Telometric User's Manual

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1 December, 2001

Contents

1 Introduction **3**
1.1 About Telometric 3
1.2 Software and System Requirements 3

2 Using Telometric **4**
2.1 Brief Overview 4
2.2 Getting Started 4
2.3 Calibrating the Image 5
2.4 Specifying Background Grayscale 5
2.5 Outlining Lanes 8
2.6 Generating Plots 8
2.7 Generating Statistics 10

A Statistical Calculations **11**

List of Figures

2.1 Sample Image 6
2.2 Calibration Input Dialog Box 7
2.3 Calibration OK Dialog Box 7
2.4 Background Subtraction OK Dialog Box 8
2.5 Sample Plots Window 9
2.6 Sample Statistics Window 10

Chapter 1

Introduction

1.1 About Telometric

Telometric is a set of NIH Image macros designed to measure telomere lengths by analyzing autoradiograph images of agarose gels. Telometric may be used to generate plots and statistical measures of telomere length frequency distributions.

1.2 Software and System Requirements

Telometric runs under NIH Image V1.61 or higher and is available for download from http://bioinformatics.fccc.edu/Software/OpenSource/Telometric. NIH Image, available for download from http://rsb.info.nih.gov/nih-image, requires Macintosh System 7.0 or later and at least 4MB of free RAM.

Chapter 2

Using Telometric

2.1 Brief Overview

Telometric consists of the following collection of macros:

- Calibrate Image
- Specify Background
- Outline First Lane
- Outline Next Lane
- Generate Plots
- Generate Statistics

Execution and usage guidelines for each macro will be presented in the remaining sections of this chapter.

2.2 Getting Started

First, you need to make sure that Telometric is available on your Mac's file system. If not, obtain a copy of Telometric (you may download a copy from http://www.fccc.edu/research/software and make it available to your Mac, e.g. put a copy on the hard drive. Next, you need to get NIH Image up and running. Once you've done this, go to the 'Analyze' menu and select 'Options'. This should bring up a settings window. Make

sure that 'User1', 'User2', and 'Angle' are selected and that 'Max Measurements' is set to at least 2000. Click OK. If you made any changes in the settings window you will be prompted that you need to close-out NIH Image and restart before the changes will be realized. If necessary, please do so. Now you're ready to load an image and the Telometric routines. To load an image, go to 'File' and open or import the autoradiograph of your choice. To load Telometric, go to 'Special' and click 'Load Macros'. Browse the file system, find Telometric, and load it. If all has gone well, you are now ready to use Telometric to calibrate the image.

2.3 Calibrating the Image

The 'Calibrate Image' macro enables you to calibrate the image using markings on the image corresponding to distances travelled by known molecular weight (MW) standards. See Figure 2.1 for example calibration markings. The 'Calibrate Image' macro must always be run before any of the other Telometric routines, excluding 'Subtract Background'. Otherwise, you will get an error message and macro execution will terminate.

To calibrate the image, start the 'Calibrate Image' macro and click the mark corresponding to the largest MW standard. You will then be prompted to enter the MW of the standard in units of kilobase pairs (See Figure 2.2). Do this, then click OK. Next, 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, press the 'option/alt' key. You should then see a window similar to that shown in Figure 2.3. If all appears well, click OK and move on to the next Telometric routine.

2.4 Specifying Background Grayscale

In addition to NIH Image's built-in methods, Telometric contains a routine for subtracting background grayscale. While proper implementation of this routine can signficantly improve results, it's use is optional and may result in highly inaccurate plots and statistics if the background region is poorly selected. Thus, proper use of this routine requires much care and good judgement.

To use Telometric's background subtraction, select an image region that you have excellent reason to believe contains a representative amount of background grayscale, then run the 'Specify Background' macro. You should then see a win-

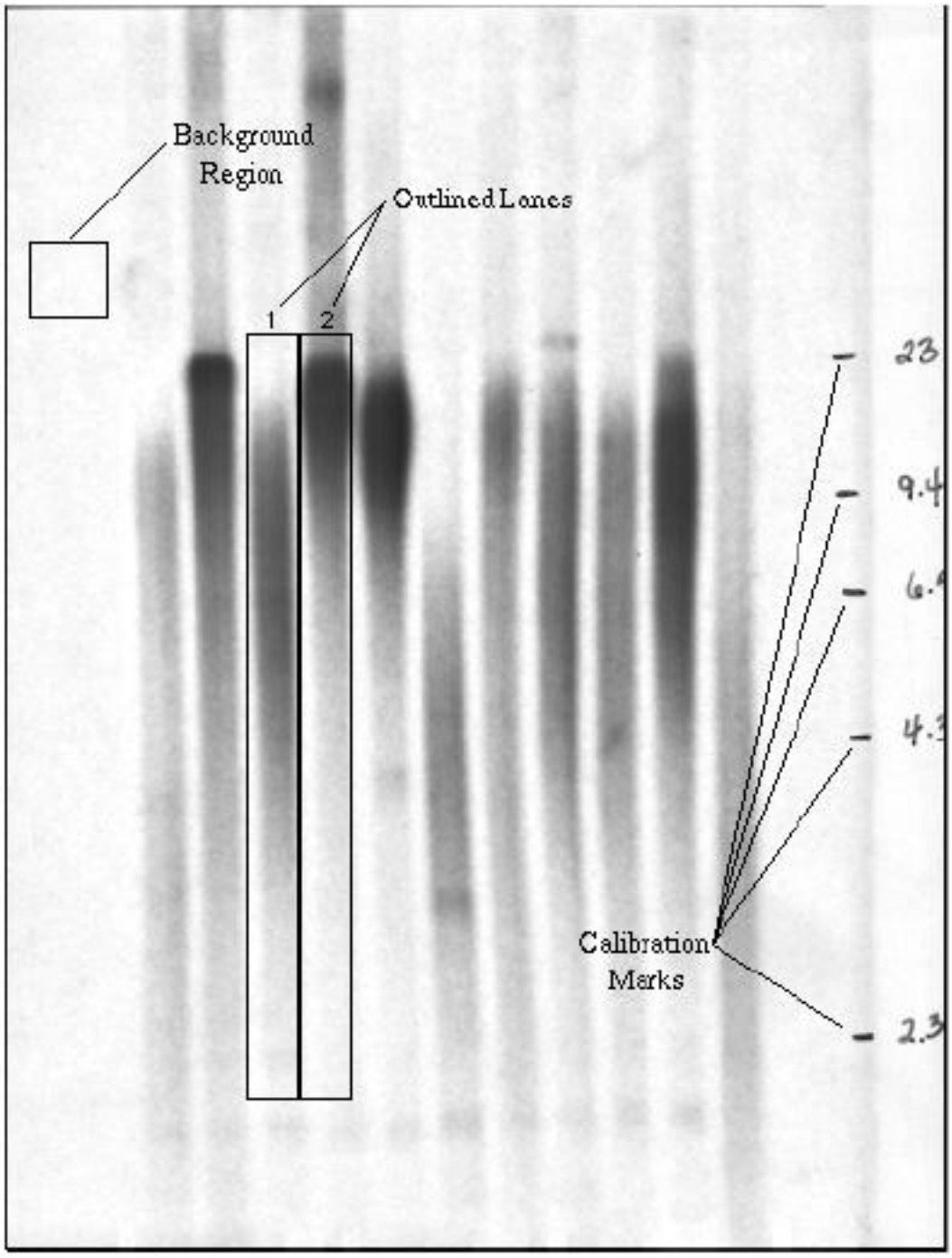

Figure 2.1: Sample Image

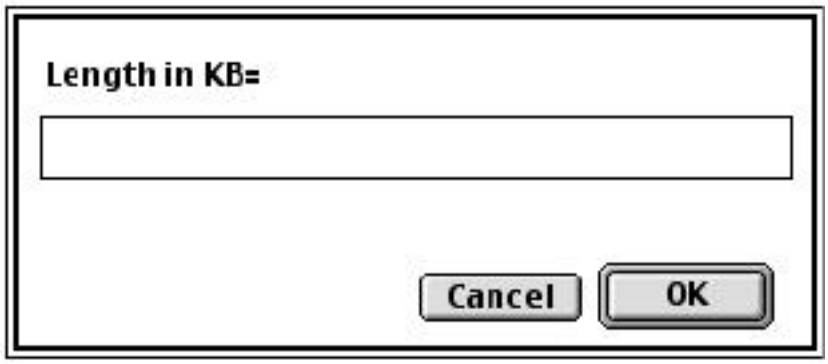

Figure 2.2: Calibration Input Dialog Box

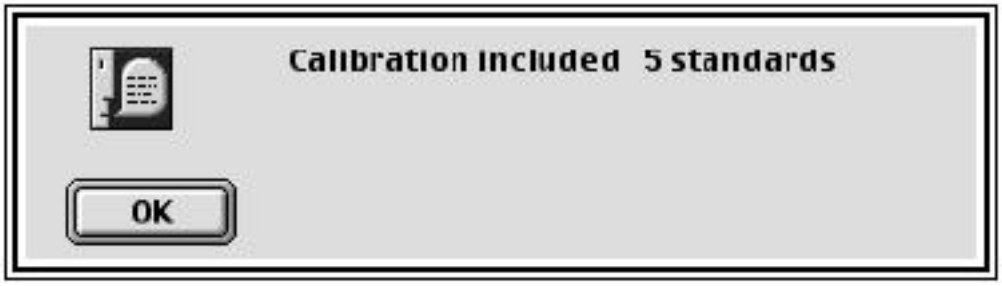

Figure 2.3: Calibration OK Dialog Box

dow like the one in Figure 2.4. A background region may be selected multiple times, with the final selection being the only one that affects subsequent calculations.

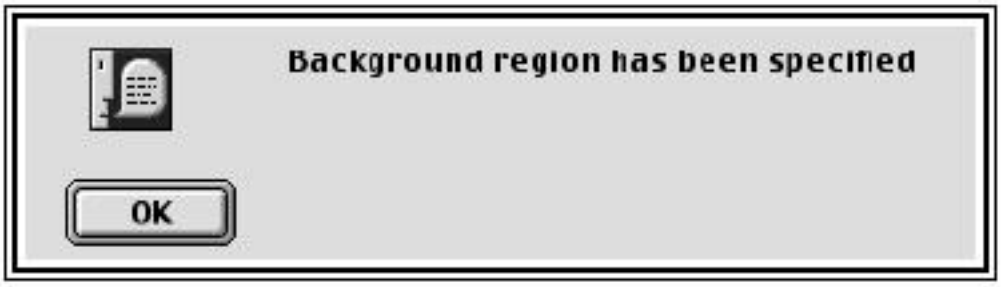

Figure 2.4: Background Subtraction OK Dialog Box

2.5 Outlining Lanes

By outlining lanes with 'Outline First Lane' and 'Outline Next Lane', 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.

To mark the first lane, click the arrow on the NIH Image pallette, then 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, run the 'Outline First Lane' macro. A new image window titled 'Duplicate' should appear with the first lane outlined. To outline each additional lane, enclose the desired area with an active region and run 'Outline Next Lane'. Once you've outlined all desired lanes, you may generate plots and/or statistics. Figure 2.1 shows an image with two outlined lanes.

2.6 Generating Plots

The 'Generate Plots' macro will create a single window containing a separate telomere length frequency distribution for each outlined lane. A telomere length frequency distribution is a graph of relative frequency (y-axis) plotted against the corresponding telomere length (x-axis). Figure 2.5 shows the plots generated for the two lanes outlined in Figure 2.1.

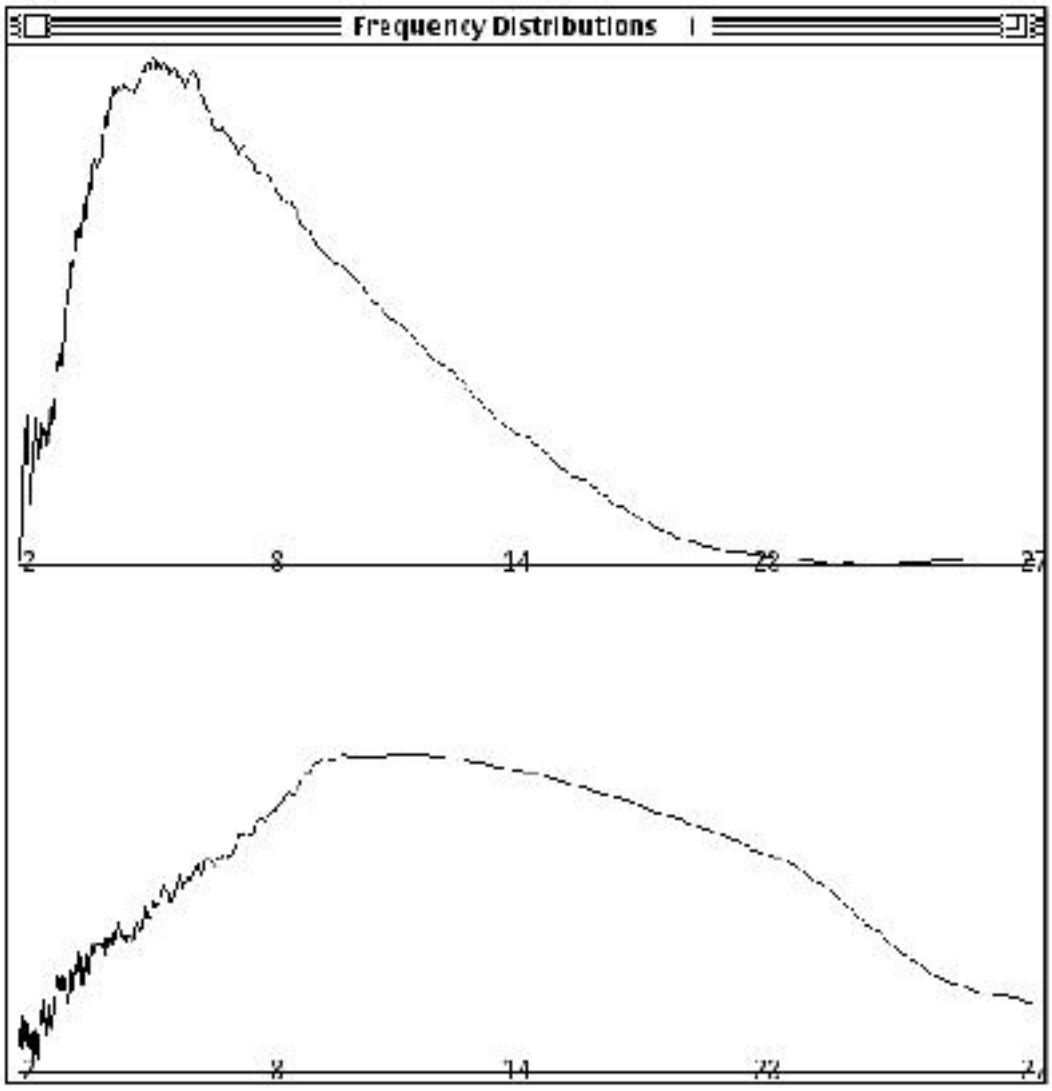

Figure 2.5: Sample Plots Window

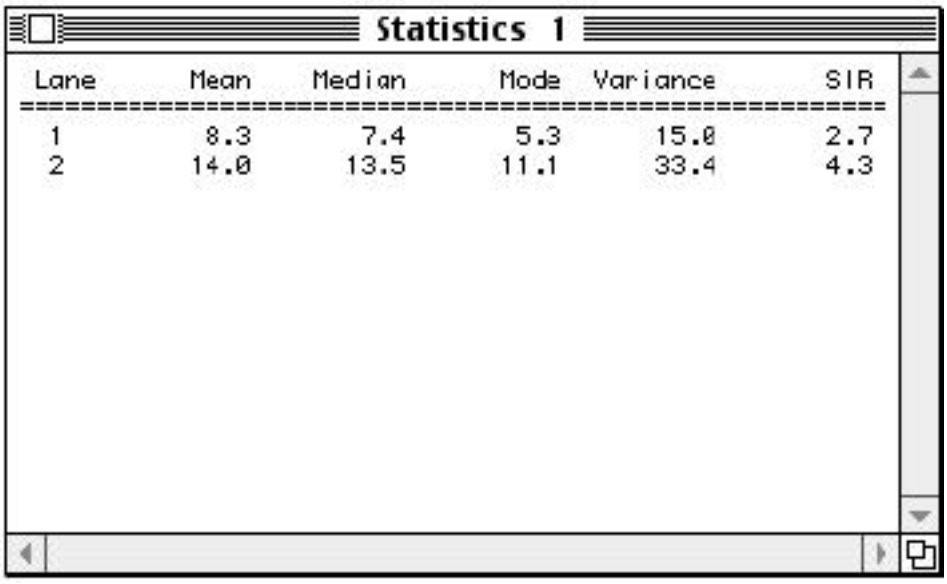

Figure 2.6: Sample Statistics Window

2.7 Generating Statistics

Running the 'Generate Statistics' macro will result in the creation of a window containing the following statistics for each outlined lane:

- $Mean =$ 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

See Appendix A for more detail concerning statistical calculations. Figure 2.6 shows a sample window containing the statistics calculated for the lanes outlined in Figure 2.1.

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.