Quantification of endocytosis as pharmacological response by fluorescence microscopy

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Introduction

Image stacks obtained by fluorescence microscopy over time and over three dimensions (X,Y and Z axis) allow us to observe internalizing vesicles (i.e. endosomes) in living cells after treatment with different drugs.
The pharmacological response can be characterized by different parameters related to the generation of endosomes. Their number, the intensity of the associated fluorescence signal or the distribution of their sizes are proposed to evaluate the pharmacological properties of a drug.
Campa et al. [1] proposed an algorithm to count the number of endosomes per cell and found differences between groups of drugs. However this algorithm needs fine tunning and provides only partial information of the pharmacological response, therefore its performance is subject to improvement.
Our approach benefits from the information contained in the third central moment (skewness) and the Laplacian of Gaussian well-known properties as a filter for spot detection.
Aim: Quantify and compare the pharmacological response of different drugs in time-lapse series of images.

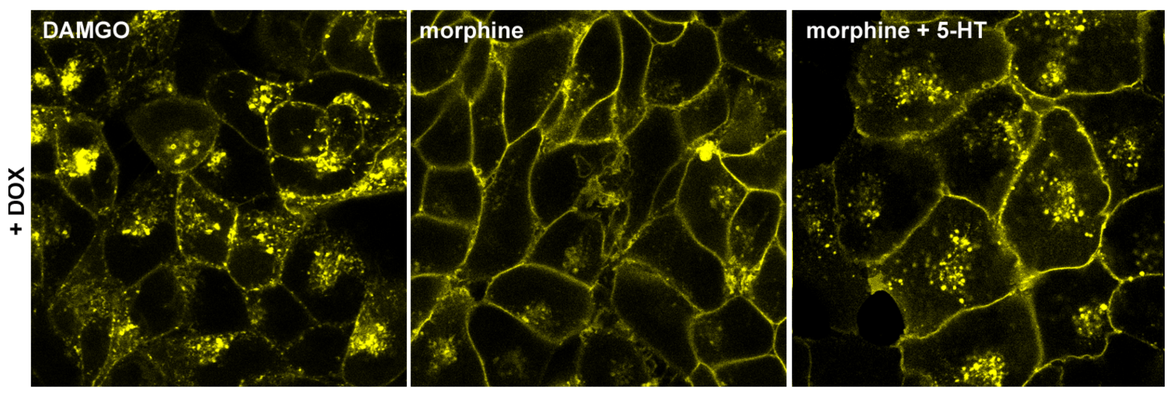


Image for cells treated with either DAMGO (10 μ M), morphine (10 μ M) or morphine + 5-HT before picture adquisition. [1].

Material and Methods

Images Stacks consisted on 7μm thick Z-stacks (11-18 planes of 0.49μm Z-step size) were acquired for 15 minutes at a rate of 1 frame per minute in an inverted epifluorescence microscope. The 16-bit resulting images had a resolution of 1004 $\times$ 1002 pixels (0.13 μm pixel size).
Fluorescence signal S(x,y) at pixel x,y from an endosome centered at position x_0, y_0 can be modeled as a 2D Gaussian function, with size parameter γ and intensity parameter T_0,

$$S(x,y) = T_0 \exp\left(\frac{(x-x_o)^2}{2\gamma^2} + \frac{(y-y_o)^2}{2\gamma^2}\right).$$

INPUT: $I(x,y,t), t = 1,..,16$, z-projection of Maximum Intensity of the 9 central planes of the image stack.

Calibration

- Estimated size parameter γ =2.07 pixels.
- Calculate B, region that separates cells from the image background for $I(x,y,t=1)$, applying Minimum Threshold.
- Find R, scale for Laplacian of Gaussian filter for $I(x,y,t=1)$, where amplification λ in B is maximum for a given γ.[2]

Algorithm

- Find B_{min}, region of minimum area after applying segmentation (Minimum Threshold) for every $I(x,y,t)$.
- Convolve every image with the Laplacian of Gaussian Filter [3] of scale R:

$$\psi(x,y) = \frac{1}{2\pi R^4}\left(2 - \frac{x^2+y^2}{R^2}\right)\exp\left[-\frac{(x^2+y^2)}{2R^2}\right].$$

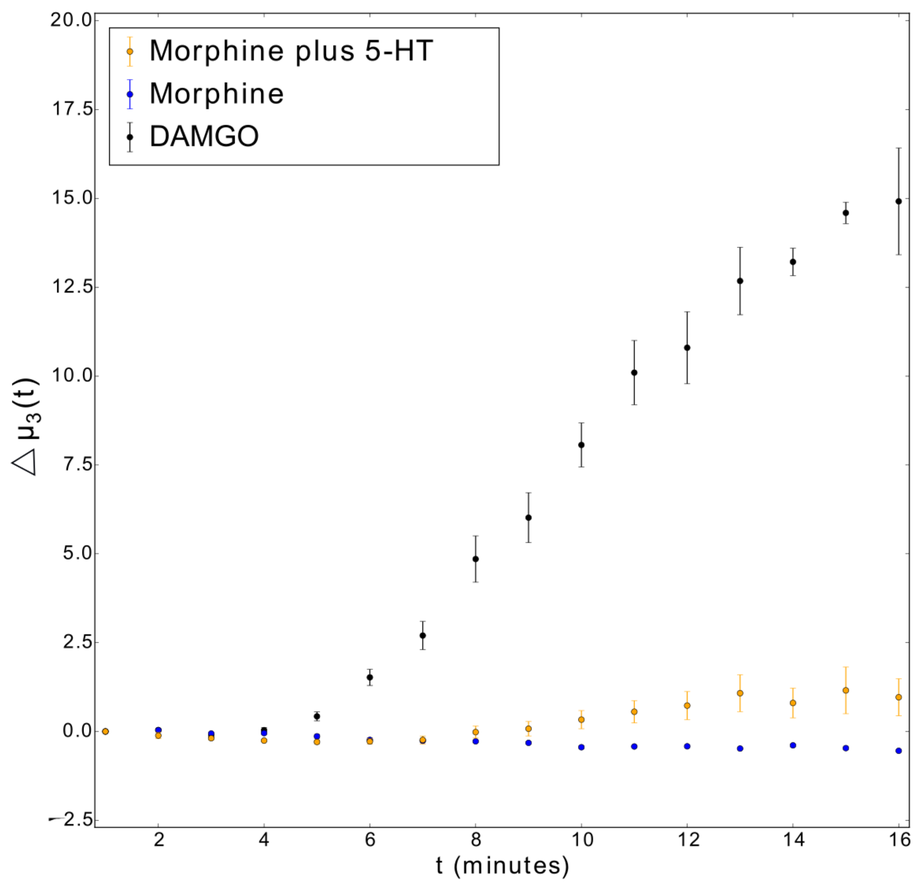
- For every convolved image $I_w(x,y,t)$, calculate

$$\mu_3(t) = \left\{\mathbb{E}\left[\left(I_w(x,y,t) - \mathbb{E}[I_w(x,y,t)]\right)^3\right], x, y \in B_{min}\right\}.$$

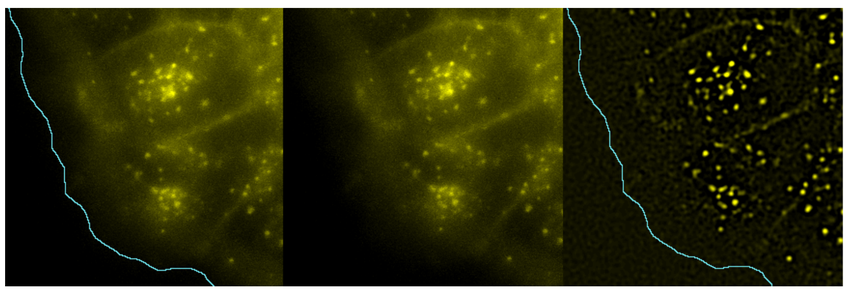
Results

We calculate $\mu_3(t)$ for five experiments of each of the three considered drugs, namely, DAMGO, morphine and morphine+5HT

$$\Delta\mu_3(t) = \frac{\mu_3(t) - \mu_3(t=1)}{\mu_3(t=1)}$$



The results show that $\Delta\mu_3$ increases over time at a different rate, with the exception of morphine in which no observable endosomes are present in the images.



DAMGO experiment closeup at t=12 minutes. (Left) Region B_{min} (in cyan) (Center) Original image, (Right) Convolution with LoG (R=1.9)

Conclusions

- The algorithm is able to differentiate between the pharmacological responses of the three drugs tested.
- The results suggest an increase both in the number of endosomes per cell and their intensity T_0, improving the discrimination power from Campa et al. [1]
- The algorithm can be easily extended to study the distribution of size of the endosomes, just varying the size parameter γ.

Bibliography

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