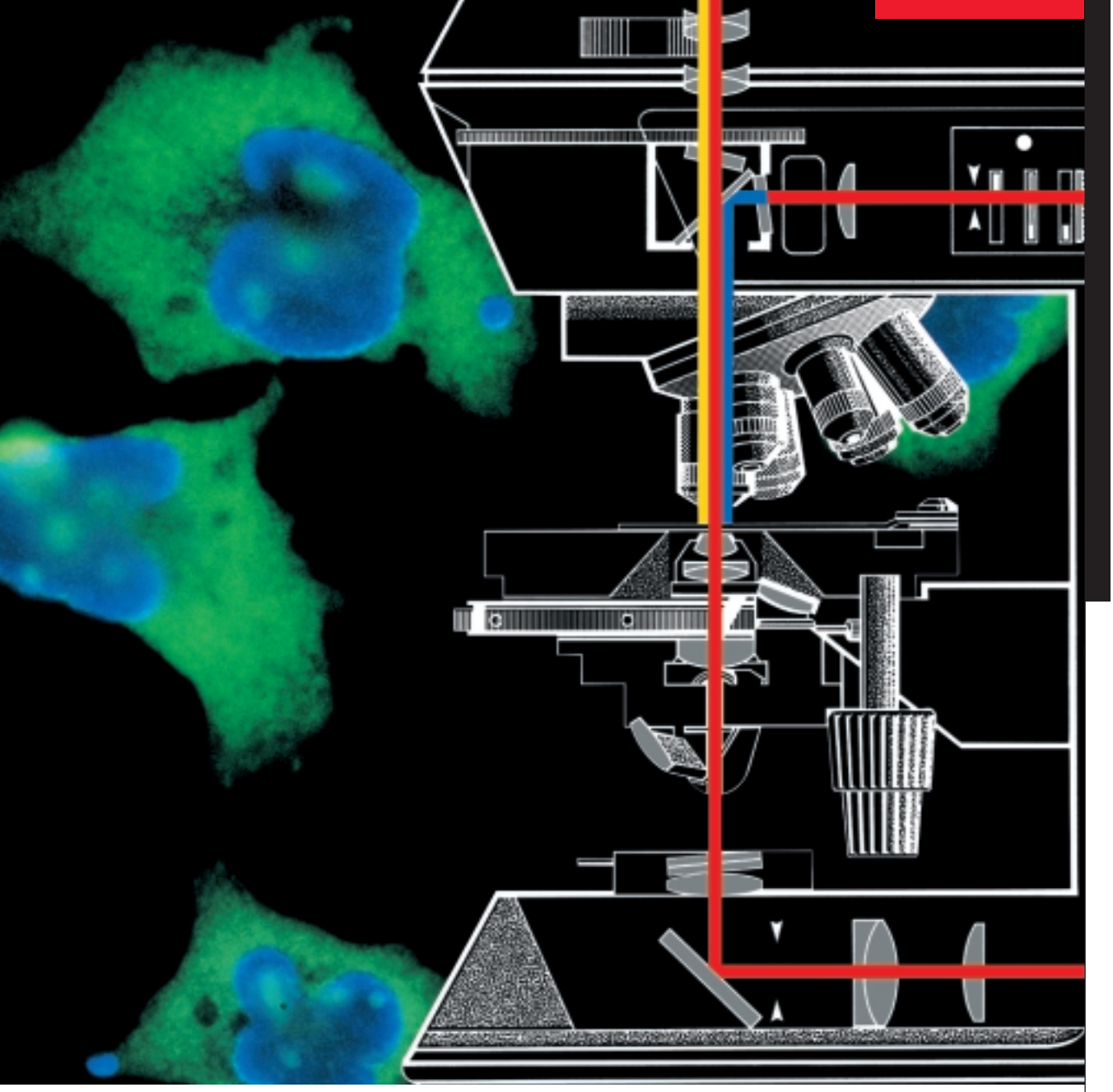
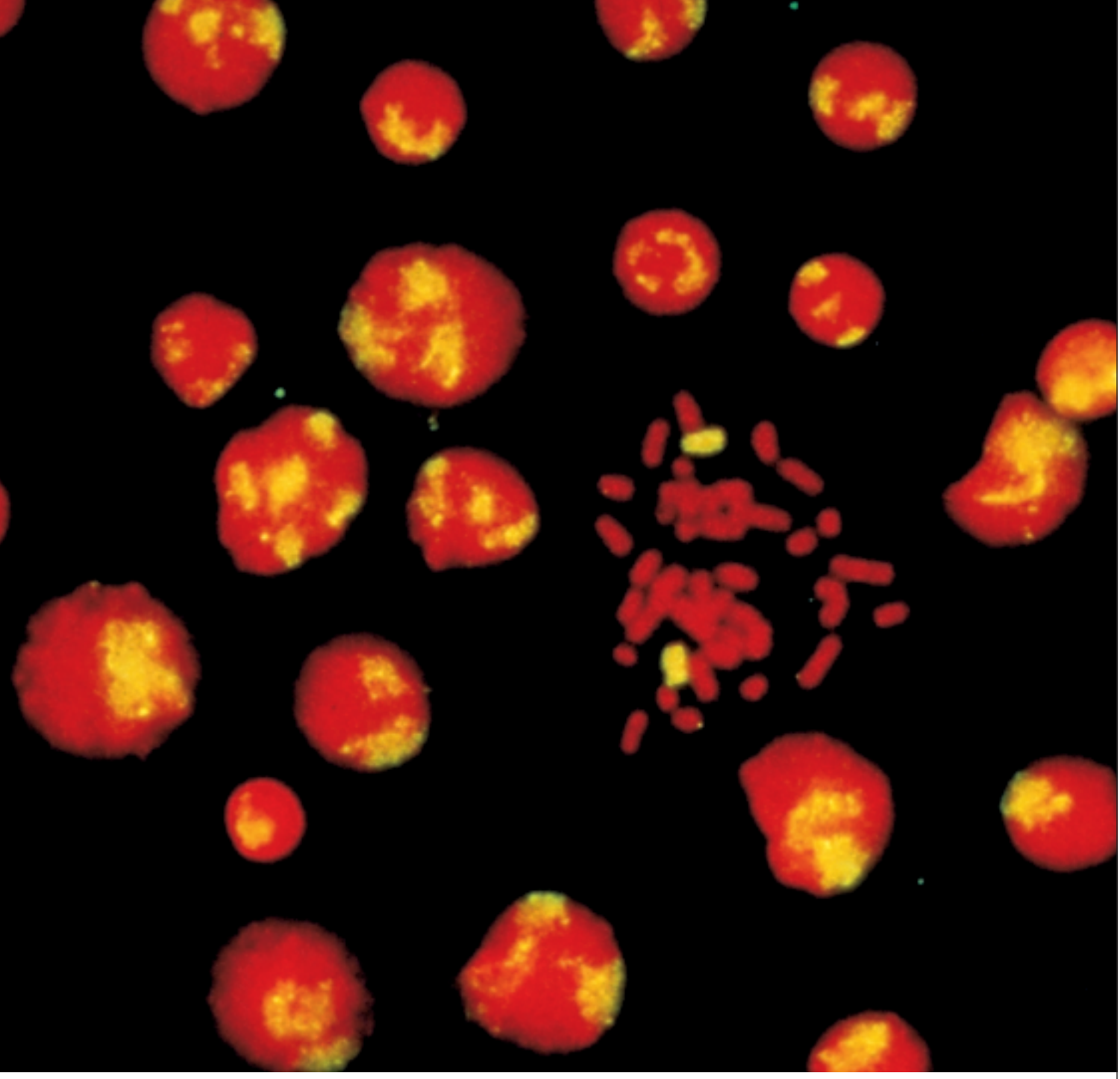


Leica Microsystems
Fluorescence Microscopy

The perfect solution for all microscope applications involving fluorescence

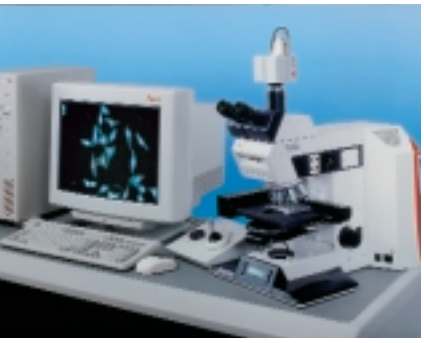
Leica



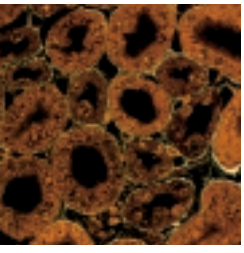
Bone marrow FISH;
FITC/TRITC

The Application

Leica DM RXA automated fluorescence microscope with Leica DM RD phototube



Leica Q550 CW Cytogenic Workstation
– Leica DM RXA
– Leica QFISH software



Fluorescence microscopy has long been established as an extremely important diagnostic tool in nearly all scientific disciplines and is now an integral part of routine diagnostics and fundamental medical and biological research.
To keep pace with the ever increasing requirements of a fluorescence microscope generated by new and constantly improved methods, e.g. in molecular genetics, Leica Microsystems dedicated to ongoing intensive research. As a result, we are able to offer state-of-the-art, application-oriented fluorescence microscopes covering the whole range of applications from the most simple routine examination to fully automatic, software-controlled multi-parameter fluorescence analysis. Top-quality Leica optics and a wide selection of filters for all classic and modern fluorescence techniques round off the performance spectrum of Leica fluorescence microscopy, making it the perfect solution for all microscope applications involving fluorescence.

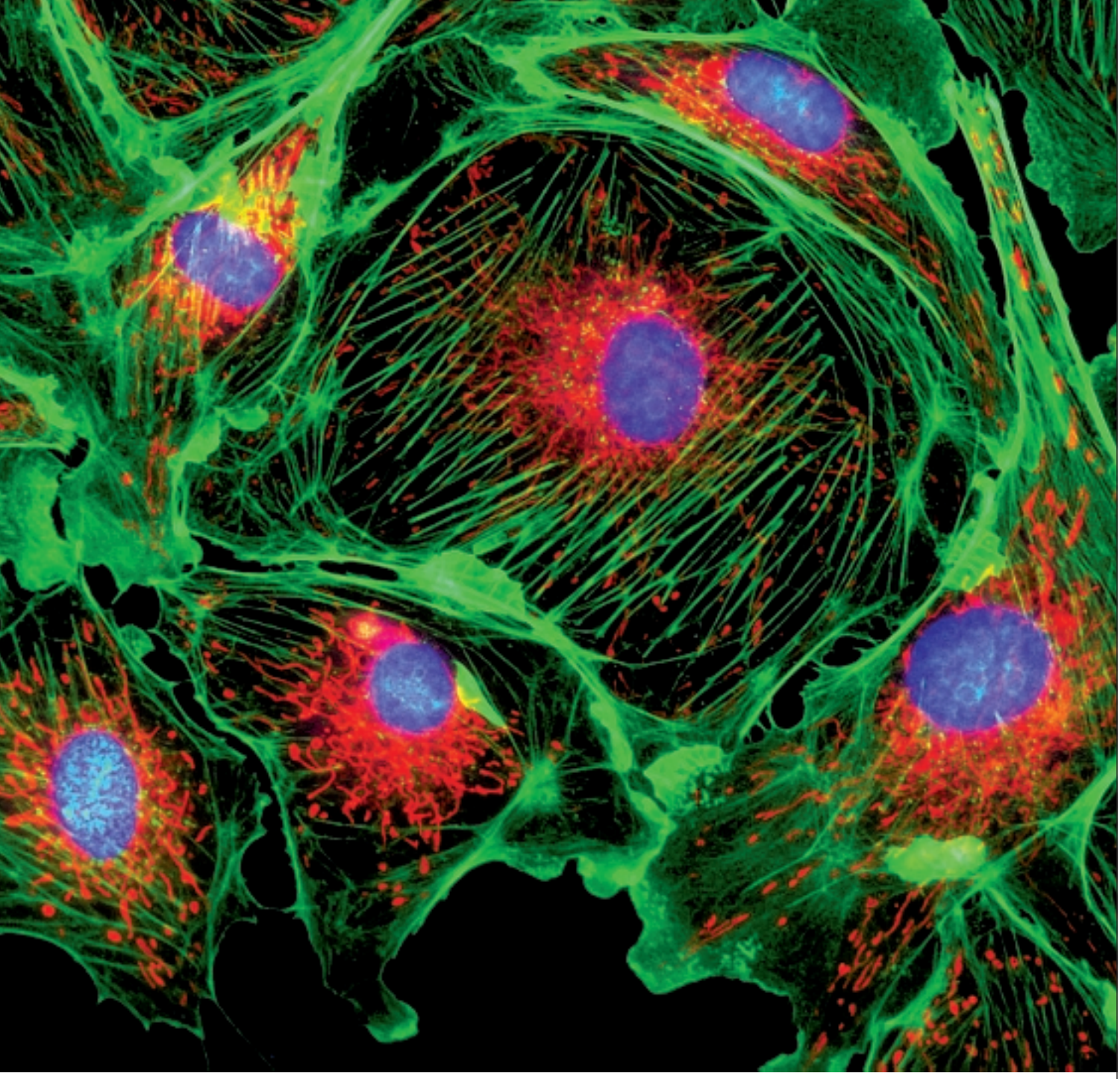
Principles of fluorescence
When irradiated with short wavelength light, fluorescent substances emit light of a longer wavelength; non-fluorescent objects, such as the background, remain dark. This property, possessed by many different materials, is known as primary or auto-fluorescence. The majority of microscopically interesting specimens, however, do not have this property.

In order to achieve the aim in fluorescence microscopy of rendering certain structures visible or of highlighting details for specific analysis, such specimens must first be stained or, in an immunochemical reaction, labelled with a fluorescent dye, known as a fluorochrome. The light emitted from a substance stained with a fluorochrome is called secondary fluorescence. Important biomedical applications of fluorescence microscopy include the classic immunofluorescence technique for detecting infectious diseases or, as examples of recent molecular-genetic developments, fluorescence in situ hybridisation (FISH) or comparative genomic hybridisation (CGH). The FISH method is used for direct localisation of genes and other DNA/RNA sequences in chromosomes or tissue (e.g. antenatal karyotyping), while CGH involves examining complete genomes for genetic changes, a method of screening which provides valuable information, particularly for tumour pathology, on all unbalanced genetic changes of the examined DNA.

Fluorescence microscopy has also been given significant new impetus by Green Fluorescent Protein (GFP). This natural fluorescing protein offers completely new prospects for cell and development of biology, in particular. For example, it can be used as a reporter gene to visualize protein expression in cells as well as the location, transport and degradation of these proteins.

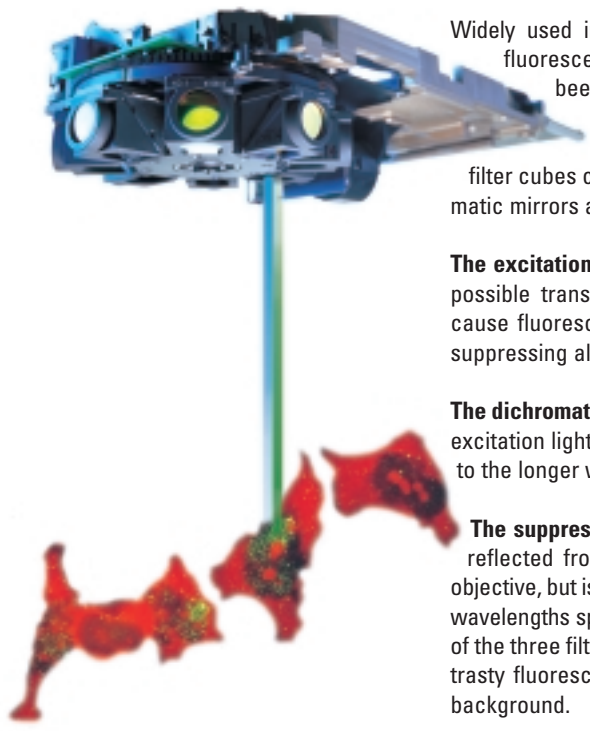
Systems and software programs
Leica Microsystems offers complete systems for the fluorescence techniques mentioned, such as the Leica Q550FW Fluorescence Workstation with universal Leica QFluoro/QFluoro Pro Imaging.
Leica QFluoro/QFluoro Pro Imaging are application software programs for high-quality digital fluorescence imaging.
Ask your Leica Microsystems agency for the Leica Q550FW brochure.

A new technique for observing extremely fine structures, which can be easily combined with fluorescence microscopy, is Leica Reflection Contrast (see illustration on the left). Please order our “Leica Reflection Contrast“ brochure.



Endothelial cells from pulmonary artery;
DAPI/BODIPY FL/MITO TRACKER RED

Incident light excitation



Maximum fluorescence intensity

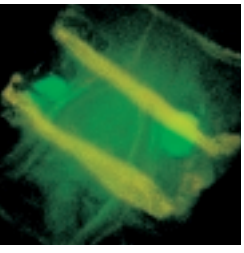
Widely used in the past, the technique of exciting fluorescence with transmitted light has now been replaced by incident light excitation. Leica Incident Light Fluorescence Illuminators accommodate up to eight filter cubes containing the excitation filters, dichromatic mirrors and suppression filters.

The excitation filter is designed to have the highest possible transmission at those wavelengths which cause fluorescence in a particular specimen, while suppressing all remaining irradiation.

The dichromatic mirror reflects the short wavelength excitation light onto the specimen, but is transparent to the longer wavelength fluorescence.

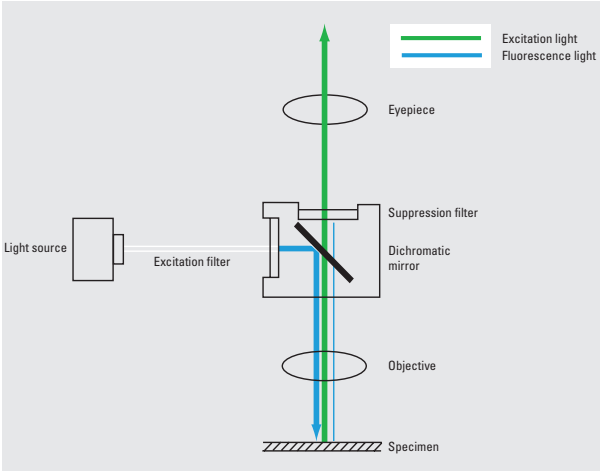
The suppression filter absorbs the excitation light reflected from the specimen which re-enters the objective, but is highly transparent to the fluorescence wavelengths specific to the specimen. The interaction of the three filter components results in a bright, contrasty fluorescence image against a dark specimen background.

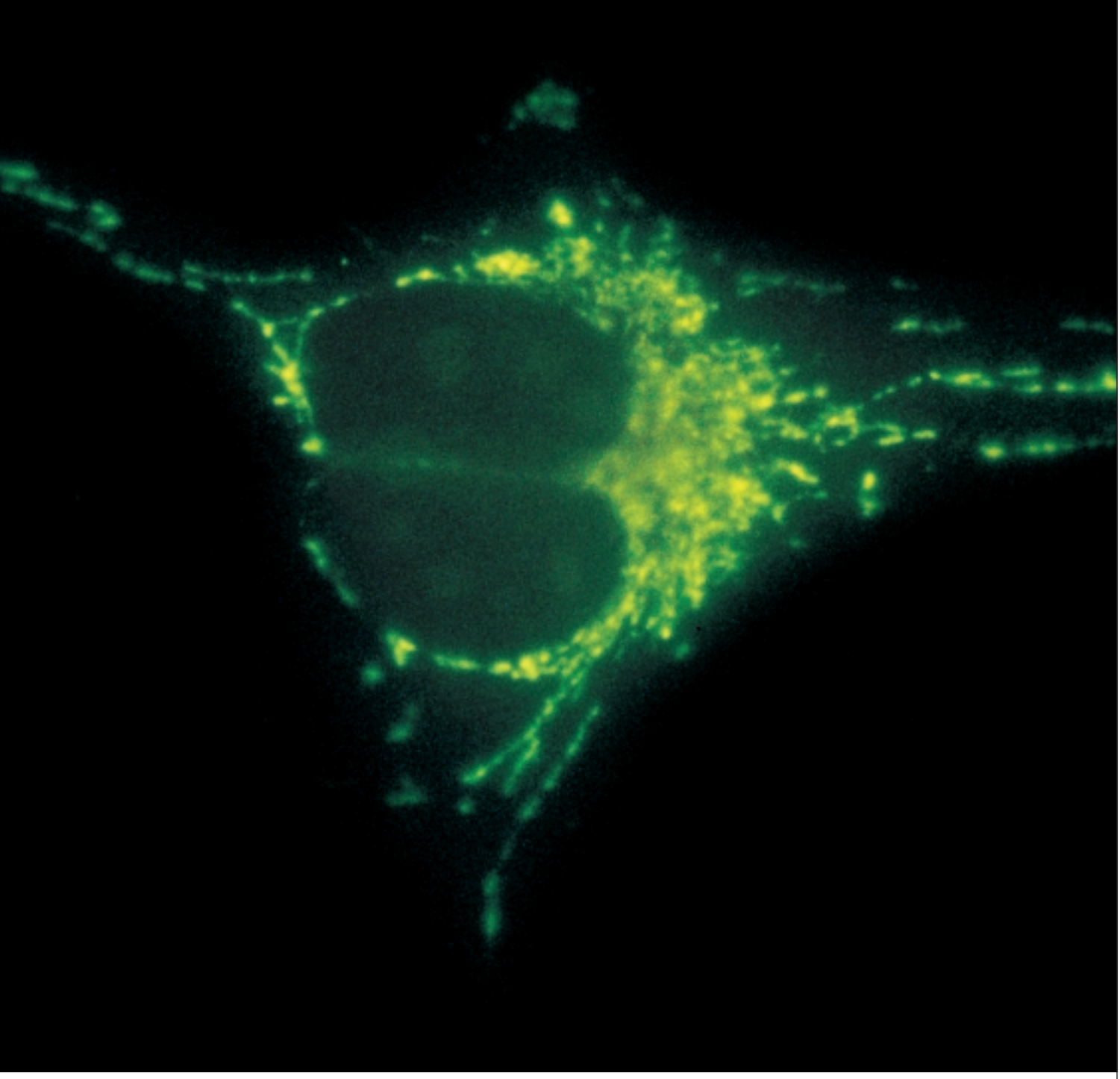
This combination of perfectly matched filters in a cube, which can easily be exchanged in a matter of seconds, explains the great ease of use of Leica incident light fluorescence systems, and is an essential criterion for the standardization of immunofluorescence tests. Incident light fluorescence microscopy has now become indispensable in clinical and biological laboratories, and is especially suitable for multi-wavelength studies and their combination with simultaneous or alternative illumination methods in transmitted light.



The immunogold technique (IGS)
With the immunogold technique, monoclone antibodies are usually marked with colloidal gold. For observation in polarized incident light, a pol filter system (see p. 8) is placed in the incident light fluorescence illuminator instead of a fluorescence filter cube system. The **polarizer** in this system polarizes the light as it comes from the source – the incident light path. It is diverted to the specimen by a neutral dichromatic mirror, which partly reflects the light to the pol filter system where it is extinguished by an analyzer in cross position. The direction of polarization remains unaffected. The light that is refracted by the gold particles in the specimen is depolarized. Therefore, it passes through the analyzser of the pol filter system, providing a high-contrast image.

Diagram of incident light excitation of fluorescence



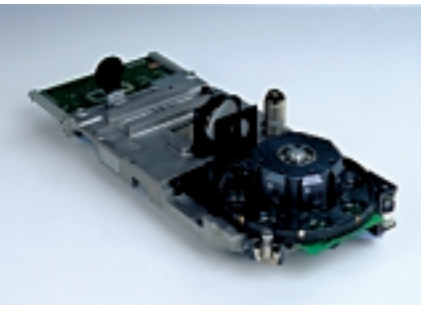


Mitochondrial DNA (wild type) in Hela cells;
FITC

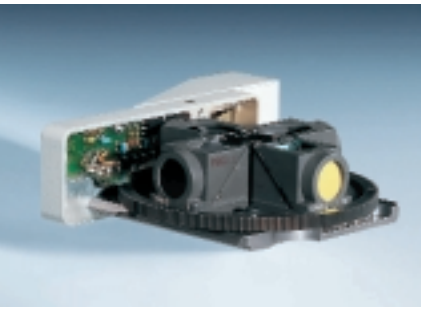
Incident light fluorescence illuminators

The ultimate in convenience

Fluorescence illuminator 3 lambda for Leica DM LS (front) and 4 lambda for Leica DM LB



Motorized fluorescence illuminator RF8 mot. for Leica DM RXA/RA



Motorized fluorescence illuminator RF4 mot. for Leica DM IRB

For the upright Leica research microscopes, DM R/E, DM RX/E and DM RXA, there are two different incident light fluorescence illuminators. With the RF4 module, up to four filter cubes, and with the RF8 module, up to eight filter cubes, can be inserted into the rotating turret.
These two illuminators are also offered with motorized filter cube change for the Leica DM RA and DM RXA microcopes (RF4 mot/RF8 mot). With the motorized version it is possible to carry out program-controlled sequential excitation for multiple stainings (e.g. FISH analysis). The motor-driven light trap ensures that the specimen is not exposed to fluorescent light for longer than necessary.
In general, there is no detectable pixel shift in the single images of the various fluorescence colors when switching between filter cubes. Incidentally, the newly designed illumination axis guarantees optimum light flux and therefore maximum brightness and homogeneity. There are two selectable settings: maximum intensity and maximum homogeneity. The incident light illuminator, which can also be retrofitted later, is integrated in the stand to ensure a constant viewing height.
The incident light fluorescence illuminator for the Leica DM IRB/E inverted research microscope offers the same design principle and performance as the above-described incident light illuminators RF4 and RF4 mot of the upright DM R microscopes. Whether manual or motor-driven, the fluorescence module is slotted into the side of the stand.

The fluorescence illuminator for the Leica DM LB laboratory microscope accommodates up to four filter cubes in horizontal positions in a rotating turret. This device and the filter cubes are identical to the corresponding components of Leica microscopes for research applications (DM R). Any image shift after a filter cube change remains under the resolving power of 35 mm film.

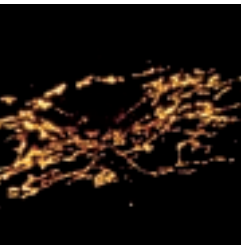
For both the upright (DM LS) and the inverted (DM IL) types of Leica routine microscope, three filter cubes can be inserted in a special slide and switched as required.
All Leica incident light fluorescence illuminators have a disengageable BG38 filter, which allows the full lamp intensity to be used for excitation (as long as a reddish specimen background is acceptable) and the most favourable tube factor for fluorescence intensity, 1x.

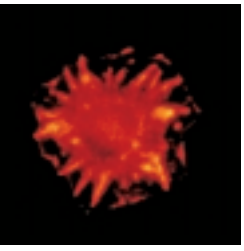
They also have a centrable field diaphragm to minimise reflections and protect the specimen. The variable aperture diaphragm is useful for adjusting contrast of reflecting samples (e.g. IGS/IGSS) or also, if necessary, for reducing excitation radiation to prevent excessive specimen fading.

All Leica microscopes can also be used, simultaneously or alternatively, for illumination techniques in transmitted light such as brightfield, darkfield, polarised light, phase and interference contrast.
Modified or entirely new investigation methods and technical advances in filter production make periodic

Leica DM IL inverted fluorescence microscope for routine examinations

Range of filter cubes*





replacement of filters a necessity. Leica Microsystems constantly updates and adapts the systems to match the latest developments in technology and newest applications on the market. For example, new high-performance systems have been added to Leica's standard filter cube range which cover all currently known requirements of multi-color fluorescence (e.g. selective excitation and observation). Thanks to Leica's zero pixel shift technology, there is no pixel shift in the single images of the various spectral ranges on the screen when switching between filter cubes.

Special filter cube combinations can also be assembled to customer specifications (with filters of other manufacturers) to comply with zero pixel shift requirements, provided that the filters of other manufacturers meet certain minimum quality standards.

The following tables show the types and characteristics of Leica filters. Bandpass filters are identified by the letters BP and the wavelength in nm. The filters are characterized either by the center wavelength and half power bandwidth (e.g. BP 525/20) or the short- and long-wave half power points (BP 450-490).

Short- and long-pass filters are identified by the letters SP and LP respectively, and the edge wavelength in mm (e.g. LP 520).

	Filter cube	Excitation range	Exitation filter	Dichromatic mirror	Suppression filter	DMLS/LSP DMIL/DMIRB DMR HCRF8	DMLB LM/LP DMR HCRF4
	A	UV	BP 340-380	400	LP 425	513 824	513 804
+	A4	UV	BP 360/40	400	BP 470/40	513 848	513 839
	D	UV + violet	BP 355-425	455	LP 470	513 825	513 805
	E4	blue	BP 436/7	455	LP 470	513 826	513 806
	H3	violet + blue	BP 420-490	510	LP 515	513 827	513 807
	I3	blue	BP 450-490	510	LP 515	513 828	513 808
	K3	blue	BP 470-490	510	LP 515	513 829	513 809
+	L5	blue	BP 480/40	505	BP 527/30	513 849	513 840
	M2	green	BP 546/14	580	LP 590	513 831	513 811
	N2.1	green	BP 515-560	580	LP 590	513 832	513 812
+	N3	green	BP 546/12	565	BP 600/40	513 850	513 841
	G/R	FITC/TEXAS RED	BP 490/20	505	BP 525/20	–	–
		blue/green	BP 575/30	600	BP 635/40	513 834	513 803
+	TX2	TEXAS RED/green	BP 560/40	595	BP 645/75	513 851	513 843
	B/G/R	DAPF/FITC/TEXAS RED	BP 420/30	415	BP 465/20	–	–
		UV/blue/green	BP 495/15	510	BP 530/30	–	–
			BP 570/20	590	BP 640/40	513 838	513 836
+	Y3	CY3 green	BP 535/50	565	BP 610/75	513 855	513 837
+	Y5	CY5 red	BP 620/60	660	BP 700/75	513 856	513 844
+	Y7	CY7 red	BP 710/75	750	BP 810/90	513 857	513 845
	GFP	GFP-blue	BP 470/40	500	BP 525/50	513 852	513 847
	FI/RH	FITC/rhodamine	BP 490/15	500	BP 525/20	–	–
		blue/green	BP 560/25	580	BP 605/30	513 854	513 846
	Pol	IGS	Polariser	Neutral	Analyzer	513 835	513 813
	Empty system		0	0	0	513 853	513 842

\+ specially for multi-parameter fluorescence
BP = bandpass filter LP = longpass filter 0 = to be fitted by customer

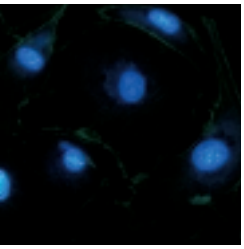
* filter cube after Ploem

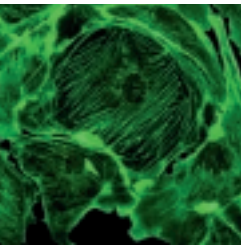
Correlation of fluorochromes and filter cubes

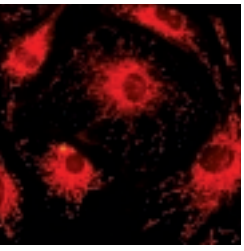
Fluorochrome	Filter cube
– Acid fuchsin	N 2.1, M 2
– Acridine blue	A
– Acridine yellow	I 3, H 3
– Acridine orange	I 3, H 3
– Acridine red	N 2.1, N 3
– Acriflavin	E 4, H 3
– Acriflavin-Feulgen-SITS (AFS)	D
– Alizarin complexion	N 2.1
– Alizarin red	N 2.1
– Allophycocyanin (APC)	Y 3, Y 5
– AMCA (Aminocoumarin)	A
– AMCA/FITC/Texas Red	B/G/R
– Aminoactinomycin D-AAD	N 2.1, N 3
– Aniline blue	A
– ACMA	E 4
– Astrazone Brilliant Red 4G	N 2.1
– Astrazone Red 6B	N 2.1
– Astrazone Yellow 7 CLL	H 3
– Astrazone Orange R	I 3, L 5
– Atabrine	E 4, H 3
– Auramine	I 3, H 3
– Aurophosphine, Aurophosphine G	I 3, H 3
– BCECF	L 5
– Berberine sulphate	H 3
– Benzoxanthen Yellow	D
– BisAminophenyl Oxdiazol (BAO)	A
– Bisbenzimide (Hoechst)	A, D
– Blancophor BA	A, D, H 3
– Blancophor SV	A
– BODIPY FL	L 5, K 3, I 3
– Brilliant Sulphaflavine FF	D, H 3
– Bromobimane (Thiolyte)	D
– Calcein	I 3
– Calcein blue	A
– Calcium Crimson	Y 3
– Calcium Green	K 3, I 3, L 5
– Calcium Orange	M 2, N 2.1
– Calcofluor White	H 3, D
– Calcofluor White standard solution	A
– Carboxyfluorescein diacetate C-FDA	I 3, L 5
– Cascade Blue	A, D
– Catecholamines (adrenalin, noradrenalin, dopa, dopamine)	D
– Chromomycin A (mithramycin, olivomycin)	E 4
– Coriphosphine O	I 3, H 3
– Coumarin-phalloidin	D
– Cy 3	Y 3
– Cy 5	Y 5
– Cy 7	Y 7
– DANS (diamino-naphtyl sulphonic acid)	A
– DAPI	A, D
– DAPI (selective)	A 4
– DAPI/FITC/Texas Red (simultaneous)	B/G/R
– Dansyl chloride	A
– DIPI	A
– DiI	Y 3
– DiO	I 3, K 3
– Diphenyl brilliant flavine 7 GFF	H 3
– Dopamine	A
– DPH (diphenyl hextariene)	A
– Eosin B	N 2.1
– Ethidium bromide	N 2.1
– Euchrysin	H 3, D
– Evans Blue	N 2.1
– Fast Blue	A
– Fast Green FC G	N 2.1, M 2
– Feulgen	N 2.1, TX 2
– FDA (fluorescein diacetate)	I 3, H 3, K 3, L 5
– FIF (formaldehyde induced fluorescence)	D, A

Fluorochrome	Filter cube
– FITC (fluorescein isothiocyanate)	I 3, H 3, K 3, L 5
– FITC/ethidium bromide	I 3, L 5, N 2.1
– FITC/phycoerithrin (PE) (simultaneous)	G/R
– FITC/Texas Red (simultaneous)	G/R
– FITC/TRITC (simultaneous)	FI/RH
– FITC (selective)	L 5
– Texas Red (selective)	TX 2
– FITC/TRITC	L 5, N 3
– TRITC (selective)	N 3
– Fluo 3	I 3, L 5
– Fluoro Gold	A
– Fluram (fluorescamine)	A
– Genacryl Brilliant Red B	N 2.1
– Genacryl Brilliant Yellow	E 4
– Generic Blue	D
– GFP (Green Fluorescent Protein)	GFP
– Granular Blue	A
– Haematoporphyrin	N 2.1
– Hoechst dye no. 33258	A, D, A 4
no. 33342	A, D, A 4
– Hydroxy-4-methylcoumarin	A
– Lissamine-rhodamine B (RB 200)	N 2.1, M 2
– Lucifer Yellow	E 4
– Magdala Red	N 2.1
– Maleimide	A
– Mepacrin	D
– Merocyanin 540	N 2.1
– Mithramycin	E 4
– MPS (methyl Green Pyronine stilbene)	A
– Nile Red	I 3, L 5, N .21
– Nuclear Fast Red	N 2.1, M 2, N 3
– Nuclear Yellow	A
– Olivomycin	E 4
– Oregon Green (488, 500, 514)	L 5
– Oxytetracycline	D
– Pararosaniline (Feulgen)	N 2.1, TX 2
– Phosphine 3 R	I 3, H 3
– Phycoerythrin (PE)	N 2.1, N 3
– Primulin O	D
– Procion Yellow	D, E 4, H 3
– Propidium iodide	N 2.1
– Pyronine B	N 2.1, M 2
– Quinacrine mustard (QM)	E 4
– Resorufin	N 2.1, Y 3
– Reverine	D
– Rhodamine B	N 2.1
– Rhodamine 123	I 3, L 5
– Serotin	A, D
– SITS (stilbene isothiosulphonic acid)	A
– SITS acriflavine Feulgen	D
– Spectrum Orange	M 2, N 2.1
– Sulphaflavine	A
– Tetracyclines: oxytetracycline, tetracycline, reverine (pyrrolidinomethyltetracycline), chlortetracycline, dimethylchlortetracycline	D
– Texas Red	TX 2
– Thiazin red R	N 2.1, M 2
– Thioflavine S	H 3, D
– Thioflavine TCN	A
– Thiolyte (bromobimane)	D, A
– TRITC (tetramethyl rhodamin isothiocyanate)	N 2.1, N 3
– TRITC (selective)	N 3
– True Blue	A
– Uranine B	H 3
– Uvitex 2 B	A, D
– XRITC	N 2.1, N 3
– Xylene orange	N 2.1, M 2

Fluorescence methods in medicine and biology

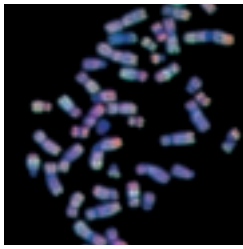


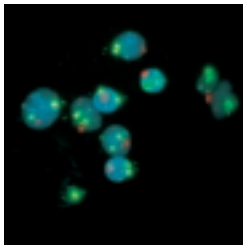


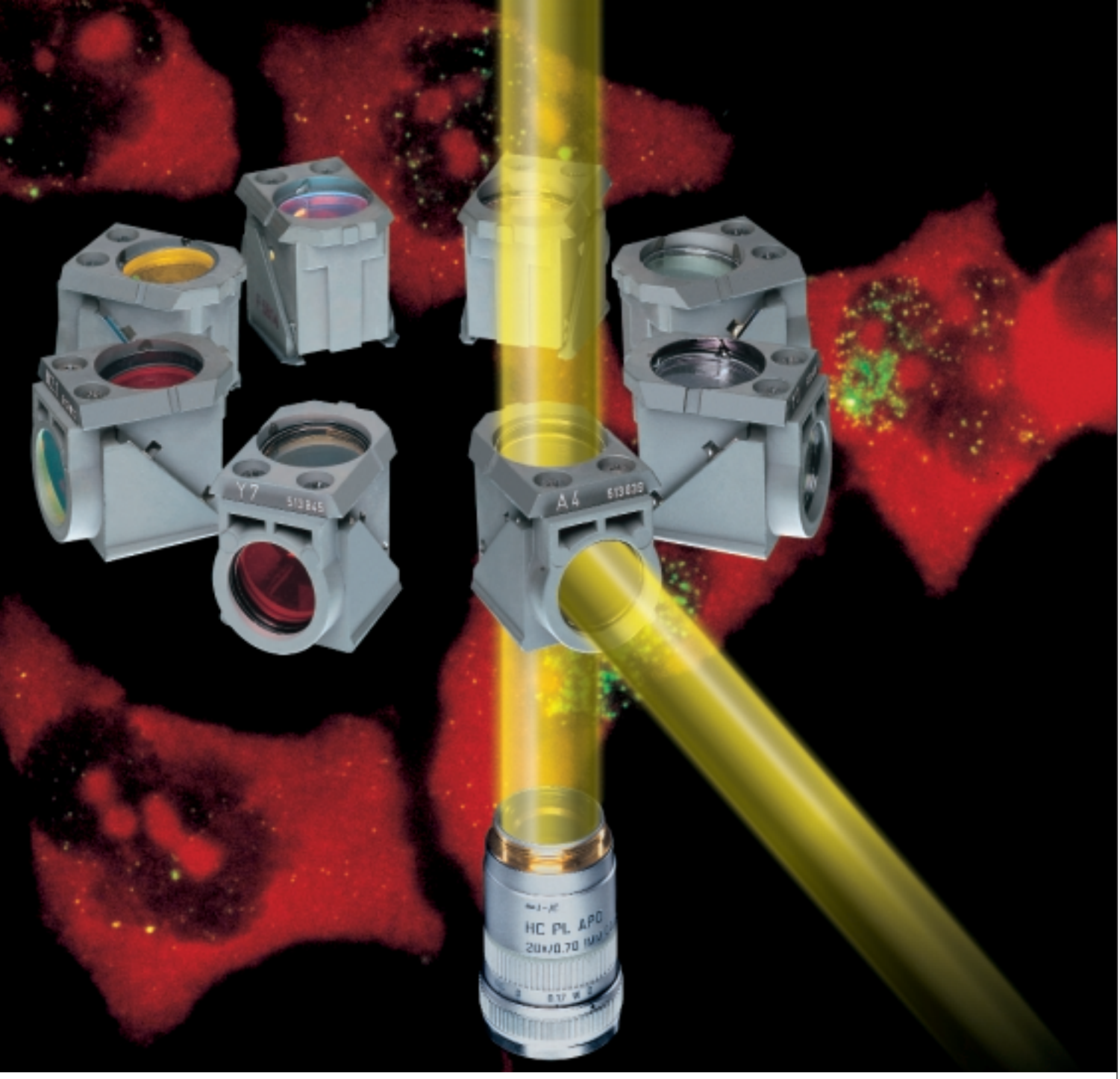


Application	Method (fluorochrome)	Filter cube
Amines, biogenic; certain catecholamines (adrenalin, noradrenalin, dopa, dopamin, serotonin)	FIF (Formaldehyde Induced Fluorescence)	D
Amines, primary	Fluram (fluorescamine)	A
Amyloid (antibody globulin)	Thioflavine S	H 3, D
Antigen-antibody reactions (evidence)	Allophycocyanin	Y 3, Y 5
	AMCA	A
	Cascade Blue	A, D
	CY 3	Y 3
	CY 5	Y 5
	CY 7	Y 7
	DAPI	A 4, A
	FITC (Fluoroscein isothiocyanate)	I 3, H 3, K 3, L 5
	TRITC (Tetramethyl rhodamine isothiocyanate)	N 2.1, N 3, M 2
	XRITC	N 2.1, N 3, M 2
	DANS (diamino naphtyl sulphonic acid)	A
	Lissamine rhodamine B 200 (RB 200)	
	Texas Red	TX 2
	Phycoerythrin	N 2.1, N 3
	FITC/Texas Red (PE)	G/R
	DAPI/FITC/Texas Red (PE)	B/G/R
	FITC/TRITC	F 1/RH
Bacteria (general)	Auramin	I 3, H 3
	Acridine yellow	I 3, H 3
	Acridine orange	I 3, H 3
	Berberine sulphate	H 3
	Coriphosphine O	I 3, H 3
	Hoechst 33258	A, D
	Hoechst 33342	A, D
Bone	Calcein	I 3
	Calcein blue	A
	Tetracycline	D
	Acid fuchsin (osteone)	N 2.1, M 2
	Xylene orange	N 2.1, M 2
Cellulose	Aniline blue	A
	Calcofluor white	D
	Coriphosphine O (cell walls)	I 3, H 3
	Euchrysin	H 3, D
	Primulin O	D
	SITS (stilbene isothiosulphonic acid)	A
Chromosomes/bands	Atebrine	E 4
Drumstick/sex chromosomes	Quinacrine	E 4
	Quinacrine mustard	E 4
Collagen	Fast Green FCF	N 2.1, M 2
Contrast staining: red with e.g. FITC staining	Evans blue	N 2.1, I 3
	DAPI	A, D, A 4
Diptheria	Coryphosphine O	I 3, H 3
DNA measurement (quantitative)	Acrindine Orange	I 3, H 3
	BAO (bisaminophenyl oxadiazol)	A
	Pararosaniline (Feulgen)	N 2.1, TX 2
Fluorescence microscopy of mononuclear cells stained	FITC/Ethidium bromide (double staining)	I 3, L 5, N 2.1
Fluorescence in situ hybridisation (FISH)	DAPI	A 4
	FITC	L 5
Comparative Genomic Hybridisation (CGH)	CY 3	Y 3
	CY 5	Y 5
	CY 7	Y 7
	TRITC	N 3
	Texas Red	TX 2
	DAPI/FITC/TX	B/G/R
	FITC/TX	G/R
	FITC/TRITC	FI/RH
Fungi/algae (in tissue)	Uvitex 2B	A, D
	Blancophor BA	A, D, H 3
Hemogram differentiation	Methyl green pyronin stilbene (MPS) (double staining)	A/N 2.1

Application	Method (fluorochrome)		Filter cube
Leucocytes/lymphocytes	Coriphosphine O		I 3, H 3
	Euchrysin		H 3, D
	Thioflavin S		H 3, D
Lipid Droplets (intracellular)	Nile Red		I 3, L 5, N 2.1
Lymphocyte differentiation (T-helpers, T-suppressor cells)	FITC/phycoerytherin (PE) (double staining)		I 3, G/R
Lymphocyte toxicity test	Carboxyfluorescein diacetate (C-FDA)/ethidium bromide (propidium iodide)		I 3, L 5, N 2.1
Lysine	Dansyl chloride		A
Membranes	DANS		A
	DiI		Y 3
	DiO		I 3, K 3
	Fluram		A
	DPH (diphenyl hexatriene)		A
	Merocyanin 540		N 2.1
Mucus	Acridine orange		I 3, H 3
	Aurophosphine G		I 3, H 3
	Coriphosphine O		I 3, H 3
	Euchrysine		H 3
	Sulphaflavine		A
Mycoplasm contamination	DAPI, H. dye no. 33258		A, D
	H. dye no. 33342		A, D
Nerve tracts (neurons) (retrograde label)	Evans blue		N 2.1
	Fast blue, Granular blue		A
	True blue		A
	Procion yellow		D, E 4, H 3
	Lucifer yellow		E 4
	SITS (stilbene isothiosulphonic acid)		A
Nucleic acids DNA, RNA (cell nuclei)		**Base specificity**	
	Ethidium bromide	low	N 2.1
	Propidium iodide	low	N 2.1
	DAPI/DIPI	A-T	A, D, A 4
	Hoechst 33258	A-T	A, D, A 4
	Hoechst 33342	A-T	A, D, A 4
	Quincacrine	slightly G-C	E 4
	Chromomycin A	G-C	E 4
	Mitramycin	G-C	E 4
	Olivomycin	G-C	E 4
	Aminoactinomycin D	G-C	N 2.1
	Pyronin B	G-C	N 2.1, M 2
	Acriflavine-Feulgen	–	D
	Pararosaniline (Feulgen)		N 2.1, TX 2
	Acridine yellow		I 3, H 3
	Acridine orange		I 3, H 3
	Berberine sulphate		H 3
	Coriphosphine		I 3, H 3
	Phosphine 3R		I 3, H 3
Nuclei and proteins	DAPI and eosin B (double staining)		A, N 2.1, M 2
Nuclear proteins	Fluram (fluorescamin)		A
Proteins/histones	Sulphaflavine		A
	Benzoxanthen yellow		D
	Thiazine red R		N 2.1, M 2
	Eosin B		N 2.1, M 2
Polychromatic sequential labelling of bone	Calcein/calcein blue		I 3/A
	Oxytetracycline/tetracycline		D
	Haematoporphyrin		N 2.1
	Xylene orange		N 2.1
	Alizarin, -red, -complexion		N 2.1
SH groups	Thiolyte (bromobimanes)		D, A
	Resorufine		N 2.1, TX 2
TBC	Acridine yellow		I 3, H 3
	Auramine		I 3, H 3
Thrombocytes	Mepacrine		D
Two-color staining method for DNA and protein in cervical cytology	Acriflavine Feulgen SITS (stilbene isothiosulphonic acid)		D



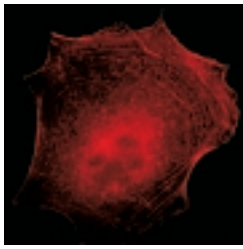


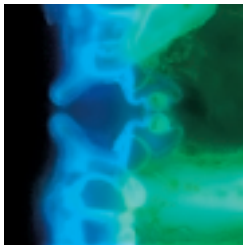


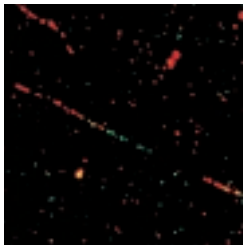
Hela cells FISH; FITC/CY 3

Fluorescence methods in industry

Application	Method	Filter cube
Detection and distribution of zinc oxide in rubber Detection of tar particles in coal Demonstration of resin, fat and fat-like substances in wood, distribution of tar oil Detection of changes in tissue and chemical reactions.	Primary fluorescence	A
Testing of glued joints, differentiation of fluorescent slag and non-fluorescent ceramic and clinker particles (cement industry). Identification of various materials (whole specimens, polished sections, thin sections), mineralogy, crystallography	Primary fluorescence	A
Demonstration of aqueous solutions of non-acid mineral oils (food and chemical processing technologies).	Fluorol G, Fluorol-green gold K	A H3
Inclusions in fibers and fiber raw material.	Primary fluorescence Rhodamine B	A N 2.1
Examination of fibers (paper industry). Detection of optical brighteners, distinction between spring and summer wood, investigation of the degree of lignification.	Primary fluorescence Thioflavine S Calceine red extra Rhodamine 6 G, 6 GD, 6 GDN	A H 3, D
Detection and investigation of fine cracks and defects in metal surfaces (semiconductor industry, steel and metal-working industry	UV Orange Fluxa concentrate F Fluxa paste Deutroflux powder Magnaflux MET-L-check	A
Investigation of plastics with embedded glass fibers, work on interface problems during pressure, strain, etc.	MET-L-check FP 91	A H 3
Demonstration and detection of impurities in zinc oxide (ceramics, electro-ceramics).	Primary fluorescence	A H 3
Detection and investigation of pores and fine cracks in concrete and minerals.	Fluorol-green gold K Tinopal Rhodamine G	A H 3 M 2
Demonstration of impurities of plastic air jets, residues in quartz filters and dirt particles in foils (plastics and fiber industry).	Primary fluorescence	A H 3 M 2
Diagnosis of wood diseases	Acridine orange	H 3

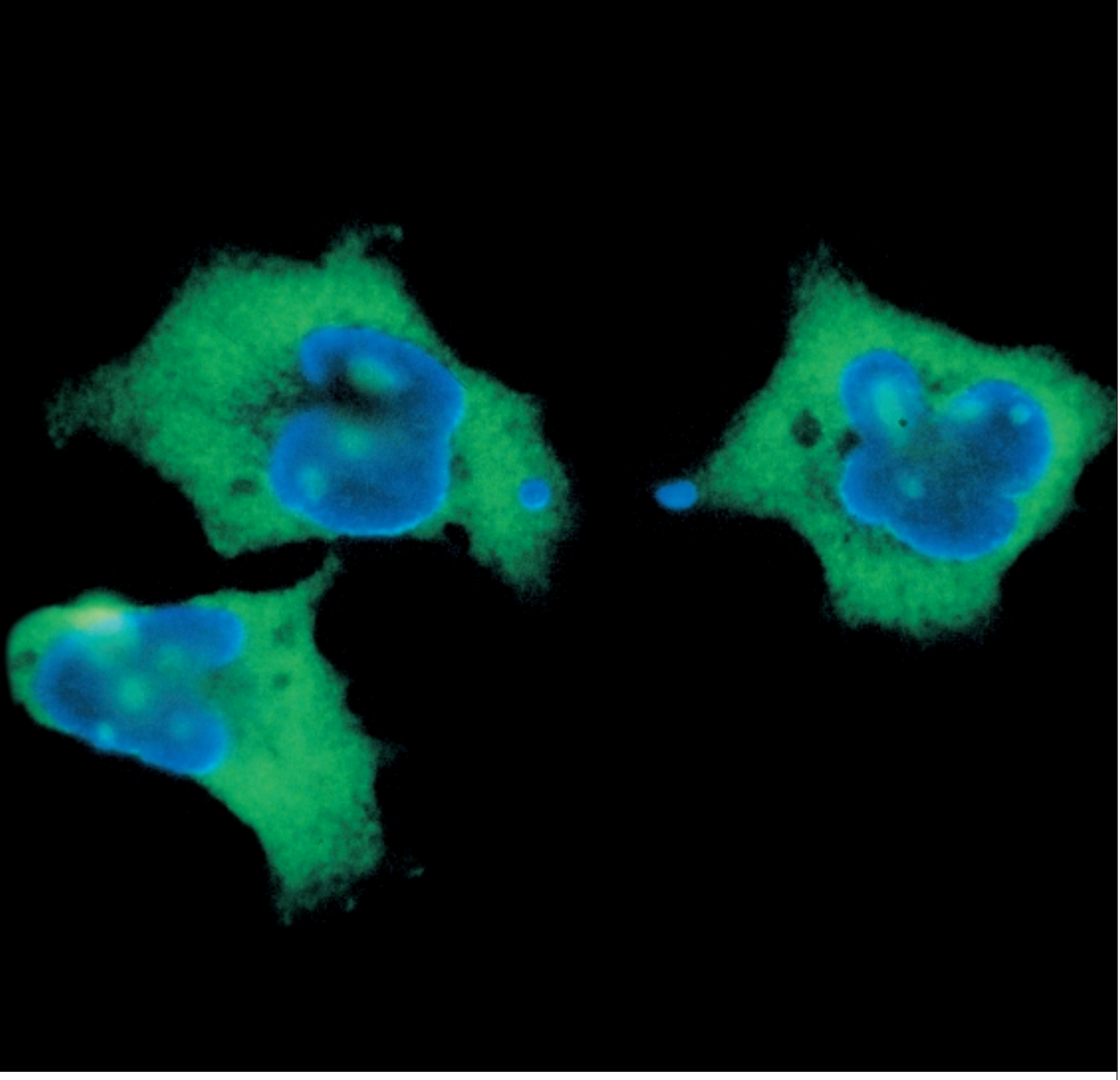






Fluorescence techniques in the electronics industry

Application	Filter cube	Magnification range
Detection of contamination on masks, wafers, pads and component groups caused by organic particles such as: Fibers, skin, dandruff, excretions, fingermarks, humidity, etc.	A, D, H 3, I 3, N 2.1	Low power High power
Detection of residual photoresist (negative)	H 3	Low power High power
Detection of residual photoresist (positive)	N 2.1	Low power High power
Detection of foreign and gel particles in photoresist	D	Low power High power
Detection of thickness variations of photoresist (striation)	D	Low power
Detection of inclusions, stains, holes, bridging, etc., after development	H 3, N 2.1	Low power High power
Detection of residues such as water, acids and chemicals	D, H 3, N 2.1	Low power High power
Checking purity and identity of chemicals	A, D, H 3, I 3, N 2.1	Low power High power



28 S ribosomal RNA in Hela cells;
FITC/DAPI

Light sources

Optimal fluorescence excitation in the specimen

Laboratory microscopes Leica DM LS (right) and Leica DM LB with 3 lambda and 4 lambda fluorescence illuminators, respectively

Adequate fluorescence intensity can only be obtained if the light source is strong in the near UV and visible short-wavelength regions. Ultra-high pressure mercury lamps and high pressure xenon lamps, two types of gas discharge lamp, are particularly suitable. The mercury lamps have a characteristic line spectrum with high intensity Hg lines.

In contrast, the xenon lamps emit a continuous spectrum in the visible region with a constant average intensity.

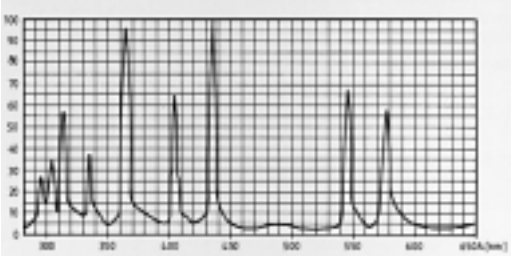
Ultra-high pressure mercury lamps are the most popular light sources for general fluorescence work due to their universal application potential, their strong ultra-violet, violet and green and adequate blue regions.

The standard light source for fluorescence microscopy is the Hg 100W ultra high pressure mercury lamp. The illumination optics of the incident light path in the microscope have been exactly matched to the size of the illuminated field of this lamp type, resulting in a significant improvement in the light flux and UV transmission.

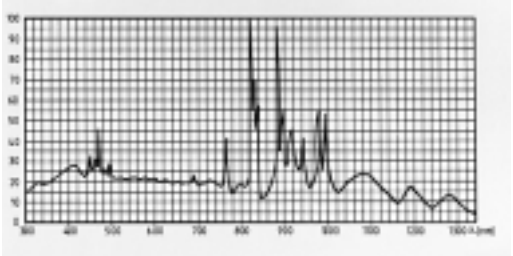
The Hg 100W and the xenon 75W high-pressure lamp are run on stabilized direct current for extremely steady burning. For routine fluorescence work in the laboratory, the more economical 50W Hg ultra high pressure lamp can be used if high luminosity is not required.

Two light sources can be coupled to the incident light path of the DM R/E and DM RX/E research microscopes. Switchable mirrors allow fast changing between e.g. a gas discharge and a halogen lamp (fluorescence and IGS observation).

Leica MZ FLIII
fluorescence stereomicroscope

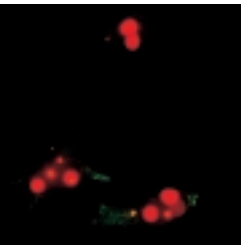


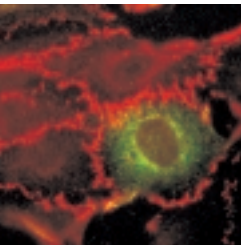
Relative spectral radiation distribution
of ultra high-pressure mercury lamps (Philips)



Relative spectral radiation distribution
of high-pressure xenon lamps (Philips)

Leica high performance objectives





High contrast and stunning clarity

Leica Microsystems offers different classes of objectives for fluorescence microscopy and photography. The performance and price of these classes are matched to the specific requirements of the particular application. For example, the N PLAN apochromat series is recommended for diagnostic tasks in clinical routine.

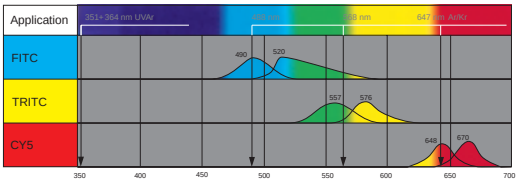
The HC(X) PL FLUOTAR semi-apochromats with chromatic correction are ideal for examining the myriad of specimens encountered in scientific microscopy. Their larger numerical apertures produce brighter fluorescence images and high optical resolution.

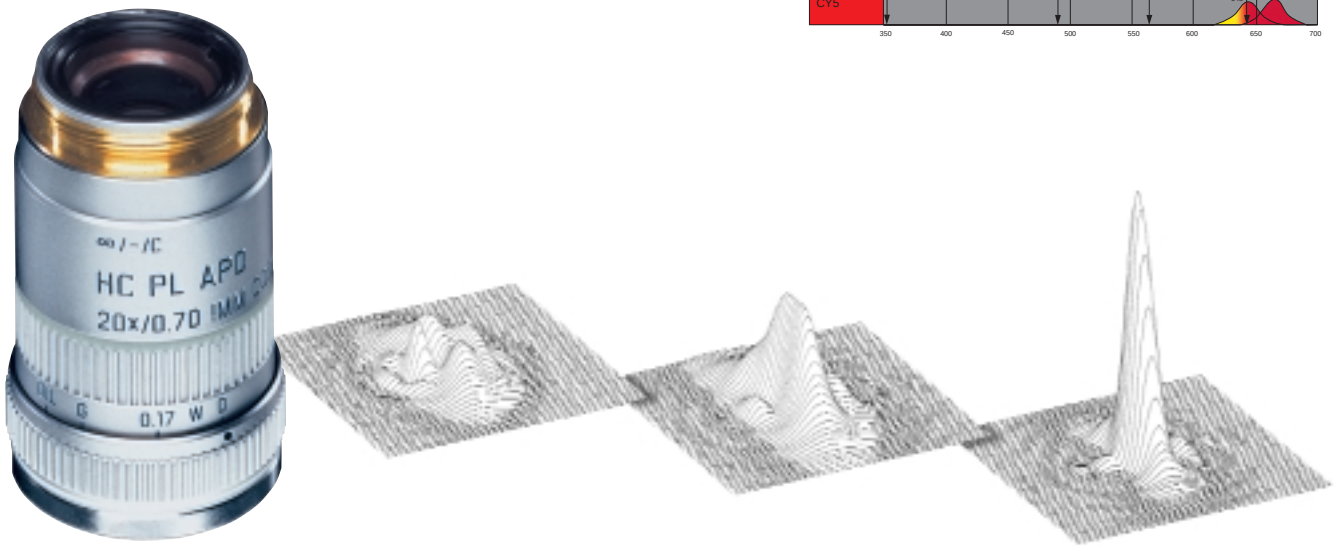
The highest requirements of modern fluorescence microscopy are met by Leica's HC(X) PLAN APOCHROMAT objectives. Besides outstanding field flattening and chromatic correction, they also feature unusually high UV-A transmission and minimal autofluorescence. The apertures of these objectives have been increased even further, resulting in phenomenally bright and vivid fluorescence images with superlative definition and contrast.

There are also special HC(X) PLAN APOCHROMAT immersion objectives (water, glycerine, OIL) in the lower and medium magnification range for specific fluorescence microscope techniques. Nearly all the brightfield objectives used for fluorescence microscopy are also available in a phase contrast version and are excellent for interference contrast observation.

There are many possibilities of combining fluorescence incident light observation with a wide variety of transmitted light contrasting techniques, either simultaneously or in alternation.

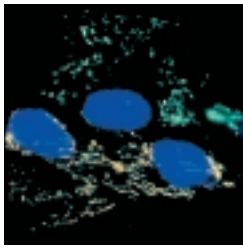
Further objectives for fluorescence microscopy are listed in the following tables.

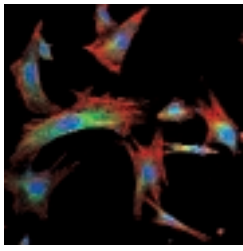


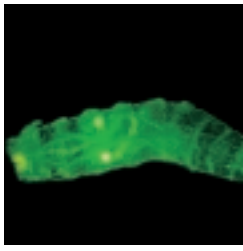


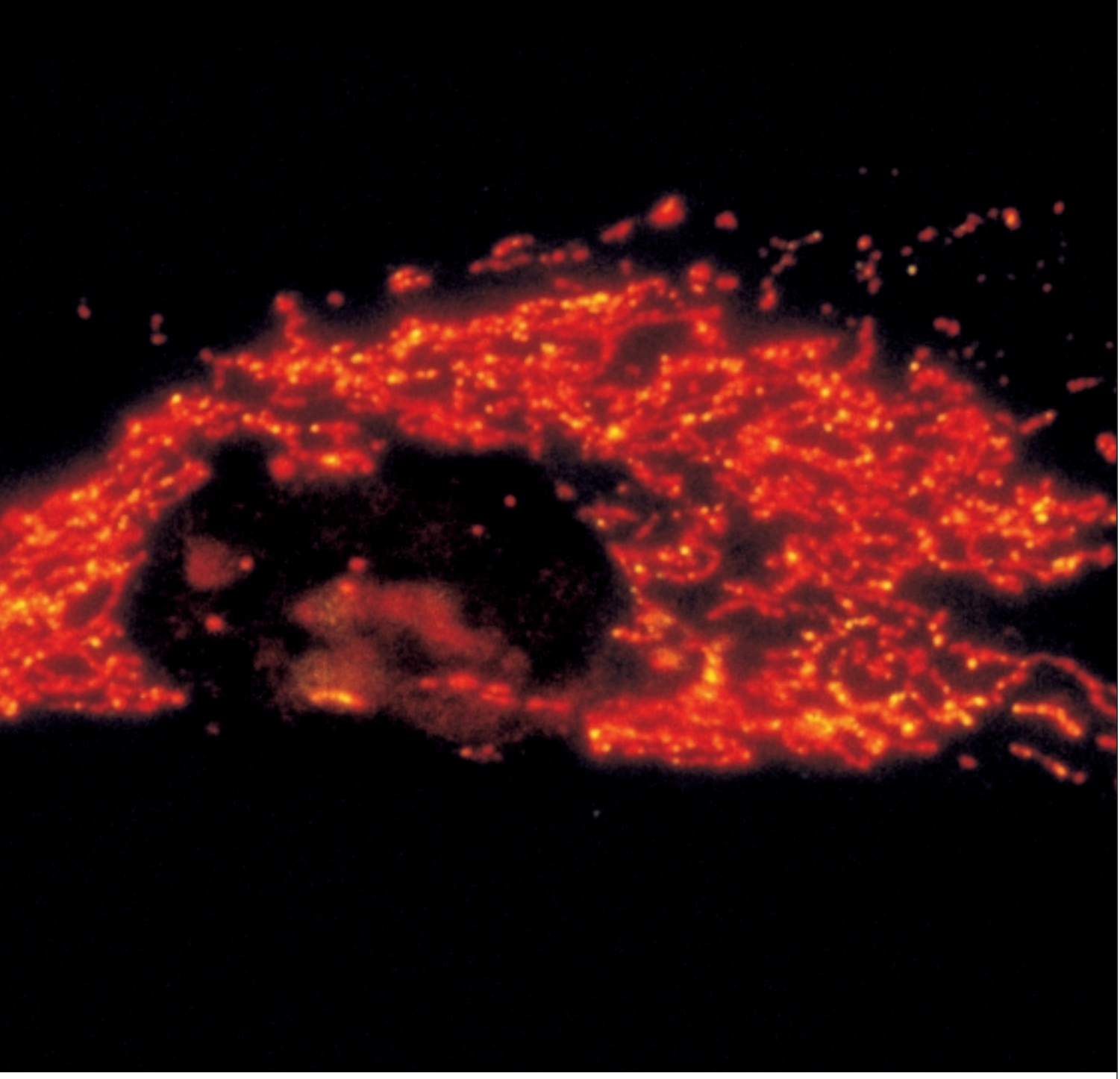
Leica objectives for brilliant fluorescence images

N PLAN objectives			
N PLAN	10x/0.25	N PLAN	10x/0.25 PH 1
N PLAN	20x/0.40	N PLAN	20x/0.40 PH 1
N PLAN	40x/0.65	N PLAN	40x/0.65 PH 2
N PLAN	50x/0.90 OIL		
N PLAN	100x/1.25 OIL	N PLAN	100x/1.25 OIL PH 3
N PLAN	100x/1.25-0.60 OIL		
N PLAN	100x/1.20-0.60 W		
HC(X) PL FLUOTAR objectives			
HC PL FLUOTAR	5x/0.15		
HC PL FLUOTAR	10x/0.50	HC PL FLUOTAR	10x/0.30 PH 1
HC PL FLUOTAR	16x/0.50 IMM	PL FLUOTAR	10x/0.50 IMM PH 2
HC PL FLUOTAR	20x/0.50	HC PL FLUOTAR	20x/0.50 PH 2
PL FLUOTAR	25x/0.75 OIL	PL FLUOTAR	25x/0.75 OIL PH 2
HCX PL FLUOTAR	40x/0.75	HCX PL FLUOTAR	40x/0.75 PH 2
PL FLUOTAR	40x/1.00-0.50 OIL	PL FLUOTAR	40x/1.00 OIL PH 3
HCX PL FLUOTAR	100x/1.30 OIL	HCX PL FLUOTAR	100x/1.30 OIL PH 3
HCX PL FLUOTAR	100x/1.30-0.60 OIL		
HC(X) PLAN APO objectives			
HC PLAN APO	10x/0.40	HC PLAN APO	10/0.40 PH 1
HC PLAN APO	20x/0.70	HC PLAN APO	20/0.70 PH 2
HCX PLAN APO	40x/0.85 CORR	HCX PLAN APO	40/0.75 PH 2
HCX PLAN APO	100x/1.35 OIL	HCX PLAN APO	100/1.35 OIL PH 3
HC(X) PL APO objectives			
HC PL APO	10x/0.40 IMM		
HC PL APO	20x/0.70 IMM CORR		
HCX PL APO	40x/1.25-0.75 OIL	HCX PL APO	40x/1.25 OIL PH 3
HCX PL APO	63x/1.32-0.60 OIL	HCX PL APO	63x/1.32 OIL PH 3
HC (X) APO U-V-I* objectives			
HCX APO	L 10x/0.30 W U-V-I		
HCX APO	L20x/0.50 W U-V-I		
HCX APO	L40x/0.80 W U-V-I		
HCX APO	L63x/0.90 W U-V-I		
HCX PL APO	40x/0.75 U-V-I		
HCX APO	100x/1.30 OIL U-V-I	HCX APO	100x/1.30 OIL U-V-I PH 3
*U-V-I = UV-Visible-IR			
Objectives with long free working distance			
N PLAN	L20x/0.40 CORR	N PLAN	L20x/0.40 CORR PH 1
N PLAN	L40x/0.55 CORR	N PLAN	L40x/0.55 CORR PH 2
PL FLUOTAR	L63x/0.70 CORR	PL FLUOTAR	L63x/0.70 CORR PH 2







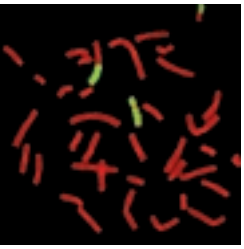


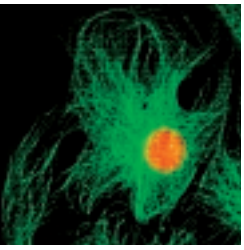
Double hybridization of mitochondrial DNA on skin fibroblasts: FITC/TRITC

Image documentation

Specifically for fluorescence microscopy

Leica DM IRB inverted fluorescence microscope for research with Leica DC100 digital camera





All new discoveries made with the aid of fluorescence microscopy need recording and documenting. One way of doing this is with conventional photomicrography, for which Leica offers various camera systems to suit different requirements and budgets.

Leica DM LD
Thanks to the modern chip technology of the new Leica DM LD microscope camera system, even the faintest fluorescence intensities and the finest specimen details are correctly exposed automatically. There are three exposure programs which can be used in either spot or integral mode for all illumination and contrasting techniques. An illuminated eyepiece graticule facilitates framing which is particularly critical in fluorescence work. A PC can easily be connected for operation, data capture, etc. (a corresponding Windows program is available).

Leica DM RD
The fully automatic Leica DM RD camera system, with integrated ergonomy viewing tube and motorized zoom, features a TV port and the possibility of connecting two cameras. The correct exposure time for any image detail can be measured with a 49-field measurement system. A highly sensitive photomultiplier acts as measuring cell. The following features ensure ergonomic operation:

- PC control
- Automatic switching of the beamsplitter in the tube
- Automatic matching of the color temperature
- Automatic flash exposure
- Automatic fading correction
- Automatic exposure series
- Automatic data capture and archiving

A completely different type of image recording is digital documentation, which is becoming in-creasingly popular for fluorescence applications in particular. Digitized recordings are displayed on the screen in brilliant quality in no time, they can be processed, stored in digital image archives, deleted or immediately used for further applications, such as printing, remote data transmission, multimedia application or internet publishing.

Leica DC 100/DC 200
For the most professional results, the image information is digitized directly at the CCD sensor of the Leica DC100/200 digital camera and displayed in real time in black and white on the screen. Thanks to progressive image scanning, the picture is constructed evenly and without flickering, enabling constant checking of definition and image area or further microscope parameters.
At the click of a mouse, the color image is then displayed on the screen in less than a second. Fluorescence exposures can be integrated for up to 13 seconds, so that even weak fluorescence can be recorded.

Leica Microsystems – the brand for outstanding products

Leica Microsystems' Mission is to be the world's first-choice provider of innovative solutions to our customers' needs for vision, measurement, lithography and analysis of microstructures.

Leica, the leading brand for microscopes and scientific instruments, has developed from five brand names, all with a long tradition: Wild, Leitz, Reichert, Jung and Cambridge Instruments. Leica symbolizes not only tradition, but also innovation.

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