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# Prostate Field Cancerization – Thinking Outside the Tumor

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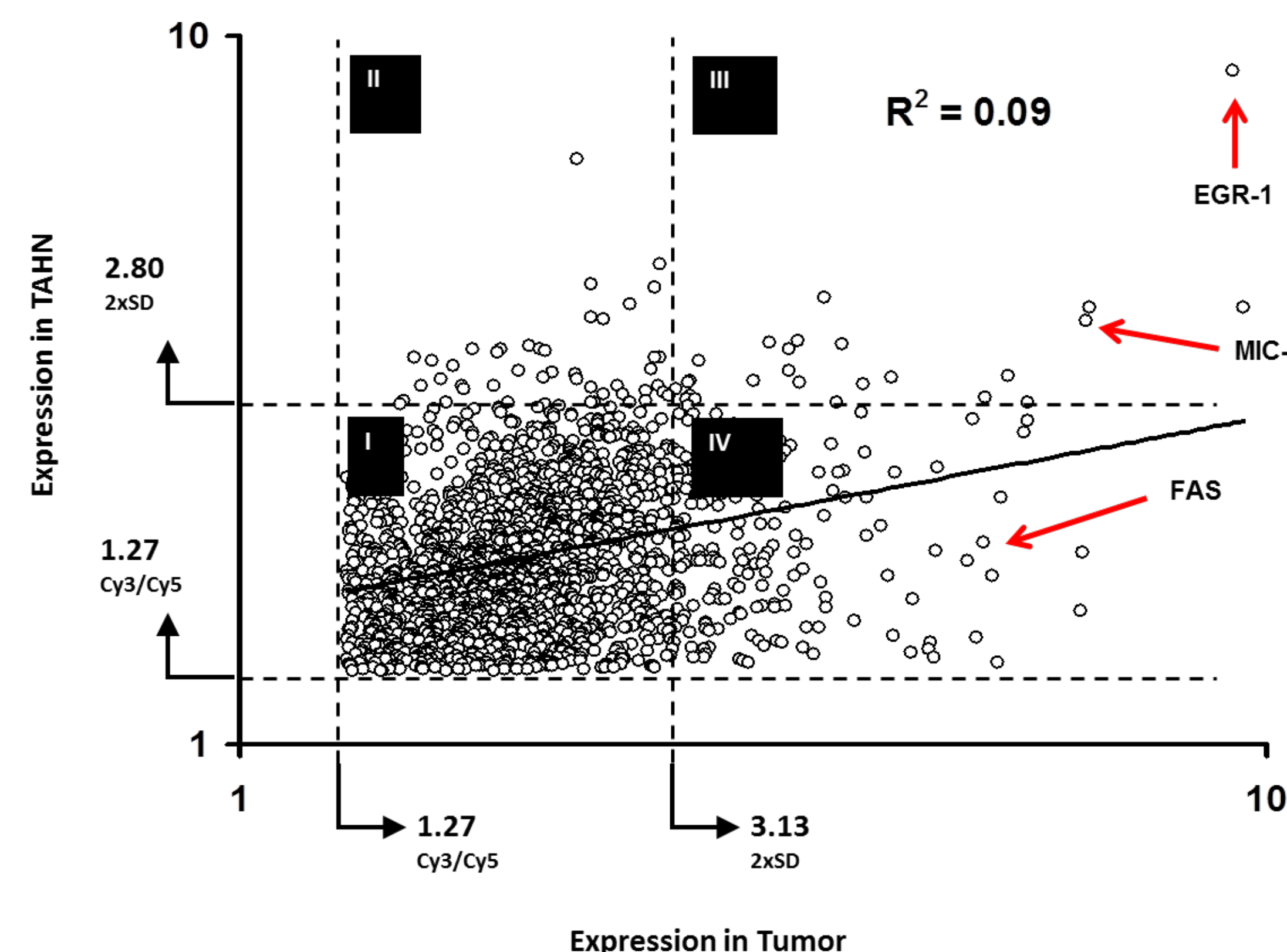
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## BACKGROUND

- Prostate cancer is the second most common cancer in American men with about 230,000 new diagnoses and 30,000 deaths annually. Diagnosis by biopsy is hampered by a 30-50% false-negative rate due to small and easily missed cancer foci.
- Field cancerization denotes genetic and/or biochemical molecular alterations in phenotypically normal cells residing in histologically normal tissues adjacent to prostate tumors and may represent a temporal record of pathways underlying oncogenesis.

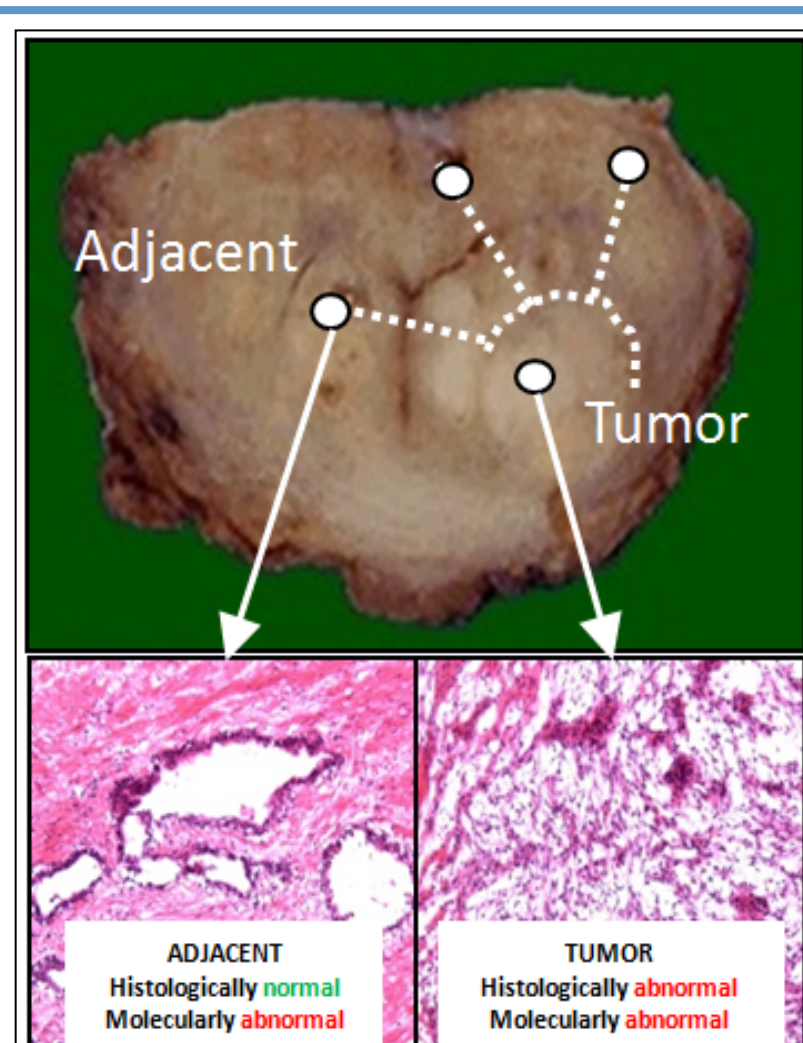


Graph 1. Scatter plot showing key transcription factor early growth response 1 (EGR-1) is up-regulated on a microarray slide at the mRNA level in tumor and tumor adjacent histologically normal prostate tissues. (Haaland CM et al. 2009; International Journal of Oncology (2009); 35:537-546)

## HYPOTHESIS and OBJECTIVE

- Hypothesis:** EGR-1 protein expression will be similarly elevated in cancerous and histologically normal adjacent tissues, which will support the concept of field cancerization. We further hypothesize that markers of field cancerization, such as EGR-1, could serve as biomarkers of disease and improve early cancer detection (diagnosis) at the time of biopsy.
- Objective:** Determine expression of EGR-1 protein in malignant and adjacent tissues.

Figure 1. Tissue sample



## EXPERIMENTAL METHODS

**Tissue Samples:** Human prostate tissues containing cancer cells (malignant) and matched adjacent tissues devoid of cancer cells (benign) from prostatectomies and matched biopsies were obtained from the Cooperative Prostate Cancer Tissue Resource (CPCTR). The present work is approved by Chapman University IRB protocol #1415H024 under biosafety level 2 (BSL2) approved practices as per Institutional, State, and Federal laws.

**Immunofluorescence Microscopy:** Immunofluorescence microscopy was performed using rabbit anti-human EGR-1 antibodies, unspecific control IgG, and goat anti-rabbit Alexa Fluor 488 (green) conjugated antibodies. Fluorescent DAPI dye (blue) was used to visualize cell nuclei.

**Quantification:** Quantitative analysis (pixel densitometry) was performed using ImageJ64 (provided by the National Institutes of Health) and graphs were generated using Microsoft Excel and Jumpy software. Two signal acquisition modes were applied: Whole image analysis and region of interest analysis.



Figure 2. CPCTR shipment

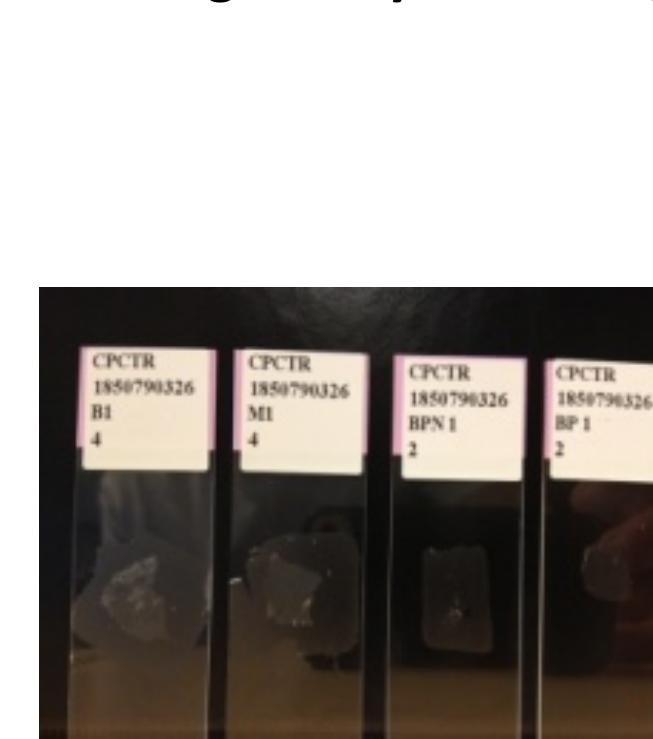


Figure 3. CPCTR slides

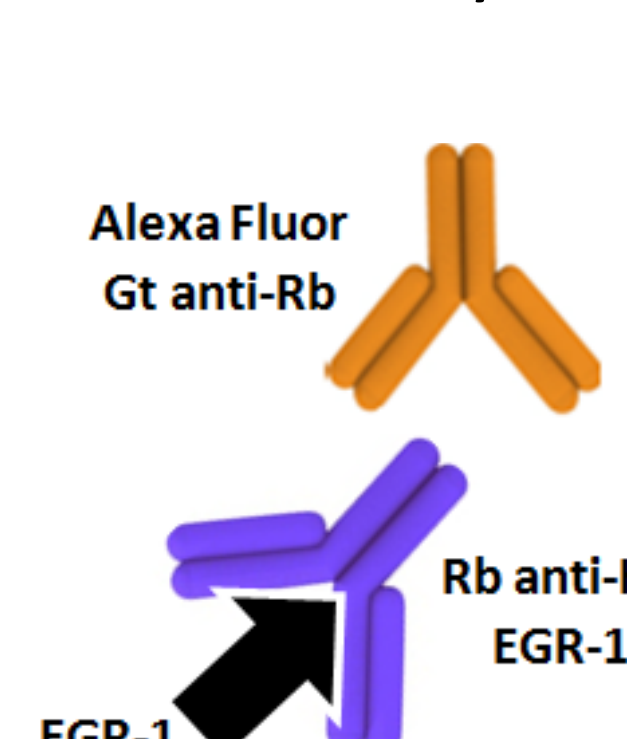


Figure 4. Antibody scheme

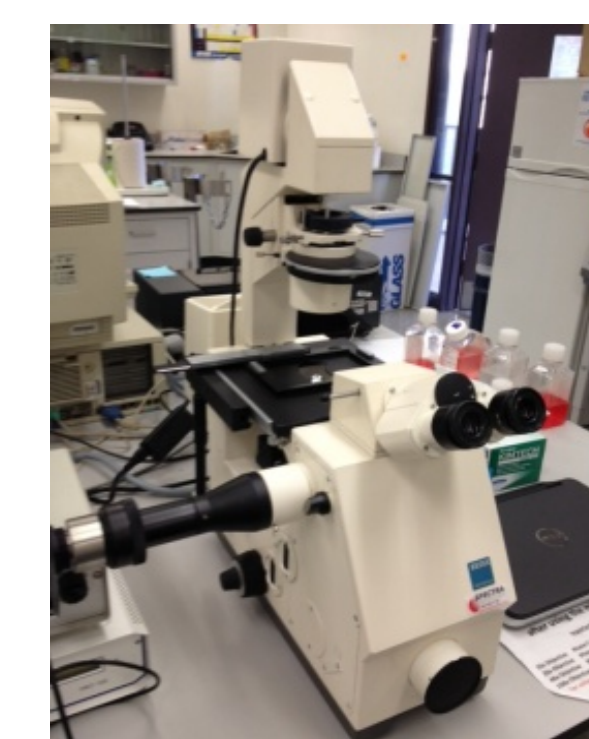


Figure 5. Microscope

## RESULTS

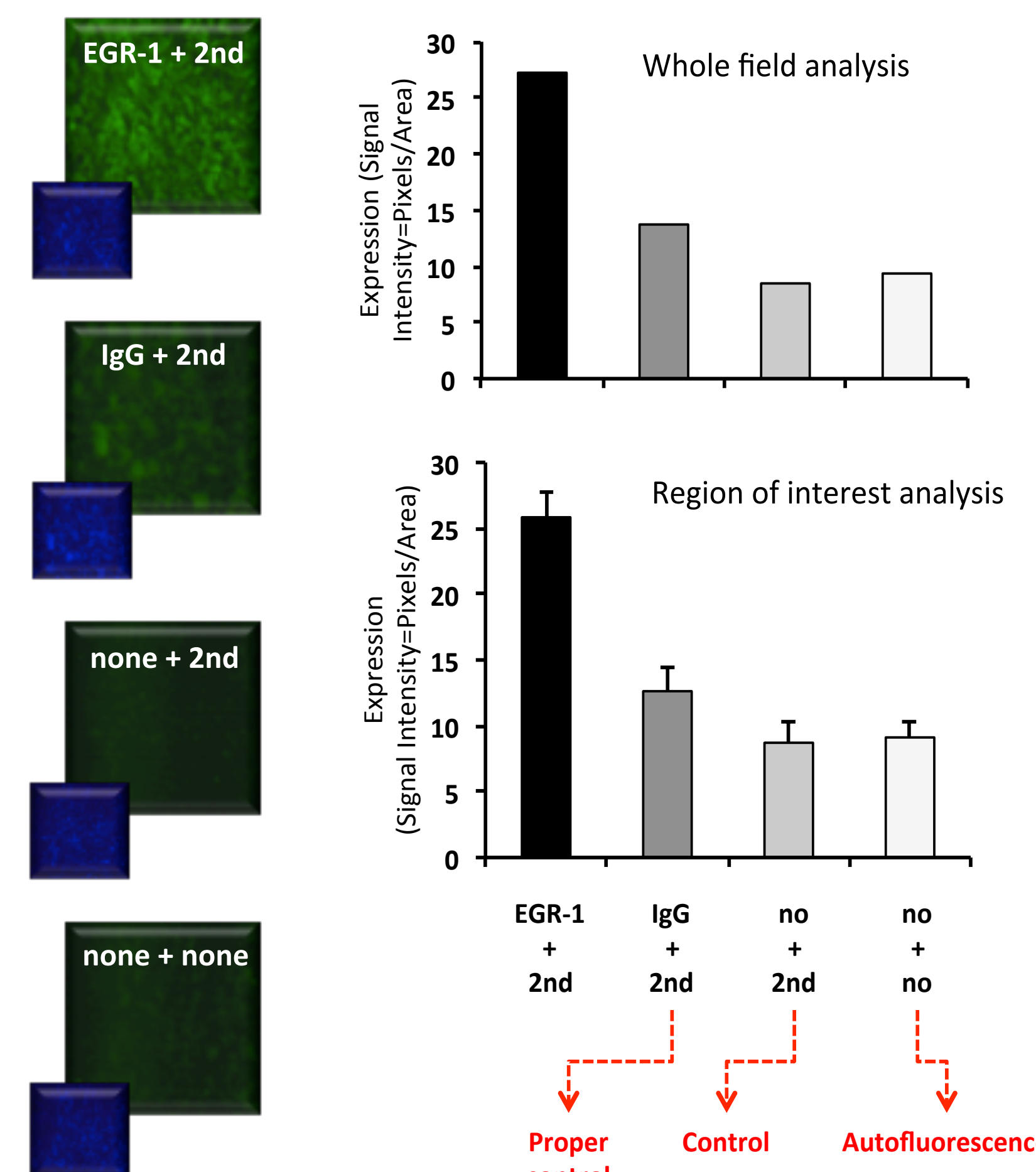


Figure 6 and Graph 2. Testing the specificity of anti-EGR-1 antibody on PC-3 human prostate cancer cells

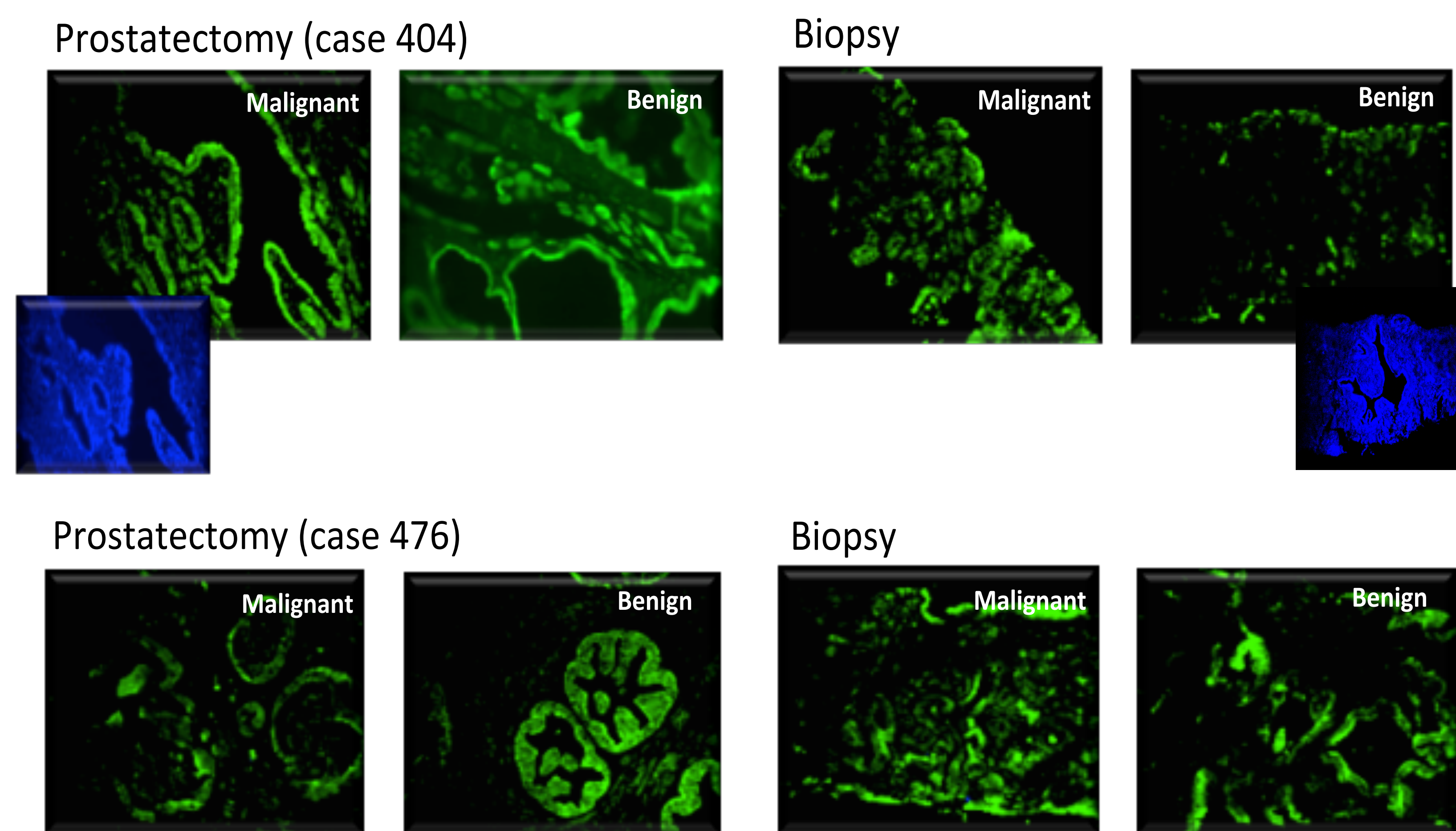


Figure 8. Representative immunofluorescent detection (green) of EGR-1 in two cases of malignant and benign tissues of prostatectomies and biopsies.

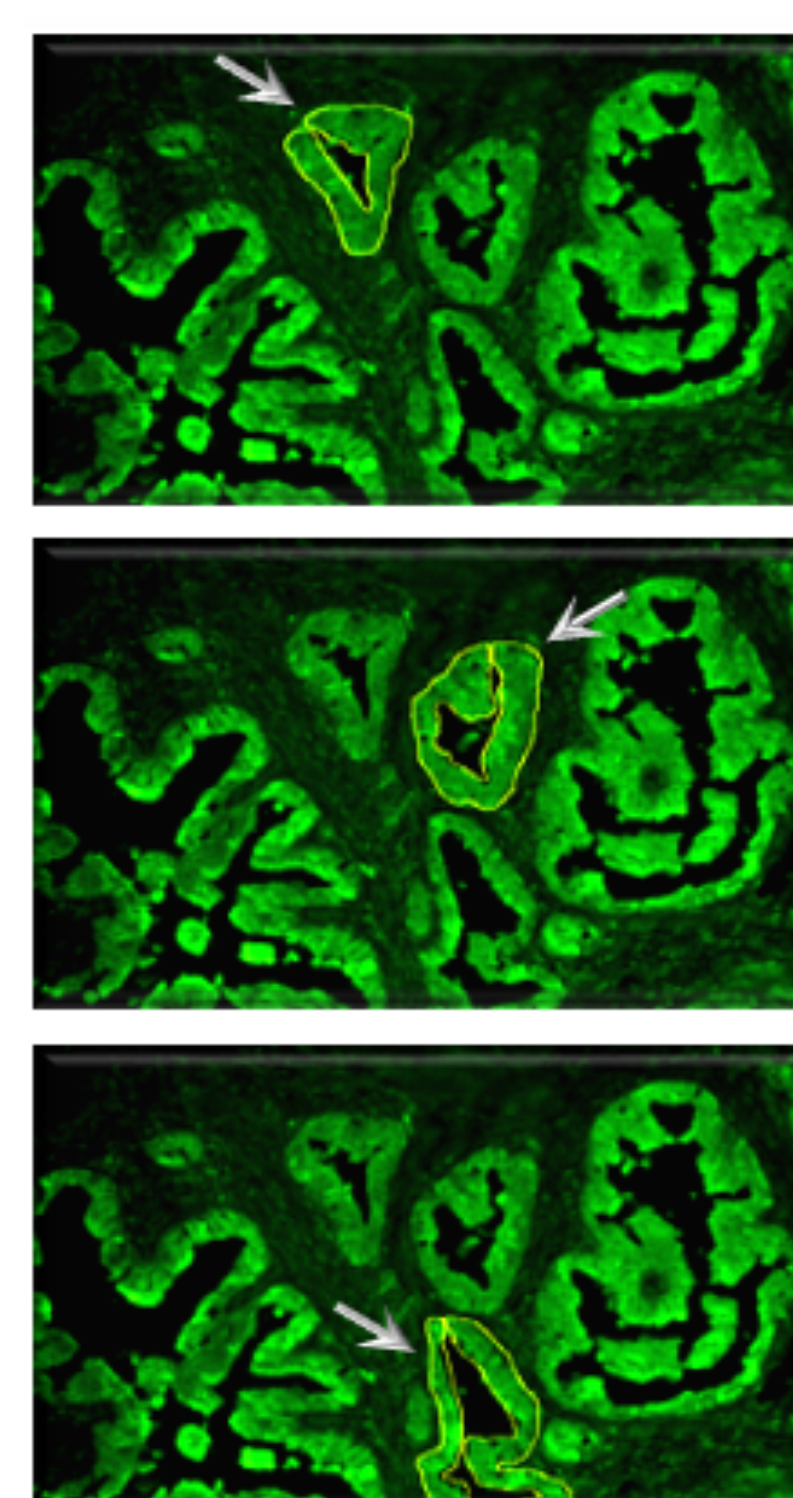
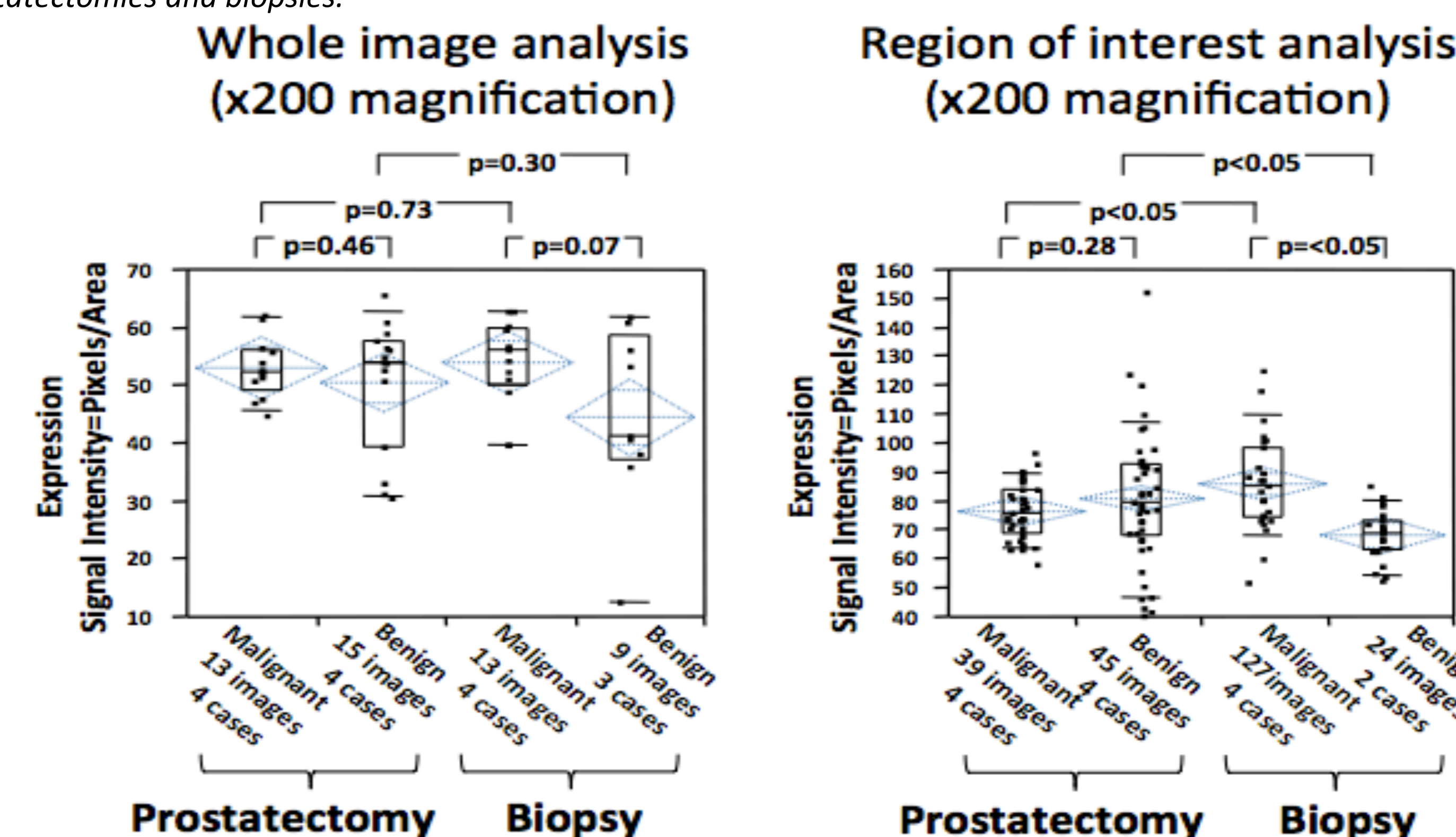


Figure 7. Depiction of the region of interest analysis mode.



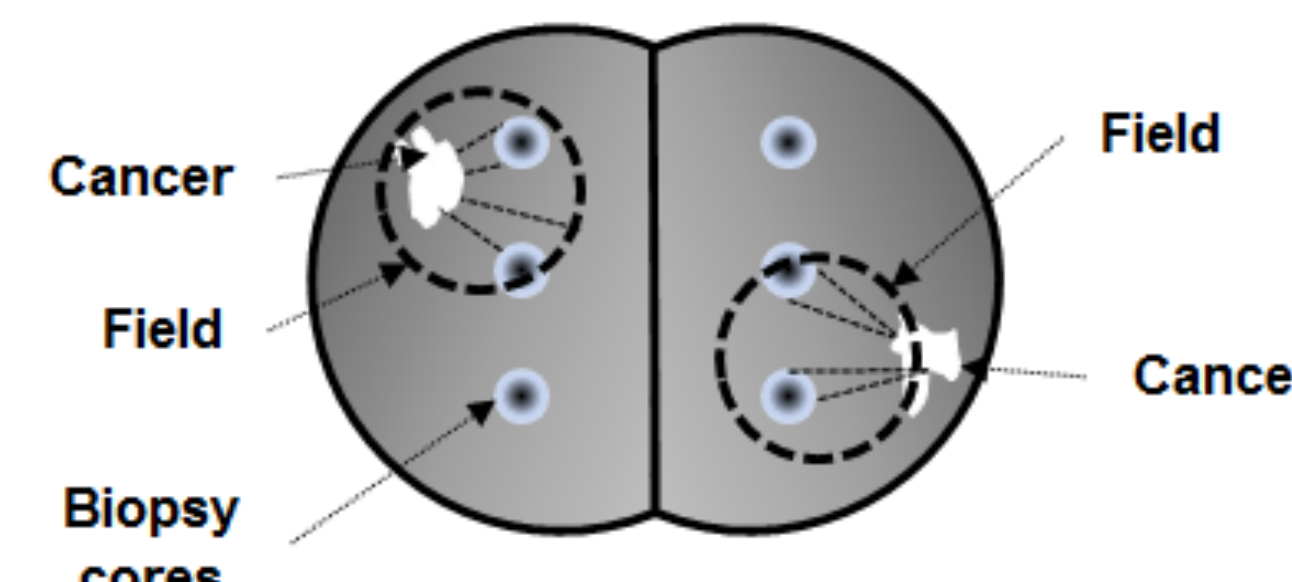
Graphs 3 and 4. Quantitative immunofluorescence data of EGR-1 in human malignant and benign prostate tissues from prostatectomies and biopsies by whole image and region of interest analyses.

## SIGNIFICANCE of RESEARCH

Markers of field cancerization:

- Potential to lower persistently high false-negative detection rate by expanding the target region.
- Potential for a new targeted repeat biopsy for patients with high serum prostate specific antigen (PSA) but negative biopsy.
- Potential to improve clinical decision making, such as surgical intervention vs. active surveillance.

Figure 9. Biopsy cores miss the two small cancer loci (white irregular structures), while the field associated with the cancer loci is detected by biopsies.



## CONCLUSIONS and FUTURE RESEARCH

- EGR-1 protein expression is similar in cancerous (malignant) and in histologically normal adjacent (benign) tissues from both prostatectomy and biopsy tissues. This supports the concept of field cancerization and indicates a potential organ-wide molecular change.
- Future research includes improvements at the conceptual and technical level:
  - Increasing the number of cases and including disease-free (age-matched) prostate tissues
  - Overcoming the autofluorescence of prostatic tissues by the use of Alexa Fluor 633-conjugated 2<sup>nd</sup> antibodies (far red) and increasing the resolution of detection by confocal microscopy
  - Design studies towards the clinical exploitation of markers of field cancerization. In particular, we are interested in developing non-invasive assessment methods using novel and upcoming technologies, including targeted nanoparticle imaging modalities

### ACKNOWLEDGEMENTS

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