Prostate Cancer Diagnosis Based on the Segmentation and Analysis of NIR Images Obtained Using Two PSMA-1 Based PDT Conjugates PSMA-1-Pc413 and PSMA-1-IR700

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Abstract

Prostate cancer is among the widespread men's diseases in the world. Prostatectomy has been the strength treatment for men with restricted prostate tumor. On the other hand, surgery, frequently can cause some side effects initiated from damage and removal of nerves and muscles surrounding the prostate. Prostate-specific membrane antigen (PSMA), a type II membrane antigen highly expressed in prostate cancer, has been an attractive target for imaging and therapy. Photodynamic therapy (PDT) is a non-invasive therapy which is used clinically in the treatment of various cancers and other diseases. The objective of this study is to segment and analyze the near-infrared (NIR) images of prostate cancer cells obtained using developed low molecular weight PSMA-targeting PDT agents, which would provide image-guidance for prostate tumor resection and allow for subsequent PDT to eliminate un-resectable or remaining cancer cells. The segmentation of NIR color images, of prostate cancer cells, is performed using Unsupervised Hopfield Neural Network Classifier (UHNNC) which gives better distribution of pixels among a predefined number of clusters. The clusters' statistics needed for analysis are given using ENVI software product for image analysis.

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The results of this study show that the combination of the segmentation results with the clusters' statistics with the appropriate PSMA, creates a better visualization of the cancerous tissues and help medical doctors for a more accurate diagnosis and surgery planning.

Keywords: Prostate cancer; near-infrared (NIR) images; molecular weight PSMA-targeting PDT agents.

1. Introduction

(As the introduction of Prostate-specific membrane antigen (PSMA) analysis, significantly additional males have been diagnosed and treated for prostate cancer [1]. Approximately 220,800 new diagnoses and 27,540 deaths from the disease are projected in 2015 among men in the United States [2]. Over 90% of men have localized tumors at initial screenings and are candidates for radical prostatectomies [3]. However, at surgery, cancer has been shown to extend outside the prostate (pathological stage C) in 20-42% of patients [4] and surgery fails to halt the disease in approximately 20% of the patients who undergo radical prostatectomy, and over a 60% recurrence rate of this disease in this population has been reported [5-7].

Although surgeons are experienced with this disease, radical prostatectomy remains an imperfect intervention. During surgery, it's difficult for surgeons to access the invasion of prostate cancer; therefore, many malignant nodules escape detection, leading to disease recurrence. A retrospective multivariate analysis by Wright *et al.* of incomplete resection of prostate cancer in over 65,000 patients who underwent radical prostatectomies [8] found that positive surgical margins were associated with a 2.6-fold increased unadjusted risk of prostate cancer specific mortality and are an independent predictor of mortality.

This study also underlined the need for surgeons to optimize surgical techniques to achieve the negative surgical margins essential for sound oncological outcomes. Surgical achievement of this without side effects is a challenge, however, because the prostate is surrounded by many nerves and muscle fibers controlling different excretory and erectile functions that are difficult but necessary to avoid. In 1983 Walsh defined nerve locations around the prostate and inspired a number of "nerve-sparing" surgical approaches, including robotic-assisted laparoscopic prostatectomy [9]. Unfortunately, the success of these approaches to mitigate side effects is mixed [10, 11] and surgical approaches to prostate cancer are still associated with significant morbidity, e.g. incontinence (3-74%) and impotence (30-90%) [12-17]. There remains an unmet clinical need to improve surgical techniques for identifying and removing cancerous tissue without damaging surrounding tissues during prostatectomy. Recently, Neuman *et al.* have shown that near-infrared (NIR) fluorescence probe YC-27 can improve the surgical treatment of prostate cancer and reduce the rate of positive surgical margins in real-time laparoscopic extirpative surgery [18].

Photodynamic Therapy (PDT) is a non-invasive therapy, which is used clinically in the treatment of cancers and other diseases [19-21]. PDT uses photosensitizers which are pharmacologically inactive until they are exposed to light in the presence of oxygen. The active drug forms reactive oxygen species such as singlet oxygen to kill nearby cells. Various agents, including porphyrins and phthalocyanines have been examined as photosensitizers [22-24]. Most photosensitizers are fluorescent and emit near-infrared (NIR) light that can be used for *in vivo*

imaging to identify the location of cancer cells and provide image-guided PDT treatment potentially leading to improved therapeutic accuracy and outcome [22]. The main challenge for PDT treatment is off-target tissue accumulation and activation of photosensitizer leading to cell death in normal tissue [25]. Development of a highly selective delivery method for photosensitizers to minimize side effects and generate better therapeutic outcomes would be optimal.

PSMA is a unique membrane bound glycoprotein originally discovered in the androgen-dependent LNCaP human prostate adenocarcinoma cell line using a monoclonal, mAb 7E11C-5.3 [26]. PSMA is overexpressed on prostate cancer. Expression of PSMA in cancer tissues correlates with the stage of disease and Gleason score [27]. PSMA expression is also higher in prostate cancer cells from hormone-refractory patients [27, 28] and increased PSMA expression has been shown to be an independent marker of disease recurrence [28-30]. In addition to being overexpressed in prostate cancer, PSMA is also expressed in the neovasculature of many solid tumors [31]. PSMA has served as a promising target for both imaging [32-34] and treatment of prostate cancer [35-37]. A peptide-based, highly negatively charged PSMA ligand (PSMA-1) was developed and used for PSMA-targeted imaging of prostate cancer [38, 39].

In this study, we present the segmentation and analysis results the NIR prostate images obtained using two PSMA-1 based PDT conjugates, PSMA-1-Pc413 Figure1.A and PSMA-1-IR700 Figure1.B. Pc413 is an analogue of second generation phthalocyanine PDT drug Pc4 which is currently in clinical trials [40], and IR700 is a commercially available near-infrared dye which has been shown to have PDT activities [41-43].
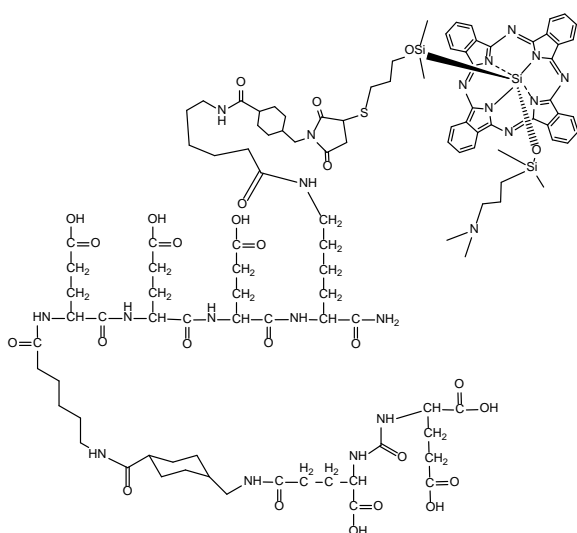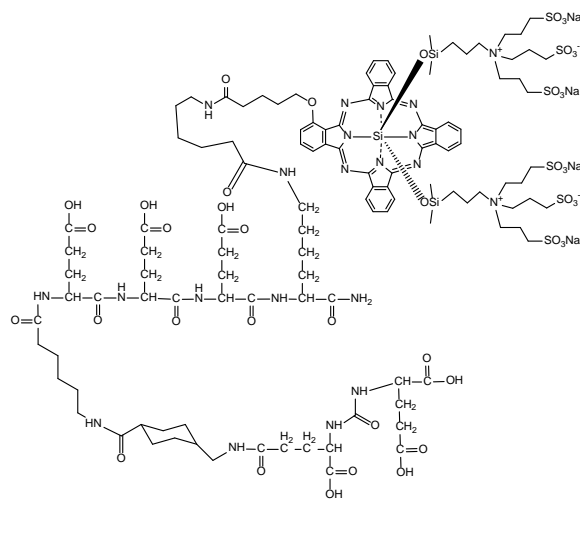

Figure1.A. Structure of PSMA-1-Pc413   Figure1.B. Structure of PSMA-1-IR700

Figure 1: Structures of PSMA-1-Pc413 Figure1.A and PSMA-1-IR700 Figure1.B.

2. Methods and Materials

PSMA targeting peptide PSMA-1 was synthesized by Fmoc chemistry as previously reported [39]. All the other

chemicals were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA. High Performance Liquid Chromatography (HPLC) was performed on a Shimadzu HPLC system equipped with a SPD-20A prominence UV/visible detector and monitored at a wavelength at 220 nm for PSMA-1-SMCC or 350 nm for PSMA-1-Pc413 and PSMA-1-IR700.

For *in vivo* NIR imaging studies, animal experiments were performed according to policies and guidelines of the animal care and use committee at Case Western Reserve University (IACUC#120024). Six to eight weeks old male athymic nude mice were implanted subcutaneously with 1×10^6 of PSMA-negative PC3flu and PSMA-positive PC3pip on the left and right dorsum respectively. Animals were ready to use when tumors reached the size at about 10 mm. PSMA-1-PDT was injected intravenously via the tail vein. Fluorescence imaging was performed using the Maestro *in Vivo* Imaging system (Perkin-Elmer, Waltham, MA). During imaging, mice were anaesthetized with isoflurane. After imaging, the mice were euthanized and tissues were harvested for *ex vivo* imaging. For *in vivo* competition experiments, mice were co-injected with 1 nmol of PSMA-1-Pc413 and 1000 nmol of ZJ-MCC-Ahx-YYYG, an analogue of PSMA-1 with similar binding affinity [38], or 1 nmol of PSMA-1-IR700 probes and 1000 nmol of PSMA-1. Different competitors were used for PSMA-1-Pc413 and PSMA-1-IR700 due to their different pharmacokinetic behaviors. Multispectral images were unmixed into their component spectra (PSMA probes, autofluorescence, and background) and these component images were used to quantitate the average fluorescence intensity associated with the tumors by creating regions of interest (ROIs) around the tumors. The two probes reported in [44] were found to be effective as theranostic probes allowing both targeted-bioimaging and targeted-photodynamic therapy of prostate cancer. The tumors were extracted 24 hours post the PDT treatment and processed by hematoxylin and eosin staining. Pathological analysis showed that the nuclei in cancer cells treated with PSMA-1-PDT probes were much smaller compared to untreated tumors, indicating that the cells were damaged as shown in Figure 2.

Hopfield Neural Network Segmentation Technique: Segmentation shows a critical role in therapeutic and biomechanical fields, such as assisting radiologists and medical doctors to improve their diagnosis. In the past few decades, researchers have proposed many effective algorithms to perform the computer aided segmentation. The successful implementation of modern mathematical and physical techniques, such as Bayesian's analysis, template matching and deformable models, greatly enhances the accuracy of segmentation results. Compared with common image segmentation algorithms, the ones used for medical images need more concrete backgrounds and must satisfy the complex practical requirements. A good survey about these techniques are discussed in [45]. In our previous work, we have UHNNC to segment different type of medical and natural color images [46, 47], the segmentation results have been appreciated with respect to the multi-dimensionality of the data type used for segmentation. This means that UHNNC gives better segmentation results as far as getting more information about the pixel of the scene under segmentation. Here, we adapt the UHNNC to segment the NIR color image, samples are shown in Figure 2, and we analyze the clusters of the segmentation results with respect to the case available medical information and tissues features. UHNNC architecture consists of a grid of NM neurons with each column representing a class and each row representing a pixel, N represents the size of the given image and M represents the number of clusters, the latter is given as a priori information. The network is designed to classify the feature space without teacher based on the compactness of each class calculated using a distance measure.
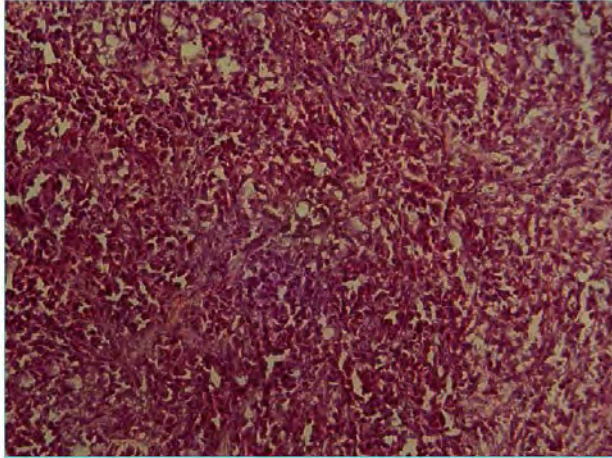
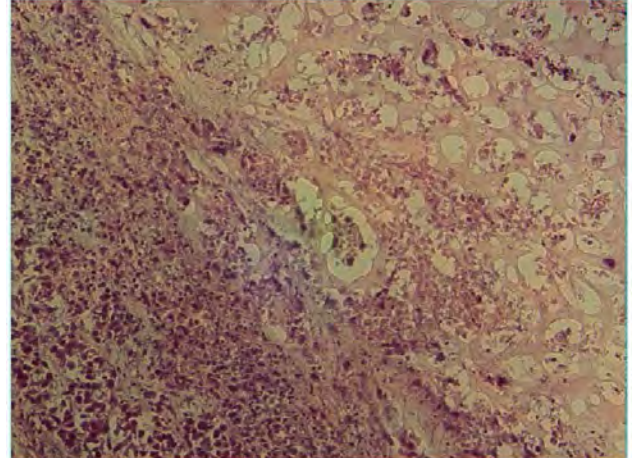Figure 2. (A) PC3pip tumor; (0.5 mg/kg PSMA-1-Pc413); (no light irradiation)

Figure 2. (B) PC3pip tumor; (0.5 mg/kg PSMA-1-Pc413); with (150J/cm2 light irradiation)
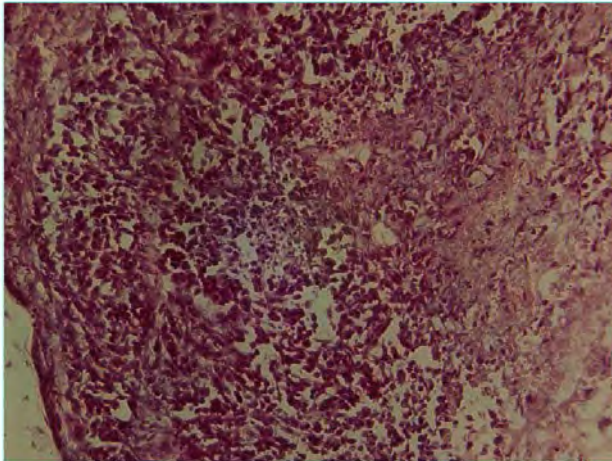
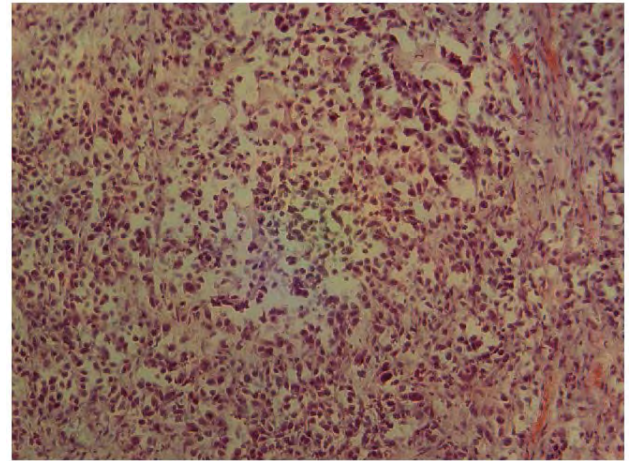Figure 2. (C) PC3pip tumor; (0.5 mg/kg PSMA-1-IR700); (no light irradiation)

Figure 2. (D) PC3pip tumor; (0.5 mg/kg PSMA-1-IR700); with (50J/cm2 light irradiation)

Figure 2: Images of the PC3pip tumors receiving PSMA-1-PC413 and PSMA-1-IR700 wit and without light irradiation.

The segmentation problem is formulated as a partition of N pixels of P features among M clusters or regions, such that the assignment of the pixels minimizes the cost-term of the energy (error) function:

$$E = \frac{1}{2}\sum_{k=1}^{N}\sum_{l=1}^{M} R_{kl}^{n} V_{kl}^{2} \qquad (1)$$

where R_{kl} is a similarity distance measure between the kth pixel and the centroid of class l, and is defined as follows:

$$R_{kl} = \|X_k - \bar{X}_l\| \qquad (2)$$

Where X_k is the P-features vector, for color images, P=3 in the RGB color space, as intensities of the kth pixel,

and $\overline{X}_l$ is the centroid of class l, and is defined as follows:

$$\overline{X}_l = \frac{\sum_{k=1}^{N} X_k V_{kl}}{n_l} \qquad (3)$$

where n_l is the number of pixels in class l. In case of n=2, the energy in (1), could be considered as the sum of squared errors, V_{kl} is the output of the kl^{th} neuron. UHNNC uses the winner-takes-all learning as the input-output function for the kth row to assign a label m to the pixel and is given by:

$$\begin{cases} V_{kl}(t+1) = 1, & \text{if } U_{kl} = Max\{U_{kl}(t), \forall\, l\} \\ V_{kl}(t+1) = 0; & \text{otherwise} \end{cases} \qquad (4)$$

the minimization is achieved by using UHNNC and by solving a set of motion equations satisfying:

$$\frac{\partial U_i}{\partial t} = -\mu(t)\frac{\partial E}{\partial V_i} \qquad (5)$$

where U_i and V_i are respectively the input and the output of the ith neuron, $\mu(t)$ is as defined in [46] a scalar positive function of time used to increase the convergence speed of the HNN as:

$$\mu(t) = t * (T_s - t) \qquad (6)$$

by applying the relation (5) to equation (1), we get a set of neural dynamics given by:

$$\frac{dU_{kl}}{dt} = -\mu(t)(R_{kl}^n V_{kl}) \qquad (7)$$

The UHNNC segmentation algorithm could be summarized in the following steps:

1. Initialize the input of neurons to random values.

2. Apply the input-output relation given in (4) to obtain the new output value for each neuron, establishing the assignment of pixel to classes.

3. Compute the centroid for each class with respect to the equation (3).

4. Solve the set of differential equation in (5) to update the input of each neuron as:

$$U_{kl}(t+1) = U_{kl}(t) + \frac{dU_{kl}}{dt} \qquad (8)$$

5. Repeat from step 2 until convergence (t = Ts), then terminate.

3. Segmentation Results

Prostate cancer has a slow rate of growth and most tumors remain organ confined for longer than other

malignancies. Because of these features, clinicians have been working to find a reliable way of detecting it early so that potentially life-saving treatments can be implemented promptly [48].

To date, prostate specific antigen (PSA) testing has provided a relatively simple means of population screening for prostate cancer. Unfortunately, however, PSA does not diagnose prostate cancer with certainty as its serum value can be elevated in both benign and malignant conditions of the prostate and not all men with prostate cancer will have high PSA levels. In addition, where prostate cancer is detected early, clinically indolent cancers may be over diagnosed, resulting in overtreatment, and men may experience side effects from untoward treatment that reduce their quality of life. There may also be unnecessary costs and burdens to our health care system. However, as active surveillance is increasingly being adopted as a first-line treatment for men with 'very low' and 'low' risk disease, the risks of overtreatment have been substantially reduced; some studies report up to 40 percent of newly diagnosed men enter this treatment pathway [48-49].

A good review of conventional histological practice in cancer diagnosis can be found in [50], where it is mentioned that few research efforts have been dedicated to the development of quantitative techniques in order to achieve accurate, robust, and reproducible diagnosis and grading in histological images. Diamond et al. [50, 51] used morphometric and texture features to identify stroma, normal, and the remaining tissue is classified into cancerous regions based on a texture feature as shown in Figure 3. [50].
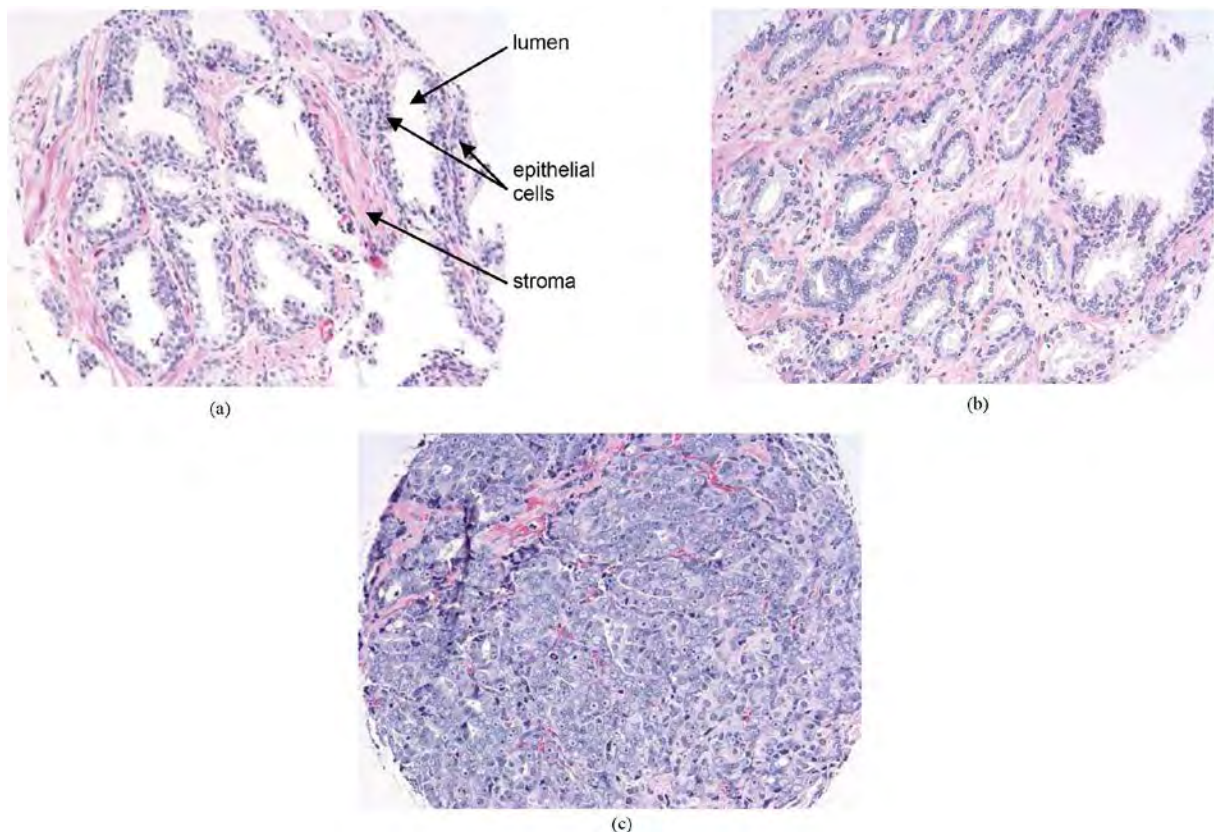

Figure 3: Samples of H&E-stained prostateTMA cores showing varying degrees of differentiation: (a) normal, (b) grade 2 (well-differentiated) cancer, and (c) grade 5 (poorly differentiated) cancer [50].

In this study, we use the population ratio between the different classes of the segmentation result to show the effect of the PSA testing as mentioned earlier with PSMA-1-Pc413 and PSMA-1-IR700. Figure 4.1 represents the control of PC3pip tumor receiving PSMA-1-PC413. Mice received 0.5 mg/kg of PSMA-1-PC413 and tumors were taken out at 48 hours post injection without light irradiation. Figure 4.2 shows the segmentation result of the raw image shown in Figure 4.1 obtained using UHNNC with 4 classes as predefined number of classes. The population ration of class1 and class2 is equal to 0.964, and the same ratio between class3 and class4 is 0.813, the most populated class is taken as a denominator of the ratio. These ration indicates also the similarity between the classes in term of density of their populations. However, these ratios are global, with respect to the image under study, their values will more significant for a Local Region of Interest (ROI) of the scene.
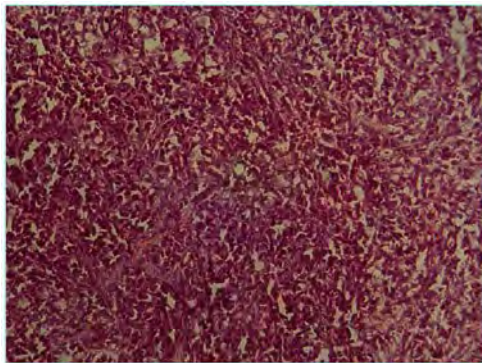
Figure 4.1: Figure2. (A) PC3pip tumor; (0.5 mg/kg PSMA-1-Pc413); (no light irradiation)
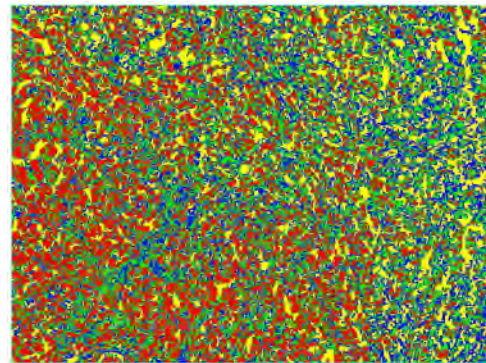
Figure 4.2: Segmentation results of the image in Figure 4.1 using the above described UHNNC. into 4 classes

Classes
- ☑ ⬛ 0: Unclassified
- ☑ 🟥 1: Class 1
- ☑ 🟩 2: Class 2
- ☑ 🟦 3: Class 3
- ☑ 🟨 4: Class 4

Class Distribution Summary
Unclassified: 0 points (0.000%)
Class 1: 216,344 points (28.147%)
Class 2: 224,200 points (29.169%)
Class 3: 180,892 points (23.535%)
Class 4: 147,176 points (19.148%)
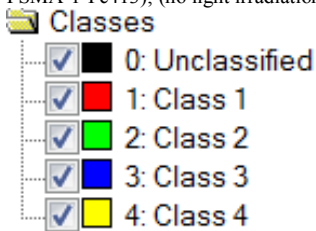
Figure 4.3 Classes Color Keys Table of the segmentation result shown in Figure 4. 2
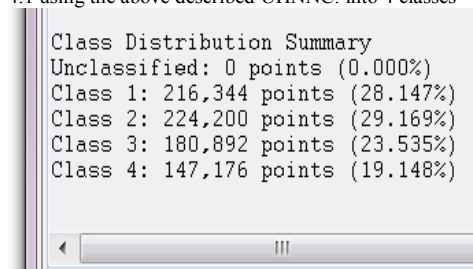
Figure 4.4 Classes Statistics Table of the segmentation result shown in Figure 4. 2
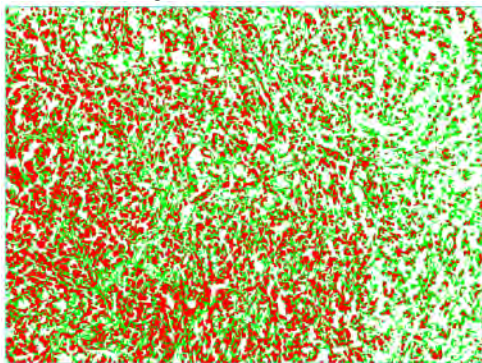

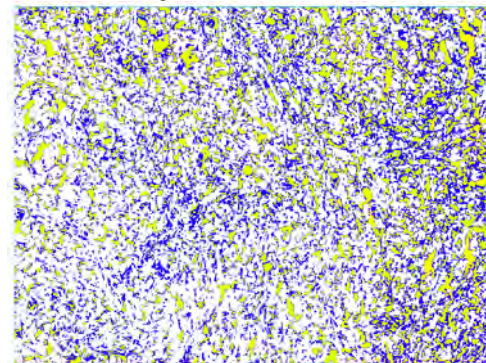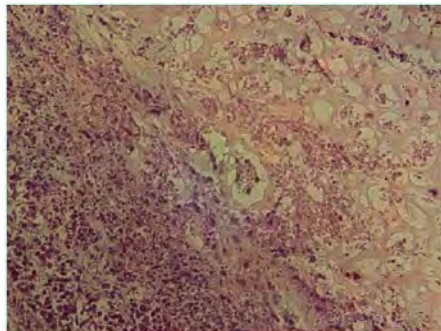Figure 4. 5 Display of Class 1 and Class 2 extracted from the segmentation result shown in Figure 4. 2

Figure 4. 6 Display of Class 3 and Class 4 extracted from the segmentation result shown in Figure 4. 2

Figure 4: Segmentation result of the raw image shown in Figure 2. A. represents PC3pip tumor; (0.5 mg/kg PSMA-1-Pc413); (no light irradiation) obtained using UHNN with 4 classes as predefined number of classes.

Figure 5.1 represents the control of PC3pip tumors receiving PSMA-1-Pc413 and treated with 150J/cm2 light. Mice received 0.5 mg/kg of PSMA-1-PC413, irradiated with 150J/cm2 light at 24 hours post injection. Tumors were taken out at 24 hours post PDT treatment. Figure 4.2 shows the segmentation result of the raw image shown in Figure 5.1 obtained using UHNNC with 4 classes as predefined number of classes. The population ration of class1 and class2 is equal to 0.621, and the same ratio between class3 and class4 is 0.788, the most populated class is taken as a denominator of the ratio. The ration of class1 and class2 indicates a considerable global change in the population of one of the two classes, and a little variation in the population of class3 and class4, as compared to the results shown in Figure 4. The results in Figure 4 and Figure 5 indicate the effect of the theranostic PSMA-1-PDT agent PSMA-1-Pc413 on the prostate cancer cells, which needs more explanation and analysis as cells interaction with the agent.

Figure 5.1: Figure2. (B) PC3pip tumor (0.5 mg/kg PSMA-1-Pc413) with (150J/cm2 light irradiation)
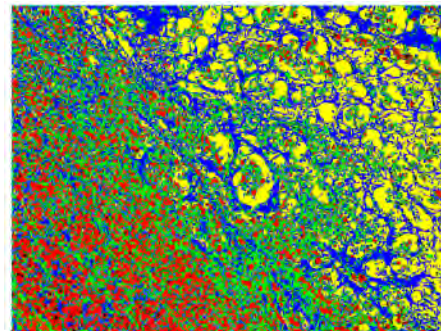

Figure 5.2: Segmentation results of the image in Figure 5.1 using the above described UHNNC. into 4 classes
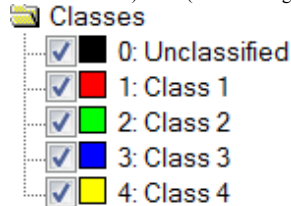

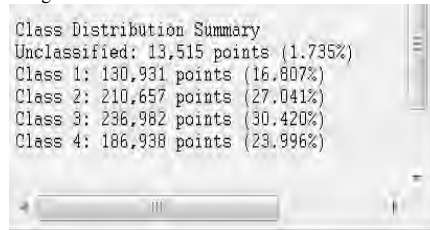Figure 5.3 Classes Color Keys Table of the segmentation result shown in Figure 5. 2

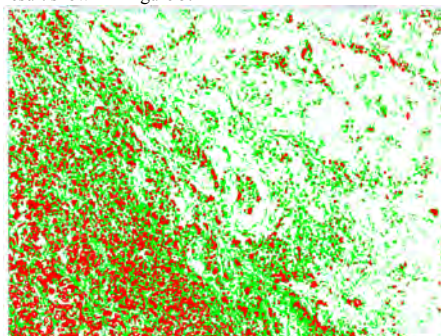Figure 5.4 Classes Statistics Table of the segmentation result shown in Figure 5. 2

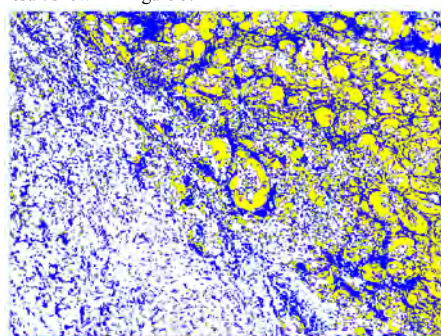Figure 5.5 Display of Class 1 and Class 2 extracted from the segmentation result shown in Figure 5. 2

Figure 5.6 Display of Class 3 and Class 4 extracted from the segmentation result shown in Figure 5.2

Figure 5: Segmentation result of the raw image shown in Figure 2. B. represents PC3pip tumor (0.5 mg/kg PSMA-1-Pc413) with (150J/cm2 light irradiation) obtained using UHNNC with 4 classes as predefined number of cluster.

Figure 6.1 represents the control of PC3pip tumor receiving 0.5 mg/kg PSMA-1-IR700 and tumors were taken out at 48 hours post injection without light irradiation. Figure 6.2 shows the segmentation result of the raw image shown in Figure 6.1 obtained using UHNNC with 4 classes as predefined number of classes. The population ration of class1 and class2 is equal to 0.897, and the same ratio between class3 and class4 is 0.886, the most populated class is taken as a denominator of the ratio. These ration indicates also the similarity between the classes in term of density of their populations.
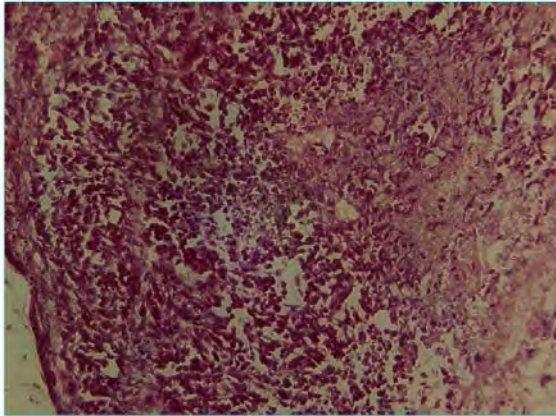

Figure 6.1: Figure2. (C) PC3pip tumor (0.5 mg/kg PSMA-1-IR700) (no light irradiation)
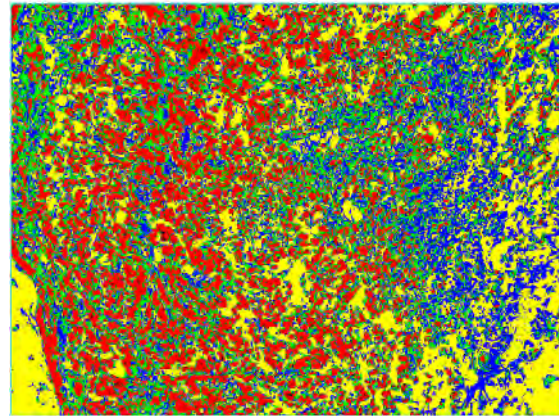

Figure 6.2: Segmentation results of the image in Figure 6.1 using the above described UHNNC. into 4 classes

Figure 6.3 Classes Color Keys Table of the segmentation result shown in Figure 6. 2

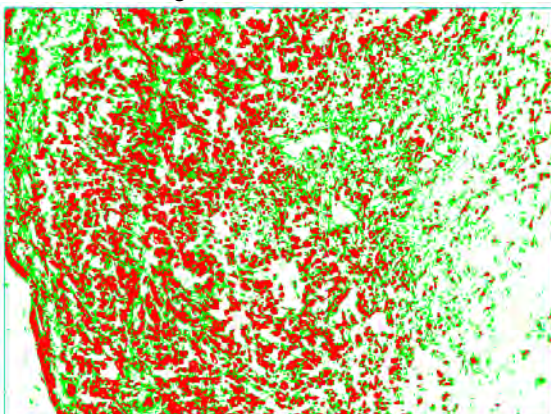Figure 6.4 Classes Statistics Table of the segmentation result shown in Figure 6. 2

Figure 6. 5 Display of Class 1 and Class 2 extracted from the segmentation result shown in Figure 6. 2

Figure 6. 6 Display of Class 3 and Class 4 extracted from the segmentation result shown in Figure 6. 2
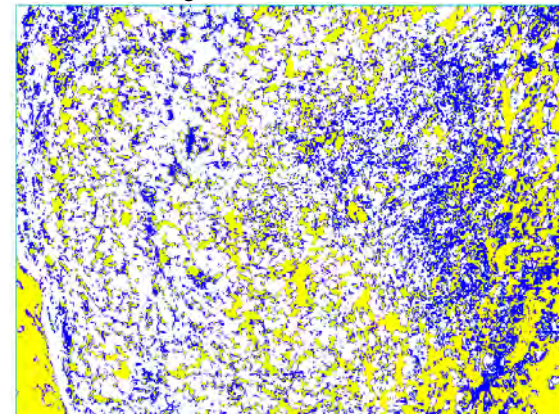
Figure 6: Segmentation result of the raw image shown in Figure 2. (C) PC3pip tumor (0.5 mg/kg PSMA-1-IR700) (no light irradiation) obtained using UHNNC with 4 classes as predefined number of cluster.

Figure 7.1 represents the control of PC3pip tumors receiving 0.5 mg/kg PSMA-1-IR700 and treated with 150J/cm2 light. Mice received 0.5 mg/kg of PSMA-1-IR700, irradiated with 50J/cm2 light at 24 hours post injection. Figure 7.2 shows the segmentation result of the raw image shown in Figure 1.1 obtained using UHNNC with 4 classes as predefined number of classes. The population ratio of class1 and class2 is equal to 0.806, and the same ratio between class3 and class4 is 0.874, the most populated class is taken as a denominator of the ratio. The ratio of class1 and class2 indicates an inconsiderable global change in the population of one of the two classes, and a little variation in the population of class3 and class4, as compared to the ratios shown with Figure 6. The results in Figure 6 and Figure 7 indicate the effect of the theranostic PSMA-1-IR700 agent on the prostate cancer cells, which needs more explanation and analysis as cells interaction with the agent.
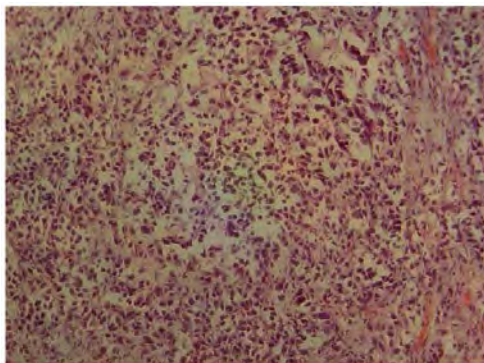

Figure 7.1: Figure2. (D) PC3pip tumor; (0.5 mg/kg PSMA-1-IR700) With (50J/cm2 light irradiation)
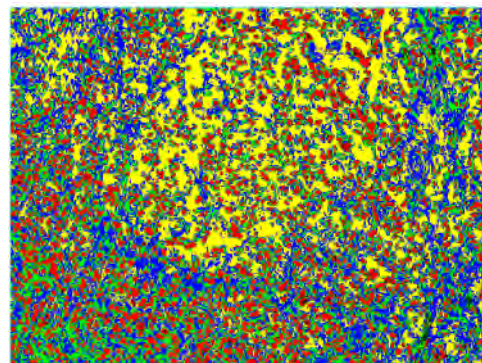

Figure 7.2: Segmentation results of the image in Figure 7.1 using the above described UHNNC. into 4 classes

Figure 7.3 Classes Color Keys Table of the segmentation result shown in Figure 7. 2

Figure 7.4 Classes Statistics Table of the segmentation result shown in Figure 7. 2

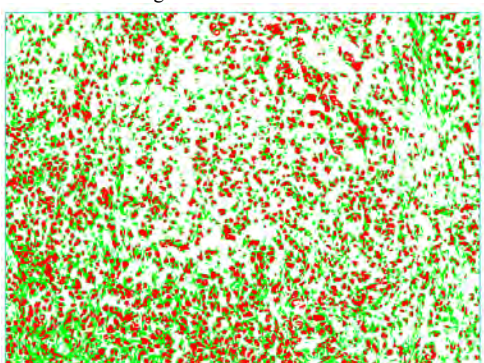Figure 7. 5 Display of Class 1 and Class 2 extracted from the segmentation result shown in Figure 7. 2

Figure 7. 6 Display of Class 3 and Class 4 extracted from the segmentation result shown in Figure 7. 2
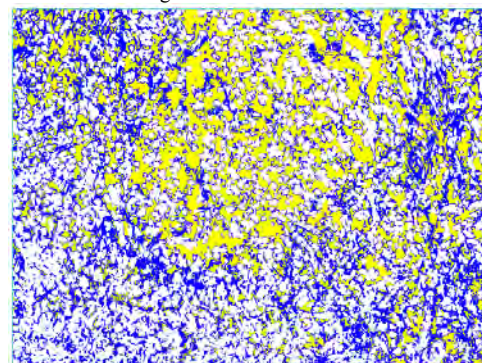
Figure 7: Segmentation result of the raw image shown in Figure. (D) PC3pip tumor (0.5 mg/kg PSMA-1-IR700) with (50J/cm2 light irradiation) obtained using UHNNC with 4 classes as predefined number of cluster.

4. Conclusion

 In this paper we have presented the use of a designed dual-functional PSMA-1-PDT conjugates to serve as anti-cancer agents. The segmentation of the *in vivo* imaging results showed that both PSMA-1-Pc413 and PSMA-1-IR700 can selectively accumulate in PSMA-positive PC3pip tumor. The analysis of these results with more affinity to small ROIs would help to achieve more accurate diagnosis.

Acknowledgments

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