

Supporting information for

Visualizing vasculature and its responses to therapies in the tumor microenvironment

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Supporting Table 1: Current window models for intravital imaging of vasculatures.

Window organ	Surgery (Y/N)	Study period	Tumor model	Blood vessels visualization method	Stabilizer /device	Microscope	Ref
Ear Skin	N	Longitudinal	n/a	FITC-dextran (2000 kDa) and TRITC-ficoll (70 kDa/400 kDa); Dextran (70 kDa/150 kDa/2000 kDa)	n/a	Upright/inverted LSCM and MPLSM	[1, 2]
Lymph node	Y	Short-term	n/a	Dextran (500 or 2,000 kDa)	Superglue fixation; Custom-built microscope stage	Upright MPLSM	[3-6]
s.c./i.m. tumor in flank	Y	Short-term	CT-26-GFP	PE-anti-CD31 (blood vessel) and PE-anti-CD49b (blood flow)	Secured to a microscope slide by sutures/heated microscope stage	Inverted LSCM and MPLSM	[7]
Dorsal skin	Y	Longitudinal	4T1-GFP or BxPC-3-DesRed s.c. tumor (inoculate at DSWC installation or 10 d)	Oxygen-sensing boron NP (hemoglobin saturation) or FITC-Dextran (2MDa)	DSWC with imaging mount	Upright/inverted WFM and LSCM	[8, 9]

Window organ	Surgery (Y/N)	Study period	Tumor model	Blood vessels visualization method	Stabilizer /device	Microscope	Ref
Dorsal skin	Y	Longitudinal	n/a or B16BL6 implanted tumor	FITC-dextran (150 K Da) or eNOSTag-GFP (endothelial cells); Cspg4-DsRed (Pericytes)/ Rhodamine PEGylated NPs (blood flow)	Adapted small and light weight-DSWC	WFM or upright MPLSM	[10, 11]
Dorsal skin	Y	Longitudinal	s.c. A-07-GFP or s.c. LLC-DsRed2 tumor	TRITC-dextran (155 KDa) or iron oxide-dextran NPs	MRI-compatible DSWC	MRI and inverted WFM or MPLSM	[12, 13]
Mammary fat pad	Y	Short-term	MMTV-PyMT orthotopic tumor	Rhodamine-dextran (70-kDa) or TRITC-Dextran (155 kDa) and FITC-dextran (10 kDa)	n/a or custom-built rubber pads or circular imaging window frame with cyanoacrylate adhesive	Inverted SDCM and MPLSM	[14-17]
Mammary fat pad	Y	Longitudinal	MTLn3- Dendra2; MTLn3-CFP; MMTV-PyMT-GFP orthotopic tumors	Fluorescent dextran (10Ka)	Coverslip mounted plastic frame (consisting of two plastic rings)	Upright LSCM and MPLSM	[18]
Mammary pad	Y	Longitudinal	MDA-MB-231-GFP; MCF-7-GFP orthotopic tumors	Alexa Fluor 647-BSA	Custom-made MRI-compatible MWC and animal holder	MRI and upright LSCM	[19]

Window organ	Surgery (Y/N)	Study period	Tumor model	Blood vessels visualization method	Stabilizer /device	Microscope	Ref
Brain	Y	Longitudinal	n/a	Texas red dextran, Evans blue, cascade blue dextran, FITC dextran (120 kDa)	Cyanoacrylate glue for fixing the skull and dental cement to cover the exposed skull	LSCM and MPLSM	[20]
Brain	Y	Longitudinal (up to 5 months)	D283-MED-Gluc and D341 s.c. tumors	TRITC dextran (2000 Kda); TRITC-BSA (68,000 MW)	Custom-built stereotactic frame. Cyanoacrylate glue, acrylic powder (1:1) to fix the coverslip-bone	Ultrasonography and MPLSM/OCT	[21]
Brain	Y	Longitudinal	n/a	n/a	Adapted transparent cranial windows with a tunable screw for controlled compression	MRI, MPLSM and CT	[22]
Brain ^{1,2,3,4}	Y	Longitudinal	n/a	n/a	Thinned skull transcranial window; Reinforced thinned skull window; Transcranial window; Implanted window; Prism-based window; Lens based window	MPLSM/ MPLSM and PET	[23]

Window organ	Surgery (Y/N)	Study period	Tumor model	Blood vessels visualization method	Stabilizer /device	Microscope	Ref
Bone marrow ⁴	Y	Longitudinal	Nalm-6 xenograft (DiD or DiR labeled); n/a	Fluorescent cyanine compounds or AlexaFluor750-ICAM-1, VCAM-1, PECAM-1 and P-selectin; Rhodamine B, Texas red and FITC-dextran (70 kDa) and CFDA-SE-RBCs	Skull window; custom-made stereotactic holder	LSCM and MPLSM	[24-26]
Bone marrow	Y	Longitudinal	T-ALL xenograft (PE-human CD45 antibody labeled)	Cy5-Dextran (500 kDa)	Calvarial bone marrow with protective intrasite gel and custom-made stabilizer	Upright LSCM and MPLSM	[27-29]
Bone marrow	Y	Longitudinal	LnCap-mCherry, Du145-mCherry, and PC3-mCherry s.c. tumors	TRITC-BSA (MW 67,000); FITC-labeled dextran (150 Kda); Quantum dots 655	Femur window (filled bilaterally with Ostron Cement-cover slide); Custom-made clamp to fix the chamber	LSCM and MPLSM	[30-33]
Lung	Y	Short-term (Up to 3 h)	n/a	Texas Red dextran (70 Kda), Red, fluorescent microspheres (blood flow)	Thoracic suction window attached to a micro-manipulator on the microscope stage	MPLSM	[34, 35]
Lung	Y	Short-term (Up to 12 h)	EO771-LG-EGFP metastasis	TRITC dextran (155 kD)	Vacuum stabilized window made of Teflon	Inverted MPLSM	[36]

Window organ	Surgery (Y/N)	Study period	Tumor model	Blood vessels visualization method	Stabilizer /device	Microscope	Ref
Lung	Y	Longitudinal (Days to weeks)	E0771–EGFP metastasis	VeCad-tdTomato (endothelia); TRITC dextran (155 kD); 2.5 µm fluorescent microspheres (blood flow)	Implantable lung window	Inverted MPLSM	[37]
Liver	Y	Short-term (up to 4 h)	n/a	Anti-mouse CD31 (PECAM-1) (endothelial cells); Anti-CD41 (platelets).	PBS-soaked Kimwipes to cover the exposed lobe; Custom-made acrylic platform	Inverted LSCM	[38, 39]
Liver	Y	Short-term (Up to 6 h)	SL4-RFP or HT29- RFP metastasis	n/a	Instant adhesive agent and a custom build-organ stabilizing system or agarose embedding	Upright MPLSM	[40, 41]
Liver	Y	Short-term	n/a	n/a	Custom-built imaging box with stabilizer	Inverted LSCM and OR-PAM	[42]
Liver, spleen, kidney, small intestine, Pancreas	Y	Longitudinal	C26–H2B-Dendra2, C26-Dendra2, C26- mCherry, and C26– LifeAct-GFP metastasis	Texas-Red dextran (70 kDa)	Abdominal imaging window with a custom-built imaging box	Inverted MPLSM	[43, 44]

Window organ	Surgery (Y/N)	Study period	Tumor model	Blood vessels visualization method	Stabilizer /device	Microscope	Ref
Pancreas	Y	Longitudinal	PANC-1 orthotopic tumor (4 weeks before window installation)	FITC-dextran (2000 Kda), TRITC-BSA	Abdominal wall window made of titanium circular mount with 8 holes on the edge	IVM	[45]
Pancreas	Y	Longitudinal	Rip1-Tag2 orthotopic tumor; KPC-EGFP orthotopic tumor	VeCad-tdTomato mice; FITC or TRITC-dextran (50 kDa)	Custom-built imaging window with cyanoacrylate, and a custom-made plastic c-clip to stabilize	MPLSM and laser speckles imaging/ MPLSM and MRI	[46, 47]
Ovary	Y	Longitudinal	ES-2-tdTomato or ES-2-eGFP s.c. tumor	FITC-dextran (500 kDa)	Custom-built imaging window with cyanoacrylate, and a custom-made plastic c-clip to stabilize	MPLSM	[48]
Mouse embryo	Y	Longitudinal	n/a	n/a	Custom-built abdominal window	MPLSM	[49]

Footnotes: Mouse models are used except for 1: ferret, 2: non-human primate, 3: pig and 4: rat. CT-26-GFP: mouse colon carcinoma; 4T1-GFP: mouse mammary carcinoma; BxPC-3-DesRed: human pancreatic adenocarcinoma; B16BL6: mouse melanoma; A-07-GFP: human melanoma; LLC-DsRed2: mouse Lewis lung carcinoma; MMTV-PyMT: (mouse mammary tumor virus-polyoma middle tumor-antigen) mouse model of breast cancer; MTLn3-Dendra2/MTLn3-CFP: highly invasive rat breast adenocarcinoma; MDA-MB-231-GFP: human breast adenocarcinoma; MCF-7-GFP: human breast adenocarcinoma; D283-MED-Gluc and D341: human medulloblastoma; Nalm-6: human B cell precursor leukemia; T-All: T-cell acute lymphoblastic leukemia; LnCap-mcherry: (Lymph Node Carcinoma of the Prostate) human prostate adenocarcinoma; Du145-mCherry: human prostate carcinoma with androgen receptor (AR) expression; PC3-mCherry: human prostate adenocarcinoma; EO771-LG-EGFP/EO771-EGFP: mouse mammary carcinoma; SL4-RFP: mouse colon carcinoma; HT29-RFP: human colorectal adenocarcinoma; C26-H2B-Dendra 2/C26-Dendra2/C26-mCherry/C26-LifeAct-GFP: mouse colon carcinoma; PNAC-1: pancreas ductal adenocarcinoma; Rip1-Tag2 mice: mouse pancreatic islet cell carcinoma; KPC-EGFP: mouse pancreatic ductal adenocarcinoma; ES-2-tdTomato or ES-2-eGFP: human ovarian carcinoma.

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