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Fluorescently Labeled Bevacizumab in Human Breast Cancer – Defining the Classification Threshold

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ABSTRACT:

In-vivo fluorescently labelled drug (bevacizumab) breast cancer specimen where obtained from patients. We propose a new structured method to determine the optimal classification threshold in targeted fluorescence intra-operative imaging.

Keywords: Imaging systems, Medical and biological imaging, Clinical applications,

1. INTRODUCTION

Guidance in human surgery and endoscopy is still performed today with a "3000-year old detector": the human eye. Fluorescence imaging using targeted fluorochromes is emerging as a paradigm shift to the surgical and endoscopic view. We published the first use of a systemically administered targeted fluorochrome in human surgery [1] and suggested the use of targeted drugs as imaging agents [2]. Many teams are actively following up on this direction since then [3].
Despite growing interest in human studies, fluorescence imaging is not an accurate method. All images and results published in thousands of fluorescence imaging papers so far (animals and humans) are based on arbitrary thresholds. Therefore the separation of malignant from healthy tissue is rather arbitrary and user-specific.
Fluorescence imaging has the potential to change the landscape of surgical and endoscopic guidance. However, empirical thresholds do not enable the full potential of the technology and may create standardization issues and difficulties with reporting accurate outcomes.
We present in-vivo in human VEGF targeted NIR fluorescence images, obtained from breast cancer patients. The specimen have been labelled (stained) in-vivo using labelled bevacizumab administered prior to surgery [4] We study fluorescence thresholds and cancer extent and derive a global threshold that differentiates malignant from non-malignant breast tissue in an optimized way [5].

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2. MATERIALS AND METHODS

The co-registration of fluorescence images and the tumor demarcation by pathologists is based on H&E slices. Affine transforms allowed a per-pixel analysis of fluorescence intensities of paraffin embedded samples and the underlying presence of malignant vs. non-malignant cells and enabled accurate correlation between pathological classification and fluorescence intensity.

For deriving a normalized global threshold all the images where normalized, so that threshold application relates to comparable images independently of gains (intensity of illumination, camera amplification), background noise (ambient light, read noise etc.) and tissue variations or amount of agent administered and patient body weight. We therefore assumed the normalized fluorescence image Si, i.e.,

$$S_{ij}(\alpha,\beta,\gamma) = \frac{F_{ij}}{\alpha * mean(F_i) + \beta * thres(F_i)} + \gamma \qquad \text{Eq.1}$$

whereby Fi,j is the fluorescence intensity value of the j-th pixel of the i-th image of the original (raw) fluorescence image, assuming a number of fluorescence images i =1..N obtained from different patients (e.g. Fig 1a). The parameters α,β modulate the normalization of image F_i by its mean value mean(F_i) and a threshold value thres(F_i); the latter indicating the value that maximizes the inter-class variance for the i-th image and was determined by the Otsu's method for each F_i image. The parameter γ adjusts the image offset, representative of a background constant value due to bias values typically present in CCD camera images. To derive a global threshold, we determined the values [α β γ] by minimizing the cost function C([α,β,γ]) of, i.e. :

$$\mathrm{C}(\alpha,\beta,\gamma) = (1 - AUC(S([\alpha,\beta,\gamma]), G)) \qquad \text{Eq.2}$$

where G is a binary image indicating the areas of malignant vs. non-malignant tissue on the image S, as obtained by the congruent H&E pathology segmentation, and AUC is the area under the ROC curve (the normalized Mann-Whitney-Wilcoxon test). The optimal [α β γ] parameters used in image normalization (Eq.1) were estimated by an unconstrained nonlinear optimization. ROC analysis was performed on data from all specimen measured and processed by Eq.1, using the image normalization parameter set [α β γ] obtained by Eq.2.

3. RESULTS

Fig.1f depicted the ROC curve for each patient and presented the sensitivity and specificity by which fluorescence intensity patterns demarcate the tumor area, as confirmed by H&E analysis. However a critical parameter in interventional fluorescence imaging relates to setting an intensity threshold on the fluorescence image in order to differentiate cancer from healthy tissue, in the absence of an H&E analysis. Today, thresholds are empirically assigned based on image appearance and may inaccurately estimate the tumor extent and surrounding tissue (see Fig.2 a – c).

The ROC analysis considered all specimen images processed by Eq.1 using image normalization based on the optimal parameter set [α β γ]. The resulting ROC curve (Fig.2e) achieved an Area Under the Curve (AUC) value of 0.97 for all 22 samples examined.

4. CONCLUSION

We hypothesized that a global threshold can be derived from a training data set for use in intraoperative imaging. Our assumption of a global threshold assumed image normalization, so that the intensity seen on different images is calibrated to the same standard.

We quantitatively study the relation of fluorescence intensity to cancer and propose method for quantitative differentiation of cancer from healthy tissues and report a sensitivity of 98% and a specificity of 79% when using labelled bevacizumab to outline the tumor mass in human breast cancer.

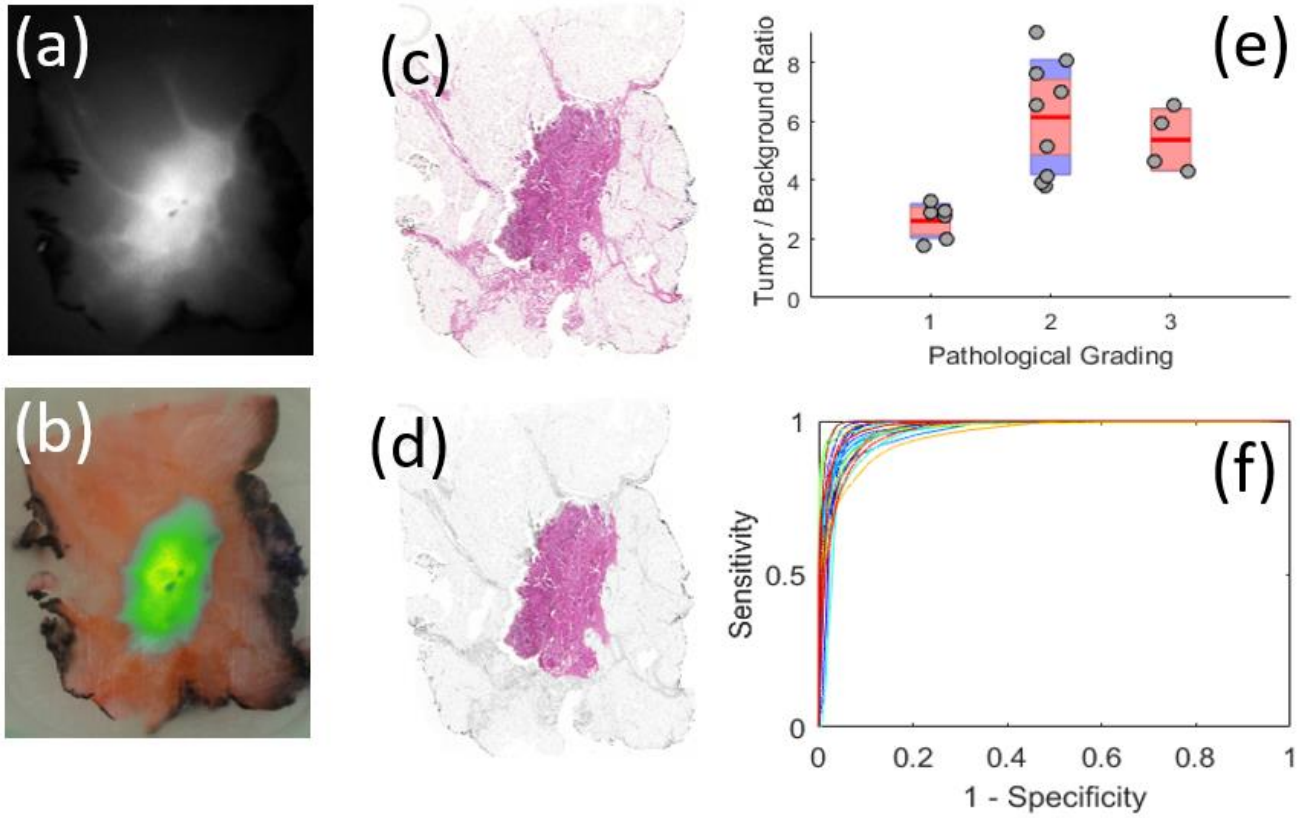

Fig. 1. Image co-registration, target-to-background analysis and spatial correlation of fluorescence and histological data. (a) Fluorescence image of the examined paraffin sample from a patient. (b) Alpha-blending overlay of pseudo colored fluorescence signal and color reflectance image. (c) Corresponding H&E staining of the same specimen. (d) Tumor location according to pathology outline. Gray color indicates background tissue. (e) Scatter plot of tumor to background ratios versus pathological grading for all patient samples; the red lines show the mean for each pathological grading group. (f) Receiver-operator-characteristics for all 22 patient samples revealing the performance of a pure value-driven binary classification in means of sensitivity vs. specificity

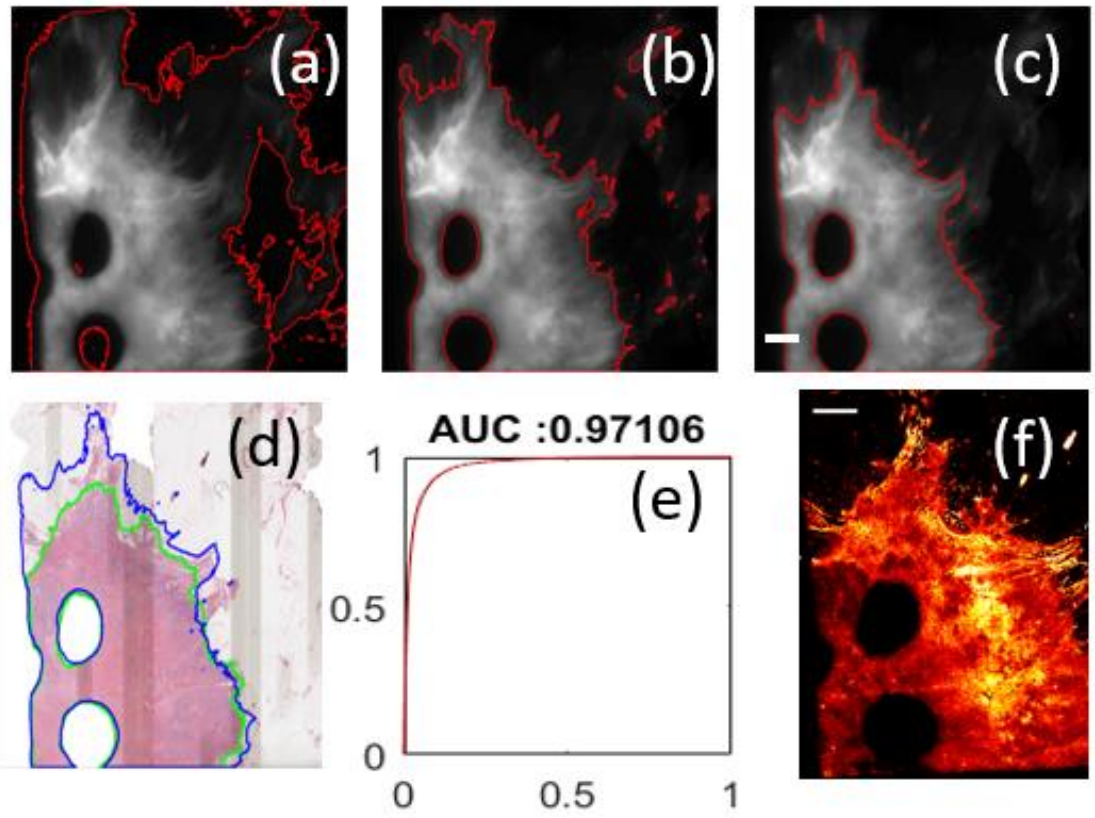

Fig. 2. Influence of threshold on segmentation. (a) – (c) Fluorescence images of paraffin embedded tissue block obtained from breast cancer stained in vivo with Bevacizumab-IRDye800CW. The region of interest (ROI) selected for different threshold levels are marked by the red line. The scale bar is 2mm (d) H&E stained slice with the gold standard segmentation by a pathologist in green and the calculated segmentation based on the fluorescence image in blue, for the threshold used in (c). (e) Receiver-Operator-Characteristics for all paraffin blocks when using the proposed global threshold achieving an AUC of 0.97. (f) Fluorescence image of 4µm-thick slice corresponding to (a) – (c) obtained from breast cancer stained in vivo with Bevacizumab-IRDye800CW. The scale bar is 2mm.

5. REFERENCES

[1] Van Dam, Gooitzen M., George Themelis, Lucia MA Crane, Niels J. Harlaar, Rick G. Pleijhuis, Wendy Kelder, Athanasios Sarantopoulos et al. "Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-[alpha] targeting: first in-human results." Nature medicine 17, no. 10 (2011): 1315-1319.
[2] Scheuer, Werner, Gooitzen M. van Dam, Michael Dobosz, Markus Schwaiger, and Vasilis Ntziachristos. "Drug-based optical agents: infiltrating clinics at lower risk." Science translational medicine 4, no. 134 (2012): 134ps11-134ps11.
[3] Whitley, Melodi Javid, Diana M. Cardona, Alexander L. Lazarides, Ivan Spasojevic, Jorge M. Ferrer, Joan Cahill, Chang-Lung Lee et al. "A mouse-human phase 1 co-clinical trial of a protease-activated fluorescent probe for imaging cancer." Science translational medicine 8, no. 320 (2016): 320ra4-320ra4.
[4] Lamberts, Laetitia E., Maximillian Koch, Johannes S. de Jong, Arthur Adams, Juergen Glatz, Marriette EG Tranendonk, Anton GT Terwisscha van Scheltinga et al. "Tumor-specific uptake of fluorescent bevacizumab-IRDye800CW microdosing in patients with primary breast cancer: a phase I feasibility study." Clinical Cancer Research (2016): clincanres-0437.
[5] Koch, Maximillian, Johannes S. de Jong, Jürgen Glatz, Panagiotis Symvoulidis, Laetitia E. Lamberts, Arthur Adams, Mariëtte EG Kranendonk et al. "Threshold analysis and biodistribution of fluorescently labeled bevacizumab in human breast cancer." Cancer Research (2016): canres-1773.