Conference of optical imaging, therapeutics, and advanced technology in head and neck surgery and otolaryngology 8207-341

Advanced micro scanning in laryngology: implications of new advanced scanning in relation to HSDI acquired signals

Morten Friis, Mette Pedersen, Klaus Qvortrup
Copenhagen Ear & Throat Research Centre, Department of Biomedical Sciences, University of Copenhagen
www.cfim.ku.dk. morten_friis@hotmail.com, qvortrup@sund.ku.dk.
The Medical Centre, Voice Unit, Østergade 18.3, DK-1100 Copenhagen K, Denmark.
www.mpedersen.dk mail to m.f.pedersen@dadlnet.dk

ABSTRACT

The objective was to provide a comprehensive overview of the advanced microscopes (light and electron) and to implicate how laryngeal science can benefit. The Core Facility for Integrated Microscopy (CFIM) has a wide range of state-of the art light and electron microscopes for users of all levels of experience and from any discipline. To explore the increasing findings 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, as well as the necessary technical assistance and support.

KEYWORDS

HSDI, laryngology, FRET, FRAP, FLIP, FLIM, TIRF, FCS, RICS, microscopy

1. INTRODUCTION

The Core Facility for Integrated Microscopy (CFIM) offers a wide range of state-of the art light and electron microscopes for users of all levels of experience and from any discipline. The scientists and the students coming to CFIM will find not only light- and electron microscopes ready to use for their research in laryngology but also the necessary technical assistance and support. The local facility is located at the faculty of Heath Sciences, the Panum institut, Copenhagen University, and is open to all members of the faculty and to members of other faculties, universities and researchers from hospitals. New users of any level of microscopy experience are welcome – However, in order to use CFIM equipment all users must receive proper training on the instruments they wish to use. Depending of the user's previous experience in microscopy, special training on demand will be necessary.

In the past, light- and electron microscopy have always been physically separated with little interaction between the disciplines. CFIM suggests the two disciplines to be combined, which undoubtedly will increase the possibility of inter-disciplinary microscopical approach to the scientific questions.

1.1 So how can laryngology and findings in high speed films benefit from advanced microscopy?

Tissue, either a sample or a brush biopsy with (living) cells – can be investigated on CFIM by electron microscopy and light microscopy. The tissue function is based on the high speed films. And the pathology related phenomena, e.g. voice development stage, allergy, infection, vocal strain and neurological disorders [1], can be analysed combined with high speed films.

1.2 Equipment at Core Facility for Integrated Microscopy

As the name indicates, CFIM is a core facility that integrates light and electron microscopy. CFIM will allow users to think broadly and select the best possible instrument(s), light and/or electron microscope(s), to provide detailed answers to their questions in laryngology at the highest resolution.

1.3 Light microscopy

The equipment at CFIM can be used for wide field fluorescence microscopy, Confocal Microscopy (z-sectioning of fixed or living specimens), Fluorescence Resonance Energy Transfer (FRET), Fluorescence Recovery after Photobleaching (FRAP), Fluorescence Loss in Photobleaching (FLIP) and Fluorescence-lifetime Imaging Microscopy (FLIM) experiments, Photoactivation and Photoconversion Experiments, Spectral imaging and Advanced Unmixing of Emission Spectra, Total internal Reflection Fluorescence (TIRF) Microscopy, Integrated Spectral Fluorescence Correlation Spectroscopy (FCS) to analyze single molecule dynamics and Raster Image Correlation Spectroscopy (RICS).
The number of fluorophores and fluorescent proteins available is increasing every day. This means that 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, which is intrinsic from biological samples.
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.

The electron microscopes at CFIM can be used for standard Transmission Electron Microscopy (TEM) and Scanning Electron Microscope (SEM imaging), 3D reconstruction of TEM images, unattended TEM sample preparation, cross-sectioning (FIB SEM) and single particle analysis and tomography analysis. (Figure 1,2)

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 nanometres (one ten billionth of a meter), 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 (a very small and very sharp needle) 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 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 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. This information is collected and used to assemble a three dimensional image of the target. (See Figure 1 and Figure 2)

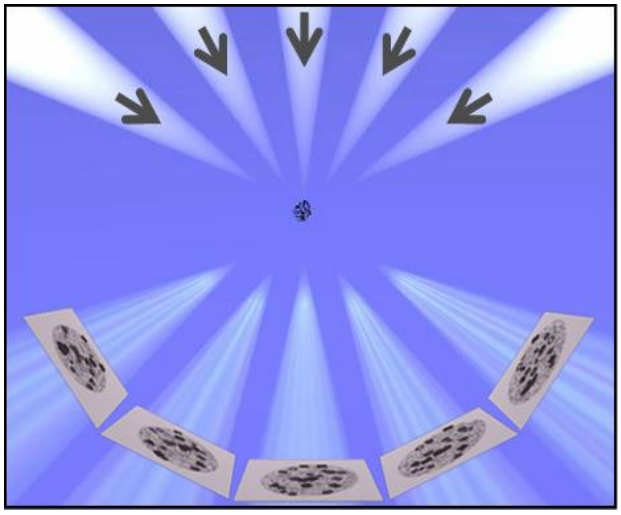


Figure 1: 3D-object => set of 2D-projections

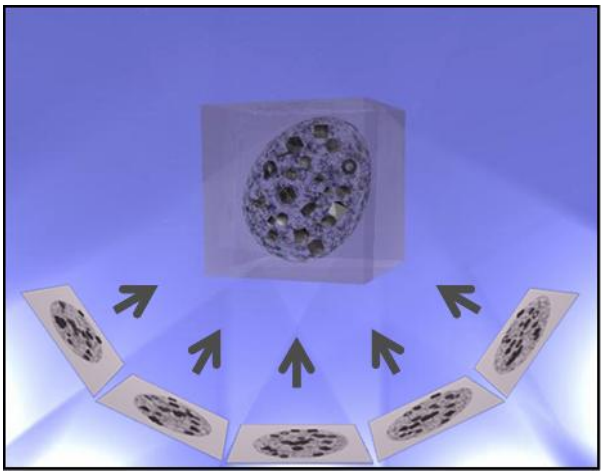
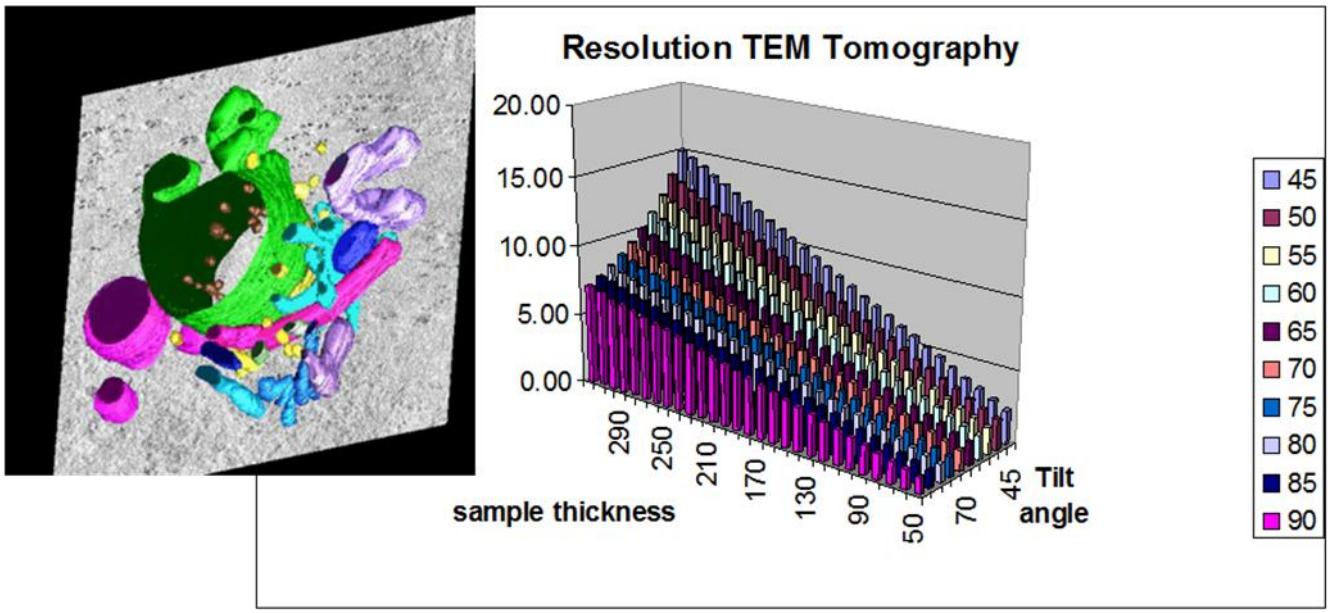


Figure 2: 2D-projections => 3D-reconstruction

Tomography is found in many disciplines and it is useful in exploring intra chordal 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. (Figure 3, 4)



$$d = \frac{\pi . D}{N} \sqrt{\frac{(\alpha + \sin\alpha\cos\alpha)}{(\alpha - \sin\alpha\cos\alpha)}}$$

D = sample thickness

α = Max Tilt angle

N = 140 images

d = resolution

Figure 3: Resolution TEM Tomography

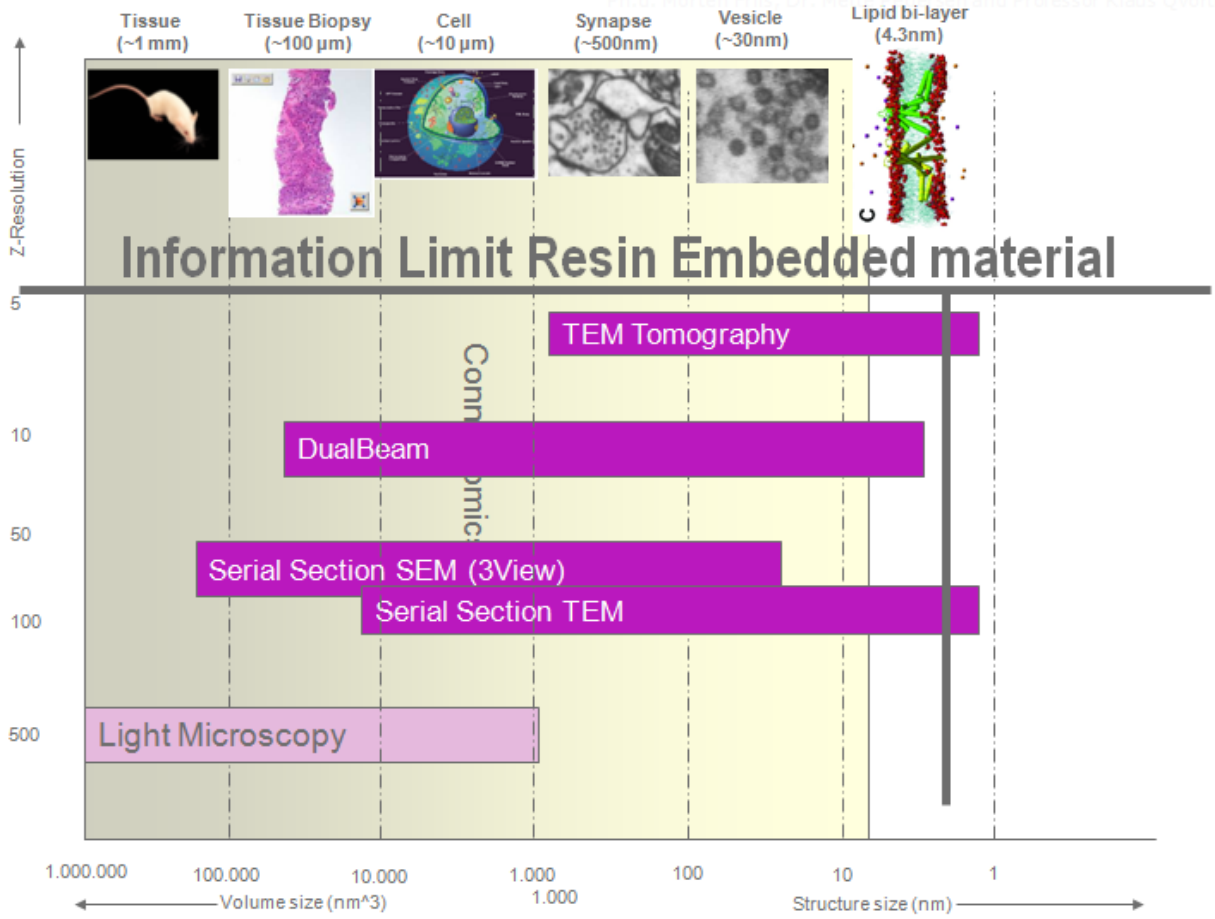


Figure 4: Information Limit Resin Embedded material

1.4 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 microscopy (SEM or stereomicroscopy). The tissue (biological specimens) needs to be optimally preserved by perfusion fixation to describe surfaces and intracellular features accurately (Glycol Solution). The Stereo Lumar v12 can be used for any of the diverse applications in conventional stereomicroscopy. It also can acquire high resolution three-dimensional images in the largest specimen field in its class, and be used for fluorescence imaging of light microscopy quality. [2-9]

2. CONCLUSION

The Core Facility – is a platform for Integrated Microscopy (CFIM) for researchers in the biomedical sciences of today and tomorrow. In the past, research groups often bought their own microscopes so that laboratory members could use them whenever they needed. Limitations in funding, space and in particular in expert support to run the microscopes, however, make it impossible for each individual research group, centre or institute to acquire all the desired equipment. Therefore combined resources and a common facility are established with access for all researchers also at the Faculty of Health Sciences at Copenhagen University, for researchers in other faculties, universities and hospitals. Such a platform opens doors for collaboration between researchers in the laryngology discipline. CFIM has become a place to meet,

learn, and discuss research questions. At Core Facility for Integrated Microscopy platforms it is possible/achievable, to make exact images that in the end will help understanding the underlying pathology based on high speed films.

3. REFERENCES

[1] Pedersen M, Eeg M. "Does treatment of the laryngeal mucosa reduces dystonic symptoms? A prospective clinical cohort study of mannose binding lectin and other immunological parameters with diagnostic use of phonatory function studies" European Archives of Oto-Rhino-Laryngology. In press (2011)

[2] Shimi T, Koujin T, Segura-Totten M, Wilson KL, Haraguchi T, Hiraoka Y. "Dynamic interaction between BAF and emerin revealed by FRAP, FLIP and FRET analyses in living HeLa cells" J Struct Biol. Jul;147(1),31-41 (2004)

[3] Rossow MJ, Sasaki JM, Digman MA, Gratton E. "Raster Image Correlation Spectroscopy in Live Cells" Nature Protocols 5,1761-1774 (2010)

[4] Millis BA, "Evanescent-wave field imaging: an introduction to total internal reflection fluorescence microscopy" Methods Mol Biol. 823, 295-309 (2012)

[5] Hyodo M, Taguchi A, Yamagata T, Desaki J. "A complex muscle fiber network in the cricothyroid muscle: a scanning electron microscopic study" Laryngoscope Apr;117(4),600-3, (2007)

[6] Tateya T, Tateya I, Bless DM, "Immuno-Scanning electron microscopy of collagen types I and III in human vocal fold propria" Laryngoscope Feb;116(2), 156-9, (2007)

[7] Martins RH, Fabro AT, Dominques MA, Chi AP, Gregório EA, "Is Reinke's edema a precancerous lesion? Histological and electron muscopic aspects" J Voice Nov;23(6),721-5 (2009)

[8] Park S, Chun HJ, Keum B, Uhm CS, Baek SK; Jung KY, Lee Sj, "An electron microscopic study—correlation of gastroesophageal reflux disease and laryngopharyngeal-reflux." Laryngoscope Jul;120(7)1303-8 (2010)

[9] Kosztyla-Hojna B, Andrzejewska A, Moskal D, Rogowski M, Falkowski D, Kasperuk J. "Morphological aspects of the euphonic voice." Folia Histochem Cytobiol. 49(1), 72-9(2011)