Chapter 7. Novel Surface Visualisation Solutions
7.1. Overview

In the previous chapters (Chapters 4-6), the main focus of the work was to develop and present 3D models that represented the different types of nano-structured surfaces, together with a visual interpretation of their initial interaction with different bacterial cell species. In this work, greater effort was devoted to showing and recreating the dynamic motions and interactions between the objects rather than emphasising the specific details and attributes associated with the surface models, such as the differences between the topographical roughness of the different surfaces.

When considering *surface modification*, the surfaces of the substrata were altered using different procedures, and these resulted in providing a diverse range of surface topographies. Therefore, it is important to have the ability to identify the differences that exist between the modified and non-modified substrate surfaces. In the case of titanium surfaces, a comparison between data sets in a graphical form is often required in order to distinguish the extent of alteration that has taken place on the surface. The extent of these differences is required to enable the multiple 3D models describing the different titanium surface topographies to be displayed.

When it comes to the 3D visualisation of nano-structured surfaces, Maya provides additional means to present the surface topography using various techniques. The powerful shading/texturing techniques, along with the tools that are used mainly for the creation of animation effects in movies and games, can be useful for the presentation of surface topographical data. The advantage of these tools is that they are already predefined and ready-to use, and no additional adjustments needs to made (e.g. developing programing code), in order to achieve the final result.

In addition, however, two techniques were developed to enable us to highlight the differences in the surfaces roughness characteristics displayed by the as-received (AR) and ECAP modified titanium surfaces. The techniques included *cross-sectional profiling* and *surface transition*. The Maya software was able to directly perform both of these techniques. A novel visualisation technique was also developed for the production of the three-dimensional surface models using displacement maps obtained from two-dimensional data that was available.
7.2 Data collection

The surface preparation and AFM scan described in this section were performed by Vy Pham, Dr Khanh Truong and other members of the Nano-biotech group at Swinburne University of Technology.

AFM scans were conducted using an Innova scanning probe microscope (Veeco/Bruker, U.S.A.). Scans were performed in tapping mode at ambient temperature and pressure, using silicon cantilevers (MPP-31120-10, Veeco/Bruker, U.S.A.) with a spring constant of 0.9 N m\(^{-1}\) and a resonance frequency of approximately 20 kHz. Scanning was performed perpendicular to the axis of the cantilever at a scan speed of 1 Hz over a 10 µm \(\times\) 10 µm scanning area.

7.3. Reconstruction of titanium surface via cross section profiling

The ability to distinguish variations in surface roughness between samples is challenging when using 2D scans. Colour maps of the surface can be used to provide some information regarding the differences in the magnitude of the surface roughness, however 2D images are not able to provide as good a description as would be obtained from, for example, 3D images of the same surfaces. These images still might not provide sufficient insight into differences in surface roughness. One solution to this problem is developing the ability to show different sections of the surface model in a sequence. This will provide opportunities for more detailed inspections of the surface topography. To achieve this, a technique was developed in Maya to allow a section-by-section inspection of the surface.

AFM scans of the various surfaces were imported into Maya via a python script and then visualised as a polygonal geometry. The surfaces were positioned and scaled on the Y-axis at 37 \(\times\) 10\(^8\) Maya units, in order to highlight the surface topographical features. This technique principally relies on the shading process of the surface colour and transparency attributes, rather than transforming the 3D model itself. The colour attribute allows a colour map to be applied, while adjusting the extent of the transparency can allow the visibility of the parts of the surface to be controlled. By
sequentially showing the 250 sections of a surface (4 nm per section) (Fig. 7.1), the variation in the surface peak density and position can be obtained.

Figure 7.1. Surface sections being sequentially displayed. During the animation process, each section (4 nm per section) appears every 5 frames until the whole surface is presented.

A “lambert” material was then applied to the surface. Such ‘materials’ can be used as a base shading nodes in Maya, allowing the addition of effects and textures to a surface. It is a node that consists of different attributes (colour, transparency, ambient colour, etc.), and allows for 3D models to be visible in the scene and the final render. A ramp texture (representing a “terrain” map) was connected to the lambert material’s colour attribute, thus colouring the surface. An additional ramp texture (with only two colour layers: black and white) was assigned using the transparency attribute (Fig. 7.2.). The black colour represents the totally visibility of the surface, while the white represents the opposite, making the object totally invisible. Therefore, animating the position of the colours will allow for a surface to appear section by section over time.
As previously mentioned (Chapter 3.), the texturing of the surfaces depends of the layout of the UV. In order for the two ramp textures to work properly, two UV sets were created. For the colour map texture, the first UV set was created using the planar mapping option for the X-axis. The second (the transparency) uses planar mapping in the Y-axis.

Figure 7.2. Graphical representation of the connections of the attributes. Two 2D texture nodes (ramp) were created (left), one for the colour map (five layers of colours representing the terrain map) and the other for the visibility of the surface sections (two colour layers, black and white). The out colour attribute of the ramp representing the colour map was connected to the lambert colour attribute (right). The out colour of the second ramp, representing the “visibility” of the surface sections, was connected to the transparency attribute of the lambert material.

The same technique was applied and the animation of transparency was developed with the same time length and speed for each of the four surfaces (Fig. 7.3). Additional animations were also created, such as camera motion. The movies were then rendered
using the mental-ray plugin in TARGA format. Each surface was rendered as separate sequence of images. The sequences were reassembled, using Adobe Premier, as a single movie. Additional information was then added, such as the colour maps and colour maps values. The movie was finally exported in MPEG4 format.
Figure 7.3. Screenshots of the Video 7.1. Cross-sectional profiling of as received (AR) and ECAP titanium in grade 2 (G2) and grade 4 (G4) (a). Whole 3D models after the profiling (b).
7.4. Transition of titanium surface roughness: received titanium into ECAP-modified

Another way to compare the surface topography of two or more surface models is to add surfaces next to each other and render the sequence, however this process can be distracting, as the simultaneous presentation requires the viewer to watch both sequences simultaneously. A new approach was therefore developed to show the roughness characteristics of the surfaces as a single 3D object using the same data files from the Cross-section profiling.

This technique was achieved by combining the two pre-visualised polygonal objects as one with Maya’s “Blend shape” tool. With selecting 2 surfaces, it transfers the attributes (in this case the AFM data values) from the first selected surface to the second surface, while still preserving the main characteristics of the second surface. Thus, the second surface contains both topographical values, represented with 0 and 1. These numbers were animated, resulting with surface transition from the AR to ECAP titanium samples. When this transition took place, the vertices were positioned according the height value of the first AFM data (0), and then translates until they reach the position values that correspond to the second AFM data (1). The surface colour map changes accordingly.

Additional animations were also developed, such as the rotation of the surface. These animations were rendered using the same procedures as described for the Cross-section surfaces described in Section 7.3.
7.5. 3D construction of nano-structured surfaces using a single SEM image

As mentioned in Section 2.2.3, AFM is the most commonly used technique to measure the topography of a surface to determine accurate depth dimensions. This data can be employed to construct 3D surface models of the surface. The ability to present data in a 3D format has great benefits, in that a greater understanding of the surface structure is provided in a format that allows effective communication to a wide audience. A drawback of the technique is that in cases where the surface topography is extremely complex, the resulting data may not be accurate.

SEM is another commonly used technique for surface imaging. SEM can be used to sample much larger fields of view, with high resolution, than AFM and is generally able to be performed over shorter times. The main drawback of SEM is that unlike AFM, traditional SEM does not measure height values in the depth dimension, and so cannot be easily utilized for the 3D visualisation of samples. However, there are reports that have utilised SEM scans for 3D surface construction based on computations based on matching points on two separate images (Samak et al., 2007).

Here, a novel technique for the effective three-dimensional reconstruction of the nanoarchitecture of a surface, based on two-dimensional electron micrographs, is presented. This technique combines the rapid data collection capability of SEM with the accurate three-dimensional measurement ability of the AFM. As a result, large areas of material surfaces can be rapidly analysed and subsequently presented as high-resolution 3D models using a displacement map application.

Displacement maps are commonly used for the application of high-resolution information using low-resolution models (Szirmay-Kalos and Umenhoffer, 2008, Lu et al., 2009, Jang and Han, 2012). These maps are usually based on a grey scale (alpha) channels, with values ranging from minimum (black) to maximum (white) height. In practise, they allow the transformation or deformation of 3D objects according to information collected from a grey-scale 2D image.

Figure 7.4 shows an example where the displacement of a flat surface (Fig. 7.4a) is controlled by a 2D texture (Fig. 7.4b) with values ranging from black=0 to white =1 (arbitrary units). In this case, if the alpha value of a given point is greater than 0, the
point is then translated perpendicular to the 2D image plane. When each point has been translated relative to each other in this manner, a proportionally accurate 3D surface structure is produced (Fig. 7.4c.). In most cases however, displacement maps are only produced for aesthetic purposes; no absolute height values can be generated (Dmitriev and Makarov, 2011). Given that most SEM systems produce grey scale images by default, electron micrographs are a prime candidate for the production of displacement maps, and when coupled with an appropriate height calibration procedure, 3D models can be produced for a surface that contain relatively accurate height values.

Figure 7.4. Application of displacement maps. Each polygonal object (e.g. a plane) consists of three-dimensional points (vertices) that are connected with lines (edges) (a). Each pixel is assigned a relative height value based on their grey-scale values; black is assigned the lowest value of 0 and white the highest value of 1. (b). During the displacement map geometry conversion each vertex is translated in 3D space according to its assigned height value, thus transforming the original, planar 2D image into a new 3D geometry (c).
7.5.1 Data collection

The surface preparation and AFM scanning described in this section were performed by Vy Pham, Dr Hayden Webb and members on Nano-biotech group at Swinburne University of Technology.

**Scanning electron microscopy (SEM)**

Micrographs of four substrate sample types, including thin titanium films, silicon wafers, polystyrene cell culture dishes and dragonfly wings were recorded using a FESEM (ZEISS SUPRA 40VP) at 3 kV at 70000× magnification. Additionally, titanium films were imaged at 30000×, 90000× and 150000× magnification. All samples were first viewed at lower magnification in order to locate clean and undamaged regions before collecting images at higher magnification.

**AFM surface calibration**

All AFM scans were conducted using an Innova scanning probe microscope (Veeco, U.S.A.). Scans were performed in tapping mode at ambient temperature and pressure, using silicon cantilevers (MPP-31120-10, Veeco, U.S.A.) with a spring constant of 0.9 N m\(^{-1}\) and a resonance frequency of approximately 20 kHz. Scanning was performed perpendicular to the axis of the cantilever at a scan speed of 1 Hz. Initially, 10 µm × 10 µm fields of view were scanned in order to identify suitable analysis regions of the surface, prior to analysis at higher resolution. Scan areas were selected to closely match with the resolution of electron micrographs, as the distance between individual sampling points can affect the calculated roughness parameters (Brune et al., 1997, Crawford et al., 2012).
7.5.2 Three-dimensional visualisation of SEM images

7.5.2.1 Construction of displacement maps

Displacement maps were constructed according to the following procedure:

- A polygonal plane was created as a reference point for the creation of 3D images from the electron micrograph. By default, the plane was a two-dimensional object.
- The resolution and dimension attributes of the plane were set according to the corresponding SEM image being used for modelling. The co-planar dimensions (i.e. height and width) were set to correspond with those of the actual areas being analysed.
- A planar UV mapping option was applied on an axis that was perpendicular to the surface.
- Data representing the depth dimension was extracted from the alpha values of each pixel in the SEM images.
- Python scripting was again utilized for the development of a simple script in order to obtain the height values at each point of the 3D surface topography. The script recorded the alpha values of each pixel as a relative translation attribute value for each vertex. The data was then stored in an empty comma-separated values file (.csv).

The csv file contacting the height values of each point was used for the calibration process. Roughness data calculated from AFM scans were used to calibrate the depth dimension scale of the displacement maps. The average value of the .csv files were set to match the average roughness ($R_\text{a}$) determined for each of the corresponding sample surfaces, and all other values were scaled proportionate to this value.

7.5.2.2 Conversion of displacement maps into 3D polygonal geometry

The process for generating the three-dimensional models from the base two-dimensional images was applied to scanning electron micrographs of titanium thin films. The results are presented in Figure 7.5.
Figure 7.5. Three-dimensional representation of a 150 nm-thick thin titanium film surface. A section of an original scanning electron micrograph, 512 × 512 pixels in size was selected for conversion into a 3D object (a). The image was assigned to a 2D polygonal plane as a displacement map, and a terrain colour map was applied (b) before depth translation according to the pixel height values. Note that the colour map values presented in (b) has been calibrated through the use of AFM roughness data. The 3D representation of the surface (c).
A lambert shading material was assigned to the polygonal plane. A new 2D file texture node was created and connected to the lambert as a displacement map attribute. The file node allows the importation of an image into Maya. A filter type can be chosen for the image, which will affect the quality of the image. However, it will also have an effect on the 3D geometry shape when the image is converted into a 3D object. For the SEM image the Gaussian filter was selected. The filter will give better image quality, resulting in a smooth 3D model (Fig. 7.6). It did not affect the main features of the object.

![Figure 7.6. Example of image filtering. A resulting 3D model without filter (left), and with Gaussian filter (right).](image)

There are two options in Maya for conversion of an image into a polygonal geometry. One is ‘convert - displacement to polygons’ and the other is ‘convert-displacement to polygons with history’. The first option converts the image, however, it is not the best choice. If the resolution was set to match the SEM image, this conversion will add extra subdivisions and which will be excessively high for Maya to compute the conversion process. The second option performs the same task, but only deforms the already defined subdivisions of the plane, without adding additional geometry. The conversion process also triangulates the subdivisions.
7.5.3 Visualisation of titanium surfaces

Three-dimensional displacement maps were successfully applied to scanning electron micrographs of the surface of 150 nm-thick sputter-coated titanium films, at 30000×, 70000×, 90000× and 150000× magnification (Fig. 7.7). For each micrograph, a subsection was chosen that measured 512 × 512 pixels (these values are arbitrary) and was subsequently imaged via the displacement map technique. The height scale was calibrated using AFM scan data. Comparative surfaces generated from AFM scans are presented in the right-hand column of Figure 7.7. The surface features visible in the SEM displacement maps appear somewhat irregular, especially in comparison to those present in the 3D AFM surfaces. This difference can be attributed to one of the drawbacks associated with AFM as an imaging technique. The size and shape of the features on a sample imaged by AFM depend not only on the geometry of the features themselves but also that of the AFM tip that is used for the analysis. This effect is known as ‘tip convolution’, and while it can be mitigated to some degree through the use of sharper tips and deconvolution algorithms, it cannot easily be eliminated (Tabet and Urban III, 1996, Shiramine et al., 2007). This is the advantage of SEM over AFM; by avoiding any physical interaction with the sample, a more accurate representation of the surface geometry can be obtained.

In the figures presented here, the height scale of the displacement maps was calibrated according to the AFM data. AFM analyses were performed on each of the samples visualised, and the average roughness \( R_a \) was calculated. Average roughness is defined as the average deviation of the height values from the mean height (Stout et al., 1993, Brune et al., 1997, Webb et al., 2012). Each pixel within the displacement maps was assigned a proportional value between 0 and 1, therefore the average deviation from the mean of the pixel values was calculated, and the height values for each pixel were scaled so that the average deviation matched the \( R_a \) of the sample.
Figure 7.7. Reconstruction of surfaces using input micrographs with various magnifications. Three-dimensional surfaces have been generated based on electron micrographs of 150 nm-thick titanium thin films at 30000× (a), 70000× (b), 90000× (c) and 150000× (d) magnifications. Colour scales have been calibrated via AFM roughness data. Three-dimensional surfaces generated based on AFM scans of corresponding surface areas are presented in the right column. The surface features in the AFM scans appear to be more regular than in SEM 3D displacement maps, due to the effects of tip convolution on AFM data.
It should be noted that the calculated $R_a$ of a surface is dependent on the sampling interval, i.e. the resolution of the AFM scan, therefore scan areas were chosen in order to match the resolution of the AFM scans to that of the electron micrographs. However, the AFM and SEM scans were not performed on the exact same scanning areas of the surface, which is another factor that influence the calibration of the height. Calibration of the height scales in this manner requires only a few time-consuming AFM scans, and the application of average roughness data can be applied to many SEM displacement maps. AFM is by no means the only technique available that can be used to calibrate the height scales; stylus and optical profilometers, for example, can also be used to record topographical data.

7.5.4. Other materials and sample types

In addition to the titanium surfaces, three different substrata surfaces were used to produce displacement maps in order to assess the versatility of the technique. The three surfaces chosen were specifically selected for their highly diverse chemical compositions and surface topographies. The surfaces included dragonfly wings, unmodified silicon wafers, and polystyrene (PS) plastic excised from standard cell culture Petri plates (Fig. 7.8). Dragonfly wings are known to have relatively large surface features (Ivanova et al., 2013, Nguyen et al., 2013), while polystyrene and silicon wafers are known to be quite smooth (Decuzzi and Ferrari, 2010, Gentile et al., 2010, Zeiger et al., 2013), and all three samples are less conductive than the previously utilized metallic titanium. In the case of dragonfly wings, the SEM displacement maps provide a clear advantage over AFM scans (Fig. 7.8a). The large feature size, and the inherent ‘stickiness’ of the epicuticular lipids that are present on the surface of the wing make it quite difficult to produce accurate AFM scans that are free from artefacts. The SEM displacement map presents a clearly defined structure.
Figure 7.8. Visualisation of material surfaces with different compositions. Three-dimensional surfaces of dragonfly (*Hemianax papuensis*) wings (a), polystyrene Petri plates (b) and silicon wafers (c) can be reconstructed from electron micrographs. Magnification in all micrographs is 70000×, and colour scales have been calibrated using AFM roughness data.

Little correlation can be drawn between the SEM displacement map and AFM scan of the PS surface. The well-defined topography on the PS surface (post gold coating) that was detected via AFM analysis was not represented well in the SEM displacement map (Fig. 7.8b). This is largely due to the highly-insulating nature of the PS surface. Build-up of electrons on the surface of the sample decreases the resolution and depth of analysis, and homogenizes the resulting micrographs (Joy and Joy, 1996). In contrast, the SEM displacement map and AFM scan of the silicon surface are also
relatively comparable; both techniques produce a smooth, feature-less surface (Fig. 7.8c).

Three-dimensional animated movie was produced to aid in comparison between the models visualised via the displacement map technique. The animation was rendered with the mental-ray plug-in within Maya, and exported as sequential images in TARGA format. The rendered images were imported into Adobe Premier for post-production, where additional information was added, such as the average roughness values \( R_a \) and the magnification values. The movie was then exported in MPEG file format.

7.5. Summary

Both techniques (Cross-section and Surface transition) were developed for the same purpose, to display and show the difference in the topography of a modified and non-modified sample. The Cross-sectional profiling presents (DVD-Video 7.1) a local comparison of surfaces roughness characteristics showing section by section of the surface model. On the other hand, the Surface transition presents a global comparison of surfaces roughness characteristics (DVD-Video 7.2). This method shows combined data sets as a whole 3D surface object, rather then a section by section. This transformation between the 3D models gives a visual perspective the relative roughness of the two surfaces, allowing for easy perception of differences in surfaces topography with the changes of the colour map providing additional qualitative insight.

The third (displacement map) technique widens the range of analytical applications that can be applied for the visualisation and assessment of surface topography. This technique is particularly applicable to scanning electron micrographs, when calibrated appropriately using data collected from AFM scans.

Displacement maps are particularly effective for visualising conductive surfaces such as titanium, or for viewing surfaces that cannot be easily examined by other topography analysis tools, such as AFM. The evaluation of four different surface types, including thin titanium films, silicon wafers, polystyrene cell culture dishes and dragonfly wings confirmed that this technique is particularly effective for visualisation
of conductive surfaces, such as metallic titanium, and is very useful for visualising surfaces that cannot be easily analysed using AFM. The speed and ease with which electron micrographs can be recorded, combined with the simple process for generating displacement maps make this technique a useful tool for the assessment of surface topography of biomaterials.

Three-dimensional animated movie show the comparison between the models visualised via the displacement map technique (DVD-Video 7.3.). The 3D models of the same surface magnifications were presented with corresponding AFM scans (AFM left, SEM right), and rotated from 0° to 360° in tandem around the Y-axis in a time range of 15 seconds.

7.5.1. Advantages and limitations of displacement map technique

The advantage of this displacement map technique is in its ability to quickly generate 3D model representations of surfaces that can be more easily analysed. To illustrate this, consider the example of few polygonal objects with a distribution of heights (Fig. 7.9a). It is not a simple task to draw conclusions on the relative heights of the objects, especially when seen from 90° (Fig. 7.9b), however, it is of course much easier to obtain knowledge on the relative heights when viewing from the side (Fig. 7.9c). The extra dimension allows the potential for insight into the height distribution to increase. The absolute heights of each polygon could also be obtained by directly measuring them, however if this were time consuming the heights of a subsection of the objects could be measured, and provided that the distribution of heights throughout the population were homogeneous and random, data from that subsection could be applied to approximate the heights of the rest of the group. Thus, the relative heights of the polygons are accurately represented, and their absolute heights are approximated in a short time. Provided one keeps in mind the limitations of the technique, this can be a highly effective tool in analysing large populations of data.
Figure 7.9 The accuracy limitation of an SEM scans: the polygon example.

There are some limitations associated with the use of displacement maps to visualize SEM images in three dimensions. Firstly, given that the displacement maps are generated from two-dimensional data, only the uppermost features can be visualized. For example, if one feature lies below another, e.g. if the angle of one larger feature causes it to overshadow a nearby smaller feature, then the lower feature cannot be visualized. As much as possible, the scan data should be collected from an angle of 90°, perpendicular to the plane of the surface.
Figure 7.10. SEM noise reduction for surface reconstruction. A high level of noise in an electron micrograph can adversely affect the final 3D geometry, resulting in a highly rough appearance (Fig. 7.10a). In order to minimize the effect of noise, a convolution algorithm can be applied. The surface presented in (Fig. 7.10b) was processed using a 3 × 3 convolution matrix to ‘smooth out’ the effect of noise. Care must be taken however, to ensure that the image is not excessively smoothed, as this will result in the loss of topographical detail. This is demonstrated in surface (Fig. 7.10c), where the same convolution matrix was applied 5 times in succession.
Similarly, the quality of the three-dimensional displacement map will be dependent on the quality of the input micrograph (Cizmar et al., 2008). Typically, SEM software allows for the manipulation of the brightness and contrast prior to the capture of the image. Ideally, for the application of displacement maps, the contrast should be set to the maximum level possible without losing the topological detail in the upper and lower extremities of the surface. Also as with any experimental technique, there is a requirement to minimize the noise with respect to the collected signal (Hiraiwa and Nishida, 2012a, Hiraiwa and Nishida, 2012b). For example, an electron micrograph with high noise levels will typically contain many bright ‘dots,’ which will subsequently appear as very tall and very narrow features in the corresponding displacement map. An example of a displacement map created from a noisy micrograph is presented in Figure 7.10a. Fortunately, this effect can often be remedied post-data collection via the application of smoothing filters. A smoothing filter adjusts the pixel values to the average of the surrounding pixels, after weighting each pixel according to a user-defined matrix. The size of the matrix, the weighting of the pixels, and the number of times the filter is applied is completely customizable to enable the production of the best image possible (Fig. 7.10b). One must be careful however, not to excessively smooth the image, as this will likely cause the topographical details to be lost (Fig. 7.10c).

Finally, the results presented here demonstrate that displacement maps cannot always be applied to the surfaces of insulating materials. Samples that are weakly or moderately insulating can be coated with a thin layer of gold or carbon (common practice for electron microscopy) (Wang et al., 2008). This process will improve the conductivity of the surface and enable micrographs of adequate quality to be obtained. The thickness of the conductive coating must be minimized as much as practically possible in order to avoid losing surface details. For strongly insulating materials however, electron micrographs do not contain sufficient topographical detail to produce useful 3D displacement maps (Nowell and Pawley, 1980).
Chapter 8. Discussion
8.1. Overview

Understanding the exact process of how bacterial cells interact with different types of nano-structured substrata can have a direct impact on many processes that influence the health of individuals. Bacterial cells are attracted to many biomaterials (Krishna Alla et al., 2011, Crawford et al., 2012) including titanium, which are widely used for the development of medical implants (Liu et al., 2004). The adhesion of bacteria to implant materials can often result in implant-related infections. Therefore, the ability to prevent bacterial attachment by, for example, altering the topography of the surface to make it less attractive to bacterial attachment has become a popular research topic among scientists in the area of nano-biotechnology (Bjursten et al., 2010, Subramani, 2010, Dreaden and El-Sayed, 2012). Prevention of adhesion of bacterial cells requires a comprehensive understanding of the physical and chemical characteristics of both the substrate surface and the bacterial cells. Extensive experimental work has been conducted in both of these areas (Mauclaire et al., 2010, Fadeeva et al., 2011, Oliveira et al., 2011).

One of the obstacles limiting our ability to fully understand bacterial cell-surface interactions is the lack of ability to observe the processes that take place in real-time. This study was primarily aimed to address this issue, and to develop the ability for researchers to obtain a greater understanding of the interactions taking place between a bacterial cell and a substrate surface in a medium that is not commonly available for scientists in the nano-biotechnology field, i.e. through the visualisation of the cell-substrate interaction.

The animations presented throughout Chapters 4-6 are depicting different scenarios related to bacterial cell-surface interactions. The interactions in each movie progressed from being simple representations to more complex, dynamic representations. As demonstrated in Chapter 4, a rudimentary dynamic process (Maya’s Dynamic menu set) can be created in order to achieve a simple dynamic behaviour and interaction among objects. The movies presented in Chapters 5 and 6 involved more complex dynamic deformation and interaction between multiple objects (such as with the bacterial cells or in the production of the extracellular polymeric substances (EPS) secreted by the cells as a strategy to assist in attachment to the surface. The Dynamic
capabilities of Maya allowed the behaviour of bacterial cells to be created through adjustment of the highly-configurable attributes of dynamic objects. This is where the developed hypothesis (stage 1 of the Scenario-based workflow) played a vital role in the visualisation process. The adjustments of the attribute strongly relied on the hypothetical assumptions being made regarding the dynamic behaviour of the bacterial cells and interactions they undertook with the substrate surfaces. As there was no experimental data available to depict the actual interaction taking place, the process of interaction that was assumed to be occurring by the researcher was an important source of information in order to achieve some level of accuracy in the depiction of the interactions.

The bacterial cells are known to interact with the surface, however the path taken by the bacteria depends heavily on the characteristics of the visualised surface topography. For example, when a 3D model of a bacterial cell falls onto the substrate surface and reaches an indentation on this surface, the cell will fall into the valley present. On the other hand, if there is an obstacle on the surface (e.g. a high peak) and the cell motion path is set towards that obstacle, the model will collide with the obstacle. Depending of the other attributes values (e.g. the values of velocity, stickiness or mass), the model would assume that the cell would either stop its movement, or bounce as a result of the interaction with the obstacle, causing it to continue its movement in another direction. These simple dynamic interactions could be considered as first steps towards the development of more accurate simulations, however there are other factors that can affect the level of accuracy of the depiction of the interaction and do not allow the extension to more complex motions, which will be discussed in the following sections.

The visualisations techniques presented in Chapter 7 allow additional aspects of 3D surface representation and reconstruction to be obtained. The process of visualisation is straightforward, as it is based on the previously developed tools that are part of Maya’s default menu. When the substrates have been modified from their original form, the 3D models of the surfaces may need to be displayed without the bacterial cells present in order to show the differences between the surface topography that occur as a result of the modification. These techniques provide an ability to show the differences in
surface roughness between different substrate, in a visual form (Cross-sections profiling – section 7.1) or as single 3D object that contains the combined data obtained from different surface scans (Surface transition - section 7.2). The third technique (section 7.3) provides a solution for a surface 3D reconstruction from a single data set (an SEM image).

For each of the movies, the 3D visualisation process followed the Scenario-based workflow presented in Chapter 3. The main purpose of these different stages was not just to visualise scientific data and produce high-quality 3D movies, but also to involve the researchers in the visualisation process. This provides an additional opportunity for researchers to visually perceive their ideas and recreate naturally occurring processes in a 3D virtual environment for which there is often no experimental data available. Most of the task mentioned above (apart from the data conversion and final post-production) can be achieved within a single platform, in this case, Autodesk Maya. This helps overcome another common problem that scientists face, where often multiple applications are required in order to accomplish 3D scientific visualisation of data (Hortolà, 2010, McGhee, 2010).

In the third stage of the methodology, two complementary steps were presented: a semi-automated step, where surface topography data is loaded into Maya and a creative step, where the interaction between a bacterial species and the surface is modelled using Maya’s Dynamic systems. The key difference between these steps is the amount of influence made towards the visualisation. For example, in the semi-automated step, most of the process – apart from selecting the input data set and the curves – is performed by the automated components of the software without requiring much human intervention. In this case, the original AFM experimental data was converted and directly imported into Maya using a simple Python script. In the creative step, hypothetical assumptions about the mechanisms taking place at various stages of the bacterial interaction and attachment processes are recreated as dynamic motions based on the available data and the assumed processes taking place. This can result in a higher level of direct involvement by the researcher in what is often an iterative process, requiring the adjustment and modification of the animation until the desired results are achieved. This results in the creation of a visualisation of process that can be used to
better communicate the hypothesis and can assist in refining the hypothesis once the resulting consequence of an attachment can be seen.

It is acknowledged that completing this third stage (especially the creative step) to a high level does require investment in learning a new skillset. Nonetheless, this is an essential and very often unavoidable requirement, regardless of the type of the applications, scientific (e.g. Avizo or Gwyddion) or non-scientific (e.g. Microsoft Word or PowerPoint). However, there is one advantage that applications from the entertainment industry, such as Maya, have over their scientific counterparts: the quantity and quality of information that can be found on-line for learning purposes. There is an abundant amount of free material and video tutorials that could be used for understanding the basic functions in these applications (Table 8.1). There are also web sites available, where researchers provide tutorials and solutions for the visualisation of scientific data at a higher artistic level, based on their experience and knowledge.

*Table 8.1.* On-line resources for 3D animation tutorials and examples.

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8.2. Maya simulation versus scientific simulation

The word *simulation* is an often-used term in the 3D visualisation process (e.g. Maya’s dynamic simulations), however, it should not be mistakenly associated with *scientific simulation* in general. Instead, the dynamics simulations in Maya are a useful tool to recreate natural motions and collisions between objects in order to capture the essence of an interaction.

The simulations in Maya are processes of interaction between objects affected by some forces, or each other, and therefore the calculations present an interpretation of the changes in object’s shape. Simulations performed with Maya’s Dynamics and nDynamics functions do not currently support options to directly import scientific data of an object’s dynamic attributes. However, there are some nodes that are already part of Maya’s Dynamic systems that do provide representation of real-world forces (for example, acceleration due to gravity has the default value of 9.8 m/s²). Based on the other attributes (mass, time, distance, etc.) forces of gravity will affect the 3D object as it would in the real world, built on the calculation and the variables assigned to those attributes. In terms of bacterial cell-surface dynamic interactions, acceleration due to gravity was used as a representation of the surface forces that pull the bacteria, since the actual magnitude of these forces are not known.

As shown for the AFM data, it is possible to create new nodes to represent additional attributes such as surface forces, and scripts that could allow the importation of additional experimental, analytical or numerical data and to use these to create more accurate and more realistic dynamic interactions of the bacterial attachment process. Developing and sharing scripts that can import scientific data directly into applications such as Maya could make these software packages more independent, less time consuming to learn, and more cost effective as an addition to existing domain-specific scientific visualisation packages. The tool developed for the 3D surface visualisation in Maya is available on the accompanying DVD (DVD-Video 8.1. demonstrates the tool’s applicability in Maya).
8.3. Limitations

When it comes to determining the limitations of the Scenario-based visualisation workflow, there are few issues that should be addressed. Those limitations mainly arise from the second (data collection) and the third (3D visualisation) stages of the workflow.

- **Limited by the data**

In spite of recent advancements in technology, it is not possible to collect all the data necessary for a complete understanding of the mechanisms taking place when a bacterial cell interacts with a surface. As mentioned before, the objects and natural processes under investigation are extremely small, and cannot be perceived with the naked eye. Therefore we rely on instruments such as microscopes to observe these processes and collect data that provides an understanding of their characteristics.

Scanning of the surface and bacterial cell samples, presents additional challenges. Depending on the type of the instrument, different aspects of the scanning process could affect the resulting data. For example, when a surface sample is scanned with scanning electron microscopy (SEM), if the surface is not sufficiently conductive, it will produce a charge, resulting in no signal, and a clear image will not be produced. Therefore, often the surface samples are coated with layers of gold in order for the surface to become conductive (Wang et al., 2008). On the other hand, if the gold layer is too thick, information of the actual surface topography could be lost. As for the cells, in some cases the SEM vacuum conditions could damage the cells.

Another common problem results from the AFM scanning procedures; an AFM scan depends strongly on the surface topography. In order for the scan to be accurate, the frequency of the contact of the AFM tip should be matched to the roughness of surface sample. In other words, if the tip moves to fast, it could miss interacting with some parts of the surface. These are just few aspects of the general problems researchers are facing when collecting data. Other problems involve the lack of ability to measure the actual dynamic motions or forces of objects (i.e. bacterial cell adhesion on the surface). It is difficult to obtain any kind of numerical data regarding the interactions.
(e.g. distance, speed, surface force, etc.) that could be useful in recreating the dynamic behaviour as a 3D representation.

Beside the processes for collecting data, another common difficulty involves data conversion. The information that derives from instruments such as AFM, is usually stored in file formats that are specific and native to that instrument. It is necessary for the data to be transformed into an appropriate format that is compatible with different software packages if further processing of the data is to be performed. For visualising the scientific data, often more time and effort needs to be devoted to developing techniques to convert raw data files into readable formats for a specific application, rather than the actual visualisation of the data. This was the case for the AFM surface topographical data, when the data had to be converted into an appropriate format (.obj or .csv) that would be recognisable by Maya. The advantage of the .csv format is that is a “human readable” text format. However, it is a less compact representative for large data sets then a binary and compressed format. With a maximum dataset size of 262,144 height values, this was not a serious limitation.

- **Limitations of the software and hardware**

Another important issue emerging from this study is the software and hardware performance. When working within applications such as Maya, the amount and the complexity (e.g. mesh resolution) of the objects with in a scene can have strong impact on the interactive performance. In other words, if the Maya scene is populated with large number of objects, the application could often “freeze”, causing the tools and options to be unusable (e.g. navigation tools, such as rotation of the camera). This is a common problem when Dynamic simulations are performed on high-resolution models.

Maya software was designed for the entertainment industry in mind. Therefore, in order to overcome the “scene complexity” issue and increase the performance in Maya, the tools and options are developed in such way so that the 3D animations should be created with as simple a geometry as possible, while the final product should make use of the most detailed representation. For example, if there is a need to animate a high-resolution model, the actual animation is created with a lower-resolution mesh. After applying additional details through different shading/texturing solutions (e.g.
bump, displacement maps, etc.) the final animation sequences are rendered. This approach was used in the animations developed in Chapters 4-6 for adding additional details to the low-resolution bacterial models, after the dynamic simulations were developed.

When it comes to scientific visualisation, accuracy is the ultimate goal. In order to achieve a level of accuracy we need to consider every detail of the object structure, along with how any type of dynamic simulation would affect or control the object's components. Regarding the bacterial cells, the membrane behaviour of the cells in the visualisation were developed based on simple dynamic deformations applied to a simple geometrical shapes. On the other hand, if there is a requirement for a simulation of the bacterial membrane to be recreated accurately, the 3D model of the membrane should contain all the components that structure the membrane (i.e. lipids, proteins, etc.). That demands a 3D visualisation on molecular level. Unfortunately, this often leads to a problem when it comes to 3D visualisation on extreme scales and massive amount of data in applications such as Maya. As there are millions of molecules, it would require for Maya to execute a great deal of calculations that would require processing power beyond the capabilities of a single desktop. It would not only limit the interactivity with in Maya scene, but can also lead to a less stable computing system. One solution is to operate Maya on a dedicated super-computer, which could process all the data within a single scene, however often these facilities are not available.
8.4. Impact of 3D visualisation on public audience

As in any research related work, it is often important and required for the complex findings and the knowledge discovery to be presented to both scientific and non-scientific audiences.

Figure 8.1. Different types of shading/texturing results on the *P. aeruginosa* cell models and the cicada wing surface. Visualised objects in Maya with applied default grey texture (a). Scientific representation of the same objects with applied colour map on the surface and a red texture (red is often associated with dying cells) (b). The artistic representation with applied SEM look a like texture.
A successful visualisation is one where important information regarding the novel findings is accessible and understandable. This is the main reason why the last stage (3D image/movie production) of the scenario-based workflow should be considered as equally important as the rest of the stages. How the final image or a movie should be produced does depend on the audience and target medium. For example, if an image needs to be created for a research publication (e.g. journal article), the image should be generated in high quality (e.g. resolution and size of an image), but should not contain any complex or additional artwork, as only the most important information should be highlighted. On the other hand, if its primary purpose is for non-scientific audience, investing time in adding more artistic effects would make the image more attractive.

Figure 8.1 shows the different options for achieving shading/texturing of the *P. aeruginosa* cell on the cicada wing surface. When the 3D animations of bacterial cells-surface interaction were developed, more time was devoted to making the cells look more “realistic” (the SEM look was often used as a resource for the development of the textures for the cells models). Numerous applications designed mainly for scientific purposes do not usually provide functionalities for adding additional effects to the visualised data, which gives another advantage to software packages from the entertainment industry over the scientific counterparts.

It is acknowledged that more effort could have been devoted to the artistic touches (i.e. adding environmental effects, background, etc.). Regardless of the style of the 3D animations, the movies did serve their purpose in their current form. They have been used as additional source of information in journal articles (Pogodin et al., 2013, Webb et al., 2013), to support presentations and have appeared on web sites including nature.com.
9. Conclusions and future directions
9.1. Summary

With a growing interest in understanding the nature of bacterial cell-surface interactions, there was a need for novel methods to be developed that enabled researchers to better understand the mechanisms taking place during these interactions. The present research investigated the way in which 2D images obtained from microscopic techniques could be used to construct 3D images, which allowed the visualisation of different substrata, based on real topographical data on the nanometre scale. In addition, these 3D images could be used as a tool for the development of plausible explanations regarding the interactions taking place when bacterial cells come into contact with substrate surfaces. A software package used extensively by the entertainment industry, Maya, was found to be a suitable tool for the 3D visualisation of bacterial cell-surface interactions. Maya’s wide range of tools and functions for 3D modelling, dynamics and animation provided the flexibility to enable the visualisation of interactions between 3D models of both bacterial cells and the substrate surfaces. The in-built programming interfaces (i.e. Python) extended Maya’s functionality in order to process data and file formats (e.g. .csv) that were not usually recognisable by the software.

The development of the bacterial cell-surface interaction animations was based on the scenario-based visualisation workflow presented in Chapter 3. It is a unified method that employs both scientific and artistic approaches. In this study, the accurate 3D visualisation of surface topographical data together with the development of a creative interpretation of the possible interaction that takes place between a cell and a surface have been realised. The development of the new Python tool has simplified the surface 3D modelling process.

The movies presented in Chapters 5 and 6 depicted interactions between two bacterial species (S. aureus and P. aeruginosa) and different types of nano-structured surface on different levels of complexity in terms of the dynamic behaviour. Additional solutions for the visualisation of a 3D surface have also been explored, along with improved ability to represent the surface topography.
It is noteworthy that interest has already been shown in these animations by both scientific and non-scientific audiences. The 3D movies that have been produced as part of this study have enabled a greater understanding of how bacteria interact with the surfaces used in this study. The available data describing the surface, together with hypothetical assumptions about the way the cells and a surface interact have been incorporated into the final 3D movie sequences.

This study opens new avenues for experimental data analysis using Maya. These include possible further improvements in tools for the visualisation of bacterial cell shapes, together with their dynamic interactions. Considering that only surface topographical data were available for use in the production of direct 3D visualisations of a surface, the next step would be the development of more accurate bacterial models. Bacterial cells are complex, and therefore it will be difficult to visualise every single component of the cell. However, often only the membrane structure is required when considering cell-surface interactions. Modelling a segment of a membrane and other cell wall components would be sufficient to enable the accurate recreation of the cell surface to be preformed.

The limiting factor is that we are not yet capable of obtaining accurate data that described bacterial cell membranes on the molecular level, however the types of molecules and their organisation are known (Huang et al., 2008). Given this knowledge, one solution would be to recreate the membrane as 3D model. It would require each type of molecule to be visualised and assembled as a geometrical object that accurately represents the membrane. The next step would require the addition of attributes to the membrane model that would allow the dynamic behaviour to be controlled. If this would be recreated in Maya, the obvious first approach would be to utilise the nParticle system to represent the atomic and molecular structure of membrane. The particles could therefore be converted into a 3D model, which would provide an enhanced representation of membrane geometrical shape. The final 3D model could be then used to develop an interaction with a 3D surface model. The dynamic interaction could be recreated based on mathematical models and algorithms of the actual process. It can be achieved by developing scripts that would process molecular data, assemble the molecules and add additional attributes. This is just one example for possible solution for the accurate 3D visualisation of a membrane interaction.
Whether applications such as Maya are suitable for achieving the steps mentioned above remains unclear. As discussed in Chapters 2 and 8, the 3D animation software packages used by the entertainment industry are designed for specific reason and not specifically for the processing of large amounts of scientific data. On the other hand, existing scientific applications have been developed for specific scientific needs. One solution could be a development of completely new “hybrid” software package, which allows the 3D visualisation of bacterial cell-surface interactions. The application should provide ability to visualise and simulate bacterial interactions with surfaces while also to have an ability to provide high-quality graphical representation of research data on the artistic level.

9.3. Conclusion

3D computer graphics and animations can be produced and used as a tool to assist in the understanding of bacterial cell-surface interactions. Future innovations in nanotechnology and computer technology will provide data that will allow for more complex and accurate 3D representations of scientific data to be produced. The advancement of the technology in both fields is occurring rapidly. With more sophisticated instruments for collecting data being developed, more information will become available regarding natural processes occurring on the nano-scale.
Bibliography


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Appendices

Appendix 3.1. Loft tool settings.
Appendix 3.2. Common render settings
Appendix 5.1. nCloth attributes. Default values of lava preset (left) and adjusted values for the *P. aeruginosa* cell body simulation (right). The values may vary scene to scene.