

## Supplementary Information

Feature name	short name	Description	Reference
Volume	vol	Volume of the cell assuming refractive index of the cell of 1.38 and refractive index of the media of 1.33.	Kasprowicz et al. (2017)
Radius	rad	Average distance from centroid of cell pixels.	
Sphericity	sph	(Surface integral of phase + cell area)/ surface area of a sphere with volume equal to the thickness of the cell.	
Length	len	Maximum distance across the cell in either vertical or horizontal direction	
Width	wid	Maximum distance across the cell perpendicular to Length.	
Velocity	vel	The current speed of the cell calculated by considering the position in the previous and following frames in addition to the current frame.	
Displacement	dis	Straight line distance between cell centroid on current frame and the frame it was first detected in.	
Tracklength	trac	Path length travelled by the cell from the frame it was first detected in to the current frame.	
DTRatio	D2T	Current tracklength/current displacement.	
Minimal box	box	Arbitrarily oriented minimum bounding box.	Toussaint (1983)
Rectangularity	rect	$\max(x, y)/(x + y)$ where $x$ and $y$ are the length and width of the minimal bounding box.	Wilson (2002)
Variance from centroid	VfC	Variance in distance to boundary pixels from cell centroid.	
Curvature	cur	Measure of boundary curvature based on the triangle inequality.	Wilson et al. (2010)
Density	den	The local density calculated as the proportion of the area around the cell containing pixels from other cells.	

Feature name	short name	Description	Reference
Area	area	The number of pixels within the cell boundary.	Wilson (2002)
AMBRatio	A2B	The ratio of pixels within the cell boundary to the number of pixels within the minimal bounding box.	
Shape descriptors from polygonal representation (4)	poly1	Mean edge length of polygonal approximation to cell.	Ramer (1972)
	poly2	Variance in edge length of polygonal approximation to cell.	
	poly3	Mean interior angle of polygonal approximation to cell.	
	poly4	Variance in interior angle of polygonal approximation to cell.	
Spatial distribution descriptors (9)	IQ1	Intensity quantiles obtained from a spatial point process based on Ripley's K function.	Baddeley et al. (2004)
	IQ2		
	IQ3		
	IQ4		
	IQ5		
	IQ6		
	IQ7		
	IQ8		
	IQ9		
First order features from cell interior pixels (3)	FOf1	Mean of pixel intensities within cell boundary.	Aggarwal and Agrawal (2012)
	FOf2	Standard deviation of pixel intensities within cell boundary.	
	FOf3	Skewness of pixel intensities within cell boundary.	
Haralick features from co-occurrence matrix (3×5)	Hf1	Energy (angular second moment).	Haralick et al. (1973)
	Hf2	Contrast.	
	Hf3	Homogeneity (inverse difference moment).	
	Hf4	Correlation.	
	Hf5	Entropy.	

**Table S1:** Table of features extracted from each cell on each frame. Where more than one feature of a particular type is produced, the number of features is shown in parentheses.

**Table S1** shows the features extracted for each cell on each frame. As the 5 Haralick features are calculated from each of 3 co-occurrence matrices to give 15 texture features, a total of 47 features are calculated for each cell on each frame. The time series for each of these features is summarised by three statistical variables as well as three variables calculated from each of the original time series and three levels of the wavelet-transformed time series, as shown in **Table S2**. This would give 15 variables for each extracted feature but, as the total descent for the feature *tracklength* is always zero, these 4 variables are not included in any analysis. One final variable, the area of the rectangle enclosing the cell’s trajectory, is calculated to give a total of 702 characteristic variables for analysis and classification.

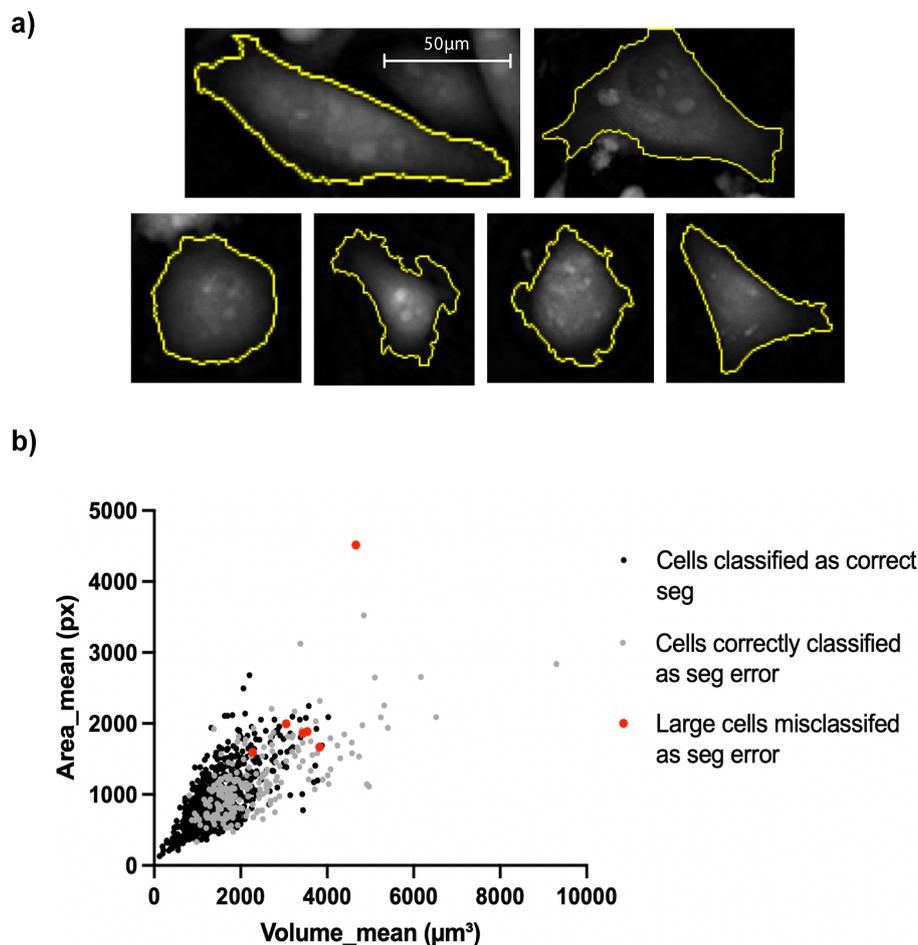
Variable	short name	Description
Mean	mean	Summary statistics from cell time series.
Standard deviation	std	
Skewness	skew	
Total ascent	asc	Elevation variables from cell time series and from 3 levels of the wavelet-transformed time series.
Total descent	des	
Maximum altitude gain	max	
Trajectory area	trajarea	Rectangular area enclosing cell time series.

**Table S2:** Table of variables extracted from each cell’s time series.

We concatenate feature and variable names throughout for brevity. Variables extracted from raw time series are denoted in the form "feature\_variable", for example "Volume\_mean" denotes a cell’s mean volume throughout its track. Variables extracted from wavelet approximated time series are denoted in the form "feature\_level\_variable", for example "Volume\_l1\_mean", "Volume\_l2\_mean", "Volume\_l3\_mean" denote a cell’s mean volume at the first, second and third wavelet approximation respectively. The Haralick features are calculated from three co-occurrence matrices: the original image vs. level 1 wavelet approximation, the original image vs. level 2 wavelet approximation, and level 1 wavelet approximation vs. level 2 wavelet approximation, denoted 01, 02 and 12 respectively. Hence Haralick features are concatenated with extracted variables in the form "feature\_cooccurrence\_variable", for example "Hf1\_01\_mean" denotes a cell’s mean energy extracted from the original image vs. level 1 wavelet approximation co-occurrence matrix.

### Segmentation Error Removal

Visual inspection showed that six large cells were misclassified as having segmentation errors. We therefore compared the area and volume of these cells with cells predicted as correct segmentation to ensure that removal of these six cells would not exclude an important subset from our data sets. This demonstrated that a subset of large cells were correctly classified as having no segmentation errors so that such cells were still represented in our test sets (**Figure S1**).



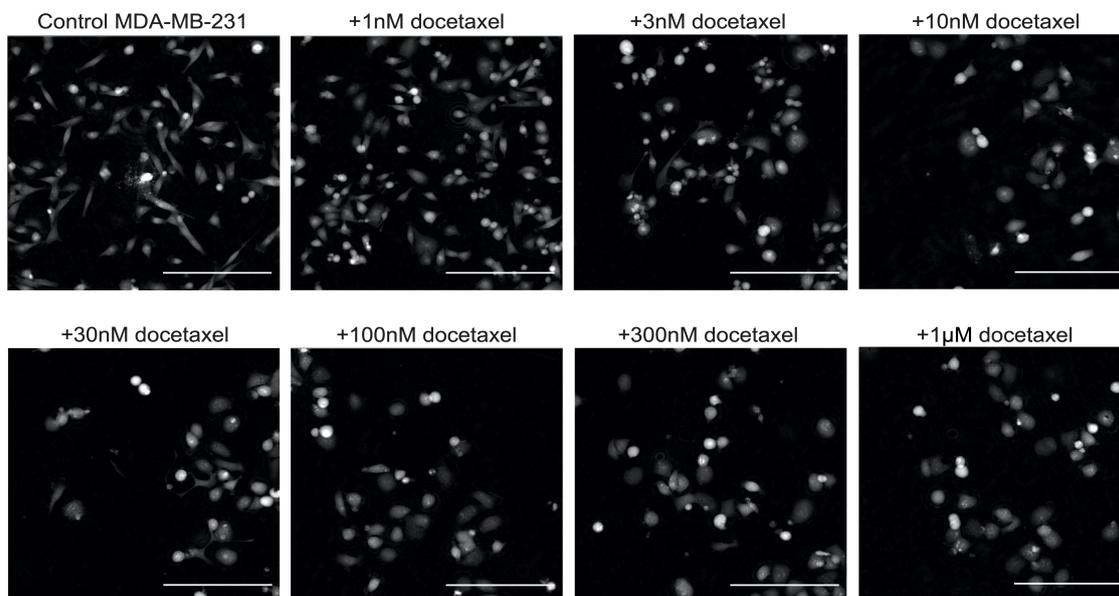
**Figure S1:** a) Images of the six giant cells from the 231Docetaxel test set misclassified as segmentation errors. All images have been equally rescaled. b) Scatter plot of mean volume vs. mean area of all 231Docetaxel test set cells, coloured according to whether the cell was predicted as segmentation error or not. Cells that were predicted as segmentation error but were then confirmed correctly segmented giant cells are coloured in red.

### Classification of Treated and Untreated Cells

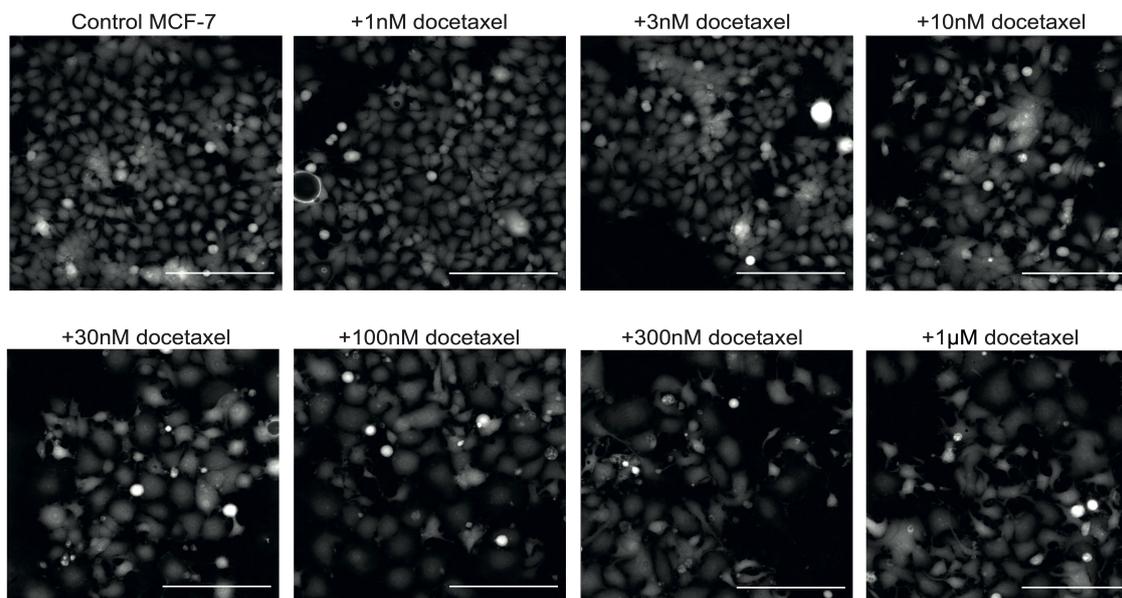
		<b>231Docetaxel</b>	<b>MCF7Docetaxel</b>	<b>231Doxorubicin</b>
<b>Train</b>	<b>LDA</b>	Untreated: 94% Treated: 96% Overall: 95%	Untreated: 100% Treated: 98% Overall: 99%	Untreated: 94% Treated: 97% Overall: 95%
	<b>SVM</b>	Untreated: 95% Treated: 98% Overall: 97%	Untreated: 92% Treated: 93% Overall: 93%	Untreated: 91% Treated: 95% Overall: 93%
	<b>RF</b>	Untreated: 100% Treated: 100% Overall: 100%	Untreated: 100% Treated: 100% Overall: 100%	Untreated: 100% Treated: 100% Overall: 100%
	<b>Ensemble</b>	Untreated: 96% Treated: 99% Overall: 98%	Untreated: 100% Treated: 99% Overall: 100%	Untreated: 97% Treated: 100% Overall: 99%
<b>Test</b>	<b>LDA</b>	Untreated: 95% Treated: 86% Overall: 93%	Untreated: 72% Treated: 74% Overall: 73%	Untreated: 80% Treated: 71% Overall: 78%
	<b>SVM</b>	Untreated: 97% Treated: 80% Overall: 93%	Untreated: 84% Treated: 70% Overall: 83%	Untreated: 82% Treated: 69% Overall: 79%
	<b>RF</b>	Untreated: 97% Treated: 78% Overall: 92%	Untreated: 86% Treated: 85% Overall: 86%	Untreated: 81% Treated: 68% Overall: 78%
	<b>Ensemble</b>	Untreated: 97% Treated: 80% Overall: 93%	Untreated: 85% Treated: 81% Overall: 85%	Untreated: 84% Treated: 71% Overall: 81%

**Table S3:** Classification accuracy scores for each data set, including results for each individual classifier as well as the ensemble. All percentages have been rounded to the nearest whole number.

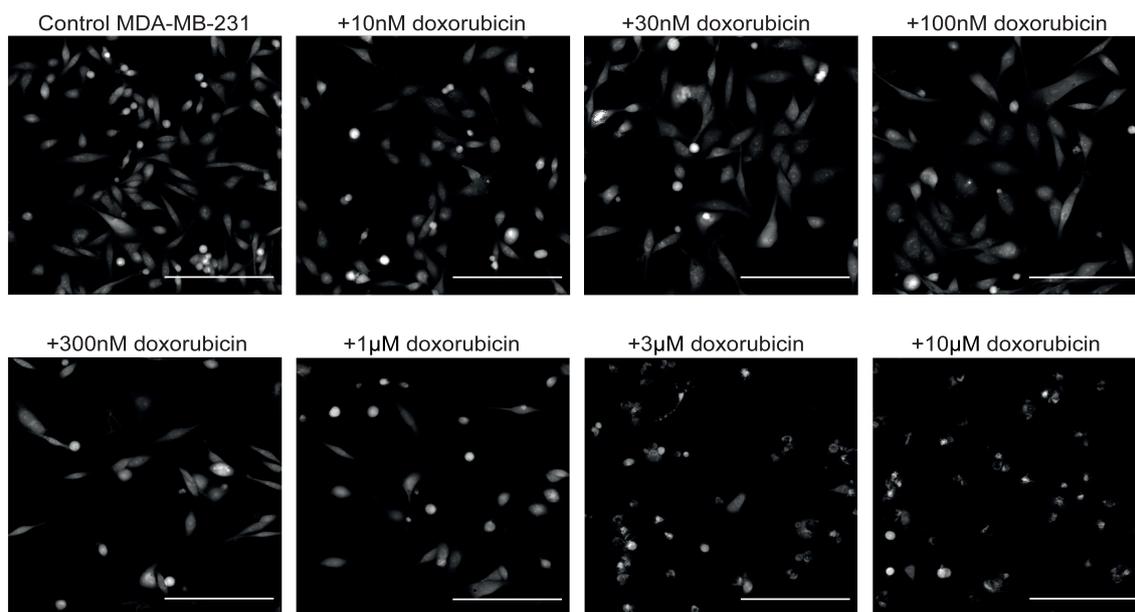
### Dose response images



**Figure S2:** Images of MDA-MB-231 cells taken 48 hours after treatment with varying concentrations of docetaxel. Scale bar = 200μm.



**Figure S3:** Images of MCF-7 cells taken 48 hours after treatment with varying concentrations of docetaxel. Scale bar = 200μm.



**Figure S4:** Images of MDA-MB-231 cells taken 48 hours after treatment with varying concentrations of doxorubicin. Scale bar = 200 $\mu$ m.

## References

- Aggarwal, N. and Agrawal, R. (2012). First and second order statistics features for classification of magnetic resonance brain images. *Journal of Signal and Information Processing*, 3:146–153.
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