CERENKOV LUMINESCENCE IMAGING ON THE PHOTONIMAGER™ SYSTEM: BIODISTRIBUTION OF THE BETA EMITTING RADIOTRACER $^{32}$P

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- Cerenkov Luminescence Imaging of $^{32}$P radionuclide distribution during an in vivo tumor study
- Discrimination of the Cerenkov signal coming from tumor using High Pass filters

INTRODUCTION:
As in vivo imaging develops at a pace, new methods are being explored to increase the effectiveness of this technology for research applications. Cerenkov luminescence imaging (CLI) is a cost effective technique which offers great potential for a wide range of preclinical applications. Imaging of Cerenkov radiation in vivo is possible because when charged radioactive particles pass through tissue at a speed higher than the phase velocity of light for that medium, they stimulate the emission of photons in the visible spectrum which can be detected by an optical imaging system. The radioisotope Phosphorus-32 ($^{32}$P) has been extensively used as a radiopharmaceutical for treatment of various cancers in the Pancreas, Liver, Brain, Lung, head and neck$^1$. In the past it has been used for the treatment of myeloproliferative chronic leukemia and erythremia$^{2,3}$.$^{32}$P is also extensively used as a metabolic tracer in many in-vitro experimental techniques. Here we demonstrate how the PhotonIMAGER™ system can be used to monitor the biodistribution of $^{32}$P in a nude mouse bearing 4T1-luc2 tumors on its left and right flanks.

MATERIALS AND METHODS:
A nude mouse (25g) was injected subcutaneously with $10^6$ 4T1-luc2 cells on each flank. The tumors were allowed to develop for seven days prior to analysis. A Biospace Lab PhotonIMAGER™ system was used to compare bioluminescence imaging (BLI) and CLI as a measure of tumor development. (Fig. 1)
Ten days post implantation, the mouse was injected intravenously with 1.6 MBq (50µCi) of $^{32}$P. The biodistribution of the radiotracer was monitored at the moment of injection and again 2, 4 and 24 hours post injection. Acquisitions of 10 minutes duration were carried out for each time point using a PhotonIMAGER™ system fitted with a 4-View™ module that allowed simultaneous recording of CLI from the ventral, dorsal and both lateral side views of the animal. (Fig. 2)

**Figure 1 (below).** Comparison of Bioluminescence & CLI in vivo – 2x 4T1-luc2 tumors visualized 7 days post subcutaneous injection.

**Figure 2 (right).** Time course monitoring of $^{32}$P biodistribution by Cerenkov Luminescence in vivo. A-T=0s; B-T=2h; C-T=4h; D-T=24h
At the 4 hour post injection time point, CLI acquisitions were performed using High Pass emission filters with cut off wavelengths at 530, 615 and 700nm in order to assess how the use of optical filters would affect the detection of Cerenkov luminescence. The 4-View module was again used to acquire Cerenkov data simultaneously from 4 views of the animal (Fig. 3).

**RESULTS:**

The direct monitoring of $^{32}$P biodistribution over time proved possible on the PhotonIMAGER™ system using Cerenkov Luminescence. The first 4 hours of incubation revealed an uptake of $^{32}$P by the soft tissues (Bladder), as well as the tumor (Fig. 2). This confirmed the preferential uptake of $^{32}$P by cancerous tissue. The distribution of $^{32}$P in the skeleton was also apparent as signal co-incident with the vertebrae and strong signal in the tail was visible. It is assumed that a substantial amount of $^{32}$P was rapidly excreted from soft tissue. This was observed 24 hours post incubation (Fig. 2D), where most of the soft tissue signal was eliminated and only traces of radioactivity remained in the spinal column, tail and skull of the animal ($^{32}$P is permanently retained in bone).

Thanks to the 4-View module it was possible to observe simultaneously the $^{32}$P biodistribution throughout the entire animal. The results correlated well with tumor specific bioluminescence data, demonstrating that Cerenkov radiation could be successfully used to monitor tumor development on the PhotonIMAGER™ system. Use of the add-on 4-View module further enhanced this study by allowing the biodistribution of the tracer throughout the whole body to be visualized in a single acquisition. The application of High Pass optical filters was shown to be a very useful tool for discriminating between the strong signal coming from the tumors and the weaker signal from the other regions of $^{32}$P uptake. (Fig 4.)

**CONCLUSION:**

Cerenkov imaging is shown to be a valid tool for complementing more commonly used BLI, FLI, SPECT and PET imaging modalities. This study successfully demonstrated how visualization of tumor onset/progression can be achieved by detecting Cerenkov luminescence from injected $^{32}$P radiolabel. The PhotonIMAGER™ system is shown to be perfectly adapted to monitor Cerenkov Luminescence in vivo.

**REFERENCES:**