Development of a high-speed optical imaging and illumination system for the Gas Dynamic Virtual Nozzles

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Abstract

Free Electron Lasers (FEL) provides a new challenge for sample delivery systems. Due to their high intensities the sample is usually destroy and thus it must be replaced at the proper frequency of the FEL. Gas Dynamic Virtual Nozzles (GDVN); provide an interesting solution to this problem. Understanding them becomes now a crucial part of the experiment .In order to do so new illumination systems must be developed to match the particular features of the jets. In this work we will develop a high-speed optical imaging and illumination system for the jets produced by GDVN

Contents

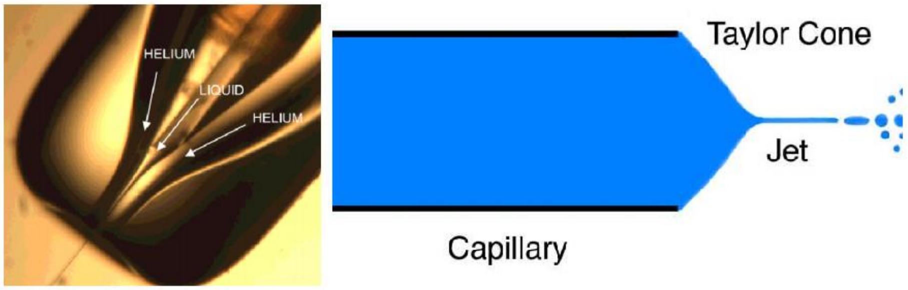

Figure 1: Working principle of a Gas Dynamic Virtual Nozzle (left), Taylor cone produce in a capillary using electrospray Ionization

1. Introduction

The high intensity of the Free Electron Lasers opens a new realm for the measuring of diffraction patterns from biological samples. Recent developments of two techniques (x-ray serial crystallography and femtosecond imaging) closely related to FELs and synchrotrons have posed an interesting challenge. Samples must be replaced at the high repetition rate of the FEL, with the proper particle density and degree of hydration without damaging them. These new requirements for new sample delivery systems must be fulfilled while keeping sample consumption low and large hit rate. Four main techniques have been devised to give an answer to this problem. A detailed review of all this systems is found in **[1]**:

Gas Dynamic Virtual Nozzles (GDVN): Figure. 1 presents a sketch of these types of nozzles. The nozzle consists of two concentric glass tubes. The inner tube delivers a constant flow of liquid while the outer has pressurized gas. By sending a liquid jet through a pressurized gradient of gas small jet diameters can be created. Liquid jets diameters as small as 300 nm have been produced. Clogging, which is a major drawback in other injector systems rarely occurs; sample consumption however is high.

Electrospray Ionization (ESI): The ESI technique charges an ionic liquid which is then forced through a capillary and place opposite to a counter electrode, with high enough voltages the emitted charged liquid forms a Taylor cone (Fig 1) and breaks into drops due to electromagnetic interaction. One major drawback of this technique is the lack of uniformity in droplet features.

Drop on Demand (DOD): DOD is a form of sample delivery system which provides great control. Here drops are produced with a piezo driven nozzle, each time the piezo is triggered a drop is produced. Matching the frequency of the nozzle to the repetition rate of the FEL can drastically reduce sample consumption. However clogging is a major drawback in this type of delivery system making them hard to use.

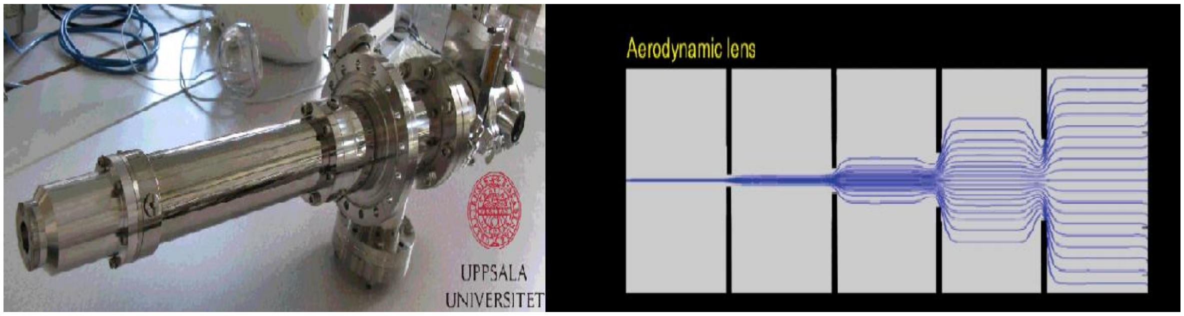

Figure 2: An aerodynamic lens currently used at LCLS and FLASH (left), working principle of Aerodynamic Lenses (Right)

Aerodynamic Lenses: Aerodynamic Lenses produce particle streams with low divergence. The working principle of these types of delivery systems is illustrated on figure 2. As a gas stream flows through a series of circular apertures, inertia cause particles to move either towards or away the center of the line depending on size and density. A review of this system can be found on **[2]**.

Each of these injector types is well suited for different types of samples. Liquid jets created by Gas Dynamic Virtual Nozzles, for example, are ideal for protein nanocristals as they can deliver the sample to the interaction region in the same solution they were prepared thus becoming quite useful at beam lines like LCLS or FLASH.

The GDVN become now a critical part of the experiment and all their properties must be clearly understood. Among these properties, the generation of microscopic streams of droplets [5] and the oscillation of the generation point of these droplets must be highlighted (Fig 3). These properties vary depending on the nozzles, as no industrial production method currently exists. Precise measurement of their properties must then be done for every GDVN.

The illumination systems currently used at the experimental stations at the Center of Free Electron Laser Science however, provide only with low quality images. In order to increase the quality of the measurements new illumination systems must be developed. This work tries to give an answer to these questions, proposing simple solutions that can be easily used in different experiments.

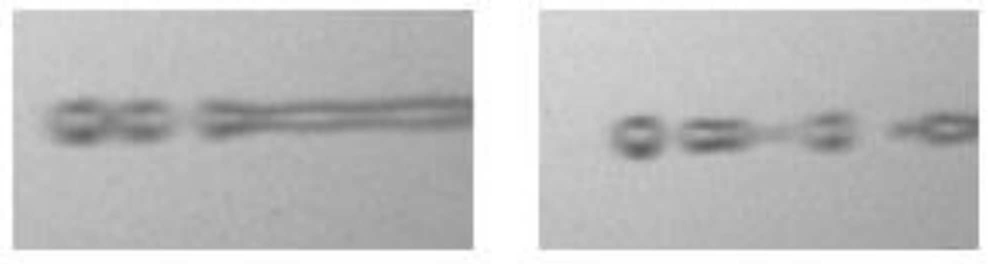

Figure 3 Point of generation of the droplet streams at different moments in time. A clear variation exists

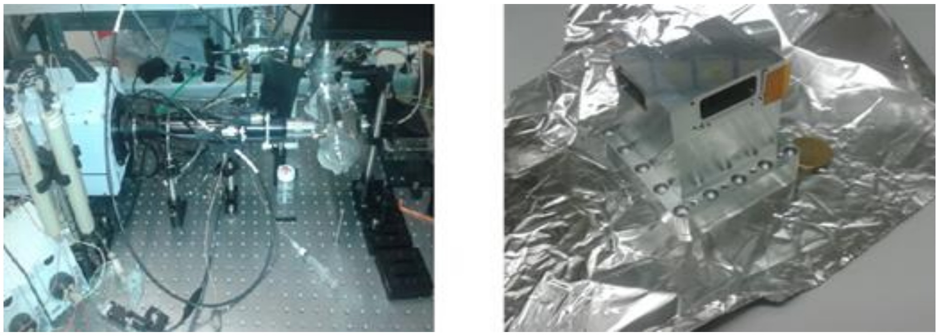

Figure 4: main nozzle testing station (left) , End part of the PETRA III beam line

2. Objective:

The main objective of this project is to improve the illumination systems that currently at used at the GDVN testing station at CFEL (center for free electron science) in DESY, and at the PETRA III beam line (Fig 4):

Currently at PETRA III beam line an LED illumination system is being used. No other optical component is utilized. This illumination provides low quality images with poor contrast and high background. Moreover, this illumination also gives as a secondary result a bright red spot witch that lowers the quality of the image even more. The result can be seen at figure 5.

In the main testing station for GDVN used at CFEL (fig 4), a pulse laser source is currently being utilize used. Two main components are used here:

-The fast camera: The camera provides high temporal resolution images with a minimum shutter speed of 1 μs and a maximum FPS of 500 000.

-This source most widely used is a pulsed laser that delivers high intensity nanosecond pulses. This source provides pulses up to 100 ns. This delivers high temporal resolution images but it fails to provide high spatial resolution images, as fringes due to interference effects appear (fig 5)

Two illumination systems will give good answer to these problems, critical illumination in the PETRA III beam line, and a pulse laser source with a diffuser to break the coherence in the testing station.

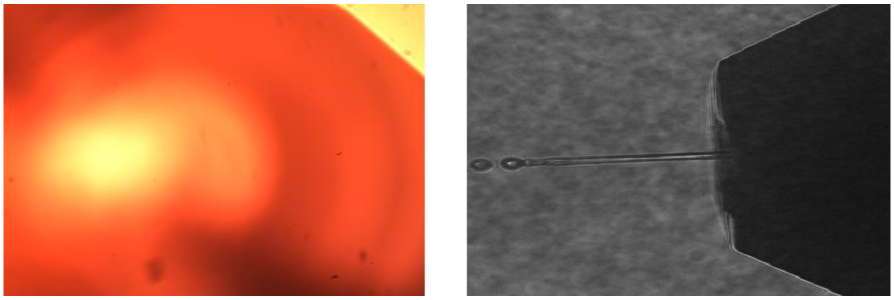

Figure 5: Image from the PETRA III beam line (left), Nozzle testing station image (Right)

3. Methodology

In order to have a methodic approach to the development of illumination systems will follow a three steps process:

First: A Control Illumination System (CIS) will be used. In our case, köhler illumination [3] will be the first to be applied as it is a widely use system in microscopy and provides high quality images.

Second: The CIS will be improved either by upgrading the system from step one, or by developing a completely new system.

Third: The upgraded illumination will be set up as a new CIS (when possible) and all the process will be repeated in a cyclical fashion.

Two details must be now highlighted as they have big impact on the fundamental procedure:

- When possible, both illuminations, CIS and the illumination under development, must be set up at the same time, to avoid any movement in the sample and to facilitate comparison. However there may be cases were this is not possible
- Set ups must be kept as simple as possible, in order to achieve flexible illuminations that can be used in many experimental stations and also in the beam line.

4. Illumination testing:

The illumination testing will be done with the following components:

- The CIS: The reason for the CIS is not clear and must be explained , as jets delivered by nozzles are not common objects studied in microscopy (their cylindrical shape makes them very rare in classical samples) strange illumination dependent effects can appear as can be seen in the following example:

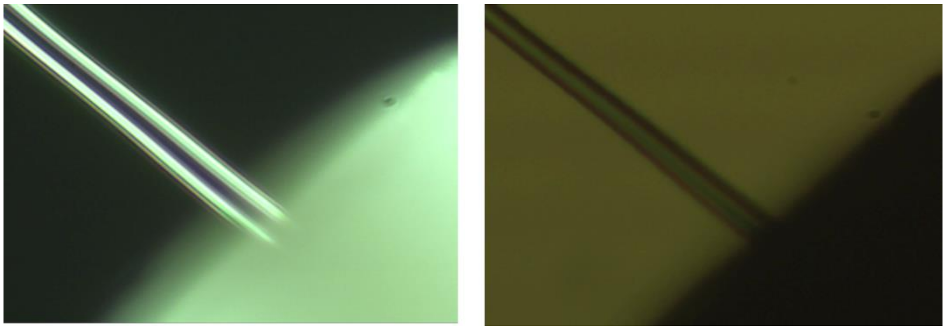

Figure 6 Perpendicular illumination gives as a result an structure of the image in bands, for bands are clearly seen (left), köhler illumination (Right)

Both images were done with the same magnification, and almost at the same time. However the left image appears to be bigger. A precise measurement confirms this behavior. This is produce by reflections at the water-air interface. A precise measurement of the jet diameter can be obtain by using the dark bands between the bright ones, this procedure agrees with all other systems used and provides excellent resolution.

- The imaging will be performed with a *20X EO M Plan Apo Long Working Distance Infinity-Corrected objective from Edmund Optics* and with the tube lens provided. We will employ a CCD microscope camera of 5 Megapixel from MOTIC to capture the images.
- And, of course, the illumination system being tested.

In order to keep a precise track of the quality of the illumination 3 different magnitudes will be recorded. First the jet diameter must be compared to the one obtained using the CIS, second the length of the jet that can be resolved, and lastly the jet size resolution.

5. Main illumination systems:

In the following section we provide a review of the most important illumination systems used.

5.1 Köhler illumination:

Maybe one of the most important illumination systems used, this set up was developed by August Köhler in 1983 [3]. In figure 7 the ray diagram of a Köhler illumination system is shown

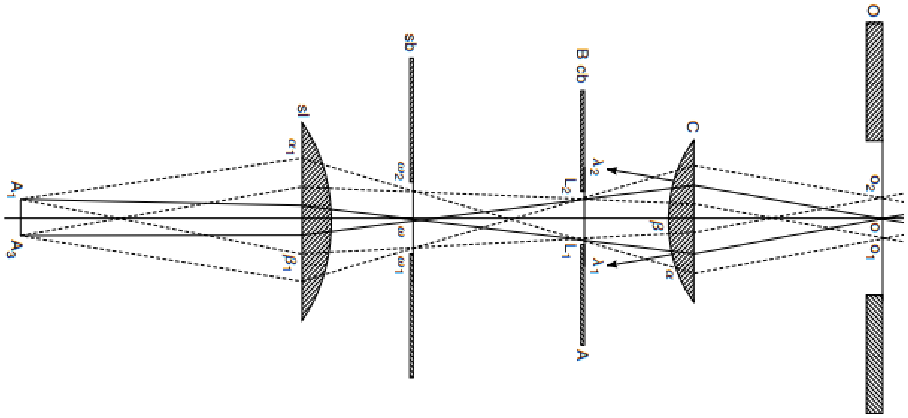

Figure 7 Köhler setup. The two lenses are the Köhler lens and the condenser lens provided even illumination. The diaphragms represented with the slides allow us to match the numerical aperture of the illumination and the objective.

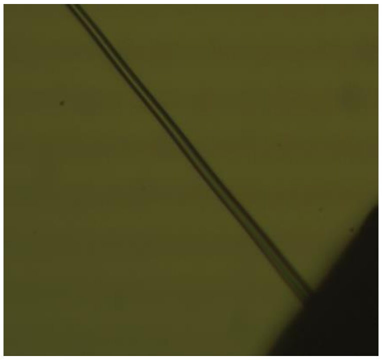

Figure 8 Köhler illumination examples

Careful tracking of the rays in this setup can show that each point in the source illuminates every point at the sample at the same time. This illumination provides then extremely even background, as can be seen in figure 8

Of course this set up can then provide a solution to the uneven illumination at the beam line in PETRA III, but due to its size , and the small space available (figure 4), it is not possible to use it here, however this setup will make an excellent control illumination as we do not face these constrains in our experimental set up

5.2 Critical illumination:

To obtain an even illumination in the sample we can employ another method. The origin of the red spot (fig 5) must be first understood. It is simply a defocused image of the source; this was seen by changing the shape of the source and by changing the focus of the objective into the source.

By changing the divergence of the rays however, we cannot avoid this effect but we can make it weaker. Critical illumination provides an answer here. Critical illumination focuses an image of a light source on to the specimen for bright illumination. Choosing then, the appropriate position of the image (a bit in front of the sample) even illumination can be obtained. For practical reasons what we did was setting up a critical illumination scheme, and then by trial we optimized it. The result can be seen in figure 9. Here we used an LED as a source, as it is done in the beam line. This provides a simple and robust answer to the problems presented at the beam line.

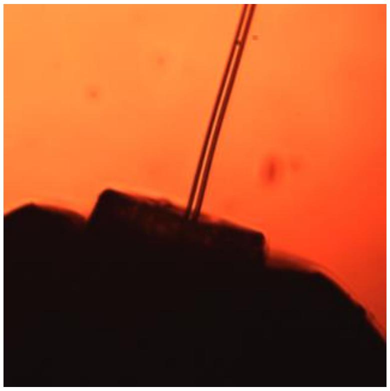

*Figure **9**: Critical illumination with an LED as a source*

Figure 10: Examples of off-axis illumination with different angles

5.3 Off-axis illumination.

Simply by moving the axis of the illumination in the critical illumination scheme we can obtain high contrast images without great difficulty. Focusing the light out of the axis of the objective, the background can be greatly decreased. It is important to note that the light observed here is reflected from the jet

The symmetry of the illumination however does not adapt itself to the object under study which gives rise to some estrange illumination effects that make the study of the jet more complicated as it provides, for example, confusing information on the jet size.

In some cases however this illumination may be useful.

5.4 Dark field illumination

One of the most common illuminations used in microscopy, dark field illumination is commonly used to increase contrast. It works by illuminating the sample with light that will not be collected by the collector lens.**[4]** provides a good review of this type of illumination A sketch of the setup is found in figure 11.

A disc called the patch stop block the central part of the beam leaving only outer ring illumination. The scattered light enters the objective lens, while the directly transmitted light simply misses the lens and is not collected due to a direct illumination block.

At a first glance it seems that this scheme provided high contrast, however , the CIS showed that using this bands as delimitation for the jet diameter is inaccurate as provides much smaller jet size. The more diffused bands seen in the image provide here the real delimitation for the jet. Varying the size of the spot better result might be obtained.

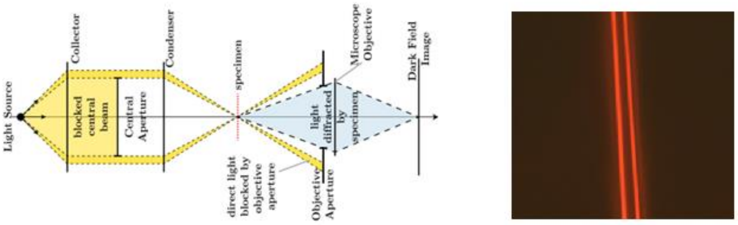

Figure 11 Dark field scheme (left) , example image done with this scheme using an LED as a source (right).

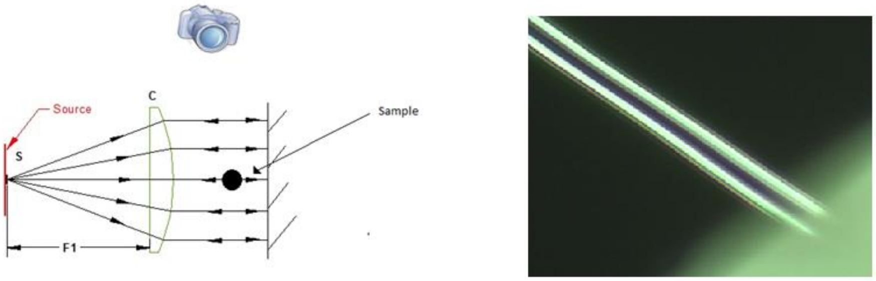

Figure 12 Perpendicular illumination set up (left), image obtain with this illumination (right)

5.4 Perpendicular Illumination.

A sketch of the setup of this illumination can be found in figure 12. Combining a collimated beam with a mirror the symmetry problem of the off axis illumination can be improved providing a symmetric image which can be studied with less difficulty. The sample is situated between the mirror and the lens, and the camera is perpendicular to the beam. This provides low background and high contrast. Due to its high resolution and its unconventional setup it will be a useful CIS for other illuminations were Köhler is not viable. The features of this type of illumination have been described in section 4 as an example of the necessity of a control illumination. We will not discuss them any further

5.6 Pulse laser illumination

All of the previous cases could be applied easily with a white light source or an LED at the same time. For pulse laser illumination a special solution is applied. Using a diffuser to break the coherent of the source in front of the sample, interference effects can be avoided and high precision measurements can be made. Here a Thor-labs diffuser was employed, but other filters can be useful to. In fig 13 the result of introducing a diffuser can be seen.

There is a dramatic reduction of the intensity that must be taken into account. Without diffuser a total electrical intensity of 0.4 A was needed. Using the diffuser the total electrical current needed is almost doubled , with a requirement of 0.7 A. A reduction of this problem may be obtained using by putting the diffuser closer to the sample and thus increasing the amount of light that arrives to it.

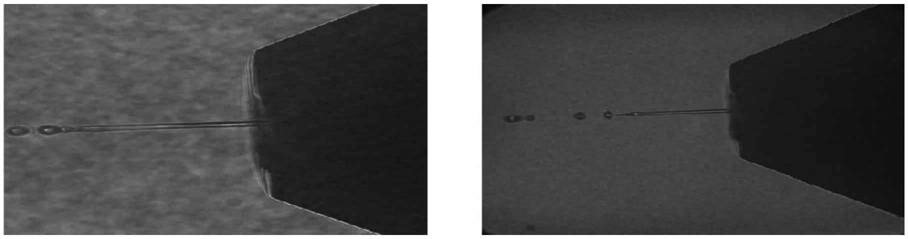

*Figure 4**3** Pulse laser light illumination without diffuser (left) and with diffuser (right). Clearly there is a reduction of interference effects and an improvement in the spatial resolution while keeping a good temporal resolution.*

6. Conclusions.

Since their installation, the illumination systems used in the beam line and in the nozzle testing station are of low quality and there has always been the need to be improve them. Different sources an illumination schemes were tested. Table 1 provides a review of them

Light sources	Properties	**Illumination schemes**	
White light	High Intensity, Incoherent, Not pulsed	Köhler illumination	Critical Illumination
Laser light	Coherent, Pulsed, Intense,	oblique illumination	Dark Field
LED	Pulsed , No spatial coherence	Off axis illumination	Perpendicular illumination

Table 1: review of the different types of illumination and light sources

Each of the sources White light, Laser Light and LED was tested with every illumination schemes mention in table 1, (i.e. Köhler illumination, oblique illumination, Dark Field, Critical Illumination, off axis illumination…) to determine which illumination was more efficient. Also new illuminations were developed that provide higher resolution, and contrast.

From all the systems studied, critical illumination and “incoherent” pulsed laser illumination provide the best answers to the experimental requirements used in the nozzle testing station and in the PETRA III beam line.

The used of a Control Illumination System proved valuable in many cases, revealing many properties of every illumination scheme.

In the next table we can see a review of the CIS employed for each illumination reviewed:

Illumination	CIS
Perpendicular Illumination	Köhler Illumination
Critical Illumination	Perpendicular Illumination
Off axis illumination	Köhler Illumination
Dark Field illumination	Köhler Illumination

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