Improved serial sectioning workflow for multi-beam SEM applications

How to produce the ideal section for Volume EM

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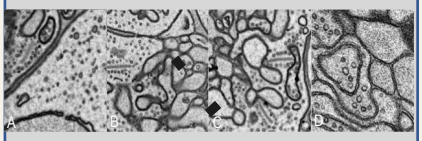
Introduction

Various techniques are used to record 3D volumes of neuronal tissue using EM [1]. Especially for larger volumes, the experiment time is a limiting factor in most of these techniques [2].
Recent developments of multi-beam scanning electron microscopes (mSEM) [3] reduce the experiment time dramatically.

Here we present our workflow for serial sectioning of tissue for mSEM applications. We modified an ultramicrotome for optimized collection of serial sections on wafer pieces. With our workflow it is possible to collect up to hundreds of wrinkle- and chatter-free sections in a short time.

Post staining procedure

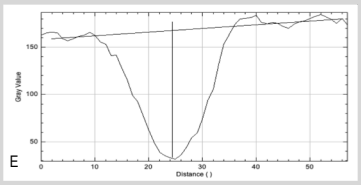


Contrast along membrane

Sample	Membrane contrast
A: ROTO	134
B: ROTO + 20 min UAC	155
C: ROTO + 20 min UAC + 20 min Pb-citrate	198
D: TEM contrast	180

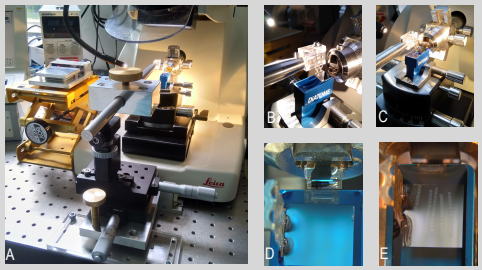
Contrast along ribbon synapse

Sample	Membrane contrast
A: ROTO	98
B: ROTO + 20 min UAC	73
C: ROTO + 20 min UAC + 20 min Pb-citrate	65
D: TEM contrast	180

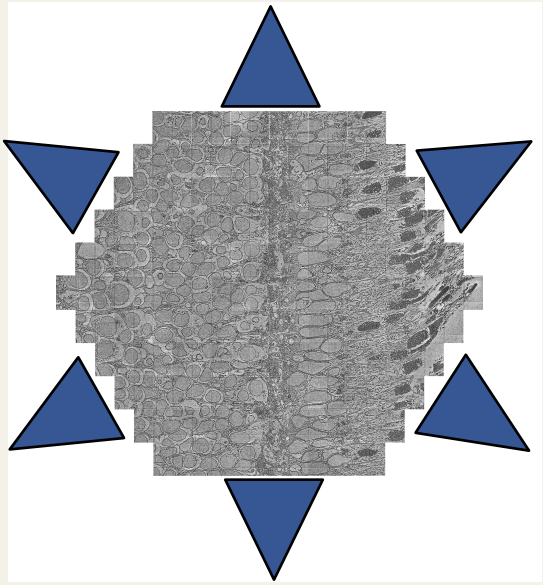


E: line plot analyses, analyzed by Fiji Software

Modification of an ultramicrotome workspace for serial sectioning



A: optimized working place on a vibration-cushioned table
B: Si wafer positioned on special holder
C: Si wafer ready for sectioning - under water surface
D: Si wafer before sectioning
E: :Si wafer after sectioning – 5 ribbons on top

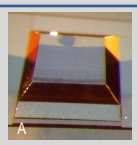


Substrate for serial sectioning

A: Si-wafer as substrate for serial sections, 4 -5 ribbons with a 25 slices on 1cm x 1cm wafer piece are possible to handle

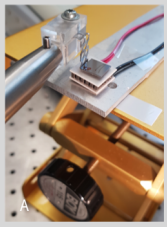
The advantages of conductive Si-wafers are:
+ Imaging conditions without tape-related issues
+ A higher packing density of the collected sections
+ Rigid and flat wafer surface allows less complicated staining procedures
+ Facilitates loading into various microscopes

Trimming procedure



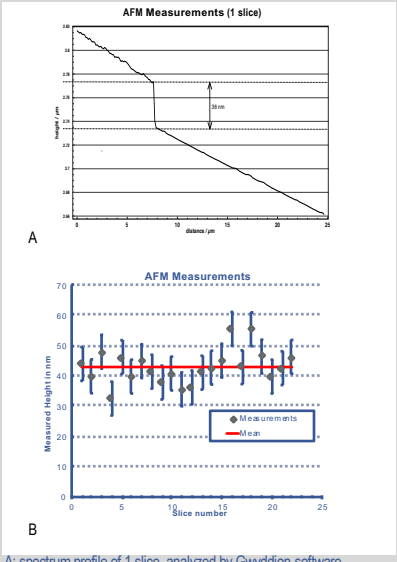
A: trimmed trapezoid with 5° on both sides

Drying procedure



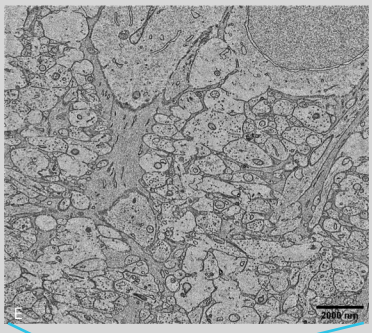
A: heating plate for drying, at 60° C less then 1min + $CHCl_3$-flow

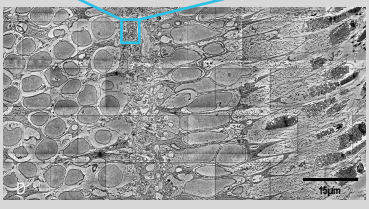
Method for quality assurance



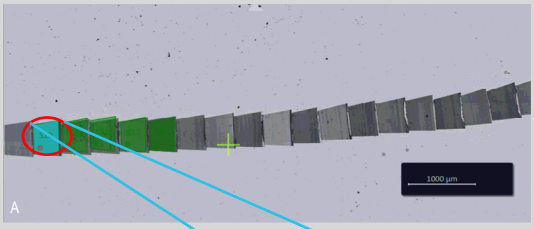
A: spectrum profile of 1 slice, analyzed by Gwyddion-software
B: spectrum profile of 24 slices, analyzed by Gwyddion-software

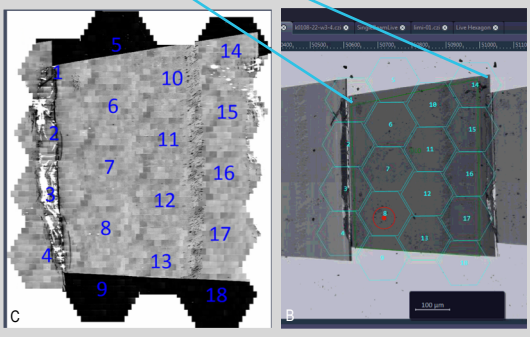
Imaging with mSEM





D: zoom1 of mFOV14
E: higher magnification of D





A: overview image with light microscope, specimen: chicken retina
B: overlay of mFOVs, 1 slice
C: 18 mFOVS recorded with mSEM

References:
[1] Christopher J. P., Lucy M. C. *Micron* (2014), **61**, 9-19
[2] Emmons, S. W. *Philos Trans B* (2015), **370**, 20140309 – 20140309
[3] Pereira, A. F., Hageman, D. J., Garbowski, T., Riedesel, C., Knothe, U., Zeidler, D., & Knothe Tate, M. L. (2016). *PLoS Computational Biology*, **12(11)**
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