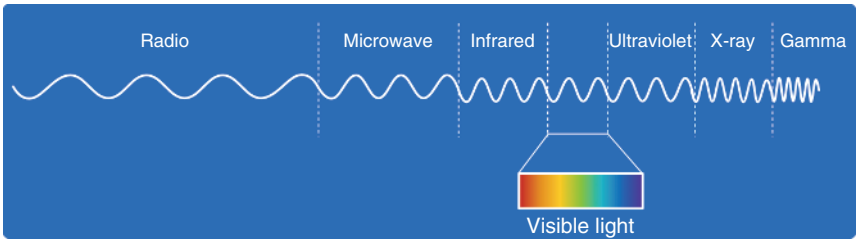

Figure 1.10 Electromagnetic waves as the light source of bioimaging.

the biomolecules can occur by breaking the covalent bonds or ionization. The accumulated damages in DNA can cause mutations of cells, resulting in cancer in somatic cells and mutagenesis in fetus. So, excess amount of exposure to X-ray is not recommended due to health concerns, especially for pregnant women.

γ-Ray has a shorter wavelength than X-ray and the higher energy allows its penetration even through bones, the hardest part of our body. The intensive γ-ray can be used for tumor treatment, which is called as γ-knife technique. To minimize the damage to normal tissue, multiple sources of γ-ray are used from different directions, and only the tumor site is focused to accumulate a high density of γ-ray. In principle, γ-ray also can be used for bioimaging in a similar way of X-ray imaging, but it is not common in practice. Instead, γ-ray-generating radioactive materials are used as imaging agents. In this case, the γ-ray is not provided from outside as in the X-ray method, but is irradiated from inside of the body, through an administered imaging probe into the target site of the body. The position of the isotope could be imaged through a γ-camera similar to an X-ray film. When CT technique is combined with γ-ray-generating radioactive isotope, a three-dimensional single-photon emission computed tomography (SEPCT) imaging is also possible. A similar, but higher performance technique is positron emission tomography (PET). In PET, instead of a direct γ-ray-generating isotope, a positron-generating isotope is used. Positron is a positively changed electron, a kind of anti-particle of electron. When a positron meets an electron, they are annihilated, generating one pair of γ-ray photons. As the two γ-ray photons travel in direct 180° providing richer information for the original position of positron, usually the spatial resolution of PET is better than SPECT. It is noteworthy that X-ray uses an external light source for the imaging, but SPECT and PET use endogenous γ-ray generated from an isotope-labeled probe (Figure 1.11). That is why SPECT and PET are called molecular imaging techniques, in contrast to X-ray imaging.

As shown earlier, electromagnetic waves with different wavelengths from visible light also can be used as the light source of various imaging techniques, when coupled with a proper detector or camera system. The different wavelengths of light render different modes of interaction with matters, and each can generate unique information for the target object. Therefore, unexplored areas of electromagnetic waves would provide novel chance of new imaging technology or modality. Terahertz light is such an emerging new source of light.

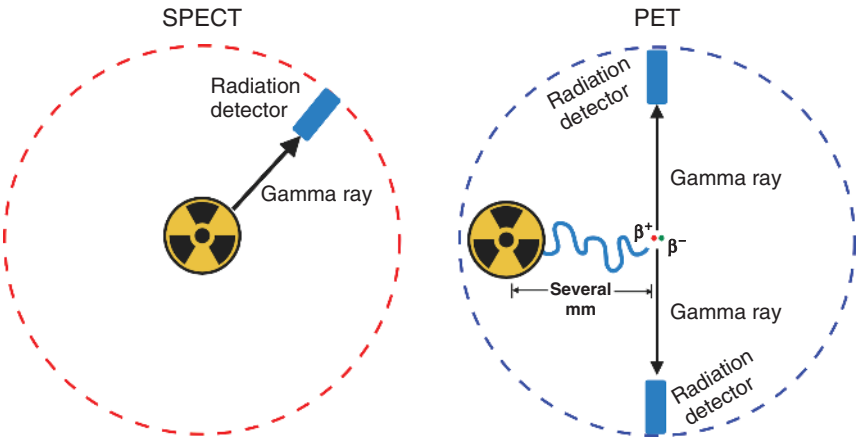

Figure 1.11 Endogenous γ-ray imaging in SPECT and PET.

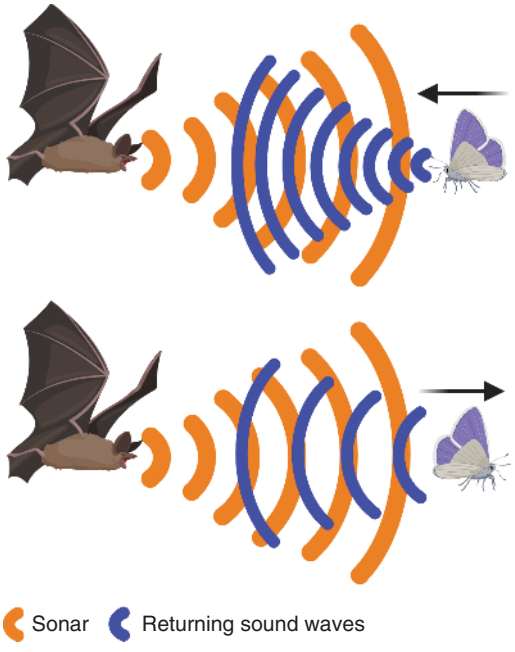

Figure 1.12 Sound imaging of bats.

Not only electromagnetic waves, sound waves or seismic waves also can generate processed images through interaction with matters. The bat's vision through ultrasonic waves would be a good example. Combining electromagnetic waves and sound waves for improved or unique imaging technique, such as photoacoustic imaging, is also a powerful visualization technique (Figure 1.12).

Electron beam is another source to provide ultrahigh-resolution imaging of materials. There are several modes of electron microscopy, such as transmission electron microscopy (TEM) and scanning electron microscopy (SEM). In TEM, an ultrathin sample is irradiated with an electron beam and the transmitted electrons

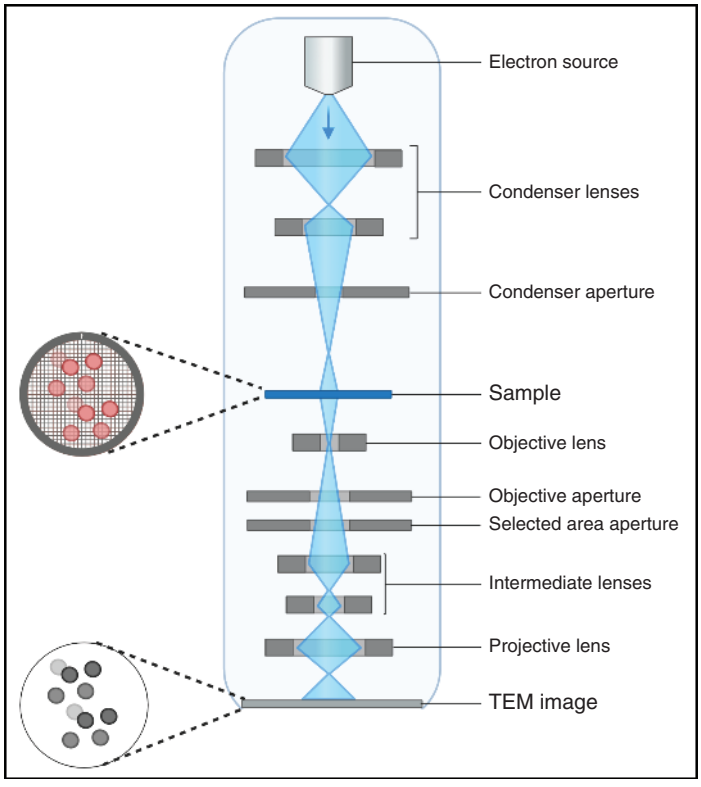

Figure 1.13 Transmission electron microscopy.

are used for the two-dimensional image construction, which is similar to X-ray imaging. In the sample area with a high electron density, the input electron beam would be scattered and may not transmit, generating the dark contrast in TEM image. Therefore, heavy metals are absorbed to the sample to enhance the contrast, and the procedure is called electron staining. The electron staining can also be achieved by an organic dye. After diaminobenzidine (DAB) is stained, through photooxidation, an electron-dense precipitate can be formed to increase the TEM contrast, which is similar to dye staining in the optical imaging.

In SEM, the incident electron beam interacts with the surface atoms of the sample and generates back scattered electrons or secondary electrons. The incident beam is focused on a sample spot and scan the surface, and the detectors are located in the same side of the input beam. As a result, SEM image shows the surface morphology with three-dimensional information especially provided by secondary electrons. The resolution of SEM image is in nanometer range, and usually TEM has higher resolution than SEM. While optical imaging suffers from the diffraction limit in sub-micrometer range, electron microscopy provides much higher resolution. By the imaging resolution scale, electron microscopy could be called as "nanoscopy," rather than microscopy. Both techniques require vacuum condition for the imaging due to the electron beam usage (Figure 1.13).

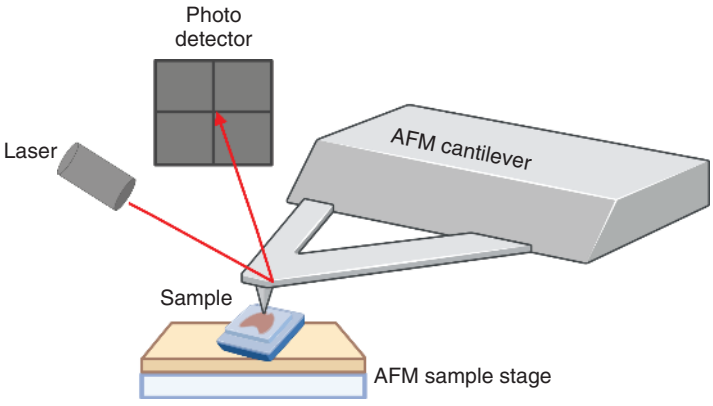

Figure 1.14 Atomic force microscopy.

Atomic force microscopy (AFM) is another nanoscopy technique. Using the physical contact force sensing, the physical probe scans the sample surface, providing the information of surface morphology. The result is similar to SEM images, but with a much higher spatial resolution. It is interesting to compare AFM with SEM as AFM does not require a light or electron beam source. Also, AFM does not require the electron stain, which may change the surface landscape. However, the physical contact of the probe with the sample surface may partially damage the sample, especially during the close-contact mode process. A modified AFM technique also allows liquid environment in addition to the vacuum condition for the imaging, and more biologically relevant samples could be imaged, such as live-cell surface imaging (Figure 1.14).

1.4 Subcellular Imaging

When the object of visualization is too far from our eyes, we use a telescope. If the object is too small, we use a microscope. Superficially, they may seem to use opposite principles, but actually they are similar in a sense that they "magnify" the "too small images" to a sensible size for naked eyes. One is for too small images due to the long distance of the object and the other is for nearby, but physically too small object. If they are similar, can we use telescope instead of microscope for the small object or vice versa? No, we cannot. What is the difference, then? The difference lies in focal distances depending on the position of the object. In a telescope, the focus is on the long distance, and in a microscope, the focus is on the sample slide right under the lens.

Now, let's focus on the microscope for visualizing small objects in biological systems. The basic unit of life is cell. For unicellular organisms, a single cell is an individual or entity. In multicellular organisms, cells gather together to make tissue, and tissues make organ, and organs assemble to make an individual body. The reason why a cell is the basic unit of life is that each cell contains the whole

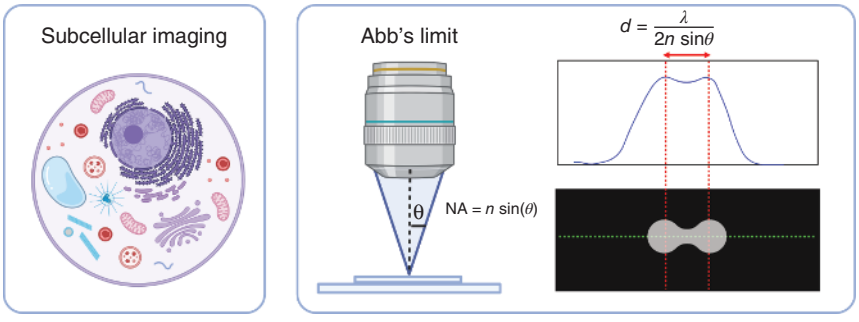

Figure 1.15 Subcellular imaging and Abbe's limit.

genomic information of the individual. In other words, starting from any single cell, in principle, we can reconstruct the whole body.

The usual size of cells in animals or plants is around 10 μm, and unicellular bacteria are about 1 μm in size. While bacteria cell structure is relatively simple, animal or plant cells have complex intracellular structure, called organelles, such as nucleus, mitochondria, lysosomes, Golgi body, and endoplasmic reticulum (ERs). The intracellular organelles are usually about 1 μm or smaller size. When light encounters an object with a similar size to the wavelength, the light path is altered by diffraction. The visible light is in 0.4–0.7 μm (400–700 nm) and if the object is about half micrometer or smaller, the image become blur. This is known as Abbe diffraction limit, named after Ernst Abbe who found it in 1873, and is considered as the physical limit of the optical resolution (Figure 1.15). Therefore, the physical size limit of a microscopic image is about ~ μm range.

To overcome the size limit, several optical and mathematical tricks were developed into "super-resolution" techniques or so-called nanoscopy, which means nanometer-resolution imaging. In addition to the size limit, organelles are usually transparent, so the optical visualization is further challenging, as it is difficult to distinguish different organelles. That is why organelle-selective dyes are widely used for vivid subcellular organelle visualization. In other words, bioimaging is a process of visualizing a biological object, otherwise invisible. Most of the cell images we have in our mind are "stained" images rather than natural cell images. For example, chromosome, as condensed form of DNA, means "color body (chromo-some)" as it is easily stained by dyes. You may have seen the change of the chromosome during the cell division, such as condensation, alignment, and division of DNA. It implies that most of the chromosome images are also obtained from DNA-stained cells rather than intact natural cells. By the same token, if there is a selective dye for each organelle, it would be possible to see specific organelle standing out from a transparent background. These selective dyes are called organelle-selective probes, and if the dyes change their colors upon binding to the target, they can be called as sensors. Therefore, the definition of probes embraces sensors. In other words, sensors are special type of probes in bioimaging, providing the information of change of the target.

1.5 Cell-Selective Imaging

In a multicellular organism or mixed bacteria community, distinctive visualization of different cells or bacteria would be critical for the study of intercellular interaction. If the different cells have unique shapes and sizes, it would be easy to discriminate them. However, in many cases, distinction of one type of cell from others is generally difficult due to their similar appearance under bright-field microscope. Even the same kind of cells may have different stages of development or death process, showing off different morphology. Considering the fact that all the cells in the same body contain exactly the same genetic information, the discrimination of their phenotypic difference is the key for the study.

To overcome the problem, cell-selective probes have been explored for a long time. Antibodies have been the most common probes for the cell distinction and are widely used. Hundreds of antibodies have been developed and validated for cell discrimination and imaging. While useful, due to their high molecular weight of 150 kDa, their access to the intracellular target in live status is intrinsically limited. Even though the binding target of antibodies is on the cell surface, they are usually functionally important enzymes or receptors. As a result, antibodies often induce functional influence in the treated cells, which is not desirable for normal cell study. Alternative solution may be a smart small molecule probe, which may complement the antibodies' weak points, especially for the intracellular target.

Not only for our own cells, we also need to distinguish and visualize foreign life forms, as our body is always interacting with them. For example, our body hosts huge numbers of bacteria as guests in similar or even higher number than our own cells, which is called the microbiome. The bacteria in the microbiome established symbiotic relationships with our body and majority of them are not harmful to us. But, if we get pathogenic bacterial infection, figuring out the identity of the bacteria would be urgent and important for making decision of the proper treatment. The morphological difference may not be informative enough to get a good discriminating information. Media-selective culturing is a standard test for the identification, but the process takes days of time, and also the identification is limited only to the known strains for their culture condition. While polymerase chain reaction (PCR)-based genetic analysis is getting more and more popular for high accuracy and sensitivity, the need for an in-site imaging probe increases for faster analysis and functional monitoring through the visual images. So far, such an efficient and practical cell-selective probe is yet to be developed.

1.6 Tissue and Organ Imaging

When cells gather to make tissues and organs, a tangible physical structure emerges, and macroscopic imaging technique is required. For diagnosis of diseases, often a biopsy (tissue sampling from live body) procedure is required for tissue imaging or biochemical testing. Usually, the tissues are stained with dyes and imaged to determine the disease status. As the test is performed outside of the body, the procedure

is called ex vivo imaging. For example, from a surgery for cancer, the excised tissue (suspected as a tumor) is processed through cryo-section or paraffin treatment, and then stained with dyes for visualizing the tumor and healthy tissue. Most likely, the sample is sent to a pathologist who has long-term training and experience to make the call if the tissue is indeed cancer or not. The procedure takes easily an hour or longer, and it is quite difficult to get the results back before the surgery procedure is over. If the sample preparation procedure becomes simpler and faster, and also a user-friendly probe is available, which does not require a pathologist for reading, it would be possible to get the results within the surgery procedure. Not only for tumors, any kind of disease symptoms such as inflammation or infection could benefit by the selective probes.

1.7 Whole-Body Imaging

If the tissue imaging can be performed without removing the tissue from the body, it would be even better. Such an optical imaging in the live body is called intravital microscopy, as a kind of in vivo imaging. The imaging for blood cell flow or extravasation is an example, and unlike the ex vivo imaging, the intravital microscopy allows repeated measurement with minimal invasiveness for long-term monitoring of diseases. Some of the imaging could be achieved from the natural tissue itself, but sometimes it is necessary to use probes to get a clear contrast.

For example, in cancer surgery, it is often difficult to discriminate the exact boundary between the tumor and normal tissue. If there is a selective probe for a tumor to show a clear boundary, it would greatly help the surgeon to decide the excision line for saving maximum healthy tissue for the patient. If the dye was colorless before binding to the tumor, but generate a strong color in the tumor, the probe could also be a sensor for the tumor and carries low background in the normal tissue. The imaging technique used in operation is called intraoperative imaging.

The eventual goal of bioimaging would be a noninvasive (without an open-up surgery) whole-body imaging without a biopsy sampling (for ex vivo imaging). The ideal probe could act as a diagnostic tool to detect disease occurrence with precise position and size information of the target. The probe should not be toxic and also could be used for body response to drug treatment as a prognostic procedure. There is huge room for improvement in the current in vivo imaging with smart probes and improved image process/analysis method.

1.8 Probes in Bioimaging

Probes help to visualize target organelles, cells, tissues, and organs with an outstanding contrast. Sensors are part of probes, and respond to the analyte or environment by changing the color or intensity. Most of the biological images are physically stained images or artificially drawn pictures, which reflect the practical importance of probes in the field. In this book, the history of probe development,

their applications in different levels of body, i.e. intracellular organelles, different cells, tissues, and whole body. In later chapters, the probe application in biological environmental changes and diseases, and various imaging techniques both for nonoptical imaging and fluorescence will be described. In perspective, design or discovery of selective probes and the future direction will be suggested.

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