Verification of Steady State in Blood Flow Experiments

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***Abstract*—** This work shows a method of examination of the numerical onset of a computer simulation, modeling an experiment with flowing red blood cells in a microfluidic device. The initial onset could have a perturbing impact to the numeric simulation, and so its length is to be determined. A method is based on comparison of the statistical distribution of cell speeds and rotations during simulations, using Kolmogorov – Smirnov test. The speed data show that a behavior of cells gets more stabilized during the simulation. Even though data about rotation do not confirm this finding, a more precise rotation characteristic is suggested.

***Keywords*—**blood flow, steady state, statistical characteristics.

I. INTRODUCTION

Recently, specialized microfluidic devices have been developed for sorting, capturing or detecting specific blood cells from blood samples [1], [2]. Design and manufacture of such devices is very time consuming and expansive [3]. Therefore, it is better to use computer simulations for their optimization, where different experimental setup can be relatively easily changed [4], [5]. Such simulation model was developed by Cell-in-fluid research group [6], [7]. The model consists of two main parts: fluid and elastic objects. A fluid is modelled by Lattice-Boltzmann method and can represent plasma or other liquid solution. Objects represent blood cells and their models are based on discretization of their surfaces. An interaction between these two parts is secured by immersed boundary method. The model and its implementation in open source software ESPResSO is in details described in [8].

To evaluate the precision of the software, and so to compare the results of numerical simulations with results of laboratory experiments, we are developing a statistical tool which could be used for such a comparison. This tool does not compare the cells one by one, but it uses a statistical approach to consider a complexity of a multi cellular system [9].

Several aspects of the cell movement are considered: Their position in the microfluidic device, their skew, their speed and their rotation. The statistical tool helps us to process the property of each cell and to determine generalized properties per simulation. The comparison between the two types of experiments (numerical or laboratory one) is done afterward by comparing that generalized information about the ensemble of the cells.

However, the comparison between the numerical and laboratory experiment should be done by comparing a steady state of the cell behavior within the both devices. The initial irregularities in the laboratory experiment can influence the movement of the cells in a manner which is not repeatable in the numerical experiment, and vice-versa. This work is focused on the examination of the numerical onset of the computer simulation. This one influences the course of the simulation at its beginning, however the length of the impacted part of the simulation is to be determined.

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II. METHODOLOGY

There are several ways how to determine the effective length of the numerical onset. In [10], the Discrete Fourier Transformation (DFT) was used, although the approach considered only the rotation data from three specific cells. The aim of the article was to get an approximative idea about the influence of the numerical onset, which explain the limited usage of the tool. To get a more founded estimation of the length of the influenced part, this method using the DFT should be applied to the totality of the cells in the simulation, eventually with an extension to the velocity data.

In this article, a different approach is used, in order to take into account a totality of the cells in the simulation, using a simpler approach which do not require the using of the DFT. Instead of it, the evolution of the speeds and rotation magnitudes of the cells was tracked, to identify whether they get stabilized during the simulation. The rotation in this concept means angular speed of the cell in respect of the center of the cell.

A simplified approach was chosen to quantify the rotation magnitude of the cells – it consists only on the simple difference between the X-velocity of two points with extremal Y-coordinate (more details in [10]). This approach appears to generate very comparable courses of the cell rotation during the simulation as the rigorous method, which is however more data-demanding.

To identify the stabilization of the cell behavior, instantaneous speeds (and rotations) of each cell were recorded regularly during the simulation. Each measurement represents a set of 50 values. The values in such a measurement were than sorted in ascending order. The recording of the speeds and of the rotation magnitudes was executed every 5000 numerical steps, which correspond to 1 ms. In such a time, a cell makes a shift of approximately one length (~4μm).

Examples of recorded speeds and rotation magnitudes are shown in Fig. 1 and Fig. 2.

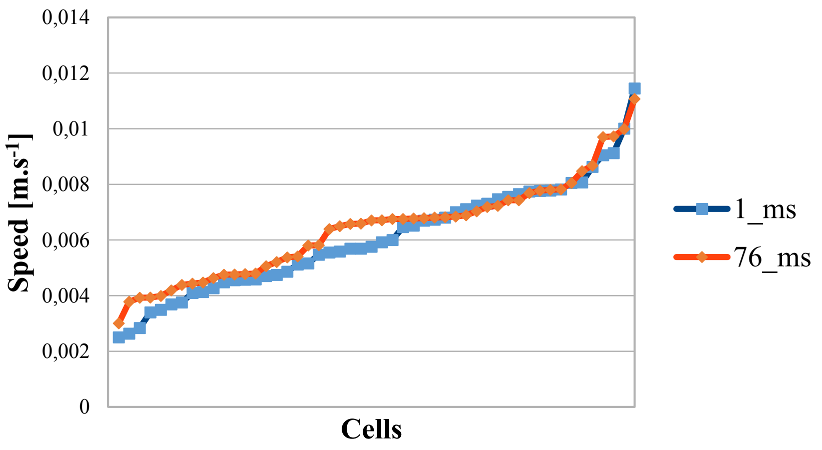

Fig. 1: Example of measured velocity magnitudes of all the cells after 1 ms of the simulation, and in the end of the simulation, after 76 ms. Values are sorted in ascending order.

The statistical distribution of those speeds (or rotations) in one moment is an object of comparison between the various moments of the simulation. This helps us to determine whether the distribution of the speeds (or rotations) get stabilized during the simulation.

The distribution of the speeds (and rotations) are compared using Kolmogorov-Smirnov (KS) test. It is a test which determines whether two compared samples come from the same distribution, even if the distributions are not common or well-known in the statistics. The smaller is the result of the KS test comparing the samples, the bigger is the probability that the two samples came from the same distribution.

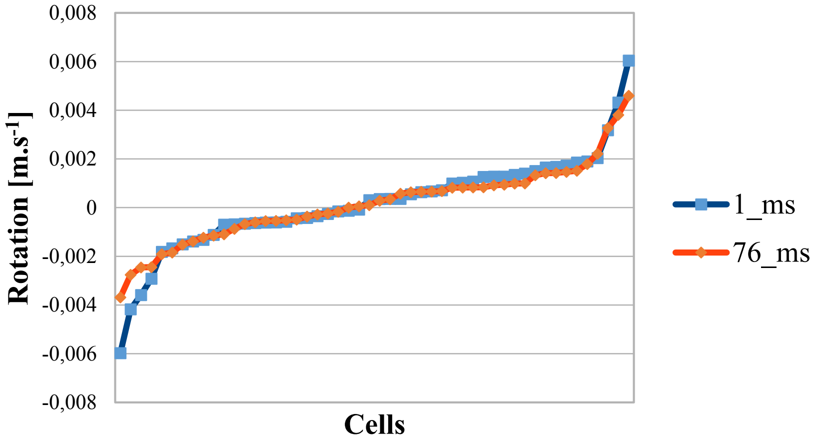

Fig. 2: Example of measured rotation magnitudes of all the cells after 1 ms of the simulation, and in the end of the simulation, after 76 ms. Values are sorted in ascending order.

During the simulation, with a total number 380 000 steps, 77 recordings of the instant speeds and rotation magnitudes were done. In order to monitor the evolution of the stability of the speed (or rotation) distribution in time, the KS test was at first done for each couple of subsequent measurements. So, the difference of the speed (or rotation) distribution was checked every 1 ms. After that, a similar comparison was done by skipping 4 measurements. It means that the difference of the speed (or rotation) distribution was checked for intervals of 5 ms. Then, the comparison between the distributions was done with skipping 9 measurements, so for the intervals of 10 ms. Finally, for each simulation, we obtained three series of the comparisons. An example of such a series is shown in Fig. 3.

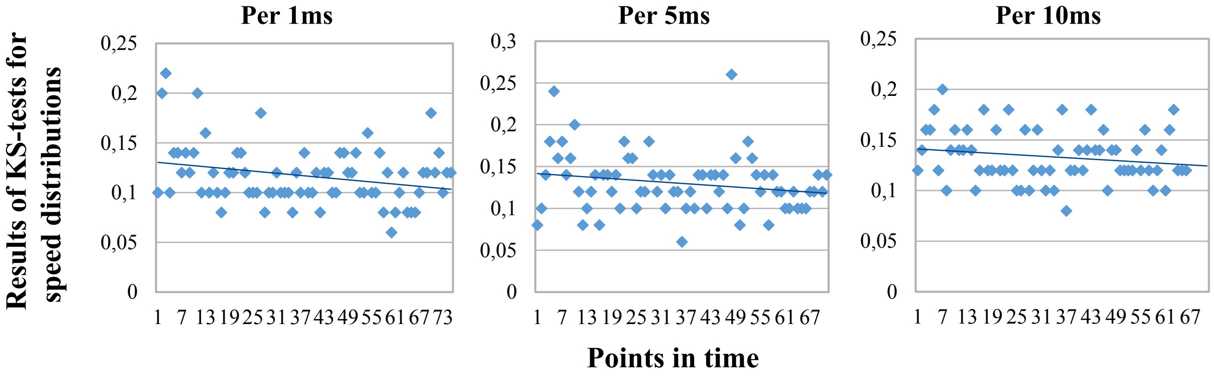

Fig. 3: Example of a comparison between different speed distributions recorded during the simulation.

Each series was than divided into two equal parts, from 0 to 38 ms and from 39 to 76 ms. After that, the average fit was evaluated for the first part and for the second part. Those two numbers signify the average resemblance of the speed (or rotation) immediate distributions in the first half and in the second half of the simulation. The improvement in the steadiness of the speed distribution is than checked by comparing the two numbers. The example is shown in the Fig. 4.

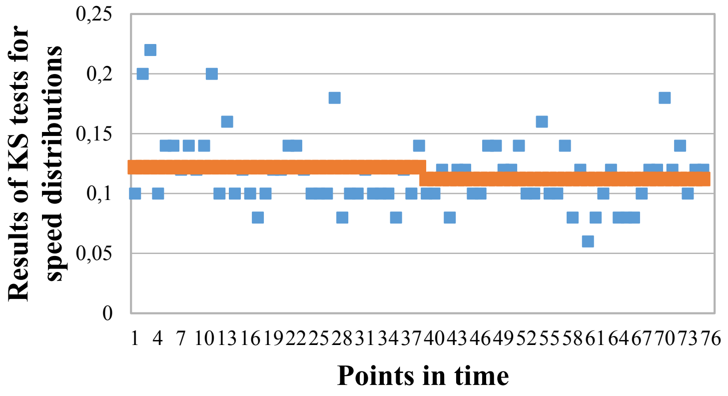

Fig. 4: Example of comparison of steadiness of the speed distribution in the first and the second half of the simulation. The average value of the results of the KS test is bigger in the first half of the simulation. It means that speed distributions in various moments are less similar to each other than in second half of the simulation.

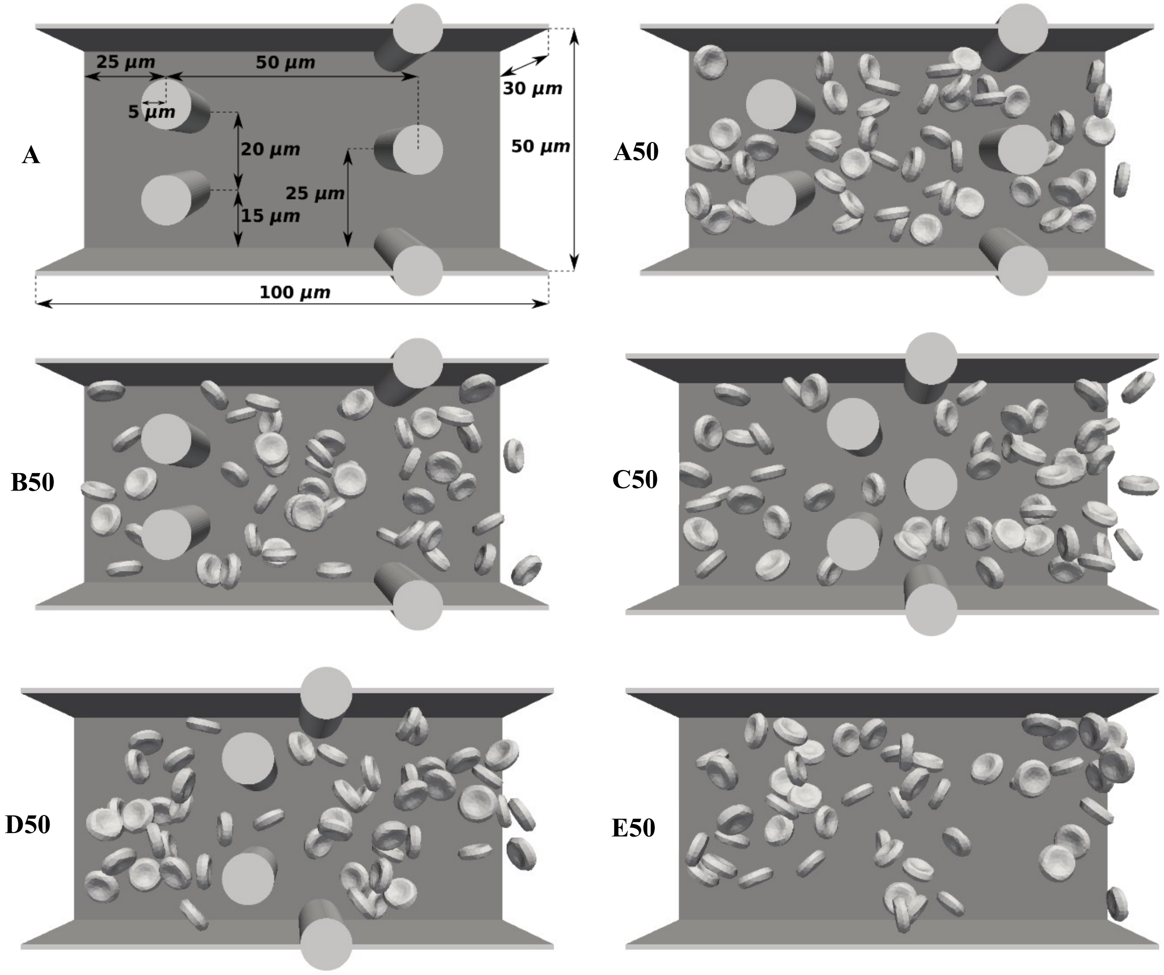

Figure 5: Five different geometries (A, B, C, D, E) used for evaluation of the numerical onset impact length. On the top left picture are sizes of the channel A, other pictures show different RBCs seedings.

Moreover, we examined the evolution of the minimal, maximal and average speed (or rotation) of the cells during the simulation. Those values should, as well, get stabilized in the time, within a range of acceptable oscillations.

III. EXAMINED SIMULATIONS

All of the examined simulations contain 50 cells, they differ only by an initial position of those cells and by a geometry of the obstacles inside of the channel. The Fig. 5 represents five different geometries of the simulation box. Each geometry was used twice to run an experiment, with two different initial seedings of the cells. Hence, ten simulations (A50a, A50b, B50a, B50b, C50a, C50b, D50a, D50b, E50a, E50b) were used to determine the length of the numerical onset impact.

IV. RESULTS

In the Table 1, we can see the results of the comparison for velocities and in the Table 2 the results for the rotations.

Table 1: The evolution of the average fit between the velocity distributions - comparisons of the velocity distributions in first and second half of the simulation ("imp." means improvement), with an offset of 1ms, 5ms and 10ms. The fit is evaluated by using Kolmogorov-Smirnov test.

	offset 1 ms			**offset 5 ms**			**offset 10 ms**		
	0-39 ms	**38-76 ms**	**imp.**	**0-41 ms**	**36-76 ms**	**imp.**	**0-44 ms**	**35-76 ms**	**imp.**
A50a	0.122	0.112	8.20%	0.134	0.124	7.46%	0.135	0.132	2.22%
A50b	0.129	0.115	10.85%	0.152	0.130	14.47%	0.144	0.127	11.81%
B50a	0.115	0.106	7.83%	0.130	0.116	10.77%	0.138	0.119	13.77%
B50b	0.113	0.107	5.31%	0.133	0.123	7.52%	0.124	0.117	5.65%
C50a	0.090	0.090	0.00%	0.133	0.116	12.78%	0.146	0.120	17.81%
C50b	0.104	0.097	6.73%	0.142	0.130	8.45%	0.148	0.139	6.08%
D50a	0.092	0.088	4.35%	0.102	0.102	0.00%	0.097	0.098	-1.03%
D50b	0.092	0.094	-2.17%	0.129	0.132	-2.33%	0.125	0.114	8.80%
E50a	0.054	0.051	5.56%	0.064	0.066	-3.13%	0.071	0.071	0.00%
E50b	0.057	0.047	17.54%	0.070	0.061	12.86%	0.081	0.066	18.52%
avg.	**0.097**	**0.091**	**6.42%**	**0.119**	**0.110**	**6.89%**	**0.121**	**0.110**	**8.36%**
avg A-D	**0.107**	**0.101**	**5.14%**	**0.132**	**0.122**	**7.39%**	**0.132**	**0.121**	**8.14%**

Table 2: The evolution of the average fit between the rotation distributions – comparison of the rotation distributions in first and second half of the simulation ("imp." means improvement), with an offset of 1ms, 5ms and 10ms. The fit is evaluated by using Kolmogorov-Smirnov test.

	offset 1 ms			**offset 5 ms**			**offset 10 ms**		
	0-39 ms	**38-76 ms**	**imp.**	**0-41 ms**	**36-76 ms**	**imp.**	**0-44 ms**	**35-76 ms**	**imp.**
A50a	0.113	0.110	2.65%	0.146	0.144	1.37%	0.138	0.144	-4.35%
A50b	0.124	0.115	7.26%	0.155	0.141	9.03%	0.155	0.146	5.81%
B50a	0.115	0.117	-1.74%	0.144	0.148	-2.78%	0.154	0.149	3.25%
B50b	0.112	0.119	-6.25%	0.149	0.158	-6.04%	0.143	0.164	-14.69%
C50a	0.108	0.114	-5.56%	0.148	0.153	-3.38%	0.155	0.161	-3.87%
C50b	0.112	0.121	-8.04%	0.144	0.150	-4.17%	0.137	0.159	-16.06%
D50a	0.113	0.120	-6.19%	0.144	0.146	-1.39%	0.139	0.149	-7.19%
D50b	0.126	0.117	7.14%	0.184	0.149	19.02%	0.162	0.142	12.35%
E50a	0.114	0.118	-3.51%	0.132	0.145	-9.85%	0.128	0.149	-16.41%
E50b	0.116	0.109	6.03%	0.161	0.132	18.01%	0.152	0.138	9.21%
avg.	**0.115**	**0.116**	**-0.82%**	**0.151**	**0.147**	**1.98%**	**0.146**	**0.150**	**-3.20%**
avg. A-D	**0.115**	**0.117**	**-1.34%**	**0.152**	**0.149**	**1.46%**	**0.148**	**0.152**	**-3.09%**

We can observe that there is a systematic improvement of the similarity of the velocity distributions along the course of the simulation. Its value is about 5% - 8%. We can note also that the absolute values of the similarity are smaller for the geometry without obstacles.

The same conclusion cannot be stated regarding the evolution of the rotation distribution similarity. The similarity of the rotation distributions is randomly improving or getting worse during the simulation, with an average improvement comparable to zero. It does not mean that the rotations are already in a steady state from the early beginning. As we can see in [10], it is not the case, the rotation is as well perturbed at the beginning of the simulation, but this method of comparing its distribution is probably not pertinent enough to show it.

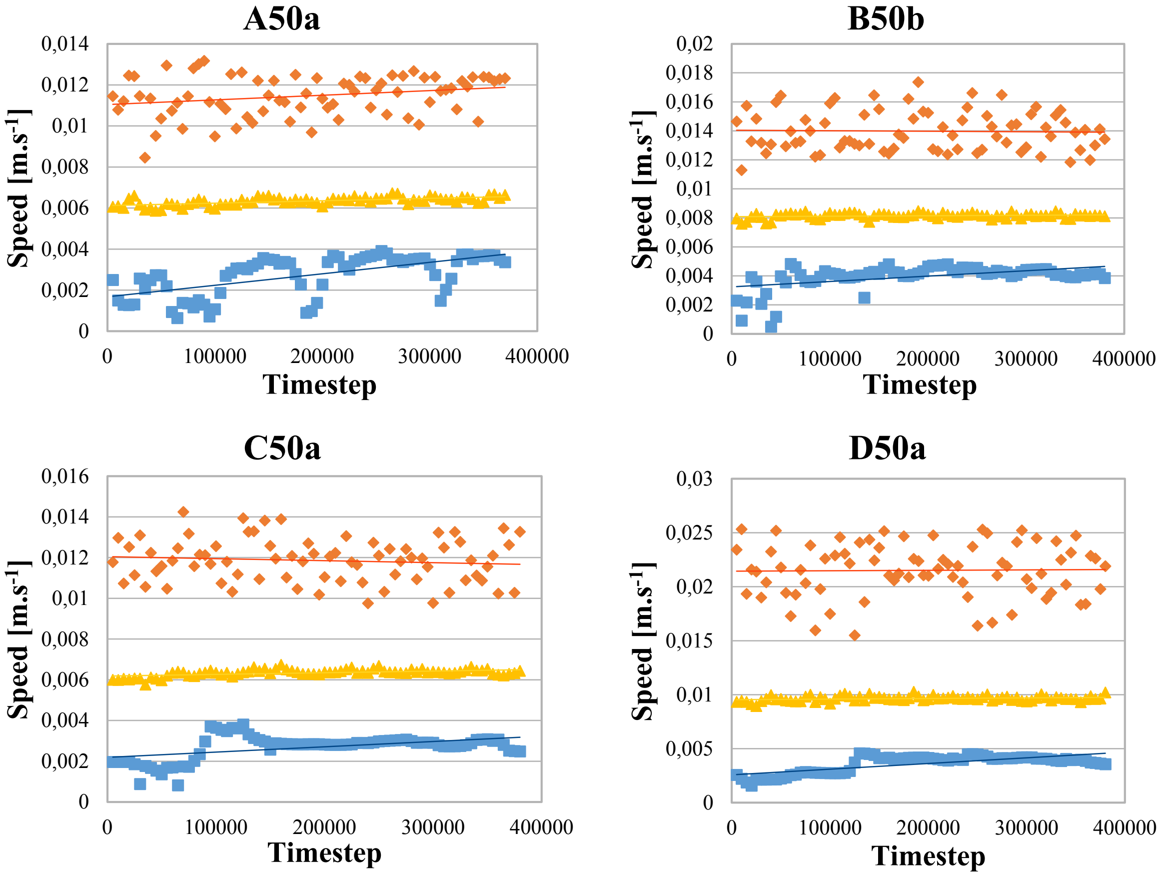

Fig. 6: Examples of evolution of the minimal (blue rectangles), maximal (red diamonds) and the average (yellow triangles) speed during the simulation.

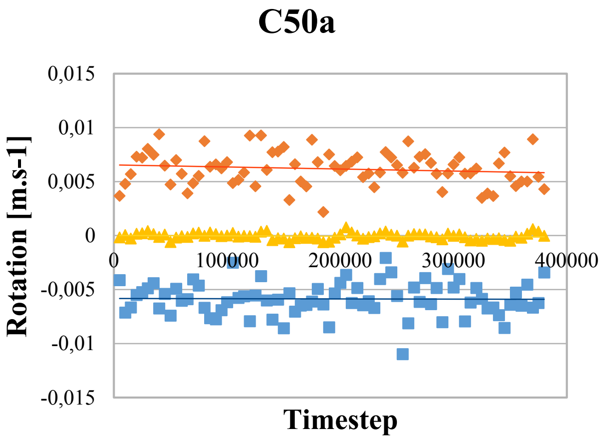

Fig. 7: Example of evolution of the minimal (blue rectangles), maximal (red diamonds) and the average (yellow triangles) rotation during the simulation.

Another point of view can be obtained by observing evolution of the extremal and average velocities. The Fig. 6 shows examples of such a graph. While the average and the maximal speed do not seem to be perturbed in the beginning of the simulation, the minimal speed is slightly different in the beginning. It takes approximately one fourth to one half of the simulation (20-40 ms) to get stabilized.

The Fig. 7 represents an example of such a graph for rotation magnitudes. The disadvantage of this approach applied to the rotations is that while the values of the speed are always positive and relatively far from zero, the values of the rotation are as negative as positive. Even if we consider only the absolute value of the rotation, we obtain a lot of values close to zero. There is not any tendency in the evolution of the minimal, maximal and average rotation, which confirms the conclusion from the observations from the Tab. 2.

V. CONCLUSION

In this article, we study the possibility to determine the numerical onset impact length in numerical models of cells in microfluidic devices. We compare the statistical distribution of the cell velocities and rotations during the simulation, using Kolmogorov-Smirnov test. The results of the comparisons of the speed distribution show that the behavior of the cells gets more stabilized in the second half of the simulation. The evolution of the value of the minimal cell speed during the simulation show that the length of the numerical onset impact is approximately 20-40 ms. However, these results are issued only from the data relative to the speed of the cells. The rotation of the cells does not manifest a stabilization of its distribution. This could mean that the applied approach is not appropriate, and another one should be used to evaluate the steadiness of the cell rotation. The Discrete Fourier Transformation is a tool which was used briefly to determine the presence of impact of the numerical onset in our previous work, and it appears that it could be more sensible to detect the evolution of the steadiness of cell rotations during simulation.

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