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UHV Microscope

Lead Beneficiary: DESY

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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

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1. Abstract

We have developed an UHV compatible sample-viewing fluorescence microscope for the identification of fluorescence markers mounted on the fixed target support frames to exactly identify the position of the frames before the measurements. The microscope consists of three main parts, the video microscope, which is partly located in the UHV chamber, the fluorescence light source and a 3D-positioning unit for adjusting the field of view and for focusing.

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2. Introduction

For rapidly scanning the pre-investigated samples at the instrument, alignment of the target holder is essential. The most versatile devices for alignment are in-line microscopes. They allow optical feedback to the user as well as automated adjustments by means of image recognition software. In-line microscopes are implemented at many instruments worldwide but mostly with limited resolution and/or vacuum compatibility.

Conventional methods for in-line microscopy are long distance microscopes outside the vacuum chamber or modified commercial microscopes in the chamber. Long distance microscopes allow imaging the sample and alignment marks through vacuum viewports and holey mirrors. Thereby, they do not enter the vacuum and no limitations of vacuum compatibility are given. On the other hand, their resolution is inherently limited by the long working distance of typically more than 160 mm. The numerical aperture (NA) at this distance can be about 0.05 limiting the resolution to not better than 4 or 5 µm. Bringing modified microscopes into the vacuum can, on the other hand, easily reach resolutions of better than a micrometre. They are, however, generally not ultra-high vacuum (UHV) compatible and limit the vacuum condition to a base pressure in the order of 10^{-5} mbar.

In this Deliverable, we report on a development of an in-line UHV compatible microscope for use at any instrument. The goal of combining high resolution imaging with uncompromised vacuum compatibility is reached by splitting the objective from the electronic imaging system by a vacuum viewport. Thereby, a UHV compatible objective can be placed in the vacuum, reaching high numerical apertures, while the non-vacuum compatible electronic components can be operated in air.

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3. Video microscope

The optical light path of the UHV fluorescence microscope is illustrated in Figure 1. The light from the sample is collected by a microscope objective, which generates a parallel light path (infinitely corrected objective) leaving the objective. This light is than focused onto the camera by using a tube lens with a focal length of 100 mm. A sapphire window placed in the parallel light path between the objective and the tube lens separates the UHV from the in-air part of the microscope without inducing any image distortions. A dichroic beam splitter unit after the sapphire widow further allows the separation of excitation and emission light path.

The microscope objective is an Olympus LMPLFLN 50x, with an NA of 0.5 and a long working distance of 10.6 mm. It has been modified such that it is compatible with operation under UHV conditions. The light transmission curve of the objective is shown in Figure 2. The mount for the microscope provides an RMS thread in order to also mount other objectives for specific applications.

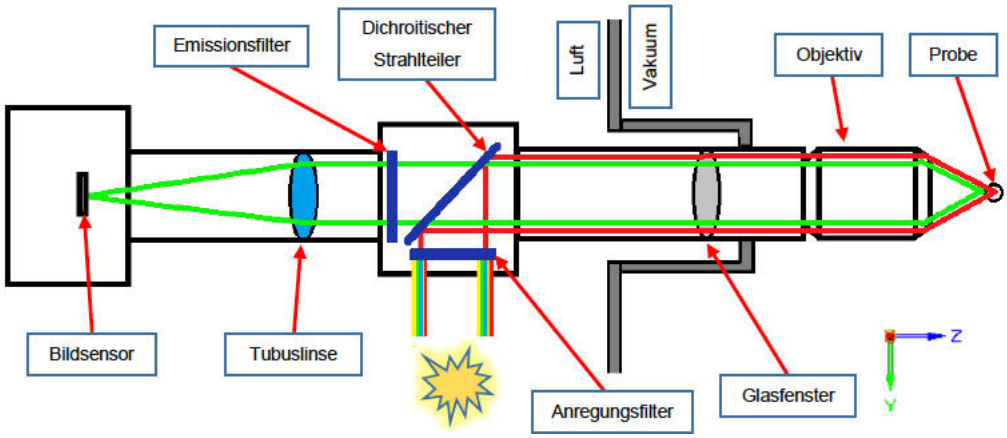

Figure 1: Optical light path of the UHV fluorescence microscope.

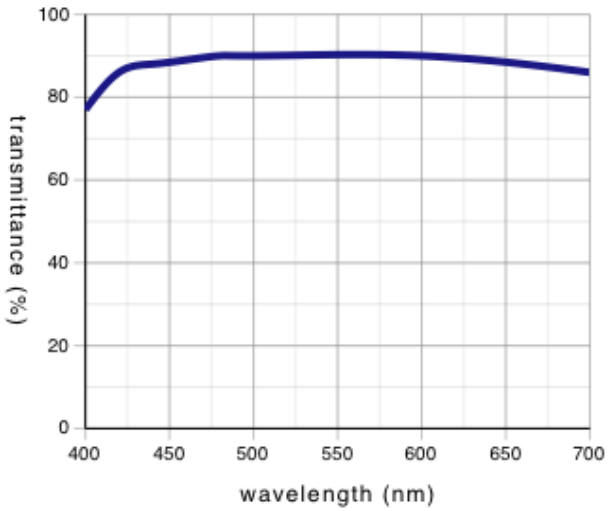

Figure 2: Transmission curve of the Olympus LMPLFLN 50x microscope objective.

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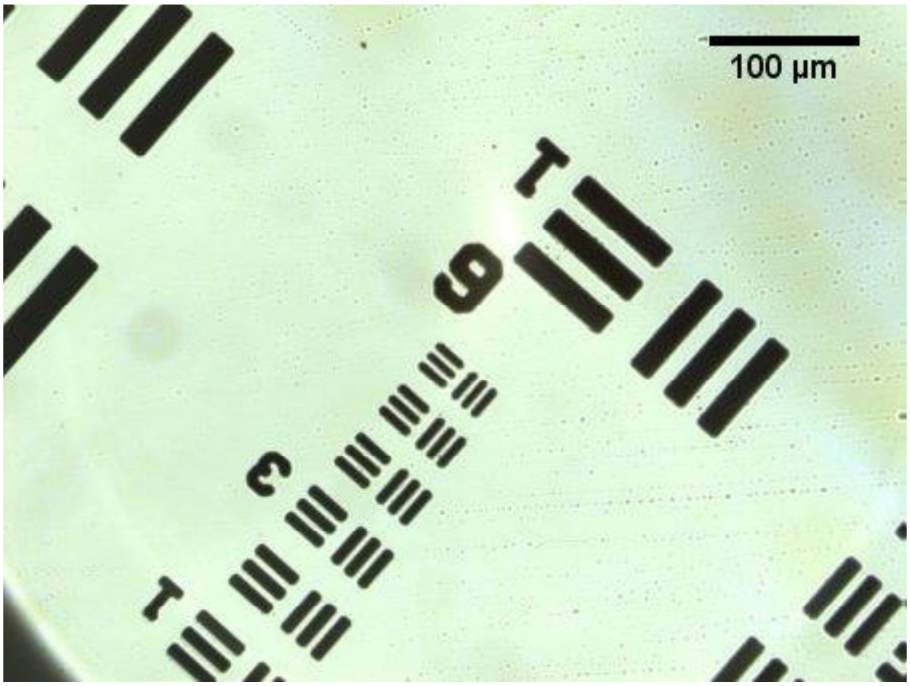

Figure 3: Micrograph of a test pattern for resolution characterization recorded with the UHV compatible fluorescence microscope.

The tube lens is an achromatic doublet lens with 1 inch diameter and a focal length of 100 mm. Due to the best compromise between weight and sensitivity an industrial camera equipped with a SONY 1/1.2'' IMX174 sensor was chosen. With a pixel size of 5.85 µm the nominal resolution of 550 nm is adequately sampled. In this configuration the microscope provides a magnification of ca. 28x with a theoretical spatial resolution of 0.55 µm. A micrograph of a test pattern for resolution characterization taken with the microscope is shown in Figure 3.

4. Fluorescence light source and dichroic beamsplitter

For excitation a SOLA SE II 365 light source was selected. The LED based white light source was chosen for its high brightness, reliability and broad spectral output with an excitation wavelength ranging from 360 nm to 650 nm. The light source is coupled into the microscope with a liquid light guide. For quick and easy adaption of the microscope to different fluorochrome standard microscope filter cubes (Nikon) can be inserted into the microscope.

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5. Motorized positioning unit

The whole imaging path is constructed as one rigid body mounted on a 3-axes positioning unit. All three axes are stepper motor driven and provide a positioning accuracy of better than 1 µm. Travel ranges in all three directions are ± 2 mm. All motors are located outside the vacuum in order to maintain a low pressure in the chamber and to avoid heating up of the motors. In order to allow movement of the whole unit together with its in-vacuum parts, an edge welded bellow is used for connecting the microscope unit to the vacuum flange. The whole positioned unit is mounted on a DN63 CF flange, which is also used for attaching the microscope unit to the sample chamber.

A 3D CAD model of the whole microscope unit is shown in Figure 4 and a photograph of the assembled setup is shown in Figure 5.

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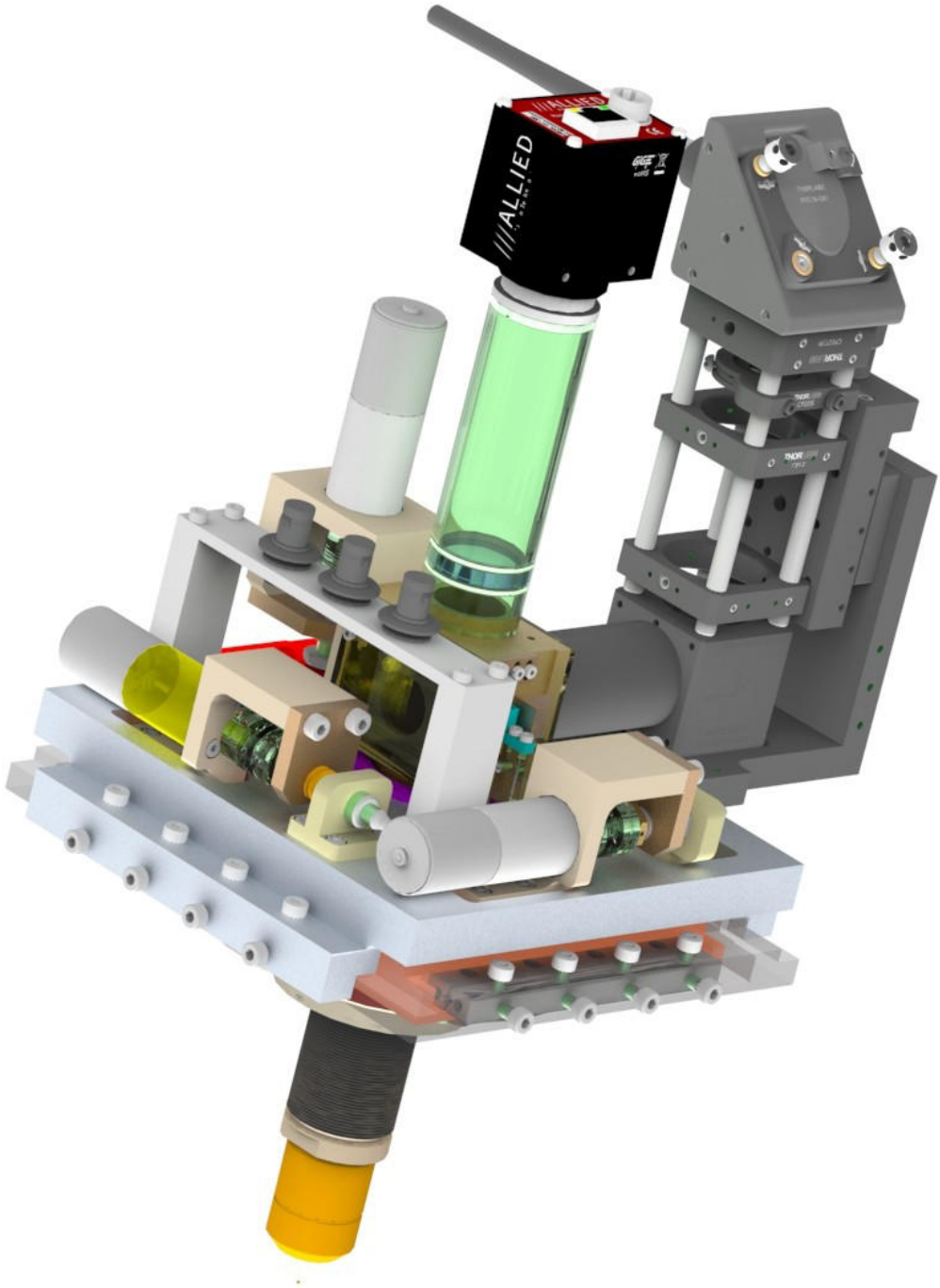

Figure 4: 3D-CAD model of the UHV sample viewing fluorescence microscope with integrated through-the-lens illumination.

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Figure 5: Photograph of the assembled UHV sample viewing fluorescence microscope mounted on at test stand. The aluminium plate holding the whole setup represents the vacuum barrier

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6. Summary

We present a UHV compatible microscope for sample characterization and alignment of fiducial marks. The system combines a vacuum compatible objective with an in-air camera system. This ensures both high resolution and UHV compatibility. The system has been setup at DESY and is optimized for small UHV chambers and the demands of synchrotron radiation facilities.

This prototype demonstrates the principle. Similar systems are under construction at the European XFEL and at the ELI institutes. While the basic concept is universal, the specific realizations at the partner institutes will be considerably different in size and geometry to meet the local requirements.

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