Automated multiscale analysis of TEM images for improved cost-effective diagnosis of cilia-disorders

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Motivation

- High resolution (~1nm), Transmission Electron Microscopy (TEM) is the gold standard and only option for several pathological diagnoses.

- However:
  - Expensive
  - Highly manual
  - Technically complex
  - Time consuming technique
  - Lack of pathologists
Goal

- To prove and demonstrate that the utilization of TEM for clinical diagnosis can be greatly enhanced and facilitated by using image analysis to:
  - automatically search for regions/objects of interest by auto steering the microscope
  - improve the quality of the information used in the diagnosis.
Why automatic searching?

Ø = 3mm
pixelsize 0.5nm
1 byte per pixel
=> 29TB image data
SPARC Project

- Clinical application: diagnosis of genetic cilia disorders

- Cilia are long hair like organelles protruding from the cell surface, whose coordinated movements are used in transport.

- Dysfunctional cilia results in chronic respiratory infections, reduced female fertility, and infertility in males.
Sample preparation for diagnostic procedure using TEM

1. Sample collection
2. Embed in plastic
3. Cut in ~50nm slices
4. Place on grid
5. Manual TEM imaging
Current diagnostic procedure using TEM

- A trained pathologist need to detect and analyze at least 50 perfectly perpendicularly cut cilia at high magnification.

- This takes on average 2 hours at the microscope.
Our strategy

- **Low mag scanning** – to automatically steer the microscope and search for areas likely to contain cilia at low magnification.
Our strategy

- Low mag scanning

- High mag image acquisition – to automatically acquire images at high magnification of locations identified in the low mag search.
Our strategy

- Low mag scanning
- High mag image acquisition
- Detection and rating of cilia — to develop techniques for automatic detection and analysis of instances of cilia in the acquired high mag images.
Our strategy

- Low mag scanning
- High mag image acquisition
- Detection and rating of cilia
- Object registration – to develop a robust registration method to combine information from several cilia and create a high resolution representation of a cilium cross section.
Our strategy

- Low mag scanning
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- Detection and rating of cilia
- Object registration
- **Result presentation** – to present the reconstruction along with a collage of the best found cilia instances, for final diagnosis.
Our strategy

- Low mag scanning
- High mag image acquisition
- Detection and rating of cilia
- Object registration
- Result presentation
- **Evaluation** – in a clinical setting and disseminate the results.
Approach

Acquisition Module
- Cilia Detection
  - LM Image
- ROI Detection
  - HM Image Set

Analysis Module
- Cilia Segmentation
  - Single HM Image
- Cilia Registration
  - HR Cilia Image
Acquisition module results
Low mag cilia detection

- Scanning and acquisition script sent to microscope
- Template matching used to find cilia candidates
Expert rating of promising cilia at medium magnification
Cilium appearance at different magnifications

At low magnification –

- Cilium has approx. **20 pixels** diameter on a 4K x 4K image covering approx. 60 sq. um region.
- Characteristic structure are not resolved and can not be used as a reliable discriminative feature.
- Shape and size remain fairly stable, at least suitable for detecting areas for high magnification imaging.
Investigating different templates

Initial trials with different templates

- Evaluating against expert rating at typical screening magnification

ROI detection for high mag image acquisition

Input LM image

Auto search result marked with set of candidate HM images
Analysis module results

Acquisition Module
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  - HM Image Set

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  - Single HM Image
  - HR Cilia Image
Cilia detection in “high” mag images

- Green-framed cilium is used as a template.
- Similarity measure taking both shape and intensity into consideration, stable in presence of noise and shape variations
- Red circles indicate the ten most similar instances
- Blue mark the following ten
- The processing time is a couple of seconds
The ten most similar objects are shown as cut-outs.
Cilia registration and HR reconstruction first example

- Refined sub-pixel image alignment by simplex search to minimize the distance measure
- We considered translation, rotation and scaling
- We utilized cubic interpolation for subpixel registration
- Energy function: feature distance between the reconstructed and the observed cilia instances

High-resolution reconstruction by feature distance minimization from multiple views of an object
ToDo: Evaluate different registration methods

Anticipated workflow
Synthetic Object Variations – A Glance

Affine Deformations

- Standard
- Radial variation
- Angular variation
- Radial + Angular variation

Non Rigid Deformations

Image Grid Variation
ToDo: determine suitable HM level

Example – Different Magnifications
What next?

• Put the whole pipeline together
• Refine the methods
• Transfer **digital ultrapathology** approach to new applications
SIMHOR - Similarity measures for hybrid object representations with applications in biomedical image processing

VR application

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Establish theoretical foundation, and explore applicability

**Theoretical development of SIMHOR**

**Hybrid object representations**

We will perform:
- Selection of descriptive and discriminative features
- Fusion of high-dimensional feature responses

**Properties of SIMHOR**

We will ensure:
- High discriminative power,
- Robustness to noise
- Appropriate (tunable) sensitivity/invariance to scale and contrast
- Sub-pixel precision, insensitivity to discretization effects
- Generalization to higher spatial and feature dimensions
- Ability to handle multimodal data for registration and matching
- Fast parallel implementations

**Application 1:**
Cilia disorder diagnosis utilizing TEM (Transmission Electron Microscopy)

Automated object detection utilizing multiscale approach
Subpixel nonrigid registration of detected instances to enhance fine structures

**Application 2:**
Virus detection and classification in TEM images

Automated acquisition, object detection and segmentation utilizing hybrid representations
Virus identification

**Application 3:**
Multimodal image registration in spatially resolved gene expression profiling

Automated registration of fluorescence and brightfield images of large-scale histopathological tissue samples
Establish theoretical foundation, and explore applicability

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A smart and easy platform to facilitate ultrastructural pathologic diagnoses

Vinnova Framework Programme Medtech4health: Medicintekniska innovationer inom vård och omsorg – 2016
Product Idea

A platform based on the MiniTEM instrument, enriched with:

- **novel image analysis methods** for user guided automated search and quantitative analysis
- an **integrated database** for diagnosis support and convenient and secure image and information handling
- an automated **report generation system**

The resulting platform will:

- significantly **strengthen the market position** of the MiniTEM
- lead to **increased usage** of diagnostic TEM
- enable **higher throughput** and quality of clinical TEM diagnoses
- lead to more informed clinical **treatment decisions**
- help overcome the **acute problem of lack of trained ultrastructural pathologists**
MiniTEM

The first and only table-top TEM with a modern and user friendly interface designed for ultrastructural analysis of biological samples. It is a generic instrument which is:

- Small
- Robust & portable
- No infrastructure requirements
- Easy to use
- Integrated camera and analysis software
- High degree of automation