Measuring Cell Wall Dimensions using the Distance Transform

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Received: 11 November 1994 Accepted: 14 February 1995

A method has been developed to measure various features of cells and cell walls. It offers possibilities for quantitative analysis of microscopic images which were not feasible before. The method is not based on a model and the only assumption required is that the middle lamella is exactly in the middle of the cell wall of two neighbouring cells. Intercellular spaces are handled correctly with this method.

Features can be measured both per individual cell and per frame. It is also possible to measure the variation of the cell wall within a single cell. Hence the variation of the cell wall thickness within cells can be compared to variation between cells.

The method is demonstrated for *Gerbera* but is generally applicable to anatomical studies of plant tissues.

Key words: *Gerbera jamesonii* Bolus ex Adlam, cell wall, image analysis, cultivar, quantitative microscopy, distance transform.

INTRODUCTION

Stem rigidity, i.e. the resistance to bending in the vase, is an important quality factor of cut flowers and therefore plays an important role in sales and export. Bending in the vase is a common postharvest disorder of cut *Gerberas* (*Gerbera jamesonii* Bolus ex Adlam) and can vary from variety to variety (Marousky, 1986). Cell wall thickness of the sclerenchyma fibres around the vascular bundles may play a role in stem rigidity.

The manual measurement of cell wall thickness is laborious, and observations are often limited to a small number of measurements at 'representative' points around the cell (Travis, Murison and Chesson, 1993). Harris, Lowry and Chapas (1981) showed that computer based image analysis is fast, precise and accurate for measuring tissue areas in sections of plant organs.

Travis *et al.* (1993) have developed a model to measure cell wall thickness in *Zea mays* internodes using image skeletonization. This approach has many advantages compared with the manual method. However, the method assumes a hexagonal model with round cells of equal size and a rigid distribution of the cell wall around the cell. It is obvious that in reality such a structure does not exist: e.g. the combination of smaller and larger cells disturbs the structure. Also the assumption of roundness of cells and the rigid distribution of the cell wall around the cell is often violated. An important factor that cannot be handled with the model is the intercellular space. Cell walls from neighbouring cells are not necessarily connected and empty space disturbs the model quite heavily. The consequences of violations of these and other assumptions of the model are not known. Besides, only the mean cell wall thickness is

obtained and not, for example, the variation in cell wall thickness.

The aim of our research is to develop a quantitative and efficient method to measure cell wall dimensions. With this method it will then be possible to test the hypothesis that differences in stem rigidity of cultivars of *Gerberas* can be predicted by differences in cell wall dimensions. The method should be suitable for routine application in variety testing to predict stem rigidity.

SLIDE PREPARATION AND IMAGE RECORDING

Tissue sources and preparation

Two cultivars which differ strongly in stem rigidity, were used in this experiment. 'Mickey' has good stem rigidity and 'Tamara' poor stem rigidity. Plants were grown in the greenhouse under conditions ensuring normal growth. Of each cultivar five scapes were harvested when the two outer rows of disc flowers were at anthesis. A scape of *Gerbera* is a leafless, determinate stem, capped by an inflorescence. Segments were cut from the stem at 15 cm below the flower head, since this is the location where bending occurs in most cases. The segments were fixed in 2% formaldehyde and 2.5% glutaraldehyde in 0.05 M potassium phosphate buffer pH 7 at room temperature overnight. The fixed segments were then rinsed, dehydrated in a graded series of ethanol and embedded in Technovit according to the manufacturer's instructions.

Transverse sections of 5 µm thickness were cut from the embedded segments using a Reichert microtome with steel knife. The sections were dried onto ethanol-cleaned slides

FIG. 1. For legend see facing page.

using a hotplate at 80 °C. Sections were stained in zinc chloride/iodine reagent according to Gerlach (1984).

Image recording

The slides were studied with a Zeiss AxioPhot microscope using a $\times 40$ Plan-Neofluar lens. For each stem the sclerenchyma fibres were studied at four different locations on the ring of vascular bundles. Directly connected to the

microscope was a JVC 3-CCD colour video-camera (model KY-F30CI). The signal of the camera was digitized by an Imaging Technology Colour-Frame-Grabber into a 24-bit full-colour image consisting of three two-dimensional images, a red, a green and a blue image with 8 bits each. Each image consisted of 256 square pixels in both the horizontal and vertical direction. The size of a pixel corresponded with $0.43 \mu\text{m}$ (calibration-factor). Each pixel was assigned a brightness level which represented the

FIG. 1. Grey level image of sclerenchyma tissue of *Gerbera* scape (A). After segmentation a binary image is obtained, showing the cells and intercellular spaces (B). The intercellular spaces are separated from the cells by their size and user-interaction (C). The intercellular spaces are added to the cell wall (D). The distance transform of image d (E). The skeleton obtained from the distance transformed image. Non border SKEPs are indicated by arrows (F). The gaps caused by pits are closed by dilation of the endpoints (G). An anchor skeletonization is performed on image g using the SKEPs as a mask (H). Image h is added to image f (I). The peripheral skeleton branches are removed by re-skeletonization without preserving SKEPs (J). The intercellular spaces are added to the skeleton (K). By multiplying the outer-contour of k with the distance transformed image e, a cell wall thickness image is obtained (L).

brightness or intensity of the image at that point where 0 was black and 255 bright. The red image had sufficient contrast and therefore the green and blue image were discarded. The red image will further be considered as a grey

level image (Fig. 1A). The image was stored on disk and analysed on a SUN-Sparcstation II computer. The image processing and analysis was controlled by the software package SCIL-Image (TPD-TNO, Delft, Netherlands).

SEGMENTATION OF CELLS, CELL WALL AND INTERCELLULAR SPACES

The EM-algorithm

The grey level histogram of the grey level image 1a (Fig. 1A) showed two distinct peaks (Fig. 2A). One fraction belonged to cell wall (low grey levels) and the other to cells and intercellular spaces (high grey levels), showing a considerable overlap. The Expectation-Maximization-algorithm (EM) (Jansen, Reinink and van der Heijden, 1993) was used to estimate the fraction of cell walls from this histogram. This procedure is based on parameter estimation in mixtures of normal distributions by maximum likelihood. An advantage of this method is that proper estimates of fractions can be obtained even when the grey level distributions of the components overlap considerably.

The grey level variance of the cell wall fraction was significantly larger than the corresponding variance of the other fraction. If two fractions with unequal variance were fitted to the histogram, a large number of pixels between the two peaks was assigned to the fraction with the highest variance (in this case, the cell wall) (Fig. 2B). Therefore a third fraction was fitted to account for the pixels which partially belong to cells (or intercellular spaces) and partially to the cell wall. These pixels were at the transition site of cells (intercellular spaces) and cell wall. This intermediate fraction showed a more or less uniform distribution in the histogram, but could nevertheless satisfactorily be described by a normal distribution (Fig. 2C).

The image was segmented by assigning each pixel to the component with the highest conditional probability for its particular grey level. The pixels of the intermediate fraction were assigned to the cell-component. Hence from grey level image 1a (Fig. 1A), binary image 1b (Fig. 1B) was obtained with value 0 for the component containing cell walls and value 1 for the component containing cells and intercellular spaces.

Separation between cells and intercellular spaces

Figure 1B was labelled, i.e. each connected component in the image was considered as a separate object which contained a unique label; each object in the labelled image could be assessed separately. An object was either an

intercellular space, a single cell or sometimes a cluster of (two) cells connected by a pit in the cell wall. The intercellular spaces were in general smaller than cells. If the area of an object was less than 150 pixels, corresponding with approximately $28 \mu\text{m}^2$, it was in most cases an intercellular space. If it was larger it was in most cases a cell. The limit of 150 pixels was used as a threshold for classification. This resulted in a misclassification of about 2% of the objects. The use of additional shape features did not significantly improve the classification.

To correct for the misclassification, we chose user-interaction at this stage. On the screen four images were displayed: the original grey-value image 1a (Fig. 1A), the labelled image containing all objects, and two additional images: one containing all objects larger than 150 pixels, and one with the smaller objects. By pointing and clicking with a mouse in the labelled image, objects were toggled from the image containing the cells to the image containing intercellular spaces, and *vice versa*.

The image with intercellular spaces was transformed into a binary image 1c (Fig. 1C). This image was added to the inverse of image 1b (Fig. 1B), using a logical OR. The result was the combination of cell wall and intercellular spaces and was stored in 1d (Fig. 1D).

CELL WALL THICKNESS

Distance transform

Distance transforms are a tool in image processing, with applications in skeletonization, medial axis transformation, convex hull extraction, clustering, matching and robot path finding. A distance transform is an operation which calculates at each pixel of an image the distance to the nearest pixel of a reference set (*in casu* the cell), distance being defined as minimum path length. The distance transformation replaces each pixel of the cell wall with an estimate of its shortest distance to the cell.

The distance transform can be computed in several ways, differing in computing time, memory requirements and relative error (Mullikin, 1992). For reasons of speed and memory requirements, we have used the 5-7-11 chamfer distance transform, dividing the results by 5.02 for an unbiased approximation of the Euclidean distance (Verwer,

FIG. 2. The histogram of a grey level image (A). The estimated distribution curves overlaid on the histogram using two fraction with unequal variance. The arrow under the histogram indicates the threshold (B). Three fractions with unequal variance are fitted (C).

1991). The distance transform of Fig. 1D was stored in Fig. 1E.

Distance skeleton

From Fig. 1E a line of single pixels was constructed forming a topological representation of the cell-wall: the distance skeleton Fig. 1F. A skeleton should lie approximately along the centre of each line-like part and satisfy, as nearly as possible in the discrete space, the definition of a line as 'that which has length without breadth' while still retaining the connectivity of the original (Hilditch, 1969). A skeleton operation is often referred to as a thinning operation, since many skeleton algorithms peel off layer by layer until a one-pixel thick line in the middle remains.

Since the skeleton corresponded with the middle of the cell wall, it represented the border between cells, dividing the intercellular spaces over neighbouring cells. Pits and inclusions in cells did however sometimes cause some imperfections in the skeleton.

Beautifying the skeleton

Ideally all points of the skeleton of the cell wall have at least two neighbours. Nevertheless some points in the skeleton image had only one neighbour. These points will be called skeleton endpoints (SKEPs). Most of these points were near the border, where cells did not fit inside the image frame. However more SKEPs occurred in the image. The cause was twofold: some SKEPs were caused by pits; others were caused by inclusions or artefacts in the cell, erroneously classified as cell walls. SKEPs caused by pits, had to be connected; SKEPs caused by inclusions, and the curves connected to them, had to be removed.

It is reasoned that SKEPs caused by pits occur in pairs, lying at opposite sides of the pit. The distance between a pair of SKEPs was in general smaller than 10 pixels (depending on magnification). SKEPs caused by cell-particles were in general single. This feature was used for their distinction.

The SKEPs were identified by a local hit-or-miss transform. All SKEPs were dilated five times using 4/8-connectivity. Dilations of SKEPs which were close together (less than 10 pixels apart), touched each other and were thus combined into a single object. The result is shown in Fig. 1G. After the dilations, an anchor skeletonization was performed. This is a special variant of skeletonization: during each thinning cycle anchor points are forced to be skeleton pixels, even if they do not meet the conditions (Verbeek and Duin, 1987). The SKEPs were used as the anchor points and the skeleton was 'anchored' through these points. If a dilated SKEP did not touch another dilated SKEP, the result of the skeletonization was the single original SKEP. If two dilated SKEPs did touch, the result was a line connecting the SKEPs. The resulting Fig. 1H contained single SKEPs and lines connecting pairs of SKEPs. Figure 1H was added (logical OR) to the skeleton image (Fig. 1F) resulting in Fig. 1I, in which the local loss of connectivity caused by pits was restored.

Now the remaining peripheral skeleton branches had to be pruned. This was done by a skeletonization of Fig. 1I without preserving endpoints (Hilditch, 1969), resulting in skeleton (Fig. 1J). The skeleton was in most cases a correct representation of the border between cells. However in some images connecting or removing curves by hand (mouse), was still necessary. Therefore user-interaction was also allowed at this stage.

Moving the skeleton outside the intercellular space

In Fig. 1J all cells were separated from one another by a completely connected skeleton. The skeleton can be regarded as the border of each individual cell. However, since the skeleton runs through intercellular spaces, it does not exactly coincide with the outer-border of a cell wall. To obtain a skeleton which indeed represents the outer-border of each individual cell wall, the following operations were performed.

The intercellular spaces were added to the skeleton using a logical OR of Fig. 1J and Fig. 1C, resulting in Fig. 1K. The contour pixels of Fig. 1K now coincided with the outer-borders of the cell wall of each individual cell.

Cell wall thickness image

By dilating Fig. 1K one step (8-connectivity), followed by a logical XOR of Fig. 1K and its dilated image, an image was obtained in which all the individual cells with their cell walls were separated from each other. The result was an image containing the contour of each individual cell, which corresponded to the outermost part of the cell wall. Multiplication of this image with the distance transformed Fig. 1E resulted in Fig. 1L. This image, containing the distance values at the contour of the objects, is called a cell wall thickness image. It contained the shortest distance of the cell wall to the inner-part of the cell, i.e. the local cell wall thickness. Since the image had a distance value of zero at the location of pits, pixels at pits could not be discriminated from non-contour pixels. This is an undesirable property for calculation of features. Therefore, the value one was added to Fig. 1E before multiplication. Now, all contour pixels had a value of one or higher, and all non-contour pixels had a value of zero.

Besides an overall cell wall thickness image, it was also interesting to have a cell wall thickness image for each individual cell. Therefore the inverse of Fig. 1K was labelled using 4-connectivity. Using 8-connected labelling would result in a single object, since the skeleton was 8-connected and hence all the cells touched each other (4-8 connectivity paradox). For each object the distance-values at the corresponding location in Fig. 1E were copied to a separate image. Also in this case the distance values were incremented by value 1. Now the same procedure was applied as described above and repeated for all objects. Since information on cells touching the border of the image was incomplete, these cells were discarded.

Notice that the value 1 was added to the distance-values, to discriminate the contour pixels from the rest of the image

and that the distance values were obtained using the 5-7-11 distance transform. Hence the local cell wall thickness was (distance value - 1)/5.02 pixels (Verwer, 1991).

FEATURE EXTRACTION

Cell wall thickness features

Due to the discrete nature of digital images, distance transformed pixels can have only a limited number of values. Therefore a histogram can be obtained, containing the number of pixels per distance (bin). A histogram can be obtained from the cell wall thickness image (Fig. 1L) or from an image containing an individual object. From this histogram, various features were derived (Table 1).

C , the total pixel-count, corresponds with the perimeter of cell walls. A more accurate estimate of the perimeter is the corner-count method as described by Vossepoel and Smeulders (1982). S , the total sum of distance-values, is related to the amount of cell wall present. However, the total cell wall area per cell is better estimated by counting the pixels of a cell wall than by using S .

A can be used as an estimate of the mean cell wall thickness. Another estimate for mean cell wall thickness is R_{50} (the median). R_{50} is less influenced by outliers than A and may therefore be preferable, since outliers are quite common. Variation of cell wall thickness can be estimated as the variance V of the histogram. For estimation of the standard deviation of the cell wall thickness, the square root of V can be used.

Of course, the bin corresponding with value zero has to be discarded from the histogram, since this bin contains the pixels not lying on the contour of the cell wall.

Cell and cell wall features

A simple but important feature is the number of cells in a frame, since a large number of small cells with thin cell walls may have the same amount of cell wall material as a few large cells. Therefore the mean cell wall thickness has to be studied in relation to the number of cells.

From each individual object, features like area, perimeter,

TABLE 1. Several features which can be extracted from a histogram. The number of bins in the histogram is n_{bins} , the distance value is $f(i)$ and the number of pixels having distance value $f(i)$ is $n(i)$

Count = $C = \sum_{i=1}^{n_{bins}} n(i)$
Sum = $S = \sum_{i=1}^{n_{bins}} n(i)f(i)$
Aver = $A = \frac{S}{C}$
Variance = $V = \frac{\sum_{i=1}^{n_{bins}} n(i)[f(i) - A]^2}{C}$
Median = $R_{50} = f(k) : \left(\sum_{i=1}^{k-1} \frac{n(i)}{C} < 0.5 \right) \wedge \left(\sum_{i=1}^k \frac{n(i)}{C} \geq 0.5 \right)$

shape-factor, bending energy, eccentricity and sphericity can be measured (Van de Vooren, Polder and van der Heijden, 1992). An object can be either the interior of a cell, the cell wall (exterior), or their combination. The combination of cell and cell wall is present in the inverse of Fig. 1K. The cell without cell wall can be obtained from the image with local cell wall thickness per individual object, by saving only the pixels having value one. The cell wall can be obtained by saving the pixels with a value higher than one. This does not necessarily yield a connected cell wall. If this is wanted, the contour of the combination of cell and cell wall can be added.

RESULTS

Of the 40 images (two cultivars of Gerbera, five stems each, four locations per stem), the fraction of cell wall was estimated, using the EM-algorithm. Also, all the histogram based features listed in Table 1 were calculated (see Table 2), both per image (40 values per feature) and per cell (1644 values per feature). Figure 3 shows that the variation within a stem is smaller than the variation within a variety. This clearly illustrates that observations per stem were not independent. Hence we used stem averages. This resulted in five means of each of the two cultivars. These averages were analysed using a one-way analysis of variance.

The cultivar 'Mickey' had significantly more cell wall, which supports the hypothesis that more cell wall increases the stem rigidity (feature EM and S). Also the cell wall thickness is larger for 'Mickey' (feature A and R_{50}). The total cell wall length as estimated by C was not significantly different between the two cultivars at the 1% level. In this case the difference in stem rigidity between the two cultivars is probably not due to a difference in total cell wall length. The variance in cell wall thickness (feature V) is significantly different between the two cultivars. This is not surprising since a thicker cell wall (and hence a larger range, since there

TABLE 2. The features described in Table 1 were calculated for total images (such as Fig. 1L) and per individual object. Besides, the fraction of cell wall as estimated by the EM-algorithm is given. The means of the two cultivars for each of the features are given. The probability levels of a two-sided Student t -test are given in the last column, where a low value (< 0.01) indicates a significant difference

Feature	Cultivar		Significance
	Mickey	Tamara	
EM	0.47	0.34	< 0.001
C -image	5800	6502	0.04
C -cell	110	97	0.03
S -image	27568	17891	< 0.001
S -cell	516	262	< 0.001
A -image	4.8	2.8	< 0.001
A -cell	4.6	2.6	< 0.001
R_{50} -image	4.6	2.6	< 0.001
R_{50} -cell	4.5	2.6	< 0.001
V -image	1.6	0.9	< 0.001
V -cell	1.1	0.6	< 0.001

FIG. 3. For each image the cell wall width in pixels (y-axis) and the fraction of cell wall in the total image (x-axis) are plotted. Small characters indicate individual images, capitals indicate stem average, and the cultivar name is the average over all stems, so 't1' is an image of stem 1 of Tamara, 'T1' is the mean of the four images of stem 1 of Tamara, and 'Tamara' is the mean over all five stems of Tamara. This figure clearly shows the difference between the two cultivars, Mickey and Tamara, but also the dependency of the observations within a stem.

are often thin spots like pits in cell walls) will often imply more variation. It can however be an interesting feature if two cultivars have similar thickness but one is more variable in thickness than the other.

It would be interesting to know if only the increase in cell wall accounts for the higher stem-rigidity, or if stems with the same amount of cell wall but with thinner cell walls are more or less rigid. This hypothesis is tested using the amount of cell wall as a co-variable in the analysis of

variance of the features for the complete image. The means for A , corrected for the amount of cell wall per image (EM), are 4.2 for 'Mickey' and 3.3 for 'Tamara', which is not a significant difference at the 5% probability level.

CONCLUSIONS AND DISCUSSION

The image analysis method has evolved during our research. We started with directly calculating the distance transform and the distance transform skeleton from the binary image (Fig. 1B). If no intercellular space is present, this method gives comparable results with the method described here and is certainly faster. However, we encountered such a large proportion of intercellular spaces, that the assumption that the skeleton coincides with the middle lamella was violated. The dramatic effect of an intercellular space of a single pixel on the distance skeleton is shown in Fig. 4. Our first approach was to use a morphological closing operation (Serra, 1982). This operation causes small intercellular spaces to disappear. The result is a small overestimate of the cell wall thickness. For a limited number of intercellular spaces, this is hardly a problem. However, when the number and the size of intercellular spaces increases, the error becomes larger. Our final approach does not suffer from this problem and gives the possibility of deriving cell wall features per individual cell.

Connecting the skeleton at pits in the cell wall was another problem which had to be solved. We chose to close these gaps by a dilation of SKEPs, followed by an anchor-skeletonization. This has evident advantages over the method proposed by Russ (1992), who suggested adding the dilated SKEPs to the skeleton and performing a complete re-skeletonization. He also proposed a watershed segmentation for separation of touching cells. It relies on the fact that eroding the binary image will usually cause touching objects to separate before they disappear. The watershed segmentation however causes over-segmentation. Removal of the newly introduced separation lines is not trivial.

FIG. 4. A representation of the cell wall at the junction of three cells. If no intercellular space is present (A) the skeleton coincides with the middle lamella. If an intercellular space of a single pixel is present (B) the distance transform also considers this pixel as background and hence false distances and skeleton are obtained. The large distance values near the branch of the skeleton in (A) are replaced by a larger number of smaller values (B), hence increasing the cell wall length and lowering the cell wall thickness.

Our method based on the distance transform has a clear advantage over the model-based method proposed by Travis *et al.* (1993). The model requires many assumptions, and the consequences of violations of these assumptions are not known. The only assumption in our method is that the middle lamella, i.e. the border line between the cell walls of two neighbouring cells is exactly in the middle of the cell wall.

Measuring cell wall features automatically has the advantage that numerous cells can be measured in a limited amount of time, hence giving robust and reliable estimates. The time needed for the analysis of a single image, which contained over 40 cells on the average, was about 45 s using a Sun Sparc Station II, including visual inspection and correction. If no user-interaction were needed, the time required for the analysis of an image could be reduced to a few seconds.

When analysing features, we have to be aware of combined effects. For example, if the number of cells is equal, an increase in thickness will correlate with an increase in the amount of cell wall. To study the true effect of thickness we have to correct for the amount of cell wall by using the latter as a co-variable in the analysis.

Since the number of features which can be measured by image analysis is large, the method described in this paper offers good possibilities to increase insight in the morphological aspects of stem rigidity. The features mentioned are only a limited set of the possible features. More features can be extracted from the histogram such as range, modus, energy, entropy and skewness and also many more features regarding size and shape of cells can be measured. Features can be measured on a per cell basis, and the variation for each of the features can be studied over the cells. Also the variation in cell wall thickness within cells can be compared to variation between cells. Depending on the interest of the researcher all kinds of morphological aspects can now be measured, both for cell walls as for cells. The method described here offers possibilities for numerical analysis of microscopic images, which were not feasible before. It might even be possible to apply construction theory to the data to determine the strength of the stem.

The method is demonstrated for stem rigidity, as a major factor in vase life of Gerbera. The data presented here are merely an illustration of the method. The results of the experiment can not be used for firm conclusions on the correlation between stem rigidity and cell wall dimensions.

In that case, larger data sets, including more cultivars, more stems per cultivar and also physiological data on stem rigidity, are necessary. Nevertheless, the experiment shows differences which are in accordance with the hypothesis that more and thicker cell walls increase the stem rigidity.

It is also possible to use the method for other purposes, such as for studies of digestibility or lodging. Its many features make it possible to gain a detailed insight of the relationship between these aspects and the anatomy of the plant.

ACKNOWLEDGEMENTS

We thank Gerda Uenk and Gerda Sabatino for preparation of the slides, Albert Vossepoel for fruitful discussions and Martin Boerma for supplying the Gerberas.

LITERATURE CITED

- Gerlach D. 1984. *Botanische Microtechnik. Eine Einführung*. Stuttgart: Georg Thieme Verlag.
- Harris PJ, Lowry KH, Chapas LC. 1981. Comparison of methods for measuring tissue areas in sections of plant organs. *Annals of Botany* 47: 151–154.
- Hilditch CJ. 1969. Linear skeletons from square cupboards. In: Merzer B, Michie D, eds. *Machine intelligence, vol. 4*. Edinburgh: University Press, 403–420.
- Jansen RC, Reinink K, van der Heijden GWAM. 1993. Analysis of grey level histograms by using statistical methods for mixtures of distributions. *Pattern Recognition Letters* 14: 585–590.
- Marousky FJ. 1986. Vascular structure of the Gerbera scape. *Acta Horticulturae* 181: 399–405.
- Mullikin JC. 1992. The vector distance transform in two and three dimensions. *Computer vision graphics and image processing: graphical models and image processing* 54: 526–535.
- Russ JC. 1992. *The image processing handbook*. Boca Raton, FL: CRC Press.
- Serra J. 1982. *Image analysis and mathematical morphology*. London: Academic Press Limited.
- Travis AJ, Murison DD, Chesson A. 1993. Estimation of plant cell wall thickness and cell size by image skeletonization. *Journal of Agricultural Science* 120: 279–287.
- Van de Vooren JG, Polder G, van der Heijden GWAM. 1992. Identification of mushroom cultivars using image analysis. *Transactions of the ASAE* 35: 347–350.
- Verbeek PW, Duin RPW. 1987. Binary Image Operations. In: *Manual of the special course on image processing for industrial applications*. Delft: CBD, D.1–29.
- Verwer BJH. 1991. Local distances for distance transformations in two and three dimensions. *Pattern Recognition Letters* 12: 671–682.
- Vossepoel AM, Smeulders AWM. 1982. Vector code probability and metrication error in the representation of straight lines of finite length. *Computer Graphics and Image Processing* 20: 347–364.