Optical and aerodynamic focusing of isolated particles for diffractive imaging experiments at X-ray free electron lasers

Dissertation

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Abstract

The highly brilliant, short and coherent pulses produced by X-ray free-electron lasers (XFELs) opened up a unique opportunity to image small objects in nature, such as protein macromolecules, clusters, viruses and nanoparticles with nearly atomic spatial resolution, and their dynamic processes can be probed with femtosecond temporal resolution. Single-particle imaging (SPI) is a coherent diffraction imaging technique at XFELs that consists of directing a stream of randomly oriented isolated bio-particles across the focus of the X-ray beam to construct high-resolution 3D structure from diffraction patterns of multiple identical particles. Since the intense X-ray pulses destroy every particle upon interaction, a constant replenishment of sample is required. However, efficiently and successively placing of the biological single particle, which are typically few tens of nanometer in size, onto the few hundred nanometer X-ray beam still remains a considerable challenge.

The main objective of this dissertation is to demonstrate an alternative aerosol focusing mechanism that efficiently and precisely delivers isolated particles into the focus of the XFEL beam, intended primary for SPI experiments. To this end, two complementary techniques were studied.

The first method dealt with aerodynamic focusing of aerosol particles using an improved aerosol injector design, which is based on a simple convergent nozzle geometry. Through high-speed optical imaging, it was shown that 300 nm virus particles can be focused down to a 3.5 µm FWHM spot, which is considerably smaller than the existing aerosol injectors can achieve. The use of this injector for SPI experiment was demonstrated in a micro-focused soft-X-ray FEL beam at the FLASH facility in Hamburg, delivering virus particles, and a hit fraction $> 18\%$ was recorded. Furthermore, delivering macromolecular nanocrystals of *Cydia pomonella* granulosis virus (CpGV), in serial femtosecond crystallography (SFX) experiments at the LCLS CXI hard X-ray instruments, extremely low background Bragg diffraction has obtained.

The second part of the dissertation reports, for the first time, the confining and concentrating of aerodynamically delivered high-speed particle beam using a structured laser illumination. In this proof-of-concept experiment, an aerosol beam from a capillary aerosol injector was delivered into vacuum against a counter-propagating laser beam. The beam was constructed with Laguerre-Gaussian or a
slowly diverging Quasi-Bessel beam that has a dark core in the middle. We call such beam an *optical funnel*. The optical force, governed by the spatial profile of the optical funnel, axially decelerate and lateral confine the particles to guide them into a convergent trajectories. Using this technique, a CpGV particle beam moving with an axial velocity of $17.4 \pm 0.93$ m/s, was transversely confined by a factor of two from its original width and its central peak particle density was increased by more than four times from the reference (laser-off) condition. Prospectively, optical guiding combined with the aerodynamic focusing may further advance single particle imaging experiments, by solving the principal problem of the precise and efficient delivery of isolated single particles to the sub-micron X-ray focus.
Zusammenfassung


Die erste Methode befasste sich mit der aerodynamischen Fokussierung von Aerosolpartikeln unter Verwendung eines verbesserten Aerosol-Injektor-Designs, das auf einer einfachen konvergenten Düsense geometrie basiert. Durch optische Hochgeschwindigkeitsbildgebung konnte gezeigt werden, dass 300 nm große Viruspartikel bis zu einem 3,5 µm FWHM-Spot fokussiert werden können, was erheblich kleiner ist, als das, was die bestehenden Aerosol-Injektoren erreichen können. Dieses Injektor wurde bei einem SPI-Experiment mit einem mikrofokussierten FEL-Strahl mit weicher Röntgenstrahlung in der FLASH-Anlage in Hamburg demonstriert, hierbei wurden Trefferquoten von 18 % für Viruspartikel erreicht. Darüber hinaus zeigten seriele Femtosekunden-Kristallographie (SFX) -Experimente am LCLS mit harten Röntgenstrahlen extrem niedrige Untergrund-Diffraktion in Kombination mit starker Bragg-Diffraktion unter Verwendung dieses Injektors. Die Probe hierfür waren makromolekulare Nanokristallen des *Cydia pomonella* Granulosis-Virus (CpGV).

Die optische Kraft basierend auf dem räumlichen Profil des optischen Trichters, führt zu einer axialen Verzögerung und einer lateralen Begrenzung der Partikel, und führt sie zu konvergenten Trajektorien. Unter Verwendung dieser Technik wurde ein CpGV-Partikelstrahl, der sich mit einer axialen Geschwindigkeit von $17,4 \pm 0.93$ m/s bewegte, in Querrichtung um einen Faktor 2 von seiner ursprünglichen Breite komprimiert und seine zentrale Peak-Teilchendichte um mehr als das Vierfache gegenüber der Referenz (Laser-Aus) erhöht. In Zukunft kann die optische Führung in Kombination mit der aerodynamischen Fokussierung Einzelpartikel-Bildgebungsexperimente weiter voranbringen, indem das Hauptproblem der präzisen und effizienten Zuführung isolierter einzelner Partikel in den Sub-Mikrometer-Röntgenfokus gelöst wird.
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<td>BB</td>
<td>Bessel beam</td>
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<td>CID</td>
<td>Coherent diffraction imaging</td>
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<td>CoNAI</td>
<td>Convergent nozzle aerosol injector</td>
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<tr>
<td>CyNAI</td>
<td>Cylindrical nozzle aerosol injector</td>
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<tr>
<td>CpGV</td>
<td><em>Cydia pomonella</em> granulosis virus</td>
</tr>
<tr>
<td>CXID</td>
<td>Coherent X-ray diffraction imaging</td>
</tr>
<tr>
<td>CW</td>
<td>Continuous wave</td>
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<tr>
<td>DOF</td>
<td>Depth of focus</td>
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<tr>
<td>FEL</td>
<td>Free-electron laser</td>
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<tr>
<td>FLASH</td>
<td>Free-Electron Laser in Hamburg</td>
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<td>FPS</td>
<td>Fluorescence polystyrene sphere</td>
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<td>FTSD</td>
<td>Fixed target sample delivery</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
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<td>GDVN</td>
<td>Gas dynamics virtual nozzle</td>
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<td>GPIs</td>
<td>Gas phase injectors</td>
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<td>GVL</td>
<td>Gate valve</td>
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<td>HPM</td>
<td>High pressure mode</td>
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<td>HWP</td>
<td>Half-wave plate</td>
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<td>ICS</td>
<td>Improved chamber setup</td>
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<tr>
<td>ID</td>
<td>Inner diameter</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical aperture</td>
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<td>LCLS</td>
<td>Linac Coherent Light Source</td>
</tr>
<tr>
<td>LG</td>
<td>Laguerre-Gaussian</td>
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<tr>
<td>LPM</td>
<td>Low pressure mode</td>
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<tr>
<td>NV</td>
<td>Needle valve</td>
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<tr>
<td>OD</td>
<td>Outer diameter</td>
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<td>OAM</td>
<td>Optical angular momentum</td>
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<td>OTs</td>
<td>Optical tweezers</td>
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<td>OVB</td>
<td>Optical vortex beam</td>
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<tr>
<td>PALM</td>
<td>Photoactivated Localization Microscopy</td>
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<tr>
<td>PBS</td>
<td>Polarizing beamsplitter</td>
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<td>PPF</td>
<td>Photophoretic force</td>
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<td>PCS</td>
<td>Primary chamber setup</td>
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<td>PS</td>
<td>Polystyrene sphere</td>
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<td>PPD</td>
<td>Projected particle density</td>
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<td>QBB</td>
<td>Quasi-Bessel beam</td>
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<td>RAV</td>
<td>Right angle valve</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>SACLA</td>
<td>SPring-8 Angstrom Compact free electron LAser</td>
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<td>SFX</td>
<td>Serial femtosecond crystallography</td>
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<td>SNR</td>
<td>Signal to noise ratio</td>
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<td>SP</td>
<td>Scroll pump</td>
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<td>SPI</td>
<td>Single particle imaging</td>
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<td>SLM</td>
<td>Spatial light modulator</td>
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<td>SPP</td>
<td>Spiral phase plate</td>
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<td>STORM</td>
<td>Stochastic optical reconstruction microscopy</td>
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<td>TEM</td>
<td>Transmission electron microscopy</td>
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<td>XFEL</td>
<td>X-ray Free-electron laser</td>
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1 Motivation

The atomic-scale imaging provided by X-ray diffraction offers a valuable tool to address key questions in the fields of biophysics, biochemistry and structural biology. X-ray crystallography is a powerful technique, which delivers macromolecular structural information of near-atomic resolution. The vast majority of high-resolution protein structures stored in the Protein Data Bank (PDB) today have been solved using this technique. With the onset of X-ray free-electron lasers (XFELs), serial femtosecond X-ray crystallography (SFX) emerged as a new method. It consists of collecting diffraction patterns from a stream of randomly oriented protein micro-crystals and nanocrystals directed across the focus of the XFEL beam. Despite the huge success of crystallography techniques, the need for growing a well-ordered crystal is often the principal bottleneck, as most complex protein molecules are difficult to crystallize.

X-ray diffractive single particle imaging (SPI) is a coherent diffractive imaging method. It uses intense femtosecond pulses from XFELs to construct the 3D structure from diffraction patterns of multiple randomly oriented isolated particles, such as protein macromolecules, viruses, bacteriophages, organelles and prokaryotes. SPI requires a high-precision injection method to deliver bio-particles into the sub-micrometer-sized X-ray focus. Ideally, the particles are isolated, monodisperse, and delivered successively, preferable synchronized with the timing signal of the XFEL pulse. Unlike X-ray crystallography, where the crystal coherently amplifies the scattering into strong Bragg-peak intensities, in transferred SPI the non-crystalline particles produce very weak and continuous diffraction patterns that can easily be overwhelmed by external signals. Therefore, the particles need to be delivered in a background-scattering free environment, such as solvent free from the injection medium.

In the past ten years a number of successful SPI experiments have been demonstrated using soft X-ray free electron-lasers such as FLASH in Hamburg [13] and hard X-ray free-electron lasers, such as LCLS at SLAC [14] and SACLA at SPring-8 [7,9]. In addition, a number of analysis tools to assist with data collection and image reconstruction have been developed [10,11]. Different sample preparation, injection and characterization techniques have been developed [3,12,14]. Despite these progresses, the achieved resolution is still far from the desired atomic length scale – SPI experiments in general are time consuming, and the data analysis is
very difficult. Precise delivery of single particles to a sub-micrometer-sized X-ray focus remains a considerable challenge. Current sample delivery efficiency (fraction of particles that are intercepted by an X-ray pulse) of SPI based on aerodynamic particle injection systems is on the order of $10^{-6}$ on average and the hit fraction (the fraction of X-ray pulses that intercept a particle) reported thus far is well below 1% [13, 14]. Low delivery efficiencies result in significant sample wastage, which renders experiments infeasible for samples that cannot be obtained in high abundance, such as human membrane proteins. The low delivery efficiency and hit fraction is mostly the result of the broad size of the particle beam relative to the X-ray beam and the stochastic nature of the injection process. In order to maximize the particle density in the interaction region, thereby (i) increasing the throughput of SPI experiments, (ii) improving the data quality, and (iii) reducing sample wastage, a method for precise guiding and positioning of particles at the X-ray focal spot is urgently needed in SPI experiments.

The force induced by an optical field is a promising route for precise “touch-free” trapping and manipulation of nanometer particles. The versatility of optical trapping is proven to be very advantageous in the sense that it is easy to produce, shape and control. Most importantly, it doesn’t interfere with the X-ray diffraction or can easily be blocked with optical filters. Furthermore, with simple controlling of the spatial and temporal profile of the laser intensity, an optical field can easily be adapted for a specific particle size, type or motion. Optical tweezers (OTs) are a non-contact method to manipulate particles using a tightly focused laser beam. Since it was first reported by Ashkin [15] forty years ago, OTs have pioneered major leaps forward in the field of life and material science, chemistry, biology and physics by allowing to detect and use the smallest forces in nature which wouldn’t have been measured otherwise.

Most OT experiments are carried out with particles submerged in a liquid medium. In addition to stabilizing the trap by suppressing the thermal motion of the particle, the liquid serves as a heat sink for the particles. However, little has been done on gas phase, in particular on light-absorbing particles. The major challenge with optical trapping of absorbing particle in air is the strong influence of the photophoretic force arising from the heating of the particle caused by the absorbed intensity. This photophoretic force is a light induced thermal force, arising when the surface of an absorbing particle is unevenly illuminated by light. The surrounding gas molecules interacting with the hotter side of the particle recoil with higher velocity than the colder side, resulting in a net force on the particle that pushes it toward the colder side. Unlike photon pressure, the magnitude of the PPF force depends on the thermal conductivity, light absorption and heat accommodation of the particle, the mean-free path of the gas molecules relative to the particle size or Knudsen number, rather than the refractive index. If it is
operated under the right pressure conditions, the PPF is orders of magnitude higher than the radiation pressure. However, as light absorbing particles are strongly pushed away from the maximum intensity of the laser, it renders absorbing particle trapping using the conventional OT’s technique infeasible.

The vital concept for guiding particles in the gas phase employed in this work is the spatial profile of the laser beam. Instead of a simple Gaussian beam, the particles are manipulated using a slowly-diverging laser beams that have a doughnut-shaped transverse intensity profile, with a dark central core surrounded by a bright intensity rings. These beams are constructed with Laguerre-Gaussian and first-order Bessel beams. We call such beam an optical funnel. The experiment consisted of directing stream of aerodynamically focused particles, contained within a low pressure helium environment, into a counter propagating and diverging optical funnel. The transverse and axial forces arising from the radiation pressure and PPF may decelerate, transversely confine and concentrate the particle beam to the intensity minimum of the laser beam. The slowly convergent nature of the optical funnel, in the direction of the particles’ motion, confines the particles into convergent-trajectories, as they traverse through the vacuum.

1.1 Outline of this thesis

In this thesis two kinds of aerosol focusing technique, in the context of coherent X-ray diffractive single particle imaging, were investigated. The first method is based on aerodynamic focusing of aerosol particles and the second technique is based on optical focusing and concentrating of aerodynamically focused particles.

In chapter 2.2, I introduce the principle of imaging single particles using CXDI techniques and the current challenges that hinder it from achieving the desired atomic resolution. In this chapter the technique of aerosol generation and transport in the context of single particle imaging will also be discussed.

In chapter 3, the theory of optical manipulation of micro- and nanometer-sized particles in the gas phase, using radiation and photophoretic forces, are introduced. An overview of the current experimental methods employed for aerosols trapping and guiding is also given. At the end, the techniques for generating structured laser illuminations which have dark-core intensity distributions is presented.

Due to the high velocity of the aerodynamically focused particles, characterizing the particle injection and studying their dynamics requires a robust, high speed and short exposure imaging setup which is capable of capturing and freezing their motion in time. In chapter 4, the techniques that have been developed to visualize aerodynamically focused particles, based on direct optical imaging will be presented. Imaging diagnostics has become an essential tool in SPI experiments, to assist with
the alignment of the dense part of the particle beam with the X-ray focus, as well as live monitoring the particle injection during diffraction experiments \[12\,13\,16\,17\]. In the laboratory optical guiding experiments, optical imaging is the primary tool used to track the motion of the individual high-speed particles and to infer the force field from their trajectories. It is also an essential tool to determine the particle densities and assess the particle beam confinement by the optical funnel.

The success of single particle imaging relies heavily on the development of new and improved injection technologies which could efficiently deliver the particles to the X-ray beam. In chapter \[5\] a new type of aerosol injector which is based on a single convergent nozzle geometry is presented. This injector can deliver an aerosol beam focused down to 3.5 \(\mu\)m for 300 nm sized particles, which is an order of magnitude smaller than any other injectors currently used for SPI. Furthermore, this aerosols beam width at the focus is comparable with most beamlines X-ray focus size ensuring maximum particle-X-ray overlap.

Chapter \[6\] presents low background femtosecond Bragg coherent X-ray diffraction achieved using aerosolized nanocrystals delivered by a convergent nozzle aerosol injector. In serial femtosecond X-ray crystallography (SFX) experiments, the weak diffraction signal from nanocrystals can easily be overwhelmed by huge scattering arising from the solvent when they are delivered with a liquid jet. In a proof-of-concept experiment at LCLS, we demonstrated a new approach for SFX by delivering the crystals as an isolated aerosol suspended in He gas. The lack of solvent medium enabled extremely low background diffraction from Cydia pomonella granulosis virus crystals.

To optically guide the particles in vacuum, two different experimental setups were constructed and implemented in the laboratory. In the first half of chapter \[7\], the detailed description of each setup is presented. The particles were delivered with an aerosol injector to a counter propagating diverging laser beam that has a dark-core in the middle. The particles dynamics were probed through an optical imaging setup (described in chapter \[4\]) constructed around the injector. The profile of the optical funnel is an important aspect of the aerosol guiding experiment as the optical trapping requires maximization of the high speed particles interaction with the lasers. In this chapter, the optical funnel formation and characterization of the setup will also be discussed in detail. In the second half of chapter \[7\], the results obtained employing these experimental techniques are presented. It demonstrates for the first time, the optical focusing and concentrating of high-speed virus and polystyrene particles in vacuum using an optical funnel constructed with Laguerre-Gaussian and Quasi-Bessel beams.
2 Coherent X-ray Diffractive Single Particle Imaging

In the first part of this chapter, an overview of coherent X-ray diffractive imaging in the context of single particle imaging is introduced. In the second part, the common techniques that are currently used to generate and deliver nano- and micrometer-sized particles to the X-ray focus in SPI experiments are presented. At the end, some of the current challenges that hinder the progress of SPI will be discussed.

2.1 Introduction

Microscopy techniques such as confocal, phase–contrast and electron microscopes have become indispensable parts of physical and life science today, and are routinely used to image organic and inorganic specimens as well as study dynamic processes. The ability of such lens-based systems to resolve fine details of a structure can be limited by the quality of the lens they use. Ultimately, there is a theoretical limit to their resolution due to diffraction, referred as \( \text{diffraction-limited resolution} \) (the Abbe diffraction limit). It is proportional to the wavelength \( \lambda \) of the light being observed and inversely proportional to the imaging objective aperture, \( d = \frac{\lambda}{2n \sin \theta} \). Where, \( n \sin \theta \) is the numerical aperture (NA), \( n \) is the refractive index of the medium and \( \theta \) is the half angle of the objective acceptance cone, Fig. 2.1. So, in order to get higher spatial resolution short wavelength illumination is required.

In optical microscopy the resolution is fundamentally limited to the visible light wavelength, for instance if a blue light centered around \( \lambda = 450 \text{ nm} \) and NA = 0.87 objective were used, the resolution will be roughly at 260 nm. This might be small enough to see most prokaryotes and cells but it is too large for most biological samples such as viruses or protein macromolecules. A few super-resolution optical microscopy techniques have been developed in recent years that bypass this diffraction limited barrier and achieve better resolution, up to 20 nm in some special cases, such as Photoactivated Localization Microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) [18–20].
Chapter 2. Coherent X-ray Diffractive Single Particle Imaging

Owing to the short de Broglie wavelength of electrons, transmission electron microscopy (TEM) is capable of imaging at atomic resolution. However, the electron beam interacts strongly with the sample. So, in order to minimize this, the specimen has to be sliced to a thickness less than 50 nm \cite{21}, this makes sample preparation difficult in TEM.

Lens-based X-ray microscopes, also known as real-space microscopes, fill the resolution gap left between the electron and optical microscopes. X-rays penetrate most objects with little damage to the sample. So, it is a method of choice to image internal structures of thick specimens. However, this very property of X-ray also makes it hard to focus using lenses compared with electrons or visible light. X-ray microscopes typically use Fresnel Zone plates as objective lenses to form a real image. A zone plate is a diffractive optical element that has circular gratings with decreasing periodicity towards its outer edge. Unlike an optical lens which focuses by refraction of the beam, zone plates use diffraction to focus the X-ray beam. Such microscopes have been used for scanning probe or full-field X-ray microscopy for direct imaging or X-ray fluorescence. However, zone plates have very short focal lengths and are also hard to manufacture with high resolution. Furthermore, they waste a portion of the X-ray beam. To date 10 nm is the best recorded image resolution obtained using zone plates \cite{22}. On the other hand, in a recent work, by scanning a sample with 16.3 keV X-ray beam focused to $8.4 \times 6.8$ nm$^2$ spot size using a pair of multilayer Laue lenses, a resolution better than 10 nm has been demonstrated \cite{23}.

In general, in lens-based imaging the quality of the lenses or misalignment in the optics causes image aberrations which limits the achievable diffraction-limited resolution. Alternatively, Coherent diffractive Imaging (CDI) is a lensless imaging technique that uses a spatially and temporally coherent monochromatic beam of X-rays, electrons or other waves to record the diffraction patterns of various crystalline or noncrystalline objects, then use algorithms to reconstruct their 2D or 3D structures from the diffraction patterns. Unlike lens-based microscopy techniques the objective lenses or zone plates that used to form the real image is removed and the diffraction pattern from the object is directly collected by a 2D detector as shown in Fig. 2.1 (b). This diffraction pattern is directly phased using software algorithm to get structural information – coherent X-ray diffractive imaging (CXDI) is a particular case of CDI that uses coherent X-ray illumination \cite{24, 25}.

CXDI is an aberration free imaging technique and the resolution is only limited by the wavelength, the extent and quality of the detector, background noise and intensity of the source. However, the absence of the lens comes with a cost that the detector now records only the amplitude of the diffraction pattern. The direct-complex phase information is lost, so reconstruction by simple Fourier transform of
2.1. Introduction

Figure 2.1: Basic configuration of lens-based and lensless imaging. (a) Conventional lens-based microscopy setup. It is a direct real space imaging technique but the resolution is limited by the depth of field and the lens aperture. (b) Typical CDI imaging setup, the resolution is limited by software and the extent of the detector. The beam stop (BS) blocks the direct X-ray beam (DB) behind the detector.

the diffraction pattern is not possible. It is commonly known as the phase problem. Non-crystalline objects produce a continuous and over-sampled diffraction patterns [26][27]. That means, the phase information is not lost rather it is uniquely encoded inside the diffraction patterns and it can be retrieved by sampling the intensity pattern at least the Nyquist sampling rate. The phases, hence the ‘real image, can be reconstructed using the Fourier-based Gerchberg and Saxton iterative algorithm [28] using only the Fourier amplitude. That means the reconstruction algorithm effectively replaces the lens to form the real image from the diffraction patterns.

CDI was first suggested by Sayre in 1980 [27]. However, weak diffraction patterns from non-crystalline samples and the aforementioned phase problem made it hard to prove the idea experimentally. In 1999 the first successful 2D CXDI experiment was carried out by Miao et al. (0.73 keV X-ray energy, resolution of 75 nm) using a non-crystalline sample consisting of an array of gold dots [29]. The first biological targets imaged using the new technique were manganese-stained Escherichia coli bacteria to a resolution of 30 nm, opening the path for imaging low signal biological objects [30]. Soon after, the CDI methods were extended to full 3D structure determination [31][32]. Many of these pioneering experiments were performed on
fixed target samples which were exposed to the X-ray beam multiple times. So, the resolution was limited by X-ray induced radiation damage to the sample. The best experimentally achieved resolution using CXDI was limited to $\sim 2$ nm on inorganic and $\sim 10–20$ nm on organic samples. In a sample deliver by a fixed target delivery technique, cooling the sample during exposure can help to minimize and slow this damage – generally to mitigate this inherent problem, ultrashort and/or intense X-ray pulses are needed [33]. Today, using powerful X-ray sources such as synchrotrons and X-ray free-electron lasers, CDI has been applied to a broad range of samples in material and life science [1–9,34,35].

2.2 Single particle imaging with X-ray free electron lasers

The development of XFELs, which are capable of delivering very short and intense coherent X-ray beams, opened up a unique opportunity to image weakly scattering non-periodic isolated particles. The pulses delivered by XFELs are short enough to mitigate the radiation damage by diffraction before destruction [36] and intense enough to give detectable diffraction signals from weakly scattering objects. The intense X-ray pulse vaporize each particle through photoionization-induced damage processes [25], therefore, at most only one diffraction pattern can be recorded from a single particle. This requires us to maintain a continuous flow of isolated particles across the X-ray beam [3], similar to the serial femtosecond crystallography (SFX) techniques [37]. In order to acquire the full data set required to determine the 3D structure of the particle, thousands of diffraction patterns must be recorded.

Each of the 2D diffraction pattern represents an Ewald-sphere slice through the 3D Fourier transform of the electron density [6]. Each particle is intercepted by the X-ray at a random orientation, therefore the diffraction patterns must then be sorted according to their relative orientations into a three-dimensional diffraction space. These is done using a software algorithms–EMC (Expansion-maximization-compression) [6][10] being the most popular one. After the orientation determination, the 3D electron density map of the particle is reconstructed using an iterative phase retrieval algorithm from the scattered intensity – the hybrid input output (HIO) algorithm is among the common one [5][10].

Despite its tremendous progresses in recent years, the achieved resolution in SPI is far from the desired atomic-length scale. The main challenges are discussed at the end of this chapter, among others isolated particle generation and deliver to the sub-micrometer X-ray focus are considered by many the principal bottle-necks.
2.2. Single particle imaging with X-ray free electron lasers

2.2.1 Efficiencies of single-particle imaging

There are two key parameters commonly used to characterize the efficiency of SPI experiments. The first parameter is known as the “hit fraction” or “hit rate”. It describes the photon usage efficiency and is equal to the fraction of X-ray pulses that intercept a particle. For femtosecond XFEL illumination, this quantity depends on the instantaneous projection of the particle number density along the X-ray beam path, and can be approximated as 

\[ H \approx \frac{f T \sigma}{(v w)} \]

where \( f \) is the rate at which particles enter the injector, \( T \) the injector transmission efficiency (ratio of the number of particles that enter and exit the injector), \( \sigma \) the effective illumination area that produces useful diffraction, \( v \) the velocity of the particles, and \( w \) the full width at half maximum (FWHM) of the particle beam. In this formulation, we assume that the particle beam has a diameter \( (w) \) smaller than the depth of focus of the X-ray beam, which is almost always satisfied in SPI experiments. We also assume that the X-ray beam diameter is significantly smaller than the particle beam diameter. We assume that \( H < 1 \), since X-ray diffraction patterns containing multiple particles illuminated simultaneously tend to complicate the diffraction analysis.

Another key parameter is known as the “delivery efficiency” – characterizes the particle consumption of the experiment, it is defined as the fraction of particles injected that are intercepted by the X-ray beam. Delivery efficiency for continuous flow of particles can be approximated as 

\[ \varepsilon \approx \frac{H F}{f} \]

where \( F \) is the XFEL pulse repetition rate, and it is assumed that \( F \ll f \). By making the concentration very high, an injector could achieve 100 % hit-rate with extremely high sample consumption \( (\varepsilon \ll 1) \). This might not be a problem if we had abundant sample, after all the photons are much more expensive than the particles. However, many samples are hard to produce in large quantities, so they also have to be delivered efficiently. The delivery efficiency of current injectors is in the order of \( 10^{-6} \) and the best recorded hit-fraction to date is 0.83 % by Daurer et al. \[14\], using 40 nm virus particles and a 150 nm X-ray focus.

In order to score a higher hit fraction and delivery efficiency, one typically needs to find an optimal compromise between the three parameters \( v, w, \) and \( T \). Importantly, for a given injector geometry, it might not be possible to vary these parameters independently of each other \[38\]. In the following sections, I will introduce some of the common aerosol sample generation and delivery techniques, and their efficiency at SPI experiments.

---


I performed the measurements. Together with R.A. Kirian, I analyzed the experimental data. With discussion and input from of other authors, I wrote the published manuscript.
2.3 Aerosol generation techniques for SPI

Most samples used in SPI experiments are non-crystalline and weakly scattering. Therefore, the diffraction patterns collected are usually very faint, in most of the cases there will only be few photons on the detector. So, it is crucial to minimize the background, for instance, by delivering the samples in the gas phase.

The first step of gas phase sample delivery is nebulization of the particles that are suspended in a solution. Nebulization or atomization refers to the process of putting liquid into gas phase. Diffraction from aggregated particles usually discarded during data analysis. So, an ideal nebulizer should deliver a high number of droplets with a controlled and narrow size distribution in a consistent manner. Furthermore, to avoid clustering of particles each droplet generated should be small enough to statistically carry only a single particle. In the following sections the common aerosol generation techniques that are used in SPI will be discussed.

2.3.1 Gas based liquid micro-drop nebulizer

Gas based nebulizers use the energy of an accelerated gas to produce the aerosol. For instance, parallel gas-sample flow neutralizers such as such as Mira-Mist (Burgener Research, Mississauga, Ontario, Canada) and concentric sample-gas flow such as Gas dynamics virtual nozzle (GDVN). In the parallel-path nebulizers, the gas and the sample flows in parallel with two different capillaries which are combined at the exit of the nozzle, cross-sectional view of such nozzle are see Fig. 2.2 (a). The aerosols are produced when the liquid surface gets in contact with the high pressure gas at the exit of the nozzle. This causes the liquid at the surface to break up into fine drops by induction and surface tension. The generated mists are carried
away by the gas stream to the relaxation chamber where most of the liquid layer will evaporate. The commercially available Mira-Mist is among the nebulizers that wok with this principle. In such a nebulizer design, the precise alignment of the gas and the liquid stream is not critical, which allows the use of bigger capillaries for the sample line. This reduces the construction complexity as well as clogging problem of the sample line and increases the life span of the nebulizer. The size of the initial droplet formed by such nebulizers varies depending on the nebulization gas type and pressure, i.e., high kinetic energy gas produces smaller drops.

In parallel-path nebulizers liquid interacts with the gas at the edge of the gas stream, which is 3 to 10 times slower than the velocity at the middle of the gas. Since the energy of the gas is related to the square of its velocity, the average drop size is relatively bigger ($5–15 \mu m$) \[39\]. Burgener updated their design by protruding the exit of the sample line to the middle of gas stream to enable the liquid to interact with the high velocity part of the gas \[39\]. The commercially available Mira-mist was used in various early SPI experiments as aerosol source \[40, 41\]. However, due to its high sample consumption and the relatively big droplet size it is not often used in recent years but still heavily used in other research areas such as chromatography and capillary electrophoresis (CE), as well as in industries.

Figure 2.3: Aerosol generation using the GDVN nozzle. (a) Axial cross-section view of the a typical melted-glass GDVN (b) Initial liquid-aerosol formed by the GDVN, which is usually injected into a small relaxation chamber just before the inlet of aerosol injector. (c) The particle-aerosol formation after the liquid buffer evaporation. Particles which are in the same drop in the initial stage most likely will form aggregates \[42\].

Seen in Fig. 2.2 (b) is the cross-section of the concentric-path geometry GDVN nozzle, the gas flows over the sample line concentrically, joined just before they exit the orifice. Here, the alignment of the liquid and the gas capillary is very crucial to maintain stable and repeatable drop generation. Low flow rate GDVNs \[43\] are extensively used in SPI experiments to produce fine liquid aerosol drops. To
generate aerosols using GDVN, first the sample to be aerosolized is suspended in buffer solution, then it is pressurized through the liquid supply line of the GDVN. At the exit, a coaxially flowing sheath gas, typically He, compresses and accelerates the liquid, acting as a virtual nozzle to create a sub-micron jet, see Fig. 2.3 (a). The jet extends to a few 100 µm in length from the exit of nozzle before it breaks up into very fine liquid drops due to an effect called Rayleigh instability \[44\]. This aerosol mist is then carried away by the gas stream to the injector, see Fig. 2.3.

The generated drop diameter \(d_d\) relates to the jet diameter \(d_j\) by \(d_d/d_j \approx 1.9\), classical Rayleigh break-up. Normally, the jet diameter is roughly equal to the inner diameter of the exit of nozzle, however in GDVN this inner wall is replaced by a smooth potential (‘virtual nozzle’) which is created by the co-flowing pressurized gas at the exit of the nozzle. This draws the liquid into a narrow cone which is considerably thinner than the exit diameter of the nozzle.

To understand the mechanism of thin-jet generation, first it is important to formulate the diameter of the jet interns of its parameters. For a GDVN operated in steady jetting regime – assuming all the energy of the gas per unit time \(\Delta P Q\) at the orifice is converted into kinetic energy \(Q \rho U^2\) of the liquid, the jet diameter of a GDVN with liquid flow rate of \(Q\) and density \(\rho\) can be expressed as \[45,46\]:

\[
d_j = 2\sqrt{\frac{Q}{\pi^2 \Delta P}} \quad (2.1a)
\]

\[
U = \frac{16Q}{\pi d_j} \quad (2.1b)
\]

Where \(U\) characteristic jet velocity and \(\Delta P\) is the pressure drop at the orifice. According to Eq. 2.1a in order to produce a smaller drop, i.e., thinner jet, the sample flow rate \(Q\) has to be reduced and the pressure gradient \(\Delta P\) has to be increased. Alternatively, at a constant liquid flow rate, the jet velocity \(\Delta P\) has to be increased to reduce the jet diameter, Eq. 2.1b. Taking this into consideration, typical SPI nebulization GDVNs, also known as low-flow rate nozzles \[47\], are constructed with a very smaller inner diameter liquid supply line \((\text{ID} < 30 \mu m)\), and narrow exit aperture nozzles. These reduce the liquid flow rate and increase the pressure gradient at the exit of the nozzle. The typical sample flow rates of SPI experiments are between 0.5–1.5 µL/min.

The reduction in the capillary ID comes with a price that such GDVNs are prone to clogging and their life span is very limited. Compared with parallel-gas atomizers such as Mira-Mist CE model nebulizer, GDVN produce very small drops. However, Mira-Mist uses a 500 µm ID liquid supply line which is, at least, 20 times bigger than the typical low flow rate GDVNs nozzle capillary ID. Therefore, it could work for a much longer period of time than GDVNs. In general, this is the...
biggest advantage of parallel-gas nebulizers that they don’t have to use smaller capillary to produce small drops.

In GDVNs, as the flow rate decreases, the jet length relative to its diameter decreases until the dripping regime is reached [45,46,48]. In the dripping mode the drops start to form shortly after the jet breakup which is right at the exit of the nozzle, causing bigger and heterogeneous drop formation [45,48]. A nozzle could change its mode from jetting to dripping after few hours of operation, either due to salt accumulation on the tip or other debris from the sample itself. As a result of this, GDVNs usually have to be monitored continuously, and the gas and sample flow need to be adjusted frequently to achieve steady aerosol generation.

The biggest advantage of gas-based liquid micro-drop nebulizers is that they can be used almost in all kind of samples and buffer conditions. However, the size of the drops they generate is still fairly big compared with most SPI samples. This results in clustering of particles as well as salt deposition on the surface of the particles after the huge buffer layer is evaporated.

2.3.2 Aerosol particle generation rate by GDVNs

In Fig. 2.3 is illustrated the evaporation process of an aerosol droplet generated by a GDVN. Drops which contain more than one particle in the initial stage of nebulization will most likely stay together and form an aggregate, after the buffer evaporates [14,42]. For a given sample flow rate \( Q_L \), the droplet generation rate of the GDVN \( (D_R) \) is the limiting factor determining the sample concentration that should be used to produce aerosol particles with a minimum amount of aggregates. Assuming monodisperse drops nebulization, the drop generation rate and the corresponding particle flow rate \( (Q_p) \) produced by a GDVN can be given by:

\[
D_R = \frac{Q_L}{V_d}, \quad \text{(drops/second)} \tag{2.2a}
\]

\[
Q_p = Q_L \times C_s, \quad \text{(particles/second)} \tag{2.2b}
\]

Where \( V_d \) is the volume of the drop and \( C_s \) is the sample concentration. The particle flow rate describes the number of particles flowing through the nozzle in a given time. This is different from aerosol particle generation or aerosol flow rate \( (P_R) \), which describes the number of single or clusters of particles generated in a given time after the buffer evaporation in Fig. 2.3(a). Increasing the concentration of the sample increases the particle flow rate, hence the aerosol particle generation rate. This is true only if \( Q_p < D_R \). However, further increasing the concentration increases the probability of containing multiple particles per drop.
For a sample uniformly dispersed in the buffer solution of concentration $C_s$ and nebulized to monodisperse drops, with each volume $V_d$, the average number of particles per drop, $\Gamma = C_s V_d$, and the number of particles occurrence ($k$) in a single drop, follows a Poisson distribution [5]:

\[
p(k) = \frac{(C_s V_d) \exp(-C_s V_d)^{-k}}{k!}
\]

In order to increase the chance of having a single particle in a drop ($k=1$), the volume of the drop, the jet diameter has to be reduced and the sample concentration should be chosen so that $Q_p \ll D_R$. Taking the occurrence of multiple particles in a single drop into consideration, the particle generation rate $P_R$ can be described by $P_R = \min(Q_p, D_R)$, $[P_R] = \text{aerosol particles/second}$.

### 2.3.3 Electrospray Ionization

Electrospray ionization (ESI) is the other class of nebulization techniques that are commonly used in SPI experiments to form aerosols. This technique was known for more than a hundred years, but it was Dole who first applied it as a method to introduce ions into the gas phase [49]. In electrospray, liquid containing the sample flows through a capillary which has a higher potential difference ($\Delta V$) with respect to a grounded counter electrode, see Fig. 2.4 [50]. To facilitate the evaporation and ionization process, conductive buffers, such as ammonium acetate, are added to the solution. Typically CO$_2$ flows over the capillary coaxially to avoid a possible corona discharge and additional air is supplied to transport the generated aerosols. Due to surface tension of the liquid, the meniscus at the tip of a capillary forms a semi-spherical shape when no external field is applied. The application of the voltage polarizes the liquid which draws the charges away from the capillary to form a new equilibrium meniscus with reduced curvature. Increasing the voltage above this threshold draws the liquid outward to form what is known as Taylor cone. Charged liquid drops are emitted from the Taylor cone with a surface charge of the same polarity with the capillary. As the droplets are carried away by the gas the solvent evaporates progressively, as a result the drop shrinks leaving more and more charges on the surface. The drop shrinks until the surface tension can’t sustain the charges, which is the Rayleigh limit, then the drop explosively dissociates to form smaller drops which also undergo a similar process to produce even smaller drops, see Fig. 2.4. These drops are carried by the gas to the neutralization chamber which use either radioactive sources such as $^{210}$Po, $^{85}$Kr or soft soft-X-ray radiation to bring the highly charged droplets to charge equilibrium [51].

The big advantage of ESI is its ability to produce a large number of extremely small monodisperse droplets, size between 40 nm – few $\mu$m in diameter, with liquid
flow rates ranging from few hundred nl/min to few µL/min. The downside of ESI is that it needs conductive polar solvents such as, SCN or THF, which could be problematic for some biological samples. Charging of the particle could also have some negative effects on some macromolecules such as proteins. Unlike GDVN, which uses Helium as a sheath gas, ESI uses CO$_2$ and excess amount N$_2$ gas or air which have a higher scattering cross section at the typical wavelength used for SPI than He. This elevates the background signal on the collected diffraction patterns of the particles. There are a number of successful SPI experiments that used the electrospray sources to produce the aerosols [3,14].

### 2.4 Sample delivery techniques for SPI

Due to the limited availability of beamtimes, it is important that the particles are delivered efficiently to the X-ray beam so that maximum number of diffraction pattern is collected with minimum sample consumption. From the standpoint of particle injection, to be able to maximize the efficiency of SPI, the following important criteria should be fulfilled by the injection system that would potentially be used for SPI:

1. The injection system should be able to deliver the particles within a minimum background scattering medium, such as solvent or gas.
2. Should be able to deliver isolated particles, i.e., one particle into a single X-ray pulse.
3. Should maintain high hit-fraction and low sample consumption or high delivery efficiency.
4. Since every exposed particle is vaporized by photon induced ionization of the
X-ray beam, a continuous flow of fresh particles should be maintained by the injector. If possible, synchronized with the X-ray pulse.

5. Easy to integrate with existing X-ray diffraction apparatus.

There is no a single particle delivery system that could fulfill all the above requirements, but a number of systems have been developed which could be optimized for the particular application. Liquid jet-injectors, gas phase injectors such as aerodynamic lens stack injectors and converging nozzle aerosol injectors, and fixed target delivery are the most widely used systems. In the following section I will discuss each of these techniques.

### 2.4.1 Liquid jet injectors

It is the development of liquid jet injector techniques that made SFX at XFELs possible, without their high throughput sample delivery all the current development wouldn’t have been possible. Liquid jet injectors have been developed in various forms, including, Gas Dynamic Virtual nozzle (GDVN) [43] and Double flow focusing [52]. At their current state, most liquid-jet injectors are not the method of choice for delivering typical SPI samples. This is because the huge background scattering arises from the solvent medium that constitute the jet overwhelms the weak diffraction signal from the small particles. However, due to their high throughput, low flow rate GDVNs can be employed for delivering larger particles, sized in the rage of the jet diameter, such as virus or single fibre-like particles [53].

The schematics of direct sample delivery using the GDVN is shown in Fig. 2.5.

![Figure 2.5: Sketch of the GDVN nozzle configuration.](image)

In GDVNs a sample suspended in a buffer solution pushed through a small diameter capillary (ID between 15 and 75 µm). At the end of the capillary a coaxially, flowing sheath gas (usually He) acts as a virtual nozzle to compress
and accelerate the liquid to create a sub-micron jet that extends few hundred micrometers from the GDVN orifice, seen in Fig. 2.5. To minimize its Free surface energy, the jet then breaks up into micro-droplets at the end of the jet [43][54].

In direct delivering of samples using the liquid jets, the background from the solvent scattering is proportional to the jet thickness. Therefore, it gives the need to make the thinnest possible jets [55]. The most interesting application of GDVN is fibre-like single particles, such as long strand DNA, amyloid fibrils. Due to the differential flow profile at the convergent parts of the GDVN, the extended filament of the fiber flow-aligns to the jet. In addition to the easy alignment of the sample to the X-ray, this give the ability to access high resolution fiber diffraction information.

2.4.2 Gas phase sample delivery techniques

Compared with other delivery techniques, gas phase injectors (GPIs) are challenging to work with, yet they are the most widely used technique at SPI experiments. Typically GPIs are known to have very low hit-fraction as well a high sample consumption. However, due to the absence or reduction of the liquid solvent that supports the particles (for instance in liquid-jet), diffraction patterns with very low background can be recorded using GPIs. Aerodynamic-lens-stack aerosol particle injectors (ALS) [3] and the recently introduced converging nozzle aerosol injectors (CNAI) [12] are commonly used systems in SPI.

Lens stack aerosol injector

As depicted in Fig. 2.6, an ALS injector consists of a series of concentric apertures mounted in a cylindrical tube of few 10s of millimeters in size. The exit of the injector is kept in high vacuum and a mist of aerosolized particles suspended in carrier gas is introduced upstream of the injector, which is at relatively higher pressure (a few 100 mbar). At the orifices, the contraction of the gas stream (the blue line in Fig. 2.6) confines the particles to the axis of the tube, and due to their high inertia the particles retain the narrow stream while the gas expands; this effect is known as an aerodynamic lens [56][57]. As the particles pass through the series of apertures, each contraction and relaxation drives more and more particles to the axis of the injector to produce a tightly collimated aerosol beam into the vacuum chamber. The size of each orifice is often chosen so that they focus only a specific size range, allowing a single ALS injector to focus a wide range of particle sizes. In principle, it could be possible to design an ALS injector optimized only for a specific size.
Figure 2.6: Aerodynamic focusing of particles to the X-ray focus. A mist of particles suspended in a gas, usually He, is introduced upstream of the injector at higher pressure. Each time the particles pass through the series of apertures, they are confined to a smaller spot along the axis. This produces a collimated aerosol beam downstream of the injector. The tick blue line indicates the gas stream and direction through the lenses. 

Depending on the size of the particles and operation conditions, an ALS injector could deliver a collimated aerosol beam of width as small as 20 µm. This beam usually has a Gaussian-like transverse particle density profile; the particle density defined as number of particles per unit area and per unit time. So, to maximize the hit fraction during SPI experiments, the dense part of the particle beam has to be precisely aligned with the X-ray focus. This is usually done by two-dimensional scanning the injector across the X-ray beam, while live-monitoring the number of diffraction patterns at each positions. ALS injectors combined with GDVNs or electrospray aerosol sources, have been extensively used in recent SPI experiments.

Convergent nozzle aerosol injectors

The CNAI is a recently introduced concept for sample delivery at SPI experiments. CNAIs use a single convergent-orifice nozzle, with an exit diameter between 100–500 µm, to focus the aerosols into the vacuum. Unlike ALS injectors, the particle beam produced by CNAI is fast, highly convergent and focused to a few micrometers spot at few 100 µm downstream of the injector. Therefore, during diffraction measurements, to maximize the hit fraction, three-dimensional scan of the injector across the X-ray is required. This is the major downside of such injectors. A detailed description of CNAI is presented in chapter 5.
2.4.3 Fixed target sample delivery

The earlier SPI experiments were performed on fixed target samples, which were exposed by the beam once or multiple times [1]. Fixed target sample delivery (FTSD) is still used in synchrotron-based X-ray crystallography and CDI experiments. Due to the relatively lower photon flux at synchrotrons, multiple exposures of a single crystal are possible, without inducing radiation damage to the sample. Similar experiments at XFELs, however, require modification. Since each X-ray shot destroys the sample, fresh samples had to be delivered uninterruptedly after each and every X-ray pulse. This is usually done by continuously scanning a sample-holder that has a pre-deposited sample across the X-ray beam using a linear motor. Due to their low background scattering, ultra-thin silicon nitride or graphene membranes are commonly used as a substrate to deposit the particles. So, the term fixed target is misleading in the context of XFEL, since the sample is not fixed at the position of the beam, instead it scanned across continuously fixed within a support structure.

Figure 2.7: Schematics of an SPI experiment based on fixed target sample delivery. The sample is deposited on an ultra-clean substrate. During the diffraction measurement, the holder with the substrate is scanned perpendicular to the X-ray beam using a motor.

Fixed-target protein serial microcrystallography has already become routine at XFELs, it has been successfully demonstrated in a variety of experiments on protein microcrystals [59][60]. However, fixed target sample delivery has been re-introduced very recently for SPI at XFELs. There are a number of promising developments in this direction, such as, on strongly scattering inorganic samples [9][61] and some on noncrystalline biological particles [7][8]. However, at the moment sample
preparation is very difficult, i.e., a method for evenly and isolatedly depositing the particles on the sample holding substrate has not yet been established. Salt deposition on the substrates caused drying of solvents, if salt buffer is used. X-ray scattering from salt crystals is much stronger and can easily mask the diffraction from the actual particles. Furthermore, during diffraction measurements, each sample holder is scanned through in a few minutes, so frequent replacement of a new holder is required, and this costs even more time if the experiment is performed under vacuum. Therefore, for FTSD to be a standard technique, these aspects of the experiment have to be improved. However, if it is successful, fixed target will guarantee very high hit-fraction as well as very low sample consumption. In the following table, the advantage of each injection techniques are summarized.
### Table 2.1: Summary of different particle delivery systems which are currently used at SPI experiments.

<table>
<thead>
<tr>
<th>Delivery type</th>
<th>Hit-fraction</th>
<th>Expected background level</th>
<th>Delivery efficiency</th>
<th>Isolated particles delivery</th>
<th>Sample preparation</th>
<th>Reliability of the operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid jet</td>
<td>moderate</td>
<td>high</td>
<td>extremely low (&lt; 10^{-6} %)</td>
<td>For relatively larger particles, it could deliver isolated particles, if they are delivered with very low concentration and sample flow rate.</td>
<td>Relatively easy, if preparing the samples in sufficiently large quantity is not a problem.</td>
<td>Frequent replacement is required due to clogging.</td>
</tr>
<tr>
<td>GDVN and DFFN</td>
<td>(1–5 %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas phase</td>
<td>very low</td>
<td>extremely low</td>
<td>extremely low (&lt; 10^{-6} %)</td>
<td>It depends on the capacity of the aerosol generator that feeds the injector to produce isolated particles.</td>
<td>Relatively easy, if preparing the samples in sufficiently large quantity is not a problem.</td>
<td>Usually runs continuously, unless the aerosol source is clogged.</td>
</tr>
<tr>
<td>ALS and CNAI</td>
<td>(&lt;1 %)</td>
<td>low</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed target</td>
<td>high</td>
<td>moderate</td>
<td>Currently, very low</td>
<td>It is extremely challenging to deposit the few 10s of nanometer-sized particles isolated from each other on the sample holding substrate.</td>
<td>challenging</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&gt; 50) %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.5 Current challenges in SPI

To image a biomolecule of size around 15 nm in diameter using $10^{13}$ photons in 4 fs pulses and at 4 keV photon energy (comparable with the output of current X-ray sources), an average of $\sim 0.1$ photons per Shannon pixel per shot are required \cite{62}. With that, the 3D structural information can be recovered using only 30,000 diffraction patterns \cite{62}. However, increasing in the background level or the presence of non-uniform background significantly increase the number of patterns required for orientation determination, if it doesn’t make it impossible.

Despite the number of progresses made over the past few years, SPI is still far behind in exploiting the full potential of the short and intense X-ray beams offered by free-electron lasers. The following are some of the main scientific and technical challenges that are currently posed in SPI experiments that hinder the ability to achieve atomic resolution structure determination.

**Scattering signal level:** The diffraction patterns collected from single particles are very weak. Furthermore, the scattering signal goes down with the size of the particles, and for most single particles, the scattering intensity to higher $q$ reduces with the forth power, $I(q) \propto q^{-4}$, where $q$ is momentum transfer. This increases the challenge of imaging small particles at higher resolution.

**Background noise:** It is one of the biggest technical problems that pose a major challenge in SPI data collection. The typically low number of scattered photons from the weakly scattering bio-particles can easily be overwhelmed by any background noise introduced during the experiment. Noise could refer to all kind of unwanted signals arising from the beamline optics, and the background signal from sample injection media, for instance, solvents, gases and detector noises. These impose difficulties for the 3D-structure and orientation determination algorithms by increasing the number of patterns needed.

**Sample heterogeneity:** In the ideal case, the diffraction patterns would be collected from multiple projections of identical particles that are kept in the same physiological conditions. However, in practice there is significant particles size variation between each and every one of them, and conformational changes could occur between the various particles. This makes the reconstruction process harder by increasing the number of patterns needed. The patterns have to be pre-sorted according to their size and possible conformation before determining their orientations. Furthermore, due to the self-amplified spontaneous emission process, free-electron lasers have significant shot-to-shot intensity fluctuations, which affect the heterogeneity of the data further in addition to sample.

**Orientation:** There is a steady improvement in the software algorithms which are currently being used for orientation as well as 3D-structure determination \cite{10}. However, their performance and reliability is limited by the quality of the data.
2.5. Current challenges in SPI

that can be offered at the moment.

**Detector:** In addition to their quality, the X-ray detectors should be able to cope with the newly developing high repetition rate FELs such as Eu-XFEL.

**Sample delivery:** Sample delivery remains the principal bottleneck in single particle imaging. Most of the challenges that have been discussed above can be traced back to the performance of the sample delivery system. The current SPI injectors have a very low hit-rate and injection media introduce significant background signal. For instance, if the injection system could guarantee high hit-rate with low background, by simply averaging multiple projections which belong to the same size and orientation, the signal-to-noise ratio could be improved significantly. The same is true for the sample heterogeneity, if there is a huge data set, then the patterns can be grouped according to the size and possible conformational states of the particles. Most importantly, the quality of the diffraction patterns determines the number of pattern needed for 3D structure determination. The better the data quality, the fewer the number of projections needed.
3 Fundamental concepts: Optical manipulation of particles in the gas phase

In the first part of this chapter the concept of radiation pressure and the light induced photophoretic force are introduced. In the second part, an overview of the various aerosol trapping and guiding techniques are presented. At the end, formation of structured optical beams that are commonly used for aerosol particle guiding will be discussed. First, I start by giving a brief introduction on the historical background of optical trapping and manipulation.

3.1 Historical background on optical trapping and manipulation

The effect of radiation pressure was first observed thousands of years ago. Chinese astronomers where the first to discover that Halley’s Comet points its tail away from the sun at all times, they knew that as early as 684 A.D. [63]. It was the famous astronomer Johannes Kepler who first tried to explain the phenomenon by attributing it to some form of solar pressure [64,65].

The idea that light, as an electromagnetic radiation, could have momentum and exert pressure on a surface was first comprehensively theorized and published by Maxwell in 1873 [66]. This was later proven experimentally by Ernest Fox Nichols and Gordon Ferrie Hull in 1901, measuring the deflection of an illuminated delicately poised vane of reflective metal hanging in an evacuated Nichols radiometer chamber [67,68]. In the 1910s, Felix Ehrenhaft discovered the Photophoretic force induced by a non-uniform temperature distribution on the surface of illuminated particles in a gaseous environment [69]. The momentum carried by photons is much smaller, therefore the effect was harder to detect or measure practically in those days. So, not much progress was made in studying radiation pressure until the invention of the lasers in 1960 [70], which produces intense and coherent light
beams. The high brilliance of the laser beams elevated the effect of radiation pressure to the extent that it could easily be detected and measured. This led to ground breaking experiments in the manipulation of microscopic objects by exerting a very controlled yet calibrated force.

In 1970, Ashkin showed in his pioneering experiment the acceleration of micrometer sized neutral particles along the direction of a slightly focused laser beam [15]. In the same work, he demonstrated a stable three dimensional trapping of transparent neutral particles using two slightly focused counter propagating laser beams. Shortly afterwards, he simplified the counter-propagation beam trapping geometry with the introduction of the single beam gradient trap, commonly known as optical tweezers [71]. Since then, single beam trapping has become the most widely used and well established technique for the trapping of atoms, micro-particles, bacteria, viruses and cells [72–75].

### 3.2 Optical forces

When Light interacts with matter, depending on the optical property of the material and the wavelength of the light employed, it could either be absorbed, reflected, refracted, transmitted or scattered while transferring its momentum in the process. These are the very properties of light that govern the optical trapping mechanism. For instance, transparent dielectric micro-particles can be polarized by the light field and manipulated by a radiation pressure arising from direct moment transfer from the photons, and a gradient force acting on the polarized particles [76]. On the other hand, light-absorbing particles in the gas phase easily heat up by the absorbed laser intensity and this results in a stronger light induced thermal force called the Photophoretic force (PPF). Particle manipulation using the PPF exploits a non-uniform heat induced on the particle to indirectly transfer the momentum of the light to the particle by its interactions with surrounding gas molecules. The gas molecules interacting with the hotter side of the particle will recoil with greater velocity, resulting in a net force that pushes the particle toward the colder side of the particle. So, in absorbing particles, the PPF dominates over the radiation pressure and if it operated at the right pressure conditions, the PPF could be up to five order of magnitude stronger than the radiation pressure for the same laser power.

In this work, semi-absorbing biological and polystyrene particles suspended in a helium gas environment were investigated. So, ideally the particles experience both radiation pressure and PPF when they interact with the laser. Note, throughout the thesis the term ‘optical force’ will be used to refer to the combination of radiation pressure and PPF, in the following sections each of these force will be detailed.
3.2. Optical forces

3.2.1 Radiation pressure

Radiation pressure (or radiation force), is associated with the transfer of the linear momentum of an electromagnetic radiation into the mechanical momentum of an object. Momentum of the light beam is transferred to an object, when either the light is absorbed or changes its direction, as it passes through or reflects off the medium. Understanding and modeling the optical force and radiation pressure relies upon the knowledge of the momentum of the light and its interaction with matter. Photons possess the properties of energy ($E$) and momentum ($p$), in free-space they are related by $E = pc$, where $c$ is the speed of light in vacuum. It is derived from the total energy of a relativistic particle, $E^2 = p^2c^2 + m^2c^4$, with photons having zero rest mass ($m = 0$). The energy and momentum carried by a photon depends only on its frequency $v$ or wavelength $\lambda$, $E = hv = \frac{hc}{\lambda}$ and $p = \hbar k = \frac{hv}{c}$, where $\hbar$ is the Planck constant and $\hbar = \frac{h}{2\pi}$ is the reduced Planck ‘constant.

On the macroscopic scale, the radiation pressure exerted on an object can be expressed by using the Maxwell-Bartoli expression, see Fig. 3.1 (a) and (b). According to Newton’s laws of motion, the change in the momentum $\Delta p$ of an object in a given time period $\Delta t$ is equivalent to the force ($F$) exerted on it, $F = \Delta p/\Delta t$. When the external force is given by light, the object exerts an equal and opposite force on the photons by changing their momentum. So, for $N_{ph}$ number of photons normally incident onto an area $A$, a momentum of $\Delta p = N_{ph}p = N_{ph}E/c$ will be transferred to the object, assuming all the light beam is completely absorbed, see Fig. 3.1 (a). Thus, the resulting radiation pressure can be described as:

$$P_{\text{rad}} = \frac{F}{A} = N_{ph} \frac{E_{ph}}{\Delta tCA} = \frac{I}{c}, \quad \text{(Nm}^{-2} \text{ or Pa)}$$

with, $I = N_{ph} \frac{E_{ph}}{\Delta tA}$, \quad \text{(W/cm}^2)$ \quad (3.1)$

Where $I$ is the intensity of the light, which is the only experimentally measurable parameter in the equation above. In the general case, when the light is incident with an angle of $\theta$, with respect to the surface normal of the object, $P_{\text{rad}} = \frac{I}{c} \cos \theta^2$. The intensity can also be expressed by the time-averaged Poynting vector $\langle \mathbf{S} \rangle$, as $I = \langle \mathbf{S} \rangle$. So Eq. 3.1 becomes:

$$P_{\text{rad}} = \frac{\langle \mathbf{S} \rangle}{c} \cos \theta^2,$$ \quad (3.2)

For a fully reflecting planar surface of incident angle $\theta$, see Fig. 3.1(b), the radiation pressure exerted on the surface then given by:

$$P_{\text{rad}} = 2 \frac{I}{c} \cos^2 \theta$$ \quad (3.3)
Chapter 3. Fundamental concepts: Optical manipulation of particles in gas phase

Figure 3.1: The momentum transfer and the resulting force (indicated by the arrows) of a single photon (propagating from left to right) on a microscopic particle. (a) Involves light absorption, (b) reflection and (c) elastic scattering. Here in addition to on axis, the resulting force can also act in the lateral direction depending of the scattering angle.

In case that the surface is not fully absorbing or reflecting, the radiation pressure can be expressed by introducing the reflection coefficient $R$ of the object in Eq. 3.2 and Eq. 3.3. Assuming no light is transmitted through the object, the general Maxwell–Bartoli expression becomes:

$$P_{rad} = \frac{I_c}{c} (1 + R) \cos^2 \theta \quad (3.4)$$

In the case of micro or nanoparticles, the radiation force acting on them can be calculated by integrating the change in the momentum of the incident electromagnetic field over the surface of the particle [77]. There are two methods that are commonly used to calculate the optical force, from the point view of classical electrodynamics.

**General methods:** applicable independent of the size and shape of the particle, such as the Maxwell’s stress tensor and Lorentz force. **Specific methods:** these approaches rely on the reduced size of the particles to simplify the calculation, such as generalized Lorenz-Mie scattering, dipole approximation and the sum of gradient and scattering optical force approximation [77].

Maxwell’s stress tensor $T$ describes the interaction between electromagnetic forces and mechanical momentum. It is applicable in various scenarios, ranging from macroscopic objects [78] to nanoparticles. Integration of the this second-order

---

*For relative permittivity $\varepsilon_r$ and permeability $\mu_r$, the Maxwell’s stress tensor given by, $T_{ij} = \varepsilon_r \varepsilon_0 \left( E_i E_j - \frac{1}{2} \delta_{ij} E^2 \right) + \frac{1}{\mu_0 \mu_r} \left( B_i B_j - \frac{1}{2} \delta_{ij} B^2 \right) \quad (N/m^2)$. Where, $E$ and $B$ are the electric and magnetic field, respectively and $i, j = 1, 2, 3$ are index of the tensor.

$\delta_{ij}$ is Kronecker’s delta function defined as: $\delta_{ij} = \begin{cases} 0 & \text{if } i \neq j, \\ 1 & \text{if } i = j. \end{cases}$
3.2. Optical forces

tensor over the surface of the particle gives the net optical force acting on it.

\[
\langle F \rangle = \int_A \langle T \rangle \cdot ndA, \quad (N)
\]

\[
= \int_V \int_V dr^3 \langle f \rangle = \int_V dr^3 \Delta \langle T(r) \rangle,
\]

Where, \( A \) and \( V \) are the surface and volume of the particle, \( n \) is the surface normal of the particle, and \( f \) is the optical force density which indicates the volumetric force across the particle. Alternatively, the electromagnetic force in microscopic or nanoparticles can be described by using the Lorentz force density \( f_g \) associated with the polarization vector \( P \), given by:

\[
f_g = P \cdot \nabla E + \frac{\partial P}{\partial t} \times B, \quad (N/m^3)
\]

\[
= \alpha (E \cdot \nabla E + \frac{\partial E}{\partial t} \times B),
\]

Where the relative polarization vector \( P = \alpha E \), \( \alpha = (\epsilon_p - 1)/4\pi \) is a scalar polarizability, where \( \epsilon_p \) is the permittivity of the particle \( \textbf{[79]} \).

From Maxwell’s equation \( \nabla \times E = -\frac{1}{c} \frac{\partial B}{\partial t} \), and the identity

\[
E \cdot \nabla E = \frac{1}{2} \nabla E^2 - E \times \nabla \times E
\]

Eq. 3.6 reduced to:

\[
f_g = \alpha \frac{1}{2} \nabla E^2 + \frac{\partial}{\partial t} (E \times B)
\]

Since the electromagnetic field oscillates extremely fast, in steady state, the time average value of the term \( \frac{\partial}{\partial t} (E \times B) = 0 \), so the Lorentz force density simplifies to:

\[
f_g = \alpha \frac{1}{2} \nabla E^2 \quad (N/m^3)
\]

Integrating the force density in Eq. 3.9 over the volume of the particle gives the total optical force acting on it. The detailed description of how to calculate the force can be found in \( \textbf{[80]} \).

\[\text{\footnotesize†}\]

\[\text{\footnotesize\textsuperscript{†}} n^2 = \epsilon_r, \epsilon_r = \epsilon_p/\epsilon_0 \text{ is the relative permittivity. For absorbing dielectric particles the complex refractive index } n^2 = \epsilon_r = \epsilon_r + i\tilde{\epsilon}_r = (n + ik)^2, \text{ where } n \text{ and } k \text{ (also known as the extinction coefficient) are the real and imaginary part of the refractive index respectively.}\]
3.2.2 Photophoretic force

When the trapping experiment is conducted in air, the gas molecules surrounding the particle affect the trapping mechanism. For instance, by introducing thermal or other fluctuations on the particle. If the particles are light-absorbing, due to anisotropic heating of the particle and the gas molecules adjacent to the particle surface, the particles start to experience strong light induced phenomena, such as thermal creep flow or photophoretic effects, in addition to the radiation pressure.

“Thermal creep flow” or “thermal slip flow” is caused by a temperature gradient along a gas associated interface, such as a surface of a particle, which results in a pressure gradient along the surface that causes thermal transportation of the gas [81]. This pressure gradient results in a force on the particle, which points in the direction of lower pressure.

The photophoretic force was first observed by Ehrenhaft in 1917. It is a light-induced thermal force on non-uniformly illuminated absorbing microscopic particles, which are suspended in a gaseous environment. Unlike the radiation pressure, the PPF arises from the interaction of a particle, which has temperature gradient across its surface, with the surrounding gas molecules. Let’s consider an absorbing airborne microscopic particle suspended in a gas chamber which is in a thermal equilibrium ‡. In this condition, the particle interacts with the surrounding gas molecules isotropically, i.e., statistically the particle undergoes the same amount of collisions in every direction, thus it experiences zero net momentum due to this collisions. However, if the particle is illuminated unilaterally, for instance by a laser, this causes non-uniform heating of its surface. Now, the surrounding gas molecules interacting with the hotter surface of the particle will recoil with higher momentum than those from the colder side, and this results in a net force on the particle that pushes it in the propagation direction of the light. This is called positive photophoretic force, it is the dominant force on strongly absorbing particles, see Fig. 3.2. In the case that the particle is weakly-absorbing or semi-transparent, it could focus the laser beam on its opposite side, as the light transmits through it. This might induce higher temperature on the opposite side of the particle than the illuminated surface, thereby forces the particle to moves toward the light source, this called negative photophoretic force.

Assuming an equal amount of energy is removed by the gas molecules from the surface of the particle as is deposited by the light, a particle would experience a photophoretic force with a factor of \((c/3v)\) times higher compared with the pressure from the photons [82], where \(c\) is the speed of light and \(v\) is the molecular velocity.

‡ At thermal equilibrium the gases molecules move in random direction and their average velocity is considered to be zero, \((v) = 0\).
3.2. Optical forces

Figure 3.2: Schematic illustration of thermal force induced by non-uniform light illumination. The absorbed laser light create temperature gradient across the surface of the particle. The surrounding gas molecules acquire higher momentum upon impact on the hotter surface of the particle than the colder side, this result in a net force that pushing the particle away from the light source, positive photophoresis.

of the gas. From this simple estimation, for a given laser intensity, the PPF on an absorbing particles can be 5 orders of magnitude stronger than the direct radiation force [82].

The momentum of a gas molecule \( p_{\text{gas}} \) at absolute temperature \( T \) is proportional to \( m_{\text{gas}}kT \) [80]. Where, \( m_{\text{gas}} \) is the mass of one molecule of the gas and \( k \) is the Boltzmann constant. So, depending on the surface temperature of the particle at the instance of the collision, the gas molecules will acquire different velocity, hence momentum. The momentum of gas molecules that have interacted with the hotter side of the particle \( p_{\text{hot}} \) and that have interact with the colder side of the particle \( p_{\text{cold}} \), can be compared by the following proportionality relation:

\[
p_{\text{hot}} \propto \sqrt{m_{\text{gas}}kT_{\text{hot}}} > p_{\text{cold}} \propto \sqrt{m_{\text{gas}}kT_{\text{cold}}}
\]  

(3.10)

Where, \( T_{\text{hot}} \) and \( T_{\text{cold}} \) are the temperature of the particle on the hotter and colder side, respectively.

Ultimately, it is the absorbed electromagnetic radiation that determines the surface temperature of the particle. However, unlike radiation pressure, which always exerts an equal amount of force on the particle for a given power of the laser, PPF is additionally affected by the density of surrounding gas molecules relative to the size of the particle. This can be better expressed by the Knudsen
number, $Kn = l/D$, where, $l$ is the mean-free path of the gas molecules and $D$ is the characteristic length, which is the diameter of the particle in this case. So, PPF calculation for a given particle size and pressure range requires a comprehensive optical and gas-kinetic analysis. This make PPF calculation extremely challenging and a single analysis technique that could calculate the PPF for the entire pressure range is yet to be established [83].

Based on the $Kn$, the photophoretic force problem can be categorized into three regimes: the free molecular flow regime ($Kn \gg 1$), the continuous flow regime ($Kn \ll 1$) and the transition flow regime ($Kn \approx 1$). In the continuous flow regime, the mean-free path of the gas is significantly shorter than the diameter of the particle, thus the gas molecules undergo more frequent collisions among themselves than with the particle. In this regime, the gas behaves as continuum fluid and obeys Navier-Stokes equations of hydrodynamics and the PPF arises from the thermal-creep flow of the gas. In the free molecular flow regime, the mean-free path is significantly longer than the particle diameter. Therefore, the gas molecules spend much more time between collisions with each other than the particle. So, the PPF force in this regime arises from individual gas molecule collision with the particle. A number of analytic methods have been developed to calculate the photophoretic in both pressure limits [83–86]. The PPF, as it is calculated from free molecular follow, increases linearly with pressure for $Kn \gg 1$ and the PPF, as it is calculated using Maxwells thermal-creeping flow, decreases with pressure for $Kn \ll 1$. However, at the transition regime ($Kn \approx 1$) a simply interpolation is often used to intersect the two regimes [83,87].

Before I formulate the PPF on an absorbing particle illuminated by a laser, let us first consider the relation between temperature of the particle surface and the surrounding gas molecules. According to the kinetic theory of gases, the kinetic energy of a gas molecule depends only on its absolute temperature $T$. The temperature $T_r$ of a gas molecule after it has interacted with a particle at a temperature of $T_s$, can be formulated with the following relation [83]:

$$T_r - T_i = \alpha(T_s - T_i) \quad (3.11)$$

Where, $T_i$ is initial temperature of the gas and $\alpha$ is the thermal accommodation coefficient. The thermal accommodation coefficient is a dimensionless quantity that characterizes the property of gas after interaction with a particle, $\alpha \leq 1$. This means, higher accommodation coefficient implies better momentum transfer between the particle and the gas.

In the following three sections, I will present PPF calculations for the three pressure regimes. Note, the formulas are adopted from [83].
3.2. Optical forces

PPF in the free molecular regime

Here, the PPF is due to the direct scattering of individual gas molecules from the surface of the particle. The differential force element \( dF \) exerted normal to the surface element \( dS \) of the particle by the gas molecules incident at temperature \( T_i \) and recoil with \( T_r \) is given by [83]:

\[
dF_{pp}^{fm} = \frac{p}{2} \left( 1 + \sqrt{\frac{T_r}{T_i}} \right) dS \quad (3.12)
\]

Where, \( p \) is the pressure. Integration of Eq. 3.12 over the surface of a spherical particle gives:

\[
F_{pp}^{fm} = \frac{\pi}{3} \alpha \frac{p}{T_i^2} \alpha^2 C_{pp}^{fm} \quad (3.13)
\]

Where, the coefficient \( G_p \) is the temperature gradient inside the particle.

PPF in the continuum regime

At high pressure, instead of individual gas molecule collision with the particles, the force is the result of the migration of the gas molecules adjustment to the unevenly heated particle surface, which is known as thermal creep [83,88]. So, a simple equation like Eq. 3.13 derived from the kinetic theory doesn’t apply any more. A creep gas flows in the direction of increasing surface temperature with tangential velocity \( v_t \), which is given by:

\[
v_t = \kappa \frac{\eta}{\rho T} \frac{dT}{dS} \quad (3.14)
\]

Where, \( T \) is the temperature and \( \frac{dT}{dS} \) is the adjacent gas temperature gradient, \( \kappa \) is the thermal creep coefficient assumed to be between 0.75 and 1.50 [88], \( \eta \) the gas dynamic viscosity and \( \rho \) is the gas mass density. The resulting photophoretic force for a spherical particle in the continuum flow is then given by [89]:

\[
F_{pp}^{co} = 4\pi\kappa \frac{R\eta^2}{M_p} G_{pp}^{co} \quad (3.15)
\]

Where, \( R \) is the universal gas constant, \( M \) the molecular weight of the gas and \( p \) is the gas pressure. There are two way to determine the value of the coefficient \( G_p \). **Case 1:** known or measurable temperature gradient, in this simple case \( G_p \) can be calculated by:

\[
G_p = \frac{1}{2} \Delta T_s \quad (3.16)
\]
Case 2: Known irradiance, this requires calculation of the inhomogeneous heat distribution inside the volume of the particle induced by the absorbed intensity. This distribution of heat source problem is expressed by a single quantity introduced by Yalamov, named the ‘asymmetry factor’ $J_1$ [90,91]. $|J_1| = \frac{1}{2}$ for fully absorbing particles. This is valid for both free molecular as well as continuum regimes. So, equation 3.15 and 3.19 can be expressed in terms of the characteristic pressure of the transition point $p^*$ as:

$$F_{ph}^{fm} = \frac{D \alpha}{2} \frac{a_p^2}{p^*} \frac{k_p}{I}$$ (3.17a)

$$F_{ph}^{co} = \frac{D}{p^*} \frac{a_p^2}{k_p} \frac{I}{I}$$ (3.17b)

Where, $k_p$ is the thermal conductivity of the particle, $I$ is the illumination intensity and $p^*$ is the optimum pressure at which the PPF is maximized, i.e., at $Kn = 1$. $D$ and $p^*$ are given by:

$$D = \frac{\pi}{2} \sqrt{\frac{\pi}{3} \frac{T_i}{\kappa c \eta}}$$

$$p^* = \frac{1}{2} \sqrt{3\pi \kappa \bar{c} \eta} \frac{1}{\bar{a}_p} = \frac{3}{\pi} \frac{D T_i}{\bar{a}_p}$$

In Eq. 3.17 above $D$ is a constant which is entirely determined by the state of the gas and $p^*$ is also constant for a given particle radius, so the photophoretic force is linear with pressure both in the continuum as a well as free molecular regime (see Fig. 3.3).

**PPF in the transition regime**

A simple analytic technique to calculate the PPF in the transition region ($F_{tr}$) has not yet been developed. So, an approximate interpolation between the PPF calculated in the two extreme pressure is used to fill the gap [83].

$$\frac{1}{F_{tr}} = \frac{1}{F_{ph}^{fm}} + \frac{1}{F_{ph}^{co}}$$ (3.18)

As seen in Fig. 3.3 and Eq. 3.17, the PPF maximizes at the transition region where $Kn \approx 1$, i.e., when the mean-free-path of the gas is in the order of magnitude with the particles size. For instance, for a 1 µm diameter particle in a Helium gas environment and room temperature, the optimum pressure $p^* \approx 190$ mbar. Practically, this pressure is too high for our experiment. First, the injector doesn’t work at such high chamber pressure; second, X-ray diffraction chambers wouldn’t
3.3. Optical trapping technique in the gas phase

Owing to the difficulty associated, little has been done on trapping particles suspended in the gas. However, in recent years, using different laser beam configurations, a handful of successful experiments have been reported. In the following section, I will give overviews on some the relevant techniques and their applications.

3.3.1 Single-beam aerosol trapping

Dielectric aerosol particle

There are two main types of single beam trap that are commonly used. The first category is illustrated in Fig. 3.4 (a). In this configuration, an upward propagating,
loosely focused Gaussian beam is used to levitate a dielectric aerosol or colloidal particle against gravity. The particle is trapped by balancing the radiation force or scattering force ($F_{\text{sca}}$), against the force of gravity ($F_{\text{grav}}$) and air drag forces ($F_{\text{d}}$), if the particle were moving. Such trapping methods commonly known as optical levitation. The second configuration is illustrated in Fig. 3.4 (b–d); here, the trap is built by focusing a Gaussian beam to an extremely small spot, using a high NA microscope objective, to create a high intensity gradient across the surface of the particle. Close to the focus, the high divergence of the beam coupled with the intensity gradient, drastically changes the momentum of the laser beam as it transmits through the dielectric particle. This results in a strong gradient force ($F_{\text{grad}}$) that pulls or pushes the particle toward the focal point of the laser and the particle is trapped at the focus by counteracting the forward scattering force and gravity, if the trap is configured vertically. This trapping configuration is commonly known as optical tweezers [15].

The optical force is proportional to the intensity gradient of the trapping laser. Once in the trap, the particle can be consider as sitting in a periodic parabolic potential-well which is created by the trapping beam gradient [93] thus, the particle dynamic can be represented by the following equation of motion for a Brownian particle in a harmonic potential.

$$m\ddot{x}(t) + \gamma \dot{x}(t) + \kappa x = F(f)$$  \hspace{1cm} (3.19)
3.3. Optical trapping technique in the gas phase

Where, the first term represents the inertial force component for a particle of mass \( m \), \( x \) is the position of the particle, \( \gamma = 6\pi \eta r \) is the drag coefficient of particle with radius \( r \), \( \kappa \) is the trap stiffness and \( F(f) \) represents the noise source initiated by Brownian motion. When the experiment is performed on a particle immersed in water, the motion of the particle is over-damped. So, the viscous force dominates and the inertial term in Eq. 3.19 can be ignored. However, when the trapping medium is air, the viscous damping is greatly reduced. Furthermore, the absence of the liquid medium, which also serves as a heat-sink for the trapped particle, will increase the thermal motion of the particle. This heavily reduce the trap stiffness and it get worse if the particle absorbs some of the light. Due to this, creating a stable 3D trap for dielectric aerosols using such a configuration in air is very difficult.

Absorbing aerosol particles

A simple Gaussian beam is not enough to create a stable trap for absorbing particles, since the particles tend to move away from the maximum laser intensity due to the photophoretic force arising from the absorbed light. As such, they require special engineering of the laser beam profile. For instance, using a doughnut-shaped laser beam that confines the absorbing particles in the intensity minimum of the beam [92] or optical bottle-beam-like traps [94,94,95].

The optical levitation of absorbing particles is a very important concept for the optical guiding and decelerating of particles, which is the main focus of this thesis work. The PPF calibration measurement of our optical trap, which was constructed using a Laguerre-Gaussian beam, was done on levitated carbon particles, see Fig. 3.5. This measurement was performed by Eckerskorn and the detail can be found in his thesis and publication [80,92].

To reduce the effect of air fluctuation and turbulence, aerosol trapping experiments are usually performed in a small trapping-cell, which the laser beam is focused into, see Fig. 3.5 (a). The aerosol particles are introduced into the cell using different techniques, such as a nebulizer or sprinkling using a brush [96,97].

3.3.2 Dual-beam aerosol trapping

In this trapping technique, two loosely focused beams are employed, see Fig. 3.6. In fact, this was the configuration used by Ashkin in his first demonstration of the optical trapping of neutral particles [15]. Unlike the optical tweezers, which relies on the gradient of the laser intensity, here the trapping mechanism is due to the force from radiation pressure or photophoretic force or a combination of...
Figure 3.5: Optical levitation of carbon particles using a divergent vortex beam. (a) The basic PPF levitation and calibration setup. (b) The vortex beam profile. (c) The principle of particle confinement in the dark core of the beam. When a particle is introduced off-axis, it will be heated only on one side, and subsequently will be pushed to the center of the doughnut. This figure is adapted from [92].

both. Therefore, the laser beam doesn’t necessarily need to be focused tightly. This increases the working distance of the trap as well as the size of the trapping volume. Furthermore, the reduced intensity of the laser in the tapping area reduces the radiation damage to biological samples.

Two loosely focused counter propagating beams arranged as illustrated in Fig. 3.6 is the most common dual-beam trapping configuration. The trapping beams can be delivered through two opposing objectives [98, 99] or optical fibers, which is also known as dual-beam fiber trapping [100]. Other configurations, such as orthogonally propagating beams or mirror-based standing wave trap, are also used [cite]. In the case of dielectric aerosols, two counter propagating Gaussian beams are typically used and the particle is trapped by counteracting the scattering, radiation forces from the two lasers and gravity. In case the particles are absorbing, two counter propagating laser beam with minimum intensity in the middle, such as LG(0,1), are commonly employed (see Fig. 3.6). Transversely, the particle is confined by the PPF \( F_{PPF} \) induced by the two overlapping laser beams pushing the particle radially inward to the dark-core. Whereas, axially, the scattering forces \( F_{scat} \) compensate each other to create a stable 3D trap [76, 101]. In air, \( F_{PPF} \gg F_{grav} \), so the effect of gravity can be ignored. In such an arrangement, by adjusting the relative power between the two lasers, the particle position in the trapping region can be adjusted.
3.4 Optical guiding techniques for aerosols

In recent years there has been growing interests in the optical guiding of aerosol particles for various applications, such as sample delivery for X-ray diffraction experiments and in environmental science to study aerosols in the atmosphere. Transparent or semi-transparent dielectric particles can be transported using a Gaussian beam, as it was shown in [102]. In this work, first they levitated aerosolized water droplets using a upward propagating laser and then gradually lowered the laser power to guide the particles down opposite to the laser, by the pull of gravity. Whereas, absorbing particles can be confined and transported inside the minimum intensity of a vortex beam. This was demonstrated by Shvedov [103]. In this work, they showed a long-range guiding of carbon nano-foam in air, using a single vortex beam as a pipeline. The PPF confines the particle in the dark core and the scattering force effectively guides the particle along the propagation direction. Particle guiding is also demonstrated on the surface of tapered optical fibers or inside of a hollow-core waveguides, such as photonic crystal fiber. The trapping force is given by the evanescent field of the guided light beam [104, 104].

In general, almost all aerosol trapping or guiding experiments were carried out on a stationary or slow particles, which were freely falling into the trap. This guaranties maximum interaction of the laser field with the particle, thus the trapping process is much convenient. Furthermore, particle imaging and tracking is very straightforward. However, in this work we were attempting to capture and
guide particles which are moving at a very high velocity, faster than 100 m/s in some cases. I will talk about this in detail in the following chapters. That means compared with standard aerosol guiding or trapping experiments, this requires careful structuring and engineering of the laser beam, so that it maximizes the interaction with the high-speed particles. So, the ability to shape and control the trapping laser beam profile is the key to our experiment. In the following section I will give an overview on the common types structured laser beams and the techniques used to generate them.

### 3.5 Structured light beams

For optical trapping and manipulation applications of micro-particles, laser beams have been shaped in various forms both temporally \[105\] and spatially \[106\], either to increase the efficiency, increase volume and throughput \[94, 94, 95\] or enable dynamic configuration of the trap \[107–110\]. In the context of this thesis, spatially structured laser illuminations with dark core in the middle are particularly interesting. In the following section, the theory and practical generation of such beams is revised.

#### 3.5.1 Optical Vortex beam

For a monochromatic laser beam propagating in the $z$ direction, the complex electric field can be expressed as \[111\]:

$$
\vec{E}(\vec{r}, t) = \psi(\vec{r})e^{kz-\omega t}
$$

(3.20)

Where $k = \frac{2\pi}{\lambda}$ is the propagation constant and $\psi(\vec{r})$ is a complex-valued amplitude representing the transverse profile of the beam and it is one of the many stable solutions of the paraxial wave equation. In cylindrical coordinates, these stable solutions are Laguerre-Gaussian mode (LG$_{p,l}$) with indices $p \geq 0$ and $l = 0, \pm1, \pm2, \pm3$...

$$
\psi(\vec{r}, \varphi) = \sqrt{\frac{2}{\pi m! n!}} \frac{\min(m, n)!}{w} (-1)^{\min(m, n)} e^{-r^2/w^2}
$$

$$
\times e^{-i(m+n+1)\varphi} e^{ikr^2/2R(z)} \left( \frac{\sqrt{2}}{w} e^{-i\varphi} \right)^{|m-n|}
$$

$$
\times L_{\min(m,n)}^{m-n} \left( \frac{2r^2}{w^2} \right)
$$

(3.21)

---

*The complex envelope $\psi(\vec{r})$ satisfies, $\nabla_\perp^2 \psi(\vec{r}) + 2ik \frac{\partial \psi(\vec{r})}{\partial z} = 0$. 

---

40
Where, $L_p^l(x)$ is the generalized Laguerre polynomials, $p = \min(m, n)$ is the radial mode index that determine the number of nodes in the radial direction of the beam and $l = \pm|m - n|$ is the azimuthal phase index (also known as topological charge), it determines the number of $2\pi$ phase shifts that occur in one full revolution around the beam center in a direction dictated by the sign of $l$. A beam with indices $p$ and $l$ is represented by $LG_{p,l}$. The beam spot size $w(z)$, Rayleigh range $z_R$ and wavefront radius of curvature $R(z)$ given by:

$$w(z) = w_0 \sqrt{1 + z/z_R^2} \quad (3.22a)$$

$$z_R = \frac{\pi w_0^2}{\lambda} \quad (3.22b)$$

$$R(z) = z + \frac{z_R^2}{z} \quad (3.22c)$$

For $LG_{0,0}$ beam, i.e., $p = l = 0$, Eq. 3.21 reduces to the fundamental Gaussian beam solution of the paraxial wave equation which has a transverse intensity profile with the maximum in the middle. For $l \neq 0$ the $\exp(-il\varphi)$ term in Eq. 3.21 introduces azimuthal angular dependence in the transverse field distribution with a phase singularity around the axis. This produces a beam that carries an intrinsic orbital angular momentum (OAM), which is known as optical vortex beam (OVB), see Fig. 3.8 (b). Each photon in such a beam carries OAM of $lh$ in addition to the spin angular momentum associated with the polarization. This means a dielectric object placed along the propagation axis of the beam will experience a torque in a direction determine by the sign of $l$. This makes such LG modes ideal for manipulation of micrometer sized particle as well as atoms. Optical vortex beams have now also gained applications in super resolution microscopy, quantum information processing, telecommunication and laser material processing. In general, a radial index $p = 0$ and azimuthal mode index $l > 0$ give rise to a beam with doughnut-shaped transverse intensity distribution with peak-to-peak intensity ring diameter ($w_{pp}$) increasing with $l$, see Fig. 3.7.

There are a number of well-established experimental techniques used to generate an LG beam. For instance, using a spiral phase plates (SPP) [112], computer generated holograms produced by spatial light modulators [113] and astigmatic mode converters [114] are the most common ones. Higher order Laguerre-Gauss modes with single ring ($LG_{0,l}$) can also be produced directly inside a laser cavity [115,116].

**Formation of Vortex beam using SPP**

One of the most common techniques used to produce an OVB is to illuminate a SPP with a Gaussian beam. SPP is a transparent optical element, constructed with
spiral phase ramp changes from 0 to $2\pi$, $l$ times around its central axis, see Fig. 3.8 (a) for $l = 1$. The insertion of such phase element in the beam path introduces a phase singularity on the central axis of the transmitted Gaussian beam. This results in cancellation of the electric field in the center of the beam to produce doughnut-shaped laser intensity with topological charge of $l$, see Fig. 3.8 (c). The generated beam intensity distribution by an $l = 1$ SPP is given by:

$$I(r, z) = I_0 \frac{4}{\pi w^2(z)} w^2(z) e^{-\frac{2r^2}{w^2(z)}}$$

(3.23)

In higher order LG modes, the well-defined peak-to-peak intensity diameter ($w_{pp}$) or peak intensity radius ($w_p = \frac{w_{pp}}{2}$) are used as a characteristics length scale to define the transverse beam width, see Fig. 3.8 (d). This is different from the fundamental Gaussian mode where $1/e^2$ intensity position of the evolving beam is used as a convention to define the beam size. So, using Eq. 3.23, we can derive a relation between the Gaussian beam waist ($w(z)$) and the higher order LG beam of order $l$ peak-to-peak intensity diameter ($w_{pp}(z)$) as:

$$w_{pp}(z) = 2w_p(z) = 2\sqrt{|l|/2}w(z).$$

(3.24)

The phase step $\Delta h$ required on a phase plate with topological charge $l$ to produce phase shift of $\Delta \theta$ is given by:

$$\Delta h = \frac{\Delta \theta}{2\pi} (n - 1)\lambda$$

(3.25)

Where, $n$ is the refractive index of the SPP material and $\lambda$ is the wavelength. For instance, in our experiment we used 532 nm laser and a SPP made of fused Silica $n = 1.5$, constructed with 16 spiral phase steps, see Fig. 3.8 and $\lambda = 532$ nm. So each of them introduces $\frac{\pi}{2}m$ phase difference, where $m = 1, 2, 3...16$ is the step number. Using Eq. 3.25, the maximum thickness on the SPP to create $\Delta \theta = 2\pi$ will be 266 nm with 16.6 nm height difference between each of the 16 successive steps.
3.5. Structured light beams

3.5.2 Quasi-Bessel beam

Bessel beams are diffraction-free solutions of the free-space Helmholtz equation in the cylindrical coordinate system, with a field amplitude described by the Bessel function of the first kind \[117\]. The electric field amplitude of an \( l \)th order Bessel beam can be described in cylindrical coordinates as:

\[
E_{l}(r, \phi, z) = A_{0}\exp(i k_{z} z) J_{l}(k_{r} r) \exp(\pm il \phi) \tag{3.26}
\]

Where \( J_{l} \) is the \( l \)th order Bessel function of first kind, \( k_{z} = k \cos \theta \) and \( k_{r} = k \sin \theta \) are the longitudinal and radial components of the free-space wavevector \( k \), with \( k = \frac{2\pi}{\lambda} = \sqrt{k_{r}^{2} + k_{z}^{2}} \). The zero-order Bessel beam has bright intensity in the middle
Chapter 3. Fundamental concepts: Optical manipulation of particles in gas phase

Figure 3.9: Transverse intensity profile of QBB for different topological charges. The beam width and divergence increase with $l$ similar to LG beams.

surrounded by concentric rings, see Fig. 3.9 (a), whereas higher-order beams ($l > 0$) has phase-singularity in the beam axis associated with the azimuthal mode term $\exp(\pm il\phi)$ in Eq. 3.26 which results in a beam with a non-diffracting dark core surrounded by bright intensity rings, see Fig. 3.9 (b)–(d) [117]. The intensity distribution of an ideal Bessel beam is given by:

$$I(r, z) = I_0 J_2^2(k_r r) \tag{3.27}$$

The most interesting future of Bessel beams, evident in Eq. 3.27, is that the intensity ($I$) of the propagating beam doesn’t depended on the $z$ component, thus it obeys $I(r, z) = I(r, 0)$. That means they have constant transverse intensity profile that doesn’t diffract or spread out while propagating in free-space. So they can be considered as diffraction-free or propagation invariant beams [118]. Furthermore, a perfect Bessel beam has infinite transverse extent and energy.

Practically, such a beam can’t be produced in the lab. However, approximate finite size Bessel beam known as ‘quasi-Bessel beam’ (QBB) that doesn’t diffract over a limited range in space can be produced experimentally [117,119]. QBBs can be created in number of ways, such as using an axicon [117], a computer generated hologram, for instance written on an SLM [106,113] or by illuminating a narrow annular slit placed on the back focal plane of a lens [118,120]. In the later technique, to generate a zero-order Bessel beam, a plane wave is used to illuminate the slit. This exploits the fact that a Bessel beam has an annular ring far-field differentiation, so the lens effectively performs the Fourier transform of the ring to form the Bessel beam in the front focal plane. Higher order Bessel beams can also be created in a similar way, but using different illuminations [121]. SLMs are the most powerful and flexible technique to produce the Bessel beam and they give dynamic control over the QBB intensity.
3.5.3 Generating high order quasi-Bessel beams using an axicon

An axicon is a refractive optical element with a conical surface (also known as conical lens) that is commonly used to generate non-diffracting beams. Both zero-order and higher-order Bessel beams can be generated by illuminating an axicon with a Laguerre-Gaussian beam of appropriate order. Despite the lack of flexibility, axicon base QBB generation is the most efficient and widely used technique, as it convert the entire incident laser intensity into the Bessel beam. Furthermore, an axicon generated QBB has smooth axial intensity (see Fig. 3.10 (b)) compared with, for instance annular slit methods, which produce rapid oscillation on the propagating beam intensity. Unlike an ideal Bessel beam intensity, given in Eq. 3.26, the amplitude of an axicon generated QBB transverse intensity depends on the position of the beam in the propagation direction, and is given as:

\[ I(r, z) = I_0 2\pi k_r \theta_2 z^{2l+1} \exp\left(\frac{-2z^2}{z_{max}^2}\right) J_l^2(k_r r) \]  

(3.28)

Where, \(I_0\) is the constant amplitude of the LG beam \[122\] and \(z_{max}\) is the propagation length of the QBB. In general, an axicon illuminated by Laguerre-Gaussian beam (LG\(_0,l\)) will generate a Bessel beam of order \(l\) \[117,118\].

Figure 3.10: Generation of a first order QBB using an axicon. (a) Configuration of LG\(_0,1\) beam focusing using an axicon lens. The QBB field exists only in the pink shaded rhombus region. (b) Calculated axial cross-sectional intensity profile of the QBB, as it propagates from left to right. In the lower inset, the variation in the propagating maximum intensity of the inner QBB ring is plotted. The maximum of this plot indicates the QBB focus, which is at \(Z_f = 400\) mm.
The idea of focusing an LG beam by an axicon is illustrated in Fig. 3.10, where the Bessel beam is depicted as set of plane waves propagates in a cone of angle given by [118] :

\[ \theta = (n - 1)\gamma \]  

(3.29)

Where, \( \gamma \) is the internal angle of the axicon and \( n \) is the refractive index of the axicon material. This produces a Bessel beam with an annular transverse spectrum of radius \( k_r \approx k(n - 1)\gamma \), that propagates a maximum distance of \( Z_{\text{max}} \). This region is shaded with pink in Fig. 3.10 (a), i.e., the rhombus formed by the intersection of the solid red and the dashed green cones. The 2D axial intensity of the QBB in the propagation direction is depicted in Fig. 3.10 (b). The propagation length \( (Z_{\text{max}} - Z_{\text{min}}) \) can be considered as the depth-of-focus (DOF) of a QBB focused by an axicon, which is analogy to the Rayleigh range of LG beams focused by a spherical lens. Using a simple geometry calculation on Fig. 3.10 (a), the \( Z_{\text{max}} \) can be estimated as:

\[ Z_{\text{max}} = \frac{k}{k_r}w = \frac{w}{\theta} \]  

(3.30a)

\[ Z_f = Z_{\text{max}} \frac{\sqrt{(2l + 1)}}{2} \]  

(3.30b)

Where \( w \) is the beam waist of the LG beam illuminated on the axicon, and \( Z_f \) is the axicon focal length, i.e., position of the maximum intensity of the QBB from the axicon, see Fig. 3.10 (b). \( Z_{\text{min}} \) indicates the distance from the axicon where the Bessel beam starts to form. Due to the dark core of high order LG beams, the two peak intensities have to propagate a distance of \( Z_{\text{min}} \), after the axicon, before they start to interfere. As seen in Eq. 3.30b the focal length of the QBB increases with the topological charge \( l \) and the beam waist \( w \), this is also true for \( Z_{\text{min}} \). However, the depth of focus is nearly independent of the topological charge [122].

The great advantage of the QBB lies on the relatively long DOF, i.e., we can create a beam which doesn’t diverge for a relatively longer distance. For example, if we compare the DOF of a LG\(_{0,1} \) beam of size \( w = 2.5 \) mm focused by a spherical lens and an axicon (angle \( \gamma = 0.5 \) degree) to same spot size \( -w_{pp} \) for the LG beam and inner ring peak-to-peak diameter for the QBB – the QBB will propagates roughly 70 times longer than that of the LG beam, with no divergence. This very crucial for our optical guiding to achieve long range interaction of the particle with the laser. However, as the beam propagates away from the QBB focus, the energy of the QBB starts to spread between the higher rings. This come at the expense of intensity in the central ring.
4 Visualizing Aerosol-Particle Injection for Diffractive-Imaging Experiments *

Delivering sub-micrometer particles to an intense X-ray focus is a crucial aspect of single-particle diffractive-imaging experiments at X-ray free-electron lasers. Enabling direct visualization of sub-micrometer aerosol particle streams without interfering with the operation of the particle injector can greatly improve the overall efficiency of single-particle imaging experiments by reducing the amount of time and sample consumed during measurements. We have developed in-situ non-destructive imaging diagnostics to aid real-time particle injector optimization and X-ray/particle-beam alignment, based on laser illumination schemes and fast imaging detectors. Our diagnostics are constructed to provide a non-invasive rapid feedback on injector performance during measurements, and have been demonstrated during diffraction measurements at the FLASH free-electron laser.

4.1 Introduction

The emergence of X-ray free-electron lasers (XFELs) has inspired the development of new particle-injection instruments capable of delivering nano- and micro-particles to the intense 0.1–5 µm focus of a few-femtosecond duration X-ray beam. Single-particle diffractive imaging is among the methods that rely on the development of such particle-beam injectors, as it requires a series of isolated molecules, viruses, cells or microcrystals to be directed across the X-ray beam. Three-dimensional diffraction intensity maps can be constructed by assembling numerous two-dimensional diffraction patterns from particles exposed in different orientations [4,58]. In this


I performed the measurements. Together with R.A. Kirian, I analyzed the experimental data. With discussion and input from of other authors, I wrote the published manuscript.
way, three-dimensional images can be formed from reproducible targets. If successful, single-particle imaging (SPI) will allow for the determination of high-resolution structures of radiation-sensitive targets [33], without the need to grow large well-ordered crystals, which is often the principal bottleneck to macromolecular structure determination.

In SPI experiments, it is important to precisely deliver the target particles to the most intense region of the focused X-ray beam in rapid succession, since each particle is completely destroyed through photoionization-induced damage processes [25]. Liquid jets formed by gas-dynamic virtual nozzles (GDVN) [43], aerodynamic aerosol focusing [3], or gas-phase supersonic jet/molecular beam injectors [123] are among the most common techniques used to deliver particles. For SPI work, gas-phase injectors are preferred since a surrounding liquid reduces contrast and increases background scatter, which makes data analysis difficult, if not impossible. Aerodynamical-lens-stack aerosol particle injectors (ALS) [56] are presently the most common injector used for SPI experiments, which can create a collimated aerosol beam when particles suspended in a carrier gas pass through a series of concentric apertures. Alternative injectors, e.g., convergent-orifice nozzles, are also under development for SPI experiments [12].

During SPI experiments, aerosol injectors must be monitored frequently in order to maintain optimal hit fraction and delivery efficiency, i.e., the fraction of X-ray pulses that intercept a particle and the fraction of particles that are intercepted by an X-ray pulse, respectively. Particle-beam diagnostics are important because XFEL facilities are costly to operate, and many samples are also costly to obtain in significant quantities. X-ray diffraction patterns themselves are the ultimate diagnostic of injection efficiency, but this diagnosis is limited by the XFEL pulse repetition rate, detector readout rate, data processing rate, and availability of the X-ray source. It is desirable to have complimentary real-time diagnostics that assist the injection optimization process, both offline as well as online, during diffraction measurements. As we show below, direct visualization of particle beams through laser illumination is a simple yet powerful means to optimize injection efficiency. In addition to improving SPI experiment efficiency, imaging diagnostics can greatly accelerate the development of new aerosol injector schemes.

Aerosolized nanoparticles are not easily visible, and particle injection environments are not always easily accessible for probing due to ancillary measurement tools. Therefore, in-situ diagnostics can be challenging to implement within existing X-ray diffraction apparatuses. Early experimental work utilized greased plates onto which aerosol particles adhere [124, 125], allowing the transverse particle beam profile to be estimated. This method is commonly employed in SPI experiments, however such particle depositions, examined under a microscope, are
not easy to interpret quantitatively. In the context of SPI work, the first detailed experimental characterization of aerodynamically focused particles was carried out by Benner et al. [125]. Here, particle velocities and positions were determined from the image charges of particles transmitted through a metal tube. Aerosol beams have also been directly imaged in the past [126–129], but so far the great utility of the approach that we emphasize here has not been integrated into SPI experiments. More generally, the determination of particle-laden flow fields has been studied extensively within the field of particle-image velocimetry (PIV) and its variants [130,131].

In this paper, we present simple direct optical imaging diagnostics for online monitoring of particle injection during XFEL experiments, as well as for general aerosol beam characterization and injector optimization. We have utilized both continuous-wave (CW) and pulsed nanosecond illumination along with high-speed cameras, and nearly real-time analysis software, that can measure particle speeds, injector transmission efficiency, and projected particle beam density profiles. We have also implemented an in-vacuum inverted microscope for imaging particles that adhere to a gel.

4.2 Theory and background

Direct optical imaging can reveal most of the key parameters needed to optimize SPI sample injection, such as hit fraction and delivery efficiency (see section 2.2.1). Different types of injectors can introduce tradeoffs – for instance, a convergent-orifice injector can create a tightly focused particle beam that approaches the size of micro-focused X-ray beams, but apparently produces particles with greater speeds than typical ALS injectors [12]. Tightly focused beams necessitate the use of an in-situ direct imaging system since one would otherwise need to perform a three-dimensional scan of the X-ray beam in order to properly position the interaction region, whereas a collimated particle beam requires only a two-dimensional scan.

In general, independent of the type of injector used, the aerosol beams we consider here are composed of fast, nearly-unidirectional, and sparsely placed small particles confined to a narrow beam in a low-pressure environment. Typically, on the order of $10^7$ particles enter the injector per second and expand into the vacuum with a speed that can reach several hundred m/s. This leads to hit fractions well below 0.1% for current injectors and nano-focused X-ray beams, thus rendering X-ray diffraction-based diagnostics inefficient, highlighting the need for complementary rapid-feedback diagnostics.
4.2.1 Direct side-view particle imaging schemes

We can classify the direct side-view imaging of particles presented here into three regimes, principally identified by three characteristic times: \( \tau = \frac{d}{v} \) – the time it takes for a particle with velocity \( v \) to move over its diameter \( d \), exposure time \( t_{\text{exp}} \) – the camera integration time or duration of the illumination pulse, and \( t_{\text{fov}} \) – the time taken for a particle to move across the full field of view (FOV). \( d \) is the diffraction-limited spot size of the particle if the particle is smaller than the resolution limit of the imaging system.

“Long exposure” imaging

In the “long exposure” mode, the particle beam is illuminated either with a continuous or pulsed light source with a very long exposure time \( (t_{\text{exp}} \gg t_{\text{fov}}) \) on the camera [126]. This mode does not allow for the determination of particle velocities, but is straightforward to implement with relatively inexpensive equipment. In many cases the resulting integrated image intensity is directly proportional to the projection of the particle density along the optical axis. However, since elastic scattering in both the Mie and Rayleigh regime scales exponentially with particle diameter (for Rayleigh scattering, the intensity scales with the sixth power of particle diameter), one must ensure that all particles are of the species of interest, and not aggregated clusters of particles (for example) that would tend to dominate the intensity profile of the image.

“Streak” imaging

Visualizing individual, fast-moving, sub-micrometer particles requires \( t_{\text{exp}} < t_{\text{fov}} \), such that the entire image is contained within the field of view. In the “streak” imaging mode, \( t_{\text{exp}} \) is chosen such that particles appear as streaks across the imaging plane \( (\tau < t_{\text{exp}} < t_{\text{fov}}) \). If \( t_{\text{exp}} \) is known and the entire streak is contained in the image, the velocity can be determined from the streak length. If the particle density is sufficiently low to avoid overlapped particle images, the number density of particles can also be determined by analyzing the intensity centroid of each streak. Ideally, the illumination source should have a well-defined top-hat temporal profile as well as uniform spatial intensity profile. This can be achieved with CW lasers, provided a fast shutter is available for either the laser or the imaging device. Longer streak lengths lead to better measurement accuracy, but also increase the chance of particle streaks overlapping and of streaks that partly fall out of the field of view. The optimum \( t_{\text{exp}} \) should be chosen according to these two factors.
“Snapshot” imaging

In the “snapshot” imaging mode, when $t_{\text{exp}} \ll \tau$, point-like particle images are produced on the detector, mitigating motion blur [132,133]. For example, particles moving at $v = 200$ m/s with $d = 1 \mu$m require a 5 ns exposure time to freeze the motion. The snapshot image can be achieved with short camera integration times or short illumination sources, e.g., pulsed lasers, flash lamps, or spark discharges [130]. We note that in the cases of streaked and snapshot imaging modes, one can determine particle positions at a resolution better than the resolution of the optical system through intensity centroid analysis, akin to super resolution microscopy molecule localization techniques [19,134]. The snapshot imaging mode has several advantages over the long-exposure imaging mode: it enables straightforward quantitative determination of particle beam density, and in principle one can infer particle volumes through integrated scattering intensity, if the system is well calibrated. The velocity and acceleration of particles can also be measured from snapshot images with the use of multiple exposures with known delays, provided that all particle images appear in the same field of view [130,135].

Provided that detector readout noise is not significant, snapshot imaging maximizes the signal-to-noise ratio (SNR) since unnecessary exposure time is avoided, and all scattered light entering the optics is focused to a single resolution element. Imaging based on continuous illumination and short camera integration time usually suffers from lower signal levels compared with pulsed illumination (assuming similar average optical power). This is due to the fact that in the latter case the intensity of a particle image is fixed by the intensity of the illumination, whereas in the former case only a small fraction of the CW laser power is used to illuminate the particle, i.e., most of the laser power is unused [130,132].

4.2.2 Transverse-plane particle imaging

Simple imaging of the transverse profile of the particle beam can be achieved with the help of a flat, sticky surface placed transverse to the particle beam propagation. The particle beam diameter can be roughly estimated from the deposition of particles on the plate, imaged either directly in-situ, or by analyzing the deposition under an external microscope [124,125]. Unlike side-view imaging techniques, this does not contain any information regarding particle dynamics, but nonetheless gives useful and rapid feedback on the performance of an injector. For instance, asymmetry of the particle beam in the transverse plane is difficult to observe with side-view imaging, but can be observed easily using this technique.
4.2.3 Laser scattering intensity

Imaging sub-micrometer particles through elastic scattering raises considerable concerns regarding scattering intensity at the detector. As we show below, a relatively modest setup can be used to image particles with diameters of a few hundred nanometers, where Mie scattering dominates. Mie scattering theory is typically applied for particle diameters down to approximately one tenth of the scattering wavelength, below which the simple Rayleigh theory becomes applicable. The latter is generally considered valid for the case \( d\pi/\lambda < 1 \), where \( d \) is the particle diameter and \( \lambda \) the wavelength of light. For a wavelength of 532 nm, this corresponds to \( d \sim 170 \text{ nm} \). In the regime \( 50 \sim 170 \text{ nm} \) both Mie and Rayleigh theories can be considered valid and yield comparable scattering cross-sections (see below). However, they differ significantly in theoretical treatment. Mie theory is based on an infinite series of spherical partial waves to describe scattering, whereas the Rayleigh approximation can be summarized as a single analytical expression. The former calculates the (complex) scattering phase functions, and therefore yields a directional scattering dependence, while Rayleigh theory assumes an isotropic scattering distribution (apart from a polarization correction). In the following basic theoretical treatment we focus on Rayleigh scattering theory, due to its mathematical simplicity and because it is a valid approximation in the size range of typical biological molecules.

The total Rayleigh scattering cross section for a sphere of diameter \( d \) and relative permittivity \( \epsilon \) is \[ \sigma = \frac{8\pi^5 d^6}{3\lambda^4} \left( \frac{\epsilon - 1}{\epsilon + 2} \right)^2 \] (4.1)

For a beam of diameter \( g \) and pulse energy \( E_0 \), the scattered energy is \( E = 4E_0\sigma/\pi g^2 \), and the number of scattered photons is \( N = E\lambda/hc \), where \( h \) is Planck’s constant, and \( c \) is the speed of light. Thus, we have

\[ N = \frac{32E_0\pi^4 d^6}{3\lambda^3 g^2 hc} \left( \frac{\epsilon - 1}{\epsilon + 2} \right)^2 \] (4.2)

The relative permittivity for proteins can vary significantly \[137\], but \( \epsilon \approx 2–4 \) is a reasonable assumption; polystyrene has \( \epsilon \approx 2.6 \). Figure 4.1 shows total scattering calculations for the Rayleigh and Mie regime for different particle diameters and laser wavelengths for the case \( \epsilon = 2.6 \). We must reduce the total scattered photon number \( N \) according to the fraction of photons observed. This results in a number of photons \( N_\Omega \) captured in the solid angle \( \Omega \) of the optical system. Neglecting polarization factors, we obtain

\[ N_\Omega = \frac{16E_0\pi^4 d^6}{3\lambda^3 g^2 hc} \left( \frac{\epsilon - 1}{\epsilon + 2} \right)^2 (1 - \cos \theta) \] (4.3)
4.2. Theory and background

**Figure 4.1:** Total number of scattered photons as a function of particle diameter for several wavelengths. Solid lines are calculated using the Rayleigh formalism, dashed lines are calculated from Mie theory. The calculation is done for 100 mJ pulses ($N_0 \approx 10^{17}$ photons) focused to a top-hat spatial intensity profile with diameter $\omega_0 = 1$ mm.

where $\theta$ (measured from the optical axis of the imaging system) is the maximum scattering angle collected by the optical system (the numerical aperture is defined as $\text{NA} = \sin \theta$). This provides a lower bound for experiments with the polarization axis of a linearly-polarized laser perpendicular to the optical axis of the imaging system.

The SNR of an imaging system depends on several factors. Since the typical size of single particle scattered intensity spans very few pixels on the detector, a pixel will collect approximately $N_\Omega$ photons from a particle. If the dominant noise sources of the imaging chip are the dark current, readout noise, background photons, and Poisson noise, the signal-to-noise ratio can be expressed as

$$SNR = \frac{N_\Omega Q}{\sqrt{N_\Omega Q + N_b Q + N_d + \sigma_r^2}}$$ (4.4)

where $Q$ is the quantum efficiency of the chip (number of electrons per photon), $N_d$ is the mean number of dark current electrons, $\sigma_r$ is the root-mean-square (RMS) readout noise (in number of electrons), and $N_b$ is the number of background photons per pixel. This estimate assumes that all photons collected by the objective are directed to a single pixel. As an example, the camera utilized in our measurements...
(Photron SA4) contains a CMOS chip that has a readout noise of 38 electrons, and a quantum efficiency of about 33% at 530 nm. Assuming that background photon levels can be reduced to nearly zero, a minimum of about \( \frac{38}{0.33} \approx 120 \) photons per pixel would be required to obtain a SNR of 1 with this chip. Factoring in the collection angle of the optics, we can roughly estimate that particles down to about 50 nm could likely be imaged with this detector. Smaller particles may require the use of single-photon detectors, such as EMCCD and SPAD detectors \[138,139\].

4.3 Experimental setup

![Diagram of experimental setup](image)

Figure 4.2: A schematic diagram of the basic direct-aerosol-imaging setup. In the left panel, the side-view imaging setup is shown with the different illumination geometries indicated by (A), (B) and (C). In the right panel, (D), the details of the in-vacuum microscope assembly are depicted, which is mounted on a three-axis translation stage and perpendicular to the particle beam injection.

Our experiment is constructed within a vacuum chamber that hosts an aerosol injector and, in some cases, X-ray diffraction detectors. For nebulization we use a gas-dynamic virtual nozzle (GDVN) \[4,43\]. The aerosol stream is delivered either by an ALS injector (Uppsala University, Sweden) or by a convergent-nozzle injector \[12\].
Side-view imaging in all three modes is implemented using a high-speed imaging configuration based on a high-frame-rate camera or on pulsed-illumination, as shown in Fig. 4.2. Imaging in the transverse plane is achieved with an inverted in-vacuum microscope that views particles as they adhere to a glass microscope slide coated with a sticky purified gel film (TELTEC, P/N DGL-20/17-X8).

### 4.3.1 Side-view imaging configuration

The key components in our side-imaging system are a high-frame-rate camera, both pulsed and CW illumination lasers, and imaging optics optimized for either a wide field of view or a high magnification. We discuss these components and their configurations below. We generally work in a quasi-dark-field imaging mode, where images are formed from scattered light without allowing the direct illumination beam to enter the optical system. For wide-field views, we use a long-working-distance (LWD) microscope (Infinity model K2, working distance 225–300 mm, depth of focus (DOF) ≈100 µm, magnification 2.13, and FOV 11.7 × 11.7 mm²) mounted outside of the vacuum chamber. For high-magnification views, a 10× infinity-corrected objective (Mitutoyo, working distance 38 mm, DOF 3.5 µm, magnification 28, FOV 850 × 850 µm²) is used, mounted on a three-axis motorized stage inside the vacuum chamber. Switching between these two configurations only involves swapping in/out the K2 objective and translating the high-magnification objective into position. The scattered light from the particle beam exits the chamber through a standard viewport and forms an image on a translatable high-frame-rate CMOS camera (Photron SA4) that is typically located about 350 mm outside of the chamber, see Fig. 4.2.

Our illumination system consists of three different optical lasers and two different illumination geometries. In the first configuration the full particle beam is illuminated with a collimated, counter-propagating CW laser (Coherent Verdi V5, 532 nm, 5 W), (A) in Fig. 4.2. The laser beam is expanded and collimated not only to illuminate the whole particle beam, but also to avoid particle deflection and damage from the tightly focused beam. This geometry allows one to introduce a second illumination source or two simultaneous viewing axes and an X-ray beam for diffractive imaging. The latter was already implemented during a SPI experiment at the FLASH FEL facility in Hamburg, as discussed in Fig. 4.4. In the second illumination configuration we use a laser beam propagating perpendicular to the particle beam direction, as show in Fig. 4.2. This can be implemented alongside a counter-propagating illumination scheme. We have utilized two short-pulse lasers, a Nd:YLF laser (Spectra Physics Empower 30, 527 nm, pulse duration 100 ns, repetition rate 1 kHz, pulse energy 20 mJ, average
power 20 W), (B) in Fig. 4.2, and a fiber-coupled diode laser (DILAS High-Power Diode Laser IS21.16-LC, 640 nm, average power 10 W), (C) in Fig. 4.2. The latter is powered by a high-speed diode driver (Dr. Heller Elektronik, UHS-500-12.8 A, repetition rate up to 1 MHz, pulse durations 10–100 ns) and mounted in oblique orientation to maximize forward scattering. The diode laser is the least expensive option and delivers a top-hat intensity profile.

### 4.3.2 Transverse-plane imaging configuration

An inverted microscope is located directly below the aerosol injector to image 2D transverse beam profiles in real time, as shown in detail in the right panel of Fig. 4.2 (D). A 5× infinity-corrected objective forms images as particles adhere to a transparent gel on a microscope slide that is manipulated with a three-axis translation stage. A polarizing beam splitter is mounted below the microscope slide, which allows scattered light to be imaged while the counter-propagating laser illuminates the particle beam for side-view imaging. The entire microscope assembly is mounted on a three-axis motorized stage so that it can be moved in and out of the interaction region during experiments, or translated along the axis of the injector to probe the particle beam at variable distances from the tip of the injector. In addition to producing transverse views of the particle beam, the microscope slide is used to protect the counter-propagating laser optics (since few particles adhere to the bare glass slide) as well as to align the laser beam to the particle beam. This alignment is done by iteratively tilting the laser beam or translating the injector while viewing the particle/laser overlap at two different distances (50 mm apart) along the axis of the injector.

### 4.4 Experimental results and discussion

#### 4.4.1 Side-view imaging

Representative images from our side-view imaging scheme are shown in Fig. 4.3 for the different imaging modes introduced in Fig. 4.2. Figures 4.3(a), 4.3(b), and 4.3(d) show images of \( d = 2 \mu m \) polystyrene-sphere particles (PS), and in Fig. 4.3(c) shows an image of \( d \approx 300 \) nm granulovirus (GV) particles. The PS particles were injected with an ALS, whereas GV particles were injected with a convergent-nozzle injector. Figure 4.3(a) shows a typical long-exposure image collected with counter-propagating CW beam illumination, which may be interpreted as a projection of the particle beam density since the particle size distribution is relatively narrow.
Figure 4.3: Experimental particle-beam images. (a) Long-exposure image with CW laser illumination and 2 s camera exposure. (b) Particle-streak image of 2 µm PS moving at 18 m/s using CW laser illumination and a 13.5 µs camera exposure time. The length $S$ and width $D$ of the particle streak are marked in the image. (c) Streak image of granulovirus particles size 200 nm $\times$ 200 nm $\times$ 400 nm moving at a speed of 240 m/s, using 100 ns diode laser illumination and a 1 ms camera exposure time (100 pulses in the single camera exposure). (d) Snapshot image of 2 µm PS particles using 100 ns pulses from the Nd:YLF laser and 20 ms camera exposure (20 pulses in the single camera exposure). The center of the red circle depicts centroid of a particle snapshot.

(< 5%). The results of streak imaging for particles moving at two different speeds are shown in Figs. 4.3(b) and 4.3(c). Figure 4.3(b) shows particle streaks recorded using counter-propagating CW beam illumination and a short integration time of 13.5 µs on the camera, while Fig. 4.3(c) is recorded with illumination by multiple
100-ns laser pulses and a long camera integration time of 1 ms. A snapshot image with $t_{\text{exp}} < \tau$ of 2 µm PS moving at approximately 18 m/s is shown in Fig. 4.3(d). Here, short illumination times (100 ns) and relatively slow particle speed lead to distinct single spots on the camera, highlighted in Fig. 4.3(d) by red circles, which are centered around the calculated centroid positions of individual particles.

In the following we demonstrate how these data can be used to reconstruct the particle-beam density and velocity distributions. Two-dimensional particle density maps generated from raw side-view images are shown in Fig. 4.4. For CW illumination in the long-exposure mode, the image intensity is directly proportional to the projected particle density, provided that only a single particle species is present. This allows for direct monitoring of the injector behavior through the observation of relative image intensities, but does not readily allow for a quantitative evaluation of particle number densities without careful calibration measurements. On the other hand, streak and snapshot imaging modes allow for direct and quantitative measurements of the particle beam density without the need for intensity calibrations, since particle image centroids (for both streaks or spots) can be determined with a precision better than diffraction limit $\text{[19,134]}$. From these centroids (seen in Fig. 4.3(d)), projected particle density maps can be produced, which allow quantitative estimates of expected hit fractions in SPI experiments.

The streak imaging technique allows for the estimation of particle velocities through evaluation of the streak length with a single pulse using a well-calibrated imaging system, as indicated in $\text{[4.3(b)]}$. A velocity measurement is also feasible using snapshot imaging if more than one illumination pulse occurs while the particle
is in the field of view, either in the same frame or successive frames. We note several pitfalls that need to be avoided for accurate determination of particle velocity distributions from side-view imaging measurements:

1. The temporal illumination intensity profile of a pulsed laser source will be reflected in the spatial intensity of the particle streak; often one might observe long, faint trails from each particle, due to a slow decay of the laser pulse intensity. An accurate determination of the velocity requires knowledge of the temporal laser profile to disentangle the spatial image of the particle. Ideally, the illumination source should have a top-hat temporal profile.

2. Particles moving out of the illuminated volume or FOV during the exposure time will appear to produce shorter streaks. This can be avoided by ensuring the illumination to be large enough to cover the entire particle beam in the FOV and ignoring streaks that lead to the edge of the image during velocity analysis.

3. Particles that move outside of the depth-of-focus of the imaging system will result in de-focused images and in some cases non-uniform streaks. If not corrected for, this will result in systematic errors in velocity estimates. However, the inclusion of image de-focus in the analysis algorithm could, in principle, reveal 3D information from a single view upon careful calibration \[142,143\]. For the narrow particle beams considered here, de-focus is typically not a significant problem and can be ignored.

Once particle densities and velocity distributions are obtained, the injector transmission efficiency can be determined by comparing the rate \((R_{\text{in}})\) at which particles enter and the rate \((R_{\text{out}})\) at which they leave the injector. \(R_{\text{out}}\) can be calculated from the expression \(n = R_{\text{out}}l/v\), where \(n\) is the total number of particles contained within a planar slab of thickness \(l\), where particles are injected at a frequency \(f\) at a velocity \(v\) in the direction normal to the slab \[12\].

### 4.4.2 Transverse-plane imaging

Poorly performing injectors sometimes generate asymmetric particle beams, analogous to astigmatisms in optical systems. This is not readily detectable in side-view imaging configurations, but is clearly visible through transverse-plane imaging with the inverted microscope discussed previously. Figure \[4.5\] shows particle-deposition images at different distances from the tip of an ALS injector, and a clear variation in particle beam asymmetry with position. For particles larger than 1 \(\mu\)m, individual particles can be detected as they adhere to the gel, allowing semi-quantitative analysis of the particle beam width on the transverse plane in real time, as shown in
Fig. 4.5(c). However, the accuracy of the analysis is limited by our understanding of how particles adhere to the gel surface—most importantly, how the likelihood of particle adherence changes with time, e.g., as particles accumulate. For particles on the order of 100 nm or smaller, a detectable particle-deposition image is obtained after a few seconds of accumulation time, depending on the concentration and size of particles.

Figure 4.5: Imaging of 2 µm PS particles from a beam focused by an ALS injector and deposition on transparent gel through an in-vacuum inverted microscope. (a) A series of images recorded at different distances from the injector tip. (b) A lateral scan of the microscope slide at a distance of 35 mm from the injector tip, recording particle distributions following a 1 min particle deposition per spot. (c) The transverse particle-beam density profile obtained in a measurement similar to (b), but under conditions where individual particles could be observed and their centroids determined. (d, e) 1 min particle depositions from a poorly performing injector, which, perhaps, is caused by dispersion in particle sizes and/or asymmetry in the particle source at the inlet of the injector.

4.4.3 Injector optimization

The presented characterization methods offer a powerful means to optimize the performance of particle injectors, both online during SPI measurements at XFEL facilities, as well as offline in the preparation laboratory. As discussed in Fig. 4.2 the 2D projected number density of the particle beam is the most important parameter
that needs to be optimized, since it scales directly with the hit fraction in an SPI experiment. The hit fraction depends on particle velocity, injector transmission, and particle beam diameter, which are ideally measured independently while developing and optimizing aerosol injectors. Figure 4.6(a) shows a typical plot for injector optimization, including the velocity and particle-beam diameter in the case of 2-µm PS particles measured 35 mm downstream from the tip of the injector, as a function of the upstream pressure of the injector. The downstream chamber pressure is maintained below 10^{-2} mbar and does not significantly effect the particle speed or beam diameter. The velocity increases linearly with the upstream pressure. However, the particle beam size exhibits a distinctive minimum around 30 µm FWHM, at an upstream pressure of 0.63 mbar. As seen from Fig. 4.6(b), the particles are moving at an average velocity of 18.49 m/s with standard deviation of 0.28 m/s at this upstream pressure. Ignoring the transmission efficiency for now, the optimum operating pressure of the injector for maximum hit fraction should be chosen such that the product of these two parameters is minimized (see Fig. 4.2), i.e., for 2 µm PS particles, the injector should be operated at 0.6 mbar for maximum hit fraction. Alternatively, one may simply measure the projected particle beam density, which automatically accounts for the contributions of velocity, transmission efficiency, and particle beam diameter. In practice, the assumption of a constant transmission efficiency is not valid over a large pressure range, and this needs to be taken into account. We note that using streak or multiple-exposure snapshot imaging allows a quantitative measure of the transmission efficiency.

Figure 4.6: Injector-performance measurement for 2 µm PS particles focused by the aerodynamic-lens-stack aerosol injector. (a) Particle velocities and projected density versus injector upstream pressure. (b) Velocity distribution of the particles at 0.65 mbar upstream pressure.
4.4.4 Integration with X-ray experiments

We demonstrated the utility of optical particle-beam imaging using a custom SPI experimental apparatus at the FLASH free-electron laser facility in Hamburg, Germany. Due to space limitations, we utilized a counter-propagating 5W CW laser, as shown in Fig. 4.7. An in-line microscope with a long-exposure CCD was placed on the same axis as the X-rays, in addition to a high-speed camera that imaged the particle beam from a viewpoint perpendicular to the X-ray beam axis. This enabled us to have, simultaneously, two orthogonal side views of the particle beam. The long exposure images from the in-line microscope were used to position of the injector for maximum hit rate, whereas images from the high-speed camera were used to position the beam with respect to the X-ray focus and to provide real-time estimates of the particle velocity and number density. As seen from Fig. 4.3.1, the counter-propagating illumination scheme leaves plenty of spaces around the interaction region for multiple views and additional diagnostics. However, it requires careful alignment of the laser with the particle beam, especially for the case of CW lasers that must be focused to smaller diameters (approximately 100 µm in this particular case) than pulsed lasers of equivalent average power. We therefore installed translation and tilting stages inside the vacuum chamber for steering and translating the laser beam. In order to mask the scattering light from the injector tip we constructed a light shielding around the objective lens. As seen in Fig. 4.7, once the CW laser is properly aligned, the average intensity from a beam of GV particles is easily visible to a typical CCD (in this case, a consumer single-lens reflex camera). The ability to immediately see a particle beam drastically reduced the time needed to align the injector, and immediately revealed the typical fluctuations in the injector transmission efficiency.

4.5 Summary and conclusion

We demonstrated the utility of direct optical imaging of micro- and nano-particle aerosol beams for the purpose of improving the overall efficiency of single-particle X-ray diffractive imaging experiments. We find that direct imaging of the particle beam is a straightforward means to quantitatively measure particle density maps, particle velocity distribution, and injector transmission efficiency, which are key diagnostics for optimizing SPI experiments at large-scale X-ray facilities where the time available for measurements is rather limited. A modest setup with an off-the-shelf CW laser of ∼1–5 W power can readily reveal the time-averaged position and width of a typical particle beam, which greatly simplifies the procedure of positioning the injector with respect to the X-ray beam. The overall brightness
4.5. Summary and conclusion

Figure 4.7: Photograph of the setup for granulovirus particle injection and visualization around the X-ray interaction region during a SPI experiment at FLASH. The focused X-ray beam passes through a 2.5 mm hole in the center of the in-line microscope objective before intersecting the particle beam. The direct X-ray beam is blocked by a beam-stop, which is mounted in front of the detector. Note that the beam-stop and detector were retracted and are not shown in this picture. This photo was taken by a digital single-lens reflex camera (Nikon, Coolpix P510) through a window on the experimental vacuum chamber.

of the particle beam is also indicative the injector performance. Remarkably, such a simple diagnostic can save many hours of effort, and corresponding facility costs, compared to “shooting blind”, i.e., when injection is optimized based on X-ray diffraction data. We also showed that pulses of well-defined duration as well as a CW laser combined with a camera with a fast shutter can simultaneously produce quantitative particle-density and velocity-distribution maps. Our side-view imaging schemes were complemented by a compact in-vacuum microscope that enables indirect particle beam imaging in the transverse plane, which readily reveals particle-beam astigmatism that is not easily observed from viewpoints that are orthogonal to the particle beam.

In the configurations considered here, we have also imaged individual 200 nm diameter particles moving at speeds of 300 m/s [12] with a modest short-pulse laser (100 ns and 10 W average power). Simple scattering estimates suggest that much smaller particles, perhaps down to few tens of nanometers, should also be visible with a sufficiently intense illumination (approximately 100 mJ pulses focused to about 1 mm diameter) and a very-high-sensitivity imaging device. Although
velocity measurements can be made from short pulses that create streaked particle images, it appears that the optimal method for determining velocities, from a signal-to-noise standpoint, is through the use of two time-delayed pulses of duration short enough to produce “snapshot” diffraction-limited particle images. Pulse durations of approximately 5 ns are required to freeze the motion of particles moving at 200 m/s for an image resolution of 1 µm, but Q-switched lasers that produce such pulses are common and relatively inexpensive.

We tested three different imaging modes that differ in terms of illumination geometry, optics, and the illumination source. Each of them can be implemented relatively straightforwardly in typical SPI experiments with only minor modifications. A counter-propagating geometry, in which the particle and laser beams oppose each other, maximizes the space available for ancillary diagnostics such as time-of-flight spectrometers, but requires a transparent shield to maintain clean beam-steering optics below the injector and unnecessarily exposes upstream particles to laser illumination. For imaging the smallest of particles, it may become necessary to operate above the damage threshold of the particles. Hence, a transverse illumination scheme would be required to avoid damaging particles prior to probing with X-rays.

Thus far, our apparatus has been used to characterize the injection process downstream of the injector, close to the interaction region of particles and X-rays. It would be advantageous to include similar imaging diagnostics at positions upstream of the injector exit, so that the aerosol formation and pre-collimation (prior to focusing) can also be monitored and de-coupled from the downstream particle-beam focusing components. Ideally, these diagnostics would be extended to include particle size measurements through careful calibrations of integrated scattering intensity, Mie scattering profiles, or other interferometric methods. Such in-situ measurements would allow us to monitor particle aggregation and evaporation rate of the liquid buffer from the initial droplets generated by the nebulization device [144].

The main message of this manuscript is straightforward: it is “relatively easy” to directly visualize a high-speed nanoparticle beam. Remarkably, this simple realization was overlooked for the first eight years of SPI development, presumably due to prevailing assumptions that Rayleigh scattering from high-speed particles would not produce detectable signals. As we see it, our manuscript dispels a long-standing perception that has hindered progress in the development of aerodynamic lenses, single-particle imaging, and presumably other fields of research.
5 Simple convergent-nozzle aerosol injector for single-particle diffractive imaging with X-ray free-electron lasers

A major challenge in high-resolution X-ray free-electron laser-based coherent diffractive imaging is the development of aerosol injectors that can efficiently deliver particles to the peak intensity of the focused X-ray beam. Here, we consider the use of a simple convergent-orifice nozzle for producing tightly focused beams of particles. Through optical imaging we show that 0.5 µm particles can be focused to a full-width at half maximum diameter of 4.2 µm, and we demonstrate the use of such a nozzle for injecting viruses into a micro-focused soft-X-ray FEL beam.

5.1 Introduction

X-ray free-electron lasers (XFELs) offer a compelling new approach to imaging a wide variety of aerosolized particles at high resolution and under conditions that are not accessible through cryogenic electron microscopy or synchrotron-based X-ray microscopy. XFELs produce intense X-ray pulses of only a few tens of femtoseconds in duration, which are sufficient to overcome the fundamental resolution-limiting effects of X-ray radiation damage [145] by making the illumination duration shorter


Together with R.A Kirian, N. Eckerskorn and M. Wiedorn, I conducted the measurements in the lab. Together with the other authors, I did the measurements at FLASH, and my main responsibility was the particle injection and instrumentation. I also contributed in the data analysis and writing the manuscript.
than the time scale for the onset of significant atomic motion \[1, 33, 146, 147\]. Diffraction patterns can be used to form images of targets without the need for lenses, and at resolutions limited, in principle, only by the X-ray wavelength \[148-149\]. Two dimensional sub-nanometer-resolution images should be achievable from single-shot diffraction patterns of irreproducible targets such as living cells and aerosol particles, and three-dimensional atomic-resolution structure determination should be possible by assembling many patterns from reproducible targets such as proteins and viruses (each of which is destroyed completely by an XFEL pulse) \[150\]. Since femtosecond pulses outrun atomic motion with timescales on the order of 10 fs, in principle, practically any target can be studied without the need for cryogenic cooling, which is usually required in electron microscopy \[151\] and X-ray microscopy \[152\] of biological samples. Time-resolved studies are also enabled, for example, by inducing structural changes with an optical laser that precedes an X-ray pulse \[153\].

Coherent diffractive imaging of aerosols was first demonstrated at the FLASH soft-X-ray FEL facility \[3\] and has recently been extended to the hard-X-ray regime at facilities such as LCLS and SACLA. A wide variety of results have emerged in recent years, including images of RNA microsponges \[154\], viruses \[4\], cell organelles \[5\], and whole cells \[16\]. Three dimensional structures have been determined from inorganic particles \[61\] and viruses \[6\]. Aerosol particulates have been studied \[155, 156\], as have superfluid helium droplets \[157\], atomic clusters \[158\], small gas-phase molecules \[123\], and metallic nanoparticles \[159\].

XFEL-based imaging is best performed on isolated, substrate-free targets, in order to avoid scattered-photon noise and reduced contrast associated with surrounding materials such as liquid solvent or solid supports. XFEL pulse repetition rates are presently 100–120 Hz, and samples, therefore, must be replaced in rapid succession. X-ray focal spot diameters are typically in the range of 0.1–5 \(\mu m\), and the rate at which X-rays intercept targets is of extreme importance since XFELs are costly large-scale facilities based on linear accelerators that are typically available to only one user group at a time. To this end, most femtosecond single particle imaging experiments have utilized aerodynamic lens stacks \[3, 56, 160\] in order to concentrate and inject particles into the vacuum environment of experimental end stations. These lens stacks consist of a series of concentric axis-symmetric apertures that cause particles to migrate toward the central streamline upon optimization of the relative magnitudes of particle inertial forces and gas drag forces. As shown by \[161\], particles in an incompressible and irrotational gas flow field generally tend to follow trajectories towards regions of higher density. Aerodynamic lens stacks are capable of producing collimated streams of protein-sized particles (of the order 5–30 nm in size) with particle beam diameters of a few hundred micrometers \[125, 162\]. For particles such as large viruses (on the order of 30–500 nm), particle beams can be produced with diameters of just a few tens of micrometers \[163, 164\].
and software are available to assist in the design of aerodynamic lens stacks [38].

Experimental hit fractions (fraction of X-ray pulses that intercept a target) are proportional to the X-ray beam cross-section, and sub-micrometer dimensions are often required due to the need for very high intensity when imaging small, weakly scattering objects. Hit fractions are determined by the X-ray beam area and the projection of the particle-beam density along the X-ray beam. The projected particle beam density is inversely proportional to both the particle beam diameter and the speed of the particles, assuming a fixed rate at which particles exit the injector. One can improve the efficiency of XFEL experiments by reducing either of these parameters. As an example, hit fractions of about 80 % have been achieved for a 5 µm X-ray beam by [5], but this fraction would drop to 0.032 % in the case of a 100 nm X-ray beam and otherwise identical conditions, assuming that the hit fraction is proportional to the area of the X-ray beam. Notably, most of the hits reported by Hantke et al. were faint hits corresponding to particles located far from the center of the X-ray beam. If the particle beam was focused to dimensions smaller than the X-ray focus, even at the expense of higher particle speeds, the fraction of faint hits could be reduced significantly. Sample delivery efficiency (fraction of injected targets that are intercepted by an X-ray pulse) is another concern, since samples are often available only in small quantities and can be expensive to produce. Delivery efficiency is proportional to the hit fraction as well as the XFEL pulse repetition rate.

In this paper, we investigate the use of a compact single-orifice aerosol injector, operating with a 1 bar pressure difference between the injector and vacuum chamber, for XFEL diffractive imaging experiments. Our design is motivated by extensive work spanning several decades [165–168] that show how sub- or super-sonic free jet expansions into vacuum from a single capillary or convergent orifice can produce particle beams with either small angular divergence or tight focus. Slowly converging capillary injectors are also under investigation, mainly for the purpose of aerosol-based printing applications [169], and recent work has shown that nanoparticles can be focused to diameters of less than 2 µm [170]. As we show here, convergent nozzles are convenient due to their compact size (in our case, only about 1 mm in diameter and 20 mm long), are simple to fabricate and operate, and can maintain targets at atmospheric pressure until they rapidly exit into vacuum in less than 1 µs. Such injectors can produce tightly focused beams of sub-micrometer particles, with focal spots of about 5 µm diameter. Our experiments performed with a 1 µm diameter soft X-ray FEL beam suggest the basic feasibility of utilizing such injectors for coherent diffractive imaging work.
5.2 Injector design and operational concept

The injector design considered here consists of a single converging nozzle orifice. Inside of the nozzle, where near atmospheric pressure is maintained, particles closely follow the convergent gas streamlines. Outside of the nozzle, the gas freely expands into vacuum, and particles of sufficient momentum continue along their initial, radially inward trajectories. This focusing scheme is defined by the angle of the internal convergent nozzle walls, and results in particle trajectories that cross the nozzle’s axis of symmetry at a nearly common point. We refer to this common crossover point as the focal point of the injector, but note that this point may vary slightly for particles that are initially located at different distances from the symmetry axis. Figure 5.1 shows the basic operational concept of the injector.

![Diagram of aerosol injector assembly and convergent nozzle](image)

Figure 5.1: (a) Schematic of the aerosol injector assembly and convergent nozzle. Liquid drops are formed in a nebulization chamber via a gas dynamic virtual nozzle, which then pass through a transport tube before reaching the convergent nozzle depicted in (b). Particle trajectories closely follow the gas streamlines within the convergent nozzle, which is at near atmospheric pressure. Upon exiting the nozzle, the pressure suddenly drops, and the ejected high-speed particles follow nearly straight line trajectories, though they may accelerate slightly upon exiting. All particles cross over the nozzle’s axis of symmetry at a common focal point that varies only slightly with the initial position of the particles at the exit orifice. The slightly curved trajectories of particles exiting the nozzle are exaggerated for illustrative purposes.
In our first experiments, described here, we used an injector with a convergence angle of 30°, and an exit orifice diameter of 100 µm. A central cross section through a 3D X-ray tomogram of the injector nozzle tip is shown in Fig. 5.2(a). Ceramic nozzles were fabricated through an injection molding process (Small Precision Tools Inc.) using a mixture of corundum (Al₂O₃) and zirconia (ZrO₂). These nozzles had an overall length of 20 mm, an inner diameter of 0.5 mm, and an outer diameter of 1 mm. Aerosolized particles were produced with a gas dynamic virtual nozzle (GDVN) [43], which typically generated droplets of about 1–1.5 µm diameter at a liquid flow rate of about 1–3 µL/min, and a gas mass flow rate of about 20 mg/min (gas and liquid pressures of about 20–50 bars are typical). The GDVN was housed in a nebulization chamber with inner diameter of about 4 cm and length of about 12 cm. The aerosolized particles passed through a metal tube of 70 cm length and 2.8 cm inner diameter, onto which the nozzle was fixed. The small ceramic tip was epoxied to a glass capillary for ease of mounting. The pressure in the nebulization chamber was monitored with a dial gauge and was typically within about 20 % of atmospheric pressure, depending on the flow rate of the liquid focusing gas in the GDVN. The pressure did not rise far above atmosphere in general, since a one way valve was used to avoid over pressurization and possible rupture of the nebulization chamber. We used helium as the carrier gas in order to minimize X-ray scattering, and because GDVNs tend to perform best with a lightweight monatomic gas. The use of helium as the carrier gas also increases the particle inertia (as compared to N₂, for example) and thereby facilitates focusing of smaller particles. Figure 5.2(b) and (c) show the injector mounted inside of the vacuum chamber, with a magnified view of the nozzle tip.

Our choice of nozzle geometry and orifice diameter was based on the components that were available to us for these first experiments. We chose an atmospheric pressure condition within the nozzle both for simplicity and to maintain samples in physiological conditions. Due to the large pressure difference between the aerosol delivery tube and the chamber, the flow through the nozzle is assumed to be choked. In this condition, the exit velocity of the helium gas is limited to the speed of sound, and further reductions in the chamber pressure below 0.4 bar will have no effect on this speed. For a 100 µm orifice, we estimate that the mass-flow rate for helium at atmospheric pressure upstream is 60 mg/min, which is similar to the typical 10–100 mg/min flow rates of our GDVN nozzles. Under this condition, the exiting gas velocity at the centerline of the nozzle reaches values near Mach 1 [165], approximately 1000 m/s for helium at standard temperature and pressure. Due to the abrupt convergence of the nozzle, particles of sufficiently large aerodynamic size may not have sufficient time to reach their terminal velocity, which is desirable since hit fractions are inversely proportional to the speed of the particle stream.
In nearly all aerodynamic focusing schemes, the parameter of greatest importance is the Stokes number, defined as \( S = \frac{v_g \tau}{D} \), where \( \tau \) is the particle relaxation time or the inverse of the proportionality constant between viscous acceleration of the particle and the difference between particle and gas velocities; \( \tau \frac{\partial^2 v_p}{\partial t^2} = v_g - v_p \), where \( v_p \) is the particle velocity [124]. The relaxation time is equal to \( \tau = \frac{\rho_p D_p^2 C}{18 \mu g f} \), where \( \rho_p \) is the particle density, \( D_p \) is the particle diameter, and the parameters \( C \) and \( f \) are correction factors that depend on particle Knudsen number (ratio of gas mean-free path to particle diameter) and Reynolds number [56]. The Stokes number for our nozzle, with 0.5 µm diameter particles of density 1.05 g/cm\(^3\), is approximately equal to 17. The critical Stokes number, at which particle beams are focused at infinity, is typically \( S \approx 1 \), although this depends on the exact nozzle geometry [56][126]. The relaxation time for a 0.5 µm diameter polystyrene particle (density \( \rho_p \approx 1.05 \text{ g/cm}^3 \)) in atmospheric helium is approximately equal to 3.5 µs, which, as we show below, is longer than the 750 ns that it takes for the particle to reach the focal point at a distance of 205 µm from the injector tip.

Similar flow fields can be achieved when scaling the present design in overall size while constraining the nozzle Reynolds number \( Re = \frac{\rho_g v_g D}{\mu_g} \approx 940 \), where \( \rho_g \approx 0.18 \text{ kg/m}^3 \) is the helium gas density, \( v_g \approx 11,000 \text{ m/s} \) is the assumed average gas velocity at the nozzle exit plane, \( D \approx \times 10^{-4} \text{ m} \) is the nozzle orifice diameter,
and $\mu_g \approx 1.9 \times 10^{-5}$ Pa s is the viscosity of helium at standard temperature and pressure. This Reynolds number is significantly larger than that of an aerodynamic lens stack consisting of several thin plate orifices, which are typically limited to $Re < 100$ in order to avoid turbulent flow conditions (Vidal-de-Miguel and de la Mora, 2012). Indeed, the work of Rao et al. (1993) showed that for values of $Re$ down to at least 15, the aerodynamic focusing effect for convergent nozzles is fairly insensitive to $Re$. An enlarged nozzle geometry would reduce the potential for nozzle clogging, increase the focal length of the converging aerosol beam, thus placing the X-ray beam further away from the nozzle end, and reduce the particle speeds. However, the particle-beam focus may increase with increasing nozzle size due to geometric aberrations, and the effects of diffusion may become significant.

5.3 Optical imaging and injector performance

Our injector was first tested through direct imaging-based measurements of particle velocities and projected particle beam density. A compact pulsed laser illumination scheme was implemented using a red diode laser of 635 nm wavelength and 10 W average power (DILAS model M1F4S22) coupled to a multi-mode fiber of 400 $\mu$m core diameter. The diode laser was pulsed with a custom built driver (Dr. Heller Elektronik) that generated 100 ns pulses with a top-hat temporal profile at repetition rates up to 100 kHz. The end of the fiber optic was situated near to the injector tip without focusing optics, and we imaged in a quasi-dark-field mode by setting the angle of the fiber optic such that the direct beam did not enter the imaging objective. We used a high frame rate CMOS camera (Photron SA4) and a 10× long working distance objective (Edmund Optic 46-144) to record images. The objective was mounted in the vacuum chamber at a working distance of about 33.5 mm, and the image projected through a window directly onto the camera sensor located outside of the chamber. This configuration proved to be sufficient for visualizing scattered light from fast moving polystyrene particles of diameter down to about 200 nm. Smaller particles can be imaged provided an imaging chip with higher sensitivity or a laser of higher intensity, e.g., with $\sim$10 W average power albeit at lower pulse repetition rate, as will be discussed in a subsequent report that details this, and other, optical imaging configurations [13]. We note that in individual exposures we can remove background signal and determine the centroid of an isolated particle at a resolution better than the imaging resolution [17]. Since the single snapshot images do indeed contain a sparse field of particles, the resolution to which we characterize the projected particle beam profile is likewise at high resolution, akin to the blink microscopy technique of photoactivated localization microscopy [19].
Figure 5.2 shows the fiber optic situated nearby the injector tip, and Fig. 5.3 shows a sum of 100 images, each with exposures by 5 laser pulses. Particle streaks in this image correspond to a 100 ns duration exposure. The velocity of the particles can be inferred from the streak length, which was typically $261 \pm 23 \text{ m/s}$ for 500 nm particles and $280 \pm 11 \text{ m/s}$ for 200 nm diameter particles. Relative particle densities can be estimated by identifying particles as elongated groups of connected pixels that fall above a configurable threshold. The centroids of identified particle streaks shown in Fig. 5.3 were assumed to be representative of particle positions, and we presume that this measure is reasonably accurate even for slightly out-of-focus particle images (the depth of focus for our objective is 3.5 \text{ µm}). The particle density maps shown in Figure 5.4 were formed from particle positions obtained in about 43000 exposures recorded at 1 kHz frame rate (processing on a desktop computer took about 5 min). The densities shown in these maps have been scaled to represent the condition in which particles enter the injector at a rate of 1 MHz, by using the known particle concentration and flow rates along with the number of laser pulses per camera exposure. Specifically, we multiplied the raw histogram counts by $1\text{MHz}/(cQN_f)$, where $c$ is the volume concentration of the sample, $Q$ is the volumetric flow rate of the liquid jet, and $N_f$ is the total number of laser flashes that contributed to the histogram. While these density maps may not be true 2D projections throughout the imaging plane, the assumption of a projection is reasonable near the focus of the particle stream. Transmission efficiencies may be calculated using the expression $n = fl/v$, where $n$ is the total number of particles expected to lie within a slab of thickness $l$ when particles are injected at a frequency $f$ at a fixed velocity $v$ in the direction normal to the slab. By comparing the calculated value of $n$ for 100 % transmission against the value of $n$ measured by the histograms, we estimate that the lower bounds on transmission efficiencies were $18 \pm 3 \%$ and $0.12 \pm 0.02 \%$ for the cases of 500 nm and 200 nm particles, respectively. We have no explanation for the large discrepancy in the transmission efficiency between the two particle sizes, but note that we have made no effort to optimize for transmission, and our particle identification algorithm has not been optimized to identify every particle (it rejects particle images with low signal-to-noise ratio, and overlapping streaks that fail to meet the maximum length criterion, for example).

The focal point of the particle beam was approximately 205 µm from the nozzle orifice, which the particles reach within about 750 ns after exiting the nozzle. This focal length is rather close to the distance of 187 µm that one would expect from a purely geometric focusing based on the 30° convergence angle of the nozzle (we presume that the longer observed distance may be attributed to the acceleration of particles upon exiting, as drawn qualitatively in Fig. 5.1). The throat diameter of the particle beam, outside of which there are few observed particles, is nearly
5.3. Optical imaging and injector performance

Figure 5.3: (a) A sum of 500 exposures of 100 ns duration laser illumination, revealing streaks from 500 nm diameter particles. (b) Particle positions determined from streak intensity centroids from 43,000 images, each with 5 laser pulses.

half the diameter of the orifice, similar to previous observations [126]. The FWHM diameter of the particle beams, determined by fitting a Gaussian profile to the focal region of the particle density maps, was 4.2 µm for 500 nm particles and 10.8 µm for 200 nm particles (see Figure 5.4). The pressure of the vacuum chamber was maintained at about 0.5 mbar. As predicted by our assumption of choked flow, we did not observe measurable differences in particle speed or density profile when this pressure was increased or decreased by a factor of 10.

Clogging is an important concern when using exit orifices of such small diameter. At the same time, it is likely that small focal spots will correspond to small orifices, so a practical compromise must be made. Cumulatively, the experiments that we have performed so far amount to about 20 h of operation, and over this time period we have encountered clogging issues twice. On the occasions that we observed these clogs, we noticed that the liquid jet was producing noticeably larger droplets than in ideal operation, suggesting that clogs may be averted by careful online observation of the aerosol droplets, perhaps through Mie scattering measurements, or by rejection of large droplets. The optical imaging results described above utilized solutions of polystyrene particles suspended in 2 mM sodium azide at a concentration of 0.04 % solids by mass and flow rates of 2 µL/min, which for our nozzle pressure of 45 bars corresponds to droplet diameters of about 1.3 µm [172]. Since these droplets are smaller than the 2 µm resolution of our optical microscope, we could not confirm the droplet size at their origin just downstream of the GDVN. For 200 nm particles,
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5.4 FEL Diffraction Experiments

We tested the feasibility of using our injector for diffractive imaging purposes at the FLASH soft-X-ray FEL facility in Hamburg, Germany. For these experiments, samples of Cydia pomonella granulovirus (CpGV)\(^{173}\) suspended in water at a concentration of \(10^{-11}\) particles ml\(^{-1}\) were used for injection. CpGV is a baculovirus that infects invertebrates such as the Codling moth (Cydia pomonella). In these viruses, a single virion, containing the viral genome, is natively embedded in an
occlusion body (OB), an in vivo grown polyhedrin protein crystal with a nominal size of $200 \times 200 \times 400 \text{ nm}^3$. The virus crystals show a narrow size/shape distribution, as can be seen in Fig. 5.5. The injector system described above was mounted to the diffractive imaging chamber, which was maintained at a pressure of about $5 \times 10^{-5} \text{ mbar}$. The laser imaging system described previously was not available during these measurements, which necessitated a time consuming scanning approach in order to overlap the X-ray and particle beam. The FLASH FEL is capable of producing pulse trains composed of an arbitrary number of pulses between 1 and 400, spaced with regular 1 $\mu$s intervals. These pulse trains repeat at 10 Hz frequency. The FEL beam was tuned to a wavelength of 13.45 nm, and produced pulses of $70 \pm 20 \mu J$ energy. X-rays were focused to a diameter of approximately 1 $\mu$m by a multi-layer-coated off-axis parabola with 27 cm focal length \cite{23, 174}. Diffraction patterns were recorded on a Princeton Instrument MTE 2048B CCD (2048 $\times$ 2048 pixels, each 13.5 $\mu$m in size, 4 s full frame readout) situated at a sample to detector distance of approximately 7 cm. The defocused direct X-ray beam was blocked by a 3–nm thick Cu beam stop.

In order to overlap the X-ray focus with the particle beam, we collected data in a many-shot mode in which the X-ray CCD integrated the diffraction signal from many shot pulse trains. Binned images were read out at a rate of 2 frames/s. We monitored hit rates on-line using a simple threshold criterion on the diffraction intensity while translating the injector via motorized translation stages. As hit rates approached 100 %, we reduced the number of pulses in each train, eventually reaching the point of single pulse operation. Approximately 2.5 h were spent on locating the particle beam. Figure 5.5 shows a single pulse diffraction pattern from
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an isolated granulovirus near the focus of the X-ray beam. The two prominent fringe spacings in this diffraction pattern correspond to 400 nm and 313 nm lengths, which agrees well with images of individual granulovirus particles. Image reconstructions were not possible due to the loss of low spatial frequency information contained in the beamstop and in the saturated regions seen in the second diffraction peak.

The injector was operated continuously for a total of 4.5 h, and over the course of the final 1.9 h of our experiment we performed several one-dimensional scans of the injector position such that the particle beam crossed the X-ray beam. These scans were performed for two different distances between the X-ray beam and the nozzle tip, which we roughly estimated were at about 300 and 450 µm downstream from the nozzle tip. We estimate that the smallest particle beam width across which we scanned the X-ray beam was 30 µm FWHM, as determined by plotting the average integrated intensity from 100 pulse exposures as a function of injector position (see Fig. 5.5 (c)). Near this location, an average hit fraction of 18% was estimated from a collection of single pulse measurements by assuming that all patterns with an integrated intensity above 3σ intercepted a particle, where σ is the standard deviation of integrated intensities for blank frames. This measure utilized a total of 99 frames that fell within a 30 µm window centered at the nominal particle beam focus. By visual inspection, about 90% of diffraction patterns appeared to arise from virus particle clusters. This is likely caused by the droplet size from the liquid jet being too large for the sample concentration. During these measurements, it is estimated that droplets of a few micrometers in diameter were produced from a nozzle running at a liquid flow rate of 3.5 µL/min, which would indeed result in more than one virus in each drop on average. Nearly all diffraction patterns exhibited a high degree of contrast and asymmetry, which suggests that most of the liquid from the initial droplets had evaporated prior to reaching the convergent nozzle exit (we would otherwise observe rather symmetric diffraction patterns consistent with nearly spherical objects).

Altogether, the data we collected during this experiment were very limited by the total available beamtime. While we observed a large fraction of aggregated particle clusters, we note that the mechanism for generating and conditioning the initial aerosol particle suspension is largely independent of the particle focusing mechanism that we describe here. An electrospray nebulization source, for example, can produce initial droplets that are about an order of magnitude smaller, and with number densities much higher, than the droplets produced by a typical GDVN.
5.5 Discussion and Conclusion

Our optical imaging experiments have shown that a simple convergent nozzle can focus low density nanoparticle beams to FWHM diameters of 4.2 and 10.8 µm, for particle diameters of 500 and 200 nm, respectively. We also demonstrated that such nozzles can be used to inject $200 \times 200 \times 400$ nm$^3$ virus particles into the 1 µm focus of a soft X-ray FEL. The question of how a convergent nozzle compares against an aerodynamic lens stack naturally arises, and we emphasize that these two injectors differ in many respects, and therefore can only be compared directly when a particular set of particle and pressure constraints are imposed. While the present work is by no means intended to conclude that convergent nozzles are better suited to XFEL diffractive imaging in general, we wish to identify the many motivations for their continued development. The size and shape of the nozzle we tested here are practically identical to that of a liquid jet nozzle, and therefore one can utilize the same basic hardware infrastructure for imaging experiments based on both liquid jet and aerosol injection, with little time needed to switch between configurations. The use of a small nozzle also suggests the feasibility of mass fabrication through injection molding techniques, as well as the consideration of acoustically pulsing the ejection of particles, which, when synchronized with the X-ray beam, could lead to significant improvements to sample delivery efficiency. Due to the small 100 µm apertures of the nozzles we tested, we were able to operate at atmospheric pressure within the nozzle, which may be advantageous for targets that must be maintained at nearly physiological temperatures and pressures.

We observed particle speeds in the range of about 230–300 m/s for particles in the range of 0.2–0.5 µm diameter, which is somewhat greater than the roughly 100 m/s speeds measured with aerodynamic lens stacks \[125\]. However, this speed increase is accompanied by a reduced particle beam diameter—if velocity increases in proportion to beam diameter, hit rates will be unaffected since this quantity scales inversely with both velocity and beam diameter. Our smallest observed beam diameter of 4.2 µm is considerably smaller than that achieved with aerodynamic lenses by more than the ratio of two of the particle velocity in the converging nozzle to that of the aerodynamic lens, suggesting that hit fractions should be higher than for aerodynamic lenses. Increased speeds may even be of advantage in some cases, for example, at high repetition rate XFELs where high energy debris from intercepted particles must be rapidly cleared away before a subsequent X-ray pulse arrives, and pre-exposure of upstream particles by the extended profile of the intense X-ray beam may cause radiation damage. In the case of tightly focused particle beams that are comparable to the size of the X-ray beam, the fraction of diffraction patterns arising from faint regions of the X-ray beam could be reduced significantly.
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The cost associated with the tight focus of a convergent aerosol beam is that maximizing the hit rate requires a three-dimensional scan of the injector position relative to the convergent X-ray focus, rather than a two-dimensional scan needed for a collimated particle beam (produced, for example, by an aerodynamic lens stack). We found this to be a significant challenge in our soft-X-ray FEL measurements. However, we have demonstrated a laser illumination system that can likely remedy this problem, with the added benefit of direct on-line imaging of the aerosol beam that would allow one to rapidly identify and diagnose injector problems, as well as optimize the sample injector for highest projected particle density [13]. It should be noted that the offline optimization of particle beam diameter alone does not necessarily optimize the hit fraction since factors such as particle velocity and transmission efficiency are also important. Direct imaging is a straightforward and non-invasive means of determining such parameters independently, although, ultimately, a projected particle density is the only measurement needed to estimate hit fractions.

The conditions that we investigated here, namely, atmospheric pressure and a small choked flow orifice, worked particularly well for particles of 0.5 µm diameter over periods of several hours. We observed that smaller particles of 0.2 µm diameter did not focus as well, and we expect this trend to continue with decreasing diameter since the focusing mechanism is strongly dependent on the particle’s momentum. In order to maintain atmospheric pressure upstream of the orifice, and simultaneously maintain the chamber pressure at sufficiently low pressure for nanoparticle imaging, it is likely that a smaller orifice is required to achieve adequate focusing. Clogging issues may arise for significantly smaller orifices, and therefore one might instead reduce the pressure in the nozzle in order to focus smaller particles.

Our present convergent nozzle injects gas into the experimental chamber at a higher mass flow rate than an aerodynamic lens stack, since lens stacks operate at lower pressures and necessitate pumping away part of the initial gas load. The increased gas density at the injector exit raises possible concerns regarding the background scatter from the evaporated buffer solution. We estimate that the total scattering from water vapor and helium is about two orders of magnitude lower than the total scattering from a 100 nm virus, assuming a 100 nm X-ray beam with 4 keV photon energy and the flow conditions presented above. Acceptable operational conditions will be strongly dependent on resolution, particle size, and X-ray beam diameter.

Our first proof-of-principle demonstration of a convergent nozzle for soft X-ray FEL-based nanoparticle imaging was very encouraging. Despite the limited time available for this study, we observed a high hit rate from a beam of viruses with a minimum diameter of 30 µm FWHM. Given this observation, combined with our
optical measurements, we believe that convergent nozzles are a promising pathway toward increased high intensity hit rates, from samples maintained at atmospheric conditions, with a relatively simple injector.
6 Femtosecond X-ray diffraction from an aerosolized beam of protein nanocrystals

We demonstrate high-resolution Bragg diffraction from aerosolized single granulovirus nanocrystals using an X-ray free-electron laser. The outer dimensions of the in-vacuum aerosol injector components are identical to conventional liquid-microjet nozzles used in serial diffraction experiments, which allows the injector to be utilized with standard mountings. As compared with liquid-jet injection, the X-ray scattering background is reduced by several orders of magnitude by the use of helium carrier gas rather than liquid. Such reduction is required for diffraction measurements of small macromolecular nanocrystals and single particles. High particle speeds are achieved, making the approach suitable for use at upcoming high-repetition-rate facilities.

6.1 Introduction

Serial femtosecond crystallography (SFX) allows the structural analysis of macromolecular crystals that may be too small or weakly scattering to study with synchrotron radiation sources. In order to record any measurable diffraction signal, such samples would require exposures far in excess of limits imposed by X-ray induced radiation damage when using conventional sources. With typical pulse energies of about 1 mJ, or $10^{12}$ photons, and durations of about 10 fs, pulses from


I conducted and analyzed the laboratory measurements. I did the measurement at LCLS with other authors, I was responsible for the particle injection and instrumentations. Together with R.A. Kirian, and various inputs from all authors, I wrote the published manuscript.
X-ray free-electron lasers (XFELs) overcome this limit by producing diffraction data before the onset of most damage processes. Furthermore, XFELs enable novel time-resolved studies with femtosecond temporal resolution, angstrom spatial resolution, all at physiological temperatures. A variety of prominent results from SFX measurements are summarized in recent reviews and special issues.

The large increase in X-ray fluence afforded by the ability to outrun damage not only increases the diffraction signal from the sample, but it also increases the diffuse scattering from the medium transporting the crystal to the beam. Many SFX measurements were, therefore, performed on microcrystals that were large enough and ordered well-enough to produce intense Bragg reflections that could be measured in the presence of the diffuse background. Such examples helped the rapid adoption of the technique. The use of such crystals, usually with volumes greater than 1 µm³, enabled a broad range of sample delivery methods to be used depending on the nature of the experiment, such as liquid microjets, viscous extrusion injectors or solid substrates. This flexibility is in stark contrast to efforts to record high-resolution coherent diffraction patterns from non-crystalline samples. Without the amplification of the diffraction signal due to periodicity, objects such as molecules, viruses, and other particles produce only weak scattering signals. Non-crystalline samples must, therefore, be delivered to the X-ray focus in a vacuum environment and in isolation from other potential scattering sources. This can be achieved, for example, through aerodynamic focusing of aerosolised particles. In certain cases, background scattering from a dense surrounding medium is highly undesirable even for experiments on crystalline samples. For example, imaging techniques have been developed to utilize the faint continuous diffraction signal in regions between and at scattering angles beyond the intense Bragg reflections due to lattice disorder or lattice truncation. The advantage and motivation for such approaches is that the continuous diffraction that can be accessed provides a direct route to solving the crystallographic phase problem without the need for prior knowledge or additional measurements.

Here, we demonstrate high-resolution X-ray diffraction from isolated protein nanocrystals delivered into the XFEL focus via a convergent-nozzle aerosol injector (CNAI). We show that the aerosol delivery produces extremely low background scattering signals compared with a conventional liquid jet. This aerosol injector has essentially the same size and form as the nozzles that are commonly used to produce liquid jets for SFX experiments and hence can be installed using standard liquid-jet mountings available at X-ray facilities. As shown in our previous work, CNAIs can produce aerosolized beams of sub-micrometer particles with a full-width at half maximum diameter < 5 µm and particle velocities on the order of a few hundred meters per second, depending on particle size and operat-
ing conditions. This high velocity may be well-suited to the MHz repetition rates of upcoming XFEL sources.

6.2 Experimental methods

This proof-of-principle experiment was performed on natural Cydia pomonella granulovirus (GV) particles of approximately $200 \times 200 \times 370$ nm$^3$ in size that consist of a central virus body surrounded by a crystalline granulin protein shell. They infect invertebrates such as the codling moth (Cydia pomonella) \[173\]. The GV particles used in this study were purified from a biopesticide solution (Certis Madex HP) using a method described elsewhere \[52\] and suspended in water at a concentration of approximately $3 \times 10^{11}$ particles/ml prior to injection. The particle concentration was measured using a NanoSight (model LM14C) particle analysis system. The volume of the particle is about $0.015 \mu m^3$, with about $2/3$ of that found as the volume of the crystalline shell \[190\], which corresponds to a diameter of approximately $300$ nm for a sphere of equivalent volume. Despite their small size, previous SFX experiments recorded diffraction to 2.1 Å resolution from such nanocrystals delivered to the X-ray beam in a gas-focused liquid jet \[190\].

Diffraction measurements were performed in the nanofocus chamber at the coherent X-ray imaging (CXI) \[191\] instrument at the Linac Coherent Light Source (LCLS). The experiment was carried out immediately after a successful liquid-jet experiment \[52\] without disruption to the X-ray beam. During that earlier experiment the beam focus was optimised by adjusting the Kirkpatrick-Baez (KB) focusing mirrors with the help of analysis of spot imprints on a gold foil. After optimisation the position of the beam was determined by placing a YAG screen in the focal plane and observing optical fluorescence with a fixed in-line microscope with a resolution of a few $\mu m$. In our experiment, the aerosol beam was initially aligned relative to this reference, and then scanned in position as described below.

The granulovirus suspension was aerosolised using a gas dynamic virtual nozzle (GDVN) \[55\] mounted in a cylindrical nebulization chamber as depicted in Fig. 6.1 (a). A GDVN uses gas flow focusing to create a liquid jet with a diameter significantly smaller than the orifice of the nozzle, and which consequently breaks up to form a mist of droplets. The liquid was pressurized to flow from the nozzle at rates between $2.7 \mu l/min$ and $3.5 \mu l/min$, producing droplets of about $2 \mu m$ diameter at a rate between $11 \times 10^6$ s$^{-1}$ and $14 \times 10^6$ s$^{-1}$, each containing on average 1.3 nanocrystals. This is equivalent to particle flow rates of $8.6 \times 10^8$–$1.1 \times 10^9$ particles/min.

The focusing gas was helium, which was set to a mass flow rate in the range of
Figure 6.1: CNAI assembly and its operation during the CXI experiment. (a) Sketch of the basic aerosol generation and transportation setup. (b) The aerosol nozzle mounted on the nozzle rod. (c) Time integrated image of a laser-illuminated stream of GV particles exiting the CNAI, recorded using the in-line microscope at the CXI instrument. This image was formed by averaging over 3.7 min, with a running median background subtracted from each frame. The CNAI tip is seen in the left portion of the image, and the approximate X-ray focal point is indicated by the star.

10–60 mg/min. The nebulisation chamber had an inner diameter of approximately 40 mm and was 150 mm in length, giving a residence time in the chamber of several minutes, and a helium pressure that stabilised at a value between 100 mbar to 1 bar. Under these conditions most of the solvent evaporated to produce nanocrystals suspended in a humid helium atmosphere [12]. Drops that contained more than one particle during the initial stage most likely formed clusters of crystals [14,42]. The aerosol flowed through conductive silicone rubber tubing (Simolex, 6.3 mm inner diameter, 30 cm length), which was coupled to a standard “nozzle rod” of the CXI beamline, which is a 1.2 m long stainless steel tube with a 6.3 mm inner diameter that is normally used to transfer liquid-jet injectors in and out of the main experimental chamber without breaking vacuum [192]. The conductive tubing along the entire particle path acted as a Faraday cage to shield external electric fields from interacting with particles that might become charged through triboelectric effects in the GDVN. The aerosol finally exited the CNAI, which was mounted at the end of the CXI nozzle rod much like a typical liquid-jet nozzle. It consisted of a ceramic injection-molded tube of 1 mm outer diameter, 500 μm inner diameter, a short converging section with a convergence angle of 15°, and a 100 μm exit aperture (further details can be found in our previous work [12]).
During the diffraction experiment we monitored the crystal injection through direct optical imaging of scattered laser light from injected particles \[13\]. A pulsed Nd:YLF laser (527 nm, \(\sim 3\) mJ per \(\sim 150\) ns pulse, 120 Hz) was focused to a \(\sim 0.8\) mm spot within the aerosol stream, and scattered light was observed through the in-line microscope available at CXI (Questar long distance microscope, model: QM-1 MK III, NA = 0.05 at 750 mm objective distance). Images were recorded using an OPAL-4000 CCD camera and stored at 30 Hz. Figure 6.1 (c) shows a 3.7 min time-averaged optical image of particles exiting the injector. We determined that particles moved at speeds of approximately 300 m/s when they exited the injector, to arrive at the X-ray interaction point within a flight-time of less than 1 \(\mu\)s. This particle speed was evaluated from the streak length of recorded particle images produced by laser illumination with a known pulse duration, conducted during laboratory characterization of the CNAI (see Fig. 6.2 (a) and Fig. 6.3). The CNAI tip is seen to the left of Fig. 6.1 (c), and the approximate X-ray focal point is indicated by the star. The particle stream could not be observed at points close to the CNAI tip because direct scattering from the tip saturated the imaging CCD.

6.3 Injector characterization and hit-fraction estimates

In order to develop and characterize the operation of the CNAIs we conducted tests of both 15° and 30° CNAIs in our laboratory. The setup differed from our previous work \[12\] by the inclusion of a narrow particle transport tube intended to replicate the delivery system used at CXI. Aerosolized GV particles were transported from the nebulization chamber to the CNAI tip using stainless steel tubing of 4 mm inner diameter and 700 mm length. The GDVN was operated at flow rates of 2.7 \(\mu\)L/min and 28 mg/min for liquid sample and helium, respectively. A GV concentration of approximately \(1.6 \times 10^9\) particles/ml was used (this was diluted by a factor of 200 from the solution that was used at the CXI experiment). This flow rate and sample concentration corresponds to the generation of drops at a rate of approximately \(1.1 \times 10^7\) s\(^{-1}\) and an entrance rate of aerosolized particles of \(7.2 \times 10^4\) s\(^{-1}\).

The imaging setup used for visualizing particles was described in detail previously \[13\]. Briefly, it was comprised of a Nd:YLF laser (Spectra Physics Empower ICSHG-30, 527 nm, approximate pulse duration 100 ns, repetition rate 1 kHz, pulse energy 20 mJ) to illuminate particles, a high-frame-rate CMOS camera (Photron SA4) and a 5\(\times\) magnification, 0.14 NA microscope objective to record images. The laser beam was collimated to a 2 mm spot, such that it illuminated particles across the entire field of view of the camera. The camera exposure time was set to 20 ms.
Figure 6.2: Laboratory characterization of a beam of GV particles focused with the 15° convergent aerosol-nozzle using a strong-magnification imaging microscope. (a) A single exposure showing streaked images of GV particles caused by the 100 ns laser illumination. The particles are moving from left to right and their streaked images have nonuniform intensity due to the relatively slow decay of the illumination laser pulses. (b) The two-dimensional rate-corrected particle density determined from the centroids of individual particle images such as the one shown in (a). (c) Gaussian fit to the particle density at the focal plane in (b).

such that each frame contained 20 pulses of the 1 kHz Nd:YLF laser illumination. A single image of particles emerging from the CNAI is shown in Fig. 6.2(a). The images are streaked due to the high velocity of the particles, and the observed intensity profile of these streaks reflects the relatively fast rise and slow decay of the Nd:YLF laser pulse. Centroid positions of individual particle streaks contained in 23,500 frames were used to produce the rate-corrected two-dimensional particle density map shown in Fig. 6.2(b). This rate-corrected density has units of particles per area per particle generation rate and is defined as

\[ D = \frac{N_p}{A \times R}, \quad (6.1) \]

where \( N_p \) is the average number of particles that fall within a spatial bin of area \( A \), and \( R \) is the rate at which particles entered the injector. Note that \( N_p \) represents the average particle counts at an instant in time and not a time-integration over
many exposures, which is appropriate because we intend to use the particle injector with femtosecond pulses. In our case, $N_p$ was computed by summing the number of particles that fell within each spatial bin, and then dividing by the number of recorded images and the number of laser illumination pulses per image.

The measured rate-corrected density $D$ may be used to estimate the optimal hit fraction that could be achieved under idealized conditions in our X-ray measurements. If an entrance rate of $R_X$ is used in the X-ray measurements, the 2D particle number density is $DR_X$. We define the effective cross-sectional area $\sigma$ such that the average number of particles intercepted by an X-ray pulse is $\sigma DR_X$. Assuming Poisson statistics, the probability of intercepting just one particle in an X-ray pulse is

$$H_1 = \exp(-\sigma DR_X)\sigma DR_X \approx \sigma DR_X$$

(6.2)

where the approximation holds to within $\sim 10\%$ error as long as $\sigma DR_X < 0.1$. We define the X-ray beam diameter as $d_X$ and the particle beam diameter as $d_p$ and estimate two limiting cases for the effective cross sectional area. The first case, $\sigma^+ = \frac{\pi}{4}(d_x + d_p)^2$, describes the optimistic limit in which a particle at the periphery of the X-ray beam produces acceptable diffraction. The second case, $\sigma^- = \frac{\pi}{4}(d_x - d_p)^2$, corresponds to the stronger assertion that an acceptable diffraction pattern requires that the entire X-ray-beam width falls within the particle (if $d_X < d_p$) or that the entire particle falls within the X-ray-beam width (if $d_p < d_X$). Finally, we arrive at two limiting hit-fraction estimates:

$$H_1^\pm \approx \frac{\pi}{4}D(d_X \pm d_p)^2R_X$$

(6.3)

The maximum rate-corrected particle density recorded in the laboratory, i. e., at the focus of the particle beam shown in Fig. 6.2 (b), was $D \approx 2.2 \times 10^{-9} \text{ µm}^{-2} \text{ s}$. Assuming the approximate values $d_X \approx 150 \text{ nm}$, $d_p \approx 300 \text{ nm}$, and $R_X \approx 11 \times 10^6 \text{ s}^{-1}$ suggests that the maximum hit fraction to be expected in our XFEL diffraction measurements is in the range $H_1^- \approx 0.04 \%$ to $H_1^+ \approx 0.4 \%$. This predicted hit fraction is much higher than the hit fraction we achieved during the CXI experiment, as discussed in the next section.

### 6.4 X-ray diffraction analysis and discussion

Diffraction measurements were conducted at a photon energy of 8 keV and an estimated average pulse energy of 4.2 mJ prior to the $\sim 30-50 \%$ beamline transmission losses [193]. The CSPAD detector was located 127.9 mm downstream from the X-ray focus. We recorded detector data frames for every X-ray pulse, at a rate
of 120 Hz for a cumulative total of 1.3 hours, which resulted in approximately 560,000 data frames.

In all of our diffraction analysis we excluded all pixels from each detector frame that had abnormally high or low variances or mean values in “dark” measurements made without X-rays, as well as a few patches of pixels for which there was obvious stray-light background. For every frame, the dark measurement was subtracted, and then a uniform common-mode electronic noise constant was subtracted from each detector panel. The common-mode offset was determined from unbonded detector pixels that are not sensitive to X-rays. The detector gain relating detector digital units to photon counts per pixel was obtained from a histogram of the pixel values, which yielded clear peaks corresponding to counts of zero, one, and two photons. Most of this analysis was performed using the Python `psana` package provided by LCLS [194].

Figure 6.3: One detector quadrant of an indexed diffraction pattern obtained from aerosolized GV crystals. The colored rings indicate the resolution from 10 Å to 3 Å, in steps of 1 Å. The gray circles in the left-hand panel indicate the expected locations of Bragg peaks as determined by auto-indexing in the CrystFEL software suite [195]. The right-hand panel shows expanded view of an individual detector tile, marked by the blue rectangle on the left. Circles in this expanded-view panel indicate peaks that are easily recognizable by eye. Notably, the predicted peak locations indicated by CrystFEL do not perfectly agree with those that the human eye notices, but this is typical of first indexing results and could be improved through the CrystFEL post-processing routines.
6.4. X-ray diffraction analysis and discussion

Figure 6.3 shows one quadrant of a recorded diffraction pattern from an aerosolized GV crystal, where the average detector dark frame and common-mode offsets have been subtracted. A total of 33 hits from GV were recorded, corresponding to a hit fraction of \( \sim 0.006 \% \). 24 patterns (73 % of hits) were indexed using the CrystFEL software suite \[195\]. Autoindexing failed on patterns that appeared to consist of multiple crystals clumped together. We expect the hit fraction for our aerosol injector to be significantly lower than a typical liquid jet (about 1-10%) because of the \( \sim 25 \)-fold higher particle speed of the aerosol beam and the \( \sim 4 \)-fold reduction in liquid flow rate. However, our recorded hit fraction was still lower than the range 0.04 – 0.4 % that we estimated from our laboratory measurements.

For comparing the background obtained using the CNAI to that typically observed in liquid-jet experiments we examined data from a previous SFX experiment \[52\] in which the exact same GV sample was injected into the X-ray beam as a liquid suspension with a GDVN. All experimental parameters were identical in both the CNAI and GDVN measurements except for the pulse energy, which was 4.6 mJ on average for the GDVN measurements.

The comparison of background scattering for the CNAI and GDVN approaches is presented in Fig. 6.4 which shows a plot of the normalized azimuthally-averaged profiles of scattered-photon counts (per pixel and per mJ of pulse energy), as a function of photon-wavevector transfer. The per-pixel standard deviations in the measurements are indicated by the gray regions in Fig. 6.4. The average profiles were divided by the average pulse energy to account for the slightly higher pulse energy in the case of the GDVN. The frames used in Fig. 6.4 were sampled uniformly from the final \( \sim 5 \) minutes of data collection, when the conditions were closest to optimal, although little difference was noticed in other measurement segments. We excluded frames that fell below 1 mJ pulse energy. We additionally excluded frames that were visually corrupt as well as those for which the X-rays obviously missed the liquid jet, which corresponded to less than 10% of the frames. After removing these outliers, we confirmed that more than 10,000 frames contributed to each of the two profiles.

As can be seen from the plots in Fig. 6.4 the liquid jet produces a background that is over 1000 times higher at a wavevector transfer of \( q = 2 \sin(\theta)/\lambda = 0.32 \) Å\(^{-1}\), corresponding to a resolution of 3.1 Å, where \( \theta \) is the Bragg angle and \( \lambda \) the wavelength. This coincides with the mean distance between oxygen atoms in water where diffuse scattering from water has its maximum. At low scattering angles the background from the liquid jet was about 200 times higher than for aerosol injection. The liquid jet for these measurements was operating at a flow rate of 20 \( \mu \)L/min. Typical liquid flow rates needed to produce a stable jet range from 5-30 \( \mu \)L/min, depending on the viscosity and surface tension of the liquid and the
Figure 6.4: Average radial intensity profiles, on a logarithmic scale, for data measured using the GDVN (labeled “Liquid Jet”) and the CNAI (labeled “Aerosol Injector”) injectors. The average per-pixel standard deviations determined from more than 10,000 frames are indicated by the vertical width of the gray regions. After averaging, the profiles and standard deviations were normalized by dividing by the average pulse energy, and then divided by the digital-to-photon conversion factor of 18.3. The horizontal axis corresponds to the wavevector transfer $q = \frac{2 \sin(\theta)}{\lambda}$ where $\theta$ is the Bragg angle and $\lambda$ is the wavelength.

Due to our convergent micro-focused particle beam, hit fractions are highly sensitive to the relative positioning of the CNAI with respect to the X-ray beam. Our initial diagnostic for particle beam positioning was direct imaging of scattered light, which allowed for the rough positioning of the CNAI. From this initial position, it was necessary to perform a subsequent two-dimensional scan of the injector position in an effort to optimize the spatial overlap between particle beam focus and X-rays. Due to the limitations of our 6-hour measurement shift, we only performed one 200 $\mu$m scan in the direction transverse to the particle beam and one 400 $\mu$m scan along the particle beam direction. It is therefore highly unlikely that we located the ideal position that maximizes the hit fraction. However, we expect that the background scatter we observed is representative of the gas and water
vapor exiting the injector because the gas expansion into vacuum is highly divergent. Direct imaging of the gas density leaving the CNAI shows that the gas plume spans volume hundreds of micrometers wide around the XFEL beam position.

Another possible culprit for our sub-optimal hit fraction is a sub-optimal aerosol transmission efficiency, which might be remedied by reducing the overall transportation tube length, increasing the particle generation rate, decreasing the particle speed, increasing the volumetric flow rate of carrier gas, or by the addition of aerodynamic lenses within the transport tube, which would maintain particles near the center of the transport tube. Although aerosol injection hit fractions tend to be relatively low in comparison to liquid jets, recent work at the CXI instrument reported hit fractions of 0.83% for aerosolized 40 nm viruses delivered with an aerodynamic lens stack aerosol injector.

Although it is convenient that our miniaturized CNAI is compatible with standard GDVN mounting hardware, the downside is that the small exit aperture, 100 μm diameter in our case, is prone to clogging. We have successfully operated our CNAIs in the laboratory for many hours without interruption, but clogging typically occurs whenever the aerosolization liquid jet misbehaves and produces large droplets for a period of a few minutes. It is, therefore, essential to ensure the formation of small droplets and continuous flow of carrier gas. In the XFEL experiment reported here, there were a total of three clogged aerosol-nozzles, each of which required ~20-30 minutes to replace. The severity of this issue could be greatly reduced by filtering out large droplets with, for example, an inline impactor, and by using electrospray ionization to produce smaller initial droplet diameters.

It must finally be noted that the GV crystals utilized here are notoriously robust and survive in nearly pure water. For crystals that dissolve, for instance, upon varying pH, it may be feasible to avoid droplet evaporation by using a humidified carrier gas, by using electrospray nebulization, or by simply placing the nebulization source close to the entrance of the aerosol nozzle to reduce the time of transport.

6.5 Conclusions

We demonstrated X-ray diffraction from aerosolized sub-micrometer protein crystals with background levels drastically lower than in typical SFX experiments utilizing liquid jets. This may be important for coherent-diffraction-imaging experiments on weakly scattering targets such as isolated proteins, viruses, or cells, as well as for the measurement of diffuse scattering or lattice-transform signals between crystalline Bragg reflections. We showed that our injector is compatible with the existing hardware at LCLS, allowing quick changes from a liquid jet to an aerosol
injection system in a single experiment. The relatively high (∼300 m/s) particle velocities may be useful for avoiding damage due to X-ray induced explosions when using new XFEL sources with pulse repetition rates up to 4.5 MHz.

While the obtained 0.006% hit fraction at LCLS was much lower than in typical liquid jet X-ray diffraction experiments, laboratory measurements suggest that this can be improved by orders of magnitude. Based on these laboratory measurements, we suspect that the low hit fractions observed in this study are a result of aerosol transport losses, clustering of particles, clogging of the aerosol-nozzle due to an under-performing GDVN nebuliser, or misalignment between the X-ray focus and particle beam focus. As we have noted, there are several possible routes to improve upon the injection strategy described here, as evidenced by other aerosol injection work performed at the same CXI instrument [14].

Above all, the lower background achieved with the aerosol nozzle somewhat offsets the lower hit fraction, since the number of required measurements depends inversely on the square of the signal to noise ratio of intensities, or directly proportional to the background counts.

This proof of principle experiment was performed on granulovirus occlusion bodies suspended in water. These protein crystals have naturally evolved to be robust against the change in the buffer conditions and dehydration caused by evaporation of the liquid layer on the crystals surface. However, most protein crystals are not stable in pure water. When working with other types of crystals, the liquid buffer evaporation rate on the surface of the crystals must be controlled, for example by controlling the relative humidity at the crystals [199][200].
7 Optically focusing and guiding of aerosolized particles at low pressure

In the first part of this thesis, a new aerodynamic particle injection system based on a convergent nozzle geometry, and an imaging diagnostics mechanism based on optical illumination have been discussed. Using this injector for X-ray diffractive experiments at the FLASH FEL facility in Hamburg, a high hit fraction was recorded. Furthermore, by employing this injector technology at the LCLS, CXI Hard X-ray instruments, extremely low background diffraction patterns were obtained using macromolecular nanocrystals of CpGV particles.

As described in section 2.2.1, SPI experiment base on aerodynamics particle injection, the hit fraction is directly related to the instantaneous particle density at the location of the X-ray focus. For a fixed rate of particles entering into the injector, the hit fraction is inversely proportional to the speed and width of the particle beam. Using the convergent nozzle aerosol injector, it was possible to produce a highly focused particle beam, down to 3.5 µm diameter (FWHM) using 300 nm virus particles. This is roughly the size of X-ray beam focus at most X-ray diffractive experiment beamlines. While matching the particle beam width with the X-ray focus size increased the hit fraction, the speed of the particles was still too high, up to 300 m/s. Aerodynamically slowing of the particles could be possible, but this would lead to a broader particle beam, i.e., it is not possible to optimize one without affecting the other. So, to maximize the hit fraction, an optimal compromise had to be found between the particle beam density and its velocity. However, by introducing an optical field into a moderately focused particle beam, these important hit-fraction parameters, particle beam width and velocity, can be optimized simultaneously, without affecting the operation of the injector. This new technique will be presented in this chapter.

In the absence of the liquid environment, which serves as a heat conductor and damp medium for the particles thermal motion in a conventional optical tweezers, manipulation of particles suspended in gas is very challenging. Typically most aerosol trapping is performed on stationary or extremely slow particles that are
floating in an enclosed chamber, which is usually kept at atmospheric pressure. The challenge becomes even greater when the experiment is performed under vacuum and the particles are moving with a very high speed. Manipulating particles in such extreme conditions requires a robust experimental setup which has an optimized trapping laser profile that maximizes particle-laser interaction, a high-speed and short illumination imaging system, and software to study the particle dynamics and a sample delivery system that can deliver a particle beam with low emittance. The setup is constructed taking these important points into consideration. In the first part of this chapter, the basic setups that have been constructed to generate, visualize and guide the aerosol particles in vacuum will be presented. The optical funnel construction and characterization setup will also be discussed. In the second part of this chapter, the results obtained in deceleration and concentrating of the particle beam in vacuum will be discussed.

7.1 Experimental setups

During the period of this PhD, two experimental setups which are based on different chamber designs were constructed, named as the primary chamber setup (PCS) and the improved chamber setup (ICS). The PCS setup is based on an older chamber design where most of the earlier proof-of-principle experiments were performed, whereas the ICS setup is based on a more simplified and improved version of the chamber. Both setups are comprised of four basic components, namely the optical beam shaping setup, particle injection system, vacuum pumping system and direct optical particles visualization. In the following sections these basic components of each setup will be presented in detail.

7.1.1 Primary chamber setup

The primary experimental set up was constructed around a vacuum chamber of 300 mm$^3$ that hosts and aerosol injection system, laser trapping optics, laser illumination optics and an optical imaging setup as shown in Fig. 4.2. The particle visualization part of the set-up is detailed in chapter 4. Briefly it is composed of high-speed camera, various illumination lasers and imaging geometries and particle analysis software that is used to track and calculate particle positions and study the dynamics. The particle generation and injection mechanism used in this setup is presented in chapters 4 and 5. To recap, the particles were delivered using the standard lens stack aerosol injector mounted vertically on xyz–translation stages (see Fig. 4.2). The particles were aerosolized using a low-flow rate GDVN nozzle.
injecting into a small nebulization chamber mounted on the top of the injector. In order to pull some of the excess He gas introduced by the GDVN, a pair of differential pumping skimmers were mounted between the entrance of the injector and the nebulization chamber (see Fig. 7.1). The He gas carries the particles from the nebulization chamber through the concentric apertures of the ALS into the vacuum chamber, to produce a collimated aerosol beam similar to Fig. 4.3. The process of producing aerosol particle using GDVN is presented section 2.3.

The ALS injector produces a well collimated and tightly focused aerosol beam with a transverse width of several 10s of micrometers. On the other hand, the optical funnel constructed using the vortex beam has a peak-to-peak diameter of 10.5 µm at the focus and a divergence of 0.92°. So, to maximize the particle-laser interaction, the axes of the particle and the laser beams should be aligned precisely all the time. This alignment procedure is described in section 4.3.2.

Vacuum pumping scheme

Efficient control of the pressure in the vacuum chamber is important for the following two reasons. First, the magnitude of the photophoretic force acting on the particles is highly dependent on the density of gas where trapping is takes place (see section 3.2.2). Therefore, the experimental chamber pressure should be optimized and maintain at a given value, with little fluctuation, during a measurement. Second, the focusing mechanism of an aerosol injector is dictated by the pressure gradient created across the inlet and outlet of the injector. Since the exit of the injector is usually maintained at high vacuum, the operation of the injector, i.e., the resulting aerosol beam width and particle velocity, is controlled merely by adjusting the pumping speed between the differential pumping skimmers. So, in order to maximize the photophoretic force and increase the reproducibility of measurement, the ability to effectively control the chamber pressure as well pumping speed is very crucial.

Two pumping schemes were established to run the experiment at two different modes, namely: low pressure mode (LPM) (chamber pressure < 10⁻⁴ mbar) and higher pressure mode (HPM) (chamber pressure > 0.1 mbar). The pumping layout is depicted in Fig. 7.1 with two scroll pumps (SP1 and SP2) and a turbo pump (TB) connected to the chamber. LPM: in this mode the chamber is evacuated by the turbo pump and the scroll pump (SP1) serves as a per-vacuum pump for the turbo. The chamber-turbo pump gate valve (GVT1) and the butterfly valve (BV1) remains open and the high pressure right angle valve (RAV1) will be closed, to form the pumping path indicated by the blue line in Fig. 7.1. HPM: here the turbo pump is vented to atmospheric pressure and only the pump SP1 though the
Chapter 7. Optically focusing and guiding of aerosolized particles at low pressure

Figure 7.1: A schematics of the pumping and optical layout in the PCS setup. The optical layout shows typical configuration for the vortex beam and Bessel beam generation inside the chamber. During experiments that use the Bessel beam, the 10× objective is placed in the position of the in-vacuum microscope. This re-image the QBB, which was created on the optical table, in front of the injector. The purified microscope sticky-covering-slide (CS) is mounted on the focal plane of the in-vacuum microscope. It used to protect the optics from particles and image their deposition as they stick to it using the in-vacuum microscope.

right angle valve (RAV1) is used to evacuate the chamber. This is done by closing GVL1 and BV1, and pumping through RAV2 and/or the needle valve (NV1). It is seen in Fig. 7.1 that the SP1 pump is connected to the chamber using two parallel lines (marked yellow for 40 mm ID hose and red for 4 mm ID steel tube). The
Experimental setups

Red-marked line together with RAV2 was used for fast pumping, whereas the yellow-marked line together with NV1, was used for slow and controlled pumping. The slow pumping scheme produced small adjustments of the chamber pressure and also stabilized the fluctuations in the chamber pressure caused by the constant fluctuation of the He gas from the GDVN.

The get valve GVL2 isolated the main chamber from upstream of the injector and atmosphere, for instance during changing the GDVNs. SP2 combined with RAV3, pumps at the differential-pumping skimmers to regulate the excess He gas that goes into the chamber, thereby controlling the operation of the injector. So during experiments, the desired chamber pressure, particle beam speed and width, maximizes the optical force, can be produced by carefully controlling the pumping at different stages with the combination of the various valves settings.

Generating the optical vortex beam

In the PCS setup most of the experiments were performed using a Laguerre-Gaussian ($LG_{0,1}$), also known as a vortex beam, which was generated by illuminating a SPP with a Gaussian beam from a 5 Watt CW laser (Coherent Verdi V5, 532 nm). The SPP used here is made of transparent fused silica and consists of 16 spiral phase steps (see Fig. 3.8). The idea of generating vortex beam using an SPP is presented in section 3.5.1.

The optical layout before and after the beam enters into the chamber is depicted in Fig. 7.2 and Fig. 7.1, respectively. On the optical table, a Gaussian beam from the laser first passes through the HWP and high power polarizing beam splitter (PBS), and then a 1:4 beam expander (BE) to fill the aperture of the SPP. The HWP together with the PBS are used to control the power of the beam. After the SPP, a doughnut-shaped beam similar to that in Fig. 7.3 is formed in the far field and propagates about 4 m on the optical table though mirrors M1 to M3. The beam is then reflected 90 degrees horizontally using mirror M1 to enter the vacuum chamber though the optical window OW1 in Fig. 7.1. Inside the chamber, the beam is refocused by the plano-convex lens (L1) to the desired beam waist and steered 90° upward by a motorized 45° mirror (KM1 in Fig. 7.1) to co-axially counter propagate against the particle beam. This establish maximum interaction of the particles with the laser beam. The lens L1 is mounted on an optical rail system, so it can be translated along the parallel beam path and adjust the focal position of the vortex beam relative to the injector tip (see Fig. 7.1). The beam size and divergence angle of the vortex beam were controlled by changing the focal length of L1 and/or adjusting the beam expander.

Fig. 7.3 shows the profile of the OVB generated using the setup described above.
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Figure 7.2: Top view of the optical table vortex beam generation and characterization setup. The optical table and experimental chamber were placed next to each other, with no physical contact between them. The half-wave plate (HWP) combined with the PBS was used to control the intensity of the beam that goes into the beam expander. The pellicle beam splitter (PL), reflects 8% of the beam to the beam profiler assembly for characterization.

To generate these, first the 2D intensity profiles of the vortex beam was measured at different $z$ positions, using a beam profiler. Then, the beam divergence was calculated from the $\frac{1}{e^2}$ intensity radius of these 2D profiles. The beam waist was calculated using the relation $w_0 = \frac{\lambda}{\pi \theta}$ from the beam divergence, then beam parameters and the evolving beam waist and peak-to-peak diameter ($w(z)$ and $w_{pp}(z)$) are approximated using Eq. 3.22a and 3.24a. The vortex beam produced has $w_{pp} = 10.5 \, \mu m$ at the focus and vortex peak intensity divergence of 0.92 ° (16 mrad).

Inside the chamber constructed an inverted dark-field microscope mounted on an $xyz$-stage directly on the axes of the injector and laser beam (see Fig. 4.2). So, during particle injection it can be freely moved in and out of the injector and laser beam axes, and image the particle beam profile in the transverse-plane. The particles were imaged as they accumulated on a glass slide coated with a “optically clear purified DGL film” (Gel-pak, DGL-20/17-X8) mounted on the front focal plane of the microscope (see section 4.3.2). In addition to imaging the particles, this microscope also served as a tool to align the particles with the laser beam. This alignment is performed by iteratively tilting the laser beam and shifting the particle beam, and maximizing the particle-laser overlap on the in-vacuum microscope at two positions which are 50 mm vertically apart (see section 4.3.2).

Few preliminarily measurements that involved the Bessel beam were performed in the primary chamber. In that case, the axicon was placed in the far-field of the
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Figure 7.3: Measured vortex beam profile. (a) The evolving beam waist \( w(z) \) and peak intensity radius \( (w_p) \), calculate using Eqs. 3.22a and 3.24 from the measured beam divergence \( \theta = \frac{\lambda}{\pi w_0} \). (b) Transverse beam profile at \( z = 2 \text{ mm} \) from the focus and in the inset plotted its cross-sectional intensity along the center of the beam.

doughnut beam, i.e., 500 mm before M1, to form a Bessel beam on the optical table. This beam is then re-imaged inside the chamber by a 10:1 demagnifying 4f lens system that was constructed by a combination of L1 and a 10× High-Power focusing objective (Thorlabs, LMH-10X-532), to form a Bessel beam few millimeters below the injector tip. Note, for measurements involving the Bessel beam, the in-vacuum microscope in Fig. 7.1 is removed from the optical path and the 10× objective is mounted at the same position. Bessel beam formation was detailed in section 3.5.2.

Drawback of the primary chamber design

In general, the PCS setup was robustly constructed, and it has led to various interesting observations and results which I will discuss them in the following sections. However, the reproducibility of these measured results were limited by the following technical reasons.

- **The pointing stability of the laser:** in the PCS the main optical table where the laser was installed and the experimental chamber where the trapping took place, were constructed independent to each other, i.e., the optical components which form the optical funnel are installed on two different optical tables that are not coupled to each other. This resulted in fluctuation of the pointing of the laser beam inside the vacuum chamber by more than \( \sigma = 30 \, \mu\text{m} \), this heavily influenced the stability of the laser beam relative to
the particle beam.

- **Vibration of the injector**: the injector was mounted on an xyz-stage which was hanging half a meter down inside a corrugated bellow. This result in an easily vibration of the injector when subjected to any movement in the lab. Furthermore, during pumping the chamber the injector moves by \( \sim 400 \, \mu \text{m} \) both lateral and vertically, this leads to extensive in-vacuum alignment.

- **Instability of the imaging setup**: as seen in Fig. 4.2 the particles image was formed by an in-vacuum objective through an optical window mounted on the door of the chamber, onto a high-speed camera. The camera was independently mounted on a movable optical table just outside of the chamber door, so it had to be moved out of the way whenever the chamber door had to be opened. Beyond frequent decalibration of the imaging FOV, this arrangement influenced the stability of the setup, thereby limited the reproducibility of the measurement results.

- **Astigmatism on the optical funnel**: during the particle-laser alignment under vacuum, astigmatism was introduced on the optical beam as the kinematic mirror KM1 in Fig. 7.1 was steered against the fixed 10× objective. This was particularly a big problem to use the Bessel beam in the PCS, as it require to be re-imaged close to the injector.

- **High particle velocity**: the particles from the ALS injector used in PCS are relatively faster and it not straightforward to slow them down to the desire velocity with a reasonable particle beam width.

- **Compatibility with existing X-ray diffractive experiments**: X-ray diffractive experiment beamline chambers require bellow \( 10^{-4} \) mbar pressure, however in order to maximize the PPF we need higher gas density in the chamber. In the configuration of the PCS setup, this is not possible. So, for future integration of this technique to X-ray diffractive experimental chambers, this vacuum requirement must be meet while maintaining optimum gas density for the PPF to work.

These result in the need for designing a better setup which mitigates most of the afore mentioned downside of the PCS. In the following section the main components of the newly constructed improved setup will be discussed.

### 7.1.2 Improved setup

This experimental setup is designed taking all the limitation of the old setup mentioned in section 7.1.1 into consideration. It is constructed around a reduced
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A chamber of size 130 mm × 32 mm × 32 mm that is mounted firmly on the optical table (see Fig. 7.4). To summarize briefly the following improvements have been made in the new setup:

- All the beam shaping elements, imaging setup and particle injection system are mounted firmly on the same optical table. This helps to reduce the uncoupled vibration of the optical elements and the injector. This improved the pointing stability of the beam at 5 m from the laser head, just before the 10× objective (MO1) in Fig. 7.4 to \( \sigma_x = 18.6 \, \mu m \) and \( \sigma_y = 11.1 \, \mu m \).

- Laser beam stabilization setup is integrated into the optical path to actively correct the beam fluctuations. This improved the beam pointing stability at 5 m from the laser head, just before the 10× objective in Fig. 7.4 to \( \sigma_x = 0.8 \, \mu m \) and \( \sigma_y = 0.6 \, \mu m \), and \( \sigma_x = 84 \, nm \) and \( \sigma_y = 72 \, nm \) after 10× objective.

- The chamber size is reduced so that all the optics can be mounted outside of the vacuum chamber.

- Cylindrical nozzle aerosol injector (CyNAI) is used to deliver the particles. Unlike ALS, in such injector the particles velocity can be controlled quite easily.

The reduced size of the chamber has two main advantages. First, it allows the optical elements that form the optical funnel and imaging setup to be mounted outside of the vacuum chamber, i.e., on the optical table. This improves the laser beam stability and makes optical alignment much convenient. Second, the entire setup can be built inside X-diffraction chambers while isolating and maintaining the small trapping cell pressure optimum for the photometric force guiding.

Seen in Fig. 7.4 is the basic ICS – the optical funnel enters into the chamber through AR coated \( \varnothing \) 1-inch optical window, and coaxially counter propagates against the particle beam. The particles were delivered using the CyNAI. The chamber has four AR coated windows in each of its four opposite sides, through which the particles are illuminated and imaged. Two side-view cameras namely, Photron SA4 coupled with 5× objective for magnified image (FOV: 2 mm × 2 mm) and Photometric high quantum efficiency camera combined with Thorlabs MVL6X12Z 6.5X Zoom Lens for wide-field image (FOV: 8 mm × 8 mm), are used to image the particles through the top and bottom optical window of the chamber, respectively. Two short-pulse lasers, Nd:YLF laser (Spectra Physics Empower 30, 527 nm, pulse duration 100 ns, repetition rate 1 kHz, pulse energy 20 mJ, average power 20 W) and a fiber-coupled diode laser (DILAS High-Power Diode Laser IS21.16-LC, 636.7 nm, average power 10 W) are used for particle illumination. The diode laser is powered by a high-speed diode driver (Dr. Heller Elektronik, UHS-500-12.8 A, repetition rate up to 1 MHz, pulse durations 10–100 ns). On the manual filter-wheel (MFW), a RazorEdge filter (Semrock, SP 532 RU, cutoff
527 nm) and a bandpass filter (Thorlabs FL635-10, center wavelength 635 ± 2 nm, FWHM = 10 ± 2 nm) are mounted to block the scattering intensity from the 532 nm trapping laser when the particles are illuminated by the Empower or DILAS laser, respectively.
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Particle injection in the new setup

Particle injection system is one of the crucial improvement made on the new setup. Instead of the lens stack aerosol injector, here the particles are delivered with a simple cylindrical nozzle aerosol injector. CyNAI basically works with the same concept as the convergent nozzle aerosol injector presented in chapter 5, except it uses a straight nozzle at the end of the injector to produce a collimated aerosol beam. Note, unlike the convergent nozzle aerosol injector which focus the particles to a small region, the CyNAI produce a collimated aerosol beam. Furthermore, in this setup to control the gas load going into the main chamber, we added a pair of skimmers upstream of the injector (see Fig. 7.5). GDVN nozzles were used to atomize the particles, the process of producing aerosol mist using the GDVN is presented section 2.3.

Figure 7.5: Simplified view of the CyNAI assembly. Aerosol mist created by the GDVN nozzle carried away by the He gas through the skimmers and focused by the 2 mm ID capillary into the vacuum chamber.

Seen in Fig. 7.5 is the CyNAI delivery system constructed with a 12.5 mm long focusing capillary tube (swagelok reducer, SS-6M0-R-2, ID = 2 mm, OD = 3 mm) mounted onto the end of a 300 mm long steel tube (OD = 6 mm and ID = 4 mm) leading from the chamber to the skimmers box. This tube transports the particles from the last skimmer to the capillary injector tip. A vacuum pump is connected at the skimmers box to pull some of the excess He gas introduced by the GDVN nozzle, thereby control the particles velocity, particle beam width, and the gas density for optimum PPF. Further control of the particle beam parameters was possible by adjusting the gap between skimmers. Note, the ICS chamber was set to operate only at high pressure mode (\( > 0.1 \text{ mbar} \)), with a pumping configuration similar as the PCS in Fig. 7.1 without the turbo pump.
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Particle-laser alignment

Due to the reduction of the chamber size, it was impossible to install the in-vacuum microscope assembly as it was in the PCS chamber. So the particle-laser alignment is performed a bit differently in the ICS. The alignment is done based on the assumption that the particle beam flows concentrically with the symmetric axis of the injector. So, precisely aligning or propagating the optical funnel concentrically through the axis of the injector should guarantee alignment of the particle beam with the laser beam.

![Image of the QBB on the injector tip. This image taken by beam alignment camera AC1. The QBB profile shown on the tip of the CyNAI](image)

To assist with the alignment, two Thorlabs CMOS cameras are put up to image the laser beam profile at two different locations (AC1 and AC2 in Fig. 7.4). AC1 installed off-axis to image the beam profile just before the beam enters into the injector. The beam profile is inferred from the scattering intensity off the injector tip (see Fig. 7.6). AC2 is installed on axis, at 1.5 m behind the chamber to image the direct beam transmitted through the injector. Since the chamber and the injector are fixed to the optical table, the alignment is done by only steering the laser beam. This is accomplished by iteratively tilting and shifting the position and pointing of the laser beam with the help of mirror M1 and M2, while monitoring the beam positions and profile on the tip and behind the injector through cameras AC1 and AC2. Initially, the alignment is performed in air then optimized by injecting particles and maximizing the scattering intensity of the particle looking through the side-view cameras.

7.1.3 Formation of the optical funnel

A typical aerosol injector that currently used for SPI experiments produce a particle beam with a transverse width $> 30 \mu m$ and velocity $> 100 \ m/s$, while the X-ray
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Figure 7.7: Optical layout for the generation and characterization of the Quasi-Bessel beam. A LG$_{0,1}$ beam is formed when the Gaussian beam from the verdi V5 laser passes through the spiral phase plate (SPP). The high power polarizing beam splitter (HPB) together with the half-wave plate (HWP), controls the power transmitted to the setup. The active laser beam stabilization setup, composed of actuated mirrors (AC1 and AC2) and position sensitive Quadrant photo diodes (QPD1 and QPD2), actively adjust the beam pointing onto the symmetric axis of the axicon. The initial quasi-Bessel beam (BB1) start to form at 270 mm in front of the axicon. A 4f-lens system composed of plano-convex lens (L1, $f = 200$) and 10× microscope objective (MO1), demagnify BB1 10× to the desire final beam (BB2) inside the chamber. The beam is characterized using the beam profiler consists of 50× microscope objective (MO2) and Ophiropt beam profiler CCD camera (SP620U). The mirror (BPM) is inserted in the beam path only when the beam was characterized. The manual filter wheel (FW) further regulates the intensity of the laser without affecting active beam stabilization setup.

beam focus size is up to two order of magnitude smaller. In order to effectively manipulate and confine a particle beam with such high momentum and spatial spread to a spot in the order of X-ray beam focus size, requires maximum interaction of the particles with the laser for relatively longer duration and/or distance. So,
the goal is to form a hollow-core beam that has divergence, propagation length and width that match with the aerosol beam. The desired beam is expected to have a funnel-like axial profile, i.e., \( \sim 5-10 \, \mu m \) inner ring diameter at the focus and \( \sim 100-150 \, \mu m \) diameter at a distance of 50–100 mm from the focus. Using a simple Laguerre-Gaussian mode only, however it is not possible to create a beam that fulfills the aforementioned criteria. For instance, if we were to create an optical funnel using LG0,1 with inner ring diameter of \( w_{pp} = 7 \, \mu m \), the beam would only propagate with Rayleigh range of 150 \( \mu m \) before it diverge quickly with divergent angle \( \theta = 34 \, \text{mrad} \) (34 degrees). Due to this, optical funnel constructed using LG modes has very small trapping volume, therefore they are limited to very slow particles. However, as the calculations in section 3.5.2 shows, using a non-diffracting Quasi-Bessel beam it is possible to realize an optical funnel that diverge very slowly for a relatively longer distance. In the following paragraphs I will present the optical setup that was constructed to create and characterize such a beam.

Figure 7.8: Intensity profile of the optical funnel at BB2 in Fig. 7.7, generated using a 1 W QBB. a) The 2D axial intensity profile of the QBB. This profile is generated by taking axial cross-section of a 3D stack composed of multiple 2D profile like in (b) measured at different z positions. b) **Top:** The 2D transverse profile of the beam at 5 mm away from the focus. **Bottom:** plot of the normalized beam intensity along the green dashed-line on the top 2D profile.
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The Basic optical setup to generate the QBB is depicted in Fig. 7.7. The QBB is created by illuminating \( l = 1 \) SPP and 0.5 degree axicon with a Gaussian beam. With no beam expander on the path, the LG doughnut beam had a \( \frac{1}{e} \) intensity beam size of \( w = 1.95 \) mm and \( w_{pp} = 2.42 \) mm on the position where the axicon was placed. From geometrical calculation using Eq. 3.30b, the Quasi-Bessel beam should be focused at \( Z_{\text{min}} = 380 \) mm and propagate for \( Z_{\text{max}} = 446 \) mm at BB1. Our measurement also agrees with these calculations, at BB1 we created a QBB with \( w_{pp} = 80 \) µm. This beam is re-imaged to the desired parameters at BB2 using a 1:10 4f lens system composed of plano-convex lens \( L_1 (f_1 = 200 \) mm) and a 10\times long working distance microscope objective \( MO_1 (f_2 = 20 \) mm) (see Fig. 7.7). The 4f telescope setup demagnified the beam roughly 10\times to \( w_{pp} = 7.8 \) µm and re-image it at 38 mm in front of the 10\times objective, inside the chamber. The generated beam at BB2 has central peak divergence of \( \sim 1.25 \) mrad and propagates for \( \sim 55 \) mm.

In Fig. 7.8(a) depicted the axial cross-section of the QBB formed at BB2. To create this profile first a series of 2D beam profiles, similar to Fig. 7.8 (b), was measured using the beam profiler at different \( z \) positions in step of \( \Delta z = 500 \) µm. Then using a software the central peak intensity cross-section, similar to the inset of Fig. 7.8 (b), extracted from each 2D profiles and stacked together according to their \( z \) positions. The intermediate intensity values are interpolated, i.e., each vertical bin in Fig. 7.8(a) has 500 µm height and its value is extracted from a single 2D profile.

![Figure 7.9: QBB characterization using 10:1 demagnifying 4f lens system. a) The QBB first peak-to-peak diameter at different distances from the objective MO1 for various 4f lenses separations, \( d \). b) Dependence of the QBB peak-to-peak diameter at the focus on the separation distance \( d \), between L1 and MO1.](image)

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Compared with the Laguerre-Gaussian beam focused to the same spot size, the Quasi-Bessel beam propagates for longer distance, with very small divergence of the central core. As seen in Fig. 7.8 closer to the focus much of the QBB intensity is concentrated in the first ring of the Quasi-Bessel beam. However, when the beam propagates away from the focus, more and more energy goes into the outer rings and eventually the QBB will collapse to form a diffused doughnut-like beam. Note, at any point along the propagation, the inner ring has higher intensity than the outer rings.

Unlike SLM, where the beam parameters (topological charge, divergence, focus size) can easily be adjusted, the beam generated using axicon has fixed beam parameters, i.e., in order to change the beam, one has to modify the optical elements. For instance, axicon with different angle to modify the propagation length, SPP with different \( l \) to change the beam size and divergence or different telescope lens arrangements to re-image the beam. However, slight modification of the beam focus position and divergence was possible by simply adjusting the gap between L1 and MO1 in Fig. 7.7. The change in the optical funnel divergence for different \( 4f \) system lenses gap is plotted in Fig. 7.9 (a), and Fig. 7.9 (b) shows the change in QBB beam size at the focus for different gap between L1 and MO1. The divergence of the beam is roughly the same for the difference separation, however the beam size tends to change significantly. In our experiment we set our \( 4f \) lenses separation 320 mm, to generate the beam shown in Fig. 7.8 (a) (\( w_{pp} = 7.8 \text{ \mu m} \) and divergence, 1.25 mrad).

### 7.1.4 Determining the laser beam focus and profile on the camera FOV

The 3D optical funnel profile measurements, described in sections 7.1.3 and 7.1.1, were done in an opened chamber, and have to be transferred to the side-view imaging camera coordinate so that the position of the particles in the laser field are accurately known during the particle injection.

To transfer the beam profile on to the camera FOV coordinate, first we scan a thin tungsten wire (\( \varnothing 5 - 25 \text{ \mu m} \)) along the laser beam symmetric axis until the wire is at the focus of the laser beam, then we translate the side-view camera to focus onto the edge of the wire. This ensures that the camera focus plane lies on the axis of the laser beam. This is important because the depth-of-focus of the imaging system as well as the trapping laser transverse width are much smaller compared with the particle beam diameter, i.e., particles that appeared defocused can be discriminated during analysis—assuming that they were not interacted with the QBB. Ones the camera’s image plane is aligned with the laser beam axis, concentrated particles were injected into the chamber and illuminated by the
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Figure 7.10: The vortex beam position in the primary chamber setup side view imaging camera FOV. This profile is extracted by time averaged of scattered intensity from gas and aerosol particles inside the chamber illuminated by the counter propagating vortex beam. During this measurement the chamber was kept at atmospheric pressure. The blue lines indicate the approximated evolving peak intensity radii \( w_p(z) \), measured from the center of the beam. These image was taken by the wide-field microscope described in section 4.3.1.

counter-propagating trapping laser. The scattering on the injected particles then showed the beam profile on the side-view camera, see Fig. 7.11. Equivalently, the beam profile could also be extracted by long-exposure imaging of the scattering from air in an open chamber, see Fig. 7.10.

For convenience, we assign the center of the coordinate system to be at the focus of the trapping laser beam. This holds for all of the analysis in the following chapter. Figure 7.10 shows the location of the vortex beam in the FOV of the wide-field imaging configuration of the PCS setup (see section 4.3.1) determined by long-exposure imaging of an open chamber with 5 watt \( \text{LG}_{0,1} \) beam. This is similar to the long-exposure particle imaging shown in Fig. 4.3(a). Similarly, Fig. 7.11 shows the position of the Quasi-Bessel beam profile in the ICS camera FOV. This profile is generated by averaging over 5000 frames each has 2 \( \mu \)m PS particle-streak.
Figure 7.11: The quasi-Bessel beam position in the improved chamber setup side view imaging camera FOV. This profile was inferred from the average scattered intensity of 2 µm particles illuminated by a counter propagating QBB. The particles were imaged through the high magnification side view imaging setup of the ICS chamber. The red dashed-lines in the figure indicate the first peaks radii of the QBB. Note: this profile was extracted from 2D projected images, therefore the dark core of the quasi-Bessel beam can’t be clearly resolved.

(similar to Fig. 4.3(c)) illuminated by the counter propagating 1 watt QBB. The accuracy of such calibration is limited by the spatial resolution of the imaging system. In the high magnification ICS imaging setup, the focus position can be determined up to ±1 and ±5 pixel accuracy in the lateral and axial direction respectively, given that one pixel spans 1.93 µm.

7.1.5 Samples used for the optical focusing

A number of different samples were investigated, the most common were polystyrene beads and *Cydia pomonella* granulosis virus (CpGV) particle (see Fig. 5.5). Both samples are ideal in the sense that they readily suspend in water, they don’t easily agglomerate and they are easy to aerosolize using GDVN nozzle. Owing
to their low density \( \rho = 1.05 \text{ g/cm}^3 \), polystyrene beads are ideal sample for optical trapping experiments. Pure PS particles are transparent at 532 nm, so to enhance their absorption we typically use fluorescent or carbon coated beads. The fluorescent polystyrene particles (FPP) are commercially available (FluoSpheres, Carboxylate-Modified Microspheres, yellow-green fluorescent) and supplied as a 2% solid suspended in water plus 2 mM sodium azide. The PS particles have specified size variation between 3 and 4 % and the typical size investigated in this work were 2 µm, 1 µm and 0.5 µm.

A CpGV consist of a single virion engulfed in an occlusion bodies, made up of well-ordered protein crystal (see sections 5.4 and 6.2). That means that, depending on the wavelength of the light being observed, a CpGV particle can be considered either as a protein crystal or a single particle. The purification and preparation procedure of CpGV was described in [52]. Since the majority of the CpGV particle consist of protein crystals, I assumed its density to be similar with that of a protein crystal \( \rho_{GV} = 1.43 \text{ g/cm}^3 \) [201]. In the following sections this density value will used to calculate the actual mass of CpGV particles and compare it with the measured masses.
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7.2 Results

In this section, I present the results obtained on focusing and concentrating stream of aerosol particles using the optical funnels constructed using a vortex and Quasi-Bessel beam. Experiential observation made using the PCS setup are presented first. This includes the deflection and deceleration of accelerated particles using a Laguerre-Gaussian beam, counter propagating against the particles. Then the most recent results achieved using the QBB is presented. It demonstrates the slowing, focusing and concentrating of PS as well as virus particle beam. In this section, the experimental and analysis techniques, followed to evaluate the effect of the optical force on the particles, are also detailed.

7.2.1 Results from the primary setup

Most of the earlier proof-of-principle observation of the light induced force such as deflection, levitation of particle beam was performed in this setup. Note most of the experiments in the primary chamber were performed using the LG optical vortex beam. The basic schematic of the experimental setup is depicted in Fig. 7.1 and Fig. 4.2 and the detail description is given in section 7.1.1. To recap briefly, the particles were delivered using the ALS injector that was arranged in the vertical orientation, and the vortex beam was generated by illuminating a spiral phase plate with a Gaussian beam. Inside the chamber, a Plano-convex lens (L1 in Figs. 7.1 or 7.2) is used to focus the beam several millimeters below the injector. The vortex beam focus size and divergence was adjusted by a beam expander placed on the optical table and/or by varying the focal length of the L1. The particle-laser alignment was performed using the procedure described in section 4.3.2 with the help of the in-vacuum microscope.

7.2.2 Particle beam deflection

The effect of the light-induced photophoretic force, on a light absorbing particle, depends on the laser beam intensity profile and the relative position of the particle with the laser beam. A simple intuitive example would be, if the trapping beam has Gaussian intensity distribution and absorbing particles introduced into the beam traveling in opposite direction. The off-axis particles would experience non-uniform illumination, hence PPF. So, they will get deflected away from the beam center, i.e., away from the maximum intensity. Particles that travels in the axis of the Gaussian beam would ideally experience a zero net radial force, and an opposite axial forces arising from the combination of radiation pressure and
PPF. In which case, depending on the laser power and velocity of the particles, they would ideally be decelerated, and eventually recoil back.

In Fig. 7.12 (a) is shown long exposure streaked-image of a 2 µm diameter PPS particle illuminated by 4 watt Gaussian beam in 5×10^{-2} mbar chamber pressure. The green dashed-lines mark the axis of the Gaussian beam, and Fig. 7.12 (c) shows the basic arrangement of the experiment. In this measurement, the particles were moving with an average speed of ∼20 m/s, in opposite direction to the laser. In Fig. 7.12 (a)(i) the particle was introduced at +50 µm away from the axis of the laser, therefore its trajectory is not strongly affected by the laser field. However, in Figs. 7.12 (a) (ii and iii) the particles were introduced within few micrometer offset from the intense part of the beam, therefore they appear to be deflected away from the beam.

Figure 7.12: Imaging the deflection of 2 µm diameter polystyrene particles by a Gaussian and vortex beam. a) Side-view image of PS particles deflected by a 4 watt Gaussian beam (ii and iii) and due to its position (i) is unaffected by the laser. The green dashed-line indicated the position of the laser beam. b) Transverse plane view of particle beam deflected by 2.25 watt vortex beam. This image is taken by the in-vacuum microscope shown in section 4.4.2. Each glowing dotes in the image is a single PPS. c) Simplified schematics of the particle beam deflections in (a) and (b).

In addition to individual particle shown in Fig. 7.12 (a), deflection of the entire particle beam was also observed, when intentionally tilting or shifting the counter propagating laser relative to the particle beam. This is depicted in Fig. 7.12 (b),
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showing the laser-on (2.25 W vortex beam) and laser-off transverse profile of 2 µm diameter FPS particles deposition, imaged by the in-vacuum microscope described in section 4.4.2. The image was taken at 44 mm below the injector tip. As shown in the figure, in the y-direction the particle beam was deflected by 170 µm opposite to the laser. Whereas, in the x-direction the particle beam was deflected toward the laser by +100 µm.

Such particle beam deflections were also imaged by the side-view imaging setup, this will be presented in the following sections. From the point of view of particle-laser interaction using PPF and radiation force outside of injector, the phenomenon that the entire particle beam is deflected by more than its width is not clear. This is because outside of the injector the transverse size of the laser beam is much smaller than the particle beam, so the laser can’t interact with all particles in the beam to induce entire particle beam deflection. Therefore, we assume that this deflection is happening inside the injector, where the particles are slower, the gas density is higher and the laser beam width is relatively larger. Additional data on the particle interaction with the beam inside the injector, which were not available at the time of experiments, are required to test this hypothesis.

Using simple Gaussian beam only, however it is not possible to trap or guide absorbing particles, as the PPF always deflected or recoiled them away. Instead, the laser beam should have dark intensity core so that the particle stay in the region of the beam where the intensity is minimum. This is analogy to an harmonic oscillator where the wall of the laser beam act as a restoring force. Furthermore, the propagating beam should have a convergent profile so that the particles follow a confined trajectory as they guide along the beam. Note, the proof-of-concept experiments were demonstrated on a particle beam that has bigger transverse width than the vortex beam diameter, so depending on the position of the individual particle with respect to the laser, deflection (similar to Fig. 7.12 (a)) can also be observed while using a vortex beam.

7.2.3 Forces on the PS particles by the vortex beam

In Fig. 7.13 (a) shown the trajectory of a 2 µm Ø FPS particle which is vertically kicked-out by a counter propagating 4 watt vortex beam in 1 mbar He environment. The trajectory is extracted from 24 consecutive frames, each has one particle streak. In this measurement, only the scattered photons from the trapping vortex beam and long camera exposure, was used to visualize the particles. This means, in contrast to using separate side illumination, this guarantees all measured particles had interacted with the trapping laser beam. The image was recorded using the wide-view setup described in section 4.3.1. The vortex beam was focused to
$w_{pp} = 10.5 \, \mu m$ at 40.6 mm below the injector. With peak intensity divergence of 16 mrad ($0.92^\circ$), the beam was expand to $w_{pp} = 1.3 \, \text{mm}$ at the exit of injector. This is smaller than the exit aperture of the injector, which is 1.5 mm, so most part of the beam is transmitted into the injector. As the gas density is higher inside the injector, this transmitted beam can influences the particles that are closer to the exit of the injector. However, since the beam is greatly diverging, the effect could be neglected in the most part of the inside of injector.

Figure 7.13: Trajectory 2 $\mu m$ FPS particle vertically kicked by a 4 watt vortex. The profile of the vortex beam is depicted in Fig. 7.3. (a) successive frames imaged by the scattered intensity of the trapping laser itself. The red line connects the intensity centroids of the particle streaks and the gray dashed-lines indicate the boundaries between the successive frames. The particle beam was moving in the $-z$ direction, whereas the laser was propagating in the $+z$ direction. (b) Shows the velocity and acceleration of the particle calculated from the trajectory in (a).

The pressure difference between the exit and upstream of the injector was adjusted to be smaller so that particles exited the injector at very low velocity. Doing so, the velocity of the particles in the $z$-direction was reduced to $0.4 \pm 0.12 \, \text{m/s}$, measured as they enter into the FOV, their laser-off velocity could be a bit higher than that. This velocity is at least an order of magnitude slower compared with the typical particle from optimized aerosol injector [13]. The reduced velocity increased the interaction time of the particles with the laser, thereby enhancing the effect of the laser on the particle and produced a measurable deflection. In Figs. 7.13 (b) depicted the $z$-component of velocity and acceleration of the 2 $\mu m$ FPS particle, calculated from the first and second time derivative of the trajectory in (a), respectively.

The $z$ component of the average particle deceleration field in the chamber is plotted in Fig. 7.14 (a). This map is generated by averaging over decelerations calculated from 160 different particle trajectories each has multiple trajectory points,
similar as Fig. \[7.13\]. The purple dashed-lines in the figure indicate the evolving peak intensity positions \(w_p(z)\) of the vortex beam in the FOV. Note, since the particles were illuminated by the trapping beam itself, hence the acceleration map resembles the laser beam profile. The number of data points in this measurement were small to create a good statistics, however the map still shows high particle deceleration closer to the vortex beam focus.

![Figure 7.14: 2 \(\mu\)m \(\varnothing\) PS particle in 5 watt vortex beam at 1 mbar pressure environment. a) 2D deceleration map of the particles induced by the vortex beam. This map is generated by averaging over multiple particle acceleration calculated from trajectories like Fig. \[7.13\]. b) The axial component of the optical force, calculated using the equation of motion given in Eq. \[7.1\]. Note, the particles were illuminated only by the trapping laser itself, therefor the deceleration as well as the optical force profile has the shape of the vortex beam.](image)

In this measurement, only the axial component of the particle motion was considered. The reason was that the resolution of the wide-view imaging setup was too low to accurately resolve the typical small lateral motion of the particles. In this setup each pixel span 11 \(\mu\)m, which in most case greater that the lateral motion of particles between two consecutive frames or illumination pulses.

Having determined the acceleration field, the resulting 2 dimensional averaged net force field, acting on the 2 \(\mu\)m PS particle, can be generated by multiplying the average deceleration map in Fig. \[7.14\](a) with the mass of the 2 \(\mu\)m \(\varnothing\) PS particles,
m_{PS} = 4.5 \times 10^{-12} \text{ g (4.51 pg)}, calculated from its density, \( \rho_{PS} = 1050 \text{ kg/m}^3 \).
The highest individual particle vertical deceleration \( (a_z) \) recorded at the focus was \( \sim 800 \text{ m/s}^2 \), this is equivalent to \( \sim 3.67 \text{ pN} \) net for force acting on the 2 \( \mu \text{m} \) particle.

The net force exerted on the particle and the optical force \( (F^{opt} = F^{PP} + F^{RP}) \), can be related using following equation of motion of a spherical particle in the presence of a Stokes’ drag force \( (F_{d,x,z}^{opt}) \):

\[
\begin{align*}
ma_x &= F_{x}^{opt} + F_{x}^{d} \\
ma_z &= F_{z}^{opt} + F_{z}^{d} - mg
\end{align*}
\] (7.1)

Where \( m \) is the mass of the particle, \( F_{x,z}^{opt} \) are the transverse and axial component of the optical force, and \( g = 9.8 \text{ m/s}^2 \) is the acceleration due to gravity.

The Stokes’ drag force on a spherical particle of radius \( r_p \) moving through a gas, is given by \[57\]:

\[
F_d = 6\pi \mu r_p(v_g - v_p),
\] (7.2)

Where \( v_g \) and \( v_p \) are the velocity of the gas and the particle, respectively and \( \mu = 1.96 \times 10^{-5} \text{ Pa s} \) is the dynamic viscosity of the helium gas at room temperature \[202\]. For micro-particle moving through gas with high Knudsen number \( (Kn \gg 1) \), such as this experiment, the particles undergo less frequent collisions with the gas molecules, therefore they slip through the gas. This leads to the reduction of the drag force and Eq. 7.2 should be corrected to account for this \[203\]. Slip-corrected drag force \( (F_{cd}^{d}) \), for a particle in high Knudsen number flow is given by:

\[
F_{cd}^{d} = \frac{6\pi \mu r_p(v_g - v_p)}{C}
\] (7.3a)

\[
C = 1 + Kn(c_1 + c_2 \cdot e^{-c_3 Kn}),
\] (7.3b)

Where \( C \) is the Cunningham slip-correction factor, introduced by Knudsen and Weber, with empirical coefficient of, \( c_1 = 1.2310, c_2 = 0.4695 \) and \( c_3 = 1.1783 \) \[57,203\].

Once the net force and the drag force on the particle are known, the optical force can be calculated using Eq. 7.1. The resulting axial optical force by the vortex beam on the 2 \( \mu \text{m} \) Ps particle is depicted in Fig. 7.14 (b). Note, since this measurement was conducted several millimeters away from the exit of the ALS injector, the gas can be considered stationary at the interaction region, i.e., \( v_g = 0 \) in Eq. 7.3. The maximum axial optical force, closer to the focus of the vortex beam, was \( F_{z}^{opt} = 5 \text{ pN} \). This force is in the same order of magnitude with the calculation made by Eckerskorn \[92\] for carbon particles.
7.2.4 Preliminary particle confinement by the vortex beam

In the PCS setup, by imaging the particle deposition through the in-vacuum microscope, few preliminary particle beam confinement by the vortex beam had been observed. This is shown in Fig. 7.15(a), where 2 µm PPS particle beam was “confined” into the dark core of a 4 watt vortex beam. The particle deposition was imaged at 10 mm below the vortex beam focus, see Fig. 7.15(a). Since the particle beam was bigger than the vortex beam, depending on the position of individual particle in the beam, either they were confined into the dark core of the beam or deflected outside of the maximum intensity ring. From this observation, I assumed that particles which formed the outer ring of Fig. 7.15(a) were deflected and those formed the inner spot were focused by the beam.

Although such kind of particle beam profile is not common, in totally different interpretation, one can argue that the particle devoid region in the Fig. 7.15(a) was created by the intense part of the laser beam “burning away” the particles collected on the microscope slide, leaving only those unexposed particles in the dark spot.
This can only be confirmed by quantitatively measurement using the side-view imaging. In PCS, it was not possible – because (1) the resolution of the imaging setup was too low to detect individual particle motion inside the vortex beam, (2) the depth of focus was too high (much bigger than the laser beam width), which makes it hard to detect out-of-focus particles which were potentially not interacted with the laser. In the ICS we improve these and other drawbacks of the PCS mentioned in section 7.1.1 and the results achieved will be presented in following section.

7.3 Results from the improved setup

In this section, I will present the recent results obtained on focusing, concentrating and guiding of virus and PS particles, using the improved setup. The detailed description of the experimental setup was given in section 7.1.2. Briefly, the particles were delivered using a CyNAI that was mounted horizontally on the optical table (see Figures 7.4 and 7.5). Unlike the PCS, all the optical elements including the imaging objectives, were mounted outside of vacuum and the particles were injected into a small chamber of 130 mm \(\times\) 32 mm \(\times\) 32 mm in size. In all of the measurements, a first order Quasi-Bessel beam with a dark core in the middle, was used to manipulate the particles. This beam was generated by illuminating a spiral phase plate followed by an axicon with a Gaussian beam (see Figs. 7.7 and 7.8). Two side-view cameras (Photron SA4, for magnified image, FOV 2 mm \(\times\) 2 mm and Photometric camera, for wide-field image, FOV 8 mm \(\times\) 8 mm), combined with three lasers that were illuminating the particle beam at different geometries, were used to image the particles. See Fig. 7.4 and table 7.2 for the imaging and illumination geometries, and their summaries.

In this setup, based on the illumination laser used and the particular application of the measurement, a single experiment typically consists of three different types of measurements.

1. **Beam profile measurement:** for this measurement, the particles were illuminated by the counter propagating QBB and the camera exposure time is adjusted to record long streaked image of the particles. A typical raw image can be seen in Fig. 4.3(b). The measured images are stacked together, after the background is subtracted and the intensity is thresholded. Then, the image stack is average to determine the QBB profile on the camera FOV (see section 7.1.4 and Fig. 7.10).

2. **Particle dynamics measurement:** here the particles were illuminated by tightly focused DILAS diode laser, operated to produce closely spaced bursts
of 100 ns pulses, so that multiple snapshots of a particle is recorded in a single frame. A typical raw image can be seen in Fig. 7.17. Velocity and acceleration of individual particles along with their trajectories can be determined with these images.

3. **Particle density measurement**: in this measurement, the particles were illuminated by the more powerful Empower laser, and a single and bright snapshots of the particles were recorded. A typical raw image can be seen in Fig. 4.3(d). The laser was focused by a cylindrical lens to a light-sheet of size $5 \text{ mm} \times 20 \mu\text{m}$, parallel to the particle beam axis, so that only in-focus particles were illuminated. The results from this measurement were used for counting individual particles and building the 2D projected particle density map, which were used to assess the particle beam confinement by the laser. The measurement types and applications are summarized in the following table.

<table>
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<tr>
<th>measurements</th>
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<tbody>
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<td>counter propagating QBB</td>
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<td>Particle dynamics</td>
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<td>Particle density</td>
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Table 7.1: Summary of measurements constituted in a single experiment.

### 7.3.1 Gas velocity in the chamber

In an ideal case, particle from aerosol injector are delivered into a high vacuum environment. At the exit of the injector, the gas expands very rapidly and the particles travel with nearly at constant velocity. However, our laboratory experiment was performed at relatively higher pressure environment ($> 0.1 \text{ mbar}$). Therefore, the particles experience a drag force, due to the relatively high gas density, that results in strong particle deceleration. This can be seen in Fig. 7.17(a), where the particle was decelerated without the presence of the laser field.

The drag force that the particles experience in the chamber is proportional to the relative velocity between the particle and the gas. So, before applying the drag force calculations in the measurements, the velocity of the He gas and particles in the chamber, need to be considered. The particles velocity can be accurately measured experimentally, using the method described in section 7.3.2 and chapter 4. However, the gas velocity cannot be measured directly, at least with the current technique.
7.3. Results from the improved setup

Figure 7.16: The He gas velocities at 0.99 mbar chamber pressure. (a) The velocity along the transverse direction and (b) the velocity along the axial direction. Where the injector exit was at $z = 0$. This simulations were done by Nils Roth using a technique described in [57].

developed in the lab. Instead, it was calculated using molecular dynamics simulation software, using the injector geometry and the measured pressures upstream and downstream of the injector as an input. The simulation solves the Navier–Stokes equations using a finite-element solver developed using COMSOL Multiphysics software [57,204]. In Fig. 7.16 shows the gas velocity field in the chamber, calculated for 0.99 mbar chamber and 2 mbar upstream pressure. As seen in the figures, close to the exit of the injector, both in the lateral and axial direction, the gas has relatively high velocity of $\sim 50$ m/s. However, it quickly decelerated further downstream of the injector. Beyond 15 mm from the exit of the injector, where the particle dynamics measurements were performed, the gas velocity can be considered as stationary. Therefore, in the following force and mass calculations, I assume the gas velocity to be zero.

7.3.2 Particle velocity and acceleration

The velocity and acceleration of a particles were determined using the “snapshot” direct optical imaging technique described in section 4.2.1. In “snapshot” imaging, the particles were illuminated by a short and high repetition laser pulses, that gives a stroboscopic image of a particle on the camera similar to the raw image in Fig. 7.17. As discussed in the previous section, the DILAS laser was used to illuminate the particles in such measurements. The advantage of this diode laser is that by modulating the driver current, the pulse duration and repetition rate can be controlled in wide ranges (repetition rate up to 1 MHz, pulse durations
Figure 7.17: Stroboscopic raw image of the CpgV particle in 1.5 W QBB, at 1 mbar and illuminated by the DILAS laser operated with 40 µs pulse period. The optical funnel has intensity distribution similar to Fig. 7.8 with peak intensity of $1.5 \times 10^6 \frac{W}{cm^2}$. The red dashed-lines indicate the QBB central ring peak intensity positions ($w_p(z)$). a) A typical laser-off particle trajectory. Due to the drag force caused by the He gas in the chamber, the particle is decelerated as it moves in the $-z$ direction. In (b) and (d) the particles are deflected by the laser. c) the particle bounced inside the minimum intensity region of the QBB. (e) the particle move from the second intensity ring of the QBB to the first before it is deflect strongly. (f) The $z$ component of an example particle trajectory, and the corresponding velocity and acceleration. In the plots, the measured data points are indicated by the circles and the continuous curve represents the polynomial approximated trajectories.

$10–100 \text{ ns})$. In such measurements, the particle concentration run through the injector is greatly reduced, so that ideally, not more than three particles trajectories are imaged in a single frame. This reduce multiple trajectory overlaps, which is hard to distinguish during analysis.

To image a particle moving at an average speed of $v_z$, $n$ times on a FOV of length $l_{FOV}$, the number of laser pulses $n_p \geq n$ in a single burst period, should fulfill the following relations:

$$n_p \geq \frac{t_{exp}}{t_s} \geq \frac{l_{FOV}}{v_z} \frac{1}{t_s} \quad (7.4)$$
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where \( t_{exp} \) the camera exposure time and \( t_s \) burst pulses separation. For the typical speed of the particles in the new setup (2–20 m/s), and \( l_{FOV} = 2 \text{ mm} \), \( t_s \) between 10 and 50 µs is proven to be enough to acquire 10–20 snapshot of a single particle in a frame.

The velocity and acceleration of the particles were determined from the particle trajectories, similar to the raw images shown in Fig. 7.17 (a–e). To extract the particle trajectory, first the intensity centroid position \( (C(x, z, t_s)) \) of each snapshot is extracted from the raw images using a software. The center of each blue circle in images indicate the centroid positions of the snapshots. Then, the particle trajectory is approximated by a third order polynomial through the measured centroid \( C(x, t_s) \) and \( C(z, t_s) \) coordinates, separately (see Fig. 7.17 (f) top plot). Finally, the velocity and acceleration of each particle trajectory was calculated by analytic first and second time derivative of the approximated polynomials, respectively (see Fig. 7.17 (f) bottom two plots). Note, some particles have complex trajectories, such as Fig. 7.17 (c) and (e), that can’t be described by a simple polynomial. In such cases, a piecewise polynomials were used to approximate the trajectories.

7.3.3 Mass and size of CpGV particles from Stokes’ drag

In the absence of the laser field in the experimental chamber, the Stokes’ drag is the only force the particles experience. This can be used to calculate the mass of a particle, if either its density or size is known. The mass of a CpGV particle and approximate radius \( (\tilde{r}_p) \), can be calculated using the following relation derived from the Stokes’ equation in Eq. 7.3 and the equation of motion Eq. 7.1 for \( v_g, F^\text{opt}, mg = 0 \):

\[
\begin{align*}
    m_{GV} a &= F_d = \frac{6\pi \mu \tilde{r}_p v_p}{C} \quad (7.5a) \\
    m_{GV} &= \frac{4}{3} \pi \tilde{r}_p^3 \rho_{GV} \quad (7.5b)
\end{align*}
\]

Where \( \tilde{r}_p \) or \( \tilde{d}_p \) corresponds to a radius or diameter of a sphere that has approximately equal volume with a CpGV particle, \( C \) is the Cunningham slip-correction factor and \( \rho_{GV} \) is the density of a CpGV particle, \( \rho_{GV} = 1430 \text{ kg/m}^3 \) (see section 7.1.5).

In Fig. 7.18 depicted the size and mass histogram of the CpGV particle calculated using the above equations. The measured was conducted at 21 mm from the exit of the injector in laser-off condition. At this region in the chamber, the gas can be considered as stationary (see Fig. 7.16). The CpGV particle were delivered into the chamber at a pressure of 0.99 mbar and room temperature of 293 K. This condition
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... gives: mean-free-path of He = 193 µm, $C = 2192$ and $Kn = 1288$. The particles were illuminated by the DILAS laser, to record multiple snapshots of the particles trajectory, similar as the raw images in Fig. 7.17 (a). The particles velocity ($v_p$) and acceleration ($a$) is calculated from the raw images, using the method described in section 7.3.2. Then, each CpGV particle approximate radius ($\tilde{r}_p$) and mass was calculated using Eq. 7.5.

Figure 7.18: Histogram showing (a) equivalent diameter and (b) the mass distribution of CpGV particles, calculated using the Stokes’ drag force in laser-off measurement. The single and double particle distributions are fitted with Gaussian functions indicated by the dashed turquoise and purple curves, respectively. The red curve indicates the sum of the two Gaussian functions.

The measured particle diameter and mass distribution are shown in Fig. 7.18, and $\tilde{d}_p = 377.4 \pm 35$ nm and $m_{GV} = 37.7 \pm 6.1$ fg ($37.7 \pm 6.1 \times 10^{-15}$ g), respectively. These values are well in agreement with the actual equivalent single CpGV diameter, $\tilde{d}_p = 370$ nm, and the actual mass of a single CpGV particle, $m_{GV} = 37.9$ fg. The actual mass is calculated using the CpGV density and its actual CpGV volume. The mass histogram in Fig. 7.18 (b) was fitted with double Gaussian function, and it showed two particle mass distributions. The first distribution was centered at a single particle mass (the dashed turquoise curve), and the second distribution was centered at twice the mass of a single CpGV particle (the dashed purple curve). The red solid-line indicated the sum of the two Gaussian fits. The second mass distribution indicate that, there are few aggregates consists of two particles. However, the majority of the aerosols (> 88%) were single particles. This confirm that most of the particles were isolated, which is crucial for the force calculations in the next section.
7.3.4 Forces on the CpGV particles by the QBB

There are two forces to be determined from this measurement. First, the net force on the particles, which was directly calculated from the measured acceleration and mass of the particle. Second, the optical force, calculated using the equation-of-motion of a micro-particle in the presence of a Stokes' drag. Note that, the particle were injected and imaged parallel to the $x, z$ plane. Therefore, the force due to gravity, that acted in the $-y$ direction, was not taken into account in the optical force calculation. Furthermore, due to the smaller mass of the particles investigated, the gravitational force was negligibly small compared with the optical and drag forces.

Figure 7.20 shows the average laser-off and laser-on deceleration field of CpGV particles in 0.99 mbar chamber pressure. In the laser-on measurement, the optical funnel used had intensity distribution similar as in Fig. 7.8 and for 0.75 W laser power used, it gives peak intensity of $0.75 \times 10^6 \text{ W/cm}^2$ at the QBB focus. The focus of the QBB was at 21 mm from the exit of the injector, and $x, z = 0$ in the figure indicates the transverse and axial positions of the QBB focus on the FOV, respectively. The laser-on acceleration map was generated by averaging over accelerations from $\sim$3000 trajectories each had up to 100 approximated trajectory points within the FOV. Note, for the same illumination period, snapshots of fast particles appear less often in the FOV than slow particles. To compensate for this bias in acceleration map generation, the time step ($\Delta t$) used to approximate each particle trajectory was chosen taking their velocity into consideration. The time step is calculated using the following relation: $\Delta t \approx \frac{z_{\text{bin}}}{v_{\text{avg}}}$, where $z_{\text{bin}}$ is the bin length in the $z$ direction and $v_{\text{avg}}$ is the average velocity of the particle. That mean that, each particle appears only once in a single bin.

![Figure 7.19: CpGV particle laser-off velocity histogram at 0.99 mbar chamber pressure. (a) In x-direction and (b) in z-direction.](image)

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In the laser-off, the particles had very small lateral motion (see Fig. 7.17(a)), moving with an average lateral velocity of $|v_x| = 6.2 \pm 5 \text{ mm/s}$. Whereas, in the axial direction the particle were moving with a velocity of $v_z = -1.72 \pm 0.42 \text{ m/s}$ (see Fig. 7.19 (a) and (b)). The maximum laser-off averaged axial deceleration, along $x = 0$ in Fig. 7.20 (a), was $\sim 1300 \text{ m/s}^2$. However, in the laser-off, the radial acceleration was nearly zero.

![Figure 7.20: Averaged axial deceleration field of CpGV particle at 0.99 mbar chamber pressure. (a) In laser-off. (b) In 0.75 W QBB, $0.75 \times 10^6 \text{ W/cm}^2$ peak intensity. The Laser was propagating in the $+z$ direction, opposite to the particles.](image)

In the laser-on averaged axial deceleration field shown in Fig. 7.20 (b), the maximum axial deceleration was $\sim 2500 \text{ m/s}^2$, which is corresponding to $\sim 0.1 \text{ pN}$ force on the particle. Whereas, the maximum axial deceleration measured on a single particle, closer to the focus, was $\sim 7700 \text{ m/s}^2$ and the maximum radial acceleration, i.e., deflection measured on a particle was $\sim 4500 \text{ m/s}^2$.

An interesting fact can be noted in the average deceleration field in Fig. 7.20 is that the particles experience considerable decelerations in the region where there is no QBB. Furthermore, the maximum deceleration observed was not even at the QBB focus, as one would expect. The photophoretic interaction of the particles with the gas molecules and the laser field is a complicated phenomenon that require the knowledge of the thermal, aerodynamic and optical property of the particles. However, the simple hypothesis for the particles deceleration outside of the QBB is that each particle might has accumulated enough thermal energy during the integrated exposure as they passed through the QBB long before reaching the QBB.
focus. Therefore, they have enough temperature gradient when they arrived at the camera FOV even they were not within the beam anymore. This could result in a strong photophoretic force, hence deceleration on the particles.

The averaged net force field in the chamber which acts on the particles, for the given laser power and pressure, can now be calculated by multiplying the laser-on averaged-deceleration field in Fig. 7.20 (b) with the mass of the CpGV particles.

Accurate determination of the optical force \( F_{opt} = F_{PP} + F_{RP} \) and mapping of the optical force field in the chamber, requires determining the 3D position of the particles inside the laser field. Since the particles were imaged only in a single 2D plane in the current setup, it is not possible to get this position information. Therefore, here, I only give the maximum optical force calculated closer to the QBB focus. In the axial direction, the maximum optical force was \( F_{opt}^z \approx 0.22 \) pN, calculated by subtracting the drag force from the net force on the particle (see Eq. 7.1). In the lateral direction, the maximum optical force was \( F_{opt}^x \approx 0.59 \) pN. The magnitude of these optical forces are smaller compared with the optical force on 2 \( \mu \)m PS particle by a 4 W vortex beam, measured at the sample chamber pressure in section 7.2.3. However, if we correct the laser power and particle surface area, which are linearly related with the force, the axial force by the QBB would be \( \sim 6.4 \) times stronger (see Eq. 3.17).

The results from the deceleration and force calculations gave the confidence that the optical force given by the QBB is indeed strong enough to deflect, confine, decelerate and even stop biologically relevant particles. In the following section I will present the results obtained in focusing and concentrating of particles using the QBB.

**7.3.5 Particle focusing and concentrating**

Particle focusing refers to the lateral confining of particle beam towards the intensity minimum region of the laser field, i.e., to a smaller spot, whereas particle concentrating is used to described the increase in particles number density due to the linear axial deceleration and/or lateral deflection of the particles by the laser field. Therefore, the total change in the projected particle density (average number of particle per unit area) could be either due to particles focusing or deceleration of the particles, or a combination of both effects. In such analysis, I compare the particle beam density and width changes between the laser-on and laser-off conditions, measured using the direct optical imaging technique described in sections 4.4, i.e., the Particle density measurement in section 7.3.
As discussed in section [7.1.2] the CyNAI produces a particle beam > 100 µm in width, whereas the QBB has lateral width of 7.8 µm closer to its focus. That means that, huge portion of the particle beam doesn’t interact with the intense part of the QBB. Therefore, in the particle density analysis, ideally, only the volume of the particle beam which was overlapped with the laser field, should be considered. To do so, a thin slab of the particle beam was illuminated by a light-sheet focused to ∼20 µm thick, and imaged with an objective lens with depth-of-focus of 14 µm, which was a factor of two bigger than the inner peak-to-peak diameter of QBB. To further select only particles which were within the volume of the QBB, the out-of-focus particles were discarded by analyzing the particles image diameters and brightness.

**Focusing polystyrene particles**

Polystyrene beads have density similar to that of water (\( \rho_{PS} = 1050 \text{ kg/m}^3 \)) and they are easy to aerosolize using the GDVN nozzles, these made them an ideal sample for this experiment. Pure PS particles are transparent at 532 nm, so to increase the absorption we typically use fluorescent or carbon coated beads. The typical sizes investigated in this work were 2 µm, 1 µm and 0.5 µm diameter PS particles.

![Figure 7.21](image)

Figure 7.21: Focused 2 µm PS particles beam using 0.75 W quasi-Bessel beam. 2D PPD map, (a) in laser-off and (b) in 0.75 W quasi-Bessel beam. (c) The averaged transverse PPD in a small area at \( z \approx 4 \text{ mm} \) from the focus of the QBB, and fitted with a Gaussian function. Note, \( N_p \) is the average number of particles that fall within each pixel at any time. It is equivalent to the total number of particles fall in each pixel, over the entire measurement, normalized by the total number of frames \( N_f \).
7.3. Results from the improved setup

In Fig. 7.21 shown 2D PPD map of 2 µm diameter FPS particle beam focused by an optical funnel constructed by 0.75 W QBB at 0.57 mbar chamber pressure. The measurement was performed at 3.7 mm from the QBB focus. The sample was suspended in water with a 2 mM sodium azide (NaNa₃) solution to a concentration of 7.5×10⁷ particle/ml and aerosolized using the GDVN nozzle. With no laser, the particles were moving with a velocity of 13.9 ± 2 m/s. The relevant experimental parameters are given in table 7.2. In Figs. 7.21 (a) and (b) depicted the laser-off and laser-on 2D PPD per µm² area, i.e., D in Eq. 6.1. Note the unit of D, it is not normalized by the particle generation rate R. This is because two measurements with the same sample flow-rate and number of frames were compared, R can be ignored without affecting the outcome. The lateral projected width of the particles density at the center of the FOV is plotted in Fig. 7.21 (c). The laser-off and the laser-on particle densities are indicated in green and magenta, receptively. By fitting Gaussian function through the measured particle densities, the FWHM particle beam of the laser on is ∼2.3 times smaller and the peak of the PPD was improved by a factor of two, compared with the laser-off condition. This density improvement alone could improve the overall hit-rate by a factor of two.

Focusing Granulovirus particles

In Fig. 7.22 shown 2D PPD of CpGV particle beam focused by an optical funnel constructed by a 0.5 W QBB (0.5×10⁶ W/cm² peak intensity) at 0.4 mbar chamber pressure. The 2D intensity profile of the optical funnel at 1 W QBB was given in Fig. 7.8. The QBB focus was located within the FOV at x, z = 0, which is 21 mm from the exit of the injector. In this measurement, CpGV particles suspended in water to a concentration of ∼5×10⁸ particles/ml, were aerosolized through the GDVN, and injected into the vacuum using the CyNAI. The relevant measurement conditions are listed in table 7.2. In the reference measurement the particle were moving with a velocity of 17.5 ± 1.1 m/s. In Fig. 7.22(a) and (b) are shown the laser-off and laser-on 2D particle density of the CpGV particle beam, respectively. Note, the two density maps are represented in the same color range. The lateral projected width of the PPD, averaged within ±20 µm region from the focus (through z = 0) and the axial cross-section of the PPD (through x = 0), are plotted in Fig. 7.22 (c) and Fig. 7.22 (d), respectively. The QBB focus position in the FOV is indicated by the dashed-line at x, z = 0. In order to estimate the particle beam width and peak density, the lateral cross-section of the PPD was fitted with a three parameter Lorentzian function. In this particular measurement, due to the high particles density close to the center of the laser-on PPD, the Lorentzian function fitted the data better than a Gaussian function that was commonly used.
Chapter 7. Optically focusing and guiding of aerosolized particles at low pressure

Figure 7.22: Focused CpGV particle beam using 0.5 W quasi-Bessel beam. 2D projected particle density map, (a) in laser-off and (b) in 0.5 W quasi-Bessel beam. (c) Lateral cross-section of the particle beam density averaged within small area at the focus of the QBB, \( z \approx 0 \). To determine the particle beam width, this points were fitted with a Lorentzian function. (d) Axial cross-section of the particle beam density averaged within small area along the axis of the QBB, \( x \approx 0 \). Note, \( N_p \) is the average number of particles that fall within each pixel at any time. It is equivalent to the total number of particles in each pixel, over the entire measurement, normalized by the total number of frames \( N_f \).

From simple FWHM calculation of the fitted Lorentzian function, the laser-on particle beam width was confined roughly by a factor of two and the particle beam peak density, at the center of the Lorentzian function, was \( \sim 3.5 \) times higher compared with the laser-off. However, as seen in Fig. 7.22(c), the actual
7.3. Results from the improved setup

<table>
<thead>
<tr>
<th>Laser power (W)</th>
<th>CpGV</th>
<th>2 µm PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Intensity (W/cm²)</td>
<td>5 × 10⁵</td>
<td>7.5 × 10⁵</td>
</tr>
<tr>
<td>Laser off average particles velocity</td>
<td>17.4 ± 0.93 m/s</td>
<td>13.9 ± 2 m/s</td>
</tr>
<tr>
<td>Chamber pressure (mbar)</td>
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<td>0.57</td>
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<tr>
<td>Injector pressure (mbar)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Skimmer pressure (mbar)</td>
<td>0.58</td>
<td>0.6</td>
</tr>
<tr>
<td>Sample flow rate (µl/min)</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Sample concentration (particles/ml)</td>
<td>~5 × 10⁸</td>
<td>7.5 × 10⁷</td>
</tr>
<tr>
<td>Particle generation rate (R, particles/s)</td>
<td>1.17 × 10⁴</td>
<td>2.25 × 10³</td>
</tr>
<tr>
<td>GDVN gas flow rate</td>
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<td>16 mg/min</td>
</tr>
<tr>
<td>The FOV center relative to the QBB focus</td>
<td>the focus was within the FOV, at x, z = 0</td>
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</tr>
<tr>
<td>Injector position relative to the QBB focus</td>
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<td>17 mm</td>
</tr>
<tr>
<td>Number of frames considered</td>
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</tr>
<tr>
<td>Frame rate</td>
<td>1 kHz</td>
<td>1 kHz</td>
</tr>
</tbody>
</table>

Table 7.2: Experimental parameters for CpGV and PS particle focusing

density of particles at the axis of the beam was much higher than the peak of the approximating function. That means, it can be assumed that the particle beam be concentrated by roughly by a factor of four. Furthermore, since we were imaging the projected particle beam density, the relatively smaller column of particle beam that had interacted/overlapped with the laser, were integrated over the entire volume of the particle beam which potentially might have not interacted with the laser. This could have reduced relative peak particle density between the laser-on and laser-off measurements depicted in Fig. 7.22(c). The overall particle density improvement can also be seen in the axial cross-section, plotted in Fig. 7.22(d).

As seen in Fig. 7.22(b), the maximum particle concentration is appear to be around ~1 mm away from the QBB focus. The first hypothesis for such high particle density above the focus is that the intensity of QBB used in this particular measurement was too high, therefore most particles were either deflected or kicked back by the laser before they arrived at the focus, see Fig. 7.23. The appearance of higher particle density above the focus could also be due to a non-uniform side illumination of the particles by the imaging laser (Empower laser). Such non-uniform illuminations could cause the particle beam at the brightest part of the
beam to appear denser, because more particles would be imaged at the brightest part of the beam. The other important point to note here is that, the bin size in the 2D density shown in Fig. [7.22] is 20 \( \mu \)m while the QBB focus was only 7.5 \( \mu \)m in diameter. Therefore, the concentration of the particles inside the dark core of the QBB could be even higher than what is shown in the particle beam profile. However, the resolution of the imaging system was not accurate enough to resolve such a small region within the QBB.

![Figure 7.23: CpGV particle beam density at 0.4 mbar chamber pressure and different laser powers. These plots are generated by taking the average of the particle density within \( \pm 50 \) \( \mu \)m region at the QBB focus and fitting a Lorentzian function through them.](image)

Ultimately, the hit-rate is determined by the particle density projected along the direction of the X-ray beam. In the laboratory, due to the orthogonal configuration of the illumination laser and the camera, we image only a horizontal slab of the PPD, i.e., only particles lay on the focal plane of the camera. So, to accurately predict the improvement in the hit-fraction, between the laser-on and the laser-off cases, the PPD in Fig. [7.22] has to be inverted or projected back into a cylindrical symmetric volume, for example using Abel transform [205]. Then, integration of this volume along the X-ray would give the full extent of the 2D PPD, and it can be uses to assess the actual improvement in the hit-fraction. That means, integrating the transverse particle density in Fig. [7.22] (c) should give the peak PPD at the
7.3. Results from the improved setup

center of the particle beam. Therefore, it is fair to assume the laser-on peak density has improved roughly, by a factor four compared with the reference measurement.

As shown in the CpGV particle confinement and concentrating above, if particles are moving with sufficiently slower speed (< 20 m/s as it is tested so far), they can be manipulated with a reduced laser power, which is always desirable to reduce the radiation damage on the particles. Seen in Fig. 7.23 is the transverse profile of a CpGV particle beam subjected to different laser powers, and measured at 21 mm from the exit of the injector. The 2D intensity profile for 1 W QBB is given in Fig. 7.3 and all the measurement conditions are similar as in table 7.2, except the laser power. The maximum confinement and concentration of the particle beam was achieved at 0.5 W and increasing the laser power reduced the peak particle density, linearly. This means that, as the laser power increases, the particles start to deflect away from the beam or recoil back long before they reach the laser beam focus or particle detection area. Figure 7.23 shows that, higher laser power is not always advantageous and the laser power should be optimized for the particular experimental conditions.

A comprehensive study on the optical property of the CpGV or FPS, and their interaction with the laser field and the surrounding gas molecules are yet to be made. However, our results show that CpGV particles focuses better by the QBB than the 2 µm diameter FPS particles. This could be either the CpGV absorbs the laser better than the FPS, i.e., stronger PPF, or its smaller size made it easier to manipulate by the laser field or a combination of both. This is because PPF scales as the square of the particle size (see Eq. 3.17), while the mass scales as cube of the particle size. Compared with the 2 µm diameter FPS, CpGV particles ~60 times smaller in cross-section but ~350 times smaller in mass. Therefore, they required ~6 times less intensity to be shifted the same distance by the laser beam, and thus they can be manipulated better and with reduced laser power.

Observation on particle beam deflection by the optical funnel

Deflection of the full particle beam was observed when the particle and laser beam axes were miss-aligned by a few tens of micrometer. This is shown in laser-off and laser-on 2D PPD of CpGV particle beam, in Fig. 7.24. The measurement was conducted at 11 mm from the exit of the injector at 0.8 mbar chamber pressure, and 2.25 W QBB. The axes of the particle and laser beam were misaligned only by 40 µm relative to each other, and at the middle of the FOV this resulted in a ∆z = 110 µm deflection of the entire particle beam. Furthermore, from other similarity measurements, the magnitude of the deflection increases with the laser power. Such deflections are not desirable for the effort to confine the particle
beam, and the particle-laser alignment must be done carefully. However, such deflections might have other application, such as in particle size sorting using their lateral deflections.

**Observation on particles acceleration outside of the optical funnel**

In Fig. 7.25 shown the trajectories of few selected CpGV particles in 1.5 W QBB and at 1.0 mbar chamber pressure. The particles were illuminated by the DILAS laser with 40 µs pulse period. The first peaks of the QBB are indicated by the two dashed red line, and the centers of the blue circles indicate the intensity centroid of the particle snapshots. In the region where the laser field exit, the trajectories shows a typical deceleration of the particles. However, it is also show that the particles being deflected and continue accelerating long after they left the interaction region. It could be that, the particles have kept the temperature gradient for significantly long period of time, and hasn’t reach thermal equilibrium in the time scale of the imaging. This might indicate that the momentum transfer is not an instantaneous and it could become problematic when trying to guide even high-speed particles.
Figure 7.25: Stroboscopic image of CpGV particles decelerated and then acceleration by the 1.5 W QBB. The laser is propagating in the positive \( z \)-direction, opposite to the particles. The two dashed red lines marks the QBB central core peak intensity positions, and the center of each blue circles indicates the centroids of the particles positions.

Therefore, in the future works it is vital to investigate a method to study this dynamic momentum transfer processes. In different hypothesis, it could also be that the particles were continuously changing mass, for instance due to evaporation or ablations by the heat induced from the absorbed laser.
8 Conclusion and Outlook

8.1 Summary

The work presented in this thesis demonstrated, for the first time, the guiding, confining and concentrating of aerodynamically focused high-speed particles in vacuum, using an optical funnel. This optical funnel was constructed with Laguerre-Gaussian or a slowly diverging quasi-Bessel beam, with a dark core in the middle. A new experimental technique, for direct mass and size measurement of nanoparticles, based on Stokes’ drag, was also demonstrated. In the other task of this dissertation, an improved aerodynamic particle injection system based on a single convergent nozzle, and an aerosol imaging diagnostics mechanism based on optical illumination, were demonstrated. The motivation of these experiments was the challenge of delivering single particles to the sub-micrometer X-ray beam focus in coherent X-ray diffractive single particle imaging. In this context, the low density polystyrene beads of various sizes, and the biologically relevant *Cydia pomonella* granulosis virus particles, were well-suited model samples for studying their laser induced dynamics, the resulting particle beam focusing and concentration, as well as their X-ray diffractions. A CpGV particle consists of a single virion body, surrounded by an occlusion bodies made up of well-ordered protein crystal of size $200 \times 200 \times 370 \text{ nm}^3$. This means that depending on the wavelength of the light being observed, it can be considered as a single particle or a protein crystal.

Employing the convergent nozzle aerosol injector in the laboratory, 300 nm virus particles were focused down to 3.5 µm FWHM spot. Using this injector technology in SPI experiment at the FLASH FEL facility in Hamburg, a hit fraction of more than 18 % was recorded. Furthermore, employing this injector in SFX experiments at the LCLS CXI hard X-ray instruments, extremely low background X-ray diffraction from aerosolized macromolecular nanocrystals of CpGV particles was obtained. This is particularly interesting for coherent diffractive imaging of weakly scattering biological specimen such as nano-crystals, cells or isolated viruses.

The experimental approach employed for the optical focusing is based on the newly introduced concept of optically guiding and transporting of absorbing aerosol particles using a doughnut-shaped laser illumination. The experiment consists
of delivering a stream of aerosolized target particles into a He gas field vacuum chamber against a counter propagating and diverging first order quasi-Bessel beam with a dark central core. The profile of the QBB, combined with the optical forces arising from photophoretic and radiation pressure, decelerates and guides the particles into a convergent trajectory which is directed towards the focus of the QBB. The effect of the optical force on the particles is governed by their velocity, the relative position in the laser field, the chamber pressure and the intensity of the Bessel beam.

A versatile experimental setup was constructed consisting of, high-speed and high-repetition rate direct-optical imaging, an optimized aerosol particle injection system and a laser beam shaping and characterization setup to generate an optimized optical funnel. With this experimental setup, snapshots of a 300 nm size particle moving with a speed of $> 300$ m/s, can be recorded, and its positions in the camera FOV can be extracted with sub-pixel accuracy. These snapshots were used to reconstruct the particle trajectory, and study the particles dynamics from the their velocities and accelerations. Furthermore, accumulating many such particle positions over long measurement periods, important aerosol beam characteristics such as instantaneous particle beam density and transverse width can be determined. As the particles were moving with very high velocity, the spatial profile of the optical funnel should be shaped such that it maximizes the particle-laser interaction time and length. Therefore, a slowly diverging and high aspect ratio quasi-Bessel beam was constructed.

In the first experiment, the velocities and accelerations of the particles were directly determined from the particle trajectories. The results from such measurements were essential to calculate the mass of the particles as well as study particles dynamics changes between the laser-off and laser-on conditions. The velocities and decelerations of particles, determined in a laser-off measurement, were used to directly calculate the masses and sizes of the particles using a slip-corrected Stokes’ drag force. For instance, the mass of a CpGV particle determined using this technique was $m_{CpGV} = 37.7 \pm 6.1 \text{ fg}$, which is very close to the ideal mass of a CpGV particle calculated from its density. Furthermore, the narrow distribution of the mass indicated that the majority of the aerosol generated were consists of isolated particles, which is vital for the particle dynamics and optical force calculations. From the laser-on measurements of this experiment, the net force field acting on the particles was calculated from the mass and averaged deceleration field of the particles. Then, the optical force induced by the Bessel beam was calculated using the equation of motion of a particle in the presence of a Stokes’ drag and the optical forces. Using only 0.75 W QBB, which has peak intensity of $7.5 \times 10^5 \text{ W/cm}^2$, it was measured a maximum CpGV particles axially deceleration of 7700 m/s$^2$ and a maximum radial deflection of 4500 m/s$^2$, which is equivalent to 0.3 pN and
0.18 pN force acting on the particle, respectively. The magnitude of these forces are indeed very small, however in the scale of the particles being investigated, they were strong enough to steer and focus the particles.

Having demonstrated the optical force is strong enough to manipulate the individual high-speed particles, the efforts were focused on applying this forces to confine and concentrate the full particle beam, which is the primary goal of the thesis. This was then the second experiment, where the particle beam density and transverse width was measured, and compared between the laser-on and the laser-off conditions. This provided an estimation of the change in particle beam concentration by the introduction of the laser field.

The first experimental evidence of biological particle focusing and concentrating, was carried out on a CpGV particle beam that was moving with an axial velocity of $17.4 \pm 0.93 \text{ m/s}$, using an optical funnel constructed by a 0.5 W QBB (5 $\times$ $10^5 \text{ W cm}^{-2}$ peak intensity). From the particle density analysis, the width of the particle beam was confined by a factor of two and the peak particle concentration, close to the QBB focus, was improved at least by a factor of 4, compared with the reference laser-off measurement. Similarly, using a 0.75 W QBB (7 $\times$ $10^5 \text{ W cm}^{-2}$ peak intensity), FPS particle beam moving with $13.9 \pm 2 \text{ m/s}$, was confined roughly by a factor of two in its lateral width and the peak particle density was also doubled. This particle density increase can be attributed to both the axial deceleration and transverse confinement of the particle beam to the dark core of the QBB, i.e., the slower the particles, the higher their density per unit time. These result also showed that, due to their smaller size, CpGV particles focus much better than 2 µm diameter FPPs.

### 8.2 Future works

#### 8.2.1 Tunable Bessel beam

In this work, maximization of particle-laser interactions was based mainly on controlling the particles’ speed and beam size while using a fixed optical funnel profile. Using an optimized capillary injector in combination with a controlled pumping scheme, aerodynamic slowing down of particles has been demonstrated. However, aerodynamically slowing of particles inherently results in a bigger particle beam and also reduces the particle transmission efficiency due to diffusion. Furthermore, different particle species exhibit distinct optical as well as aerodynamic properties so they could focus differently, for example in the resulting particles velocity and beam width. Therefore, besides optimizing the injector for each condition, it is also desirable to have a flexible optical funnel where the optical parameters (divergence,
in the current optical funnel generation setup, which is based on a fixed SPP and
axicon, slight tuning of the optical parameters was possible. For instance, using an
axicon with different angle to modify the propagation length; SPP with different $l$
to change the beam size, (see Fig. 3.9), or different telescope lens arrangements to
demagnify the beam size and divergence. However, these require modification of the
various optical elements as well as rigorous and time consuming optical and particle
alignments. Alternatively, by replacing the SPP and axicon with a single SLM, most
of the above parameters can be tuned actively for a specific condition. Therefore,
in the future work, integrating an SLM into the setup will be advantageous.

8.2.2 Possible improvements on the experimental setup

The reduction of the chamber size has greatly helped to simplify and stabilize the
overall experimental setup, by allowing the construction of all the sensitive optical
elements outside of the vacuum. However, the reduced size of the chamber comes
with a few cost. The in-vacuum microscope, which was imaging the particle beam
in the transverse plane inside the primary vacuum chamber, doesn’t fit any more in
the improved chamber. Particle-laser alignment requires transverse plane imaging
of the relative position of the particles and laser beam at two different axial points.
In the PCS setup, this was done by simultaneously imaging the transverse profile
of the particle and laser beam using the in-vacuum microscope. However, due to
space limitation, this is not possible in ICS. As a result, the alignment had to be
done using the side view-imaging setup, only on a single axial plane.

Particle-laser alignment is an important aspect of the experiment, and it has to be
done very precisely. It was observed that, a few tens of micrometers misalignment
between the particle and the laser beam results in a huge deflection of the particle
further beam downstream of the injector, seen Fig. 7.24 in section 7.3.5. Such
deflections can pose a challenge in confining the particle beam into a defined
position, such as the X-ray focus. So, in the future a better live particle-laser
alignment system should be established.

In addition to the particle-laser alignment, mounted on the axis of the laser
and particle beam, the in-vacuum microscope gave a protection for vacuum optics
from the direct particle depositions in the PCS setup. In the new setup, the
major practical challenge of the optical guiding experiment was imposed by the
arrangement of the $\varphi$ 1 inches optical window, through which the QBB enters
into the chamber, against the particle beam. As seen in Fig. 7.4, the injector is
mounted directly 50 mm in front of this entrance window, without any protective
element in between. So, during injection the particles quickly accumulate on this window surface, and it distorts, attenuates and eventually blocks the QBB from transmitting into the chamber. In addition, this particle deposition also increases the background scattering signals in the recorded particle optical images, making data analysis difficult. Frequent venting the chamber and cleaning of the optical windows between each measurement was therefore required. In addition to taking valuable time, this might affect the repeatability of a measurement. To minimize this effect, the chamber optical windows were cleaned after each measurement, which is a laser-on followed by its reference laser-off measurement. So, to mitigate this problem a method to cover the optical window should be considered.

The particle injection and aerosol generation systems are another aspect of the experiment that requires improvement. Due to the unstable nature of the gas and particle flow rate from the aerosol source, the GDVN in this case, the particles and gas density could fluctuate between and during measurements. Since the PPF as well the aerodynamic focusing dependent on the gas density, such fluctuation could greatly influence the reproducibility of the results. In the CpGV particle mass calculation, which was the smallest particle studied, it was measured that the majority of the aerosol generated were isolated single particles, \( \geq 90\% \). However, in future work to study even smaller particles, the droplet generated by the GDVNs might be too big to produce isolated particles. Therefore, other aerosol generation techniques such as electrospray ionization should be investigated. Furthermore, the direct aerosol imaging setup should be upgraded so that it could also give additional information about the particles, such as their size and type, in addition to their snapshot positions. The process of solvent evaporation rate from the surface of the particles, also needs to be studied.

### 8.2.3 Optical simulation

The current experimental setup, including the imaging and trapping optics, was constructed mainly to study the particles’ dynamics a few millimeters downstream of the injector. Inside the injector, where the majority of particle-laser interaction takes place, was not possible to study experimentally. As was shown in Fig. 7.24, a huge deflection of the CpGV particle beam has been observed when there was a misalignment between the particle and laser beams. In this measurement, the particle beam center was deflected by more than 10 times the width of the QBB beam at the focus. This means that, closer to the QBB focus, there was no interaction between the laser and the particles. Furthermore, such a huge deflection requires strong particle-laser interactions. So, the most likely place this could happen is inside of the injector, where the gas density is higher and the laser beam
transverse profile is relatively bigger, see Fig. 7.8. So, to better understand this phenomenon, a comprehensive optical and aerodynamic simulation that models the interaction of the particles with the laser, the gas profile, and particles dynamics inside and outside injector should be established.

To date, most studies involving photophoretic forces are performed on stationary or very slow particles, so the rate of the optical momentum transfer to the particle was not crucial and it was overlooked. However, due to the speed of the particles investigated in this work, it is important to understand how fast the effect of the photophoretic force occurs on a particle after they have exposed to the laser, i.e., how long does it takes the light momentum to transfer from the laser to the particle and then to the surrounding gas molecules. Furthermore, it is also important to understand how long the particle keeps their temperature gradient, before they go back to thermal equilibrium conditions. To efficiently manipulate the particles and guide them through a predictable trajectories, this important studies has to be made in the future.

In an ideal case, once a particle is in the optical funnel it should decelerated axially and deflected laterally only in the region where the laser field exists. However, in several laboratory measurements, particle acceleration was observed long after leaving the interaction region, see Fig. 7.25 in section 7.3.5. This might indicate that the particles have kept the temperature gradient on their surface for significantly long period of time and hasn’t reach thermal equilibrium in the time scale of the illumination duration, i.e., the momentum transfer by the PPF might not be instantaneous or ultra-fast process. This is purely speculation – to better understand such observations, the rate of momentum and heat transfer to and by the particles to the gas molecules should be studied. In the future, a comprehensive simulation that comprises optical, aerodynamic and heat transfer, should be conducted on the particles. Furthermore, the experimental setup need to be modified to measure the surface temperature of the particles, at least for bigger particles.

8.2.4 Radiation damage on the particles induced by the QBB intensity

In order to create the desired temperature gradient across the particles surface, a huge portion of the optical funnel’s intensity has to be absorbed by the particles. A peak QBB intensity higher than $1 \times 10^6 \, \text{W/cm}^2$ could induce significant radiation damage on the particles if it is absorbed completely, and the effects are yet to be studied. A simple visual inspection of exposed particles under the microscope can give some information on the integrity of the particle shape. However, ultimately, the high-resolution image and the molecular structure of a laser-on and laser-off
particles should be determined and compared. High-resolution imaging technique such as coherent X-ray diffractive imaging can be used to assess the extent of the radiation induced changes on the particles.

**8.2.5 Integrating the optical guiding of particles to the X-ray diffraction instrument**

Integrating the well-established aerodynamic focusing of aerosol particles with the optical guiding technique could open up a new platform for efficient delivery of micro and nanoparticles in X-ray diffractive single particle imaging experiments. The “hit-fraction” is the key parameter used to describe the efficiency of SPI at XFELs. For SPI based on aerosol injectors, it is determined by the particle beam velocity and width. In order to increase the hit-fraction, one has to reduce the velocity of the particles and/or reduce the particle beam width. As was presented in chapter 5, aerodynamically reduction of the particle beam width was possible, but it requires to accelerate the particles using a smaller-exit nozzle geometry. This resulted in more tightly focused but faster particles. On the other hand, aerodynamically slowing of the particles leads to a bigger particle beam. So, it is inherently impossible to change one without affecting the other. However, through the optical deceleration and confining technique demonstrated in this thesis, the important hit fraction parameters can be optimized simultaneously without affecting the operation of the injector. This can greatly improve the sample delivery efficiency of SPI experiments.

The major challenge of integrating this setup at SPI experiments lays on the stringent pressure requirements of the photophoretic trapping experiments. Typically, X-ray diffractive experimental chambers require pressures well below $10^{-4}$ mbar, whereas we want to conduct the optical trapping experiments at pressures higher than 0.1 mbar. This problem might be mitigated by performing the optical focusing experiment in an isolated trapping cell, which is built independently inside the X-ray diffraction chamber. That means that, all the optical elements can be constructed inside the X-ray diffraction chamber, and the particles can be injecting and optically focused inside the small chamber, which is kept at the required pressure.

The optical focusing has improved the projected particle density on CpGV particle at least by a factor of four, which is a huge improvement. However, since the amount of particles introduced into the injector are still the same, the improvement in the sample delivery efficiency (the number of particles intercepted by the X-ray), is negligibly small. That means, the vast majority of the particles are flowing past the beam between the X-ray pulses. Therefore, in the future this technique may also be further improved to operate synchronized with the repetition
rate of the X-ray pulses, such that only few particles (or ideal only one particle) are precisely delivered to the focus per X-ray pulse. This way the particle delivery efficiency can also be improved.

### 8.3 Prospect of optical guiding of particles for X-ray diffractive SPI

The results presented in this thesis open up a new prospect for efficient delivery of aerosolized micro and nanoparticles to the focus of X-ray beam for coherent diffractive single particle imaging experiments. The CpGV particle focusing using the optical guiding would ideally improve the hit fraction at X-ray diffractive SPI experiments at least by a factor of four. At the current state of SPI, this would be a great improvement on the efficiency of the experiment, by allowing more data to be collected in the usually limited beamtime period.

To this date, no one has reported such a high-speed particle beam of any kind confining and/or concentrating using a laser, neither in air nor vacuum. In addition to particle guiding for SPI experiments, in the future this technique could also have various interesting applications, such as in trapping and transporting of aerosol particle for atmospheric chemistry, aerosol science and mass spectrometry experiments, or for laser deflection based aerosol particle size selection and sorting.
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Relevant publications


Additional publications


Additional publications


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