

Product survey: Microscopes

# Slipping Through the Barrier

For more than 350 years microscope developers have approached the optical diffraction barrier in tiny steps. In the last ten years, however, they have taken gigantic measures, that have led them even beyond the barrier.

**B**y the help of microscopes, there is nothing so small, as to escape our inquiry; hence there is a new visible world discovered to the understanding. These farsighted words, which Robert Hooke, one of the trailblazers of compound microscopes, wrote down in his famous book *Micrographia* in 1664, are more relevant than ever, today. Especially the last decade has seen a tremendous progress towards the visualisation of ever smaller details inside living cells with optical microscopes. Novel microscopic methods are currently springing up like mushrooms after a warm rainfall, which makes it hard for non-experts to keep track of all the new visualisation techniques that have emerged in the last couple of years.

All of them, however, are on the same quest: to find the holy grail of microscopy, an objective that has been keeping microscope developers and manufacturers busy since the days of Robert Hooke and Antonie van Leeuwenhoek. Their main goal is to push the spatial resolution of optical microscopes to the very limit. According to Ernst Abbe's law, the spatial resolution is limited to approximately half the wavelength of light. In the case of visible light, ranging from 400 to 750 nanometres, this so called diffraction barrier restricts the resolution of any optical microscope to about 200 nanometres in the optical plane (x,y-axis) and 450 nanometres along the optical axis (z-axis). Since Abbe's law is fundamental, it cannot be broken – that doesn't mean, however, that the diffraction barrier may not be circumvented.

## Optical Loopholes

Like clever tax payers, who always find loopholes in fiscal laws, biophysicists working on super- or high-resolution optical microscopes have developed several tricks to slip through Abbe's diffraction barrier, pushing the current resolution limit to approximately 20 nanometres. Probably the

two most prominent super resolution techniques are Photo-Activation Localization Microscopy (PALM) and Stimulated Emission Depletion Microscopy (STED).

Eric Betzig, now at the Janelia Farm Campus at the Howard Hughes Medical Institute in Virginia, USA, already published the fundamental theories for PALM in 1995, in the journal *Optical Letters*.

However, the way from theory to practical application is often a bumpy road and it was almost ten years before he and his colleagues came up with a PAL-Microscope that worked. The high resolution power of PALM is based on the blinking and bleaching of single fluorescent molecules that are switched on and off by photoactivation. The blinking molecules are imaged and their exact positions are determined with nanometric accuracy. Repeated photoactivating and bleaching

leads to a map, containing the discrete positions of each fluorescent molecule that is converted into a high-resolution image of the sample.

Stefan Hell, now at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, may tell a similar story to Eric Betzig. Though he presented the theoretical background of STED microscopy as early as 1994 in *Optical Letters*, most experts were rather doubtful at that time as to whether his theory would ever work in practice. But Hell proved all sceptics wrong. His STED microscope has caused a big buzz in the last two years and even *Nature*, which had rejected Hell's manuscript of his preliminary STED microscope in 1999, celebrated the STED microscope in 2008 as 'Method of the year'.

As with PALM, the on and off switching of fluorescent molecules is at the heart of STED microscopy. In contrast to PALM, however, a whole ensemble of fluorescent molecules is turned on and off with two different laser beams. A regularly focused excitation beam is superimposed by a dough-

nut-shaped depletion beam leaving a non-depleted spot at the centre of the excitation profile with a diameter of approx. 20 nanometres. Since both excitation and depletion beams obey Abbe's law, the diffraction barrier is not broken. It's the non-linear reduction of fluorescence by stimulated depletion that squeezes the non-depleted fluorescent spot beyond the diffraction limit.

An international team centred around John Sedat, Mats Gustafsson and David Agard from the University of California, San Francisco, USA and Heinrich Leonhardt from the Ludwig Maximilians University, Munich, Germany, recently came up with another high resolution microscope that is causing a lot of excitement among Life Science researchers, called Structured Illumination Microscope (SIM).

SI microscopy, which is based on a concept published by Mats Gustafsson in 2000 in the *Journal of Microscopy*, follows a somewhat different approach to PALM and STED. Objects placed under a SI Microscope are illuminated with multiple interfering beams of light, showing a periodic intensity pattern that encodes high resolution spatial information from the sample into multiple moiré images. The moiré patterns are computationally reconstructed and transformed into a real image of the sample.

## Manageable sales numbers

Though the SI Microscope reaches a resolution of 'just' 100 nanometres, it has two major advantages over its rivals PALM and STED: it is able to detect three different wavelengths in the same sample and the resolution is enhanced in lateral (x,y) and axial (z) directions, making it particularly suitable for three-dimensional microscopy.

Both STED and SI Microscope are already commercially available. Since they are brand new and rather expensive, the sales numbers are still pretty modest. Currently, the SIM, also called OMX for Optical Microscope experimental, has taken the lead with seven units sold against one STED microscope unit. But only time will tell as to which high resolution microscope(s) will finally conquer the laboratories.

HARALD ZÄHRINGER



Robert Hooke's compound microscope.

## Microscopes

Company	Name of product	Resolution	Contrasting Technique(s)
<b>Carl Zeiss MicroImaging</b> Göttingen, Germany www.zeiss.de/micro <b>Contact:</b> Phone: +49(0)551 5060 660 micro@zeiss.de	Primo Star	Depends on the used objective, e.g. Plan-Achromat 4x - 100x	Transmitted Light, Bright Field, Darkfield, Phase Contrast
	Axio Scope	150x	Transmitted Light, Brightfield, Reflected Light, Darkfield, Phase Contrast, Fluorescence, DIC, PlasDIC
	Axio Observer	150x	Transmitted Light, Brightfield, Reflected Light, Darkfield, Phase Contrast, Fluorescence
	Stemi DV4	Depends on objective 2,4 - 64x	Transmitted Light, Brightfield, Reflected Light, Darkfield
	Stemi 2000	Depends on objective 6,5 - 50x	Transmitted Light, Brightfield, Reflected Light, Darkfield
<b>GeSiM</b> Grosserkmannsdorf, Germany www.gesim.de <b>Contact:</b> Steffen Howitz or Frank-Ulrich Gast Phone: +49-351-2695-322 info@gesim.de	MicCell (microscope accessory: PDMS-based microfluidic system with standard chip-to-world connections; sizes 22 x 22, 22 x 50, 25 x 75 mm)	n/a (depends on microscope)	n/a (depends on microscope)
	µCP 2.1 (microscope accessory: motorized z-axis for automatic microcontact printing)	Resolution of printed structures can be as small as 0.1 µm	n/a
<b>ibidi</b> Martinsried (Munich), Germany www.ibidi.de <b>Contact:</b> uraedler@ibidi.de Phone: +49(0)89 520461731	EVOS Microscope	4x, 10x, 20x, 40x objectives	Phase contrast
<b>Intas Science Imaging Instruments</b> Göttingen, Germany www.intas.de <b>Contact:</b> info@intas.de Phone: +49(0)551 50 50 50	Nuance FX Multi-Spectral Microscopy System	The Nuance has for each 1.4 Megapixels a spectral resolution of 420 - 720 nm in 1 nm steps. Each pixel generates a full spectrum of up to 300 datapoints. 10x, 20x and 40x Zeiss Plan Achromat Objectives	Multi Spectral Imaging, Brightfield, Fluorescence
<b>Keyence Germany</b> www.Keyence.de www.digitalmikroskop.de	Keyence BZ Fluorescence Microscope	Maximum of Light Microscopy	Light Microscopy, Fluorescence Microscopy, Brightfield Observation, Darkfield Observation, DIC Contrast, OTC Contrast
	Keyence VK Color Laser-Scanning-Microscope	1nm resolution	Confocal Microscopy
	Keyence VHX digital microscope	54 Megapixel CCD-Chip	Light Microscopy, D.I.C., Polarized Light
<b>Leica Microsystems</b> Wetzlar, Germany www.leica-microsystems.com <b>Contact:</b> Phone: +49(0)6441-29-0	Leica DMI6000 B	■ Enlargement: 1.25-100x (objectives) ■ Resolution: up to 230 nm ■ Observation area: 250 mm - 20 mm	Fluorescence stimulation, External filterwheels, Fully automated, inverted contrast method, Different CCD- and EM-Gain-Cameras
	Leica M205 FA	■ Enlargement: 0.5 - 2x (objectives), zoom factor 7.8 - 160x with 1x objective ■ Resolution: up to 476 nm ■ Observation area: 1.44 - 29.5 mm	Fluorescence stimulation, Different transmitted and reflected contrast methods, Different CCD- and EM-Grain-Cameras (coloured and monochrome)
	Leica TCS SP5 X	■ Enlargement: 5 - 100x (objectives) ■ Resolution: up to the optical resolution barrier ■ Observation area: up to 8.192 x 8.192 pixel-picture size, 22 mm frame	Variable white-light lasers in 1nm steps for the excitation, Confocal detection including up to 5 simultaneous photomultipliers
<b>MDS Analytical Technologies</b> Wokingham, Berkshire, UK www.moleculardevices.com <b>Contact:</b> James Ford Phone: +44(0)118 944 8000 infobox@moldev.com	ImageXpress Micro	Depends on Objective used: 1x - 100x available	Fluorescence, 300 Watt Xenon Lamp excitation and filter cubes for excitation and emission
	ImageXpress Ultra	Depends on Objective used: 4x - 100x available	Fluorescence, Laser Excitation up to four channel excitation and emission standard with 488 and 635 nm Lasers 405, 532 and 561 nm laser options
<b>Nikon</b> Mikroskope/Optische Messtechnik Düsseldorf, Germany www.nikoninstruments.eu <b>Contact:</b> Ulrike Will Phone: +49(0)211-9414214 lmikroskope.messtechnik@nikon.de	50i/55i	Objective 1...100x	Brightfield, Darkfield, Phase Contrast, Fluorescence, simple Polarisation
	80i	Objective 1...100x	Brightfield, Darkfield, Phase Contrast, Fluorescence, simple Polarisation, DIC
	90i	Objective 1...100x	Brightfield, Darkfield, Phase Contrast, Fluorescence, simple Polarisation, DIC
	TS-100 invers	Objective 4...40x	Brightfield, Phase Contrast, Hoffmann-Modulation-Contrast, Fluorescence
	Ti-E/U/S invers	Objective 2...100x	Brightfield, Phase Contrast, „external“ Phase Contrast, Hoffmann-Modulation-Contrast, simple Polarisation, DIC, Darkfield, TIRF, Fluorescence

Application(s)	Miscellaneous, Specialities, Generally	Price [EUR]
Education and Routine	<ul style="list-style-type: none"> <li>■ Halogen, optional LED</li> </ul>	On request
From Routine to Entry-Level Research in BioMedical applications	<ul style="list-style-type: none"> <li>■ Adaptable allround-stand</li> </ul>	On request
Cell observation, cell manipulation and cell analysis and Live Cell Imaging	<ul style="list-style-type: none"> <li>■ Fully integrated research platform</li> </ul>	On request
Education and Routine	<ul style="list-style-type: none"> <li>■</li> </ul>	On request
Routine	<ul style="list-style-type: none"> <li>■</li> </ul>	On request
Cell perfusion (e.g. shear stress, microelectrode arrays), cell adhesion tests, single-molecule/single-particle handling, laser tweezers, kinetic measurements, electrophoresis, etc.	<ul style="list-style-type: none"> <li>■ Available for many inverted and upright microscopes</li> <li>■ All external control devices in one "Fluid Processor" box</li> <li>■ Many accessories</li> <li>■ GUI-based software for interactive and automatic operation</li> <li>■ Fully modular and customizable</li> </ul>	On request
μCP device mounted on top of an inverted microscope; μCP or NIL under microscope control	<ul style="list-style-type: none"> <li>■ Allows reproducible and accurate microcontact printing and nanoimprinting lithography (NIL) <ul style="list-style-type: none"> <li>■ Printing pressure pneumatically controlled</li> <li>■ Includes casting station for PDMS stamps</li> </ul> </li> <li>■ Customized Teflon-coated micro- or nanostructured silicon masters available <ul style="list-style-type: none"> <li>■ Peripherals: computer and compressor</li> </ul> </li> </ul>	On request
Cell routine work Manipulation on cells Stem cell workdocumentation	<ul style="list-style-type: none"> <li>■ No oculars</li> <li>■ Directly connected via a camera to a screen</li> <li>■ CMOS camera integrated</li> <li>■ Images stored on an SD card <ul style="list-style-type: none"> <li>■ Small footprint</li> </ul> </li> <li>■ Can be placed in a hood <ul style="list-style-type: none"> <li>■ Ergonomic design</li> </ul> </li> </ul>	Around 10.000,-
Detections and separation of overlapping but spectral different Fluorophores or Brightfield Chromogens. Multiplexed Stains will be unmixed from Autofluorescence or Brightfield Counterstains. FISH FRET IHC Tissue Pathology	<ul style="list-style-type: none"> <li>■ Compute pure spectrum</li> <li>■ Real Component Analysis</li> <li>■ Colocalization measurement tool</li> </ul>	79.000,-
Life Science Microscopy of all kind	<ul style="list-style-type: none"> <li>■ Integrated dark room <ul style="list-style-type: none"> <li>■ Fully automatic usage, easy-to-use</li> </ul> </li> <li>■ Z-Stack and 3-D-Observation <ul style="list-style-type: none"> <li>■ Haze Reduction Function</li> </ul> </li> </ul>	Starting from 47.000,-
Material Microscopy, R&D-purposes, Quality control	<ul style="list-style-type: none"> <li>■ Easy-to-use <ul style="list-style-type: none"> <li>■ Roughness Measurements</li> </ul> </li> <li>■ Profile Measurements</li> </ul>	Starting from 70.000,-
Material Microscopy, R&D-purposes, Quality control	<ul style="list-style-type: none"> <li>■ Highest depth of field</li> <li>■ Quick 3-D model and measurement,</li> </ul>	Starting from 35.000,-
Cell biology, Biochemistry, Developmental Biology	<ul style="list-style-type: none"> <li>■ Optional fully automated Multi-Colour-TIRF with controlled penetration into the evanescent field <ul style="list-style-type: none"> <li>■ Deconvolution &amp; 3D-Imaging</li> </ul> </li> </ul>	On request
Developmental Biology, Phytology, Zoology	<ul style="list-style-type: none"> <li>■ FusionOptics for highest resolution on maximum definition</li> <li>■ FluoCombi III for a parallax-free image with microscope-objectives</li> </ul>	On request
Each scientific division using confocal microscopes	<ul style="list-style-type: none"> <li>■ Fully automated absorption of excitation spectrums</li> <li>■ Variable laser allows optimal excitation of dyes</li> <li>■ Flexible excitation and detection to avoid fluorescent signal cross-talk <ul style="list-style-type: none"> <li>■ Usage of yet not excitable dyes possible</li> </ul> </li> </ul>	On request
Automated Microscopy and High Content screening	<ul style="list-style-type: none"> <li>■ Options include Transmitted LightEnvironment control (temp, CO2 and humidity) <ul style="list-style-type: none"> <li>■ Fluidics Robot</li> <li>■ Robotics solution available</li> </ul> </li> </ul>	On request
Automated Microscopy and High Content screening	<ul style="list-style-type: none"> <li>■ True Point Scanning confocal instrument with adjustable pinhole</li> <li>■ Robotics solution available</li> </ul>	On request
Upgradable microscope for clinical and biomedical application	<ul style="list-style-type: none"> <li>■ Also LED-version</li> </ul>	4.100,- to 19.000,-
Microscope for laboratory, research + imaging for biomedical application	n.a.	7.400,- to 35.000,-
Microscope for research + imaging for biomedical application	n.a.	18.000,- to 65.000,-
Routine cell culture	n.a.	2.800,- to 13.000,-
Biomedical imaging: Fluorescence microscopy, Live cell imaging (incl. FRET; FRAP, PA-GFP, TIRF, CLSM) immune histology IVF	n.a.	12.000,- to 120.000,-

## Microscopes

Company	Name of product	Resolution	Contrasting Technique(s)
<b>Nikon</b> Contact see page 58	FN-1	Objective 1.6...100x	Brightfield, DIC, simple Polarisation, Schattenwurf-Reliefkontrast, Fluorescence
	SMZ-1500	0.75...11.25x Zoom, 0.5...1.6x Objective	Brightfield, Darkfield, OCC-Contrast, simple Polarisation, coaxial illumination
	SMZ-800/1000	0.8...8x Zoom, 0.5x...2x Objective	Brightfield, Darkfield, OCC-Contrast, simple Polarisation, coaxial illumination
	AZ-100	Objective 0.5x - 5x, Zoom 8:1	Brightfield, DIC, simple polarisation, OCC-contrast, fluorescence, coaxial illumination, LED
	BioStation IM	10x - 80x	Phase contrast, fluorescence
	BioStation CT	2x - 40x	Phase contrast, fluorescence (LED)
	eC1plus	4x - 100x	3-channel acquisition, transmission detector
	A1	4x - 100x	4-channel acquisition, transmission detector, true spectral detection
	A1-R	4x - 100x	4-channel acquisition, transmission detector, true spectral detection
<b>Olympus Life Science Europe</b> Hamburg, Germany www.olympus-europa.com Contact: Esther Ahrent Phone: +49(0)40 23773-0 info@olympus-europa.com	BX2 Upright Research Microscopes	Optimised for each magnification	Darkfield, Brightfield, Phase contrast, Polarisation, DIC, Fluorescence
	IX2 Upright microscopes	Optimised for each magnification	Darkfield, Brightfield, Phase contrast, Polarisation, DIC, Fluorescence
	SZX2 Stereo microscopes	Optimised throughout zoom range (max 900 LP/mm)	Darkfield, Oblique, Brightfield, Phase contrast, Polarisation, DIC, Fluorescence
	FV1000 Confocal Laser Scanning microscope	Peerless optical and wavelength resolutions	Confocal fluorescence, DIC, Brightfield, Phase contrast, Darkfield, Polarisation
	FV1000MPE Multiphoton system	Multiphoton resolution and clarity	Deep fluorescence
	LV200 Luminoscope	Peerless resolution for bioluminescence microscopy	Brightfield, DIC, Luminescence, Fluorescence
	MVX10 Macroscope	Excellent resolution throughout zoom range	Fluorescence, Brightfield, DIC, Darkfield
	LEXT OLS4000 measuring confocal laser scanning microscope	120 nm X/Y; 10 nm Z	Polarisation-based confocal, Laser DIC, Brightfield
	cell^M and cell^R	Optimised for each magnification	Fluorescence, Darkfield, Brightfield, Phase contrast, Polarisation, DIC
	scan^R	Optimised for each magnification	Fluorescence, Brightfield, DIC
	dotSlide	Optimised for each magnification	Brightfield
<b>Partec</b> Görlitz, Germany www.partec.com Contact: Ivana Guskowski Phone: +49(0)3581 87460 i.guskowski@partec.com	CyScope Plus Malaria - LED Fluorescence Microscope	20x, 40x and 100x oil immersion lenses, 10x wide field / 18 mm binocular	-
	CyScope Plus TB - LED Fluorescence Microscope	20x, 40x and 100x oil immersion lenses, 10x wide field / 18 mm binocular	-
	CyScope Plus HP - High Power LED Fluorescence Microscope	20x, 40x and 100x oil immersion lenses, 10x wide field / 18 mm binocular	Polarization microscopy and other techniques

**Addendum:** our product survey in the last issue of *Lab Times* covering 2D Electrophoresis Systems omitted the Wita company products.

## 2D Electrophoresis Systems

Company	Name of product	Short description	Gel format/Number of gels per run?
<b>Wita</b> Teltow, Germany www.wita.de Contact: H.-R. Graack Phone: +49(0)3328-394933 graack@wita.	WITAvision	Equipment for 1st and 2nd dimension of NEPHGE 2-DE including gel casting stand for casting of 1st dim gels, and transfer moduls for easy to handle transfer of 1st dim. Gels to the 2nd dim. Apparatus, User training included	1st dim.: tubing gels 0.9 - 1.5 mm diameter, 70, 225, or 410 mm in length  2nd dim.: gels 225 x 300 mm, 410 x 300 mm, modular 1 - 10 simultaneous individual 1D gels, and 1 - 5 individual 2D gel plates (Pentapack)

Microscopes		
Application(s)	Miscellaneous, Specialities, Generally	Price [EUR]
Fixed-stage-microscope for physiological applications	n.a.	16.000,- to 50.000,-
n.a.	n.a.	7.800,- to 27.000,-
n.a.	n.a.	3.000,- to 25.000,-
Multi-purpose-mono-zoom-microscope	n.a.	10.000,- to 42.000,-
Long-term time-lapse imaging	■ Long-term time-lapse imaging system	50.000,-
Monitoring of sensitive cells, IVF, Stem cells, Cell screening, Development	■ Automated cell-culture and observation system	250.000,-
Standard Confocal Imaging	■ Confocal imaging for everyone	70.000,-
Advanced Confocal Imaging including true spectral detection	■ Highest sensitivity	200.000,- to 400.000,-
Fast Confocal Imaging incl. techniques like FRAP, FRET, Photo-activation	■ Resonant and hybrid scanner	300.000,- to 500.000,-
Routine to advanced research microscopy including water immersion	■ World-leading optics ■ Ergonomic design	On request
Routine to advanced research microscopy	■ Multiport design ■ World-leading optics	On request
Routine to advanced stereo microscopy	■ Refractive aberration correction ■ Highly flexible	On request
Advanced confocal microscopy with dual scanner and TIRFM options	■ Dual scanners ■ Optical bench principle	On request
3 World-leading multiphoton microscope systems	■ Simultaneous imaging and stimulation	On request
Unrivalled sensitivity and resolution in bioluminescence microscopy	■ Complete bioluminescence system	On request
Research macro zoom fluorescence microscopy	■ Macro to micro zoom fluorescence	On request
Advanced optical metrology and 3D surface analysis (405nm laser)	■ Accurate and repeatable optical metrology	On request
Advanced high-speed live cell imaging	■ Capture every cellular event	On request
High-speed screening for multiple fluorescence markers	■ Modular advanced multi-component screening	On request
Flexible virtual microscopy for clinical and research applications	■ Virtual microscopy, global discussion	On request
Malaria Parasites Diagnosis with ready-to-use Malaria test slides	■ Battery Operation - UV (365 nm) LED ■ USB CCD camera ■ White light LED ■ Made in Germany	2.620,-
TB Diagnosis using Auramine staining	■ See above except Royal Blue (455 nm)	2.620,-
Immunofluorescence, Cell Biology, Fluorescence and Light Microscopy	■ High Power Blue 470 nm LED ■ USB CCD camera ■ White light LED ■ Made in Germany	3.460,-

## 2D Electrophoresis Systems

Miscellaneous, Specialities, Generally	Price [EUR]
<ul style="list-style-type: none"> <li>■ Individual gel cooling</li> <li>■ Gel casting within the apparatus</li> <li>■ Gradient gel possible</li> <li>■ DIGE possible</li> </ul>	Depending on purchase of number of modular parts of the equipment. Demonstration and price information on request