A novel computational method to quantify and analyse osteoclastic bone resorption.

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Footnote: Source files of "OsteoPro" can be obtained free at:

www.navis.demoscene.gr/osteopro1-source.rar

ABSTRACT

Bone destruction is a common feature of arthritis. Bone is resorbed by bone resorbing cells, termed osteoclasts. In medical research, quantification of the amount of bone resorbed areas is vital in understanding the resorptive capacity of the osteoclast under certain pathologic conditions, and its response to various treatments and pharmacological inhibitors. Validated image analysis algorithms and procedures, therefore, have become critical for elevating the quality of bone resorption assays results. As in all computational experimental methods in biology the pressure increases to make analysis transparent and reproducible. In this paper we present “OsteoPro” a novel software which has been designed specifically address those issues. “OsteoPro” is a "turnkey" application that functions with minimal human interaction, by making use of morphological operations and blob analysis to classify structures according to their hue, saturation and size. In these experiments we have cultured osteoclasts on dentine slices, and the amount of bone resorption was analysed with the “OsteoPro” software using the techniques described in this paper. Finally “OsteoPro” is compared and contrast with other generic image processing suites, and further enhancements of the procedures used are also discussed.

Keywords: Image analysis, bone resorption, osteoclasts, bilateral filtering, blob analysis.

INTRODUCTION

Bone resorption is carried out by osteoclasts which are large multinucleated cells formed by fusion of monocyte/macrophage precursors [1] and are of haematopoietic origin [2]. In medical research, functional evidence of osteoclast differentiation is obtained by a bone resorption assay system in which cells are cultured on dentine slices as described previously [3]**.** Dentine provides a smooth-surface mineralised substrate for the identification of bone resorption pits, which are only formed by osteoclasts. Since the 2000-2010 has been declared the bone and joint decade a great number of experimental work on bone and joints has produced a plethora of data in medical research of various disciplines; such as orthopaedics, rheumatology and cell biology. Recently, a great number of newly discovered growth factors and cytokines have been associated with bone resorption and an equal amount of pharmacological inhibitors remains to be tested. Data related to biological functions are of immense importance in the design of drugs and need to be analysed efficiently. The quantification of such results is therefore of immense importance, however, to date, there are no particular tools available for accurate interpretation of results. Researchers employ different methods for counting the extent of bone resorption such as point counting [4] or image processing suites such as ADOBE and Image J which are not particularly suitable for such functions. As an increasing need of using image processing suites for measuring biological functions develops, we designed this software that enable us to perform accurate measurements on quantification of bone resorption. “OsteoPro” is a "turnkey" application that functions with minimal human interaction, by making use of morphological operations and blob analysis to classify structures according to their hue, saturation and size.

METHODS

Osteoclasts were generated from synovial fluid macrophages of rheumatoid arthritis patients, and cultured on dentine slices as previously described [3]. These slices provide a smooth-surface mineralized substrate for the assessment of resorption pits. Dentine slices at the end of the culture period were rinsed in phosphate-buffered saline and left overnight in 1M ammonium hydroxide. The dentine slices were then sonicated to remove cell debris and stained with 0.5% toluidine blue. Resorption pits were examined by light microscopy using an Olympus microscope BX40 and images were taken using a Nikon Coolpix 4500 digital camera.

1. Image Processing

There are three sequential steps in the processing chain. Firstly, the image goes through a bilateral edge preserving smoothing filter [5,6]. The aim of this operation is to suppress noise and other small fluctuations that may exist. Most importantly, smoothing suppresses isolated, high frequency components of the image that are the result of specular reflection of camera lights on the surface of the dentine. In simple kernel filters, like the Guassian low-pass, a weighted average of pixels in the neighborhood is calculated, with weights dependant upon the distance from the central pixel. The outcome is an image with colors that deviate slowly over space. However, there is no provision for pixels that belong to an edge (where there is a 'step' transition), hence edges are also consequently blurred. The idea behind BiLateral filtering is that smoothing occurs within smooth regions only, with the edges preserved. Hence, similar colours are blended together (retaining overall shading) while perceptual boundaries and shapes are retained.

This initial step is performed only once, when the image is read. The smoothed image is passed to the second stage in which resorption pits (foreground) and dentine (background) separation is attempted, by means of thresholding [7].

Thresholding is based upon a simple concept:

$$f(T(\overrightarrow{P[i]}) > \delta) \rightarrow P'[i] = 1$$
$$else$$
$$P'[i] = 0$$

where $\overrightarrow{P[i]}$ is the 3 component (red, green, blue) color vector at *i*. *i* is a variable running through all pixels of image, P' is the output binary image, and T the transfer vector function which maps the color space into a scalar value, that is tested against the intensity threshold (δ). While there is not a single, generic solution that could handle all possible conditions (mainly color, depth, coverage of dye and lighting conditions), it can safely be assumed that we process well lit images, with bright, saturated purple colors for pits and white for dentine. Therefore a transfer function can be set so that the distinction between foreground and background is based on the saturation level of the 'purple' component of the color:

$T[(\overrightarrow{rgb})] = \max(0, \min(1, 0.5*(r+b)-g))$. $\overrightarrow{rgb}$ the colour vector, all components $\in$ [0..1].

2. Threshold calculation

One needs to find δ in order to segment T. There are plenty of mechanisms to do so, even though it is not always guaranteed that the images can be accurately segmented. This can

be determined by calculating the grayscale histogram of T: A graph presenting the sum of pixels with the same intensity values found in the image. At this point, T should produce a bi-modal histogram [8], i.e. one with two well defined clusters, one of which represents the bone intensities (low value) and another cluster the dyed areas (high value). “OsteoPro” can suggest a threshold (a value in between the peaks of the histogram clusters, a local minimum). The user can then slightly adjust it in real-time by means of a slider, until satisfied with the result. Once the threshold is set, P' holds a number of foreground and background elements. The coverage of foreground pixels over the image is easily calculated as: $\frac{\sum_{i=1}^{total\_pixels} P'[i]}{total\_pixels}$.

3. Blob (binary large object) Analysis

Blob analysis [9] is a process for tagging with a unique ID all different non-connected image elements (blobs). One can use blob analysis to detect blobs in an image and make selected measurements of those blobs. A loop processes all elements in P': If a pixel is background, or foreground and tagged, it is ignored if a pixel is foreground and untagged, a unique ID is set and a 'bucket-fill paint' algorithm is applied: For each neighboring untagged foreground pixel, tag pixel with ID and repeat recursively until no new pixel that falls into this category is found (this process is called blob labeling). The end result is a new map Pblob, where the separated structures can be identified by their relative size (the sum of pixels with a given ID over total number of pixels in image). In “OsteoPro”, the relative size of all blobs can be calculated, sorted, and then manually selected through a size 'band-pass' filter defined by two sliders. Blob analysis provides with a very

intuitive tool that can be used to isolate structures of interest on the images, or to exclude unwanted segments of noise. The latter is possible by allowing only structures with a size over, for instance, 10% of the overall foreground mass to take part in the coverage calculations, thus targeting 'salt & pepper' type of noise that was not eliminated by the bilateral filtering.

4. Software Specifications

"OsteoPro" was developed using C++ and OpenCV, an open source image processing library developed by Intel. "OsteoPro" requires a fast Pentium family PC (600+ MHz, 4mb hard disc free space), and basic OpenGL 1.0 support. All .dll's needed are included in the package, so no external libraries must be supplied by the user.

RESULTS

Images of resorbed dentine slices have been processed using “OsteoPro” Fig 1. It can be seen that the algorithm performs with satisfactory results when compared to what the end result would be like if it had been processed manually. Indeed this is the only measure one could use in order to estimate efficiency in a 'real-life' situation: How does a robot-vision algorithm contrast to the human perception. There is little point in trying to assess the effectiveness of “OsteoPro” by testing it against procedurally generated images, with set shapes or signal-to-noise ratios, because this configuration does not represent a real-life situation. In reality, there are many more complexities, colour and structure irregularities to take into consideration. One need also to take into account that if invalid data is entered in a computer program, the resulting output will also be invalid. The user should expect close to flawless results only if the dye is set on a well cut bone surface and photographed using top-notch hardware (lens, quality of electronics on camera etc.) with sufficient lighting.

DISCUSSION

The need for a tool like “OsteoPro” derives from the inability, in terms of ease of use, reproducibility, and throughput capacity, of the previously used methods to deal with the demands of such problems. These methods are normally put together by biology researchers in haste, without the necessary means (time or resources to consult more specialized personnel), and fall mainly into the categories of completely manual work and semi-automated work.

In completely manual work that involves the use of commercial 'drawing' software such as PhotoShop. The user draws around the regions of interest, by using own perception, completely unassisted by any automation. The resorption pits are then filled with black colour, against white background. The coverage (resorption area) is then calculated by looking into the histogram (provided by the software), or by requesting a 'Count black/white pixels on screen' operation. This method could be considered to yield the best results in terms of accuracy (since humans possess a superior ability to identity and classify abstract structures). However, it is a very slow and tedious process; with an estimated average time of 20 minutes for each dentine. Also, it makes it extremely difficult to measure the sizes of individual structures. One would need to repeat the process as many times for a single image, and do the calculations by hand.

In semi-automatic work with generic image processing suites, such as ImageJ may provide limited solutions. Although this is a step forwards compared to the previous

method, there are still drawbacks. It is required that the several different 'steps' of the algorithm described have to be batched, if the process is to be performed automatically, or applied manually and sequentially. Hence, the user has to have some background in image processing and be able to also program/manipulate the software itself. Furthermore, a subsystem is needed to be able to keep a log of operations or capture snapshots of the original image with the estimated structures overlayed-all of which are built into “OsteoPro”. It can be seen that several hurdles have to be overcome, usually by scientists specialized in fields other than image processing, for whom a 'turnkey' application that keeps its internal mechanism unexposed and provides with a clear input/output interface is required.

We believe that “OsteoPro” is a major step towards this direction and with further enhancement of the procedures used this software can be extensively used in medical imaging especially those data that frequently require high degrees of throughput, and need advanced data management and statistical analysis. “OsteoPro” has great potential for development to be used in assessing bone resorption and possibly inflammation from MRI scans and Power Doppler images. Recently data have indicated that high-frequency ultrasound and power Doppler are sensitive tools for evaluation of disease activity and assessment of response to therapy [10]. Power Doppler imaging may also have the potential to predict those patients most at risk of accelerated joint destruction. We believe that “OsteoPro” will play a prominent role in standardizing the use of these imaging technologies.

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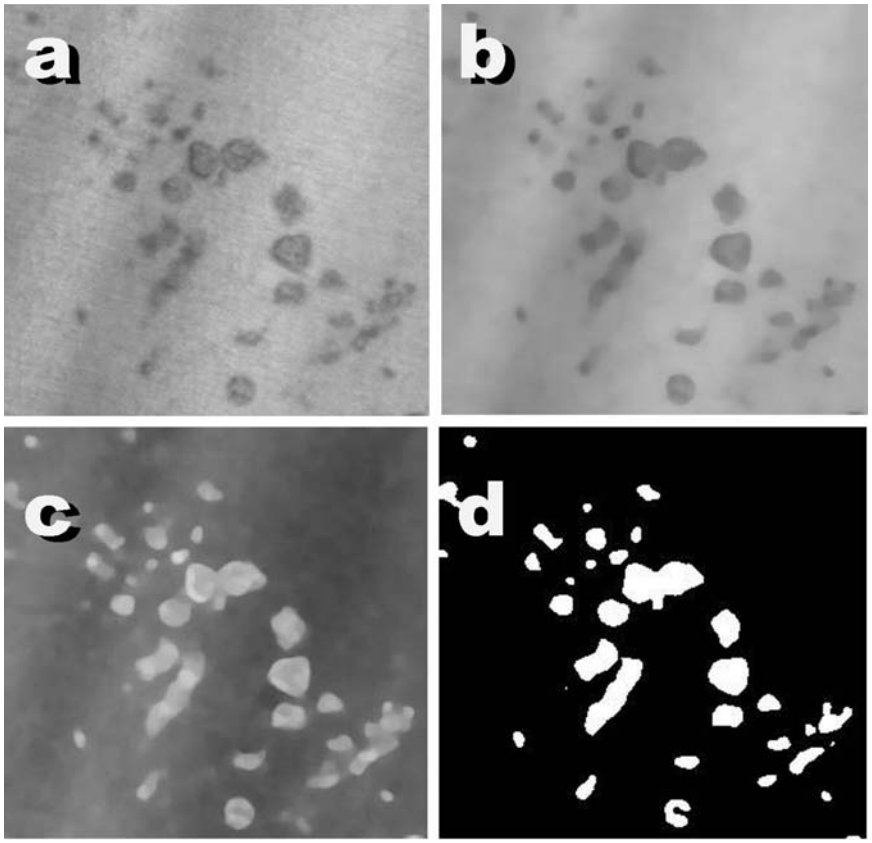
FIGURE LEGENDS

Figure 1. Images of resorbed dentine slices before **(a)** and after process using OsteoPro features, **(b)** bilateral edge preserving smoothing filter, **(c)** hue saturation domain and **(d)** thersholding in which a pits (foreground) and dentine (background) separation is achieved.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