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Introduction. Developed in the 70s, the Fluorescence Recovery After Photobleaching (FRAP) technique is based on the progressive increase of fluorescence intensity in a photobleaching area obtained after an illumination with a LASER beam. This enhancement corresponds to the gradual arrival (through gap junctions) of intact fluorescent molecules towards the targeted zone. This widely used method is principally dedicated to study fluorescent constituents mobility in cellular membranes and gap junctional intercellular communication (GJIC) at microscopic scale.

Purpose. The final addressed question is to assess the relevance to use GJIC characteristics to discriminate different cancer cell lines. With this aim in view, we have proposed a model-based approach in which some parameters could be potentially used as decision statistics. As proof of concept, we have tested the applicability of a compartmental model to describe differences between gap-FRAP responses of two human head and neck carcinoma cell lines (FaDu and KB).

Methods and Materials. Cx43, a protein of the connexin family responsible for GJIC, distribution and intercellular communication of FaDu and KB cells were performed in monolayer cultured cells and spheroids. Six experiments were performed for each case and data were collected through an imaging system composed of a macroscope combined to a fluorescence excitation source (Hg) and a CCD camera. The pixel intensities were measured in three concentric Regions of Interest (ROI) every 15 seconds for 15 minutes on each image. The measured values were assumed to be proportional to the mean amount of photons emitted in each ROI. After normalization with respect to the fluorescence intensity values before photobleaching, the kinetics of fluorescence recovery were plotted.

Modeling method. To study gap-Fluorescence Recovery After Photobleaching (gap-FRAP), the perturbation-relaxation kinetic equation is commonly used but is sometimes unable to describe some parts of the fluorescence response. A new behavioral model is proposed to study fluorescence recovery. The latter is based on a three-compartment representation (one compartment for each ROI) and the rates between each compartment represent the flow coefficients of the different gap junctions. This model provides a set of differential equations for which the associated continuous-time second-order transfer function was identified using the Simplified Refined Instrumental Variable in Continuous-time (SRIVC) algorithm. The algorithm

returns three estimated parameters (a static gain and two time constants) and their standard deviations.

Results. Two model parameters have allowed us to discriminate gap junctions functionalities. Indeed, parameters of KB cells, which are positive for Cx43 expression, are significantly superior to those of FaDu cells in culture 2-D and 3-D. No significant differences were observed for KB cells data independently of culture type confirming negligible contribution from underlying layers during fluorescence restitution in Z plan by confocal microscopy.

Conclusions. Our study exemplifies the contributions brought by dynamic models of biological phenomena to diagnostic applications in biomedicine.