Bench ●Marks!

SUIKER: Quantification of Antigens in Cell Organelles, Neurites and Cellular Sub-structures by Imaging

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Abstract

Quantification of fluorescence colocalization and intensity of strongly overlapping cells, e.g., neuronal cultures, is challenging for programs that use image segmentation to identify cells as individual objects. Moreover, learning to use and apply one of the large imaging packages can be very time- and/or resource-demanding. Therefore, we developed the free and highly interactive image analysis program SUIKER (program for SUperImposing KEy Regions) that quantifies colocalization of different proteins or other features over an entire image field. The software allows definition of cellular subareas by subtraction ("punching out") of structures identified in one channel from structures in a second channel. This allows, e.g., definition of neurites without cell bodies. Moreover, normalization to live or total cell numbers is possible. Providing a detailed manual that contains image analysis examples, we demonstrate how the program uses a combination of colocalization information and fluorescence intensity to quantify carbohydrate-specific stains on neurites. SUIKER can import any multichannel histology or cell culture image, builds on user-guided threshold setting, batch processes large image stacks, and exports all data (including the settings, results and metadata) in flexible formats to be used in Excel.

Multichannel fluorescent microscopy allows the study of cells, but also the setup of quantitative *in vitro* assays, if combined with appropriate imaging software. Often, the function of a molecule is revealed by its association with another molecule or a specific cellular compartment. Fluorescence colocalization can be quantified in various ways, however normalization of the respective data affects the results and can cause severe distortions (Dunn et al., 2011).

Several commercial software applications have therefore been optimized for the evaluation of fluorescent images. However, their adaptation to user-specific requirements is often time-consuming. Flexible options are offered by open-source image analysis packages like ImageJ or Fiji, where specific macros can be written to suit the analysis needs. These require knowledge of a programming language but some large publicly funded projects, like the CellProfiler initiative (Carpenter et al., 2006), have started to provide cell researchers with ready-to-use solutions. For work on assay setup and optimization (*vs* large scale screening), it is important for the software to visualize any image change in real-time upon tuning of analysis parameters (slopes, thresholds, area definitions, etc.). For some research fields, e.g., involving cells that grow complex neurite networks, further features are required: (i) image evaluation without segmentation to individual cells, and (ii) the ability to subtract subareas from one another (Hoelting et al., 2016; Krug et al., 2013; Stiegler et al., 2011). The latter function would allow the evaluation of neurites without the cell bodies or of cytoplasm without the nucleus (Falsig et al., 2004, 2006; Henn et al., 2011; Kleiderman et al., 2016) or of the cell membrane without the cell interior. In the course of our work on incorporation of unnatural sugars into the cellular plasma membrane, we noticed the need for these two features to quantify fluorescent molecules, their colocalization, and intensity on neurites.

The program SUIKER[1] was therefore written to quantify colocalization of different proteins or other features in a cell. The only required input is a microscopic image that stores information in separate (≤ 3) color channels.

The different channels, as defined by the fluorescence filters of the microscope, can be assigned to the red-green-blue (RGB) channels used by computers. The program will use the channel assigned to blue (ch1) as indicative of nuclei. This information is used to find and count nuclei and to quantify cell numbers. It can be optionally used to further segment cellular subregions in an image. The other two channels (ch2 and ch3) will be interpreted as representing two different features of the cell as defined by respective specific stains (e.g., antibodies or organelle-selective dyes). The program will count the number of pixels that show overlap between ch2 and ch3. The calculated values are given as absolute numbers and as average per cell.

The number of stained pixels is not the only parameter that defines the signal produced by a protein or by other cell features. The pixel intensity is also important. Therefore, the program yields combinations of pixel number and intensity as output for multiple image regions. The definition and automatic recognition of image regions are explained in the manual[2] using the example of carbohydrate-specific stains on neuronal surfaces. Labeled sugars can end up both in the cell body and in the neurites. To differentiate between these parts of the cell, SUIKER gives the user the possibility to define the cell body as "that part of the cell surrounding the nucleus". The size of such "soma areas" can be set and interactively visualized. These "soma areas" are then used to differentiate signals originating from the neurites or from the cell body. This feature can be used to "punch out" one image feature from another (e.g., the cytoplasm without the nucleus).

SUIKER gives the user a large range of settings enabling background corrections in all channels, discriminating nuclei on account of their intensity and size, and enhancing the signal in any channel. These functionalities are visualized in real-time and they enable the user not only to assess the colocalization but also to create signal-enhanced pictures. Another example of a potential application could be assessing the colocalization of a protein with mitochondria, the Golgi apparatus, or the cell membrane (Delp et al., 2018; Gutbier et al., 2018; Nyffeler et al., 2018; Schildknecht et al., 2013).

A major image processing strategy implemented in SUIKER is that it does not require segmentation of the image to identify individual cells (impossible for neuronal networks). The obligation to treat cells as individual objects in some programs may create artefacts and it can lead to exclusion of large parts of the available information. SUIKER works independently of cell recognition, on the entire image field, and normalizes to cell number afterwards. This can be advantageous for strongly superimposed cells, e.g., of the nervous system, for stained tissues, or for other superimposed structures, such as the mitochondrial network, the cytoskeleton or phagocytosed cells (Hirt et al., 2000; Latta et al., 2000; Schildknecht et al., 2011).

The optimal image processing parameter, established based on a few training images by interactive visual control (observer-controlled data exploration), can then be fixed and stereotypically applied to large image stacks (unbiased comparison of experimental situations). All data (complete with all settings, metadata and results) will be exported directly into an Excel file. The optimized, multi-channel picture will also be exported automatically with all quantification data and metadata, so that all requirements for data transporting and display can be met (Fritsche et al., 2017; Hartung et al., 2019; Leist and Hengstler, 2018; Pamies et al., 2018).

SUIKER is freely available[1]. Its easy-to-use graphical interface allows intuitive use and supports users regardless of their computational skills. A detailed user manual[2] can be found in the supplementary information to this article.

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Conflict of interest
The authors declare no conflict of interest.