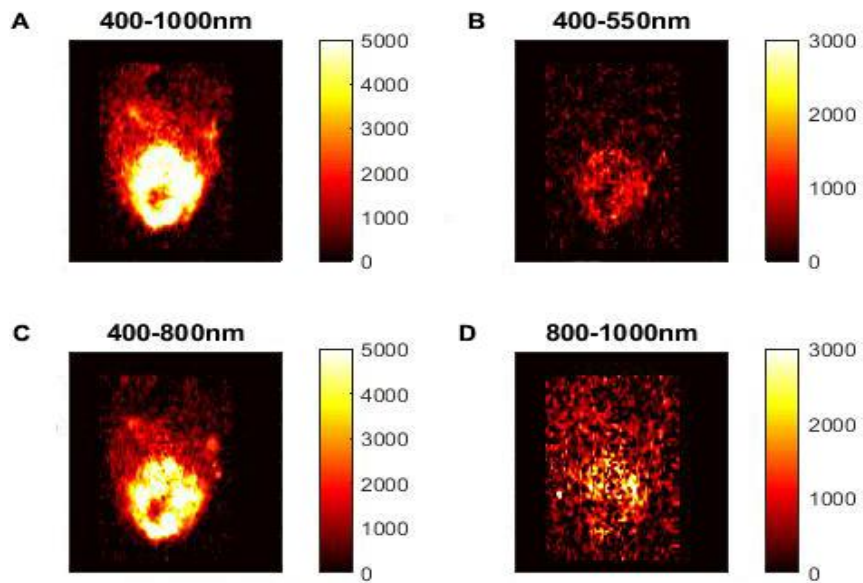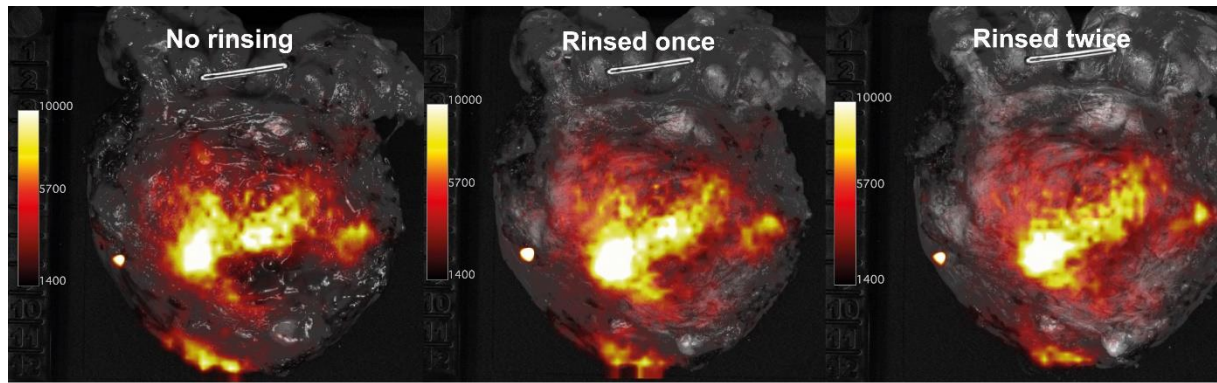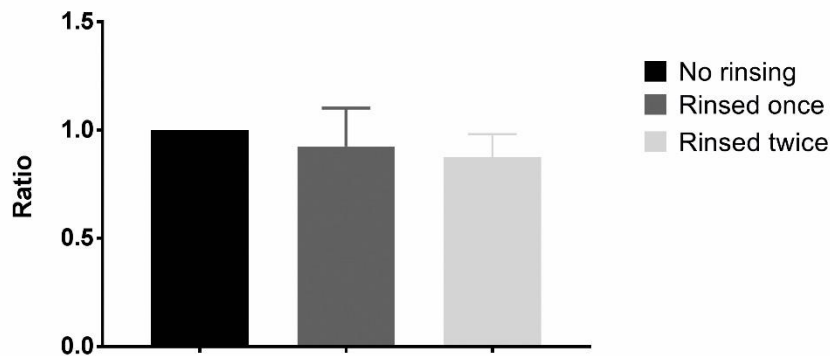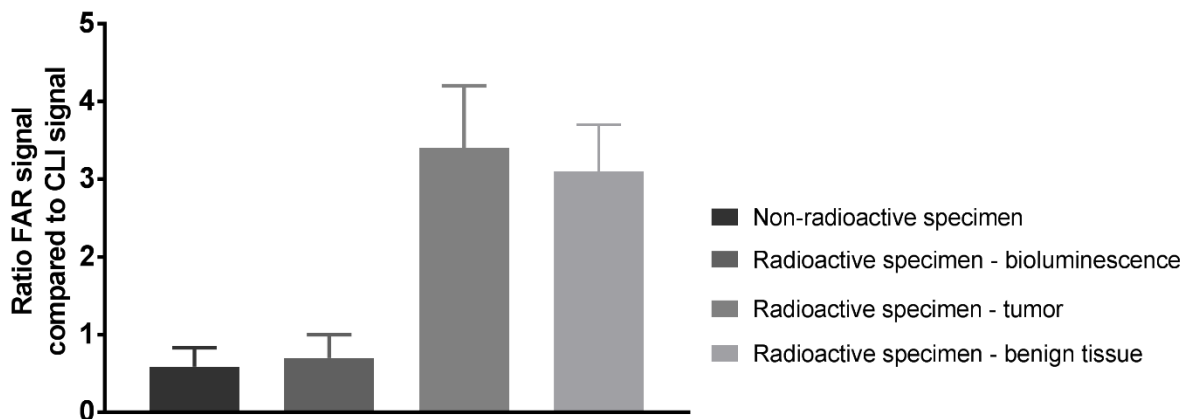Supplemental data

Supplemental Figure 1. Cerenkov image of the non-radioactive base imaged with an exposure time of 150s, 8x8 binning with A) no optical filter, B) a 550nm shortpass filter, C) a 800nm shortpass filter and D) the result of the subtraction of C from A, resulting in the theoretical signal present in the 800-1000nm spectrum. The signal of the filters resulted in a reduction of 87%, 20% and 80% for B, C, and D, respectively compared to the signal of the original non-filtered image (A). Thus, indicating that chemiluminescence was still present, and that differentiation using this 800nm shortpass filter in prostate specimens is not helpful for clinical decision-making.
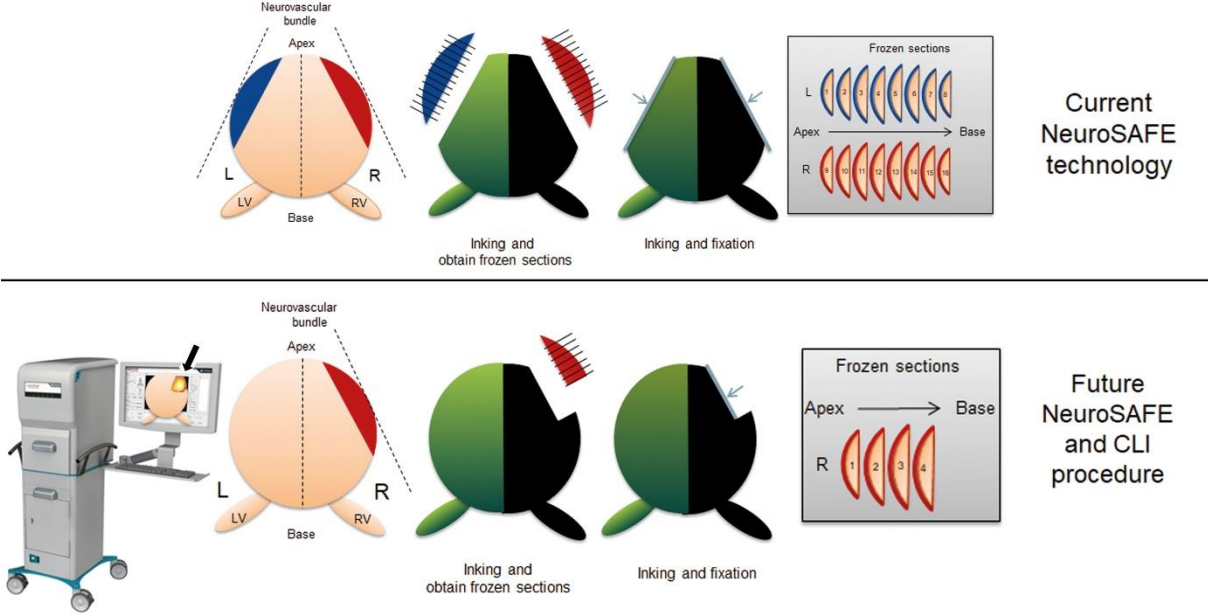
Supplemental data

Supplemental Figure 2. Effect of rinsing the prostate on the intensity level at the prostate base. First images shows the prostate directly after removal, without additional rinsing. The second image is taken 5 minutes later, after removal of possible contamination of radioactive urine and blood by using 500mL of sodium chloride. The third images is taken again 5 minutes later after rinsing it twice with sodium chloride. Images are acquired without an optical filter and the same scaling is applied. The bar chart displays the ratio between the average intensity level after rinsing it once or twice compared to the intensity level where no rinsing is applied.

Supplemental Figure 3. A bar chart showing the effect of the application of flexible autoradiography (FAR) in different specimens. The signal is displayed as a ratio compared to the unfiltered CLI image without FAR. The FAR is applied in non-radioactive and in radioactive specimens. In the latter, the three bar charts display the signals originating from areas with tumor, benign tissue and areas where diathermy was applied. Each bar chart displays the average ratio and standard deviation measured 3 different specimens.

Supplemental data

Supplemental Figure 4. Schematic overview of the current NeuroSAFE procedure (top) and potential future NeuroSAFE procedure if combined with CLI (bottom). Top: currently the neurovascular bundles are inked left (L) and right (R) in a different color. From these bundles frozen sections are obtained. The sections are assessed by a pathologist for the presences of tumor in the resection margin near the bundle. Bottom: by using the information from CLI certain areas of interest could be identified and subsequently evaluated with NeuroSAFE (see black arrow). These areas can then be inked and frozen sections obtained. By focusing only on areas at risk based on CLI, less sections need to be made and assessed by NeuroSAFE, thus making the procedure faster. As a CLI acquisition of both neurovascular bundles only takes 5 minutes.

Supplemental data

Supplemental Table 1. Agreement between CLI and histopathology at three regions of the prostate. The regions are divided into a PSM, a CSM (tumor ≤1mm from the surface), and a NSM (tumor >1mm away from the surface) based on histopathology. CLI is divided into the presence of hotspots (yes/no) and categorized by Likert scores (1-3: likely NSM, 4-5: likely PSM). The colors of the cells represent the agreement as follows: if hotspot yes and a NSM on histopathology then there is no agreement (red), if it is a PSM on histopathology then green. Orange represents the CSM, the agreement depends on the definition: if the agreement excludes the CSM, then the CSM is counted as a NSM on histopathology. When including the CSM in the agreement, then the CSM is counted as a PSM on histopathology. The agreement between CLI and histopathology is given at every region and overall, excluding and including CSM.. LS= Likert Score, PSM= Positive surgical margin, NSM= negative surgical margin, CSM= close surgical margin, CLI= Cerenkov luminescence imaging, x= no lesions in this category.

Location	Based on Histopathology	Based on CLI				
		Hotspot yes	Hotspot no	Hotspot yes LS 1-3	Hotspot yes LS 4-5	Hotspot no
Base	**NSM**	14	x	15	x	x
	CSM	2	x	1	1	x
	PSM	4	x	4	x	x
	Agreement excluding CSM	4/20 =20%		15/20=75%		
	Agreement including CSM	(4+2)/20=30%		(15+1)/20=80%		
Mid-gland	**NSM**	3	9	1	2	9
	CSM	6	x	2	4	x
	PSM	x	x	x	x	x
	Agreement excluding CSM	9/18 =50%		(9+1+2)/18=67%		
	Agreement including CSM	(6+9)/18=83%		(9+1+2+4)/18=89%		
Apex	**NSM**	9	x	4	5	x
	CSM	11	x	x	11	x
	PSM	6	x	x	6	x
	Agreement excluding CSM	6/26 =23%		(6+4)/26=38%		
	Agreement including CSM	(6+11)/26=65%		(6+4+11)/26=81%		
Overall	**Agreement excluding CSM**	31%		60%		
	Agreement including CSM	59%		83%		