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Micropilot: automation of fluorescence microscopy–based imaging for systems biology

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Note: Supplementary Software and Supplementary Videos 1–4 are available on the Nature Methods website.

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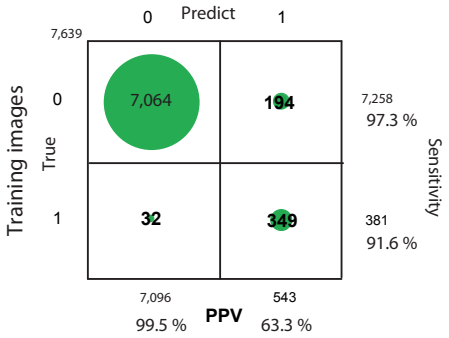
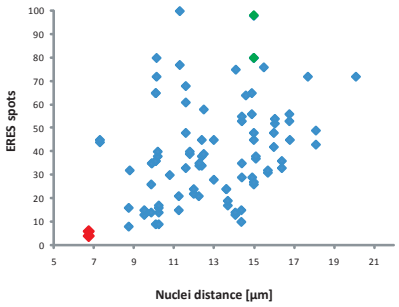
Supplementary Figure 1

Classifier training and measurements of *YFP-SEC31* HeLa cells.

a



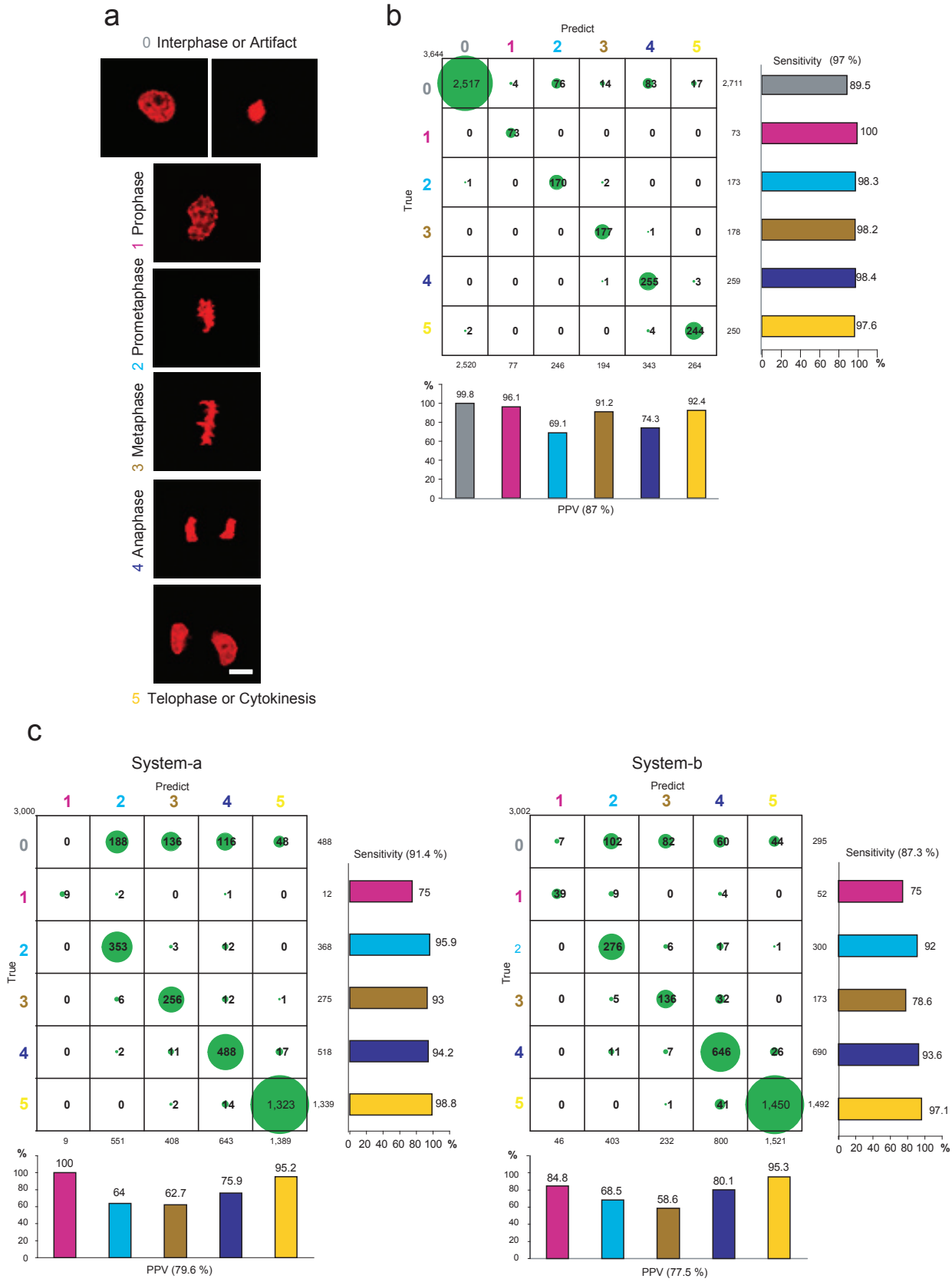
b



(**a**) Confusion matrix of the trained classifier shows true class assignments by human observer horizontally against the predicted vertically for the two different classes. At edges of the matrix the total numbers of the cell classes are given (overall total number in the left upper corner) corresponding to the PPV = TP / (TP + false positives) and the sensitivity = TP / (TP + false negatives). (**b**) Number of ERES of 91 high-resolution early anaphases to late telophases are plotted versus distance of nuclei (red rhombs correspond to nuclei in left and green rhombs to nuclei in right image in **Fig 2c**).

Supplementary Figure 2

Example images and classification of the live *H2B-tubulin* HeLa cells (scrambled siRNAi).



(**a**) H2B-mCherry example images representing the heterogeneous default class interphase or artifact (index 0 or gray) with two different morphologies. The five depicted mitotic phases represent the class prophase (index 1 or purple), prometaphase (index 2 or turquoise), metaphase (index 3 or brown), anaphase (index 4 or blue), and telophase (index 5 or yellow). White bar represents 10 µm. (**b**) Confusion matrix of the training results with SVM (Radial Base Function (RBF) kernel, RBF width = 0.1, penalty parameter = 16, ratio of class weights: 1:10:10:10:10:10) which shows true class assignments by human observer horizontally and predicted class assignment vertically for the six different classes. On the right and bottom edges of the matrix, the total numbers of the cell classes are given (overall total number in left upper corner). The graph on the bottom shows the PPV of the different classes. The bars on the right correspond to the sensitivity in percent, and the mean for both value sets is in parentheses. For training procedure, we enriched very rare phenotypes such as prophases (frequency < 1 %) or anaphases by selective cell picking using the 'train+'-module in Micropilot. (**c**) Confusion matrices of the prediction results (as described in 1.5.9) of confocal standard system-a and system-b using live cell samples made six months after training. Due to the high ratio of interphases to mitotic phases, the interphase (class 0) class was not predicted nor validated. The results from the offline training microscope (system-a) and the online control microscope (system-b) showed only a slight reduction of 4 % and 2 % for the mean class prediction sensitivity and PPV, respectively

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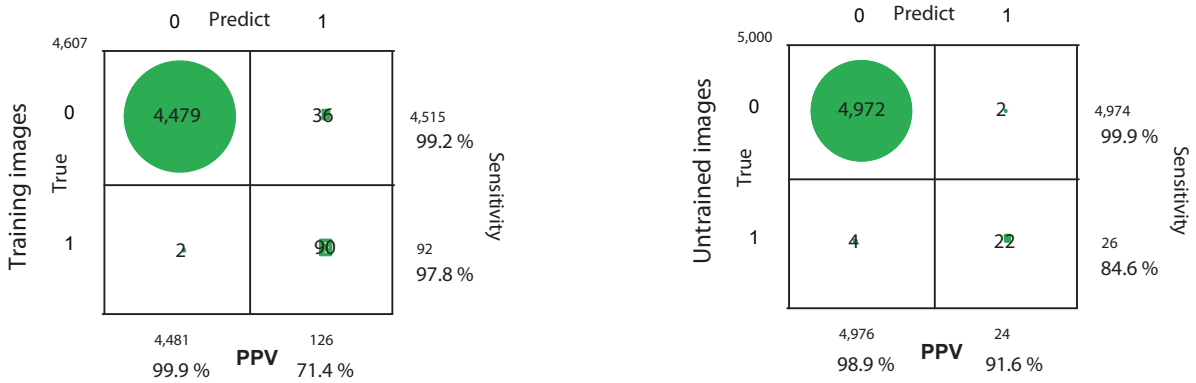
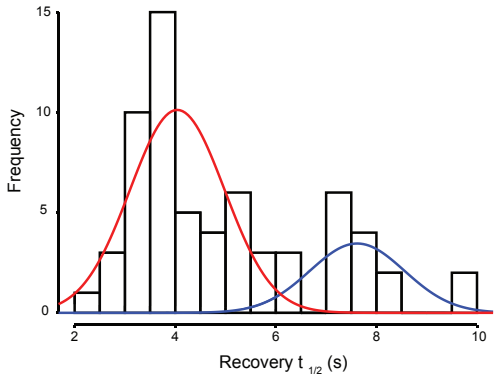
Supplementary Figure 3

Classification and normal recovery rate distribution of the automatic FRAP on *CBX1-EGFP* cells.

a



b



(**a**) Confusion matrix of the classification show true class assignments by human observer horizontally against the predicted vertically for the two different classes. At edges of the matrix the total numbers of the cell classes are given (overall total number in the left upper corner) corresponding to the PPV and the sensitivity. (**b**) Histogram of the $t_{1/2}$ recovery of *CBX1-EGFP* of all prophase cells and the fitting of normal distribution for late (red) and early (blue) using normal mixture modeling.

Supplementary Table 1 Pseudo code examples for different microscope systems.

Please read the tables step-by-step following the arrows according to the Fig. 1b.

(**a**)

Synchronous (adhoc) communication using windows registry
(similarly implemented for Zeiss 510, Olympus ScanR, or Perkin Elmer Ultraview).

Microscope	Micropilot
Write image path Set windows registry image path parameter after image capture in low-resolution: Software\ Settings\OnlineImageAnalysis\macro\path String Value = C:\repository\experiment_001\image--T001--Z01--C01.tif	
Listener on and wait Set the listener: Software\ Settings\OnlineImageAnalysis\macro\code String Value = 1 and wait	**Read listener** Read every 150ms the windows registry key for new incoming images in: Software\ Settings\OnlineImageAnalysis\macro\code
	Read Image If the listener parameter: Software\ Settings\OnlineImageAnalysis\macro\code String Value = 1 Then read: Software\ Settings\OnlineImageAnalysis\macro\path String Value = C:\repository\experiment_001\image--T001--Z01--C01.tif
	Classification Classify image performing segmentation, feature extraction, and class prediction
Continue prescan If listener: Software\ Settings\OnlineImageAnalysis\macro\code String Value = 0 Then capture the next image position and jump back to 'Write image path'	**Set listener** If no positive cell found, set listener: Software\ Settings\OnlineImageAnalysis\macro\code String Value = 0 to skip position for complex imaging Else: Set xy-position offsets of the object of interest found relative to the image origin: Software\ Settings\OnlineImageAnalysis\macro\xoff String Value = 120 Software\ Settings\OnlineImageAnalysis\macro\yoff String Value = -240 Set listener for complex imaging: Software\ Settings\OnlineImageAnalysis\macro\code String Value = 2
Complex imaging If listener: Software\ Settings\OnlineImageAnalysis\macro\code String Value = 2 Read: Software\ Settings\OnlineImageAnalysis\macro\xoff String Value = 120 Software\ Settings\OnlineImageAnalysis\macro\yoff String Value = -240 Move accordingly to xy values and deploy predefined complex imaging. If finished go back to 'Continue prescan'	

(**b**)

Asynchronous (list) communication using socket
(implemented for Leica SP5).

Leica SP5 (LAS/Matrix Screener)	Micropilot
Send image path CAM server sends image path after new image capture: /relpath:FinalerTest\MitoExperimente--2009_10_22_14_05_20\slide--S00\well--U00--V03\field--X03--Y00\image--L0000--S00--U00--V03--J00--X03--Y00--T0000--Z00--C00.ome.tif	**Read image** Read CAM command for image path at the specified address and port: /relpath:FinalerTest\MitoExperimente--2009_10_22_14_05_20\slide--S00\well--U00--V03\field--X03--Y00\image--L0000--S00--U00--V03--J00--X03--Y00--T0000--Z00--C00.ome.tif (eventually filter out command if not applicable, e.g. autofocus images or complex imaging acquisition)
Continue prescan and go back to 'Send image path'	**Classification** Perform segmentation, feature extraction, and class prediction.
Add position to list Listen to CAM add list commands and hold position list meanwhile	**Send position** If positive cell found send CAM command to add a position: /cli:CConrad /app:matrix /cmd:add /tar:camlist /exp:ips-file /ext:none /slide:0 /wellx:0 /welly:3 /fieldx:3 /fieldy:0 /dxpos:120/dypos:-240
Complex imaging Receive CAM start command and stop prescan to run complex imaging for list positions If finished jump back to 'Continue prescan'	**Execute list** If maximum number of positive cells is reached Or If timer is down (counting from first positive cell found) Then send CAM start command with timelapse parameters: /cli:CConrad /app:matrix /cmd:startcamscan /runtime:600 /repeattime:60 Clear list of position: cli:CConrad /app:matrix /cmd:deletelist

(**c**)

Synchronous (adhoc) communication using socket (implemented for µManager).