Preparation of 2D Sequences of Corneal Images for 3D Model Building

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ABSTRACT

A confocal microscope provides a sequence of images, at incremental depths, of the various corneal layers and structures. From these, medical practitioners can extract clinical information on the state of health of the patient’s cornea. In this work we are addressing problems associated with capturing and processing these images including blurring, non-uniform illumination and noise, as well as the displacement of images laterally and in the anterior posterior direction caused by subject movement. The latter may cause some of the captured images to be out of sequence in terms of depth. In this paper we introduce automated algorithms for classification, reordering, registration and segmentation to solve these problems. The successful implementation of these algorithms could open the door for another interesting development, which is the 3D modelling of these sequences.

Keywords: Artificial neural networks; Confocal microscopy; Classification; Registration; Segmentation; Z-ring adapter.
1. Introduction

The cornea is the clear outer layer covering the front of the eye comprising a collection of cells, fibrils and proteins that constitute a very highly organized structure. The cornea must remain transparent to allow light to enter the eye and the curvature of its outer surface accounts for much of the focusing power required to properly form images[1-3]. The flexible crystalline lens then further refracts the incoming light and provides the remaining focusing power required to achieve a sharp image on the light sensitive retina. The cornea also works as a protective membrane to the human eye, among other things helping to screen out the ultraviolet wavelengths which are found in sunlight and prevent the lens and the retina from being damaged by these wavelengths [4]. The cornea joins smoothly with the non-transparent conjunctiva and has lateral dimensions, on average of, 12.6 mm in the horizontal direction and 11.7 mm in the vertical direction. The thickness of the cornea is non-uniform, ranging from about 520 µm at the centre to about 650 µm, at the periphery. The cornea has a tear film on its front surface and three main internal layers separated by two thin membranes. The outermost layer is the Epithelium, which is separated by Bowman’s membrane from the central Stroma layer, which is separated in turn by Descemet’s membrane from the innermost endothelial layer as shown in Fig. 1.

A number of Injuries, dystrophies and ocular surface diseases (Keratoconus, Lattice Dystrophy, Dry Eye, Conjunctivitis, etc.) may lead to opacities in the cornea, which can severely impair vision. In addition some diseases of the cornea cause severe pain and chronic discomfort [3]. The slightest changes in the shape of the corneal can clearly diminish visual performance of the eye.

The various layers and structures of the cornea can be studied in vivo using a confocal microscope to provide a sequence of images at different depths, as illustrated in Fig. 2. The use of the confocal microscope, such as that shown in Fig. 3, has provided a better understanding of the microstructure of the cornea’s cells (normal, postsurgical and diseased), and, from these images, Ophthalmologists can extract clinical information on the status of the cornea. To the best of our knowledge, the analysis of these images are currently based on manual inspection or aided by semi-automatic methods. Individual corneal images in these sequences are often noisy and sometimes dark or contain no data [6, 7]. Images display non-uniform illumination caused by factors such as: the spherical shape of the corneal layers, which causes non-uniform reflection of the illumination light from the different areas of the cornea, and the different attenuation of light along the different paths of illumination.

The confocal microscope enables images of the cornea to be scanned at different depths (default layer separation 5 µm, minimum separation 1 µm) and immediately viewed for diagnostic purposes. During a scan, the instrument locates the rear of the cornea (no signal back from the vitreous humour) and then steps forward in 5 µm steps until it reaches the front surface of the cornea (no signal back from the tear layer). This cycle is repeated 3 times during a 20 s scan which provides about 350 images [6].

Although the confocal microscope, as shown in Fig. 3, has a head rest supporting chin and forehead, one of main problems with these types of images is caused by movements of the eye during the scanning process. Respiration, cardiac pulse and other factors cause images of adjacent layers to be displaced laterally with respect to each other and may cause images in the capture sequence to be out of sequence in terms of depth. This also means that the difference in depth between captured layers is not necessarily uniform or the same as the instrument setting. The amount of movement varies from patient to patient and from scan to scan. The confoscan4 confocal microscope used in the current
The study can optionally incorporate a z-ring adapter detachable contact element as shown in Fig. 4. The z-ring adapter maintains a small constant pressure on the cornea, so that it and the connected objective lens follow the movement of the cornea in the anterior posterior direction [9]. This increases image stability to give a more precise location along the z-axis through measuring the position of each frame along the z-axis [10, 11]. Despite improvement in image stability provided by the z-ring adapter, movement is not entirely eliminated.

Scarpa et al. [12] have constructed a stack of registered 2D images to create a 3D image volume that can be viewed from x, y, or z directions. The 3D stack view is affected by the quality of the 2D Images, and it’s difficult to maintain the full resolution of the viewed images as they are affected by the viewing angle. Also, it is not easy to use the 3D image stack to compare the pathology of the cornea at different times.

Fig. 4. Z-ring adapter (z-CS4) [10].

We are working in collaboration with a group of specialists from more than one hospital and a centre of ophthalmology to achieve more accurate methods to make diagnostics which will improve standards of patients care. Our aim is to work towards creating a system which can provide 3D model structures from these corneal images. The importance of 3D models is that they potentially present a better visualisation of the anatomy of corneal layers, with more accurately identified diseased areas. Also 3D models provide relationships of anatomic and pathologic structures.

The organization of the paper is as follows:

![Fig. 5. The stages of a system to generate 3D models from an image sequence from a confocal microscope. The first 4 stages are described in this research.](image)

2. Materials

The confocal images used in the remainder of this study were downloaded from [8] and consist of 3 folders from subjects known as 1, 2 and 3 containing sequences, from the Epithelium to the Endothelium, of 144, 85 and 127 images respectively. These images were obtained using a ConfoScan 4 confocal microscope (Nidek Technologies) with an image field of 460×350μm at 40X magnification. The images are of size (768×576) pixel and are saved in JPEG format. These three sets of images were acquired using a z-ring adapter [12], so all the images are assumed to be in the right sequence.

To validate the classification methods, we have used a different set of corneal images, which were provided by Tennent Institute of Ophthalmology, Gartnavel General Hospital (Glasgow). These images contain sequences from the Epithelium to the Endothelium and were obtained using a ConfoScan 4 confocal microscope (without z-ring adapter) with an image field of 460×350μm at 40X magnification. The images are of size (768×576) pixel and are saved in TIF format.

3. Methods

The basic steps of our automated system are shown in Fig. 5. Firstly, classify individual images in the sequence into Epithelium, stroma and Endothelium classes. Secondly, order the images in each class in depth sequence. In this work, for test purposes, only the stroma images have been
subjected to the stages after classification, because they are the most numerous. Thirdly, register adjacent images with each other to remove the effect of lateral displacement from the centre of images. Fourthly, segment the image sequence so that individual objects (cells) are labelled consistently throughout the sequence. Each of these steps is now described in detail.

3.1. Classification Algorithm

Ruggeri Pajaro [7] used Artificial Neural Networks (ANN) to classify confocal microscope corneal images according to the related layers based on two types of features derived from the shape of the cells processed. In the first they used means of Hu variables (central moments) of binarized images and in the second Zernike moments, extracting the description of the cell shape without the need to create binary images. With a set of 37 corneal images, they trained the ANN with both methods, and then validated with a set of 46 corneal images; the results obtained with second method were better than those obtained with the first method.

In our work, the classification of the images includes a pre-processing stage, a feature extraction stage and then the classification stage as indicated in Fig. 6. All stages were implemented using MATLAB.

Pre-processing was applied because the images from the confocal microscope suffer from non-uniform illumination and noise. The Confoscan-4 microscope source of our images is a slit based scanning system and the image is subject to additive, out-of-focus, illumination. A recent paper by J W McLaren at al.[13] measured the illumination of a uniformly scattering solution and then subtracted a scaled version of this from images of the eye. We apply a high-pass DCT filter (second-order Butterworth with cut at channel number 10) to improve the uniformity of illumination and standardise the image contrast (by applying a linear contrast stretch to image histograms between 0.1% and 99.9% of the area) after Gaussian smoothing with (σ = 2.2 pixels) to reduce noise. Image binarization is done using the Otsu method [14-17]. These operations were applied in the different combinations shown in Fig. 6 in order to explore their effects on the classification performance. Features for classification were extracted from the central 477×477 pixel parts of the images, because the outer parts of the original images are dark and noisy as in the examples in Fig. 2.

a) Texture Analysis

As can be seen in Fig. 2, the three layers differ in the shape and texture of the corneal structures, showing endothelial cells, Stroma keratocytes, Epithelium cells, nerve fibres, etc. Hence it was decided to use texture-based feature extraction to obtain numerical features that might provide representative descriptions of the image content. In this work, four statistical methods were used for texture analysis of corneal images. These are: six first-order image histogram (FOS) measures (Mean value, Standard deviation, Skewness, Kurtosis, Energy) [18-20]; nine grey-level co-occurrence matrix (GLCM) measures (Contrast, Correlation, Energy and Homogeneity, Entropy, mean of row, Standard deviations of row, Absolute value and Inverse difference moment) [20-22] all calculated using distances d = 7, 9, 11, 13, 15, 17 and 21 and angles θ = 0º, 45º, 90º and 135º; the value of a distance d is dependent on texture type, as it requires a small values for fine texture and a large values for coarse textures [23]; fourteen Law’s masks and texture energy measures (TEM) calculated from E5L5, S5L5, W5L5, R5L5, E5S5,
E5E5, E5R5, E5W5, S5R5, S5W5, S5S5, W5R5, W5W5, R5R5 [24-26]; sixteen grey run-length matrix (GRLM) measures with 8 quantization levels (Short runs emphasis, Long runs emphasis, Grey level non-uniformity and Run length non-uniformity, all calculated in the 4 directions $\theta = 0^\circ$, $45^\circ$, $90^\circ$ and $135^\circ$) [27, 28].

b) Classification

Artificial Neural Networks (ANNs) are widely used for classification purposes in many different applications including engineering, finance, health and medicine because they have proved to have powerful capabilities [29, 30]. In our work, we have used a cascade-forward back propagation network to classify the corneal images into three classes (Endothelium, Epithelium and Stroma). The inputs used for the neural network are the statistical features (FOS, GLCM, TEM and GRLM) extracted from the set of images specified in Section II.1. The classification involves three steps: a) Select sample images from the subjects 1, 2 and 3 comprising 9 Endothelium, 30 Epithelium and 45 Stroma images and extract 32 different types of feature data by combining the eight types of pre-processing with the four statistical measures; b) Divide the image data into two sets with 80% of the data used to train the neural networks with numbers of hidden nodes ranging from 5 to 20. c) Use the remaining 20% of the data to test each trained neural network 10 times and calculate some measures through the confusion metrics: True positives rate (TPR), False positives rate (FPR), Accuracy (ACC), True negatives rate (TNR), Positive predictive value (PPV), Negative predictive value (NPV), and False discovery rate (FDR) [31]. The standard deviation of each measure has also been calculated to monitor the variation in the results.

c) Results

Space precludes showing all the results but Table 1 gives one set of performance measures for different numbers of hidden nodes. It can be seen that the accuracy ranges is from 0.829 to 0.994, with best accuracy (and other measures) with 8 hidden nodes. Table 1 summarises the best accuracy results from ANNs with different numbers of hidden nodes for each of the 32 combinations of pre-processing methods and types of extracted features. The first four columns of the results in Table 1 apply to grey scale images and the second four columns of results apply to binary images. It is worth noting that GLCM gave the highest accuracy in the majority of cases in

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<th>Table 1 - Summary of the best average accuracies of ANNs for each combination of pre-processing and type of feature.</th>
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To validate the different methods of classification, the optimised ANN from each method was used to classify another 72 images (7 Endothelium, 35 Stroma, and 30 Epithelium) from the Tennent Institute of Ophthalmology. The best results, reported in Table 2, were obtained from the trained neural network combined with DCT filter, Gaussian smoothing, contrast Otsu’s threshold and with the GCLM texture measure. The result was an overall accuracy of 97.22% with just two Stroma image misclassified as Epithelium. These results, with 100% of Endothelium, 97.22% of Stroma and 100% of Epithelium images correctly classified, are better than the results of previous work (84% correctly classified as Stroma) [7]. It should be kept in mind that different image sets are used for both works.
3.2. Image Ordering Algorithm

As was mentioned in Section 1, the z-ring increases image stability to give a more precise location along the z-axis and providing a sequence of images in the right order. However, not all confocal microscopes are equipped with a z-ring adapter. This fact forces us to find a method to sort and reorder confocal microscope images in the right sequence.

Sorting the images in the z-sequence, whether manually or automatically is only possible because neighbouring image in the correct sequence have structural similarities. The size of each cell in the z direction is larger than the nominal z interval between captured images, which indicates that the same objects (cells) appear in more than one image. From this observation, we assume that the next image in the sequence should be the one similar. We have used the Structural Similarity Index Measure (SSIM) [32], and the basic principle is to sort the images so that the next image is the one with the largest SSIM to the current image. To reduce the processing time we reduced the sizes of the images by using the approximation sub-band after applying a multilevel 2D wavelet transform (DWT), without much loss of significant information [33]. Fig. 8 indicates the main steps of this algorithm.

![Diagram of Image Ordering Algorithm]

Fig. 8: The main steps of the image ordering algorithm

To assess the method we constructed two disordered sequences of images: 51 Stroma images from subject 1 in the order 015, 030, 022, 027, 041, 020, 021, 017, 023, 024, 063, 026, 018, 028, 029, 016, 031, ..., 036, 045, 038, ..., 040, 019, 042, ..., 044, 037, 046, ..., 062, 025, 064, 065; and 69 Stroma images from subject 2 in the order 007, 012, 009, 010, 011, 008, 013, ..., 018, 030, 020, ..., 024, 046, 026, 027, ..., 042, 073, 044, 044, 045, 025, ..., 072, 043, 074, 075. We applied a DWT from one to four levels to the sequence and in each case sorted the images into the correct sequence using SSIM method. Table 3 summarises the results of applying the SSIM method and the advantage of using the DWT to decrease the time required to order the sets of images. The same procedure was applied to the 91 stroma images from subject 3 arranged in the order 007, 012, 009, 010, 011, 008, 013, 020, 015, ..., 019, 014, ..., 030, 077, 090, 033, ..., 076, 031, 078, ..., 089, 032, ..., 097. In this case the method failed overall, although sub sequences were sorted correctly. It was later found that this failure was associated with occasional large lateral shifts between some pairs of images in subject 3 of up to about 138 pixels. It was observed by analysing all the data that the SSIM was successfully applied in all cases where the displacement was 16 pixels or less and failed where the displacement was 29 pixels or more. There were no cases of displacements between 16 and 29 pixels.

3.3. Registration Algorithm

There are several factors (respiration, cardiac pulse, etc.) which cause movements of the cornea relative to the confocal microscope. The process of registering two images is based on finding common features in the images which will enable a transformation to be found to properly align the images. The authors of [11], applied an automatic registration procedure on pairs of images, using a normalized correlation method; the procedure failed for 3% of images, and they mention that the shift were mainly located in the x direction.

The authors of [34] registered enhanced dental radiograph images, using a Phase-Only Correlation (POC) method and corrected non-linear distortions based on a Thin-Plate Spline model.

In order to estimate these movements between neighboring pairs of images, we have used the Speeded-Up Robust Features (SURF) algorithm [35] to obtain matching points and hence to estimate the shifts along the x and y axes. For
comparison the Scale Invariant Feature Transform (SIFT) was also applied in a similar way. This type of registration method (SIFT) works well when the two images are from the same depth but shifted [6].

In the results of this work two particular sources of error are present: spuriously matched points and duplicate matched points. These are visible in the samples of results shown in Fig. 9.

We define our original SURF algorithm, named SURF_Algorithm_1, as follows:
- \( I_a = \text{image}(i), I_b = \text{image}(i+1) \)
- Use “SURF” function to compute Points\_a, descriptor\_a & Points\_b, descriptor\_b.
- Match the two sets of SURF descriptors descriptor\_a and descriptor\_b.
- Extract matched points with coordinates \( x_1, y_1 \) and \( x_2, y_2 \).
- Calculate the shift\_x = median(x2-x1) and the shift\_y = median(y2-y1).

To filter the results to remove errors due to spuriously matched points and duplicate matched points as shown in Fig. 9 and get accurate matching between each pair of images as shown in Fig. 10, we define SURF_Algorithm_2 as follows:

- (F) \( I_a = \text{image}(i), I_b = \text{image}(i+1) \)
- Use “SURF” function to compute Points\_a, descriptor\_a & Points\_b, descriptor\_b.
- Surf function provides matrix dis\_mat(N,M) constants Euclidean distance b/w descriptor\_a (64xN) and descriptor\_b(64,M).
- (L) Find, Extract and determine number (k) of the best matches points that has a smallest distance between the matches points by \([r \ c] = \text{find_best}(\text{dist\_mat}, k)\)
- Extract matches points coordinates \( x_1, y_1 \) and \( x_2, y_2 \).
- Calculate \( \Delta y, \ pos\_y = \text{size}(find(\Delta y>0)) \) & \( \neg y = \text{size}(find(\Delta y<0)) \)
- If \( pos\_y > neg\_y \) (delete matched points with \( \Delta y<0 \)) else (delete matched points with \( \Delta y>0 \))
- If No. of matches > 2
  - \( \text{Shift\_x} = \text{median}(\text{sort(matches\_points, \Delta x})) \)
  - \( \text{Shift\_y} = \text{median}(\text{sort(matches\_points, \Delta y})) \)
  - Registe image(i+1) with (shift\_x, shift\_y) related to image(i)
  - \( i = i+1; \text{goto (F)} \)
  - Else
  - \( k=2^k; \text{go to (L)} \)
a) Results

To assess the use of the SURF and SIFT Algorithm [35, 36] and their replacements for registration, tests were made taking four different types of corneal images and then adding noise and known random shifts along the x and y axes.

As a practical example we used 51 ordered Stroma images 15 to 65 from subject 1. Two different amounts of Gaussian noise (10% and 20% of maximum image intensity) were added to the images along with random shifts along the x and y directions. Our comparisons between the four methods were in terms of the accuracy of registration and the time taken to complete the process. Results are shown in Table 4 with registration errors divided into minor differences (<=2 pixel) and major differences (>=3 pixel).

![Table 4: Comparison of use of original SIFT and SURF algorithms, SIFT algorithm_2 and SURF algorithm_2 to registering sets of 51 images (original images, images with 10% of Gaussian added noise and images with 20% of Gaussian added noise) after making random shifts to each image.](image)

Fig. 10: Two examples showing results of applying SURF_Algorithm_2.

The results are encouraging and show that the modified algorithms that filter the results of both original algorithms work well. These results indicate the following:

1) The registration methods can tolerate the presence of significant amounts of noise, which is important because the images of some layers will be darker and contain a higher percentage of noise than others. Also the enhancement of images during pre-processing (DCT filter and Gaussian smoothing) reduces the number of point matches between the images.

2) In our experience, the results from the original SIFT algorithm are more accurate than those from the SURF algorithm, but SURF is faster. However, for algorithm_2, both methods provide accurate results and SURF has the advantage of being faster.

3) The extra complexity of algorithm_2 does not cause much increase in processing time.

3.4. Object Labeling

In the sequences of correctly registered images, objects appear in several images and our purpose here is to identify the distinct objects within each image and label them consistently so a particular object has the same label in all the images in which it appears. This is important as it enables us to get an estimation of the depth of objects, which is important for any 3D modelling system we develop in the future; Fig. 11 indicates the main steps of this algorithm. We implemented this algorithm using MATLAB and C, and its main steps are as follows:

1) Pre-process the images by applying a high-pass DCT filter (second-order Butterworth with cut at channel number 10) to improve the uniformity of illumination, apply Gaussian smoothing with (σ = 2.2 pixels) to reduce noise, standardise the image contrast (by applying a linear contrast stretch to image histograms between 0.1% and 99.9% of the histogram area) and binarize using the Otsu method [14-16] removing small (noise) objects.
2) Apply connected component labelling to the first image in the sequence and store the details of each object.
3) Identify corresponding labelled objects in neighbouring images in the sequence and update their labels for consistency and uniqueness.
4) For visualisation, the interior of each object is filled with the same colour in all images it appears.
5) Painting with original or filtered intensities, the aim here is to generate an image registering and labelling which approximates reality.

![Image of the main steps of the Object Labeling algorithm](image)

Fig. 11: The main steps of the Object Labeling algorithm

a) Results

The proposed method was applied to the sequence of 51 images registered as described in section (2.3). We identified corresponding objects in the set of the sequence images in two different ways using the centre coordinates and the boundaries of objects, with generally similar results in the two cases. Fig. 12 presents some results using our proposed method, where column (A) shows registered images, column (B) shows binarized images; and column (C) shows objects segmented with colour. The centre coordinate approach to identifying corresponding objects in neighbouring image is simpler than the boundary approach but may not be as reliable in the following circumstances:

1) If, as a result of the noise say, a single object is seen split into two pieces in another image, the centre coordinates approach results in two new objects, whereas a boundary based approach may recognise the true situation.
2) A new object overlapping a different object (with different shape and size) in a neighbouring images may be identified as the other object by the centre coordinates approach whereas a boundary based approach may recognise the true situation.

![Image of a sequence of images](image)

Fig. 12: A sample from a sequence images, Subject1_015 to subject1_020. (A) Registered images, (B) pre-processed and binarized images, (C) Labelling indicated by colouring; and (D) Restoring original intensity.

4. Conclusions and Future Work

A confocal microscope can provide an in vivo sequence of images at different depths of the various corneal layers from which Ophthalmologists can extract clinical information on the state of the corneal. However, there are difficulties encountered during the automatic analysis of images. These are non-uniformly illuminated, noisy and sometimes dark. Due to subject movements, the images suffer lateral translations and may not be in the expected anatomical sequence. In this work, we have investigated novel applications for corneal image classification, image ordering, image registration, and labelling of objects within images. A simple but promising 3D model of the colour segmented objects in a sequence of confocal images is shown in Fig. 13.

Several points from our experiences are noteworthy and can be briefly described as follows:

1) We have addressed the classification problem by using an artificial neural network and considering four different statistical texture features applied to original corneal images and pre-processed images. The best performance was achieved with the Otsu thresholding and GLCM texture measure with an average accuracy of 0.994 using an ANN with 8 hidden nodes. This result was obtained by training and
testing the ANN on 84 corneal images. To validate the different methods of classification, the optimised ANN from each method was used to classify another 72 images (7 Endothelium, 35 Stroma, and 30 Epithelium) from the Tennent Institute of Ophthalmology. The best result (only two images misclassified) reported in Table 2. We can observe from the results given in Table 1 that the GLCM texture measures provide the optimum performance in the majority of the cases. The results may be less sensitive to the use of the DCT filtering because our system extracted the features from the middle part of the image as we explained in section (3.1).

2) Also in this work, we have addressed the problem of automatically reordering the images into their correct positions in the sequence. We have applied the SSIM method to compare the similarity between the images on the three different sets of images and used the DWT until 4 levels to compress the image size and decrease the time spent ordering the set of images by a factor of more than 20. We proved that this method provides correct and fast images reordering within 16 pixel shift in x, y-axis between each pair image. In the future we intend to continue work to reorder the images and resolve problems that prevent correct image ordering.

3) One of our aims is to provide efficient registration of a sequence of correctly ordered images. Starting with the well-known SIFT and SURF algorithms we have added a filter algorithm to remove mismatches and thereby improve the registration performance. RANSAC is often used in conjunction with SIFT and SURF to remove the mismatches in image registration. The assessments of all algorithms (original and modified) were carried out by taking four different types of corneal images and then adding noise and known random shifts along the x and y axes for testing registration. Two different amounts of Gaussian noise (10% and 20% of maximum image intensity) were added to the images with random shifts along the x and y axes. The results are encouraging and show that the method works well with corneal images and the added filtering improves performance with little effect on processing time. We have summarized results in Table 4.

4) The task of Segmentation corresponding objects (cells) in different images is the last step before building a 3D model. In this task, we addressed the problem of identifying views of all individual objects (cells) in the sequence of images. Two approaches based on comparing the centres or boundaries of objects in different images were considered applied to 51 Stroma images. Encouraging results were obtained allowing a simple 3D model of the Stroma to be constructed.

5) This work has some limitations. The performance of the labelling algorithm needs to be improved when dealing with objects (cells) splitting in two or more objects in subsequent images or the case when different object appear in the same position in different images. These issues will be tackled in the future work.

6) Our long-term aim is not to visualise the whole image in 3D. Instead we would like to develop. In the near future, a diagnostic system that will extract medical/statistical features from the corneal cells and track their evolution over time. It’s a feedback that we received from the clinicians we work with. Hence, it’s important for us to detect and extract features from the region of interests (RoIs) in 2D and then develop a technology to visualise the detected RoI to 3D (neglecting the background).

Fig. 13: Capture of 3D models created by ImageJ software [37]. (A) Labelling indicated by colouring; and (B) Restoring original intensity.
ACKNOWLEDGMENT

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