34  Near-Infrared Spectroscopy Imaging of Biological Materials and Systems

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CONTENTS

34.1  Introduction .......................................................................................................................... 715
34.2  Newly Developed NIR Imaging Systems .............................................................................716
  34.2.1  Portable NIR Imaging Device (D-NIRs)........................................................................717
  34.2.2  A High-Speed and Wide-Area Monitoring NIR Imaging System with a Novel
          NIR Camera (Compovision) ............................................................................................718
  34.2.3  Imaging-Type Two-Dimensional Fourier Spectroscopy (ITFS) System ..................718
34.3  Examples of NIR Imaging Studies of Biological Materials and Systems ............................719
  34.3.1  Pharmaceutical Tablet Monitoring by D-NIRs ........................................................ 719
  34.3.2  Quantitative Analysis of a Model Tablet Containing Three Different
          Components Using the High-Speed Wide-Area Monitoring System with a
          Newly Developed NIR Camera ...................................................................................720
  34.3.3  Noninvasive, High-Speed, NIR Imaging of the Biomolecular Distribution in
          Fertilized Fish Eggs ..................................................................................................721
  34.3.4  Nonstaining Blood Flow Imaging Using Optical Interference due to Doppler Shift.....726
34.4  Future Prospects ...................................................................................................................728
References ......................................................................................................................................729

34.1  INTRODUCTION

In the last two decades or so, the developments of new NIR instruments such as Fourier transform (FT)-NIR spectroscopy and NIR spectroscopy imaging have extended the application of NIR spectroscopy dramatically (Ciurczak and Drennen 2002, Siesler et al. 2002, Roberts et al. 2004, Ozaki et al. 2006, Ozaki 2012, Workman and Weyer 2012, Jue and Matsuda 2016). Chemical imaging data such as NIR imaging data can be represented as a three-dimensional cube spanning one

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wavelength and two special dimensions (Lewis et al. 2004, Gowen et al. 2007, Grahn and Geladi 2007, Šašić and Ozaki 2010, Adu-Baffour 2012, Ishikawa et al. 2014, Salzer and Siesler 2014, Strlic 2018). This data construct is called a hypercube. To handle these hypercubes, one uses a variety of chemometric methods such as principal component analysis (PCA). NIR imaging collects data in a spatially resolved manner, so that using NIR imaging, one can learn from where on a sample a NIR spectrum is obtained. Therefore, information about the special distribution of sample components can be derived. These spatially resolved data provide qualitative and quantitative insights into the functionality of heterogeneous samples such as pharmaceuticals tablets, polymers, and agricultural and biological materials.


1. Studies of polymer crystallization, blending, and degradation.
2. Diffusion process of a solvent into a polymer.
4. Imaging analysis of cells and tissues.
5. Studies of distributions and functions of biological molecules in biological systems.
7. Quantitative and qualitative analysis and quality control of pharmaceutical tablets. This is concerned with PAT (process analytical technology) and QbD (quality by design).
8. Monitoring of water diffusion process into a pharmaceutical tablet.

The strong points of NIR imaging compared with IR and Raman imaging may be as follows:

i. NIR imaging is useful for probing thick samples or bulk materials with little or no sample preparation required.
ii. It is easier to study and analyze an aqueous dispersion with NIR imaging than IR imaging.
iii. One can use an optical fiber for NIR measurement, while it is not easy to use it for IR measurement.

There is another category of NIR imaging, that is, functional NIR spectroscopy (fNIR or fNIRS). It has been used for functional neuroimaging. Using fNIR, brain activity can be monitored through hemodynamic responses associated with neuronal behavior (Surhone 2009, Haas 2010, Cui et al. 2011). NIR is a noninvasive imaging method involving the quantification of chromophore concentration resolved from the measurement of NIR light attenuation or temporal or phasic changes. The region from 700 to 900 nm is the region of the optical window in which skin, tissue, and bone are mostly transparent to NIR light while hemoglobin (Hb) and deoxygenated hemoglobin (deoxy-Hb) are stronger absorbers of light. Differences in the absorption spectra of deoxy-Hb and oxy-Hb allow the measurement of relative changes in hemoglobin concentration through the use of light attenuation at multiple wavelengths (Surhone 2009, Haas 2010, Cui et al. 2011).

The purpose of this chapter is to outline state-of-the-art of NIR imaging. Recent progress in NIR imaging devices and the applications of NIR imaging in biological sciences are described. We do not treat fNIR in this chapter.

34.2 NEWLY DEVELOPED NIR IMAGING SYSTEMS

Recently, several kinds of novel NIR imaging systems have been developed for a variety of purposes from industrial online monitoring to biomedical applications (Ishigaki et al. 2016a, 2016b, 2018a, 2018b, Ishikawa et al. 2012, 2013a, 2013b, 2013c, Murayama et al. 2013, 2015, 2018, Puangchit et al. 2017). Although positive features such as high sensitivity, high wavelength, and spatial resolutions...
are essential qualifications, issues concerning high-speed measurements and portability are also primary concerns in the development of the instruments (Ishikawa et al. 2012, 2013a, 2014). In this session, we introduce three kinds of new NIR imaging devices: a highly sensitive portable NIR imaging device (Ishikawa et al. 2012, 2013c, Murayama et al. 2013, 2015, 2018), a device with high-speed, wide-area monitoring over a broad NIR wavelength region (Ishikawa et al. 2013b, 2013c, 2016b, Puangchit et al. 2017, Ishigaki et al. 2018a), and an imaging-type two-dimensional Fourier spectroscopy (ITFS) system (Ishigaki et al. 2018b, Qi et al. 2015).

### 34.2.1 Portable NIR Imaging Device (D-NIRs)

D-NIRs is a compact (151×93×120 mm³) portable NIR imaging instrument and enables one to develop images with high speed, high wavelength resolution (approximately 1 nm), and maximum spatial resolution (0.025 mm per pixel) (Ishikawa et al. 2012, 2013a). This system is suitable not only for PAT but also for other various industrial applications. The optical system of D-NIRs depends on a polychromator type of spectrometer, P-NIRs, which is equipped with a developed 640-element high-density photodiode array detector and a charge amplifier (Ishikawa et al. 2012, 2013a). It is noted that P-NIRs can obtain spectra in the 1000–1600 nm region with a 1 nm spectral resolution. Figure 34.1 depicts the imaging unit of D-NIRs consisting of light sources and a pair of Galvano mirrors. D-NIRs uses three halogen lamps of 5 W. The Galvano mirrors move to the direction of x- and y-axes, respectively, by two motors, and then, two-dimensional spectra data in the whole spectral region between 1000 and 1600 nm are obtained by a line scanning system. The design based on the combination of lamps and mirrors not only contributes to the portability of D-NIRs but also enables high-speed data acquisition at approximately 50 s per 100 pixels (in the case of 1 mm spatial resolution). The maximum spatial resolution of D-NIRs achieves 0.025 mm per pixel due to the special mechanical design for focus. Its maximum measurement speed is 5 ms per pixel.

**FIGURE 34.1** Schematic diagram of the imaging unit of the developed NIR imaging device (D-NIRs). The red lines indicate the diffuse-reflected light from the object. A: Galvano mirror and motor (x-axis); B: Galvano mirror and motor (y-axis); C: halogen lamps; D: focus lens 1; E: focus lens 2; and F: optical fiber. (Copyright: Ishikawa et al. 2012.)
34.2.2 A HIGH-SPEED AND WIDE-AREA MONITORING NIR IMAGING SYSTEM WITH A NOVEL NIR CAMERA (COMPVISION)

Figure 34.2 shows a novel NIR imaging device with a newly developed hyperspectral camera (Compovision) by Sumitomo Electric Industries, Ltd (Ishikawa et al. 2013b, 2013c). This imaging system consists of a linear moveable stage, a halogen light source, and the NIR camera. The characteristic features of the camera include high-speed and wide-area monitoring over a broad NIR wavelength region thanks to a newly developed indium gallium arsenic (InGaAs) detector (Ishikawa et al. 2013b, 2013c). The detector is equipped with InGaAs and gallium arsenic antimony (GaAsSb) type II quantum wells (QWs), laminated on an indium phosphide (InP) substrate. Thus, one can measure an NIR spectrum in the 1000–2350 nm region of a 150 × 200 mm² area (approximately 100,000 pixels) within 2–5 s. Commercial NIR imaging devices measure into approximately 10 × 10 mm² area with several minutes. Thus, the performance of high-speed monitoring of the NIR imaging system is potentially powerful for various purposes from industrial process monitoring to biomedical applications. A wide-area NIR image that can be rapidly obtained is very attractive for a variety of practical applications.

34.2.3 IMAGING-TYPE TWO-DIMENSIONAL FOURIER SPECTROSCOPY (ITFS) SYSTEM

ITFS uses the so-called phase-shift interferometry (Ishigaki et al. 2018b, Qi et al. 2015). The system adopts a characteristic mechanism of optical interference that gives spatial phase differences to the objected light from samples by a partial movable mirror as shown in Figure 34.3. The interference occurs only in the case where each light ray gathers to the same point in ITFS, and the interference between lights coming from out of the in-focus plane is not observed in alternate current component in the interferogram, detected as a direct current element. Therefore, by changing the focal plane, three-dimensional spectral data can be acquired using this system (Ishigaki et al. 2018b, Qi et al. 2015).

NIR measurements can be performed in both the reflectance and transmission modes by changing the positional relationship of the optical components such as light source and detection parts. In the former, we used the system consisting of a hyperspectral imaging unit (AOI Electronics Co., Ltd., NT00-T011), an InGaAs camera (Hamamatsu Photonics K.K., C10633-13), a halogen lamp, and an objective lens at 8× magnification. In the transmission mode, it was composed of a hyperspectral imaging unit (AOI Electronics Co., Ltd., NT00-T012), the same halogen lamp, an NIR camera (Sumitomo Electric Industries, Ltd., CVN800), and an objective lens at 4× magnification.

FIGURE 34.2 Outline view of the imaging instrument including the developed NIR camera. (Copyright: Ishikawa et al. 2013b.)
The spatial resolution with an objective lens at 8× magnification is about 8 µm. The system covers the region of 1000–2500 nm, and the wavelength resolution is 10 nm. The number of pixels in the two-dimensional image was approximately 81,000 points (319(X)×255(Y)). Of note is that the instrument adopts the wave-front split-type interferometer (Ishigaki et al. 2018b, Qi et al. 2015).

34.3 EXAMPLES OF NIR IMAGING STUDIES OF BIOLOGICAL MATERIALS AND SYSTEMS

NIR imaging has recently been used in pharmaceutical industry, agriculture, food research and technology, biology, and biomedical research (Lewis et al. 2004, Gowen et al. 2007, Grahn and Geladi 2007, Šašić and Ozaki 2010, Adu-Baffour 2012, Ishikawa et al. 2014, Salzer and Siesler 2014, Strlic 2018). For the applications in the pharmaceutical industry, for example, each pharmaceutical process such as blending, graduation, drying, and coating, the possibility of predicting quantity of components, evaluating the particle size, and determining the distribution of mixing components have been investigated (Lewis et al. 2004, Šašić and Ozaki 2010, Ishikawa et al. 2012, 2013b, 2014, Salzer and Siesler 2014, Murayama et al. 2013, 2015, 2018). In this chapter, two examples of applications to pharmaceutical industries utilizing novel types of NIR imaging devices are presented, and moreover, new types of biological applications and applications to studies of embryo development are introduced (Ishigaki et al. 2016b, 2018a, 2018b, Puangchit et al. 2017).

34.3.1 PHARMACEUTICAL TABLET MONITORING BY D-NIRs

To demonstrate the performance of D-NIRs as a PAT and QbD tool, the visualization of the distribution of tablet components was examined (Ishikawa et al. 2012, 2013b, Murayama et al. 2013, 2015, 2018). Figure 34.4 depicts a second-derivative image (a) and a standardized image (b) for the distribution of talc in the tablet developed by a peak at 1391 nm (Ishikawa et al. 2012). The standardized image was prepared by the mean and standard deviation of second derivative at 1391 nm to enhance the pattern observed in the image. Of note is the homogeneity of the component in the

![Optical conformation of the imaging-type two-dimensional Fourier spectroscopy system (ITFS; AOI Electronics Co., Ltd., NT00-T011). (Copyright: Ishigaki et al. 2018b.)](image-url)
tablet. Moreover, one should be recognized that D-NIRs has high portability, which enhances the potential as a PAT tool.

NIR images obtained by D-NIRs have also potential for the monitoring dissolution process of a tablet. Figure 34.5a and b shows NIR spectra and their second-derivative spectra of the tablet at a point of \(x\) (4.5 mm) and \(y\) (1.5 mm) during dissolution process, respectively (Ishikawa et al. 2013a). The intensity of a broad feature in the 1400–1600 nm region increases with the increase in bandwidth (Figure 34.5). Note in the second-derivative spectra that three bands appear at 1451, 1432, and 1420 nm in this region. However, a major reason for the intensity increase is water absorption because it increases with elapsed time. Figure 34.5c exhibits NIR images of the point mapped at 0, 120, and 240 min by the absorbance of 1361 nm due to ascorbic acid. One can clearly observe the dispersion of water into the tablet.

Figure 34.6 displays NIR images built by the intensity ratio of two bands at 1361 and 1354 nm of the second-derivative spectra obtained by D-NIRs during the dissolution process (Ishikawa et al. 2013a). The images demonstrated that the concentration of ascorbic acid in the tablet changes gradually due to water penetration. These studies successfully presented that D-NIRs is an attractive tool for pharmaceutical applications.

### 34.3.2 Quantitative Analysis of a Model Tablet Containing Three Different Components Using the High-Speed Wide-Area Monitoring System with a Newly Developed NIR Camera

In many cases of high-speed NIR imaging devices, the improvement of quantitative accuracy of sample spectra obtained at line or in line is important to use them for various industrial situations. We demonstrated the practical potential of high-speed NIR imaging devices through the quantitative analysis for tablets using NIR spectra combined with chemometrics (Ishikawa et al. 2013b, 2013c). The diffuse reflectance (DR) NIR spectra of tablets containing ascorbic acid, talc, and cellulose were measured for the 1000–2300 nm region, and their images were developed. To evaluate the quantitative accuracy, moving-window PLS (MWPLS) was employed to determine the regions of interest. A root mean square error of prediction (RMSEP) of less than 4.1 wt% and an \(R^2\) of more than 0.91 using one PLS factor were obtained as quantitative results (Ishikawa et al. 2013b). Although the quantitative accuracy of cellulose is relatively low \((R^2=0.83, \text{RMSE}=4.71 \text{ wt%})\) comparing with others, it was found that the standard normal deviate (SNV) model is useful in such cases. Finally, the SNV model provided a better prediction of \(R^2\) (0.86), and thus, one can say that its quantitative accuracy is almost as good as that obtained by a conventional NIR spectrometer.

For NIR imaging, sample tablets including talc, ascorbic acid, and cellulose were prepared. The sample contained a 3% food coloring reagent rather than the same proportion of cellulose.
As shown in Figure 34.7a, each sample consisted of three separate parts that differed in component concentration. Figure 34.7b depicts images developed by the prediction results over each component concentration in the tablets. The contrast of each NIR image corresponds to the concentration of each component, and the homogeneity within the separated area of each tablet is demonstrated.

34.3.3 Noninvasive, High-Speed, NIR Imaging of the Biomolecular Distribution in Fertilized Fish Eggs

NIR imaging allows one to analyze the distribution of biomaterials and their molecular mechanisms in embryonic development of Japanese medaka fish nondestructively and noninvasively without staining using NIR imaging (Ishigaki et al. 2016a, 2016b, 2018a, 2018b, Puangchit et al. 2017). The high-speed and wide-area monitoring system was used in this research, enabling ultra-high-speed imaging; using this system, it is possible to acquire microscopic imaging data only in a few seconds.
Figure 34.8 demonstrates the schematic view of the microscopic NIR imaging system (Ishigaki et al. 2018a). NIR measurements were performed in transmission mode. In order to regulate the optical path length, eggs were sandwiched between two glass slides with pinchcocks and spacers at 5 mm thickness. The objective lens at 5× magnification was placed in front of the camera and the spatial resolution was about 6.8 µm. The distributions of biomolecules were examined by mapping the intensities of NIR bands resulting from lipids, proteins, and water in two dimensions. The NIR imaging study succeeded in visualizing the structures of eyes, lipid bilayer membranes, micelles, and water-structure differences at the interface of different substances constituting different structures on the egg (Ishigaki et al. 2018a). Moreover, this investigation provided new insights on the metabolic mechanisms of lipids and membrane functions from the biased distribution of lipoproteins and the presence of unsaturated fatty acids in the egg membrane (Ishigaki et al. 2018a).

Figure 34.9a depicts an optical image of a fertilized medaka egg on the first day after fertilization (Ishigaki et al. 2018a). Soon after fertilization, the egg was structurally inhomogeneous. Many small oil droplets distributed throughout the egg began to coalesce and fuse into larger droplets. The cytoplasm at the same time began to form a blastodisc that would later transform into an embryonic disk, as shown in Figure 34.9b.

Figure 34.10a shows averaged NIR spectra in the 1000–2200 nm region of the three major parts (yolk, oil droplets, and embryo) of a fertilized medaka egg measured on the first day and the day
just before hatching (JBH) (Ishigaki et al. 2018a). Figure 34.10b and c depicts second-derivative spectra of (a) in the 1100–1820 and 1800–2150 nm regions, respectively. The spectra from yolks and embryos have similar spectral patterns; however, the spectra from oil droplets have several characteristic peaks due to lipids. A band at 1217 nm arises from a second overtone of the C-H stretching mode of CH₂ groups, and two bands at 1716 and 1773 nm are attributed to the first overtone of the C-H stretching modes of CH₂ groups in hydrocarbons and aliphatic compounds (Ishigaki et al. 2018a). A broad feature centered at approximately 2050 nm in the yolk and embryo spectra (Figure 34.10c) originates from a combination of an N-H stretching mode and amide II. It is noted that the spectral shapes in the 1400–1500 and 1880–1980 nm regions due to water differ significantly between oil droplets and other parts of the eggs as shown in Figure 34.10b and c.

FIGURE 34.8 The microscopic NIR imaging system with a prepared slide of a medaka fish egg. (Copyright: Ishigaki et al. 2018a.)

FIGURE 34.9 An optical image of a fertilized medaka egg on (a) the first day and (b) the fifth day after fertilization. (Copyright: Ishigaki et al. 2018a.)
Figure 34.11a shows visible images of a medaka fish egg from the first day to the day JBH, and Figure 34.11(b)–(f) exhibits NIR images developed by plotting the second-derivative intensities of some notable bands and scores extracted from PCA. In Figure 34.11b, the shape of the egg membrane, the contour of oil droplets, and the outline of the embryo appear by plotting the intensity at 1767 nm. The selected wavelength is located in the middle of the two bands between egg membrane (1730 nm) and oil droplets (1773 nm), which are both attributed to the first overtone of the C-H stretching modes of aliphatic compounds, such as fatty acids and hydrocarbons (Ishigaki et al. 2018a). The fact that these images obtained with the information of C-H stretching in CH2 groups showed clear membrane structures is consistent with the reported biomaterial constituents of cell membranes. Moreover, on the third day after fertilization, eye structures become clear. Eyes include a large amount of sphingolipids and glycerolipids with long carbon chains, which helps to clarify their structures in NIR images without staining.

Figure 34.11c displays the images developed by the C-H stretching band at 1716 nm of CH2 groups included in aliphatic compounds. Of note is that this band has large contributions from the sp2 CH2 group in unsaturated fatty acids. Therefore, Figure 34.11c shows the distribution of particularly unsaturated fatty acids in various kinds of fatty acids, and the structures of oil droplets and egg membrane are highlighted. Using the characteristic peaks due to saturated or unsaturated
structures, the distributions of different fatty acids can be selectively visualized. Of interest in these images is that the high oil contents within oil droplets and the thin-layer structure of the egg membrane are clearly visualized using this band. A terminal sp$_2$ CH$_2$ group exists at the ends of the molecules, and there are fewer sp$_2$ CH$_2$ than sp$_3$ CH$_2$ in fatty acids, which draw the membrane structure as shown in Figure 34.11b.

Images in Figure 34.11d were prepared based on the intensity of the band at 1564 nm arising from the first overtone of N-H stretching mode of amide groups (Ishigaki et al. 2018a). The structure of the egg membrane is clearly depicted because the second-derivative spectra obtained from the egg membrane show the significant peak. In cell membranes, proteins are embedded in the membrane structure and bound to lipids. These proteins are called membrane proteins, and these components are thought to be present in the image. Figure 34.11d appears to show that lipoproteins

FIGURE 34.11  (a) Visible images of medaka eggs from the first day after fertilization to the day just before hatching, and near-infrared images of eggs based on band intensities in second-derivative spectra at (b) 1767 nm (aliphatic compounds), (c) 1716 nm (unsaturated fatty acids), (d) 1564 nm (proteins), and (e) 1460 nm (water), using (f) scores calculated by projecting PC1 loadings onto imaging data to identify the contribution of weakly hydrogen-bonded water species. (Copyright: Ishigaki et al. 2018a.)
are heterogeneous in oil droplets. Heterogeneous components can also be seen in Figure 34.11b obtained by using a lipid band. The positions of these heterogeneous components overlap between Figure 34.11b and d, and these very likely are lipoproteins. Furthermore, the structures of eyes are shown in Figure 34.11d. For example, the vitreous body of the eye contains dense collagen, and collagen fibers have many amino groups. Therefore, eyes seem to be highlighted.

Figure 34.11e displays the images developed by plotting band intensities at 1460 nm due to strongly hydrogen-bonded water species. The images do reflect the slight differences in hydrogen-bonding states between the egg yolk and embryo, especially in the early stages of development. The concentration of strongly hydrogen-bonded water species, for example, is low in embryonic parts. It is very likely that this is caused by differences in the concentrations of proteins and lipids between the yolk and blastodisc. The yolk is a dense packet of energy and materials such as proteins. Water strongly interacts with dense materials in the yolk, and the environments surrounding water may be different between the yolk and embryo (Ishigaki et al. 2018a).

These results indicated the potential for NIR imaging in exploring the biological functions and metabolic systems of cells and embryos.

### 34.3.4 Nonstaining Blood Flow Imaging Using Optical Interference due to Doppler Shift

Ishigaki et al. further expanded the nonstaining NIR imaging of developing fish egg embryos. They not only obtained NIR images, but also detected interference signals caused by the Doppler effect with the ITFS system described above (Ishigaki et al. 2018b).

Using this system, absorption spectra of the egg substances were observed in the NIR region, and the information about the molecular composition of the embryo can be acquired noninvasively in situ. Of interest is that light reflected by a moving structure, which is associated with heart beat and blood flow, is also observed to show a slightly shifted frequency due to the Doppler shift. A beat signal is generated by heterodyne interference between the shifted and non-shifted frequency components. Figure 34.12a and b shows the interferograms obtained from the yolk part (static part) and from the heart part (moving part) of a medaka fish egg on the fifth day after fertilization, respectively (Ishigaki et al. 2018b). The interferogram from the static part exhibited the normal center burst, but the one obtained from the moving part, on the other hand, showed a noise-like pattern in addition to the center burst. The spectrum calculated by Fourier transformation of the interferogram from the heart part (Figure 34.12b) revealed three peaks at 3768, 1884, and 1256 nm (Figure 34.13). These new peaks, characteristic of the motion such as heart beat and blood flow,
seemed to be appearing in addition to those arising from molecular vibrations. In order to confirm their origin, the wavelength of the peaks $\lambda [\mu m]$ after Fourier transformation of the interferogram (Figure 34.12b) was calculated. The number of periodic noise-like waveforms were counted as 19 during 6-s measurement, corresponding to the frequency as 3.2 Hz. The optical path difference given by the partial movable mirror was $250 \sqrt{2} \mu m$ in 30 s. Therefore, $\lambda$ was calculated as $\lambda [\mu m] = 250 \sqrt{2} [\mu m]/3.2 [Hz] \times 30 s$, resulting in $\lambda = 3.68 \mu m$. It shows good agreement with the new peak as shown in Figure 34.13a. Other two peaks (1.88 and 1.26 $\mu m$) in Figure 34.13b, on the other hand, correspond to the first and second overtones of the fundamental mode (3.68 $\mu m$), respectively.

Figure 34.14(A) and (B) shows the images developed by plotting the intensities in two dimensions at the wavelength of the second overtone (1256 nm) and the first overtone (1884 nm), respectively, by

![Figure 34.13](image-url)

**FIGURE 34.13** Spectral information calculated by Fourier transformation of the data in Figure 34.12 (b) in the wave number region of (a) 2000–15,000 nm and (b) 1000–2500 nm. (Copyright: Ishigaki et al. 2018b.)

![Figure 34.14](image-url)

**FIGURE 34.14** Blood flow images of a medaka fish egg on the fifth day after fertilization obtained by plotting (a) detected light intensity from the sample, (b) absorbance, and (c) relative intensity calculated as the light intensity divided by the white light reference at (A) 1260 and (B) 1860 nm. (Copyright: Ishigaki et al. 2018b.)
using the detected light intensity (a), absorbance (b), and relative intensity (c) (Ishigaki et al. 2018b). It is noted that the location where the flow of red blood cells linked with the motion of the heart beat occurs is successfully visualized, and blood flow images can be created without staining.

Absorbance spectra obtained in a transmission mode show a large contribution due to water. Of note is that two dips originated from the heart beat are observed at ~1880 and 2260 nm. Figure 34.15 depicts NIR images developed by plotting the light intensity at 2260 nm (a) and absorbance at 1880 (b), 1940 (c), and 2360 nm (d). Figure 34.15a and b displays the exact part of the heart and the blood vessel stretched over the yolk in addition to the heart part (Ishigaki et al. 2018b). Figure 34.15c is produced using the water signals and scattering effect. Figure 34.15d is developed by the combination of the C–H stretching and bending modes of hydrocarbons and aliphatic compounds. The yolk has relatively high absorption due to the vibrational mode of C–H groups included in hydrocarbons and aliphatic compounds, and the yolk structure is highlighted in Figure 34.15d.

The technique described here has a variety of applications in biology and biomedical sciences such as cardiogenesis and differentiation of induced pluripotent stem (iPS) cells into cardiomyocytes. One may be able to catch the slight beating between them through optical interference in the very early stages of cardiogenesis and differentiation of iPS cells. It enables identification of the beat position and evaluation of the potential for proper differentiation of cells into cardiomyocytes.

### 34.4 FUTURE PROSPECTS

NIR imaging has become a powerful analytical tool; it has been utilized in various application fields. One of the possible new directions may be the use of NIR imaging for basic science. The investigations of embryo development described in this chapter are one good example. The NIR imaging studies of various kinds of cells including iPS cells hold considerable promise. NIR imaging may become a basic analytical tool in biology. As for practical applications, new attractive applications can be expected for almost all fields including pharmaceutical industry, food and agriculture, and medical sciences. Higher performance with more rapid measurement is expected to be developed. Although NIR imaging has been used for a wide range of art, historic, and archaeological materials, it is also starting to be used for studies of cultural heritage—the identification and quantitative characterization of heritage materials.

Recent advances in NIR instruments are of particular note. Potable NIR imaging systems should become more popular and popular. One can use it from characterizations, quality assessment, and growing monitoring in a field. Miniaturization of portable imaging systems is now in progress,
so that the working places of portable NIR imaging devices should become wider and wider. Last but not at least, three-dimensional NIR imaging systems are now realistically expected.

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