US 20070020697A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2007/0020697 A1

Cualing et al. (43) Pub. Date: Jan. 25, 2007

(54) VIRTUAL FLOW CYTOMETRY ON IMMUNOSTAINED TISSUE-TISSUE CYTOMETER

(76) Inventors: **Hernani Cualing**, Lutz, FL (US); **Eric E. Zhong**, East Brunswick, NJ (US)

Correspondence Address:
HERNANI CUALING
18804 CHAVILLE RD.
LUTZ, FL 33558 (US)

(21) Appl. No.: **11/492,167**

(22) Filed: **Jul. 25, 2006**

Related U.S. Application Data

(60) Provisional application No. 60/701,774, filed on Jul. 25, 2005.

Publication Classification

(51) **Int. Cl.**

G01N 33/567	(2006.01)
G06F 19/00	(2006.01)
C12M 3/00	(2006.01)

(52) **U.S. Cl.** **435/7.2**; 435/287.2; 702/21

(57) **ABSTRACT**

The invention provides an automated method of single cell image analysis which determines cell population statistic, applicable in the field of pathology, disease or cancer diagnosis, in a greatly improved manner over manual or prior art scoring techniques. By combining the scientific advantages of computerized automation and the invented method, as well as the greatly increased speed with which population can be evaluated, the invention is a major improvement over methods currently available. The single cells are identified and displayed in an easy to read format on the computer monitor, printer output or other display means, with cell parameter such as cell size and staining distribution at a glance. These output data is an objective transformation of the subjective visible image that the pathologist or scientist relies upon for diagnosis, prognosis, or monitoring therapeutic perturbations. Using our novel proposed technology, we combine the advantages provided by the clinical standard tool of flow cytometry in quantifying single cells and also retain the advantages of microscopy in retaining the capability of visualizing the immunoreactive cells. Unlike flow cytometry however, the invention uses commonly available formalin fixed immunostained tissue and not fresh viable cells. To accomplish this aim, we resort to new and improved advanced image analysis using a unique, useful, and adaptive process as described herein. The method uses multi-stage thresholding and segmentation algorithm based on multiple color channels in RGB and HS I spaces and uses auto-thresholding on red and blue channels in RGB to get the raw working image of all cells, then refines the working image with thresholding on hue and intensity channels in HS I using an adaptive parameter epsilon in entropy mode, and further separates different groups of cells within the same class, by auto-thresholding within the working image region. The Immunohistochemistry Flow cytometry (IHCFLOW) combination results in a new paradigm that is both useful, novel, and provides objective tangible result from a complex color image of tissue.

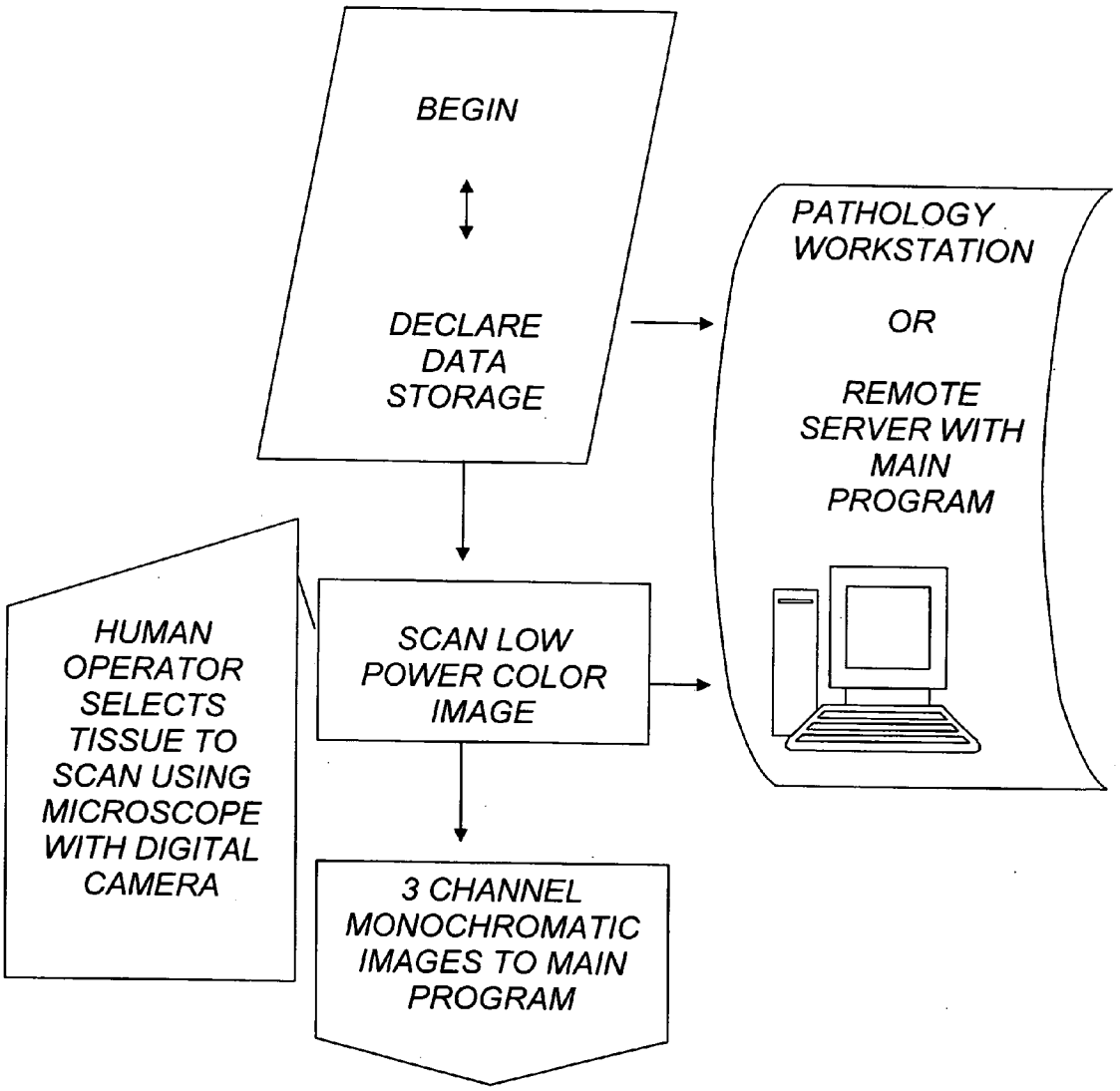
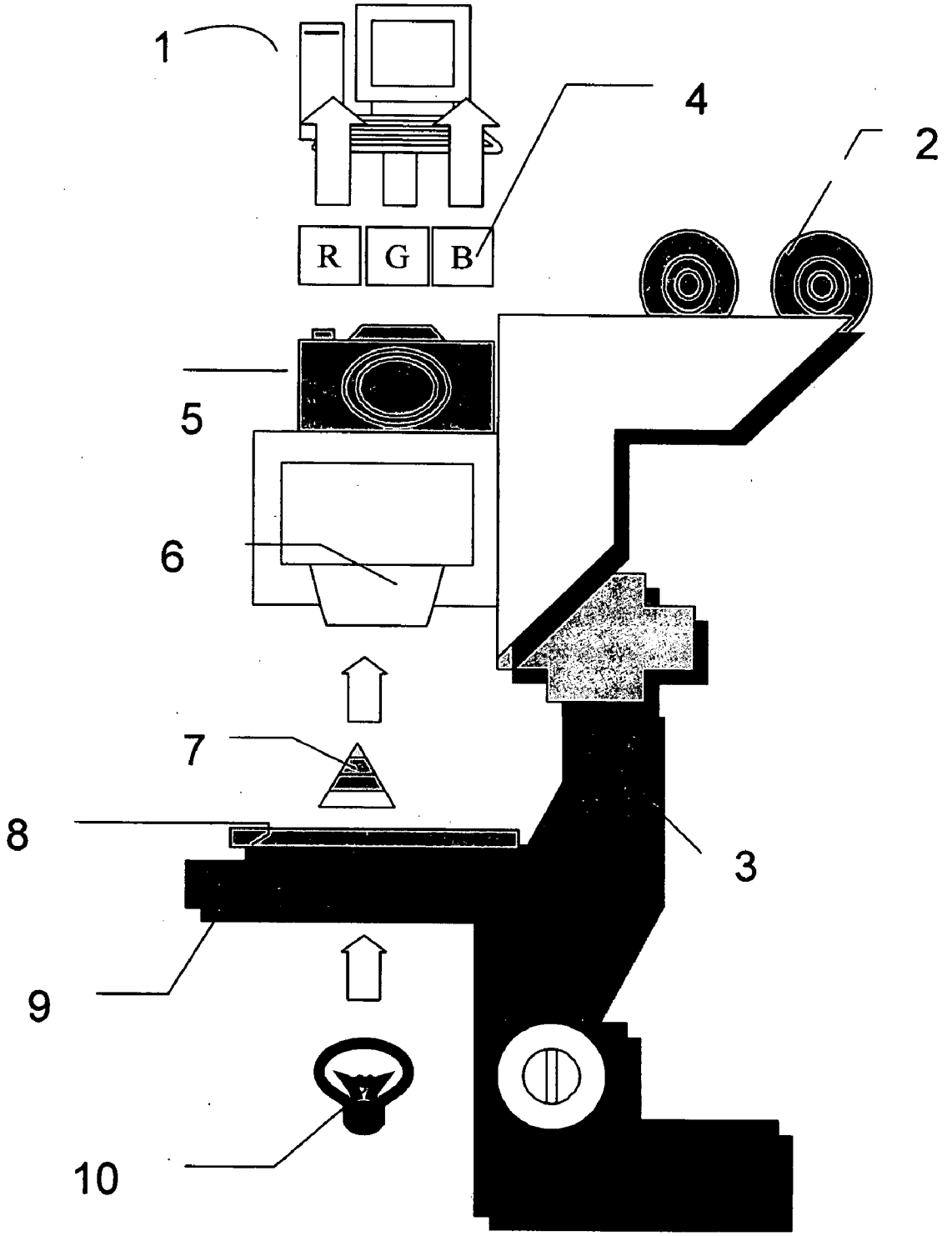

BEGIN
DECLARE DATA STORAGE
PATHOLOGY WORKSTATION
OR
REMOTE SERVER WITH MAIN PROGRAM
HUMAN OPERATOR SELECTS TISSUE TO SCAN USING MICROSCOPE WITH DIGITAL CAMERA
SCAN LOW POWER COLOR IMAGE
3 CHANNEL MONOCHROMATIC IMAGES TO MAIN PROGRAM

FIG. 1

FIG. 2



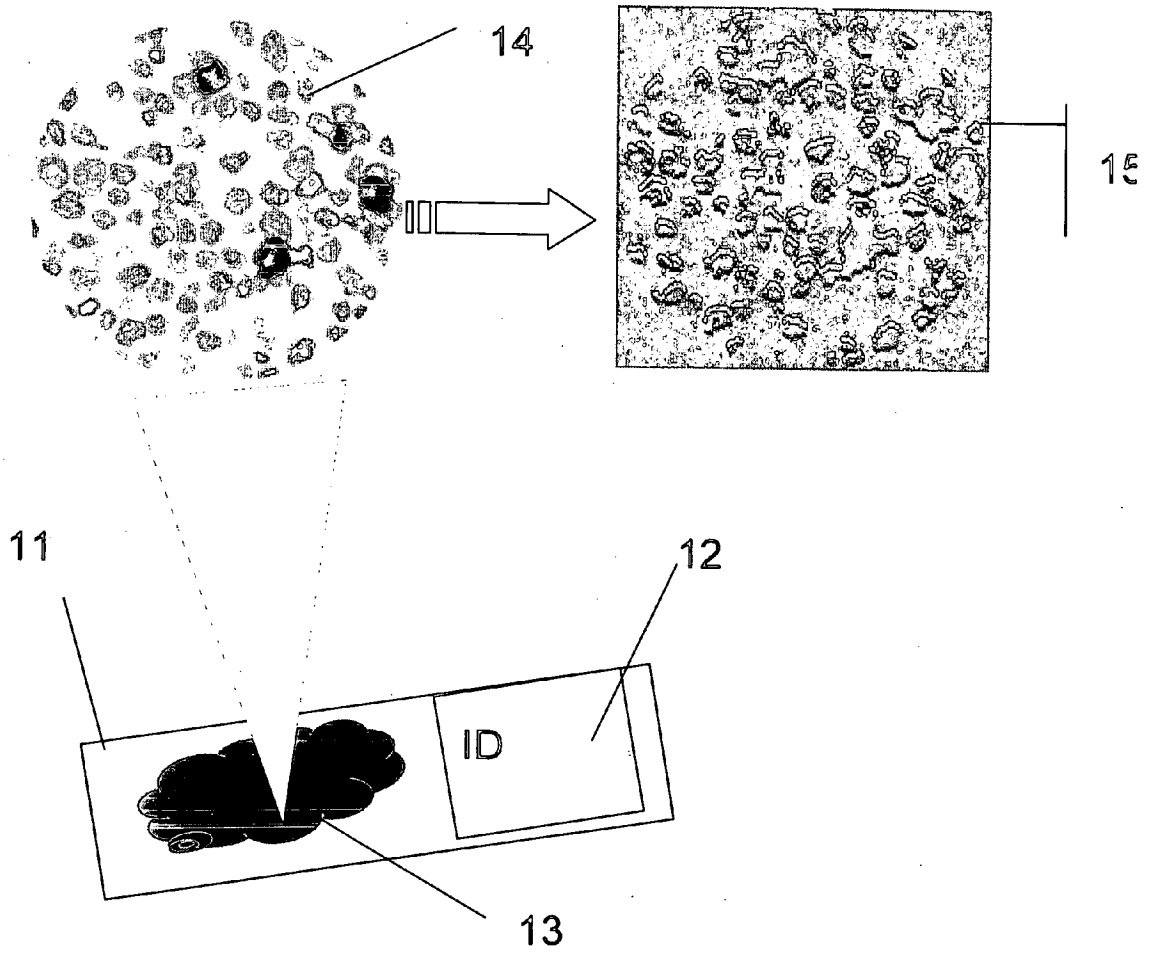


FIG. 3

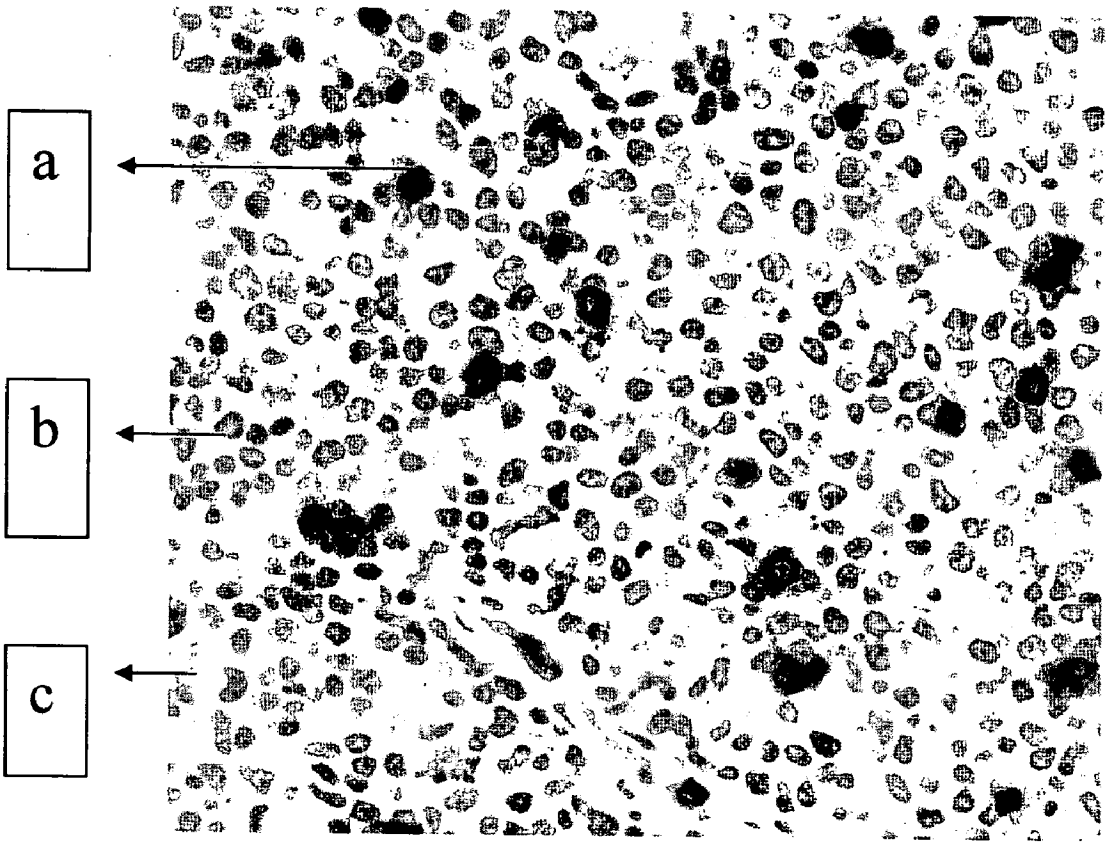


FIG. 4

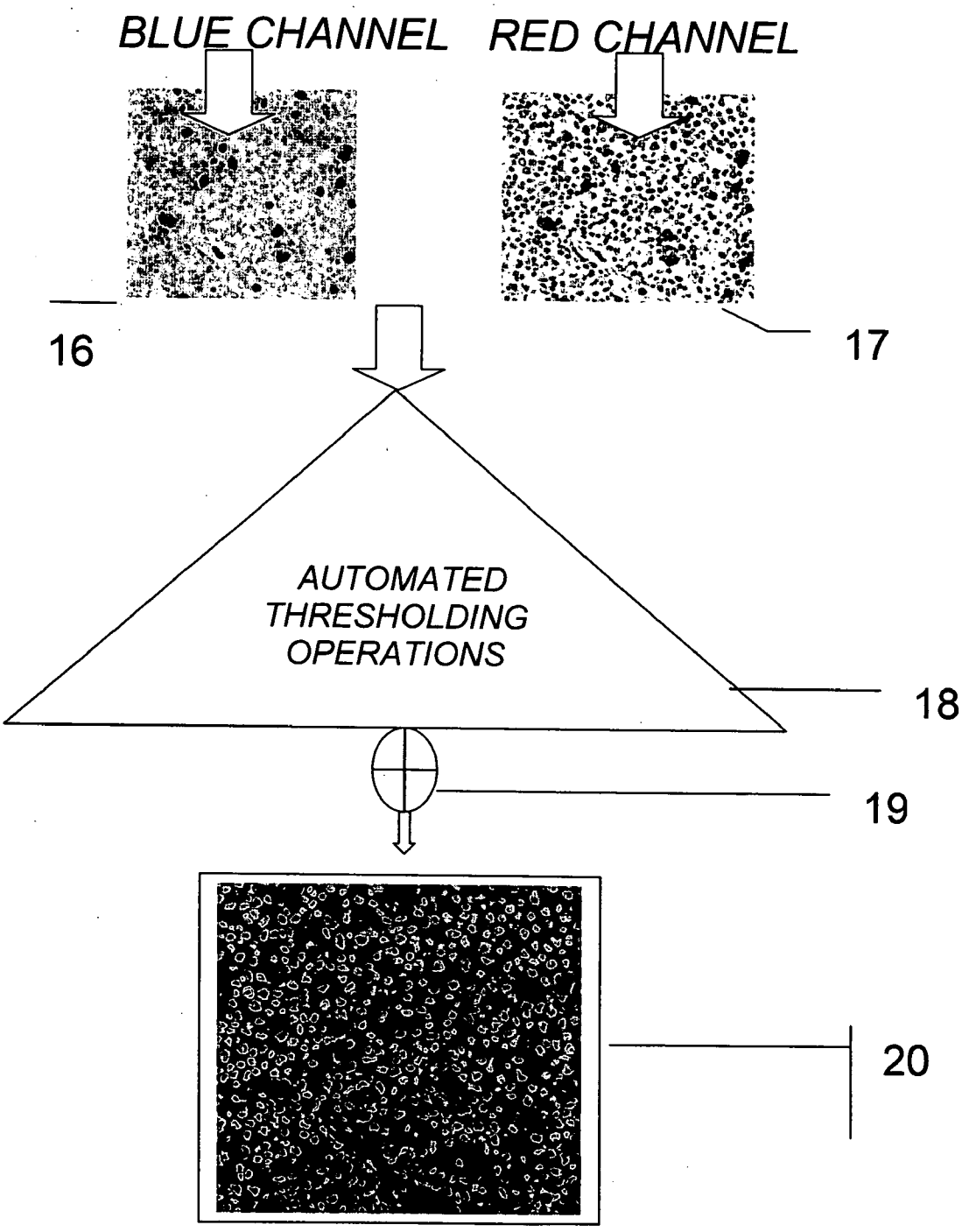
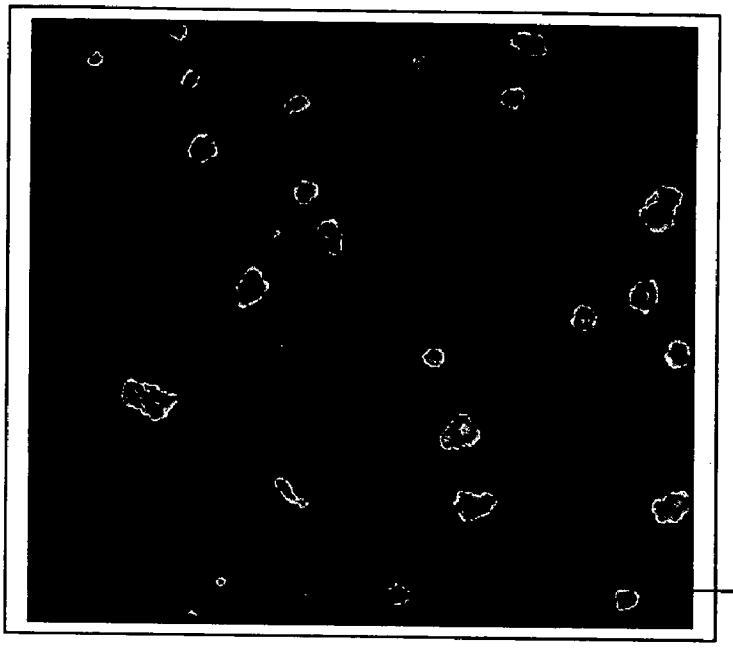

BLUE CHANNEL
RED CHANNEL
16
17
AUTOMATED
THRESHOLDING
OPERATIONS
18
19
20

FIG. 5



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NUMERATOR

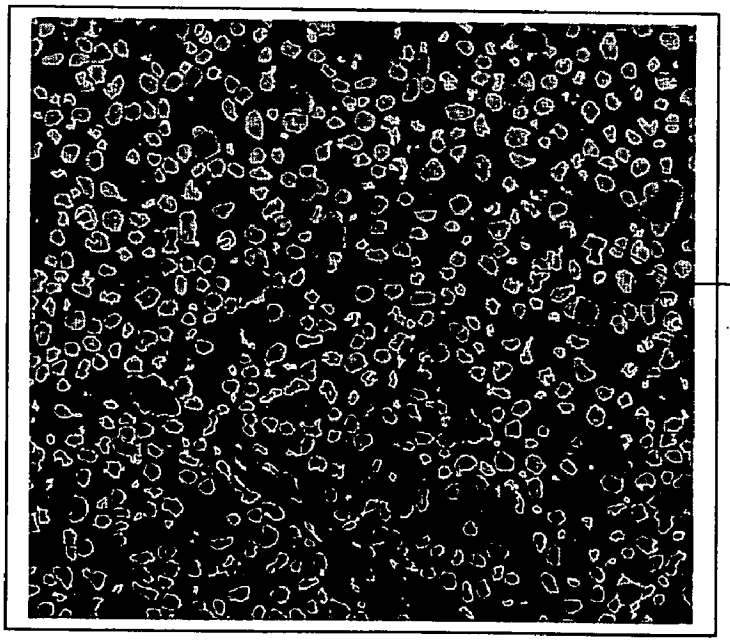

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FIG. 6

DENOMINATOR