EUCALL

The European Cluster of Advanced Laser Light Sources

Grant Agreement number: 654220

Work Package 6 – HIREP

Deliverable D6.8
Integration of sample stages with microscope

Lead Beneficiary: European XFEL

Authors: Carsten Deiter, Mihail Cernaianu, Daniele Magaronne, David Watts, Alexander Pelka and Joachim Schulz

Due date: 30.09.2018
Date of delivery: 29.09.2018

Project webpage: www.eucall.eu

Deliverable Type	
R = Report DEM = Demonstrator, pilot, prototype, plan designs DEC = Websites, patents filing, press & media actions, videos, etc. OTHER = Software, technical diagram, etc.	DEM
Dissemination Level	
PU = Public, fully open, e.g. web CO = Confidential, restricted under conditions set out in Model Grant Agreement CI = Classified, information as referred to in Commission Decision 2001/844/EC	PU

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

Abstract / Executive Summary

This deliverable summarizes the work of the HIREP work package by demonstrating operation of the entire work flow. A prototype integrated sample environment has been assembled and tested at the European XFEL instrument SPB/SFX. The setup combines a sample scanner using the HIREP standard sample flames, an in-vacuum microscope for sample imaging and alignment and the high precision stages. The output from the Targeter software to specify samples can be used for positioning.

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

Contents

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

1. Introduction

The scope of the HIREP work package in the EUCALL collaboration is to unify sample characterization and positioning in the participating facilities. This enables exploiting synergies in user support and simplifies the access of external user groups to the advanced light sources.

In this final deliverable, we demonstrate the feasibility to combine the developed hardware into an integrated instrument. This instrument is on the one hand specific to the demands of the facility, in this case the European XFEL. On the other hand, it uses standard components available to all facilities and accepts the standard sample frame developed within the HIREP work package, thereby allowing external user groups to measure their targets and samples not only at the European XFEL but at all participating laser light sources.

The setup has been integrated into the Single Particles, clusters and Biology and Serial Femtosecond Crystallography (SPB/SFX) instrument of European XFEL. This instrument is optimized for crystallography and coherent scattering on biological structures. The fixed target scanner is one of its main sample delivery setups.

2. Final integration of the high precision sample stages with sample holders and UHV microscope

2.1 High Precision Stage

The high precision stage (see Figure 1) has been designed and set up at the SPB/SFX instrument. The used piezo motors of the company SmarAct offer a reproducibility of the positioning of 80 nm. In combination with the mechanical components of the stage and the used encoders an absolute accuracy of 1 µm is achieved. The resistibility against electromagnetic pulses (EMP) for piezo motors of this working principle has been proofed at the DRACO laser at HZDR at a dedicated test beam time in May 2018.

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

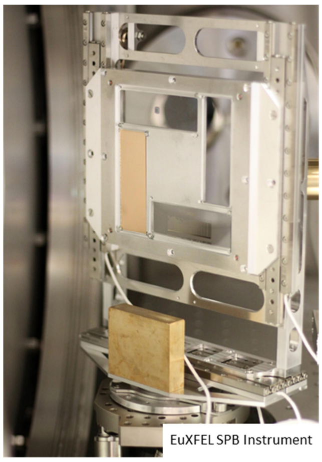

Figure 1.

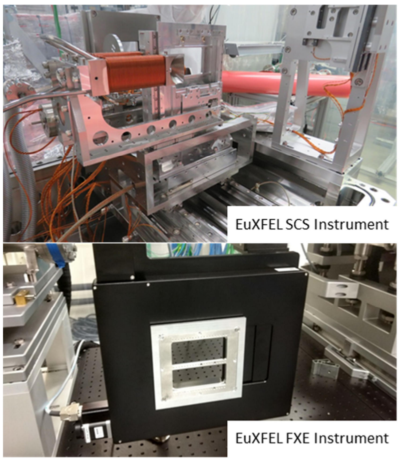

Figure 2.

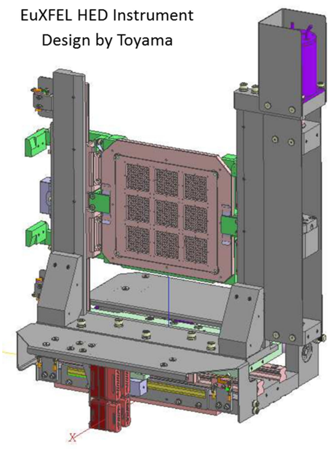

Figure 3.

2.2 Frame System

The standard frame system as it is described in Deliverable D6.1 has been introduced into three of the six instruments of the European XFEL (SPB/SFX, FXE & SCS) (see Figures 1 & 2). Two more instruments (MID (same as SCS) & HED (see Figure 3)) will follow when they go online in 2019.

Into two analysis tools the frame system has been implemented, so that users can inspect their samples and targets easily. For this purpose the target localization microscope (see Figures 4 & 5) and the confocal microscope in the user labs of the European XFEL have been equipped with a fixture to carry all possible inner frames. The electron microscope and the dual beam SEM / FIB will follow in 2019.

Furthermore, the frame system will be implemented in the shock compression experiment of the ID24 end station of the European Synchrotron Radiation Facility and in the ELIMAIA instrument at ELI-Beamlines in 2019.

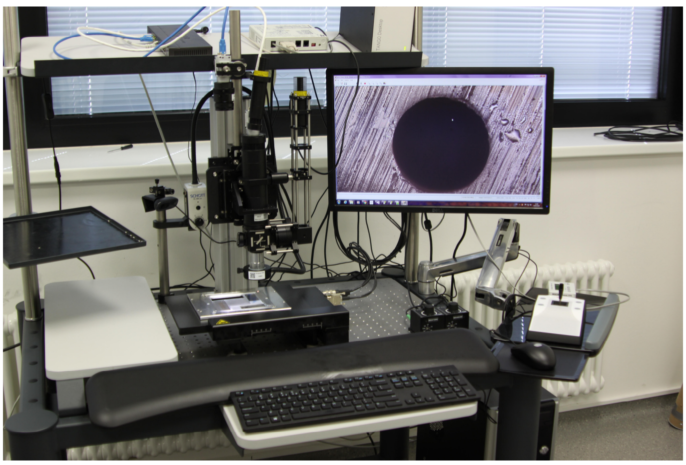

Figure 4.

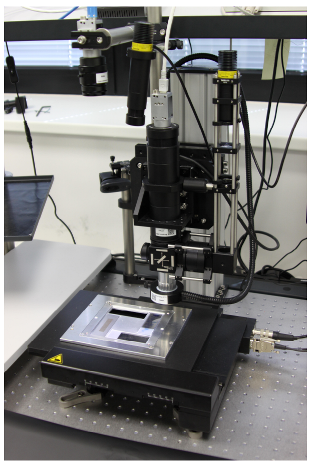

Figure 5.

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

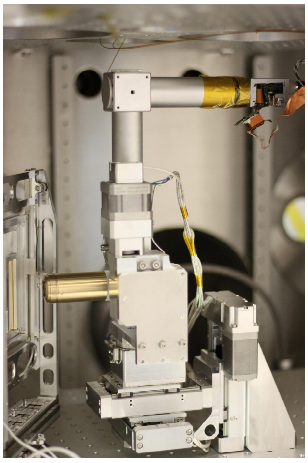

Figure 6.

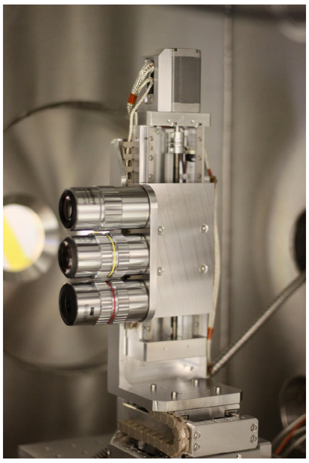

Figure 7.

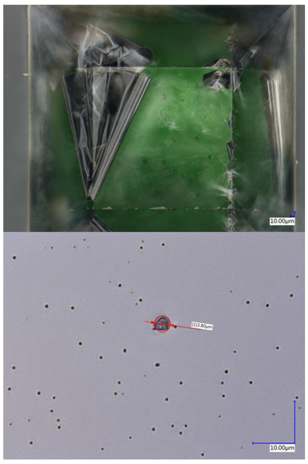

Figure 8.

2.3 UHV microscope

The vacuum inline microscope (see Figure 6) can be equipped with different objectives for changing magnification and filed of view. A side view microscope is equipped with a three objectives changer unit (see Figure 7) for automatic adaption. The optical system in the objectives is modified, so that the x-ray beam can pass the optical path collinearly. The picture is then reflected by a beam splitter by 90° up into the CMOS camera which is coupled to a massive heat sink to prevent overheating of the camera inside the vacuum chamber. The optical performance can be varied by the choice of the objective between high resolution (~1 µm) and overview mode (5 x 5 mm² field of view) (see Figure 8).

2.4 Integration of all three components at the SPB instrument

The integration of all three components, frame system, stage and microscope, was set up at the SPB/SFX instrument. To proof the performance of the system x-ray focus imprints have been performed at positions with equal distance on a grid like pattern. For the coarse alignment of the frame in the interaction region the inline optical laser was used (see Figure 9). The fine alignment was performed using the microscope and the fiducials mounted to the inner target frame. In Figure 9 one can see the microscope slide with the gold film used for the imprints on the right hand side from the spot of the alignment laser. The imprints have been analzsed using a video microscope at different magnifications with a resolution of 400 nm and an accuracy of the microscope's translation stage of ~1 µm.
In Figure 10 we show the overview image of the grid of imprints. Figure 11 shows a detailed micrograph of the hole that a 10 µm focus has imprinted into the gold film. The achieved accuracy of the positioning process is better than the accuracy of the microscope used for the test and hence fulfils the requirements described in Milestone M6.1

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

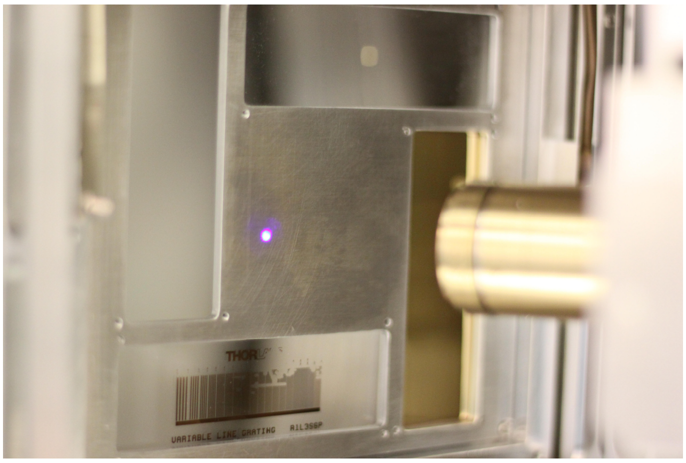

Figure 9.

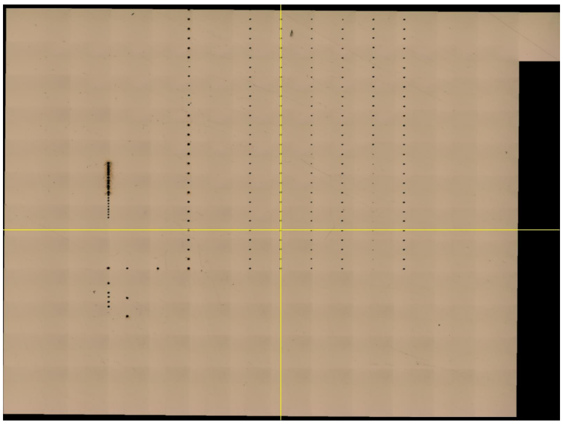

Figure 10.

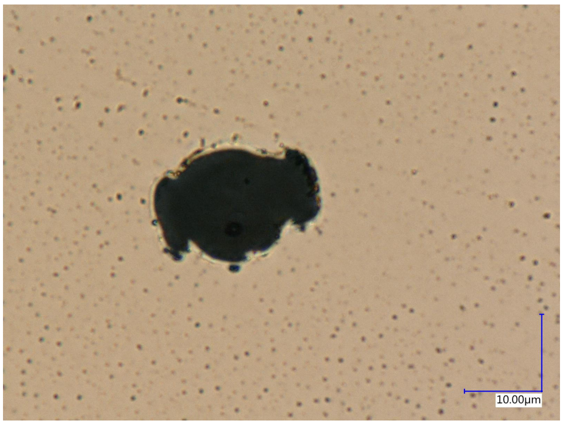

Figure 11.

3. Integration of the output of the sample identification software with the sample stage control software

The target identification software "Targeter" writes the achieved target positions in respect to the coordinate system of the target frame's fiducials into the data base record linked to the identification of the frame. To position the frame in an x-ray or laser experiment at one of the end stations using the standard frame system it is necessary to feed the needed information into the target stage control computer. Either a direct download of the data base record is initiated or the data is transported using the XML file format. The format of the XML representation of the data base record is described in Deliverable D6.7.

For this task a script has been developed to process the XML file line by line and to extract the target's ID and position to remotely control the end station's control computer and to position the target frame with its sample manipulation system (high precision stage). The source code is written in python 3 and contains the call for pseudo functions for the

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

communication with the control computer. Since these functions vary from end station to end station and depend on the used control system and software, the code has to be written individually by the participating facility. The code can be found in the Annex of this document.

4. Summary

We have shown the integration of all major achievements of the HIREP work package into one workflow:

- the microscope for the imaging of the target frames (see Deliverable D6.7)
- the software for the detection of the individual targets with XML output (also D6.7)
- the extraction of the target information out of the XML file for the transfer to the control computer
- the high precision stage with EMP hardness and acceptance of the standard frame system
- the UHV inline microscope with alignment laser for the course and fine alignment of the target frame in the interaction point of the x-ray or laser experiment
- the interplay of all components using the example of x-ray focus imprints in a gold film on glass at the SPB instrument at the European XFEL

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

Annex

python 3.2 code with call of pseudo functions for the communication with the end station's control computer:

```
import sys
import os
from string import *


DATASET = []
FILE=open("storage/emulated/0/Qpython/first_steps/SampleXYZ.xml","r")
TARGETSET = []
POSITION = 0
TARGET = 0

while 1:
    LINE = FILE.readline()
    LINE = LINE.strip()
    if LINE == "":
        break
    if "<target>" in LINE:
        print("TARGET detected")
        TARGET = 1
    if "</target>" in LINE:
        print("END of TARGET detected")
        TARGET = 0
    if "<position>" in LINE:
        print("POSITION detected")
        POSITION = 1
    if "</position>" in LINE:
        print("END of POSITION detected")
        POSITION = 0
    if POSITION == 1 and TARGET == 1:
        if "<x>" in LINE:
            XPOS = LINE[3:-4]
            print("XPOS = "+XPOS)
        if "<y>" in LINE:
            YPOS = LINE[3:-4]
            print("YPOS = "+YPOS)
        if "<z>" in LINE:
            ZPOS = LINE[3:-4]
            print("ZPOS = "+ZPOS)
    if TARGET == 1 and "<ID>" in LINE:
            ID = LINE[4:-5]
            print("ID = "+ID)
            TARGETSET.append([ID, XPOS, YPOS, ZPOS])
            print("TARGET added to LIST")

print("processing of target sequence begins:")
for LINE in TARGETSET:
    TARGET_ID = int(LINE[0])
    TARGET_POS_X = float(LINE[1])
    TARGET_POS_Y = float(LINE[2])
```

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220

```
    TARGET_POS_Z = float(LINE[3])
    print("moving STAGE to position ID = " + LINE[0] + ", XPOS = " +
LINE[1] + ", YPOS = " + LINE[2] + ", ZPOS = " + LINE[3])
    #move_stage_to(TARGET_ID, TARGET_POS_X, TARGET_POS_Y, TARGET_POS_Z)
    print("waiting for stage to arrive at position")
    #wait_for_stage_to_arrive()
    print("stage arrived - trigger sent to experiment")
    #set_trigger_to_experiment()
    print("waiting for experiment to be finished")
    #wait_for_experiment()
    print("experiment finished")
print("target sequence ended")
```

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 654220