Non-Interferometric Techniques for X-ray Phase-Contrast Biomedical Imaging

Paul Claude Diemoz, Alberto Bravin, Paola Coan, and Luigi Rigon

CONTENTS

50.1 Introduction .................................................................................................................................................. 999
50.2 General Considerations .......................................................................................................................... 1000
50.3 Analyzer-Based Imaging .......................................................................................................................... 1001
  50.3.1 The Rocking Curve .......................................................................................................................... 1001
  50.3.2 A Simple Model of AB Image Formation .......................................................................................... 1002
  50.3.2.1 The Sample Introduces Refraction, But No USAXS .................................................................. 1003
  50.3.2.2 The Sample Introduces USAXS, But No Refraction ............................................................... 1003
  50.3.2.3 The Sample Introduces Both Refraction and USAXS ............................................................. 1003
  50.3.3 Phase Retrieval ................................................................................................................................. 1004
  50.3.3.1 The Sample Introduces Refraction, But No USAXS ............................................................... 1004
  50.3.3.2 The Sample Introduces USAXS, But No Refraction ............................................................... 1004
  50.3.3.3 The Sample Introduces Both Refraction and USAXS ............................................................. 1004
  50.3.3.4 Other Approaches to Phase Retrieval ....................................................................................... 1004
  50.3.4 Requirements ..................................................................................................................................... 1005
  50.4 Edge Illumination ..................................................................................................................................... 1005
  50.4.1 The Illumination Curve ..................................................................................................................... 1007
  50.4.2 Phase Retrieval and Sensitivity ....................................................................................................... 1008
  50.4.3 Coherence Requirements ................................................................................................................ 1010
  50.5 Computed Tomography ....................................................................................................................... 1010
  50.6 Applications to Biomedical Imaging ..................................................................................................... 1011
    50.6.1 Breast .............................................................................................................................................. 1011
    50.6.2 Musculoskeletal Phase-Contrast Imaging ..................................................................................... 1013
    50.6.3 Lung .............................................................................................................................................. 1013
    50.6.4 Neuroimaging ................................................................................................................................. 1014
    50.6.5 Vasculature, Circulation, and Other Tissues ................................................................................. 1015
  50.7 Conclusion ............................................................................................................................................... 1017
  References ....................................................................................................................................................... 1018

50.1 Introduction

The name of Arthur H. Compton is associated to the inelastic scattering of an X-ray photon by an electron. The Compton Effect is actually a wonderful example of the particle nature of X-rays (see Chapter 1). However, Compton was fully aware that X-rays have a wave nature as well. In fact, his Nobel lecture, held on December 12, 1927, bears the evocative title “X-rays as a Branch of Optics,” and it clearly states at the very beginning that “there is hardly a phenomenon in the realm of light whose parallel is not found in the realm of X-rays” (Compton 1927, p. 174). Despite this early awareness, in the first century after X-ray discovery, the wave nature of X-rays has only occasionally been exploited for imaging.

The late development of X-ray phase-contrast imaging (XPCI) is possibly related to the relatively weak interaction of X-rays with matter: actually, in the complex refraction index, $n = 1 - \delta + i\beta$, both the refractive index decrement, $\delta$, and the imaginary part, $\beta$, are very small: for example, in mammography (performed with X-rays in the 15 to 25 keV energy range), $\delta$ is of the order of $10^{-6}$ to $10^{-7}$, for soft tissues, while $\beta$ can be quoted in the range $10^{-6}$ to $10^{-10}$ (Henke et al. 1993). As a consequence, the deviations suffered by X-ray photons are in the microradians scale, and, thus, too small to be detected, unless specific phase-sensitive techniques are used. Most of these techniques require intense and highly coherent X-rays, and this is why XPCI has increasingly expanded in the last two decades, together with the availability of third generation synchrotron radiation sources. Several XPCI techniques have been progressively introduced, including: Bonse–Hart (or crystal) X-ray
interferometry, analyzer-based phase-contrast imaging (ABI) (see Section IV, Chapter 53), propagation-based phase-contrast imaging (PBI), edge illumination (EI), Talbot (or grating) X-ray interferometry, single grid, and speckle tracking.

All these techniques have been reviewed by Pelliccia, Morgan, and Kitchen in Chapter 49 of this Handbook. Here we would like to focus our attention on three non-interferometric methods (namely PBI, ABI, and EI) and their applications to medical imaging. The theory of PBI has been treated in detail in the previous chapter (Chapter 49). Thus, in the next sections, after some general consideration, we will focus on the basic principles of ABI and EI, illustrating the algorithms for image processing. Examples of application of PBI, ABI, and EI to breast, musculoskeletal, lung, neuro-, and vascular imaging are then provided in the second part of this chapter.

50.2 General Considerations

In a sense, PBI is the simplest phase-contrast method, since it just requires placing the detector at a suitable distance, \(z_{od}\), downstream from the sample, instead of immediately behind it (where a conventional, absorption-based image would be measured). However, to be effective, this method requires the X-ray beam to have a high degree of coherence and the detector to have sufficient spatial resolution, in order to capture the fringes arising upon propagation. The specific requirements for PBI have been comprehensively treated in the literature (Cloetens et al. 1996; Kotte and Birch 1999; Gureyev et al. 2008, 2009), and go beyond the scope of this chapter. Here, however, we would like to highlight the importance of the object-to-detector distance, \(z_{od}\), which should be compared to \(a^2/\lambda\), where \(\lambda\) is the X-ray wavelength, and \(a\) is the size of the smallest detail of interest in the sample. When \(z_{od} \ll a^2/\lambda\) (near-field region) a characteristic profile is recorded with one positive and one negative peak, in correspondence with the detail edges. Further, if \(z_{od} \sim a^2/\lambda\) (intermediate region: Fresnel diffraction or holographic regime), the object image is slightly distorted, presenting an interference pattern with several oscillations in correspondence to each edge. Finally, if \(z_{od} \gg a^2/\lambda\) (far-field region: Fraunhofer diffraction), the intensity pattern at the detector plane has less resemblance to the object, but can still be used for imaging in the case of small samples and highly coherent beams (Nugent 2010). In medical X-ray imaging, typical values for \(a\) and \(\lambda\) are \(10^{-3}\) m and \(10^{-6}\) m, respectively, which makes \(a^2/\lambda\) of the order of 1 meter.

Arguably, so far the most interesting applications in medical imaging have been obtained in the near-field region. The edge-enhanced images obtained in this case can be used without further processing, since the improved visualization of the detail outline is often sufficient to highlight its presence, even in the case of low absorption contrast. Alternatively, phase retrieval procedures can be utilized (see Chapter 49) with a twofold advantage. First, quantitative parametric images can be obtained, especially when the sample meets particular conditions (e.g., weak absorption, or a nearly constant \(\delta/\beta\) ratio). Second, and possibly more importantly, phase retrieval can improve the visualization of the detail (particularly in the case of noisy images), since the edge enhancement is somehow converted to an area contrast which makes the image interpretation easier (Chen et al. 2013).

In contrast to PBI, ABI and EI require the introduction of particular devices in the imaging system. Although quite different in the requirements and implementation, ABI and EI are conceptually similar, in that the contrast is created by selectively accepting or rejecting the X-ray photons, according to the deviations that they have experienced in traversing the sample. Moreover, both ABI and EI are sensitive to the same physical characteristics of the sample and produce parametric images, where a pixel-by-pixel map of these characteristics is provided. More specifically, both techniques can provide three different parametric images: the first is related to the absorption of the sample, and is, thus, similar to conventional X-ray imaging; the second is based on refraction, and is sometimes referred to as a differential phase-contrast image (because the refraction angle is proportional to the gradient of the phase shift, as detailed in Equation 50.1 below); the third allows visualizing X-ray scattering at small or ultra-small angles, and can be considered as a form of dark field imaging.

Although it could be noted that refraction, small-, and ultra-small-angle scattering share the same physical origin (Davis 1994), a schematic distinction is outlined in the following for X-ray energies and spatial resolutions typical of medical imaging applications.

Refraction is observed when an object is much larger than the detector pixel size and is well resolved by the imaging system: thus, a well-defined deviation angle, \(\Delta \theta_{ref}(x, y)\), can be associated to a given point \((x, y)\) of the object plane, where the detector corresponds to a certain detector pixel in the image plane. In the projection approximation (see Chapter 49), the deviation, \(\Delta \theta_{ref}(x, y)\), with respect to the original direction, \(z\), can be expressed as:

\[
\Delta \theta_{ref}(x, y) \approx \frac{1}{k} \sqrt{\left(\frac{\partial}{\partial x} \phi(x, y)\right)^2 + \left(\frac{\partial}{\partial y} \phi(x, y)\right)^2} \approx \frac{1}{k} |\nabla_{xy} \phi(x, y)|
\]

(50.1)

where \(k = 2\pi/\lambda\) is the wavenumber, and \(\nabla_{xy} \phi(x, y)\) is the gradient of the phase shift, \(\phi(x, y)\), in the object plane. Typically, refraction can be observed at the boundaries of some particular feature in the sample under investigation, where the phase shift, \(\phi(x, y)\), is characterized by sudden variations. Another classical example is that of a wedge-shaped object, which introduces a constant non-zero value for the spatial derivatives of the phase shift, and hence a constant deviation, \(\Delta \theta_{ref}\), across the object.

In a sense, scattering can be considered as a series of multiple refractive events generated by small internal structures that cannot be resolved by the imaging system (Rigon et al. 2003). In particular, small-angle X-ray scattering (SAXS) arises from diffuse photon deviations in the milliradian range, which originate from structures at the nanometer scale, while ultra-small-angle X-ray scattering (USAXS) is due to diffuse photon deviations in the microradian scale, which originate from larger structures spanning from hundreds of nanometers up to several micrometers.

The ability of ABI and EI to produce three parametric images based on absorption, refraction, and scattering (which is shared also by other XPCI techniques, such as Talbot—or grating—interferometry) is very important, since each of these images
(or likely their combination) can provide additional information compared to conventional X-ray imaging.

### 50.3 Analyzer-Based Imaging

Analyzer-Based Imaging (ABI) is a phase-sensitive technique, which relies on an analyzer crystal placed between the sample and the detector (see Section IV, Chapter 53). The analyzer acts as an angular band-pass filter. Its angular pass window is typically 1–20 microradians wide, and is, thus, commensurate with the tiny deviations experienced by the X-ray photons traversing a biological sample in medical imaging (in the energy range of 10–100 keV). Therefore, a photon, which has been deviated in the microradians range, has a different probability to be collected by the detector compared to the undeviated X-rays. As a result, the analyzer transforms the tiny angular deviations induced by the sample into measurable intensity modulations on the detector, providing extra contrast in addition to X-ray absorption.

The first ABI pioneers were probably Förster et al. (1980), who developed what they called the “X-ray Schlieren method” using a double crystal diffractometer for the investigation of shell targets in laser fusion experiments. The technique was then seldom employed (Podurets et al. 1989; Ingal and Beliaevskaya 1995; Davis et al. 1995a,b), until, when re-discovered by Chapman et al. (1997), it quickly spread among the synchrotron radiation X-ray imaging community, particularly in the biomedical field. As is often the case in science, Chapman and co-workers’ discovery was characterized by a certain amount of serendipity: their original idea was to use the analyzer crystal as an extraordinarily selective anti-scattering grid to obtain very clean (absorption-based) images. However, they immediately realized that in this configuration the analyzer crystal could yield additional (phase) contrast (Chapman et al. 1996).

Actually, raw AB images typically incorporate different pools of phase-contrast (in addition to absorption), due to refraction, SAXS, and USAXS occurring in the sample. Since the angular selection of the crystal has a bandwidth of the order of 1 to 100 microradians, SAXS is fundamentally rejected by the analyzer crystal, thus creating the so-called extinction contrast (Chapman et al. 1997). On the other hand, USAXS is commensurate to the pass window of the analyzer system and can, thus, contribute in several different ways to the image contrast, as will be described in detail in the following.

#### 50.3.1 The Rocking Curve

ABI is usually performed with a monochromatic and highly collimated X-ray beam, synchrotron radiation being a natural choice. The essential elements of an ABI setup are the monochromator and the analyzer crystals, which are placed before and after the sample, respectively. Several choices are possible in designing an ABI system, concerning the crystal number, arrangement, type, and order of diffraction. To begin with, both the monochromator and the analyzer can be either single or double crystals, and can exploit either Bragg or Laue diffraction, or both (Ingal and Beliaevskaya 1995; Chapman et al. 1996; Kitchen et al. 2011). Possibly, the simplest setup is shown in Figure 50.1a, where single crystals in Bragg geometry are used as a monochromator and as an analyzer (Sztrókai et al. 2012). Of course, this implies that the sample should be oriented at right angles with the diffracted beam, thus inclined by twice the Bragg angle with respect to the horizontal (incident) direction. An alternative setup is shown in Figure 50.1b,
where double crystals in Bragg geometry are used as a monochromator and as an analyzer (Arfelli et al. 2017a). While this configuration brings in some complication in the precise alignment of a four-bounce system, it does not require one to slant the sample stage. Another option is using a double crystal monochromator and a single crystal analyzer, which, however, requires the inclination of the detector stage (Figure 50.1c; Zhong et al. 2000).

In the absence of the sample, the intrinsic rocking curve, \( R(\theta) \), can be obtained by measuring the beam intensity diffracted by the analyzer crystal as a function of the analyzer misalignment angle, \( \theta \) with respect to the Bragg angle, \( \theta_B \). This measure is typically carried out by means of two ion chambers, placed before and after the analyzer. The peak reflectivity is found at \( \theta = 0 \), when the analyzer is perfectly aligned with the monochromator at the Bragg angle, \( \theta_B \).

The rocking curve is actually the angular filter that selectively weights the probability that each photon is diffracted towards the detector. Thus, its shape and its width are fundamental characteristics of an ABI system.

Ideally the rocking curve should be narrow, in order to maximize the SAXS rejection and, thus, the extinction contrast, and steep, since the slope of the rocking curve is responsible for refraction contrast, as demonstrated later in the next section. On the other hand, a narrow pass window means a reduced intensity on the detector; moreover, ABI systems based on narrow and steep rocking curves are particularly prone to mechanical and thermal instabilities. Thus, the choice of the crystal type, geometry, and diffraction order must take these drawbacks into account. It is worth noting that, for a given crystal system, the width of the rocking curve is inversely proportional to the energy (Caciuffo et al. 1987). This partially compensates the \( 1/E^2 \) signal loss characteristic of phase-contrast effects (Gureyev et al. 2009).

Prior to image acquisition, the intrinsic rocking curve, \( R(\theta) \), is measured and a working point, \( \theta_k \), is chosen on the rocking curve. The choice of the working point deeply affects the characteristics of the image and the balance among the different sources of contrast that it incorporates.

Figure 50.2 shows an example of a rocking curve (obtained for Si(111) crystals in a non-dispersive Bragg configuration at 17 keV). Five possible working points of \( \theta_k \), with \( A \in \{1, 2, 3, 4, 5\} \) are also shown. In particular, \( A = 1 \) and 5 correspond to the toes of the rocking curve, \( A = 2 \) and 4 to the flanks and \( A = 3 \) to the peak.

This nomenclature for the working points is arbitrary and is not a standard in ABI literature, but is introduced here for convenience, as it will be heavily used in the next paragraph.

### 50.3.2 A Simple Model of AB Image Formation

Before proceeding, a clarification is in order. Since the analyzer crystal is only sensitive to deviations that fall in its diffraction plane \( yz \), we define \( \Delta \theta_{ref}(x, y) \) as the projection of the refraction angle, \( \Delta \theta(x, y) \), on the \( yz \) plane. In other words, ABI is basically insensitive to the \( x \) component of the gradient of the phase shift. Thus, only the \( y \) component contributes to \( \Delta \theta_{ref}(x, y) \), and Equation 50.1 simplifies to:

\[
\Delta \theta_{ref}(x, y) = \frac{1}{k} \frac{\partial}{\partial y} \phi(x, y).
\] (50.2)

In ABI literature, and in the following, the distinction between \( \Delta \theta_{ref}(x, y) \) and \( \Delta \theta(x, y) \) is often taken for granted, and the expression refraction angle is used when referring to \( \Delta \theta(x, y) \).

A simple model of ABI is given in the following. For the sake of clearness, three different cases are considered, depending on the characteristic of the sample: namely, the cases in which (1) the sample introduces refraction, but no USAXS; (2) the sample introduces USAXS, but no refraction; and (3) the sample introduces both refraction and USAXS. It is worthwhile to note that, in each of the cases above, the sample could also absorb X-rays (absorption contrast) and introduce SAXS, which simply leads to the aforementioned extinction contrast.

![Rocking curve with five possible working points, \( \theta_k, A \in \{1, 2, 3, 4, 5\}, A = 1 \) and 5 correspond to the toes of the rocking curve (~20% of relative intensity), \( A = 2 \) and 4 to the flanks (~50% of relative intensity), and \( A = 3 \) to the peak (100% of relative intensity). The full width at half maximum (FWHM) of the rocking curve is indicated with \( \Delta \theta_{ref} \).](image)

**FIGURE 50.2** Rocking curve with five possible working points, \( \theta_k, A \in \{1, 2, 3, 4, 5\}, A = 1 \) and 5 correspond to the toes of the rocking curve (~20% of relative intensity), \( A = 2 \) and 4 to the flanks (~50% of relative intensity), and \( A = 3 \) to the peak (100% of relative intensity). The full width at half maximum (FWHM) of the rocking curve is indicated with \( \Delta \theta_{ref} \).
### 50.3.2.1 The Sample Introduces Refraction, But No USAXS

In this case, the photons exiting the object plane xy at the point (x, y) are either subject to SAXS (extinction contrast) or are refracted at a well-defined refraction angle, $\Delta\theta(x, y)$ (see Equation 50.2), with respect to the incident direction, z. Let $I(\theta_{i};x, y)$ be the image that can be measured on the detector plane, where $\theta_{i}$ represents the working point chosen on the rocking curve. In the case in which USAXS is negligible, a simple expression can be written for $I(\theta_{i};x, y)$ (Chapman et al. 1997):

$$I(\theta_{i};x, y) = I_{R}(x, y) \cdot R(\theta_{i} + \Delta\theta_{i}(x, y))$$  \hspace{1cm} (50.3)

where $R(\theta_{i})$ is the derivative of the rocking curve $R(\theta)$ at the angular position $\theta_{i}$. Of note, the first-order Taylor expansion is particularly accurate when the working point $\theta_{i}$ is chosen on the flanks of the rocking curve ($\theta_{2}$ and $\theta_{4}$) in Figure 50.2), where the linear approximation appears adequate.

A closer look at Equation 50.4 can simply demonstrate how the choice of the working point, $\theta_{i}$, affects the measured intensity. Consider, for instance, images collected at $\theta_{i}$. Since refraction is negligible, the intensity collected by the detector plane, where $\theta_{i}$ is chosen at the flanks of the rocking curve ($\theta_{2}$ and $\theta_{4}$), will be dominated by absorption and extinction contrast. On the other hand, if $\theta_{i}$ is chosen at the toes of the rocking curve ($\theta_{1}$ and $\theta_{3}$) in Figure 50.2), where $R'(\theta_{i})$ is maximum, refraction contrast is highlighted and typically dominates on absorption and extinction contrast.

### 50.3.2.2 The Sample Introduces USAXS, But No Refraction

In the absence of refraction, the intensity collected by the detector with the analyzer crystal set to the working point, $\theta_{i}$, can be written as:

$$I(\theta_{i};x, y) = I_{R}(x, y) \cdot \int R(\theta_{i} + \Delta\theta_{i})f(\Delta\theta_{i};x, y)d(\Delta\theta_{i})$$  \hspace{1cm} (50.5)

where $\Delta\theta_{i}$ is the stochastic scattering angle, and the normalized probability density function, $f(\Delta\theta_{i};x, y)$, represents the USAXS distribution for photons exiting the object plane at the point (x, y) (Rigon et al. 2003). Since refraction is negligible, $f(\Delta\theta_{i};x, y)$ is centered forward; this fact can be used, for small scattering angles, $\Delta\theta_{i}(x, y)$, to simplify the integral in Equation 50.5 by performing the second-order Taylor expansion of $R(\theta)$, yielding:

$$I(\theta_{i};x, y) = I_{R}(x, y) \cdot \left[ R(\theta_{i}) + \frac{1}{2} R''(\theta_{i})\sigma^{2}_{\Delta\theta_{i}}(x, y) \right]$$  \hspace{1cm} (50.6)

where $R''(\theta_{i})$ denotes the second derivative of the rocking curve, $R(\theta)$, at the angular position, $\theta_{i}$, and $\sigma^{2}_{\Delta\theta_{i}}(x, y) = \int (\Delta\theta_{i})^{2}f(\Delta\theta_{i};x, y)d(\Delta\theta_{i})$ represents the second moment of the USAXS distribution. As a consequence of Equation 50.6, in order to emphasize the contrast due to USAXS, the working point, $\theta_{i}$, should be chosen at the peak ($\theta_{1}$ in Figure 50.2) or at the toes of the rocking curve ($\theta_{2}$ and $\theta_{4}$) where $R'(\theta_{i})$ reaches local extrema. In particular, when the working point is chosen at the peak, USAXS contributes to decreasing the measured intensity, similarly to absorption and extinction contrast, as $R''(\theta_{i})$ is negative. On the contrary, when the working point is chosen at the toes of the rocking curve, USAXS contributes to increase the measured intensity, as $R''(\theta_{i})$ are positive. Actually, in this case some scattered photons are accepted with greater likelihood than the photons in the unperturbed beam, which is mostly rejected by the analyzer crystal. Thus, the scattering detail will appear brighter than the background. Exploiting this reverse contrast is sometimes referred to as dark field imaging. Finally, choosing the working point at the flank of the rocking curve, ($\theta_{2}$ or $\theta_{4}$), where $R'(\theta_{i})$ vanishes, will suppress USAXS contrast.

### 50.3.2.3 The Sample Introduces Both Refraction and USAXS

In the general case when the sample features both refraction and USAXS (as well as absorption and SAXS), the intensity, $I(\theta_{i};x, y)$, reaching the detector with the analyzer set at the angle, $\theta_{i}$, can be written as (Rigon et al. 2007a,b):

$$I(\theta_{i};x, y) = I_{R}(x, y) \cdot \int R(\theta_{i} + \Delta\theta_{i}(x, y) + \Delta\theta_{s})$$

$$f(\Delta\theta_{i};x, y)d(\Delta\theta_{i}),$$

\hspace{1cm} (50.7)

which can be readily reduced to Equation 50.3 (in the case of pure refraction) or to Equation 50.5 (pure USAXS). Again, the integral in Equation 50.7 can be solved by performing a second-order Taylor expansion for $R(\theta_{i} + \Delta\theta_{i}(x, y) + \Delta\theta_{s})$ around $\theta_{i}$ and utilizing the properties of the USAXS distribution. The result is:

$$I(\theta_{i};x, y) \cong I_{R}(x, y) \cdot \left[ R(\theta_{i}) + R'(\theta_{i})\Delta\theta_{i}(x, y) \right.$$

$$\left. + \frac{1}{2} R''(\theta_{i})\Delta\theta_{i}^{2}(x, y) + \sigma^{2}_{\Delta\theta_{i}}(x, y) \right].$$

\hspace{1cm} (50.8)

Obviously, as previously noted for Equations 50.4 and 50.6, also the expression for $I(\theta_{i};x, y)$ in Equation 50.8 is accurate for small angles, $\Delta\theta_{i}(x, y)$ and $\Delta\theta_{s}$, while it becomes imprecise in the case of large refractive and/or scattering deviations. Actually, the use of the Taylor expansion has been proposed here because it simplifies the approach to image formation and to phase retrieval (discussed in the next paragraph). However, there are several different ABI models, both for image formation and for phase retrieval, which do not perform the Taylor expansion of the rocking curve. These models will be treated at the end of the next paragraph.
50.3.3 Phase Retrieval

In ABI, images collected at different working points on the rocking curve can be combined to obtain parametric images which represent maps of the phase shift (or of other related physical quantities) introduced by the sample. Some simple phase retrieval methods are reviewed in the following. In parallel with the previous paragraph, the three cases in which (1) the sample introduces refraction, but no USAXS; (2) the sample introduces USAXS, but no refraction; and (3) the sample introduces both refraction and USAXS are presented.

50.3.3.1 The Sample Introduces Refraction, But No USAXS

The first example of these phase retrieval methods can be found in the diffraction-enhanced imaging (DEI) algorithm, introduced by Chapman et al. (1997), for samples that cause refraction, but no USAXS. DEI consists of combining the two images acquired on the flanks of the rocking curve, at the angular positions, \( \theta_2 = -\Delta \theta_2/2 \) and \( \theta_1 = \Delta \theta_2/2 \), \( \Delta \theta_2 \) being the full width at half maximum (FWHM) of the rocking curve (see Figure 50.2). Equation 50.4 can be specifically rewritten with \( \Delta \theta_2 \), providing a map of the refractive structures, which cannot be imaged directly by the imaging system because of its limited spatial resolution. In this way, we obtain a system of two independent equations, which can be solved to yield the unknowns \( I_R(x, y) \) and \( \Delta \theta_2(x, y) \):

\[
I_R(x, y) = \frac{I_2(x, y)R'(\theta_2) - I_4(x, y)R'(\theta_1)}{R(\theta_2)R'(\theta_1) - R(\theta_1)R'(\theta_2)}, \tag{50.9}
\]

\[
\Delta \theta_2(x, y) = \frac{I_4(x, y)R(\theta_2) - I_2(x, y)R(\theta_1)}{I_4(x, y)R'(\theta_2) - I_2(x, y)R'(\theta_1)}. \tag{50.10}
\]

As a matter of fact, Equations 50.9 and 50.10 are applied on a pixel-by-pixel basis, giving two parametric images: \( I_R(x, y) \) is called the apparent absorption image, and accounts for the absorption and extinction properties of the sample, while \( \Delta \theta_2(x, y) \), the so-called refraction image, provides a map of the refraction angle. Since the latter is proportional to the vertical gradient of the phase shift (see Equation 50.2), \( \Delta \theta_2(x, y) \) is sometimes dubbed the differential phase-contrast image.

50.3.3.2 The Sample Introduces USAXS, But No Refraction

A modified version of the DEI algorithm can be applied for samples that cause negligible refraction but considerable USAXS (Rigon et al. 2003). Usually, these samples are characterized by a large amount of very fine refractive structures, which cannot be imaged directly by the imaging system because of its limited spatial resolution. In this case, two possible instantiations of Equation 50.6 can be obtained, acquiring two images at the peak and at one toe of the rocking curve, respectively, thus at the angular positions \( \theta_1 \) (or \( \theta_2 \)) and \( \theta_1 \) (see Figure 50.2). The system of two independent equations represented by Equation 50.6 with \( A = 1, 3 \) can be solved to yield the unknowns \( I_d(x, y) \) and \( \sigma^2_{\Delta \theta_6}(x, y) \):

\[
I_d(x, y) = \frac{I_2(x, y)R''(\theta_1) - I_4(x, y)R''(\theta_2)}{R(\theta_1)R''(\theta_2) - R(\theta_2)R''(\theta_1)), \tag{50.11}
\]

\[
\sigma^2_{\Delta \theta_6}(x, y) = 2 \frac{I_4(x, y)R(\theta_1) - I_2(x, y)R(\theta_2)}{I_2(x, y)R''(\theta_1) - I_4(x, y)R''(\theta_2)}. \tag{50.12}
\]

The first parametric image is again the apparent absorption, \( I_d(x, y) \), while the second gives a map of the square width, \( \sigma^2_{\Delta \theta_6}(x, y) \), of the USAXS distribution, and is usually called the USAXS image.

50.3.3.3 The Sample Introduces Both Refraction and USAXS

A generalized DEI (GDEI) approach can also be used with samples introducing both refraction and USAXS (Chou et al. 2007; Rigon et al. 2007a,b). In this general case, three images are required in input and three parametric images are produced in output. The three input images can be represented as three different instantiations of Equation 50.8. In principle, any choice of three different working points on the rocking curve is allowable; however, the best results are generally obtained by utilizing the two flanks and the peak position (Rigon et al. 2007b), such as with \( A = 2, 3, 4 \). The solution of the three-equation system represented in this case by Equation 50.8 provides three parametric images, namely the apparent absorption image, \( I_d(x, y) \), the refraction image, \( \Delta \theta_6(x, y) \), and the USAXS image, \( \sigma^2_{\Delta \theta_6}(x, y) \). A lengthy but straightforward calculation gives the results reported in Equations 50.13 to 50.15:

\[
I_d = \frac{I_2(R''R'' - R'R'' - R''R'') + I_4(R'R'' - R''R'') - R_4(R''R'') + R_2(R''R'') - R_4(R'R'') + R_2(R''R'')} {R_2(R''R'' - R'R'') - R_4(R''R'') + R_2(R''R'') - R_4(R''R'') + R_2(R''R'') - R_4(R''R'') + R_2(R''R'') - R_4(R''R'') + R_2(R''R'') - R_4(R''R'')} \tag{50.13}
\]

\[
\Delta \theta_6 = \frac{I_2(R'R'' - R''R'') + I_4(R''R'') - R_4(R'R'' - R''R'') + I_4(R''R'') - R_4(R'R'' - R''R'') + I_4(R''R'') - R_4(R'R'' - R''R'')} {I_2(R'R'' - R''R'') - I_4(R''R'') + I_4(R''R'') - R_4(R'R'' - R''R'') + I_4(R''R'') - R_4(R'R'' - R''R'')} \tag{50.14}
\]

\[
\sigma^2_{\Delta \theta_6} = \frac{2 I_2(R'R'' - R''R'') + I_4(R''R'') - R_4(R'R'' - R''R'') + I_4(R''R'') - R_4(R'R'' - R''R'') + I_4(R''R'') - R_4(R'R'' - R''R'')} {(\Delta \theta_6)^2} \tag{50.15}
\]

where, in order to keep the notation more compact, the spatial variables \((x, y)\) have been omitted, and \( R(\theta) \), \( R'(\theta) \), and \( R''(\theta) \) have been dubbed \( R_2 \), \( R_4' \), and \( R_4'' \), respectively. Equations 50.13 to 50.15 define the GDEI algorithm.

50.3.3.4 Other Approaches to Phase Retrieval

As mentioned above, not all the phase retrieval methods require approximating the rocking curve with its Taylor expansion. Actually, avoiding Taylor expansion allows for extending the range of validity of the phase retrieval methods, at least in principle, since in this case the refraction and scattering angles are not
required to take only small values compared to $\Delta \theta_p$ (the rocking curve FWHM). For instance, in the case of samples introducing refraction, but no USAXS, the so-called extended DEI algorithm (Hu et al. 2008) utilizes a simple Gaussian model for the rocking curve, $R(\theta)$, producing parametric images similar to the apparent absorption and to the refraction images. Similar approaches, that rely on Equation 50.3 rather than on the approximated Equation 50.4, can be found also in Maksimenko (2007) and Kitchen et al. (2007), where more refined models are used for the rocking curve, $R(\theta)$. More recently, Arfelli et al. (2017b) developed a non-Taylor algorithm that deals with the general case of samples introducing both refraction and USAXS, relying on Equation 50.7 rather than on the approximated Equation 50.8. In this case, three images are required in input and three parametric images are produced in output (as in GDEI); moreover, the intrinsic rocking curve, $R(\theta)$, is modeled as a Gaussian with standard deviation, $\sigma_{\theta_c}$. Although any choice of three different working points is legitimate, the two flanks and the peak position are used in the following. The solutions of the three-equation system represented by Equation 50.7 (with $A = 2, 3, 4$) are calculated as:

$$I_R = I_A \frac{\sigma_{\theta_c}}{\sigma_{\theta_c}} \exp\left[\frac{(\theta_A + \Delta \theta_2)^2}{2\sigma^2}\right] \quad (50.16)$$

$$\Delta \theta_2 = \frac{1}{2} \frac{D(\theta_1^2 - \theta_2^2)}{D(\theta_2 - \theta_3) + C(\theta_1 - \theta_2)} \quad (50.17)$$

$$\sigma^2_{\Delta \theta_2} = \frac{(\theta_2 - \theta_3)(\theta_1 - \theta_3)(\theta_1 - \theta_2)}{D(\theta_2 - \theta_3) + C(\theta_1 - \theta_2)} - \sigma^2_{\theta_c} \quad (50.18)$$

where $\sigma^2 = \sigma^2_{\theta_c} + \sigma^2_{\Delta \theta_2}, C = -2\ln(I_2/I_A), \text{ and } D = -2\ln(I_A/I_2)$. Other approaches for phase retrieval in ABI are based on the concept of local rocking curve, such as the rocking curve that can be measured after the object on a pixel-by-pixel basis on the image plane $xy$. The local rocking curve is then compared, also on a pixel-by-pixel basis, with the intrinsic rocking curve, which is measured in the absence of the object. Typically, measuring these rocking curves requires the acquisition of a few different images. The measured (local) rocking curve will appear of lower intensity (compared to the intrinsic rocking curve, because of absorption, shifted due to refraction), and broader (as an effect of USAXS). The area (and/or the height), the angular shift, and the angular broadening of the local rocking curve (as compared to the intrinsic rocking curve) can be regarded as parametric images measuring apparent absorption, refraction, and USAXS, respectively. This approach is in principle more accurate than the methods relying on the Taylor expansion of the rocking curve. Its accuracy, however, depends on the number of collected images (which ranges from 3 up to 30), and on the width of the angular interval covered by these images. In the so-called Multiple Image Radiology (MIR) approach, such parametric images are evaluated by means of numerical and statistical methods (Pagot et al. 2003; Wernick et al. 2003). Alternatively, the local rocking curve can be analytically fit on a pixel-by-pixel basis (Oltulu et al. 2003; Nesterets et al. 2006; Zhifeng et al. 2007; Kitchen et al. 2010). Such fitting methods are proven to be more robust than GDEI and MIR when the object introduces large refraction or scattering angles as compared to the width of the rocking curve (Diemoz et al. 2010). However, they also require more computational power: curve fitting the local rocking curve for each pixel can be rather lengthy, for instance, in a standard mammographic image of 18 cm $\times$ 24 cm with a pixel size of 50 microns, which contains almost 20 million pixels.

### 50.3.4 Requirements

ABI can provide extraordinary sensitivity to sub-microradian deviations in the sample; however, its performance implies stringent requirements in terms of mechanical stability of the imaging system. The latter can be achieved with a state-of-the-art setup, which includes optical tables, precision motors, and possibly active feedback systems (Rhoades et al. 2013).

As detailed above, ABI is only sensitive to angular deviations which lie in the crystal diffraction plane $xyz$. However, ABI sensitivity can be extended to both transverse directions ($x$ and $y$) by means of a so-called Bragg magnifier (Modregger et al. 2007), which consists of crystals with mutual perpendicular diffraction planes.

ABI implementation on a conventional X-ray tube is hampered by the need of a highly collimated and temporally coherent (monochromatic) beam. Attempts of applying ABI to conventional sources have so far produced limited X-ray intensity on the sample, causing a correspondingly long exposure time (Vine et al. 2007; Parham et al. 2009). However, several research and development projects are focused on this target. A technical discussion of some challenges and pitfalls can be found in Höhnicke et al. (2012).

### 50.4 Edge Illumination

The edge illumination (EI) XPCI technique was first developed at the Elettra synchrotron in Trieste (Italy) in the late 1990s, as an alternative method to the ABI technique that would not involve the use of a perfect crystal (Olivo et al. 2001). Similarly to ABI, the EI technique is based on detecting the refraction angles undergone by the beam in passing through the sample. The elimination of the need to use a crystal, however, significantly simplifies the setup and relaxes the requirement in terms of mechanical stability and beam coherence, making the technique applicable to tabletop setups employing X-ray tubes (Olivo and Speller 2007a,b).

The working principle of EI is schematized in Figure 50.3, in the case of its simplest implementation using two slits, which is the one usually employed in synchrotron setups (Olivo et al. 2001, 2012, 2013). The beam is collimated by a slit placed before the sample (the so-called *pre-sample slit*), featuring an aperture typically on the order of a few to tens of micrometers. A second slit (the *detector slit*) is set in front of the detector and aligned with a row of detector pixels. The two slits are partially misaligned with respect to each other, so that the collimated beam produced by the first slit is incident onto the edge of the detector aperture: in this situation, part of the beam will be able to reach the detector, while the remaining part will be stopped by the slit. In order to...
obtain this partial illumination condition, typically the first slit is shifted rather than the second, to prevent moving the detector as well. When a sample is introduced in the collimated beam, the beam can be refracted, thus shifting the position of the beamlet on the detector slit by the quantity $y = z_{od} \tan(\Delta \theta_y)$, where $z_{od}$ is the distance between the sample and the detector slit, and $\Delta \theta_y$ is the component of the refraction angle in the direction orthogonal to the slits. Since refraction angles for X-rays are typically very small (on the order of a few microradians or a fraction of a microradian), the expression for the displacement can be accurately approximated by $\Delta y \sim z_{od} \cdot \Delta \theta_y$ (this quantity is usually on the order of a few micrometers or less, for propagation distances $z_{od}$ on the order of 1 m). This beam shift has the effect of either increasing or decreasing the counts on the detector, depending on whether the beam is moved towards the aperture or towards the absorbing part of the slit (see Figure 50.3). The method thus encodes the refraction angles produced by the sample into a measurable intensity modulation on the detector. In particular, the boundaries of the various object structures, where refraction mainly takes place, will produce positive or negative signal peaks, exactly like in ABI.

A two-dimensional image can then be obtained via a scan of the sample in the direction orthogonal to the slits, to cover the full extent of the object. Pasting together the single lines produced by each step of the scan provides the final image of the sample. In addition to the refraction signal, mainly highlighting the sample structures as mentioned above, attenuation and scattering also play a role in producing image contrast in EI. As in conventional X-ray imaging, attenuation of the sample causes a reduction in the flux of photons reaching the detector and, therefore, a drop in the measured intensity. As highlighted in a previous section on ABI, the so-called USAXS can be interpreted as refraction produced by sample structures on a scale smaller than the aperture size, which are too small to be resolved (Rigon et al. 2003; Endrizzi et al. 2014). Various points in each pixel are then refracted by a different angle, with this distribution of angles resulting in a broadening of the beamlet (Endrizzi et al. 2014). The contribution of USAXS to the image contrast will be analyzed in more detail in the next sections.

When an extended beam such as that produced by an X-ray tube is used to irradiate the object, the EI principle can be replicated over the whole field of view by replacing the two slits with two masks featuring several apertures, thus making the sample scan unnecessary (Figure 50.4a) (Olivo and Speller 2007a). In this case, the period of the detector mask is usually chosen to match the pixel size, so that every detector aperture corresponds to a row or column of pixels on the detector. The effect of the beam divergence can be taken into account by simply scaling down the period of the sample mask.

Eliminating the need for the sample scan has the advantage of making the image acquisition much faster, as different areas of the samples can be imaged at the same time by different aperture pairs. However, a procedure called dithering is often used in order to increase the sampling step in the direction orthogonal to the slits. This consists in a scan of the sample along the same direction, in steps of a fraction of the mask period. Various frames are then collected and recombinated, in order to create a new image with improved spatial resolution (Olivo and Speller 2007b; Ignatyev et al. 2011). The effective sampling step of this image will then be equal to the period of the pre-sample mask, divided by the number of dithering steps. For instance, for a typical sample mask period of 50 µm, using five dithering steps enables reaching an effective spatial resolution of about 10 µm.

It has to be mentioned, however, that there exists a limit to the spatial resolution achievable by this procedure, which is mainly dictated by the geometry of the setup (Diemoz et al. 2014).

It is important to highlight that, although masks with several apertures are used, the mask period is made sufficiently large to prevent the mixing and interference of adjacent beamlets, and thus each aperture pair acts independently from the neighboring ones. The presence of several apertures, in fact, has the scope of replicating the EI principle over the whole field of view, thus making the acquisition faster. While so far masks of a few square centimeters have been typically used in lab-based setups (Olivo et al. 2013), large masks of dimensions up to $20.48 \times 1.28$ cm$^2$ have been recently designed and exploited (Astolfo et al. 2016a), thus significantly enlarging the field of view available for imaging.

It can be noted that other implementations of the EI technique are possible, in addition to the one presented above. For instance, the presence of the second mask is not necessary if a detector with sharp boundaries between pixels is used. In this case, in fact, the EI principle can be reproduced by simply aligning the beamlets created by the first mask with the edge between pixels, thus exploiting this edge to create the image contrast (Krejci et al. 2010). In yet another implementation, the second mask can be removed when a high resolution detector is available. In this case, in fact, the shift of the beam due to refraction, the drop in the total intensity due to absorption, and the beam broadening due to scattering can be retrieved by fitting (for instance...
with a Gaussian function) the values of the various pixels illuminated by each beamlet (Vittoria et al. 2014, 2015). The usual implementations of EI employing linear apertures are only sensitive to refraction in one direction (that orthogonal to the aperture). However, two-dimensional sensitivity can be achieved if L-shaped masks are used, which are sensitive to beam displacements in two directions. This solution was implemented in both synchrotron (Olivo et al. 2009) and laboratory setups (Kallon et al. 2015): it offers the advantage of an improved sample visualization and of an easier integration of the refraction signal to obtain the phase map, at the cost of increased setup complexity and longer exposure times.

50.4.1 The Illumination Curve

The signal in EI can be rigorously modeled by following a wave optics treatment based on Fresnel-Kirchhoff diffraction integrals. Numerical methods based on this model have been developed and used to accurately simulate EI images (Munro et al. 2010a; Vittoria et al. 2013). In the following, however, we will concentrate on a formalism based on the so-called illumination curve, which significantly simplifies the rigorous wave optics formalism and, importantly, enables a simple way to perform the phase retrieval.

We will first consider a single monochromatic component of the beam, and later sum the resulting monochromatic signals over the whole spectrum, in order to take into account beam polychromaticity. The case of a single aperture pair and sample scanning is also considered for simplicity, as, from a mathematical point of view, this implementation of EI is equivalent to the one employing masks, as long as the various apertures are sufficiently separated to prevent mixing of the individual beamlets. Moreover, we will only consider the signal in the direction orthogonal to the apertures (y), as in the other direction (x) the signal is not affected by the presence of the masks (Diemoz et al. 2014). Let \( \rho_{\text{ref}}(y; E) \equiv \frac{\partial N}{\partial y \partial E} \) indicate the distribution of the beam incident upon the detector slit in the absence of the sample, defined as the number of photons per unit of length and unit of energy, produced by a pre-sample slit centered at \( y = 0 \). The monochromatic signal measured by the corresponding detector pixel will be equal to the integral of the beam within the detector aperture, such as (Diemoz et al. 2013a,b):

\[
I_{\text{ref},E}(y_e) = \int_{y_e}^{y_e+d} d y \rho_{\text{ref}}(y; E) \equiv C_E(y_e) \cdot I_{0,E} \tag{50.19}
\]

where \( y_e \) is the position of the lower edge of the detector aperture, and \( d \) the aperture size. \( I_{0,E} = \int_{-\infty}^{+\infty} d y \rho_{\text{ref}}(y; E) = \frac{\partial N}{\partial E} \) is the density of photons at energy \( E \), and \( C_E(y_e) \) describes the fraction of those photons falling within the detector aperture, as a function of the misalignment between the two slits. An example of this function is reported in Figure 50.4b. This is usually referred to as the illumination curve, in analogy to the rocking curve encountered in the case of the ABI technique. The similarity between this model for EI and the rocking curve model for ABI is substantial, as the two models are formally equivalent from a mathematical point of view. This similarity, as we will see in the following, can be exploited to intuitively understand the variation of intensity in EI due to refraction, as well as in the development of phase retrieval methods.

In a geometrical optics approximation, the illumination curve is simply equal to the convolution between the magnified presample slit, the detector slit, and the source distribution projected onto the detector plane (Olivo and Speller 2007b; Diemoz and Olivo 2014). It can be shown that geometrical optics is a good approximation for EI implementations employing non-microfocal X-ray sources (Munro et al. 2010a; Diemoz et al. 2013b; Diemoz and Olivo 2014). When a highly coherent beam is employed, instead, diffraction effects become non-negligible, and accurate wave optics simulations are necessary to determine the shape of the illumination curve (Diemoz et al. 2013a). Importantly, however, this function can also be easily measured, by simply scanning one slit with respect to the other and by measuring the detected intensity at each position.

When a non-scattering sample is introduced, the beam on the detector slit can be attenuated and spatially shifted, so that the detected signal becomes, at a specific step \( p \) of the sample scan:

\[
I_{\text{sum},E}(p) = I_{0,E} T(p; E) C_E(y_e - z_{od} \Delta \theta_e(p; E)) \tag{50.20}
\]

Here, we have implicitly assumed that both the transmission \( T \) and refraction angle \( \Delta \theta_e \) are approximately constant.
within the size of the sample aperture, so that the beam is rigidly attenuated and shifted, while its shape is left unchanged. We see that, once a working position, \( y_s \), has been chosen on the illumination curve, the contrast is determined by the shift on the illumination curve caused by refraction (in addition to the trivial dependence upon transmission). The dependence upon refraction becomes even more obvious if the refraction angle is small, so that the illumination curve can be linearly approximated:

\[
I_{\text{sum},E}(p) \approx I_{0,E}T(p;E) \cdot \left[C_E(y_s) - z_{od}C'_E(y_s)\Delta\theta_s(p;E)\right]
\]

(50.21)

where \( C_E(y_s) = \partial C_E(y_s)/\partial y_s \). The change in signal, therefore, is to first approximation proportional to the propagation distance, to the refraction angle, and to the steepness of the illumination curve at the chosen point. This means that the observable contrast can be tuned by changing the working position on the illumination curve: the refraction signal will be maximized, in particular, at the slopes of the illumination curve, where its first derivative is largest.

The detected signal in the polychromatic case can be simply expressed as the incoherent sum of the monochromatic intensities, weighted by the detector response \( f(E) \):

\[
I_{\text{sum}}(p) = \int dEf(E)I_{\text{sum},E}(p)
\]

\[
= \int dEf(E)I_{0,E}T(p;E)C_E(y_s) - z_{od}\Delta\theta_s(p;E)).
\]

(50.22)

If the slits are totally absorbing at the used energies and diffraction effects can be neglected (such as in tabletop setups using non-microfocal sources, where convolution with the projected source distribution effectively washes out any diffraction peak), the illumination curve shows no dependence upon the energy. This was also shown experimentally using a photon-counting energy-resolving detector (Endrizzi et al. 2015).

As in the case of other XPCI techniques such as grating interferometry (Pfeiffer et al. 2006), the signal in the polychromatic case is often expressed through the use of a modified version of Equation 50.20, considering effective transmission, \( T_{\text{eff}} \), and refraction angle, \( \Delta\theta_{\text{eff}} \), which are obtained as a weighted average over the whole spectrum, such as:

\[
I_{\text{sum}}(p) = I_0T_{\text{eff}}(p)C(y_s) - z_{od}\Delta\theta_{\text{eff}}(p))
\]

(50.23)

where \( I_0 = \int dEf(E)I_{0,E} \). A comprehensive model for polychromaticity was developed in Munro and Olivo (2013). We will just mention here that, in the special case where the illumination curve is independent of the energy and can be linearly approximated, the effective transmission is equal to \( T_{\text{eff}}(p) = \int dEf(E)T(p;E) \), while the effective refraction angle is equal to \( \Delta\theta_{\text{eff}}(p) = \int dEf(E)\Delta\theta_s(p;E) \). The effective energies corresponding to the transmission and refraction angles are in general different, because the absorption and refraction coefficients have a different dependence upon the energy and because the effective refraction angle also depends on the transmission at the various energies. Furthermore, the effective energies are in general a function of the position in the sample, as regions with higher attenuation will cause a shift towards higher energies (i.e., beam hardening).

As described in previous sections for ABI, a scattering sample will introduce a finite distribution of angles, \( f(\Delta\theta_{\text{eff}}) \), around the mean refraction angle, \( \Delta\theta_{\text{eff}} \), due to the presence of refracting structures in the sample on length scales smaller than the sample mask aperture. Equation 50.23 needs then to be rewritten as:

\[
I_{\text{sum}} = I_0T_{\text{eff}}\int d(\Delta y_{\text{eff}})f(\Delta y_{\text{eff}})C(y_s - \Delta y_{\text{eff}} - \Delta y_{\text{eff}})
\]

\[
= I_0T_{\text{eff}}C(y_s - \Delta y_{\text{eff}})
\]

(50.24)

where, for simplicity of notation, we have discarded the dependency of the various quantities upon the sample position, \( p \), and where \( \Delta y_{\text{eff}} = z_{od}\Delta\theta_{\text{eff}} \) and \( \Delta y_{\text{eff}} = z_{od}\Delta\theta_{\text{eff}} \). The measured (local) illumination curve, \( C(y_s - \Delta y_{\text{eff}}(p)) \), is given by the convolution of the intrinsic illumination curve with the scattering distribution: this function will, thus, appear broader as an effect of the scattering.

The effect of sample scattering on the image contrast is different, depending on the chosen working position. At the top of the illumination curve, scattering will appear as a decrease in the measured signal: photons previously falling into the detector aperture, in fact, can be thrown out of it because of scattering. At the tails of the illumination curve, instead, a contrast reversal will be observed: in fact, scattered photons can be sent into the aperture, thus increasing the number of counts on the detector. At the slopes of the illumination curve, the effect of scattering will be minimum.

### 50.4.2 Phase Retrieval and Sensitivity

We will describe in this section only the retrieval methods based on the illumination curve formalism. Other phase retrieval methods were previously developed in Munro et al. (2012, 2013a) for both synchrotron radiation and laboratory implementations of EI. However, these models require precise knowledge of the setup parameters and make some additional assumptions, such as that of ideal (perfectly absorbing and sharp) optical elements. Moreover, they do not take into account that the shape of the beam can be affected by diffraction in the case of highly coherent beams (Diemoz et al. 2013a). The illumination curve model, instead, enables a rather practical and accurate way to perform phase retrieval, as all experimental parameters are incorporated in the illumination curve, which can be experimentally measured.

If the contribution from scattering can be neglected, two input images are needed in order to separate attenuation and refraction. These are usually chosen at the left and right slopes of the illumination curve, like in many of the algorithms developed for the ABI technique (Chapman et al. 1997; Diemoz et al. 2010), as (1) at these positions the illumination curve is almost linear, and (2) the sensitivity to refraction is here maximized.

The dependence upon the sample transmission can be factored out by taking the ratio between the two images at the right (\( I_{\text{sum},r} \)) and left (\( I_{\text{sum},l} \)) slopes, such as (cf. Equation 50.23):
A relationship can, thus, be established between the ratio of the two images and the refraction angle, where the function, $R$, can be calculated numerically from the experimental measure of the illumination curve. The refraction angle can then be extracted by inverting Equation (50.25) (Diemoz et al. 2013a,b; Munro et al. 2013b):

$$
\Delta \theta_{\text{eff}} = \frac{1}{z_{\text{sd}}} R^{-1} \left( \frac{I_{\text{sum,+}}}{I_{\text{sum,-}}} \right)
$$  \hfill (50.26)

and the transmission can be obtained by combining Equations 50.23 and 50.26:

$$
T_{\text{eff}} = \frac{I_{\text{sum,+}}}{I_s C(y_{e,+}) - R^{-1}(I_{\text{sum,+}}/I_{\text{sum,-}})}
$$  \hfill (50.27)

A similar algorithm based on a linearization of the illumination curve, which was inspired by the classic DEI algorithm in ABI (Chapman et al. 1997), was proposed in Munro et al. (2013b). This linear approximation holds if the refraction angles are sufficiently small, and is more easily verified at the slopes of the illumination curve, which are almost linear. Under this assumption, the transmission and refraction angle can be easily calculated from the sum and difference of the images at the two slopes of the illumination curve, such as:

$$
T_{\text{eff}} = \frac{I_{\text{sum,+}} + I_{\text{sum,-}}}{2C(y_{e,+})}
$$  \hfill (50.28)

$$
\Delta \theta_{\text{eff}} = \frac{C(y_{e,+}) I_{\text{sum,+}} - I_{\text{sum,-}}}{z_{\text{sd}} C(y_{e,+}) I_{\text{sum,+}} + I_{\text{sum,-}}}
$$  \hfill (50.29)

where it is assumed that the two EI images are taken at symmetric positions, $C(y_{e,+}) = C(y_{e,-})$ and $C'(y_{e,+}) = -C'(y_{e,-})$.

The uncertainty on the calculated values $\Delta \theta_{\text{eff}}$ and $T_{\text{eff}}$ can be estimated analytically by propagating the noise in Equations 50.26 and 50.27 (the same result would be obtained using Equations 50.28 and 50.29). The assumption is made that the noise in the input images is purely statistical (Poissonian), that the detector is a photon counter, so that $\sigma(I_{\text{sum,+}}) = \sqrt{I_{\text{sum,+}}}$, and that two symmetric positions on either side of the illumination curve are chosen. The uncertainties on $\Delta \theta_{\text{eff}}$ and $T_{\text{eff}}$ are then (Diemoz et al. 2013a,b):

$$
\sigma(\Delta \theta_{\text{eff}}) \approx \frac{\sqrt{C(y_{e,+})}}{z_{\text{sd}} \sqrt{2T_{\text{eff}}} \rho_{\text{eff}}(y_{e,+}) - \rho_{\text{eff}}(y_{e,+} + d)}
$$  \hfill (50.30)

$$
\sigma(T_{\text{eff}}) \approx \frac{\sqrt{2T_{\text{eff}}}}{2I_s C(y_{e,+})}
$$  \hfill (50.31)

where $\rho_{\text{eff}}(y) \equiv \int dE \rho_{\text{eff}}(y; E)$ is the number of photons per unit of length in the beam incident onto the detector edge. In particular, the value $\sigma(\Delta \theta_{\text{eff}})$ can be considered as an estimate of the refraction sensitivity of a given EI imaging system, for a fixed number of photons (or equivalently a fixed dose to the sample). In fact, angles smaller than this value are likely to go undetected in the image, as they fall below the noise level, while angles larger than this value will likely lead to detection of the corresponding object structures. Equation 50.30 can then be exploited to study analytically the dependence of the angular sensitivity upon the various parameters of the setup, such as focal spot size, aperture sizes, setup distances, position on the illumination curve, and so on, which can be useful to guide the design and optimization of EI setups. In Figure 50.5, as an example, we present the variation of the sensitivity as a function of the focal spot size, in the case of the laboratory setup described in Diemoz et al. (2013b). Furthermore, Equation 50.30 potentially enables a comparison with the sensitivity of other XPCI techniques, along the lines of Diemoz et al. (2012).

If the sample scattering is not negligible, three input images are instead required for the phase retrieval. Endrizzi et al. (2014) developed a method based on modeling both the illumination curve and the object scattering distribution as Gaussian functions. Under this assumption, the signal, $I_s$, measured at each of the three mask positions, $y_i$, with $i = 1, 2, 3$, is (c.f. Equation 50.24):

$$
I_s(y_i) = T_{\text{eff}} \frac{A}{\sigma \sqrt{2\pi}} \exp \left( -\frac{(y_i - y_{\text{sd}}\Delta \theta_{\text{eff}})^2}{2\sigma^2} \right)
$$  \hfill (50.32)

where $A$ is a constant, $\sigma^2 = \sigma_{\text{sc}}^2 + \sigma_{\text{ref}}^2$, $\sigma_{\text{sc}}$ is the standard deviation of the illumination curve, and $\sigma_{\text{ref}}$ is the (unknown) standard deviation of the scattering distribution $f(\Delta \theta_{\text{eff}})$. This system of three equations in three unknowns can be solved analytically. In particular, if one image is acquired at the top of the illumination curve, the solution can be obtained from two other images at the sides.

![FIGURE 50.5 Variation of the sensitivity as a function of the source dimensions, for a typical EI laboratory setup. The difference between the two profiles is due to the fact that, by neglecting diffraction effects, geometrical optics provides an inaccurate value for the beam shape $\rho_{\text{eff}}$, in the case of small focal spots. (Reprinted with permission from Diemoz, P.C. et al. Sensitivity of laboratory based implementations of edge illumination X-ray phase-contrast imaging. Applied Physics Letters 103:244104. With the permission of AIP Publishing. Copyright 2013b, American Institute of Physics.)](image-url)
curve, such as $y_2 = 0$, and the two other images are acquired at symmetric positions on the slopes, such as $y_1 = -y_1$, it can be found that (Endrizzi et al. 2014):

$$T_{\delta \theta} = \frac{2y_1}{A} \sqrt{\frac{\pi}{D + C}} e^{(1/D - C)^2} \left( \frac{(D - C)^2}{2^D - D + C} \right)$$

(50.33)

$$\delta \theta_{\delta \theta} = \frac{1}{z_{\text{red}}} \frac{y_1}{2} \frac{D - C}{D + C}$$

(50.34)

$$\sigma_\delta^2 \sigma_\theta^2 = \frac{2y_1^2}{D + C} - \sigma_k^2$$

(50.35)

where $C = -2 \ln(I/I_2)$ and $D = -2 \ln(I_2/I_1)$. An improvement on the algorithm above was proposed in Endrizzi and Olivo (2014), in order to take into account an illumination curve with an offset different from zero. This is an experimentally relevant case: offsets between 5% and 30% are typically observed in laboratory EI setups, mainly arising due to partial transmission through the masks at the higher energies of the spectrum. In this work, an iterative procedure is employed to correct the retrieved values to account for this effect. An alternative setup based on the use of asymmetric masks was also recently proposed and applied, in order to implement the three-image retrieval but without the need to move the masks during acquisition (Endrizzi et al. 2016). Instead, the data necessary for the retrieval are obtained by replacing the mask movement with a scan of the sample in the direction orthogonal to the mask lines. Polychromaticity and beam hardening have been shown to produce an effect on the measured signal, and in particular to create ghost signals in the absorption and scattering channel, if not properly accounted for. A new algorithm that can prevent such beam hardening artefacts was developed in Vittoria et al. (2015).

Finally, a retrieval method was recently developed that requires only a single input image acquired at one slope of the illumination curve (Diemoz et al. 2015), which presents important advantages for applications where a short acquisition time is crucial (such as dynamical imaging and computed tomography). This method is based on a linearization of the illumination curve and on the additional assumption that the sample is quasi-homogeneous, thus the $b/\beta$ ratio is constant across the field of view. This assumption has been used extensively in the framework of the PBI technique, and in particular it was at the basis of the method developed in Paganin et al. (2002). Importantly, it is usually satisfied in the case of biological soft tissues, as these are typically very similar to each other in terms of elemental composition. As in the original Paganin algorithm, the image processing only consists in applying an appropriate Fourier filter to the experimental image. Due to the low-pass nature of the filter, the method is very stable with respect to high frequency noise (Diemoz et al. 2015).

50.4.3 Coherence Requirements

EI does not have strict coherence requirements. This directly comes from the incoherent nature of the technique: in fact, the working principle of EI and the generation of contrast in the image can be described by using models based on geometrical optics (Munro et al. 2010a; Diemoz et al. 2013b; Diemoz and Olivo 2014), where the X-rays are treated like particles traveling along straight lines. It was shown that geometrical optics is rather accurate when applied to EI laboratory setups (Munro et al. 2010a; Diemoz and Olivo 2014), while the accuracy decreases in synchrotron setups, due to the high coherence of the radiation and the need to take into account wave diffraction effects (Diemoz et al. 2013a).

The technique does not require a beam with high temporal coherence (i.e., monochromaticity) (Munro et al. 2010b; Diemoz et al. 2013b), as different energies in the spectrum only have the effect of producing refraction angles of different amplitude; however, all contributing to the image contrast. All X-ray energies are, therefore, efficiently exploited in a given setup, as long as they are (1) sufficiently high to traverse the mask apertures (typically built on a graphite or silicon substrate), and (2) sufficiently low to prevent transmission through the absorbing lines in the masks (typically built in gold). In practice, the above conditions do not pose strong limitations on the usable range of energies, which is typically of several tens of kiloelectronvolts.

High spatial coherence in the beam is also not required. In fact, the transverse coherence length was calculated to be on the order of 0.5 μm for a typical laboratory implementation of EI (Endrizzi et al. 2014), which is much smaller than both the period and aperture size of the masks. However, the focal spot should not be too large, in order to (1) prevent mixing of individual beamlets at the detector plane, and (2) prevent loss in phase sensitivity. In fact, it was shown that angular sensitivity decreases with increasing source size, although slowly, as seen in Figure 50.5 (Diemoz et al. 2013b).

50.5 Computed Tomography

In this section, we will review the main methods for computed tomography (CT) reconstruction jointly for the ABI and EI techniques, as the physical quantities measured or retrieved at every rotation angle are the same in the two cases. Typically, at every rotation angle several images are acquired and retrieval algorithms described in previous sections are applied to retrieve the absorption, refraction, and scattering images. These sets of projections are then used as input to the CT reconstruction, which exploits the fact that these parametric images are equal to line integrals of fundamental properties of the sample, so that conventional CT reconstruction algorithms such as filtered back projection (FBP) can be used (see Section III, Chapter 33). Specifically, the apparent absorption image is related to the line integral of a modified linear attenuation coefficient, $\mu$, in which extinction contrast, due to SAXS rejection, is included (Dilmianian et al. 2000). Scattering parametric projections correspond to line integrals of the second moment of a local scattering function (Rigon et al. 2008), which has been dubbed local angular impulse response function by Brankov et al. (2006). Such a second moment is equivalent to the linear diffusion coefficient introduced by Bech et al. (2010) in developing tomographic methods for a different XPCI technique, grating interferometry, which produces parametric images similar to ABI and EI (Pelliccia et al. 2013).
Three-dimensional (3D) distributions of \( \mu \) and of this linear diffusion coefficient can, thus, be easily reconstructed.

Two different CT acquisition geometries, however, need to be distinguished regarding the reconstruction of the refraction signal (Figure 50.6): (1) the sample rotation axis is parallel to the direction, \( y \), along which the system is sensitive to refraction, and (2) the sample rotation axis is orthogonal to the sensitivity direction, \( y \). The refraction image obtained at each projection corresponds to the line integral of the gradient along \( y \) of the refractive index decrement, such as \( \partial \delta / \partial y \) (Dilmanian et al. 2000; Brankov et al. 2006).

In the former geometry (also referred to as out-of-plane geometry), this quantity is rotationally invariant (with respect to a rotation in the \( x-z \) plane), and, therefore, its 3D distribution can be reconstructed by using conventional CT algorithms (Dilmanian et al. 2000; Hagen et al. 2014a). We note that, in principle, the 3D map of \( \delta \) can also be reconstructed, for example by integrating the refraction angle maps along \( y \) before CT reconstruction, or by integrating along \( y \) the reconstructed 3D map of \( \partial \delta / \partial y \). However, this is usually avoided, as integration of the refraction image typically results in severe streak artefacts, due to propagation of noise and other artefacts from the input image (Wernick et al. 2006). A third possibility exists, in this CT geometry, which consists in acquiring a single image per rotation angle in a linear region of the rocking curve (for ABI) or of the illumination curve (for EI), and in applying CT reconstruction without prior phase retrieval. This procedure is justified under the assumptions that the scattering signal is negligible, and that the refraction angles are small, so that the rocking curve or illumination curve can be linearly approximated. In this case the absorption and refraction signals cannot be separated, but it can be shown that the reconstructed quantity is a well-defined linear combination of the quantities \( \mu \) and \( \partial \delta / \partial y \) (Diemoz et al. 2011; Hagen et al. 2014a).

In the second CT geometry, where the sensitivity direction \( y \) is contained within the rotation plane (also called for this reason in-plane geometry), the quantity \( \partial \delta / \partial y \) is not rotationally invariant and, therefore, cannot be reconstructed directly. One possibility is to integrate the refraction angle image to obtain the phase map at every angle, and then use this set of projections to reconstruct the 3D distribution of \( \delta \) through conventional CT algorithms. It can be shown that, by using the FBP algorithm, the integration step can be incorporated within the reconstruction step by simply replacing the usual ramp filter with the so-called Hilbert filter. This method was first proposed for phase-contrast CT using visible light (Faris and Byer 1988), and was later extended to the X-ray regime for both ABI (Huang et al. 2006) and EI (Hagen et al. 2014b) techniques. It can be noted that, unlike in the out-of-plane geometry, in this geometry the streak artefacts from integration are generated in the reconstruction plane, and, thus, tend to cancel each other in the CT reconstruction, providing artefact-free slices. In order to extract the refraction map at every rotation angle, two or more projections usually need to be acquired, at different positions of the analyzer crystal (ABI) or at different mask misalignments (EI). However, an extension of the previous method was developed, which does not involve the movement of the optical elements during the acquisition, but simply requires a rotation over 360° instead of 180°. It is based on the idea that projections acquired at angles \( \theta \) and \( \theta + 180° \) contain the same absorption information, but, since the sample is rotated by 180° with respect to the refraction sensitivity direction of the imaging system, perfectly opposite refraction signals, like images acquired at the two slopes of the rocking curve (ABI) or of the illumination curve (EI). Each pair of projections can, thus, be used as inputs for the phase retrieval, and the obtained \( \Delta \theta \) projections then used to reconstruct the 3D map of \( \delta \) via FBP with the Hilbert filter. This reversed projections method was first developed for ABI (Wang et al. 2007) and was recently demonstrated to be applicable also for EI (Hagen et al. 2016). Another method that has been described for reconstruction of \( \delta \) in the in-plane geometry is the so-called gradient vector field approach (Maksimenko et al. 2005; Gasilov et al. 2014). This consists of first reconstructing, from a set of \( \Delta \theta \) projections, the two components of the gradient of \( \delta \) in the reconstruction plane, and then in using these as inputs for the calculation of \( \delta \). One of the advantages of this method is the flexibility in the choice of the CT reconstruction algorithm to be used for the calculation of the gradient of \( \delta \), and in the algorithm for the calculation of \( \delta \) itself from its gradient. A comparison of the gradient vector field approach with methods involving (a) integration of the refraction, regularization, and FBP with ramp filter, and (b) FBP with Hilbert filter can be found in Gasilov et al. (2014).

50.6 Applications to Biomedical Imaging

50.6.1 Breast

The distinctive high sensitivity of XPCI in soft tissue visualization and low-absorbing feature detection soon found one of the
most natural and interesting application fields in breast imaging. Mammography, which is the primary imaging method in national screening programs and for the clinical work-up of symptomatic patients (Albert et al. 2009), is an intriguing diagnostic case because of its challenging requirements. Detection of breast cancer relies on the recognition of different kinds of breast changes, including subtle architectural distortions, masses, skin thickening, and microcalcifications. The small differences in attenuation of X-rays between normal and malignant tissue, as well as the small dimensions of microcalcifications, which make it hard to distinguish their benign or malignant nature, result in a difficult diagnosis, including an important number of false positive and false negative cases because of limited sensitivity (92%–93%) and specificity (87%–91%) (Kemp Jacobsen et al. 2015; Ohuchi et al. 2016). The situation is made more complicated by the fact that the breast (i.e., the glandular tissue) is one of the most radiosensitive organs and, thus, the radiation dose level in mammography needs to be as low as possible to limit the risk-benefit ratio of the examination. The first studies in which XPCI was applied to excised breast tissue samples and was proven to be able to enhance the contrast of mammographic images dated back to the end of the last century (Arfelli et al. 1998, 2000; Pisano et al. 2000). Since then, many works have been published in the field presenting theoretical, technical, methodological, and image processing developments validated on both phantoms and human tissues and by correlation with conventional clinical methods (e.g., histology, mammography, magnetic resonance imaging). The most important achievements and results of XPCI for breast cancer detection have been extensively reviewed in recent years (Keyriläinen et al. 2010; Coan et al. 2013).

Clinical trials have been carried out at the Elettra synchrotron radiation facility in Trieste, Italy (Castelli et al. 2007, 2011; Longo et al. 2014) using the PBI planar radiography technique, involving 71 patients who had previously been diagnosed with questionable or suspicious breast abnormalities on the basis of combined digital mammography and ultrasonography. The results highlighted that normal structures and abnormal findings were depicted with higher image quality with respect to conventional digital mammography (Figure 50.7). As a consequence, PBI mammography was able to clarify cases of questionable or suspicious breast abnormalities from conventional digital mammography.

In the past 5 to 10 years, XPCI research in breast imaging has particularly focused on the demonstration of low-dose breast CT.

Recent ex vivo studies using PBI have been carried out at the Shanghai Synchrotron Radiation Facility; 14 carcinoma and eight adenoma specimens have been examined using PBI-CT, with a 100% matching with histopathological findings (Jian et al. 2015).

Synchrotron radiation mammography is also being tested at the Australian Synchrotron Radiation facility, which showed the presence of edge enhancement also in images acquired using a Hamamatsu CMOS flat panel with a 100 µm pixel size (Nesterets et al. 2015) and with mean glandular doses between 4.7 mGy and 10.8 mGy, depending on the imaging parameters, for phantoms of 5 cm diameter. These and other research programs along the same pathways (Longo et al. 2016; Sarno et al. 2016) are preparing the ground for clinical trials in PBI-CT. In particular, Longo et al. (2016) present PBI-CT images of 9 to 10 cm uncompressed breast tissues acquired with a voxel size of 120 µm³ produced with a limited number of angular projections (300) and a minimum dose level (mean glandular dose about 4 mGy) that is only 2- to 4-times higher than that of a two-view mammography for a 2 cm compressed breast (i.e., 1–2 mGy).

There are several studies where the ABI method is used for imaging in vitro breast samples with the same goal of identifying mammographic signs in the images. Comparison of the same high resolution (∼20 lp/mm) CT slices with microscopy of histological images suggests that ABI-CT imaging may provide “histopathology” of the breast, both in partial (Keyriläinen et al. 2008) and in full and large (150 mm diameter) organs (Sztrókay et al. 2012).

By combining XPCI with an image reconstruction method known as equally sloped tomography, Zhao et al. (2012) imaged a full human breast using ABI and identified a malignant cancer with a voxel size of (92 µm³) using a radiation dose (2 mGy) equivalent or lower than that of dual-view mammography.
ABI-CT was also used to investigate the visibility of typical post-therapeutic tissue changes in Breast Carcinoma (Grandl et al. 2016). Accurate and quantitative density maps were retrieved from the ABI-CT data by using an advanced mathematical algorithm. Images depicted the different tissue types with an excellent correlation to histopathology, showing the potential of the method to become a unique diagnostic tool in the prediction of tumor response to neoadjuvant chemotherapy.

In recent years, the EI technique has emerged as a new method for high sensitivity and low-dose breast imaging. Importantly, EI can also be implemented with laboratory sources such as conventional X-ray tubes, and thus bears the potential for a future application in a clinical environment. In Olivo et al. (2013), projection images of 2-cm thick breast tissues were acquired using a laboratory EI setup with a detector pixel size of 85 µm, and with entrance air kerma values down to 1 mGy, which is within the acceptable limit for the entrance dose in mammography. All images showed a clear increase in detail visibility (in terms of both tissue definition and microcalcification detection) in the EI images with respect to absorption images, thanks to an increase in the image contrast of a factor of 5. Using synchrotron X-rays, a photon-counting detector and a recently developed single image phase retrieval method based on EI (Diemoz et al. 2015), Diemoz et al. (2016) imaged 2-cm and 4-cm thick breast tissue samples with a 55 µm pixel size and a mean glandular dose for each acquisition of only 0.12 mGy, thus an order of magnitude smaller than a clinical mammogram.

50.6.2 Musculoskeletal Phase-Contrast Imaging

The visualization of articular cartilage and of its degeneration, the study of the effect of therapies, or of the status of the healing of bone implants call for high resolution and sensitive imaging. The portfolio of standard clinical techniques includes conventional radiology and CT, ultrasound, and Magnetic Resonance Imaging (MRI); unfortunately, none of these methods fulfill all the clinical requirements. In reported experimental studies, most of them performed at synchrotrons, XPCI has shown its potential for providing an early and precise visualization of osteoarthritis (OA) and rheumatoid arthritis (RA), whose diagnosis implies the analysis of both soft tissue (i.e., cartilage) and subchondral bone details. In fact, conventional imaging techniques are sensitive only to advanced OA or RA stages when therapeutic strategies are less effective. In this field, most of the research was performed using the ABI technique, due to its higher sensitivity with respect to PBI. A review of the key results in this field has been recently published (Olubamiji et al. 2014). Early studies (Mollenhauer et al. 2002), confirmed by more recent ones demonstrated that the ABI technique could be a powerful tool in diagnostic orthopedics. Non-invasive detection of cartilage abnormalities, especially in the initial stages of degenerative joint disease (or early in its progression) was proven on small samples and on excised human femoral heads. The evaluation by ABI of the quality of the bone ingrowth into the implant surface was shown to be more sensitive than conventional radiography (Wagner et al. 2006).

Application on excised portions and full human joints using ABI-CT demonstrated the ability of this technique to visualize internal architectural properties of the cartilage matrix in human cartilage samples (Muehleman et al. 2004). Fine cartilage anatomical features were visualized, and a comparison with histology was also performed for osteoarthritic and healthy tissues (Coan et al. 2010a). AB images allowed differentiating osteoarthritic from normal samples in analogy to histopathological criteria. In a thumb image, it was possible to visualize articular cartilage, tendons, and other soft tissues (Muehleman et al. 2009). A preliminary test of ABI on large intact synovial joints, such as cadaveric human knee joints, has been carried out by Li et al. (2009). Images showed simultaneous high soft tissue and bone contrast, and clearly depicted the articular cartilage, cruciate ligaments, loose connective tissue, menisci, and chondrocalcinosis. Proof-of-principle studies of in vivo application of ABI were first performed by Coan et al. (2010b): in vivo ABI radiographs and ABI-CT of guinea pigs knees were used to investigate the development of OA. Images gave strong evidence of the ability of ABI in depicting both anatomic structures in complex systems (as living organisms) and clinical signs of osteoarthritis, with high contrast, high spatial resolution, and at an acceptable radiation dose.

Further studies (Pratt et al. 2015) have also shown the feasibility of 10 to 20 micron voxel size imaging to investigate the cortical porosity in live rat in longitudinal studies; however, the radiation dose (2.5 Gy) remained the limiting factor in the protocol.

Some groups have started exploring the possibility of applying the ABI technique on microfocus X-ray tubes (Muehleman et al. 2009). Images of an intact human knee showed the articular cartilage edge of the femoral condyle, even when superimposed by the tibia. Using the PBI technique, bone imaging was demonstrated by Cooper et al. (2011) at very high resolution (1.4 µm pixel size), with the 3D visualization of osteon morphology and the quantitative assessment of murine articular cartilage and bone in a longitudinal study of collagen-induced arthritis (Li et al. 2014). In another study, lumber facet joint degeneration, which is believed to be an important cause of low back pain, was examined by ABI-CT in a rat model. In particular, the degeneration process and the morphological changes in the subchondral bone were delineated at an unprecedented high resolution (Cao et al. 2016b).

Using PBI, a clear demonstration of the effective possible transferability of this research in clinics has been the high resolution image (46 micron pixel size) of a full cadaveric knee (Horng et al. 2014). In a single image the cartilage and the subcondral bone were visualized at high resolution in two intact knees, and compared with images acquired at a clinical CT scanner and using a 3T MRI (Figure 50.8). PBI-CT presents soft tissue contrast surpassing that of conventional CT with a clear discrimination of ligamentous, muscular, neural, and vascular structures using a dose down to 50 mGy. In addition, phase-contrast images show cartilage and meniscal calcifications that are not perceptible on conventional CT or on MRI.

Using the EI technique, Marenzana et al. (2012) demonstrated visualization of the cartilage tissue in a laboratory setup. In this study, they imaged excised samples of tibial bone and cartilage from rats (1–2 mm thickness) and showed that cartilage thickness and surface defects can be depicted both with the sample in air or in saline solution.

50.6.3 Lung

In conventional radiography, the lung is a weakly absorbing organ, and pathologies are seen as opaque masses. In early ABI
fibrosis, have been imaged in a mice model in vivo (Donnelley et al. 2011). It has been observed that the projection image of the lung has a strong speckle pattern, and this was interpreted to arise from multiple refraction in alveoli, and to variable focusing of the X-ray beam (Kitchen et al. 2004). The speckle pattern has been used for studying respiratory development and pathology, and to quantitatively measure airway dimensions and changes in their size during respiration (Kitchen et al. 2015). Vessel stenosis and individual alveoli were imaged in excised rat and mice tissues (Zhang and Luo 2011). ABI has been used to detect atelectasis in the injured lung (Connor et al. 2011), and imaging of the lung has been used as an example in the development of a variant of the ABI method, where a Laue-type analyzer crystal splits the transmitted beam to direct and diffracted beams, which are recorded simultaneously at several rocking angles. Absorption, refraction, and scattering images are then retrieved by an iterative algorithm (Beltran et al. 2011).

Kitchen et al. (2014) studied how changes in positive end-expiratory pressure (PEEP) alter the distribution of ventilation within the lung immediately after birth in newborn rabbits. Initiating ventilation with 10 PEEP (i.e., with PEEP = 10 cm H₂O) resulted in a uniform increase in functional residual capacity throughout the lung, whereas initiating ventilation with 5 PEEP or 0 PEEP preferentially aerated the upper right quadrant rather than both lower quadrants. With ventilation at 10 PEEP, the distribution of air at end-inflation was uniform across all quadrants and remained so, regardless of the PEEP level. Thus, uniform distribution of ventilation can be achieved by initiating ventilation with a high PEEP.

Broche et al. (2016) studied the recruitment/derecruitment (R/D) of oxygen during respiration as a function of PEEP in a model of mechanical ventilated lungs at acinar length scales (Figure 50.9). Data showed that cyclic R/D of neighboring airspaces can occur as a result of dynamic opening/closure of airways and acini, provided that mechanical interdependences exist between neighboring terminal lung units.

50.6.4 Neuroimaging

The visualization of neurological tissues at the microscopic and submicroscopic scale is at the frontiers of neuroimaging. Conventional CT and clinical functional MRI, which have opened new windows in anatomical and functional imaging for a better diagnosis and characterization of brain abnormalities and diseases, are limited in resolution to several dozen or even hundreds of microns. Therefore, despite the deep insights offered by these methods, their sensitivity and spatial resolution are insufficient to study the neuronal structures at cell level. Using PBI, important developments in the simultaneous submicrometric 3D imaging of the microvascular network and the neuronal system in a mouse spinal cord have been reported (Fratini et al. 2015). In that work, the microcapillary network and the micrometric nerve fibers, axon-bundles, and neuron soma have been imaged, paving the way to preclinical investigations of neurodegenerative pathologies and spinal cord injuries.

The morphology of the microvasculature on digital slices was studied by XPCI without contrast agents and matched with histological findings in both normal and injured spinal cord in a rat model (Hu et al. 2015; Cao et al. 2016a). Quantitative analysis...
performed on 3D images revealed a significant decrease in the number and volume of vascular networks in the pathological cases; this observation was especially relevant to vessels with a diameter $<50\ \mu m$. Similar experimental methods have been focused on the study of other vascular diseases, like, for instance, in the detection of intramedullary artery pathologies (Cao et al. 2016b) and in the delineation of the cerebrovascular anatomy at the micrometer level without any need for contrast agents (Zhang et al. 2015). By using an innovative phase retrieval method applicable to a single distance PBI image, PBI-CT results showed demarcated tissue borders at the gray/white matter boundaries of a rat brain (Beltran et al. 2011), and the visualization of subtle details in the brainstem, including the ventral cochlear nucleus, spinal tract of the trigeminal nerve, and inferior cerebellar peduncle. This single image approach has clear benefits in terms of radiation dose and acquisition time with respect to the multi-imaging modalities so far used in brain imaging by the other XPCI techniques.

Another important application concerns the detection of core pathological features of Alzheimer’s disease. One of the most important efforts in neuroimaging research is in fact the visualization of amyloid plaques, a hallmark feature of Alzheimer’s disease and of other neurological pathologies, to evaluate the progression of the disease, but also to facilitate its diagnosis. Because of their very small dimension and low radiographic contrast, amyloid plaques are not visible in conventional X-ray absorption-based imaging. Proof-of-principle studies with synchrotron radiation ABI (Connor et al. 2009) in micro-CT mode were performed on the brains of Alzheimer’s disease model mice, demonstrating its potential in visualizing the amyloid plaques as small nodules in the cortex and hippocampus of the brain (Figure 50.10). More recently, similar findings have been shown also using the PBI technique (Astolfi et al. 2016b).

50.6.5 Vasculature, Circulation, and Other Tissues

Imaging of vasculature and circulation is traditionally based on X-ray absorption imaging, where the image contrast is provided by a previously injected compound containing a biocompatible heavy element, like for instance iodine, barium, or gadolinium.

Gas-filled microbubbles are a standard contrast agent in ultrasoundography. Arfelli et al. (2010) demonstrated that the same product can be used as an XPCI contrast agent: in fact, multiple refraction and scattering from micrometer-size bubbles determine strong contrast in ABI. PBI was applied in an ex vivo experiment to visualize microvessels in a mouse model (Xi et al. 2011), and to study in vivo angiogenesis in subcutaneous tumors (Tang

FIGURE 50.9 Alveolar R/D occurring alternately in neighboring lung units over short time scales $\sim 1$ min, in an injured lung at 6 PEEP. (a–c) 3D renderings of aerated lung regions obtained by segmentation of synchrotron PBI-CT images at (a) 0 s and (b) 84 s in a 2.5 mm thick slice, in injured lung at 6 PEEP; (c) R/D map quantified using image registration between T1 (a) and T2 (b). Squares delineate regions of interest magnified in panels (d)–(k), with (d), (f), (h), (j) computed from two successive images acquired at 0 and 84 s (T2–T1) and (e), (g), (i), (k) from the subsequent time interval between 84 and 159 s (T3–T2). ∗: Bronchioles; †: Recruiting airspaces; and §: Derecruiting airspaces. (Adapted from Broche, L. et al. 2016. Critical Care Medicine 45(4):687–694. April, 2017, Figure 1. Reproduced with permission.)
**FIGURE 50.10** Slice images of the brain of a transgenic mouse imaged using ABI-CT (a). Zoomed-in views of the boxed regions of (a) are presented in (b). In (c) the corresponding histology is reported. The numbered arrows point to nodules. (Adapted from Connor, D.M. et al. 2009. *Neuroimage* 46:908–14. Figure 3. Reproduced with permission.)

**FIGURE 50.11** CT image and histological section of an excised pathological rat liver. Fibrosis was induced by administrating human albumin. (a) One reconstructed ABI-CT slice. (b) Histological section that corresponds to the area where the CT image is acquired. The two images in (c) and (d) are the enlarged views of the white rectangle regions in (a) and (b), respectively. The 3D vessel microstructure image of mild hepatic fibrosis. (e) 3D vessel image. (f) The structure of the vessel inner wall. (g) The enlarged view of the white rectangle region in (f). (Adapted from Duan, J. et al. 2013. *PloS ONE* 8:e78176. Figures 3 and 4. Reproduced with permission.)
et al. 2011). Both ABI and PBI techniques were used for imaging gastric cancer samples (Tang et al. 2012). Gaseous contrast agents have been employed in other XPCI applications: ambient air in the high resolution post-mortem imaging of the vascular tree in liver tissue CT imaging of mice (Laperle et al. 2008), and CO₂ to image microvasculature in rat kidneys (Lundstrom et al. 2012). The absorption and phase-contrast images of P-selectin-targeted microbubbles (MBP) were obtained and compared in vitro (Tang et al. 2016). MBP, control IgG-targeted microbubbles, and unbound microbubbles were tested for binding specificity on thrombi expressing P-selectin. XPCI clearly visualized the microbubbles, otherwise invisible with absorption contrast imaging.

ABI-CT was successfully used in the quantitative evaluation of vessel microstructures from different stages of hepatic fibrosis in rats and to characterize the various stages of fibrosis progression using high resolution 3D vessel morphology (Duan et al. 2013) (Figure 50.11).

Brandlhuber et al. (2016) used PBI-CT for the detection and characterization of early changes after ischemia-reperfusion in a standardized rat liver transplantation model. They found that X-ray PBI of histological liver specimens can detect ischemia-reperfusion-induced tissue necrosis and provide detailed 3D information complementary to standard histopathologic findings.

Using PBI, Tang et al. (2013) first reported the use of fluorescent carboxyl microspheres (FCM) as radio lucent embolic agents for embolizing hepatic portal veins. The fluorescent characteristic of FCM could help to determine their approximate location easily. Additionally, the microspheres were found to be fairly good embolizing agents for portal vein embolization.

Scaffolds of a wide range of intricate organs (esophagus, lung, liver, and small intestine) from different animal models were imaged using PBI and EI in the frame of the development of new protocols in regenerative medicine (Hagen et al. 2015). Both synchrotron (PBI) and laboratory (EI) techniques were able to perfectly delineate the sample microarchitecture and to detect major anatomical features, such as the esophageal mucosal-submucosal separation, pulmonary alveoli, and intestinal villi (Figure 50.12).

### 50.7 Conclusion

Over the past two decades, X-ray phase-contrast imaging has undergone extensive and impressive advancements. The literature has seen a rapid increase in the number of publications reporting the results of both technical developments and applications, in particular those in the biomedical fields. While synchrotron X-rays have been largely used as Gold Standard radiation to test and validate new setups and the potential of the techniques for new scientific cases, XPCI has also become a paradigm in laboratory X-ray biomedical imaging, opening the pathway towards its clinical application.

The theoretical and technological developments of XPCI have been vast. Advanced experimental configurations, acquisition modalities, and image processing tools have been designed and implemented. This has allowed (1) extending the range of applications towards higher X-ray energies (and, therefore, thicker samples), (2) diminishing the imaging time (through, e.g., new algorithms that need a reduced number of images to perform the phase retrieval), (3) reducing the radiation dose delivered to the samples, and (4) increasing the sensitivity of the technique.

Following the fervent interest that XPCI attracted in the medical community since its first experiments on biological tissues, scientists all around the world have focused on demonstrating and exploiting the diagnostic relevance of XPCI in a broad range of pathologies. In vitro and in vivo biomedical studies of breast, joints, cartilage, lung, central nervous system, vasculature, and other tissues (e.g., liver, esophagus, kidney) have been conducted on both animal and human excised specimens and full samples. Results of the first clinical study in mammography performed at the Elettra synchrotron facility showed that XPCI mammography (using the PBI technique) can increase the diagnostic
accuracy with respect to conventional absorption-based X-ray radiography.

Presently XPCI has a twofold application in biomedicine. High resolution XPCI (i.e., at micrometer or nanometer scale) is used to perform a so-called “virtual 3D histology” of tissue portions to obtain images of histological or even higher resolution of structural changes induced by diseases or treatments. The other direction is to go towards lower spatial resolutions (up to tens of micrometers) and develop protocols for fast and low-dose in vivo XPCI.

The impressive developments carried out worldwide have made XPCI the preclinical imaging reference in the visualization of several pathologies. Nevertheless, apart from the pilot projects already mentioned, there are still some technical factors limiting the immediate translation of the method in the clinical practice. Every XPCI technique has its own requirements and limitations with regard to the beam coherence, the mechanical and/or thermal stability, the spatial resolution, and the field of view of the imaging system. In fact, PBI, although very simple in the instrumentation, requires, in order to be fully exploited, a highly spatially coherent beam and a high resolution imaging system, while ABI demands a highly temporally coherent beam and is prone to mechanical and thermal instabilities. In contrast, EI is a fundamentally incoherent method, and is not particularly demanding in terms of stability. Thus, EI is arguably the most promising from a translational point of view among the XPCI techniques reviewed in this chapter. although it is rather limited, at the moment, in the field of view. The application of XPCI techniques to clinical imaging has been mainly restricted by the exam duration and/or the delivered radiation doses, even though important improvements for low-dose imaging have been recently developed and experimentally confirmed.

Moreover, for different reasons, XPCI techniques can only use part of the beam delivered by a conventional polychromatic and divergent X-ray source (a quasi-monochromatic beam is required in ABI or part of the beam is masked like in EI, a highly spatially coherent beam is required by PBI). In this scenario, the technology of compact X-ray sources that has been under development over the past years and is nowadays on the market too (e.g., Lyncean Technologies, http://www.lynceantech.com) is of uttermost importance. These new sources aim at delivering quasi-monochromatic X-ray beams with flux densities that are between those provided at large synchrotron radiation facilities and by clinical X-ray tubes. These new machines associated with refined image processing tools may revolutionize the diagnostic use of XPCI and extend the exploitation of the method for a large variety of scientific and industrial applications.

REFERENCES


Handbook of X-ray Imaging


Munro, P.R., K. Ignatyev, R.D. Speller, and A. Olivo. 2010a. The relationship between wave and geometrical optics models of coded aperture type x-ray phase contrast imaging systems. *Optics Express* 18:4103.


