A PROPOSED DECISION-TREE WORKFLOW TO IDENTIFY TATTOO PIGMENT ON REFLECTANCE CONFOCAL MICROSCOPY IMAGING

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Abstract

Purpose:
Tattoos can obscure or alter lesion structure on both clinical and dermoscopic examination, complicating pattern recognition and risk stratification. When evaluating a lesion on tattooed skin, clinicians must weigh the benefits of a biopsy against the risks of damaging the tattoo to minimize the risks of both unnecessary biopsies or missed pathology.[1, 2]

Reflective confocal microscopy (RCM) may offer a superior approach to identifying benign lesions and avoiding unnecessary biopsies on tattooed skin by differentiating between endogenous structures (keratin, melanin, collagen) and exogenous tattoo pigment.[1] Therefore, we propose the following functional decision-tree workflow for distinguishing exogenous tattoo pigment from endogenous bright signals using RCM.

Methods:
The workflow was developed through a single-patient, multi-site analysis to delineate tattoo pigment from nevus and scar architecture. Dermatoscopic landmarks were used for spatial alignment with RCM to delineate bright foci. This proposed workflow builds upon the literature offering strategies for tattoo pigment recognition.[1, 3]

Results:
The proposed workflow effectively uses dermoscopy-RCM image-to-image correlation to map tattoo pigment to confocal signals and provide layer-specific algorithms to separate acellular ink from cellular elements.

Conclusion:
A formalized workflow using RCM to evaluate lesions on tattooed skin may reliably allow for identification of benign lesions by differentiating tattoo pigment from endogenous signals. Our proposed decision tree uses a layer-first, architecture-aware approach to distinguish brightness from exogenous tattoo pigment versus endogenous structures and may help reduce unnecessary biopsy or enable targeted sampling when lesions occur in tattooed skin.

Keywords

Reflectance confocal microscopy; tattoo pigment; dermoscopy; noninvasive imaging; decision tree; workflow

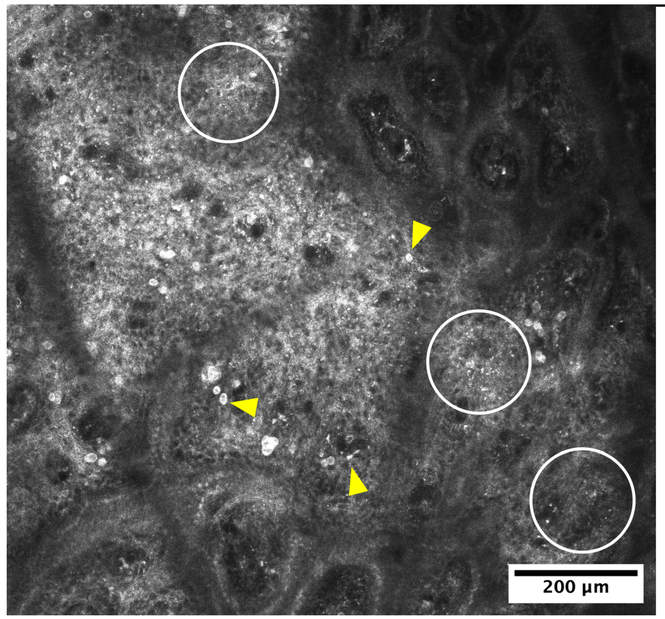
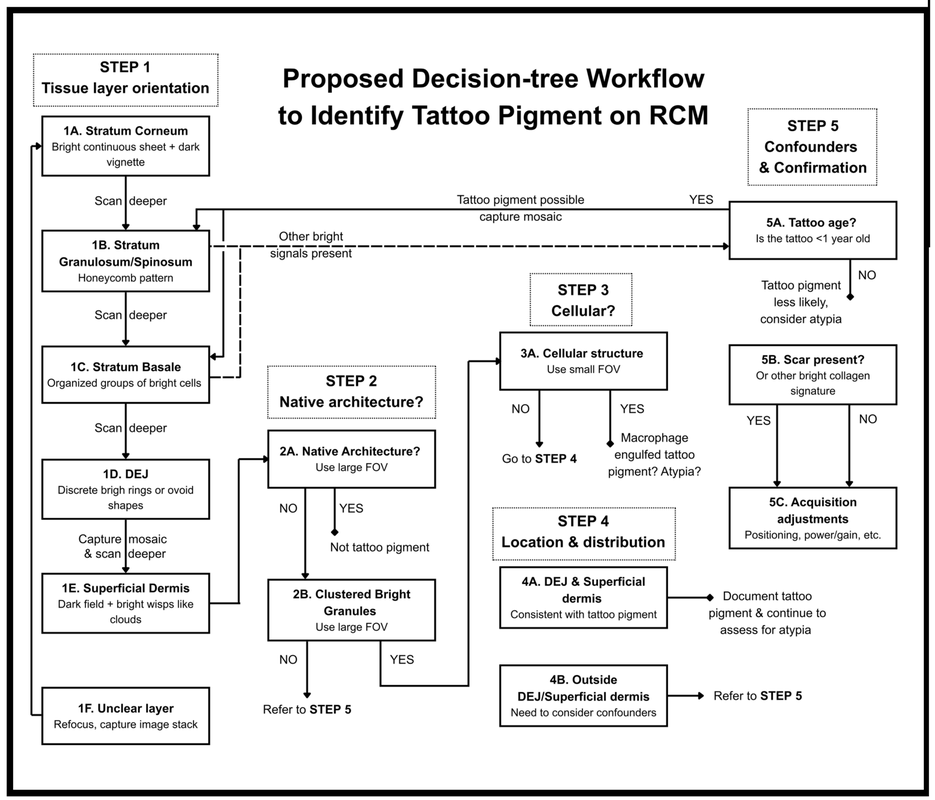


Figure 1.
RCM mosaic of tattoo pigment granules notable for coarse acellular granularity (white circles) with occasional round/ovoid shapes, consistent with sequestered tattoo pigment (yellow arrows)

Figure 2.
Proposed layer-first, architecture-aware decision-tree workflow for the identification of tattoo pigment in RCM



Appendix: Decision Tree

Start by placing the RCM field over tattooed skin with the lesion centered in the tissue window.
Step 1: Determine tissue layer orientation and capture mosaic image at each layer
A. Stratum Corneum: Most superficial optical section, is there a near-continuous sheet of bright signal with a dark peripheral vignette?
1. If yes, this is endogenous keratin rather than ink; capture mosaic and scan deeper into the tissue
2. If no, continue scanning for the most superficial layer

B. Stratum Spinosum/Granulosum: Does the bright signal create a honeycomb pattern?
1. If yes, this is consistent with bright keratinocyte cytoplasm surrounding a dark nuclei rather than ink; capture mosaic and scan deeper into the tissue
2. If there are other bright signals that do not participate in native architecture, refer to Step 5 before continuing
3. If no, return to more superficial tissue to orient depth, then continue

C. Stratum Basale: Do the bright signals appear as organized groups of bright cells?
1. If yes, this is consistent with bright basal keratinocytes, caps of the dermal papillae; capture mosaic, then make small adjustments to increase depth, the DEJ is typically within 20 µm
2. If there are other bright signals that do not participate in native architecture, refer to Step 5 before continuing
3. If no, return to more superficial tissue to orient depth, then continue

D. Dermoepidermal Junction (DEJ): Do the bright signals appear as discrete bright rings or ovoid shapes?
1. If yes, this is consistent with ringed dermal papillae; this is the beginning of the papillary dermis, where tattoo pigment should be visible; capture mosaic then go to Step 2
2. If no, return to more superficial tissue to orient depth, then continue

E. Superficial Dermis: A darker field with bright wisps like clouds?
1. If yes, this is consistent with the reticular dermis, where tattoo pigment should be visible; capture a mosaic then go to Step 2

F. If the layer is unclear, re-focus and capture image stacks to clarify the tissue architecture, then reassess at Step 1A

Step 2: Does the bright signal form native architecture?
A. Using a larger field of view (FOV), observe for native architecture patterns typical for DEJ and superficial dermis
1. If yes, endogenous structure rather than ink
2. If no, go to Step 2B

B. Using a larger FOV, observe for clustered, bright course granules within the dermal papillae and superficial dermis
1. If yes, go to Step 3A
2. If no, refer to Step 5

Step 3: Does it look cellular?
A. Using a smaller FOV, observe for cellular structure
1. If yes, consider macrophage engulfed tattoo pigment and evaluate for atypia
2. If no, acellular bright granules in clusters, go to Step 4

Step 4: Is the location and distribution typical for tattoo pigment?
A. Acellular bright granules in clusters found within the superficial dermis and DEJ, this is consistent with tattoo pigment; document and continue to assessment of atypia
B. Acellular bright granules in clusters found outside of the superficial dermis and DEJ, go to Step 5

Step 5: Confounders and Confirmation
A. Age of tattoo impacts the location of tattoo pigment
1. Is the tattoo <1 year old?
   a. If yes, tattoo pigment is likely to be present within the spinous/granular layers; capture mosaic and complete Step 1B-D, then continue to Step 2
2. Is the tattoo older than 1 year?
   a. If yes, tattoo pigment is less likely to be present within the epidermis; consider evaluating for atypia

B. Presence of scar tissue?
1. Bright collagen signature in fibrotic tissue seems to mask and interfere with the identification of tattoo pigment
   a. If yes, go to Step 5C
   b. If no, continue
2. Acquisition adjustments: Consider patient positioning, tissue window, power/gain adjustments, use the published International expert recommendations on image acquisition for in vivo reflectance confocal microscopy of cutaneous tumors to inform best practices for RCM image acquisition [26]

References

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The authors declare no conflicts of interest related to this work. | **Regulatory requirements:** This case report is IRB/COMIRB-exempt because it involves a single patient description without systematic investigation or intent to generate generalizable knowledge.