1 CELL SEGMENTATION AND NUCLEAR REGION DESIGNATION

To automatically identify the cells present in the full field of view, Otsu's global threshold is applied to the Optical Phase Difference (OPD) images after normalization. The original OPD image is masked with the binary matrix generated after global threshold segmentation and all the background pixels are set to zero. To avoid that non desired objects present in the image, such as debris or dirties, to be processed as cells, the phase volume of each object is computed and those volume below a predetermined threshold are discarded. Each cell is then cropped to its bounding box region and a four-level segmentation (L=4) is performed with MATLAB *multitresh* function followed by *imquantize* from the Image Processing Toolbox. The number of levels ($L = 4$) is justified by the Histogram-based Valley Estimation Method for determining the number of clusters for an image to be properly segmented as described in Huang et al. (2011). Within the cell region, three clusters are presented (as shown in figure S1) while the fourth one refers to the background pixels (not shown in the histograms). Furthermore, the search-based algorithm from *multitresh* function does not converge for $L > 4$ and has lower values of the metrics coefficient for $L < 4$, indicating that the different classes are not optimally separated.

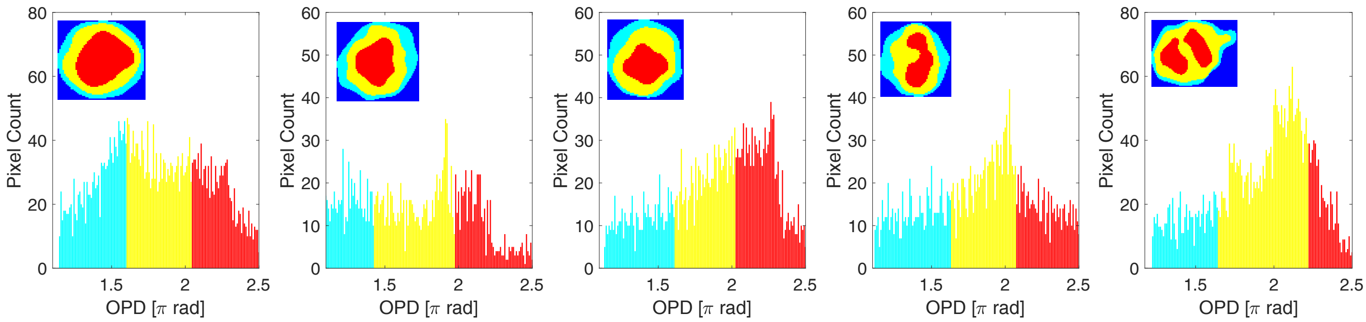

Figure S1. Histograms presenting the pixels count within the cell region. Each colour represents a class corresponding to a cell region (shown on the top). The nucleus is designated to the last segmentation class coloured in red, the cyan and yellow regions are assigned to the cytoplasm.

In both single and multiple threshold segmentation, the thresholds are estimated based on Otsu's global segmentation (Otsu, 1979). Briefly, it works by searching the thresholds that minimizes the weighted within-class variance (called intra-class variance), while maximizes the between-class variance (called inter-class variance). For an image represented in G gray levels $(0, 1, ..., G-1)$, the number of pixels at level i is denoted by f_i; then the total number of pixels equals $N = f_0 + f_1 + ... + f_{G-1}$. For a given gray level image, the occurrence probability of gray level i is given by:

$$p_i = \frac{f_i}{N}, \quad p_i \geq 0, \quad \sum_{i=0}^{G-1} p_i = 1 \tag{S1}$$

If the image is segmented into L levels (classes) $(C_0, C_1, ..., C_{L-1})$, $L-1$ thresholds $(t_0, t_1, ..., t_{L-2})$ must be selected. The cumulative probability w_l and mean gray level μ_l for each class C_l are respectively given by:

$$w_l = \sum_{i \in C_l} p_i \quad and \quad \mu_l = \sum_{i \in C_l} i \cdot p_i / w_l, \; l \in 0, 1, ..., L-1 \tag{S2}$$

Therefore, the mean intensity of the whole image μ_T, the between-class variance σ_B^2 and within-class variance σ_W^2 are respectively determined by:

$$\mu_T = \sum_{i=0}^{G-1} i \cdot p_i = \sum_{l=0}^{L-1} \mu_l w_l \tag{S3}$$

$$\sigma_B^2 = \sum_{l=0}^{L-1} w_l(\mu_l - \mu_T)^2 = \sum_{l=0}^{L-1} w_l \mu_l^2 - \mu_T^2 \tag{S4}$$

and

$$\sigma_W^2 = \sum_{i=0}^{G-1} w_i(\mu_i \mu_T)^2 - \sigma_B^2 \tag{S5}$$

The optimal thresholds are determined by minimizing the within-class variance(σ_W^2), while maximizing the between-class variance (σ_B^2).

The nuclear region is defined as the pixels from the last segmentation level (coloured in red in figure S1), because they are in agreement with the expected morphology for the nucleus corresponding to each cell maturation stage.

Supplementary Material

REFERENCES

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Otsu, N. (1979). A threshold selection method from gray-level histograms. *IEEE Transactions on Systems, Man, and Cybernetics* 9, 62–66. doi:10.1109/TSMC.1979.4310076