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Post-acquisition image analysis of fluorescence images - comparing the signal-background ratio (SBR) to the contrast-noise ratio (CNR)

INTRODUCTION

- SBR commonly used to quantitate fluorescence images - endpoint in (pre)clinical studies
- Cut-off value normal *vs.* tumor tissue based on marginal evidence
- Alternative measure CNR: quantifies absolute difference in fluorescence signal strength of tumor and background using the standard deviation (SD) of the of the background signal – info on image quality
- CNR has been suggested to be superior to TBR, but head-to-head comparisons between CNR and SBR are scarce

AIMS

- Compare SBR and CNR as quantitative parameters for post-acquisition image analysis

MATERIALS AND METHODS

- Randomly selected, representative sample of 271 intraoperative and *in vitro* images
- Image characteristics
  - Different sources: animal and human studies
  - Different tumor types
  - Different fluorescent agents
  - Different imaging systems
- Standard image assessment (ImageJ, National Institute of Health, Bethesda, USA)
- Calculation of SBR and CNR of each image:
  - $SBR = \frac{mean\ signal\ target\ lesion}{mean\ signal\ background}$
  - $CNR = \frac{(mean\ signal\ target\ lesion - mean\ signal\ background)}{SD\ background}$
- Selection of ROI: 3 approaches (figure 1a and 1b)
  (1) ROI from the region surrounding the tumor ROI within the same anatomical structure
  → subtraction tumor ROI from overlapping background with ROI manager in ImageJ
  (2) the darkest region adjacent to the tumor
  (3) the lightest region adjacent to the tumor

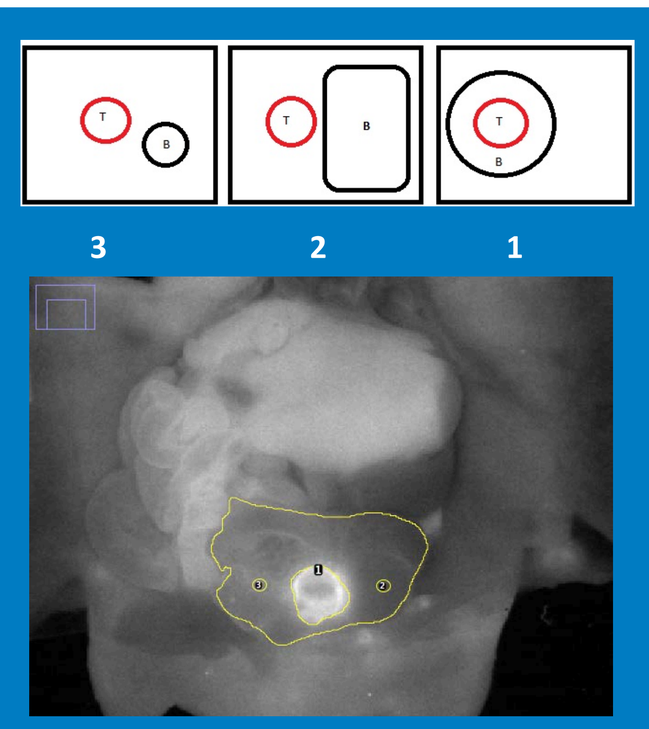

Figure 1. Schematic (top panel) and in vivo representation of ROI selection (bottom panel)

RESULTS

- Background selection crucial (figures 2)
- CNR and SBR linearly related (figure 3)
  - CNR = (SBR-1) × (Background ⁄ SD Background)
- No greater dynamic range CBR at high background and high SD (figure 3)
- Tissue specific differences CNR and SBR - differences in contrast / autofluorescence
  - Intraoperatively obtained sentinel lymph nodes (n=9)
    - Average SBR=11.8 vs average CNR=6.8
    - No difference in identification
  - Liver metastases (n=4)
    - Average CNR = 5.21vs. average TBR = 2.2
    - CNR identified all 4 meta's, TBR only 2 meta's
  - Small (mm-size) peritoneal ovarian cancer metastases (n=95)
    - Average CNR = 3.7 vs. average TBR = 2.0
    - CNR (>3) identified 65% of lesions, TBR (>2) only 28% of lesions

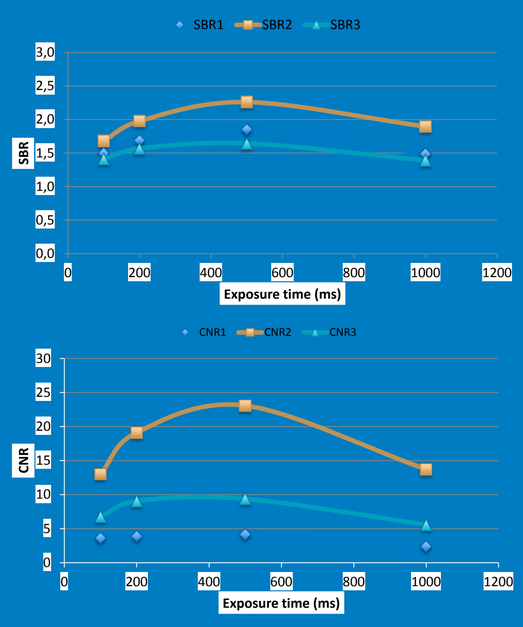

Figure 2. Influence of background selection for SBR (top panel) and CNR (lower panel); note that numbering is similar as in fig. 1

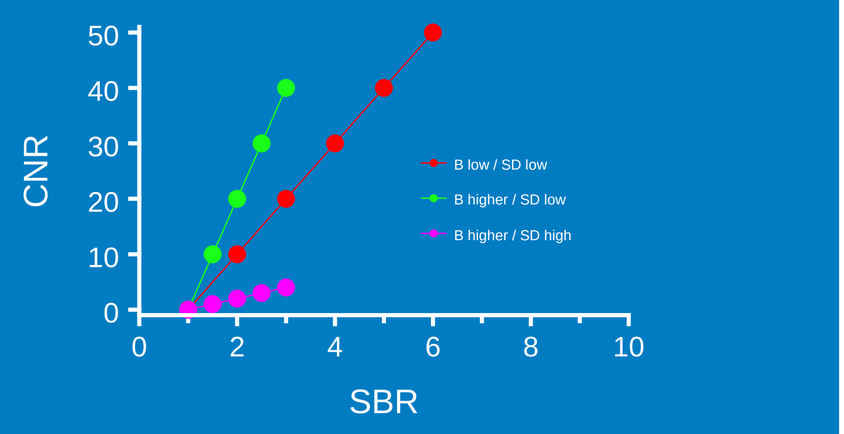

Figure 3: Relationship between SBR and CNR at different signal strengths for lesion and background

Discussion and Conclusions

- Selection of background and tumor ROI most important determinants in the quantification of fluorescence images irrespective of the use of SBR or CNR as summary measure
- CNR provides additional quantitative information over TBR - added value for low contrast and low signal strength images
- Consensus on standardization and characterization of imaging devices when reporting quantitative image analyses are needed