Fluorescence Endoscopy
in Veterinary Medicine
Fluorescence Imaging

Fluorescence imaging is becoming increasingly popular as a visual tool in surgery as clinical studies in animals pave the way for applications in human medicine. KARL STORZ thus offers OPAL1® technologies for near infrared (NIR/ICG) and autofluorescence (AF) imaging as well as for photodynamic diagnosis (PDD). Each technology also holds great potential for enhanced diagnosis and treatment of disease in veterinary patients.

OPAL1® Technology NIR/ICG
Near infrared (NIR) fluorescence cholangiography with indocyanine green (ICG)

In 1852, George Gabriel Stokes described the mineral Fluorite as emitting blue light following exposure to UV-light. He termed the phenomenon as “Fluorescence” and substances with this property as “Fluorophores”.

The ability to emit fluorescence is very common in nature. The sensitivity of delocalized electrons in aromatic ring structures is responsible for this. The light energy excites the delocalized electrons into a higher state of energy. When the electrons return to the ground state, the light energy is emitted as fluorescence. The emitted fluorescence is lower in energy as the exciting light energy is partly lost as heat.
Aromatic ring structures are the main components of biological substances such as DNA, proteins and sugars. Since the 1960’s their ability to emit fluorescence has been used for fluorescence imaging in life sciences and in medicine.

The oldest known approved near-infrared (NIR) fluorescent dye in medicine, ICG, has been discovered as a tool to visualize the anatomy and the lymphatic system as well as to detect tumors and to assess perfusion. Therefore, a wide range of applications are possible.

ICG is a drug which has been approved for use in humans by the FDA since 1959 for cardiac and liver function testing. The tricarbon dye has an excitation maximum of $\lambda_{\text{em}} = 805$ nm and an emission maximum of $\lambda_{\text{ex}} = 835$ nm. This results in tissue detection depth for NIR fluorescence of up to 1 cm.

ICG is usually intravenously administered where it binds to plasma proteins (albumin), thereby remaining in the bloodstream due to size exclusion. From the bloodstream, ICG is transported to the liver, where it is excreted via the bile into the duodenum. This exclusive excretion into bile makes it an ideal tool for the delineation of the extra-hepatic biliary tree.
The OPAL1® technology for NIR/ICG based on the IMAGE1 S™ platform has already been used for several different indications in veterinary medicine. The four main indications are:

- Blood perfusion assessment
- Lymphatic mapping
- Visualization of anatomical structures (e.g., bile duct imaging)
- Tumor identification

The following list is a selection of the veterinary applications of the NIR/ICG technology to date:

- Identification of vascular integrity
- Lymphatic mapping for oncology
- Evaluation of lymphatic disease (chylothorax, lymphangiectasia, assessment of lymphedema)
- Biliary function and anatomy (obstruction, leakage)
- Tumor margin localization
- Identification of metastasis (primarily liver)
- Lung segmentectomy
- Ureteral identification
- Skin flap/graft viability
The versatile usage of the OPAL1® technology based on the IMAGE1 S™ platform is demonstrated by the following examples:

➢ **Perfusion Assessment**

![Perfusion assessment of a thoracoscopic thoracic duct ligation with NIR/ICG.](image)

Fig. 3: Perfusion assessment of a thoracoscopic thoracic duct ligation with NIR/ICG.

➢ **Framing of Hypervascularized Tumors**

![Framing of Hypervascularized Tumors](image)

Fig. 4: Perfusion assessment of hepatic mass in a dog with NIR/ICG.

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1 Images courtesy of: Michele A. Steffey, DVM, DACVS, University of California at Davis, School of Veterinary Medicine, USA

2 Images courtesy of: Jeffrey J. Runge, DVM, DACVS, University of Pennsylvania, School of Veterinary Medicine, USA
Detecting a Metastasis with OPAL1® Technology for NIR/ICG

Fig. 5: Detection of metastasis in the cranial lung lobe of a dog.

Visualizing the Lymphatic System

Fig. 6: Visualization of the lymphatics associated with the cisterna in the region of the abdominal aorta of a dog.

2 Images courtesy of: Jeffrey J. Runge, DVM, DACVS, University of Pennsylvania, School of Veterinary Medicine, USA
OPAL1® Technology for NIR/ICG

1. **IMAGE1 S™ camera system**
   - brilliant FULL HD image quality
   - NIR/ICG mode + SPECTRA A lead to optimal illumination + contrast enhancement with a display in the cyan spectral range

2. **NIR/ICG telescope**
   - telescopes for optimal fluorescence excitation & detection, which can be used for white light and NIR/ICG applications
   - standard endoscopes can also be used with the NIR snap-on filter

3. **Camera head**
   - 3-chip FULL HD camera head with high resolution and optimal NIR light sensitivity

4. **D-LIGHT P light source (Xenon light source)**
   - best daylight spectrum
   - NIR fluorescence with backlight
   - no additional security measures (vs. Laser)

5. **Autoclavable fiber optic light cable**
   - optimal light transmission in the white light and NIR spectral range

**Footswitch**
- fast switch between white light and fluorescence mode

**Literature**, see pages 18 and 19
The identification and total removal of malignant tissue determines the life expectancy of a cancer patient. Many researchers try to overcome this problem by developing specific tumor markers. One of the most promising substance groups are the protoporphyrin IX-producing (PPIX) drugs. 5-aminolevulinic acid (5-ALA) and its approved derivative Hexvix® from Photocure (Cysview® in USA) and Gliolan® from Medac are precursors of PPIX in the heme biosynthesis. The substances are internalized and metabolized by all cells in the body. Malignant cells have a metabolic defect, which results in the accumulation of PPIX in the cells. Since PPIX has fluorescent properties, malignant tissue can be visualized by enhancing the PPIX fluorescence (PPIX absorption maximum $\lambda_{\text{Em}} = 420$ nm, PPIX fluorescence emission maximum $\lambda_{\text{Ex}} = 630$ nm).

Fig. 7: 5-ALA, Hexvix® or Gliolan® are converted into precursors of PPIX in the heme biosynthesis.
Indications

Two primary indications for photodynamic diagnosis (PDD) with PPIX precursors are known: 5-ALA or Gliolan® is used for glioma identification in neurosurgery. Hexvix® (Cysview® in USA) is distilled into the bladder for bladder cancer detection (Fig. 8).

➢ Demarcation of Bladder Tumors via Photodynamic Diagnosis

Fig. 8: Fluorescence diagnosis of a bladder tumor with Hexvix®

➢ Visualization of Glioblastoma via Fluorescence

Fig. 9: Fluorescence diagnosis of a glioblastoma with Gliolan®

1 Images courtesy of PD Dr. med. Carsten Kempkensteffen, Charité University Medical Center Berlin, Germany

4 Images courtesy of Prof. Potapov, Burdenkow Neurosurgical Institute Moscow, Russia
OPAL1® Technology for PDD

1. **IMAGE1 S™ camera system**
   - PDD mode + SPECTRA A and SPECTRA B lead to optimal illumination and contrast enhancement with a display in the cyan spectral range

2. **PDD telescope**
   - Telescopes for optimal fluorescence excitation & detection, which can be used for white light and PDD applications

3. **Camera head**
   - 1-chip FULL HD HX camera head with high resolution and optimal PDD light sensitivity
   - Standard endoscopes can also be used with the PDD snap-on filter

4. **D-LIGHT C light source (Xenon light source)**
   - Best daylight spectrum
   - PDD fluorescence with backlight
   - No additional security measures (vs. Laser)

5. **Fluid light cable**
   - Optimal light transmission of white light and fluorescence

**Footswitch**
- Fast switch between white light and fluorescence mode

**Literature**, see page 20
Another application of fluorescence for diagnosis is the intrinsic autofluorescence (AF) of tissue components. AF focuses on the differentiation between healthy and malignant tissue in bronchoscopy and laryngoscopy. The underlying principle is simple: AF detects the green fluorescence of flavins in the healthy mucosa. Malignant tissues like bronchial or laryngeal carcinomas are identified by the lack of fluorescence since their compactness blocks the autofluorescence of the underlying healthy mucosa.

Fig. 10: Principle of autofluorescence
AF in Veterinary Medicine

Autofluorescence (AF) facilitates early differentiation of malignant changes from benign tissue. The autofluorescence method is based on the fact that light with a certain wavelength induces fluorescence. Pathological findings appear as dark areas against an apple-green background (normal tissue).

Blue light and specialized equipment visualize information that remains undetected in the conventional light mode. For this purpose, the light of a specific spectral composition is introduced into the body via an almost loss-free light guide system. The major advantage of this technology is that marker substances are not required.

Fluorescence Imaging with Endoscopes

A) White light

B) AF mode

5 Images courtesy of Dr. Stanzel, Lung Clinic Hemer, Germany
Autofluorescence

1. **IMAGE1 S™ camera system**
   - brilliant FULL HD image quality

2. **Camera head**
   - 1-chip FULL HD HX camera head with high resolution and optimal AF light sensitivity

3. **Endoscopes or fiberscopes**
   - all telescopes with the AF snap-on filter are suitable for both white light applications as well as optimal fluorescence excitation & detection

4. **D-LIGHT C/AF**
   - best daylight spectrum
   - filters are variably adjustable
   - no additional security measures (vs. Laser)

5. **Fluid light cable**
   - optimal light transmission of white light and fluorescence

**Footswitch**
- fast switch between white light and fluorescence mode

**Literature**, see page 21
D-LIGHT P
modifiable for various fluorophores

Modified D-LIGHT P VET M1, with integrated special filter, high-performance light unit for perfusion assessment, autofluorescence, and standard endoscopic diagnosis, including a 300 Watt Xenon bulb and KARL STORZ light cable connection, power supply 100-125/220-240 VAC, 50/60 Hz, for use with snap-on filters and special endoscopes for autofluorescence in veterinary medicine including:

- Cold Light Fountain D-LIGHT P
- Mains Cord
- One-Pedal Footswitch, digital, one-stage
- Demo Card Fluorescence Imaging

66100M2  Same, with two integrated special filters

66100M3  Same, with three integrated special filters

Filters are available for the following fluorophores:

- GFP, tdTomato/DsRed, CY5, Methylene blue, 5-ALA/PDD, ICG/NIR, AF, RFP/mCherry, CY7, Fluorescein blue
Overview: Fluorophores and Suitable System Combinations

(Date: 01.02.2020, subject to change)

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<tr>
<th>Available Dyes</th>
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<td>tdTomato/ DsRed/ tRFP</td>
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<td>RFP/ mCherry</td>
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<td>AF</td>
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<td>fluorescein blue</td>
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<tr>
<td>ALA/PDD</td>
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Please note:
Should you have any queries or require further information on the use of fluorescence in veterinary medicine, please contact VET@KARLSTORZ.com.
Snap-on Filters
for use with standard eyepieces and D-LIGHT P light source

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<td>Snap-on Filter for RFP/mCherry</td>
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<td>Snap-on Filter for GFP</td>
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<td>Snap-on Filter for tdTomato</td>
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<td>Snap-on Filter for AF</td>
</tr>
</tbody>
</table>

Check out our schedule of upcoming hands-on training courses at http://go.karlstorz.com/eventsVET
Literature – NIR/ICG

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Notes
Shaping the Future of Endoscopy with you