SUPPLEMENTAL RESULTS

Statistical correlation analysis

To analyze the correlation between the occurrence of abnormal morphologies and cases of cell death, and prometaphase length we used *Kendall's τ* and *Spearman's ρ* (which are suited for non-normally distributed data). We determined the correlation coefficients between prometaphase length and ratio *r* (number of post-prometaphase phenotypes / total number of post-prometaphase time steps). Scatter-plots of the data revealed that the correlation coefficient was influenced by several cases with $r=0$ (Supplemental Fig. S8). Since these cases occur, e.g., when prometaphase is cut off at the end of an image sequence, they were excluded for the correlation analysis. We obtained values of $\tau=0.38$ and $\rho=0.51$ for the manually annotated data, and $\tau=0.27$ as well as $\rho=0.38$ for the automatically annotated nocodazole data. For the RNAi experiments we yielded even higher values of $\tau=0.63$ and $\rho=0.73$ for the manually annotated data, and $\tau=0.43$ as well as $\rho=0.57$ for the automatically annotated data. All results proved to be highly significant (Table 2B). Note, that even when including the exceptional cases, in most cases the correlations were still significant (Supplemental Table S13).

Statistical analysis of the treatment effects on other phase lengths

To detect effects of the treatments on other phase durations, we first performed two-sided *Mann-Whitney U* tests for all phases to determine whether a shift between the treated and control distributions existed. If so, one-sided tests were performed to determine the direction of the shift (i.e., phase shortening or prolongation).

Nocodazole experiments. We found a significant shortening of interphase for all concentrations in the manually annotated data and for *low* and *medium* concentrations in the automatically annotated data, while abnormal interphase was shortened significantly only in the manually annotated data. However, this does not necessarily indicate true differences in interphase length since cells under nocodazole treatment were strongly delayed in prometaphase while the total duration of the time-lapse experiments was equal. Consequently, many interphases were not recorded until the end and may appear shorter. Late anaphase was significantly prolonged for the manually annotated data. For telophase we detected a significant shortening for all concentrations in the manually and automatically annotated data. This effect was caused by alterations between telophase and abnormal telophase. Finally, a significant prolongation was detected for abnormal telophase for *low* and *high* concentrations in the manually annotated data and for *high* concentrations in the automatically annotated data (Supplemental Table S14).

RNAi experiments. For the RNAi treated experiments we found a significant shortening of abnormal interphase and telophase for the automatically annotated data (Supplemental Table S15) which occurred due to the same reasons as described above. Also, we detected a significant prolongation of early anaphase and late anaphase in the automatically annotated data and a significant prolongation of abnormal telophase in the manually annotated data. Note that here the sample numbers were comparatively low. For both types of experiments the statistical tests could not be performed for apoptosis and abnormal early anaphase due to small sample numbers.

SUPPLEMENTAL NOTE

1) Validation of the tracking parameters c_1 and c_2

Supplemental Figure S6A shows the distribution of sizes for all anaphase nuclei (which usually constitute the daughter cell nuclei shortly after mitosis) and the size distribution of interphase nuclei (which is the most frequent class). It can be seen that apart from a few outliers, almost all anaphase nuclei fall below the threshold defined by c_1, while nearly all interphase nuclei are above the threshold. Consequently, the value for c_1 is well suited to exclude interphase cells from being considered as daughter cells. At the same time almost all anaphase cells are considered as daughter cells.

Supplemental Figure S6B shows the distribution of the sibling distances, i.e. the distances of daughter chromosome sets directly after a splitting event for the ground truth data set. The plot reveals that the distribution of sibling distances is concentrated around its median and c_2 is well chosen with regard to the distribution.

2) Detailed description of the static image features

The numbers of used features per feature type are given in Supplemental Table S9. The features based on size and shape (**1**) include the area A (number of pixels of the segmented object) and the contour length p of a segmented object. Furthermore, the circularity c ($c=p^2/A$) and Feret's diameter (calliper length) which is the maximum distance between any two contour pixels. Additional edge-related features are computed by applying Laplace and Sobel filters to the image and subsequent thresholding. The features based on geometric moments (**2**) include, e.g., the distance of the gravity center to the bounding box center, and the ratio of the second

order central moments μ_{20} and μ_{02}. The wavelet-based features (Chang and Kuo 1993) (**3**) are based on a recursive subdivision of the image into different frequency channels. These features are computed for four subdivision cycles. The Zernike moments (Zernike 1934) (**5**) use complex Zernike polynomials as moment basis set. We compute the Zernike moments up to degree 12 and use the moment's magnitudes as features, which are invariant to rotation (as proposed, e.g., in Boland et al. 1998), resulting in 49 features. The granularity features (**7**) consider the difference of gray values of pixel pairs in a certain distance under a certain angle. As feature values the mean and standard deviation of the maximum differences are computed over the whole image. Here, distances of one to ten pixels considering eight directions were used. Gray scale invariants (Burkhardt and Siggelkow 2001) (**8**) combine sets of neighboring pixels using local kernel functions of different scales, which is followed by integration over the whole image. We used two different kernels with radii of 2, 4, 16, and 32 pixels. Haralick texture features (Haralick 1979) (**10**) are based on co-occurrence matrices of pixel pairs with a certain distance under a certain angle. We used distances of one to five pixels and four different angles resulting in 20 co-occurrences matrices. For each of such matrices 13 features were computed, including, e.g., contrast, entropy, and angular second moment. Note that only the wavelet features were computed for both the most informative slices as well as for the projected images.

3) Experimental results using weighted SVMs

The unbalanced data problem (here, much more samples for the interphase class than for all other classes) can also be dealt with using weighted SVMs. To determine the performance using weighted SVMs we repeated the five-fold cross validation on the RNAi data set. The weights were determined for class *i* as the number of samples

for the largest class (here interphase) divided by the number of samples for class *i*, as proposed, e.g., in (Wang et al. 2004). The result is shown in Supplemental Table S12 (compare to Supplemental Table S4). It can be seen that for most classes with small sample size the accuracy decreased (partially significantly, e.g., for early anaphase by 9.1% and abnormal interphase by 8.9%). Only for prometaphase and metaphase the accuracy increased, but only by 0.1%. The accuracy for interphase increased by 1.0%, which resulted in an overall accuracy of 95.6%. Consequently, the weighted SVM scheme did not significantly improve the accuracies for classes with small sample numbers in our case.

SUPPLEMENTAL TABLES

Experiment	Total no. of cells	Correctly segmented	Under-segmented	Over-segmented	Accuracy [%]
Noco. Control	4015	3989	26	0	99.4
Noco. Low	2263	2252	10	1	99.5
Noco. Medium	3298	3296	2	0	99.9
Noco. High	5020	4774	175	71	95.1
Total No.	**14596**	**14311**	**213**	**72**	**98.1**

Table S1. Evaluation of the segmentation accuracy for all nocodazole treatments based on one randomly picked sequence per treatment. In particular, the proportion of undersegmetations and oversegmentations has been quantified: 74.7% of the errors are undersegmentations and 25.3% are oversegmentations.

A

Experiment	No. of tracks	No. of links	Errors by segm.	Errors by mitosis detect.	Errors by corresp. Finding	Accuracy [%]
Noco. Control	21	5000	3	0	2	99.9
Noco. Low	14	2450	1	3	0	99.8
Noco. Medium	19	3600	0	2	0	99.9
Noco. High	27	5900	25	4	0	99.5
Total No.	**81**	**16950**	**29**	**9**	**2**	**99.8**

B

Experiment	Total No.	TP No.	FP No.	PPV [%]	Sensitivity [%]
Noco. Control	103	99	5	95.2	96.1
Noco. Low	25	23	2	92.0	92.0
Noco. Medium	32	31	1	96.9	96.9
Noco. High	28	27	10	73.0	96.4
RNAi	30	28	0	100.0	93.3
Total No.	**218**	**208**	**18**	**92.0**	**95.4**

Table S2. Evaluation of tracking accuracy. (**A**) Overall accuracy of tracking (including mitosis detection errors) based on the same four sequences as in Table S1. The errors have been subdivided into errors caused by (1) segmentation, (2) mitosis detection, and (3) correspondence finding. (**B**) Mitosis detection accuracy for all treatments based on 22 sequences (including the four sequences used in (**A**)). Total: total numbers of occurring mitoses, TP: true positives (correctly detected), FP: false positives, PPV: positive predictive value (PPV = TP/(TP+FP)) and the sensitivity (sensitivity = TP/Total).

			Interphase	Prophase	Prometa-phase	Metaphase	Early ana-phase	Late ana-phase	Telophase
Nocodazole	Man	Control	15649	123	111	107	43	94	556
		Low	9041	75	516	151	19	39	225
		Medium	7748	127	644	133	23	76	504
		High	9276	218	2474	211	7	18	176
	Auto	Control	40812	703	419	312	84	290	1798
		Low	4421	69	313	77	5	23	134
		Medium	2961	49	177	40	5	32	134
		High	5502	217	859	161	18	41	172
RNAi	Man	Control	6181	54	50	46	18	48	227
		Treated	2787	54	1619	52	4	9	57
	Auto	Control	9644	112	194	64	25	63	471
		Treated	6242	97	3917	25	34	32	244
Total number			**120264**	**1898**	**11293**	**1379**	**285**	**765**	**4698**

			Abnormal interphase	Cell death	Abnormal early anaphase	Abnormal late anaphase	Abnormal telophase	Number of image sequences
Nocodazole	Man	Control	347	0	0	2	10	**4**
		Low	675	0	9	12	99	**4**
		Medium	1614	0	8	17	136	**4**
		High	4797	133	26	41	518	**4**
	Auto	Control	1873	4	9	14	175	**14**
		Low	1018	0	5	4	52	**2**
		Medium	757	0	0	2	32	**2**
		High	789	0	5	4	90	**2**
RNAi	Man	Control	699	0	0	1	11	**2**
		Treated	373	115	3	4	56	**2**
	Auto	Control	1665	3	0	9	20	**4**
		Treated	686	184	0	5	78	**4**
Total number			**15293**	**439**	**65**	**115**	**1277**	**48**

Table S3. Number of available samples per class for different data sets. “Man”: manually annotated training set, “Auto”: automatically annotated test set, for the nocodazole as well as the RNAi experiments. In the last column for each data set the number of underlying image sequences is given.

True Class	Classifier Output											
	Inter	**Pro.**	**Pro-meta**	**Meta**	**Ana. 1**	**Ana. 2**	**Telo.**	**Inter ***	**Cell dth.**	**Ana. 1***	**Ana. 2***	**Telo. ***
Inter.	**3825**	3	0	0	0	0	16	87	0	0	0	0
Pro.	30	**68**	6	0	0	0	0	4	0	0	0	0
Prom.	0	3	**1646**	10	0	2	1	0	3	0	0	4
Meta.	0	0	9	**85**	1	0	3	0	0	0	0	0
Ana.1	0	0	4	1	**15**	2	0	0	0	0	0	0
Ana.2	0	0	1	0	0	**54**	2	0	0	0	0	0
Telo.	21	0	2	0	0	3	**244**	10	0	0	0	4
Inter.*	99	2	0	1	0	0	6	**959**	1	0	0	4
Cell d.	1	0	10	1	0	1	0	1	**100**	0	0	1
Ana.1*	0	0	3	0	0	0	0	0	0	**0**	0	0
Ana.2*	0	0	3	1	0	1	0	0	0	0	**0**	0
Telo.*	2	0	8	0	0	0	7	6	0	0	0	**44**
Accur. [%]	**97.3**	**63.0**	**98.6**	**86.7**	**68.2**	**94.7**	**86.0**	**89.5**	**87.0**	**0.0**	**0.0**	**65.7**

Table S4. RNAi data: Confusion matrix for 5-fold cross-validation on the training set (the interphase samples have been reduced to 1000 samples per sequence). Ana. 1 and Ana. 2 denote early Anaphase and late Anaphase, the asterisk denotes the abnormal morphology classes. The low accuracies for the classes abnormal early and late anaphase is due to the very low number of available samples together with a high intra-class variability.

	Nocodazole				RNAi	
	Control Man-Auto	**Low Man-Auto**	**Medium Man-Auto**	**High Man-Auto**	**Control Man-Auto**	**Treated Man-Auto**
n_1, n_2	57, 180	44, 37	68, 21	120, 45	27, 66	29, 115
p-value	0.04	0.06	0.92	0.28	0.25	0.02

Table S5. Results of *Mann-Whitney* test on the prometaphase length distributions of manually annotated data against the automatically annotated data for nocodazole and RNAi experiments, two-sided test with alternative hypothesis: "true shift is not equal to 0", n_1, n_2 are the numbers of samples.

	Nocodazole		RNAi	
	Manual	**Automatic**	**Manual**	**Automatic**
Mean length μ	2.00	2.43	2.04	2.94
Stddev of lengths σ	1.24	1.51	0.81	2.61
Significance threshold (μ + 2 σ)	**4.48**	**5.46**	**3.65**	**8.17**
Threshold in minutes (rounded)	**31 min**	**38 min**	**26 min**	**57 min**

Table S6. Means and standard deviations of prometaphase lengths determined for control experiments (in time steps), and significance thresholds to determine prolonged prometaphases (in time steps and minutes).

		Including cases with r = 0				**Excluding cases with r = 0**			
		Nocodazole		RNAi		Nocodazole		RNAi	
		Man	**Auto**	**Man**	**Auto**	**Man**	**Auto**	**Man**	**Auto**
Prolonged	Low	0.23	0.27	0.17	0.30	0.41	0.41	0.90	0.68
	Medium	0.32	0.31			0.49	0.36		
	High	0.35	0.25			0.69	0.41		
Normal	Low	0.16	0.17	0.04	0.15	0.31	0.44	0.20	0.40
	Medium	0.09	0.31			0.27	0.38		
	High	0.14	0.11			0.51	0.30		
Control		0.02	0.12	0.07	0.11	0.25	0.24	0.19	0.30

Table S7. Mean values for ratio r (number of post-prometaphase steps with abnormal phenotypes / total number of post-prometaphase time steps) for all experiments. In the left part, all cases were used for computing the mean (including cases with a ratio of r = 0), in the right part, only samples with a ratio of r > 0 were considered.

		Nocodazole				RNAi	
		Control	**Low**	**Medium**	**High**	**Control**	**Treated**
Man	n	57	44	68	120	27	29
	p-value	$2.04 \cdot 10^{-10}$	$5.32 \cdot 10^{-4}$	$1.06 \cdot 10^{-10}$	$9.22 \cdot 10^{-11}$	$9.71 \cdot 10^{-8}$	0.06
Auto	n	180	37	21	45	66	115
	p-value	$1.76 \cdot 10^{-15}$	$8.61 \cdot 10^{-5}$	$1.84 \cdot 10^{-7}$	$1.52 \cdot 10^{-7}$	$5.68 \cdot 10^{-11}$	$2.01 \cdot 10^{-10}$

Table S8. Results of *Shapiro-Wilk* normality test on the prometaphase length distributions of manually and automatically annotated data for the nocodazole and RNAi experiments; n is the number of samples.

	Index	**Description**	**Number**
Computation based on MIP images	1.	Features based on size and shape	12
	2.	Geometric moments-based	5
	3.	Mean intensity	1
	4.	Tree structured wavelets-based (Chang & Kuo 1993)	4
	5.	Zernike moments (Zernike 1934)	49
	6.	Dynamic features (mean intensity, size, shape)	10
Computation on most informative slices	7.	Granularity (local differentiation)	20
	8.	Gray scale invariants (Burkardt & Siggelkow 2001)	8
	9.	Haralick texture features (Haralick 1979; Theodoridis & Koutroumbas 1999)	260
	10.	Tree structured wavelet-based (Chang & Kuo 1993)	4
	11.	Standard deviation	1
	12.	Dynamic features (standard deviation)	2
		Total Number	**376**

Table S9. Feature types and feature numbers extracted for each type. 81 features have been computed based on the maximum intensity projections, and 295 features have been computed based on the most informative slices.

Set	1	2	3	4	5	6	7	8	9	10	11	12	Ova.	Avg.
(a)	98.0	61.7	95.7	82.4	71.7	90.8	83.5	93.3	91.7	30.2	37.5	64.4	**93.8**	**75.1**
(b)	98.0	65.2	95.5	82.4	68.5	92.1	82.6	93.3	91.7	30.2	41.7	62.8	**93.7**	**75.3**
(c)	98.0	67.8	95.4	82.6	70.7	90.8	84.7	93.5	91.7	25.6	41.7	63.4	**93.9**	**75.5**

Table S10. Classification accuracies for all classes resulting from 5-fold cross-validation on the training set using different feature sets: (a) features from MIP image, (b) features from the most informative slice of 3D image, (c) combination of both feature sets. The column numbers 1-12 represent the classes (1) inter-, (2) pro-, (3) prometa-, (4) meta-, (5) early ana-, (6) late ana-, (7) telo-, and (8) abnormal interphase, (9) cell death, (10) abnormal early ana-, (11) abnormal late ana-, and (12) abnormal telophase. The column “Ova.” provides the overall accuracies and the column “Avg.” the average accuracies. The interphase samples have been reduced to 1000 samples per sequence for reasons of computation time; the grey tones represent the accuracy ranking within the three groups, where dark gray indicates the lowest accuracy and white the highest accuracy.

True Class	Classifier Output											
	Inter.	**Pro.**	**Pro-meta.**	**Meta.**	**Ana. 1**	**Ana. 2**	**Telo.**	**Inter.***	**Cell death**	**Ana. 1***	**Ana. 2***	**Telo.***
Inter.	**19494**	45	1	1	1	0	143	242	0	0	0	4
Pro.	163	**452**	22	0	0	0	3	11	0	0	0	0
Prom.	8	21	**5214**	110	3	3	4	9	6	12	6	18
Meta.	1	1	88	**586**	11	0	6	0	2	0	1	4
Ana.1	0	0	11	10	**83**	2	1	0	0	7	0	0
Ana.2	0	0	2	1	4	**260**	7	0	1	0	9	0
Telo.	171	0	4	6	1	5	**1475**	21	0	0	3	59
Inter.*	478	6	8	2	1	0	43	**7891**	1	0	0	75
Cell d.	4	0	15	3	0	1	0	1	**221**	0	2	1
Ana.1*	0	0	20	0	9	0	0	0	0	**14**	3	0
Ana.2*	0	0	19	3	1	13	3	0	2	1	**32**	3
Telo.*	10	0	35	4	0	2	125	124	0	0	2	**528**
Accur. [%]	**97.8**	**69.4**	**96.3**	**83.7**	**72.8**	**91.6**	**84.5**	**92.8**	**89.1**	**30.4**	**41.5**	**63.6**

Table S11. Confusion matrix for the combined classifier trained on the nocodazole and the RNAi data using five-fold cross validation (the interphase samples have been reduced to 1000 samples per sequence). Overall classification accuracy **94.0%**.

True Class	Classifier Output											
	Inter.	**Pro.**	**Pro-meta.**	**Meta.**	**Ana. 1**	**Ana. 2**	**Telo.**	**Inter.***	**Cell death**	**Ana. 1***	**Ana. 2***	**Telo.***
Inter.	**8811**	5	0	0	1	0	29	122	0	0	0	0
Pro.	35	**64**	6	0	0	0	0	3	0	0	0	0
Prom.	2	3	**1648**	8	0	2	1	2	2	0	0	1
Meta.	0	0	8	**86**	1	0	3	0	0	0	0	0
Ana.1	1	0	5	1	**13**	2	0	0	0	0	0	0
Ana.2	0	0	1	1	0	**52**	3	0	0	0	0	0
Telo.	32	0	2	0	0	3	**234**	8	0	0	0	5
Inter.*	196	2	1	1	0	0	3	**864**	1	0	0	4
Cell d.	2	0	10	1	0	0	0	1	**100**	0	0	1
Ana.1*	0	0	3	0	0	0	0	0	0	**0**	0	0
Ana.2*	0	0	4	0	0	1	0	0	0	0	**0**	0
Telo.*	6	0	7	0	0	0	7	5	0	0	0	**42**
Accur. [%]	**98.3**	**59.2**	**98.7**	**87.8**	**59.1**	**91.2**	**82.4**	**80.6**	**87.0**	**0.0**	**0.0**	**62.7**

Table S12. Confusion matrix for weighted SVM classifier trained on the RNAi data using five-fold cross validation. Overall classification accuracy **95.6%**.

A		Nocodazole		RNAi	
		Manual	**Automated**	**Manual**	**Automated**
n		110	136	15	69
Kendall	**τ**	0.38	0.27	0.63	0.43
	p-value	$9.45 \cdot 10^{-9}$	$8.63 \cdot 10^{-6}$	$1.27 \cdot 10^{-3}$	$4.19 \cdot 10^{-7}$
Spearman	**ρ**	0.51	0.38	0.73	0.57
	p-value	$7.81 \cdot 10^{-9}$	$2.52 \cdot 10^{-6}$	$9.44 \cdot 10^{-4}$	$2.07 \cdot 10^{-7}$

B		Nocodazole		RNAi	
		Manual	**Automated**	**Manual**	**Automated**
n		272	261	51	171
Kendall	**τ**	0.33	0.24	-0.03	0.11
	p-value	$5.53 \cdot 10^{-13}$	$2.17 \cdot 10^{-7}$	0.39	0.03
Spearman	**ρ**	0.41	0.31	-0.04	0.13
	p-value	$8.17 \cdot 10^{-13}$	$1.71 \cdot 10^{-7}$	0.40	0.05

Table S13. Correlation coefficients τ and ρ for prometaphase length and ratio r. (**A**) **Excluding** cases with ratio r = 0. (**B**) **Including** cases with ratio r = 0. Additionally, the p-values for the significance tests and numbers of measurements **n** are given.

		Manual			Automatic		
		Control - Low	**Control - Medium**	**Control - High**	**Control - Low**	**Control - Medium**	**Control - High**
shortened	Interphase						
	n_1, n_2	184, 142	184, 172	184, 142	545, 70	545, 48	545, 65
	p-value	$9.83 \cdot 10^{-3}$	$1.26 \cdot 10^{-10}$	$3.80 \cdot 10^{-3}$	$5.05 \cdot 10^{-3}$	$9.63 \cdot 10^{-3}$	0.88
	Telophase						
	n_1, n_2	104, 52	104, 113	104, 52	358, 44	358, 40	358, 45
	p-value	$1.93 \cdot 10^{-4}$	$1.32 \cdot 10^{-4}$	$3.47 \cdot 10^{-6}$	$9.88 \cdot 10^{-5}$	$4.78 \cdot 10^{-3}$	0.05
	Abnormal interphase						
	n_1, n_2	5, 24	5, 36	5, 157	55, 27	55, 18	55, 24
	p-value	$6.57 \cdot 10^{-4}$	0.04	$3.85 \cdot 10^{-3}$	0.94	0.94	0.55
prolonged	Late anaphase						
	n_1, n_2	95, 38	95, 72	95, 19	262, 18	262, 24	262, 31
	p-value	0.01	NA	0.01	0.24	0.07	0.42
	Abnormal Telophase						
	n_1, n_2	5, 28	5, 40	5, 87	99, 27	99, 18	99, 30
	p-value	0.04	0.13	$8.44 \cdot 10^{-3}$	0.59	0.46	0.02

Table S14. Results of *Mann-Whitney* test for all phases (**nocodazole** experiments), one-sided test with alternative hypothesis: “true shift is greater than 0” for prolonged phases and “true shift is smaller than 0” for shortened phases, n_1, n_2 are the numbers of samples, NA resulted for a test for which all samples were identical.

			Manual	Automatic
			Control - Treated	**Control - Treated**
shortened	Telophase	n_1, n_2	51, 9	90, 54
		p-value	0.95	0.03
	Abnormal interphase	n_1, n_2	14, 14	27, 32
		p-value	0.14	$2.72 \cdot 10^{-6}$
prolonged	Early anaphase	n_1, n_2	19, 5	24, 22
		p-value	NA	0.02
	Late anaphase	n_1, n_2	48, 9	62, 27
		p-value	NA	0.02
	Abnormal Telophase	n_1, n_2	3, 6	13, 35
		p-value	0.01	0.29

Table S15. Results of *Mann-Whitney* test for all phases (**RNAi** experiments), one-sided test with alternative hypothesis: "true shift is greater than 0" for prolonged phases and "true shift is smaller than 0" for shortened phases, n_1, n_2 are the numbers of samples, NA resulted for a test for which all samples were identical.

Total no. of cells	**Correct**	**Under-segmented**	**Over-segmented**	**Partly / not segmented**	**Accuracy [%]**
4008	3981	27	0	0	99.3

Table S16. Evaluation of the segmentation accuracy for six image sequences of NRK (normal rat kidney) cells (acquired with the LSM 510 Meta point-scanning microscope).

No. of tracks	No. of links	Errors by segm.	Errors by mitosis detect.	Errors by corresp. finding	Accur. of linking [%]	Mitoses found	Mitosis detection accur. [%]
66	4073	5	2	6	99.7	14/16	87.5

Table S17. Evaluation of the tracking accuracy for six image sequences of NRK (normal rat kidney) cells (acquired with the LSM 510 Meta point-scanning microscope).

True Class	Classifier Output						
	Inter.	**Pro.**	**Prom.**	**Meta.**	**Ana. 1**	**Ana. 2**	**Telo.**
Inter.	**3020**	30	0	0	0	0	82
Pro.	18	**51**	3	0	0	0	1
Prom.	3	3	**34**	1	1	0	1
Meta.	2	1	8	**9**	0	0	2
Ana.1	2	0	1	0	**26**	6	0
Ana.2	0	0	0	1	5	**27**	9
Telo.	63	0	0	1	0	7	**135**
Accur. [%]	**96.4**	**69.9**	**79.1**	**40.9**	**74.3**	**64.3**	**65.5**

Table S18. Confusion matrix for classification of NRK cells (acquired with the LSM 510 Meta point-scanning microscope). Overall classification accuracy **92.9%**.

Total no. of cells	Correct	Under-segmented	Over-segmented	Partly / not segmented	Accuracy [%]
9226	9206	5	0	15	99.8

Table S19. Evaluation of the segmentation accuracy for four image sequences acquired with the LSM 5 LIVE line-scanning microscope (HeLa cells).

No. of tracks	No. of links	Errors by segm.	Errors by mitosis detect.	Errors by corresp. finding	Accur. of linking [%]	Mitoses found	Mitosis detection accur. [%]
32	8431	4	0	0	99.9	22/22	100.0

Table S20. Evaluation of the tracking accuracy for four image sequences acquired with the LSM 5 LIVE line-scanning microscope (HeLa cells).

True Class	Classifier Output						
	Inter.	**Pro.**	**Prom.**	**Meta.**	**Ana. 1**	**Ana. 2**	**Telo.**
Inter.	**7724**	1	0	0	0	0	74
Pro.	7	**41**	0	0	0	0	0
Prom.	1	2	**58**	5	0	0	0
Meta.	1	0	7	**56**	2	0	0
Ana.1	1	0	0	3	**19**	5	2
Ana.2	2	0	0	0	2	**44**	10
Telo.	53	0	0	0	0	5	**242**
Accur. [%]	**99.0**	**85.4**	**87.9**	**84.8**	**63.3**	**75.9**	**80.7**

Table S21. Confusion matrix for classification of four image sequences acquired with the LSM 5 LIVE line-scanning microscope (HeLa cells). Overall classification accuracy **97.8%**.

A					
Method	**Correct**	**Under-segmented**	**Over-segmented**	**Partly / not segmented**	**Accuracy [%]**
Global Otsu	10055	12	1516	3007	68.9
K-Means clustering	10787	1184	1298	1327	73.9
Our method	14311	213	72	0	98.1

B					
Method	**No. of links**	**Errors by segm.**	**Errors by mitosis detect.**	**Errors by corresp. finding**	**Accuracy [%]**
Cell Profiler	16956	31	43	3	99.6
MTrack2	16942	29	43	2	99.6
Our method	16950	29	9	2	99.8

Table S22. Comparison of our segmentation and tracking approaches with other approaches based on four images sequences with different treatments (low, medium, and high nocodazole concentration, and control sequence). The total number of analyzed cell nuclei was 14596. (**A**) Segmentation accuracies of our approach in comparison with global Otsu and K-Means clustering (3 clusters). (**B**) Tracking accuracies of our approach compared to tracking approaches in CellProfiler and ImageJ (MTrack2).

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