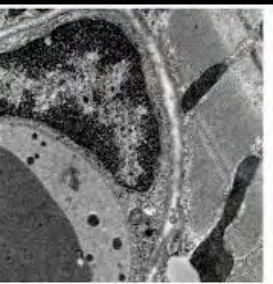
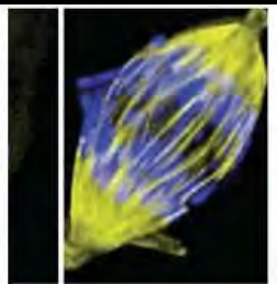
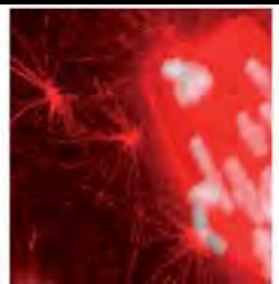
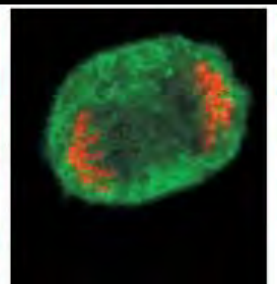
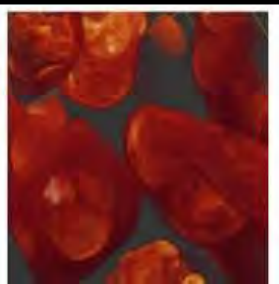
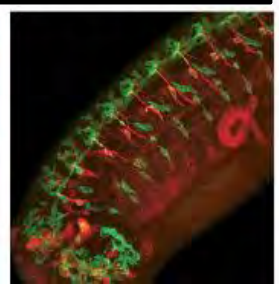
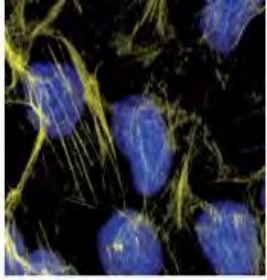
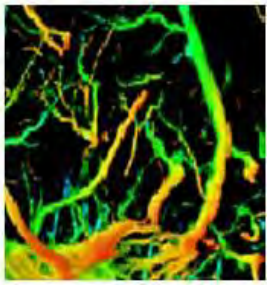
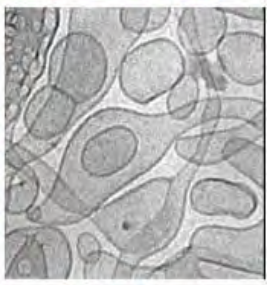
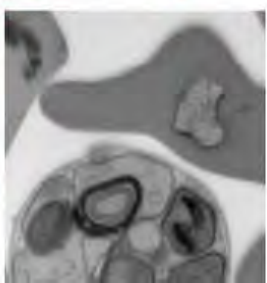
ADVANCED SCANNING IN LARYNGOLOGY PART I- IFOS 2013 SEOUL

Implications of New Advanced Scanning

M. Pedersen
FRSM PhD et h.c. Ear Nose Throat specialist
Delegate from the Danish
Ministry of science. COST 2103
The Medical Centre
Østergade 18 3.
DK - 1100 Copenhagen Denmark
Phone: +45 33137700
+45 33159600
Mobile +45 31126184
FAX: +45 33137705
E-mail: m.f.pedersen@dadlnet.dk
Url// www.mpedersen.org

Student co workers:
Anders Jønsson
Sanila Mahmood



ABSTRACT

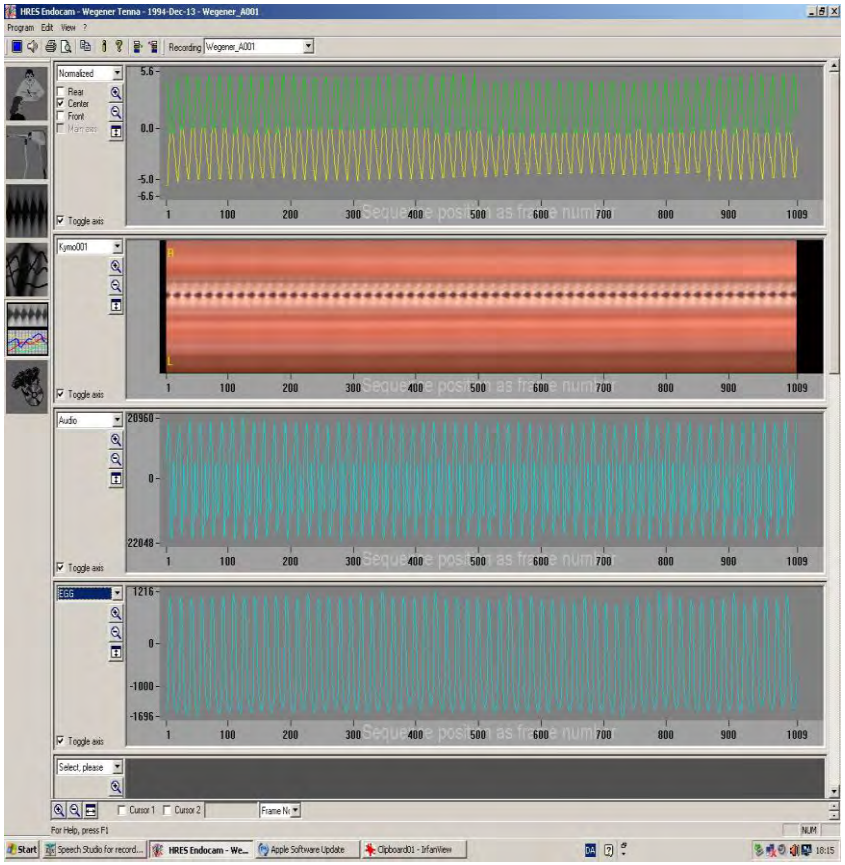
- **Purpose:** To explore the increasing findings available with the high-speed film, researchers need to know more about the underlying pathology **(tissue changes at cellular level).** The scientists need to have access to state-of-the-art light and electron microscopes ready for use in their research and the necessary technical support.
- **Methods:** ***Light microscopy*** can be extremely sensitive, detecting single molecules. Fusion of antibodies with a fluorophore is a particularly powerful method that can visualize a single component of interest through the highly specific binding of the antibody with its fluorophore. Since the discovery of the green fluorescent protein and the first experiments in molecular biology with fusion proteins comprising green fluorescent protein and a protein of interest, the method has improved tremendously. The amount of fluorophores and fluorescent proteins available are abundant, and so are different setups for visualisation of cells and tissue. These depend partly on the researchers ability to overcome the excitation and emission cross-talk between the fluorophores. ***Electron microscopy*** is a useful alternative to light microscopy in some situations. The great advantage of electron microscopy is the resolving power which is many orders of magnitude greater than light microscopy. The main difference is that instead of a light, an electron beam is the energy source illuminating the sample. There are many varieties of electron microscopy, each with their advantages and disadvantages, but common to them all is that the information is stored in the electron beam and interpreted to give a high resolution image. Examples are given.

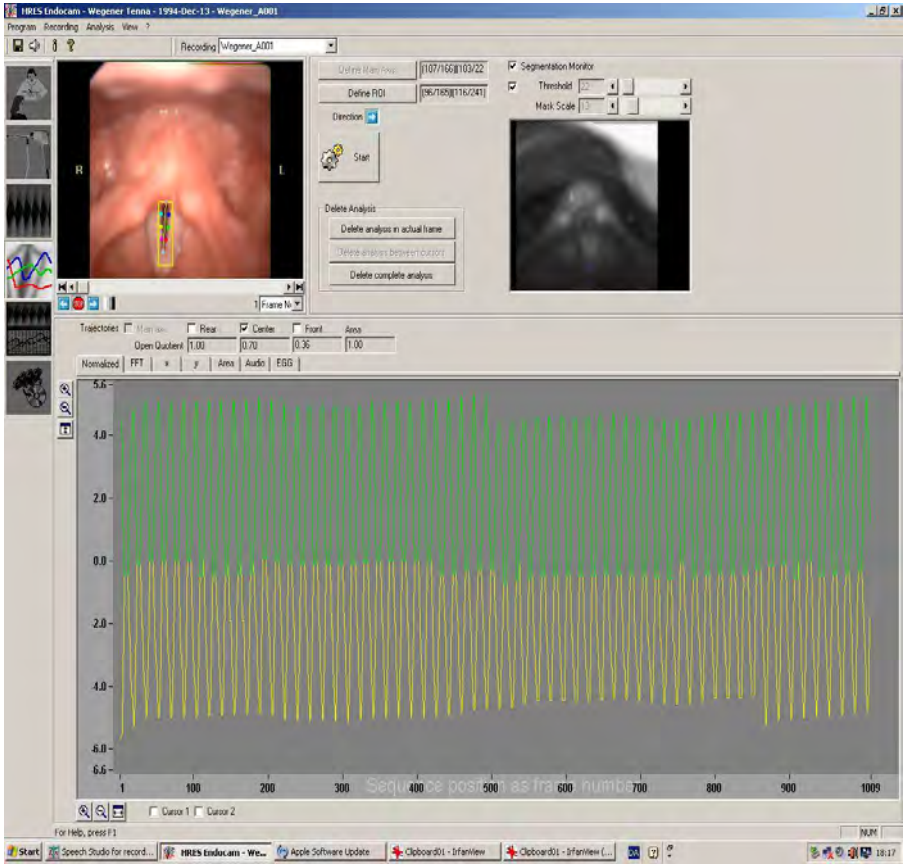
- **Keywords:** light microscopy, electron microscopy, laryngology

SO HOW CAN LARYNGOLOGY AND FINDINGS IN HIGH SPEED FILMS BENEFIT FROM ADVANCED MICROSCOPY?

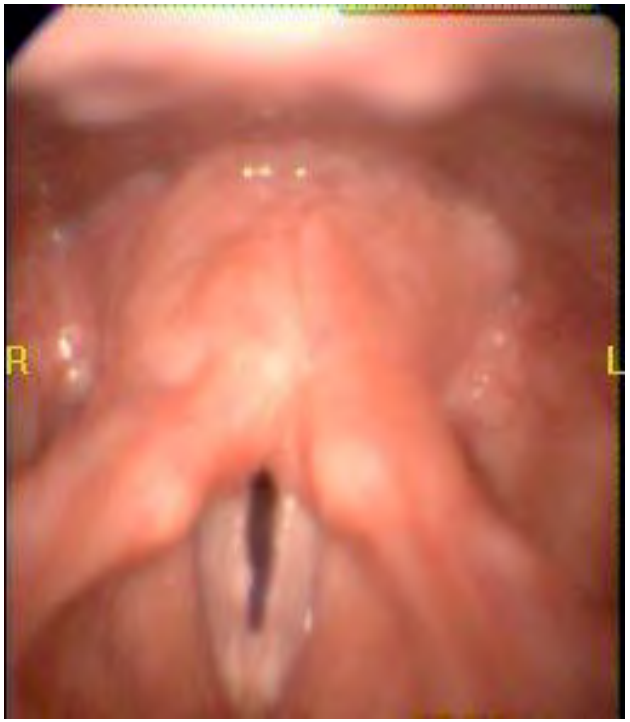
- The tissue can be **investigated** by electron microscopy and light microscopy.
- The tissue **function** can be made with high speed films and the pathology related phenomena, e.g. allergy, infection, vocal strain and neurological disorders should be analysed combined with high speed films.

HIGH SPEED FILMS

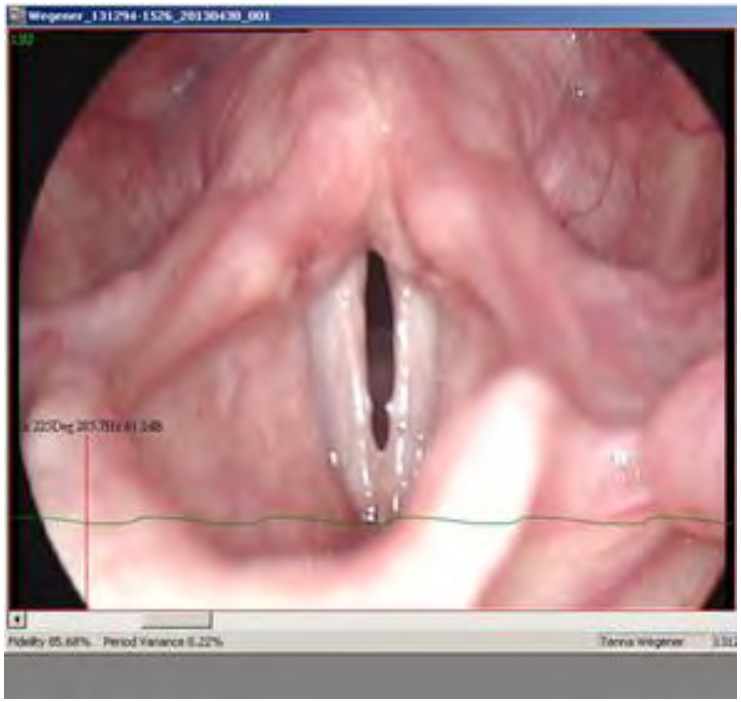




HIGH SPEED / VIDEOSTROBOSCOPY

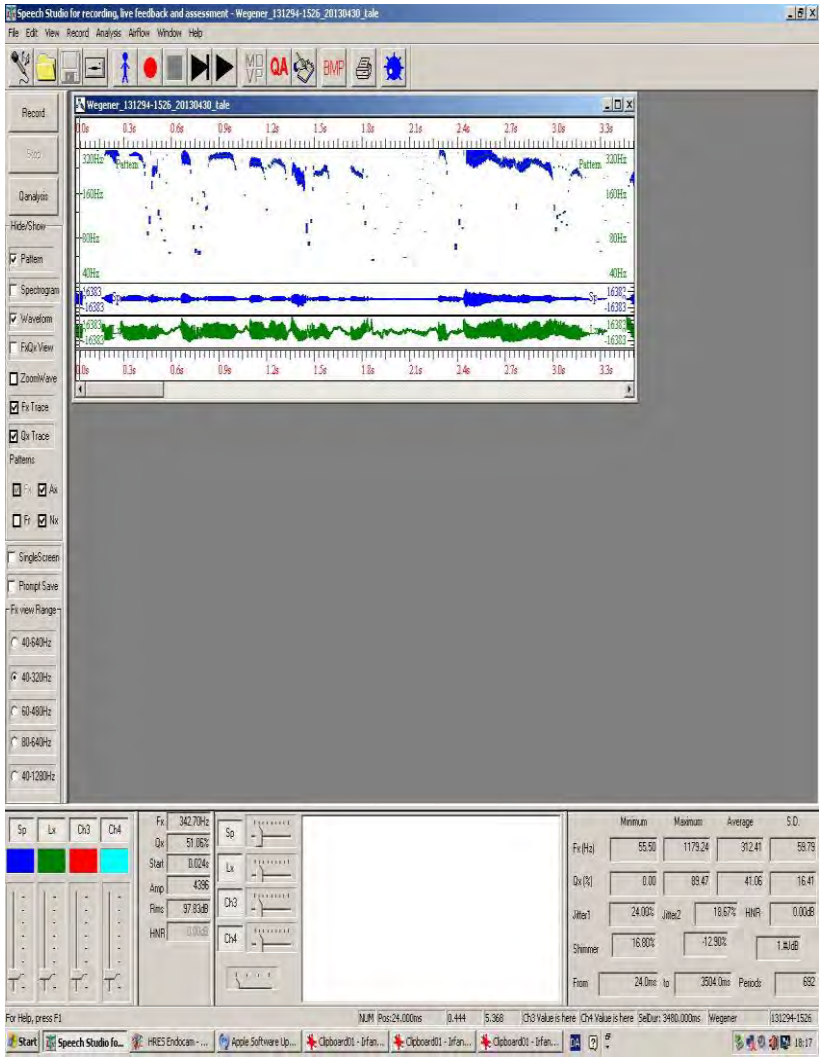


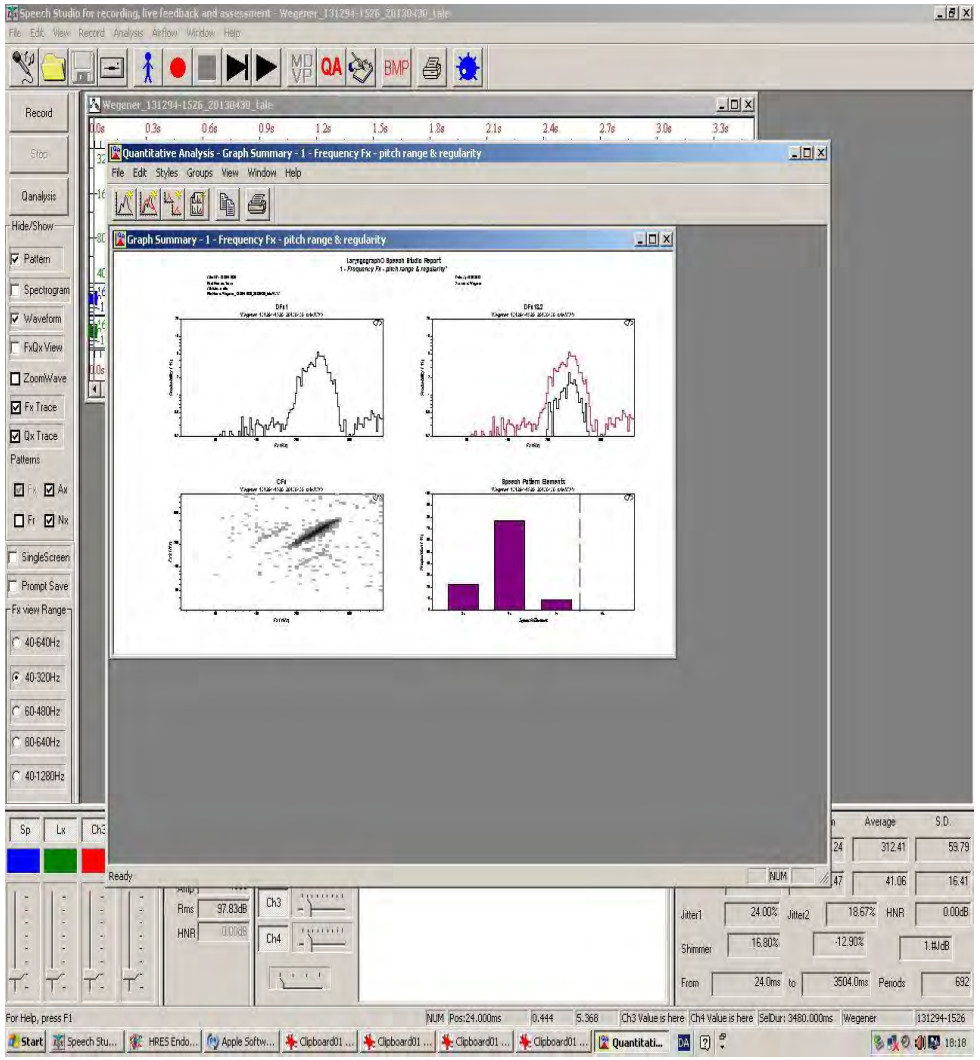
High Speed film



Videostroboscopy

MDVP BY LARYNGOGRAPH LTD





LIGHT MICROSCOPY

The equipment used for wide field fluorescence microscopy:

- Confocal Microscopy (z-sectioning of fixed or living specimens)
- Fluorescence Resonance Energy Transfer (FRET),
- Fluorescence Recovery after Photo bleaching(FRAP)
- Fluorescence Loss in Photobleaching (FLIP)
- Fluorescence-lifetime Imaging Microscopy (FLIM) experiments,
- Photoactivation and Photoconversion Experiments
- Spectral imaging
- Advanced Unmixing of Emission Spectra
- Total internal Reflection Fluorescence (TIRF) Microscopy
- Integrated Spectral Fluorescence Correlation Spectroscopy (FCS)
- Raster Image Correlation Spectroscopy (RICS).

LIGHT MICROSCOPY

- The number of fluorophores and fluorescent proteins available is increasing every day.
- Therefore, the factor that limits the number of proteins that can be labelled in samples with different fluorophores has a capability to overcome the excitation and emission cross-talk between them.
- Overlapping fluorescent signals can be separated, like the ones originated by Green Fluorescent Protein (GFP) and Yellow Fluorescent Protein (YFP), or even separate fluorescent labels from autofluorescene.

LIGHT MICROSCOPY

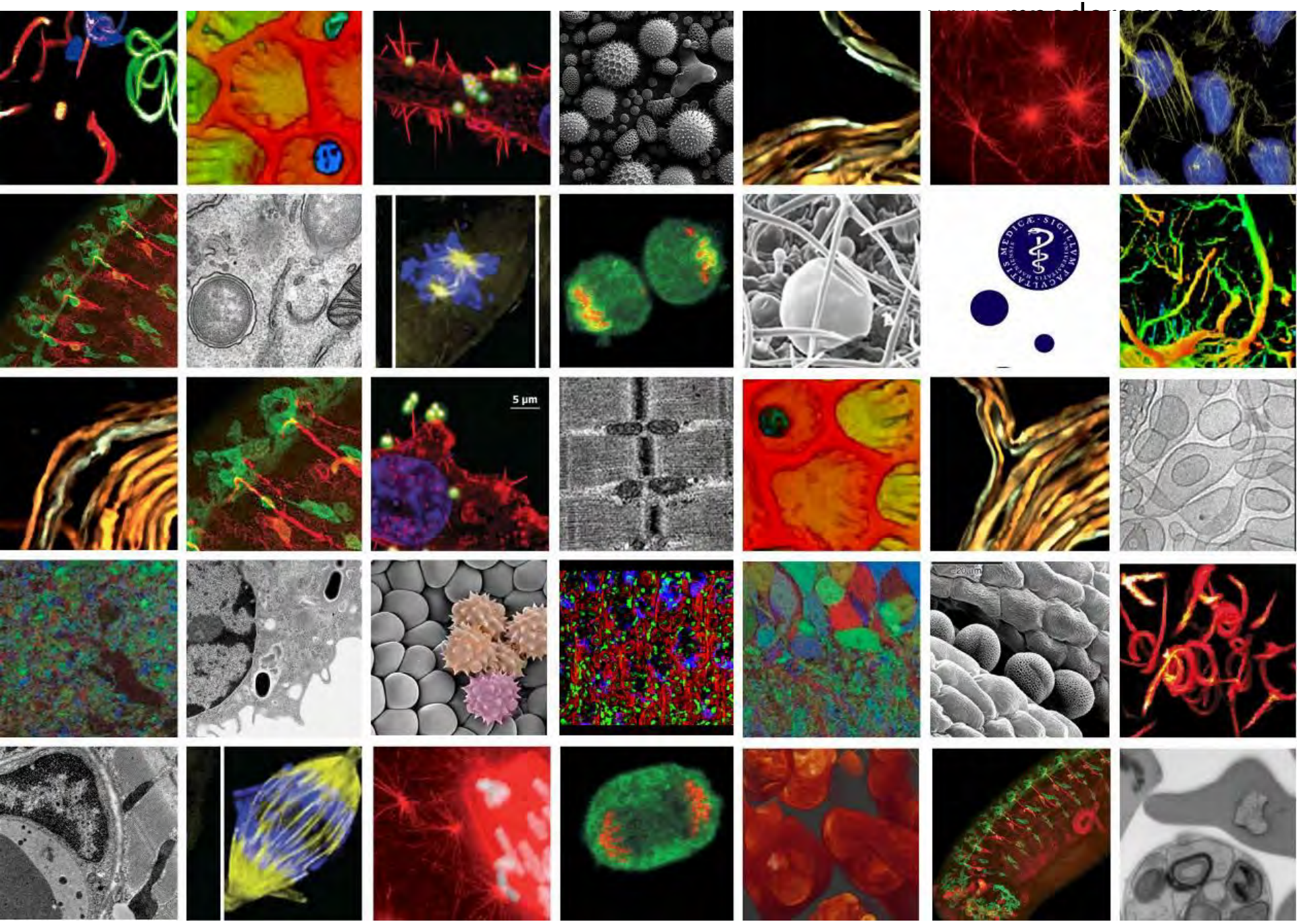
In order to perform Emission Fingerprinting, it is needed to

1. Acquire a lambda stack of the sample of interest.
2. Load reference spectra for each of the fluorescence emitting elements in the sample.
3. Ask the software to perform the unmixing of the lambda stack.

ELECTRON MICROSCOPY

The electron microscopes can be used :

- Transmission Electron Microscope(TEM)
- Scanning Electron Microscope(SEM imaging)
- 3D reconstruction of TEM images
- Unattended TEM sample preparation
- Cross-sectioning (FIB SEM)
- Single particle analysis
- Tomography analysis.



Examples of electron microscopy from Department of Biomedical Sciences, University of Copenhagen.

ELECTRON MICROSCOPY

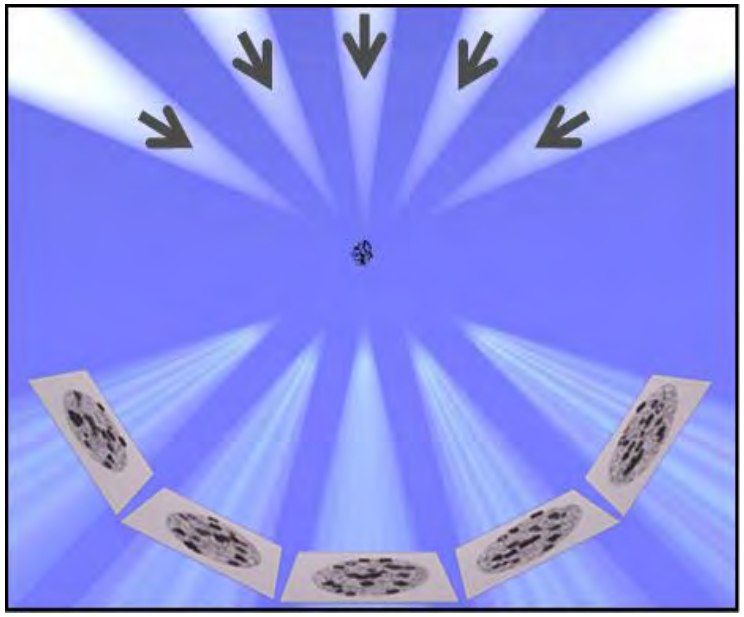
- Electron and ion microscopes use a beam of charged particles instead of light, and use electromagnetic or electrostatic lenses to focus the particles.
- They can see features as small as one-tenth of a nanometer, including individual atoms.
- Scanning probe microscopes allow researchers to image, characterize and even manipulate material structures at exceedingly small scales including features of atomic proportions.
- Scanning probe microscopes use no lenses, but a very sharp probe that interacts with the sample surface. It maps various forces and interactions that occur between the probe and the sample to create an image. Scanning electron microscopy is a high-resolution tool perfect for rendering the surface of a specimen.

ELECTRON MICROSCOPY

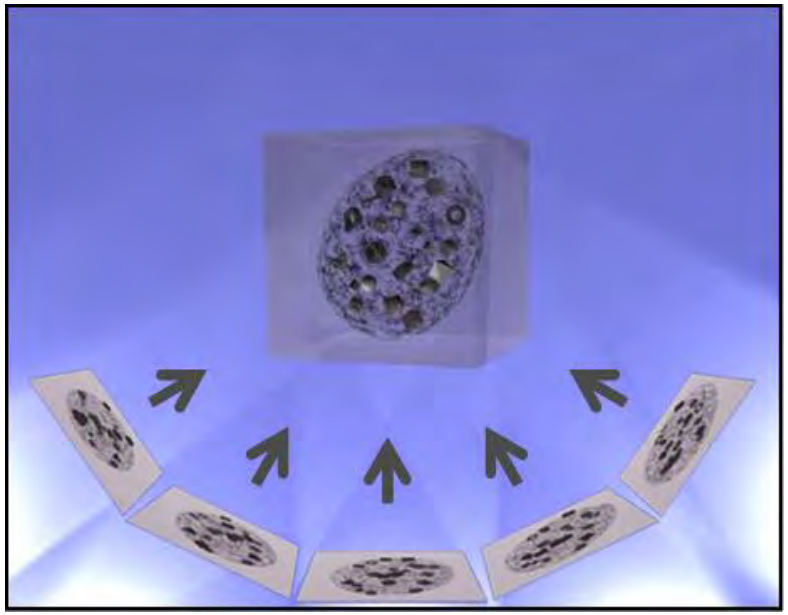
- Electron Tomography (ET) is a tomography technique for obtaining detailed 3D structures of subcellular macromolecular objects.
- Electron tomography is an extension of traditional transmission electron microscopy (TEM) and uses a transmission electron microscope to collect the data. In the process, a beam of electrons is passed through the sample at incremental degrees of rotation around the centre of the target sample.
- The information is collected and used to assemble a three dimensional image of the target.
- Tomography is found in many disciplines and it could be useful in exploring intra cordal structures of the vocal chords or laryngeal tissue-blocks e.g. of the arytenoid region.
- Current resolutions of ET systems are in the 5-20 nm range, suitable for examining supra-molecular multi-protein structures.

THE PRINCIP OF ELECTRON TOMOGRAPHY

3D-object => set of 2D-projections



2D-projections => 3D-econstruction



THREE-DIMENSIONAL IMAGING

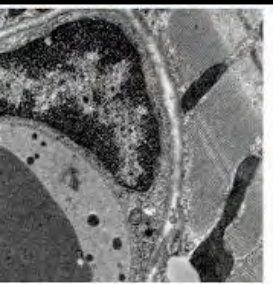
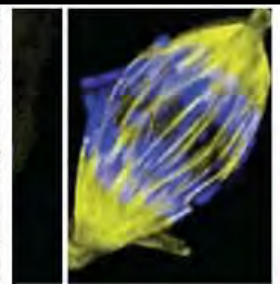
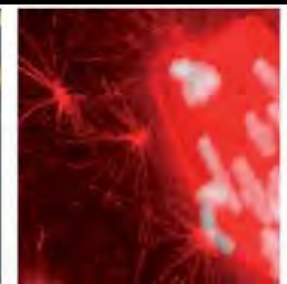
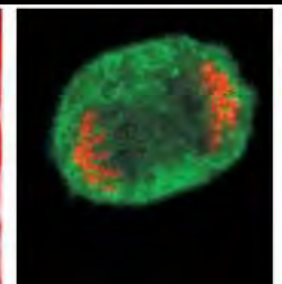
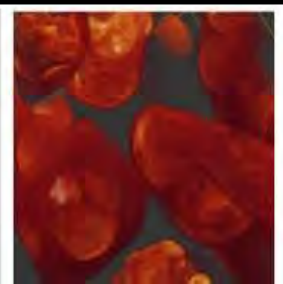
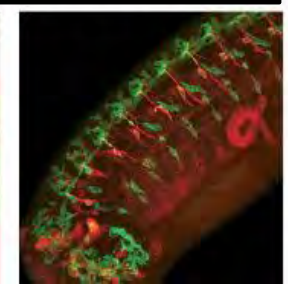
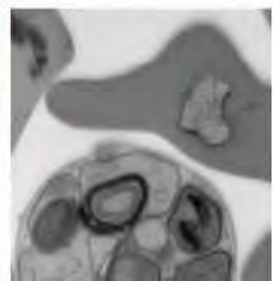
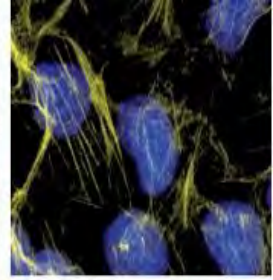
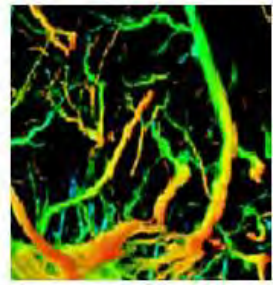
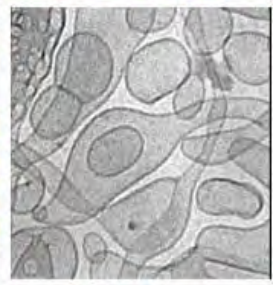
- Three-dimensional reconstruction enables visualization of the size, shape and spatial relationship between anatomical structures.
- Three-dimensional imaging can be computer generated or directly visualized by stereomicroscopy.
- The tissue needs to be optimally preserved by perfusion fixation to describe surfaces and intracellular features accurately (Glycol Solution).
- The Stereo Lumar v12 microscope can be used for any of the diverse applications in conventional stereomicroscopy. It also can acquire highresolution three-dimensional images in the largest specimen field in its class, and be used for fluorescence imaging of light microscopy quality.

ACKNOWLEDGEMENTS

- Thanks for earlier presentations at COST 2103 symposium 2011 to Department of Biomedical Sciences, University of Copenhagen (Klaus Qvortrup, Morten Friis).

ADVANCED MICRO SCANNING IN LARYNGOLOGY PART II

Implications of New Advanced Scanning and Optical Coherence Tomography (OCT)


WHAT IS OCT?

- Optical coherence tomography (OCT) is an optical signal acquisition and processing method. It captures micrometer-resolution, three-dimensional images from within optical scattering media (e.g., biological tissue).

- Optical coherence tomography is an interferometric technique, typically employing near-infrared light. The use of relatively long wavelength light allows it to penetrate into the scattering medium.

- Confocal microscopy, another similar technique, typically penetrates less deeply into the sample.

WHAT IS OCT?

- **Depending on the properties of the light source** (superluminescent diodes, ultrashort pulsed laser and supercontinum lasers have been employed), optical coherence tomography has achieved sub-micrometer resolution (with very wide-spectrum sources emitting over a ~100 nm wavelength range).

- A relatively recent implementation of optical coherence tomography, frequency-domain optical coherence tomography, provides advantages in signal-to-noise ratio, permitting faster signal acquisition.

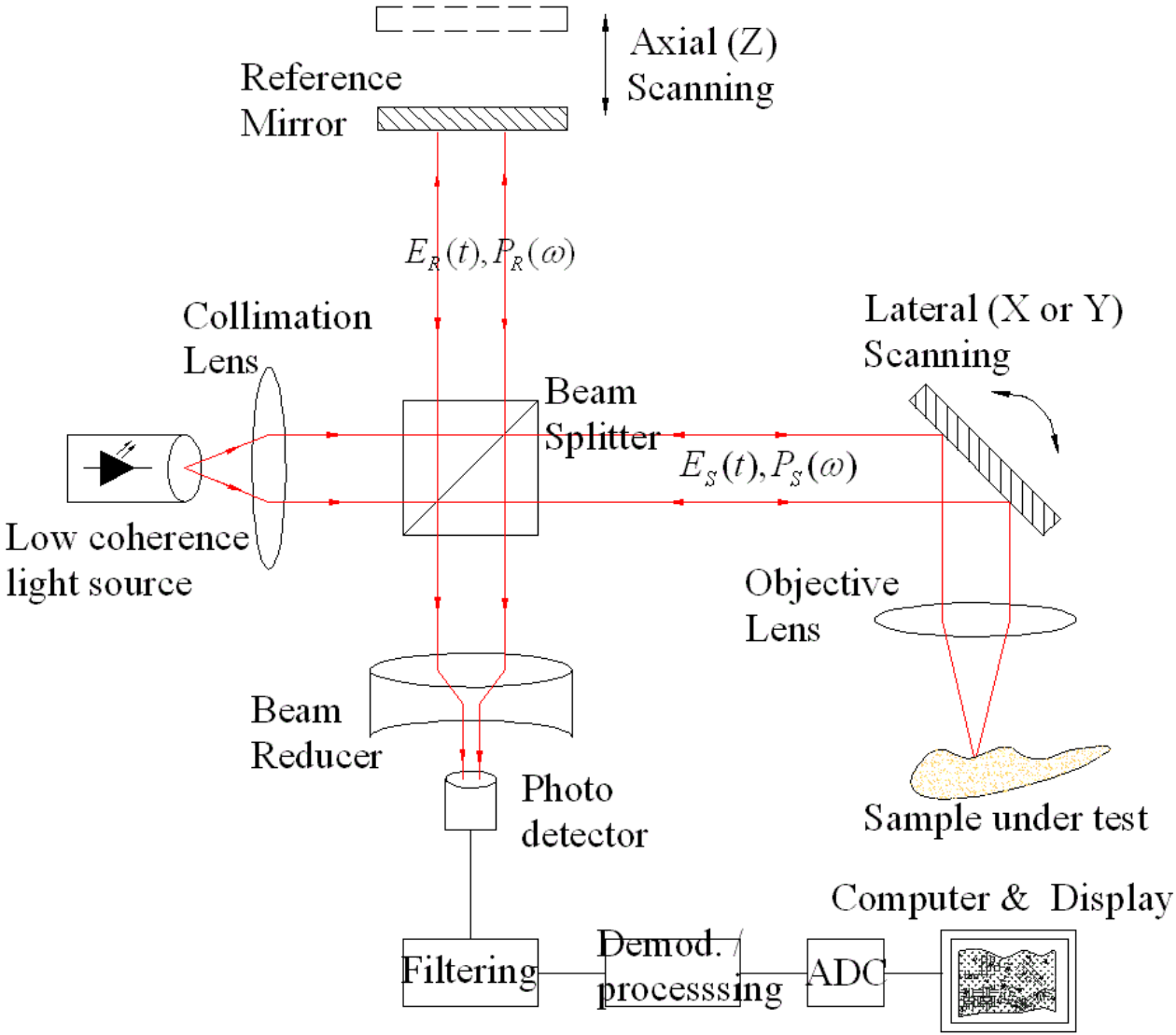
- **Commercially available** optical coherence tomography systems are employed in diverse applications, including art conservation and diagnostic medicine.

PRINCIPLE

The principle OCT is white light or low coherence interferometry.

The optical setup typically consists of an interferometer with a low coherence, broad bandwidth light source.

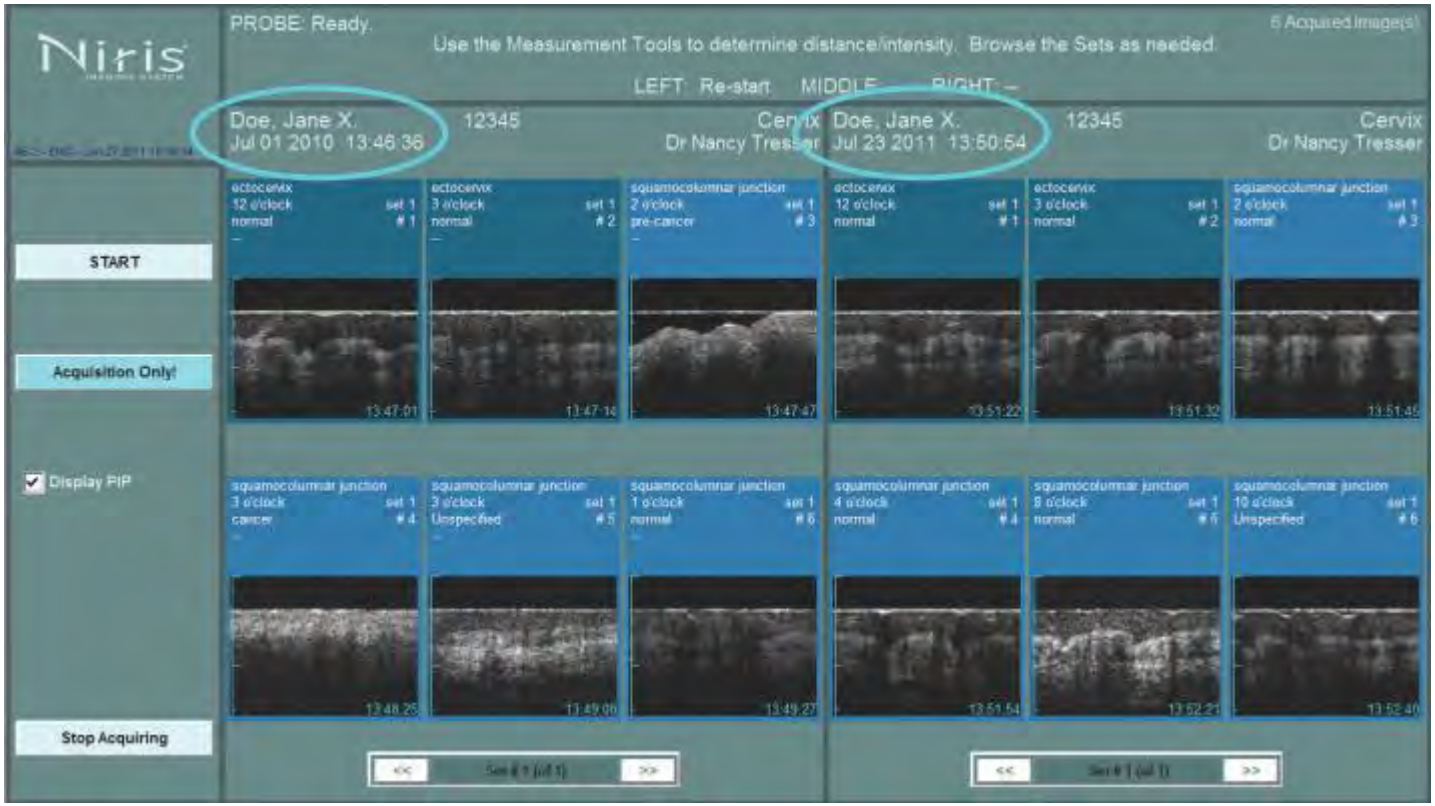
Light is split into and recombined from reference and sample arm, respectively.



EXAMPLE OF COMMERCIALLY AVAILABLE OCT EQUIPMENT

- A presentation of three different tissues

NIRIS


Niris
PROBE: Ready.
Use the Measurement Tools to determine distance/intensity. Browse the Sets as needed.
6 Acquired Image(s)
LEFT: Re-start
MIDDLE
RIGHT: –
Doe, Jane X.
Jul 01 2010 13:46:36
12345
Cervix
Dr Nancy Tresser
Doe, Jane X.
Jul 23 2011 13:50:54
12345
Cervix
Dr Nancy Tresser
START
Acquisition Only!
Display PIP
Stop Acquiring

EXAMPLES IN THE LITTERATURE

Model Based 3D Segmentation and OCT Image Undistortion of Percutaneous Implants

Abstract. Optical Coherence Tomography (OCT) is a noninvasive imaging technique which is used here for in vivo biocompatibility studies of percutaneous implants. A prerequisite for a morphometric analysis of the OCT images is the correction of optical distortions caused by the index of refraction in the tissue. We propose a fully automatic approach for 3D segmentation of percutaneous implants using Markov random fields. Refraction correction is done by using the subcutaneous implant base as a prior for model based estimation of the refractive index using a generalized Hough transform. Experiments show the competitiveness of our algorithm towards manual segmentations done by experts.

Oliver Müller[1], Sabine Donner[2,3], Tobias Klinder[4], Ralf Dragon[1], Ivonne Bartsch[3], Frank Witte[3], Alexander Krüger[2,3], Alexander Heisterkamp[2,3], and Bodo Rosenhahn[1,*]

[1] Institut für Informationsverarbeitung, Leibniz Universität Hannover, Appelstraße 9a, 30167 Hannover, Germany
{omueller,rosenhahn}@tnt.uni-hannover.de
[2] Laser Zentrum Hannover e.V., Hollerithallee 8, 30419 Hannover, Germany
[3] CrossBIT, Center for Biocompatibility and Implant-Immunology, Hannover Medical School, Feodor-Lynen-Straße 31, 30625 Hannover, Germany
[4] Philips Research North America, Briarcliff Manor, NY 10510, USA

EXAMPLES IN THE LITTERATURE

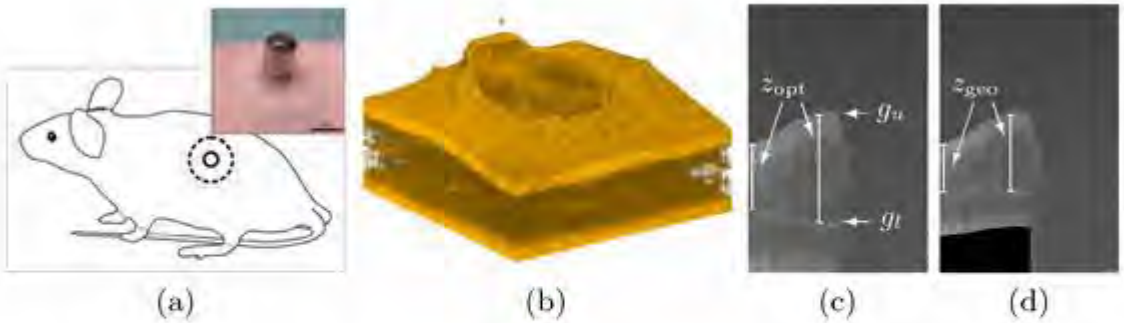


Fig. 1. (a) Location and photo of a percutaneous implant, (b) OCT dense 3D scan volume rendering (percutaneous pin is not visible), (c) single OCT B-scan (cropped at half) showing the distorted baseline, and (d) corresponding undistorted result

EXAMPLES IN THE LITTERATURE

- **Optical coherence tomography visualizes microstructure of apple peel**

- Pieter Verboven, Alexandra Nemeth, Metadel K. Abera, Evi Bongaers, Dirk Daelemans,Pascale Estrade, Els Herremans, Maarten Hertog, Wouter Saeys, Els Vanstreels, Bert Verlinden,Michael Leitner, Bart Nicolaï

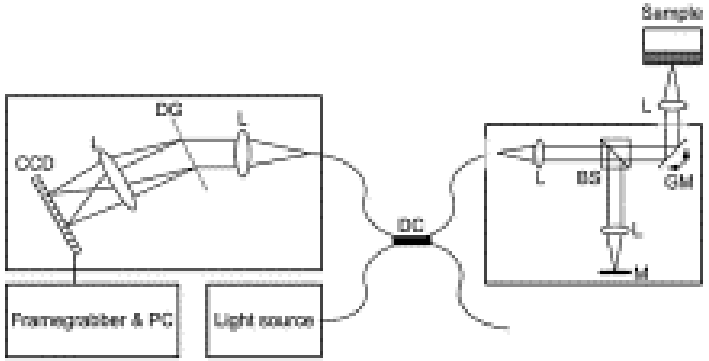
- BIOSYST-MeBioS, KU Leuven, Willem de Croylaan 42 - box 2428, 3001 Leuven, Belgium, RECENDT, Research Center for Non Destructive Testing GmbH, Science Park 2/2, OG, Altenberger Straße 69, A-4040 Linz, Austria, Department of Microbiology & Immunology, Kapucijnenvoer 33 blok i - box 1030, 3000 Leuven, Belgium, VCBT, Flanders Centre of Postharvest Technology, Willem de Croylaan 42 - box 2428, 3001 Leuven, Belgium, Skyscan Bruker Micro-CT, Kartuizersweg 3B, 2550 Kontich, Belgium, Visualisation Sciences Group, Avenue Kennedy 87, Mérignac Cedex 33708, France

NKT LIGHT SOURCES

CTO
Christian Vestergaard Poulsen

- In the article the prototype spectral-domain (SD)-OCT system (henceforth referred to as PSD-OCT) was operated with a supercontinuum light source (**Koheras SuperK Versa, NKT Photonics,** Denmark) centred around 860 nm and a spectral bandwidth of 160 nm.

- Fig. 1. Schematic diagram of a spectral-domain OCT system. The dashed boxes represent portable and independent modules. DC: directional coupler; BS: beamsplitter; (G)M: (galvanometer) mirror; L: lens; DG: diffraction grating.



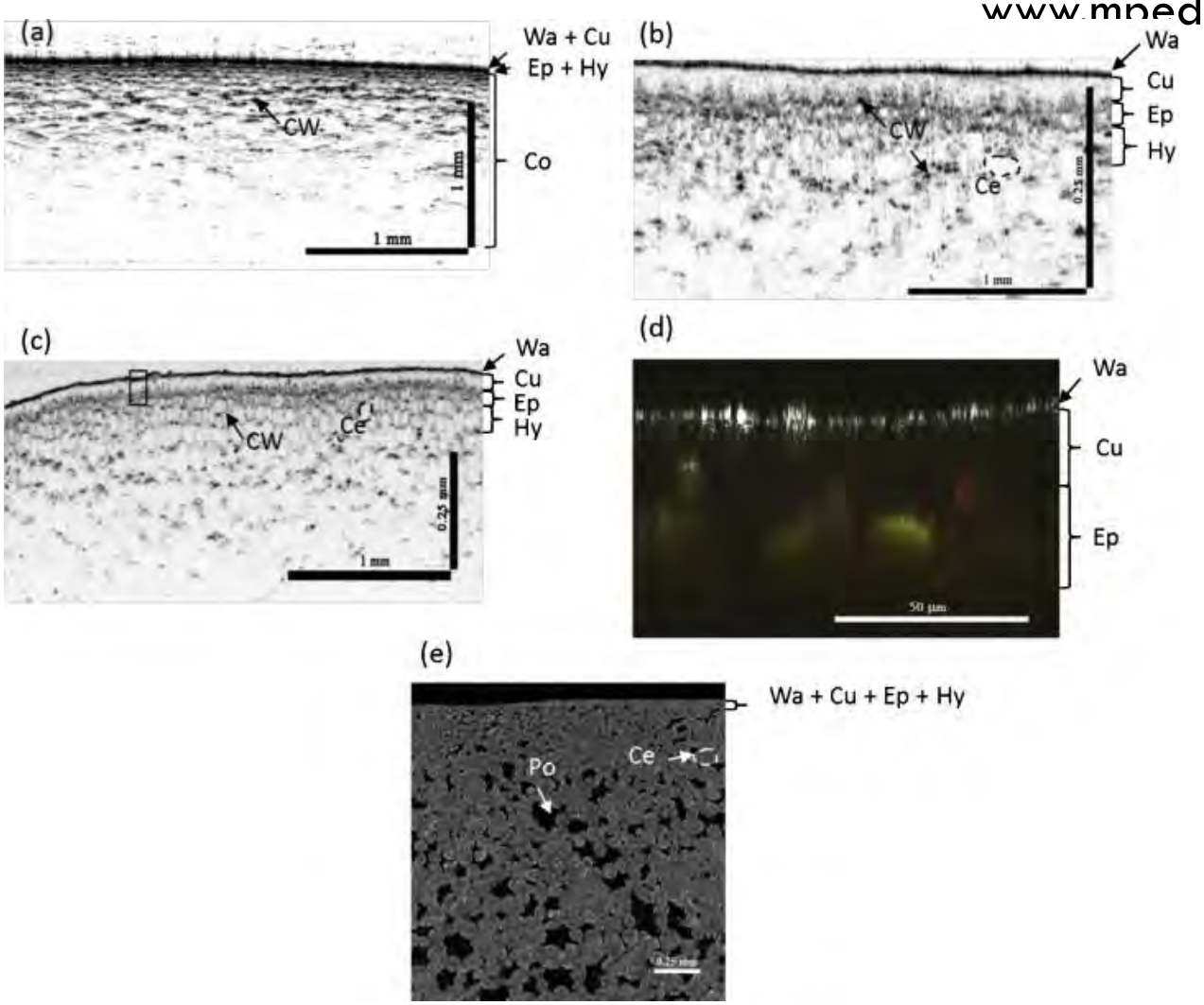


Fig. 2. Cross-sectional images of 'Braeburn' apple peel obtained with different OCT methods, confocal microscopy and micro-CT: (a) CSD OCT 1300 nm (axial pixel resolution 5 µm); (b) TD OCT 800 nm (axial pixel resolution 2 µm); (c) PSD OCT 800 nm (axial pixel resolution 1.25 µm); (d) confocal microscopy (pixel resolution 0.215 µm, region of image corresponds to rectangular zone in figure (c)); (e) cross section of a micro-CT image (pixel resolution 4.8 µm). (vertical scales in a, b and c are optical length). Legend: Wa: wax; Cu: cuticle; Ep: epidermis; Hy: hypodermis; Co: cortex; CW: cell wall; Ce: cell; Po: pore.

European Conferences on Biomedical Optics (ECBO)

12 - 16 May 2013
Messe Munchen, Munich, Germany

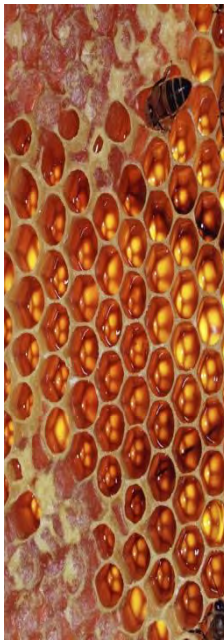
- Examples of use

European Conferences on Biomedical Optics (ECBO)

12 - 16 May 2013
Messe Munchen, Munich, Germany

- EM1B.3 • 11:06
- Miniaturized Endoscopy Using Micro-Optic Imaging Fiber Bundle for Minimally Invasive Optical Biopsy

- A spatio-spectral suppression of honeycomb patterns is demonstrated to restore micro- endoscopic images based on fiber bundle. **Both the background and patterned object became homogeneous due to effectively reduced artifacts with minimal blurring at the edges.**



- Cheon Yang Lee, Yeong-Mun Cha, **Ji-hyun Kim**, Jae-Ho Han
- Brain and Cognitive Engineering, Korea Univ., Republic of Korea.

European Conferences on Biomedical Optics (ECBO)

12 - 16 May 2013
Messe Munchen, Munich, Germany

- ETu2B.6 • 11:45
- GPU Accelerated Real-Time Ultrahigh-Resolution Optical Coherence Tomography Based on Multi-Superluminescent Diodes for Non-Invasive Skin Imaging

- **We demonstrated a cross- sectional imaging rate of 98 frames/s** for wide tissue area scanning. Multi-superluminescent diode source with 220-nm bandwidth at 1375-nm and the compute unified device architecture were utilized to achieve real-time OCT.

- **Ji-hyun Kim**, Jaehong Aum, Jae-Ho Han, Jichai Jeong
- Department of Brain and Cognitive Engineering, Korea Univ., Republic of Korea; Department of Computer and Radio Communication Engineering, Korea Univ., Republic of Korea.

European Conferences on Biomedical Optics (ECBO)

12 - 16 May 2013
Messe Munchen, Munich, Germany

- ETu2C.22 • 11:33
- Highly Accurate Segmentation for Functional Anatomy of Neuro-Sensory Tissue Images Using Double Sided Kernels Based on Gaussian Mixture Model

- **Efficient image segmentation is demonstrated to differentiate anatomy of neuro-sensory tissue images using an adaptive method based on statistical clustering.** Functional layers are accurately distinguished by fast and reliable two-sided morphological classification under speckle noise.

- Yeong-Mun Cha, Cheon-Yang Lee, **Ji-hyun Kim**, Jae-Ho Han
- Department of Brain and Cognitive Engineering, Korea Univ., Republic of Korea.

- We are on our way to understand new technics in our field. Still much work has to be done.
- Concept for Tremor Conpensation for a Handheld OCT-laryngoscope as presented by Sabine Donner is one of the important future aspects, but she is only working on animals yet.
- Other problems are the focus and artefacts. Still OCT seems to be one of the future methods of diagnosis of mucosal problems in the larynx, especially in the arytenoid regions.
- Many years ago we synchronized stroboscopy with Electroglottography and made hard- and software for phonetograms. It is nice to follow the tecnological development.

Thank you for your attention

Union of the European Phoniatricians