

Your Vision, Our Future

CONFOCAL LASER SCANNING BIOLOGICAL MICROSCOPES

FV300 FLUOVIEW





FV300 CONFOCAL LASER SCANNING MICROSCC PERFORMANCE FOR THE PERSONAL USER

The FV300 is the ideal choice of laser scanning microscopes for personal users. Its optical system is fully integrated, from scanner to microscope, and not only delivers outstanding optical sectioning, but also ensures the easy, flexible expandability required for any future upgrade.

With its wide choice of options and configurations, including the Olympus inverted, upright and fixed-stage upright microscope platforms, the FV300 offers excellent versatility as well as top-class laser scanning performance.

- Highest image quality (12 bit, 2048x2048 pixel resolution) with economical cost
- · Easy operation, with user-friendly software
- Simultaneous capture of 2 fluorescence and 1 transmitted light detector images
- Capable of the most demanding tasks, with a direct and efficient optical system
- Optical system chromatically corrects aberrations from UV to NIR (near infrared red) spectrum
- Fiber illumination system separates fluorescence and transmitted light sources from the microscope body for improved temperature stability











- Optical fiber for laser introduction
- 2 Beam collimator3 Polarizer
- Dichromatic mirror
- **5** Excitation dichromatic mirror
- A Y galvanometer mirror scanners
 Pupil lens
 Collector Lens
 Pinhole turret

- Emission beam splitter slider
- Barrier filter slider
 Photo multiplier

Software Graphical User Interface

Ultimate ease of operation and monitor display.

Dye selection display

When a fluorescence dye is chosen, the laser and light path settings are selected automatically, with each of the selected fluorescence dyes displayed graphically on the monitor.



Innovative scanning method for improved performance





ZoomIn scanning

Zoom scanning can be conducted over any designated rectangular area.

Since only the region of the targeted, zoomed-in area is acquired, scan time and laser exposure of the specimen is minimized.



Point scanning

The ultimate in fast scanning, the point scan enables accurate quantitation of intensity changes during rapid physiological events.



Line scanning

A single line may be scanned, oriented at any angle in the XY plane. This fast scanning

quantitation of physiological events such as Calcium waves or sparks.

Versatile display options

Exchange between condensed and full image display modes can be performed with a single touch. Individual panel layouts can be changed at will, and the panel in use can be placed in any desired position.

Simplified toolbar

A newly designed toolbar with various dedicated buttons has greatly improved ease of use. The user can execute a succession of selected processes with one-click operation for each.

Flexible setting of scanning size, zoom, movement and rotation

The observation field and scanning area are both displayed graphically. Settings can be confirmed while scrolling through the zoom ratios. The "pan" button lets the operator move the image acquisition area at will, and rotation scanning of images is also possible.





Free line scanning

Intensity changes may be measured over a given period of time along the length of a

freely drawn line, such as the trace of an axon or along a cellular junction.

Clip scanning

By cropping the image, selected areas can be cut out of complex image stacks.





Preset the conditions for image acquisition and loading

Storage of Acquisition Settings enables immediate, one-touch recall of all the relevant experimental settings and conditions. Adding new conditions or altering existing ones is guick and easy.



AOTF: flexible control of the laser intensity to meet the specific demands (optional)

The laser exposure will be limited within the scanning area by default, minimizing unnecessary bleaching of the specimen. Option includes:

• Any laser intensity for any excitation area ("Region of Excitation" mode)

• Multiple laser applications

•AOTF controller that provides easy link with external equipment



Easy exchange between display methods

Independent navigation bars for each image window enable the display method to be changed quickly and easily.

Image tool bar

X-Y-Z scanning operations and timelapse observations both produce multiple images, which can be displayed in sequence simply by clicking the sequential mode button. Channel selection and image zooming are also available on the same menu.

Thumbnail display

Data stored in the gallery window are displayed as thumbnails for easy viewing.



Tiling display function for see-at-aglance comparison of multiple images

The FV300's live tiling function, which is especially valuable in time course experiments, allows observations of multiple images or changes in the specimen while the experiment is in progress. Images in series (e.g. XYt or XYZ) can be freely displayed.

Sequential scanning to prevent cross talk

Sequential scanning may be used to minimize the fluorescence cross talk often seen between channels in multicolored samples. This is achieved by exciting each fluorochrome independently, one dye at a time. With the AOTF function, line sequential scanning is available as well.

* Once optimized: steps 1-4 can be performed easily

Excited by only HeNe633







4 Composition

Human colon crypt





Scanning unit set-up monitor display





Actin (Green) Alexa 488 APC gene product (Red) Alexa 568 Christine Anderson, Laboratory of Prof. Ray White, Hunstsman Cancer Institute, Utah

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Time Course

Using different scanning modes to chart time-lapse changes efficiently.



High-speed (4 frames/sec) image acquisition

For the high speed observation of the sample, Fluoview is capable of scanning 4 frames per second in a fast scanning mode at an image size of 512X512. By limiting the image size, the frame rate will be even faster. This scanning mode is suitable for living cell observation.

Versatile line scanning modes have many uses

The wide variety of the line scanning modes (linear/slant/free-line) enables flexible analysis of rapid time-lapse experiments.

Superior slice patching system

In combination with the unique fixed stage & nosepiece focusing BX61WI microscope, the FV300 provides a highly effective system for slice patching. This unique set-up has a small footprint for increased room in a space-limited cage. The remote control microscope options minimize the danger of accidentally touching the delicate experimental settings. Olympus also offers ideal non-cover glass long working distance water immersion objectives and an optional XY translation stage that moves the entire confocal microscope system while the sample and other experimental hardware remains in a fixed position.

Highly precise time-lapse analysis

Fluoview's wide dynamic range of 12-bit or 4096 grey levels provides enough sensitivity to detect even the slightest changes in intensity. The user can designate multiple regions of interest (ROI) by using drawing tools. The fluorescence intensity or the ratio can be analyzed with the intuitive GUI driven program.



Calcium wave in Xenopus oocyte, Calcium Green staining, fluorescence pseudo-colored fluorescence image after injection of inositol 3-trisphospate Japan Science and Technology Corporation, Exploratory Research for Advanced Technology, Mikoshiba cell control project, Prof. Aya Muto





Calcium wave in isolated cardiac myocyte Dr. Sandor Gyorke Texas Technical University

Calcium sparks in isolated cardiac myocyte Dr. Sandor Gyorke Texas Technical University



Patch clamp



Image acquisition



ROI designation



Intensity versus time measurement

Immersion-type LUMPLFL objectives

The 40X water immersion objective in this series has a 3.3mm working distance and an extremely fine tip which is suitable for micromanipulation using a fixed stage upright microscope. It has a large N.A. (0.8) and is also ideal for confocal observations. When using the BX61WI fixed stage & nosepiece focusing upright microscope with water immersion objectives, confocal imaging can be used to monitor time-lapse fluorescence changes in thick specimens such as brain slices.



Long working distance, non-cover glass water immersion objective

C

FRET

Hardware and software support to optimize the environment for FRET.



Ratio changes when cameleon is manifested on the HeLa cell and stimulated by histamine then inhibited by cyproheptadine.

Cameleon genes provided by Dr. Miyawaki Atsushi in Brain Research Institute. Equipment: FV300 and HeCd laser Time period : 4 seconds.



CFP Fluorescence wavelength 485nm



YFP Fluorescence wavelength 530nm



CFP/YFP FRET

Calcium ion concentration in a live HeLa cell using a cameleon (split type) indicator. Energy transfer between CFP and YFP is proportional to bound calcium. The time series shows the increase of calcium ion density caused by stimulation of histamine and the effect of blocking by proheputajin.

The 440nm diode laser can be added for CFP/YFP FRET imaging.

A 440nm diode laser is optionally available for CFP/YFP imaging. The 440nm laser line ideally excites CFP, with minimal disturbance to YFP, and is therefore suitable for CFP/YFP FRET experiments. The high performance LSM objectives, PLAPO40XWLSM and PLAPO60XWLSM, are precisely corrected in this wavelength range, and ensure the highest measuring reliability.

*For simultaneous observation of CFP and YFP, 440nm and 515nm laser lines are required.

Ratio imaging to analyze 2-wavelength images

Using time course software, the ratio image can be continuously displayed in pseudocolor. At the same time, the intensity of each channel can be monitored graphically. The analysis process is presented as an intuitive flow chart. (optional time course software: TIEMPO)

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Input/output of external trigger signal

The optional time course software gives control over the input/output trigger signal by GUI. It is suitable for combined experiments such as those involving patch clamping.

PAPP for FRAP Application

PAPP: Programmable Acquisition Protocol Processor Easy, reliable flow of experiments for fluorescence recovery after photobleaching.

AOTF and PAPP function for effective FRAP (Fluorescence Recovery After Photobleaching)

Fluorescence recovery after photobleaching can be analyzed on any designated area by means of the AOTF-equipped laser combiner. During the processes of photobleaching and recovery, the PAPP function enables time scales to be freely and easily programmed to suit different experiment purposes.



Mouse; hippocampal neurons; fluorescence of GFP

Living neurons expressing GFP were maintained in culture and fluorescent images were obtained. Subsequently, FRAP analysis was performed on the same cell to determine the diffusion rate of GFP proteins into the dendritic spines. Rapid fluorescence recovery (within seconds) was observed. Shigeo Okabe

Department of Anatomy and Cell Biology Tokyo Medical and Dental University



New PAPP (Programmable Acquisition Protocol Processor) makes it easy to program a wide range of experiments

Using the new PAPP function, which is included in standard software, the experiment protocol is created by describing the individual steps or phases within the experiment. Users can specify detailed conditions and parameters for each step. This function enables users to construct complex experimental protocols with minimal effort. PAPP is suitable, for example, for FRAP experiments that require more flexibility.



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Multi-point time lapse system

High-magnification multi-point time lapse observation of living cells.



Introduction of DsRed2 expression vector into brain of 14th day mouse embryo through womb electroporation. Taking a specimen slice from the 15th day embryo and observing the living nerve stem cell and nerve cells subsequently born from it.

Image courtesy of: Dr. Kazunori Nakajima Dr. Hidenori Tabata School of Medicine, Keio University



Using multi-point software*, it is possible to acquire an XYt, an XYZ or and XYZt image series at multiple positions through automated software control of the motorized XY stage.

Wide-ranging specimen observations for improved experiment throughput

Use of a motorized XY stage allows the analysis of time lapse changes in many points scattered over a wide area. The system is therefore effective for work with thick specimens, such as observing changes in the states and movements of stem cells using a brain slice, or analyzing expression mechanisms at the individual level in an embryo. In wide-ranging tasks such as analyzing cell functions using GFP, the system provides many different kinds of data at the same time, enabling a higher overall level of experiment efficiency even in long-lasting observations. In addition, using separate chambers at the same time makes it possible to perform different experiments at the same time. These are just some of the ways in which this system dramatically improves the throughput of experiments requiring long-duration observations.



Features

1. Measure up to 254 points

A variety of scan conditions can be set for each point, such as XYZ coordinates, the Z-axis acquisition range and the detector sensitivity.

2. Up to 5 X 5 adjacent fields of view registered automatically

Since adjacent fields of view are registered automatically, it is possible to broaden any given field while maintaining a high magnification level.

3. High-precision XY stage scanning

A wide area can be observed with highly precise position reproduction. Errors from repetition are not accumulated. (High-precision XY stage complies exclusively with the "PROSCAN" model from PRIOR Scientific)

4. Flexible scanning conditions

The system combines scan flexibility with time lapse imaging for monitoring changes in the specimen over time.

3D Imaging

Using multiple 3D images to obtain accurate 3D structure analysis.



Easy Z axis operation and setting

The upper and lower limit of Z scanning can be specified interactively by actually scanning the sample or by direct input of the numerical value.



Acquire X-Y-Z images and display X-Y cross-sectional images quickly and continuously in increments of 0.01*µm

Thanks to the precision driving mechanism that enables 0.01µm step control within the BX61, BX61WI and IX81 motorized microscopes, high-quality continuous cross-sectional images can be acquired. The 3D function also provides extended focus projections, red/green stereo views, topographic projections and 3D animations for exploring the structure of the sample. Multi-plane images can be created from an XYZ image series, enabling easy measurement and observation of horizontal and vertical cross sections. Other useful procedures include 3D image cropping, series animation and simple volume measurement.

 * 0.025 μm is the smallest increment for other microscope combinations.









Topographic projection Height of 3D structure indicated by color.

Colocalization

Analyzing the degree of intensity overlap between channels.



Physiolink

Analyzing the state of a cell interior by synchronizing electrophysiological and confocal image data. (Optional software).



Link with patch clamping data

- With PCs linked through a LAN, Physiolink can synchronize electrophysiological and confocal image data simultaneously.
- With the same time stamp recorded in the two PCs, it is possible to access and analyze an image and its patch clamping data corresponding to the Physiolink software time scale.
- The patch clamping graph and Physiolink software are interlocked and activated concurrently.
- Physiolink software complies with the FV300's high-speed scanning, enabling msec analysis.



Connecting two PCs by LAN is required.

Dr. Stuart Thompson, Department of Biological Sciences, Hopkins Marine Station, Stanford University.

Pacemaker neuron: Sea-slug (nudibranch)

Applications Gallery





Mouse hippocampal neurons GFP: postsynaptic density protein Rhodamine-phalloidin: actin Hippocampal neurons expressing a GFP-tagged postsynaptic density protein were fixed and stained with rhodamine-phalloidin to visualize the localization of cytoplasmic actin filaments. In dendrites, actin filaments are concentrated in the postsynaptic sites. *Shigeo Okabe*

Department of Anatomy and Cell Biology Tokyo Medical and Dental University



Lucifer Yellow: retina ganglion cell TexasRed: dopamine-operated amacrine cell Prof. Shigetada Nakanishi Dept. of Biological Sciences, Kyoto Univ. Faculty of Medicine



Lucifer yellow injected visual interneurons of swallowtail butterfly

Extended focus is used for every 100µm on 383µm Z-range image and displayed by overlapping pseudo colors Mituyo Kinoshita, Pr. Kentaro Arikawa Laboratory of Neuroethology, Graduate School of Integrated Science, Yokohama City University



Purkinje cell in the rat cerebellum FITC: vesicular GABA transporter VGAT Cy3: vesicular glutamate transporter VGLUT1 Pr. Masahiko Watanabe Department of Anatomy, Hokkaido University School of Medicine





Structure of PtK2 cell Nucleus: DAPI (Blue) Actin: FITC (Green) Mitochondria: Mito Tracker (Red) Microtubules: Cy5 (White)



Rat tongue taste bud DAPI: Nuclei FITC: TrkB, high-affinity receptor for brain-derived neurotrophic factor Texas Red: Protein Gene Products Pr. Shigeru Takami Department of Anatomy, School of Health Science, Kyorin University



Human Colon Crypt Alexa 488 and To-Pro 3 Christine Anderson, Prof. Ray White's Laboratory, Huntsman Cancer Institute, U. Utah

Fluorescent Proteins



C elegans expressing beta-integrin fused to GFP Dr. Xioping Xhu and Dr. John Plenefisch University of Toledo, Dept. of Biology



GFP-labeling of Drosophila adult brain with staining of mushroom bodies Assistant Prof. Aigaki Cytogenetics Tokyo Metropolitan University, Science Dept.



GFP-labeling of Drosophila adults



Expression of DsRed in a zebrafish embryo Extended focus image of 5µmx30 slice Pr. Yasuhiro Kamei, Pr. Shunsuke Yuba Institute for Molecular and Cellular Biology Osaka University



Coexpression of EGFP and DsRed in a zebrafish embryo Extended focus image of 10µmx28 slice Pr. Yasuhiro Kamei, Pr. Shunsuke Yuba Institute for Molecular and Cellular Biology Osaka University





Apoptosis of Tabacco hybrid plant cells Dr. Wataru Marubashi Laboratory of Plant Breeding and Cell Engineering, School of Agriculture,Ibaraki University



Isolated Zinnia mesophyll cells Keisuke Obara Pr. Hiroo Fukuda Department of Biological Sciences, Graduate School of Science, The University of Tokyo

Fluorescence Dyes and Filters



Specifications

	Item	Specifications					
Laser light source	Visible light laser source	Select from the following laser, to mounted on laser combiner Multi-line Ar laser (458nm, 488nm, 515nm, Total 40mW), Ar laser (488nm,10mW), Kr laser (568nm, 10mW), HeNe (G) laser (543nm,1mW), HeNe (R) laser (633nm,10mW), LD405 (405nm, 25mW), LD440 (440nm, 5.3mW)					
	Laser combiner	Each laser light path is equipped with a continuously variable neutral density filter or AOTF All laser lines are combined to apsis along the same fiber optic					
Scanning unit	Scanning method	Galvanometer mirror scanners (both X and Y)					
	Field number	20 (10 with use of LD405 laser)					
	Pinhole	5-position pinhole turret					
	Image memory and scanning speed	Standard scanning mode: 256 x 256 (0.45s) - 2048 x 2048 (10.835s) Bi-directional high-speed scanning mode: 512 x 512 (0.25s) (Simultaneous scanning of up to 2 channels)					
	Image channel	Selectable from 2-channel (fluorescence) or 2-channel (fluorescence) + 1-channel (transmitted light) 3-channel (fluorescence) using virtual channel					
	Selection of filters according to staining	Manual selection					
	Scanning modes	XY, XYZ, XYT, XYZT, XZ, XT, XZT, point, Line-t, free line-t, line-z, free line-z, Clip, ZoomIn					
	Image depth resolution	12-bit (=4096 grey levels)					
	Zoom	1X-10X					
	Z-drive	Step motor/Minimum step 10nm (BX61, BX61WI and IX81 combination), 25nm (other microscope combination)					
Microscopes	Upright	BX51, BX61, BX51WI, BX61WI					
	Inverted (special laser safe frame)	IX81FVSF, IX71FVSF (side port)					
External trans-	Transmitted light illumination unit	External halogen light source connected to microscope via fiber cable					
mitted light unit	Transmitted light detector	External detector unit with built-in photomultiplier Connected to microscope frame via fiber cable					
Fluorescence illumination unit		Connect to external mercury light source and microscope via fiber cable Standard equipment of FV300-BX51, FV-300-BX61, FV300-BX51WI, FV300-BX61WI					
PC with system control boards		PC-AT compatible machine/OS: Windows XP (English version)/ 1GB memory (can be expanded to a maximum of 4GB) CPU: Pentium 4, over 2.8GHz, Special I/F board/image capture: PCI bus Graphic board: G450_Dual 32MB Hard disk: 80GB 7200rpm_ID (ATA100) with DVD-ROM Monitor: Two 19" LCD monitors are recommended, each able to display 1280x1024 images in full color (16.77 million colors) LAN: On board					
Fluoview application	Image acquisition	Scanning condition setting: image size, scanning speed, zoom, panning etc. Real-time image calculation: Kalman filtering, peak integration,					
software	Hardware control	Laser, scanning unit, microscope					
	Image display	Each image display: Single-channel side-by- side, merge, cropping, tiling, series (Z/T) pass and continuous LUT: Individual color setting, pseudo-color, Overlay: Lines, text, scale bar, etc					
	Image processing	Individual filter: Average, Low-pass, High-pass, Sobel, Median, Prewitt, 2D Laplacian, edge enhancement etc. Calculations: Inter-image, mathematical and logical, DIC back ground leveling					
	Image analysis	Overview of fluorescence intensity within an area, histogram, perimeter measurement for user-assigned area, time-lapse measurement, etc.					
	3D visualization	3D animation, left / right stereo pairs, red / green stereoscopic images and cross section					
	Others	Graphic-based help, PAPP (Programmable Acquisition Protocol Processor), time course software (optional), trigger IN/OUT function (optional), Multi point software (optional)					
Power consumption		Microscope (115V 6A/230V 3A), scanning unit+PSU (115V 3.5A/230V 2A), computer & monitor (115V 4.5A/230V 10A), Ar laser (115V 10A/230V 5A), Mult-linei Ar laser (115V 10A/230V 5A), Kr laser (230V 20A), HeNe laser each (115V 0.4A/230V 0.2A), LD laser (405nm, 440nm: 100V 0.9A/230V 0.5A)					

Objectives for BX and IX (using U-UCD8, IX-LWUCDA and U-DICTS)

Description	NA	W.D	Cover glass thickness	Immersion	Correction ring	Condenser for BX U-UCD8A optical element	Condenser for IX IX-LWUCDA optical element	U-DICTS position
UPLSAPO 4X	0.16	13	_					
UPLSAPO 10X	0.40	3.1	0.17			U-DIC10	IX2-DIC10	normal
UPLAPO 10XO3	0.40	0.24	0.17	Oil		U-DIC10	IX2-DIC10	normal
UPLAPO 10XW3	0.40	0.43	0.17	Water		U-DIC10	IX2-DIC10	normal
UPLSAPO 20X	0.75	0.6	0.17			U-DIC20	IX2-DIC20	normal
UPLAPO 20XO3	0.80	0.19	_	Oil		U-DIC20	IX2-DIC20	normal
UPLSAPO 40X	0.90	0.2	0.11-0.23		0	U-DIC40	IX2-DIC40	normal
UPLFLN 40XO	1.30	0.2	0.17	Oil		U-DIC40	IX2-DIC40	normal
PLAPON 60XO	1.42	0.15	0.17	Oil		U-DIC60	IX2-DIC60	BFP1
UPLSAPO 60XO	1.35	0.15	0.17	Oil		U-DIC60	X2-DIC60	normal
UPLSAPO 60XW	1.20	0.28	0.15-0.2	Water	0	U-DIC60	X2-DIC60	normal
UPLSAPO 100XO	1.40	0.12	0.17	Oil		U-DIC100	X2-DIC100	normal

Objectives for fixed stage upright microscopes (using WI-UCD, WI-DICTHRA)

-		-		
Objective	N.A.	W.D.	DIC prism	Revolving nosepiece
MPL5X	0.10	19.60	—	WI-SSNP, WI-SRE2
UMPLFL10XW	0.30	3.30	U-LDPW10H	WI-SSNP, WI-SRE2
UMPLFL20XW	0.50	3.30	U-LDPW20H	WI-SSNP, WI-SRE2
LUMPLFL40XW	0.80	3.30	U-LDPW40H	WI-SSNP, WI-SRE2
LUMPLFL60XW	0.90	2.00	U-LDPW60H	WI-SSNP, WI-SRE2
LUMPLFL40XW/IR	0.80	3.30	U-LDPW40H	WI-SSNP, WI-SRE2
LUMPLFL60XW/IR	0.90	2.00	U-LDPW60H	WI-SSNP, WI-SRE2
LUMPLFL100XW	1.00	1.50	U-LDPW60H	WI-SSNP, WI-SRE2
XLUMPLFL20XW	0.95*	2.00	U-LDPXLU20 HR	WI-SNPXLU

* Note: These conditions are not met in confocal microscopy

500

(unit: mm)

FV300-IX dimensions

1310 Ê 1130 1200 2330 Depth: 990

370 1130 1200 2330

Different types of laser combiners

Selectable from ND filter or AOTF combiner. The shutters and light intensity can be controlled via the Fluoview computer. * Laser combiner for AOTF is required for multi-line Argon laser.



Laser combiner with Ar+HeNe (Red) / (Green) lasers Laser combiner with Multi Ar+HeNe (Red) / (Green) lasers •Installation stand is not included in the unit. * Please consult your Olympus dealer for additional laser combinations.





*Direct fiber connection to scan unit.

External transmitted light detector and fluorescence illumination system



External transmitted light detector system



Fluorescence illumination system Standard configuration for FV300-BX51/FV300-BX61/ FV300-BX51WI/FV300-BX61WI combination



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Specifications are subject to change without any obligation on the part of the manufacturer.



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OLYMPUS CORPORATION Shinjuku-ku, Tokyo, Japan OLYMPUS EUROPA GMBH OLYMPUS AMERICA INC. Two Corporate Center Drive, Metville, NY 11747-3157, U.S.A.
 OLYMPUS SINGAPORE PTE LTD.

 491B River Valley Road, #12-01/04 Valley Point Office Tower, Singapore 248373
 491B River Valley Road, #12-01/04 Valley Point Office Fower OLYMPUS UK LTD. 2-8 Honduras Street, London EC1Y OTX, United Kingdom.

OLYMPUS AUSTRALIA PTY. LTD. Gilby Road, Mt. Waverley, VIC 3149, MelDourne, Ausurana.
 OLYMPUS LATIN AMERICA, INC.
 6100 Blue Lagoon Drive, Suite 390 Miami, FL 33126-2087, U.S.A.

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ISO14001

Certification



(unit: mm)

1310

Depth: 990



LD405 laser unit