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A Short Note on the Interference Layer Microscopy / Sparavigna, A.C., Giorcelli, M.. - ELETTRONICO. - (2017).
[10.2139/ssrn.3070405]

Availability:

This version is available at: 11583/2693885 since: 2017-12-01T08:30:50Z

Publisher:

SSRN Electronic Journal, Elsevier

Published

DOI:10.2139/ssrn.3070405

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A short note on the interference layer microscopy

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The interference layer microscopy is a specific technique of microscopy that requires the deposition on the specimen of a thin layer of a transparent dielectric material to enhance the contrast of the observed images. In white light, the different components of the specimen appear as having different and distinctive colors. In this manner, the specimen can be rendered in beautiful colored micrographs. Here, we shortly discuss this technique. We also propose an analysis of a colored image obtained by this technique, analysis made by means of a processing based on RGB histograms.

Keywords: Microscopy, Interference Light Microscopy, Composites, Color Metallography, Image Segmentation, Image Processing.

Today, the optical microscopy is still important for the inspection of the micro-structures of a great range of materials. It is also the fundamental support for a further microscopy, made by means of some scanning microscopes, such as the electron scanning microscopes for instance.

The optical microscopy can be used in transmission or reflection modes. Transmission mode is suitable for transparent specimens. In this microscopy, the contrast of the specimen arises from differences in the absorption of the light through its different regions. The transmission mode can be improved, in the case of birefringent specimens, by means of the use of polarized light. The reflection mode is given by the reflected-light microscopy, used for materials such as metals, ceramics and composites, which are giving non-transparent specimens. The observed contrast of the resulting micrograph, that we can record from this microscopy, is given by the variations in the surface topography and differences in reflectivity of the different regions.

In reflected light, the surface features are revealed by some specimen preparation techniques. One of this technique is the etching. It works because of a selective chemical attack of the surface of the specimen. As explained in [1], etching is used for the study of alloys with more than one phase, because it creates contrast between different regions through differences in topography or reflectivity. Etching is also used to evidence high-energy sites, such as boundaries and defects. The etching is obtained by means of a reagent, suitably chosen for the specimen under consideration.

Another method for enhancing the contrast is that of using vapor-deposited interference films on the specimen. In 1960 Werner Pepperhoff demonstrated that the microstructural contrast can be evidenced without etching [2-4]. On the surface of the specimen, a suitable material is deposited by using vacuum processes, to produce a thin, low-absorption, dielectric film with a high refractive index. The small differences in intensity and in optical phase angles which exist between the microstructural constituents are usually either invisible or barely visible under bright-field illuminations. The introduction of a thin interference film amplifies these minor differences by successive light reflection at the specimen surface, rendering the structure visible [2].

In Pepperhoff's microscopy, the sample is polished and coated with an interference film that can be examined by monochromatic light: the contrast is increased as the amount of light is reduced by interference [2]. Used materials are ZnTe, TiO₂, ZnSe and ZnS. In ceramography, that is, in the case of ceramic materials, since the components are exhibiting a low index of refraction and a minimal light absorption, this technique is used on a limited scale [5]. Let us also note that, when a white

light is used, this microscopy, which is defined as “interference light microscopy” [6], causes the different components to appear colored. Actually, the thin oxide films produce interference film color contrast between the different phases that are present in the specimen [7]. In [8], the theory, techniques for optimizing the performance and for the quantification of the results are explained. We can observe the effect of the deposition by means of some files that we can find in the Internet Archive, a non-profit internet library. Images are from NASA/Glenn Research Center, concerning a qualitative analysis of the “color metallography”, obtained by means of the interference light microscopy. Identifiers of images are ranging from C-1994-4961 to C-1994-4968, Rights: Public Domain, Year 1994. The web site address of the first image is <https://archive.org/details/C-1994-4961>. The ceramic composite is made of Silicon Carbide, Silicon, Carbon and Niobium Silicide. The specimen is shown in the Figure 1. On the left, we find the specimen seen in the optical bright field microscopy. In the middle, we have the specimen interference layered with lead oxide. Finally, on the right, we see a backscatter electron image of the composite.



Figure 1: Thanks to the images provided by the NASA/Glenn Research Center, we can see a micrograph obtained by means of the interference layer microscopy. On the left, it is shown the specimen in an optical bright field microscopy. In the middle, we have the specimen, interference layered with lead oxide. Finally, on the right, we see a backscatter electron image.

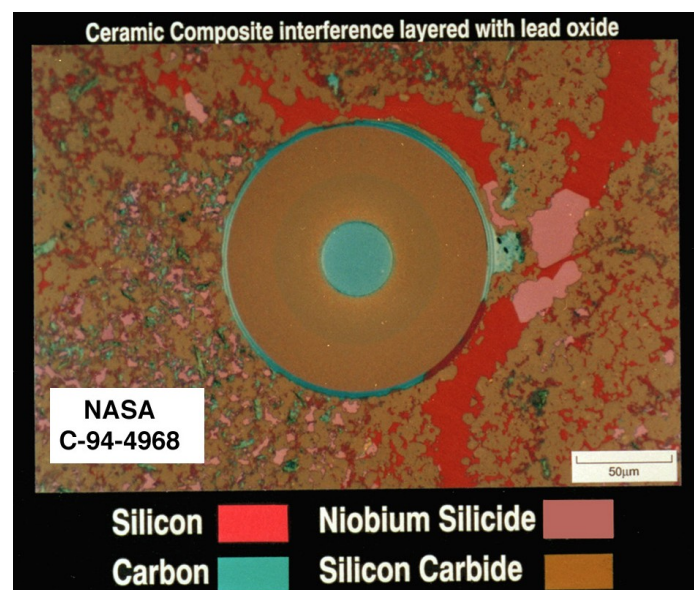


Figure 2: In the image provided by NASA/Glenn Research Center, we can see the components and the related colors from the interference layer microscopy. The bar in the image corresponds to 50 microns.

In the Figure 2, we have the correspondence between colors and components.

In optical bright field microscopy, the specimen seems recorded as in a grey-tone image, but it is not so. Both images (left and middle panels in Figure 1) are RGB images.

Are the two images giving the same information? That is, is the use of an interference layer deposited on the specimen relevant or not for a further image processing? Here, to answer the question we use the RGB histograms, that is the histograms of the three color tones (red, green and blue). The answer is important, because, at a first glance and as remarked in [7], the use of a thin film on the specimen appears as “particularly suitable to automated image analysis”.

Let us remember that in a colored image, each pixel can be characterized by three color-tones, the values of which are ranging from 0 to 255. An RGB histogram is giving the frequencies of the values of the three color tones appearing in the image.

In the Figure 3, we see the micrograph on the left of the Figure 1 and its RGB histogram. It is obtained by means of GIMP, the GNU Image Manipulation Program. The same is given in the Figure 4 for the image in the middle of Fig.1.

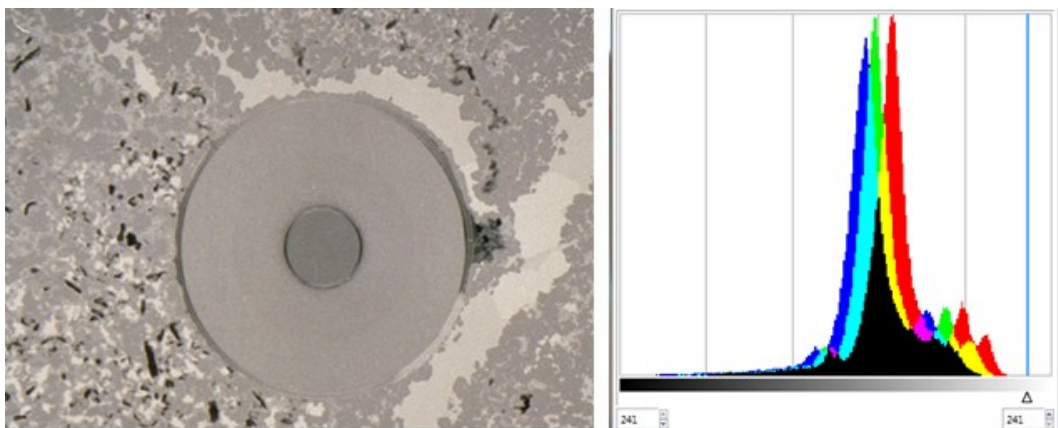


Figure 3: Image in the panel on the left of Figure 1 and its RGB histogram of the color tones.

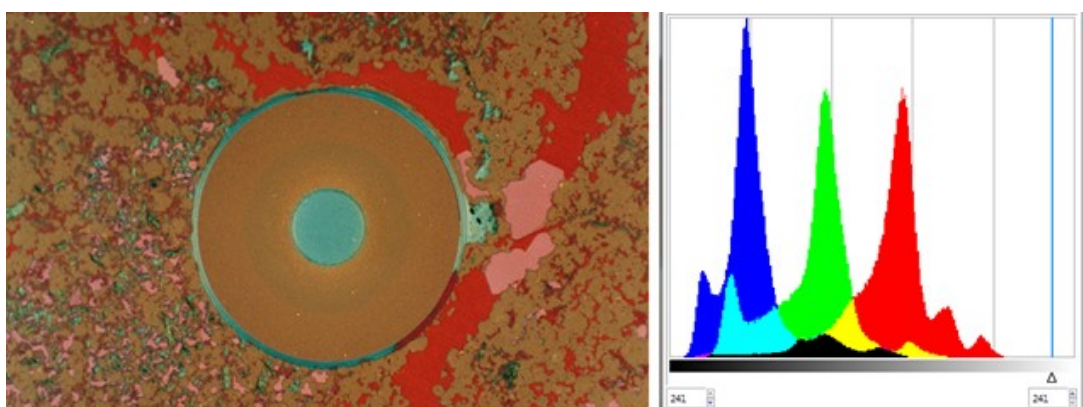


Figure 4: Image from the micrograph in the middle of Figure 1 and related RGB histogram.

It is evident from the Figures 3 and 4 that the histograms are different. However, to see how much the information contained in the two images is truly different, we need a further investigation and statistical analyses of the results. A possibility is that of using the image entropy [9-11], but here we can follow a different approach.

We know from Figure 2, that the components of the composite material are four. From the RGB histograms, we see four peaks too. By means of these peaks we can try to subdivide, or, as told in image processing, to “segment” the image according to the components. Let us concentrate, for instance, on the Silicon component and examine the histograms in the Figures 3 and 4. For the Figure 3 we use the red channel, and make a simple segmentation by means of the Threshold tool of GIMP. The result is given in the Figure 5: selecting the part of the histogram highlighted by the blue band, we are evidencing the pixels rendered in red in the image on the left, whereas the other pixels are black. The image is therefore segmented in red domains in a black background. If we compare these red domains with the red domains in the Figure 2, we have that the segmentation has evidenced the Silicon component of the material.

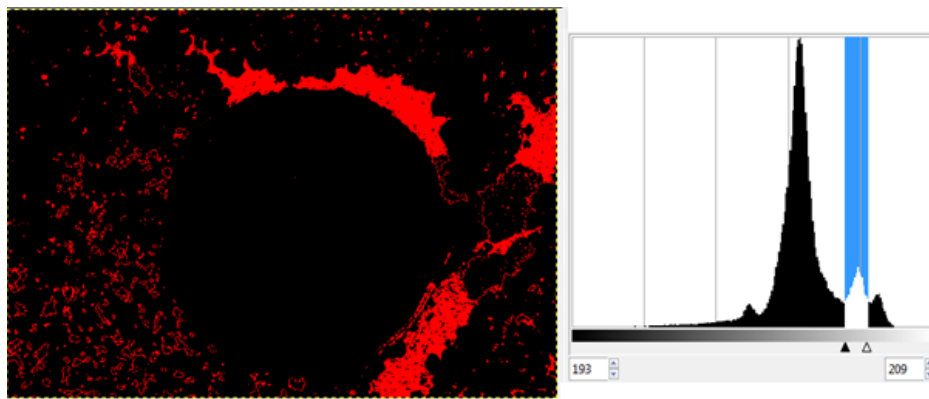


Figure 5: Silicon component, after the segmentation (red channel) of the image in the Figure 3.

We can repeat the same approach for the other components, obtaining the Figure 6. Actually, we have segmented in four parts the histogram of the red channel. Comparing with the Figure 2, we can propose that the four different segments are corresponding to the four components as in the Figure 7. Of course, we can use the approach also for the image in the Figure 4. In this case, we obtain the best results using the blue channel and its histogram (the segmentation is given in black and white to have a better visualization of the image). The result is given in the Figures 8 and 9.

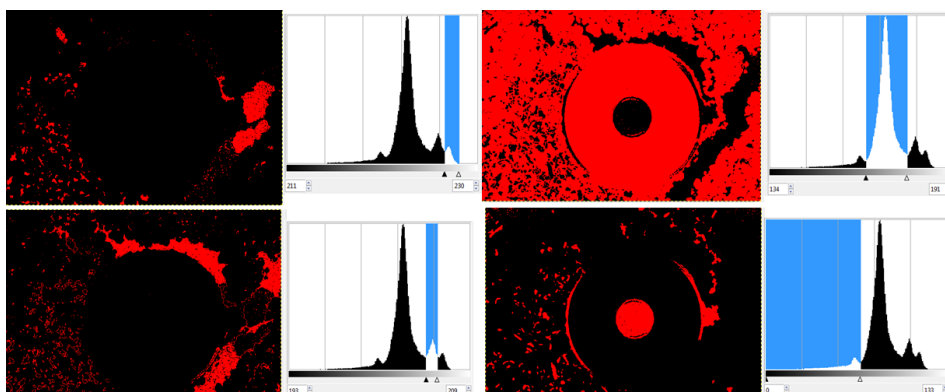


Figure 6: Using the approach explained in the text, that produced the Figure 5, we obtain the segmentation in four parts of the histogram of the red channel, in the case of the sample without the coating.

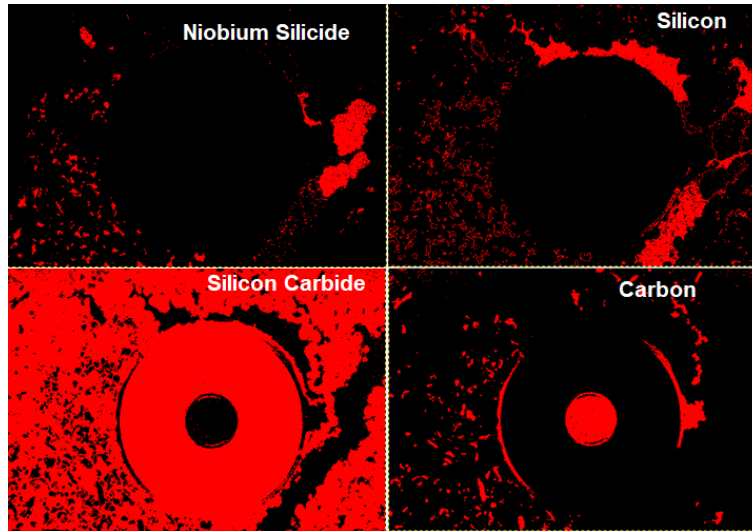


Figure 7: Comparing the results given in the Figure 6 and the map in Figure 2, we are proposing this segmentation of the four components, in the case of the sample without the coating.

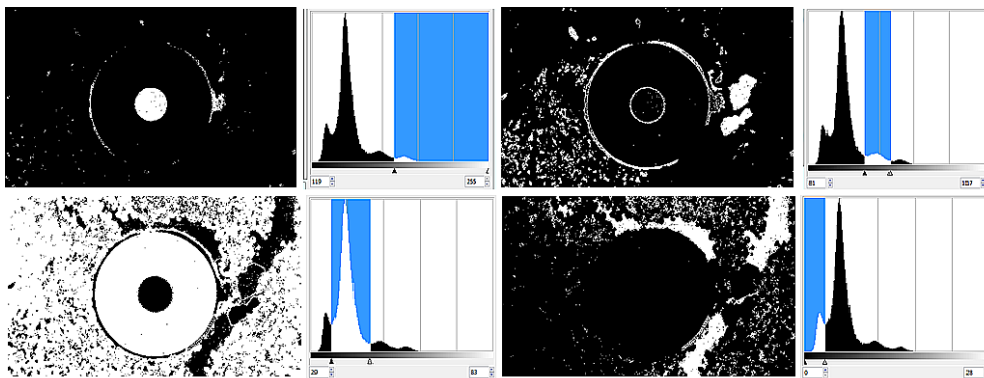


Figure 8: Using the same approach of Figures 6 and 7, we obtain the segmentation in four parts of the histogram of the blue channel, in the case of the sample with the coating (Fig.4).

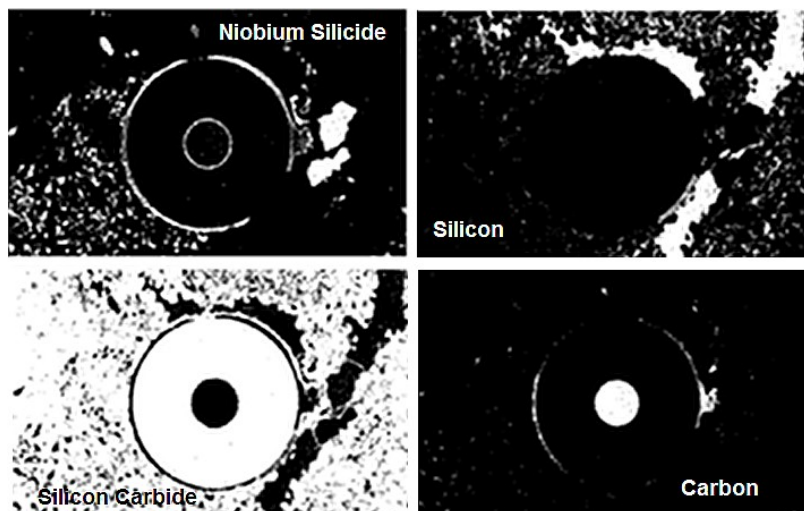


Figure 9: Comparing Figures 8 and 2, we are proposing the segmentation of the four components, in the case of the sample with coating.

If we compare the images in Figures 7 and 9, we see small differences. For instance, the domains of the Niobium Silicide are different in size. The segmentation in Figure 9 give more domains than segmentation in Fig.7. In the case of Carbon, it is the Figure 7 which is giving more domains. Actually, it seems that segmentation of the micrograph of the sample without coating is giving a result which is in good agreement with the map of Figure 2.

In this preliminary work, we have used a segmentation which is based on the subdivision of the histograms in some parts, for investigating the effect of the use of interference layers to improve the micrographs for automated analyses. Further studies are required, on other images of specimens recorded with and without the interference layer, to have a more general view of the problem. Also an improvement of the image segmentation could be relevant for continuing the analysis of the interference layer microscopy, in particular for what is concerning the use of RGB primary colors.

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