FLUORESCENCE LIFETIME MICROSCOPY REVEALS SYNTHETIC IDENTITY AND BIOLOGICAL FUNCTION OF LIPOSOMAL DOXORUBICIN

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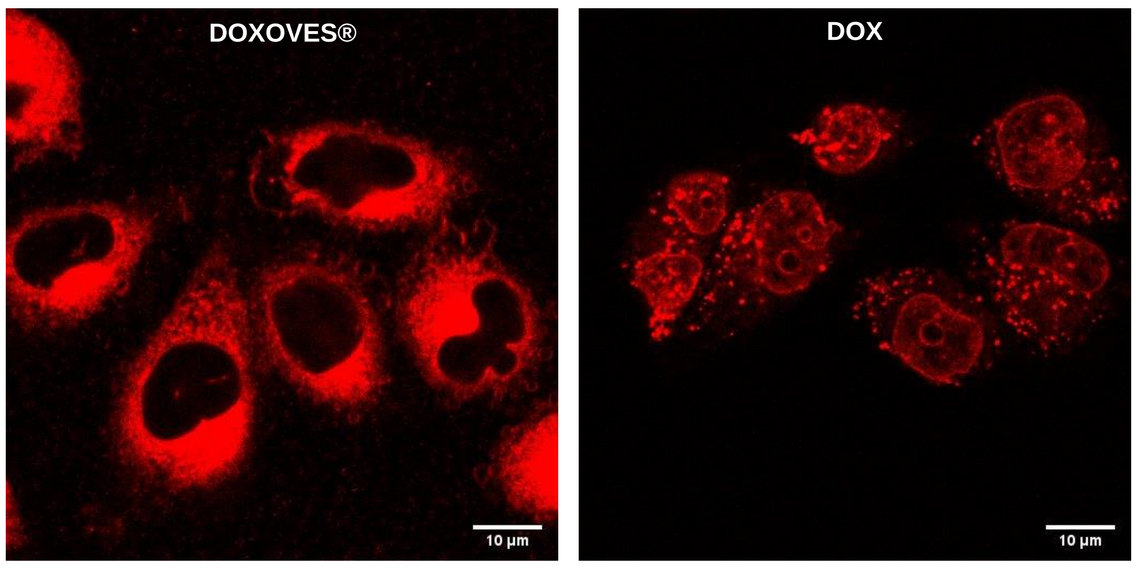
OBJECTIVES

- Knowing the form in which **encapsulated Doxorubicin** is administered to cells with a **label-free** procedure
- Monitoring stability conditions of a liposomal formulation in a non-invasive way

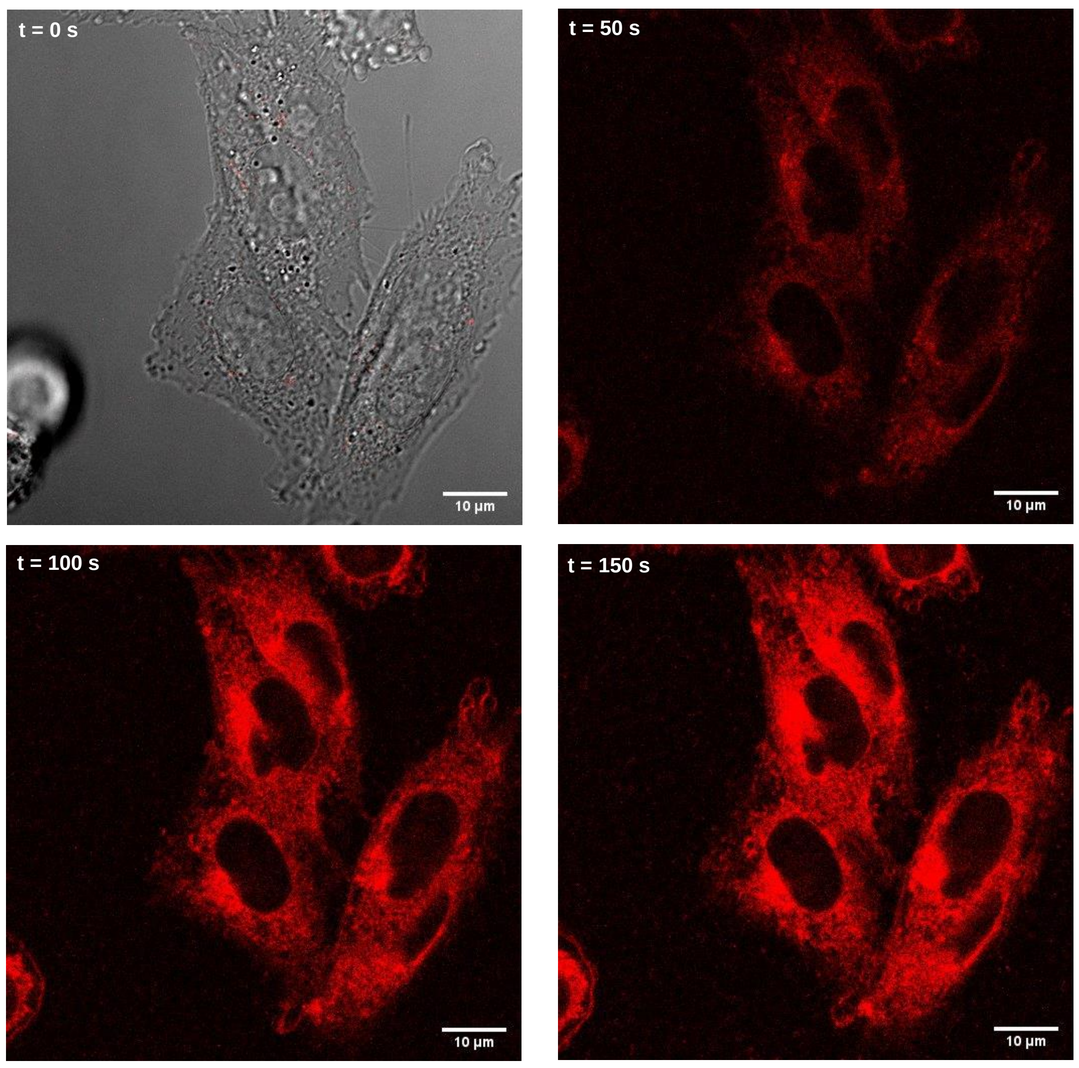
EXPERIMENTAL OBSERVATIONS

Confocal microscopy shows different cellular localization for liposomal Doxorubicin (DOXOVES®) with respect to molecular Doxorubicin (DOX).



Left: Encapsulated Doxorubicin is located predominantly in the cytoplasm.
Right: Molecular Doxorubicin reaches the nuclei leading cells to death.

DOXOVES® uptake is immediate (in seconds) and generates a significantly greater signal than autofluorescence.



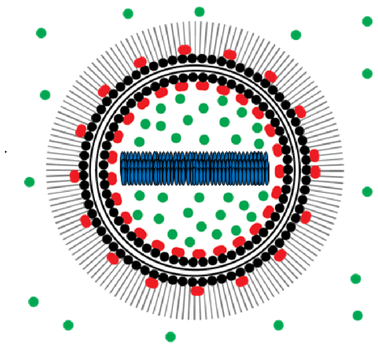
Confocal images of CHO cells observed during treatment with DOXOVES®.
Autofluorescence is already subtracted since t = 0 s
(corresponding to drug's administration).

RESULTS

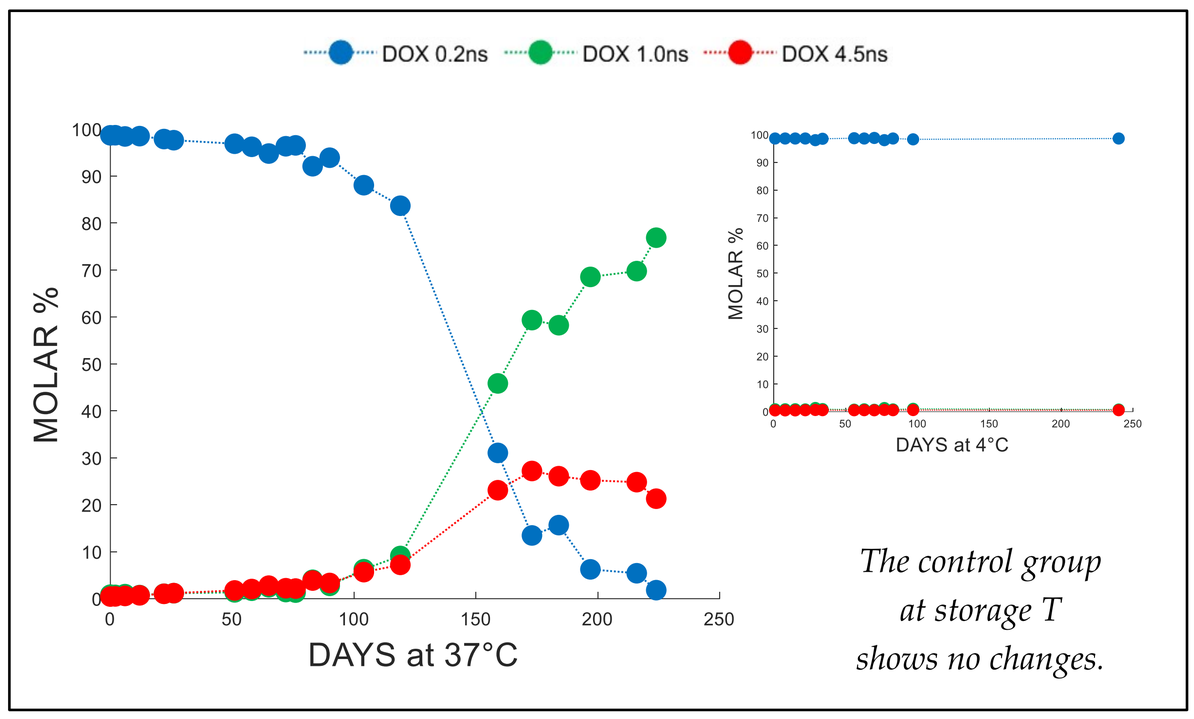
We resolved the **phasor-FLIM** signature of DOXOVES® into the contribution of three co-existing fluorescent species (each with its characteristic mono-exponential lifetime):

- **crystallized DOX** (0.2 ns),
- **free DOX** (1.0 ns),
- **DOX bound to lipids** (4.5 ns).

On the right a schematic representation of DOXOVES® based on phasor-FLIM results.



We quantified the changing in terms of molar fractions under altered temperature conditions.

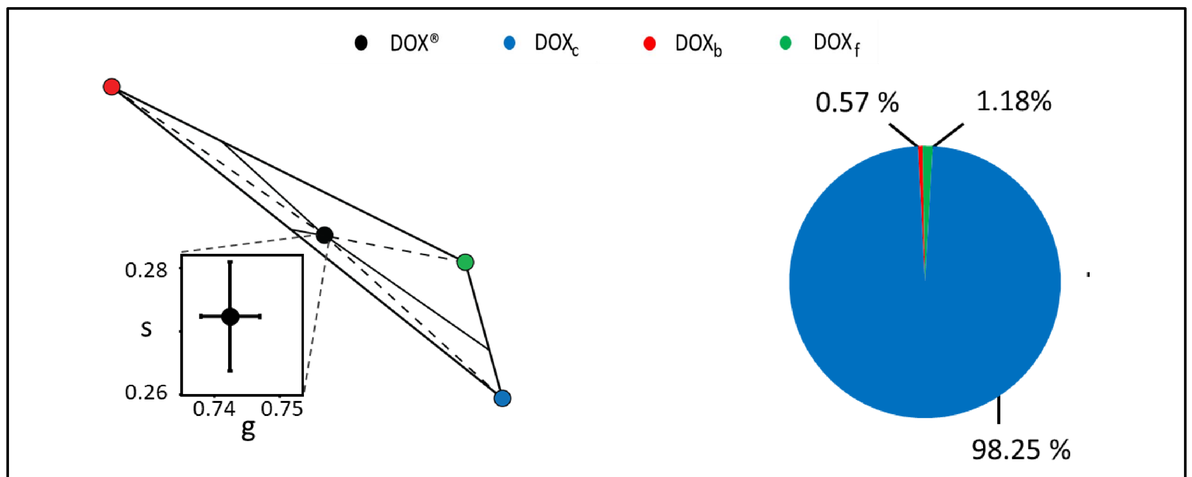


The control group at storage T shows no changes.

METHODS

The supramolecular organization of DOXOVES® was investigated with **nanoscale sensitivity** using a phasor approach to Fluorescence Lifetime Imaging Microscopy.

We investigated DOXOVES®**:** a formulation of 85 nm-diameter PEGylated liposomes loaded with Doxorubicin, exploiting its **intrinsic fluorescence.**



Left: Phasor Plot. *Right: Molar fractions.*
From the phasor position of DOXOVES® we geometrically obtained the fractional-intensity contributions of the three species.
After correction by QY and ε of each pure species, we calculated molar fractions.

SCAN FOR REFERENCE

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Nano 2022 Innovation Conference & Exhibition Rome, 19-23 September