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Endoscopic filter fluorometer for detection of accumulation of Protoporphyrin IX to improve photodynamic diagnostic (PDD)

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Abstract: Photodynamic diagnostic (PDD) is an optical enhancement option for the endoscope to support the detection of cancer, for example in the bladder. In real application PDD efficiency suffers due to the complex accumulation of the photosensitizing drug inside the tumor and the associated processes of heme syntheses to create the fluorescent components needed. To optimize the diagnostic outcome of PDD it would be helpful to predict the optimal time for diagnosis based on measurable precursors. In a previous cell study, we proposed a new filter fluorometer to image the accumulation of the precursors Coproporphyrin III (CP-III) and Uroporphyrin III (UP-III) that metabolize to Protoporphyrin IX (PP-IX) later. This accumulation process can be used to predict the optimal time slot for diagnostic imaging. Therefore, a new filter system was designed to distinguish between CP-III and PP-IX. In this work we tested this filter system in combination with a standard PDD endoscopic imaging system. Goal of this study was to prove the technical feasibility in a non-patient setup to prepare a later clinical study.

Keywords: bladder cancer; cystoscopy; filter; fluorescence; photodynamic diagnostic; urology.

Introduction

Photodynamic diagnosis (PDD) is an enhancement approach used in endoscopic procedures to increase the detection rate of cancer cells. One application is the detection of non-muscle-invasive cancer (NMIBC) of the urinary bladder. Bladder cancer is the fourth most prevalent cancer for both sexes in Europe and North America [1]. Diagnosis only with withe light cystoscopy (WL) can be challenging. PDD can increase the detection rate of NMIBC [2] due to the visible fluorescence of 5-aminolevulinic acid (5-ALA) induced porphyrins. For PDD in the bladder an intravesical instillation of the ALA based pharmaceutical HEXVIX (Ipsen Pharma, France) is needed 1 h before the diagnostic procedure [3]. Metabolically active cells like cancer cells accumulate this fluorophore and convert it into Protoporphyrin IX (PP-IX). PP-IX emits red fluorescence when exposed to blue light and is used as marker in PDD.

For successful application of PDD in clinical routine timing is a major factor to achieve valuable results [4]. According to the instructions of use of HEXVIX, there is a time window between one and 3 h after instillation to perform the diagnosis [4]. But the accumulation process is individual leading sometimes to insufficient fluorescence intensity with a potentially large effect on specificity [5].

The metabolic process creates two more porphyrins as precursors, Coproporphyrin III (CP-III) and Uroporphyrin III (UP-III) that appear before the highest presence of PP-IX. Both have a fluorescence peak approx. 10 nm below the peak of PP-IX. These precursors can serve as indicators to predict the optimal time slot for PDD. This can lead to a higher efficacy of PDD.

This principle could already be shown on CP-III and PP-IX solutions in a prior work [6] and on cell models in unpublished work. Special optical filters were used during fluorescence imaging to separate the individual fluorescence of the precursors and PP-XI. To achieve that a complex and sensitive filter fluorometer was built [7]. In a next step the filter setup was redesigned following a methodical and user integrated approach [8] to allow application in a clinical setting [9]. This new filter system including two optical bandpass filters mounted into slider (FB620-10, FB630-10 Thorlabs, Germany) was already tested in a laboratory setting using a 405 nm laser light source to induce fluorescence. In this work we now describe the first tests of this new filter system in a clinical environment and in combination with the real PDD equipment as a preparation of a later clinical study on patients.

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Methods

Test setup

The new filter system described in [9] can be placed between the endoscopic camera and the endoscopic optic. Standardized C-mount adapters are used to ensure system compatibility. The filter system includes a slider with a 620 nm filter to detect CP-III and UP-III and a second 630 nm filter to detect PP-IX. In the center of the slider an aperture allows the use of standard imaging without additional filter. The filter system is shown in Figure 1.

A plastic box was used to create a dark test environment. Inside the box, test samples of CP-III and PP-IX can be placed on a holder. A hole in the side wall allows insertion of the endoscope optics.

This setup was placed inside a standard urology surgery room including an endoscopy and PDD system (Image 1, D-Light, KARL STORZ, Germany). A HYSTERO 4MM 30 DEG cystoscope in combination with an H3-P camera head (both KARL STORZ, Germany) were used for imaging. The test setup is shown in Figure 2.

Testing

In a first test cycle a CP-III sample was placed inside the test box. The cystoscope connected to the imaging system was inserted into the test box from the side hole. The tip of the optic was placed in a distance of 20 mm in front of the test sample. White light images, PDD images and PDD images with filter one (F1) and filter two (F2) were acquired. The slider of the filter system was used to change between the acquisition modes. Then, the test sample was exchanged by a PP-IX sample and the procedure was repeated. All images were directly stored on the endoscopic imaging system.

Since the images of the first test cycle appeared quite dark we placed the camera and light cable directly inside the test box with the sample for the subsequent testing. With that the light intensity could be increased. The light source was placed in a distance of 20 mm from the sample and the camera in a distance on 100 mm. All acquired images were exported form the endoscopic system for later evaluation.

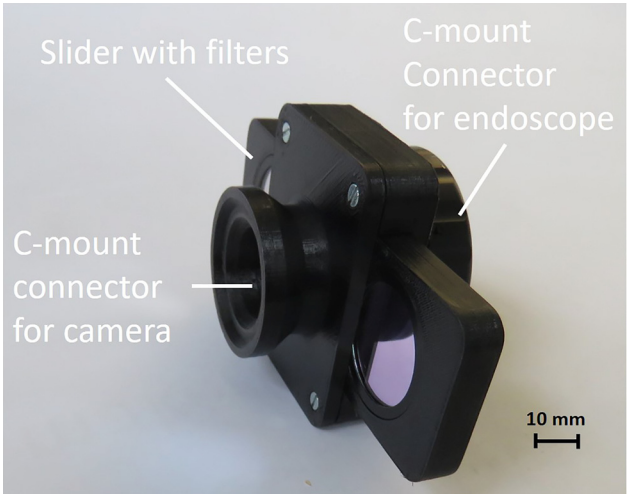

Figure 1: Filter system including slider with two filters to separate the fluorescence wavelength of Coproporphyrin III (CP-III) and Protoporphyrin IX (PP-IX).

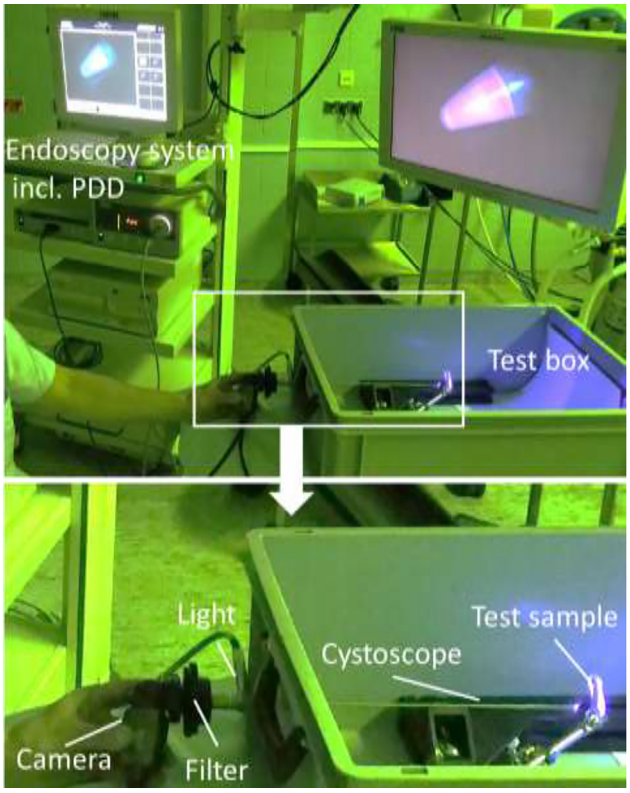

Figure 2: Test setup–top: overall setup incl. Photodynamic diagnosis (PDD) system and box, down: details of the box setup.

Post processing and evaluation

Following an evaluation approach described in [9] the images were analysed in ImageJ first. Since some of the images appear dark, a change in intensity of the fluorescence as expected was hard to see visually. Contrary to the priory used methods in a laboratory setting, a postprocessing was needed for evaluation. In a first step the Red-Green-Blue (RGB) channels were separated and subsequently only the red channel used for further processing. After adjusting the windowing level, a rectangular region of interest (ROI) of each image was selected and the intensity profile of the ROI was computed. The maximum intensity level was chosen for comparison.

Results

Eight images were acquired in the first test cycle using the cystoscope. The white light image was used for targeting, the PDD mode showed fluorescence of the used samples.

The filtered images of the CP-III were dark for both filter types, but the sample showed still an observable fluorescence. At the filtered images of the PP-XI sample the fluorescence structure was not visible. For this reason, the postprocessing was applied as described before. After splitting the RGB channels and separating the red channel the sample structure could be identified in all images. Computation of the intensity profile allows a comparison of the fluorescence. As expected, the intensity was higher when using filter one for CP-III and higher when using filter two for PP-IX (Figures 3 and 4).

The filtered images of the second test cycle showed high fluorescence under all conditions. Nevertheless, the post processing was applied to achieve comparable results. Again, the intensity was higher when using filter one for CP-III and higher when using filter two for PP-IX (Figures 5 and 6).

Discussion

Our tests could demonstrate the feasibility of our filter system to distinguish between CP-III and PP-IX. The light intensity of a standard PDD light source is lower compared to the laser system we have used before [9]. Even without an endoscopic optic the light is still sufficient to detect the differences in fluorescence. When the cystoscope is used, the light intensity is reduced dramatically. This leads to a low fluorescence of the samples. The light has to pass the cystoscope and the fluorescent emission has to pass the way back through the cystoscope again. This leads to a reduction of intensity resulting in dark images. Nevertheless, we were able to detect the sample structure and analyse the intensity by a simple postprocessing. For preparation of a clinical study we now have to prove feasibility in a filled artificial bladder under more real conditions.

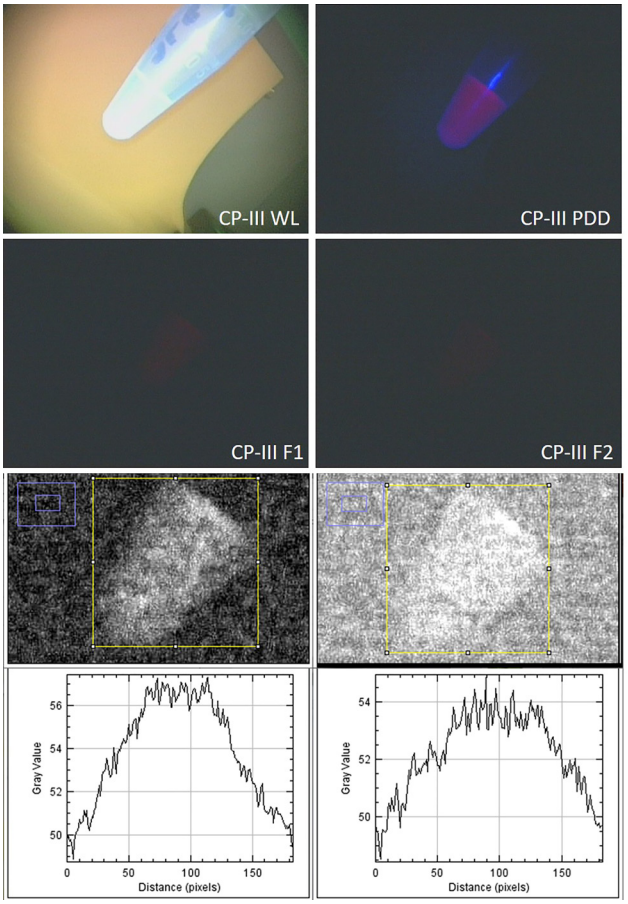

Figure 3: First test cycle, images of the CP-III sample with cystoscope. White light mode, PDD mode, filter 1, filter 2, postprocessed and intensity profile.

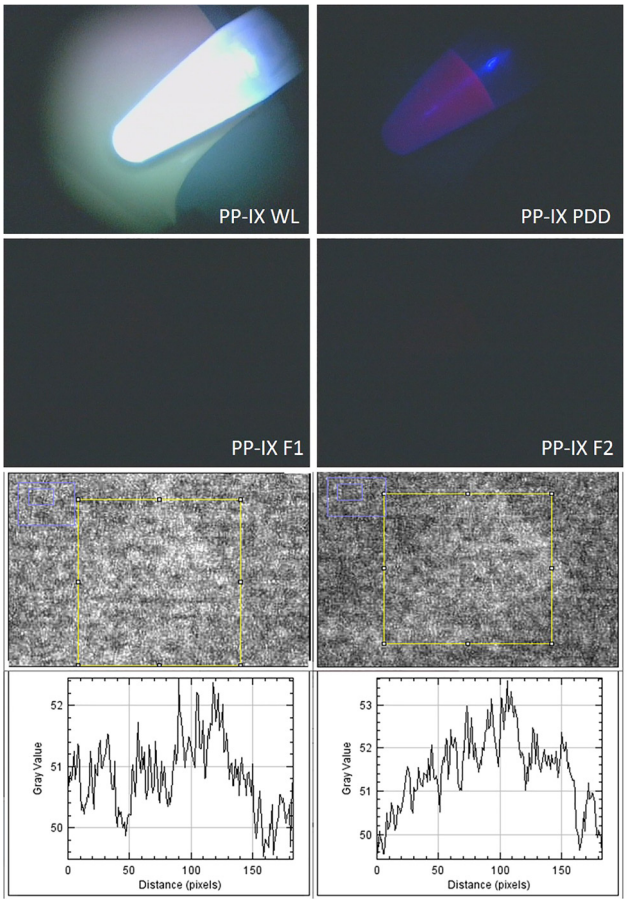

Figure 4: First test cycle, images of the PP-IX sample with cystoscope. White light mode, PDD mode, filter 1, filter 2, postprocessed and intensity profile.

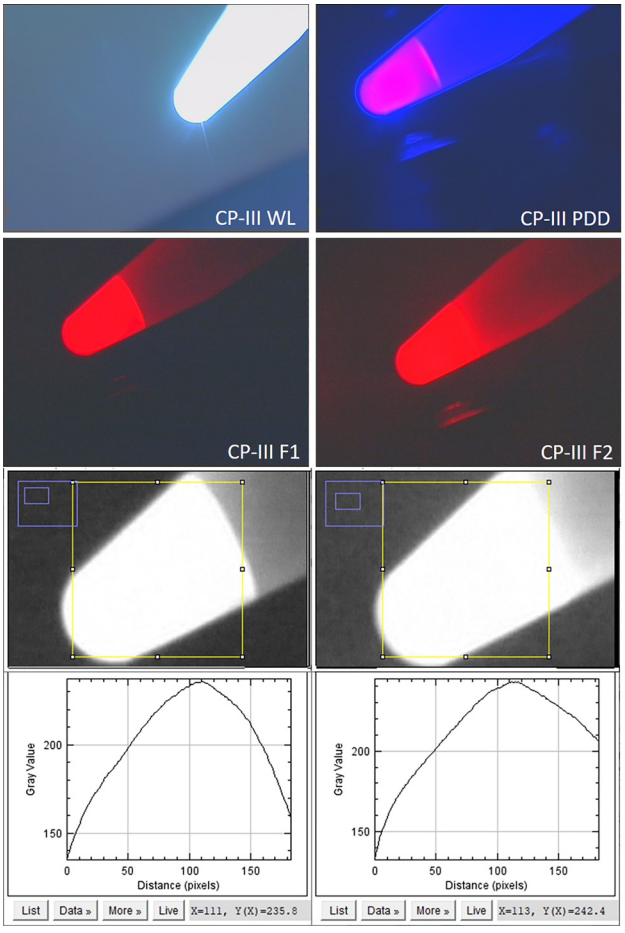

Figure 5: Second test cycle, images of the CP-III sample. White light mode, PDD mode, filter 1, filter 2, postprocessed and intensity profile.

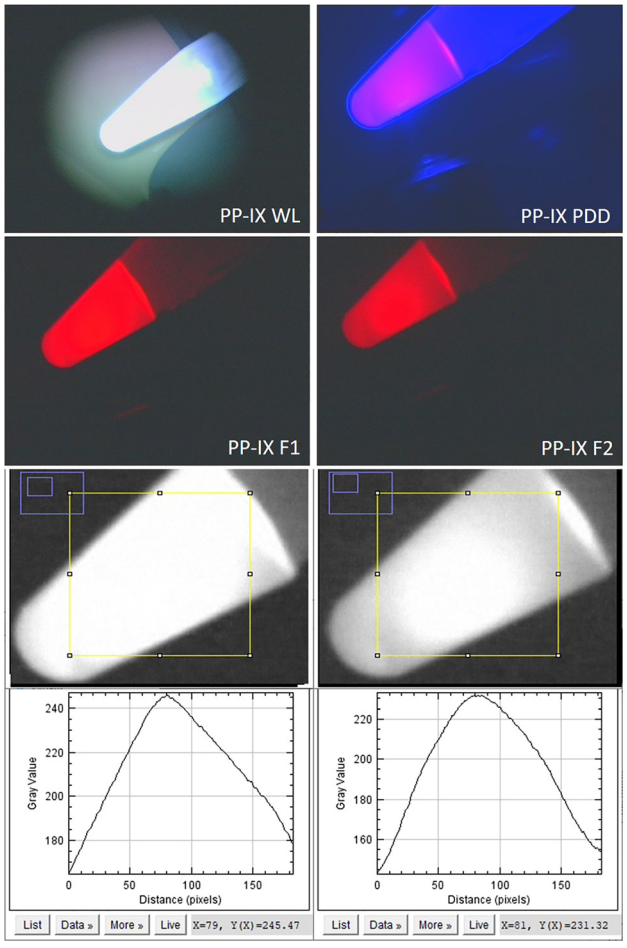

Figure 6: Second test cycle, images of the PP-IX sample. White light mode, PDD mode, filter 1, filter 2, postprocessed and intensity profile.

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Informed consent: Informed consent was obtained from all individuals included in this study.

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