

Intravital Microscopy (IVM)

In Vivo Live Cell
Imaging Platform



- All-in-One Intravital Microscopy

IVIM Technology:

Intravital microscopy enables dynamic 3D cellular-level imaging of various biological processes in living animals *in vivo*. It enables scientists to directly verify hypotheses derived from *ex vivo* or *in vitro* observations in natural physiological *in vivo* microenvironments. Using intravital microscopy, *in vivo* visualizations of gene expression, protein activity, cell trafficking, cell-cell / cell-microenvironment interaction and various physiological responses to stimuli have been accomplished providing novel insights, which have been impossible to obtain with conventional static 2D observation of *ex vivo* or *in vitro* samples. However, up to now, individual users have had to improvise the required functions for each of the intravital imaging applications for the various organs with conventional standalone microscopes, which resulted in non-optimal imaging performance and limited applicability.

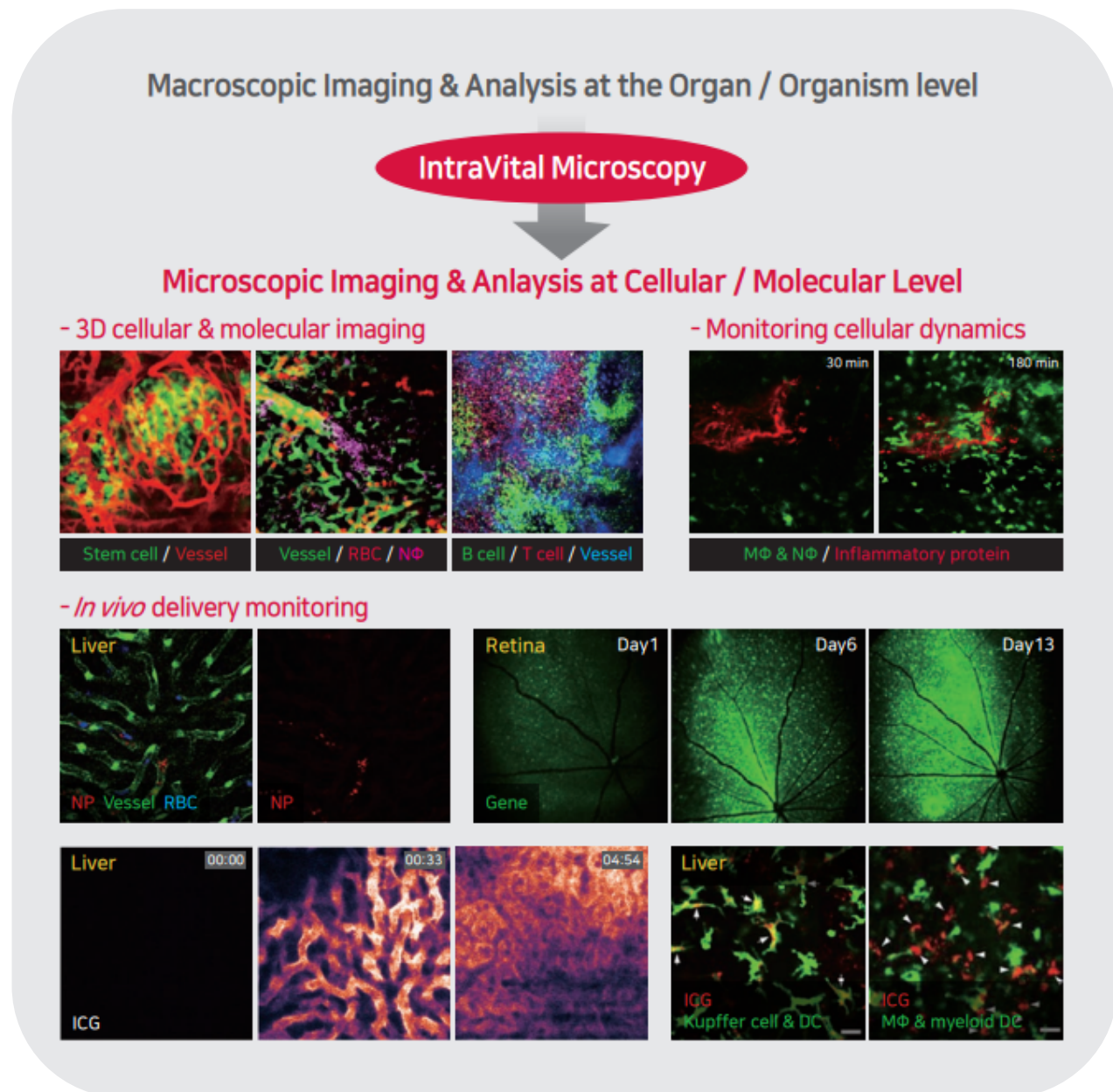


IVIM Technology's All-in-One intravital confocal / two-photon microscopy system (IVM C/M/CM/MS) is extensively optimized and carefully engineered to provide superb performance in the intravital imaging of live animal models *in vivo*.

- IVIM Technology's IVM series integrates expertly selected best-suited optical, optomechanical, and optoelectrical components, ensuring optimal imaging performance for a wide range of intravital imaging applications.
- Key indispensable functionalities for intravital imaging are fully integrated into the All-in-One system with attentive design for smooth and easy operation.
 - Body temperature monitoring and automated regulation system
 - Anesthesia apparatus optimized for integration into the animal stage
 - Animal motion stabilization apparatus customized for various organs
 - Implantable imaging window apparatus customized for longitudinal observation of various organs

The world's first All-in-One intravital microscopy platform from IVIM Technology is a key solution that can explore complex dynamic behaviors of numerous cells inside a living body and serve as the next-generation core technology to elucidate unknown pathophysiology of various human diseases and discover new cures for them.

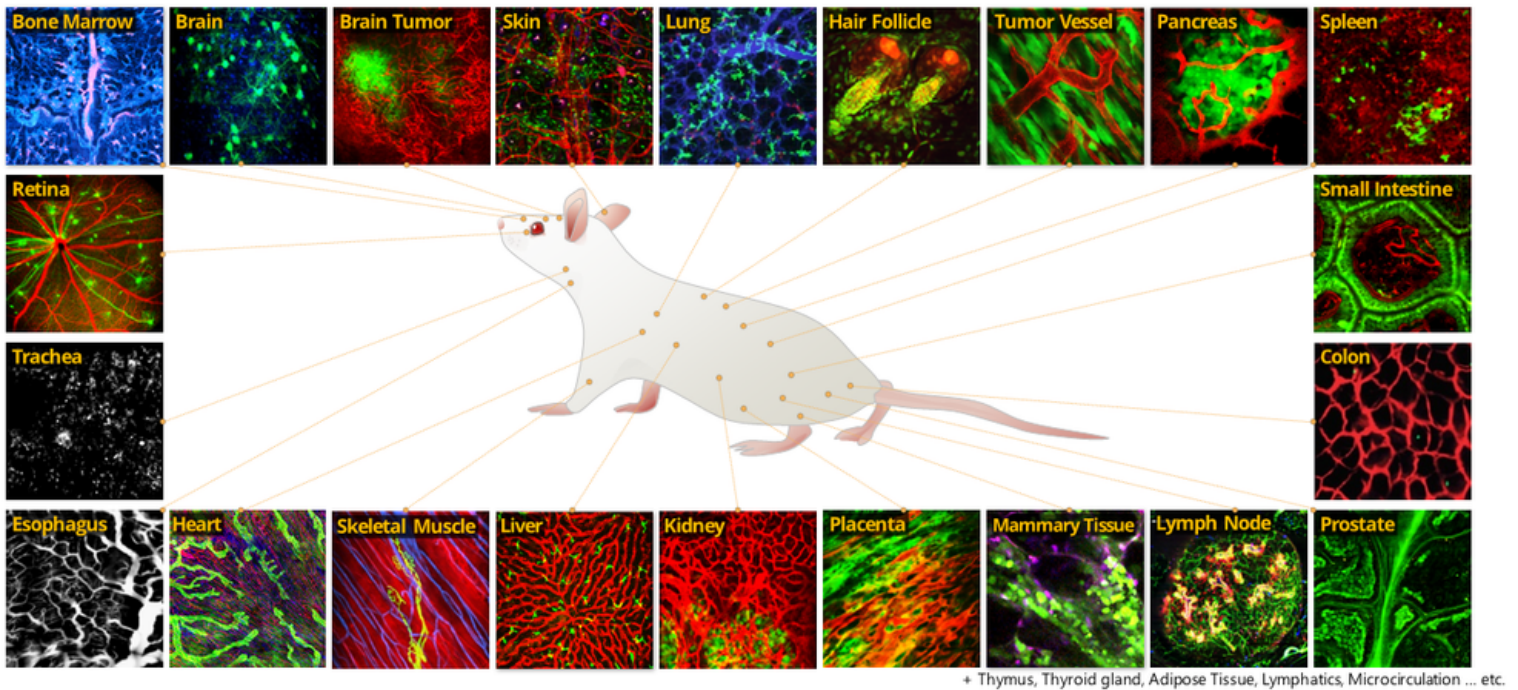
Why Intravital Microscopy (IVM)?



- IntraVital Microscopy (IVM) enables dynamic real-time imaging of various cellular-level dynamics such as cell trafficking, cell-cell / cell-microenvironment interaction, and various physiological responses to stimuli inside the living body *in vivo*, providing novel insights into the processes of human disease development.
- For drug development, IVM enables direct analysis of drug delivery to target tissues and cells and the efficacy of new therapeutic candidates by the intended MOA (Mode of Action) at a microscopic cellular level in various preclinical models of human diseases

Why IntraVital Microscopy (IVM)?

Unique Solution for In Vivo Live Cell Imaging



IVIM Technology's All-in-One intravital confocal / two-photon microscopy system (IVM-C/M/CM/ MS) has been designed to provide expandability and flexibility for highly diverse intravital imaging application of various tissues and organs including the brain, liver, spleen, kidney, pancreas, lung, heart, gastrointestinal tracts, retina, skeletal muscle, bone marrow, peripheral lymph node, prostate, thymus, thyroid gland, adipose tissue, blood and lymphatic vessels, etc.

In addition, to handle various imaging needs raised by researchers for a wide range of biomedical studies, detailed experimental protocols have been established for high-quality intravital image acquisition.

Subsequent quantitative analysis of various cellular-level dynamics and physiological alterations have been established.

For drug discovery and development applications, IVIM Technology's All-in-One intravital microscopy system can serve as a highly valuable and versatile tool.

- Target identification and validation in natural in vivo microenvironments
- Confirmation of MoA (Mode of Action) and PoC (Proof of Concept) of new therapeutics by direct in vivo cellular observation
- Monitoring of *in vivo* drug delivery, distribution, retention and *in situ* efficacy at target tissues and organs

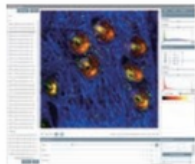


Why Intravital Microscopy (IVM)?

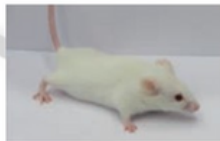
All-in-One Platform

Conventional Approach

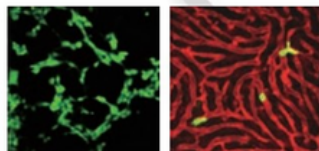
Image processing



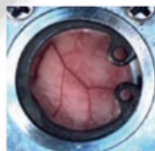
Animal manipulation



In vivo fluorescence labeling



Imaging chamber



Individual user have had to improvise the required functions for each of the intravital imaging applications with conventional standalone microscope.

- High degree of technical difficulty for non-expert individual user
- Non-optimized intravital imaging performance
- Limited applicability to various organs
- Difficult to standardize intravital imaging procedure
- Difficult to establish universal protocol for experiment
- Limited reproducibility and productivity



IVIM's All-in-One Packaged Platform

New!

All-in-One
IntraVital Microscopy



IVIM Technology's All-in-One intravital confocal / two-photon microscopy system (IVM-C/M/CM/MS) is extensively optimized and carefully engineered to provide superb performance in the intravital imaging of live animal models, *in vivo*.

All-in-One IVM Platform

- All-in-One single-box packaged IntraVital Microscopy (IVM) system
- Fully integrated with key functionalities for imaging of live animal model
- Unified animal stage to accommodate various accessories for wide-range of intravital imaging applications
- User-friendly design for easy and smooth operation

Optimized *In Vivo* System

- Sub- μm *in vivo* imaging resolution
- Ultra-high speed *in vivo* imaging (Max. 100 fps @ 512x512 pixels)
- 4-color simultaneous confocal / two-photon microscopic *in vivo* imaging
- Integrated animal motion compensation

IVIM Technology's Confocal / Two-Photon Technologies

Enable High-resolution *In Vivo* Imaging

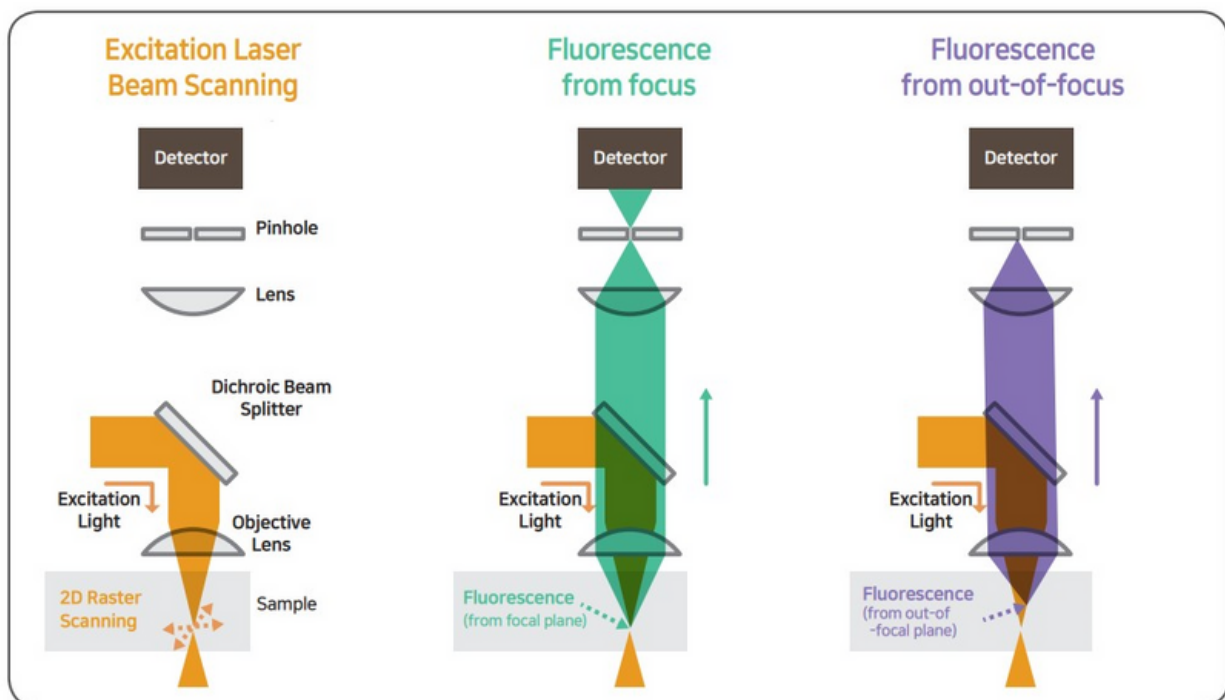
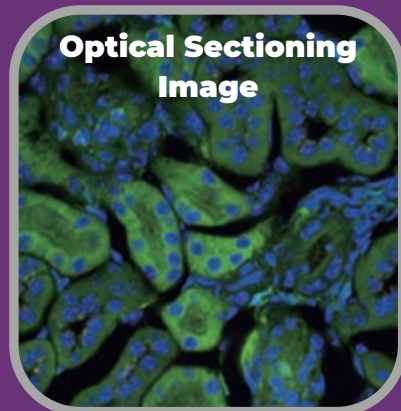
Confocal Microscopy (IVM Technology: IVM-C, CM)

- High-resolution optical sectioning imaging of *in vivo* tissue
 - Rejection of fluorescence signal from out-of-focal volume by confocal pinhole
 - High contrast, low background, high quality *in vivo* imaging
- Ultrafast precise Raster scanning of multi-color excitation laser-beam focus
 - Video-rate imaging of fast cellular-level dynamics in live tissue *in situ*
- Ideal for high-sensitivity multi-color intravital imaging of *in vivo* tissue

Widefield Image



Optical Sectioning Image

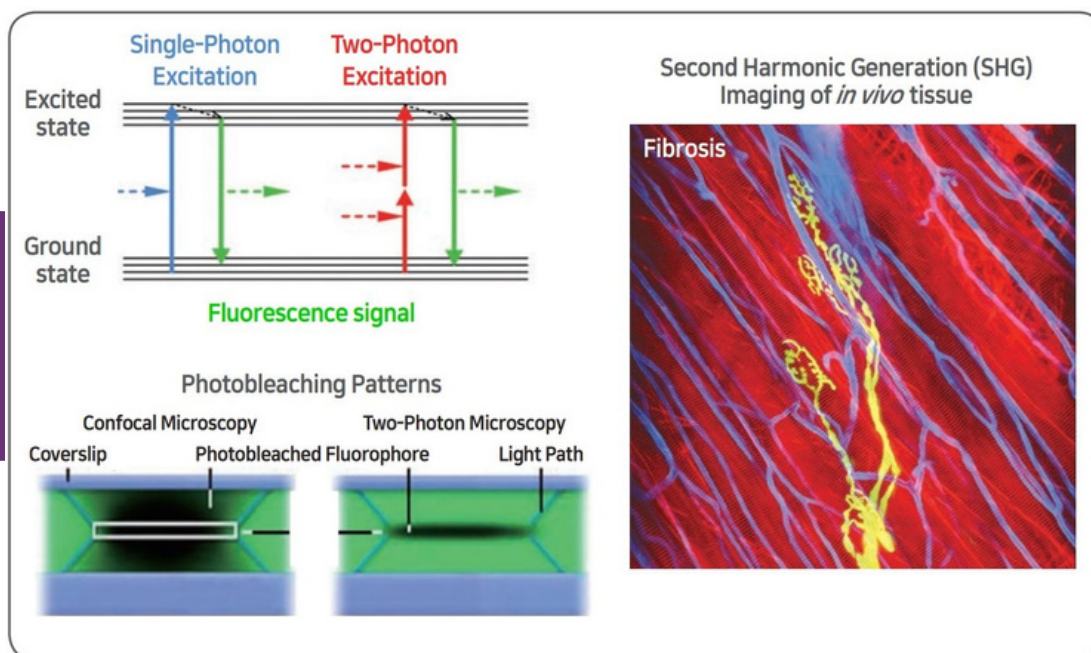


IVIM Technology's Confocal / Two-Photon Technologies

Enable High-resolution *In Vivo* Imaging

Two-Photon Microscopy (IVM Technology: IVM-C, CM)

- High-resolution optical sectioning imaging of *in vivo* tissue
 - Fluorescence signal is intrinsically generated only at the focus
- Deeper tissue imaging with longer-wavelength near-infrared (NIR fs-pulse laser for two-photon/multi photon excitation
- Capable of label-free, non-linear multi-harmonic generation imaging (SHG, THG)
- No photo-bleaching at non-focal plane: reduced phototoxicity
- Ideal for long-term 3D intravital imaging of *in vivo* tissue



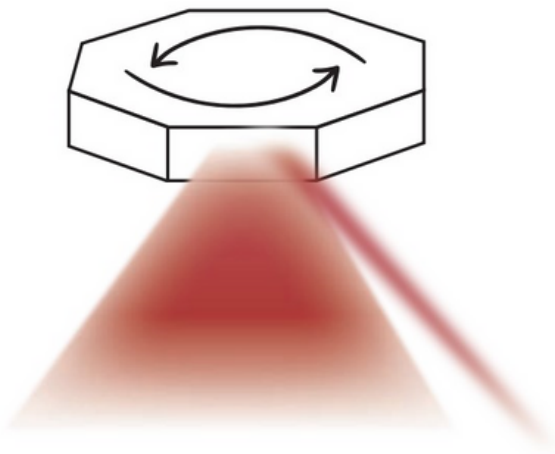
Confocal Microscopy

- Single-photon excitation
- Point scanning + Pinhole
- Optical sectioning:
 - Fluorescence signal from out-of-focus is blocked
- Imaging Depth:
 - 100-200 μm
- Continuous -wave solid-state laser with flexible choice of wavelength at the range from ultraviolet (UV) and visible (VIS) near-infrared (NIR)
- Descanned confocal detector
- Easy & efficient, multi-color 3D intravital imaging

Two-Photon Microscopy

- Two-photon excitation
- Point Scanning + No Pinhole
- Optical sectioning
 - Fluorescence signal is intrinsically generated only at the focus
- Imaging depth:
 - 250-1000 μm
- Femto-second pulsed laser tunable at near-infrared (NIR) wavelength range
- Non-descanned detector (NDD)
- Deeper-tissue 3D intravital imaging

IVIM Technology's Key Advantage 1: Ultrafast Scanning with Uniform Illumination

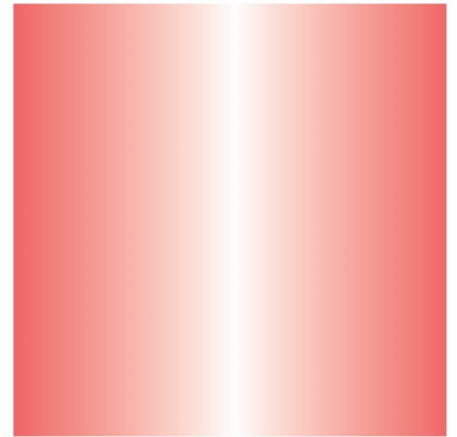


Ultrafast Uniform Laser-beam Scanning

IVIM Technology's Ultrafast
Polygon Scanning

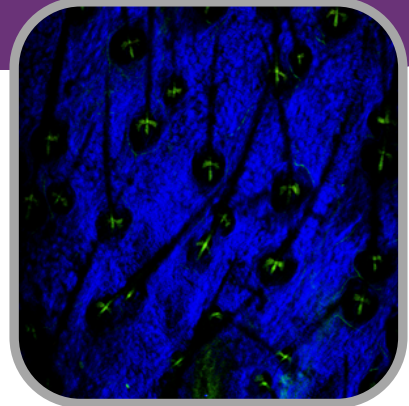
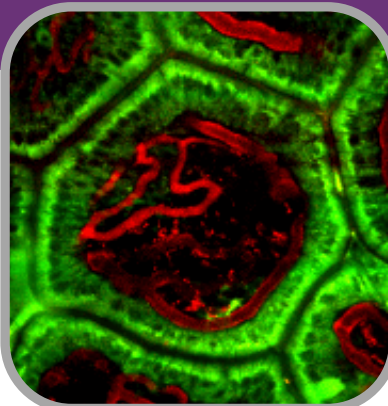
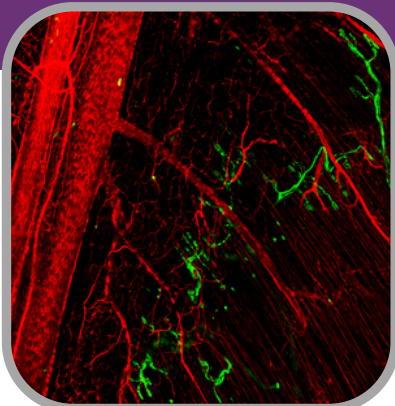


Conventional Resonant
Galvo Scanning



IVIM Technology's All-in-One intravital microscopy system is equipped with ultrafast rotating polygonal mirror scanner

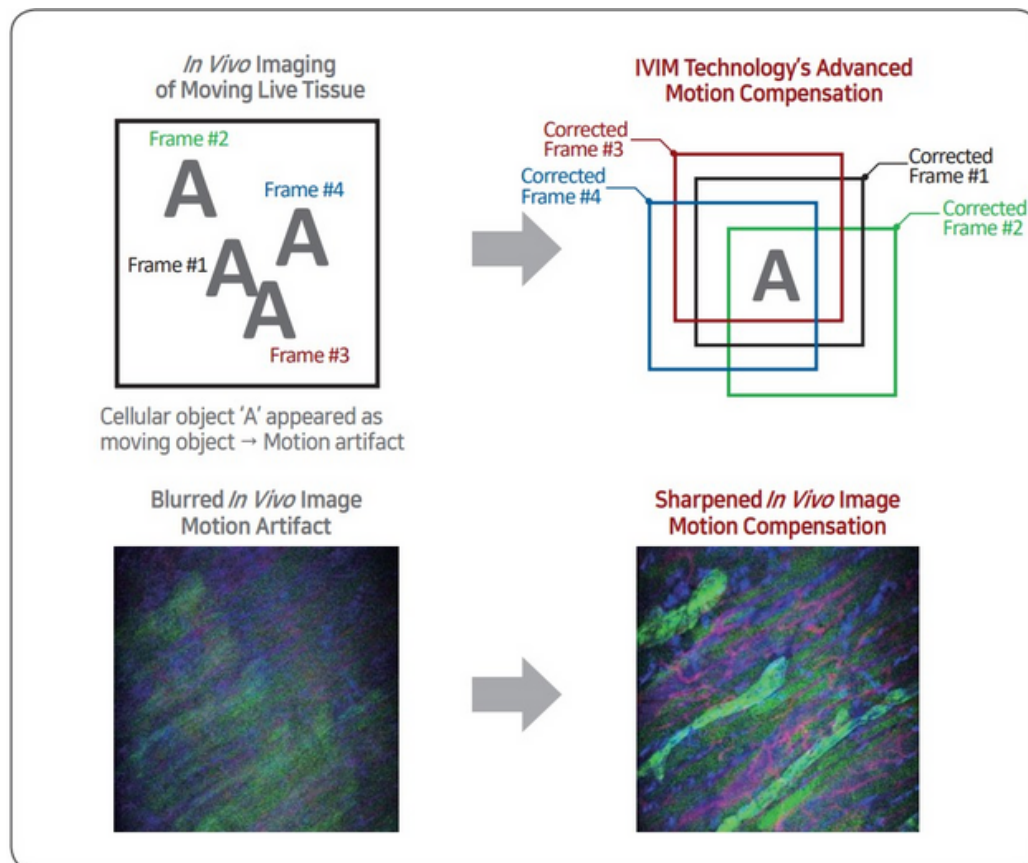
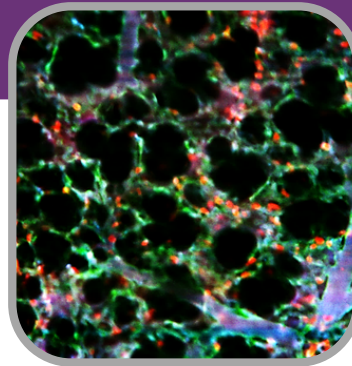
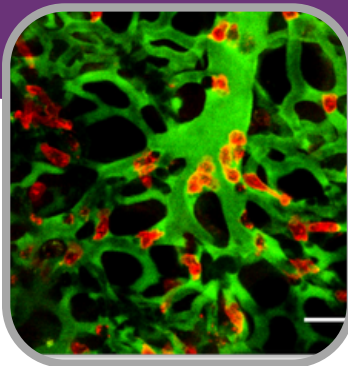
- Enables ultra-high speed *in vivo* imaging (max. 100 fps @ 512x512 pixels)
- Achieves uniform excitation illumination over entire imaging field of view (FOV)
 - No reduced fluorescence signal and signal-to-noise ratio (SNR) at center area of FOV
 - No excessive photobleaching at edge area of FOV
 - Uniform high signal-to-noise ratio over entire FOV
 - Improved image quality without wasting excessive photons



IVIM Technology's Key Advantage 2: Animal Motion Compensation

IVIM Technology - Integrated Motion Artifact Compensation

- Automatic hassle-free high-precision motion compensation
- Immediate acquisition of motion-compensated imaging results by GPU-assisted parallel computing for acceleration of algorithm processing
- Synergistic effect with ultrafast intravital imaging
- Guarantee optimal results in wide-range of spatiotemporal tissue movement from slowly moving tissues (e.g. abdominal organs such as liver, kidney, spleen) to rapidly moving tissues (e.g. thoracic organs such as heart, lung)



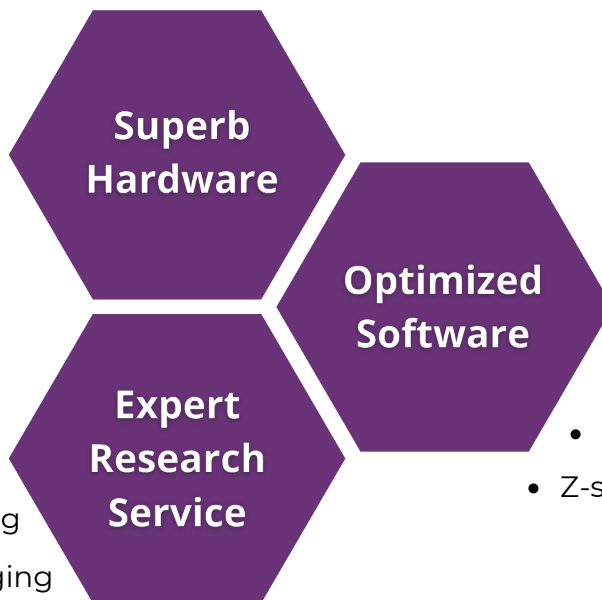
IVIM Technology's Key Advantage 3: All-in-One Packaged Platform

Intravital Microscopy IVM-C/M/CM/MS

- Inhalation anesthesia
- IMaging chamber & holder
- *In vivo* animal stage

Expert Solution for Intravital Imaging

- Consulting for intravital imaging
- Optimization for intravital imaging
- Modeling & intravital imaging
- Data processing



IVM Engine & Viewer

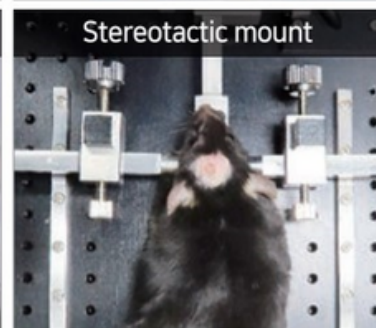
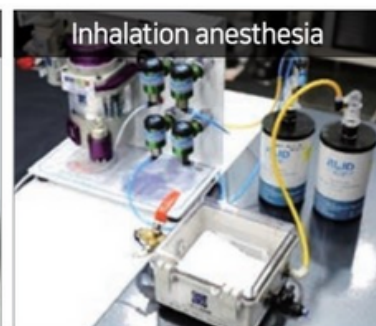
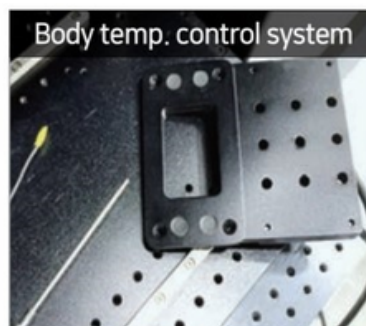
- Animal Motion Compensation
- Z-stack / Mosaic / Multi-position

Hardware

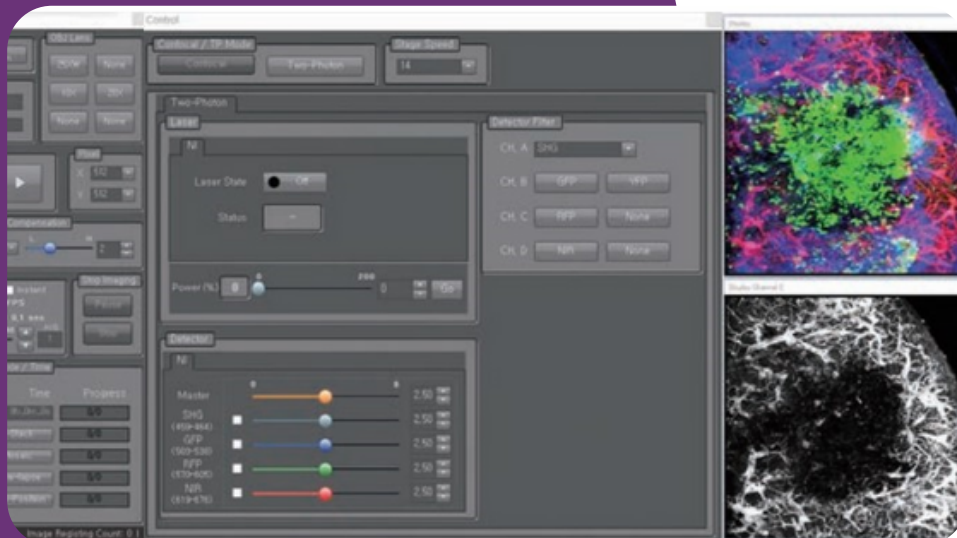
All-in-One Intravital Microscopy



Accessories for Intravital Imaging



IVM Engine



IVM Viewer



Research Service

Step
1

Consulting for
In Vivo live Cell Imaging

Sample Demonstration &
Test Imaging

Optimization of
Experimental Plan

Step
2

Creating Animal Models
of
Various Human Diseases

IVM Imaging
of Various Organs
in Mouse Model

Image Processing
& Analysis
in Cellular Level

IVIM Technology's Product Lines:

- IVM-C (Confocal)
- IVM-M (Two-Photon)
- IVM-CM (Confocal & Two-Photon)
- IVM-MS 9Two-Photon Smart Ver.)
- Research Service - Intravital Imaging



Key features of Intravital Microscopy (IVM)

- Superb performance and unique functionality for *in vivo* imaging of living animal
 - Ultra High-speed Imaging (max 100 fps-512x512 pixels)
 - 4D Animal Motion Compensation (X, Y, Z & Time)
 - Automatic, Hassle-free, GPU-accelerated processing

World's 1st All-in-One Packaged IntraVital Microscopy for live animal model

- Single box-type IVM System
- Fully integrated for *in vivo* imaging
- *In vivo* maintenance unit / *in vivo* animal stage
 - Monitoring & homeostatic regulation of animal vitality
- 4-color simultaneous imaging (confocal / Two-Photon Mode)

IVIM Technology's All-in-One IntraVital Microscopy system has been verified to be used without any restriction on various organs

IVIM Technology's Product Lines:

IVM-C (Confocal)

IVM-C is the All-in-One IntraVital Confocal Microscopy System, optimized for *in vivo* imaging experiments. Especially, because it is equipped with 4 different wavelength lasers and 4 high-sensitivity confocal detectors, IVM-C is the optimal system to observe highly diverse, dynamic multi-cellular behaviors in live animals simultaneously with 4 different fluorescence colors.

- Easy & highly efficient multi-color simultaneous imaging
- High flexibility in selection of laser / detector wavelength
- Customizable design for modifications according to the customer's requests and accommodations for future updates
- Applicability for conventional *ex vivo*, *in vitro* and *in vivo* imaging

IVM-M (Two-Photon)

IVM-C is the All-in-One IntraVital Confocal Microscopy System, optimized for *in vivo* imaging experiments. Especially, because it is equipped with 4 different wavelength lasers and 4 high-sensitivity confocal detectors, IVM-C is the optimal system to observe highly diverse, dynamic multi-cellular behaviors in live animals simultaneously with 4 different fluorescence colors.

- Easy & highly efficient multi-color simultaneous imaging
- High flexibility in selection of laser / detector wavelength
- Customizable design for modifications according to the customer's requests and accommodations for future updates
- Applicability for conventional *ex vivo*, *in vitro* and *in vivo* imaging

IVIM Technology's Product Lines:

IVM-CM (Confocal & Two-Photon)

IVM-CM is the All-in-One IntraVital Confocal / Two-Photon Microscopy System, optimized for *in vivo* imaging experiments. Especially, because both of the confocal and two-photon microscopes are integrated into a single-box packaged system, IVM-CM provides the ultimate versatile functionality of the IVM-C and IVM-M in one system.

- Dual-mode IVM-C (Confocal) / IVM-M (Two-Photon) imaging capability
- One-click automated transition between confocal and two-photon imaging mode

IVM-MS (Two-Photon Smart Ver.)

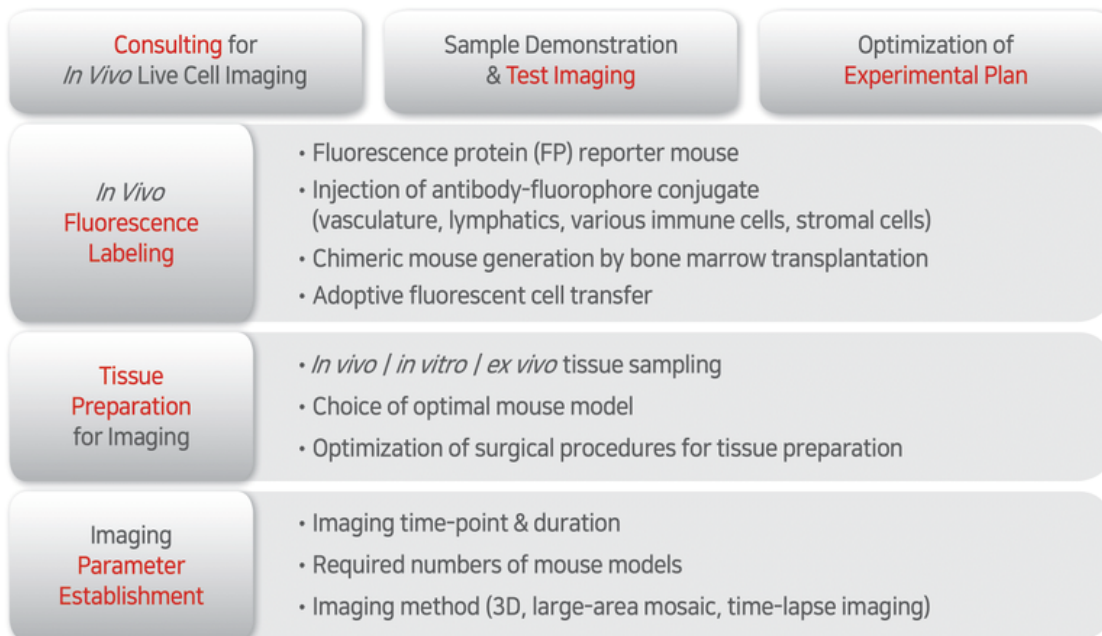
IVM-MS is the All-in-One IntraVital Two-Photon Microscopy System, optimized for *in vivo* imaging experiments and equipped with a new compact high-efficiency fs-pulse laser module. Especially, because it integrates a compact high-stability maintenance-free fs-pulse laser into a single box, the IVM-MS is the ideal solution for customers in need of a two-photon microscope with limited resources of space and budget.

- Less expensive, fully-automated fs-laser system
- Space saving with no additional fs-laser box configuration
- Simple hand-free turn-key operation of the fs-laser for two-photon excitation
- Easy maintenance and management without liquid cooling system requirements

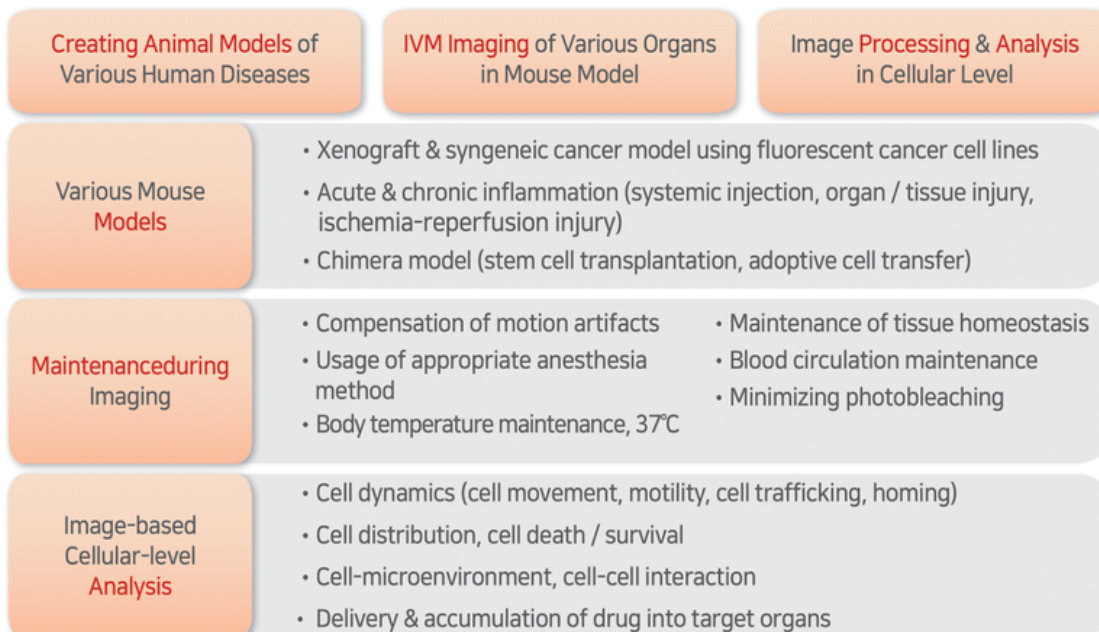
IVIM Technology's Product Lines:

Research Service - Intravital Imaging

Step1 : Consulting, Testing & Planning



Step 2 : Intravital Imaging & Analysis



IVIM Technology's Product Lines:

Research Service - Intravital Imaging

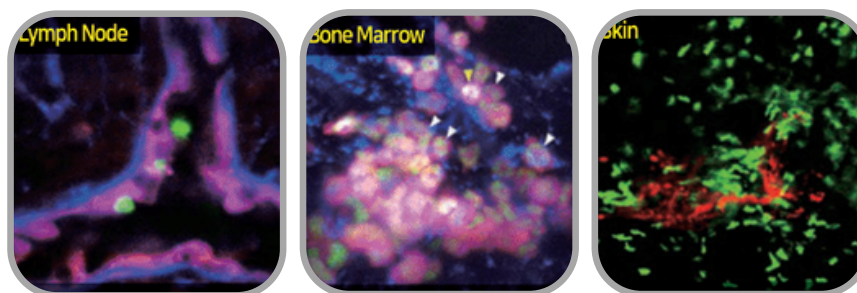
Key Research Service

In vivo 4D cell imaging, tracking & interaction monitoring

- *In vivo* visualization of dynamic molecular & cellular mechanisms
- *In vivo* imaging analysis of novel drug compound efficacy & action
- *In vivo* imaging of drug delivery to target tissues & cells
- *In vivo* imaging of various organs in mouse model (liver, lymph node, spleen, skin, retina, lung, brain, colon, pancreas, small intestine, prostate, kidney, heart, trachea, esophagus, bone marrow, thymus, etc.)

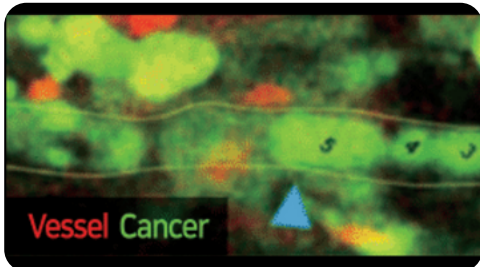
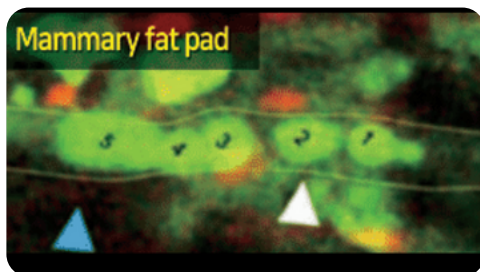
Live Cell Intravital Imaging

- *In vivo* cell dynamics of migration, interaction, activation, etc.
- *In vivo* tie-lapse, multi-position, mosaic, z-stack imaging

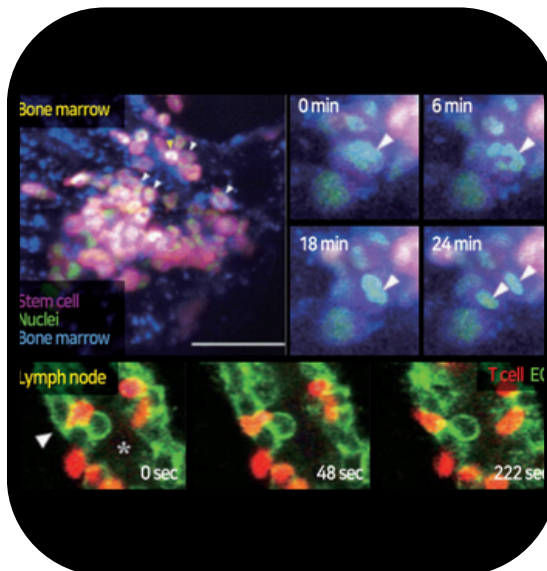


In Vivo Drug Efficacy Monitoring

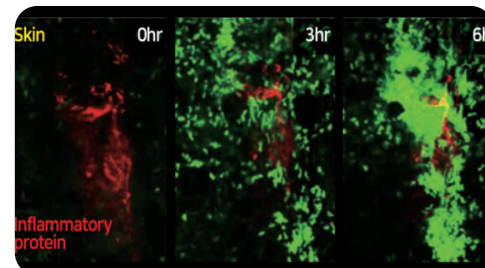
Tumor Metastasis



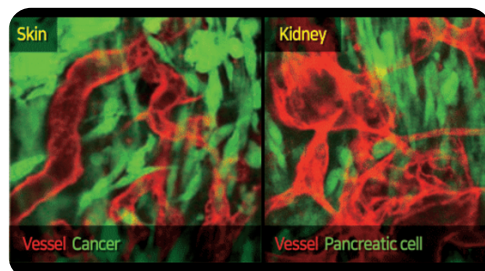
Proliferation & migration



immune cell recruitment



Angiogenesis



IVIM Technology's Key Application 1:

In Vivo 4D Dynamic Live Cell Imaging

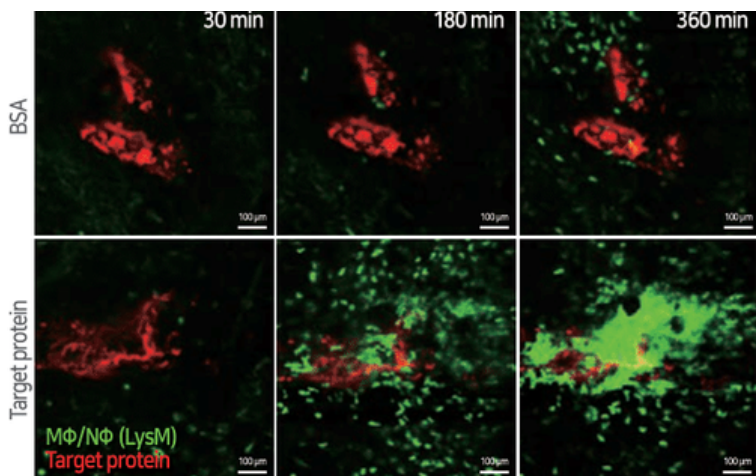
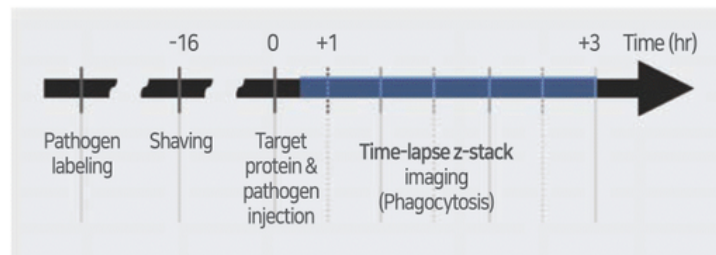
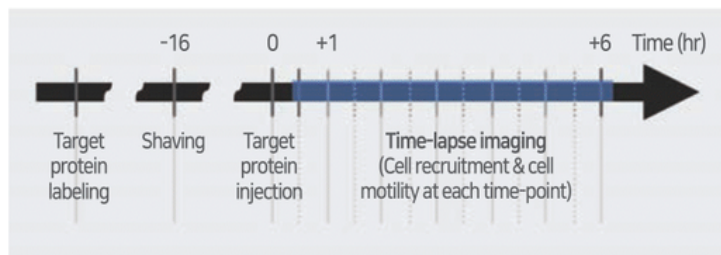
Drug Target Identification & Validation (Anti-Inflammation)

Imaging Target & Imaging Method

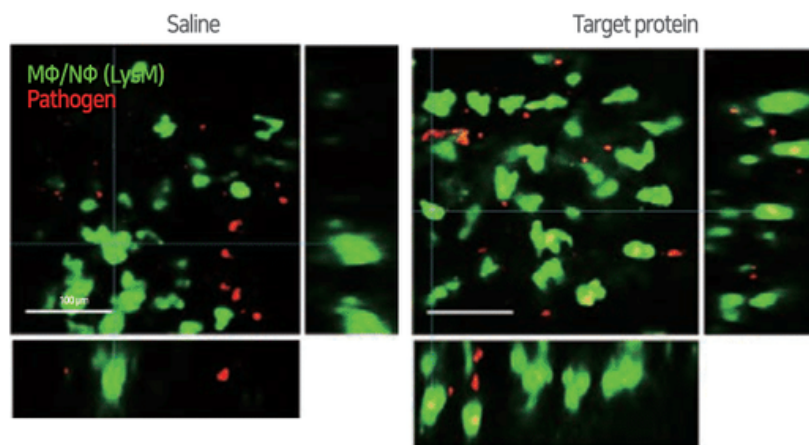
- Objective: Identification & validation of anti-inflammatory drug target
 - Monitoring of pro-inflammatory effect of target protein
 - Monitoring of pro-/anti-inflammatory effect of target overexpression & inhibition
- Quantification: Changes in Immune Cellular Dynamics
 - Immune cell recruitment & motility : Immune cell number & movement
 - Phagocytic efficacy : Colocalization of fluorescent signal of immune cell & pathogen
- Imaging Method: Intravital time-lapse, z-stack imaging o animal Skin

Fluorescent Labeling

Transgenic mice : Intrinsic fluorescent protein (GFP) in immune cells



Journal of Cell Biology, 216(7):2201-2216 (2017)

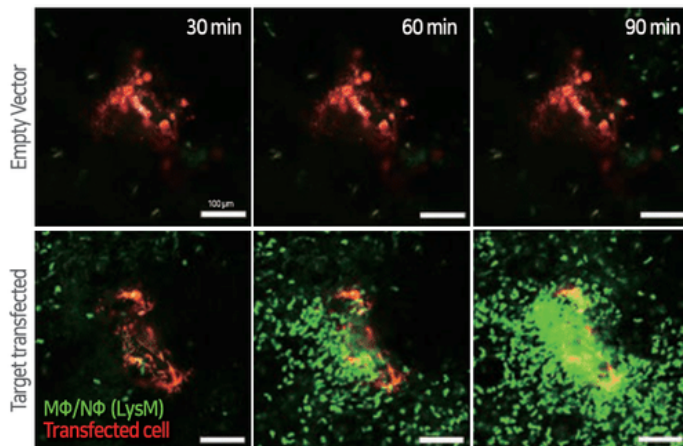
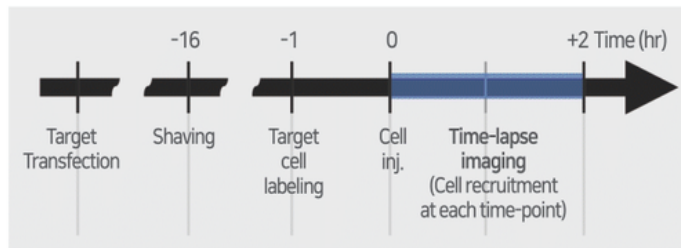


Nature Microbiology, 2:16191 (2016)

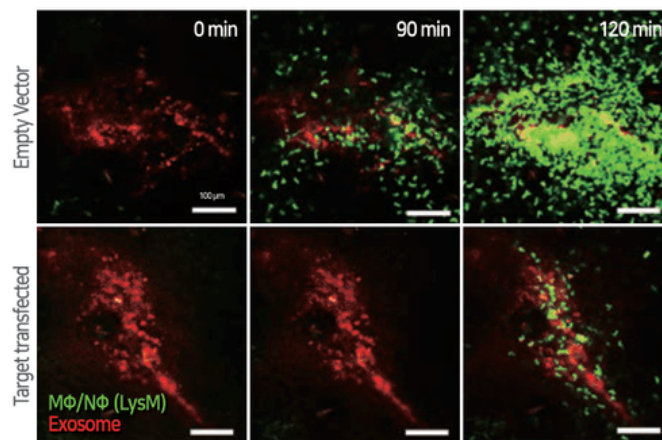
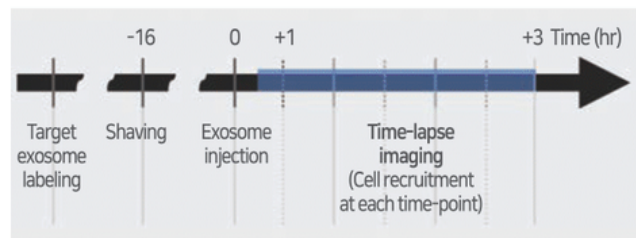
IVIM Technology's Key Application 1:

In Vivo 4D Dynamic Live Cell Imaging

Drug Target Identification & Validation (Anti-Inflammation)

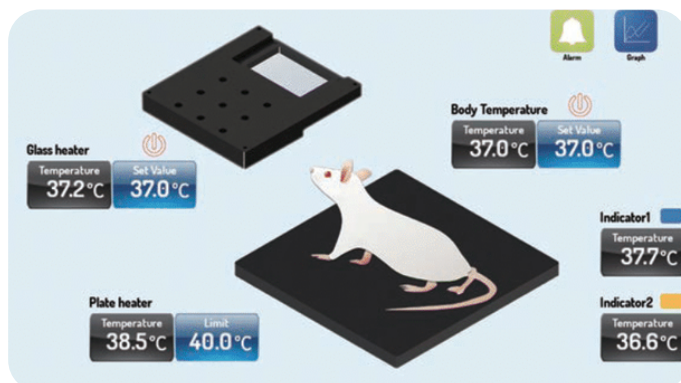
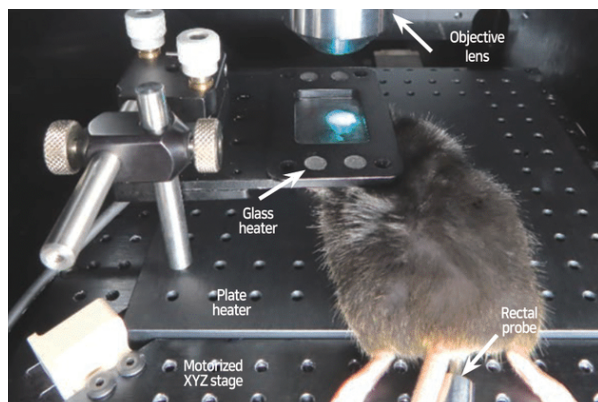


Journal of Cell Biology, 216(7):2201-2216 (2017)



Journal of Cell Biology, 216(7):2201-2216 (2017)

Recommended Solutions



Imaging System

IVM-C

Imaging Tool

In Vivo animal stage

4 Channel body and tissue temperature control system

- User-friendly S/W
- Temperature control with feedback from rectal probe
- Glass heater & plate heater
- 2 temperature indicators for monitoring tissue temperature

IVIM Technology's Key Application 2:

Repetitive Intravital Imaging

Drug Efficacy Monitoring (Anti-cancer Drug)

Imaging Target & Imaging Method

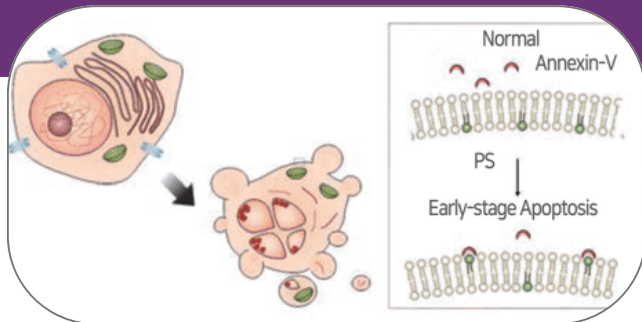
Objective : Validation of anti-cancer lead compounds

Quantification 1 : Changes in tumor cell apoptosis

- Apoptotic cell numbers: The number of Annexin-V+ cells / Total

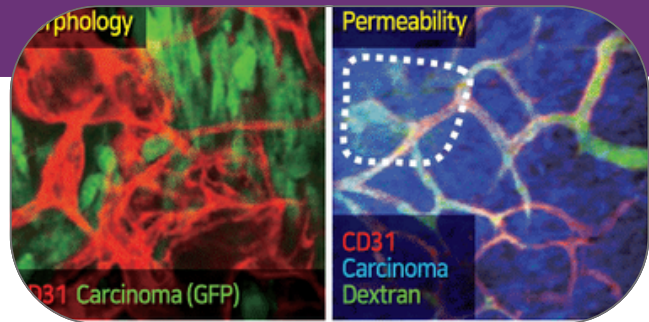
Quantification 2 : Changes in tumor vessel characteristics, vessel normalization

- Vessel dilation : Average vessel diameter
- Vessel density : Area ratio, CD31+ vessel area / Total area
- Vessel permeability : Area ratio, Dye leak area / Tissue area



Characteristic of apoptotic cells

- Cell shrinking, chromatin condensation, nuclear fragmentation
- Loss of membrane integrity (exposure of phosphatidylserine)
- Membrane blebbing, shedding of apoptotic bodies



Characteristic of tumor vessels

- Abnormal vascular morphology
- Increase in vessel diameter (vessel dilation)
- Increased vascular permeability

Imaging Method:

- Repetitive intravital imaging of identical site
- Z-stack, mosaic imaging of tumor cells or vasculatures

Fluorescent Labeling

Cancer cell labeling:

- Intrinsic fluorescent protein (GFP/RFP) expressing stable cancer cell line
- FP expression in cancer cells: Nuclei (H2B) only, or cytosol & nuclei of cells

Vessel (vascular endothelial cell) labeling:

- Intravenous injection of anti-CD31 antibody conjugated with fluorescent dye

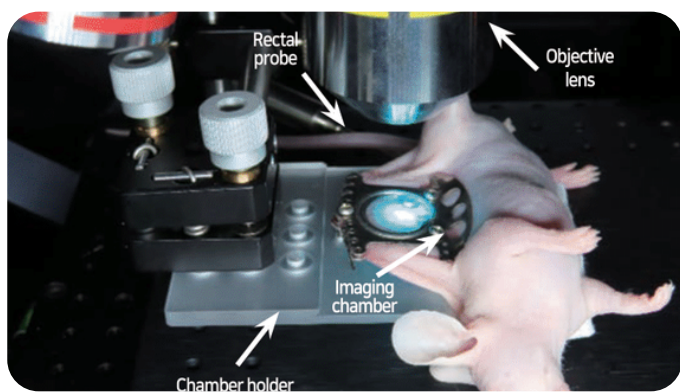
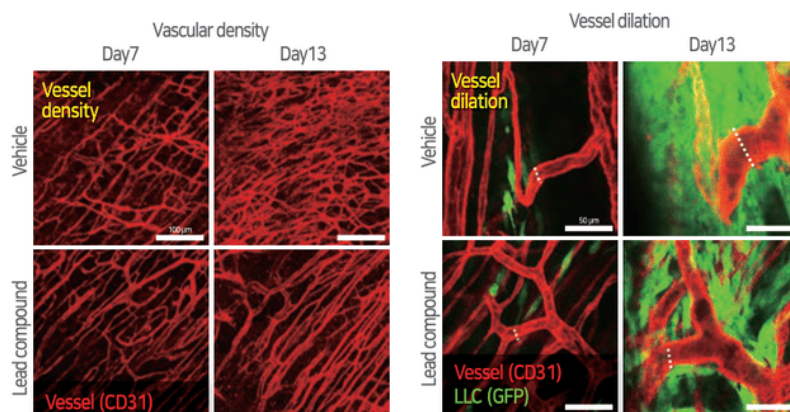
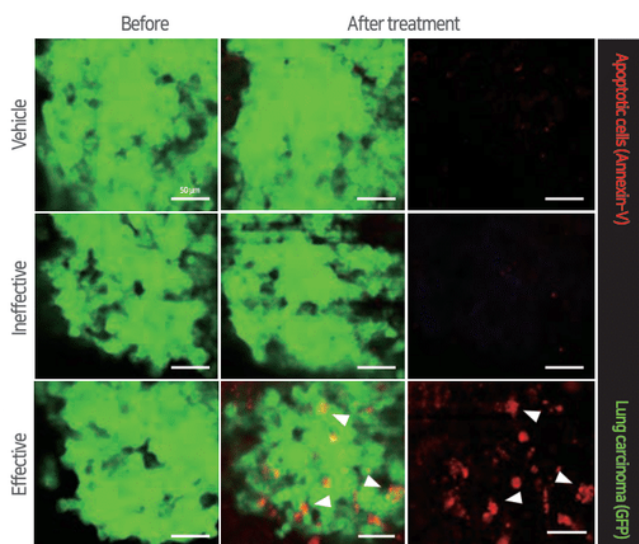
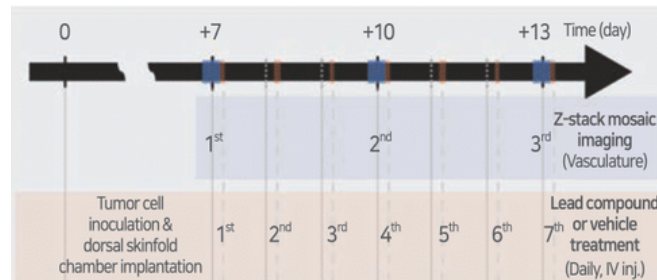
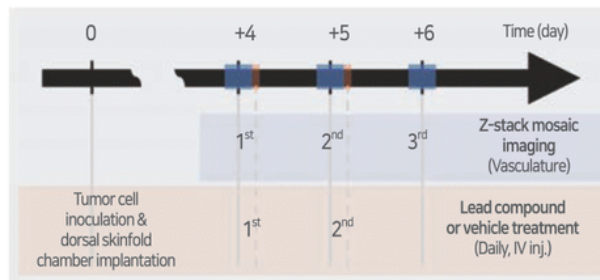
Apoptotic cell labeling:

- Intravenous injection of Annexin-V conjugated with fluorescent dye

IVIM Technology's Key Application 2:

Repetitive Intravital Imaging

Drug Efficacy Monitoring (Anti-cancer Drug)



Imaging System

IVM-C

Imaging Tool

In Vivo animal stage

4 Channel body and tissue temperature control system

Imaging chamber & holder

Dorsal skinfold chambers for each imaging organ (tumor, surrounding microenvironment)

IVIM Technology's Key Application 3:

Cellular-level imaging of various tissues (Brain)

Optimized for intravital imaging with *In Vivo* / *Ex Vivo*

Identification & validation of drug targets

Objective : Validation of anti-cancer lead compounds

Quantification: Immune cell distribution & target uptake in brain tissue

- Target cell numbers in parenchyma, vessel
- Target cell motility changes before and after treatment
- Colocalization of immune cell & target material

Fluorescent Labeling

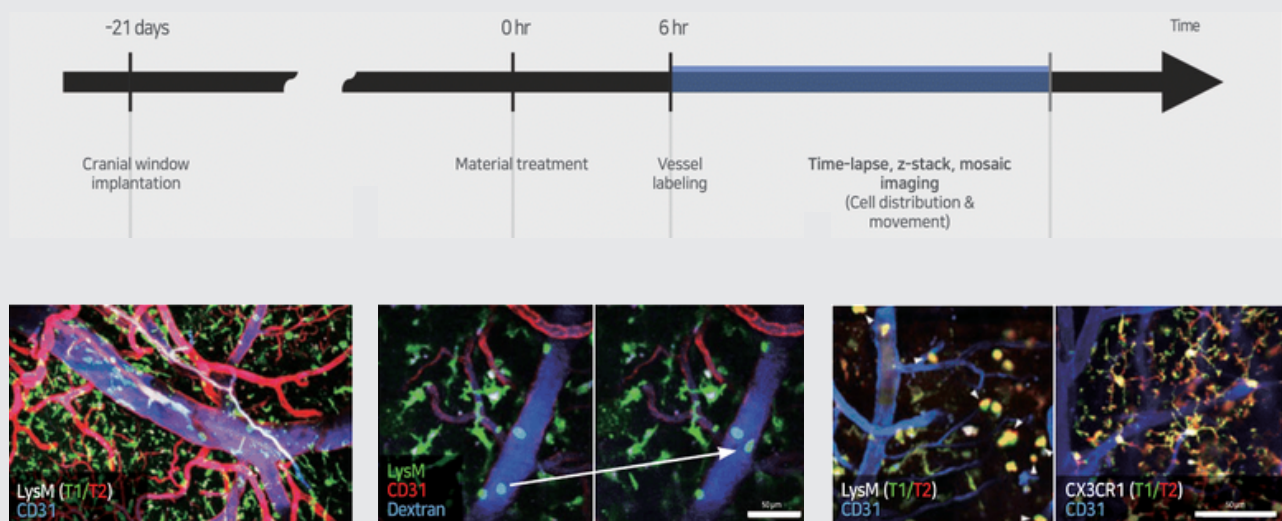
Transgenic mice : Intrinsic fluorescent protein (GFP) in immune cells

Vessel (vascular endothelial cell) labeling :

- Intravenous injection of anti-CD31 antibody conjugated with fluorescent dye
- Dextran conjugated fluorescent dye

Imaging Method:

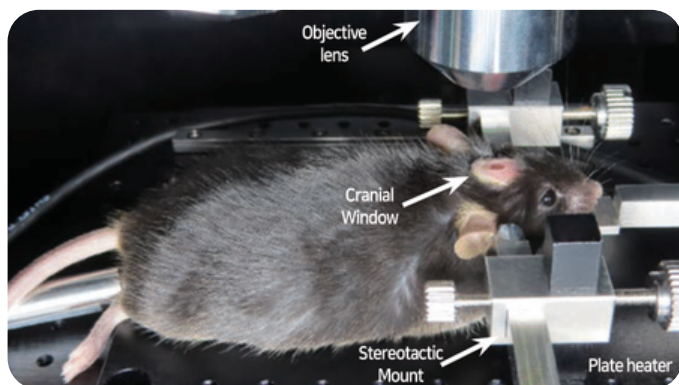
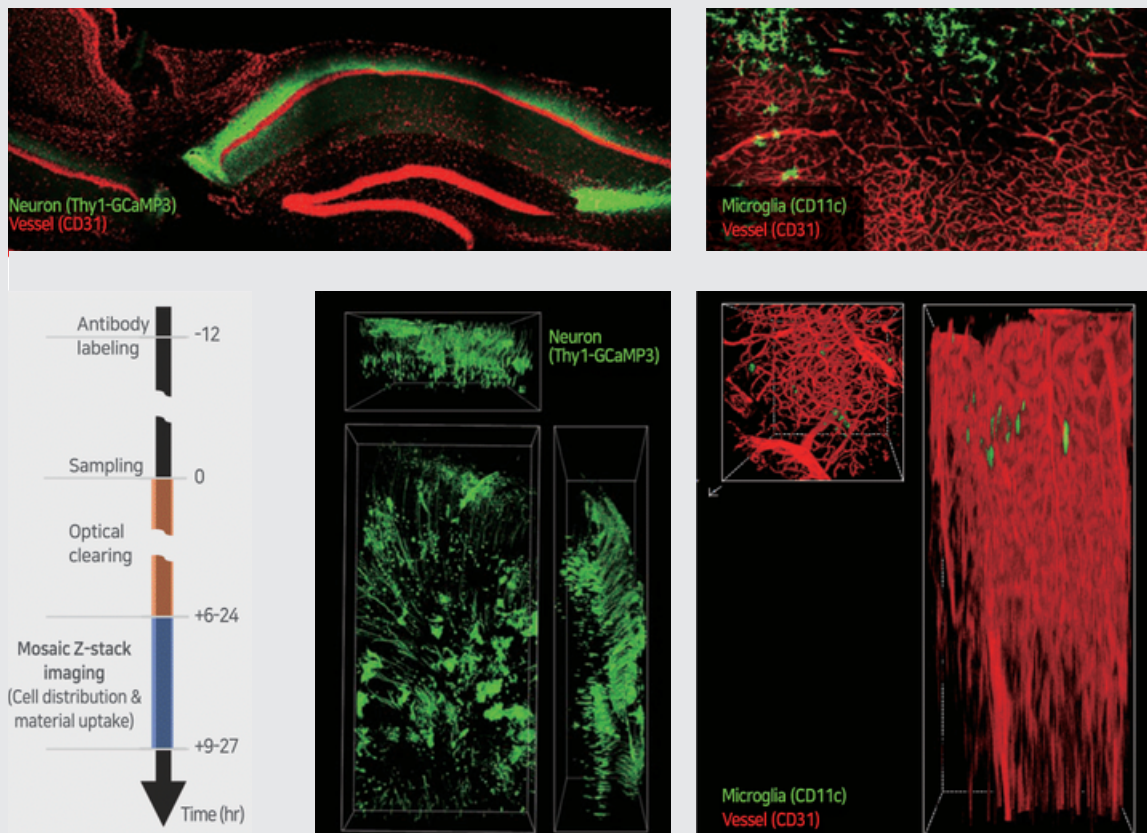
- Repetitive intravital imaging of identical site
- Z-stack, mosaic imaging of tumor cells or vasculatures



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Imaging System

IVM-C

Imaging Tool

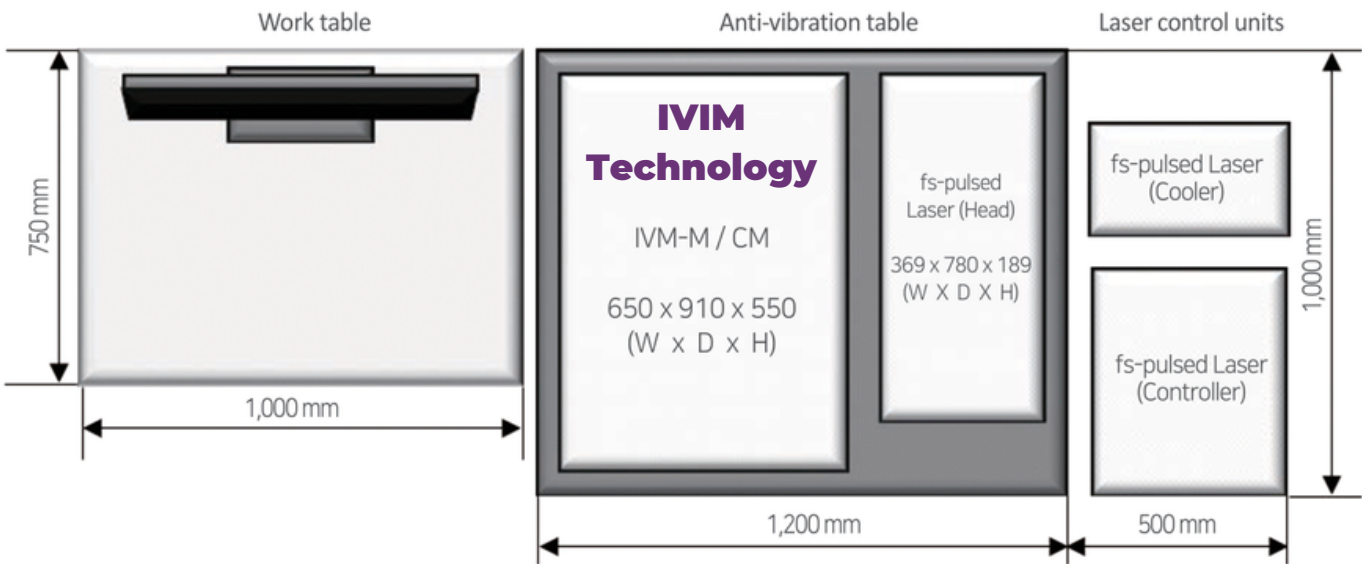
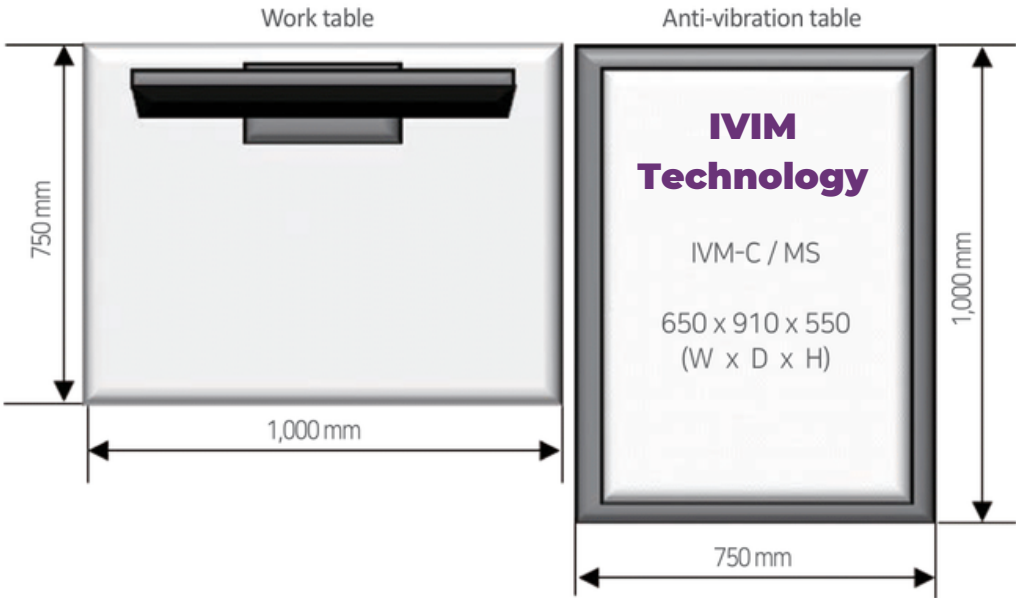
In Vivo animal stage

4 Channel body and tissue temperature control system

Imaging chamber & holder

- Cranial window for brain imaging
- Stereotactic mount

Specifications



Specifications

IVM Series (IVM-)

Laser	Confocal Laser Unit	<ul style="list-style-type: none"> Max. 4 laser unit (405, 420, 445, 473, 488, 505, 514, 532, 561, 633, 642, 660, 685, 705 730, 785 nm)
	Tunable Two-Photon Laser Unit	<ul style="list-style-type: none"> Ti: Sapphire laser Wavelength : 690-1050 nm, Pulse width < 75 fs, Rep. rate: 80 MHz Avg. power > 2.5 W, Dispersion compensation: 0 to -49,000 fs²
	Compact Two-Photon Laser Unit	<ul style="list-style-type: none"> Air cooled fs-fiber laser system Wavelength : 920 nm, Pulse width < 100 fs, Rep. rate: 80 MHz Avg. power > 1 W, Dispersion compensation: 0 to -30,000 fs
Fluorescence Detector	Confocal Detector	<ul style="list-style-type: none"> Wavelength: 185 - 900 nm (DAPI, CFP, GFP, YFP, RFP, Cy5, Cy5.5, etc.) 4 Ultra-broadband high SNR PMTs (UV to Near IR, Ultra High Sensitivity, Low Dark Current) 25-2000 μm variable pinhole (16 steps)
	Two-Photon Detector	<ul style="list-style-type: none"> Wavelength: 185 - 760 nm (DAPI, CFP, GFP, YFP, RFP, Cy5, Cy5.5, etc.) 4 High quantum efficiency PMTs (UV to Near IR, Ultra High Sensitivity, Low Dark Current)
	Variable Emission Filter (Optional)	<ul style="list-style-type: none"> 6 or 2 emission filters can be mounted on each of four detectors
Scanner Imaging Head	Scanner	<ul style="list-style-type: none"> Polygonal mirror (Fast axis scanning, Max. 66 kHz) Galvano scanner (Slow axis scanning, Max. 200 μs/step)
	Objectives	<ul style="list-style-type: none"> Max. 6 objectives are mountable on motorized turret (1X - 100X) Compatible for commercial objectives (RMS or M25)
	FOV	<ul style="list-style-type: none"> 100 x 100 μm² to 10 x 10 mm²
Image	Pixel Resolution	<ul style="list-style-type: none"> Max. 2,048 x 2,048 pixels
	Imaging Speed	<ul style="list-style-type: none"> 30 fps @ 512 x 512 pixels (Max. 100 fps), 15 fps @ 1,024 x 1,024 pixels (Max. 50 fps)
	3D Stage	<ul style="list-style-type: none"> Travel Range: 50,000 x 50,000 x 75,000 μm (XYZ) Micromanipulation (Max. 0.2 μm resolution)
Sample Stage	Specimen Holder	<ul style="list-style-type: none"> Flexible-design universal specimen holder can be mounted <ul style="list-style-type: none"> U-shape window bracket for imaging inner organs (optional) Homeothermic warming system with plate heater and body temperature probe (optional) Small animal inhalation anesthesia system (optional) Long term imaging holders for transplanted window chamber <ul style="list-style-type: none"> Stereotactic mount for cranial window Ring-type window holder for abdominal imaging window
		<ul style="list-style-type: none"> A single glass slide or culture dishes
Control Unit	Joy Dial	<ul style="list-style-type: none"> 3-axis independent control of stage position & translation speed Motorized turret control for objective lens change
Motion Correction	4-D <i>In Vivo</i> Imaging Motion Compensation	<ul style="list-style-type: none"> XY motion compensation : Averaged image acquisition with motion artifact compensation Z motion compensation : Image-based sample Z position adjustment for long-term intravital microscopic imaging & sample tracking (Feedback-loop automatic stage control) T motion compensation : Image-based image XY position adjustment for long-term intravital microscopic imaging & sample tracking (Feedback-loop automatic stage control) Combination of above three compensation for 4D in vivo motion compensation
	Image Display	<ul style="list-style-type: none"> Independent 4 single channel display (RGBA channel) Overlay channel display (Selection among RGBA channel)
Image Adjustment	<i>In Vivo</i> Image Acquisition	<ul style="list-style-type: none"> Averaged image with motion artifact compensation Continuous real-time video recording and display
	<i>In Vivo</i> Imaging Mode	<ul style="list-style-type: none"> Mosaic imaging (XY), Z-stack imaging (Z), Time-lapse imaging (T) Time-lapse imaging at Multi-position (T-M) Time-lapse & Z-stack imaging (TZ) Time-lapse & Z-stack imaging at Multi-position (TZ- M)
	Image Adjustment	<ul style="list-style-type: none"> Contrast / Brightness control, Histogram / Level adjustment Pseudo color setting, Channel splitting, Color mapping Zoom, Cut, Crop, Rotation, Invert, Annotation



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