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“Measurement of Microvascular Blood Flow in skin and Skeletal Muscle Using Ultrasound Contrast Agents and a Negative-Bolus Indicator-Dilution Technique”

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A thesis submitted in conformity with the requirements for the degree of Master of Science, Graduate Department of Medical Biophysics, University of Toronto.

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Measurement of Microvascular Blood Flow in skin and Skeletal Muscle Using Ultrasound Contrast Agents and a Negative-Bolus Indicator-Dilution Technique


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Abstract

Introduction: Clinical measurement of blood flow at the capillary level remains a challenging problem. The introduction of microbubble contrast agents and their specific ultrasound detection schemes may make measurements at this vessel level possible. We describe a method for quantitative microvascular blood flow measurement, utilizing ultrasound imaging and microbubble contrast agents, with emphasis on application in reconstructive microsurgery. The method is tested in bench-top phantoms, and an in-vivo model of skin perfusion.

Methods: Microbubble agents are inherently susceptible to disruption by high-power ultrasound pulses. Disruption was combined with ultrasound imaging to measure flow based on indicator-dilution theory. Implementation on a clinical scanner allowed in-vitro testing in vessel phantoms (7mm and 100μm diameter) while in-vivo measurements were done on a porcine skin model and compared to results obtained by radioactive microsphere injection.

Results: In-vitro, ultrasound flow measurement and actual flow were found to be well correlated ($r=0.98$), while in-vivo good agreement was also found between the ultrasound technique and microsphere injection ($r=0.78$).
# Chapter 1: Blood Flow Measurement

## 1.1 Introduction

## 1.2 Terminology: What is blood flow?

## 1.3 Motivation - Why measure blood flow?

1.3.1 Microvascular surgery & tissue flaps:

1.3.2 Current free flap monitoring options:
- Physical examination:
- Tissue temperature:
- Dye staining & fluorometry:
- Tissue \( O_2 \) tension:
- Tissue pH:
- Plethysmography:
- Ultrasound & laser Doppler:
- Electromagnetic flowmetry:

1.3.3 Therapeutic intervention options:
- Surgical options:
- Pharmacologic interventions:

## 1.4 Function and morphology of the microcirculation

## 1.5 The principles of flow measurement using indicator-dilution

1.5.1 The mean transit time (MTT):

1.5.2 The residue function:

1.5.3 Drawbacks and errors in conventional indicator-dilution measurements:
- Flow is time invariant:
- Tracer is injected as an instantaneous, well defined bolus into the region of interest:
- The tracer is entirely intravascular:
- Tracer is well mixed and uniformly distributed in the blood:

## 1.6 Survey of existing microvascular blood flow measurement techniques

1.6.1 Laboratory methods:
- Labeled microsphere injection
- Tracer clearance

1.6.2 Clinical methods:
- Medical nuclear imaging
- CT
- MR flow detection:

## 1.7 Ultrasound microbubble contrast agents:

1.7.1 Development

1.7.2 Theory

1.7.3 Unique properties: Microbubble resonance and destruction

1.7.4 Standardization of output power: The mechanical index
1.8 Overview of microbubble-specific imaging methods: ........................................ 57
  1.8.1 Introduction to Doppler techniques: .................................................. 57
  1.8.2 Pulse wave Doppler detection: ......................................................... 58
  1.8.3 Clutter rejection .................................................................................. 62
  1.8.4 Color Doppler flow mapping: ............................................................... 62
     1.8.5 Power Doppler flow mapping: ......................................................... 64
  1.8.6 Detecting capillary flow ........................................................................ 65
  1.8.7 Harmonic imaging: ............................................................................ 66
  1.8.8 Pulse-inversion: .................................................................................. 69
  1.8.9 Summary of detection methods ............................................................ 73

1.9 Summary ..................................................................................................... 73

1.10 Appendix A: Pulse wave Doppler signal processing ................................. 76

1.11 Appendix B: Color Doppler velocity estimators ........................................ 80
  1.11.1 The phase shift estimator ........................................................................ 80
  1.11.2 The cross-correlation estimator ............................................................ 82

Reference List .................................................................................................. 86
Chapter 2: **Negative bolus indicator-dilution measurement of microvascular blood flow.**

2.1 **Introduction** .................................................... 90

2.2 **Background** .................................................. 90
   2.2.1 Microbubble contrast agents as vascular indicators: .............. 90
   2.2.2 Flow measurement with a negative bolus of indicator: A theoretical model. .............. 91

2.3 **Methods & Materials** ....................................... 98
   2.3.1 In-vitro ......................................................... 98
      (1) Signal power vs microbubble concentration: .................. 101
      (2) Flow measurement: ........................................... 103
   2.3.2 In-vivo ........................................................ 103
      2.3.2.1 Canine model ............................................. 103
         (1) Steady microbubble concentrations from IV infusion: .......... 104
         (2) Signal power vs microbubble concentration: ................. 104
      2.3.2.2 Porcine skin flap model .................................... 104
      2.3.2.3 Microsphere flow measurement: ............................ 107

2.4 **Results** ...................................................... 108
   2.4.1 In-vitro ......................................................... 108
      (1) Signal power vs microbubble concentration: .................. 108
      (2) Flow measurement: ........................................... 108
   2.4.2 In-vivo ........................................................ 110
      (1) Steady microbubble concentrations achieved with IV infusion: .......... 110
      (2) Signal power vs. microbubble concentration: ................. 110
      (3) Flow measurement: ........................................... 113

2.5 **Discussion** ................................................... 118
   2.5.1 Power Doppler is flow dependent: .................................. 120
   2.5.2 Harmonics and pulse inversion imaging performed poorly in-vivo: .............. 122
   2.5.3 Choice of agent influences flow measurement: .......................... 122
   2.5.4 Relative flow measurement: .................................... 124

2.6 **Conclusions** .................................................. 126

**Reference List** .................................................. 127
Chapter 3: Future work

3.1 Introduction ........................................................................................................ 129

3.2 Changes needed for clinical implementation ..................................................... 129
3.2.1 Bubble destruction and bubble detection: ..................................................... 129
   3.2.1.1 Optimizing bubble destruction ............................................................. 130
   3.2.1.2 Bubble size distribution ..................................................................... 131
   3.2.1.3 Optimizing bubble detection ............................................................. 134
3.2.2 Reducing data acquisition time ...................................................................... 135
   3.2.2.1 Estimating initial slope ...................................................................... 136
   3.2.2.2 Flash-echo real time imaging .............................................................. 136

3.3 Estimating absolute blood flow .......................................................................... 137
3.3.1 Normalizing vascular volumes ...................................................................... 138
3.3.2 Microbubble scintigraphy ............................................................................ 141

3.4 Estimating microvascular anatomy and flow distribution based on the residue
curve: .................................................................................................................... 141

3.5 Summary: .......................................................................................................... 144

Reference List .......................................................................................................... 146
Chapter 1  

Blood Flow Measurement

1.1 Introduction

Primary diseases of the circulation, such as ischemic heart disease, stroke and peripheral vascular disease, are the leading causes of death and morbidity in the developed world. The common pathology to these disorders is insufficient blood flow at the most basic level of the circulation - the capillaries - where nutrient delivery and exchange take place and end organ dysfunction begins. While several non-invasive techniques have been developed for blood flow measurement in large caliber vessels, detection and quantification of flow in vessels smaller than 100μm (the microcirculation) remains difficult. A great deal of research effort has been directed at finding clinically practical methods for the detection and measurement of microvascular blood flow, but the results thus far have been less than satisfactory. Many existing techniques are based, in one form or another, on the principles of indicator-dilution. Strict application of the indicator-dilution method in its classical form, however, is restricted by its highly invasive nature to select applications in the laboratory and critical care setting. Modifications of indicator-dilution for application in MR, CT and nuclear medicine trade off invasiveness for either accuracy or, in the case of the latter, repeatability. The most widely used imaging modality, clinical ultrasound, although safe, non-invasive, portable and widely available, has not, until recently, had an available tracer suitable for application in microvascular blood flow measurement. The introduction of microbubble ultrasound contrast agents and their specifically designed detection methods may finally make the application of indicator-dilution principles at the bed side possible and practical.

This work describes an approach to microvascular blood flow quantification using ultrasound and microbubble contrast agents, based on indicator-dilution theory and the unique properties of microbubbles - a method that will be seen to offer several advantages over existing flow measurement techniques. This chapter introduces the subject of microvascular blood flow measurement, and its
application to a particularly interesting surgical field which constitutes our motivation. It includes a thorough discussion of the principles of indicator-dilution flow measurement theory and concludes with a review of the subject of microbubble contrast agents, ultrasonic detection and image formation. In chapter 2 the principles of flow quantification with microbubbles and ultrasound imaging are developed and then tested in bench-top phantoms as well as in-vivo models of skin and muscle perfusion. It concludes with a discussion of the current limitations of this flow measurement technique. Finally, chapter 3 is a discussion of the implications and future directions of this method, with emphasis on clinical application.

1.2 Terminology: What is blood flow?

There is often a great deal of confusion surrounding the concept of blood flow and the term "perfusion". Despite a great deal of debate, there is little agreement between physicists and physiologists as to the true meaning of either. To some, perfusion means bathing of the tissue in blood, or in other words, simple movement of blood into and out of a given block of tissue. To others, perfusion means the general process of nutrient delivery and waste removal, a process which is only partly dependent on actual blood flow[11]. The former can be measured by cutting the venous drainage channels and allowing the fluid to collect in a graduated cylinder under a stopwatch, while the latter cannot. It is the quantity that can be measured with a graduated cylinder and a stopwatch that we are interested in, and to avoid confusion, the term perfusion will be abandoned in favor of the more precise term "blood flow".

In the study of fluid mechanics, which is based on vector field theory, "flow" refers to "mass flow rate" which is a measure of flux - that is, the mass of a fluid that passes through a defined area per unit time, and has units of \( \text{kg/m}^2\text{/sec} \). In this setting, flow is a field property which is continuous, conserved, and scalable. In physiology, and in this work, 'flow' refers to 'volume flow rate', and is
defined as the volume of an incompressible fluid (whole blood) that flows into a given mass of tissue per unit time, and therefore, has the dimensions \( m^3/kg/sec \) or, as frequently rescaled to, \( mL/100g/min \) \(^2\). This is not the same quantity as defined by the mathematician, and its value is dependent on the size and orientation of the region used for its measurement. It is important to realize, if only for a moment, that it makes no sense to talk about comparing volume flow rates without specifying the region in which it is to be measured. What is implied, often unwittingly, is that the region of measurement is given and unchanged between comparative measurements, either confined to the entire organ, or, in the case of external detection, to the imaging region of interest.

1.3 Motivation - why measure blood flow?

Insufficient blood flow is the common functional basis for the most frequent and lethal diseases affecting the industrialized population. While the list of disorders involving the circulation is long, the most important three are by far ischemic heart disease, affecting the coronary circulation, stroke, affecting the cerebral circulation and peripheral vascular disease, involving primarily the blood supply of the limbs. With an estimated prevalence of 12,000,000 in the US alone, ischemic heart disease is the single largest killer in North America, accounting for just over 1 in every 5 deaths and responsible for an annual mortality of over 475,000\(^1\). Strokes rank just behind heart disease and cancer as the third leading cause of death, causing 1 in every 15 deaths for a total mortality of 160,000 per annum\(^3\). Peripheral vascular disease affects between 10 - 20% of the population between the ages of 55 and 75 years and, over a lifetime, approximately 15 to 20% of those affected will progress to critical limb ischemia, requiring either surgical revascularization or amputation\(^4\). The societal implications of these ischemic diseases alone,

\(^{1}\) Mortality figures quoted are for the United States for the year 1996.\(^{10}\)
in terms of morbidity, mortality and health care burden are, therefore, tremendous.

The term ischemia refers to lack of oxygen specifically caused by inadequate blood supply. The etiology of ischemia is, in general, diverse but in the overwhelming majority of cases it is caused by atherosclerotic disease in the macrocirculation (vessel lumens > 100μm)\textsuperscript{[8]}. By reducing the cross-sectional area of supply vessels, atherosclerotic plaques cause an absolute reduction in end-organ flow at baseline state, or limit the capacity of the circulation to provide additional reserve flow when demand for oxygen increases. Once the obstruction has progressed beyond the ability of the circulation to compensate the result is ischemia, pain, organ dysfunction, tissue necrosis and ultimately, in the case of vital organs such as the heart and brain, death.

Qualitative blood flow detection at baseline activity or during physiologic stress testing has, therefore, been used to detect the presence of ischemic disease, grade its severity and provide decision criteria for medical and surgical intervention\textsuperscript{[6]}. Once treatment is instituted, repeat blood flow measurement can provide an objective assessment of treatment success, particularly following surgical revascularization, and may directly contribute to reduction in morbidity and mortality.

While the primary pathology lies in the macrocirculation, blood flow measurement at this level may not reflect actual cellular blood supply. Several factors are responsible for this discrepancy, the most important of which include: (1) multiple intervening autoregulatory mechanisms that shield capillary flow from fluctuations in large vessel flow, (2) participation of collateral, parallel blood supply networks, and (3) the presence of undetectable disease in the microcirculation\textsuperscript{[6]}. This phenomenon has been best studied in the heart where, since 1962, the gold-standard diagnostic test for the presence and grading of disease has been coronary angiography. In this invasive test, radiographic-opaque dye is injected directly into the coronary circulation while real-time, dynamic X-Ray images of the myocardium are taken, providing high resolution, two-dimensional maps of dye distribution. While images obtained in this way can accurately pin-point the location of large primary lesions, they have been shown to be
poor predictors of functional impairment and disease severity. X-Ray angiography underestimates the anatomic extent of disease in up to 50% of cases when compared to postmortem findings\(^7\), and functionally in as many as 95% of cases when compared to physiological studies of coronary flow reserve performed with intra-coronary ultrasound Doppler\(^8\). Studies such as these have led to the realization that angiography, and large vessel flow measurement in general, do not directly evaluate end-organ function and tissue ischemia\(^6\). Even if flow measurements in large supply vessels could be made with reasonable accuracy, for instance using implantable electromagnetic probes or the non-invasive ultrasound Doppler flowmeter, they provide no information about the spatial distribution of blood flow and the presence of focal disease within the organ itself. The ultimate measure of ischemia, therefore, must be made at the basic units of the microcirculation where oxygen exchange actually takes place - the capillaries.

Cancer research is another field where blood flow measurement has recently found application. Ranked behind cardiovascular disease, cancer is the second largest killer in the industrialized world and is responsible for about 25% of all deaths in North America\(^9\). Investigators are beginning to understand the importance of the blood supply in tumor development and metastasis and the potential of new anti-cancer agents, such as Endostatin, to cause tumor regression by targeting and inhibiting angiogenesis (the formation of new blood vessels) has been the target of much research and media interest. Naturally, blood flow measurement may find application both in cancer research and treatment by providing insight into the process of new vessel formation, aiding tumor detection and diagnosis, and in the direct evaluation of treatment efficacy.

While the significance of microvascular blood flow and its impact on organ dysfunction in the heart, brain and in tumors is clear, this thesis is concerned with flow measurement in transplanted skin and skeletal muscle. Of fundamental importance to the field of Plastic and Reconstructive surgery, this application and its relevance are discussed in the next section.
1.3.1 Microvascular surgery & tissue flaps:

Reconstructive surgery is the specialized surgical branch concerned with the repair of complex wounds, which often result from extensive trauma, burns, and the surgical ablation of cancer and infection. Complex wounds are characterized by either: (1) exposed vital structures such as bone, nerve, tendons, blood vessels or visceral organs, (2) extensive loss of skin or soft tissue, (3) compromised healing ability of the wound site due to either prior irradiation, diabetes mellitus or peripheral vascular disease, and (4) compound tissue loss involving bone, joints, muscle, skin, nerve, tendon or fascia. The goals of reconstructive surgery are to restore function, form and aesthetics to the affected region by replacing, as much as possible, missing tissue components with similar tissue. The replacement tissue is called a “flap” and may contain any combination of skin, fat, muscle, bone, fascia, intestine or any other tissue which may be used for reconstruction. The defining characteristic of a flap, which distinguishes it from a graft, is that the flap survives the transfer based on its intrinsic blood supply (figure 1.1), while a graft must be nourished extrinsically from the wound site.

When a flap is completely detached from its distant donor bed and moved into the wound site the procedure is known as a free tissue transfer, or a “free flap”. Free flaps require that their intrinsic blood supply, supported by only two or three feeder vessels typically 2 to 3 mm in diameter, be anastomosed to local vessels at the wound site using microsurgical technique (figure 1.2). The ability of the intrinsic blood flow to meet the cellular metabolic needs of the tissue and the adequacy of the local blood supply determine whether the flap will survive the transfer. Understanding the anatomy of the circulation in donor tissues and recipient site and the intrinsic and extrinsic physiologic factors that regulate it form the basis of the clinical practice and research investigation of microsurgery. In experienced hands, free flap reconstruction is currently a feasible option in almost every part of the body, with an overall success rate of 90-95%. Still, 5-10% of all flap reconstructions ultimately fail,
Figure 1.1: The anterolateral thigh flap as an example of a skin flap suitable for microsurgical transfer. The anatomic dissection, the donor bed and feeding artery and vein (pedicle) are shown. This flap is ideal when large areas (up to 12x38cm) of skin are required, and can be sensate if the nerves are included in the repair. (a. = artery, n. = nerve, modified from Serafin, D: Atlas of Microsurgical Composite Tissue Transplantation, 1996, W.B. Saunders Co.)
Figure 1.2: The use of the transverse rectus abdominis myocutaneous (TRAM) flap for post-mastectomy breast reconstruction. (a) The vascular anatomy of the abdominal wall which allows the harvesting of both the muscle and its overlying skin territory. (b) The rectus abdominis muscle with its inferior pedicle. (c) The dissection of the muscle with a large, overlying skin island. The entire skin territory is supplied by small perforating branches from the rectus muscle. The muscle is taken in order to carry the blood supply to the skin but also adds additional bulk to the flap which may be used to advantage in contouring. (d) The flap is completely detached, rotated and contoured into position and the pedicle is anastomosed to local vessels. TRAM flap breast reconstruction has become a very popular option in light of the controversy surrounding silicone breast implants. (Modified from Serafin, D: Atlas of Microsurgical Composite Tissue Transplantation, 1996, W.B. Saunders Co.)
and the consequences to the patient can be devastating, often resulting in more complex defects that require repeat surgery, prolonged hospitalization and sub-optimal secondary reconstruction with further functional and anatomic deficits. It is thus of primary importance to prevent extensive tissue loss with the timely recognition and treatment of compromised flaps. To understand how microvascular blood flow monitoring may contribute to successful flap reconstruction, it is necessary to understand the physiological changes that take place during free tissue transfer.

Elevation and detachment of the flap from its original bed disrupts the homeostatic balance present in normal tissue. Division of the arterial and venous pedicles causes immediate sympathetic denervation and a transient ischemic insult to the tissue. Once the flap is set in position and vascular connections are re-established with the local blood supply, a series of circulatory events are set in motion that, over a period of days and weeks, attempt to restore homeostasis. The key events in this chain, based on Kayser and Hodges, are summarized below:

10-24 hrs: A reduction in arterial blood flow is observed in the period immediately following surgery. Congestion and tissue edema in the first 24 hours are marked by dilatation of arterioles and capillaries.

1-3 days: Gradual appearance of vascular anastomoses between the flap and its recipient bed and decrease in tissue edema are accompanied by a slow recovery of total blood flow in the transplanted tissue.

3-7 days: Newly formed anastomoses between the flap and wound bed turn functionally significant and the vessels become reoriented along the longitudinal axis of the pedicle.

7-14 days: Circulatory function between the flap and its bed is well established and hemodynamic pattern approaches the normal, preoperative state. Total blood flow during this period may surpass normal levels and stays elevated until the 3rd post-
operative week.

3-4 weeks: Microvascular anatomic pattern resembles the pre-operative state and total blood flow returns to normal. The flap at this point has achieved 90% of its final, mature circulatory state.

For the transfer to succeed, the donor tissue must be able to tolerate the short period of absolute ischemia intra-operatively as well as a longer period of relative ischemia during the first 24 hours post-operatively. Tolerance to ischemia is determined by resting metabolic activity and depends on tissue type: Skin and subcutaneous tissue, for example, exhibit much greater resistance to lack of oxygen than does skeletal muscle. Reducing tissue temperature has a direct affect on metabolic rate in general, so that cold ischemia is uniformly better tolerated than warm ischemia. Table 1 summarizes the estimated ischemic tolerance of several common flap constituents:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Warm</th>
<th>Cold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin &amp; subcutaneous fat</td>
<td>4-6 hr</td>
<td>&lt; 12 hr</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>&lt; 2 hr</td>
<td>8 hr</td>
</tr>
<tr>
<td>Bone</td>
<td>&lt; 3 hr</td>
<td>24 hr</td>
</tr>
</tbody>
</table>

Once the ischemic tolerance of the tissue is exceeded, irreversible cellular damage takes place to the extent that reestablishment of blood supply may not succeed in reperfusing the tissue. The failure of tissue to perfuse after prolonged ischemia, even after the restoration of adequate blood supply, is called the “no-reflow” phenomenon and was first described in 1968 in association with cerebral ischemia in rabbits[12]. Since then the no-reflow phenomenon has been observed in heart, kidney,
muscle, skin and subcutaneous tissue as well as in free flaps\textsuperscript{11}. The mechanism of no-reflow has not been fully elucidated, but endothelial cell damage secondary to the accumulation of toxic metabolites, particularly oxygen derived free radicals, is believed to play a major role. Toxin accumulation leads to swelling and retraction of vascular endothelium cells, allowing fluid to leak into the extravascular space and exposing basement membrane collagen. Exposed collagen initiates the coagulation cascade, beginning with platelet adhesion, aggregation and fibrin deposition, leading to thrombosis, obstruction of blood flow and microcirculatory collapse\textsuperscript{10}. The initial ischemic insult usually arises in the main arterial and venous pedicles. It may be due to a long delay between flap harvest and anastomosis, extrinsic compression of the pedicle or intrinsic reduction in flow from local tissue reaction and thrombi, or a systemic drop in blood pressure. The end result, however, is the same - inadequate capillary blood flow. Successful salvage of a failing free flap, whether by surgical or pharmacologic means, therefore, requires that rapid intervention take place prior to the onset of irreversible damage. The importance of microvascular monitoring in this setting becomes obvious: Not only could flow measurement be used to continuously monitor the health of the flap post-operatively and alert the surgeon at the earliest onset of vascular compromise, it could also be used to assess the intrinsic blood supply of the wound bed pre-operatively and help to optimize the planning and tissue selection stage. To be useful in such a setting, microvascular blood flow measurement must be: (1) applicable at the bedside or operating room, (2) performed in real-time, (3) accurate, reliable and reproducible, (4) repeatable as necessary without causing cumulative tissue damage or radiation exposure, (5) neither destructive nor highly invasive, and (6) able to assess the entire flap, including those used for reconstruction deep to the skin surface (buried flaps)\textsuperscript{10,11}.

A review of the methods currently used to monitor tissue transfers is presented in the following section. Methods specifically designed to assess flow in the microcirculation are discussed in section 1.6.
### 1.3.2 Current free flap monitoring options:

Several methods have been developed to monitor free flaps post operatively. None of these involves the direct measurement of blood flow, and it is worth noting that as a whole, they have not been found to be particularly reliable and effective in predicting tissue viability. For this reason, no method has yet supplanted the role of physical examination by the surgeon\(^\text{[10]}\).

1. **Physical examination:**

   This is the oldest and most subjective of all assessment techniques. By observing and touching the flap, color, capillary blanching and tissue warmth can be assessed, and by stabbing the flap with a short needle or the tip of a blade, the presence or absence of dermal bleeding can be noted. Failed flaps exhibit blue, mottled coloring, sustained capillary blanching and a surface that is cool to the touch. Dermal bleeding may be slow or absent. Unfortunately, these signs are late manifestations of ischemia that have proven grossly unreliable and irreproducible in a clinical setting\(^\text{[12]}\). Often, clinical exam can only confirm that a flap has progressed beyond its critical ischemia time, and is of little use as a monitoring tool.

2. **Tissue temperature:**

   Temperature monitoring, either at the skin surface or with a deep probe for buried flaps, is a simple, inexpensive and continuous method of monitoring the course of a free flap. However, temperature may be affected by a host of conditions other than vascular compromise, including anxiety, pain and shock, and is subject to environmental fluctuations. Animal experiments have shown that a compromised flap may continue to be warmed by underlying tissue and will not manifest a significant drop in temperature for as long as 24 hours after the onset of ischemia\(^\text{[9]}\). For this reason, temperature monitoring is not used to assess free flaps, with the only exception being replanted digits. Since digits
are supplied by a terminal vascular loop they do not undergo heat exchange with underlying tissue and for this particular application, surface temperature recording has been found to be a sensitive and reliable monitoring tool\cite{10}.

(3) **Dye staining & fluorometry:**

Fluorescine (resorcinolphthalein) is an injectable dye that has been used to assess flap viability for over 50 years. Typically it is injected as a bolus into the circulation and is allowed to leak through the capillaries into the interstitial space. 20 to 30 minutes post injection, the flap is exposed to ultraviolet light and any dye present in the tissue will fluoresce, giving visual, qualitative confirmation of the presence or absence of capillary blood flow\cite{12}. When the test is performed 18 hours postoperatively, the areas of viable tissue and of skin staining have been found to be well correlated, but not when performed 1 hour after flap elevation and anastomosis\cite{13}. Because the test relies on optical detection methods, it has minimal tissue penetration and is suitable for application on skin flaps only. Since Fluorescein remains in tissue anywhere from 8 to 24 hours post administration, the bolus test is not suitable for continuous or even repeated application. Furthermore, fluoresceine administration is not without drawbacks, with side effects including nausea, vomiting and hypotension\cite{10}. A more objective variant of the bolus test is *perfusion dermofluorometry*, a technique that measures tissue fluorescence via a fibre-optic light guide, to obtain a quantitative index which is related to capillary flow. Since smaller doses of fluorescein are used, this test is more appropriate for frequent administration, but is still limited for use on skin flaps only\cite{9}.

(4) **Tissue O$_2$ tension:**

Tissue oxygen partial pressure reflect blood flow, transcapillary exchange and the rate of uptake and utilization in cells. Oxygen tension can be measured with a transcutaneous sensor consisting of a
silver anode and a platinum cathode. When the electrodes are heated to 42°C, the current between them is proportional to the number of oxygen molecules reduced at the cathode, and provides an indirect measure of blood flow. Although this technique can provide quantitative and continuous reading, and has been shown to correlate with ultimate flap survival, it has proven to be a disappointing clinical monitoring tool. Not only is the transcutaneous sensor limited to use on skin flaps only, it has been found to be highly sensitive to residual anaesthetic gas in the first, and critical, 24 hours post operatively. A modification of this technique by Golde and Mahoney involves the implantation of a disposable opto-chemical pO₂ microprobe, or optode, which can be used to continuously monitor buried flaps with minimal tissue invasion. The utility of pO₂ tension as a clinical monitoring tool, however, remains to be demonstrated.

(5) Tissue pH:

As tissue becomes ischemic, it switches from aerobic to anaerobic metabolism, the result of which is the local build up of lactic acid and a reduction in tissue pH. Thus, much like O₂ tension, pH may reflect tissue oxygenation, metabolic activity and the rate of removal of waste products at a single point within the flap. pH monitoring has been studied in animal models with mixed results, and although it is possible to obtain nearly continuous and rapid monitoring with this technique, it also has not found wide spread clinical utility.

(6) Plethysmography:

This is a technique that measures fluid volume by detecting variations in infrared light absorption through the skin, and can be used to detect the presence or absence of a pulsatile wave form in tissue. This method can be combined with oxymetry, the measurement of hemoglobin oxygen saturation by differential light absorption, and is used routinely in modern anaesthesia practice to
monitor systemic oxygenation. Its use in monitoring free flaps, however, has not been validated\textsuperscript{13}. 

(7) **Ultrasound & laser Doppler:**

The essence of Doppler based techniques is the measurement of red blood cell velocity in a small volume of tissue. Their physical foundation lies in the phenomenon observed by C.J. Doppler in 1842, which is that the frequency perceived by a stationary observer of a cyclical energy wave emitted from a moving source, is dependent on the relative velocity between the emitter and source\textsuperscript{14}. To take advantage of the Doppler phenomenon, a source at the skin surface is used to transmit wave energy into tissue at a given frequency, $f_o$, and reflections from moving scatterers (in this case red blood cells) are picked up and analyzed for their frequency shift. In the case of uniform velocity, this frequency shift, also known as the Doppler frequency, $f_d$, is given by the Doppler equation:

$$f_d = \frac{2v \cos \theta}{c} f_o. \tag{6}$$

where $v$ is the velocity of the red blood cells, $\theta$ is the angle between the direction of flow and the direction of wave propagation, and $c$ the propagation speed of the wave in tissue. This equation is only an approximation for the case when $v << c$, which turns out to be reasonable in the case of blood flow for both sound\textsuperscript{15} and light waves\textsuperscript{16}. Thus the frequency content of the signal received from the tissue reflects the distribution of blood velocities within the sample volume while the intensity, or power, of the signal reflects the number of scatterers present. Neither of these quantities, alone or in combination, is a direct measure of actual blood flow, since that would require a-priori knowledge of vessel geometry, including the cross-sectional area and angular orientation.

In the case of ultrasound Doppler, the waves used to interrogate the tissue are mechanical sound
waves with frequencies on the order of 1-10 MHz. At these frequencies, the penetration depth of ultrasound in tissue is about 10 - 15 cm\textsuperscript{14}, so that practically any vessel can be examined. Signal to noise considerations, however, limit the sensitivity of conventional ultrasound Doppler to flow in large vessels only and its primary use is the qualitative detection of a pulsatile wave form in the main vascular pedicle of the flap and not microvascular flow measurement\textsuperscript{11,12}. A simple, self-contained ultrasound Doppler probe can also be used in the pre-operative planning stage to locate and trace the paths of the major blood vessels required for free flap transfer.

Laser Doppler, on the other hand, utilizes electromagnetic waves in the near visible spectrum, and its penetration is limited to approximately 1.5 mm below the skin surface with a region of interest volume on the order of 1 mm\textsuperscript{3}\textsuperscript{11}. The wavelengths of light used allow detection of blood velocity in capillaries, but because of the low penetration depth this is restricted to the subdermal capillary plexus only. The laser Doppler can provide continuous, quantitative readings, and has been shown by some to be well correlated with ultimate skin flap survival\textsuperscript{17}. However, it has also been shown to be highly sensitive to tissue motion and external vibration which is severe enough to limit its clinical usefulness\textsuperscript{10,12}.

In summary, although the Doppler techniques have shown the most promise as flap monitoring tools, they have drawbacks which make them less practical than anticipated: They are each restricted to measuring very small volumes within the flap, neither actually measures true blood flow and while ultrasound Doppler is restricted to the assessment of large vessels, laser Doppler only reflects blood flow in most superficial 1 mm of an exposed flap.

(8) **Electromagnetic flowmetry:**

The electromagnetic flowmeter is an implantable probe that is placed as a cuff around a large blood vessel, and measures flow based on Faraday’s law of electromagnetic induction. In brief,
Faraday’s law states that a conductor moving within a perpendicular magnetic field generates an electrical potential, whose magnitude is proportional to the velocity of the conductor. Blood acts as a moving, or flowing, conductor by virtue of free, charged ions (Na⁺, Cl⁻, HCO₃⁻) carried in plasma[18]. The cuff of the electromagnetic flowmeter places a transverse magnetic field across the blood vessel and houses two opposing electrodes that must be in firm contact with the vessel wall. The flow of blood in the presence of the magnetic field causes an electric potential to develop across the conductive electrodes and the current measured can be calibrated to reflect total blood flow continuously and reproducibly[19].(figure 1.3). The drawbacks of the electromagnetic flowmeter are that it is (1) invasive, requiring implantation at the time of surgery and must either be left in-situ permanently or require re-operation for removal, (2) that it is sensitive to probe motion, since the conductive electrodes must be placed firmly against the vessel wall yet be slack enough not to constrict the vessel[21], and finally, (3) because it evaluates flow in the main pedicle only, it does not reflect ischemic changes at the capillary level until they progress to the critical stage and affect flow upstream.

1.3.3 Therapeutic intervention options:

A monitoring system is of limited use unless suitable therapeutic interventions exist. In the case of failing free flaps, treatment options may be broadly classified as either surgical or pharmacologic:

(1) Surgical options:

Surgical intervention is aimed at the relief of vascular obstruction in the main pedicle (arterial or venous) of the flap. Obstruction to flow may be due to external causes, such as compression of the artery or vein by neighboring structures, tight skin closure or vessel kinking, or due to internal factors,
Figure 1.3: The electromagnetic flowmeter. (a) Electrolytes in plasma flow through a perpendicular magnetic field maintained by the flowmeter probe, inducing a current in a second, mutually perpendicular pair of electrodes in contact with the vessel wall. (b) An actual EM flowmeter probe, designed to be used as a cuff around a blood vessel. (Modified from: Granger DN, Bulkley GB. Ed. Measurement of Blood Flow: Applications to the Splanchnic Circulation. 1981. Williams & Wilkins. Baltimore).
such as poor microsurgical technique resulting in endothelial trauma and thrombosis\textsuperscript{[9]}. Once vascular compromise is recognized and is believed to be amenable to surgical correction, the patient is urgently taken back to the operating room and the surgical site explored for obvious sources of obstruction. The anastomotic region is resected, the thrombi thoroughly irrigated out and the vessels re-approximated. On occasion, it may be necessary to find an alternate local blood vessel, one that may offer a higher flow rate or a more favorable orientation. The literature indicates that up to 60 - 80\% of failing free flaps and digital replants may be salvaged by expedient surgical exploration\textsuperscript{[12]}.

\textbf{(2) Pharmacologic interventions:}

Pharmacologic control of flap physiology is a complex field that has been researched extensively. What follows is a very brief overview of the most promising options, based on Kayser and Hodges\textsuperscript{[10]}:

Intravenous anticoagulants, such as Dextran and Heparin, decrease platelet aggregation and the tendency of blood to thrombose, thereby theoretically increasing flap circulatory patency rate. Thrombolytic agents, such as Streptokinase and Urokinase are able to dissolve existing thrombi in-situ, and may have direct clinical application in failing flaps. The benefits of such therapy, however, must be cautiously weighed against the increased risk of post-operative hemorrhage, which may be life-threatening.

Smooth muscle relaxants, including the calcium channel blockers and other antihypertensive drugs, are able to act on vascular smooth muscle to cause vasodilatation and improve blood flow. Their use has been associated with increased flap survival in animal models, and further research is underway to study these and similar agents, including adrenergic receptor blockers, for use in humans free tissue transfers.

Anti-inflammatory agents, such as corticosteroids, aspirin and allopurinol, target free radical formation and the associated inflammatory cascade that leads to cellular damage and circulatory
collapse. Although these agents have been shown to improve flap survival in animals, their use for this purpose in humans is not yet indicated.

Leeches may not be considered pharmaceutical agents, but their use can be valuable in salvaging flaps under certain conditions. Medical grade leeches, *Hirudo medicinalis*, can be applied to a swollen tissue flap to relieve congestion due to inadequate venous drainage, which may otherwise lead to flap failure. Leeches supplement their mechanical action by injecting *birudin*, a naturally occurring anticoagulant, at the site of application, as well as *hyaluronidase*, which breaks down connective tissue and facilitates the drainage of blood and the spread of anticoagulant. The use of leeches may be associated with infection, most often with *Aeromonas hydrophila* which is the enteric organism leeches use to digest red blood cells.

At present, there is not sufficient data to suggest pharmacologic manipulation can replace urgent surgical exploration as primary treatment for failed tissue transfer. Its role may be, instead, to maximize flap survival by acting as an adjunct to surgery.

1.4 Function and morphology of the microcirculation

In the last section the importance of the microcirculation in reconstructive flap surgery was examined, as were techniques for the indirect evaluation of blood flow at that level. The reason that indirect methods have been used thus far in clinical practice is that detection of blood flow at the level of the microcirculation is notoriously difficult. To understand why this is so, we must briefly review the anatomy and function of the circulation:

Like other systems in the body, the circulation performs many interrelated tasks. Its primary function, however, is to service the needs of the tissues - to deliver essential nutrients, remove waste products, transport hormones as a means of intercellular communication and participate in thermal regulation. While most physiology textbooks divide the circulation into the *systemic* and *pulmonary*
circuits, for the purposes of this discussion it is useful instead to look at the circulation as made up of conduit vessels and functional exchange, or nutritive units (capillaries). The function of the conduit vessels is to transport blood under high pressure from the pumping source, the heart, via the arterial network to the end organs of the circulation, the capillaries, then back to the heart via the low pressure venous system. The arterial network begins with large, elastic arteries, such as the aorta and the iliac arteries, that transport blood from the left ventricle to the next generation of vessels which are the muscular arteries, whose walls are circumferentially lined with a thick coat of vascular smooth muscle cells. The elastic arteries dampen and smooth out the cardiac pulsations, acting as capacitors by virtue of their highly compliant walls, and maintaining forward flow during diastole\(^2\). A large nutrient artery to a typical organ will branch 6 to 8 generations until it becomes arterioles, reducing its diameter down from several millimeters to approximately 20\(\mu\)m\(^2\). The arterioles are the smallest branches of the arterial network that are completely encased by smooth muscle cells, and it is these muscular walls that allow the arterioles to close off completely or dilate several fold to act as control valves, regulating the local distribution of blood flow according to the needs of the tissue. Arterioles may branch an additional 2 to 5 generations to become metarterioles, also known as terminal arterioles, which may be distinguished from their progenitors by a discontinuous smooth muscle cell covering. The metarterioles transport blood to the capillaries, which are lined by a thin, single layer of endothelium cells and are about 5-10\(\mu\)m in diameter. Each capillary bed contains true capillaries, where substrate exchange with the interstitial space may take place, and larger channels called shunts, that allow blood to bypass the capillary circulation and quickly return to the venous system without undergoing nutrient exchange. The entrance to each capillary has a muscular component known as the precapillary sphincter, which acts to regulate the distribution of blood flow between the functioning exchange bed and the shunt network\(^2\). The layout of a typical terminal vascular loop is shown in figure 1.4. Shunts may also exist on a larger scale, and their flow is regulated by the action of the muscular arteries. Once the blood has
Figure 1.4: Anatomy of the microcirculation. (a) A terminal vascular loop of arterioles, metarterioles, capillaries and shunts. The distal most cuff of smooth muscle cells is referred to as the precapillary sphincter and acts to regulate the distribution of blood flow between nutrient and shunt vessels. (b) The typical, parallel arrangement of capillaries in skeletal muscle differs from the "branching tree" pattern found in other organs such as the heart, lungs and skin. (Modified from: Fung YC. Biomechanics: Circulation. 2nd Ed. 1997, Springer, New York)
traversed the capillaries, it is collected into venules, which in turn coalesce into larger veins to finally return the blood to the heart. The walls of the veins, although much thinner than their arterial counterparts, are also muscular and allow them to constrict or dilate, to act as volume reservoirs. Table 2 summarizes a few of the key hemodynamic parameters in the hierarchy of the circulation.

<table>
<thead>
<tr>
<th>Table 2: Relevant hemodynamic parameters of the circulation(^{[22,24]})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
</tr>
<tr>
<td>Arteries</td>
</tr>
<tr>
<td>Arterioles</td>
</tr>
<tr>
<td>Capillaries</td>
</tr>
<tr>
<td>Venules</td>
</tr>
<tr>
<td>Veins</td>
</tr>
<tr>
<td>Vena Cavae</td>
</tr>
</tbody>
</table>

† The remaining 16% blood volume is divided between the pulmonary circulation (9%) and the heart (7%).

Active tissue, in particular skeletal muscle, may require as much as 20 - 30 times its resting blood flow during peak activity. The heart, however, is only able to increase its output by a factor of five\(^{[23]}\). It is therefore not possible to meet the metabolic demands of a particular organ or tissue simply by increasing the blood supply everywhere in the body. Instead, the microcirculation continuously monitors local tissue needs by sensing oxygen levels and the accumulation of various byproducts of respiration, such as pH, p\(\text{CO}_2\), and potassium. Local factors combine with circulating hormonal messengers and neural pulses conducted by the sympathetic fibers of the autonomic nervous system to control vascular smooth muscle tone and balance the distribution of blood flow\(^{[10]}\). Even under resting conditions this distribution is not static. Neighboring capillary beds undergo cyclical fluctuations in
blood flow, known as vasomotion, and alternately shut down for periods of 20 seconds and up to a minute or more\textsuperscript{[251]}. Vasomotion has been observed in many tissues, including skeletal muscle and skin, but its function is not well understood\textsuperscript{[250]}.

Organ blood flow is, therefore, determined almost exclusively by local tissue needs, and total cardiac output is controlled by the sum of all tissue flows. The significance of this control mechanism is that increased demand for blood flow may be met by two means: (1) increased total blood flow, and (2) redistribution of flow away from shunts and into true capillaries. The inverse of this is also true, it is possible that the metabolic needs of the tissue are not met despite high total blood flow, if most of the flow is directed through shunt channels. This is the underlying physiological reason why total pedicle blood flow is not a reliable measure of cellular ischemia.

1.4.1 Challenges to flow detection

Although we wish to measure flow at the capillary level, it has proven difficult to do so, particularly with non-invasive means. For imaging techniques, the problem lies in the dimensions of the microcirculation, which, for skin and skeletal muscle, are summarized in table 3:
Table 3: Capillary morphology \[^{[16,23]}\]

<table>
<thead>
<tr>
<th></th>
<th>MUSCLE</th>
<th>SKIN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow(^1)</strong></td>
<td><strong>Minimum</strong></td>
<td>3-5 cc/min/100g (20°C)</td>
</tr>
<tr>
<td></td>
<td>2-5 cc/min/100g (Rest)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Maximum</strong></td>
<td>100 cc/min/100g (Exercise)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 cc/min/100g (40°C)</td>
</tr>
<tr>
<td>Capillary Diameter(^1)</td>
<td>7 µm</td>
<td>10-20 µm</td>
</tr>
<tr>
<td>Capillary Density(^2)</td>
<td>1300 /mm(^2)</td>
<td>250 /mm(^2)</td>
</tr>
<tr>
<td>Mean Inter-Capillary Distance(^2)</td>
<td>34 µm</td>
<td>150 µm</td>
</tr>
<tr>
<td>Mean Red Cell Velocity(^2)</td>
<td>700 µm/sec</td>
<td>600 µm/sec</td>
</tr>
<tr>
<td>Mean Vascular Volume(^2)</td>
<td>5 %</td>
<td>n/a</td>
</tr>
</tbody>
</table>

\(^{[1]}\) Kayser MR, Hodges PL, 1995.
\(^{[2]}\) Fung YC, 2nd Ed.

At 5-10µm diameter, and average length of 1mm, capillaries lie below the visual resolution limit of conventional imaging modalities, including very high frequency ultrasound systems with axial resolution on the order of 50µm. Likewise, for velocity sensitive techniques such as Doppler and MR, individual capillary flow, moving at <1mm/sec and totaling less than 0.05mL/day, is too slow to be detected over tissue noise in-vivo\[^{[24]}\]. Finally, since capillaries are subject to vasomotion cycles and local control mechanisms, flow in a given capillary bed is not uniform either spatially or temporally. For these reasons it is neither possible nor practical to measure blood flow in an individual capillary\[^{[20]}\]. What is needed instead is a method that measures bulk volume flow rate in a large ensemble of capillary beds within a given tissue, where temporal and spatial averaging is not only meaningful but also independent of the geometrical properties of individual vessels.

Indicator-dilution provides us with a means to do exactly that. The theory is introduced in the next section.
1.5 The principles of flow measurement using indicator-dilution

Indicator-dilution methods combine tracer kinetics and the principles of mass transport to measure total blood flow independent of vessel geometry. This technique has its origin in the central volume principle, described by G.N. Stewart in 1894, but was not formally established for intravascular indicators until the work of Meier and Zierler, published in 1954\[2,18,27,28\]. To understand how flow is measured with this principle, consider a simple organ, represented by a well-mixed chamber (figure 1.5) with a single inlet and a single outflow pipe, with a fixed total volume, \( V \), and fixed volume flow rate, \( Q \).

If we inject an amount, \( m_0 \), of an intravascular dye into the inlet pipe as a sudden bolus, we can define a new quantity \( h(t) \) as the rate at which the dye leaves the system, normalized to the amount injected. That is, \( h(t) dt \) is the fraction of dye that leaves the chamber between time \( t \) and \( t + dt \). Since all injected dye must eventually leave,

\[
\int_0^\infty h(t)dt = 1
\]  
(18)

If we are able to measure the concentration of dye in the outflow (venous) pipe, \( c_r(t) \), then the amount of dye leaving the system between \( t \) and \( t + dt \) is simply the product of the volume of fluid leaving in that time interval and the instantaneous outflow dye concentration, or \( Qc_r(t)dt \). Thus \( h(t) \) is the ratio of this amount to the total amount of dye injected:

\[
h(t) = \frac{Qc_r(t)}{m_0}.
\]  
(24)

Using equations (2) & (3), and assuming flow is time-invariant, we get:

\[
\int_0^\infty h(t)dt = \int_0^\infty \frac{Qc_r(t)}{m_0} dt = \frac{Q}{m_0} \int_0^\infty c_r(t)dt = 1
\]  
(25)

or
Figure 1.5: Classic indicator-dilution. (a) An organ represented by a fixed volume, well-mixed chamber with single inflow and outflow pipe (artery and vein) and a constant flow rate, $Q$. (b) A sudden, bolus injection of tracer into the flow inlet will be modified by the presence of the chamber, and sampling of tracer concentration, $c_r(t)$, at the outflow pipe shows a delayed and widened profile compared with the well-defined bolus.
Equation (5) is the fundamental indicator-dilution relation, and states that for bolus injection of tracer in a steady-state system, flow can be obtained from the area under the venous outflow concentration curve.

1.5.1 The mean transit time (MTT):

The transit time is simply the time required for a given dye particle to travel through the system from the point of injection to the point of detection. Since \( t \) is, by definition, the transit time of all dye particles leaving the chamber between time \( t \) and \( t + dt \), \( h(t) \) must represent the frequency distribution (probability density) of transit times for a given flow system\(^{27}\). The average of all such times, or the Mean Transit Time is then defined as:

\[
\bar{t} = MTT = \int_0^\infty t h(t) dt
\]  

(31)

If we represent the system as the sum of all possible paths from the inflow pipe to the outflow pipe (figure 1.6) and assume the dye mixes uniformly with all fluid in the system, then the time required for dye particles to traverse the \( k \)th path is its transit time, \( t_k \). If the volume of each path is \( V_k \), then the flow rate, \( Q_k \), is by definition \( V_k / t_k \). That is, \( V_k = t_k Q_k \).

The fraction of tracer in each path is distributed according to relative flow, and since \( h(t_k)\Delta t \) is the fraction of dye particles that take the \( k \)th path, \( Q_k = h(t_k)\Delta t Q \) where \( Q \) is the total flow rate in the system. In other words \( V_k = t_k h(t_k)\Delta t Q \). For a continuous distribution of transit times, this
Figure 1.6: For the purpose of transit time analysis, the organ (or chamber) can be broken down into an ensemble of many separate paths. The transit time $t_k$ is the time it takes a tracer particle to completely traverse the $k^{th}$ path. Paths which have equal lengths, and hence equal transit times, can be considered as one path without loss of generality.
becomes \( dV = \theta h(t)Qdt \), which can be integrated to obtain the total system volume:

\[
V = \int \theta h(t)Qdt = \int \theta h(t)dt
\]

\[
\frac{V}{Q} = \int \theta h(t)dt
\]  

(44)

The right side of equation (44) is the same as the Mean Transit Time (MTT), the average time required for dye to exit the system following a sharp bolus injection. Substituting equation (3), we get

\[
\frac{V}{Q} = \frac{Q}{m_o} \int tc_v(t)dt
\]

and substituting equation (5), we get:

\[
\frac{V}{Q} = \frac{\int tc_v(t)dt}{\int c_v(t)dt}
\]  

(46)

Equation (46) and the second line of equation (44) form the Central Volume Principle: which states, in words, that in a well mixed system the ratio of fluid volume to flow rate is equal to the normalized first moment of the venous outflow concentration, which is also the mean transit time\(^{28,29}\). This can also be said intuitively: the mean flow rate in a system is the volume of fluid within it, divided by the amount of time it takes that fluid to flow out.

1.5.2 The residue function:

In the derivation of indicator-dilution theory presented above, the only measured quantity was, \( c_v(t) \), the venous outflow concentration. Obtaining this quantity in-vivo, however, requires identification and cannulation of the complete venous drainage of the system under study, which would not only prove highly invasive, but often impossible since most organs and tissues have multiple, parallel venous drainage pathways. It is often easier, and less invasive, to measure the tissue concentration of dye
by external means. This is most often done with a gamma camera placed over the patient following the administration of radioactively-labeled tracers, or alternatively with radio-opaque dyes and dynamic CT scanning. As outlined below, measured tissue tracer concentration must be treated differently than the outflow concentration\[27,30].

Let \( m(t) \) be the absolute amount of tracer present in the region of interest at a time \( t \) post injection. We can now define the Residue Function, \( R(t) \), as the fraction of injected tracer remaining in the system at time \( t \) (figure 1.7), so that \( R(t) = m(t)/m_0 \). The fractional rate of tracer leaving the system is then \( -\frac{dR(t)}{dt} \), which is also, by definition, equal to \( h(t) \):

\[
-\frac{dR(t)}{dt} = h(t) \tag{55}
\]

We can obtain the classic form of the residue function by integration:

\[
R(t) = 1 - \int_0^t h(t)dt \tag{56}
\]

(having made the assumption that all tracer is initially present in the region of interest, or \( R(0) = 1 \).) If we substitute equation (55) into equation (6) for the MTT, we get \( MTT = -\int tR'(t)dt \), and integration by parts yields the equivalent relationship

\[
MTT = \int_0^t R(t)dt \tag{59}
\]

From the Central Volume Principle, we can obtain the ratio of flow rate to vascular volume based on equation (59):
Figure 1.7: The residue function, $R(t)$, is used in place of the outflow concentration, $c_v(t)$, whenever the external detector is placed over the organ instead of its venous outflow. $R(t)$ measures the amount of tracer left inside the organ, and is related to the outflow concentration through an integral relationship (equation 14). $c_a(t) = \text{arterial tracer concentration}$
\[ Q/V = 1/MTT = \int_0^1 R(t) \, dt \]

This equation is less useful than would first be suggested, since it does not result in a measurement of flow, but rather, flow in relation to vascular volume, which is generally an unknown, variable quantity. A better way to obtain actual flow, independent of volume, is to abandon the Central Volume Principle, and substitute equation (3) into equation (55) to get:

\[ Q = \frac{m_o h(t)}{c_r(t)} = \frac{m_o}{c_r(t)} \frac{dR(t)}{dt}. \]

That is, the flow rate is linearly proportional to the slope of the residue curve at any given time \( t \), divided by the venous concentration at the same time\(^{28} \). In practice, for the reasons mentioned above, it is often not possible to measure the venous outflow concentration, and it is also quite difficult to estimate the actual total amount of tracer injected into the system. If, however, a fixed time is chosen, for instance \( t = 0 \), and the injection method and amount are carefully controlled and reproduced, it may be possible to make comparative measurement of flow rate, based on the initial slope of the residue curve alone, and assuming that \( c_r(0) \) is held constant by the system and is independent of flow rate.

In chapter 2, the negative-bolus variation on indicator-dilution is described, where such an assumption will be shown to be valid.
1.5.3 **Drawbacks and errors in conventional indicator-dilution measurements:**

In the course of the derivation presented above, several assumptions were made whose validity determines the overall accuracy of indicator-dilution flow measurement. These assumptions are now listed and discussed:

(1) **Flow is time invariant:**

If flow is an unknown function of time, it must be included in the integral of equation (4) and the subsequent mathematical analysis breaks down. In-vivo, flow is often under the influence of cardiac pulsation, and time-invariance cannot be assumed. If however, flow fluctuations occur rapidly in comparison to the time required for flow measurement, or are of sufficiently small magnitude, the errors introduced by time variance will be small.\(^{2,18}\) Fortunately, by the time blood reaches the capillary circulation, the cardiac pulsations have been sufficiently dampened by the arterial tree so as not to pose a significant source of error to flow measurement.\(^{21}\) Low frequency fluctuations, however, such as result from vasomotion, will contribute significantly to measurement errors. Low frequency cycles in flow may be approximated by cyclical functions with an unknown, fixed amplitude, which would then allow numerical integration with the measured residue function to obtain an estimate of flow rate. An example of such an analysis is given by Bassingthwaighe\(^{31}\) but is beyond the scope of this discussion.

(2) **Tracer is injected as an instantaneous, well defined bolus into the region of interest:**

If the tracer is not injected as an ideal (arterial concentration, \(c_a(t) = \delta(t)\)) bolus and if it spreads significantly by the time it reaches the region of interest, \(h(t)\) no longer represents the impulse response, or probability density, of transit times.\(^{28}\) The instantaneous relationship between \(h(t)\) and \(c_r(t)\) is lost and must be replaced by a convolution with the arterial input function, which is not known,
\[ c_r(t) = \frac{Q}{m_o} h(t) * c_a(t). \]

This is further complicated by the recirculation of tracer after initial injection, which further acts to alter the input function\(^2\). Recirculated tracer and bolus spreading result in significant flow measurement errors. Interestingly, flow can still be calculated from equation (14) - a simple statement of conservation of tracer mass which is unaffected by the arterial input function.

(3) **The tracer is entirely intravascular:**

Every equation of indicator-dilution assumes the conservation of tracer mass between the input and output side of the flow system. While it is easy enough to assumed that no exogenous tracer is spontaneously destroyed or created within the body, it is another matter to assume that no tracer is lost from the intravascular space due to diffusion into surrounding tissue or escapes via parallel, unsampled venous channels. Most radiologic tracers are, in fact, quite diffusible across vascular endothelium and are distributed in the interstitial fluid according to their tissue affinity\(^3\). Measurement of flow using diffusible tracers must be based on the *Fick principle*, which relates tissue tracer concentration, \(c(t)\), measured externally, with arterial concentrations, \(c_a(t)\), and tissue partition coefficient, \(\lambda\), as summarized by the single partition Key equation\(^4\):  

\[
\frac{dc(t)}{dt} = -\frac{Q}{\lambda V} [c(t) - \lambda c_a(t)].
\]

(4) **Tracer is well mixed and uniformly distributed in the blood:**

Uniform distribution and thorough mixing is the most basic requirement of indicator-dilution theory, and is at the heart of the *Central Volume Principle*. It means that the dye must have uniform access to all parts of the system in proportion to flow, so that the venous concentration is determined only by the distribution of transit times. Under controlled flow conditions in a closed circuit,
A comparison of positive-bolus indicator-dilution using Cardio-green dye injection against electromagnetic and timed-collection flow measurements has demonstrated consistent and significant overestimation of true flow rate, which was inversely proportional to the amount of mixing taking place.\textsuperscript{[34,35]} Errors on the order of 100-200\% were noted in-vitro, being higher at high flow rates (up to 10L/min)\textsuperscript{[34,35]}. In the best of circumstances, correlation between the estimated and true flow rate was at the $r = 0.880$ level for dye injection, and was significantly worse than the performance of the electromagnetic flowmeter.

Likewise, inconsistencies in exercise-induced changes in myocardial flow rate measured with indicator-dilution in-vivo have also been attributed to incomplete mixing following intra-arterial injection of tracer.\textsuperscript{[35]}

The errors encountered in the use of conventional indicator-dilution for myocardial blood flow measurement are summarized by Bassingthwaighte\textsuperscript{[28]}. The author notes that not only was indicator observed to streamline along one side of a coronary artery without mixing post bolus injection, but that it was not possible to simultaneously inject tracer into both the right and left coronary arteries and obtain uniform mixing of dye throughout the vascular volume of the heart. Finally, since coronary venous blood is collected via two distinct venous drainage paths, the coronary sinus and the Thebesian veins, complete sampling of venous dye outflow was not even possible.

Indicator dilution is, therefore, most suited for use in isolated organs with well defined, easily accessible, single arterial input and venous output vessels\textsuperscript{[30]}. In such a setting, dye injection would be expected to provide a good estimate of total organ blood flow (conduit + nutritive) assuming complete mixing has taken place. Measurement errors, however, would compound quickly when studying organs with more complex vascular patterns. While multiple arterial inputs may not, in isolation, affect the accuracy of flow measurement, except by degrading uniform mixing and bolus spread, multiple venous channels present a more fundamental problem. An important example of such a setting involves flow measurement in a volume of interest within a well perfused organ. Such a volume, depending on its
size, will typically not be nourished as the vascular territory of one nutrient artery and vein, but rather as a syncytium, fed and drained through hundreds of arterioles and veins too numerous and small for direct bolus injection or sampling. Although external measurement of the tissue residue function may address the complex venous drainage issue, the problem of achieving an ideal arterial bolus injection remains.

Indicator-dilution remains a sound, well-founded method for microvascular blood flow detection, whose use, however, must be tempered by proper interpretation based on consideration of the theoretical principles and sources of error listed above. In one form or another, it is the basis of most techniques used for microvascular blood flow measurement in use today, some of which are reviewed in the next section.

1.6 Survey of existing microvascular blood flow measurement techniques

The most widely used techniques for microvascular blood flow detection are now reviewed, in order to illustrate the principles of flow measurement and to explain why these methods are not suitable for clinical monitoring of microsurgical free flaps. What distinguished this section from section 1.3.2, is that the methods described below attempt to directly measure true flow, as opposed to the indirect techniques aimed at predicting flap survival presented earlier. These are divided into techniques appropriate for research applications (but not exclusively so) and those that are well suited to clinical application.

1.6.1 Laboratory methods:

(1) Labeled microsphere injection

The microsphere technique is considered the gold-standard in microvascular flow measurement in the laboratory\cite{18,36,37}. The microspheres are a suspension of uniformly sized (typically 15\textmu m, but 9,
20 and 50μm diameter may also be used), inert polymer beads that are injected into the circulation via a left ventricular catheter. The beads may be tagged with radioactive tracers, or colored to facilitate detection. The assumption is that post-injection, the beads are well mixed with blood and then distribute uniformly throughout the circulation, in direct proportion to regional blood flow. Because the beads are larger than the capillary diameter, they become trapped at the pre-capillary sphincter level and do not go on to recirculate. The dose of microspheres injected is typically on the order of 10⁵/kg and is calculated to cause deposition in approximately 10% of capillaries. Larger concentrations may begin to significantly affect hemodynamic parameters, while lower doses are associated with decreased precision due to stochastic variation. The distribution of microspheres in tissue represents the distribution of blood flow at the instant of injection into the left ventricle. To turn this into a quantitative measure, the number of microspheres deposited in tissue must be determined, and this is done according to the labeling method selected: colored beads are counted under microscopic examination or via a Coulter counter, while radioactive-labeled spheres are counted by a Gamma detector. In either case, the organ under study must be sacrificed and sectioned into small portions so that the number of microspheres per unit mass of tissue can be obtained. Since the total number of microspheres injected is known, the method provides a measure of tissue blood flow as a fraction of total, instantaneous cardiac output as distributed through the sections of the organ. To turn this into an absolute measure, in mL/min/100gm of tissue, a calibrated, sham organ is placed into the arterial circulation, usually in the form of a standardized withdrawal pump connected to the femoral artery. A few seconds prior to ventricular injection, the pump is turned on and withdraws a fixed volume of blood at a fixed flow rate. This blood is transferred to a counting vial, the number of microspheres collected is determined and used to calibrate all other tissue measurements against the set flow rate of the withdrawal pump (figure 1.8).
Figure 1.8: Determining flow by the microsphere technique. A known number of labeled microspheres is injected into the arterial circulation via a left ventricular catheter. The circulating microspheres become trapped at the precapillary sphincter (in the case of 10µm microspheres) and are removed from the circulation after one pass. Trapped microspheres are then counted within the tissue of interest. A calibrated withdrawal pump in the femoral artery is used to convert the data to absolute flow measurement. (From: Pang CY, et al. PRS 1984)
The size of the microspheres determines the level of the circulation at which flow detection takes place. The smaller beads determine flow at the capillary level, while larger spheres determine arteriolar flow only. The use of several, differently labeled spheres allows multiple, sequential injections and measurements to be made\cite{14}.

The disadvantages of this technique, however, severely limit any clinical usefulness: (1) Since the beads cannot pass the pulmonary circulation, they must be injected directly into the left ventricle via a carotid artery catheter - a highly invasive and often risky proposition. (2) Accurate detection of counts requires sectioning of the tissue after injection. Since the method is destructive it is not suitable for clinical use. Alternatively, an external Gamma camera may be used to obtain counts non-invasively, but requires prolonged exposure times. (3) Absolute flow determination requires intra-arterial catheter placement - again a highly invasive and occasionally risky procedure. (4) Measurement cannot be made in real time. (5) Repeat measurements may significantly affect hemodynamics (including shock), result in cumulative radiation dose to the patient, and are only possible with different isotope labeled microspheres. And finally, (6) the trapping of microspheres in the circulation is a binary probability process governed by a Poisson distribution which is superimposed on the distribution of blood flow in tissue. At low flow rates, the variance of the trapping process becomes very high and single study microsphere data becomes difficult to interpret\cite{18,37}.

(2) Tracer clearance

The measurement of tracer clearance, or washout, also provides absolute flow rate, based on the Fick Principle described in equation (73). A diffusible, externally detectable tracer, such as \( ^{133}\text{Xe} \), \( ^{85}\text{Kr} \), \( ^{22}\text{Na} \), \( ^{99m}\text{Tc} \), is deposited by direct injection into the tissue to be studied. The rate of disappearance of the tracer is measured externally by a Gamma counter, and, if the movement of tracer is not diffusion limited, reflects the capillary flow in the region of interest. Determination of mixed venous tracer
concentration by venipuncture calibrates the measurement and turns it to absolute flow. Alternatively, tracer can be deposited during the wash-in phase that follows systemic administration, either by injection, or in the case of $^{133}$Xe, by inhalation, with subsequent determination of clearance rate.\textsuperscript{[18,38]}

The limitations of clearance techniques are based mostly on the complexity of equipment required and operator skill. In addition, the presence of residual tracer and background radiation limit measurement frequency to every 12-24 hour only, which clearly cannot be performed in real-time. As with all radioactive-labeled media, repeat exposure results in cumulative dose to the patient, which is probably undesirable.\textsuperscript{[10,12]}

1.6.2 Clinical methods:

(1) Medical nuclear imaging

These are simply clinical implementations of tracer methods described above, relying on measurement of either tracer deposition by wash-in or tracer clearance by washout. Although these techniques are currently the clinical gold standard in blood flow measurement, they are not suitable for monitoring purposes for the same reasons outlined above for tracer techniques. On occasion, Technetium 99m bone scans may be used to assess the viability of vascularized bone transfers, but not in the immediate post-operative period.\textsuperscript{[12,18]}

(2) CT

\textit{Computerized Tomography} is a cross-sectional imaging modality in which tissue contrast is obtained on the basis of differential attenuation of X-rays. Linear slice projections are obtained at multiple different acquisition angles and are then back-projected to form a two-dimensional image. In flow measurement, CT is used as the detection method, providing quantitative tracking of slice tracer content, or residue, following the administration of some form of tracer or contrast agent. Labeled
Xenon is a common tracer used for cerebral flow quantification by the wash-in technique and is administered by inhalation. Tracer accumulation in brain tissue is tracked with fast CT data acquisition, and the concentration of Xenon in expired air, combined with blood hematocrit determination, is taken to represent the arterial input function.\textsuperscript{39,40} Alternatively, iodinated contrast agents can also be used for flow detection when combined with ultrafast CT data acquisition for near real-time bolus tracking. Most X-Ray contrast agents, however, have been found to be highly permeable in vascular endothelium and tracer loss in tissue becomes a significant source of error.\textsuperscript{40} In general, CT offers few advantages for flow detection over other imaging modalities, and is subject to the same fundamental restrictions as any application of indicator-dilution. In particular, the high levels of ionizing radiation exposure associated with CT scanning precludes repeated, frequent application for real-time flow monitoring.

(3) **MR flow detection:**

The use of magnetic resonance imaging for flow measurement has generated a great deal of interest over the years. MR offers several distinct advantages as an imaging modality, including the flexibility of image acquisition, access to all regions of the body, inherent high soft tissue contrast and freedom from ionizing radiation. Flow measurements can be performed using exogenously administered MR contrast agents, such as protein-bound Gadolinium,\textsuperscript{41,42} but when used merely as external detector MR is subject to the same restrictions as other applications of indicator-dilution. The more interesting techniques, however, combine the magnetic properties of blood with the inherent flow sensitivity of conventional MR imaging to create endogenous tracers for flow measurement:

MR image formation is, in general, a two step process: The first involves the excitation, or saturation, of tissue hydrogen protons into a 'spin' state using a radio frequency electromagnetic pulse, while the second is the reception and localization step, which forms the image from the emitted RF signal through the use of external electromagnetic field gradients.\textsuperscript{43} Images acquired this way are
inherently sensitive to flow by two distinct mechanisms: (1) Time of flight (TOF) effects and (2) Spin-phase phenomena.\[^{64}\]

Time of flight effects on flowing blood may result in either a decrease or increase in signal intensity depending on the velocity of flow and the pulse sequence used. In a conventional spin-echo sequence, tissue is exposed to consecutive 90° and 180° excitation pulses in order to form an image. If moving protons do not remain within the selected slice long enough to receive both pulses, the result is an attenuated signal, and the degree of attenuation is directly proportional to the velocity of blood within the voxel.\[^{43}\] TOF effects are also at the root of the 'bolus tracking' flow detection technique. This sequence utilizes two excitation pulses, a 90° pulse and a 180° pulse that are spatially separated into two slices, one downstream of the other, so that only blood flowing through both slices can experience coherent excitation. Flowing blood, therefore, gives a strong signal while the signal from all other stationary tissue is suppressed. The downstream, 180° excitation pulse effectively labels or 'creates' a bolus of tracer, while the upstream, 90° pulse images it to form a qualitative flow map. The protons tagged by the initial pulse are mostly bound in free water molecules and are thus not strictly intravascular tracers. Since water is free to diffuse out of capillaries and distribute in tissue, adjusting the time interval between the distinct excitation pulses allows different classes of blood vessels to be imaged: short times, on the order of hundreds of milliseconds visualize flow in the larger arteries only (MR angiography), while longer delay times of approximately one or two seconds allow tagged protons to diffuse into tissue and provide some measure of capillary blood delivery.\[^{38}\]

A second class of flow sensitive effects are spin-phase dependent. The spins are the electromagnetic property of protons that allows them to be detected and imaged with MR. The spin phase is the angular velocity or rate of precession, and is directly dependent on the magnetic field strength. When linear magnetic field gradients are used for slice selection and for localization, spin phase becomes a function of proton position. When blood flows through the gradient, its position
becomes a function of time, and so proton phase changes depending on velocity. The faster blood moves through the gradient, the more phase it accrues and vice versa, the result of which is a dispersion of phase across the voxel leading to signal loss. Once again, then, signal intensity is used to form images that code for blood velocity.

These examples serve only to illustrate the basic principles of MR flow detection. Many different strategies are in use or are being developed and the field of MR flow mapping is ever expanding. Of particular interest is the conversion of MR velocity maps into quantitative images of flow, a step which will likely depend on knowledge of geometry. More information on MR based flow measurement can be found in the references.

1.7 Ultrasound microbubble contrast agents:

It is the aim of this work to introduce ultrasound microbubble contrast agents as intravascular indicators for microvascular blood flow measurement. This section deals briefly with the historical development and function of microbubble agents, and the unique properties which set them apart from most other radiologic contrast agents. Since indicator-dilution requires quantitative tracer detection, this section concludes with an overview of microbubble-specific ultrasonic imaging methods.

1.7.1 Development

The ‘discovery’ of microbubble based ultrasound enhancement is consistently attributed to the work of Gramiak and Shah in 1968. During a routine echocardiographic study of a patient with aortic valve regurgitation, the cardiologists noted immediate opacification of the left ventricle and ascending aorta following the direct injection of tracer (indocyanine green) via an intra-cardiac catheter, a finding that was later reproduced with injections of 5% Dextrose in water, saline, and even the patient’s own blood. Visual and experimental evidence soon confirmed that the enhancement was
due to minute air bubbles introduced into the blood stream, likely created by agitation of the solution or cavitation at the catheter tip during rapid injection. When injected intravenously, these bubbles had a life span measured in fractions of seconds and could not survive passage through the lungs, yet despite that provided added detail to ultrasonic images of right-sided cardiac abnormalities.

To be truly useful in imaging the systemic circulation and the left cardiac chambers in particular, the bubbles needed to be made small enough to pass through the pulmonary capillaries and stable enough to remain in suspension longer than a few seconds. It was not until the 1980s that commercial development of contrast microbubbles began in earnest, and the first issue to be tackled was the poor stability of small gas microbubbles. By encapsulating the gas with an external shell, the microbubbles could be made stable enough to survive for prolonged periods in the circulation, while controlling the particle size allowed them passage through pulmonary and systemic capillaries.

The first agent approved for clinical use by the FDA and stable enough to opacify the left ventricle after intravenous administration was Albunex® (Molecular Biosystems Inc., San Diego, CA), an air-filled bubble encapsulated by a shell of human albumin and a mean diameter of 3.5µm. Levovist® (SHU508A, Schering AG, Germany) is another of the first generation agents, made of crystalline galactose stabilized by a small amount of fatty acid and a 3.5µm mean particle size. Typically, a suspension of microbubbles would be prepared just prior to administration, a process which varied according the specific agent used, then injected into the circulation via a peripheral vein. Following bolus injection, the agents would produce echo enhancement that would last several minutes and reach as far as the abdominal organs. Although the performance of these agents was a marked improvement over the original free bubble agents, their longevity in-vivo left much to be desired.

Second generation agents take advantage of the reduced solubility of heavier-than-air gases in plasma to prolong the life-span of microbubbles in the circulation. Optison® (FS069, Mallinckrodt Inc.) is a perfluoropropane-filled bubble with an albumin shell similar in size to the original Albunex but
producing greater enhancement of blood. **DMPI** (DuPont Merck Inc., Boston, MA) is, likewise, a perfluoropropane based agent whose bi-layered lipid shell imparts it great stability both in-vitro and in-vivo, sufficient, in fact, to produce steady enhancement following administration by a slow infusion.\(^{[49]}\)

Other agents awaiting clinical approval include **Quantison**\(^{®}\) (Andaris, Nottingham, UK) and **SHU563A** (Schering AG, Germany). Due to their size, contrast agents are unable to leak through vascular endothelial gaps and thus remain purely intravascular while in the body. Their distribution in blood is similar to red blood cells and when administered in clinical doses have been found to have no effect on the hemodynamic state.\(^{[50]}\)

Despite their increased stability, microbubble contrast agents do not circulate in the body indefinitely. The encapsulated gas will eventually diffuse out to the plasma, a process which is enhanced by the high pressures and shear rates present in the left ventricle and aorta, and be exhaled through the lungs or excreted by the kidneys, leaving the remaining shell material to be taken up by circulating macrophages and the reticulo-endothelial system of the liver and removed from the circulation.

1.7.2 Theory

To understand why gas microbubbles are effective as contrast agents it is necessary to review to basic principles of ultrasonic image formation. The material presented here follows the discussions by Jensen\(^{[15]}\) and Hill.\(^{[51]}\)

Gray-scale ultrasound images are formed by the transmission and reflection of longitudinal pressure waves in tissue, a method originally based on sonar echo-location developed for submarine warfare. Acoustic energy is generated by a piezoelectric crystal vibrating at ultrasonic frequencies (>20kHz, but typically 2-20MHz), and transmitting short pulses of pressure waves from the skin surface into the body. As the pulses propagate, they are partially reflected and scattered from local variations in acoustic impedance (defined as the product of material density and speed of sound) caused by tissue
inhomogeneities. The backscattered echoes reflect back to the skin surface where they are received by the transducer and translated into an electrical signal. Because the acoustic pulses are attenuated by the medium in which they travel, the backscattered echoes may be several orders of magnitude weaker than the transmitted pulses, and must undergo amplification prior to further processing. Furthermore, because attenuation depends on the distance, or depth, traveled through the tissue, non-constant, or time gain compensated (TGC) amplification is used to increase the gain for pulses reflected by deeper structures. The time delay, \( \Delta t \), between pulse emission and echo return is measured by the scan converter, and if the velocity of propagation of sound in tissue is assumed constant \( (c \approx 1540 \text{ m/s}) \), the distance of the reflecting surface from the transducer can be calculated \( (d = \frac{1}{2} c \cdot \Delta t) \). By measuring the backscattered echoes down a single line of sight, a simple tracing of echo amplitude versus tissue depth can be formed - making the most basic of ultrasound images known as an ‘A’ (‘Amplitude’) scan. If the line of sight is rapidly translated, or scanned, in one direction along the skin surface, and pixel brightness is used to encode echo amplitude according to a fixed gray-scale map, a two-dimensional, or ‘B’ (‘Brightness’) mode image of the underlying tissue can be formed (figure 1.9). Instead of mechanically moving the probe across the body, most modern ultrasound scanners make use of transducer array technology, in which the hand held probe is made up of as many as 256 thin, rectangular piezoelectric crystals stacked side to side and individually controlled. By only firing a few of the crystals at a time the beam can be formed and electronically scanned across the width of the probe, and a two-dimensional image within a limited window can be formed without the need for physical translation of the probe (figure 1.10). The beam profile (both for transmit and receive) is determined by the ability of the transducer to focus ultrasound energy and this is achieved both mechanically and electronically in most linear array configurations: in the cross-sectional, or imaging, plane, focusing is done electronically by adjusting the time delays within the group of elements forming the beam, while the thickness of the beam in the out-of-plane, or elevation, direction is determined by a fixed acoustical lens.
Figure 1.9: (a) Pulse-echo imaging is performed by transmitting short pulse bursts into the tissue and listening for echoes reflected from tissue interfaces. When an echo is received, its amplitude is displayed by a pixel brightness value on the screen, while the distance to target is determined from the time delay. This is known as 'B-mode' imaging. (b) The block diagram of a simple 2-dimensional B-mode imaging system.
Figure 1.10: Array transducers, such as the linear one shown in (a) are used in most modern scanners. (a) By activating only a subset of elements, beam positioning and translation can be controlled. (b) Adding a graduated delay to each element in the group allows the beam to be steered to various scan angles. Focusing is achieved in three dimensions: (c) the out-of-plane, or elevation, focus is fixed by a mechanical, acoustic lens on the face of the transducer. (d) In-plane focusing is controlled electronically by adding delays to the transducer elements as shown. Focusing can be separately achieved for both transmission and reception.
The scattering of ultrasound from a target is determined by the acoustic properties of both the particle and the medium surrounding it. More specifically, it is determined by the difference in acoustic impedance between the two media. For normal reflection from a smooth surface boundary, the ratio of reflected to incident power is the *intensity reflection coefficient*

\[ R_i = \left( \frac{Z_2 - Z_1}{Z_2 + Z_1} \right)^2 \]  

(77)

where \( Z_1 \) and \( Z_2 \) are the acoustic impedances of the two media. For point, or Rayleigh, scatterers, which are characterized by diameters much smaller than the incident wavelength \((r \ll \lambda)\), the relationship between scattered and incident pressure intensity is more complex, and can be calculated from the *scattering cross section*, where \( I_s = I_i \sigma_w \). The reason it is called a *cross-section* is that it has the dimensions of \( m^2 \) and represents the equivalent area of reflector that would intersect a beam of intensity \( I_i \), and reflect the power, or intensity \( I_s \). Since the scattered wave is spherical, it is further attenuated by distance from the scatterer to transducer, raised to the second power. The scattering cross section for a single, uniform particle can be calculated from the Rayleigh scattering model\(^{52}\)

\[ \sigma_w = \frac{4\pi}{9} \left( \frac{2\pi f_0}{\lambda} \right)^4 r^5 \left( \frac{\kappa - \kappa}{\kappa} \right)^2 \left( \frac{3(\rho - \rho)}{2\rho + \rho} \right)^2 \]  

(85)

where \( \kappa_1 \) & \( \kappa \) are the adiabatic compressibilities of the scatterer and medium, respectively, and \( \rho_1 \) & \( \rho \) represent the respective densities. (The Rayleigh model presented here is actually inappropriate for predicting microbubble behavior, since it fails to take into account the effects of the shell, thermal interactions, and, most importantly, bubble oscillations and resonance. Some of these factors have been
added to the model over the years, including the more complex models of Trilling\textsuperscript{[53]} and Hoff\textsuperscript{[54].} In general, the actual acoustic field scattered from a group of particles is extremely complex and difficult to calculate, but the average reflected field intensity can be obtained by linear superposition of the individual scattering cross section - an approximation which is valid as long as the ensemble is sparse.\textsuperscript{[51]}

An important implication of the linear superposition principle is that signal power is linearly proportional to the number of scatterers, provided they are sparsely distributed. Table 4 shows typical values of acoustic impedance encountered in the human body.

<table>
<thead>
<tr>
<th>Table 4: Tissue acoustic impedance\textsuperscript{[15]}</th>
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<tbody>
<tr>
<td><strong>Medium</strong></td>
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<tr>
<td>Water</td>
</tr>
<tr>
<td>Muscle</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>Blood</td>
</tr>
<tr>
<td>Bone</td>
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<td>Air</td>
</tr>
</tbody>
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Table 5 shows estimated scattering cross section, calculated from equation (17), for solid, liquid, and gas based scatterers in water, for a 5\textmu m diameter particle insonated with a frequency of 5Mhz.
These data point out two important facts about ultrasound in tissue: (1) Interfaces between tissues with large impedance differences act as strong reflectors and severely limit imaging of deeper structures. For instance, at the interface between bone and muscle, 50% of the ultrasound energy is reflected back, and at the interface between air and fat, 99.95% of the energy is reflected, a fact which essentially restricts ultrasound imaging to soft tissue structures only. (2) Tissue-gas interfaces present an impedance mismatch that is several orders of magnitude greater than that between any two tissues and results in very high intensity echoes. It is, therefore, the gas content of microbubbles that is responsible for the 10-20 dB enhancement of backscatter echo seen with contrast administration.

On conventional gray scale images blood vessels are displayed as black (or hypoechoic) areas, surrounded by a bright ring of echoes. This is because although red blood cells scatter acoustic energy, blood as a whole is a poor reflector of ultrasound and the returning echoes are often 30 dB or more weaker than echoes from surrounding tissue. Since gray scale maps of B mode imaging are optimized to display soft tissue detail, and the echoes from vessel walls and blood lie at the opposite extremes of the display dynamic range, the vessel walls appear very bright and blood appears black on the monitor. This fact limits the amount of blood flow detail that can be presented in a conventional ultrasound examination. The addition of microbubble contrast agents to the circulation, even in minute quantities, results in up to 20 dB of enhancement in backscattered echo and greatly improves the detection of blood.
1.7.3 Unique properties: microbubble resonance and destruction:

When the scattering cross section of microbubble contrast agents is actually measured, it is found to be greater than predicted on the basis of geometrical size alone. This is because, just like free air bubbles in a liquid medium, microbubbles are not passive reflectors of sound but undergo oscillations when driven by the compressions and rarefactions of a propagating acoustic pulse. Just like other oscillators, microbubbles possess a natural resonance frequency, \( f_r \), at which their oscillations become greatly magnified and energy return is maximized. This resonance frequency depends on resting bubble size and the properties of both the gas and the shell, and can be estimated using the model by de Jong, et al.\(^{55,56}\)

\[
f_r = \frac{1}{2\pi r} \sqrt{\frac{3\gamma}{\rho_o} \left( P_o + \frac{\pi S_r}{3\gamma r} \right)}
\]

(90)

where \( \gamma \) is the adiabatic gas constant, \( S_r \) the shell elasticity and \( P_o \) and \( \rho_o \) the mean pressure and density of the medium, respectively. It is a fortunate coincidence that for the typical size distribution and shell material of most contrast agents, the resonant frequency lie within the diagnostic ultrasound frequency range of 1-20MHz. As an example, according to this model, a 5\(\mu\)m Albunex particle will resonate at 4.2MHz while an 8\(\mu\)m particle will resonate at 2.1MHz.\(^{52}\) The effect of resonance on scattering cross section is shown in the Hoff model\(^{54}\)

\[
\sigma_s = \frac{4\pi r^2}{\left[ \left( \frac{f_r}{f} \right)^2 - 1 \right] + \left[ \frac{f_r}{f} \right]^\delta^2}
\]

(95)

where \( f \) is the insonation frequency, \( f_r \) the resonance frequency, and \( \delta \) represents the viscous and thermal damping constants. According to this model, scattering cross section rises sharply and peaks
at the resonance frequency, with an amplitude that is three or four orders of magnitude larger than at non-resonant frequencies (figure 1.11).

An important consequence of bubble resonance is that it leads to non-linear oscillations, typified by unequal bubble compression and expansion cycles. The reflected ultrasound waves are, therefore, not of a uniform, single frequency but many different frequencies which are integer multiples, or harmonics, of the fundamental transmit frequency. Non-linear behavior can be used to form images of contrast agents and will be described further in section 1.8.7.

An additional consequence of non-linear oscillation, crucial to this application, occurs when bubbles are further overdriven by very intense pressure cycles, the resulting high amplitude expansions and contractions disrupt the relatively stiff bubble shell and allow the gas to diffuse out and dissolve in plasma. This process may require a period of only a few microseconds in the case of air-based bubbles, or hundreds of milliseconds for the less soluble perfluorocarbon agents, but the result is loss of contrast enhancement and the physical destruction of the bubbles.\textsuperscript{49,57,58} Although at sufficient high incident pressures bubbles can be disrupted at almost any transmit frequency, lower frequencies are, in general, more effective.\textsuperscript{58} Since incident energy is maximum at the focus and drops off rapidly with distance, bubble disruption, for the most part, is restricted to the beam focal zone. If the ultrasound beam is scanned rapidly across tissue, the destruction zone can be expanded to encompass a region of interest determined by the width of the scan, the depth of the image and the elevation thickness of the beam.

The first observations of ultrasound mediated bubble destruction led most investigators to believe that microbubble contrast agents were an imaging dead-end. How could an agent provide sustained contrast enhancement if it were being rapidly destroyed by the very imaging modality used to detect it? It turns out, however, that the destruction of microbubbles can be a very valuable imaging tool. One of the reasons is that bubble disruption is accompanied by transient, high intensity echo signals known variously as “Transient power scattering” or “Stimulated acoustic emission”.\textsuperscript{49,59}
Figure 1.11: Bubble resonance as predicted by the Hoff model (equation 19). Scattering cross-section peaks sharply at the resonance frequency which can be used to gain additional image contrast. (From Goldberg BB, Ed. Ultrasound Contrast Agents. 1993)
Since our current understanding of the physics of microbubble disruption is limited, little is actually known about the mechanism of stimulated acoustic emissions. One possible explanation, however, is the ultrasound-induced fragmentation of a single contrast agent microbubble into multiple, smaller, and very short-lived free gas bubbles.\[^{60}\] Since these new bubbles are not subject to mechanical damping by the shell, their summed scattering cross section may be many times greater than that of the single, encapsulated bubble, and would explain the transient echo peak. Regardless of the mechanism, the added backscatter echo intensity gained by this process has enabled imaging of the circulation with a level of detail never before seen with conventional techniques, including, for example, imaging of renal blood flow in 40μm microvessels.\[^{49}\]

1.7.4 Standardization of output power: The mechanical index

Since microbubble destruction is heavily dependent on the incident pulse pressure, imaging techniques that rely on bubble destruction must have a consistent way to record and control scanner output power. Transmitted power is directly governed by the voltage sent to the transducer crystals, but signal voltage has little to do with the amount of energy incident on a target deep within tissue, since it must undergo multiple transformations before reaching its target: Choice of piezoelectric crystal, backing material and the transmit frequency determine the efficiency with which the transducer converts electrical voltage to mechanical pressure. Similarly, the choice of impedance matching material between the crystal surface and skin determines the fraction of energy that is actually transmitted to the skin surface, while tissue type, target depth and transmit frequency determine the degree of pulse attenuation. Because this process is so complex, it is not possible, short of direct hydrophone measurement, to obtain exact values of the incident pressure in-situ. What the scanner does, instead, is report the Mechanical Index (MI), which is an industry-wide standardized measure equal to
where $P$ is an estimate of absolute peak pressure, in MPa, based on the output voltage and normal values of tissue attenuation (where the subscript $sp\text{sp}$ denotes spatial peak $\&$ temporal peak), and $f_0$ is the transmit center frequency, in MHz. Thus for a 2MHz ultrasound pulse, a mechanical index of 1.0 implies a peak pressure of approximately 1.4MPa at the beam focus. The true purpose of the MI is to provide a measure of the cavitation potential (the propensity to form spontaneous bubbles, or 'tear' the medium) of an ultrasound examination, and as an index of a possibly harmful biological effect, it is subject to upper limits set by the FDA.

The MI plays a major role in the way microbubbles are imaged by ultrasound, and this is the subject of the next section.

### 1.8 Overview of microbubble-specific imaging methods:

The three techniques described below represent the most recent developments in imaging technology. Combined with microbubble contrast agents, their application allows detection of blood flow with resolution not previously available with conventional imaging modalities: Power Doppler is a motion-based detection scheme that is not a true contrast-specific modality, while harmonic and pulse inversion imaging were designed to take advantage of the non-linear behavior of oscillating bubbles and are thus contrast-specific.

#### 1.8.1 Introduction to Doppler techniques:

Doppler techniques are used to assess the motion of targets in an ultrasound field. There are
several Doppler modes available on modern scanners, and these include the basic *continuous wave* (CW) and *pulse wave* (PW) spectral Doppler, and the more advanced 2D color flow mapping modes: color and power Doppler. Detailed treatments of the subject of Doppler detection in general can be found in Jensen, Wells, and Ferrera and we limit this discussion to a basic review of the principles required to understand how microbubbles are detected. To understand power Doppler imaging, we must first understand the principles of pulse wave Doppler velocity detection:

### 1.8.2 Pulse wave Doppler detection:

Classic Doppler motion detection refers to the frequency shift observed between an emitted acoustic pulse and the echo reflected from a moving target (figure 1.12), a shift which is proportional to the relative velocity of target and source, and is given by the Doppler equation:

\[
\Delta f = f_D = \frac{2v}{c-v} f_0
\]

\[= -\frac{2v}{c} f_0, \quad \text{(for } v \ll c)\]  

(102)

where \( f_D \) is the Doppler shift frequency, \( f_0 \) is the transmit frequency and \( v \) is the target velocity with respect to the transducer. Since mean propagation velocity of sound in tissue is \( c = 1540 \text{ m/s} \) and maximum blood velocity encountered in the body is \( |v|_{\text{max}} \leq 1 \text{ m/s} \), this approximation is generally valid. Target velocity can, therefore, be calculated from the frequencies of the received echo and the transmit pulse. Motion detection based on frequency analysis, however, requires very long pulse durations that significantly degrade axial resolution, and is exquisitely sensitive to frequency-dependent pulse attenuation in tissue. For these reasons it is limited for use in the simplest of Doppler detection techniques, *continuous wave* Spectral Doppler.
Figure 1.12: The Doppler principle. (a) The perceived frequency of sound transmitted at given frequency $f_0$ is altered by a reflector moving toward the emitter, so that the received sound is of higher frequency. The difference between the emitted and received frequencies is known as the Doppler frequency, $f_R$. The velocity of the reflector with respect to the emitter is $v \cos \theta$. (b) The effect is similar, but reversed, for a reflector moving away from the emitter. In this case the received sound is of lower frequency.
Another way to detect motion is to forsake the Doppler principle and instead measure the echo time delay that results from target motion between two successive ultrasound pulses. In pulse-wave Doppler detection, a small region of interest is interrogated with multiple, short pulses of ultrasound transmitted down a fixed line-of-sight. The pulses are emitted at a set pulse repetition frequency, or PRF, and are reflected by moving scatterers (RBC's or microbubbles) within the sample volume (see figure 1.13). The received backscattered echoes then undergo a process of quadrature demodulation, and fixed time point sampling, which results in a single velocity estimate (or a distribution of velocity estimates for multiple moving scatterers) for the sampled volume. Quadrature demodulation and signal reduction by fixed time point sampling are treated in detail in appendix A at the end of this chapter.
Figure 1.13: Pulse wave Doppler detection. (a) Very short pulse bursts are transmitted into tissue. The difference in time delays between successive echos indicates target motion between pulse transmissions. (b) Block diagram of a simple, directional PW Doppler detector. The directional information is obtained from quadrature demodulation, and the sample and hold unit is required to compare successive echo signals in order to obtain target velocity.
1.8.3 Clutter rejection

Difficulty arises when Doppler methods are used to interrogate blood flowing in real tissue, since real tissue contains stationary, as well as moving targets other than blood, all of which reflect ultrasound energy. Since the normal scattering coefficients of vessel walls and other tissue boundaries are on the order of 10 to 100 times larger than blood, the reflected signal is strong enough to saturate the detector and completely mask the weak blood flow information. This high amplitude, low velocity signal component, called “clutter”, must be removed from the signal prior to Doppler detection, and this is achieved with a suitable high pass filter, also known as a wall filter. The filter cut off frequency depends on the transmit frequency, $f_o$, the PRF and the conditions imposed by the tissue being imaged.

Clearly, any signal from blood flowing with velocity below the cut off velocity is also removed, so that too high a cut off results in the loss of significant blood flow signal, whereas too low a cut off increases the clutter content in the signal and reduces blood-tissue contrast. The aortic wall, for example, moves with a peak velocity of 5mm/sec. In order to image flow in the aorta without wall motion artifact, the filter cut off must be set to the frequency equivalent of at least 5mm/sec, with the result that blood flowing with velocity less than 5mm/sec is not detected. Even away from large, moving structures the wall filter cannot be turned off, since even slight tissue motion, such as resting muscle vibration and operator’s hand tremor are sufficient to mask slow blood flow. In general then, using a wall filter to remove clutter involves an inherent trade off between low flow detection and tissue contrast, a fact which limits the usefulness of conventional Doppler techniques for sensing flow in the microcirculation.

1.8.4 Color Doppler flow mapping:

Two dimensional flow mapping is a natural extension of the pulsed wave detection scheme described in the previous section. To form two-dimensional images, a tissue cross section is divided into multiple vertical lines of sight, and each line of sight is divided into many sample, or range, gates
along its depth axis. A velocity estimate is obtained for each depth, and is coded for amplitude and
direction using a color map. Typically, red indicates movement toward the transducer and blue away
from the transducer, while velocity magnitude is coded by the respective color intensity. The color
pixels are overlaid on a conventional, gray-scale B-mode image to allow correlation with anatomic
structure.

Since many sample volumes must be interrogated to produce a two dimensional image, each
velocity estimation must be based on a smaller number of pulse echoes so that images can still be
formed with a high frame rate and in real-time. The most important element in color flow mapping is,
therefore, the velocity estimator that replaces conventional PW Doppler processing. Several estimators
have been devised for this purpose,[15,62-64] the two most important ones being the phase-shift and cross-
correlation detectors. The front-end of the estimators are identical: a train of ultrasound pulses is directed
at a small sample volume, and the target velocity is estimated from small changes in consecutive
backscattered echoes, and a single, mean value is returned for each sample volume. A mathematical
description of both these estimators may be found in appendix B at the end of the chapter. [15]

Both the phase-shift and cross-correlation techniques require that echoes from stationary tissue
be removed prior to velocity estimation. In PW Doppler, a high pass filter was used to process the
signal and remove the high amplitude stationary, and near-stationary components. In color Doppler
flow mapping, clutter suppression is performed by delay line canceling whose function is to subtract
consecutive echoes. The assumption is that the echoes from stationary targets always appear at the
same time point and have the same shape at each pulse-echo, while moving targets do not. Subtracting
two pulses, aligned so that their transmission times coincide, results in an RF echo that is free of high
amplitude, stationary signal which can then processed by the velocity estimator. It has been shown that
this type of filtering, known as finite impulse response filtering, results in a velocity dependent reduction in
signal amplitude as well as distortion of the frequency spectrum.[15] This is an important consideration
for the detection of blood flow and will be discussed further in chapter 2.

1.8.5 Power Doppler flow mapping:

Whereas color flow maps display color coded estimates of mean velocity in individual depth samples, power Doppler images are formed from estimates of the total energy present in the signal from moving blood. The energy, or power, of the signal is coded as a single color intensity map overlaid on a standard B-mode image to indicate the presence and location of blood flow regardless of direction. Since the scattered power depends on the scattering cross section, which in turn, is linearly dependent on the number of scatterers, the power estimate is an indicator of the number of moving scatterers in the sample volume, but is theoretically, independent of scatterer velocity and direction.[15,62,63] The power Doppler image, therefore, is not prone to velocity aliasing artifacts. The energy, or power, $P$, of an analytic signal can be estimated from its autocorrelation function:

$$P = R_{ss}(0) = \sum S^*(i)S(i) = \sum I^2(i) + Q^2(i)$$

where $S, I, Q$, are the analytic signal components described in appendix A. In order to truly represent blood flow, the signal processed by the power estimator must originate from blood only, which requires that it first pass through a very efficient stationary target, or clutter, filter. The advantage of power estimation comes from two sources: (1) Freedom from velocity bias allows longer pulse durations to be transmitted, which increases the inherent detector sensitivity to low flow, and (2) the implementation of better clutter filters, with maximum suppression of stationary targets, sharper drop off and flatter response past the cut off frequency.[59] The tradeoff is high sensitivity to tissue motion, leading to what are called “flash artifacts” - saturation of the entire color image - caused by even minute motion of tissue with respect to the transducer. Power Doppler image acquisition must be, therefore, carried out under highly stable, motion free conditions.

Contrast agents have a direct effect on power Doppler images by enhancing the amount of
energy scattered from blood and improving not only the signal to noise ratio but also the detectibility of small blood volumes, an effect which is independent of motion. The clear advantage of power Doppler flow mapping, then, is an output signal which is directly proportional to the concentration of scatterers and improved detection of slow flow, which is inherently velocity independent except for the action of the clutter filter. Having said that, the sensitivity of power Doppler to very slow motion is still not adequate to detect capillary blood flow. This implies that microbubbles flowing in capillaries can not be detected based on their motion alone.

1.8.6 Detecting capillary flow

In essence, both color and power Doppler detection are based on the decorrelation of two consecutive pulse-echoes. In the extreme case, echoes from completely stationary targets will be identical, or perfectly correlated. If the targets move between pulse transmission, consecutive echoes will not be the same, and the degree of difference is related directly to the amount of motion. In color Doppler, the degree of decorrelation is directly mapped to a color intensity value which codes for velocity. In power Doppler, the degree of decorrelation is used only to compare with a threshold value (the clutter filter) and all signal above the threshold is integrated to give an energy value which is displayed as a color intensity.

Consecutive echoes from red blood cells and other linear scatterers decorrelate because of relative motion due to flow. Echoes from microbubbles, however, decorrelate for two distinct reasons: (1) relative motion due to blood flow, and (2) non-linear oscillation and ultrasound-mediated bubble destruction between pulses. Partial or complete destruction of contrast bubbles between two consecutive pulses is equivalent to motion as far as the Doppler detector is concerned, and the more extensive the disruption, the greater the decorrelation and the higher the estimated velocity. Thus, even stationary bubbles can be detected and displayed, both in color and power modes, provided the incident

64
pressure is sufficient to cause disruption.\[^{[49]}\] This is the key to imaging flow in the capillaries: Bubbles that are invisible because their flow rate is below the stationary filter detection threshold are made visible by turning up the transmit power output and inducing bubble destruction.

Detection, therefore, becomes a question of maximizing the rate of bubble destruction. As discussed in section 1.7.3 this can be achieved either by increasing the total acoustic energy incident on the bubble or by changing the properties of the contrast agent itself. The incident acoustic energy is determined primarily by the ultrasound scanner settings, and these include the actual power output of the transducer, the number of pressure cycles (duration) of each pulse and the number of pulses (ensemble length) per image frame, as well as the matching of transmit frequency to the bubble resonance frequency.\[^{[58]}\] Although all these parameters can be changed on a modern scanner, they cannot necessarily be changed independently of each other, and often an optimal compromise must be found to maximize bubble destruction. The properties of the contrast agent, on the other hand, are not selectable but depend strictly on the type of agent used. In general, rigid shell air agents are more sensitive to acoustic disruption and decorrelate rapidly, since their shell is more likely to crack under pressure fluctuations and once it does, the air inside dissolves quickly in plasma.

1.8.7 Harmonic imaging:

The equations governing the behavior of acoustic fields, including the fundamental wave equation and Lord Rayleigh's scattering theory, are all based on the small perturbation assumption, in which the propagating wave is of sufficiently low amplitude so as not to disturb the local material properties of the medium through which it travels.\[^{[51]}\] Under this assumption, wave attenuation, scatter and reflection are linear processes, an important consequence of which is that the backscattered signal frequency is equal to the incident frequency. For contrast agents, linear oscillations imply that bubble diameter varies directly with the external pressure cycles. This assumption quickly breaks down,
however, as the power output of the scanner is raised and the amplitude of the ultrasound wave increases until at very high transmit power, wave propagation and reflection exhibit non-linear properties. This is particularly true for contrast microbubbles. Bubble oscillations exhibit marked non-linear components, distinguished by unequal compression and expansion phases, at even moderately elevated incident pressures.\cite{1,2} This type of oscillation leads to the emission of acoustical signal with multiple, harmonic frequency components which are integer multiples of the fundamental, or incident, frequency $f_o$. The second harmonic is thus found at $2f_o$, while higher harmonics may be found at $3f_o, 4f_o, \ldots nf_o, \ldots$ albeit at decreasing magnitude. Because the microbubbles exhibit strong non-linear behavior, the harmonic components arising from contrast agents are as much as 35dB higher than the harmonic components arising from tissue at most transmit pressure, and this provides the contrast between agent and tissue.\cite{3} In harmonic imaging, the backscattered echo signal is put through a band pass filter, centered around twice the transmitted frequency, to reject all but the second harmonic signal component which is then used to form the ultrasound image.

Harmonic imaging can be implemented on existing scanners using the same hardware and software components with the addition only of a second harmonic band pass filter. However, receiving signal at twice the transmit frequency requires that high performance, broadband transducers be used in the manufacture of the scan head. The gain in image contrast, however, comes at the expense of image resolution.\cite{4,5} If the transmit and receive passbands (centered about $f_o$ and $2f_o$, respectively) overlap, linear echoes originating in tissue will spill over into the second harmonic signal and mask echoes arising from microbubbles, a problem that becomes prominent at low transmit power when non-linear behavior is minimal. If the transmit and receive bandwidths are narrowed in order to reduce band overlap and increase contrast, the axial resolution will be degraded (figure 1.14). This tradeoff between resolution and contrast, therefore, limits the ability of harmonic imaging to image the microcirculation.
Figure 1.14: Contrast/resolution tradeoff is inherent to harmonic imaging. Pulse transmission is at a frequency of $f_a$, while reception is at $2f_a$. Overlap between the two pass-bands degrades the contrast between microbubbles (non-linear) and tissue (linear) echos. Reducing the overlap requires narrow-band pulse transmission & reception and, therefore, degrades imaging resolution.
The clear advantage of harmonic imaging, however, is that the separation of microbubble and stationary tissue signals is not made on the basis of velocity, and therefore does not come at the expense of detection of slow, microvascular flow. Harmonic imaging can, therefore, be combined with decorrelation detection methods to form harmonic color and power Doppler images, which have been shown to be highly sensitive to the presence of bubbles without being overwhelmed by clutter signal or sacrificing low flow detection. [49]

1.8.8 Pulse-inversion:

Pulse inversion Doppler, like harmonic imaging before it, was specifically developed to take advantage of the non-linear properties of bubble oscillation. [67]

Pulse inversion images are formed by pairs of ultrasound pulses, \( p_1(t) \) and \( p_2(t) \), that are transmitted into tissue separated by a suitable time delay, \( T \), or pulse repetition period. The two pulses are identical, except that one is an inverted version of the other, \( p_2(t) = -p_1(t - T) \). In the case of a linear, stationary scatterer, the respective echoes, \( e_1(t) \) and \( e_2(t) \), from each of these pulses, would also be inverted images of each other, and their arithmetic sum, \( e = e_1(t) + e_2(t) \), would be zero. Echoes scattered from bubbles, however, or any other non-linear reflector, will not be exact, inverted replicas of each other, and their sum would not be zero (figure 1.15). Thus, adding the corresponding echoes from an inverted pulse pair, with suitable time shifting, provides a means of separating the microbubble signal from stationary, linear tissue clutter. The resulting signal can then be processed in the usual way to form a B-mode image, and this is known as pulse inversion imaging. Because there is no separation of the transmit and receive spectra into fundamental and harmonic bands, there is no compromise between spectral overlap (and hence contrast) and bandwidth (or axial resolution). The method is, however, sensitive to tissue motion, since even linear scatterers and reflectors, if moving, will produce echoes that
Figure 1.15: Basic pulse inversion imaging: A pulse and its inverted copy are transmitted in succession. Linear reflectors give rise to inverted echo pairs which sum to zero when properly time shifted. Non-linear targets, such as microbubble contrast agents, do not give rise to identical, inverted pulse-echos, so that their sums are not zero. This is the basis of pulse inversion image formation. Target motion between pulse pairs, however, may also give rise to a non-zero echo pair sum, so that some form of motion suppression is required.
are not only inverted but also phase offset by an amount equal to the distance moved during the pulse repetition period, and will therefore not sum to zero. At higher transmit pressures, non-linear propagation in tissue becomes considerable and results in non-linear scattering of the ultrasound from even stationary targets, which further reduces the contrast between agent and tissue.

To overcome motion clutter, pulse inversion detection can be combined with pulse wave (PW) Doppler processing to form what is known as pulse inversion Doppler (PID). In PID, an alternating sequence of normal and inverted pulses are sent into the tissue at a fixed pulse repetition frequency, or PRF, and the returning echoes are analyzed by the scanner. In conventional PW Doppler processing, sequential echoes are compared to determine the amount of target movement between pulses, based on either time-shift, or phase-shift calculations. The same processing can be carried out on the pulse inverted echoes, but noting that two consecutive echoes from linear, moving targets are phase shifted not only by an amount due to target translation, but also an added 180° phase shift that is due to the pulse being inverted in sign. The resultant Doppler shift for linear scatterers, calculated by the Doppler processor, is

\[ f_d = \frac{2v}{c} f_o + \frac{1}{2} PRF \]  

(124)

where, as previously, \( v \) is scatterer velocity (relative to the transducer), \( c \) is the speed of sound in tissue and \( f_o \) the transmit center frequency. Using a similar argument, the echoes from moving non-linear targets can be broken into even and odd function components, and it can be shown that the corresponding Doppler shift frequencies are

\[ f_d^{\text{odd}} = \frac{2v}{c} f_o + \frac{1}{2} PRF \]

\[ f_d^{\text{even}} = \frac{2v}{c} f_o \]  

(128)

Equations (128) and (124) would indicate that the frequency content of the signal from moving, linear
targets is displaced to the right in the Doppler spectrum by $\frac{1}{2} PRF$ which, incidently, is also the Nyquist sampling limit for PW Doppler, while the useful component of the bubble echo ($f_{d}^{max}$) has a Doppler shift frequency which is undisplaced and identical to the one obtained by conventional Doppler processing. If we impose the condition that the maximum scatterer velocity is limited such that

$$\frac{2 \nu_{max}}{c} f_0 \leq \frac{1}{4} PRF$$

(131)

then $|f_{d}^{max}|_{max} \leq \frac{1}{4} PRF$ so that the desired signal from bubbles lies inside the range $f \in [-\frac{1}{4} PRF, \frac{1}{4} PRF]$ while the entire clutter signal lies outside this range. A low pass filter, with a passband of $\pm \frac{1}{4} PRF$, can then be used to separate the two and provide clutter suppression. The obvious advantage of this scheme is that this filtering process does not eliminate the low velocity, and hence low flow, components in the signal. The penalty comes instead at the high end of the velocity scale, since the revised Nyquist velocity limit is halved to $\frac{1}{4} PRF$ from the conventional limit of $\frac{1}{2} PRF$. PID would thus appear to be an optimal technique for detection of flow in microcirculation, particularly at the low end of the transmit power scale, where non-linear propagation and scattering from tissue are negligible.
1.8.9 Summary of detection methods

The key obstacle to microvascular flow measurement has been the inability of conventional ultrasound imaging to detect microbubbles flowing at the very slow rates found in capillaries. This inability was based primarily on the strategies used to provide separation of tissue, or clutter, signal from blood signal. Three techniques optimized for microbubble detection and available on commercial scanners have been described, all of which have the potential to overcome these limitations. Power Doppler imaging combined with high transmit powers utilizes rapid microbubble decorrelation to detect contrast agent signal over stationary tissue, and gives an output signal that reflects the concentration of bubbles. Harmonic and pulse-inversion imaging utilize the non-linear oscillations of bubbles to separate their echoes from linear tissue reflections. Their output signal is a gray-scale, amplitude map of the distribution of bubbles. The greatest promise for optimal contrast agent detection, however, lies in the combination of these modalities, including harmonic power Doppler, and pulse-inversion Doppler, which are, unfortunately, not yet available on most clinical scanners.

1.9 Summary

This chapter opened with an introduction to the clinical problem of tissue ischemia, in diseases such coronary artery disease, stroke and peripheral vascular disorders, to illustrate the need for microvascular, and particularly capillary, blood flow measurement.

Another clinical problem where tissue ischemia may play a vital role and result in significant morbidity is surgical wound reconstruction with free tissue transfer. The topic was reviewed with particular attention to the role of the microcirculation and blood flow in the physiology of tissue auto-transplantation. The review of physiology concluded with a discussion of the microvascular changes that may lead to flap failure, in order to emphasize the need for real time post-operative monitoring and early surgical intervention. Existing monitoring techniques, which are not based on true blood flow measurement, were described and shown to be unreliable.
The difficulty in measuring microvascular blood flow was shown to be the result of two important factors: (1) the size of vessels of interest and the flow rate within them which are too small for conventional detection schemes, and (2) autoregulation and redistribution of flow by shunting that serve to uncouple capillary flow from flow in the larger, detectible vessels.

Indicator-dilution flow measurement is a means to overcome the limitations imposed by the microcirculation. The theoretical principles of indicator dilution, including the central volume principle, were derived to show how flow can be measured independent of vessel geometry using: (1) an intravascular indicator, (2) an intra-arterial bolus injection and (3) quantitative detection of outflow indicator concentration. Indicator-dilution theory can also be implemented using cross-sectional imaging modalities, where outflow concentration is not measured, providing proper analysis is performed using residue curves. Although the theory is sound, in practice it has been found to be associated with flow measurement errors on the order of 100 - 200%. The sources of these errors, based on violations of the basic assumptions of indicator-dilution, were explained in section 1.5.3.

The remainder of this chapter was devoted to reviewing ultrasound imaging and microbubble contrast agents so that we may understand how they can be used to perform flow measurement. The contrast enhancing properties of gas-based microbubbles, their non-linear oscillations and resonance phenomena were reviewed, including the significance of acoustic mediated bubble destruction. In order to use contrast agents as intravascular tracers, their concentration must be detectible and measurable. To that end, three ultrasound imaging techniques were described, power Doppler, harmonic imaging and pulse inversion, that take advantage of these unique properties to form images of microbubbles. Unlike conventional images, these modalities do not rely entirely on the motion of targets and are thus able to detect microbubbles even under capillary flow conditions.

With this information in mind we can now outline the proposed solutions to the problem of microvascular flow measurement: regional flow measurement will be performed using the principles of indicator-dilution. The problems of tracer diffusion and incomplete mixing will be solved with the use
of intravenously administered microbubble contrast agents, and the problem of intra-arterial bolus injection will be resolved with ultrasound mediated bubble destruction. Finally, detection of microbubble concentration and its separation from tissue noise will be addressed using non-linear ultrasound imaging modalities. These are the principles of "negative-bolus indicator dilution" flow measurement, and the implementation and experimental verification of this technique are the subject of the next chapter.
1.10 Appendix A: Pulse wave Doppler signal processing

For the sake of discussion, we can simplify the analysis by assuming a solitary, linear scatterer moving with a fixed velocity $v$ away from the transducer.

A simple, $n$ cycle emitted ultrasound pulse can be represented by a gated sinusoid:

$$e_i(t) = \text{rect}(t) \sin(2\pi f_o t)$$

$$\text{rect}(t) = \begin{cases} 
1, & 0 \leq t \leq \frac{n}{f_o} \\
0, & \text{otherwise}
\end{cases}$$

The received echo from this pulse, reflected by a linear scatterer initially a distance $d_o$ away, will be an attenuated version of $e_i(t)$, frequency shifted to account for the Doppler effect and delayed by a time factor of $\frac{2d_o}{c}$, which is the time required by the pulse to travel to the target and back:

$$r_i(t) = a \cdot e_i(\alpha t - t_o)$$

$$= a \cdot \text{rect}(\alpha t - t_o) \sin(2\pi \alpha t - \frac{2\pi}{f_o})$$

$$\alpha = 1 - \frac{2v}{c} \quad \text{and} \quad t_o = \frac{2d_o}{c}$$

(143)

where $\alpha$ is the Doppler frequency shift term, $a$ is an amplitude term accounting for attenuation and receive sensitivity and $t_o$ is the time delay due to the initial distance between transducer and target.

Subsequent echoes must also account for any target movement that takes place in the interval between pulse transmission. For example, the second pulse, $e_2(t)$, is generated at a time $t = T = \frac{1}{PRF}$ after the first, and during that time the target moves a distance $d = \nu T$ further away from the transducer, introducing an additional time delay of $\Delta t = \frac{2\nu T}{c}$. In general, by the $i^{th}$ pulse, $e_i(t)$, the scatterer...
moves a distance \( d = v(i - 1)T \) with a corresponding time delay of \( \Delta t = \frac{2v}{c} (i - 1)T \). (Strictly speaking, the scatterer actually moves a little further by the time the pulse catches up to it, for a total of
\[
d = \frac{c}{c-v} \left( d_o + [i-1]vT \right).
\]
Thus the true time delay is
\[
\Delta t = \frac{2}{c-v} (d_o - [i-1]vT) = \frac{2}{c} \left( \frac{1}{1 - \frac{v}{c}} \right) (d_o - [i-1]vT)
\]
which is approximately as stated when \( v \ll c \).) The \( i^{th} \) returned echo can be written as
\[
\begin{align*}
    r_i(t) &= a \cdot e_{i} \left( \alpha t - t_o - \frac{2v}{c} (i-1)T \right) \\
    &= a \cdot \text{rect} \left( \alpha t - t_o - \frac{2v}{c} (i-1)T \right) \sin \left( 2\pi f_o \left[ \alpha t - t_o - \frac{2v}{c} (i-1)T \right] \right)
\end{align*}
\] (157)
Substituting equation (27), we have
\[
\begin{align*}
    r_i(t) &= a \cdot \text{rect} \left( \alpha t - t_o - \frac{2v}{c} (i-1)T \right) \sin \left( 2\pi f_o \left[ \alpha t - t_o - \frac{2v}{c} (i-1)T \right] \right) \\
    &= a \cdot \text{rect} \left( t \right) \sin \left( 2\pi f_o \left[ \alpha t - t_o - \frac{2v}{c} (i-1)T \right] \right)
\end{align*}
\] (158)
Note that \( t \) represents pulse time, measured from the generation of each pulse, which is shifted in comparison to absolute time by an amount equal to the pulse repetition period. The equation above is the radio frequency (RF) echo signal received at the transducer as a result of the \( i^{th} \) pulse. In order to extract the target velocity, \( v \), the signal must undergo demodulation by multiplication with the center-frequency oscillator. The (in-phase) mixer function is
\[
m(t) = \sin \left( 2\pi f_o \left[ t - t_o \right] \right)
\] (161)
Multiplying equations (31) and (30), and using the appropriate trigonometric identity\(^2\), we get a new signal

\[
I_i(t) = r_i(t)m(t) = a \cdot \text{rect}_i(t) \cos \left(2\pi f_0[\alpha + 1]t - 2\pi f_0(t_o - 2\pi f_0(i - 1) \frac{2v}{c} T\right) + \\
+ a \cdot \text{rect}_i(t) \cos \left(2\pi f_0[\alpha - 1]t - 2\pi f_0(i - 1) \frac{2v}{c} T\right) \tag{162}
\]

The first term of the mixed signal equation (32) is a high frequency component, with frequency

\[f = (\alpha + 1)f_o = (2 - \frac{2v}{c})f_o\]

which can be suppressed by a suitably matched low pass filter. The result of low pass filtering is the demodulation signal:

\[
\text{LPF}\{I(t)\} = I_{LP}(t) = a \cdot \text{rect}_i(t) \cos \left(2\pi f_0[\alpha - 1]t - 2\pi f_0(i - 1) \frac{2v}{c} T\right) \tag{164}
\]

Note that this equation contains two time scales: \(t\), which is the RF, or \textit{fast}, time and \(iT\), which is a sampled version of pulse, or \textit{short}, time. By sampling the demodulated signal once, at a time \(t = t_i\) fixed with respect to the pulse time, the term \(2\pi f_0(\alpha - 1)t_i\) becomes a constant phase term, and we can write

\[
I_{LP}(i) = a \cdot \text{rect}_i(t_i) \cos \left(2\pi f_0 \frac{2v}{c} iT + \phi\right) \tag{169}
\]

\[
\phi = 2\pi f_0[\alpha - 1]t_i + 2\pi f_0 \frac{2v}{c} T
\]

The pulse wave demodulation and sampling process described in equations (31) through (34) collapses each RF echo signal to a single point value, given by equation (34). An ensemble of sequential echoes

\[^2\] \(2\sin \phi \sin \psi = \cos(\phi + \psi) - \cos(\phi + \psi)\)
from a series of pulses, therefore, results in a slowly varying sampled function, whose frequency is

\[ f = \frac{2|v|}{c} f_0 \]  

which, interestingly, is the same as the Doppler shift frequency derived earlier. Target velocity is thus obtained from the frequency content of the demodulated, sampled signal.

Unfortunately, equations (34) and (35) are ambiguous with regard to the sign of the quantity \( v \) which implies that the direction of flow is lost in the process of detection. Velocity direction can be recovered using a quadrature demodulation process. In equations (31) and (32) the received RF signal was multiplied with a mixer function in-phase with the center-frequency oscillator to obtain \( I(t) \). The RF signal can also be mixed with a function that is exactly \( \pi/2 \) out of phase with the master oscillator, to obtain an out-of-phase, or quadrature, component, \( Q(t) \). In this case the mixer function is

\[ m(t) = \sin(2\pi f_0 [t - t_0]) \]

and the result after multiplication, low pass filtration and sampling is

\[ Q_Lp(t) = a \cdot \text{rect}(t) \sin \left(2\pi f_0 \frac{2v}{c} iT + \phi \right) \]  

Having both the in-phase, \( I(iT) \), and the quadrature, \( Q(iT) \), components allows unambiguous calculation of velocity and its sign, with in the principal range of the trigonometric functions, i.e. - as long as \( 0 \leq 2\pi f_0 \frac{2v}{c} T < \pi \). This condition sets the maximum detectable, or Nyquist velocity, to

\[ 3 \text{ Not to be confused with flow.} \]

\[ 4 \text{ Using } 2 \sin \phi \cos \psi = \sin(\phi + \psi) + \sin(\phi - \psi) \]
In reality, the sample volume will contain not one, but multiple scatterers moving at different velocities. The analysis presented above is still valid when the signals from multiple scatterers are combined by superposition, provided the ensemble is sparse enough to maintain linearity. The demodulated signal will contain multiple frequency components corresponding to the various velocities present within the sample volume, so that the frequency, and hence, velocity distribution can be obtained from the Fourier transform of the analytic signal \( S(t) = I(t) + jQ(t) \). The result of the Fourier analysis can be displayed as the familiar Doppler velocity spectrum, or sonogram.

1.11 Appendix B: Color Doppler velocity estimators

The velocity estimator forms the core of color Doppler signal processing. Several different estimation algorithms have been devised.

1.11.1 The phase shift estimator

The first class are the phase-shift estimators. Phase shift processing begins with the analytic signal \( S(t) = I(t) + jQ(t) \), where the components \( I(t) \) and \( Q(t) \) are given by equations (34) and (37) respectively. Using these equations, we can write

\[
S(i) = I(i) + jQ(i) = a \cdot \text{rect}_c(iT)e^{j(2\pi f_o \frac{2\pi}{c} iT + \phi_c)}
\]  

(184)

so that velocity can be calculated from the time derivative of the phase in equation (39):
\[
\frac{d\phi}{dt} = 2\pi f_o \frac{2v}{c}
\]

Since the signal is discrete, the derivative of the phase must be estimated from two adjacent samples,

\[
\frac{d\phi}{dt} = \frac{\Delta\phi(i)}{\Delta i} = \phi(i + 1) - \phi(i)
\]

(186)

Now the phase of the signal \( S(i) = I(i) + jQ(i) \) can be obtained in the standard way

\[
\phi(i) = \arctan\left(\frac{Q(i)}{I(i)}\right)
\]

(188)

and by combining equations (41) and (42)\(^5\) we can obtain the estimate

\[
\Delta\phi = \phi(i + 1) - \phi(i) = \arctan\left(\frac{Q(i + 1)}{I(i + 1)}\right) - \arctan\left(\frac{Q(i)}{I(i)}\right) = \arctan\left(\frac{I(i)Q(i + 1) - I(i + 1)Q(i)}{I(i)I(i + 1) + Q(i)Q(i + 1)}\right)
\]

(189)

So that, finally, we obtain an estimate of velocity for a pair of consecutive echoes:

\[
v = \frac{c \cdot PRF}{4\pi f_o} \arctan\left(\frac{I(i)Q(i + 1) - I(i + 1)Q(i)}{I(i)I(i + 1) + Q(i)Q(i + 1)}\right)
\]

(190)

and estimates from several echo pairs can be averaged together to increase the precision of the final estimate. Expression (44) can also be written in terms of the discrete autocorrelation function of the analytic signal, which is defined as:

\[
R(m) = \frac{1}{N - 1} \sum_{i=0}^{N-2} S^*(i)S(i + m)
\]

(191)

\(^5\) And using the identity \( \arctan \alpha - \arctan \beta = \arctan \left( \frac{\alpha - \beta}{1 + \alpha \beta} \right) \)
where $S^*$ denotes complex conjugate. (Note that this expression is an estimate of the autocorrelation function for a limited sample of data). From equation (45) we have

$$R(l) = \frac{1}{N-1} \sum_{i=0}^{N-2} \left[ (I(i)I(i+1) + Q(i)Q(i+1)) + J[I(i)Q(i+1) - I(i+1)Q(i)] \right]$$

and combined with equation (44), it can be shown that the averaged velocity estimate over an ensemble of echo pairs is

$$\bar{v} = \frac{c \cdot PRF}{4 \pi f_o} \arctan \left( \frac{\Im \{ R(l) \}}{\Re \{ R(l) \}} \right)$$

(194)

where $\Re \{ \}$ and $\Im \{ \}$ denote the real and imaginary parts, respectively. Equation (47) is the basis of the autocorrelation approach to velocity estimation. The maximum detectible velocity depends on the uniqueness of the inverse trigonometric function $\arctan$, which has a range of $\pm \pi$. The resultant velocity limit is, therefore

$$|v|_{\text{max}} = \frac{c \cdot PRF}{4 \pi f_o}$$

which is equivalent to the pulse wave Doppler detection limit. Velocities greater than this maximum simply get aliased, or wrap around, within the detectible range.

1.11.2 The cross-correlation estimator

Another approach to the detection and quantification of target motion involves direct analysis of the RF, or time domain, echo signal by means of the cross-correlation method. Once again a given sample volume of blood is interrogated by a series of regularly spaced, short ultrasound pulses. The echoes are reflected by groups of red blood cells within the sample volume, such that each of these ensembles makes a unique contribution, or signature, to the returned RF signal, as illustrated in figure 1.16. As the blood flows the ensembles move along with the flow and the shape of RF echo changes from pulse to pulse. If, however, the pulse repetition period, $T$, is short enough so that the ensemble
Figure 1.16: Velocity estimation via the cross-correlation technique. If a group of scatterers remains sufficiently coherent over short intervals, it will have a recognizable echo 'footprint' that can be tracked with successive ultrasound pulses. Group motion is estimated in a similar manner to the PW Doppler technique, except that to increase the speed of computation, fewer echoes and faster algorithms are used. Estimating the maximum of the cross-correlation function between successive echo pairs is akin to sliding the echoes back and forth on paper until they more or less line up. Scatterer velocity is then estimated from the amount of sliding required, or the maximum-to-maximum distance.
of red blood cells stays coherent between consecutive pulses, the signature of a particular ensemble will remain recognizable but will be time shifted with respect to the beginning of each pulse. If the time shift, $\Delta t$, is measured, it can be used to directly calculate the distance, $d = \frac{1}{2} \Delta t \cdot c$ the ensemble moved and hence its velocity, $v = \frac{d}{T} = \frac{\Delta t \cdot c}{2T}$. A simple way to perform this function would be to draw two consecutive RF echoes on transparencies, lay them on top of each other and then slide one on the other until they appear to match, then calculating the time delay from the amount of shifting required. The mathematical equivalent to the slide-and-match method is the cross-correlation technique. The cross correlation function gives a measure of the match, or degree of agreement, between two signals time shifted with respect to one another, and for two received echoes, $r_j(t)$ and $r_2(t)$, with $t$ measured from the start of each corresponding pulse, is defined as

$$R_{1,2}(\tau) = \lim_{T \to \infty} \int_{-T}^{T} r_j(t) r_2(t + \tau) dt$$

or in the case of discrete, time limited signal, can be approximated as

$$R_{1,2}(n) = \frac{1}{N} \sum_{k=-N}^{N} r_j(k) r_2(k + n)$$

(207)

Calculating the time shift is then simply a matter of searching for the value of $n$ that gives the maximum $R_{1,2}(n)$. In order to find this value with reasonable computational efficiency, the search is restricted to discrete segments of the echo for which the cross-correlation function is computed, and the true optimal time shift is calculated from a polynomial fit to the discrete data. The time shift can be calculated not only for pairs of consecutive echoes, but also between any pair of echoes obtained from
the same sample volume and this strategy can be used to increase the precision of the velocity estimate.

The advantages of the cross-correlation technique are the increased precision and improved performance under low signal-to-noise conditions which means shorter acquisition times and increased image frame rate. The tradeoff is the heavy computational burden associated with the multiple calculations of cross-correlations and the maximum value search algorithm.\textsuperscript{[15]}
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89

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UMI
Chapter 2  **Negative bolus indicator-dilution measurement of microvascular blood flow**

2.1  **Introduction**

Having discussed the importance of blood flow for medical and surgical applications and the shortcomings of existing measurement techniques, we now describe a novel approach to microvascular blood flow measurement which is based on the administration, destruction and detection of ultrasound microbubble contrast agents. The technique is established on a modification of indicator-dilution theory discussed in chapter 1, and on the unique properties of microbubble contrast agents that allow their use as intravascular indicators. These properties are examined in detail in the next section, as are the mathematical principles of flow measurement using the destruction of indicator. The theory is then tested and validated in a bench top flow loop, as well as in an animal model of skin perfusion. This chapter concludes with a discussion of the results and possible sources of error from signal processing, detection and geometrical considerations.

2.2  **Background**

2.2.1  **Microbubble contrast agents as vascular indicators:**

Ultrasound microbubble contrast agents possess unique properties which make them ideal tracers for flow measurement: (1) they are safe for intravenous injection, (2) are uniformly distributed in blood, (3) remain intravascular after administration without affecting hemodynamic parameters, (4) microbubble concentration can be measured by integrated ultrasound echo signal intensity, and finally (5) exposure to ultrasound energy causes microbubble destruction. This latter property, which is
unique among all radiologic contrast agents, can be used in place of intra-arterial injection as a means of altering agent concentration in a target organ. Positive bolus injection is replaced with confined destruction of contrast agent by a properly focused ultrasound transducer positioned at the skin surface, and this sudden, confined absence of agent we have called a "negative bolus." The microbubbles specific imaging schemes reviewed in chapter 1 - Power Doppler, harmonic and pulse-inversion imaging - are able to detect bubble destruction and their sensitivity to very small concentrations of agent is maximized by imaging intermittently and at high transmitted pulse pressures (high MI).

Although the early contrast agents proved too short lived for anything but bolus injection, recently introduced second generation agents, including DMP115 and SHU563ATM, are sufficiently stable to survive several cycles through the circulation and can achieve steady-state intravascular concentrations supported only by slow intravenous administration of dilute agent.

In summary, in microbubble contrast agents we have an intravascular indicator, a sensitive detection method, and a non-invasive means of instantaneously changing indicator concentration in any tissue in the body accessible by ultrasound. The following section describes how these properties can be used in flow measurement.

### 2.2.2 Flow measurement with a negative bolus of indicator: A theoretical model.

Ultrasound mediated destruction and detection of microbubbles can be combined with indicator-dilution principles to measure microvascular blood flow. It is instructive to examine how we may do this using a simplified model: consider a single, straight tube with plug flow of velocity \( v_o \), and volume flow rate \( Q \). A microbubble contrast agent at constant rate and concentration \( C_o \) is added to the fluid, and an ultrasound transducer is placed over the vessel (figure 2.1). At time \( t = 0 \) an image of the vessel cross section is taken by the transducer, and the ultrasound energy destroys the microbubbles in the region defined by the imaging plane and the out-of-plane (elevation) thickness of
the ultrasound beam, $E$. If our imaging modality is able to reject all signal not due to microbubble destruction with ideal efficiency, a second image taken immediately after the first would have an integrated signal power $S(0) = 0$, since no bubbles are present within the region of interest. If we wait a short period of time $\Delta t$, fresh bubbles will flow into the region of interest and their number will equal the negative-bolus residue function, $R(t)$:

$$\begin{align*}
R(\Delta t) &= Q\Delta t C_n & (0 \leq \Delta t < \frac{E}{v_n}) \\
R(\Delta t) &= QC_n \frac{E}{v_n} & (\Delta t \geq \frac{E}{v_n})
\end{align*}$$

where the second term reflects the fact that once sufficient time is allowed for fresh agent to completely transverse the ultrasound beam elevation thickness $E$, the number of microbubbles in the region of interest remains constant thereafter and equal to the product of concentration and region volume. If we acquire a single image at this time, and if $S(\Delta t)$ is linearly proportional to $R(\Delta t)$, then:

$$S(\Delta t) = R(\Delta t)\Psi = Q\Delta t C_n\Psi$$

where $\Psi$ is the ultrasound transfer function, accounting for the transmitted signal power, beam attenuation, bubble scattering cross-section as well as transmit and receive sensitivities. Signal intensity, therefore, increases with the time interval between images, in this case linearly, at a rate proportional to flow, $Q$, until a plateau level determined by equation (1) is reached.

To carry this model further, consider now a collection of $N$ straight, parallel vessels, each with plug flow $Q_n$, flow velocity $v_n$, and cross section area $\sigma_n$, for $n = 1, \ldots, N$. Without loss of generality, assume $v_1 > v_2 > \ldots v_{N-1} > v_N$. We now define the transit time $t_n = \frac{E}{v_n}$, as the time required by the
Figure 2.1: Performing negative-bolus indicator-dilution in a simplified setting - a single, large vessel filled with flowing contrast microbubbles. An imaging transducer with a beam elevation thickness $E$ is shown directly over the vessel. $m(t)$ represents the number of microbubbles present in the imaging region of interest. (a) The initial image is taken at $t=0$ and generates a negative-bolus, or void, of agent, characterized by $m(0)=0$. Waiting a short time $t=\Delta t$ allows fresh agent to flow into the region of interest as shown in (b). The number of microbubbles is directly proportional to the flow rate and the time interval. (C) Waiting longer than $t=E/v_o$ allows the region to be completely filled with fresh agent between images, so that $m(t)$ remains constant thereafter.
Figure 2.2: Negative-bolus indicator-dilution applied to a distribution of flow velocities, represented here by $n$ straight vessels with uniform flow velocities $v_i$, and filled with a constant concentration of contrast agent. (a) Generation of a negative-bolus at $t = 0$. (b) At $t = \Delta t$, the negative-bolus will have washed out of the faster flowing vessels, but only partially out of the remaining vessels (equation 3).
microbubbles in vessel $n$ to transverse the elevation thickness $E$ of the ultrasound beam, and it follows then that $0 < t_1 < t_2 < \ldots < t_N$. Again, at $t=0$, a negative bolus is created by the ultrasound transducer in a region of interest encompassing all vessels. If we image after a time interval $\Delta t$, such that $t_k < \Delta t < t_{k+1}$, for some $1 < k < N$, the negative bolus will have washed out of vessels 1 through $k$, but still be in transit in vessels $k+1$ through $N$ (figure 2.2). Summing the contributions from all vessels, the residue function is then:

$$R(\Delta t) = \sum_{i=1}^{k} C_i E \sigma_i + \sum_{i=k+1}^{N} Q_i \Delta t C_i, \quad \text{for} \quad t_k < \Delta t < t_{k+1}$$

where the first sum is constant and equal to microbubble content of the first $k$ vessels (concentration $\times$ imaged vessel volume, $E \sigma_i$). For the special case $0 < \Delta t < t_1$ (i.e. the first segment of the washout curve), $k = 0$ and equation 3 becomes:

$$R(\Delta t) = \sum_{i=1}^{N} Q_i \Delta t C_i$$

which, assuming once again a linear relationship between $S(t)$ and $R(t)$, determines the signal intensity over this short time segment:

$$S(\Delta t) = \sum_{i=1}^{N} Q_i \Delta t C_i \Psi_i, \quad 0 < \Delta t \leq t_1$$

Taking the derivative of equation 5 with respect to time, assuming that flow is time invariant and that
the transfer function $\Psi$ is approximately uniform across the region of interest, we get

$$\left. \frac{dS(t)}{dt} \right|_{t < t_i} = \Psi C_0 \sum_{i=1}^{N} Q_i$$  \hspace{1cm} (44)

which is the relationship between the signal intensity, or washout, curve and total flow $Q = \sum_{i=1}^{N} Q_i$. In the limiting case of $N \to \infty$, as the individual transit times $t_i$ become continuous and $t_i \to 0$, we can rewrite equation 6 as:

$$Q = \frac{1}{C_0 \Psi} \frac{dS(0)}{dt}$$  \hspace{1cm} (49)

In practice, $C_0$ and the transfer function $\Psi$ are not known in-situ and, therefore, the absolute flow rate cannot be measured using equation 7. However, if we assume that the baseline concentration of agent is constant for the duration of the experiment and that the determinants of the transfer function remain unchanged, we can calculate the relative flow rate, $\bar{Q}$, which we now define as:

$$Q \approx \bar{Q} = \frac{dS(0)}{dt}$$  \hspace{1cm} (53)

In other words, relative flow rate is equal to the initial slope of the negative bolus washout curve.

This principle is illustrated in figure 2.3, where cross-sectional Power Doppler images of agent flowing in a latex vessel taken at different time increments are shown. Image brightness increases incrementally with increased time delay and approaches a plateau enhancement level determined by baseline agent
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97

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concentration and vessel volume. Under real flow conditions, $S(t)$ has been found to fit an exponential saturation model of the form:

$$S(t) = A(1 - e^{-\beta t}) \quad (55)$$

Thus, according to equation 8, if $S(t)$ is linearly proportional to $R(t)$, relative flow is:

$$Q = \frac{dS(0)}{dt} = A \cdot \beta \quad (58)$$

Equation 10 forms the basis of the negative-bolus flow measurement technique, and the following sections describe the experimental testing and validation of the theory presented.

### 2.3 Methods & Materials

#### 2.3.1 In-vitro

These experiments aim to test the principles of negative-bolus flow measurement in simple geometrical setups where precise flow determination is possible. Two phantoms (figure 2.4) were constructed for this purpose: A large vessel model, 15cm long, made of thin walled latex tubing (¼" Penrose surgical drain, 7mm outer diameter under flow conditions) and a small vessel model, 8cm long, consisting of approximately 40 hollow fibers of 100μm internal diameter (C-DAC Artificial Kidney model 90SCE, CD Medical, Miami, FL). Mean flow velocity and equivalent volume flow rates per unit tissue mass are shown in Table 2.1.
**Figure 2.4:** In-vitro set up. Contrast agent was pumped from a stirred reservoir, via a peristaltic pump into a second mixing chamber which also served as a bubble trap. The solution was then gravity driven into a small tank holding one of two phantoms: a 7mm latex vessel, or a bundle of microfibers. A constant height bottom reservoir maintained constant flow for the duration of the experiment.
Table 2.1: Estimated phantom flow parameters

<table>
<thead>
<tr>
<th></th>
<th>7mm phantom</th>
<th>100μm phantom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (mL/100gm/min)</td>
<td>430-4300</td>
<td>210 - 1000</td>
</tr>
<tr>
<td>Mean velocity (mm/sec)</td>
<td>0.7 - 7.2</td>
<td>120-600</td>
</tr>
</tbody>
</table>

Volume flow per unit tissue mass was estimated by calculating the effective vascular volume of each phantom (ROI volume occupied by vessel lumen for the large vessel, ROI volume bounded by outer diameter for the small vessel bundle) and assuming an equivalent tissue density of 1g/1cc. The large vessel phantom was, therefore, used to simulate flow velocities compatible with the slow moving blood of capillaries (<1mm/sec) while the microvessel model allowed us to measure blood flow in unresolvable vessels. In general, bench top models of the microcirculation are extremely difficult to construct, so that realistic velocities and flow rates could not be simultaneously achieved in a single phantom. The vessels were immersed in a water bath coated with ultrasound absorbing material to facilitate ultrasonic imaging.

Steady flow was supplied by a reservoir system designed to maintain a constant driving pressure head for the duration of the experiment, while flow rate was adjusted by varying the height of the upper reservoir. True flow rate was determined by volume collection of effluent from the bottom reservoir over a period of 10 minutes. All water was distilled and gas-desaturated for a minimum of 48 hours to minimize background echoes from particulate matter and free air bubbles within the flow loop.

Data acquisition and image analysis were performed with a commercial digital ultrasound scanner (HDI5000) using a curvilinear, low frequency scan head (C5-2) fixed in position over the vessel phantom at a distance of 7cm from and 60° angle to the direction of flow. Computer software provided by the manufacturer (ATL Inc. Bothell, WA) provided algebraically correct spatial averaging and integration of signal power measurements without the errors introduced by the use of log-compressed, non-linear video data. Scanner imaging parameters were set and held constant throughout
the experiment: frame averaging was turned off, focal depth was set to coincide with vessel position, PRF and wall-filter cut-off frequency were minimized to increase sensitivity to low flow, and the MI, sensitivity and line density settings were maximized to increase incident acoustic pressure and microbubble destruction.

(1) \textit{Signal power vs microbubble concentration:}

To obtain flow measurement using equation 10, a linear relationship must exist between scattered ultrasound echo intensity, $S(t)$, and scatterer concentration, $R(t)$. While this has been well established for sparsely distributed, solid scatterers in a low power acoustic field, it has not, however, been verified in the non-linear regime of gas microbubbles undergoing destruction by high pressure ultrasound pulses. This experiment was thus designed to establish this relationship by measuring the Power Doppler echo intensity obtained from different concentrations of flowing agent.

DMP 115 (DuPont, Boston, MA), a lipid shell perfluorocarbon gas contrast agent with mean bubble diameter of 5\mu m, was chosen for these experiments after preliminary experiments showed it to be highly stable in solution with long in-vitro half life (figure 2.5). To obtain accurate concentrations of microbubbles, a closed mixing chamber was supplied with a solution of water and a solution of continuously stirred contrast agent (0.1mL/L), via separate digital IV pumps. The steady-state agent concentration (0 - 0.1mL/L) was varied by changing the individual rates of each IV pump (0 - 1000cc/hr), while the total flow rate in the phantom vessel was kept constant (1000cc/hr). The digital pumps were calibrated using timed collection prior to each experiment to ensure accurate dilution. Power Doppler images of flowing agent were obtained at a 1Hz frame rate at two power output settings (maximum available $MI=1.1$, and intermediate $MI=0.6$). 10 images were acquired at each measurement and transferred to a PC to determine average integrated signal power for each concentration and power
Figure 2.5: DMP115 is a highly stable contrast agent in-vitro. A plot of Power Doppler signal power against time shows a loss of only 5dB after nearly 2 hours had elapsed since agent preparation. Measurements were made in the 7mm phantom, under continuous, steady flow using a 0.05mL/L DMP115 solution.
Flow was measured in both the large and small vessel phantoms using a solution of DMP115 (0.05mL/L) and a sequence of Power Doppler images acquired at maximum transmit power (MI=1.1). To perform negative bolus indicator-dilution images were triggered at seven different time intervals: 0.5, 1, 2, 4, 8, 15 and 30 seconds apart. At each triggering interval 10 individual images were acquired and averaged, for a total acquisition time of approximately 10 minutes per flow measurement. Images were transferred to a PC for calculation of integrated power, and iterative least-squares was used to fit the theoretical model (equation 9) to the data using a statistical software package (Microcal Origin 4.1, Microcal Software, Northampton, MA). Flow calculated from the fitted curves using equation 10 was compared to actual flow rate obtained by timed collection.

2.3.2 In-vivo

Two animal models were used for in-vivo testing. A canine model was used to establish that the conditions necessary for negative-bolus flow measurement are satisfied in-vivo, while a porcine model of skin perfusion was used to conduct actual flow measurements:

2.3.2.1 Canine model

A 25kg mongrel was intubated under general anaesthesia and allowed to ventilate spontaneously. Anaesthesia was induced with an intramuscular barbiturate and maintained with an inhaled mixture of oxygen and Halothane. The dog was placed on a warming blanket in the supine position with legs abducted to expose the medial aspects of both thighs. Animal care conformed to the guidelines of the Canadian Council on Animal Care (CCAC) and the protocol approved by our institutional review board.
(1)  *Steady microbubble concentrations from IV infusion.*

This experiment was designed to establish that IV infusion of contrast agent could achieve and maintain a constant intravascular concentration of microbubbles in-vivo. DMP115 in saline (1mL/250mL) was infused via a digital IV pump into a paw vein while Doppler signal enhancement in the femoral artery was tracked with a PW Spectral Doppler unit (Interspec Corporation, Conshohoken, PA) using a 10MHz pencil probe set to the lowest possible output power. The quadrature signals from the Doppler unit were fed into a separate computer equipped with an analogue-to-digital data acquisition board (National Instrument) for real-time integrated signal power analysis.

(2)  *Signal power vs microbubble concentration.*

The linear relationship between signal power and microbubble concentration must be established in-vivo as well as in-vitro before flow measurement can take place: DMP115 in saline (1mL/250mL) was once again infused via a digital IV pump into a paw vein. The IV infusion rate was varied (100 - 500 mL/hr) to obtain different intravascular concentrations of agent. Re-establishment of steady-state was verified after every change in the infusion rate using an independent PW Spectral Doppler unit (set up as above) over the left femoral artery. Power Doppler images (MI=1.1) of the right femoral artery and vein were obtained with an HD15000 digital scanner using a curvilinear C5-2 probe. Image acquisition was synchronized to ECG and triggered every 2nd QRS complex. Ten images were acquired at each infusion setting and averaged together to obtain integrated power.

**2.3.2.2 Porcine skin flap model**

The porcine buttock skin flap is an established model for human skin perfusion. The flap is an island of skin and subcutaneous tissue that is surgically isolated from all its tissue attachment except for a single vascular pedicle at its proximal end. Blood is supplied only via this vascular base and by
designing the flap longer than the base can functionally support, a gradient of blood flow is formed from the base to the distal tip (figure 2.6). Thus flow can be measured in skin ranging from normal to ischemic. Castrated Yorkshire pigs (12-14kg) were anaesthetized with intramuscular ketamine, then intubated and mechanically ventilated. Anaesthesia was maintained with an inhaled mixture of oxygen and nitrous oxide (50/50) and a warming blanket was used to maintain constant body temperature. An intravenous line was established through an ear vein to infuse isotonic saline (2mL/min) and for the introduction of contrast agent. A catheter was placed in the femoral artery via a cut-down procedure and used to monitor mean arterial blood pressure for the duration of the experiment. The animal protocol was approved by the Hospital for Sick Children Animal Care Committee.

A total of three buttock skin flaps were used, each measuring 8 x 18 cm. Once raised, the flaps were sutured back in their original position for support and to prevent skin contraction. Steady intravascular concentration of microbubbles was maintained with an IV infusion of DMP115 (1.8mL/250mL Saline at 200mL/hr). The negative bolus Power Doppler image sequence described in section Chapter 2.3.1.02, was acquired through an HD15000 scanner using a linear array transducer (L7-4) held fixed in position by an articulated arm. A 2cm tall, 2% agar standoff was used to approximate the elevation focus of the transducer to the skin surface and cross sectional flow measurements were made at 1.5 cm intervals along the length of the flap.
Figure 2.6: The porcine buttock skin flap. (a) The design of the rectangular flap on the flank of a pig. The axial blood supply of the flap is shown. (b) The skin and subcutaneous fat is surgically elevated from all tissue attachment except at the base where the pedicle enters the tissue. The pedicle is left intact. (c) The flap is then sewn back in position in preparation for flow measurement. Both ultrasound and microsphere measurements were made in a total of twelve, 1.5cm wide segments.
2.3.2.3 Microsphere flow measurement:

Microsphere flow measurement principles were reviewed in chapter 1. Briefly, the method involves the introduction of uniformly sized (15μm), radioactive-isotope labeled polystyrene beads into the circulation via a left ventricular injection catheter. The fundamental assumptions are that the microspheres are well mixed with blood and are then distributed to different organs in direct proportion to blood flow at the time of injection. Because the beads are too large to pass through capillaries they become stuck at the precapillary level and are removed from the circulation in the first pass. The tissue is then sacrificed and cut into pieces which are placed in a gamma counter to estimate the total number of microspheres trapped. If the total number of injected microspheres is known, the count number gives an estimate of blood flow relative to total cardiac output. To make the method absolute, a sham organ is introduced into a major artery in the form of a catheter attached to a precision withdrawal pump. At the time of injection, the pump withdraws a fixed volume of arterial blood at a predetermined flow rate, and this blood sample is then used to normalize all other tissue measurements. The microsphere technique, therefore, gives an instantaneous measure of absolute capillary flow (mL/min/100gm) in a given tissue sample at the time of injection.

Following ultrasound flow measurement described in the previous section, cobalt 57-labeled microspheres, 15.7 ± 0.5μm diameter (New England Nuclear, Boston, Mass), were prepared according to standard protocol and injected (~120,000/kg) into the left ventricle via a carotid artery catheter. Reference blood samples were obtained from the femoral artery via a withdrawal pump and transferred to counting vials. The skin flap was cut into 1.5 cm wide transverse segments and the radioactivity in the samples determined in a gamma counter (1282 Compugamma CS, LKB Wallac, Finland). At the conclusion of the flow study the pig was sacrificed with an overdose of pentobarbital (100mg/kg).
2.4 Results

2.4.1 In-vitro

(1) Signal power vs microbubble concentration:

The relationship between microbubble concentration and integrated Power Doppler signal intensity for two transmit power settings is shown in figure 2.7. As in the case of a low power ultrasound field incident upon linear scatterers, scattered power is linearly proportional to microbubble concentration ($r = 0.98$) with no evidence of attenuation over the range of agent concentration $0 - 0.1\text{mL/L}$. Integrated intensity, therefore, provides an accurate measure of relative agent concentration, but without knowledge of in-situ microbubble scattering cross section and beam attenuation, absolute agent concentration cannot be known.

(2) Flow measurement:

In figure 2.8 three sample negative bolus washout curves are shown for three different flow rates, as measured in a cross section of the large vessel phantom using the Power Doppler mode. The residue function curves fitted to this data are also shown, and the initial slope of these curves (representing relative flow rate) and six others are plotted against true flow rate in figures 2.9 & 2.10, for the large and small vessel phantoms, respectively. Both are linear and highly correlated to true flow rate ($r = 0.991$ and $r = 0.937$).
Figure 2.7: Signal power versus microbubble concentration in-vitro. Integrated Power Doppler signal power (in linear units) is plotted against the concentration of DMP115 at both maximum (MI=1.1) and intermediate (MI=0.6) transmit power. The relationship is linear with no evidence of attenuation for agent concentration below 0.1mL/L. Error bars shown are +/- 1 Std. Dev.

Figure 2.8: Negative-bolus washout curves obtained in the 7mm vessel phantom. Three sample curves are shown for three different flow rates. The data points represent averaged, integrated signal power, while the smooth curves represent the best fit to the mathematical model.
2.4.2 In-vivo

(1) Steady microbubble concentrations achieved with IV infusion:

Constant intravascular microbubble concentration is central to the application of the negative bolus technique in-vivo. Figure 2.11 shows a sample Spectral Doppler power trace obtained over the femoral artery of a dog during IV infusion of DMP115. Steady Doppler enhancement, and hence intravascular concentration of agent, were achieved within 2 minutes of the start of infusion, and were sustained until the infusion was discontinued. Enhancement levels of up to 20 dB were achieved with infusion rates of 0.8mL of contrast agent per hour. Similar tracings (not shown) were obtained in the porcine model, but obtaining comparable levels of enhancement required higher infusion rates (1.44mL/hr).

(2) Signal power vs. microbubble concentration:

A linear relationship between integrated Power Doppler signal power and microbubble infusion rate (and hence intravascular concentration) was obtained in the dog (figure 2.12) confirming the results obtained in-vitro. No attenuation effects were noted at infusion rates used (100 - 500mL/hr).
Figure 2.9: Relative flow calculated from the washout curves is well correlated to the flow rate obtained by timed collection in the 7mm vessel phantom. DMP115 was used, at a concentration of 0.05mL/L. Error bars shown are +/- Std. Error from the model fit.

Figure 2.10: Similar results obtained in the 100μm vessel phantom. Although the vessels were not visually resolved, flow estimation was still possible.
Figure 2.11: Steady intravascular concentration of DMP115 is sustained with a slow intravenous infusion in-vivo. Pulse wave, spectral Doppler power measured in the femoral artery of a dog receiving a slow infusion of contrast agent (0.4mL/hr) is plotted against time. Steady intravascular concentration is evidenced by the constant level of signal enhancement starting just prior to the three minute point.

Figure 2.12: Signal power versus DMP115 concentration in-vivo. Integrated Power Doppler signal power at maximum (MI=1.1) transmit pressure varies linearly with microbubble infusion rate, as measured in the femoral artery and vein of a dog. No evidence of attenuation is seen at infusion rates under 0.8mL/L. Error bars shown are +/- 1 Std. Dev.
(3) Flow measurement:

Figure 2.13 shows Power Doppler images of porcine skin blood flow at 5 different time interval settings, clearly demonstrating incremental signal enhancement due to the wash-in of fresh contrast agent. Three sample negative bolus washout curves obtained from similar images in a porcine skin flap are shown in figure 2.14. As in-vitro, the data was used to fit residue function curves which are also shown in the figure. The flow rate obtained with the microsphere technique is compared to the relative flow obtained via the negative bolus method in figures 2.15-17. Flow has been normalized to the flow rate measured at the base of the flap and the paired data are shown for different sections along the length of the flap, from proximal to distal, for three skin flaps. Correlation levels between the microsphere and ultrasound data varied from $r = 0.59$ to $r = 0.82$, but more importantly, both methods agreed on the distance at which flow became undetectable. Normalized ultrasound derived flow pooled from all three flaps is plotted against microsphere derived flow in figure 2.18, with an overall correlation rate of $r = 0.77$. Most of the data points lie above the $y = x$ line, indicating that the negative bolus technique tends to overestimate flow as measured by microsphere injection.
Figure 2.13: Images of porcine skin. (a) Pre-contrast image, for anatomical orientation, showing the tissue layers imaged in cross-section. The region of interest alone is shown in (b) where a series of power Doppler images obtained at maximum MI are shown for two different cross sections of the porcine skin flap: one proximal and one distal with respect to the base. Negative-bolus tracking is again demonstrated in the incremental enhancement in color intensity seen as the time interval between images increases. The enhancement is less pronounced distally, where blood flow is reduced.
Figure 2.14: Sample negative-bolus washout curves in the porcine skin perfusion model, measured at three different points along the length of the flap. The smooth curves represent the best fit to the mathematical model.
Figure 2.15: Ultrasound and microsphere measured flow rates, normalized to the first (base) segment of the flap, plotted against the distance from the base. The results of linear regression analysis are shown.

\[ Q_{us} = 0.728 Q_{ms} + 0.267 \]
\[ (r = 0.579) \]

Figure 2.16: Similar results for the second of three skin flaps raised.

\[ Q_{us} = 0.879 Q_{ms} + 0.146 \]
\[ (r = 0.819) \]
Figure 2.17: Similar results for the third and final flap raised.

\[ Q_{us} = 1.66 \, Q_m + 0.02 \]
\[ (r = 0.797) \]

Figure 2.18: Pooled results for all three porcine skin flaps. There is a systematic bias toward overestimation of flow by the ultrasound technique which is evident after comparison with the line of identity, \( y=x \).
2.5 Discussion

Measurement of blood flow using conventional indicator-dilution is often complicated by the breakdown of the principle assumptions on which the theory is based (chapter 1), often leading to significant errors in flow estimation: (1) To minimize invasiveness, tracer is often injected some distance from the organ under study and most often in the venous circulation. The resultant uncertainty in the tracer input function leads to significant bolus spread error.\(^\text{[7,8]}\) (2) Most radiological tracers leak through vascular endothelium and become sequestered in tissue. Intravascular indicator mass is therefore not conserved.\(^\text{[9]}\) (3) Incomplete mixing of tracer with blood after bolus injection has been estimated to cause as much as 100-200\% error in flow measurement.\(^\text{[10,11]}\) Furthermore, conventional positive-bolus indicator-dilution is not suitable for tissues that are supplied by multiple arterial input and venous output networks, such as skin and muscle.

As we shall see, most of these important issues can be addressed directly by the negative-bolus technique: (1) Bolus spread error is completely eliminated. Since the negative-bolus is created in the same region and by the same transducer that measures the residue function the result is the absence of a wash-in phase and a near ideal bolus profile. (2) Unlike other radiological tracers, microbubbles remain entirely intravascular during their lifetime. No errors are introduced due to loss of indicator and the measurements made reflect only blood supply, not diffusion or exchange across capillaries. (3) Injecting agent into the venous circulation means the microbubbles undergo thorough mixing with blood both in the right and left ventricles, as well as undergo several circulation cycles prior to arriving at the site of flow measurement. Administration of contrast agent and bolus injection become two separate processes. Because positive bolus injection into the arterial inflow is replaced with the creation of a negative bolus right in the region of interest, and because contrast agent is distributed uniformly in blood, the problem of flow measurement in organs with multiple inflow paths and supply networks.
is eliminated. This, however, does mean that since the ultrasound beam geometry is planar in nature (elevation thickness $\ll$ height and width) the blood flow measurement may depend on transducer orientation, particularly in longitudinally oriented tissue such as skeletal muscle. The effect of beam orientation on the flow estimate has yet to be investigated.

In light of this discussion it is perhaps surprising that the correlation between the ultrasound technique and the gold-standard microsphere technique is not better than it is ($r = 0.77$). The difficulty in comparing blood flow rates obtained with the two techniques is that they do not measure the same quantity: In any tissue blood flow may be divided into *nutrient* (capillary) flow, which is destined for nutrient exchange with cells, and *conduit* flow (feeding and draining) or *shunt* flow, which do not participate in exchange. The negative bolus technique is sensitive to microbubbles in all blood vessels and, therefore, measures total blood flow, while 15$\mu$m microspheres measure only nutrient flow.$^{[12,13]}$ The presence of large feeding or draining vessels in the region of interest would then be expected to lead to large discrepancies between the two techniques and this was confirmed by visual examination of the images used to generate the data in figures 2.15-17. In regions where large conduit vessels are absent (such as the distal half of the flap) the two methods would be expected to be in closer agreement, which is again confirmed by the data. Microvascular shunts, however, would remain a source of discrepancy, since shunts are present throughout the length of the flap and usually in great concentrations within skin.$^{[14,15]}$ We must also keep in mind that the ultrasound derived measurement is an averaged quantity acquired over several minutes, while microsphere injection provides only an instantaneous, single time point measurement which is, therefore, subject to large stochastic variation$^{[12,13,16]}$ and contributes further to differences between the techniques.

Signal processing considerations and the mechanical properties of contrast agents may also play a role in the accuracy of flow measurement and these are discussed in the next three sections:
2.5.1 Power Doppler is flow dependent:

Theoretically, the expected enhancement from a vessel as $t \to \infty$ depends only on the product of intravascular agent concentration and vessel volume. If circulating microbubble concentration is constant and vessel geometry is fixed the asymptotic enhancement should remain fixed and independent of flow rate. The asymptotic enhancements obtained from the Power Doppler wash-out curves in-vitro, however, exhibit a clear flow dependence, decreasing as flow rate drops (figure 2.8). When the experiment was repeated using tissue harmonic imaging, the washout curves obtained did converge asymptotically and exhibited true flow independence (figure 2.19), while using two-pulse pulse inversion Doppler showed some flow dependence, but not as marked as for Power Doppler (figure 2.20). The explanation may lie in Doppler signal processing: Power Doppler uses a velocity threshold to separate moving from non-moving scatterers. This so-called wall filter removes the portion of the signal lying below the velocity threshold, and although the threshold can be minimized, it cannot be turned off. Thus, in slower moving flows, a proportionately larger fraction of the signal is removed by the wall filter, and the result is a flow dependent artifactual reduction in enhancement. Harmonic imaging does not distinguish moving from non-moving targets and in the absence of a clutter filter, the signal is preserved and true flow independence is obtained. Harmonic imaging performed well in-vitro, being highly correlated with true flow rate ($r = 0.975$ and $r = 0.94$ for the large and small vessel phantoms, respectively). On the other hand, pulse inversion imaging, although lacking a true wall filter, has an inherent velocity-dependent amplitude component, (see section 1.10) and the resultant wall-filter-like artifact is noted in the figure.

Given the high level of correlation between Power Doppler measurements and true flow rate, the significance of the wall-filter artifact in-vitro appears minimal. Its effect in-vivo, however, where very slow flow is much more likely, may be more pronounced.
Figure 2.19: In-vitro washout curves obtained with harmonic imaging. These curves, unlike those obtained with Power Doppler (figure 2.8) converge asymptotically, because harmonic imaging provides velocity-independent detection.

Figure 2.20: Washout curves obtained with pulse inversion imaging in-vitro. Compared with Power Doppler derived curves (figure 2.8) these show less asymptotic dependence on flow rate, however, unlike harmonic imaging (figure 2.19), there remains a residual wall filter artifact, as can be seen from the lack of asymptotic convergence.
2.5.2 **Harmonics and pulse inversion imaging performed poorly in-vivo:**

While the performance of harmonic imaging in-vitro compares well with Power Doppler, it would appear from the above discussion to be superior as a flow quantifying modality based strictly on signal processing considerations. The question, then, is whether this imaging modality is sufficiently sensitive to microbubbles to allow measurement of blood flow in-vivo in the absence of a wall filter. To address this issue, negative bolus flow measurements were carried out in the canine thigh muscle model using the three imaging modalities available: Power Doppler, tissue harmonics and pulse inversion Doppler. With harmonics and pulse inversion Doppler, pre-contrast background subtraction was carried out to separate contrast from the stationary tissue signal. A sample washout curve obtained with each method is shown in figure 2.21. As before, the Power Doppler data fit the theoretical model (equation 9). However, the pulse-inversion data, although demonstrating incremental echo enhancement, failed to converge to the residue function model while the tissue harmonic data did not show incremental echo enhancement at all. It would seem, then, that the lack of an advanced wall filter in the latter two modalities, combined with the presence of significant clutter signal in tissue means that neither method in its current state is suitable for quantitating microvascular flow in muscle and skin.

2.5.3 **Choice of agent influences flow measurement:**

A different approach to the wall filter problem may involve the choice of contrast agent: The Power Doppler image intensity is estimated from the time-domain decorrelation of the RF echo from insonated microbubbles. This decorrelation comes from two sources: (1) motion of bubbles within the ROI due to blood flow - which is determined by the hemodynamic state of the tissue, and (2) the destruction of microbubbles due to ultrasound energy - which is determined by the properties of the microbubble and the imaging parameters. If the latter contribution to the Power Doppler spectrum could be maximized, the relative signal energy removed by the wall filter and hence the wall-filter artifact
Figure 2.21: Both harmonic and pulse inversion imaging fail in-vivo. Negative bolus "washout" curves measured in canine thigh muscle are shown, as obtained with three different microbubble detection schemes. While the power Doppler data fit the theoretical model quite well, the least-squares fit failed to converge for the pulse inversion data. Measurements made with harmonic imaging did not demonstrate incremental signal enhancement at all.
would be reduced. Using an air-based microbubble contrast agent can do just that: Air is highly soluble in plasma, particularly more so than perfluorocarbon gas, so that once the shell is disrupted the air-based agent disappears quickly. Rapid disruption means increased decorrelation and should result in a more broadband Doppler spectrum. This hypothesis was tested in-vitro using the air based agent SHU563A (Schering AG, Berlin). Loss of asymptotic enhancement measured with Power Doppler at different flow rates are compared for DMP115 and SHU563A (figure 2.22). The air based agent, as predicted, demonstrates significantly less flow-dependent filter artifact than the slower decorrelating, perfluorocarbon agent. Raising the PRF and wall-filter cut off frequency - effectively raising the threshold velocity - increases the effect of the wall filter for both agents.

These data demonstrate that air-based agents can, therefore, minimize the error introduced by the wall-filter while maintaining the sensitivity of Power Doppler to low microbubble concentrations in-vivo.

2.5.4 Relative flow measurement:

In section 2.2.2, it was pointed out that this ultrasound technique, in its current implementation, can measure relative changes in tissue flow only, since conversion to absolute flow requires knowledge of in-situ acoustic attenuation and scattering cross-section. This may not necessarily prove to be a significant drawback, however, since absolute flow measured in units of mL/min/100 gm may not be, on its own, a reliable predictor of tissue viability and ischemia. Simply put, absolute flow rate means little without the knowledge of the normal physiologic value, and since what constitutes normal physiology may vary significantly between patients, “normal” values must be calibrated on an individual basis. To be meaningful, then, flow measurements obtained with this technique must be compared with either serial measurements made in the same region or with measurements made elsewhere but under similar attenuation conditions - i.e. similar tissue composition and depth and identical imaging parameters. Such circumstances include the measurement of flow along the length of a flap, as was
Figure 2.22: The air based agent SHU563A is less affected by the power Doppler wall filter. Loss of steady-state (asymptotic) signal power is plotted against absolute flow rate, for SHU563A and the perfluorocarbon agent DMP115, as measured with intermittent power Doppler. The perfluorocarbon agent shows a greater dependency on flow rate, which becomes pronounced for very low flow. For both agents, the effect is enhanced when the wall filter cut-off frequency and the PRF are increased.
done with the porcine skin model, or comparison of flow against either an immediately adjacent region or a mirrored region in the unaffected side of the body.

2.6 Conclusions

This work lays down the theoretical foundation for quantitative flow measurement in the microcirculation of skin and muscle using the unique properties of ultrasound contrast agents and the principles of indicator dilution. We have shown experimentally that the conditions necessary for such measurement can be satisfied in-vitro as well as in-vivo: (1) A linear relationship exists between agent concentration and integrated ultrasound signal power, which enabled the measurement of residue curves directly from the pre-processed ultrasound data. (2) Constant intravascular concentrations of microbubbles can be maintained in-vivo by slow intravenous infusion of agent, and (3) The negative bolus technique can measure relative flow rate accurately in bench top phantoms and in an animal model of skin flap perfusion. Although the principles of flow measurement outlined here were tested in skin and skeletal muscle only, the theory is directly applicable in any other tissue accessible by ultrasound. This method provides a relative measure of total blood flow in a defined region of interest, and allows comparison of flow rates while both agent concentration and tissue attenuation (depth and tissue type) are similar. It does not, at this time, provide a measure of absolute blood flow, nor a means of separating true capillary, or nutrient, flow from shunted blood flow. Finally, our detection method of choice, power Doppler, was shown to have a velocity-dependent measurement bias introduced by the process of clutter suppression. In the absence of clutter suppression, however, flow measurement in-vivo became exceedingly difficult. The solution to this problem may lie in the use of air-based contrast agents and in new microbubble detection schemes, such as pulse inversion Doppler and harmonic power Doppler, that could offer both high sensitivity to small bubble concentrations and true flow velocity independence.
Reference List


9. Doriot PA, Dorsaz PA, Dorsaz L, Rutishauser WJ. Is the indicator dilution theory really the adequate base of many blood flow measurement techniques? *Medical Physics* 1997; V 24(12); pp. 1889-1898.


Chapter 3. **Future Work**

3.1 **Introduction**

In the previous chapter we introduced a technique for blood flow measurement in the microcirculation, whose principles were derived from indicator-dilution theory and the properties of ultrasound microbubble contrast agents. In this final chapter the future direction of this technique will be outlined, with particular attention paid to clinical application at the bed-side for the purpose of tissue monitoring. The unresolved issues include the precision of flow measurement, the optimization of the processes of bubble destruction and detection and the reduction of measurement time. The chapter concludes with a discussion of possible avenues for absolute blood flow measurement and additional anatomic information which may be gleaned from the residue curve.

3.2 **Changes needed for clinical implementation**

Currently, two obstacles make the negative bolus technique difficult to apply at the bed-side: (1) the reproducibility of the process of bubble destruction and of bubble detection are unknown, as is measurement precision, and (2) the lengthy acquisition time required for each flow measurement, during which the transducer must be rigidly held and the local hemodynamic state must remain steady. Both must be addressed before this technique can be used at the bed-side:

3.2.1 **Bubble destruction and bubble detection:**

The negative-bolus indicator-dilution method consists of two processes that should, in theory, be distinct. The first is the destruction of bubbles, which generates the negative bolus, and the second is the detection of agent as it refills the region of interest. Currently, both of these processes are intimately linked, since the disruption of bubbles with high pressure ultrasound also allows their detection and quantification with power Doppler. In fact, with the software and hardware available on
our clinical scanner at the time, this was the only way to perform negative-bolus flow measurement. The processes of bubble disruption and detection, however, have different requirements and should be considered separately.

3.2.1.1 Optimizing bubble destruction

As discussed in chapter 1, ultrasound mediated bubble disruption is a complex process that is currently not well understood. We know that it depends on both the rate and total amount of acoustic energy incident upon the bubble, and on the mechanical nature of the shell material and gas content of the bubble itself. To increase the precision of negative-bolus flow measurement, bubble destruction must be optimized to consistently reproduce, as closely as possible, the ideal bolus profile \( C_{\text{arterial}}(t) \rightarrow \delta(t) \): that is, the negative bolus must be formed instantaneously, uniformly and completely across the entire region of interest. This can be achieved by optimizing the shape and attributes of the incident ultrasound pulse as well as by altering contrast agent properties as discussed in chapters 1 and 2. Most commercial scanners allow the user direct or indirect control over pulse amplitude, transmit center frequency, the number of cycles, and the number of pulses sent down a line of sight to form a single image frame. Each can affect the rate and completeness of bubble destruction, but these parameters cannot always be set independently of each other. The estimated MI has an upper limit imposed by FDA regulations for each particular imaging applications, so that increasing the pulse duration or ensemble length may cause a machine compensated decrease in pulse amplitude to maintain the MI within the allowed limits. Similarly, there exists a tradeoff between pulse duration, ensemble length, depth and region of interest size as determined by the desired image frame rate. Therefore, an optimum setting that will maximize bubble destruction under the given constraints of MI and frame rate must exist, but it remains unknown since our understanding of the physical principles that govern bubble disruption is limited. Several investigators are now attempting to shed light on this issue by
studying the behavior of microbubbles in high intensity acoustic fields. New mathematical models of bubble oscillation that can account for the complex behavior of the shell, thermal and viscous effects, as well as the interactions of ensembles of bubbles in a pulse-wave acoustic field are under development.\textsuperscript{11,25} These models can be used to predict the theoretical response of bubbles to different pulse forming parameters and may allow calculation of optimal scanner settings. Another interesting development has been the use of optical microscopy and very high speed photographic equipment to study bubble disruption. Early experiments have shown that the disruption process is indeed complex, and often involves fragmentation of the shell and the formation of transient, free gas bubbles in the medium.\textsuperscript{31} Without the dampening action of the shell, the small bubble fragments, although short lived, have greatly enhanced scattering properties and may be responsible for the stimulated acoustic emission phenomenon. These optical studies may eventually be used to directly measure the effect of various scanner and pulse forming settings on the rate and completeness of bubble destruction in-vitro.

3.2.1.2 Bubble size distribution

Another agent property that is of significance to the process of destruction and detection is the size distribution of the bubbles themselves. It is well known that once prepared for injection, a dose of microbubbles does not contain uniformly sized particles (unlike radioactive microsphere preparations) but rather has an inherent size distribution, centered around some mean value.\textsuperscript{11,41} Since both the scattering cross section and the resonance frequency are highly dependent on bubble diameter, having a non-uniform bubble size implies that not all bubbles respond similarly to the destruction and detection pulses. In other words, not all the injected bubbles will participate in the generation of a negative bolus, because the transmit settings are not optimized for their destruction. If the bubbles do not ‘pop’ and are also not detected, their presence will not influence the flow estimate, since they are essentially invisible to the measurement technique. If the bubbles are not ‘popped’ but still undergo
resonance behavior that results in a detected signal, they will then bias the flow estimate. To see why this should happen, consider a flow system made up of two flow pipes with equal concentrations of contrast agent, $C_0$, one with flow rate $Q_1$ and the other with flow rate $Q_2$. Let them both be imaged simultaneously, but assume that only the bubbles in the first pipe are destroyed. At a time $t = \Delta t$ after negative-bolus creation the number of fresh contrast agent present in the region of interest will be

$$m(t) = C_0(\Delta t + E\sigma_2)$$

(6)

where $E\sigma_2$ is the vascular volume of the second pipe, defined by the beam elevation thickness and the pipe cross-sectional area, and is constant, since no bubbles are destroyed. According to the model, the system flow rate is given by the time derivative of equation (1), which gives $Q \approx C_0Q_1$, which is an underestimate of the true flow rate $Q = Q_1 + Q_2$ (figure 3.1). In general, if only a fraction, $\varepsilon$, of bubbles are destroyed but all bubbles are still detected, the resulting flow underestimate will be $\varepsilon$th of the true flow rate, or $\varepsilon Q$. This problem may not be of much significance, however, when measuring relative flow, as long as $\varepsilon$ is constant for a given agent and affects all flow measurements. If it is not, the flow estimate will vary with the particular size distribution of the batch of contrast agent used. Although the mean bubble diameters have been estimated for various agents, for instance 5μm for DMP115,[5] the actual size frequency distribution is often not known. Even if it were known in-vitro, that distribution will likely change significantly once the bubbles are administered in-vivo due to the filtering action of the pulmonary capillary beds and the premature destruction of small, unstable bubbles by intra-cardiac mechanical stress and shear forces. The effect of pulmonary filtering and disintegration of small bubbles in the circulation is likely to narrow the distribution of bubbles about their mean diameters and make the bubbles more uniform in size - a hypothesis which remains to be proved.
Figure 3.1: To illustrate the effect of incomplete microbubble destruction, two vessels with different flow rates are shown imaged under the same transducer. In the top vessel bubbles are both imaged and disrupted, whereas in the bottom vessel bubbles are imaged but not destroyed. The latter vessel does not, therefore, contribute to the flow measurement, resulting in a systematic underestimation.
3.2.1.3 Optimizing bubble detection

This discussion concerns three fundamental issues: (1) Obtaining images that are clutter free, (2) eliminating flow dependent bias in the detection method and (3) verifying experimentally that microbubbles can, in fact, be detected in capillaries.

In order to equate the echo signal power with the negative bolus residue function, as was done in chapter 2, there must be no significant clutter component in the scanner output signal. This is clearly not the case with either harmonic or pulse inversion imaging, where tissue motion and non-linear components are present in the output signal. An attempt to deal with the clutter signal with simple background image subtraction had failed since the variance in the speckle images was likely much greater than the actual signal arising from microbubbles, and the result was an image dominated by noise. On the other hand, clutter rejection in power Doppler introduced a velocity bias into the flow estimate. The ideal detection method must, therefore, have perfect clutter rejection, and at the same time provide a measure of agent concentration that is not based on its flow rate.

The newer ultrasound imaging modalities may approach that ideal detection model. Harmonic power Doppler employs clutter suppression based on the linear behavior of non-microbubble targets, so that the wall filter in the Doppler processor may either be eliminated or greatly minimized to reduce the velocity dependent signal suppression. Pulse inversion Doppler would appear to offer even greater advantage, since clutter suppression comes at the expense of high velocity detection, leaving the low velocity, and hence low flow, range free from bias. Testing of these new modalities for use in flow measurement will likely form the next step in development.

The third issue is the confirmation of capillary microbubble detection. While theoretically the three bubble-specific imaging modalities described in sections 1.8 through 1.10 are able to detect the presence of microbubbles in the microcirculation, and although clinical evidence exists for contrast agent detection in 40μm renal arterioles,[5] this has not been demonstrated unequivocally in capillaries.
The flow images obtained in the porcine skin flap show incremental enhancement of detected signal power as late as 20 seconds after the generation of the negative bolus, implying that for an approximate beam elevation thickness of 1.5mm, flow of velocity under 750μm/sec was being imaged - a velocity consistent with capillary flow. Such low velocities could also result from laminar flow in larger vessels with very slow flow near the vessel wall, so this evidence cannot be taken as absolute confirmation. Some evidence for single bubble detection comes from experiments in bubble counting using very dilute concentrations of contrast agent (under 1000 bubbles/mL) with harmonic power Doppler and very high MIs, allowing individual bubbles flowing in a bench top vessel loop to be detected and displayed. These bubbles were, however, flowing at greater than physiologic velocities, conditions which do not necessarily mimic capillary flow.

A possible way to confirm the detection of microbubbles in capillary flow would employ confocal, intravital optical microscopy and ultrasound scanning. Using such a setup, and an infusion of a very dilute solution of contrast agent, optical confirmation of ultrasound detection of solitary bubbles could be obtained under physiologic conditions. This confirmation is essential for a rigorous demonstration of the validity of negative-bolus indicator-dilution microvascular flow measurement.

3.2.2 Reducing data acquisition time

The implementation of negative-bolus flow measurement described in chapter 2 is based on power Doppler imaging and requires very high transmitted pressures to detect slow moving contrast agent. The high MI means that every image acquired also generates a de-novo negative bolus, so that the residue function curve cannot be tracked in real time, but must instead be reconstructed in a piece wise manner. Combined with the averaging of 10 acquired frames for noise reduction, a single washout curve tracked for 30 seconds requires roughly 10 minutes of data acquisition. During this time, both subject and ultrasound transducer must be kept relatively motionless, and hemodynamic state must
remain constant - conditions which would make this acquisition sequence difficult to apply at the bedside.

Two possible schemes for reducing acquisition time will now be suggested: One is based on the current image acquisition protocol, while the other proposes an altogether different acquisition sequence that takes advantage of newer microbubble detection modalities.

3.2.2.1 Estimating initial slope

Based on negative-bolus indicator-dilution theory, volume flow rate is estimated from the initial slope of the received integrated signal power curve. In chapter 2 the slope estimate was not obtained from the data itself, but rather from a theoretical model that was fitted to the data (equations 9 and 10), taking into account all points acquired up to 30 seconds post negative bolus generation. Curve fitting was used in combination with data averaged over several images in order to reduce the inherent variability in ultrasound images, but this was responsible for the prolonged acquisition time. If it were possible to reduce the noise in the data, perhaps through better detection schemes, and higher agent concentrations, the initial slope of the signal power curve could be estimated directly from the first and second image frames. For instance, estimating initial slope based on a time delay of only one and two seconds, even with 10 frame image averaging, would reduce the data acquisition time from 10 minutes to under 30 seconds. Of course, it is the first few seconds of negative-bolus washout that contain the fewest number of fresh microbubbles and hence are subject to the maximum stochastic variance, and lowest signal-to-noise ratio, so that precision will be degraded.

3.2.2.2 Flash-echo real time imaging

Perhaps the most promising approach to real-time flow measurement involves the complete separation of negative bolus creation and bubble detection. One strategy requires the use of two separate, confocal, ultrasound transducers (and scanners) set up over the region of interest - one
optimized for bubble destruction, and the other for continuous bubble detection at low transmitted pressures.\(^7\) The added cost and bulk of a second ultrasound scanner and difficulties in aligning two transducers over the same region of interest have limited the development of this technique. \(^8\) A better approach, available on a few commercial scanners, has been the use of specially designed imaging sequences, for instance flash-echo imaging by Toshiba, in which the first frame of a sequence is acquired at very high transmit pressures, generating a negative-bolus, while subsequent frames are acquired continuously at a lower MI. Although this allows continuous, and therefore, real time imaging, the problem is that conventional imaging under low transmitted pressures cannot detect bubbles in the microcirculation. Pulse inversion Doppler, however, allows detection of very slow moving microbubbles even at low transmitted pressures and can be used with the flash-echo sequence to track negative-bolus washout in real time.\(^9\) The calculation of a single blood flow measurement could, therefore, be carried out in under 30 seconds, instead of the previous 10 minutes, and be repeated on a minute by minute basis, as required. Several measurements, however, may still need to be averaged together to give a more precise estimate of mean blood flow. The advantages of this type of detection are multiple, including the reduction of data acquisition time, velocity-independent flow detection with pulse-inversion Doppler, and the ability to independently optimize the technique for both bubble destruction and for bubble detection.

Early trials of canine myocardial blood flow measurement with flash-echo and pulse inversion Doppler have been carried out in our lab, and although publication is pending, early results show great promise for the future of real-time flow measurement.

### 3.3 Estimating absolute blood flow

It is possible that absolute determination of blood flow, in mL/100g/min, may be desirable for some applications. An ultrasound based detection technique cannot directly provide absolute
measurements of agent concentration, since neither tissue attenuation and scattering cross-sections can be measured in-situ. It may be possible to address this problem indirectly using two different approaches, one based on the normalization of vascular volumes, and the other based on bubble counting, or scintigraphy:

3.3.1 Normalizing vascular volumes

The problem of absolute flow measurement stems from the inability of the detection method to estimate absolute indicator concentration in-vivo. This is, in fact, common to all imaging methods that rely on tissue attenuation to form images, including both ultrasound and CT scanning.

Recalling chapter 2, equation 7, absolute volume flow rate is

$$Q = \frac{1}{C_0} \frac{dS(0)}{dt}$$

but neither $C_0$, the baseline concentration, nor $\Psi$, the transfer function, are known. We can estimate these quantities by examining a small sub-region within the imaging volume that can be considered to be entirely vascular, i.e. a large, resolvable artery or vein intersecting the scan plane. Let this be region 1, and assume its cross-sectional area, $\sigma_1$, can be measured directly from the ultrasound image. The measured, integrated received signal power from region 1 is $S_1(t_\omega)$, where $t_\omega$ is the time at which the microbubble concentration may be taken as approximately equal to baseline, or $C_0$. $S_1(t_\omega)$ is given by the product of the vessel volume (area $\times$ elevation thickness, $E$) and microbubble concentration, multiplied by the transfer function:

$$S_1(t_\omega) = C_0 \cdot \sigma_1 E \cdot \Psi$$

$$\therefore C_0 \Psi = \frac{S_1(t_\omega)}{\sigma_1 E}$$

(24)
Thus, by locating a large, perpendicular vessel within the region of interest, measuring its area and integrated signal power, along with an estimate of the beam elevation thickness, the correction factor given by equation 3 can be used to convert relative flow to absolute flow, in the immediate vicinity of the calibration vessel (figure 3.2). This is similar in concept to the normalization of tracer clearance flow measurement with a blood sample drawn from a peripheral vein or artery, and is routinely used for CT flow measurement[8] and has been suggested for ultrasound measurement.[9] The accuracy of this correction depends not only on the accuracy of measurement of beam thickness, which is in itself an estimate,[10,11] but also on the assumption that $\Psi$ remains constant within the region of interest. To minimize variation in attenuation, and hence $\Psi$, the correction must be applied piecewise on regions that are at the same tissue depth as the vessel used for calibration and is, therefore, limited by the availability of suitable, large vessels near or within the region of interest.
Figure 3.2: Estimating *absolute* flow by normalizing vascular volumes. Images of porcine skin flap are shown: (a) initial image, showing a large artery or vein in cross section, which is evident even at the shortest imaging interval. (b) 15 second image, with the cross sectional area of the vessel shown. The integrated signal within the circle, its area, and the estimated beam elevation thickness can be used to correct for absolute flow.
3.3.2 Microbubble scintigraphy

Inferring microbubble concentration from the bulk property of average energy scattering, or integrated signal power, serves the purposes of relative flow measurement well, but has two significant drawbacks: (1) The relationship is linear only over a small range of bubble concentrations, and (2) it is ambiguous due to the uncorrected effects of beam attenuation. A possible solution to this problem may be to completely bypass integrated signal power measurement and instead count the passage of individual bubbles through the region of interest. By counting actual bubble popping - which is a binary event - an absolute measure of bubble concentration can be obtained that is independent of attenuation. This is analogous to radioactive decay counting, or scintigraphy, as performed by gamma counters used in nuclear medicine. The absolute microbubble count can be used directly to obtain absolute volume flow rate using negative-bolus indicator-dilution. Early experiments with microbubble scintigraphy, or echoscintigraphy, have been described, where very dilute concentrations of microbubbles (under 1000 bubbles/mL), were used to improve the accuracy of vessel lumen measurements. In its current form, however, due to the very low concentrations of agent used, image formation by scintigraphy requires very prolonged frame acquisition and integration, taking as long as 30 minutes to form an image, so that flow imaging or bolus tracking is not yet feasible. As microbubble detection technology improves, however, it is possible that echoscintigraphy will become a practical means of obtaining absolute flow measurement in-vivo.

3.4 Estimating microvascular anatomy and flow distribution based on the residue curve:

In Chapter 2, the shape of the in-vivo residue curve was approximated by the empiric model

\[ R(t) = A(1 - e^{-\beta t}) \]

as used by Wei, et al. The shape of the residue curve, however, can be determined analytically in cases where vessel geometry and flow velocity distribution are known. For example, in the simple case of plug flow in a straight vessel and beam elevation thickness \( E \), the residue curve was
derived in Chapter 2, and is given by

\[ R(t) = \begin{cases} 
\pi r^2 v_0 C_0 t & 0 < t < E/v_0 \\
\pi r^2 v_0 C_0 & E/v_0 \leq t 
\end{cases} \] (29)

For a straight vessel of radius \( r_0 \) and a laminar, parabolic flow velocity profile

\[ v(r) = v_0 \left[ 1 - \left( \frac{r}{r_0} \right)^2 \right] \] (31)

it can be shown, using a similar derivation, that the residue curve is

\[ R(t) = \begin{cases} 
\pi r_0^2 t, & 0 \leq t \leq E/v_0 \\
(2\pi r_0 + 1) \frac{E}{2} - \frac{E^2}{2v_0 t}, & E/v_0 < t 
\end{cases} \] (32)

as is shown in figure 3.3. In general, for a large ensemble of parallel, straight vessels of uniform cross section area, \( \sigma \), and a flow velocity distribution \( \omega(v) \), the residue function will be given by the solution of

\[ \frac{dR(t)}{dt} = C_0 \sigma \int_{r_{\text{min}}}^{E/v} v \omega(v) dv \] (35)

Thus the distribution of flow velocities, which may be a reflection of the vascular anatomy in the region of interest, will have direct bearing on the shape of the washout curve. Conversely, it may be possible to infer information about the morphology of the microcirculation from the shape and behavior of the washout curve. This type of deduction may, at some point in the future, prove valuable in differentiating normal, ordered microvascular flow, from the chaotic, random flow found in rapidly growing neoplastic tissue.
Figure 3.3: Deriving the residue curve from a priori knowledge of the flow velocity distribution. (a) The expected residue curve for uniform, or plug, flow profile is shown, based on equation 3.4. (b) The expected residue curve as it would appear for a vessel with a parabolic flow profile, based on equation 3.6. (calculated for $r=5\text{mm}$, $E=1.5\text{mm}$, $v_o=30\text{mm/sec}$).
3.5 Summary:

In chapter 2 a new approach to measuring blood flow in the microcirculation of skin and muscle was described and then tested against accepted gold-standards. For the purposes of testing, the method was implemented using available imaging equipment and software, a process which necessitated prolonged acquisition times. The data acquisition time is, in my opinion, the greatest obstacle to clinical implementation, particularly at the bedside, since it places great demands on both the patient, the operator and the circulatory system. The focus of future work, therefore, must be directed toward solving this problem. Possible strategies for reducing data acquisition times were described, and the one showing greatest promise involves real-time imaging using a combination of “flash-echo” frame sequences and pulse-inversion Doppler, an approach which is currently being investigated in our lab. The advantages of flash-echo and pulse inversion Doppler are not only the reduced acquisition time but also the ability to separately control and optimize the bubble destruction and detection processes and the freedom from velocity-dependent detection bias. Clinical implementation of the negative-bolus technique will likely be based on this approach.

The utility of flow measurement could be further increased if it were possible to convert relative flow measurement to absolute, physiological blood flow in mL/100gm/min. A practical means of doing so is by normalization of vascular volumes, a method routinely used in other medical imaging techniques. Bubble echoscintigraphy, on the other hand, is a completely new approach to absolute flow quantification which is not, unfortunately, clinically feasible at this time, but is of great interest for future development.

The problem of practical, bed-side measurement of blood flow is by no means solved. With this work, however, we have shown that intravenous administration of microbubbles and negative-bolus
tracking with ultrasound can provide accurate relative flow measurement in experimental conditions. With further research and development in the directions outlined above, this method could provide a valuable clinical tool in many medical fields, including microvascular and reconstructive surgical applications.
Reference List


