Detecting Treatment Failure in Rheumatoid Arthritis with Time-Domain Diffuse Optical Methods

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Biomedical Engineering
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Abstract

Rheumatoid arthritis (RA) is the most common type of inflammatory arthritis, and affects approximately 1% of the population in Canada. While the disease has no cure, early treatment within the first 3–6 months of onset is known to substantially reduce disease progression and improve patient prognosis. Nevertheless, identifying which therapy will elicit the appropriate treatment response depends on a time-consuming, trial-and-error approach. Thus, there is a strong clinical motivation to develop treatment monitoring methods which signal the need for treatment adaptation as early as possible; this helps ensure that patients reap the benefits of effective early treatment, and mitigates the risk of irreversible joint damage. The limitations of current monitoring methods include subjectivity, low sensitivity, high cost, and operator dependence. Diffuse optical methods offer an objective, sensitive, low-cost, and operator-independent solution for monitoring RA disease activity. Previous diffuse optical methods used continuous-wave and frequency-domain techniques to identify joint inflammation; however, little work has explored time-domain (TD) methods. TD techniques typically provide richer information content, which may be leveraged to increase sensitivity to subtle changes in RA disease activity. This dissertation investigates the prospects of two TD diffuse optical methods for RA treatment monitoring. First, a contrast-enhanced near-infrared spectroscopy technique was used to monitor joint blood flow (BF) changes in a longitudinal study of a rat model of inflammatory arthritis. However, the study found no significant difference in joint BF between controls and rats with induced arthritis. Second, a novel TD diffuse optical imaging (DOI) method for monitoring RA disease activity was developed and assessed in silico; this method was then implemented experimentally and tested on disease-mimicking phantoms. Spatiotemporal Fourier components extracted from simulated TD-DOI images were strongly correlated with a measure of virtual RA disease activity, and components acquired by the experimental TD-DOI system could clearly distinguish between phantoms that mimicked different RA disease activities. These findings suggest that TD-DOI has the potential to be a sensitive treatment monitoring tool for RA, and future work should test its efficacy in RA patients.
**Keywords:** rheumatoid arthritis, treatment monitoring, diffuse optical imaging, near-infrared spectroscopy, time-domain, time-resolved, single-pixel, Monte Carlo simulation, dynamic contrast-enhanced, blood flow
Summary for Lay Audience

Rheumatoid arthritis (RA) is a common, long-term inflammatory disease which primarily affects joints. The disease has no cure, but starting treatment early after diagnosis can substantially improve patient outcomes over the long term. Since patients with RA respond differently to the various available treatments, identifying the correct treatment for an individual patient requires a time-consuming, trial-and-error approach. Therefore, there is a need for treatment monitoring methods that can easily determine if a patient is responding to treatment as early as possible to avoid further joint damage and help ensure that patients reap the benefits of effective early treatment.

Current monitoring methods are either subjective, expensive, have low sensitivity, or are highly dependent on the person operating the monitoring equipment. Optical techniques are an objective, sensitive, and relatively low-cost alternative for treatment monitoring which do not depend on an experienced equipment operator. Previous optical imaging methods have been used to identify joint inflammation; however, there has been little work focused on methods using time-domain (TD) acquisition even though it typically provides more information, and might be more effective at identifying subtle changes in joint inflammation.

This dissertation investigates the prospects of two TD optical methods for RA treatment monitoring. First, an optical technique for measuring joint blood flow (BF) was used to track BF changes in a rat model of inflammatory arthritis. However, this study did not find any significant difference in joint BF between rats with induced arthritis and healthy rats. Second, a TD diffuse optical imaging (DOI) method for RA treatment monitoring was developed and assessed using computer simulations; this method was then implemented experimentally and tested on physical models that mimic different stages of RA. Simulated TD-DOI images could be used to track different levels of virtual disease, and images acquired by the TD-DOI imaging system could distinguish between the physical disease models. TD-DOI has the potential to be a sensitive treatment monitoring tool for RA patients, and future research should test this technique in RA patients.
Co-Authorship Statement

Chapter 2 of this dissertation was adapted from a published journal article, while chapters 3 and 4 were adapted from unpublished manuscripts which are in preparation for submission. As first author of these manuscripts, I was responsible for all data acquisition and analysis, and contributed significantly to the interpretation of results and manuscript preparation. Dr. Mamadou Diop secured the funding for the presented works and supervised all projects, while also providing guidance and greatly assisting in data analysis, interpretation of results, and manuscript preparation. Each of the listed co-authors contributed to the work and approved manuscript submission; additional individual contributions are listed below.

Chapter 2 was adapted from work which was published in 2020 by Seva Ioussoufovitch, Laura B. Morrison, Lise Desjardins, Jennifer A. Hadway, Keith St. Lawrence, Ting-Yim Lee, Frank Beier, and Mamadou Diop in the Journal of Biomedical Optics (volume 25, issue 1) under the title “Quantification of joint blood flow by dynamic contrast-enhanced near-infrared spectroscopy: application to monitoring disease activity in a rat model of rheumatoid arthritis”. Aside from the aforementioned contributions, Mamadou Diop was responsible for the study design and provided the system used for data acquisition. Laura B. Morrison, Lise Desjardins, and Jennifer A. Hadway were responsible for animal care and assisted with data acquisition.

Chapter 3 is an unpublished work titled “Time-domain diffuse optical imaging technique for monitoring rheumatoid arthritis disease activity: theoretical development and in silico validation”, which is in preparation for submission by Seva Ioussoufovitch and Mamadou Diop. Aside from the aforementioned contributions, I was responsible for the experimental design, and the creation of the virtual models used for data simulation.

Chapter 4 is an unpublished work titled “Time-domain diffuse optical imaging technique for monitoring rheumatoid arthritis disease activity: experimental validation in tissue-mimicking finger phantoms”, which is in preparation for submission by Seva Ioussoufovitch and Mamadou Diop. Aside from the aforementioned contributions, I was responsible for the experimental design, developing the system used for data acquisition, and creation of the imaging phantoms.
Dedication

I dedicate this work to my family.

To my parents, thank you for the sacrifices you have made, the worries you have carried, and the love you have always provided. I am eternally grateful for the life you have given me, and feel privileged to be the son of such amazing people.

To my sisters, brother-in-law, and grandparents, thank you for the endless encouragement, affirmation, and kindness you have provided over all these years. To my niece and nephews, it has been a joy watching you grow into the individuals you are today; thank you for always putting a smile on my face.

To Lily, I’ve been lucky to grow alongside you over so many years. Thank you for your constant support and for always believing in me, especially when I didn’t believe in myself.

To my dog Riley, thank you for being an endless source of happy memories, and teaching me that it really is possible to relax anywhere.

To my partner Nat, thank you for helping me become a better person every day. Your honesty, compassion, and commitment to doing right by others is a constant source of inspiration. Your openness, thoughtfulness, and sense of adventure make each day a special one. I am so grateful that our paths crossed. Thank you for being my companion.

Finally, to all my family and friends, thank you for the memories and experiences we have shared. Each one of you has added something unique and beautiful to my life, and I am immeasurably lucky to be surrounded by so many good people.
Acknowledgements

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I owe a hefty debt of gratitude to wonderful folks at the Lawson Health Research Institute who made my research possible. Thank you to Lise Desjardins, Laura Morrison, and Jennifer Hadway for your generous support throughout all animal experiments, and for your joyful company during those long experiment days. Thank you to both Lynn Keenliside and Dr. Daniel Milej for sharing your wide breadth of technical knowledge and offering assistance at many different stages of this work.

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I would also like to acknowledge all of the past and present graduate students who have contributed to both my research and my graduate school experience overall. Dr. Lawrence Yip was a constant source of technical know-how, and was absolutely essential in developing the final version of the TD-DOI system presented in this work. Natalie Li was a fantastic sounding board throughout the development of the TD-DOI data analysis, and always willing
to engage in healthy scientific debate. David Cohen was a lovely coding companion and his Monte Carlo expertise was a key asset behind the simulations underlying much of my \textit{in silico} work. Thank you to all of the past and present members of both the Translational Biophotonics lab and the Lawson Optics group for their friendship, kindness, and great company throughout this journey. From lab Olympics to Halloween costume contests to conference adventures, you have given me some of the most memorable moments of my graduate career. I would also like to thank the countless other individuals who have brightened my graduate school experience over the last six years; this includes the Biomedical Engineering Graduate Student Committee which provided an avenue to meet new people and occasionally escape from the lab.

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<th>Abbreviation</th>
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<tr>
<td>ACPA</td>
<td>Anticitrullinated Peptide Antibodies</td>
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<tr>
<td>ACR</td>
<td>American College Of Rheumatology</td>
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<tr>
<td>AIA</td>
<td>Adjuvant-induced Arthritis</td>
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<tr>
<td>APR</td>
<td>Acute-phase Reactant</td>
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<tr>
<td>AUC</td>
<td>Area Under The Curve</td>
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<tr>
<td>bDMARD</td>
<td>Biologic DMARD</td>
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<td>BF</td>
<td>Blood Flow</td>
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<tr>
<td>CCD</td>
<td>Charge-coupled Device</td>
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<tr>
<td>CDAI</td>
<td>Clinical Disease Activity Index</td>
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<tr>
<td>CR</td>
<td>Conventional Radiography</td>
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<td>CRP</td>
<td>C-reactive Protein</td>
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<td>CS</td>
<td>Compressive Sensing</td>
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<td>Conventional DMARD</td>
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<td>Computed Tomography</td>
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<tr>
<td>CW</td>
<td>Continuous-wave</td>
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<tr>
<td>DAS28</td>
<td>Disease Activity Score In 28 Joints</td>
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<td>DCE</td>
<td>Dynamic Contrast-enhanced</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>DE</td>
<td>Diffusion Equation</td>
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<td>DI</td>
<td>Disease Index</td>
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<tr>
<td>DMARD</td>
<td>Disease-modifying Antirheumatic Drug</td>
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<tr>
<td>DMD</td>
<td>Digital Micro-mirror Device</td>
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<tr>
<td>DOI</td>
<td>Diffuse Optical Imaging</td>
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<tr>
<td>DOT</td>
<td>Diffuse Optical Tomography</td>
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<tr>
<td>DTOF</td>
<td>Distribution Of Times-of-flight Of Photons</td>
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<tr>
<td>ESR</td>
<td>Erthrocyte Sedimentation Rate</td>
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<td>EULAR</td>
<td>European League Against Rheumatism</td>
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<tr>
<td>FD</td>
<td>Frequency-domain</td>
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<td>FFT</td>
<td>Fast Fourier Transform</td>
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<td>FOI</td>
<td>Fluorescence Optical Imaging</td>
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<td>FOV</td>
<td>Field-of-view</td>
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<td>Humane Endpoints</td>
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<td>Heart Rate</td>
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<td>ICG</td>
<td>Indocyanine Green</td>
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<td>IRF</td>
<td>Instrument Response Function</td>
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<td>LED</td>
<td>Light-emitting Diode</td>
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<tr>
<td>log-HR</td>
<td>Log Hazard Ratio</td>
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<td>Abbreviation</td>
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<tr>
<td>MC</td>
<td>Monte Carlo</td>
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<td>Photoacoustic Imaging</td>
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<td>PAT</td>
<td>Photoacoustic Tomography</td>
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<td>PCA</td>
<td>Principal Components Analysis</td>
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<td>PMT</td>
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<td>RA</td>
<td>Rheumatoid Arthritis</td>
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<td>RAMRIS</td>
<td>Rheumatoid Arthritis MRI Scoring System</td>
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<td>RF</td>
<td>Rheumatoid Factor</td>
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<td>ROI</td>
<td>Region Of Interest</td>
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<td>RTE</td>
<td>Radiative Transfer Equation</td>
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<td>SNR</td>
<td>Signal-to-noise Ratio</td>
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SPC  Single-pixel Camera

T2T  Treat To Target

TCSPC  Time-correlated Single-photon Counting

TD  Time-domain

TNF  Tumour Necrosis Factor

TPSF  Temporal Point-spread Function

TR  Time-resolved

tsDMARD  Targeted Synthetic DMARD

US  Ultrasonography

WOO  Window Of Opportunity
Chapter 1

Introduction

1.1 Clinical Rationale

1.1.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a long-term autoimmune disease and the most common form of inflammatory arthritis. In Canada, approximately 1% of the population currently lives with RA, with double the disease prevalence in females compared to males [1]. Further, the burden of the disease on the Canadian economy was estimated to be over $420 million in 2010 [2]. RA is typically characterized by inflammation in the joints—most commonly, hands, wrists, and feet—which causes pain and stiffness; if left untreated, this inflammation can lead to permanent joint damage. Additionally, RA is frequently associated with comorbidities including cardiovascular, lung, and neuropsychiatric disease [3], as well as fatigue, pain, depression, and a substantial reduction in overall quality of life [4, 5].

Though the cause of RA is not known, both genetic and environmental factors contribute to its development. A notable example of the former are alleles of HLA genes (e.g., HLA-DRB1) whose association with RA has been documented for over 50 years and continues to represent one of the most significant genetic risk factors for the disease [6, 7]. Examples of the latter include smoking [8], periodontitis [9], viral infections [10], and microbiome characteristics [11]. In fact, the interaction of environmental factors with genetic risk factors—along with their contribution to epigenetic modifications [12]—has been clearly implicated in the development of autoantibodies known to precede RA symptoms; these include anticitrullinated peptide anti-
Figure 1.1: Simplified diagram of a healthy joint and its associated tissues (left) alongside an inflamed joint (right) which illustrates tissue-specific effects typically observed in RA.

Autoantibodies (ACPA) and rheumatoid factor (RF) [7]. Following autoantibody development, the time delay before the appearance of clinical symptoms is highly variable. Autoantibodies have been identified in individuals’ serum up to 14 years before symptoms appeared; at the same time, some individuals develop symptoms only several months after autoantibody appearance [13].

Clinically, RA presents as pain and swelling in multiple joints, and—though larger joints may be involved in the disease (e.g., ankle, knee, elbow)—these symptoms primarily affect the wrists, as well as metacarpophalangeal (MCP), metarsophalangeal, and proximal interphalangeal (PIP) joints [7]. Using the PIP joint as an example, Fig. 1.1 illustrates the main joint tissues, and summarizes how they are impacted by the progression of RA. The capsule encompassing the joint is composed of an outer fibrous membrane and the inner synovial membrane. This structure contains synovial fluid which helps reduce friction between the cartilage-lined bones on either side of the joint cavity. On the outside, the capsule is surrounded by larger soft tissues—including the tendon, subcutis, and skin—as well as smaller...
tissues such as vessels and nerves (not shown). In RA, autoantibodies bind their target autoantigens within the synovial membrane to form immune complexes. By triggering a variety of biologic pathways—including activation of endothelial cells and increased expression of adhesion molecules [14]—these complexes then stimulate the infiltration of the synovial compartment by immune cells as well as the release of pro-inflammatory cytokines (e.g., tumor necrosis factor (TNF), interleukin-6) [7]. As a result, the volume of synovial fluid in the joint increases (i.e., joint effusion) and—driven in part by the proliferation of fibroblast-like cells [15]—an expanded, hyperplastic synovial membrane develops (i.e., synovitis). The same processes which affect the joint’s synovial membrane can also cause inflammation of the synovial membrane which surrounds tendons (i.e., tenosynovitis). All of these effects have previously been demonstrated in RA patients using contrast-enhanced magnetic resonance imaging (MRI) [16, 17, 18]. In addition, the aforementioned changes within the joint are also known to cause an increase synovial oxygen consumption and create hypoxic conditions [19]. This acts as a signal for angiogenesis, a known hallmark of RA, and increased blood flow (BF); however, newly formed vessels are typically unable to adequately address the drastically increased oxygen demand [19, 20]. This has been supported by observed decreases in synovial membrane capillary density which result in an overall decrease in the tissue’s blood volume fraction [21]. Further attraction of the immune cells to the synovial compartment is caused by the aforementioned release of pro-inflammatory cytokines which promote the inflammatory response. In fact, large increases in leukocyte and protein content within the joint [22, 23] are associated with the, typically clear, synovial fluid becoming turbid and yellow [24]. Increased cytokine levels can also activate chondrocytes within the joint, and cause fibroblasts to activate macrophage differentiation into osteoclasts; these degrade cartilage and resorb bone, respectively [25]. Cartilage water content has also been reported to increase [22, 24]. Overall, the biological cascades briefly described above clinically manifest as chronic joint pain and swelling and, if left unmanaged, can result in irreversible and disabling joint damage [7].
1.1.2 Clinical Management of RA

Despite the lack of a cure for RA, therapeutic advances over the last few decades have made it possible to achieve tight disease control using disease-modifying antirheumatic drugs (DMARDs). The modern treatment paradigm for RA is commonly referred to as a treat to target (T2T) approach, and is characterized by the setting of a treatment target (typically clinical remission) and the adaptation of a patient’s treatment until the target is achieved [26]. In particular, treatment adaptation remains a critical pillar of RA management due to the well-known variability in patient clinical response to treatment [27]. As outlined by American College of Rheumatology (ACR) guidelines, patients receiving their first DMARD treatment are typically given conventional DMARD (csDMARD) therapy; in recent years, methotrexate (MTX) monotherapy has been specifically recommended over other therapies (e.g., hydroxychloroquine, sulfasalazine) [28, 29]. Yet, even in the case of MTX—a DMARD considered to be the cornerstone of modern RA therapy [25]—at least 1 in 3 patients are expected to demonstrate insufficient treatment response (i.e., experience treatment failure). In response to initial treatment failure, a number of alternative therapies can be prescribed. This includes MTX combination therapy with a TNF inhibiting biologic DMARD (bDMARD) (e.g., etanercept), a non-TNF bDMARD (e.g., abatacept), or a targeted synthetic DMARD (tsDMARD) (e.g., tofacitinib). Further, addition of glucocorticoids such as prednisone is often considered on a patient-by-patient basis to alleviate symptoms in the short-term. If subsequent treatment failure occurs, patients are often assigned to a different class of bDMARDs or tsDMARDs than they previously received until an adequate treatment response is achieved.

With this in mind, it is important to note that effective early treatment within the first 3–6 months of disease onset is well known to significantly improve patient prognosis [30, 31, 32]. Though the typical “the earlier the better” principle with regards to treatment initiation is also valid for RA [33], it is widely accepted that a therapeutic window of opportunity (WOO) exists during which RA is much more susceptible to treatment. For example, over two separate
clinical trials which followed 738 and 533 RA patients, van Nies et al. found that patient likelihood of achieving DMARD-free sustained remission—as characterized by the steepness of log hazard ratio (log-HR) curves plotted against symptom duration—decreased non-linearly with symptom duration [31]. Specifically, the steepness of the log-HR curves decreased sometime after 15–20 weeks of symptom appearance; thus, patients who initiated treatment before this decrease were disproportionately more likely to achieve remission than their counterparts.

1.1.3 Established Diagnostic and Monitoring Tools

Even though early diagnosis and treatment initiation have very clear benefits for long-term patient well-being, no diagnostic criteria currently exist for RA. Notably, accurate diagnosis of RA is inherently complicated by the myriad of differential diagnoses such as osteoarthritis, psoriatic arthritis, systemic lupus erythematosus, and gout [34]. Despite the lack of diagnostic criteria, the 2010 ACR RA classification criteria—a standard method for stratifying patients with RA within clinical trials—can be used as a diagnostic aid and are relatively well-aligned with clinical diagnostic practices [35]. These criteria use a scoring system which classifies the presence of RA using a patient’s joint involvement (presence of swollen or tender joints identified using physical examination or imaging), symptom duration, and results of laboratory testing for both autoantibodies (RF, ACPA) as well as acute-phase reactants (APRs). APRs refers to the concentration of proteins known to accompany inflammation; this is usually assessed by looking at the levels of C-reactive protein (CRP) as well as the rate at which red blood cells settle i.e., erythrocyte sedimentation rate (ESR). Most patients with RA return abnormal results for one of the aforementioned laboratory tests. In particular, patients with elevated levels of RF and/or ACPA (i.e., seropositive patients) are typically designated as having a poor prognosis; however, over 30% of RA patients are seronegative for these autoantibodies and 5–15% of tests are expected to be false positives (e.g., patients with other inflammatory conditions) [36]. Similarly, up to 40% of RA patients may have normal APR levels [36]. As a result, the 2010 ACR classification criteria only recommend laboratory testing as a supplement
to characterizing joint involvement. Even then, these criteria have a reported sensitivity and specificity of only 82% and 61%, respectively, which highlights the challenge of achieving an accurate, early diagnosis.

Following diagnosis, T2T necessitates follow-up assessments of patient disease activity to identify treatment response and adjust therapy accordingly. For this purpose, well-established composite measures such as the clinical disease activity index (CDAI) and disease activity score in 28 joints (DAS28) are often used in the clinic since they provide a single score which can be used to track disease activity over time. Aside from using assessments similar to those outlined by the 2010 ACR criteria, these measures also incorporate assessments of physical function and patient global assessments of disease activity in order to guide treatment. Nevertheless, though these methods are the current standard of care, they are insensitive to potentially predictive, subclinical inflammation, and—considering the repetitive, longitudinal nature of treatment monitoring—their effectiveness may be limited by their subjectivity and variability [37, 38].

Historically, conventional radiography (CR) has been an important method of diagnosing and monitoring RA disease progression both in the clinic and in clinical trials [39]. In fact, DMARD efficacy is traditionally based on their ability to slow radiographic progression (i.e., damage observed on radiographs). Due to its widespread availability, relatively low cost, and excellent ability to visualize bone, CR remains particularly well-suited for the assessment of articular damage such as bone erosion and narrowing of the joint space (Fig. 1.2). As such, CR is still one of the most commonly used tools for assessment of disease progression [40, 39]. Nevertheless, CR uses ionizing radiation and primarily provides information about joint damage which is both irreversible and occurring at later stages of the disease [41]. Both of these drawbacks ultimately limit its usefulness for routine monitoring and early diagnosis. Compared to CR, computed tomography (CT)—which is the volumetric extension of CR—subjects the patient to higher levels of ionizing radiation, increases cost, and offers limited advantages for RA disease assessment; thus, its usefulness as an RA assessment tool remains very limited.
Figure 1.2: Examples of articular damage in RA patients visualized with CR. (a) depicts an RA patient with bone erosion in the second MCP joint (arrow, bare area). (b) depicts an RA patient with advanced disease with erosive changes affecting the second, third, and fifth MCP joints. Reproduced with permission from Springer Nature [40].

On the other hand, MRI does not require the use of ionizing radiation, and is able to produce high resolution images with substantially better soft-tissue contrast than CR. As outlined in the characteristics of the rheumatoid arthritis MRI scoring system (RAMRIS)—a well-established scoring system developed by the international Outcome Measures in Rheumatology Clinical Trials (OMERACT) working group—MRI can be used to not only score bone erosion, but also bone edema which is a likely precursor to the erosions [42]. Moreover, with intravenous administration of a contrast agent such as gadolinium, MRI can be used to directly score both joint synovitis and tenosynovitis (Fig. 1.3). In fact, MRI has been found to be sensitive to subclinical inflammation which can occur in the earliest phases of RA [43]. In an examination of 1790 joints from 179 patients with early arthritis, Krabben et al. found a high level of association between swelling determined by physical exam and inflammation determined using MRI [44]. Further, MRI detected inflammation in a high proportion of joints without clinical swelling: for example, 57–58% of joints where low-grade inflammation was detected on MRI
Figure 1.3: Example of inflammation in an RA patient visualized with MRI. Bilateral MR images of the hand and wrist of a 33-year-old woman with early inflammatory arthritis with a disease duration of 3 months. Top: Contrast-enhanced axial T1-weighted fat-saturated MR image shows grade 2 tenosynovitis of the flexor tendons of the second and third digits on the right hand (arrows). Bottom: Contrast-enhanced coronal T1-weighted fat-saturated MR image at the MCP joint level (arrows) demonstrates absence of MCP joint synovitis. Bilateral interphalangeal joint synovitis and left radiocarpal joint synovitis were noted (dashed arrows). Reproduced with permission from The Radiological Society of North America (RSNA ®) [18].

were not considered clinically swollen. MRI has also been found to be more predictive of radiographic progression than other typical disease activity measures [45], and—when used in conjunction with the ACR 2010 classification criteria—MRI helps improve identification of RA compared to using the criteria alone [46]. Despite the clear advantages and benefits of MRI, it is an expensive form of advanced imaging with many contraindications (e.g., metal implants) and the requirement of an advanced operator. Thus, as of 2019, disease activity measures requiring this imaging modality did not meet the ACR’s minimum standard for regular use in RA assessment [47].

By comparison, ultrasonography (US) is substantially more widespread and less expensive than MRI, while also having greater sensitivity to measures of joint inflammation than CR [48]. OMERACT has recently outlined definitions of RA pathophysiologies which can be detected...
with US including synovitis, tenosynovitis, and bone erosions [49]. The European league against rheumatism (EULAR) has also recommended the use of US for improving diagnostic certainty, more accurate assessment of inflammation, and assessment of clinical remission [41]. In particular, power doppler US (PDUS)—a mode of US typically used to estimate blood flow—has garnered interest for RA disease assessment. In a sample of 94 patients presenting with arthritic symptoms, PDUS was found to independently improve RA classification when combined with the ACR 2010 classification criteria [50]. PDUS also had a sensitivity and specificity of 85.7% and 82.8%, respectively, for detecting disease relapse over 1 year in a sample of 85 RA patients. Moreover, MRI of the same patients was found to not be predictive of relapse [51]. Nevertheless, there is still a lack of standardization with regards to US methodologies and standards for guiding RA treatment [52]. Further, due to the variability introduced by different US machines, settings, and operators, US is known to suffer from suboptimal reproducibility which can limit its usefulness for routine assessment [53, 54]. Indeed, as of 2019, no disease activity measures requiring US met the ACR’s minimum standard for regular use in RA assessment [47].
1.2 Diffuse Optical Methods

1.2.1 Light Propagation in Tissue

Diffuse optical methods are most often referenced within the context of monitoring biological tissues in vivo using light. In general, there are two key interactions that dictate how light propagates through tissue: absorption and scattering. Absorption can occur when a photon strikes a molecule with energy equivalent to the energy gap between the molecule’s ground state and one of its excited states. The first characterization of this process was conducted in non-scattering media by Pierre Bouguer in 1729, and then summarized by Johann Heinrich Lambert, in 1760, as

\[ A = -\log \left( \frac{I}{I_0} \right) = \mu_a l, \]  

(1.1)

where \( A \) is the absorbance, \( \frac{I}{I_0} \) is the proportion of absorbed light, and \( l \) is the path length of the light in the non-scattering medium (equivalent to the thickness of the medium). August Beer subsequently re-framed Eq. (1.1) to outline how individual light absorbing compounds within the medium (i.e., chromophores) contributed to \( A \); this is commonly referred to as the Beer-Lambert Law:

\[ A = \sum_{i=1}^{n} \varepsilon_i c_i l, \]  

(1.2)

where \( \varepsilon_i \) and \( c_i \) are the molar extinction coefficient and concentration in solution of the \( i \)th chromophore, respectively.

While the expressions above were derived for non-scattering media, biological tissues have strong optical scattering. Specifically, as light propagates through tissue, it is strongly scattered due to mismatches in the refractive index \( n \) of various biological components, and due to the presence of cellular structures (e.g., nuclei, mitochondria) whose size is close to the light’s wavelength \( \lambda \). Under the assumption of spherical scattering particles, individual light photon scattering events can be modelled using Mie theory or, if the scattering particles are substan-
tially smaller than $\lambda$, the simpler Rayleigh theory. The direction of photon scatter is typically described by the anisotropy $g$ which is defined as the mean cosine of the scattering angle, and is an intrinsic property of the medium through which the light is propagating. $g$ can range from -1 to 1; these limits correspond to a high prevalence of backward or forward scattering, respectively (0 indicates isotropic scattering). Generally, $g \approx 0.9$ for biological tissues which implies that individual photons are preferentially scattered in the forward direction of their propagation. Nevertheless, in the high-scattering environment of biological tissue, the aggregation of many individual forward-scattering events results in a quick loss of initial photon directionality which, in turn, causes photon trajectories to appear as a random walk. This “diffusion-like” propagation of light is one of the defining features of diffuse optical methods.

Notably, if continuous photon propagation through multiple scattering events is typical within a medium, this also implies that the medium is associated with a relatively low probability of photon absorption. In other words, the appearance of diffuse light propagation is indicative both of a medium’s relatively strong optical scattering and relatively weak optical absorption. Indeed, in biological tissue the typical mean photon path preceding an absorption event (10–100 mm) is approximately 2–3 orders of magnitude greater than the typical mean photon path between scattering events (0.1 mm) [55]. The reciprocals of these paths are known as the absorption coefficient $\mu_a$ and scattering coefficient $\mu_s$, respectively, and are defined as the probability of either event occurring per unit path length. Given the aforementioned loss of photon directionality expected to occur in biological tissue, it is also common to consider the mean path travelled by a photon after which its movement can be modelled as a random walk; the reciprocal of this distance is known as the reduced scattering coefficient $\mu'_s$ and is defined as

$$\mu'_s = \mu_s(1 - g).$$

(1.3)

In summary, the interaction of light with highly scattering media such as tissue is dictated by four basic optical properties intrinsic to the medium: $\mu_a$, $\mu_s$, $g$, and the index of refraction $n$. Under the condition that $\mu_a \gg \mu_s$, Eq. (1.1) may serve as a sufficiently accurate description of
light propagation. Nevertheless, since $\mu_s \gg \mu_a$ in biological tissue, it is important to consider that a non-negligible proportion of light will typically be scattered such that it is not detectable by a typical experimental measurement. Delpy et al. outlined this effect simply by introducing the modified Beer-Lambert law in 1988 [56]:

$$A = \mu_a l + G,$$

(1.4)

where $G$ is a term describing the proportion of light lost due to scattering, and we note that the path length $l$ is no longer equivalent to the thickness of the medium since scattering causes photons to travel a tortuous path prior to detection. For clarity, $A$ can be referred to as attenuation instead of absorbance since this communicates its dependence on phenomena other than absorption more explicitly. An important consequence of Eq. (1.4) is that the effects of absorption and scattering are intermingled when acquiring simple experimental measurements of $A$; accounting for or separating these effects is one of the main challenges of using diffuse optical methods.

A typical approach to addressing this ill-posed problem is by modelling light propagation within the medium, and then comparing the model’s results to those obtained by experimental measurement. The Monte Carlo (MC) method is a numerical approach which models light propagation in biological tissue by calculating individual, step-by-step photon trajectories using probability distributions. While the MC method is a gold-standard technique for this type of modelling, its statistical nature requires a large amount of photon trajectories to be simulated; thus, it is computationally time-consuming. The radiative transfer equation (RTE) is the analytical counterpart to MC simulations, and is derived from Maxwell’s equations to enable accurate modelling of photon transport in biological tissue:

$$\frac{1}{v} \frac{\partial L(r, \hat{s}, t)}{\partial t} = -\hat{s} \cdot \nabla L(r, \hat{s}, t) - \mu_t L(r, \hat{s}, t) + \mu_s \int_{4\pi} L(r, \hat{s}', t) P(\hat{s}' \cdot \hat{s})d\Omega' + S(r, \hat{s}, t)$$

(1.5)

where $v$ is the speed of light in the medium, $L(r, \hat{s}, t)$ is the light radiance in the direction $\hat{s}$ at
position \( \mathbf{r} \) and time \( t \), \( \mu_t = \mu_a + \mu'_s \), \( P(\hat{s}' \cdot \hat{s})d\Omega \) is the phase function describing the probability of scattering from \( \hat{s} \) into \( d\Omega \) around \( \hat{s}' \), and \( S(\mathbf{r}, \hat{s}, t) \) is the energy produced from a light source \([55]\). While accurate, Eq. (1.5) includes six independent variables which makes it difficult to solve. Instead, given the aforementioned diffuse nature of light propagation in biological tissue, the RTE is often simplified using the diffusion approximation. Specifically, under the condition \( \mu'_s \gg \mu_a \), it is assumed that the radiance will be isotropic after sufficient scattering. The result of applying this simplification is known as the diffusion equation (DE):

\[
\frac{1}{v} \frac{\partial \Phi(\mathbf{r}, t)}{\partial t} + \mu_a \Phi(\mathbf{r}, t) - \nabla \cdot [D \nabla \Phi(\mathbf{r}, t)] = S(\mathbf{r}, t), \tag{1.6}
\]

where \( \Phi(\mathbf{r}, t) \) is the fluence rate and \( D = \frac{1}{3(\mu_a + \mu'_s)} \) is the diffusion coefficient. Solutions to Eq. (1.6) have been developed for certain regular geometries (e.g., semi-infinite medium \([57]\)). Nevertheless, analytical solutions for complex geometries (e.g., human finger) are typically not possible unless critical geometric simplifications (e.g., modelling the finger as a cylinder) are introduced.

### 1.2.2 Tissue Optical Spectroscopy

The main endogenous chromophores of interest within biological tissue are water (H\(_2\)O), melanin, lipids, deoxyhemoglobin (Hb), and oxyhemoglobin (HbO). Note that the latter two chromophores are simply forms of hemoglobin which are either unbound from or bound to oxygen, respectively. In 1977, Frans Jöbsis observed that light in the near-infrared (NIR) wavelength range from 650–950 nm could penetrate relatively deep into tissue due to the low absorption of these chromophores in that spectral region (Fig. 1.4). Specifically, shorter wavelengths outside of this region are highly absorbed by Hb while longer ones are highly absorbed by water. This “optical window” is the foundation of diffuse optical spectroscopy, which is more commonly known as NIR spectroscopy (NIRS).

Generally, NIRS is based on injecting NIR light from some source into a medium of in-
Figure 1.4: Absorption coefficients (mm\(^{-1}\)) of the main chromophores in the subcutis tissue of a human finger in the NIR range. Chromophore concentrations taken from [58].

Measurements obtained with NIRS can be used to monitor endogenous chromophore concentrations \textit{in vivo}. In turn, these chromophore concentrations can be used to determine clinically important physiological parameters such as tissue oxygen saturation (SO\(_2\)):

\[
SO_2 = \frac{[HbO_2]}{tHb},
\]  
(1.7)
where the square brackets denote the concentration of a chromophore, and $tHb = [HbO_2] + [Hb]$ is the total hemoglobin concentration. Measurements of exogenous chromophores which are introduced into the tissue can also be used to gain insight into tissue physiology. For example, Indocyanine green (ICG)—a synthetic dye which has been used in medicine since the 1950s [59]—has strong absorption in the NIR range, and has been used as a tracer in dynamic contrast-enhanced NIRS studies to quantify tissue BF [60, 61].

1.2.2.1 Dynamic contrast-enhanced NIRS

Dynamic contrast-enhanced (DCE) NIRS is a technique that is capable of quantifying regional tissue BF with the aid of a contrast agent. Briefly, the technique involves injecting a bolus of an exogenous intravascular tracer, and linking the time-dependent changes in its arterial concentration $C_a(t)$ and tissue concentration $Q(t)$:

$$Q(t) = BF \cdot R(t) * C_a(t),$$

where $*$ is the convolution operator, and $R(t)$ (i.e., the impulse residue function) represents the fraction of tracer in the tissue at time $t$ after the bolus injection at $t = 0$ [62]. A typical measurement involves injecting ICG into a peripheral vein, measuring $C_a(t)$ using an instrument known as a dye densitometer, and measuring $Q(t)$ with NIRS [60, 61]. Next, a deconvolution algorithm can be applied to the measurements of $C_a(t)$ and $Q(t)$ to extract $BF \cdot R(t)$, and—since $R(0) = 1$ by definition—BF can be recovered. It is important to note that a typical bolus-tracking experiment can last approximately 40 s; thus, DCE-NIRS is only suitable for providing discrete measurements of tissue BF.

1.2.2.2 Modes of NIRS Acquisition

NIRS data are measured using three modes of acquisition: continuous-wave (CW), frequency-domain (FD), and time-domain (TD) (Fig. 1.5, right); notably, TD is also often referred to
Figure 1.5: Common types of NIRS probe configurations (left) and modes of acquisition (right). Schematic of “banana patterns” which show the tissue volume sampled in the transmission and reflectance geometries. As a rough rule of thumb, the mean light penetration depth in the reflectance geometry—where the source and detector are separated by $\rho$—is of order $\rho^2$. Input and detected light intensities for CW, FD, and TD are depicted in (1), (2) and (3), respectively, in order of increasing information content per measurement. Reproduced from [63]. © IOP Publishing. Reproduced with permission. All rights reserved.

as time-resolved (TR). CW-NIRS uses sources which emit a light intensity that is constant in time, and measures the attenuation of the light after it has probed the medium of interest. FD-NIRS uses sources whose intensity is modulated in time. Thus, as this light travels through a medium, it experiences a phase shift in addition to attenuation. Notably, the phase shift is proportional to the mean photon path length, and this additional information can be used to better disentangle the contributions of scattering and absorption to the overall light attenuation. TD-NIRS uses sources that emit laser light in extremely short pulses on the order of picoseconds to femtoseconds. A detector with high temporal resolution, combined with specialized electronics, is typically used to detect the arrival time of each individual photon. The photon arrival times are then aggregated into a histogram known as a distribution of times-of-flight of photons (DTOF). Notably, measurements acquired using FD and TD are directly related to one another through the Fourier transform: performing a Fourier inversion of many superimposed FD measurements can be used to recover a TD measurement which closely resembles a DTOF [64].

The abundance of information provided in a DTOF can be leveraged to calculate various
TD features which provide insight about the medium probed by the detected light. For example, temporally summing the entire DTOF gives the total number of detected photons, which is directly linked to overall light attenuation. Alternatively, separate sections of the DTOF can be binned to better localize the effects of light attenuation. In a reflectance configuration, later-arriving photons at the tail end of the DTOF will have travelled further—and likely deeper—into the tissue. Thus, these can be separately binned to increase a measurement’s depth sensitivity [65]. In a transmission configuration, early-arriving photons, which have experienced fewer scattering events, can increase a measurement’s sensitivity to an area more closely localized to the line of sight between the light source and detector [66]. It is important to note that experimentally measured DTOFs are dependent on the temporal characteristics of the instrumentation used to measure them. Specifically, a DTOF can be represented by the convolution of the temporal characteristics of the instrumentation and the probed medium. These are known as the instrument response function (IRF) and temporal point-spread function (TPSF), respectively:

\[ DTOF(t) = IRF(t) * TPSF(t). \]  

As shown by Eq. (1.9), the IRF causes temporal dispersion of the DTOF compared to the true TPSF, and attempting to directly remove the effects of the IRF by performing a deconvolution is quite challenging because the problem is ill-posed [67, 68]. Nevertheless, it is possible to extract features of the DTOF from which the effects of the IRF can be removed in a straightforward manner. In general, the \( k \)th normalized statistical moment \( m_k \) of a temporal distribution \( g(t) \) can be defined as

\[ m_k = \langle t^k \rangle = \frac{\int_{-\infty}^{\infty} t^k g(t) dt}{\int_{-\infty}^{\infty} g(t) dt}. \]  

\( m_1 \) of a DTOF is equivalent to the average time it takes photons to travel through a medium (i.e., mean photon time-of-flight), and is directly linked to mean photon path length. The second centralized moment of a DTOF—i.e., the variance \( V \) calculated as \( V = m_2 - m_1^2 \)—is related to the medium’s optical properties and has been shown to increase measurement sensitivity to
deeper tissues [69]. Further, the statistical moments of the IRF, TPSF, and DTOF are known to have an additive relationship (e.g., $m_{1}^{DTOF} = m_{1}^{IRF} + m_{1}^{TPSF}$) which makes it simple to account for the effects of different instrumentation.

All in all, in practice, the three modes of NIRS data acquisition can be listed in order of richness of information as CW, FD, and TD; however, it is noteworthy that this order is equally representative of the cost of each mode’s associated instrumentation.

### 1.2.3 Diffuse Optical Imaging

The concepts underlying NIRS can be readily extended to diffuse optical imaging (DOI) in multiple spatial dimensions. In its simplest form, DOI of a sample can be achieved by acquiring multiple NIRS measurements using a single source and detection probe sequentially placed at different locations on the sample, and displaying the resulting data in a manner that captures the spatial relationship of the measurements. Substantially more advanced imaging techniques have also been developed to provide more holistic visualizations of a medium’s optical properties. Diffuse optical tomography (DOT) involves emitting and detecting NIR light at many different locations on a sample, and using tomographic reconstruction techniques to create either a two-dimensional or three-dimensional distribution of the sample’s optical properties.

DOI can be implemented using instrumentation that maintains contact with the medium (e.g., probes); however, for practicality, it is often desirable to use equipment which enables non-contact imaging. For illumination, various opto-mechanical elements can be used to redirect light to specific locations on a sample without requiring the need for time-consuming probe placement. Examples include translation stages which re-position light emitted from an optical fibre [70], scanner systems based on a galvanometer [71], and systems which reflect light using an array of small mirrors known as a digital micro-mirror device (DMD) [72]. Similarly, array-based detectors such as charge-coupled device (CCD) cameras can be used to measure the light exiting from a multitude of locations on the sample in a single image. Nevertheless, especially for light detection, the advantages offered by instrumentation which enables
non-contact DOI are associated with additional hardware expenses. This becomes particularly evident when coupled with the costs associated with the various modes of acquisition (see Sec. 1.2.2.2). For example, a CCD camera used for CW-DOI [71] can be purchased for hundreds to several thousand USD while a streak camera capable of TD-DOI detection typically costs hundreds of thousands USD.

1.2.3.1 Single-pixel Cameras

In an effort to reduce cost without sacrificing the benefits of non-contact imaging, DOI systems may employ non-contact configurations which only use a single, cheaper “bucket” detector with no spatial resolution (e.g., PMT). To acquire spatially-varying information in this single-pixel detector configuration, a sample is scanned by spatially redirecting the position of the light source and sequentially acquiring a measurement for each new position. In general, this acquisition scheme can be represented as the inner product of the vectorized forms of an image that would have been obtained with an array detector ($f$) and some arbitrary illumination pattern ($\phi$):

$$y_i = \phi_i f,$$

where $y_i$ is the measurement recorded by the system’s single-pixel detector for the $i$th illumination pattern. The most basic type of acquisition that uses this design, which is known as a single-pixel camera (SPC), is raster scanning: a sample is scanned point-by-point in a rectangular pattern and the detector’s measurement is assigned to each point in the image accordingly. Alternatively, spatial light modulators such as DMDs can be used to project a series of more complex illumination patterns onto the sample. For example, a scan can be performed by using illumination patterns taken from a well-known mathematical basis such as a Hadamard basis—this is referred to as a basis scan. The reconstructed image $\hat{f}$ can then be recovered as

$$\hat{f} = \Phi^{-1}y,$$
where each row of the matrix $\Phi$ is a single illumination pattern $\phi$. The use of an SPC also makes it possible to implement compressive sensing (CS) acquisition which enables image reconstruction using fewer measurements than a basis scan while also reducing measurement noise. Nevertheless, a comprehensive treatment of CS is outside of the scope of this thesis. For reference, an overview of the topic has previously been provided by Duarte et al. [73].

1.2.4 Diffuse Optical Methods in RA

As discussed in Sec. 1.1.1, early RA disease activity is associated with a host of pathological changes in the joint including increases in synovial fluid and synovial membrane volumes, synovial fluid turbidity, hypoxia, and angiogenesis. Each of these changes alter the optical properties of the joint and, as such, the ability of several diffuse optical methods to monitor RA disease activity has been investigated.

The first application of diffuse optical methods towards detection of a rheumatic condition was reported by Prapavat et al. in 1995 [74, 75]. In one work, a joint-mimicking optical imaging phantom was created using Intralipid—a fat emulsion—to simulate biological tissue scattering, and India ink to simulate tissue absorption. The other work was an in vivo pilot study in which measurements were acquired from the PIP joint of four healthy participants and four patients with a rheumatic condition. In both cases, a CW-DOI system with single point laser illumination and a CCD camera for detection was used to acquire intensity profiles in a transmittance configuration. For the in vivo study, illumination and detection were oriented on the dorsal and palmar sides of the finger, respectively. Motivated by Prapavat et al.’s further work which showed significant differences in the in vitro optical properties of various healthy and pathologic joint tissues [76, 77], Scheel et al. used a similar CW-DOI system (Fig. 1.6) to image 22 RA patients (72 PIP joints) and 8 healthy controls (64 PIP joints) at 675 nm [78]. Specifically, the study focused on determining whether joints were inflamed at baseline and after a follow-up period (mean time of 6 weeks). These results were then compared to the results of a clinical examination for reference. Joint circumference measurements were also acquired
concomitant to clinical examination. In a follow-up investigation, the acquired images were analysed using various classification methods and it was found that, when combined with joint circumference measurements, a Gaussian process regression classifier could correctly track inflammation changes with approximately 80% sensitivity, 90% specificity, and an overall error rate of 14% [79]. Nevertheless, an error rate of 22.2% could also be achieved with the same classifier using only the information provided by joint circumference measurements, and the authors noted a general inability to accurately determine joint inflammatory status without reference to a baseline measurement. To improve classification, the same group then developed a CW-DOT instrument which was used to reconstruct the spatial distribution of $\mu_a$ and $\mu'_s$ within PIP joints from 13 RA patients (78 PIP joints) by sequentially scanning a finger along its sagittal plane [70, 80]. While this approach was able to detect changes in joint optical properties as a result of inflammation (e.g., elevated $\mu_a$ inside the joint cavity), it was only able to achieve sensitivities and specificities of 76% and 78% when compared to US assessments [81]. Given the superior ability of FD at separating the effects of optical absorption and scattering (Sec. 1.2.2.2), this system was later superseded by an FD-DOT system combined with a laser
scanner to capture the finger’s surface geometry [82]. Using heuristics extracted from DOT image data obtained from 99 RA patient fingers and 120 fingers from healthy volunteers, image classification based on machine learning was able obtain sensitivities and specificities between 93.8% and 100% [83, 84]. High-performing heuristics which were extracted from the spatial distributions of three-dimensional $\mu_a$ and $\mu_s'$ maps (Fig. 1.7) included optical property variance within the joint, as well as values from a Gaussian mixture model or fast Fourier transform (FFT) analysis. From their results, the authors concluded that the pathological changes associated with RA likely cause the PIP joint to have an optical profile more similar to the tissue surrounding it. Recently, Kim et al. have also evaluated the use of a third-order simplified spherical harmonics model—a higher order approximation to the RTE than the DE—to reduce the lengthy reconstruction time required for their FD-DOT images [85]. Using the data from Hielscher et al.’s previous 2011 study, they were able to maintain a sensitivity and specificity of 88% and 93%, respectively, while reducing reconstruction time by 100-fold.

Figure 1.7: Diagram depicting orientation of FD-DOT images using a photo, FD-DOT cross-section of $\mu_a$, and MR image (left), along with slices from tomographic reconstructions of $\mu_a$ and $\mu_s'$ from healthy volunteers and RA patients (right). Adapted from [82] with no permission required. © 2011 IEEE
Despite these successes, cost and complexity remain drawbacks of an FD-DOT approach, and limit its critical translation. Thus, a variety of alternative diffuse optical methods have also been investigated. Lasker et al. used a two-wavelength (765 and 832 nm) CW-DOT system to image a small number of healthy volunteers and RA patients [86] while also inducing a controlled hemodynamic response by inflating a pressure cuff around each subject’s forearm. Specifically, cuff inflation was targeted to occlude either the subject’s venous or arterial blood flow. This approach—termed dynamic DOT—allows clearer investigation of the joint’s vascularity and its response to a perturbation when compared to typical DOT. Indeed, dynamic traces of light transmission at 765 nm, as well as changes in Hb, HbO, and tHb concentrations were found to show complex but distinct patterns when comparing data from two RA patients with data from a healthy subject. Meier et al. applied a similar approach using a simpler CW-NIRS system in a transmittance configuration which acquired PIP joint spectra over 500–1000 nm from 67 RA patients (77 PIP joints) before and during venous occlusion [87]. In this work, comparison of light attenuation between 720 and 810 nm suggested the presence of lower inflamed joint oxygenation, and stronger hemodynamics were observed in inflamed joints in response to cuff inflation (i.e., faster accumulation of blood). A follow-up study in 59 RA patients, as well as 10 patients with arthralgia (i.e., joint pain), measured optical transmission before and during venous occlusion using a new two-wavelength (660 and 810 nm) CW-DOI system capable of simultaneously assessing the PIP, MCP, and wrist joints of both hands (Fig. 1.8) [88]. When compared with US as a diagnostic reference standard, optical measurements had a 83%, 70%, and 39% sensitivity as well as 64%, 74%, and 87% specificity for detecting inflammation in the PIP, MCP, and wrist joints, respectively. A further follow-up study in 46 RA patients (1003 joints), in which the LED illumination in the system was replaced with a laser light source, found an overall sensitivity of 60% and specificity of 89% across all joints when compared to US [89]. Notably, swollen and tender joint assessments performed by a clinician only had sensitivities of 46% and 50%, and specificities of 86% and 78% when compared to the same US standard. Nevertheless, a recent study of 484 RA patients which
used a similar optical system and measurement methodology concluded that, when compared to the DAS28 clinical assessment, this approach was not sensitive to different levels of baseline disease activity on a patient level [90].

Motivated by the potential sensitivity of hemodynamics to joint inflammation, Rajaram et al. used a 3-wavelength (760, 802, 830 nm) TD-NIRS system to quantify and compare a variety of physiological changes occurring at various stages of disease activity in an animal model of RA [91]. Specifically, changes in Hb and HbO₂ concentration, as well as SO₂, were measured, and DCE-NIRS was used to quantify changes in joint BF. When compared across the same four animals, joint BF was the only parameter which was significantly affected over the approximately 20 day period of joint inflammation.

Lighter et al. also investigated multiple physiological properties of the joint using a 5-wavelength (650, 710, 730, 830, and 930 nm) CW-DOT system. Specifically, this study looked at the inter- and intra-subject variation of SO₂, tHb, water concentration, and scattering in healthy volunteers. Consistent with the previously mentioned observations by Hielscher’s group, the joint cavity of healthy participants showed lower scattering amplitudes and tHb
values than the tissue around it; SO$_2$ and water concentrations distinguished the healthy joint cavity with less clarity. The main observation from this work was that inter-subject variation was significantly higher than intra-subject variation. Lighter et al. then conducted a follow-up study in 21 RA patients using simpler multi-wavelength CW-DOI; however, unlike previous DOI work, this study was conducted with the illumination and detection on the palmar and dorsal sides of the finger, respectively [92]. Focusing on generalized changes in the spatial variation of light transmittance in and around the joint cavity, Lighter et al. obtained sensitivities and specificities up to 77.9% and 90.9% by analyzing changes in the spatial Fourier transform of light transmission profiles through PIP joints.

Milanic et al. conducted an investigation on the prospects of using even more light wavelengths to distinguish between multiple disease activity states [24, 93]. Specifically, they tested \textit{in silico} hyperspectral (600–1100 nm) CW-DOI’s ability to distinguish between simulated disease activity states caused by the individual and combined effects of joint effusion and synovial membrane proliferation. Nevertheless, the conclusions of this work were somewhat limited by the modelling of the finger using a simplistic geometry (e.g., cylinders, ellipsoids). A follow-up study was conducted using a virtual finger model which was based on a realistic tissue geometry derived from a high-resolution MRI image, and these results were compared to images obtained with a real-world hyperspectral (400–1000 nm) CW-DOI system for reference [94]. Ultimately, the authors concluded that the spectral ratio between transmittance through the joint and transmittance through its surrounding tissue may be used to distinguish between multiple simulated RA disease activity states. Nevertheless, the presented results also implied that the same distinction could be made using a single wavelength CW-DOI system.
1.2.5 Related Optical Techniques

1.2.5.1 Fluorescence Optical Imaging

As mentioned in Sec. 1.2.1, a molecule may absorb an incident photon if the photon’s energy is sufficient to elevate an electron of the molecule to an excited state. One possible outcome of this interaction is that the elevated electron relaxes to ground state by releasing energy through heat and fluorescence—that is, production of another lower energy photon. Notably, fluorescence is typically shifted to longer wavelengths compared to excitation light (i.e., Stokes shift). Some contrast agents such as ICG emit a fluorescence spectrum within the NIR range; these agents can be used in fluorescence optical imaging (FOI) for visualizing some of the hemodynamic changes which typically occur in an inflamed joint (Fig. 1.9). With this in mind, Schafer et al.

![Image](imageurl)

Figure 1.9: Example of FOI with ICG of two RA patients at baseline (left) and after 12 months of treatment (right). Note the substantial reduction in contrast enhancement of the PIP, MCP, and wrist joints following treatment. Reproduced from [95] under the terms of the Creative Commons Attribution license (CC BY 4.0: http://creativecommons.org/licenses/by/4.0/).

tested the ability of ICG-based FOI to detect synovitis in 18 RA patients (36 PIP, 36 MCP, and 18 carpal joints) [96]. Compared to an MRI reference standard for synovitis, the FOI method
had a 67% sensitivity and 77% specificity. This finding is in line with other reports which linked visible perfusion enhancement with the presence of early inflammatory arthritis in both FOI [97] and tomographic FOI systems [98]. More recently, FOI was investigated in a RA cohort of 35 patients before and after 12 months of DMARD therapy [95]. While a reduction in FOI enhancement corresponded with a reduction in mean DAS28 disease activity observed at follow-up, significant reductions were also observed in patients labelled as treatment non-responders. Further, as mentioned in Sec. 1.2.4, comparable sensitivities and specificities have been obtained using CW-DOI systems without the need for intravenous contrast agent injection. Notably, recent work has aimed to address the latter limitation by investigating—in a mouse model of RA—the possibility of contrast self-administration methods such as subcutaneous injection and oral delivery [99].

### 1.2.5.2 Photoacoustic Imaging

Photoacoustic imaging (PAI) relies on the creation of acoustic waves within a medium as a result of light absorption which—due to a local thermoelastic expansion—creates a localized pressure change; this is known as the photoacoustic effect. Briefly, PAI is a hybrid imaging modality which relies on irradiating a sample with pulsed light, and detecting the resulting ultrasonic waves (i.e., photoacoustic waves) using US transducers. Thus, PAI maintains the advantage of strong optical contrast while simultaneously having resolution far superior to DOI due to its US detection. Sun et al. first presented absorption coefficient maps recovered from in vivo photoacoustic tomography (PAT) images of a distal interphalangeal joint [100]. Since then, dual-modality imaging—which combines results from functional PAI measurements with morphological images from US—has been investigated in both animal models of RA [101] as well as RA patients [102, 103]. In the latter, significant group differences in raw PAI response as well as measurements of hemoglobin content and hypoxia were found between RA patients and healthy volunteers.
1.3 Thesis Motivation, Objectives, and Outline

There currently exists a need to develop more sensitive treatment monitoring tools which can identify RA treatment failure earlier in order to prevent patients from missing the benefits of effective treatment within the WOO, and prevent irreversible joint damage (see Sec. 1.1). A variety of methods are currently used for measuring RA disease activity; however, each one has drawbacks such as subjectivity (clinical examination), low sensitivity (laboratory tests and CR), high cost (MRI), and sub-optimal reproducibility (US).

Diffuse optical methods offer an objective, sensitive, relatively low-cost, and operator-independent solution for monitoring RA disease activity. Of all diffuse optical methods investigated to date, FD-DOT has achieved the highest sensitivity and specificity for identifying the presence of inflammation (i.e., RA diagnosis) [82, 83, 84]; however, for the purposes of treatment monitoring, detection of subtler, earlier physiological changes may offer substantial patient benefits. Further, DOT is associated with increased costs, complicates multi-joint evaluation, and lengthens examination times compared to simpler, non-tomographic diffuse optical methods. CW-DOI has shown sensitivity to the presence of clinical inflammation, and the potential to distinguish between a few types of RA disease activity [92, 94]. Yet, outside of DOT, there has been little work towards investigating the use of more information-rich modes of acquisition than CW.

Joint BF—measured using DCE TD-NIRS—has already demonstrated a higher sensitivity to joint inflammation in an animal model of RA than a variety of other physiological parameters [91]. A general investigation of TD-DOI—which may be more practical to implement clinically than DCE TD-NIRS—could also reveal additional TD features with increased sensitivity to RA.

Given the above, the goal of my doctoral research was to assess the prospects of using non-DOT TD-based diffuse optical methods to monitor RA under a range of disease activities. This goal has been addressed through several objectives:
1. Assess the sensitivity of joint BF, as measured with DCE TD-NIRS, to disease activity and DMARD treatment in a rat model of chronic RA.

2. Investigate the sensitivity of TD-DOI to differences between a wide range of RA disease activity states in silico.

3. Test the sensitivity of an experimental TD-DOI system to differences between solid phantoms of multiple RA disease activity states.

A summary of the chapters included within this dissertation, which address the objectives outlined above, is provided below.

1.3.1 Chapter 2: Quantification of joint blood flow by dynamic contrast-enhanced near-infrared spectroscopy: Application to monitoring disease activity in a rat model of rheumatoid arthritis

This chapter presents a longitudinal study wherein joint BF was monitored in a rat model of chronic RA throughout the development of the induced disease. It was adapted from work which was previously published in 2020 by Seva Ioussoufovitch, Laura B. Morrison, Lise Desjardins, Jennifer A. Hadway, Keith St. Lawrence, Ting-Yim Lee, Frank Beier, and Mamadou Diop in the Journal of Biomedical Optics (volume 25, issue 1) under the title “Quantification of joint blood flow by dynamic contrast-enhanced near-infrared spectroscopy: application to monitoring disease activity in a rat model of rheumatoid arthritis” [104].

1.3.2 Chapter 3: Time-domain diffuse optical imaging technique for monitoring rheumatoid arthritis disease activity: theoretical development and in silico validation

This chapter presents an investigation of TD-DOI’s ability to distinguish between 80 states of RA disease activity simulated in silico. A publication based on the data presented in this
chapter and titled “Time-domain diffuse optical imaging technique for monitoring rheumatoid arthritis disease activity: theoretical development and in silico validation” is in preparation by Seva Ioussoufovitch and Mamadou Diop.

1.3.3 Chapter 4: Time-domain diffuse optical imaging technique for monitoring rheumatoid arthritis disease activity: experimental validation in tissue-mimicking finger phantoms

This chapter presents an experimental study conducted using a TD-DOI system to assess its ability to distinguish between RA disease-mimicking solid phantoms under varying experimental conditions. A publication based on the data presented in this chapter and titled “Time-domain diffuse optical imaging technique for monitoring rheumatoid arthritis disease activity: experimental validation in tissue-mimicking finger phantoms” is in preparation by Seva Ioussoufovitch and Mamadou Diop.

1.3.4 Chapter 5: Conclusion

This chapter summarizes the findings of the chapters above and outlines the future prospects of TD-based diffuse optical methods for use in RA treatment monitoring.
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Chapter 2

Quantification of joint blood flow by dynamic contrast-enhanced near-infrared spectroscopy: Application to monitoring disease activity in a rat model of rheumatoid arthritis

This chapter was adapted from work which was previously published under a Creative Commons Attribution license (CC BY 4.0: http://creativecommons.org/licenses/by/4.0/) in 2020 by Seva Ioussoufovitch, Laura B. Morrison, Lise Desjardins, Jennifer A. Hadway, Keith St. Lawrence, Ting-Yim Lee, Frank Beier, and Mamadou Diop in the Journal of Biomedical Optics (volume 25, issue 1) under the title “Quantification of joint blood flow by dynamic contrast-enhanced near-infrared spectroscopy: application to monitoring disease activity in a rat model of rheumatoid arthritis” (DOI: 10.1117/1.JBO.25.1.015003).

2.1 Introduction

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease that afflicts about 1% of the population and is associated with pain [1], reduced quality of life [2], and disability [3]. Fortunately, the introduction of a novel treatment paradigm that combines treat-to-target
strategies [4] with early use of disease-modifying antirheumatic drugs (DMARDs) [5] has significantly improved mid- and long-term patient outcomes over the past two decades. However, DMARD treatment failure, which is currently identified only after 3–6 months of therapy, still occurs in 30% of RA patients [6, 7]. After failure, these patients must undergo an iterative process where they are assigned to new therapies and wait again for 3–6 months before treatment efficacy can be reliably assessed. This process is drawn-out, costly, and results in patients losing the benefits of effective early treatment while having an increased risk of developing irreversible joint damage [8].

Treatment response is currently assessed using a combination of clinical examination and patient self-assessment. Though this approach is the current standard of care, it can be ineffective for repetitive, longitudinal assessments because of its subjectivity and variability [9, 10]. Furthermore, clinical examination and patient self-assessment are unlikely to detect subclinical changes in inflammation which could be early indications of treatment response. As such, clinical examination is often supplemented with laboratory tests, radiography, or ultrasonography. However, though these additional tools can be effective at aiding diagnosis, their usefulness for long-term monitoring is limited by low sensitivity (radiography and laboratory tests) [11] and suboptimal reproducibility (ultrasonography) [12, 13, 14]. Given the above limitations, there is currently a need to find more sensitive and objective techniques that can detect RA treatment failure within the first 3 months of therapy.

In recent years, there has been a growing interest in investigating the ability of near-infrared (NIR) optical methods to assess RA disease progression. This is in part due to the ability of NIR techniques to provide quick and objective measurements at a relatively low cost. In the case of RA, NIR methods generally attempt to monitor disease progression by measuring downstream effects of joint hypoxia which is well-known to play a central role in the maintenance and progression of the disease [15, 16]. It is well established that chronic inflammation, which is a key feature of RA, causes hypertrophy of the synovial lining; the thickness of the synovial membrane increases from 1–3 to over a dozen cell layers in RA [16]. This hypertrophic cell
mass induces an increased metabolic demand that exceeds supply, leading to the development of hypoxic regions within the synovium [15, 17]. In fact, it has been shown (using invasive probes) that the partial pressure of oxygen is lower in inflamed joints than in healthy joints [18]. The presence of chronic hypoxia then acts as a signal both for the formation of new blood vessels (i.e., angiogenesis) [17] and increased tissue blood flow (BF) [19, 20].

Previous studies using NIR techniques have largely focused on assessing changes in oxygen saturation and blood content within the joint since these characterize hypoxia and angiogenesis, respectively, and are the downstream effects of chronic inflammation. Some examples of NIR techniques that have been applied to RA disease monitoring include photoacoustic tomography [21, 22, 23, 24], diffuse optical spectroscopy [25, 26, 27], contrast-based fluorescence imaging [28, 29, 30], and diffuse optical tomography [31, 32, 33, 34]. Using these approaches, RA disease progression can be assessed by measuring increases in blood volume, variations in blood oxygen saturation, intensity of contrast agent fluorescence, and total hemoglobin concentration in the joint. As mentioned, increased tissue BF—defined as the volume of blood flowing through a mass of tissue per unit time (mL/min/100g)—is another physiological response to chronic hypoxia; in fact, joint BF has yet to be thoroughly investigated as a potentially highly sensitive marker of RA disease activity. Thus, we hypothesized that BF can be a surrogate marker of changes in joint hypoxia and RA disease progression. We previously developed a dynamic contrast-enhanced time-resolved near-infrared spectroscopy (DCE TR-NIRS) technique for measuring joint BF and showed—in a rabbit model of RA—that joint BF is more sensitive to inflammatory arthritis than changes in hemoglobin concentration and oxygen saturation [35]. These findings suggest that joint BF may be a more sensitive biomarker of inflammatory arthropathies than those previously investigated with other NIR techniques. Thus, the objective of the current work was to investigate whether joint BF, as measured with DCE TR-NIRS, can track longitudinal changes in joint inflammation during disease induction and DMARD treatment in a rat model of RA.
2.2 Methods

2.2.1 Instrumentation

The TR-NIRS system was built in-house using a pulsed diode laser (LDH-P-C-810; PicoQuant, Germany) connected to a PDL 828 laser driver (PicoQuant). The laser emission was centered at 805 nm and the pulse repetition rate was set to 80 MHz. The laser output was coupled into an emission fiber ($\phi = 400 \mu m$, NA = 0.22; Fiberoptics Technology, Pomfret, CT, United States) that guided light to the ankle joint. Light transmitted through the ankle joint was collected with a fiber optic bundle ($\phi = 3 \text{ mm}$, NA = 0.55; Fiberoptics Technology) that was coupled to a hybrid photomultiplier detector (PMA Hybrid; PicoQuant) and a time-correlated single photon counting module (HydraHarp 400; PicoQuant). A 3D-printed probe-holder was used to ensure good contact between the ankle joint and the emission and detection probes. Instrument response functions (IRFs) were acquired by placing the emission and detection probes into a light-tight box containing a piece of paper, positioned 2mm away from the emission fiber, which acted as a light diffuser to fill the numerical aperture of the detection probe.

2.2.2 Phantom experiments

Since the DCE TR-NIRS technique relies on accurate estimation of changes in tissue absorption, we assessed the system’s ability to measure absorption changes in a tissue-mimicking phantom made with Intralipid and India Ink. A cuvette (10 mm x 10 mm x 40 mm) was filled with 3.5 mL of 0.8% Intralipid created by diluting a 20% stock solution of Intralipid with water. We then placed the emission and detection probes transversely across the cuvette, secured the entire setup using a 3D-printed holder, and acquired a baseline measurement with the TR-NIRS system. Next, we began adding 0.02 mL increments of an India Ink solution with a known absorption coefficient ($\mu_{a,\text{ink}}$). Note that $\mu_{a,\text{ink}}$ was determined by measuring light transmittance through an India ink dilution to determine the ink’s molar absorption coefficient (see
Figure 2.1: Experimental timeline. Baseline measurements (B1–B3) were acquired within a 10-day period before and on the day of arthritis induction in the experimental group (day 0). Starting on day 20, animals in the experimental group were treated with the DMARD Enbrel® (etanercept) every 5 days.

Sec. 2.2.4 for details). The volume of increments was chosen so that the investigated changes in absorption would cover a range similar to what had been measured during preliminary animal experiments (0.005–0.040 cm\(^{-1}\)). After adding each 0.02 mL increment, the solution was mixed with a glass stirring rod and left to settle for 60 s before the next set of measurements. Each set of TR-NIRS measurements consisted of 100 distribution of times-of-flight of photons (DTOFs) acquired over 30 s.

### 2.2.3 Animal model & experiments

Animal experiments were conducted under an animal care protocol approved by the local ethics committee. All experimental procedures were conducted while the animals were under anesthetic. Anesthesia was induced in an airtight chamber with 5% isoflurane gas and maintained with 2% isoflurane by mask.

The study included 12 adult male Lewis rats: 4 rats in the control group and 8 rats in the experimental group (experimental timeline is shown in Fig. 2.1). Arthritis was induced using the adjuvant-induced arthritis (AIA) model [36]. Prior to the start of the study, animals were allowed to acclimate for 5–7 days, after which three baseline BF measurements were acquired.
over a 10-day period (Fig. 2.1). After the third baseline measurement was acquired on day 0, rats in the experimental group received a subcutaneous injection of a solution prepared using heat-killed lyophilized mycobacteria butyricum suspended in incomplete Freund’s adjuvant to induce polyarticular arthritis [37]; rats in the control group received saline injections. Thereafter, ankle joint BF was measured every 5 days until the end of the study on day 40 or until rats reached pre-determined humane endpoints (HEP) based on pain assessment by a veterinarian. AIA was allowed to progress until day 20 (Pre-treatment phase). Starting on day 20, the Treatment phase of the study began: rats that had not reached HEP were treated with intramuscular injections of the DMARD etanercept (Enbrel®: 0.5 mL/kg) every 5 days. Treatments were administered on each measurement day within the Treatment phase immediately after BF measurements. Rats in the experimental group were studied in separate cohorts of 2; the entire study protocol (e.g. induction, treatment) was repeated 4 times with cohorts of 2 rats for a final sample size of 8 in the experimental group. Rats in the control group were studied simultaneously in one cohort of 4 animals. Weight measurements and qualitative written notes describing animal appearance, behavior, gait, and swelling in the paws or joints were recorded for each rat on and between study days by two certified veterinary technicians (L. Morrison, L. Desjardins). These observations were used in consultation with a veterinarian to determine the appearance of first symptoms of induced inflammation and to assess whether an animal had reached HEP. Evidence of arthritis included visible swelling in paws or joints, weight loss (>5% of initial body weight), reluctance to ambulate, decreased social interaction, and abnormal posture.

The experimental setup for the BF measurements is shown in Fig. 2.2. During each measurement, animals received a tail vein bolus injection of the optical contrast agent Indocyanine green (ICG). A dye densitometer (DDG-2001; Nihon Kohden, Japan) was attached to one paw to measure the arterial concentration of ICG. The dye densitometer also measured the animal’s heart rate (HR) and arterial oxygen saturation which were recorded and used to assess animal condition throughout the experiment. TR-NIRS measurements were acquired with the emis-
Figure 2.2: Image of TR-NIRS probe placement on a rat ankle for joint BF measurement. A dye densitometer, placed on the contralateral paw, was used to measure the time-dependent arterial concentration of the BF tracer (Indocyanine Green) while TR-NIRS probes were used to measure its tissue concentration.

sion and detection probes positioned transversely across the rat ankle joint on the contralateral paw (Fig. 2.2). Each joint BF measurement was started by acquiring 10 s of data before a bolus of ICG solution (0.2 mg/kg) was injected into the rat tail vein. For each BF measurement, we acquired 400 DTOFs over 120 s to obtain the tissue ICG concentration curve. On every measurement day, joint BF was measured on both sides of the animal (i.e., right and left ankles); measurements were repeated twice to mitigate potential unsuccessful measurements. Typical reasons for unsuccessful measurements included data loss due to DDG instrument failure, failed ICG injections due to poor catheter placement, and loss of probe contact (both DDG and TR-NIRS) during acquisition.

2.2.4 Data analysis

The TR-NIRS measurements were analyzed using an in-house software developed in MATLAB 2017a (The MathWorks Inc., Natick, MA, 2017). For each measurement, the difference in mean photon time-of-flight \( \langle t \rangle \) between the DTOFs acquired prior to contrast introduction
(India Ink for phantom experiments; ICG injection for animal experiments) and the system’s IRF was used to compute the optical pathlength $p$:

$$ p = \frac{c}{n} (\langle t \rangle_{DTOF} - \langle t \rangle_{IRF}) \tag{2.1} $$

In Eq. 2.1, $c$ is the speed of light in vacuum, and $n$ is the refractive index of tissue ($n = 1.4$). The optical pathlength was then used, in combination with the modified Beer-Lambert Law, to determine changes in the absorption coefficient over time (i.e., $\Delta \mu_a(t)$):

$$ \Delta \mu_a(t) = \ln \left( \frac{I(t)}{I_0} \right) \div p \tag{2.2} $$

In Eq. 2.2, $I(t)$ and $I_0$ are the detected light intensities after and prior to introduction of the contrast agent, respectively. Note that light intensities were computed as the sum of the total number of photons in each DTOF and that, for animal experiments, changes in pathlength due to ICG injection were deemed negligible as discussed in Sec. 2.4. For phantom experiments, the measured $\Delta \mu_a$ were compared to expected $\Delta \mu_a$ for validation. The expected $\Delta \mu_a$ were computed using Eq. 2.3. The molar absorption coefficient of the ink ($\epsilon_{a,ink}$) was calculated by determining the molar extinction coefficient $\epsilon_{e,ink}$ from a transmission measurement through India ink of known dilution and multiplying it by the ratio $\frac{\epsilon_{a,ink}}{\epsilon_{e,ink}} = 0.885$ [38] as suggested by Spinelli et al. [39]

$$ \Delta \mu_{a,expected} = \ln(10) \epsilon_{a,ink} \Delta c \tag{2.3} $$

For joint measurements, it was noted that the geometry of the joint, along with its expected structural changes during disease progression, substantially complicates the use of typical analytical solutions of light propagation in tissue and fitting approaches to determine optical properties (see Sec. 2.4 for detailed discussion).
Instead, use of the modified Beer-Lambert Law to compute changes in absorption provides a robust method that is immune to the challenges posed by the joint morphology. Furthermore, the measured $\Delta \mu_a(t)$ was converted into an ICG tissue concentration curve using Eq. 2.4:

$$Q(t) = \frac{\Delta \mu_a(t)}{\ln(10) \cdot \varepsilon_{ICG}},$$  \hspace{1cm} (2.4)

where $Q(t)$ is the time-dependent ICG concentration in the ankle joint, and $\varepsilon_{ICG}$ is the extinction coefficient of ICG at 805nm [40]. The measured tissue and arterial ICG concentration curves were subsequently used to compute joint BF, using a previously reported deconvolution algorithm [41]. Note that this method of computing BF has been previously tested and validated using phantom and animal experiments [42, 43]. As mentioned in Sec. 2.2.3, four perfusion measurements (two per ankle joint) were obtained for each animal in order to account for potentially unsuccessful data collection. If both repeat measurements from one ankle were available, the BF value for that ankle on that day was calculated as the mean of the two measurements. It is noteworthy that in approximately 10% of cases, one out of the two repeat measurements was missing and only the non-missing value was used.

### 2.2.5 Statistical analysis

Statistical analysis was conducted using SPSS Statistics 25 (IBM Corp., Armonk, NY, 2017) and power analysis was performed using G*Power software [44]. For the phantom experiments, agreement between expected and measured changes in $\mu_a$ was investigated using linear regression. Regression analysis was performed using a $y$-intercept fixed at 0 with the assumption that $\Delta \mu_{a, observed} = \Delta \mu_{a, expected} = 0$ prior to the addition of any India ink. Prior to analysis, regression assumptions of linearity and homoscedasticity were confirmed. For the linearity assumption, a visual inspection of scatter plots of raw observed versus expected values was used to confirm a linear relationship between the two variables. Data homoscedasticity was confirmed by generating scatter plots of residuals versus values predicted by the regression.
model and visual confirmation of consistent variance of the residuals for all predicted values. Furthermore, assumption of normality in the dataset was confirmed using the Shapiro-Wilk test.

For the in vivo experiments, statistical analysis was only conducted for timepoints which contained data from all animals, to account for animals reaching HEP before study completion. Though this approach limited the scope of the statistical analysis, it was necessary in order to avoid any potential survivorship bias within the dataset. A three-way repeated measures analysis of variance (ANOVA) was conducted with time and measurement side (i.e., left or right) as the within-subjects variables, and subject group as the between-subjects variable (i.e., control or experimental group). Prior to analysis, normality of the dependent variable and sphericity were confirmed—using the Shapiro-Wilk test and Mauchly’s test of sphericity, respectively—to ensure no assumptions inherent to ANOVA were violated. In addition, visual inspection of boxplots and histograms of BF data within each group revealed no significant outliers. Upon discovering a significant effect, differences were uncovered using a post-hoc Tukey’s honest significant difference test to account for multiple comparisons. A post-hoc test in G*Power was then used to assess the power of the findings using an effect size equal to the partial $\eta^2$ value determined from the ANOVA.

The potentially confounding effects of HR on joint BF were investigated using correlation analysis. Since joint BF could be affected by arthritis induction and DMARD treatment, as well as other temporal factors, the effect of HR on joint BF could only be isolated on a per day basis. Thus, for timepoints with no missing data, each animal’s raw daily BF measurements were correlated with their corresponding HR to generate a correlation coefficient between BF and HR for each day. Since each correlation coefficient was derived using only 4 comparisons (one for each raw measurement), the resulting coefficients were prone to error and difficult to interpret in isolation; instead, all obtained correlation coefficients were averaged with the assumption that the presence of an underlying trend in the data would skew the average correlation coefficient.
Figure 2.3: Comparison of expected and measured changes (mean±SD) in the absorption coefficient of a tissue-mimicking solution (0.8% Intralipid) caused by incremental addition of India Ink. One hundred measurements were acquired using the time-resolved near-infrared spectroscopy system and absorption coefficients were computed using the modified Beer-Lambert Law. Note that absorption changes measured during subsequent animal experiments were within this range.

2.3 Results

2.3.1 Phantom experiments

A simple linear regression with the y-intercept set to 0 (see Sec. 2.2.5 for details) was conducted to compare $\Delta \mu_a$ measured in tissue-mimicking solution to the expected $\Delta \mu_a$ due to India ink addition. There was a significant linear relationship between measured and expected $\Delta \mu_a$ ($F(1,9) = 5773, p<.05$), and a strong relationship between the variables ($R^2 = 0.99$) with a slope of 0.96 within the tested 0.005–0.040cm$^{-1}$ absorption range (Fig. 2.3).

2.3.2 Animal experiments

Figure 2.4 shows typical arterial and tissue ICG concentration curves measured during an animal experiment. The dynamic inflow and washout of the tracer can be clearly identified on both
Figure 2.4: Arterial and tissue ICG concentration curves measured by the dye densitometer and the TR-NIRS system, respectively. ICG was injected into the rat’s tail vein at the 10 s mark.

curves, with the tissue curve showing a slower rise and subsequent clearing of ICG compared to the arterial curve.

The results of the statistical analysis from the first day of the baseline period (B1) to day 15 are summarized in Table 2.1. In all cases, the BF data was not significantly different from a normal distribution ($p=\text{ns}$; ns: not significant) and did not violate the assumption of sphericity ($p=\text{ns}$). Only time and group were found to have a significant main effect on joint BF (Table 2.1) with effect sizes of partial $\eta^2 = .400$ and $\eta^2 = .365$, respectively. These effects were interpreted as follows: BF increased over time regardless of group assignment, and experimental group BF was higher than control group BF regardless of measurement time. Figure 2.5A and 2.5B show the data relevant to the statistically significant effects denoted in Table 2.1: a BF time course averaged over measurement side and group, and group BF values averaged over time and measurement side. As seen in Fig. 2.5A, a post-hoc Tukey test for the effect of time on ankle joint BF revealed a significant difference between BF on the first day of baseline (B1) and BF on day 10 at the $p<.05$ level. In addition, since statistical analysis revealed that measurement side was not implicated in any interaction and did not have a significant main effect on BF (Table 2.1), time courses for each group averaged over measurement side are pre-
Table 2.1: Summary of three-way repeated measures ANOVA with time and measurement side as the within-subject variables, and group as the between-subjects variable. Analysis was conducted on data from the first day of the baseline period (B1) to day 15 post-induction of arthritis.

<table>
<thead>
<tr>
<th>Variable(s)*</th>
<th>F-statistic</th>
<th>p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>time<em>group</em>side</td>
<td>$F(5,50) = 0.149$</td>
<td>$p=ns$</td>
</tr>
<tr>
<td>time*side</td>
<td>$F(5,50) = 0.659$</td>
<td>$p=ns$</td>
</tr>
<tr>
<td>time*group</td>
<td>$F(5,50) = 1.773$</td>
<td>$p=ns$</td>
</tr>
<tr>
<td>group*side</td>
<td>$F(1,10) = 0.135$</td>
<td>$p=ns$</td>
</tr>
<tr>
<td>time</td>
<td>$F(5,50) = 6.670$</td>
<td>$p&lt;.05$</td>
</tr>
<tr>
<td>group</td>
<td>$F(1,10) = 5.736$</td>
<td>$p&lt;.05$</td>
</tr>
<tr>
<td>side</td>
<td>$F(1,10) = 0.129$</td>
<td>$p=ns$</td>
</tr>
</tbody>
</table>

*indicates interaction between multiple variables  
**ns: not significant

presented in Fig. 2.5C to facilitate interpretation of the uncovered main effects. We note that the primary effect of interest in this work was the presence of a two-way interaction between time and group which would have manifested as a significant difference between the time courses in Fig. 2.5C. The presence of this effect would have been interpreted as a difference in joint BF between the experimental and control group over time. To further investigate the lack of this effect (Table 2.1), post-hoc power analysis was calculated for the two-way interaction between time and group using an effect size of partial $\eta^2 = .151$ and yielded a power of 0.21.

Confounding effects of HR on measured joint BF values were investigated by correlating raw BF measurements with their corresponding HR and averaging across all animals and days as described in Sec. 2.2.5. This analysis revealed a weak positive correlation ($r = .1370$) between HR and BF measurements.

To further investigate the variations in joint BF, longitudinal measurements from each individual rat were examined (Fig. 2.6); time courses for raw data as well as data averaged across measurement side are presented. Consistently elevated joint BF levels throughout the
Figure 2.5: BF (±95% confidence interval) for time courses averaged by group and measurement side (A), and boxplots of BF for each group averaged over time and measurement side (B). Asterisks indicate a significant difference at the p<.05 level between two timepoints (A) or two groups (B). To facilitate interpretation of main effects, BF (±95% confidence interval) time courses for each group averaged over measurement side are shown in (C). Time courses (A) and (C) show 3 baseline measurements (B1 – B3; grey), followed by 3 measurements post-arthritis induction in the experimental group (after B3: day 0; orange). Note that, to avoid survivorship bias, statistical analysis was only conducted on timepoints that included all animals in each group; thus, data from the Treatment phase is not shown.

Pre-treatment phase were only observed for rats 1 and 2; these animals had the highest incidence of ankle BF above the typical baseline threshold of approximately 15 mL/min/100g. For 4 out of 8 rats in the experimental group, joint BF on day 5 was higher than their typical baseline values. In the control group, joint BF on day 10 for was higher than typical baseline values for 3 out of 4 rats. Rats 3 & 4 were the only individuals in the experimental group who completed the entire study period; these rats also showed very minimal variation in their joint BF compared to other rats in their group and their BF times courses are generally more similar to those of the rats in the control group. In contrast, for rats 1 & 2 joint BF decreased once treatment was initiated; however, only a few treatment timepoints are available since these rats reached HEP quickly. Looking at individual side measurements, there is no clear trend in the inconsistency between measurement sides across animals. For the experimental group, average BF time courses among rats are more similar within each cohort than between cohorts. This similarity is also present in the occurrence of first symptoms and reaching of HEP (Fig. 2.6).
Figure 2.6: Scatter plots of individual BF values (mL/min/100g) for each rat; average BF values are shown as black markers, while right and left ankle measurements are indicated using red and blue markers, respectively. Time courses show 3 baseline measurements (B1 – B3; grey), followed by measurements post-arthritis induction in the experimental group (after B3: day 0; orange) and after DMARD treatment was started (days 20 – 40; blue). Experimental and control group data are enclosed by red and blue borders, respectively. For the experimental group, HEP and appearance of first symptoms are indicated by solid and dashed vertical lines. Rats in the experimental group were studied in cohorts of 2 while those in the control group were studied in one cohort of 4; experimental group data are subdivided by black lines to delineate cohorts. Cohort numbers for both groups are indicated at the top right (C1–C5).
2.4 Discussion

This work sought to investigate whether joint BF, as measured with DCE TR-NIRS, could track longitudinal changes in joint inflammation during disease induction and DMARD treatment in the AIA rat model of RA. AIA was the first-described animal model of RA and remains widely used for preclinical assessment of RA treatments [45]. The model is typically characterised by rapid onset (e.g., 10 days post-injection) and progression of polyarticular arthritis; however, the inflammation tends to subside after a month of disease activity. While the AIA rat model does not exhibit the chronic disease progression characteristic of human RA, which takes place over months, it shares many of the relevant biological features such as swelling, joint destruction, cell infiltration, and T-cell dependence. As well, treatment with etanercept—a common biologic drug that is currently used to treat RA patients—is known to be active in this model and has been associated with slight reductions in arthritic and radiographic scores [36, 46]. It is important to note that rats tend to develop antibodies against etanercept; thus, when considering the difference in body weight, response to the drug is limited to higher treatment doses (e.g. 4 – 10mg every 3 – 5 days) [46, 21] than what is typically used in RA patients (50mg weekly).

For proper quantification of joint BF, DCE TR-NIRS relies on an accurate measurement of the dynamic $\mu_a$ changes in a tissue bed following an ICG bolus injection. These measurements can be used to compute a tissue ICG concentration curve which can then be combined with an arterial ICG concentration curve to calculate BF. As such, we conducted tissue-mimicking phantom experiments to ensure that our TR-NIRS system could accurately measure changes in $\mu_a$. Figure 2.3 shows a strong linear relationship ($R^2 = 0.99$) with a slope of 0.96 between expected and measured $\mu_a$ changes in a tissue-mimicking phantom whose absorption coefficient was modulated using various concentrations of India Ink; these results confirmed our system’s ability to quantify static changes in $\mu_a$. Next, the ability of the TR-NIRS system to track dynamic changes in absorption was confirmed through preliminary animal experiments
during which representative arterial and tissue ICG concentration curves were obtained. Tissue ICG concentrations were approximately 100 times lower than the concentrations of the tracer in arterial blood (Fig. 2.4) which was similar to what we previously reported for tissue concentration values in the rabbit knee joint [35].

Following system validation, experiments were conducted in 12 rats: 4 rats in the study’s control group and 8 rats in the AIA experimental group. To account for potential injection effects, rats in the control group received saline injections to match any injections administered to the experimental group. Due to the severity of the AIA model, 50% of rats in the experimental group reached HEP before day 20 post-induction of arthritis. Thus, to avoid survivorship bias, we limited the temporal scope of our subsequent statistical analysis to timepoints which contained data from all animals, i.e., all baseline timepoints and the first 3 timepoints of the Pre-treatment phase. The only significant effects found in this study were that both time and group had a significant main effect on joint BF with effect sizes of $\eta^2 = .400$ and $\eta^2 = .365$, respectively (Fig. 2.5A and 2.5B). Interestingly, the implication of the main effect denoted in Fig. 2.5B is that BF differed between rats in both groups regardless of when it was measured (i.e., even at baseline). To further investigate this possibility, we compared mean BF time courses of both groups along with their 95% confidence intervals (Fig. 2.5C); since measurement side did not have a significant effect on BF, we averaged time courses across both ankles to facilitate data interpretation. First, BF appears consistent between both groups and among the baseline timepoints (Fig. 2.5C); this is an important result as it confirms the ability of our DCE TR-NIRS technique to reliably quantify joint BF. Second, once arthritis is induced (Pre-treatment), BF in the experimental group is substantially higher than in the control group on day 5 after which BF in the control group rises to match that of the experimental group. These trends in Fig. 2.5C are consistent with the the significant effect seen in Fig. 2.5A: regardless of AIA, joint BF changes as the study period progresses. Furthermore, Fig. 2.5C suggests that the significant effect described by Fig. 2.5B is largely driven by the discrepancy in BF between both groups on day 5. Nevertheless, this single discrepancy was not substantial enough for the
time courses in both groups to be statistically different from one another (i.e., no significant interaction between time and group).

As mentioned in Sec. 2.1, it is well-established that chronic inflammation, which is a key feature of RA, leads to the development of hypoxic regions within the synovium [15, 17], and that the presence of hypoxia acts as a potent signal for angiogenesis [17] and increased tissue BF [19, 20]. Based on this known pathophysiology of inflammatory arthritis and the results from our previous study in a rabbit model of inflammatory arthritis [35], we expected to find an interaction between time and group in this study. More specifically, we anticipated an increase in joint BF in the experimental group compared to the control group during the Pre-treatment phase.

One possible reason that this study did not find a significant difference between control and experimental group time courses was the introduction of measurement error due to the variation in animal HR during different BF measurements. However, only a very weak correlation ($r = .1370$) was noted between HR and joint BF, suggesting that the potentially confounding effect of varying HR throughout an experiment had a negligible effect on the BF results. Another reason could be the relatively small sample size. Based on the effect size observed here for the two-way interaction between time and group (partial $\eta^2 = .151$), post-hoc power analysis revealed that, in regards to this two-way interaction, our study only had a power of 0.21. To reach a power of 0.8 with the aforementioned effect size, our study would require a sample size of 74; this is a notable difference from the large effect size we previously measured in an inflammatory mono-arthritis rabbit model [35] where only 4 subjects were needed to detect a statistically significant difference between control and inflamed joints at the $p<.05$ level with a power of 0.8. In particular, this discrepancy between both studies highlights the challenge of modelling human disease with animal models.

Aside from differentiating between groups during the Pre-treatment phase, this study sought to investigate whether treatment with etanercept affects joint BF in the AIA model post-induction of arthritis. Since etanercept is known to be active in this model of arthritis [36, 46], we ex-
pected to see decreased joint BF in response to etanercept treatment. However, because no statistical analysis could be performed for timepoints in the Treatment phase, we compared treatment response between control and experimental group rats on a case-by-case basis (Fig. 2.6). Only rats from cohort 1 and 2 progressed into the Treatment phase and only rats from cohort 1 showed evidence of BF decrease in response to treatment. In particular, their BF dropped from the elevated Pre-treatment values on days 15 & 20 to values similar to cohort 2 and control rats on days 25 and 30. Rats in cohort 2 did not have elevated Pre-treatment BF values and their BF remained relatively constant throughout the study. In fact, aside from a slight elevation in BF in Rat 3 on day 5, the time courses of the rats in cohort 2 looked quite similar to those of the control group. Consultation of recorded animal well-being metrics revealed that rats 3 & 4 had the latest appearance of first symptoms (Fig. 2.6) and, while both rats exhibited some paw swelling, they did not have the gait issues that were typically seen in the other cohorts. Thus, the relatively mild disease induction in cohort 2 rats may have played some role in making it difficult to distinguish between them and control group rats on the basis of joint BF. Importantly, the approach that is typically reported in the literature is to administer treatment on the first day of inflammatory symptoms. In contrast, our study was designed so that treatment was only administered at a set timepoint regardless of first symptom appearance. Since some variability in disease progression is expected and was observed between rats, it is possible that experimental group rats received treatment while in a different state of disease progression which may have confounded the Treatment phase results. Due to these factors as well as the small experimental group sample size available from the Treatment phase, the conclusions regarding the relationship between joint BF and DMARD treatment response remain limited.

We also examined the Pre-treatment phase of Fig. 2.6 for any consistent differences between the experimental and control groups. Four out of 8 rats in the experimental group showed BF increases on day 5, while increases from baseline only occurred on day 10 in 3 out of 4 rats in the control group; this is consistent with the BF difference on day 5, as shown
in Fig. 2.5C. A striking observation from Fig. 2.6 was that, for the entire study period, time courses among rats within the same cohort were more similar than rats from different cohorts. Figure 2.6 also revealed that rats within the same cohort experienced first symptoms within a day of each other while first symptom occurrence had a standard deviation of 2.20 days when all cohorts were combined. Together, these observations suggest that rats in the same cohort had similar disease progression and that the heterogeneity in the response to arthritis induction between cohorts may have had an important influence on the BF time courses. This observation is further confirmed by the temporal consistency between first symptom appearance and HEP within each cohort. To further investigate this possibility, we used SPSS Statistics 25 to perform a hierarchical cluster analysis on the experimental group’s BF values from the first day of the baseline period (B1) to day 15 post-induction of disease. Since no outliers were present in the dataset, Ward’s method was used as the cluster method and squared Euclidean distance as the similarity metric; the elbow method was subsequently used to identify the optimal number of clusters [47]. The analysis revealed that 4 distinct clusters were present in the data: one for each cohort in the experimental group. To confirm the stability of the solution we randomized the order of the data and re-ran the analysis 3 times. In addition, we also performed k-means clustering on the original dataset and assumed that 4 clusters were present in the data. For all analyses, it was determined that the same 4 clusters were present in the data, with each cluster corresponding to our original cohort assignments. This provides further evidence that a main source of inconsistency in the study is that animals were divided into small cohorts whose arthritis was induced using 4 different mycobacterium butyricum solutions. Though every effort was taken to ensure that all solutions were prepared using the same protocol, it is possible that slight variations in injected solution volume or preparation (e.g., mixing of bacteria with Freund’s adjuvant) contributed to varied disease induction among the experimental group.

As shown in this study, temporal variability in inter-subject disease and treatment response made it difficult to interpret the link between changes in BF and disease severity. Cases like this benefit particularly from the ability of DCE TR-NIRS to reliably quantify tissue perfusion
in absolute units, which makes it possible to compare a single subject’s values over a long range of timepoints to uncover potential physiological relationships. It is important to note that other optical techniques, such as diffuse correlation spectroscopy (DCS), can noninvasively monitor tissue perfusion \([48, 49, 50]\); for example, DCS generally provides a BF index whose proportionality to absolute BF is a function of tissue optical properties and tissue geometry. Importantly, RA progression is associated with synovial lining hypertrophy, typically leading to bone resorption, membrane inflammation, edema, and other general morphological changes to the joint tissue which makes modelling the joint geometry quite challenging \([45]\). Considering that these changes vary from subject to subject, accurate quantification of tissue optical properties during disease progression and treatment with DCS could be very difficult. Furthermore, failing to account for these changes would likely confound the relationship between BF indices measured at different timepoints. There are systems that combine DCS with TR-NIRS or frequency domain NIRS to make it possible to quantify the necessary optical properties for continuous DCS measurement on a day-by-day basis \([48, 51, 52]\); however, these approaches still typically rely on the use of analytical solutions to the diffusion equation for simple tissue geometries (e.g., slab geometry). As mentioned in Sec. 2.2.4, a key advantage of our DCE TR-NIRS technique is that it only requires the ability to quantify changes in absorption to measure joint BF. This circumvents the challenge of needing an analytical solution to the diffusion equation for a joint structure with a complex shape that changes both in time and across subjects, and is not well approximated by regular geometrical shapes.

While DCE TR-NIRS has unique advantages that make it suitable for longitudinal joint BF monitoring, a potential source of error while using this technique is the occurrence of dynamic changes in optical pathlength caused by changes in absorption due to the passage of ICG through the joint. To address this, we estimated the change in optical pathlength for five datasets and found that, on average, pathlength varied by \(\pm 0.5\%\) over a 120 s measurement period. When these differences were propagated forward in the analysis, they only resulted in a \(\pm 1\%\) change in recovered BF values. Since these changes are negligible, for computational...
efficiency, $p$ was estimated using the mean pathlength measured prior to ICG injection. Another limitation of DCE TR-NIRS is that it requires the use of a contrast agent (e.g., ICG) which may limit clinical translation. However, it has been previously shown that changes in tissue oxy- and deoxy-hemoglobin concentrations in muscle following venous occlusion can be used to quantify BF in lieu of a contrast agent [53]. While the ability of the latter method to quantify joint perfusion has yet to be tested, it offers an interesting alternative which could be readily implemented with TR-NIRS instrumentation and should be an area of future research.

Despite the advantages of the DCE TR-NIRS technique, the small sample size of this exploratory work, issues with animal survivorship, and sources of error linked to disease heterogeneity among the experimental group limit this study’s conclusions regarding the link between BF, arthritis induction, and DMARD treatment response in the AIA model. Overall, we found the use of the AIA model in a continuous, longitudinal study design challenging due to the severe and rapid onset of the disease which often occurred in the 5-day periods between subsequent BF measurements. We also conducted a subset of experiments to investigate mycobacterium dose and disease severity but could not determine a reliable dose for inducing slower and milder arthritic progression. Considering the mismatch between this relatively rapid disease progression and the relatively low overarching temporal resolution of our data (i.e., 1 measurement every 5 days), our time courses may have missed otherwise important variations in BF and disease activity. While the temporal frequency of our BF measurements was part of the original study design which was formulated based on results from our previous study [35], it was ultimately a limitation of the study presented here and should be amended in future work. The authors also acknowledge the potentially confounding effect of anesthesia induced by isoflurane on BF in rodents [54, 55]. Though anesthetic concentrations were tightly managed to consistent levels during experiments, future work may benefit from investigating whether varying isoflurane concentrations or using other anesthetics such as ketamine has an effect on joint perfusion. Future work would also benefit from exploring longitudinal BF changes in milder models of rheumatoid arthritis with slower symptom onset, such as
pristane-induced arthritis [56], to allow a more robust investigation of the relationship between DMARD treatment and joint BF.

2.5 Conclusion

In this work, we used our quantitative joint perfusion technique (DCE TR-NIRS) to monitor joint BF in a longitudinal rat model of RA. Joint BF was measured in each animal’s left and right ankles before treatment (Pre-treatment phase) and after treatment with the DMARD etanercept (Treatment phase) in an experimental group of 8 rats with AIA. Four additional rats served as controls throughout the study. Time and group had separate significant effects on joint BF; however, there was no significant interaction between time and group despite a large difference in average BF values on day 5. Measurement side did not have a significant effect on joint BF. Statistical analysis of the Treatment phase was limited since 50% of the animals reached humane endpoints before starting treatment. Comparison of individual animal time courses between the experimental and control group revealed no consistent trend in treatment response; effects may have been masked by heterogenous disease response to AIA in the experimental group. Future work will focus on exploring longitudinal BF changes in milder models of rheumatoid arthritis with slower symptom onset to allow a more robust investigation of the relationship between DMARD treatment and joint BF.

2.6 Acknowledgements

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Chapter 3

Time-domain diffuse optical imaging technique for monitoring rheumatoid arthritis disease activity: theoretical development and in silico validation

This chapter was adapted from work in preparation for submission by Seva Ioussoufovitch and Mamadou Diop.

3.1 Introduction

Rheumatoid arthritis (RA) is a common chronic inflammatory disorder associated with fatigue, pain, depression, and reduced quality of life [1, 2]. While the disease has no cure, early treatment within the first 3 months of disease onset has been shown to significantly improve patient prognosis [3, 4, 5]. Unfortunately, at least 1 in 3 RA patients fail their first treatment and current monitoring methods—typically based on clinical examination, patient self-assessment, and laboratory testing [6]—can require 3–6 months to detect this failure [7, 8]. As a result, these patients not only incur unnecessary expenses and side effects, but also lose the benefits of effective early treatment and are placed at a higher risk of developing irreversible joint damage [9]. This has created a need for reliable monitoring tools that can detect subclinical indications of treatment failure [10, 11].
Magnetic resonance imaging and ultrasonography—the most commonly discussed techniques for this purpose—suffer from high cost and suboptimal reproducibility, respectively [12, 13, 14]. Diffuse optical imaging (DOI) has been proposed as a low-cost, objective alternative, and its sensitivity to RA disease activity has been demonstrated [15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. Notably, images from a single-wavelength continuous-wave (CW) system were previously used in combination with joint circumference to track changes in inflammation with an error rate of 14% [15, 16]. Subsequent work has leveraged a variety of alternative DOI implementations to further improve sensitivity and specificity to joint inflammation. Notably, dynamic contrast-enhanced CW-DOI [20], CW-DOI combined with cuff occlusion [21], multi-spectral CW diffuse optical tomography (DOT) [22], and tomographic frequency-domain (FD) DOT [17] have all been investigated. Importantly—using heuristics extracted from the spatial distribution of tomographically-reconstructed optical absorption and scattering values—follow-up investigations based on the latter work demonstrated that image classification based on machine learning could be used to obtain sensitivities and specificities between 93.8% and 100% [18, 19]. Despite this success, DOT is associated with increased costs, complicates multi-joint evaluation, and lengthens examination times compared to its non-tomographic counterparts. Thus, recent work has focused on augmenting simpler DOI approaches. Notably, Lighter et al. applied Fourier transform analysis to multi-spectral CW-DOI data to obtain sensitivities and specificities up to 77.9% and 90.9% [23]. However, this work was aimed at early RA diagnosis as opposed to treatment monitoring, and primarily focused on providing a binary distinction between healthy and inflamed joints instead of tracking more subtle changes in disease activity. Furthermore, Dolenec et al. previously showed—in a virtual model—that additional spectral information provided by hyperspectral CW-DOI could be used to distinguish between multiple simulated states of RA disease activity [24]. Similarly, we have recently leveraged the additional information provided by time-domain (TD) DOI to distinguish between four simulated states of RA disease activity (None, Mild, Moderate, and Severe) [25, 26].
In TD acquisition, the number of detected photons and their travel time in the tissue are both measured and represented using a distribution of times-of-flight of photons (DTOF). In comparison, CW only measures light attenuation while FD acquisition measures both the attenuation and phase shift of the detected light. In practice, DTOFs typically capture more information than CW and FD measurements, and this additional information could be leveraged to provide greater insight into changes in RA disease activity (i.e., to detect treatment response). This was reinforced by our previous findings that suggest that TD-DOI has the potential to distinguish between several simulated states of disease activity [25, 26].

Changes in RA disease activity are defined by alterations in multiple physiological parameters whose individual effects on TD-DOI remain unclear. For example, progression of RA disease activity has been associated with 1) increased synovial fluid and synovial membrane volumes [27], 2) changes in synovial fluid optical properties [28], 3) hypoxia [29], 4) decreased blood volume fraction in the synovial membrane [30], and 5) increased water concentration in the cartilage [31, 28]. Though this list is not exhaustive, investigating the TD-DOI contrasts produced by each of these physiological changes is a good starting point for identifying the technique’s clinical utility. To this end, we previously investigated the individual effects of the aforementioned 5 RA-associated physiological changes on TD-DOI [32]. The investigation was conducted *in silico* to facilitate the use of a more realistic tissue geometry and to enable more precise alteration of physiological parameters than in a phantom or human study, respectively. Yet, this preliminary work only assumed a single baseline disease activity and, most importantly, did not include the anticipated effects of a real-world TD-DOI imaging system. More specifically, it is well-known that TD-DOI measurements will include contributions from both the imaged medium (i.e., temporal point-spread function, TPSF) and the imaging system (i.e., instrument response function, IRF).

In this work, we aim to address these limitations by conducting a systematic *in silico* investigation of TD-DOI’s ability to track changes in physiological parameters known to be associated with RA disease activity under multiple baseline disease activities. Furthermore, we
present a new data analysis pipeline to recover these changes even after accounting for the effects of noise and an IRF. Motivated by Lighter et al.’s successful identification of proximal interphalangeal (PIP) joint inflammation using intensity-normalized, transverse-averaged dorsal optical transmission measurements [23], our simulations were done using a similar PIP joint imaging geometry, and we conducted all analyses on TD-DOI images averaged in the finger’s transverse direction and normalized by the total number of detected photons. Further, we investigated the effects of the 5 aforementioned RA-associated physiological changes by simulating their effects on TD-DOI data, obtained from a realistic finger joint, at 4 different baseline disease activities.

3.2 Methods

3.2.1 Simulations

For reference, tissue-specific changes in physiological parameters associated with RA disease activity are summarized in Table 3.1. Simulations were performed using virtual phantoms based on a realistic tissue geometry derived from a segmented MRI image of a healthy adult finger [33]—details of the segmentation have been previously described [25] (Appendix A). The segmented finger was composed of 7 tissue types (skin, subcutis, tendon, synovial membrane, synovial fluid, cartilage, and bone) and was used as the base tissue geometry for a model with no RA disease activity (i.e., “None”). Additional tissue geometries for “Mild”, “Moderate”, and “Severe” models of disease activity were created by linearly transforming transverse

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Change</th>
<th>Affected Tissue(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joint volume (G)</td>
<td>Increase</td>
<td>Synovial Fluid, Synovial Membrane</td>
</tr>
<tr>
<td>$\mu_\text{a}$ and $\mu'_\text{s}$</td>
<td>Increase</td>
<td>Synovial Fluid</td>
</tr>
<tr>
<td>Oxygen saturation (SO$_2$)</td>
<td>Decrease</td>
<td>Subcutis, Synovial Membrane</td>
</tr>
<tr>
<td>Blood volume fraction (BVF)</td>
<td>Decrease</td>
<td>Synovial Membrane</td>
</tr>
<tr>
<td>Water concentration ([H$_2$O])</td>
<td>Increase</td>
<td>Cartilage</td>
</tr>
</tbody>
</table>
slices of the None geometry to incrementally increase the volume of the synovial fluid and synovial membrane [25]. These four geometries served as 4 possible states of the “Joint volume” parameter in Table 3.1.

Optical properties for various RA disease activities were derived by considering changes to the remaining parameters in Table 3.1. Our previous models of None and Severe RA disease activity were used to bound the range of possible values for each physiological parameter [25] (Appendix A). The value of each parameter for Mild and Moderate disease activity were estimated by linearly interpolating between its values for None and Severe. Specifically, Mild and Moderate RA disease activity were defined as 33 and 66%, respectively, within the 0–100% disease activity range bounded by None and Severe.

To create one virtual phantom, each of the five physiological parameters in Table 3.1 is assigned a value that corresponds to one of the four disease activities. That is to say that a virtual phantom is created by converting a combination of 5 physiological parameters into a tissue geometry with tissue-specific optical properties [25] (Appendix A). To investigate the effects of changes in individual physiological parameters under different baseline disease activities, we assembled 20 sets of virtual phantoms. Here, a set of virtual phantoms is defined as a group of 4 phantoms for which 4 of the 5 physiological parameters are fixed to the same baseline disease activity, while the value of the remaining physiological parameter is sequentially set to one of the four disease activities for each individual phantom. For example, one set of virtual phantoms was composed of four virtual phantoms with different $\mu_S$ values while all the other parameters were set to values that correspond to Mild disease activity. Therefore, investigating the effects of changes in the 5 physiological parameters under 4 possible baseline disease activities, resulted in 20 sets of virtual phantoms containing 4 virtual phantoms per set (80 virtual phantoms total).

MCXLAB was then used to simulate TD-DOI of the phantoms [34]. An $8 \times 8$ grid of light source positions was defined on the dorsal side of each phantom’s PIP joint over an area ($\approx 12 \times 24$ mm$^2$; $L \times W$) that was centered around, and encompassed, the joint cavity. Next,
by enabling one source at a time, the joint was raster scanned to create an image: for each source position, $10^8$ photons were simulated and a single TPSF of all of the photons transmitted through the palmar side of the finger was recorded. TD-DOI was simulated at 800 nm for all the phantoms as this wavelength corresponds to one of the transmittance peaks in PIP joints [24] and approximately matches the wavelength of the light source in the prototype TD-DOI system that will be used for future experimental validation [26]. Simulated TD-DOI data was organized as 3D data structures with spatial information on the x-y plane and TPSFs on the z-axis i.e., each image pixel represented a TPSF; hereafter, we refer to this structure as a TPSF image. Similar to Lighter et al.’s approach, we averaged each image in the transverse direction and normalized it by its total number of photons [23]. As a result, each of the 80 virtual phantoms was associated with a transverse-averaged, normalized TPSF image (Fig. 3.1).

The large amount of multi-dimensional information within each image makes a straightforward comparison between images quite challenging. To address this, we used Principal Components Analysis (PCA) to identify the largest sources of variance between all 80 images—this type of approach is often used to build classifiers for information-rich imaging data [35]. PCA was applied using the pca function in MATLAB 2020b (The MathWorks Inc., USA) and by treating the value of each time bin in the TPSF image as a variable. The result of the PCA
analysis revealed that the first principal component (PCA-1) accounted for 95% of the total explained variance between all 80 TPSF images. Further, the sensitivity of PCA-1 to changes in individual physiological parameters agreed with our anticipated sensitivity of TD-DOI to each physiological parameter [32]. To further quantify this sensitivity, the values of PCA-1 were z-score normalized and the mean change in these values for each virtual phantom set was calculated. If changes to a parameter’s disease activity did not produce a consistent direction of change in PCA-1 values (e.g., consistent decrease in PCA-1 with worsening disease activity), we assumed that our implementation of TD-DOI was insensitive to RA-associated changes in that physiological parameter. Importantly, in sets where PCA-1 changed consistently it always decreased as disease activity worsened. Given this consistency and the large amount of variance explained by PCA-1 alone, its values will be referred to as a disease index (DI) hereafter, and a decrease in DI will be interpreted as an indicator of worsening RA disease activity. Further, DI will be used as a quantitative reference metric against which more intuitive and generalizable TD-DOI features (e.g., statistical moments, Fourier components) will be compared.

To create a dataset more representative of experimental acquisitions, each TPSF image was convolved with a randomly-scaled IRF image acquired using an in-house prototype TD-DOI system. Specifically, each pixel’s TPSF was convolved with the IRF of the corresponding pixel in the IRF image:

\[ I_{DTOF}(x_n, t) = I_{IRF}(x_n, t) \ast I_{TPSF}(x_n, t) \] (3.1)

where each image has a spatiotemporal dependence \((x, t)\), \(n\) is the index of a pixel located at \(x\) in the discrete image, \(t\) is the photon time-of-flight, and \(\ast\) is the convolution operator. Thereafter, 10 versions of the resulting DTOF images were created by adding simulated Poisson noise 10 separate times using MATLAB’s `imnoise` function, which resulted in a total of 800 DTOF images (10 for each virtual phantom).
3.2.2 Feature extraction

3.2.2.1 Temporal feature recovery

For simplicity, we start by focusing on the relationship between the DTOF, IRF, and TPSF located at a single image pixel. As shown by Eq. (3.1), it is well-known that the IRF causes temporal dispersion of the DTOF compared to the true TPSF [36], and attempting to directly remove the effects of the IRF by performing a deconvolution is quite challenging because the problem is ill-posed [37, 38]. Nevertheless, it is possible to extract certain DTOF features from which the effects of the IRF can be removed in a straightforward manner. Common examples are the curves’ statistical moments since these are known to have an additive relationship:

\[ m^{DTOF}_1 = m^{IRF}_1 + m^{TPSF}_1 \]  
\[ (3.2) \]

and

\[ V^{DTOF} = V^{IRF} + V^{TPSF}, \]  
\[ (3.3) \]

where \( m_1 \) is the first normalized moment, and \( V \) is the second centralized moment [39]. Alternatively, temporal Fourier components can be recovered from each curve based on the well-known convolution property of the Fourier transform:

\[ F_i[DTOF] = F_i[IRF] \times F_i[TPSF], \]  
\[ (3.4) \]

where \( F_i \) represents the Fourier transform operator in the temporal dimension. We note that in an experimental setting, it is a scaled version of the IRF that is typically measured (i.e., \( C_1 IRF \) where \( C_1 \) is an unknown positive scaling factor). In the case of Eqs. (3.2) and (3.3), the nature of the moment analysis inherently discards \( C_1 \), and thus it is of no concern. In the case of Eq. (3.4), the effect of \( C_1 \) can be removed if each of the curves is first normalized by its area under the curve (AUC); therefore, only the Fourier components of the normalized TPSF can
be recovered once Eq. (3.4) is inverted. With this in mind, we computed \( m_{1}^{\text{DTOF}} \), \( V^{\text{DTOF}} \), and \( F_{i}[\hat{\text{DTOF}}] \)—where the hat symbol indicates a curve normalized by its AUC—from each pixel of the DTOF images. Next, we inverted the relations in Eqs. (3.2), (3.3), and (3.4) to estimate each pixel’s \( m_{1}^{\text{TPSF}} \), \( V^{\text{TPSF}} \), and \( F_{i}[\hat{T\hat{P}\hat{S} F}] \).

### 3.2.2.2 Spatial Fourier analysis

Inversion of the relations described in Eqs. (3.2), (3.3), and (3.4) allows for the recovery of TPSF features at each image pixel. However, previous work has demonstrated the utility of using changes in the spatial distribution of image information to track RA disease activity [18, 19, 23]. Therefore, we applied the spatial Fourier decomposition proposed by Lighter et al. for CW-DOI [23] to the simulated TD-DOI data to assess how the spatial Fourier components of various features of the TPSF image vary with changes in RA disease activity.

A one-dimensional discrete fast Fourier transform (FFT) was implemented using the MATLAB function `fft` and applied to the \( m_{1}^{\text{TPSF}} \) and \( V^{\text{TPSF}} \) images to extract their spatial Fourier components. All components were then normalized by the value of the images’ DC component (i.e., first Fourier coefficient) except for the DC component itself. This ensured that the extracted non-DC spatial Fourier coefficients only depended on the spatial distributions of the features within the \( m_{1}^{\text{TPSF}} \) and \( V^{\text{TPSF}} \) images [23].

We noted in Sec. 3.2.2.1 that the process of creating \( F_{i}[\hat{T\hat{P}\hat{S} F}] \) images requires that the curve at each pixel be normalized by its AUC. Although this process does not affect the relative relationship between the temporal Fourier components at each pixel, it does alter the relationship between the temporal Fourier components of the different pixels in the image i.e., the spatial variation that would have been present in \( F_{i}[T\hat{P}\hat{S} F] \) images prior to pixelwise AUC normalization. For example, the aforementioned normalization results in the value of the DC temporal Fourier component at each pixel to be equal to 1. Thus, prior to proceeding with the spatial Fourier analysis of the \( F_{i}[T\hat{P}\hat{S} F] \) images, an additional processing step was required to re-establish the spatial variation that existed in the images prior to the pixelwise AUC
normalization.

First, let’s consider the temporal summation of TD images which contain $T$ discrete time bins. In general, the pixelwise relationship between temporally-summed DTOF, IRF, and TPSF images is

$$
\sum_{t=1}^{T} I_{DTOF}(x_n, t) = \sum_{t=1}^{T} I_{IRF}(x_n, t) \times \sum_{t=1}^{T} I_{TPSF}(x_n, t). 
$$

(3.5)

This relation can be easily confirmed by considering the relationship—shown in Eq. (3.4)—between the DC temporal Fourier component of each curve, which is equal to the total number of photons in that curve. Equation (3.5) shows that to recover the spatial variation of interest—contained in $\sum_{t=1}^{T} I_{TPSF}(x_n, t)$—we must correct for the effects of the IRF $\sum_{t=1}^{T} I_{IRF}(x_n, t)$ at each pixel. We also know that the measurement of the IRF must scaled by some unknown constant $C_1$ (as discussed in Sec. 3.2.2.1). Thus, we multiply Eq. (3.5) by $C_1$ and attempt to isolate $\sum_{t=1}^{T} I_{TPSF}(x_n, t)$:

$$
\frac{\sum_{t=1}^{T} I_{DTOF}(x_n, t)}{C_1 \sum_{t=1}^{T} I_{IRF}(x_n, t)} = \frac{\sum_{t=1}^{T} I_{TPSF}(x_n, t)}{C_1}.
$$

(3.6)

As mentioned, $C_1$ is unknown and cannot be directly separated from the image described by the right side of Eq. (3.6). However, we note that this image is just a scaled version of our desired spatial variation. Further, we know that we are interested in TPSF images which have been normalized by their total number of photons (see Sec. 3.2.1); in other words,

$$
\sum_{n=1}^{N} \sum_{t=1}^{T} I_{TPSF}(x_n, t) = 1
$$

where $N$ is the total number of pixels in the image. Thus, if we simply normalize the image on the right side of Eq. (3.6) by its total number of photons—

$$
\sum_{n=1}^{N} \sum_{t=1}^{T} I_{TPSF}(x_n, t)/C_1
$$

—we recover our spatial variation of interest:

$$
\frac{C_1 \sum_{t=1}^{T} I_{TPSF}(x_n, t)}{C_1 \sum_{n=1}^{N} \sum_{t=1}^{T} I_{TPSF}(x_n, t)} = \sum_{t=1}^{T} I_{TPSF}(x_n, t),
$$

(3.7)

Let $\sum_{t=1}^{T} I_{TPSF}(x_n, t)$ be a column vector with $N$ rows. Now, let $\Psi$ be a matrix of $N$ rows where each row contains the normalized temporal Fourier components of an image pixel (i.e., $F_t[TPSF]$). If we scale each row of $\Psi$ using its associated row in $\sum_{t=1}^{T} I_{TPSF}(x_n, t)$, we can
recover the two-dimensional Fourier transform of the normalized TPSF image $I_{TPSF}(x, t)$ as

\[
F[I_{TPSF}(x_n, t)] = F_x \left[ \Psi \odot \sum_{i=1}^{T} I_{TPSF}(x_n, t) \right],
\]

where $\odot$ denotes element-wise multiplication, $F_x$ is the one-dimensional spatial Fourier transform, and $F$ is the two-dimensional spatiotemporal Fourier transform. It is important to note that even though the result of Eq. (3.8) suggests that Fourier inversion may be used to recover the original normalized TPSF image, this is typically infeasible in an experimental setting due to the distortion of many higher-order Fourier components by experimental noise.

### 3.2.3 Statistical analysis

Following extraction of each feature’s spatial Fourier components from all the DTOF images, the ability of the components to track the expected variation between TPSF images was assessed. Specifically, we performed a correlation analysis between DI (see Sec. 3.2.1) and the components. Note that the magnitude and phase of the Fourier components were assessed separately and, to avoid duplicate results, symmetric Fourier components were removed prior to analysis.

In addition, we quantified the sensitivity of each component to individual physiological changes. For each set of virtual phantoms, phantoms were treated as the between-subjects variable and repetitions created by addition of Poisson noise (see Sec. 3.2.1) were treated as the within-subjects variable. Generalized eta squared ($\eta^2_G$)—a measure of effect size—was then computed using the values of each component within each phantom set [40].

### 3.3 Results

Recall that—as described in Sec. 3.2.1—virtual phantoms were organized into sets with the same baseline disease activity within which the value of only one physiological parameter was altered. Figure 3.2 quantifies the change in DI caused by these simulated physiological
changes under various baseline disease activity conditions. For example, the number in the 2nd row and 4th column shows, within a set of phantoms with Mild baseline disease activity, the average change in DI in response to a change in $\mu_{SF}$ disease activity. Note that changes in DI are presented on a z-score normalized scale for ease of interpretation. In 12 of the 20

![Heatmap of mean intra-set change in z-score normalized DI values for various physiological parameters (see Table 3.1) at different states of baseline disease activity. Sets in which DI values did not maintain a consistent direction of change were excluded from the analysis.](image)

sets of phantoms, changes in the physiological parameters were not associated with consistent changes in DI. In particular, changes in SO$_2$ and H$_2$O did not consistently affect DI regardless of the baseline disease activity. Overall, $G$ caused the largest decrease in DI, and this decrease became slightly larger as baseline disease activity worsened. Similarly, the changes caused by $\mu_{SF}$ became larger with worsening baseline disease activity; however, DI was not sensitive to changes in $\mu_{SF}$ when baseline disease activity was None. $BVF$ caused a relatively modest decrease in DI, but only at None baseline disease activity (1st row, 3rd column).
Figure 3.3 shows the results of the correlation analysis between the spatial Fourier components of the TPSF image features and their respective DI. Higher correlation coefficients correspond to spatial Fourier components that accurately tracked DI despite the addition of simulated noise and IRF to the original TPSF data from which the DI was derived. Note that images of \( F_i[TPSF] > 1.6 \text{ GHz} \) were excluded from Fig. 3.3 since these images did not have a single component with \( |R| > 0.5 \). All components derived from images of \( V \) also had low correlations (\( |R| < 0.5 \)) [41]. For \( m_1 \) images, the magnitude of the spatial DC and 0.06 mm\(^{-1}\) components had very high (\( |R| \geq 0.9 \)) and high (\( 0.7 \leq |R| < 0.9 \)) negative correlations, respectively [41], and the phase of their 0.06 mm\(^{-1}\) spatial frequency also had a high positive correlation.
correlation. Many $F_i[TPSF]$ image components were highly correlated with DI. Notably, for both magnitude and phase, the 0.06 mm$^{-1}$ spatial frequency of most $F_i[TPSF]$ images < 1.6 GHz had very high correlation coefficients. As well, the 0.13 mm$^{-1}$ magnitude and DC phase of many components was highly correlated with DI. Considered together, 64 components were strongly correlated with DI ($|R| > 0.8$) and 7 components had extremely high correlation coefficients ($|R| > 0.99$); this clearly suggests that it is possible to extract individual features from DTOF images which accurately track DI changes derived from TPSF images.

To further clarify the utility of various components in a clinical setting, we focused on the components’ ability to track individual physiological changes (see Sec. 3.2.3). In particular, we chose to focus on components’ ability to track changes in $\mu_S$ at a Mild baseline disease activity (Fig. 3.4): the sensitivity profile in Fig. 3.2 suggests that this is the smallest physiological change detectable with TD-DOI under this baseline disease activity (which is most likely to be clinically relevant) [42]. Figure 3.4 quantifies this ability using the effect size—measured as generalized eta squared ($\eta^2_G$)—of changes in $\mu_S$ on each component. For clarity, only $\eta^2_G \geq 0.26$ values—which typically correspond to a large effect size [43, 44]—are shown. Most notably, the phase of the $F_i[TPSF]$ 0.06 mm$^{-1}$ spatial Fourier component was associated with very large $\eta^2_G$; for both the 0.24 and 0.33 GHz $F_i[TPSF]$ images, it had the largest effect size ($\eta^2_G = 0.91$).

### 3.4 Discussion

Novel monitoring techniques that are sensitive to subtle, subclinical changes in RA disease activity could substantially reduce current delays in detecting RA treatment failure, and help ensure that patients reap the benefits of effective early treatment while avoiding irreversible joint damage. To this end, various DOI techniques have been proposed [15, 16, 17, 18, 19, 23]; however, these techniques have typically focused on making the binary distinction between healthy and inflamed joints. Preliminary sensitivity to subtler changes in RA disease activity
has been demonstrated—in virtual models—by using DOI approaches that capture additional information from the spectral [45, 24] or temporal domains [25, 26]. Building upon the latter approach, we have presented a systematic *in silico* investigation that highlights the potential of TD-DIO to track changes in RA disease activity. Starting with a realistic tissue geometry—based on an adult PIP joint—we individually simulated 5 major physiological changes associated with RA (Table 3.1) at 4 different levels of disease activity to create 80 virtual finger phantoms i.e., 80 states of disease activity. These phantoms were further grouped into sets of virtual phantoms, each containing 4 phantoms among which only one physiological parameter was changing.

Employing a DOI imaging geometry which was previously tested for early RA diagnosis [23], we simulated TD-DIO of each virtual phantom, and used PCA to compare the resulting images. PCA-1 accounted for 95% of the variance between the TD-DIO images of the 80 simulated states of disease activity, and its value consistently decreased as disease activity
worsened. As such, we selected this component as a “disease index” (DI). Then, we used it to estimate the sensitivity of TD-DOI to changes in each of the RA-associated physiological parameters by calculating the mean z-score normalized change in DI within each set of virtual phantoms (Fig. 3.2). This analysis revealed that TD-DOI was mostly sensitive to changes in joint volume, and had lower or negligible sensitivity to changes in other physiological parameters. Understandably, changes in [H\(_2\)O] only affected joint cartilage so—given the relatively small volume of the affected tissue—it is unsurprising that their impact was difficult to detect with TD-DOI. Physiological changes affecting the optical properties of the synovial membrane (SO\(_2\), BVF) and synovial fluid (\(\mu_{SF}\)) may have been obscured for similar reasons. Nevertheless, TD-DOI still demonstrated moderate sensitivity to changes in \(\mu_{SF}\); this was likely because the optical properties of the synovial fluid change more as a function of RA disease activity than those of the synovial membrane [46]. Though changes in SO\(_2\) were also simulated within the subcutis—which makes up much of the total finger volume—the low blood volume fraction in this tissue (0.8%) resulted in negligible changes to its optical properties as a function of SO\(_2\) [46]. In addition to the tissue-specific factors mentioned above, low sensitivity to SO\(_2\) could have been caused by the choice of an imaging wavelength (800 nm) close to the isosbestic point of hemoglobin. To test the generalizability of these findings to other wavelengths, a subset of simulations were repeated at 750 and 830 nm, to sample a wide range of the near-infrared region that is typically used for tissue optical imaging. Specifically, simulations were conducted with Mild baseline disease activity since it is most likely to represent the state of early diagnosis prior to treatment [42]. Using the same methodology as in Sec. 3.2.1, PCA analysis was conducted on the normalized TPSF images at these additional wavelengths; each wavelength’s respective DI explained 92% of the total variation in their datasets. We then performed a simple linear regression between their DI values and the ones derived from 800 nm data and found a strong linear relationship (\(R^2 = 0.99\)) in both cases with slopes of 0.99. Overall, this suggests that the aforementioned trends should not be strongly influenced by the choice of wavelength in the near-infrared.
Though the DI provides an excellent way to distinguish between the simulated images, it is an abstract metric that is challenging to interpret and difficult to generalize to TD-DOI images of different finger geometries or captured under different experimental conditions. Therefore, we focused on extracting more intuitive and generalizable TD-DOI features, and used the DI as a quantitative reference metric to assess their sensitivity to RA disease activity. Further, to confirm the future utility of these features in an experimental setting, we modified our TD-DOI images prior to feature extraction by adding Poisson noise and convolving each image with a randomly-scaled IRF from an in-house TD-DOI system. Given the important influence of an IRF on TD data [37, 38], we focused on extracting image features from which the effects of the IRF could be easily removed: statistical moments and temporal Fourier components (see Sec. 3.2.2.1). Motivated by previous work—which showed notable changes in the spatial distribution of image information in response to changes in RA disease activity [17, 23]—we also performed spatial Fourier decomposition on extracted TD-DOI features. Despite the addition of noise and the influence of an IRF, the spatial Fourier components of many extracted features remained highly correlated with DI (Fig. 3.3). Further, the 0.06 mm\(^{-1}\) phase of lower temporal frequency Fourier components (\(\leq 0.4\) GHz) was most sensitive to small, likely subclinical, physiological changes (Fig. 3.4).

Using CW-DOI, Lighter et al. found that changes in 0.05 mm\(^{-1}\) and 0.1 mm\(^{-1}\) spatial frequencies were the most accurate at detecting early inflammation, and that the length scales corresponding to these frequencies approximately matched the size of dips observed in the intensity profiles of inflamed PIP joints [23]. These findings are consistent with the results of our simulations: both the 0.06 mm\(^{-1}\) and 0.13 mm\(^{-1}\) spatial frequencies of many features were most sensitive to small changes in simulated RA disease activity (Fig. 3.4). The spatial frequency corresponding to the width of the joint area in the current work was 0.09 mm\(^{-1}\) which is between 0.06 mm\(^{-1}\) and 0.13 mm\(^{-1}\). As such, our results suggest that the sensitivity of a given spatial frequency component to RA disease activity is related to how closely it matches the width of the joint cavity. Further, it appears that we can expect changes in RA...
disease activity to produce changes across a band of spatial frequencies.

Overall, the TD-DOI features revealed that lower frequency temporal Fourier components had substantially higher sensitivity than any of the extracted statistical moments (Figs. 3.3 and 3.4). One potential explanation for this observation is the well-known, considerable sensitivity of statistical moments to noise [39]; this is especially true when calculating higher-order statistical moments, and is consistent with the poorer sensitivity of \( V \) to RA disease activity compared to \( m_1 \). Indeed, without the addition of Poisson noise, the 0.06 mm\(^{-1}\) and 0.13 mm\(^{-1}\) spatial frequencies of \( V \) were found to have a high correlation with DI and, in the case of \( m_1 \), these frequencies also became more strongly correlated than originally observed. As for the \( F_i[TPSF] \) features, Poisson noise is known to have a greater effect on higher frequency temporal Fourier components [47]; this effect can clearly be seen in the tendency of \( R \) and \( \eta_G^2 \) values to decrease with increased \( F_i[TPSF] \) frequency (Figs. 3.3 and 3.4).

It is important to note that temporal Fourier decomposition provides insight into the relative sensitivity that is expected when using other DOI modes of acquisition. Specifically, the DC temporal component of \( F_i[TPSF] \) (i.e., the total number of photons) is equivalent to a typical CW-DOI measurement, while the remaining \( F_i[TPSF] \) measurements are equivalent to FD-DOI measurements taken at various modulation frequencies. From this perspective, our results show that the sensitivity of both CW and FD to RA disease activity is enhanced when looking at changes in spatial frequency components aside from the DC spatial component. Nevertheless, the DC spatial components of some \( F_i[TPSF] \) frequencies still demonstrated relatively high correlations with DI (Fig. 3.3) and sensitivity to small DI changes (Fig. 3.4). FD instruments for diffuse optical applications typically operate in the 0.1 – 1 GHz range [48]; for small tissue volumes (e.g., human finger), numerical simulations have identified 0.4 GHz as an optimal modulation frequency [47]. Hielscher et al. acquired FD-DOT images of PIP joints at DC, 0.3 GHz, and 0.6 GHz, and found that DC images had considerably lower Youden indices (a statistical measure of diagnostic performance) than their counterparts [17]. A similar trend can be noted in the phase of various spatial frequencies in Fig. 3.4: the largest \( \eta_G^2 \) values generally
occurred at $F_l[TPSF]$ frequencies slightly greater than the DC frequency, though the exact frequency varies depending on the spatial frequency component under consideration.

Overall, the results of this study confirm the previously demonstrated benefits of utilizing spatial frequency analysis to enhance sensitivity to changes in RA disease activity [19, 23]. Further, we have observed that utilizing this analysis on low temporal frequency $F_l[TPSF]$ features extracted from TD-DOI images appears to yield the greatest sensitivity, and that this sensitivity is primarily related to increases in joint volume and changes in the optical properties of the synovial fluid. In particular, our results suggest that FD-DOI and TD-DOI may be capable of tracking the latter, likely subclinical, changes with greater sensitivity than previously tested CW-DOI [23]. Additionally, since TD-DOI is able to simultaneously assess changes across a band of $F_l[TPSF]$ frequencies, it may offer a more robust confirmation of potential changes in disease activity compared to a single-frequency FD system. Of course, we note that this same information could be obtained by using an FD-DOI system to acquire multiple images at several modulation frequencies.

While encouraging, we note that these results were obtained in silico using a finger model which may not fully capture the true tissue geometry and physiological changes present in an in vivo human finger. For simplicity, this model could not take into account localized changes in physiology and relied on assumptions, sourced from the literature, to define average changes to tissues as a function of simulated RA disease activity. Further, we limited our work to studying the individual effects of only 5 RA-associated parameters whose effects were limited to an area encompassing the PIP joint. As a result of these simplifications, we anticipate that the high sensitivity of the TD-DOI features presented herein to RA disease activity may be more pronounced than they would be in an in vivo setting. Future work will aim to experimentally validate these findings in both solid tissue-mimicking phantoms and humans using an in-house TD-DOI system.
3.5 Conclusion

This work presents a systematic *in silico* study that highlights the potential of TD-DOI to track changes in RA disease activity. Specifically, we investigated TD-DOI’s sensitivity to 5 RA-associated physiological changes under 4 baseline disease activities. In total, TD-DOI images of 80 disease activity states—each modelled using a virtual finger phantom based on an adult PIP joint—were compared both with and without the addition of Poisson noise and the effects of an IRF. A disease index (DI) based on the variance of the former images revealed that TD-DOI is primarily sensitive to changes in joint volume, but also has sensitivity to changes in the optical properties of the synovial fluid. In contrast, low to negligible sensitivity was observed to changes in oxygen saturation, blood volume fraction, and water concentration within the joint. Furthermore, many spatial Fourier components of features extracted from TD-DOI images which incorporated the influence of Poisson noise and the IRF remained highly correlated with DI. In particular, the phase of the 0.06 mm\(^{-1}\) spatial frequency of TD-DOI images’ low frequency temporal Fourier components (\(\leq 0.4\) GHz) was both strongly correlated with DI and highly sensitive to small physiological changes.

3.6 Acknowledgments

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Chapter 4

Time-domain diffuse optical imaging technique for monitoring rheumatoid arthritis disease activity: experimental validation in tissue-mimicking finger phantoms

This chapter was adapted from work in preparation for submission by Seva Ioussoufovitch and Mamadou Diop.

4.1 Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune condition which has been linked to substantial reductions in overall quality of life including fatigue, depression, and pain [1, 2]. Despite the lack of a cure, patient prognosis significantly improves if treatment with disease-modifying anti-rheumatic drugs is initiated early (i.e., within 3 months of symptoms) [3, 4, 5]. Nevertheless, at least 1 in 3 RA patients are still expected to fail their first treatment, and current monitoring methods—based on clinical examination and patient self-assessment—typically require 3–6 months to detect this failure [6, 7]. These patients risk developing irreversible joint damage while simultaneously losing the benefits of early treatment [8] and, as such, there is a need for reliable, quantitative monitoring tools that can detect subclinical indications of treatment response [9, 10]. Nevertheless, ultrasonography and magnetic resonance
imaging—which are the most commonly discussed techniques for this purpose—suffer from suboptimal reproducibility and high cost, respectively [11, 12, 13].

Diffuse optical imaging (DOI) is an alternative approach that is sensitive to and provides objective measures of RA disease activity at a relatively low cost. Previous work was able to track joint inflammation changes with an error rate of 14% using a combination of single-wavelength continuous-wave (CW) DOI images and joint circumference measurements [14, 15]. A variety of alternative DOI implementations have subsequently been used to further improve DOI sensitivity and specificity to joint inflammation. Some implementations have focused on using CW-DOI assisted by other techniques (e.g., contrast enhancement [16], cuff occlusion [17]), while others used a tomographic approach (i.e., diffuse optical tomography; DOT) [18]. Alternative modes of acquisition have also been tested: heuristics extracted from the spatial distribution of images captured with frequency-domain (FD) DOT [19] were paired with machine learning image classification to obtain sensitivities and specificities between 93.8% and 100% [20, 21]. Despite this success, DOT approaches for assessment of joint inflammation have become less popular; this is due in part to their longer examination times (particularly for multi-joint evaluation) as well as increased costs compared to typical DOI. Instead, more recent works have focused on other DOI approaches. For example, Lighter et al. previously applied a spatial Fourier transform analysis to multi-spectral CW-DOI data to distinguish between inflamed and healthy joints [22]. Further, Dolenec et al. distinguished between several virtual states of joint disease activity using the additional information provided by hyperspectral CW-DOI [23]. This latter work is particularly relevant to RA treatment monitoring, since greater sensitivity to subtle changes in disease activity may allow earlier detection of treatment failure. Similarly, we have investigated how the additional information provided by the time-domain (TD) mode of acquisition can be used to distinguish between multiple simulated RA disease activity states [24, 25] (Chapter 3).

Compared to CW methods—which measure the attenuation of light intensity—and FD methods—which measure both the light attenuation and its phase shift at a specific frequency—
TD techniques measure the number and travel time of detected photons. The resulting distribution of times-of-flight of photons (DTOF) contains more information than is captured using the simpler CW and FD modes of acquisition, and this information may be leveraged to provide greater insight into the physiological state of the tissue being imaged.

We recently demonstrated that spatiotemporal Fourier components extracted from simulated TD-DOI data—which was obtained from a realistic tissue distribution—are sensitive to hallmarks of RA disease activity such as changes in joint volume and optical properties. In particular, unlike the swelling caused by joint volume changes, changes in joint optical properties are not directly measured in the clinic, and could potentially be an important subclinical biomarker. While our previous in silico investigation showed promising results, it is unclear whether these results can be replicated under experimental conditions. In addition, a key advantage of the component extraction method was its theoretical ability to account for the effects of the TD-DOI system (i.e., instrument response function; IRF); this remains to be tested using a real-world TD-DOI device.

Motivated by the findings of our in silico investigation of TD-DOI sensitivity to RA disease activity, this work presents the development of an in-house TD-DOI system, and demonstrates its ability to track changes in disease-mimicking solid phantoms independently of changes to the system’s IRF. Specifically, we assessed whether changes in the spatiotemporal Fourier components of experimental, intensity-normalized, transverse-averaged dorsal optical transmission measurements could be used to distinguish between hemi-cylindrical phantoms that mimic various states of RA disease activity in a proximal interphalangeal (PIP) joint.

### 4.2 Methods

#### 4.2.1 System design

A schematic of the TD-DOI system is shown in Figure 4.1. Note that the system uses a single-pixel camera detection scheme (see Sec. 4.2.2) to help avoid the typically high cost of a spatial
array of time-resolved detectors. The output of a pulsed diode laser (LDH-P-C-810; Pico-Quant, Germany) emitting at 805 nm is attenuated by a variable neutral density filter (NDF; NDC-50C-4M-B, Thorlabs Inc., NJ) and transmitted into a microscope objective (RMS10X, Thorlabs Inc.) that couples it into a multimode fiber (P400-2-VIS-NIR, Ocean Optics Inc., FL). Light from the fiber is sent to a digital micromirror device (DMD; AJD-4500, Ajile Light Industries, Canada) whose micromirrors reflect individual segments of light towards or away from the system’s imaging platform. Thus, it is possible to ensure that emission light only travels through a region of interest (ROI) located on the object being imaged, and avoids detector saturation due to light travelling through empty areas on the imaging stage. Before reaching the object on the imaging platform, light reflected by the DMD passes through a black foamboard screen which blocks any unwanted diffraction orders [26]. Light which is diffusely transmitted through the object on the platform is collected by a series of lenses (2 × FRP232, ACL50832UB, FRP232, 2 × FRP125, Thorlabs Inc.), focused into a homogenizing rod (63-089, Edmund Optics, NJ), and passed into a light pipe. Finally, the collected light is sent into a photomultiplier tube (H7422P-50, active area $\varnothing = 5$ mm, Hamamatsu Photonics, Japan) coupled to a time-correlated single-photon counting (TCSPC) module (SPC-130; Becker & Hickl GmbH, Germany).

### 4.2.2 Data acquisition

Single-pixel imaging is based on using a sequence of spatially resolved patterns to measure a series of light intensity correlations between each pattern and an object of interest [27]. This type of imaging is within the same class of multiplexing as raster scanning; however, under the right conditions, it can offer improved acquisition times and reduce noise [28]. The configuration in Fig. 4.1 is known as structured illumination: the object is illuminated with an $N$-sized stack of patterns $\Phi$ and a corresponding $N$-sized stack of measurements $Y$ is detected. Since, for each projected pattern, the system measures a distribution of times-of-flight of photons (DTOF) instead of a simple light intensity measurement, $Y$ can be represented as a matrix.
Figure 4.1: Schematic of the TD-DOI system used in the study. Light from a pulsed near-infrared laser is attenuated by a neutral density filter (NDF) and coupled into an emission fiber using a microscope objective (L1). Light from the emission fiber is then projected by a digital micro-mirror device (DMD) onto an object on the imaging platform, before being collected by a series of lenses (L2 – L8), and focused through a homogenizing rod onto a photomultiplier tube (PMT) coupled to a TCSPC module.
whose rows contain the measured DTOFs (one DTOF for each displayed pattern per row). Thus, if we choose our sampling patterns from a known basis and represent each 2D projected pattern as a vector with $N$ elements (resulting in $\Phi_{N \times N}$), we can recover the time-resolved distribution of light $\hat{I}$ transmitted through our object:

$$\hat{I}(x, y, t) = \Phi^{-1} \mathbf{y}$$ (4.1)

In this study, we used Hadamard basis patterns ranked from lowest to highest spatial frequency; when used to reconstruct spatially-varying DTOF data, these have previously been shown to match or outperform other basis choices [29]. In addition, we relied on the commonly used approach of differential Hadamard single-pixel sampling [30, 31] to physically implement pattern projection on the DMD and increase imaging noise suppression [32, 30]. Unless noted otherwise, a 1 s collection time was used for each pattern’s DTOF.

With the above in mind, we note that the spatial extent of our reconstructed image—i.e., the system’s field-of-view (FOV)—is the same as the size of the patterns projected by the system’s DMD. For clarity, we will hereafter denote the system FOV for each acquisition as “$(S_x \times S_y; N_x \times N_y)$” where $S_{x,y}$ and $N_{x,y}$ correspond to the physical size and number of elements of a projected pattern, respectively, along the corresponding axis (Fig. 4.1).

### 4.2.3 Liquid phantoms

To validate the TD-DOI system’s ability to accurately measure DTOFs in a turbid medium, we compared measurements acquired with the device in liquid homogeneous tissue-mimicking phantoms to measurements obtained using standard time-resolved reflectance near-infrared spectroscopy (TR-NIRS).

An opaque PVC container ($20 \times 20 \times 10$ cm; $L \times W \times H$) with a plexiglass bottom (2 mm thickness) was used to contain the liquid phantoms. The scattering and absorbing compounds used to tune the phantoms’ optical properties were Intralipid–20%® (Fresenius Kabi
AG., Germany) and India ink, respectively. The container was filled with 400 mL of diluted 0.8% Intralipid (IL) solution—resulting in a phantom thickness of 1 cm—and a baseline image \((7 \times 8 \text{ cm}; 16 \times 16)\) was acquired with the TD-DOI system. Following the baseline measurement, 0.2 mL increments of a 1 : 500 India ink dilution were added to the phantom. After each addition, the solution was magnetically stirred for 60 s, allowed to settle for 60 s, and another image was acquired; this process was repeated for a total of 10 India ink additions. An instrument response function (IRF) image was then acquired by imaging a white piece of paper placed on the bottom of the emptied phantom container.

To obtain TR-NIRS measurements, the system’s emission fiber (Fig. 4.1) was placed into a 3D-printed probe holder suspended above the phantom using a Manfrotto articulated arm (Vitec Imaging Solutions, Italy). A detection fiber was then added to the probe holder—at a 21 mm separation from the emission fiber—and coupled directly into the TD-DOI system’s light pipe. The phantom container was filled with 800 mL of 0.8% Intralipid solution and the probe tips were positioned on the solution’s surface. The previous experiment was then repeated using 0.4 mL increments of the same India ink dilution. Following the phantom experiment, an IRF was acquired by placing the emission and detection probes on either side of a box containing a piece of white paper to diffuse light. For each acquisition in the TR-NIRS configuration, 128 DTOFs were measured with a collection time of 300 ms per DTOF.

### 4.2.4 Simulations

Prior to solid phantom fabrication, we created virtual references of our planned solid phantoms and conducted Monte Carlo simulations to establish a reference for the anticipated differences between their TD-DOI images. Previous work which modelled RA disease activity as a function of five tissue-specific physiological parameters—joint volume, synovial fluid optical properties, oxygen saturation, blood volume fraction, and water concentration—was used as a reference for virtual phantom geometry and optical properties (Chapter 3). Note that each of the physiological parameters could be assigned to 1 of 4 RA disease activity values defined in
previous work: “None”, “Mild”, “Moderate”, and “Severe”.

Surface models of the virtual phantoms were created using the free open-source 3D modeling suite Blender [33]. Figure 4.2 shows a wireframe of the None surface model which mimics a finger with a healthy PIP joint. Note that the model is composed of 3 parts: a hemi-cylindrical outer shell which contains a hemi-cylindrical inclusion, and a rectangular base. The inclusion mimics the joint cavity whose volume is expected to change as a function of RA disease activity; thus, surface models of states with worsening RA disease activity (i.e., Mild, Moderate, Severe) have larger inclusion dimensions compared to the None model (Fig. 4.2). Inclusion dimensions were derived from the geometries of RA disease activity of virtual finger models.
Specifically, the volume of each reference phantom’s inclusion was tuned to approximately match the summed volume of the synovial fluid and synovial membrane in its virtual finger counterpart.

Virtual phantoms were created by pairing a surface model—which represents a state of disease activity for “joint volume”—with tissue-specific optical properties derived by setting all other physiological parameters to 1 of 4 disease activity states. The optical properties of the inclusion were set to the volume-weighted average of synovial membrane and synovial fluid optical properties, while the rest of the phantom’s properties were set to those of subcutis tissue [24] (Appendix A). The 4 possible absorption $\mu_a$ and reduced scattering coefficients $\mu'_s$ for the phantoms’ inclusions are shown in Table 4.1.

Table 4.1: Optical properties of the virtual phantom inclusions (mm$^{-1}$) for 4 simulated RA disease activity states

<table>
<thead>
<tr>
<th>Disease activity</th>
<th>$\mu_a$</th>
<th>$\mu'_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.0088</td>
<td>0.2920</td>
</tr>
<tr>
<td>Mild</td>
<td>0.0142</td>
<td>0.4552</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.0170</td>
<td>0.5426</td>
</tr>
<tr>
<td>Severe</td>
<td>0.0189</td>
<td>0.5920</td>
</tr>
</tbody>
</table>

While we expected TD-DOI to be sensitive to changes in model geometry, we also wanted to confirm its sensitivity to geometry-independent optical property changes; these are more representative of subclinical disease activity which may typically be missed in the clinic. As such, two sets of virtual reference phantoms were created. The first set of 4 phantoms was created by matching surface model and optical property disease activities (e.g., None surface model with None inclusion optical properties) and iterating over the four possible disease activity states. The second set of 4 phantoms was created by fixing the surface model to a Mild value and pairing it with the 4 different sets of optical properties.

MMCLAB was used to simulate TD-DOI of the phantoms [34]. All simulations were conducted at the source wavelength of the TD-DOI system (Fig. 4.1). First, the Blender add-on “BlenderPhotonics” was used to generate a tetrahedral volume mesh of each virtual phantom’s surface model [35]. Each mesh was generated using a maximum tetrahedron volume of
100 mm$^3$ and inspected to ensure no air gaps were present between the 3 parts of the phantom. Next, a 4×8 grid of light source positions was defined on the top of each phantom over an area ($\approx 12 \times 24$ mm$^2$; $L \times W$) that encompassed, and was centered around, the inclusion. The joint was then raster scanned to create an image: $10^6$ photons were simulated for each source position, and the transmittance of photons exiting through the bottom of the phantom’s rectangular base was recorded. Simulated TD-DOI data was organized as a 3D data structure with spatial information on the x-y plane and an array of temporal point-spread functions (TPSFs) on the z-axis i.e., a TPSF image with one TPSF per image pixel. Similar to our previous work, each TPSF image $I_{TPSF}$ was averaged in the transverse direction, and then each image pixel was convolved with an IRF $I_{IRF}$ image pixel in order to create a DTOF image $I_{DTOF}(x_n, t)$ (Chapter 3):

$$I_{DTOF}(x_n, t) = I_{IRF}(x_n, t) \ast I_{TPSF}(x_n, t)$$ \hfill (4.2)

where each image has a spatiotemporal dependence $(x, t)$ and $n$ is the index of each discrete image pixel. For this purpose, four different IRFs were acquired by imaging a white piece of paper with the TD-DOI system: IRFs 1 – 3 were acquired under the same conditions but with different temporal offsets while IRF 4 was acquired using an additional piece of paper to induce an additional temporal shift and broadening (Fig. 4.3). The TPSF image acquired from each phantom was convolved with each one of the four different IRFs to create four different DTOF images per phantom. Following the creation of each DTOF, simulated Poisson noise was added to the DTOF using the `imnoise` function in MATLAB 2020b (The MathWorks Inc., USA).

### 4.2.5 Solid phantoms

Solid phantoms matching the virtual phantoms described in Sec. 4.2.4 were created using a modified version of a previously published protocol [36] which itself was a modified version of the protocol published by Sekar et al. [37]. Phantoms were made of transparent silicone...
rubber (Parts A and B of PlatSil® SiliGlass, Polytek, PA) mixed with various amounts of an absorber (Silc Pig Black Silicone Pigment, Sculpture Supply Canada, Canada) and a scatterer (Glass spheres, 440345, Sigma-Aldrich, MO). The black silicone pigment used in this work was sourced from a different manufacturer than the pigment in the previous protocol [36], and was found to be less absorptive than expected; thus, we used an initial pigment dilution ratio of 1 : 568 as opposed to 1 : 2272. Additionally, we increased the mass of Part B by 3.0 g to account for material left on container walls. Since the additional degassing step prior to mixing recommend by Naglic et al. [36] was found to have a negligible impact, phantom constituents were mixed together immediately after the protocol’s first vacuum step as originally described by Sekar et al. [37]. Finally, our last vacuum step was reduced from 8 min to 3 min; we found that this time was sufficient to remove all gas bubbles in the mixture while helping avoid accidental polymerization.

Since some of our optical properties of interest (Table 4.1) fell outside of the previously published linearity range for the phantom constituents ($\mu_a$: 0.01 – 0.1 mm$^{-1}$; $\mu_s'$: 0.5 – 2.5 mm$^{-1}$) [37] and the constituents are likely to have non-negligible inter-batch variability [36],
we created a series of reference phantoms to characterize the relationship between constituent concentrations and expected optical properties. Reference phantom creation and optical property estimation are detailed in Appendix B. Ultimately, the linear relationships between constituent concentrations and optical properties (Fig. B.1) were used to identify which absorber and scatterer concentrations should be used for solid phantom creation.

Figure 4.4 shows a solid phantom of the None surface model described in Sec. 4.2.4 along with dimensions; in addition, solid phantoms of the other surface models, along with the dimensions of their inclusions, are shown. In total, 2 sets of 4 solid phantoms were created to match the 2 sets of 4 virtual phantoms described in Sec. 4.2.4. An image (15.5 × 28.4 mm$^2$; 4 × 8) of each solid phantom was then acquired using each of the 4 TD-DOI system configurations used to acquire the four different IRFs in Sec. 4.2.4; thus, each solid phantom was imaged 4 times. Images were centered on the location of the inclusion and, for each system

<table>
<thead>
<tr>
<th>Inclusion Dimensions</th>
<th>Height (mm)</th>
<th>Width (mm)</th>
<th>Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.8</td>
<td>10.2</td>
<td>7.9</td>
</tr>
<tr>
<td>Mild</td>
<td>6.2</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Moderate</td>
<td>7.9</td>
<td>15.6</td>
<td>12.1</td>
</tr>
<tr>
<td>Severe</td>
<td>8.3</td>
<td>17.4</td>
<td>13.2</td>
</tr>
</tbody>
</table>
configuration, phantoms were imaged in a random order. All images were then averaged in the phantoms’ transverse direction to match the DTOF images simulated in Sec. 4.2.4.

4.2.6 Data analysis

4.2.6.1 Liquid phantoms

For TR-NIRS measurements, DTOFs obtained over an acquisition were averaged to obtain a single DTOF with improved signal-to-noise ratio (SNR). Liquid phantom optical properties were then estimated using the diffusion approximation [38]. First, the averaged DTOF from the baseline phantom acquisition was fit with a theoretical DTOF produced by convolving the model solution with the system’s IRF. The fitting range was set to 80% and 1% of the peak value on the leading and falling edges, respectively [39], and the DTOF was fit for $\mu_a$, $\mu'_s$, and an amplitude term. DTOFs from all subsequent acquisitions were then fit in the same way except that $\mu'_s$ was fixed to the value estimated from baseline. Changes in absorption due to India ink addition (i.e., $\Delta\mu_a$) were calculated by subtracting estimated $\mu_a$ values from the $\mu_a$ obtained at baseline.

For TD-DOI acquisitions, changes in phantom absorption were estimated using the modified Beer-Lambert Law,

$$\Delta\mu_{a,j} = \ln\left(\frac{I_j}{I_{IL}}\right) \div p_j$$

(4.3)

where $I_{IL}$ is the light intensity measured at baseline, while $I_j$, $\Delta\mu_{a,j}$ and $p_j$ are the detected light intensity, absorption change, and phantom optical pathlength measured for the $j$th India ink addition. Note that $p_j$ was determined by multiplying the difference in mean photon time-of-flight $\langle t \rangle$ between the DTOFs of the $j$th measurement and the system’s IRF by the ratio of the speed of light in vacuum $c$ to the refractive index of tissue ($n = 1.4$):

$$p_j = \frac{c}{n} (\langle t \rangle_{DTOF,j} - \langle t \rangle_{IRF}).$$

(4.4)

The above analysis was applied on a pixel-by-pixel basis to each TD-DOI image so that a
A value was obtained at each image pixel for each India ink addition. Agreement between \( \Delta \mu_a \) measured using TR-NIRS and the mean \( \Delta \mu_a \) of TD-DOI images was investigated using linear regression. Prior to analysis, regression assumptions of linearity and homoscedasticity were confirmed.

### 4.2.6.2 Solid phantoms and simulations

Prior to further analysis, DTOF images were normalized by their sum of photons to ensure results were independent of incident light intensity. Spatiotemporal Fourier component extraction—which has been described in detail in our previous work (Chapter 3)—was then applied to both the DTOF images from virtual (Sec. 4.2.4) and solid phantoms (Sec. 4.2.5). A two-way repeated measures analysis of variance (ANOVA) without replication was applied to each of the spatiotemporal Fourier components acquired from each set of 4 phantoms. For this analysis, system configuration was treated as the within-subjects variable, phantoms as the between-subjects variable, and we assumed that no interaction was present between the two variables. For each spatiotemporal component, further analysis was only performed in the presence of a significant between-subjects effect \( (p < 0.05) \) and no significant within-subjects effect. If this was the case, the effect size measure generalized eta squared \( (\eta^2_G) \) was calculated for that component [40].

### 4.3 Results

Figure 4.5 compares \( \Delta \mu_a \) values of a liquid tissue-mimicking phantom as measured using “gold-standard” TR-NIRS and using the TD-DOI system. A simple linear regression revealed a strong relationship \( (R^2 = 0.99) \) between both sets of values with a linear relationship that had a slope of 1.06 with a y-intercept \( \approx -0.003 \). This indicated that—while \( \Delta \mu_a \) measurements from both systems agreed strongly in general—the TD-DOI system tended to slightly underestimate \( \Delta \mu_a \). Nevertheless, these results still suggest that the TD-DOI system can measure
Figure 4.5: Comparison of $\Delta \mu_a$ measured in a homogeneous tissue-mimicking solution (0.8% Intralipid) using time-resolved near-infrared spectroscopy (TR-NIRS) and the TD-DOI system; absorption changes were caused by incremental addition of India ink. For TD-DOI system measurements, mean image values are shown along with errorbars which represent the standard deviation (SD) of $\Delta \mu_a$ within each image.

DTOFs within a turbid medium with reasonable accuracy.

Figures 4.6 and 4.7 show the generalized eta squared ($\eta^2_G$) values from the simulation and solid phantom experiments, respectively. For simplicity, only $\eta^2_G \geq 0.26$ values—typically corresponding to a large effect size [41, 42]—are shown. For both sets of virtual phantoms, there was a multitude of spatiotemporal Fourier components capable of distinguishing between phantoms with high sensitivity. For the first set of phantoms, wherein both phantom geometry and optical properties were manipulated, the magnitude and phase of the $0.04 \text{ mm}^{-1}$ spatial Fourier component tended to have the largest $\eta^2_G$ values (Fig. 4.6A). In the second set of phantoms, wherein the phantom geometry was set to Mild and only optical properties were manipulated, the magnitude of the $0.04 \text{ mm}^{-1}$ spatial Fourier component also tended to have the largest $\eta^2_G$ values; however, the $0.04 \text{ mm}^{-1}$ phase appeared less sensitive (Fig. 4.6B). In addition, DC spatial phase and the $0.07 \text{ mm}^{-1}$ spatial magnitude of many Fourier components could also distinguish between both sets of phantoms with relatively high sensitivity.
Figure 4.6: Heatmap of generalized eta squared ($\eta^2_G$) for spatiotemporal Fourier components extracted from normalized DTOF images of two sets of virtual phantoms. (A) shows values for phantoms with geometries ranging from None to Severe while (B) shows values for phantoms with a Mild geometry; in both cases, phantom optical properties were varied from None to Severe RA disease activity (see Sec. 4.2.4 for more details). Only components with $\eta^2_G \geq 0.26$ are shown.
Figure 4.7: Heatmap of generalized eta squared ($\eta^2_G$) for spatiotemporal Fourier components extracted from normalized DTOF images of two sets of solid phantoms which match the sets of virtual phantoms shown in Fig. 4.6. Only components with $\eta^2_G \geq 0.26$ are shown.
On average, there was a reduction in component sensitivity to differences between solid phantoms compared to their virtual references (Fig. 4.7). Nevertheless, for the first and second set of phantoms, the 0.04 mm\(^{-1}\) phase and magnitude of many temporal components appeared to retain most of their sensitivity, respectively. Interestingly, for the first set of phantoms, the DC magnitude of multiple Fourier components actually appeared more sensitive to differences between phantoms than expected (Fig. 4.7A).

Figure 4.8 highlights which components had a large effect size ($\eta^2_G > 0.26$) in all phantom sets. Aside from three exceptions, only the 0.04 mm\(^{-1}\) spatial frequency of many components was sensitive to the differences between all 4 phantom sets.

Figure 4.8: Binary heatmap highlighting spatiotemporal Fourier components with $\eta^2_G \geq 0.26$ across all 4 tested phantom sets.

4.4 Discussion

We have presented a TD-DOI system based on a single-pixel camera architecture, and shown that this system is capable of clearly distinguishing between solid phantoms which mimicked
a range of RA disease activity states. Previous work which used DOI to measure RA disease activity has primarily focused on classifying individual joints as either healthy or inflamed for the purposes of RA diagnosis [14, 15, 19, 20, 21, 17, 22]; however, RA treatment monitoring would obviously benefit from the ability to track subtler changes in joint disease activity. Clinical treat-to-target approaches rely on the detection of treatment response or failure as a signal for adjusting each patient’s therapy. Thus, through trial and error, treatment adjustment is iterated until an effective therapy is found for each individual patient. Higher sensitivity to subtle, subclinical changes in disease activity should reduce the time needed to identify treatment failure, and thereby reduce the risk of patients missing the benefits of early treatment and potentially incurring irreversible joint damage.

Sensitivity to differences between a few RA disease activity states has previously been demonstrated in silico using hyperspectral CW-DOI [23]. Additionally, Van Onna et al. previously found modest correlations between indices derived from CW-DOI measurements, results of clinical examinations, and synovitis graded using US and MRI [17]. Lighter et al. also found low correlations between CW-DOI measurements and US-graded synovitis [22]. Nevertheless, our previous in silico work suggested that spatiotemporal Fourier components extracted from TD-DOI images should have greater sensitivity to changes in RA disease activity than CW-DOI alone (Chapter 3). Thus, we sought to investigate whether results obtained using an experimental TD-DOI system also demonstrated greater sensitivity to RA disease activity in disease-mimicking solid phantoms.

First, we conducted a preliminary validation of the reported TD-DOI system by comparing its ability to quantify changes in $\mu_a$ to gold-standard TR-NIRS. Even though different types of data analyses were used to determine $\Delta \mu_a$ in liquid tissue-mimicking phantoms, we found very strong agreement between both methods as well as a relatively low standard deviation over the TD-DOI system’s FOV (Fig. 4.5).

Previous DOI-based approaches have used the relative contrast between the joint cavity and its surrounding tissue as a metric of inflammation [19, 22]. Since constructing a phantom
which accurately mimics the complex geometry of a human finger is highly challenging, we designed a simplified model focused on simulating this type of contrast. The resulting phantom was composed of an inclusion—representing the joint—which was embedded in a shell that represented the tissue surrounding the joint. Though TD-DOI is highly sensitive to changes in tissue geometry associated with worsening RA disease activity, we also previously noted its sensitivity to geometry-independent optical property changes (Chapter 3). Notably, sensitivity to these geometry-independent changes is of particular interest since they represent the type of—likely subclinical—change which may be missed during a typical clinical examination. Thus, we created two sets of phantoms: one in which both the geometry and optical properties of each phantom were varied, and one in which only the optical properties were varied.

To establish expectations for our experimental measurements, TD-DOI was first simulated on virtual references of the planned solid phantoms (Fig. 4.2). Further, to better mimic a realistic experimental acquisition, all simulated data was convolved with one of four experimentally acquired IRFs (Fig. 4.3) prior to data analysis. The sensitivity profile of extracted spatiotemporal Fourier components was noticeably different between both sets of phantoms (Fig. 4.6). Nevertheless, the 0.04 mm$^{-1}$ and 0.07 mm$^{-1}$ spatial frequency bands were generally the most sensitive to differences between phantoms.

Lighter et al. previously found that magnitude changes at 0.05 mm$^{-1}$ and 0.1 mm$^{-1}$ were relatively accurate indicators of early inflammation in the joints of RA patients [22]. Similarly, we previously found that—across a range of temporal frequencies—both magnitude and phase changes at 0.06 mm$^{-1}$ were highly correlated with worsening RA disease activity. In both cases, the spatial frequencies most affected by disease activity changes were either similar to or larger than the length scales matching the size of the joint area. In the present work, the length of the joint cavity was varied between 8–13mm which corresponds to a spatial frequency range of 0.08–0.13 mm$^{-1}$; these frequencies are about twice as large as those found to be the most sensitive to the differences between the virtual references (Fig. 4.6). In other words, differences between phantoms were more evident when looking at changes in spatial frequencies whose
length scales exceeded the size of the phantom inclusion. This observation is in alignment with the expectation that differences between phantoms are more obvious when comparing light transmittance through both the joint cavity and its surrounding tissue as opposed to relying on changes in the joint cavity alone.

Using a modified version of a previously published protocol [37], we created solid phantoms which matched the dimensions and optical properties of the virtual phantoms (Fig. 4.4). The optical properties necessary for our phantom were outside of the linearity range previously tested in the publication that reported the protocol; further, others have noted substantial batch-to-batch variation in the constituents typically used for these phantoms [36]. As such, we created a series of reference disks and characterized their properties using gold-standard TR-NIRS in order to extend the published linearity range of the phantom constituents beyond previous work, and identify the constituent concentrations necessary for our phantoms (see Appendix B).

Despite the use of four different IRFs during image acquisition, multiple spatiotemporal components derived from TD-DOI images of the solid phantoms could still be used to clearly differentiate between phantoms. Nevertheless, on average, there was a reduction in component sensitivity compared to the virtual references (Fig. 4.7). The majority of components which showed sensitivity to differences in both the virtual references and solid phantoms remained associated with the 0.04 mm\(^{-1}\) spatial frequency (Fig. 4.8). Interestingly, at this spatial frequency, phase changes in the 0.4–1.0 GHz range were more sensitive to the solid phantom set which incorporated geometry changes while magnitude changes across a broad range of temporal frequencies were more sensitive to changes in optical properties alone.

Overall, images captured by the presented TD-DOI system could be used to differentiate between solid phantoms mimicking different states of RA disease activity. As predicted with virtual references of the solid phantoms, spatiotemporal Fourier components associated with the 0.04 mm\(^{-1}\) spatial frequency demonstrated the most consistent sensitivity to differences between phantom sets. Thus, the results of this study confirm that data captured with the
presented TD-DOI system can be combined with spatiotemporal Fourier component analysis (Chapter 3) to identify simulated changes in RA disease activity even when the system’s IRF is altered. While there was an overall reduction in sensitivity when comparing results from simulations and experimental work, the system was capable of capturing expected differences in the 0.04 mm\(^{-1}\) spatial frequency band. Nevertheless, we note that the simplified geometry of the tested phantoms is not truly representative of the complex changes in tissue geometry and physiological changes expected to be present in an \textit{in vivo} human finger. Future work will aim to test the TD-DOI system’s ability to track longitudinal changes in RA disease activity within a patient cohort and correlate these differences with other methods of monitoring RA (e.g., clinical examination, US, MRI).

4.5 Acknowledgments

This work was supported by a Discovery Grant (RGPIN-2023-05561) from the Natural Sciences and Engineering Research Council (NSERC), as well as start-up funds from Western University’s Schulich School of Medicine and Dentistry and the Lawson Health Research Institute. Seva Ioussoufovitch is supported by an NSERC CGS-D and the Collaborative Program in Musculoskeletal Health Research at Western University. The authors thank Dr. Lawrence Yip for his significant technical contribution and support in the development of the TD-DOI system used for this project.
Bibliography


Chapter 5

Conclusion and Future Directions

As detailed in Sec. 1.3, the goal of my doctoral research was to assess the prospects of using TD-based diffuse optical methods to monitor RA under a range of disease activities. This goal was achieved through several objectives:

1. Assess the sensitivity of joint BF, as measured with DCE TD-NIRS, to disease activity and DMARD treatment in a rat model of chronic RA

2. Investigate the sensitivity of TD-DOI to differences between a wide range of RA disease activity states \textit{in silico}

3. Test the sensitivity of an experimental TD-DOI system to differences between solid phantoms of multiple RA disease activity states

Chapter 2 presented a longitudinal study of joint BF changes in a rat model of chronic RA. DCE-NIRS was used to monitor ankle joint BF every 5 days for up to 40 days in rats with adjuvant-induced arthritis and healthy controls. After 20 days of disease progression, rats with arthritis were treated with a DMARD once every 5 days for an additional 20 days. There was a notable difference in average joint BF between the experimental and control group 5 days following disease induction; however, this difference was not statistically significant. Further, comparison of individual BF measures between rats did not reveal a clear response to DMARD treatment. A key limitation of this study was the heterogeneous disease response of the rats in the experimental group: examination of individual joint BF time courses clearly revealed large differences in joint BF changes between individual animals. Ultimately, we concluded
that joint BF time courses could not be used to reliably distinguish between rats with induced arthritis and controls.

Chapter 3 presented an *in silico* investigation focused on characterizing the ability of TD-DOI to distinguish between many different states of RA disease activity. Using several realistic tissue geometries as well as tissue-specific optical properties derived from the literature (Appendix A), we simulated TD-DOI of 80 virtual finger phantoms which encompassed changes in 5 RA-associated physiological parameters across 4 levels of disease activity. First, a disease index—which accounted for 95% of the variability between the TD-DOI images—was derived. Second, after adding Poisson noise and convolving the images with a randomly-scaled experimental IRF, we presented a method of extracting spatiotemporal Fourier components from which the effects of the IRF were removed. Finally, we found that these components were both highly correlated with disease severity, and that the spatiotemporal phase of components at 0.06 mm$^{-1}$ and near 0.4 GHz was most sensitive to small changes in RA disease activity. While the results of this investigation were promising, and suggested that TD-DOI could capture metrics which were more sensitive to RA disease activity than the closest related CW-DOI metrics, the observed conclusions were ultimately limited by the *in silico* nature of the work.

Chapter 4 presented a preliminary experimental validation of the findings in the *in silico* study by using images from a TD-DOI system to distinguish between RA disease-mimicking solid phantoms under varying experimental conditions. Notably, the system was constructed using an SPC architecture to increase cost-effectiveness. Due to the complexity of mimicking the realistic geometry of a finger, we designed our phantoms based on a simplified two-part model meant to broadly mimic the joint cavity and its surrounding tissue. Further, we created two separate phantoms sets: one which simulated changes in both optical properties and joint geometry, and one which simulated changes in optical properties under identical joint geometries. In particular, the latter set sought to demonstrate the system’s ability to track the types of physiological changes which cannot be measured in the clinic using typical clinical examination. TD-DOI of virtual references of the planned solid phantoms was simulated to
establish expectations for the experimental measurements. Following a validation of the system’s ability to track basic optical changes across its FOV, each solid phantom was imaged multiple times using different IRFs. Despite the influence of different IRFs and experimental noise, a multitude of spatiotemporal Fourier components extracted using the method presented in Chapter 3 could clearly distinguish between the phantoms within each set. While, on average, components extracted from experimental images performed worse than the expectations established using virtual references, general trends between simulations and experimental results were consistent. Magnitude and phase changes at 0.04 mm$^{-1}$ were the most sensitive to simulated changes in the solid phantoms. However, unlike the in silico investigation, changes in the magnitude of these components—across a broad range of temporal frequencies including the DC component—were the most sensitive to changes in the second phantom set (i.e., changes to optical properties alone). Nevertheless, we note that the conclusions of this study were limited by the simplified design of the phantoms, which are not truly representative of the complex geometrical and physiological changes expected to be present in an in vivo human finger. Furthermore, both this study and the in silico study only focused on investigating models of the PIP joint. Though the PIP joint is often affected in RA, MCP and wrist joints are also very commonly affected.

Given the above, it appears that the spatiotemporal Fourier analysis of TD-DOI images is a potentially effective solution for RA treatment monitoring. Future work should focus on assessing the abilities of the experimental system presented in Chapter 4 to distinguish between different types RA disease activity in vivo. Specifically, this system could be used to measure the PIP, MCP, and wrist joints of RA patients. Similar to previous work [1], these joints could also be concomitantly assessed using quantitative clinical examination (e.g., DAS28), and quantitatively scored using imaging modalities such as US and MRI. Correlations between the various joint assessments would then be used to highlight the level of agreement between TD-DOI and other typical methods of measuring RA disease activity. Further, it would be highly desirable to conduct these assessments in the same patients using a longitudinal study
model [2]; this would clarify the predictive ability of TD-DOI measures compared to clinical examination and other imaging methods.

Notably, the conclusions of Chapter 3 and Chapter 4 are based on results that were acquired under highly controlled conditions. In a clinical study, there are many uncontrolled variables that could influence RA disease activity within the same patient: these include body temperature, unrelated medications, and the time of day at which measurements are acquired—patient disease expression is known to oscillate daily, and symptoms are typically exacerbated during the morning hours [3]. The influence of these variables on our proposed optical indices of disease activity will require additional investigation, and may require the modification of imaging protocols in order to limit their effects (e.g., only imaging in the morning). In addition, to maximize the clinical utility of DOI, substantial consideration should be given to DOI of joints outside of the hand. Given the importance of hand joints in early RA, all diffuse optical methods investigated to date have been designed for hand imaging; however, in a clinical assessment of RA, the involvement of large joints—such as the elbow, shoulder, and knee—is also an important contributor to a patient’s overall disease activity [4]. As such, the system presented in Chapter 4 may benefit from future modifications that make it possible to acquire images in a reflectance geometry capable of assessing large joints (Fig. 1.5). Further, given the differences in imaging geometry as well as overall joint structure, the effectiveness of the analysis presented in Chapter 3 would have to be re-assessed for larger joints.

Clinical data acquired with TD-DOI would provide critical insight into the potential benefits of acquiring more information-rich DOI data than has previously been explored. Analysis of components extracted from the DC temporal frequency band—equivalent to the data that would be obtained with CW-DOI—would directly highlight how much additional benefit is provided by considering the temporal variation in light propagation through different parts of the joint (i.e., using FD-DOI or TD-DOI). The in vivo spatiotemporal Fourier component maps acquired by such a study could also be analysed using machine learning approaches in order to investigate the presence of highly sensitive spatiotemporal component combinations. Similar
strategies—which have applied various algorithms such as \( k \)-nearest-neighbours and support vector machines to hundreds of extracted image features—have been successful in boosting the sensitivity and specificity of other diffuse optical methods [5, 6, 7]. In addition, the resulting make-up of these component combinations could be analysed to compare the clinical utility of acquiring measurements at just a single temporal frequency (i.e., performing FD-DOI) compared to the TD-DOI approach of simultaneously measuring a multitude of temporal frequency bands.

In conclusion, this dissertation has assessed the potential of two TD diffuse optical methods for measuring changes in RA disease activity, with the goal of monitoring RA treatment response. Despite promising results from previous studies [8], the results of the longitudinal animal study described in Chapter 2 suggest that joint BF measurement using DCE-NIRS is not effective for RA treatment monitoring. On the other hand, the in silico investigation described in Chapter 3 suggests that spatiotemporal Fourier components extracted from TD-DOI images may be highly sensitive to small changes in RA disease activity. Further, the experimental single-pixel TD-DOI system presented in Chapter 4 was able to use these components to clearly distinguish between solid phantoms of different RA disease activity states. As such, these results suggest that spatiotemporal Fourier analysis of TD-DOI images has the potential to be a sensitive treatment monitoring tool which could help identify RA treatment failure earlier than current methods, and help patients maximize the benefits of effective early treatment.
Bibliography


Appendix A

Optical properties and tissue geometries for simulating RA

The material in this section was adapted from work which was previously published under SPIE copyright in 2021 by Seva Ioussoufovitch and Mamadou Diop in the Proceedings of SPIE, Optical Tomography and Spectroscopy of Tissue XIV (volume 11639) under the title “In silico experiments of time-resolved near-infrared light transport through human fingers with simulated rheumatoid arthritis” (DOI: 10.1117/12.2576899). The citation recommended by the copyright holder is provided below.

Seva Ioussoufovitch, Mamadou Diop, ”In silico experiments of time-resolved near-infrared light transport through human fingers with simulated rheumatoid arthritis,” Proc. SPIE 11639, Optical Tomography and Spectroscopy of Tissue XIV, 116390J (5 March 2021); https://doi.org/10.1117/12.2576899

A.1 Tissue geometry

Tissue geometries were derived from the middle finger of an MRI image of a healthy human hand [1]. First, 3D Slicer [2, 3] was used to crop the finger from the MRI image and manually segment three of its larger tissues: bone, tendon, and subcutis. Additional tissues, which could not be reliably segmented due to limited image resolution (0.5 mm × 0.5 mm × 0.5 mm), were then artificially added based on reference MRI images of healthy hands [4]. Note that—
facilitate the addition of these finer tissues—the segmentation was oversampled by a factor of 2 compared to the original image; this resulted in a segmentation resolution of $0.25 \text{ mm} \times 0.25 \text{ mm} \times 0.25 \text{ mm}$. After identifying bone surfaces on either side of the joint cavity, we manually covered them with a layer of cartilage (0.25 mm average thickness) [5, 6] and denoted the remaining space as synovial fluid. A hollow shell (0.25 mm thickness) was subsequently created around the synovial fluid and designated as the synovial membrane. Lastly, we created a hollow shell (1.25 mm thickness) [7] around the subcutis and designated it as skin. Transverse slices of the final segmentation were then linearly transformed in MATLAB 2020a to create 3 additional models which simulated worsening states of RA disease activity. As shown in Figure A.1, a total of 4 models were created (None, Mild, Moderate, Severe).

Figure A.1: 3D views of the original finger segmentation (top) used to create models for simulations along with transverse slices of the 4 simulated states of disease activity (bottom). Tissues types (SM - synovial membrane; SF - synovial fluid), light sources (red), and detectors (blue) are indicated using arrows in the 3D views. Tissue types for slice views are indicated with colors, and finger diameter is shown at the top of each slice; volume expansion factors relative to the “None” model are shown for synovial membrane (SM) and synovial fluid (SF). Slice views were taken at the location shown in the second 3D view (top right).
A.2 Optical properties

Tissue optical properties were sourced with the help of previous joint simulation studies and review papers [8, 9, 10, 11]. Absorption coefficients ($\mu_a$) were calculated by selecting a base $\mu_a$ value (Table A.1) and adding additional absorption based on various tissue parameters (e.g., presence of chromophores). Reduced scattering coefficients ($\mu'_s$) and refractive indices ($n$) were either assumed or directly sourced from the literature (Table A.1). Several exceptions to this approach are noted in the Tables below. Values of various tissue parameters were adjusted according to changes expected due to the presence of RA [9] (Table A.2). Final optical properties at 800 nm for None and Severe RA disease activity are shown in Table A.3; optical properties of intermediate disease activity states can be determined by linearly interpolating between these two endpoints.

Table A.1: References for each tissue’s base absorption coefficient $\mu_a$, reduced scattering coefficient $\mu'_s$, and refractive index $n$.

<table>
<thead>
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<th>Tissue</th>
<th>References</th>
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<td>Skin</td>
<td>No base $\mu_a$; $\mu'_s$ from average parameters in Jacques (Table 2)[11]; $n$ = 1.44 (typical value)</td>
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<tr>
<td>Subcutis</td>
<td>No base $\mu_a$; $\mu'_s$[12]; $n$ = 1.44 (typical value)</td>
</tr>
<tr>
<td>Tendon*</td>
<td>All properties from porcine tendon[13]</td>
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<tr>
<td>Synovial membrane</td>
<td>Base $\mu_a$ and $\mu'_s$ from mucous membrane[14]; $n$ = 1.44 (typical value)</td>
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<tr>
<td>Synovial fluid**</td>
<td>All properties from water[15] or synovial fluid from arthritic knee joint[8]</td>
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<tr>
<td>Cartilage***</td>
<td>Base $\mu_a$ and $\mu'_s$,[16] $n$[11]</td>
</tr>
<tr>
<td>Bone</td>
<td>Base $\mu_a$ and $\mu'_s$[17] $n$[18]</td>
</tr>
</tbody>
</table>

*Tendon $\mu_a$ taken directly from reference without adjusting for chromophore concentrations.

**Synovial fluid properties were calculated as $P_{SF} = P_{Water}(1-D)+P_{ASF}D$ where $P$ is an optical property ($\mu_a$, $\mu'_s$, or $n$) for synovial fluid (SF), water, or arthritic synovial fluid (ASF) and $D$ is the “disease activity fraction” parameter (Table A.2).

***Cartilage refractive index was calculated as a linear combination of the refractive indices of dry tissue mass and water; thus, this value changed based on tissue water concentration (Table A.2).

<table>
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<th>Parameters</th>
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<td>Subcutis</td>
<td>S/Bl/W+F/cF</td>
<td>(21/0.8/95/68) [12]</td>
<td>15 [23]/0.8/95/68</td>
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<tr>
<td>Synovial fluid</td>
<td>D**</td>
<td>0</td>
<td>100</td>
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<tr>
<td>Cartilage</td>
<td>W</td>
<td>75 [25]</td>
<td>90 [26, 8]</td>
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</table>

*Skin and bone parameters were set to the same values for all models.

**Disease activity fraction represents the weighting given to optical properties obtained from arthritic synovial fluid compared to the optical properties of water in the calculation of a particular model’s synovial fluid optical properties (see Table A.1 for equation).

Table A.3: Absorption coefficient $\mu_a$, reduced scattering coefficient $\mu'_s$, and refractive index $n$ at 800nm used for the 2 disease models (None and Severe). Properties were derived using the information specified in Tables A.1 and A.2.

<table>
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*Skin, bone, and tendon optical properties were set to the same values for all models.

**Changes in oxygen saturation between models resulted in negligible optical property changes due to the low blood volume fraction in this tissue.
Bibliography


Appendix B

Assessment of solid phantom constituents

The material in this section was adapted from an appendix of the work in Chapter 4 which is currently in preparation for submission by Seva Ioussoufovitch and Mamadou Diop. Additional details regarding the creation of reference phantoms (see Sec. 4.2.5) as well as the estimation of their optical properties and the relationship between these properties and phantom constituent concentrations are provided here.

Similar to phantoms created by Sekar et al. [1], we created cylindrical reference phantoms with large diameters (85 mm) and an average thickness of 18.37 ± 0.84 mm. Once all the phantoms were created, we re-oriented the TR-NIRS configuration described in Sec. 4.2.3 so that measurements were acquired in a transmittance geometry, and measured each phantom 3 times in a random order; system IRFs were measured before and after the reference phantom measurements. Absorption $\mu_a$ and reduced scattering $\mu'_s$ coefficients of each reference phantom were then estimated using a semi-infinite slab model of the diffusion equation with extrapolated boundary conditions [2, 3]. A nonlinear optimization routine built using MATLAB’s `lsqnonlin` function was used to fit the DTOFs measured for each phantom with a theoretical DTOF produced by convolving the model solution with the system’s IRF. Fitting was performed from 80% of each curve’s rising edge to 1% of each curve’s falling edge, and was conducted in two rounds to reduce crosstalk between $\mu_a$ and $\mu'_s$. First, data from 4 phantoms which did not contain any absorber were fit for only $\mu'_s$ and an amplitude term. A linear fit was applied to the resulting $\mu'_s$ values to determine an initial estimate for the relationship between scatterer concentration and $\mu'_s$. Second, data from the remaining phantoms—which contained both absorber and scatterer—was fit for $\mu_a$, $\mu'_s$, and an amplitude term; however, the initial guess for $\mu'_s$ was
set using the previously determined relationship. Fig. B.1 shows the final $\mu_a$ and $\mu'_s$ estimates for all phantoms as a function of their absorber and scatterer concentrations, respectively; the equations of their respective lines of best fit are also indicated.

Figure B.1: Linearity relationships between phantom absorber concentration and $\mu_a$ (top), as well as phantom scatterer concentration and $\mu'_s$ (bottom).
Bibliography


Curriculum Vitae

Education

**Doctor of Philosophy in Biomedical Engineering.**
Musculoskeletal Health Research Specialization
The University of Western Ontario, London, ON
- Thesis: Detecting Treatment Failure in Rheumatoid Arthritis with Time-Domain Diffuse Optical Methods
- Supervisor: Dr. Mamadou Diop

**Bachelor of Medical Science.**
Honors Specialization in Medical Biophysics (Clinical Concentration)
The University of Western Ontario, London, ON
- The University of Western Ontario Gold Medal
- Dean’s Honor List

Scholarships & Awards

**SPIE Student Travel Grant**
Travel Grant ($1000)
SPIE

**London Imaging Discovery Day Magna Cum Laude Presentation Award**
Presentation Award
The University of Western Ontario

**NSERC Canada Graduate Scholarship – Doctoral**
National Scholarship ($105 000)
The University of Western Ontario

Honorable Mention
Presentation Award
Imaging Network Ontario 2019

**Top 3 Presentation – Western Engineering 3MT**
Communication Award ($100)
The University of Western Ontario

**SPIE Student Travel Grant**
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Bronze Excellence in Leadership Award
Issued in recognition of 75 hours of service within the London, ON community
The University of Western Ontario

Western Scholarship of Excellence
Entrance Scholarship ($2000)
The University of Western Ontario

Publications & Presentations

Peer Reviewed Journal Manuscripts
Published


Refereed Abstracts & Conference Presentations
Published & Presented


Disease Induction and Treatment. Poster at London Imaging Discovery Day, June 14, London, ON.


**Non-Refereed Contributions**


**Positions**

**Marketing Associate**  
*University Consulting Group (UCG)*  
Aug 2022 – Oct 2023

**Academic Peer Reviewer**  
*Journal of Biomedical Optics*  
2022

**Consultant**  
*University Consulting Group (UCG), Western Chapter, London, ON*  
Oct 2021 – Apr 2022

**Research Assistant**  
*Translational Biophotonics Lab, Western University, London, ON*  
Sept 2017 – Oct 2023

**Collaborative Specialization in Musculoskeletal Health Research Trainee**  
*Western University, London, ON*  
Sept 2017 – Oct 2023

**Collaborative Specialization in Musculoskeletal Health Research Summer Student**  
*Lawson Health Research Institute, London, ON*  
May – Aug 2017

**Process Optimization Intern**  
*City of London, London, ON*  

**Volunteer Research Assistant**  
*Assistant to Dr. Mamadou Diop PhD, Lawson Health Research Institute, London, ON*  
May 2014 – Apr 2017
Community Involvement

Councillor – Biomedical Engineering
Graduate Engineering Society, London, ON
Nov 2018 – Aug 2023

Student Representative
Biomedical Imaging Research Centre (BIRC), London, ON
Oct 2018 – Aug 2023

Finance Committee Member
Society of Graduate Students (SOGS), London, ON
Oct 2018 – Aug 2023

Councillor – Engineering Science
Society of Graduate Students (SOGS), London, ON
March 2018 – Sept 2019

Organizing Committee Member
London-Middlesex Children’s Water Festival, ON

Administrative Volunteer
Heart & Stroke Foundation, London, ON
Nov 2012 – April 2013

Academic Committee Member
Western Science Students’ Council, London, ON
Sept 2012 – April 2013

Society & Association Memberships

Canadian Arthritis Trainee Association (CATA) 2018 – 2020
Optical Society of America (OSA) 2018 – 2023
SPIE 2018 – 2023