

# Cholesterol dependent uptake and interaction of doxorubicin in MCF7 breast cancer cells

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## INTRODUCTION

Doxorubicin, an anthracycline antibiotic, is used as a cytostatic drug in cancer chemotherapy, such as breast cancer, bronchial carcinoma and lymphoma, and has been studied for several decades [1]. The drug is taken up by cells due to passive diffusion through their membrane and finally intercalates in DNA strands, where it causes chromatin condensation and initiates apoptosis. Due to its fluorescence properties, doxorubicin can be localized within the cells, e.g. by different methods of fluorescence microscopy. Here, fluorescence spectra, images and decay kinetics are used to examine the uptake of doxorubicin and its dependency on membrane dynamics, in particular as a function of cellular cholesterol content which has been shown to have a high impact on membrane stiffness and fluidity [2].

## CONCLUSION

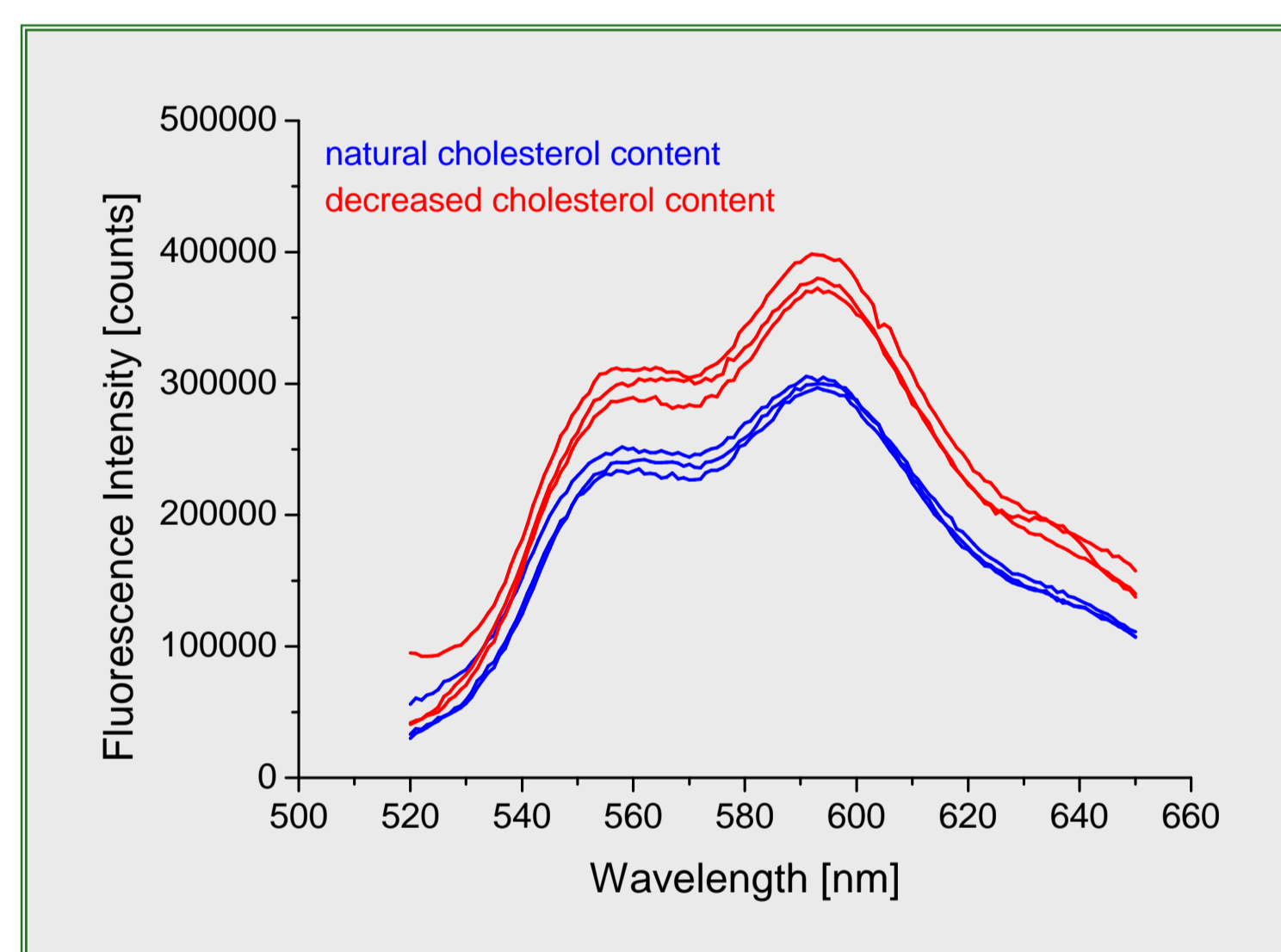
A combination of fluorescence spectroscopy, fluorescence imaging and fluorescence decay kinetics may be useful to measure cellular uptake, intracellular distribution and intermolecular interactions of the chemotherapeutic drug doxorubicin in cancer cells to monitor early steps of apoptosis. In particular, uptake of doxorubicin and drug response (assessed by changes in fluorescence lifetime) were related to cholesterol dependent membrane fluidity. For further steps towards clinical application, cell monolayers may be replaced by 3-dimensional cell cultures whose physiology and morphology is closer to the in vivo situation.

## MATERIALS AND METHODS

The cellular uptake of doxorubicin was measured in MCF7 cells after 2 h and 24 h incubation time (2  $\mu$ M in culture medium) as a function of cholesterol content. Cholesterol reduction was induced by treating cells with 4 mM methyl- $\beta$ -cyclodextrin (M $\beta$ CD) for 15 min. prior to incubation with doxorubicin. For microscopic measurements, a diode laser with high repetition pulses (LDH 470 with driver PDL 800-B, Picoquant, Berlin, Germany; wavelength: 470 nm; pulse energy: 12 pJ, pulse duration: 55 ps, repetition rate: 40 MHz; average power: 0.5 mW) was adapted to a fluorescence microscope (Axioplan 1, Carl Zeiss Jena, Germany) by fibre optics for epi-illumination of whole cells. Fluorescence images were recorded by an electron multiplying (EM-) CCD camera with Peltier cooling and a sensitivity below  $10^{-16}$  W/Pixel (DV887DC, ANDOR Technology, Belfast, U.K.) using a long pass filter for  $\lambda \geq 520$  nm. For fluorescence decay kinetics and lifetime images (FLIM) a time-gated image intensifying camera (Picostar HR 12; LaVision, Göttingen, Germany) with a temporal resolution of 200 ps was used in a sampling mode (time range: 8 ns; exposure time: 1 second per channel). Data were fitted as mono-exponential curves, and median values as well as median absolute deviations (MADs) of fluorescence lifetimes were determined. A grating spectrometer (Jobin Yvon, JY.3 447) operated at a spectral resolution of 10nm was used for recording fluorescence spectra of cell suspensions in a glass cuvette.

## RESULTS

To examine cholesterol dependent cellular uptake and drug response of MCF7 cells either as suspensions or as monolayers on object slides, incubated with doxorubicin, we determined fluorescence spectra, fluorescence lifetimes and fluorescence images. Fluorescence spectra, depicted in Figure 1, for 3 independent measurements of cell suspensions show lower fluorescence intensities than cells upon cholesterol depletion. It is assumed that after cholesterol depletion cell membranes were more fluid, and that the uptake of doxorubicin was, therefore, enhanced. While fluorescence lifetimes (Figure 2) of cells with natural or decreased cholesterol content were almost the same after 2 h incubation with doxorubicin, they appeared shortened upon cholesterol depletion after 24 h incubation time.



In Figure 3, phase contrast, fluorescence intensities and fluorescence lifetime images (FLIM) are depicted. While fluorescence of doxorubicin is well located in the nucleus, its lifetime shows a similar behaviour as depicted in Figure 2. This indicates possible changes of intermolecular interaction due to an increased uptake of doxorubicin and, consequently, a more rapid apoptotic process.

⇒ Figure 1. Fluorescence spectra of MCF7 cells incubated with doxorubicin [2  $\mu$ M, 24 h]; spectra of 3 suspensions of  $10^6$  cells each with natural or decreased cholesterol content obtained during one series of experiments are compared;  $\lambda_{\text{ex}} = 470$  nm.

⇒ Figure 2. Fluorescence lifetime of MCF7 cells with natural (blue bars) or decreased (red bars) cholesterol content after incubation with doxorubicin [2  $\mu$ M] for 2 and 24h.  $\lambda_{\text{ex}} = 470$  nm; medians and median absolute deviations (MADs) of 15 measurements each are indicated.

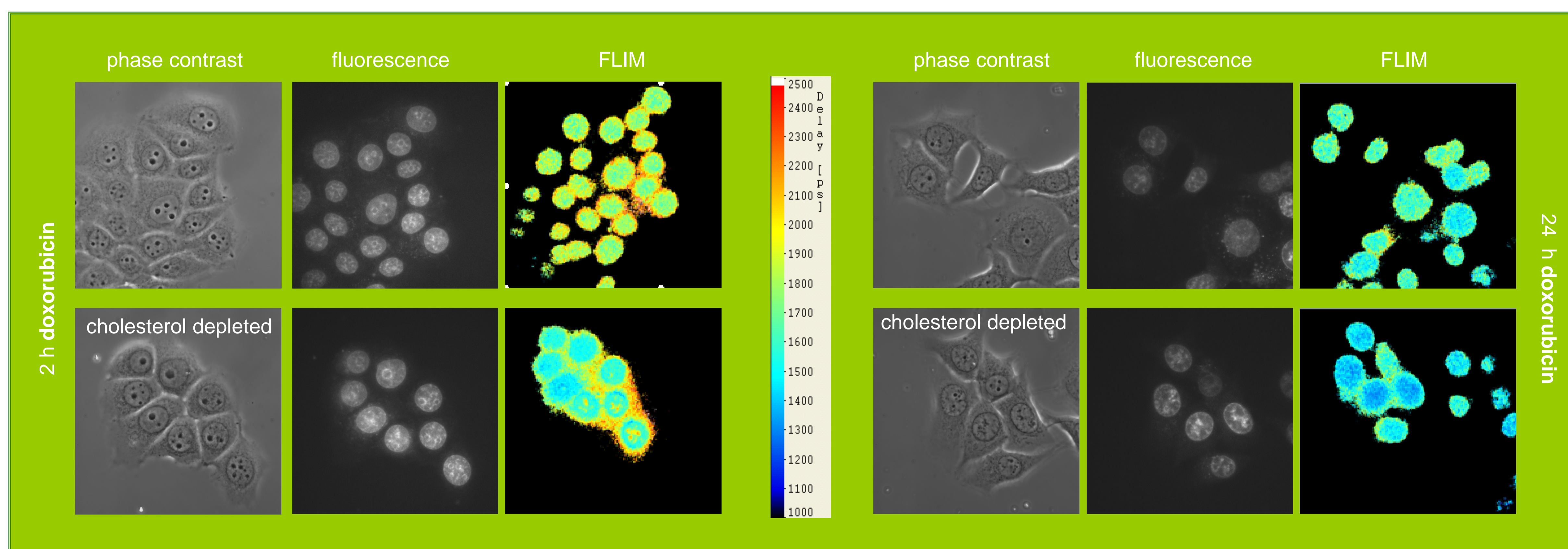
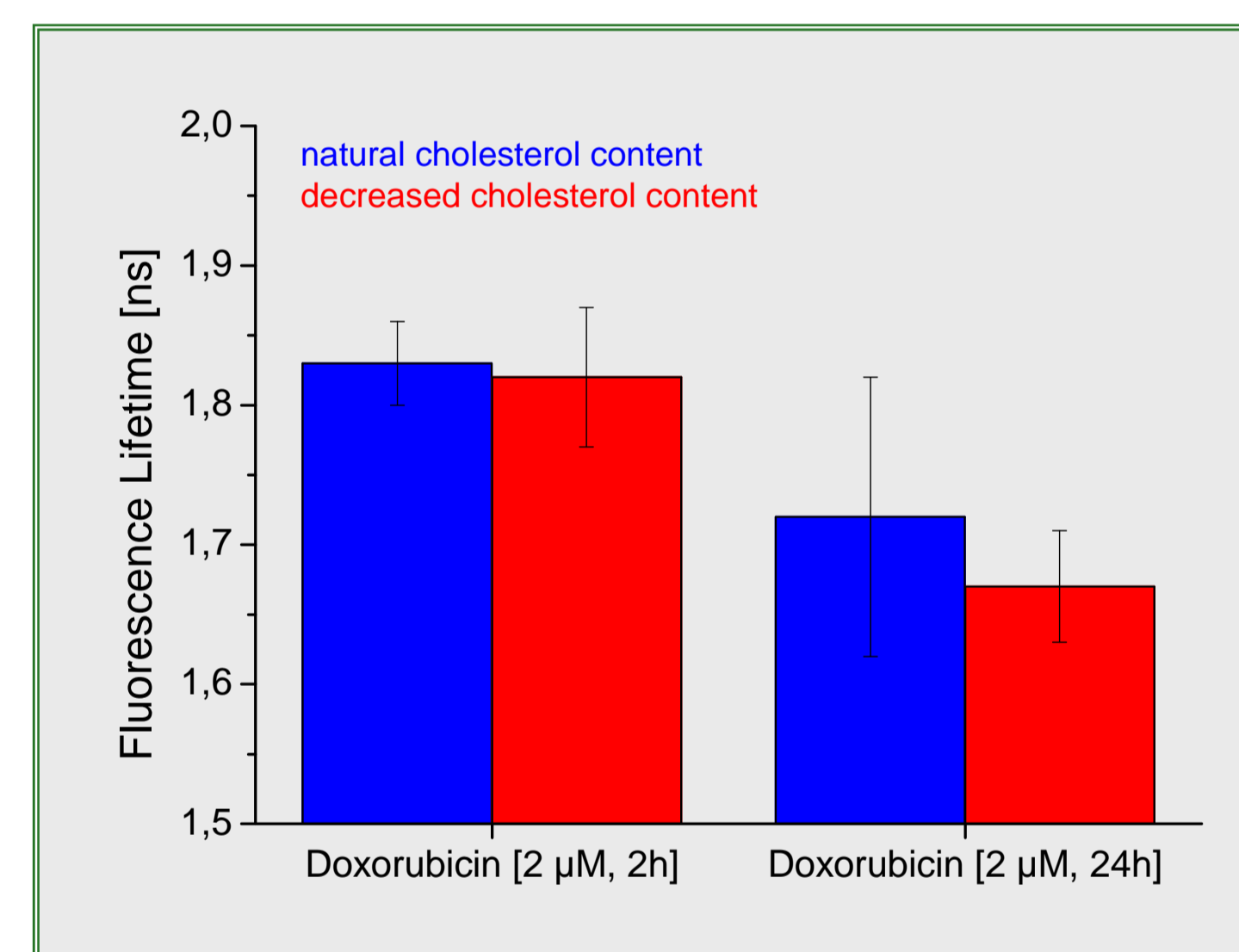


Figure 3. Phase contrast images, fluorescence intensities and effective fluorescence lifetimes  $\tau_{\text{eff}}$  in picoseconds of MCF7 cells incubated with doxorubicin [2  $\mu$ M] for 2 h or 24 h; cells with natural and cells with decreased cholesterol content are compared. Excitation wavelength: 470 nm; fluorescence recorded at  $\lambda \geq 520$  nm; image size: 140  $\mu$ m x 140  $\mu$ m each.

## SELECTED REFERENCES

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