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The scripts are optimized to find *S. cerevisiae* in phase contrast images image acquired under Köhler illumination with a Plan Apo λ 40x/0.95 OFN25 Ph2 DM Nikon objective.

High level overview of script:

When an experiment is about to begin, the loadTimeCourse() method is called which sends the time course data to the Arduino, how many LEDs should be on and with what pwm intensity at each time.

Data from an experiment is then collected through a continuous iteration of the following instructions:

- The computer sends a message to the Arduino to stop the sampling pump. Yeast settle to the bottom of the sampling pump.
- The acquireImages() method is called, which makes the microscope take a fluorescent and non-fluorescent image at every stage position specified in the stagePositionList.
- The sampling pump is turned back on.
- The generateBG() method is called which makes the microscope take an image at each stage position three, five, or seven times, as specified by the bgSamples variable. For each pixel location, the median pixel intensity of the acquired set of images is used to create a composite image. The composite image is an image of the PDMS channel without any flowing yeast, because it is assumed that a for the majority of the images acquired there was not a yeast at that location. This assumption is most valid when the cell density is low and the value of bgSamples is high.
- The getData() method is called which analyzes those images. This method calls:
    - the backgroundArithmetic() method which subtracts the background images from the initially acquired images, so that the yeast from the initially acquired images are all that is left.
    - the alignImages() method is then called which translates the fluorescent image to ensure that it most closely matches the non-fluorescent image. This is especially useful when the sample moves slightly between the acquisition of the two types of images.
    - the processAndThreshold() method finds the regions of interest in the non-

fluorescent image which correspond to cells.

- the analyzeROIs() method maps each of the regions of interest from the non-fluorescent image to the fluorescent image, and then saves data about the pixels within the ROIs to the Results Table, a table that can be automatically generated when FIJI's Analyze Particles function is called.
- the saveResults() method saves data from the results table in a comma separated value file.