HOW CAN THE ENHANCED SENSITIVITY AND FAVOURABLE NOISE CHARACTERISTICS CONFERRED BY ELECTRON MULTIPLICATION IMPROVE FLUORESCENCE-GUIDED SURGERY?

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Introduction

Fluorescence-guided surgery for intracranial tumor resection relies on optical contrast from the endogenous molecule, protoporphyrin IX (PpIX). Exogenous administration of the precursor δ-aminolevulenic acid (ALA) overloads the heme cellular pathway and leads to selective accumulation of PpIX in neoplastic tissues. Studies have shown that fluorescence contrast in high-grade tumors is sufficiently high, such that lesions can be detected intra-operatively using a conventional broad-beam surgical microscope modified for fluorescence imaging with blue light excitation [1, 2] (Fig. 1).

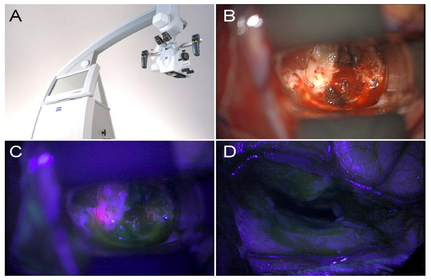

Figure 1. (A) Surgical microscope modified for fluorescence imaging. During resection: (B) white-light image of the cavity, (C) fluorescence image displaying a high level of PpIX fluorescence (pink), (D) area of low contrast enhancement with no observable fluorescence.

However, spectral imaging of fluorescence in biological tissue often results in very faint light levels, which can be troublesome. For sufficient fluorescence detectability, long integration times are often required. In *in vivo* surgery, time is very expensive and every minute counts.
A simple yet efficient way to improve detectability while reducing integration time with electron multiplication will be advanced in this short study.

Hypothesis

Here, we hypothesized that upgrading the camera in this particular setting would yield better detectability while allowing to acquire images at a greater speed.

For that to be true, the camera used in the experiment must significantly contribute to the background signal. If that is not the case, changing the camera won't impact significantly the signal-to-background ratio. The influence of the background noise on the camera's performances is shown in *Fig.2*

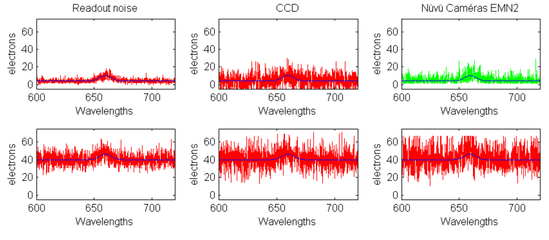

Figure 2. Simulation of the influence of the background intensities on a camera's performance for 1 pixel for a CCD and an EMCCD.

Methods

An experimental setup was made to simulate the optical conditions of *in vivo* surgery (see *Fig. 3*). A liquid phantom mimicking the optical properties of brain tissues was made with bovine hemoglobin (Sigma Aldrich Co. St-Louis, MO) and intralipid solution. For simplicity and photostability, Alexa 647 was used as the fluorophore instead of PpIX. The Alexa 647 concentrations were chosen to yield light intensities similar to biologically relevant PpIX concentrations. Three concentrations were made: 10ng/mL, 1ng/mL and 100pg/mL. A 635nm laser was used for excitation (7404, Intense Co., North Brunswick, NJ). Spectral acquisition of the fluorescence between 600nm and 720nm was allowed by a LCTF (VariSpec VIS, Perkin Elmer, Waltham, MA). All optical components were connected onto a neurosurgical Zeiss OPMI 1-FC microscope (Carl Zeiss Microscopy, Germany). Part of the acquisitions was made with a PixelFly CCD camera (PCO, Germany) and part with the EMN2 EMCCD camera (Nüvü Caméras, Quebec, Canada). Both cameras' main specifications are resumed in *Table 1*. All CCD images were obtained with 2x2 binning. Fluorescence spectra were then obtained from a 2mm x 2mm region of interest (ROI). Signal-to-background ratio (SBR) was measure as the ratio between fluorescence signal and background signal in the same image.

	CCD camera	Nüvü Caméras' EMN2
Sensor size	1392x1040	**512x512**
Quantum efficiency	65% @ peak	**92% @ peak**
Dark noise	1 ē/pixel/s @ 23° C	**< 0.001 ē/pixel/s @ -85° C**
Readout noise	7 ē rms @ 12MHz	**< 1 ē @ 20MHz gain > 100**
Pixel size	6.45µm x 6.45µm	**16µm x 16µm**

Table 1. Camera specifications

Figure 3. Experimental setup

Experimental Results

Alexa 647's detectability was measured by normalizing spectrum intensities. We can see in *Fig.4* that the detectability with the EMCCD is great at both concentration while the CCD has some problems at 1ng/mL. Only background was detected at 100pg/mL

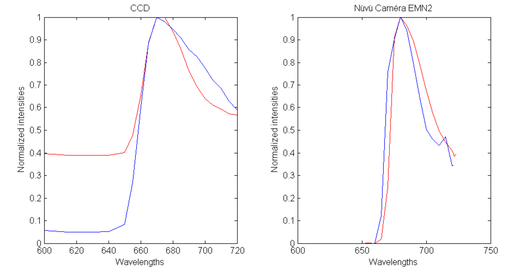

Figure 4. Detectability of Alexa 647 at different concentration for CCD and Nüvü Cameras' EMCCD. 10ng/mL in red, 1ng/mL in blue

A performance index I=SBR/t_{exp} was also measured to take into account the SBR and the exposure time. As a high SBR and a short exposure time are preferable, a higher Performance Index is desirable. Indexes measured for both cameras at 10ng/mL and 1ng/mL are shown in *Fig.5*.

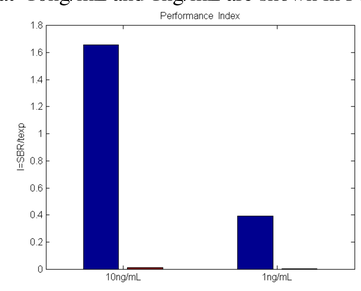

Figure 5. Multiparametric index k for Nüvü Caméras' EMN2 (blue) and the CCD camera (red).

Discussion

From the analyzed spectra, the background signal decreases and the detectability increases when using the EMCCD camera. That means that a part of the background signal detected with the CCD detector was due to its own noise: most likely its readout noise (7ē) and dark noise (1ē/s). Therefore, it is not surprising that the background signal lowered when we used the EMN2, with its readout noise and dark noise of less than 1ē. The EMCCD and CCD total noise equations are shown here:

$$SNR_{CCD} = \frac{S * QE(\lambda) * t_{exp}}{\sqrt{S * QE(\lambda) * t_{exp} + B * QE(\lambda) * t_{exp} + t_{exp} * D + \sigma^2}}$$

$$SNR_{EMCCD} = \frac{S * QE(\lambda) * t_{exp}}{\sqrt{F^2(S * QE(\lambda) * t_{exp} + B * QE(\lambda) * t_{exp} + t_{exp} * D + C) + \frac{\sigma^2}{G^2}}}$$

,where S is the fluorescence signal, QE is the quantum efficiency of the camera, B is the background signal, D is the dark signal, σ is the readout noise, G is the electron multiplication gain, F is the excess noise factor and C the clock induced charges.

The CCD camera's performances was limited by the amount of light available. Nüvü's EMN2 collected enough light to perform at full capacity. In fact, while imaging at full frame, the EMCCD camera was limited by its own speed, meaning that it could have still performed well if it were able to go faster. Using ROI, we were able to detect spectrum with good SBR while imaging at 85fps, as we can see in *Fig.6*.

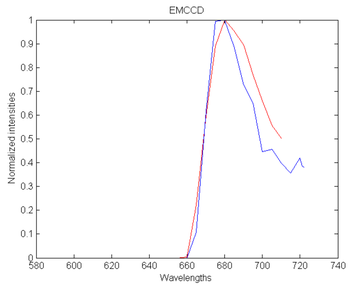

Figure 6. . Detectability of Alexa 647 at increased frame rates for Nüvü Cameras' EMCCD. 10ng/mL at 85fps in red, 1ng/mL at 50 fps in blue

As we can see in *Fig.5*, the Perfromance Index was between 150 and 530 times higher for the EMCCD than for the CCD, which confirms the EMCCD yields higher SBR while imaging faster. The difference in efficiency between the two detectors was even greater as the concentration lowered.

Conclusions

- Nüvü Cameras' EMCCD device was capable of achieving image acquisition in this particular experiment in up to 100 times faster than the CCD camera while allowing superior signal-to-background ratio.

- Upgrading the detector lowered the background signal in this experimental setup, meaning that the background was not dominated by its shot noise, but by the noise of the CCD camera itself. *In vivo* high sensitivity spectroscopy can benefit from improved detectors.

- The improved fluorescence detectability with Nüvü Caméras' EMCCD will allow surgeons to perform fluorescence guided tumor resection on different types of tumor with better sensitivity and in real time.

References

[1] Stummer, W. et al., Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial, Lancet Oncol. 7, 392-401 (2006).
[2] Stummer, W. et al., Extent of resection and survival in glioblastoma multiforme: identification of and adjustment for bias, Neurosurgery 62, 564-576 (2008).

Acknowledgements

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