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(54) **SCORING ESTROGEN AND PROGESTERONE RECEPTORS EXPRESSION BASED ON IMAGE ANALYSIS**

BILDVERARBEITUNGSBASIERTE BERECHNUNG EINES SCORES FÜR DIE EXPRESSION VON ÖSTROGEN- UND PROGESTERON-REZEPTOREN

NOTATION DE L'EXPRESSION DES RECEPTEURS OESTROGENIQUES ET DE LA PROGESTERONE SUR LA BASE D'UNE ANALYSE D'IMAGE

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Description

[0001] This invention relates to a method, an apparatus and a computer program for scoring Oestrogen and Progesterone Receptors expression (ER and PR) in histological image data to provide information for clinical treatment and prognosis. It is particularly (but not exclusively) relevant to providing clinical information for breast cancer management.

[0002] Breast cancer is a common form of female cancer, and it also occurs in the male albeit with lower frequency: Once a lesion indicative of breast cancer has been detected, tissue samples are taken and examined by a histopathologist to establish a diagnosis, prognosis and formulate a treatment plan. However, pathological analysis of tissue samples is a time consuming process. It entails interpretation of images by human eye. In the main this is a reproducible and reliable process but can be subjective. There can be inaccuracies in the observation of the same sample by different observers and even by the same observer at different times. For example, two different observers assessing the same ten tissue samples may give different opinions for three of the slides - 30% error. The problem is exacerbated by heterogeneity, i.e. complexity of some tissue sample features.

[0003] The hormone receptor status of breast cancer cells provides useful information for treatment and prognosis since the presence of some of these receptors can provide a means for controlling cell growth via chemotherapy. In general, cancers in which the cells express ER in their nuclei have a better prognosis and can respond to hormonal manipulation for example using the anti-oestrogen drug Tamoxifen. ER and PR are steroid hormone receptors. ER and PR expression can be observed in many tissue types, and in both tumour and non-tumour material. In the breast they are expressed by normal epithelium and in approximately 70% of breast cancers. They are used in breast cancer assessment because steroid hormone status guides selection of therapy. Almost 75% of breast cancers expressing ER will respond to this therapy, whereas less than 5% not expressing ER will respond. In general, cancers that are ER positive will also be PR positive. However, carcinomas that are PR positive, but not ER positive, may have a poorer response to endocrine therapy. Histological images produced for ER and PR determination are indistinguishable visually, though they are obtained from specimens stained by applying different antibodies to a specimen tumour section. Staining of specimen tissue slides is obtained by using antibodies specific to respective receptors, i.e. one antibody for ER and a different antibody for PR. Because ER and PR are both steroid receptors, their associated staining will be similar and so the same process can be used to extract both.

[0004] US Pat No. 5,202,931 discloses detecting ER and PR by a specimen staining technique using immunoperoxidase followed by counterstaining. Two monochromatic optical filters are used to separate images of stained receptor areas and nuclear areas. Optical density measurements yield intensity values related to the quantity of hormonal receptor in the specimen.

[0005] C Rothman et al. disclose ER evaluation by spectral image analysis in Histology and Histopathology, Vol. 15, pages 1051-1057, October 2000, "Potential Use of Image Analysis of the Quantitative Evaluation of Oestrogen Receptors in Breast Cancer". Specimens were stained by the immunoperoxidase method followed by haematoxylin counterstain. Image pixels were compared with a linear combination of reference spectra. Products of ER spectra with pixel intensity gave net intensity of ER staining.

[0006] Techniques for ER analysis are disclosed by R L Camp et al., in Nature Medicine, Vol. 8, No. 11, November 2002, "Automated Subcellular Localisation and Quantification of Protein Expression in Tissue Microarrays". Image thresholding is first used to create a binary mask (pixels equal to 1 or 0 only) in which pixel value 1 locates a target signal from a tumour. Fluorescent tags are applied to separate tumours from stroma and define subcellular compartments. Distribution of a target antigen is then assessed according to its co-localisation with these tags. Target expression is delineated as the sum of its intensity divided by total size. Another technique involves subtracting a slightly out-of-focus image from an in-focus image, which is said to improve assignment of pixels to a particular subcellular compartment (nucleus, membrane or cytoplasm). Finally, each pixel is assigned to a specific subcellular compartment on the basis of a ratio between two compartment-specific markers.

[0007] Computer-aided detection of breast cancer nuclei in connection with ER and PR is disclosed by F Schnorrenberg et al. in IEEE Trans. Information Technology in Biomedicine, pages 128-140, Vol. 1, No. 2, June 1997. This reference describes the use of an algorithm to process a image of a stained tissue specimen in order to make a histogram of the image bimodal: i.e. the histogram has a first mode indicating cell nuclei and a second mode indicating background. To achieve this it applies to the histogram iteratively a receptive field filter in combination with a non-linear Gaussian difference squashing function, which ensures that detection is predominantly dependent on geometry as opposed to intensity. Detected nuclei are then classified according to staining intensity.

[0008] O. Ferrer Roca et al. disclose ER and PR determination in Analytical Cellular Pathology Vol. 9, pages 151-163, 1995. The paper is entitled "Immunohistochemical Correlation of Steroid Receptors and Disease-free Interval in 206 Consecutive Cases of Breast Cancer: Validation of Telequantification Based on Global Scene Segmentation". Immunohistochemical quantification of images of stained specimens was based on pixel densitometric measurements by area with a 550 nm filter of 20nm bandwidth. The measurements obtained percentage positive area and staining intensity.

[0009] R. J. Sklarew et al. disclose determination of ER and PR in Cytometry (the journal of the society for Analytical

Cytometry of the United States) Vol. 12, page 207-220, 1991, "Comparison of Microscopic Imaging Strategies for Evaluating Immunocytochemical (PAP) Steroid Receptor Heterogeneity". Specimens were not tissue samples but instead obtained from cell cultures. Measurements were made on stained specimens to determine integrated optical density, cell nucleus projected area and cell nucleus perimeter, nuclei being identified by use of a computer mouse to indicate areas of an image of the specimen on a monitor.

[0010] L. True, "Quantitative Immunohistochemical Assay of Estrogen Receptors by Image Analysis", Laboratory Investigation, Vol.56, no.1, 1987, discloses determination of ER expression from imaged tissue samples based on pixel frequencies of each grey level within a "positive" range that are used to calculate an integrated optical density.

[0011] It is an object of the invention to provide an alternative form of objective measurement of ER and PR to support a pathologist's diagnosis and patient treatment.

[0012] The present invention provides an automated method of scoring Oestrogen or Progesterone Receptors expression (ER or PR) from image data obtained from histological specimens stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, the method including obtaining digitised colour image data from a histological specimen by means of a microscope and a camera, **characterised in that** the method also includes using computer apparatus to count image pixels having intensities below a predetermined intensity threshold, to score ER or PR by comparing the number of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR scores, and by assigning a score of such a range in which the number of image pixels appears.

[0013] The invention provides the advantage that it is an objective procedure for scoring ER or PR.

[0014] The image data may be transformed to a different image space having an intensity image plane, the step of counting image pixels with intensities below the predetermined intensity threshold being implemented with pixels in the intensity image plane.

[0015] The step of scoring ER or PR may provide one of two contributions to a composite ER or PR score, the method further including:

- a) determining from blobs in images, the images being based on the obtained image data, a proportion of total blob area which is also brown blob area,
- b) obtaining another of the two contributions to the composite ER or PR score by comparing the brown blob area proportion with predetermined proportions having assigned scores, and
- c) combining the two contributions and obtaining the composite ER or PR score.

[0016] The method may include determining the brown blob area proportion by the steps of:

- a) selecting pixels having intensities below a prearranged intensity threshold and remapping their intensities to increase their contrast,
- b) transforming pixels having intensities at or above the prearranged intensity threshold into a contrast-free background,
- c) converting the remapped image data into thresholded binary images from which total blob area and brown blob area are discernible respectively, and
- d) expressing brown blob area as a proportion of total blob area.

[0017] In a preferred embodiment, the present invention provides an automated method of scoring Oestrogen and Progesterone Receptors expression from image data obtained from histological slides stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, **characterised in that** the method includes:

- a) providing one of two contributions to a composite ER or PR score by counting image pixels having intensities below a predetermined intensity threshold and comparing the number of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR score contribution values,
- b) providing another of the two contributions to the composite ER or PR score from blobs in images, the images being based on the obtained image data, by determining a proportion of total blob area which is also brown blob area, and comparing the brown blob area proportion with predetermined proportions having assigned ER or PR score contribution values, and
- c) combining the two contributions and obtaining the composite ER or PR score.

[0018] The method may include determining a hue for the image data and deriving a correction for the composite ER or PR score obtained from the first and second contributions if the hue indicates a degree of blueness or brownness which renders such correction appropriate.

[0019] In another aspect, the present invention provides an apparatus for automated assessment of histological specimens stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, the apparatus

incorporating a microscope and a camera for use in obtaining digital colour image data from a histopathological specimen, and computer apparatus for processing the image data, **characterised in that** the computer apparatus is programmed to count image pixels having intensities below a predetermined intensity threshold, to score ER or PR by comparing the number of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR scores, and by assigning a score of such a range in which the number of image pixels appears.

[0020] The computer apparatus may be programmed to transform the image data to a different image space having an intensity image plane and to implement the step of counting image pixels with intensities below the predetermined intensity threshold with pixels in the intensity image plane.

[0021] The ER or PR score may be one of two contributions to a composite ER or PR score, the computer apparatus being further programmed to:

- a) determine from blobs in images, the images being based on the obtained image data, a proportion of total blob area which is also brown blob area,
- b) obtain another of the two contributions to the composite ER or PR score by comparing the brown blob area proportion with predetermined proportions having assigned scores, and
- c) combine the two contributions and obtain the composite ER or PR score.

[0022] The computer apparatus may be programmed to determine the proportion of total blob area which is brown blob area by:

- a) selecting pixels having intensities below a prearranged intensity threshold and remapping their intensities to increase their contrast,
- b) transforming pixels having intensities at or above the prearranged intensity threshold into a contrast-free background,
- c) converting the remapped image data into thresholded binary images from which total blob area and brown blob area are discernible respectively, and
- d) expressing brown blob area as a proportion of total blob area.

[0023] In a further aspect, the present invention provides an apparatus for automated assessment of histological specimens stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, the apparatus incorporating a microscope and a camera for photographing a histopathological specimen to obtain colour image data, and computer apparatus for processing the image data, **characterised in that** the computer apparatus is programmed to:

- a) provide one of two contributions to a composite ER or PR score by counting image pixels having intensities below a predetermined intensity threshold and comparing the number of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR score contribution values,
- b) provide another of the two contributions to the composite ER or PR score from blobs in images, the images being based on the obtained image data, by determining a proportion of total blob area which is also brown blob area, and comparing the brown blob area proportion with predetermined proportions having assigned ER or PR score contribution values, and
- c) combine the two contributions and obtain the composite ER or PR score.

[0024] The computer apparatus may also be programmed to determine a hue for the image data and to derive a correction for the composite ER or PR score obtained from the first and second contributions if the hue indicates a degree of blueness or brownness which renders such correction appropriate.

[0025] In another further aspect, the present invention provides a computer programme for use in automated scoring of ER and PR from digitised colour image data obtained by means of a microscope and camera from a histological specimen stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, **characterised in that** the programme includes instructions for counting image pixels having intensities below a predetermined intensity threshold and for obtaining an ER or PR score by comparing the number of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR scores and by assigning a score of such a range in which the number of image pixels appears.

[0026] The computer programme may include instructions for transforming the image data to a different image space having an intensity image plane and for counting image pixels with intensities below the predetermined intensity threshold using pixels in the intensity image plane. It may further include instructions for treating the ER or PR score as one of two contributions to a composite ER or PR score and also instructions for obtaining another of the two contributions to the composite ER or PR score by:

- a) determining from blobs in images, the images being based on the obtained image data, a proportion of total blob area which is also brown blob area,
- b) obtaining the other of the two contributions to the composite ER or PR score by comparing the brown blob area proportion with predetermined proportions having assigned scores, and
- c) combining the two contributions and obtaining the composite ER or PR score.

[0027] The computer programme may include instructions for determining the proportion of total blob area which is brown blob area by:

- a) selecting pixels having intensities below a prearranged intensity threshold and remapping their intensities to increase their contrast,
- b) transforming pixels having intensities at or above the prearranged intensity threshold into a contrast-free background,
- c) converting the remapped image data into thresholded binary images from which total blob area and brown blob area are discernible respectively, and
- d) expressing brown blob area as a proportion of total blob area.

[0028] In an additional aspect, the present invention provides a computer programme for use in automated scoring of ER and PR from image data obtained from histological slides stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, **characterised in that** the programme includes instructions for:

- a) providing one of two contributions to a composite ER or PR score by counting image pixels having intensities below a predetermined intensity threshold and comparing the number of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR score contribution values,
- b) providing another of the two contributions to the composite ER or PR score from blobs in images, the images being based on the obtained image data, by determining a proportion of total blob area which is also brown blob area, and comparing the brown blob area proportion with predetermined proportions having assigned ER or PR score contribution values, and
- c) combining the two contributions and obtaining the composite ER or PR score.

[0029] The computer programme may include instructions for determining a hue for the image data and deriving a correction for the composite ER or PR score obtained from the first and second contributions if the hue indicates a degree of blueness or brownness which renders such correction appropriate.

[0030] In order that the invention might be more fully understood, embodiments thereof will now be described, by way of example only, with reference to the accompanying drawings, in which:-

Figure 1 is a block diagram of a procedure of the invention for scoring oestrogen and progesterone receptors expression; and

Figure 2 is a block diagram showing part of the procedure of Figure 1 in more detail.

[0031] Referring to Figure 1, there is illustrated a procedure 10 of the invention for scoring the expression of ER and PR in tissue samples presented as histopathological slides of carcinomas of the breast. The procedure 10 requires data from histological slides in a suitable form. Sections are taken (cut) from breast tissue samples (biopsies) and placed on glass slides. Slides are stained using a standard peroxidase-conjugated streptavidin-biotin method with 3,3'-diaminobenzidine as a chromogen to detect a reaction for c-erbB-2, ER and PR. Using this method, a positive reaction is seen as a brown stain. Staining for ER and PR is seen in the nuclei of tumour cells and any normal breast luminal epithelial cells. This standard immunohistochemical protocol is widely applied to the detection of a broad range of antibodies across all subspecialties in pathology. For example, the same methodology is used to score a broad range of markers e.g. proliferation markers (MIB-1, Mcm-2, Mcm-5; cell cycle regulators (cyclins A, D and E) and growth factor receptors (EGFR) that are being investigated as prognostic and therapeutic adjuncts in a range of tumours that include colorectal cancer; melanoma; prostate cancer, and brain tumours.

[0032] In the present example, image data were obtained by a pathologist using Zeiss Axioskop microscope with a Jenoptiks Progres 3012 digital camera. Image data from a slide is a set of digital images obtained at a linear magnification of 40 (i.e. 40X).

[0033] To select images, a pathologist scans the microscope over a slide, and at 40X magnification selects a region (referred to as a tile) of the slide which appears to be most promising in terms of an analysis to be performed. This region is then photographed using the microscope and digital camera referred to above. The digital camera produces a digitised

image in three colours, i.e. red, green and blue (R, G & B) intensity values for each pixel each in eight bits and therefore in the range 0 to 255: each image is an electronic equivalent of a tile. Three intensity values are obtained for each pixel in a pixel array to provide a colour image as a combination of R, G and B image planes. The image data from the tile is stored in a database 12 for later use. ER and PR receptors expression are determined using a feature detection process 14: this provides a score 18 for input to a diagnostic report at 20.

[0034] ER and PR are measured from brown stain characteristics (blob colour intensity and area ratio) in a specimen produced using tissue staining processes mentioned above. The objective of the procedure 10 is to quantify the ER and PR positivity in a tumour: this uses the fact that the more oestrogen or progesterone positive a specimen is the greater the intensity and area of its brown stain.

[0035] In a prior art manual procedure, a clinician places a slide under a microscope and examines a region of it at magnification of x40 or others for indications of ER and PR. The prior art manual procedure for scoring ER and PR involves a pathologist estimating by eye the percentage of positive response as well as staining intensity. The process described below in this example replaces the prior art manual procedure with an objective procedure.

[0036] Referring now to Figure 2, the process 14 is shown in more detail. At 30 an input red, green and blue (RGB) digital colour image (initial image designated I) is a starting point for obtaining a score for stained area proportion. The image I has three eight-bit values in the range 0 to 255 per pixel for the R, G and B image planes. Images of histological slides such as the image I show objects referred to as "blobs" having a variety of colours, shapes and sizes, some of which are of interest for ER and PR scoring and some are not. Blobs appearing brown are of interest for ER/PR measurement. The "stained area proportion" measurement is the sum of the areas of brown stained blobs divided by the sum of the areas of all blobs in the image.

[0037] It has been found that pixels of interest for the purposes of assessing staining are those with relatively low intensity values. The next stage 32 is therefore to transform the initial image I so that an intensity range 0-100 of its darker pixels in each of the three R, G and B image planes is transformed by a mapping to fill the full eight-bit intensity range 0 to 255: this means that a pixel intensity value of 100, which is less than half but more than one quarter of the 255 maximum intensity value, becomes transformed to 255. It also has the effect of transforming pixels in I with intensity greater than 100 to 255. If a pixel has R, G and B all equal to 255, it appears white in the image.

[0038] In this example, specimen images were processed in an automated manner by computer using computer software referred to as "Matlab[®]" produced by Mathworks Inc., an American corporation. This software provides a variety of image processing functions. Blobs are identified in the initial image I using the Matlab "imadjust" function, which maps an image's intensity values to a new range. Imadjust is used as previously indicated to remap darker pixel intensity values of all three I image planes R, G and B in the range 0 to 100 to fill the full intensity range of 0 to 255: this increases the contrast of darker regions of the image as well as transforming into a contrast-free white background those pixels with relatively high intensities of 100 and above in all image planes. This transformation is shown in Table 1 below.

Table 1: Transformation from Image I to Image K

Input image I: R, G or B pixel value P_I	Output image K: R, G or B pixel value P_K
$0 \leq P_I \leq 100$	$P_K = 255P_I/100$
$P_I \geq 100$	$P_K = 255$

[0039] The syntax of imadjust for mapping the input image I to an output image K is:

$$K = \text{imadjust}(I, [\text{low_in high_in}], [\text{low_out high_out}]) \quad (1)$$

where low_in and high_in are the chosen dark intensities 0 and 100 in the input image I which are mapped to low_out and high_out 0 and 255 in the output image K at 32. Because only relatively darker pixels are retained, it is assumed that they approximately correspond to all blobs whether stained or not. A bitmap image is consequently obtained at 34 using another Matlab function referred to as "im2bw", which creates from the RGB image K a binary bitmap image denoted L with pixel values 0 and 1 derived using a luminance threshold of 0.9.

[0040] To derive L, the image K is firstly converted from red/green/blue (RGB) to a one colour image. In this example the conversion of K is by transformation into a different image space hue/saturation/value (HSV). Value is also referred to as brightness, luminance or intensity. The RGB to HSV transformation is well known and described in more detail on page 77 of Image Based Measurement Systems, F. Van Der Heijden. It will not be described further. It is not essential to use HSV, one could select one of the image planes or use another known transformation such as principal component

analysis (see e.g.

[0041] Jackson J.E., 'A User Guide to Principal Components' pp 1-25, John Wiley & Sons, 1991). In the present example a one colour image is derived by setting to zero the hue H and saturation S components of pixels in the transformation of K, and the value (luminance) component V is retained as a greyscales image. V has values in the range 0 to 1.0. All pixels with a value greater than or equal to 0.9 are then set to 1 and those less than 0.9 are set to 0: this creates the binary bitmap image L in which pixels of blobs are 0 and background pixels are 1.

[0042] The sum of the areas of all blobs in image I collectively (i.e. whether stained brown or not) is denoted by S1. S1 is computed by inverting the bitmap image L (exchanging 1 for 0 pixel values and vice versa) to form an image L⁻¹ (or "not(L)" in Matlab) in which blob pixels are value 1; pixels in L⁻¹ which are not blob pixels are 0. The value 1 pixels in L⁻¹ are then counted at 34 to provide S1, which is given in Matlab by the instruction:

$$\text{i.e. } S1 = \text{sum}(\text{sum}(\text{not}(L))) \quad (2)$$

[0043] The sum of the areas of all blobs stained brown in image I denoted S2 is now required. Here again the image K (see at (1) above) is converted at 36 to a single colour greyscales image, but for this purpose the blue image plane of the image K is selected: this is because trials showed that the blue image plane contains more brown staining information and fewer spurious details than the green and red planes. Using the im2bw Matlab function, as before the single colour image pixel values (in this case the blue image plane) are converted from the range 0 to 255 to 0 to 1 by division by the maximum of the pixel values, normally 255. A second binary bitmap image M is obtained from the 0 to 1 pixel value equivalent of the single colour image by thresholding: i.e. all pixels with a value greater than or equal to a threshold of 0.85 are set to 1 and those less than 0.85 are set to 0. This threshold provides for removal (merging into background) of blob pixels originally bluish and retention of those originally brownish. The sum S2 of the areas of all blobs stained brown is computed at 36 by inverting the bitmap image M to form image M⁻¹ (or "not(M)"), and counting the pixels which are equal to 1 in M⁻¹ and which are therefore blob pixels; here again pixels in M⁻¹ which are not brownish blob pixels are 0. S2 is given by the Matlab instruction:

$$S2 = \text{sum}(\text{sum}(\text{not}(M))) \quad (3)$$

[0044] The percentage W of blobs or cells stained brown is then computed at 38 by dividing S2 by S1 and multiplying by 100:

$$\text{i.e. } W = 100S2/S1 \quad (4)$$

[0045] The score for proportion staining is denoted as SC1 and is obtained at 38 by comparing W to the thresholds mentioned above. For this example, the score for proportion of brown staining of cells in ER and PR detection in breast cancer is tabulated in Table 2 below. The thresholds in Table 2 are largely as used in manual assessment, except that the thresholds for scores 0 and 1 have been changed slightly from the manual equivalents, as this proved to be more efficient than the manual equivalents using a specific set of trial images in testing the invention.

Table 2: Score for Proportion of Brown Staining:

W: % of cells stained brown	Proportion Score SC1
0 - 0.3%	0
0.3% < W < 1%	1
1-10%	2
11-33%	3
34-66%	4
67-100%	5

[0046] The next stage 40 is to obtain a score SC2 for cell staining intensity. A study of a set of trial images with different staining intensity for the purposes of the invention has shown that the occurrence frequency of relatively darker pixels (intensity value ≤ 100) in the RGB initial image I varies sufficiently with intensity staining to provide a measurement technique in this regard. In particular, strong staining is characterised by a relatively high occurrence frequency of darker pixels, but this frequency is zero (no darker pixels) when there is no staining. For weak to moderate staining, the frequency is intermediate. Consequently, in accordance with the invention, the next step is to form a histogram showing pixel intensity occurrence frequency: to achieve this, the RGB initial image I undergoes an HSV transformation of the kind described earlier. Pixel intensities in the V (value or intensity) image plane of the transformed image are then analysed to produce the histogram. The histogram has an abscissa axis with 256 pixel intensity values or bins and an ordinate axis showing number of pixels with the intensity associated with the respective bin each case. Based on these observations, it was decided to sum the numbers of pixels contained in a sufficient number of the lower valued (darker) pixel intensity bins to give a statistically significant result. The number of bins is not very critical and in this example the bins that were chosen were bins 1 to 100 inclusive: this corresponds to counting pixels with intensities below a intensity threshold equal to the intensity associated with bin number 101. Here again Matlab functions were used such as "imhist" that returns the histogram as bin counts and bin locations as x (ordinate) values. A Matlab routine was written to carry out the summation of pixels in bins 1 to 100.

[0047] The score SC2 for intensity staining was derived using thresholds derived from analysis of a set of images and partly from the value of SC1. Denoting the total number of pixels in bins 1 to 100 collectively as NP(1:100), SC2 was 3 if NP(1:100) was greater than 100,000. SC2 was 2 if NP(1:100) was in the range 2001 to 100,000, and it was 1 if the number of pixels was found to be in the range 400 and 2000. If the number of pixels was less than 400, SC2 would be 0 if SC1 was 4 or less and 1 if SC1 was 5. This is tabulated below in Table 3.

Table 3: Score for intensity of staining

NP(1:100): Number of Pixels in Bins 1 to 100	SC1	SC2	Staining
> 100,000	Any	3	Strong
2,001 to 100,000	Any	2	Moderate
400 to 2,000	Any	1	Weak
< 400	5	1	Weak
< 400	0 to 4	0	None

[0048] Having obtained SC1 and SC2, they are summed at 42 to provide an overall intermediate score in the range 0 to 8. The overall score is subject to the condition that if SC1 or SC2 is 0, the overall score is also set to 0. This is because a zero percentage of brown blobs implies zero staining intensity and vice versa. In principle one could obtain an estimate of ER or PR using SC 1 or SC2 alone, but results are better when SC2 is combined with SC1 as described above.

[0049] The overall 0-8 intermediate score may be taken as the final score, or it may be converted to an overall final score in a more conventional range 0 to 3 as set out in Table 4 below.

Table 4: Overall Final Score

Overall Intermediate Score	Overall Final Score	Endocrine Treatment Prospects
0	0	Failure to respond
2 or 3	1	Small Chance of Response
4, 5 or 6	2	50% Chance of Response
7 or 8	3	High Chance of Response

[0050] The procedure of the invention is rapid. One image takes on average 9 seconds to process. It has been evaluated for an overall number of 97 images for ER and 92 images for PR. Results produced by use of the invention with these images have been compared to assessments made manually by a pathologist, and are very encouraging. Over 80% of scores obtained as described above agreed with a pathologist's overall final score, and over 90% either agreed with the pathologist's score or differed by not more than 1.

[0051] It has been found that a scoring failure may result from confusion between slides stained very dark blue and those stained very brown. To counteract this, a correction for the overall final score has been developed based on image

coloration. The correction involves transforming the RGB initial image I to a hue, saturation, value (HSV) image of the kind described earlier. Colour perception usually refers to three quantities that are hue, saturation and brightness: hue refers more specifically to a dominant wavelength or colour in an image and thus it is of interest for discriminating between blue and brown.

[0052] Hue provides a check on whether blobs treated as being brown are indeed brown, or instead dark blue as sometimes happens with badly stained images. A parameter called "rep" is obtained by summing the values of pixels of blobs thought to be brown. Blobs thought to be brown are indicated by pixels with value 1 in the thresholded binary image M^{-1} (in Matlab code "not(M)") previously defined. A hue or H image plane of I is derived at 44 for the initial image I by HSV transformation as aforesaid and rejection of the S and V values. Each pixel value in H of I is then multiplied by a respective pixel value in the same location in M^{-1} to yield HB of I. All non-blob pixels in HB of I are 0 and all blob pixels have respective values as in H of I. The required parameter rep is now obtained at 44 once more by summing the pixel values in HB of I: this sum - i.e. rep - was observed to vary considerably depending on whether blobs have been stained predominantly blue or predominantly brown. A small value of rep is usually characteristic of bluish slides while a large value of rep is characteristic of brownish slides. Rep therefore provides a test of blueness or brownness enabling a correction to be applied to an overall intermediate score in the range 0 to 8: the correction increases or decreases the overall intermediate score according to what rep indicates the associated slide staining colour to be.

[0053] For example, a small value of rep such as 0.05 indicates bluish images not containing many brown pixels. An overall intermediate score of 7 for an image associated with a small value of rep is corrected at 46 by being decreased by 1 or 2 to reflect the relative paucity of brown pixels which its rep value indicates: such an image is unlikely to merit an overall intermediate score of 7 (overall final score 3) but is more likely to merit an overall intermediate score of 6 or 5 (overall corrected final score 2). It has been found that this correction improves results for both ER and PR. For PR in particular, 88% of corrected overall final scores agreed with the pathologist's manual result, and none differed from the latter by more than 1. Since pathologist's manual results can be up to 30% in error as mentioned earlier, the difference may not be statistically significant. However, these results were obtained using a limited data set. It is desirable to verify the procedure of the invention using more images, laboratories and staining procedures.

[0054] The example of the invention in the foregoing description can clearly be implemented by an appropriate computer program comprising program instructions embodied in an appropriate carrier medium and running on a conventional computer system. As described, much of the program requirements are commercially available in Matlab, and can easily be augmented. The carrier medium may be a memory, a floppy or compact or optical disc or other hardware recordal medium, or an electrical signal. Such a program is straightforward for a skilled programmer to implement without requiring invention, because it involves well known computational procedures.

Claims

1. An automated method of scoring Oestrogen or Progesterone Receptors expression, ER or PR, from image data obtained from histological specimens stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, the method including obtaining digitised colour image data from a histological specimen by means of a microscope and a camera, **characterised in that** the method also includes using computer apparatus to count (40) image pixels having intensities below a predetermined intensity threshold, to score ER or PR by comparing the number NP(1:100) of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR scores, and by assigning a score SC2 of such a range in which the number NP(1:100) of image pixels appears.
2. A method according to Claim 1 **characterised in that** the image data is transformed to a different image space, HSV, having an intensity image plane V and the step of counting (40) image pixels with intensities below the predetermined intensity threshold is implemented with pixels in the intensity image plane V.
3. A method according to Claim 1 or 2 **characterised in that** the step of scoring (40) ER or PR provides one SC2 of two contributions, SC1, SC2 to a composite ER or PR score, and the method further includes:
 - a) determining (38) from blobs in images, L^{-1} , M^{-1} , the images being based on the obtained image data, a proportion W of total blob area S1 which is also brown blob area S2,
 - b) obtaining another, SC1, of the two contributions, SC1, SC2, to the composite ER or PR score by comparing (38) the brown blob area proportion W with predetermined proportions having assigned scores, and
 - c) combining the two contributions, SC1, SC2, and obtaining the composite ER or PR score.
4. A method according to Claim 3 **characterised in that** it includes determining the brown blob area proportion W by

the steps of:

- a) selecting pixels having intensities below a prearranged intensity threshold and remapping (32) their intensities to increase their contrast,
- b) transforming (32) pixels having intensities at or above the prearranged intensity threshold into a contrast-free background,
- c) converting (34, 36) the remapped image data into thresholded binary images, L^{-1} , M^{-1} , from which total blob area $S1$ and brown blob area $S2$ are discernible respectively, and
- d) expressing brown blob area $S2$ as a proportion of total blob area $S1$.

5. An automated method of scoring Oestrogen or Progesterone Receptors expression, ER and PR, from image data obtained from histological slides stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, **characterised in that** the method includes:

- a) providing one, $SC2$, of two contributions, $SC1$, $SC2$, to a composite ER or PR score by counting (40) image pixels having intensities below a predetermined intensity threshold and comparing the number $NP(1:100)$ of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR score contribution values,
- b) providing another, $SC1$, of the two contributions, $SC1$, $SC2$, to the composite ER or PR score from blobs in images, L^{-1} , M^{-1} , the images being based on the obtained image data, by determining (38) a proportion W of total blob area $S1$ which is also brown blob area $S2$, and comparing (38) the brown blob area proportion W with predetermined proportions having assigned ER or PR score contribution values, and
- c) combining the two contributions, $SC1$, $SC2$, and obtaining the composite ER or PR score.

6. A method according to Claim 5 **characterised in that** it includes determining a hue (44) for the image data and deriving a correction (46) for the composite ER or PR score obtained from the first and second contributions, $SC1$, $SC2$, if the hue indicates a degree of blueness or brownness which renders such correction appropriate.

7. Apparatus for automated assessment of histological specimens stained to indicate Oestrogen or Progesterone Receptors expression, ER or PR, a positive reaction for ER or PR being indicated by a brown stain, the apparatus incorporating a microscope and a camera for use in obtaining digital colour image data from a histopathological specimen, and computer apparatus for processing the image data, **characterised in that** the computer apparatus is programmed to count (40) image pixels having intensities below a predetermined intensity threshold, to score ER or PR by comparing the number $NP(1:100)$ of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR scores and by assigning a score $SC2$ of such a range in which the number $NP(1:100)$ of image pixels appears.

8. Apparatus according to Claim 7 **characterised in that** the computer apparatus is programmed to transform the image data to a different image space, HSV, having an intensity image plane V and to implement the step of counting (40) image pixels with intensities below the predetermined intensity threshold with pixels in the intensity image plane V .

9. Apparatus according to Claim 7 or 8 **characterised in that** the ER or PR score (40) is one, $SC2$, of two contributions, $SC1$, $SC2$, to a composite ER or PR score and the computer apparatus is further programmed to:

- a) determine (38) from blobs in images, L^{-1} , M^{-1} , the images being based on the obtained image data, a proportion W of total blob area $S1$ which is also brown blob area $S2$,
- b) obtain another, $SC1$, of the two contributions, $SC1$, $SC2$, to the composite ER or PR score by comparing (38) the brown blob area proportion W with predetermined proportions having assigned scores, and
- c) combine the two contributions, $SC1$, $SC2$, and obtain the composite ER or PR score.

10. Apparatus according to Claim 9 **characterised in that** the computer apparatus is programmed to determine the proportion W of total blob area $S1$ which is brown blob area $S2$ by:

- a) selecting pixels having intensities below a prearranged intensity threshold and remapping (32) their intensities to increase their contrast,
- b) transforming (32) pixels having intensities at or above the prearranged intensity threshold into a contrast-free background,

- c) converting (34, 36) the remapped image data into thresholded binary images , L^{-1} , M^{-1} , from which total blob area S_1 and brown blob area S_2 are discernible respectively, and
- d) expressing brown blob area as a proportion of total blob area.

5 11. Apparatus for automated assessment of histological specimens stained to indicate Oestrogen or Progesterone Receptors expression, ER or PR, a positive reaction for ER or PR being indicated by a brown stain, the apparatus incorporating a microscope and a camera for photographing a histopathological specimen to obtain colour image data, and computer apparatus for processing the image data, **characterised in that** the computer apparatus is programmed to:

- a) provide one, SC_2 , of two contributions, SC_1 , SC_2 to a composite ER or PR score by counting (40) image pixels having intensities below a predetermined intensity threshold and comparing the number $NP(1:100)$ of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR score contribution values,
- 15 b) provide another, SC_1 , of the two contributions, SC_1 , SC_2 , to the composite ER or PR score from blobs in images, L^{-1} , M^{-1} , the images being based on the obtained image data, by determining (38) a proportion W of total blob area S_1 which is also brown blob area S_2 , and comparing (38) the brown blob area proportion W with predetermined proportions having assigned ER or PR score contribution values SC_1 , and
- 20 c) combine the two contributions, SC_1 , SC_2 , and obtain the composite ER or PR score.

25 12. Apparatus according to Claim 11 **characterised in that** the computer apparatus is also programmed to determine a hue (44) for the image data and to derive a correction (46) for the composite ER or PR score obtained from the first and second contributions, SC_1 , SC_2 , if the hue indicates a degree of blueness or brownness which renders such correction appropriate.

30 13. A computer programme for use in automated scoring of Oestrogen or Progesterone Receptors expression, ER or PR, from digitised colour image data obtained by means of a microscope and camera from a histological specimen stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, **characterised in that** the programme includes instructions for counting (40) image pixels having intensities below a predetermined intensity threshold and for obtaining an ER or PR score by comparing the number $NP(1:100)$ of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR scores and by assigning a score SC_2 of such a range in which the number $NP(1:100)$ of image pixels appears.

35 14. A computer programme according to Claim 13 **characterised in that** it includes instructions for transforming the image data to a different image space, HSV, having an intensity image plane V and for counting (40) image pixels with intensities below the predetermined intensity threshold using pixels in the intensity image plane V .

40 15. A computer programme according to Claim 13 or 14 **characterised in that** it further includes instructions for treating the ER or PR score as one, SC_2 , of two contributions, SC_1 , SC_2 , to a composite ER or PR score and also instructions for obtaining another, SC_1 , of the two contributions to the composite ER or PR score by:

- a) determining (38) from blobs in images, L^{-1} , M^{-1} , the images being based on the obtained image data, a proportion W of total blob area S_1 which is also brown blob area S_2 ,
- 45 b) obtaining the other, SC_1 , of the two contributions, SC_1 , SC_2 , to the composite ER or PR score by comparing (38) the brown blob area proportion W with predetermined proportions having assigned scores, and
- c) combining the two contributions, SC_1 , SC_2 , and obtaining the composite ER or PR score.

50 16. A computer programme according to Claim 15 **characterised in that** it includes instructions for determining the proportion W of total blob area S_1 which is brown blob area S_2 by:

- a) selecting pixels having intensities below a prearranged intensity threshold and remapping (32) their intensities to increase their contrast,
- b) transforming (32) pixels having intensities at or above the prearranged intensity threshold into a contrast-free background,
- 55 c) converting (34, 36) the remapped image data into thresholded binary images , L^{-1} , M^{-1} , from which total blob area S_1 and brown blob area S_2 are discernible respectively, and
- d) expressing brown blob area as a proportion W of total blob area.

17. A computer programme for use in automated scoring of Oestrogen or Progesterone Receptors expression, ER and PR from image data obtained from histological slides stained to indicate ER or PR, a positive reaction for ER or PR being indicated by a brown stain, **characterised in that** the programme includes instructions for:

- a) providing one, SC2, of two contributions, SC1, SC2, to a composite ER or PR score by counting (40) image pixels having intensities below a predetermined intensity threshold and comparing the number NP(1:100) of image pixels so counted with predetermined pixel number ranges corresponding to respective ER or PR score contribution values,
- b) providing another, SC1, of the two contributions, SC1, SC2, to the composite ER or PR score from blobs in images, L⁻¹, M⁻¹, the images being based on the obtained image data, by determining (38) a proportion W of total blob area S1 which is also brown blob area S2, and comparing (38) the brown blob area proportion W with predetermined proportions having assigned ER or PR score contribution values, and
- c) combining the two contributions, SC1, SC2, and obtaining the composite ER or PR score.

18. A computer programme according to Claim 17 **characterised in that** it includes instructions for determining a hue (44) for the image data and deriving a correction (46) for the composite ER or PR score obtained from the first and second contributions, SC1, SC2, if the hue indicates a degree of blueness or brownness which renders such correction appropriate.

Patentansprüche

1. Automatisiertes Verfahren zur Bewertung der Expression von Östrogenrezeptoren, ER, oder Progesteronrezeptoren, PR, aus Bilddaten, die von histologischen Proben erhalten sind, die zum Kenntlichmachen von ER oder PR gefärbt wurden, wobei eine positive Reaktion auf ER oder PR durch eine braune Färbung angezeigt wird, wobei das Verfahren den Erhalt von digitalisierten Farbbilddaten von einer histologischen Probe mit einem Mikroskop und einer Camera umfasst,

dadurch gekennzeichnet, dass das Verfahren ferner die Verwendung einer Computervorrichtung umfasst, um Bildpixel mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert zu zählen (40), um ER oder PR zu bewerten durch Vergleichen der Anzahl so gezählter Bildpixel mit vorgegebenen Bereichen der Pixelanzahl, die den jeweiligen ER- oder PR-Bewertungen entsprechen, und durch Zuordnen einer Bewertung eines solchen Bereichs, in dem die Anzahl von Bildpixeln auftritt.

2. Verfahren nach Anspruch 1, **dadurch gekennzeichnet, dass** die Bilddaten in einen unterschiedlichen Bildraum mit einer Intensitätsbildebene transformiert werden und der Schritt des Zählens (40) von Bildpixeln mit Intensitäten unter dem vorgegebenen Intensitätsschwellenwert mit Pixeln in der Intensitätsbildebene durchgeführt wird.

3. Verfahren nach Anspruch 1 oder 2, **dadurch gekennzeichnet, dass** der Schritt der Bewertung (40) von ER oder PR einen von zwei Beiträgen zu einer zusammengesetzten ER- oder PR-Bewertung liefert und das Verfahren ferner umfasst:

- a) Ermitteln (38) eines Anteils an der gesamten Fleckfläche, der auch eine braune Fleckfläche ist, aus Flecken in Bildern auf der Basis der erhaltenen Bilddaten,
- b) Erhalt des anderen Beitrags der beiden Beiträge zu der zusammengesetzten ER- oder PR-Bewertung durch Vergleichen (38) des Anteils an brauner Fleckfläche mit vorgegebenen Anteilen mit zugeordneten Bewertungen und
- c) Kombinieren der beiden Beiträge und Erhalt der zusammengesetzten ER- oder PR-Bewertung.

4. Verfahren nach Anspruch 3, **dadurch gekennzeichnet, dass** es die Ermittlung des Anteils der braunen Fleckfläche durch folgende Schritte umfasst:

- a) Auswählen von Pixeln mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert und Neuabbildung (32) ihrer Intensitäten zur Erhöhung ihres Kontrasts,
- b) Transformieren (32) von Pixeln mit Intensitäten am oder über dem vorgegebenen Intensitäts-Schwellenwert in einen kontrastfreien Hintergrund,
- c) Umwandeln (34, 36) der einer Neuabbildung unterzogenen Bilddaten in mit einem Schwellenwert verglichene binäre Bilder, aus denen die gesamte Fleckfläche bzw. die braune Fleckfläche erkennbar sind, und

d) Ausdrücken der braunen Fleckfläche als Anteil an der gesamten Fleckfläche.

5. Automatisiertes Verfahren zur Bewertung der Expression von Östrogenrezeptoren, ER, oder Progesteronrezeptoren, PR, aus Bilddaten, die von histologischen Präparaten auf Objektträgern erhalten sind, die zum Kenntlichmachen von ER oder PR gefärbt wurden, wobei eine positive Reaktion auf ER oder PR durch eine braune Färbung angezeigt wird,

dadurch gekennzeichnet, dass das Verfahren umfasst:

- a) Erzeugen eines Beitrags von zwei Beiträgen zu einer zusammengesetzten ER- oder PR-Bewertung durch Zählen (40) von Bildpixeln mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert und Vergleichen der Anzahl so gezählter Bildpixel mit vorgegebenen Bereichen der Pixelanzahl, die den jeweiligen Werten des ER- oder PR-Bewertungsbeitrags entsprechen,
- b) Bestimmen des anderen Beitrags der beiden Beiträge zu der zusammengesetzten ER- oder PR-Bewertung aus Flecken in Bildern auf der Basis der erhaltenen Bilddaten durch Ermitteln (38) eines Anteils an der gesamten Fleckfläche, der auch eine braune Fleckfläche ist, und Vergleichen (38) des Anteils an brauner Fleckfläche mit vorgegebenen Anteilen mit zugeordneten Werten des ER- oder PR-Bewertungsbeitrags und
- c) Kombinieren der beiden Beiträge und Erhalt der zusammengesetzten ER- oder PR-Bewertung.

6. Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** es umfasst:

Bestimmen eines Farbtons (44) für die Bilddaten und Ableiten einer Korrektur (46) für die zusammengesetzte ER- oder PR-Bewertung, die aus dem ersten und dem zweiten Beitrag erhalten ist, wenn der Farbton einen Grad an blauem Farbton oder an braunem Farbton anzeigt, der eine solche Korrektur richtig macht.

7. Vorrichtung zur automatisierten Bewertung von histologischen Proben, die zum Kenntlichmachen der Expression von Östrogenrezeptoren, ER, oder Progesteronrezeptoren, PR, gefärbt wurden, wobei eine positive Reaktion auf ER oder PR durch eine braune Färbung angezeigt wird, wobei die Vorrichtung ein Mikroskop und eine Camera zur Verwendung zum Erhalt von digitalen Farbbilddaten aus einer histopathologischen Probe sowie eine Computervorrichtung zur Verarbeitung der Bilddaten aufweist,

dadurch gekennzeichnet, dass die Computervorrichtung so programmiert ist, dass sie Bildpixel mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert zählt (40), um durch Vergleichen der Anzahl so gezählter Bildpixel mit vorgegebenen Bereichen der Pixelanzahl, die den jeweiligen ER- oder PR-Bewertungen entsprechen, und durch Zuordnen einer Bewertung eines solchen Bereichs, in dem die Anzahl von Bildpixeln auftritt, ER oder PR zu bewerten.

8. Vorrichtung nach Anspruch 7, **dadurch gekennzeichnet, dass** die Computervorrichtung so programmiert ist, dass sie die Bilddaten in einen unterschiedlichen Bildraum mit einer Intensitätsbildebene transformiert und den Schritt des Zählens von Bildpixeln mit Intensitäten unter dem vorgegebenen Intensitäts-Schwellenwert mit Pixeln in der Intensitätsbildebene durchführt.

9. Vorrichtung nach Anspruch 7 oder 8, **dadurch gekennzeichnet, dass** die ER- oder PR-Bewertung (40) ein Beitrag von zwei Beiträgen zu einer zusammengesetzten ER- oder PR-Bewertung ist und die Computervorrichtung ferner so programmiert ist, dass sie folgende Schritte durchführt:

- a) Ermitteln (38) eines Anteils an der gesamten Fleckfläche, der auch eine braune Fleckfläche ist, aus Flecken in Bildern auf der Basis der erhaltenen Bilddaten,
- b) Erhalt des anderen Beitrags der beiden Beiträge zu der zusammengesetzten ER- oder PR-Bewertung durch Vergleichen (38) des Anteils an brauner Fleckfläche mit vorgegebenen Anteilen mit zugeordneten Bewertungen und
- c) Kombinieren der beiden Beiträge und Erhalt der zusammengesetzten ER- oder PR-Bewertung.

10. Vorrichtung nach Anspruch 9, **dadurch gekennzeichnet, dass** die Computervorrichtung so programmiert ist, dass sie den Anteil der braunen Fleckfläche an der gesamten Fleckfläche durch folgende Schritte ermittelt:

- a) Auswählen von Pixeln mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert und Neuabbildung (32) ihrer Intensitäten zur Erhöhung ihres Kontrasts,
- b) Transformieren (32) von Pixeln mit Intensitäten am oder über dem vorgegebenen Intensitäts-Schwellenwert

in einen kontrastfreien Hintergrund,

c) Umwandeln (34, 36) der einer Neuabbildung unterzogenen Bilddaten in mit einem Schwellenwert verglichene binäre Bilder, aus denen die gesamte Fleckfläche bzw. die braune Fleckfläche erkennbar sind, und

d) Ausdrücken der braunen Fleckfläche als Anteil an der gesamten Fleckfläche.

11. Vorrichtung zur automatisierten Bewertung von histologischen Proben, die zum Kenntlichmachen der Expression von Östrogenrezeptoren, ER, oder Progesteronrezeptoren, PR, gefärbt wurden, wobei eine positive Reaktion auf ER oder PR durch eine braune Färbung angezeigt wird, wobei die Vorrichtung ein Mikroskop und eine Camera zum Photographieren einer histopathologischen Probe zum Erhalt von Farbbilddaten sowie eine Computervorrichtung zur Verarbeitung der Bilddaten aufweist, **dadurch gekennzeichnet, dass** die Computervorrichtung so programmiert ist, dass sie folgende Schritte durchführt:

a) Erzeugen eines Beitrags von zwei Beiträgen zu einer zusammengesetzten ER- oder PR-Bewertung durch Zählen (40) von Bildpixeln mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert und Vergleichen der Anzahl so gezählter Bildpixel mit vorgegebenen Bereichen der Pixelanzahl, die den jeweiligen Werten des ER- oder PR-Bewertungsbeitrags entsprechen,

b) Bestimmen des anderen Beitrags der beiden Beiträge zu der zusammengesetzten ER- oder PR-Bewertung aus Flecken in Bildern auf der Basis der erhaltenen Bilddaten durch Ermitteln (38) eines Anteils an der gesamten Fleckfläche, der auch eine braune Fleckfläche ist, und Vergleichen (38) des Anteils an brauner Fleckfläche mit vorgegebenen Anteilen mit zugeordneten Werten des ER- oder PR-Bewertungsbeitrags und

c) Kombinieren der beiden Beiträge und Erhalt der zusammengesetzten ER- oder PR-Bewertung.

12. Vorrichtung nach Anspruch 11, **dadurch gekennzeichnet, dass** die Computervorrichtung ferner so programmiert ist, dass sie einen Farbton für die Bilddaten bestimmt (44) und eine Korrektur für die zusammengesetzte ER- oder PR-Bewertung ableitet (46), die aus dem ersten und dem zweiten Beitrag erhalten ist, wenn der Farbton einen Grad an blauem Farbton oder an braunem Farbton anzeigt, der eine solche Korrektur richtig macht.

13. Computerprogramm zur Anwendung bei der automatisierten Bewertung der Expression von Östrogenrezeptoren, ER, oder Progesteronrezeptoren, PR, aus digitalisierten Farbbilddaten, die mit einem Mikroskop und einer Camera von histologischen Proben erhalten sind, die zum Kenntlichmachen von ER oder PR gefärbt wurden, wobei eine positive Reaktion auf ER oder PR durch eine braune Färbung angezeigt wird,

dadurch gekennzeichnet, dass das Programm Befehle enthält zum Zählen (40) von Bildpixeln mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert und zum Erhalt einer ER- oder PR-Bewertung durch Vergleichen der Anzahl so gezählter Bildpixel mit vorgegebenen Bereichen der Pixelanzahl, die den jeweiligen ER- oder PR-Bewertungen entsprechen, und durch Zuordnen einer Bewertung eines solchen Bereichs, in dem die Anzahl von Bildpixeln auftritt.

14. Computerprogramm nach Anspruch 13, **dadurch gekennzeichnet, dass** es Befehle enthält zur Umwandlung der Bilddaten in einen unterschiedlichen Bildraum mit einer Intensitätsbildebene und zum Zählen (40) von Bildpixeln mit Intensitäten unter dem vorgegebenen Intensitäts-Schwellenwert unter Verwendung von Pixeln in der Intensitätsbildebene.

15. Computerprogramm nach Anspruch 13 oder 14, **dadurch gekennzeichnet, dass** es ferner Befehle zur Behandlung der ER- oder PR-Bewertung als einen von zwei Beiträgen zu einer zusammengesetzten ER- oder PR-Bewertung sowie Befehle zur Ermittlung des anderen Beitrags der beiden Beiträge zur zusammengesetzten ER- oder PR-Bewertung durch folgende Schritte enthält:

a) Ermitteln (38) eines Anteils an der gesamten Fleckfläche, der auch eine braune Fleckfläche ist, aus Flecken in Bildern auf der Basis der erhaltenen Bilddaten,

b) Erhalt des anderen Beitrags der beiden Beiträge zu der zusammengesetzten ER- oder PR-Bewertung durch Vergleichen (38) des Anteils an brauner Fleckfläche mit vorgegebenen Anteilen mit zugeordneten Bewertungen und

c) Kombinieren der beiden Beiträge und Erhalt der zusammengesetzten ER- oder PR-Bewertung.

16. Computerprogramm nach Anspruch 15, **dadurch gekennzeichnet, dass** es Befehle zur Ermittlung des Anteils der braunen Fleckfläche an der gesamten Fleckfläche durch folgende Schritte enthält:

- a) Auswählen von Pixeln mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert und Neuabbildung (32) ihrer Intensitäten zur Erhöhung ihres Kontrasts,
- b) Transformieren (32) von Pixeln mit Intensitäten am oder über dem vorgegebenen Intensitäts-Schwellenwert in einen kontrastfreien Hintergrund,
- c) Umwandeln (34, 36) der einer Neuabbildung unterzogenen Bilddaten in mit einem Schwellenwert verglichene binäre Bilder, aus denen die gesamte Fleckfläche bzw. die braune Fleckfläche erkennbar sind, und
- d) Ausdrücken der braunen Fleckfläche als Anteil an der gesamten Fleckfläche.

17. Computerprogramm zur Anwendung bei der automatisierten Bewertung der Expression von Östrogenrezeptoren, ER, oder Progesteronrezeptoren, PR, aus Bilddaten, die von histologischen Präparaten auf Objektträgern erhalten sind, die zum Kenntlichmachen von ER oder PR gefärbt wurden, wobei eine positive Reaktion auf ER oder PR durch eine braune Färbung angezeigt wird,

dadurch gekennzeichnet, dass das Programm Befehle zur Durchführung folgender Schritte aufweist:

- a) Erzeugen eines Beitrags von zwei Beiträgen zu einer zusammengesetzten ER- oder PR-Bewertung durch Zählen (40) von Bildpixeln mit Intensitäten unter einem vorgegebenen Intensitäts-Schwellenwert und Vergleichen der Anzahl so gezählter Bildpixel mit vorgegebenen Bereichen der Pixelanzahl, die den jeweiligen Werten des ER- oder PR-Bewertungsbeitrags entsprechen,
- b) Bestimmen des anderen Beitrags der beiden Beiträge zu der zusammengesetzten ER- oder PR-Bewertung aus Flecken in Bildern auf der Basis der erhaltenen Bilddaten durch Ermitteln (38) eines Anteils an der gesamten Fleckfläche, der auch eine braune Fleckfläche ist, und Vergleichen (38) des Anteils an brauner Fleckfläche mit vorgegebenen Anteilen mit zugeordneten Werten des ER- oder PR-Bewertungsbeitrags und
- c) Kombinieren der beiden Beiträge und Erhalt der zusammengesetzten ER- oder PR-Bewertung.

18. Computerprogramm nach Anspruch 17, **dadurch gekennzeichnet, dass** es Befehle enthält zur Bestimmung eines Farbtons (44) für die Bilddaten und zur Ableitung einer Korrektur (46) für die zusammengesetzte ER- oder PR-Bewertung, die aus dem ersten und dem zweiten Beitrag erhalten ist, wenn der Farbton einen Grad an blauem Farbton oder an braunem Farbton anzeigt, der eine solche Korrektur richtig macht.

Revendications

1. Procédé automatisé de notation de l'expression des récepteurs d'oestrogène ou de progestérone, ER ou PR, à partir de données d'image obtenues de spécimens histologiques marqués pour indiquer ER ou PR, une réaction positive pour ER ou PR étant indiquée par une marque marron, le procédé comprenant l'étape consistant à obtenir des données d'image en couleur numérisées d'un spécimen histologique au moyen d'un microscope et d'une caméra, **caractérisé en ce que** le procédé comprend en outre l'étape consistant à utiliser un appareil informatique pour compter (40) des pixels d'image ayant des intensités inférieures à un seuil d'intensité prédéterminé, pour noter ER ou PR en comparant le nombre de pixels d'image ainsi comptés à des plages de nombres de pixels prédéterminés correspondant à des notations ER ou PR respectives, et en attribuant une notation d'une telle plage dans laquelle le nombre de pixels d'image apparaît.
2. Procédé selon la revendication 1, **caractérisé en ce que** les données d'image sont transformées en un espace d'image différent ayant un plan d'image d'intensité et l'étape consistant à compter (40) les pixels d'image avec des intensités inférieures au seuil d'intensité prédéterminé est mis en oeuvre avec des pixels dans le plan d'image d'intensité.
3. Procédé selon la revendication 1 ou 2, **caractérisé en ce que** l'étape consistant à noter (40) ER ou PR fournit l'une de deux contributions à une notation ER ou PR composite, et le procédé comprend en outre les étapes consistant à :
 - a) à partir de pâtés dans des images sur la base des données d'image obtenues, déterminer (38) une proportion de la surface de pâté totale qui est également une surface de pâté marron,
 - b) obtenir une autre des deux contributions à la notation ER ou PR composite en comparant (38) la proportion de surface de pâté marron à des proportions prédéterminées ayant des notations attribuées, et
 - c) combiner les deux contributions et obtenir la notation ER ou PR composite.
4. Procédé selon la revendication 3, **caractérisé en ce qu'il** comprend l'étape consistant à déterminer la proportion

de surface de pâté marron par les étapes consistant à :

- a) sélectionner les pixels ayant des intensités inférieures à un seuil d'intensité pré-arrangé et re-mapper (32) leurs intensités pour accroître leur contraste,
- b) transformer (32) les pixels ayant des intensités supérieures ou égales au seuil d'intensité pré-agencé en un arrière-plan sans contraste,
- c) convertir (34, 36) les données d'image re-mappées en images binaires de seuil dans lesquelles la surface de pâté totale et la surface de pâté marron sont respectivement discernables, et
- d) exprimer la surface de pâté marron en proportion de la surface de pâté totale.

5. Procédé automatisé de notation de l'expression des récepteurs d'oestrogène ou de progestérone, ER ou PR, à partir de données d'image obtenues de préparations histologiques marquées pour indiquer ER ou PR, une réaction positive pour ER ou PR étant indiquée par une marque marron, **caractérisé en ce que** le procédé comprend les étapes consistant à :

- a) fournir l'une de deux contributions à une notation ER ou PR composite en comptant (40) les pixels d'image ayant des intensités inférieures à un seuil d'intensité prédéterminé et comparer le nombre de pixels d'image ainsi compté à des plages de nombres de pixels prédéterminés correspondant à des valeurs de contribution de notations ER ou PR respectives,
- b) à partir des pâtés dans des images sur la base des données d'image obtenues, fournir une autre des deux contributions à la notation ER ou PR composite en déterminant (38) une proportion de la surface de pâté totale qui est également une surface de pâté marron, et en comparant (38) la proportion de surface de pâté marron à des proportions prédéterminées ayant des valeurs de contribution de notations ER ou PR attribuées, et
- c) combiner les deux contributions et obtenir la notation ER ou PR composite.

6. Procédé selon la revendication 5, **caractérisé en ce qu'il** comprend l'étape consistant à déterminer une teinte (44) pour les données d'image et l'étape consistant à dériver une correction (46) pour la notation ER ou PR composite obtenue à partir des première et deuxième contributions si la teinte indique un degré de bleu ou de marron qui rend ladite correction appropriée.

7. Appareil d'évaluation automatisée de spécimens histologiques marqués pour indiquer l'expression des récepteurs d'oestrogène ou de progestérone, ER ou PR, une réaction positive pour ER ou PR étant indiquée par une marque marron, l'appareil incorporant un microscope et une caméra destinés à être utilisés pour obtenir des données d'image en couleur numériques d'un spécimen histopathologique, et un appareil informatique pour traiter les données d'image, **caractérisé en ce que** l'appareil informatique est programmé pour compter (40) des pixels d'image ayant des intensités inférieures à un seuil d'intensité prédéterminé, pour noter ER ou PR en comparant le nombre de pixels d'image ainsi comptés à des plages de nombres de pixels prédéterminés correspondant à des notations ER ou PR respectives, et en attribuant une notation d'une telle plage dans laquelle le nombre de pixels d'image apparaît.

8. Appareil selon la revendication 7, **caractérisé en ce que** l'appareil informatique est programmé pour transformer les données d'image en un espace d'image différent ayant un plan d'image d'intensité et pour mettre en oeuvre l'étape consistant à compter les pixels d'image avec des intensités inférieures au seuil d'intensité prédéterminé avec des pixels dans le plan d'image d'intensité.

9. Appareil selon la revendication 7 ou 8, **caractérisé en ce que** la notation ER ou PR (40) est l'une de deux contributions à une notation ER ou PR composite, et l'appareil informatique est en outre programmé pour :

- a) à partir de pâtés dans des images sur la base des données d'image obtenues, déterminer (38) une proportion de la surface de pâté totale qui est également une surface de pâté marron,
- b) obtenir une autre des deux contributions à la notation ER ou PR composite en comparant (38) la proportion de surface de pâté marron à des proportions prédéterminées ayant des notations attribuées, et
- c) combiner les deux contributions et obtenir la notation ER ou PR composite.

10. Appareil selon la revendication 9, **caractérisé en ce que** l'appareil informatique est programmé pour déterminer la proportion de la surface de pâté totale qui est une surface de pâté marron par les actions consistant à :

- a) sélectionner les pixels ayant des intensités inférieures à un seuil d'intensité pré-arrangé et re-mapper (32) leurs intensités pour accroître leur contraste,

b) transformer (32) les pixels ayant des intensités supérieures ou égales au seuil d'intensité pré-agencé en un arrière-plan sans contraste,

c) convertir (34, 36) les données d'image re-mappées en images binaires de seuil dans lesquelles la surface de pâté totale et la surface de pâté marron sont respectivement discernables, et

d) exprimer la surface de pâté marron en proportion de la surface de pâté totale.

11. Appareil pour l'évaluation automatisée de spécimens histologiques marqués pour indiquer l'expression des récepteurs d'oestrogène ou de progestérone, ER ou PR, une réaction positive pour ER ou PR étant indiquée par une marque marron, l'appareil incorporant un microscope et une caméra pour photographier un spécimen histopathologique pour obtenir des données d'image en couleur, et un appareil informatique pour traiter les données d'image, **caractérisé en ce que** l'appareil informatique est programmé pour :

a) fournir l'une de deux contributions à une notation ER ou PR composite en comptant (40) les pixels d'image ayant des intensités inférieures à un seuil d'intensité prédéterminé et comparer le nombre de pixels d'image ainsi compté à des plages de nombres de pixels prédéterminés correspondant à des valeurs de contribution de notations ER ou PR respectives,

b) à partir des pâtés dans des images sur la base des données d'image obtenues, fournir une autre des deux contributions à la notation ER ou PR composite à partir de pâtés dans des images en déterminant (38) une proportion de la surface de pâté totale qui est également une surface de pâté marron, et en comparant (38) la proportion de surface de pâté marron à des proportions prédéterminées ayant des valeurs de contribution de notations ER ou PR attribuées, et

c) combiner les deux contributions et obtenir la notation ER ou PR composite.

12. Appareil selon la revendication 11, **caractérisé en ce que** l'appareil informatique est également programmé pour déterminer une teinte (44) pour les données d'image et pour dériver une correction (46) pour la notation ER ou PR composite obtenue à partir des première et deuxième contributions si la teinte indique un degré de bleu ou de marron qui rend ladite correction appropriée.

13. Programme informatique destiné à être utilisé dans la notation de l'expression des récepteurs d'oestrogène ou de progestérone, ER ou PR, à partir de données d'image en couleur numérisées obtenues au moyen d'un microscope et d'une caméra à partir d'un spécimen histologique marqué pour indiquer ER ou PR, une réaction positive pour ER ou PR étant indiquée par une marque marron, **caractérisé en ce que** le programme comprend des instructions pour compter (40) des pixels d'image ayant des intensités inférieures à un seuil d'intensité prédéterminé et pour obtenir une notation ER ou PR en comparant le nombre de pixels d'image ainsi comptés à des plages de nombres de pixels prédéterminés correspondant à des notations ER ou PR respectives et en attribuant une notation d'une telle plage dans laquelle le nombre de pixels d'image apparaît.

14. Programme informatique selon la revendication 13, **caractérisé en ce qu'il** comprend des instructions pour transformer les données d'image en un espace d'image différent ayant un plan d'image d'intensité et pour compter (40) les pixels d'image avec des intensités inférieures au seuil d'intensité prédéterminé en utilisant des pixels dans le plan d'image d'intensité.

15. Programme informatique selon la revendication 13 ou 14, **caractérisé en qu'il** comprend en outre des instructions pour traiter la notation ER ou PR en tant que l'une de deux contributions à une notation ER ou PR composite, et également des instructions pour obtenir une autre des deux contributions à la notation ER ou PR composite par :

a) à partir de pâtés dans des images sur la base des données d'image obtenues, déterminer (38) une proportion de la surface de pâté totale qui est également une surface de pâté marron,

b) obtenir l'autre des deux contributions à la notation ER ou PR composite en comparant (38) la proportion de surface de pâté marron à des proportions prédéterminées ayant des notations attribuées, et

c) combiner les deux contributions et obtenir la notation ER ou PR composite.

16. Programme informatique selon la revendication 15, **caractérisé en ce qu'il** comprend des instructions pour déterminer la proportion de la surface de pâté totale qui est une surface de pâté marron par :

a) sélectionner les pixels ayant des intensités inférieures à un seuil d'intensité pré-arrangé et re-mapper (32) leurs intensités pour accroître leur contraste,

b) transformer (32) les pixels ayant des intensités supérieures ou égales au seuil d'intensité pré-agencé en un

arrière-plan sans contraste,

c) convertir (34, 36) les données d'image re-mappées en images binaires de seuil dans lesquelles la surface de pâté totale et la surface de pâté marron sont respectivement discernables, et

d) exprimer la surface de pâté marron en proportion de la surface de pâté totale.

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17. Programme informatique destiné à être utilisé dans la notation automatisée de l'expression des récepteurs d'œstrogène ou de progestérone, ER ou PR, à partir de données d'image obtenues de préparations histologiques marquées pour indiquer ER ou PR, une réaction positive pour ER ou PR étant indiquée par une marque marron, **caractérisé en ce que** le programme comprend des instructions pour :

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a) fournir l'une de deux contributions à une notation ER ou PR composite en comptant (40) les pixels d'image ayant des intensités inférieures à un seuil d'intensité prédéterminé et comparer le nombre de pixels d'image ainsi compté à des plages de nombres de pixels prédéterminés correspondant à des valeurs de contribution de notations ER ou PR respectives,

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b) à partir des pâtés dans des images sur la base des données d'image obtenues, fournir une autre des deux contributions à la notation ER ou PR composite en déterminant (38) une proportion de la surface de pâté totale qui est également une surface de pâté marron, et en comparant (38) la proportion de surface de pâté marron à des proportions prédéterminées ayant des valeurs de contribution de notations ER ou PR attribuées, et

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c) combiner les deux contributions et obtenir la notation ER ou PR composite.

18. Programme informatique selon la revendication 17, **caractérisé en ce qu'il** comprend des instructions pour déterminer une teinte (44) pour les données d'image et pour dériver une correction (46) pour la notation ER ou PR composite obtenue à partir des première et deuxième contributions si la teinte indique un degré de bleu ou de marron qui rend une telle correction appropriée.

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Fig.1.

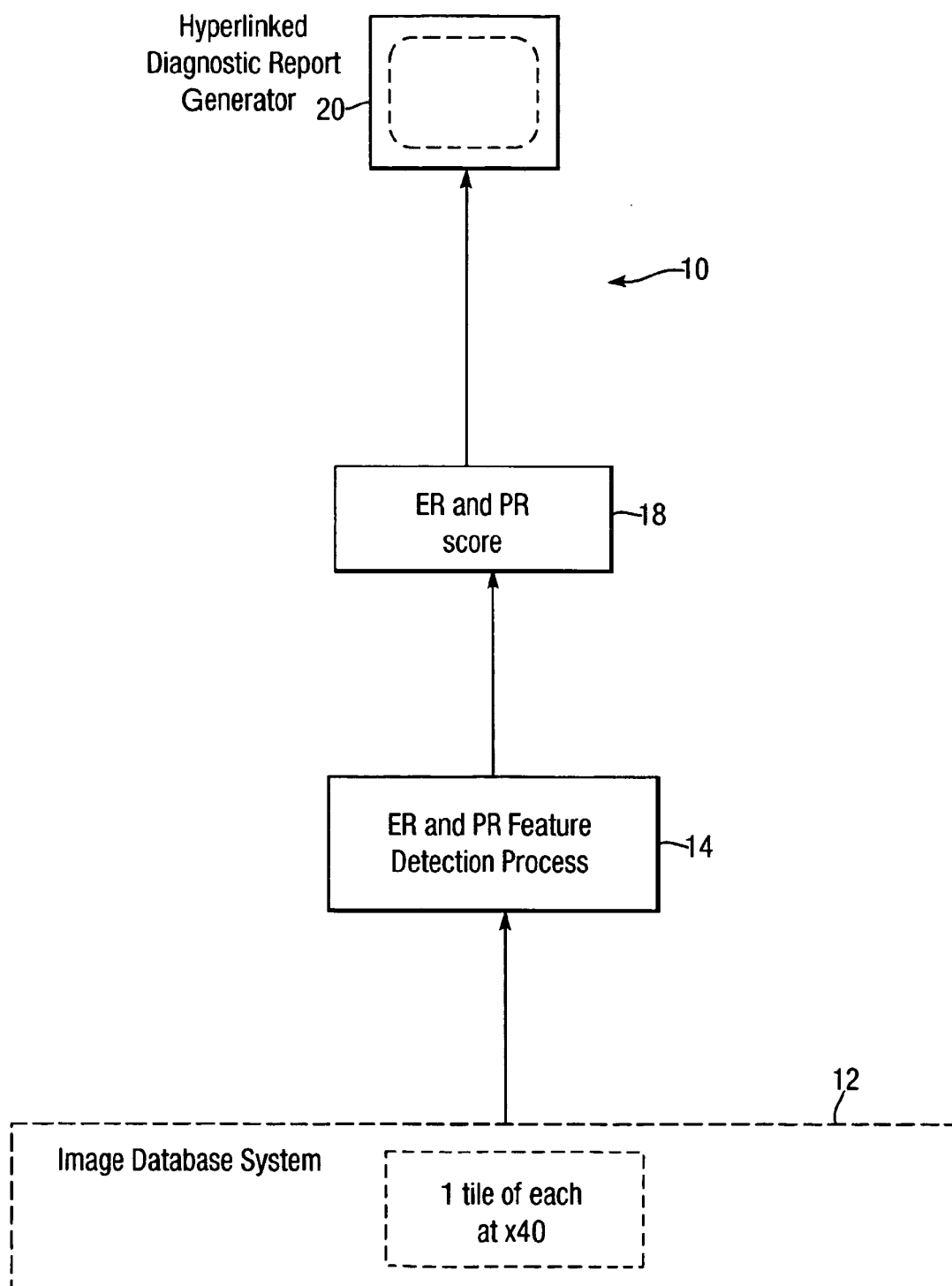
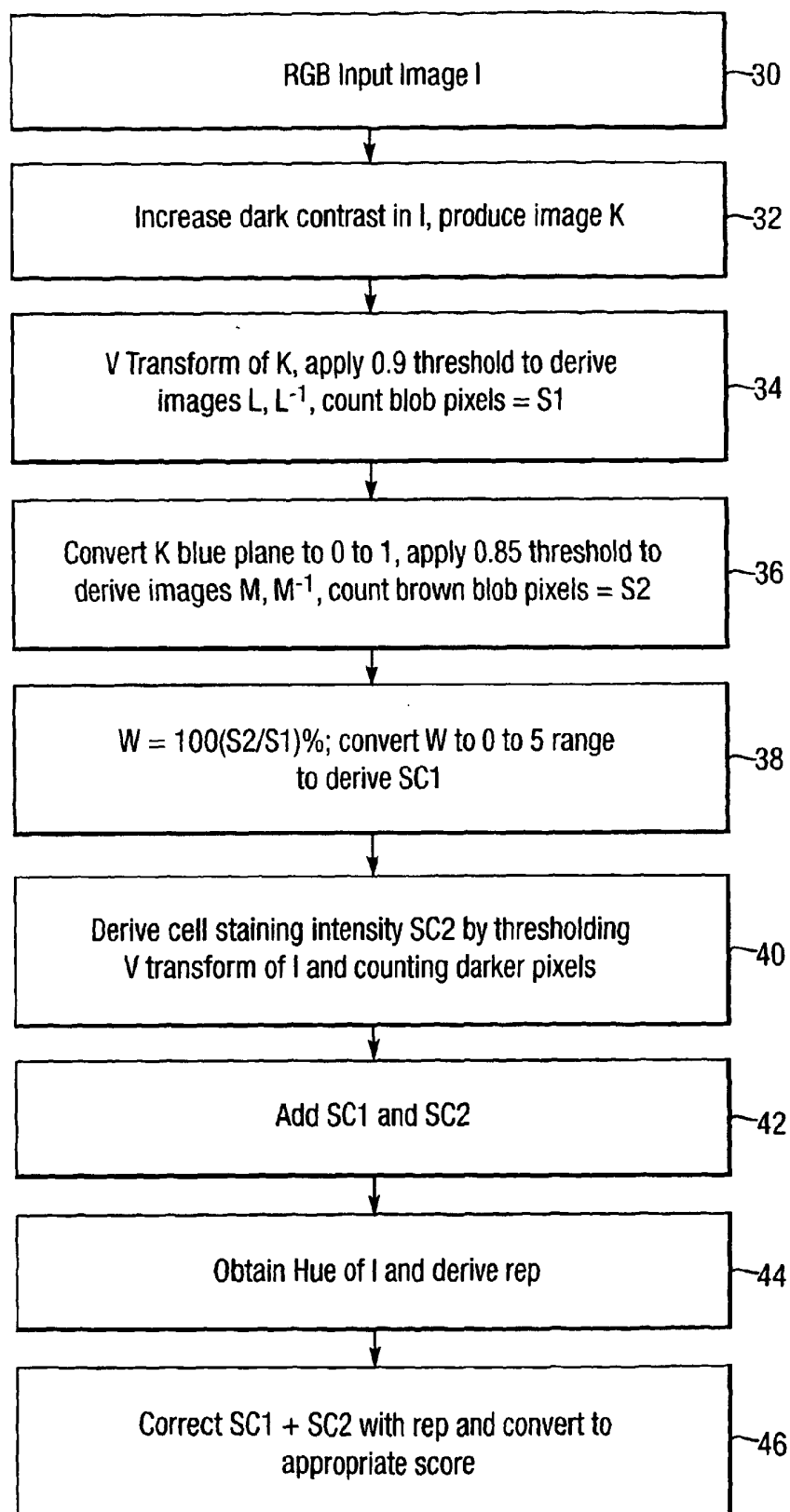


Fig.2.



REFERENCES CITED IN THE DESCRIPTION

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