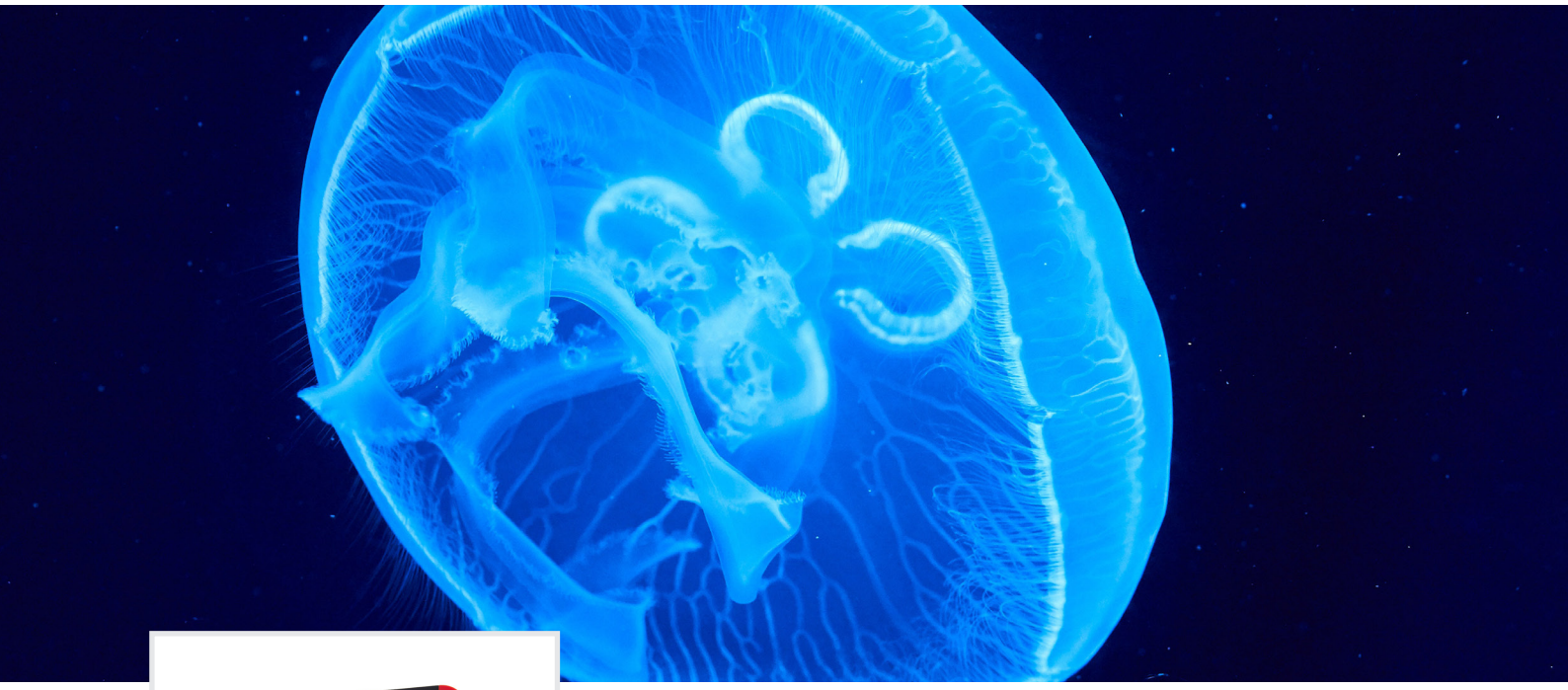

Challenge

Non-invasive imaging of a patient-derived orthotopic mouse model of pancreatic cancer expressing GFP

Solution

In vivo imaging with the UVP iBox Studio

In Vivo Imaging with GFP: From Blue Squeezates to Green Mice

Introduction

The natural glow of organisms has captivated observers since ancient times[1]. Sprinkled about Chinese texts, one can find passing references made to glowing organisms as in The Odes of Pin from 1500-1000 BCE[‡,1,2]: "Our paddocks seem crowded with deer, / glowing intermittently are the fireflies. / Such thoughts while they filled us with fear, / we tried, but in vain, to keep out." Nearly 3000 years would pass before researchers would discover the essence of the intermittent glow. In 1885, French Pharmacologist, Raphael Dubois, produced heat-labile and heat-sensitive extracts from an elaterid beetle—or firefly—of the Pyrophorous genus, which he called luciferin and luciferase, respectively. When combined, these extracts would emit a blue glow. For most of the next century, researchers would discover similar instances of this chemical luminescence from other organisms—in the presence of oxygen and luciferase, luciferin would oxidize to produce a blue light[3] (Figure 1). Still, the provenance of a certain bioluminescence would evade researchers for nearly another century.

While most luminescent extracts matched in hue to that which was observed in vivo, some did not. Organisms such as *Aequorea Victoria*, or jellyfish, emitted a green glow. This glow could be coaxed by mechanical or electrical stimulation, and was visibly green under an ultraviolet (UV) mineralite[4]. In 1962, in a scientific tour de force, Shimomura and colleagues harvested the green-glowing organs of over 10,000 jellyfish, from which only 5 mg of partially purified protein was produced[5]. These extracts, or "squeezates", luminesced blue like other bioluminescent

extracts. However, Shimomura determined that these extracts were not enzymatic as they did not react with luciferin, the reaction did not require oxygen like the luciferin-luciferase pair (Figure 1A), and instead, only required calcium—he identified the simplest luminescent system to date (Figure 1B). Importantly, his blue luminescent extract, which he called aequorin, would fluoresce green when exposed to UV light. He went on to make a prescient concluding remark about the green co-purified protein in his 1962 paper[5]:

> "...it is reasonable to suppose that the greenish quality results from a light-filtering effect and fluorescence of the green protein which is highly concentrated together with aequorin in the photogenic cells. If this supposition is correct, perhaps the green protein has some biological significance, through its influence on the quality of the light of bioluminescence."

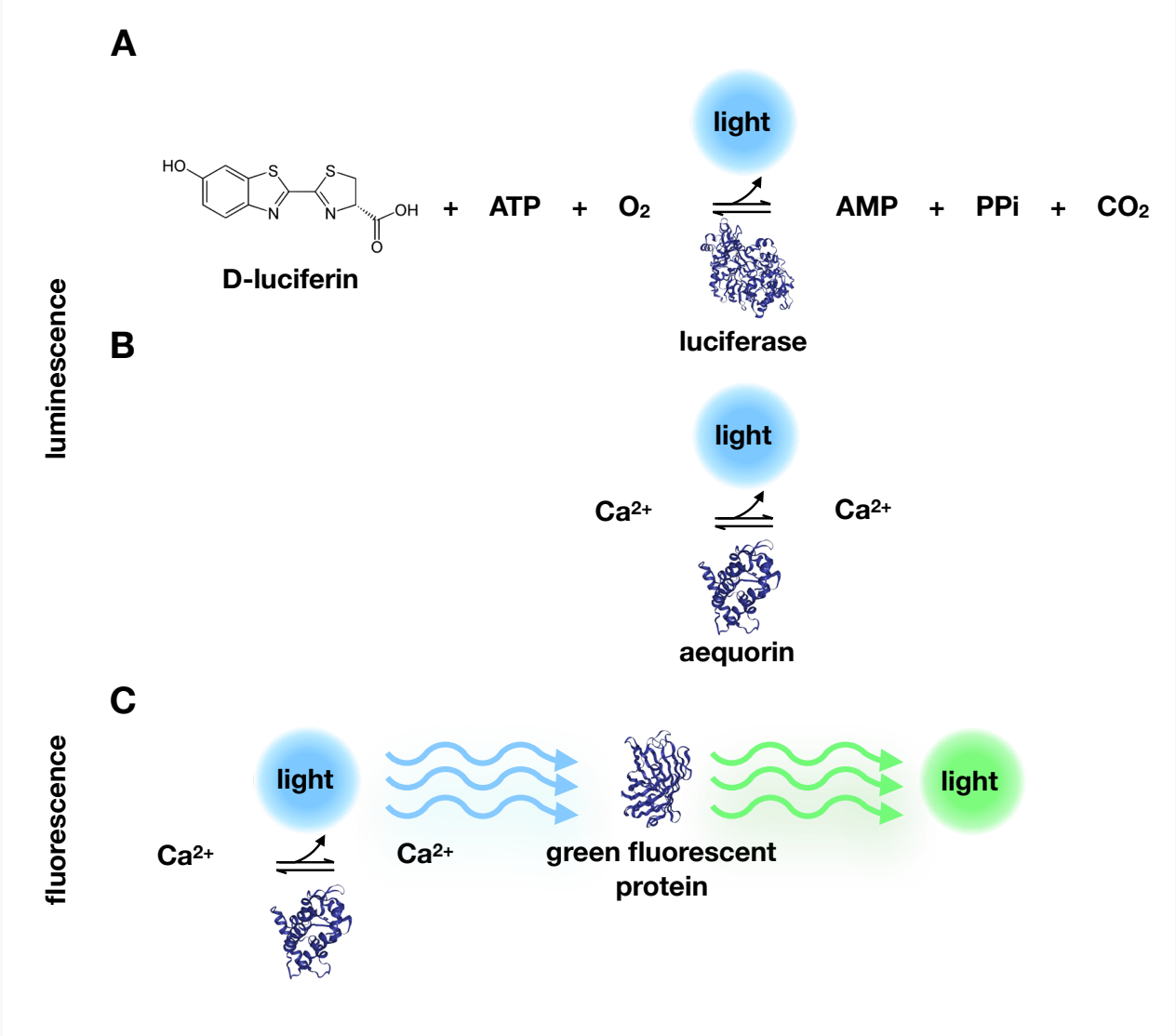

Figure 1: Bioluminescence and fluorescence reactions. A) Before Shimomura's work, bioluminescence reactions were known to require ATP, O_2 and the D-luciferin substrate. D-luciferin is oxidized during the enzymatic reaction with luciferase, which produces light. Shimomura and colleagues identified a simpler system, that only required calcium and aequorin. B) In addition, they discovered that the blue light emitted from calcium binding to aequorin could be absorbed by another protein that would emit green light. This protein was therefore called, green fluorescent protein. Protein structures[‡‡] in this image were retrieved from the protein data bank (PDB): luciferase (2D1R), aequorin (1EJ3), and green fluorescent protein (1EMB).

Indeed, Shimomura would later demonstrate that aequorin, when excited with UV, would have an emission spectrum that overlapped with the absorption spectrum of the green fluorescent protein (GFP), and that the green fluorescence was the result of an intermolecular energy transfer between the two proteins[6] (Figure 1C). For his contributions, Shimomura would be one of three scientists to receive the 2008 Nobel Prize in Chemistry[7]. Today, the in vivo imaging community has been a major benefactor from the discovery of GFP, the cloning and characterization of this protein[8], as well as the expansion of the fluorescent protein color pallete[9,10]. These fluorescent proteins have brought light to darkness; making the invisible, visible.

Below we demonstrate the exquisite detail with which the UVP iBox Studio can perform in vivo imaging of an orthotopic mouse model of pancreatic cancer labeled with GFP.

Orthotopic Mouse Model

For a detailed protocol of animal care and use, see Lwin et al (2018)[11]. Mice were anesthetized with a cocktail of ketamine (100mg/kg), xylazine (10mg/kg), acepromazine (3mg/kg) and placed atop a pre-calibrated Analytik Jena warming plate (part no. 95-0538-01). GFP was excited with blue epi-LED illumination and a GFP emission filter (part no. 38-0352-01) was mounted in the filter wheel. The image was captured with the following settings: exposure time – 2.1 s, binning – 1x1, brightness/aperture 61%, focus 74%, and histogram – Auto. A white light image was captured with the following settings: exposure time – 70 ms, binning – 1x1, brightness/aperture 20%, focus 74%, and histogram – Auto. The GFP fluorescent image was pseudocolored green, and both the white light and fluorescent image were composited, to form an image overlay. Image capture and post-processing was performed using our VisionWorks software package version 9.0.

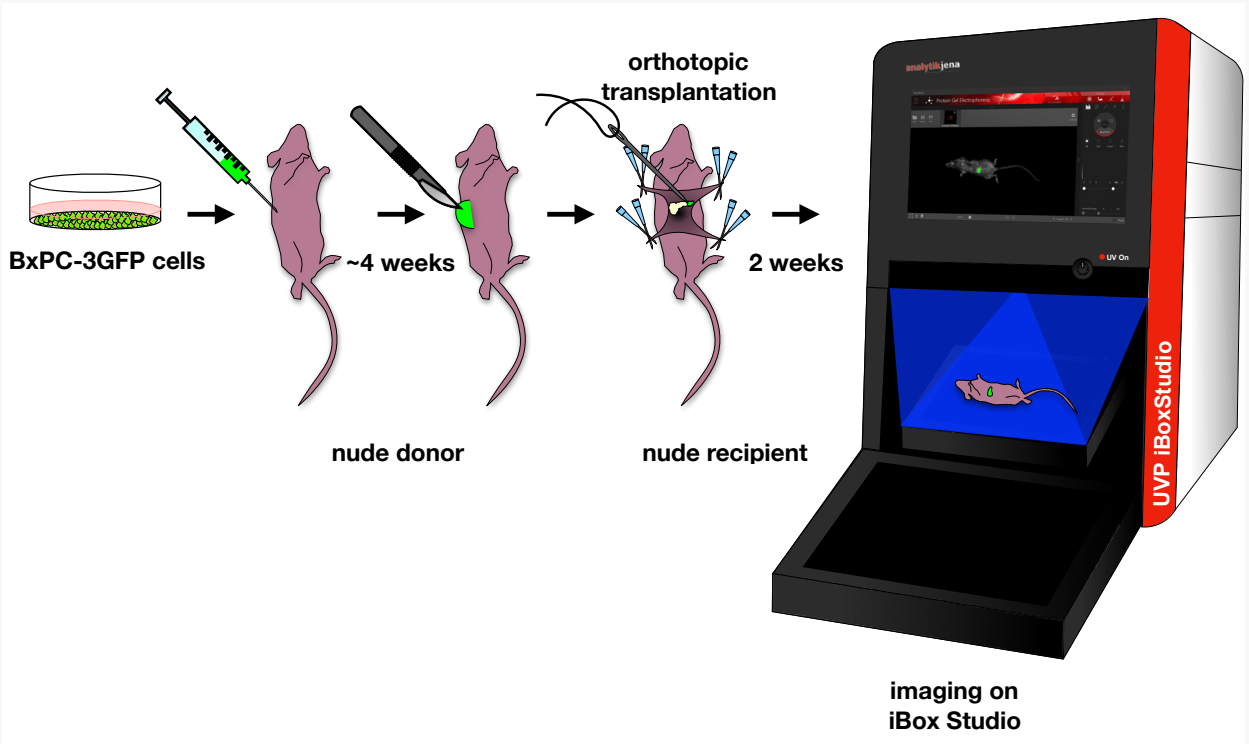

Figure 2: Orthotopic mouse model of pancreatic cancer. BxPC-3GFP human pancreatic cancer cell lines were maintained under selection. A nude donor mouse was injected with these cells. A piece of the GFP tumor was excised from the donor and implanted onto the tail of the pancreas in a nude recipient. Two weeks after implantation, the mouse was imaged on a UVP iBox Studio.

Results and Conclusion

Non-invasive imaging two weeks after orthotopic transplantation, revealed that GFP signal is still robust despite passing through several layers of tissue (Figure 3). This experiment highlights not only the striking detection capability of our instrument to visualize a deep tumor, but the fine detail that can be captured with our high sensitivity, deeply cooled, low noise detector.

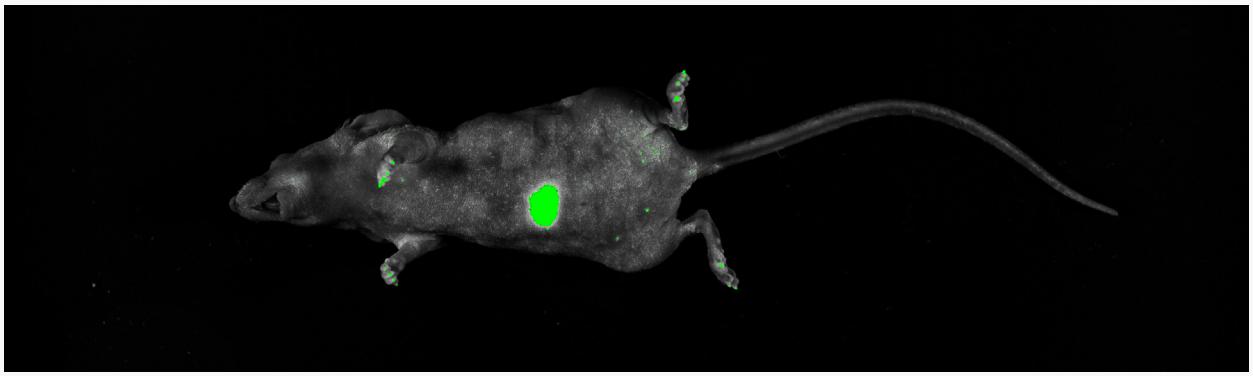

Figure 3: Imaging of GFP orthotopic mouse model. Imaging was performed on the UVP iBox Studio. The mouse was anesthetized according to Lwin et al (2018). The mouse was placed on top of warming plate during capture, GFP was excited using standard blue overhead LED illumination and a green (GFP) emission filter. The image was post-processed and composited using Analtyik Jena's VisionWorks software package ver. 9.0.

The UVP iBox Studio is equipped to handle any in vivo imaging application. We have outfitted this instrument with white, blue, green, and red epi-LED illumination. In addition, for custom applications that require more intense lighting, an external xenon-arc lamp can be added. As researchers begin to move toward deep tissue non-invasive imaging, we have made the UVP iBox Studio near-IR ready—researchers can upgrade to near-IR lasers to excite 700/800 nm dyes. For users using gaseous anesthesia, the UVP iBox Studio is configured with gas ports, to accommodate an external anesthesia system, which we offer as an add-on feature. Lastly, our filter wheel is easily accessible, so that researchers can outfit their UVP iBox Studio with emission filters from our extensive library or order custom filters for specialized applications at any time. The customizability of our instrument combined with its sensitivity and easy-to-use software interface, make it an excellent option for our researchers needing a high quality in vivo imaging solution.

References

1. Harvey, E. N. A history of luminescence from the earliest times until 1900. 44, (American Philosophical Society, 1957).
2. The Book of Poetry: Book XV. The Odes of Pin: III. Tung Shan. Available at: https://www.sacred-texts.com/cfu/bop/bop159.htm. (Accessed: 1st October 2019)
3. Dubois, R. Fonction photogenique des Pyrophores. CR Seances Soc Biol Fil 37, 559–62 (1885).
4. Davenport, D. & Nicol, J. C. Luminescence in hydromedusae. Proceedings of the Royal Society of London. Series B-Biological Sciences 144, 399–411 (1955).
5. Shimomura, O., Johnson, F. H. & Saiga, Y. Extraction, Purification and Properties of Aequorin, a Bioluminescent Protein from the Luminous Hydromedusan, Aequorea. Journal of Cellular and Comparative Physiology 59, 223–239 (1962).
6. Morise, H., Shimomura, O., Johnson, F. H. & Winant, J. Intermolecular energy transfer in the bioluminescent system of Aequorea. Biochemistry 13, 2656–2662 (1974).
7. The Nobel Prize in Chemistry 2008. Available at: https://www.nobelprize.org/prizes/chemistry/2008/summary/. (Accessed: 30th September 2019)
8. Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. & Prasher, D. C. Green fluorescent protein as a marker for gene expression. Science 263, 802–805 (1994).
9. Campbell, R. E. et al. A monomeric red fluorescent protein. PNAS 99, 7877–7882 (2002).
10. Shaner, N. C. et al. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nat Biotechnol 22, 1567–1572 (2004).
11. Lwin, T. M. et al. Tumor-Specific Labeling of Pancreatic Cancer Using a Humanized Anti-CEA Antibody Conjugated to a Near-Infrared Fluorophore. Ann Surg Oncol 25, 1079–1085 (2018).
12. Head, J. F., Inouye, S., Teranishi, K. & Shimomura, O. The crystal structure of the photoprotein aequorin at 2.3 A resolution. Nature 405, 372–376 (2000).
13. Brejc, K. et al. Structural basis for dual excitation and photoisomerization of the Aequorea victoria green fluorescent protein. Proc.Natl.Acad.Sci.USA 94, 2306–2311 (1997).
14. Nakatsu, T. et al. Structural basis for the spectral difference in luciferase bioluminescence. Nature 440, 372–376 (2006).

‡ As translated by John Legge in The Books of Poetry and reinterpreted by Dr. Hu Shih as written in E. Newton Harvey's 1957 book, A History of Luminescence from the Earliest Times Until 1900.

‡‡ Protein structures were retrieved from the PDB and reprinted here per their usage policy.

Headquarters
Analytik Jena US
2066 W. 11th Street
Upland, CA · USA

Phone +1 909 946 3197
Fax +1 909 946 3597

info@us.analytik-jena.com
www.analytik-jena.com

en · 11/2019