Dual Color Imaging from a Single BF₂-Azadipyrromethene Fluorophore Demonstrated *in vivo* for Lymph Node Identification

Niamh Curtin,¹ Dan Wu,¹ Ronan Cahill,² Anwesha Sarkar,² Pól Mac Aonghusa,³ Sergiy Zhuk,³ Manuel Barberio,⁴ Mahdi Al-Taher,⁴ Jacques Marescaux,⁵ Michele Diana,^{4,5,6} Donal F. O'Shea^{1*}

¹Department of Chemistry, Royal College of Surgeons in Ireland, RCSI, 123 St Stephen's Green, Dublin 2, Ireland.

²UCD Centre for Precision Surgery, School of Medicine, University College Dublin, Ireland;

Department of Surgery, Mater Misericordiae University Hospital, Dublin, Ireland.

³IBM Research – Ireland, Damastown Industrial Estate, Mulhuddart, Dublin 15, Ireland.

⁴IHU-Strasbourg, Institute of Hybrid Image-Guided Surgery, Strasbourg, France.

⁵IRCAD, Research Institute against Cancer of the Digestive System, Strasbourg, France.

⁶ICube Lab, Photonics Instrumentation for Health, Strasbourg, France.

Supporting Information

Legends for Movies S1-5

Movie S1: Fluorescence intensity map showing syringe containing **1**, injection site and ICG reference card as shown in Figure 10.

Movie S2: Lymphatic mapping with 1 using 800 nm emission as shown in Figure 11.

Movie S3: Lymphatic mapping with **1** using 700 nm emission as shown in Figure 12.

Movie S4: Lymph node mapping at 800 nm using **1** in porcine colorectal tissue using Stortz clinical laparoscopic instrument.

Movie S5: Lymph node mapping at 800 nm using **1** in *ex vivo* human colorectal tissue using Stryker laparoscopic instrument as shown in Figure 13.

Figure S1: Fluorescence spectrum of J-aggregated 1

Figure S2: Stability of 1 in 2.5 mM PBS/EL solution

Figure S3: Imaging 1 with a Stortz clinical 800 nm laparoscopic system

Figure S4: NMR spectra for 1.



Figure S1. Fluorescence spectrum of J-aggregated 1 with excitation at 800 nm.



Figure S2. Fluorescence spectra of **1** at 2.5 mM PBS/EL upon storage for 1 week. Day 1 (black trace), day 2 (red trace), day 3 (green trace), day 7 (yellow trace).



Figure S3. Imaging with a Stortz clinical 800 nm laparoscopic system in a porcine model showing colonic lymph channel and lymph node. Panel A and B: while light image showing injection of **1**. Panel C: fluorescence imaging showing injection site and lymphatic channel. Panel D: fluorescence image showing lymph node. See SI Movie S4 for video.



Figure S4: ¹H NMR (400 MHz, DMSO-*d*₆) of **1**





¹H COSY (DMSO-d₆) of $\mathbf{1}$

