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# Cell deformability heterogeneity recognition by unsupervised machine learning from in-flow motion parameters†

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Cell deformability is a well-established marker of cell states for diagnostic purposes. However, the measurement of a wide range of different deformability levels is still challenging, especially in cancer, where a large heterogeneity of rheological/mechanical properties is present. Therefore, a simple, versatile and cost-effective recognition method for variable rheological/mechanical properties of cells is needed. Here, we introduce a new set of in-flow motion parameters capable of identifying heterogeneity among cell deformability, properly modified by the administration of drugs for cytoskeleton destabilization. Firstly, we measured cell deformability by identification of in-flow motions, rolling (R), tumbling (T), swinging (S) and tank-treading (TT), distinctively associated with cell rheological/mechanical properties. Secondly, from a pool of motion and structural cell parameters, an unsupervised machine learning approach based on principal component analysis (PCA) revealed dominant features: the local cell velocity ( $V_{\text{Cell}}/V_{\text{Avg}}$ ), the equilibrium position ( $Y_{\text{Eq}}$ ) and the orientation angle variation ( $\Delta\phi$ ). These motion parameters clearly defined cell clusters in terms of motion regimes corresponding to specific deformability. Such correlation is verified in a wide range of rheological/mechanical properties from the elastic cells moving like R until the almost viscous cells moving as TT. Thus, our approach shows how simple motion parameters allow cell deformability heterogeneity recognition, directly measuring rheological/mechanical properties.

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## Introduction

Single cell deformability represents a straightforward indicator of changes in many cellular activities, such as signal transduction, gene expression or apoptosis.<sup>1–3</sup> These functions are strictly related to the cell state, as healthy or pathological. In particular, a high cellular heterogeneity has been associated to deformability and rheological and mechanical properties of cancer diseases, representing a distinctive marker for the detection of different degrees of cancer invasiveness.<sup>4</sup> In fact, such morphological alterations and cytoskeletal changes are indicative of malignant transformations of cancer cells usually marked by high replication and motility.<sup>2,5–8</sup> However, the recognition of such highly variable deformability levels is a daunting challenge.<sup>9</sup>

During the last years, numerous techniques identified heterogeneous rheological/mechanical properties existing

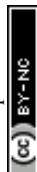
both within and between different cell lines.<sup>2,5,10</sup> For example, single-cell optical stretcher studies on three different breast cancer cell lines showed that MDA-MB-231 cells and MDA-MB-436 cells, which are both highly-invasive epithelial breast cancer cell lines, are mechanically more different from each other than from non-malignant epithelial MCF-10A cells.<sup>10</sup> Instead, by means of the atomic force microscopy technique, a local cell deformability was measured examining the role of the cytoskeletal actin disassembly in the softening process of highly invasive cancer cells, such as MDA-MB-231.<sup>11</sup> Moreover, results of micropipette aspiration experiments confirmed that alterations in cell viscoelastic properties induced by cancer are highly correlated with changes in the actin cortex and microtubule content and arrangement.<sup>12</sup> However, all of these techniques face some obstacles such as low cell throughput, mechanical contact of the used probe leading to possible adhesion and active cellular responses or special preparation procedures. Moreover, none of the presented techniques allow variable deformability levels to be recognized and measured among different cell types in a sample.

Trying to overcome the aforementioned drawbacks, a variety of microfluidic systems enabled alignment,

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Here, we present a combination of motion dynamics features useful for a completely label-free measurement of a wide range of deformability levels for different cell types with distinct rheological and mechanical characteristics. To measure these properties, we decided to change the deformability of cells with two treatments to destabilize the cytoskeletal structures mainly involved in the rheological/mechanical response of suspended cells. In particular, we refer to cytochalasin-D (CytD) and nocodazole (Noco) to destabilize the actin cortex and microtubule network, respectively, for the used breast cell lines (MCF-10A and MDA-MB-231) (Fig. 1). What we expect is randomly distributed or poorly interconnected proteins with a more irregular and compliant state of cells. To measure the resulting different levels of deformability, we imposed a viscoelastic-based compression force on cells into the so-called compression region (Fig. 1, S1 and Table S1†).<sup>34</sup> The microfluidic channel was designed to be small enough to guarantee alignment and deformation of cells in a contactless manner ( $\sim 20$   $\mu\text{N}$ , ESI† Table S1). After compression release, cells exit the section at the channel centre line, with the major axis aligned to the gradient-axis (z-axis, Fig. 1) arriving at an enlarged observation region, properly conceived to allow the in-flow motion investigation (Fig. 1 and S1†).<sup>34</sup> By tracking the centroid position of the deformed cells, we further defined the in-flow cell lateral equilibrium position ( $Y_{\text{Eq}}$ , ranging from 0.5 to 1, identified

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by the channel centre line and wall respectively, Fig. 1) and the local cell velocity, evaluated with respect to the average fluid velocity ( $V_{\text{Cell}}/V_{\text{Avg}}$ ). This allows us to improve the cell classification, so far dependent on the cell orientation angle ( $\varphi$ , Fig. 1) with its own variation ( $\Delta\varphi$ ) and the morphological parameters –CD and AR– occurring along the observation region (Fig. 1). In particular, both  $V_{\text{Cell}}/V_{\text{Avg}}$  and  $Y_{\text{Eq}}$  were chosen since they are standard references of deformability, being well-established that highly deformable cells move faster towards the centre line than less deformable cells. However, only the combination of such dynamic and morphological parameters reveals the possibility to coherently classify different cell classes and to mechanically characterize them. In general, to reduce parameters sets, unsupervised machine learning approaches are widely used in literature for cell classification.<sup>41</sup> Among them, we assessed principal component analysis (PCA) as a machine learning approach, to verify whether it is suitable for the distinction of cell clusters grouped depending on a specific combination of parameters associated to different rheological and mechanical cell responses (Fig. 1). As a result, a new master curve for inner cell viscosity measurement is proposed, representing such a viscosity change as a function of the described motion regime and perceived deformation.

## Materials and methods

### Viscoelastic alignment and compression forces

To induce deformation of single cells under viscoelastic flow conditions, we use a highly biocompatible polyethylene oxide (PEO, 4MDa, Sigma Aldrich). Fluid rheological properties were previously investigated and properly characterized.<sup>34</sup> We decided to work with the so-called PEO 0.5 concentration, which corresponds to a polymer concentration of 0.53 wt%. We calibrated the initial pressure and velocity conditions in the microfluidic device, as well as the channel geometry to align and deform cells in a contactless way. We computed viscoelastic compressive force ( $F_{\text{EMax}}$ ) values coming from the channel walls (see ESI† Table S1), supposing that the cell is already at its own equilibrium position where the competing viscoelastic and drag forces are balanced.<sup>34</sup>

### Experimental setup for the in-flow measurement

The setup includes a pressure pump (P-pump, Dolomite Microfluidics), round shaped flexible fused silica capillary tubing (Molex), an *ad hoc* designed microfluidic chip, a fluid connector (N-333, IDEX) and an inverted microscope (X81, Olympus) with a CMOS camera (ORCA FLASH 4.0, Hamamatsu Photonics K.K.). Briefly, a pressure pump pushes the sample volume through the capillary in the microfluidic device inlet, while at the end of the chip a reservoir collects the cells (ESI† Fig. S1). The chip is made of two separate parts made with polymethyl methacrylate, which are placed together by magnetic forces. The main part was machined using a standard CNC based micromilling technique (Minitech Machinery) to develop microfluidic duct sections

of different height, width, and length, while the cover part was simply used to close the chip (ESI† Fig. S1).<sup>34</sup> In particular, the third section has the smallest height ( $W \times H$ :  $100 \times 25 \mu\text{m}$ ) implying that a well-defined viscoelastic compression over a certain time period on each passing cell is applied, enabling precise cell deformation in flow. Then, a subsequent enlarged section ( $W \times H$ :  $200 \times 90 \mu\text{m}$ ) is conceived for the observation and analysis of the compressed cells (ESI† Fig. S1).

## Cell culture

We have investigated MCF-10A and MDA-MB-231 cells. MCF-10A cells were donated by S. Piccolo (Istituto FIRC di oncologia molecolare, IFOM, Milan, Italy) and cultured in a mammary epithelial basal medium (MEBM) supplemented with a mammary epithelial growth media (MEGM) bullet kit (Lonza). MDA-MB-231 cell lines were kindly donated by Daidone's group and Dr P. F. Cammarata (Institute of Molecular Bioimaging and Physiology, IBFM-CNR, Cefalù (PA), Italy), respectively. MDA-MB-231 cells are cultured in a 1:1 mixture of Dulbecco's modified essential medium (DMEM, Euroclone) and Ham's F-12 medium (Microtech) supplemented with 10% FBS, 1% non-essential amino acid mixture and 100 U ml<sup>-1</sup> penicillin/streptomycin.

Finally, each investigated cell type is diluted in 500  $\mu\text{l}$  of viscoelastic medium to reach a final cell concentration of *circa* 50 cells per  $\mu\text{l}$ . No evidence of mycoplasma infection is present.

Additionally, target-specific drugs are employed to induce cytoskeletal perturbations and cell deformability changes. We decided to test CytD from Sigma-Aldrich at 37 °C for 30 minutes, at a final concentration of 30  $\mu$ M in serum free medium to inhibit actin polymerization and disrupt main actin filaments. For microtubules, cells were treated with Noco to destabilize the microtubules, which consequently promotes their depolymerization. Noco (Sigma-Aldrich, M1404) was dissolved in 1 ml of dimethyl sulfoxide and 100  $\mu$ g of the dissolved drug was further dissolved in 5 ml of culture medium. From this, the necessary volume was added to the cells to obtain the needed concentrations, while cells were adherent. The treatment duration was 30 minutes.

For immunostaining, cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 15 min at room temperature, then rinsed twice with PBS. Permeabilization –with 0.1% Triton X-100 (Sigma-Aldrich) for 5 min– was performed. Thus, the actin cortex was stained at the level of F-actin components with Alexa 488 phalloidin (Invitrogen) at 1:200 dilution for 1 h. The microtubule network, instead, was stained with Beta III Tubulin monoclonal antibody overnight at 4 °C with Alexa543 anti-mouse secondary antibody. For the image acquisition of the actin cortex and microtubule network, we used a 63× oil immersion objective (NA 1.4) of a confocal laser scanning microscope (LSM 710, Zeiss) equipped with argon and HeNe laser lines at wavelengths of 488 and 543 nm, respectively. Image



Out of such parameters, the cell deformation (CD), cell aspect ratio (AR) and angle variation ( $\Delta\varphi$ , at the beginning and the end of the tracing region) are calculated as follows:

$$\text{AR} = d_1/d_2 \quad (1)$$
$$\text{CD} = 1 - c = 1 - 4\pi A/P^2 \quad (2)$$

$$|\Delta\varphi| = |\varphi_e - \varphi_b| \quad (3)$$

In the specific case of tumbling motion of oblate cells, we computed the variation of the cell orientation angle as follows:<sup>45</sup>

$$T_{\text{rev}} = 2\pi(\text{AR} + \text{AR}^{-1})\dot{\gamma}^{-1} \quad (4)$$

$$\tan(\varphi(t)) = AR \cdot \tan(2\pi t/T_{\text{rev}}) \quad (5)$$

where  $T_{\text{rev}}$  indicates a complete period over which a tumbling rotation occurs (0.3 s) and  $\dot{\gamma}$  is the shear rate. Of note, in the limit of  $\dot{\gamma} \sim 15 \text{ s}^{-1}$ , the symmetry remains allowing to re-write eqn (5) also with a minus sign since  $\varphi \sim -\varphi$ .

$\varphi(t)$  can be re-written as a function of  $\mu'/\mu$ , as follows:<sup>40</sup>

$$\tan(\varphi(t)) = (A + B/(A^2 - B^2)^{1/2}) \cdot \tan(2\pi t/T_{\text{rev}}) \quad (6)$$

where  $A \propto \dot{\gamma}$  and  $B = f(\dot{\gamma}, \text{AR}, \mu'/\mu)$ . From the known oscillation ( $\varphi(t)$ ) of the cell in tumbling motion, we can retrieve the  $B$  parameter and then the value of  $\mu'/\mu$ .<sup>40</sup>

In particular, for a tank-treading cell motion, eqn (6) can be expressed as a stationary angle, only dependent on the fixed  $\dot{\gamma}$  and AR:<sup>40</sup>

$$\varphi_{\text{stationary}} = 1/2 \arccos (-A/B) \quad (7)$$

## PCA and statistical analysis

In-flow cell analysis was performed over the full cell tracing length ( $\sim 0.3$  s of measurement in the tracing region). The lateral equilibrium positions ( $Y_{\text{Eq}}$ ) of individual particles/cells were determined by measuring the cell centroid coordinates and comparing them with the observation channel coordinates. We fixed 0 and 1 as the two wall extremes, placing the channel centre line at 0.5 along the channel width ( $y$ -axis direction-ESI† Fig. S4). Then, we estimated  $V_{\text{Cell}}$  as the difference between the local cell velocity and the local fluid velocity from velocity COMSOL simulation (ESI† Fig. S4), at the same  $y$ -coordinate. Such velocity values were then divided by the average fluid velocity ( $V_{\text{Avg}}$ ).<sup>42</sup>  $V_{\text{Cell}}$  gives a measure of how much the cell lags the fluid during the alignment process, and it tends to go to zero as long as the cell reaches the channel centre line and the alignment is reached at the equilibrium positions.<sup>43</sup>

We measured the dynamic in-flow cell parameters, such as the major axis ( $d_1$ ), minor axis ( $d_2$ ), area ( $A$ ), perimeter ( $P$ ) and orientation angles ( $\varphi$ ) in the range of  $[-90^\circ$  to  $90^\circ]$  (ESI† Fig. S2 and S3), at each 0.03 s.

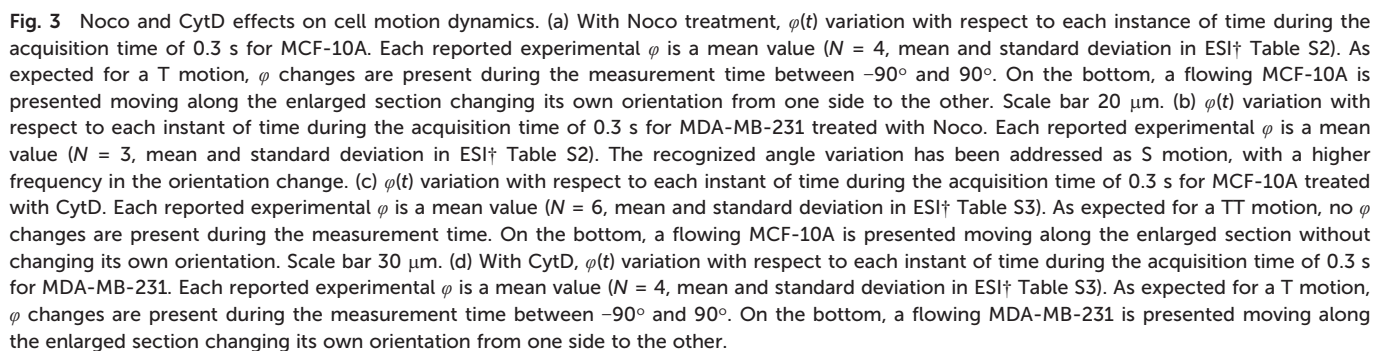
For the unsupervised machine learning based on PCA, we used Matlab R2020a (Mathworks Inc.). In particular, the analysis returns the PC scores and variances. We chose the PCs whose variance combination reproduces the highest possible number of data. In our case, the two chosen PCs cover around 80% of the cumulative variance of data. Moreover, the scree plot suggests that three main features are necessary for the data clustering (elbow rule), since only components with eigenvalues equal to or higher than 1 are usually of interest (ESI<sup>†</sup> Fig. S6).

For the statistical analysis, significance was indicated by  $p$  values ( $^{ns}p > 0.05$ ;  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ) computed by the application of a Kruskal–Wallis statistical test with Microsoft Excel 2019.



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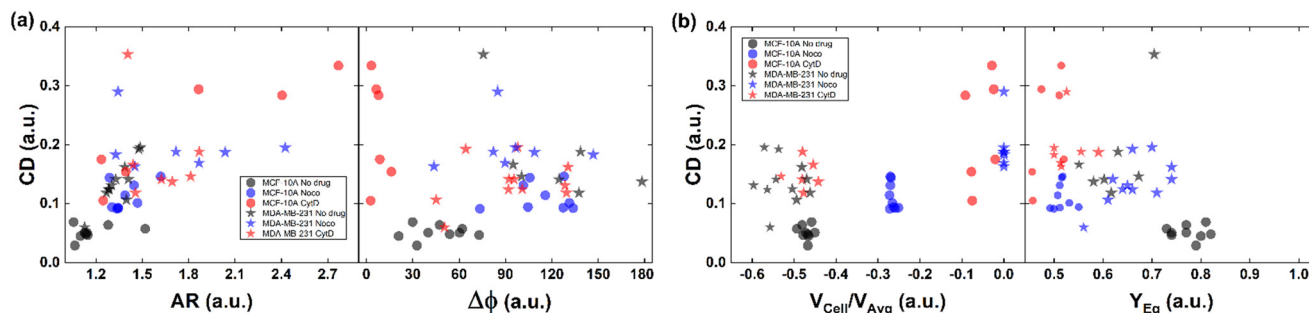




All things considered, compared to no drug conditions,<sup>34</sup> cells express higher CD and AR at CytD and Noco. In particular, MCF-10A is more sensitive to the actin cortex destabilization, with two-times higher CD entities compared to the no drug condition. Instead, MDA-MB-231 increases in deformability (CD  $\sim 0.334$ ) at microtubule destabilization, probably being the most effective response of MDA-MB-231 (Fig. 2d and f). On the other hand, comparable values of CD in the CytD and no drug cases have been found. At this point, we retrieved the cell classes characterized by the motion regimes and specific rheological/mechanical properties. Hereby, we noticed that cell groups are not distinguished, using only CD, AR and  $\Delta\varphi$ . This is reasonable, since the same CD and  $\Delta\varphi$  values could be associated to multiple in-flow

However, understanding which of the computed in-flow parameters are dominant in the rheological/mechanical description of cells was necessary. Thus, we decided to extract such relevant parameters to identify different cell clusters. For this, we implemented an unsupervised machine learning by using PCA to test the ability of the parameter selection for cell motion and deformability recognition. As shown, the cells described by the aforementioned morphological and





**Fig. 4** CD variation with respect to AR,  $\Delta\phi$ ,  $V_{Cell}/V_{Avg}$ , and  $Y_{Eq}$  for MCF-10A and MDA-MB-231 under no drug and treated conditions for each analysed cell. (a) CD variation with respect to AR and  $\Delta\phi$  is plotted for each cell. Increasing CD and AR are present for the treated cells. MCF-10A deforms more at CytD expressing lower values of  $\Delta\phi$  ( $\leq 50^\circ$ ) related to a high CD range of (0.15–0.35) while MDA-MB-231 still shows  $50^\circ \leq \Delta\phi \leq 180^\circ$  after CytD treatment, in the same range of no drug. MCF-10A deforms less at Noco expressing slightly equal values of  $\Delta\phi$ , AR and CD typical of a T motion. (b) CD variation with respect to  $V_{Cell}/V_{Avg}$  and  $Y_{Eq}$  is shown for each cell. It is verified that an increase of the CD values corresponds to a decrease of the  $V_{Cell}/V_{Avg}$  since cells tend to move at the same velocity of the fluid, without lagging it anymore. According to the  $V_{Cell}/V_{Avg}$  changes, the  $Y_{Eq}$  values decrease as cells move faster towards the centre line ( $Y_{Eq} \sim 0.5$ ) as the CD increases. The total number of analysed cells  $N_{Tot} = 49$ . The Kruskal–Wallis test performed on the experimental data is reported in Table S4.†

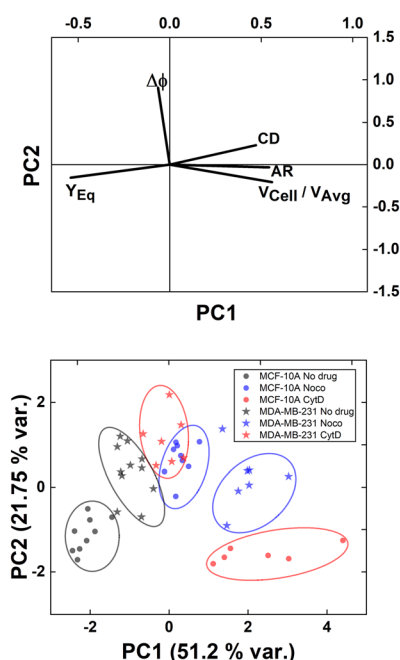
dynamic parameters form distinct clusters of data. In particular, the loading plot (Fig. 5, on the top and S6†) shows the principal component subspace of the original feature space spanned by the peaks of the  $V_{Cell}/V_{Avg}$ , CD, AR,  $\Delta\phi$  and  $Y_{Eq}$ . The biplot of cell population shows six cell clusters that can be separated from each other by forming well-defined

clusters (Fig. 5, on the bottom). Both for the loading and biplot representation, the two PCs, which returned a variance capable of covering almost 80% of the cumulative variance, are presented. The comparison of the loading and biplot lets us consider the dominant parameters  $V_{Cell}/V_{Avg}$ ,  $Y_{Eq}$  and  $\Delta\phi$  in precisely dividing cells. In fact, these are the only features characterized by eigenvector coefficients greater than 0.4 and useful for the identification of the principal components.

Of note, these clusters are separated depending on the dynamic motion behaviour ranging from R and T until S and TT. In particular, by comparing the diameter of each cluster with the distance among couples of clusters, the most separated clusters are the R-cases with respect to the T, S, and TT as well as T and S with respect to TT. Of note, the T-cases represented by the no drug MDA-MB-231, the CytD MDA-MB-231 and the Noco MCF-10A are not separated from each other, indicating comparable rheological/mechanical properties among the lines moving in the same way (ESI† Table S5). Less separated are the clusters S and TT. This is reasonable if we think about that the S behaviour is an intermediate among T and TT, where highly deformed cells ‘swing’ moving fast towards the centre line but with still high angle variation ( $50^\circ \leq \Delta\phi \leq 180^\circ$ , ESI† Table S5).

Thus, the reduction of parameters, set to a combination of only three, helped in re-plotting cell populations in a three-dimensional representation. This shows relevant differences among clusters only related to motion regimes, each of which is associated to a scaling deformability level as low or high (Fig. 6– on the right). In fact, deformability properties span from rigid cells (low deformability) moving as R, going through cells flowing as T dynamics, until highly soft cells (high deformability) travelling with S and TT motions (Fig. 6).

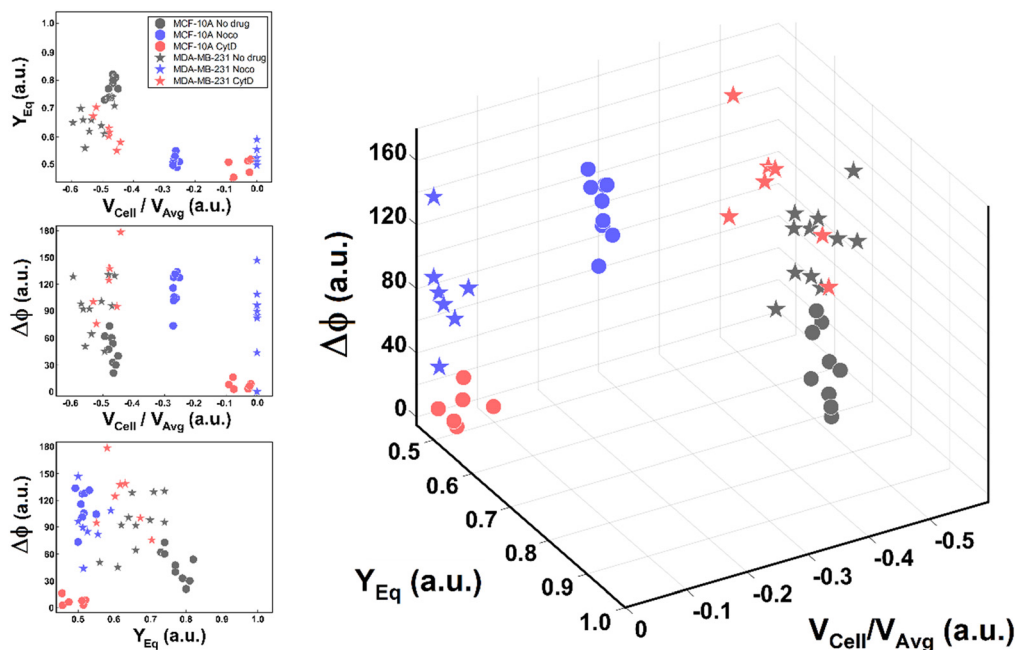
Structural modifications at the actin cortex and microtubule network level revealed modified MCF-10A in-flow behaviour ascribed to altered cell state conditions instead; for the S case, we performed a non-linear



**Fig. 5** PCA results on cell populations with respect to the parameter set. On the top, the loading plot representing the eigenvector scores, from the velocity through the morphological parameters until the orientation angle and the equilibrium position. On the bottom, the biplot with the data scores represented with respect to the highlighted eigenvectors. The most discriminant features are  $V_{Cell}/V_{Avg}$ ,  $Y_{Eq}$  and  $\Delta\phi$ . Such PCA reveals a precise cluster distinction among cell classes, confirmed by the performed computation of the separation degree among clusters (ESI† Table S5).

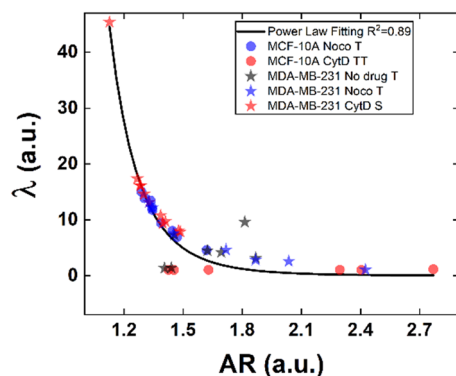






**Fig. 6** Three-dimensional representation of cell classes depending on  $V_{\text{Cell}}/V_{\text{Avg}}$ ,  $Y_{\text{Eq}}$  and  $\Delta\phi$ . Cell classes are distinguished depending on the motion dynamics which range from R to TT according to deformability characteristics indicated by the applied treatments and cell state as healthy or pathological. On the left, the combination of the different parameters on two-dimensional plots is presented for each analysed cell. Only these motion dynamics parameters satisfy the necessity of a cell classification. The total number of analysed cells  $N_{\text{Tot}} = 49$ . The Kruskal–Wallis test performed on the experimental data is reported in Table S4.†

extrapolation procedure to collect  $\lambda$  data from the known AR (Fig. 7). From our outcome, we were able to definitely consider suspended cells as a viscoelastic medium with the viscosities as the expression of a resistance opposed by cells to the imposed fluid-flow condition, as it would be for pathological cells like cancer cells. In fact, modified MCF-10A resembles motion dynamics characteristics typical of MDA-MB-231 cells. Such evidence further supports the possibility to correlate a heterogeneous deformability to the identified cell classes, creating a completely new way to read and measure rheological and mechanical properties of suspended cells simply relying on in-flow motion features.



**Fig. 7** Internal cell viscosity estimation from in-flow deformation-dependent dynamics. We correlate  $\mu'/\mu$  with AR depending on the T, S and TT motions. Low values of  $\mu'/\mu$  correspond to high values of AR and *vice versa*, in good agreement with the power-law curve.

Since the inner content of suspended cells can be modelled as a viscoelastic fluid, with the intracellular concentrations of protein filaments dissolved in a water-like liquid,<sup>52,53</sup> only below a certain length time-scale the resulting behaviour is ascribable to purely viscous effects. In particular, at stimuli frequencies less than  $0.5 \text{ s}^{-1}$  as the ones we used during compression, such viscous behaviour is dominant.<sup>54</sup> Taking advantage of this, we used the previously presented cell classification to extract an estimation of the inner cell viscosities from the computation of  $(\lambda = \mu'/\mu)$  using the T, S and the TT data (eqn (6) and (7)). The R case has been not considered in this computation, the reason is that this motion, which permits the cells to avoid energetically costly shape changes, is a sign of cell elasticity.<sup>47</sup> Then, the combination of morphological parameters such the AR with  $\Delta\phi$  leads to the identification of a master-curve describing the deformability property of cells in terms of inner viscosity. Very low values of  $\lambda$  correspond to low values of  $\Delta\phi$ , inversely correlated with high AR values, in agreement with previously demonstrated data showing  $\lambda \leq 0.5$  typical for cells going towards the channel centre easily.<sup>55</sup>

Whereas,  $0.5 \leq \lambda \leq 10$  classically describes an intermediate condition, for which it is known that cells move more slowly to the centre.<sup>55</sup> Both the mentioned ranges well match our outcome, precisely correlating for the first time the motion regimes with rheological and mechanical properties of cells. In fact, TT defines  $\lambda \leq 0.5$  while S and T describe  $0.5 \leq \lambda \leq 10$  for pathological cells of heterogeneous deformability.





## Conclusions

The proposed microfluidic approach based on viscoelastic compression forces has successfully enabled the recognition of heterogeneous cell deformability properties in a completely label-free manner. Our approach aimed to compress cells at forces at least greater than  $10^1$   $\mu\text{N}$  to cover a certain CD range ( $\text{CD} \sim [0-0.45]$ ), capable of returning all of the motion regimes of interest. Thus, the approach appears to be highly versatile and generalizable for other cell samples, despite different cell diameter dimensions. In fact, scaling the compression force with respect to the cell diameter, a change in PEO concentration and/or applied pressure drop would be necessary to still obtain a value of  $\sim 10^1$   $\mu\text{N}$  for compression and the desired CD range.

Overall, we demonstrated a simple and easy-to-use way to compress and analyse cells by unsupervised machine learning. We introduce a set of motion parameters namely  $V_{\text{Cell}}/V_{\text{Avg}}$ ,  $Y_{\text{Eq}}$  and  $\Delta\varphi$  capable of recognizing different levels of cell deformability in a wide range of measurable rheological/mechanical properties. As a benchmark in terms of deformability measurement, we used the  $V_{\text{Cell}}/V_{\text{Avg}}$  estimation coupled with the  $Y_{\text{Eq}}$ , since it is well-established that less deformable cells move slower than more deformable cells towards the centre line. By the CytD and Noco administration, we noticed that no relevant differences are present in terms of structure organization, CD and AR between MCF-10A at no drug and after Noco treatment, as well as for MDA-MB-231 between no drug and CytD treatment. Of note, motion dynamics succeeded in distinguishing such cell samples, by identifying more rigid no drug MCF-10A as R, while modified Noco MCF-10A as T which is then more deformable. On the other hand, the T regime was confirmed for no drug MDA-MB-231 and modified CytD MDA-MB-231, supporting the fact that no relevant contribution comes from the actin compartment in compression. As a result, we demonstrate how the approach allows to detect the intrinsic heterogeneity of cells depending on the deformability characteristics. In particular, by the application of an unsupervised machine learning with the PCA approach, distinct cell clusters are identified varying the motion features. These describe the regimes spanning from R, T, S until TT corresponding to gradual levels of deformability from the lowest (R) to the highest (TT). Thus, the combination of  $V_{\text{Cell}}/V_{\text{Avg}}$ ,  $Y_{\text{Eq}}$  and  $\Delta\varphi$  gives the complete description of the rheological and mechanical properties of cells since this is not allowed with only classical descriptors such as CD and AR. Such an approach also opens the possibility to quantify such properties as inner cell viscosity. The future development of our approach has a great potential application in rapid, label-free, cost-effective and non-invasive disease diagnostics towards a personalized healthcare management.

## Author contributions

FC and DD contributed to the conceptualization of the research project. MIM, DD and VP developed the methodology. MIM implemented the measurement and

performed the FEM computation and the data analysis. MIM wrote the paper, and the review-editing was carried out by FC, MIM, VP, DD and PAN. The project supervision was done by FC.

## Conflicts of interest

There are no conflicts to declare.

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