

# In vivo and ex vivo 3D visualisation of the vascular morphology of a thick nodular melanoma - a case report

Glanzer A<sup>1</sup>, Eyssele T<sup>1</sup>, Mayer O<sup>1</sup>, Schuh S<sup>1</sup>, Welzel J<sup>1</sup>

<sup>1</sup>University Hospital Augsburg, Department of Dermatology and Allergology, Augsburg, Germany

## Introduction

Malignant melanoma has the highest metastasis rate of all skin tumors. Tumor thickness is considered a prognostic marker for the risk of metastasis. Therefore, it is remarkable that the patient in this case report has no detectable metastasis despite a tumor thickness of 13 mm.

## Methodology

At first, the patient's medical history was assessed and a physical examination was performed. The melanoma was photographed clinically and dermoscopically and examined in vivo using non-invasive imaging techniques such as D-OCT and LC-OCT. The melanoma was promptly excised and histologically processed. The histological preparation was analyzed using light sheet microscopy which allows the ex vivo assessment of blood and lymph vessels in three dimensions. Laboratory determination of the tumor marker S100 beta protein and sonography of the lymph nodes showed no pathological findings.

## Results and conclusion

Clinically, a 5 cm x 3 cm, asymmetrical, sharply defined, heavily pigmented, centrally ulcerated lump was found on the right side of the thorax. Dermoscopically, an atypical network with a blue-grey veil and irregular stripes at the edges were visible. Curved, linear and tortuous vessels were observed (Fig.1). LC-OCT showed a chaotic architecture, inflammatory cells and adhesion of lymphocytes to the vessel wall (rolling). Vessel diameter was up to 50  $\mu\text{m}$  and erythrocytic flow rate was moderate to high. In general, a high vessel density, polymorphic vessels and a pronounced vascular network could be recognised. With regard to the vascular morphology D-OCT revealed "blobs", "serpiginous vessels", "curves", "lines" and "branching". In D-OCT and dermoscopy, the vascular pattern and distribution were irregular, the vessel diameter was medium and the vessel density high (Fig.2). Light sheet microscopy visualised lymphatic vessels with podoplanin staining (magenta) and blood vessels with CD31 single domain antibody (nanobody) (cyan) in the entire sample in 3D and 2D sections. Interestingly, several cells could be detected in the lymphatic vessels. If these cells were confirmed as melanoma cells, lymphatic vessel invasion may have occurred if further staining and tests confirm this. The aim of this case report is to use diagnostic imaging to visualise tumour vessels in and ex vivo. Light sheet microscopy is a special histologic procedure that could provide more precise information about the blood and lymph vessels (Fig.3).

## Objectives

The aim of analyzing blood and lymph vessels of this melanoma using modern imaging methods such as line-field confocal optical coherence tomography (LC-OCT), dynamic optical coherence tomography (D-OCT) and light sheet microscopy is to find further prognostic features in addition to tumor thickness.

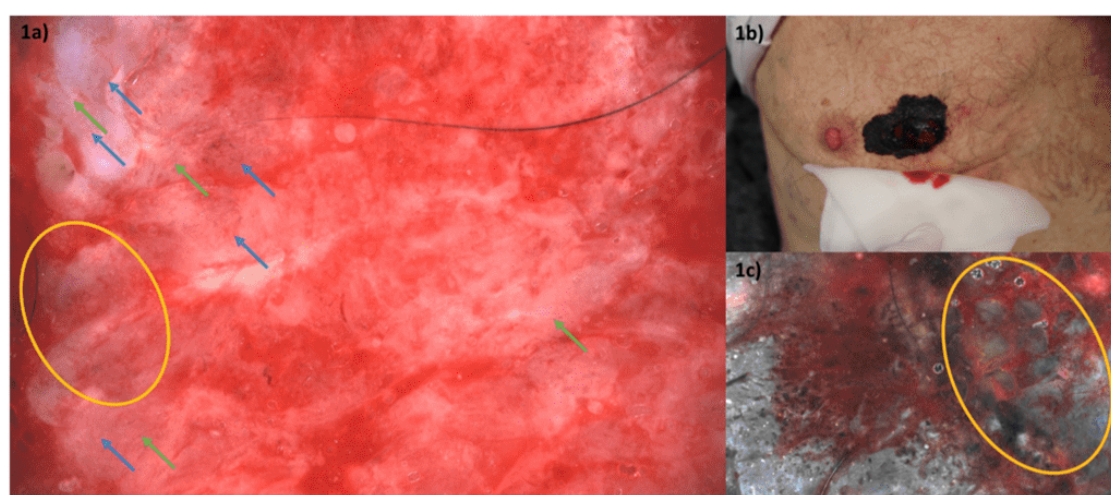


Fig. 1. Dermoscopic images (1a, 1c) and clinical picture (1b) of the melanoma. Dermoscopically, an atypical network with a blue-grey veil (yellow circle) is visible (1c). Additionally, the vessel morphology shows curves (blue arrows), lines (green arrows) and a tortuous architecture in the ulcerated bloody area (1a).

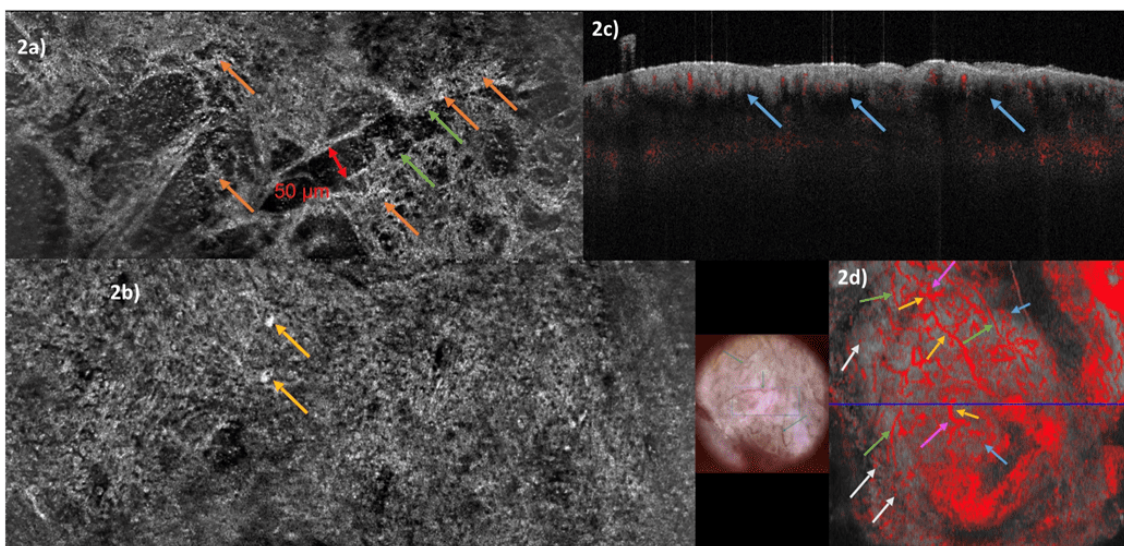


Fig. 2. LC-OCT (2a, 2b with dermoscopy) and D-OCT (2c, 2d) images of the skin lesion. LC-OCT (2a) shows a chaotic architecture and inflammatory cells (orange arrows). Adhesion of lymphocytes to the vessel wall can be recognized (rolling) (green arrows). The vessel diameter is up to 50  $\mu\text{m}$  (red double arrow). Furthermore, scattered plump pagetoid melanoma cells (yellow arrows) can be identified (2b). Dermoscopy shows a lot of serpiginous vessels (green arrows) in 2b. In the vertical view of D-OCT the dermoepidermal junction (DEJ) can be described as blurred and an acanthosis and enlarged papillary structures can be observed in this area (blue arrows) (2c). In the horizontal view of D-OCT the vascular pattern and distribution of the vessels are irregular and the vessel density is high (2d). With regard to the vessel morphology „blobs“ (white arrows), „serpiginous vessels“ (yellow arrows), „curves“ (blue arrows), „lines“ (green arrows) und „branching“ (purple arrows) can be seen.

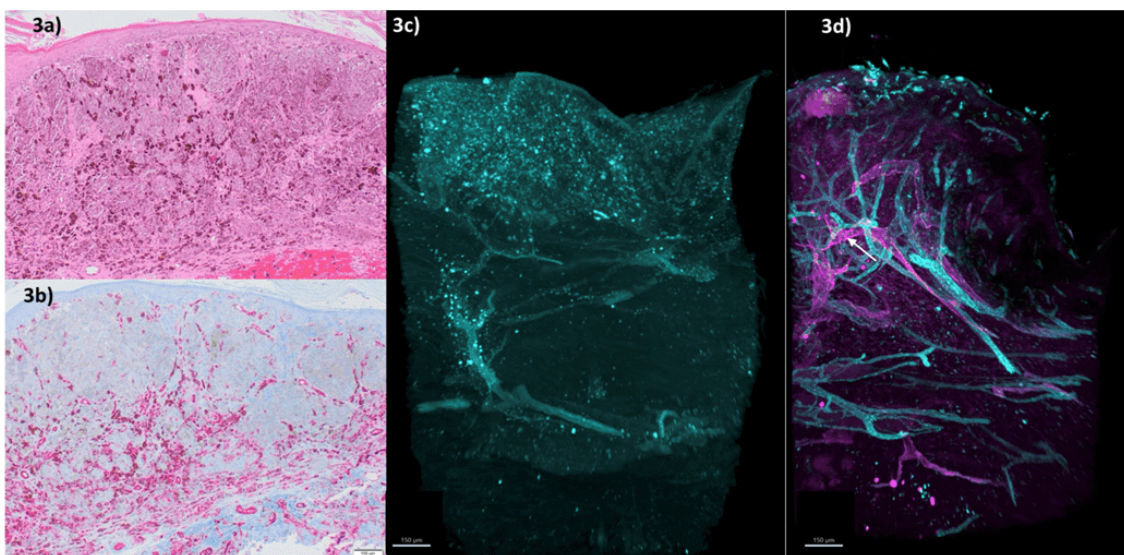


Fig. 3. Histology of the melanoma, tumor thickness > 13 mm with pagetoid atypical melanocytes, extensive polymorphic cells and atypical mitosis (3a). The tumour cells are all positive for Melan A staining (3b). Light sheet microscopy of the melanoma and prominent vessels in the dermis in Fluorescein Haematoxylin Eosin staining (3c). Fig. 3d Light sheet microscopy shows lymph vessels with podoplanin staining (magenta color) and blood vessels with a CD31 single domain antibody (nanobody) (cyan color). The vessel architecture is displayed in 3D reconstruction and on 2D slices. In lymphatic vessels several big cells (white arrow) can be detected. To prove that these are atypical melanocytes invading a lymph vessel needs to be shown with further staining and tests.