MODELING ULTRASOUND IMAGING OF 
RED BLOOD CELL AGGREGATION IN SHEAR FLOW

by

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A thesis submitted in conformity with the requirements 
for the degree of Doctor of Philosophy 
Graduate Department of Electrical and Computer Engineering 
and Institute of Biomedical Engineering 
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ABSTRACT

Modeling Ultrasound Imaging of Red Blood Cell Aggregation in Shear Flow
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The primary focus of this thesis is to gain an understanding of the effects of red blood cell (RBC) aggregation on the backscattered ultrasound signal from blood flow. In pursuit of this goal, this study consists of three distinct parts.

First, an efficient and accurate method of simulating pulsed ultrasound imaging of tissue was implemented and validated, which calculates the backscattered ultrasonic signal by summing contributions from basic scattering units called “acoustic voxels”.

Second, a model of aggregation was developed, which takes into consideration the effect of shear rate, hematocrit, aggregate structure (as described by a fractal dimension), and cell adhesion strength on aggregate size. Simulations of blood flow in a large diameter tube using this model show that variations in average aggregate size across the tube diameter and downstream of the tube entrance arise due to the effect of flow streamlines having distinct shear histories.

Finally, the results of an initial simulation study of the relationship between aggregate size distributions and packing statistics (as measured by RBC concentration variance) are presented. It is shown that the concentration variance is strongly affected by hematocrit, aggregate size and size distribution, as well as by aggregate shape.

By combining these three parts, the backscattered ultrasound signal from blood flow in a large diameter tube was simulated. Good qualitative agreement between these simulation results and the experimental results reported by other investigators was obtained. In particular, these simulations yielded useful insights into understanding the aggregation-related “black hole” phenomena observed in ultrasound imaging of blood flow.
Acknowledgements

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- My parents, whose pleasure and pride in me have been familiar and affirming companions.
- The various communities and communities of faith in which I have found a sense of belonging, - from Sanctuary, the Navigators, the Institute of Biomedical Engineering (IBME), the Graduate Christian Fellowship and Calvary Church, I have had the privilege to call many of you my brothers, my sisters, my mentors, my friends. I have been touched in mysterious and wonderful ways by the gifts, the presence of grace, that you have been to me.

Financial support from the Natural Sciences and Engineering Research Council of Canada, the University of Toronto, the Government of Ontario and the Sumner Foundation is gratefully acknowledged.

This pilgrimage of scientific investigation into the mysteries of Creation has been a gift, a journey shared with others at times, and at many moments, a joyful enterprise...

*I will give thanks to thee, for I am fearfully and wonderfully made; Wonderful are thy works, and my soul knows it very well. (Psalm 139:14, NASB)*

... One day when I was sitting quiet and feeling like a motherless child, which I was, it come to me: that feeling of being part of everything, not separate at all. I knew that if I cut a tree, my arm would bleed. And I laughed and I cried and I run all around the house. I knew just what it was. (The Color Purple, Alice Walker)

A distant call: Do not forget to hear the cry of the poor and the suffering, it seems to be saying. May I be, somehow, a redemptive and hope-giving presence to others. In awareness of my frailties and failures, I offer up, with fear and trepidation, this work and my life...

*Ad Majorem Dei Gloriam*
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Vector quantities are represented by **boldface** characters.

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<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$\alpha$</td>
<td>Collision efficiency (percentage of successful aggregate collisions)</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wavelength of incident ultrasound</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Viscosity of blood</td>
</tr>
<tr>
<td>$\mu_p$</td>
<td>Viscosity of plasma</td>
</tr>
<tr>
<td>$\sigma_b$</td>
<td>Differential backscattering cross-section of a particle</td>
</tr>
<tr>
<td>$\sigma_m$</td>
<td>Differential backscattering cross section for a symmetric $m$-dimensional scatterer</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Shear stress</td>
</tr>
<tr>
<td>$\tau_r$</td>
<td>Shear stress at radial distance $r$ from tube axis</td>
</tr>
<tr>
<td>1-D</td>
<td>One-dimensional</td>
</tr>
<tr>
<td>2-D</td>
<td>Two-dimensional</td>
</tr>
<tr>
<td>3-D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>$A$</td>
<td>Hamaker constant (in joules), used to describe interparticle (van der Waals) attractive forces</td>
</tr>
<tr>
<td>$a_0$</td>
<td>Radius of a sphere having the same volume as an RBC</td>
</tr>
<tr>
<td>$a_i, a_j$</td>
<td>Radii of aggregates consisting of $i$ and $j$ RBCs</td>
</tr>
<tr>
<td>BSC</td>
<td>Backscattering coefficient</td>
</tr>
<tr>
<td>$c$</td>
<td>Speed of sound in tissue</td>
</tr>
<tr>
<td>CVI</td>
<td>Chronic venous insufficiency</td>
</tr>
<tr>
<td>CW</td>
<td>Continuous wave</td>
</tr>
<tr>
<td>D</td>
<td>Tube Diameter</td>
</tr>
<tr>
<td>$d_F$</td>
<td>Volume-length correlation (fractal) dimension of an aggregate</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
</tbody>
</table>
f(.)  Shorthand notation denoting a rate equation (Eq. 2.2.1)
$F_A$  Aggregation adhesive force
$F_H$  Hydrodynamic forces exerted on an aggregate by shear flow
$g_1, g_2, g_3, g_4$  Intermediate results for the Runge-Kutta algorithm
$G$  Shear rate
$G(r)$  Shear rate at radial distance $r$ from tube axis
Hct, $H, h$  Hematocrit
$i_{ave}$  Average aggregate size for a voxel
$i_{max}$  Maximum aggregate size (# of RBCs), used in Eqn. 4.1.1
$j$  $\sqrt{-1}$
$k$  Wave vector for single-frequency plane wave
$k$  Particle size-dependent scaling factor in the calculation of collision efficiency $\alpha$ (Chapter 2, 4)
$k$  Ratio of occurrence of aggregates consisting of $i$ particles to occurrence of aggregates consisting of $i-1$ particles (Chapter 5)
$k'$  Scaling factor used in the calculation of the hydrodynamic force exerted on an aggregate, $F_H$
$k_1, k_2$  Constants used to calculate the viscosity in the Casson model
$K_{ip}, K_{ij}(t)$  Collision kernel - rate of collisions between aggregates of $i$ and $j$ RBCs
$m$  Particle packing dimension, may take on non-integer values
MC  Monte Carlo
MD  Molecular dynamics
$M_{max}$  Maximum aggregate size (in # of RBCs) for a given aggregate size distribution
$MSE$  Mean square error
$N$  Number of scatterers insonated
$n$  Power law exponent for velocity and shear rate profiles (Chapter 4)
$n_{ave}$  Average number of particles per voxel
$n_i, n_j$  Concentration of aggregates of $i$ and $j$ RBCs, respectively
$\Delta n_{i\rightarrow j}$  Change in concentration of aggregates formed from the
collision of aggregates consisting of \( i+j \) particles

\( n^n \)
Shorthand notation denoting \((n^1, n^2, \ldots, n^n, \ldots)\)

\( n_{i+j}^n \)
Concentration of aggregates formed from the collision of aggregates consisting of \( i+j \) particles at time \( t_n \)

\( N_{\text{check}} \)
Number of voxels used in checking for scatterer overlap

\( N_s \)
Number of signal samples used to calculate the \( \text{MSE} \)

\( N_v \)
Number of scatterers contained within a voxel

\( N_{v_i} \)
Number of scatterers in the \( i \)-th voxel

\( N_{\text{voxel}} \)
Number of voxels in tissue sample

\( p(i) \)
Probability of occurrence of an aggregate consisting of \( i \) particles

\( p_i \)
Probability of occurrence of a single particle aggregate

\( \mathbf{R} \)
Vector position of a voxel center with respect to the transducer

\( R \)
Tube radius

\( r \)
Radial distance from tube axis

\( \Delta r_{\text{avg}} \)
Average vector position of the scatterers within a voxel with respect to the voxel center

\( \text{RBC} \)
Red blood cell

\( \text{RF} \)
Radio frequency

\( \mathbf{R}_i \)
Vector position of \( i \)-th particle with respect to the transducer

\( r_i \)
Radial distance of voxel \( i \) from tube axis

\( s \)
seconds

\( t \)
Time

\( t_n \)
Time (i.e. after the \( n \)-th time step \( \Delta t \))

\( \Delta t \)
Time step used in aggregation simulations (Chapter 2, 4)

\( v(r) \)
Velocity at radial distance \( r \) from tube axis

\( v(r_i) \)
Velocity of voxel \( i \) at radial distance \( r \) from tube axis

\( \text{Var} \)
Variance in number of scatterers per voxel

\( v_{\text{max}} \)
Peak blood flow velocity

\( v_p \)
Particle volume in Bascom's backscattering model (Eqn. 2.3.1)

\( V_p \)
Particle volume
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta V$</td>
<td>Voxel volume</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell</td>
</tr>
<tr>
<td>$x(t)$</td>
<td>Backscattered ultrasound signal</td>
</tr>
<tr>
<td>$x_i$</td>
<td>The $i$-th sample of the simulated backscattered RF signal $x$ obtained by the particle method (Chapter 3)</td>
</tr>
<tr>
<td>$(x, y), (x_i, y_i)$</td>
<td>Circle centre coordinates, coordinates for the $i$-th circle</td>
</tr>
<tr>
<td>$x_f(t)$</td>
<td>Wideband (pulsed) signal at time $t$</td>
</tr>
<tr>
<td>$x_{var}$</td>
<td>Voxel index along $x$-axis (Appendix B)</td>
</tr>
<tr>
<td>$y_i$</td>
<td>The $i$-th sample of the simulated backscattered RF signal $y$ obtained by the voxel method (Chapter 3)</td>
</tr>
<tr>
<td>$y_{var}$</td>
<td>Voxel index along $y$-axis (Appendix B)</td>
</tr>
<tr>
<td>$z$</td>
<td>Voxel position along tube axis</td>
</tr>
<tr>
<td>$z_f$</td>
<td>Final voxel position along tube axis</td>
</tr>
<tr>
<td>$z_0$</td>
<td>Voxel position along tube axis at time $t_0$</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction

Red blood cell (RBC) aggregation is a reversible process in which RBCs clump together to form aggregates or chain-like structures called rouleau. Although RBC aggregation is an intrinsic physiological process, it is also associated with various pathological conditions in which extensive rouleau networks and large aggregates may form. It is a distinct process from platelet aggregation or coagulation and clotting, and, due to its effect on viscosity, is an important contributing factor to the non-Newtonian flow behaviour of blood. In the past 3 decades, many studies have been undertaken to gain a better understanding of the role of RBC aggregation in physiological function, pathological conditions, its effect on hemodynamics in the circulatory system, as well as the mechanisms and kinetics of aggregation.

Measurement of the backscattered ultrasound signal from blood is one of the techniques which has been used in recent years to study RBC aggregation. When ultrasound is directed at a blood vessel, the resulting echo signal is dependent on the size, concentration and packing arrangement of the RBCs and the aggregates which caused the scattering. It is hoped that gaining more insight into the relationship between the backscattered ultrasound signal and aggregation may lead to the development of better methods for the diagnosis of disease, as well as to a better understanding of hemodynamics in the circulatory system, and is the subject of this research.
In this introduction, a brief survey of various aspects of RBC aggregation and of the methods used in past aggregation studies will be presented. This chapter concludes with statements of the purpose and objectives of this research and an outline of the thesis organization.

1.1 Red Blood Cell (RBC) Aggregation

The main components of blood are RBCs, white blood cells (WBCs) and platelets, suspended in plasma, which contains protein macromolecules - fibrinogen, globulins and albumin. Cellular elements (RBCs, WBCs and platelets) make up on average about 45% of the volume of whole blood in humans, and RBCs make up by far the largest proportion by volume and number of these elements - at a normal human hematocrit (volume fraction) of 45%, there are about 5 million RBCs per mm³, compared to about 7000 WBCs and 400,000 platelets. Human RBCs are deformable biconcave disks about 8 µm in diameter and 2 µm at their thickest location, and have average volumes of about 90 µm³ (Shung and Thiemie, 1993). Rouleaux are formed when RBCs approach each other closely and the broad RBC surfaces become aligned to form straight or branched chains, similar in appearance to stacks of coins.

Good evidence has been presented to support the view that aggregation arises when macromolecules, which are present in plasma, become adsorbed into RBC surfaces and bridge the surfaces of adjacent RBCs (Chien et al., 1970; Chien 1975; Chien, 1976; Fabry 1987). RBCs suspended in saline (in which macromolecules are absent) do not display aggregate formation. In experiments using human RBCs and dextran macromolecules, it was observed that, for stable aggregation to occur, molecules of sufficient length (i.e. molecular weight) and concentrations were required (Chien et al., 1970). Transmission electron micrographs from these experiments showed that the surfaces of adjacent RBCs followed each other in curvature with a uniform intercellular distance, which altered as the length of macromolecules used was changed. Another theory of aggregation hypothesizes that the number of polymers or macromolecules become depleted near particle surfaces, which results in a polymer concentration gradient and hence a positive surface energy and attraction between particles (Gast and Leibler, 1986; Joanny et al., 1979). Although the results of the above-cited experimental studies of the effect of macromolecule concentration and weight on
aggregation are not necessarily incompatible with the depletion layer theory of aggregation, the macromolecular bridging model seems most compelling.

The strength with which RBCs adhere to one another depends on the macromolecule weight and concentration, the shape and deformability of the RBCs, electrostatic surface charges (Chien, 1981), ionic charges in the suspending medium, and the types of macromolecules and plasma proteins involved in aggregation (Weng et al., 1996). Aggregate breakup occurs when any external forces acting on the RBC surfaces overcome the adhesive forces. The degree to which RBCs and aggregates interact with each other can be affected by the hematocrit, mixing due to shear forces, as well as the structure and size of the aggregates formed (Chien, 1975).

It has been well-established that RBC aggregability varies greatly among different animal species (Usami et al., 1969; Chien et al., 1971; Amin and Sirs, 1985; Schneck, 1990; Wickham et al., 1990; Popel et al., 1994; Weng et al. 1996). For example, horse blood displays strongly aggregating tendencies, pig and human blood aggregate moderately, and negligible aggregation is observed for sheep blood (Schneck, 1990; Weng et al, 1996). These variations may be due to different aggregating mechanisms, aggregating molecules, plasma protein concentrations, and RBC characteristics, such as shape and deformability (Weng et al, 1996). It is known, for example, that fibrinogen is the most important macromolecule for aggregation in human blood, but fibrinogen concentration does not correlate well to the degree of aggregation in other species (Schneck, 1990; Weng et al., 1990). Attempts have been made to understand these different aggregating tendencies from the perspective of physiological function. Popel et al. (1994) presented data which purported to show that higher degrees of aggregation were characteristic of more athletic species, while Wickham et al. (1990) hypothesized that reduced aggregation in seals was an adaptive response to reduced cardiac response during long dives.

1.1.1 Clinical significance - physiological function of RBC aggregation
The primary function of the circulatory system is to transport nutrients, metabolites, hormones and other materials to and from tissues. Vascular hindrance and blood viscosity are the major determinants of circulatory resistance, and therefore, of nutrient transport (Chien, 1987). Blood vessel radius is the predominant factor in the determination of vascular
hindrance. Vessels with small diameters, such as arterioles, capillaries and venules, contribute the most to vascular hindrance and flow resistance in physiological conditions, although severe narrowing of large vessels (arteries and veins) may lead to significant increases in flow resistance in vascular disease. The arterioles are the major resistance vessels, as they consist of smooth muscle and can undergo marked changes in diameter.

Human whole blood is a non-Newtonian fluid - its viscosity, the ratio of the applied shear force to the resulting shear rate of the fluid, is not constant. Instead, blood displays shear-thinning behaviour, which means that its viscosity decreases with shear rate. The non-Newtonian behaviour of blood is due primarily to the deformability of RBCs and to RBC aggregation. At low shear rates, aggregation is enhanced, resulting in high blood viscosity, while at high shear rates, aggregates are broken up and RBCs deform and align themselves in the direction of flow, thus causing a decrease in viscosity (Chien, 1970; Cokelet, 1987). Platelets, being much smaller than RBCs, and WBCs, being far less numerous than RBCs, probably make insignificant contributions to blood viscosity. Besides RBC aggregation and deformation, blood viscosity is also dependent on hematocrit and plasma viscosity, and inversely dependent on temperature.

RBC aggregation occurs for “normal” physiological conditions in the absence of any obvious diseased state, which suggests that it serves as a means of viscosity regulation, and hence, circulatory perfusion. Under physiological flow conditions, the shear stresses in the arteries and arterioles are sufficiently high to prevent extensive aggregation and maintain low apparent viscosity. Aggregation is unlikely in capillaries since the capillary diameters are comparable to those of the RBCs, and the highest shear stresses are normally found in the capillaries (Chien, 1987). The most likely sites of aggregation are in the postcapillary venules and veins because they have the lowest shear stresses in the circulatory system. For blood flow in small tubes, it has been observed that aggregation causes RBCs to concentrate towards the tube center, which increases the size of the cell-free layer next to the wall, decreases the apparent viscosity and would therefore enhance perfusion (Vicaut, 1995; Cokelet, 1987). It has been hypothesized then, as a means of viscosity regulation, that a moderate level of aggregation reduces in vivo viscosity, and a high level increases viscosity (Vicaut, 1995).
1.1.2 Clinical significance - pathological aspects of RBC aggregation

The degree of aggregation is dependent on the levels of macromolecules in plasma, which are sensitive to disease and stress, RBC deformability, as well as the blood vessel shear stress, which depends on such disease-affected factors as vessel geometry, size and distensibility. Hyperaggregating states are found in diabetes, venous insufficiency, myocardial infarction, hypertension, and nephrotic syndrome (Razavian et al., 1992; Vicaut, 1995). Aggregation may also be important in atherosclerosis (Kitamura et al., 1995) and thrombus formation (Fatkin et al., 1997).

In the case of sialic acid deficiency, the degree of RBC aggregation is elevated, accompanied by an elevation of viscosity at low shears. In the case of sickle cell disease, RBC rigidity is increased, which results in decreased viscosity at low shear rates since alignment of RBC surfaces is hindered, and aggregate formation is therefore reduced. Increased viscosity occurs at high shear rates because the increased RBC rigidity prevents the cells from deforming in alignment with the flow. Since oxygen transport is dependent on hematocrit but inversely dependent on viscosity, oxygen transport at high shear rates would be reduced if normal hematocrit levels were maintained. It has been found that sickle cell patients, in fact, have reduced hematocrits - this may be a possible compensatory mechanism to decrease the viscosity and increase oxygen transport (Chien, 1987).

In chronic venous insufficiency (CVI), low shear stresses in the post-capillary venule levels lead to enhanced aggregation. A self-propagating cycle may then arise in which shear stresses are further reduced and slower venous return occurs. Interstitial leakage into the capillaries then causes local hemoconcentration and increased plasma fibrinogen, which leads to increased aggregation and decreased tissue oxygenation (Zuccarelli et al., 1995).

1.2 Methods of studying RBC aggregation

Aggregation of RBCs has been studied by means of a variety of techniques such as erythrocyte sedimentation rate (Copley et al., 1975; Chien et al., 1970; Rampling and Whittingstall, 1986; Fabry, 1987), optical methods (Chien et al., 1970; Schmid-Schonbein et al., 1976a, 1976b; Shiga et al., 1983; Snabre et al., 1987; Goldsmith, 1993; Chen et al., 1995; Weng et al., 1996), viscometric methods (Chien et al., 1970; Rampling and
As its name implies, the erythrocyte sedimentation rate (ESR) consists of observing the rate at which RBCs sediment - the larger the aggregates formed, the greater the rate of sedimentation (that is, the shorter the time required for sedimentation to occur). Optical methods have made use of light microscopy, light transmission or reflection or laser light scattering, to observe aggregates and study aggregation kinetics. Optical methods have often been applied to suspensions which have been subjected to controlled shear or flow conditions. Electron microscopy has also been used. Viscometric methods entail viscosity measurements of blood samples as functions of applied shear force or apparent shear rate.

Experiments with blood of different species, macromolecules (e.g. dextran or fibrinogen), and hematocrits, under various flow conditions, and with RBCs in various suspending mediums (e.g. plasma or saline) have been used in conjunction with many of these methods. One of the primary drawbacks of all the above methods is that a blood sample is required - an invasive process that may be inconvenient in a clinical setting. Direct microscopic observation of aggregates can only be done for very low hematocrits, while other optical methods, ESR and viscometric techniques are indirect methods of studying the degree and process of aggregation. Moreover, none of the above techniques can be used for \textit{in vivo} study of aggregation kinetics.

In contrast, ultrasound techniques may lead to non-invasive methods for carrying out quantitative \textit{in vivo} studies of RBC aggregation. Past experimental investigations of RBC aggregation with ultrasound have made use of B-mode imaging (Yuan and Shung, 1989; Sigel \textit{et al.}, 1992; Kim \textit{et al.}, 1989; Shehada \textit{et al.}, 1994; Kitamura \textit{et al.}, 1995), the Doppler power spectrum (Shung \textit{et al.}, 1992; Cloutier \textit{et al.},1996) and the measurement of the backscattered echo signal (Boynard and Lelievre, 1990; Razavian \textit{et al.}, 1991; Mo \textit{et al.}, 1994) These ultrasound methods have been applied to small blood samples, \textit{in vitro} tube flow arrangements, as well as \textit{in vivo} situations (Machi \textit{et al.}, 1983; Kitamura and Kawasaki,
Chapter 1. Introduction

1997) to investigate the influence of such factors as shear rate, flow conditions, hematocrit, macromolecule concentration and time on aggregation.

1.3 Motivations and Objectives

Modeling the backscattering of ultrasound from tissue is important for two reasons. First of all, in order to improve ultrasound imaging system design, it is important to be able to realistically simulate the effects of tissue insonation. Secondly, an understanding of the relationship between changes in tissue characteristics and the backscattered signal may lead to improved diagnostic methods.

The primary objectives of this research are (1) to develop a theoretical model which describes the process of RBC aggregation in a shear flow, and (2) to gain a better understanding of the relationship between the ultrasound signal backscattered from blood, the size and concentration of RBC aggregates in blood, and the flow conditions which contribute to aggregation. The insights obtained from this research may lead to improved ultrasound methods for the quantitative measurement of aggregation and the noninvasive diagnosis of pathological conditions associated with high degrees of aggregation. A better understanding of the role that various physical parameters play in aggregation kinetics might be obtained, as part of fundamental studies of hemodynamics in the circulatory system. Some secondary objectives are to validate a previously developed model for simulating the backscattered ultrasound signal from tissue and to explain some experimental results obtained by past investigators.

1.4 Organization of thesis

This research consists of three distinct parts: (1) the implementation and validation of a voxel-based method for performing computer simulations of the backscattered ultrasound signal from tissue, (2) the development of a model of shear-dependent RBC aggregation in a large diameter tube, and (3) an investigation into the influence of RBC and aggregate size,
shape and size distribution on the scatterer packing statistics, and consequently, on the backscattered ultrasound signal.

Chapter 2 describes the background of the thesis, and consists of a discussion of methods for simulating continuous wave (CW) ultrasound imaging of tissue, a description of previous models of RBC aggregation kinetics, and a survey of past ultrasound studies of blood flow and aggregation. The computer simulation work for validating the voxel method for the simulation of pulsed ultrasound imaging is described in Chapter 3. The implementation of the particle and voxel methods are presented, the simulation results are presented, and the two methods are compared. A theoretical model of RBC aggregation in shear flow is presented in Chapter 4. The simulation method is described, and the simulation results are presented, discussed and compared to past experimental results. Chapter 5 consists of a study of the relationship between aggregate size and scatterer packing statistics. The results of this study are used for simulating ultrasound imaging of RBC aggregation, as described in Chapter 6. Finally, a summary of contributions, conclusions and suggestions for future work are presented in Chapter 7.
Chapter 2
Background

If we look more deeply at a particular aspect - whether this is the growth cycle of the potato or the meaning of a single word of the Bible - we can touch the mystery through it...
When we train our intelligence onto a single subject, we enter the world of wonder and contemplation.
Our whole being is renewed when we touch the light of God hidden at the heart of things...
We shouldn't read just what is useful; we should also try to understand for its own sake, because it is the gratuity of the light which is stimulating.
(Community and Growth, Jean Vanier)

In this chapter, theoretical background describing methods of simulating ultrasound imaging of tissue and previous models of describing aggregation kinetics are presented. As well, models and simulation approaches for aggregation and particle packing statistics are discussed, as are the results of a number of experimental studies of blood of relevant to the study of aggregation.

2.1 Simulating CW ultrasound imaging of tissue - particle and voxel methods

Simulation methods provide an important means for the development and design of modern ultrasound imaging systems. Especially important is the use of a realistic model of tissue so that the imaging performance can be studied and optimized at the design stage and thereby avoid the costs of experimental investigations. In addition, a good tissue model could help provide additional information of diagnostic significance through improved interpretation of the backscattered signal.

The RF signal that results from the backscattered ultrasound waves from tissue depends on the detailed tissue structure, attenuation, ultrasound beam characteristics, pulse shape and the transducer/receiver characteristics. For computer simulations of ultrasound
backscattering from tissue, the 'exact' particle approach is commonly used (Routh et al., 1987; Zhang et al., 1994; Hunt et al., 1995). In this approach, the tissue compressibility and density variations, which are often assumed to be the primary cause of scattering, are represented by particles, and the RF signal is simulated by adding together the backscattered signal contributions from each individual scatterer within the tissue, with appropriate account being taken of the transducer/receiver characteristics. See Fig. 2.1 below. The computational burden for simulating scattering from realistic tissue volumes can be high. For example, a 1 mm$^3$ sample volume of 40% hematocrit blood contains approximately $4.5 \times 10^6$ red blood cells.

Figure 2.1 Schematic illustrating the particle approach: backscattering contributions of individual scatterers are summed at the observation point. $\mathbf{R}_i$ denotes the vector position of the $i$-th particle with respect to the observation point.

In contrast, the approximate voxel approach recognizes that individual scatterers may be too small relative to the incident ultrasound wavelength ($\lambda$) to be resolved, and therefore divides the tissue into basic scattering units called 'acoustic voxels', which may contain many individual scatterers (Mo and Cobbold, 1992). The RF signal is then simulated by adding together the backscattered signal contributions from each voxel, whose scattering strength is determined by the number of scatterers contained within it. Depending on the selection of voxel size, the computation time can be reduced significantly from that required by the 'exact' method. For example, at a hematocrit of 40% and a typical wavelength of 308
μm, a λ/10 voxel contains on the order of 16 scatterers in 2-D and 130 scatterers in 3-D, and consequently, the number of calculations required to simulate the backscattered signal would be reduced by about 10 and 100 times, respectively.

Figure 2.2(a) shows a possible two-dimensional distribution of scatterers, which is divided into voxels as illustrated in Fig. 2.2(b). Figures 2.2(c) and 2.2(d) show the replacement of the particles by 'equivalent scatterers'. It can be seen in Figs. 2.2(c) and 2.2(d) that an 'equivalent scatterer' can be placed either at the geometric center of the voxel or at the location of the voxel’s 'center of phase'. The center of phase is that voxel point for which the equivalent scatterer's returned signal has a phase which is equivalent to that of the signal obtained by summing the backscattered contributions from the individual particles. Our use of the term ‘center of phase’ is intended to draw attention to the fact that it is the phase of the returned signal from the voxel that is of interest, rather than simply the ‘center of mass’. The simplest voxel method places equivalent scatterers at the voxel geometric centers, while a higher order method places equivalent scatterers at the voxel centers of phase.

Figure 2.2. Illustrating the particle and voxel approaches. (a) original particle distribution; (b) subdivision into voxels; (c) equivalent scatterers placed at voxel geometric centers, voxel scattering strength assigned according to number of particles in each voxel; (d) equivalent scatterers placed at actual voxel centers of phase.
Chapter 2. Background

The voxel method has been used to model the backscattered signal for continuous wave (CW) Doppler ultrasound systems, and the maximum error between the voxel and particle methods was evaluated analytically to be less than 5% for a voxel size of $\lambda/20$ (Mo and Cobbold, 1992). However, when wideband transmit signals are used, the errors incurred are more difficult to evaluate analytically, and have not been previously addressed.

2.1.1 Particle and voxel methods - theory

In the 'exact' particle method, the Born approximation allows the backscattered signal from a particle distribution insonated by a plane wave to be calculated from (Bascom and Cobbold, 1995)

$$x(t) \approx \sum_{i=1}^{N} \frac{\sqrt{\sigma_b} e^{j2k \cdot R_i}}{|R_i|},$$

(2.1.1)

where $N$ is the total number of particles insonated, $\sigma_b$ is the differential backscattering cross-section of a particle, $k$ is the wave vector associated with the incident, single frequency plane wave, $R_i$ is the vector position of the $i$-th particle with respect to the transducer (see Fig. 2.1) and the time dependence ($e^{j\omega t}$) is assumed and has been omitted for notational simplicity.

In the voxel method (Mo and Cobbold, 1993), all the scatterers contained in a voxel are replaced by an equivalent scatterer positioned at the geometric center (please refer to Fig. 2.2(a), 2.2(b), and 2.2(c)), and the backscattered contribution from a single voxel insonated by a plane wave can be expressed as

$$x(t) \approx \frac{\sqrt{\sigma_b} e^{j2k \cdot R}}{|R|} N_v,$$

(2.1.2)

where $R$ is the vector position of the voxel center and $N_v$ is the number of scatterers contained within it. The backscattered signal at a particular time $t$ is then just the summation of the contributions from all insonated voxels.

A higher order voxel model, which takes into consideration the arrangement of scatterers within the voxel, has been discussed by Bascom and Cobbold (1995). Using a Taylor series expansion for the exponential term in Eq. 2.1.1, they showed that a second-order approximation for the contribution from a single voxel can be expressed as
where $\Delta r_{\text{avg}}$ is the average vector position of the scatterers within the voxel with respect to the voxel center. Thus, in this model, the scatterers contained by a voxel are replaced by a single equivalent scatterer placed at the ‘center of phase’, rather than the voxel’s geometric center (compare Fig. 2.2(c) and 2.2(d)). Comparison of Eq. (2.1.3) with (2.1.2) shows that the contribution from a voxel is dependent on the phase associated with the voxel’s ‘center of phase’, rather than on the phase of its geometric center.

The above expressions apply for insonation by a single frequency plane wave. Noting that the wave vector $k$ and the associated phase shift $k \cdot r$ will be different for other frequencies, the higher order voxel approach has been extended to wideband systems (Bascom and Cobbold, 1995), resulting in

$$x(t) \approx \sqrt{\frac{\sigma_b}{|R|}} N_v \sqrt{\frac{1}{4\pi}} \cdot \frac{e^{j k \cdot R} - e^{-j k \cdot (2 \cdot \Delta r_{\text{avg}})}}{|R|},$$

(2.1.3)

where $x_r(.)$ represents the transmitted signal, $\overline{k} = k / |k|$, $c$ is the speed of sound in the tissue, and the time-shift term corresponds to the transmit-receive delay. Equation 2.1.4 can also be used for the simplest voxel approach in which an equivalent scatterer is placed at the center of the voxel ($\Delta r_{\text{avg}}=0$), as well as for the particle approach, in which case, $N_v=1$, $|R|$ is the transducer-particle separation and $\Delta r_{\text{avg}}=0$. The full details of the derivations of Eqs. 2.1.1 to 2.1.4 may be found in the reference to Bascom and Cobbold (1995).

### 2.1.2 Particle method - implementation in past simulation studies

In the particle method, it is necessary to create a tissue distribution by generating as many random non-overlapping positions as there are scatterers. In the simplest particle method algorithm, a scatterer position is generated and then checked for overlap with all other successfully placed scatterers. The newly generated position is rejected if overlap occurs, and accepted otherwise. As the scatterer concentration increases, it becomes progressively more difficult to place scatterers: since the probability of overlap between a newly generated particle position and previously placed scatterers increases, the number of attempts needed to
successfully place the particle increases. Zhang et al. (1994) have shown that the number of iterations required to create a distribution rises exponentially with scatterer concentration. At high concentrations (i.e. hematocrits of 40% or 45%), it becomes very likely that the algorithm may become "trapped" while trying to place a particular scatterer, i.e., a particular scatterer configuration will arise for which it is impossible to place the desired number of particles.

Particle method simulation studies by other investigators have made use of various scatterer placement strategies to reduce the amount of checking required to generate non-overlapping positions. These strategies involve either checking small localized regions adjacent to the particle to be placed (Zhang et al., 1994), or placing particles in a regular lattice and then adding pseudo-random displacements to the particle positions (Hunt et al., 1995). Figure 2.3 illustrates a voxel-based strategy used to generate particle positions that will be presented in Chapter 3, together with two other schemes. Besides computation time, memory overhead is also an important factor which must be minimized if possible in the simulation of large tissue volumes. The method of Zhang et al. required considerable memory overhead while the method described by Hunt et al., which assumed point scatterers (i.e. no overlap problems), would incur a much higher computational overhead if modified for particles of non-zero size.

2.2 RBC aggregation kinetics - previous models

General aspects of particle aggregation, (though not specifically concerned with RBCs) have been modeled recently by Elimelech et al. (1995), Shamlou and Titchener-Hooker (1993), and Muhle (1993). Elimelech et al. have discussed and reviewed equations that describe particle collision rates under the influence of Brownian motion and shear flow conditions. In addition, since hydrodynamic or viscous effects are important in determining whether a collision results in the formation of a new aggregates, they also presented equations that describe the efficiency of collision-based aggregation. Equations for predicting how the maximum floc (aggregate) size is limited by shear under laminar and turbulent flow conditions have been proposed by Muhle (1993).
Figure 2.3. Illustrating strategies for generating non-overlapping particle positions. (a) voxel-based method; (b) grid method used by Zhang et al. (1994): the grid square containing the center of a newly generated particle is determined and only the surrounding 24 grid squares are checked for overlapping particles; (c) randomized lattice method used by Hunt et al. (1995): point-sized particles are placed at lattice intersections and random displacements are then added.

In 1988 Murata and Secomb presented a model of RBC aggregation in shear flow. This was based on a general rate equation that describes particle collision rates and aggregate degradation rates. The general effect of shear rate on average aggregate size (the number of RBCs per aggregate) was investigated, including qualitative observations of trends in rouleau growth with time for various shear rates. A ‘sticking probability’, which serves a similar
purpose to the collision efficiency in Elimelech et al.'s work, was incorporated into the model. However, the model of Murata and Secomb is limited by the fact that the actual aggregation mechanism was not considered, nor were the collision and degradation rates made a direct function of the aggregate size and structure. The general rate equation used by Murata and Secomb has also been adapted for use in the work of Samsel and Perelson (1982, 1984) and Chen and Huang (1996).

Aspects of these previous models which were useful for the development of the shear-dependent aggregation model presented in Chapter 4 are described in greater detail below.

2.2.1 Aggregation kinetics - theory

An increase in the number of aggregates of a given size results from the collision and coalescence of smaller aggregates and the breaking down of larger aggregates. On the other hand, a decrease in number is due to collisions with other aggregates to form larger aggregates, as well as degradation into smaller aggregates. The number of aggregates formed per unit time from the collisions between aggregates of \(i\) and \(j\) RBCs in a shear flow is given by Murata and Secomb (1988) and Elimelech et al. (1995):

\[
\frac{\Delta n_{i+j}}{\Delta t} = \alpha K_{ij} n_i n_j,
\]

where \(K_{ij}\) is the rate of collisions between aggregates of \(i\) and \(j\) RBCs, \(n_i\) and \(n_j\) are the concentrations of aggregates of \(i\) and \(j\) RBCs, \(\alpha\) is a collision efficiency, and in which only two-body collisions are assumed to occur.

The collision rate of aggregates in simple laminar shear flow depends on the shear rate, and the sizes, concentrations and structures of the colliding aggregates. It is assumed that the aggregate structures can be characterized by a fractal dimension (Meakin, 1988). In addition, for the shear rates of interest in this thesis, it is assumed that collisions due to Brownian motion are negligible (Murata and Secomb, 1988; Elimelech et al., 1995). According to Elimelech et al. (1995), the collision rate \(K_{ij}\) is given by

\[
K_{ij}(t) = \frac{4G}{3} \left( a_i + a_j \right)^3 = \frac{4Ga_s^3}{3} \left( 1^{i^{d_s}} + j^{d_s} \right)^3,
\]
where \( G \) is the shear rate, \( d_\tau \) is a volume-length correlation fractal dimension, \( a_o \) is the equivalent radius of an RBC, and it has been assumed that
\[
a_i = a_o^{1/d_\tau}, \quad a_j = a_o^{1/d_\tau}
\]
(2.2.3)
are the equivalent spherical radii of a fractal aggregate consisting of \( i \) or \( j \) RBCs. The fractal dimension used here is defined as the quotient of the logarithm of the aggregate volume and the logarithm of the aggregate’s characteristic length (usually taken to be the aggregate’s longest dimension). For a given size, the characteristic length of an aggregate increases as the fractal dimension is decreased. An aggregate with a fractal dimension of 3 is spherical in shape and more compact than an aggregate containing the same number of RBCs with a fractal dimension of about 1, in which the individual RBCs line up in a rouleau-like structure. A less compact, less spherical aggregate \((d_\tau < 3)\) presents a larger cross-sectional area than an aggregate of the same size but higher fractal dimension, which consequently increases the rate of collisions. It should be emphasized here that spherical particles are assumed.

Because of misalignment of RBCs, repulsive surface charges, hydrodynamic resistance between particles, and insufficient number of macromolecule bridges formed, not all collisions result in aggregation (Chien, 1981; Fabry, 1987; Skalak and Zhu, 1990). These factors can be incorporated into aggregation models by multiplying the collision rate by a ‘sticking probability’ or ‘collision efficiency’. Murata and Secomb (1988) hypothesized that aggregates fail to stick together when they are unable to remain in close proximity for at least some minimum period of time such as that required for macromolecular bridging to occur. Since increasing the shear rate reduces the amount of time aggregates spend in proximity to each other, they proposed that the sticking probability was inversely proportional to shear rate. Elimelech et al. (1995) presented a collision efficiency equation based on hydrodynamic interactions, in which it becomes increasingly difficult to force liquid out from between particles as they approach each other closely. The fractal form of their collision efficiency can be written as
\[
\alpha = k \left( \frac{A}{36\pi \mu G a_o^3 i^{3/d_\tau}} \right)^{0.18}, \quad 0<\alpha \leq 1
\]
(2.2.4)
where \( k \) is a particle size-dependent scaling factor, \( A \) is the Hamaker constant (~\( 10^{-20} \) J for aqueous suspensions, as suggested by Elimelech et al.), and \( \mu \) is the fluid viscosity. This
equation describes the probability of sticking for two equally sized aggregates of similar structure (same fractal dimension) consisting of \( i \) primary particles. When all collisions are successful it has a maximum value of one. It should be noted that both the sticking probability of Murata and Secomb's model and the collision efficiency presented by Elimelech et al. are inversely dependent on the shear rate.

Both the collision efficiency and the aggregation adhesive strength depend on the number of macromolecule bridges formed, which in turn, depends primarily on the macromolecule concentration and weight (size). The effect of macromolecule concentration and size has been extensively studied, particularly in Chien's experimental work with dextran macromolecules (Chien, 1975), and these suggest that aggregation models can account for the concentration dependence by introducing another multiplicative scaling factor in Eq. (2.2.4).

Since aggregate breakage occurs when shear forces are greater than the bonding forces, it can be assumed that the maximum aggregate size is limited by the shear rate (Snabre, 1987). Muhle (1993) has proposed that the hydrodynamic force exerted on an aggregate containing \( i \) RBCs is proportional to the aggregate size, the primary particle (i.e., RBC) size, the viscosity and shear rate. The fractal form of his expression for the force is

\[
F_H = 4k' a_i a_o \mu G = 4k' i^{1/d_F} a_o^2 \mu G ,
\]

where \( k' \) is a scaling constant and use has been made of Eq. (2.2.3).

2.3 RBC aggregation - packing statistics and concentration variance

It is well established that the backscattered ultrasound power from a distribution of Rayleigh scatterers (i.e. the scatterers are much smaller than the wavelength of the insonating ultrasound) is directly dependent on the variance of the number of scatterers within an elemental volume or voxel, rather than on the scatterer concentration itself (Mo and Cobbold, 1992, 1993). This dependence of backscattered power on concentration variance enables us to understand and qualitatively explain why the backscattered power has been observed to initially increase with hematocrit, reach a peak in the range of 15-20% and decrease with hematocrit thereafter (Shung et al., 1984; Mo et al., 1994). There are very few scatterers at
low hematocrit, and consequently local variations in concentration would be small. Scatterers are tightly packed at high hematocrits, and, while the scatterer concentration may be high, the variations in concentration would be small because of the close packing. At hematocrits intermediate to these extremes, there is far more freedom for various packing arrangements to arise, which results in increased local concentration variances and higher backscattered power. The exact arrangement of particles in a system can be dependent on the size and shape of the aggregates formed, as well as the physical processes of aggregate formation and dissociation (Kolb and Jullien, 1984; Mills, 1985; Meakin, 1988).

### 2.3.1 A fractal packing model

Various attempts have been made to explain the relationship between the backscattered ultrasonic power from blood and the manner in which RBCs are packed (Twersky, 1978; Lucas and Twersky, 1987; Bascom and Cobbold, 1995). In the model presented by Bascom and Cobbold (1995), it was proposed that a fractional packing factor may be used to represent local fluctuations in the scatterer concentration. In their work, the backscattering coefficient (the power backscattered per steradian from a unit volume of scatterers for an incident plane wave of unit amplitude) was modeled by

\[
BSC = \sigma_m \frac{h}{\nu_p^m} \frac{(1 - h)^{m+1}}{(1 + h[m - 1])^{m-1}},
\]

where \(\sigma_m\) is the differential backscattering cross section for a symmetric \(m\)-dimensional scatterer, \(\nu_p\) is the scatterer volume, and \(h\) is the hematocrit. The parameter \(m\), which is referred to as the packing dimension, is related to the physical dimension of the scatterer and can be considered as a measure of symmetry in the way that a scatterer is packed or correlated with other scatterers. A geometric interpretation of \(m\) would visually represent a scatterer with \(m=1\) as an infinite plane, a scatterer with \(m=2\) as a cylinder, and with \(m=3\) as a sphere. However, the parameter \(m\) may also take on non-integer, fractional or fractal, values to represent scatterers with shapes and symmetries somewhere in between those of integer dimensions. The packing arrangements of RBCs in blood are affected by such factors as flow conditions and aggregation, and thus, this model gives insight into the results of experimental backscattering studies by Mo et al. (1994) and Shung et al. (1984). As the fractal packing
dimension is increased from 1 to 3 (i.e. as the degree of packing symmetry is increased), the peak for the plotted curve of BSC as a function of hematocrit decreases in magnitude and shifts to lower hematocrits. Various flow conditions, such as turbulence, and RBC aggregation can cause local changes in the scatterer density, and can be represented by changes in the packing dimension $m$, and therefore, in the backscattered power.

2.3.2 Simulation approaches

Using the collision rate, collision efficiency and shear force equations in Section 2.2, simulations can be carried out to track the change in concentration of aggregates of different sizes over time. However, the exact spatial arrangement of particles, and hence, the concentration variance, cannot be obtained easily from these simulations. Molecular dynamics (MD) and Monte Carlo (MC) type simulations have been used by other researchers to model spatial distributions of particles and study the packing statistics of these distributions in liquid and gas simulations (Allen and Tildesley, 1987; Chen and Doi, 1989; Dickinson and Euston, 1992; Elimelech et al., 1995).

Monte Carlo type simulations take a stochastic approach to modeling particle distributions. Basically, MC simulations sample the ensemble of possible particle spatial distributions in order to obtain the most statistically likely distribution. The physical laws governing the particle interactions limit the possible arrangements, and various strategies may be employed to efficiently sample the most likely distributions. Such simulations make extensive use of random numbers in their ensemble sampling procedures: beginning with some initial particle arrangement, each particle is selected either sequentially or randomly and perturbed by some random spatial displacement. After a particle is perturbed, some measure of energy of the system is calculated, based on the particle interaction laws. If a particle perturbation decreases the system energy, the move is accepted and the particle position is updated. Otherwise, the perturbation is accepted with some probability which is dependent on the magnitude of the energy increase. A single MC cycle is completed when every particle has been perturbed, although not necessarily when every perturbation has been accepted. Generally, many MC cycles are required to allow the system to "evolve" and to ensure that an equilibrium system state has been reached (Elimelech et al. suggest that on the order of $10^5$-$10^6$ cycles are needed).
Molecular dynamics type simulations are deterministic in nature, since the particle distribution is obtained by solving a set of Newton's equations of motion for each particle. Thus, the MD method lends itself to the simulation of time-dependent processes. Starting with particle positions and velocities at time $t$, the particle positions are calculated for some later time $t + \Delta t$. When a collision of particles occurs, the changes in particle velocities are calculated using conservation of energy and momentum equations. By repeating this step-by-step procedure, the arrangement of particles in the distribution can be determined at any time.

A major difficulty in the implementation of these simulation methods, especially with the MD method, is that the physical properties of the particles, their motion and their interactions must be known quite precisely. As well, both of these methods can be very computationally intensive, since either all particles must be perturbed in a single simulation cycle (the MC method) or all particle pair interactions must be calculated (the MD method).

2.4 Past ultrasound studies of blood flow and aggregation

There are currently no direct methods of quantitatively measuring aggregate size distributions at high hematocrits (Chien et al., 1970; Rampling and Whittingstall, 1986; Chen et al., 1995). Shehada et al. (1994) conducted their study by measuring the backscattered ultrasound power from blood flow, which is an indirect measure of aggregate size and RBC packing. In the B-mode ultrasound imaging studies of Yuan and Shung (1989), Mo et al. (1991) and Shehada et al. (1994), a ‘black hole’ was observed to form downstream of the tube entrance, and this consisted of a hypoechoic region in the center of the tube surrounded by a hyperechoic region. It was hypothesized that the ‘black hole’ effect can be understood in terms of shear and time-dependent RBC aggregation. At any point downstream of the tube entrance, the blood flow may be specified by some velocity profile and corresponding shear rate profile across the tube cross-section. The backscattered ultrasound power can therefore be plotted as a function of velocity or shear rate. Figure 2.4 shows a typical plot of the backscattered ultrasound power (echogenicity) versus shear rate as reported by Shehada et al. (1994).

It can be observed from Fig. 2.4 that the backscattered ultrasound power is low for very low shear rates, increases to a peak at moderately low shear rates, and then decreases for high shear rates. Figure 2.4 can be compared to Fig. 2.5, which displays the experimental
results of Copley et al. (1976), as obtained with the ESR technique, another indirect measure of aggregate size. The difference in flow conditions, fibrinogen content and other factors may account for the different shear rates at which the peaks occur, nonetheless the qualitative agreement between the two sets of results is still good. If the backscattered ultrasound power is assumed to be dependent on the degree of aggregation as a first approximation, we can hypothesize from the results in Figures 2.4 and 2.5 that very little aggregation occurs at very low shear rates and high shear rates, but peaks at moderate shear rates. It is expected that very little aggregation should occur at high shear rates, since the shear forces should be sufficient to overcome the macromolecular bridges between RBC surfaces. At low shear rates, cells would move very little with respect to each other, and therefore, few RBCs would be adequately aligned for aggregation to occur. At moderate shear rates then, it is likely that aggregation is enhanced because RBC mixing is increased while the shear forces are insufficient to cause significant disaggregation. The distinct aggregation peak at moderate shear rates which is evident in all of these results was also hypothesized by Chien (1976) to be the result of increased mixing.

Shehada et al. proposed that the hypoechoic 'black hole' corresponded to regions of low shear and minimal aggregation, while the surrounding hyperechoic ring was believed to correspond to the range of aggregating-enhancing shear rates. The regions surrounding the hyperechoic ring extending up to the tube walls display low backscattered power, and are believed to correspond to disaggregating shear rates. These hypotheses are tested in this thesis, with a comparison between the 'black hole' experiment results and the shear-dependent aggregation simulation results presented in Chapter 4.
Fig. 2.4. Experimental results from Shehada et al. (1994), showing the backscattered ultrasound power (echogenicity) versus shear rate for mean flow velocities of 1.3, 2.4, 5.3 cm/s, Hct=28, at 60D from the entrance of a tube with a diameter of 2.54 cm.

Fig. 2.5. Experimental results reported by Copley et al. (1976) for fresh human blood as measured using the erythrocyte sedimentation rate (ESR) versus shear rate.
Chapter 3
Accuracy of the Voxel Method for Pulsed Excitation

In this chapter, the implementation of the particle and voxel technique for 1-D and 2-D simulations of backscattered ultrasound for wideband Gaussian-shaped transmit signals will be described, and the accuracy of the voxel technique with respect to the particle method will be examined. The effect of hematocrit and voxel size on the range of errors, together with the accuracy of a higher order voxel model, are also discussed.

3.1 Implementation of the particle and voxel methods: assumptions and methodology

In our study, distributions of stationary scatterers were insonated at normal incidence by plane waves to produce simple A-line backscattered signals. The following assumptions and approximations were used:

1. The separation between transducer and tissue was assumed to be sufficiently large relative to the sample volume size so that the incident wave can be considered to be plane and the receiver can be regarded as a point.
2. The effects of attenuation and frequency dependent scattering were ignored.
3. The particles are weak scatterers, so that multiple scattering effects could be ignored.
4. The scatterer distribution is spatially invariant.
5. The particle concentration (i.e. the number of particles in an arbitrary small volume) is Gaussian distributed (see discussion below in 3.1.1. Creation of scatterer distributions).
Chapter 3. Validation of the voxel method for pulsed imaging

6. The particles are identical (monodisperse) and non-deformable.
7. The particles are sufficiently small so that Rayleigh scattering could be assumed.

As presented in Chapter 2, Eq. 2.1.4 represents the backscattered signal obtained with the higher order voxel approach for wideband systems (Bascom and Cobbold, 1995),

\[ x(t) \approx \sqrt{\sigma_b} N_r \frac{x_r}{|R|} \left[ t - 2 \frac{k \cdot (R + \Delta r_{avg})}{c} \right], \tag{2.1.4} \]

where \( x_r(.) \) represents the transmitted signal, \( \overline{k} = k / |k| \), \( c \) is the speed of sound in the tissue, and the time-shift term corresponds to the transmit-receive delay. For all the simulations presented in this chapter, Equation 2.1.4 was used to implement the simplest voxel approach (\( \Delta r_{avg}=0 \), equivalent scatterer placed at the voxel center), as well as the particle approach (\( N_r=1 \), \( |R| \)=transducer-particle distance, \( \Delta r_{avg}=0 \)). The total backscattered signal was calculated by summing the contributions from each element.

Both 2-D and 1-D simulations were performed using the particle and the voxel approaches. In the particle method 1-D simulations, the scatterers were represented by a row of infinite slabs whose thickness (2.0 \( \mu \)m) corresponded approximately to the thickness of an RBC. These slabs were imbedded in plasma, such that the thickness of the plasma layers were exponentially distributed and the mean was inversely related to the hematocrit. These assumptions are the same as those used by Routh et al. (1987) and subsequently, by Mo et al. (1994). For the 2-D particle simulations, the scatterers were assumed to be infinite cylinders with radii of 2.8 \( \mu \)m, which corresponds to the radius of a sphere whose volume is the same as an RBC.

The transmitted ultrasound pulse was assumed to be a symmetrical Gaussian-shaped sinusoid with center frequency of 5 MHz, and a 6 dB bandwidth of 2 MHz, which correspond to a duration of about 1 \( \mu \)s. This is a fairly realistic wideband pulse which has been used in previous studies (Zhang et al., 1994; Hunt et al., 1995). Bandwidths of 4 and 5 MHz were also used for some simulations (e.g. see Fig. 3.3). For the 2-D simulations, the incident beam was assumed to be uniform over the sample volume width of 6\( \lambda \) (1.8 mm). Also, the sample volume was assumed to have a uniform response along its length. The length was adjusted by keeping the transmit pulse length fixed and varying the receive window. Although a
variety of sample volume lengths have been investigated, the results for 10\(\lambda\) (3 mm) will be emphasized. Two steps were involved in the simulations: the creation of a distribution of scatterers (either particles or voxels), and the calculation of the backscattered signal. Both of these will now be described.

### 3.1.1 Creation of scatterer distributions

For the particle approach, the primary problem is to create a distribution of non-overlapping particle positions in a reasonably efficient manner. A good way of doing this is to divide the sample size \(V\) into voxels of equal size \((\Delta V)\), then determine the number of particles in each voxel, and finally, determine the position of each particle such that overlap is avoided. The voxel size should be sufficiently large so that each voxel contains on the average several particles, yet it must be small enough so that the computational burden incurred from having to repeat the placement of particles in non-overlapping positions does not become excessive. This process is described below for the 2-D simulations.

For a hematocrit \(H\), the average number of scatterers per voxel is given by

\[
N_v = H\Delta V/V_p,
\]

where \(V_p\) is the particle area. Furthermore, based on the Percus-Yevick packing theory for 2-D Rayleigh scatterers (dimension\(<\lambda\)) it has been previously established (Twersky, 1978) that the 'local' particle density has a variance that is proportional to

\[
(1 - H)^3 [1 + H]\]

This is the same expression as that presented by Bascom and Cobbold (1995) for a packing dimension \(m\) of 2. By using the above equations, both the average number of scatterers per voxel and the variance can be calculated for a given hematocrit. By assuming that the number of particles in each voxel is Gaussian distributed, a random number of particles (representing the voxel scattering strength) was generated for each voxel within the sample 'volume' by using a standard algorithm (Press et al., 1988). A distribution of non-overlapping particles was then created by converting the voxel distribution one voxel at a time, using the procedure described next.

The scattering strength assigned to a given voxel was taken to be the desired number of non-overlapping positions to be generated. The particle positions were assumed to be uniformly distributed throughout the voxel. Since the particles are of finite size and each particle position corresponds to the particle center, each newly generated position must be
checked for overlap with particles already placed in the same voxel, as well as for overlap with particles in adjacent voxels. If overlap occurred, the newly generated position was rejected and another position was generated. This procedure was repeated until either the particle was successfully placed within the voxel or a maximum number of attempts to place the particle had been made. If the number of attempts to place a given particle exceeded some allowable maximum, then fewer particles than required had been successfully placed. In this event, the placement process for this voxel was repeated. If the number of repeats for a particular voxel reached some maximum value, then the last generated distribution was accepted and the scattering strength for that voxel was changed to the number of particles successfully placed. The maximum number of particle-placement attempts (i.e. the number of attempts to place a given particle) used in the simulations was 150, and the maximum number of voxel-filling attempts (i.e. the number of times the entire process of generating and checking particle positions was attempted) used was 75. Both of these parameters were determined by trial and error, by varying them one at a time and checking whether the algorithm was successful in placing the correct number of particles for a range of scatterer concentrations and voxel sizes.

At high concentrations (i.e. hematocrits of 40% or 45%), it becomes very likely that the algorithm may become “trapped” while trying to place a particular scatterer, i.e., a particular scatterer configuration will arise for which it is impossible to place the desired number of particles. By placing an upper limit on the number of attempts to place any one scatterer and repeating the entire placement process when the upper limit is reached, the algorithm is able to avoid becoming “trapped” in an undesirable scatterer configuration, and the likelihood of successfully placing all the particles in the voxel is increased.

This voxel-based method for generating a random distribution of scatterers is quite efficient because it is quicker to generate non-overlapping particle positions over small localized regions than over large regions - a newly generated particle needs to be checked for overlap only with particles in the same voxel and in immediately adjacent voxels, rather than with all generated particle positions in the scatterer distribution. As mentioned in Chapter 2, particle method simulation studies by other investigators have also made use of various scatterer placement strategies to reduce the amount of checking required to generate non-overlapping positions. The voxel-based method described above and the strategies of Zhang
et al. (1994) and Hunt et al. (1995) are shown in Figure 2.3. The main advantage of the voxel-based method is that it enables us to specify the local scatterer concentration variance, which is perhaps the most important factor to be considered in studying the backscattered signal from blood (Mo and Cobbold, 1992). The drawbacks of the methods of Zhang et al. and Hunt et al. have been pointed out in Chapter 2.

3.1.2 Calculating the backscattered signal

For both the particle and the voxel approaches, the first step is to compute the transmit-receive time for each particle or voxel. The backscattered RF signal from the entire sample volume was then obtained by using Eqn. (2.1.4) for each scattering element and summing together the contributions from all elements that are received at the same instant of time.

Voxel sizes of $\lambda/10$ and $\lambda/20 (\lambda=300 \mu m)$ and various hematocrits between 10 and 45% were used. Approximately 250 to 300 realisations for each voxel size-hematocrit pairing were performed for the 2-D simulations. This choice was made by determining the range of realisations over which fluctuations in the RF signal power variance stabilized to less than 10% of the average value for that range.

3.1.3 Comparison measure

It is well-established that the backscattered RF signal from a random distribution of scatterers with a high number density approximates a Gaussian process and that the envelope values are Rayleigh distributed (Wagner et al., 1986). Histograms of the simulation results were calculated to confirm these findings. The signal envelopes were calculated by subtracting the means from the signals and then forming the complex analytic signal with the use of the Hilbert transform.

The accuracy of the voxel method was determined by comparing the RF signal with that obtained using the particle method. Specifically, we used the mean squared error ($MSE$), defined by

$$MSE = \frac{\sum_{i=1}^{N_x} (x_i - y_i)^2}{\sum_{i=1}^{N_x} x_i^2},$$

(3.1)

where $x$ and $y$ are the RF signals obtained by the particle and voxel methods, respectively, $N_x$ is the number of signal samples, and $x_i$ is the $i$-th sample of the particle method signal. The
MSE is a measure of the fractional difference between the RF signal sample obtained with the voxel method and its corresponding particle method sample, averaged over all the samples for that data set. Values of MSE which approach zero indicate that two data sets are more closely identical. Because the MSE does not have an upper bound, it is technically not a measure of the average percentage error between the voxel method data and the "real" particle method data, although it may intuitively be thought of in this way.

The MSE's between the two RF signals was used because this is a more rigorous measure of the discrepancy than that obtained by using the MSE's between either the signal envelopes or the signal powers. Any discrepancy between RF signals would be a direct result of replacing particles by equivalent scatterers at the geometric centers of the voxels. On the other hand, because of the averaging nature of envelope data and signal power, discrepancy information may be masked.

3.1.4 Higher order voxel method

The backscattered signal depends on the variations in voxel scatterer concentration and on the particular arrangement of scatterers in each voxel. In the simplest voxel method the effect of scatterer arrangement is ignored, and all scatterers are assumed to be concentrated at the voxel center. For the higher order voxel method, a first order account is taken of the scatterer distribution by assuming that all scatterers are located at the center of phase. Figure 2.2(c) shows voxels of varying scattering strength, with all scatterers placed at the voxel centers (i.e. the lower order voxel method). Figure 2.2(d) also shows voxels of varying strength but with the scatterers displaced from the voxel centers (i.e. the higher order voxel method). It is clear that the backscattered signals produced by (c) and (d) will differ. The center of phase position ($\Delta r_{avg}$) depends on both the hematocrit and the voxel size. In order to study these dependencies particle distributions were created and the centers of mass were calculated.
3.2 Results and comparison of methods\textsuperscript{1}

3.2.1 Code validation

The code for generating voxel distributions was tested by examining the actual variances of the number of particles per voxel of the generated distributions, plotting them as a function of hematocrit, and then comparing them with those based on the Percus-Yevick theory. For both 1- and 2-D simulations, good agreement was obtained for voxel sizes of $\lambda/5$, $\lambda/10$ and $\lambda/20$ in the higher hematocrit range. When the average number of scatterers per voxel is small (low hematocrits) the distribution of scatterers can no longer be approximated as Gaussian, and, as expected, only fair agreement was obtained. As the voxel size is increased for a particular hematocrit, the average number of scatterers per voxel is increased and the agreement improves. The scheme used for generating particle positions was tested by checking that the correct number of scatterers had been placed in non-overlapping positions.

To validate the backscattered signal simulation code a number of test cases were used. In the simplest, small numbers of scatterers were placed at known intervals. Well-separated scatterers were easily resolvable, while the backscattered contributions from scatterers separated by less than half a pulse length overlapped with each other and did not produce easily resolvable signals.

3.2.2 Higher order voxel method

Figure 3.1 shows the center of phase displacements for various hematocrits with the assumption that the scatterer positions are uniformly distributed over the voxel (i.e. a homogeneous tissue sample is represented). It should be noted that for a particular voxel size, the distribution becomes narrower with increasing hematocrit. This is expected since, for a voxel containing a larger number of particles, it becomes more likely that a scatterer on one side of the voxel's geometric center will be "neutralized" by a scatterer located at an equal distance from the other side. Consequently, at high hematocrits the center of phase is more likely to be near the geometric center. In general, the assumption of an isotropic distribution may not be valid. For example, blood flow in the presence of RBC aggregation

\textsuperscript{1} The results of these simulations have also been presented elsewhere (Lim et al., 1996), and are reprinted with permission from Elsevier Science.
is likely to result in anisotropy, in which case *a priori* knowledge about flow conditions and the arrangement of RBC's would be needed to accurately model the distribution of the center of phase displacement.

![Figure 3.1. Distribution of the center of phase of a λ/20 voxel for three different hematocrits.](image)

To exactly implement the higher order voxel method for a single simulation realisation, it is necessary to calculate $\Delta r_{\text{avg}}$ for each voxel. This requires that the actual particle positions be known. Consequently, for generating a scatterer distribution, there is no computational reduction over the particle method, although there will be a small saving in calculating the backscattered signal. A much more efficient approach would be to estimate the standard deviation of the center of phase (the mean will be zero), and then to assign a $\Delta r_{\text{avg}}$ to each voxel based on a Gaussian distribution function. However, without *a priori* knowledge of the actual arrangement of particles, the best average performance for a single realisation of the voxel method would be obtained by placing the equivalent scatterer at the voxel geometric centers (setting $\Delta r_{\text{avg}}=0$), i.e., by using the simpler voxel method.
3.2.3 Sample volume length and hematocrit

For all the simulations, the region containing scatterers was assumed to be surrounded by a medium with no scatterers. Consequently, the received RF signal will also contain components that arise from the near and far-wall boundaries. Figure 3.2 shows a typical simulated received signal from a region of length $15\lambda$, together with the Gaussian-shaped transmitted waveform (inset). The large boundary signals are clearly evident, while the

![Figure 3.2](image.png)

Figure 3.2. Typical RF signal obtained from 2D simulations, showing large boundary signals due to the transmit pulse entering and exiting the sample volume. The reduced amplitude in the middle section is due to significant destructive interference when the pulse is entirely within the sample volume. The inset displays the Gaussian-shaped transmitted pulse with a center frequency of 5 MHz and a 2 MHz, -6 dB bandwidth.

smaller amplitude central portion is due to significant destructive interference among backscattered contributions when the pulse has completely entered the scattering region. Evidently, if the scatterer region is shorter than the transmit pulse, the resulting signal consists entirely of boundary signals. To avoid the boundary artifacts the scatterer region was made sufficiently long compared to the sample volume length, i.e. the gated portion of the received signal used for MSE calculations.
Chapter 3. Validation of the voxel method for pulsed imaging

It is important not to use a scatterer region length that significantly exceeds the sample volume length, since this would increase the computation time required to place the scatterers. To determine the best value, we used a 1-D model to study how the boundary signal influenced the $MSE$ for several different sample volumes, using a voxel size of $\lambda/20$. The length of each sample volume was always chosen to be exactly equal to the scatterer region length, for reasons of computational efficiency. Furthermore, the received signal was gated from the time of arrival of the leading edge at the scatterers, to the time at which the leading edge exited from the scatterers, i.e. the leading edge boundary signal was included. Figure 3.3(a) shows that when the sample volume length was less than the effective pulse length (defined as the length for which the pulse envelope amplitudes is $>1\%$ of the peak amplitude) of about $4\lambda$, the $MSE$ decreased as the hematocrit was increased. For a critical sample volume length of $7.5\lambda$, which is approximately twice the effective pulse length, it was found that the $MSE$ was roughly constant with hematocrit. For sample volume lengths greater than this, the $MSE$ was found to increase with hematocrit. However, for a fixed hematocrit, the $MSE$ did not vary much for sample volumes longer than the critical length. Thus, we decided to choose as our ‘standard’ condition a sample volume length of $10\lambda$, a scatterer region length of $10\lambda$, and to sample the received signal 1 pulse length ($4.0\lambda$) from the start (i.e. the leading edge boundary signal was not included). The leading edge boundary signal was not included from this point on because we were primarily interested in sample volumes surrounded by tissue having the same average acoustic properties.

It was found that when a single or small number of voxels was used, very little destructive interference between backscatter contributions occurred, and edge effects dominated. As the hematocrit increased, the centers of phase of the few voxels which contributed most to the signal more closely approached the voxel geometric centers, and the error between voxel and particle approaches decreased. For sample volume lengths much longer than the pulse, destructive interference among backscatter contributions becomes important.
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Figure 3.3. Mean Square Error (MSE) between particle and voxel method realisations: (a) 1-D simulations plotted as a function of hematocrit for a voxel size of $\lambda/20$ and varying sample volume lengths. (b) 1-D simulations plotted as a function of hematocrit for a voxel size of $\lambda/20$ and sample volume length of $15\lambda$ for varying pulse bandwidths. The voxel size and sample volume length are calculated for a center frequency of 5.0 MHz. Each point is the average of 300 realisations, and has the standard error shown.
Since destructive interference arises from the phase differences between scatterers, it is expected that a wideband ultrasound pulse will accentuate these differences in the case of the voxel approach, which assumes that the phase of an incident ultrasound wave is essentially the same over all the particles within it. For instance, a $\lambda/20$ voxel at the center frequency of 5 MHz corresponds to a $\lambda/14$ voxel at 7 MHz and a $\lambda/33$ voxel at 3 MHz. Thus, significant phase differences between particles and the voxel center can occur for frequency components higher than the center frequency (i.e. smaller wavelengths). As the hematocrit is increased, more backscatter contributions with phase errors will be summed and, as a result, for sample volume lengths greater than the critical value, the $MSE$ should increase, as shown in Fig. 3.3(a).

The influence of phase differences was examined by investigating the effect of increasing the transmitted pulse bandwidth for a fixed voxel size and a long sample volume length ($15\lambda$). The results of Fig. 3.3(b) demonstrate that for a given hematocrit the $MSE$ increased when the bandwidth was increased from 2 MHz to 5 MHz.

### 3.2.4 Voxel size and hematocrit

For sample volumes longer than the critical length, the $MSE$ trend as a function of hematocrit was found to be independent of length. Consequently, 2-D results for a sample volume length of $10\lambda$ are presented in this section for a 2 MHz bandwidth pulse.

The $MSE$ can be expected to decrease with decreasing voxel size. This is because the number of scatterers within each voxel decreases, i.e., the voxel approach more closely resembles the particle approach. Shown in Fig. 3.4 are typical simulated RF signals (single realizations) produced by the particle and voxel approaches for a 30% hematocrit and voxel sizes of $\lambda/20$ and $\lambda/10$. Qualitatively, these clearly show that the performance of the voxel approach improves as the voxel size is decreased. The $MSE$ values obtained for $\lambda/10$ and $\lambda/20$ voxel sizes are plotted as a function of hematocrit in Fig. 3.5, with each point representing the average $MSE$ of 300 realisations. This shows in a quantitative manner the reduction in $MSE$ as the voxel size is reduced.
Figure 3.4. Typical gated RF signals obtained from 2-D simulations at a 30% hematocrit, using particle and voxel methods with a voxel size of (a) λ/20; (b) λ/10.
Figure 3.5. Mean Square Error (MSE) between particle and voxel method realisations: 2-D simulations plotted as a function of hematocrit (scatterer concentration) for $\lambda/10$ and $\lambda/20$ voxel sizes. Each point is the average of 300 realisations, with standard error plotted.

### 3.2.5 Computational burden for 2-D

The number of particle positions that need to be generated and the number of positions that must be compared in order to create a distribution of a fixed size are useful measures with which to compare the computational burden of the voxel approach and various implementations of the particle method.

Since the voxel approach generates a single number for each voxel (i.e. the scattering strength) regardless of scatterer concentration and does not need to check for particle position overlap, the sum of the number of generated scatterer positions and the number of positions compared is constant and does not vary with hematocrit for a fixed sample volume size. This is shown for two voxel sizes ($\lambda/10$ and $\lambda/20$) in Fig. 3.6(a). Also displayed is the computational burden of three particle method implementations: the voxel-based method used in this work, the grid method used by Zhang et al. (1994) and a simple “exhaustive search” method. In the “exhaustive search” method, a newly generated particle position is compared to all previously placed particles, while the other two implementations attempt to
limit the number of comparisons, at the cost of extra memory storage overhead. For all three particle method implementations, the computational cost of successfully placing a particle increases exponentially as the scatterer concentration is increased. As expected, the "exhaustive search" method displays the worst performance. Although the voxel-based method reduces the number of comparisons to only about the same order of magnitude as that by Zhang et al's grid method, less memory overhead is required and it provides the advantage of being able to directly specify localized variations in scatterer concentration, which is highly desirable for investigations of the relationship between tissue characteristics and the backscattered signal (cf. Chapter 5 and 6). The grid method would require modification and added memory overhead to achieve the same control over the concentration variance.

Figure 3.6(b) compares the number of computations to create a tissue distribution and to calculate the backscattered signal required by the voxel method and the voxel-based particle method. The results for two voxel sizes are shown: when a larger voxel size is used in the simulations (e.g. \( \lambda/10 \)), the calculation of the backscattered signal requires fewer computations, at the cost of decreased accuracy.

It can be observed that the voxel method reduces the computational burden of the particle method both in the generation of scatterer distributions and in the calculation of backscattered signals. The area of greatest potential time savings is in the generation of scatterer distributions, since the need to generate non-overlapping particle positions is eliminated. Depending on the level of accuracy needed, the total simulation time (distribution generation and backscattered signal calculation) can be reduced by using larger voxel sizes. But for larger voxel sizes the assumption that the phase of the insonating pulse is constant over each voxel becomes an increasingly poor approximation, and as a result the accuracy of the voxel method decreases.
Figure 3.6. (a) Number of computations required to produce a tissue distribution, plotted as a function of hematocrit for particle and voxel methods. (b) Number of computations to generate tissue distribution and to simulate backscattered signal, plotted as a function of hematocrit for particle and voxel methods. Each point is the average of 400 realisations for a $\lambda/2$ by $\lambda/2$ (5.0 MHz center frequency) sample volume, with the standard deviation shown.
3.3 Chapter summary

It has been shown that the voxel approach can be used in place of the particle method with a high degree of accuracy, while considerably reducing computation time. The accuracy of the voxel approach improves as the voxel size is decreased, while the computation time is still considerably less than that required by the exact particle method. A voxel size of \( \lambda/20 \) (5 MHz ultrasonic insonation) produces excellent results, with average realisation mean squared errors (MSE’s) in the range of 0.05 to 0.15, while reducing the number of required computations for 2-D simulations by up to a factor of about 100 at physiologically relevant hematocrits.

The voxel approach requires considerably fewer computations than the exact particle approach in both generating tissue distributions and simulating the backscattered signal. At physiologically relevant hematocrits (40-50%), the bulk of the computational burden for the exact particle method is devoted to generating non-overlapping particle positions, whereas the number of computations required to generate a tissue distribution is independent of scatterer concentration for the voxel approach.

For 3-D simulations then, it can be inferred from the 2-D simulations that the voxel approach should be able to reduce appreciably the total computation time required to generate tissue distributions and calculate the backscattered signal. Figure 3.7 compares the estimated average number of computations required per 3-D voxel as a function of hematocrit with the 2-D voxel-based method results. The number of computations was estimated by summing together the number of generated particle positions (whether accepted or rejected), the number of comparisons between particle positions, and the number of calculated backscattered signal contributions (equal to the number of successfully placed particles) for a single 3-D voxel. Further details are given in the caption for Fig. 3.7. Since the number of computations in 2-D was reduced by a factor of 100 and the results in Fig. 3.7 show that the average number of computations per 2-D voxel is about 10 times less than for 3-D at most hematocrits, it is estimated that the voxel approach might be able to reduce computation by at least 1000 times at high scatterer concentrations, thus making simulations with realistic tissue volumes tractable.
Figure 3.7. Average number of computations required per voxel to generate a scatterer distribution for 2-D and 3-D voxels. Each point for 3-D is the average of generating 500 single voxels of size $\lambda/20$, with standard deviation shown. The 2-D results are the average per voxel results from Fig. 8.
Chapter 4
Simulating RBC Aggregation in a Shear Flow

In this chapter, a model of RBC aggregation in shear flow is described, and the results of computer simulations based on this model are presented and compared to published experimental results.

4.1 Description of theoretical model

The aggregation equations presented in Chapter 2, with slight modifications, provide the basis for the model to be presented. The assumptions and hypotheses incorporated into the model are also summarized.

4.1.1 Model equations

The number of aggregates consisting of \( i+j \) RBCs formed per unit time from collisions between aggregates of \( i \) and \( j \) RBCs in a shear flow is given by

\[
\frac{\Delta n_{i+j}}{\Delta t} = \alpha K_{ij} n_i n_j,
\]

(2.2.1)

where the concentrations of aggregates of \( i \) and \( j \) RBCs are denoted by \( n_i \) and \( n_j \), and \( \alpha \) is a collision efficiency for two-body collisions. In addition, \( K_{ij} \) is a collision rate given by
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\[ K_\dot{g}(t) = \frac{4G}{3} \left[ a_i + a_j \right]^{\prime} = \frac{4Ga_o^3}{3} \left[ i^{t/d_F} + j^{t/d_F} \right]^{\prime} , \]  

where \( G \) is the shear rate, \( d_F \) is the volume-length correlation fractal dimension, \( a_o \) is the equivalent radius of an RBC, and the equivalent spherical radii of a fractal aggregate consisting of \( i \) or \( j \) RBCs is given by

\[ a_i = a_o i^{1/d_F} , \quad a_j = a_o j^{1/d_F} . \]

It should be noted that, in general, the collision kernel \( K_\dot{g} \) is time-dependent. However, this time dependence has not been incorporated into the simulations described, although it should not be ignored in future aggregation models.

As a simplifying assumption in our first-order model, we have assumed that RBCs can be represented by spheres, and consequently, rouleau structures cannot be exactly simulated without further modifying this fractal formula. A rouleau-like structure, in which spherical particles line up behind each other, may still be simulated with a fractal dimension \( d_F \) which is close to 1. Since the types of aggregate structures formed may depend on shear rate and size (the number of particles), the fractal dimension \( d_F \) may also be expressed as a function of these parameters. In this work, this shear rate and size dependence was not modeled, and it is to be understood that \( d_F \) in Eqs. (2.2.2) and (2.2.3) represents an average fractal dimension. The fractal dimension may also be a function of the RBC binding forces, which depends on the adhesion surfaces between RBCs. For example, the adhesive forces are strongest when the broad RBC surfaces are aligned with each other, providing the maximum surface area for bonding. Since we have modeled RBCs as spheres, it is hypothesized that the binding forces between RBCs are essentially equal in strength. In our model then, aggregate shape and structure (as characterized by the fractal dimension) are taken to be independent of adhesive strength.

The 'sticking probability' or 'collision efficiency' \( \alpha \) in Eq. (2.2.1) is inversely dependent on the shear rate and aggregate size:

\[ \alpha = k \left( \frac{A}{36\pi\mu G a_o^3 t^{3/d_F}} \right)^{0.18} , \quad 0 < \alpha \leq 1 \]

where \( k \) is a particle size-dependent scaling factor, \( A \) is the Hamaker constant (~10\(^{-20}\) J for aqueous suspensions, as suggested by Elimelech et al., 1995), and \( \mu \) is the fluid viscosity.
This collision efficiency essentially describes the ratio of the interparticle attractive forces due to van der Waals forces to the force of the hydrodynamic interactions. Murata and Secomb (1988) reasoned that the sticking probability was inversely dependent on the shear rate because two aggregates have to be within close proximity of each other for a sufficiently long time in order for stable macromolecular bridging to occur: as the shear rate increases, the interaction time is reduced and the probability of sticking decreases. Elimelech et al. (1995) explained the collision efficiency's inverse dependence on aggregate size in terms of hydrodynamic interaction: it becomes increasingly difficult to force the liquid out from between two particles approaching each other as the size of the particles increase. This equation describes the probability of sticking for two equally sized aggregates of similar structure (same fractal dimension) consisting of $i$ primary particles. When all collisions are successful it has a maximum value of one. For collisions of unequally sized aggregates, the collision efficiencies can apparently become very low (Elimelech et al., 1995), so Eq. (2.2.4) represents an upper limit on the sticking probability of collisions involving aggregates of all sizes when used to calculate the collision of two single RBCs ($i=1$).

The fractal form of Muhle's (1993) expression for the shear force exerted on an aggregate of $i$ RBCs is given by

$$F_H = 4k' a_i a_o \mu G = 4k' i^{1/d_F} a_0^2 a_i \mu G,$$

where $k'$ is a scaling constant and use has been made of Eq. (2.2.3) to obtain the second form. The hydrodynamic force on the surface of an aggregate changes as the aggregate rotates in a shear flow, and may be incorporated into $k'$, but this was not modeled in our work. It follows from Eq. (2.2.5) that when the applied shear force is just equal to the aggregation adhesive force $F_A$, the aggregate size will be a maximum. Thus, an aggregate will contain, on the average, at the most $i_{max}$ RBCs when equilibrium has been reached and this is given by

$$i_{max} = \left( \frac{F_A}{4k' a_0^2 \mu G} \right)^{d_F},$$

where, strictly speaking, $d_F$ depends on $i_{max}$. However, in this first order model, as previously mentioned, we have assumed $d_F$ to be constant, independent of shear. It should be noted that at high shear rates the particles will all be disaggregated. If we assume that complete
disaggregation occurs at a minimum shear rate of $G_{\text{max}}$, i.e. $i_{\text{max}}=1$, then the scaling constant $k'$ can be determined provided the other parameter values (except $d_p$) are known.

Although the macromolecule bridging theory of aggregation has been assumed, it should be noted that the depletion-layer theory could also have been represented by our model implementation.

### 4.1.2 Summary of model assumptions and parameter ranges

The assumptions in our model and simulations are as follows:

1. only two-body collisions were considered [Eq.(2.2.1)];
2. aggregates consisting of RBCs modeled as spheres could be characterized by a fractal dimension [Eq. (2.2.3)];
3. the dependence of the fractal dimension on shear rate, aggregate size and adhesive forces was not modeled;
4. the macromolecular bridging model of aggregation was assumed;
5. the rotation of aggregates in shear flow was not modeled;
6. in both single streamline and large tube simulations, RBCs were considered to be sufficiently sheared at the tube entrance to be completely disaggregated;
7. aggregates were assumed to break down into randomly distributed sizes due to shear;
8. the shear rate within a voxel is assumed to be uniform;
9. tube flow was considered to be laminar and axisymmetric, and the velocity profile was considered to be the same at all distances downstream of the tube entrance;
10. the time required for aggregation binding to occur was assumed to be much less than the time required for a distribution of RBCs to reach an aggregated steady state;
11. the collision efficiency [Eq. (2.2.4)] between single RBCs was used for collisions between aggregates of all sizes;
12. the hematocrit (total number of RBCs per voxel) was assumed to be constant across the tube (Mo et al., 1991).

The parameter ranges used in our simulations are as follows:

1. a shear rate range of $10^{-3}$ to $10^2$ s$^{-1}$ was used;
2. a hematocrit range of 25% to 45% was used;
3. disaggregation shear stresses of 1 to 2 dynes/cm² was used;
4. a fractal dimension range of 1.7 to 2.5 was used;
5. RBC aggregates were considered to be disaggregated for shear rates higher than 10 s⁻¹;
6. a power law velocity profile with exponents that ranged from 2.4 to 3.2, and mean flow velocities in the range of 1.3 to 5.3 cm/s were used in the tube flow simulations;

### 4.2 Simulation method

The general approach to the simulation work was to trace small volumes, or voxels, of initially disaggregated RBCs travelling along shear flow streamlines, and to track aggregate growth and changes in the aggregate size distribution in these voxels over time. Figure 4.1 illustrates this approach. A voxel i is shown as it travels along a streamline at a radial distance ri from the tube axis. At time t₀, the voxel is at a distance z=z₀ from the tube entrance. At a later time t=t₀+Δt, the voxel is at a distance z=z₀+Δt*v(ri). With the use of a conventional 4th order Runge-Kutta algorithm (Press et al., 1988), the number of new aggregates formed during each increment in time was calculated by using the collision rate and sticking probability relations given by Eqs. (2.2.1), (2.2.2) and (2.2.4), and then the aggregate size distribution was updated. For a given shear rate, aggregate degradation by shear forces was then determined as described below, and the size distribution was again updated before moving to the next time step. More details may be found in Appendix A.

Aggregate breakage was simulated by the following procedure. For the shear flow streamline being considered, the maximum aggregate size was calculated from Eq. (2.2.6). It was assumed that breakage occurred for all aggregates larger than the maximum size. Considering each aggregate in turn, a number from one to (i_max-1) was randomly generated. This generated number represented the size of a fragment being broken from the aggregate under consideration. The number of aggregates corresponding to this fragment size was then incremented, and this fragment size was subtracted from the size of the original aggregate that was considered. If the remainder was smaller than the maximum aggregate size, it was assumed to be a stable aggregate and the number of aggregates of this size was incremented.
Fig. 4.1. Schematics of the simulation approach. In (a), the state of aggregation is tracked in a voxel as it travels along a laminar flow streamline. The aggregate size distribution, rather than the spatial distribution, was tracked for each voxel. The radial position of the voxel in the tube determines its transit velocity and shear rate. Tracking voxels of varying radial positions, allows the construction of plots of average aggregate size versus radial position and aggregate size versus shear rate at any axial position in the tube. The velocity profile $v(r)$ and shear rate profile $G(r)$ (or shear stress profile $\tau(r)$) are assumed to be constant with axial position, as illustrated in (b). The velocity and shear rate profiles shown are only for illustrative purposes.
If the remainder was still larger than the maximum aggregate size, the above procedure was repeated until the remaining aggregate fragment was smaller than the maximum size. This procedure assumes that the manner in which aggregates break into smaller aggregates due to shear is dependent on the maximum size allowable for that given shear rate, and that the aggregate bonds are equally breakable. Chen and Huang (1996) proposed an alternative aggregation model which also considered the aggregate bonds to be equally breakable.

For the range of shear rates of interest to us, the voxel size was chosen to be small enough such that the shear rate would be essentially uniform across the entire voxel, and large enough such that the maximum aggregate size was limited by the shear rate rather than voxel size. It was further assumed that the total number of RBCs (single and aggregated) in the voxel remained constant. Cubic shaped voxels with sides of 60 μm were used. Based on a RBC volume of 90 μm³, these could contain 2400 RBCs at 100% packing. At a physiological hematocrit of 40%, such a voxel would contain approximately 1000 RBCs.

Initially, two-byte integer arithmetic was used in the computer implementation. However, since round-off errors caused difficulties, floating point numbers were subsequently used to represent the number of collisions and number of aggregates of each size. As a result, non-integer number of aggregates of each size were obtained and these were interpreted as representing the average size obtained from many voxels under the influence of the same shear rate. To test this interpretation, multiple realizations were performed with all simulation parameters held constant, except for the random number generator seed used for the aggregate breakage procedure. The average aggregate sizes and size distributions, tracked over time, showed almost no variation (σ<10⁻⁶) between realizations. This justified our use of single realisations for any set of simulation parameters for obtaining average values.

4.2.1 Choice of Simulation Parameters
The selection of an appropriate time step size was important for reasons of speed and accuracy. The chosen time step must be sufficiently small relative to the time required for the whole distribution of RBCs to go from a disaggregated state to an equilibrium aggregate state, and also so that the numbers of collisions do not exceed the number of particles available. On the other hand, the time step must not be so short as to approach the time
required for aggregates to “stick” upon collision as well as to unnecessarily prolong the simulation time. Cokelet (1987, 1980) reported results of experiments by Copley et al. (1975) and Schmid-Schonbein (1976) which dispersed RBCs at very high shear rates and then sheared them at steady state values between 0 and 10 s⁻¹. It was found that anywhere between about 10 s to several minutes were required for normal human blood to reach an aggregated steady state. The time required for aggregates to adhere after approaching each other sufficiently closely was assumed to be very small compared to this and small relative to the time step duration. Time steps between 0.1 and 1 second were tested and a step of 0.25 s was chosen as a good compromise between speed and accuracy. Specifically, for times of less than 0.25 s it was found that the accuracy was not significantly improved.

For shear rate \((G)\) dependence, a range of \(10^{-3}\) to \(10^{3}\) s⁻¹ was chosen, primarily to cover the range of prior ultrasound in-vitro studies (Cloutier et al., 1996; Shehada et al., 1994; Yuan and Shung, 1989). While physiological human hematocrits are in the range of about 37% to 54%, for the purposes of comparison to past experimental work, a range of 25% to 45% was used. It seemed reasonable to choose a fractal dimension \((d_f)\) range of 1.7 to 2.5 since similar ranges for aggregation processes have been presented in the literature (Kolb and Jullien, 1984; Mills, 1985; Snabre et al., 1987; Meakin, 1988), though these were not for RBCs. As mentioned earlier, the dependence of the fractal dimension on shear rate and aggregate size was not modeled in our simulations.

The collision efficiencies were calculated from Eq. (2.2.4). In order to simplify the problem of dealing with the collision efficiencies of various aggregate sizes, it was assumed that the efficiency for aggregates was the same as that of a single RBC. The viscosity \(\mu\) in this equation was assumed to be the viscosity of plasma \(\mu_p\), and taken to be about \(10^{-3}\) Pa-s. For a shear rate of 1 s⁻¹ and range of values for the scaling factor \(k\) from 0.1 to 1.0 (Eq. 2.2.4), the collision efficiencies ranged from 4 to 40%. For a shear rate of 0.01 s⁻¹ the efficiencies ranged from 9 to 90% for the same range of values for \(k\). Actual collision efficiencies for RBC aggregation are not known, but these values seem reasonable since collisions are very likely to be successful at low shear rates, and RBCs are less likely to be aligned properly or have sufficient time to align themselves properly for bridging at high shears (Chien, 1975; Fabry, 1987). It will be shown later in the results section that the
collision efficiency affects the rate at which the average aggregate size is reached, but not the size itself.

A range of values for the minimum shear force required for disaggregation was obtained from prior experimental work for both normal human blood (Chien, 1981; Snabre et al., 1987; Skalak and Zhu, 1990) and normal porcine blood (Cloutier et al., 1996). Taking a value for the surface area of the flat side of a RBC to be about $5 \times 10^{-7}$ cm$^2$ and multiplying it by disaggregation pressures in the range of 1 to 2 dynes/cm$^2$ (obtained from the experimental work cited above) gives an adhesive force ($F_a$) range of $5 \times 10^{-7}$ to $1 \times 10^{-6}$ dynes, which was used in the simulations. It should be pointed out that for pathological blood or different bridging molecules, the range of shear forces required for disaggregation can be considerably higher (Chien, 1981; Snabre et al., 1987). The shear rate at which RBCs are disaggregated appears to depend on many factors (macromolecule concentration, type and length, animal species, flow conditions), and has been estimated to range from about 10 s$^{-1}$ (ultrasound studies of normal porcine blood by Shehada et al., 1994; erythrocyte sedimentation rate studies of normal human blood by Copley et al., 1976; viscosity studies of normal human blood by Chien, 1975) to about 50 s$^{-1}$ (viscosity studies of normal human blood, Schmid-Schonbein et al., 1968). The scaling constant $k'$ in Eq. (4.1) was calculated by assuming that RBCs are completely disaggregated ($i_{max}=1$) for shear rates above 10 s$^{-1}$. Taking an upper bound value for $F_a$ ($=1 \times 10^{-6}$ dynes), setting $a_s=2.78 \mu$m, which is the radius of a sphere having the same volume as a RBC (~90 $\mu$m$^3$), and taking $\mu=7.5 \times 10^{-3}$ Poise at this shear rate (Cho and Kensey, 1991), yields $k' \approx 4.3$. For a typical value of $d_r=2.4$, $(k')^{d_r} \approx 33$. The viscosity $\mu$ used to obtain $i_{max}$ in Eq. (4.1) was calculated from the Casson model presented in Cho and Kensey (1991):

$$\mu = \frac{\tau}{G} = \left(\frac{\sqrt{k_1} + \sqrt{k_2 G}}{G}\right)^2$$

(4.2)

where $\tau$ is the shear stress, $G$ is the shear rate, the yield stress $k_1$ is taken to be 0.05 dyne/cm$^2$ and the constant $k_2$ is 0.04 dyne s/cm$^2$. 

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4.2.2 Simulations Performed

Because of the complexity of the model and the number of parameters whose values could only be approximately estimated, it was important to first examine the sensitivity of the model to parameter changes. The simplest way of doing this was to examine a single voxel as it started from an initially disaggregated state and moved under constant shear conditions towards an equilibrium state. Thus, the first set of simulations performed consisted of tracking a single voxel and determining the effect of changing one parameter at a time (shear rate, hematocrit, bond strength, fractal dimension) on the average aggregate size, using the parameter ranges specified above. Sample computation times on a Pentium processor (133 MHz clock speed) ranged from about 300 s to 600 s for a single streamline simulation corresponding to following a voxel for a duration of 300 seconds.

A second set of simulations were performed, in which the aggregation kinetics in a long, large diameter (2.5 cm) rigid tube were modeled. The purpose of these simulations was to compare the results to the experimental studies of Shehada et al. (1994). A power law velocity profile,

\[ v(r) = v_{\text{max}} [1 - (r/R)^n] \]

(4.3)

was assumed for the blood flow, where \( r \) denotes the radial position in the tube, \( v_{\text{max}} \) is the maximum velocity, \( R \) is the tube radius, and \( n \) is the power law exponent. The corresponding shear rate profile is given by

\[ G(r) = v_{\text{max}} n (r^{n-1}/R^n) \]

(4.4)

If the mean flow velocity mean \( v_{\text{mean}} \) is known, the maximum flow velocity \( v_{\text{max}} \) may be obtained from (Shehada, 1992)

\[ v_{\text{mean}} = v_{\text{max}} n/(n+2) \]

(4.5)

In our simulations, the average aggregate size and size distribution in a number of voxels across the tube cross-section were tracked as the voxels traveled down the tube along laminar streamlines, as illustrated in Fig. 4.1. The actual spatial positions of aggregates within the voxels were not tracked. It was assumed that the tube flow was axisymmetric, so only a single row of voxels on the tube's symmetry plane needed to be tracked. Shehada et al. (1994) worked with mean flow velocities in the range 1.3 to 5.3 cm/s, and measured velocity profile exponents that ranged from 2.4 to 3.2 for porcine whole blood. Our simulations were performed using the same ranges of velocities and \( n \) values. In the experimental study of
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Shehada et al. (1994), it was observed that changes in the velocity profile due to the non-Newtonian effects of aggregation occurred as the blood traveled downstream from the tube entrance. These velocity profile changes were not modeled in the simulations: once selected, the velocity profile exponent was assumed to be constant at all points along the tube. It was anticipated that this would not affect the results appreciably, since the maximum velocity was calculated to change by less than 13% over an $n$-range from 2.4 to 3.2 for the same mean velocity range. By tracking the average aggregate size in 118 voxels taken across the tube diameter, cross-section profiles of average aggregate size were obtained at various distances downstream of the tube entrance. The blood was assumed to be fully disaggregated at the tube entrance. Since each voxel was associated with a single shear rate, plots of average aggregate size versus shear rate were also obtained. A typical simulation to obtain a plot of aggregate size versus shear rate at a distance of 60D downstream of the tube entrance took about 3 to 4 hours of computing time on a Pentium processor (133 MHz).

Further simulation details and sample calculations may be found in Appendix A.

4.3 Results, discussion, comparison to past experimental results

For all the results presented in this section, the following parameter values were used in the simulations: the time step $\Delta t=0.25$ s, the Hamaker constant $A=10^{-20}$ J (used in Eq. (2.2.4)), plasma viscosity $\mu_p=10^{-3}$ Pa·s (used in Eq. (2.2.4)), RBC equivalent radius $a_0=2.78$ μm, and blood viscosity $\mu$ (for use in Eq. (4.1)) was calculated from Eq. (4.2) with $k_1=0.05$ dyne/cm$^2$ and $k_2=0.04$ dyne·s/cm$^2$. All other parameter values which were used are as indicated in the figure captions.

4.3.1 Single voxel simulations

Figure 4.2 shows that the rate at which the average aggregate size approaches equilibrium, but not the size itself, increases with hematocrit ($Hct$). This is reasonable since the increasing concentration primarily affects the rate of collision, but the aggregate size itself is constrained

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1 The results of these simulations have also been presented elsewhere (Lim et al., 1997), and are reprinted with permission from Elsevier Science.
by the shear rate. At high shear rates, RBCs tend to remain disaggregated or form stable aggregates which are quite small because of low sticking probabilities and high shear forces, while at lower shear rates, large aggregates result from increased mixing (higher collision efficiencies). These effects are illustrated in Fig. 4.3. Figure 4.3 also shows that aggregates reach their maximum shear-limited size much more quickly for high shear rates than for low shear rates (e.g. compare the curves for $G=1.0 \text{ s}^{-1}$ and $G=0.05 \text{ s}^{-1}$) - although low shear rates are conducive to high collision efficiencies, RBCs also tend to move very slowly with respect to each other (low collision rates), thus increasing the time required to reach the maximum aggregate size. Increased bond strengths (Fig. 4.4) and more compact aggregate structures (higher fractal dimensions - Fig. 4.5) lead to larger average aggregate sizes (i.e. aggregates containing more RBCs) as a direct result of Eq. (4.1), since both decrease the susceptibility of aggregates to breakage by shear forces. Finally, Fig. 4.6 shows that the collision efficiency parameter $k$ does not change the average aggregate size at equilibrium, but does increase the rate at which equilibrium is reached.

These results compare well to the qualitative trends found in other theoretical work (Murata and Secomb, 1988; Chen and Doi, 1989) and also with the experimental work reported by Kitamura et al. (1995). Murata and Secomb found that the average aggregate size and the time to reach an equilibrium size both decreased with increasing shear. Using 'molecular dynamics' type simulations of aggregating spheres, Chen and Doi also found a similar relationship between aggregate size, time to reach equilibrium and shear rate. In addition, they found that increasing the hematocrit without changing the shear rate increased the rate at which the equilibrium aggregate size was reached, but did not change the final size. The experimental results of Kitamura et al. showed that the steady-state average aggregate size was independent of hematocrit, and that the steady-state average aggregate size was reached more quickly with increasing hematocrit. Kim et al. (1989) have also reported experimental results, but these are somewhat more difficult to interpret because ultrasound echo intensity is used as a measure of aggregate size. While the echo intensity is indeed related to aggregate size, it also depends more directly on concentration variance, which is a function of factors such as aggregate size, hematocrit and flow conditions (Mo et
Fig. 4.2. Single voxel simulation, showing the change in the average aggregate size versus time, starting from an initially disaggregated state, for different hematocrits. Shear rate $G=1.0 \text{ s}^{-1}$, $d_p=2.0$, $F_A=1.0\times10^{-6} \text{ dynes}$, $k=1.0$.

Fig. 4.3. Single voxel simulation showing the effects of different shear rates, $G$: Hct=45, $d_p=2.0$, $F_A=1.0\times10^{-6} \text{ dynes}$, $k=1.0$. 
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Fig. 4.4. Single voxel simulation illustrating the effects of varying adhesive strengths, $F_A$: $G=1.0 \text{ s}^{-1}$, Hct=45, $d_F=2.0$, $k=1.0$.

Fig. 4.5. Single voxel simulation showing effect of varying fractal dimensions, $d_F$: $G=1.0 \text{ s}^{-1}$, Hct=45, $F_A=1.0 \times 10^6$ dynes, $k=1.0$. 
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4.3.2 Large diameter tube simulations

Figure 4.7 shows the average aggregate size versus tube radial position for mean flow velocities, obtained at a distance of 60 tube diameters (60D) downstream from the tube entrance. Distinct peaks in aggregate size are observable along streamlines around the tube axis, with smaller average aggregate sizes occurring at the tube center and towards the tube walls. The shape of the plots can be understood in terms of the different shear histories experienced by voxels that lie on different flow streamlines. The streamlines near the tube center are associated with high flow velocities, which give little time for aggregation to occur, and because of the low shear rates, there will be little mixing and low collision rates. On the other hand, voxels near the tube walls experience high shears which break down any aggregates that may form despite the long transit times in these regions. At intermediate distances between the tube center and walls, moderate shears enhance mixing and moderate
flow velocities allow sufficient time for a high degree of aggregation to occur. It will be noted that as the flow velocity increases, the size distribution peak reduces because the shorter transit times do not allow significant aggregation to occur. The plots for 1.4 and 2.4 cm/s, in contrast to that for 5.2 cm/s, show that the growth in aggregate size is enhanced by the longer transit times and by the moderately low shear rates away from the tube center which increase the amount of mixing between RBCs and aggregates. Figure 4.8a shows the corresponding plots of average aggregate size versus shear rate. Note the low degree of aggregation at very low shear rates (associated with the high velocities near the tube center, short transit times and low collision rates) and at high shear rates (associated with the low velocities near the tube walls), as well as the enhanced degree of aggregation for a range of shear rates between 0.1 and 5 s⁻¹. It can be observed that the peak shifts to higher shear rates as the average flow rate is increased. Since the average transit time of a bolus diminishes at higher flow rates, only those streamlines associated with a high shear will allow equilibrium to be achieved (see Fig. 4.3). In Fig. 4.9a, the radial profiles of average aggregate size are shown at several distances downstream of the tube entrance for a mean flow velocity of 2.4 cm/s. As the distance from the tube entrance is increased, the region of low aggregation near
the tube center surrounded by the regions of enhanced aggregation becomes more distinct. These profiles also show the time dependence of aggregation: as the distance from the tube entrance is increased, the transit time increases for all shear streamlines, and the average aggregate size for each streamline also increases. It can again be observed that the equilibrium aggregate size is shear dependent, as evidenced by the shape of the aggregate size profile, and that the rate of aggregate growth is shear dependent and is non-uniform.

While there are no direct measurements of aggregate size at high hematocrits in shear flow to which these simulation results can be compared, the ‘black hole’ experiments discussed in section 2.4 lend themselves readily to qualitative comparison. Although the backscattered ultrasound power is directly related to local variations in the RBC concentration, it can still be a useful indirect indicator of aggregate size; it seems intuitively obvious that the packing of RBCs becomes less uniform as the average aggregate size increases. This will be discussed further in Chapter 5. Figure 2.3 shows the backscattered ultrasound power (echogenicity) versus shear rate as reported by Shehada et al. (1994). Comparison of the simulation results of Fig. 4.8a and the experimental observations of Fig. 2.3 shows good general qualitative agreement. The general shapes and trends of the results suggest that the ‘black hole’ effect can be understood by the shear histories as elucidated above. In addition, the observation of Shehada et al. that the ‘black hole’ became more distinct downstream of the tube entrance is also in agreement with the simulation results shown in Fig. 4.9a. The model predicts that the ‘black hole’ should eventually become narrower and possibly disappear at very great distances downstream of the tube entrance if steady conditions prevail (Fig. 4.9b). This is because the increased distance allows sufficient time for large aggregates to form in the center (very low shear) regions of the tube. However, the experimental results of Shehada et al. (1994) showed that for certain conditions the black hole diminished and disappeared within 60 tube diameters, which is not predicted by the model. This can probably be accounted for by the fact that the idealized laminar flow used in our simulations does not exist in experimental conditions. The assumption that the shear is constant over a given voxel does not take into consideration the viscous effects of adjacent streamlines which give rise to the development of the flow. Any changes in velocity and shear profiles will be further enhanced by aggregation. Lateral migration of particles due to viscous and inertial effects (Goldsmith, 1993), as well as secondary flow components,
probably encourage mixing between flow streamlines, resulting in a more uniform average aggregate size profile across the tube, and a faster disappearance of the black hole in the experimental situation.

The shape of the aggregate size-shear rate curves and tube profiles were found to change little with changes to the hematocrit, adhesive strength $F_a$, and fractal dimension $d_f$, which was expected since the single voxel simulation results showed that the time required for aggregates to reach their equilibrium size did not vary much with these parameters (Figs. 4.2, 4.4 and 4.5). However, as previously noted, the time for aggregates to reach equilibrium size was found to depend strongly on the collision efficiency parameter $k$ (Fig. 4.6). As illustrated in Fig. 4.8b, for the large tube flow simulations this dependence resulted in a significant change in the average peak aggregate size and shear at which it occurs. For low collision efficiencies ($k << 1$), only aggregates under the influence of higher shear rates had sufficient time to reach their equilibrium size and hence the aggregate size versus shear rate peak shifted to higher shears as the collision efficiency parameter was decreased. Further study is needed to obtain data on actual collision efficiencies in RBC aggregation, but in light of the good qualitative agreement between results, our choice of parameter values seems reasonable.

Figures 4.8a and 2.3 can also be compared to Fig. 2.4, which displays the experimental ESR (erythrocyte sedimentation rate) results of Copley et al. (1976). This method, which is dependent on aggregate size and density, measures the rate at which RBCs sediment under the influence of gravity while being sheared. We can again observe similarities between the simulation and experimental results in the way that the degree of aggregation depends on the shear rate. The difference in flow conditions, fibrinogen content and other factors may account for the different shear rates at which the peaks occur, nonetheless the qualitative agreement between the two sets of results is still good. The distinct aggregation peak at moderate shear rates which is evident in all of these results was also hypothesized by Chien (1976) to be the result of increased mixing. It must again be emphasized that a direct linear relationship between these results cannot be inferred.
Fig. 4.8. Large tube simulation showing average aggregate size versus shear rate for (a) mean flow velocities of 1.4, 2.4, and 5.2 cm/s: Hct=28, \( d_f=2.20 \), \( F_a=1.0\times10^{-6} \) dynes, \( k=1.0 \), and for (b) collision efficiency k values of 0.25, 0.5 and 1.0: mean flow velocity=2.4 cm/s, Hct=28, \( d_f=2.20 \), \( F_a=1.0\times10^{-6} \) dynes.
Fig. 4.9. Large tube simulation showing the average aggregate size profiles for a mean flow velocity of 2.4 cm/s at distances of (a) 20D, 40D, 60D from the tube entrance and (b) 60D, 240D and 2400D from the tube entrance: Hct=28, $d_f=2.20$, $F_r=1.0 \times 10^6$ dynes, $k=1.0$. 
4.4 Chapter summary

A model has been developed for RBC aggregation in a shear flow and this has enabled us to study the general influence of shear rate, adhesion strength, collision efficiency, hematocrit, and aggregate fractal dimension on the average aggregate size and the process of aggregation itself. It has also been shown that this model can be useful for explaining and understanding some interesting experimental results. It should be emphasized that this is a first order model and that many assumptions were made both concerning the processes involved and the parameter values used. For example, it was assumed that the fractal dimension was independent of the aggregate size and shear rate. While we recognize the difficulty of justifying such an assumption, it is clear that the development of a more sophisticated model would require a better understanding of the quantitative relationships. Perhaps more importantly, lateral migration of particles across flow streamlines due to viscous and inertial effects, and changes in velocity and shear profiles have not been considered, which severely limit the accuracy of the shear flow model used in this work. In spite of these limitations, the model shows a good deal of promise as a basis for achieving a better understanding of a complex hemodynamic process and it could lead to the development of quantitative methods of measuring aggregation.

I would have to talk about the occasional sense I have that life is not just a series of events causing other events as haphazardly as a break shot in pool causes the billiard balls to careen off in all directions, but that life has a plot the way that a novel has a plot, that events are somehow or other leading somewhere, that they make sense.

(Frederick Buechner)
Chapter 5

Studying the Relationship between RBC Aggregation and Packing Statistics

The model of shear-dependent RBC aggregation presented in the previous chapter is useful for helping us gain an understanding of how the average aggregate size and the aggregate size distributions are affected by different variables such as shear rate and aggregate bond strength. However, those simulations give us very little indication of how the aggregate size and size distribution may correspond to the particular spatial arrangements of the aggregates, i.e. to the way they are packed. This chapter presents the method and results of an initial simulation study of the relationship between aggregate size distributions and packing statistics.

A survey of the general literature on the packing of particles revealed that one of the most frequently addressed problems was determining the geometric arrangement for the densest packing of spheres (Coffman, 1991; German, 1989). This problem is quite different in nature from the one we wish to address, which is essentially that of determining what variations in particle packing are possible. The packing problem we are considering is much more difficult to model because a given size distribution of aggregates generally does not have one unique packing arrangement, although there may be preferred aggregate orientations. A possible further complication is the dependence of aggregate shape on the process by which aggregation occurs (Meakin, 1988).

Figures 5.1-5.5 schematically illustrate the effect of a number of parameters on the packing of particles. Figure 5.1 shows sample particle arrangements for low, medium and
high hematocrits. As was discussed briefly in section 2.3, large local variations in scatterer density are most likely to occur at medium hematocrits, whereas low scatterer densities at low hematocrits and close packing of scatterers at high hematocrits make such variations less likely. Intuitively, it is expected that larger gaps between aggregates, and consequently, larger variations in concentration, will arise as the average aggregate size increases, and this is illustrated in Fig. 5.2. Aggregate shape is also expected to have an effect on the packing of particles. The average aggregate size (i.e. the number of particles per aggregate) is the same for both of the aggregate distributions shown in Fig. 5.3, but the average aggregate fractal dimension is different - the distribution consisting of compact aggregates has a higher average fractal dimension than the distribution consisting of long rouleau-like structures. The concentration variance may increase if long-chain aggregates form networks with large gaps between the rouleaux, but it is also possible that the variance may decrease if the rouleaux become aligned with each other (e.g. in the direction of blood flow), thus leading to a more uniform and symmetrical arrangement. When there is a distribution of aggregate sizes, the number of RBCs in any given aggregate can vary depending on the distribution chosen. We will define $M_{\text{max}}$ as the maximum number of RBCs that can occur in any aggregate that is a member of a particular set having a given distribution of sizes. Figures 5.1-5.3 depict tissue samples in which all the aggregates contained within each sample are the same size (i.e. the size distribution is a delta function). In real life, a range of aggregate sizes are likely to arise in any sample. An aggregate size distribution may be characterized by its size range (the number of different aggregate sizes contained within a sample) and by its shape (the curve traced out by plotting the frequency of occurrence of the aggregate sizes in the sample), as well as by its average size. Two tissue samples having the same average aggregate size are shown in Fig. 5.4. One of the samples has a delta function size distribution (i.e. all of the aggregates are the same size) whereas the other contains a range of sizes, which is more heavily weighted towards smaller aggregates. As illustrated in this figure, it might be
Chapter 5. Studying the relationship between RBC aggregation and packing statistics

Fig. 5.1. Schematic illustrating the effect of hematocrit on concentration variance.

Fig. 5.2. Schematic illustrating the effect of aggregate size (# RBCs per aggregate) on concentration variance.

Fig. 5.3. Schematic illustrating the effect of aggregate shape on concentration variance. The rouleau-like aggregates in (a) have a smaller fractal dimension than the compact aggregates in (b).
expected that an aggregate distribution consisting of many aggregate sizes would decrease the variations in scatterer concentration, due to smaller aggregates "filling in" the gaps between larger aggregates. Figure 5.5 shows one sample in which each aggregate size appears with the same frequency (uniform distribution) whereas the other contains a higher proportion of single particles and doublets (an approximately exponential distribution). It
seems intuitively obvious then, that the size distribution shape will also have an effect on packing statistics.

5.1 Description of simulation method

The Molecular Dynamics (MD) and Monte Carlo (MC) techniques described in Chapter 2 are simulation methods which were initially considered for this study of aggregation packing, since they may be used to create spatial distributions of aggregates (Chen and Doi, 1989; Dickinson and Euston, 1992; Elimelech et al., 1995). Initially, the Monte Carlo method appeared to be more promising than the Molecular Dynamics method in terms of computational burden and modeling complexity. While both methods can track changes in spatial position of a distribution of particles, the MC method does not need to calculate all the forces involved in every particle interaction as in the MD method. The MC method models particle interactions with a higher level of abstraction than the MD method through the use of potential well functions, which can incorporate the effect of many factors, such as adhesive strength, by adjusting the size and shape of the potential well functions. However, simple MC methods can generally only be used to obtain equilibrium results (Elimelech et al., 1995). For this reason, as well as not knowing the exact nature of the particle interactions, it is difficult to make quantitative comparisons between MC simulation results and experimental results. Although some MC preliminary simulations were carried out, ultimately neither the MC nor the MD method was chosen for the simulations described in this chapter due to the drawbacks pointed out above.

5.1.1 Simulation method

As a first-order study, our simulation approach in this section of work was to create distributions of randomly placed, identical, finite-sized, and incompressible aggregates consisting of circles (or infinite rods) in 2-D and spheres in 3-D, and then to calculate the concentration variance as the measure of packing. In these simulations, the effect of aggregate size (the number of particles contained in an aggregate), hematocrit, aggregate size distribution width (i.e. the range of aggregate sizes) and distribution shape (i.e. the concentration of each aggregate size) on distribution packing was investigated. It is hoped
that future simulation studies will use particles which more closely approximate the deformable, biconcave discoid geometry of RBCs.

The measure of packing used in this work was the local concentration variance, the variation in the number density of particles when small volumes (or areas) of the distribution are examined. This packing measure is the most useful for our purposes because of the direct relationship between the concentration variance and the backscattered ultrasound power. The local concentration variance was calculated in the following manner: the tissue distribution was first subdivided into voxels and non-overlapping particle positions were then generated. The characteristic dimension of these subvoxel areas or volumes was chosen to be in the range of $\lambda/10$ to $\lambda/20$. For each non-overlapping particle position generated, the voxel containing this particle was determined, and then the number of particles contained by that voxel was incremented. After the required number of particle positions was generated, the average number of particles per voxel was calculated, and the variance in the number of particles per voxel could subsequently be obtained.

To reduce the computation needed to generate non-overlapping particle positions, a voxel-based overlap checking method was used. In this method, a newly generated particle position was checked for overlap with only those particles which had already been successfully placed in the same voxel or the immediately surrounding voxels. In this way, most of the unnecessary particle position comparisons could be avoided. Although this method is superficially similar to the voxel-based method described in section 3.1.1, it differs in that the number of particles to be placed in each voxel is not a priori known, as it is in the latter procedure. By using smaller voxels, the amount of overlap-checking could be further reduced - a 2-D voxel having a characteristic dimension of $\lambda/20$ can contain at most 10 particles, whereas a $\lambda/40$ voxel can contain a maximum of 3 particles. For all simulations described in this chapter, $\lambda/40$ voxels were used to create the tissue samples, while $\lambda/10$ voxels were used to calculate the local concentration variance. For further simulation details, please refer to Appendix B.

When investigating the effect of a particular parameter such as hematocrit or size distribution width (i.e. $M_{max}$), multiple realisations were performed since a single realisation represents only one particular arrangement of particles. Multiple realisations were obtained by starting with a different seed for the random number generator used to create the particle
distribution. Therefore, for a given set of simulation parameters (hematocrit, tissue sample size, size distribution, voxel size), we calculated the local concentration variance for each realisation, calculated the variance averaged over all the realisations, and were then able to obtain a standard deviation for this average variance. For both 2-D and 3-D simulations, the number of realisations performed was generally in the range of 200-300, and most frequently 250. This range of realisations was chosen by performing a large number of realisations (>500) in the preliminary simulations described below, plotting the local concentration variance as a function of realisation, and then determining the range of realisations over which fluctuations in the variance stabilized to less than 10% of the average value for that range.

A uniform random number generator was used to generate the particle positions - although the packing of RBCs will, in reality, have preferential orientations and arrangements due to their biconcave shape and macromolecular bridging, as well as flow conditions, this would require us to be able to exactly model these interactions. Without a priori knowledge of the exact forces acting on the particles, and with the simplifying assumption of identical, incompressible particles, the simplest starting point was to assume that particles could be placed anywhere within the distribution volume as long as they did not overlap with each other.

5.1.2 Preliminary simulations

The first simulations performed were of distributions of completely disaggregated particles. To test the correctness of the simulation algorithm, the concentration variances were obtained and plotted as a function of hematocrit, and these curves were then compared to the theoretical curves derived from the model of Bascom and Cobbold (1995). In Bascom and Cobbold's fractal model, changes in particle packing arrangements due to particle shape, aggregation, and flow conditions may be represented by changes in the fractal dimension, \( m \). It was expected that these preliminary simulations would correspond most closely to uniform flow conditions, so the 2-D and 3-D simulation results were compared to theoretical curves for \( m=2 \) and \( m=3 \).

These initial 2-D and 3-D simulations were carried out while experimenting with tissue sample dimensions of \( \lambda/4 \) to \( \lambda \). The reason for this was to determine the minimum
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Tissue sample size necessary for the simulations to run correctly: a small tissue sample size reduces the computation time required for a simulation, but "boundary effects" may become important as the tissue sample becomes comparable in size to the particles. The particle positions generated by the simulation represent the location of the particle centres, and thus must be checked to ensure that the particle itself is completely within the tissue sample. This results in a "particle-free" zone around the inside of the boundary, which increases in size as the sample size is reduced, or conversely, as the particle size is increased. These boundary effects obviously affect the packing of the particles and the calculation of the average concentration variance for the tissue sample: as a sample decreases in size, the sample's perimeter-to-area ratio increases, and voxels along the sample edges more heavily weight the variance calculation. For a characteristic particle radius of 2.78 μm (the radius of a sphere having the same volume as a typical RBC - 90 μm³), a sample dimension of λ (0.308 mm for an acoustic velocity of 1540 m/s and ultrasound centre frequency of 5 MHz) was found to give correct results. All the 2-D simulations described in this chapter use λ×λ sample areas, while a λ×λ×λ/10 sample volume was used for all 3-D simulations.

5.1.3 Simulations with aggregates and polydisperse distributions

The next step in this study was to move from distributions of single, disaggregated particles to distributions of aggregates of varying sizes. Due to the high computational burden of 3-D simulations, all simulations described in this section were performed only for 2-D tissue samples. We started by investigating monodisperse distributions consisting entirely of doublet, triplet or quadruplet aggregates so as to study the effect of aggregate size. Tissue samples of 2-, 3- and 4-particle aggregates were created at hematocrits between 2 and 40. For a given hematocrit, the variance was expected to increase as the average aggregate size was increased. It was assumed that aggregates were chain-like in structure, in which each particle just touches the preceding one (i.e. the centres of two contiguous particles in a chain are separated by exactly 2 particle radii). Two-particle aggregates were created in a straightforward manner: the position of the first particle was randomly generated, checked for overlap with already-placed particles, and the centre of the second particle was then placed 2 radii away from that of the first particle at some randomly generated angle. The position of the second particle was then checked for overlap with other particles. The particle positions
for 3-particle aggregates were determined in the following manner: the first two particles in the aggregate chain were placed as described above. The third particle was then placed 2 radii away from that of the second at a random angle which was constrained only by the requirement that the particle not overlap with the first particle in the aggregate chain or any of the other particles in the tissue sample. A similar procedure was used for aggregates larger than 3 particles: any particle in the aggregate chain was placed at a random angle to the immediately preceding particle in the chain, and was checked for overlap with the previously-placed particles in the aggregate as well as in the tissue sample.

After the simulation of monodisperse distributions, polydisperse distributions (i.e. distributions consisting of aggregates of different sizes) were considered. As for monodisperse distributions, it was hypothesized that the variance should increase as the average aggregate size was increased. However, it is not known exactly what kind of RBC aggregate size distributions actually exist in vivo although a number of in vitro (Chen et al., 1995; Chen et al., 1996) and theoretical studies (Samsel and Perelson, 1984) have been made. The aggregate size distributions (both the range of aggregate sizes and the frequency of occurrence of each size) depend on the actual flow conditions and particle interactions, as well as the morphology of the aggregates formed. Size distributions can be obtained from the shear-dependent aggregation model presented in Chapter 4, and will be incorporated into the work presented in Chapter 6, but the scarcity of in vivo or in vitro studies on aggregate size distributions suggests directions for future experimental and theoretical work.

As a simple starting point, size distributions were created in which the frequency of occurrence of an aggregate size is a constant fraction $k$ of the immediately preceding aggregate size:

$$p(i) = p_1 k^{(i-1)}, \ i=1,2...$$

(5.1.1)

where $p(i)$ is the probability of finding an aggregate having $i$ particles and $p_1$ is the probability of occurrence of a single particle aggregate. The sum of the probabilities $p(i)$ must always equal 1, which enables us to calculate $p_1$ once the maximum aggregate size $M_{\text{max}}$ is chosen:

$$p_1 = \frac{(1-k)}{(1-k^M_{\text{max}})}.$$  

(5.1.2)

From the form of Eq. (5.1.1), it can be seen that the shape of the size distributions was taken to be exponential. Size distribution ranges (i.e. maximum aggregate size) of 3, 5, 7 and 9,
and $k$ values of 0.25, 0.50, and 0.75 were used over the hematocrit range of 2 to 40. As $k$ approaches 1, the size distribution more closely approximates a uniform (flat) distribution over the selected size range and the average aggregate size increases. As $k$ approaches 0, the distribution becomes more heavily weighted towards smaller aggregates and the average aggregate size of the tissue sample decreases. A maximum aggregate size of 9 was chosen because the computational time required to create a tissue sample increases considerably as the size distribution range is increased beyond this.

As an introductory investigation into the effect of aggregate shape on concentration variance, simulations of monodisperse distributions of 3-particle and 4-particle aggregates were performed. Two general aggregate geometries were tested: straight, or almost-straight, chains (fractal dimension $m$ closer to 2) and more compact clumped aggregates ($m$ closer to 3). In contrast to the simulations described above involving multiple-particle aggregates, the generation of particle positions in these simulations was more heavily constrained. In the almost straight-chain aggregates, the angle described by the lines running through the centres of any three particles had to be at least 120 degrees ($2\pi/3$ radians), whereas the angle for the clumped aggregates could be no more than 90 degrees ($\pi/2$). As usual, no overlap between any particles in an aggregate or with any other particle in the tissue sample was allowed. These simulations were also performed over the hematocrit range of 2 to 40. The purpose of these simple and fast simulations was to see if the shape of small aggregates could have an appreciable effect on particle packing. The effect of the shape of large aggregates in mono- and polydisperse distributions is a possible direction for a more in-depth future study.

### 5.1.4 Summary of simulation assumptions

The following assumptions were used:

1. The particles are nondeformable and incompressible - the particles cannot overlap each other.
2. The particles are represented by circles in 2-D and by spheres in 3-D.
3. No particular packing arrangement is assumed - individual particle positions are taken to be uniformly distributed throughout the tissue sample.
4. Aggregates are modeled as flexible linear chains in which each particle just touches the preceding one (i.e. the particle centers are separated by exactly 2 particle radii).
5.2 Simulation results

5.2.1 Preliminary results: single particles

The results from the 2-D and 3-D simulations of monodisperse distributions of single particles are shown in fig. 5.6. The variance is plotted as a function of hematocrit for the 2-D and 3-D simulation results as well as the curves derived from theory. Excellent agreement between the simulation and theoretical curves were obtained over the entire simulated hematocrit range for the 2-D case. Excellent agreement is also obtained for the 3-D curves up to a hematocrit of about 24, beyond which the results begin to diverge. The discrepancy in results at higher hematocrits is probably due to tissue sample boundary effects which arise from the simulation method. As the hematocrit is increased, the particle-free zone at the sample boundaries probably becomes more pronounced, which would affect the calculation of the local concentration variance. Modifications to the simulation method to reduce the size of the particle-free zone should be experimented with in future work. Of special note for both the 2-D and 3-D results is the fact that the variance peaks at moderate hematocrits - about 22 for 2-D and 12 for 3-D. Experimental results plotting the backscattered power from blood under a number of different flow conditions as a function of hematocrit display similar dependence (Bascom and Cobbold, 1995). Since these results were obtained from RBCs suspended in saline, the packing dimension $m$ was affected only by particle shape and flow conditions, and not by aggregation. Curves with $m$ in the range of 2.17 to 2.57 were fitted to these results, and these curves would nicely fill the gap between the 2-D ($m=2$) and 3-D ($m=3$) simulation curves.
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5.2.2 Simulation results: aggregates and polydisperse distributions

Figure 5.7 is a plot of the variance versus hematocrit for monodisperse distributions of single, 2-, 3- and 4-particle aggregates, as well as the theoretical curve. The error bars, which are shown only for the 3-particle aggregate curve, represent one standard deviation. It is evident that an increase in average aggregate size leads to an increase in the concentration variance. However, it should be noted that this increase in variance with aggregate size is not uniform over all hematocrits: e.g. for the 3-particle curve, the variance at a hematocrit of 22 is about 2.85 times that at the same hematocrit for the single particle curve, whereas the ratio of variances at a hematocrit of 40 is about 2.45. The greatest increases in variance from the single particle curve for all the other curves appear to occur at moderate hematocrits. This can be explained by the dependence of variance on hematocrit, since the possible particle arrangements at high hematocrits seem to be far more constrained than for moderate hematocrits, and many of the most likely particle arrangements at low hematocrits display only small variations in concentration. It can also be noted from the results that changes in the average aggregate size do not appear to affect the hematocrit at which the peak variance
occurs - all curves appear to display peaks at a hematocrit of close to 22. Although the curves are not simply scaled versions of each other, it can still be noted that the variance peak for the 2-, 3- and 4-particle curves are roughly 2, 3, and 4 times the value of the variance peak for single RBCs. The effect of average aggregate size on variance are corroborated by the experimental results presented by Shung et al. (1984). Shung et al. measured the backscattered ultrasound power from saline suspensions of bovine and human RBCs. No aggregation occurs when RBCs are suspended in saline, so any changes in backscattered power from uniformly stirred suspensions are due only to changes in RBC size. Human RBCs are approximately twice the volume of bovine RBCs, and therefore a saline suspension of human RBCs effectively simulates a monodisperse suspension of doublet bovine RBC aggregates. The backscattered power was observed to increase accordingly, which corresponds to an increase in concentration variance. In these experimental results, the peak value of the backscattered power for the human RBC suspension was observed to be approximately twice that of the bovine RBC suspension, although a linear relationship between aggregate size and power (variance) at all hematocrits cannot of course be inferred.

Figure 5.8 shows results obtained for a hematocrit of 25 and various aggregate size ranges and \( k \) values. The variance is plotted as a function of aggregate size range and each curve represents a different \( k \) value. It can be observed that the variance increases with aggregate size range, since the average aggregate size is not being held constant. Since the aggregate size distributions are assumed to be exponential, the frequency of occurrence of large aggregates is quite low, and the variance appears to eventually level off. The effect of changing the size distribution shape by adjusting the \( k \) value is quite easy to understand: as the \( k \) value is increased, the frequency of occurrence of larger aggregates increases, and the variance correspondingly increases. Figure 5.9 plots the variance as a function of hematocrit for a number of different aggregate size ranges and \( k \) values. As expected, increasing the size range while keeping the \( k \) value constant results in an increase in variance, and increasing the \( k \) value while holding the size range constant also results in an increase in the variance. It is again interesting to note that the variance peak does not appear to shift for different aggregate distributions. Yet, according to Bascom and Cobbold’s fractal model (1995), a change in the average packing dimension \( m \) of the tissue sample results in a shift in the hematocrit at which
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Fig. 5.7. Simulation results, investigating the effect of aggregate size: monodisperse distributions, 250 realisations, \( \lambda \times \lambda \) 2-D tissue sample, \( \lambda/10 \) voxel for variance calculation, typical standard deviations shown.

Fig. 5.8. Simulation results, showing the effect of size distribution: Hct=25, 250 realisations, \( \lambda \times \lambda \) 2-D tissue sample, \( \lambda/10 \) voxel for variance calculation.
the variance peaks. It is not clear as to why this discrepancy arises, nor whether it is of any particular importance. Taking into account the size of the standard deviations, it is possible that the peaks occur over a hematocrit range of 17 to 27: i.e. there may be shifts in the peaks, but they may not be measurable in our simulations. Figure 5.10 shows the spatial arrangement of aggregates from two typical realisations, with the tissue sample characteristics shown in the caption. It is certainly obvious that the packing of particles changes as the size distribution characteristics are changed.
Fig. 5.10. Typical simulated tissue samples, polydisperse distributions: Hct=25, λ×λ 2-D tissue sample, (a) k=0.75, range=3, (b) k=0.75, range=9.
Figure 5.11 shows plots of variance versus hematocrit for monodisperse 3-particle distributions. The curves for tissue samples consisting of straight-chain aggregates and dense aggregate clumps are shown. Although slight differences between the 2 curves can be observed, they do not appear to be significant - they are far smaller than the standard deviations shown. Similar trends are observed for monodisperse 4-particle distributions (Fig. 5.12). For small aggregates, at least, it appears that aggregate shape does not appreciably affect the packing statistics. It is anticipated, however, that aggregate shape may have a very important influence on the packing dimension in the case of large aggregates. Typical realisations illustrating the spatial arrangements of straight-chain and clumped 3-particle aggregates are shown in Fig. 5.13. Figure 5.14 show corresponding realisations for 4-particle aggregates. Some differences in packing between the straight-chain and clumped aggregates can be observed.

5.3 Chapter summary

In this exploratory investigation, simulations have been carried out to assess the effect of hematocrit, aggregate size, aggregate shape, and size distribution on the local concentration variance.

In studies of the effect of hematocrit on the packing of single disaggregated particles, excellent agreement was obtained between the simulation results and theory for both 2-D and 3-D simulations. It is only at hematocrits higher than those at which the concentration variance peaks that both the 2-D and 3-D simulation results begin to diverge from the theoretically predicted results.

The simulation of 2-D monodisperse distributions of aggregates consisting of 2, 3, and 4 particles showed, as expected, that the variance increases as the aggregate size increases. The hematocrit at which the variance peaks does not seem to be affected by the aggregate size for monodisperse distributions.

The shape of small (3- and 4-particle) aggregates does not appear to appreciably affect the concentration variance, although monodisperse distributions consisting of straight-chain or almost-straight-chain 3- and 4-particle aggregates appear to yield lower variances than denser, clumped aggregates for the entire hematocrit range simulated. This is possibly
due to the straight-chain aggregates becoming aligned with each other, resulting in a more uniform spatial distribution of particles and a decrease in the concentration variance. Further studies are needed to investigate the effect of the shape of larger aggregates on particle packing.

The effect of the aggregate size distribution on packing was investigated. As the aggregate size range (and the average aggregate size) of a tissue sample increases, the concentration variance also increases. As the aggregate size distribution changes from an exponential distribution to closer to a uniform distribution, the variance increases. This is as expected since the number of large aggregates increases, as well as the average aggregate size, which leads to looser packing and larger gaps between aggregates.

Although 3-D simulations were used only for distributions of single particles, it is expected that a study of the effects of aggregate size, shape and aggregate size distribution on the packing of 3-D tissue samples will show similar trends to those obtained with the 2-D simulations.
Chapter 5. Studying the relationship between RBC aggregation and packing statistics

Fig. 5.11. Simulation results, showing the effect of aggregate shape as a function of hematocrit for 3-particle aggregates: 250 realisations, $\lambda \times \lambda$ 2-D tissue sample, $\lambda/10$ voxel for variance calculation, standard deviations as shown.

Fig. 5.12. Simulation results, showing the effect of aggregate shape as a function of hematocrit for 4-particle aggregates: $\lambda \times \lambda$ 2-D tissue sample, $\lambda/10$ voxel for variance calculation, standard deviations as shown.
Fig 5.13. Typical simulated tissue samples, monodisperse distributions of 3-particle aggregates: Hct=10, λ×λ 2-D tissue sample, (a) straight-chain aggregates, (b) compact aggregates.
Fig. 5.14. Typical simulated tissue samples, monodisperse distributions of 4-particle aggregates: Hct=10, λ×λ 2-D tissue sample, (a) straight-chain aggregates, (b) compact aggregates.
Chapter 6
Simulating Ultrasound Imaging of RBC Aggregation

The obscure we see eventually. The completely apparent takes a little longer.
(Edward R. Munson)

The final step in this work was to coordinate the voxel method for simulating ultrasound imaging, the shear-dependent aggregation model, and the results of our study of the relationship between aggregation and packing statistics. Due to the complexity of the overall problem, the purpose of this phase of the work was to make a qualitative comparison of the simulated images to some of the results of the "black hole" whole blood experiments described by Shehada et al. (1994).

6.1 Description of simulation method

Using the shear-dependent aggregation model presented in Chapter 4, aggregate size distributions were calculated along streamlines spanning the tube cross-section, at a number of points downstream of the tube entrance. These aggregate size distributions were then used to determine the local concentration variances across the tube, and the backscattered ultrasound was then simulated by applying the voxel method presented in Chapter 3. The variations in backscattered power across the tube cross-section were then compared to Shehada et al.'s (1994) experimental results.
6.1.1 Creating particle distributions

Regarding the shear-dependent aggregation simulations described in Chapter 4, we used 118 streamlines across the diameter of a 2.5 cm tube. Axi-symmetric laminar flow with streamlines parallel to the tube axis was again assumed, enabling aggregate size distributions to be calculated at any point along each streamline. A hematocrit of 28% and voxels having λ/5 dimensions (60 μm) were used in these simulations. In order for simulated backscattered signals to be compared to the tube flow ultrasound experiments by Shehada et al. (1994), size distributions were calculated at distances of 20, 40 and 60 tube diameters from the tube diameter, and mean flow velocities of 1.4, 2.4 and 5.2 cm/s. As for the simulations described in Chapter 4, a power law velocity profile was used, with an exponent of 3.0. Figure 6.1 shows aggregate size distributions obtained along a number of streamlines at distances of 20 {column (a)} and 60 tube diameters {column (b)} from the tube entrance. At a particular distance from the tube entrance, the variations in size distributions across the tube can be explained by the shear-dependence of aggregation. The streamlines closer to the tube walls experience high shears, which effectively limit the maximum aggregate size despite the long transit times associated with the low flow velocities near the tube walls. Thus, the size distributions consist mainly of single particles, doublets or triplets (rows vi and vii). Near the tube center, low shears allow the possibility that large aggregates can be formed, but only after a sufficient amount of time due to the decreased frequency of particle collisions. Therefore, distributions which are heavily skewed toward smaller aggregate sizes result (row i), although large aggregates do form (row ii). At intermediate tube radii, the shear rates are low enough to enhance the frequency of particle collisions, but not so high as to limit the range of aggregate sizes. As a result, the size distributions along intermediate streamlines are less heavily skewed towards small aggregates than for the streamlines near the tube walls or tube center (rows iii, iv, and v). By examining the size distributions obtained at 20D and 60D from the tube entrance, the effect of transit time can be observed. Near the tube center (rows i and ii), the increased transit time allows a greater number of larger aggregates to form, although the size distributions are still heavily skewed towards small aggregates. At
Fig. 6.1. Simulation results showing aggregate size distributions at various radial distances, Hct=28, at (a) 20D from the tube entrance, and (b) 60D from the tube entrance.
intermediate radii (rows iii and iv), the size distributions become more uniform in shape (i.e. the relative frequencies of occurrence of most aggregate sizes begin to approximate each other). Finally, as the tube walls are approached, increased disaggregation due to higher shear rates leads to increasingly skewed size distributions (rows v, vi, and vii).

6.1.2 Calculating local concentration variance

The size distributions obtained from the shear-dependent aggregation simulations were for 3-D tissue samples, which posed a problem. Since the computational burden was already very
heavy for creating 3-D volumes of single particles, performing a satisfactory number of realisations (i.e. ~ 250) for calculating the concentration variance of polydisperse 3-D distributions would have taken an unacceptable amount of time on our computer equipment (Pentium processor, 166 MHz). It was decided that 2-D tissue samples would be simulated, and that these would then be used for simulating 2-D A-line scans. The problem that needed to be solved then, was to determine how the aggregate size distributions corresponding to 3-D volumes could be used to create the 2-D tissue volumes from which the concentration variances for the backscatter signal simulations were to be calculated.

The simplest solution, which was the chosen method, was to obtain the aggregate sizes for the 2-D tissue sample by taking the cube root of the 3-D tissue sample aggregate sizes, then squaring them, and finally rounding them to the nearest integer: e.g. an aggregate size of 125 in the 3-D tissue sample translates to a 2-D aggregate size of 25, while a 3-D aggregate size of 32 is transformed into a 2-D aggregate size of 10. It can be noted that this mapping of aggregate sizes from the 3-D sample to the 2-D sample was nonlinear and not 1 to 1: e.g. aggregate sizes of 1 and 2 for a 3-D sample are mapped to an aggregate size of 1 for the 2-D sample, whereas 3-D aggregates of sizes 118 to 124 translate to a 2-D aggregate of size 24. To determine the frequency of occurrence of an aggregate size in the 2-D sample, the probabilities of occurrence of each aggregate size in the 3-D sample were added to the probabilities of occurrence of their transformed 2-D aggregate sizes. For example, the probabilities for 3-D aggregates consisting of 118 to 124 particles were summed together to give the probability for 2-D aggregates consisting of 24 particles.

Another possible solution would have been to perform the transformation by using the fractal dimensions for 2-D and 3-D aggregates. For example, if the average fractal dimension of the 3-D sample’s aggregates is \( m \) (\( m=3 \) in the method described above) and the average fractal dimension of the 2-D sample’s aggregates is \( n \) (\( n=2 \) above), then the transformation from the 3-D aggregate size \( i \) to the 2-D aggregate size \( j \) can be described by the equation \( j=\lceil i^{(n/m)} \rceil \), where the square brackets \( [\ ] \) denote the function which returns the largest integer less than or equal to the real number \( i^{(n/m)} \). As was already mentioned, the solution which was chosen used \( m=3 \) and \( n=2 \). The primary motivation for choosing these values of \( m \) and \( n \) was to simplify the problem: aggregate shape depends on factors such as RBC shape, aggregation mechanisms, flow conditions and aggregate size, so it is likely that
aggregates of different sizes have different fractal dimensions. Therefore, to accurately simulate a tissue sample, it is necessary to know exactly how the aggregate fractal dimension varies with these factors. More research must be performed to obtain the needed fractal dimension data before this level of modeling accuracy can be achieved.

Using the transformed size distributions obtained with the simplest solution, the average variance for each of the 118 streamlines was calculated by creating random 2-D tissue samples, according to the methodology described in Chapter 5. For each streamline, 250 realisations were performed, using a $\lambda \times \lambda$ tissue sample, and a $\lambda/10$ voxel for calculating the variance.

### 6.1.3 Simulating ultrasound imaging

Once the concentration variances for each of the streamlines were calculated, the backscattered signal was then simulated. At a fixed radial position (depth) and distance away from the tube entrance, the tissue sample was assumed to have constant characteristics - i.e. all insonated voxels at that depth were assumed to have the same concentration variance. The tissue sample to be simulated was divided up into lines of voxels parallel to the tube axis, and the voxel scattering strength was determined by using a Gaussian distributed random number generator. The average number of scatterers per voxel was determined by the hematocrit and voxel size, and the variance was given by the calculated value of the streamline (one of the 118 streamlines) that was closest in depth (radial distance) to the line of voxels being insonated.

As for the 2-D simulations of the backscattered signal described in chapter 3, a simple unfocused piston transducer was used, with a symmetrical Gaussian-shaped sinusoid pulse (center frequency of 5 MHz and 6 dB bandwidth of 2 MHz) 1 $\mu$s in duration, and an incident beam which was assumed to be uniform over the sample volume width of $6\lambda$ (1.8 mm). As well, the sample volume was assumed to have a uniform response along its length, which was adjusted by keeping the transmit pulse length fixed and by varying the receive window. The sample volume length was adjusted so as to encompass the width of the tube (2.5 cm). Equation 2.1.4 was used to calculate the backscattered signal. The aggregate size distributions were calculated at 3 distances (20D, 40D and 60D) from the tube entrance for each flow velocity (1.4, 2.4, 5.2 cm/s), so 250 A-lines were calculated for each flow velocity-
distance pair. The rf data was then converted into B-mode data by taking every 16 samples and summing together their absolute values. The average of the 250 B-mode lines was then found, the logarithm calculated and then multiplied by 10 to transform the final trace into decibels (dB).

The effect of attenuation with depth on the backscattered power was considered. Shehada et al. (1994) estimated that the attenuation of 28% disaggregated whole porcine blood at 20°C should be about 1.6 dB/cm at 7.0 MHz. Shehada et al. also found that this attenuation varied in a nearly linear manner over a 1.5 cm depth. Using these findings, the effect of attenuation was incorporated into our simulations by adding a linear correction factor of 1.6 dB/cm to the B-mode data across the whole diameter. Because the effect of RBC aggregation on attenuation is unknown, it should be stressed that this correction factor should be considered to be a rough estimate of the true value. However, the contribution of aggregation to the attenuation in the low MHz range is not expected to be very significant.

6.2 Results and discussion

Figure 6.2 shows simulated backscattered data obtained for a flow velocity of 2.4 cm/s and at points 20D, 40D and 60D downstream of the tube entrance. No attenuation factor has been added in this figure, and the data has been corrected such that the lowest value is set to 0 dB. For ease of comparison, all data in Figs. 6.2-6.7 has been similarly corrected without added attenuation unless otherwise noted. In Fig. 6.2, very high echogenicities can be observed near the tube walls, which occur due to insonating pulse entering into and exiting from the tissue sample of the insonating pulse. The echogenicity across the tube diameter is asymmetric; i.e. the echogenicity at a point on the side of the tube axis closest to the near wall is higher than at a point an equal distance away from the tube axis on the side closer to the far wall. This can be accounted for by the $1/|R|$ dependence of the backscattered signal, as described by Eq. 2.1.4, where $R$ is the transducer-scatterer separation. Time-gain (also known as depth-gain) compensation is usually employed in modern ultrasound imaging systems, but was not applied to these backscattered signal simulations, since it was also not used in the experiments of Shehada et al. (1994). It can be observed that a “black hole” becomes more distinct (narrower and “deeper”) as the distance from the tube entrance increases. As already
hypothesized in Chapter 4, this is most likely due to the shear histories of the different streamlines, where particles at radial positions intermediate to the tube center and tube walls have had sufficient time to aggregate significantly. This figure may be compared to the experimental results of Shehada et al. (1994) in Fig. 6.3, which show the echogenicity profiles across the tube for a mean flow velocity of 2.4 cm/s at distances of 35D, 45D and 60D from the tube entrance, obtained by ensemble averaging 10 consecutive digitized B-mode images. According to Shehada et al., acoustic reverberation effects from the near wall caused artifacts, which masked the RBC backscattered signals from the near wall to a depth of about 0.5 cm below. It also may have caused some interference with the signal just below this depth, resulting in an asymmetry in the echogenicity profile. Asymmetry due to the $1/|\mathbf{R}|$ dependence of the backscattered signal and attenuation effects may be present, but is not obvious. Similar to the simulation results, Fig. 6.3 shows that the "black hole" becomes more distinct as the distance from the tube entrance increases. Quantitatively, the "black hole" in the experimental result for 60D appears to be approximately twice the depth of that of the simulation results at its deepest. Figure 6.4 shows the simulated echogenicity profile at 60D from the tube entrance with and without the -1.6 dB/cm attenuation correction. It can be readily observed that the effect of the attenuation correction factor is to increase the asymmetry of the echogenicity profile. Shehada et al. left their echogenicity data in uncorrected form since the degree to which attenuation was affected by aggregation was unknown.

Figure 6.5 shows the echogenicity profiles at 60D from the tube entrance for flow velocities of 1.4, 2.4 and 5.2 cm/s. Similar to the experimental results of Shehada et al. (1994), it can be observed that the black hole is more distinct for the slower flow velocities, which can again be explained as a result of shear rate histories. At higher flow velocities, the transit time of particles is insufficient for significant aggregation to occur over the distances of interest, and higher shear rates than at low flow velocities also enhance disaggregation forces. Notice the manner in which the echogenicities at intermediate distances between the tube walls and tube center increase, as the average flow rate is decreased, in contrast to Fig. 6.2 where the echogenicity increases are due to increases in distance from the tube entrance. Figure 6.2 demonstrates the effect of transit time on echogenicity, whereas Fig. 6.3 shows the
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Fig. 6.2. Simulation results showing the average backscattered power profiles across the tube for a mean flow velocity of 2.4 cm/s, Hct=28, 250 realisations, at 20D, 40D and 60D from the entrance of the tube.

Fig. 6.3. Experimental results from Shehada et al. (1994) showing the echogenicity profiles across the tube for a mean flow velocity of 2.4 cm/s, Hct=28, at 35D, 45D and 60D from the entrance of the tube. The crosshatched region indicates the locations where reverberation caused major artifacts.
Fig. 6.4. Simulation results showing the average backscattered power profiles across the tube for a mean flow velocity of 2.4 cm/s, Hct=28, 250 realisations, at 60D from the tube entrance, with and without correction for attenuation of 1.6 dB/cm.

Fig. 6.5. Simulation results showing the average backscattered power profiles across the tube at 60D from the tube entrance, Hct=28, 250 realisations, for mean flow velocities of 1.4, 2.4 and 5.3 cm/s.
effect of changing shear rate profiles.

Finally, Fig. 6.6 shows a plot of the B-mode data for all three flow velocities at 60D as a function of shear rate - the shear rates were easily calculated from the radial distances and the power law velocity (shear rate) profile. The high echogenicity artifacts near the tube walls (and therefore incorrectly interpreted as associated with high shear rates) have been cropped. Figure 6.6 shows good qualitative agreement with Shehada et al.'s echogenicity (B-mode) data, shown in Fig. 6.7. The relative positions of the echogenicity peaks and shape of the curves are similar, although there are discrepancies in actual magnitudes between the two figures.

### 6.3 Chapter Summary

Two-dimensional simulations have been carried out, integrating the voxel method for calculating the backscattered ultrasound signal, the shear-dependent aggregation model, and the study of aggregate size distributions and packing statistics. Although it should be emphasized that these simulations made use of a first-order model of shear-dependent aggregation and a method of determining packing statistics which did not model the effect of flow conditions, realistic results having reasonable qualitative agreement with experimental data were still obtained. Suggestions for further work will be made in the following chapter.

It is clear from the simulation work presented in this chapter that the relationship between aggregation and backscattered ultrasound signal is very complex. Aggregation size distributions are dependent on flow conditions, aggregating mechanisms and structures, and particle packing is in turn dependent in a nonlinear way on aggregate size and aggregate size distributions. It can be hypothesized that tissue samples having the same average aggregate size but different size distributions can display very dissimilar packing statistics and backscattered ultrasound signals. This makes the task of extracting aggregation information only from the backscattered signal a very difficult one.

*It is not that representation now dominates or effaces the referent, but rather that it now self-consciously acknowledges its existence as representation - that is, as interpreting (indeed creating) its referent, not as offering direct and immediate access to it.*

(The Politics of Postmodernism, Linda Hutcheon)
Fig. 6.6. Simulation results showing the average backscattered power as a function of shear rate, 60D from the tube entrance, Hct=28, 250 realisations, for mean flow velocities of 1.4, 2.4 and 5.3 cm/s.

Fig. 6.7. Experimental results from Shehada et al. (1994), showing the backscattered ultrasound power (echogenicity) versus shear rate for mean flow velocities of 1.4, 2.4, 5.2 cm/s, Hct=28, at 60D from the entrance of a tube with a diameter of 2.54 cm. Data has been corrected such that the lowest value is set to 0 dB.
Chapter 7

Conclusions

Lady Bracknell: I have always been of the opinion that a man... should know either everything or nothing. Which do you know?
Jack (after some hesitation): I know nothing, Lady Bracknell.
Lady Bracknell: I am pleased to hear it. I do not approve of anything that tampers with natural ignorance. Ignorance is like a delicate exotic fruit; touch it, and the bloom is gone.
(The Importance of Being Earnest, Oscar Wilde)

7.1 Conclusions

Although past investigators (Angelson, 1980; Mo and Cobbold, 1993; Bascom and Cobbold, 1995) have established that the backscattered ultrasound signal is directly related to local variations in particle concentration, the exact relationship between the local concentration variance (and therefore, the backscattered signal) and RBC aggregation is a complex one. Aggregate size, shape and size distribution all affect the packing of RBCs, and are themselves dependent on such factors as flow conditions, the aggregation mechanism, RBC size and shape, and the hematocrit. This study has been an initial attempt to provide a better understanding of the relationship between the ultrasound signal and RBC aggregation. It has hopefully provided the foundation from which a more accurate model of the effects of RBC aggregation on the ultrasound signal can be obtained, and this may ultimately enable additional information of diagnostic value to be extracted.

A brief discussion of the hypothesized mechanisms and clinical significance of RBC aggregation was presented in Chapter 1, as well as a survey of various methods used for studying aggregation. From the discussion of the clinical significance, it was clear that the
study of aggregation is important as part of fundamental studies of hemodynamics in the circulatory system.

Methods for simulating CW ultrasound imaging of tissue, previous models of RBC aggregation kinetics, models relating the backscattered ultrasound signal to scatterer packing, and the results of past ultrasound studies of blood flow and aggregation were discussed in Chapter 2. The purpose of these discussions was to provide the background for the work presented in the rest of this thesis. The simple but efficient voxel method was described and compared to the particle method, and the theoretical basis for applying the voxel method to the simulation of pulsed imaging was presented. Bascom and Cobbold’s (1995) fractal packing model for describing the relationship between the backscattered ultrasonic power from blood and the manner in which RBCs are packed was briefly discussed. Of particular relevance to the work of this thesis was the capacity of the fractal model to explain the impact of flow conditions, RBC and aggregate shape on the backscattered signal in terms of packing symmetries. Finally, the results of past ultrasound studies were presented, with special emphasis on the experiments with blood flow in a long, large-diameter tube performed by Shehada et al. (1994), which have come to be referred to as the “black hole” experiments. This study is important for illustrating the time- and shear rate dependence of aggregation and was the primary source of comparison for many of the simulation studies in this thesis.

Chapter 3 presented the work done to validate the voxel method for simulating pulsed ultrasound imaging of tissue. The relationship between the accuracy of simulation results, computational burden and choice of voxel size was investigated. Considerable reductions in computational burden over the particle approach were achieved for 2-D simulations, and preliminary simulations were performed to show similar reductions for 3-D tissue volumes.

A theoretical model of shear-dependent aggregation was presented in Chapter 4. This simple first-order model incorporates equations describing particle collision rates, collision efficiency or sticking probability, and disaggregating shear force, in order to track changes in aggregate size distributions over time for a given shear rate. The effect of hematocrit, shear rate, aggregating adhesive strength, aggregate shape (fractal dimension) and collision efficiency on aggregate size was investigated. This model was then used to simulate blood flow in a long, large-diameter tube and qualitative comparisons of the results were made to
the work of Shehada et al. (1994). Although direct quantitative comparisons could not be made, good qualitative agreement was obtained, demonstrating the usefulness of this model.

A simple simulation study of the relationship between RBC aggregation and packing statistics was presented in Chapter 5, as an initial attempt to bridge the gap between aggregation and the backscattered ultrasound signal. In this chapter, small tissue sample volumes were simulated to investigate the impact of aggregate size, shape and size distribution on RBC packing statistics (and hence, on the backscattered signal). Although it is assumed that the shape and orientation of aggregates is heavily dependent on flow conditions, this initial study made no attempt to directly track aggregate formation due to the complexity of the problem. It is to be emphasized that the shear-dependent aggregation model presented in Chapter 4 tracked changes in aggregate size distributions only in a statistical manner, rather than in a direct tracking approach. Other simulation approaches are proposed in the last section of this chapter. Despite its shortcomings, this study yielded results which were useful for illustrating the importance of hematocrit and aggregate size distribution characteristics (in particular, maximum aggregate size) to RBC packing.

Finally, Chapter 6 endeavoured to integrate the disparate parts of this thesis: aggregate size distributions were obtained from simulations of blood flow in a long, large-diameter tube, packing statistics were calculated from simulations of tissue sample volumes, and the backscattered ultrasound power from blood was then simulated using the calculated packing statistics. The results of these simulations were compared to some experimental results obtained by Shehada et al. (1994), and good qualitative agreement was found.

### 7.2 Summary of contributions

The contributions of this thesis are as follows:

1. A computationally efficient and accurate method for simulating the backscattering of pulsed ultrasound from tissue has been implemented and validated.
2. A first-order model of RBC aggregation in shear flow has been developed which incorporates the effects of shear rate, aggregate adhesion strength, collision efficiency, hematocrit, and aggregate fractal dimension. This model has been shown to be useful for explaining and understanding some interesting experimental results.
3. An approximate initial study of the effect of aggregate characteristics on particle packing, and hence, the backscattered ultrasound signal, has been carried out. Average aggregate size, hematocrit, aggregate size distribution, and aggregate shape were the most important factors studied.

4. Aggregate distributions in laminar tube flow have been simulated using the shear-dependent aggregation model. This was used to determine spatial variations in packing, and this enabled the backscattered ultrasound signals to be simulated. The good qualitative agreement between the simulated signals and experimental results suggests that the approach and assumptions used in this approximate model are reasonable.

7.3 Suggestions for future work

7.3.1 Simulating ultrasound imaging of tissue
The backscattered ultrasound signal has been simulated in this work by assuming tissue insonation by a simple unfocused piston transducer, an incident beam which is uniform over the sample volume width (i.e. no beam apodization), and a symmetrical Gaussian-shaped sinusoid pulse. While the objective of demonstrating in principle the validity of the voxel method was attained, more realistic tissue imaging simulations would perhaps make use of linear array transducers whose beams can be steered and focused (Crombie et al., 1997), more realistic incident pulses, and processing techniques widely used in modern medical ultrasonic imaging systems such as time-gain compensation (TGC).

7.3.2 Modeling of RBC aggregation in shear flow
The theoretical model of RBC aggregation in a shear flow developed in this work is a first-order model, leaving much room for further refinement. Some aspects which may be incorporated into this aggregation model include an explicit dependence on the macromolecule concentration and weight, non-Newtonian effects of aggregation on the flow velocity profile, and the dependence of aggregate fractal dimension on shear rate, aggregate size and adhesive strength.

The dependence of the fractal dimension (aggregate shape) on shear rate, aggregate size and adhesive strength is of particular interest because it is the structure of the aggregates
which affect the manner in which they pack and therefore affect the non-Newtonian behavior of blood. The fractal dimension itself may convey information about the aggregation process (Kolb and Jullien, 1984; Mills, 1985; Meakin, 1988). For example, at very low shears RBCs may "roll" over each other to possibly fill gaps and form very dense, compact aggregates, or they may stick immediately upon collision and form very irregular, branched networks of aggregates. As well, the shape and size of aggregates and their orientation in a shear flow would affect the rate and manner in which they break up.

As mentioned in section 2.2.1, the effect of macromolecule concentration and size on aggregation has been extensively studied by Chien (1975), and it is possible that this relationship might be incorporated into the aggregation model in a straightforward manner.

In applying this model to simulations of blood flow in a large tube, it was assumed that the velocity profile did not change (i.e. that the velocity and shear were constant over the width of each voxel) and that the small volumes of RBCs and aggregates that were tracked traveled along laminar streamlines. Goldsmith has shown that lateral migration of RBCs across streamlines is possible due to a tubular pinch effect which arises from fluid inertia, as well as due to particle deformation (Goldsmith, 1993). The lateral migration is appreciable for flow in small tubes (tube diameters on the order of 10 times the diameter of a RBC or less), which suggests that the simple assumption of laminar flow is inadequate when applying the aggregation model to simulations of flow in small tubes. As well, the viscous effects of adjacent streamlines which give rise to the development of laminar flow should be considered. Although it has been shown through MRI studies (Mo et al., 1991) that the hematocrit is constant over the tube, lateral migration of particles might still be incorporated into the model by ensuring that the rate at which particles (the number of RBCs, whether aggregated or not) enter a given voxel from adjacent streamlines exactly equals the rate at which particles exit the voxel so that the hematocrit (mass) is conserved.

7.3.3 Packing statistics and aggregation

In the study of aggregation and packing statistics presented in this thesis, only a few 3-D simulations were completed due to their computationally intensive nature. Although many useful insights were gained from the results of the 2-D simulations, a more complete investigation would use 3-D simulations to study the effect of aggregate size, shape and size
distribution. To study the effect of the shape of large aggregates on packing, another 2-D simulation which might be performed is the creation of monodisperse distributions of aggregates consisting of more than 4 RBCs.

Of fundamental interest is the effect of the shape of individual RBCs on packing statistics - RBCs differ in shape between species, which may affect the degree of aggregation and the aggregate structures which are characteristic of those species. Simple computer simulations using non-spherical particles could be performed.

It was assumed in the study of aggregation packing statistics presented in this thesis that the positions of the aggregates were uniformly distributed. Since particle packing realistically depends on flow conditions, molecular dynamics (MD) type simulations, though extremely computationally intensive, should be used to obtain more accurate results.
References


References


Appendix A

Simulating RBC Aggregation

Equation (2.2.1) gives the rate equation for the formation of aggregates of \(i+j\) RBCs from collisions between aggregates of \(i\) and \(j\) RBCs:

\[
\frac{\Delta n_{i+j}}{\Delta t} = \alpha K_{ij} n_i n_j. \tag{2.2.1}
\]

To track the progress in concentration of each aggregate size along a single shear streamline, a 4th-order Runge-Kutta algorithm (Press et al., 1988) was used. The concentration of aggregates formed from collisions between aggregates of \(i\) and \(j\) RBCs at time \(t_n\) (i.e. after the \(n\)-th time step \(\Delta t\)) is denoted by \(n_{i+j}^n\). The concentration at time \(t_{n+1}\), \(n_{i+j}^{n+1}\), is calculated from

\[
n_{i+j}^{n+1} = n_{i+j}^n + \frac{g_1}{6} + \frac{g_2}{3} + \frac{g_3}{3} + \frac{g_4}{6}
\]

where

\[
g_1 = f(t_n, n^n) \Delta t, \tag{A.2}
g_2 = f(t_n + \Delta t/2, n^n + g_1/2) \Delta t, \tag{A.3}
g_3 = f(t_n + \Delta t/2, n^n + g_2/2) \Delta t, \tag{A.4}
g_4 = f(t_n + \Delta t, n^n + g_3) \Delta t. \tag{A.5}
\]

The rate equation (Eq. 2.2.1) is denoted in Eqs. A.2 to A.5 by the shorthand notation \(f(\cdot)\), and \(n^n\) is shorthand notation denoting \((n_1^n, n_2^n, \ldots, n_i^n, \ldots, n_j^n, \ldots, n_{i+j}^n, \ldots)\); i.e. the concentrations of each aggregate size at time \(t_n\). It can be observed that this method makes use of “trial” results at different points across the time step \(\Delta t\) to obtain the “real” results at the end of the time step.

A.1 Single streamline simulations

In these simulations the value of \(G\), the shear rate, is fixed. This sample calculation will use \(G=1.0\ \text{s}^{-1}\) and \(d_f=2.4\).

Consider a voxel of dimensions \(\lambda/5 \times \lambda/5 \times \lambda/5\), where \(\lambda=clf\). For hematocrit \(h\), the voxel initially contains \(n_i \Delta V = h \Delta V / V_p\) disaggregated particles, where \(n_i\) is the concentration (\# aggregates/m\(^3\)) of aggregates consisting of 1 particle, \(\Delta V\) is the voxel volume and \(V_p\) is the...
particle volume. For \( c=1540 \text{ m/s}, f=5 \text{ MHz}, \Delta V=(\lambda/5)^3, V_p=90\times10^{-18}, \) and \( h=30\%, n_i^0\times \Delta V=779 \text{ particles.} \)

From Eq. 2.2.2, \( K_{ij} = \frac{4Ga_o^3}{3} [i^{l/d_F} + j^{l/d_F}]^3 \) gives us

\[
K_{1,1} = \frac{4(1)(2.78 \times 10^{-6})^3}{3} [1 + 1]^3 = 2.29 \times 10^{-16} \text{ m}^3/\text{s},
\]

\[
K_{1,2} = K_{2,1} = \frac{4(1)(2.78 \times 10^{-6})^3}{3} [1 + 2^{1/2.4}]^3 = 3.65 \times 10^{-16} \text{ m}^3/\text{s},
\]

\[
K_{1,3} = K_{3,1} = \frac{4(1)(2.78 \times 10^{-6})^3}{3} [1 + 3^{1/2.4}]^3 = 4.92 \times 10^{-16} \text{ m}^3/\text{s}, \text{ and so on.}
\]

From Eq. 2.2.4,

\[
\alpha = k \left( \frac{A}{36\pi \mu_p G a_o^3 i^{3/d_F}} \right)^{0.18} = (1.0) \left( \frac{10^{-20}}{36\pi 10^{-3} (1.0)(2.78 \times 10^{-6})^3 (1.0)} \right)^{0.18} = 0.372.
\]

From Eq. 4.2, the blood viscosity \( \mu \) at this shear rate is

\[
\mu = \frac{(\sqrt{k_1} + \sqrt{k_2} G)^2}{G} = \frac{(\sqrt{0.05} + \sqrt{0.04(1.0)})^2}{(1.0)} = 0.1794 \text{ dyne/cm}^2 = 1794 \text{ dyne/m}^2.
\]

The shear-limited maximum aggregate size at this shear rate is (from Eq. 4.1)

\[
i_{\text{max}} = \left( \frac{F_A}{4k'a_o^2 \mu G} \right)^{d_F} = \left( \frac{10^{-6}}{4(4.3)(2.78 \times 10^{-6})^2 (1794)(1.0)} \right)^{2.4} \approx 31.
\]

We only need to consider collisions between aggregates of up to 31 RBCs in size for these simulation parameters.

At time \( t_0 \), the voxel contains only aggregates consisting of single RBCs; i.e. \( \Delta Vn_i^0 = 779 \) aggregates, \( \Delta Vn_2^0 = 0, \Delta Vn_3^0 = 0, \ldots \Delta Vn_{31}^0 = 0 \). For the first time step \( t_1 \), evaluating \( g_i = f(n_i^0 n^0) \Delta t \) yields:

\[
\Delta V\Delta n_{i+1} = \Delta V\Delta n_2 = \Delta V\alpha K_{i,n_i^0,n_i^0} \Delta t/2
\]

\[= (\lambda/5)^3 (0.372)(2.29 \times 10^{-16})[(779)^2/((\lambda/5)^3)](0.25/2) = 27.65,
\]

which is the change in the number of aggregates of size 2 from collisions between single RBCs, and the factor of 2 is due to collisions between aggregates of the same size. The change in number of aggregates of size 1 is given by
\[ \Delta V \Delta n_1 = -2 \Delta V \Delta n_2 = -55.3. \]

To evaluate \( g_s(t, \Delta t/2, n^n + g_s/2) \), we calculate trial solutions for the number of aggregates of size 1 and size 2 using the results of \( g_s \) (i.e. \( \Delta V n_1^0 + \Delta V \Delta n_1/2, \Delta V n_2^0 + \Delta V \Delta n_2/2 \)), and calculate solutions for \( \Delta V \Delta n_1, \Delta V \Delta n_2, \ldots, \Delta V \Delta n_3 \). The solutions for \( g_2 \) and \( g_4 \) are similarly calculated. For the first time step, this yields:

\[
\begin{align*}
g_1: \Delta V \Delta n_1 &= -55.3, \quad \Delta V \Delta n_2 = 27.6, \\
g_2: \Delta V \Delta n_1 &= -26.4, \quad \Delta V \Delta n_2 = 12.2, \quad \Delta V \Delta n_3 = 0.6, \\
g_3: \Delta V \Delta n_1 &= -27.1, \quad \Delta V \Delta n_2 = 13.1, \quad \Delta V \Delta n_3 = 0.3, \\
g_4: \Delta V \Delta n_1 &= -52.8, \quad \Delta V \Delta n_2 = 24.5, \quad \Delta V \Delta n_3 = 1.2,
\end{align*}
\]

and from Eq. A.1, the solutions for \( \Delta V n_i^t, \Delta V n_i^t, \ldots, \Delta V n_3^t \) are obtained:

\[
\begin{align*}
\Delta V n_1^t &= \Delta V n_1^0 + (-55.3)/6 + (-26.4)/3 + (-27.1)/3 + (-52.8)/6 = 779 + (-35.8) = 743.2, \\
\Delta V n_2^t &= 17.1, \quad \text{and} \\
\Delta V n_3^t &= 0.5.
\end{align*}
\]

This procedure is repeated for as many time steps as are required.

The aggregate size distribution for a voxel may be examined by plotting the frequency of occurrence of a particular aggregate size versus the range of possible aggregate sizes (e.g. Fig. 6.1). The average aggregate size for a voxel at time \( t_n \) may be calculated from:

\[
i_{\text{ave}} = \frac{\Delta V \sum_{i=1}^{\text{max}} n_i^t}{\Delta V \sum_{i=1}^{\text{max}} n_i^t} = \frac{\Delta V n_1^0}{\Delta V \sum_{i=1}^{\text{max}} n_i^t}
\]

and plots of average aggregate size \( i_{\text{ave}} \) versus time can then be constructed (e.g. Fig. 4.2).

In the sample calculation above, the average aggregate voxel size after one time step is \( i_{\text{ave}} = (743.2)+(2\times17.1)+(3\times0.5)/(743.2+17.1+0.5) = (779/760.8) = 1.02 \text{ RBCs/aggregate}. \)

**A.2 Large tube simulations**

In these simulations, the value of \( G \) is determined by the radial position of the voxel under consideration and the shear rate profile (Eq. 4.4). Consider cubic voxels with \( \lambda/5 \approx 61 \mu \text{m} \) sides. If we consider 60 voxels evenly spaced across half of the tube diameter (119 voxels in total across the tube diameter of \( D=2.5 \text{ cm} \)), the distance between voxels is about 210 \( \mu \text{m} \).

Supposing we want to track the progression of aggregation in the 20th voxel from the tube center (i.e. the streamline at a radius \( r=19\times210 \mu \text{m}=3.99 \text{ mm} \) up to an axial distance
60D (=1.5 m) from the tube entrance, we start by calculating the flow velocity $\nu$ and shear rate $G$ associated with this streamline.

From Eqs. 4.3, 4.4 and 4.5, and assuming a power law exponent $n=2.8$ and a mean flow velocity of 2.4 cm/s, we obtain

\[
\nu_{\text{max}} = \nu_{\text{mean}}(n+2)/n = (2.4)(4.8)/2.8 = 4.11 \text{ cm/s},
\]

\[
\nu(r) = \nu_{\text{max}}[1-(r/R)^n] = 4.11[1-(3.99\times10^{-3}/1.25\times10^{-2})^{2.8}] = 3.94 \text{ cm/s}, \text{ and}
\]

\[
G(r) = \nu_{\text{max}}n(r^n/R^n) = (4.11\times10^{-2})(2.8)((3.99\times10^{-3})^{1.8}/(1.25\times10^{-2})^{2.8}) = 1.18 \text{ s}^{-1}.
\]

For the voxel to travel a distance of 1.5 m downstream of the tube entrance, a transit time of $1.5/\nu(r) = 1.5/(3.94\times10^{-2}) = 38.07$ s, which would require 152 time steps for $\Delta t = 0.25$ s. The aggregate size distribution at 60D ($n^{i52} \Delta V$, $n^{i52} \Delta V$, ...) and average aggregate size $i_{\text{ave}}$ may now be computed following the procedure described in section A.1 with $G=1.18 \text{ s}^{-1}$.

If this procedure is repeated for the other 118 voxels across the tube diameter, we can obtain the aggregate size distribution and average aggregate size for each voxel. The average aggregate size may then be plotted as a function of radial distance from the tube center (e.g. Fig. 4.6) or as a function of shear rate (e.g. Fig. 4.8) since each voxel is associated with both a radial distance and a shear streamline.
Appendix B
Packing Simulations

B.1 Finding the variance of a simulated tissue sample

The basic algorithm for carrying out the simulations described in Chapter 5 is described below (2-D case, single particle aggregates). Specific data structures are not described, but appropriate data structures are assumed for storing particle positions and the number of particles found in each voxel.

1. Consider a $\lambda \times \lambda$ box.

   The box dimensions are calculated from $\lambda = c/f$. For $c=1540$ m/s, and $f=5$ MHz, $\lambda=308$ $\mu$m. The coordinates of the box’s vertices are $(x,y)=(0,0), (\lambda,0), (0,\lambda), (\lambda,\lambda)$.

   The particles to be placed are circles, with radii $a_0=2.78$ $\mu$m (the radius of a sphere having a volume equivalent to a human RBC).

2. For a given hematocrit $h$ (area fraction), calculate $N$, the number of circles to be generated:

   Hematocrits under consideration range from 0 to 40. If we consider the case of $h=20$, then the number of circles to be placed is $N = \frac{h}{100} \times \frac{\lambda \times \lambda}{\pi a_0^2} \approx 781$.

3. Generate circle centre coordinates $(x,y)$ until $N$ non-overlapping circles are obtained.

   No part of any circle may be outside of the box: i.e. the circle centre $x$- and $y$-coordinates are individually generated using a uniform random number generator (Press et al., 1988) such that the $x$- and $y$-coordinates are uniformly distributed over $[a_0, \lambda-a_0]$.

   No circle may overlap with any other circle. This entails checking that a newly generated circle centre $(x_i, y_i)$, the $i$-th circle to be placed, is no closer than $2a_0$ from the centres of the $(i-1)$ circles which have already been placed. An efficient algorithm for doing this is described in section B.2. See figure B.1.
4. **Subdivide the box into voxels for calculating the average number of circles per voxel.**

Voxels with dimensions of $\lambda/10$ give us a reasonable number ($N_{\text{voxel}}=10\times10=100$) of voxels with which to calculate the concentration variance, and are large enough so that they will contain, on the average, more than one particle even at hematocrits less than 10%. For the case of $h=20$, the average number of particles per voxel, $n_{\text{ave}}$, is 7.81 ($N/100$).

5. **Calculate the number of circles per voxel, and then the variance.**

Having stored the circle centre coordinates $(x,y)$, the number of circles per voxel $N_{v}^{l}$ (where $l=1..N_{\text{voxel}}$) may be easily found by checking each circle's coordinates with the voxel boundaries. A circle is considered to be contained within a voxel if its centre coordinates are inside the voxel's boundaries. See figure B.2.

The variance in the number of circles per voxel, $\text{Var}$, is then found by calculating

$$\text{Var} = \frac{1}{N_{\text{voxel}}-1} \times \sum_{l=1}^{N_{\text{voxel}}} (N_{v}^{l} - n_{\text{ave}})^{2}$$  \hspace{1cm} (B.1)

where $N_{\text{voxel}}$, $N_{v}^{l}$ and $n_{\text{ave}}$ have been defined above. By carrying out multiple realisations for a given $h$, the variance can be calculated for each realisation and an average variance (and standard deviation for the average variance) can be found.

By repeating steps 1 to 5 for hematocrits in the range of 0 to 40%, graphs of variance versus hematocrit can be constructed (e.g. Figure 5.6). Tissue distributions consisting of aggregates containing more than 1 particle are more complicated to implement (as described in Chapter 5), but the basic algorithm and constraints are the same (i.e. no particle may overlap with any other).

**B.2 Checking for particle overlap**

A voxel-based method for determining whether a newly generated circle $(x_{i},y_{i})$ overlaps any of the $(i-1)$ circles which have already been generated is described below. A tissue sample of dimensions $\lambda\times\lambda$ m is assumed, as are appropriate data structures which store the circle positions and the number of circles stored in each voxel. In this example, $\lambda = \frac{c}{f} = 308 \, \mu m$ for $c=1540 \, m/s$ and $f=5.0 \, MHz$, and $a_{0} = 2.78 \, \mu m$. 
1. The $\lambda \times \lambda$ tissue sample is assumed to be subdivided into voxels having dimensions of $\lambda/40$.

Voxels of this size can contain at most $(\lambda/40)^2/(\pi a_0^2) \approx 2$ circles. There are $N_{\text{check}} = 40 \times 40 = 1600$ non-overlapping voxels of this size in the tissue sample.

2. Determine the voxel $j (j=1..N_{\text{check}}$) which contains the generated circle position $(x_i, y_i)$.

Calculating $x_{\text{vox}} = x_i/(\lambda/40)$ and $y_{\text{vox}} = y_i/(\lambda/40)$ and rounding down to the nearest integers allows us to obtain the index $j = (y_{\text{vox}} - 1) \times 40 + x_{\text{vox}}$ for the appropriate voxel within the voxel grid (see figure B.3).

3. Determine the voxels which are immediately adjacent to voxel $j$.

The 8 voxels adjacent to voxel $j$ are voxels $(j-1), (j+1), (j+39), (j+40), (j+41), (j-41), (j-40)$ and $(j-39)$. If voxel $j$ is close to the edge of the tissue sample, the number of adjacent voxels will be reduced.

4. Check the circles in the adjacent voxels for overlap with circle position $(x_i, y_i)$.

Only circles within the immediately adjacent voxels will potentially overlap with the newly generated circle position $(x_i, y_i)$. An overlap occurs when $(x_i, y_i)$ comes within a distance $2a_0$ of another circle centre. Since each voxel contains at most 2 circles, and there are a maximum of 8 adjacent voxels, at most 16 previously generated circle positions need to be checked. If an exhaustive checking algorithm were employed (i.e. a newly generated circle position is compared to every previously generated circle), up to an average of about 580 comparisons per new circle would need to be made for a hematocrit of 30% and $\lambda \times \lambda$ tissue sample.
Figure B.1 Schematic of a simulated tissue sample with $N=46$ non-overlapping particles.

Figure B.2 Tissue sample from fig. B.1 subdivided into voxels ($\lambda/4$, for illustrative purposes). In this example, $n_{\text{ave}} = (46/16) = 2.875$, and $Var = 21.75/15 = 1.45$ (for $N_i = 3, 2, 5, 3, 5, 2, 3, 1, 2, 5, 2, 3, 2, 2, 3, 3; i=1..16$).

Figure B.3 Schematic illustrating voxel-based method of checking for particle overlap. Only particles within the immediately adjacent voxels (in this example, voxel # 42, 43, 44, 82, 84, 122, 123, 124) need to be checked.