Fluorescent probes used in intraoperative imaging

Application Note

Also referred to as fluorophores or simply fluors, fluorescent probes are molecules that absorb light of a specific wavelength (excitation light) and emit light of a different wavelength (emitted fluorescence).

When an electron of a fluorescent particle absorbs a photon of excitation light, the energy level of that electron is raised to an excited state. Some of this energy is dissipated, but the remaining energy is emitted as a photon.

The photon typically carries less energy, meaning it has a longer wavelength, which enables the emitted fluorescence to be differentiated from the excitation light. The excitation and emission light from a fluorescent probe are cyclical and the probe can continue to be excited until it eventually becomes irreparably damaged.

Applications in molecular imaging

Fluorescent probes have a wide range of applications, including their use as a molecular imaging tool during surgical operations in order to increase the amount of visual information a surgeon has access to.

Significant advances made in the field of biomedical optics over the last decade could have a great impact on patient care. In particular, near-infrared fluorescence (NIRF) imaging has emerged as an intraoperative technology that has the potential to revolutionize surgical operations by providing surgeons with real-time image guidance for procedures such as angiography, lymph node mapping and tissue resection.

NIR light (the 700-900 nm region) can penetrate several millimetres of tissue, to reveal structures underlying the tissue surface. In terms of signal detection, NIRF dyes provide significant advantages compared to visible fluorescent dyes, offering higher resolution imaging, a greater imaging depth and a higher signal-to-background ratio.

In a fluorescence-based angiography procedure, a commercial system is used in combination with indocyanine green (ICG) to allow a surgeon to see cerebrovascular blood flow and to establish vessel patency using a video monitor or a surgical microscope.

NIRF-based lymph node mapping has the potential to reduce the requirement for radical resection, where the lymph nodes and blood supply are removed along with an organ. In breast cancer surgery, for example, unaffected lymph nodes are removed in almost 50% of cases. The ability to map, intraoperatively, which nodes are involved, could reduce the need for this overtreatment.

With regard to tumor resection in general, a significant challenge surgeons face when relying on palpitation and visual assessment only, is distinguishing between cancerous tissue and healthy tissue. NIRF imaging could potentially be used to highlight the location of tumorous tissue and lower the frequency of positive margins and the requirement for further surgeries.

Currently, only two fluorophores, ICG and methylene blue (MB), have been approved by the Food and Drug Administration for use in the clinical setting. In a 2014 review article, Sven Meiog (Leiden University Medical Center) and colleagues describe the use of these probes for assessing patients with pancreatic cancer.

In cases where the cancer has spread to the liver, it has been shown that ICG accumulates around these secondary cancers, creating a fluorescent rim. This is probably because hepatocytes in the area near a liver tumor become compressed and ICG is retained in compressed hepatocytes.

Among patients without pre-detected liver metastases who had pancreatic resection carried out, NIRF imaging using ICG showed that 16% had micrometastases that were at least 1.5 mm in length. NIRF imaging could therefore reduce the number of ineffective pancreatic resections being performed, by identifying liver metastases that have previously gone undetected. Meiog and team also demonstrated the accumulation of MB in insulinomas of the pancreas.

Current developments

Recently, researchers have focused on creating new NIRF probes that have improved photostability and emit a stronger fluorescence signal. In 2014, Jianlin Yuan (Fourth Military Medical University, Shaanxi, China) and colleagues published a review article in the International Journal of Nanomedicine describing some of the potential NIRF probes that are currently being developed for biomedical imaging applications.

The team look at the research efforts being made to modify several categories of NIRF dyes including the cyanines, rhodamines, cyanines, BODIPY-based dyes, phthalocyanines, squaraines and porphyrin derivatives.

Detection systems

Obtaining live images highlighted by fluorescent dyes requires a combination of filters, lenses and cameras. For intraoperative imaging during surgery, the most frequently used devices are silicon-based charge-coupled device (CCD) cameras, although these are associated with disadvantages. Although, these cameras offer a low readout noise and high resolution they also demonstrate low quantum efficiency (QE) at 800 nm and slow readout times.

Alternatively, there are the complementary metal oxide semiconductor (CMOS) sensors, which offer an increased frame rate and reduced power consumption, but these have a high readout noise and also suffer from low QE at 800 nm.

However, the newly developed scientific CMOS (sCMOS) sensors offer all the desirable features, providing low readout noise and a high QE. The Zyla 4.2 PLUS is the latest sCMOS device from Andor, a global leader in the manufacture of high performance light measuring solutions. Offering an additional 10% boost in QE, this sensor delivers excellent coverage of NIR wavelengths and is highly optimized for use with a wide range of common fluorescent probes.

With industry-fastest USB 3.0 frame rates and a response linearity of 99.8%, the Zyla 4.2 PLUS offers unparalleled quantitative accuracy and is ideally suited to a number of high-resolution microscopy applications.

- http://www.nature.com/subjects/fluorescent-probes
- http://www.hindawi.com/journals/bmri/2014/890230/
- http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3956734/
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