PROTOCOL TITLE: High Resolution Imaging for early and better detection of bladder cancer.

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DATE: March 14, 2017

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POPULATION: Patients aged 18 years and above with anticipated diagnosis of superficial bladder cancer.

NUMBER OF CLINICAL SITES: One; LBJ general Hospital
STUDY DURATION: 4 years

SUBJECT DURATION: 10 minute imaging session at the time of cystoscopy with bladder biopsy.
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|          |               | • Section **Study Design**: Added “normal bladder tissue and”.
|          |               | • Section **Duration of Subject Participation**: Added: “and/or”.
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|          |               | • Section **Sample Size**: Added: “comparing normal bladder tissue with cancerous bladder lesions”.
|          |               | • Section **Sample Concerns**: Added: “normal tissue”.
|          |               | • Section **Data Analysis and End-Point/Statistical Considerations**: Added: “normal tissue”.
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General Information

Bladder cancer is the 6th most common cancer in United States. 563,640 are the estimated number of people living with bladder cancer in United states currently and it accounts for 4.4% of all new cancer cases in United States. The spectrum of disease ranges from superficial low grade tumors that are prone to recurrence, to high grade potentially lethal malignancies resulting in 15,210 estimated deaths in 2013, responsible for 2.6% of all cancer deaths in the US. Tumors are multifocal throughout the bladder and they are prone to recurrences. Surveillance for bladder cancer includes endoscopic inspection of the bladder via the urethra using a cystoscope. Visual appearances of cancerous lesions can range from large bulky papillary masses projecting into the lumen of the bladder to small, subtle, flat lesions that are difficult to detect but just as dangerous, if not more so. Conventional settings such as use of white light cystoscopy often result in the detection of 3D lesions such as papillary; it often fails to distinguish flat carcinogenic lesions from normal such as in condition like carcinoma in situ. Apart of white light cystoscopy, fluorescence cystoscopy and Narrow Band Imaging (NBI), have been introduced and are being investigated to improve the detection rates for cancerous lesions.

Once the suspicious lesions are visualized, current practice requires a tissue biopsy to establish the diagnosis of cancer. This is particularly important given the high rate of false positive diagnoses based on visual appearance. Infection, inflammation, iatrogenic manipulation and anatomic variation can all result in lesions on the bladder wall surface that mimic bladder cancer and carcinoma in situ (CIS). This is evidenced by reports suggesting unnecessary biopsy rates of 31-39%. These unnecessary biopsies add increased risks for the patient and resource depletion to the healthcare system. The ability to more accurately characterize suspicious appearing lesions at the cellular level could significantly decrease morbidity and healthcare costs.

High-resolution imaging can image epithelial features, such as increased nuclear-cytoplasmic ratio. We hypothesize that high resolution imaging of visually suspicious lesions in the bladder may improve the ability to discriminate flat lesions such as in early neoplasia from inflammatory and iatrogenic lesions, leading to a higher specificity and reducing the number of unnecessary biopsies performed. The goal of this proposal is to develop, optimize and validate a High Resolution Imaging System in the bladder that displays images in real-time, providing automated diagnostic criteria for bladder cancer screening. This technology has already been approved for study in the detection of oral cancers at UTHSCH (IRB #: HSC-DB-10-0708).

Background Information

Bladder cancer has many presentations based on both histologic grade and anatomic stage. Papillary tumors grow over time and occupy space within the lumen of the bladder, becoming bulky and potentially obstructive if left undiagnosed and untreated. Bladder cancers also have the potential to grow into the muscular wall of the bladder and thus carry the threat of local and distant metastatic spread. As the depth of penetration increase so does the mortality risk from the disease. A disease of field defects resulting from environmental exposures and age, bladder cancer can manifest in urothelial
cells throughout the bladder thus making this a multifocal disease. Furthermore, it is a disease of frequent recurrences, mandating routine surveillance and vigilant follow-up to prevent disease progression.

Cystoscopy is the gold standard for bladder cancer detection and screening. The procedure involves inserting an endoscope through the urethra and inflating the potential space of the bladder with irrigation fluid. The bladder is visually inspected for gross visible tumor or mucosal discoloration and irregularities. While detection of bulky papillary tumors is generally straightforward, small papillary lesions can often times go unnoticed. This is thought to contribute to the 70 percent recurrence rate for bladder cancer. High-grade carcinoma in situ (CIS) also presents a diagnostic challenge. It generally presents as a small flat lesion on the bladder wall with varying degrees of redness or subtle discoloration. Some studies estimate up to 85% of CIS foci are missed at the time of standard cystoscopy. These lesions are particularly troublesome because of their health threat, with an estimated 41% risk of progression and 26% risk of death at 15 years.

In order to increase the sensitivity in detection of these lesions, several adjuncts to white light cystoscopy are being investigated. Fluorescence cystoscopy involves the use of photoactive porphyrins in the irrigation fluid, which then accumulate preferentially in cancerous cells and produce a red hue when illuminated with blue light. Narrow band imaging (NBI) uses shorter wavelengths of light to preferentially highlight the hemoglobin in highly vascular structures such as CIS tumors, thereby increasing detection rates. While these techniques certainly improve the sensitivity of bladder cancer detection, they can further confound the specificity of screening. The differential diagnosis of an erythematous or flat lesion in the bladder as seen on standard white light cystoscopy includes infection, benign inflammation, iatrogenic manipulation, anatomic variant with increased vascularity and malignancy. The inability to accurately distinguish between these entities results in an estimated false positivity rate of 31% for white light cystoscopy and 39% for fluorescence cystoscopy. Therefore, once a lesion is seen on surveillance, it must then be biopsied to obtain pathologic confirmation of the presence of cancer prior to instituting therapy. These biopsies carry procedural risks to the patient, societal costs of consumption of limited resources and the potential for delayed diagnosis, which particularly threatens underserved populations and those who face barriers to reliable follow-up. Screening test having low specificity insinuates that many patients are being biopsied, and thus being subjected to these risks, unnecessarily.

Objectives

The primary objective of this study is to develop, optimize and validate a Standard High Resolution Micro-endoscope (HRME) and Line-Scanning Confocal High Resolution Micro-endoscope (LSC-HRME) as a tool to be used in the screening and early diagnosis of superficial bladder cancer.

In this study, we will inspect the bladders of high risk patients with standard of care cystoscopy. Visually suspicious lesions by cystoscopy will be stained with Proflavine and then imaged with sub-cellular resolution using a high resolution imaging sub-system. The lesion will then be biopsied per standard of care for definitive histologic diagnosis. Finally, the high-resolution images will be assessed and compared with the pathology findings in order to develop image analysis algorithms for future diagnostic predictions.

We hypothesize that high resolution epithelial imaging will improve specificity by better discriminating between inflammation and cancer.
Tool Information

**Standard High Resolution Micro endoscope (HRME)**

This is a high-resolution imaging device developed to directly visualize the changes in epithelial morphology of mucosal lesions. Recent advances in *in vivo* confocal microscopy and optical coherence tomography (OCT) have demonstrated the potential of optical imaging to provide clinical images from living subjects of intact tissues with sub-cellular resolution. This High Resolution Micro endoscope (HRME) uses a miniature fiber optical bundle to produce high-resolution images of the tissue it comes into direct contact with. This device produces images of sufficient resolution to investigate nuclear-to-cytoplasmic ratios, cellular organization and architecture, and other important tissue parameters. The opposite end of the fiber bundle is then imaged with a standard microscope objective onto a CCD camera, producing the final high-resolution image of the tissue being interrogated. This system uses inexpensive light emitting diodes (LEDs) as an illumination source.

**Line-Scanning Confocal HRME (LSC-HRME)**

The line-scanning confocal high-resolution microendoscope (LSC-HRME) is an improved, confocal version of the standard HRME. Like the standard HRME, it is a fiber-based fluorescence microscope. It uses the same coherent fiber bundle with a circular field of view of 720 μm. The difference is that the LSC-HRME uses digital light processing (DLP) technology and a rolling shutter configuration to achieve confocal imaging, which improves optical sectioning and image contrast.

A schematic of the line-scanning confocal HRME is shown in Figure 1. Illumination patterns are programmed using a DLP LightCrafter 4500 (Texas Instrument, Dallas, Texas, USA). A 10x objective is used to focus the projected patterns on the proximal end of the fiber. A camera lens is coupled with the objective to relay the fiber image on the camera.

![Schematic of LSC-HRME](image)

During image acquisition, a TTL signal is sent from the DLP to trigger the camera exposure. Upon each trigger signal, a pattern sequence programmed in the DLP software is synchronized to the rolling shutter and scanned across the CMOS sensor. Each pattern in the sequence is aligned with the rolling shutter aperture through fine adjustment to ensure confocal imaging. To capture real time videos, the camera is triggered repeatedly and displayed in real time at a
frame rate of 7 – 8 frames/second. The optical system, as shown in the dashed box in Figure 1, is housed in a portable enclosure approximately 15” x 13” x 6” in size.

Figure 2 shows images of ex vivo bladder tissue taken using the standard HRME and the LSC-HRME. The confocal image demonstrates improved rejection of out-of-focus signal when compared with the standard HRME. The contrast improvement is most significant in the area enclosed by the white box, where the confocal image shows a vessel bifurcation that is not easily seen in the standard HRME. These ex vivo images suggest that the confocal HRME can potentially improve visualization of the nuclei and vascular architecture, facilitate automated algorithm diagnosis and assist in clinical decision-making.

![Figure 2. Ex vivo imaging of bladder tissue using the standard and confocal HRME. The confocal image demonstrates enhanced rejection of background signal and thus improved contrast.](image)

**Contrast Agent to be used**

A contrast agent is needed to adequately visualize the cell structures using high resolution imaging devices. The contrast agent that will be used in this protocol is proflavine. Several types of optical contrast agents have been used to improve detection of dysplastic and cancerous lesions in the gastrointestinal tract and cervix. The most frequently used contrast agents are acetic acid, toluidine blue, acrilavine (a mixture containing proflavine and acrilavine), fluorescein, tetracycline and crystal violet.\(^5^,^7\) The Standard HRME and Line-Scanning Confocal HRME devices works best with a fluorescent contrast agent.

Fluorescent topical contrast agents have been used in conjunction with novel optical instrumentation for imaging of dysplastic and cancerous tissues in luminal organs. Potentially suitable agents in humans are fluorescein, acrilavine, proflavine, quinacrine, or tetracycline. The contrast agent can be applied systemically (fluorescein, tetracycline) or topically (all others) by using a cotton swab or a spraying catheter, in case of endoscopy. In human studies so far, the most commonly used contrast agents have been intravenously delivered fluorescein at a concentration of 10% (colon, esophagus, stomach) and topically applied Acriflavine at a concentration of 0.2% (stomach, and colon).\(^6^,^8^,^9\)

Proflavine, the topical fluorescent dye which will be used in this protocol is a dye in the family of aminoaacidines.\(^10\) Proflavine is the major component of Acriflavine. Acriflavine is a commercially available mixture of proflavine (3,6-diaminoacridine) and its N-methyl quaternary salt, euflavine. Acriflavine hydrochloride has been used topically for a number of in vivo imaging studies and is routinely used in Europe and Australia during endoscopy of the esophagus and colon.\(^6^,^9\) This compound was originally used as a topical antiseptic, and to pack open surgical wounds, but has largely been replaced
now by antibiotics in the post-antibiotic era.\textsuperscript{11} Proflavine is also a component of Triple Dye (a mixture containing brilliant green, Proflavine hemisulfate, and crystal violet) which is still routinely used in the United States as a topical antiseptic to treat umbilical cord stumps in newborn human infants.\textsuperscript{12} Proflavine is unlikely to cause harm to unborn fetuses, but there is no data available on risks to pregnant women.

For imaging purposes, proflavine will be diluted to a concentration (prepared at OP pharmacy) many times lower than what would be used for antiseptic treatment. A typical imaging procedure using proflavine involves the application of a 0.05% solution in buffered saline to the epithelial surface, allowing it to incubate for less than 1 to 2 minutes and imaging can be performed. Experimental data collected on \textit{ex vivo} oral tissue specimens has also shown a very rapid uptake of the dye and limited diffusion. Proflavine is highly desirable as a contrast agents for cancer imaging purposes because of its ability to highlight cell nuclei, while leaving cytoplasmic regions relatively dark. This allows assessment of nuclear architecture, which can be particularly important for grading intraepithelial neoplasia and cancer. Other fluorescence contrast agents lack this ability, or require intravenous administration, which is not practical for cancer screening.

We will use proflavine in its pure form, instead of the acriflavine mixture. Both proflavine and acriflavine have been used clinically as antibacterial agents, and other acridine derivatives have found use as antimalarial (chloroquine) and anticancer (tamoxifen) agents. These and most other biological effects of acridine compounds are attributed to their ability to bind reversibly, but non-covalently to DNA by intercalating between adjacent base pairs. The nature of this interaction results in structural modifications to the DNA, including unwinding and lengthening, which have been shown to affect transcription and replication processes. The capacity for acridine compounds including proflavine and acriflavine to exhibit mutagenic activity has been well established in bacteria, virus, and yeast assays, where frameshift mutations in repetitive DNA regions appear to be the dominant event. In cultured human cells, simple acridines including proflavine have demonstrated clastogenic effects, but do not show a wide range of other mutagenic activities.\textsuperscript{13,14} While it is established that intercalation alone is insufficient to induce mutagenesis, it remains unclear how elements of other processes such as recombination are involved in acridine mutagenesis. As of 2007, no evidence had been reported to demonstrate that simple acridines were carcinogenic in either animals or humans.\textsuperscript{15} In comparison with results established in \textit{in vitro} systems, there is relatively little data on mutagenic and carcinogenic effects of acridines from \textit{in vivo} experiments in whole animals.\textsuperscript{13}

In addition, it has been established in bacteriophage and yeast model systems that acridines can produce distinct and enhanced mutative effects following photoactivation by visible light.\textsuperscript{14,16,17} It is in this capacity that proflavine and several other acridines have been studied for their potential as photodynamic therapy drugs. In a brief report by Regan and Setlow, no effect on the DNA of human cells was observed following incubation with proflavine while isolated from light. However, the combination of proflavine and light exposure resulted in a measured reduction in the average molecular weight of DNA from human skin fibroblasts in culture.\textsuperscript{18} Interestingly, a return to the pre-illumination molecular weight was measured within 2 hours of exposure, highlighting the additional significance of DNA repair following mutagenesis.

Researchers in the genotoxicity field recognize that the results of \textit{in vitro} studies cannot be reliably extrapolated to \textit{in vivo} systems, and the subsequent carcinogenicity of most acridine compounds has not been established at present. The most recent review by the World Health Organization’s International Agency for Research on Cancer (IARC), classified proflavine as a \textit{group 3} carcinogen, meaning that “The agent is not classifiable as to its carcinogenicity to humans”.\textsuperscript{19} Based on the data available, the IARC summarized that “Proflavine is mutagenic in viral and bacterial systems. It increased
the number of chromatid breaks and induced sister chromatid exchanges in mammalian cells. The available experimental results were inadequate for an evaluation of the carcinogenicity of proflavine in experimental animals, and no data were available from human studies.”

Against this background, it is clearly prudent to use acridine compounds including proflavine with caution. However, demonstrated mutagenesis is not typically considered sufficient reason to avoid exposure to a particular compound. Caffeine can be either mutagenic or anti-mutagenic depending on the dose. In Europe, Asia, and Australia, acriflavine has been used for several years as a contrast agent in investigational gastrointestinal imaging studies, similar to the imaging procedures to be performed in this protocol. The research team leading those studies acknowledged that “the use of Acriflavine should be carefully considered because of the little but residual risk of possible mutagenic activity”. In similar fashion, compounds including methylene blue, crystal violet, and toluidine blue are routinely used to enhance visual contrast between normal and neoplastic tissues in the gastrointestinal tract and oral cavity, despite recognition that these agents interact with DNA and uncertainty existing over possible carcinogenicity. In vitro assays have been reported in which photoactivated methylene blue was more than twice as mutagenic as proflavine under identical conditions. Methylene blue is commonly used in the urinary tract, instilled intravesically or intravenously as an intraoperative tool. The mutagenic effect of toluidine blue was also confirmed in vitro. In neonatal care, Triple dye, a combination of brilliant green, proflavine hemisulfate, and gentian violet is routinely used as a topical antibacterial agent on the umbilical stump of newborn babies, with a recent review of the practice categorizing toxicity as rare.

Extrapolation of data from in vitro assays and the limited studies in in vivo mammalian systems to human subjects is clearly difficult and subject to different interpretation. However, quantitative comparisons with reported clinical usage of the type outlined above can provide us with guidance. We propose the use of proflavine solution at 0.01% (w/v), a concentration which is an order-of-magnitude lower than that of the proflavine component in commercial triple dye, 0.11% (w/v) [Kerr Triple Dye, VistaPharm]. Investigational in vivo human studies of confocal microscopy for gastrointestinal cancer currently use topical acriflavine at 0.05% concentration. The additional exposure to light which will occur during imaging can also be compared to that received by newborn babies undergoing phototherapy for jaundice. The high-resolution fiber-optic microendoscope to be used in this protocol delivers 0.5 mW of 455 nm light to the tissue through a 0.8 mm diameter fiber-optic bundle, corresponding to an irradiance level of 100 mW/cm2. The American Academy of Pediatrics defines intensive phototherapy as a spectral irradiance of at least 30 W/cm2 per nanometer over the 430-490 nm spectral band, equivalent to a total irradiance of 1.8 mW/cm2. Although the irradiance level is over 50-times higher with the fiber microendoscope system, a typical imaging session of 30 minutes (including imaging for routine care) is approximately 50-times shorter than a typical 24 hour (1440 minutes) phototherapy incubation, leading to an equivalent light dose in each scenario.

Proflavine, the investigational dye, for this study will be purchased in powder form from Sigma-Aldrich Chemicals by Dr. Kortum’s fund. The liquid dye will be prepared in the OR Pharmacy. In this protocol, dilute proflavine, at a concentration of 0.01% (w/v) will be used as a contrast agent to stain the bladder tissue prior to imaging with the high resolution micro endoscope. Proflavine reconstitution instructions are as follows:

1) Prepare a solution of 0.01% (w/v) by dissolving 100 mg of proflavine in 1000 mL of sterile water.
2) Heat the solution to approximately 40 degrees C. Shake or vortex the solution until the proflavine is completely dissolved.
3) The solution should be stored in a light proof vial at 4 degrees C and protected from light (i.e., solution is stable for up to 14 days).

At the time of cystoscopy, when the surgeon is ready for HRME imaging, the bladder will be drained of irrigant through the cystoscope. A 60cc Leur-Lock syringe will then be used to inflate the bladder under direct vision with a total of approximately 240-300cc of the proflavine solution depending on anatomic variations in bladder capacity. Stability testing of proflavine has been performed demonstrating that this formulation is stable at room temperature and when refrigerated for at least 3 months.

**Phase of the study**

Pilot study

**Study Population**

This study will enroll 100 subjects aged 18 years and older who have a suspicious bladder lesion or clinical presentation identified by a physician who recommends further evaluation with cystoscopy and bladder biopsy or who are undergoing cystoscopy as part of their routine clinical care.

**Eligibility**

A. **Inclusion Criteria**
   All participants in this study will be chosen independent of age, sex, and ethnic background meeting following criteria:
   1. Diagnosed with clinically suspicious bladder lesion or clinical finding; or who are undergoing cystoscopy as part of their routine clinical care
   2. Age 18 years and older
   3. Must be willing and able to participate and provide written informed consent
   4. Women of childbearing age who have the possibility of being pregnant must have a negative pregnancy test prior to participation

B. **Exclusion Criteria**

A patient will be excluded if:
1. Patient with sufficient evidence of cognitive impairment that limits the subject's ability to understand the protocol, provide informed consent, or to comply with the protocol procedures.
2. Women with the possibility of having the pregnancy.
3. Patients having acute infection.
4. Person with Lidocaine sensitivity.

**Study design**

This is a cross-sectional, non-interventional study that will collect high resolution images of normal bladder tissue and suspicious bladder lesions from 100 patients who present to a study site for clinical evaluation. Subjects will be consented and enrolled in the pre-procedural outpatient clinic
appointment or the pre-procedural holding area prior to the administration of any anesthetic medication.

**Duration of Study**

The study will run for 4-years.

**Duration of Subject Participation**

A subject’s participation will typically be limited to the duration of the surgical procedure planned for cystoscopy and/or bladder biopsy. After informed consent and after cystoscopy procedure which is a usual standard of care, participants will undergo a 10-minute imaging session. At the end of the procedure, the participant’s involvement in the study from the procedural perspective will end.

It is at the physician’s discretion to repeat cystoscopy or biopsy based upon the patient’s clinical status. For each repeat procedure, the patient will be consented again for participation and will be considered as a “New patient” in the study. But from data collection and analysis aspect, the participant will be included in the study for study duration and participant’s imaging and tissue sample data will be documented as follow-up visits.

**Subject Enrollment**

Potential subjects will be identified from patients who are visiting LBJ Houston for evaluation of bladder cancer or a suspicious bladder lesion/clinical symptomatology or from patients who are undergoing cystoscopy as part of their routine clinical care. Clinicians and staff at the clinics will be made aware of the study and trained on the enrollment and study procedures. Research personnel (referred to as study coordinator from this point forward) will be available at LBJ during enrollment. The study coordinator will review appointment schedules and information available at the time of patient appointments to identify potential subjects and clinician will assess their eligibility to participate in the study. The clinician will then ask for patient’s participation and after patient’s interest the study coordinator will apprise the patient in detail about the study. The study coordinator will inform patient that non-participation will not influence their clinical management. The study coordinator will then calls for patient’s participation and administer the informed consent contingent upon patient’s willingness. After having informed consent, clinician will reassess patient’s eligibility for the study after consent and will make changes if necessary.

The screening for eligibility by clinician does not require any testing. Before initiating any study procedures, signed informed consent will then again be taken. All procedures will be done as a part of standard of care guidelines.

An enrolled subject in this study will be assigned a “Unique study identification number”. The unique study ID will be used in the future for all study data related procedures.

A subject code log that associates names to unique study identification numbers will be maintained by the research staff at UT Medical School, Department of Urology, Houston. Only De-identified data, with password protection will be shared with Rice University for image analysis for the research purposes of this study.
Study Procedures

All the study procedures require one visit of the patient.

I. The clinician will examine the bladder with standard cystoscopy, and indicate any abnormal areas on the bladder examination data sheet (attached) and rate each area as (1) normal, (2) abnormal-not suspicious for cancer (low-risk), (3) abnormal-suspicious for cancer (high-risk). (Appendix A)

II. As a part of standard care of procedure, biopsy will be taken after cystoscopy procedure will be done.

III. After biopsy procedure, bladder will be inflated with proflavine solution prepared at OP pharmacy to stain the inner wall of bladder.

IV. Stained area will be then analyzed with the HRME and/or LSC-HRME by placing the HRME probe for approximately 20 seconds. High-resolution images will be collected, by placing the HRME probe in light contact with the epithelial surface at those locations appearing abnormal under cystoscopy. The LSC-HRME will use digital light processing (DLP) technology and a rolling shutter configuration to achieve confocal imaging. The probe will undergo high-level disinfection by Cidex Plus before and after each patient measurement.

V. Once the imaging is complete, the bladder will be drained off the proflavine solution, and then re-inflated with sterile water or saline as per standard surgical procedure.

VI. The biopsied tissue will then be sent to pathology for analysis, once again as standard clinical care.

VII. The research coordinator will follow up by telephone with the participant 2-5 days after the procedure to monitor symptoms of infection or bleeding or any other adverse events from the procedure.

VIII. Images obtained will be compared against the pathology results of the biopsied tissue taken from the same region. For the high-resolution images obtained with the HRME, morphological features such as nuclear diameter, nuclear density, and nuclear-to-cytoplasmic ratio, and fluorescence intensity will be evaluated. Based on these features, classification algorithms will be developed to separate imaged tissue into 2 diagnostic categories: normal non-neoplastic mucosa vs. dysplasia and cancer. Algorithm performance will be optimized relative to the gold standard of histopathology diagnosis obtained from the biopsies.

Sample size

Even though it is a pilot study, we have chosen a sample size of 100 patients which we feel will provide us the sufficient number to develop an image algorithm for comparing normal bladder tissue with cancerous bladder lesions bladder cancer detection.

Samples Concerns

Bladder tissue will be collected as a part of standard of care at the time of the cystoscopy and HRME and/or LSC-HRME will be used to capture images to determine early detection of cancerous lesions with more sensitivity. The image samples will be coded. Image analysis will be conducted in Dr. Kortum's
laboratory located at Rice University, Houston. No specimens will be stored for this study. After the image analysis results, tests for this protocol is completed, the imaging data will be stored at Dr. Kortum’s laboratory. Research related procedures and data collected during the screening will be handled as per The University of Texas health science center, Houston guidelines. Password protected document showing relationship to unique study ID’s and personal information will be kept under Principal Investigator at Department of Urology, University of Texas Medical School, Houston. Due to the preliminary and qualitative nature of this study, and the low-risk to participating subjects, we will be using a convenient sample of 100 subjects. Based on our previous experiences with the same device, an exploratory study of 100 patients is proposed in order to obtain some preliminary data regarding the appearance of normal tissue, inflamed tissue, benign lesions and malignant bladder lesions when illuminated by the HRME and/or LSC-HRME system.

Retention and Dropout withdrawal

Given the very short follow up, minimal dropout or loss to follow up is expected in this cohort among patients who clinically require follow-up.
If patient chose to drop-out, he will be replaced to attain a required sample size. For purpose of tracking the feasibility of conducting a trial with HRME, reasons for drop out or withdrawal will be documented.

Data Analysis and End-Point/Statistical Considerations

The purpose of this study is to explore the potential of the HRME system as a tool for distinguishing normal from abnormal bladder tissues and to study the optical properties of benign inflammatory and potentially malignant lesions of the bladder mucosa. Due to the preliminary and qualitative nature of this study, and the low-risk to participating subjects, we will be using a convenient sample of 100 subjects. This number was derived based on our clinical experience and preliminary testing of the HRME system in oral cavity studies performed at Rice, UT and MDACC (IRB number – HSC – DB-10-0708). In keeping with the strict exploratory study design on the effectiveness of this new instrumentation, we are not anticipating testing for statistical significance. As such, no sample size calculation is needed at this time. An exploratory study of 100 patients is proposed in order to obtain some preliminary data regarding the appearance of inflamed tissue, benign lesions and malignant bladder lesions when illuminated by the HRME system. Should our initial results look promising, this data will be used to calculate an appropriate sample size for a larger clinical trial.

Risks

The procedures for this study should not expose subjects to risks that are any greater than the risks encountered during routine cystoscopic examinations and bladder biopsies. Cystoscopy is considered a very safe and routine procedure. But some complications include urethera swelling, mild infection etc. As with any bladder procedure such as cystoscopy, bladder perforation is a potential risk of the imaging procedure, and will be monitored clinically during the procedure. However, the force and pressure exerted during imaging of the bladder is expected to be far less than that involved with bladder biopsy and tumor resection, and therefore excess risk of bladder perforation is not anticipated. Thus, safety monitoring will only focus on unanticipated problems and serious adverse events related to study participation that jeopardize the safety or rights of subjects. The Principal Investigators for this protocol will be responsible for accurate implementation of the protocol. Care will be taken to avoid these complications. Any clinical deviations from the protocol will be reported to Dr. Nadeem Dhanani, who
will determine the appropriate course of action. Any instrumental deviations from this protocol will be reported to Dr. Rebecca Richards-Kortum, who will determine the appropriate course of action.

**Loss of Confidentiality**

The loss of confidentiality regarding research information is a possibility; although, the risk is very small.

**Benefits**

A. Subject: Future subjects seeking screening of Bladder cancer, HRME as a diagnostic tool will serve as less invasive technique for early and accurate detection of bladder cancer. It will benefit the subject economically and psychologically from the procedural complications.

B. Society: The predominant benefit of the study is to the society because it will provide insight about the diagnosis of bladder cancer at a very early stage. This tool will increase sensitivity and specificity for early bladder cancer detection. Hence, it will dramatically lower the rate of biopsies conducted.

C. Risk to benefit ratio: The risks are minimal and mitigated in this protocol. The benefit of offering minimally invasive techniques for bladder cancer far exceeds the risk.

**Costs to subjects**

Patients will not be responsible for any costs associated with Proflavin and HRME use. Consulting and screening procedure will be done as a part of standard of care guidelines.

**Payment to subjects**

Patients will not be paid for participating in this study.

**Unanticipated Problems**

The Office for Human Research Protections (OHRP) considers unanticipated problems to be any incident, experience, or outcome that meets all of the following criteria:

1. Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the IRB-approved research protocol and informed consent; and (b) the characteristics of the subject population being studied;

2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

3. The research places subjects or others at a greater risk of physical, psychological, economic, or social harm than was previously known or recognized.

An incident, experience, or outcome that meets the three criteria above generally will warrant consideration of substantive changes in order to protect the safety, welfare, or rights of subjects or others. Examples of corrective actions or substantive changes that might need to be considered in response to an unanticipated problem include:

1. Changes to the research protocol initiated by the investigator prior to obtaining IRB approval to eliminate apparent immediate hazards to subjects.
2. Modification of inclusion or exclusion criteria to mitigate the newly identified risks
3. Implementation of additional procedures for monitoring subjects
4. Suspension of enrollment of new subjects
5. Suspension of research procedures in currently enrolled subjects
6. Modification of informed consent documents to include a description of newly recognized risks
7. Provision of additional information about newly recognized risks to previously enrolled subjects.

Adverse Event Reporting

Adverse events will be reported in accordance to all applicable ICH, IRB guidelines, rules & regulations.

Serious Adverse Events
Some, but not all, unanticipated problems may result in Serious Adverse Events (SAE). An SAE will be defined as an unanticipated problem that results in any of the following outcomes:
1. Death while on protocol.
2. Life threatening event (subject at immediate risk of death at the time of the event)
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant disability or incapacity
5. Medically important event that may jeopardize the subject or may require interventions to prevent one of the outcomes above.

Reporting Procedures

Clinical Investigators are responsible for detecting, documenting, and reporting unanticipated problems and resulting SAEs that occur between the time of enrollment and study completion or termination. Unanticipated problems and serious adverse events directly related to study participation must be reported to the IRBs according to local guidelines, to the laboratory site, and other clinical sites.

Unanticipated Problems as Serious Adverse Events

Any unanticipated problem that is also an SAE and is directly related to study participation must be reported to the investigators at all sites. All SAEs will be reported by phone, fax, or email. Deaths and life-threatening events must be reported within 24 hours of the time the clinical site becomes aware of the event. All other events must be reported within 72 hours. Other supporting documentation for the event may be requested by the local IRBs and should be provided as soon as possible.

Other Unanticipated Problems

All unanticipated problems must be reported and documented within 72 hours, regardless of their severity. Reporting of unanticipated problems will include the following information:
1. A detailed description of problem
2. Reason(s) why the event being reported qualifies as an unanticipated problem
3. A description of actions that have been taken or are proposed in response to the unanticipated problem

Follow-up of Subjects after Unanticipated Problems
All subjects who experience unanticipated problems will be evaluated by a study clinician and followed until the event resolves or stabilizes, regardless of whether the unanticipated problem resulted in an SAE. The duration of follow-up may differ by event.

**Halting Rules**

The study will be temporarily halted for any of the following reasons:
1. An unanticipated problem that is fatal or life-threatening
2. Two or more serious adverse events resulting from unanticipated problems that are not fatal or life-threatening

The clinical investigators will be responsible for reporting data related to halting criteria to the Principal Investigator at Rice University within 1 business day. When a halting rule is confirmed, enrollment will be stopped immediately, and the local IRBs will be informed within 1 business day. The study may be allowed to continue, to continue with modification, or it may be terminated by the local IRB or the Principal Investigator.

**Data Safety and Monitoring**

This study requires taking several images of bladder mucosa which take approximately ten minutes or less. No specific tests or evaluations are required as part of this study, other than the imaging procedure described above. The subjects will undergo routine evaluation in the immediate post-operative period according to standard of care for their pre-cancer or cancer. Research personnel will review the subject's clinical chart to document the patient's status at the first post-operative evaluation and at approximately 6 months after surgery. Subjects will be considered off-study after completion of the imaging evaluation during surgery. The Principal Investigator will be responsible for data safety and monitoring.

**Clinical Monitoring**

The purpose of clinical monitoring is to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and ICH Good Clinical Practice guidelines, and that the integrity of study data is maintained. Yearly reports will be sent to each IRB, updating the board on subject enrollment and protocol deviations. SAE’s and unanticipated problems will be reported within 72 hours of occurrence.

The clinical principal investigator performing the procedure will monitor the participants for any immediate adverse events. If any event is noted during the procedure this event will be treated with standard of care therapy. Both PI’s will be informed within 24 hours of the event. If any sensitivity is detected at the follow up phone call by study coordinator, both PI’s will be informed within 24 hours. The PI’s will inform CPHS of any sensitivity meeting SAE criteria within 24 hours of becoming aware of the event.

**Data Handling and Record keeping**
The study coordinator will gather research data. No personally identifiable information will be entered in research records. Subjects will be identified only by their unique study identification number in research records. Subject code log sheet will be kept under direct supervision of Principal investigator.

A Data collection Form
Protocol-specific data will be collected on Data collection form. The completed dataset should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from UT CPHS.

B Record Retention
To enable evaluations and/or audits from Health Authorities/UT CPHS, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all Data collection forms, and detailed records of procedure. To comply with international regulations, the records should be retained by the investigator in compliance with regulations. After telephonic follow up, the Research Coordinator will document the occurrence of any unexpected adverse events, or expected adverse events with higher than expected severity, and their resolution on a detailed log in the regulatory binder.

Ethics & Good Clinical Practices

The investigators will ensure that this study is conducted in full conformity with the principles set forth in the Belmont Report, “Ethical Principles and Guidelines for the Protection of Human Subjects of Research,” as drafted by the U.S. National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and the ICH E6.

Institutional Review Boards

The following institutional review boards (IRBs) will have oversight of this study:
  Rice University IRB
  University of Texas Health Science Center at Houston IRB
  Harris Health IRB
The clinical principal investigator at each site will obtain IRB approval of the protocol prior to the enrollment of any subjects. Any amendments to the protocol will be approved by all the IRBs before they are placed into use. Continuing IRB Review applications will be submitted according to the schedules and requirements of each IRB.

Informed Consent

Subjects will be identified from those visiting in the Department of Urology-Surgery at LBJ hospital, Houston. The investigator or his designated personnel must explain to each subject the objectives, nature, procedures, duration, potential risk and benefits of the study. Each subject must be informed that participation is voluntary, patient can withdraw from study at any time and that withdrawal of consent will not affect his subsequent relationship with the physician. Signed informed consents will be obtained from the subjects prior to the start of any study procedures. The consent process will be documented in the subjects’ medical and research records.
Documentation of the consent process will include the following elements:

1. Date and time of consent;
2. Topics discussed with the subject (e.g. risk, benefits, etc.)
3. Confirmation that the consent was reviewed, that the subject’s questions were answered, and that a signed copy of the consent was provided to the subject.

For non-English speaking subjects, all the procedures required for Informed consent and documentation will follow guidance provided by the Office of Human Research Protections (Department of Health and Human Services) and local IRBs. The consent form will be updated or revised whenever important new safety information is available, whenever the protocol is amended, or whenever any new information becomes available that may affect participation in the study.

**Subject Confidentiality**

Subjects’ confidentiality will be protected as far as permitted by law. Personally identifiable information will only be present in the subject’s medical records, the consent forms, and the subject code log that links subject names to study identification numbers. Only clinicians and the study coordinator at the subject’s clinical site will have access to those records. Study monitors may also periodically have access to identifiable information during monitoring visits. These visits will occur on the site and any identifiable information will not leave the clinical site. All efforts will be made to ensure patient confidentiality and assurance of HIPAA compliance. Immediately after obtaining any specimens and HRME images, subjects will be assigned a unique protocol-specific code that will be used for all further data management. All other study materials, such as research documents, study database, and research laboratory results and records, will contain study identification numbers rather than personally identifiable information. The names of participants will not be released to any outside organizations or to persons not involved with the investigation. Participant names will not be revealed in written reports or publications detailing the research findings.

**Quality Control and Assurance**

The clinical principal investigators at their respective clinical sites will be responsible for monitoring the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all study data. The clinical principal investigators are required to keep accurate records to ensure that the conduct of the study is fully documented. A data quality assurance program will be established and will consist of the following elements:

1. Verification of research data entries against source documentation, such as medical records, and pathology reports, during periodic clinical monitoring visits; and
2. Periodic study database review and reports.

Decision of the clinical Investigator will prevail in case of any clinical and procedural discrepancy in the data and decision of the technical PI will prevail in case of any technical issues with HRME.

Participating subjects will be notified upon the completion of the study.

**Amendments in the protocol**

If it is necessary to amend the protocol, the proposed amendment will be submitted to UT- CPHS, Rice IRB, Harris health IRB for approval. Amendments in the protocol are subject to be in effect only after the approval from all IRB’s. The principal investigators are responsible for distribution of the approved documents to the staff.
Publications

Principal investigator holds the right to publish the research results. Only results not identifying patients will be published to maintain high research ethics.

APPENDIX – A

Figure 1: High Resolution Micro-endoscope (HRME). Schematic of fiber bundle microscope system (left) and illustration of relative size of imaging probe (right).

Figure 2: Working of endoscopic probe in Panel A and illuminations of tissues with the use of HRME technique for early cancer detection.
Figure 3: HRME in a travel case with top removed.

Figure 4: Powered on HRME and attached Optical fiber
Figure 1. Schematic of the line-scanning confocal HRME platform. Solid arrows show directions of scanning on the DLP, CMOS and fiber surface; dashed arrows show the data flow among the laptop, DLP and camera. The dashed box indicates the optical system enclosure.

Figure 2. Ex vivo imaging of bladder tissue using the standard and confocal HRME. The confocal image demonstrates enhanced rejection of background signal and thus improved contrast.
References

17. Iwamoto Y, Mifuchi I, Yielding LW. Photodynamic mutagenic action of acridine compounds on yeast Saccharomyces cerevisiae. Mutat Res 1985; 158;169-175.