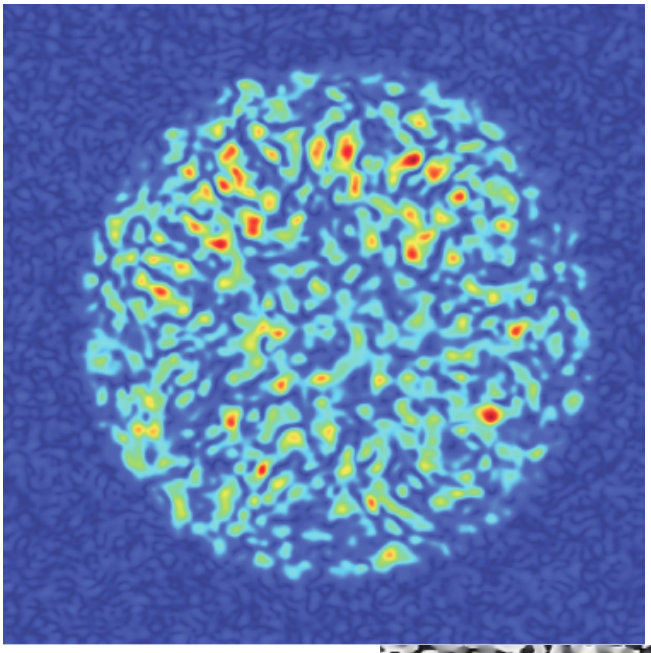
SSOM

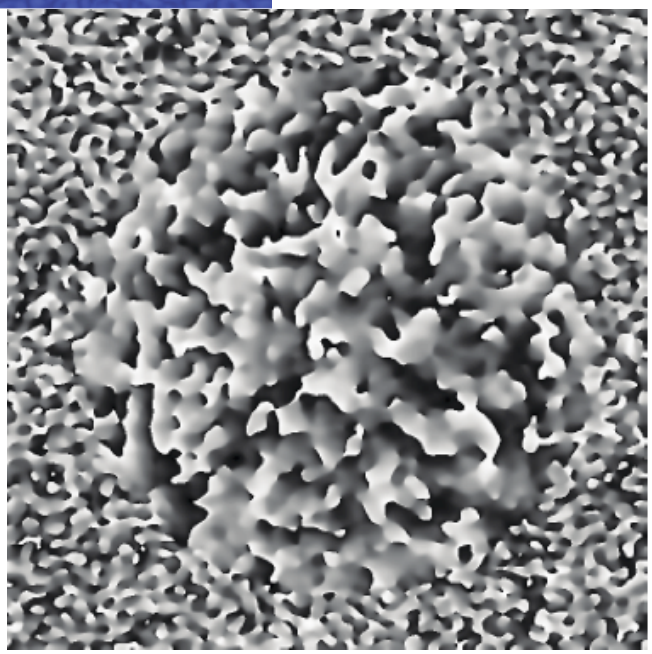
Swiss Society for Optics and Microscopy

Société Suisse pour l'Optique et la Microscopie

Schweizerische Gesellschaft für Optik und Mikroskopie

Mitteilungsblatt / Bulletin d'information 3+4 / 2012





Endomicroscopy with multimode fibers

# SWISS SOCIETY FOR OPTICS AND MICROSCOPY

www.ssom.ch

# BOARD MEMBERS 2012-2013

## *President*

**Dr. Markus Dürrenberger**
Zentrum für Mikroskopie, Pharmazentrum, Universität Basel, Klingelbergstrasse 50, CH-4056 Basel
Tel. +41 61 267 14 04, Fax +41 61 267 14 10
markus.duerrenberger@unibas.ch

## *Treasurer*

**Gianni Morson**
Zentrum für Mikroskopie, Pharmazentrum, Universität Basel, Klingelbergstrasse 50, CH-4056 Basel,
Tel. +41 61 267 14 06 , Fax +41 61 267 14 10
gianni.morson@unibas.ch

## *Redactor*

**Dr. Reto Holzner**
Apfelbaumstrasse 2, CH-8050 Zürich Tel. +41 44 312 15 63,
reto.holzner@hispeed.ch

## *Webmaster*

**Dr. Nadine Matthes**
Solvias AG**,** Römerpark 2**,** CH- 4303 Kaiseraugst,
Tel. +41 61 845 65 07**,** nadine.matthes@solvias.com

## *Optics Section Vice President*

**Prof. Dr. Beat Neuenschwander**
Technik und Informatik, Berner Fachhochschule, Pestalozzistrasse 30, CH-3400 Burgdorf, Tel. +41 34 426 42 20, Fax. +41 34 423 15 13,
beat.neuenschwander@bfh.ch

## *Microscopy Section Vice President*

**Dr. Marco Cantoni**
EPFL SB CIME-GE , MXC 135 (Bâtiment MXC) , Station 12 , CH-1015 Lausanne, Tel +41 21 693 48 16, Fax +41 21 693 44 01,
marco.cantoni@epfl.ch

## *Nanotechnology Section Vice President*

**PD Dr. Harry Heinzelmann**
CSEM, Nanoscale Technology & Biochemical Sensing, Jaquet-Droz 1, CH-2007 Neuchâtel , Tel. +41 32 720 55 33 , Fax +41 32 720 57 50,
harry.heinzelmann@csem.ch

## *Optics Section Board*

**Dr. Markus Rossi**
Heptagon Oy, Moosstrasse 2, CH-8803 Rüschlikon, Tel. +41 44 497 30 03, Mobile +41 79 470 24 91, Fax +41 44 497 30 01,
markus.rossi@heptagon.fi

**Prof. Dr. Markus W. Sigrist**
Institut für Quantenelektronik, ETH-Hönggerberg HPF D 19, CH-8093 Zürich, Tel. +41 44 633 22 89, Fax +41 44 633 12 30,
sigrist@iqe.phys.ethz.ch

**Dr. Berthold Schmidt**
R&D Advanced Devices, Bookham, Postfach, Binzstrasse 17, CH-8045 Zurich, Tel. +41 44 455 85 04, Fax +41 44 455 85 86,
berthold.schmidt@bookham.com

**Prof. Dr. Hans Peter Herzig**
Institut de Microtechnique, Rue A.-L. Breguet 2, CH-2000 Neuchâtel, Tel. +41 32 718 32 70, Fax +41 32 718 32 01,
hanspeter.herzig@unine.ch

**Dr. Martin Forrer**
Fisba Optik AG, Rorschacher Str. 268, CH-9016 St.Gallen, Tel. 071 282 31 81, Fax 071 282 33 02 , martin.forrer@fisba.ch

**Prof. Dr. Andreas Ettemeyer**
Labor für Technische Optik, Interstaatliche Hochschule für Technik Buchs NTB, Werdenbergstrasse 4, CH-9471 Buchs, Tel. +41 81 755 34 87, andreas.ettemeyer@ntb.ch

**Prof. Dr. Christophe Moser**
Laboratory of Applied Photonics, EPFL-LAPD, Devices. Station 17, BM 4111Tel. +41 21 69 36110 (35185), christophe.moser@epfl.ch

## *Microscopy Section Board*

**Dr. Patrick Schwarb**
Novartis Forschungsstiftung, FMI, Maulbeerstrasse 66, WRO-1066.2.16, CH-4058 Basel, Mob +41 79 353 49 65
schwarb@imagic.ch

**Dr. Dimitri Vanhecke**
Bio-Nanomaterials, Adolphe Merkle Institute, Université de Fribourg, Balmweg 30, CH-3007 Bern,Tel: +41 76 430 60 66,
dimitri.vanhecke@unifr.ch

**Dr. Roger Wepf**
Elektronenmikroskopie-Zentrum, ETH Zürich, HPI F 13, Wolfgang-Pauli-Str. 14, CH-8093 Zürich, Tel. +41 44 633 45 58
roger.wepf@emez.ethz.ch

**Dr. Robin Schäublin**
EPFL SB CRPP Groupe Matériaux, Paul Scherrer Institut, ODGA, CH-5232 Villingen-PSI, Tel. +41 56 310 40 82, Fax +41 56 310 45 29, robin.schaeublin@psi.ch

**Dr. Lorenz Holzer**
Hochleistungskeramik , EMPA, Überlandstr.129, CH-8600 Dübendorf, Tel. +41 44 823 44 90, lorenz.holzer@empa.ch

**Dr. Sousan Abolhassani**
Lab. for Materials Behaviour OHLD/104, Paul Scherrer Institut, CH-5232 Villigen PSI, Tel. +41 56 310 21 91 Fax +41 56 310 22 05,
sousan.abolhassani@psi.ch

**Dr. Rolf Erni**
Electron Microscopy Center, EMPA,Überlandstr. 129, CH-8600 Dübendorf, Tel. +41 44 823 40 80, Fax +41 44 823 40 80,
rolf.erni@empa.ch

## *Nanotechnology Section Board*

**Prof.Dr. Christian Schönenberger**
Institut für Physik, Universität Basel, Klingelbergstrasse 82, 4056 Basel, Tel: +41 61 267 36 90, Fax : +41 61 267 37 84
christian.schoenenberger@unibas.ch

**Dr. Jens Gobrecht**
Paul Scherrer Institute, Laboratory for Micro- and Nanotechnology, CH - 5232 Villigen-PSI, Tel: +41 56 310 2529, jens.gobrecht@psi.ch

## *Biomedical Photonics working group*

**PD Dr. Martin Wolf**
Clinic of Neonatology, University Hospital Zurich, Frauenklinikstr. 10, CH-8091 Zurich, Tel. +41 44 2555346, Fax: +41 44 2554442
martin.wolf@alumni.ethz.ch

# *Table of content*

**SSOM**

# *From the President*

Dear members,

the Swiss Society for Optics and Microscopy has traditionally an excellent relationship to international organizations. This year Roger Wepf has been elected as president of the EMS. Congratulations. He is after Ueli Aebi the second SSOM-member being president of the EMS.
Christophe Moser and Harry Brandenberger contributed the scientific articles published in this issue. Thanks to the authors.
If you feel that you have also an interesting contribution, please contact me our redactor (Reto Holzner).
Our webpage still lacks originality. It should live also from your contributions. If you have a picture of the month, a publication or an activity in your scientific field, please contact our webmaster (Nadine Matthes).
The latest news about events you will find always on the SSOM homepage (www.ssom.ch).

I wish you an interesting lecture.

Markus Dürrenberger
President SSOM

# *Endomicroscopy with multimode fibers*

## Introduction

Endomicroscopy is an endoscopic imaging modality which allows imaging biological material in vivo. There are a number of approaches to make minimally invasive, i.e small diameter, endomicroscopes. They are either based on a bundle of small diameter optical fibers (up to 100,000) which deliver illumination and at the same time collect backscattered light [1-4]. The fiber-to-fiber pitch is approximately 3 micrometers which limits the lateral resolution of this technique. Another approach is to use a single mode fiber. At the distal end of the fiber, a small lens is positioned to focus the output from the fiber. By scanning the fiber tip with actuators, an image is formed by collecting the scattered light fiber point by point with surrounding multimode fibers. This technique, called scanning fiber endoscopy (SFE), yields sub-micrometer resolution with a reported minimum diameter of 1.0 mm [5]. Yet another method is to use a small CMOS/CCD camera directly at the tip of a flexible shaft that also delivers illumination. Fiber bundles (FB) have nevertheless remained the main-stay in small flexible endoscopes and provide sufficient sampling resolution in many applications. However, the low mechanical flexibility of the FB deters the use of high core count bundles. For this reason, alternative technologies such as SFE and custom CCD and CMOS image sensors are in development to promise higher resolutions and better mechanical properties. However, expense and technical complication surrounding color, packaging and electronic characteristics, have thus far prevented their adoption. When optical zoom is added to any endoscopes to dynamically change the field of view, for example to form a large field of view for navigation and a small field of view for detailed imaging, the size of actuators increases the footprint of the endoscope (Pentax endoscope 10 mm in diameter and 1 mm field of view). A comprehensive review of these technologies is described in detail in [5].

## Multimode fiber endoscope: principle of operation

We have recently proposed a new approach [6,7] based on a single multimode fiber, which combines the passive feature of fiber glass bundles with the high resolution and wide field of view of active endoscopes. Within this year, several other groups have successfully applied wavefront optimization based on iterative methods in order to achieve imaging through a multimode optical fiber [8-12]. The field is thus very dynamic and results to fast advances in ultra-thin endoscopes based on multimode fibers.

We have experimentally demonstrated a record ultrathin endoscope of diameter 0.5 mm, capable of imaging a field of view of 200 µm with sub-micrometer resolution. The image is formed without moving parts by external electronic means only.

The working principle of the endoscope is illustrated in figure 1. A phase only spatial light modulator (SLM) produces a “specific” modulated wavefront to the input of the multimode fiber such as to create a clean focal spot at the output of the fiber. A multimode fiber supports many optical modes and as such the output wavefront is typically a speckle pattern. However, there exist an input wavefront to a multimode fiber such that the output is a clean, speckle free, focus. This can be intuitively understood by a time reversing argument: a multimode fiber guide is a linear system with an input and output satisfying Maxwell’s equation. Maxwell’s equation remains the same if the time is reversed meaning that if the output speckles wavefront is time-reversed, the reverse propagation produces the input.

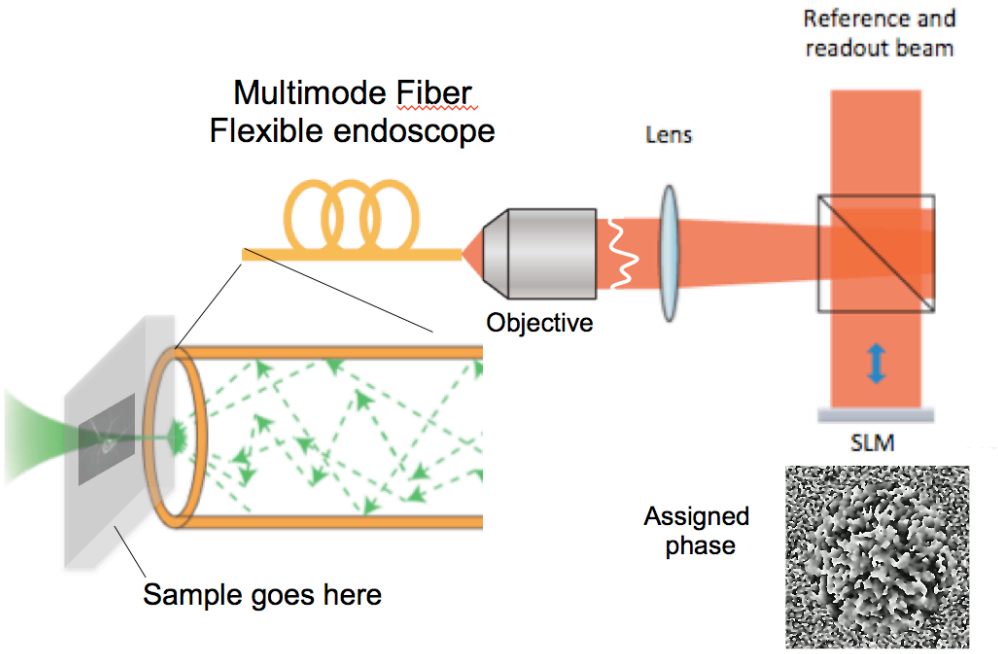



**Figure 1**: working principle of the multimode fiber endoscope: a phase only spatial light modulator (SLM) produces a "specific" modulated wavefront to the input of the multimode fiber such as to create a focal spot at the output of the fiber. The "specific" wavefront is determined by producing a known focus at the distal end of the fiber and recording its wavefront as illustrated in figure 2.

Fortunately, time reversal is possible in optics: the "specific" wavefront is determined by producing a known focus at the distal end of the fiber and by recording the interference between the wavefront coming out of the multimode fiber with a known coherent reference beam as illustrated in figure 2. The wavefront phase is then extracted from the interference image. Time reversal is then achieved by presenting the wavefront phase to the SLM and then illuminating the SLM with the known reference beam.

Time reversal is named optical phase conjugation in the optical domain. It has been proposed and experimentally implemented as a technique for suppressing the turbidity through a scattering medium [13]. This system requires a single image to calculate the correct wavefront to produce a desired output (e.g. a focus spot). In order to obtain N focal spots covering a desired field of view, N phase images are required.

Fig. 3 shows the result of phase conjugation following the set-up of Fig. 2. The step-index fiber parameter is the following: length = 1 m, numerical aperture N.A=0.22, wavelength= 1064 nm. This fiber supports 1870 modes. The laser beam is focused with the use of a 40x microscope objective (left in Fig 2.) onto the fiber input facet. The beam waist, w0, of the excitation spot is equal to 2.25μm.

The speckle pattern generated at the output of the fiber is presented in Fig. 3(a). The speckles are both randomly and uniformly distributed over the whole fiber facet indicating that a large number of the supported modes are excited and interfere at the output. The phase of the optical field, calculated by the captured hologram is presented in Fig. 1(b). The phase image is assigned to the SLM which is illuminated by the readout beam. The resulting modulated wavefront propagates back through the multimode fiber. A very sharp focus spot is observed at the other end of the fiber. The contrast of the focus, which is defined as the peak value over the mean of the background, is evaluated to be ~1800.

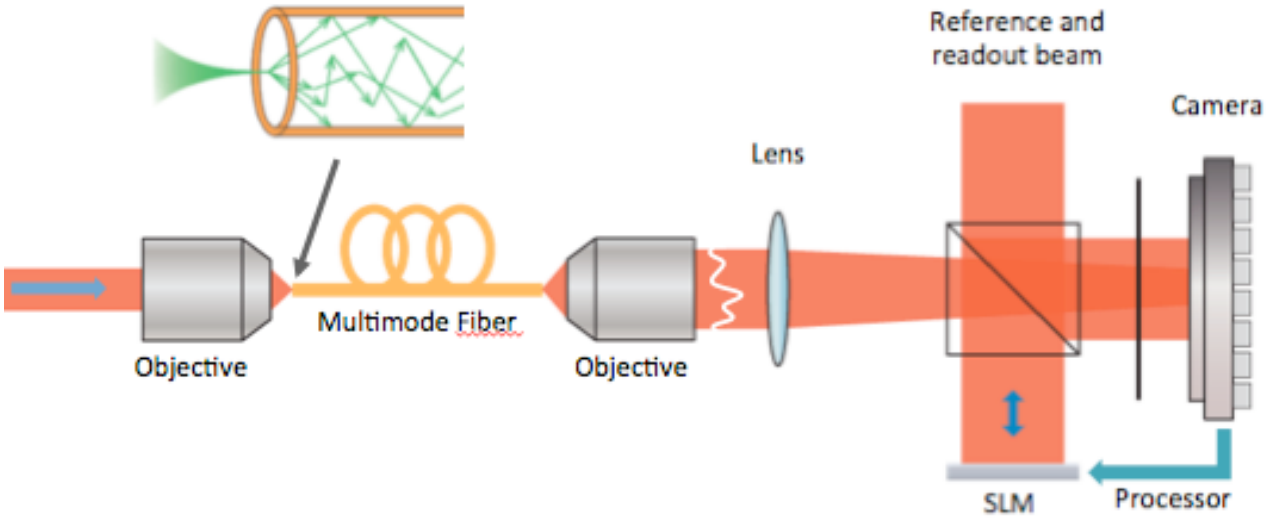



**Figure 2**: A focal spot generated by a microscope objective (lef) serves as the input to the multimode fiber. at the distal end of the fiber and recording its wavefront as illustrated in figure 2.

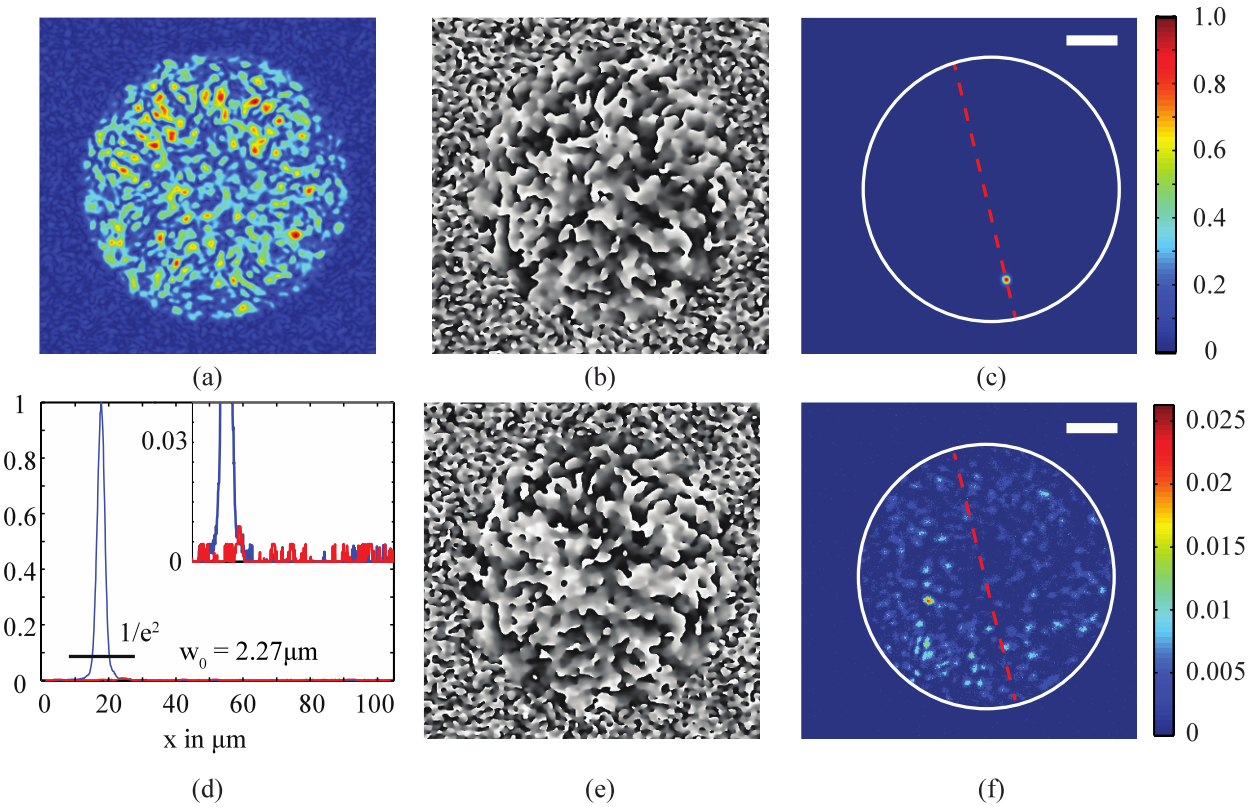



**Figure 3**: Focusing through a multimode fiber using digital phase conjugation. (a) Reconstructed speckle pattern at the output of the fiber as a result of a focus propagating along the multimode fiber. (b) Calculated phase. (c) Phase conjugated focus point at the input of the fiber. (e), (f) Random phase pattern and the corresponding image at the output of the fiber. (d) Profiles along the red dashed line drawn in (c) and (f). In blue the profile after phase conjugation and in red the profile without. (d) Inset. Detail of the plot where the limits of the y-axis are set from 0 to 0.04. The white circle in (c) and (f) defines the multimode fiber core. Scale bar in (c) and (f) is 20µm.

On the contrary, if the beam is modulated with a random phase pattern, as depicted in Fig. 1(e) no focusing is observed. The field gets scrambled while propagating, generating a new random speckle pattern at the other end of the fiber. This result demonstrates that digital phase conjugation is an efficient way for producing an intense focus through multimode optical fibers.

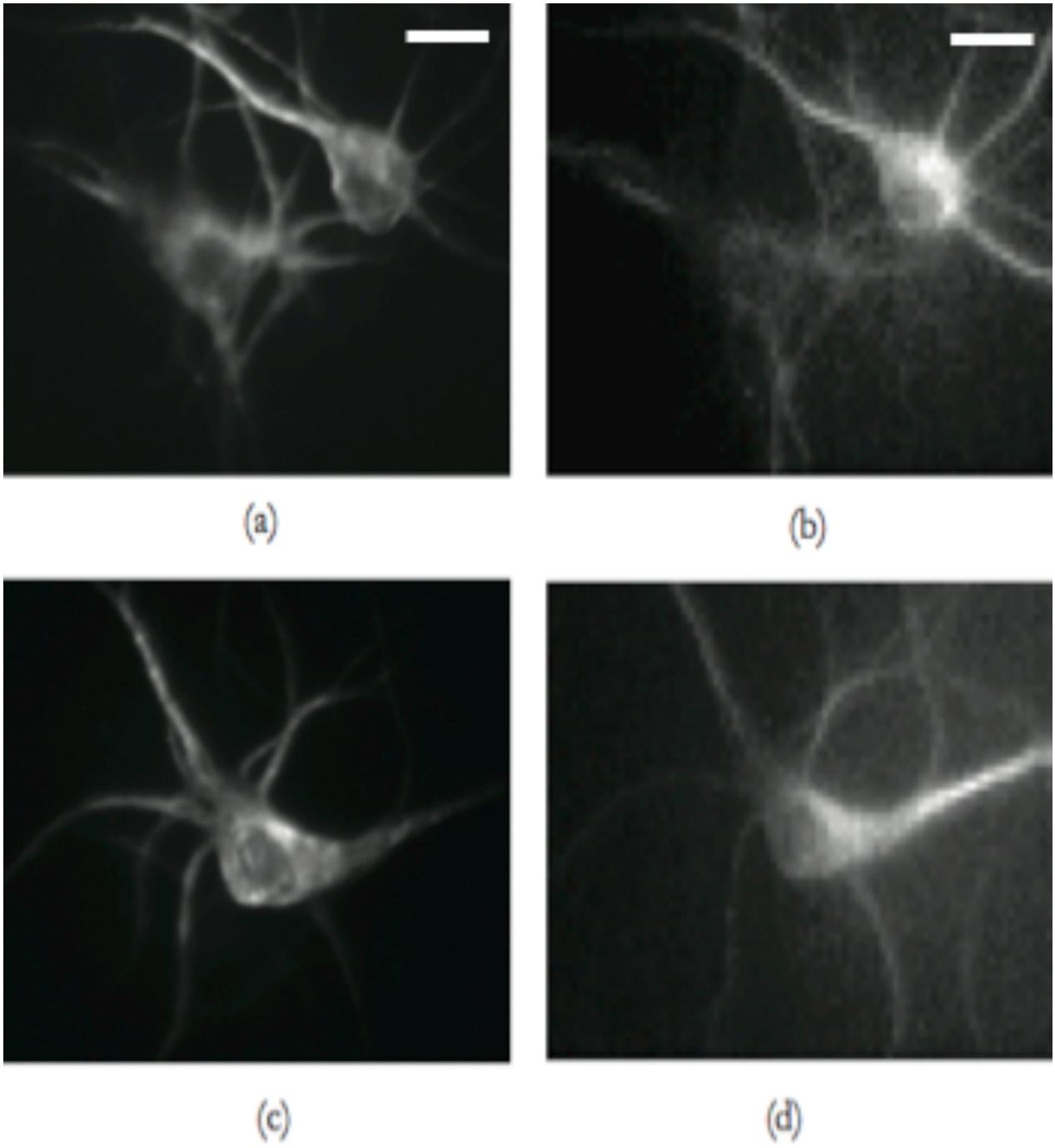



**Figure 4**: Images of fluorescently stained neuronal cells acquired with the multimode fiber endoscope compared against conventional images acquired with a microscope objective. (a) – (c) widefield fluorescent image of neurons and (b) (d) fluorescent images with the multimode fiber endoscope . Field of view is 60um by 60um and scale bars in all images are 10μm.

In order to produce an array of 2D focal spots on the distal end of the endoscope, a phase pattern is calculated for each spot and saved in the computer's memory. The phase patterns are then projected sequentially onto the SLM and the readout beam picks up the phase information on the SLM which will give just the right wavefront to focus the beam at the intended location at the distal end of the fiber. The endoscope is thus well suited for providing fluorescence images point by point. At each focal spots, the fluorescence generated by the sample under test is collected by the same large aperture multimode fiber, which is guided to the proximal end of the fiber and then detected by a single detector or a camera as illustrated in Fig. 2.

In order to obtain resolution, below 1 μm, a new endoscope was built with a green light source (532 nm) and a multimode fiber with 200 μm core, N.A=0.37. The endoscope imaging capability is shown in Fig. 4. Widefield reflection fluorescent images are shown in the left column (a-c). The images in the right column (b-d) are fluorescent images obtained with the multimode fiber endoscope. The quality of the acquired images can potentially lead to effective diagnosis that depends on cellular phenotype.

These images have a field of view of 60 x 60 μm at a distance of 200 μm from the distal end of the endoscope. The focusing distance can be adapted to any depth simply by supplying the corresponding phase pattern to the SLM. Thus it is possible to adapt the effective focal length of the endoscope by pure electronic means. This feature is useful, for example, to navigate the endoscope to an appropriate location with a long focal length and then revert to a high resolution, small focal length to obtain sub-micrometer resolution images.

**Conclusions, challenges and outlook**

We have demonstrated that, by using digital phase conjugation to focus and scan the light in a multimode fiber, we have achieved sub-micrometer resolution fluorescent imaging of fluorescently stained neuronal cells. The quality of the images offers an opportunity for direct diagnosis based on cellular phenotype. The combination of high resolution, ultra-thin diameter, adaptable focal length in a passive endoscope offers unique opportunities compared with SFE, FB and micro CMOS/CCD endoscope systems for minimally invasive high-resolution endoscopic imaging through direct tissue penetration.

A major challenge for multimode fiber endoscopes is the modal dispersion dependence on the spatial conformation (bending) of the fiber. This requires that the fiber transmission property be computed in real time for the fiber endoscope to retain its flexible capability. Several research groups including ours are currently working on this challenge. One possible solution to facilitate early acceptance is to make the fiber rigid, therefore avoiding bending.

*Christophe Moser, Ioannis N. Papadopoulos, Salma Farahi, Demetri Psaltis*

School of Engineering
EPFL, 1015 Lausanne
christophe.moser@epfl.ch

**References**

[1] Fujikura [FAI:Image Fiber] www.fujikura.com.

[2] Sumitomo [Optical Fiber Bundle/Image Guide] http://www.sumitomoelectricusa.com

[3]A. Osdoit, M. Genet, A. Perchant, S. Loiseau, B. Abrat, and F. Lacombe, Endoscopic Microscopy, Proc. SPIE 6082, 608208 – 608210 (2006).

[4] F. Schwarz, A. Le Nevez, M. Genet, A. Osdoit, and F. Lacombe, Endoscopic Microscopy IV, Proc. SPIE 7172, 71720G – 11 (2009).

[5] C.M.Lee, C.J. Engelbrecht, T.D. Soper, F. Helmchen, E. Seibel “scanning fiber endoscopy with highly flexible 1 mm catheterscopes for wide-field, full-color imaging” J. Biophoton. 3, No5-6, 385-407 (2010).

[6] I. Papadopoulos, S. Farahi, C. Moser, D. Psaltis “ focusing and scanning light through a multimode optical fiber using digital phase conjugation”, Opt. Exp. Vol. 20, 10, pp. 10583-10590 (2012).

[7] I. Papadopoulos, S. Farahi, C. Moser, D. Psaltis “ High resolution lensless endoscope based on digital scanning through a multimode optical fiber”, submitted to Biomedical optics express (2012).

[8] S. Bianchi and R. Di Leonardo, “A multi-mode fiber probe for holographic micromanipulation and microscopy,” Lab Chip 12(3), 635–639 (2012).

[9] R. Di Leonardo and S. Bianchi, "Hologram transmission through multi-mode optical fibers," Opt. Express 19, 247–254 (2011).

[10] Choi et al, “Scanner-free and wide-field endoscopic imaging using a single multimode optical fiber”, Phys. Rev. Lett. 109, 203901 (2012).

[11] T. Čižmár and K. Dholakia, "Shaping the light transmission through a multimode optical fibre: complex transformation analysis and applications in biophotonics," Opt. Express 19, 18871–18884 (2011).

[12] T. Cizmar, K. Dholakia, “Exploiting multimode waveguides for pure fibre-based imaging”, Nat. Comm., 3:1027 (2012)

[13] Z. Yaqoob, D. Psaltis, M. S. Feld, and C. Yang, "Optical phase conjugation for turbidity suppression in biological samples," Nature Photonics 2, 110–115 (2008).

Die

SSOM

Swiss Society for Optics and Microscopy

Société Suisse pour l'Optique et la Microscopie

Schweizerische Gesellschaft für Optik und Mikroskopie

gratuliert

# Dr. Roger Wepf ETH Zürich, zur Wahl zum EMS Presidenten.

**During emc2012 held in Manchester, September 16 - September 21, the General Assembly of the European Microscopy Society (EMS) elected a new Executive Board for a four-year mandate. Dr. Roger Wepf, ETH Zurich is now president.**

It is our distinct honor and pleasure, on our personal behalf and on behalf of *Imaging & Microscopy*, to extend our most sincere congratulations on the election of Dr. Roger Wepf as President of the EMS. We are also conveying sincere wishes for success in the execution of this high and responsible function.

Former president of the EMS Paul Midgley (left) and current president Roger Wepf (right) more

# *Correlative Microscopy holder for forensic applications*

The examination of documents is one of the classic topics in forensic science. The patterns formed by crossing streaks are typically investigated by using light microscopes (LM) and electron microscopes (EM). However, the images from the two types of microscope look completely different and are difficult to correlate and interpret.

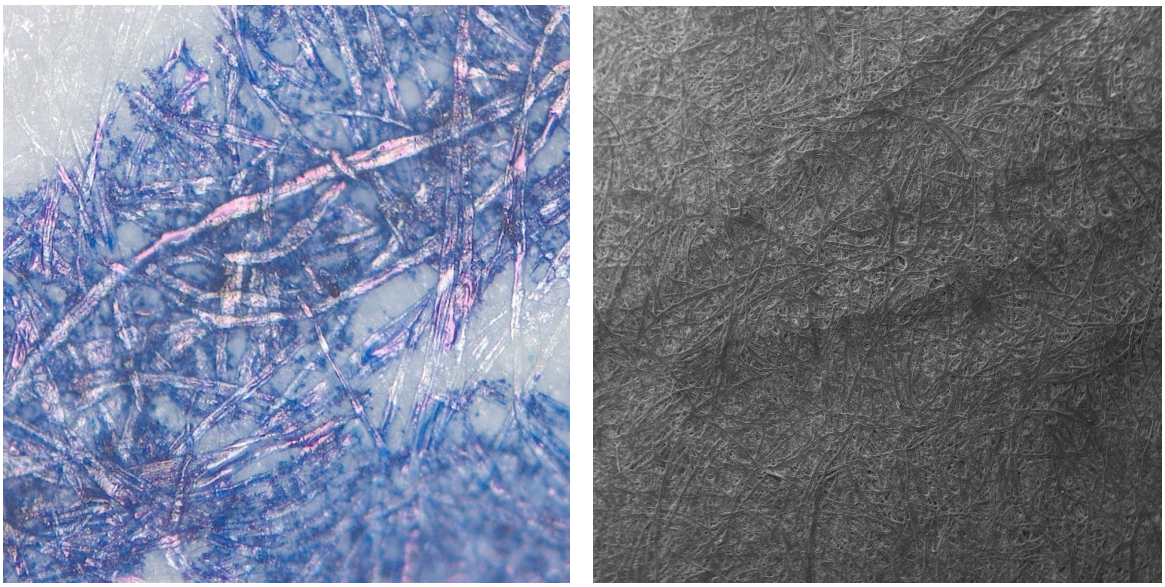


**Fig. 1**: Streak crossing under light and electron microscope, same position (courtesy of Urkundenlabor, KaPo Zürich)

The Urkundenlabor (document library) at the Forensic Institute, Canton Zurich Police, uses a tool called "Shuttle & Find" from Carl Zeiss to interpret the information from both microscopes. The problem was that the available sample holders did not fit to these type of applications: Based on ideas from users, Gloor Instruments has solved this problem by developing a special holder who covers the requirements.

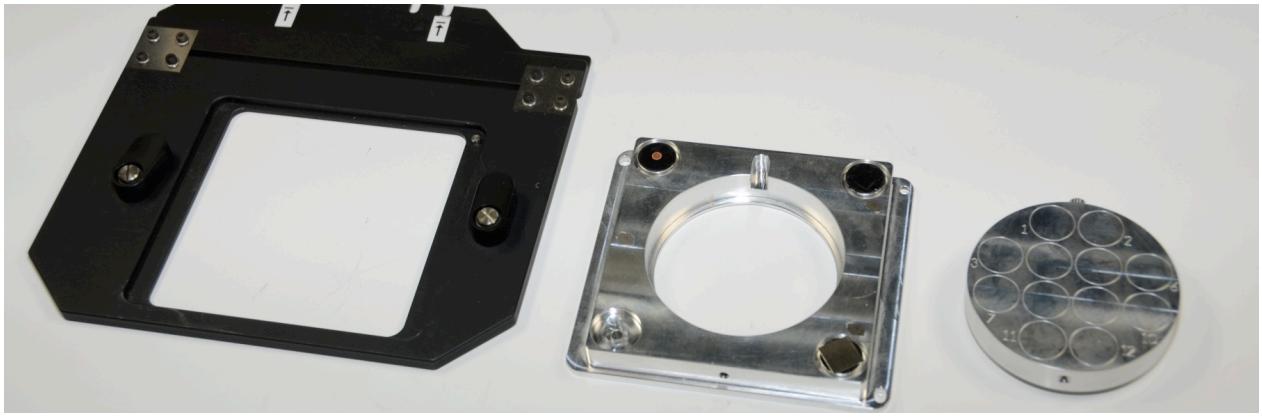


**Fig. 2**: Holder system consisting of an LM adapter, a holder frame with markers and a core disc with samples attached to the surface. In the corner of the holder, stub positions are available for reference samples.

"Shuttle & Find" consists of a sample holder and software that can be used with different microscopes. The holder has three markers that are used for automatic registration between the LM and EM. The samples are attached to a central disc, which can be removed for coating purposes (see Fig. 2, right). The disc fits very precisely in its housing, thus allowing the disc to be reproducibly repositioned in the holder. Furthermore, separate adapters for LM and EM enable a simple transfer from one system to the other.

The images from the microscopes together with the relevant coordinate parameters can easily be transferred between the microscopes. This enables not only a simple relocation, but also the simultaneous display of the two overlaid images:

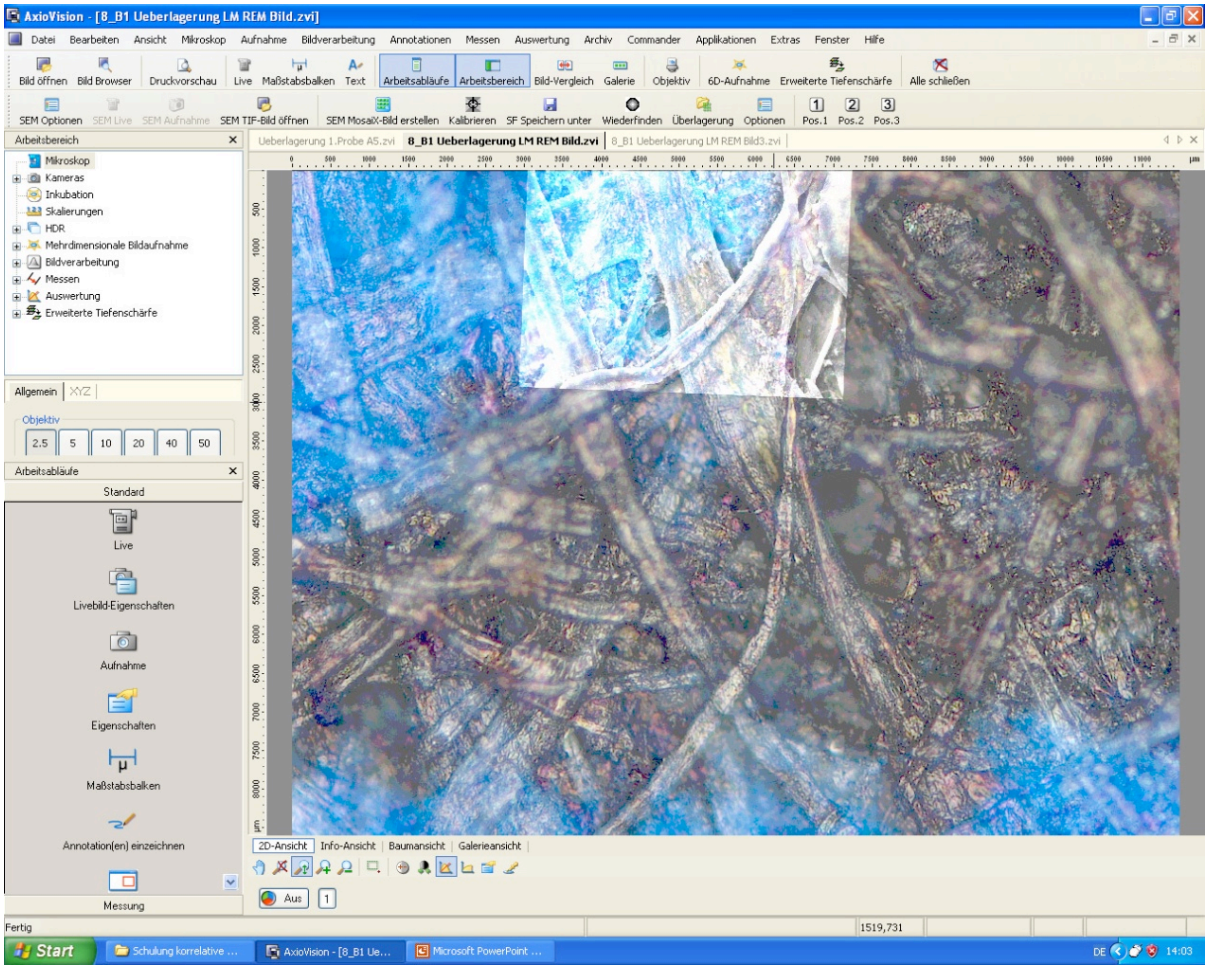



**Fig. 3**: Overlaying the EM and LM image

The core discs have laser-engraved numbers which makes it easy to archive the document samples.

*Harry Brandenberger*
*Gloor Instruments AG*

Redaktion: Dr. Reto Holzner
Apfelbaumstrasse 2
8050 Zürich

Tel. 044 312 15 63
reto.holzner@hispeed.ch

Adressänderungen : Bitte direkt an Gianni Morson
Gianni.Morson@unibas.ch

Redaktionsschluss : 15. Mai, 15. November

Die SSOM ist Mitglied bei der

Schweizerischen Akademie der Naturwissenschaften
Schweizerischen Akademie der Technischen Wissenschaften

Druck: Druckerei Dietrich AG, Pfarrgasse 11, 4019 Basel

Der Druck wurde unterstützt von der Schweizerischen
Akademie der Naturwissenschaften

sc | nat
Member of
the Swiss Academy of Sciences

GLOOR INSTRUMENTS

www.gloorinstruments.ch


# We Focus On Your Image

## TENGRA – 5.3 Megapixel Bottom-Mounted TEM CCD Camera

Olympus Soft Imaging Solutions has many years of experience serving the needs of the global EM community. By focusing on exceptional image quality for both Life and Materials Sciences, we have developed an acclaimed product range of high-quality side- and bottom-mounted TEM cameras over the years. Now it's time to complete this line-up with the TENGRA, our new 5.3 Megapixel TEM CCD bottom-mounted camera.

Boasting a superb price-performance ratio, this great camera meets the expectations of customers buying high-end TEM CCD cameras today – the combination of high resolution, high sensitivity, superior contrast, high frame rates, and comparably large field of view. With its excellent image quality, flexible navigation tools, digital live streaming, live image transfer, plus various other features, the TENGRA meets both stringent quality demands and market requirements. The TENGRA is of course also completely integrated into iTEM, our TEM imaging platform.

For further information:
info.osis@olympus-sis.com, www.soft-imaging.net

OLYMPUS

Your Vision, Our Future

Nikon

# See the evolution!

**NI-series upright research microscopes – versatile imaging for all bioscience and medical research on one platform**

- **Modular** – tailor your imaging system requirements
- **CFI Plan Apo Lambda series** – for superior optical performance
- **Motorised capabilities** – for effortless imaging
- **Simple one-touch controls** – for speed and ease of use
- **NIS-Elements 4.0 software** – for improved image acquisition and analysis

ECLIPSE Ni

www.nikoninstruments.eu

turning ***vision*** into information