Graz University of Technology

Software for PCO FLIM Camera

User Manual

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pco.

This software has been created entirely in Python and offers a simple user interface for the PCO Flim camera. It includes the basic tools for analyzing the fluorescence of a material. This document explains the software functions and their overall operation.

Buttons and interface:

Set the camera parameters:

On the right-hand side strip, you can enter all the parameters needed for the camera to function. Here's a list of parameterizable data and their uses:

-Exposure time: duration during which the camera will capture data for 1 acquisition.

-Wave form: whether the modulation signal is rectangular or sinusoidal. (none = no signal)

-Number of phases: Number of phases wanted during a period of the modulation signal (2, 4, 8, 16). For example if number phase = 2, there will be one image at 0 degrees and one at 180 degrees.

-Symmetry correction: If twice selected, 2 images will be taken for each phase (one with tap A and one with tap B). If “single” is selected just one image will be taken for each phase.

-Phase order: If "ascending" is selected, images will be captured in ascending order, otherwise, it will be captured in the reverse order.

-Selected Tap: If both are selected, images will be captured by alternating between the two taps.

-Background correction: If “yes” is selected, the values set and stored during background calibration are subtracted from each image pixel to eliminate background noise. Furthermore a mask will be applied to set the background to 0. (The threshold for this mask can be adjust in the menu “Calibration”)

-Frequency: Enter the frequency of the modulation signal according to the expected lifetime.

-Apply filters: If ticked, a Wiener filter and a Blur filter will be applied to the images which allows to eliminate the dead pixels and improve the signal on noise ratio.

Live acquisition:

By clicking on the button “Start Live”, you will be able to place the component under the camera and will see the camera return to the screen. This image has not been processed, it’s a raw image.

Launch acquisition:

By clicking on the button “Launch acquisition”, you will display the 3 graphs representing respectively the intensity, the phase angle and the modulation of each pixel of the component. This will also bring up 3 sliders, whose usefulness will be explained below.

Acquisition mode

Once the acquisition has been launched, you'll be able to use a variety of features:

Scale adaptation:

3 slides appear under the buttons. You can move the green circles to adapt the scales for each graph. You can for exemple move the max and min circles around the mean value to be able to see more precisely on the graph the variation around the mean value. The right green circle set the new maximum of the scale and the left one set the new minimum. Click on “Apply changes” to see the graphs changed.

Region of interest:

If you click and drag on a graph, you can draw a rectangle and thus obtain the minimum, maximum and average values for this surface. You can also adjust the size and the placement of the rectangle. The values will be displayed on the data table at the bottom of the interface.

Save graphs:

Next to each graph, a button appears to save each graph as an image among your

documents.

Data table:

There are three buttons: “Intensity”, “Phase” and “Modulation”. If you click on that button you'll see the values corresponding to each of the graphs in the central column for the entire image, and in the right-hand column the values for the selected area, if any.

There is also a button “Export data”, it allows you to export the table as an excel file. You can save it and use it directly in excel.

Menu

Background calibration:

You have to remove the component and leave the camera in the same conditions as during acquisition. The camera will capture 150 images and average them. Then the final image will be stored in the software. If the background correction is selected this image will be subtracted from the images captured during acquisition to remove background noise. You can cancel the background calibration if you think there might be a mistake during the calibration or if you change background. You click on “cancel background calibration” it will set the matrix to 0.

When background correction is selected, it also applies a mask on the image making all the background at the value 0. You can adjust the detection threshold in the menu “Adjust threshold” which means that each pixel under the defined threshold will be set to 0. (By default, the threshold is set to 0.08).

Lifetime calibration:

This function is used to refine the program if you have a component for which you know perfectly the lifetime. You place this component under the camera, then a menu will appear asking you to select an area where there is only the component (to avoid averaging the background). Then an average of the data will be calculated only in the area, then we can calculate the phase angle and the modulation. With those values, we can calculate an expected lifetime and compare it to the actual lifetime. We obtain a ratio for the modulation and one for the phase angle. Then when you launch a new acquisition, the phase values will be multiplied by the established coefficient and the

same for the modulation. If you are not sure that the calibration has been correctly set, you can check the coefficient in “Help”. If calibration has been carried out incorrectly you can set the parameter to 1 by clicking on ‘Cancel lifetime calibration”.

Fitting:

You can create a new fitting in the "Fitting" menu. This will ask you to select the model you want, then enter the parameter corresponding to the components you have. When you click on "calc", the program will capture an image and then ask you to select a zone to calculate the desired data. You can give conditions to the parameters or fix them, then click on "Fit" to display the curve, the parameters found and the calculation points on a graph. Then you can close this window.

In the "Use fitting” window, you'll find a menu of available equations (for which fitting has been performed). By selecting one of them, you can launch an acquisition and display the fitted parameter selected for the image.