

# nandb: AN OPEN-SOURCE R PACKAGE FOR NUMBER AND BRIGHTNESS ANALYSIS WITH A NOVEL AUTOMATIC DETRENDING ALGORITHM

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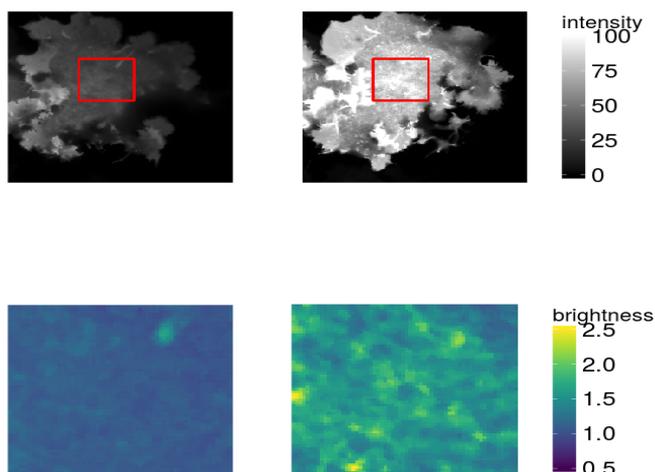
## 1. NUMBER AND BRIGHTNESS

"Number and brightness" (N&B) [1] is a fluorescence fluctuation spectroscopy (FFS) technique to measure the average number and oligomeric state of *motile* (freely diffusing) molecules in a given pixel of an image. For the technique to work, one needs to accurately measure intensity fluctuations over time.

## 2. DETRENDING

Bleaching causes artificial intensity variation so this needs to be corrected for before proceeding with N&B calculations. This is the “detrending” step. Here we propose a new automatic method that can automatically choose an appropriate detrending procedure based on properties of the data. Previous detrending methods have relied on an arbitrary choice of detrending parameter (the size of the “box car” or the exponential filtering parameter). Our novel method uses exponential filtering but does not require the user to select the parameter. The idea is to resimulate the images as having come from immobile molecules only; then, by enforcing the well-known property that immobile particles give brightness 1, the exponential filtering parameter is determined.

## 3. nandb



nandb is an R [2] package for general-purpose N&B analysis. It includes the aforementioned automatic detrending algorithm, functions for performing N&B analysis in high throughput (using parallel computing), creation of N&B videos and nice visualisation of N&B images. It is the first free, open source software of its kind, facilitating new levels of use as well as mutability and interoperability with the rest of the R landscape.

Figure 1. Example plots from the software

[1] Digman, M. A., Dalal, R., Horwitz, A. F., and Gratton, E. (2008). “Mapping the number of molecules and brightness in the laser scanning microscope.” *Biophys. J.*, 94(6), 2320–2332.

[2] R Core Team (2016). “R: A Language and Environment for Statistical Computing.” *R Foundation for Statistical Computing*, Vienna, Austria.