Determining the feasibility of Cerenkov Luminescence Imaging in surgical oncology

Determining the Feasibility of Cerenkov Luminescence Imaging in Surgical Oncology

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PREFACE

Ever since my first understanding of cancer I am intrigued by the once unspeakable disease that now has one of the highest impact on death rates in the western countries. Cancer is a disease that has been studied for so many years in which a lot of knowledge about the mechanisms of cancer is gathered. However, still we are not fully capable to control the disease let alone to diminish its impact. This has triggered me to delve more into the world of oncology and that is also the reason why I landed in the NKI-AvL during my second clinical internship. This experience, and the present I received at the end of the internship, a wonderful book called 'The emperor of all maladies', made me decide to stay involved with oncology.

For my graduation internship I decided to go back to the NKI-AvL because Professor Ruers had a challenging and ambitious research project that I was able to cooperate in. The scope of this project was to see if Cerenkov luminescence imaging could be used to image tumors during surgery. This project was brought to the NKI with the arrival of Dr. Jeffrey Steinberg who experienced working with Cerenkov imaging at his previous job in Singapore. Due to some delay in his own activities in the animal facility he, together with Professor Ruers, suggested this new research project.

During the past year I have worked with pleasure on this project and my clinical activities in the NKI-AvL. As a rookie in the hospital a lot of help and supervision was available to guide me through this internship and enabled me to deliver this master thesis. Therefor a special word of thanks goes out to Jeffrey Steinberg. He was the enthusiastic driving force behind this project but also my closest supervisor and collaborator.

I would also like to thank the other members that were involved in this project. First of all Theo Ruers from whom I learned that research has to be purposeful, implementable but above all clinical relevant. Next to that I would like to thank Dick Sterenborg whose knowledge and critical view were essential for the results of this project. I also am very thankful for the involvement of Linda de Wit van der Veen. She was always kindly available to help me with all my practical and clinical problems even though she was busy enough for herself. Furthermore I want to thank Linda Janssen Pinkse for her work and cooperation in this project.

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I especially want to express my gratitude to all the patients who were willing to cooperate with the different experiments I have performed. Despite their sickness they showed there patience and willingness to help out a student and his research project.

Another thanks goes out to my friends at 'the beach' who employed me throughout my study making sure I didn' t have to live in poverty and I could pay for all the necessary expenses.

At last, but not least, I would like to thank my mom and dad for lovingly taking me in during my internships and giving me all the space resources to complete my study.

Robert Poel

ABSTRACT

Background: Surgical oncology is still one of the main treatments for curing cancer. For a successful surgery however, a clean and radical resection is required. This can only be obtained if there is precise knowledge on were the tumor is situated and what parts of the tissue are malignant and which are not. To localize a tumor a lot of techniques are available. Peroperative determination if a tissue is malignant or benign is still a challenge. To be able to do this a technique is required that is fast, implementable in the OR and foremost is tumor specific. Cerenkov Luminescence Imaging (CLI) combines optical imaging with tumor specific PET tracers such as ¹⁸F-FDG. Within biological tissue these tracers send out a very faint luminescence which can be detected with highly sensitive EMCCD cameras. This will make it a promising technique that might be able to visualize tumors during surgery.

Purpose: In this study we are trying to determine if CLI is a feasible technique for peroperative tumor visualization.

Methods: Different in vitro studies are performed to determine the characteristics of Cerenkov luminescence and to predict the sensitivity of CLI. Different cameras have been tested to be able to come up with the best CLI setup with the highest sensitivity. The technique was tested in 5 subjects who had a superficial tumor and subsequently had undergone an 18F-FDG PET/CT scan. Images with the CLI setup, containing an EMCCD camera, where made 4 cm from the skin where the tumor was situated. Thereafter the obtained images were processed to see if the Cerenkov light from the tumor was detected.

Results: We were able to visualize an activity of 3.5 kBq/ml in vivo. On average a tumor takes up around 10 kBq/ml. However, the biological tissue absorbs the majority of the light. We were not able to detect any light coming from the ¹⁸F-FDG during the in vivo studies.

Conclusion: Image guided surgery based on Cerenkov Imaging is theoretically very promising. However, the reality is that the intensities of the signal are too weak to use for in vivo imaging during surgery.

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LIST OF ABBREVIATIONS

| μSv | microsievert |
|------------------|---|
| ¹¹ C | carbon-11 |
| 124 | iodine-124 |
| 131 | Iodine-131 |
| ¹⁸ F | fluorine-18 |
| ³² P | phosporus-32 |
| ⁶⁸ Ga | gallium-68 |
| ⁸⁹ Zr | zirconium-89 |
| 90Y | yttrium-90 |
| ALARA | as low as reasonably achievable |
| AvL | Antoni van Leeuwenhoek |
| Bq | becquerel |
| сс | cubic centimeter |
| CCD | charge-coupled device |
| CL | Cerenkov luminescence |
| CLI | Cerenkov luminescence imaging |
| CR | Cerenkov radiation |
| СТ | computed tomography |
| CTE | charge transfer efficiency |
| DFO | desferrioxamine |
| ECLI | endoscopic Cerenkov luminescence imaging |
| EGF | epidermal growth factor |
| EMA | European medicines agency |
| EMCCD | electron multiplying charge-coupled device |
| FDA | food and drug administration |
| FDG | fluorodeoxyglucose |
| FITC | fluorescein isothiocyanate |
| GBq | Giga becquerel |
| ICCD | intensified charge-coupled device |
| ICG | indo cyanine green |
| ICRP | international commission on radiological protection |
| | |

| IVIS | in vitro imaging system |
|-------|--|
| kBq | kilobecquerel |
| keV | kiloelectronvolt |
| kg | kilogram |
| LED | light emitting diode |
| MBq | megabecquerel |
| MIPAV | medical imaging processing, analysis and visualisation |
| mL | millilitre |
| mm | millimetre |
| MOS | metal oxide semiconductor |
| MRI | magnetic resonance imaging |
| msV | milisievert |
| NIR | near infrared |
| NKI | Nederlands kanker instituut (Netherlands cancer institute) |
| nm | nanometre |
| NPS | nanophotosensitizers |
| OR | operating room |
| PACS | picture archiving and communication system |
| PET | positron emission tomography |
| PSM | positive surgical margins |
| PSMA | prostate-specific membrane antigen |
| QE | quantum efficiency |
| RALP | robot-assisted laparoscopic prostatectomy |
| ROI | region of interest |
| SD | standard deviation |
| SNR | signal to noise ratio |
| SUV | standard uptake value |
| sV | sievert |
| VEGF | vascular endothelial growth factor |

1 INTRODUCTION

Begin July the central bureau of statistics published their data on mortality and its causes over 2014. This was picked up by most media with the main message "Cancer still the number one cause of death in the Netherlands". Ever since 2007 cancer has the dubious honour of topping the mortality list in the Netherlands. The mortality rates are increasing every year while the mortality rates of heart attacks are declining since the seventies. This does not mean that oncology lacks the development which is present in cardiology as expressed by the lower mortality rates. The main reason is the ever growing life expectancy which causes an inevitable growth in developing cancer. This causes the absolute number of cancer mortality to increase but the numbers of people that are cured shows an even higher increase. This trend of growing number of cancer rates will last for a couple of decades until it will be surpassed by dementia.

Oncologic surgery was one of the first effective treatments of cancer and was performed already centuries ago. Today a diversity of treatments is available from which most of them are the most effective when they are applied in a multi-modal fashion. However, surgery is still the obvious choice of treatment for solid solitary malignant tumours. Despite the fact that surgery is a risky business it offers patients good prospects for long term survival. Still today the majority of cancer cures are accomplished with surgery.

1.1 RATIONALE FOR THIS STUDY

For oncologic surgery with curative intentions the most important outcome is a clean resection to make sure there will be no recurrent disease. To make sure all malignant tissue will be removed the surgeon has to know exactly where the malignant tissue is situated. This can be achieved by different types of imaging that take place prior to the surgery. During the procedure the surgeon has to navigate to the tumour based on his anatomical knowledge. In this, he can be assisted by radio guidance, by placing a radioactive seed in the tumour, or by surgical navigation. However, when the surgeon has reached the site of the tumour he must determine what is malignant tissue and what is not. From now on the surgeon mainly relies on his visual and tactile feedback. This is a difficult task since a lot of tumours cannot be visually distinguished from the surgeon will benefit from a technique that makes it possible to distinguish malignant from benign tissue.

Techniques that are currently used for tumour assessment such as CT (Computed Tomography), MRI (Magnetic Resonance Imaging), Ultrasound and PET (Positron Emitted Tomography) are not suited for intraoperative use because they are either too bulky for the OR, too slow, not specific enough or lack the resolution that is required. The ideal technique will be one that can visualize the tumour in real time with a high specificity and a high resolution. Optical imaging seems the most suited for this job.

However, the difficulty is to specifically distinguish malignant tissue. For many years they are trying to develop fluorescence and bioluminescence agents that are able to highlight malignant tissue. Up to now, hardly any of these have found their way into clinical practice.

Recently Robertson et al. discovered that that it is possible to image Cerenkov light derived from radioactive decay in biological tissues[1]. This Cerenkov light arises when charged particles exceed the speed of light in a biological tissue. Clinically used PET radiotracers such as 18F produce these charged particles. This led to a new technique called Cerenkov luminescence imaging (CLI) that can possibly be used for several biomedical applications. The advantage over fluorescent and bioluminescent agents is that this new technique provides clinically used and widespread available tumour specific tracers. In this thesis we try to answer the question whether CLI is a feasible technique for intraoperative assistance during oncological surgery.

1.2 RESEARCH QUESTIONS AND SUBQUESTIONS

Based on the rationale in section 1.1 the main research question for this study can be formulated as:

Is it clinically feasibile to develop a novel image guided surgery application based on Cerenkov Luminescenc imaging in oncological surgery?

To perform a structuered investigation and to be able to provide a solid statement on behalf of this question, a series of subquestions have been formulated that should be answered throughout the study:

- What are the possibilities and limatations of CLI?
- What are the requirements for an intraoperative CLI application?
- How sensitive should a CLI system be for in vivo human applications?
- What is the most suitable camera for CLI?
- Are we able to image Cerenkov luminescence from an in vivo tumor in humans with radioactive uptake?
- Are there other possible applications of CLI in surgical oncology?

1.3 PLAN OF APPROACH

On the department of surgery, by the knowledge of Jeffrey Steinberg, it was known that Cerenkov Luminescence imaging could be performed using a highly sensitive camera. Money was made available so that the department could buy such a camera to start up a new route towards improving surgery. However, the opinions in different departments were divided on the feasibility of a clinical valuable application. It was our task to investigate the feasibility of CLI and its possible applications in oncological surgery prior to the purchase of a camera. To proof that CLI is feasible we wanted to image Cerenkov radiation from a radiotracer taken up by an actual tumor in a human being in vivo. In order to do so we have divided this investigation into a number of consecutive steps:

- The first step was to study the available literature. We did this to learn about the possibilities and the limitations of CLI were. We wanted to know how sensitive such a system could be and if this type of imaging was already performed in humans. Furthermore we wanted to know for what possible applications CLI could be used. To do this we needed information on the characteristics of the Cerenkov phenomenon. At last we wanted to know how others performed their studies with CLI so that we were able to reproduce these or could come up with the best method to perform CLI.
- The next step was to determine what the requirements were a CLI application for oncological system has to fulfill. We had to determine which radiotracers we could use. We should determine how much signal could obtained from a tumor on how much activity we could administer and what the consequences were for radiation safety. Next to that we had to resolve where the surgeon wants to use CLI and then what ahis requirements are for such an application.
- The third step was to determine what camera we could use. What are the options and which one is the most sensitive and the most suitable for this application. To proof that CLI is feasible we wanted to have the best suitable camera available. To determine which camera that is, we wanted to test them for ourselves and compare them with each other.
- Step four was to determine what the possibilities of the most suitable camera are. Are we able to detect Cerenkov luminescence in an own build setup in vitro. We want to determine what the least detectable activity is in an optimal setting and how long the exposure time has to be. Furthermore, we wanted to test the difference of different isotopes and we wanted to test the attenuation by tissue.
- Based on the first four steps we would be able to give a well-reasoned assumption on whether we are able to detect Cerenkov radiation due to radiotracer uptake in an actual tumor. To proof the theory we set up a pilot study in which we want to image tumors in vivo of patients who are administered with a radioactive tracer. The result of this pilot study should give an answer on whether CLI is feasible in a clinical application

2 CERENKOV RADIATION

2.1 DISCOVERY

Cerenkov luminescence or radiation is light generated when charged particles travel through a dielectric medium with a velocity exceeding the phase velocity of light in that particular medium. These charged particles can either be positrons, electrons or α particles and are often derived from radioactive decay. When this occurs in a dielectric medium such as water or biological tissues a faint bluish light arises along the path of the charged particle. This phenomenon can be observed as blue glow in the water coolers of nuclear reactors (Fig 1).



Figure 1

The typical blue glow, known as Cerenkov luminescence that can be seen with the naked eye in nuclear power plants.

The phenomenon of the blue light caused by the Cerenkov luminescence was first noticed by Pierre and Marie Curie shortly after their discovery of radioactivity [2]. In the biography of Marie Curie they speak of a bluish glow from glass vessels containing radium, that could be seen in the dark [3].(Fig. 2) This is undoubtedly the Cerenkov radiation.

"Don't light the lamps!" Marie said in the darkness. Then she added with a little laugh:

"Do you remember the day when you said to me: 'I should like radium to have a beautiful colour'?"

The reality was more entrancing than the simple wish of long ago. Radium had something better than "a beautiful colour"; it was spontaneously luminous. And in the sombre shed, where, in the absence of cupboards, the precious particles in their tiny glass receivers were placed on tables or on shelves nailed to the wall, their phosphorescent bluish outlines gleamed, suspended in the night.

"Look . . . Look!" the young woman murmured.

She went forward cautiously, looked for and found a strawbottomed chair. She sat down in the darkness and silence. Their two faces turned toward the pale glimmering, the mysterious sources of radiation, toward radium—their radium. Her body leaning forward, her head eager, Marie took up again the attitude which had been hers an hour earlier at the bedside of her sleeping child.

Her companion's hand lightly touched her hair.

She was to remember for ever this evening of glow-worms, this magic.

Figure 2

Fragment from "Madame Curie" - by Eve Curie – translated by Vincent Sheehan. This is probably the first description of visible Cerenkov radiation [3].

After the Curie's, other scientists also encountered Cerenkov luminescence. Mallet, in 1926 even made photographs of the spectrum and found out that there was a difference with fluorescence but could not find an explanation [4]. The phenomenon of the Cerenkov luminescence was first described by Vavilov and Cherenkov [2]. The two Russian scientists worked in 1932 in the Lebvedev Physical institute in the former Leningrad. Cherenkov worked under his supervisor Vavilov and studied the luminescent glow of uranyl salt solutions under the influence of gamma rays from radium. On one day in 1933 it happened that his research set up contained a pure solvent instead of a salt solution. It turned out this caused the same glow and thus it was not originated from the luminescence from the uranyl salt. They tried to find the explanation of the glow and found that it could not be due to luminescence. In 1934 they published the characteristics for the new phenomenon [5]. Eventually they discovered that electrons where the source of the Cerenkov radiation. Confirmation that moving electrons where the source for the radiation was found when they reproduced the experiments in a magnetic field. It turned out that the Cerenkov radiation was anisotropic and could be altered by changing the magnetic field. This discovery was the key for

researchers Franck and Tamm to come up with the theory for the Cerenkov phenomenon [6]. They explained the characteristics of the Cerenkov radiation and stated a formula to calculate the number and angle of photons depending on the energy of the charged particle and the refractive index of the medium. This is the so called Frank Tamm formula. In 1958 Cerenkov, Frank and Tamm received the Nobel Prize for Physics for their discovery. A timeline on the history of Cerenkov luminescence can be found in appendix A.

2.2 APPLICATIONS

The most prominent development following the discovery of Cerenkov radiation was the Cerenkov Counter [2]. This is basically a large water tank with photomultiplier detectors to detect the photons. The device is capable to detect high velocity charged particles like a Geiger Muller counter. The advantage, however, is that it is way faster and in addition is able to determine the direction the particle travels and its primary energy. In the following years Cerenkov radiation was mainly used in particle physics. The light coming from high energy particles formed an ideal method of detecting high energy nuclear interactions. This led to a lot of knowledge of neutrinos. It is also used in neutrino astronomy like the detection or cosmic air showers [7]. This can be done with big size Cerenkov counters as the SuperKamioKande, a 40 by 50 meter water basin covered by photomultipliers in an underground cave in Japan.

The first appearance of Cerenkov radiation in medical biology in 1971 was the study into diagnosis of eye tumours after the administration of Phosphorus 32 (³²P). This ³²P is incorporated preferentially in dividing and thus malignant tissue. The idea was that the Cerenkov light originating in the vitreous humour would be detected by the patient [8]. This idea was based on the discovery of cosmic rays causing flashes in astronaut' s eyes during the early space missions.

Although Cerenkov in his Nobel prize speech mentioned that the usefulness of Cerenkov radiation would extend rapidly in the future it was until 2009 where Robertson described a totally knew biomedical application for the light phenomenon [1], [9]. He was the first one to perform optical imaging based on Cerenkov luminescence derived from PET tracers in mice. He was hereby helped by the high sensitivity camera systems that were developed for low light imaging as in fluorescence and bioluminescence. Since then, Cerenkov luminescence imaging has quickly emerged as a preclinical molecular imaging modality and a lot of new applications in clinical research were developed based on CLI such as cancer imaging [10]–[13], therapy monitoring [14]–[16], radionuclide detection [17], dose calibration for radiotherapy [18], [19] and fluorophore excitation [20]–[22]. In the last 6 years a lot of knowledge is gained on the use of different radiotracers, and on how to make the technique more sensitive. This led to the understanding that we have the

possession of a compact, high resolution molecular device which could have a lot of potential in the medical clinic. Currently a lot of efforts are done to translate the applications from a preclinical setting into applications that can be used in oncology. Cerenkov based Image guided surgery is one of those prestigious ideas.

2.3 PRINCIPLES OF CERENKOV RADIATION

Cerenkov radiation or Cerenkov luminescence is light that arises when a charged particle moves through a dielectric medium with a speed exceeding the speed of light *c*, in that particular medium. The first thing that should be noticed is that particles are exceeding the speed of light. The concept of particles exceeding the speed of light was already described independently by Oliver Heaviside (1888) [23], Lord Kelvin (1901) [24] and Arnold Sommerfeld (1905) [25]. They all described how an electromagnetic cone forms round the moving particle by the disturbance of the magnetic field. Analogous to how Mach described the sonic boom, where particles exceed the speed of sound. However, due to Einstein' s theory of relativity (1905) this earlier work was neglected for several years until it was adopted by Franck and Tamm to explain the light found by Vavilov and Cerenkov.

It turned out that particles can indeed travel with a speed higher than the speed of sound. However, this can only take place in a dielectric medium. A dielectric medium is a medium with a high polarizability, optical transparency and has insulating properties. Lots of media are dielectric such as water but also organic tissues.

The speed of light in vacuum is determined by the parameters permeability and permittivity. This is the well-known constant c (299 792 458 m/s). The wave velocity in a medium is dependent on the polarizability of the materials in the medium. This is also called the phase velocity the higher the polarizability the slower the velocity of light is in the material. The ratio between the speed of light in vacuum and the phase velocity of light we call the refractive index. The refractive index thus determines the speed of light in that particular material. In water where the refractive index is 1.33 (n = 1.33) the speed of light is only c/1.33 = 0.75 c. This means that a particle can travel faster than light in this medium without compromising the special theory of relativity.

Charged particles can travel statically through a medium. When this occurs in a polarizable medium the molecules of that medium along the path of the charged particle are excited to higher states. When these molecules relax back to their ground state these molecules emit some photons in the form of electromagnetic waves. If the charged particle moves slower than the phase velocity n/c these electromagnetic waves do not interact with each other and extinguish slowly. However, if the particle moves faster than the electromagnetic waves, the waves cross each other and add up at

certain places constructively leading to a coherent radiation. This radiation is directed from a certain angle with respect to the path of the particle. This particular radiation is called the Cherenkov radiation.



charged particles. These can either be beta particles like positrons and electrons, alpha particles or subatomic particles as the muon.



Whenever a charged particle moves through a dielectric medium it instantly polarizes the surrounding molecules. When the particle travels further, instantaneous depolarization occurs and a weak electromagnetic wave is spread out.



The electromagnetic wave travels with the speed of light (c/n) in that particular medium. When speed of the particle is lower than c/n there is no interference of the electromagnetic waves. If the particle exceeds the speed of light the electromagnetic waves cross and constructively interfere in a conic shape leading to an observable effect: Cherenkov Radiation



The angle of the cone and thus the direction of the emitted light depends on the speed or energy of the particle and the refractive index of the medium.

Figure 3

The principle of the production of Cerenkov radiation.

2.3.1 WHEN DOES CERENKOV LIGHT OCCUR?

From the definition of Cerenkov radiation we know that there are three conditions that have to be met. There will have to be charged particles. They have to travel in a dielectric medium. At last they have to exceed the phase velocity. Charged particles may result from radioactive decay such as positrons, β and α particles or as a result of internal conversions due to gamma or X-rays. A dielectric medium is an insulating and optical transparent medium but most of all it means that the molecules making up the material are polarizable. Water and many other liquids are dielectric and thus so are organic tissues.

The last condition is the most difficult one. The speed of the particle has to exceed the phase velocity of the medium. This thus depends on the kinetic energy of the particle and the refractive index of the media. For moving objects we know the famous formula of the relativity theory by Einstein, $E = mc^2$. In developing its special relativity Einstein found for the kinetic energy of a moving body the following formula:

$$E_k = MC^2 \left(\frac{1}{\frac{v^2}{c^2}} - 1\right) \tag{1}$$

With this formula the velocity of the particle can be calculated in terms of c, the speed of light when the kinetic energy of the particle is known. Furthermore, the phase velocity can be calculated in terms of c by the following formula:

$$v' = \frac{c}{N} \tag{2}$$

This means that for a certain refractive index n, there is a threshold for the kinetic energy where the particle will exceed the speed of light, and thus Cerenkov light arises. For a positron in water, which has a refractive index of 1.33, this threshold is 263 KeV. However, if the refractive index increases the threshold decreases. In figure 4 the threshold energy for Cerenkov is plotted against the refractive index. For β particles like positrons and electrons, which have a very low mass these thresholds can be easily overcome. α particles, however, have too much mass and therefor their energy threshold is many times higher to obtain a speed higher than the phase velocity. In practice α particles will not directly be the source of Cerenkov radiation.



Figure 4

On the left a curve describes the threshold of the energy at which Cerenkov radiation occurs against the refractive index. The threshold in human tissue lies around the 230 keV. On the right we see the light production relative to the energy of the particles. Adopted from Mitchell et al. [26].

2.3.2 CHARACTERISTICS OF CERENKOV LUMINESCENCE

How many light there will be produced depends first of all on the amount of charged particles that are traveling faster than the phase velocity. Next to that it depends on how much light will be produced by each particle. The amount of light produced by a charged particle is determined by the distance the part travels at superluminal speed. Which in turn depends on the initial energy of the particular particle. As a particle moves through a medium it gradually loses its energy. As long as this energy stays above the Cerenkov threshold, Cerenkov light will be emitted. The higher the initial energy was the longer the particles energy will stay above the threshold and more light will be emitted. The production of light can be described by the formula provided by Frank and Tamm:

$$\frac{dN}{dx} = 2\pi\alpha \left(1 - \frac{1}{\beta^2 n^2}\right) \int_{\lambda 1}^{\lambda 2} \frac{1}{\lambda^2} d\lambda$$
(3)

This gives the number of photons within the spectral range $\lambda 1 - \lambda 2$, for a given distance x that is travelled at superluminal speed [27]. Since the kinetic energy of a particle gradually decreases the

intensity of the produced light is directly proportional to the energy of the particle as can be seen in figure 4 [<u>26</u>].

The energy of a particle depends on the principle of how this particle is originated. This can be done through excitation of certain gamma or X-rays. In this way the energy and direction of the charged particle is determined by the excitation beam. The direction of the particles and thus the emitted light are also dependent on the direction of the excitation. Another possibility for having charged particles is radioactive decay. In this case the particles are transmitted isotropically from a radioactive source. The energy of the charged particles is dependent on this source. We take Fluorine 18 (¹⁸F), a widely used positron emitter in nuclear medicine, as an example. ¹⁸F sends out positrons with a yield of 97%. The energy of these positrons form a parabolic spectrum around 250 Kev with a maximum of 633 keV (Fig. 5). The Cerenkov threshold in water (n = 1.33) is 263 keV. Therefor 47 % of the emitted positrons will travel at superluminal speeds and thus form Cerenkov light. By using the Frank and Tamm formula we can calculate the amount of light that is produced per travelled distance for a certain initial energy and a wavelength range. Mitchell et al calculated that an endpoint positron of 633 keV will emit 16 photons per mm in the visible spectrum of 400-800 nm [26]. The distance that this positron will travel at superluminal speed in water is 2.1 mm [28]. This means that a ¹⁸F derived endpoint positron in water produces 34 Photons. However, as we can see from the energy spectrum we expect positrons with less energy than 633 keV and therefore overall less photons than 34 will be produced per positron.

To determine the average production of photons per decay, Mitchell et al performed a Monte Carlo simulation. A point source with an energy spectrum of positrons emitted by ¹⁸F was modelled in a volume of water. Based on the Frank and Tamm formula the pathway of positrons travelling at superluminal speeds was modelled. The distance from the origin had a root mean square (RMS) of 0.3 mm. Since the number of photons can be calculated from distance travelled at superluminal speed they determined that for ¹⁸F an average of 1.4 photons are created per decay, in the visible spectrum. If the refractive index is higher, the Cerenkov threshold is lower and more photons will be produced. In biological tissue with a refractive index of 1.4 the photon production will be 2.4 per decay. If we take another radioactive source with a different and an overall higher energy spectrum also more photons per decay will be formed. Yttrium 90 (⁹⁰Y) which has an endpoint energy of 2.23 MeV will produce 57 photons per decay and thus produces 40 times as much light as ¹⁸F (Fig. 4 and Fig. 5). Evidently the travelled path at superluminal speed will also be longer. The RMS of travelled

distance at superluminal speed for Y⁹⁰ is 2 mm.



Figure 5

The energy spectra of ¹⁸F and ⁹⁰Y are given. ¹⁸F has a relative small energy range with an average around 250 keV. Less than half of the positrons have the energy to produce Cerenkov radiation. ⁹⁰Y has a wider spectrum with higher energies. Consequently more positrons will be above the Cerenkov threshold and more light will be produced per positron. Adopted from Mitchell et al. [26].

From the Frank and Tamm formula also the frequency spectrum can be determined. Unlike most fluorescent or emission spectra which have specific peaks, the spectrum of Cerenkov radiation is continues. However, since the refraction is wavelength dependent the intensity of the wavelengths is inversely proportional to the square of the wavelength.

$1/\lambda^2$

(4)

This means that the higher frequencies, or lower wavelengths are more intense than the lower frequencies. The larger part of the light thus appears in the ultraviolet range. This is why visible Cerenkov radiation appears as a bluish glow. The wavelength spectrum measured by different researchers can be seen in figure 6.



Figure 6

Different measurements on the wavelength of the Cerenkov induced luminescence. In all graphs we slightly see the relation to the inverse square of the wavelength. Reproduced from [29]–[31]

3 CERENKOV LUMINESCENCE IMAGING USING CLINICALLY USED RADIOTRACERS

3.1 CONCEPT OF CERENKOV LUMINESCENCE IMAGING

In 2009 Robertson et al. found a new scope for the Cerenkov effect. They discovered that Cerenkov radiation also occurs with positron emitting radiotracers in biological tissue, but more importantly that this radiation could be imaged optically. This resulted in a new molecular optical imaging modality based on FDA (Food and Drug Administration) and EMA (European Medicines Agency) approved positron emitting radiotracers with extensive possibilities. This technique is now known as Cerenkov luminescence imaging. Since the first publication a lot of research has been done by different groups scattered throughout the world. It started with in vitro and preclinical small animal studies but in only a few years' time this has led to numerous possible clinical and preclinical applications. The overall conclusion of most publications is that the technique is at least ' feasible' and more outspoken 'very promising'.

The concept of Cerenkov Luminescence Imaging is to inject PET radiotracers into a human body or an animal subject. These radiotracers tend to spread to specific tissues in a the body. The PET tracers send out high energetic positrons which will produce Cerenkov luminescence in biological tissue. When this takes place in a completely dark environment, the emitted photons can be detected through the tissue by means of a high sensitivity super cooled CCD camera.

To bring a new imaging technique into clinical practice the first question is where it can be of added value compared with currently used modalities. In this chapter we try to answer this question by presenting an overview of the broad range of possible application different researchers have explored over the past years.

3.2 POSSIBLE APPLICATIONS FOR CERENKOV.

In the last six years many applications have been suggested. These applications have different scopes, different purpose and/or different methods. Concerning the scope you can use Cerenkov on animals, patients or on specimens. Next to that the purpose to use Cerenkov radiation makes a big difference. You can either use it for diagnostics, therapy monitoring, treatment response, image guidance or as excitation for photo therapy. Lastly there are different methods of how Cerenkov imaging can be performed for instance in vitro or ex vivo, planar or tomographic. It is important that the choices that are made are matched with each other in order to come up with a successful

clinical application. Tomographic optical imaging in humans is for instance improbable due to penetration properties of optical signals. For this project we focused on Cerenkov imaging in humans that could assist in oncological surgery.

3.2.1 ANIMALS VS PATIENTS

There is a lot of difference in using Cerenkov imaging for animals or for patients. The most obvious reason is that the for imaging patients there are a lot of regulations that do not apply to small anmals because they are euthanized afterwards. This is reflected primarily in the amount of radioactivity that can be administered. The administration doses for patients have strict regulations that can be neglected in small animals. Especially in mice the dose weight ratio can become very high which will result in a dose per kilogram bodyweight that is over 100 times higher than is used in human. Next to that the we are measuring light as a signal. Light has a very poor ability to penetrate through biological tissue. This will make the technique feasible for imaging small animals that lack thick layers of tissue. For imaging in a larger species such as humans, detecting light is more difficult and imaging of deeper structures can only be done invasively. At last, there is the available imaging system. For imaging mice, the commercially available IVIS (In Vitro Imaging System) can be used (Fig. 7). This is a very sensitive precisely tuned and tailored system to perform low light imaging on mice. The mice can be sedated and be imaged five at a time in a light tight cabinet. For patients, such a device is not available and other solutions have to be found. Problems that arise here are to obtain a light tight system that is as sensitive as the IVIS system keeping the size and the convenience of the patient in mind.

3.2.2 OBJECTIVES

Since the discovery of CLI it is clear that it is a promising new modality for molecular imaging with the use of PET tracers and as an additional advantage could image β^- radiation or electron emission. It would especially be suitable for preclinical mouse studies. This was independently stated by the pioneers of Cerenkov Imaging, [1], [32], [33]. Hu et al. even explored the options for 3 dimensional Cerenkov imaging or Cerenkov luminescence tomography [34].

Besides the straightforward applications as in vivo tumour imaging, treatment response and therapy monitoring that are also used in PET imaging, there are numerous other applications suggested or explored such as: In vitro assays [33], plant studies [33], imaging of pure β min emitters [30], Reporter gene expression [30], source depth measurements [30], microfluidic chip measurements [35], Imaging of α emitting radionuclides [36], imaging Cerenkov radiation excited by external beam radiation [19], excitation of quantum dots or other luminescent material by CR for diagnostic

or therapeutic purposes[21], [22], [30], negative contrast imaging[37], selective bandwidth quenching of the CR spectrum[17], [38] and CR imaging of positron distribution in proton therapy[18].



Figure 7

The in vivo imaging system which is designed especially for optical imaging with low light levels in small animals. It consists of a light tight cabinet with a high sensitivity super cooled CCD camera with different lenses, band filters and special lights to excite fuorophores. On our case we do not use the lights. Reproduced from www.perkinelmer.com.

In 2010 Ruggiero et al. were the first to mention that optical imaging methods have to be translated into the clinic so that endoscopy and surgery could benefit from it [39]. Shortly after that, from the same group of the Memorial Sloan Kettering hospital, Holland et al, published a paper on intraoperative Cerenkov luminescence imaging [12]. He performed intraoperative CLI on mice with Zirconium 89 (⁸⁹Zr) DFO-trastuzumab. The data he published really captures the imagination of the application(Fig. 8). However, it was performed on mice and not on a clinical scale. Nevertheless, he

supported the development of CLI as a possible clinical tool in surgical procedures for defining tumour margins.



Figure 8

Cerenkov image guided surgery performed on a mouse by Holland et al. A her2/neu positive tumour was injected with Zr89-DFO-Trastuzumab 144 hours later resection of the tumour was performed. A) A background CLI image. B) CLI image prior to the surgical incision. C) CLI image after exposure of the tumour just prior to the excision. D) Resected tumour in the left upper corner. The incision site lacks a CLI signal. E) CLI of the excised tumour alone. F) Postoperative CLI after closing the excision. Adopted from Holland et al. [12]

In 2012, Liu et al. were the first to explore the possibilities of CLI through endoscopy [11]. Endoscopy seemed one of the most potential applications for clinical translation because the modality already uses optical imaging and a natural dark environment can be acquired. They demonstrated it was possible to detect tumour tissue in vivo with a fibre based endoscope attached to an EMCCD (Electron Multiplying Charge-Coupled Device) camera. A technique they named endoscopic CLI (ECLI). Again this study was performed in mice.

A year later Chin et al., who studied hybrid imaging agents, published a review paper where they

compared CLI to fluorescence [40]. In this review he pointed out what the current weaknesses for CLI where namely ; low light, ambient light distortion, low penetration power and the necessary radioactivity. Nonetheless he stated that it is a very exciting research line and that these limitations could be overcome by technical developments.

In 2013 the first CLI on humans was performed. Spinelli et al. detected light emitted from the thyroid of a patients treated for hyperthyroidism with Iodine-131(¹³¹I) [41]. This was the first proof that CLI was actually possible in patients. (Fig. 9) This result suggested the application of monitoring radiation dose of therapy with β emitters in superficial organs. This would especially be relevant for β^- emitters since there is currently no efficient way of imaging them.

In that same year Thorek et al. also performed CLI in humans [42]. He had performed a pilot study on four patients and they showed that they were able to image a positive axillary node in a patient with lymphoma after injection of ¹⁸F-FDG. With this result they demonstrated it was feasible to detect tumours with CLI using diagnostic doses of ¹⁸F-FDG. They also stated that for establishing the value of CLI for clinical use, they required larger studies with possible more sensitive equipment and higher energy positrons. From the article it is not really clear how they exactly obtained their results. This is also questioned in a response to this article made by Spinelli and Boschi [43]. Despite the fact that a lot of the criticism could be refuted, the results of Thorek is difficult to explain next to the other published data concerning CLI. Even today a reproduction of Thoreks work has not been published.

In 2014, Jarvis et al. published its results on the visualization of the surface dose due to radiation therapy [19]. By use of CLI he was able to image the surface dose on human subjects in real time. They proposed that Cerenkov video imaging could detect errors in everyday clinic and therefor improve the safety and quality of radiation therapy.

The latest published study performed in humans is a feasibility study for the ECLI system for detecting gastro-intestinal disease. Hu et al. performed endoscopic CLI on four patients after ¹⁸F-FDG administration [44]. They found elevated signals in cancerous tissue compared with normal tissue. This result will make the application of CLI for endoscopic diagnostic procedures very feasible.

Thyroid I-131, Spinelli et al.



Colon 18F-FDG, Hu et al.





Breast External Beam RT, Jarvis et al.





Cerenkov imaging that is performed on humans. In the upper left corner we see a patient with who is treated with ¹³¹I for hyperthyroidism. 550 MBq was administered and after 24 hours a 2 minute acquisition with a EMCCD camera was made. A clear elevated intensity can be seen at the position of the thyroid gland. Adopted from [41]. On the upper right we see a malignant lymph node in the axilla that is imaged with an ICCD camera. 470 MBq of ¹⁸F-FDG was administered and after 68 minutes a 5 minute acquisition was made. Image B and D show the left axilla which shows elevated intensity corresponding to the PET examination (E). Adopted from [42]. In the lower left corner we see the results of an endoscopic approach of CLI. With a fiberscope coupled to an EMCCD camera malignant tissue (A and C) is compared to benign tissue (B and D). For this study this

patient was administered with 9.25 MBq/kg ¹⁸F-FDG was administered. Adopted from [44]. In the lower right corner we see an image from a study from Jarvis et al. He imaged the dose on the surface of a breast that is radiated with an external beam. This high energetic radiation causes Cerenkov radiation in human tissue that can be real time monitored with an EMCCD camera during the treatment. Adopted from [19]

In the most recent years Cerenkov radiation has been explored for therapeutic purposes. This was initiated by the work to overcome the problems of the penetrability of Cerenkov light. It was demonstrated that fluorescent probes as small molecules and quantum dots could be excited by Cerenkov radiation [45]. This meant that the spectrum of Cerenkov radiations that peaks in the blue and green wavelengths could be changed to narrow band high penetrable wavelengths without compromising the tissue specificity of the used radioisotope.

Ran et al. were the first to describe the concept of Cerenkov radiation as a tissue specific light source [21]. He used Cerenkov radiation derived from administered ¹⁸F-FDG as photo activation of luciferin to have activation at specific malignant sites deep in vivo where external photo activation was not possible.

In a recent review published by Grimm, the results of the group from Achelifu was discussed [22]. They studied the activation of titanium dioxide nanophotosensitizers (NPS) by Copper-64 (⁶⁴Cu). When he injected both in fibrosarcoma tumours in mice, the disease showed a complete regression in 30 days. They repeated this with a slightly different approach by injecting the NPS and ¹⁸F-FDG intravenously. Also this study showed good result. Again there is a remark that this work was performed on mice and that NPS as were used here have a long way to go before they can be used in clinical practice.

From the literature it can be concluded that CLI has a large potential. Many researchers describe the possible uses in clinical practice. The current results however, are very poor and as with all optical imaging the translation from small animals to life-size humans is difficult due to the attenuation of light by tissue. To make a successful start into clinical translation more easily adaptable applications should be developed first. Such small steps are for instance made with the ECLI. It might not have a large impact on clinical relevance but foremost it is likely to succeed since an optical guided procedure and system are already implemented in endoscopic examinations and it makes use of the naturally dark environment in the colon. For assistance in oncological surgery can be thought of margin detection as the principle application. For this only small specimens have to be imaged and there is no need to look in deeper layers of the tissue.

3.3 RADIOTRACERS

As it appears from the literature there are a lot of properties that make CLI very difficult. The biggest challenge perhaps is that the Cerenkov light is produced in very low quantities, resulting in a weak signal. It should be noticed that a lot of research is currently performed on enhancing the CLI signal. Next to that this weak signal has to stand out against the background. A problem we also encounter with other nuclear modalities and with fluorescence optical imaging. The amount of light and also the signal to background ratio is determined by the type of radiotracer that will be used. Today there are a lot of different radiotracers available for different purposes. To have an overview of what radiotracers are suited for CLI and which one is the best option for a specific purpose this paragraph gives an overview of the clinically used radiotracers and their most important properties for CLI.

It has been made clear that Cerenkov radiation arises when radioactive decay occurs in water or biological tissue. There are a lot of radionuclides used in nuclear medicine, over 40 different isotopes. But there are a handful of properties that make these isotopes suitable for Cerenkov imaging.

- 1. The first and most important factor is that the radioisotope has to emit high velocity charged particles. With high velocity we mean they have to possess a certain energy that enables the particle to move faster than the phase velocity or medium specific speed of light. In general these include all isotopes that emit either positrons or electrons of sufficient energy. α particles typically are too heavy to be able to reach speeds like that. However, certain isotopes which initially decay into α particles or γ rays might produce daughters that emit high energy β particles. However, the forming of the daughters might cause a delay in obtaining a signal. This means that radioactive isotopes that produce Cerenkov radiation are the ones that decay trough β particles or produce daughters that will decay by β emission.
- 2. The second important factor is that it has a sufficient amount of yield and energy. The amount of light that is produced by Cerenkov radiation is relatively low and to make a light source visible, especially within a tissue, an intended number of photons is required. The amount of photons that are produced depend mainly on the number of charged particles that are emitted. This can be determined by the activity and the yield. Furthermore the energy of the charged particles is important. The higher the energy the more photons it will produce before it drops under the Cerenkov threshold. For positrons in biological tissue with a refractive index of 1.4 this threshold is 230 keV. Therefor isotopes with a high yield of

 β emissions and a high initial energy of those β particles are particularly suitable for Cerenkov imaging.

- 3. The half-life of an isotope is also important for imaging purposes. Since we want to image light that is emitted by radioactive decay the signal is directly associated to the activity of the radioactive source. We have to make sure that there is enough activity at the right site in a human or animal body or even in a specimen at the time of the imaging. The half-life of the isotope should not be too low in order to keep the activity on an intended level for imaging and to give the pharmacokinetics the time to reach the targeted parts. Next to that you also want a half-life that is not too long to make sure the radioactivity is cleared from a subject within a reasonable time. On the contrary, there are some circumstances a longer half-life could be beneficial. If the radioisotope probes can stay at its target for a prolonged time the majority of the activity, that is not specifically bound, will leave the body by excretion. This could reduce the total activity in the whole body while maintaining a high activity at the targets of interest. For instance in intraoperative Cerenkov imaging this could reduce the radiation dose to the surgeons. Due to this diversity in applications we accept a broad range of half-lives.
- 4. The radioisotope that you want to image should have a clinical value. This means that the information of the distribution of the isotope through the body or specimen gives you relevant information. The isotope therefor has to possess some properties to bind to certain elements or structures that are interesting to visualize. These properties can either be part of the isotope itself or come from a molecular labelling structure it can bind or chelate to. Radiotracers suitable for CLI are thus the ones with specific binding or distribution capacities.
- 5. The last important property which is one of the important benefits of CLI is that the radiotracers are FDA or EMA approved and already used in the clinic. Based on these properties a list of isotopes is composed that seem to be suitable for Cerenkov imaging (Table 1).

| Radioisotope | Half life | Medical use | ß Decay yield Other decay | Other decay | Beta Daughters | Max energie β (KeV) | FDA/EMEA approved |
|----------------|-----------|---|---------------------------|-------------|-------------------|------------------------|----------------------|
| Actinium 225 | 10,0 d | Targeted alpha therapy | 0 | α 100% | Yes | | No |
| Bismuth 213 | 46 min | Targeted alpha therapy | 0,979 | α 2,1 % | Yes | 1422,75 | No |
| Bismuth 212 | 60,55 min | None | 0,64 | α 36% | Yes | 2254 | No |
| Caesium 137 | 30 yr | Blood sterilisation | 1 | | | 1175 | No |
| Carbon 11 | 20,39 min | Tumor imaging, therapy monitoring | 0,9976 | EC 0,24 % | | 960,5 | Yes |
| Chromium 51 | 27,7 d | Red blood cell labeling | 0 | EC 100 % | | | Investigational |
| Cobalt 57 | 272 d | Estimate organ size | 0 | EC 100 % | | | No |
| Cobalt 60 | 5,27 yr | Sterilisation, External beam RT | 1 | | | 313,13 | Investigational |
| Copper 64 | 12,7 h | PET imaging, cancer therapy | 0,563 | EC 43,6% | | 653 | No |
| Copper 67 | 2,6 d | Cancer therapy | 1 | | | 575 | No |
| Dysprosium 165 | 120 min | synovectomy treatment | 1 | | | 1286 | No |
| Erbium 169 | 9,4 d | Relieving arthritis pain | 1 | | | 351 | No |
| Fluorine 18 | 109 min | Tumor imaging, positron emitter | 0,973 | EC 3,7 % | | 633,5 | Yes |
| Gallium 67 | 78 h | Tumor imaging | 0 | EC 100 % | | | Yes |
| Gallium 68 | 68 min | Tumor imaging, positron emitter | 0,891 | EC 10,9 % | | 1899,1 | Yes |
| Holmium 166 | 26 h | diagnosis and treatment of liver cancer | 1 | | | 1854,9 | No |
| Indium 111 | 2,8 d | specialist diagnostic studies | 0 | EC 100 % | | | Yes |
| lodine 123 | 13 h | Thyroid function diagnostics | 0 | EC 100 % | | | No |
| lodine 124 | 4,18 d | Tracer | 0,228 | EC 77,2 % | | 2137,6 | No |
| lodine 125 | 90 q | Cancer brachytherapie and diagnosis | 0 | EC 100 % | | | Yes |
| lodine 131 | 8 d | Thyroid cancer treatment and organ function | 1 | | | 606,31 | Yes |
| Iridium 192 | 74 d | Internal Radiotherapy source | 0,9624 | EC 4,76% | | 675,12 | No |
| 23 | 46 d | Spleen metabolism | 1 | | | 465 | Investigational |
| | | | | | | | |
| Radioisotope | Half life | Medical use | β Decay yield | Other decay | Beta Daughters | Max energieβ (KeV) | FDA/EMEA approved |
|----------------|------------|--|---------------|-------------|----------------|-----------------------|-------------------|
| Lead 212 | 10,6 h | Targeted alpha therapy, AlphaRT | 1 | | | 573 | No |
| Lutetium 177 | 6,7 d | Gamma imaging, beta RT | 1 | | | 498,3 | Yes |
| Nitrogen 13 | 9,97 min | Positron emitter | 1 | | | 1200,3 | Yes |
| Oxygen 15 | 122,24 sec | Positron emitter | 666'0 | EC 0,1% | | 1731,9 | No |
| Palladium 103 | 17 d | Implant seeds, brachytherapy | 0 | EC 100% | | | No |
| Phosphorus 32 | 14 d | Cancer imaging and therapy | 1 | | | 1710,99 | Investigational |
| Potassium 42 | 12 h | Potassium exchange coronary blood | 1 | | | 3525,4 | Investigational |
| Radium 223 | 11,43 d | Cancer therapy | 0 | α 100% | Yes | 1372 | Yes |
| Rhenium 186 | 3,8 d | Bone cancer pain relief | 0,9253 | EC 7,47 | | 1069,5 | No |
| Rhenium 188 | 17 h | Coronary artery radiation | 1 | | | 2120,4 | No |
| Rubidium 82 | 86 sec | Myocardial perfusion imaging | 1 | | | 3150 | Yes |
| Samarium 153 | 47 h | Cancer painrelief | 1 | | | 808,2 | Yes |
| Selenium 75 | 120 d | digestive enzym production studies | 0 | EC 100% | | | Investigational |
| Sodium 24 | 15 h | electrolyte studies | 1 | | | 1392,9 | No |
| Strontium 89 | 50 d | Pain relief cancer | 1 | | | 1495,1 | Yes |
| Technetium 99m | 6 h | lmaging | 0,000037 | IC 99,99% | | 436 | Yes |
| Thallium 201 | 73 h | Heart condition imaging | 0 | EC 100% | | | Yes |
| Xenon 133 | 5 d | Pulmonary ventilation studies | 1 | | | 346,4 | Yes |
| Ytterbium 169 | 32 d | cerebrospinal fluid studies in the brain | 0 | EC 100% | | | No |
| Yttrium 90 | 64 h | Cancer brachytherapie | 1 | | | 2280,1 | Yes |
| Zirconium 89 | 78,4 h | Immuno PET | 0,223 | EC 77,7% | | 902 | No |

Table 1

List of isotopes that are used in medical applications and there characteristics that are of interest for Cerenkov luminescence imaging. These factors include the half-life, the type and yield of decay and the endpoint energy of the derived β particles. Some isotopes produce daughters that result in β particles and are thus also capable of producing Cerenkov radiation.

Within CLI there are various applications. Not every isotope will be suited for each application. The amount of luminescence that can be produces is of interest for all CLI functions. Factors as the half-life and the possible tracers that it can bind to determine if an isotope can be used for a certain application. Currently with CLI as a new modality in nuclear medicine the applications are depended on the radiopharmaceuticals that are available. A list of different tracers the isotopes can be combined with can be found in appendix B.

To determine which of these radioisotopes are suited for Cerenkov imaging we can divide them in two different groups. Radiopharmaceuticals for therapeutic purposes and radiopharmaceuticals that are used for diagnostics. These two groups have a lot of different properties and also their own reason why optical imaging would be relevant. The most important factor to determine whether a radioisotope is suitable for Cerenkov imaging is the purpose of the imaging. In our case we want to use it for visualizing malignant tissue during surgery. Important factors for us art thus: A high production of Cerenkov radiation, tumour specific distribution and a relative short half-life. To determine how much light an isotope will produce exactly per amount of activity, several researchers have performed Monte Carlo simulations based on the formulas determined by Frank, Tamm and Jelley [26], [36], [46], [47]. (Table 2). In this table we can see how much photons are produced per decay for a collection of isotopes. It should be noted that the different studies set different conditions in which the photons are included. They don't take all the wavelengths into account. The studies performed by Gill and Mitchell determined the amount of photons produced in water (n = 1.33) in the range of 400-800 nm. Also they included all the photons that will be produced in 1 month after the decay so also the Cerenkov radiation produced by daughters is taken into account. Ackerman and Graves had a somewhat smaller range with 400-700 nm. The study performed by Beattie et al shows much lower amounts. Despite the fact he simulated the production of photons in tissue (n = 1.4) the narrow bandwidth of photons he included (550-570) nm) determined the low values. (Table 2)

For the purpose of visualizing tumours during surgery Carbon-11 (¹¹C), Gallium-68 (⁶⁸Ga) and ⁹⁰ Y will be the best options since they produce much luminescence, have proper half-lives and are FDA and EMA approved. Additionally C¹¹ and ⁶⁸Ga can be combined with tumour specific tracers.

25

| Radioisotope | Mitchell et al. | Ackerman and Graves | Beattie et al. | Gill et al. |
|---------------|-----------------|------------------------|----------------|-------------|
| Actinium 225 | | 0,0 (40,2) | 1,01 | 13 |
| Astatine 211 | | | | 0,01 |
| Bismuth 212 | | 22,5 (44,8) | | 13,1 |
| Bismuth 213 | | 11,4 (40,2) | | 31,4 |
| Carbon 11 | | | 0,55 | 6,87 |
| Cesium 137 | | | | 2,27 |
| Cobalt 60 | | | | 1,92 |
| Copper 62 | | | | 72,9 |
| Copper 64 | | | 0,05 | 0,56 |
| Copper 67 | | | | 0,17 |
| Fluorine 18 | 1,4 | | 0,13 | 1,32 |
| Gallium 68 | | | 2,56 | 33,9 |
| Holmium 166 | | | | 27,5 |
| Indium 111 | | | | 0,001 |
| Indium 114 | | | | 34,6 |
| Iodine 124 | | | 0,37 | 8,97 |
| Iodine 131 | | | 0,07 | 0,67 |
| Iridium 192 | | | | 0,8 |
| Lutetium 177 | | | | 0,14 |
| Molybdenum 99 | | | | 9,27 |
| Nitrogen13 | 14 | | 1,01 | 13,2 |
| Oxygen 15 | | | 2,33 | 30,9 |
| Phosphorus 32 | 32 | | | 28,1 |
| Radium 223 | | | | 28 |
| Rhenium 186 | | | | 5,83 |
| Rhenium 188 | | | | 34,9 |
| Rubidium 82 | | | | 80,8 |
| Samarium 153 | | | | 1,45 |
| Sodium 22 | | | | 1,54 |
| Strontium 89 | | | | 21 |
| Thormium 227 | | | | 23,5 |
| Uranium 230 | | 0,0 (8,5) | | 0,01 |
| Yttrium 90 | 57 | | 3,7 | 47,3 |
| Zirconium 89 | | | 0,12 | 2,29 |

Table 2

This table gives an overview of four studies that are conducted to determine the amount of Cerenkov radiation that is produced per decay. This is based on performed Monte Carlo simulations. The different studies used a different range for the wavelength and are therefore not comparable with each other. However, the studies per se give a good estimation on the relative difference in light production between different isotopes. The study performed by Ackerman et al. was done to determine the amount of light produced by the intrinsic isotope and the total through all the daughters. This number is given in brackets.[26], [36], [46], [47]

4.1 DIFFICULTIES IN SURGICAL ONCOLOGY

One of the biggest challenges in surgical oncology is often to make sure all of the malignant tissue is removed without compromising functional and cosmetic outcomes. To achieve this, a surgeon should be very well aware where the primary tumour and possible metastasis are located within the body. Besides the location of the tumour, its location with respect to critical organs is very important. The next step is to make sure the surgeon exactly knows what tissue belongs to the tumour and what not. In other words he has to make a distinction between malignant and benign tissue.

Over the past decades several techniques are developed for locating a tumour. In most cases this is done based on imaging that took place days or weeks before the surgery. This can either be CT, MRI, PET-CT or Ultrasound imaging. With these modalities the location and often the spatial orientation can be recovered. Based on these images and the anatomical knowledge of the surgeon a plan is made prior to the surgery. The difficulty with this method is that the patient' s position between imaging and surgery can be changed and additionally the position of the tumour can change during surgery. Furthermore the time between them could have caused proliferation or shrinkage of the tumour. Several techniques are currently performed to improve this method. One of the most recent applications is surgical navigation. This tracks and registers the position of the patient and the tools of the surgeon and displays it on an imaging based model. However, in cases as breast and rectal cancer the organs are very mobile during surgery and lack the proximity of rigid anatomical landmarks which are important for navigation and intra-operative guidance. There are numerous ways to solve this including; intraoperative ultrasound guided resection, the placement of guide wires or markers, the use of dyes, cryoprobe assisted localization or the use of injected radiopharmaceuticals [40], [48]–[50]. With the help of radiography and or gamma probes the exact location of the tumour can be determined during the surgery.

This means that the first step, localizing a tumour, is pretty well solved. However, all these techniques do not solve the second step of discriminating tumour tissue from healthy tissue. To do this surgeons still depend on their visual and tactile input. For some tumours this is a difficult task since they have almost the same visual and textural properties as the surrounding healthy tissue. Also in patients that have recurrent disease or who underwent extensive chemo-radiotherapy, determining the borders of the tumour is even more difficult due to a disturbed anatomy and

fibroses caused by prior surgery or radiation. It follows that resection in these cases is hampered and will impede optimal tumour resection.

4.2 POSITIVE SURGICAL MARGINS (PSM)

A non-clean resection means that there are positive surgical margins. In the literature researchers have reported positive margin rates of 20-40% in breast conserving treatment of primary breast tumours [48], 16-27% in radical prostatectomy [51], [52], and up to 28 % in rectal cancer [53]. It should be noted that these are not the current numbers representative for the NKI-AvL (Netherlands Cancer Institute – Antoni van Leeuwenhoek) , but findings in other centres in the Netherlands and other western countries. PSMs are a typical risk factor for local recurrence. Positive margins in patients who received breast conserving surgery will give a fivefold increased risk of local recurrence [54]. In prostatectomy PSM will lead to a fivefold increased risk of additional treatment [51]. Besides the additional treatment in the form of surgery or radiation therapy, PSMs will lead to uncertainty and anxiety for the patient. Next to that the additional treatment comes with extra costs, risks and more discomfort for the patient. It should be noted that the assessment of PSMs is not an exact science and therefore not always properly diagnosed. Improper diagnosis will results in an even higher risk for local recurrence [55]–[57], and hence a worsened patient outcome. This provides even more reason to prevent positive surgical margins.

One of the approaches to obtain clean margins is to do intraoperative margin assessment. Several techniques have been explored over the last decades such as: Intraoperative Specimen Radiography, frozen section analysis and intraoperative touch preparation cytology (imprint cytology)[58]–[60]. Of these only the latter showed promising results but is not widely implemented because it is not applicable to most of the tumours and requires on site pathology personnel.

Another approach is to develop a technique that enables the surgeon to distinguish tumour tissue from healthy tissue even when the visual and textural appearance cannot be distinguished. We can already do this with different types of imaging like MRI or PET-CT. In this case we need an imaging device that is very tumour specific and next to that has a very high sensitivity and resolution to detect small tumour manifestations. For practical reasons it also has to be easy to handle, easily implementable and fast. And with fast we mean at least near real time. The one technique that seems very suitable for this kind of image guidance is optical imaging.



Figure 10

The new hallmarks of cancer. The properties that define malignancy. They consist of the 6 originally hallmarks proposed in 2000 completed with four new processes that characterize malignant tissue. Adopted from [63]

4.3 OPTICAL IMAGING

Optical imaging systems have the advantage of being free of ionizing radiation, being easy to handle, having fast acquisitions and a high resolution. Next to that they are relatively inexpensive and can be easily implemented in a surgery setting. Real time technologies really puts the images directly in the hands of the surgeon. The big question is how we can distinguish tumour tissue. The answer to this question can be found in the hallmarks of cancer: 10 different properties that define malignancy. (Fig. 10) These properties can be used to make a distinction between benign and malignant tissue.

There are different forms of optical imaging that can be used for optical imaging. Autofluorescence is the intrinsic fluorescence of tissue that is excited by a certain wavelength of light. An advantage is that no exogenous agents have to be administered. It is shown that they found robust correlations for elevated red fluorescence and decreased green fluorescence in tumour tissue in the oral cavity [61]. However, the technique did not show an advantage over the naked eye and furthermore the specificity of the detection remains uncertain because it is not clear what the exact contribution of the individual components are [62].

In conventional fluorescence they use probes that emit between 400-600 nm that will give high levels of nonspecific background in tissue. Furthermore they have little optical penetration and a lot of absorption by haemoglobins. Since optical imaging is all about the signal to background ratio, and for this purpose tumour to background ratio, conventional fluorescence in not a good choice for image guided surgery.

Near infrared (NIR) fluorescence was a crucial step in optical imaging and especially for imaging in vivo. The optical imaging window where the least absorption and scattering takes place in tissue lies between 700 and 900 nm. (Fig. 11) Probes with these wavelengths thus have a better penetration, less scattering and less autofluorescence and therefor provide a better signal to background ratio. There are different approaches to form suitable fluorophores for NIR fluorescence [62]:

- Non targeted fluorophores as fluorescein and indo cyanine green (ICG) which have excellent fluorescent properties but do not have the ability to bind to a specific tissue.
- Non-targeted activatable fluorophores. These are non-specific markers that are delivered in a quenched state. Due to cleavage of certain enzymes they can be activated to make them more specific to tissues where the intended enzyme is situated. However, this has not led to tumour specific agents yet.
- Targeted organic fluorophores are NIR fluorescent agents that have the ability to provide molecularly specific detection. The agents are foremost conjugated to specific target ligands or monoclonal antibodies. Different types of fluorophores like Cy 5.5, Alexa Fluor and IRdye800CW are targeted to epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) or her2/neu receptors. These agents show excellent signal to background ratio and this can even be increased by using specific quenching.
- Quantum dot nanoparticles are small inorganic crystals. They can provide high signal intensities and can be designed to peak at a certain wavelength. Furthermore they can be targeted to multiple biomarkers. Unfortunately the quantum dot nanoparticles show a high level of toxicity that forms a large obstacle for their way into the clinic.

Techniques like fluorescence seem very suitable for tumour localization and intraoperative margin assessment. Although the recent years has seen an increase in the number of commercially available fluorophores, translation towards the clinical setting is still very limited. Their appearance in clinical practice is hampered by lacking FDA or EMA approval and limited standardization of production, imaging and quality control [64].



Figure 11

In this graph we can see the different absorption coefficients in human tissue against the wavelength. The region between 700 and 900 nm has we see a lower absorption by bound and unbound haemoglobin and water. This region is also known as the optical window. In light blue we see the spectrum of Cerenkov luminescence which shows a very low yield in this particular window. Reproduced from [65]

4.4 OPTICAL AND MOLECULAR IMAGING COMBINED.

The question that remains is on how we will be able to detect malignant cells by optical imaging. A good example for tumour specific imaging can be found in nuclear imaging and PET in particular. They are capable of detecting malignancies using highly tumour-specific radioactive tracers that are readily available for clinical use. The mainstay tracer for oncology is ¹⁸F-FDG, which makes use of the increased metabolism due to the limitless replicative potential. It is used to localise lesions, monitor disease burden and therapy efficacy. Unfortunately, the technology used for the detection of radioactivity is less suitable for intraoperative use because it lacks the resolution, the speed and the ease of use we need for image guidance during surgery.

CLI provides us with the means of combining optical imaging with molecular imaging through clinical available radiopharmaceuticals. It actually combines the best of both worlds. From the optical imaging point of view we have the advantage that it is a sensitive technique with a high resolution. The optical devices are relatively inexpensive, easy to operate and can be easily implemented into clinical practice due to their small profile. Furthermore they are capable of fast acquisitions and able to provide real time or near real time imaging data.

The radioactive molecular imaging probes provide us with a wide range of clinically available and

approved probes. These radiopharmaceuticals are widely used and produced on large scale and therefore relatively inexpensive. This means that no large costly studies into pharmacokinetics and toxicology are required and there can be a rapid translation into clinical practices. Also, performed studies have proven that CLI can provide a quantative measure and that it shows good correlation with PET studies [1], [10], [39], [66].



Figure 12

This graph shows the emission intensity of the different wavelengths of Cerenkov luminescence compared with fluorescent and bioluminescent agents. We clearly see that most of the Cerenkov signal is located in the ultraviolet region. Additionally the intensity peak is a factor 1000 lower than the other agents. Adopted from [40].

These characteristics make CLI very promising as a new technique for image guided surgery. However, there are also some limitations that have to be overcome before CLI can be actually applied in the clinic. First of all, the signal derived from the Cerenkov effect is very low, more than three orders of magnitude less than luminescent markers. (Fig. 12) This results in two additional challenges. First of all the signal is so low that it can be overshadowed by any glimmer of ambient light. This requires that images have to be made in total darkness. For in vivo imaging in humans this will not be easy and especially in a surgical setting this will be very difficult to accomplish. Secondly, this means that to obtain a sufficient signal, lots of radiotracer should be administered. Liu et al. calculated that for her endoscopic system an amount of 2.1 GBq (giga Becquerel) should be administered to obtain detectable concentrations in a small head and neck tumour [11]. Since the administration preferably has to take place several hours before the surgery, decay determines that the administered dose should be even higher. This will lead to very high doses for the patients but also for the OR personnel who have to follow much stricter rules for exposure.

The low signal intensity will become even a bigger problem when performing imaging in vivo. This is caused by the nature of the spectrum. The yield is proportional to the inverse square of the wavelength. (Eq. 4) As a result the spectrum peaks at 180 nm with the majority of the light in the ultraviolet region and only a fraction of the light in the optical imaging window (700-900 nm). Thus, with performing CLI in practice we face the challenge of having a low signal of which only a small portion is usefull when used in vivo.

5 EMCCD CAMERAS

In order to make Cerenkov radiation visible we need a very sensitive camera which provides a very high signal to noise ratio in a very low light environment. For this purpose we use an EMCCD camera. EMCCD stands for electron multiplying charge-coupled device. It is an innovative design for amplifying low light signals above the read noise floor of already sensitive CCD sensors without compromising the quantum efficiency. Together with low costs and a high resolution this makes it better candidate than other high sensitive cameras as for instance the intensified CCD. The low light capabilities of EMCCD are primarily of use in astronomy and biomedical research. In astronomy it is used to look at faint stars. Biomedical applications are photon counting and different types of spectroscopy among others. More recently EMCCDs have broken into the field of fluorescence microscopy and small animal imaging.

A CCD chip is a piece of technique that is extensively used in digital imaging. It enables the shift of a charge to different areas of a chip for further processing. For instance, the conversion from charge to digital values. The chip consists of two parts; an imaging part and a storage part. The upper surface of the imaging part consists of a silicon photoactive region. Here incident photons are transformed into photoelectrons. Under this layer each pixel of the chip is represented by a capacitor well. Often this is a metal oxide semiconductor (MOS) capacitor. It stores and accumulates charge proportional to the amount of incident photons during an acquisition. The storing part consists of the same amount of capacitor wells as the imaging part. After an exposure the charges of each capacitor well are shifted to the storing area. At this time the image can be read out one line at the time and each pixel is converted from an analogous to a digital signal. (Fig. 13) At the same time of the readout the imaging part can start a new exposure.



Figure 13

Working principle of a CCD chip. On the image section the photons are collected during the exposure time. During the readout the charge in the capacitor wells which represent the pixels are shifted towards the store section. From the store section they are read out per line and then per pixel converted to a digital signal. Adopted from www.andor.com

In electron multiplying an additional gain is built in between the readout and the conversion to a digital signal. This is done by a technique called the electron avalanche effect. This gain can be set digitally and is capable of multiplying the charge by a thousand. The exact placement of this gain results in the suppressing of certain noise factors leading to a higher signal to noise ratio [67].

5.1 NOISE SOURCES

With low light imaging it comes all down to signal to noise ratio. When imaging something it is the goal to elevate the signal above the noise floor. There are different types of noise that have different natures and contribute at different moments in the process (Fig. 14). For an EMCCD camera we have the following types of noise[68]:

Shot Noise (*N_{shot}*): This is a property of the quantum nature of light. It appears due to statistical fluctuation of the number of photons that are emitted by the source. It can be seen as the natural variation in the signal and this cannot be removed.

- Dark Current (N_{dc}): Is caused by thermally generated electrons in the photoactive part of the CCD chip. This current also has its own stochastic variance known as the dark noise. The contribution of dark current can be greatly reduced by lowering the temperature of the chip to temperatures of minus 80 degrees Celsius. For these low temperatures the dark current drops from 300 electrons per second per pixel at 20 degrees to lower than 0.001 electrons per second per pixel at minus 80 degrees.
- Readout Noise(N_r): Is the noise that is generated by the electronic circuitry that converts the charges to a digital signal. The readout noise has different contributors as the reset noise, output amplifier noise, excess noise, quantization noise and clock induced charge. It cannot be eliminated but the different contributors can be suppressed due to sophisticated designs. Within an EMCCD chip we can divide the different contributors by noise generated before reaching the gain amplifier as charge transfer noise (N_{ct}) and the remaining readout noise (N_{r0}).
- Total noise (N_{tot}) : The total noise that is generated by an EMCCD is thus:

$$N_{tot} = (N_{tot}^2 + N_{dc}^2 + N_r^2)^{1/2}$$
⁽⁵⁾

The shot noise and the dark current are both dependent on the integration time of the acquisition. Therefor we can group them as N_{τ} . Furthermore the charge transfer part of the readout noise depends on the electron multiplying gain. This gives us the following total noise:

$$N_{tot} = (N_{\tau}^2 + N_r^2)^{1/2} \tag{6}$$

With:
$$N_r = [N_{r0}^2 + (G \times N_{ct})^2]^{1/2}$$
 (7)



Figure 14

Here we see a schematic of an EMCCD camera. When the signal is collected through the lens of the camera the signal only contains shot noise. With every part of the signal processing in the CCD chip the signal is adjusted or noise is added. In the first step the signal is multiplied with the quantum efficiency (QE) of the CCD at the same time dark current is added. When the pixels are read out the charge transfer noise is added. When the EM-gain is applied the signal will be amplified at the cost of some more noise. Eventually when the signal from the CCD chip is converted to digits output noise is generated.

5.2 SIGNAL TO NOISE RATIO

If we want to image very low light intensities we besides a sensitive system a system with a high discriminative power of the signal. This is indicated with the signal to noise ratio:

$$SNR = \frac{Signal}{Noise} \tag{8}$$

We already know how the noise of an EMCCD system is constructed. However, also the signal depends on the integration time and the electron multiplying gain. This results in the following signal to noise ratio:

$$SNR = \frac{G \times S \times \tau}{(N_t^2 + N_r^2)^{1/2}} \tag{9}$$

Since we know from equation (7) that only a part of the noise is affected by the gain G, we can see that increasing the gain higher than unity will result in a higher signal to noise ratio. In addition, activating the gain will also result in excess noise produced by the electron multiplying procedure. Thus the signal to noise ratio will only benefit when the amount of remaining readout noise is higher than the noise due to the gain. Furthermore, as the charge packages begin to grow above 100 thousands of photons per pixel a problem arises with the charge transfer efficiency. Small amounts of charges are left behind during amplification eventually leading to horizontal artefacts in the image. The EMCCD camera will therefor only be beneficial for signals that are higher than the clock induced charge and at the same time is not high enough to cause problems with the CTE.

To obtain the best signal to noise ratio we want to have a back illuminated highly sensitive CCD chip with a high quantum efficiency over a large wavelength range. It should have a thermoelectric cooling system to reduce the dark current as much as possible. It should have an electron multiplying gain to reduce most of the readout noise and be able to do acquisitions as short as possible. At last it should have a sophisticated design in order to minimize the clock induced charge and electron multiplying derived noise and artefacts.

6 PRECLINICAL STUDIES

At the beginning of this project we gained a lot of knowledge on Cerenkov imaging from the literature. We have seen that it is a very useful technique in preclinical research. We are aware of the possible opportunities for in vivo imaging and image guidance. We also have proof that Cerenkov imaging is possible in living human subjects. However, we are also very well aware of the limitations of in vivo imaging with Cerenkov. Because there is so little known about the practical use of CLI in humans the goal of our research was to determine if Cerenkov imaging can be a possible application for intraoperative assistance in oncological surgery.

6.1 CERENKOV LUMINESCENCE ATTENUATION BY TISSUE

Our biggest concern was if there was enough light produced to detect a tumour especially in vivo. Due to the continuous spectrum of the Cerenkov light the penetration trough tissue is more difficult to predict. It is therefore valuable to know how much light is attenuated by tissue. These first experiments were carried out in an earlier phase of this study by another technical medicine student [69]. These experiments were performed on the IVIS 200 system that was available in the animal lab of the NKI. The camera is provided by a back illuminated grade one CCD chip with a high quantum efficiency over the visible and NIR spectrum. The CCD has 2048 by 2048 pixels (1920x1920 effective) and is peltier cooled to minus 90 degrees Celsius. Due to the strict regulations with radioactive sources in the mouse facility of the NKI we were only able to work with closed sources of ¹⁸F and we could only use mouse derived tissue to test the penetration depth.



Figure 15

Schematic of the setup for the mouse fat attenuation experiment. An Eppendorf tube containing ¹⁸F-FDG is placed on the floor of the IVIS 200. In between the lens and the vial a light blocking plastic cone with a hole is placed. On top of the hole a plastic cylinder containing the mouse fat is positioned. Reproduced from [69].

For this experiment 2 vials with activity were created. One Eppendorf tube with 6.97 MBq ¹⁸F-FDG, and another with 25.47 MBq ¹⁸F-FDG. Mouse fat was created from mice in the animal facility of the NKI. The mouse fat was moulded into plastic cylinders with different thicknesses of fat: 1.5, 3, 8 and 12 mm. The Eppendorf tubes were placed in the IVIS 200 and 7 different images were made with FOV 6.5 cm and binning 8x8 (Fig. 15):

- Image of the active tube alone
- Image of the active tube with an empty plastic cylinder on top.
- Image of the active tube with different layers of mouse fat on top; 1.5, 3, 8 and 12 mm.

| | Average radiance | (p/s/cm²/sr) |
|------------------------|------------------|--------------|
| Thickness of fat layer | 6.97 MBq | 25.47 MBq |
| 0 mm | 3.85E+05 | 1.31E+06 |
| 1.5 mm | 1.70E+05 | 4.91E+05 |
| 3 mm | 1.08E+05 | 2.62E+05 |
| 8 mm | 9.57E+04 | 1.22E+05 |
| 12 mm | 8.44E+04 | 1.23E+05 |
| Background | 1.46E+04 | 1.46E+04 |

Table 3

In this table we see the measured radiances at different thicknesses of mouse fat in between the two vials containing ¹⁸F-FDG. Adopted from [69].

This same experiment was repeated with ⁶⁸Ga. This isotope will provide more photons per decay. In this case only one Eppendorf tube was filled with an initial activity of 6.79 MBq. Additionally 2 thicknesses were added to have enough data to present the attenuation of mouse fat in a graph. For this purpose the measured radiances at different time points are corrected for the decay of the ⁶⁸Ga (half-life: 68 min). The graphs can be seen in figure 16.





On the left we see the attenuation of the Cerenkov luminescence derived from ⁶⁸Ga through different thicknesses mouse fat. On the right we see the same data on a logarithmic scale. We would expect a more convex line instead of a concave graph this is caused by the nature of the wabelength spectrum of the Cerenkov luminescence.

We see that the graph of the radiance against the thickness of the mouse fat describes an exponential decrease. This can be expected by uncharged ionization radiation as in photons.

However, on a semi-logarithmic scale on the right we see that the relation between radiance and tissue thickness is not exponential and not even shows a convex shape as you expect with a pointsource. This is influenced by the scatter that occurs in the tissue. Scatter tends to attenuate even more light with a thicker layer of tissue. However, the inverse exponential nature of the spectrum of Cerenkov has even more influence on the attenuation curve. Because the majority of the radiance consists of short wavelengths with low penetrative power the attenuation ratio is the highest in the beginning. This gives the logarithmic curve a downwards dent. Eventually this leads to a loss of signal of 95% in the first 3 mm of tissue, 65% in the second 3 mm of tissue and 40 % in the third 3 mm of tissue. This means that roughly 1 % of the signal will survive 1 cm of prepared mouse fat.

6.2 ABSOLUTE UPTAKE VALUES OF RADIOPHARMACEUTICALS IN TUMOURS AND ORGANS

The Cerenkov light derived from radioactive decay is very limited. As stated in the introduction the amount of light depends mainly on the activity, the energy spectrum of the corresponding isotope and the refractive index of the medium. Since the amount of light is directly proportional to the activity it is important to know how much activity is taken up by a tumour. In standard clinical practice the uptake in a tumour is determined by the SUV (standard uptake value). The SUV is a simple quantative analysis for the uptake of PET tracers in human tissue. The SUV is calculated as the ratio between: (1) the radioactivity concentration (c(t)) determined by the PET scan and (2) the administered activity divided by bodyweight. Adjusted for time and decay.

$$SUV = \frac{c(t)}{Injected \ activity \ (t)} /_{Bodyweight}$$
(10)

It presents the uptakes of a certain tissue relative to an imaginary situation were the activity is equally distributed over the whole body. This means it also provides information on the amount of activity against the background and thus the tumour to background ratio. This is of course an important parameter in optical imaging but for the experiments where we want to determine the sensitity we are interested in what the actual activity in a human tumour is. Therefor we took the parameter (c(t)) from different PET-CT scans of different tumors in different patients.

METHODS

In the database of the nuclear medicine department we have selected patients who underwent an ¹⁸F-FDG PET-CT scan who were known to have primary breast tumours, breast metastasis, and colorectal cancer. For each group we included 10 patients. Evaluation of the images was performed on the Osirix imaging processing software. To determine the activity we drew a region of interest (ROI) around the tumour based on the PET and CT data. From this ROI we determined the mean activity concentration in Bq/mL, the max activity in Bq/mL and the volume of the tumour in mL. We also did this for patients who received a ⁶⁸Ga-Dotatate PET-CT scan. Since we determined this is a potentially valuable isotope for CLI. ⁶⁸Ga-Dotatate is a fairly different radiopharmaceutical than ¹⁸F-FDG with another distribution pattern and a lower administration dose.

Because the ⁶⁸Ga scans take place less frequently we studied all types of tumours that were present in the available patients. We examined the tumours in the same way as we did for the ¹⁸F-FDG scans. Additionally since the distribution of ⁶⁸Ga accumulates highly in certain organs we determined the uptake in the thyroid, salivary glands, liver, spleen, kidneys and bladder. We did this to determine which organs could interfere with Cerenkov imaging.

6.2.1 RESULTS

For the ¹⁸F-FDG we selected 10 primary breast tumours, 10 breast metastasis and 10 colorectal tumours. Additionally we included 2 lung tumours and 4 liver metastasis. We also measured the uptake in the liver.

The average mean uptake concentration in primary breast tumours was 7.5 kBq/mL (\pm 3.8). For breast metastasis this was comparable; 7.1 kBq/mL (\pm 4.1). In colorectal cancer the average mean uptake was slightly higher with concentrations of 12.2 KBq/ml (\pm 3.2). The overall uptake concentration including the liver metastasis and the lung tumours is 8.9 kBq/ml (\pm 4.0)(Table 4).

For the ⁶⁸Ga we selected 12 patients. In these 12 patients we found 31 tumours including 11 in the liver, 8 in lymph nodes throughout the body, 6 in the pancreas, 4 metastasis in the bone and further in the ileum the breast and in the lung. In addition we have measured the uptake values of the following organs in all twelve patients; Thyroid liver, spleen, kidneys and the bladder. We could only find the uptake of the salivary glands in 8 patients.

We saw an average mean uptake concentration of 12.9 kBq/mL (\pm 7.8) in liver tumors.4.7 kBq/mL (\pm 2.2) for lymph nodes and 16.2 kBq/ml (\pm 15) for pancreas tumours. For all tumours together we had an average mean uptake of 10.6 kBq/ml (\pm 9.5).

The uptake average uptake in the organs was low for the thyroid and the salivary glands, moderate for the liver and the kidneys but high for the spleen and exceptionally high 35,4 kBq/mL in the bladder.

| | Absolute uptake values | s of 18F-FDG and G | ia68 in kBq/ml in PET- | CT examinations | ; |
|-------------|------------------------|-----------------------|----------------------------|--------------------------|--------------------------|
| Radiotracer | Region | mean concentration | Standard deviation of mean | Maximum concentration | Minimum concentratior |
| | Primary Breast tumor | 7,47 | 3,78 | 13,2 | 3,2 |
| | Breast metastasis | 7,1 | 4,11 | 16,9 | 3,2 |
| 18F-FDG | Colorectal cancers | 12,22 | 3,25 | 16,7 | 6,8 |
| | Liver metastasis | 7,4 | 1,04 | 8,9 | 6,5 |
| | All tumors | 8,88 | 4,07 | 16,7 | 3,2 |
| | Liver tumors | 12,89 | 7,81 | 35 | 7,9 |
| | Lymph nodes | 4,67 | 2,2 | 7,5 | 2,2 |
| | Pancreas tumors | 16,15 | 14,97 | 46 | 4,9 |
| | All tumors | 10,64 | 9,47 | 46 | 2,2 |
| 68Ga | Thyroid | 2,59 | 0,87 | 3,8 | 1,6 |
| | Salivary glands | 2,76 | 0,83 | 3,6 | 1,5 |
| | Liver | 6,73 | 2,42 | 10,5 | 2,6 |
| | Spleen | 12,67 | 5,34 | 23,6 | 5,4 |
| | Kidney | 7 | 1,52 | 9 | 4,5 |
| | bladder | 35,41 | 20,51 | 69,1 | 6,2 |

Table 4

Absolute uptake values that are determined in PET/CT images. The mean concentration of activity of 10 tumours in 10 different patients are given. In the right two columns the uptake of the most active and least active tumour are shown. In the ⁶⁸Ga-Dotatate scans also organs with high physiological uptake have been included.

On average the tumour uptake of ¹⁸F-FDG lies slightly under 10 kBq/mL and for ⁶⁸Ga slightly over 10 kBq/mL. This gives us an idea of the order of magnitude we should at least be able to detect. Because we also want to measure in vivo we have attenuation due to the tissue. This means that produced Cerenkov Luminescence can be detected only partially. Converted back to in vitro studies this means we should be able to detect even lower concentrations than 10 kBq/mL. What we also can see from this data is that despite the fact ⁶⁸Ga is administered in lower doses than ¹⁸F, pproximately 80 MBq versus around 190 MBq, the absolute dose in the tumour is slightly higher. As we look at equation 10 we can see that this will result in a SUV that is more than 2 times higher than that in ¹⁸F-FDG. Together with the fact that there is very high uptake in certain organs this means that there is a relative low uptake in the overall healthy tissue. This means that for tumours outside the proximity of the high uptake organs there will be a high tumour to background ratio which is an advantage for optical imaging.

6.2.2 DISCUSSION

From these results it is made clear that we have to be able to detect very small activities. If we want to perform in vivo imaging we should be able to detect activities of at least lower than 10 kBq/ml. To determine if this was possible we first consulted the literature. Many articles are published on in vitro Cerenkov imaging with known activities. In these articles we looked what the lowest activity was they used or that they could detect. This information can be seen in table 5.

| Study | lsotope | Activity (µCi) | Activity (KBq) | acquisition time (seconds) | Measuring device | distance |
|-----------------------|---------|----------------|----------------|----------------------------|------------------------------|----------|
| Robertson et al. 2009 | 18F | 100 | 3700 | 10 | IVIS 100/200 | |
| | 13N | 10 | 370 | 10 | IVIS 100/200 | |
| | 18F | 1 | 37 | 60-300 | IVIS System | |
| | 64Cu | 3 | 111 | 60-300 | IVIS System | |
| | 1311 | 1 | 37 | 60-300 | IVIS System | |
| | 90Y | 0,2 | 7,4 | 60-300 | IVIS System | |
| | 177Lu | 2 | 74 | 60-300 | IVIS System | |
| | 111In | 5 | 185 | 60-300 | IVIS System | |
| | 1311 | 100 | 3700 | 180 | IVIS Kinetic | |
| | 1241 | 1,75 | 64,75 | 30 | IVIS 100 | |
| | 1311 | 2,5 | 92,5 | 30 | IVIS 100 | |
| | 18F | 1,3 | 48,1 | 300 | IVIS 100 | |
| | 90Y | 1 | 37 | 30 | IVIS 100 | |
| | 18F | 810 | 30000 | 30 | IVIS 200 | |
| | 68Ga | 810 | 30000 | 30 | IVIS 200 | |
| | 1241 | 0,4 | 15 | 60 | IVIS 200 | |
| | 18F | 1,05 | 39 | 60 | IVIS 200 | |
| | 1311 | 3,57 | 132 | 60 | IVIS 200 | |
| | 68Ga | 0,16 | 6 | 60 | IVIS 200 | |
| | 89 Zr | 61 | 2660 | 30 | IVIS 200 | |
| | 18F | 2,7 | 100 | 300 | Stanf. Phot. ICCD Mega10Z | 8 cm |
| | 68Ga | 0,5 | 18,5 | 300 | Andor ixon ultra 888 endosc. | 5mm |
| | 18F | 5 | 185 | 300 | Andor ixon ultra 888 endosc. | 5mm |
| | 68Ga | 6400 | 236800 | 30 | Andor ixon ultra 888 endosc. | 5 mm |

Table 5

Collection of all the studies that have published their results on in vitro CLI. The important factor is what the lowest activity was they were able to measure. This activity is given in the third and fourth column. The show the perspective also the isotope the acquisition time and the imaging modality are given. The lowest activity of ¹⁸F that is measured is 37 kBq by Liu et al. [1], [14], [26], [29], [33], [34], [39], [42], [44], [66], [70]

In general these studies use high activities for their experiments and especially higher than the amounts of activity we have found as the uptake in human tumours. There results that came the closest were the 37 kBq of ¹⁸F measured by Liu et al and 39 kBq by Park et al [33], [66]. This is still 4 times higher than the limit we need to be able to detect. Really low activities could be detected for ⁹⁰Y by Liu and Iodine-124 (¹²⁴I) and ⁶⁸Ga by Park. However, according to Gill et al. these emit respectively 35.8, 6.8 and 25.7 as much photons as ¹⁸F [<u>47</u>]. Furthermore it is not really clear if the

experiments in these publications tried to find the minimal detectable activity. In many cases they used convenient measurable amounts like the 37 kBq from Liu et al. which comes down at 1 μ Ci. Most of the in vitro experiments are performed in one of the types of IVIS systems by PerkinElmer. Thorek et al was the first to perform in vivo measurements with an ICCD (Intensified Charge-Coupled Device) standalone camera. He managed to detect an activity of 100 kBq this is even 10 times as much as we should detect [42]. The last three publications are from a Chinese group who are developing the ECLI. In this case a fibre scope is attached to the camera which has a smaller aperture lens. Such a construction leads to a lower intensity than otherwise will be obtained. Due to the lack of inromation of the numerical aperture not good comparison can be made. Furthermore in all of the publications they talk about the absolute activity and they do not concern the concentration of the activity which is an important factor of how intense the luminescence should be.

Besides the in vivo Cerenkography performed by Thorek et al. We cannot determine from the literature if it is feasible to image the activity actually taken up by a human tumour. To determine what the detection limits concerning the activity are we performed our own series of tests.

6.3 MINIMAL DETECTABLE ACTIVITY CONCENTRATION OF ¹⁸F-FDG AND ⁶⁸GA.

Like previous experiments we performed Cerenkov imaging on the IVIS 200 in the animal facility of the NKI. We performed the imaging on Eppendorf tubes filled with ¹⁸F-FDG. We made a dilution series of 8 Eppendorf tubes to determine the minimal detectable activity concentration.

6.3.1 METHODS

The IVIS system has several options to make acquisitions. The different acquisition settings will result in changes in the sensitivity of the system, a change in background noise or on total SNR (Signal to Noise Ratio). These settings were thus of importance for this experiment. There are five parameters that can be set on the IVIS 200:

- Field of view: There are 5 different settings for the field of view; A,B,C,D and E. They represent a field of view between 4 cm for A and 25 cm for E. Concurrently with changing the FOV the object distance gets smaller as the field of view gets smaller. The closer the object is to the lens, the more light will be detected by the camera and your signal will be higher.
- F/stop: This represents the aperture of the lens. It controls both the amount of light and the depth of field of the acquisition. The higher the f-stop number the smaller the aperture and the less light will be detected by the camera. The choice for a low f/stop number will result

in a high sensitivity but comes at the cost of a small depth of field. The lowest f/stop number is f/1.

- Binning: With binning you can artificially increase the pixel sizes. This will give a higher sensitivity because you will catch more signal per pixel but it also comes at the cost of the spatial resolution. Binning options are 1x1, 2x2, 4x4, 8x8 and 16x16. A binning of 2x2 means that each pixel is build-up of 4 components. A binning of 4x4 means that each pixel consists of 16 components. The SNR will have a quadratic improvement with each binning step.
- Exposure time: The amount of time the shutter of the camera is open to make an image. This number can be chosen from 0.5 seconds. Naturally the longer the exposure time the more signal will be detected.
- Emission filters: Different types of filters can be placed in front of the CCD camera lens. This is especially useful for fluorescence and spectral imaging. For bioluminescence and also for Cerenkov imaging the filter will be left open to obtain the best images.

For our experiment we want to have an acquisition with the most optimal setting to detect a small activity of ¹⁸F. We thus need best possible sensitivity and the highest signal to noise ratio but the acquisition cannot be too long and the spatial resolution must be sufficient enough to distinct the shape of the activity. We therefor take the smallest field of view that is possible with the smallest object distance and we also take the lowest f/stop number to obtain the most signal. We tested different binning options and exposure times to determine what the best acquisition settings are for CL detection.



Figure 17

A dilution series is prepared with an initial activity of around 1 MBq in 1ml in the first vial. All the other vials are filled with 500 μL of water. From the first vial 500 μL is pipetted and mixed with the second vial. We repeat this for the second vial were we take 500 μL and mix it with the third vial. We do this up to the last vial and after we mixed this we remove 500 μL. In the end we add 500 μL of water to each vial to obtain the activity that is shown under the vials per mL We made a dilution series of 8 Eppendorf tubes of 1mL. We started with 1 MBq and diluted this eight times. After the dilution the highest activity was calibrated at 450 kBq. Consequently every following tube has half the concentration than the previous tube with the lowest concentration of approximately 3.5 kBq. (Fig. 17)

The Eppendorf tubes were placed in two rows of 4 tubes so that they were able to fit on the 6.5 cm field of view of the IVIS 200. Acquisitions were made with exposure times of 5, 10, 20, 40, 60, 120 and 300 seconds for binning of 2x2, 4x4, 8x8 and 16x16. For the f/stop we took f/1 and we did not use any filters.

Other than in the previous experiments we are not interested in the amount of photons we measure but the amount of signal we detect and if this can be distinguished from the background. Therefor we want to determine the number of counts and the signal to noise ratio of the different tubes for different acquisition times and different binning settings. To be more precise we want to determine the minimal detectability. Theoretically this can be defined as the difference of the signal and the background hast to be at least 3 times higher than the standard deviation of the background. Therefor the signal to noise ratio we use is determined by the following equation:

$SNR = \frac{signal}{noise} = \frac{mean \ counts \ per \ pixel \ of \ object-mean \ counts \ per \ pixel \ of \ background}{Standard \ deviation \ of \ counts \ per \ pixel \ of \ background}$ (11)

The data to calculate the SNR are obtained by drawing the ROIs in Living Image software provided by Xenogen. (Fig. 18) The SNR will be calculated for each vial for each acquisition. Since the acquisitions are made at different moments in time the SNRs are corrected for decay in order to make a fair comparison between the different acquisitions.



Figure 18

Method of determining the signal to noise ratio. An ROI is drawn on the activity in the vial and an ROI is drawn in the background. From these ROIs the mean number of counts is given and the standard deviation which determines how noisy the signal is. The mean of the background is subtracted from the average signal from the activity. This is then divided by the standard deviation of the background.

6.3.2 RESULTS

From the results we could confirm that as expected the relation between the activity and the SNR is linear as well as the relation between exposure time and the SNR. We could also confirm the quadratic relation between the binning and the SNR. This means that we obtained the highest signal to noise ratio for the higher activities, the longer exposure times and the highest binning. We obtained a SNR of 737 for the 450 kBq/ml vial an exposure time of 300 seconds and 16x16 binning. (Fig. 19) More interesting was the activity that was the least detectable to determine the limits of the imaging device. Theoretically the SNR has to exceed 3 for the signal to be detectable. In 4 of the 28 acquisitions the SNR for the least active vial exceeded this threshold. In the 300 second acquisition with 8x8 binning and for the 60, 120 and 300 second acquisition with 16x16 binning. This means that we are practically able to detect activity concentrations of 3.5 kBq per mL but at the cost of long exposure times and extensive binning.



Figure 19

Image of the dilution series containing ¹⁸F performed on the IVIS 200. This is a 300 second acquisition with the highest amount of binning 16x16. We can distinct all the vials from the background including the one with the least activity. The signal to noise ratio for this particular vial was 13.85.

6.4 DETERMINING THE BEST STANDALONE CAMERA AND ITS MINIMAL DETECTABILITY

Since we want to determine the clinical feasibility of CLI we cannot use a system as the IVIS 200 but we have to make use of another type of commercially available camera. This camera has to be a standalone camera and preferably an EMCCD camera. As mentioned in <u>chapter 5</u> EMCCD cameras will give the best performance for low light imaging as is the case in CLI. We made a selection of commercially available EMCCD cameras and a few other types of cameras that have been used in CLI and made a comparison based on their specifications. Because we wanted to know what the minimal detectable activity was we needed the most sensitive camera which provides the best SNR. Important are thus the factors that determine the sensitivity and the noise of the camera. The most important specifications are on the one hand the QE which determines the sensitivity of the CCD chip and on the other hand the mechanisms that contribute to the total noise floor. These mechanisms are the amount of chip cooling that will have an effect on the dark current, the readout noise and the clock induced charge. Furthermore the gain possibilities and the resolution of the chip are of importance.

| Camera | Resolution Pixel size | Pixel size | Cooling | Quantum efficiency > 80 % | Spectral range | Dark Current | Readout Noise | CIC | Max gain |
|--------------------------------|-----------------------|------------|--------------------|---------------------------|----------------|--------------|---------------|-------|----------|
| Andor iKon M-934 CCD | 1024x1024 | 13 µm | -80 AC / -100 LC | 380 - 900 nm | 200-1100 nm | 0.0003 | 13,6/~ | ł | ł |
| Andor ixon ultra 888 | 1024x1024 | 13 µm | -60 AC / -75 LC | 420 - 790 nm | 300 - 1100 nm | 0.0005 | 130/<1 | 0.005 | 1000 |
| Falcon Blue Emccd | 1004x1002* | 8 µm | -20 AC | z | 180 - 1100 nm | <1 | 27/<1 | ł | 1000 |
| Hamamatsu ImagEMx2 | 1024x1024 | 13 µm | -50 AC / -65 LC | 450 - 750 nm | 300 - 1100 nm | 0.001 | 15/<1 | 0.01 | 1200 |
| Horiba Efis EMCCD † | 1004x1002 | 8 µm | -20 AC | s | 180 - 1100 nm | <1 | 27/<1 | ł | 1000 |
| Nuvu EM-N2 1024 | 1024x1024 | 13 µm | -110LN ** | 430 - 790 nm | 250 - 1100 nm | 0.0004 | ~/< 0.1++ | 0.002 | 5000 |
| Nuvu HNu 1024 | 1024x1024 | 13 µm | -85 AC / -90 LC | 430 - 790 nm | 250-1100 nm | 0.0004 | ~/< 0.1++ | 0.002 | 5000 |
| Photometrics Casll1024 | 1024x1024 | 13 µm | -60 AC | 460 - 760 nm | 250 - 1050 nm | 0.005 | 45/<1 | 0.01 | 1000 |
| Photometrics Evolve 512 Delta | 512x512 | 16 µm | -75 AC / -75 LC*** | 380 - 790 nm | 300 - 1100 nm | 0.003 | 75/<1 | 0.002 | 1000 |
| Princeton instr. PI-MAX4 1024 | 1024x1024 | 13 µm | -20 AC | s | 280 - 900 nm | 2.5 | 90/<1 | ł | ł |
| Princetoninstr. ProEm+ 1024B | 1024x1024 | 13 µm | -65 AC / -75 LC | 430 - 800 nm | 280 - 1100 nm | 0.002 | 40/<1 | 0.01 | 1000 |
| Rolera EMC2 | 1004x1002 | 8 µm | -50 AC | s | 200-1100 nm | 0.06 | 20/<1 | ł | z |
| Stanford Photonics XR Mega 102 | 1024x1024 | 12.5 µm | -20 AC | ł | 300 - 750 nm | 1-2/framettt | 15/~ | ł | 100000 |
| 6 9 | | | | | | | | | |

* Resolution: The 1004x1002 sensor are made by Texas Instruments.

** Not specified at what conditions

*** Liquid cooled at 20° ambient air.

[†] Same camera as Falcon Blue

++ Not certain if readout noise is better or more extensive calculation

 $^{++}$ 1-2 per frame detected at full speed, 30 FPS $^{\sim}$ A box filled with ' $^{\sim}$ ' means that the specification is unknown or not applicable

Table 6

Overview of different commercially available EMCCD cameras, one ICCD camera and one CCD camera. All the important parameters for low light imaging with high sensitivity and readout speed without gain. The second number is at full gain. Clock induced charge is part of the noise floor of EMCCDs. It is measured in electrons per pixel per frame at optimal electrons per frame. Displayed numbers are based on optimal cooling and gain. Read noise is measured in the amount of electrons per frame. The first number is obtained at full low noise are compared. Cooling is in minus degree Celsius for air cooled (AC) at 20° degree ambient air, water cooled (WC) at 10° coolant and liquid nitrogen cooled (LN). This numbers displayed are based on the maximum readout speed. Quantum efficiency shows the range in spectrum where the QE is higher than 80 % derived from QE curve. The boxes with a '~' mean that QE never exceeds 80 % but is 65 % max. Dark current is measured in electrons per pixel per second. Only for the XR Mega 10Z it is determined in cooling and full gain. Gain is the amount of multiplication of electrons in EMCCD and in photons for ICCD. The CCD camera doesn't have a gain. As we can see from table 6 there are two types of chips manufactured. The chips made by Texas instruments show inferior specifications than the other chips. They are not cooled to extreme low temperatures and besides they lack the information on the QE. The rest of the chips show to have a similar quantum efficiency. The big difference is made in the cooling possibilities and the performances on the different types of noise. We have selected 4 cameras that in our opinion promised the best performance for low light imaging (Fig. 20):

- Andor iXon ultra 888
- Princeton ProEM+ 1024b
- Nüvü Hnü 1024
- Hamamatsu ImagEMx2

We have tested these four cameras in our own build setup in a similar way as we did in the previous experiment on the IVIS. In this way we tried to determine what camera is the best and at the same time determine the limit for Cerenkov luminescence detection.



Andor iXon ultra 888



Hamamatsu ImagEM x2



PI ProEM 1024b



Nuvu Hnu 1024

Figure 20

The 4 different EMCCD cameras that were tested.

6.4.1 METHODS

6.4.1.1 SETUP

A dark cabinet was available at the NKI. We adjusted the cabinet in such a way that an EMCCD camera could be placed with the lens facing downwards to the direction of the desired specimen. Furthermore we made sure that the cabinet was perfectly light tight to make sure no ambient light could reach the cameras sensor. (Fig. 21)



Figure 21

Schematic of the setup that was built to perform CLI with a standalone EMCCD camera. In a dark cabinet the camera is placed on a shelf with the lens sticking trough this shelf pointing downward. The specimens are placed on a dark plateau to position them as close to the lens as possible. The camera has a wire connection with a computer placed outside the cabinet that can operate the camera.

6.4.1.2 CAMERA LENS

To make a sensitive optical imaging system the lens is very important. The lens is mostly the determinant on how much signal can be detected by the system. Especially with the low amounts of light coming from CL it was very important to catch as much light as possible. This means that we had to provide a lens that has a low f/stop number or a high aperture, combined with a short imaging distance. The f-number is has a quadratic relation with the opening of the lens and thus the amount of light that is collected. An f-number that is twice as high will result in a four times smaller aperture. (Fig. 22) Next to that it is also important that the specimens can be imaged from nearby. The distance of an object to the lens also has a quadratic relation. To catch as much light as possible

you want the object to be as close to the lens as possible. Therefor a short focus distance is required to obtain a short object distance without compromising the sharpness of the image.





A representation of the different f/stop numbers that indicate the aperture. We see that a quadratic relation between the f/stop number and the area of the aperture. This means that a twice as low f/stop number leads to a four time increase in signal. Adopted from <u>www.gotosnapshot.com</u>



Figure 23

Here we see a schematic of the CCD sensor and different image projection surfaces of different lens formats. The sensor in red, is 13 by 13 mm. You would think this would have the best fit with a ½". However, lens formats are measured by the diagonal of the square the circle just fits in. This means that for this CCD chip we need a 1" lens format to make use of the entire CCD chip.

For our experiments we selected the best option that was available in house and was compatible with all the EMCCD cameras (C-mount). We had the opportunity to use a Pentax TV lens with a focal distance of 8.5 mm and a maximal aperture of f/1.5. This led to a minimal object distance of

20 cm. However, the format of the lens, a manufacturing parameter of the housing combined with the mount, was a 2/3". This meant that the CCD chip which have a size of 13x13 mm are not fully utilized. (Fig. 23) Preferably we would like a 1" format lens.

6.4.1.3 ACQUISITION

The cameras were delivered with the corresponding software to operate the camera. Besides the options that were available in the IVIS system the different EMCCD systems provided a lot more options to set up different acquisitions. There are different readout modes, you can choose not only times for the exposure but also the rate of readout. Furthermore you can choose the speed of the storage shift on the chip and the pixel rate of the analogue to digital converter. Additionally the temperature of the chip can be set and there are different options to perform corrections. These settings will have a huge effect on the resulting image. Especially the noise levels can be tweaked for any other acquisition setting.

It was not a simple task to find out what the optimal acquisition options are because many of the settings are interdependent. For instance the speed of the readout should be matched with the gain and the exposure time. Also for all cameras the setting options were slightly different and the behaviour of each camera was unique. Some cameras were more prone to noise than others and other cameras showed artefacts if the settings were not fully optimized.

The performance of the camera and the ease of use was very important. Next to that we wanted to know which camera could provide us with the highest SNR when imaging a certain amount of radioactivity. Therefor we did not compare the cameras in the exact same settings but we tested them all to their limits to compare their overall performance. This means that all the parameters that were not camera dependent were kept the same: the used lens, the distance between the lens and the specimens, the exposure time and the amount of binning. Although the activity in the vials of the dilution series was not always the same we can easily correct for this since it shows a linear relation.

With each camera we have performed a series of acquisitions on the dilution series with ¹⁸F and ⁶⁸Ga. We chose the acquisition settings that after extensive testing showed the best performance. As with the experiments on the IVIS we compared different acquisition times but additionally we also made acquisitions with different EM gains. To compare the different acquisitions for a camera with each other we compared the SNR of the highest active vial. Next to that we determined the lowest detectable activity for the best 60 second acquisition and the best 300 second acquisition.

6.4.2 RESULTS

6.4.2.1 ANDOR IXON ULTRA 888

With the Andor camera we were able to obtain nice images without any gain. This is the so called conventional mode. For longer acquisitions however, a lot of 'cosmic rays', saturated pixels due to gamma rays falling directly on the camera chip, appeared. The software had no options to remove this data from the image. In the conventional modes these dots could be ignored. However, when the EM-gain was increased to a certain amount the cosmic rays resulted in a horizontal striking pattern. This is an artefact caused by charge transfer efficiency problems. A phenomenon known to EMCCD' s when pixels in the gain register pack to many electrons [<u>67</u>]. As a result acquisitions with long exposure times and high EM-gain are useless. (Fig. 24)





Raw data images of the dilution series made with the Andor EMCCD camera. We can see a lot of incident gamma rays which will disturb the images significantly. Especially when the EM-gain is applied, upper right, this will lead to horizontal patterns that distort a lot of the data. On the lower left we see an image made with ⁶⁸Ga. Due to higher light production lower activities can be used which lead to less gamma rays and consequently a better distinction with the background. Also there is less binning required (1x1 instead of 4x4) which results in a higher resolution.

6.4.2.2 PRINCETON INSTRUMENTS PROEM + 1024B

The performance of the ProEm for the ¹⁸F dilution series was a little bit poor, and definitely less than the Andor. An explanation for this performance can be that there was some degree of light leakage into the light tight cabinet. This can be seen by the brighter circle in the centre which represents the aperture of the lens. For the ⁶⁸Ga dilution series no such thing could be detected. These resulted in very clear images of the activity up to low levels of activity. The striking artefacts caused by CTE problems was not present in this camera.(Fig 25)



Figure 25

Selection of the results made with the Princeton instruments EMCCD camera. Above we see the ¹⁸F dilution series. Only a few of higher active vials can be detected. Only several hundreds of kBq/ml can be distinguished. We can also see a bright circle in the middle of the images. This is the projection surface of the 2/3" lens. This means that there was some ambient light present during the acquisitions. The images with the ⁶⁸Ga again show more clear images and the vial of 21 kBq/ml can be distinguished.

6.4.2.3 NÜVÜ HNÜ 1024

The Nüvü camera was the one which had the best specifications and therefor the expectations of the performance were high. Unfortunately the company that manufactured the camera is a new kid on the block and this was reflected by the provided software that gave a lot of problems. As a consequence we were not able to perform exposure times longer than 30 seconds and even these
were rare. Added to this the images turned out to be very blurry. Were the other cameras give a clear outline of the activity containing vials the images acquired with the Nüvü just represented intense blobs. In a later stadium when the camera was already returned to the company it turned out that the distance from the flange to the CCD chip was not correct and therefore not compatible with a C-mount lens. (Fig. 26)



18F - 10 seconds -EM = 1000

68Ga - 30 seconds - EM = 1000

Figure 26

Results made with the Nüvü EMCCD camera. Directly noticeable is that the images are not as sharp as the ones made with other cameras. This was of caused by an incorrect flange distance from the chip to the flange. However, despite the fact only a maximum of 30 second acquisition could be made 8 different blobs can be distinguished and also the signal to noise ratios were pretty high.

6.4.2.4 HAMAMATSU IMAGEMX2

Due to a limited availability of the Hamamatsu camera we only performed the dilution series with the F¹⁸. The images acquired with the Hamamatsu camera were very noisy. This led to overall small signal to noise ratios but nevertheless we were able to visually detect the lowest activity. Also here we found the horizontal pattern due to the CTE problems but to a lesser extent. The limited amount of time we had the camera at our disposal might have compromised that we used an optimal acquisition. (Fig. 27)



18F - 300 seconds - Em = 598

Figure 27

Images made of the ¹⁸F dilution series with the Hamamatsu EMCCD camera. The images both show a very noisy pattern. This was also reflected in the signal to noise ratios. Also in this camera some horizontal artefacts can be seen.

6.4.2.5 COMPARING THE DIFFERENT CAMERAS

18F - 60

598

To compare the different cameras we selected the best performances of the camera in comparable settings. We took the highest signal to noise ratio we measured with the corresponding activity in the vial and we selected the minimal detectable vial with its SNR and the corresponding activity. We did this for both a 30 second and a 300 second exposure time. The binning was set at 4x4. To give a simpler comparable outcome measure we took the ratios of the SNR and the activity of the highest SNR and the lowest detectable activity and averaged them.

The ¹⁸F dilution series experiments were all performed with similar settings except that the exposure time for the Nüvü camera is shorter. For the ⁶⁸Ga Dilution series several parameters are different which make them hard to compare with each other. If we look at the table 7 with all the results we see a lot of unexpected outcomes. Concerning the ¹⁸F dilution series which should be comparable we expect the SNR of a 300 second exposure time to be the fivefold of the SNR of a 60 second exposure time. This is not the case and even differs among the different cameras from 1.8 for the Princeton instruments to 10.3 for the Hamamatsu camera. There is also no correlation between the activity in the vial and the measured SNR which should be directly proportional to each other. Also the quadratic relation between the SNR and the binning settings is not present.

The results of the Ga⁶⁸ dilutions are even more disturbing. The Andor camera provides a better SNR for the 60 second exposure time compared with the 300 second exposure time. These inconsistencies make the results very difficult to interpret and makes it hard to make a statement on which camera has the best performance. What we can say is that we are at least able to detect activities in the range of a few tens of kBq of activity.

Based on the results of the ¹⁸F dilution the Nüvü camera seems to provide the best SNR values. Even with a six time shorter exposure time the SNRs are roughly 10-30 times higher than its competitors. However, due to malfunction in the design the images are very blurry and are not as reliable as the others. The fact that ¹⁸F gives a better outcome than ⁶⁸Ga is also strange. This is probably due to unreliable values measured for the lowe activities which are basically blurs. Also disturbing is the fact that the Princeton instruments camera had the poorest performance in the F¹⁸F dilution series but provided the best images and the highest SNRs when ⁶⁸Ga was imaged.

It is thus impossible to determine which camera has the best performance to image. However, from our own experiences and based on the results we have to most trust in the Andor or the Princeton Instrument camera. Since we have found a method to minimize the adverse effects of the Andor camera we decided to use the Andor camera for the following experiments.

| SNR Activity SN | | | | 68-0 | Gallium | | 18-Fluor | | | | | | | | | |
|--|-----------------------|--------|-------------|------|---------|------|----------|-----|------|------|-------------|------|--------------|--|--|--|
| Andor 60* 300* 5,99 7,05 0,15 0,22 15,57 18,71 0,59 0,87 33,34 27,12 60 300 3,17 0,31 4,91 17,55 0,17 2,14 35,39 9,21 23,29 Hamamatsu 60 300 60 300 3,17 0,11 37,04 0,92 34,03 10 8,34 0,04 9,33 2,04 23,29 Nuvu** 30 300 3,17 0,11 37,04 0,92 34,03 10 8,34 0,04 9,33 2,43 111,66 Prioreton Instruments 60 3,42 0,08 42,97 1,34 36,39 60 4,41 0,85 5,83 1,71 4,30 | | | | | Highe | | | | | | Highe | | SNR/Activity | | | |
| Andor 300* 7,05 0,22 18,71 0,87 27,12 300 4,91 0,17 35,39 2,04 23,25 Hamamatsu 60 300 60 300 | | | | | | | | | | | | | | | | |
| 300* 7,05 0,22 18,71 0,87 27,12 300 4,91 0,17 35,39 2,04 23,25 Hamamatsu 60 300 60 300 60 60 2,95 0,77 7,25 3,07 3,10 Nuvu** 30 300 3,17 0,11 37,04 0,92 34,03 10 8,34 0,04 9,33 2,43 111,60 Princeton Instruments 60 3,42 0,08 42,97 1,34 36,39 60 4,41 0,85 5,83 1,71 4,30 | | 60* | 5,99 | 0,15 | 15,57 | 0,59 | 33,34 | 60 | 3,17 | 0,31 | 17,55 | 2,14 | 9,21 | | | |
| Hamamatsu 300 | | 300* | 7,05 | 0,22 | 18,71 | 0,87 | 27,12 | 300 | 4,91 | 0,17 | 35,39 | 2,04 | 23,29 | | | |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | | 60 | | | | | | 60 | 2,95 | 0,77 | 7,25 | 3,07 | 3,10 | | | |
| Nuvu** 300 300 300 300 300 Princeton Instruments 60 3,42 0,08 42,97 1,34 36,39 60 4,41 0,85 5,83 1,71 4,30 | | 300 | | | | | | 300 | 1,29 | 0,02 | 6,79 | 2,64 | 32,00 | | | |
| 300 300 <td></td> <td>30</td> <td>3,17</td> <td>0,11</td> <td>37,04</td> <td>0,92</td> <td>34,03</td> <td>10</td> <td>8,34</td> <td>0,04</td> <td><i>9,33</i></td> <td>2,43</td> <td>111,66</td> | | 30 | 3,17 | 0,11 | 37,04 | 0,92 | 34,03 | 10 | 8,34 | 0,04 | <i>9,33</i> | 2,43 | 111,66 | | | |
| Princeton Instruments | | 300 | | | | | | 300 | | | | | | | | |
| 300*** 3,77 0,08 45,45 1,32 40,20 300 3,30 0,42 12,86 1,67 7,78 | Princeton Instruments | 60 | 3,42 | 0,08 | 42,97 | 1,34 | 36,39 | 60 | 4,41 | 0,85 | 5,83 | 1,71 | 4,30 | | | |
| | | 300*** | 3,77 | 0,08 | 45,45 | 1,32 | 40,20 | 300 | 3,30 | 0,42 | 12,86 | 1,67 | 7,78 | | | |
| * Binning 1x1 | | * | Binning 1x1 | | | | | | | | | | | | | |

** 512x512 CCD chip and out of focus

Binning 2x2

Table 7

An overview of the results for the 4 different EMCCD cameras for the ⁶⁸Ga and ¹⁸F dilution series. From each 60 second and 300 second acquisition the SNR and the activity of the most intense and the least detectable vial are taken. For an easy comparable parameter we combined the SNRs of the both vial and corrected them for the activity. This resulted in a mean SNR per MBq which can be found in the last column of both the ⁶⁸Ga and ¹⁸

7 CLINICAL PILOT STUDIES

7.1 HUMAN IN VIVO CLI STUDY

7.1.1 RATIONALE FOR THE STUDY

In the first experiments we have proven in vitro that we are able to detect activities, in accordance with the actual uptake in human tumours. This is of course a favourable setting with transparent substances and vials. Translation of this technique from a preclinical setting into the operating room has led to a is another challenge. One of the biggest issues is the spectral character of the Cerenkov light. Blue light is generally well attenuated by perfused tissue, thus the penetration depth of this light is in the order of a few millimetres. For a superficial tumour in the skin, like the mouse tumour models often used in a preclinical setting, this is not a problem and enough light will reach the CCD camera. Obviously, humans are much bigger than mice and tumours are not always located in the skin. Imaging of Cerenkov light in-vivo will be a much tougher assignment. This means that, before we start using a CCD camera in a surgical setting, we have to prove that we are able to make clinically relevant images with the available techniques. With clinical relevant images we mean exactly comparable to actual uptake activity in real human tissue. To do this we want to image actual tumour in living human beings.

This is not an easy task since cancer patients with administered activity are required and subsequently the tumour has to be accessible with the EMCCD camera. To prevent invasive methods and additional doses of radiopharmaceuticals we selected a special group of patients for this pilot study. Patients with relatively superficial tumours that are already scheduled for diagnostic or follow-up ¹⁸F-FDG or ⁶⁸Ga-Dotatate PET imaging are asked to participate in this study.

7.1.2 METHODS:

PATIENT SELECTION:

Eligible patients are patients of the NKI-AvL which will undergo a routine ¹⁸F-FDG PET-CT scan. The inclusion criteria are:

- Scheduled for a routine ¹⁸F-FDG PET-CT scan.
- Patients with a superficial tumour. (< 2 cm from surface)
- Any tumour type can be included however, it must show sufficient uptake during the PET-CT scan.
- Written informed consent should be provided.

• Patients ≥ 18 years old

The exclusion criteria are:

• Melanomas with high melanin levels as determined by visual assessment prior to the PET/CT scan because melanin can absorb a lot of light.

Patients will be selected after they are planned for a PET-CT scan. This planning occurs for the majority of the patients one or two weeks prior to the PET-CT scan. If patients are scheduled the researcher will screen their dossier to determine if they have a superficial tumour. This will be done based on the PET-CT indication, clinical dossier and when available prior CT, PET-CT or MRI scans. When patients are eligible they will be contacted by telephone to confirm if they really have a tumour at the moment and are up for a PET-scan. They are informed on the study and asked to join, additionally patient information will be sent by post or e-mail. The decision to join the study and informed consent will be asked at the day of the investigation, prior to the PET-CT scan. When a patient decides to participate, informed consent will be signed.

Taking into account possible errors and the fact that we want to measure tumours at different depths in the patient we have set the maximum sample size at 12 patients in total. After each patient an evaluation is made on the feasibility of the camera set-up. If it is necessary the imaging protocol will be modified. When sufficient data is acquired to answer the question within the 12 patients the study will not be continued.

7.1.2.1 ROOM PREPARATION HOW TO MAKE IT DARK

For the imaging of the patients a special room was available near the nuclear department. This room had only a small window to the hallway and was therefore relatively perfect to eliminate as much light as possible. To make to room as dark as possible the window was covered with aluminium foil on the outside and a darkening cloth on the inside. The slits around the door were covered by an extra layer of insulation strips on the inside of the door frame. Additionally, the slits were covered on the inside by more darkening cloth. In the room all tiny light sources such as LEDs from different devices were covered with black aluminium tape. The fluorescent tube lights were off for at least an hour in advance of the experiments to minimize the afterglow.

The light elimination was checked by making images with the EMCCD camera with a high gain from every corner of the room. The most prominent light source left was the keyhole in the door which was also covered with black aluminium tape. However, there was still some degree of light in the room but it was not clear where exactly this came from. It might have been photons in the NIR region coming through the suspended ceiling.

To make sure this remaining light did not distort the imaging on the patients. We covered the imaging area with a double darkening cloth to reduce the remaining light by at least a 100 fold.(Fig. 28)

7.1.2.2 STUDY PROCEDURE

Patients in this study will receive a PET-CT scan as part of their standard clinical care. For the PET-CT examination they will be administered with ¹⁸F-FDG. Directly after the scan they are taken to the prepared light tight room. Here they are positioned on a patient bed and the Cerenkov imaging is performed.



Figure 28

Schematic of the setup in which the CLI is performed on patients for the in vivo experiments. The patients that were administered with ¹⁸F-FDG were lying on an examination bed. The camera which was placed on a shelf attached to a trolley could be placed over the tumour. The area between the lens and the patient was shielded with two layers of darkening cloths. All patients will undergo the following procedures:

- Standard clinical care:
 - o Patients are administered with ¹⁸F-FDG.
 - o Patients will receive a PET-CT scan as usual.
- Procedures related to this study:
 - After the PET-CT scan is finished patients are taken to a prepared light tight room at the nuclear medicine department.
 - In the CLI room patients will be placed on an examination bed with the site of the tumour uncovered and accessible for the camera.
 - The camera will be positioned in the direction of the tumour.
 - Darkening cloths are positioned around the camera and the tumour location.
 - Multiple acquisitions will be performed in total darkness while the patient remain still. Acquisition times vary from 1 to 300 seconds.
 - When the imaging is finished, the patient is ready to go home.

The CLI examination will take place roughly two hours after the administration of the radiopharmaceutical. The total CLI procedure will take no more than 30 minutes.

STUDY PARAMETERS

Based on the PET-CT scan the absolute uptake of the concerned tumour will be determined in the same way as is done in chapter 6.2. Additionally the distance from the surface of the tumour to the surface of the skin will be determined. This will be used to correlate the acquired signal to the depth and the activity.

The images will be analysed and the number of counts per pixel will be determined for two regions of interest (ROI) for each tumour (Fig 28).



Figure 29

Timeline of the study procedure as it will be carried out for patients that will have an ¹⁸F-FDG PET scan. This includes a fasting period of 6 hours prior to administration and an exposure period of an hour before the PET-CT scan.

The first ROI will represent the healthy tissue around the tumour and thus can be seen as background. The second ROI will be located on top of the tumour and will represent the tumour signal. The number of counts derived from the tumour should be related to the radioactivity in the tumour and the depth of the tumour within the tissue. From the mean number of counts in each ROI and the standard deviations (SD) the signal to noise ratio and thus the detectability can be determined. The signal will be the mean number of counts in ROI 2 and the noise will be the SD in the number of counts in ROI 1. The signal to noise ratio will be the following:

$$SNR = \frac{signal}{noise} = \frac{Mean ROI 2 - Mean ROI 1}{SD ROI 1}$$
(12)

If the SNR is larger than 3, the tumour can theoretically be distinguished from the background.



Figure 30

If the dashed red circle represents the tumour the blue square will be the imaging area that is captured with the camera. From these images the counts per pixel will be determined for 2 ROIs: ROI 1 will be measured a few cm outside the tumour area and consist of healthy tissue . ROI 2 will be measured at the site of the tumour.

The ultimate goal eventually is to visualize a tumour based on its Cerenkov luminescence. Therefor we will perform image processing to select the signal from the background noise and other disturbing factors such as incident gamma rays. Thereafter this processed image is overlaid with a light image that shows the view of the imaged area. This will lead to an intensity map of the Cerenkov signal over the normal photo just like is done for the images of the IVIS. This will be done independently by two researchers by different methods:

Matlab

The raw data that was made with the camera software is loaded into Matlab. First the cosmic rays are removed by selecting a threshold that will eliminate all pixels above that value. These pixels are replaced with the average of their surroundings. After that the image is smoothed. The noise floor is removed by providing another threshold that eliminates all pixels below a certain value. What is left is the signal caused by CLI. Of this signal an intensity colormap is produced which is overlaid with the light image.

ImageJ

The raw data is opened in ImageJ. The image is processed by using successively the 'Remove Outliers', algorithm and the despeckle function to get rid of the cosmic rays. Subsequently the image is smoothed. The image is then merged with the light image in MIPAV (Medical Image Processing, Analysis, and Visualization). This program enables to set an intensity scaled colormap for one of the datasets.

| Pat. Nr. | Tumor type | Distance to surface | Injected dose | SUVmax | Mean activity conc. | SNR |
|----------|--------------------------------|------------------------|---------------|--------|------------------------|------|
| CLI-01 | In-transit metastasis melanoma | 4 mm | 181 MBq | 8.3 | 4.6 KBq/mL | 1.66 |
| CLI-02 | Recurrent melanoma | 0 mm | 179 MBq | 2.3 | 3.6 KBq/mL | 1.3 |
| CLI-03 | In-transit metastasis melanoma | 0 mm | 213 MBq | 11.7 | 7.7 KBq/mL | <1 |
| CLI-04 | Metastasis melanoma | 13 mm | 450 MBq | 23 | 40 KBq/mL | <1 |
| CLI-05 | Recurrent melanoma | 0 mm | 210 MBq | 6.9 | 7.3 KBq/mL | <1 |

Table 8

Characteristics of the 5 included patients for the in vivo CLI study. The distance of the tumour to the surface, the SUVmax and the mean activity are based on the PET-CT scan that was made prior to the CLI. The SNR was determined on the CLI images.

7.1.3 RESULTS:

For this study we have included a total of 5 patients. The main reason only 5 patients were included is that the trial period of the camera ended before we could include more patients. The relevant characteristics of the patients can be seen in table 8. Among these 5 patients were patients with ideal conditions to perform CLI on. Ideal conditions are tumours that are very superficial with a high uptake value. Especially the third subject seemed very promising with a tumour directly at the surface of the lower leg and with high uptake values with an average of 7.7 kBq/ml and a SUVmax of 11.7. On all of the patients acquisitions up to 300 seconds were performed. In patient number two were the tumour was located on the hand there was light leakage through the darkening cloth which resulted in a useless result. From then on we used two separate darkening cloths to prevent any light from contaminating the imaging.

In none of the images with acquisition shorter than 300 seconds any signal could be detected. For the 300 second acquisition in the first patient we did detect a signal. As you can see from the SNR

which is 1.66, it is not very intense. We were able to visualize the signal in the image. (Fig. 32)By means of image processing a signal could be discovered. The both independent processing methods came to a similar result. Next to that it is also in the area of where the tumour is situated. However, the shape of the signal is not conform the shape of the tumour as can be seen on the PET-CT images.(Fig. 31)

The images of the second patient had a lot of light distortion which can be seen in figure 33. Although there is positive signal to noise ratio this is probably caused by a higher reflectance on the tumour site than on the site were the background was measured. Due to the clear leakage of light this data is not representative. In the remaining three patients no signal could be detected at all. The intensities that can be seen in the images are clearly some form of noise that appears. This means that if there is a signal we are not able to distinguish it from the noise levels.(Fig. 33) More detail in Appendix C.



Figure 31

Max intensity projection image based on the PET data of subject CLI-01. The blue square indicates the imaging area. The intense spot is the uptake of the ¹⁸F-FDG in the melanoma.



Figure 32

On the left we see the light images made of the imaging area that is seen in figure 31. The red circle indicates where the tumour is located. In the middle we see a merged image of the light image and the CLI image processed with Matlab. On the right we see the merged image of the light image and the CLI image processed in ImageJ.



Figure 33

Merged images the light image and the processed CLI image. The CLI images have an exposure time of 300 seconds and a binning of 8x8, CLI 1-3, or 4x4, CLI 4 and 5. The red circles indicate the location of the tumour. In none of the images there is a correct correlation between the obtained signal and the location of the tumour.

7.1.4 DISCUSSION

The results show that only in the first patient there was some signal detected that could be derived from Cerenkov luminesnce. However, it is highly likely that the signal is derived from some other source. First of all the shape of the signal does not exactly match the shape and position of the tumour. Next to that we found much higher activities of ¹⁸F in more superficial tumours in patient 3 and 5 in which we were not able to detect any signal here. This means that if we have detected Cerenkov radiation in the first patients we should have definitely detected it in patient 3 and 5. Moreover during the first two patients only one darkening cloth was used to cover the imaging area. In the second case it is very clear that there is light leakage since the intensity of the dark image corresponds to the brighter areas of the light image. This makes it more likely that the intensity is caused by reflectance of some light leakage.

The conclusion is thus that we are not able to detect any activity. In the first two cases a possible explanation could be the light leakage. It is evident that any slight amount of light will overshadow the Cerenkov luminescence signal. In the last three cases light leakage could not be detected in the images. However, the histogram data tells us that in any case there was at least some ambient light in the data. (Fig. 34)



Figure 34

A method to check if there is ambient light distorting the acquisition is by looking at the histogram of the counts. We see two histograms with the same range from 500-700. On the left we see an acquisition that is made with the lens cap on so there is no ambient light at all. We see a very high peak around the offset of 500. On the right a histogram is shown of an acquisition that was contaminated with light leakage. We can see that each pixel starts with an offset of at least 600 counts. Furthermore it can be explained why we can' t detect any activity by the results of our previous experiments. In an in vitro experiment in ideal settings with near perfect darkness and transparent phantoms we were only able to barely detect an activity concentration of around 20 kBq/ml. The activities in these patients at the moment of the PET-CT scan are more than twice as low. Due to the decay the activities would be even lower at the moment of the CLI experiments. Next to that the activity was situated in human tissue covered by the epidermis which causes a lot of absorbance and scattering of the Cerenkov light. Based on the experiments in mouse fat this will lead to a loss of 95% in the first 3 mm of tissue. This means that we are dealing with an intensity that is approximately 50 times lower than the lowest intensity we were able to detect so far.

7.1.4.1 COSMIC RAYS

The cosmic rays showed to have a lot of influence on the CLI imaging. Especially when longer exposure times or the gain was used. These cosmic rays are caused by the gamma rays that are coming from the patient which is at that moment very active. In experiments with the second subject were the tumour was situated at the hand we positioned a few lead blocks in between the camera chip and the patient' s body to eliminate most of the incident rays. This was however, not an option for the other measurements since it was not possible to shield the chip from most of the patient' s body.

Eventually we came up with a solution to remove the cosmic rays by making two exposures at the same setting and remove all pixels which had a too large intensity difference between the two datasets. This led to twice as long acquisitions which was especially inconvenient for the longer exposure times. Furthermore the longer exposure times resulted in so many incident rays that a lot of data was useless. To solve this we did multiple shorter exposures and added them later when the cosmic rays were removed.

7.2 PROSTATE EX VIVO CLI STUDY

7.2.1 RATIONALE FOR THIS EXPERIMENT:

Besides imaging tumours through the skin performing CLI on a resected specimens is also of interest. Imaging of radioactive tumour specimens is to a certain extent also similar to a realistic clinical situation since it consists of actual human tissue and it contains an actual tumour. Furthermore it provides an actual uptake of the radiopharmaceutical in the tumour. Imaging of specimens has some advantages over in vivo imaging through the skin. First of all you will have direct access to the tumour because it is not covered by large amounts of skin or blocked by other

organs. Also you are not dependent on melanoma' s or other superficial tumours but any type of tumour that is resected can be included. Next to that you are able to image them in a light tight cabinet were you are better able to prevent ambient light from distorting the image. Lastly you are able to image with longer exposure times and do more acquisitions since you don' t have to worry about the convenience for the patient.

On the other hand imaging resected specimens is more problematic because you have to administer additional activity to the patient resulting in an increased dose. This is of course not preferred. Next to you will bring a radioactive patient into the OR which causes a dose to all the personnel who are involved with the patient during and around the surgery.

Ex vivo measurements on resection specimens can be of clinical relevance in for instance perioperative margin assessment. However, the radiation safety issues have a significant impact. In the Netherlands and most of the western countries the total effective annual dose a surgeon can receive is only 1 mSv. When performing surgery on a patient who is injected with a PET tracer like ¹⁸F-FDG this will cause a significant dose per surgery for the surgeon and other personnel standing directly next to the patient. The consequence is that a surgeon can only perform a small amount of these surgeries a year. This makes the technique not applicable clinically.

A solution for this problem could be robotic surgery where the surgeon is positioned several meters away from the patient for the majority of the procedure. In the NKI mainly prostatectomies are performed by robotic surgery. This is a so called RALP (Robotic Assisted Laparoscopic Prostatectomy) procedure. Intraoperative margins assessment is a very welcome development for prostatectomies since positive margins are a serious issue [51]. Furthermore, new radiopharmaceuticals are under development for prostate cancer based on ⁶⁸Ga and DOTATATE, an isotope with a lot more light production then ¹⁸F [71]. In our opinion this could be the field of the first clinical application of CLI we wanted to test the feasibility of Cerenkov imaging on a resected prostate.

In agreement with the urologist and the radiation safety officer we were authorized to do a pilot in one patient.

7.2.2 METHODS

For this study we need one patient that will undergo a prostatectomy. For the cause of the study the patient will have to be administered with ¹⁸F choline. To prevent additional administration of a radiopharmaceutical the patient receives a PET-CT scan with ¹⁸F choline as part of the standard clinical care. This PET-CT scan will be planned directly prior to the surgery. The patient was selected when it was included for surgery by the involved.

Inclusion criteria:

- Had to be scheduled for RALP performed by involved urologist.
- Prostatic tumour should be clearly present. Preferably near the surface of the prostate.
- There must be an indication to do a Choline PET-CT scan.
- The Choline PET-CT scan could be planned directly prior to the surgery.
- Patient must agree to participate in this pilot.
- Patients \geq 18 years old.

Exclusion criteria are:

• Sentinel node procedure with radioactive sources.

Choline PET-CT scans are normally made at specific days and in specific time slots because it has to be ordered from an outdoors company. The choline can only be delivered from 11:00 o' clock. The standard days for choline scans in the NKI-AvL are Wednesday and Thursday. The RALP procedures are often planned on one day with 3 to 4 slots a day starting at 8:00. This means that only the third slot that will start around 12:30 provides a proper connection with a PET-CT scan that will start at 11:00. Unfortunately this is also the timeslot the more complex surgeries with sentinel node procedures are planned.

7.2.2.1 STUDY PROCEDURE

The patient will undergo a PET-CT and a RALP procedure on the same day. The prostate that will be resected during the surgery will be imaged within the light tight cabinet before it will be delivered to the pathology.

The patient will undergo the following procedures:

- Patient is hospitalized in the morning.
- Administration of the ¹⁸F choline directly prior to the scan
- ¹⁸F choline PET-CT scan
- Scan is send directly to PACS (Patient Archiving and Communication System) server, patient is send to holding.
- The PET-CT scan will be reported by nuclear physician
- Start surgery
- Removal of the prostate

- Prostate is prepared for CLI
- CLI is performed on the Prostate
- Prostate is delivered to the pathology department



Figure 35

Timeline scheme of the ex vivo specimen experiment. Above the timeline we see the schedule for the patient. The procedure starts with a PET scan with ¹⁸F-choline. Directly after the PET scan the patient is prepared for surgery. After the resection of the prostate the prostate is imaged before it is delivered to the pathology department.

7.2.2.2 CERENKOV IMAGING

The resected prostate and possible active lymph nodes will be prepared for imaging on the OR. The specimens are rinsed with saline to reduce the amount of blood and urine from the specimen. The specimens are placed in a black container tray. The specimen is imaged on the Cerenkov Imaging setup that is installed in the specially prepared room. Acquisitions will be made in the light tight cabinet in the prepared light tight room. Specimens are measured in their container without the lid. Both sites of the prostate will be imaged to make sure that most of the surface will be covered. Several acquisitions will be made on the resected prostate. Total time of the procedure will be 30 minutes. After the imaging procedure the specimens will be delivered to the Pathology department were it will be routinely processed.

The goal of the imaging is to see if we can detect the Cerenkov light derived from the ¹⁸F-Choline that is taken up by the tumour. We do this with the setup containing a high sensitive EMCCD camera. The image will be processed in the same way as described in the previous study. For correlation of the obtained Cerenkov images the ¹⁸F choline PET-CT images will be used and the pathology examinations will be consulted.

7.2.3 RESULTS

The patient that was included for this pilot had 2 foci in the prostate that was diagnosed based on MRI imaging. The foci had a size of around 1 cm³. The foci that was clearly present was situated at the right posterior surface of the prostate near the rectum. The patient was administered 160 MBq ¹⁸F choline at 11:18. During the PET scan an SUV max of 5.5 was found with a mean activity concentration of 11 kBq/ml (Fig. 36).



Figure 36

On the left we see an MRI image of the prostate of the subject. In the red circle the tumour nr. I is visible. On the right we see the PET fusion images made prior to the surgery. On the transverse view above we see two spots with elevated uptake. Based on the patholical reports we know that the left spot in the middle of the surface correlates with the tumour. The rest of the activity is caused by some local haemorrhages. The tumour is clearly situated a little bit to the right at the dorsal side of the prostate.

At one side of the prostate, the side the bladder was attached to we can see a signal that correlates to the position of the prostate. In almost all acquisitions from 10 to 300 seconds we could distinguish the same pattern (Fig. 37). However, on the other side of the prostate, the side where the tumour was situated no signal can be detected at all.



Figure 37

CLI images of the resected prostate. Above we see the images made of the more ventral side of the prostate corresponding to images A from figure 38 and figure 39. On the left a raw image is shown from an acquisition with a 300 second exposure time. Right of that 3 merged images of the light and processed CLI images are shown for three different exposure times. A clear signal can be seen that correlates to the position of the prostate. The lower three images are form the dorsal side of the prostate. They correlate to images B from figure 38 and figure 39. Already in the raw image no signal can be detected. Also the processed merged images show no signal but only some artefacts and a little bit of light leakage from the right were the door of the cabinet is located.

7.2.4 DISCUSSION

We did see activity on the side imaged from position A (Fig. 39) but on the other side we did not detect any activity at all. The places where we saw the activity was the side that was situated towards the bladder. Based on the imaging the first lesion was situated at the other side near the rectum. This was confirmed by pathological examination. The pathologist found 2 significant lesions. The first lesion, lesion I corresponded with the lesion that was visible in the MRI and PET-CT scans. Another lesion was found near the right seminal vesicle with ingrowth into the seminal vesicle. This is lesion II in the images.

If we would have detected radioactivity from the tumour uptake it should have shown in image B and then most likely at the marked areas. However, no signal was detected at this side at all. We can thus conclude that we were not able to image the tumours within the prostate. Even though at some places there was only a minimal surgical margin.

At side A there was a significant signal detected. This was already clearly visible in the raw unprocessed images. The signal had such high levels that it was already visible with an acquisition of 10 seconds. Prior experiences have taught us that this signal must be caused by an activity of at least a few hundreds of kBq/mL. Next to that the signal should come from the surface of the prostate.



Figure 38

Schematic sagittal section of the prostate. The two positions of malignant tissue based on the pathology examination are displayed as well as the orientation of the prostate relative to the images A and B from figure

39.

The signal is not derived from light leakage. Based on the shapes of the histogram we could tell that in the acquisitions there was no ambient light present. Furthermore, if the elevated signal was caused by reflection it should have had another pattern and be more intense at shiny surfaces. Other light sources are not present inside the cabinet during the acquisitions. This means the signal must have come from Cerenkov Luminescence caused by radioactivity. The question is why there is such a high concentration of ¹⁸F at that particular position. It could have been contamination from leaked blood or leaked urine. However, this is very unlikely since the activity in the blood and thus also in the urine at the moment of surgery, at least 2 hours after administration, is negligible. Other than in ¹⁸F choline the distribution out of the blood is very rapid. That the activity of the blood and urine was not high was confirmed by measurements with a dose meter. After the surgery the collected blood and urine were measured but no elevated activities could be detected. Furthermore,

if it was activity contamination by blood or urine it should be all over the specimen and on the surface of the black container tray. This was not the case.

To determine what could have caused the signal we tried to find a correlation between the images and the prepared specimens together with the pathologist. At the places where we detected a signal we could not see any malignant tissue. We could also not find any other structures that might explain the presence of radioactivity. We did notice that there was a correlation between the signal and a pattern of rough edges on the surface of the prostate. Closer inspection suggested that this pattern could be caused by the electrosurgical knife during the surgery. However, this was not enough to explain the presence of activity.



Figure 39

Full color images of the both sides of the resected prostate. On the left we have the ventral side A where the signal was detected at its surface. On the right the dorsal side B is displayed. From this side the malignant areas I and II should are the most exposed. The green circles represent the positions of the tumors.

A possible explanation for the activity is that due to electro surgery the leaked urine is vaporized and left all the ¹⁸F at the surface of the prostate. Since all the fluid vaporized and only the activity is left the concentration has increased severely leading to a detectable concentration on the surface of the prostate. There are of course more places of coagulated tissue were no activity is found. The active parts however, are situated at the side of the bladder were the urethra was excised and therefor a lot urine spill is assumed at that location.

Another possibility is the occurrence of chemiluminescence. In certain chemical reactions energy in the form of electrons and photons is released. This can occur when a conversion of choline to hydrogen peroxide takes place. It might be possible that the heat of the coagulation triggered such a reaction.

8 RADIATION SAFETY

As we learned Cerenkov imaging is dependent on radioactive sources. Since we are looking into an intraoperative use we want to use the technique in patients. This requires a lot of activity that has to be administered to those patients. The administered doses have to be at least equal to the amounts that are used in PET imaging. As mentioned earlier this such an activity cannot indiscriminately be administered. Although it is not a big issue for that particular patient. The patient can receive this dose since it is of use for his clinical care and there will be more benefit then it does harm. However, for the personnel that is involved in the administration, imaging, transport and surgery of this patient this naturally does not apply. All the persons will receive a dose via the radioactive patient and for them it can only do harm. The should keep the received dose as low as reasonably possible conform the ALARA (As Low as Reasonably Achievable) principle. The workers on the nuclear medicine department are specially trained to deal with radioactivity and can receive an excepted annual dose. The bottleneck is the OR personnel. They are not specially trained as a radioactive worker are limited to low annual dose while at the same time they have to stand next to the radioactive patients throughout the entire surgery which will result in a high dose. Depending on the dose they receive per surgery they are restricted to a certain number of these types of surgery a year. If a surgeon cannot perform a certain amount of surgeries a year the technique will not be valuable enough. The surgeon is not able to specialize in and optimize the surgery and relatively little patients can be treated with a new invested technique.

The dose a surgeon will receive during a surgery with a radioactive patient is thus an important parameter for the success of CLI.

8.1 RADIATION REGULATION

The protection of people against radiation in the Netherlands is regulated conform the 'kernenergiewet' and the 'besluit stralingsbescherming'. In these laws dose limits are described for all employees. These limits are defined as the annual effective dose a person can receive. This is not a measurable variable but it can be calculated from different other physical parameters and weigh factors as stipulated in directive ICRP 60. It is a measure for the amount of radiation that is received by the whole body corrected for the type and energy of radiation and the amount of damage it can do to any part of the body. It is measured in Sievert. The naturally received background dose in the Netherlands for any person is 2.5 mSv/year. This is derived from naturally present radon and radiation from space. The dose limits set by the 'kernenergiewet' are defined as the annual dose that is received from ionizing radiation and artificial radiation sources in addition to the background radiation. There are different categories of dose limits for employees:

| ٠ | Standard worker > 18 years old: | 1 mSv/year |
|---|---------------------------------|-------------|
| ٠ | Exposed workers > 18 years old: | |
| | o A-worker | 6 mSv/year |
| | o B-worker | 20 mSv/year |
| • | Exposed worker < 18 years old: | 6 mSv/year |

Surgeons belong to the standard worker category and cannot receive more than 1 mSv a year. Exposed workers are employees that need to work with radiation for their profession. An example of an A-worker is a nuclear medicine physician. An example of a B-worker is an intervention radiologist.

These regulations are the same for the whole EU and in the US and Canada they also apply a dose limit of 1 mSv a year.

8.2 DETERMINING THE RECEIVED DOSE

To determine what the received dose will be for surgeons that operate on a radioactive patient we consulted the literature. The most information can be found on sentinel node procedures that make use of technetium 99 (99Tc). This is a widely used technique with radioactivity during surgery. Such a procedure differs from a possible CLI procedure in that there is only a small amount of activity administered locally. Furthermore ⁹⁹Tc is a pure game emitter other than the PET tracers that will be used in CLI. These procedure thus lead to a very low dose of less than 2 µSv per procedure. This means that one can do up to 500 procedures a year [72], [73]. Other literature were ¹⁸F was used as a source provided more useful data. Radiation doses between 18.6 and 238 µSv were measured. The amount of dose really depends on the administered dose, length of the surgery and the period between the administration and the surgery. These parameters are very different in the reviewed articles. To have a proper indication of the expected doses in a CLI procedure in the NKI an estimation was calculated based on relevant parameters (Table 9.). This led to an estimation of 170 µSv per procedure for the surgeon. Based on these numbers a surgeon will exceed the dose limit in already 6 procedures. This was a dramatic conclusion because this suggested that it was not feasible to implement CLI into surgery. Of course the estimations are a worst case scenario and it is actually an overestimation. The real doses would be lower. To have even more realistic numbers we received data from Lightpoint Medical who were conducting clinical trials at the moment. They provided us with de dose measurements of all the OR personnel of the first three patients were they used CLI in breast conserving surgery. They showed dose rates between 18 and 40 μ Sv/hour for the surgeons. For a two hour surgery this will result in between 36 and 80 µSv per procedure. This is a more positive result than the estimations calculated by the radiation safety officer. Furthermore, the

administered dose they used was 5 MBq/kg which is twice as high as the doses we use in the NKI. The actual doses thus will probably be significantly lower. To give a more precise estimation of what the received dose for a surgeon in the NKI will be we performed dose measurements on patients at the nuclear department who were administered with clinical doses of ¹⁸F-FDG and ⁶⁸Ga-Dotatate. We also wanted to know what the received dose will be directly at the surface of a patient.

| | lsotope | administered dose (MBq) | Time between administration and surgery (Min) | Duration OR (Min) | Dose tempo surgeon (μSv/h | Dose per procedure (μSv) | Procedures per year |
|---|---------|----------------------------|---|----------------------|------------------------------|--------------------------------|------------------------|
| Radioguided surgery | Tc99 | 30,00 | 60,00 | 180,00 | 0,63 | 1,89 | 529,10 |
| The sentinel node in surgical oncology | Tc99 | 15,00 | 60,00 | 60,00 | 1,80 | 1,80 | 555,56 |
| Nallay et al | 18F | 287,50 | 228,00 | | 37,50 | | |
| Povoski et al | 18F | 699,00 | 179,00 | 235,00 | 61,00 | 239,12 | 4,18 |
| Heckathorne et al | 18F | 353,00 | 79,00 | 48,50 | 23,00 | 18,40 | 54,35 |
| Piert et al. | 18F | 44,00 | 222,50 | 247,50 | 5,55 | 22,76 | 43,95 |
| Andersen et al. | 18F | 45,00 | 65,00 | 15-450 | 13,20 | 3,30 | 303,03 |
| Estimations NKI-AvL | 18F | 200,00 | 90,00 | 120,00 | 85,00 | 170,00 | 5,88 |

Table 9

Overview of a literature study into the received dose to surgeons when performing surgery on a radioactive patient. The first two studies show the results for Tc99 which is used in sentinel node procedures. Next 5 different studies that have published received doses due to patients administered with ¹⁸F. The last row shows the calculated estimation done by the radiation safety officer for a plausible scenario.

8.2.1 METHODS

We have measured patients who received clinical doses of ¹⁸F-FDG and ⁶⁸Ga-Dotatate in the context of a PET-CT scan. We measured the dose tempo with a dosimeter. We have measured on the surface of the patients on the right and left flank in between the hip bone and the ribs and from the front 30 cm from the bellybutton. The measurements were done prior to the PET-scan and directly after the PET study.

The data will be plotted against the time obtain a curve that represents the dose flux for any time after the administration of the radioactivity. This will be done for the three different measurement positions and for both the ⁶⁸Ga and the ¹⁸F.

8.2.2 RESULTS

We have measured seven patients who were administered with ⁶⁸Ga and 10 patients who received ¹⁸F-FDG. In all the patients we measured twice on each position. Once prior to the scan and once

directly after the scan. In one patient who received a second scan for a late uptake study a third measurement was done. The constructed dose flux curves can be seen in figure 40 and figure 41.



Figure 40

In this graph the dose rates of the measurements performed on the 7 patients who received a dose of ⁶⁸Ga-Dotatate are plotted against the time after administration. The measurements were taken 30 cm from the front the patient' s body. The two measurements on the same patient are connected with a continues line. The dashed line represents a dose tempo vs time curve based on the interpolated averages of the individual measurements.

For the ¹⁸F the doses flux at the flank were 3 times as high as the dose flux at 30 cm. Furthermore there was not difference in the right or left flank. In the ⁶⁸Ga the difference between the flanks and at 30 cm in front of the body was even bigger. There was also a difference between the left and the right flank were the right flank dose flux was approximately 1/3 higher. The measured dose fluxes were averaged before a curve was constructed. In between the measurements of the patients administered with ¹⁸F went to the bathroom to empty there bladder. This sanitary interference will cause 20% of the administered activity to leave the body. The curve is corrected and this is seen by

the downward step the curve takes.



Figure 41

In this graph the results of the ¹⁸F-FDG administered patients are plotted just like in figure 34. The measurements were taken 30 cm from the front the patient' s body. The dashed line also represents the dose tempo vs time curve. In this case we see a steep fall in dose tempo in between the two measurements. This is caused by the loss of activity due to emptying of the bladder. This causes a 10 % drop in the initial activity [74]. The one measurement at 200 minutes from administration is from a patient who also received a late uptake PFT-CT scan

8.2.3 DISCUSSION

As best estimation for the distance a surgeon on average is from a patient during surgery we take a distance of 30 cm. Based on the constructed curves we can easily determine the dose someone will receive when he stands 30 cm from the patient for a specific time frame. If we take the same situation as done in the calculations; 2 hour surgery 90 minutes after administration, we can tell the surgeon will receive an effective dose of 56 μ Sv per procedure. This results in 17.85 procedures a year. This is a pretty accurate estimation of the received dose. However, this is still too high and real dose measurements should give a decisive answer.

8.3 DOSE MEASUREMENTS DURING RALP

A solution to circumvent these problems is to create more distance between the surgeon and the patient by doing CLI in robotic surgeries. Since we have done one pilot patient with a RALP

procedure we could measure what de doses were for the personnel around the surgery. We have provided the 6 persons who will stand the closest to the patient with a personal dosimeter during the surgery.

An estimation of the time and distance with respect to the patient as a radioactive source for the whole procedure was made from prior observations (Table 10).

| Minutes | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 | 105 | 110 | 115 | 120 | | |
|----------------------------|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------|-----------|-----|-----|-----|-----|-----|--|-------|
| Surgeon 1 | | | | | | | | | | | | | | | | | | | | | | | | | | >4 m |
| Surgeon 2 | | | | | | | | | | | | | | | | | | | | | | | | | | >3 m |
| Anesthesiologist | | | | | | | | | | | | | | | | | | | | | | | | | | >2 m |
| Anesthesiologist assistant | | | | | | | | | | | | | | | | | | | | | | | | | | >1 m |
| Circulating Nurse | | | | | | | | | | | | | | | | | | | | | | | | | | 30 cn |
| Scrub Nurse | | | | | | | | | | | | | | | | | | | | | | | | | | |

Table 10

This table shows the amount of time at what distance the different OR attendants are positioned from the patient during a RALP procedure.

An estimation of the received dose for the surgeons will be lower than 15 μ Sv/procedure. For the anaesthesiology and the circulating nurse this will be up to 30 μ Sv/procedure. The scrub nurse will receive the highest dose but this will be lower than 55 μ Sv/procedure. These assumptions are made on the dose measurements we did before. Eventually the following personnel wore a dosimeter:

- Radiation safety officer
- Researcher
- Anaesthesiologist assistant
- Surgeon
- Circulating nurse
- Scrub nurse

Furthermore measurements with a dose flux meter were performed to determine the activity at different positions from the patient and other possible radioactive material. The following measurements were performed:

- At different distances from the patient during transport, in holding, during surgery and at the recovery.
- The collected urine.
- The collected blood.
- The resected Specimens.
- The collected waste from the surgery.

8.3.1 RESULTS

The patient was administered at 11:18 with 160 MBq ¹⁸F-FDG. The surgery started at 12:30 and ended at 14:35. In this time span the surgeon received a dose of 7 μ Sv. The highest dose was received by the scrub nurse who has to stand next to the patient for almost the whole procedure. She received a dose of 22 μ Sv. This means that the surgeon can perform 142 such procedures a year based on these measurements (Table 11). The scrub nurse however, is the limiting factor with 45 procedures a year. This is already a lot more than was earlier determined for a standard operating procedure without robotic assistance.

| Function | Received dose (µSv) | Max procedure per year |
|----------------------------|---------------------|------------------------|
| Surgeon | 7 | 142 |
| Anesthesiologist assistant | 9 | 111 |
| Circulating nurse | 4 | 250 |
| Scrub nurse | 22 | 45 |
| Radiation safety officer | 3 | 333 |
| Researcher | 1 | 1000 |

Table 11

The doses measured with the personal dosimeters during the RALP procedure. The dose is given in micro Sievert. In the right column the amount of procedures per year that can be performed based on these doses is given

9 DISCUSSION, RECOMMENDATION AND CONCLUSION

9.1 DISCUSSION

The results of our study show that it was not possible to measure Cerenkov radiation in a human being. (7) At least not in the way we have performed the measurements. Although we were able to measure clinical relevant activities in vitro, imaging these same amounts in tissue was another challenge. The attenuation of light by tissue is too high and this effect is enhanced by the nature of the Cerenkov lights wavelength spectrum (6.1). Since the majority of light is in the ultraviolet and blue area the larger part of the light is blocked by even the slightest amount of tissue. We saw already that in prepared mouse fat 95% of the light is lost in the first 3 mm. And this tissue did not even contain blood or other absorbers that can be found in human tissue. Expected is thus that the actual attenuation is even higher.

To be able to image Cerenkov radiation in tissue we need thus a much higher concentration of activity. With the IVIS 200 we were able to detect a concentration of 3.5 kBq/ml in the most ideal conditions and a long exposure time with maximum binning. (6.3). This can be seen as the most sensitive method of detecting Cerenkov radiation today. This means that if we want to image in tissue we need an activity that is 20 times higher around 70 kBq/mL. In the NKI-AvL the concentrations in tumours are around 10 kBq/ml after a standard administration dose.(6.2) Obtaining higher concentrations thus requires a very high administration dose. Increasing the administration doses is an unwanted development since the policy is to reduce the doses as much as possible. Moreover this will lead to an increased dose to the bystanders which already is an issue.(8) In the published studies were they performed CLI on humans all the researchers used a higher administration dose than we did for our studies. We used the standard administration dose that is used for PET studies. This dose is around 180 MBq and higher for larger persons. This comes down to about 2.5 MBg/kg. In the studies by Thorek and Hu they used respectively an equivalent dose of 6.5 MBq/kg and 9.25 MBq/kg. [42], [44] This resulted in much higher uptake values in the tumour. In the study by Thorek the uptake value was 50 KBq/ml [42]. This was 5 Times higher than in our experiments. Even though they required a 5 minute acquisition to image the tumour in the axilla.

An intraoperative clinical application using ¹⁸F-FDG is far away at this moment. The most sensitive cameras that are currently available can only slightly detect activity with acquisitions that take several minutes and only after administration of high amounts of ¹⁸F-FDG. (<u>6.4</u>) Also elevating the signal by using the optimal lens and imaging from up close did not lead to the required improvements. We

were able to improve the images by using more sophisticated acquisitions and by image processing. However, for image guided surgery this is not sufficient enough. For this purpose we want to have fast acquisitions that are near real time with a clear signal to background ratio and a high a resolution.

Besides the problems we have with the sensitivity the ambient light forms a problem. In a system such as the IVIS 200 that are designed for small animals or specimens it is not difficult to eliminate all the light. In this case you build a cabinet with light tight materials and a perfect seal to close the door. Of course you are able to build such a design on a larger scale that will fit humans. It is of course not very patient friendly to put patients in a dark box. Also in a surgical setting this is not an option. Another option is that you are able to make the whole OR dark. In practice this is very difficult since any small LED on any device should be eliminated because any spec of light can disturb the measurements. Furthermore, temporarily eliminating all the light in the OR seems like a bad idea when a whole OR team is present with all kinds of equipment that keep the patient alive that have to be constantly monitored. The best option is to try to seal of only a small area of the patient that you want to image. However, it will not be easy to come up with a design that can give a light tight seal between the lens and any part of the patient that meets the quality standards for sterile OR equipment. Such a system is currently under development by Lightpoint Medical from the UK. Another option to eliminate light is to use the technique during scopic procedures. In this case you bring the lens of the camera inside the patient's body. Turning of the light inside the body can be harmlessly done. However, it is not really clear if there will be no light at all. When performing scopic surgery on the abdomen a thin skin might transmit some of the light from the OR room. Next to that the holes that provide the entrance of the scope and other equipment should be sealed of light tight.

Image guided surgery using ¹⁸F-FDG is currently not feasible. In order to make such an application useful a large tumour to background ratio, a high signal and a sensitive system are required to be able to detect malignant tissue in near time and behind a layer of tissue. The Cerenkov radiation derived from ¹⁸F-FDG is able to provide a good tumour to background ratio in the same way as it does with PET imaging. The intensity of the signal or the sensitivity of the imaging equipment is the limiting factor. To meet the demands for image guided surgery several orders of magnitude in sensitivity have to be gained.

9.2 RECOMMENDATIONS

To improve CLI it is obvious that the sensitivity of the imaging system can be improved. The current available CCD cameras are already very sophisticated and highly sensitive. The development of these types of cameras might result in even lower noise floors. These are developments CLI will definitely benefit from it. The question is how much improvement this will give. Probably not in the orders we need. More important is to find methods to eliminate all the ambient light.

The other way of improving CLI is by cranking up the signal. This can be easily achieved by using higher doses of activity. However, as stated before this is not a desired development. Obtaining a higher dose in a tumour can also be achieved by different strategies. Some possibilities are the use of more tumour specific tracers or a more local method of administration. Although the development of new pharmaceutical precisely what we want to overcome with CLI, it could be interesting to explore the possibilities of obtaining higher uptake values with the current radiopharmaceuticals.

The easiest way to get more Cerenkov signal from a tumour is to use a different type of isotope that produces more photons per activity. Although ¹⁸F-FDG seemed the most ideal radiopharmaceutical for CLI because it is widely used and tumour specific for a broad range of tumours, the amount of Cerenkov light that is produced is poor. There are a lot of isotopes that are more suitable for CLI. However, they have to be tumour specific and preferably FDA and EMEA approved. The best options seems to be Gallium-68. This isotope produces 20 to 45 more photons per decay according to the simulation studies [46], [47]. In vitro studies with gallium showed an intensity that was approximately 12 times higher than ¹⁸F [<u>37</u>]. Furthermore, this isotope can be attached to different types of tracers and can be easily produced in house with a Germanium-68/Gallium-68 generator. In response to the poor results with ¹⁸F in our experiments the focus of upcoming work should really be on ⁶⁸Ga.

There are also other techniques that could enhance the CLI signal. One of the strategies is to enhance the intensity of detectable luminescence by shifting the peak wavelength from the blue regions to the red and infrared regions [20], [27], [75]. The principle is based on a fluorophores that are induced by the Cerenkov luminescence. This works with approved fluorophores as for instance the fluorescein isothiocyanate (FITC). This will significantly improve the penetration of the luminescence through tissue without losing the tumour specificity of CLI. However, while shifting this peak to longer wavelengths it must obey to the conservation of energy meaning the intensity of the signal is not enhanced but more photons are able to penetrate the tissue. This approach thus will only slightly solve the penetration problem.

A second approach is the use of quantum dot nanoparticles. They can be used conform the same principle as the fluorophores. They are induced by the tumour specific radioactive compound. The

difference with these nanoparticles is that depending on their design can be induced by photons or gamma radiation [70], [76]. The most promising part is that they possess high quantum yields and thus can deliver a very high intensity. Mice studies have shown a 50 fold increase in sensitivity in vivo [70]. The downside here is that most quantum dot nanoparticles are cytotoxic and although new more biocompatible approaches are being developed quantum dots are far from approval for clinical use [62].

CLI for image guided oncology surgery seems to be far from achievable. There are more opportunities for other intraoperative applications. A possible application is on site margin detection. The immediate knowledge on the surgical margins can be very valuable. Such an application solves already a large part of the difficulties that are presented for image guidance. First of all it can take place ex vivo and thus in a specialized cabinet where all ambient light can be blocked. Furthermore we don' t need near real time speed. For an application like this a five minute acquisition will be no problem. Lastly you only want to look at the surface of the specimen. The lack of penetration by the Cerenkov light would be beneficial for this purpose. An extension to this technique could be to do in vivo margin detection. By imaging the resection area you might be able to detect any residual malignant tissues. If you are able to image the whole resection area in one acquisition this will not take much time but if any residue can be detected you directly know where extra tissue should be removed.

9.3 CONCLUSION

Image guided surgery based on Cerenkov Imaging is theoretically very promising. However, the reality is that the intensities of the signal are too weak to use for in vivo imaging during surgery. Also, there are currently no possibilities to sufficiently enhance the signal besides the use of tremendously high activities. In the quest for bringing CLI in the clinic further research should focus on high emitting isotopes of which ⁶⁸Ga seems to be the most logical choice. More specifically, to bring CLI into the OR an application that focuses on perioperative margin detection is most likely to succeed. A good starting point can be the margin detection of resected prostates. In this type of surgery immediate knowledge of surgical margins is very relevant. Also the radiation safety issue can be bypassed since most prostatectomy's are performed using the RALP procedure. To conclude, a specific radiotracer for prostate cancer with ⁶⁸Ga will be available in the near future.

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12 APPENDIX B: MEDICAL USES OF DIFFERENT ISOTOPES

| Radioisotope | Label | Medical use | Targeted areas |
|----------------|------------------|--------------------------------------|---|
| Actinium 225 | | | Her2/neu expressed breast |
| | Trastuzumab | Targeted Alpha Therapy | cancer cells |
| | DOTMP | Targeted Alpha Therapy | Bone |
| | None | Targeted Alpha Therapy | Bone and liver |
| | EDTA | Targeted Alpha Therapy | |
| | Citrate | Targeted Alpha Therapy | |
| | DTPA | Targeted Alpha Therapy | |
| | HEHA | Targeted Alpha Therapy | |
| | DOTA | Targeted Alpha Therapy | |
| Bismuth 213 | DTPA | Targeted Alpha Therapy | |
| | DOTA | Targeted Alpha Therapy | |
| | PSMA | Targeted Alpha Therapy | |
| Carbon 11 | Choline | Tumor Imaging | Breast, prostate |
| | Methionine | Tumor Imaging | Brain |
| | | Cardiology studies, tumor | Heart, prostate, liver, |
| | Acetate | Imaging | kidneys, Bladder, Brain |
| | Thymidine | Molecular imaging | Endogeneous compounds |
| Copper 64 | Somatostatine | Tumor Imaging | SSR, neuroendocrine tumors |
| | Bombesin | Tumor Imaging | BB2 receptors, Prostate, GI, Breast |
| | ATSM | Beta Emitting Radiotherapy | hypoxic tumor cells |
| Dysprosium 165 | Ferric hydroxide | Arthritis therapy | Synovic joints |
| Fluorine 18 | FDG | Tumor Imaging | Metabolic active tissue |
| | Thymidine | Tumor Imaging | Prolifirating tissue, DNA synthesis |
| | Misonidazole | Tumor Imaging | hypoxic tumor cells |
| | | | Cellular membrane |
| | Choline | Tumor Imaging | phospholipids |
| | Dopa | Neurologic imaging, Tumor Imaging | Dopamine Receptors, Amino acid receptors, NETs |
| Gallium 68 | Dotatate | Tumor Imaging | Somatostatine receptor |
| | Dotatoc | Tumor Imaging | Somatostatine receptor |
| | | | BB2 receptors, Prostate, GI, |
| | Bombesin | Tumor Imaging | Breast |

| Radioisotope | Label | Medical use | Targeted areas |
|---------------|----------------|----------------------------|----------------------------|
| Holmium 166 | | | |
| | DOTMP | Beta Emitting Radiotherapy | Bone Marrow |
| | PLLA-ms | Poto Emitting Padiothoropy | Liver |
| | PLLA-IIIS | Beta Emitting Radiotherapy | Liver |
| | ACAC-ms | Beta Emitting Radiotherapy | Kidneys |
| lodine 124 | | Cardiology studies, tumor | Ischemic tissue, neurolog |
| | MIBG | Imaging | tumors |
| | Na | Diagnosis and dosimetry | Thyriod |
| | IAZG | Hypoxic studies | Hypoxic tissue |
| | FIAU | Tumor imaging | reporter genes |
| Lutetium 177 | EDTMP | Targeted Alpha Therapy | Bone tumors |
| | Dotatate | Targeted Alpha Therapy | Neuroendocrine tumors |
| Nitrogen 13 | | Myocardial perfusion | |
| | ammonia | imaging | Heart |
| Phosphorus 32 | | Tumor imaging and tumor | |
| | Sodium | treatment | Prolifirating tissue |
| | | Tumor imaging and tumor | |
| | Chromic | treatment | Prolifirating tissue |
| Potassium 42 | | Tumor Imaging and | |
| | none | distribution studies | Potassium |
| Rhenium 186 | | | _ |
| | HEDP | Beta Emitting Radiotherapy | |
| | Sulphide | Radiosynoviorthesis | Joint disease |
| Rhenium 188 | | | . . |
| | HEDP | Beta Emitting Radiotherapy | Bone tumors |
| | DIAGA | | Design and Lides |
| | DMSA | Beta Emitting Radiotherapy | Bone and kidney |
| | UDD (liniadal) | Data Emitting Dadiatharany | Llanata calullar carcinama |
| | HDD (lipiodol) | Beta Emitting Radiotherapy | Hepatocelullar carcinoma |
| | Lexidronam | Beta Emitting Radiotherapy | Bone tumors |
| | Lexidionani | | bone tumors |
| | EDTMP | Beta Emitting Radiotherapy | Bone tumors |
| Sodium 24 | none | Electrolyte studies | Electrolytes |
| Yttrium 90 | none | Radiosynoviorthesis | Joint disease |
| | hone | Radioembolization | Liver |
| Zirconium 89 | Trastuzumab | Tumor Imaging | HER2 |
| | Cetuximab | Tumor Imaging | EGFR |
| | PSMA | Tumor Imaging | Prostate |
| | Bevacizumab | Tumor Imaging | VEGF |
| | cU36 | Tumor Imaging | CD44 |

13 APPENDIX C: DETAILED IMAGES STUDY SUBJECTS

13.1 CLI-01



On the left; transverse, coronal and sagittal planes of the PET –CT fusion images. The upper right shows the maximum intensity projection of the PET scan. The blue square indicates the CLI exposure area. The lower right images shows the result of the CLI imaging. The red circle indicates the position of the tumor.

13.2 CLI-02



On the left; transverse, coronal and sagittal planes of the PET –CT fusion images. The upper right shows the maximum intensity projection of the PET scan. The blue square indicates the CLI exposure area. The lower right images shows the result of the CLI imaging. The red circle indicates the position of the tumor

13.3 CLI-03



On the left; transverse, coronal and sagittal planes of the PET –CT fusion images. The upper right shows the maximum intensity projection of the PET scan. The blue square indicates the CLI exposure area. The lower right images shows the result of the CLI imaging. The red circle indicates the position of the tumo

13.4 CLI-04



On the left; transverse, coronal and sagittal planes of the PET –CT fusion images. The upper right shows the maximum intensity projection of the PET scan. The blue square indicates the CLI exposure area. The lower right images shows the result of the CLI imaging. The red circle indicates the position of the tumor.

13.5 CLI-05



On the left; transverse, coronal and sagittal planes of the PET –CT fusion images. The upper right shows the maximum intensity projection of the PET scan. The blue square indicates the CLI exposure area. The lower right images shows the result of the CLI imaging. The red circle indicates the position of the tumor.



Aan dhr. R. Poel Afd. HOD <u>Alhier</u>

Datum: 13-04-2015 Kenmerk: PTC15.0501/N15HCL

Besluit NL52499.031.15

Geachte heer Poel, beste Robert,

Hierbij zend ik u het besluit van de Protocol Toetsingscommissie van het Antoni van Leeuwenhoek (PTC) inzake het protocol NL52499.031.15, getiteld 'Human Cerenkov luminescence imaging of superficial in-vivo tumours after administration of 18F-FDG (N15HCL).

De PTC verleent haar goedkeuring aan genoemd onderzoek. Voor de overwegingen bij het besluit verwijs ik u naar het bijgevoegde oordeel.

De PTC wijst u erop dat definitieve toestemming van de Raad van Bestuur/Directie nodig is voordat tot uitvoering van het onderzoek kan worden overgegaan.

Ik hoop u hiermee naar behoren te hebben geïnformeerd.

Met vriendelijke groet, namens de PTC, Prof. dr. E.F. Smit, voorzitter

Voor deze, Deraick M. de Kock Mw.

Ambtelijk secretaris

C.c.,

-CCMO Trialsecretariaat, alhier (digitaal) Prof. dr. T.J.M. Ruers (digitaal)

Plesmanlaan 121 1066 CX Amsterdam Website: www.avl.nl KvK-nummer: 40530817 Deutsche Bank IBAN NL79DEUT0548711089 INGbank NL22INGB0002989455 Protocol Toetsingscommissie Emailadres secretariaat: PTC@nki.nl Tel: + 31 (0)20-512 2857 |Fax: +31 (0)20- 669 1449



Aan dhr. R. Poel Afd. HOD <u>Alhier</u>

Kenmerk PTC15.0533/N15HCL Telefoon 020-512 2862 E-mail ac.bakker@nki.nl Datum 16-04-2015

Betreft: Autorisatie voor uitvoering onderzoek NL52499.031.15

Geachte heer Poel, beste Robert,

Conform het besluit van de Protocol Toetsingscommissie van het Antoni van Leeuwenhoek (PTC) d.d. 13 april 2015 deel ik u mede dat de Raad van Bestuur toestemming verleent voor uitvoering van de studie protocol NL52499.031.15, getiteld *Human Cerenkov luminescence imaging of superficial in-vivo tumours* after administration of 18F-FDG (N15HCL).

Met vriendelijke groet, namens de Raad van Bestuur

Prof. dr. E.E. Voest

Directeur Zorg en Zorgontwikkeling

C.c.: Trialsecretariaat, alhier (digitaal) Prof. dr. T.J.M. Ruers (digitaal)

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