AN AUTOMATED THRESHOLDING ROUTINE FOR V++

Lloyd Donaldson[1] and Mattias Moëll[2]

[1]Forest Research, Private Bag 3020, Rotorua, New Zealand, email: lloyd.donaldson@forestresearch.co.nz
[2]Center for Image Analysis, Lägerhyddv. 17, 752 37 Uppsala, Sweden, email: mattias@cb.uu.se

We use V++ to measure wood cell dimensions on images from our confocal microscope. We prepare 60 µm thick cross-sectional slices of wood with a microtome, stain with safranin, oven dry the sections and mount them in immersion oil. We then image the sections with a Leica TCS confocal microscope acquiring single optical sections from near the upper surface of the section, at a wavelength of 600 nm. Confocal fluorescence images can be subject to uneven brightness if the section is not flat or is of uneven thickness, due to differential absorption of light coming from the focal plane. During acquisition its important to avoid images with small dim areas as the thresholding procedure will fail with this type of image. We are still trying to develop a routine that will successfully correct this type of illumination problem but at present we find it is easier to control the problem during acquisition of the image (Donaldson & Lausberg, 1998).

Because our images vary in overall brightness we can't use a "typical" threshold to segment the images for object measurement. We also find that manual thresholding yields significant bias among operators and is time consuming. We have therefore developed an automated routine for thresholding the images without any user input, by measuring the average brightness of the cell walls in the image. We have found that the optimum threshold varies in proportion to the average brightness. Our thresholding routine uses a simple regression equation to calculate the optimum threshold from the average brightness of the image. Details of how we measured the relationship between average brightness and optimum threshold will be published elsewhere (Moëll & Donaldson, in preparation). It is possible that this relationship may vary depending on the content of the image, but it appears to be robust for our wood images.

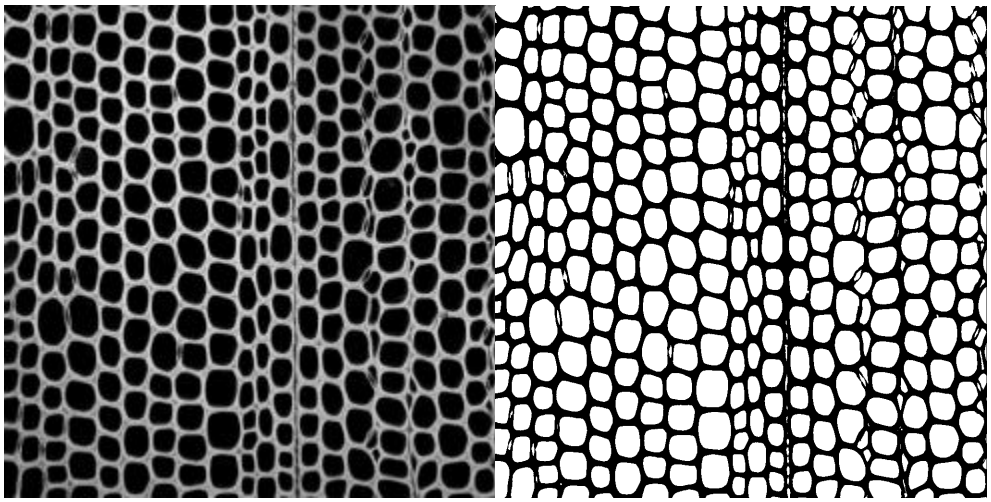

Fig. 1. A typical confocal slice of radiata pine and its binary image segmented using the average brightness threshold. The average brightness of the cell wall object in the image is 120 grey levels and the optimum threshold is 69 grey levels.

Average brightness is calculated by excluding the majority of the dark background pixels using an arbitrary limit of 5 grey levels as follows:

```
{Calculate average brightness}
Img2 := ( Img>5 ) ;
Img3 := Mask( Img,Img2 ) ;
Sum := SumOf( Img3 ) ;
{Count number of pixels >5}
Sum2 := SumOf ( Img2 ) ;
{Calculate new average}
CWAvg := (Sum/Sum2) ;
```

Where "Img" is the image to be segmented. We then calculate the optimum threshold as follows:

```
{Calculate the deviation}
Dev := ( 3.165+(0.606*CWAvg)+(-0.00174*(CWAvg*CWAvg)) ) ;
{Threshold the image}
BinImage := ( Img<=(CWAvg-Dev) ) ;
Show( BinImage,'Binary' ) ;
Writeln( CWAvg ) ;
Writeln( Dev ) ;
```

The binary image can then be measured and the data sent to Microsoft™ Excel for further processing.

Table 1. Typical cell dimensions for radiata pine earlywood.

Mean wall thickness	3.7	µm
Tangential diameter	26.8	µm
Radial diameter	33.3	µm
Lumen area	773.5	μm^2
Cell area	1244.4	μm^2
Wall area	470.9	μm^2
Coarseness	0.7	
Density	567.6	kgm^3
Perimeter (lumen)	105.3	µm
Perimeter (wall)	141.1	µm
Circularity	1.2	
Eccentricity	1.3	

We have compared the average brightness technique with a number of other procedures but have found the average brightness procedure to be fast and accurate over a range of image quality. We use this procedure to measure wood quality and, by measuring samples wet and then dry, we can study the shrinkage behaviour of individual cells.

References.

Donaldson, L.A., M.J.F. Lausberg 1998: Comparison of conventional transmitted light and confocal microscopy for measuring wood cell dimensions by image analysis. IAWA J. 19: 321-336.

Moëll, M., Donaldson, L.A. 2000: Comparison of segmentation methods for digital image analysis of confocal microscope images to measure tracheid cell dimensions. IAWA J. (In preparation).

