EXAMPLE 3
Oct 14, 2021
Matthias Schmidt

Data provided by S. Schymura, Helmholtz-Centre Dresden Rossendorf (HZDR).
The data was collected in the framework of research on
"Effects of surface roughness and mineralogy on the sorption of Cm(III)
on crystalline rock"
published in Demnitz et al. J. Haz. Mat. 423 (2022), DOI: 10.1016/j.jhazmat.2021.127006

---

Pre-processing of the data-set
---

Before image-registration with Correlia can be performed the data has to be pre-processed.
This involves
- import of the data (which does not necessarily have to be in a common image format)
- calibration of the length scale in physical units
  (telling the software the size of a pixel in microns)
- saving the data in tagged image file format (tiff)

** LM.png
   - open the file
   - using the line tool, draw a line along the x-axis (from 0.0 to 1.54mm)
   - open FIJI menu Analyze->Set Scale
   - the "distance in pixels" is about 2600 (taken from the line)
   - the "known distance" is "1540" (which we know from the scale attached to the image)
   - "unit of length" should be set to "microns"
   - now the image is calibrated
   - using the rectangle selection tool crop the image to the plain data
   - save the image as "LM.tif"
   - you may close the image window

** Topo_2120x1457_645nm.txt
   - this data is a "text image" and has to be imported into FIJI
   - for that click File->Import->Text Image and browse to find the file
   - this will open a 32bit image on your screen
   - calibrate the image using Analyze->Set Scale
   - the pixel size is known to be 645nm so "distance in pixels" is 1
     and "known distance" is 0.645 ("unit" is microns)
   - since this image decodes a height profile let's subtract the background:
     - select a mask over the entire image with CTRL-A
     - measure the average value "Analyze->measure"
       which results in a minimum of -37219.422
     - add this value to the image (Process->Math->Add)
     - correct brightness and contrast (Image->Adjust->Brightness Contrast)
   - add a look-up table (click on Lut button and select e.g. "fire")
   - for convenience convert image to 8bit RGB (Image->type->RGB Color)
   - save image as tiff (make sure not to use spaces in the file name!)

** Sq_2120x1457_645nm.txt
   - this data again is a "text image" and has to be imported into FIJI
   - for that click File->Import->Text Image and browse to find the file
   - this will open a 32bit image on your screen
   - calibrate the image using Analyze->Set Scale
   - the pixel size is known to be 645nm so "distance in pixels" is 1
     and "known distance" is 0.645 ("unit" is microns)
   - adjust brightness and contrast such that edges are white and well pronounced
     (Image->Adjust->Brightness Contrast)
   - (optionally) convert to 8bit grey-scale image (Image->type->8bit)
   - save the image as tiff (make sure not to use spaces in the file name!)

** AR_53x42_25Âµm.tif
   - open the auto-radiogrphy data using File->Open
   - this will show you a very small (53x42 pixels!)
     16bit false-coloured image on the screen

   - (click on this image window in order to put the focus on it)
   - calibrate the image using Analyze->Set Scale; 1 pixel equals 25 microns
   - set look-up-table to grey (for that click on LUT button)
   - save image as tiff

** ÂµTRLFS_intensity_51x51_20Âµm.txt
   - this data again is a "text image" and has to be imported into FIJI
   - for that click File->Import->Text Image and browse to find the file
   - this will open a 32bit image on your screen
   - calibrate the image using Analyze->Set Scale: 1pixel equals 20 microns
   - convert image to 16bit
   - save image as tiff


Image-registration with Correlia
--------------------------------

- for convenience close all open images in FIJI
- start the correlia plug-in (Plugins->Registration->Correlia)
- a dialogue with radio-buttons will open; select "new project" and click OK
- select the processed and calibrated light-microscopy LM.tif
  image as canvas (base image) for the new project
- the "Edit Image Properties" dialogue opens up:
  - name the image properly (e.g. "light microscopy")
  - "acquisition date" should be 2021-07-12
  - "setup" could be the maker of the microscope
  - "experimenter" probably is "Stefan Schymura"
  - in "additional information" put useful information for you colleagues working
    on the same sample
- OK the dialogue
- two screens open up: the Correlia viewer and the GUI

- describe the project (GUI-menu->Project->Project Properties)
  - title: HZDR test data
- OK the dialogue

- save the project (GUI-menu->File->Save project as)

- import topography map
  - either click in the "+" in the bottom left corner of the GUI or
    GUI-menu->Project->Add image to project
  - select the calibrated topography map (e.g. Topo_2120x1457_645nm_8bitRGB.tif)
  - the image properties dialogue opens: title "topography"
    and if known enter the other information

- the Correlia viewer displays an overlay of the light micrography with the topography map
- select "topography" from the image list in the Correlia GUI
- pre-align the image using the arrows in the "image alignment" field in the GUI
- hint: clicking the centre will change from corse ("C") to fine ("F")
- after pre-alignment we can try to improve the alignment automatically by clicking
  "auto" in the Correlia GUI
- select "translation" and "rotation" (scale we trust to be correct)
- this should result in a proper overlay

- set topography map to "invisible" by clicking on the eye next to "topography"
  in the image list in the GUI
- import roughness data (Sq_2120x1457_645nm_8bit.tif) as before and name it "roughness"
- now roughness and light microscopy are viewed
- since roughness and topography are captured during the same measurement
  we can use the coordinates of the topography map:
  - select "topography" in the GUI image list
  - click the marriage icon right of the image preview
  - a check-box dialogue opens -> select "roughness"
  - now the roughness map is registered
- for convenience we can colour the roughness map in orange by clicking on the clour-box in
 the GUI image list

- set roughness map to invisible

- import the auto-radiography data (AR_53x42_25mum_16bit.tif)
- set colour to blue
- manually align (auto-alignment will not work because there is not enough structure in the microscopy)
- (x0,y0) should be about (0,43)

- set auto-radiography map to invisible
- import the fluorescence data (ÂµTRLFS_intensity_51x51_20mum_16bit.tif)
- set colour to red
- manually align (auto-alignment will not work because there is not enough structure in the microscopy)
- (x0,y0) should be about (0,31)