Cell Tracking for Assessment of In-Vitro Uptake Kinetics in Ultrasound-Mediated Drug Delivery using Fibered Confocal Fluorescence Microscopy

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***Abstract*— Local drug delivery in oncology aims at depositing high doses of anticancer agents locally while limiting their toxic side effects. Biological barriers, such as cell plasma membranes, hinder their delivery and requires strategies to address this challenge. For this purpose, the use of ultrasound (US) waves allows enhancing plasma membrane permeabilization.**

Previously [1], we demonstrated the feasibility to monitor in real-time, with Fibered Confocal Fluorescence Microscopy, the intracellular delivery of a hydrophilic model drug mediated by US, and to quantify the pharmacokinetic parameters derived from a two-compartment model. Here we propose to correct for cellular displacement, which has not been taken into account previously, and improve the accuracy of the resulting pharmacokinetic parameters by cell tracking.

I. Methods

The proposed post-processing pipeline, applied on 3 example data sets, consisted of: 1) image pre-processing using an anisotropic filter (the Smallest Univalue Segment Assimilating Nucleus filter - SUSAN); 2) cell-nucleus detection using a Radial Symmetry Transform (RST) algorithm [2]; 3) frame-by-frame tracking [3] of detected nuclei using an Iterative Closest Point algorithm, followed by manual verification to exclude exceptions; 4) fitting of a two-compartment model, with an extracellular and an intracellular compartment separated by a plasma membrane, to the fluorescence intensity data collected for each individual nucleus: $I(t) = A[1-\exp(-k(t-T))]$; 5) statistical analysis of the resulting pharmacokinetic parameters (asymptote A, uptake onset T and uptake rate *k*). Data are reported as the median with the interquartile range (IR). The improvement of the accuracy was evaluated using the unpaired nonparametric Mann-Whitney test (significant when $p<0.05$).

II. Results

With application of the cell-nucleus tracking, 93% of the 370 nuclei showed a fluorescence signal well described by a two-compartment model. In addition to removing biases, by incorrectly estimating the nucleus signal, the distribution of pharmacokinetic parameters was found to be more homogeneous (Table 1), thus increasing their precision. No significant difference in the uptake rates was found, when the manual verification step was skipped, indicating the potential for a fully -automated pipeline, with thus reproducible outcomes.

TABLE I. Values of pharmacokinetic parameter

	Uptake rates (1/k)	*No tracking*	*Tracking*
Dataset 1 (n=168)	Median	3’00”	2’02”
	IR	3’09”	1’15”
Dataset 2 (n=95)	Median	3’19”	2’19”
	IR	3’08”	1’32”
Dataset 3 (n=107)	Median	3’24”	2’27”
	IR	4’05”	1’28”
All datasets (n=370)	Median	3’16”	2’14”
	IR	3’29”	1’29”
	p-value	< 0.0001	

III. Conclusion

The proposed automatic post-processing methodology improved the accuracy of pharmacokinetic parameters assessed in a cell population of more than one hundred cells. This will help studying kinetic aspects of US-mediated plasma-membrane permeabilization in local drug delivery.

References

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