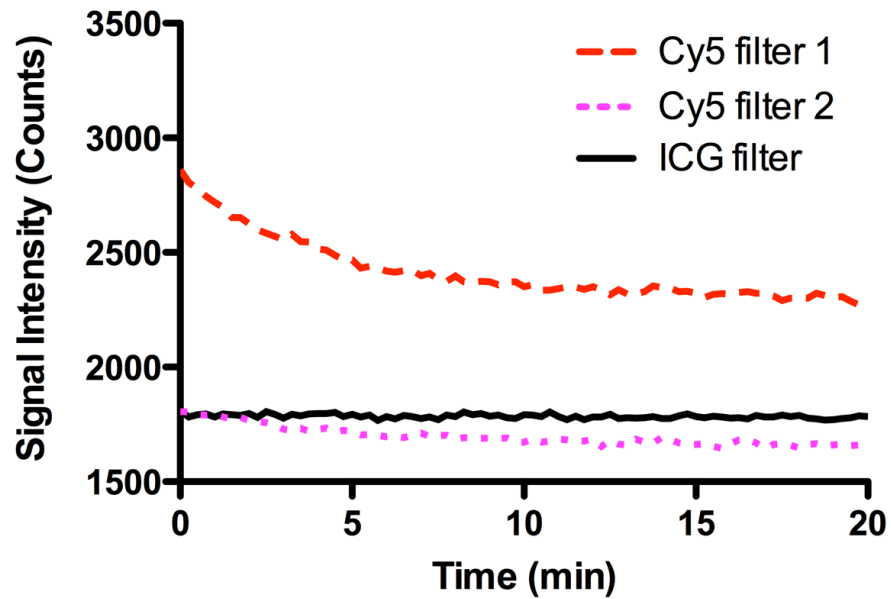


## Supplemental Data

### Supplemental Figure



**Supplemental Figure 1. Photobleaching of endogenous fluorophores at Cy5 wavelengths.** Dynamic imaging of the saphenous vein region was performed in female C57BL/6J-Tyr<sup>c-J</sup> albino mice under three different conditions to test for photobleaching effects. Cy5 filter setting 1: 300 ms exposure, 100% LED intensity, Cy5 filter setting 2: 200 ms exposure, 70% LED intensity, ICG filter: 500 ms exposure, 100% LED intensity.

## Supplemental Video Legends

**Supplemental Video S1. Evans blue dye is not lymphatic specific.** Imaging of a region downstream from the s.c. injection site in the right rear paw. After injection of 20  $\mu\text{L}$  2% Evans blue, uptake of dye into popliteal vein is immediately seen. At 2 min, a massage of the injection site shows uptake of the dye into the collecting lymphatic vessels. Images are acquired at 1 frame per 1 s at 25x magnification and is shown at 10x normal speed.

**Supplemental Video S2. P20D680 is lymphatic specific.** Imaging of a region downstream from the s.c. injection site in the right rear paw. After s.c. injection of 20  $\mu\text{L}$  of 25  $\mu\text{M}$  P20D680, some uptake of tracer is seen in the downstream collecting lymphatic vessels, but is not apparent in the popliteal vein. At 2 min, a massage of the injection site shows increased uptake of the dye into the collecting lymphatic vessels. Images are acquired at 1 frame per 1 s at 25x magnification and is shown at 10x normal speed.

**Supplemental Video S3. Evans blue dye is immediately apparent in the systemic circulation after s.c. injection.** Imaging of a region on the contralateral leg from the s.c. injection site in the right rear paw. Shortly after injection at  $t = 0$  min Evans blue dye is seen in the saphenous vein indicating direct venous uptake at the injection site. Signal continues to increase throughout the 30 min imaging period. Images are acquired at 1 frame every 15 s at 25x magnification.

**Supplemental Video S4. Sensitivity of the imaging system to programmed infusions of P40D800 into the blood.** Imaging of the saphenous vein of a mouse with a tail vein catheter implanted for stepwise infusions of P40D800. Each infusion is of 10  $\mu\text{L}$  of 1  $\mu\text{M}$  P40D800 and they begin at  $t = 0$  min and are repeated every 3 min until  $t = 45$  min. A noticeable consistent increase of signal is seen at each infusion. Images are acquired at 1 frame every 15 s at 25x magnification.

**Supplemental Video S5. Stimulation of lymphatic function in mice under anesthesia by a massage protocol.** Imaging of a region downstream from the s.c. injection site in the right rear paw (5  $\mu\text{L}$  of 100  $\mu\text{M}$  P40D680). Each massage is performed with a cotton applicator with slight pressure on the injection site once per second for 10 s and they begin at  $t = 5$  min and are repeated every 5 min until  $t = 50$  min. No signal from P40D680 is seen immediately after injection at  $t = 0$  min. With each massage signal increases are seen inside the downstream collecting vessels with steady contractility evident. Images are acquired at 1 frame per 5 s at 6x magnification.

**Supplemental Video S6. Blood signal in mice under anesthesia after s.c. injection and utilization of a massage protocol.** Imaging of a region on the contralateral leg from the s.c. injection site in the right rear paw (5  $\mu$ L of 200  $\mu$ M P40D800). Each massage is performed with a cotton applicator with slight pressure on the injection site once per second for 10 s and they begin at  $t = 5$  min and are repeated every 5 min until  $t = 50$  min. No P40D800 signal is seen immediately after injection at  $t = 0$  min, signal becomes first apparent at around  $t = 15$  min and continues to increase throughout the experiment. Images are acquired at 1 frame per 15 s at 25x magnification.