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Modeling the hematocrit distribution in microcirculatory networks: A quantitative evaluation of a phase separation model

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Abstract

Objective—Theoretical models are essential tools for studying microcirculatory function. Recently, the validity of a well-established phase separation model was questioned and it was claimed that it produces problematically low hematocrit predictions and lack of red cells in small diameter vessels. We conducted a quantitative evaluation of this phase separation model to establish common ground for future research.

Methods—Model predictions were validated against a comprehensive data base with measurements from four mesenteric networks. A Bayesian data analysis framework was used to integrate measurements and network model simulations into a combined analysis and to model uncertainties related to network boundary conditions as well as phase separation model parameters. The model evaluation was conducted within a cross-validation scheme.

Results—Unlike the recently reported results, our analysis demonstrated good correspondence in global characteristics between measurements and predictions. In particular, predicted hematocrits for vessels with small diameters were consistent with measurements. Incorporating phase separation model parameter uncertainties further reduced the hematocrit validation error by 17% and led to absence of red-cell-free segments. Corresponding model parameters are presented as alternatives to standard parameters.

Conclusion—Consistent with earlier studies, our quantitative model evaluation supports the continued use of the established phase separation model.

Keywords

Bayesian inference; flow simulation; microcirculatory measurements; phase separation effect; quantitative model evaluation

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Introduction

Red blood cells constitute the most frequent cell type in blood with a volume fraction (hematocrit) typically about 40–45%. These cells have a high oxygen carrying capacity and are critical for the continuous and adequate supply of oxygen to tissue. Because of their size and number, red blood cells strongly influence the rheological properties of blood and thereby its distribution across the microvasculature [20,25,29]. Experimental studies have reported a highly heterogeneous distribution of hematocrit across the microvasculature as well as a reduced average capillary hematocrit relative to the hematocrit of the feeding arteriole. Theoretical modeling has provided a quantitative basis for interpreting the mechanisms underlying these phenomena [19,26]. As blood flows through microvascular networks, it encounters a series of branch points, usually either converging or diverging bifurcations. At diverging branch points, red blood cells and plasma may be distributed disproportionally between daughter vessels and the resulting discharge hematocrits in daughter branches may differ between each other and also from the mother vessel - the “phase separation effect” [18]. Red blood cells tend, on average, to enter the branch with higher flow rate, resulting in a strong correlation between hematocrit and flow velocity [18,38]. At the network level, this correlation leads to a reduction in average capillary discharge hematocrit relative to the feed hematocrit - the “network Fåhræus effect” [19]. Furthermore, the phase separation effect may cause hematocrit to increase in arterioles, if the arterioles give rise to a series of smaller side branches. This effect, in turn, increases the probability that red blood cells travel along longer pathways through the network - the “pathway effect” [17]. Accordingly, the interaction between the phase separation effect and the architectural heterogeneity of microvascular networks results in large heterogeneities in microvessel hematocrit [17,26] as illustrated in Figure 1.

In vivo and *in vitro* measurements and also theoretical considerations have guided the development of mathematical models of the phase separation effect [6,10,12,18]. These phase separation models have been incorporated into continuum models for simulating blood flows through microvascular networks. Continuum models provide a pragmatic approach for modeling blood flows in larger microvascular networks based on current understanding of microcirculatory flow phenomena [13]. Such models have been used, for example, to study hematocrit distributions in microvascular networks [16,28,29], to simulate blood flow in large, anatomically accurate microvascular networks [11,15], and as part of bottom-up simulations of the blood oxygenation-level dependent (BOLD) signal acquired with functional magnetic resonance imaging (fMRI) [7].

In the present study we performed a quantitative evaluation of the phase separation model developed by Pries and colleagues [18,22,28], herein referred to as the “PS model”. This model was originally used to determine the influence of a series of physiological variables on red blood cell distribution at bifurcations. The PS model, an asymmetric three-parameter logistic function, was fitted to comprehensive *in vivo* measurements in rat mesentery. The analysis showed that the diameters of segments constituting the bifurcation, inlet hematocrit, and also the hematocrit profile as influenced by the upstream bifurcation all affected the parameters of the logistic function and hence the phase separation effect [18]. This parametric description was later adopted in simulations of hemodynamics in mesentery

networks [28]. Network flow simulations were compared against a comprehensive data set with measurements of hematocrit and flow direction in three mesentery networks with a total of 1895 vessel segments. While considerable variation between local segment hematocrit measurements and model predictions was observed, a good correspondence between measurements and model predictions was observed for global characteristics of the network hemodynamics such as the mean segment hematocrit and also the distribution of hematocrit. The model simulations also predicted hematocrit to decrease with vessel segment diameter [26] consistent with experimental measurements in the mesentery of the cat [14] and the rat [19]. The PS model has since been used in a wide range of modeling efforts and is perhaps the most widely used phase separation model. Recent applications of the PS model include large scale simulations in which the model successfully has been used to simulate flows in large anatomically correct vascular networks [11,15]. Guibert and colleagues analyzed a primate cerebral cortex network spanning an 18 mm³ volume with close to 16,000 vessel segments [11]. In comparative analyses of the PS model against another phase separation model (Dellimore and colleagues [6]), they showed that the two models did not lead to drastically different hematocrit distributions except for very low hematocrits [11]. Lorthois and colleagues similarly used the PS model for flow simulations in a large human cortical network with more than 10,000 vessel segments [15]. With regard to hematocrit the large scale simulations yielded a “hematocrit distribution [that] is in qualitative agreement with the available experimental data” [15].

However, a recent analysis by Gould and Linninger questioned the validity of the PS model [10]. They introduced a new phase separation model which was compared against the PS model and a recent model developed by Guibert and colleagues [12]. Model comparison was based on analyses at single bifurcation level as well as at network level. In contrast to previous analyses e.g. [11,15,21,24,26,28,29], they reported “deficiencies in prior models, including inconsistent plasma-skimming trends”, that the PS model yielded “problematically low hematocrit predictions”, concluding that “improperly formulated phase separation laws drastically impact predicted oxygen distribution to the tissue, especially how the prediction of RBC-free capillaries leads to drastically low oxygen tension” and that the “large fraction of RBC-free vessels in Model 1 [PS model] steady state raises concerns with its consistency of oxygen predictions”. It was further concluded that the more simplistic model by Gould and Linninger performed “no worse” than the PS model. Unfortunately, the single bifurcation analysis was based on inaccurately transcribed model coefficients, and re-analysis with an appropriate model parameterization demonstrated that the PS model provided a better fit to the single bifurcation data [1]. Indeed, a recent *in vitro* study of phase separation in micro channels beyond 20 µm concluded that “These data clearly show that the “ad hoc” law proposed by Gould and Linninger is inappropriate for parametrizing the phase separation effect. By contrast, they are consistent with the empirical description of Pries et al.” [31].

Of note, Gould and Linninger based part of their analysis on a network representation that they derived from a photomontage of a mesentery network, originating for a data base of seven networks [26]. The PS model has previously been shown to provide plausible hematocrit predictions on these networks. Importantly, network simulations predicted average segment discharge hematocrit relative to the discharge hematocrit in the feeding

arteriole to decrease from about 1.0 at 10 μm to about 0.25 at 4 μm and hence reduced but non-zero hematocrit even for small vessel diameters [26]. Unfortunately, the disagreements between these early findings and the recent analysis results by Gould and Linninger were not discussed.

Given this recent debate, there is an urgent need to evaluate whether the PS model produces physiologically realistic predictions of hematocrit distributions in microvascular networks. The objective of the present study was therefore to conduct a formal quantitative evaluation of model predictions against experimental measurements in four rat mesenteric networks, including the network analyzed by Gould and Linninger (“network 1”, Figure 1A). Such an assessment is needed in order to establish a common ground for future analyses of flow in microvascular networks. We adopted a Bayesian probabilistic data analysis framework as a means of integrating the experimental measurements and network model simulations into a combined and statistically coherent analysis [30]. This analysis framework allowed us to take into account the inevitable presence of uncertainties or errors, for example measurement errors and uncertainties related to the unknown pressure and hematocrit boundary conditions. Furthermore, we considered the parameters of the PS model as uncertain parameters, since, as previously discussed [18,29], the original data used to derive the empirical description of the phase separation effect showed a fairly large scatter. Since PS model parameters were assigned a degree of uncertainty, our analysis setup allowed these to deviate from fixed values based on previous fits, and we further illustrate how the PS model may benefit from the resulting parameter estimates obtained by the Bayesian analysis. Because inference over the unknown/uncertain boundary conditions and PS model parameter requires the use of measurements for model calibration, a performance evaluation may be over-optimistic if model performance is assessed using the calibration data. To avoid this over-optimism we further embedded the performance evaluation within a resampling scheme. Our analysis demonstrated good global correspondence between the experimental measurements and the simulation model’s predictions, supporting its continued use in simulations of microvascular blood flows.

Materials and Methods

Our analysis set up was built around a flow simulation model which was evaluated by comparing its predictions to experimental measurements. Unknown parameter values of the simulation model were inferred by Bayesian inference and the evaluation of the model’s predictions was conducted within a cross-validation framework. In the following, we provide details on the individual elements in our analysis set up.

Experimental data

The experimental data originate from observations of the mesenteric microvasculature in rats. Here we provide an overview of the data and refer to previous reports for detailed information regarding experimental set up, data acquisition, and data quantification [18,19,28,29].

The experimental data originate from four mesenteric microvascular networks with observations from all vessel segments within the areas studied ($\sim 30\text{-}50\text{ mm}^2$) [19,28,29].

Vessel segments were grouped into arterioles, capillaries, and venules by an algorithm that classifies vessel type without requiring flow information [34]. The diameter and length of each vessel segment as well as the topological connectivity were available for all networks. The number of segments for networks 1–4 were 389/392/383/546, the number of interior nodes were 252/248/248/352, and the number of boundary nodes were 22/40/22/36. Measurements of hematocrit and blood flow direction were available in all segments in all networks, whereas measurements of blood flow velocities were available in all segments in networks 2–4. To support the network-oriented analysis in the network without velocity measurements (network 1), we generated a series of target velocity measurements as follows. First we obtained local polynomial regression fits [4] of velocity as a function of vessel diameter for arterioles and venules based on available measurements from networks 2–4. In network 1 we then assigned target velocity measurements to larger arteriolar and venular vessel segments (diameter > 25 μm) based on these polynomial regression fits. Using target velocities in network 1 resulted in predicted velocities in correspondence with the observed velocities in networks 2–4 (see Supplementary material A).

Simulation model

To model blood flow we used a one-dimensional simulation model which, over the past decades, has been developed to describe blood flow in rat mesentery networks and further has been used to simulate blood flow in a series of other network types ranging from simplistic artificial networks to anatomically accurate large human intra-cortical vascular networks [11,15,24,26–29].

The simulation model is partly based on physical principles regarding flow-pressure relationships and mass conservation at bifurcations, and partly on empirical descriptions of rheological effects. The microcirculatory network is represented by interconnected cylindrical tubes, and flow conservation at bifurcations is used together with Poiseuille's law to predict blood flow patterns throughout the network. Rheological phenomena, for example variable blood viscosity with diameter and hematocrit (Fåhræus-Lindqvist effect), reduction in tube hematocrit relative to discharge hematocrit (Fåhræus effect), and the disproportional distribution of red blood cells at bifurcations (phase separation effect) are included in the model via a series of empirical descriptions [28].

Typical microcirculatory networks have many boundary nodes due to the difficulty in acquiring complete networks or tissue regions. Hence, pressure or flow and hematocrit boundary conditions needs to be specified for these boundary nodes. After specifying appropriate boundary conditions the blood flows and hematocrit distributions throughout the network can be simulated by an iterative approach [28]. Iterations are required since the rheological descriptions impose interdependence between blood flow and hematocrit (and hence flow resistance) and amounts to alternating between computation of network blood flow and network hematocrit. Convergence analysis of the iterative procedure is available in the Supplementary material B.

In the following section we will review the phase separation model, and refer to previous reports for additional information on the remaining rheological models [20,22,28,29].

Phase separation model

Comprehensive experimental measurements guided the development of PS model and allowed for quantitative assessment of the influence of red cell flow distribution on the feed hematocrit, distribution of blood flow in daughter vessels, segment diameters, and bifurcation “history” (type of upstream branch) [18]. The relationship between fractional red cell flow FQ_E^α and fraction blood flow FQ_B^α in daughter branch α was described by

$$FQ_E^\alpha = \begin{cases} 0 & , \text{ if } FQ_B^\alpha < X_0 \\ 1 & , \text{ if } FQ_B^\alpha > 1 - X_0 \\ \frac{1}{1 + \exp\left(-A - B \logit\left(\frac{FQ_B^\alpha - X_0}{1 - 2X_0}\right)\right)} & , \text{ otherwise} \end{cases} \quad (1)$$

where $\logit(x) = \ln[x/(1-x)]$, while the three coefficients A , B , and X_0 are specific to individual bifurcations and describe asymmetry, nonlinearity, and critical volume flow fraction below which only plasma enters the branch, respectively. Relationships between the parameters A , B , and X_0 and relevant physiological variables (mother vessel diameter D_F , daughter vessel diameters D_α , D_β , feed hematocrit H_F) were further established

$$A = \theta_A \frac{D_\alpha^2/D_\beta^2 - 1}{D_\alpha^2/D_\beta^2 + 1} (1 - H_D)/D_F \quad (2)$$

$$B = 1 + \theta_B (1 - H_D)/D_F \quad (3)$$

$$X_0 = \theta_{X0} (1 - H_D)/D_F \quad (4)$$

with θ_i being parameters with numerical values of -13.29 , 6.98 , and 0.964 , respectively, obtained from linear regression analysis [22]. The θ parameters are all with dimension μm . The effect on phase separation of a side branch upstream of the studied bifurcation was further assessed and a significant effect on the asymmetry parameter (A) was found for those cases where the side branch was located within 10 vessel diameters of the bifurcation. However, this was the case for only 12% of the studied bifurcations, and the available data did not provide a sufficient basis for quantifying the effect. In principle, such a “history” effect could be represented in future models for phase separation, based on additional experimental data and/or theoretical analyses. The parametric description of the phase separation effect eq. 1 in conjunction with the linear relationships eqns. 2–4 (and earlier versions) has constituted a model of the phase separation effect that could be used in

subsequent flow simulation analyses in networks for which the parameters A , B , and X_0 were not available. Instead, these parameters could conveniently be estimated from available physiological variables via eqns. 2–4.

Model calibration

The flow simulation model is specified by the network topology, the segment morphology (diameter and length), the parameters of the empirical rheological descriptions, and the network boundary conditions [28]. Network topology and segment morphology were available from experimental measurements in all four networks while the pressure or flow and hematocrit boundary conditions on the other hand were unknown. However, the integration of experimental measurements with model simulations within a network-oriented analysis has proven as an effective means of estimating these unknown boundary conditions in a procedure where boundary conditions are adjusted to achieve good correspondence between experimental measurements and model predictions [28,30].

Unfortunately, such network-oriented analysis is complicated by the inevitable presence of measurement errors as well as simulation model errors. Measurements are corrupted by various sources of errors including measurement quantification errors [5,29], and such errors hinder a perfect match between measurements and model simulations even if the model was exact. The empirical rheological descriptions used in the simulation model are approximations used to represent complex physical processes. While these descriptions have proven useful in presence of an incomplete understanding of the physics underlying the complex rheological phenomena, the approximations also represent a significant source of error [29]. Furthermore, the rheological descriptions are governed by a series of parameters with numerical values obtained by fits to empirical data resulting in uncertainty which, in turn, renders these parameters uncertain [29]. Finally, the challenge of specifying appropriate values for the unknown boundary conditions also contributes to the mismatch between measurements and model predictions.

To overcome the challenge of integrating experimental measurement and model simulations in a combined analysis, we recently developed a Bayesian probabilistic data analysis framework for calibration of microvascular flow models [30], Figure 2 provides a conceptual overview. In this framework, experimental measurements y , model predictions $\hat{y}(x)$, and model parameters x are treated as random variables governed by probability distributions (Figure 2C). Specifically, we considered the experimental measurements (blood flow velocity, flow direction, and hematocrit) as being variables governed by uncertainties (errors) and similarly the unknown pressure and hematocrit boundary conditions as well as the three parameters (θ_A , θ_B , and θ_{X0}) of the phase separation model eqns. 2–4 to be governed by uncertainties. This probabilistic description allows incorporation into the analysis of assumptions regarding deviations between measurements and model predictions via a likelihood function, and assumptions regarding unknown model parameters and their uncertainties via their prior distributions (Figure 2C). For mathematical details regarding measurement uncertainty as well as hematocrit and pressure boundary condition uncertainties (likelihood and pressure and hematocrit priors) we refer to our previous report [30]. To model uncertainty governing the parameters of the phase separation model we used

broad “uninformative” priors specified by $\theta_A \sim \text{unif}(-30, 0)$, $\theta_B \sim \text{unif}(0, 10)$, and $\theta_{X0} \sim \text{unif}(0, 1.4)$. Broad priors were deemed appropriate due to the relatively large number of observations in the data set. The widths of the priors were chosen such that the “standard” parameter values (-13.29 , 6.98 , and 0.964 , respectively) were well within these uniform distributions, and yet allowed the inferred parameter values to deviate significantly from these standard values. We further extended our previous analysis setup and used a more flexible noise model to describe blood flow velocity errors and hematocrit errors (error model details and residual analysis are provided in the Appendix section).

The probability distributions (priors and likelihood) can be combined by use of probability rules to yield information on quantities of interest. For example, Bayes’ rule yields distributions over unknown parameters (Figure 2C). Unfortunately, an exact analytical solution of the posterior distribution governing the uncertain model parameter (boundary conditions, phase separation model parameters, and noise model parameters) is not available. Instead we used Markov chain Monte Carlo (MCMC) sampling to generate a sequence of samples from the posterior distribution (Figure 2D) [8,30,36]. MCMC generates the sequence of samples while exploring the parameter space, and the samples are drawn such that they mimic samples from the posterior distribution. The resulting samples from the posterior distribution were used to represent distributions over the uncertain model parameters (Figure 2D). Additionally, model predictions (of blood flow velocity, flow direction, and hematocrit) were obtained by propagating the individual parameter samples through the flow simulation model, thereby propagating the uncertainties, as represented in the posterior probability distribution governing pressure and hematocrit boundary conditions and also phase separation model parameters, to uncertainties in the simulation model’s predictions (Figure 2D). Transitions in the Markov chains were generated using the DREAM(ZS) algorithm [35–37]. The interested reader is referred to the cited references for further details regarding Bayesian inference and MCMC sampling [3,8,36].

Quantitative model evaluation

To evaluate the simulation model’s predictions, we compared its predictions $\hat{y}$ to the experimental measurements y and used the mean squared velocity error $\sum_j [y_{\text{vel},j} - \hat{y}_{\text{vel},j}(x)]^2 / n_{\text{vel}}$, the mean squared hematocrit error $\sum_j [y_{\text{hct},j} - \hat{y}_{\text{hct},j}(x)]^2 / n_{\text{hct}}$, and the percentage of segments in which predicted and measured flow direction did not agree $100 \sum_j I[y_{\text{dir},j} \neq \hat{y}_{\text{dir},j}(x)] / n_{\text{dir}}$ (reversed flows) as evaluation metrics. To guard against potential over-optimism due to overfitting to the calibration data, we implemented the quantitative model evaluation within a cross-validation scheme. The measurement data set was randomly split into halves with approximately the same number of segment measurements in each split. The simulation model was calibrated on samples in the first half, and the quantitative evaluation metrics were estimated using the other half of the samples. This procedure was repeated with the halves of the data set switched, and the evaluation metrics from the two procedures were then averaged. The resampling procedure was repeated 50 times leading to 50 estimates of the evaluation metrics.

Results

We first present results concerning the evaluation of the ability of the PS model to produce physiologically plausible hematocrit predictions and demonstrate that the PS model is capable in producing hematocrit prediction in good agreement with the experimental measurements. To investigate the effect of modeling the uncertainty governing the PS model parameters, we used two different calibration setups. In these we considered two different settings for the prior distributions governing the PS model parameters; (i) Dirac prior (standard values), and (ii) broad “uninformative” prior. After presenting results of the quantitative model evaluation we provide results regarding the inferred PS model parameter values and illustrate how these inferred parameters as well as the standard parameter values influence the behavior of the PS model and the resulting hematocrit predictions.

Model performance

Figure 3 shows the quantitative model evaluation of the two PS parameter prior distributions, and Table 1 provides corresponding summary statistics. The overall model performance is consistent with previous reports with regard to the calibration error [24,30]. Across all evaluation metrics, the validation errors exceed calibration errors - a characteristic frequently observed in cross-validation analyses. Focusing on the validation errors, we observed decreased errors (mean squared error) in velocity predictions (Figure 3A) medians: 0.697/0.650 ($\theta_{\text{standard}}/\theta_{\text{inferred}}$) and flow direction predictions (% reversed flows) (Figure 3C) medians 2.6/2.5, although with scatter across individual resampling splits. With regard to hematocrit predictions (Figure 3B), we observed a prominent reduction of 17% in median hematocrit error: 0.0289/0.0239 (mean squared error), when associating the PS model parameters with uncertainty. Hematocrit error decomposition analysis is available in Supplementary material C.

Hematocrit prediction at single segment level

Figure 4 shows a segment-by-segment comparison between measured and predicted values of hematocrit for the network-oriented analysis. We observed considerable scatter and variability in individual segment predictions across the different resampling splits. A total of 25 unique red-cell-free segments out of 1710 segments in total were observed across all 100 resampling split-halves in the analysis with standard parameterization of the PS model. Of these, however, only seven segments were stable red-cell-free segments across all resampling splits, and the average rate of red-cell-free segments was 0.9 %. This relatively low number of red-cell-free segments was further reduced when accounting for PS model parameter uncertainties (Figure 4B). In this analysis, red-cell-free segments were absent. Residual analysis is presented in the Appendix section.

Measured and predicted values of hematocrit were further compared by grouping segments according to vessel diameter in Figure 5. Overall an increase in median hematocrit with diameter was observed for both measurements and the two different variants of model simulations. Given the relative large scatter observed within individual diameter bins we observed good correspondence between measurements and medians of the predictions. The distributions representing measurements are generally broader than those representing

predictions, with the model with inferred phase separation model parameters having narrowest distribution for small diameters.

Spatial representations of hematocrit predictions are shown in Figure 6 for two networks. Heterogeneous distributions are observed in accordance with the experimental measurements (Figure 1B,C). Furthermore, segments with low predicted hematocrit also tend to have low measured hematocrit (Figure 1B,C vs. Figure 6) as also apparent from the scatterplots in Figure 4.

Posterior distributions of phase separation model parameters

Figure 7 shows the inferred posterior distributions governing the PS model parameters (model with broad priors) and Table 2 provides statistics summarizing these distributions. We observed heterogeneity across the inferred distributions corresponding to model calibrations conducted on individual split-halves (Figure 7A–C, thin gray lines). The variability between the distributions inferred from different calibration splits of the data results from the interaction between random variables including pressure and hematocrit boundary conditions, PS model parameters, and errors between model simulations and experimental measurements. Despite this variability between individual split-halves, the inferred distributions appear well localized (identifiable parameters) for both the individual split-halves and also for the frequency histogram representing samples pooled across all resampling splits (dark gray). The standard values of θ_A and θ_B (-13.29 and 6.98 , respectively) are well within the probability masses, with posterior medians of -10.11 and 5.89 , respectively. In contrast, we observed that the posterior distributions of θ_{X0} deviate significantly from the standard value of 0.964 (Figure 7C) with posterior median values of 0.09 . While Figure 7 shows dispersion in the marginal distributions, the posterior distribution of the PS parameters is also governed by correlation between the individual parameters (Table 3). Such correlation between parameters can appear because of lack of information in the data or because of the mathematical structure of the model equations.

Post hoc evaluation of posterior summary statistic

While the posterior distribution governing model parameters allows for quantification of uncertainties, we acknowledge that many investigators resort to using point estimates of “optimal” parameter values in simulation models. To evaluate the quality of such point estimates, we performed quantitative model evaluations with PS model parameter fixed according to the medians of the posterior distribution (Table 2), ($\theta_A = -10.11$, $\theta_B = 5.89$, and $\theta_{X0} = 0.09$) corresponding to the posterior distribution estimated using broad prior.

Besides different PS model parameter settings, the analysis setup was identical to the above model evaluation using a Dirac prior with standard PS model parameters ($\theta_A = -13.29$, $\theta_B = 6.98$, and $\theta_{X0} = 0.964$). When fixing PS model parameters according to the posterior summary statistics we observed decreases in errors according to all evaluation metrics in comparison to the model with standard PS parameters, Table 4 vs. Table 1 θ_{standard} (Dirac prior). A graphical representation, with the same format as Figure 3, corresponding to the quantitative evaluation of the post hoc model is available in Supplementary Figure S2, segment-by-segment comparison between measured and predicted values of hematocrit is

available in Supplementary Figure S3, and plots of hematocrit as grouped by segment diameter are available in Supplementary Figure S4).

We further quantified the plausibility of the inferred parameter distributions and conducted an analysis without using flow simulations and the Bayesian analysis. This analysis was thereby designed to be independent of issues related to sampling in the Bayesian framework as well as hematocrit errors that accumulate throughout the network in simulations. Further details are provided in Supplementary material D, and this analysis provided additional support to the inferred parameter values.

Significance of the inferred PS model parameter values

The inferred values of the threshold parameter θ_{X0} differ from the standard value. As presented above the inferred values lead to a substantial reduction in global hematocrit error (Figure 3 and Tables 1,4). We therefore further investigated how network simulations may benefit from the inferred parameter values and in particular the reduced θ_{X0} value. Figure 8 shows a spatial representation of network 4 in the vicinity of the main feeding arteriole. The main arteriolar trunk gives rise to daughter arteriolar branches with relatively large vessel diameters as well as daughter branches with relatively small vessel diameters. The first bifurcation (marked with *) is relatively unbalanced with respect to daughter branch diameters (7.4 μm , 23.1 μm) and also measured blood flow fractions (2.6%, 97.4%). The simulation model using standard PS parameter values predicts a substantial decrease in hematocrit of the small daughter branch whereas the inferred parameter values leads to a hematocrit predictions in better agreement with the measurement. The pattern of predictions with reduced hematocrit in small diameter side branches is, in turn, observed at consecutive bifurcations giving rise to a series of vessel segments with low hematocrit. Average predicted blood flow fractions in the small daughter branch of the bifurcation marked with * was 3.7%/2.7% ($\theta_{\text{standard}}/\theta_{\text{inferred}}$). Hence, the simulation model using standard PS parameters attempts to correct for the reduced hematocrit by increasing the fractional blood flow into the small branch (and thereby increase fractional red cell flow). However such compensatory flow increase is limited by corresponding increases in error between the measured and predicted blood flow velocity.

Post hoc analysis of inferred parameter distributions

Additional analyses were conducted at single bifurcation level to interpret how the PS model is influenced by the inferred parameter distribution, its dispersion, and also the correlations between parameters (Figure 7 and Table 3). To represent a variety of bifurcation configurations we considered two bifurcations previously reported [18], and one bifurcation marked with * in Figure 8. These three bifurcations were selected to represent three different bifurcation geometries. The objective of the single bifurcation analysis was: i) qualitatively to understand how the uncertainties, which is represented in the posterior distribution (Figure 7) as well as the correlation between the inferred parameters (Table 3), translate into uncertainties in the PS model's predictions, and ii) to illustrate that characteristics of the PS model such as asymmetries and non-linearities are maintained also for the inferred PS model parameters. The PS model was evaluated using: (i) the standard values $\theta_A = -13.29$,

$\theta_B = 6.98$, and $\theta_{X0} = 0.964$ together with eqns. 2–4, and (ii) samples of θ_A , θ_B , and θ_{X0} drawn from their joint distribution (marginal distributions represented in Figure 7) together with eqns. 2–4. Figure 9 shows that the considerable dispersion, which governs the distributions in Figure 7, and also the correlation between PS parameters samples (Table 3) both translates into minor uncertainties when samples are propagated through the non-linear PS model. Furthermore, the inferred parameters lead to a reduced slope of the sigmoid transition and hence dampen the non-linearity of the PS model. Finally, the reduced θ_{X0} values inferred with the broad prior reduce the threshold at which the model predicts cell-free flow for low blood flow rates (Figure 9 insets).

According to Figure 9C,F the differences between the standard and inferred PS model parameters only lead to small differences in the fractional red cell flow as predicted by the PS model. However, when considering the ratio of daughter hematocrit to mother hematocrit H_d/H_F , Figure 10, we observe large dynamics in this quantity at low fractional blood flows and accordingly a large difference between model predictions using standard and inferred parameter values.

Discussion

This study demonstrates that the incorporation of the PS model into simulation models of network hemodynamics results in predictions that show good global agreement with experimental measurements. Our quantitative model evaluation was based on a data base comprising four mesentery networks with a total of 1710 vessel segments, for which measurements of vessel lengths and diameters, network architecture, flow directions and hematocrits were available in all four networks, and blood flow velocity measurements were available in three out of the four networks. Since the simulation model's unknown pressure and hematocrit boundary conditions as well as the phase separation model parameters were inferred using the *in vivo* measurements, we conducted the quantitative model evaluation within a resampling framework in order to avoid potential over-optimism in the evaluation metrics.

The use of standard parameters in the PS model (Figure 3, θ_{standard}) lead to model performance in agreement with previous analyses with regard to velocity errors, hematocrit errors, and flow direction errors. Taking PS model parameter uncertainties into account further reduced the hematocrit error by 17% (Figure 3 and Table 1). Segment-to-segment comparison between hematocrit measurements and model predictions showed considerable scatter (Figure 4) consistent with previous analyses [28–30]. Such scatter is inevitable because of the accuracy of measurement of key variables such as vessel diameter is limited by the resolution of optical microscopy, and because microvascular networks are heterogeneous in structure, and variations in geometrical and hemodynamical conditions at each bifurcation cannot be fully described in terms of a few parameters. Previous analyses have demonstrated the simulation model's capability of providing global network characteristics with a high degree of similarity between experimental measurements and model predictions albeit the considerable scatter at segment level [28]. Similarly, our analysis demonstrated good agreement between measured and predicted hematocrit as

grouped by vessel diameter as shown in Figure 5. Quantitatively, there was good agreement between median measurements and median predictions within the diameter groups. Importantly, for small vessels below 10 μm we observed a high degree of similarity between measurements and model predictions based on standard parameters of the PS model (θ_{standard}) with a median relative hematocrit of 0.7 for both. This agreement between measured hematocrit and predicted hematocrit is consistent with previous reports of both experimental data and model simulations [19,26] but in disagreement with the recent analysis by Gould and Linninger (Figure 9 in ref. [10]), who reported substantial discrepancies between data and model predictions. Considerable scatter between segment hematocrit was observed within individual diameter groups (Figure 5). Vessel segments with relatively low hematocrits were present both among the measurements and the simulations, whereas the simulation model also predicted hematocrit to be numerically zero for 0.9 % of the segments on average when using standard parameterization of the PS model. Of these only seven segments were stable zero red cell segments across all individual resampling split. This result is in agreement with previous analyses, which reported a limited number red cell-devoid vessel [28,29], but again in disagreement with the recent analysis by Gould and Linninger reporting “many capillaries without any RBCs.” [10]. Furthermore, allowing the PS model parameters to adapt to the network data at hand, by appreciating the uncertainty governing these parameters [18,29] and modeling these as uncertain parameters, led to absence of red-cell-devoid vessels (Figures 4 and 5).

Early analysis indicated that the empirical description of the phase separation phenomena as derived from *in vivo* measurements (PS model) was adequate to predict the distribution of plasma and red blood cells across microvascular networks [28]. As already discussed, the PS model also led to satisfactory correspondence between measured and modeled global hemodynamic characteristics across the entire network, albeit with considerable segment-to-segment differences between measurements and simulations [28]. A later quantitative analysis of the deviations between measurements and model predictions decomposed these deviations into contributions from error sources defined by measurement error (estimated from double measurements), model input error (segment length, diameter, and parameters of the phase separation model), and rheological model error [29]. Subsequent analyses have primarily focused on reducing the viscosity model’s contribution to the overall simulation model error by establishing and evaluating a series of *in vivo* viscosity models [24,29] which, in turn, has revealed the significant difference between *in vivo* and *in vitro* resistances and pointed to the role of the endothelial surface layer in flow resistance [13,24].

Network-oriented analysis of microcirculatory measurements has been an important tool in the development of these *in vivo* viscosity descriptions because of the limited availability of direct *in vivo* viscosity measurements [24,29]. On the other hand, comprehensive *in vivo* measurements from single bifurcations have guided the development of the PS model [18,22]. Model parameters were estimated by a sequential approach. First, the three-parameter logistic function eqn. 1 (A , B , X_0 coefficients) was fitted to each individual bifurcation, and second the three parameters θ_A , θ_B , and θ_{X_0} were found by fitting the relationships eqns. 2–4 to the A , B , X_0 coefficients. In this discussion, the model parameters in eqns. 2–4 are deliberately denoted by θ_i instead of the fitted numerical values to underscore the distinction between model structure and model parameter fits. While the fits

were statistically significant, the data also exhibited a considerable scatter [18]. To take this uncertainty into account, we considered the PS model parameters as uncertain parameters. Similarly to previous analyses which combined network-oriented analysis with parameter optimization to adjust parameters of *in vivo* viscosity descriptions, we here combined network-oriented analysis with Bayesian inference to infer the posterior distributions of PS model parameters. Interestingly, this network-oriented analysis (Figure 7, Table 2) provided posterior distributions for θ_A and θ_B in good agreement with the single bifurcation fits [18,22], whereas the inferred distributions for θ_{X0} deviated substantially from the single bifurcation fit. Since the X_0 parameter in eq. 1 defines the flow threshold below which only plasma enters a downstream branch, reductions in θ_{X0} leads to a PS model that is less aggressive with respect to generating red cell-devoid daughter branches. Furthermore, since $(1 - 2X_0)$ defines the fractional blood flow range for which the PS model exhibits the transition from 0 to 1 in fractional red cell flow, reductions in θ_{X0} effectively decrease the slope of this transition which, in turn, results in decreased non-linearity of the PS model. These effects are evident when comparing the predictions of the PS model using the standard parameters and the inferred parameters, respectively (Figures 8–10), and also by the reduced scatter in hematocrit predictions (Figure 4 and 5).

The quantitative model evaluation was conducted within a resampling framework where the validation performance represents the simulation model's ability to generalize to data which were not already used for model calibration. Hence, the quantitative model evaluation (Figure 3, Table 1) supports the validity of the inferred parameter distributions because of the decreases in hematocrit validation error. The post hoc analysis of the network data set at the single bifurcation level (Supplementary material D) provided further support to the validity of the inferred parameter distributions, since the inferred parameter values also lead to error reduction at single bifurcation level.

The present analysis demonstrates improved model performance if PS model parameters are treated as uncertain parameters (Figure 3, Table 1) or fixed at values differing from standard parameter values (Table 4). It is important to note, however, that the original single bifurcation analysis [18] and the present network-oriented analysis are complementary rather than in disagreement. The original single bifurcation analysis was based on a comprehensive data set with multiple perturbed flows measured at individual arteriolar bifurcations. This data supported the development of an empirical description of phase separation in single bifurcations and this description, in turn, also proved useful when later applied in network modeling [28]. On the other hand, our present analysis represents an adaptation of the PS model parameters to the network data at hand. Hence, instead of being “optimal” in explaining comprehensive measurements from isolated individual bifurcations, the inferred parameter distributions (Figure 7) support the observed blood flows and hematocrits in highly interconnected vessels segments in the four microcirculatory networks. It is important to note that the PS model, in the network-oriented analysis, acts in concert with the other rheological models (and their parameters) and also with the boundary conditions. The inferred distributions are therefore not believed to be universal but rather represent the result of an effort to obtain good correspondence between model simulations and the particular experimental network data at hand. The present data base only has a single measurement of segment blood flows and hematocrits available for individual vessel

segments. Future comprehensive data sets with multiple perturbations of vessel flow and hematocrit could guide further development of phase separation models.

In pure simulation studies, it may also be appropriate to consider uncertainty in the fitted PS model parameters due to the empirical nature of the model and also to study the impact of the simulated flow patterns on particular model parameter choices. Possible strategies could be to adopt statistical averaging, similar to the MCMC analysis in the present study, or noise perturbation analysis [29]. In such analyses, PS model parameters could be varied within conceivable ranges informed by, for example, our present analysis (Figure 7) and previous fits [18]. While statistical averaging and noise perturbation analysis allow for uncertainty quantification, we also recognize that “optimal” values also may be useful in future network simulations, in which the investigator prefers to use fixed parameter values. To evaluate the quality of point estimates summarizing the posterior distribution represented in Figure 7 we therefore performed a quantitative post hoc model evaluation based on PS model parameters fixed according to statistics summarizing the posterior distribution (Table 2). Since model performance increased according to all evaluation metrics (Table 4 vs. Table 1 θ_{standard}), the point estimates available in Table 4 may be used as alternatives to the standard PS model parameters in future network simulations.

The main objective of the present study was to evaluate the ability of the PS model to produce physiologically plausible hematocrit predictions. To achieve this goal we adopted the Bayesian analysis framework in order to infer appropriate values for unknown/uncertain pressure and hematocrit boundary conditions. Additionally, the Bayesian analysis allowed us to treat the PS model parameters as uncertain parameters. This, in turn, pointed towards PS model parameters that deviate from standard parameter values. Whereas our focus has been on the PS model, our analysis setup also facilitates a quantitative comparison of different phase separation models, e.g. between the PS model and the models of Dellimore and colleagues [6] and Gould and Linninger [10]. These models are also governed by unknown/uncertain parameters, which could be treated similar to the PS parameters in the present analysis. Additionally, variants of models, in which parameters depend on local bifurcation characteristics, as it is the case for the PS model (e.g. feed hematocrit, vessel diameters), could be constructed based on the single bifurcation data set. However, such comprehensive analysis is beyond the scope of the present study.

By demonstrating physiologically plausible network hematocrit predictions and good correspondence between global hematocrit characteristics of model predictions and experimental measurements, our results are consistent with a series of studies [11,15,21,24,26,28,29] but in disagreement with the recent analysis by Gould and Linninger [10]. We will attempt to comment on this disagreement in the following. In network 1, the main arteriolar inlet and the main venous outlet reside in close vicinity as indicated in Figure 1A,B. While being potentially unclear from the photomontage this fact has been evident in a series of other representations, e.g. refs. [16,21,34]. Unfortunately, to the best of our interpretation of Figures 6 and 9 in ref. [10], the representation used by Gould and Linninger disregarded the main arteriolar inlet. Instead, blood was assumed to enter the network at the main venous outlet, and then to distribute towards capillaries partly via the main venular trunk and venular branches, and partly via the main arteriolar branch which was considered

as a side branch to the main venular trunk. As recently illustrated in computer simulations, the PS model (as well as other phase separation models) may exhibit non-physiological behavior when applied to special network configurations [12]. Indeed, Figures 8–10 illustrate that the PS model can lead to low predicted hematocrits in small bifurcation branches when bifurcation branch diameters and flows are highly asymmetric. While we observed this behavior for a subset of vessel segments (Figure 4), we did not observe this to be a general problem in our present analysis of *in vivo* mesentery networks (e.g. Figures 4–6). On the other hand, we may speculate that the network representation adopted by Gould and Linninger could lead to such non-physiological behavior, since both the main venular trunk and also the venular branches have large diameters (Figure 1) and would therefore be expected to carry large flow fractions. Assuming blood to enter the network through the main venular trunk is indeed expected to yield problematic hematocrit predictions because of the resulting non-physiological bifurcation configurations and flow distributions. As illustrated in Figures 8–10, the PS model can produce low hematocrit in small daughter branches receiving small fractional flows. This model property coupled with reversed flow in the large venular vessels is thus expected to yield hemoconcentration in large vessels at the expense of lack of hematocrit in small daughter branches. Indeed, Gould and Linninger observed hemoconcentration in the main “arteriolar trunk from left to right” (main venular trunk) and many capillaries without red cells (Figure 6C in [10]). Another factor which could potentially contribute to the disagreement is the selection of appropriate boundary conditions. Given the inevitable presence of boundary condition uncertainty, we combined experimental measurements and model predictions in a network-oriented analysis using a Bayesian probabilistic approach [30] in order to infer appropriate values for unknown pressure and hematocrit boundary conditions.

Model performance evaluation is an important aspect of the iterative cycle of model building. Theoretical models are subject to continued development and improvement guided by experimental data. By validating a theoretical model against experimental data, inadequate aspects of models can be identified, the model can be refined, and this iterative cycle may lead to model improvement as well as provide novel insights into the rheology of blood in microcirculatory systems [9,24,29,33]. Because of the uncertainties governing pressure and hematocrit boundary conditions and also PS model parameters, we used a probabilistic Bayesian data analysis framework for model calibration [30]. By adopting this formal analysis strategy, honoring the inevitable presence of parameter uncertainties, we reduce the possibility that poor model performance merely reflects the model’s sensitivity to inappropriate numerical values assigned to uncertain parameters (e.g. boundary conditions). However, this strategy may also necessitate the use separate data for performance evaluation [2]. We therefore used cross-validation in which model calibration and model evaluation was based on separate parts of the data. We have chosen split-half resampling that splits data into halves of approximately sample size. This allows for two independent estimates of model performance which are influenced by the inferred PS model parameters and network boundary conditions. These performance estimates were in turn averaged to provide robust estimates of the evaluation metrics. The split-half procedure was repeated 50 times. This number of repetitions was judged as appropriate given the computational complexity of the analysis and the level of scatter between individual repetitions (Figure 3). Regarding

performance metrics we used both global characteristics and segment-to-segment characteristics to compare predictions to experimental measurements. A global comparison represents the simulation model's ability to reproduce global characteristics of the measurements. The global analysis of hematocrit as grouped by diameter in Figure 5 demonstrates good correspondence between the medians for all models vs. measurements given the scatter within individual diameter groups. The segment-to-segment comparisons in Figures 3 and 4 and in Tables 1,4 represent the model's ability to reproduce the observed data at the scale of the measurements. Considering the evaluation metrics in Figure 3 there is an agreement between calibration performance and validation performance, in the sense that model performance is consistently improved when taking PS model parameter uncertainties into account. Residual analysis can reveal systematic unmodeled behavior and is important for model diagnostics. The residual analysis (Appendix section) shows a similar match between theoretical residuals and observed residuals of two models (θ_{standard} , θ_{inferred}), and it is hence judged, that the assumptions regarding residual structure do not impose significant bias in the comparison of these two models. It remains a subjective consideration how the individual metrics for model evaluation should be balanced against each other when comparing a model's performance to our expectations. Regardless these considerations, the global evaluation (Figure 5 and 6) as well as the local segment-to-segment evaluation (Figures 3, 4 and Tables 1,4) clearly demonstrate that the PS model cannot be rejected based on a criterion which rejects phase separation models exhibiting non-physiological behavior with a large fraction of red-cell-free vessels and a large error in average hematocrit for small vessels [10].

In summary, network-oriented analysis combining theoretical modeling with experimental measurements has proved to be a versatile tool for interpreting experimental measurements and for studying mechanisms underlying microcirculatory flow phenomena [9,17,23–25,28,29,33]. In the present study, we conducted a formal quantitative evaluation of the PS model as a component of a network flow simulation model. Good correspondence between measured and modeled hematocrit characteristics was observed, and hence the currently available data basis supports the continued use of the PS model in network simulations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Error model and residual analysis

Blood flow velocity errors and hematocrit errors (ε_{vel} and ε_{hct}) are assumed to be distributed according to exponential power distributions [3], uncorrelated, and with identical error variances within each observation type (i.e. homoscedasticity within observation type)

$$p_i(y_i|x, \beta_i, \sigma_i) \propto \prod_{j=1}^{n_i} \frac{\omega(\beta_i)}{\sigma_i} \exp \left[-c(\beta_i) \left| \frac{y_{i,j} - \hat{y}_{i,j}(x)}{\sigma_i} \right|^{2/(1+\beta_i)} \right], \quad i \in \{\text{vel, hct}\} \quad (5)$$

with the noise model parameters σ_i and β_i being standard deviation and kurtosis parameters, respectively, and $\omega(\beta_i)$ and $c(\beta_i)$ are calculated as functions of the kurtosis parameter

$$\omega(\beta_i) = \frac{\Gamma^{1/2}[3(1+\beta_i)/2]}{(1+\beta_i)\Gamma^{3/2}[(1+\beta_i)/2]}, \quad (6)$$

$$c(\beta_i) = \left\{ \frac{\Gamma[3(1+\beta_i)/2]}{\Gamma[(1+\beta_i)/2]} \right\}^{1/(1+\beta_i)}. \quad (7)$$

The error model parameters σ_i and β_i were inferred as part of the Bayesian inference procedure, and estimated posterior statistics of error model parameters are reported in Table 5. Noise variances corresponding to the model with standard PS parameterization (Dirac prior) exceed the noise variances of the models, in which PS parameters were inferred (broad prior), in accordance with the evaluation metrics in Figure 3. The exponential power distribution resembles the Gaussian distribution if the kurtosis parameter $\beta = 0$. As the kurtosis parameter approaches 1, the corresponding probability distribution will be characterized by tails that asymptotically approach zero more slowly than a Gaussian distribution (heavy tails). The median kurtosis parameters governing the velocity error distributions are close to 1 (double exponential distribution) and have rather narrow credible intervals, providing evidence for heavy-tailed velocity errors. On the other hand, the median kurtosis parameters governing the hematocrit error distributions have more intermediate values and broader credible intervals. Figure 11 provides a graphical evaluation of model residuals in terms of quantile-quantile plots. Overall we observe a similar behavior in the residuals across the two settings of the prior distribution governing PS model parameters. The velocity quantile-quantile plots show discrepancy in a rather limited number of residuals (1318 scatter points / segments in total), and the hematocrit quantile-quantile plots show rather good correspondence between theoretical and observed quantiles (1710 scatter points / segments in total). The velocity residual histograms exhibit a small tendency for

skewness, whereas the histograms representing hematocrit residuals appear symmetric. A more advanced variant of the error model incorporating a skewness parameter is available [32]. However, such increased error model flexibility comes at a cost of more parameters to be inferred. Since Figure 11 illustrates rather good overall correspondence between the error models and the observed errors, we deemed the used error models as appropriate. Additionally, the residuals behave similarly across the two settings of the PS model prior, and hence possible discrepancies between model and data are not judged to impose bias in the comparison between the two models.

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Perspectives

Theoretical modeling of microcirculatory flow patterns has proved as a versatile tool for interpreting experimental measurements and for studying mechanisms underlying microcirculatory flow phenomena. This paper presents a formal quantitative evaluation of the ability of a widely used modeling approach, incorporating a model of the phase separation effect, to predict physiologically realistic network hematocrits. A validation against current available comprehensive measurements from mesenteric networks supports the continued use of the modeling approach including the phase separation model.

Figure 1.

A: Photomontage of a mesenteric microvessel network (network 1). **B:** Measured hematocrit in network 1. **C:** Measured hematocrit in network 4. Arrows indicate main arteriolar inlets and venular outlets. Segment diameters are doubled relative to the figure scale in **B** and **C** to enhance visibility.

Figure 2.

Approach to combine experimental measurements and model simulation in a network-oriented analysis. **A:** Experimental measurements comprise measurements of blood flow directions y_{dir} and hematocrit y_{hct} as well as network topology and morphology in four networks and also measurements of blood flow velocities y_{vel} in three of the four networks.

B: The flow simulation model yields predictions $\hat{y}$ of blood flow directions, hematocrit, and blood flow velocities throughout the networks. The flow simulation model requires prescription of appropriate values for pressure boundary conditions ζ_p , and hematocrit boundary conditions ζ_{hct} . The simulation model also incorporates a series of empirical rheological descriptions, which, in turn, are parameterized by fitted parameters θ , for example the parameters of the phase separation model $\{\theta_A, \theta_B, \theta_{X0}\}$.

C: Experimental measurements and flow model simulations are combined within a Bayesian probabilistic analysis framework [30]. The probabilistic approach allows the uncertain boundary conditions and also the phase separation model parameters to be associated with uncertainty instead of being ascribed with fixed parameter values. $x = \{\zeta_p, \zeta_{hct}, \theta_A, \theta_B, \theta_{X0}, \beta, \sigma^2, \gamma\}$

concatenates the uncertain parameters, with $\{\beta, \sigma^2, \gamma\}$ being parameters of the hematocrit, velocity, and flow direction likelihood functions. Priors $P(\cdot)$ governing pressure boundary conditions (mmHg) and hematocrit boundary conditions varies with vessel type and vessel diameter, and shown are example priors governing a single boundary node J . A broad prior governing the θ_{X0} parameter of the phase separation model and a prior governing the

kurtosis parameter β_{vel} of the velocity error model are also shown. The “distance” between the experimental measurements and model simulations is quantified by the likelihood function $P(y|x)$, which, in turn, is governed by the set of parameters $\{\beta, \sigma^2, \gamma\}$. Bayes’ rule yields the posterior distribution governing the uncertain parameters, with $P(y)$ being the model evidence. This posterior distribution cannot be inferred by analytical means, and instead we use MCMC sampling **D** for posterior inference. MCMC sampling provides samples from the posterior distribution, and a series of samples corresponding to the parameters in **C** are shown. The samples can be used to summarize the posterior distribution, and marginal distributions are shown as a summary example. The samples can further be propagated through the simulation model **B** to evaluate how, for example, boundary condition uncertainty propagates to uncertainties in the simulation model’s predictions (predictions for a single vessel i are shown). This results in multiple predictions of hemodynamic variables throughout the networks, as represented by the stack of hematocrit predictions.

Figure 3.

Quantitative evaluation of model predictions in four mesenteric networks based on split-half resampling. Dots mark individual resampling splits (50 in total), outlines represent density estimates, and red lines represent medians. Results corresponding to models with two different prior distributions (Dirac, broad) governing the phase separation model parameters are shown. θ_{standard} corresponds to the analysis with the Dirac prior (standard values for phase separation model parameters), and θ_{inferred} corresponds to the analysis with the broad prior.

Figure 4.

Scatter plots comparing measured and predicted hematocrit in four mesenteric networks (1710 segments). Black dots mark mean model predictions (based on validation segments) and gray lines mark predictive range across the 50 resampling splits. θ_{standard} corresponds to the analysis with the Dirac prior (standard values for phase separation model parameters), and θ_{inferred} corresponds to the analysis with the broad prior.

Figure 5.

Comparison of measured and predicted hematocrit as grouped by vessel diameter in four mesenteric networks (1710 segments). Hematocrit was normalized by the measured main inlet hematocrit for the individual networks. Black dots mark measurements and median (validation) predictions. Outlines represent density estimates. θ_{standard} corresponds to the analysis with the Dirac prior (standard values for phase separation model parameters), and θ_{inferred} corresponds to the analysis with the broad prior.

Figure 6.

Spatial representation of predicted hematocrit in network 1 (**A-B**) and network 4 (**C-D**). Segment hematocrits represent averages across all resampling split-halves. Hematocrit distributions are shown as insets. Segment diameters are doubled relative to the figure scale to enhance visibility. θ_{standard} corresponds to the analysis with the Dirac prior (standard values for phase separation model parameters), and θ_{inferred} corresponds to the analysis with the broad prior.

Figure 7. Marginal posterior distributions representing the inferred phase separation model parameters (θ_A , θ_B , and θ_{X0}). The red dashed lines represent prior distributions, the thin gray lines represent density estimates based on models calibrated on individual split-halves (100 splits-halves in total), and the dark gray frequency histograms represent samples pooled across all 50 resampling splits.

Figure 8.

A: Spatial representation of network 4. **B:** Zoom (rotated) on section in the vicinity of the main feeding arteriole marked in **A**. Arrows indicate measured flow directions. Measured hematocrit and average predicted hematocrit are reported in the format: measurement/ $\theta_{\text{standard}}/\theta_{\text{inferred}}$, with θ_{standard} and θ_{inferred} corresponding to the analyses with the Dirac prior (standard values) and the broad prior, respectively. Bold numbers mark daughter segments of bifurcations for which the standard PS model parameters leads to low hematocrit prediction in comparison to predictions when using the inferred parameter values. Red, green, and blue colors represent arterioles, capillaries and venules respectively. Segment diameters are doubled relative to the figure scale to enhance visibility.

Figure 9.

Single bifurcation evaluation of the inferred posterior distribution of the phase separation model coefficients (Figure 7) illustrating the extent to which the uncertainties represented by the marginal distributions in Figure 7 and between parameter correlations in Table 3 affect the phase separation model at single bifurcation level. Bifurcation configurations:

$H_D = 0.49/0.42/0.43$, $D_F = 20/7.5/27.7$, $D_\alpha = 16.5/6/7.4$, and $D_\beta = 17.5/8/23.1$ (bifurcation I / bifurcation II / bifurcation III). Dotted lines represent model prediction using standard model parameters in eq. 2–4, while dashed lines represent mean predictions obtained with the broad prior (surrounding lines represent 95% credible intervals).

Figure 10.

Fractional discharge hematocrit versus fractional blood flow for bifurcation III (marked with * in Figure 8). Dotted lines represent model prediction using standard model parameters in eq. 2–4, while dashed lines represent mean predictions obtained with the informative prior (surrounding lines represent 95% credible intervals).

Velocity residuals **Hematocrit residuals** **Figure 11.**

Residual analysis. Quantile-quantile plots comparing theoretical quantiles against observed quantiles. Theoretical quantiles were calculated by use of the error model Eq. 5 and MCMC chain samples of the noise model parameters σ and β (estimated from calibration samples). Observed quantiles were based on residuals calculated from validation samples. The dots represent averages across all MCMC chain iterations and all resampling splits. Insets show histograms of observed (validation) residuals overlaid by the theoretical error probability distributions. θ_{standard} corresponds to the analysis with the Dirac prior (standard values for phase separation model parameters), and θ_{inferred} corresponds to the analysis with the broad prior.

Table 1

Validation performance for two parametrizations of the phase separation model; θ_{standard} corresponds to the analysis with the Dirac prior (standard values for phase separation model parameters), and θ_{inferred} corresponds to the analysis with the broad prior. Format: median (mean) [95% credible interval]. Medians, averages, and quantiles calculated across resampling splits.

PS parameters	Velocity error	Hematocrit error	Wrong flow dir.
θ_{standard}	0.697 (0.738) [0.650 0.969]	0.0289 (0.0290) [0.0286 0.0294]	2.6 (2.6) [2.3 3.0]
θ_{inferred}	0.650 (0.698) [0.609 0.967]	0.0239 (0.0239) [0.0234 0.0242]	2.5 (2.5) [2.2 2.9]

Table 2

Estimated posterior statistics for the phase separation model parameters, summarizing inferred parameter values θ_{inferred} corresponding to the analysis with broad prior. Statistics are estimated from MCMC chain samples pooled across chain iterations from all individual calibration splits. Format: median (mean) [95% credible interval]. Standard parameters: $\theta_A = -13.29$, $\theta_B = 6.98$, and $\theta_{X0} = 0.964$.

Posterior statistics		
θ_A	θ_B	θ_{X0}
-10.11 (-10.15) [-15.71 -4.94]	5.89 (5.88) [3.82 7.98]	0.09 (0.10) [0.01 0.27]

Table 3

Pair-wise correlation between inferred phase separation model parameters, calculated from MCMC chain samples. Format: median (mean) [95% credible interval].

$\text{corr}(\theta_A, \theta_B)$	$\text{corr}(\theta_A, \theta_{X0})$	$\text{corr}(\theta_B, \theta_{X0})$
-0.822 (-0.815) [-0.882 -0.723]	0.175 (0.180) [0.032 0.317]	-0.549 (-0.538) [-0.699 -0.377]

Table 4

Post hoc model evaluation based on phase separation parameters fixed according to posterior statistic ($\theta_A = -10.11$, $\theta_B = 5.89$, and $\theta_{X0} = 0.09$). Format: median (mean) [95% credible interval]

Velocity error	Hematocrit error	Wrong flow dir.
0.648 (0.698) [0.607 0.972]	0.0237 (0.0237) [0.0233 0.0241]	2.5 (2.5) [2.2 2.8]

Table 5

Estimated posterior statistics of the error model parameters. θ_{standard} corresponds to the analysis with the Dirac prior (standard values for phase separation model parameters), and θ_{inferred} corresponds to the analysis with the broad prior. Format: median (mean) [95% credible interval].

PS parameters	Parameter			
	σ_{vel}^2	β_{vel}	σ_{hct}^2	β_{hct}
θ_{standard}	0.44 (0.44) [0.35 0.54]	0.99 (0.98) [0.94 1.0]	0.026 (0.026) [0.021 0.030]	0.55 (0.55) [0.29 0.83]
θ_{inferred}	0.42 (0.42) [0.34 0.52]	0.99 (0.98) [0.94 1.0]	0.021 (0.021) [0.018 0.024]	0.42 (0.43) [0.19 0.69]