We claim:

1. A method for generating a brightfield type image that resembles a brightfield staining protocol comprising the steps of:

acquiring image data of two or more fluorescent images of a fixed area on a biological sample;

analyzing the image data utilizing, at least in part, featured based information or pixel intensity data information to generate mapping parameters wherein said mapping parameters comprises a nonlinear estimation model;

applying said mapping parameters to the fluorescent images;

transforming the two or more fluorescent imaging into a brightfield color space; and

generating a brightfield type image.;

~~2. The method of claim 1~~ wherein the nonlinear estimation model is defined as:

$$R = 255 \exp(- a[Dye1]*z[Dyel] - a[Dye2]z[Dye2] -... a[Dyen]z[Dyen])$$

$$G = 255 \exp(- b[Dyel]*z[Dyel] - b[Dye2]z[Dye2] -...)$$

$$B = 255 \exp(- c[Dyel]*z[Dyel] - c[Dye2]z[Dye2] -...);$$

and wherein R, G, and B are resulting red, green and blue pixel values in the brightfield type image;.

and z is a scaling coefficient for a fluorescent dye quantities observed at a given pixel location; and

a, b, and c are the extinction coefficients corresponding to the brightfield color space. and wherein the triples ,a[Dyen], b[Dyen], c[Dyen], are a constant times the extinction coefficients of the nth dye in the virtual stain as defined using a preselected or desired color.

23. The method of claim 12 wherein the constants [Dyen] are chosen such that:

min(exp(-a[Dyen]*z[Dyen]),exp(-b[Dyen]*z[Dyen]),exp(-c[Dyen]*z[Dyen])) = 1/255.

34. The method of any preceding claim claim 1 or claim 2, further comprising the step of applying a sharpening transformation correction to the brightfield type image.

45. The method of claim 34 wherein said sharpening transformation correction comprising a convolution filter whose kernel is the matrix:

$$\begin{bmatrix} -0.25 & -0.25 & -0.25 \\ -0.25 & 3.00 & -0.25 \\ -0.25 & -0.25 & -0.25 \end{bmatrix}.$$

56. The method of any preceding claim, wherein the brightfield type image corresponds to an immunostained type image having a red, green, and blue three channel color space.

67. The method of any preceding claim, wherein at least one image of the two or more fluorescent images is of autofluorescence.

78. The method of any preceding claim, wherein the acquiring a brightfield image step comprises the steps of sequentially staining the biological sample with two or more histochemical or immunohistochemical stains.

89. The method of any preceding claim, wherein the feature based information comprises one or more features selected from a group consisting of nuclei, epithelia, and stroma.

910. The method of any preceding claim, further comprising the step of applying the mapping parameters to two or more fluorescent images of a second fixed area wherein the second fixed area is from the same or a different biological sample.

1011. The method of any preceding claim, further comprising the step of pathological diagnostics using said brightfield type image.

1112. The method of claim 1011 wherein the pathological diagnostics is for cancer.

1213. The method of any preceding claim, further comprising the step of quantitative analysis using said brightfield type image.

1314. The method of claim 1213 wherein the step of quantitative analysis comprises identifying molecular pathways as a function of one or more morphological structures selected from a group consisting of nuclei, epithelia, and stroma.

1415. An image analysis system for generating a brightfield type image that resembles a brightfield staining protocol of a biological sample, using fluorescent images comprising:

a digital imaging device adapted to acquire two or more fluorescent images of a fixed area on a biological sample;

a processing device configured to;

analyzing the image data utilizing, at least in part, featured based information or pixel intensity data information to generate mapping parameters wherein said mapping parameters comprises a nonliner estimation model;

applying said mapping parameters to the fluorescent images;

transforming the two or more fluorescent imaging into a brightfield color space; and

generating a brightfield type image; and

a display device for displaying said brightfield image;.

~~16. The system of claim 15~~ wherein the nonliner model is defined as:

R = 255 exp(- a[Dyel]*z[Dyel] - a[Dye2]z[Dye2] -... a[Dyen]z[Dyen])

G = 255 exp(- b[Dyel]*z[Dyel] - b[Dye2]z[Dye2] -...)

B - 255 exp(- c[Dyel]*z[Dyel] - c[Dye2]z[Dye2] -...);

and wherein R, G, and B are resulting red, green and blue pixel values in the brightfield type image;~~.~~

and z is a scaling coefficient for a fluorescent dye quantities observed at a given pixel location; and

a, b, and c are the extinction coefficients corresponding to the brightfield color space~~.~~ and wherein the triples ,a[Dyen], b[Dycn], c[Dyen], are a constant times the extinction coefficients of the nth dye in the virtual stain as defined using a preselected or desired color.

15~~17~~. The system of claim 14~~16~~ wherein the constants [Dyen] are chosen such that:

min(exp(-a[Dyen]*z[Dyen]),exp(-b[Dyen]*z[Dyen]),exp(-c[Dyen]*z[Dyen])) = 1/255.

16~~18~~. The system of ~~any one of claims 15 to 17~~ claim 14 or claim 15, further comprising applying a sharpening transformation correction to the brightfield type image.

17~~19~~. The system of claim 16~~18~~ wherein ~~—~~said sharpening transformation correction comprising a convolution filter whose kernel is the matrix:

$$\begin{bmatrix} -0.25 & -0.25 & -0.25 \\ -0.25 & 3.00 & -0.25 \\ -0.25 & -0.25 & -0.25 \end{bmatrix}.$$

1820. The system of any one of claims 1415 to 1719, wherein the processing device is further configured to store mapping parameters from one or more previously analyzed biological samples.