Materials Science-Poland, Vol. 23, No. 2, 2005

New possibilities of light microscopy research resulting from digital recording of images

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New research possibilities related to recent improvements in light microscopy are discussed. New research microscopes are fully automated and motorized. All functions, such as those connected with focusing or stage positioning, can be controlled with a PC. Techniques which combine images recorded at different locations on *X*, *Y* and *Z* axes are presented. The first technique – combining high resolution single images, made in *X* and *Y* axes on the surface of a specimen, creates overview images of specimens. This is particularly important in quantitative materialography because the technique produces a significant decrease in the edge effect. Examples of applications of PC controlled *X-Y* stage and stage management software in the study of a duplex steel, made from Ni-base superalloy and welding are presented. Automatic alignment of images made in applications of this technique in the study of a zinc layer on cast iron and thermal barrier coatings on a heat-resisting alloy are shown.

Key words: *digital imaging; extended focal imaging; motorized stage; image analysis software*

1. Introduction

Progress in light microscopy has been made since the invention of the first simple microscope in 16th century. The main improvements made in recent decades have had a great influence on obtaining images rich in detail. The most important examples are: refinement of microscope illuminators; introduction of new lenses and multilayer non-reflective coatings; study of a new generation of universal objectives for passive light and reflection for greater fields of vision and working distances. These achievements have an influence on more effective correction of optical system defects. Thus, obtaining a sharp image with more contrast and constant beam intensity over the whole

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field of vision becomes possible [1]. Moreover, the application of systems for changing magnification, zoom systems, and enriching a microscope with a number of useful devices for sharp images in all observation outputs, i.e. infinity-corrected optics [2, 3], is conducive to digital image recording. In addition, improvements to all mechanical systems and increasing the rigidity of microscopes is of significance, because these all affect ease of use and are propitious for the introduction of automatization and management of the stage.

It is important to emphasize that all the described changes have significance for the digital recording of images. The latest digital cameras, combined with powerful computer software, offer image quality that is comparable with traditional silver halide film photography. Moreover, digital cameras are also easier to use and offer greater flexibility of image manipulation and storage. Digital imaging is increasingly applied to image capture for microscopy – an area demanding high resolution, colour fidelity and careful management of, often, limited light conditions [4]. The quality of the final image, both digital and film, is dependent on the quality of the original microscopic image.

2. Experimental

All digital images presented in this paper have been produced using an Olympus DP70 digital camera system coupled to an inverted stage metallurgical microscope Olympus GX71 with infinity-corrected optics. The microscope has a mechanical z-drive and mechanically operated stage. It characterises by super-wide field, zooming and a mirror cube turret for BF, DF, DIC and polarized light. Combining the microscope with the DP70 Olympus digital camera enables sharp images to be recorded, because the DP70 can gather extremely high resolution images, equivalent to 12.5 million pixels in only 3 seconds [5] as well as high sensitivity coupled with high -speed processing. The images obtained can have 36-bits of RGB colour depth which is essential for recording subtle colour gradations and intensities, and, as a result true, the natural colours of images.

The microscope is equipped with image analysis software analySIS supplied by the German company Soft Imaging System GmbH. The software offers image acquisition, evaluation and analysis, data archiving and the generation of professional reports. The motorized stage of the microscope enables analySIS modules to automate the processing. The microscope stage can be correctly controlled by the image analysis software. The Stage is a module for automatic service of the microscope stage which results in the operator having full control of all three axes: x, y and z. Two modules of this software using the motorized stage and the appropriate software represented here to give evidence of the possibilities of modern microscopy.

3. Results

3.1. Application of combining single images made in *X*-*Y* axes

The Stage Manager module from the analySIS provides the following possibilities:

• creating an overview image of the specimens by combining single images with high resolution – the resolution of the overview image can be adjusted (Fig. 1);

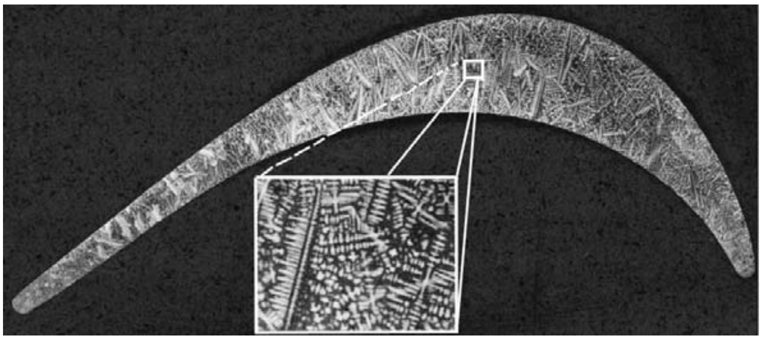

Fig. 1. An overview image of a dendritic microstructure turbine blade made from Ni-base superalloy René 77 (magnification 50×)

• defining individual stage positions for the acquisition of the series of images – the motorized microscope stage can be executed at the different positions (the positions and travel paths of the stage are defined in the Stage Manager – integrated into the analySIS [6]);

• making an estimation of inhomogeneity structure on the cross-section and longitudinal view of products (Fig. 2);

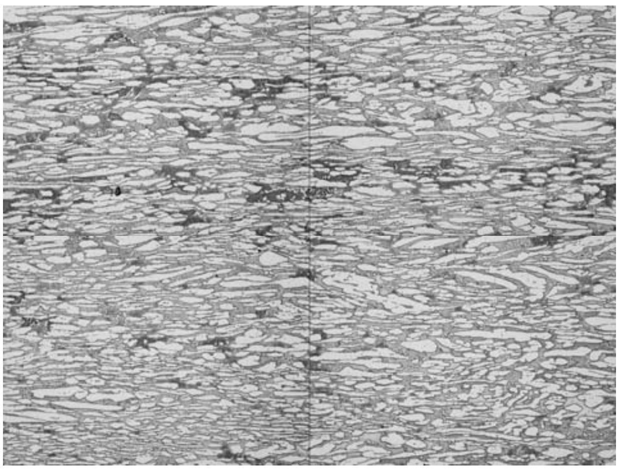

Fig. 2. A fragment of the overview image of duplex steel 2205 (magn. 200×)

- quantitative evaluation of materials microstructure;
- carrying out researches of materials macrostructure; the overview image is a high resolution image of a larger object (Fig. 3).

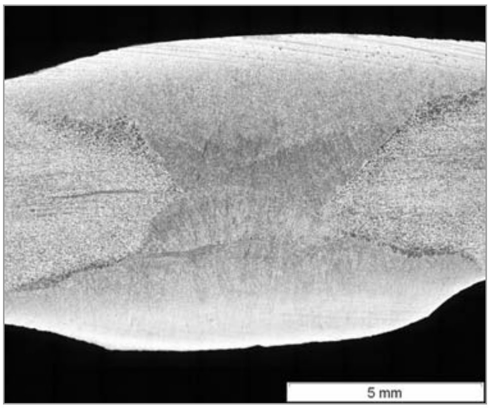

Fig. 3. Double-sided headwave weld of duplex steel made by welding with coated electrode Avesta 2205 AC/DC (magnification 1:1)

In the overview image only the object areas which have already been scanned can be displayed. The system positions individual images from which the overview image is composed in edge to edge mode. All individual images which belong to the current stage path are represented by frames.

A resulting digital overview image of the duplex steel is illustrated in Fig. 2. The importance of being able to combine single images is worth emphasizing in this case, for the reason that the duplex steel microstructure is characterized by two basic grain sizes. Using a higher magnification revealed fine grey grains of ferrite, but the measuring frame will cut long bright grains of austenite which were not taken into consideration in the analysis and evaluation. However, if one wants to avoid cutting grains of austenite by frame and use a smaller magnification, grains of ferrite are not visible. Thus an overview image avoids an edged effect and to observe through a microstructure both kinds of grains [7].

Figure 3 shows an example of weld overview made with the Stage Manager. It is an image consisting of 4 (2×2) single frames. An auxiliary grid has been removed to show the whole weld.

A MosaiX and a Panorama image acquisition module from Axio Vision Carl Zeiss [8] appears to be similar to the Stage Manager module.

3.2. Application of automatically aligning images made in *Z* axis direction

In many cases Extended Focal Imaging eliminates a restricting problem of light microscopy – limited depth of focus. The microscope's own depth of focus is only capable of focusing a limited range of height. The remaining parts of the image are then blurred, i.e. details which are visible on images taken with different focus settings are normally not visible in sharp details on a single image. Electronic image processing gives the solution to this problem. A series of individual images of the specimen is needed, which are obtained by adjusting the microscope focus for each image, i.e. separately focusing each image at different height. The module extracts the sharp details from each individual shot to determine a final image that is rich and sharp in all details, i.e. it seems to have unlimited depth of focus. To generate an EFI image from a focused series, the EFI software goes through the whole image series pixel by pixel. EFI determines which the image which contains the best focused pixel(s) of every image in the series [6]. Moreover, the EFI module can automatically align images that show a lateral displacement, for example if the images were taken with a stereo microscope.

Selected digital images with limited depth of focus at different locations of the images recorded at varying focus levels are shown in Figs. 4a–c, and in Fig. 4d – the reconstructed image which is of better quality and sharpness than the primary images.

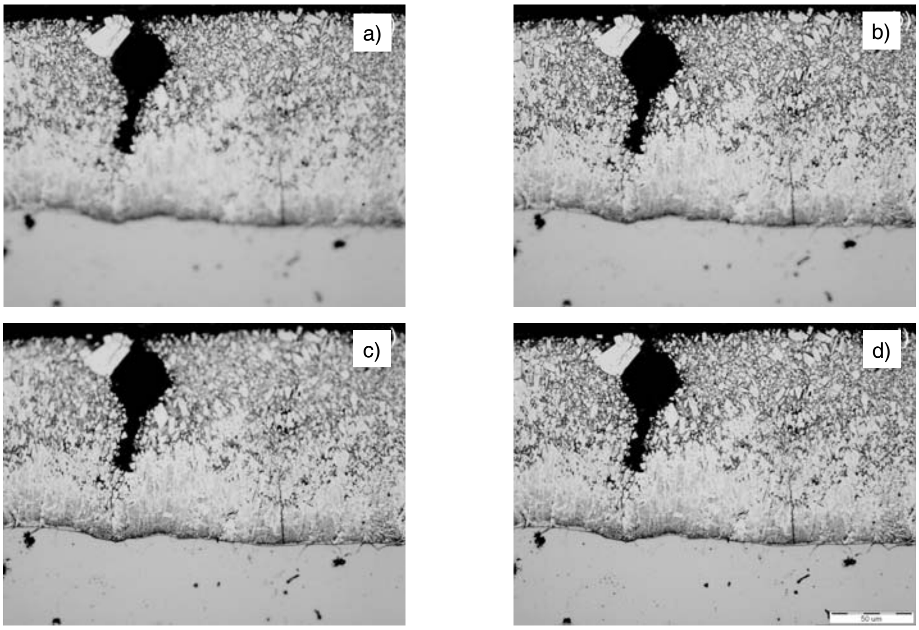

Fig. 4. Example EFI procedure – zinc layers on the cast iron (magnification 50×)

When used in a live mode, the EFI module provides both a live image as well as a partially reconstructed one. The program is able to focus interactively the missing details of the image which are not yet in focus and then add them to the current image.

The EFI has greater importance at higher magnifications. In spite of correct preparation of specimens to be examined at high magnification it is difficult to obtain a sharp image of the microstructure over the whole field of vision. The EFI module is very useful for observing surface layers of products and materials with phases having different properties which make it difficuot to obtain a structure without relief. Figures 5a–c present selected digital images with a limited depth of focus and Fig. 5d – a reconstructed image of another inhomogeneous material, a thermal barrier coating on a heat-resisting alloy, René 77.

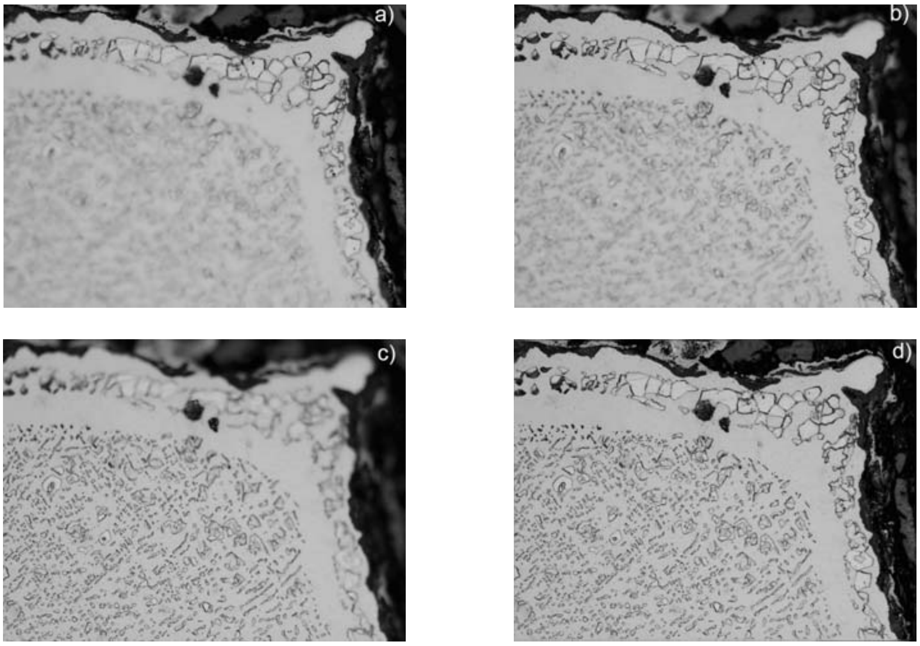

Fig. 5. Acquisitions of images at various focus levels and the reconstructed image following EFI procedure – Ni-based superalloy René 77 after aluminizing and oxidation (magnification 1:1)

On the basis of the whole series of images the EFI module provides an option to determine a height map. The height map can be used to create a 3-dimensional representation of the object's surface. The described module enables precise measurements of the roughness of the surface [6]. This is important for fracture surfaces permitting quantitative fractography research and in wear mechanism research. Moreover, creating a height map makes light microscopy similar to a confocal laser microscopy because of the reconstruction abilities. The latter also provides height maps of an object's surface. Classical light microscopy does not permit the construction of a single objective with a large depth of focus coupled with high resolution of the specimen details, i.e. true colour, high-quality images rich in detail, because the depth of focus is inversely proportional to the numerical aperture.

The appropriate software and microscopic equipment, i.e. the digital camera and motorized stage, can solve the problem of the limited depth of focus. A DeepView technique, Axiovert 200 MAT from Carl Zeiss company [9, 10] seems to be similar to the EFI module.

4. Conclusions

The examples presented show that digital recording of high resolution images increases the range of possible image analysis and accuracy of microstructural evaluation; without these techniques complete investigations practically are not possible.

The analysis of digital images essentially increases the possibilities of quantitative materialography and fractography applications. Thus the digital microscope ensures high performance and a diverse programme of examination.

Moreover, the digital form of images stored in the computer memory makes them especially suitable for various numerical transformations. The recorded images are immediately available and ready to be processed electronically, by e-mail or archive. Thus, the perfect complement to a digital camera for image archiving, measuring, analyzing or direct reporting is network-compatible image management software.

Acknowledgements

The work was supported by Polish State Committee for Scientific Research under grants Nos. 6 T08 2003 C/06083 and 6 T08 2003 C/06084.

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Received 6 September 2004
Revised 21 December 2004