Translational Research In
Ophthalmology and Clinical Medicine

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Translational Research Collaborations with ORNL

- Automated Retinal Diagnostics - 2004
- MEMS Applications for Ocular Surgery – 2004
- Electrochemical Quantification of Drugs – 2007
- Ophthalmic Telemedical Network – 2007
- EC Nanosensor Fabrication, CNMS - 2008
What is *Translational Research*?
An Image-Based Method for Automated, Computer-based Screening of Retinopathy

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Early Detection and Treatment

- Early detection of eye disease due to diabetes, glaucoma, and age-related macular degeneration has a significant impact on the prevention of blindness. By 2025, we will need to screen 1 Million patients every day, worldwide for diabetic retinopathy.

- Currently, technology consists primarily of “store and forward” digital retinal photography with image analysis by certified techs at reading centers.

- We are developing an automatic approach for diagnosing retinal pathology using an archive of patient image and metadata.
  - Leverage image content
  - Estimate of disease probability
  - High-throughput, low-cost, broad-based screening

Fundus image showing manifestations of non-proliferative diabetic retinopathy including exudates, hemorrhages, and microaneurysms.
Example: CBIR Interface for Semiconductor Analysis

- **User-defined query image**
- **Defect mask**
- **Query results list**
- Visually similar population of images representing related state
- **Similarity metric**
- **User settings**
  - Flexibility provides query capabilities to many users
- **Process statistics**
  - Group statistics used to predict state of query
Schematic process flow for image indexing and retrieval from a CBIR library.
Retinopathy Image Search and Analysis

Process Flow

fundus camera ➔ electronic image ➔ image analysis ➔ feature analysis

Diagnosis ➔ CBIR library

“vision threatening disease likely. recommend referral for evaluation”

patient database
CBIR and Retinal Diagnostics

We need to understand (in an automated sense) where the important structures of the eye reside

Image constraints
- Limited to color and red-free imagery

Requirements
- Vascular segmentation
- Optic disk detection
- Macula region detection
- Lesion detection
- Lesion characterization (feature extraction)
- Create an image index
Optic Nerve Detection Algorithm

Prior probability

Training data

Fundus image

Vascular segmentation

Features

Bayes classifier

Likelihood map

Detected optic nerve

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Macula Localization Algorithm

- Detected optic nerve
- Segmented vasculature
- Applied parabolic model
- Macula located along horiz. raphe
CBIR and Retinal Diagnostics
Optic Nerve and Macula Detection, Foveal Localization
CBIR and Retinal Diagnostics

Lesion Detection

- Detection of lesions is being approached by multiple methods including
  - Morphological image processing (geodesic reconstruction)
  - Multi-level histogram equalization
  - Non-linear filtering and cluster analysis
- Still have work to do in this area, recall there are several types
  - Flame hemorrhages
  - Dot hemorrhages
  - Micro-aneurysms
  - Exudates
  - Drusen
  - Cotton-wool spots
Ocular Imaging and Analysis

Lesion Labeling

• We now have 1,800 hand-labeled images of DR from a dataset of 17,000 images

• This population is being used to:
  – Provide ground-truth test data
  – Drive lesion segmentation process
  – Develop and evaluate predictive capabilities
Ocular Imaging and Analysis

Lesion Detection

- Lesions are segmented with a morphological reconstruction method which works well with regions of varying contrast.

- The technique tends to over-segment so an automatic post-process of classifying blobs into “keep” or “reject” categories is under development.

Bayesian Framework

Query image, $q$

Retrieved images, $k_i$

Features based on lesion population and localization

$S(q, v_j) = 1 - \frac{D(q, v_j)}{M^{1/L}}$

$P(\omega_i | v) = \frac{p(v, \omega_i)}{\sum_{j=1}^{C} p(v, \omega_i)} = \frac{k_i}{k} = \frac{\sum_i S(q, v)^w}{\sum_k S(q, v)^w}$

Frequency distribution of states, $\omega_i$, in the visually similar population
Results

- To test the system, we perform a hold-one-out (HOO) statistical validation.
- The images at right show the posterior probability estimate for three examples of stratified disease from the archive.
Integrated Network

- Patient data entry
- Image data submission
- Diagnostic report
- Remote web clients

**SITE 1**

**SITE 2**

- Automatic retina analysis
- Automatic report generation
- CBIR index tree generation and maintenance
- Object feature extraction and reduction
- Data verification for archive submission and update

**DICOM network**

**Commercial Database**

**Commercial PACS**

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Oak Ridge National Laboratory
U. S. Department of Energy

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Ph: (865) 574-8521, E-mail: tobinkwjr@ornl.gov
Trypan Blue-Assisted Membrane Peeling
Optical Coherence Tomography
Relative size of membrane forceps to ERM and retina
Micro-Machined Glass Microarray

Designer has control of spacing, height, aspect ratio, angle

Glass has excellent biocompatibility

Glass creates a very stiff & durable spike

Spikes 7μm pitch, 12 μm height
SEM of glass arrays with 90°, 45° and 30° spikes

90 degree spikes

45 degree Cut

30 degree Cut
Micro Needle Imprinting into a Synthetic Polyethylene Membrane
Electrochemical Quantification of Serum Propofol Levels for Target Controlled Infusion Anesthesia (TCIA)

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University of Memphis

Tim McKnight, Ph.D.
Oak Ridge National Laboratory
Voltammetric electrochemical sensors

Electroactive product of the oxidation of DIP

Figure 1. 10^{-3} M H_2SO_4: 10^{-3} M DIPP
100mV/s, 10 cycles, 413b3.png
WE-Glassy Carbon
AE- Pt wire, REF - Ag/AgCl
DIPP from 0.01M solution in 0.1M NaOH/water

Figure 2. 10^{-3} M H_2SO_4 - only after 10 cycles of growing - rest of of polymerized DIPP
100mV/s, 10 cycles, 413b2.png
WE-Glassy Carbon
AE- Pt wire, REF - Ag/AgCl
DIPP from 0.01M solution in 0.1M NaOH/water

Therapeutics-Image Guided
Cyclic Voltammetry of Propofol

Figure 17. $10^{-2} \text{M} \text{H}_2\text{SO}_4 + 10^{-5} \text{M} \text{DIPP} + 10^{-5} \text{M}$ sodium lauryl sulfate, stirring
acum. at 0.8V, strip 100mV/s to -0.3V, DIPP32.opj
WE-Glassy Carbon
AE-Pt wire, RE - Ag/AgCl
DIPP from 0.01M solution in 0.1M NaOH/water

Figure 18. $10^{-2} \text{M} \text{H}_2\text{SO}_4 + 10^{-5} \text{M} \text{DIPP}$, stirring
acum. at 0.8V, strip 100mV/s to -0.3V, DIPP33.opj
WE-Glassy Carbon
AE-Pt wire, RE - Ag/AgCl
DIPP from 0.01M solution in 0.1M NaOH/water

Optimizing cycling time and conditions
Cyclic Voltammetry of Propofol Redox Reactions at the Electrode Surface

Electron sink electrode (Anode).

Electron source electrode (Cathode).

Oxidation or de-electronation.
P = reductant (electron donor)
Q = Product

Reduction or electronation.
A = oxidant (electron acceptor)
B = Product

In principle any species which can be oxidised or reduced can be detected amperometrically.
Carbon Nanofiber Electrochemical Array
Oak Ridge National Laboratory
CNMS Carbon Nanofiber “Forest Electrode”

1A. Oxide Growth
1B. Metalization
1C. Resist Spin
1D. Lithography
1E. Catalyst Deposition
1F. Lift-off
1G. Fiber Synthesis
1H. Resist Spin and Lithography
2A. Metalization
2B. Resist Spin
2C. Lithography
2D. Chrome Etch
2E. Silica Etch (6:1 HF BOE)
2F. Resist Strip/Chrome Etch
2G. Metalization
3A. Anodic Bonding

High surface area material

20 µm

Nanofiber electrode
Interconnect structure

10 mm
5 μm diameter gold discs 50 μm center to center distance hexagonally arranged
Vertically Aligned Carbon Nanofibers: VACNFs
Coated VACNFs

Gold particle by electrodeposition

Polypyrrole coated
Electrochemical Microarray
Coated VACNFs: Hybridization of CYP-oligomers to complementary cDNA

Confocal microscopy, 90 ° 30 °
Cell Culture
Gene Transfer to Living Cells

4 hours

48 hours
Nanoscale Tools for Transcriptome Profiling of Intact Cells

Internalized CNFs in a Living Cell
Heat to 72 ºC, add Taq polymerase and dNTP’s to copy oligonucleotide

Denature DNA or cDNA by heating to 94ºC

Cool to 48-65 ºC and add gene-specific primers

Copy templates by extending primers at 72 ºC

Heat to 72 ºC, add Taq polymerase and dNTP’s to copy oligonucleotide

Repeat: 30 cycles

Amplified sequence

2^{30} = 6 million copies
Quantification of IGF-1 message in RPE cells and IGF-1 transfected RPE cells expressing high levels of an IGF-1 transgene

Standard RT-PCR

\[ \Delta C_T = (25.2 - 18.1) = (~2^7) \]

\[ 2^7 = 128 \text{-fold difference in concentration of IGF-1 transcript} \]
VACNF Electrochemistry

EC species

cDNA
Nanopipes

Internal electrode *
There’s More Than One Way to Skin a Cat
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Research to Prevent Blindness

930 Friends

Fight for Sight

Mid-South Lions