Optimised reflection imaging for surface roughness analysis using confocal laser scanning microscopy and height encoded image processing

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Abstract. Quantitative surface measurement is an important field in engineering. Due to the complexity of surface topography, accurate surface characterisation requires three-dimensional (3D) surface measurements not provided by many current measurement systems. A non-destructive and versatile technique for quantifying 3D surface features is Confocal Laser Scanning Microscopy (CLSM). However, there is little documentation on standard CLSM hardware settings required to capture images of suitable quality for 3D surface measurements. Understanding the complex relationship between CLSM settings, specimen properties and image quality is crucial to optimising the acquisition process for quantitative 3D surface measurements. The response of image quality to variations in CLSM hardware settings and specimen properties has been investigated in the study. Through the investigations, criteria have been developed to select optimal CLSM hardware settings to minimise image noise, eliminate image distortion, maximise contrast and resolution for reliable and accurate 3D numerical surface measurements. A reliable 3D image analysis system has been developed for image processing and surface measurement of engineering surfaces and small particles. The image analysis system developed in Matlab for the confocal system provides a new means to quantitatively characterise a wide range of engineering surfaces with accuracy and efficiency.

1. Introduction
Confocal laser scanning microscopy (CLSM) is highly suited to the inspection and analysis of three-dimensional (3D) surface structures that is both non-contacting and un-intrusive to the specimen [1,2]. The technique is recognised for significantly improved lateral (xy) and axial (z) resolution for image sectioning [2,3]. Reflection imaging has been successfully applied for the visual inspection and in particular the measurement of surface structures including semi-conductor, engineered and worn metallic surface [1,4,5,6] and wear debris [7,8].

The key benefit of CLSM is the confocal aperture that reduces light from above and below the focal plane (background light) contributing to image formation, enhancing both lateral and axial spatial resolution by significantly improving image contrast [1,2,9]. Compiling a series of 2D images captured at (z) step size through a specimen into a 3D data set, provide pixel intensity and sub-micron spatial information well suited to quantitative surface measurements. For engineered and worn metal surface research, 3D reflection images are compressed into 2D maximum brightness images (MBI) or 2D height encoded images (HEI) [7]. The 2D MBI is compiled from the brightest pixel in each (z) column at each (x_i, y_i) coordinate of a 3D data set, providing sub-micron spatial and intensity information useful for visual inspection, contrast measurements and basic 2D numerical analysis of
surface structures. The HEI contains height information rather than intensity, providing sub-micron spatial information \((x, y, z)\) well suited to 2D and 3D numerical surface analysis \([7, 8, 10]\).

Three dimensional measurements involve a high degree of accuracy when capturing information \([11]\). Important aspects in obtaining suitable quality images for quantitative measurements are reducing image noise, enhancing visible contrast to maximise available resolution \([3,12]\). Image distortion caused by scanning system accuracy, lens aberration and specimen properties that degrade the quality of images \([2]\) are also issues to consider in optimising the imaging process for surface analysis. Many CLSM parameter settings contribute to 3D image quality, governing the structural information extracted from 3D data sets \([2,3]\). Variables fundamental to CLSM optics and the electronics of system control each contributes to the quality captured images \([2,3,12]\). As the specimen is part of the optical system \([2]\), specimen optical properties and surface topology interact with light to provide several modes of image contrast including phase shift, interference, reflectivity, fluorescence etc \([13,2]\), all of which need to be distinguish from reflection data.

Understanding the complex relationship between CLSM settings, specimen properties and image quality are crucial to optimising the acquisition process for quantitative 3D surface measurements. This research investigated the CLSM image acquisition process and analysis system for 3D numerical analysis of engineered and worn metal surface structures. The response of image quality to variations in CLSM hardware settings and specimen properties were tested. The effects image quality has on the accuracy and reliability of the HEI generated surface measurements were also highlighted. Key objectives were to determine optimal CLSM hardware settings for reliable and accurate 3D numerical surface measurements regardless of surface reflectivity or surface finishes.

2. Methodology

2.1. Study of hardware settings for high quality images for numerical analysis

Hardware setting and resolution were tested for the Bio-Rad Radiance2000 CLSM using the Nikon LU Plan 50× lens (0.8 NA), 488 nm laser and a ¼ wave polarisation plate above the objective. Axial resolution was measured using a front surfaced aluminium optical flat. Lateral resolution was tested using aluminium 1200, 1800, 2400 and 3600 sinusoidal line/mm holographic diffraction grates to determine zooming that eliminated liasing in captured MBI. These grates also confirmed smaller confocal aperture size improved contrast for optimal lateral resolution in MBI. Resolution for HEI is substantially less and the sinusoid surface structures could not be faithfully reconstructed as 3D surface maps. Minimum zoom for the HEI was determined using a 600 line/mm ruled diffraction grate (≈ 0.25 um high sawtooth profile). Image distortion associated with scanning system error and specimen induced height encoding error were key aspects focused on. With 512×512 pixel frame size, most zoom settings above 1.5 for the LU Plan 50× exhibited no field curvature. Image distortion resulting from 25 Hz, 50 Hz, 166 Hz, 500 Hz and 750 Hz line scan rates were also tested using the 600 line/mm grate providing a measure of x–y scale distortion and scanning system instability. Both the optical flat and 600 line/mm grating were essential in identifying height encoding error resulting from specimen optical properties and optical effects caused by surface structures. Pixel statistics including mean intensity \((\mu)\), standard deviation \((\sigma)\) and coefficient of variance \((CV = \sigma /\mu)\) were obtained from capture MBI histograms. CV was a useful measure of image quality indicating noise levels associated with varied laser, PMT gain, scan rate, frame averaging, confocal aperture and specimen reflectivity. Variable tests involved imaging the optical flat in addition to stainless steel, brass and copper surfaces polished to a stylus roughness of ≈ 0.015 um. High zoom during testing reduced large field of view surface variations that can affect CV. Height encoded images were used to identify the influence each variable had on 3D HEI construction and numerical surface analysis.

2.2. Numerical analysis of surface roughness using computer image analysis techniques

In order to develop a computer assisted CLSM surface roughness measurement technique, a set of image processing and analysis techniques have been developed or used in the following two steps.
(1) Maximum brightness & height encoded images formation

Key techniques for this step include: (i) noise reduction to remedy the effects of light interference, (ii) the formation of MBI and HEI using a series of 2D image and (iii) filtration which separates long wave (waviness) and shortwave (roughness) components from profiles [14]. The above image processing has been implemented in Matlab to generate high quality 3D images readily for numerical analysis which is briefly discussed as follows.

(2) Image analysis using numerical descriptors

Using the combination of 3D CLSM imaging and HEI processing, 3D spatial information of surfaces are easily stored for 3D measurements. The technique has been successfully developed and employed for both 2D and 3D numerical analysis of engineered metal surface, worn metal surface and for the analysis of small metal wear particles [8]. An important consideration in characterising surface properties is the selection of appropriate numerical parameters. In addition the complexity of surface topography cannot be quantified with any single parameter. Therefore a broad selection of 2D and 3D parameters [15] has been used in the development for the numerical analysis of images captured of engineered and wear particle surfaces.

3. Results and Discussion

The 50× lens FWHM axial resolution was approximated at 0.8 µm using the smallest confocal aperture setting of 0.7 mm. With appropriate zoom each holographic grate was resolved except the 3600 line/mm. The 2400 grate required 6.4 zoom or more to eliminate liaising in the MBI when aligned horizontally or vertically. Ideal contrast and line definition was achieved using 0.7 mm aperture setting for vertical grate orientation and 2.2 mm for horizontal orientation. With a 0.7 mm aperture, imaging the 600 line/mm ruled grating with zoom 1.8 was sufficient in generating MBI without liaising, when orientated vertically and horizontally in the field of view. Slightly lower zoom may have been sufficient, and would confirm a factor of 4 between zoom required to image the 600 or 2400 line/mm graters. Height encoding images of the 600 line/mm grate found zoom 6 or more was required to generate HEI resembling the saw tooth structure and was only achieved by aligning the structure to slope down to the microscope back (lines horizontal). Figure 1 illustrated the HEI and MBI quality at four positions 90° apart. Not shown in Figure 1 are the effects of removing the ¼ wave plate, which rotated the ideal position 90° clockwise to slope down to the right (lines vertical). This effect identifies the significant interaction of plane polarised laser light with surface structures, particularly near detailed edges. To alleviate this issue a rotatable ¼ wave plate and algorithms for the HEI software will further be investigated. In addition the need for a 0.7 mm and 2.2 mm aperture to obtain ideal image definition when imaging the 2400 line/mm grate in vertical and horizontal alignment will also be further investigated. Slow scan rates were found to have the most significant effect on MBI and particularly HEI. The 25 Hz, 50 Hz and 166 Hz produced scan jitter through a (z) series of 2D images and slight
shrinking in the width of the imaged periodic structures, when compared to images captured using 500 Hz. The 500 Hz scan rate with Kalman 4 frame averaging produced the best quality images without jitter. Measurements of the vertically and horizontally aligned 600 line/mm grate agreed well with the manufacturer specified 1.666 um peak to peak line spacing. Since the 500 Hz scan speed was used for CLSM servicing and pixel calibrations, this setting was used throughout testing. Using Kalman 4 frame averaging, 500 Hz scan rate, 0.7 mm confocal aperture and zoom 999, optimal settings for laser power and PMT gain were determined for imaging the aluminium optical flat, brass, stainless steel and copper surfaces. Laser power ranging from 1.5, 1.75, 2.5 and 3% respectively for the different metals provided the lowest CV reading, with higher CV indicating greater pixel intensity variation due to noise. PMT gain ranging from 0.27, 0.45, 0.05 and 0.06% respectively for each metal surface also provided the lowest CV values. Although image noise could be minimised by optimal selection of laser and PMT gain, final height encoding of these images found they had little influence on the HEI software locating each surface consistently within the same height range. From these test it was determine for the CLSM in use that PMT settings below 0.005% and the lowest laser power should be avoided as instability of these setting caused HEI error.

4. Conclusion
This is the first time that the development of comprehensive techniques for surface analysis using CLSM has been systematically conducted. The capability and resolution of CLSM have been thoroughly studied in the project in relation to suitable images for quantitative analysis. Key settings of the CLSM for obtaining appropriate images for the purpose have been carefully examined and tested. Values of the key settings along with other important settings have been recommended for numerical analysis. A set of image processing techniques for noise control, profile filtration and 3D image reconstruction have been developed and implemented in the system together with a comprehensive set of 2D and 3D numerical descriptors. This development has provided a new means to quantitative surface measurement using confocal laser scanning microscopy.

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