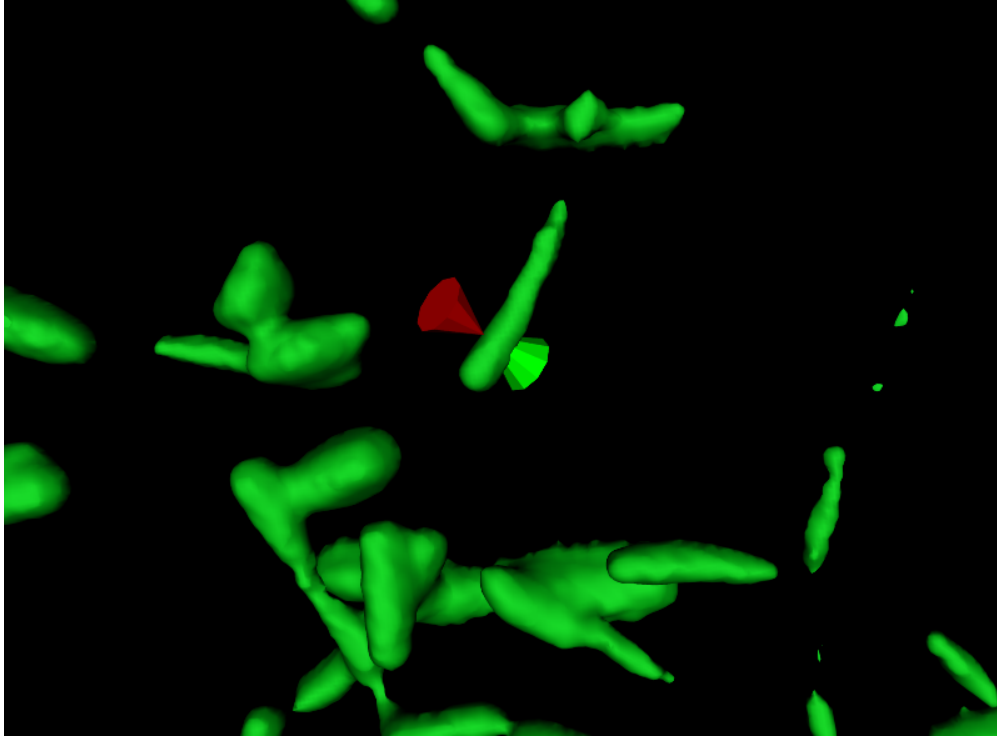
In the next image, the data has been rotated counter-clockwise approximately 90 degrees, and rotated away from the user (into the screen) approximately 45 degrees.

3.1.2 Magnification

Zooming in and out can be accomplished either with the mouse wheel (up to zoom in, down to zoom out) or by right-clicking and dragging with the mouse. Dragging up zooms in, dragging down zooms out. Zooming with the mouse wheel generally results in larger magnification steps. Zooming with the right mouse button gives you smoother magnification and slightly more control over how far it zooms in and out.

This screenshot shows the original data from above, magnified.





3.1.3 Moving Data

Especially when magnifying data, in order to get the best view it is very useful to translocate the rendered data. In other words, moving the data up/down or left/right in order to center a particular section of interest within the Visualization Window. This can be accomplished with a third mouse button. Typically this button is in the middle of the mouse, and in mice with wheels, may be accessed by pressing down on the wheel itself. For mice with more than three buttons, this action is mapped to whichever button is assigned to be mouse button 3. The first image below shows the sample data set as was shown previously.

The second image shows the data after being moved over to the right. Notice that neither the orientation nor the magnification level has changed.

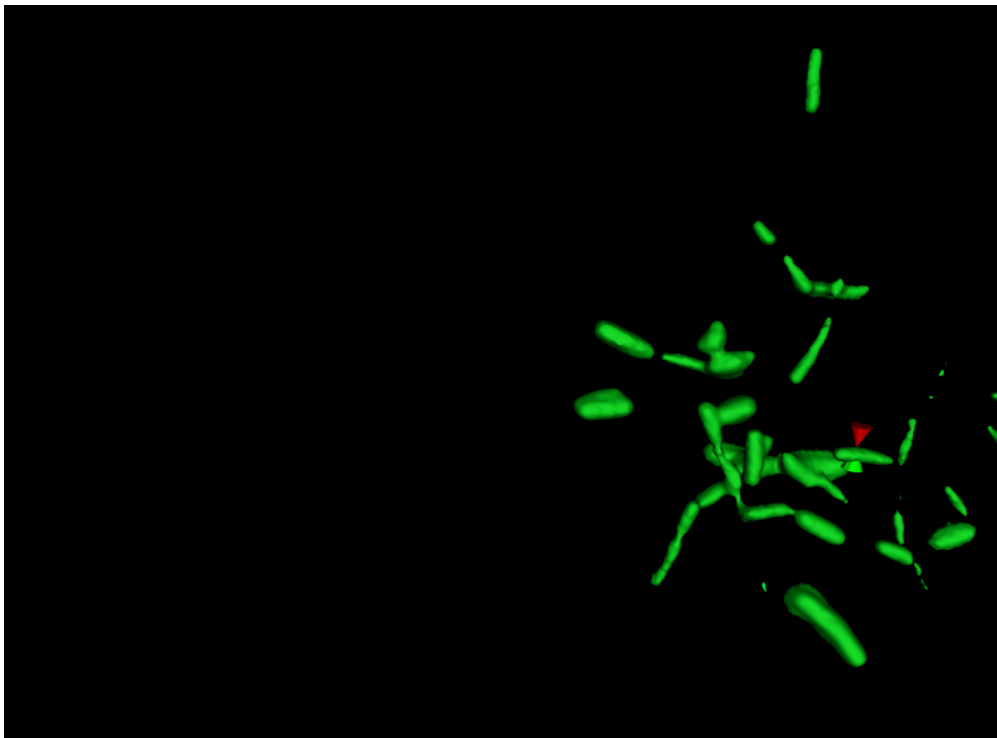
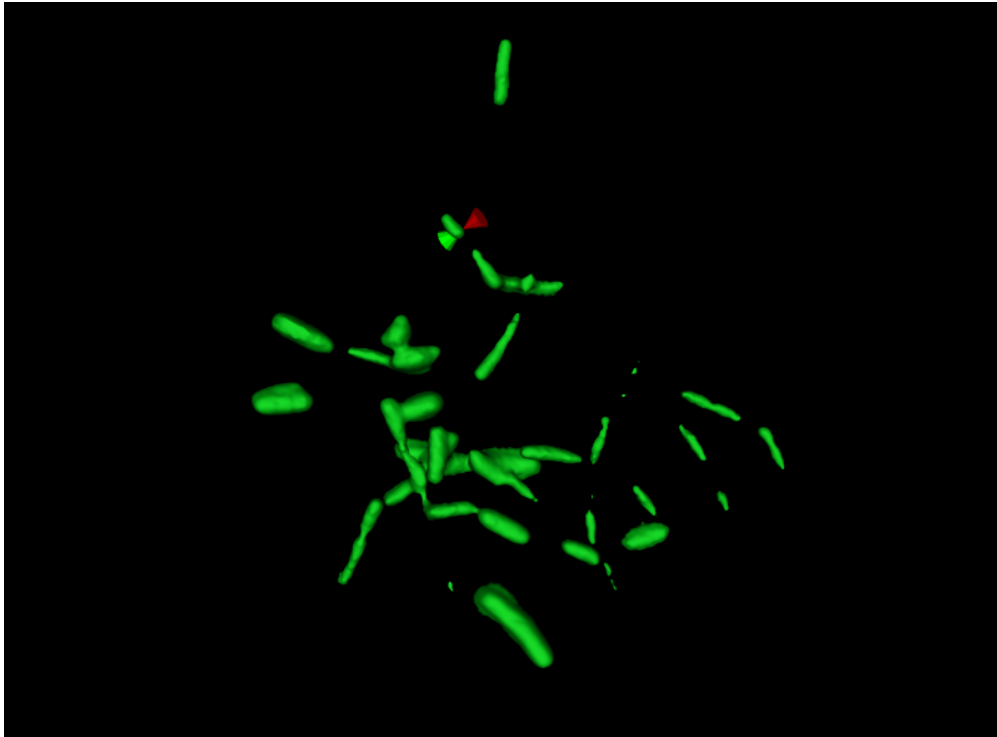
3.1.4 Rendering Settings

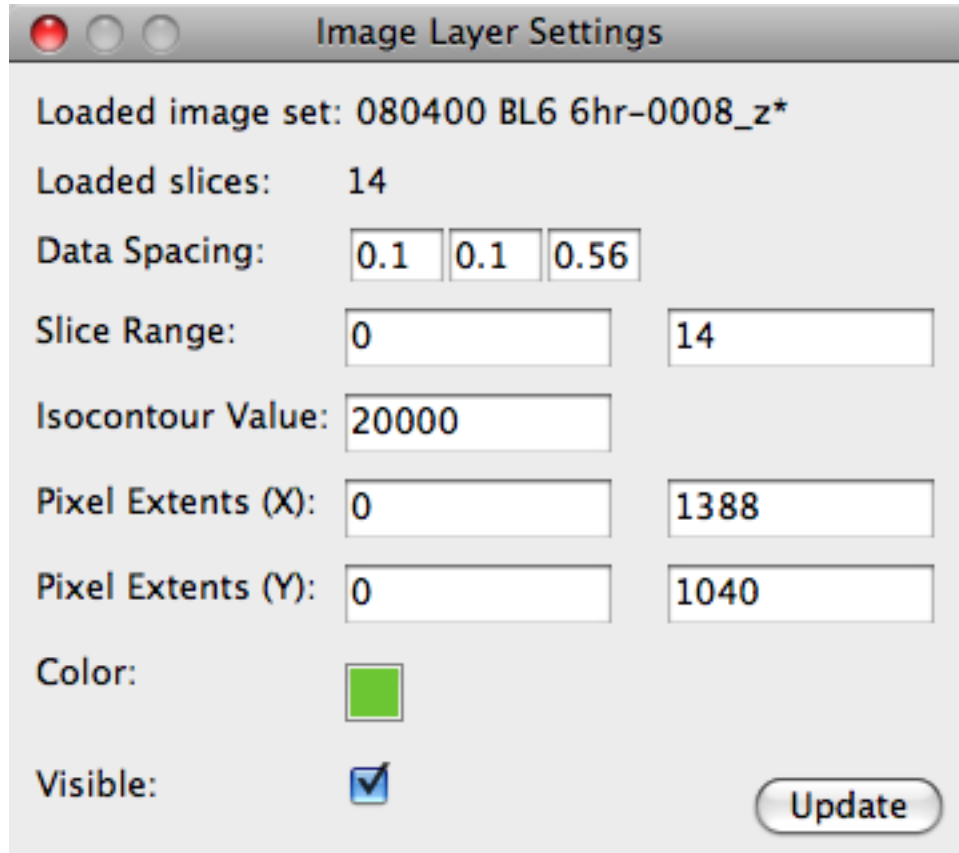
ProkaryMetrics creates the 3D reconstruction of the fluorescent image data using a number of default settings that are user-modifiable through a settings dialog shown below:

Loaded image set This uneditable field simply represents the set of files the image data is coming from. It assumes the files have a consistent naming scheme with some static part and a variable part indicating the image number. ProkaryMetrics extracts the largest static part of the name to create this field. The asterisk (*) at the end indicates what follows in the naming scheme is variable.

Loaded slices This indicates how many images were loaded to create the 3D rendering.

Data Spacing In order to accurately render the image data, ProkaryMetrics needs to know the approximate conversion factor from micrometers to pixels. In the example above, the loaded data has a horizontal (X) and vertical (Y) resolution of 0.1 micrometers/pixel, and a slice thickness of 0.56 microns.





Slice Range This setting allows you to modify the range of images that are used to create the 3D rendering. For example, if your first two and last two image slices are very noisy and should be excluded, you would change the slice range values to 3 and 12 to only render using those images.

Target Pixel Intensity ProkaryMetrics creates the 3D rendering by connecting pixels of a specified value/intensity (or range of values) to make a surface in 3D. In the example dialog, the value used is 20000. The large value indicates it is a 16-bit image as opposed to an 8-bit image. Pixels in 16-bit images can take a values in the range $(0, 2^{16}-1)$ or $(0, 65535)$. Similarly, 8-bit images have pixel values in the range $(0, 2^8-1)$, or $(0, 127)$.

Color This button pops up a color-chooser that allows the user to specify the overall color the rendered image data should have. The default, as seen above, is green.

Visible Allows the user to make the rendered image data visible or not visible.

3.2 Image Sets as Layers

ProkaryMetrics allows users to load more than one set of images at a time. Each image set is rendered within the Visualization Window as if it was the only image data being rendered. So if you load two sets of image data taken from the same sample but perhaps with different fluorescent colors, the two data sets will be rendered exactly as they appeared under the microscope, i.e. intermingled.

Each image set is separate in terms of settings, but you will need to tell ProkaryMetrics which is “active”.

This can be done through the View >> Select Image Layer menu item. This pops up a dialog with a list of the loaded image sets. One item can be selected at a time, and simply clicking on the item will change the currently active image set to the one selected. Then, when you choose the View >> Image Layer Settings menu item, the settings for the correct image set will be displayed.

Note that the Select Image Layer dialog does not prevent interacting with any other windows in ProkaryMetrics, so if you are frequently switching between image sets, you can keep the dialog up at all times.

3.3 Marking and Recording Bacteria

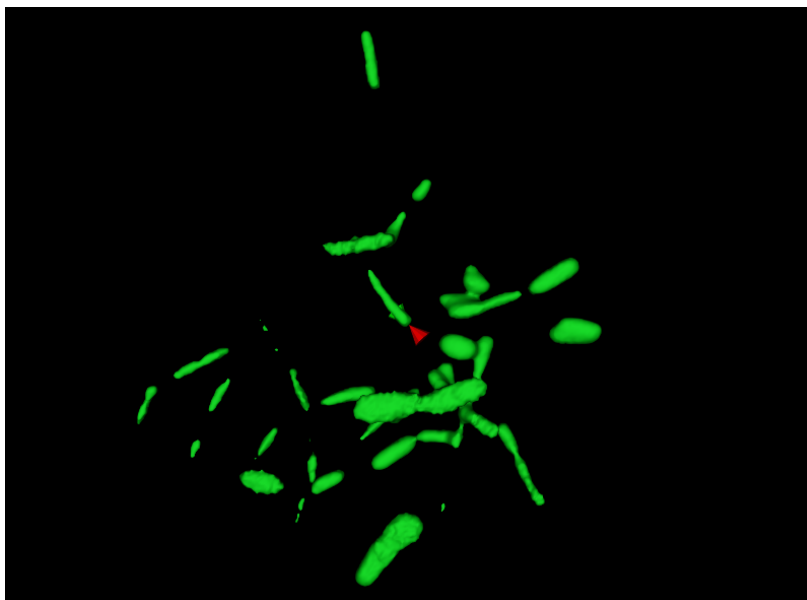
In order to perform measurements and analysis, ProkaryMetrics currently requires users to mark the visible bacteria within the loaded image data. In the future, we hope to partially automate the process.

The process of recording bacteria begins by marking the rendered image data with end and middle points. Currently ProkaryMetrics expects a bacillus-like morphology for the bacteria and supports three separate forms: coccoid, bacillus, and filament. Functionally, these correspond to one, two, and three or more marker(s) that are required to record each morphological type.

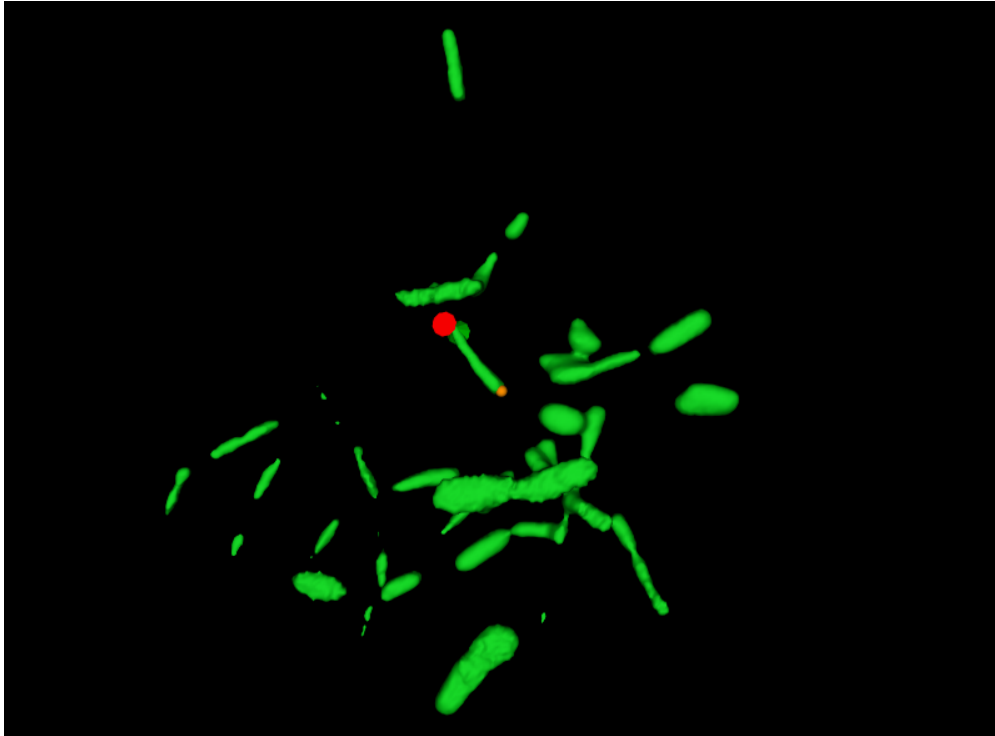
To begin (after loading some image data), make sure the Visualization Window has the focus by clicking once anywhere within it. Notice that the third status bar section indicates that you are currently in **Exploring Mode**. This means that the mouse will be used to manipulate the view of the data as was discussed previously. If you now press the ‘x’ key, the status bar display will change to indicate you are now in **Recording Mode**. In this mode, the mouse is used to place and remove the markers that will be used to record the position of bacteria.

Note again, that the mouse cursor is displayed within the Visualization Window as two opposing cones that attach to the nearest surface and follow the contours in the data. This allows you to click directly on the image data, and place a marker exactly on the surface of bacteria.

In the image below, we are in Recording Mode, and the cursor is positioned at one end of a bacterium, ready to place a marker.



In this next image, a single marker has been placed at the surface of the image data by clicking once. You will notice that the marker is a small sphere with a default color of orange. Both the marker radius and color can be changed with a settings dialog that will be described later. Finally, the cursor has been moved over to the other end of the bacterium in preparation to place the second marker.



Here we have placed the second marker at the other end of the bacterium, and since this is a bacillus, requiring two markers, we are ready to record the markers as representing a single bacterium.

Once you have placed the appropriate markers, move the mouse down to the Actions Panel beneath the Visualization Window, and click on the **Record Bacterium** button. This removes the markers you just placed, and replaces them with a rendering of a single bacterium with the endpoints in the locations you marked.

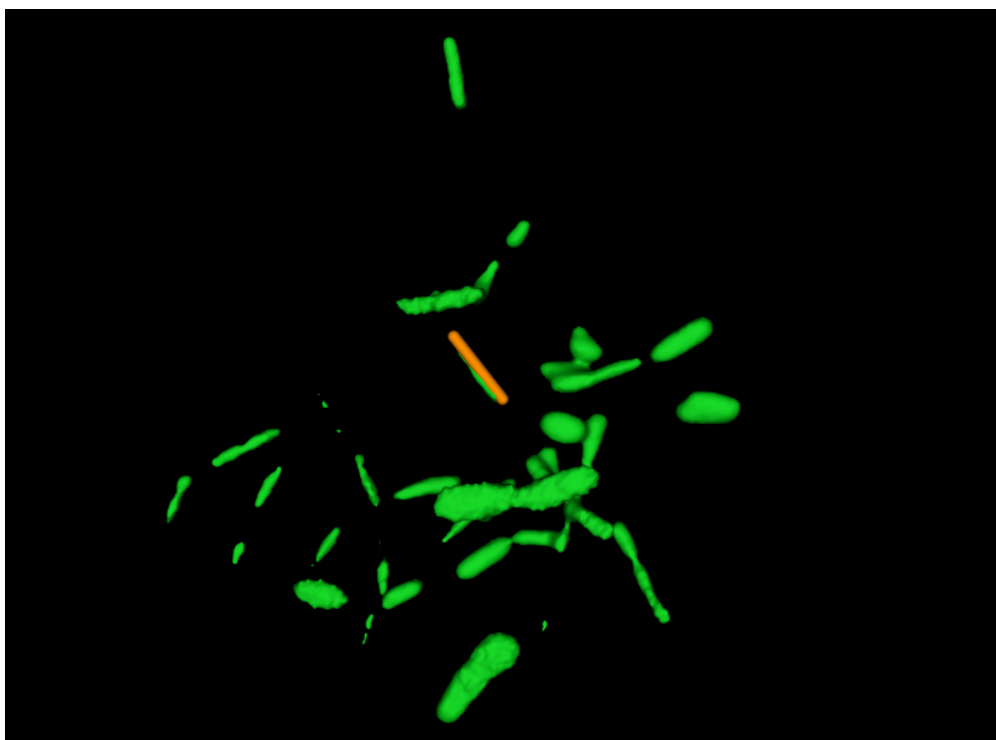
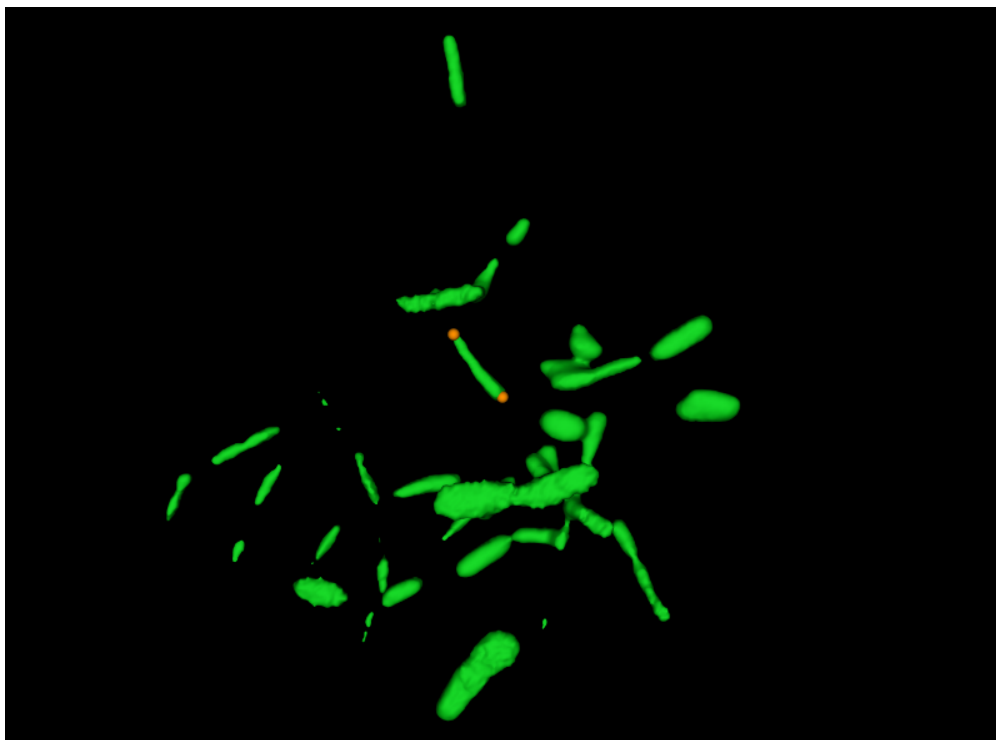
Note that when you click on the record button, the focus is taken away from the Visualization Window, and the keyboard shortcuts will no longer work until the Visualization Window has focus again. So if you think you will need to rotate the rendered data before placing more markers it is good practice to toggle back to the Exploring Mode (x key) before clicking on the Record button. If the rendered data is in good position to continue placing markers, go ahead and leave it in Recording Mode.

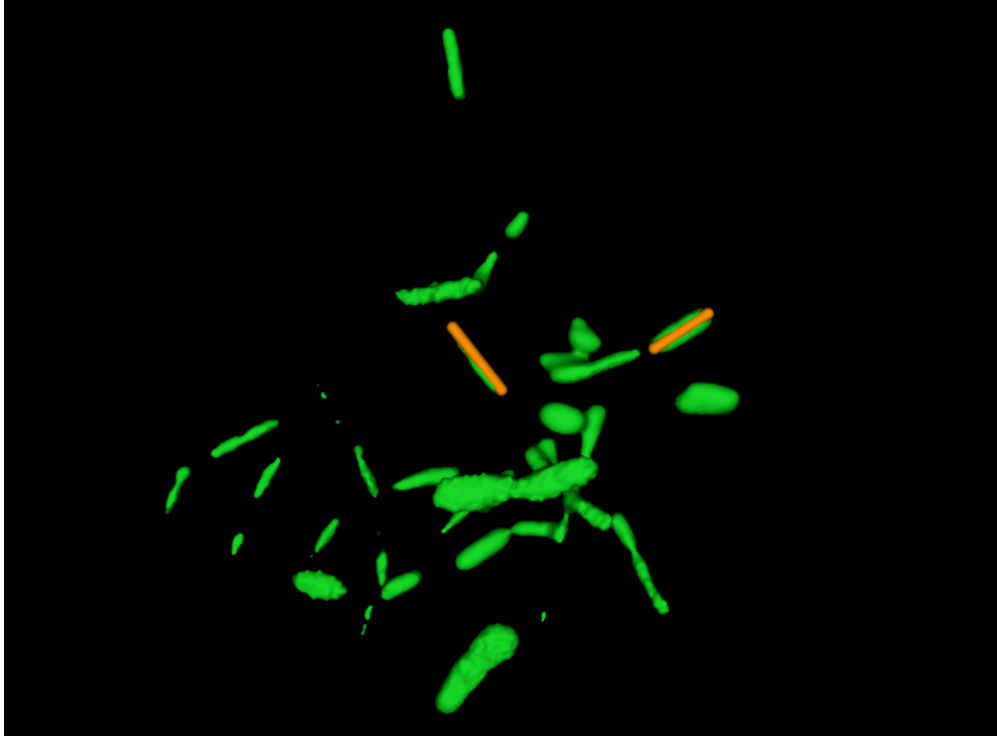
In this final image, we have recorded a second bacterium.

Note that (except perhaps for very small communities) it is not necessary to record every single bacterium within the sample in order to gather enough data for analysis. In fact, understanding how much of the community *is* necessary is an ongoing area of research.

3.3.1 Deleting Markers and Recorded Bacteria

To delete a placed marker, simply move to cursor on or very near it, and right-click with the mouse.



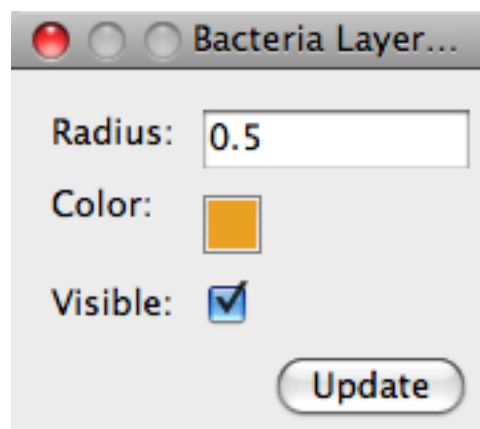


To delete the most recently recorded bacterium, click the **Undo** button in the Actions Panel. If you continue to click the Undo button, it will remove recorded bacteria in reverse order in which they were added.

To delete any specific bacterium, move the mouse cursor on or very near it and press the **D** key.

3.3.2 Marker and Bacteria Settings

Selecting the View >> Bacteria Layer Settings menu item will show the following dialog:



Radius Determines the size of the placed markers as well as the recorded bacteria that are rendered.

Color Pops up a color-selection dialog that allows the user to change the default color of markers and recorded bacteria.

Visible Allows the user to set whether markers and recorded bacteria are visible or not.

3.4 Capturing Screenshots

To export an image of the current state of the Visualization Window, click on the Tools >> Take Screenshot menu option. ProkaryMetrics supports saving screenshots as PNG, JPEG, TIFF, PNM, PostScript, or BMP.

3.5 Keyboard Commands Reference

- A** Toggle anti-aliasing mode. Anti-aliasing greatly improves image quality at the expense of smooth interaction. Depending on the graphics card and processor speed of your computer, turning this mode on may make interacting with your data extremely slow. For most computers it is only recommended for taking high-resolution screen captures for publication or magnification (e.g. posters).
- D** Delete the recorded bacterium nearest to the cursor. This is only effective to a certain radius around the cursor, so you should place it fairly close, if not directly on, the bacterium you want to delete.
- I** Toggle the clipping box. This key displays and hides a wireframe box that is drawn such that it initially contains within it all the rendered image data. The box also includes spherical handles that you can grab with the mouse and drag to expand or decrease the volume of the box. If a plane of the box crosses over any rendered image data, that data is made invisible. Expanding the box will reveal again any previously hidden image data. This allows you to focus on specific regions of your data, which can be helpful especially in large or especially noisy data.
- X** Toggle between Exploring mode and Recording mode.

ANALYTICS

After reconstructing microscopy data, the second aim of ProkaryMetrics is to provide the tools for quantitative analysis of the visualized communities. This section provides information on each of the tools provided by ProkaryMetrics and how to use them.

4.1 Data Display Panel

Before describing the available quantitative analysis tools, the data display panel needs to be introduced. This panel (normally hidden) is a simple text display that ProkaryMetrics uses to provide the user with numerical information relating to the performed analyses. For some tools this information is supplementary to the visual representation of the results, for other tools the data display panel is the only means to provide the results to the user.

To access the panel on a Windows machine, hold down the Ctrl button and press the D key. On a Mac, hold down Command (or apple) and press the D key.

4.1.1 Quick Reference

Windows: Ctrl-D

Mac OS: Apple-D (Command-D)

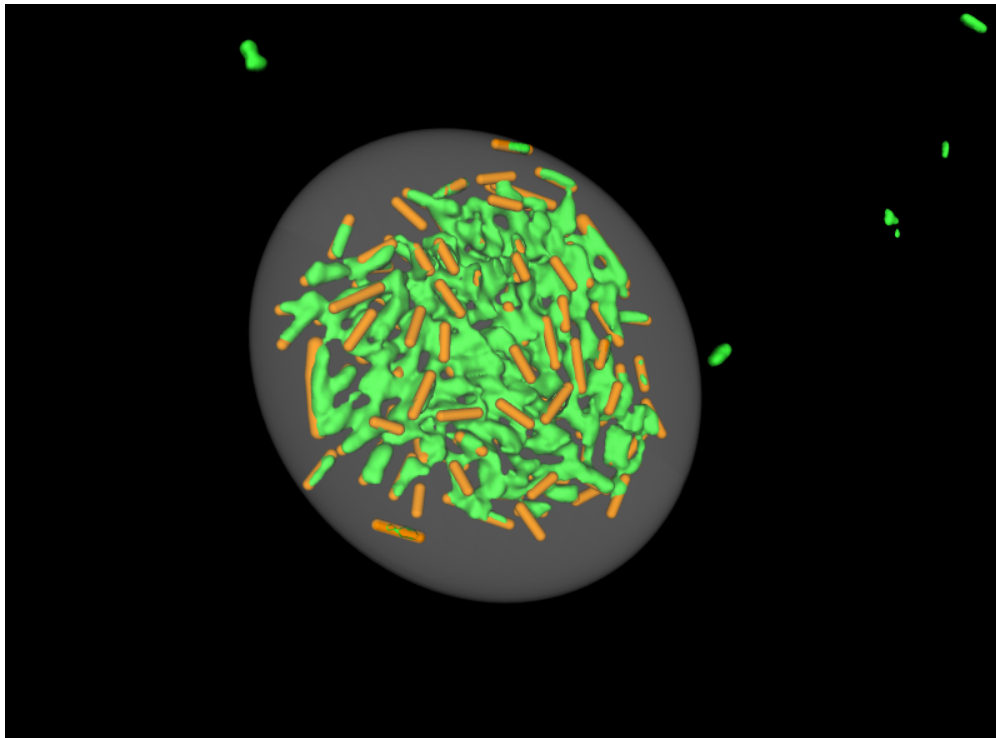
4.2 Volume Estimation

A useful method for understanding the amount of space occupied by a microbial community is to fit an ellipsoid (also known as a 3D ellipse, or a generalized sphere) to the community itself. ProkaryMetrics can use either recorded bacteria or, if you want a quick estimation of volume, any markers that are placed. In that second case, just toggle into Recording Mode and place markers on the reconstructed data near the extents of the community. Note that if you are doing this, ProkaryMetrics will provide a better estimation if the markers are not right up against the edge of the data, but somewhat inward a bit.

4.2.1 Performing the Fit

To actually perform the volume estimation, click on the Tools >> Volume Estimation >> Fit Ellipsoid menu option. The only requirement to using this tool is that you have at least nine (9) markers placed. This can either be actual markers (as in the second option from above) or recorded bacteria that required at least 9 markers to create. For example, if you have 3 bacilli (2 markers each) and one filament that required 3 markers, the recorded bacteria make up 9 markers in total. When you click the Record Bacterium button, ProkaryMetrics visually represents the bacterium as the bacillus-like shapes that appear, but internally represents the bacterium as a set of 3D points corresponding to the markers you placed.

When you select this menu option, ProkaryMetrics performs the necessary calculations and then displays a mostly transparent ellipsoid surrounding the data:

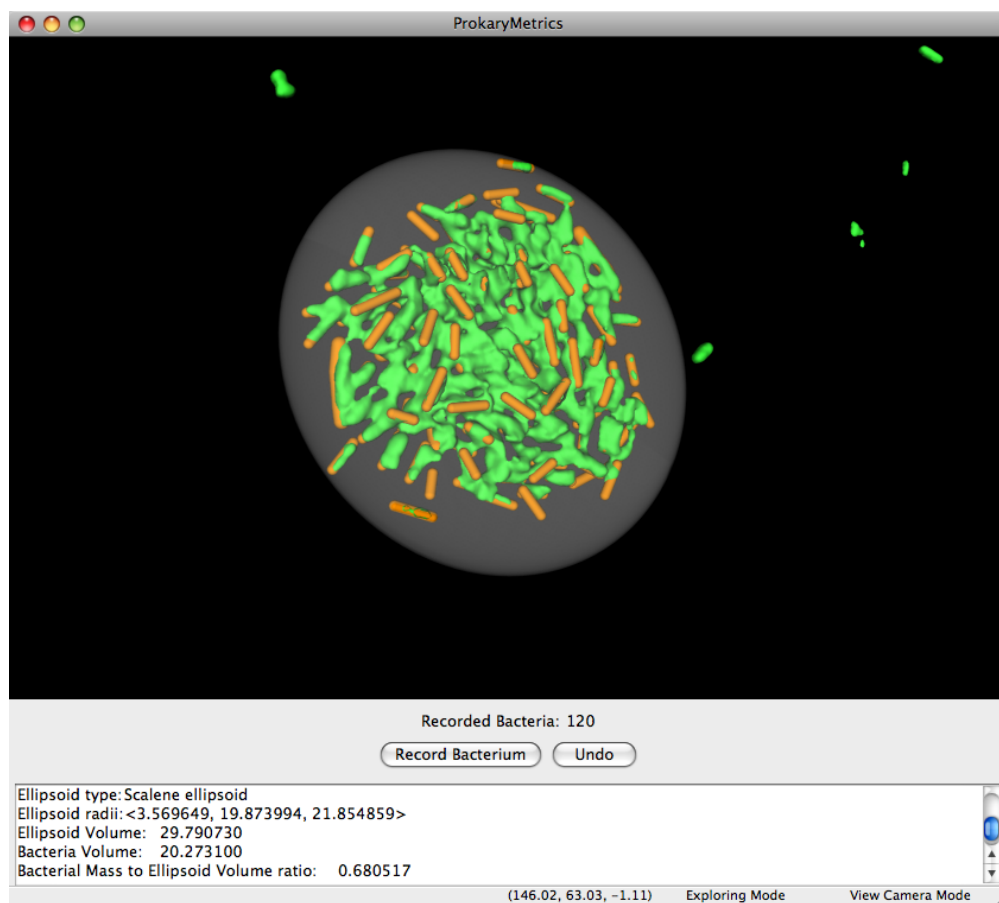


The data display panel provides additional numeric information as seen below:

ProkaryMetrics provides the user with additional quantitative information including a broad classification of the ellipsoid type (here Scalene because two of the three radii are essentially equal), the numeric volume of the fitting as well as that of the recorded bacteria, and finally the ratio of the two.

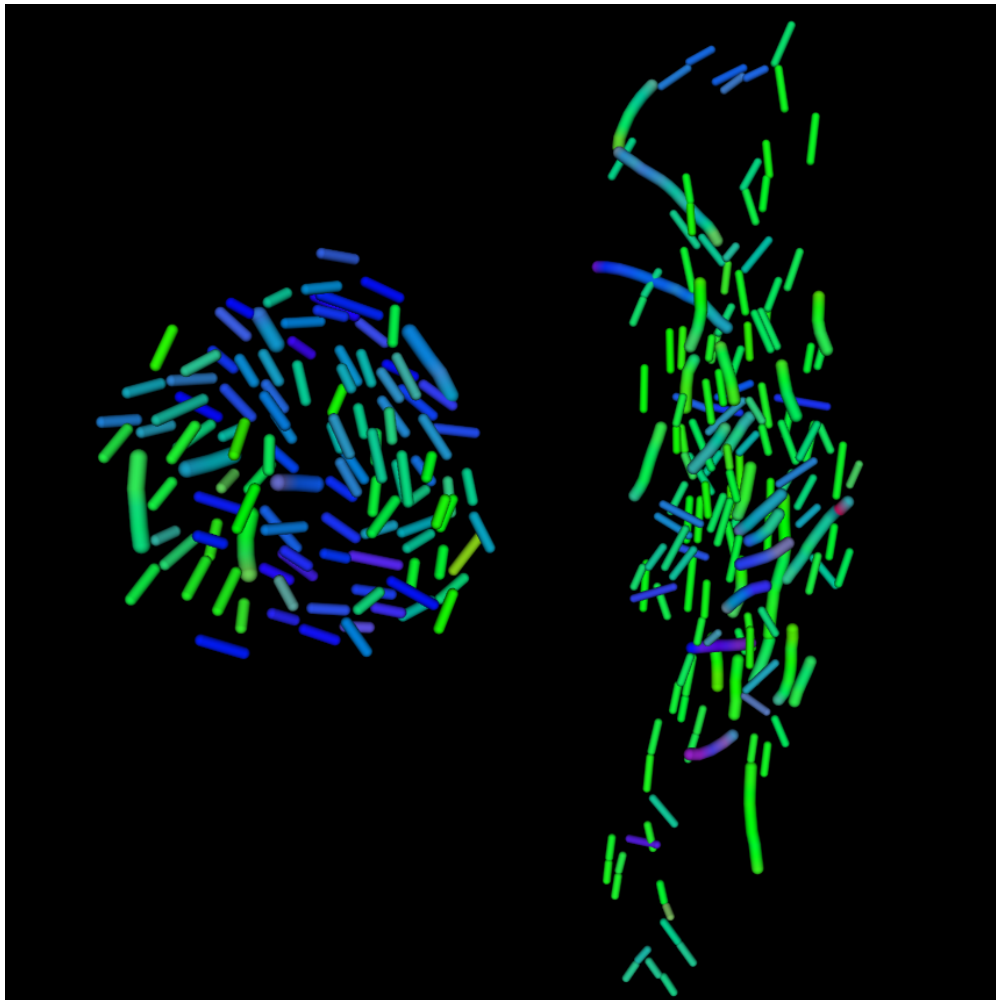
4.2.2 Toggle visibility

If you want to continue marking bacteria, the rendered ellipsoid will interfere with the cursor tracing the surface of the rendered image data. So to hide the ellipsoid, select the Tools >> Volume Estimation >> Toggle Ellipsoid Visibility. To see it again, simply click the menu item again.



4.3 Bacteria Orientation

Another interesting and useful bit of quantitative data is calculating how the bacteria are oriented within the 3D space that they occupy. For each bacterium, ProkaryMetrics calculates the main axis vector and compares that to the X, Y, and Z axes to get a number that describes how much the bacterium deviates from each of the axes. Those numbers are then used to color the bacteria, associating Red, Green, and Blue with the deviations from X, Y, and Z respectively.



ProkaryMetrics provides three coloring schemes to map X/Y/Z to colors: RGB, GBR, and BGR. The figure above shows the BGR coloring, and gives a good example of the utility in comparing orientations between data sets. In the community on the left, the bacteria are split between those with the major orientation component along the X (blue) axis and the Y (green) axis. Whereas with the community on the right, the clear majority of bacteria are aligned along the Y (green) axis. Note that a few of the bacteria have a significant portion of their orientation in the Z axis, and they have a reddish component, making the bacteria either purple for those with the other major component along the X axis, or yellow for those with a major component along the Y axis.

4.3.1 Performing the Orientation Visualization

To color the bacteria using orientation information, as seen in the figure above, select the Tools >> Orientation >> Color Bacteria by Orientation submenu, and choose one of the available coloring schemes as listed previously. You can return the bacteria to their original colors through the View >> Image Layer Settings dialog box. Additionally, the orientation information can be exported to a CSV through the File >> Export menu.

Descriptive statistics can also be calculated for the overall orientation information. Select the Tools >> Orientation >> Calculate Orientation Stats menu item. The results are shown in the Data Display Panel and give the mean, median, standard deviation, and first and third quartiles.

4.4 Community Distance Information

The final analytic tool available is calculating the average distance among the community members. This calculation is meant to give a metric for comparing how densely packed the bacteria are within the community. The community distance currently has no visual aspect, but the results are output to the Data Display Panel. To perform the calculation, select the Tools >> Calculate Community Distance Stats menu item. The results give the mean, median, standard deviation, and first and third quartiles.

INDICES AND TABLES

- *genindex*
- *modindex*
- *search*