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IMAGE ANALYSIS: A PERSPECTIVE

The history of biomedical imaging is comparatively short. In 1895, Wilhelm Conrad Röntgen discovered a new type of radiation, which he called the x-ray. The discovery caused a revolution in medicine, because for the first time it became possible to see inside the human body without surgery. Use of x-rays in medical centers spread rapidly, but despite their vast popularity, little progress was made for over half a century. Soon after the discovery of x-rays, materials were discovered that exhibited visible-light fluorescence when illuminated by x-rays. With such materials, the quantum efficiency of film-based x-ray imaging could be improved and the exposure of patients to radiation thus reduced. Contrast agents were introduced around 1906 to allow imaging of some soft tissues (namely, intestines), which show low x-ray contrast. For about six decades, x-ray tubes, film, and x-ray intensifying materials were improved incrementally, but no fundamental innovation was made.

After World War II, the next important development in biomedical imaging finally arrived—ultrasound imaging. The medical technology was derived from military technology: namely, sonar (sound navigation and ranging), which makes use of sound propagation in water. Applying the same principles to patients, sound echos made visible on oscilloscope-like cathode ray screens allowed views into a patient's body without the use of ionizing radiation. The mere simplicity of creating sound waves and amplifying reflected sound made it possible to generate images with analog electronics—in the early stages with vacuum tubes. Electronic x-ray image intensifiers were a concurrent development. X-ray image intensifiers are electronic devices that are based on a conversion layer that emits electrons upon x-ray exposure.

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These electrons are collected and amplified, then directed on a luminescent phosphor. Here, the image is formed with visible light and can be picked up by a video camera. Electronic intensifiers made it possible to further reduce patient exposure to x-rays and speed up the imaging process to a point where real-time imaging became possible. At this time, video cameras could be used to record x-ray images and display them instantly on video screens. Interventional radiology and image-guided surgery became possible.

The next major steps in biomedical imaging required an independent development: the evolution of digital electronics and the microprocessor. Milestones were the invention of the transistor (1948),[1] the integrated circuit as a prerequisite for miniaturization (1959), and the first single-chip microprocessor (1971).[20] Related to these inventions was the first integrated-circuit random-access memory (RAM; 1970).[62] Although the microprocessor itself was built on the principle of the programmable computer devised by Conrad Zuse in 1936, the miniaturization was instrumental in accumulating both computing power and memory in a reasonable space. Early digital computers used core memory, which got its name from small ferrite rings (cores) that could store 1 bit of information because of their magnetic remanence. Core memory was already a considerable achievement, with densities of up to 100 bits/cm^2. Early RAM chips held 10 times the memory capacity on the same chip surface area. In addition, integrated-circuit RAM did away with one disadvantage of core memory: the fact that a core memory read operation destroyed the information in the ferrite rings. Consequently, read and write operations with integrated-circuit RAM were many times faster. For four decades, integration density, and with it both memory storage density and processing power, has grown exponentially, a phenomenon known as *Moore's law*. Today's memory chips easily hold 1 trillion bits per square centimeter.*

The evolution of digital electronic circuits and computers had a direct impact on computer imaging. Image processing is memory-intensive and requires a high degree of computational effort. With the growing availability of computers, methods were developed to process images digitally. Many fundamental operators[15,18,24,32,36,43,64,72] were developed in the 1960s and 1970s. Most of these algorithms are in common use today, although memory restrictions at that time prevented widespread use. A medical image of moderate resolution (e.g., 256×256 pixels) posed a serious challenge for a mainframe computer with 4096 words of core memory, but today's central processing units (CPUs) would effortlessly fit the same image in their built-in fast cache memory without even having to access the computer's main memory. A convolution of the 256×256-pixel image with a 3×3 kernel requires almost 600,000 multiplications and the same number of additions. Computers in the 1970s were capable of executing on the order of 100,000 to 500,000 instructions per second (multiplication usually requires multiple instructions), and the convolution above would have cost several

*Today's most dense memory chips pose one challenge reminiscent of the information loss in core memory: Since the information is stored as a charge in a capacitor, which tends to leak and discharge slowly, the information needs to be read and rewritten several thousand times per second, a process known as *memory refresh.*

seconds of CPU time. On today's computers, the same convolution operation would be completed within a few milliseconds.

The availability of early mainframe computers and minicomputers for data processing enabled new revolutionary imaging modalities. In 1917, mathematician J. Radon stipulated that a manifold can be represented (transformed) by an infinite number of line integrals.[60] Almost 50 years later, when mainframe computers became widely accessible, A. M. Cormack developed an algorithm based on Radon's idea,[13,14] which in turn helped G. Hounsfield develop the computed tomography (CT) scanner.[37] Cormack and Hounsfield shared a Nobel prize in 1979 for development of the CT scanner. In fact, CT was a completely new type of imaging modality because it *requires* computed data processing for image formation: The x-ray projections collected during a CT scan need to be reconstructed to yield a cross-sectional image, and the reconstruction step takes place with the help of a computer.[42] Other imaging modalities, such as single-photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) also require the assistance of a computer for image formation.

Another important development in biomedical imaging resulted from the use of radioactively labeled markers. One such example is indium pentetreotide, a compound that acts as an analog for somatostatin and tends to accumulate in neuroendocrine tumors of the brain.[69] Indium pentetreotide can be labeled with radioactive ^{111}In, a gamma emitter. Another example is fluorodeoxyglucose, a glucose analog. Fluorodeoxyglucose accumulates at sites of high metabolic activity. When fluorodeoxyglucose is labeled with ^{18}F, it becomes a positron emitter. Radiation emission becomes stronger near active sites where the radiolabeled markers accumulate, and with suitable devices, tomographic images of the concentration of the radioactive compounds can be gathered. The use of positron emitters that create gamma rays as a consequence of electron–positron annihilation events was proposed in 1951[78] and eventually led to positron emission tomography (PET).[6] With radiolabeled physiologically active compounds (radiopharmaceuticals), it became possible to obtain images of physiological processes. These imaging methods not only improved the diagnosis of carcinomas, but also helped in our understanding of physiological processes, most notably brain activity. Functional imaging has become a key tool in medical diagnosis and research.

Subsequent research and development aimed at the improvement of image quality (e.g., improvement of resolution, better contrast, less noise). Current trends also include an increase in three-dimensional images and the involvement of computers in image processing and image analysis. A detailed overview of current trends is given in section 1.3.

1.1. MAIN BIOMEDICAL IMAGING MODALITIES

A number of fundamentally different methods of obtaining images from tissue, called *imaging modalities*, emerged during the historical development of biomedical imaging, and the information that these modalities provide differs among modalities.

It is outside our scope here to provide a detailed description of the physical and engineering foundations of the modalities, but a short overview is provided for completeness.

X-ray Imaging X-ray imaging is a projection method. The patient is illuminated by x-rays, high-energy photons that penetrate the body. Some of the x-rays are absorbed in the tissue. X-rays predominantly follow a straight path. The absorption process can be described by the *Lambert–Beer law*:

$$\ln I(x,y) = \ln I_0 - \int_s \mu(\vec{r})\, d\vec{r} \tag{1.1}$$

where I is the x-ray intensity that passes through a patient's body, I_0 the incident x-ray intensity, $\mu(\vec{r})$ the x-ray absorption coefficient at any spatial location $\vec{r}$, and the integration takes place along a straight line s, which intersects with the x-ray film at (x,y). At this location, the film is blackened by the x-rays, and the more x-rays that pass through the body, the higher the optical density of the film. At the end of this process, the film contains a two-dimensional distribution of optical density that relates to the tissue distribution inside the patient. If path s passes through bone, for example, the optical density at the end of that path is lower than that of a neighboring path, s', that traverses only soft tissue. In the case of film-based x-ray imaging, the film needs to be digitized with a film scanner to obtain a digital image. Filmless x-ray imaging with a digital detector is becoming more common.

Computed Tomography Computed tomography (CT) is an x-ray-based imaging method used to obtain a two- or three-dimensional map of absorbers inside the imaged object. The principle behind CT is to collect many projections, following equation (1.1), at various angles θ relative to the imaged object. One projection consists of measured attenuation values along parallel beams that are displaced a distance t from the center of rotation. When the incident beam intensity is known, the line integral along s can be represented by the computed attenuation p at detector position t and angle θ. Let us assume that the Fourier transform of the absorption map $\mu(x,y)$ is $M(u,v) = \mathcal{F}\{\mu(x,y)\}$, where the symbol $\mathcal{F}$ denotes the Fourier transform and u and v are the axes of the frequency-domain coordinate system (a detailed explanation is provided in Chapter 3). It can be shown that the one-dimensional Fourier transform of the projection with respect to t, $\mathcal{F}\{p(t,\theta)\}$, is identical to a one-dimensional cross section of the Fourier transform of the absorber map $M(u,v)$ subtending an angle θ with the u-axis. This relationship is known as the *Fourier slice theorem*. In CT, the projections $p(t,\theta)$ are obtained during the scanning process, but the absorption map $\mu(x,y)$ is unknown. The purpose of the scanning process is therefore to obtain many projection scans $p(t,\theta)$, to perform a Fourier transform, and to enter them at the angle θ into a placeholder $M(u,v)$, thereby filling as many elements of $M(u,v)$ as possible. The cross-sectional slice $\mu(x,y)$ is then obtained by computing the inverse Fourier transform of $M(u,v)$. Other reconstruction methods also exist (a comprehensive overview of CT reconstruction techniques is presented by Kak and

Slaney[42]), as well as reconstruction algorithms for beams that are not parallel but fan- or cone-shaped. To obtain projections at different angles, a CT scanner contains an x-ray source and a detector array mounted on opposite sides of a large ring (the gantry). The patient is placed in the center of the ring and the source-detector system rotates around the patient, collecting projections. The patient can be moved in the axial direction on a patient tray. The patient tray not only allows patient positioning but also the acquisition of three-dimensional images.

Magnetic Resonance Imaging Magnetic resonance imaging (MRI) is another modality that requires the use of a computer for image formation. In a strong magnetic field, protons orient their spins along the magnetic field. The magnetic moments are not perfectly aligned, but rather, precess around the external field lines with an angular frequency that is proportional to the external field. The precession frequency is known as the *Larmor frequency*. With an externally introduced radio-frequency (RF) signal in resonance, that is, at the Larmor frequency, the orientation of the electron spins can be manipulated, but after cessation of the RF signal, the spins return to their original position. During this process, the spins emit a weak RF signal (echo) that can be picked up by an antenna. The time is takes for the spins to return to their original position depends on the tissue. Magnetic gradients allow us to change the precession frequency and precession phase angle along the spatial axes, and the spatial origin of a RF echo component can be reconstructed by Fourier analysis of the signal. In fact, the task of any MRI pulse sequence (i.e., the sequence of RF signals that manipulates spin precession) is to fill a frequency-domain placeholder, called a *k-space matrix*, with data. Inverse Fourier transform of the *k*-space matrix yields the cross-sectional image. Depending on the pulse sequence, different information can be obtained from the tissue. Three tissue constants are the relaxation times T_1 and T_2 and the proton density (water content). These tissue constants can vary strongly between different types of soft tissue, and for this reason, MRI provides excellent tissue–tissue contrast.

Ultrasound Imaging Ultrasound imaging makes use of the physics of sound propagation in tissue. Sound waves propagate at a certain, tissue-dependent velocity. At the interface between two tissues, some of the sound is reflected, and the sound echo can be picked up by a receiver. The round-trip time of the echo can be translated into the depth of the echo source because the speed of sound is known. An *A-mode scan* (the echo strength as a function of depth) is obtained by emitting a short burst of sound into the tissue and recording the echos for a short period of time. Sound generation and recording are carried out by transducers made of a piezoelectric material, that is, crystals that deform under the influence of an electric field and that generate an electrostatic field when deformed. An A-mode scan can be represented as a thin line on a screen where the intensity depends on the echo strength. By directing the incident sound wave in different directions, a *B-mode scan* can be obtained. A B-mode scan consists of several parallel or fan-shaped A-mode scans. It is also possible to record A-mode scans as a function of time, which is referred to as an *M-mode* (motion

mode). Although ultrasound imaging could be performed with purely analog circuits, today's ultrasound devices use digital signal and image processing. One disadvantage of ultrasound imaging is its widely qualitative nature, although size measurements are possible with moderate accuracy. However, ultrasound can quantitatively measure motion (notably, blood flow) through the Doppler effect.

***Single-Photon Emission Computed Tomography* (*SPECT*)** SPECT is analogous to CT but uses a radiation source internal to a patient rather than the external x-ray generator used in CT. The radiation source is usually a radiopharmaceutical that emits gamma rays. External cameras obtain projections of the radiation strength analogous to the projection $p(t,\theta)$ in CT. Radiation is kept weak, and highly sensitive cameras need to be used to capture as many radioactive events as possible. Since the radioactive events are random processes, the image has a very high noise component. Therefore, SPECT image reconstruction methods are optimized for noisy images. Furthermore, the resolution is much lower than that of CT. However, SPECT allows us to image the accumulation of radioactive tracers at physiologically active sites. Whereas CT provides information about the structure of tissue, SPECT provides information about function and physiological activity.

***Positron Emission Tomography* (*PET*)** Like SPECT, PET uses radiolabeled markers to image physiological activity. Whereas SPECT uses primarily gamma emitters, the radiolabeled compounds in PET are positron emitters (such as ^{18}F, ^{15}O, ^{124}I, and ^{89}Zr). The positron (i.e., antimatter electron) collides with an electron a short distance from its emission site. In this annihilation event, two high-energy gamma photons are emitted in opposite directions. These photon pairs are captured in a detector ring. The occurrence of photon pairs is critical for PET imaging, because the time-of-flight difference, together with the location of the registering detectors, allows us to determine the exact location of the annihilation event in the scanned slice. Moreover, spontaneous background events can be excluded; only coincident gamma pairs are recorded. A sufficient number of recorded events create an image of spatially resolved physiological activity. Like SPECT images, PET images have low spatial resolution and low signal-to-noise ratio.

Visible-Light Imaging Visible-light imaging with conventional digital cameras or specialized scanning elements also play an important role in biomedical imaging. Often, visible-light imaging is found in conjunction with microscopy. A large number of techniques provide information about the imaged object, such as a cell layer or tissue sample. Tissue can be stained (histology) or labeled fluorescently. Fluorescent markers can provide physiological information on the microscopic level in a complementary fashion to SPECT and PET, which provide physiological information on a macroscopic level. Confocal microscopy allows us to obtain three-dimensional volumetric images.

1.2. BIOMEDICAL IMAGE ANALYSIS

Biomedical image analysis is a highly interdisciplinary field, being at the interface of computer sciences, physics, medicine, biology, and engineering. Fundamentally, biomedical image analysis is the application of image processing techniques to biological or medical problems. However, in biomedical image analysis, a number of other fields play an important role:

- *Anatomy*. Knowledge of shape, structure, and proximity to other anatomical objects can help identify features in images and determine abnormalities.
- *Physiology*. Physiology plays a role in functional imaging, where functional imaging should be defined very broadly, ranging from blood flow imaged with Doppler ultrasound to cell physiology imaged with microscopy and fluorescent probes. The term *functional imaging* is often used for modern methods to image physiological processes by means of functional MRI, PET, or SPECT.
- *Physics of the imaging modality*. Depending on the imaging modality, the image values represent fundamentally different properties of the imaged object. Examples include x-ray attenuation in CT, optical light scattering and absorption in microscopy and optical coherence tomography (OCT), and magnetic spin relaxation time constants in MRI.
- *Instrumentation*. Even within the same modality, images of the same object can be markedly different. One example is x-ray imaging and CT, where the anode voltage and the introduction of beam-hardening filters influence the apparent x-ray density, whereas beam collimation determines the amount of haze and blur in the image. Another example is the exposure time and source brightness in optical modalities (microscopy, OCT), which may produce contrast in different regions of the object. In addition, every imaging instrument introduces some amount of noise.
- *Medical application*. The medical application in diagnosis or intervention provides the foundation and motivation for biomedical image analysis. The selection of an imaging modality and of possible image processing steps depends on many medical factors, such as the suspected disease or the type of tissue to be imaged.

In addition, several fields of computer science exist on top of image processing that play a role in biomedical image analysis, most notably artificial intelligence and computer modeling. Artificial intelligence approaches find their ways into biomedical image analysis in the form of fuzzy logic,[2,26,48] evolutionary computing,[9,73] computer learning,[44,59] and artificial neural networks.[28,79,82] Computer models play a key role in advanced segmentation techniques and in the description of time-course dynamics.[35,50,70]

Biomedical image analysis consists of four distinct stages, where each stage is generally a prerequisite for the next stage, but at any stage the chain can end to

allow a human observer to make a decision or record results. These stages are image acquisition, image enhancement and restoration, image segmentation, and image quantification.

1.2.1. Image Acquisition

The first stage is to gather information about the object, such as a suspect tissue in a patient. In the context of an image, the information is spatially resolved. This means that the image is a map of one or more tissue properties on the nodes of a discrete rectangular grid. The grid nodes coincide with integer coordinates, and the image value on an integer coordinate is termed a *pixel* (picture element) or *voxel* (volume element) in three-dimensional images. The image values are stored in finite memory; therefore, the image values themselves are also discrete. In many cases, image values are limited to integer values. Noninteger values can be used as image values if a floating-point representation for a pixel is used, but floating-point values have limited precision as well.

The image values themselves generally have physical meaning. To name a few examples, photography and microscopy provide image values that are proportional to light intensity. Computed tomography provides image values that are proportional to local x-ray absorption. In magnetic resonance imaging, the image values can represent a variety of tissue properties, depending on the acquisition sequence, such as local echo decay times or proton density.

The goal of the image acquisition stage is to obtain contrast. To use x-ray imaging as an example, let us assume a patient with a suspected clavicular hairline fracture. X-rays passing through the clavicle are strongly attenuated, and film optical density is low. X-rays along neighboring paths that lead though soft tissue are less attenuated, and the optical density at corresponding locations of the film is higher. Contrast between bone and surrounding soft tissue is generally high. Some x-rays will pass though the fracture, where there is less bone to pass through, and the corresponding areas of the x-ray film show a slightly higher optical density—they appear darker. In this example it is crucial that the contrast created by x-rays passing through the intact clavicle and through the fracture is high enough to become discernible. This task is made more difficult by the inhomogeneity of other tissue regions that are traversed by the x-rays and cause unwanted contrast. *Unwanted contrast* can be classified as noise in the broader sense (in a stricter sense, noise is a random deviation of a pixel value from an idealized value), and distinguishing between contrast related to the suspected disease—in this case the clavicular fracture—and contrast related to other contrast sources leads immediately to the notion of the signal-to-noise ratio. *Signal* refers to information (i.e., contrast) related to the feature of interest, whereas *noise* refers to information not related to the feature of interest.

The human eye is extremely good at identifying meaningful contrast, even in situations with poor signal-to-noise ratios. Human vision allows instant recognition of spatial relationships and makes it possible to notice subtle variations in density and to filter the feature from the noise. A trained radiologist will have no difficulty in identifying the subtle shadow caused by a hairline fracture, a microscopic region of

lower optical density in a mammogram, or a slight deviation from the normal shape of a ventricle, to name a few examples. However, these tasks may pose a considerable challenge for a computer. This is where the next steps of the image processing chain come into play.

1.2.2. Image Enhancement

Image enhancement can serve two purposes: to improve the visibility of features to allow a human observer (radiologist) to make a more accurate diagnosis or better extract information, or to prepare the image for the next processing steps. The most common image enhancement operators are:

- *Pixel value remapping*. This includes linear or nonlinear contrast enhancement, histogram stretching, and histogram equalization.
- *Filtering*. Filters amplify or attenuate specific characteristics of an image, and filters make use of the pixel neighborhood. Filters that operate on a limited pixel neighborhood (often based on the discrete convolution operation) are referred to as *spatial-domain filters*. Other filters use a specific transform, such as the Fourier transform, which describes the image information in terms of periodic components (frequency-domain filters). To name a few examples, filters can be used to sharpen edges or smooth an image, to suppress periodic artifacts, or to remove an inhomogeneous background intensity distribution.

A specific form of image enhancement is *image restoration*, a specific filtering technique where a degradation process is assumed to be known. Under this assumption, the image acquisition process is modeled as the acquisition of an idealized, undegraded image that cannot be accessed, followed by the degradation process. A restoration filter is a filter designed to reverse the degradation process in such a manner that some error metric (such as the mean-squared error) is minimized between the idealized unknown image and the restored image. The restored image is the degraded image subjected to the restoration filter. Since the idealized image is not accessible, the design of restoration filters often involves computer simulations or computer modeling.

Whether for enhancement or restoration, filter design is a critical step in image processing. Typically, the degradation process introduces two components: blur and noise. In some cases, such as microscopy, inhomogeneous illumination may also play a role. Illumination may change over time, or motion artifacts may be introduced. Unfortunately, filters often require balancing of design criteria. Filters to counteract blurring and filters for local contrast enhancement tend to amplify the noise component and therefore reduce the signal-to-noise ratio. Conversely, noise-reducing filters negatively affect edges and detail texture: Whereas these filters increase the signal-to-noise ratio, image details may get blurred and lost. Moreover, filter design goals depend on the next steps of the image processing chain. To enhance an image for a human observer, the noise component plays a less critical role, because the human eye can recognize details despite noise. On the other hand, automated processing

such as segmentation requires that the image contains as little noise as possible even at the expense of some edge and texture detail.

1.2.3. Image Segmentation

Image segmentation is the step where an object of interest in the image is separated from the background. Background in this definition may include other objects. To perform image segmentation successfully, the object of interest must be distinguishable from background in some way: for example, by a difference in image intensity, by a delineating boundary, or by a difference in texture. Sometimes, a priori knowledge such as a known shape can help in the segmentation process. The goal of this process can be either a *mask* or an outline. A mask is an image where one pixel value (usually, 1) corresponds to an object pixel, while another pixel value (usually, 0) corresponds to background. An *outline* can be a parametric curve or a set of curves, such as a polygonal approximation of an object's shape. There seems to be almost no limit to the complexity of segmentation approaches, and there is certainly a trend toward more complex segmentation methods being more application-specific. An overview of the most popular segmentation methods follows.

Intensity-Based Segmentation When the intensity of the object of interest differs sufficiently from the intensity of the background, an intensity threshold value can be found to separate the object from its background. Most often, an object is a more or less convex shape, and pixel connectivity rules can be used to improve the segmentation results. Intensity-based segmentation methods that make use of connectivity are region growing and hysteresis thresholding. Intensity thresholds can be either global (for the entire image) or locally adaptive. To some extent, intensity-based thresholding methods can be applied to images where the object texture (i.e., the local pixel intensity distribution) differs from background, because the image can be filtered to convert texture features into image intensity values. The latter is a good example of how image filtering can be used in preparation for image segmentation.

Edge-Based Segmentation Sometimes, objects are delineated by an intensity gradient rather than by a consistent intensity difference throughout the object. In such a case, a local contrast filter (edge detector) can be used to create an image where the outline of the object has a higher intensity than the background. Intensity-based thresholding then isolates the edge. Images containing the edge of the object are prerequisites for parametric segmentation methods such as boundary tracking and active contours. Parametric shapes (i.e., lines, circles, ellipses, or polygonal approximations of a shape) can also be extracted from the edge image using the Hough transform.

Region-Based Segmentation Some segmentation approaches make more extensive use of local similarity metrics. Regions may be similar with respect to intensity or texture. In fact, a feature vector can be extracted for each pixel that contains diverse elements, including intensity, local intensity variations, or directional variations. Similarity could be defined as the Euclidean distance between feature

vectors. Unsupervised region-based methods are region splitting and region merging or region growing. Region splitting starts with the entire image as one region and subdivides the image recursively into squares with dissimilar regions, whereas region growing starts at the pixel level and joins similar regions. The two methods can be combined to form split-merge segmentation, where splitting and merging alternate.

Clustering If feature pixels are already separated from the background, clustering is a method to group the feature pixels into clusters. Assignment of individual pixels to clusters can be based on the Euclidean distance to the cluster center or on other similarity metrics. Most widely used are the *k*-means clustering method, where each pixel is assigned to exactly one cluster and fuzzy *c*-means clustering, where cluster membership is continuous (fuzzy) and to some degree, each pixel belongs to multiple clusters.

Neural Networks In medical imaging, a widely used approach is to train artificial neural networks with feature vectors that have been manually assigned to classes. Once a neural network has been trained, it can then segment similar images in an unsupervised manner. Although unsupervised segmentation is desirable, some manual interaction can greatly facilitate the segmentation task. Examples of limited manual interaction are the placement of seed points for region growing, and crude object delineation for active contour models and live-wire techniques.

If the segmentation result is a binary mask, some postprocessing may improve the segmentation result. Examples of postprocessing steps are removal of isolated pixels or small clusters, morphological thinning and extraction of a single pixel-wide skeleton, morphological operations to reduce boundary irregularities, filling of interior holes or gaps, and the separation of clusters with weak connectivity.

1.2.4. Image Quantification

Similar to the way that a radiologist uses an image to assess the degree of a disease for a diagnosis, image quantification encompasses methods to classify objects or to measure the properties of an object. Image quantification requires that the object be segmented. The goal of image quantification is either to classify an object (e.g., as diseased or healthy) or to extract a continuous descriptor (e.g., tumor size or progression). The advantage of computerized image quantification is its objectivity and speed.

Examples of continuous variables include the measurement of intensity, density, size, or position. As an example, bone mineral density is a crucial determinant of bone strength, and people (especially women) lose bone mineral with age, a condition that may lead to osteoporosis. X-ray imaging techniques (quantitative CT and dual-energy x-ray absorptiometry) are particularly suited to measuring bone mineral density.[29] The degree of osteoporosis and with it the risk of a patient to suffer spontaneous fractures is often determined by comparing bone density to an age-matched distribution.[25] Today, this measurement is usually highly automated, with unsupervised segmentation of

the bone examined (e.g., vertebrae or the calcaneus) and subsequent determination of the bone mineral density, measured in milligrams of calcium hydroxyapatite per milliliter of bone, from the x-ray attenuation.

Examples of classification include healthy/diseased or healthy tissue/benign lesion/malignant lesion. A typical example is the classification of suspicious masses in mammograms as benign or malignant. This classification can be based on the shape of the segmented lesion[61] or on the texture.[67] To illuminate the classification process, let us look at texture analysis, a process that is representative of a large number of similar approaches to classifying lesions in x-ray mammograms. Sahiner et al. propose segmenting the lesion and extracting its outline, then transforming a narrow band of pixels perpendicular to the outline onto a rectangular region where descriptive metrics can be extracted from the texture.[67] In their study a total of 41 scalar values were extracted from each image using methods based on the co-occurrence matrix and run-length analysis. The 41 values formed a descriptive feature vector, and a classification scheme based on computer learning (Fischer's linear discriminant classifier[45]) was used by radiologists to produce a malignancy rating from 1 to 10.[10] The generation of high-dimensional feature vectors and the use of artificial intelligence methods to obtain a relatively simple decision from the feature vector is very widespread in image quantification.

1.3. CURRENT TRENDS IN BIOMEDICAL IMAGING

Once computers started to play an instrumental role in image formation in modalities such as CT and MRI, the next step was indeed a small one: to use the same computers for image enhancement. Operations such as contrast enhancement, sharpening, and noise reduction became integrated functions in the imaging software. A solid body of image processing operations has been developed over the last 30 years, and many of them provide the foundation for today's advanced image processing and analysis operations. A continuous long-term goal, however, remains: to use computers to aid a radiologist in diagnosing a disease. Much progress toward this goal has been made. As mentioned above, established computer-aided imaging methods to determine bone density and therefore indicate the degree of osteoporosis are in clinical use. CT scans can be used to find colon polyps and help diagnose colon cancer. Optical coherence tomography has rapidly been established in ophthalmology to diagnose retinal diseases. Yet there are many more areas where increasing computing power combined with more elaborate computational methods hold some promise of helping a radiologist with the diagnosis, but the trained observer proves to be superior to computerized image analysis. An example is computerized mammography, where a lot of progress has been made but no single method has entered mainstream medical practice. With the vision of computer-aided radiology, where computers provide an objective analysis of images and assume tedious parts of the image evaluation, advanced biomedical image analysis is—and will remain for a long time—an area of intense research activity.

Progress in image analysis is aided by a dramatic increase in the memory and processing power of today's computers. Personal computers with several gigabytes of memory are common, and hard disks have reached beyond the terabyte limit. Storing and processing a three-dimensional image of $512 \times 512 \times 512$ bytes is possible with almost any off-the-shelf personal computer. This processing power benefits not only computerized image analysis but also image acquisition. Volumetric imaging modalities generate images of unsurpassed resolution, contrast, and signal-to-noise ratio. Acquisition speed has also improved, giving rise to real-time imaging that allows motion measurements, for example, of the heart muscle.[54,89]

As the availability of tomographic scanners increases, multimodality imaging becomes more popular. Clinicians and researchers aim to obtain as much information on the tissue under examination as possible. Predominantly, one imaging modality that provides a high-resolution image of the tissue or organ (normally, CT or MRI) is combined with a functional imaging modality, such as PET.[51] Multimodality imaging is particularly popular in cancer diagnosis and treatment, because it is possible to place a radioactive label on tumor-specific antibodies. PET, and to a lesser extent, SPECT, are used to image antibody uptake by the tumor, whereas the exact localization of the tumor is found by combining the PET or SPECT image with MRI or CT (see pertinent reviews[33,41,53,55]). Multimodality imaging produces two or more images generally with different resolution and probably with different patient positioning between images. The different images need to be matched spatially, a process called *image registration*. Dual-modality imaging devices are available (e.g., a combined PET/CT scanner), but software registration is most often used, and new registration techniques are an active field of research.[71]

Another recent field of study is that of optical imaging techniques, more specifically tomographic imaging with visible or near-infrared light. A prerequisite for this development was the introduction of new light sources (specifically, lasers) and new mathematical models to describe photon propagation in diffusive tissues.[12,23] Unlike x-ray and CT imaging, visible light does not travel along a straight path in tissue because of the high scattering coefficient of tissue and because of tissue regions with different refractive index. Optical coherence tomography (OCT)[39] is often considered the optical equivalent of ultrasound imaging because the image is composed of A-mode scans. OCT has a low penetration depth of a few millimeters, but it provides good spatial resolution. Optical coherence tomography has found wide application in dermatology and ophthalmology (see reviews[22,38,63,68,84]), but its poor signal/noise ratio calls for advanced image enhancement methods. Optical transillumination tomography, the optical equivalent of CT, faces major challenges because of refractive index changes along the light rays. Progress has been made to use optical transillumination tomography to image bone and soft tissues,[74,87] but spatial resolution and contrast remain limited. Attempts have been made to correct the refractive index mismatch in software[30] and to reject scattered photons,[11,34] but major improvements are needed before this modality enters medical practice. The third major optical tomography method is diffuse optical tomography.[27] Its main challenge is the mathematical modeling of light-wave propagation, which is a

prerequisite for image reconstruction.[16] Presently, diffuse optical tomography requires crucial improvements in spatial resolution and signal-to-noise ratio before it becomes applicable in biomedical imaging. These challenges notwithstanding, optical imaging methods enjoy strong research efforts because they promise fast and radiation-free image acquisition with relatively inexpensive instrumentation.

A special focus of imaging and image analysis is the brain. Brain imaging studies have been driven in part by the availability of MRI, which does not expose study subjects to ionizing radiation, and in part by functional imaging techniques, which make it possible to localize areas of brain activity.[40,83] Another important subject is the development of anatomical brain atlases, which allow mapping of images with high interindividual variability onto known anatomical models.[3,56] Although our understanding of the brain is still rudimentary, biomedical imaging has helped enormously to find the loci of brain activity, to understand cognitive functions, and to link images to disease (see pertinent articles and reviews[5,7,46,52,66]).

On the general image processing side, new methods and operators of higher complexity also tend to be more application- and modality-specific. Three recent articles highlight the challenges: Masutani et al.[50] review image modalities and image processing methods specifically for the diagnosis and treatment of liver diseases; Hangartner[31] demonstrates how a key step in segmentation—threshold selection—affects the quantitative determination of density and geometry in CT images; and Sinha and Sinha[70] present MRI-based imaging techniques for breast lesions. All three examples have in common the fact that the methods and conclusions cannot readily be translated into other modalities or applications. The main reason for this very common phenomenon is the inability of computers to understand an image in the same way that a human observer does. A computer typically examines a limited pixel neighborhood and attempts to work its way up toward more global image features. Conversely, a human observer examines the entire scene and discovers features in the scene in a top-down approach. The problem of image understanding, allowing computers to recognize parts of an image similar to the way that a human observer does, has been approached with algorithms that involve learning[8] and, more recently, with a top-down analysis of statistical properties of the scene layout[57] and with imitation of the human visual system through genetic algorithms.[88] Image understanding is not limited to biomedical imaging but also affects related fields of computer vision and robotics and is therefore another area of intensive research.

Related to image understanding is the problem of image segmentation. Meaningful unsupervised image segmentation requires certain image understanding by the computer. The scope of most image segmentation algorithms is limited to special cases (e.g., where the object of interest differs in intensity from the background). The main reason is the extreme variability of medical images, which makes it difficult to provide a consistent definition of successful segmentation. Learning algorithms, artificial neural networks, and rule-based systems are examples of state-of-the art approaches to segmentation.[17,90] More recently, new methods to compare segmentation algorithms objectively have been proposed,[81] and a database with benchmark segmentation problems has been created.[49] These examples illuminate the present search for a more unified segmentation paradigm.

The development of new filters is another area of research. Early spatial- and frequency-domain filters used fixed filter parameters. Subsequently, filter parameters became dependent on the local properties of the image. These filters are called *adaptive filters*. Many recently developed filters are tuned toward specific modalities, with examples such as a noise filter for charge-coupled-device (CCD) cameras,[21] an adaptive filter to remove noise in color images,[47] a speckle reduction filter for optical coherence tomography,[58] or a fuzzy filter for the measurement of blood flow in phase-contrast MRI.[76] Novel filters are highly sought after because a good filter can often make possible an otherwise impossible segmentation and quantification task.

A final example for emerging areas in image processing is the use of image data for modeling. The use of finite-element models to predict bone strength[80] is a particularly good example because CT image values depend strongly on mineral content, and it is hypothesized that bone strength and mineral content are strongly related. However, those models need further improvement before they become useful in clinical practice.[4] Image-based computational fluid dynamics can be used to compute blood flow and wall shear stress in arteries that are frequently affected by arteriosclerosis.[86] One recent example is a study by Sui et al.[75] in which MR images of the carotid artery were used to calculate wall shear stress. Experiments with cell culture indicate that shear stress gradients enhance cell proliferation and therefore contribute to arteriosclerosis,[85] and image-based flow simulations are a suitable tool to further elucidate the disease and perhaps aid in the prediction and early diagnosis of arteriosclerosis.[77]

1.4. ABOUT THIS BOOK

The overall aim of this book is to provide the reader with a comprehensive reference and self-study guide that covers advanced techniques of quantitative image analysis with a focus on biomedical applications. The book addresses researchers, professionals, teachers, and students in all areas related to imaging. Ideally, the reader has some prior basic knowledge of image processing. Any reader who has an interest or involvement in imaging may use this book for a conceptual understanding of the subject, and to use equipment and software more efficiently. The reader will gain an overview of advanced image analysis techniques that were recently established or are still in active research, and the book illuminates the inner workings of image processing software, which often comes with such imaging devices as scientific cameras, microscopes, CT scanners, and optical coherence tomography devices. Furthermore, readers will gain the ability to understand and use the algorithms in a meaningful way and the ability to design their own algorithms based on understanding gained from this book. Readers with programming experience in C, C++, Python, Java, Matlab, or other languages can use this book to implement and refine custom algorithms for advanced image processing and unsupervised analysis. A survey of software programs for image analysis and image visualization is provided in Chapter 14, and the focus is placed on free software such as ImageJ and OpenDX, which the reader can freely download and put to immediate use.

Each chapter provides the mathematical background for an image processing operator with the purpose of explaining how it works. We then proceed to applications and limitations and to their realization in software. For key algorithms, a pseudocode implementation is provided. The pseudocode can be translated readily into many programming languages and is also well suited to explain the "inner workings" of an image processing operator. In addition, each chapter includes application examples in the biomedical field. Although the application focus is biomedical, the methods described are not restricted to the biomedical field. Some areas in which advanced image analysis plays a key role are satellite imaging, oceanography, environmental and geological sciences, soil sciences, anthropology, forensic sciences, and astronomy.

It was mentioned that a basic understanding of image processing is beneficial for readers of this book. Several books are available that provide such a basic understanding. Two notable examples are an image processing handbook by Russ[65] and a medical imaging book by Dougherty.[19] The main strength of the first book derives from the numerous examples from various imaging-related fields, and the main strength of the second book is its combined coverage of medical imaging modalities and medical image processing.

In the present book, the topics follow the outline given in Section 1.2. Chapter 2 provides an overview of established and fundamental image processing operators and can be used to review the main topics of basic image processing. In Chapter 3 we introduce the Fourier transform and image filtering in the frequency domain. The subject of Chapter 3 is fundamental and established, yet its extremely widespread use and importance warrants detailed coverage in a separate chapter. There is considerable overlap between Chapters 2 and 3 and the two book examples mentioned above.

In Chapter 4 we introduce the wavelet transform and explain filters that use the wavelet transform. Unlike the Fourier transform, the wavelet transform retains spatial information and gives rise to new and very powerful image filters. Since the introduction of the wavelet transform, wavelet-based filters have rapidly found their way into mainstream image processing. In Chapter 5 we explain the concepts and examples of adaptive filters. Spatial-domain filters were introduced in Chapter 2, but conventional filters have fixed parameters. Adaptive filters adjust their filter parameters to local image properties and can achieve a superior balance between noise removal and detail preservation.

Chapters 6 and 7 present two different approaches to segmentation: active contours and the Hough transform. Active contours (the two-dimensional versions are referred to as "snakes") are physical models of energy functionals that use image features as an external energy term. An active contour behaves like a rubber band that snaps onto prominent image features such as edges. Active contours are generally used as supervised segmentation methods that yield a parametric representation of a shape. The Hough transform (Chapter 7) is a popular method used to find parametric shapes in images, such as lines, circles, or ellipses. The strength of the Hough transform is that the shape does not need to be complete, and the transform can be used to find the shape even if the image is strongly corrupted by noise.

Chapters 8, 9, and 10 present advanced techniques for image quantification. In Chapter 8 we explain methods for texture analysis and texture quantification. Texture

refers to local gray-scale variations in an image, and texture information can be used for feature extraction, segmentation, and for quantitative analysis that is, for example, related to a diagnosis. Analogously, in Chapter 9 we explain methods used to describe and classify the shape of segmented objects. Texture and shape analysis are very powerful tools for extracting image information in an unsupervised manner. In many images, structures exhibit some apparent self-similarity; that is, a shape or texture repeats itself on smaller scales. Self-similarity is related to the fractal dimension, and fractal methods have been used widely to quantify medical images. In Chapter 10 we provide an introduction, overview, and in-depth analysis of fractal methods for shape and texture quantification.

Chapters 11 and 12 are more focused on special biomedical problems. In Chapter 11 we explain the principles of image registration, that is, methods used to match the exact resolution and spatial position of two images that were taken with different modalities or with the same modality when a patient shifted. As multimodality imaging grows even more popular, image registration gains in importance. Image compression, storage, and transportation are covered in Chapter 12. Medical imaging produces an exponentially increasing volume of data through a growing number of medical procedures and higher resolution. With archiving required and telemedicine evolving, approaches to handling increasing data volumes are introduced in Chapter 12.

Chapter 13 covers image visualization, an important step in preparing an image for analysis by a human observer. The image can be two- or three-dimensional, or it can be a time-course sequence. Depending on the application, brightness and contrast manipulation, false coloring, and three-dimensional rendering can emphasize important aspects of an image and facilitate the analysis task of a human observer.

Chapter 14 provides a link to the practical application of topics covered in the book. A number of software programs for image processing, analysis, and visualization are presented briefly. Two popular packages, ImageJ and OpenDX, are covered in more detail, as is Crystal Image, the author's software on the accompanying DVD. This software was used to create most of the examples in this book and to test the algorithms covered in the book. Most of the software in Chapter 14 is free and can be downloaded and put to use immediately.

With these elements, the book provides a solid and in-depth foundation toward understanding advanced image analysis techniques and developing new image analysis operators.

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