

## AUTOMATED METHOD FOR IMAGE ANALYSIS OF RESIDUAL PROTEIN

### CLAIM OF PRIORITY

This application is a continuation of U.S. patent application Ser. No. 09/612,022, filed Jul. 7, 2000 (now U.S. Pat. No. 6,330,349), which claims benefit of priority from U.S. Provisional Application No. 60/143,181, filed Jul. 9, 1999 and is a continuation-in-part of U.S. patent application Ser. No. 08/758,436, filed Nov. 27, 1996 (now U.S. Pat. No. 6,215,892), which claims the benefit of priority from U.S. Provisional Patent Application No. 60/026,805, filed Nov. 30, 1995.

### TECHNICAL FIELD

The invention relates generally to light microscopy and, more particularly, to automated analysis of cellular specimens containing stained markers.

### BACKGROUND OF THE INVENTION

Alkaline phosphatase concentrations usually increase in blood neutrophils of normal pregnant women. However, the maternal neutrophil alkaline phosphatase (NAP) in Down's syndrome pregnancies (women with trisomy 21 fetuses) differs from NAP found in normal pregnancies. NAP from women with Down's syndrome pregnancies is characterized by: (1) an increase in NAP enzyme activity over that found in normal pregnancies, (2) NAP thermal stability, (2) NAP stability in urea, (3) a significant decrease in reactivity with anti-liver-type alkaline phosphatase (AP); (4) low reactivity with anti-placental-type AP or anti-intestinal-type AP antibodies; (5) altered response to AP enzyme inhibitors; and (6) marked dispersion of NAP lead citrate reaction products or anti-NAP antibody colloidal gold-labeling in neutrophil cytoplasm, as detected by electron microscopy. These characteristics suggest that neutrophils of a woman with a trisomy 21 fetus contain two AP isoenzymes: the liver/bone type AP and an atypical AP that is related to the early placental form. Thus, the non-specific alkaline phosphatase isoenzyme can be an "enzyme marker" to diagnose Down's syndrome pregnancies.

The histochemical measurement of maternal NAP has a high detection rate for the prenatal detection of Down's syndrome pregnancies. However, because the histochemical method is laborious and subjective to use, the method has not gained widespread acceptance in prenatal screening programs.

Some automated methods have been developed. Tafas et al. have developed an image analysis method for the measurement of neutrophil alkaline phosphatase (NAP) and established a correlation of urea-resistant fraction of NAP (URNAP)/NAP scoring between the manual and automated methods for prenatal screening of Down's syndrome (U.S. Pat. No. 5,352,613 to Tafas et al.; Tafas et al., *Fetal Diagn. Ther.* 11 (4):254-259, 1996). Measurements ("scores") obtained by manual and automated methods correlate, but the automated scoring is threefold faster. However, a less laborious and subjective automated image analysis method could have benefits in the medical arts.

### SUMMARY OF THE INVENTION

The invention provides an automated method for the measurement of a residual component of a cellular protein. A specimen (sample) to be stained with a cytochemical stain is obtained from a subject. At least one subsample is treated

before being stained, such that the treatment may affect the measurable protein level. At least one subsample is not treated, to serve as a control. The untreated subsample and the treated subsample are stained together, for uniformity of staining. An apparatus automatically selects a position in each subsample for candidate objects of interest and obtains a low magnification color digital image of the candidate objects of interest. The apparatus automatically filters the pixels of the candidate object of interest with a low pass filter and morphologically processes the candidate object of interest pixels to identify artifact pixels, identifying the candidate objects of interest from the remaining candidate object of interest pixels in the subsample not identified as artifact pixels. The apparatus is adjusted to high magnification for automatically acquiring a high magnification image of the subsample, at the location coordinates corresponding to the low magnification image, for each candidate object of interest. The apparatus automatically transforms pixels of the high magnification image in the first color space to a second color space to differentiate high magnification candidate objects of interest pixels from background pixels, identifying objects of interest from the candidate object of interest pixels in the second color space. The protein level is scored in the untreated and the treated subsamples. The value Delta ( $\Delta$ ) is determined ( $\Delta = [\text{protein level in the treated subsamples}] / [\text{protein level in the untreated subsamples}]$ ) as a measurement of the residual component of the cellular protein.

In particular embodiments, the method is for the measurement of maternal neutrophil alkaline phosphatase after treatment with or without urea or heat (diagnostic for Down's syndrome pregnancies), measurement of leukocyte acid phosphatase after treatment with or without tartrate (diagnostic for hairy cell leukemia), and measurement of leukocyte esterase after treatment with  $\alpha$ -naphthol butyrate with or without fluoride (diagnostic for leukemia).

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view of an apparatus for automated cell image analysis.

FIG. 2 is a block diagram of the apparatus shown in FIG. 1.

FIG. 3 is a block diagram of the microscope controller of FIG. 2.

FIG. 4 is a plan view of the apparatus of FIG. 1 having the housing removed.

FIG. 5 is a side view of a microscope subsystem of the apparatus of FIG. 1.

FIG. 6 shows a slide carrier.

FIG. 6a is a top view of a slide carrier for use with the apparatus of FIG. 1.

FIG. 6b is a bottom view of the slide carrier of FIG. 6a.

FIG. 7 shows views of an automated slide handling subsystem.

FIG. 7a is a top view of an automated slide handling subsystem of the apparatus of FIG. 1.

FIG. 7b is a partial cross-sectional view of the automated slide handling subsystem of FIG. 7a taken on line A—A.

FIG. 8 shows end views of the input module of the automated slide handling subsystem. FIGS. 8a-8d illustrate the input operation of the automatic slide handling subsystem.

FIGS. 9a-9d illustrate the output operation of the automated slide handling subsystem.