(or descending) order by the corresponding absolute difference of the number of pixels from the median pixel count.

[0010] In one aspect, the act of sorting the identified one or more connected components includes ordering a calculated joint probability that the connected component represents (i) a cell and optionally (ii) that the cell is alive. The connected components having the greatest joint probability can be sorted for further evaluation of the focus quality estimate using a machine learning module.

[0011] In one aspect, the act of sorting the identified one or more connected components includes determining one or more of a circularity or a brightness of each connected component, and sorting the connected components based on a comparative circularity and/or brightness determined for each of the connected components.

[0012] In one aspect, methods of autofocusing an imaging system can include forming a z-stack of a particular pixel dimension (e.g., 32 x 32 or other appropriate size) for each connected component. In one aspect, the machine learning module includes an artificial neural network that receives the pixel z-stack for each connected component as an input. In one aspect, the artificial neural network includes a plurality of feature identification layers having a design pattern of a convolution layer, a linear layer, and a max-pooling layer. In one aspect, the convolution layer includes a 3 x 3 convolution layer, the linear layer includes a ReLu nonlinearity function, and/or the max-pooling layer includes a 2 x 2 max-pooling layer or average pooling layer.

[0013] In one aspect, the artificial neural network includes a long-short-term-memory (LSTM) process layer following the plurality of feature identification layers, and the LSTM layer processes the z-stack for each connected component in a bi-directional manner. In one aspect, the artificial neural network includes a final linear layer that is combined with an output of the LSTM layer to define the focus quality estimate.

[0014] Embodiments of the present disclosure additionally include computer systems configured to autofocus an imaging system. In one aspect, a computer system configured to autofocus an imaging system includes 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 any one or more of the methods disclosed herein.

[0014a] Embodiments of the present disclosure additionally include a method of performing automated cell viability counting, comprising: capturing an image at a target focus position; 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.

[0014b] Embodiments of the present disclosure additionally include 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.

[0015] Embodiments of the present disclosure may additionally include methods of performing automated cell viability counting that include the acts of obtaining an image and

defining a set of one or more tiles based on the image, processing the one or more tiles using a convolutional neural network, and ~~constructing~~ generating a plurality of pseudo-probability maps based on output of the convolutional neural network. The plurality of pseudo-probability maps may include at least one or more seed likelihood images. The acts may further include generating one or more masks based on the one or more seed likelihood images. The one or more masks may define pixel locations for one or more objects represented in the one or more seed likelihood images. The one or more masks may indicate/provide a cell count.

[0016] In one aspect, methods of performing automated cell counting include performing connected-component analysis on the one or more seed likelihood images to determine the cell count.

[0017] In one aspect, the plurality of pseudo-probability maps further comprises one or more whole cell likelihood images, and the methods of performing automated cell counting include 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.

[0018] In one aspect, the image is captured at a target focus position.

[0019] In one aspect, the convolutional neural network is trained with a set of training data comprising images from multiple focal planes relative to the focus position, preferably ranging from -2 to +2 z-position, to increase a robustness of the method and to reduce a sensitivity of the method to autofocus output accuracy. The set of training data may comprise ground truth output including one or more whole cell binary masks and/or one or more seed masks. The images of the set of training data may comprise pre-processed images.

[0020] In one aspect, the image comprises a 2592 x 1944 monochrome 8-bit image having a pixel size of 0.871 μm. Other types of images (e.g., with different dimensions, bit values, and/or pixel size) may also be utilized.

[0021] In one aspect, methods of performing automated cell counting further include performing one or more pre-processing operations. The one or more pre-processing operations may be performed based on the image to generate a pre-processed image. The set of one or more tiles may be decomposed from the pre-processed image, or the one or more pre-processing operations may be performed on the one or more tiles.