Response to Referee #1

Global stability of flowing red blood cell trains

Bryngelson, Freund

We are grateful for the referee's efforts in improving the quality of this paper. We quote the comments of the referee and discuss changes made to the paper in response to these comments in the following.

Referee #1: The authors have developed a method for simulating the motion of multiple red blood cells in a tube, using spherical harmonics to describe the shape of each cell and a boundary integral method to compute the coupled motion of the cell and the surrounding fluid. This method is well suited for exploring the stability properties of motions in which the cells are initially placed in a symmetric train-like array on the tube axis. The authors examine the effects of several parameters, including tube diameter and cellular volume fraction, internal viscosity and volume. The typical modes of instability involve either a tilt of individual cells with respect to the axis or a staggered displacement of alternate cells in the train. These predictions are consistent with observed behaviors. The work is thoroughly done and carefully described.

1. P. 1. The origin of Fig. 1 is unclear. If it was obtained by the methods described in this manuscript, then that should be stated.

   The caption is now rewritten to explain that these visualize simulation results using methods discused later in the paper.

2. P. 5. The variables ϕ and θ are not defined. Presumably they label material elements in the membrane. Are they the spherical coordinates of a material element if the cell is expanded to a sphere of radius r_o? Also, there is a conflict of notation with ϕ as particle volume fraction (P. 3).

   The ϕ (changed to ψ in the revision to avoid the noted conflict of notation) and θ are the spherical reference coordinates, now defined more clearly on P. 5.

3. P. 5. How is the surface traction ($\Delta\boldsymbol{\sigma}$) calculated? Presumably the membrane stresses (tension, bending and transverse forces) are calculated using the stated constitutive model, and the spatial derivatives of these stresses can be obtained analytically in terms of the coefficients in equation (2). Some explanation is needed.

   The referee is correct, and we should have explained this more thoroughly. The tractions are derived from a local force and torque balance, and the necessary derivatives are computed via the spherical harmonic expansion. We have also added more details regarding the form of the constitutive model and the numerical methods for its evaluation, along with the references to sources with additional details.

4. P. 6. "transform is unitary"

   This error in the text has been fixed: "transform in unitary" $\rightarrow$ "transform is unitary".

Response to Referee #2

Global stability of flowing red blood cell trains

Bryngelson, Freund

We are grateful for the referee's efforts in improving the quality of this paper. We quote the comments of the referee and discuss changes made to the paper in response to these comments in the following.

The paper, which focuses on the flow of trains of red blood cells in a straight microchannel, considers a yet non-tackled problem: the three-dimensional study of the global stability of elastic membranes encapsulating an internal fluid, while accounting for cell-wall and cell-cell interactions. The only study that presently exists on the topic is the one conducted by the authors, in which they had recently studied the stability of a train of cells in the two-dimensional case. Being aware of the limit in relevance of the first study, the flow of cells being an intrinsically three-dimensional problem, the authors now present the results of the very first stability analysis of models of red blood cells in flow. Generalizing the 2D stability study to 3D has necessitated deep reformulation of the stability analysis. After having very well introduced the manuscript, the authors clearly explain the physical and mathematical issues that play a key role in the stability study. What is of great interest is that they then conduct a comprehensive parametric study and systematically analyze the influence of all the governing parameters of the problem. The study is thus exhaustive and highly relevant.

1. Red blood cell modeling:

   - It could be of interest to specify that the channel is cylindrical as the reader can infer it from Fig 1.

     We agree and updated the manuscript to reflect this.

   - What is the reference shape retained for the red blood cells? The at-rest equilibrium shape is mentioned to be the biconcave shape: is it the reference shape? Many recent studies have suggested that the reference shape is rather a nearly spherical shape. Have the authors looked at the effect of the reference shape on the results of the stability analysis?

     Indeed the reference shape is the biconcave at-rest geometry, which is now stated explicitly. We have not systematically investigated the effect of varying the reference shape, though we also anticipate it not to be principally important for the high strain rates considered.

   - How are bending effects taken into account in the constitutive modeling of the wall?

     The tube wall is rigid, which is now stated explicitly in the manuscript.

   - It would be interested to provide the value of the parameter C of the Skalak law. Many authors have encountered numerical instabilities when the parameter C is much higher than 1, as it is presently the case ($C \approx 7.6$). Have the authors encountered such issues? Have they extended the stability analysis varying C (i.e. the dilatation modulus E_d)?

     In our model we have $C = 16.1$, which is now noted in the manuscript, though no numerical instabilities have been detected. Our results are insensitive to doubling C, which is stated on page 4.

- Are the bending, dilatation and shear moduli really fully uncorrelated, as stated in the manuscript? Two of them naturally are, but one can wonder whether a thin-shell approach combined with the Skalak et al law could allow to correlate the 3rd moduli to the first 2. Since the 3 moduli are associated with one another through the wall thickness, it could for instance be interesting to determine the equivalent thickness associated with the chosen values of the parameters and compare it with the literature.

  The bending modulus we use is based upon experimental measurements. We have not delved into the rich literature on constitutive modeling of cellular membranes, though we note here that it is significantly non-uniform in its structure through its thickness. It would seem that some accounting for the structure would be a greater priority than considering a thickness parameter alone. We emphasize that the dilatation modulus is chosen to keep the membrane area approximately constant, as a model for the near incompressibility of actual red blood cell membranes. Further, this combination of models and moduli have been shown to quantitatively reproduce important observables of actual whole blood flow, such as its famous non-monotonic effective viscosity [Zhao 2010].

2. Numerical methods:

   - It is unclear why the authors considered a computing box that is bigger than the cylindrical channel. Can you elaborate on the benefit to solve for flow velocities outside the channel?

     The formulation of the triply-periodic PME scheme, with the standard approach of solving the mesh component using FFTs, is built upon rectangular domains. The flow outside the tube is a simple consequence of this choice. The domain is chosen to be slightly larger than the tube diameter such that the no-slip tube boundary does not intersect with itself in those periodic images. This is now stated in the manuscript.

   - What is U? the unperturbed velocity in the channel?

     U is the (unperturbed) mean flow velocity in the tube, as stated on page 5.

   - It would be useful to detail the complete set of boundary conditions that is imposed.

     The boundary condition summary in figure 2 is augmented to address this. The computation of the cell membrane surface tractions is now also better explained.

   - Information on the channel mesh is provided, but none on the meshing of the cells. Is the mesh the same whatever the cell-to-channel radius ratio?

     It is now explained that the tube-wall mesh is stretched as appropriate for the choice of D and L. The spherical harmonic discretization of the cells quantified on page 5.

   - Is the numerical method used to correct the volume energy-preserving?

     The volume-correction procedure is not formally energy preserving, as it is based upon a kinematic variational description. However, these changes are sufficiently small as to be unimportant for the formation of the base states, for which the volume drifts less than 1% even without the volume correction procedure. Further, our stability analysis is wholly independent of the volume correction, which is also stated on page 6.

   - Stokes flows naturally have symmetries (e.g. axial symmetry here), which can lock the simulations in non-stable equilibrium configurations. Have perturbations been applied in the DNS simulations? If you, what is the shape/type of this perturbation?

The symmetry of the flow allows the (possibly unstable) equilibrium state to form in our simulations, and thus our stability analysis proceeds as time-stationary (with respect to the base flow velocity U). The agreement between the amplification rates of our linear analysis and the associated DNS (see, e.g. figure 4) ensures that the simulations are not locked in a quasi-stable state. Another advantage of posing the problem this way is that it replicates the flow of cells that have been 'focused' into a train by external factors (see section 6).

3. Stability analysis formulation:

- How is the perturbation imposed in practice in the study?

  The perturbation is applied by adding the corresponding eigenvector to the base cell shapes and scaling it by magnitude ε. This is now stated more clearly in the manuscript.

- Can one consider the problem to be solved mathematically in the case of an infinite supply of energy, which is, of course, never the case in reality?

  Indeed the problem is solved invariant to the energy of the perturbation involved, which was quantified in table 1. One must choose between many possible constraints when forming the perturbation basis, for which we choose a geometrically-constrained measure. We emphasize that a strain-energy-invariant approach is inappropriate for our analysis since zero-strain-energy perturbations (e.g. rotations and translations) can potentially be amplifying. This is stated on page 6.

- Why is there a ratio of 2.2 between the squares of the norms of the vectors $\mathbf{x}$ and $\mathbf{s}$?

  The norms based on the collocation points $\mathbf{x}$ and spherical harmonics $\mathbf{s}$ differ by a constant factor of about two due to the non-unitary property of the transformation and are quantified by Parseval's theorem.

- The definitions of the vectors $\mathbf{x}_b$ and $\mathbf{s}_b$ are missing.

  These vectors are the base cell locations in physical and spherical harmonic coordinates, respectively. We have added this description to the manuscript.

- The sentence "this measure is not unique, however, and is not expected to be for such a complex system" would need to be clarified. Further explanation on the concept of an energy-based metric would need to be added.

  Indeed no measure would be unique, as noted in the previous comment regarding a normalization of the perturbation subject to a constant strain energy. This is now clarified in the manuscript.

- It would be useful to detail where equation (11) comes from and how it is related to equation (4).

  We agree and have added further detail regarding how, using the new numbers, (12) follows from (5).

- What is the computational time required to construct the matrix $\mathbf{A}$?

  The computational time required to construct $\mathbf{A}$ can be cast in terms of time steps of a typical simulation, which is now quantified in the manuscript: about 5 hours.

4. Results:

   - Some results of capsule shape are provided in configurations that one can assume to be "steady-state" as no information is provided on the associated time (e.g. Fig 6 - 7). How is "steady-state" presently defined? Are steady states ever reached?

     The flow is said to be in a steady state when the wall-normal velocity is less than $10^{-4}U$ for all cell collocation points, which is discussed in section 5 A. Of course, the flow is never perfectly steady, though this threshold is sufficiently small that our results are independent of it for our choice of the δ that constructs $\mathbf{A}$. The times associated with these states vary depending upon the flow configuration, and so we quote the terminating condition instead.

   - Many authors have indicated how the perturbation brought by a cell on the baseline flow quickly decays in Stokes flow conditions and tends to be negligible when one moves away of the cell by a cell diameter. One could then expect the cell train to be stable when the cell-cell distance is about 2 diameters. Is it something that is seen? The present results do not provide information on the question, as it appears that, even in the case, the cell-cell distance is smaller than 2 diameters.

     We do not consider cases with such small cell packing fractions, as these conditions are not typically seen in the microcirculation or achieved in microfluidic devices (see, e.g. sections 1 and 6), and thus restrict our study to these cases. While spanwise perturbations to such a flow, for certain parameter combinations and tube diameters, might decay, it is unclear if this behavior is robust to the full basis of possible membrane perturbations, such as we consider here.

   - Fig 8: are the high modes a consequence of the way the cell wall is modeled (membrane model)? Would a proper accounting of the wall thickness "kill" these high fluctuation modes?

     Our model is a standard and well formed choice with a calibrated bending resistance. Any model that linearizes to this same form, should of course show a similar behavior. The role of additional terms in other models is potentially interesting, but obviously beyond our scope. That said the short wave-length do decay rapidly regardless (see figure 9).

   - I would suggest to add an extra sub-section on the middle of p.19 with title "Cell-cell and cell-wall interactions"?

     This is a good idea. We have added this additional subsection.

5. Extra question: Can the study and the images of the transient cell shapes (e.g. Fig 12) provide information or introspect on the stability of a capsule train flowing through a bifurcated channel?

   The cell–cell pairing seen for some cases may indeed have implication for flows through bifurcated vessels, though we emphasize that it is challenging to draw definitive conclusions based upon the results presented. We have added a brief comment at the end of section 6.

6. Minor points:

   P3: non-model

   P3: the the minimum streamwise

P7: replace the semi-column by a comma in (i; j, k) and (j, k; q, r)?

P12: with the their respective

Fig 14: invert the order of the upper and lower figures and they appear in the reverse order.

P.21: provides as an estimate

P.21: thus, is seems

We have gladly corrected the manuscript to fix these typos and implement these suggestions.