
3D SINGLE-CELL SHAPE ANALYSIS OF CANCER CELLS USING GEOMETRIC DEEP LEARNING

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ABSTRACT

1 Aberrations in cell geometry are linked to cell signalling and disease. For example, metastatic
2 melanoma cells alter their shape to invade tissues and drive disease. Despite this, there is a paucity
3 of methods to quantify cell shape in 3D and little understanding of the shape-space cells explore.
4 Currently, most descriptions of cell shape rely on predefined measurements of cell regions or points
5 along a perimeter. The adoption of 3D tissue culture and imaging systems in medical research has
6 recently created a growing need for comprehensive 3D shape descriptions of cells. We have addressed
7 this need using unsupervised geometric deep learning to learn shape representations of cells from 3D
8 microscopy images of metastatic melanoma cells embedded in collagen tissue-like matrices. We used
9 a dynamic graph convolutional foldingnet autoencoder with improved deep embedded clustering to
10 simultaneously learn lower-dimensional representations and classes of 3D cell shapes from a dataset
11 of more than 70,000 drug-treated melanoma cells imaged by high throughput light-sheet microscopy.
12 We propose describing cell shape using 3D quantitative morphological signatures, which represent a
13 cell's similarity to shape modes in the dataset, and are a direct output from our model. We used the
14 extracted features to reveal the extent of the cell shape landscape and found that the shapes learned
15 could predict drug treatment (up to 86% accuracy) and cell microenvironment, and are explainable.
16 In particular, we found strikingly similar deep learning shape signatures between cells treated with
17 microtubule polymerisation inhibitors and branched actin inhibitors. Finally, we implemented our
18 methods as a Python package for ease of use by the medical research community.

19 **Keywords** 3D, geometric, deep learning, single-cell, shape

20 Introduction

21 A central principle in biology is that the shape of living things is connected to their function. Shape is determined
22 by the interaction of evolution with physical laws [1, 2], and aberrations in shape can be a cause or sign of disease
23 [3]. This connection between shape and function holds at every spatial scale from molecules to cells, to organs, and
24 organisms[4].

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26 Due to the importance of shape, biologists have developed many descriptors that attempt to measure and describe it.
27 Traditionally, we understand shape using predetermined or “classical” features. These are pre-selected, mathematically
28 defined measures of geometry and often require a preconceived idea of which features are important for the task at hand.
29 Geometric descriptors include region-based shape descriptors such as area, perimeter, and eccentricity [5, 6, 7, 8, 9],
30 as well as boundary features such as the relative position of consecutive points along a 2D perimeter [10, 11]. In
31 the context of studying living cells in 2D images, these geometric approaches have been remarkably successful at
32 identifying major categories of cell shape, and linking cell shapes to the molecular signalling pathways that control
33 geometry [8, 12, 3, 13], or respond to changes in geometry [14, 15, 16, 17].
34

35 While measuring shape in 2D images has been illuminating, cells exist in 3D environments and their 3D geometry
36 is a rich source of information about the inner workings of cells and tissues. For example, recent pioneering work
37 has demonstrated that shape motifs extracted from 3D images can be linked to lipid membrane signalling and cancer
38 signalling pathways [14], and changes in 3D nuclear morphometry are linked to chromatin organisation [18]. At the
39 scale of developing tissues, the discovery of new 3D geometries has explained how cells pack stably into curved
40 organs [19]. Now, the development of high throughput light-sheet imaging [20, 21] has made it possible to image
41 large numbers of cells in 3D with sub-cellular resolution. This has led to a growing need for accurate 3D shape
42 representations. We have addressed this need by building upon recent advances in deep learning (DL), and the field of
43 computer vision.
44

45 Robust representation of the shape of 3D objects is a major goal in the field of computer vision [22], and there has
46 been rapid progress in this domain. Supervised and unsupervised methods of shape description have been built on
47 large-scale labelled datasets of 3D models of everyday objects [22, 23, 24]. State-of-the-art methods have analysed
48 these 3D models as either 3D binary voxelised objects, or point clouds for tasks of classification [25, 26, 27, 28]
49 and unsupervised representation learning [29, 30, 31, 32, 33, 34, 35]. Point cloud representation is an alternative
50 approach to representing 3D images with regular dense voxels. Point clouds are composed of unordered and
51 irregular sets of points, $\mathbf{P} \in \mathbb{R}^{N \times 3}$. Point clouds are scattered collections of points in 3D space and are arguably
52 the simplest shape representation. These are easily obtainable once a segmented mask has been created and are
53 memory-efficient when compared to 3D images. 3D images grow in memory depending on the size of the object
54 or the number of voxels, however, point clouds may be represented by a fixed set of points so that larger objects
55 use the same amount of memory as smaller objects. This means that methods using point cloud representation are
56 easily scalable. This data representation is the most commonly used in 3D shape analysis primarily because this
57 is the raw data of object surfaces received from LIDAR sensors in applications of autonomous driving, robotics,
58 and scene understanding. This has led to in-depth research into DL architectures which explore local geometric
59 information, using convolution, graph, and attention mechanisms on point clouds for improvements in 3D shape analysis.
60

61 DL on point cloud data often involves representing point clouds as graphs and then performing geometric deep learning
62 (GDL) on these graphs [25, 36, 27, 37, 38]. In computer science, graphs are data structures that consist of nodes and
63 edges. DL on graphs requires adaptations as the data structure is non-Euclidean and makes regular operations such as
64 convolution rather elusive. GDL is a branch of deep learning that consists of methods to generalise DL methods to
65 non-Euclidean domains such as graphs [39].
66

67 Feature or representation learning are techniques that enable a machine to automatically learn representations needed
68 for downstream tasks including clustering or classification. Autoencoders are DL algorithms that are commonly
69 used for representation learning tasks. In general, autoencoders are machines that consist of an encoder and a
70 decoder part where the encoder maps the input to a lower-dimensional space and the decoder attempts to reconstruct
71 the input. Autoencoders are trained by minimising the difference between the input and the reconstructed data.
72 Graph-based autoencoders learn lower-dimensional representations of graph-structured data and have been used to
73 learn representations of 3D shapes [32, 31, 40].
74

75 Motivated by the need to extract information embedded in 3D shape, and by the effectiveness of GDL approaches in
76 generating 3D shape representations, we have used artificial intelligence to reveal the geometric landscape of biological
77 cells. In this study, we build on the progress made in computer vision and use neural networks to automatically learn
78 high-quality shape representations for single cells in 3D microscopy images. To our knowledge, this represents the
79 first unsupervised approach to the description of 3D cell morphology. After the generation of high-quality 3D shape
80 representations we use our GDL features to predict drug treatments, and explore and explain the features learned. Finally,
81 the methods have been coordinated in an open-source Python package for ease of use for the research community.

82 The code and links to all repositories used in this study are freely available at <https://github.com/DeVriesMatt/cellshape>.
83

84 Results

85 Data acquisition and processing

86 To create a dataset of cell and nuclei 3D shapes that exist in a human cell line, we used light-sheet microscopy to image
87 more than 70,000 WM266.4 metastatic melanoma cells embedded in tissue-like collagen matrices. To sample a wide
88 range of the possible shapes that cells can make we treated cells with inhibitors of the cytoskeleton (Figure 1A), as
89 well as inhibitors of cells signalling pathways. These cells were embedded in an environment that spanned from
90 mechanically rigid and flat (within $7 \mu\text{m}$ of the coverslip), to soft and 3D (greater than $7 \mu\text{m}$ away from the coverslip).
91 Coverslip height was identified using a z profile of the nucleus intensity as described in [41]. Based on previous works
92 on 3D cell shape analysis and collagen dispensed on rigid substrates, we treat these as two different environmental
93 classes, namely proximal and distal [41].
94

95 In order to focus on shape data, we created binary masks of each cell and nucleus by thresholding the whole volume by
96 active contour segmentation [42]. Touching cells and nuclei were separated by the watershed-based methods described
97 in [41] (Figure 1B). Our GDL method requires point cloud data. As such, 3D voxel masks were then converted to mesh
98 objects using marching cubes and 2048 points were sampled from the surfaces of each mesh object to give a point cloud
99 representation of each cell and nuclei (Figure 1C). We have created a Python package for point cloud generation called
100 'cellshape-helper'. We have implemented a number of GDL algorithms for shape representation learning on point
101 clouds and made these available as a python package called 'cellshape-cloud'. Point clouds were pre-processed by
102 mean centring and scaling to assist in training our GDL model.

103 An automated method to profile cell shape in 3D using geometric deep learning

104 Towards the goal of creating an automated method to profile single-cell shapes in 3D, we used GDL models. Our
105 GDL model, dubbed Dynamic Graph Convolutional FoldingNet (DFN) (Figure 1D) (detailed description in), is a
106 combination of three techniques and is intended to learn shape features and classes simultaneously. DFN consists of an
107 autoencoder to learn shape representations in an unsupervised manner with an additional clustering layer that groups
108 cells into classes based on these features. A point cloud representation of a cell or nuclei is passed into the encoder part
109 of the autoencoder, this encoder part is known as a dynamic graph convolutional network [25] (light blue in Figure 1D).
110 Here, the point cloud goes through a series of operations known as edge convolution. Edge convolution is an operation
111 which takes in a point cloud, creates a k -nearest neighbour graph on that point cloud, and then performs 2D convolution
112 on the edges or adjacency matrices of that graph and outputs a new lower-dimensional set of points. This part is called a
113 dynamic graph convolution as a new graph is constructed at every layer of the network. The output of this encoder is a
114 lower-dimensional representation, \mathbf{Z} , of the input point cloud. This lower-dimensional representation passes through
115 two branches, each with different purposes. In order to learn representative features, we need to be able to reconstruct
116 from this lower-dimensional space to the original point cloud. To do this, we use a decoder in the form of a FoldingNet
117 [31] (light orange in Figure 1D). The FoldingNet takes in the lower dimensional representation concatenates it with
118 points sampled from a 2D plane and performs multi-layer perceptron operations on this to output a reconstructed point
119 cloud. This part is trained on how similar the input and output point clouds are. In order to learn shape classes, a
120 clustering layer takes in the lower dimensional feature vector, performs k -means clustering to get an initial estimate of
121 shape classes and then gives a probability score of how likely a particular feature representation of a 3D cell is to belong
122 to a cluster or shape class (grey in Figure 1D). In order to avoid confusion, we refer to the k in the k -nearest neighbours
123 graph algorithm as k_g and the k in the k -means clustering algorithm as k_c . Cluster centres and encoder weights are
124 learned in a way which places most of the probability mass where the clustering layer is confident that certain cells
125 belong to certain classes. Initially, the DFN was trained on the ShapeNet dataset for 250 epochs and then we continued
126 training on our point cloud representations of both cells and nuclei for another 250 epochs using Adam optimiser with
127 e^{-6} weight decay. We used a batch size of 16 with an initial learning rate of e^{-4} and an exponential learning rate decay
128 scheduler. The DFN model was set to extract 128 features from each point cloud (see Methods Section).

129 Exploring the cell shape feature landscape

130 Our trained DFN model without the clustering layer was first used to extract 3D shape features from all cells in our
131 dataset. We visualised the extent of the cell shape landscape by performing UMAP [43] on the extracted features to
132 form a two-dimensional embedding space. We sampled two-dimensional locations along this projection and plotted

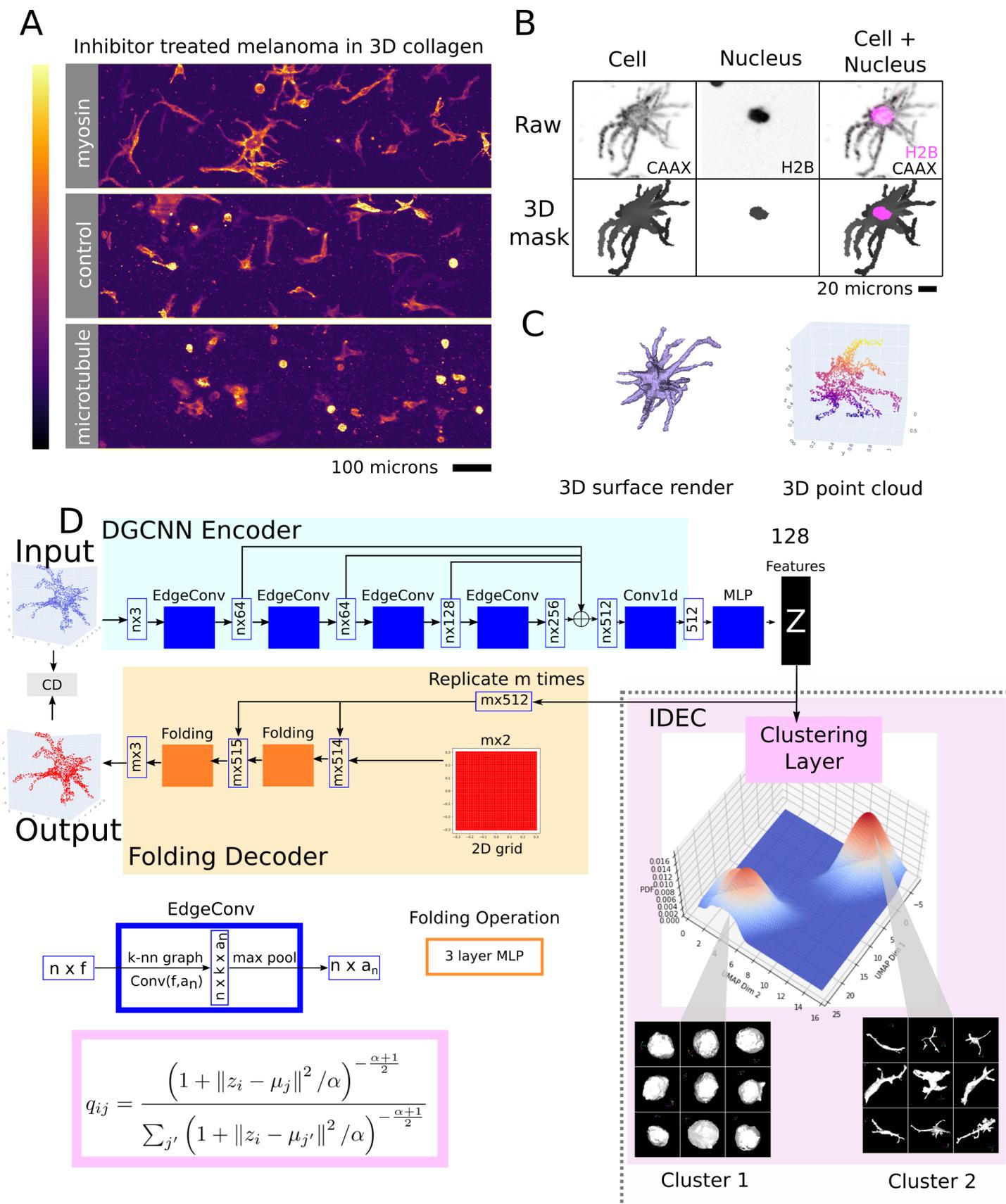


Figure 1: Learning shape features and classes simultaneously through geometric deep learning and deep clustering. (A) Single-cells embedded in a collagen matrix were treated with a variety of small molecule drugs and imaged in 3D in high-throughput by stage scanning oblique plane microscopy (ssOPM). Maximum intensity projections (XY view) of cells treated with DMSO (control), Blebbistatin (myosin) and Nocodazole (microtubule) are shown. Intensity is the grayscale intensity of the CAAX-GFP membrane marker. (B) The cells were labelled with transgenes expressing Histone-2B (which labels the nucleus) and CAAX-GFP (which labels cell membranes). Nucleus and cell label intensities (Raw) and their segmentation masks (3D mask) are shown for a single cell. (C) We used the marching cubes algorithm to create a mesh object of vertices, faces, and normals and then sampled 2048 points from this mesh object to create a point cloud for each cell cytoplasm and nucleus. The 3D surface render and 3D point cloud for a cell are shown. (D) A dynamic graph convolutional foldingnet autoencoder was used to learn a lower-dimensional representation of the input cell shape. This consisted of a Dynamic Graph CNN as an encoder (light blue) and a FoldingNet decoder (light orange). In order to learn representations and shape classes simultaneously, improved deep embedded clustering adds a clustering layer to the feature representations (light purple). This maps the representations of each cell to a specific shape class and gives a morphological signature for each cell in terms of the different shape classes that exist in the dataset. We show a dotted box around this component of the model as it is not always used and is an addition to the autoencoder.

133 rendered images of cell masks which generated the features projected in UMAP space (Figure 2A). Furthermore, we
134 zoom in on locations where feature representations were dense to show common shapes that exist in the dataset. The
135 first dimension of the UMAP seems to incorporate eccentricity, with round cells having low values for this dimension
136 and more eccentric cells having high values. After visualising the extent of the cell shape landscape, we looked at
137 the distribution of cells within this landscape. We estimated the probability density function using kernel density
138 estimation of the UMAP embedding of the latent space (Figure 2B). This revealed three peaks representing common
139 cell shapes or shape modes in our dataset. These peaks represented small round cells, large round cells, and a large
140 group of cells that ranged from elongated to protrusive. A subset of cells in our dataset had been treated with small
141 molecule inhibitors, including myosin and microtubule inhibitors. We visually assessed the effect of these on cell
142 shapes in 3D. The cell shape feature landscape demonstrated that cells treated with different inhibitors cause different
143 distributions of embedding (which represent shape distributions). For example, Control cells (Figure 2C) and cells
144 treated with Blebbistatin (myosin inhibitor) (Figure 2D) are able to take on a larger range of shapes than cells treated
145 with Nocodazole (microtubule inhibitor) (Figure 2E). Nocodazole-treated cell shapes are confined to rounder-like cells
146 with few protrusive cells.

147

148 The cells in our dataset are embedded in a 3D collagen matrix at a variety of depths. To understand how differences in
149 the physical environment influence cell shape we visualised our UMAP space based on the different environments. We
150 coloured each embedding location in UMAP space according to the cell's distance from the coverslip (Figure 2F). This
151 showed that cells close to the rigid coverslip (proximal) and cells embedded in collagen (distal) are separated in shape
152 space and that the geometry and physical properties of the microenvironment have a major influence on cell shape. The
153 distribution of cells proximal to the coverslip (fewer than 7 microns) showed two peaks or shape modes with one of
154 these being more spread out than the other (Figure 2G). A similar analysis of the distribution of the UMAP embedding
155 for cells distal to the coverslip (greater than 7 microns) showed one major density peak with three smaller peaks (Figure
156 2H). Interestingly, the embedding location of the greatest peak for distal cells is almost non-existent in cells proximal to
157 the coverslip. It is also evident that the cells' environment impacts their ability to take on different shapes.

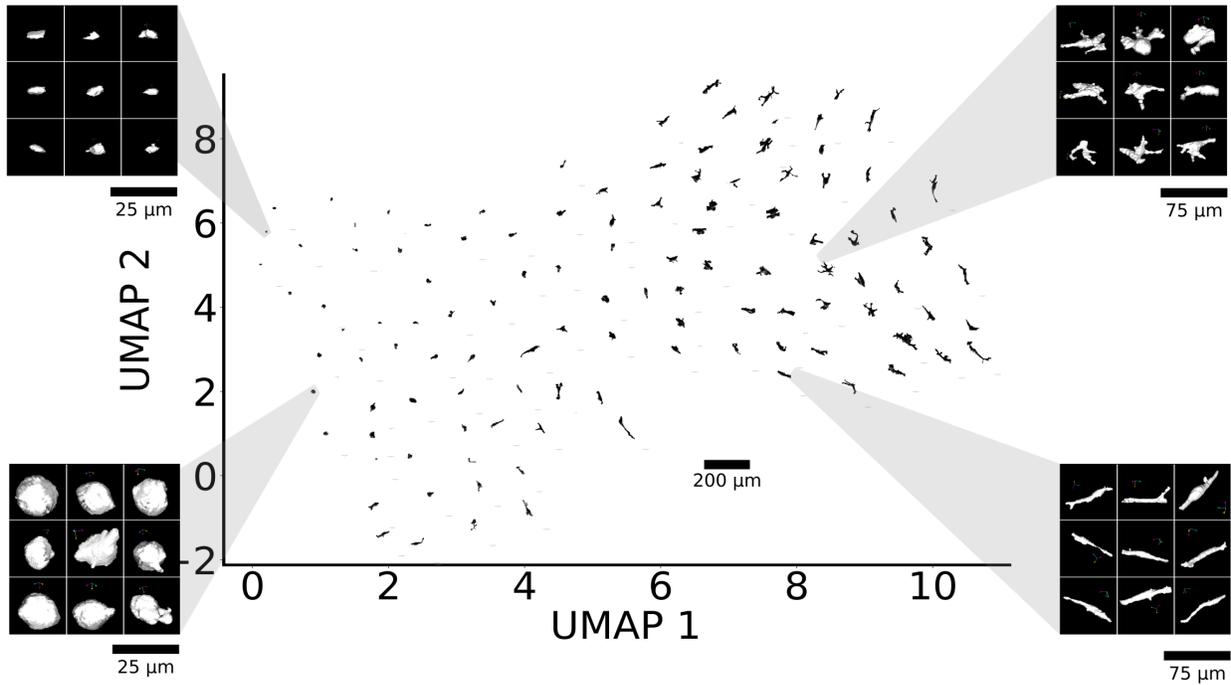
158

159 **Deep embedded clustering learns shape features and classes simultaneously**

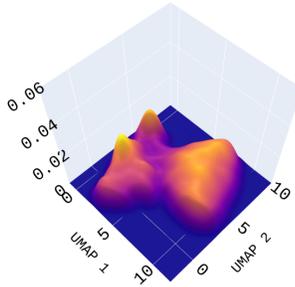
160 Many studies have used deep learning to cluster or organise everyday 3D objects by their shape [31, 44, 45, 46]. These
161 tasks use datasets that have distinct semantic (or human meaningful) classes. For example, the ModelNet [22] dataset
162 includes chairs, aeroplanes, baths, beds, lamps and cars. In contrast, in problems of phenotypic classification within a
163 dataset of cells, we do not have labelled phenotypes for every single cell. Furthermore, we did not know the number
164 of semantic or phenotypic classes in our dataset, or even if any distinct classes exist. An interesting solution given
165 this lack of prior knowledge has been to automatically learn feature profiles and classify images into classes without
166 predefined labels [47, 48]. We wanted to explore the different classes of cell shape in our dataset and propose jointly
167 learning feature representations and shape clusters, simultaneously.

168

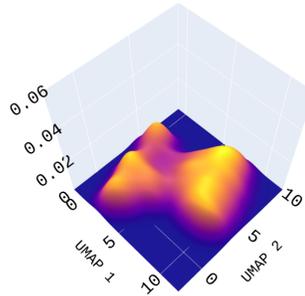
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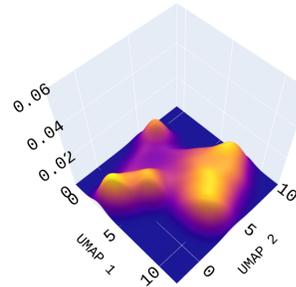
B All



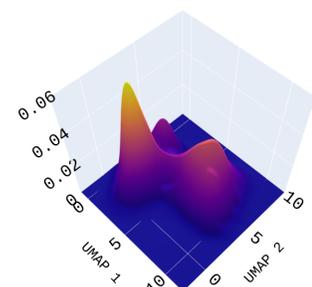
C DMSO



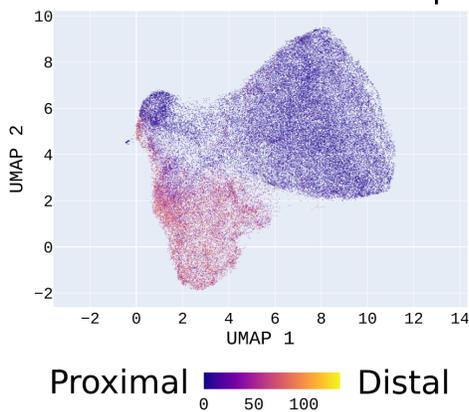
D Blebbistatin



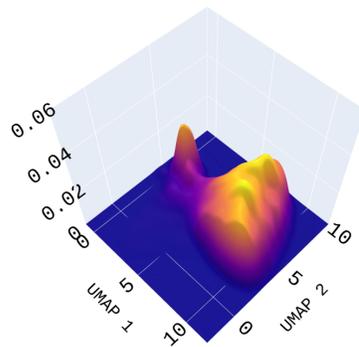
E Nocodazole



F Distance from coverslip



G Proximal



H Distal

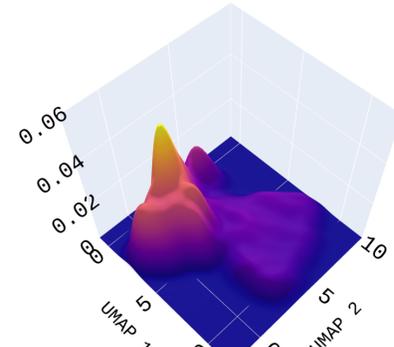


Figure 2: **Dynamic graph convolutional foldingnet reveals the cell shape landscape in a cancer cell line.** We performed UMAP on the 128 features extracted using the DFN without the addition of the clustering layer. (A) Rendered sample images of cell masks are plotted in UMAP. (B-E) The probability density of the UMAP was estimated using kernel density estimation. DMSO treated cells (C) had different UMAP distributions than Blebbistatin (D) treated cells and Nocodazole (E) treated cells. (F) The points on the UMAP were coloured by their distance relative to the coverslip. The microenvironment impacts the ability of cells to take on different shapes. (G) Cells on the coverslip tend to be flatter and more spread out. (H) Cells embedded in collagen (further away from the coverslip) are more round.

169 After training the DFN autoencoder, we performed improved deep embedded clustering (IDEC) [49] by adding a
170 clustering layer to the output of the encoder part. This clustering layer (grey in Figure 1D) takes the embedded feature
171 space, \mathbf{Z} , as input and outputs a soft-label over the shape classes found according to a Student's t-distribution (purple
172 box in Figure 1D). The soft-label is interpreted as the probability of assigning an input cell i to shape cluster j . For
173 example, if there are k_c clusters in the dataset, each cell is assigned k_c soft-labels (one for each cluster) and each
174 soft-label contains scores from zero to one reflecting how similar the cell is to each of the different cluster centres (with
175 one being most similar)(Figure 3A). Since the 3D shape of cells in a fixed assay is a relatively continuous distribution
176 with an indefinite number of distinct classes (cells may be very similar in shape, yet almost never the exact same), it is
177 more natural to describe a shape by a signature of how similar it is to a number of different exemplar classes. For
178 example, we may wish to describe the 3D shape of a cell to be 30% similar to shape cluster 0, 10% similar to shape
179 cluster 1, 5% similar to shape cluster 2 for shape classes $i = 0, 1, \dots, k_c$, such that the sum of similarity percentages add
180 to 100 ($\sum_{i=0}^{k_c} k_c^{(i)} = 100$, where $k_c^{(i)}$ is shape class i). This can be defined as a quantitative morphological signature for
181 the 3D shape of a cell (3DQMS). Therefore, our k_c -dimensional vector of soft-labels can be used as a 3DQMS. The
182 number of clusters was chosen as a hyper-parameter. However, this could also be estimated by prior knowledge, or
183 through more formal methods such as the elbow, average silhouette or gap statistic methods when undertaking the
184 initial k -means clustering. The clustering layer is packaged in a tool called 'cellshape-cluster' and can easily be
185 imported into our 'cellshape-cloud' autoencoder models.

186

187 For our experiments, we selected five clusters and the DFN-IDEC output a soft-label for each cell of the five shape
188 classes found. These five clusters approximated the five most different groupings of 3D cell shape features in our
189 dataset. We sampled from the dataset for cells which had the highest values of soft-label for each of the five classes to
190 see classes ranging from round, to elongated, to protrusive and block shapes cells (Figure 3B). Using this soft-label,
191 we were also able to examine the 3DQMS for cells treated with different inhibitors. We can further inspect how
192 Blebbistatin and Nocodazole-treated cells make up the different clusters by projecting the features using UMAP and
193 fitting a kernel density on this UMAP projection (Figure 3 C), similar to Figure 2. Cells treated with Blebbistatin
194 explore four of the five shape clusters (Figure 3C), labelled 1-4 in Figure 3A. These shape clusters are large and
195 protrusive when compared with shape cluster 0 which is the round cluster. Nocodazole-treated cells tend to mostly fall
196 within shape cluster 0 (Figure 3I). Next, we standardised and averaged the 3DQMS for each cell by their treatment and
197 performed hierarchical clustering on these to find four classes of treatment effects on shape (Figure 3D). We noted that
198 two distinct inhibitors of myosin activation (Blebbistatin and H1152) grouped together, indicating that the 3DQMS will
199 be a powerful tool for using cell shape to identify drugs that act on similar pathways. The other classes of treatment
200 effects on shape included Nocodazole, which inhibits the polymerisation of microtubules, CK666, which prevents actin
201 branching and then control cells grouped with those treatments that do not have significant effects on cell shape. To
202 visualise the variability in 3DQMS within each treatment and differences between treatments, we took the average
203 3DQMS for each well and embedded these in two dimensions using UMAP (Figure 3E). This again showed the two
204 distinct myosin inhibitors grouped together.

205

206 When clustering on the shape classes (hierarchical clustering on the columns of Figure 3D), we saw that shape cluster 0,
207 which is the first column in Figure 3D, was the main driver of variation between the treatments. Thus, we examined the
208 soft-label distribution for shape cluster 0 in these four main classes of treatment effects on shape (Figure 3F). Cells
209 treated with Blebbistatin tended to have more cells with low-scoring soft-labels in cluster 0 (Figure 3F), compared
210 to the DMSO and CK666 treated cells (Grey box in Figure 3F and F, respectively). In contrast, cells treated with
211 Nocodazole tended to have much higher soft-label scores for cluster 0 (Figure 3F). A cell's environment impacts the
212 shape space, as seen in Figure 2, and thus affects the 3DQMS of different treatments. For cells distal to the coverslip,
213 the distribution of soft-label scores of cluster 0 for cells treated with Blebbistatin tends to flatten out slightly (Magenta
214 box in Figure 3F). All distributions shift to the right (higher scores for shape cluster 0) for distal cells (Magenta box in
215 Figure 3F), whilst this distribution shifts to the left for proximal cells across all treatments (Purple box in Figure 3F).
216 Cells treated with Nocodazole that were distal to the coverslip mostly scored very high for shape cluster 0 (Magenta in

217 Figure 3F). Finally, the feature landscape is affected by the clustering layer such that each peak roughly represents a
218 shape cluster (Figure 3A).
219

220 **Learned shape features predict cell treatment**

221 We explored the possibility to distinguish between cells with different small molecule inhibitor treatments based
222 purely on cytoplasm and nuclei shape features. Many studies in computer vision and 3D shape representation learning
223 test the effectiveness of their representations with transfer classification tasks. This involves using a trained model
224 to extract features from a dataset and then using these features to train a linear support vector machine (SVM)
225 classifier [31, 44, 45, 46]. These tasks generally involve classification between objects from distinct semantic classes
226 or categories, for example, cars, chairs, and aeroplanes [22]. Our dataset is comprised of a single cell type and did
227 not have such distinct semantic classes, so to test our shape representations we predicted our drug treatment labels.
228 Following similar procedures to previous researchers [31, 44, 45, 46], we used the trained DFN to extract features
229 from each cell. These features were then used to train a linear SVM. For all experiments, we use one-versus-one
230 SVM classifiers with an L2 penalty ($C = 1$), balanced class weights, and intercept scaling during training. We tested
231 cytoplasm features and nuclei features alone and then combined them for ablation studies (Figure 4A). We performed a
232 10-fold cross-validation for each experiment and report mean accuracies.
233

234 To compare the relative importance of cell and nuclear for predicting drug treatments we tested the accuracy of
235 classifiers using only cell features, only nuclear features or the combination of both cell and nuclear feature sets. We
236 also analysed prediction accuracies for different environments (Figures 4 B and C). To do this comparison we tested
237 one-versus-one classification accuracies between Blebbistatin and Nocodazole-treated cells. We used these treatment
238 sets because our deep clustering of our dataset showed that these two treatments had the most different 3DQMS profiles.
239

240 When using cytoplasm shape features alone, we were able to predict the cells' treatment with an accuracy of 79%
241 for cells proximal to the coverslip, 82% for cells distal to the coverslip and 80% for all cells regardless of the
242 microenvironment. This is compared to the 74% accuracy when using shape features extracted from the nucleus only
243 for proximal cells, 76% for distal cells and 74% for all cells. When we combined these two sets of shape features,
244 accuracies increased to 86%, 82% and 83% for proximal, distal and all cells respectively. This difference in accuracy
245 between combined and individual data sets suggests that not only are the shapes of the cell and nucleus affected by
246 these treatments but so too are the relationship between them (Figure 4A).
247

248 Based on the combination of cell and nuclear features giving the best prediction accuracy we tested the prediction of all
249 of our treatments in a pairwise fashion (Figures 4B and C). Due to the strong influence of environment on shape (Figure
250 4A), we made these predictions separately for cells in soft collagen environments (distal to the coverslip), and cells in
251 rigid environments (proximal to the coverslip). In the proximal setting, we saw a general trend for higher accuracy of
252 one-versus-one predictions (Figure 4B) than those in the distal setting (Figure 4C). When looking at all cells in both
253 settings, we see accuracies between the two settings (Figure 4D). We also visualised the difference between the two
254 settings directly as the difference between 2D and 3D prediction accuracies (Figure 4D). For distal settings cells treated
255 with Nocodazole, Blebbistatin and H1152 all had one-versus-one classification accuracies across all other treatments
256 higher than 62%. On average, cells treated with Nocodazole had the highest classification accuracies (Figure 4C).
257 We noted that Blebbistatin and H1152 were both distinguishable from control (66% for H1152 vs No Treatment and
258 70% for Blebbistatin vs No Treatment in both environments, shown in Figure 4D) but difficult to distinguish from one
259 another (56% accuracy).
260

261 In the distal setting, many of the remaining treatments had one-versus-one classification accuracies of less than 0.6,
262 indicating they occupy a similar region of shape space. Unexpectedly, we did notice that Binimetinib compared to our
263 "No Treatment" control was as equally distinguishable as the arp2/3 inhibitor, CK666 (equally 63% in proximal cells,
264 62% and 61% in distal cells, and equally 61% when looking at all cells). This was notable because Binimetinib is a
265 MEK1/2 inhibitor without major connections to regulating the cytoskeleton, whereas the primary effect of CK666 is to
266 prevent the formation of branched actin structures.
267

268 Consistent with the distal setting, Nocodazole, Blebbistatin and H1152 had the highest one-versus-one classification
269 accuracies in the proximal setting (Figure 4B). Also consistent with the distal setting, we saw that the targeted therapy
270 Binimetinib was as distinguishable from controls as CK666. Strikingly the classification accuracy of Binimetinib

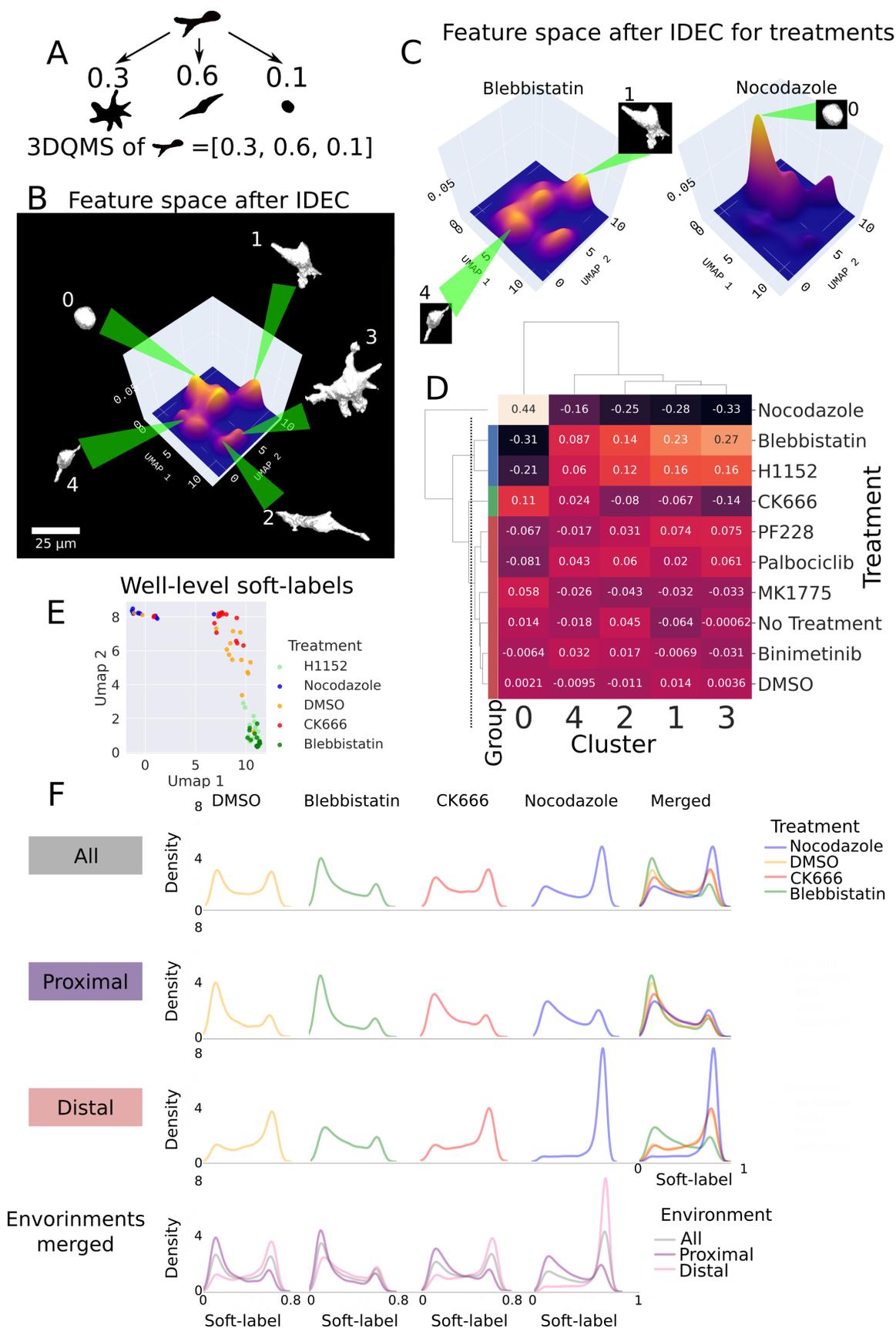


Figure 3: Improved deep embedded clustering learns quantitative morphological signatures for 3D shapes of cells. We added a clustering layer to a trained DFN model to learn the shape clusters that exist in the dataset. (A) Schematic example of Improved deep embedded clustering (IDEC) soft label assignment to a cell in the case of three clusters. (B) The clustering layer outputs a distribution of soft-labels across the five classes. We show the five exemplar shape classes found and the kernel density approximation of the features extracted. (C) Shows the UMAP projection of the features extracted from the DFN-IDEC model for Blebbistatin and Nocodazole-treated cells. (D) Each cell is assigned a 3DQMS which is a five-dimensional vector that describes how similar it is to each shape cluster. We standardised and averaged the 3DQMS for each treatment and performed hierarchical clustering to reveal four classes of the treatment effects on cell shape. The dotted line shows the threshold for hierarchical clustering. (E) Shows a UMAP projection of the 3DQMS for different treatments. (F) We show the distribution of soft-label scores for cluster 0 for Blebbistatin, DMSO, CK666, and Nocodazole-treated cells respectively (All). Similarly, we show this for cells proximal to the coverslip (Proximal). (D'-H') And for cells distal to the coverslip (Distal).

271 was increased in the proximal setting (Figures 4B, C and E), suggesting that the shape effect of the MEK inhibitor
272 is more pronounced in rigid environments. This does not appear to be a general effect of the cell cycle inhibitory
273 effect of Binimetinib, for two reasons. First, cells treated with the CDK4/6 cell cycle inhibitor, Palbociclib, were not
274 readily distinguishable from control. Second, In contrast to Binimetinib, there was no obvious interaction between
275 shape and environment on the effect of treatment with Palbociclib (Figure 4D). Taken together, the lack of general
276 effect of cell cycle inhibition suggests components of the cytoskeleton may be direct targets of MEK1/2 phosphorylation.
277

278 Finally, following the observation that Blebbistatin and H1152 were distinct from control but hard to discern from
279 one another, we developed a classification “interference test”. To do this, we measured the change in the correct
280 classification of Blebbistatin-treated from control cells, in three-way comparisons between DMSO, Blebbistatin
281 and each other treatment. We found that in one-versus-one comparisons, the SVM classified Blebbistatin correctly
282 from a pool of Blebbistatin and control cells with an accuracy of about 65% (65% of true Blebbistatin-treated cells
283 were predicted as Blebbistatin). This is different to the accuracies reported above as those were the accuracies for
284 both treatments involved in the classifier. This accuracy showed little change in three-way comparisons between
285 Blebbistatin, DMSO and iterations of most other treatments. For example, introducing MK1775 into the classification
286 (Blebbistatin:MK1775:DMSO) had little effect (0.02 interference), but introducing H1152 (Blebbistatin:H1152:DMSO)
287 had a large impact on classification accuracy (0.18 interference) (Figure 4E). Blebbistatin and H1152 act at separate
288 points in the Rho-Rock-Myosin II activation cascade, indicating that our extracted shape features and classification
289 interference test can be used to group drugs with similar mechanisms of action (Figure 4F).
290

291 **Interpreting the learned features**

292 Feature interpretation allows biologists to inspect DL models. Ultimately we want to know what the models are
293 seeing in the data and learn from them. DL models may be learning redundancies or focusing on information that is
294 not important to the task at hand. Alternatively, the models may learn something which is not already known, thus
295 providing new information on possible biomarkers.
296

297 Given that the DFN is able to automatically extract features which classify myosin and microtubule inhibitors based on
298 cell shape (Figure 4), we sought to interpret the cell shape features learned by our method. These methods can be easily
299 extended to nuclei shape features. We interpreted our features depending on two criteria, first we discovered intuitive
300 explanations for the DL features that were the most powerful drivers of variation in cell shape space by relating them to
301 manually crafted, ‘classical’ features.
302

303 Our second approach was a task-specific approach, where we decoded the DL features that contributed the most to
304 successful discrimination between cell treatments in different classification tasks (see Methods Section). Here, we
305 compared features important for classifying Blebbistatin versus Nocodazole-treated cells.
306

307 Naturally, there existed a correlation between the DL features. Therefore, we performed principal component analysis
308 on the DL features to obtain 128 principal components (PC) ordered by the magnitude of their singular values. We then
309 correlated the principal components with our classical features of geometry (Figure 5A). Green boxes in Figure 5A
310 show the classical features with the highest absolute correlation to each PC. PC0 encodes many of the classical features

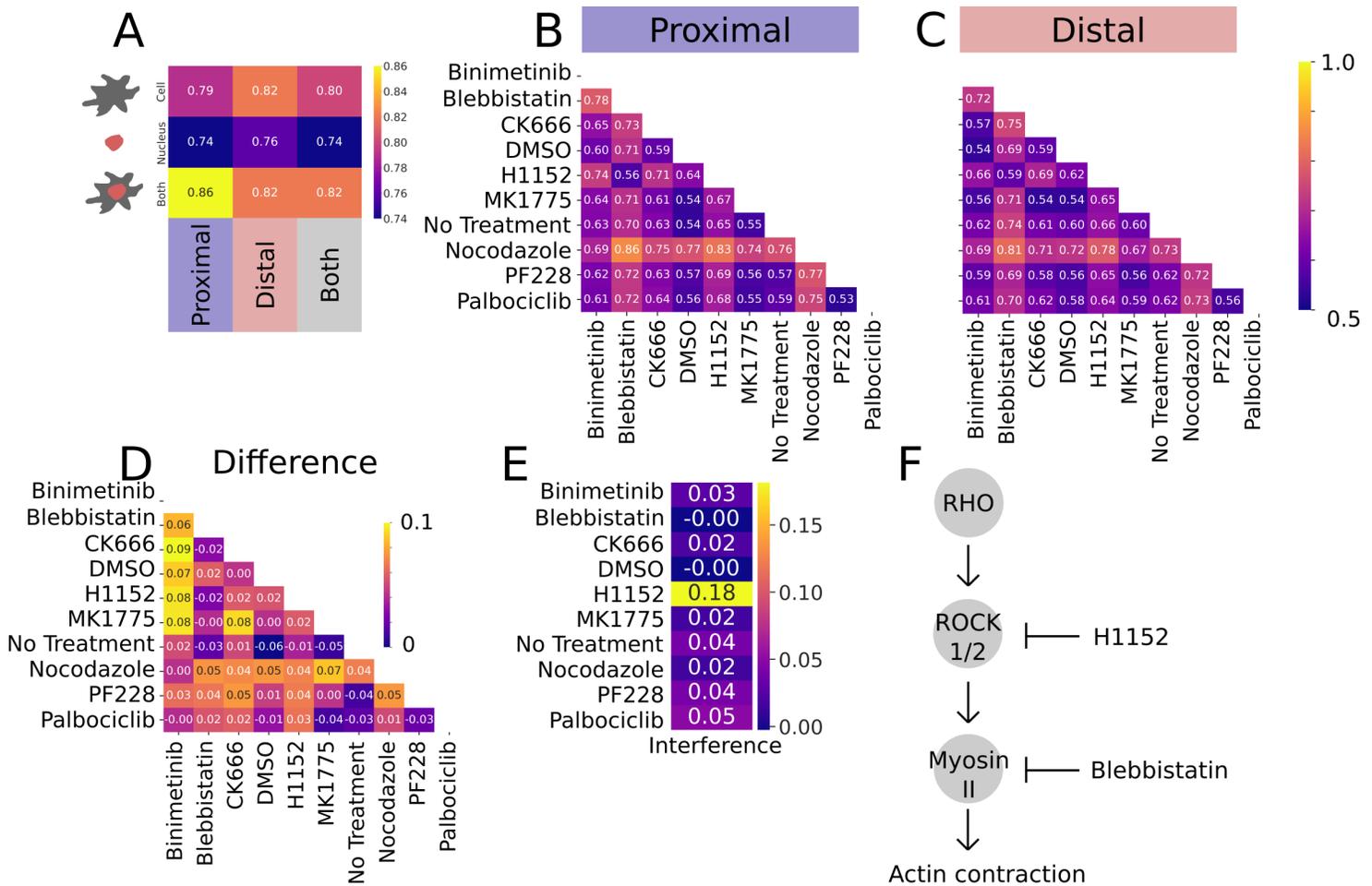


Figure 4: Features extracted using the DFN accurately predict small molecule treatments. (A) Features were extracted from the cytoplasm, and nuclei of cells treated with Nocodazole and Blebbistatin and used to train a linear support vector machine (SVM) to predict treatment. We tested the different feature sets using cytoplasm features only, nucleus features only and combining these two. We also tested predictions in different environments from cells proximal to the coverslip, to distal from the coverslip to all cells (both proximal and distal). (B-C) We used an SVM to predict treatment for all cells using the combined cell and nucleus feature set in (B) proximal cells, and (C) distal cells. (D) Shows the difference in accuracies between proximal and distal cells. (E) Shows the interference effect of each treatment on the accurate classification of Blebbistatin when compared to DMSO. The treatment with the strongest interference effect was H1152, which acts above Blebbistatin in the RHO-ROCK1/2-MyosinII pathway.(F) A schematic of the RHO-ROCK1/2-MyosinII pathway and the target of Blebbistatin and H1152.

311 with major axis, surface area and sphericity being the highest. PC2, which explains 10.85% of the variation in the
 312 data, has a maximum correlation coefficient of 0.14 with classical features (minor axis) and does not correlate highly
 313 with any classical features suggesting that the DFN may be capturing features which are not explained by our classi-
 314 cal features. The first two PCs account for 42.47% of the variation in the data with the first 10 PCs accounting for 82.24%.
 315

316 We assessed how the treatments differ in terms of their PC loadings by taking the average PC loadings for cells treated
 317 with Blebbistatin, Nocodazole, H1152, CK666 and DMSO (control) (Figure 5B). For most of the first 10 PCs, we
 318 see that cells treated with Blebbistatin and cells treated with H1152 (Blebbistatin and H1152 which both act in the
 319 Rho-Rock-Myosin activation cascade) have similar average PC loadings. Interestingly, cells treated with Nocodazole
 320 and cells treated with CK666 have similar signs of average PC loadings for most PCs. These two groups (Blebbistatin
 321 and H1152 being one and Nocodazole and CK666 being another) tend to have opposite average PC loadings.
 322

323 The second, task-specific approach, revealed that PC0, PC3, and PC9 were most important in classifying between
324 Nocodazole and Blebbistatin treated cells (See Methods Section). Similar to methods in [50], the correlations shown in
325 Figure 5A are visually confirmed by sampling cell shapes from the dataset with a range of values for different principal
326 components (Figure 5C-E shows this for PC0, PC3, and PC9). To assess the relationship between classical features and
327 the PC of the DL features, we fit local regression curves using weighted linear least squares regression (lowess) (Figure
328 5C-D). We calculated confidence intervals around the lowess fits by using bootstrapping to provide an estimate of the
329 spread of the curve. We show the 65% confidence intervals.

330

331 The first PC, PC0, represents a combination of classical features including the major axis, surface area, and the
332 sphericity of the cell. We see a positive linear relationship between PC0 and both surface area and major axis
333 when fitting local regression curves between PC0 and these features and a negative relationship between PC0 and
334 sphericity (Figures 5C). PC3 is most correlated with axial extent, distance from the coverslip and the minor axis. These
335 relationships are more complex (Figure 5D). Similarly, PC9 has complex relationships to the volume to surface area
336 ratio and the equivalent diameter of 3D cells (Figure 5E).

337

338 PC1, which explains 18.67% of the variation of the DL features correlates most with roll which is an orientation
339 parameter representing the angle of rotation when looking at the origin along the x-axis (calculated using MATLAB's
340 regionprops3 function). The orientation of cells, whilst important to geometry, may not be of interest in some biological
341 contexts. As a step toward a rotationally invariant method to extract 3D shape features, we used a "pose correction"
342 approach to first align the cells before extracting the features (Section). Cells were pose corrected to align them
343 according to their principal axes and obtain canonical poses (See Methods Section). Figure 5F and G show two cells
344 before and after alignment respectively.

345

346 We visually expressed how DL features may capture classical features by performing UMAP on the DL features and
347 colouring each point by the corresponding classical feature value. We do this for DL features extracted from both
348 unaligned (Figure 5H-H//) and aligned cells (Figure 5I-I//). This revealed a clear pattern of organisation of roll values
349 suggesting that this feature is being incorporated by the DL features of the unaligned dataset (Figure 5H). However,
350 after pose correction to align the cells by their principal axes, the effect of roll was mitigated (Figure 5I). Figure 5H/-H//
351 show how classical features; sphericity, eccentricity and polarity, are all incorporated by the DL features. Similarly,
352 after aligning the cells, we still see these features being incorporated (Figure 5I/-I//).

353 Discussion

354 Deep learning has been applied in the context of 3D microscopy for tasks of segmentation [51, 52, 53], restoration and
355 denoising [54], and classification. However, few methods explore the possibility of utilising deep learning to learn the
356 shape feature profiles of single-cells in 3D. We have developed an automatic tool to simultaneously learn shape features
357 and shape classes of 3D objects and applied this to a metastatic melanoma cell line. Our method assigns 3D quantitative
358 morphological signatures to each cell. This signature measures how similar the shape of each cell is to the shape classes
359 found in the dataset. We used these extracted features to distinguish between cells treated with inhibitors of myosin and
360 microtubules and show that these inhibitors are environmentally dependent. We were also able to use pose correction as
361 a practical method to remove learning of rotational variance. Finally, we provide intuitive explanations of the learned
362 features by correlating them to classical measures of geometry and by visualising uncorrelated embeddings of these
363 features (in the form of principal components). This showed that our model automatically learns features which capture
364 the traditional measures of geometry, and the low correlation of our second principle component with traditional
365 measures suggested we are capturing information that is previously missed.

366

367 Building a deep learning model requires design choices about input data, model architecture and hyperparameters. We
368 discuss this in more detail to allow repeatability and improvements to our methods in further studies.

369

370 The first decision is the choice of input data. Since the creation of 3D object datasets such as ModelNet [22], the two
371 broad approaches which have been used to represent the input data are to use voxels (the 3D counterpart to pixels in 2D
372 images), or point clouds. Recently point clouds have been the dominant approach [38, 28, 55]. One reason for this is
373 practical. In particular, point cloud data is abundant and is the type of data generated for raw object surfaces when using
374 devices like LIDAR sensors. Additionally, point cloud data is a very simple 3D representation of 3D shape, consisting
375 of a 2D matrix of positions of points. We had the choice of both voxel and point cloud representations and tried both
376 approaches when representing human cells. For learning representations on 3D voxel grids, we utilised 3D ResNet

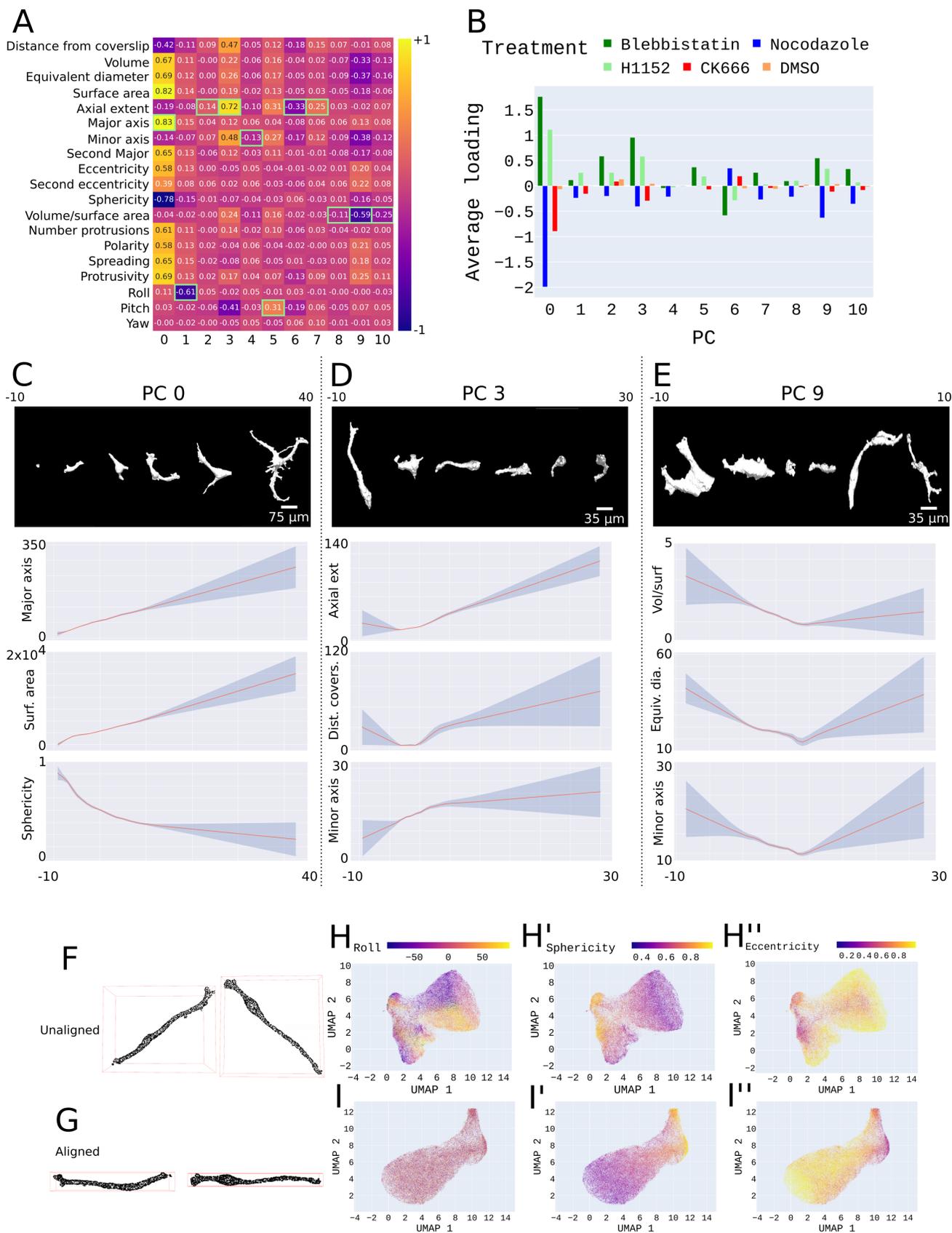


Figure 5: **Interpreting learned features and a step towards rotational invariance with pose correction.** (A) Pearson correlation coefficients between principal components of extracted cell shape features and classical features. Green boxes show the classical features with the highest absolute correlation to each principal component. (B) Average PC loadings for cells treated with Blebbistatin, Nocodazole, H1152, CK666, and DMSO. (C-E) Interpreting the cell shape features extracted from the DFN, by continuously varying PC (PC0, PC3, PC9) values. Images are sampled from binned encodings of the respective PC (PC0, PC3, PC9) value. We show a lowess fit with a 65% confidence interval between (C) PC0 and major axis, surface area (Surf. area), and sphericity. (D) Shows the sampled images for the range of PC3 values. Panels show lowess fit with a 65% confidence interval between PC3 and axial extent, distance from the coverslip (Dist. covers.), and minor axis. (E) Shows the same for PC9- the lowess fit of PC9 with volume to surface area ratio (Vol/Surf), equivalent diameter (Equiv. dia.), and Minor axis. (F) Before pose correction to align cells to their principal axis. (G) After pose correction to align cells shown in (F). (H-H//) Scatter plot of UMAP embedding of cell shape features on unaligned data with each point coloured by their corresponding roll, eccentricity, sphericity, and polarity value. (I-I//) shows the same for the aligned cells.

377 architectures and subsequently packaged our methods as a Python package called 'cellshape-voxel' for researchers
378 to use freely. Using performance on classification tasks as a guide, we found that autoencoders using point cloud data
379 were superior and have reported on these results. Our choice of 2048, as the number of points, was based on previous
380 works [31, 25]. Future work could explore methods of describing cells by either a few critical points or alternatively, a
381 constant density of points. For example, a cell could be described by its centre of mass and points along its protrusions.
382 While decreasing the detail by describing cells by much fewer points, computational efficiency would significantly
383 increase.

384

385 A second key design decision is the selection of an autoencoder. As previously discussed, geometric deep learning
386 is the branch of deep learning that deals with graph or manifold (unstructured) data such as graphs created on point
387 clouds. Geometric deep learning has predominantly been used on point cloud data to produce state-of-the-art results for
388 classification and representation learning tasks [38, 40]. Our model incorporated edge convolution [25] as the primary
389 operator in our encoder part of our autoencoder as this has proved successful in representation learning tasks [55].
390 FoldingNet is a novel folding-based decoder designed to assist in representation learning on point clouds [31]. This
391 decoder backbone has been used extensively in the literature primarily as it is simple, and has shown promising results
392 across several tasks [32, 40]. Thus, we incorporate a folding-based decoder in our model. Looking beyond the shapes
393 of cells, future research could utilise our models to explore the shapes or networks of collections of cells in 3D cancer
394 models such as tumour spheroids or organoids. Each cell in these populations of cells could represent a point with the
395 entire population being represented as a point cloud. We can then use our methods to extract features from these point
396 cloud representations of cells in populations, describing 3D cancer models as networks.

397

398 A third important decision is a method to classify unlabelled data into classes. This is a challenging task with methods
399 proposed for common 2D benchmark datasets [56, 48]. To solve this problem for biological cells, we took the approach
400 of deep embedded clustering. Deep embedded clustering is a way to simultaneously learn feature representations
401 and cluster assignments or classes. This method involves clustering with Kullback–Leibler divergence on feature
402 representations from an autoencoder [57]. This method can easily be incorporated into any autoencoder architecture
403 and offers an interpretable output in the form of a soft-label which describes the probability of assigning data input
404 to each cluster class. This, in turn, allows the assignment of data input to be continuous across classes rather than
405 discrete. Cell shape morphology is a continuous variable with no two cells being identical in shape, and rather similar
406 to each other or similar to exemplar shapes [58]. Describing cell shapes by a continuous similarity score to exemplar
407 shapes offers a more natural solution than discrete binning into certain shape classes [8]. Thus, we have opted for
408 deep embedded clustering to learn the 3D quantitative morphological signature (3DQMS) of cell shapes. Using these
409 3DQMS, we found four groups of treatment effects on the shape which grouped two distinct inhibitors of myosin
410 activation (Blebbistatin and H11522). This showed that our 3DQMS will be a powerful tool for using cell shape to
411 identify drugs that act on similar pathways.

412

413 Finally, the choices of hyperparameters are critical decisions in any deep learning pipeline. These include training
414 configurations such as batch size, and learning rate, as well as optimisation algorithms and model architectural
415 hyperparameters. These include the number of clusters in clustering algorithms, the number of neighbours to use when
416 constructing k-nearest neighbour graphs, and the number of features to extract. Hyperparameters are commonly chosen
417 through trial and error, or grid searches across a range of combinations with the best combination chosen according to

418 performance on the task at hand. We chose our hyperparameters by trial and error on transfer learning classification on
419 the ModelNet40 dataset, with guidance from previous work on which our model is based [31, 25].

420

421 A major theme in recent research into 3D shape representation is how to deal with differences in the rotation of objects
422 with an otherwise similar geometric form [32, 59]. Orientation, composed of measures of roll, pitch and yaw, is
423 generally considered part of the formal geometric description of an object. In some settings, a focus on orientation
424 is useful, but in others it is undesirable. For example, earlier attempts at distinguishing everyday objects found that
425 accurate classification of cars can drop from 70% to 20% depending on the orientation [59]. In these settings where the
426 task is the classification of 3D objects based on shape, we generally do not expect an object to change its identity
427 depending on its orientation. This means that for classification tasks, sensitivity to orientation is likely to be undesirable.
428 However, there are settings where orientation is helpful. For example, in biological contexts like embryonic and
429 postnatal development, the establishment of planar cell polarity results in the alignment of stereocilia. Orientation is a
430 meaningful feature for the study of the shape of stereocilia in development. Similarly, orientation can reveal important
431 information about a cell's microenvironment, for example, alignment in the orientation of cancer cells migrating can
432 indicate polarisation of the extracellular matrix. Because orientation can be important and useful, our feature extraction
433 captures orientation by default (Figure 2 and Figure 5). However, we note there are also many applications in biology
434 where orientation is not of interest. For example, orientation may not be of interest when studying the shapes of human
435 cells in suspension, or free-living marine organisms. In this study, we addressed this using pose correction techniques
436 based on works in [60] (Figure 5). It is worth noting that pose correction caused a decrease in the extracted features'
437 ability to distinguish between treatments. Pose correction has been used in 2D shape analysis in biology [11], and has
438 been a standard approach for circumventing the confounding effect of rotation in 3D shape datasets [22]. Recently, a
439 number of groups have also developed rotationally invariant methods, which are directly incorporated into their models,
440 to learn 3D shape representations [32, 59] that could also be applied to cells.

441

442 Explainability is an important feature to consider when building deep learning models in any application, especially
443 in medical research. We have considered ways of interpreting learned features, however, other methods may prove
444 beneficial. When dealing with 2D pixel data of images, applications exist that overlay heatmaps to visually express
445 where the model is looking when making certain decisions or classifications [61]. These have recently been extended to
446 deal with 3D voxelised data and are commonly used in medical image analysis tasks [62]. These methods have been
447 explored for use on point cloud data [63] showing that the methods may be reliable on point cloud data. Future work
448 could incorporate class activation maps on point cloud data of cells to visually represent why certain cells are being
449 grouped together.

450

451 While our problem is fundamentally focused on an unsupervised feature representation of 3D cell shape, we have
452 applied it in the context of cells treated with different small molecule inhibitors. We have taken advantage of this by
453 using features automatically extracted using our DFN model, to train a linear support vector machine (SVM) that
454 could distinguish between cells treated with myosin inhibitors from those treated with microtubule inhibitors with
455 accuracies up to 86%. Furthermore, we showed how both cell and nuclei shape information is important in combination
456 to predicting treatment, suggesting that these drugs not only affect cell shape and nuclei shape individually but also the
457 relationship between the shapes of these components. Finally, we found that predicting treatment from cell shape
458 was dependent on the environment with 86% accuracy between Blebbistatin treated cells in a proximal setting and
459 82% in distal settings. Future work could explore supervised techniques to classify the treatment of a cell directly.
460 Finally, treatments may have a significant effect on shape, but the temporal aspect of the treatment effect is not captured
461 in a dataset of fixed cells. Understanding the temporal shape signatures or patterns in cell shape dynamics could
462 allow a more accurate understanding of the effect of treatment on cell shape. Extending these methods to incorporate
463 time-lapse sequences of cells could be done by utilising recurrent neural networks on sequential shape features extracted.

464

465 Finally, a major component of our work is its accessibility to the wider biological and medical community. Packing our
466 methods in open source Python packages with adaptability and ease of use allows further research on connecting 3D
467 shapes to function across a variety of domains. Our Python package offers adaptability and growth with the plan to
468 implement new methods continuously.

469

470 **Methods**

471 **Melanoma cell preparation**

472 Cells used in this study were WM266.4 harbouring CAAX-EGFP (donated from the Marshall lab), with the addition of
473 an ERK-KTR-Ruby construct (addgene #90231) and a Histone2B-iRFP670 construct (addgene #90237).

474 **Collagen preparation**

475 Collagen hydrogels were prepared to a final concentration of 2mg/mL. Briefly, hydrogel solutions of dH₂O, 5xDMEM,
476 HEPES (7.5 pH), and Rat Tail Collagen IV (Corning) were prepared on ice to a final collagen concentration of 2mg/mL.
477 Cells were re-suspended in hydrogel solution at a concentration of 4 E4 cells per 100 μ l, and 100 μ l of this solution
478 dispensed into each experimental well on a 96 well plate. After dispensing cells, the plates were incubated at 37 degrees
479 Celsius for 1 hour, and 100 μ l of DMEM was added to each well.

480 **Treatments and cell fixation**

481 Treatments were added to cells 24 hours after seeding in collagen hydrogels. After 6 hours of treatment, cells were
482 fixed in 4 percent paraformaldehyde for 30 minutes at room temperature. Final concentrations for treatments were:
483 Binimetinib (2 μ M), Palbociclib (2 μ M), MK1775 (1 μ M), Blebbistatin (10 μ M), H1152 (10 μ M), PF228 (2 μ M),
484 CK666 (100 μ M), Nocodazole (1 μ M) and DMSO 1 in 1000. Concentrations were calculated including the 100 μ l
485 volume of the collagen hydrogel.

486 **Microscopy setup and image acquisition**

487 OPM imaging was performed on a modified version of the OPM system described in [21, 41]. The primary microscope
488 objective was a 60X/1.2NA water immersion objective, the secondary objective was a 50X/0.95NA air objective and
489 the tertiary objective was a 40x/0.6NA air objective. The OPM angle was 35 degrees.

490
491 A single sCMOS camera (pco Edge) was used in Global Reset acquisition mode with 1280 \times 1000 pixels. A motorised
492 filter wheel (FW103H/M, Thorlabs) was used to switch between filters for multichannel imaging. An OPM volume was
493 acquired for iRFP (642 nm excitation and 731/137 emission filter (Semrock Brightline)) before the stage returned to
494 the start position prior to the acquisition of the EGFP volume (488 nm excitation and 550/49 emission filter (Semrock
495 Brightline)). Finally, a collagen scattered light volume (488 nm illumination and no emission filter) was acquired from
496 the same start position. The laser illumination and camera exposure time were both 4 ms. The stage velocity was 0.16
497 μ m ms^{-1} and image acquisition was triggered every 1.4 microns of stage travel. For each field of view, the x-y stage
498 covered 4000 μ m and three regions were imaged for each well. Prior to analysis, raw frames were compressed using
499 jetraw compression software (jetraw, Dotphoton). Volumes were then de-skewed into standard xyz coordinates [21]
500 and binned such that the final voxel size was 1x1x1 μ m³. Image reslicing was performed using bi-linear resampling
501 similar to the methods described in [41].

502 **Segmentation**

503 Segmentation of cells and nuclei was by active contour segmentation. Segmentation was performed with a threshold set
504 at the mean volume grey value plus the standard deviation of the volume grey values and finding connected components
505 (minimum connected component sizes were 50 μ m³ and 512 μ m³ for nuclei and cells respectively). These connected
506 components defined sub-regions which were fully segmented.

507 **Outlier removal**

508 The three repeated experiments produced a segmented dataset of more the 7×10^4 single cells. Similar to [41], we
509 removed cells and nuclei that expressed low CAAX-GFP transgene, as this caused inaccurate segmentation. We
510 automatically removed cells with a maximum intensity less than the mean of all cell maximum intensities minus the
511 standard deviation of all cell maximum intensities. We also removed cells based on their size. That is, if the cell volume
512 $< 512 \mu$ m³ or the nuclei volume $< 50 \mu$ m³, the cell was removed from the dataset.

513

514 Due to cells being densely packed within each well, there were cases where the segmentation methods grouped two
515 cells as one. To avoid this, we removed cells from the dataset that contained two nuclei within the segmented cell mask.
516

517 After the above outlier removal and quality control, there was an additional round of quality control during the data
518 analysis stage. Cells with a mean CAAX-GFP intensity of fewer than 70 units were removed from the study, and cells
519 with a mean nuclear Histone-2B of fewer than 5 units were also removed from the study.

520 Classical shape measurements

521 Similar to previous our previous works [41], cell and nuclei classical shape measurements were calculated by the
522 `regionprops3` function in MATLAB. Further measurements were derived from these outputs as described in [41].

523 3D rendering

524 3D renders of intensity images are generated as a 3D projection with trilinear interpolation by using the volume viewer
525 2.01 Fiji plugin [64]. 3D renders of cell masks are presented as 3D surface representations using isosurfaces in napari
526 [65].

527 Dynamic graph convolutional foldingnet

528 The DFN follows the design of the FoldingNet [31] with a dynamic graph convolutional neural network (DGCNN) as
529 the encoder [25]. This encoder takes a 3D point cloud as input, constructs a local neighbourhood (k -nearest neighbour)
530 graph on these points and applies convolution-like operations on the edges of connecting neighbouring points. They
531 call these operations edge convolution (EdgeConv). [25] shows translation-invariant properties of EdgeConv operations.
532 For our experiments, we have chosen $k_g = 20$ for our graph construction. We remove the final linear layer from the
533 original architecture and replace it with one that outputs a feature vector of length 128. The decoder takes the feature
534 vector, \mathbf{Z} , as input and concatenates it with points sampled from a plane in 2D space. This is then passed through a
535 series of two folding operations (defined in [31]) to output a reconstructed point cloud. The number of reconstructed
536 points, m , does not need to be the same as the input number of points, n . The Chamfer distance (CD) [66] is commonly
537 used to compare two PCs. We used the extended CD presented in [31] as our reconstruction error between input point
538 cloud S and reconstructed point cloud \hat{S} . The CD is defined in Equation 1.

$$d_{CH}(S, \hat{S}) = \max \left\{ \frac{1}{|S|} \sum_{\mathbf{x} \in S} \min_{\hat{\mathbf{x}} \in \hat{S}} \|\mathbf{x} - \hat{\mathbf{x}}\|_2, \frac{1}{|\hat{S}|} \sum_{\hat{\mathbf{x}} \in \hat{S}} \min_{\mathbf{x} \in S} \|\hat{\mathbf{x}} - \mathbf{x}\|_2 \right\} \quad (1)$$

539 Initially, the DFN was trained on the ShapeNet dataset for 250 epochs and then we continued training on our point
540 cloud representations of both cells and nuclei for another 250 epochs using Adam optimiser with e^{-6} weight decay. We
541 used a batch size of 16 with an initial learning rate of 0.001/16 and an exponential learning rate decay scheduler. The
542 DFN model was set to extract 128 features from each point cloud. All algorithms were implemented in PyTorch.

543 Deep embedded clustering

544 Deep embedded clustering (DEC) [57] is a specialised clustering technique that simultaneously learns feature rep-
545 resentations and cluster assignments using autoencoders. Following the algorithm described in DEC, we propose
546 learning autoencoder parameters θ which map the 3D shapes into embedded feature space \mathbf{Z} as well as k cluster centres
547 $\{\mu_j \in \mathbf{Z}\}_{j=1}^k$ of the embedded feature space \mathbf{Z} . This is done in two phases:

- 548 1. parameter initialisation with an autoencoder (through either ‘cellshape-voxel’ or cellshape-cloud tools) and
- 549 2. parameter optimisation through simultaneous autoencoder reconstruction and minimisation of the Kullback-
550 Leibler (KL) divergence between a target distribution and a distribution of soft-labels.

551 The second step is done through the addition of a clustering layer (CL) on the features to refine them by learning
552 features that are optimised to represent the 3D shape as best as possible as well as grouping similar and separating
553 dissimilar objects. This part of the model works by initialising cluster centres using the k -means clustering algorithm
554 on the embedded feature space outputted from a pre-trained autoencoder. These cluster centres are kept as trainable
555 parameters. The clustering layer then assigns soft-labels to each input by mapping features to clusters (q) based on the

556 Student’s t-distribution as a kernel that represents the similarity between a feature vector (\mathbf{Z}) and a cluster centre (μ):

$$q_{ij} = \frac{\left(1 + \|z_i - \mu_j\|^2 / \alpha\right)^{-\frac{\alpha+1}{2}}}{\sum_{j'} \left(1 + \|z_i - \mu_{j'}\|^2 / \alpha\right)^{-\frac{\alpha+1}{2}}} \quad (2)$$

557 This can be interpreted as the probability of assigning input i to cluster j , hence why this is a soft assignment. Soft-
558 assignments with high probabilities are considered trustworthy and thus DEC designs a target distribution which raises
559 this to the second power to place more emphasis on these confident assignments. Following DEC, we define the target
560 distribution as:

$$p_{ij} = \frac{q_{ij}^2 / f_j}{\sum_{j'} q_{ij'}^2 / f_{j'}} \quad (3)$$

561 We can then define the clustering loss as the Kullback–Leibler divergence between p and q :

$$L = \text{KL}(P\|Q) = \sum_i \sum_j p_{ij} \log \frac{p_{ij}}{q_{ij}} \quad (4)$$

562 The features at cluster centres become representations of ‘example’ or ‘template’ shapes of each shape class. [57]
563 proposed training an autoencoder in the first phase (parameter initialisation) and then abandoning the decoder in the
564 second phase to only fine-tune the encoder through the clustering loss alone. Variants of DEC since then have shown
565 that this kind of fine-tuning may distort the embedded space and weaken its representativeness of the input [67, 68].
566 Thus, we follow the procedure in [49] and have added the decoder back to the second phase of training and optimised
567 both the reconstruction loss and the clustering loss together with a final loss defined as:

$$L = L_r + \gamma L_c \quad (5)$$

568 where $\gamma \geq 0$ defines the magnitude that the clustering loss adds to the final loss.

569 Predicting small molecule treatments

570 Following similar procedures to [31, 69, 70, 71, 55], we used the trained DFN to extract features from each cell. These
571 features were then used to train a linear support vector machine (SVM). For all experiments, we use one-versus-one
572 SVM classifiers with an L2 regularisation parameter ($C = 1$), balanced class weights, and intercept scaling during
573 training. We tested cytoplasm features and nuclei features alone and then combined them for ablation studies (Figure
574 4A). We performed a 10-fold cross-validation for each experiment and report the mean accuracy. We used scikit-learn
575 for these methods [72, 73].
576

577 Feature importance

578 Extreme gradient boosting (XGBoost) is a machine learning algorithm which uses gradient boosted decision trees for
579 classification and regression tasks [74]. A benefit of using gradient boosting is that it is relatively simple to explore
580 which features are important for the task at hand. Importance provides a score that indicates how valuable each feature
581 was in the construction of the boosted trees in the model. The more a feature is utilised to make a classification, the
582 higher its importance. This is based on the number of times a feature is used in a tree. We use this for interpreting which
583 features are important for our classification tasks. We trained an XGBoost classification model to classify Blebbistatin
584 and Nocodazole-treated cells based on the principal components of the cell shape features extracted using the DFN
585 without the addition of the clustering layer. For methods involving XGBoost, we used the open-source Python package
586 ‘xgboost’ [74].

587 Aligning 3D shapes to a common axis

588 As a step towards rotational invariance, we propose converting the point cloud representations of 3D cells to their
589 PCA-based canonical poses. PCA calculates 3 orthogonal bases or principal axis of point cloud data. This enables us to
590 align original point clouds to the world Cartesian plane [60]. We briefly discuss how the canonical pose is calculated by
591 following directly from [60]. We perform PCA on a given point cloud, $\mathbf{P} \in \mathbb{R}^{n \times 3}$, by:

$$\frac{\sum (\mathbf{P}_i - \bar{\mathbf{P}}) (\mathbf{P}_i - \bar{\mathbf{P}})^T}{n} = \mathbf{E} \mathbf{\Lambda} \mathbf{E}^T, \quad (6)$$

592 where $\mathbf{P}_i \in \mathbb{R}^3$ is the i^{th} point of \mathbf{P} , $\bar{\mathbf{P}} \in \mathbb{R}^3$ is the mean of \mathbf{P} , \mathbf{E} is the eigenvector matrix composed of eigenvectors
593 ($\mathbf{e}_1, \mathbf{e}_2, \mathbf{e}_3$) (principal axes), and $\Lambda = \text{diag}(\lambda_1, \lambda_2, \lambda_3)$ are the corresponding eigenvalues. By aligning the principal
594 axes to the three axes of the world coordinate, we obtain the canonical pose as $\mathbf{P}_{can} = \mathbf{P}\mathbf{E}$.

595

596 [60] goes on to prove the rotational invariant property of \mathbf{P}_{can} . We calculate the canonical pose for each point cloud
597 representation of a cell and use this as input to our DFN.

598 Software availability

599 The described software is available as a Python package which can be installed through pip and is avail-
600 able at <https://github.com/DeVriesMatt/cellshape>. The main package is made up of four inde-
601 pendent sub-packages available at <https://github.com/Sentinal4D/cellshape-cloud>, <https://github.com/Sentinal4D/cellshape-voxel>, <https://github.com/Sentinal4D/cellshape-cluster>, and <https://github.com/Sentinal4D/cellshape-helper>.

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