Telometric 1.2 User's Guide

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Introduction

1.1 About Telometric

Telometric is a software package that enables users to assess the sizes and distribution of telomeric fragments represented in autoradiograph images of agarose gels. The hybridization from multiple matching telomeric probes is corrected for and simple statistics and plots may be generated for each selected lane. Telometric may be downloaded from http://bioinformatics.fccc.edu/software/OpenSource/telometric/telometric.shtml. For a more comprehensive discussion of Telometric that is beyond the scope of this user's guide, see 'Telometric: A Tool Providing Simplified, Reproducible Measurements of Telomeric DNA from Constant Field Agarose Gels', *BioTechniques* 31:1314-1318.

1.2 Java Preliminaries

Telometric 1.2 requires the Java 2 Runtime Environment (JRE) and Java Advanced Imaging (JAI). Installations of the JRE and JAI may be obtained from http://java.sun.com/j2se/1.3/jre/ and http://java.sun.com/products/java-media/jai/downloads/download.html, respectively.

Chapter 2

Using Telometric

2.1 Brief Overview

A typical Telometric session might consist of the following activities:

- Starting Telometric
- Loading an image
- Calibrating the image
- Outlining lanes
- Specifying background intensity
- Specifying probe hybridization
- Generating and saving statistics
- Generating plots of molecule size distribution and intensity

Detailed information on the execution of each step is given in the sections that follow.

2.2 Getting Started

The manner in which you start Telometric may be system dependent. If you locate the file telometric.jar with Windows Explorer or the Windows file finder, it may

be possible to start Telometric by simply double-clicking the icon associated with telometric.jar. If this does not work, or if you're using a Linux machine, you may start Telometric by going to the command line, switching to the directory that contains telometric.jar, and typing java -jar telometric.jar. At this point you should see Telometric's main interface (See Figure 2.1). Note that the only toolbar buttons that are active are those that allow you to 'open an image' and 'exit'. Other buttons will become active when the appropriate prerequisites have been met.

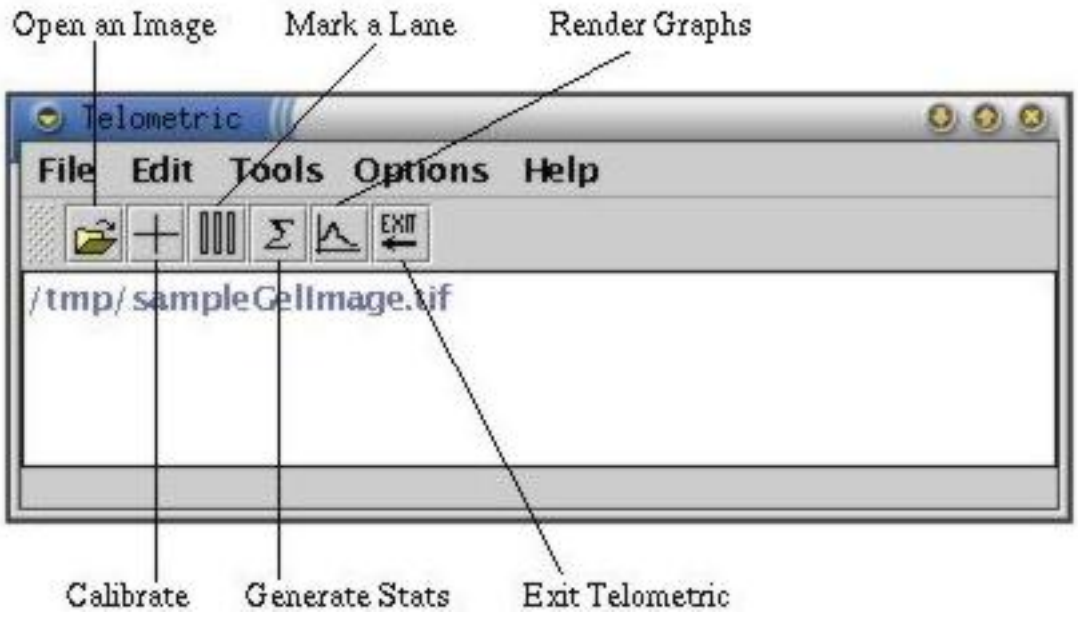


Figure 2.1: Telometric's Central User Interface

2.3 Loading an Image

To load an image, click the 'Open Image' button on the toolbar, or go to File, Open Image. Telometric will open all file types supported by the Java Advanced Imaging package. See http://java.sun.com/products/java-media/jai/forDevelopers/jaifaq.html for more information regarding supported file formats.

2.4 Calibrating the Image

Telometric's calibration feature enables you to calibrate the image using markings on the image corresponding to distances traveled by known molecular weight (MW) standards. See Figure 2.2 for example calibration markings.

To calibrate the image, click the 'Calibrate' toolbar button and click the mark corresponding to the largest MW standard. You will be prompted to enter the MW of the standard in units of your choosing (See Figure 2.3). After entering the MW and clicking OK you may click on the remaining standard markers in descending order of MW and enter the MW for each. After you've entered the MW of the smallest standard, click the 'Calibrate' toolbar button a second time. You should see a window similar to that shown in Figure 2.4. You may now proceed to mark the lanes.

2.5 Outlining Lanes

By outlining lanes you tell Telometric which portions of the image to analyze. Thus, it is up you to specify lane locations and to specify which portion of each lane that you want analyzed.

You will automatically be in lane marking mode once the image as been calibrated and you've clicked the 'Calibrate' button a second time (see previous section, 'Calibrating the Image). To mark the first lane, move the cursor over the image and, with the left mouse button pressed, stretch an active region rectangle over the desired portion of the first lane that you wish to outline. Once you are satisfied with the size, shape, and location of the this region, click the 'Mark a Lane' button or press the spacebar. To outline each additional lane, press the left mouse button and move the automatically generated outline to the desired location then mark the lane by clicking the 'Mark a Lane' button or pressing the spacecbar. Figure 2.2 shows an image with two outlined lanes. Once you've outlined all desired lanes, you may generate plots and statistics. However, you may first want to specify a background region.

2.6 Specifying Background Grayscale

While proper implementation of Telometric's background subtraction feature can significantly improve results, it's use is optional and may result in highly inaccu-

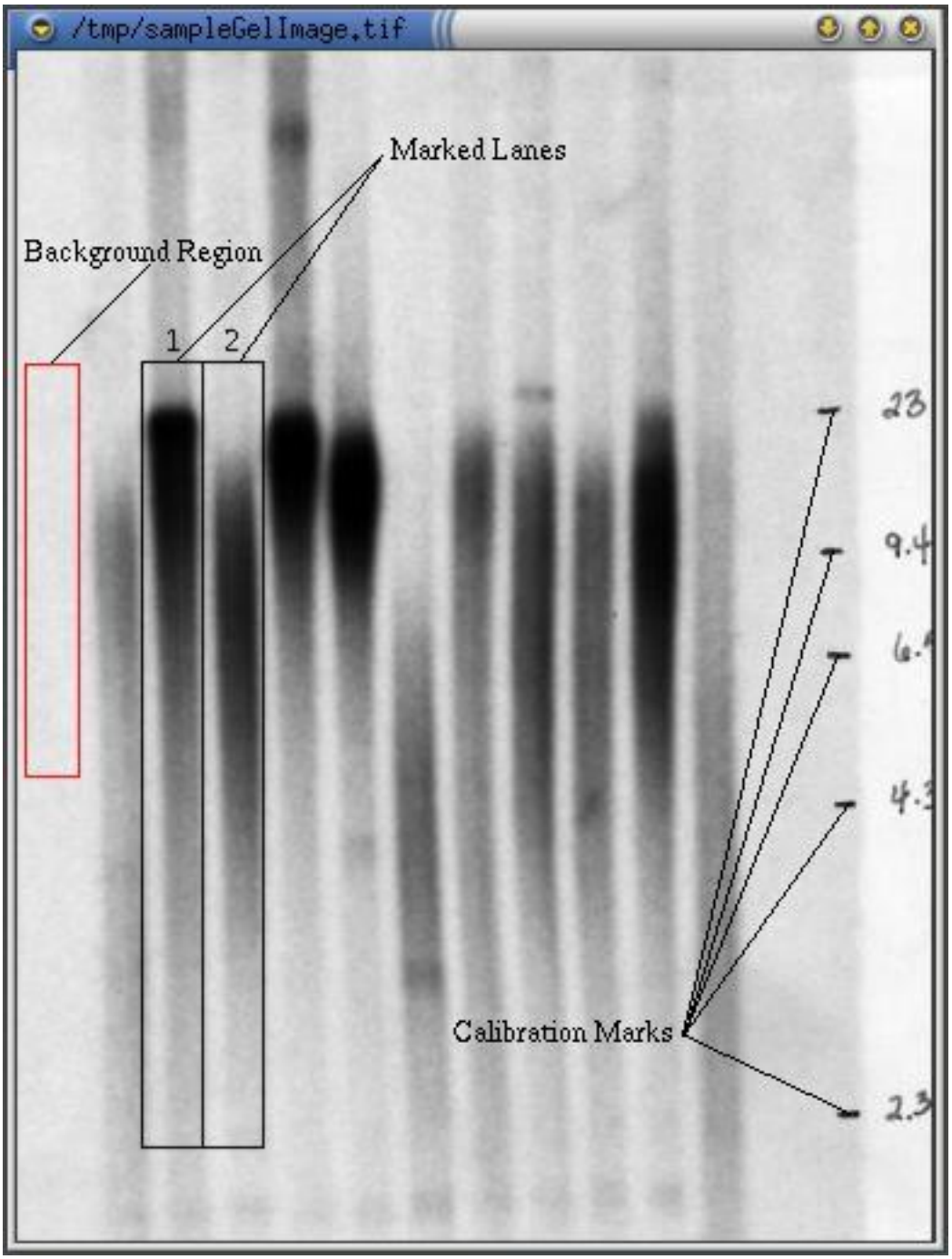


Figure 2.2: Sample Image

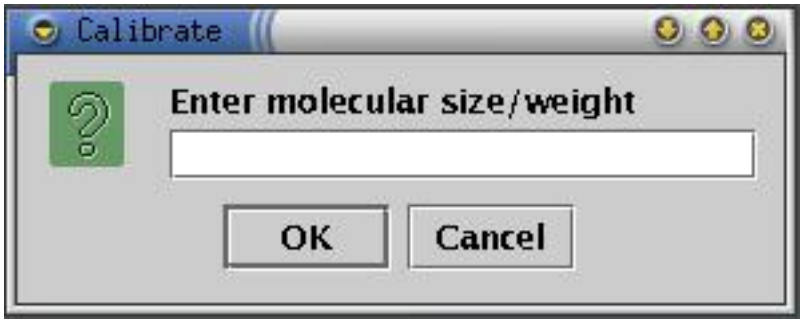


Figure 2.3: Calibration Input Dialog Box

Figure 2.4: Calibration OK Dialog Box

rate plots and statistics if the background region is poorly selected. Thus, proper use of this routine requires much care and good judgment.

To use the background subtraction feature, go to 'Tools' and then select 'Remove Background'. Using the cursor, outline an image region that you have excellent reason to believe contains a representative amount of background grayscale (See Figure 2.2 for a representative background region). After releasing the mouse button you should see a window like the one in Figure 2.5. You may specify multiple background regions. However, the last specified region will be the only one that affects subsequent calculations.

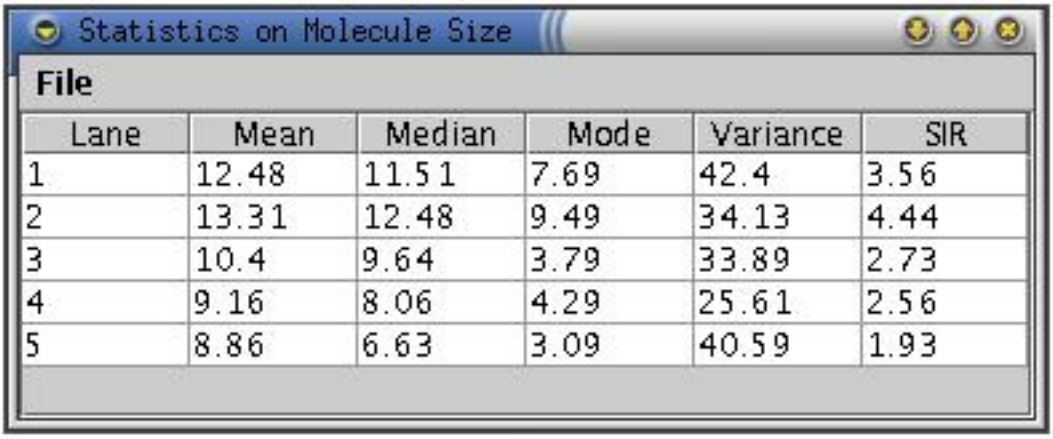
Figure 2.5: Background Subtraction OK Dialog Box

2.7 Specifying Probe Hybridization

Under the 'Options' menu you specify whether probe hybridization was performed to Duplex Repeats (default) or G-Strand Overhangs.When 'to Duplex Repeats' is selected, Telometric assumes a fixed a probe length, but with the amount of probe attached increasing with increasing molecular weight. When 'to G-Strand Overhangs' is selected, Telometric assumes that the same amount of probe hybridizes to each molecule, regardless of molecular weight.

2.8 Generating Statistics

Clicking the 'Generate Stats' toolbar button will result in the creation of a window containing the following statistics for each outlined lane:



Lane	Mean	Median	Mode	Variance	SIR
1	12.48	11.51	7.69	42.4	3.56
2	13.31	12.48	9.49	34.13	4.44
3	10.4	9.64	3.79	33.89	2.73
4	9.16	8.06	4.29	25.61	2.56
5	8.86	6.63	3.09	40.59	1.93

Figure 2.6: Sample Statistics Table

- $Mean =$ mean telomere length
- $Median =$ median telomere length
- $Mode =$ most frequently occurring telomere length
- $Variance =$ variance of telomere lengths
- $SIR =$ semi-interquartile range of telomere lengths

Statistics may saved in a tab-delimited file by clicking 'File' on the Statistics window, and then clicking 'Save Stats'. See Appendix A for more detail concerning statistical calculations. Figure 2.7 shows a sample statistics table.

2.9 Generating Plots

Clicking the 'Render Graphs' toolbar button will create two figure windows. The 'Grayscale Profiles' window contains plots of grayscale intensity versus vertical distance (top to bottom) for each outlined lane. The 'Copy Number Profiles' window contains plots of relative frequency versus molecule size for each outlined lane. See Figure 2.6 for a sample copy number profile.

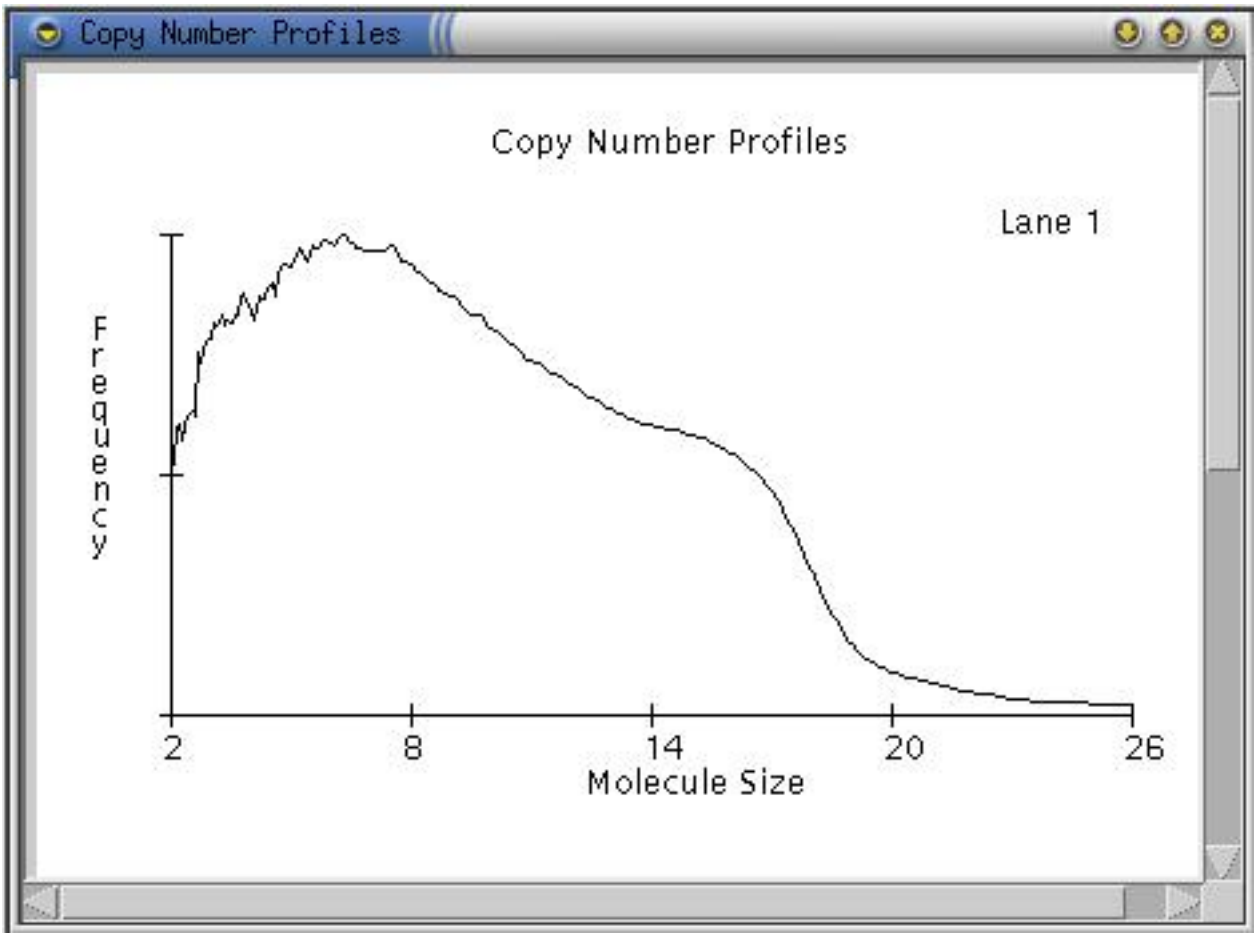


Figure 2.7: Sample Copy Number Profile

Appendix A

Statistical Calculations

Telometric obtains telomere length (TL) data by first obtaining the average grayscale intensity for each row of pixels in an outlined lane. These intensities are then used to obtain a relative frequency for each TL. Since the TL spacing may be non-uniform, a second data set of relative frequencies is generated using linear interpolation at uniformly spaced TL intervals. The statistics are then calculated as follows:

$$Mean = \sum_{i=1}^{n} \frac{\gamma_i L_i}{\sum_{i=1}^{n} \gamma_i}$$

$$Median = L_j \text{ such that } \sum_{i=1}^{j} \frac{\gamma_i}{\sum_{i=1}^{n} \gamma_i} = \frac{1}{2}$$

$$Mode = \max_i \frac{\gamma_i L_i}{\sum_{i=1}^{n} \gamma_i}$$

$$Variance = \sum_{i=1}^{n} \frac{\gamma_i (L_i - Mean)^2}{\sum_{i=1}^{n} \gamma_i}$$

$$SIR = \frac{P_{75} - P_{25}}{2}$$

where n is the total number of uniformly spaced TL points, L_i is the i_{th} TL, γ_i is the relative frequency of L_i, P_{75} is the TL at the 75th percentile, and P_{25} is the TL at 25th percentile.