## Basic research and Healthcare 4.0: which relationship? A case study ... at the nanoscale

A number of imaging methods based on fluorescence readouts (e.g. fluorescence fluctuations or lifetime) are currently used to increase the amount of quantitative information that can be extracted from optical microscopy measurements. This in turn stimulates continuous efforts in the development and implementation of new deep-learning algorithms for the analysis and interpretation of optical signatures. These new tools are expected to open new perspectives in molecular biophysics and related fields, nanomedicine above all. To photograph the state of the art, a case study within the nanomedicine research activities developed at NEST is presented here, which is of interest for the ISAAC initiative.

In detail, the supramolecular organization of Doxorubicin within the standard Doxoves<sup>®</sup> liposomal formulation (DOX<sup>®</sup>) is quantitatively investigated for the first time using a label-free method based on visible light and the phasor approach to fluorescence lifetime imaging (phasor-FLIM). First, the phasor-FLIM signature of DOX<sup>®</sup> is resolved into the contribution of three co-existing fluorescent species, namely: crystallized DOX, free DOX, and DOX bound to the liposomal membrane. Then, the exact molar fractions of the three species are derived by combining phasor-FLIM with quantitative absorption/fluorescence spectroscopy on pure standards. The proposed methodological platform paves the way to similar studies on the supramolecular organization of encapsulated luminescent drugs/molecules at any level, from production to processing within living matter.