Confocal microscopes for imaging skin cancers: translation from laboratory to market to clinic

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Memorial Sloan-Kettering Cancer Center (MSKCC)  
Charles DiMarzio  
Northeastern University  
Christian Costa  
Lucid Inc.

Laboratory prototype  
Mass. General Hospital, Harvard Medical School

Vivascope 3000  
Lucid Inc.

Melanoma \textit{in vivo}  
MSKCC, Dermatology Service

Basal cell carcinoma \textit{ex vivo}

Melanin autofluorescence  
Northeastern Univ., ECE Department
Recent advances

TECHNOLOGY (Lucid, MSKCC, Northeastern Univ.)
Smaller, simpler, lower-cost instruments
Vivascope 3000
Line-scanning confocal microscopes, endoscopes

CLINICAL STUDIES (MSKCC, Worldwide centers)
Screening and diagnosis *in vivo* - melanoma, basal cell carcinoma
Intra-operative imaging-guided surgery - melanoma, Mohs surgery
Image-guided biopsy
Confocal mosaicing microscopy *ex vivo* - Mohs surgery

MACHINE LEARNING-BASED IMAGE ANALYSIS (Northeastern Univ.)
Classification algorithms for automated detection of morphology
Why skin cancers?

Among the highest-incidence in the USA and worldwide

Similar to breast cancer, skin cancer touches almost all of us, directly or indirectly
Skin cancers – per year, in the USA alone

National Cancer Inst., American Cancer Society, 2009 statistics

1.2 million new cases
800,000 basal cell carcinomas (BCCs)
300,000 squamous cell carcinomas (SCCs)
100,000 melanomas

5.5 million biopsies
BCCs: 2 million biopsies, 40% positive
SCCs: 2 million biopsies, 15% positive
Melanomas: 1.5 million biopsies, 7% positive

80% ~ 4.3 million biopsies are normal or benign
cost ~ $2.2 billion/year, to detect “normal”

Better detection with confocal imaging
VISUAL EXAMINATION
Sensitivity = 43-86%, specificity = 71-94%

DERMOSCOPY
Sensitivity = 79-96%, specificity = 69-99%

SPECTROSCOPY
MelaFind (MELA Sciences, Inc.): Sens = 98%, spec = 9.5-45%
SIAscscopy (Astron Clinica Ltd.): Large clinical trial in progress
SkinSpect (Spectral Molecular Imaging, Inc.): New in market
Confocal images show nuclear and cellular detail

Normal human skin *in vivo*: dark nuclei within bright cytoplasm

Melanoma skin cancer *in vivo*
Imaging skin cancers in vivo

1992-1997
Rajadhyaksha, Anderson & Webb
MGH / Harvard Medical School

Applied Optics 1999

2008
Fox, Eastman
Lucid Inc.

VivaScope 3000
Imaging and mosaicing ex vivo
- for real-time surgical pathology at-the-bedside

VivaScope 2500

XY mosaics

Display magnification: 2X-30X

BCC in Mohs surgical excision

William Fox & Jay Eastman, Lucid Inc.
Imaging skin cancers *in vivo*

**LABORATORY Technology R&D**
- Phase I studies

**MARKET Product development**

**PRE-CLINICAL Phase II studies**
- Phase II Feasibility studies
- Sensitivity & specificity

**MULTI-CENTER CLINICAL TRIALS**
- Phase III studies

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**1992-1997**
- Rajadhyaksha, Anderson & Webb
- MGH / Harvard Medical School

**1997-2002**
- Zavislans, Greenwald, Fox, Rajadhyaksha & Eastman
- Lucid Inc.

**2003-2007**
- Gonzalez & Anderson - MGH / Harvard

**2007-2010**
- Fox & Eastman
- Lucid Inc.
- & Dermatology groups worldwide

*Applied Optics* 1999
Imaging and mosaicing *ex vivo*
- for real-time surgical pathology at-the-bedside

**LABORATORY**
Instrumentation, Phase I studies

- 1998-2001
  - Rajadhyaksha, Gonzalez, Flotte
  - MGH / Harvard Medical School
  - Derm. Surgery 2004

**PRE-CLINICAL**

- 2002-2006
  - Gareau, Karen, Nehal, Rajadhyaksha
  - MSKCC
  - British J. Dermatol. 2009
  - J. Biomed. Optics 2009

- 2007-2009
  - Sensitivity & specificity

**MULTI-CENTER CLINICAL TRIALS**

- 2010 -
  - Fox & Eastman
  - Lucid Inc.

- Sensitivity & feasibility study
Worldwide technology dissemination and clinical trials

> 250 VivaScopes, > 200 published papers
Diagnosing melanoma *in vivo*

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<thead>
<tr>
<th></th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
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<tr>
<td><strong>CONFOCAL:</strong></td>
<td>85-92%</td>
<td>84-69%</td>
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<tr>
<td><strong>DERMOSCOPY:</strong></td>
<td>62-91%</td>
<td>39-31%</td>
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20 Pathologists: 87% (55-100%)
Dermatopathologists: 90.4%

94% (83-100%) 86.5%

351 DERMOSCOPIC EQUIVOCAL lesions

RCM score ≥ 3, AUC = 0.852

Diagnosing basal cell carcinoma *in vivo*

**Sensitivity:** 92%,  **Specificity:** 97%

- elongated nuclei, monomorphic nuclei
- polarized (highly oriented) nuclei
- pleomorphic, basaloid epidermis
- increased vascularity, inflammatory infiltrates in dermis

Mapping of melanomas to guide surgery

27 successful cases: Lentigo maligna melanomas, amelanotic melanomas
Mosaicing microscopy in Mohs surgical excisions

Toward rapid pathology at-the-bedside to guide surgery

Micronodular BCC tumors

Sensitivity: 96.6%
Specificity: 89.2%

45 mosaics (160 sub-mosaics) – blinded examination and subsequent correlation to Mohs pathology

Gareau et al., JBO 2009
Karen et al., BJD 2009
Imaging of residual tumor in post-biopsy wounds \textit{in situ}
Toward intra-operative imaging to guide Mohs surgery

47 patients
BCCs, SCCs, AKs, SKs, melanomas
Contrast agent – aluminum chloride, 35% for 1 minute

Scope et al., British J. Dermatol., accepted, 2010
Line scanning confocal microscopy  
? Smaller, simpler, lower-cost technology  
To accelerate worldwide dissemination in diverse healthcare settings

MSKCC            Daniel Gareau, Bjorg Larson, Gary Peterson, Sanjee Abeytunge  
Northeastern Univ.        Peter Dwyer, Yogesh Patel, Charles DiMarzio

Human skin  &  oral mucosa  
in vivo

Preliminary Images
Machine learning-based classification algorithms
? Automated detection of morphology to aid diagnosis

Northeastern Univ. Sila Kurugol, Dana Brooks, Jennifer Dy
MSKCC Alon Scope, Juliana Braga, Itay Klaz, Allan Halpern

LOCALIZATION OF DERM-O-EPIDERMAL JUNCTION IN CONFOCAL IMAGES

Algorithm vs. clinical expert accuracy: classification ~ 90%, localization ~ 15 um

Sila Kurugol et al., JBO, submitted

FUNDING SUPPORT: Communications and Digital Signal Processing Center & Center for Integrative Biomedical Computing (NIH NCRR BTRC P41)
Future: translation to routine clinical utility

Translational / Clinical opportunities and challenges

Real-time observation of large volumes of tissue
Reading black-and-white images without stains
Image understanding and interpretation – new paradigms for pathology?
Determining clinical utility – new paradigms?
Improving sensitivity and specificity – more importantly, specificity
Imaging to guide biopsy and pathology – directed “intelligent biopsy”
Early detection for prognosis – new paradigms?
Future: translation to routine clinical utility

Technological opportunities and challenges

- Smaller, simpler, lower-cost confocal microscopes
- Integration with IT and Telepathology –
  access to pathologists, access to world

Endogenous and exogenous contrast agents (stains)
example: autofluorescence
(Kerimo and DiMarzio @ Northeastern Univ.)

Multimodal imaging – combining with other modes
example: confocal and Raman spectroscopy
(Mahadevan-Jansen @ Vanderbilt Univ.)
Fluorescence Imaging of Melanin

Conventional Fluorescence

Excited state

Absorption

Ground state

fluorescence

Multiphoton Absorption

Virtual states

Real states: $S_1, S_2, S_3$

Excited State Absorption

Sepia Melanin granules

Brightfield Image

15 µm

Melanin absorption spectrum

$S_1$, $S_2$, $S_3$
Fluorescence Imaging of Melanin

Control: Pure Melanin (Sepia)

Hair Melanin

Lung Alveoli

Lung Melanin

"Green" three-photon fluorescence

"Red" One-photon fluorescence

"Green" two-photon fluorescence Elastin

"Red" Confocal Reflectance Alveoli


Images: Joe K.
Photobleach rate reduced in nitrogen atmosphere.

Laser power dependence studied in nitrogen.

454nm laser excitation

“red” on image linear fit

700nm CW laser excitation

“green” on image 3rd order fit

Submitted: JBO
Conclusions

Melanin emission enhanced by ~1000 fold

Step-wise three photon excitation

Photobleach rate is reduced in nitrogen atmosphere

Two emissive components

Hair melanin and Lung melanin mapped

Both NIR and visible enhanced emission is detected

Commercial confocal with melanin detection, easy add-ons:
1) filter
2) Beamsplitter
3) Additional detector