# CORRECTION OF PHOTOBLEACHING FOR THE ASSESSMENT OF PHARMACOKINETIC PARAMETERS USING DYNAMIC FLUORESCENCE MICROSCOPY

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*M. Derieppe, C. Bos, M. de Greef, C. Moonen, B. Denis de Senneville*


## ABSTRACT

Local drug delivery in oncology aims at depositing high doses of anticancer agents while limiting their toxic side effects. Biological barriers, such as cell plasma membranes, hinder their delivery and requires strategies to address this challenge.

Previously [1], we demonstrated the feasibility to monitor in real-time, with dynamic fluorescence microscopy, the intracellular delivery of a hydrophilic model drug mediated by ultrasound (US), and to quantify the pharmacokinetic parameters derived from a two-compartment model. We evaluate here the impact of the photobleaching (PB) effect experimentally, and compute the PB-corrected uptake kinetics.


## 1. METHODS

US-mediated uptake of Sytox Green model drug, a fluorescent intercalating dye, was monitored using Fibered Confocal Fluorescence Microscopy [1]. Cell-nuclei were first detected and tracked over all acquired images [2]. Fluorescence signal enhancement was then fitted with a 2-compartment model, representing an extracellular and an intracellular compartment separated by a plasma membrane, as follows:

$$I(t) = A[1 - e^{-k(t-T)}] \tag{1}$$

with $A$ the asymptote, $T$ the uptake onset, $k$ the uptake rate.

A 3-compartment model was then performed to represent PB as an efflux rate $k_{pb}$ of the fluorescence in the inner cell compartment, as follows:

$$I(t) = \frac{A \times k}{k - k_{pb}} \times [e^{-k_{pb}(t-T)} - e^{-k(t-T)}] \tag{2}$$

After 6 minutes, the acquisition was interrupted for 9 minutes to stop PB, while the fluorophore concentration further equilibrated (Fig. 1). Then, a second 6-minute acquisition served to evaluate the PB rate. This estimate was then used as a fixed parameter in the 3-compartment model. The kinetic parameters were compared using the Mann-Whitney test (significance: $p < 0.05$).

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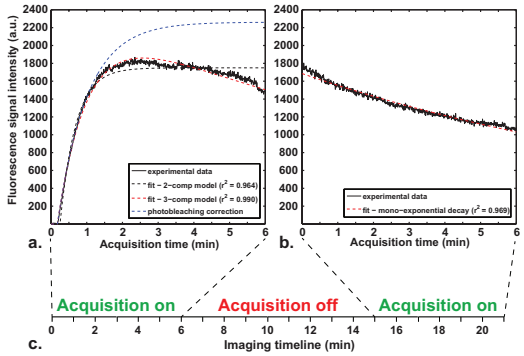


**Fig. 1**. Representative fluorescence signal enhancement of the uptake (a) and evaluation of photobleaching (b).

## 2. RESULTS

PB rates displayed a significant increase ($p < 0.001$) of $1.0\ 10^{-3}\ s^{-1}$ ($4.4\ 10^{-4}\ s^{-1}$, $n = 85$), $2.1\ 10^{-3}\ s^{-1}$ ($6.4\ 10^{-4}\ s^{-1}$, $n = 103$) and $2.8\ 10^{-3}\ s^{-1}$ ($1.1\ 10^{-3}\ s^{-1}$, $n = 73$) at 25 %, 50 % and 75 % of laser power, respectively. In a representative experiment, from which data in Fig. 1 are taken, application of the 3-compartment model, with the PB rate as an input, modified significantly the estimated uptake rate ($p < 0.001$), from $1.2\ 10^{-2}\ s^{-1}$ ($1.1\ 10^{-2}\ s^{-1}$, $n = 62$), using the 2-compartment model, to $6.2\ 10^{-3}\ s^{-1}$ ($4.7\ 10^{-3}\ s^{-1}$, $n = 62$) using the 3-compartment model.

## 3. CONCLUSION

This study demonstrates that processing of photobleaching is critical for the assessment of pharmacokinetic parameters in dynamic fluorescence imaging.

## 4. REFERENCES

[1] M. Derieppe et al., "Real-time assessment of ultrasound-mediated drug delivery using fibered confocal fluorescence microscopy," *MIB*, vol. 15(1), pp. 3–11, 2013.

[2] M. Derieppe et al., "Tracking of cell nuclei for assessment of in vitro uptake kinetics in ultrasound-mediated drug delivery using fibered confocal fluorescence microscopy," *MIB*, vol. 16(5), pp. 642–651, 2014.