

STEP 3: SEGMENT IMAGES AND EXTRACT FEATURES USING CELLPROFILER

To extract morphological features from the brightfield and darkfield images and to determine the ground truth DNA content we used the imaging software CellProfiler.

- a. Open CellProfiler (we used version 2.1.1).
- b. Load the provided CellProfiler project (**Supplementary Code 2**) using File > Open Project.
- c. Specify the images to be analyzed by dragging and dropping the folder where the image montages that were created in step 2 (**Supplementary Data 4**) are located into the white area inside the CellProfiler window that is specified by 'File list'.
- d. Click on 'NamesAndTypes' under the 'Input modules' and adjust the names of the image channels as they were exported from IDEAS and specified in step 2 f. Then click on Update
- e. Analyze the images by adding analysis modules (visit www.cellprofiler.org for tutorials on how to use CellProfiler). In the provided CellProfiler pipeline (**Supplementary Code 2**), we defined a grid that is centered at each of the 15x15 single cell images. We extracted features for the darkfield images (granularity, radial distribution, texture, intensity) that we did not segment since the darkfield image is recorded under a 90° angle and does not necessarily depict the physical shape of the cell. Next, we segmented the brightfield images without using any stains, but by smoothing the images (CellProfiler module 'Smooth' with a Gaussian Filter) followed by an edge detection (CellProfiler module 'EnhanceEdges' with Sobel edge-finding) and by applying a threshold (CellProfiler module 'ApplyThreshold' with the MCT thresholding method and binary output). We close the obtained objects (CellProfiler module 'Morph' with the 'close' operation) and use them to identify the cells on the grid sites (CellProfiler module 'IdentifyPrimaryObjects'). To filter out secondary objects (such as debris), which are typically smaller than the cells, on the single cell images we measure the sizes of secondary objects (if there are any) and neglect the smaller objects. Then we extract features for the segmented brightfield images (granularity, radial distribution, texture, intensity, area and shape and Zernike polynomials). In a last step, we extract the intensity of the PI images

that we use as ground truth for the DNA content of the cells. The complete CellProfiler pipeline with the parameters used in our analysis can be found in **Supplementary Code 2**.

- f. Specify the output folder by clicking on 'View output settings' and selecting an appropriate 'Default Output Folder'.
- g. Extract the features of the images by clicking on 'Analyze Images'. The extracted features from the brightfield and darkfield images as well as the intensity of the PI images in .txt-format are provided as **Supplementary Data 5**.