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Primer Review

Atomic force microscopy comes of age

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AFM (atomic force microscopy) analysis, both of fixed cells, and live cells in physiological environments, is set to offer a step change in the research of cellular function. With the ability to map cell topography and morphology, provide structural details of surface proteins and their expression patterns and to detect pico-Newton force interactions, AFM represents an exciting addition to the arsenal of the cell biologist. With the explosion of new applications, and the advent of combined instrumentation such as AFM–confocal systems, the biological application of AFM has come of age. The use of AFM in the area of biomedical research has been proposed for some time, and is one where a significant impact could be made. Fixed cell analysis provides qualitative and quantitative subcellular and surface data capable of revealing new biomarkers in medical pathologies. Image height and contrast, surface roughness, fractal, volume and force analysis provide a platform for the multiparameter analysis of cell and protein functions. Here, we review the current status of AFM in the field and discuss the important contribution AFM is poised to make in the understanding of biological systems.

Introduction

AFM (atomic force microscopy) is an exciting analytical tool that was originally developed for the characterization of nanometre-scaled semiconductor devices, but is increasingly used in biological and biophysical research due to its unique analytic capabilities (reviewed in Parot et al., 2007). Since its inception there has been a pioneering decade of intense instrument development followed by improvements in sample preparation (El Kirat et al., 2005), interpretation (Horber and Miles, 2003) and data analysis (Horber, 2002). The ability of AFM to generate image contrast, utilizing its unique contact probe methodology, is becoming an established method in cell biology (for a detailed technology review, see Gerber and Lang, 2006; detailed information has been gathered from a variety of cell types in the following: Parpura et al., 1993; Ohnesorge et al., 1997; Butt et al., 2007). Its potential for high sensitivity, high-throughput operation in fluid, and for force detection are major considerations for its continued integration into mainstream cellular and molecular analyses.

AFM studies, using both fixed and live cells, have revealed novel molecular resolution information about membrane structures, cell organelles and the cytoskeleton (for reviews, see Henderson et al., 1992; Hoh and Hansma, 1992; Lal and John, 1994). Visualization of induced conformational alterations in renal physiology (Henderson and Oberleithner, 2000) and membrane proteins expressed in oocytes (Lal and Yu, 1993) and the demonstration of erythrocyte pore formation induced by the presence of lanthanide cations (Grandbois et al., 2000) highlight the diversity of research possibilities using AFM. Structures including actin filaments (Henderson et al., 1992), the interior structures of synaptic vesicles (Tojima et al., 2000) and subsurface features on cardiac myocytes (Davis et al., 2001) were all imaged early in the development of AFM. Using AFM to characterize force interactions has led to the quantification of, for example, the unbinding events between a cell probe and a second cell, or a ligand/molecular probe and a host receptor. The ever increasing diversity of exciting biological applications using AFM ensures its complete integration in cell biology research (Ikai, 2008).

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Key words: atomic force microscopy (AFM), force interaction, imaging, surface topography, systems biology.
Abbreviations used: AFM, atomic force microscopy; CLSM, confocal laser scanning microscopy; HCA, hierarchical cluster analysis; SCFS, single cell force spectroscopy; SEM, scanning electron microscopy; SMFS, single molecule force spectroscopy.

Hybrid instrumentation

The development of AFM instruments combined with other (light) microscopes and/or other SPM (scanning probe microscopy) instruments represents a future path of instrument development, which will result in an explosion in applications for biomedicine and clinical translational studies. AFM and CLSM (confocal laser scanning microscopy) technologies are particularly complementary, and are more frequently being used to image biological systems (Pfister et al., 2005; Doak et al., 2008). The application of different high-resolution correlative microscopies has, and will continue to, facilitate the accumulation of new insights into the morpho-functional and structural organization of cells and membranes (Haupt et al., 2006).

The mechanisms by which living cells sense mechanical forces and how they respond and adapt to their environment can also be analysed. A critical first step has been to develop a new technology that integrates AFM with TIRF (total internal reflection fluorescence) and FSD (fast-spinning disc) confocal microscopy to investigate cellular behaviour at the subcellular level, thus providing high spatial and temporal resolution (Trache and Lim, 2009). Other necessary considerations for application development include the selection of cantilever tips with spring constants that are appropriate to the cell type under analysis, as well as scanning parameters such as the resonant frequency employed (Murphy et al., 2006). The flexibility of the AFM and its combination with light microscopy techniques, namely DIC (differential interference contrast microscopy) and CLSM, have allowed the organization of adhesion molecules on the surface and their correlation with structures involved in binding to be elucidated (Poole et al., 2004). The labelling of specific molecules on the surface of cells and their correlation with adhesive status is a major future direction for AFM, and technological developments including increases in scanning speed will allow these processes to be monitored in real time in live, dynamic environments (Kassies et al., 2005; De Jong et al., 2006).

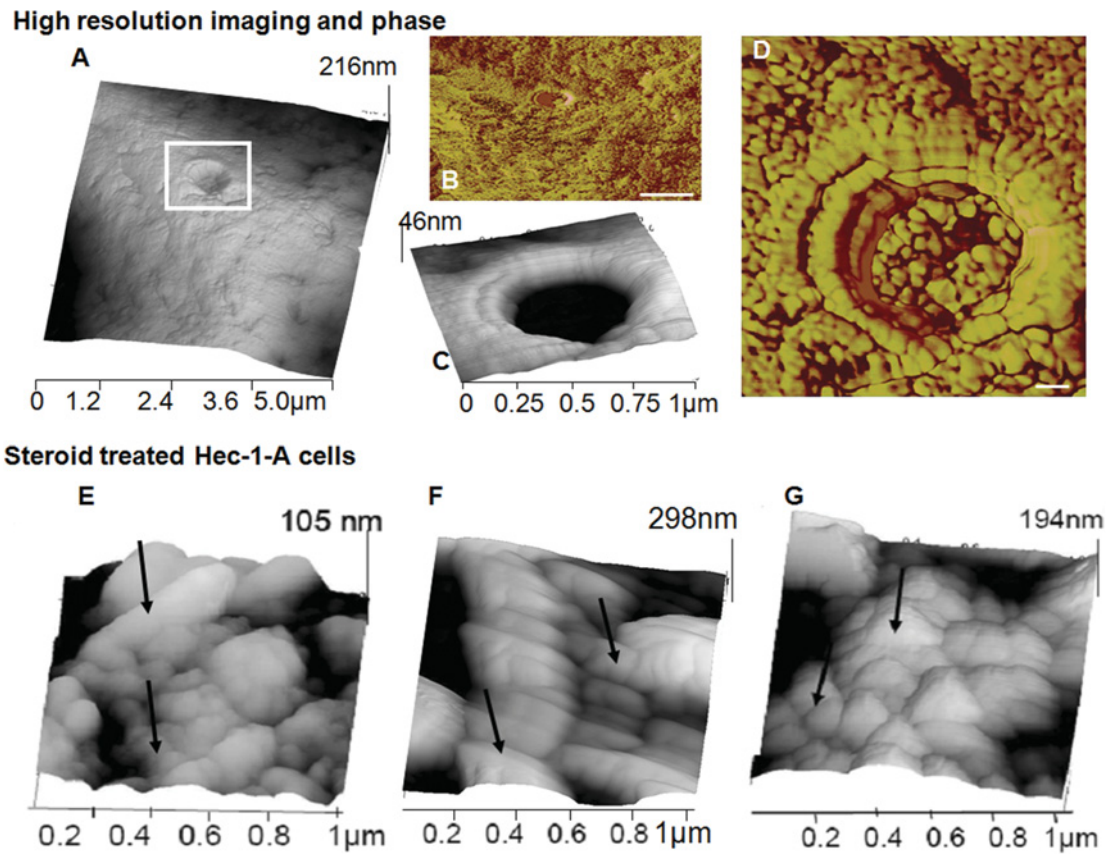
Cell imaging

AFM was quickly recognized as having major potential applications in histology and cytology owing to its ability to visualize materials in non-vacuous environments (air or liquid) without the need for coating, staining or freezing of samples, yet producing images comparable with SEM (scanning electron microscopy) and TEM (tunnelling electron microscopy) (Ushiki et al., 1996; Miklaszewska et al., 2004; Tiribilli et al., 2005; Haupt et al., 2006; Iakovleva et al., 2009). AFM allows a very flexible analytical approach currently providing greater membrane (fixed cell) and intracellular (live cell) detail, thus offering distinct advantages over current techniques such as SEM. AFM techniques can offer novel insights into a diverse range of tissues and cells, as well as biological processes that impact physiological processes resulting in disease. Despite the intrinsic difficulty of molecular scale imaging of soft samples, direct imaging of cells, without the need for sample coating, became a reality in the mid-1990s (Oberleithner et al., 1995). Stable, reproducible, high-resolution images of erythrocytes appeared alongside studies of dynamic actin filaments in glial cells (Zhang et al., 1995; Matzke et al., 2001). Epithelial cells are larger and have greater height profiles than fibroblasts and represent a challenging cell type for AFM imaging. It is critical therefore that the appropriate scanning parameters for imaging tissue types that contain both epithelial and endothelial cell layers are recognized. For example, the use of AFM in toxicological and pharmacological studies of ocular cells, both fixed and live cell imaging, offered their own benefits (Sinniah et al., 2002). No differences in the height and topographical contact mode images of live epithelial cells were observed when collected in air or in liquid. In contrast, images obtained from fixed cells were far more suitable for the investigation of the epithelial cell membrane characteristics. Indeed, the use of tapping mode AFM and marginal fixation to stiffen cells were both required even for the analysis of endothelial cells, when whole cell were observed (Braet et al., 2001a, 2001b).

Currently, the enhanced AFM resolution obtained with fixed cells allows more detailed subcellular analysis. AFM imaging at intermediate resolution can reveal novel structural information as can be seen with the pore-like structure at the epithelial cell surface shown in Figure 1. Such structures can then be further resolved through accurate spatial positioning of the cantilever probe, to permit high-resolution data to be captured. When used in conjunction with phase imaging, high-resolution images of epithelial cell membranes can be obtained (Figures 1A–1D).

Figure 1 | High-resolution AFM imaging applications at the epithelial cell surface

High-resolution AFM imaging together with simultaneous phase imaging revealed the pore-like structures on the surface of fixed epithelial cell. Substructure detail revealed heterogeneous surface structures measuring from <50 nm to >100 nm both within the pore and surrounding it (**A**–**D**, with composite phase images shown in **B** and **D**). Steroid-hormone-treated endometrial epithelial cells (Hec-1-A) showed the effects of oestrogen (**F**) and progesterone (**G**) treatment compared with an untreated control sample (**E**). Nanoscale structures (indicated by black arrows) are characteristic of the topographical transformations induced by treatment. Scale bars, (**B**) 200 nm and (**D**) 100 nm. (**G**) is reproduced with permission from Francis et al. (2009).



Such nanoscale observations can be used to complement existing genetic and protein data, leading to a more detailed understanding of the formation and function of such structures. It is at the nanoscale that events such as protein–protein interactions, enzymatic conversions and the stochastic behaviour of single molecules can be observed. Approx. 30 % of the genes in the human genome code for membrane proteins, and such proteins are notoriously difficult to crystallize. Using phase imaging, AFM offers the opportunity to image such assembled molecular structures *in situ*, as resolutions can reach 50–150 nm, enabling subunit structure to be characterized.

Steroid-hormone-treated endometrial epithelial cells (Hec-1-A) revealed nanoscale changes when compared with untreated controls (Francis et al., 2009). Distinct surface shape and morphological transformation occurred following progesterone treatment (Figure 1G), with circular structures measuring 100–150 nm protruding from the surface, highlighting its convoluted nature. These densely packed molecular-scale features appear structurally distinct from oestrogen-treated cells, where both larger surface features and smaller, rounded, structure measuring between 100–400 and 50–100 nm respectively.

Single-cell AFM analysis has the potential to complement other imaging modalities such as multi-cell SEM in clinical cell biology. Detailed nanoscale analysis of single cells appears likely to permit the detection cellular defects (Figures 1E–1G) which may reflect early stages in the development of a pathology, thus allowing early intervention and treatment (Soon et al., 2007). The potential for AFM analysis of single cell dynamics and architecture will permit new insights into molecular events that result in more gross phenotypes observed at the level of cell and tissue organization (Kusick et al., 2005; Soon et al., 2007).

Successful imaging of live cells is influenced by many variables, including culture conditions, cell morphology, surface topography, scan parameters and choice of cantilever, and still requires a number of obstacles to be overcome before its mainstream adaptation in biological sciences (Trache and Meininger, 2008; Dulebo et al., 2009; Goksu et al., 2009). Faced with these challenges, live cell imaging in physiologically relevant conditions is a driving force behind AFM developments, as it will offer a step change in cell biology due to the fact that no other available method can offer dynamic imaging and force spectroscopy at such high resolution.

Biological surface science

Biological surface science is concerned typically with the characterization of interfaces, at which most reactions in biology occur (Kasemo, 2002). Cells membranes form the biological interfaces where complex networks of receptors, glycoproteins and glycolipids interact with antibodies, lectins, bacterial antigens, hormones and other external/environmental factors. Surface-area geometries at biological interfaces enhance reaction turnover rates, and unique organic micro-environments permit specific affinities and reactions. These interactions are increasingly relevant in disease as their roles become understood (Trosko, 2006; Upham and Stick, 2006).

Through their cell membrane, cells are inherently sensitive to local mesoscale, microscale and nanoscale patterns of chemistry and topography in their surroundings. Gaining more knowledge of the cell surface, and the effect that exogenous agents have on their surrounding micro-interface is crucial. Characterizing a cells reaction to interactions with external bodies and their effect in causing cell surface alterations, for example in malignant and virally transformed cells has a huge clinical relevance (Wardle, 1999).

It is essential that materials used in medical devices have appropriate structural and mechanical properties and do not provoke severe bodily reactions (Wilson et al., 2005). These requirements dictate the use of various surface treatments, creating the correct surface properties for functionality and biocompatibility, which is crucial for tissue regeneration and integration (Wilson et al., 2005). Approaches are required for the fabrication and characterization of such surfaces (Velzenberger et al., 2008). The application of specialized coatings to the surface of medical devices can enhance and modify properties such as adhesion, anti-adhesion, lubricity, hydrophobicity/hydrophilicity and biocompatibility (von Recum and van Kooten, 1995). AFM is currently being used in the characterization of modified and unmodified surfaces for use in regenerative medicine and implantable structures (Schneider et al., 2007). Applications include the investigation of sample topography, morphology and mechanical properties, all of which are essential in understanding, for example, nanostructured polymeric and metallic surfaces used in vascular stent applications (Pareta et al., 2009). Furthermore, measuring a surface's elastic modulus through nanoindentation as well as determining surface roughness is essential in characterizing resistance to enzymatic degradation and other significant factors which may determine the longevity of such implantable devices (Schneider et al., 2007; Jiang et al., 2008).

AFM offers opportunities to undertake cell surface analysis on intact cells (fixed or live) as the cantilever tip has now developed into a multi-functional probe. Tip functionalization by the attachment to cells and molecules through specific linkage mechanisms enables its conversion to a 'laboratory on a tip' that can detect many biophysical and chemical variables (Kienberger et al., 2006a, 2006b; Ebner et al., 2007; Barattin and Voyer, 2008) while scanning membranes. Functional probes convert the AFM tip into a tool for detecting biological, chemical and physical interactions. Single receptor ligand pairs [SMFS (single molecule force spectroscopy)] as well as cell–cell adhesive interactions [SCFS (single cell force spectroscopy)] can now be probed, therefore it is possible to directly correlate structural and functional information using AFM (Muller, 2008).

An alternative approach to intact membrane analysis is the use of supported planar membrane bilayers mounted or absorbed on to surfaces (Lin et al., 2007). The flattened membrane bilayers can then act as natural anchors for membrane proteins, thereby permitting analysis using AFM (Singh and Keller, 1991) and contributing essential understanding of protein/ligand interactions at the cell membrane surface. Dynamic real-time measurements of membranes and model supported lipid bilayers are an exciting new development. For example, amylin formation, a process involved in Type II diabetes mellitus, has been visualized on the surface of planar membrane surface using AFM in both contact and tapping mode to give nanoscale resolution (Cho et al., 2008). Quantitative analysis of mechanisms and factors controlling vesicle rupture, domain shape and size, phase transformations and other model biological interactions have also been made (Goksu et al., 2009).

Molecular imaging

The use of AFM for observing and manipulating structures such as DNA (Guthold et al., 1999, Ueda et al., 1999), collagen molecules/fibrils (Bigi et al., 1997a, 1997b; Guthold et al., 1999) and chromosomes in the 1990s demonstrated that AFM compares favourably with other methods (Ushiki et al., 1994). AFM is currently used in a variety of biological and biomedical research, including investigating DNA, RNA, nucleic acid–protein complexes, chromosomes and ligand–receptor binding. The analysis of single molecules is possible on the membranes of intact cells, stripped cell membranes and in reconstituted systems, and has allowed the forced unfolding of multidomain proteins to be studied (Staple et al., 2008, 2009). For example, experimental set-ups have been used to image as well as extend/unfold single protein and polysaccharide filaments at the *Lactobacillus rhamonosus* cell surface showing the magnitude and timing of intracellular stress propagation, using AFM and integrin associated particle tracking by defocused fluorescence microscopy (Rosenbluth et al., 2006, 2008; Francius et al., 2009).

Living cells are complex systems, and their use in SMFS and SCFS studies often requires strong expertise in the field and technological advances in AFM equipment (Linke and Grutzner, 2008; Alsteens et al., 2009). Accurate data acquisition and its interpretation in such a system depend heavily on the surface chemistry and the cell linkage to the cantilever as well as the quality of the tip itself. These factors can all suffer alterations during experiments and therefore a fundamental understanding is needed before such specialist experiments can be undertaken (Dufrene and Hinterdorfer, 2008).

High-level technical expertise and very specialized equipment are still restricted to a few laboratories. In addition the technique requires the use of recombinant substrates or genetic manipulation to limit the number of receptors expressed at the surface. Coupled with this is the difficulty in recognizing individual target receptor–ligand interactions within a heterogeneous cell surface environment. Further advances in both protein engineering and AFM force spectroscopy are needed to permit the full exploitation of this technique (Linke and Grutzner, 2008). Furthermore, a more detailed understanding of the physiological implications of such *in vitro* experiments is needed in order to understand how mechanosensing and mechano-signalling events may proceed *in vivo*. With these advances in place, pertinent biological and medical questions requiring advanced understanding of ligand–receptor interactions will be answered.

Torsional mode AFM is a new development that permits a more detailed understanding of protein unfolding, and has been used to observe force plateaus characteristic of single repeat unit sequential stretching (Staple et al., 2009). Such experiments on individual flexible macromolecules have placed emphasis on the analysis of elasticity and conformational changes in polymer chains and individual macromolecular chains following environmental or mechanical stresses (Giannotti and Vancso, 2007). Away from membrane protein/complexes, AFM has been used to study subcellular complexes such as DNA protein complexes associated with gene regulation and DNA damage/repair (Davies et al., 2005; Ke et al., 2008; del Arroyo et al., 2009). For example, AFM offers advantages in analysing transcription complexes in that it offers higher resolution than currently achievable with cryo-SEM, and that DNA–protein complexes can be studied overcoming inherent problems with EM (electron microscopy)-associated DNA staining, where contrast cannot easily be achieved in many cases.

Surface roughness

The calculation of the roughness of a cell surface (R_a) provides novel information regarding the effect of external stimulation, developmental stage, origin and type on membrane topography (Girasole et al., 2007; Francis et al., 2009). Ra is calculated for a whole image by averaging the values collected from image component lines and can demonstrate differences between surfaces. The use of Ra as a measurement of cell surface status is likely to reflect the status of the cell surface prior to fixation. Ra measured across a cell surface reflects the natural variation of such a complicated biological interface; measurements over the cytoplasm and nucleus can demonstrate region specific differences which group closely when subject to stringent statistical analysis. Furthermore Ra analysis demonstrates that surface roughness can be used to monitor the response of cells to signalling molecule (see Figure 1).

Force interactions

AFM provides a step-change in approaches to studying the dynamics of force interactions associated with intermolecular mechanisms, where nano-indentation is a powerful tool for measuring nano- and micro-scale mechanical properties such as 'adhesion mapping' of whole cells under physiological conditions with high temporal and force resolution. The small probe size of contact tips allows local mechanical properties in small, thin, and heterogeneous samples to be studied (Ebenstein and Pruitt, 2004).

Cells have the ability to sense mechanical stimuli in their immediate environments through mechanosensitive ion channels, integrin receptors and tyrosine kinases (Banes et al., 1995). The use of the nano-indentation allows mechanical stress to be simultaneously applied and monitored, permitting derivation of specific information regarding elasticity and mechanical properties of the cell. The retraction of the nano-indentor also allows the determination of the adhesive nature of the surface allowing insights into cytoskeletal reorganization (Kuznetsov et al., 1997; Jaasma et al., 2006; Butt et al., 2007). Chemically induced changes in cellular protein expression levels and their effect at the cell surface as well as cellular morphology and polarity changes can also be monitored using this technique (Rotsch and Radmacher, 2000). Dynamic force spectroscopy is a valuable technique to explore the energy landscape of molecular interactions (Garcia-Manyes et al., 2005). Nano-indentation, SCFS and SMFS provide a broad range of possibilities, which when coupled with synthetic chemistry and light microscopy represent a future direction for cell surface structure/function analysis (Puech et al., 2006; Parot et al., 2007; Muller, 2008). SCFS and SMFS techniques have both enhanced the scope of force spectroscopy significantly, and have coincided with AFMs emergence as a powerful tool to investigate the forces and motions associated with biological molecules and enzymatic activity (Neuman and Nagy, 2008). Excitingly SCFS is likely to allow the effects of glycosylation of cell surface proteins on cell–cell adhesion and inter-cellular mechanical properties to be elucidated (Thie et al., 1998). AFM now sits alongside optical tweezers and magnetic tweezers as a force spectroscopy technique.

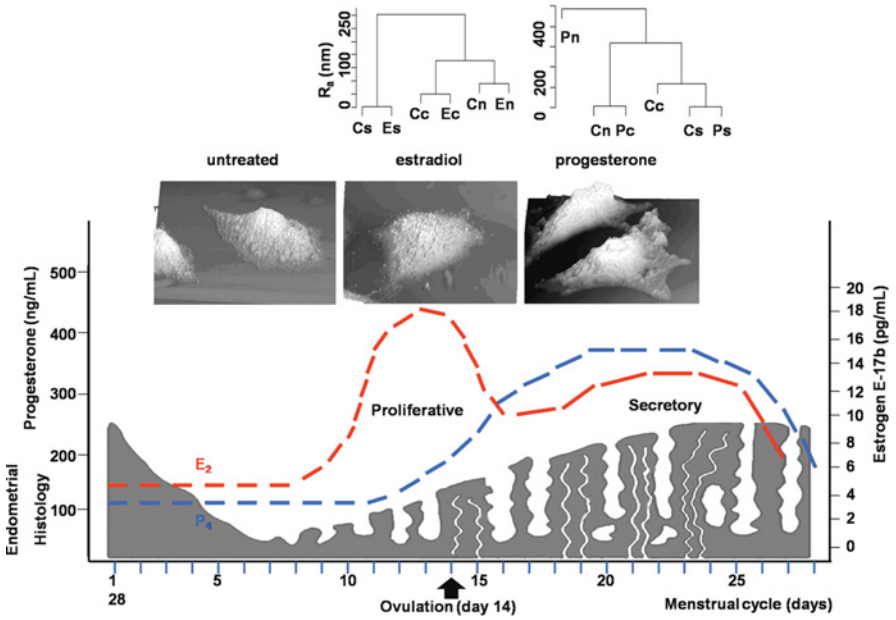
Modelling and data analysis

A current hurdle in the evaluation of cellular morphology is the lack of simple visualization strategies that offer comparative, intuitive, analysis and that operate within open-source environments of image, surface, and volume applications. Of the limited examples available, plug-ins for ImageJ platform exist that allow for the import of proprietary image, volume and surface data into a single application (Rajwa et al., 2004). It is essential therefore that common open-source platforms are developed in order to handle the combined datasets that will be produced by hybrid instrumentation, for example AFM–CLSM systems.

Surface roughness (R_a) analysis provides a method of assessing the surface composition for example the distribution of surface and transmembrane proteins. The relationship between group data distributions can be visualized using HCA (hierarchical cluster analysis) where the maximum, minimum and quartiles are calculated for each group to yield data points that summarize the frequency distribution. Given these data points, the Euclidean distance can then be calculated between all pairs of groups. The resulting distance matrix then serves as input to a dendrogram building algorithm (complete linkage) using the R statistical computing environment. Fractal dimension is another efficient criterion to characterize morphological complexity of objects and cells. The

Figure 2 | *In vitro* model of the human menstrual cycle

The endometrium is driven by the 28-day menstrual cycle to produce a primed receptive surface for embryo implantation. Oestrogen (E2, red) and progesterone (P4, blue) are important factors in driving the morphological changes in cells of the endometrium (stromal and epithelial). Endometrial epithelial cells (Hec-1-A) were grown in the presence of oestradiol (10^{-7} M) and progesterone (10^{-6} M) for 48 h, fixed and processed for imaging (see Francis et al., 2009). AFM was used to obtain images of epithelial cells capturing morphological, topographical and surface changes resulting from exogenous stimulation. HCA (top row) of surface roughness (R_a) data, captured at the time of cell imaging, revealed significant quantitative differences between the cell surfaces in response to progesterone treatment over both cytoplasmic and nuclear areas (Pc v Cc and Pn v Cn). C, control; E, oestradiol; P, progesterone. R_a data collected above s, substrate; c, cytoplasm; n, raised nuclear area.

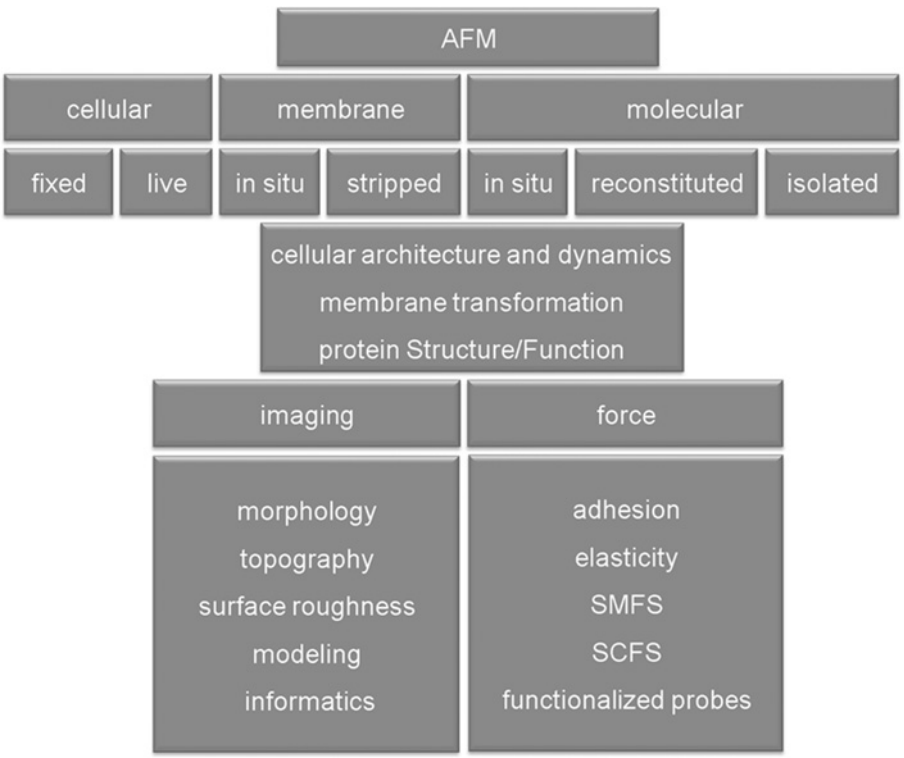


modelling of AFM height data by assigning fractal dimension and other fractal characteristics has huge potential for the correlation of morphological characteristics with stage, origin and maturation of single and multiple cells originating from different pathological samples (Cross, 1994; Smith et al., 1996).The self similar features seen in cells are sufficient for analytical description by fractal analysis (Nonnenmacher et al., 1994). Fractal analysis has proved an effective quantitative descriptor of real cytoplasm morphology in feline oocytes and neonatal cardiac fibroblasts, without introducing any bias or shape approximation (Fuseler et al., 2007). The application of image analysis and non-Euclidian geometrical fractal analysis, along with box counting dimension, the Richardson, Minkowski and Sholl dimension have all been used for individual cells to date and now further development is needed. These factors indicate its reliable application as an objective cellular classification method highlighting its potential for providing insight into the regulation of cell behaviour and gene expression during development and disease.

Force manipulation involves the precise placement of the AFM tip in distinct cellular regions. With a lateral resolution of 1–5 nm and a vertical resolution of 0.1–0.5 nm, it is possible to probe the micro- and nano-mechanical structures on the cell surface with forces in the pico-Newton range. This allows the spatial distribution mapping of cell structures and their mechanical properties in an integrated whole cell system (Hansma, 2001; Kuznetsova et al., 2007). A force curve is the product of instrument loading force relative to the sample indentation for each incremental step in the movement of the cantilever towards and away from the sample. Cantilever spring constant, deflection-sensitivity and the defining of

Figure 3 | AFM modalities and its contribution to life sciences and clinical research

AFM imaging and force modalities are currently being used in many aspects of biosciences research with applications in the study of cellular architecture and dynamics, membrane transformation and protein structure/function; AFM has a broad experimental application at the cellular, membrane and molecular levels.



the zero force enable the conversion of the cantilever deflection at each increment into force as the basis for the estimation of Young's modulus and surface adhesion. Approach and retraction curve manipulation can be related to cell micromechanical properties and adhesion respectively. The Hertzian or Sneddon model variations are theoretical framework models for calculating Young's modulus (Kuznetsova et al., 2007). The interactions involving macromolecules and other cell surface factors provide the force curve with a characteristic adhesive interaction/retraction shape (Lehenkari and Horton, 1999; Simon and Durrieu, 2006; Wu et al., 2009).

Biomedical applications

The powerful application of AFM, and its different modalities, in biomedical research has been outlined above. There is also a huge potential for AFM to be used in clinical diagnostics, where the use of AFM, in combination with more conventional analytical approaches, could inform decisions related to recommendations for treatments. Data such as surface roughness (Girasole et al., 2007) could indeed be used as a novel cell surface biomarker, which when coupled with conventional biomarker analysis (histology, cytology, immune histo- or cyto-chemistry, fluorescence *in situ* hybridization, etc.) could better inform clinical decisions. We have recently reported on the analysis of cells derived from the endometrium, the lining of the uterus that is the site of embryo attachment (Francis et al., 2009). *In vitro* treatment with physiologically relevant concentrations of oestrogen and progesterone is an accepted model for studying cell development and response through the menstrual cycle (Horne et al., 2006). Figure 2 shows an enhanced data set (Francis et al., 2009) demonstrating the use of AFM in studying the response of endometrial epithelial cells to exogenous steroid-hormone exposure, and demonstrates how responses at both the cell and membrane levels can

be revealed using AFM through qualitative (image) and quantitative (surface roughness) analyses of fixed cells. Further developments have seen this approach being able to differentiate between endometrial cells originating from patients with endometriosis when compared with control samples (L.W. Francis, D. Gonzalez, C. J. Wright, L. A. Joels, J. O. White and R. S. Conlan, unpublished data).

Concluding remarks

The ability of AFM to simultaneously detect morphological, topographical, roughness and micromechanical induced changes demonstrates its utility for capturing multiple parameter measurements in a single analysis (Figure 3). AFM can be readily applied to monitoring the action of signalling molecules. For example, the cellular response to treatment with the steroid hormones oestradiol and progesterone is shown in Figures 1 (high resolution) and 2 (low resolution). Furthermore, with the 'magnification' range available with AFM, it offers exciting opportunities for consecutively imaging, in true three dimensions, of biological surfaces at macro, micro and molecular resolution.

A major current direction in biological research, systems biology, aims to characterize the integrated function of complex, multicomponent biological systems, to understand physiological and pathophysiological processes. Integration of data from cell, tissue and whole-organism physiological experiments together with molecular data derived from 'omics' approaches is set to supply models of such complex systems through defining inter-relationships using high-level computational multiparameter modelling (Rose and Oakley, 2007). Outcomes from integrated data modelling inform rationalization of the experimental approach to test predicted outcomes. The contribution of AFM to such systems-based approaches will provide valuable additional qualitative and quantitative parameters to feed conceptual frameworks.

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