

SYSTEMS AND METHODS FOR FACILITATING CLONE SELECTION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] Priority is claimed to U.S. Provisional Patent Application No. 62/774,154, filed November 30, 2018, the entire contents of which are hereby incorporated herein by reference.

FIELD OF DISCLOSURE

[0002] The present application relates generally to clone selection techniques, and more specifically relates to techniques for determining whether a cell colony has formed from a single clone/cell.

BACKGROUND

[0003] In various cell line development processes, clone selection requires knowledge of whether a cell colony formed from a single clone cell. For example, individual cells may be inoculated into separate wells (e.g., of a 96-well plate) by a flow cytometry technique such as a fluorescence-activated cell sorting (FACS) technique, followed by incubation of the cells for a suitable time period (e.g., 14 days). Throughout the incubation period, digital images of the wells are captured at suitable time intervals, such as every day, or every seven days, etc. An analyst reviews the well images captured at the end of the incubation period (e.g., the 14th day) in order to identify cell colonies and, if a particular well contains a colony, the analyst may also review one or more earlier images of that same well (e.g., an image captured on the first day of incubation) to determine whether the colony formed from only a single clone. If the analyst can confirm that the colony formed from a single clone, that particular sample may be forwarded to one or more additional stages of the cell line development process. If not, the sample may be discarded.

[0004] This process is very time consuming and tedious. It is not unusual, for example, for a single project to require hundreds of man-hours spent analyzing microscopic images. Moreover, it can be difficult for an analyst to accurately identify a single cell within a well when attempting to identify a progenitor of a colony. For example, lack of focus on an imaged object may result in a false positive (i.e., incorrectly labeling an object as a single cell). As another example, doublets or "stacked" cells can be difficult to distinguish from single cells. False positives (and/or false negatives) can also result from other factors, such as debris, shadows created by the well wall, and/or aberrations on the inner periphery of the well wall.

SUMMARY

[0004a] According to a first aspect of the present invention, there is provided a method for facilitating clone selection, the method comprising:

generating, by a first imaging unit, a plurality of time-sequence images of a well containing a medium, the plurality of time-sequence images including a first time-sequence image generated at a first time and a second time-sequence image generated at a second time later than the first time;

detecting, by one or more processors analyzing the first time-sequence image, one or more candidate objects depicted in the first time-sequence image;

for each of the one or more candidate objects, determining, by the one or more processors analyzing an image of the candidate object using a convolutional neural network, whether the candidate object is a single cell;

detecting, by the one or more processors analyzing the second time-sequence image, a cell colony depicted in the second time-sequence image;

determining, by the one or more processors, whether the cell colony was formed from only one cell based at least in part on whether each of the one or more candidate objects was determined to be a single cell; and

generating, by the one or more processors, output data indicating whether the cell colony was formed from only one cell.

[0004b] According to a second aspect of the present invention, there is provided one or more non-transitory computer-readable media storing instructions that, when executed by one or more processors, cause the one or more processors to:

receive a plurality of time-sequence images of a well containing a medium, the plurality of time-sequence images being generated by a first imaging unit, and including a first time-sequence image generated at a first time and a second time-sequence image generated at a second time later than the first time;

detect, by analyzing the first time-sequence image, one or more candidate objects depicted in the first time-sequence image;

for each of the one or more candidate objects, determine, by analyzing an image of the candidate object using a convolutional neural network, whether the candidate object is a single cell;

detect, by analyzing the second time-sequence image, a cell colony depicted in the second time-sequence image;

determine whether the cell colony was formed from only one cell based at least in part on whether each of the one or more candidate objects was determined to be a single cell; and

generate output data indicating whether the cell colony was formed from only one cell.

[0004c] According to a third aspect of the present invention, there is provided a system comprising:

a visual inspection system including

a stage configured to accept a well plate, and

a first imaging unit configured to generate images of wells within the well plate on the stage, wherein each image corresponds to a single well; and

a computer system including

one or more processors, and

one or more memories storing instructions that, when executed by the one or more processors, cause the computer system to

command the first imaging unit to generate a plurality of time-sequence images of a well containing a medium, the plurality of time-sequence images including a first time-sequence image generated at a first time and a second time-sequence image generated at a second time later than the first time,

detect, by analyzing the first time-sequence image, one or more candidate objects depicted in the first time-sequence image,

for each of the one or more candidate objects, determine, by analyzing an image of the candidate object using a convolutional neural network, whether the candidate object is a single cell,

detect, by analyzing the second time-sequence image, a cell colony depicted in the second time-sequence image,

determine whether the cell colony was formed from only one cell based at least in part on whether each of the one or more candidate objects was determined to be a single cell, and

generate output data indicating whether the cell colony was formed from only one cell.

[0005] Embodiments described herein relate to systems and methods that improve upon traditional visual inspection techniques used for clone selection. In particular, in some embodiments, an automated visual inspection system captures a series of digital images, for each well within a well plate, at intervals over an incubation period (e.g., days or weeks). Depending on the embodiment, the images may be captured only on the first and last days of the incubation period, on a daily basis, or on some other suitable time schedule. The system may identify candidate object colonies that came from a single cell. Based on identified candidate objects, the system may move the position of a well for further image capture. A cell from a well identified as comprising a colony that came from a single cell may be transferred to a new culture environment and cultured. As used herein “well” refers to any laboratory-scale cell culture environment that permits optical inspection of its contents. While wells on multi-well plates are discussed by way of example herein, it will be appreciated that wherever a “well” and a “well plate” are mentioned, unless stated otherwise, these terms are contemplated to encompass any suitable laboratory-scale cell culture environment permitting optical inspection of its contents.

[0006] For a given well, a computer image processing technique may be applied to a later image (e.g., an image captured on the last day of the incubation period) to determine whether the well contains a cell colony. For example, a convolutional neural network (CNN) may be used to classify an object within the well medium as a cell colony. The CNN may include any suitable number of convolutional layers for two-dimensional convolution (e.g., to detect features such as edges within images), pooling