Investigation of the Spread of Indocyanine Green in the Lymphatic System of the Prostate: An Experimental Study on Rats

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Investigator: Gasanov Denis

Scientific Supervisor: Prof. Robert Molchanov

Dnipro State Medical University
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Lymph node dissection is an important component of radical prostatectomy in patients with prostate cancer. However, its performance is associated with a complication—lymphocele, which may require additional treatment. Limiting the extent of lymph node dissection through sentinel lymph node assessment represents an attractive alternative to extended dissection. One of the effective methods for identifying sentinel lymph nodes is intraoperative fluorescence imaging using the ICG/NIR technology [1]. Nevertheless, the patterns of ICG distribution within the lymphatic system of the prostate and the impact of inflammatory processes on this distribution remain insufficiently studied.

Objective

To determine the dynamics of ICG distribution in sentinel lymph nodes and to evaluate the impact of prostatic inflammation in a rat model.

Tasks:

1. To investigate the characteristics of ICG distribution in the prostate and lymph nodes under normal conditions.
2. To assess the effect of inflammation on the dynamics of ICG accumulation.
3. To perform histological verification of morphological changes in the prostate and lymph nodes.

Materials and Methods

Ethical Considerations

The experimental study was conducted in compliance with the principles of humane treatment of laboratory animals, as regulated by:

- The Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV, dated February 21, 2006),
- The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, March 18, 1986)

The study was reviewed and approved by the Local Ethics Committee of Dnipro State Medical University (Protocol No. 23, dated December 18, 2024).

The experiment was conducted on 14 male Wistar rats, aged 6–7 months, weighing 210–306 g. The animals were divided into two groups: Group I – without inflammation (n = 9), and Group II – with induced prostatic inflammation (n = 5)

Under general anesthesia induced by intraperitoneal administration of thiopental sodium (40 mg/kg), the rats underwent a midline laparotomy (from the xiphoid process to the pubic

symphysis), which provided optimal exposure of the prostate and regional lymph nodes. Following exposure, indocyanine green (ICG) was injected directly into the prostate.

The dose of ICG was 0.02 mg/kg body weight (average single dose ≈ 0.004 mg in 0.03 ml). The tracer was administered intraprostatically: 0.01 ml into the apical, middle, and basal segments of each lobe. In total, the injection volume was 0.09 ml, distributed among the right and left dorsal, and ventral prostate lobes. In connection with the most prominent inflammatory changes in these regions [2]. The dynamics of ICG accumulation were recorded using the IC-Flow Imaging System PC 6200 at predefined time points (0, 15, 30, 45, and 60 minutes; in some cases, 75 and 90 minutes). Fluorescence intensity was analyzed in relative units using the Fiji/ImageJ software package.

Prostatitis was modeled using a standard method by intrarectal administration of a mixture of turpentine (meta- or ortho-xylene) with 10% dimethyl sulfoxide (DMSO) in a 1:4 ratio. The mixture was vigorously shaken for one minute to obtain a stable emulsion and introduced into the rectum using an atraumatic semi-rigid catheter (3 mm diameter, 25 mm length). A single dose of 1 ml was administered at a depth of 20–25 mm from the anal verge, corresponding to the anatomical location of the prostate. Clinical manifestations of prostatitis developed by day 28.

Euthanasia

At the end of the experiment, animals were sacrificed by anesthetic overdose under thiopental sodium anesthesia. This approach complies with international FELASA (Federation of European Laboratory Animal Science Associations) recommendations for the humane termination of rodent experiments. Following termination of the experiment, tissue samples were harvested for further morphological and histological evaluation.

Image Analysis

Quantitative fluorescence analysis was performed using Fiji/ImageJ software (https://imagej.net/ij/), an open-source package with preinstalled plugins. Regions of interest (ROIs) were drawn around the fluorescent areas, and signal intensity was measured in arbitrary units (a.u.).

Statistical Analysis

Statistical analysis was carried out using the R software package (version 4.2.0; packages *stats*, *ggpubr*), a free and open-source solution. Normality of distribution was assessed using the Shapiro–Wilk test. For within-group longitudinal comparisons, the Friedman test was applied, followed by pairwise Wilcoxon signed-rank tests. Between-group comparisons at each time point were performed using the Mann–Whitney U test. All analyses used non-parametric methods due to non-normal distributions.A p-value of <0.05 was considered statistically significant.

Results

Raw data are provided in Table1, 2

Table 1. Raw Data: Group I (Healthy Prostate) – Weight, ICG Appearance, and Fluorescence Intensities

No.	Weight (g)	Lymph Node* Appearance Time (min)	Prostate** Fluorescence Intensity (a.u.)	Lymph Node Fluorescence Intensity (a.u.)
1***	271	--	15'–1.5 30'–1.7 45'–2.8 60'–2.0	--
2	259	23	15'–0.57 30'–0.58 45'–0.58 60'–0.95 75'–0.86	30'–2.5 45'–3.1 62'–3.75 75'–2.93 90'–3.76
3	234	31	15'–1.01 30'–0.94 45'–0.80 60'–0.88	30'–1.10 45'–1.30 60'–4,5
4	224	30	15'–1.60 30'–1.57 45'–1.31 60'–1.27	30'–1.69 45'–1.16 60'–1.48
5	284	18	15'–3.94 30'–3.00 45'–3.50 60'–5.85 75'–2.06	30'–15.00; 45'–14.47; 60'–23.75
6	306	15	15'–1.26 30'–1.09 45'–1.59 60'–1.70	15'–4.05 30'–6.10 45'–7.70 60'–12.14
7	288	16	15'–5.00 30'–1.75 45'–5.77 60'–4.49	15'–2.05 30'–2.17 45'–9.42 60'–4.80
8	210	33	15'–1.46 30'–1.52 45'–0.74 60'–0.75	30'–1.88 45'–2.18 60'–2.63
9	208	32	15'–2.64 30'–1.04 45'–1.85 60'–1.73 75'–4.14	15'–0.96 30'–1.60 45'–1.30 60'–5.85

Table 2. Raw Data: Group II (Prostatitis) – Weight, ICG Appearance, and Fluorescence Intensities

No.	Weight (g)	Lymph Node Appearance Time (min)	Prostate Fluorescence Intensity (a.u.)	Lymph Node Fluorescence Intensity (a.u.)

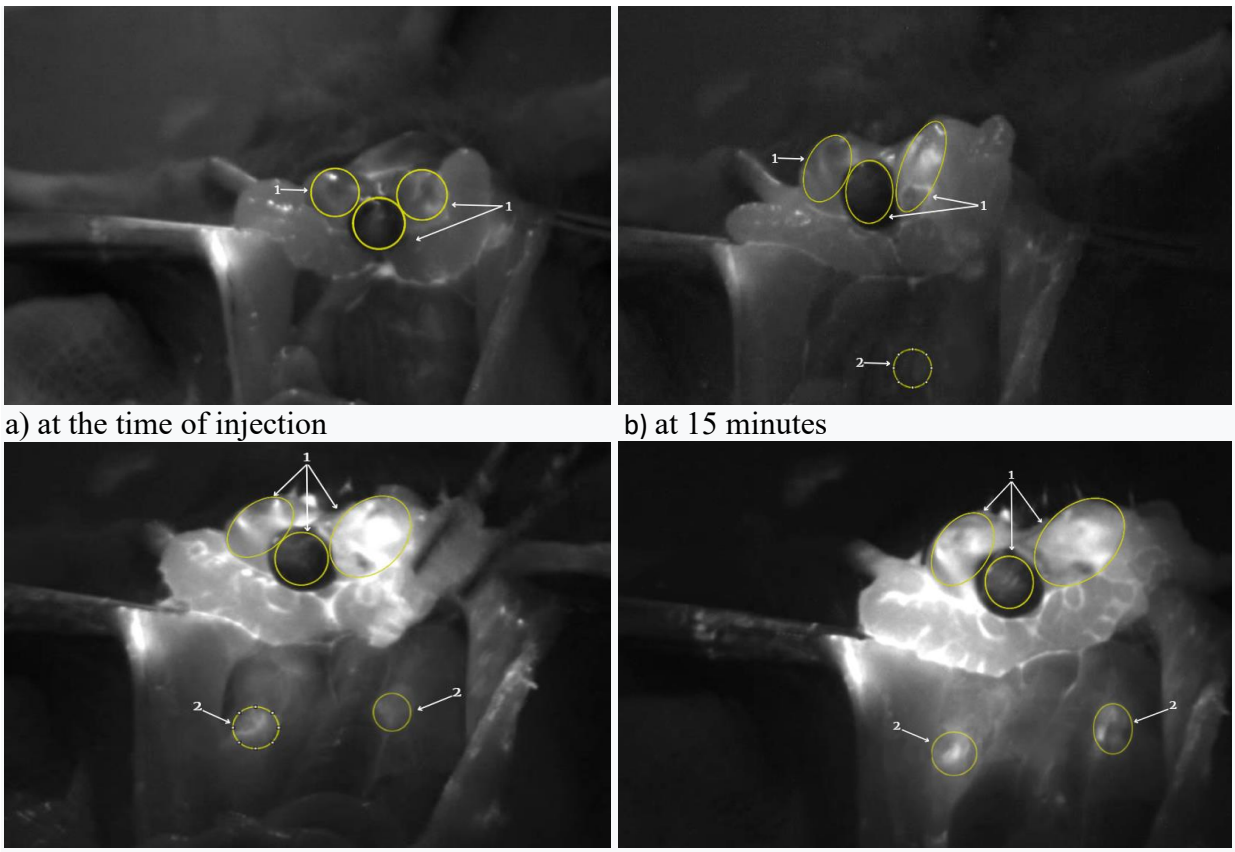
1	250	32	15'–0.88 30'–3.26 45'–1.49 60'–1.81	30'–13.42 45'–8.13 60'–8.50
2	225	30	15'–1.63 30'–1.33 60'–4.70	15'–1.82 30'–4.39 45'–11.46 60'–18.75
3	235	28	15'–9.44 30'–4.00 45'–4.03 60'–3.35	30'–2.70 45'–2.93 60'–2.10
4	286	32	15'–1.91 30'–2.00 45'–1.04 60'–4.91	15'–2.23 30'–2.50 45'–1.85 60'–5.93
5	230	17	15'–1.19 30'–2.06 45'–4.35 60'–6.26	15'–0.66 30'–1.53 45'–3.42 60'–9.44

*-The time when we can see the first illuminated lymphatic node

**- The intensity of the glow at the moment of injection of ICG was taken as 1.

*** In the case 1 we did not succeed to identify sentinel lymph node, but we include it for statistic for ICG spreading in prostate

Fig. 1 illustrates the stages of ICG accumulation in the prostate and regional lymph nodes.



a) at the time of injection b) at 15 minutes

c) at 30 minutes d) at 60 minutes

Figure 1. Stages of contrast agent accumulation (a,b,c,d). Legend: 1 – prostate; 2 – lymph node.

Histological verification of the prostate and sentinel lymph nodes was performed.

In the group of animals with experimentally induced inflammation, histological examination of the prostate tissue revealed pronounced interstitial edema. The stroma appeared indistinct due to marked plasma impregnation. In most microscopic fields, the glands were cystically dilated. In less affected areas of the prostate tissue, the amount of stroma was greater and the edema was less pronounced. The glandular contours were irregular, with small papillary projections. Signs of secretory activity were either absent or unevenly reduced, and the cytoplasm of the glandular epithelium appeared darkened (Fig. 2a, b). In regions with a higher content of stromal elements and capillaries, focal perivascular inflammatory infiltrates were observed, composed of segmented neutrophils with occasional lymphocytes and macrophages (Fig. 2c).

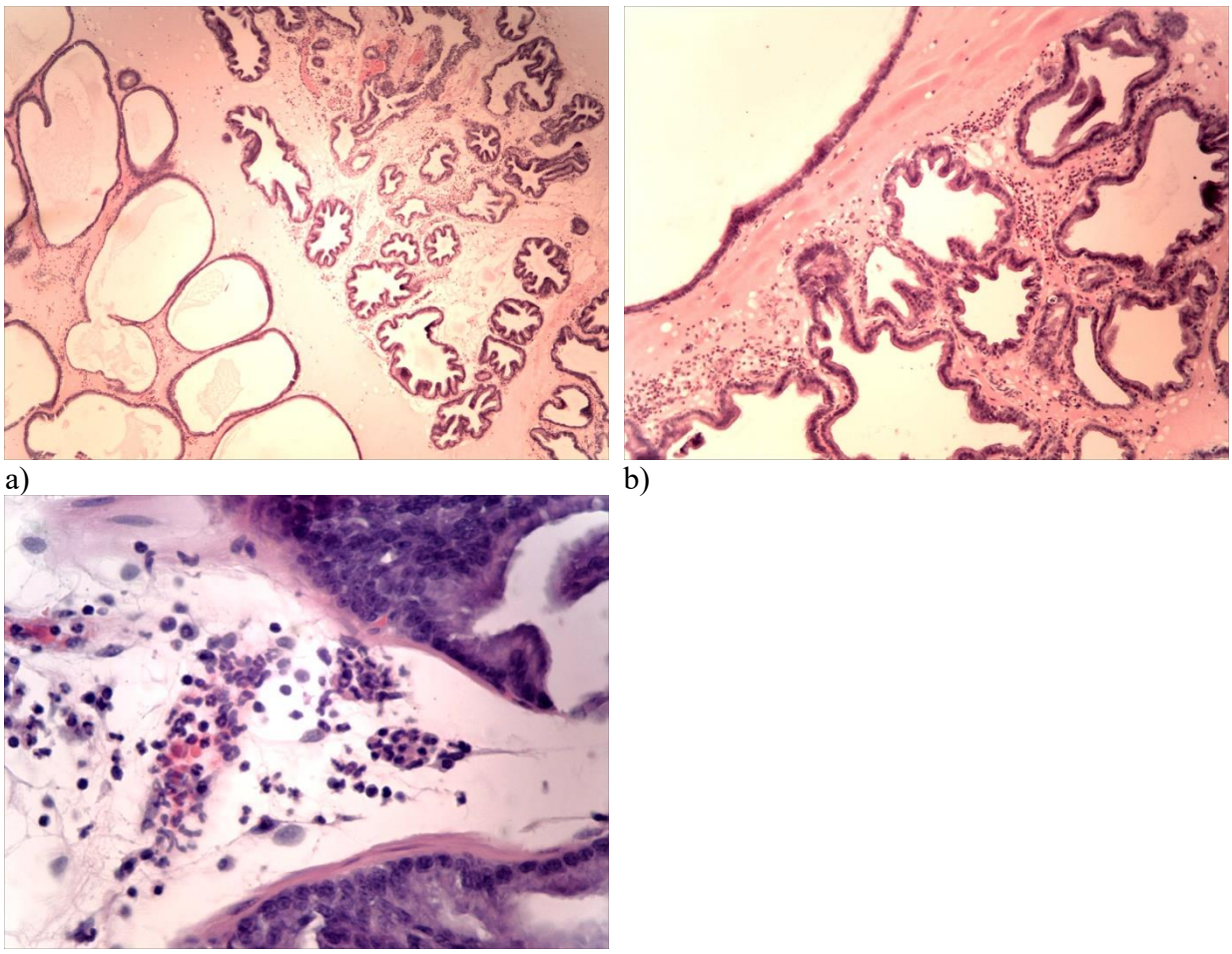
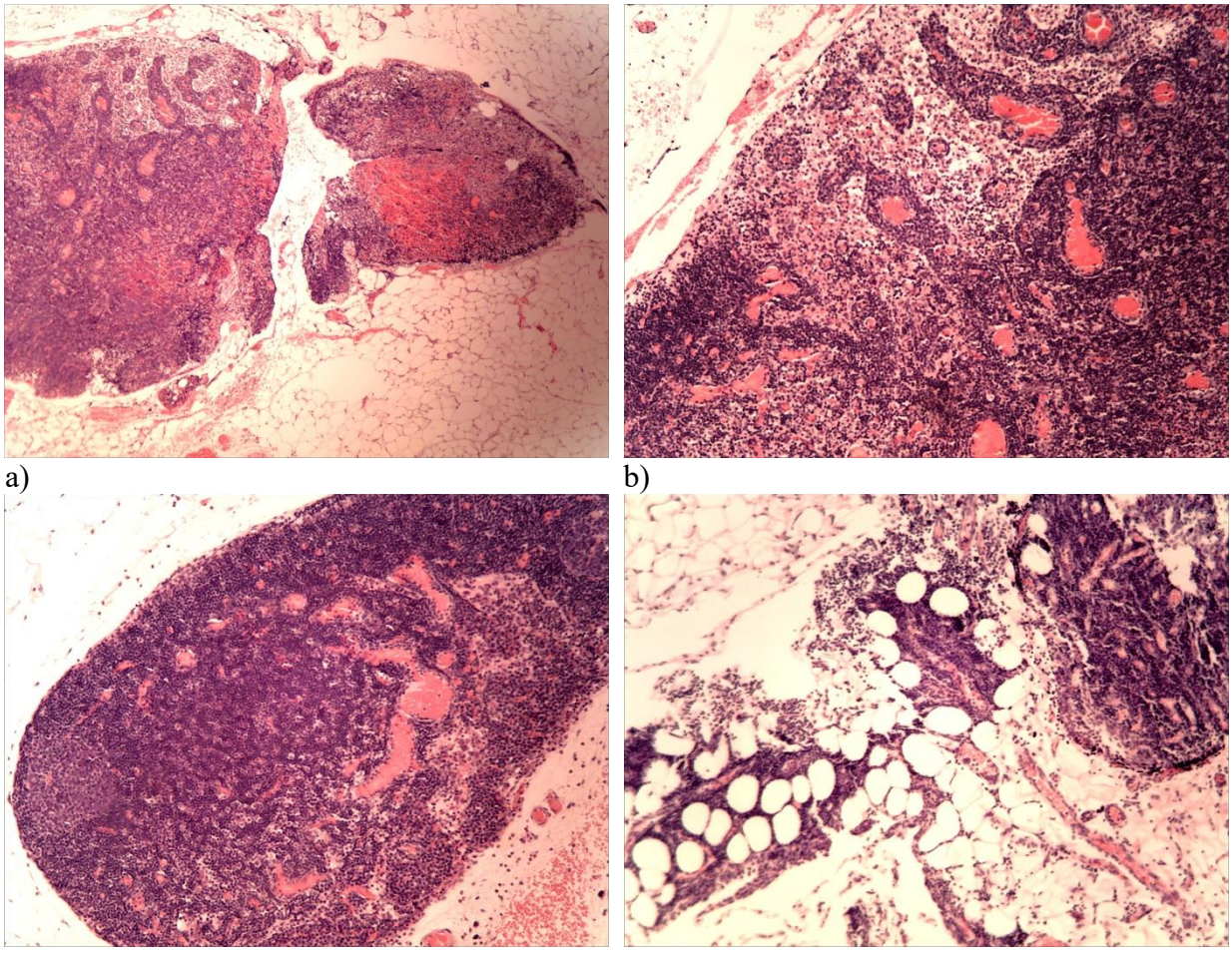


Figure 2. Prostate. a) Interstitial edema, plasma impregnation of the stroma, cystically dilated glands. ×40. Hematoxylin and eosin staining. b) Irregular glandular contours, papillary projections, reduced signs of secretory activity, inflammatory infiltrates. ×100. Hematoxylin and eosin staining. c) Perivascular inflammatory infiltrate. ×400. Hematoxylin and eosin staining.

In the lymph node tissue, pronounced congestion was observed. The sinuses were dilated and filled with histiocytes (Fig. 3a, b). Irregular hyperplasia of lymphoid follicles and infiltration of lymph node tissue with hypertrophied macrophages were detected (Fig. 3c). In some lymph nodes, marked liposclerosis with substantial replacement of lymphoid tissue by adipose tissue was identified (Fig. 3d).



a) b) c) d)

Figure 3. Lymph nodes. a) Congestion, hemorrhages, sinus histiocytosis. ×40. Hematoxylin and eosin staining. b) Same findings. Histiocytes in dilated sinuses. ×100. Hematoxylin and eosin staining. c) Congestion, hyperplasia of lymphoid follicles, infiltration with hypertrophied macrophages. ×40. Hematoxylin and eosin staining. d) Pronounced liposclerosis of lymphoid tissue. ×40. Hematoxylin and eosin staining.

Statistical Analysis Results

Descriptive statistics were computed separately for the two experimental groups: Group I (rats without inflammation) and Group II (rats with induced prostatic inflammation). Group II generally exhibited higher mean fluorescence intensity in both prostate and lymphatic tissues, with increased variability at later time points.

Fluorescence intensity was recorded at predefined time points after ICG injection.

Prostate tissue:

- In both groups, fluorescence gradually increased.
- Group II showed enhanced fluorescence from 30 minutes onward, with the most pronounced difference at 60 minutes.

Lymphatic nodes:

- Group II showed consistently higher intensity values at 30, 45, and 60 minutes.
- This indicates enhanced ICG transport via lymphatics in inflammation.

1. Normality Testing (Shapiro–Wilk Test)
Normality of fluorescence intensity distributions was tested at each time point using the Shapiro–Wilk test. Many variables had p-values < 0.05, indicating non-normal distributions, especially in Group II. This suggests the appropriateness of non-parametric statistical tests (Tab. 3) .

Table 3. Shapiro–Wilk Normality Test Results

Tissue	Time Point	Group	W-statistic	p-value
Prostate	15 min	I	0.852	0.07926
Prostate	15 min	II	0.657	0.00323
Prostate	30 min	I	0.785	0.01386
Prostate	30 min	II	0.932	0.61099
Prostate	45 min	I	0.914	0.34309
Prostate	45 min	II	0.893	0.39824
Prostate	60 min	I	0.716	0.00219
Prostate	60 min	II	0.972	0.89046
Prostate	75 min	I	0.903	0.44745
Lymph Node	30 min	I	0.783	0.01898
Lymph Node	30 min	II	0.736	0.02219
Lymph Node	45 min	I	0.818	0.04401
Lymph Node	45 min	II	0.875	0.30849
Lymph Node	60 min	I	0.752	0.00850
Lymph Node	60 min	II	0.931	0.60000

2. Repeated Measures (Friedman Test)
The Friedman test was used to evaluate time-based changes in fluorescence within groups (Tab.4).

Table 4. Friedman Test for Repeated Measures – Prostate Fluorescence Intensity

Group	Tissue	Friedman Stat	p-value
I	Prostate	10.68	0.0136
II	Prostate	-	-
I	Lymph Node	-	-
II	Lymph Node	-	-

- The Friedman test for Group I – Prostate yielded a p-value of 0.0136, indicating statistically significant changes in fluorescence over time within this group.
- The test could not be applied to other tissues/groups due to insufficient repeated observations.

3. Wilcoxon Signed-Rank Test Results

The test was applied to assess whether fluorescence intensity in the prostate changed significantly over time within each group (Group I: healthy animals, Group II: animals with prostatitis) (Tab.5).

Table 5. Wilcoxon Test Results (Prostate Fluorescence)

Group	Time 1	Time 2	W-statistic	p-value
I	15 min	30 min	10.0	0.2626
I	15 min	45 min	13.0	0.3008
I	15 min	60 min	17.0	0.5703
I	30 min	45 min	16.0	0.7792
I	30 min	60 min	11.5	0.2031
I	45 min	60 min	14.0	0.3594
II	15 min	30 min	7.0	1.0
II	15 min	60 min	5.0	0.625
II	30 min	60 min	3.0	0.3125

Based on the results, none of the within-group time comparisons for prostate fluorescence intensity showed statistically significant differences ($p > 0.05$). This suggests that although some fluctuation in intensity was observed, it was not consistent or strong enough to confirm a systematic trend between individual timepoints when tested pairwise.
However, the Friedman test (reported earlier) showed a significant overall change across all timepoints in Group I, indicating that while no two specific timepoints differ significantly, the overall pattern does suggest dynamic accumulation over time.

The Wilcoxon signed-rank test was also applied to lymph node fluorescence intensity data to determine whether there were statistically significant changes in signal over time within each experimental group. Due to limited data in some time points, only time pairs with sufficient samples ($n \geq 5$) were included in the analysis.

Table 6. Wilcoxon Test Results (Lymph Node Fluorescence)

Group	Time 1	Time 2	W-statistic	p-value	Significant
I	30 min	45 min	17.0	0.9453	No
I	30 min	60 min	8.0	0.1953	No
I	45 min	60 min	7.0	0.1484	No
II	30 min	60 min	4.0	0.4375	No

The Wilcoxon signed-rank test results showed no statistically significant differences in fluorescence intensity between any pair of time points in either Group I (healthy animals) or Group II (animals with prostatitis). This suggests that while lymph node fluorescence was observed and varied between subjects, there was no consistent pattern of change over time within individual animals.

4. Between Group Comparison (Mann–Whitney U)

Used to compare Group I and Group II at each time point (Tab. 7).

Table 7. Mann–Whitney U Test – Between Groups (Prostate and Lymph Node at Each Time Point)

Tissue	Time Point	U-statistic	p-value
Prostate	15 min	21.00	0.89810
Prostate	30 min	11.00	0.14685
Prostate	45 min	12.00	0.41399
Prostate	60 min	9.00	0.08292
Lymph Node	30 min	17.50	0.76939
Lymph Node	45 min	11.00	0.91852
Lymph Node	60 min	14.00	0.43512

- No statistically significant differences ($p < 0.05$) were observed between the groups at any time point.
- However, the prostate fluorescence at 60 minutes shows a trend toward significance ($p \approx 0.083$), which aligns with earlier analyses.
- This suggests a possible effect of inflammation on fluorescence accumulation over time.

Dynamic of fluorescence intensity in prostate and lymph nodes depicted in Fig.4, 5

Figure 4: Prostate Fluorescence Intensity (Mean ± SD)

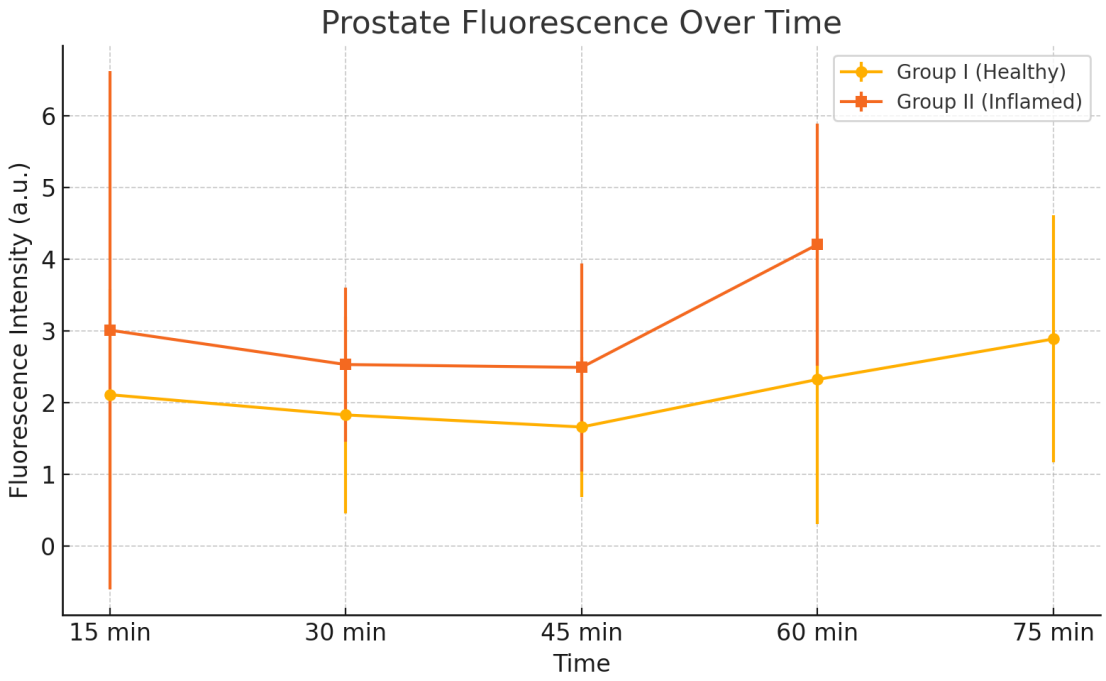
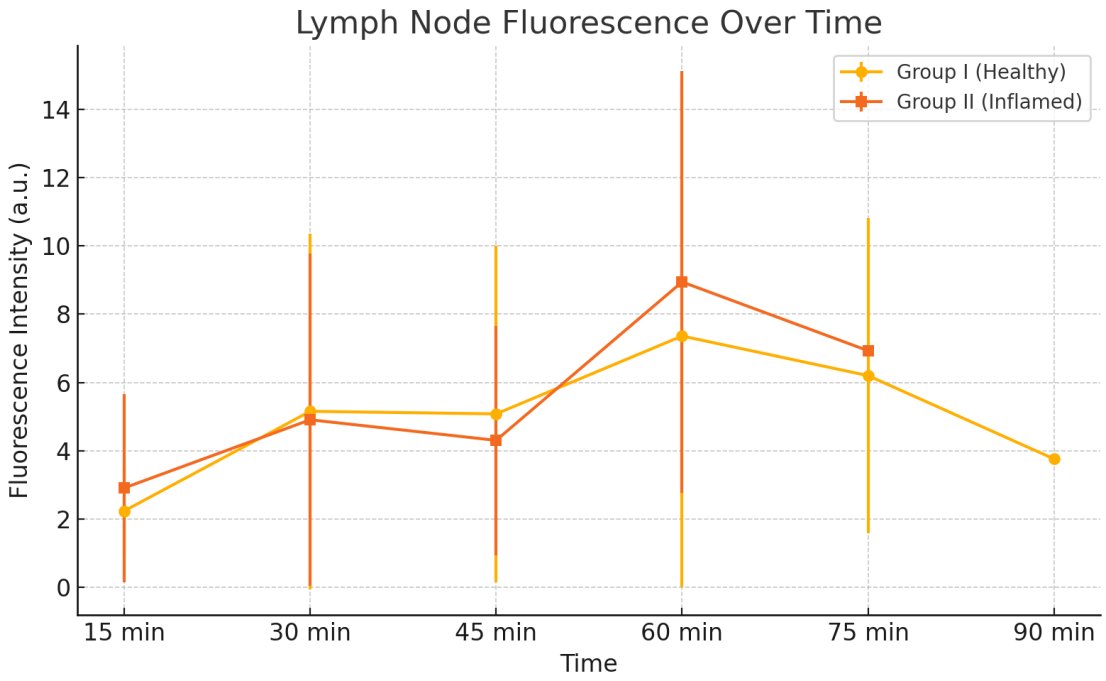


Figure 5: Lymph Node Fluorescence Intensity (Mean ± SD)



Conclusions

1. The ICG distribution in the prostate under normal conditions (Group I) changes significantly over time (Friedman p = 0.0136), indicating dynamic accumulation.
2. Between-group comparison revealed a trend toward increased fluorescence in Group II (inflammation) at 60 minutes (Mann–Whitney p = 0.083), suggesting inflammation enhances ICG uptake.
3. The data suggest that inflammation enhances the accumulation and spread of ICG within both the prostate and regional lymphatic system. Although not statistically significant at the 0.05 level, observable trends support the hypothesis that inflammation improves intraoperative lymph node visualization.
4. The findings support the utility of ICG for intraoperative lymphatic mapping and suggest inflammation alters tracer dynamics, relevant for prostate cancer surgery planning.

References

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