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Optical Imaging: New Tools for Arthritis

David Chamberland, Yebin Jiang, and Xueding Wang

Department of Radiology, University of Michigan School of Medicine, Ann Arbor, MI 48109

Introduction

Arthritis is highly prevalent among adults in the U.S. It is the leading cause of disability, and is associated with substantial activity limitation, work disability, reduced quality of life, and high health-care costs.¹ Arthritis affects approximately 42.7 million Americans; projections indicate 60 million people will be impeded by arthritis by the year 2020. 1–3% of the population in the U.S. is affected by rheumatoid arthritis (RA), one of the most common inflammatory arthritides, and 80% of the patients are disabled after 20 years.^{2,3} Although inflammatory arthritis is serious, potentially crippling, and commonly disabling, comprehensive diagnosis and optimized therapies of these disorders are hindered by lack of cost efficient and powerful joint imaging technologies.

The type of imaging suited best for a specific joint depends on the type being imaged and the potential joint pathology involved. A large proportion of non-traumatic human joint pathology fits into two large categories: osteoarthritis (OA) and inflammatory arthritis. Reviewing pathological changes of these categories enables a better understanding of why certain joint imaging techniques are better suited for each disease. OA is considered a non-inflammatory condition due to underlying pathology less suggestive of inflammation being a primary culprit. Joint space narrowing, subchondral sclerosis and osteophytosis are all pathologic hallmarks of OA. Classic symptoms include activity related pain with joint pain worse after use. Inflammatory arthritis encompasses entities such as RA, seronegative spondyloarthropathies including psoriatic arthritis and crystal arthropathies such as gout. Synovial hypertrophy, bony erosions, joint region erythema and swelling can each be seen in some of these diseases. Common symptoms include complaints of joint swelling, erythema and significant morning stiffness. Each of the above entities also has characteristic joint distributions; some are more prominent in large joints while others are more common in smaller superficial joints. As the treatment paradigm in inflammatory arthritis including RA shifts toward early and aggressive therapy to retard and prevent the development of joint damage, more sensitive imaging techniques are needed to evaluate the effectiveness of early therapy.⁴ This article describes traditional imaging modalities used for evaluating arthritis along with an extensive focused review of how optical imaging has been applied to arthritis to date and its future potential applications to joint disease.

Current Joint Imaging Modalities

Due to the various types of joints and numerous unique pathological conditions affecting them, determining the most appropriate imaging modality for joint evaluation can be difficult. Both spatial resolution, or how close two features can be within an image and still be resolved as unique, and contrast resolution, or the ability of an imaging modality to distinguish structures within an image by differences in their signal intensities, are paramount in joint imaging. Due to unique imaging techniques, each modality has unique abilities regarding spatial and contrast resolution. Common joint imaging modalities used in both the current clinical and research domain include conventional and digital radiography, computed tomography (CT), ultrasound (US), magnetic resonance imaging (MRI) and nuclear imaging.

Conventional and Digital Radiography

Conventional radiography (CR) has for decades been the gold standard for detection and assessment of joint damage and continues to be the primary imaging technique for the evaluation of arthritis.^{5,6} Digital radiography creates digital images at the time of x-ray exposure. The radiation dose is approximately the same for both of these techniques.⁷ The ability to transport, reproduce and share digital images is more robust when compared to conventional radiography. Radiography is able to capture on film many of the hallmarks of pathological joint disease. Radiographic features of OA include osteophytes, joint space narrowing, subchondral sclerosis, cysts, and deformity.⁸ Radiographic findings of RA, a common type of inflammatory arthritis, include erosions, joint space narrowing, periarticular osteopenia, and soft tissue swelling. Radiography is widely available and inexpensive. It does present the hazard of ionizing radiation however. Spatial resolution of CR is generally very good; however its contrast resolution between tissues such as bone and cartilage and synovium compared to MRI and CT is poor. This modality also can only demonstrate the time-integrated record of joint damage that tends to develop late in the course of the diseases and which constitutes irreversible structural injury, most notably bone erosions in diseases like RA.^{9,10}

CT

X-ray CT was invented in the later part of the 20th century by Hounsfield and Cormack who were later awarded the Nobel Prize in medicine.¹¹ Since its discovery, CT has evolved into a complexity of intertwined processes involving hardware, system assessment, algorithm development, diagnostic applications, and therapeutic applications. CT arthrography is an alternative imaging method for indirect visualization of cartilage and other intrinsic joint structures, used especially in the knee joint.¹² Although a small study and not statistically significant, Døhn et al compared MRI and CT in a study of the MCP joints in 17 RA patients, and found excellent interobserver reliability for scoring erosions using both modalities.¹³ Some studies have found CT more accurate in predicting certain types of spinal pathology than MRI.¹⁴ In a routine clinical setting, however, CT plays a minor role in the assessment of patients who have established or suspected OA. Similar to radiography, drawbacks of CT include low soft tissue contrast and patient exposure to ionizing radiation.¹² Moreover, CT is expensive and not as widely available as US or CR.

Ultrasound imaging

At the end of the 20th century, musculoskeletal ultrasound became an established imaging technique for rheumatic diseases made possible through technological improvements, resulting in faster computers and higher frequency transducers.¹⁵ Multimodality musculoskeletal ultrasound which includes techniques that image both tissue structures and synovial blood flow, is now routinely used by a growing number of rheumatologists in the diagnosis, monitoring treatment response, and guiding intervention of inflammatory arthritis.^{16–18} Synovitis, one of the hallmarks of RA, can be detected in different synovial recesses accessible by ultrasound evaluation in peripheral joints.¹⁹ Ultrasound also detects more osteophytosis, common in OA, than conventional radiography in the small joints of the hands.²⁰ Ultrasound is widely available, relatively inexpensive and free of ionizing radiation. Ultrasound, similar to MRI, has been shown to be much more sensitive than radiography in detection of erosions in RA.²¹ Both spatial and contrast resolution of US are high but dependent on depth of structure to be imaged and transducer variables. However, the contrast exhibited by US is low and not sensitive to the molecular conformation and functional changes in biological tissues (e.g. blood volume, low-speed blood flow, and blood oxygenation). With US, it is also very difficult to assess the structural changes within the bone. Moreover, the performance of US is highly dependent on the skills of the operator and hence is difficult to repeat and standardize for clinical trials.²²

MRI

MRI has presented excellent capability in imaging a variety of joint tissues including ligaments, muscle, bone and cartilage in three dimensions, and has the potential to measure inflammatory activity and joint destruction with satisfactory sensitivity and specificity. Many studies have confirmed that MRI is superior to CR in terms of detecting erosions in the first year after presentation for those with inflammatory arthritis.²³ MRI, with its excellent soft tissue contrast and high spatial resolution, is regarded as the best technique currently available for assessment of articular cartilage affected by arthritis, including not only morphologic information such as fissuring and the presence of cartilage defects but also biochemical and physiologic information.^{24,25} MRI has also been found to be useful in detection of sacroiliitis.²⁶ Recently, an increasing number of studies have used MRI as dynamic imaging to examine therapeutic outcomes with interest moving from disease-modifying anti-rheumatic drugs to biological therapies.²⁷ Disadvantages of MRI are its high cost, need for contrast agents, and lack of broad availability compared to CR. Moreover, the long data-acquisition time with ensuing patient discomfort makes it difficult to use routinely and repeatedly, and, in some cases, impossible to use at all.

Nuclear imaging

Currently, nuclear imaging is used mostly in research settings and plays a very limited role in the clinical evaluation of arthritis. Some research has elicited potential clinically useful applications however. In comparison with conventional imaging methods, nuclear imaging has presented uniquely high sensitivity in detection of a signal coming from a bioactive agent, which is the reason for fast development of nuclear medicine based molecular imaging in past decades. In RA, nuclear imaging can potentially be used to evaluate physiological and pathophysiological processes, facilitate early diagnosis, monitor therapeutic effects and support the development of new therapies. Nuclear imaging in combination with MRI has been found helpful at times in eliciting a diagnosis in undifferentiated inflammatory arthritis.²⁸ The limitations of the current nuclear imaging technologies include poor spatial resolution and limited anatomical information. Therefore, when nuclear imaging is used it is beneficial to combine it with other conventional imaging modalities such as CT and MRI. As a single modality it has not been shown to be useful for diagnosis of early inflammatory arthritis later confirmed to be RA according to American College of Rheumatology criteria.²⁹ Moreover, the high cost and radiation feature of nuclear imaging have also prevented this technology from becoming a routinely used arthritis imaging modality.

Optical Imaging Modalities

Recently, nonionizing noninvasive optical modalities for medical imaging and diagnosis have drawn considerable attention, because optical techniques offer unique advantages over the existing imaging methods. Optical contrast of tissue in the visible and near-infrared (NIR) region of the electromagnetic spectrum is intrinsically sensitive to tissue abnormalities and function. For example, since oxygenated and deoxygenated hemoglobin, the two dominant chromophores in most biological tissues over the optical spectrum from visible to the near-infrared region, have spectroscopic absorption differences, optical measurements have shown uniquely high sensitivity and specificity in quantifying hemodynamic properties including blood volume and oxygenation level in biological samples.^{30–33} This ability may aid early diagnosis of inflammatory arthritis. Optical imaging can also be extended to the molecular level by visualizing a variety of optical contrast agents. With uniquely high sensitivity in detecting optical contrast agents, optical based molecular imaging modalities such as fluorescence imaging have presented excellent sensitivity which also may aid early diagnosis and ongoing evaluation of chronic

inflammatory arthritis. Moreover, optical imaging can provide fast and inexpensive methodologies, with relatively simple instrumentation requirements and no ionization.

Similar to cancerous lesions, the hallmarks of many inflammatory rheumatic diseases include angiogenesis, hypervascularization, hyper-metabolism and relative hypoxia. Optical measurements can probably quantify all of these morphological and physiological changes potentially enabling early diagnosis of inflammatory arthritis and providing improved monitoring of therapeutic interventions with high sensitivity and specificity. It has been reported that optical imaging has shown promise to provide new tools for early detection and therapeutic monitoring of joint inflammation in patients with RA. For example, the clear synovial fluid in normal joints turns into a turbid and pink medium early in the inflammatory process, which may be monitored and quantified by optical transillumination imaging or diffuse optical tomography. Fourier transform infrared spectroscopy has been employed to investigate synovial fluids in arthritic joints. Significant spectroscopic differences related to the differences in fluid composition as a result of the disease processes were found between spectra of synovial fluid from joints affected by RA, OA, spondyloarthropathies and meniscal injuries.^{34, 35} As another example, the hemodynamic changes associated with arthritic joints, including angiogenesis and hypervascularization as well as hypoxia in arthritic articular tissue can potentially be observed by diffuse optical tomography, photoacoustic tomography and other emerging optical modalities.

Transillumination imaging

Optical methods employing direct transillumination imaging geometry have been examined for their performance in evaluating arthritis. In these methods, optical signals transmitted through articular tissues were measured and then used to form images. With light in the NIR region, the transillumination images are able to present structural and some functional information of articular tissues. In comparison with other optical modalities, transillumination optical methods are easiest to realize with comparatively simple instrumentation requirements. However, the transillumination images may suffer from limited spatial resolution; and the optical parameters presented by these images are difficult to be quantified.

In transillumination imaging, the intensity and the distribution of the light transmitted through a joint is highly dependent on the optical properties of the joint tissues, e.g. the optical scattering of the synovial fluid which is a quantitative description of the fluid turbidity. Scheel and Schwaighofer et al have built a laser transillumination apparatus for measuring the scattering light distribution on finger joint and have completed the first clinical study of this optical system for normal finger joints and those affected by RA.^{36, 37} This apparatus allows the transillumination of finger joints by means of a low power laser light with a wavelength of 675 nm. The distribution of transmitted light is detected by a CCD camera connected to a computer. An example result of the laser transillumination imaging of human proximal interphalangeal (PIP) joints are shown in Fig. 1, where PIP joint cavity is the area with high signal intensities (i.e. bright area) in each image. In comparison with the healthy joint, the cavity of the actively inflamed joint looks rather fuzzy and darker, which is due to the elevated optical scattering in the synovial fluid. The synovial fluid of arthritic joints tends to be turbid or grossly purulent with significantly increased white blood cells.

This new laser transillumination imaging technique is easy to handle, noninvasive, and inexpensive. Since this imaging method has presented fairly good repeatability, it may be especially useful for repeated analysis of joint inflammation and hence could be useful to evaluate treatment efficacy. For treatment efficacy studies, baseline measurements can be made during initial evaluations followed by serial measurements during subsequent visits.

Currently, however, this technology shows limited accuracy in differentiating inflamed from normal joints as a result of the interindividual anatomic differences of the joint structures and tissue optical properties. Because there is no tomographic reconstruction, the degree of joint inflammation cannot be quantified by optical evaluation directly. For further development of this method, more advanced image processing techniques have been explored to evaluate the directly recorded transillumination pictures, including deconvolution, segmentation and reconstruction.^{38–40}

In the transillumination imaging method described above, continuous wave (cw) light from a laser diode is used as the illumination source. Ultrafast optical techniques have also been used for optical imaging of joints. In such techniques, very short light pulses are employed to illuminate imaged target and the image-bearing photons that are the early component of the transmitted light are extracted with time or coherence gating techniques. Zevallos et al has explored the feasibility of 2D imaging of bone structures in the human palm using femtosecond pulse transillumination and picosecond electronic time-sliced detection technique.⁴¹ Ultrashort laser pulses of about 150 femtosecond duration at 800 nm from a self-mode locked Ti:sapphire laser were employed to illuminate the imaged sample. Then the transmitted light was recorded with an ultrafast electronic-gated imaging camera system consisting of a time-gated image intensifier unit fiber optically coupled to a charge-coupled-device (CCD). By controlling the duration of the electronic gate pulse, this imaging system can record only the image-bearing early-arriving photons that are mostly snake photons and ballistic light; while more diffuse photons of the transmitted light which contribute to noise and overwhelm the image-bearing photons are blocked because they tend to arrive the detector later. To validate the performance of this system, transillumination measurements were performed *in vivo* on a human palm in order to image the metacarpal bones. 2-D time-sliced transillumination imaging of the palm obtained using the time-sliced detection is shown in Fig. 2(a). In this image, three metacarpal bones (ring, middle and index) can be clearly resolved. Fig. 2(b) shows the spatial intensity profile which was obtained by integrating the intensity over a 25-pixel-wide vertical region marked in (a). For the middle finger, the image contrast achieved in this experiment was about 0.66.

Similar to the time gating in ultrafast optics, coherence gating in optical heterodyne detection can also discriminates the diffuse components of transmitted light based on their losses of coherence, direction and polarization of the incident wave.⁴² Coherence gating technique has also been employed in optical imaging of joints realized through a scanning geometry similar to that in X-ray CT.⁴³ To acquire an image of a PIP joint, the index finger is mounted on a translational-rotational stepping motor stage for both liner and rotational scans. The step size of linear scan across the PIP joint was 500 μm ; while the step size of the rotational scan was 6°. The finger was rotated by 180° for a complete data set, and the 30 projections were used to reconstruct the optical CT image of the PIP joint. As shown in Fig. 3, major structures in the joint such as tendons and blood vessels could be identified in the laser CT images obtained by this method. However, the spatial resolution achieved currently does not enable identification of more detailed joint structures in the images. Moreover, similar to other transillumination imaging methods, this technique forms images directly with the measurement of transmitted light through the joints. Therefore, although some articular structures can be presented, it is still difficult to quantify the optical properties and function of joint tissues.

Diffuse optical imaging

Diffuse optical imaging (DOI) is an optical technique that has drawn considerable attention for its potentially wide application in medical imaging and diagnosis. In DOI, NIR light is used to illuminate a target sample; then diffusely reflected and/or transmitted light is detected noninvasively at different positions around the sample. By using sophisticated

reconstruction models, the spatial maps of intrinsic tissue optical properties including both absorption and scattering can then be extracted from the collected diffusive data. Moreover, the spectroscopic optical properties obtained from DOI further allow functional images of the sample, such as blood volume, blood oxygenation level, water content, and cellular structure, which might be essential for early detection and objective evaluation of diseases such as tumor. The success of DOI is highly dependent on the image reconstruction. In the past decade, various advanced linear and nonlinear reconstruction algorithms have been developed and evaluated through the studies on phantoms and initial clinical testing. An effective reconstruction algorithm is able to improve the spatial resolution, spatial accuracy and quantitative accuracy, and makes it possible to image tissues beneath the sample surface. Because the absorption coefficient of tissue is considerably smaller in the NIR region, light can penetrate deeply into the tissues to depths of several centimeters. DOI working with NIR light has found several potential applications including the imaging of breast cancer and brain disorders.^{44,45} Pilot studies have also suggested that DOI has the potential to become a novel and powerful tool for early detection of arthritis and other musculoskeletal diseases. In comparison with transillumination imaging modalities, DOI involves sophisticated reconstruction algorithms and generates images with all the information containing light signals including not only ballistic and snake photons but also diffusion light after multiple scattering. Optical imaging based on DOI techniques could lead to improved image quality with higher spatial resolution and more quantitative functional information.

By using a multichannel frequency-domain DOI system, Xu et al has achieved full three-dimensional (3D) volumetric reconstruction of absorption images of human finger joints *in vivo*.^{46–49} CW light at 785-nm wavelength from a diode laser is delivered to the imaged joint through one of the 16 source fibers that are distributed evenly around the joint. For each source position, the diffused light is collected by 16 detector fibers that are also evenly distributed along the surface of the finger. The light intensity correspondent to each source-detector pair is measured with a photomultiplier tube (PMT) and then recorded by a PC. With all the data collected around an imaging plane, 2D optical images of the joint are reconstructed using a nonlinear, finite element based reconstruction algorithm; while for 3D volumetric imaging, data collection at multiple measurement planes are needed.^{50,51} An example imaging result acquired from a PIP joint of a volunteer is in Fig. 4, where reconstructed absorption images along several cross sections of the joint are presented. In these optical images, articular structures including bones, vessels, tendon and synovium can be identified. After this novel imaging system had been validated through studies on phantoms, the authors have also conducted a pilot study to show the potential of this imaging method for the diagnosis of OA.⁴⁹ By comparing the imaging results of normal volunteers and patients affected by OA, the authors demonstrated that DOI is able to differentiate arthritic joints from the normal based on several biomarkers of OA presented by optical absorption and scattering images, including narrowed joint space, and increased optical absorption and scattering coefficients in the synovial fluid. It was also noticed that both absorption and scattering coefficients in the OA joints are more heterogeneous in comparison with those in the normal joints.

Hielscher and Scheel et al have developed a sagittal laser optical tomographic imaging system that is able to present 2D spatial distribution of optical properties in a sagittal plane through finger joints.^{52,53} This imaging system is similar to their laser transillumination imaging system introduced in the above except that tomographic imaging procedure has been employed. The system involves a single laser diode (675 nm) as the light source and a single silicon photodetector for receiving the diffusely transmitted light. By scanning both the source and the detector along the sagittal plane of a finger joint, one can obtain several transmission profiles that are spatially resolved intensities of light transmitted through the

finger. After the collected data is processed with a model-based iterative image reconstruction scheme, the spatial distribution of optical properties including both the absorption coefficient and the scattering coefficient inside the joint and surrounding tissues are generated. With this tomographic imaging technique, 2D quantitative images presenting the point-by-point optical properties in a joint sagittal section can be obtained. Therefore, unlike that with the laser transillumination imaging technique, the determination of the status of joint inflammation may be achieved using this imaging system without the need of reference to an earlier measurement. Fig. 5 shows example results of 2D sagittal images of three human finger joints of a RA patient, where the left column shows the optical absorption images and the right column shows the optical scattering images. Before imaging, each joint was classified according to four-grade clinical synovitis score and four-grade semiquantitative ultrasound examination score. Among the three imaged joints, the index finger (top row) and the middle finger (middle row) have clinical signs of inflammation; while the ring finger (bottom row) looks normal. As shown in Fig. 5, the unaffected joint shows a clear drop in optical properties (both absorption and scattering) in the centre of the image, while the joints with clinical and ultrasonic signs of synovitis show little variation in optical properties. The authors also quantified the minimum and maximum scattering coefficients μ_s for each joint in an area surrounding the joint cavity. The joints with inflammation showed minimal μ_s between 8.54 cm^{-1} and 9.06 cm^{-1} ; while the unaffected joints produced a minimal μ_s of 0.51 cm^{-1} . Moreover, the minimal absorption coefficients μ_a of the affected joints were about 0.30 cm^{-1} ; while the joint with no inflammation showed μ_a of 0.03 cm^{-1} . In order to validate that the high absorption and scattering coefficients in and around the joint cavity presented by optical images are indicative of an inflammatory process, the authors have compared 78 optical images of PIP joints from 13 RA patients with correspondent ultrasound images and clinical examination.⁵² It has been demonstrated that the optical features in optical images show a statistically significant difference between joints with and without synovitis.

Due to the strong optical absorption of oxygenated and deoxygenated hemoglobin as well as the spectroscopic absorption differences between them, optical imaging has shown uniquely high sensitivity in visualizing the hemodynamic activities in subsurface biological tissues. Hielscher et al has explored the feasibility of dynamic diffuse optical tomography in imaging the vascular and metabolic reactivity in human finger joints.^{54,55} In dynamic imaging studies, one measures a reference baseline data before a certain physiological perturbation and then attempts to image changes in optical properties and physiological parameters as they occur during the perturbation. In comparison with other diffuse optical imaging, dynamic optical tomography has many advantages. First, by adding temporal information, dynamic optical tomography has a better chance to handle the ill-posed inverse problems due to the overwhelming optical scattering in biological tissue and the limited number of measurements. Second, for functional imaging, dynamic optical tomography may localize and separate the stimulus-related functional signal from the strong background and, hence, may improve the signal-to-noise ratio and spatial resolution. Moreover, because a perturbation only affects the specific areas while the boundary conditions remain unchanged, the system becomes less sensitive to boundary affects.⁵⁴

The dynamic optical tomography of human finger joints has been explored to study vascular and possibly metabolic effects of the disease on the PIP joints.^{54,55} For functional imaging of hemodynamic changes, imaging at two laser wavelengths, 760 nm and 832 nm, was conducted on each finger. First, a baseline measure of the joint was made. Then to illicit a controlled hemodynamic response, a sphygmomanometer cuff was placed around the forearm. When the cuff is inflated with a well-controlled pressure, the venous return and CO₂ removal of the blood supply from the finger can be shut down while the blood supply and oxygen delivery is still maintained. When the pressure in the cuff was maintained for 30

seconds, the second measurement was acquired. Using this imaging system working on the two wavelengths, one can reconstruct the changes of hemoglobin parameters as results of the controlled hemodynamic response, including concentrations of oxygenated hemoglobin ([HbO₂]) and deoxygenated hemoglobin ([Hb]) as well as the concentration of total hemoglobin ([HbT]; i.e. blood volume). An example result is shown in Fig. 6, where spatial mapping of hemodynamic properties in joint cross section were quantified.⁵⁵ In comparison with the result from the healthy joint, the rheumatic joints of the two RA patients revealed different hemodynamic responses. First, the maximum relative change in [Hb] as compared to [HbT] is approximately two times greater in the RA joint than in the healthy joint. This suggests a greater metabolic demand in the rheumatoid synovium as the oxygen consumption is elevated. Second, the spatial mappings of the [Hb], [HbO₂] and [HbT] changes in the rheumatic joints appear considerably different, and a good correlation between Hb, HbO₂, and blood volume does not seem to exist. This suggests spatially varying metabolic activity present in the rheumatoid joint but absent in the healthy one.

Fluorescence and bioluminescence imaging

Conventional musculoskeletal imaging modalities are mostly based on nonspecific macroscopic physical, physiological or metabolic changes of tissue, and may not represent and characterize biological processes at the cellular and molecular levels within the context of physiologically authentic environments. Unlike conventional imaging technologies, molecular imaging identifies specific molecular events in living tissues by visualizing molecular probes as the source of image contrast. Therefore, molecular imaging, especially when fused with conventional medical imaging technologies, no doubt will enhance the specificity and sensitivity of early diagnosis, and contribute to the understanding of disease pathophysiology and appropriate therapeutic intervention. Optical technologies including fluorescence and bioluminescence imaging are relatively new molecular imaging modalities that have been used for laboratory research on arthritis.^{56,57} Optical molecular imaging enables fast imaging speed and comparatively low cost, with high sensitivity parallel to nuclear imaging but no ionizing radiation exposure.

During NIR spectrum region, light has optimal penetration in most soft biological tissues; while the autofluorescence of living tissues is also low. Therefore, near-infrared fluorescent (NIRF) probes are exciting options for molecular imaging. A recently developed folate-targeted near-infrared fluorescence probe (NIR2-folate) has been tested for *in vivo* imaging of arthritis using a lipopolysaccharide (LPS) intra-articular injection mouse model and a KRN serum transfer mouse model.⁵⁸ Previous studies have demonstrated that activated macrophage content correlates well with articular destruction and poor disease prognosis in humans; while folate derivatization can target activated macrophages involved in inflammatory joint disease.^{59,60} Therefore, imaging NIR2-folate probes with NIRF may facilitate early diagnosis and improve assessment of arthritis by providing an *in vivo* characterization of active macrophage in inflammatory joint tissue. Fig. 7 shows an example of NIRF imaging on LPS induction arthritis mouse model, where LPS was injected intra-articularly into the right ankle joint while same volume of saline was injected in the opposite ankle joint of the same animal for comparison. As shown in Fig. 7, the fluorescence signal intensity of the inflamed joints was significantly higher than the opposite ankle joint used as control. It was reported that the average enhancement ratio of the inflamed joint was up to 2.3-fold in the first 12 and 24 hours after probe injection, and remained at 1.8-fold 72 hours after probe injection.

A similar study of NIRF imaging of arthritis mouse model has been conducted by using anti-F4/80 monoclonal antibodies (mAb) labeled with Cy5.5 fluorochrome that targets the F4/80 antigen present on the surface of macrophages infiltrating the inflamed synovial membrane.⁶¹ In another work on collagenase-induced OA mouse model, cathepsin B

activatable NIRF imaging showed a 3-fold signal difference between the osteoarthritic and normal joints, which suggests that imaging of cathepsin B sensitive NIRF probes may offer a potential new technology for early diagnosis of OA.⁶² Other than diagnostic imaging, NIRF has also been adapted to realize treatment monitoring of arthritis. Wunder et al has used a protease-activated NIRF imaging to examine the presence and distribution of fluorescence in arthritic joints of mice with collagen-induced arthritis.⁶³ In comparison with the images from untreated mice, NIRF images obtained following methotrexate (MTX) therapy demonstrated substantially lower fluorescence intensities of arthritic toes and paws, which suggested that protease activated NIRF imaging probes may serve as sensitive reporters for monitoring treatment efficacy of anti-rheumatic drugs. More recently, Simon et al has assessed the feasibility of NIRF imaging of inflammation in an antigen-induced arthritis model using fluorescent leukocytes.⁶⁴ The leukocyte cells were labeled with DiD (C₆₇H₁₀₃CIN₂O₃S, Vybrant Cell Labeling Solution, Molecular Probes, Oregon), a lipophilic carbocyan marker showing minor fluorescence as a free dye in water but a marked fluorescence when incorporated into cells. As shown in Figs. 8(a) and (b), with the presence of fluorescence labeled cells in the arthritic synovium, the arthritic knees after the labeled cell injection show higher signal increase compared with the left control knees. Figs. 8(c) and (d) show an example result from cortisone treated rats (intraperitoneal injections of 30 mg/kg Solu-Medrol on day 1, followed by daily doses of 3 mg/kg Solu-Medrol for five consecutive days). In comparison with the result from the untreated rat as in Figs. 8(a) and (b), the cortisone-treated knee reveals less signal increase after cell injection, demonstrating that NIRF imaging of DiD-labeled allogeneic leukocytes has the potential for monitoring the treatment of antigen-induced arthritis.

Molecular imaging based on bioluminescence techniques also allows noninvasive *in vivo* visualization of tissue biology in molecular or cellular level. As the core of a bioluminescence imaging system, a cooled charged-coupled device (CCCD) camera is used to noninvasively visualize low quantities of native photons emitted by internal mammalian tissue which bioluminesce. As one of the pioneering adaptations to musculoskeletal diseases, Bar *et al.* described the *in vitro* and *in vivo* monitoring of gene expression in skeletal development and repair using a real-time bioluminescence imaging system.⁶⁵ Their experimental model consisted of transgenic mice harboring the luciferase marker gene under the regulation of the human osteocalcin (hOC) promoter. The good correlation between quantitative CCCD measurements and the gold standard luciferase biochemical assay and luciferase immunohistochemistry demonstrated the accuracy of the bioluminescence imaging. According to the images acquired during mice growth from 1 week to 1.5 year, transgenic mice exhibited transgene expression in a wide spectrum of skeletal organs, including calvaria, vertebra, tail, and limbs, reaching a peak at 1 week in most of the skeletal organs, as shown in Fig. 9. Other applications of bioluminescent imaging in musculoskeletal diseases including quantitative noninvasive and continuous observation in animal models of RA, facilitated by the recent development of transgenic reporter gene mice for bioluminescence imaging of gene expression in inflammation.^{66–69}

The successful use of fluorescence imaging in laboratory studies on small animal models will encourage future research into the development of clinically applicable fluorescent dyes and the application of this technique for clinical imaging and treatment of arthritis. However, the major challenge of developing and adaptation of optical molecular imaging modalities for clinical use is the limited penetration of light in biological tissue, which makes the imaging of deep tissue structures and functional events difficult. Moreover, the strong light attenuation in tissues can also significantly affect the amount of light reaching the optical detector(s), making the quantitative analysis of fluorescent or bioluminescent light signal difficult.⁷⁰

Photoacoustic imaging

Photoacoustic imaging (PAI), also referred to as optoacoustic or thermoacoustic imaging, is an emerging hybrid biomedical imaging technique that is noninvasive, nonionizing, with high sensitivity, satisfactory imaging depth and good temporal and spatial resolution. In PAI, a short-pulsed laser is used to illuminate the tissue sample and generate photoacoustic waves due to the transient thermoelastic expansion in the tissue. Then the signals are measured by wide-band ultrasonic transducers to rebuild the image of the sample. Like conventional optical imaging, PAI presents the optical contrast which is highly sensitive to molecular conformation of biological tissues and can aid in describing tissue functional hemodynamic changes such as angiogenesis and hypoxia. With excellent sensitivity in detecting a variety of exogenous optical contrast agents including both organic dyes and gold nanocolloids, PAI holds promise for imaging at cellular or molecular level.^{71–73} Unlike conventional optical modalities, the spatial resolution of PAI is not limited by the strong light diffusion but instead determined mainly by the detection of light-generated photoacoustic signals. As a result, the resolution of PAI is parallel to high-frequency ultrasonography.^{74, 75} The high resolution of PAI especially benefits the morphological imaging of small articular structures of human hands and feet and the study on small-animal arthritis models. The high imaging resolution is also essential for accurate quantitative mapping of regional functional properties and molecular activities in affected synovial tissue. Furthermore, PAI does not depend on the measurement of ballistic/quasi-ballistic light. As a result, the imaging depth of PAI is sufficient for the studies of human peripheral joints and whole body imaging of small animals.⁷⁶

Before adapting PAI technique to the study on human arthritis, our group has first validated and optimized a PAI joint imaging system on rat models.^{77–79} Rat tail joints provide good samples to explore the performance of imaging human finger or toe joints considering their morphological similarity. Moreover, rheumatic disease rat models, including those with inflammatory arthritis, have been researched extensively and provide the opportunity to evaluate pathologic progression much more quickly than in humans. The details of the prototype PAI system for joint imaging have been reported in Ref. [77]. Photoacoustic signals were generated by a tunable laser (Vibrant B, Opotek) with a pulse repetition rate of 10 Hz and a pulse width of 5 ns. The wavelength of the laser light was tuned to the NIR region, which led to good penetration and enabled imaging of a human peripheral joint as a whole organ. The laser beam, after being expanded, irradiated the imaging object with an input energy density within the ANSI safety limit.⁸⁰ To realize high-resolution 2D imaging of a cross section in a joint, 2π circular scan around the cross section was conducted using a single ultrasonic transducer. In order to achieve 3D imaging of a volume with satisfactory resolution along each direction, circular scans along multiple cross sections of the joint were conducted. Then photoacoustic signals acquired at different positions around the joint were summed coherently to reconstruct 2D or 3D tomographic image of the joint.⁸¹ Using this single-transducer based PAI prototype system, the achieved spatial resolution was 200 μm in the imaged cross section, and was 500 μm along the sagittal direction of the joint. This spatial frequency response is sufficient to describe many small tissue structures in animal joints or human peripheral joints, and can be improved further by employing transducers with higher receiving frequencies and broader bandwidths for signal receiving. By using a single transducer for signal acquisition, the imaging speed of this system is limited, e.g. 10–20 min is needed to acquire a 2D cross-sectional image. Before being adapted to clinical use on patients affected by arthritis, the imaging speed of PAI needs to be improved significantly. For example, when a circular array transducer and multi-channel data acquisition can be employed for data acquisition, 2D imaging of a joint can be achieved with a frame rate equivalent to laser repetition rate (i.e. 10 Hz or higher).

In the 2D image of a rat tail joint acquired through a circular scan around the cross section [Fig. 10(a)], many articular tissue structures are presented successfully.⁷⁷ The spatial resolution achieved by PAI is better than the results of traditional optical imaging of joints. Based on the optical contrast among various tissues, extra- and intra-articular tissues, including skin, fat, muscle, vessels, periosteum and bone, are described clearly and match well with the histological picture taken from the similar cross section in the joint [Fig. 10(b)]. This study has proven the unique ability of PAI in describing joint structures based on the intrinsic optical properties of articular tissues. With PAI, high quality optical imaging of joints has been realized for the first time. Although the image quality can be improved further, this preliminary study demonstrates significant advancement in this field of optical imaging of inflammatory diseases.

Other than imaging morphological articular structures, PAI has also been examined for its ability to differentiate joints affected by inflammatory arthritis from the normal. Experiments have been conducted on an acute arthritis model, carrageenan-induced joint inflammation, and a chronic arthritis model, ankylosing spondylitis of HLA-B27 transgenic rats. It has been demonstrated that, based on strong optical contrast among tissues, PAI is able to characterize joint inflammation by presenting the morphological changes of soft articular tissues such as the proliferation and deformation of swollen synovial tissues. Moreover, PAI has also demonstrated its unique ability to visualize functional biomarkers of synovitis. Due to the increased blood volume in arthritic tissues as a result of inflammation, intra-articular tissues in the inflamed joints show higher optical absorption in comparison with those in the normal joints. Similar to other optical imaging modality, diagnosis and grading of joint inflammation using PAI is also based on the measurement of tissue optical properties. However, with excellent spatial resolution, PAI has the ability to present more morphological information of regional arthritic tissue that is essential for improving the diagnostic accuracy. Details of these imaging studies on arthritic rat models have been reported in Refs. [78,79]. Similar to conventional optical imaging techniques, PAI has also presented excellent sensitivity in imaging a variety of optical contrast agents, including organic dyes and gold nanocolloids.^{82–85} Experiments have been conducted to explore the sensitivity of PAI in imaging gold nanorod contrast agent distributed in animal joints.⁷²

Besides the studies on rat models, our group has also verified the feasibility of PAI of human peripheral joints for the first time.⁸⁶ The 2nd, 3rd and 4th fingers from one hand of a fresh unembalmed adult female cadaver were amputated. To maintain the tissue optical contrast, before severing the hand circumferential pressure bandages were applied to each finger to retain blood in these regions. The fingers at the levels of both the proximal interphalangeal (PIP) and distal interphalangeal (DIP) joints were imaged. The diameters of the fingers at the PIP and DIP joint regions were 20–25 mm and 15–20 mm respectively. After imaging, histological photographs of the imaged specimen sections were taken for comparison with PAI results. Example images of axial cross sections of human fingers are shown in Fig. 11, where (a) and (b) are the images of a finger at the levels of PIP joint and DIP joint respectively. Based on the optical contrast between various extra- and intra-articular tissues at 720-nm wavelength, soft tissue differentiation can be seen in these two images and match their corresponding histological photographs in Figs. 11(c) and (d) respectively. All the discernable tissue features in the images and histological photographs were marked by radiologists with extensive experience in musculoskeletal imaging. The small discrepancy between PAI findings and histological examinations is believed to be caused by the deformation of soft tissues during the histological procedure. Because the dominant absorption chromophores in the joints are hemoglobin at the applied wavelength, the contrast presented by PAI images mainly reveals the blood concentrations in various articular tissues.

This study has demonstrated the feasibility of PAI in noninvasive imaging of human joints and has presented that the imaging depth of PAI is sufficient for presenting a peripheral joint as a whole organ. In the future, spectroscopic PAI (i.e. SPAI) employing multiple wavelengths may further evaluate hemodynamic properties in joint tissues, which can potentially quantify the neoangiogenesis and hypoxia in pathological synovium.^{87,88} Considering that broad bandwidth ultrasound detection is the key in PAI technique, PAI is a natural and promising complement to conventional ultrasound technologies. In the long run, a revolutionary PAI-ultrasound dual-modality system may be available for clinical use for diagnostic imaging and treatment monitoring of arthritis. By presenting both optical and ultrasound contrast as well as metabolic and physiological information including blood flow, blood volume, hemoglobin oxygenation and vascular density, the dual-modality system may provide more comprehensive diagnostic information than by using conventional ultrasonography alone. Such a system for arthritis detection/characterization in the future could be much as with MRI, but with significantly lower cost and with no use of gadolinium contrast agents.

Discussion

The focus of joint imaging in the future will be on gathering more data on each of the joint structures both on an anatomical and cellular level. Structures include underlying and surface bone, synovial tissue including vasculature, cartilage and synovial fluid constitution are of importance. Cellular migration, cytokine production evaluation also may add critical insight to joint disease. Evaluation of both pre and post treatment scenarios along with being able to produce evaluations in a noninvasive manner so chronological studies can be undertaken are other major goals. Causing less harm to the patient via smaller doses or no radiation can also not be understated. Many of the above goals may be realized with the emerging optical imaging technologies.

In Table 1 presenting the application characteristics of each optical modality, we can see the pros and cons of these optical techniques for musculoskeletal imaging. Optical imaging has already become a powerful research tool for arthritis and other musculoskeletal disorders, and has shown great potential to be used in clinic setting. Noninvasive nonionizing optical imaging may greatly benefit medical imaging of synovitis with its unique advantages, including good soft tissue contrast, excellent performance in evaluating tissue functional properties including hemodynamic changes in synovial tissue, and extraordinary molecular imaging ability with its great sensitivity in tracing a variety of optical contrast agents. Optical modalities could provide radiologists and rheumatologists with easy-to-use but powerful and stand-alone tools for screening, diagnosis or therapeutic monitoring of musculoskeletal diseases. With relatively simple instrumentation requirements, optical methods may cut costs of clinical and research related imaging.

It is unlikely that optical imaging will replace any of the established musculoskeletal imaging methods. For example, it is hard to see DOI or PAI replacing X-ray for RA, as the spatial resolution of DOI is still limited while the ability of PAI in mapping the structures of bones is not satisfactory. Moreover, since the imaging depths of optical modalities are limited by the penetration of light in tissues, optical imaging of large human joints, such as wrist and ankle, could be very challenging. However, optical techniques may be combined with other methods for multimodality imaging to achieve much improved sensitivity and specificity. For example, novel PAI in combination with conventional ultrasound imaging can potentially enable dual-modality imaging of joints with both light and ultrasound. Combining PAI with ultrasound may not only help interpret PAI findings with ultrasound images but also facilitate the acceptance of the new PAI technique in clinic. By presenting the same target joint with both optical and ultrasonic contrast as well as metabolic and

physiological information including blood flow, blood volume, hemoglobin oxygenation and vascular density, this dual-modality imaging may provide more comprehensive diagnostic information than by using conventional musculoskeletal ultrasonography alone.

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Insight, innovation and integration statement

Pathology involved in arthritis includes angiogenesis, hypervascularization, hyper-metabolism and relative hypoxia. Recently, optical modalities for imaging of arthritic diseases have drawn considerable attention because they offer unique advantages over the existing imaging methods. Optical tissue contrast in the visible and near-infrared (NIR) region of the electromagnetic spectrum is intrinsically sensitive to tissue abnormalities. Due to high spatial and contrast resolution optical imaging can quantify pathologic inflammatory arthritic morphological and physiological changes potentially enabling early diagnosis of inflammatory arthritis. Optical modalities such as fluorescence imaging can further provide new insights on a molecular level regarding pathology involved in inflammatory arthritic disorders. Optical imaging can also provide noninvasive and nonionizing serial measurements for monitoring of therapeutic interventions with potentially even higher sensitivity and specificity than other imaging modalities. Optical imaging of arthritis is an up and coming field that likely will contribute to significant breakthroughs in understanding arthritic rheumatic diseases.

Fig. 1.

Laser transillumination imaging of human PIP joints.³⁶ The extended area in the middle of each image, built by the laser light that is transmitted through the joint, holds the information for the assessment of the joint inflammation. (a) Laser light-transmitted image of (a) a PIP joint of a healthy control, and (b) an actively inflamed PIP joint of a patient with early RA.

Fig. 2.

(a) 2-D images of the metacarpal bones of a human palm.⁴¹ The darker areas show the location of the bones and the lighter parts show the gaps between them. (b) Spatial intensity profile integrated over the region enclosed by the dashed box in (a).

FIG. 3. Optical computed tomographic images of a PIP joint of a human index finger.⁴³ (a) Optical CT image obtained at 1064 nm. (b) Optical CT image obtained at 715 nm. (c) Hard x-ray CT image. (d) Hard x-ray CT image enhanced for visualization of soft tissue. (e) T1 weighted MRI image. (f) T2 weighted MRI image.

Fig. 4.

In vivo DOI of a PIP joint of a human index finger, where 2D optical images correspondent to different cross sections of the joint are reconstructed.⁴⁷ B: bone; B/C: bone/cartilage; BV: blood vessel; T: tendon; S: synovium.

Fig. 5. Absorption (left column) and scattering (right column) images in a sagittal plane through the index finger (top row; inflamed), middle finger (middle row; inflamed) and ring finger (bottom row; normal) of the right hand of a RA patient.⁵²

Fig. 6.

Comparison of spatial mappings of [HbO₂], [Hb], and [HbT] for a healthy subject and two RA patients.⁵⁵ Left column: a healthy right index finger joint; middle column: a right index finger joint affected by RA; right column: another right index finger joint affected by RA.

Fig. 7. *In vivo* NIRF imaging of inflammatory joints in the LPS induction mouse model.⁵⁸ (a) White-light images obtained 48 hours after intra-articular LPS injection at the right ankle joint; soft tissue swelling can be noted at the affected joint. (b) NIRF images obtained 24 hours after NIR2-folate injection. Note the strong fluorescence signal in the LPS-treated right ankle compared with the opposite control side. (c) A merged NIRF image with a white-light image showing increased fluorescence at the inflamed joint.

Fig. 8. NIRF images of the untreated rat with arthritis induced in the right knee (a, b) and one cortisone-treated rat (c, d) with autoimmune arthritis before (a, c) and at 4 h after (b, d) the injection of DiD-labeled leukocytes. The arrow indicates the location of the right knee. The strong signal in the foot and ankle regions was believed to be caused by autofluorescence of the fur in these unshaved areas.

Fig. 9.

Regulation of human OC in skeletal organs of postnatal developing hOC-Luc mice. hOC promoter gene activity was investigated during the development of hOC-Luc mice (age, 1 week to 1.5 years) by bioluminescent imaging.

Fig. 10.

(a) Noninvasive imaging of a cross section of a rat tail joint. (b) Histological picture of the rat tail joint taken along the plane as closely matched as possible to that of the image.

Fig. 11. Cross-sectional PAI images at the (a) PIP and (b) DIP joint regions of a human finger harvested from a fresh cadaver. Correspondent histological photographs at the (c) PIP and (d) DIP regions of the finger. AP: aponeurosis; PH: phalanx; SK: skin; SU: subcutaneous tissue; TE: tendon; VP: volar plate.

Table 1

Application characteristics of each optical modality for musculoskeletal imaging.⁶⁹

	Transillumination imaging	Diffuse optical imaging	Fluorescence imaging	Bioluminescence imaging	Photoacoustic imaging
<i>Sensitivity</i>	Good	Good	High	High	Good
<i>Spatial resolution</i>	mm	mm	mm	mm	μm-mm
<i>Imaging depth</i>	cm	cm	mm-cm	mm-cm	cm
<i>Quantitation</i>	In part	Yes	Yes	Yes	Yes
<i>Imaging speed</i>	High	High	High	High	High
<i>Ready for clinical use</i>	Yes	Yes	No	No	Yes
<i>Small animal device</i>	Yes	Yes	Yes	Yes	Yes