Typhoon™ FLA 9000 biomolecular imager

Typhoon FLA 9000 (Fig 1) is a versatile laser scanner for biomolecular imaging applications including sensitive and quantitative measurements of radioisotopic labels by storage phosphor, chemifluorescent Western blots, and multiplex fluorescence as well as digitization of colorimetric stains (e.g., Coomassie™ Blue and silver-stained gels).

The system supports both 2-D Difference Gel Electrophoresis (DIGE) and Amersham™ ECL™ Plex™ Western blotting systems.

Fig 1. Typhoon FLA 9000 is a high performance biomolecular imager for sensitive and quantitative measurements.

Typhoon FLA 9000 delivers:

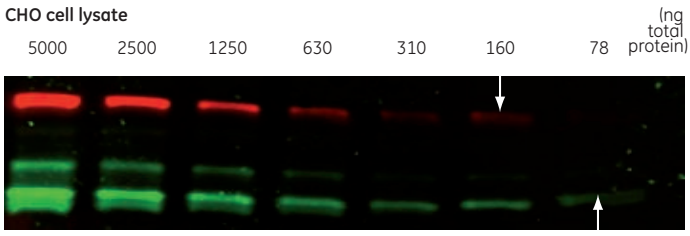
- **Versatility:** image radioisotope-, multifluorescent-, chemifluorescent-, and colorimetric-labeled samples
- **High resolution and quantitation:** a pixel resolution of up to 10 µm and a linear signal response over five orders of magnitude provides precise quantitation in gels, blots, tissue sections, and arrays
- **High sample throughput:** A scanning area of 40 × 46 cm enables simultaneous imaging of up to 20 gels or blots, measuring 10 × 8 cm in size. This facilitates comparisons among blots, and reduces workload and waiting time.
- **Flexibility:** optimized performance for new applications by adapting the system with stages, detectors, filters, and lasers
- **2-D DIGE imaging:** simultaneously image two 2-D DIGE gels for differential expression studies
- **Visible and infrared fluorescence imaging:** optional near infrared excitation for imaging IRDye™ and other infrared dyes

Typhoon FLA 9000 is a variable mode laser scanner with modular access to the optical components and excitation sources (Fig 2), providing both versatile and flexible imaging for precise quantitation of proteins, nucleic acids, and other biomolecules.

The system provides several imaging modes such as fluorescence, filmless autoradiography, and digitization of colorimetrically stained gels (e.g., Coomassie Blue and silver stain). Chemiluminescence imaging is possible but for detection of low abundance proteins by chemiluminescence, the ImageQuant™ imager series is recommended.

Fig 2. Filters are easily exchanged by the user.

The system is optimized for: 2-D DIGE imaging for differential protein expression studies, Amersham ECL Plus chemifluorescent imaging for quantitative protein detection by Western blotting, and multifluorescent Amersham ECL Plex imaging for precise quantitation of two or more proteins in the same blot (Fig 3).



Sample: CHO cell lysate
Membrane: Hybond™ LFP
Target protein: β-tubulin (Cy™5, red)
ERK 1/2 (Cy3, green)
Detection: **Primary antibodies:**
Mouse anti-tubulin and rabbit anti-MAP kinase ERK1/2
Secondary antibodies:
ECL Plex goat anti-rabbit IgG-Cy3
ECL Plex goat anti-mouse IgG-Cy5

Imaging:	**Excitation**	**Emission filter**
Cy3:	532 nm	BPG1 (570DF20)
Cy5:	635 nm	LPR (665LP)

LOD: β-tubulin in 160 ng CHO cell lysate (Cy5)
ERK 1/2 in 78 ng CHO cell lysate (Cy3)

Fig 3. Multiplex detection of endogenous proteins by Amersham ECL Plex Western blotting. Tubulin and ERK1/2 were targeted in a dilution series of CHO cell lysate by using mouse anti-tubulin and rabbit anti-MAP kinase ERK 1/2 primary antibodies. Secondary antibodies were ECL Plex anti-mouse Cy5 (red) and anti-rabbit Cy3 (green). Imaging was performed with Typhoon FLA 9000 using separate detection channels. Arrows indicate the limits of detection (LOD) in each detection channel. The minimal crosstalk and low background mean that it is possible to reliably quantitate specific signals relative to a housekeeping protein.

Fig 4. (A) The Phosphor Stage, **(B)** Multi Stage, **(C)** Low Fluorescent Glass Plate Stage, and **(D)** Fluor Stage are designed to accommodate a variety of sample formats and imaging modes.

Table 1. Typhoon FLA 9000 specifications

Detection modes:	Fluorescence, phosphorimaging, digitization, and chemiluminescence
Excitation wavelengths:	473 nm (blue LD laser), 532 nm (green SHG laser), 635 nm (red LD laser), 685 nm (optional near IR LD laser), and 785 nm (optional near IR LD laser)
Radioisotopes:	^{3}H, ^{11}C, ^{14}C, ^{125}I, ^{18}F, ^{32}P, ^{33}P, ^{35}S, ^{99m}Tc, and other sources of ionizing radiation
Dynamic range:	5 orders of magnitude
Bit depth:	16-bit
Scanning area:	40 × 46 cm
Pixel sizes:	10, 25, 50, 100, 200 µm, and prescan 1000 µm
Standard filters:	IP (Phosphorimaging), LPB (510LP), LPG (575LP), LPR (665LP), BPB1 (530DF20), and BPG1 (570DF20)
Optional filters:	BPFR700 (R715), BPFR800 (R810), DBR1 (530DF20/665LP), and DGR1 (570DF20/665LP)
Dimensions (W × H × D):	900 × 400 × 800 mm
Weight:	97 kg
Line frequency:	50/60 Hz
Temperature:	15°C to 30°C
Humidity:	20% to 70% (no condensation)
Supply voltage:	100 - 240 VAC ± 10%
Power consumption:	Approx. 0.3 kVA

Technical features

Optimal choice of filter, stage, laser and PMT

Filters are easily accessed and exchanged without tools to attain optimal imaging conditions. The system accommodates up to four computer-controlled filter positions at any time. Custom filters can be easily installed by the user.

Stages (Fig 4) give the correct positioning and stability for optimal imaging of a range of sample types. Samples that can be scanned include agarose and polyacrylamide gels, membranes, DIGE gels, radioisotope-labeled samples using a phosphorimaging plate, as well as microplates and glass slides with the titer plate (TP) plug-in. The system can simultaneously scan two DIGE gels, each measuring up to 21.5 × 27.5 cm with the Low Fluorescent Glass Plate stage. The stages are easily removed from the system for cleaning.

The system can be equipped for dual simultaneous fluorescence detection by the addition of a second photomultiplier tube (PMT). Each PMT is selected for optimal response to the detected emission wavelength. The standard bialkali PMT 1 is suitable for phosphorimaging and dyes

excited by blue (e.g., Cy2), green (e.g., Cy3), and red (e.g., Cy5 or Alexa Fluor™ 633) light (Fig 5) whereas the optional multialkali PMT 2 is optimal for dyes excited by far red and infrared light such as IRDye680 or IRDye800. In experiments using a secondary antibody labeled with IRDye 680, the performance of Typhoon FLA 9000, in terms of sensitivity, dynamic range and linearity of response was shown to be similar to that of the Odyssey™ Infrared Imaging System from LI-COR™ (Fig 6).

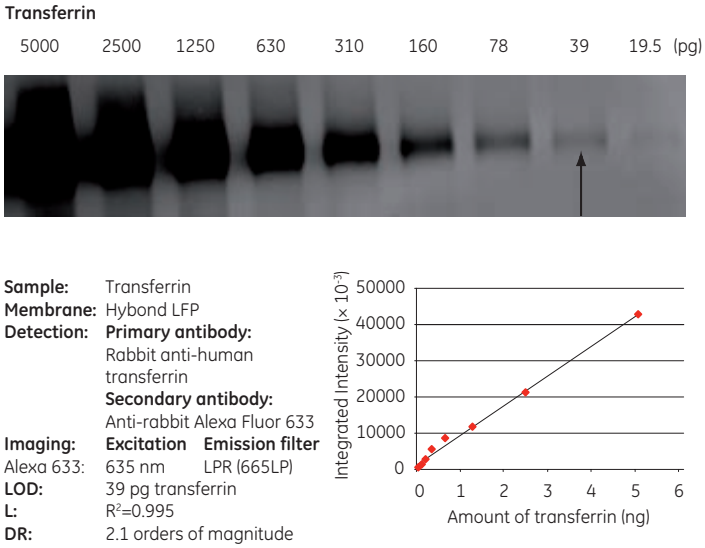


Fig 5. A two-fold dilution series of transferrin starting at 5 ng was subjected to Western blotting and detected with a rabbit anti-transferrin primary antibody and anti-rabbit Alexa Fluor 633 secondary antibody. Results demonstrate detection in the red wavelength region with a linear signal response. Arrow indicates the limit of detection (LOD). The linear dynamic range (DR) was 2.1 orders of magnitude, and the linearity of the response (L) was R^2=0.995.

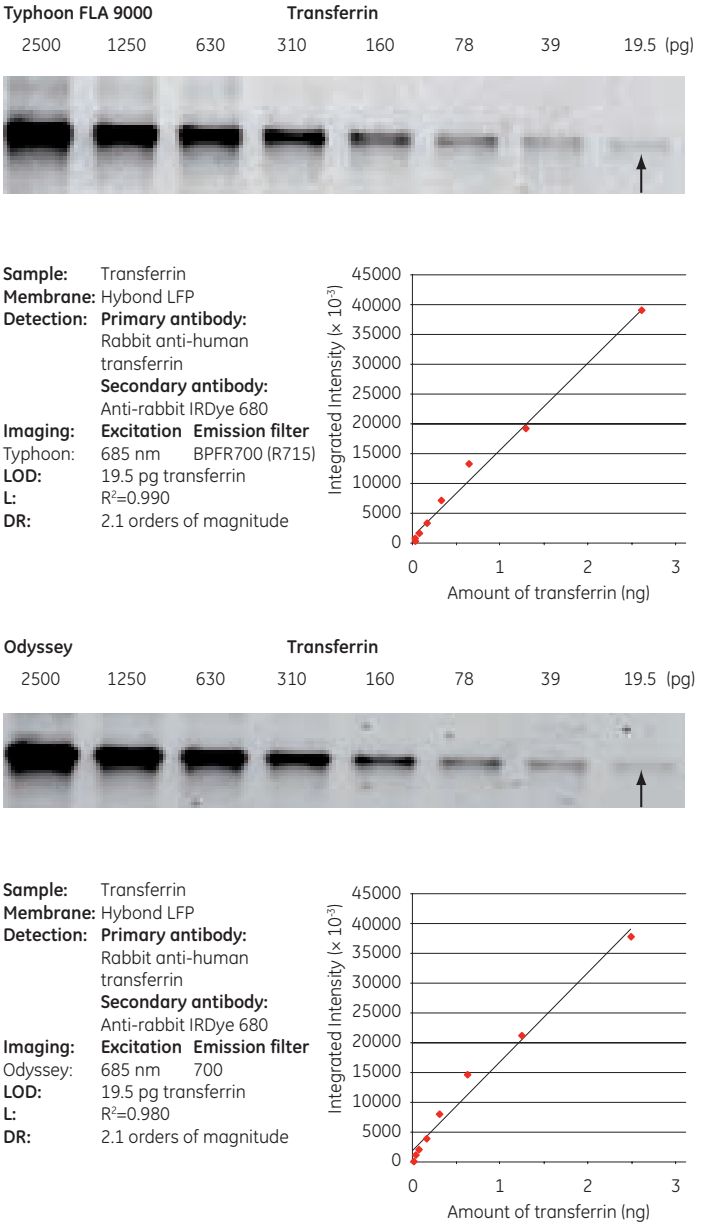


Fig 6. A two-fold dilution series of transferrin starting at 2.5 ng was subjected to Western blotting and detected with a rabbit anti-transferrin primary antibody and anti-rabbit IRDye 680 secondary antibody. Imaging by Typhoon FLA 9000 showed similar performance for detection in the near infrared wavelength region compared to the Odyssey Infrared Imaging System from LI-COR. Arrows indicate the LOD. The experiments were performed at GE Healthcare laboratories according to the manufacturers' instructions.

Scanning is rapid and detection is sensitive for laser-induced fluorescence, radioisotopic imaging by storage phosphor, and digitization. A fast 1000 µm prescan function gives a rapid overview of the sample for selecting the correct settings. At a pixel size of 200 µm, a 10 × 15 cm sample is scanned in two minutes. The system provides a linear signal response over five orders of magnitude. This, together with digitization of the image with up to 16-bit resolution, provides a suitable basis for the precise quantitation of proteins, DNA and other labeled molecules.

Lasers can be exchanged in the field to accommodate new applications and fluorophores. The system can house up to four lasers simultaneously, from a choice of five laser excitation wavelengths (473, 532, 635, 685, and 785 nm).

Optimal detection of chemifluorescent Western blots

Laser scanning systems are not optimal for imaging chemiluminescence. Typhoon FLA 9000 does, however, perform well with Amersham ECL Plus by imaging its stable chemifluorescent signal, which is emitted upon excitation by the 473 nm laser. This provides a means to obtain optimal imaging performance from a chemifluorescent reagent.

Ettan™ DIGE system

Ettan DIGE system is an integrated solution for accurate quantitation of changes in protein expression. Typhoon FLA 9000 is a fully optimized part of Ettan DIGE system with DeCyder™ 2D Differential Analysis Software (Fig 7).

The strengths of Typhoon FLA 9000—high sensitivity and broad dynamic range for measuring low and high abundant proteins in one scan (Fig 8)—make it highly suited for 2-D DIGE applications, enabling the user to detect and accurately quantitate subtle changes in protein expression. By generating overlaid, multichannel images for each gel with minimal crosstalk, Typhoon FLA 9000 exploits the multiplexing potential of CyDye™ DIGE fluors to remove experimental variation between gels. Images are analyzed using DeCyder 2D to accurately and confidently measure very small differences in protein abundance.

Imaging

For the detection of radioactivity, fluorescence and chemifluorescence, emitted light is collected and transformed to an electrical signal by a photomultiplier tube (PMT). The electrical signal is then converted into digital information by A/D conversion for image display and analysis.

Detection of radioactivity

Samples containing radioactive probes are exposed to a storage phosphor screen. Light is emitted from the screen in proportion to the amount of radioactivity in the sample upon laser-induced stimulation.

Fluorescence

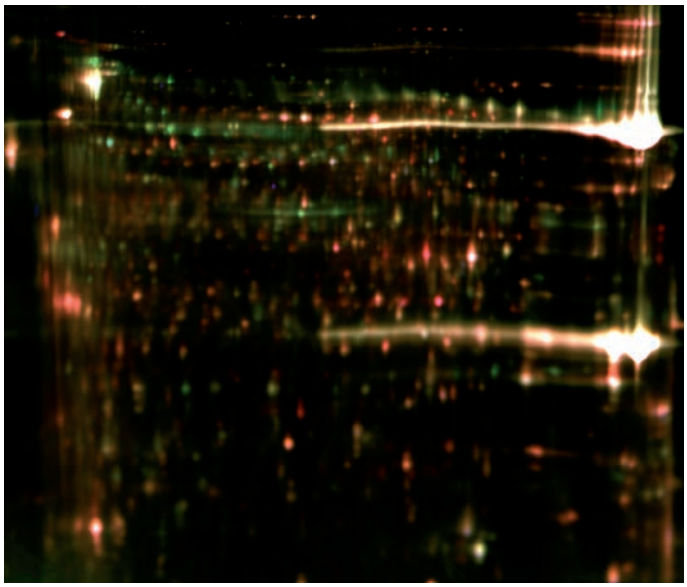
Upon excitation, light is emitted from a fluorescently labeled sample in proportion to the amount of labeled compound in the sample. Multiple fluorescent wavelengths can be detected with minimal crosstalk for comparative expression experiments. See Table 3 for emission filters.

Chemifluorescence

Upon excitation, light is emitted from a fluorescent product generated in an enzyme-catalyzed reaction, in proportion to the amount of labeled compound in the sample.

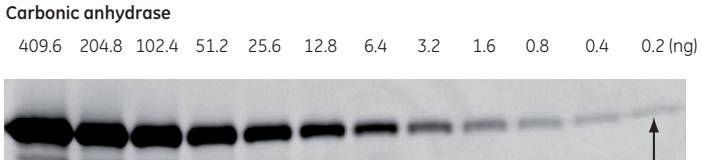
Digitization

Excitation light passes through the sample and excites a fluorescent plate. The emitted light from the plate passes through the sample again and is collected and converted to an electrical signal. The method is suitable for documentation of colorometrically stained gels.



Sample:	CHO cell lysate, expressing monoclonal antibody (MAb) grown under various culture conditions	
Gel:	Large precast DIGE Gel (within glass cassette)	
Imaging:	**Excitation**	**Emission filter**
Cy2:	473 nm	BPB1 (530DF30)
Cy3:	532 nm	BPG1 (570DF20)
Cy5:	635 nm	LPR (665LP)

Fig 7. CHO cells expressing MAb were grown in different culture media. 2-D DIGE was used to analyze the expression of host cell proteins as part of efforts to improve MAb process understanding. Image analysis was performed using DeCyder 2D software.



Sample:	Carbonic anhydrase	
Label:	CyDye DIGE fluor, Cy3 minimal dye	
Gel:	12% acrylamide Tris-glycine	
Imaging:	**Excitation**	**Emission filter**
Cy3:	532 nm	BPG1 (570DF20)
LOD:	0.2 ng carbonic anhydrase	
L:	R^2=0.998	
DR:	3.3 orders of magnitude	

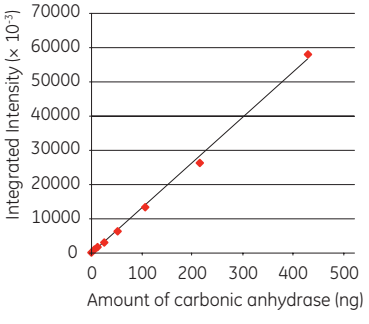


Fig 8. Different concentrations of carbonic anhydrase were labeled with CyDye DIGE fluor, Cy3 minimal dye and subjected to 1-D electrophoresis. The gel was imaged with Typhoon FLA 9000. The detection limit (LOD) was 0.2 ng carbonic anhydrase and the linear DR was 3.3 orders of magnitude. Arrow indicates the LOD.

Chemiluminescence

The light detection path is scanned across the sample without illumination. The emitted chemiluminescence from each scanned point is collected and transformed to an electrical signal by a PMT. The electrical signal is then converted into digital information by A/D conversion for image display and analysis.

Data storage

Data are stored either in linear 16-bit grayscale TIFF (.TIF file format) or in square root encoded 16-bit TIFF (.GEL file format). The .GEL format encoding provides higher dynamic resolution than .TIF at lower signal levels to exploit the low signal detection capability of the phosphorimaging technology.

Image analysis

Designed for seamless data transfer and quantitative gel and blot analysis, we provide image analysis software for use with Typhoon FLA 9000 (Table 2).

Table 2. Image analysis software

Software	Analysis
ImageQuant TL	1-D gel electrophoresis, dot blots, arrays, colony counting, and user-defined gel analysis
DeCyder 2D	Differential high-resolution 2-D DIGE analysis including Extended Data Analysis
ImageMaster 2D Platinum	2-D gels, including single stain and 2-D DIGE

Table 3. Emission filters

Filter type	Wavelength range (nm)	Detection examples
IP	BP390	Phosphorimaging
LPB (510LP)	≥ 510	Cy2, ECL Plus, SYBR™ Green, FAM™, FITC, Alexa Fluor 488, SYPRO™ Ruby, SYPRO Orange, GFP
BPB1 (530DF30)	515 to 545	Cy2 DIGE Fluor, ECL Plex Cy2
LPG (575LP)	≥ 575	Cy3, Deep Purple™, HEX, Alexa Fluor 532 and 555, SYPRO Red
BPG1 (570DF20)	560 to 580	Cy3 DIGE Fluor, ECL Plex Cy3
LPR (665 LP)	≥ 665	Cy5, Alexa Fluor 633, TOTO™ 3, DiD, Cy5 DIGE Fluor, ECL Plex Cy5
BPFR700 (R715)	713 to 726	Alexa Fluor 700, IRDye680, IRDye700
BPFR800 (R810)	814 to 826	Alexa Fluor 790, IRDye800

Imager performance

	Typhoon 9400/9410	Typhoon FLA 9000	Typhoon Trio/Trio+	Typhoon FLA 7000
Storage Phosphor				
^{3}H, ^{11}C, ^{14}C, ^{125}I, ^{18}F, ^{32}P, ^{33}P, ^{35}S, ^{99m}Tc, and other sources of ionizing radiation	++++	++++	++++	++++
Macroarray (radiolabeled)	++++	++++	++++	—
Fluorescence—Proteins				
CyDye DIGE Fluors				
Cy2	++++	+++	++++	—*
Cy3	++++	++++	++++	—*
Cy5	++++	++++	++++	—*
ECL Plex Fluors				
Cy2	++++	+++	++++	—*
Cy3	++++	++++	++++	—*
Cy5	++++	++++	++++	—*
Protein Stains				
Deep Purple Total Protein Stain	++++	++++	++++	++++
SYPRO Ruby	++++	++++	++++	+++
NanoOrange™ (solutions)	+++		+++	
Pro-Q Diamond (phosphorylated proteins)	++++	+++	++++	++
Pro-Q Sapphire 532 (Histidine-tagged proteins)	++++	+++	++++	++
ELISA				
AttoPhos		++++		+++
Fluorescence—Nucleic acids				
Cy3 and Cy5	++++	++++	++++	+++
Alexa Fluor 532 and Alexa Fluor 633	++++	++++	++++	+++
Nucleic acid stains				
Ethidium Bromide (post stain)	++++	+++	++++	+++
Vistra Green, SYBR Gold, SYBR Green I and II	++++	++++	+++	+++
PicoGreen, RiboGreen	+++		+++	
Chemifluoresence (enzyme-catalyzed)				
Amersham ECL Plus Western blotting	++++	+++	+++	++
ECF, AlkPhos direct ECF	++++	+++	++++	++
DDAO Phosphate	++++	++++	++++	+++
Other applications				
Cy2	++++	+++	++++	++
Cy3	++++	++++	++++	+++
Cy5	++++	++++	++++	+++
Fluorescein, FAM, FITC, Alexa Fluor 488	++++	++++	++++	+++
TET, HEX, ROX, TAMRA	++++	+++	++++	++
Green fluoresecent protein	+++	+++	+++	++
Chemiluminescence	+	+	+	—
Amersham ECL Amersham ECL Plus Amersham ECL Advance™				

++++ Superior performance +++ High performance ++ Good performance + Acceptable performance — Not compatible

Ratings are based on overall system performance including model-specific features, versatility, and sensitivity (limit of detection). Blank fields indicate that data are not available.
* Multiplex experiments (e.g., 2-D DIGE and Amersham ECL Plex) cannot be performed on Typhoon FLA 7000. CyDye DIGE Fluors and Amersham ECL Plex conjugates can be imaged in single probe experiments on Typhoon FLA 7000 (i.e., experiments where there is only one dye or conjugate on the gel or membrane).

Ordering information

System	Quantity	Code no.
Typhoon FLA 9000*	1	28-9558-08

*Includes 473 nm, 532 nm, and 635 nm lasers, filter tray, IP filter, LPB filter, LPG filter, LPR filter, BPB1 filter, BPG1 filter, Fluor Stage, Membrane Weight, Phosphor Stage, Low Fluorescent Glass Plate Stage, Multi Stage, and TP plug-in. Fluorescent plate for digitization, capture software, USB cable, mains cables (EU and USA), User Manual and Getting Started Guide.

Upgrades and accessories	Quantity	Code no.
Fluor Stage Set *Fluor Stage, Membrane Weight*	1	28-9589-04
Multi Stage Set *Multi Stage, TP plug-in*	1	28-9564-19
BAS-IP MS 2040 E *Phosphorimaging plate, 20 × 40 cm, multipurpose*	1	28-9564-74
BAS-IP MS 2025 E *Phosphorimaging plate, 20 × 25 cm, multipurpose*	1	28-9564-75
BAS-IP MS 3543 E *Phosphorimaging plate, 35 × 43 cm, multipurpose*	1	28-9564-76
BAS-IP SR 2040 E *Phosphorimaging plate, 20 × 40 cm, high resolution*	1	28-9564-77
BAS-IP SR 2025 E *Phosphorimaging plate, 20 × 25 cm, high resolution*	1	28-9564-78
BAS-IP TR 2040 E *Phosphorimaging plate, 20 × 40 cm, for Tritium detection*	1	28-9564-81
BAS-IP TR 2025 E *Phosphorimaging plate, 20 × 25 cm, for Tritium detection*	1	28-9564-82
FLA Image Eraser	1	28-9564-73
685 nm laser upgrade *685 nm laser, BPFR700 filter, PMT2, installation, and testing*	1	28-9610-22
785 nm laser upgrade *785 nm laser, BPFR800 filter, PMT2, installation, and testing*	1	28-9610-25
685 + 785 nm laser upgrade *685 nm and 785 nm lasers, BPFR700 filter, PMT2, BPFR800 filter, installation, and testing*	1	28-9610-32

Related literature	Code no.
Typhoon FLA 7000 biomolecular imager, Data file	28-9610-73

Minimum computer requirement

OS: Windows™ XP™ SP3 (32-bit) or Windows Vista™ Business SP1 (32-bit), RAM: more than 1 GB, Processor: Intel™ Core 2 Duo processors, Hard disk: more than 80 GB, USB Ports: USB 2.0, Optical drive: DVD-ROM or Super Multi Drive, Monitor: 1280 × 1024 pixel resolution or higher

Please contact your local sales representative for the latest recommended computer configuration.

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com/quantitative_imaging

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imagination at work

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