A1r/A1 — the ultimate confocal microscope
Capturing high-quality images of cells and molecular events at high speed, Nikon’s superior A1 confocal laser microscope series, with ground breaking technology, enables you to bring your imaging aspirations to life.

**A1 with high performance and A1R with additional high-speed resonant scanner**


**Dynamics**

A high-speed resonant scanner allows imaging of intracellular dynamics at 30 frames per second (fps). Moreover, image acquisition of 230 fps is possible.

**Interaction**

Simultaneous imaging and photo activation with the proprietary hybrid scanner reveal intermolecular interaction. Analysis software for FRAP and FRET is provided as standard.

**Spectrum**

Fast spectral image acquisition for 32 channels at a maximum of 16 fps is possible. New real-time spectral unmixing and the V-filtering functions expand the range of use of spectral images.

**Image Quality**

Fluorescence efficiency is increased by 30 percent, and S/N ratio of images is also increased. With diverse new technologies such as the VMS pinhole unit, superior image-quality has been achieved.
A1R’s hybrid scanner for ultrahigh-speed imaging and photo activation

A1R incorporates two independent galvano scanning systems: high-speed resonant and high-resolution non-resonant. This allows ultrafast imaging and photo activation imaging required to unveil cell dynamics and interaction.

**Ultrahigh-speed imaging**

**World’s fastest 230 fps (512 x 64 pixels)**

A resonant scanner with ultrahigh resonance frequency of 7.8kHz is simultaneously mounted with a non-resonant scanner that is capable of high-resolution (4096 x 4096 pixels) image capture.

<table>
<thead>
<tr>
<th>Method</th>
<th>Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D scanning</td>
<td>10,000fps</td>
</tr>
<tr>
<td>2D scanning</td>
<td>230 fps</td>
</tr>
<tr>
<td>Full frame scanning</td>
<td>30 fps (512 x 512 pixels)</td>
</tr>
</tbody>
</table>

**Stable, high-speed imaging**

The Nikon original optical clock generation method is employed for high-speed imaging with a resonant scanner. Stable clock pulses are generated optically, offering images that have neither flicker nor distortion even at the highest speed.

**High-speed data transfer with fiber-optic communication**

The fiber-optic communication data transfer system can transfer data at a maximum of four giga bps—40 times faster than the conventional method. This allows transfer of image data (512 x 512) in five modes at more than 30 frames per second.

**High-speed imaging of wide field of view**

When a non-resonant scanner is being used for high-speed image acquisition, the field of view of the scanned image is reduced to avoid overheating of the scanner (motor). Resonant scanners do not suffer overheating. Therefore, the field of view of the scanned area is approximately five times larger.

**High-speed photo activation imaging**

**Simultaneous photo activation and imaging**

Simultaneous photo activation and fluorescence imaging is conducted using non-resonant and resonant scanners. Because the resonant scanner can capture images at 30 fps, image acquisition of high-speed biological processes after photo activation is possible.

**Optical path in the A1R**

**Continuous variable hexagonal pinhole (page 12)**

**Low-angle incidence dichroic mirror (page 12)**

**Ultrahigh-speed imaging**

**A1R’s hybrid scanner for ultrahigh-speed imaging and photo activation**

**Dynamics & Interaction**

**What is a hybrid scanner?**

This mechanism allows flexible switching or simultaneous use of two galvano scanners (resonant and non-resonant) with high-speed hyper selector.

**High-speed photo activation imaging**

**Simultaneous photo activation and imaging**

Simultaneous photo activation and fluorescence imaging is conducted using non-resonant and resonant scanners. Because the resonant scanner can capture images at 30 fps, image acquisition of high-speed biological processes after photo activation is possible.

**High-speed imaging of photo activation**

Imaged at video rate (30 fps) while photo activating the point with a 405nm laser.

**Points within the cell and changes of fluorescence intensity**

(from the point laser to the activated point: red, blue, violet)

**What is a hybrid scanner?**

This mechanism allows flexible switching or simultaneous use of two galvano scanners (resonant and non-resonant) with high-speed hyper selector.
Photos courtesy of: Dr. Kenta Saito and Prof. Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University

Imaging laser wavelength: 457nm, Image size: 512 x 512 pixels, 30 fps

Yellow Cameleon 3.60 increases, the CFP fluorescence intensity decreases, and the YFP fluorescence intensity increases.

from the region of interest (ROI). Along with the increase of calcium ion concentration in the cell, the intermolecular FRET efficiency between CFP and YFP within


observed. The (blue) emission of CFP and the (yellow) emission of YFP are shown as green and red channels respectively. The graph displays fluorescence

Image of a zebra fish labeled with four probes

HeLa cells expressing Yellow Cameleon 3.60 were excited with 457nm laser light. After stimulation with histamine, calcium ion concentration dynamics were

Kaede changes fluorescence colors irreversibly from green to red due to fluorescence spectral conversion when it is exposed to light with a spectrum from ultraviolet to violet.

While imaging a HeLa cell expressing Kaede with green and red fluorescence using 457nm and 550nm lasers as excitation lights, Kaede in a ROI is continuously activated with the 457nm laser for photo conversion. The dispersion of Kaede red fluorescence (red by photo conversion) is observed. The horizontal axes of the two graphs indicate time and the vertical axes indicate fluorescence intensity (pixel intensity). The green line and red line in the graph respectively indicate the intensity change of Kaede green and red fluorescence in the ROI.

FRET (Förster Resonance Energy Transfer)

FRET is a physical phenomenon that occurs when there are at least two fluorescent molecules within a range of approximately 10nm. When the emission spectrum of a fluorescent molecule overlaps with the absorption spectrum of another fluorescent molecule and the electric dipole directions of the two molecules correspond, radiationless energy transfer from a donor molecule to an acceptor molecule may occur.

Kaede photo conversion fluorescence protein

Photo conversion protein is a fusion protein of the CFP variant and the PA-GFP variant. When the PA-GFP variant is activated with violet to ultraviolet light, it changes blue fluorescence to green fluorescence due to intermolecular FRET from CFP to PA-GFP.

Photos courtesy of: Dr. Tomoki Matsuda and Prof. Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University

Activation laser wavelength: 405nm, Imaging laser wavelength: 488nm/561nm, Image size: 512 x 512 pixels, 1 fps

While imaging a HeLa cell expressing photo conversion protein with blue and green fluorescence using 457nm laser as excitation light, the PA-GFP subset in an ROI is continuously activated with the 405nm laser for photo conversion. The activated part observed in blue fluorescence (shown in grayscale) and the dispersion of photo converted fluorescence indicated by the green (frame no. 3 to 5) in the images is observed.

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HeLa cells expressing PA-GFP are excited with 488nm laser light. Directly after photo-activation (using 405nm laser light) of a region of interest, green emission (shown in grayscale) by photo-activation of PA-GFP is observed and the subsequent fluorescence of the photo-activated protein is recorded at high speed. Please note that photo-activation (with the 405nm laser) and image acquisition (with the 488nm laser) are performed simultaneously. Both 488 and 561 nm recordings are displayed. Graph shows fluorescence intensity (vertical) versus time (horizontal).

Activation laser wavelength: 405nm, Imaging laser wavelength: 457nm, Image size: 512 x 512 pixels, 1 fps

Kaede photo conversion fluorescence protein

While imaging a HeLa cell expressing Kaede with green and red fluorescence using 457nm and 550nm lasers as excitation lights, Kaede in a ROI is continuously activated with the 457nm laser for photo conversion. The dispersion of Kaede red fluorescence (red by photo conversion) is observed. The horizontal axes of the two graphs indicate time and the vertical axes indicate fluorescence intensity (pixel intensity). The green line and red line in the graph respectively indicate the intensity change of Kaede green and red fluorescence in the ROI.

Four-color imaging

Standard four-channel detector eliminates the necessity of an additional fluorescence detector after purchase and allows easy imaging of a specimen labeled with four probes.

Image of a zebra fish labeled with four probes

NT-JOF

The graph indicates the changes of fluorescence intensity in each ROI. The blue line indicates the changes of fluorescence intensity of the CFP channel. The green line indicates the changes of fluorescence intensity of the PA-GFP channel. The red line indicates the change of fluorescence intensity of the PA-GFP variant.

Ultrahigh-speed imaging and hybrid scanners allow advanced imaging of cell dynamics and molecular interactions.

High-speed imaging (high time resolution imaging) from a video rate of 30 fps (33ms time resolution) to 420 fps (2.4ms time resolution) is possible. In addition, X-t scanning mode enables ultrahigh-speed imaging of dynamics with 64fps time resolution. Simultaneous photo activation during such high-speed imaging is also possible.

Kaede changes fluorescence colors irreversibly from green to red due to fluorescence spectral conversion when it is exposed to light with a spectrum from ultraviolet to violet.

Photo conversion protein

Photo conversion protein is a fusion protein of the CFP variant and the PA-GFP variant. When the PA-GFP variant is activated with violet to ultraviolet light, it changes blue fluorescence to green fluorescence due to intermolecular FRET from CFP to PA-GFP.

The A1R’s high-speed imaging and hybrid scanners allow advanced imaging of cell dynamics and molecular interactions.
**Spectrum**

**Enhanced spectral detector**

Nikon's original spectral performance is even further enhanced in the A1 series, allowing high-speed spectral acquisition with a single scan. In addition, new functions including a V-filtering function are incorporated.

**Fast 32-channel imaging at 16 fps**

New signal processing technology and high-speed AD conversion circuit allow acquisition of a 32-channel spectral image (512 x 512 pixels) in 0.5 second. Moreover, acquisition of 512 x 64 pixels images at 16 frames per second is realized.

**Faster spectral unmixing**

Nikon's original algorithms and high-speed data processing enable fast and accurate unmixing during image acquisition in less than a second. Coupled with high-speed spectral imaging, an image with no crosstalk can be created in real time.

**Simultaneous excitation of four lasers**

Three user-defined laser shields allow simultaneous use of four lasers selected from a maximum of nine colors, enabling broader band spectral imaging.

**V-filtering function freely utilizes 32 channels**

With the V-filtering function, up to four desired spectral ranges can be selected from 32 channels and total intensity of each range is adjusted individually, as if separating colors and controlling four PMTs by using optical filters. It allows acquisition of the desired spectral range, providing flexibility to handle any new fluorescence probes.

**Diffraction Efficiency Enhancement System (DEES)**

With the DEES, unpolarized fluorescence light emitted by the specimen is separated into two polarizing light beams P and S by a polarizing beam splitter. Then, P is converted by a polarization rotator into S, which has higher diffraction efficiency than P, achieving vastly increased overall diffraction efficiency.

**High-efficiency fluorescence transmission technology**

The ends of the fluorescence fibers and detector surfaces use a proprietary anti-reflective coating to reduce signal loss to a minimum, achieving high optical transmission.

**Accurate, reliable spectral data: three correction techniques**

Three correction techniques allow for the acquisition of accurate spectra.

- **Inter-channel sensitivity correction**, which adjusts offset and sensitivity of each channel.
- **Spectral sensitivity correction**, which adjusts diffraction grating spectral efficiency and detector spectral sensitivity, and correction of spectral transmission of optical devices in scanning heads and microscopes.
- **Multi-anode PMT sensitivity correction**

**High-quality spectral data acquisition**

**Optical fiber**

The wavelength resolution is independent of pinhole diameter.

**DEES system**

High diffraction efficiency is achieved by matching the polarization direction of light entering a grating to the polarizing light beam S.

**Polarization rotator**

Wavelength resolution can be varied between 2.5/6/10nm with three gratings. Each position is precisely controlled for high wavelength reproducibility.

**Multiple gratings**

Wavelength resolution can be varied between 2.5/6/10nm with three gratings. Each position is precisely controlled for high wavelength reproducibility.

**32-channel detector**

A precisely corrected 32-PMT array detector is used. A three-mobile-shielding mechanism allows simultaneous excitation by up to four lasers.

**Pre-correction**

**Post-correction**

**Characteristics of grating**

![Diffraction efficiency (%)](image)

**Diffraction efficiency (%)**

![High-efficiency fluorescence transmission technology](image)

**Diffraction Efficiency Enhancement System (DEES)**

With the DEES, unpolarized fluorescence light emitted by the specimen is separated into two polarizing light beams P and S by a polarizing beam splitter. Then, P is converted by a polarization rotator into S, which has higher diffraction efficiency than P, achieving vastly increased overall diffraction efficiency.

**Diffraction efficiency (nm)**

![High-quality spectral data acquisition](image)

**High-quality spectral data acquisition**

**Brightfield**

**Specimen: HeLa cell**

**Fluorescence reagent: anti-tubulin/Alexa488 (microtubule), Histone H2B-GFP (chromosome)**

Specimen courtesy of: Dr. Tokuko Haraguchi, Kobe Advanced ICT Research Center, NICT
**Image Quality**

**Key Nikon innovations for improving image quality**

A best ever image quality is realized by an increased light sensitivity resulting from comprehensive technological innovations in electronics, optics and software.

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**Low-angle incidence dichroic mirror realizes 30% increase in fluorescence efficiency**

With the A1 series, the industry’s first low-angle incidence method is employed on dichroic mirrors. High transmission rate of an average 98% and a 30% increase of fluorescence efficiency are realized.

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**VAAS pinhole unit transcends the existing concept of a confocal microscope**

It is commonly known that reducing pinhole size to eliminate flare light from non-focal plane causes darker images. The VAAS (Virtual Adaptable Aperture System) provides a new confocal microscopy that can eliminate flare while retaining image brightness.

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**Principle and features**

**Conventional confocal microscope**

Small pinholes reduce flare but darken images, while large pinholes brighten images but increase flare.

**VAAS pinhole unit**

By the deconvolution of the light that passes through the pinhole and the light that doesn’t pass through the pinhole, flare can be eliminated while using a large pinhole.

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**Effects**

1. Acquisition of brighter images with less flare is possible.
2. Different sectionings (slice thicknesses) can be simulated via deconvolution after image acquisition.
3. Images of both focal plane and non-focal plane can be acquired with a single scan, boosting speed and reducing damage to live cells.

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**DISP improves electrical efficiency**

Nikon original DISP (Dual Integration Signal Processing) technology has been implemented in the image processing circuitry to improve electrical efficiency, preventing signal loss while the digitizer processes pixel data and resets. The signal is monitored for the entire pixel time resulting in an extremely high S/N ratio.
The remote controller allows the regulation of major settings of laser, detector, and scanner with simple operation using push buttons and dials.

**4-channel detector unit with changeable filters**

In combination with four lasers, simultaneous observation of four fluorescence labels is possible as standard. Each of three filter wheels can mount six filter cubes that are commonly used for a microscope, and are all easily changeable by users. This combines modularity, flexibility with user friendliness.

**Spline Z scans for real-time display of cross-sectional images**

High-speed image acquisition in the Z direction as well as the XY direction is possible. By using the piezo motorized Z stage, arbitrary vertical cross-sectional view can be achieved in real time without acquiring a 3D image.

**Easy operation by remote controller**

The remote controller allows the regulation of major settings of laser, detector, and scanner with simple operation using push buttons and dials.

### CLEM (Controlled Light-Exposure Microscopy)

During lengthy time-lapse imaging, cellular apoptosis caused by the exposure to light is a problem. The CLEM senses the fluorescence signals and controls the on/off of the laser exposure depending on signal intensity. This reduces laser exposure and alleviates the problem of cellular apoptosis.

<table>
<thead>
<tr>
<th>Non-CLEM</th>
<th>CLEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis occurs after a lapse of one hour</td>
<td>Apoptosis doesn't occur even after a lapse of 2.5 hours</td>
</tr>
</tbody>
</table>

**NIS-Elements C**

Superior operability based on analysis of every possible confocal microscope operation pattern realizes easy operation without an instruction manual, satisfying both beginners and experienced confocal users. Taking advantage of the hybrid scanner, it enables a complicated sequence of experiments such as photo activation to be carried out with simple setting and operation.

**Simple image acquisition**

- Basic operation
  - Parameters for basic image acquisition are integrated in a single window, allowing simple image acquisition.
- Optical setting
  - By simply selecting a fluorescence probe, an appropriate filter and laser wavelength are set automatically. Microscope setup is also conducted automatically.

**Diverse application**

- Parameter setting for photo activation
  - Timing and imaging parameters for photo activation are set intuitively.
- Multidimensional Image acquisition
  - Acquisition of images with a free combination of multidimensional parameters including X, Y, Z, t and \( \lambda \) is possible.

**Reliable analysis functions**

- Real-time ratio display
- Deconvolution
- High-speed 3D rendering
- Multidimensional image display (nD Viewer)
- Synchronized display of multidimensional images (View synchronizer)
- Diverse measurement and statistical processing
- Powerful image database function
- Colocalization and FRET
Either A1 standard scanner set or A1R scanner set can be chosen.

### System components

- **A1 Scanning Head**
- **A1R Scanning Head**
- **4-detector Unit**
- **4-laser Unit**
- **Diascopic Detector Unit**
- **3-laser Unit EX**
- **4-laser Power Source Rack**
- **SU4FVI Adapter Set**

#### Recommended objective lenses

- **CFI Plan Apochromat 10x**
  - NA 0.45, W.D. 4.0mm
- **CFI Plan Apochromat 20x**
  - NA 0.70, W.D. 1.0mm
- **CFI Plan Apochromat 40x**
  - NA 0.95, W.D. 1.4mm
- **CFI Plan Apochromat VC 60x**
  - NA 1.20, W.D. 0.27mm
- **CFI Apochromat TIRF 60x**
  - NA 1.49, W.D. 0.13mm
- **CFI Apochromat TIRF 100x Oil**
  - NA 1.49, W.D. 0.12mm
- **CFI Plan Fluor 10x**
  - NA 0.50, W.D. 1.2mm
- **CFI Fluor 20x**
  - NA 0.75, W.D. 1.3mm
- **CFI Fluor 40x**
  - NA 1.30, W.D. 0.22mm
- **CFI Fluor 100x Oil with iris diaphragm**
  - NA 0.5-1.30, W.D. 0.20mm

#### Recommended filters

<table>
<thead>
<tr>
<th>Excitation Laser</th>
<th>Channel 1</th>
<th>Channel 2</th>
<th>Channel 3</th>
<th>Channel 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>488/561/647</td>
<td>515/35</td>
<td>585/65</td>
<td>700/75</td>
<td></td>
</tr>
<tr>
<td>405/488/546/647</td>
<td>450/50</td>
<td>515/30</td>
<td>585/65</td>
<td>700/75</td>
</tr>
<tr>
<td>457/492/514</td>
<td>482/35</td>
<td>540/30</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

For filters other than the above, please consult your local Nikon representative.
Diverse peripherals and systems for pursuit of live cell imaging

CFI Plan Apochromat VC series objectives

Motorized stages

Confocal microscope with Perfect Focus System

Multi-mode imaging system—A1 with TIRF system

Stage incubation system INU series

Specifications

<table>
<thead>
<tr>
<th>Specifications</th>
<th>A1R</th>
<th>A1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Input/output port</strong></td>
<td></td>
<td></td>
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<tr>
<td>Laser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method:</td>
<td></td>
<td></td>
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<tr>
<td>A0 (Acoustic) device or drive current control</td>
<td>A0 (Acoustic) device or drive current control</td>
<td></td>
</tr>
<tr>
<td>Control power control for each wavelength, Return mask, ROI exposure control</td>
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<td></td>
</tr>
<tr>
<td>Laser unit</td>
<td>Standard: U4-IF unit</td>
<td>Optional: C-UD/EX 3-laser unit EX</td>
</tr>
<tr>
<td>Filter cube</td>
<td>6 fiber cubes commonly used for a microscope mountable on each of three filter wheels</td>
<td>Recommended wavelengths: 455/50, 482/35, 525/25, 595/50, 700/75, 540/30, 515/30, 585/65</td>
</tr>
<tr>
<td>Scanning head</td>
<td>Scanning: 4 fps (512 x 512 pixels)</td>
<td>Standard image acquisition: Scanner: non-resonant scanner 3-axis, resonance frequency 7kHz</td>
</tr>
<tr>
<td>Number of channels: 32 channels</td>
<td>Number of channels: 32 channels</td>
<td></td>
</tr>
<tr>
<td>Spectral image acquisition speed</td>
<td>4 fps (256 x 256 pixels), 1000 fps</td>
<td>Scanner: non-resonant scanner A2: Pixel size: max. 400 x 400 pixels</td>
</tr>
<tr>
<td>Maximum wavelength and resolution</td>
<td>455/50: 1000 (1000 x 1000), 488/35: 1000 (1000 x 1000)</td>
<td>Scanning speed: 4 fps (512 x 512 pixels) to 230 fps (512 x 64 pixels), 7500 1-frame/sec (512 x 512 pixels)</td>
</tr>
<tr>
<td>Wavelength range variable from 250nm to 1050nm</td>
<td>Scanning speed: 4 fps (512 x 512 pixels) to 230 fps (512 x 64 pixels), 7500 1-frame/sec (512 x 512 pixels)</td>
<td></td>
</tr>
<tr>
<td>Unmixing: High-speed unmixing, Precision unmixing</td>
<td>Unmixing: High-speed unmixing, Precision unmixing</td>
<td></td>
</tr>
<tr>
<td>Z-step: 5 steps</td>
<td>Z-step: 5 steps</td>
<td></td>
</tr>
<tr>
<td>Compatible microscopes</td>
<td>SCUPE E1-tilt inverted microscope, SCUPE E2500 E inverted microscope, SCUPE 1M upright microscope, SCUPE EMP fixed stage microscope</td>
<td></td>
</tr>
<tr>
<td>Software</td>
<td>Display/Image processing: 2D analysis, 3D volume rendering/orthogonal, 4D analysis, spectral unmixing</td>
<td>Display/Image processing: 2D analysis, 3D volume rendering/orthogonal, 4D analysis, spectral unmixing</td>
</tr>
<tr>
<td>Control computer</td>
<td>OS: Microsoft Windows® XP SP3 English version</td>
<td>Control computer: OS: Microsoft Windows® XP SP3 English version</td>
</tr>
<tr>
<td>Options</td>
<td>Motorized XY stage, High-speed Z stage, VIVAS, CLEM</td>
<td>Options: Motorized XY stage, High-speed Z stage, VIVAS, CLEM</td>
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<tr>
<td>Installation condition</td>
<td>Temperature: 5°C to 35°C, Humidity: 65% (RH) or less (non-condensing)</td>
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</tr>
</tbody>
</table>
Dimensions and weight

LU4 4-laser unit 438(W) x 301(H) x 690(D)mm Approx. 35kg (without laser)
LU-LR 4-laser power source rack 438(W) x 400(H) x 800(D)mm Approx. 20kg (without laser power source)
Scanning head 276(W) x 163(H) x 364(D)mm Approx. 13kg
Controller 360(W) x 580(H) x 600(D)mm Approx. 40kg
A1-DU4 4-detector unit 360(W) x 199(H) x 593.5(D)mm Approx. 16kg

Power source

Controller Input voltage: 100–240VAC ±10% 50–60Hz
Current rating: 5A @100VAC
Overcurrent protection: main breaker 15A

LU-LR 4-laser power source rack Power source for Ar laser and control circuit: 100VAC, 15A/115VAC, 15A/230VAC, 7.5A, 50/60Hz (breaker 15A)
Power source for lasers except Ar laser: 100VAC, 3A/115VAC, 3A/230VAC, 1.5A, 50/60Hz (breaker 5A)

The AOTF incorporated into the 4-laser unit and the AOM optionally incorporated into the 3-laser unit are classified as controlled products (including provisions applicable to controlled technology) under foreign exchange and trade control laws. You must obtain government permission and complete all required procedures before exporting this system.