CLAIMS

What is claimed is:

1. A method of autofocusing an image, comprising:

optionally, receiving a sample holder at an imaging system;

optionally, based at least in part on the sample holder, automatically determining a z-axis step size for a nominal focus range;

receiving a plurality of images over the nominal focus range;

generating input data based on the plurality of images, the input data being generated via one or more image processing operations;

utilizing the input data as input to a machine learning module configured to provide target focus position output responsive to input generated from images associated with one or more nominal focus ranges;

obtaining a target focus position based on output of the machine learning module; and

configuring the imaging system to capture an image using the target focus position.

2. The method of claim 1, wherein the one or more image processing operations comprise connected component analysis, and wherein the input data comprises one or more connected components.

3. The method of claim 1, further comprising:

identifying one or more connected components within each image;

sorting the identified connected components based on a number of pixels associated with each connected component;

generating a focus quality estimate of at least a portion of the sorted connected components using the machine learning module; and

identifying a target focus position based on the focus quality estimate of the sorted connected components.

4. The method of claim 3, wherein capturing a plurality of images comprises capturing a 1280 x 960 8-bit image at each z-axis step over the nominal focus range.

5. The method of any one of claims 1-4, wherein each z-axis step comprises a pre-determined size automatically determined based on a sample holder detected at the imaging system.

6. The method of claim 3 or claim 4, wherein identifying one or more connected components in each image comprises:

thresholding each image to obtain a resultant binary image for each image;

applying one or more morphological operators to the resultant binary image; and

defining one or more connected components within the resultant binary image.

7. The method of claim 6, wherein thresholding is based on a difference between minimum and maximum images for the nominal focus range.

8. The method of claim 6 or claim 7, wherein applying the one or more morphological operators comprises applying one or more of a morphological closing, a morphological opening, and/or a foreground hole fill.

9. The method of claim 8, wherein the morphological closing comprises a 2 x 2 morphological closing.

10. The method of claim 8 or claim 9, wherein the morphological opening comprises a 2 x 2 morphological opening.

11. The method of any one of claims 8–10, wherein the foreground hole fill comprises an 8-connected foreground hole fill.

12. The method of any one of claims 6–11, wherein identifying one or more connected components in each image comprises:

measuring first and second binary moments of each connected component;

fitting a corresponding ellipse with equivalent moments to each connected component; and

creating a second binary image comprising the corresponding ellipses.

13. The method of claim 12, further comprising measuring each connected component in the second binary image and removing any connected component having an ellipse minor axis less than 10 μm, preferably less than 7 μm, more preferably less than 5 μm.

14. The method of any one of claims 3–13, wherein sorting the identified one or more connected components comprises:

counting the number of pixels in each connected component;

calculating a median pixel count over the one or more connected components; and

sorting the one or more connected components based on a corresponding absolute difference of the number of pixels from the median pixel count.

15. The method of claim 14, wherein the one or more connected components are sorted in ascending order by the corresponding absolute difference of the number of pixels from the median pixel count.

16. The method of any one of claims 3-15, wherein sorting the identified one or more connected components comprises:

determining one or more of a circularity or a brightness of each connected component; and

sorting the one or more connected components based on a comparative circularity and/or brightness determined for each of the connected components.

17. The method of any one of claims 3-16, further comprising forming a 32 x 32 pixel z-stack for each connected component.

18. The method of any one of claims 3-17, wherein the machine learning module comprises an artificial neural network receiving the 32 x 32-pixel z-stack for each connected component as an input.

19. he method of claim 18, wherein the artificial neural network comprises a plurality of feature identification layers having a design pattern of a convolution layer, a linear layer, and a max-pooling layer.

20. The method of claim 19, wherein the convolution layer comprises a 3x3 convolution layer.

21. The method of claim 19, wherein the linear layer comprises a ReLu nonlinearity function.

22. The method of any one of claims 19-21, wherein the max-pooling layer comprises a 2 x 2 max-pooling layer.

23. The method of any one of claims 19-22, wherein the artificial neural network comprises a long-short-term-memory (LSTM) process layer following the plurality of feature identification layers.

24. The method of claim 23, wherein the LSTM layer processes the z-stack for each connected component in a bi-directional manner.

25. The method of claim 23 or claim 24, wherein the artificial neural network comprises a final linear layer, wherein the final linear layer is combined with an output of the LSTM layer to define the focus quality estimate.

26. A method of autofocusing an image, comprising:

receiving a sample holder at an imaging system;

based at least in part on the sample holder, automatically determining a z-axis step size for a nominal focus range;

receive a plurality of images over the nominal focus range;

obtaining a target focus position; and

~~configuring the imaging system to capture an image using the target focus position.~~

~~27. A computer system configured to autofocus an imaging system, comprising one or more processors and one or more hardware storage devices having stored thereon computer-executable instructions that when executed by the one or more processors configure the computer system to perform the method of any one of claims 1-26.~~

~~28. One or more hardware storage devices storing instructions that are executable by one or more processing devices of an imaging system to configure the imaging system to perform the method of any one of claims 1-26.~~

~~29.~~1. A method of performing automated cell viability counting, comprising:

capturing an image at a target focus position~~, the target focus position being calculated as in any one of claims 1-26~~;

spatially downsampling the captured image to form a downsampled image;

decomposing the downsampled image into a plurality of tiles;

storing the plurality of tiles in a tensor array;

processing the tensor array using a convolutional neural network;

constructing a plurality of pseudo-probability maps based on an output of the convolutional neural network;

thresholding the plurality of pseudo-probability maps to generate binarized location maps and binarized mask maps;

generating a segmented downsampled image by segmenting the downsampled image using the binarized location maps for seeding and the binarized mask maps for delimitating cell areas, wherein the segmented downsampled image indicates/provides a cell viability count; and

displaying a representation of the cell viability count.

~~30.~~2. The method of claim ~~29~~1, wherein the convolutional neural network is trained with images from multiple focal planes relative to the focus position, preferably ranging from -2 to +2 z-position.

~~31. The method of claim 29, wherein the image comprises a 2592 x 1944 8-bit image having a pixel size of 0.871 µm.~~

~~32.~~3. The method of claim ~~29~~ 1 or claim ~~30~~2, wherein spatially downsampling the captured image comprises downsampling the captured image by a factor of 2 via pixel decimation, optionally resulting in a 1296 x 972 downsampled image.

~~33.~~4. The method of any one of claims ~~29–32~~1-3, wherein decomposing the downsampled image into a plurality of tiles comprises:

reflecting the downsampled image to extend its edges by a plurality of pixels, forming a reflected image; and

decomposing the reflected image into the plurality of tiles with pixel overlap.

~~34.~~5. The method of claim ~~33~~4, wherein reflecting the downsampled image extends its edges by 4-12 pixels, or 6-10 pixels, or about 8 pixels.

~~35.~~6. The method of claim ~~33–~~4 or claim ~~34~~5, wherein the reflected image is decomposed into 80-190 tiles, or 90-180 tiles, or 100-170 tiles, or 110-160 tiles, or 120-150 tiles, or about 130 tiles, optionally with size of 128 x 128 pixels with pixel overlap.

~~36. The method of any one of claims 33–35, wherein the tensor array comprises a 130 x 1 x 128 x 128 tensor array.~~

~~37.~~7. The method of any one of claims ~~29–36~~1-6, wherein the convolutional neural network comprises a U-net convolutional neural network.

~~38.~~8. The method of any one of claims ~~29–37~~1-7, wherein the output of the convolutional neural network is stored in a 130 x 4 x 128 x 128 tensor array.

~~39.~~9. The method of any one of claims ~~29–38~~1-8, wherein constructing the plurality of pseudo-probability maps comprises converting the output of the convolutional neural network into an 8-bit format tensor and image stitching the 8-bit format tensor.

~~40.~~10. The method of claim ~~39~~9, wherein converting the output into the 8-bit format tensor comprises multiplying the 130 x 4 x 128 x 128 tensor array by a multiplier, optionally wherein the multiplier is about 255.

~~41.~~11. The method of claim ~~39–~~9 or claim ~~40~~10, wherein image stitching the 8-bit format tensor comprises constructing 4 full size pseudo-probability maps from the tensor array.

~~42.~~12. The method of claim ~~41~~11, wherein the output of the convolutional neural network comprises pseudo-probability maps associated with (i) a location of live cells, (ii) a mask of live cells, (iii) a location of dead cells, and (iv) a mask of dead cells.

~~43.~~13. The method of any one of claims ~~29–42~~1-12, wherein thresholding comprises applying a threshold value greater than 50% to 90% pseudoprobability, or about 75% pseudoprobability.

~~44.~~14. The method of any one of claims ~~29–42~~1-13, wherein the downsampled image is segmented by watershed transform applied on a distance map computed from respective locations of live/dead cells and delimitated by the respective mask of live/dead cells.

~~45.~~15. The method of any one of claims ~~29–44~~1-14, further comprising ellipse fitting cells within the segmented downsampled image.

~~46.~~16. The method of claim ~~45~~15, wherein ellipse fitting comprises identifying one or more objects ~~as in any one of claims 6–13~~in an image.

~~47.~~17. The method of claim ~~46~~16, wherein ellipse fitting comprises measuring object size, constructing a histogram of object size, pixel intensity, and calculating object circularity.

~~48.~~18. A computer system configured to perform automated cell viability counting, comprising one or more processors and one or more hardware storage devices having stored thereon computer-executable instructions that when executed by the one or more processors configure the computer system to perform the method of any one of claims ~~29–47~~1-17.

~~49.~~19. A method of performing automated cell viability counting, comprising:

obtaining an image;

defining a set of one or more tiles based on the image;

processing the one or more tiles using a convolutional neural network;

generating a plurality of pseudo-probability maps based on an output of the convolutional neural network, the plurality of pseudo-probability maps comprising at least one or more seed likelihood images; and

generating one or more masks based on the one or more seed likelihood images, the one or more masks defining pixel locations for one or more objects represented in the one or more seed likelihood images, the one or more masks indicating/providing a cell count.

~~50.~~20. The method of claim ~~49~~19, further comprising performing connected-component analysis on the one or more seed likelihood images to determine the cell count.

~~51. The method of any one of claims 49–50, wherein:~~

~~the plurality of pseudo-probability maps further comprises one or more whole cell likelihood images, and~~

~~the method further comprises generating one or more segmented images based on at least the one or more whole cell likelihood images and the one or more masks, wherein the one or more segmented images indicates/provides a cell count.~~

~~52. The method of any one of claims 49–51, wherein the image is captured at a target focus position, the target focus position being calculated as in any one of claims 1–25.~~

~~53.~~21. The method of any one of claims ~~49–52~~19-20, wherein the convolutional neural network is trained with a set of training data comprising images from multiple focal planes relative to a focus position, preferably ranging from -2 to +2 z-position.

~~54.~~22. The method of claim ~~53~~21, wherein the set of training data comprises ground truth output including one or more whole cell binary masks and/or one or more seed masks.

~~55. The method of any one of claims 53–54, wherein the images of the set of training data comprise pre-processed images.~~

~~56. The method of claim 49, wherein the image comprises a 2592 x 1944 8-bit image having a pixel size of 0.871 μm.~~

~~57.~~23. The method of any one of claims ~~49–56~~19-22, further comprising performing one or more pre-processing operations, wherein the one or more pre-processing operations are performed based on the image to generate a pre-processed image, and wherein the set of one or more tiles is decomposed from the pre-processed image, or the one or more pre-processing operations are performed on the one or more tiles.

~~58. The method of claim 57, wherein the one or more pre-processing operations are performed based on the image to generate a pre-processed image, and wherein the set of one or more tiles is decomposed from the pre-processed image, or the one or more pre-processing operations are performed on the one or more tiles.~~

24. The method of ~~any one of claims 57–58~~claim 23, wherein the one or more pre-processing operations comprise a downsampling operation, a background removal operation, and/or a pixel or voxel intensity normalization operation.

25. A method of autofocusing an image, comprising:

optionally, receiving a sample holder at an imaging system;

optionally, based at least in part on the sample holder, automatically determining a z-axis step size for a nominal focus range;

receiving a plurality of images over the nominal focus range;

generating input data based on the plurality of images, the input data being generated via one or more image processing operations;

utilizing the input data as input to a machine learning module configured to provide target focus position output responsive to input generated from images associated with one or more nominal focus ranges;

obtaining a target focus position based on output of the machine learning module; and

~~59.~~ configuring the imaging system to capture an image using the target focus position.

~~60. The method of claim 59, wherein the downsampling operation utilizes an averaging filter.~~

~~61. The method of claim 60, wherein the downsampling operation reduces image size by a factor of at least 2.~~

~~62. The method of any one of claims 57-61, wherein the one or more pre-processing operations comprise a background removal operation.~~

~~63. The method of claim 62, wherein the background removal operation comprises:~~

~~estimating a background by calculating a per-pixel local mean within a radius of each pixel; and~~

~~subtracting the background from the image.~~

~~64. The method of claim 62, wherein the background removal operation comprises:~~

~~calculating a respective minimum value in each image region of the image or a downsampled image; and~~

~~fitting a surface through the respective minimum values.~~

~~65. The method of any one of claims 57-64, wherein the one or more pre-processing operations comprise a pixel or voxel intensity normalization operation.~~

~~66. The method of claim 65, wherein the pixel or voxel intensity normalization operation comprises a global normalization operation.~~

~~67. The method of claim 65, wherein the pixel or voxel intensity normalization operation comprises a kernel-based normalization operation.~~

~~68. The method of any one of claims 57-67, wherein at least some of the one or more pre-processing operations are performed as a batch process on the one or more tiles provided as input to the convolutional neural network.~~

~~69. The method of any one of claims 49-68, wherein defining the set of one or more tiles comprises:~~

~~reflecting the image or pre-processed image to extend its edges by a plurality of pixels, forming a reflected image;~~

~~decomposing the reflected image into the set of one or more tiles with pixel overlap; and~~

~~storing the set of one or more tiles into a tensor array.~~

~~70. The method of claim 69, wherein reflecting the image or pre-processed image extends its edges by 4-12 pixels, or 6-10 pixels, or about 8 pixels.~~

~~71. The method of any one of claims 69-70, wherein the reflected image is decomposed into 80-190 tiles, or 90-180 tiles, or 100-170 tiles, or 110-160 tiles, or 120-150 tiles, or about 130 tiles, optionally with size of 128 x 128 pixels with pixel overlap.~~

~~72. The method of any one of claims 69-71, wherein the tensor array comprises a 130 x 1 x 128 x 128 tensor array.~~

~~73. The method of any one of claims 49-72, wherein the convolutional neural network comprises a U-net convolutional neural network.~~

~~74. The method of any one of claims 49-73, wherein the output of the convolutional neural network is stored in a 130 x 4 x 128 x 128 tensor array.~~

~~75. The method of any one of claims 49-74, wherein generating the plurality of pseudo-probability maps comprises converting the output of the convolutional neural network into an 8-bit format tensor and image stitching the 8-bit format tensor.~~

~~76. The method of claim 75, wherein converting the output into the 8-bit format tensor comprises multiplying the 130 x 4 x 128 x 128 tensor array by a multiplier, optionally wherein the multiplier is about 255.~~

~~77. The method of claim 75 or claim 76, wherein image stitching the 8-bit format tensor comprises constructing a plurality of full-size pseudo-probability maps from the tensor array.~~

~~78. The method of claim 77, wherein the plurality of pseudo-probability maps comprises (i) one or more live cell seed likelihood images, , (ii) one or more live whole cell likelihood images, (iii) one or more dead cell seed likelihood images, and (iv) one or more dead whole cell likelihood images,.~~

~~79. The method of any one of claims 49-78, wherein generating the one or more masks comprises applying a threshold value greater than 50% to 90% likelihood, or about 75% likelihood.~~

~~80. The method of claim 79, the threshold value corresponding to a threshold value of 192 (0.75 x 255) in pixel intensity in the plurality of pseudo-probability maps.~~

~~81. The method of claim 79 or claim 80, wherein generating the one or more masks further comprises applying connected-component labeling to detect connected regions.~~

~~82. The method of any one of claims 49-81, wherein the one or more masks are generated utilizing a deep learning algorithm.~~

~~83. The method of any one of claims 51-82, wherein generating the one or more segmented images comprises applying a watershed transform on a distance map computed from the pixel locations of the one or more objects represented in the one or~~

more seed likelihood images and delimitated by the one or more whole cell likelihood images.

84. The method of any one of claims 49–83, further comprising determining the cell count based on the output of the convolutional neural network.

85. The method of any one of claims 49–84, further comprising displaying the cell count.

86. The method of any one of claims 51–85, further comprising performing one or more feature identification operations using the one or more segmented images.

87. The method of claim 86, wherein the one or more feature calculation operations comprises ellipse-fitting cells within the one or more segmented images.

88. The method of claim 87, wherein ellipse-fitting comprises one or more of measuring object size, constructing a histogram of object size or pixel intensity, or calculating object circularity.

89. The method of any one of claims 49–88, wherein the image comprises a monochrome image.

90. A cell counter configured to perform automated cell counting, comprising one or more processors and one or more hardware storage devices having stored thereon computer-executable instructions that, when executed by the one or more processors, configure the cell counter to perform the method of any one of claims 49–89.

91. A flow cytometer configured to perform automated cell counting, comprising one or more processors and one or more hardware storage devices having stored thereon computer-executable instructions that, when executed by the one or more processors, configure the cell counter to perform the method of any one of claims 49–89.