tuning-based techniques were facilitated with these machines and studies involving ultra-small samples (crystals, single fibres, or tiny liquid aliquots) or very large unit cells were enabled. As a result, micron-sized protein crystals as well as huge multi-macromolecular biological structures (of large viruses, for example) also became accessible.

8.1.5.4. *New national SR machines*

Today a variety of enhanced national SR machines are being proposed and/or built. In the UK there is the DIAMOND 3 GeV machine and in France there is SOLEIL. The SLS in Switzerland, the country's first SR light source, is under construction. These machines are more tailored to the bulk of a country's user needs, distinct from the special provisions at the ESRF. The different countries' SR needs, of course, have many aspects in common, with some historical biases. The new sources are, in essence, characterized by high brilliance, *i.e.*, low emittance. The 2 GeV high-brilliance SR source ELETTRA in Trieste, the MAXII machine in LUND and the Brazilian Light Source are already operational. In many ways, national sources like the SRS, LURE, DORIS and so on fuelled the case and specification for the ESRF. Now the developments at the ESRF, including high harmonic emission of undulators *via* magnet shimming (Elleaume, 1989) and narrow-gap undulator operation (Elleaume, 1998), are fuelling ideas and the specification of what is possible in these new national SR sources. Table 8.1.5.1 compares the parameters of the mature SRS of 1997 (from Munro, 1997) with the proposed design for DIAMOND (Suller, 1994). A shift of emphasis to high brilliance is again clear, as the applications of SR involving small samples dominate. Likewise, a 3 GeV machine energy is indicative of the need to include a provision of high photon energies for many applications, including, obviously, access to short-wavelength absorption edges. The extent to which undulators, for <3 GeV, will reach the hard X-ray region at high brilliance (*e.g.* around 1 Å wavelength) will depend on the minimum undulator magnet gaps realizable, along with magnet shimming to improve high harmonic emission. Moreover, longer wavelengths in protein crystallography are being explored on lower-energy SR machines (*e.g.* <3 GeV) at >1.5 Å, even 2.5 Å (Helliwell 1993, 1997*a*; Polikarpov *et al.*, 1997; Teplyakov *et al.*, 1998), and even softer wavelengths are under active development to utilize the S *K* edge for anomalous dispersion (Stuhrmann & Lehmann, 1994). Such developments interact closely with machine and beamline specifications. At very short ($\sim$0.5 Å) and ultra-short ($\sim$0.3 Å) wavelengths, a high machine energy yields copious flux output; pilot studies have been conducted in protein crystallography at CHESS (Helliwell *et al.*, 1993) and at the ESRF (Schiltz *et al.*, 1997).

8.1.5.5. *X-ray free electron laser (XFEL)*

In terms of the evolution of X-ray sources, mention should be made of the X-ray free electron laser (XFEL); it now seems feasible that this will yield wavelength output well below the visible region of the electromagnetic spectrum. At DESY in Hamburg (Brinkmann *et al.*, 1997) and at SLAC (Winick, 1995), such considerations and developments are being pursued. Compared to SR, one would obtain a transversely fully coherent beam, a larger average brilliance and, in particular, pulse lengths of $\sim$200 fs full width at half-maximum with eight to ten orders of magnitude larger peak brilliance. Such a machine is based on a linear accelerator (linac)-driven XFEL utilizing a linear collider installation (*e.g.*, for a high-energy physics centre-of-mass energy capability of 500 GeV). For this machine there is a 'switchyard' distributing the electrons in a beam to different undulators from which the X-rays are generated in the range 0.1 to $\sim$12 keV. The anticipated r.m.s. opening angle would be 1 mrad and the source diameter would be 20 µm. This source of X-rays would then compete in time resolution with laser-pulse-generated X-ray beams [see Helliwell & Rentzepis (1997) for a survey of that work and a comparison with synchrotron radiation] and would also have higher brilliance.

8.1.6. SR instrumentation

The divergent continuum of X-rays from the source must be intercepted by the sample cross-sectional area. The crystal sample acceptance, as seen above, is a good way to illustrate to the machine designer the sort of machine emittances required. Likewise, the beamline optics, mirrors and monochromators should not degrade the X-ray beam quality. Mirror surface and shape finish have improved a great deal in the last 20 years; slope errors of mirrors, even for difficult shapes like polished cylinders, which on bending give a toroidal reflecting surface, are now around 1 arc second (5.5 µrad) for a length of 1 m. Thus, over focusing distances of 10–20 m, say, the focal-spot smearing contribution from this is 55–110 µm, important for focusing onto small crystals. Choice of materials has evolved, too, from the relatively easy-to-work with and finish fused quartz to silicon; silicon having the advantageous property that at liquid-nitrogen temperature the expansion coefficient is zero (Bilderback, 1986). This has been of particular advantage in the cooling of silicon monochromators at the ESRF, where the heat loading on optics is very high. An alternative approach with the rather small X-ray beams from undulators is the use of transparent monochromator crystals made of diamond, which is a robust material with the additional advantage of transparency, thus allowing multiplexing of stations, one downstream from the other, fed by one straight section of one or more undulator designs. For a review of the ESRF beamline optics, see Freund (1996); for reviews of the macromolecular crystallography programmes at the ESRF, see Miller (1994), Branden (1994) and Lindley (1999), as well as the *ESRF Foundation Phase Report* (1987). See also Helliwell (1992), Chapter 5.

Detectors have been, and to a considerable extent are still, a major challenge. The early days of SR use saw considerable reliance on photographic film, as well as single-counter four-circle diffractometers. Evolution of area detectors, in particular, has been considerable and impressive, and in a variety of technologies. Gas detectors, *i.e.*, the multiwire proportional chamber (MWPC), were invented and developed through various generations and types [Charpak (1970); for reviews of their use at SR sources, see *e.g.* Lewis (1994) and Fourme (1997)]. MWPCs have the best detector quantum efficiency (DQE) of the area detectors, but there are limitations on count rate (local and global) and their use at wavelengths greater than $\sim$1 Å is restricted. The most popular devices and technologies for X-ray diffraction pattern data acquisition today are image plates (IPs), mainly, but not exclusively, with online scanners [Miyahara *et al.* (1986); for a recent review, see Amemiya (1997)], and charge coupled devices (CCDs) (Tate *et al.* 1995; Allinson, 1994; Westbrook & Naday, 1997). Image plates and CCDs are complementary in performance, especially with respect to size and duty cycle; image plates are larger, *i.e.*, with many resolution elements possible, but are slower to read out than CCDs. Both are capable of imaging well at wavelengths shorter than 1 Å and with high count rates. Both have overcome the tedium of chemical development of film! Impressive performances for macromolecular crystallography are described for image plates (in a Weissenberg geometry) by Sakabe (1983, 1991) and Sakabe *et al.* (1995), and for CCDs by Gruner & Ealick (1995). Other detectors needed for crystallography include those for monitoring the beam intensity; these must not interfere with the beam collimation, and yet must monitor the beam downstream of the collimator (Bartunik *et al.*, 1981); also needed are fluorescence

detectors for setting the wavelength for optimized anomalous-scattering applications.

An area-detector development is the so-called pixel detector. This is made of silicon cells, each 'bump bonded' onto associated individual electronic readout chains. Thus, extremely high count rates are possible, and large area arrays of resolution elements may be conceived at a cost. These devices can then combine the attributes of large image-plate sensitive areas with the fast readout of CCDs, along with high count-rate capability and so on. Devices and prototypes are being developed at Princeton/Cornell (Eikenberry *et al.*, 1998), Berkeley/San Diego (Beuville *et al.* 1997), Imperial College, London (Hall, 1995), and by Oxford Instruments and the Rutherford Appleton Laboratory ('IMPACT' detector programme).

8.1.7. SR monochromatic and Laue diffraction geometry

In the utilization of SR, both Laue and monochromatic modes are important for data collection. The unique geometric and spectral properties of SR render the treatment of diffraction geometry different from that for a conventional X-ray source.

8.1.7.1. *Laue geometry: sources, optics, sample reflection bandwidth and spot size*

Laue geometry involves the use of the polychromatic SR spectrum as transmitted through the beryllium window that is used to separate the apparatus from the machine vacuum. There is useful intensity down to a wavelength minimum of $\sim\lambda_c/5$, where λ_c is the critical wavelength of the magnet source. The maximum wavelength is typically $\geq$3 Å; however, if the crystal is mounted in a capillary, then the glass absorbs the wavelengths beyond ~2.5 Å.

The bandwidth can be limited somewhat under special circumstances. A reflecting mirror at grazing incidence can be used for two purposes. First, the minimum wavelength in the beam can be sharply defined to aid the accurate definition of the Laue spot multiplicity. Second, the mirror can be used to focus the beam at the sample. The maximum-wavelength limit can be truncated by use of aluminium absorbers of varying thickness or a transmission mirror (Lairson & Bilderback, 1982; Cassetta *et al.*, 1993).

The measured intensity of individual Laue diffraction spots depends on the wavelength at which they are stimulated. The problem of wavelength normalization is treated by a variety of methods. These include:

(i) use of a monochromatic reference data set;

(ii) use of symmetry equivalents in the Laue data set recorded at different wavelengths;

(iii) calibration with a standard sample, such as a silicon crystal.

Each of these methods produces a 'λ curve' describing the relative strength of spots measured at various wavelengths. The methods rely on the incident spectrum being smooth and stable with time. The bromine and silver *K* absorption edges, in AgBr photographic film, lead to discontinuities in the λ-curve. Hence, the λ-curve is usually split up into wavelength regions, for example $\lambda_{\min}$ to 0.49 Å, 0.49 to 0.92 Å, and 0.92 Å to $\lambda_{\max}$. Other detector types have different discontinuities, depending on the material making up the X-ray absorbing medium. Most Laue diffraction data now recorded on CCDs or IPs. The greater sensitivity of these detectors (expressed as the DQE), especially for weak signals, has greatly increased the number of Laue exposures recordable per crystal. Thus, multiplet deconvolution procedures, based on the recording of reflections stimulated at different wavelengths and with different relative intensities, have become possible (Campbell & Hao, 1993; Ren & Moffat, 1995*b*). Data quality and completeness have improved considerably.

The production and use of narrow-bandpass beams, *e.g.* $\delta\lambda/\lambda \leq 0.2$, may be of interest for enhancing the signal-to-noise ratio. Such bandwidths can be produced by a combination of a reflection mirror used in tandem with a transmission mirror. Alternatively, an X-ray undulator of 10–100 periods should ideally yield a bandwidth behind a pinhole of $\delta\lambda/\lambda \simeq 0.1$–$0.01$. In these cases, wavelength normalization is more difficult, because the actual spectrum over which a reflection is integrated is rapidly varying in intensity; nevertheless, high-order Chebychev polynomials are successful (Ren & Moffat, 1995*a*).

The spot bandwidth is determined by the mosaic spread and horizontal beam divergence (since $\gamma_H > \gamma_V$) as

$$(\delta\lambda/\lambda) = (\eta + \gamma_H)\cot\theta, \tag{8.1.7.1}$$

where η is the sample mosaic spread, assumed to be isotropic, γ_H is the horizontal cross-fire angle, which in the absence of focusing is $(x_H + \sigma_H)/P$, where x_H is the horizontal sample size, σ_H is the horizontal source size and P is the sample to the tangent-point distance. This is similar for γ_V in the vertical direction. Generally, at SR sources, σ_H is greater than σ_V. When a focusing-mirror element is used, γ_H and/or γ_V are convergence angles determined by the focusing distances and the mirror aperture.

The size and shape of the diffraction spots vary across the detector image plane. The radial spot length is given by convolution of Gaussians as

$$\left(L_R^2 + L_c^2\sec^2 2\theta + L_{\rm PSF}^2\right)^{1/2} \tag{8.1.7.2}$$

and tangentially by

$$\left(L_T^2 + L_c^2 + L_{\rm PSF}^2\right)^{1/2}, \tag{8.1.7.3}$$

where L_c is the size of the X-ray beam (assumed to be circular) at the sample, $L_{\rm PSF}$ is the detector point spread factor,

$$L_R = D\sin(2\eta + \gamma_R)\sec^2 2\theta, \tag{8.1.7.4}$$

$$L_T = D(2\eta + \gamma_T)\sin\theta\sec 2\theta, \tag{8.1.7.5}$$

and

$$\gamma_R = \gamma_V\cos\psi + \gamma_H\sin\psi, \tag{8.1.7.6}$$

$$\gamma_T = \gamma_V\sin\psi + \gamma_H\cos\psi, \tag{8.1.7.7}$$

where ψ is the angle between the vertical direction and the radius vector to the spot (see Andrews *et al.*, 1987). For a crystal that is not too mosaic, the spot size is dominated by L_c and $L_{\rm PSF}$. For a mosaic or radiation-damaged crystal, the main effect is a radial streaking arising from η, the sample mosaic spread.

8.1.7.2. *Monochromatic SR beams: optical configurations and sample rocking width*

A wide variety of perfect-crystal monochromator configurations are possible and have been reviewed by various authors (Hart, 1971; Bonse *et al.*, 1976; Hastings, 1977; Kohra *et al.*, 1978). Since the reflectivity of perfect silicon and germanium is effectively 100%, multiple-reflection monochromators are feasible and permit the tailoring of the shape of the monochromator resolution function, harmonic rejection and manipulation of the polarization state of the beam. Two basic designs are in common use. These are the bent single-crystal monochromator of triangular shape (Fig. 8.1.4.1*a*) and the double-crystal monochromator (Fig. 8.1.4.1*b*).

8.1.7.2.1. *Curved single-crystal monochromator*

In the case of the single-crystal monchromator, the actual curvature employed is very important in the diffraction geometry. For a point source and a flat monochromator crystal, there is a

references