Fluorescence Endoscopy
in Veterinary Patients
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 ability 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.

Fig. 1: Principle of fluorescence/Stokes shift
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.
Indications in Veterinary Patients

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.](image1)

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

➢ **Framing of Hypervascularized Tumors**

![Perfusion assessment of hepatic mass in a dog with NIR/ICG.](image2)

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

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.

A) White light

B) Metastasis showing accumulation of ICG.

Visualizing the Lymphatic System

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

A) White light

B) Lymphatic vessels showing ICG fluorescence.

Images courtesy of: Jeffrey J. Runge, DVM, DACVS, University of Pennsylvania, School of Veterinary Medicine, USA

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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
   - endoscopes in various sizes and dimensions

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 16 and 17
OPAL1® Technology for Photodynamic Diagnosis (PDD)

Fluorescence imaging with 5-aminolevulinate acid (5-ALA)

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-aminolevulinate 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 $\lambda_{Em} = 420$ nm, PPIX fluorescence emission maximum $\lambda_{Ex} = 630$ nm).

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

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

![Image](image1.png)

A) White light  
B) PDD mode with OPAL1® technology based on IMAGE1 S™

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

- Visualization of Glioblastoma via Fluorescence

![Image](image2.png)

A) White light  
B) PPIX-accumulating glioblastoma (red)

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. TRICAM® SL II camera system
   - brilliant image quality

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

3. TRICAM® PDD 3-chip camera head
   - for photodynamic early diagnosis PDD in conjunction with light source D-LIGHT C

4. D-LIGHT C light source (Xenon light source)
   - best daylight spectrum
   - filters are variably adjustable
   - no additional security measures (vs. Laser)

5. Fluid light cable
   - optimal light transmission in the white light and NIR spectral range

Footswitch
   - fast switch between white light and fluorescence mode

Literature, see page 18
Another application of fluorescence for diagnosis is the intrinsic autofluorescence (AF) of tissue components. The OPAL1® technology AF focuses on the differentiation between healthy and malignant tissue in bronchoscopy and laryngoscopy. The underlying principle is simple: The OPAL1® technology AF detects the green fluorescence of flavins in the healthy mucosa. Malignant tissues like bronchial or laryngeal carcinoma are identified by the lack of fluorescence since their compactness blocks the autofluorescence of the underlying healthy mucosa.

Fig. 10: Principle of autofluorescence
OPAL1® Technology for 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 Various Endoscopes

Fig. 11: Application examples for AF bronchoscopy

5 Images courtesy of Dr. Stanzel, Lung Clinic Hemer, Germany
OPAL1® Technology for AF

1. TELECAM SL II FI camera system
   - brilliant image quality

2. AF videobronchoscope
   - telescopes for optimal fluorescence excitation & detection, which can be used for white light and AF applications

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

Literature, see page 19
D-LIGHT P
modifiable for various fluorophores

66100 M1  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

66100 M2  Same, with two integrated special filters

66100 M3  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
Snap-on Filters
for use with standard eyepieces and D-LIGHT P light source

60 1000 36 Snap-on Filter for RFP/mCherry
60 1000 37 Snap-on Filter for GFP
60 1000 38 Snap-on Filter for tdTomato
60 1000 39 Snap-on Filter for CY7
60 1000 41 Snap-on Filter for CY5/methylene blue
60 1000 40 Snap-on Filter for ICG
20 1000 33 Fluorescein Barrier Filter
20 1000 34 Snap-on Filter for 5-ALA/PDD
20 1000 35 Snap-on Filter for AF

Please note:
Fluorescence with the above named substances may not be compatible with the IMAGE1 S™ platform. Please contact KARL STORZ for further information.

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

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Rutger M. Schols, Nicole D. Bouvy, Ronald M. van Dam, Ad A. M. Masclee,
Cornelis H. C. Dejong, Laurents P. S. Stassen
Springer Science+Business Media New York 2013

W. B. van Leeuwen, Stephan Hruby
2013 Published by Elsevier B.V. on behalf of European Association of Urology
http://www.europeanurology.com/article/S0302-2838(13)01460-7/abstract

Wachter D, Behm T, von Eckardstein K, Rohde V.
Neurosurgery. 2013 Sep;73(1 Suppl Operative):ons67-72; ons72-3.
doi: 10.1227/NEU.0b013e318285b846.

Nynke S. van den Berg, Renato A. Valde’s-Olmos, Henk G. van der Poel and
Fijs W.B. van Leeuwen
Journal of Nuclear Medicine, published on March 14, 2013

J PlastReconstrAesthet Surg. 2013 Ma

Jeschke S, Lusuardi L, Myatt A, Hruby S, Pirich C, Janetschek G.

[8] Laparoscopic fluorescence angiography with indocyanine green to control the perfusion of gastrointestinal anastomoses intraoperatively.
Carus T, Dammer R.
SurgTechnol Int.2012 Dec 30;XXII. pii: sti22/44.
[9] Indocyanine green fluorescence endoscopy for visual differentiation of pituitary tumor from surrounding structures.
Zachary N. Litvack, Gabriel Zada, and Edward R. Laws Jr.
J Neurosurg / February 24, 2012

[10] Clinical applications of indocyanine green (ICG) enhanced fluorescence in laparoscopic surgery.

Tummers QR, Verbeek FP, Prevo HA, Braet AE, Baeten CI, Frangioni JV, van de Velde CJ, Vahrmeijer AL.
SURG INNOV 1553350614535857, first published on June 5, 2014

Schols RM, Bouvy ND, van Dam RM, Masclee AAM., Dejong CHC., Stassen LPS.

Wachter D, Behm T, von Eckardstein K, Rohde V.
Neurosurgery. 2013 Sep;73(1 Suppl Operative):ons67-72; ons72-3.
doi: 10.1227/ NEU.0b013e318285b846.

Arezzo A, Arolfo S, Mistrangelo M, Mussa B, Cassoni P, Morino M.
Minimally Invasive Therapy. 2013;Early Online:1–4


Witjes JA, Gomella LG, Stenzl A, Chang SS, Zaak D, Grossman HB.


Karl A, Weidlich P, Buchner A, Hofmann T, Schneevoigt B, Stiefl Ch., Zaak D.
In: © 2014 Karl A et al. Brochure


In: © 2014 European Association of Urology. Published by Elsevier B.V.

José Piquer, Jose L. Llácer, Vicente Rovira, Pedro Riesgo, Ruben Rodriguez, Antonio Cremades.

[8] 5-Aminolevulinic Acid-derived Tumor Fluorescence: The Diagnostic Accuracy of Visible Fluorescence Qualities as Corroborated by Spectrometry and Histology and Postoperative Imaging
Stummer W, Tonn J-Ch., Goetz C, Ullrich W, Stepp H, Bink A, Pietsch T, Pichlmeier U.
Literature – AF

Kraft M, Betz CS, Leunig A, Arens C.

Gabrecht T, Lovisa B, van den Bergh H, Wagnières G.

Arens C, Reussner D, Woenkhaus J, Leunig A, Betz CS, Glanz H.


[5] Cell migration leads to spatially distinct but clonally related airway cancer precursors.

Nakao M, Oguri T, Miyazaki M, Hiji kata H, Yokoyama M, Kunii E, Uemura T, Takakuwa O, Ohkubo H, Maeno K, Niimi A
In: © 2013, Spandidos Publications