Development of the new infrared imaging for avoiding of cancer recurrence after radical prostatectomy and Partial nephrectomy

Part 2

kidney cancer

This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG), grant FR-22-195.

Over the past two decades, the incidence of the kidney cancer has increased by 2% worldwide. 90-95% of renal malignancies are so- called renal-cell carcinoma (RCC). As for kidney sarcoma and Wilms tumor, they are much rear. Current guidelines recommend PN as the treatment of choice for the majority of patients diagnosed with a small renal mass (Campbell et al 2009, Ljungberg et al 2019). PN provides the same oncological effectiveness as radical nephrectomy, with the added benefit kidney survival and of minimizing the risk of chronic kidney disease (Touijer et al 2010). After the cancerous portion along with some healthy portion is removed from the kidney, the material is sent for examination, where it is frozen for express histo-morphological examination to determine whether there remains any tumor in the nephrectomy site.

Shota Rustaveli National Science Foundation of Georgia (SRNSFG), grant FR-22-195.

This intra-operative diagnosis is known as the ‘FS diagnosis” (Breda at al. 2007. Sidana at la. 2014). During this express examination, surgery is suspended and the surgeon is awaiting a response of the express examination, to which the next action of the surgeon is greatly dependent. If the tumor is detected by this examination at the margin, which was supposed by the surgeon as healthy tissue, the surgeon will be informed about incomplete removal of the tumor. The implication of this finding is a potential increased probability for local or distant recurrence. This is also associated with possible radiation risk, used for future treatment (Breda at al. 2007, Sidana at al. 2014). Such a patient is subject to special observation in the postoperative period.

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It is noteworthy, that FS is not characterized with high accuracy. Therefore, in the course of surgery, the surgeon does not have complete information about the negative margins of cancer, and it is appropriately common for cancerous outgrowths to remain in the nephrectomy site. This significantly increases the risk of cancer recurrence. Full information on the presence of cancerous residue in the nephrectomy site is obtained by the means of a routine histo-morphological examination performed after the accomplishment of the surgery. The recurrence rate of cancer in patients diagnosed with positive margins in the removed specimen was 16%, whereas in the case when diagnosis give of negative answer the recurrence was only 3% (Wood at al. 2018).

On the other hand at the beginning of surgery, the blood vessels supplying the kidney are clamped, which for a long time is inadmissible. This can cause renal ischemia and irreversible changes of nephrons in the kidney. Ischemia time (so-called “warm ischemia”) should be limited whenever possible: 30– 35 min should be the expected maximum duration to prevent long- term renal dysfunction. FS is time labor and time consuming, sometimes it may take more time than allowable.

Therefore, as patient follow-up and adherence to oncology principles depend heavily on the outcome of the express examination, new alternative methods are looked for that would significantly reduce the burden on the pathologist and improve diagnostic accuracy. For example, Resonance Imaging Method with 7 Tesla Magnetic field (7T MRI) was used to evaluate surgical edges and pseudo capsules of small kidney masses (Van Oostenbruggeat al. 2019). The disadvantages of this method are as follows: it is complicated, expensive, and impossible to widely use in clinics, in addition, it requires both a highly qualified radiologist and a doctor who has undergone special training.

Shota Rustaveli National Science Foundation of Georgia (SRNSFG), grant FR-22-195

In the frame of the proposed project, we developed a new method of infrared (IR) imaging that ismuc h faster, more reliable, than existing methods (and cheaper). This method allows accurate determination of the margins of cancerous formations in isolated specimens. The method needs only a few minutes, versus FS, and requires also much less time in comparison with other existing methods. This ensures that no more cancerous outgrowths remain in the nephrectomy site. As a result, the surgeon will be able to reach the negative margins.

Methods

Experimental unit consists of: infrared radiation source, holder for cancerous tissue, and CCD camera. Rays emanating from the infrared source pass through tissue (kidney). Rays after passing the tissue, containing the information about optical density inhomogeneity, fall into the infrared-sensitive matrix. The output of the CCD camera will be connected to the computer, where our computer program converts electrical signals coming from the CCD camera into visual images –IR images.

In the figure 1 is shown experimental setup for IR investigations. The setup is placed in darkness to avoid error caused by external room illumination

Experiment set up

Figure 1. Experimental set up. Kidney specimen is placed on Petri dish. LEDs are placed under the specimen - shown with arrow. CCD camera (shown with arrow) is connected to PC. IR passing through kidney tissue carries information about optical density inhomogeneity caused by existence of cancerous formations.

Methods

The CCD camera matrix contains a certain number of pixels. The intensity of infrared radiation falling on each pixel is determined by the intensity after passing from the investigated biological tissue. Therefore, the distribution of the intensities on the CCD matrix corresponds to the pattern of the intensities of the IR passing from the biological tissue. As it is well known, a CCD camera converts the intensity of an infrared ray falling on each pixel into an electrical signal of the corresponding magnitude, the value of which is proportional to this intensity. Thus signals are transmitted from the CCD camera to the computer.

In the case of homogeneity of the study tissue, the intensity of the infrared rays passing from all points of the tissue will be the same. If the tissue is heterogeneous, for example, it contains both cancerous and healthy tissue because the optical densities of these tissues are different, the intensities of the infrared light passing from them will be different from each other (Partsvania 2014, Partsvania 2016, Partsvania 2017). In particular, because tumor tissue is optically denser than non-tumor tissue, the intensity of the infrared rays after it's passing will be less than that of healthy tissue.

Results

34 experiments were provided on the kidney specimen obtained after partial nephrectomy.

Noncancerous kidney tissue IR image is characterized with homogenies distribution of illumination brightness at all area. Figure 2 illustrates this statement.

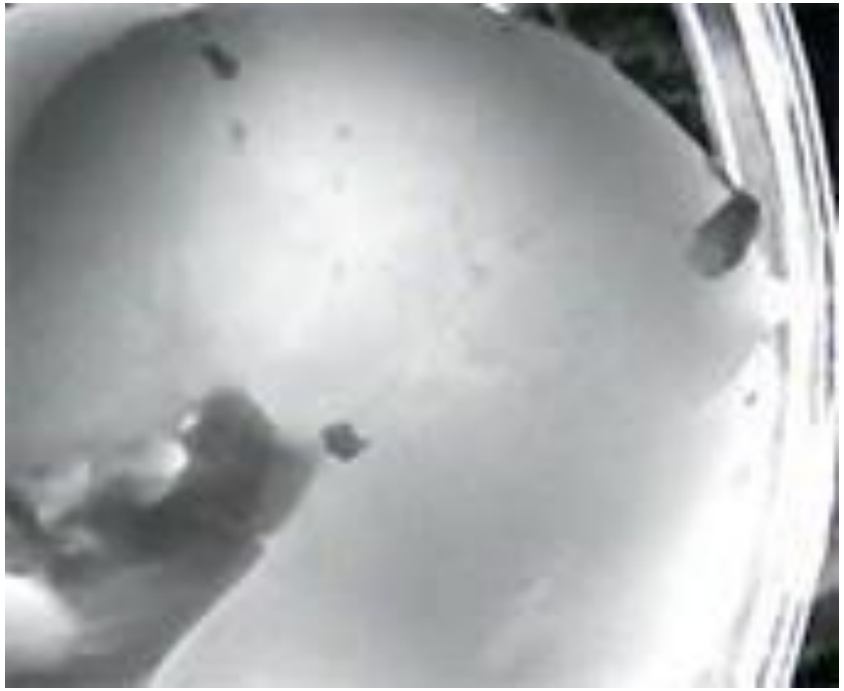


Figure 2. IR image of the noncancerous tissue placedon the Petri dish.

Results

Experiments revealed that the optical density of kidney cancerous tissue is much higher than that of healthy tissue. Therefore, the cancerous tissue is observed as areas of high darkness, while the healthy tissue is observed as areas with high illumination in the IR image. To illustrate this, let us refer to Figure 2, where the margins between cancerous and healthy tissues are sharply defined.

Positive Surgical Margins (PSMs): The presence of PSMs after partial nephrectomy can influence outcomes. While the implications of PSMs are debated, they are generally associated with a higher risk of recurrence and poorer oncological outcomes. Identifying risk factors for PSMs is essential for improving surgical techniques and patient management strategies

Results

Here we see specimen obtained after partial nephrectomy and IR image of the specimen

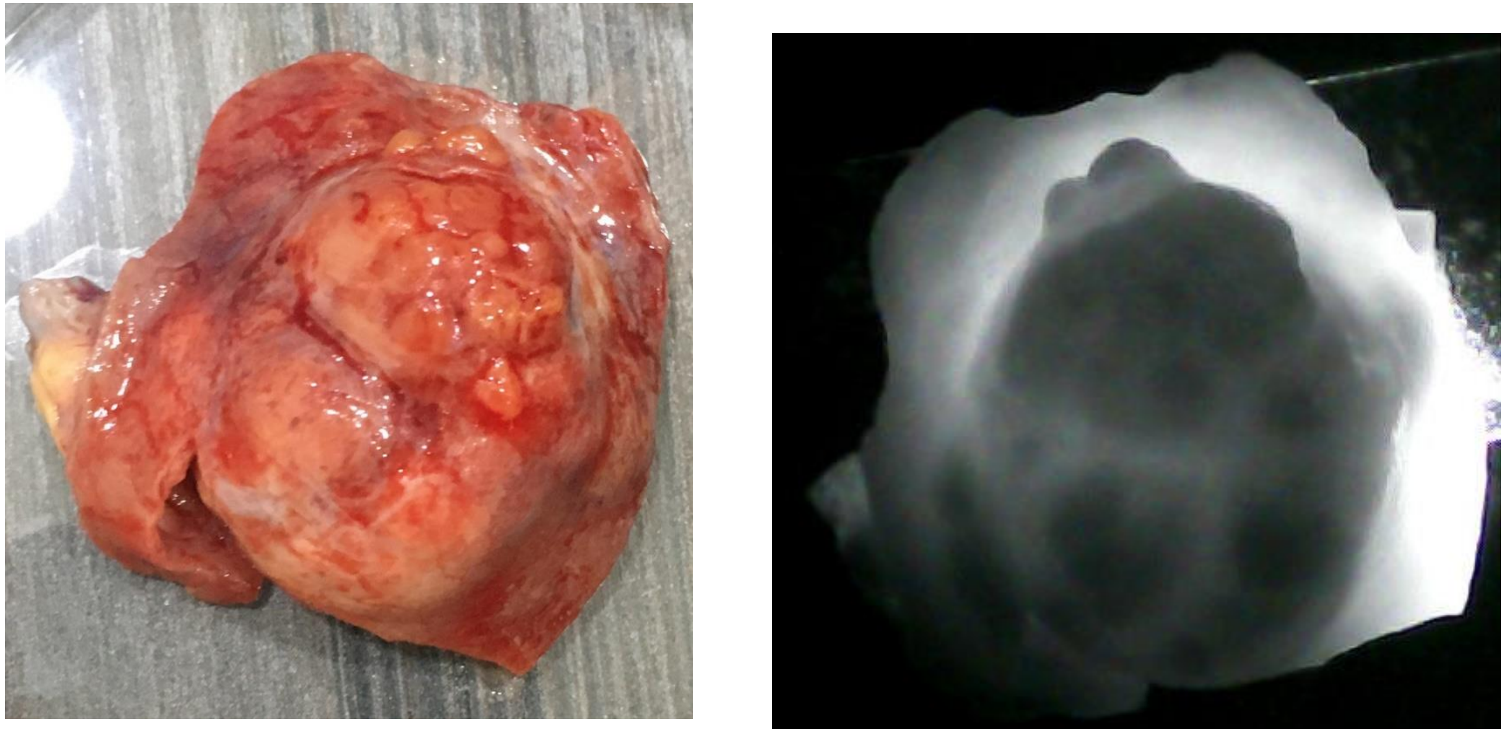


Figure 3. Left image is photography of isolates cancerous kidney tissue containing also portion of healthy tissue. Right image is IR image of this tissue. Margins between malignant and noncancerous tissues is sharply evident. Noncancerous tissue has much higher brithness, while malignant portion is observed as dark areas on the IR image.

Another case of specimen obtained after partial nephractomy and IR image of the specimen

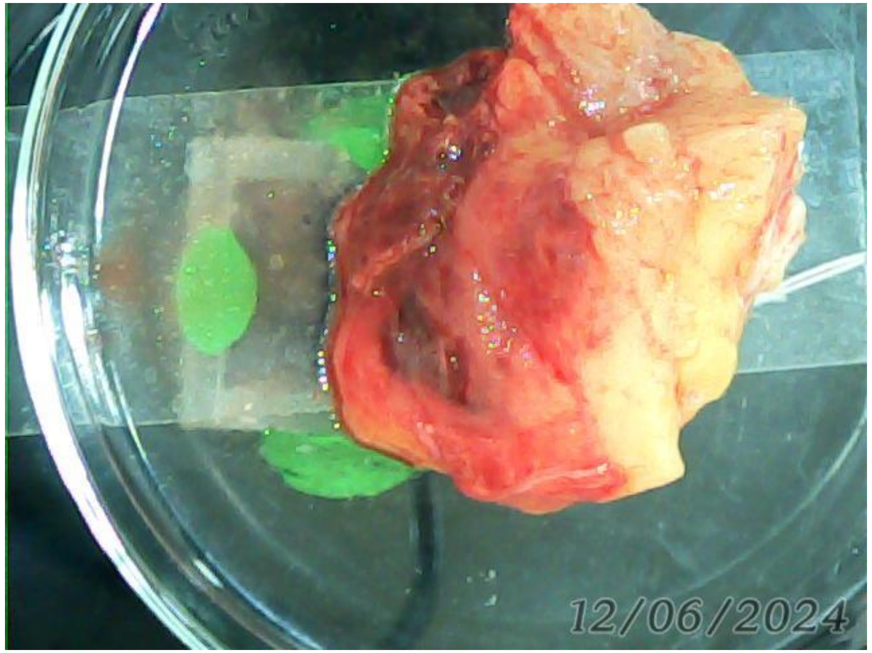
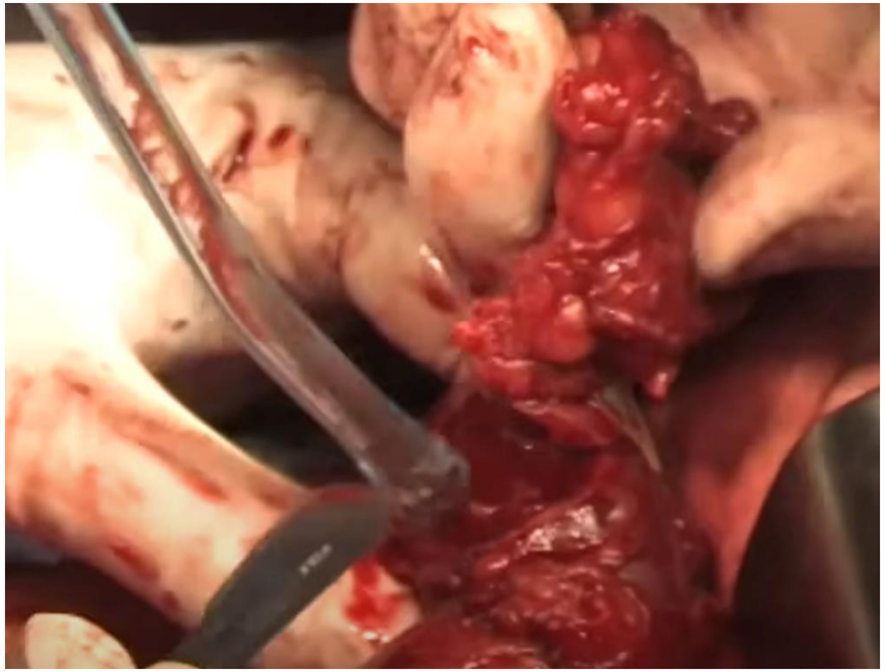
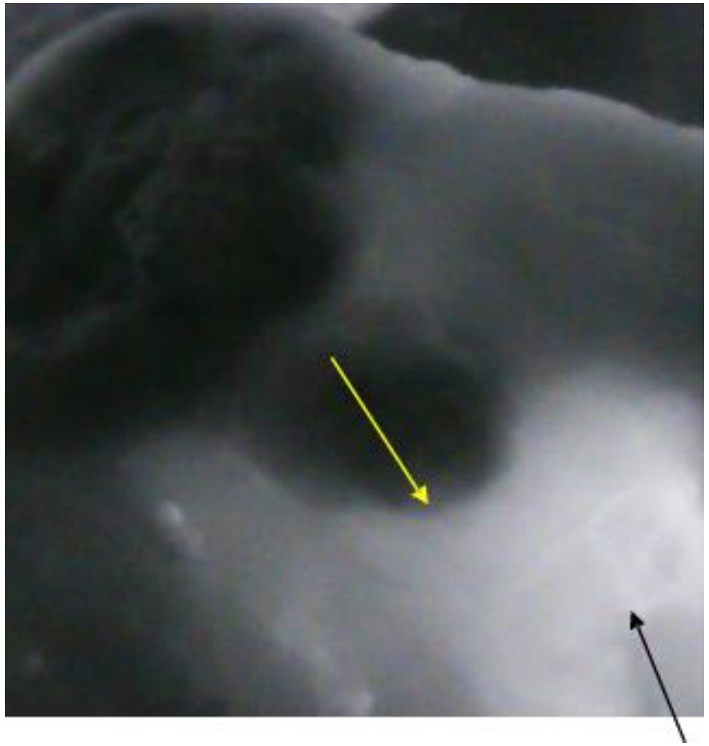


Figure 4. Left image - photography of isolates cancerous kidney. Right image is IR image of this tissue. . Noncancerous tissue has much higher brashness, while malignant portion is observed as dark areas on the IR image.

partial nephrectomy

Here is shown moment of cutting maliglant tissue during open partial nephrectomy and IR image of operated portion of kidney.





On the IR image we can notice that malignant area appears as much darker and healthy area as brighter. Yellow arrows indicates boundary between malignant and healthy areas. Black arrow shows healthy tissue.

Here is shown also isolated kidney specimen, however not all adjacent tissue was healthy. Blue arrow indicated cut portion of tissue, which was regarded as healthy one by surgeon, assessing visually. However IR examination revealed malignancy on cut tissue.

Figure 6. IR image of operated kidney tissue. Yellow arrow indicates main portion of the tumor. Black arrow indicates heathy parts and blue arrow indicates growing up into helathe portion cancer.

Kidney cancer

Portion of isolated kidney during patial nephnactomy.

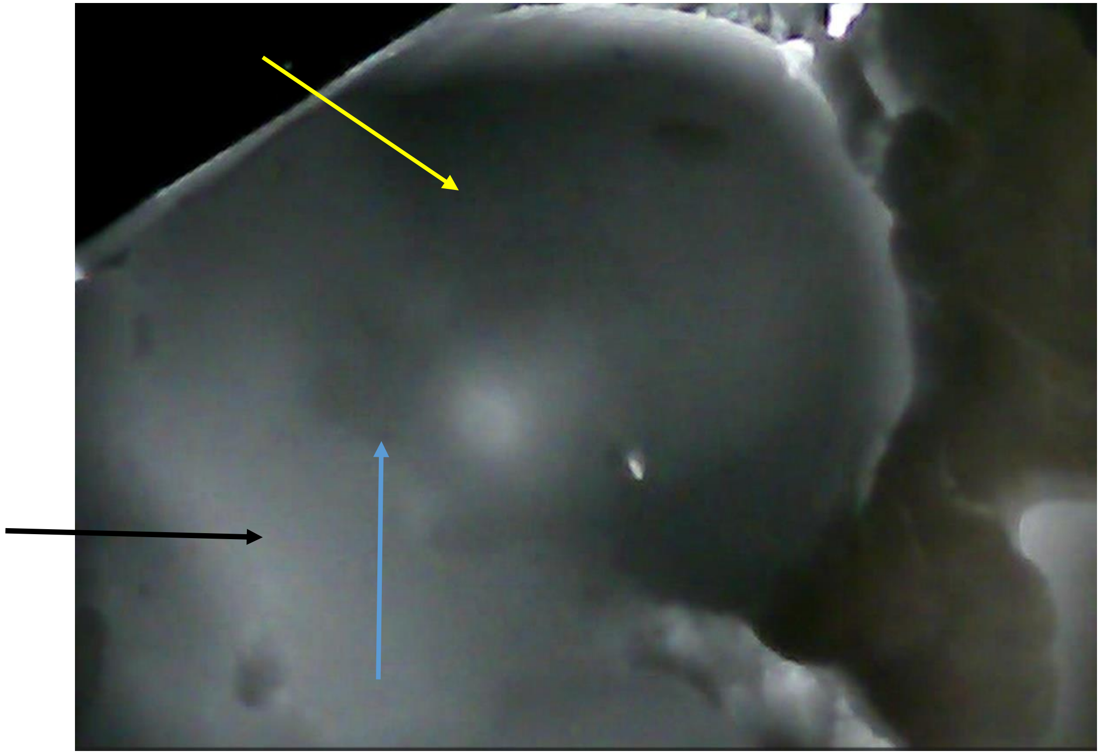


Figure7. Black arrow shows healthy area and yellow arrow – a malignant area. Tip of the blue arrow is at one of points of the margin between healthy and malignant areas.

Software administration

Developed software was administrated for determining cancerous (C) and healthy (H) portion images on the IR image and thereby find clear margins between them.

After surgery portions H and C were processed with well known methods to obtain formalin-fixed paraffin embedded tissues (FFPE) in specific containers. Then slices for microscopic investigations were cut from these FFPEs. So we obtained The slices mounted on the slide glass (SMSG) as for C as for H . We studied microscopic histo-pathological images of these SMSGs and determined their appearance. We used histo-morphological investigation of C and H tissues to determine their correspondence to the brigthnesses on the IR images.

ოპერაციის შემდეგ

The results clearly gives opportunity to distinguish between malignant and healthy tissues IR images.

Results

- **Background.** The current gold standard in nephrectomy is partial nephrectomy (PN), due to its ability to preserve kidney function while removing cancerous tissue. On the other hand, at the beginning of surgery, the blood vessels supplying the kidney are clamped. Research indicates that the duration of warm ischemia is crucial. Intraoperative frozen section (FS) examination is the prevailing technique for assessing surgical margins during PN. Yet, its limitations, such as long time and high false-negative rate, raise questions about its reliability and efficacy in ensuring optimal patient outcomes. This motivated us to look for a simpler and significantly faster approach.
- **Methods**. The sharp contrast in the IR images is produced by the IR imaging technique, which makes use of optical density variations between tumor and healthy tissue. After undergoing a radical nephrectomy, the malignant kidneys were checked. After removing the malignant tissue and a portion of the surrounding healthy tissue, the IR technique was used to evaluate the samples. Using the program we developed, we examined the specimens' infrared images. A histomorphological examination of tissue samples taken from those locations was then used to determine if malignancy was present or not.
- **Results**. According to experiments, in the infrared image, healthy tissue appears as areas with high illumination, but malignant tissue appears as areas with high darkness. Our software outlines the regions on the infrared image that belong to both healthy and malignant sections, calculates their average brightness, and determines the ratio of the malignant area's average illumination (RAI) to the healthy area's illumination. Determined the 95% probability interval for RAIs to occur, which falls between 0.25 and 0.41. After that, a histomorphological analysis was used to determine where the malignancy was. We investigated the compliance between the histomorphological appearance and the patterns of brightness and darkness in the infrared image.
- **Conclusions**. The IR imaging technique offers significant promise for improving the accuracy and efficiency of margin assessment during kidney cancer surgeries. The IR imaging technique can provide immediate feedback on the tumor boundaries, which could potentially reduce the duration of warm ischemia during surgery. Subsequent investigations should be focused on verifying the technology in further clinical trials and investigating its integration into the surgical process, which could result in its acceptance as a standard instrument for intraoperative decision-making in kidney cancer operations.

Resuts

On the other hand, we knew from which place the specimen I was taken. we knew also the intensity of the illumination of this specimen in the IR image . Our method can substitut FS method successfully.

შედეგები

- სურათზე X ნაჩვენებია ერთერთი ასეთი სპეციმენი მიღებული სრული ნეფრაქტომიის შემდეგ.
- მარცხენა სურათზე არის ამოწრილი ნაწილის ფოროსურათი, ხოლო მარჯვნივ მისი IR გამოსახულება. ამ სემთხვვაში ფარდობა ??? ტოლია.
- კვლევის შემდეგ ეტაპზე ვიკლვევდით ნაწილობრივი ნეფრატომიის ოპერაციის შემდეგ თირკმელის ამოჭრილ ნაწილებს. დგინდებოდა განათებულობათა ფარდობის მნიშვნელობა ზმოთ მითითებული მეთოდის გამოყენებით და ვადგენდით საზღვრისარსებობას 95% ალბათობით. სურათზე 2 არის ერთერთია ასთი IR გამოსახულება.
- ამის შემდეგ,   თირკმელის ამოჭრილი ეს ნაწილი იჭრებოდა ნაწილებად, მუშავდებოდა ცნობილი მეთოდით FFPE-ბის მისაღებად და სათანადოდ ინომრებოდა, რათ გვცოდნოდა მისი აღების ადგილი. ეს საშუალება გვაძლევდა განგვესაზღვრა თუ მოცემულ კონტეინერში იყო ავადმყოფი თუ ჯანმრთელი ქსოვილი.   შემდეგ Then slices for microscopic investigations were cut from these FFPEs და გამოიკვლეოდა მათი ჰისტო-მორფოლოგია. ე.ი. ცალცალკე ვიკლვევდით C და H შესაბამის კონტეინერში მყოფი სპეციმენების ჰისტო-მორფოლოგიებს.
- სურათზე 3 ნაჩვენებია ერთერთი თირკმლის C და H შესაბამის კონტეინერში მყოფი slices mounted on the slide glass (SMSG)-ის ჰისტომორფოლოგიური სურათები. კვლევებმა დაადგინა, რომ მუქი არეები IR გამოსახულებებზე მართლაც შეესაბამებოდა ავადმყოფ ქსოვილსხოლო ნათელი არეები კი ჯანმრთელს.

Conclusion

IR imaging method occurs reliable and effective method for detecting positive margins after partial nephrectomy in remaining kidney tissue. It determines with 95% probability successful removal all malignant tissues from the kidney during partial nephrectomy . Besides it consumes a few minutes versus warm or could ishemias and provides reliable savety of the kidney after operation.

Histology

kidney cancer exhibits diverse aggressiveness levels influenced by various biological factors. Understanding these differences is crucial for effective diagnosis and treatment planning.

The aggressiveness of partial nephrectomy, particularly in relation to tumor characteristics and surgical techniques, can significantly impact patient outcomes, including renal function and long-term health.

Impact of Aggressiveness on Renal Function

Acute Kidney Injury (AKI): Partial nephrectomy inherently carries a risk of AKI, which can lead to long-term renal function deterioration. Studies indicate that the duration of AKI is critical; longer AKI periods correlate with increased risks of chronic kidney disease (CKD) and cardiovascular events. Specifically, the risk ratios for long-term mortality associated with AKI duration are 1.42 for ≤2 days, 1.92 for 3–6 days, and 2.28 for ≥7 days. This suggests that managing AKI effectively post-surgery is vital to preserving renal function.

Surgical Technique: The aggressiveness of the surgical approach—such as the duration of ischemia during the procedure—also affects outcomes. Research shows that longer warm ischemia (over 20 minutes) and cold ischemia (over 35 minutes) are associated with higher rates of AKI.

Moreover, aggressive nephron-sparing techniques can help maintain renal function, but they may still lead to a modest decline in overall kidney health, especially in patients with solitary kidneys.

Tumor Location and Aggressiveness: The aggressiveness of tumors plays a crucial role in determining surgical strategies. Centrally located tumors tend to exhibit higher aggressiveness compared to peripheral ones, leading to worse prognoses. These tumors are often associated with higher ISUP grades and increased lymph node involvement, which complicates surgical management and may necessitate more aggressive interventions.

This circumstances motivated us to investigate postoperative kidney specimens using IR imaging technique.

In the experiments, kidney tissue obtained throughout parcial nephrasctomy were utilized. It was provided 36 experiments on isolated kidney specimens for studying tumor agressivnness using IR imaging technique.

It is well known that a certain technique is used to cut the kidney tissue in order to detect the aggressiveness. For this purpose a formalin-fixed paraffin embedded tissues (FFPE) are obtained by processing the specimens. After this a microtome is used to cut slices from FFPE for microscopic analyses. The slices mounted on the slide glass (SMSG) are obtained after a well-known processing step. It is easy to understand that FFPE and SMSG are complementary. Their localized cancer distributions are one and the same.

Here is shown FFPE of one of the kdney specimen, and SMSG taken from this FFPE.

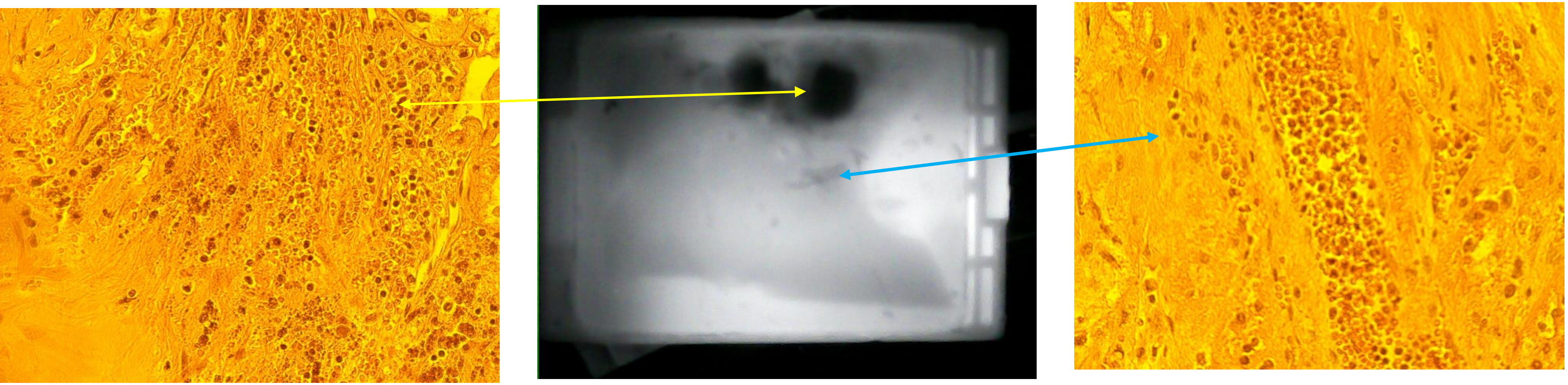
Figure 8 . One of the FFPE, and SMSG.

Experiment setup

Figure 9. FFPE is placed between CCD camera. The LEDs (indicated by the arrow) are placed below the prostate tissue. The CCD camera, indicated by the arrow, is placed above the FFPE.

K 10 18.5:23 21.5:14

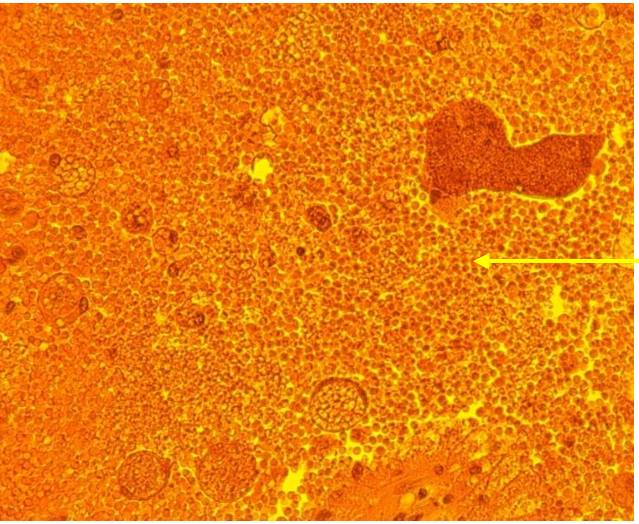
Present example demonstrarte existence of at least two aread of different agressivnesses of tomors. We can dsitingwich them on the IR image. This difference is proven by corresponding histo-morphological investigations shown on the images below. s as well th

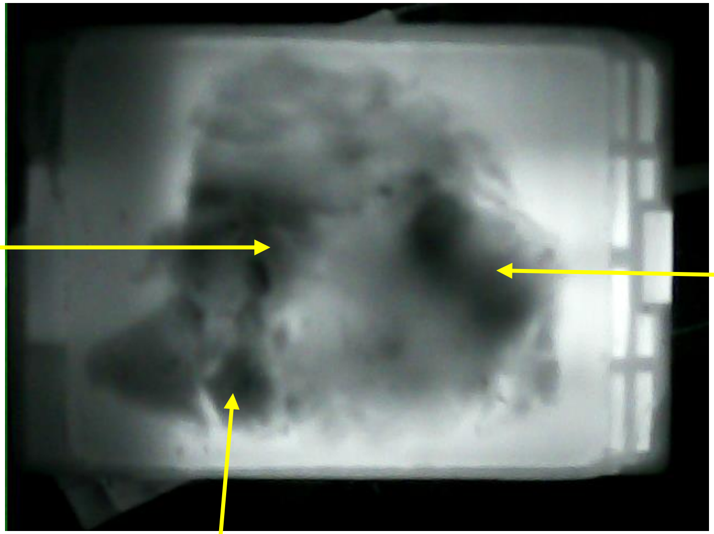


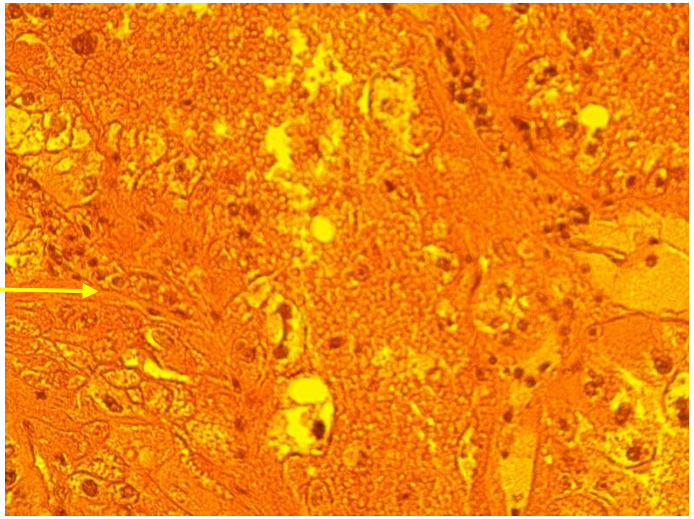
Figue 10. Middle picture is IR image of on of kidney FFPE. Left and right fictures represent histo-morphological images of the points which are connected to these pictures with arrows.

20:14 2) 30:4 3) 30:13.5

In this slide is demonstrated the case when only one aggressiveness is present in the specimen.







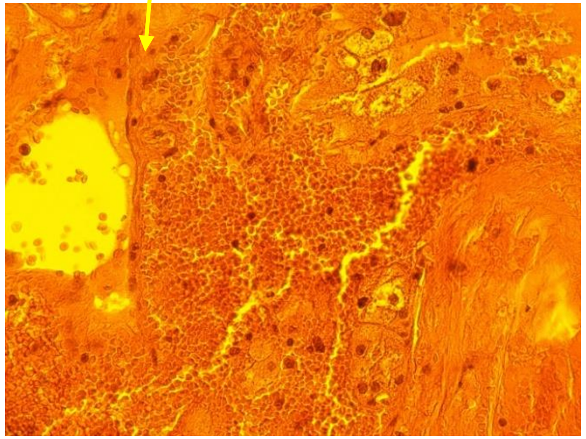


Figure 11. Kideney FFPE and Histomorphological images Taken from different points of the corresponding SMSG

Arrows connect histo-morphological images to the points from which they are taken.

Histo-morphological image of one cancerous point and it’s fluorescence image.

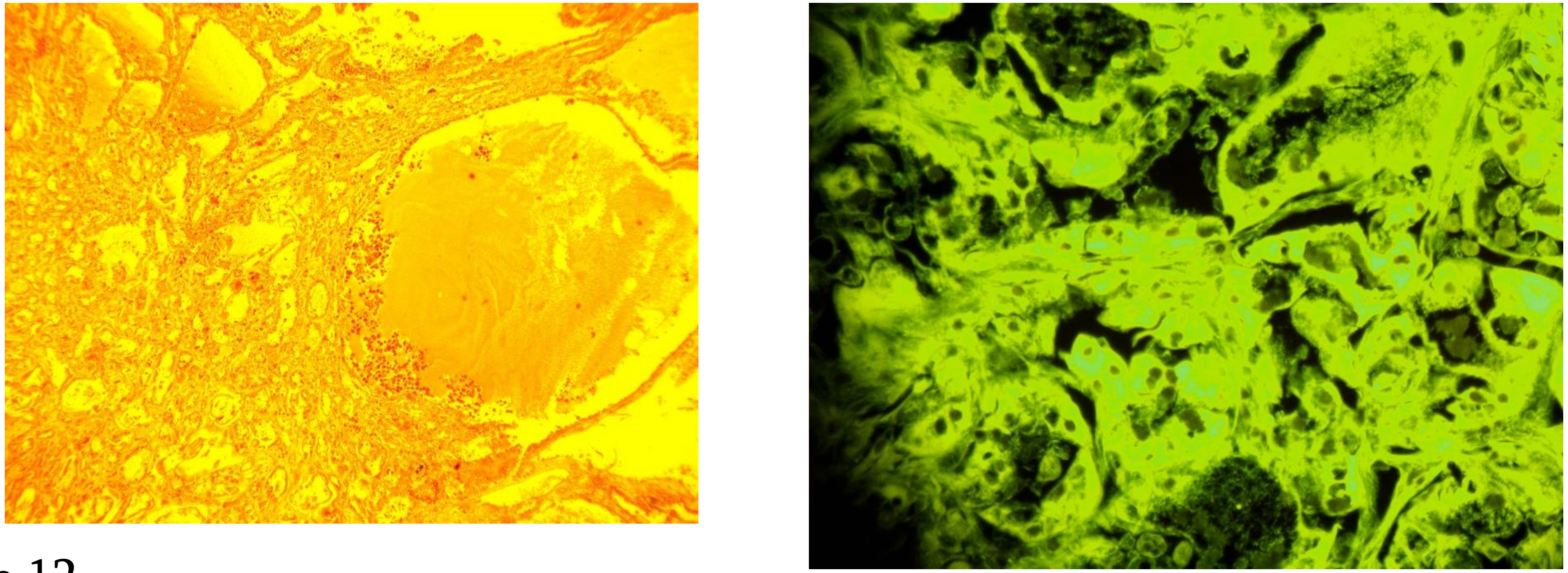
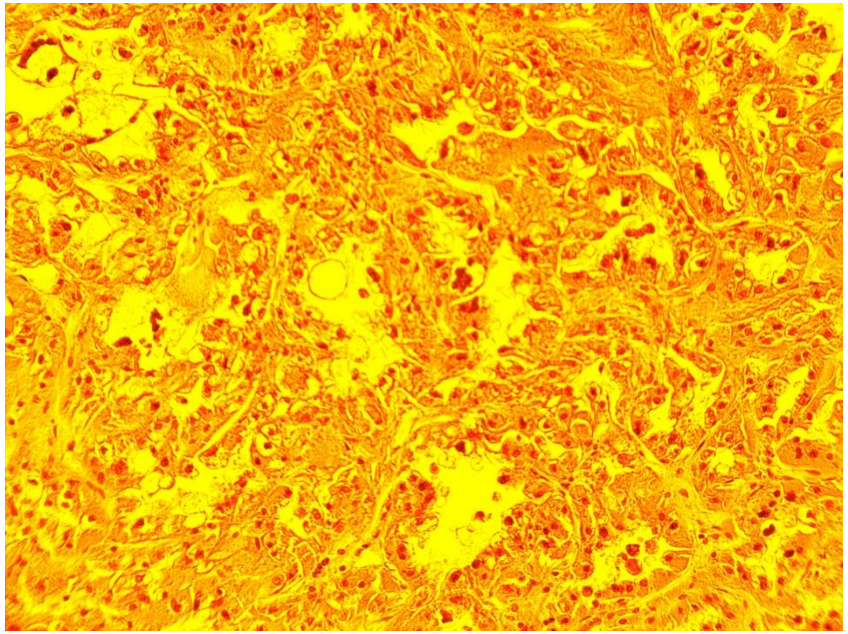
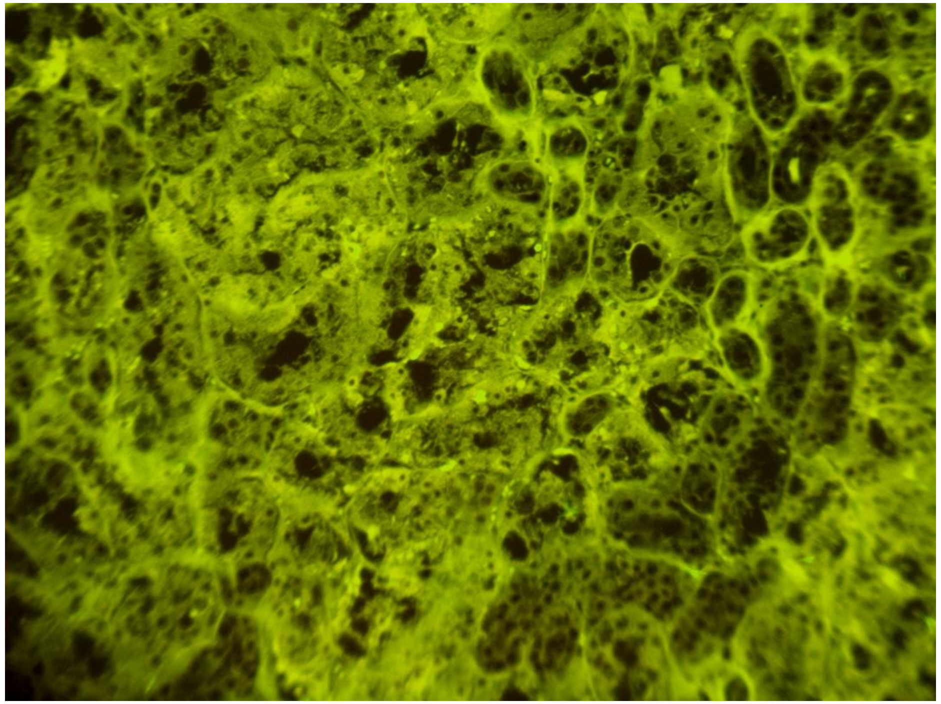


Figure 12.

Histo-morphological image of one cancerous point and it's fluorescence image. It is interesting that cnacerous tissue does not fluorescencing.





Kidney FFPE specimen IR image and histo-morphological pictures taken from different points. Thes pictures rae connected with arrow to their correspond cites.

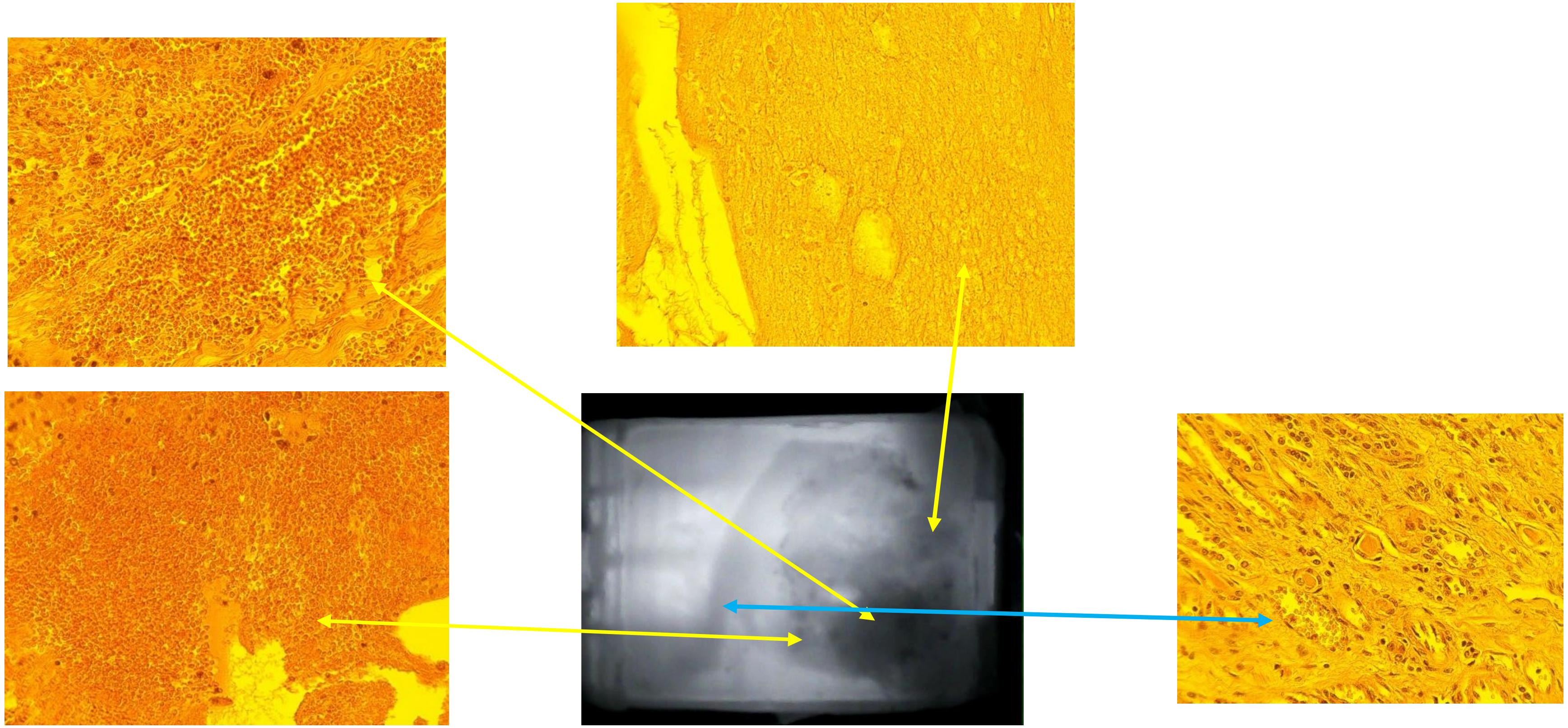
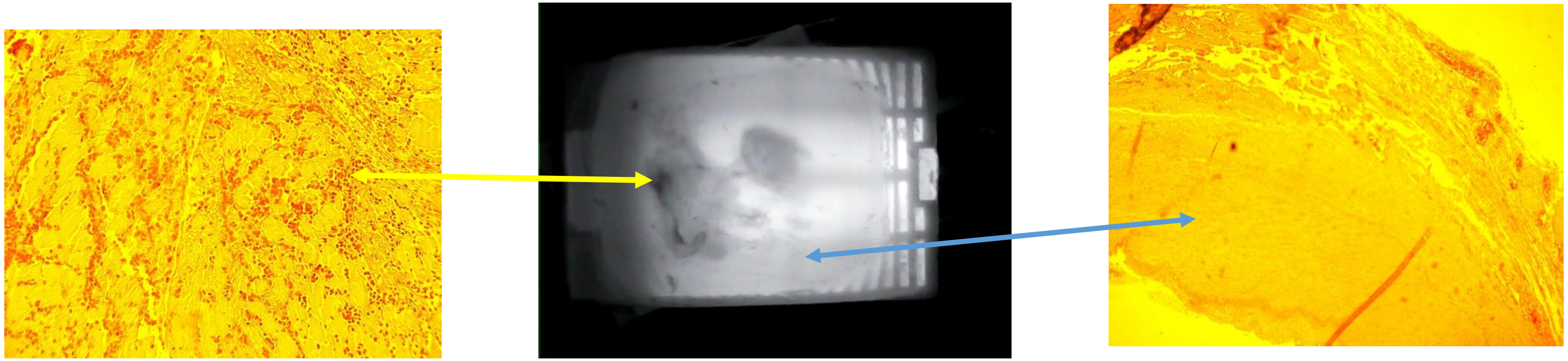


Figure 13

Example of maliglant and healthy tissue.



Left - histo-morphologicl image of cancerous cite, which appears as dark point on the IR image. On the right - histo-morphologicl image of healthy tissue .These histo-morphologicl images are connected with arrowt to correpondin points on the IR image.

Figure 14

Conclusion

IR imaging method gives opportunity to distinguish between tumors os different agressivnesses in a kidney. It csn and therby can be successfully used for investigating biopsy matherials taken befor surgery and therby.

Determining the aggressiveness of kidney cancer before surgery enhances treatment decision-making, informs surgical planning, provides prognostic insights, and facilitates personalized treatment approaches. This proactive strategy ultimately aims to improve patient outcomes and quality of life while minimizing unnecessary interventions.