CONCLUSIONS

INFLAMMATORY DISEASES AND COSMETIC SECTION
State of the art: reflectance confocal microscopy for inflammatory skin conditions

- Inflammatory skin diseases as well as pigmentary disorders are spreading indications for RCM
- New topics as acne, rosacea, scalp diseases, melasma, vitiligo, etc. are now under study
- Therapeutic follow-up is one of the most promising indications
- New and more detailed multicenter studies are really wellcome

What RCM adds to trichoscopy?

- Using RCM, information about adnexal structures distribution and density, hair shaft integrity and dimension, location and amount of inflammatory cells in different layers can be obtained
- RCM can be useful for non-invasive, cicatricial and non-cicatricial alopecias differentiation
- The main RCM criteria giving a real time discrimination between scarring and non-scarring alopecias is represented by the detection of an active inflammatory process prevalently involving the adnexal epithelium and the DEJ (interface dermatitis) associated with different degree of dermal sclerosis typically located around adnexal structures
- In this specific dermatology field, RCM can be considered as an intermediate step between dermoscopy and horizontal histology for the evaluation and therapeutic management of scalp inflammatory diseases

Live microscopy of vesiculous conditions

- In vesicobullous disorders, RCM allows an easy identification of the blisters and of their split levels
- Excellent correlation between RCM and Tzanck’s test and/or histopathology
- RCM may support the clinical diagnosis and indicate to the physician the appropriate patient management and/or the need for further investigation
- The handheld device allows a rapid examination of several skin lesions in real time, and a better access to difficult anatomic locations (folds, scalp, nose, eyelids, ears and mucosae)

Evaluation and algorithms of early aging signs

- Young skin is characterized by regular polygonal keratinocytes and thin reticulated collagen fibers.
- With aging, more irregularly shaped keratinocytes and areas with unevenly distributed pigmentation and increased compactness of collagen fibers are observed.
- In the elderly, thinning of the epidermis, marked keratinocyte alterations, and huddles of collagen and curled fibers, corresponding to elastosis, were present
Monitoring rosacea treatments

- Reflectance confocal microscopy allows detection and quantification of demodex mites in facial skin of rosacea patients non-invasively in vivo
- Number of mites are higher in rosacea patients than in healthy controls
- RCM enables the quantification of mites over time on the same site in rosacea patients and is therefore also helpful in therapy monitoring
- Numbers of mites were significantly lower after treatment of rosacea
- Treatment monitoring, also in newer therapies like topical ivermectin, might help to understand pathogenic mechanisms

Subtyping psoriasis and monitoring antipsoriatic treatments

- Confocal microscopy is useful for the diagnosis of different types of psoriasis.
- There are some differences between plaque and guttate psoriasis, and also between unstable and stable plaque psoriasis.
- Unlike histology, confocal microscopy can evaluate biological changes over time at the same point.
- Antipsoriatic treatments can be assessed by confocal microscopy.
- By detecting subclinical changes we may predict the clinical response or even detect non-responders
CONCLUSIONS

NON MELANOCYTIC TUMORS SECTION
Reflectance Confocal Microscopy for non-melanocytic tumors: The state of the art

- RCM represents an accurate tool in the armamentarium for NMSC management optimizing their best treatment option and reducing unnecessary biopsies.
- RCM represents a step forward for monitoring non-invasive treatment.
- RCM features for benign non-melanocytic lesions are crystal-clear for diagnoses.

Vascular patterns in non-melanoma skin tumors

- Leucocyte rolling is characteristic for vascular structures.
- Increased density, elongation of blood vessels and accelerated blood flow in the dermal papillae related to tumoral angiogenesis.
- Vascular morphologies categorised in six types: curved linear, straight linear, branching, tubular/canaliculair, round and polymorphic vessels.
- Real time assessment of vascular images increases diagnostic accuracy of non melanocytic skin tumors.

Defining basal cell carcinoma subtypes

- With RCM it is possible to determine the subtype of BCC.
- Prospective large scale studies are required.

Differentials in progression of squamous neoplasia: actinic keratosis, SCC in situ and invasive squamous cell carcinoma

- RCM is applicable for the evaluation of all stages of squamous neoplasia and for monitoring of treatment response.
- AK are characterized by parakeratosis, atypical honeycomb pattern and solar elastosis.
- SCC in situ/Bowen is characterized by more pronounced atypia resulting in disruption of the honeycomb pattern, architectural disarray and increased vasculature with oval to s-shaped vessels.
- Invasive SCC is characterized by atypical honeycomb pattern in the epidermis and tumor nest of varying refractility in the dermis.
- Overlying Hyperkeratosis represents the main limitation in the evaluation of squamous neoplasia.
Monitoring photodynamic therapy for superficial and nodular BCCs

- RCM is a valuable non-invasive tool in monitoring the therapeutic response after PDT in BCCs, avoids invasive and unnecessary skin biopsies, preserving its good cosmetic outcome.
- Biological changes following PDT can be monitored in vivo over time by RCM.
- Non-invasive detection of BCCs in subclinical stage can be performed, accelerating retreatment for very early recurrences.

Monitoring cryotherapy for field cancerization

- All patients’ lesions presented improvement both in epidermal atypia (evaluated as extension of atypical honeycomb pattern) and dermal collagen rearrangement.
- The order of sequencing therapies was irrelevant to the success of the combination treatment.
- The combination of different non-invasive imaging technologies aid in confirming clearance.

Monitoring cryotherapy for superficial and nodular BCCs

- Dendrites seem to be destroyed immediately after cryosurgery.
- Intense edema with tumor nest detachment is the immediate most frequent finding.
- BCC refractile islands usually maintain their architecture for more than 1 month after cryosurgery.
- BCC islands are surrounded and invaded by multiple inflammatory cells usually one to two weeks after cryosurgery.
- “Pseudocystic structures” (“empty” tumor nests) can be observed two to three months after treatment.
Monitoring BCC Clearance Following Laser Ablation

- RCM has been accepted as a standard diagnostic modality for dermatology.
- RCM assists in triage BCC for treatment planning.
- There are inherent limitations using RCM to monitor post ablation sites. Most evident are the depth and mimicking features of scars and BCC.
- Analysis of pre- and post-ablation RCM features of BCC in combination with clinical and dermoscopy findings may help to establish therapeutic and surveillance guidelines for RCM guided laser ablation.

Monitoring medical treatments for field cancerization

- RCM allows the morphological study of field cancerisation due to its cellular resolution of the epidermis and upper dermis.
- RCM can be utilised in the investigation of the impact in vivo of new drugs in field cancerisation.
- RCM has clinical application to monitor treatment response to topical medications, PDT or other modalities.
CONCLUSIONS

MELANOCYTIC SECTION
Melanocytic lesions: situations in which non-invasive sub-architectural (microscopic level) information would help my practice

- Reflectance confocal microscopy (RCM) offers high-resolution, noninvasive skin imaging and can help avoid obtaining unnecessary biopsy specimens.
- It can also increase efficiency in the surgical setting by helping to delineate tumor margins.
- Diagnostic criteria and several RCM algorithms have been published for the differentiation of benign and malignant tumors.
- Indications, limitations and future directions of RCM will be defined the following years.

Reflectance Confocal Microscopy for melanocytic tumors: The state of the art

- RCM represents a step forward towards a more and more accurate diagnosis of melanoma.

RCM patterns of common acquired nevi and dermoscopic correlates

- The majority of nevi with Ringed or Meshwork RCM patterns correlate with reticular dermoscopic pattern.
- Nevi with Clod RCM pattern mostly correlate with light-homogenous or globular dermoscopic pattern.
- Nevi with mixed RCM pattern correlated most frequently with complex dermoscopic patterns.
- Most dermoscopically-homogenous nevi could be categorized into specific RCM patterns.
**Subtyping melanomas and progression**

- Distinct melanoma types exist in relation to patient characteristics, tumor morphology, histopathologic aspects and genetic background.
- Melanomas with a predominantly dendritic cell population ('dendritic-cell melanomas') typically were thin by Breslow index,
- Melanomas typified by roundish melanocytes were smaller in size than dendritic cell MMs, but thicker by Breslow index, and predominantly occurred in patients with a high nevus count.
- Melanomas characterized by dermal nesting proliferation usually were thick by Breslow index at the time of diagnosis, although frequently smaller in size compared with the other types.

**RCM versus histopathology for nevi and melanoma**

- Horizontal RCM is superior to vertical histopathology at detecting asymmetry and peripheral rim of nests, and thus less likely to miss superficial melanoma arising in a nevus.
- Pagetoid cells and small melanocytes can lead to diagnostic pitfalls on RCM that are easily avoided with immunohistochemistry on histopathology.
- Histopathology is needed to diagnose melanocytic tumors (such as blue, desmoplastic, and Spitz) in which DDX between nevus and melanoma relies on dermal features not visualized on RCM, such as maturation, contour and mitotic activity.

**RCM phenotyping melanoma in a high-risk population**

- We revalidated previous RCM melanoma classification in Familial and multiple primary melanoma patients:
  - Dendritic
  - Round cells
  - Dermal nests
  - Combined
  - Non-classifiable

  Dendritic cell melanomas in older patients, more solar exposition and sun damaged skin, Less nevi, less familial melanoma and more multiple primary sporadic cases
- Round cell melanoma are younger, more nevi, more familial and more phototype I
- No specific associations for CDKN2A mutations
- Combined-type and non-classifiable type melanomas presented the higher and lower RCM scores, respectively, the same way as occurred with tumour thickness. They may represent opposite sides of a morphological spectrum.
CONCLUSIONS

FURTHER CLINICAL APPLICATIONS IN SKIN ONCOLOGY
Confocal Application in Practice Everyday (CAPE) 2016: Near & Far!

- **Near**: Obtain image at exam table & evaluate immediately → avoid bx if benign or proceed to definitive therapy if malignant
- **Far**: Image captured by confocalist & transmitted electronically to expert confocalist for sign out anywhere in the world (Teleconfocal!)
- **Far**: Via store & forward technology have access to confocal specialists for additional opinions

Tweaking the diagnoses of collision tumours

- Collision tumors can be a diagnostic challenge
- A collision of SK and BCC is the most common on sun-damaged skin
- RCM can allow the recognition of the different tumors composing the collision

Facial Macules

- Pigmented facial macules are a clinical and dermoscopic challenge
- Reflectance confocal microscopy (RCM) offers a valuable information in the non-invasive differential diagnosis of facial pigmented macules
- RCM may improve the management of lentigo maligna (LM) by margins mapping before surgery
- RCM is a useful technique for surveillance of LM after treatment

Monitoring inoperable lentigo maligna

- RCM in addition to dermoscopy of inoperable LM is:
  1. The best tool to delimitate and to indicate proper management
  2. Allows the non-invasive monitoring during therapy
  3. Compared to histopathology achieves
     - Sensitivity 100%
     - Specificity 93%
  4. However, inflammation and sun-damage can pose a challenge: could simulate LM
Intraoperative RCM of Mohs micrographic surgery wounds

- Reflectance confocal microscopy can be used to image surgical wounds intraoperatively
- Use of video RCM can accurately detect residual skin cancer at the margins
- Use of aluminum chloride intraoperatively can enhance detection of residual tumor
- Further studies can be done to improve efficiency and reliability as well as tumor contrast

Ex vivo confocal for dermatology and mohs micrographic surgery: limitations and strengths

- FCM enables a real-time tissue imaging with cellular resolution directly in freshly excised skin sample
- A 20x20 mm mosaic could be stitched together being able to assess most of Mohs surgery samples
- Rapid "bedside pathology" during Mohs surgery and many other intraoperative pathology
- Time and cost saving
- Limitations and strengths as any other medical technique

Tips and tricks for ex vivo FCM good imaging

- Parameters which should be considered: laser wavelength, laser power, optical filters, optical properties of the fluorescent dye, pH value, solvent, concentration
- Challenges: toxic side effects, time window of fluorescence, photo bleaching, quenching, imaging artifacts
- Acridine orange was found to be the best fluorescent dye for ex vivo imaging (concentration 1.2 mg/ml)

Ex vivo confocal microscopy: a new pathologic scenario in skin and visceral oncology

- HIGH POTENTIAL
  (i) Intra-operative diagnosis; (ii) MarginsTumor; (iii) thickness
  (iv) Time sparing; (v) Cost-effective; (vi) Various tumors
- NEED FOR STANDARDS
  Ideal cutting technique
  Diagnostic algorithms
- STAININGS
  Greater choice of stains
  Long-term fluorescent dyes
- IMMUNOHISTOCHEMISTRY
  Specific antibodies