

Identifying Active **Melanocyte**

Based on *In Vivo*
Harmonic Generation Microscopy

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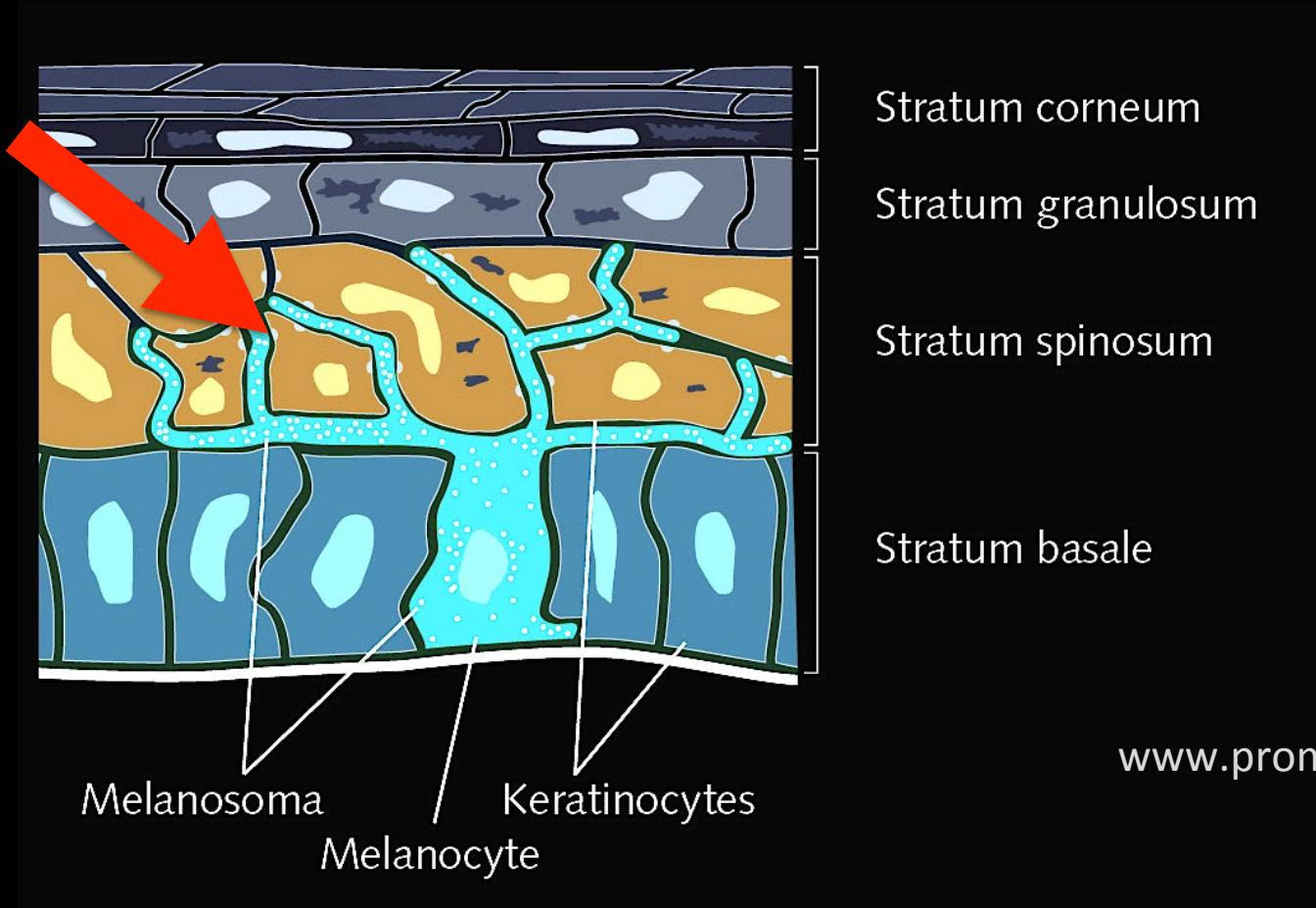
[Molecular Imaging Center | National Taiwan University]

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Clinical Scenario

- When I was a medical student...



Clinical Scenario

- When I was a resident in pathology...



Why Active Melanocyte?

- Producing melanin
- Endogenous marker for making diagnosis of melanocytic lesions

Hashemi. *J Am Acad Dermatol.* 2012
Segura. *Arch Dermatol.* 2007

- Various diseases
 - Hypermelanosis (melasma, solar lentigo)
 - Hypomelanosis (vitiligo, albinism)
 - Melanoma
 - Most fatal skin cancer

Question

- Can I see melanocytes,
or even active melanocytes
with dendrites and melanin clearly
by **in vivo skin scanning**, but
without time-consuming invasive biopsy?

Current Pitfall

- Identification of melanocytes and Langerhans cells (LC)
 - Difficult for the common used *in vivo* microscopy such as confocal microscopy

Hashemi . *J Am Acad Dermatol.* 2012

Segura. *Arch Dermatol.* 2007

Agero. *J Am Acad Dermatol.* 2006

Why Harmonic Generation?

- THG-enhanced nature of melanin

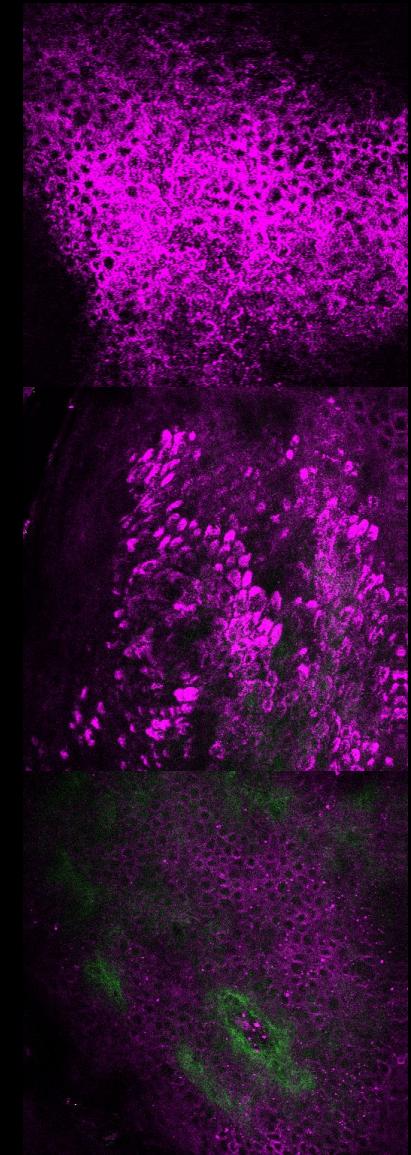
Chen *IEEE J Sel Topics Quantum Electron.* 2010

- Melanin quantification

Tsai. *Focus On Microscopy.* 2014

- HGM has high diagnostic accuracy in non-melanoma pigmented skin tumors

Tsai. *J Biomed Opt.* 2014

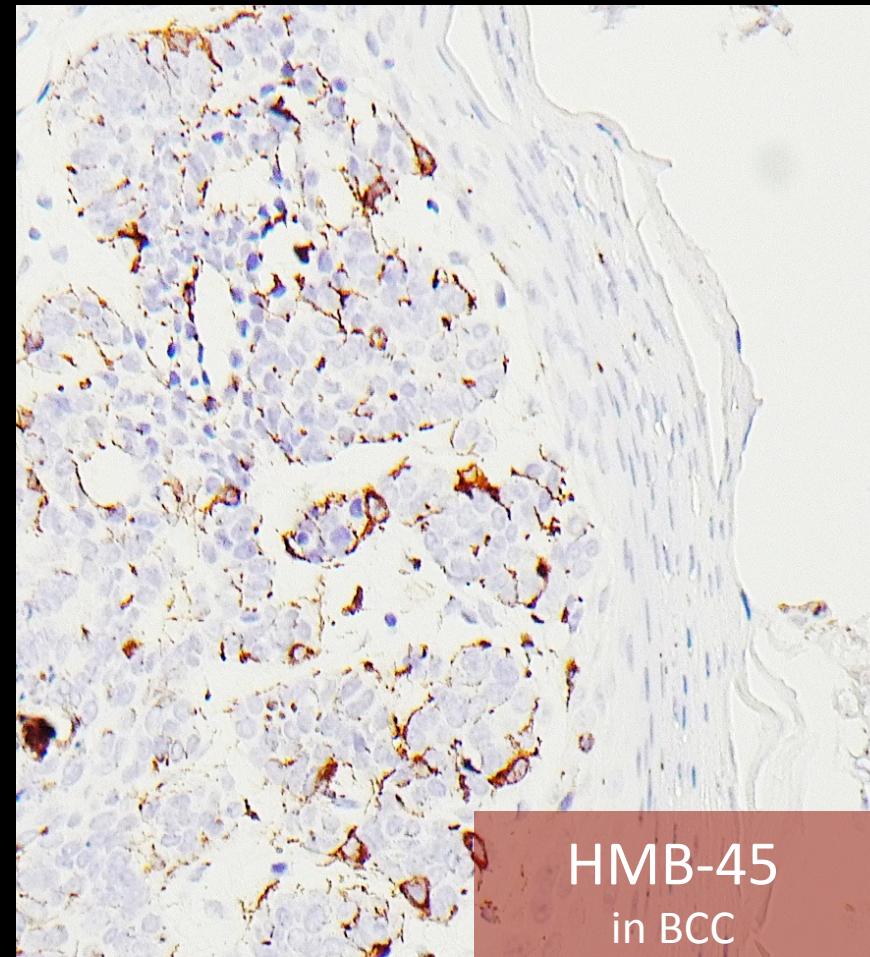


Medical Fact

- Dendritic cells in skin
 - 2 types

Florell. *Am J Dermatopathol.* 2001

1. Active melanocyte
 - HMB-45+ in melanocyte
 - Focal area
 - Pagetoid spread in melanoma, nevi (no atypia)

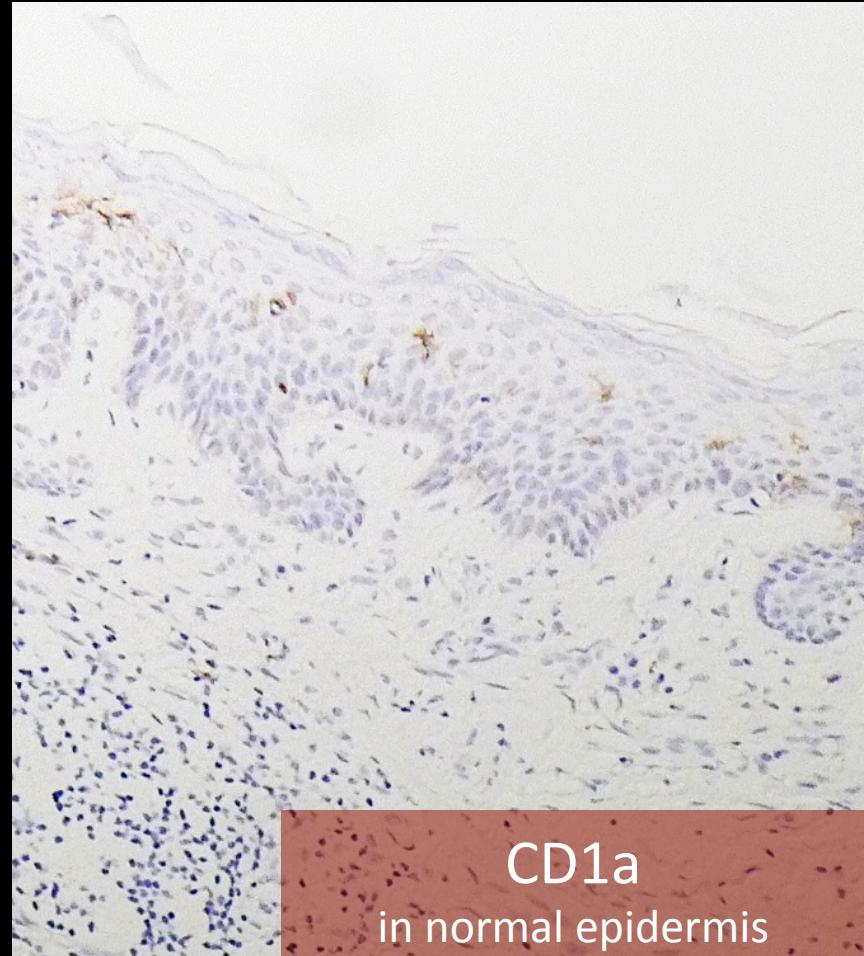


Medical Fact

2. Langerhans cell
 - CD1a+ (also in cortical thymocytes)
 - Superficial epidermis
 - In stratum spinosum

Agero. J Am Acad Dermatol. 2006

*Fitzpatrick's Dermatology
in General Medicine. 2008*

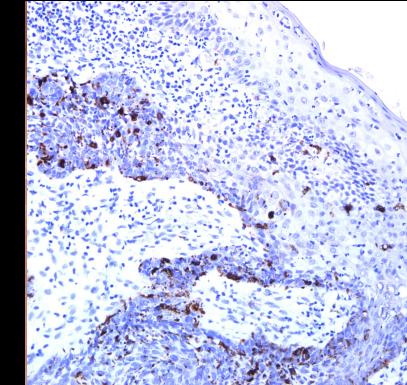


Study Protocol

Case | 17 patients, 6 ex vivo / 11 in vivo, 34 to 85 y/o
melanoma (1) / pigmented BCC (8) / SK (4) / nevus (4)



HGM | Cr:forsterite laser / 1230 nm / pulse width
100 fs / repetition rate of 110 MHz / output 500 mW ≤
30 min, accumulated energy < 180 J in each volunteer



~~2-3 sections with the most dendritic cell-like signal~~



Tissue Prove | By IHC staining
HMB-45 (active melanocyte) / CD1a (LC)
Observe suprabasal / basal layer and lesion

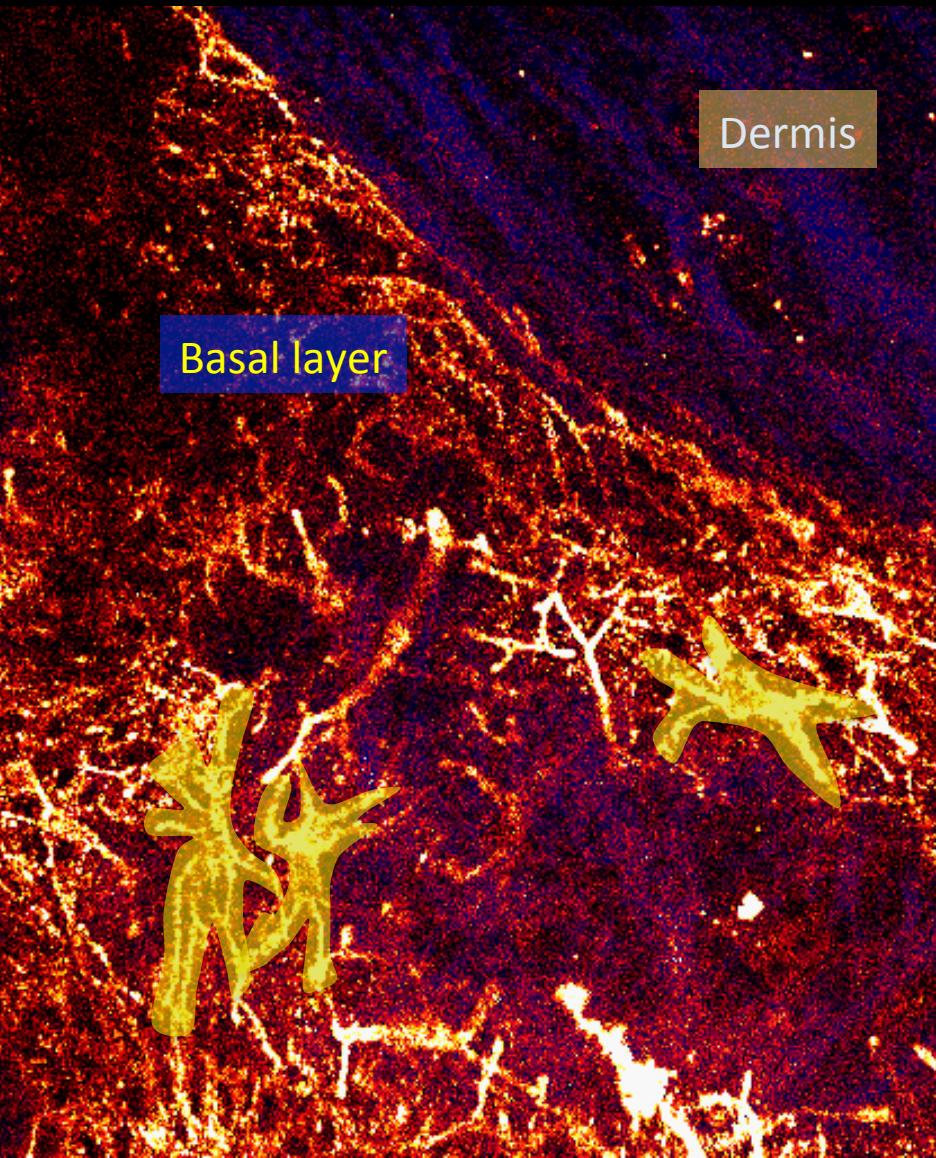


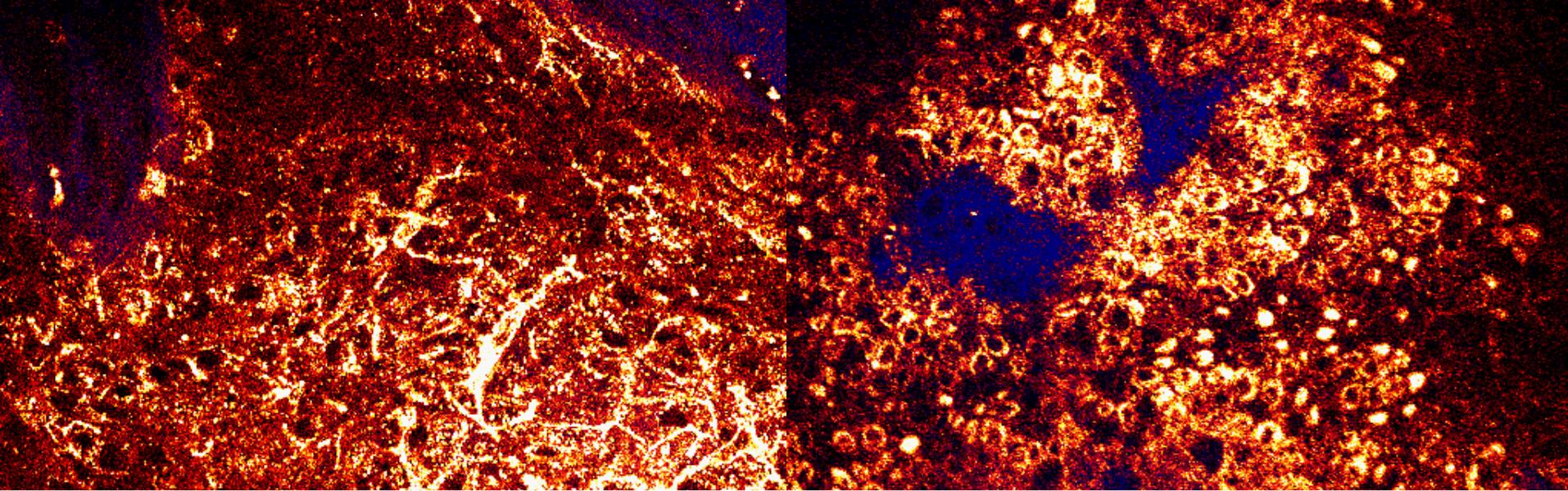
Interpretation and Analysis | By two doctors
HGM vs. IHC / statistical analysis

Study Result

- Agreement between 2 observers
 - Dermatologist and pathologist
 - Near 100% in more than 700 training images
 - Inconsistent data → Discard

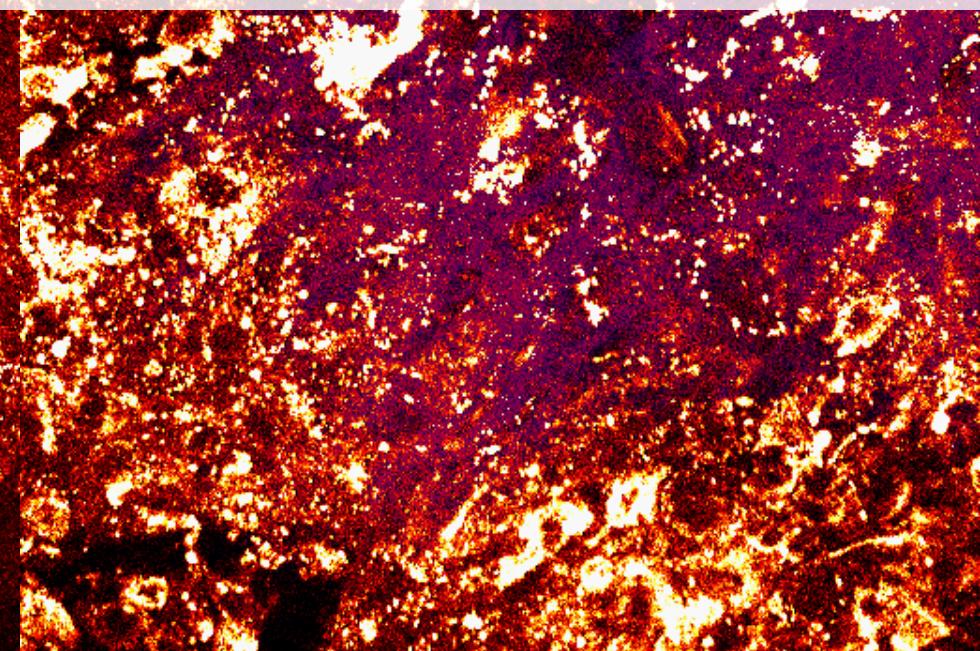
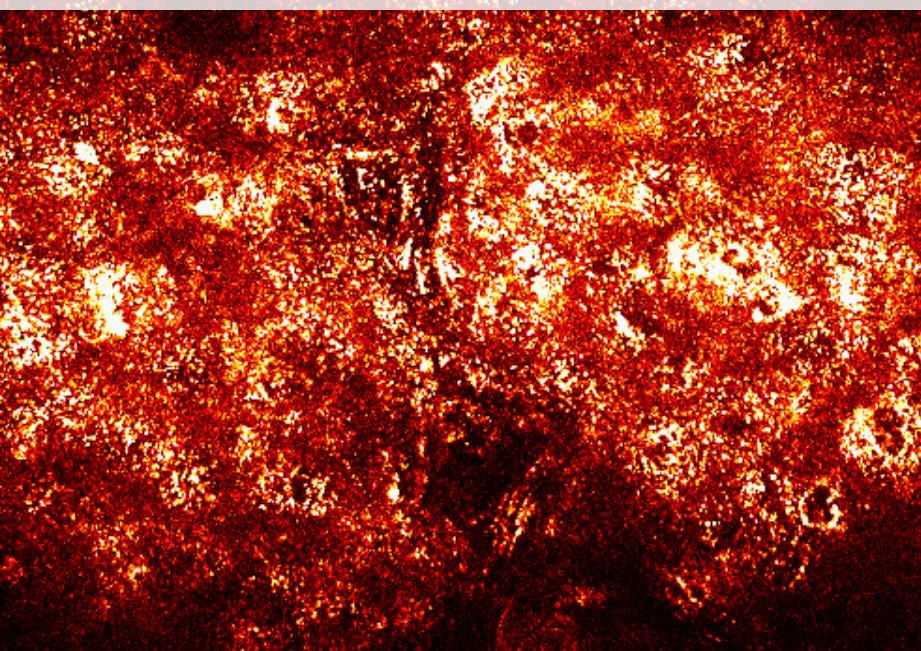
Dendritic Cell Identification



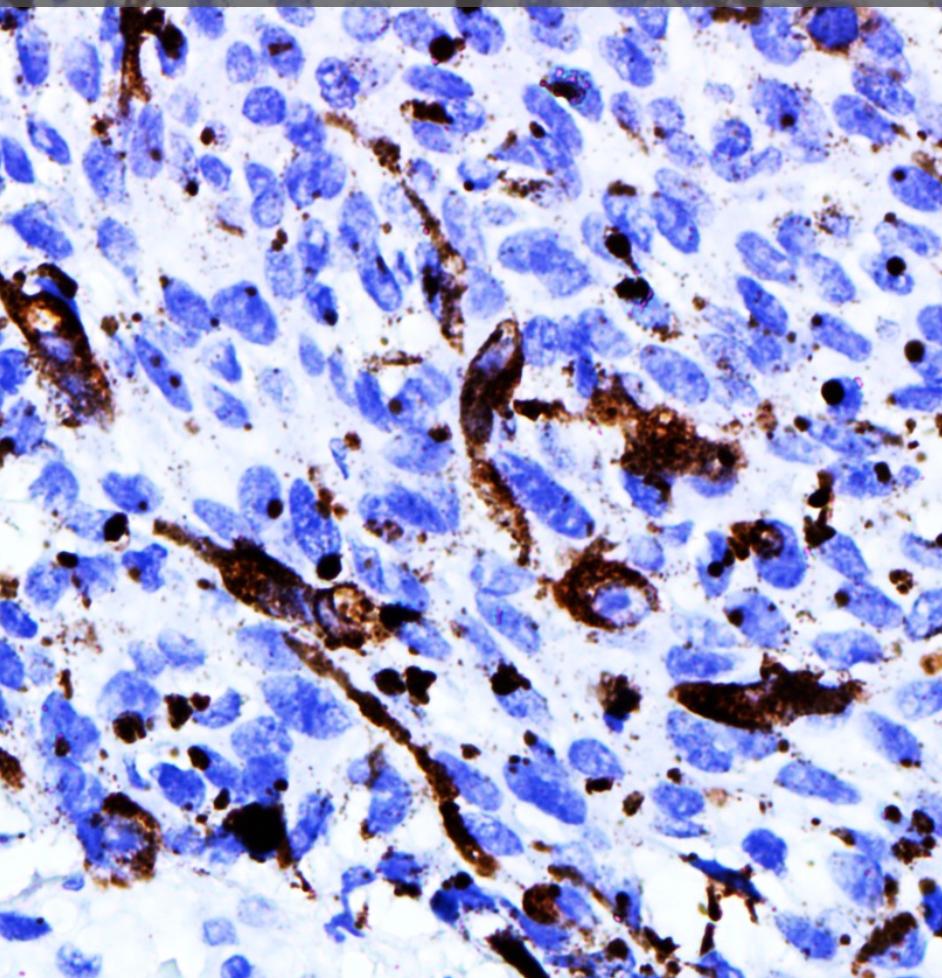


↑ Basal Cell Carcinoma
↓ Seborrheic Keratosis

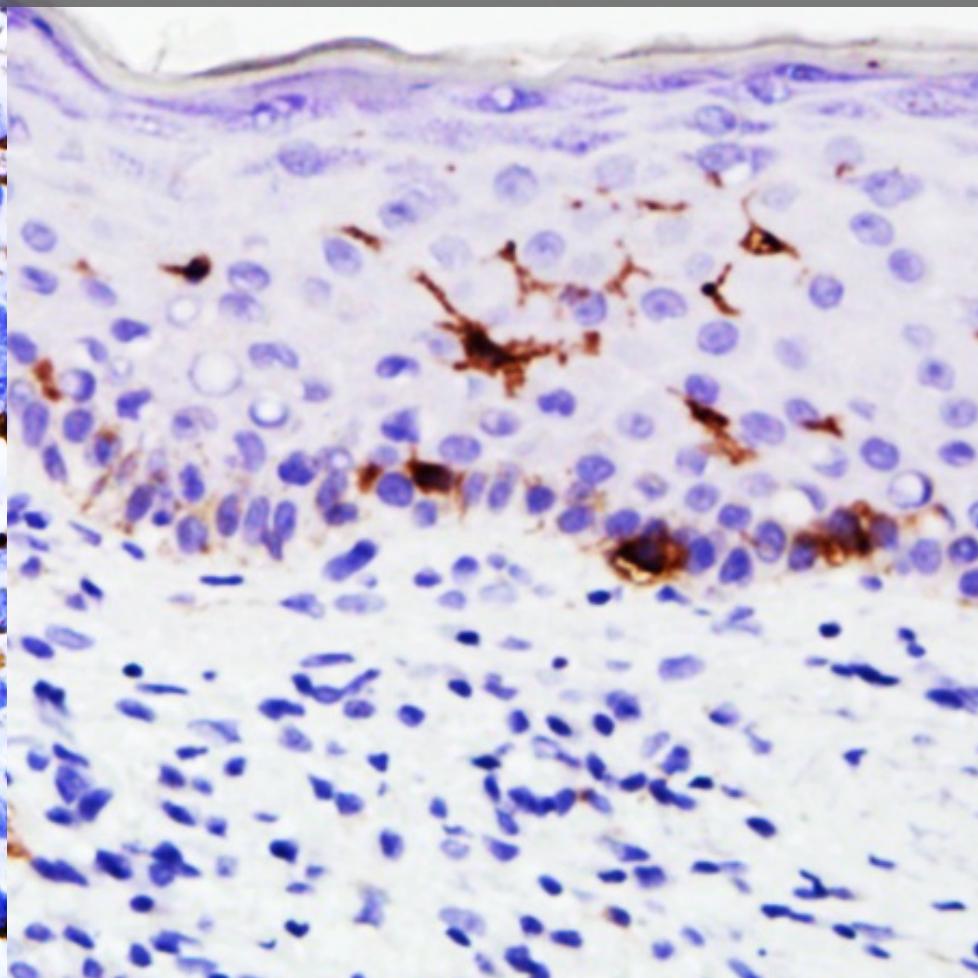
Intradermal Nevus
↑
Melanoma ↓



Evaluation of Special Staining



Melanocyte
HMB-45

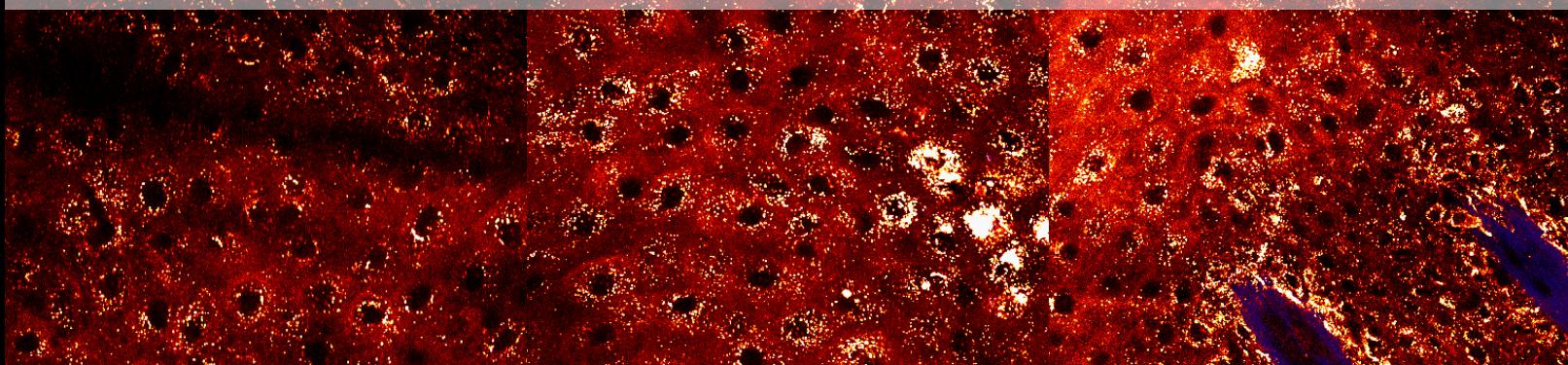


Langerhan's cell
CD1a

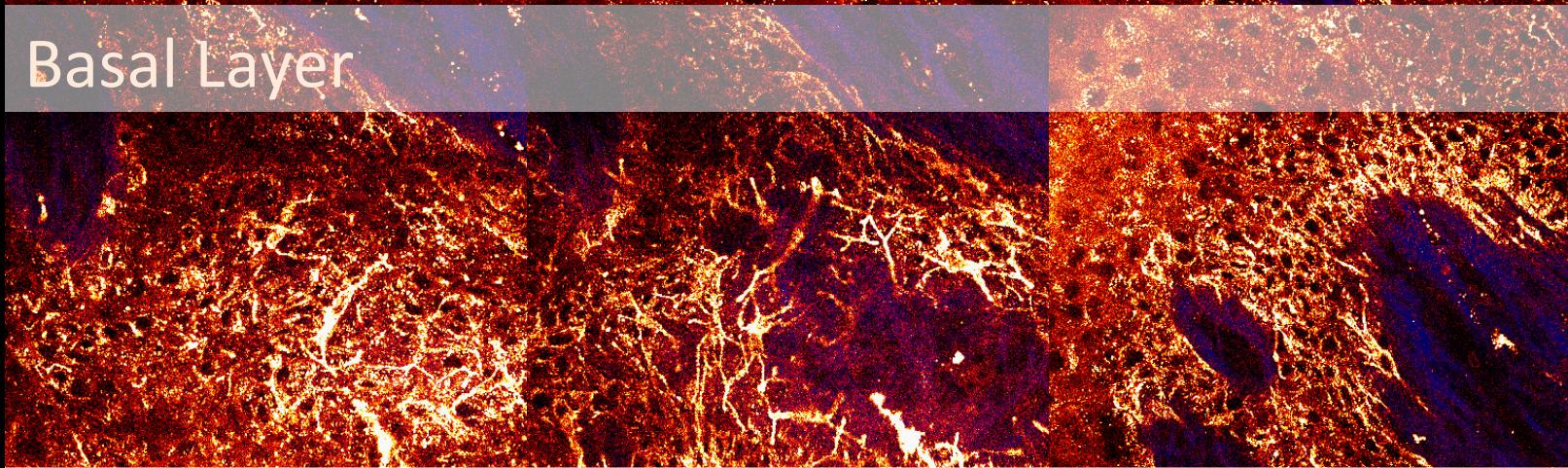
Summary of Results

- Dendritic cell-like signal in THG
 - Suprabasal 17.6%, basal and lesion 58.8%
 - 100% in basal layer and lesion of BCC
- HMB-45 (melanocyte)
 - Suprabasal 11.8%, basal and lesion 64.7%
 - 100% in basal layer and lesion of BCC
- CD1a (Langerhan's cell)
 - Suprabasal 100%, basal and lesion 17.6%

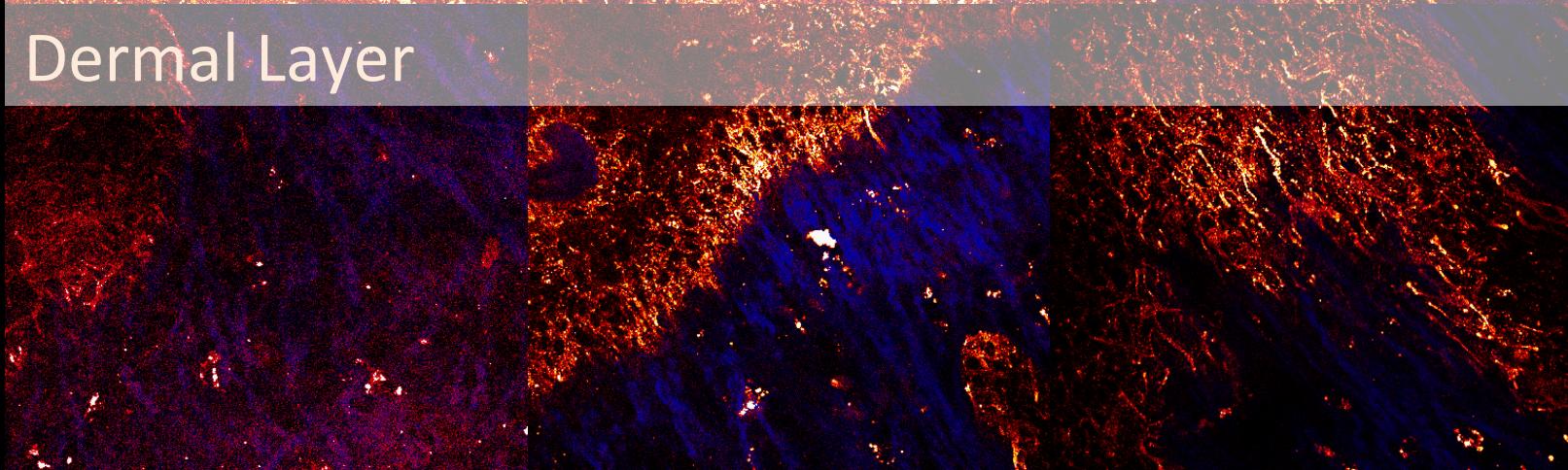
Suprabasal Layer



Basal Layer



Dermal Layer



Good Consistency

- **Consistency test for reliability**
 - Dendritic cell signal vs. active melanocyte
 - Kappa statistic = 0.807 → Good agreement
 - Dendritic cell signal vs. Langerhan's cell
 - Kappa statistic = -0.339 → Poor agreement
- Dendritic cells in HGM are more related to active melanocyte but not Langerhans' cell

High Sensitivity and Specificity

- **Statistics**
 - Dendritic cell signal vs. active melanocyte
 - Accuracy = 90.9%
 - Sensitivity = 95.0%
 - Specificity = 84.6%
 - PPV = 90.5%
 - NPV = 91.7%
- THGM has high accuracy to detect active melanocytes

Discussion

- First study of *in vivo* imaging demonstrate active melanocyte clearly by THGM
→ Potential clinical application
(Teledermatology / Telemedicine)

Comparison

	Melanocyte	Langerhan's cell
Third Harmonic Generation	Active – clear!	No
Reflectance Confocal	Yes	Yes

Hashemi. *J Am Acad Dermatol.* 2012
Segura. *Arch Dermatol.* 2007

Limitation

- Small case numbers
- Histopathological validation by melasma, vitiligo or albinism (usually no biopsy)

Conclusion

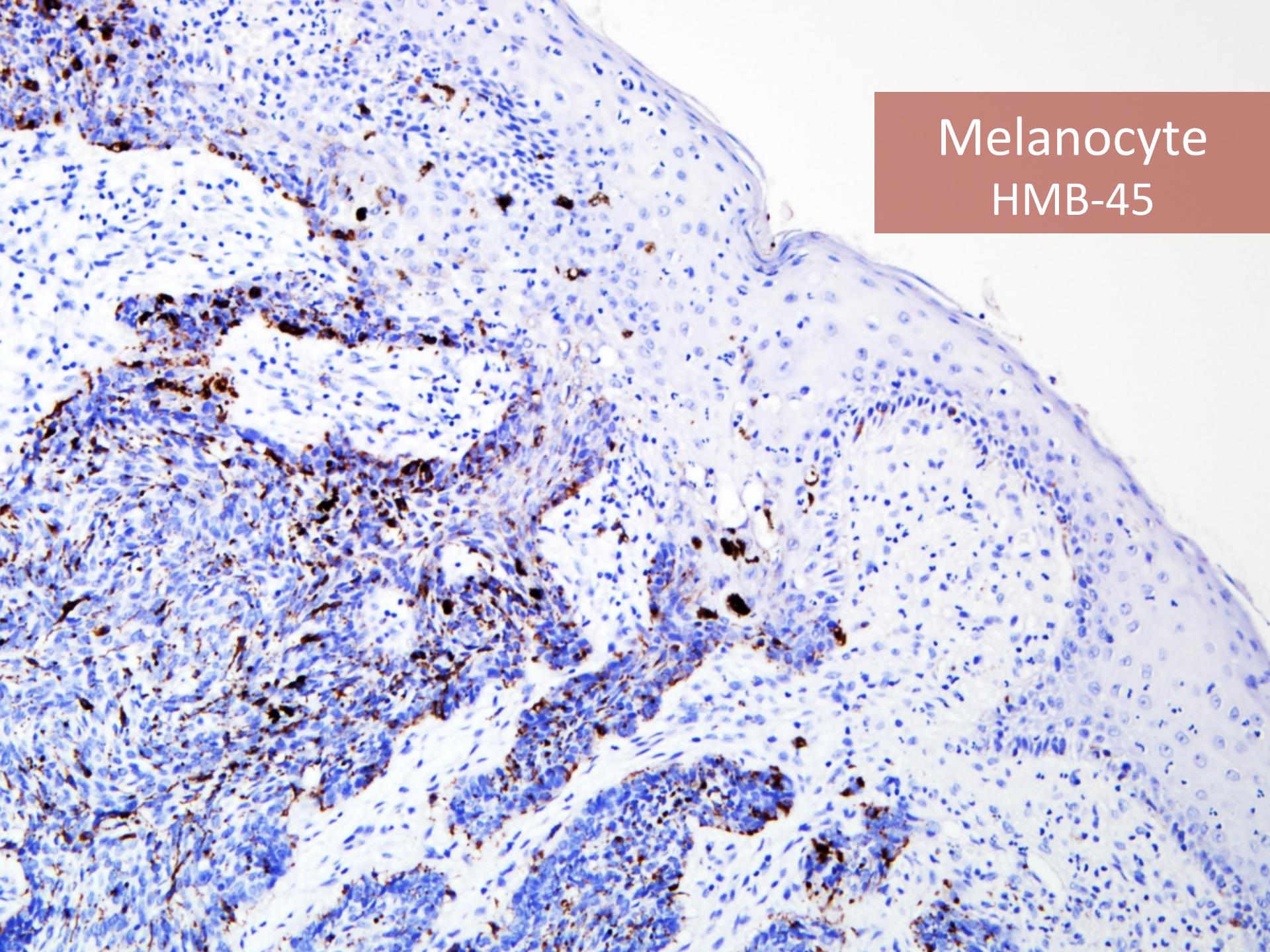
you for your attention!

Active Melanocyte
Dendritic-like THG bright cells in THGM image
THG enhancement nature of melanin
Histopathological and IHC proved
Potential of making differential diagnosis
of cutaneous lesions

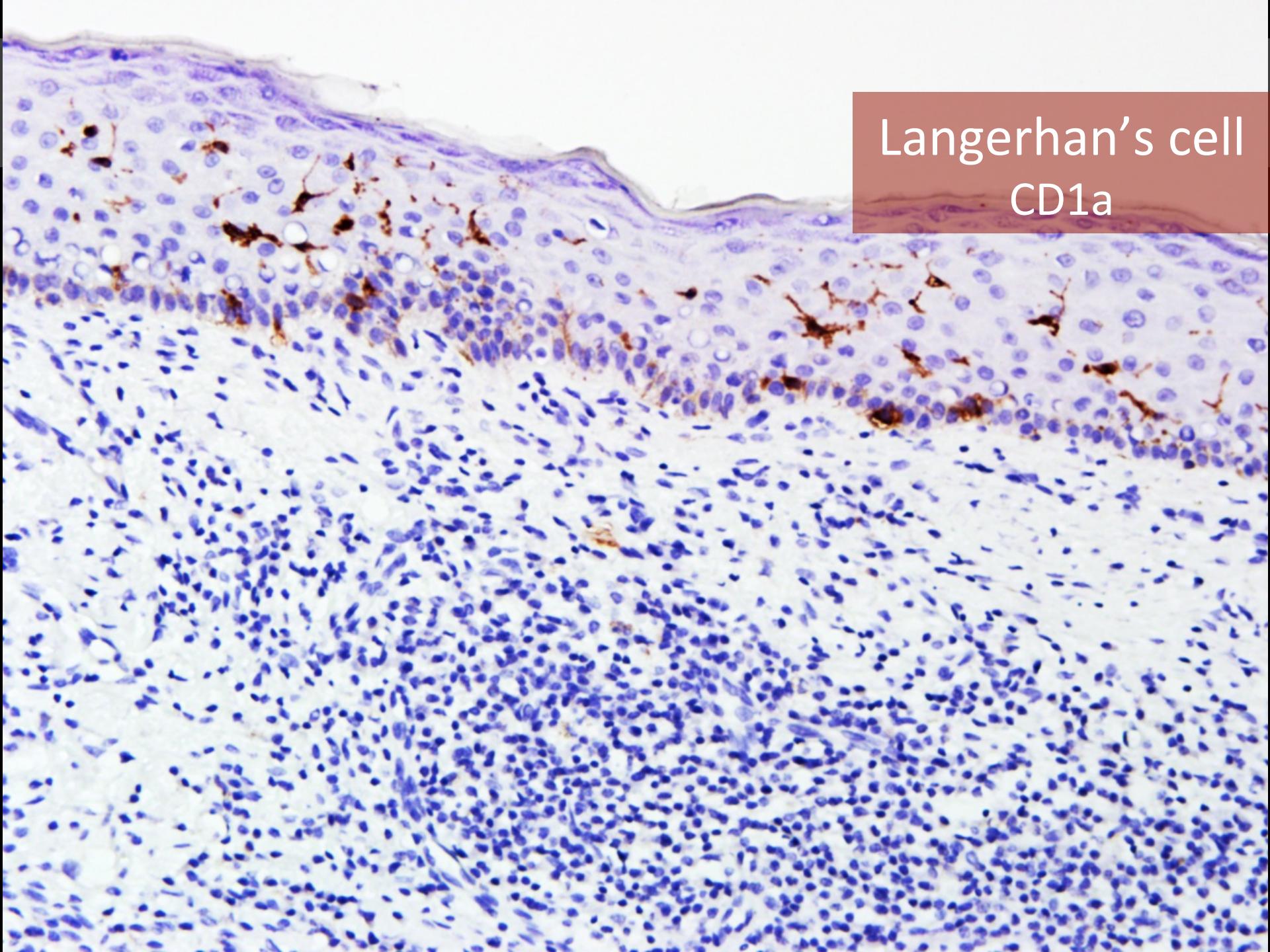
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Sun Lab | Molecular Imaging Center | National Taiwan University | Taiwan

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Melanocyte
HMB-45

This immunohistochemical image shows a cross-section of skin tissue. The epidermis at the top contains numerous small, dark brown-stained cells, which are Langerhan's cells. These cells have irregular, somewhat star-shaped nuclei and are surrounded by a dense layer of blue-stained keratinocytes. Below the epidermis is the dermis, which contains a more uniform distribution of blue-stained cells.

Langerhan's cell
CD1a

Harmonic Generation

- Dendritic cell-like THG signal presents

	Suprabasal	Basal Lesional	Dermis
Melanoma	1/1(100%)	1/1(100%)	1/1(100%)
BCC	2/8 (25%)	8/8 (100%)	0/8 (0%)
SK	0/4 (0%)	0/4 (0%)	0/4 (0%)
Nevus	0/4 (0%)	1/4 (25%)	1/4 (25%)

HMB-45 (Melanocyte)

	Suprabasal	Basal Lesional
Melanoma	1/1 (100%)	1/1 (100%)
BCC	1/8 (12.5%)	8/8 (100%)
SK	0/4 (0%)	0/4 (0%)
Nevus	0/4 (0%)	2/4 (50%)

CD1a

	Suprabasal	Basal Lesional
Melanoma	1/1 (100%)	0/1 (0%)
BCC	8/8 (100%)	2/8 (25%)
SK	4/4 (100%)	0/4 (0%)
Nevus	4/4 (100%)	1/4 (25%)

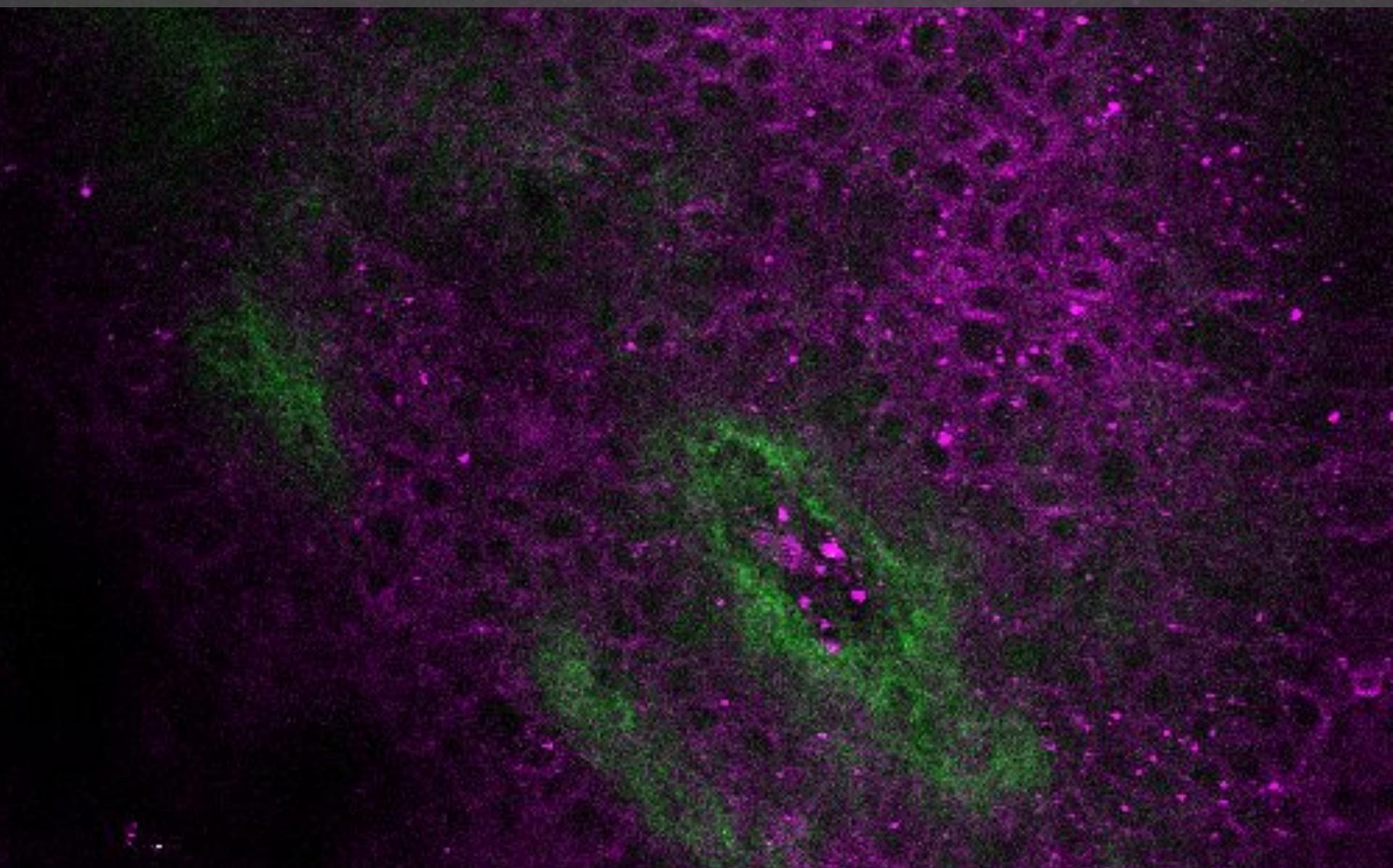
Look into 8 BCC Cases

	THG		HMB-45		CD1a	
Case	SB	B/L	SB	B/L	SB	B/L
1 (S01)	+	+	+	2+	1+	-
2 (S02)	-	+	-	2+	2+	2+
3 (S03)	-	+	-	1+	2+	-
4 (S11)	-	+	-	2+	2+	-
5 (S12)	-	+	-	1+	1+	-
6 (P13)	few	+	-	2+	1+	-
7(P33)	-	+	-	1+	1+	-
8 (P39)	-	+	-	2+	2+	1+

Other Cases

	THG		HMB-45		CD1a	
Case	SB	B/L	SB	B/L	SB	B/L
9 (S04)	+	+	+	+	1+	-
10 (P07)	-	-	-	2+	2+	1+
11 (P34)	-	-	-	-	2+	-
12 (P42)	-	NA	-	-	2+	-
13 (P43)	-	-	-	-	2+	-
14 (P17)	-	-	-	-	2+	-
15 (P31)	-	-	-	-	2+	-
16 (P36)	-	-	-	1+	2+	-
17 (P38)	-	-	-	-	2+	-

Case of Vitiligo



Melanin Quantification

- Melanosome in melanocyte
 - Membrane-bound organelle where melanin biosynthesis takes place
 - Eumelanosomes and pheomelanosomes
 - Eumelanin or pheomelanin
- Eumelanin
 - Dominant component of human epidermal melanin
 - More than 90% of total epidermal melanin

Alaluf 2002

Melanin Quantification by HGM

1. THG and TPEF *in vivo* imaging of mouse melanocytes
 - Correlation between THG and TPEF
2. Imaging different concentration of melanin solution by 2PF
 - Correlation between melanin concentration and TPEF
 - $TPEF = 68 * \text{melanin concentration} + 390.8$
3. Bridging two correlations and get the empiric correlation between melanin concentration and THG enhancement ratio

Liu 2013

HGM criteria for differential diagnosis of non-melanoma pigmented skin lesions

Basal cell carcinoma	Sensitivity / CI (%)	Specificity / CI (%)
Peripheral palisading cells	95 (84-99)	100 (96-100)
Proliferation of polymorphous basaloid cells	98 (88-100)	90 (82-95)
Elongated cells/nuclei	88 (75-95)	92 (85-96)
Collagen changes	67 (52-79)	98 (92-99)
Nevus		
Monomorphous cell nests	98 (90-100)	96 (89-99)
Normal epithelial stratification	98 (90-100)	88 (80-94)
Elongation of rete ridges	74 (61-84)	81 (71-88)
Seborrheic keratosis		
Proliferation of monomorphous melanocytic cells	97 (83-99)	98 (93-99)
Acanthotic epidermis	97 (83-99)	100 (96-100)

Sensitivity and specificity for differential diagnosis of three kinds of pigmented tumors

- Based on these HGM features with histopathological diagnostic scales
 - Overall sensitivity is **92%** and specificity is **96%** for direct judgment
 - Overall sensitivity is **94%** and Specificity is **100%** for **presence of 2 criteria**

	Sensitivity / CI (%)		Specificity / CI (%)	
	Direct judgment	Presence of 2 criteria *	Direct judgment	Presence of 2 criteria *
BCC	95 (72-99)	93 (69-98)	99 (86-100)	100 (88-100)
Nevi	93 (72-98)	96 (77-99)	94 (79-98)	99 (84-100)
SK	87 (58-94)	93 (64-99)	96 (83-99)	100 (89-100)

*Two criteria are with the highest sensitivity and specificity