

focus on Microscopy & Microtechniques

Frequency domain (Wavelet) investigation of OCT images of skin

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In this article we investigate the use of wavelet transform on the images produced by an optical coherence tomography (OCT) to obtain further information from the OCT images transformations in different levels of decomposition with two wavelet mother functions, in frequency domain. To transform the image from the spatial domain to the frequency domain, wavelet transformation of the image was used as it was found that the images obtained from the wavelet transform include more details than those obtained from Fourier transform. The OCT system employed for imaging was an en-Face time domain OCT which uses a dynamic focus scheme (Figure 1). With dynamic focus, the coherence gate is synchronised with the confocal gate; hence, the transverse resolution is conserved throughout the depth range and an enhanced signal is returned from all depths. Therefore, higher resolution images than those of standard OCT can be obtained. This OCT system is especially designed for use in applications where a high lateral resolution and a large depth range are required. The system uses a super luminescent diode (SLD) with a central wavelength of 1300 nm and a spectral bandwidth 54 nm.

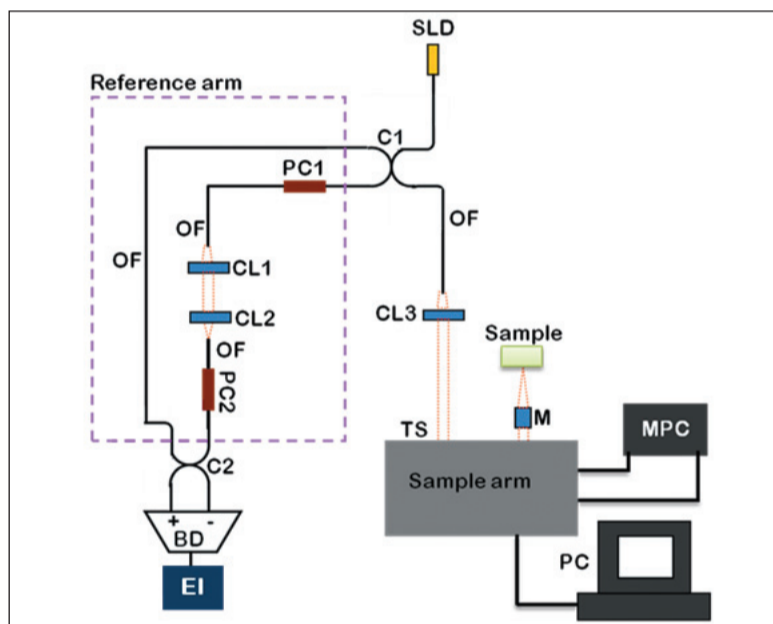


Figure 1. Dynamic focus time domain OCT (DF-OCT) optical set-up. SLD: super luminescent laser diode, BD: balance detection photodetector unit; EI: electronic conditioning signal interface; C1,2: 2 x 2 coupler, CL1,2,3: collimator lens, MPC: mirror positioning controller, PC1,2: polarisation controller; TS: translation stage, M: microscope objective, OF: optical fibre.

We utilised two wavelet transformation approaches: discrete wavelet transform (DWT), and stationary wavelet transform (SWT). The DWT is not a time-invariant transform which means that even with periodic signal extension, the DWT of a translated version of a signal X is not, in general, the translated version of the DWT of X . To restore the translation invariance, which is a desirable property lost by the classical DWT, SWT is employed. We applied the stationary and discrete wavelet transformations in different levels of decomposition with two wavelet mother functions, Daubechies and Symlet, more precisely sym4 and dB1, to the OCT images of fingertip of a 29 years-old Asian male (skin type III). The OCT images are cross section (B-scan) images. The results of imaging are illustrated in Figure 2 and 3. Of notice, that the images are enhanced using histogram equalisation before the wavelet transformations.

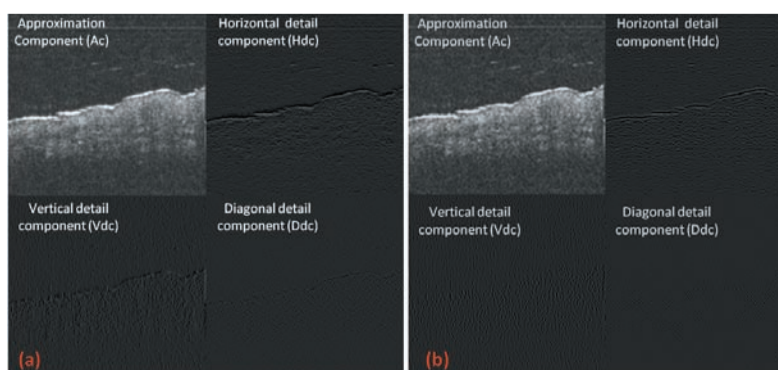


Figure 2. Application of stationary wavelet transform on the fingertip OCT image with (a) dB1 level2, (b) sym4 level2

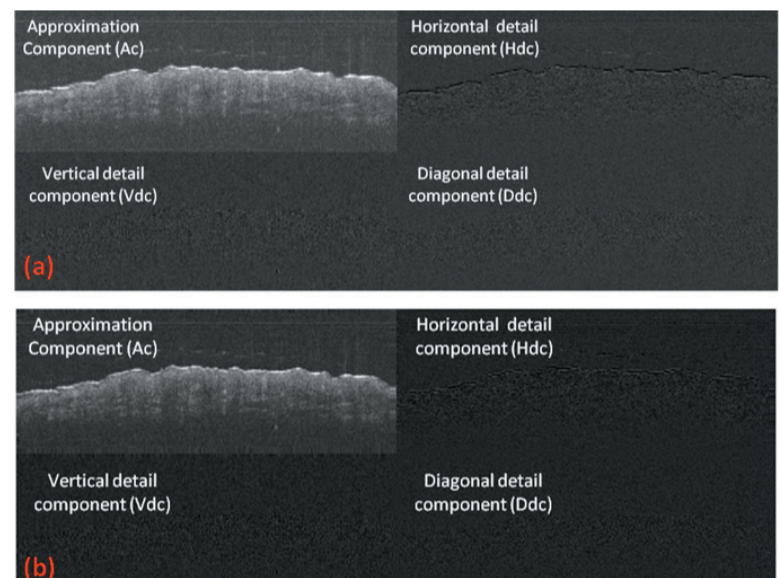


Figure 3. Application of discrete wavelet transform on the fingertip OCT image with (a) dB1 level2, (b) sym4 level2

We then applied Canny edge detector with the threshold value 0.4 to compare the information obtained from the components of SWT and DWT with two different wavelet mother functions. In Figure 4 and 5, the gradient images of approximation, horizontal and vertical components are demonstrated.

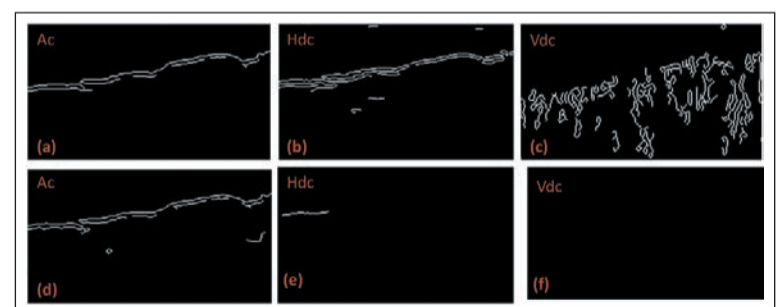


Figure 4. Canny edge detector with threshold 0.4, applied on the SWT components. Gradient image of (a) approximation component obtained with dB1 level2, (b) horizontal component obtained with dB1 level2, (c) vertical component obtained with dB1 level2, (e) approximation component obtained with sym4 level2, (f) horizontal component obtained with sym4 level2, (g) vertical component obtained with sym4 level2.

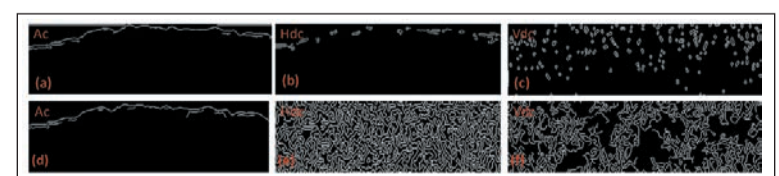


Figure 5. Canny edge detector with threshold 0.4, applied on the DWT components. Gradient image of (a) approximation component obtained with dB1 level2, (b) horizontal component obtained with dB1 level2, (c) vertical component obtained with dB1 level2, (e) approximation component obtained with sym4 level2, (f) horizontal component obtained with sym4 level2, (g) vertical component obtained with sym4 level2.

Looking at the images in *Figure 2 (a and b)*, one can see the sweat ducts in the vertical detail component image. There are indications of sweat duct and sweat gland in the horizontal detail component images of dB1 produced by either DWT or SWT. Stratum corneum can be detected easily from the horizontal detail component image produced by either dB1 or Sym4. The images produced by wavelet mother function dB1 seem to be more informative than those produced by Sym4. The vertical and horizontal components obtained from the DWT compared to those from SWT do not carry significant information. (see *Figure 3 (a and b)*). In both images produced by stationary and discrete wavelet functions, the diagonal detail component image does not include much useful information. Using SWT, with dB1, the gradient images of the horizontal and vertical components are more informative than their approximation component. With SWT, sym4 does not demonstrate many details in the images. With DWT, this is the case with dB1. The horizontal and vertical components produced by sym4 are more informative than those by dB1. We only demonstrated the results of the transformation in the second decomposition level as with this level of decomposition the best results were obtained. The information that was obtained in the gradient image is helpful for the investigation of skin structure.

The preliminary results presented here, showed that the wavelet components and their gradients can add more details to the OCT skin images. This eventually helps the dermatologist to make a better decision in diagnosis.

References:

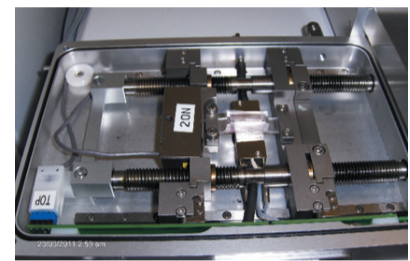
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Controlling Stress in Human Collagen Growth

Linkam Scientific Instruments have been chosen by the Physics Department of the University of Liverpool to study the effects of controlling stresses applied during the growth of collagen secreted from human fibroblast cells. The research goal of Senior Research Assistant, Dr Caroline Smith, and her colleagues at the University of Liverpool is to advance the treatment of tendon injuries by developing a method of growing oriented human collagen. To achieve this and to gain a fundamental understanding of the process, Dr Smith is using the optical technique of reflection anisotropy spectroscopy to monitor the extent to which collagen, grown by mouse fibroblast cells on elastomeric substrates, is oriented when subjected to a regular uniaxial stress mounted in a Linkam TST350 tensile stage.

The ability to choose a wide range of cyclic speeds to relate back to the growth of collagen is very useful. Initially, Dr Smith thought about making her own TST but having a small unit that could control the cyclic stress and also measure stress/strain curves at the same would be very difficult to manufacture in-house. Hence, the Linkam unit was purchased.

The TST350 stage is built with two precision ground stainless steel lead screws to maintain perfect uniform vertical and horizontal alignment. The sample jaws move in opposite directions to maintain sample in both reflected and transmitted microscope fields of view. Temperature control and accuracy is excellent with a range from -196°C to 350°C with 0.01°C control and up to 30°C/min rates, with virtually no temperature feedback to the measurement of force. Samples can be quickly loaded into the jaws and a test run can be performed in seconds. The data feedback from the force transducer, designed and built in-house, can be used to display an online plot of the force/distance when the jaws are moving at constant speed or when speed is varied to maintain a constant force. The speed of the jaws, force applied and distance moved can all be varied relative to the cell temperature. The sample chamber is sealed and can be controlled with various inert gases via the precision click fit valves built onto sides of the stage.



Circle no. 461

Temperature Controlled Sample Holder and Fibermetric

Phenom-World launches the Temperature Controlled Sample Holder and improved Fibermetric application for the Phenom™ G2 desktop scanning electron microscopes. Prolonging the viewing time of vacuum-sensitive and vulnerable samples with the new Temperature Controlled Sample Holder and execute more analysis on your fibre materials with the improved Fibermetric application. With these two launches Phenom-World further extends the usability of the Phenom G2 desktop SEM's. Giving the opportunity to image vacuum-sensitive and vulnerable samples without damaging the sample structure by using the Temperature Controlled Sample Holder. Secondly the new version of Fibermetric is able to take the analysis of fibres and filter to an even higher level. In close cooperation with its preferred development partner Deben, Phenom-World has developed a Temperature Controlled Sample Holder to study vacuum-sensitive and vulnerable samples. This active sample holder is designed to control the temperature of the sample between -25°C and +50°C.

With the use of the Temperature Controlled Sample Holder, the temperature of the sample is manipulated and therefore the humidity around it can be controlled. This enables imaging of moistures and water containing samples as well as reducing the effect the electron beam has on beam sensitive samples. This results in an extended viewing time, without noticeable vacuum artifacts. The Temperature Controlled Sample Holder can be retrofitted to all versions of the Phenom G2 system.



The improved Fibermetric allows measurements and analysis on complicated fibre structures, ranging from spunbond and electrospun fibers to the melt blown type of fibers. The Fibermetric application provides accurate size information from micro and nano fibre samples. Through further automation of several important features, the Fibermetric has become more user-friendly and guarantees a fast return on investment. The automated features that have the most effect on this are the high number of measurements, the automated feature and fibre size detection, and the analysis of the data points. The large range of fibres size allows Fibermetric to be used in a wide variety of applications, like investigation control.

Circle no. 462

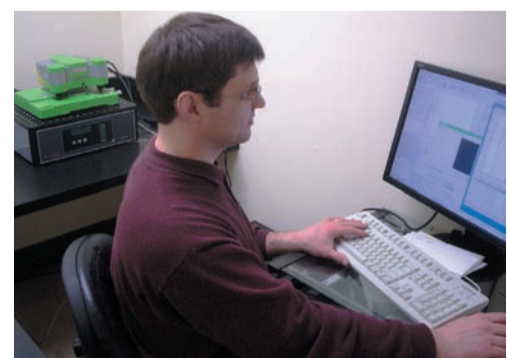
High Volume Single Molecule Force Spectroscopy Results

JPK Instruments reports on the work from the Pharmaceutical Sciences Department of the Medical School at the University of Nebraska. The Department has selected the JPK ForceRobot® 300 system to extend their studies applying atomic force microscopy, AFM, in the measurement of single molecule force spectroscopy.

Professor Yuri Lyubchenko heads a research group at UNMC in genomics. Their goal is to unravel the role of the DNA dynamics at different levels as key mechanisms for various DNA functions including gene regulation, DNA recombination and the mismatch repair with the major focus on DNA recombination.

Lyubchenko's group has published widely using AFM as both a direct and complimentary tool to advance their understanding of biological processes, some of which are relevant to human health. Single molecule force spectroscopy is used to study interactions between peptides and proteins involved in the development of these neurodegenerative diseases. AFM helps to bridge the gap of various techniques, which are capable of looking at the complex processes of protein misfolding and aggregation. However, it has the drawback of being very time and labour consuming to make measurements. This has been solved by the introduction of JPK's ForceRobot® 300 system into the group.

One of the key researchers in the group is Dr Alexey Krasnoslobodtsev. Speaking about his research, Krasnoslobodtsev said: "AFM is my instrument of choice in my work towards a better understanding of these protein misfolding and aggregation phenomena. By measuring strength of interactions between protein molecules, it is possible to detect pathological misfolded conformations of proteins, which are capable of triggering aggregation. Such conformations are characterised by elevated propensity to interact with one another. It is hoped that better understanding the mechanisms underlying self-assembly of proteins and peptides into nano-aggregates of various sizes and morphologies would eventually facilitate the development of efficient therapeutic and diagnostic tools for diseases associated with protein misfolding."



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