Abstract (English)

Based on the success of artificial intelligence (AI), especially in its application for classification of digital images in the world wide web, its utilisation for automated diagnostics of medical image data has become a major focus. Despite great results with prediction tasks involving big data, a naïve application of deep learning, i.e., use without any prior knowledge about the respective domain, may not be the optimal solution when there are only small amounts of data for the prediction task at hand, which is usually the case in medical studies and biological experiments. Therefore, it may be beneficial to integrate prior information, abbreviated prior, in deep learning or use a more traditional prior-based learning approach.

In this work, **novel macroscopic and microscopic imaging biomarkers for computed tomography (CT) and multiphoton microscopy (MPM) are identified** by developing image processing and learning techniques for biomarker research in **pneumonology, oncology, and muscle research**. From a biological point of view, we were able to improve **fracture discrimination** in CT by detailed analysis of muscle and lipid mixing in the thigh compared to using known bone imaging biomarkers alone. Also, our approach of combining the assessment of macroscopic tumour spread and distribution with machine learning allowed better **survival prediction for cancer patients** than using known biomarkers or naïve applications. We present a calibration method for CT that helped to **preserve the predictive performance of known imaging biomarkers** even when large technical variation and bias induced by the image acquisition process are prevalent. Lastly, we present an AI system that was able to **predict various single muscle fibre properties** from MPM images with better performance than previously known biomarkers.

From a technological point of view, a continuum of learning methods between the old world, i.e., statistics or traditional machine learning with handcrafted features, and the new, i.e., deep learning and meta-learning, is utilised. Our motivation to use prior information leads to novel hybrid learning-based biomarker systems combining prior knowledge with AI. We show that **prior information about a task can effectively be integrated to improve predictive performance of learning algorithms compared to naïve approaches**. Within this work, a prior can be the engineering and integration of suitable features based on biological knowledge about a task, the choice of a suitable data representation for an AI system, or physics knowledge about the nature of noise and artifacts. Our results further indicate that the **representation of the data input to a learning algorithm can be more important than the learning algorithm itself**, and that a suitable data representation for an AI can be different from that for the human observer, e.g., a radiologist. In the final chapter, an **AI is presented that simultaneously determines the required complexity of its neural network architecture, the data representation, and the degree of prior knowledge integration** using meta-learning. It is shown that the integration of priors into deep learning with simultaneous optimisation of the data representation provides the best results, better than naïve deep learning models without priors but also better than models that solely rely on priors.

Altogether, this work presents novel biomarker models for different medical disciplines by combining AI with priors, effectively refining the prior knowledge by AI and regularising complex AI models by priors.

Zusammenfassung (Deutsch)

Aufgrund des Erfolgs der künstlichen Intelligenz (KI), insbesondere bei der Klassifizierung digitaler Bilder im World Wide Web, ist ihre Nutzung für die automatisierte Diagnostik medizinischer Bilddaten in den Fokus gerückt. Trotz großartiger Ergebnisse bei Vorhersagen mit großen Datenmengen, ist eine uninformierte Anwendung von Deep Learning, d. h. dessen Verwendung ohne jegliches Vorwissen über den jeweiligen Bereich, möglicherweise nicht die optimale Lösung, wenn nur wenige Daten für die jeweilige Vorhersageaufgabe vorliegen. Dies ist bei medizinischen Studien und biologischen Experimenten in der Regel der Fall. Daher kann es von Vorteil sein, Vorinformationen, auch bekannt als *Priors*, in Deep Learning zu integrieren oder einen klassischeren, auf Vorwissen basierenden Lernansatz zu verwenden.

In dieser Arbeit werden **neue makroskopische und mikroskopische Bildgebungs-Biomarker für die Computertomographie (CT) und Multiphotonenmikroskopie (MPM) identifiziert**, indem Bildverarbeitungs- und Lerntechniken für die Biomarkerforschung in der Pneumologie, Onkologie und Muskelforschung entwickelt werden. Aus biologischer Sicht konnten wir die **Frakturdiskriminierung** mittels CT durch eine detaillierte Analyse der Muskel- und Lipidmischung im Oberschenkel im Vergleich zur alleinigen Verwendung bekannter Knochenbiomarker verbessern. Auch unser Ansatz, die Quantifizierung der makroskopischen Ausbreitung und Verteilung von Tumoren mit maschinellem Lernen zu kombinieren, ermöglichte eine **bessere Überlebensvorhersage für Krebspatienten** als bekannte Biomarker oder uninformierte Anwendungen. Des Weiteren wird eine Kalibrierungsmethode für die CT vorgestellt, die dazu beiträgt, die **Vorhersagekraft bekannter Biomarker zu erhalten**, selbst wenn eine technisch-bedingte Variation und Verzerrung durch den Bildaufnahmeprozess existiert. Schließlich präsentieren wir ein KI-System, das in der Lage war, **verschiedene Eigenschaften einzelner Muskelfasern auf MPM-Bildern mit besserer Leistung als bisher bekannte Biomarker vorherzusagen**. Aus technologischer Sicht wird ein Kontinuum von Lernmethoden zwischen der alten Welt, d.h. Statistik oder dem traditionellen maschinellen Lernen mit „handgefertigten“ *Features*, und der neuen Welt, dem Deep Learning und Meta-Learning, genutzt. Unsere Motivation, Vorinformationen zu nutzen, führt zu neuartigen hybriden lernbasierten Biomarker-Systemen, die Vorwissen mit KI kombinieren. Wir zeigen, dass ***Priors* über eine Aufgabe effektiv integriert werden können, um die Vorhersageleistung von Lernalgorithmen im Vergleich zu uninformierten Ansätzen zu verbessern**. Im Rahmen dieser Arbeit kann ein *Prior* die Entwicklung und Integration geeigneter *Features* sein, die auf biologischem Wissen über ein Problem basieren, die Wahl einer geeigneten Datendarstellung für ein KI-System, oder physikalisches Wissen über die Natur von Rauschen und Artefakten. Unsere Ergebnisse deuten außerdem darauf hin, dass die **Repräsentation der Daten, die in einen Lernalgorithmus eingegeben werden, wichtiger sein kann als der Lernalgorithmus selbst**, und dass eine geeignete Datenrepräsentation für eine KI eine andere sein kann als für den menschlichen Betrachter, z.B. einen Radiologen. Im letzten Kapitel wird eine **KI vorgestellt, die die erforderliche Komplexität ihrer neuronalen Netzwerkarchitektur, die Datenrepräsentation und den Grad der Integration von *Priors* mit Hilfe von Meta-Learning selbst bestimmt**. Es wird gezeigt, dass die Integration von *Priors* in Deep Learning bei gleichzeitiger Optimierung der Datenrepräsentation die besten Ergebnisse liefert, besser als uninformierte Deep Learning-Modelle, aber auch besser als Modelle, die ausschließlich auf *Priors* basieren.

Diese Arbeit präsentiert innovative Biomarker-Modelle für verschiedene medizinische Fachbereiche, die KI und Vorwissen miteinander kombinieren. Einerseits wird hierbei das Vorwissen durch Einsatz von KI verbessert, andererseits werden komplexe KI-Modelle durch das Vorwissen regularisiert.

1. Introduction and State-of-the-Art

1.1. Macro- and Microscopic Imaging Technologies for Biomarker Identification

1.1.1. Computed Tomography (CT) for Macroscopic Imaging

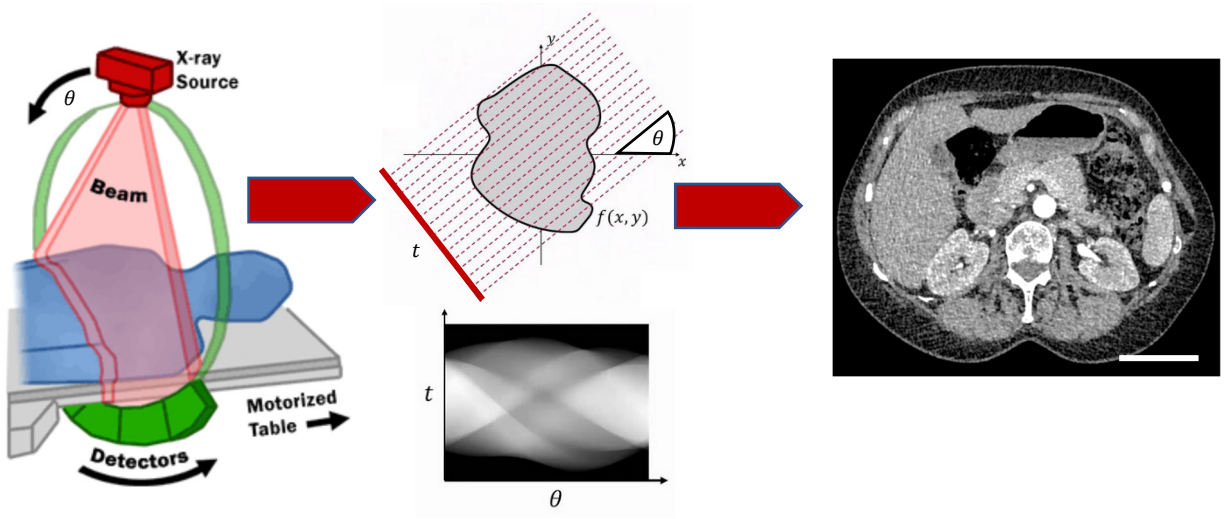

Figure 1. Schematic drawings of CT image acquisition. Information about the attenuation of photons by the object is collected in the detectors (left) and used to reconstruct the object f(x,y) (centre, top) slice-wise from its sinograms (centre, bottom), i.e., the attenuation profile with offset t from the detector origin for all rotation angles θ. For simplification, parallel beams were assumed in the central image. An exemplary axial CT image of the abdomen is shown on the right. Bar: 10cm. Modified from U.S. Food & Drug Administration[1] and Van Aarle et al.[2].

Computed tomography (CT) is an x-ray-based diagnostic imaging modality. CT utilises that the radiodensity of biological tissue, i.e., the relative inability of electromagnetic radiation to pass through it, is dependent on the material's physical density and its mass attenuation coefficient[3]. The latter is a function of the atomic number of the material and the photon energy. The attenuation of x-ray photons can thus be used to determine the radiodensity and, accordingly, also tissue distribution and tissue properties within objects. As shown in (Fig. 1., left), a CT gantry, consisting of a photon emitter and a detector, rotates around an object, and the attenuation of x-ray photons after passing the object is measured in the detector. By rotating the gantry, attenuation profiles (projections) for different gantry angles can be obtained. The projection information over all angles is termed sinogram (Fig. 1, centre bottom). The operation that enables the generation of a sinogram from a 2D image is known as Radon transform[4], and the inverse Radon transform, accordingly, is the required operation to solve the CT image reconstruction problem. Applying image reconstruction on a sinogram yields an axial 2D image (Fig. 1, right), and by movement of the motorised table, a 3D image, i.e., volume, of the object can be generated. The radiodensity shown in CT images is usually provided in the Hounsfield scale, in which distilled water at standard pressure has a Hounsfield unit (HU) of 0 and air of -1000 HU.

A common reconstruction algorithm is the filtered back projection (FBP) which is a practical and fast solution for the ill-posed inverse Radon transform problem[5]. FBP can be derived by the central slice theorem that links 1D projections and the corresponding axial 2D image by Fourier and inverse Fourier transforms, thereby enabling reconstruction of 2D images from the sinogram in a simple and fast process[3]: the projection for each angle (of the sinogram) is convolved with a reconstruction kernel, and the results are integrated over all angles in 2D. The reconstruction kernel is selected specifically for the

radiological examination at hand as a trade-off between noise- and detail-level, with the hard kernels providing sharper images with more details, and soft kernels providing images with less noise and artifacts[3]. More advanced algorithms for image reconstruction in CT are based on iterative reconstruction (IR) which involves iteratively solving an optimisation problem to estimate the image from the projection data. This method results in improved image quality, but it also requires a longer reconstruction time compared to FBP. IR minimises the difference between the measured projection data and the estimated projections based on the current estimate of the image, improving the image estimate with each iteration[6,7]. IR can incorporate prior information, such as model-based knowledge of anatomy or structure, to improve the image estimate, reduce noise and artifacts, and speed up convergence. Besides the reconstruction algorithm, further important influencing factors for the image formation are the tube voltage which determines the energy spectrum of the photons, and the tube current which determines the photon quantity. Higher tube current results in lower statistical noise as more photons arrive at the detector, but it also leads to higher radiation exposure. Higher tube voltage is needed if the object would otherwise attenuate the photons too much, but this also results in lower image contrast.

CT is by far the most widely used tomographic imaging modality with approximately 300 million scans per year worldwide compared to 95 million magnetic resonance imaging (MRI) scans[8]. It is commonly the imaging method of choice in oncology, pneumonology, in the emergency department, and for the assessment of bone structures or calcium deposits. Compared to MRI, its more frequent application in oncology is based on the lower cost and wider availability of CT while yielding sufficient diagnostic information for most cancer types. In pneumonology, it is based on the better imaging capabilities to assess slight density differences of air-filled organs, like the lungs. In the emergency room, especially the significantly faster image acquisition compared to MRI is the deciding factor for CT. Finally, CT is superior for the assessment of bone structures or calcium deposits, e.g., in the coronaries, as a result of its imaging mechanism based on attenuation.

Variants of CT are used in different clinical and research scenarios. Contrast medium is injected in around 40% of all CT examinations[8] to increase the contrast of structures of interest thus enabling, e.g., the indirect assessment of tumour metabolism[9,10] or the diagnosis of cardiovascular diseases[11]. Dual-energy CT[12] uses photons from two energy spectra to exploit the high energy dependence of the mass attenuation coefficient of materials with high atomic numbers. It, thereby, allows material decomposition to assess kidney stones, gout, or iodine content and even enables the generation of synthetic images like virtual non-contrast, i.e., the contrast medium is virtually removed from the images. Current research and also an emerging clinical application field is photon-counting CT in which the detector measures single photons and their energy (energy-resolving) instead of the sum of all energies of the photons reaching the detector (energy-integrating)[13]. In photon-counting CT, commonly four energy bins are used for the incoming photons, and the number of photons in each of these bins is counted. This allows material decomposition, in analogy to dual-energy, in each scan and yields even more multi-spectral information. Furthermore, this technique provides increased contrast-to-noise ratio (CNR) and spatial resolution. It can reduce noise and various artifacts, like beam hardening, and enables to discriminate photons with energies below the lowest energy bin, resulting in an elimination of electronic noise. To enable photon-counting CT, a novel detector was constructed which replaces the common scintillation detector by a semi-conductor detector that directly transforms photons into electric pulses according to their energy. Lastly, for preclinical research, micro-CT can be employed, which offers higher resolution and is often used for *in vitro* and *in vivo* small animal imaging[14].

1.1.2. Label-free Multiphoton Microscopy (MPM) for Microscopic Imaging

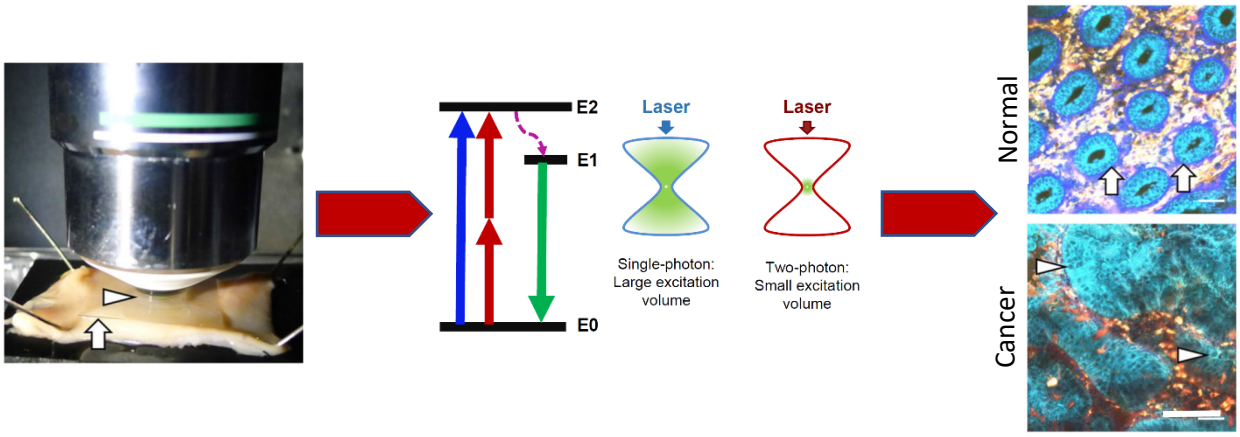

Figure 2. Schematic drawings of label-free MPM image acquisition. A colorectal tissue sample is examined by MPM (left) for oncological diagnostics. The sample is covered by a coverslip (arrow) and a water drop (arrowhead) is used as immersion. (centre) Two photons are absorbed to excite a fluorophore. When the electron falls back to the ground state, fluorophore-specific light Is emitted. The excitation volume for two-photon microscopy is smaller than for single-photon applications due to excitation based on the simultaneous absorption. (right) MPM enables to differentiate normal colon tissue from cancer. Arrows and arrowheads indicate the epithelial cells of normal and cancer tissue, respectively. Images were acquired at 780nm excitation and using bandpass filters of 417/60nm (blue: epithelial cells and fibrous tissue), 480/40nm (green: immune cells and epithelial cells), and 629/56nm (red: immune cells). Bar: 100µm. Modified from Matsui et al.[15] and Kreiss et al.[16].

Multiphoton microscopy (MPM) is an advanced optical microscopy technology that utilises a pulsed laser as excitation source[17]. In this approach, two laser photons of lower energy are simultaneously absorbed for the excitation of a single fluorophore. This fluorophore can either be an exogeneous marker that is added to the sample by fluorescent staining[18] or a native molecule in the sample. When the excited electron falls back to its ground state, light is emitted from the fluorophore, which can then be recorded. One main advantage of this technique over conventional fluorescence microscopy is that the excitation is naturally confocal and without simultaneous light emission from out-of-focus planes. This is due to the constraint of spatially-confined simultaneous photon absorption. In addition, since the probability of scattering increases for photons of higher energy, MPM manages to produce images deeper in the tissue by using photons of lower energy (Fig. 2).

MPM also allows second-harmonic generation (SHG) imaging by using short laser pulses[18]. Samples with second order susceptibility properties are polarised by the electric field component of the incident light. This polarisation can be described in a Taylor series. If the electrical field is strong enough, the otherwise negligible second term of this series induces a secondary wave at exactly twice the frequency. In comparison to two photon-induced fluorescence, SHG photons have twice the energy as incident photons[19,20]. Therefore, the SHG signal can not only be separated from light of the source but also from fluorescence by using appropriate dichroic mirrors and filters. The application of SHG, however, is restricted to biomolecules without inversion symmetry such as tubulin, collagen-I, and myosin-II[21]. As a main advantage, SHG is sensitive to the orientation of the examined probes. This can, for example, be used to analyse muscle fibres, more specifically the structural state of myofibrillar myosin polymers[22].

Most optical microscopy methods use exogenous markers for the examined samples. However, these can interfere with the probe's biology or its intrinsic binding homeostasis. Label-free approaches utilise the non-linear excitation of native fluorophores, e.g., FAD or NADH, in combination with the SHG of native biomolecules. These label-free variants are particularly interesting as they enable the use of MPM for endoscopic examinations, i.e., a so-called multiphoton endomicroscopy[23].