Table 9.3.2.1
Summary of various algorithms

The algorithms are, from top to bottom: error reduction, solvent flipping, hybrid input–output, difference map, averaged successive reflections, hybrid projection–reflection and relaxed averaged alternating reflections. A reflection is defined by its associated projection as $\mathbf{R} = 2\mathbf{P} - \mathbf{I}$, where $\mathbf{I}$ is the identity projection (Marchesini, 2007).

Algorithm	Iteration $\rho^{(n+1)} =$
ER	$\boldsymbol{P}_s\boldsymbol{P}_m\rho^{(n)}$
SF	$\boldsymbol{R}_s\boldsymbol{P}_m\rho^{(n)}$
HIO	$\begin{cases}\boldsymbol{P}_m\rho^{(n)}(\boldsymbol{r}), & \boldsymbol{r}\in S\\ (\boldsymbol{I}-\beta\boldsymbol{P}_m)\rho^{(n)}(\boldsymbol{r}), & \boldsymbol{r}\notin S\end{cases}$
DM	$\{\boldsymbol{I}+\beta\boldsymbol{P}_s[(1+\gamma_s)\boldsymbol{P}_m-\gamma_s\boldsymbol{I}] -\beta\boldsymbol{P}_m[(1+\gamma_m)\boldsymbol{P}_s-\gamma_m\boldsymbol{I}]\}\rho^{(n)}$
ASR	$(1/2)[\boldsymbol{R}_s\boldsymbol{R}_m+\boldsymbol{I}]\rho^{(n)}$
HPR	$(1/2)\{\boldsymbol{R}_s[\boldsymbol{R}_m+(\beta-1)\boldsymbol{P}_m]+\boldsymbol{I}+(1-\beta)\boldsymbol{P}_m\}\rho^{(n)}$
RAAR	$[(1/2)\beta(\boldsymbol{R}_s\boldsymbol{R}_m+\boldsymbol{I})+(1-\beta)\boldsymbol{P}_m]\rho^{(n)}$

been demonstrated in two dimensions, although the total efficiency of the optic used was of the order of 1% (Chao *et al.*, 2009) at the highest spatial frequencies. Thus, at the cost of throughput and ease of use, a diffraction microscope provides increased X-ray efficiency and resolution.

The following sections first report on the current standard single-particle phase retrieval techniques and then on recent experiments in CXDM, which establish the state of the art in whole-cell imaging by diffractive methods. Images of dry yeast at 11 nm resolution are presented, which represent the highest resolution X-ray images of whole cells currently on record. The effects of radiation damage are discussed and Sayre's idea of using stereoscopic viewing as a means of obtaining quick and low-dose three-dimensional information is explored (Sayre, 2008).

9.3.2. Phase retrieval from single-particle diffraction data

The problem of phase retrieval is solved through successive application of constraints on the recovered object in the data and object spaces. Using the language of convex optimization, the mathematical operators which act on the data are projectors. The projector in the reciprocal (data) space forces the Fourier components to have the correct magnitude, while in object space finite support is enforced. To calculate the Fourier magnitude projector, one first needs to propagate the object density, ρ, to the data space by a Fourier transform, then replace the estimated magnitudes $|\tilde{\rho}|$ with the measured ones, $I^{1/2}$, and finally propagate back to real space. Using these transforms one simplifies the calculation of the projection, which becomes an element-wise operation on each recovered Fourier component. The forward $\mathcal{F}$ and inverse $\mathcal{F}^{-1}$ transforms must be incorporated into the operator defined in real space $\mathbf{P}_m$,

$$\mathbf{P}_m = \mathcal{F}^{-1}\tilde{\mathbf{P}}_m\mathcal{F}, \tag{9.3.2.1}$$

where the measured Fourier magnitudes are enforced in Fourier space by $\tilde{\mathbf{P}}_m$. Using the Fourier basis, one simply replaces the estimated magnitudes $|\tilde{\rho}|$ with the measured ones $I^{1/2}$, $[\tilde{\mathbf{P}}_m\tilde{\rho}(\boldsymbol{k}) = I(\boldsymbol{k})^{1/2}\tilde{\rho}(\boldsymbol{k})/|\tilde{\rho}(\boldsymbol{k})|]$. Similarly, in the object space the finite support constraint is applied on a per pixel basis through multiplication by the support mask. The corresponding projector is

$$\mathbf{P}_s\rho = S\cdot\rho.$$

Table 9.3.2.1 lists the combination of projections used by the most popular algorithms.

The violation of the support constraint is used as an error metric to monitor the convergence towards the solution. The solution should have zero density outside the support mask, so the error can be defined as the total density outside the support area,

$$\varepsilon_s^2(\rho) = |||\rho - S\rho|||^2 = ||[I-\mathbf{P}_s]\rho||^2. \tag{9.3.2.2}$$

Alternatively, the error metric can be defined in the data space as the difference between the measured and calculated magnitudes,

$$\varepsilon_m^2(\rho) = |||\mathcal{F}\rho| - I^{1/2}||^2 = ||[I-\mathbf{P}_m]\rho||^2. \tag{9.3.2.3}$$

In reality, the measured intensities are subject to noise which prohibits exact compliance with the constraints, so the error metrics cannot drop to zero. Although in most cases the algorithm can locate the global minimum, random noise will force fluctuations around the minimum. Once the algorithm reaches this steady-state regime, any particular iterate chosen as the solution would have a misleading degree of detail. On the other hand, the average of many fluctuating iterates would have reduced intensity in those Fourier components which are not reliably phased. The ratio of the average Fourier magnitude to the measured magnitude provides a measure of the stability, or reproducibility, of the retrieved phase information. This ratio as a function of spatial frequency is the phase retrieval transfer function (PRTF),

$$\mathrm{PRTF}(\mathbf{q}) = \frac{|\mathcal{F}[\langle\psi\rangle]|(\mathbf{q})}{[I_m(\mathbf{q})]^{1/2}}, \tag{9.3.2.4}$$

where $\mathcal{F}[\langle\psi\rangle]$ is the Fourier transform of the final averaged image and I_m is the measured intensity pattern. The PRTF is analogous to the differential phase residual of electron microscopy and, following Chapman *et al.* (2006), the resolution of a reconstruction is chosen as the spatial frequency at which the PRTF falls below a value of 0.5.

9.3.3. High-resolution imaging of yeast

The X-ray dose required to image a given volume of protein is nearly independent of energy above the oxygen K edge. At the same time, the photon flux required to image the same volume increases with E^2 because of the energy dependence of the scattering cross section (Howells *et al.*, 2009). For this reason, it is advantageous to use the lowest energy commensurate with the desired resolution of 5–10 nm. Commercially available charge-coupled device (CCD) detectors can easily provide a scattering angle of 0.1 radians [a 1 inch detector placed 5 inches from the sample (1 inch = 2.54 cm)], which results in a half-period resolution of 8 nm when using 750 eV X-rays. Furthermore, a cell with a diameter of 3 µm would have an oversampling ratio (number of intensity samples per speckle) of at least ten in this geometry if the detector has 20 µm pixels.

In this particular case, a Princeton Instruments CCD (PI-MTE:1300) is placed 136 mm downstream of a freeze-dried yeast cell using the CXDM instrument on Beamline 9.0.1 of the Advanced Light Source (ALS). The yeast cell is illuminated by a coherent beam of 750 eV X-rays defined by a 5 µm pinhole located 25 mm upstream. The incident intensity of 4×10^6 photons s^{-1} μm^{-2} is high enough to cause rapid structural changes to the cell (discussed in the next section), so the sample requires pre-irradiation for about 30 minutes prior to collection of the final data set intended for reconstruction. The