Automatic workflow for data analysis of fluorescence microscopy images at a single cell level

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In the present work, we demonstrate a simple approach to automated analysis of data from DUV fluorescence microscopy measurements. Our aim is to establish universal and consistent image analysis procedure that will provide comprehensive information on individual cell's behavior.

In fluorescent microscopy, having high background signal or low signal to noise ratio represent frequent obstacles in data analysis, that usually impose the need of manual analysis, which results in selecting few representative cells per sample. We address the problem in following general steps. First, it is necessary to adapt initial images for the detection of the cells' contours in order to separate cells' interior areas from the background. Afterwards, the full background is reconstructed from the background mask, enabling the analysis of the intrinsic fluorescence signal of the studied biological system. This step is of great importance because the initial distribution of fluorescence and its differences throughout the sample originate from several sources, which should be appropriately distinguished. Finally, when the real signal is obtained, the signals from target groups of samples are corrected on the relevant control samples and the results can be evaluated.

We illustrate this procedure on the influx of antibiotics in Gram-negative bacteria *Escherichia coli*, followed by Deep UV Fluorescence microscopy at DISCO beamline (synchrotron Soleil). The process we follow is the time-course accumulation of the antibiotics molecules revealed by their intrinsic fluorescence under DUV excitation at the individual cell's level and the dependence of the level of expression on modulation of input and output membrane carriers. The automated analysis procedure is carried out on every sample and the results are presented and discussed. Additionally, potential improvements are highlighted.

References

[1] B. Cinquin, Scientific Reports **5**, 17968 (2015)