Computerized image analysis of morphologically transformed and nontransformed Syrian hamster embryo (SHE) cell colonies: application to objective SHE cell transformation assay scoring

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We have developed an automated image analysis system that provides comparable classification of morphologically transformed SHE cell colonies to the current visual classification method used in the in vitro SHE cell transformation assay. Visual classification of morphologic transformation in this assay has been shown to accurately predict the carcinogenic potential of chemical, biological and physical agents. The image analysis system is quantitative, based on measuring features of colony color, texture and growth patterns. A linear combination of feature measurements produces a classification process that agrees with visual assessment 93% of the time. All identifiable sources of error are explored and the method is found to be robust in analyzing nearly 500 colonies from a variety of studies performed over a one year period. The high degree of correlation between the visual classification and the objective measurements of the image analysis system validates the reproducibility of the visual scoring process and serves as a basis for automation of the assay.

Introduction

The Syrian hamster embryo (SHE*) cell transformation assay is an in vitro cell culture system which predicts the carcinogenic potential of chemical, biological and physical agents compared to bioassays with 80–90% accuracy (reviewed in 1–3). The SHE cell transformation assay was initially described in 1963 by Berwald and Sachs (4). Since that time it has been used by a number of investigators worldwide to test the carcinogenic potential of over 500 chemical, biological and physical agents as well as for basic research into the mechanisms of neoplastic transformation (1–3, 5–16). As an in vitro model system for neoplastic transformation, SHE cells undergo multistage transformation beginning with normal cells which, following carcinogen exposure, undergo a block in cellular differentiation which is phenotypically identified as morphological transformation (11,12). Morphological transformation is followed by additional mutations leading to cells which are immortal, tumorigenic, and finally metastatic and fully malignant (5,7,10–14). These changes correlate with mutations in a large number of genes including oncogenes, tumor suppressor genes, growth factors, etc. (10,11). Based on the collective body of data, the process of neoplastic transformation of SHE cells is an accurate and relevant in vitro recapitulation of the in vivo neoplastic process, which probably accounts for the high concordance between morphological transformation in SHE cells in vitro and rodent carcinogenic potential in vivo. It has recently been established that the induction of morphological transformation, the phenotypic endpoint used most commonly in the SHE assay for assessing the carcinogenic activity of a chemical, involves alterations in the expression of at least one tumor suppressor gene, the \( H19 \) gene (11,12). Re-expression of this gene suppresses the tumorigenicity of the fully neoplastic SHE cells. This indicates that correction of the carcinogen-induced block in cellular differentiation is able by itself to suppress tumorigenicity irrespective of additional later stage mutations which have occurred. As such, this use of morphological transformation as the quantitative endpoint for the SHE carcinogen assessment assay has both practical and biologically relevant advantages.

The SHE cell transformation assay, and a variety of alterations on the basic assay, have been described in detail by multiple investigators (11). One particular modification of the standard SHE cell transformation assay which has improved the assay performance is the reduction of the cell culture media from pH 7.3 to pH 6.7 (2,17). This change results in an assay which is easier to perform and more predictive than the standard SHE cell transformation assay (1). In particular, under reduced pH conditions virtually all SHE cell isolates and fetal bovine serum lots support carcinogen-induced morphological transformation. These improvements also aid in the scoring of the assay, since morphologically transformed colonies under pH 6.7 culture conditions are more pronounced in phenotype and easier to identify (2,17). The reasons for the improvement in assay performance as a result of lowering the culture media pH are numerous and include: (i) alterations in the cellular protein phenotype including changes in the number and types of protein expressed and the phosphorylation status of multiple proteins; (ii) alterations in gap junctional communication by changes in connexin43 gene expression; (iii) changes in cellular calcium metabolism such that growth and differentiation factor-induced calcium signaling is blocked, thus maintaining stem cells in an undifferentiated state longer and providing more targets for morphological transformation; and (iv) improvements in the clonal cellular growth characteristics resulting from constant mitogenic signaling by hydrogen ions (reviewed in 3).

One criticism of the SHE cell transformation assay is the subjective nature of the identification of the morphologically transformed phenotype in scoring the assay (discussed in 2). Morphological transformation is a subtle change in cellular and colony growth characteristics and training is necessary for users of the assay to correctly identify morphologically transformed colonies from nontransformed colonies. In an effort to develop an objective scoring methodology of the SHE assay, we have utilized computerized image analysis to measure the factors which expert scorers use in differentiating morphologically transformed colonies from nontransformed colonies.

*Abbreviations: SHE, Syrian hamster embryo; ROI, region of interest; RGB, red, green and blue; HSI, hue, saturation and intensity; HITP, hue, intensity, texture and pattern; DMSO, dimethyl sulfoxide; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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The development and application of this technology to the SHE assay is the subject of this report.

Materials and methods

**Chemicals**

The procedures used for isolation and culturing of SHE cells, and the conduct and scoring of the transformation assay were recently published as part of a comprehensive protocol for the conduct of the SHE assay at pH 6.70 (17). Briefly, SHE cells were isolated from 13 day embryos; cultured in pH 6.7 LeBecuf’s Modified Dulbecco’s Modified Eagle’s Medium (LMDMEM) (Quality Biologicals Inc., Gaithersburg, MD) containing 20% fetal bovine serum (FBS) (HyClone) maintained at 37°C in an atmosphere of 10% carbon dioxide and 95% relative humidity. Analyses were performed on colonies from archived SHE cell transformation assays. SHE cell transformation assays were performed on either wild-type SHE cells or SHE cell clones plated at a final colony density of ~35 colonies/60 mm culture dish on a viable, metabolically active, yet replication impaired feeder layer of 40,000 cells/60 mm dish with 40 dishes per treatment group. Following plating, the cells were incubated overnight and then exposed for 7 days to the indicated carcinogen. Following carcinogen exposure the media was removed and SHE cells were fixed with methanol and stained with Giemsa stain. Colonies were scored for morphological transformation. Morphological transformation was assessed visually by two expert graders based upon previously published criteria (17). The MT colonies primarily exhibit extensive, random-oriented, three dimensional growth with colonies being composed of criss-crossed, stacked, basophilic cells. Treated yet nontransformed cells demonstrate oriented, monolayer growth patterns.

**Acquisition of colony images**

Images of individual colonies were obtained using a 3 chip CCD color camera (DXC-3000P, SONY) mounted on a microscope (Axioplan, Zeiss) equipped with a 1.25X objective and 1.0X Optivar. The camera was color balanced by calibrating black with the light path closed and calibrating white with the tungsten-halogen lamp voltage set to 3 volts, the light path open and no sample; automatic gain was turned off. Images were acquired into computer memory (MacIntosh 950, Apple Computer Co.) by averaging 16 frames using a color framegrabber (Pixelpipe, Perceptics Corp.) and an image analysis software package (ONCOR Image, ONCOR Inc.) controlled by a graphic user interface program written by the authors in HyperCard (Apple Computer). The images have a spatial resolution of 600 x 480 pixels and color resolution of 24 bits. Approximately 500 images of colonies from several experiments were obtained and analyzed in batch mode. A few of the images were manually edited using a graphics package (Photoshop, Adobe Inc.) to eliminate artifacts and other colonies in the field of view around the colony of interest.

For most experiments images were taken of all the colonies judged by visual grading to be morphologically transformed and a random sampling of nontransformed colonies usually in the ratio of two nontransformed to every transformed colony. In one experiment all of the colonies were imaged from the culture dishes. The source of the target cells were usually wild type SHE cells; however, clonal SHE cell isolates were used in two experiments in order to determine if scoring represented selection of a subpopulation of cells as opposed to a cell transformation process. Statistical comparisons were performed using appropriate tests (IMP, SAS Inc.).

**Analysis of images**

Based on discussions with expert graders, the analysis program was designed to measure three features of the individual colonies: (i) color, (ii) texture and (iii) growth patterns. Visual graders utilize a slight change in color due to increased basophilia, a grainy texture due to cell stacking and a complete lack of the ‘flowing’ pattern of organized cell growth, to identify morphologically transformed colonies. Visually categorizing a given colony as transformed or nontransformed is difficult since the degree to which a transformed colony exhibits each of these features is variable. The final visual judgment is a subjectively weighted combination of these features. An additional complicating feature, not highly correlated with transformation, is cell density. Clonal cell growth rates are highly variable resulting in differences in the number of cells/colony. Some of this variability is related to inherent differences in growth rates of various cell types and some is related to the differences in the cytotoxic susceptibility of individual target cells to applied chemicals. Some colonies are so sparse that they are categorized as ‘too small to score’.

A computer program was written to provide continuous measures of color, texture and pattern for each colony and then mimic the visual grading base on a weighted linear combination of these features. The program first removes any color contribution from the background. Background color can arise from residuals, uneven illumination or optical properties of the glass cover slip. Variation in lamp temperature not compensated by the camera circuitry. To remove this background color, areas associated with the colony are removed and a maximization filter is then applied to calculate a smooth background image. The original color image was normalized to this calculated background image to produce an image with a flat ‘white’ background.

The region of interest (ROI) was defined as an area around the center of the colony where cell density was >50%. Large black areas saturated with stain were eliminated from analyses since measurement within these regions would be meaningless; this was important for only a few of the larger colonies. The following is a brief description of how the ROI was established. A spatial filter was used to define the area of the colony that had at least 50% cell coverage. This area was measured and a circular ROI with the equivalent area was positioned at the centroid of the colony to ensure a radially symmetrical sampling of an area with significant cell coverage. All the intensity measurements were normalized to the area of the ROI in order to make measurements independent of colony size. Sparserly populated colonies were excluded from analysis by setting a threshold on size of the ROI to approximate the process used in visual scoring.

To measure changes in colony color, the image was converted from red, green and blue (RGB) color space to hue, saturation and intensity (HSI). The intensity image was inverted for easier visualization of the intensity variations. The average values of HSI were calculated for the area within the ROI (Figure 1a,b). Texture was measured by calculating the difference between the maximum and minimum values within a 3 x 3 neighborhood and calculating the resulting image’s average grey level for the area within the ROI (Figure 1c,d). Pattern was determined by looking for small local areas of common edge directions within the ROI. A Kirsch edge direction operator (18) was applied to the blue image which had been enhanced by an adaptive contrast enhancement process. Since the edge directions of the cells within the colonies are highly variable, the edge directions were smoothed using directional filters to create grey level images representing the consensus of that direction. An image with no local consensus of a particular direction would have an intensity of 0 in that local region. Any strong directional pattern would result in a large positive value for the filter aligned with that direction and a low value for the image aligned at 90 degrees to that image. Absolute differences in the intensities of the two image pairs at 90 degrees to each other were calculated and combined as a measure of the overall pattern of the colony (Figure 1e,f).
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Fig. 2. Distributions of measured features (a–e) across all colonies analyzed by class. The best combination of features to discriminate classes was determined by nominal logistic regression to be hue, intensity, texture and pattern (HITP). The horizontal lines on each plot represent a threshold for classification. Throughout the paper a square marker indicates visual classification of the colony as transformed and the diamond marker indicates nontransformed.
colony and whose area is based on cell density. The transformed circular ROI whose position is based on the centroid of the measured features in each class. These images show the colonies have nearly the average values for each of the color, texture and pattern for a transformed (Figure 1a,c,e) and the nontransformed (Figure 1b,d,f). The distributions of the computer scores for the two classes are shown in Figure 3. A threshold of 1.0 was chosen to separate the two classes since that value produces no overlap between the two classes. Thus, colonies with HITP scores <1.0 are classified as transformed, whereas colonies with HITP scores ≥1.0 are classified as nontransformed. The overall agreement between visual scoring and the computer scores is 93% (Table I). The cross-correlation of the individual features confirms the visual perception that they are only weakly related (Table II). All of the errors were reviewed with expert graders and no obvious misclassifications were detected; e.g., all of the errors were also difficult to classify visually. The progression of changes in the appearance of colonies associated with HITP scores is shown in Figure 4 for colonies of average size. The range in colony appearance for transformed colonies with similar HITP scores (~3 to ~4) are shown in Figure 5a,c,e and for nontransformed cells (HITP scores of 4 to 5) in Figure 5b,d,f.

Day-to-day variation
The day-to-day variation in analyzing individual colonies was determined by repetitively imaging five colonies over the 4 day period required to acquire images and analyze all the colonies from one study. This introduced variations in camera calibration, lamp color and orientation of the colonies (rotation and translation) as well as any potential effects of light exposure on the dye. Variations with the day of sampling appeared random; the reproducibility of the HITP computer scores is shown in Table III. Compared to the variation of the entire data set taken over the same 4 days, the analytical variability is significantly smaller indicating the analytical method is capable of measuring biological variability among colonies (see Figure 6 and Table IV). The results show no significant classification differences in HITP values across days by Tukey-Kramer comparisons at the P = 0.05 significance level. Since every colony within this study was analyzed, colonies are generally more grey or purple and darker than the normally blue nontransformed colonies (Figure 1a versus 1b). In the image analysis system this results in lower hue values and higher intensity values for the transformed colonies; saturation values remain fairly consistent between transformed and nontransformed colonies. The average intensity of the ‘texture’ derived image is lighter for transformed colonies (Figure 1c versus 1d), while the average intensity of the ‘pattern’ derived image is greater for the nontransformed colonies (Figure 1e versus 1f).

Classification of colonies by individual and linear combinations of features
The ability of the individual features to discriminate the transformed from non-transformed classes of colonies are shown in Figure 2a–e for the entire data set of nearly 500 colonies. The combination of features that best discriminate the classes determined by a nominal logistic regression is shown in Figure 2f. Colony area (data not shown) and saturation were insignificant features compared to hue, intensity, texture and pattern (HITP) and were eliminated from consideration. The HITP combination of features was highly significant (P<0.0001) and the regression explained >73% of the total variation. The computer score defined by this regression is:

\[
\text{HITP Score} = 0.14 \times \text{Hue} - 0.092 \times \text{Intensity} - 0.042 \times \text{Texture} + 0.11 \times \text{Pattern} - 9.28
\]

The distributions of the computer scores for the two classes are shown in Figure 3. A threshold of 1.0 (Table IV). All of the errors were reviewed with expert graders and no obvious misclassifications were detected; e.g., all of the errors were also difficult to classify visually. The progression of changes in the appearance of colonies associated with HITP scores is shown in Figure 4 for colonies of average size. The range in colony appearance for transformed colonies with similar HITP scores (~3 to ~4) are shown in Figure 5a,c,e and for nontransformed cells (HITP scores of 4 to 5) in Figure 5b,d,f.

\[
\text{Area} = 0.036 \times \text{Hue} - 0.070 \times \text{Intensity} - 0.056 \times \text{Texture} + 0.029 \times \text{Pattern} + 0.100
\]

In summary, each of the three features (color, texture and pattern) were transformed into grey level images whose intensities, when normalized to the ROI, provide a continuous value proportional to each feature independent of colony size.

### Results
The images in Figure 1 visually depict the measurement of color, texture and pattern for a transformed (Figure 1a,c,e) and a nontransformed (Figure 1b,d and f) colony. These two colonies have nearly the average values for each of the measured features in each class. These images show the circular ROI whose position is based on the centroid of the colony and whose area is based on cell density. The transformed colonies are generally more grey or purple and darker than the normally blue nontransformed colonies (Figure 1a versus 1b). In the image analysis system this results in lower hue values and higher intensity values for the transformed colonies; saturation values remain fairly consistent between transformed and nontransformed colonies. The average intensity of the ‘texture’ derived image is lighter for transformed colonies (Figure 1c versus 1d), while the average intensity of the ‘pattern’ derived image is greater for the nontransformed colonies (Figure 1e versus 1f).

### Table I. Comparison of visual and computer classification of nontransformed (N) and transformed (T) SHE colonies

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>T</th>
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<tr>
<td>Visual classification</td>
<td>367</td>
<td>7</td>
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<tr>
<td>Computer classification</td>
<td>93%</td>
<td>7%</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>96</td>
</tr>
<tr>
<td>Total</td>
<td>393</td>
<td>103</td>
</tr>
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</table>

### Table II. Cross-correlation of colony features measured by image analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Area</th>
<th>Hue</th>
<th>Texture</th>
<th>Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>0.293</td>
<td>0.339</td>
<td>0.461</td>
<td></td>
</tr>
<tr>
<td>Hue</td>
<td>1.000</td>
<td>0.042</td>
<td>-0.726</td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>0.427</td>
<td>0.025</td>
<td>-0.787</td>
<td></td>
</tr>
<tr>
<td>Pattern</td>
<td>-0.038</td>
<td>0.685</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HITP</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The ability of the individual features to discriminate the transformed from non-transformed classes of colonies are shown in Figure 2a–e for the entire data set of nearly 500 colonies. The combination of features that best discriminate the classes determined by a nominal logistic regression is shown in Figure 2f. Colony area (data not shown) and saturation were insignificant features compared to hue, intensity, texture and pattern (HITP) and were eliminated from consideration. The HITP combination of features was highly significant (P<0.0001) and the regression explained >73% of the total variation. The computer score defined by this regression is:

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\]
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Fig. 4. Images of representative colonies with progressively increasing HITP scores (lowest scores are more likely transformed; highest are more likely nontransformed; cutoff between classes is 1.0). The HITP scores represented by images are (a) -7.6, (b) -4.1, (c) -1.8, (d) 1.8, (e) 4.7, (f) 7.4, (g) 10.7, (h) 13.9 and (i) 18.4.

this data set is unbiased to operator subsampling of nontransformed colonies.

Study-to-study variation
Results were obtained for three studies performed over a year’s time where wild type target cells were treated with benzo[a]pyrene. The dishes were stained and stored for months until they were subjected to image analysis over the period of several weeks. The results shown in Figure 7 and Table IV show no significant classification differences in HITP values across studies with benzo[a]pyrene even when studies were performed and stained at different times and stored for long periods of time.

Target cell dependence
To test whether computer scores would show the same class related differences for ring-cloned clonal target cell populations as well as wild type target cells, colonies from two clonal populations were exposed to benzo[a]pyrene and analyzed (see Figure 8 and Table IV). While only small numbers of colonies were sampled, the transformed colonies were identified correctly. The mean computer score of the nontransformed clonal colonies, however, were significantly closer to the threshold than the wild type nontransformed colonies. This suggests the possibility that the subpopulations of cells giving rise to these clones may be more susceptible to transformation than wild type cells. In any case, the clonal subpopulations show narrower distributions of HITP values than do the wild type, indicating that much of the scatter in wild type HITP scores is due to differences in target cell types.

Test material dependence
Finally, the data set was analyzed to determine if the computer scoring was influenced by the test substance. As shown in Figure 9 and Table IV, treatment with a negative control (dimethyl sulfoxide [DMSO]), and three transforming chemicals (benzo[a]pyrene, lead acetate and 12-O-tetradecanoylphorbol-13-acetate [TPA]) resulted in the correct classification of colonies with no measurable differences in group means within each class. The differences between classes were preserved even though the original experiments had been performed and the stained plates stored over the span of >1 year.
Variations due to colony size at two different HITP scores. Colonies in a, c, and e have HITP scores within the range of -2.5 to -4 and colonies b, d, and f have HITP scores in the range of 4 to 5.

Table III. Reproducibility of HITP computer scores for five different colonies

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-2.5 ± 1.5</td>
</tr>
<tr>
<td>2</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>3.5 ± 1.3</td>
</tr>
<tr>
<td>4</td>
<td>7.2 ± 1.3</td>
</tr>
<tr>
<td>5</td>
<td>9.6 ± 1.2</td>
</tr>
</tbody>
</table>

Discussion

We present here a method to objectively measure features of colonies that have been associated with cell transformation in the low-pH SHE assay. A linear combination of these feature measurements can be shown to correlate well with expert visual assessment. Repetitive analysis of individual colonies demonstrates that the method is capable of measuring biological differences among the colonies. Being able to classify transformed cells from both clonal as well as treated wild type target cells is consistent with the current transformation model, in which cells express specific phenotypic changes due to the influence of chemical exposure. Importantly, this provides evidence that previous conclusions from visual grading can be regarded with confidence and serves as a basis for future automation of the assay.

The mathematical model is based on the correlation of selected feature measurements with visual assessment and is not based on any mechanistic model to predict why chemical exposure causes the measured changes. For instance, we do not know the chemical basis for the color difference and can only speculate as to the cause of texture or pattern differences (disruption of cell-cell communication, etc.). Further, we have no biological basis for using linear combinations of features. However, with this technique we accurately mimic the visual classification of the colonies. While our data fit this two-stage model reasonably well, what we are modeling is the visual perception used in scoring rather than the actual biological transformation process. Because the training data set is large \( n = 500 \), the number of measured features is small \( n = 4 \) and the results are consistent regardless of chemical or date.
Analysis of three different studies using benzo[a]pyrene as a test material are compared. These studies were performed at different times over the course of a year. Sources of error include variables in target cell isolates, culturing conditions, material exposure, fixation and staining, visual scoring, image acquisition and computer scoring. Small differences that may exist do not interfere with the agreement between visual and computer scoring.

Transformed colonies using clonal target cells were identified by both visual and computer scoring. The means and distributions of the HITP scores for nontransformed colonies from clonal target cells appear to represent a subset of the nontransformed wild type with scores shifted slightly toward the transformed. These clones were isolated based on their higher rate of transformation (visual assessment) after benzo[a]pyrene exposure.

The agreement between visual classification and computer scoring are independent of the materials tested. These studies were performed at various times over the period of 1 year. While the number of materials is small, all identifiable sources of error are contained in these studies.

From the standpoint of automation, the greatest drawback of the assay is the requirement that we obtain images of individual colonies at low magnification (12.5×). Manual selection of colonies on culture plates under a microscope is time-consuming and monotonous. Overlapping colonies are difficult to separate and colonies at the edges can be inaccessible or distorted by the edge of the plate. Solutions to these problems can be engineered, but would require some modification in culture conditions and some level of revalidation of the assay. Analysis of colonies with no magnification using a macro lens on the camera (data not shown) did not have sufficient resolution to: (i) eliminate the contribution of background illumination (light coming from spaces around the cells) or (ii) measure differences in texture or pattern. This is consistent with human experience as visual scoring usually involves inspection of the colonies at magnifications ranging from 13–40×.

The characterization of mean values and variance of the transformed and nontransformed populations from our data set can be used to determine the experimental parameters for practical use. Most of our data set was enriched for transformed colonies by randomly subsampling the nontransformed colonies. The complete data set contains colony images in the ratio of 1:4 transformed: nontransformed, making the differences easier to demonstrate. If we model the analysis of our data set as a single step transformation resulting in two gaussian distributions, we can easily see the differences in the mean values and the overlap of the two populations (Figure 3).

In practice, it is estimated from visual grading that strong transforming agents increase the transformation from a background rate of 0.1–0.5% to a maximum of 2–5%. These
numbers are based on using a visual mental threshold to classify colonies as transformed or nontransformed. Using such a threshold will increasingly underestimate the actual number of transformed colonies as the degree of overlap between the two classes increases. In scoring the colonies visually, the effect is that only the colonies with marked differences are counted as transformed. Those with only slight differences were once counted as ‘altered’ but this category was abandoned because it had limited diagnostic power due to the overlap with nontransformed colonies. This may explain the advantage in sensitivity of the low-pH assay in that the differences between the two categories is greater and the overlap is reduced. Because image analysis provides continuous measures of the features and combined HTP values, we can use the distribution information and mathematical modeling techniques to more accurately predict actual transformation rates assuming different models.

The implications for practical use of an image analysis system to objectively score the SHE assay are readily apparent: (i) because of the overlap and low frequency of transformation, analyzing large numbers of colonies will still be required to provide a statistically significant increase in transformed colonies over background; (ii) a subtraction or deconvolution routine may work better than a simple threshold to calculate accurate transformation rates; (iii) automation of the image acquisition process will be the rate limiting step in throughput; and (iv) enhancement of the transformation process by optimizing growth conditions (low pH, etc.) or eliminating insensitive cell types may provide better sensitivity. This work demonstrates that we can mimic the visual scoring of SHE cell transformation based on the measurement of relatively subtle changes in the color, texture and pattern of SHE colonies grown at low pH. The work also supports that visual scoring of the MT phenotype is based on consistent changes in colony morphology and can be accomplished reproducibly by trained individuals. Until automated image acquisition issues can be addressed, visual scoring of the SHE assay will remain the most time and cost efficient method of choice.

References


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