12th Meeting of the International Confocal Working Group (ICG)
October 4th, 2013 (Friday) 18:00-21:00

AGENDA:
18:00  Introduction  
S. González

SKIN CANCER SESSION:
10´presentation + 3´Q&A

18.05-18.18  Effectiveness of RCM for melanoma diagnosis in the skin cancer diagnostic workflow  
Witkowski A, Farnetani F, Longo C, Pellacani G  
Department of Dermatology, University of Modena and Reggio Emilia, Italy

18.18-18.31  The Role of Reflectance Confocal Microscopy as an Aid in the Diagnosis of Collision Tumors  
Moscarella E1,2, Rabinovitz H3,4, Oliviero MC3,4, Longo C2, Zalaudek I2,5, Lallas A2, Argenziano G2, Pellacani G1  
1Department of Dermatology, University of Modena and Reggio Emilia, Modena, Italy.  
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5Department of Dermatology, Medical University of Graz, Austria.

18.31- 18.44  Prospective differentiation of clinically difficult to distinguish nodular basal cell carcinomas and intradermal nevi by non-invasive Reflectance Confocal Microscopy  
Department of Dermatology, Radboud University Nijmegen Medical Centre, Nijmegen.  
* contributed equally to this work

18.44- 18.57  Nodular lesions and confocal microscopy: light and dark  
Longo C1, Farnetani F2, Moscarella E1,2, Ciardo S2, Zalaudek I1, Argenziano G1, Pellacani G2  
1Dermatology and Skin Cancer Unit, Arcispedale Santa Maria Nuova, (Istituto di Ricovero e Cura a Carattere Scientifico-IRCCS) Reggio Emilia,  
2Department of Dermatology, University of Modena and Reggio Emilia, Italy

18.57- 19.10  In Vivo Reflectance Confocal Microscopy to Optimise the “Spaghetti” Technique for Defining Surgical Margins of Lentigo Maligna  
Cinotti E1, Perrot JL1, Labeille B1, Champin J2, Douchet C3, Parrau G2, Seguin P2, Cambazard F1  
1Department of Dermatology,  
2Department of Maxillofacial and Plastic Surgery,  
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Debarbieux S¹, Perrot JL², Erfan N³, Ronger-Savlé S⁴, Labeille B², Cinotti E², Depaepe L⁵, Cardot-Leccia N⁶, Lacour JP³, Thomas L¹, Bahadoran P³,⁷,⁸
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19.23-19.36 In vivo reflectance confocal microscopy of cutaneous T cell lymphomas: evaluation before and after photochemotherapy
Venturini M, Sala R, Zanca A, Calzavara-Pinton PG
Department of Dermatology, University of Brescia, Italy

INFLAMMATORY CONDITIONS AND COSMETICS SESSION:
19.36-19.49 Demodex mite density in rosacea patients - Therapy monitoring by confocal laser scanning microscopy
Sattler EC, Maier T, Hoffmann V, Ruzicka T, Berking C
Department of Dermatology and Allergology, Ludwig-Maximilian University Munich, Germany

19.49-20.02 Lichen Sclerosus: Correlation of Histopathological Features and Dermoscopy with in vivo Reflectance Confocal Microscopy
Arzberger E¹, Drabeni M², Massone C¹, Hofmann-Wellenhof R¹, Komericke P¹
¹Department of Dermatology and Venereology, Medical University of Graz, Austria, ²Department of Dermatology, Bellinzona Regional Hospital, Bellinzona, Switzerland

20.02-20.15 Dermoscopic and confocal microscopy changes in melanocytic nevi after laser treatment for hair removal
Pampín Franco A, López Estebarranz JL, Gamo Villegas R
Dermatology Unit, Hospital Universitario Fundación Alcorcón, Madrid, Spain.

UPDATE SESSION:
20.15-20.22 Update - exvivo confocal arena
Presented by J Malvehi
Melanoma Unit, Hospital Clinic, Barcelona, Spain

20.22-20.32 Update - multicentric clinical trial on Inflammatory conditions
Presented by Ardigo M.
Department of Dermatology, San Gallicano Institute, Rome, Italy

20.32-20.35 Initiative “Survey on Practical Aspects of RCM”
Presented by P. Bahadoran P
Dermatology department, Hôpital Archet 2, Nice, Centre de Recherche Clinique, CRC, Nice, INSERM U 1065, Equipe 1, Nice, France

20.35-21.00 Confocal microscopy: state of the art and experts' consensus
Presented by G Pellacani
Department of Dermatology, University of Modena and Reggio Emilia, Italy
ABSTRACTS

Effectiveness of RCM for melanoma diagnosis in the skin cancer diagnostic workflow
Witkowski A, Farnetani F, Longo C, Pellacani G
Department of Dermatology, University of Modena and Reggio Emilia, Italy

The Role of Reflectance Confocal Microscopy as an Aid in the Diagnosis of Collision Tumors
Elvira Moscarella, MD;1,2 Harold Rabinovitz, MD;3,5 Margaret C Oliviero, ARNP;3,5 Caterina Longo, MD;2 Iris Zalaudek,2,4 MD; Aimilios Lallas, MD;2; Giuseppe Argenziano, MD;2 Giovanni Pellacani, MD.1
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Background: Collision tumors have been defined as the association of two or more different neoplasms within the same lesion. The association of a benign neoplasm with a malignant neoplasm is of particular significance and warrants diagnostic accuracy.

Objective: To test the concordance of diagnosis with dermoscopy, reflectance confocal microscopy and histopathology.

Methods: Twenty-four histologically confirmed collision tumors were included in the study and retrospectively evaluated by means of dermoscopy and confocal microscopy.

Results: There was good to excellent concordance of diagnoses for benign-benign lesions with dermoscopy, confocal microscopy, and histopathology. For lesions with a malignant component, confocal microscopy either performed equivocally or was better than dermoscopy. The malignancy most commonly detected was basal cell carcinoma (n=13), followed by melanoma (n=5); and squamous cell carcinoma in situ (n=4). Seborrheic keratoses were the most common benign neoplasm found in association with collision tumors (n=18), followed by nevi (n=7). The presence of a SK component was frequently a challenge dermoscopically, in collision tumors involving BCC and SCC. In 6 cases the appearance of these neoplasms was suggestive of the diagnosis irritated SK.

Limitation: The main limitation was the retrospective design of the study.

Conclusion: The dermatoscope and the reflectance confocal microscope, when used in conjunction are valuable tools aiding in the diagnosis of collision tumors.

Prospective differentiation of clinically difficult to distinguish nodular basal cell carcinomas and intradermal nevi by non-invasive Reflectance Confocal Microscopy

L.Hoogedoorn1,4, M. Peppelman1,4, P.E.J. van Erp1, M.J.P. Gerritsen1. * contributed equally to this work.
1. Department of Dermatology, Radboud University Nijmegen Medical Centre, Nijmegen.
Clinical differentiation between a nodular basal cell carcinoma (nBCC) and a benign intradermal nevus can be difficult. Even with additional dermoscopic evaluation, a correct diagnosis may not be easy. Currently, histopathological examination of a biopsy is the gold standard to differentiate between these lesions. However, this is an invasive technique and can cause sampling errors. The present study with clinically difficult to distinguish nodular lesions, demonstrates that in routine patient care, reflectance confocal microscopy allows prospective, non-invasive, in vivo differentiation between nBCCs and intradermal nevi. Subsequently, unnecessary biopsies of benign lesions in cosmetic areas can be avoided.

**Nodular lesions and confocal microscopy: light and dark**

Caterina Longo¹, MD, PhD, Francesca Farnetani², MD, Elvira Moscarella¹,², MD, Silvana Ciardo², BS, Iris Zalaudek¹ MD, Giuseppe Argenziano¹, MD, Giovanni Pellacani², MD ¹Dermatology and Skin Cancer Unit, Arcispedale Santa Maria Nuova, (Istituto di Ricovero e Cura a Carattere Scientifico-IRCCS) Reggio Emilia, Italy ²Department of Dermatology, University of Modena and Reggio Emilia, Italy

**Background:** Nodular lesions poses diagnostic challenge since nodular melanoma may simulate all kind of melanocytic and non-melanocytic lesions. Reflectance confocal microscopy is a novel technique that allows the visualization of skin at nearly histologic resolution although limited laser depth penetration hamper the visualization of deep dermis.

**Methods:** 140 nodules were retrospectively evaluated by means of confocal microscopy in blind from histopathologic diagnosis. At the end of the study the patients’ codes were broken and the evaluations were matched with histopathologic diagnosis before performing statistical analysis.

**Objective:** We sought to assess whether the diagnostic accuracy of confocal microscopy compared to histopathology for the diagnosis of nodular lesions, and to identify possible limitations of this technique.

**Results:** The study consisted of 140 nodular lesions (23 “pure” nodular melanomas, 9 melanoma metastasis, 28 BCCs, 6 invasive SCC, 32 naevi, 14 Seborrheic keratosis, 17 dermatofibroma, 5 vascular lesions and 6 other lesions). Confocal microscopy correctly diagnosed 121 out of 140 lesions (86.4%); eight out of 140 (5.7%) lesions revealed discordance between histopathology and confocal microscopy. Eight out of 140 (5.7%) cases were not evaluable by means of confocal microscopy due to the presence of ulceration or hyperkeratosis and three cases showed a non specific pattern. Interestingly, confocal microscopy reached a 96.5% sensitivity and 94.1% specificity (AUC: 0.970) (CI95%: 0.924-1.015) (p<0.001) for the diagnosis of melanoma.

**Conclusions:** Despite the limited laser depth penetration of confocal microscopy, this imaging tool represents an effective instruments in diagnosing nodular lesions; however, fully ulcerated lesions or when a marked hyperkeratosis is present, biopsy should be always performed. Prospective studies on difficult to diagnose nodules should be performed to further analyze the pros and contra of RCM in skin cancer diagnosis.

**In Vivo Reflectance Confocal Microscopy to Optimise the “Spaghetti” Technique for Defining Surgical Margins of Lentigo Maligna**

Elisa Cinotti, MD,¹ Jean Luc Perrot, MD,¹ Bruno Labeille, MD,¹ Julie Champin, MD,² Thomas Alix, MD², Catherine Douchet, MD,³ Gregory Parrau, MD,² Pierre Seguin, MD, PhD,² Frédéric Cambazard, MD, PhD,¹ ¹Department of Dermatology, ²Department of Maxillofacial and Plastic Surgery, and ³Department of Pathology, University Hospital of Saint Etienne, Cedex 2, 42055 Saint-
**BACKGROUND** Lentigo maligna (LM) presents a therapeutic challenge for surgeons because of its location in aesthetic areas and its difficult determination of margins.

**OBJECTIVE** To investigate a new procedure combining the "spaghetti" technique with *in vivo* reflectance confocal microscopy (RCM) to define the margins of LM more accurately and allow strict histological control.

**METHODS AND MATERIAL** Thirty-three consecutive patients with LM of the head underwent a RCM-guided delineation of the margins followed by the "spaghetti" technique. If the excision was not in sano, a new excision of the affected area was performed in the same manner, until a tumor-free margin was reached. Finally, the excision of LM and the reconstruction was performed.

**RESULTS** The excision of the first “spaghetti” in a tumor-free area was obtained in 28 out of 33 patients; in 4 cases a second “spaghetti” was necessary and in one case 3 spaghetti were performed. The average length of the spaghetti was 96 mm.

The average steps to obtain free-tumor margins were 1.18 against 1.55 for the “spaghetti” technique alone and 1.63 to 1.9 for the “staged excision” techniques according to the different series. The final histological analysis confirmed the accuracy of the MCIV: the average minimum margin was 2.7 mm. A follow up of 27 patients on an average of 10 (range 4-25) months did not show any recurrence.

**CONCLUSION** This procedure allows an accurate definition of the surgical margins of LM, with a low rate of multiple excisions, sparing tissue in functional and/or aesthetic areas. These results should be confirmed on the basis of a larger series with a longer follow-up.

**Reflectance confocal microscopy of mucosal pigmented macules: a review of 56 cases including 10 macular melanoma.**

Sébastien Debarbieux MD¹, Jean Luc Perrot MD², Nouran Erfan MD³, Sandra Ronger-Savlé⁴, Bruno Labelle MD², Elisa Cinotti MD², Lauriane Depaepe MD⁵, Nathalie Cardot-Leccia⁶, Jean Philippe Lacour MD PhD⁵, Luc Thomas MD PhD¹, Philippe Bahadoran MD PhD³,⁷,⁸

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**Background** : Although most mucosal pigmented macules are benign, it can be clinically challenging to rule out an early melanoma in some cases. Reflectance confocal microscopy (RCM) is a non-invasive imaging technique useful to discriminate between benign and malignant skin lesions.

**Objective** : The purpose of our study was to describe the confocal aspects of benign and malignant mucosal pigmented macules with histopathological correlations.

**Methods** : We retrospectively reviewed the confocal images of 56 labial or genital pigmented macules including 10 macular melanoma

**Results** : In benign macules, the most frequently observed pattern was a ringed pattern characterized by round or polycyclic papillae, with hyperpigmentation of the basal layer; another pattern was characterized by sparse bright dendritic cells in the basal layer, the basal epithelial cells being otherwise usually less reflective. This pattern was variously observed according to the location (very frequent on the lip, frequent on the vulva, rare on
the glans). The presence of roundish cells, a high density of roundish or dendritic cells with atypias, intraepithelial bright cells were clues in favour of malignancy.

**Limitations**: According to the retrospective nature of the study, we evaluated the recorded images chosen by the physicians that performed the RCM examination for each case.

**Conclusion**: RCM seems to be a valuable tool to non invasively differentiate benign from malignant mucosal pigmented macules and target biopsies in cases of equivocal features.

**In vivo reflectance confocal microscopy of cutaneous T cell lymphomas: evaluation before and after photochemotherapy**

Venturini M, Sala R, Zanca A, Calzavara-Pinton PG
*Department of Dermatology, University of Brescia, Italy*

Primitive cutaneous T lymphomas (CTCL) are an heterogeneous group of non-Hodgkin lymphomas characterized by a dominant skin-homing T-cell clone. The clinical presentation of cutaneous T-lymphoma is variable depending on the subtype and on the stage. MF is the most frequent variant of CTCL, usually arising in mid to late adulthood, clinically defined by a progressive evolution of erythematous patches, plaques and tumors over year. Because of the variegate clinical expressions of MF, especially in the early stage, it represents often a diagnostic challenge for clinicians and it can require years before a correct diagnosis is made.

Although traditional histopathology is considered the gold standard in the diagnosis of MF, frequently the findings of early stages may be non-specific and resemble chronic inflammatory dermatoses; consequently could be needed multiple skin biopsies to establish the diagnosis of MF.

In vivo reflectance confocal microscopy (RCM) is a non-invasive technique tool for real-time imaging of epidermis and superficial dermis in vivo, generating horizontal skin sections at high resolution close to conventional histology.

RCM has been previously reported to be useful in the in vivo evaluation of skin tumors and inflammatory cutaneous diseases.

The aim of this study was to investigate the utility of in vivo RCM in describing morphological features of CTCL and in noninvasively evaluating morphologic changes after treatment with photochemotherapy (Psoralen-UVA therapy).

**Demodex mite density in rosacea patients - Therapy monitoring by confocal laser scanning microscopy**

Sattler EC, Maier T, Hoffmann V, Ruzicka T, Berking C
*Department of Dermatology and Allergology, Ludwig-Maximilian University Munich, Germany*

Demodex mites are thought to play a pathogenic role in Demodex-associated skin diseases. Confocal laser scanning microscopy allows the detection and quantification of Demodex mites *in vivo*. In rosacea patients significantly higher numbers of *Demodex folliculorum* were seen compared to skin healthy controls using CLSM.

Our recent data demonstrate the use of CLSM as a non-invasive method in monitoring the density of Demodex mites in 25 rosacea patients before and after therapy. As CLSM leaves the skin unaltered – in contrast to other diagnostic methods like SSSB or skin biopsy or skin scraping - , it allows the repeated examination of the same site of skin over time. The absolute number of mites as well as the number of mites per follicle differed significantly pre- and posttreatment (p-value <0.0001).

**Lichen Sclerosus: Correlation of Histopathological Features and**
Dermoscopy with in vivo Reflectance Confocal Microscopy

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Introduction & Objectives: Lichen sclerosus (LS) is a chronic inflammatory mucocutaneous disorder of unknown aetiology, predominantly affecting the anogenital region. An extragenital involvement may be present in 15-20\% of patients. In vivo reflectance confocal microscopy (RCM) is a non-invasive imaging technique for the study of lesions on the skin and mucosa.

The objectives in this study were to examine and describe the characteristics of genital and extra-genital LS using RCM and to compare them with histopathological features and dermoscopy.

Materials & Methods: Four cases of LS, three genital and one extragenital (trunk), were analysed by dermoscopy, histopathology and RCM.

Results: Clinically all cases showed an ill-defined hypopigmented partially atrophic macule in genital area and a plaque on the trunk. Numerous teleangiectatic vessels and superficial haemorrhage were observed in three cases. i) Dermoscopy showed white, scar like areas in all LS. In RCM these areas correspond to epidermal hyperplasia. The histopathologic counterpart was epidermal acanthosis. ii) However white areas, seen in dermoscopy, may be in part due to hyalinosis in the upper dermis, which was present in all cases in histopathology. In RCM the dermis shows hyperreflecting inhomogeneous thickened plump fibres and dilated papillae with increased perivascular tissue. iii) An increased number and size of vessels in dermoscopy and histopathology correlated with increased vessels identified in all cases in RCM. We differentiated between dotted, lacunar and numerous tortuous vessels on dermoscopy, the same three lesions showed button hole-, ectatic - and tortuous superficial vessels in RCM. iv) Dermoscopy showed tiny grey dots in three cases. In comparison, an extensive infiltration of hyperreflective large nucleated cells in the upper dermis was revealed in RCM. On histopathology inflammatory infiltration in the upper dermis was detected in all cases, increase of fibroblasts in two cases, elastophagocytosis and histiocytes with focal giant cells were observed only in the extragenital case.

Conclusions: We demonstrated a good correlation between RCM and both dermoscopic and histopathological findings in LS. To validate our observations further studies concerning RCM features of LS are necessary.

Dermoscopic and confocal microscopy changes in melanocytic nevi after laser treatment for hair removal

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Hair removal by laser or light systems has become a very common practice between women and men. We report the changes in nevi of two patients after laser and intense pulsed light for epilation. Clinical, dermoscopic, confocal microscopy and histopathologic findings are described.

A 40 year-old man that was under digital dermoscopy surveillance because of atypical nevi syndrome presented with changes in a melanocytic nevi on his left knee. He had received intense pulsed light treatment for hair removal on his legs a month ago. On physical examination we observed a 0'6 cm pigmented macule with eccentric hyperpigmentation and blue-greyish color of the 80\% of the area of the lesion by dermoscopy. By confocal microscopy examination pagetoid cells were not observed, Regular honeycomb and edged papillae were found and also focally aggregated round to triangular cells, correlating to melanophages on the upper dermis. Excision was performed, and histologic examination
revealed intense oedema in papillary dermis, fibrosis, capillary neoformation and pigment incontinence in papillary dermis, with isolated melanocytes with residual nevocellular nests in the dermoepidermal junction. Cellular atypia and malignancy evidence were absent.

A 33 year-old man with atypical nevi syndrome presented with changes in several nevi on his back. He was taking laser sessions for depilation on this location during last year. On physical examination, we observed clearance of multiple melanocytic nevi. Dermoscopy showed blue-greyish areas on several nevi. Confocal microscopy examination of two lesions was performed, and no atypical cells were observed. Edged and non-edged papillae were seen, and scattered small bright particles and focally aggregated round to triangular cells correlating to lymphocytes and melanophages, were seen on the upper dermis. We excised one of these lesions, and a compound nevi with architectural distortion without cellular atypia and with focal regression were observed.

Laser and light systems for epilation are very widespread. Depending on its wavelength, laser light will be absorbed by different skin structures. Usually the melanin containing in the hair follicle is the main target, but if the laser light is applied directly on a pigmented lesion, it can be absorbed by the melanin containing in melanocytes with thermal injury of the lesion. There are few reports on the scientific literature describing clinical, dermoscopic and histologic changes on melanocytic nevi after laser treatment. To our knowledge, there are no reports detailing changes on these lesions by confocal microscopy examination. We would like to stand out that confocal microscopy could offer an important diagnostic tool to rule out malignancy in these cases in which the thermal injury caused by laser treatment could provide atypical clinical or dermoscopy features.