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Quantifying modes of 3D cell migration

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Abstract

Although it is widely appreciated that cells migrate in a variety of diverse environments *in vivo*, we are only now beginning to use experimental workflows that yield images with sufficient spatiotemporal resolution to study the molecular processes governing cell migration in 3D environments. Since cell migration is a dynamic process, it is usually studied via microscopy, but 3D movies of 3D processes are difficult to interpret by visual inspection. In this review, we discuss the technologies required to study the diversity of 3D cell migration modes with a focus on the visualization and computational analysis tools needed to study cell migration quantitatively at a level comparable to the analyses performed today on cells crawling on flat substrates.

Keywords

3D cell migration; image visualization; image analysis

Introduction

In vivo cells move in a wide variety of 3D environments, from hard bone to the gaps between muscle fibers and the regions adjacent to wounds. Many studies have now shown that the physical environment is a key regulator of cell structure and function and that cells interact with their environment using a variety of mechanisms. Indeed, otherwise similar cells can move in different environments using completely different migration modes [1–3]. Nonetheless, because of technological challenges, particularly in imaging, most molecularly-detailed cell migration research has been carried out in 2D environments, with cells migrating on flat glass or plastic surfaces.

Cell migration is fundamentally a dynamic process of morphological extension and retraction. Therefore, to directly study the molecular mechanisms underlying 3D cell migration, we need to be able to image cells at the length and time scales of protrusions and retractions. These scales will vary depending on the system, but in general are about one micrometer spatially in all three dimensions and less than ten seconds temporally [4]. Advances in microscopy, particularly the development of high resolution light sheet

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microscopes, are now beginning to allow us to image cells at these spatiotemporal scales in 3D [5–7] (reviewed in [8–11]).

Microscopy alone, however, will not enable widespread access to the study of the molecular underpinnings of 3D cell migration. Methods from the fields of fluorescence probe and biosensor design, 3D culture [12], image databases [13–15], and 3D image visualization and analysis will need to be integrated to complete the experimental workflow. Whereas 2D cell migration was largely studied without automated image analysis, we anticipate that for 3D cell migration automated analysis will be indispensable. Not only do light sheet microscopes simply produce too much data for manual analysis, 3D images displayed on a 2D screen are difficult to interpret. Furthermore, the diversity and complexity of 3D cell migration will likely benefit from quantitative analysis and modeling. In this review, we describe the diversity of 3D cell migration modes and discuss the visualization and image analysis technologies needed to study the regulation of these modes at the subcellular scale. We assess the current state of these technologies as applied to cell biology and speculate about technological progress in the near term.

Diversity in 3D cell migration

On flat surfaces cells typically move by extending lamellipodia, which are thin sheetlike actin-based protrusions at their leading edge, by adhering to the surface via focal adhesions, and by retracting their backs. A working model for generalized lamellipodial migration on 2D surfaces was first proposed by Abercrombie [16] more than three decades ago and has since been extensively studied [17] and modeled [18].

The role of the substrate in 2D lamellipodial migration has often been ignored, perhaps because cells are usually imaged in a 2D plane parallel to the surface and within this plane the physical environment is highly symmetric. This environment is isotropic, spatially and temporally homogeneous, and usually even similar from lab to lab. Even though the role of substrate mechanics has been explored, and surface topography [19], stiffness [20], and adhesivity [21] have been found to modulate lamellipodial migration, most migratory processes have been studied without considering the role of environmental parameters in 2D.

The physical environment, however, greatly affects cell characteristics and behaviors [3, 22]. For example, on hard surfaces melanoma cells adopt a flat and elongated morphology (Figure 1A), whereas in a 3D collagen matrix these same cells tend to adopt a rounded morphology with prominent membrane blebs (Figures 1B and 1C). Blebs are pressure-driven protrusions, in which the membrane detaches and balloons away from the actin cortex [23]. 3D environments used in migration studies are diverse, ranging from relatively simple microfabricated devices and *in vitro* preparations of extracellular matrix proteins to highly complicated *in vivo* tissues. Many of these environments vary even at the subcellular scale, and in some cases are actively altered by the cell. In 3D, migration and mechanics of the environment may thus not be as easily separable as with 2D migration, and Abercrombie-style models for 3D modes of migration will likely need to explicitly consider the role of the environment.

Several characteristics of the physical environment, including rigidity, adhesivity and geometry, have been shown to regulate migration mode [3]. For example, differing levels of confinement are associated with distinct migration modes. A few eukaryotic cell types have been observed to move even without nearby surfaces via swimming [24, 25]. In contrast, on hard and flat surfaces most cells use an adhesion-centric mode of migration. When confined in one dimension between two parallel, planar surfaces to which they are non-adherent, it was found that in 19 out of 20 cell types tested some cells migrate via a “stable bleb” mode that is associated with global cortical flow [2, 26]. Whereas cells on a surface need adhesions to exert forces on their environment, confined cells can move by pushing off the opposing walls [27]. Confining cells in two dimensions by placing them in tunnels yields even more migration modes. In tunnels, some cells move by constructing a thick wall of actin at their fronts [28], and others act as an osmotic engine and move by controlling fluid flow through the tunnel [29]. Finally, cells confined in all three dimensions can sometimes escape by first degrading the walls with matrix metalloproteinases [30].

In more complicated environments, cells have been shown to migrate in different though possibly interrelated ways [31]. In matrigel, cells can move via myosin contractility at the cell rear [32], and in some types of collagen matrices cells move via a nuclear piston [1, 33]. In zebrafish embryos cells migrate via blebbing [34, 35], and *in vivo*, leukocytes migrate by extending actin-based protrusions that do not require focal adhesions [36]. Upon changes in the environment, cells can switch between modes, and this plasticity has often been hypothesized to play a role in cancer cell metastasis [22, 37, 38]. Indeed, mathematical modeling suggests that different motility modes are optimal for different environments [39, 40]. Cells in heterogeneous conditions may dynamically employ multiple modes of migration, which would blur the distinction between migration phenotypes.

Understanding 3D cell migration requires understanding how the migration strategy of a cell changes with the environment. However, it also requires understanding how these modes are enabled by cytoskeletal structure and dynamics and by the spatiotemporal organization of signaling pathways (Figure 1D). Image analysis has played a key role in interrogating the latter aspects in 2D migration [41, 42] and will continue to be important in the study of 3D migration (Table 1). In future, image analysis of the dynamic interaction between cells and their environment will need to be performed.

Visualizing 3D cell migration

The first step in the interpretation and analysis of an image is visualization. Visualization is critical not only for data inspection, but also for troubleshooting experimental workflows, and designing and validating automated analyses. Image visualization, though closely related to microscopy, is not itself a form of imaging and is subject to a different set of possible artifacts and biases. Whereas a 2D image can be straightforwardly visualized by displaying it on a 2D screen, 3D images require adequate projections that permit an accurate and intuitively graspable representation of a 3D image volume on a 2D screen. The complexities of visualizing images of 3D cell migration behavior are potentiated by the necessity to also present dynamic information.

A typical light sheet microscopy image is enormous in size. The example image shown in Figure 2 is 512 million voxels (see the Glossary), whereas a computer screen typically only has about 1 million pixels. The problem of data size is amplified when such image volumes are acquired over time and for multiple channels. The movie associated with Figure 2 is 120 frames in duration, but it is routine to acquire movies of more than 2500 frames with these microscopes [7]. Thus, a single light-sheet movie can easily exceed 1 TByte in size, which is manifold more than the memory of a computer – even a node within a computer cluster – can hold. Visualization then requires specialized hardware and software architectures that dynamically load the movie for display.

Even when specialized architectures are available, how can one interpret the content of an image when only 1/500th of it can be seen at any one time? One could simply scroll through the volume and examine individual z-planes. However extraordinary visual memory would be required to reconstruct the relevant features of the 3D scene [43], as is illustrated by Movie S1. The problem of limited visual memory could be partially alleviated by displaying orthogonal slices through the volume. Figure 2A shows three such slices (XY, XZ, and YZ) through the same retinal pigment epithelial cell shown in Movie S1. But even with orthogonal slices, viewers must still mentally assemble the 3D cellular structure. Alternatively, the impression of a 3D scene can be evoked by creating a rendering of the image. A rendering is a 2D image of a 3D model. Figures 2B and C show two different renderings of this same cell. Many different rendering algorithms have been developed for 3D images, each of which focuses on visualizing a different aspect of the image [44]. (Boxes 1 and 2 describe the two major categories of rendering algorithms.)

To counter image manipulation, many journals have enacted standards for the publication of 2D images. There is no standard or optimal way to display a 3D image volume in a 2D figure, and different visualization algorithms lead to different interpretations of the raw data. This problem is analogous to p-hacking, where multiple statistical tests are performed until a test yields a low p-value [45]. A possible plan to reduce p-hacking is the selection of statistical analyses before the experiment is performed. However this suggestion is likely untenable for cell biology where experimental results may be too unpredictable for prescribed analyses and researchers are unlikely to agree to restrict the ways in which they may look at their data. Indeed, instead of restricting researchers, publication readers could be empowered by providing them with the raw image data and diverse interactive visualization tools all readily accessible from the publication website [46].

The human retina is essentially a 2D screen, yet via a combination of depth cues we are able to construct a 3D model of the observed world [47]. Renderings make direct use of some of these depth cues to create the impression of a 3D scene. The 3D image visualization literature is dominated by the fields of computer graphics, which often aims to use depth cues to produce computer-generated scenes that appear realistic; and by medical imaging, which tends to use depth cues more abstractly to render scenes, such as MRI images, that are not directly observable [44]. Depth cues that can be displayed on a 2D computer screen include occlusion, perspective (relative size of objects and textures, foreshortening, position relative to the horizon), depth of field (range over which the image is in focus), shading (object color or brightness due to relative lighting), and shadowing. Movement allows for

the recovery of more depth information, which is referred to as structure from motion, and most rendering software packages take advantage of this by allowing users to interactively rotate 3D volumes.

Commercial packages for the visualization of 3D cell biology data include Imaris (Bitplane, Inc.) and Amira (FEI, Inc.) as well as tools packaged with some 3D microscopes. ImageJ/Fiji [48], a commonly used open source image processing platform, has basic 3D rendering features. Other open source packages with 3D visualization tools include Vaa3d [49, 50], Icy [51], and BioImageXD [52]. Many more specialized packages have also been developed, such as MorphographX [53], which was designed for the analysis of 3D plant images. Professional illustration and animation software may also become increasingly useful for cell biologists and were recently described in a review of visual analysis for the structural biology community [54]. An emerging alternative to the direct visualization of 3D images is the construction of 3D cell models based on experimental parameters and mathematical rules of cell organizational principles [55]. For example, CellPack provides algorithms to assemble many arbitrarily shaped objects such as proteins into a cell shape specified by the user [56]. However, to construct models from experimental parameters or even to effectively compare models with data, quantitative analysis is required.

Image analysis of 3D cell migration

The need for automatically quantifying cell biological parameters in images is now well appreciated [42]. Automation not only increases efficiency, it also allows for acquisition of more complete data sets and enhances reproducibility across labs. While an automated analysis is not necessarily unbiased, its biases can be far more easily investigated than biases in manual analysis. Quantification enables statistical hypothesis testing, which is the formal assessment of how well something is known. It also drives model development by providing quantitative experimental parameters and by forcing researchers to carefully describe what feature or behavior is under study. Importantly, automated quantification also allows the study of phenomena that would be too subtle or complex to study via direct observation.

Many 2D analyses of cell migration have been undertaken, and 3D cell migration has been analyzed at the cellular scale [57, 58], providing insight into how the environment affects cell migration strategies over hundreds of micrometers. However, analyzing 3D migration at the subcellular scale brings a new set of challenges, even apart from the need for visualization. Not only does the cell's structure and function need to be quantified, but also the structure and dynamics of the local environment and the cell's interactions with that environment. Additionally, most 3D microscopes have worse axial than lateral resolution and consequently have non-isotropic resolution. This complicates image segmentation and any form of geometric analysis of the extracted structures. Even usually straightforward image filters have to be redesigned to account for the directionally variable bandwidth of the signal. Light sheet microscopy can remedy the resolution and sampling issues, but we have yet to establish methods for mitigating the artifacts that arise with this imaging modality. For example, in light-sheet microscopy, streaks downstream of local scattering, refraction, or absorption create uneven, yet nonrandom illumination that for some applications may be difficult to ignore or correct [11].

Despite the challenges associated with 3D analysis, 3D-DIAS, which was probably the first software package for the 3D analysis of cell migration, was launched more than 20 years ago [59–61]. This package was under continuous development for many years, but has mostly been used to characterize amoeboid cells moving on 2D surfaces. More recently, numerous groups have begun to address the challenges of segmenting and mathematically describing 3D cell shapes. The neuroscience community has used various techniques to segment the highly branched morphologies of neurons [62–64]. Others have used spherical harmonics or spherical wavelets to characterize 3D cell shape, but these methods have not yet been applied to biological questions [65–68]. Among the first findings linking molecular processes to 3D cell shape, one study employed cell segmentation and surface curvature analysis in combination with the measurement of local fluorescence intensity to establish roles for the molecular motor myosin II in organizing branching morphogenesis [69]. Few algorithms have been published that use fluorescence images to characterize the 3D structure of the cytoskeleton, though one study recently released software to quantify 3D cytoskeletal networks [70]. Several groups have developed algorithms to describe environmental structure, such as collagen network geometry [71] and pore size [72–75]. Measuring cell traction exerted on 3D environments has remained technologically challenging, but forces have begun to be inferred for cells in matrigel [76], in confinement [77], and within tissues [78]. In general, tools to quantify the morphodynamics of a migrating cell, its interactions with the environment, and the underlying organizational variations in cytoskeleton, adhesion, and signaling, however, remain in a nascent state.

With the advent of light-sheet microscopy and the enormous amounts of data these instruments generate, the pressure for robust computational image analysis tools is steadily increasing. Light-sheet microscopy was first demonstrated by interrogating developmental processes [79, 80]. Hence, 3D automated analysis at the tissue scale has seen much greater progress than at the subcellular scale. Sophisticated nuclear tracking algorithms have been developed for lineage tracing in large developing embryos [81, 82], and nuclear segmentation has been used for cell cycle profiling *in vivo* [83]. Algorithms for 3D membrane segmentation and tracking have also been developed [53, 84, 85], though analysis of large-scale systems is still often performed in 2D [53, 86].

Future work in the 3D analysis of cell migration will require cell biologists to forge connections with previously unrelated subfields of mathematics and computer science. The computer vision field, from which many algorithms have been adapted for the 2D analysis of cell migration, will likely also remain the mainstay for 3D analysis [42]. Finding the cell surface is a preliminary step in many analyses. In 2D the cell surface is usually mathematically represented as a closed loop, but in 3D it is conveniently represented as a triangle mesh (Figure 3A). Algorithms to render, process, and analyze triangle meshes are most often developed by the computer graphics field. Even seemingly simple 3D analyses may map to ongoing research problems in computer graphics. Consider the problem of measuring the polarization of a cortically localized biosensor on the surface of a cell in 3D. Just as we can calculate the mean and variance of a one-dimensional distribution, we can calculate the mean and variance of a fluorescence intensity distribution defined on a cell surface (termed the Fréchet mean and variance [87]). In this case, the mean would describe

the location of the biosensor, and the variance would describe how narrowly it is polarized about that location. Calculating these statistics requires measuring shortest paths along the surface. On a 2D plane the distance between two points is just the length of the straight line that connects them, which we can calculate from the Pythagorean theorem (Figure 3B). The distance between two points along a curve, regardless of whether the curve is in 2D or 3D, is similarly the length of that curve between the two points (Figure 3C). However, the shortest path between two points on a surface with varying curvature is more difficult to calculate, since the local curvature affects path length. While a classic problem first tackled by cartographers in the 17th and 18th centuries, the computationally efficient calculation of shortest paths on meshes remains a subject of research even today (Figures 3D and 3E) [88, 89].

Not only is the computer science of the cell surface more complicated in 3D than in 2D, the mathematics is more complicated as well. The mathematics of curves and surfaces, such as cell shapes, is described by differential geometry [90]. Curvature, a fundamental concept in differential geometry, can be readily understood for 2D analysis. For a curve in 2D, curvature is defined everywhere along the curve as the reciprocal of the radius of the osculating (“tangent”) circle (Figure 3B). In 3D however, each location on a 2D surface is associated with two principal curvatures, which can be combined or considered separately [69]. Figures 3G and H show two different ways of combining them. The need to incorporate methods from new fields for 3D quantification likely extends beyond cell surface analysis and will require cell biology to absorb new bodies of literature and to form new connections with other fields.

Concluding Remarks

Technological advances in microscopy are beginning to allow us to image cells migrating in diverse 3D environments at the subcellular scale. Unlike for 2D migration where there is essentially one environment – flat, hard surfaces of infinite extension – in 3D systems there are many different environments ranging from relatively simple to highly complex. This diversity of environments coupled with the many ways in which cells interact with their environment may be the origin of the multitude of new cell migration modes described over the past few years. As the field becomes more quantitative we anticipate that numerous additional migratory modes and migration regulation mechanisms will be discovered (see Outstanding Questions).

On their own, however, microscopy advances will not be sufficient to make progress in understanding the behavior of migrating cells. While a 2D movie is interpretable by eye, 3D movies are far more difficult to grasp. Rendering algorithms must be used that condense 3D images into 2D snapshots and highlight the information necessary to gain new understanding of a particular process. Combining multiple types of renderings would give researchers better insight into their own data. Along with this need for multi-modal data representation comes the search for new means of communicating 3D cell biology that fully convey the complexity of a data set.

Contemporary 3D microscopes also produce far too much data to manually interpret even with the aid of visualization algorithms. Computer algorithms and mathematics may then no longer serve merely as aides to a researcher's intuition, but as the main vehicle that drives discovery. In many fields of science they already play that role. In structural biology the mathematics required to measure and render molecular forms and dynamics is essential to advances in the field. In 3D cell migration research, these partner disciplines may soon provide verifiable insight into processes that are obviously outside the grasp of a researcher's intuition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Computer Graphics	Subfield of computer science that develops algorithms to display visual scenes
Computer Vision	Subfield of computer science concerned with training computers to interpret images
Image Segmentation	Subdivision of an image into multiple regions, usually to facilitate understanding or further analysis
Isoluminant	Having uniform perceived brightness
Isosurface	3D equivalent of contour lines on a topographical map. More formally, a level set of a continuous function that interpolates a 3D image
Mesh	Spatial network of vertices joined by edges. In computer graphics most meshes are polygon meshes, which are composed of vertices, edges and polygonal faces, with the faces understood to represent the surface of an object
Rendering	2D image of a 3D image-derived-model. In a surface rendering the model is composed of only surfaces, whereas in a volume rendering it is composed of 3D elements
Scene	Objects, including surfaces and volumes, arranged in a 3D configuration
Voxel	A "pixel" in a 3D image. (Voxel combines volume and pixel; pixel combines picture and element.)

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- In 3D environments cells move using diverse modes of migration.
- Advances in microscopy now allow imaging at the spatial and temporal scales relevant to migration.
- Visualization of 2D cell migration movies is straightforward. However, visualization of 3D migration movies requires rendering, i.e. projecting a subset of the 3D information onto a 2D screen in order to evoke the impression of a 3D volume.
- Computational methods for image analysis are required for tackling the size and complexity of 3D image data, but have so far been little used to interrogate 3D migration. Existing algorithms from fields such as computer vision, computer graphics, and statistics, may be adaptable to the study of 3D cell migration.

Text Box 1: Surface Rendering

In a surface rendering, a 3D image is modeled as a collection of surfaces. These surfaces are then displayed by using depth cues to create the illusion of a 3D scene. Surfaces are mathematically represented as meshes (nets) with triangular faces (Figure 3A). Since the graphics in video games and computer-generated movies are principally surface renderings, many such algorithms have been developed and almost all computers have specialized hardware to perform the required computations.

A straightforward approach to generating a 2D surface from a 3D image is to extract an isosurface, which is the 3D equivalent of a contour line on a topographical map with terrain height corresponding to image intensity. The intensity level of an isosurface can be chosen manually or via an automated algorithm. In practice, most microscopy images would yield very noisy and rugged isosurfaces without preprocessing. Figure 2B shows an isosurface that approximates the cell surface of the vimentin-labeled RPE cell shown in Figure 2A. It was generated by applying a median filter to the raw image and then creating an isosurface at an intensity level slightly below the cell's cytoplasmic background fluorescence. Figure 2C shows another isosurface of the same image, created at a higher intensity level that indicates the locations of brighter vimentin fibers and punctae.

Surfaces can be colored to display additional information, such as surface curvature or local fluorescence intensity. However, the apparent brightness of a surface location will vary both with the local coloring cue and with rendering effects such as surface lighting and shading. For example, in Figure 1A the surface of a primary melanoma cell in collagen appears mottled even though it was colored white. This same surface is shown colored by the mean curvature of the boundary in Figure 1B. One way to decouple rendering effects from a surface colored by data is to use an isoluminant colormap as shown in Figure 1C. However such colormaps tend to be less vibrant than other options and perhaps for this reason are infrequently used. (Colormaps adapted from [91, 92].)

Figure I.
Surface Rendering

Text Box 2: Volume Rendering

In a volume rendering, a 3D image is modeled as a volume. Volume renderings tend to be less computationally efficient than surface renderings, but can be used to create realistic displays of a greater variety of scenes. To understand volume rendering, imagine viewing a 3D image through a window. The image can be thought of as a cube of voxels, whereas the window can be pictured as a screen of pixels. A volume rendering is created by first extending from each pixel a ray normally outwards through the cube of voxels. For each ray the intersected voxels are next determined. Finally, a predefined mathematical function uses information about the voxels associated with each ray, such as order of intersection, intensity, and opacity, as well as information about the scene, such as direction and strength of light sources, to calculate the brightness and color of each pixel in the rendering.

The most commonly used volume rendering is the maximum intensity projection, which for each pixel is simply defined as the maximum intensity along the ray associated with the pixel. A maximum intensity projection of the RPE cell shown in Figure 2 is displayed in Figure IIA (scale bar 10 μm), and a rotation of this rendering is shown in Movie S2. Because of the human visual system's ability to discern structure from motion, the vimentin architecture is more apparent from this rotation than from any of the still renderings. More complex volume rendering algorithms model the effects of lighting or allow voxels closer to the pixel screen to occlude pixels that are farther away. A volume rendering in which bright pixels occlude the pixels behind them is shown in Figure IIB. Volume renderings can also be used to highlight particular information in the 3D image, for example in Figure IIC voxel intensities in a specified intensity range appear uniformly dark gray.

Figure II.
Volume Rendering

Outstanding Questions

- **2D vs 3D.** Which aspects of the knowledge we gained from studying 2D migration will carry over to 3D migration?
- **Migration Modes.** How do cells protrude, retract, and exert forces on their environment in 3D, i.e. can we develop Abercrombie-style models for the predominately 3D migration modes? Are there yet undiscovered migration modes? How does the environment drive the selection of modes?
- **Plasticity.** How are the migration modes interrelated? Are there situations where the cells can use multiple modes at once, or do cells usually just use one mode? What sets the frequency of plasticity?
- **Polarity.** How is polarity established in 3D?
- **Environment.** How do cells rearrange their environment in order to more easily migrate through it?
- **Adhesions.** How are adhesions organized in 3D? Under what circumstances do cells form focal adhesions in 3D and how is adhesion maturation and turnover governed? Does the adhesion clutch exist in 3D?
- **Cytoskeleton.** How is the actin cytoskeleton organized in the absence of a flat surface to guide its formation? Do other cytoskeletal networks play a different role in 3D migration than they do in 2D migration?
- **Signaling.** Are the same signaling pathways activated in 3D migration as in 2D migration? How does 3D cell morphology affect signal transduction in space and time?

Figure 1.

Cell morphology is environmentally dependent. (A) Primary human melanoma cell from a mouse xenograft on a hard, flat surface. Actin is labeled with GFP-Tractin and imaged via epi-fluorescence microscopy. (B) A cell from the same tumor as in A embedded in 3D collagen, cytosolically labeled with GFP, and imaged via light-sheet microscopy. (C) A cell from a melanoma cell line (MV3) embedded within 3D collagen, labeled with GFP-tractin, and imaged via light-sheet microscopy. (D) Interacting aspects of 3D cell migration that image analysis may aid in elucidating (see Table 1). All scale bars are 10 μm .

Figure 2.

Surface and volume renderings of 3D images. (A) A 3D image of a vimentin-labeled retinal pigment epithelial cell acquired via light-sheet microscopy (see [7]). Three orthogonal slices with the dashed red lines indicating the locations of the other planes. (Scale bar: 10 μm)

Movie S1 shows the entire z-stack. (B) For the same cell, surface rendering of an isosurface that roughly corresponds to the cell boundary. (C) Surface rendering of an isosurface created at a higher intensity level showing vimentin fibers and punctae.

Figure 3.

Computer science and mathematics of 3D cell morphology. (A) Part of the surface of a primary melanoma cell represented as a triangle mesh. (Scale bar: 1 μm) (B) In 2D the distance between two points (blue dots) is simply calculated as the length of the straight line connecting them. (C) The distance between two points along a curve in either 2D or 3D is the length of the curve that connects the two points. (D) Some approximate shortest paths, originating at the white dot, on the surface of a sphere. The color of the partially transparent surface shows the relative distance from the white dot. (Shortest paths here and in E were calculated using the *Fast Marching Toolbox* for Matlab written by Gabriel Peyré.) On a sphere, shortest paths are arcs of great circles and so in general can be analytically calculated. (E) Shortest paths on the surface of a primary melanoma cell. (F) For a curve in 2D, curvature anywhere along the curve can be simply understood as the reciprocal of the radius of the osculating (“tangent”) circle. Here two red osculating circles with black radii are shown along the gray line. (G) Mean curvature of the boundary for the same surface as in E. (H) Gaussian curvature of the boundary. (All renderings in this figure were performed in Matlab.)

Table 1

Measures for the analysis of 3D cell migration.

Cell Morphology and Motion
- Mathematical representation of the cell surface
- Protrusion/retraction detection and classification
- Spatial and temporal characteristics of protrusions/retractions
- Relationship of protrusions/retractions to overall cell motion
Environment
- Composition and remodeling
- Structure and dynamics of components such as fibers
- Interactions with other cells
- Forces exerted by and on cells
Cytoskeletal Structure and Dynamics
- Composition and structure
- Assembly/disassembly and intracellular movement
- Intracellular forces
Regulation via Signaling
- Spatial localization
- Temporal hierarchy of signals
- Causality of signals

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