1

INTRODUCTION

The present work deals with the properties and development of automated phase plate application in Transmission Electron Microscopy (TEM). In this chapter, the problem is motivated at the example of the imaging requirements for specimen of current research interest. The relevance of TEM is illustrated and the difficulties to generate contrast for transparent, beam-sensitive specimen are pointed out. A possible solution to the problems is the application of physical phase plates for contrast generation, which is however impeded by other negative effects.

With the context provided in this overview, the essential questions are formulated and the chapter organisation of the thesis is presented.

STRUCTURE-FUNCTION RELATIONSHIP

The efficiency and function of organic electronics is not only dependent on the properties of the materials used, but also on their structural composition. For instance, in the active layer of organic photovoltaic cells, excited states of electron-hole-pairs, so-called excitons, are generated by absorption of incident photons. The excitons need to be separated into electrons and holes and subsequently transported to the respective electrodes in order to contribute to a usable electrical current [71].

During its short lifetime, the exciton is able to travel a certain characteristic distance before it is annihilated by recombination. This distance is called diffusion length. In typical materials used for organic photovoltaic cells, this distance is in the order of 2–10 nm [84, 71].

Distinct constituents of organic electronics may differ in their electron-affinity as an intrinsic material property. In the context of organic electronics, materials with lower and higher electron affinity are called donor and acceptor materials. Both form the active layer of an organic photovoltaic cell, since brought into contact, electrostatic forces are generated in the interfacial area, which help separate the excitons. For an efficient charge separation, such an interface has to be present within the diffusion length of an exciton.

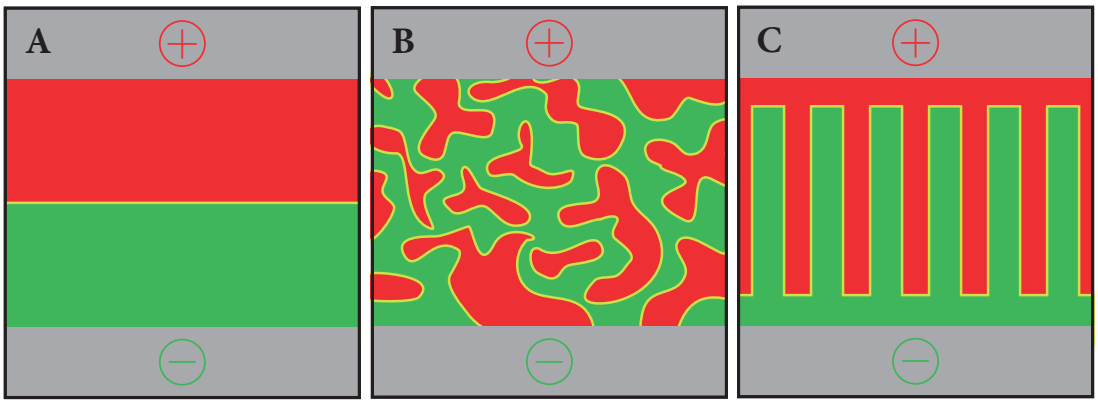

Figure 1.1: Schematic morphologies of active layers of organic photovoltaic cells. The size of the interfacial area (yellow) between electrode-connected regions of donor and acceptor materials increases from planar configuration (**A**), bulk heterojunction (**B**) to the theoretical optimum, a fine interdigitation (**C**) in a comb structure with the width of the fingers twice the exciton diffusion length [71]. Donor- and acceptor-rich regions are marked in green and red, electrodes in grey.

After charge separation, holes and electrons subsequently need to be transported to cathode and anode contacts, which requires an uninterrupted percolation path of low series resistance to the respective electrodes. The existence of such a pathway can be guaranteed for planar configurations of the active layer, as can be seen in figure 1.1 (**A**). However, as efficient absorption of photons requires a larger bulk thickness, many of the generated excitons cannot reach the interfacial area and recombine before separation. This results in a small likelihood of a successful charge separation and therefore a poor power conversion efficiency.

By replacing the 2D planar donor-acceptor interface with a 3D bulk heterojunction, shown in figure 1.1 (**B**), the size and distribution of the interfacial area can be optimized, but this only results in better power conversion efficiency, if uninterrupted electrical connections to the electrodes with low series resistance can be maintained [85]. An ideal configuration, a finely interdigitated comb structure, is shown in figure 1.1 (**C**) [71].

Variation of the process parameters during manufacture of the organic solar cells influence various morphological properties, such as domain sizes, level of donor-acceptor intermixture, degree of crystallinity or preferential location of the domains, which all have an influence on the device performance. In order to better approximate the ideal comb structure, the influence of process parameters on the resulting morphology need to be

understood for a target-driven optimisation of a manufacturable structure. Therefore, the possibility of direct visualisation of the resulting morphology can provide key insights to understanding the structural reasons for a certain device performance and provide hints for improvement.

For characterisation of the domain sizes, the relevant dimensions are in the order of the exciton diffusion length, i. e. about 2–10 nm. Distinct crystalline features, such as characteristic π–π-stacking distances can be useful for material identification within the active layer, which usually requires a better point resolution of about 1–20 Å, depending on the properties of the materials. Ideal conditions would be able to image both the fine and the coarse resolution scale equally efficient.

Comparable to organic electronics, biological systems are mainly composed of a selection of light elements with low atomic numbers. Constructed after the blueprints encoded in the DNA, miniature machines, so-called macromolecular complexes serve as building blocks of life. The individual shape of the nanometer-sized macromolecular complexes is adapted to the specific function. Similar to organic electronics, structure determination helps understanding the key mechanisms of certain diseases or how living organisms work in general. The required point resolution for such deductions is also in a range of a few Å.

RESOLUTION

In 1878 Ernst Abbe formulated his famous equation, which relates the attainable resolution of an optical system to its numerical aperture and the wavelength λ of the illuminating wave [1]. Restricted to visible light with a wavelength of a few hundred nm, the maximal attainable point resolution of a general object is limited to about 200 nm, even for a perfect, i. e. aberration free optical system with maximal numerical aperture. To attain higher resolution, shorter wavelengths are needed. Non-visible light of higher energy, such as X-rays, offers smaller wavelengths in the range of 10 nm–10 pm, but due to the resulting decreased interaction with matter, construction of usable lenses becomes more and more difficult.

The wavelength of sufficiently accelerated electrons can be several orders of magnitude smaller than that of photons for which acceptable optics can be manufactured. Using a suitable optical system, which allows to form images using electrons, a much higher resolution is therefore feasible. The first steps towards implementation of such an optical system were the experiments of Ernst Ruska, who managed to create a magnetic field of

suitable shape to focus electrons into a spot, originally with the aim to improve the performance of cathode ray tubes used in oscilloscopes [68]. Shortly after, with optimisations of the shape of the magnetic field, the aberrations could be controlled well enough to demonstrate its use as a lens suitable for imaging. Combining two of such lenses, an optical system was demonstrated, which allowed to surpass the resolution attainable by visible-light microscopy.

PHASE OBJECTS AND PHASE CONTRAST

A theoretical investigation by Hans Boersch [8] analysed the attainable contrast in TEM and noted that atoms imaged with electrons at medium acceleration voltages essentially appear as transparent *phase objects*, where the individual atoms in the sample offer a locally varying refraction index to the electron wave. After passing through the sample, the object's information is almost exclusively encoded in a small modulation of the phase of the beam, which unfortunately results in negligible contrast on a detector, if an aberration-free optical system is used for imaging.

From light optics, such specimen were already known as phase objects. A solution to imaging such objects with nonvanishing contrast was offered by Frits Zernike [88]. For contrast generation, an additional phase shift of $\pi/2$ needs to be introduced in between the part of the wave having interacted with the sample and the unscattered reference wave. This can be realized using an additional optical element with two areas of different optical thickness, where the optical pathway trough one region is an odd multiple of $\lambda/4$ longer than the other.

This so-called *phase plate* needs to be inserted in a diffraction plane, where there is a spatial separation between scattered and unscattered beams. For parallel illumination, the unscattered or *zero-order beam* is focused into a spot, while beams having interacted with the specimen are scattered to higher angles. Using this spatial separation, the phase-shifting effect can be applied to either scattered or unscattered beams, if phase plate structures of suitable size and shape can be manufactured.

Boersch suggested to adapt Zernike's phase contrast method for TEM [8]. Unlike structured glass phase plates for photons, the $\lambda/4$ plate equivalents for electron microscopy make use of an electrostatic potential to provide a phase shift. The inner potential of a thin film material of suitable thickness can be source of an approximately homogeneous potential generating a phase shift of $\pi/2$. By cutting out a hole in such a film, a discontinuity in the

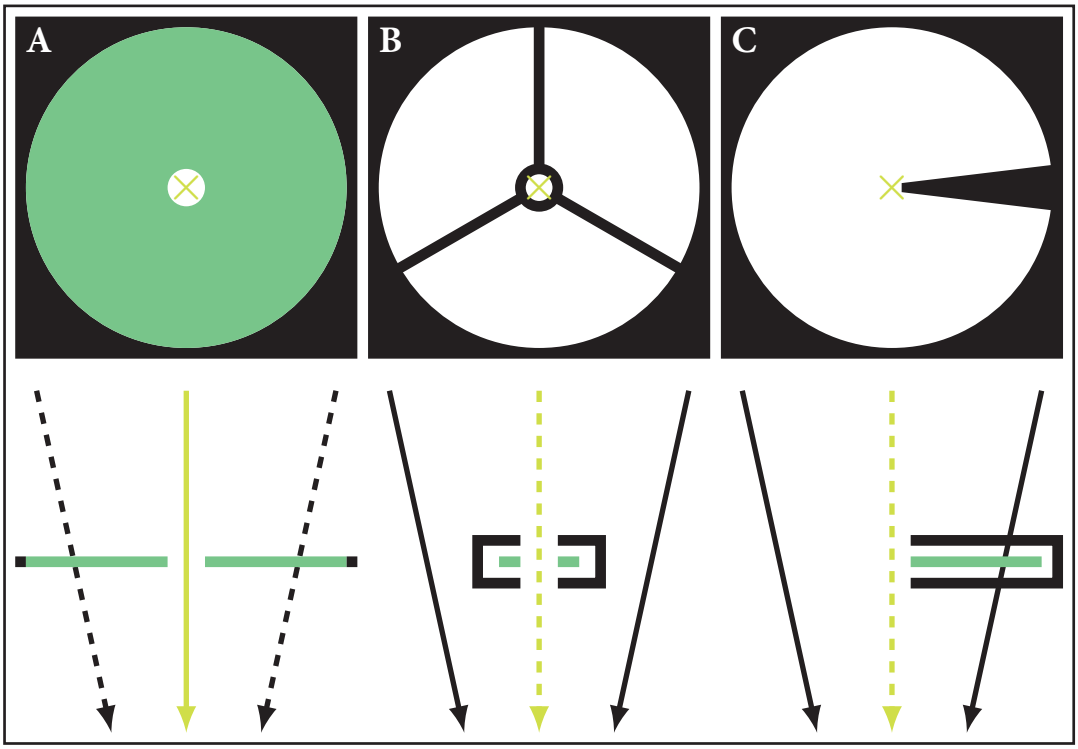

Figure 1.2: Phase plates suitable for TEM in plan (upper row) and orthogonal (lower) views through the aperture center in the diffraction plane. Zernike film phase plate in (**A**) consisting of a thin matter foil of suitable thickness with cut-out hole for the zero-order beam (×). Electrostatic phase plate design proposed by Boersch in (**B**) and its optimized successor, the Zach phase plate with smaller electron-obstructing area in (**C**). Therein, the phase-shifting potential is generated by an applied voltage. The elements responsible for the phase shift are highlighted in green. The supporting structure is kept at ground potential (solid black). For an additional phase shift (⇢) of $\pi/2$ between scattered and unscattered beams, either the unscattered beam (**B**–**C**) or all scattered beams (**A**) have to be phase shifted relative to the respective unshifted beams (→).

potential distribution is realized and the necessary relative phase shift is generated, if the reference wave is allowed to pass through this cut-out hole and all scattered electrons pass through the matter foil. This is depicted in figure 1.2 (**A**).

Alternatively, miniature electrode assemblies generating a localized electrostatic field can be used to adjust the phase of the reference wave. Compared to the film phase plates, this has the advantage that the scattered electrons, which carry all the specimen information, are not subjected to another scattering process in the phase plate, avoiding a negative influence on the attainable point resolution. Furthermore, the amount of phase shift is tunable with variation of the applied potential.

Boersch also noted that lens aberrations, most importantly defocus and spherical aberration, influence the phase of the electrons and can be used to enable phase contrast transfer. This is especially true for small real space object distances or high spatial frequencies in reciprocal space, corresponding to large scattering angles [8, 73]. While this is the most important source of phase contrast transfer in standard TEM images, it is impeded by the resulting oscillating transfer function. This complicates quantitative and often even qualitative image interpretation, due to unavoidable contrast inversions and transfer gaps. Therefore, the transferred contrast always suffers from dislocation, i. e. the relative position of an object feature is not faithfully reproduced in the image. This is especially true for low spatial frequencies, as their transfer demands relatively large intended aberrations and therefore large contrast dislocations, which obscure the true shape of an object.

To obtain an unaberrated phase contrast image using conventional methods, a so-called exit-wave reconstruction has to be performed, wherein the information from many differently aberrated images is combined [58, 64]. However, to reproduce the true object properties in such a reconstruction, a precise knowledge of the actual aberration parameters for each of the individual images is necessary, which can be difficult to measure accurately. For beam-sensitive specimen, the amount of exposures at the same sample position is limited and therefore only a small set of differently aberrated images can be acquired. Depending on the required range of accurately reconstructed spatial frequencies, a certain amount of measuring points are required, and the dose budget has to be split accordingly. Eventually, this results in poor signal-to-noise ratios in the individual images, which ultimately prohibits a faithful reconstruction.

Using ideal physical phase plates and suitable imaging conditions, unaberrated phase contrast images can be acquired in a single exposure. If an

electrostatic phase plate with adjustable phase shift is used, a full exit-wave reconstruction can be realized in only three images [33].

3D IMAGING AND DOSE PROBLEM

Even though the samples in TEM have to be relatively thin to allow passage of the beam through the sample, many applications still require 3D object information. Unlike techniques restricted to accessing surface information, in TEM the electron-beam is influenced by the whole bulk of the sample. In a single image, a projected view of the specimen potential is generated. Using electron-tomography, the 3D object information can be reconstructed, if a sufficient amount of views at different projection angles is available. For general specimen, such views can be generated by acquisition of a tomographic tilt series. Depending on the required resolution, this requires acquisition of about 30 to more than 100 individual images at the same specimen position, which exposes relatively small sample areas to a high electron dose.

Unfortunately, typical samples of organic specimen such as biological macromolecules and organic electronics degrade if exposed to the intense electron beam, i. e. their spatial conformation changes. For imaging, this means only a certain dose budget is available, which ultimately limits the attainable signal-to-noise ratio.

A standard approach to reduce the structure-changing effects of the electron exposure is maintaining the specimen at cryogenic temperatures. While this can increase the sustainable dose of a delicate specimen by an order of magnitude [35], it usually does not enable recording of high spatial resolution with sufficient signal-to-noise ratio on its own.

To increase the signal-to-noise ratio with biological macromolecules, the so-called single-particle approach is widely used. Therein, the ability of biological systems to produce large amounts of virtually identical particles is exploited. The attained signal-to-noise ratio in a 2D projection image then only has to allow a reasonable determination of the particle's relative rotation and translation parameters [38, 29]. By taking the combined information of several thousand single particles gathered from hundreds of images and leveraging the signal from the different angular views present in the dataset, low-noise 3D maps with resolutions better than 3 Å can be generated [3, 12].

While usual fabrication steps of organic electronics involving vapour deposition or solution processing may result in locally ordered structures

due to self-assembling properties of the individual materials, the general bulk morphology is non-repetitive, i. e. no identical copies of regions can be expected to occur. Therefore, no single-particle approach is feasible to increase the signal-to-noise ratio while maintaining low-dose conditions and only the acquisition of tomographic tilt series allows reconstruction of the 3D object information. To limit the sample degradation and maximize the signal-to-noise ratio, the dose budget has to be transferred into image contrast most efficiently. As phase plates optimize the contrast transfer, they can be an enabling technology to extend the applicability of such advanced acquisition schemes also on dose-sensitive samples.

Similarly, also the single-particle approach can benefit from phase plate application. Typically, biological macromolecules are imaged close to their native state, i. e. embedded in a vitrified aqueous solution. Due to the low difference of atomic numbers between sample and background, the available signal is rather small, and conventionally requires use of an excessive amount of intended defocus to produce recognizable contrast. For small macromolecules with dimensions of e. g. 10–20 nm, this often means the apparent contrast dislocation is larger than the dimensions of the actual particle, which limits the practical use of this approach. Phase plate application is a potential key technology to enable imaging of such delicate objects.

1.1 CHALLENGES OF PHASE PLATE USE

Theoretically, the superior imaging properties of phase plate microscopy demand its application to all kind of TEM problems. So far, the practical application of phase plates has been impeded by a couple of detrimental effects. For instance, the requirement to impose different phase shifts to zero-order and scattered beams can often only be approximated, due to the small extent of the zero-order beam focus in the diffraction plane. Especially if an electrostatic phase plate is used for generating the phase shift, current micromanufacturing techniques fail to produce sufficiently small electrodes that the obstructions of the opaque electrode support material are negligible.

For certain phase plate geometries involving a cut-out hole, such as Zernike film and electrostatic Boersch phase plates, only spatial frequencies exceeding a certain, so-called *cut-on frequency* are subject to the necessary phase shift of $\pi/2$. The main effect is a sudden, step-like onset of contrast transfer for the film phase plate and the obstruction of a certain

range of spatial frequencies corresponding to the radius of the cut-out hole for the electrostatic phase plate (cf. figure 1.2 (**A**) and (**B**)), which both introduce image distortions. The detrimental effects can be alleviated if smaller physical phase plate structures are used.

Since the manufacturing technology does not allow a further significant minimisation of the electrostatic phase plate structure, they can be inserted into an optically magnified diffraction plane for a relative size reduction compared to the unmagnified conditions. In the KRONOS instrument, this has been realized in an additional column element, the Diffraction Magnification Unit (DMU).

Intense electron-beams can channel adsorption of residual molecules stemming from imperfect vacuum conditions to a surface [27, 26]. The deposited contaminations create poorly conducting patches on the surface of a phase plate, which subsequently accumulate a charge and influence the electrostatic potential distribution.

In the electrode assembly of an electrostatic phase plate, insulating materials need to be used for construction. Irradiated with highly accelerated electrons, the electron-obstructing structure is partially penetrated and inelastic interactions occur within the material, which can generate positive and negative charges [82]. Due to the low charge carrier mobility present in insulators, the generated charges are trapped and permanently influence the phase-shifting behaviour of the phase plate. Most detrimental effects of contamination and charge deposition can therefore be avoided, if the phase plate is handled with the utmost care, and the physical structure never exposed to the intense zero-order beam.

Finally, to generate the desired phase-shifting effect, the physical phase plates need to be aligned very precisely relative to the zero-order beam. Manual alignment can be very tedious and time consuming, especially if suboptimal electron-optical alignment does not allow side-effect free variation of individual parameters of the optical system. Time consuming, tedious and delicate handling tasks are often better left to machines, rather than human operators. Fully automated phase plate application could therefore provide a key contribution to establish phase contrast microscopy as a standard tool in TEM.

1.2 GOALS AND OUTLINE

Phase plate microscopy potentially enables superior imaging of phase objects. Towards establishing this technology as a standard technique in TEM, this thesis contributes insights to the following questions:

- Can an automatic application of a phase plate be realized?
- How can the necessary functionality be integrated in standard automated image acquisition software?

As phase plate microscopy requires tight control of the beam conditions in the diffraction plane, where the phase plates are installed, further points of interest for a successful realisation are:

- What requirements have to be met by the electron-optical alignment to allow automated phase plate microscopy?
- What are the consequences of introducing a DMU to the optical setup? Does it provide a better environment for phase plate application?
- Are there alternative means to reach a similar goal with standard electron-optics?

Unlike human operators, who need a continuous visual feedback for manual phase plate alignment, automation algorithms can precisely determine the current positioning error and compute a matching correction from a static exposure. Such a discrete alignment scheme potentially imposes a lower dose on the phase plate structure. An additional aspect is therefore:

- Can automated phase plate alignment routines help prevent phase plate degradation and increase their effective lifetime?

To answer the questions, this thesis is laid out as follows: In chapter 2, the theoretical mechanisms of contrast formation in TEM are described. In the following chapter 3, an introduction of the concepts of automated image acquisition is given. The source of electron-optical alignment problems are identified and appropriate means for controlling the beam in the diffraction plane are presented. Afterwards, several methods to determine the current phase plate position are shown. All aspects are combined in an automatic alignment routine, whose performance is evaluated.

The experimental results acquired using electrostatic phase plates and evaluation of the DMU properties are given in chapter 4. With the development of a new imaging mode for film phase plates, which is described

in chapter 5, the implementation of automated alignment strategies are demonstrated on beam-sensitive samples. The results are presented in chapter 6.

Finally, the implications of the different phase plate technologies, microscope properties and benefits from automation are discussed in chapter 7.

2

CONTRAST FORMATION IN TEM

This chapter begins with a description of the main structural components of a transmission electron microscope and introduces the names and location of the most important lenses and principal planes. Then, the interaction of an electron wave with matter, such as a specimen or a thin film phase plate is discussed. Afterwards, the image contrast transfer of the optical system is discussed using the idealized properties of a pure phase object. Some more attention is given to the properties of phase contrast transfer using various phase plate designs. Finally, the known limitations of physical phase plates and possible remedies are discussed.

2.1 STRUCTURAL COMPONENTS OF A TRANSMISSION ELECTRON MICROSCOPE

In most transmission electron microscopes the same principal building blocks can be found in a similar sequential configuration. The individual components are stacked in a column, with an evacuated beam pipe in the centre. Electron-source and camera are located at top and bottom of the column, respectively. In between, round electron-lenses, deflectors and other beam-shaping electron-optical elements are arranged in specialized groups, such as condenser, objective and projective, which are responsible for a certain, often specialized task. An annotated image of a modern transmission electron microscope used in this work is shown in figure 2.1.

In the electron gun, free electrons are extracted from a pointed tip using thermal or tunnelling effects. Important properties of the electron-source are the attainable radiant intensity, the effective source size and the width of the energy distribution of the emitted electrons, which determine spatial and temporal coherence parameters of the illumination. Modern field emission gun (FEG) electron-sources offer both high spatial and temporal coherence, with the energy spread ΔE of a thermally assisted FEG in the range of 0.2–0.7 eV, which can be further reduced by an integrated monochromator [45].

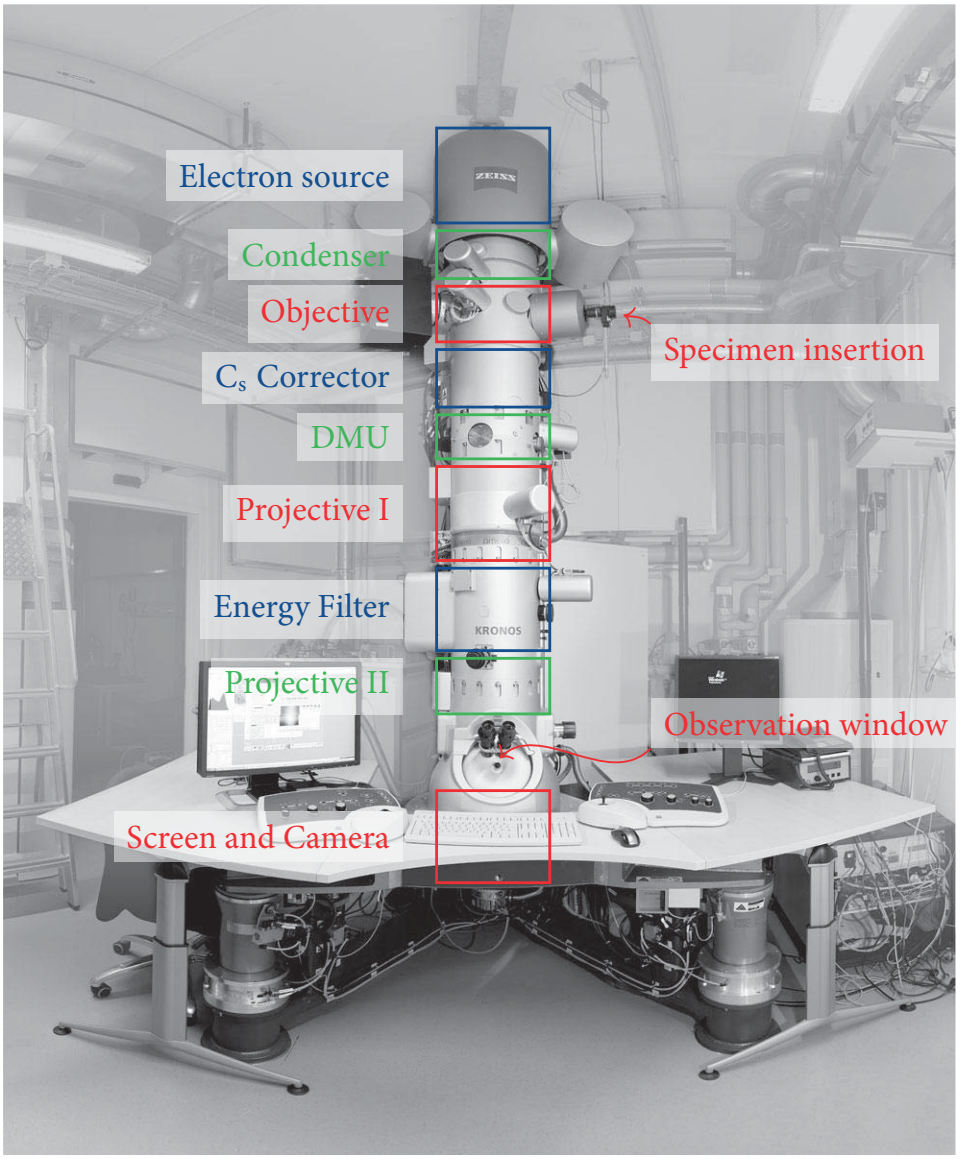

Figure 2.1: Annotated view of a specialised transmission electron microscope used in this work. From top to bottom, the electrons are emitted from the electron-source, conditioned to provide a sample illumination of the desired parameters, interact with the sample and are projected to a screen or other detector. Many high-performance instruments feature additional electron-optical elements to enhance certain optical properties, such as correcting for the spherical aberration C_s or provide devices allowing analytical studies, such as imaging energy filters and spectrometers. Additional to the provisions of standard instruments, this microscope features an accessible magnified diffraction plane in a dedicated unit (DMU) for phase plate application. The height of this instrument is about 4.5 m and the column diameter about 35 cm. Image courtesy of Levin Dieterle/iL.

Subsequently, the electrons are accelerated to the desired high-voltage and fed into the condenser. The resulting electron wavelength is given by

$$\lambda = \frac{h}{\sqrt{2m_0 eU\left(1 + \frac{eU}{2m_0 c^2}\right)}}$$

with electron wavelength λ, Planck's constant h, m_0 the electron rest mass, e the elementary charge, U the acceleration voltage and c the speed of light in vacuum.

In the condenser system, the parameters of the illuminating plane wave, such as the size and intensity of the illuminated area at specimen level can be varied by choosing different excitations of lenses arranged in a single or double zoom system. The condenser is also responsible for projecting a beam cross-over, i. e. a demagnified image of the electron-source in the front focal plane of the objective prefield lens. This ensures parallel (plane-wave) specimen illumination. Details of the resulting ray paths can be seen in the schematic overview shown in figure 2.2.

The specimen is immersed in the magnetic field of a Riecke-Ruska-type single-field condenser-objective lens. In the ray diagrams shown in this work, it is represented by two separate lenses, the objective prefield and the objective lens. The objective prefield lens in front of the specimen is responsible for forming the illuminating beam. The objective lens located thereafter receives the electrons scattered by the specimen and provides the first, most critical refraction. Since in the entire microscope, only the objective lens needs to deal with large scattering angles, its performance is decisive for the fidelity of the optical system.

During its passage through the thin specimen, the electron-wave interacts with the specimen potential and undergoes elastic and inelastic scattering events. After passing through the bulk of the specimen, the so-called *exit* or *object wave* is formed.

The objective is responsible for focusing rays diffracted into a certain angle to a certain position in the back focal plane. It can be shown that this property forms a reciprocal space representation of the object wave in the back focal plane, which corresponds to the Fourier transform of the specimen's potential [45, 28]. Of further note is the correspondence of shifts in the diffraction plane to tilts in the object plane and vice-versa.

In the back focal plane, electrons scattered to higher angles are focused at larger distances with respect to the optical axis. By introduction of an aperture in this plane, the angular distribution of scattered electrons can