Biofotónica en el diagnóstico de cáncer cutáneo

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Melanoma Unit, Dermatology Department, Hospital Clínic Barcelona, Spain
Conflicts of interest

Consultant: Derm Spectra, Scibase

Research collaboration: Mavig, Scibase, Agfa Heathcare, Dermtech, Derma Instruments, Canfield, 3Gen, Diagnoptics

Editor of dermoscopy and confocal books
Modern diagnostic tools for early detection of melanoma

“Diagnosing melanoma by simple visual examination with the ABCD rule is incorrect in almost 1 out of every 3 melanoma diagnoses”

“Early diagnosis of melanoma by dermatologists is NOT saving many lives”


Liu W et al. Rate of growth in melanomas: characteristics and associations of rapidly growing melanomas. Arch Dermatol. 2006
1840 – January 14, 1905

Abbe is credited by many for discovering the resolution limit of the microscope, and the formula (published in 1873)

0.2 micrometers
The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner "for the development of super-resolved fluorescence microscopy".
Resolution is limited by penetration

- 0.25 mm: Confocal Multiphoton
- 1.0 mm: OCT
- 10 mm: Sonography

- Epidermis
- Dermis
- Hypodermis
Non-invasive methods for skin examination

- Total body photography
- Dermatoscopy
- Digital dermatoscopy
- Wood’s lamp
- Multispectral imaging
- Hyperspectral imaging
- Electrical impedance spectroscopy
- Tissue elastography
- Reflex transmission imaging
- X-ray fiber diffraction
- Raman spectroscopy
- Optical Coherence Tomography
- Sonography
- Photoacoustic microscopy
- Confocal scanning laser microscopy

Other methods:
- Melanoma sniffing dogs
- Epidermal genetic information retrieval
- Digital dermatoscopy
- Wood’s lamp
Electromagnetic radiation in medical diagnosis

Herman C. Clinical, Cosmetic and Investigational Dermatology. November 2012
Modern diagnostic tools for early detection SKIN CANCER

Population

- General population
- High risk-patients

End users

- Patients
- Nurses or general physicians
- Dermatologists
- Specialized centers
Qualitative methods

Systems that use different light to obtain images from the tissue.
Type I, II, III.
Passive methods, Available

- Total Body Photography
- Dermoscopy
- Digital Dermoscopy
Qualitative methods

Systems that use different light to obtain images from the tissue.
Type I, II, III.
Passive methods, Available

- Total Body Photography
- Dermoscopy
- Digital Dermoscopy

- Reflectance Confocal Microscopy
- Optical Coherence tomography
- Multiphoton tomography
- RAMAN+RCM
- Photoacoustic microscopy
- Multi-Hyperspectral imaging

High resolution color imaging in 10 minutes

Comprehensive body imaging from head to toe (85% coverage)

Private storage of all images and date marked for easy comparison
3D Total Body surface
New technologies in TBP

New technologies in TBP

New technologies in TBP

New technologies in TBP and TB dermoscopy

Multi-Hyperspectral imaging

A total of 14 LMs and 5 LMMs in 19 patients were included. HIS analysis matched the histopathological analysis in 18/19 (94.7%) cases while in 1/19 (5.3%) cases HIS showed lesion extension not confirmed by histopathology (false positives). Compared to clinical examination, HIS defined lesion borders more accurately in 10/19 (52.6%) of cases (wider, n=7 or smaller, n = 3) while in 8/19 (42.1%) cases lesion borders were the same as delineated clinically as confirmed histologically. Thus, HIS is useful for the detection of subclinical LM/ LMM borders.
Hyperspectral: visual representation of distribution of colours

- Visual interpretation
- Computer vision analysis

Hyperspectral images in Melanoma: Diagnoptics project
New imaging technology in skin cancer

- In vivo confocal microscopy
- Multiphoton microscopy
- Optical coherence tomography
Resolution and penetration

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[Graph showing comparison of resolution and penetration among different imaging techniques: Dermoscopy, Sonography, OCT, Confocal reflectance/fluorescence, Multiphoton tomography.]

- **Resolution**
  - Dermoscopy
  - Sonography
  - OCT
  - Confocal reflectance/fluorescence
  - Multiphoton tomography

- **Penetration**
  - Epidermis
  - Dermis
  - Hypodermis

DEPTH

RESOLUTION

<table>
<thead>
<tr>
<th>Epidermis</th>
<th>Dermis</th>
<th>Hypodermis</th>
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<tbody>
<tr>
<td>0.25</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
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Reflectance confocal microscopy
Principles of Confocal Imaging

- The pigment **melanin** within the epidermis has a high refractive index, in fact higher than keratin.
- The confocal microscope images keratinocytes in the epidermis, erythrocytes and leukocytes in capillaries within the papillary dermis and collagen bundles within the dermis to a **depth of 100-200 µm at the 830 nm wavelength**.
Hand held RCM

Fast examination (1-5 minutes)
Facial areas
Resolution is limited by penetration

- Dermoscopy
- Sonography
- OCT
- Confocal reflectance/fluorescence
- Multiphoton tomography

Epidermis | Dermis | Hypodermis
Multiphoton tomography

Simultaneous excitation of endogenous fluorophores by two or more photons of low energy in the NIR

M. Kaatz, K. König. Multiphotonenmikroskopie und In-vivo-Multiphotonentomographie in der dermatologischen Bildgebung. Hautarzt 2010

Multiphoton tomography

Combination of MPT and FLIM (fluorescence life time excitation). 200 microm resolution

Federica Arginelli et al. High resolution diagnosis of common nevi by multiphoton laser tomography and fluorescence lifetime imaging. Skin res and technol 2013
Federica Arinelli et al. High resolution diagnosis of common nevi by multiphoton laser tomography and fluorescence lifetime imaging. Skin res and technol 2013
In Vivo Multiphoton Microscopy of Basal Cell Carcinoma

Mihaela Balu, PhD; Christopher B. Zachary, MD; Ronald M. Harris, MD; Tatiana B. Krasieva, PhD; Karsten König, PhD; Bruce J. Tromberg, PhD; Kristen M. Kelly, MD

Federica Arginelli et al. High resolution diagnosis of common nevi by multiphoton laser tomography and fluorescence lifetime imaging. Skin res and technol 2013
HIGH-DEFINITION OPTICAL COHERENCE

OCT is a noninvasive, in vivo imaging method, which captures high-resolution (1 μm), 3D images of biological tissue.

OCT is an interferometric technique using relatively long-wavelength light in the near-IR portion of the spectrum, which is able to penetrate into the scattering medium (deeper than CSLM).

HIGH –DEFINITION OPTICAL COHERENCE

An image is generated not by the light intensity directly like in dermoscopy but by the strength of the interference signal.

Result is:
1. Scattered light is blanked out
2. Depth selection becomes possible

Courtesy of Marc Boone
HIGH DEFINITION OPTICAL COHERENCE

• Lower resolution than Reflectance confocal microscopy
• Higher depth of tissue examination (500 microm)
• 3 dimensional reconstruction

Courtesy of Marc Boone
Resolution is limited by penetration

- Dermoscopy
- Sonography
- OCT
- Confocal Multiphoton

<table>
<thead>
<tr>
<th>Epidermis</th>
<th>Dermis</th>
<th>Hypodermis</th>
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Depth: mm

- 0.25
- 1.0
- 10

Resolution vs. Depth
Multimodal microscopy

RESOLUTION

DEPTH

Epidermis  Dermis  Hypodermis
Multimodal microscopy

Superdepth, superresolution, and superb contrast
Photoacoustic microscopy

Pulsed laser + US detector

Photoacoustic microscopy

PAM/OCT imaging system (PAT)

Photoacoustic microscopy

PAM/OCT imaging system

Photoacoustic microscopy

PAM/OCT imaging system

Wang LV. Multiscale photoacoustic microscopy and computed tomography. Nat Photonics. 2009 Aug 29;3(9):503-509
Photoacoustic microscopy

PAM/OCT imaging system

Quantitative methods using automatised algorithms

Systems that use different physical measures to obtain information from the tissue. The system is providing an automatised analysis and final classification to the end user.

- Multi-Spectral Imaging (Melafind)
- Electrical impedance Spectroscopy (EIS) (SCIBASE)
- Raman
MelaFind (Multi-Spectral Imaging)

Noninvasive, fully automatic, computer-vision diagnostic system (type 2)

Designed as an aid to detection of early melanoma and developed to identify PLs that should be considered for biopsy to rule out melanoma.
Multi-Spectral Imaging

MelaFind acquires digital multispectral images of a PL in 10 different spectral bands, from blue (430 nm) to near infrared (950 nm).

MelaFind uses automatic image analysis and statistical pattern recognition to help identify lesions to be considered for biopsy to rule out melanoma.
MelaFind

1. Emits multiple wavelengths of light
2. Captures lesion images
3. Analyzes images
4. Separates pigmented lesions from melanoma
The Performance of MelaFind

A Prospective Multicenter Study

Gary Monheit, MD; Armand B. Cognetta, MD; Laura Ferris, MD, PhD; Harold Rabinovitz, MD; Kenneth Gross, MD; Mary Martini, MD; James M. Grichnik, MD, PhD; Martin Mihm, MD; Victor G. Prieto, MD, PhD; Paul Googe, MD; Roy King, MD; Alicia Toledano, ScD; Nikolai Kabelev, BCSc; Maciej Wojton, MS; Dina Gutkowicz-Krusin, PhD

Setting: Three academic and 4 community practices in the US

Patients: 1632 lesions (including 127 melanomas—45% in situ—with median Breslow of invasive lesions, 0.36 mm)

Trial Registration: clinicaltrials.gov Identifier: NCT00434057

MelaFind® - P090012

This is a brief overview of information related to FDA’s approval to market this product. See the links below to the Summary of Safety and Effectiveness Data (SSED) and product labeling for more complete information on this product, its indications for use, and the basis for FDA’s approval.

**Product Name:** MelaFind®
**PMA Applicant:** MELA Sciences, Inc.
**Address:** 50 South Buckhoult Street, Suite 1, Irvington, NY 10533
**Approval Date:** November 1, 2011

**What is it?** An optical imaging and analysis device used in the detection of melanoma among atypical skin lesions.

**How does it work?** The device uses light to image the skin through a layer of isopropyl alcohol to generate a positive or negative result based on predefined image analysis algorithms.

**When is it used?** The device is used when a dermatologist chooses to obtain additional information on atypical skin lesions for a decision to biopsy.

**What will it accomplish?** MelaFind will provide the dermatologist with additional information about atypical skin lesions. This additional information may help a dermatologist find additional melanomas that may not have been found without the use of the device.

**When should it not be used?** There are no contraindications.

**Additional information:** Summary of Safety and Effectiveness and labeling will be available online.
Overview

- EIS is a measure of the overall resistance within the tissue at alternating currents of various frequencies.
- EIS is measured by applying an unnoticeable and harmless alternating electrical current onto the skin and measure the response.
- The frequencies used in the applied signal relate to clinically relevant properties, such as composition of intra and extra-cellular environments, cell shape and size, and cell membrane composition.
- The CE marked technology platform consists of a handheld probe with a disposable electrode connected to a device analysing the signals.
  - Aside from the disposable electrode, other parts of the device are made of standard components.
- Algorithm development based on >4,000 lesions with 500 Malignant Melanoma measurements in International Multicenter Melanoma Training Studies (IMATS).
- The system gives on-screen results within seconds.

Small and portable device, with a screen where the results are shown within seconds.
Principle of microinvasive impedance

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The pins penetrate into the stratum corneum</td>
</tr>
<tr>
<td>2. Impedance is measured in the viable skin under stratum corneum</td>
</tr>
<tr>
<td>3. Alternating current is transmitted from one electrode bar to another at 35 predefined frequencies that relate to clinically relevant properties in the skin</td>
</tr>
<tr>
<td>4. Amplitude and phase shift in the receiving signal are measured at the receiving electrode bar for each of the 35 frequencies</td>
</tr>
<tr>
<td>5. In order to cover the lesion in both width and depth, the measurement is performed in 10 permutations covering both shallow measurements between neighbouring electrode bars as well as deeper measurements between more distant electrode bars</td>
</tr>
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</table>
Electrical impedance Spectroscopy (EIS)

Measurement process – three simple and painless steps with results within seconds

1. Clean and moisten the skin
2. Perform a reference measurement close to the lesion
3. Perform lesion measurement(s)

On screen results (benign or malignant)…

…within seconds
The patented microinvasive electrode

Electrical impedance Spectroscopy (EIS)

The electrode consists of 5 goldplated electrode bars

On the surface of each electrode bar there are 45 microscopic spikes (length ~150 μm and width ~40 μm)
# Pivotal study – overview

SciBase International Melanoma Pivotal Study

<table>
<thead>
<tr>
<th>Purpose</th>
<th>The final SIMPS pivotal study was performed with the objective to provide scientific evidence of the accuracy of the SciBase system in detecting Malignant Melanoma. As the study is designed to provide scientific evidence of the methods' accuracy, it also provides the basis for a regulatory approval in the US</th>
</tr>
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<tbody>
<tr>
<td>Design overview</td>
<td>The study was run as an international, multicenter, prospective, non controlled and non randomised clinical trial conducted at both private and academic dermatological centers. Prior to initiation, the study was approved by national and local ethics committees and carried out in accordance with international conference of harmonization of good clinical practice (ICH-GCP)</td>
</tr>
<tr>
<td>Geography</td>
<td>The study was run in both Europe and the US with 22 participating clinics in UK, Germany, Sweden, Hungary, Austria, Spain and the US (17 clinics in Europe and 5 clinics in the US)</td>
</tr>
<tr>
<td>Size</td>
<td>In total 2,400 lesions were included in the study, which is the largest prospective study ever conducted in melanoma detection</td>
</tr>
</tbody>
</table>
Pivotal study – top line results *

Median Breslow thickness: 0.4 mm

- Age Groups 30 years and above
- SCC = Squamous cell carcinoma
- BCC = Basal cell carcinoma

<table>
<thead>
<tr>
<th>Exclusion Reason</th>
<th>No. Lesions</th>
<th>Source</th>
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<tbody>
<tr>
<td>Lesions included</td>
<td>2416</td>
<td></td>
</tr>
<tr>
<td>Signed Informed Consent Form Missing</td>
<td>1</td>
<td>0,04%</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>17</td>
<td>0,7%</td>
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<tr>
<td>Not eligible (i.e. inclusion/exclusion)</td>
<td>61</td>
<td>2,5%</td>
</tr>
<tr>
<td>Major Protocol Violation</td>
<td>29</td>
<td>1,2%</td>
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<tr>
<td>Measurement not acquired</td>
<td>60</td>
<td>2,5%</td>
</tr>
<tr>
<td>Coverage***</td>
<td>98</td>
<td>4,1%</td>
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<tr>
<td>Not eligible histopathology (preparation quality)</td>
<td>8</td>
<td>0,3%</td>
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<tr>
<td>Missing histopathology*</td>
<td>39</td>
<td>1,6%</td>
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<tr>
<td>Inaccurate mapping of histopathology†</td>
<td>7</td>
<td>0,3%</td>
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<tr>
<td>No Consensus†</td>
<td>44</td>
<td>1,8%</td>
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<tr>
<td>Poor Reference Quality**</td>
<td>95</td>
<td>3,9%</td>
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<tr>
<td>Device failure</td>
<td>14</td>
<td>0,6%</td>
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<tr>
<td>Eligible Lesions</td>
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</table>

Electrical Impedance Spectroscopy (EIS)
Sir C.V. Raman
Nobel Prize of Physics in 1930
Vibration profiles specific of some molecules in the tissue can be induced by photons. These methods have been introduced for the diagnosis of skin tumors.


IR and RAMAN

IR and Raman spectroscopy provide details regarding the chemical composition and molecular structure of substances in cells and biological tissues, and they are considered to be vibrational spectroscopic techniques.

IR spectroscopy measures absorbed radiation, and can serve as a visualization tool to aid the pathologist in evaluating tissue specimens.


Confocal Raman microspectroscopy has been used to study structures in up to 200-μm depth. The combination of Raman spectroscopy and Confocal Scanning Laser Microscopy (CSLM) offers the ability to analyze sections and layers of the skin without physically dissecting the tissue. The feasibility of these methods for the diagnosis of skin cancers


A revolutionary advancement in skin cancer detection

Verisante's Aura™ is a novel, multimodality imaging and spectroscopy system designed to aid in the detection of skin cancer. This system provides valuable information by identifying spectral changes associated with the biochemistry of skin cancer cells in less than a second; providing immediate results.

Early Detection Saves Lives
Early Detection Saves Lives

Jointly developed by the BC Cancer Agency and the University of British Columbia, and refined and tested at the Skin Care Centre at Vancouver General Hospital, this patent protected technology has already been used in a human clinical study spanning six years on approximately 1,000 lesions. Results published in the peer-reviewed journal Cancer Research showed that for sensitivities between 95% and 99%, specificities ranged between 66% and 24%. According to an Australian study, the sensitivity of clinical diagnosis for malignant melanoma is approximately 33.8%.

Verisante’s Aura™ will help to automate the current process of diagnosis, allowing rapid scanning of the 20 – 40 skin lesions on “at risk” individuals. No longer will patients need to suffer through long wait times to see a dermatologist, as scans may be accomplished quickly by trained technicians or assistants. Verisante’s Aura™ will greatly aid healthcare professionals, delivering significant clinical impact through improved patient outcomes and reduced wait times.

The Verisante product development team is led by award-winning pioneers in the field of cancer imaging. In addition, the Company is proud to have world renowned experts in cancer detection involved with our technical and medical team.

Aura™ has been approved for sale in Canada, the EU and Australia.

The Facts on Skin Cancer

- Skin cancer is the most common form of cancer, and most rapidly increasing
- Every hour, one person in the United States dies of melanoma
- 50% of people over the age of 65 in the US will be affected by skin cancer
- One in six Canadians will develop skin cancer during their lifetime
- Survival rate of patients where the disease is detected early is 99%
- Survival rate of patients with advanced stage melanoma skin cancer is 15%
- The treatment of advanced stage melanoma costs 2200% more than early stage melanoma
- The annual treatment cost of treating skin cancer in the United States is estimated at USD $3.0 billion
For sensitivities between 95% and 99%, the specificities ranged between 15% and 54%. Our findings establish that real-time Raman spectroscopy can be used to distinguish malignant from benign skin lesions with good diagnostic accuracy comparable with clinical examination and other optical-based methods.
Spectral results plotted for lower frequency range only (500–1,055 cm\(^{-1}\))
Variability according to lesion diagnosis.

The y-axis scales for A and B are different. AK, actinic keratosis; AN, atypical nevus, BN, blue nevus; CN, compound nevus; IN, intradermal nevus; JN, junctional nevus; MM, malignant melanoma; SK, seborrheic keratosis.
Lesion classification by Raman spectroscopy based on PC GDA analysis. Skin cancer (actinic keratosis, including MM, BCC, SCC, AK, n = 232) from benign skin disorders (including atypical nevi, blue nevi, compound nevi, intradermal nevi, junctional nevi, seborrheic keratosis, n = 286; A), melanoma (n = 44) from benign pigmented skin diseases (including atypical nevi, blue nevi, compound nevi, intradermal nevi, junctional nevi, seborrheic keratosis, n = 286; B), and melanoma (n = 44) from seborrheic keratosis [(n = 114); C].

D–F
<table>
<thead>
<tr>
<th>Department</th>
<th>Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>Director</td>
<td>J. Malvehy</td>
</tr>
<tr>
<td>Research Coordinator</td>
<td>S. Puig</td>
</tr>
<tr>
<td>Dermatology</td>
<td>C. Carrera, A. Vilalta, A. Bennassar</td>
</tr>
<tr>
<td>Dermatopathology</td>
<td>A. Alós, A. Díaz</td>
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<td>Surgery</td>
<td>R. Rull</td>
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<td>Radiotherapy</td>
<td>C. Conill</td>
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<tr>
<td>Immunotherapy</td>
<td>R. Vilella, J. Mila</td>
</tr>
<tr>
<td>Nuclear Med.</td>
<td>S. Vidal</td>
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<td>Image Dx.</td>
<td>O. Chirife, M. Sánchez, R. Vilana</td>
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<td>M. Oncology</td>
<td>A. M. Arance</td>
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<tr>
<td>Genetics</td>
<td>C. Badenas, J. A. Puig, M. Milà, R. Cervera</td>
</tr>
<tr>
<td>Research Fellows</td>
<td>I. Alarcon, T. Martinez, V. Martins</td>
</tr>
<tr>
<td>Research nurses</td>
<td>P. Iglesias, D. Gabriel, M. Dominguez, Abel Caño</td>
</tr>
<tr>
<td>Psychologist</td>
<td>M. González</td>
</tr>
</tbody>
</table>
"I fear the day technology will surpass our human interaction. The world will have a generation of idiots."

- Albert Einstein
Animal diagnostic accuracy in medicine
A 75-year-old white Caucasian man presented to the dermatology clinic after his pet dog licked persistently at an asymptomatic lesion behind his right ear. The patient was previously unaware of the lesion.
Pigeons (*Columbia livia*) as Trainable Observers of Pathology and Radiology Breast Cancer Images

Richard M. Levenson\(^1\)*, Elizabeth A. Krupinski\(^2\), Victor M. Navarro\(^3\), Edward A. Wasserman\(^4\)

\(^1\) Department of Pathology and Laboratory Medicine, University of California Davis Medical Center, Sacramento, California, United States of America, \(^2\) Department of Psychological and Brain Sciences, The University of Iowa, Iowa City, Iowa, United States of America, \(^3\) Department of Radiology & Imaging Sciences, College of Medicine, Emory University, Atlanta, Georgia, United States of America

* levenson@ucdavis.edu (RML); ed-wasserman@uiowa.edu (EAW)
Results of training with breast histopathology samples at different magnifications and rotations.

A) When first trained with 4 magnification images the birds performed at chance levels of accuracy, but quickly learned to discriminate. Subsequently, when the birds were exposed to higher magnifications samples, their performance commenced at accuracies above chance (but below their final performance at lower magnification they had previously been exposed to), and improved further with training.

B) Introducing rotated versions of the training stimuli did not significantly affect performance at any of the magnifications.
Examples of benign (left) and malignant (right) masses in mammograms. Subsequent biopsy established histopathology ground-truth.

The birds proved to be similarly capable of detecting cancer-relevant microcalcifications on mammogram images. However, when given a different (and for humans quite difficult) task—namely, classification of suspicious mammographic densities (masses)—the pigeons proved to be capable only of image memorization and were unable to successfully generalize when shown novel examples.
Nevus en crecimiento