Of cell shapes and motion: the physical basis of animal cell migration

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ABSTRACT

Motile cells have developed a variety of migration modes relying on diverse traction force generation mechanisms. Before the behavior of intracellular components could be easily imaged, cell movements were mostly classified by different types of cellular shape dynamics. Indeed, even though some types of cells move without any significant change in shape, most cell propulsion mechanisms rely on global or local deformations of the cell surface. In this review, focusing mostly on metazoan cells, we discuss how different types of local and global shape changes underlie distinct migration modes. We then discuss mechanical differences between force generation mechanisms, and finish by speculating on how they may have evolved.

1. Local cell shape changes: leading edge protrusions in migrating cells

In most animal cells, migration is associated with cell polarization: the formation of a morphologically and biochemically distinct front and rear. Early studies have described different types of protrusions at the cell front, including flat lamellipodia, finger-like filopodia, and rounded blebs (Fig. 1) (Trinkaus, 1973). However, for many years, investigations of cell migration have mainly focused on the formation, regulation, and function of lamellipodia, the predominant protrusion type in cells migrating on 2D substrates. Lamellipodia are thin (~ 200 nm in height) sheet-like protrusions generated by a branched actin network pushing out against the plasma membrane (Fig. 1a). The pathways controlling the polarized formation of lamellipodia at the cell leading edge, as well as the forces driving their extension have been extensively characterized (some recent reviews include: Holz and Vavylonis, 2018; Krause and Gautreau, 2014; Rottner and Schaks, 2019). Lamellipodia formation is often accompanied by the formation of filopodia, thin (~200 nm in diameter) tubular protrusions that can elongate up to ~30 μm at the front of lamellipodia but that can also form independently (Fig. 1b) (Jacinto and Wolpert, 2001; Rottner and Schaks, 2019). In migrating cells, both lamellipodia and filopodia have been proposed to play a sensory role in addition to their direct role in migration, probing substrate properties and driving path finding (Davenport et al., 1993; Leithner et al., 2016; Wong et al., 2014).

Over the last ~15 years, progress in 3-dimensional (3D) imaging has demonstrated that, as suggested by early studies (Trinkaus, 1973), cells migrating in 3D display a much broader array of protrusions than their 2D counterparts. In particular, in addition to lamellipodia and

filopodia, cells migrating in 3D matrices and in tissues can display pressure-driven blebs at the leading edge (Fig. 1c) (Bergert et al., 2012; Blaser et al., 2006; Diz-Muñoz et al., 2010; Liu et al., 2015; Ruprecht et al., 2015; reviewed in: Ridley, 2011). Contrary to most other protrusion types, blebs do not grow as a result of actin pushing against the plasma membrane but are initially actin-free; they arise due to hydrostatic pressure in the cytoplasm pushing against the plasma membrane at sites of weakened cortex or disrupted membrane-to-cortex attachment. As a result, blebs initially expand as spherical bulges of plasma membrane where the actin cortex reassembles over time (reviewed in: Charras and Paluch, 2008; Paluch and Raz, 2013). Some cell types also form large cylindrical protrusions termed lobopodia (Fig. 1d) (Petrie and Yamada, 2012; Petrie et al., 2012), because they have been first identified in lobose amoeba (Kudo, 1966). Similar to blebs, lobopodia formed by mammalian cells in matrices appear to be primarily pressure-driven (Petrie et al., 2012).

The type of protrusion formed is sometimes used to categorize migration modes, and different protrusions roughly correlate with different modes of propelling force generation (see Section 3 below). Lamellipodia are generally associated with mesenchymal migration, where cells display spread morphologies with strong attachments to the substrate, whereas blebs are often considered a signature of amoeboid motility, with rounded cell bodies and low substrate attachment (Lämmermann and Sixt, 2009). However, recent progress, in particular in investigating 3D migration, has highlighted that such classifications are too restrictive, and that migration modes exist over broad spectrum that cannot be easily reduced to differences in cell morphology (Boekhorst et al., 2016; Fritz-Laylin et al., 2018). For instance, thin sheet-like protrusions, morphologically resembling lamellipodia, have been reported in a variety of rather rounded cells migrating in 3D matrices and in vivo (Diz-Muñoz et al., 2010; Fritz-Laylin et al., 2017a). Furthermore, while some cell types exclusively display one protrusion type, many others can switch between protrusions depending on internal and external cues (Bergert et al., 2012; Boekhorst et al., 2016; Helvert et al., 2018; Liu et al., 2015; Petrie and Yamada, 2016). Such migration plasticity is thought to be important during cancer cell dissemination. Interestingly, other cell types, particularly in early development, can display blebs, lamellipodia, and other protrusion types simultaneously (Diz-Muñoz et al., 2010; Trinkaus, 1973; Tyson et al., 2014).

2. Global cell shape: a look beyond the leading edge

While studies of cell morphology during cell migration have overwhelmingly focused on leading edge protrusions, analysis of global cell shape can provide a valuable complementary and more holistic view. Cell shape is a summary readout of a myriad of microscopic intracellular and extracellular processes (Fig. 2a). Intracellularly, the shape of individual cells is largely determined by the architecture of actomyosin networks (Chalut and Paluch, 2016; Pollard and Cooper, 2009) and extracellularly by a cell's contacts with neighboring cells (De Palo et al., 2017; Farhadifar et al., 2007; Messal et al., 2019) and the microenvironment (Albert and Schwarz, 2014, 2016; Théry et al., 2007). Clearly, the dimensionality of the space cells inhabit is an important determining factor for both cell shape and migration mode. During migration in 1D and 2D, cell shape is generally relatively simple. For instance, cells migrating in 1D microchannels, on 1D adhesive tracks, or on suspended nanofibers mimicking fibrillar environments, adopt elongated rod-like shapes (Bergert et al., 2012; Doyle et al., 2009; Guetta-Terrier et al., 2015; Maiuri et al., 2015). On 2D substrates, some cell types, such as fish epithelial keratocytes display fan-like shapes that remains essentially stationary while steady cytoskeletal flows drive movement (Keren et al., 2008). Other cell types, e.g. migratory fibroblasts or amoeboid cells like *Dictyostelium discoideum*, adopt more dynamic morphologies (Mogilner and Keren, 2009; Xiong et al., 2010). Nonetheless, in 3D extracellular matrix environments and in vivo, migrating cells display a much larger variety of shapes (Driscoll and Danuser, 2015; Minina et al., 2007; Paluch et al., 2016).

Despite biochemical processes and membrane fluctuations occurring at subsecond timescales, global cell shape, as defined by the cell outline without considering the many small short-lived local fluctuations, is much more stable, often persisting over minutes and hours. Moreover, even for migrating cells displaying a large heterogeneity of shapes, dimensionality reduction analysis of morphological features (Box 1) indicates that global cell shape is surprisingly low dimensional. This suggests that cell shapes are constrained and that migrating cells cannot adopt just any type of morphology. For example, in fish epithelial keratocytes the projected cell area is either rounded 'D' shaped or elongated 'canoe' shaped with variability in aspect ratio and left–right asymmetry of the side lobes (Keren et al., 2008); while in *Dictyostelium* most of a cell's shape variability is described by elongation (stretching), pseudopod splitting, and polarization (front heavy, pear shaped) (Tweedy et al., 2013a). These strong shape constraints can subsequently be used to inform biophysical models of cell motility. For example, Tweedy et al. (2013) developed a Meinhardt-type model, based on simple reaction-diffusion of three molecular species with protrusion activating and inhibiting roles, to describe

Dictyostelium migration. After demonstration that the simulated shape modes match actual *Dictyostelium* behavior, the model was used to predict that shape defects alone can drastically impair chemotaxis accuracy, a prediction that was then experimentally confirmed (Tweedy et al., 2013a).

Cell shape is often directly related to cell function. For instance, epithelial cells tile densely to form quasi-2D sheets for covering and protecting tissues and organs, and neurons have long protrusions that allow them to form 3D neural networks with other neurons. In cell migration, fibroblasts are strongly elongated and bifurcated, aiding their capacity to heal wounds by interleaving with other cells and allowing them to more efficiently remodel the extracellular matrix during wound healing (Pattabiraman and Rao, 2010). Moreover, the shapes cells use to move can be a strategy for accurate chemotaxis in social *Dictyostelium discoideum* amoeba. In shallow, difficult to sense, noisy chemical gradients amoeba use cycles of pseudopod splitting, for choosing from few directions, and elongation to implement a biased random walk. In contrast, in steep, easy to sense, strong gradients, cells polarize in the correct direction and move nearly ballistically (Fig. 2b) (Aquino et al., 2014; Tweedy et al., 2013a).

Shape characteristics can also be used as an indicator of gene expression (Sero et al., 2015; Yin et al., 2013). For example, automated, shape-based classification of motile *Drosophila* haemocytes indicates that shape characteristics correlate with gene expression levels, in particular that depletion of the tumor suppressor PTEN leads to populations with overrepresented rounded and elongated cells (Yin et al., 2013). This may be an appropriate approach to take if, for example, creating automated assistance for pathologists when classifying metastatic melanoma. Of particular note is the observation by Yin and co-workers (Yin et al., 2013) that genes regulate switch-like shape transitions as opposed to the shapes themselves. Hence, once a low dimensional representation or basis set has been found, transitions between shape attractor states can be deduced (Kimmel et al., 2018), or sequences of shapes can be learned from the data and used to predict long-time cell dynamics during cell migration (Tweedy et al., 2019).

Taken together, cell shape analysis provides a global view linking molecular details to global cell parameters. More specifically, models of cell shape dynamics have been used to predict the relation between speed and persistence (Maiuri et al., 2015), lamellipodial curvature and the relationship between speed and morphology (Keren et al., 2008), and even cellular strategies for accurate chemotaxis (Tweedy et al., 2013a). Cell shape can also inform about

gene expression (Yin et al., 2013), and hence be used for screening (via morphological profiling) to infer cell function and health (Marklein et al., 2018). Such morphometric screening could be particularly important for identifying cells undergoing epithelial-to-mesenchymal transitions, potentially leading to fast migrating metastasizing cells.

Notably, cell shape appears to be sometimes more conserved than the biochemical processes that control it (Yin et al., 2014). For instance, the sperm cells of the nematode *C. elegans* move in a crawling fashion with shapes reminiscent of crawling cells with large lamellipodia. Yet, nematode sperm motility is not driven by actin but by a cytoskeleton-like component specific to these cells, major sperm protein (Nelson et al., 1982). Major sperm protein polymerizes like actin, but the filaments formed are not polarized and no motor-like proteins have been identified. Yet, nematode sperm cells display protrusions at the leading edge and rear retraction, just like crawling fibroblasts. The physical basis of force generation, in particular for the contractions driving rear retraction in this system is not fully understood (Ananthakrishnan and Ehrlicher, 2007). However, recent studies indicate that propelling force generation relies on a molecular clutch mechanism similar to that found in cells relying on actomyosin-driven motility, but driven by an entirely different set of biochemical components (Havrylenko et al., 2014).

3. From deformation to motion: generating cellular propelling force

The relationship between shape, force and motion has fascinated thinkers for many centuries. In the 4th century BC, Aristotle theorized that objects move at speeds proportional to the force exerted and thus require continuous force to remain in motion (Stinner, 1994). Of course, this has since long been disproven and Newton's first law teaches us that inertia causes an object to maintain a constant velocity unless acted upon by a force (Newton, 1726). However, at microscopic scales and speeds inertial forces become negligible compared to the viscosity of water. In such a situation, mathematically characterized by a low Reynolds number (which measures the ratio of inertial to viscous forces), inertia cannot maintain motion. Thus, at microscopic scales, Newton's laws converge with Aristotle's theory and small objects like cells stop moving as soon as the forces that propel them stop (Purcell, 1977). Therefore, cellular motion relies on the constant generation of propelling forces.

In most metazoan cells, traction forces are primarily generated by cellular actin networks, with flagellar beating-driven motion of sperm cells a notable exception. A spectrum of mechanically

distinct modes of force generation and force transmission have been described in migrating cells. Inside cells, force generation can be accomplished through polarized growth of actin filaments, leading to retrograde actin flows in lamellipodia (Case and Waterman, 2015), or through myosin-driven contractions that can lead to retrograde flows of the actomyosin cortex (Salbreux et al., 2012) and to bleb expansion at the cell front (Paluch and Raz, 2013).

Intracellularly generated forces must be transmitted to the substrate to generate the traction forces that propel the cell forward. The canonical mode of force transmission relies on adhesion points between the cell and the substrate, and has been extensively studied in the context of mesenchymal, lamellipodia-driven migration. At the front of the lamellipodium, the cell is anchored to the substrate via focal adhesions (Fig. 3a). These consist of the transmembrane receptor integrin-complexes that bind extracellular matrix components on the outside of the cell and the actomyosin complex (indirectly, via talin proteins) inside the cell. Cell movement is then achieved through a well-described protrusion-adhesion-retraction cycle (Abercrombie, 1980; Ananthakrishnan and Ehrlicher, 2007): 1) pushing forces at the cell front are generated by actin-polymerization and lead to protrusion of the lamellipodium; 2) retrograde actin flow is linked to the substrate via focal adhesions, which leads to forward movement of the cell; 3) retraction of the cell posterior then occurs due to rear contraction and release of focal adhesions at the back of the cell. In effect, focal adhesions enable cell migration by coupling the retrograde flow of lamellipodial actin to the underlying substrate (Fig. 3a) (reviewed in: Case and Waterman, 2015; Elosegui-Artola et al., 2018). This mechanism has been termed the 'molecular clutch', as it allows for dynamic and differential engagement between the actin network and the substrate, allowing a cell to sense and react to its environment (Chan and Odde, 2008; Mitchison and Kirschner, 1988).

Despite the fact that most migration studies have focused on focal-adhesion driven migration, increasing evidence indicates that many cells migrating in 3D rely on weak or no adhesion at all (reviewed in: Paluch et al., 2016). This observation is in line with the greater variability in migration modes displayed in complex 3D environments, e.g. the more diverse spectrum of leading edge protrusions (see section 1 and (Caswell and Zech, 2018)). Although adhesive migration does not strictly rely on a lamellipodium or on integrin molecules—adhesions could in principle form at other protrusions or under the cell body and consist of other adhesive receptors (reviewed in: Case and Waterman, 2015; Elosegui-Artola et al., 2018)—multiple other lines of evidence have suggested that many fast-moving cells migrate in an adhesion independent manner (reviewed in: Paluch et al., 2016). Unequivocal evidence that cells can

migrate in the absence of focal adhesions came from a study of primary mouse dendritic cells that were either pan-integrin or talin knock-out (Lämmermann et al., 2008), both of which are absolutely essential for focal adhesion formation. Strikingly, *in vivo* imaging of knock-out and wildtype cells, co-injected into live mice, revealed no discernible difference between cell speed, directional persistence, or the efficiency of cells to localize to lymph nodes, ~1 cm away from the injection site (Lämmermann et al., 2008). Therefore, non-adhesive migration mechanisms must exist that allow cells to efficiently migrate, including in complex *in vivo* environments.

Several mechanisms have been proposed to explain force transmission during non-adhesive migration (reviewed in Paluch et al., 2016). The mechanism that is currently supported by the strongest experimental and theoretical evidence is friction-driven migration (Bergert et al., 2015). In this migration mode, cells rely on non-specific friction between the retrograde flow of the actomyosin cortex and the extracellular substrate to generate traction forces (Fig. 3b). By combining experiments in microfabricated confinement devices with theoretical modeling, Bergert et al (2015) quantitatively characterized how changing friction affects migration velocity and traction force generation. Other proposed mechanisms for non-adhesive migration (reviewed in Paluch et al., 2016) include 'chimneying', where cells push off perpendicularly against the substrate (Hawkins et al., 2009; Malawista and Chevance, 1997; Yip et al., 2015), and 'interdigitation', where cellular protrusions intercalate into structural features of the substrate (Tozluoğlu et al., 2013). Due to the difficulty of direct mechanical measurements on cells within 3D environments, it is not easy to unambiguously identify which specific force generation mechanism is used by cells migrating *in vivo*. Nonetheless, quantitative studies in confined environments in vitro should help identify key regulators of force generation on non-adhesive substrates, allowing for the investigation of the role of these regulators during migration in specific environments *in vivo*.

4. Mechanical differences between adhesive and non-adhesive migration

Even though friction generation and focal adhesion formation both rely on chemical interactions between the cell and the substrate, these force generation mechanisms are fundamentally different in a number of ways (summarized in Table 1). First of all, while adhesion-based migration functions both on 2D substrates and in 3D confinement, friction-based migration is only possible in 3D confinement (Lämmermann et al., 2008; Paluch et al.,

2016). This is due to differences in strength and duration of the molecular bonds underlying cell-substrate interactions in the two migration modes. Indeed, for a cell to maintain prolonged contact with a 2D substrate, a requirement for efficient propelling force generation, cell-substrate interactions must be strong enough to counteract Brownian motion. Integrin-ECM bonds within focal adhesions are sufficient to provide such stable attachment. Furthermore, individual integrin-ligand interactions behave as catch-bonds, where bond life-time is prolonged by force (Kong et al., 2009), and the focal complexes further strengthen in response to force due to clustering and cooperativity (Choquet et al., 1997; Galbraith et al., 2002; Riveline et al., 2001). In contrast, during friction-based migration, individual bonds are thought to be significantly weaker and shorter-lived. This follows from observations that non-adhesive migration is displayed by cells that, when placed on a 2D substrate, do not maintain substrate contact and cannot migrate even when they are polarized and form leading edge protrusions (Bergert et al., 2015; Lämmermann et al., 2008). Placing cells in 3D confinement counters Brownian motion and allows for the prolonged cell-substrate contacts required for efficient force generation. Intriguingly, *Dictyostelium* can migrate efficiently on 2D substrates but does not appear to rely on specific adhesion molecules (Loomis et al., 2012; Weber et al., 1995). Recent studies suggest that non-specific van der Waals interactions might be sufficient to maintain substrate attachment in *Dictyostelium* (Loomis et al., 2012). How exactly such interactions generate sufficient forces to counteract Brownian motion, and why non-specific interactions are not sufficient to maintain surface contact in other cell types, is not well understood.

The differences in cell-substrate bonds lifetimes between adhesion- and friction-based migration have important consequences for how migration efficiency depends on substrate interactions. Focal adhesions can outlive the dwell time of the cell itself at a given location, such that cell movement usually depends on active disassembly of adhesions at the rear (Gupton and Waterman-Storer, 2006). As a result, there is an optimal adhesion strength for cell migration, where too strong adhesions stall actomyosin flow and therefore migration (Gardel et al., 2010; Gupton and Waterman-Storer, 2006). In contrast, during friction-driven migration, cell-substrate interactions are very transient (Hawkins et al., 2011) and do not appear to interfere with retraction of the cell rear, as evidenced by a minimal traction force requirement that is only just sufficient to resist the external drag force of the surrounding medium (Bergert et al., 2015). As a result, experimental evidence and theoretical modeling suggest that

increasing friction leads to a plateau of migration speed and no slowing down is expected even at very high friction (Bergert et al., 2015).

Another important difference between the two migration modes is the spatial distribution of the traction forces. During adhesive migration, forces exerted by focal adhesion are distributed such that they exert a negative force dipole (i.e. from the periphery towards the cell center), effectively pulling themselves forward and contracting the underlying substrate (Schwarz and Safran, 2013). In contrast, during non-adhesive migration, several studies suggest that the force dipole is positive (i.e. from the center outwards), with the confined cell exerting pushing forces that expand the substrate underneath it (Bergert et al., 2015; Yip et al., 2015) (Fig. 3).

The distinct mechanics of cell-substrate interactions during adhesive and non-adhesive migration likely affect how mechanosensing can influence cell movement. During adhesive migration, cells have been shown to sense and respond to a variety of substrate physical properties. For instance, many cell types migrating on 2D substrates can follow gradients in substrate stiffness, due to mechanosensitive behavior of focal adhesions (this process, termed durotaxis, has been extensively reviewed elsewhere (Plotnikov and Waterman, 2013; Haeger et al., 2015)). Whether cells migrating without focal adhesions can direct migration in response to stiffness gradients has, to our knowledge, not been investigated, possibly due to the difficulty in creating a stiffness gradient in confined environments. However, other mechanical parameters have been shown to influence migration directionality in confinement. For instance, assays investigating cell migration in tapered microchannels of decreasing cross-section have shown that increasing confinement can lead to cell arrest, repolarization and reversal of migration direction (Mak et al., 2011; Wang et al., 2018). Interestingly, a portion of cells persistently migrate into even strongly tapered channels, and the relative proportions of cells reversing direction and persistently migrating strongly depended on the cell type investigated (Mak et al., 2011; Wang et al., 2018). A number of recent studies have highlighted that the nucleus, which is significantly stiffer than the cytoplasm, can act as a physical barrier during migration through highly constricted spaces (reviewed in (McGregor et al., 2016)). It is possible that the behavior of cells migrating in increasing confinement depends on the physical properties of their nuclei, although the actin cytoskeleton also directly contributes to controlling migration patterns in tapered channels (Wang et al., 2018). Interestingly, it has also been shown that levels of hydraulic resistance can direct cell migration in microchannels. When cells migrate in confined environments like microchannels, or like microcapillaries in vivo, the fluid ahead of them only partially flows around or permeates through the cell (Bergert et al., 2015;

Stroka et al., 2014), while most of the fluid is pushed forward as the cell moves. This generates hydraulic resistance to migration. A study using a microfluidic chip with channel bifurcations with different levels of hydraulic resistance, has shown that HL-60 neutrophils polarize towards the path of least resistance, a process termed "barotaxis" (Prentice-Mott et al., 2013) A recent study has identified the mechanosensitive channel TRPM7 as a key molecular sensor mediating barotactic behavior (Zhao et al., 2019). How exactly such mechanosensing behaviors affect cell migration in narrow spaces *in vivo*, such as micro-capillaries or tracks through interstitial tissues (Paul et al., 2017), remains to be investigated.

Finally, there is likely a significant difference in energetic efficiency between the two migration modes. As any active biological process, cell migration costs energy. ATP is consumed both during actin filament extension (reviewed in: Dominguez and Holmes, 2011; Rottner and Schaks, 2019) and myosin mediated contraction (Holmes, 2008), which lie at the basis of both adhesive and friction-driven migration. During adhesive migration, additional energy is consumed by kinases (notably by focal adhesion kinase (FAK)) and GTPases that regulate processes such as focal adhesion assembly, disassembly, and coupling to the flowing actin network (reviewed in: Case and Waterman, 2015; Gardel et al., 2010; Kleinschmidt and Schlaepfer, 2017; Schoenherr et al., 2018; Sun et al., 2016). In contrast, because adhesion-independent migration is thought to rely on spontaneously occurring transient interactions, no energy is expected to be required to initiate or break the bonds. While to our knowledge there have been no direct comparative measurements of energy consumption between adhesive and adhesion-free migration, cell velocity or the amount of stress exerted on the substrate could be used as a proxy for migration efficiency. Strikingly, while focal adhesions generate forces that are orders of magnitude higher than strictly required for translocation (del Alamo et al., 2007), efficient translocation is achieved during friction-driven migration at forces on the same order of magnitude as the theoretical minimally required force, given by the media's viscous drag resisting forward translocation (Bergert et al., 2015). Together with the observation that cells migrating without focal adhesions are generally much faster than their adhesive counterparts (Table 1), this further suggests that friction-based migration is energetically more efficient than focal-adhesion driven movement.

It is of note that most metazoan *in vivo* environments are predicted to be both highly crowded, thereby confining cells, and to feature abundant ligands, generally ECM components, for integrin binding. Therefore, both adhesive and adhesion-free migration remain viable options for *in vivo* cell migration.

5. Concluding remarks and speculations

Many cells have the capacity of directed motion, which is essential for a multitude of physiological and pathological processes, including development, immune-response, and metastasis. Most well studied cell culture systems display adhesion-based migration in standard (2D) laboratory conditions. Additionally, while such 2D conditions preclude non-adhesive migration, a recent extensive study has shown that physical confinement leads to efficient migration in the absence of adhesive substrates in a wide variety of cell lines (Liu et al., 2015). These include transformed, tumoral, and healthy primary cells from 3 separate mammalian species and various tissue origins. Furthermore, actin-based, yet adhesion-independent cell migration has further been observed in diverse cells from multiple metazoan species (e.g., Neilson et al., 2011; Ruprecht et al., 2015), as well as in a range of distantly related eukaryotes, including the chytrid fungus *Batrachochytrium dendrobatidis*, the amoeboflagellate *Naegleria gruberi*, and the parasitic protozoan *Trichomonas vaginalis* (Fritz-Laylin et al., 2010, 2017b; Kusdian et al., 2013). The type of migration displayed by these distant species, termed α-migration, is characterized by 3D lobose pseudopods rich in branched actin networks (Fig. 1e; note that these are different from lobopodia mentioned above and in Fig. 1d) (Fritz-Laylin et al., 2017b). Based on the phylogenetics of essential genes for pseudopod-based motility, it appears that α-migration was present in the last eukaryotic ancestor (Fritz-Laylin et al., 2017b). Although integrins are also very ancient proteins, with certain domains found even in prokaryotes, their function in cell adhesion has so far only been observed in the Amorphea (unikont) lineage (Johnson et al., 2009; Sebé-Pedrós et al., 2010), which does not encompass *Naegleria* or *Trichomonas*. This argues that non-adhesive migration likely predates migration driven by focal adhesion.

As discussed above, the amount of force required to mediate a constant velocity is much higher in adhesive migration than during adhesion-independent migration. This has led to the speculation that bleb-based, adhesion-independent migration may require less energy than focal adhesion-based motility. Interestingly, during collective strand migration of metastatic cells, it has been shown that leader cells, which display strongly polarized mesenchymal morphologies and remodel the extracellular matrix to facilitate strand migration, display higher glucose uptake than followers (Zhang et al., 2019). Furthermore, leader cell lifetime is directly related to cellular energy levels. This further suggests that strongly adhesive mesenchymal-like

migration has high energy requirements. Moreover, because focal adhesions depend on integrin-ligand binding, cells that rapidly move between different types of tissue, including many immune and metastatic cancer cells, would potentially need to adapt their integrin repertoire to adhere to each specific environment. Taken together, there are strong indications that, in the absence of further constraints, adhesion-independent cell migration is more efficient in terms of energy, genetics, and regulation than focal adhesion-based migration. Therefore, despite being overlooked in many experimental settings, it is tempting to speculate that non-adhesive migration is the default cell migration mode for many cells. In this scenario, adhesive migration would represent a specialized form of migration that has evolved more recently and possibly in specific circumstances, e.g. during migrating against flow in vasculature or in unconfined environments. Nonetheless, focal adhesions have been observed in cells migrating through dense matrices and it is likely that in the complex 3D environments faced during migration *in vivo*, cells would combine or alternate adhesive and friction-based mechanisms for force generation.

Acknowledgements

The authors acknowledge funding from the Medical Research Council UK (EKP), The European Research Council (EKP), The Wellcome Trust (DLB), the Leverhulme Trust (EKP, WP), the Herchel Smith Postdoctoral Fellowship Fund (WP), and BBSRC grant BB/N00065X/1 (RGE).

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Box 1: Cell morphometric analysis

This Box outlines the typical steps involved in automated analysis of cellular shapes and lists some of the analysis tools available.

1. Segmentation of cell shapes

The starting point of morphometric analysis (Box Fig. 4A) of cell shape is of course image segmentation, i.e. the transformation of cell shape into a form that can be computationally processed (Box Fig. 4B,C). A wide variety of segmentation algorithms have been developed. A frequently used segmentation algorithm is thresholding (Box Fig. 4C). In its simplest form, image pixels above or below a certain intensity value are interpreted as being located within the cell (Burger, Wilhelm and Burge, 2010). More advanced thresholding algorithms, such as adaptive thresholding (Pappas, 1992), allow for local changes of the threshold value. While these methods have proven successful for images where intensity inside the cell differs strongly from its surrounding, thresholding-based segmentation fails in cases where only the cell boundary, e.g. the membrane, is labeled. In this case, an alternative approach has often proven successfully: the active contour approach. Here, a deformable representation of the cell contour deforms, typically using the information of the intensity of the cell membrane. Active contour methods are well established to analyze 2D cell shapes (Ray et al., 2002) and within the last few years, 3D versions have been developed (Machado et al., 2019; Smith et al., 2017). While thresholding and active contour approaches can be implemented easily, manual detection of cell shapes is often out-performing them.

Recent developments in machine learning methods have significantly improved cell segmentation. Two of the most common tools are the Weka (short for Waikato Environment for Knowledge Analysis) toolbox (Arganda-Carreras et al., 2017), allowing for the training of a classifier and segmentation from a limited number of manually set annotations, and U-Net (Falk et al., 2019), a user-friendly tool for the segmentation of biomedical data by a training of a neural network. For both tools, Fiji (Schindelin et al., 2012) plugins exist ("Trainable Weka Segmentation" plugin and the "U-Net segmentation" plugin) that allow for an easy and open-source implementation of machine and deep learning approaches to segmentation.

After segmentation, cell shapes are typically represented as either a binary mask for which pixels inside of a cell have the value 1 and outside of the cell, 0, or as a set of points that

parametrize the cell surface (Zhang and Lu, 2004). In 2D, the connection of neighboring points allows for a clear representation of the cell perimeter. In 3D, surface reconstruction algorithms are required to generate a closed surface.

2. Quantification of cell shapes

The next step in morphometric analysis is the extraction of cell shape parameters, a limited number of features that describe general morphological properties. To compare the shapes of many cells, for example to detect subpopulations, the features should be independent of each other, independent of any translation, rotation and reflection of the cell shape and should uniquely describe a shape. A detailed overview of methods for shape feature extraction can be found in (Mingqiang et al., 2008). The most common approaches are:

- A straightforward method to quantify a cell shape are standard measures, such as area, convex area, perimeter, circularity, etc. in 2D, or volume, convex volume, surface area, sphericity, etc. in 3D. While a wide range of such features can be defined (Bakal et al., 2007) and they can be clearly interpreted, two different features are not always independent of each other. Additionally, distinct cell shapes can be described by the same set of features and a reconstruction of shape from the features is not possible.
- Closely connected to standard shape measures are so-called landmark points (Pincus and Theriot, 2007), recurring points, e.g. locations of specific cell protrusions, that can be uniquely defined for each shape. This approach has been successfully applied in situations where relatively comparable shapes are studied, e.g. at a more macroscopic scale in botany and anatomy, or to describe the protrusions of nerve cells (Radojevie et al., 2015). However, because it focuses on the quantification of pre-defined recurrent features, it is not generally applicable to the investigation of animal cell shapes where morphology can vary strongly and without any clearly identifiable features.
- A method that allows for a unique description of essentially any cell shape is the so-called Fourier descriptor (Kuhl and Giardina, 1982). This tool has been developed for the characterization of 2D shapes and is based on the Fourier transform of the x- and y-component of the cell outline. Each resulting mode is described by four harmonics. The harmonics are not independent of rotations and require an alignment of the images. Alternatively, it is also possible to study the mode amplitudes that are independent of the cell orientation but do not allow for complete shape reconstruction (Tweedy et al.,

2013b). Importantly, even complex cell shapes can be relatively accurately reconstructed using a subset of Fourier descriptor modes (Box Fig. 4D). For this reason, Fourier descriptors are often used to describe cellular shapes (Diaz et al., 1989; Pincus and Theriot, 2007; Tweedy et al., 2013b) and for a wide range of other systems, such as prehistoric stone artefacts (Cardillo, 2010) and cereal grains (Mebatsion et al., 2012).

- While a few methods have been developed to apply Fourier descriptors in 3D (Van Der Maaten and Hinton, 2008), they are significantly more complex mathematically and computationally. An alternative approach more suitable for the quantification of 3D cell shapes is the use of moment invariants. These are scalar values that characterize the images by summing up pixels with weight functions. Examples for 2D moment invariants are Hus moments, complex moments, Zernike moments, Legendre moments and Radial Chebyshev moments (Flusser et al., 2016; Mingqiang et al., 2008; Zhang and Lu, 2004). A summary of 3D invariants can be found in (Flusser et al., 2016). While moment invariants can be calculated for any cell shape in 2D or 3D, they are often not independent of each other and lack intuitive interpretation. For this reason, the choice of the right method to extract shape features depends on the specific problem. Moment invariants have been applied to study the shape of spermatogonia (Liyun et al., 2009) and invasive cancer cells (Alizadeh et al., 2016).

3. Analysis of high-dimensional morphometric data

The quantification methods outlined above typically generate high-dimensional datasets of cell morphological features. To analyze such datasets, morphometric studies use dimensional reduction methods. Examples of dimensional reduction approaches are the principal component analysis (PCA) (Box Fig. 4E) (Jolliffe, 2002), t-distributed stochastic neighbor embedding (tSNE) (Van Der Maaten and Hinton, 2008) and SPRING (Weinreb et al., 2018). For instance, PCA has been successfully used to investigate *Dictyostelium* chemotactic migration (Tweedy et al., 2013b) (see main text and Fig. 2B) or the dynamics of neuronal growth cone dynamics (Goodhill et al., 2015).

Low-dimensional representations of cell shape features allow for direct visual identification of sub-populations of cells with distinct shapes. For a more quantitative investigation of the existence of such subpopulations, clustering algorithms (Jain, 2010) such as k-means (Hartigan and Wong, 1979) and hierarchal clustering (Bakal et al., 2007) can be used. After identifying

clusters of specific cell shapes, a new cell can be classified to predict to which population it belongs. To this aim, algorithms such as decision trees (Safavian and Landgrebe, 1991), Bayes classifier (Hand and Yu, 2001) and support vector machines (Cortes and Vapnik, 1995) can be applied.

While the described combination of dimensional reduction and clustering tools has only been applied to morphological cell features in recent years, similar data analysis pipelines have been extensively applied to other types of data sets, such as RNA sequencing data (Wagner et al., 2018) and even to characterize the motile dynamics of cells (Kimmel et al., 2018).

4. Deep learning tools

An alternative to the pipeline described above that does not require segmentation and feature extraction to identify clusters and classify cells, are machine and deep learning tools (Chen et al., 2016). For example, a recent study (Buggenthin et al., 2017) has shown that a deep neural network can efficiently predict lineage choice in differentiating primary hematopoietic progenitors, based only on microscopy data of cell shapes and dynamics. While very powerful for classification and predictive power, such approaches give only limited information as to which specific morphometric features are particularly important for a specific cell behavior. Nonetheless, the strong predictive power of such approaches could make them particularly relevant for biomedical screening and diagnosis.

Figures and Tables

Figure 1: Protrusions in migrating cells. Cartoons of the side and top view of cellular protrusions (highlighted in green) displayed by migrating cells.

Figure 1: Global cell shape and behavior. A) Cell shape is a summary readout of a myriad of microscopic intracellular and extracellular processes. B) Shape space is low-dimensional. Principal component analysis (PCA) performed on Fourier spectra (see Box 1) of cell shapes from hundreds of cells in a wide range of chemical gradients reveals that 90% of shape variability in D. discoideum can be accounted for with the first three principal components, corresponding to elongation (PC 1), pseudopod splitting (PC 2) and polarization (PC 3) in the spatial domain. Example trajectories in the PC 1 and PC 2 shape space for one low- and one high-signal-to-noise ratio (SNR) cell, corresponding to chemotaxis in shallow and steep chemical gradients, respectively. Cell outlines from the two trajectories are superimposed in their correct positions in the principal component space. This shows that cell behavior is a run-and-tumble like stereotypical behavior in shallow gradients, with cell shape alternating between elongation and pseudopod splitting. Images taken from (Tweedy et al. 2013).

Figure 2: Adhesive versus non-adhesive migration. Cartoons of cells migrating in an adhesive (A) and non-adhesive (B) fashion. On the left is a side view of the migrating cell and its surrounding environment. On the right is a zoomed in cartoon of the molecular dynamics and interactions thought to underlie each type of migration.

Box Figure 3: Cellular morphometric analysis A) Pipeline of automated cell morphometric analysis. B) Image of a cell. C) Example segmentation of the cell displayed in Panel B, obtained with a random forest segmentation algorithm in Fiji, using the Weka plugin (Arganda-Carreras et al., 2017; Schindelin et al., 2012). D) Cell mask (gray) and reconstruction of the contour using the first five (blue) and 20 (orange) modes of the Fourier descriptor. E) PCA of general cell shape features. Experimental data from Irene Aspalter.

Table 1

Migration mode	Adhesive	Non-adhesive
Protrusion type	Usually lamellipodia	Usually blebs
Propelling force generation	Filament extension / actin flow	Cortex flow
Force transmission	Focal adhesion	Friction, protrusion intercalations, etc
Substrate interaction	Specific	Non-specific
Duration of cell-substrate interactions	Longer than dwell time	Shorter than dwell time
Speed-substrate interaction strength relationship	Bell curve	Plateau
Environment	2D surfaces and 3D environments	3D confinement
Migration speed*	~0.1 – 1 μm/min	~1 – 10 μm/min
Stresses exerted on substrate**	~10^2 – 10^5 Pa	~1 Pa
Actin flow profile	Mainly in lamellipodium	At the cortex all along the cell body, max velocity in cell center
Force dipole	Contractile	Expansile

Table 1: Comparison of key features of focal-adhesion based and focal adhesion-independent cell migration. * For adhesive migration: (Liu et al., 2015; Maiuri et al., 2012); non-adhesive migration: (Liu et al., 2015). ** For adhesive migration: (Balaban et al., 2001; Dembo and Wang, 1999; Galbraith and Sheetz, 1997; Legant et al., 2010); non-adhesive migration (Bergert et al., 2015).

Figure 1

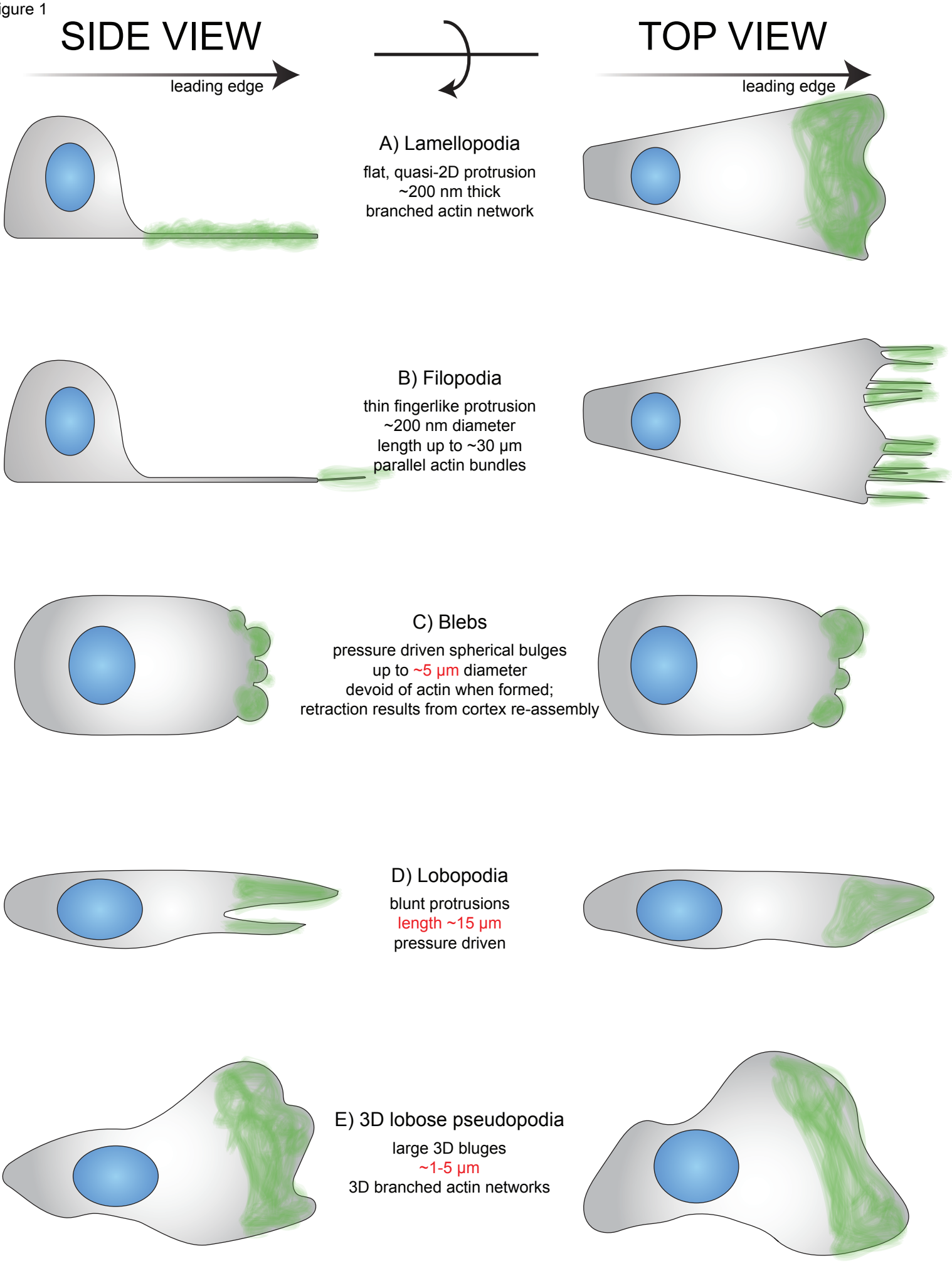
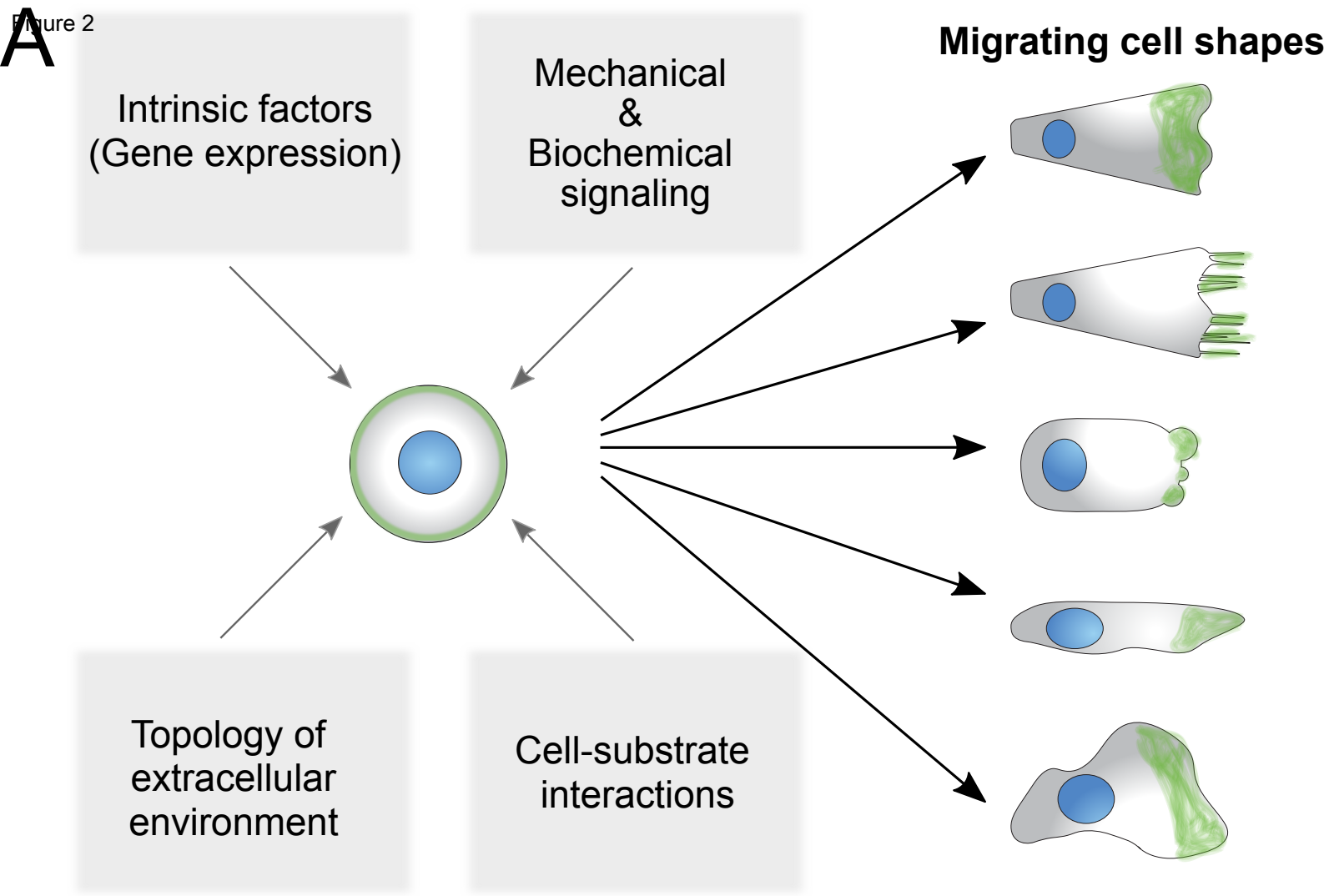
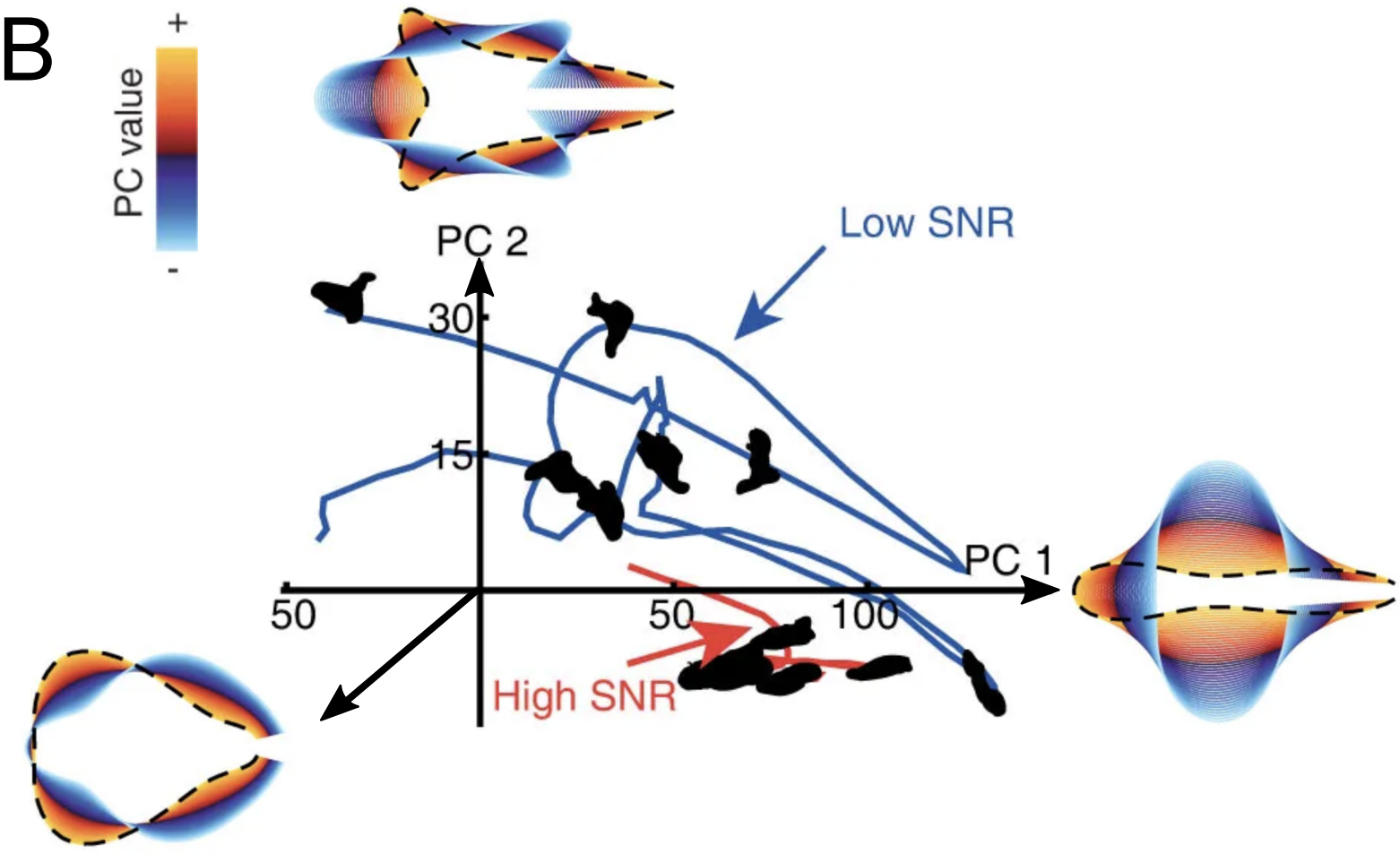

SIDE VIEW
leading edge
TOP VIEW
leading edge
A) Lamellopodia
flat, quasi-2D protrusion
~200 nm thick
branched actin network
B) Filopodia
thin fingerlike protrusion
~200 nm diameter
length up to ~30 µm
parallel actin bundles
C) Blebs
pressure driven spherical bulges
up to ~5 µm diameter
devoid of actin when formed;
retraction results from cortex re-assembly
D) Lobopodia
blunt protrusions
length ~15 µm
pressure driven
E) 3D lobose pseudopodia
large 3D bluges
~1-5 µm
3D branched actin networks

Figure 2


A
Intrinsic factors
(Gene expression)
Mechanical
&
Biochemical
signaling
Migrating cell shapes
Topology of
extracellular
environment
Cell-substrate
interactions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PC value
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-
PC 2
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15
50
50
100
PC 1
Low SNR
High SNR

Figure 3

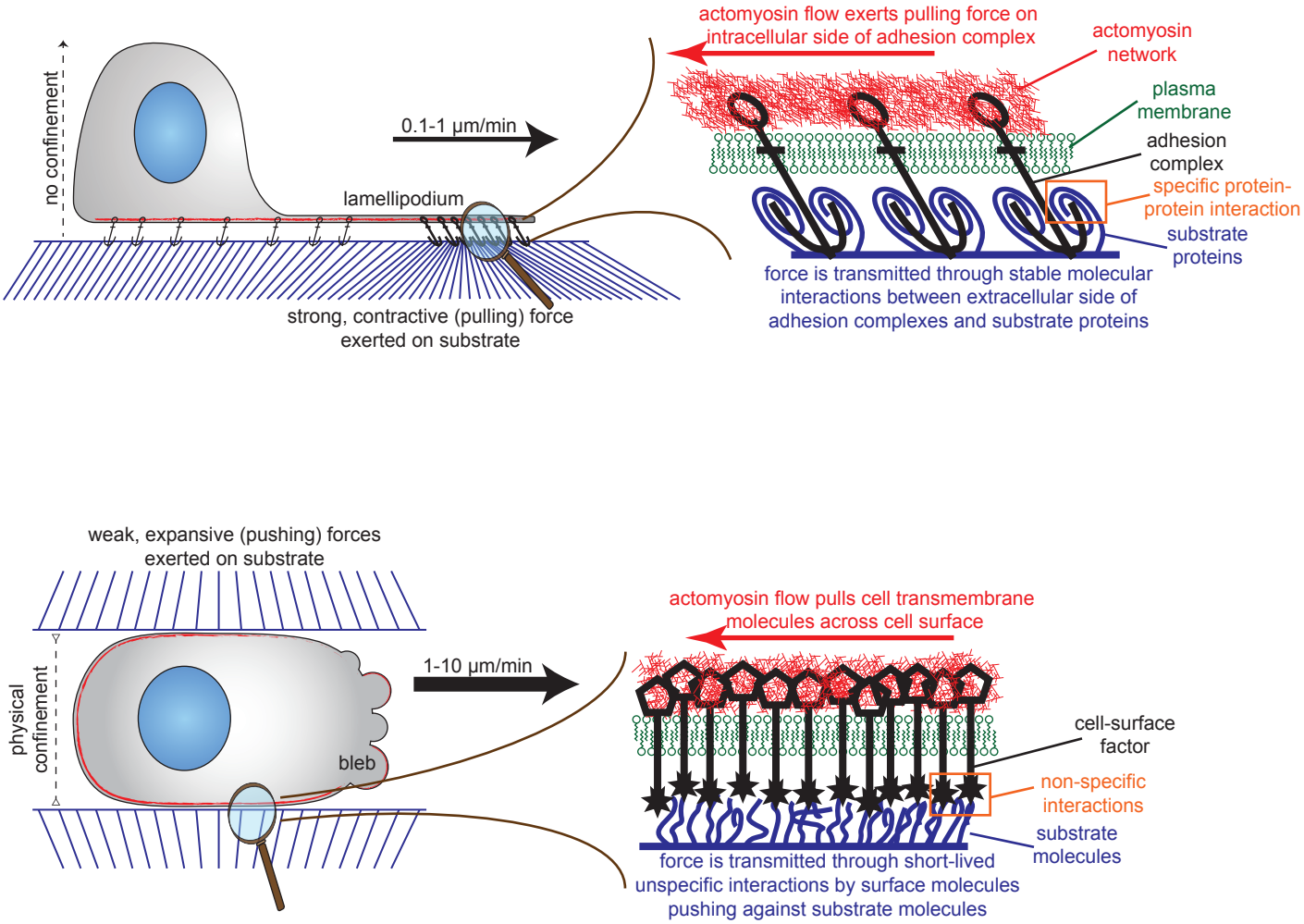
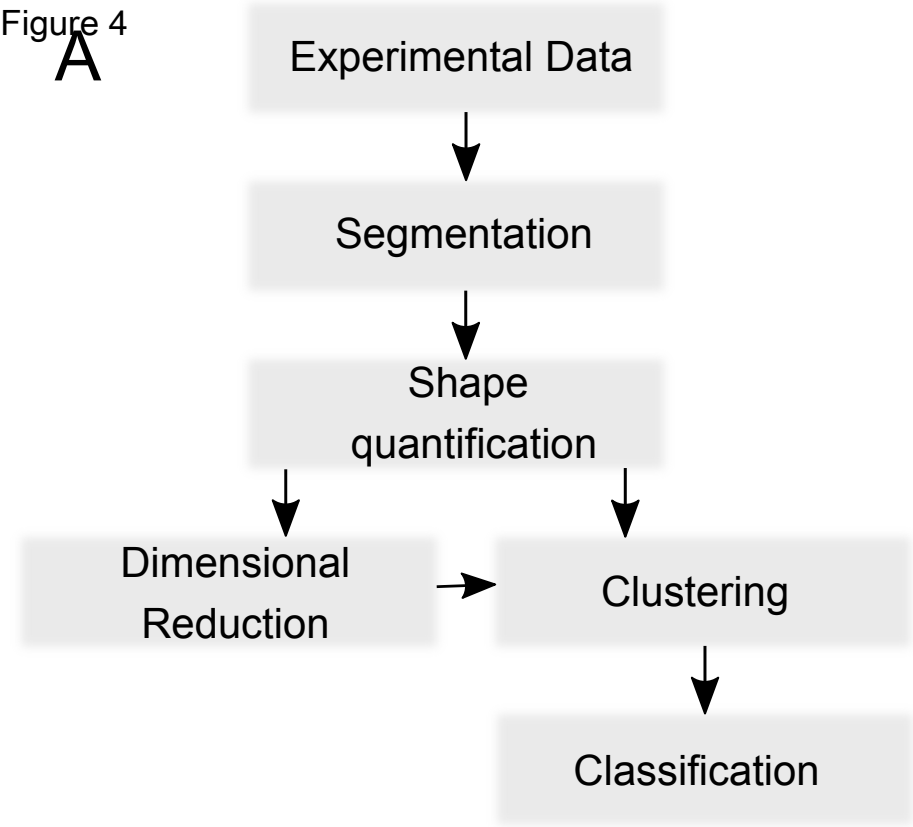
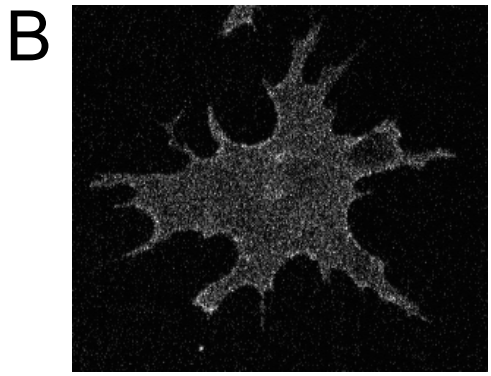
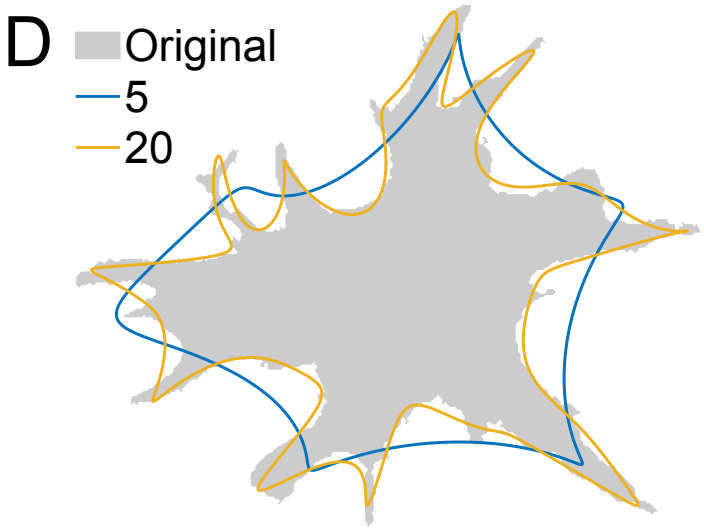

no confinement
0.1-1 µm/min
lamellipodium
strong, contractive (pulling) force
exerted on substrate
actomyosin flow exerts pulling force on
intracellular side of adhesion complex
actomyosin
network
plasma
membrane
adhesion
complex
specific protein-
protein interaction
substrate
proteins
force is transmitted through stable molecular
interactions between extracellular side of
adhesion complexes and substrate proteins
weak, expansive (pushing) forces
exerted on substrate
physical
confinement
1-10 µm/min
bleb
actomyosin flow pulls cell transmembrane
molecules across cell surface
cell-surface
factor
non-specific
interactions
substrate
molecules
force is transmitted through short-lived
unspecific interactions by surface molecules
pushing against substrate molecules

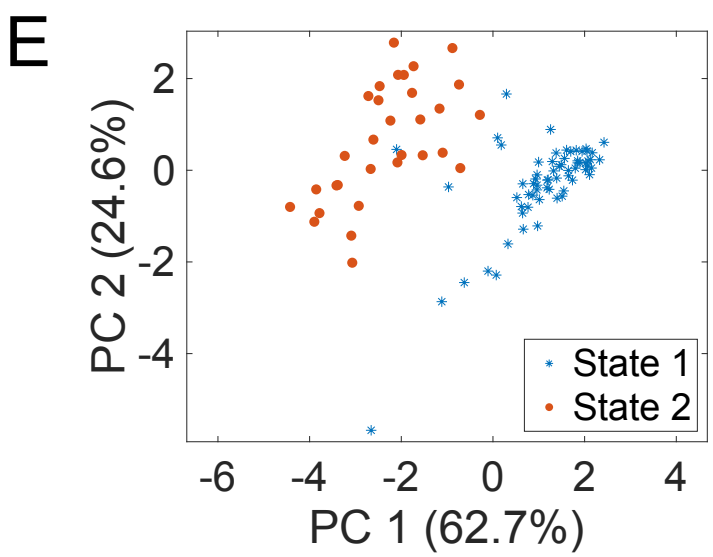
Figure 4


A
Experimental Data
Segmentation
Shape quantification
Dimensional Reduction
Clustering
Classification


B


C


D
Original
5
20


E
PC 2 (24.6%)
PC 1 (62.7%)
State 1
State 2
2
0
-2
-4
-6
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4