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# Software to stitch images from microscope Leica DM4000M at Petra III beamline P06

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In this paper I will present the work I have done during the Desy Summer Student Programme 2009 in the team of beam line P06 at the PETRA III source. The program presented in this document is the combined work of Kamila Biernacka and Dorota Szepietowska. I have also worked with second project which pertain to evaluated and improve the script *KEMP2* that is used to process fluorescence XAS datasets.

# MICROSCOPE IMAGES STITCHING User's Guide

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The *Microscope Images Stitching* is a program which is helpful in combining microscope images to produce a comprehensive, high-resolution sample image.

The beam line P06 will provide visualization by micro and nano analysis using X-ray techniques like: X-ray fluorescence (XRF), X-ray absorption spectroscopy (XAS), X-ray diffraction (XRD), imaging (absorption, phase contrast) and tomographies.

The Leica DM4000M microscope will be used to take images of the sample. Then after stitching, obtained map of the sample will be helpful to focus beam in correct place of the sample. There are many different programs dedicated to combine images but most of them require nearly exact overlaps between images and identical exposures to produce seamless results, users should make as many pictures as it is required to cover whole sample.

The *Microscope Images Stitching program* use encoder values from Sensor Control Display and in this case user can take picture only this parts of the sample which are important for measurement. The map of the sample is created even not whole area is photographed.

To take a photo users should use *Leica Application Suite (LAS),* which combines microscope, digital camera and integrated image archiving software into a common micro-imaging environment. LAS combine the visualization, enhancement, measurement and documentation of digital images.

The *Microscope Images Stitching* was written in the scripting language Python. Python is available for free and it runs on all major operating systems such as Windows, Linux/Unix, OS/2, Mac OSX among others. *Microscope Images Stitching* program is installed on P06 laboratory computer but it could be installed on any other computer in simply way. It requires some of freely available Python's modules like: SciPy+NumPy, Matplotlib, PIL, which are easy to install even for inexperienced users.

*Microscope Images Stitching* program contains two scripts. The script *Record Encoder Values* is used to read encoder values of taken images from Sensor Control Display. This data is saved to .txt file. The *Leica Application Suite* can save images in .tif and .jpg files, the program is able to work with both types of this files. Also LAS create the .xml file for each image which consist all important information about picture, like: magnification, real length of one pixel which are important in the second part of the program. After the all images are taken, user can choose the directory where images, .xml files and .txt files are going to be

saved. Default path to this directory on Windows based computers is: C:\Documents and Settings\All Users\Documents\Leica Application Suite\Images.

The main purpose of the second script *Image* is to combine microscope images. In the beginning user is asked about the preferred extension of the images files, then the program takes the encoder values from .txt files, real length of one pixel and magnification from .xml files. The encoder values are giving information about the center position of the image and are used to calculate the range of main box and the exact position of the images. The value of real length of one pixel (XMetresPerPixel) is necessary to calculate the real width and height of the image in meters. The program uses information about the magnification to select subset of the offset parameters, which should be taken to obtained the correct position of the images.

X and Y center coordinates are used to calculate the minimum and maximum values of X and Y coordinates for each image. Then the program chooses the lowest and highest values from all Xmin, Xmax, Ymin and Ymax values, which are used to define the size of main box. The main box defines the relative figure coordinates as left bottom corner (0,0) an right upper corner (1,1). The program uses function `axes` to calculate the correct position of the images. The axes command defines the shape of each image as: [left, bottom, width, height] in relative figure coordinates.

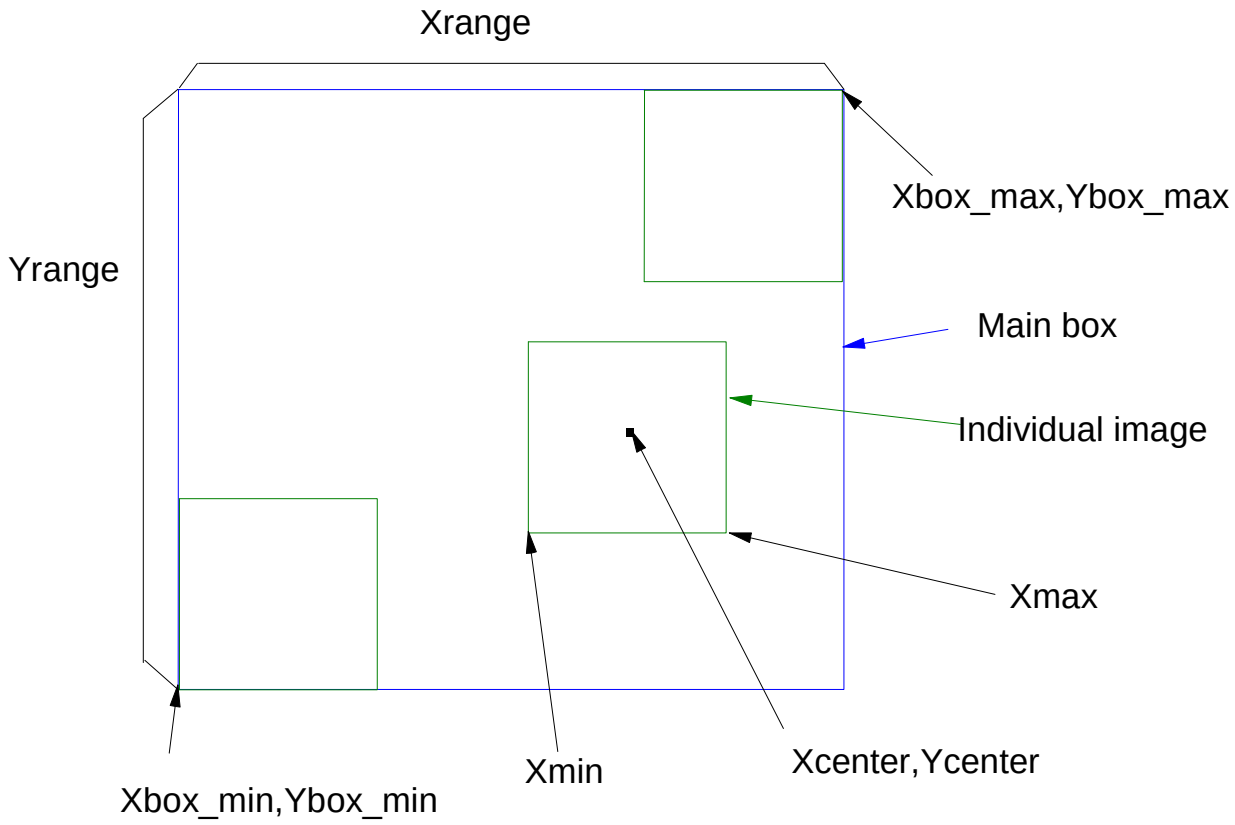


Fig. 1 Coordinates of main box and individual images.

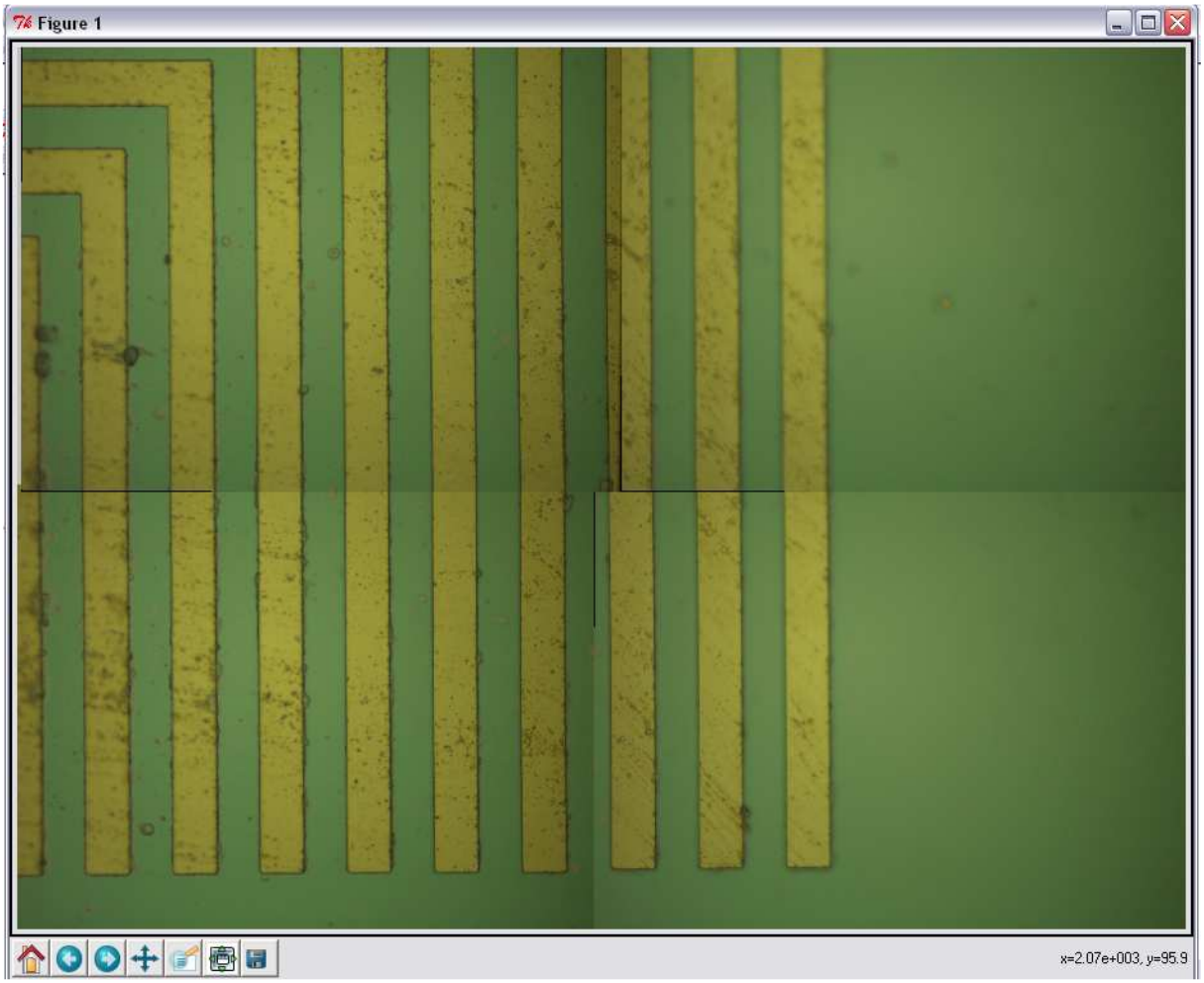


Fig. 2 Combined four images with the aid of the *Microscope Images Stitching* program.

During calculations of all necessary X and Y coordinates, program have to include the offset of X and Y values and the correction of magnification. Also all of the images are rotated in angles of 0.39 degree. The offsets and the rotation are caused by the not proper alignment of the microscope camera. Unfortunately rotation cut the part of the image which is rotated, the result of this action is visible in the Fig. 1. The other disadvantage of *Microscope Images Stitching* program is that user can only take photos of sample in one magnification. The program doesn't stitch images in appropriate way when they were taken with different magnification.