

## MOVING FROM COLOUR TO MULTISPECTRAL DIGITAL IMAGING IN OPTICAL MICROSCOPY

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Optical microscopy has greatly benefited from the last innovations in digital photography. It is now commonplace to manage huge databases of numerical images for archiving and retrieving of microscopical documents. For such kind of use, digital still video cameras based on large CCD sensors with megapixel resolution are the users first choice.

For sake of image analysis however, it appears that most microscopists do not often realize what are there technical requirements for proper imaging of microscopical objects. Too often, general public instruments are used instead of scientific imaging tools. It is the purpose of this contribution to show how a scientific use of colour measurements brings interesting improvements to phase identification in materials microscopy.

Still video and standard video cameras for personal use are mono-CCD cameras. This means that colour is obtained at the expense of resolution by using one pixel out of two for green measurements and one out of four for red and blue measurements. In other words, most pixel values are interpolated. To remedy to this 3-CCD professional colour cameras offer full resolution for each channel by means of an optical prism. Digitization is then operated on each individual channel allowing for real time (40ms) colour imaging. For materials microscopy video frame rates are often useless because with a few exceptions no movements are perceptible in the scene. Therefore, the simplest and most flexible way to build colour information is to rely on successive images taken with a single (black and white) CCD camera and adequate filters. These can be either R, G, B tristimulus filters or liquid crystal tunable filters or even interference filters with narrow bandwidth. Some manufacturers do offer such technology as rotating filter wheels integrated within or separated from the camera body.

A simple but important advantage offered by sequential filters with respect to 3CCD is the fact that integration timing (exposure) can be optimised for each filter (as long as this feature is offered by the CCD camera). This is particularly true for the blue region ( around 450 nm) where the sensitivity of silicon based CCD's severely drop. Table 1 compares exposure times with conventional 3-CCD cameras and with sequential filters technology, as defined when imaging iron sulfides in optimal conditions.

| <b>Filter</b> | <b>3-CCD TV</b> | <b>Single CCD and sequential filters</b> |
|---------------|-----------------|--|
| <b>Blue</b>   | 40 ms           | 3000 ms                                  |
| <b>Green</b>  | 40 ms           | 280 ms                                   |
| <b>Red</b>    | 40 ms           | 40 ms                                    |

*Table 1. Comparison between exposure times for 3-CCD and sequential filter technology.*

More interestingly, the sequential filter technology allows one to be freed from the human colour perception and rendering problem of the broadcasting applications and to investigate truly spectral properties for individual phases.

Using interference filters with narrow bandwidth (10 nm) fills the gap between spectrophotometric databases (Criddle and Stanley) and imaging, opening the way to computer assisted phase identification under the optical microscope.

The mode of operation for optimal acquisition (except integration time settings) has been described elsewhere (Pirard et al.) and a dedicated software has been made available (Ulysse : <http://www.ulg.ac.be/mica/ulyssse>) for monospectral and RGB imaging. It can be easily extended to multispectral problems.

The benefits of using multispectral imaging is shown here on images from a nickel ore. Figure 1 shows how the scene looks like under a blue filter with 3000 ms integration time as compared to the same scene with a 438±5 nm filter and 700 ms integration time. Figure 2 displays the scatterplots for 500 pixels taken as representative of each phase within the image.

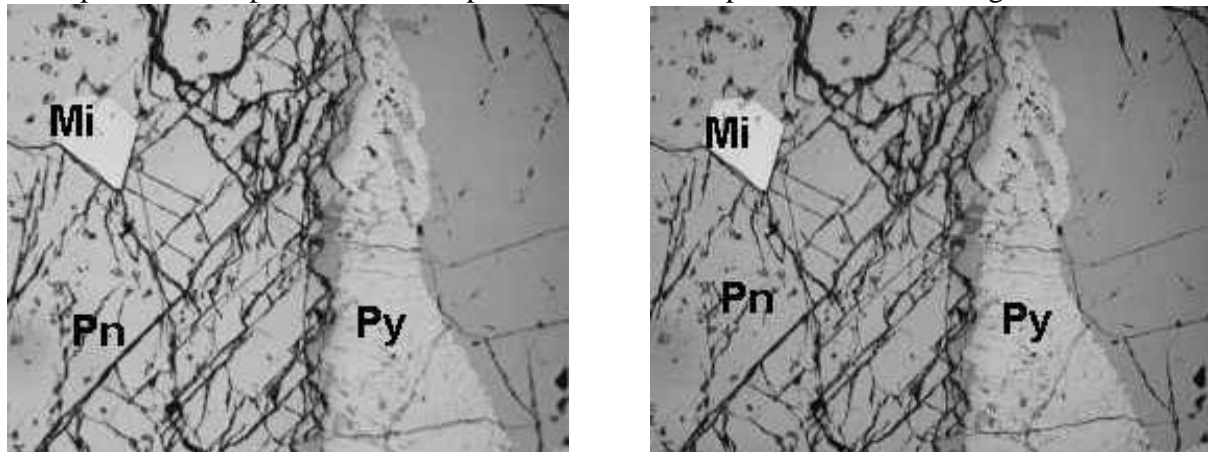


Figure 1. Images of a nickel sulfide ore taken with a blue filter with optimal integration time and the same scene viewed at 438 nm (Mi=Mispickel; Pn=Pentlandite; Py=Pyrite).

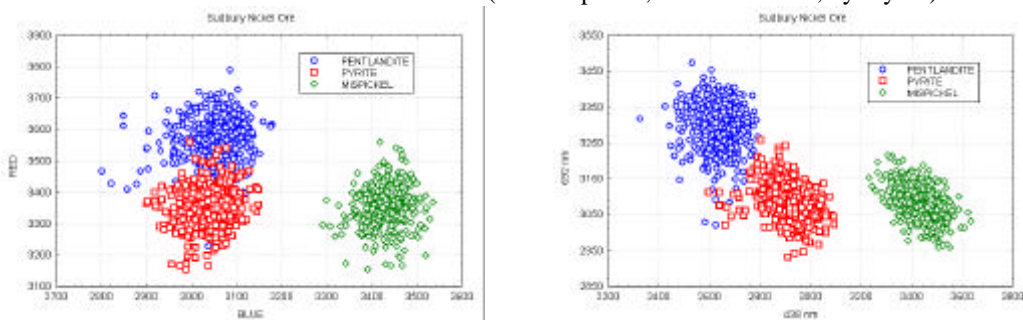


Figure 2. Scatterplots for groups of 500 pixels taken within each phase (cf. Fig. 1).

The first one corresponds to the Red vs. Blue space, whether the second one is the 438 nm - 692 nm spectral space. In order to quantify the improvements of (438 nm - 498 nm - 692 nm) multispectral imaging over conventional RGB, Mahalanobis distances have been computed between the minerals for both spectral spaces. Values are given in table 2.

| Mineral pairs           | 438 nm - 498 nm - 692 nm<br><i>Mahalanobis distances</i> | RGB<br><i>Mahalanobis distances</i> |
|-------------------------|--|-------------------------------------|
| Pentlandite - Pyrite    | 33.11  | 10.98                               |
| Pyrite - Mispickel      | 78.69  | 70.62                               |
| Pentlandite - Mispickel | 188.42   | 85.05                               |

Table 2. Mahalanobis distances indicating the superior discrimination between phases with multispectral imaging.

#### References :

**CRIDDLE A. and STANLEY C.**, 1993, Quantitative Data File for Ore Minerals : 3<sup>rd</sup> Ed., Chapman & Hall, London.

**PIRARD, E., LEBRUN, V. and NIVART J-F**, 1999, Optimal acquisition of video images in reflected light microscopy, European Microscopy and Analysis, V60, pp. 9-11