Electronic Supplementary Material (ESI) for Analyst.

Identification and characterization of different tissues in blood vessel by multiplexed fluorescence lifetimes

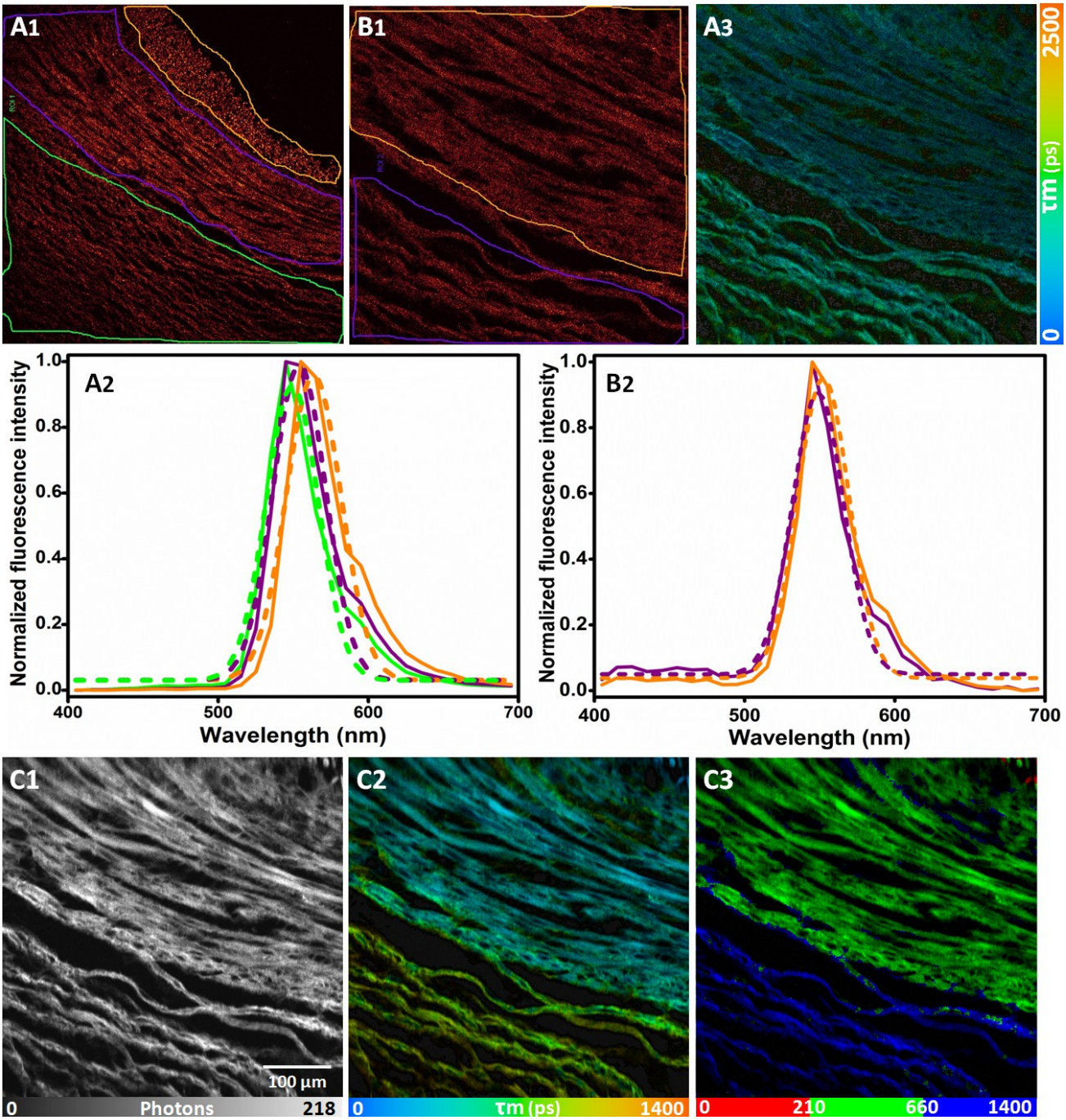
Teng Luo[a], Danying Lin[a], Ting Zhou[a], Yuan Lu[b], Shaoxiong Liu[c], Junle Qu[*a]

[a] Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China

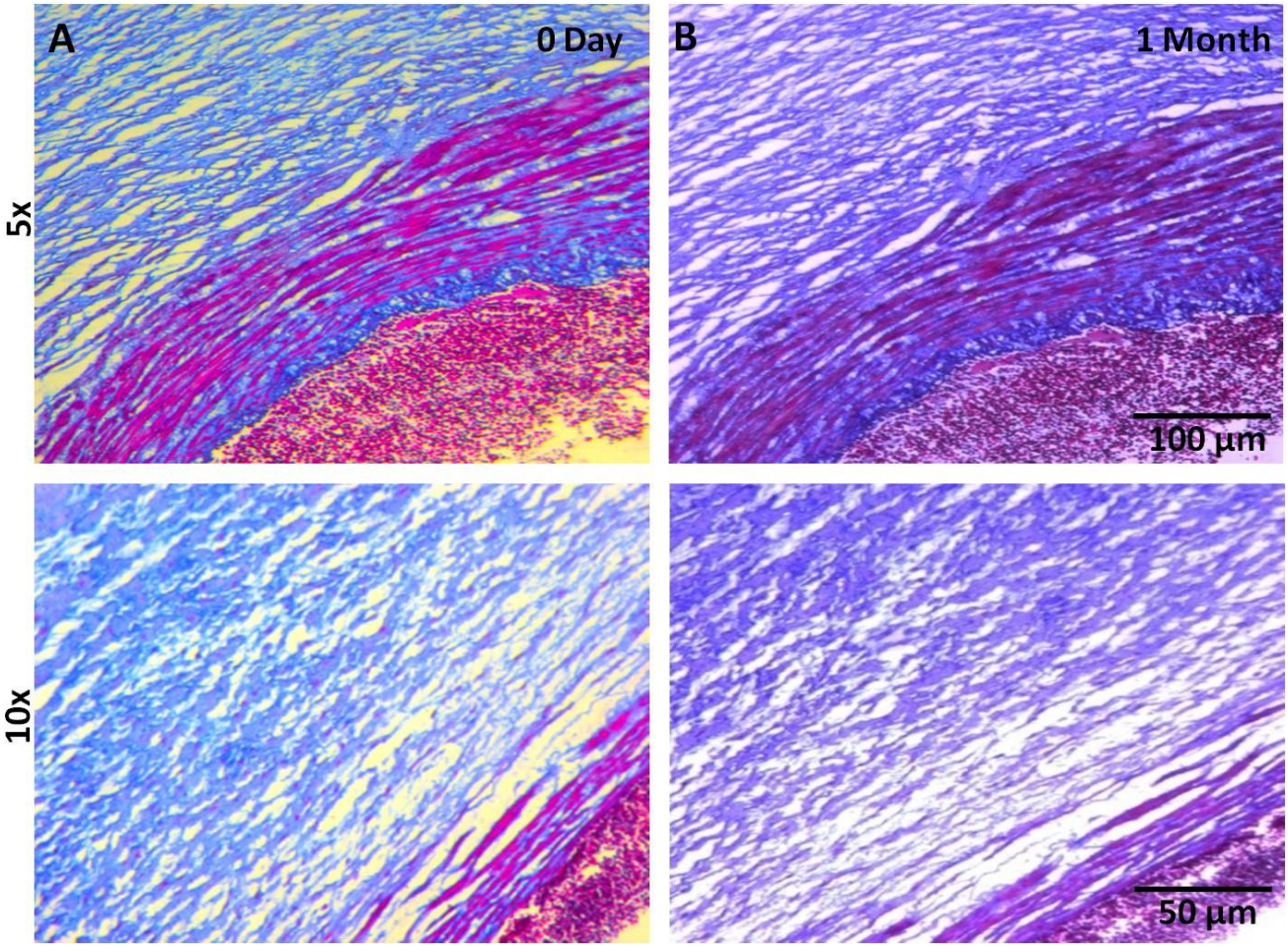
[b] Department of Dermatology, The Sixth People's Hospital of Shenzhen, Shenzhen 518052, China

[c] Department of Pathology, The Sixth People's Hospital of Shenzhen, Shenzhen 518052, China

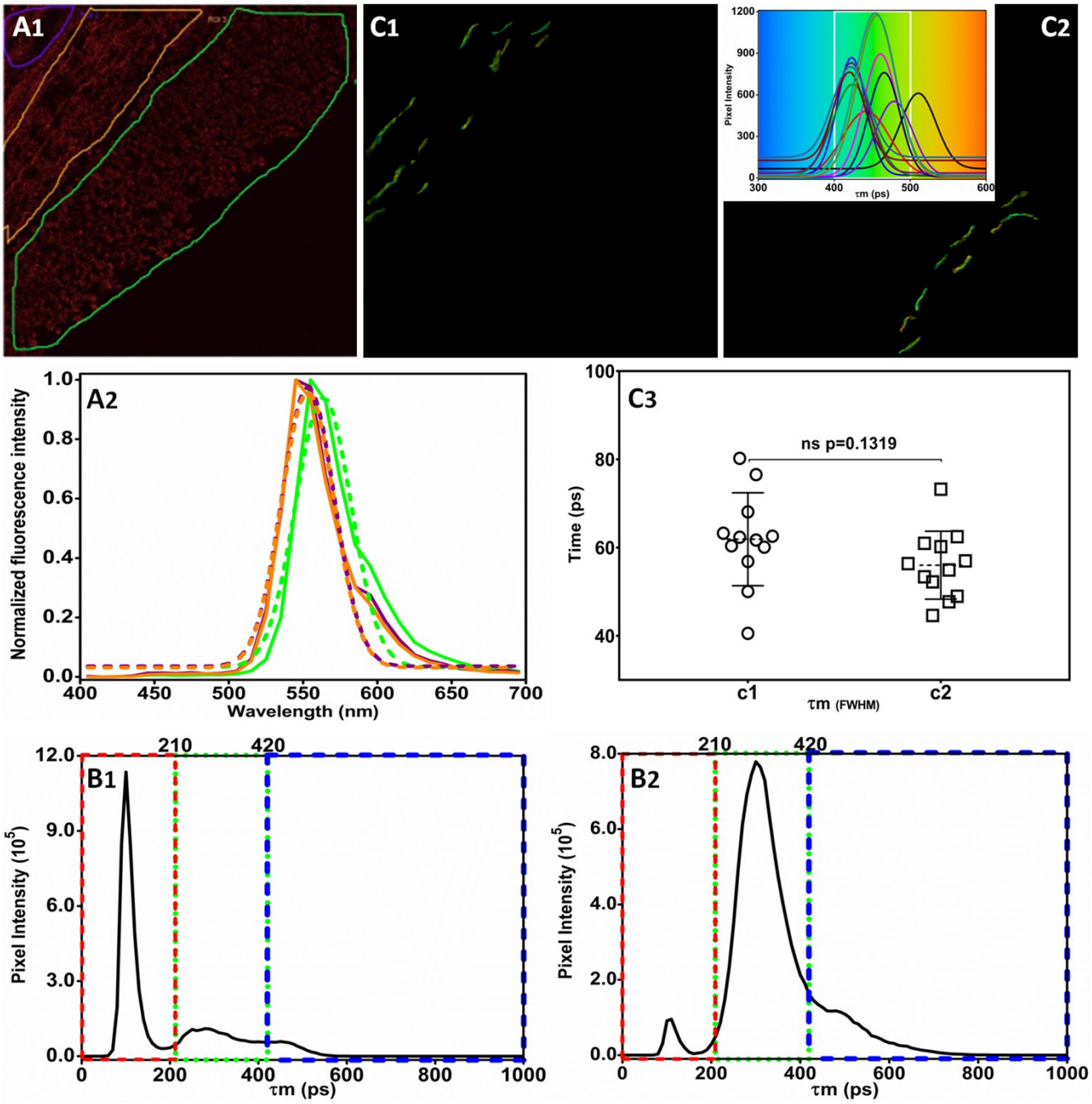
Supporting Information



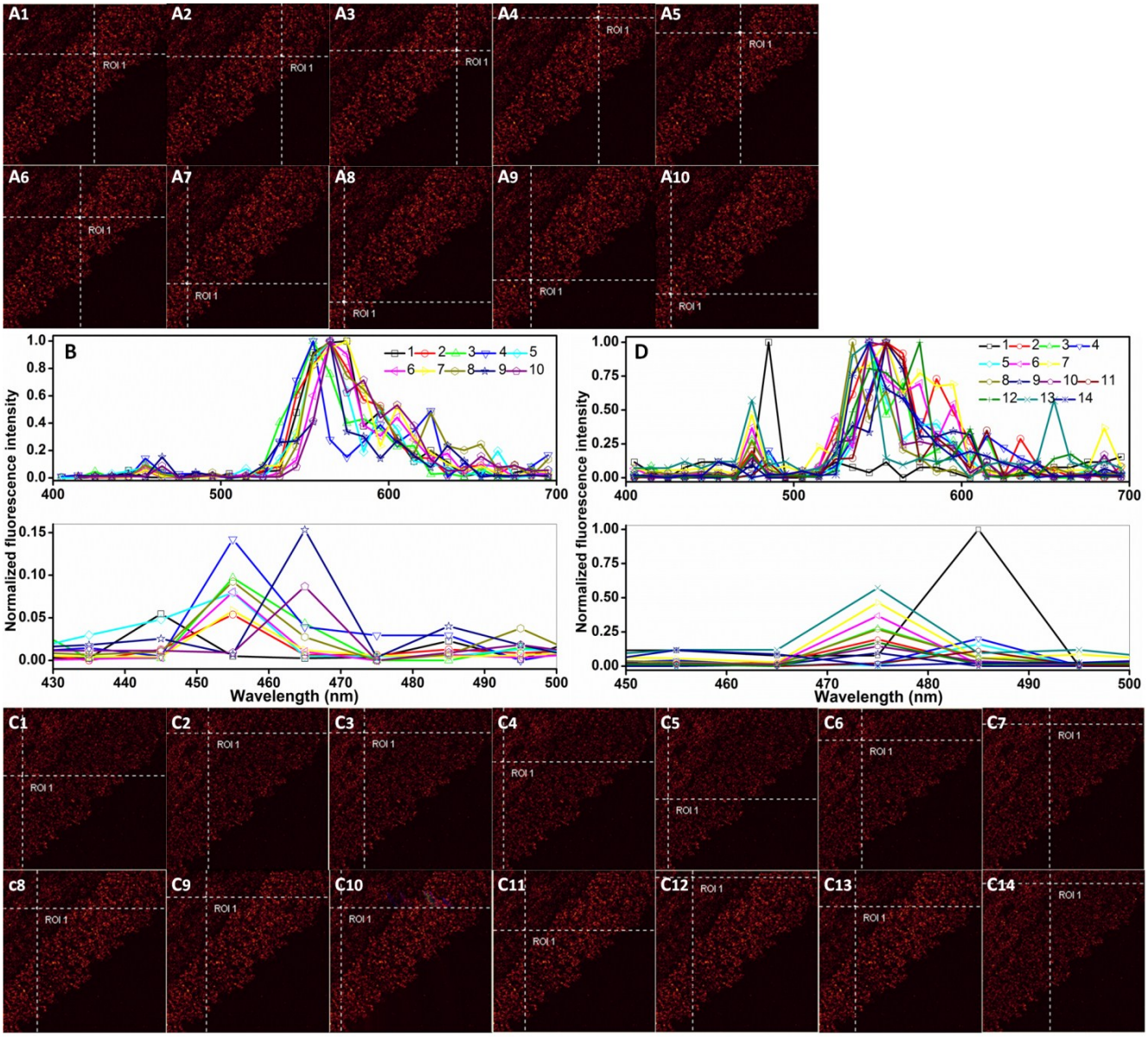
Supplementary Fig. 1 H&E fluorescence intensity image (A1) and spectra (A2) corresponding to Fig. 1B1. H&E fluorescence intensity image (B1) and spectra (B2) corresponding to Fig. 1C. Autofluorescence lifetime image corresponding to Fig. 1E1 (B3). Compared with CT, the emission peak of erythrocytes is red-shifted by ~13 nm (orange line). H&E fluorescence (C1), lifetime (C2) and lifetime trichrome (C3) images corresponding to Fig. 1C1.



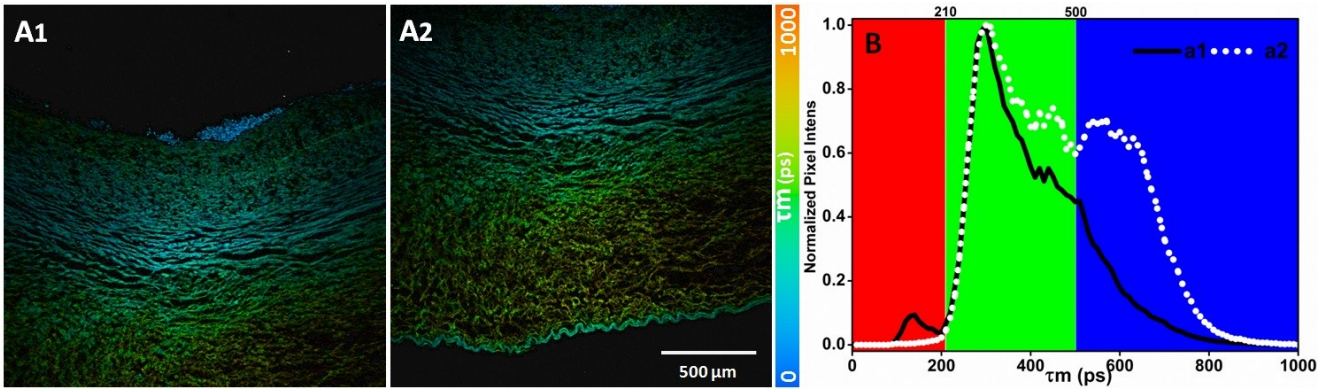
Supplementary Fig. 2 Masson's trichrome staining of an umbilical artery. (A) Smooth muscle and collagen can be clearly seen in the prepared tissue section. Smooth muscles appear red; collagen fibers appear blue; nuclei appear blue-black; and erythrocytes appear orange. (B) After one month, the chromatic contrast between smooth muscle, collagen fibers, and erythrocytes had decreased.



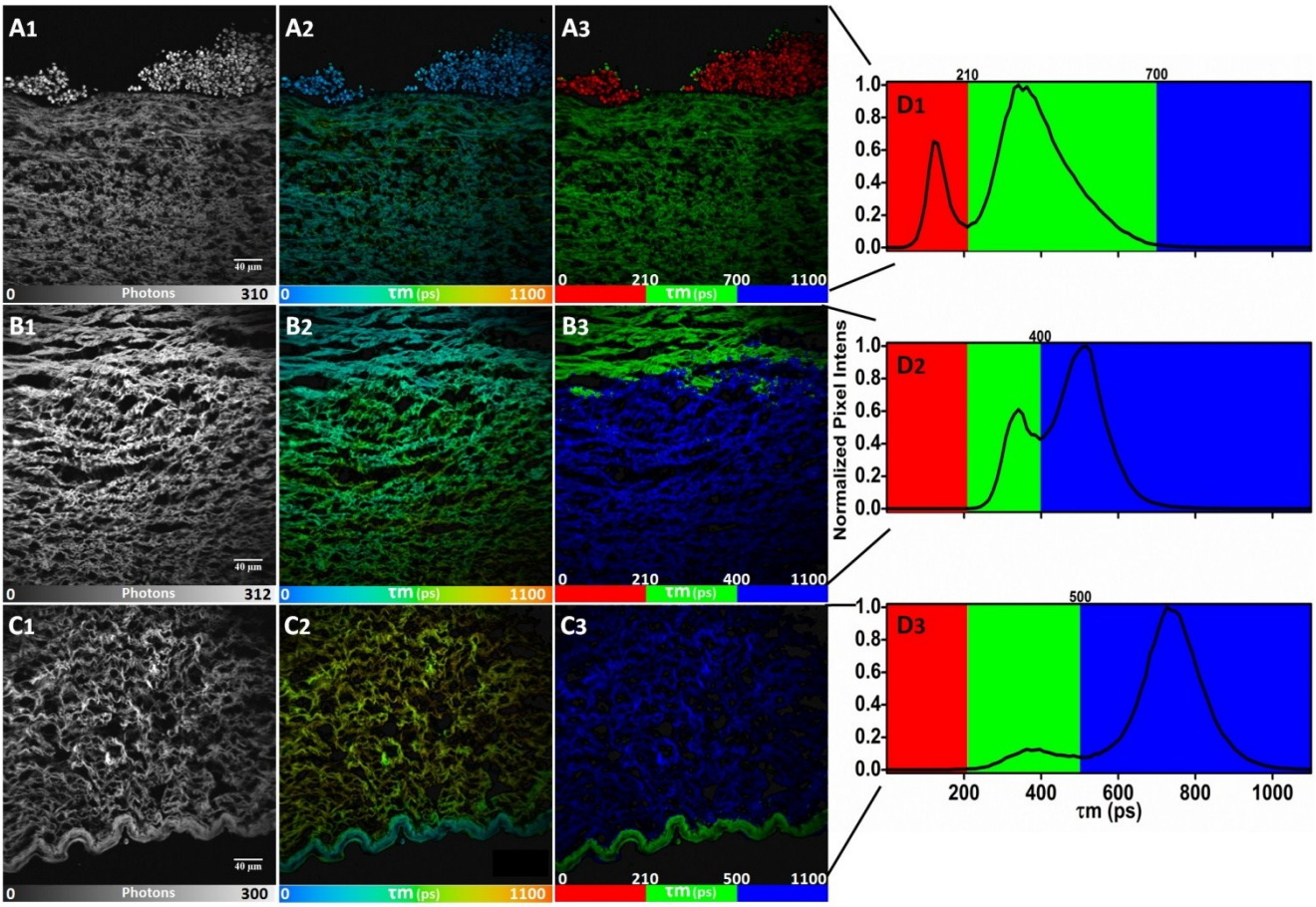
Supplementary Fig. 3 Weigert staining like elastic fibers specific imaging by multiplexed fluorescence lifetimes of eosin. H&E fluorescence intensity image (A1) and spectra (A2) corresponding to Fig. 2C. (B1 and B2) τm histogram corresponding to Fig. 2C3 and D3, respectively. Both histograms can be divided into three regions: 0–210, 210–420 and 420–1000 ps. Red, green and blue colors were assigned to the corresponding regions extracted from the τm histogram to obtain a trichrome lifetime image. (C1 and C2) H&E fluorescence lifetime images of H&E-stained elastic fibers in Fig. 2C3 and D3, respectively. The corresponding τm histogram insets C2. Elastic fibers (cyan) can be observed through autofluorescence (> 425 nm) of H&E-stained tissue sections (yellow arrows, in Fig. 2) under direct UV excitation (340–380 nm). (C3) Comparisons of the full width at half maximum (FWHM) of τm (τm_{FWHM}) of H&E for randomly selected in C1 and C2 (n=12).



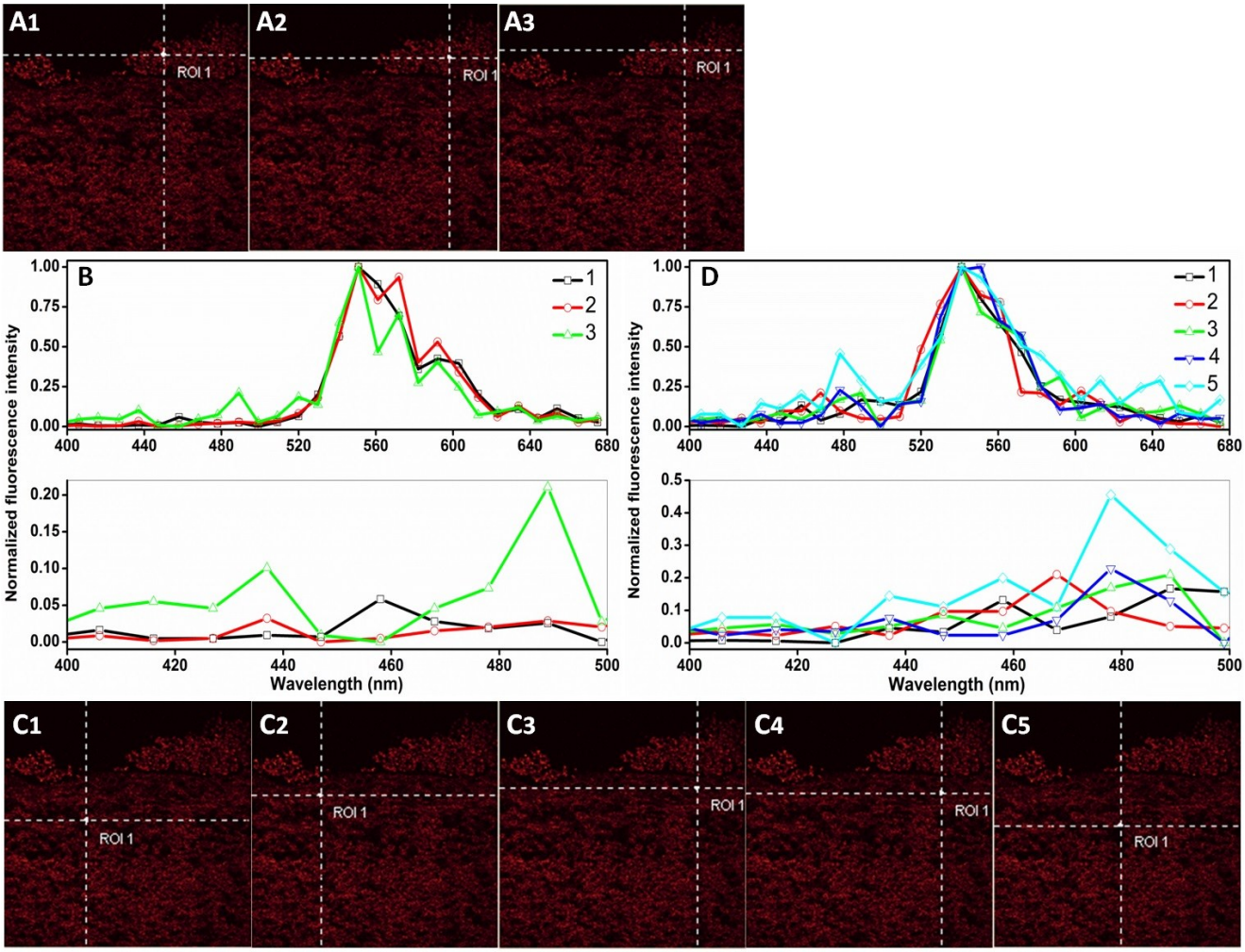
Supplementary Fig. 4 Identification of elastic fibers (EFs) by autofluorescence (AF). The AF spectra of H&E-stained erythrocytes (A1–A10), and the AF spectra of H&E-stained elastic fibers (C1–C14). The spectra correspond to B and D, respectively. AF emission peak of erythrocytes is located between 440 and 470 nm.



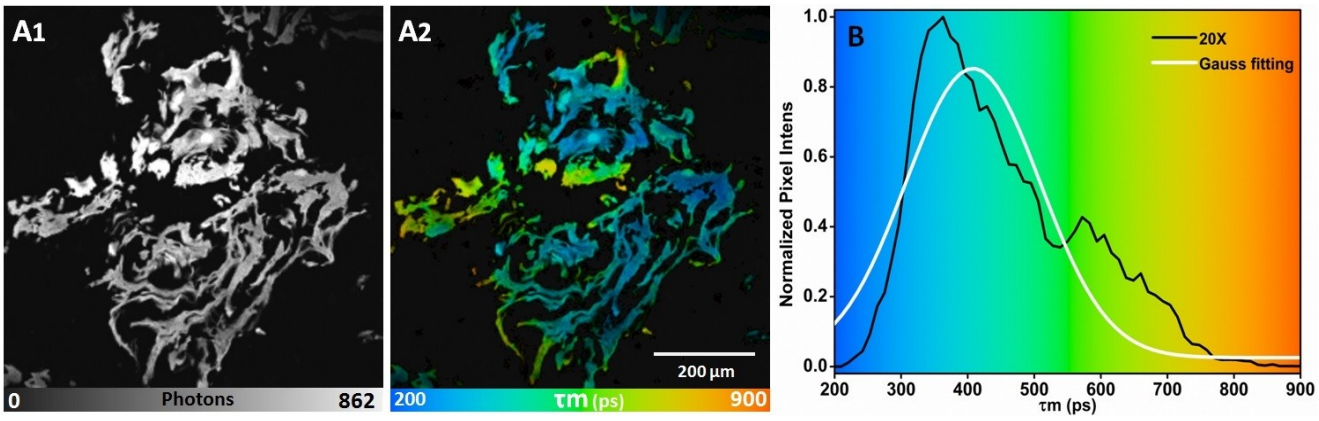
~~Supplementary Fig. 5 Fl~~uorescence lifetime trichrome images of an H&E-stained umbilical artery. Due to the limited field of view, Fig. 3A2 is split over A1 and A2. (B) The τm histogram and the corresponding division ranges. The histogram can be divided into three regions: 0–210, 210–500 and 420–1000 ps. Red, green and blue colors were assigned to the corresponding regions extracted from the τm histogram to obtain a trichrome lifetime image.



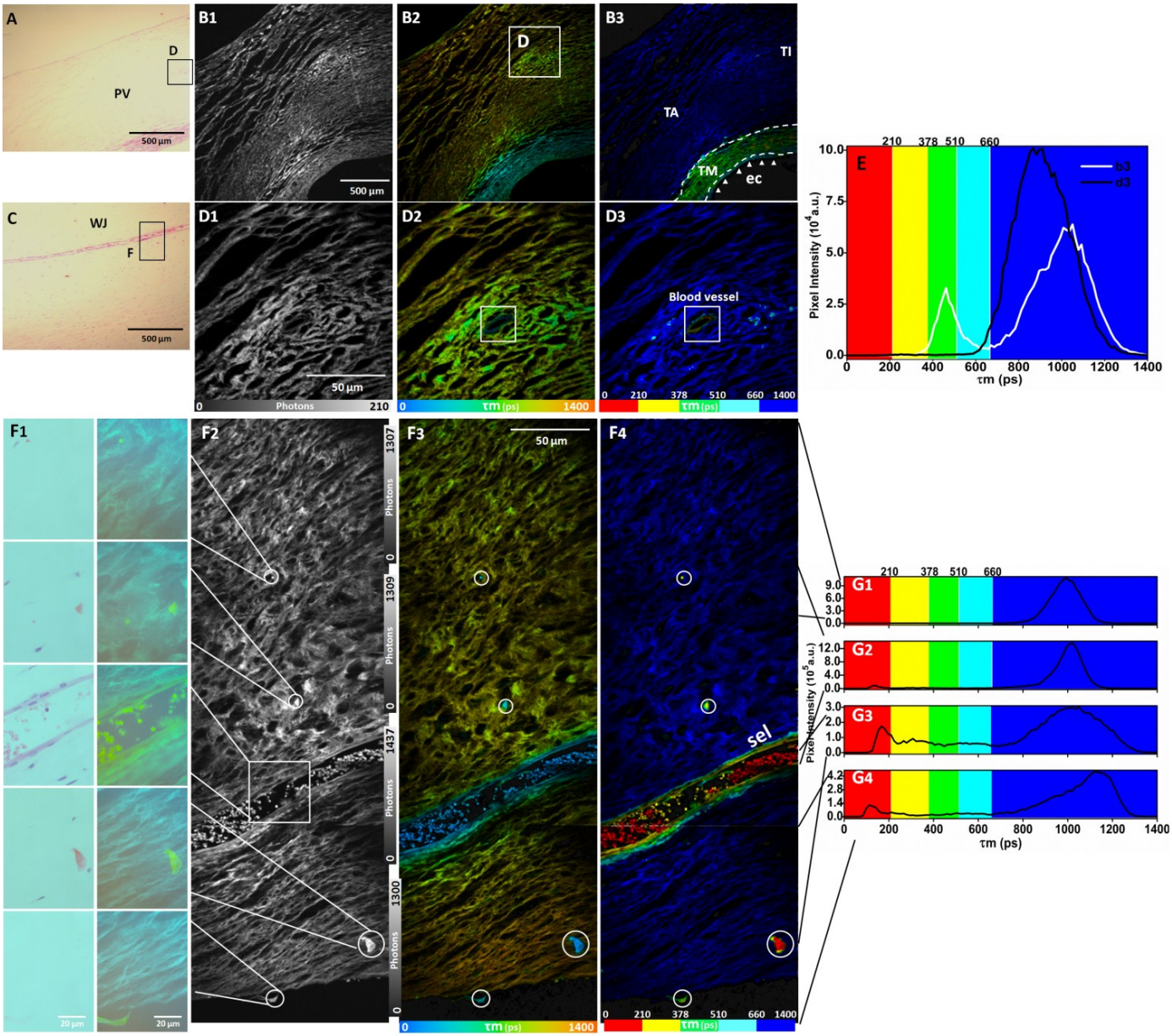
Supplementary Fig. 6 (A–C) H&E fluorescence intensity (A1, B1 and C1), images with continuous false-color coding (A2, B2 and C2) and trichrome images (A3, B3 and C3) corresponding to the overlaid images Fig. 3B1 and B2, B3 and B4, B5 and B6, respectively. (D) The τm histograms and the corresponding segmentation correspond to A3 (D1), B3 (D2) and C3 (D3), respectively.



Supplementary Fig. 7 The autofluorescence (AF) spectra emitted by erythrocytes (A, B) and elastic fibers (C, D) in the selected regions of interest in Fig. 3B1.



Supplementary Fig. 8 H&E Fluorescence intensity (A1) and lifetime images (A2) of H&E-stained collagen from bovine achilles tendon. τm histograms of A1 and A2 (B). The black line represents the normalized measurement data and the white line is the Gaussian fitting.



Supplementary Fig. 9 H&E fluorescence lifetime pentachrome images of umbilical vein and placental microvessel, obtained by using the same lifetime segmentation in Fig. 3D. (A1) Bright field H&E, (B1) H&E fluorescence intensity, (B2) lifetime with continuous pseudocolor coding and (B3) lifetime with pentachrome coding images of umbilical vein. The position of C1 is shown as region A in C. (D) corresponds to the rectangles in A1 and B2. H&E fluorescence intensity (D1), lifetime (D2) and lifetime pentachrome (D3) images of region D in B2. The rectangles in D2 and D3 represent the perivascular region (PV) surrounding the umbilical blood vessels. (E) The τm histogram and the divided regions of H&E fluorescence lifetime for B3, D3. Bright field H&E of placental microvessels embedded in the proteoglycan-rich gelatinous Wharton's jelly (WJ), which is shown as region F in C. Bright field images and H&E fluorescence intensity images under UV excitation (F1), H&E fluorescence intensity (F2), fluorescence lifetime (F3) and lifetime pentachrome (F4) images of placental microvessels. The circular areas in F2 may be residual impurities from the process of preparing H&E-stained sections, and correspond to their bright field H&E and UV fluorescence images. (G1–G4) τm histograms of F4. E and G represent the τm histograms (0–1400

ps) of D3 and F3, respectively. E and G can be divided into five segments according to the τm peaks, cross-points and τm_{FWHM} (0–210, 210–378, 378–510, 510–660 and 660–1400 ps), represented as the five colors red, yellow, green, cyan and blue, respectively.