In Vivo Optical Imaging-Guided Targeted Sampling for Precise Diagnosis and Molecular Pathology

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Research Article

Keywords: optical imaging, reflectance confocal microscopy, precision biopsy, cancer diagnosis, molecular profiling, optical coherence tomography

Posted Date: June 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-585266/v1

Version of Record: A version of this preprint was published at Scientific Reports on November 30th, 2021. See the published version at https://doi.org/10.1038/s41598-021-01447-4.

Abstract

Conventional tissue sampling used in disease and cancer diagnosis can lead to misdiagnoses and repeated biopsies, and the tissue processed for histopathology suffers from poor nucleic acid quality/quantity for molecular profiling. Targeted micro-sampling of tissue can ensure accurate diagnosis and molecular profiling in the presence of spatial heterogeneity and complexity, especially in tumors, and facilitate acquisition of fresh tissue for molecular analysis. In this study, we explored the feasibility of a 1-mm precision biopsy approach guided by high-resolution reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) imaging, and reflective metallic grids for accurate spatial targeting and sampling in 7 skin cancers lesions in 6 patients. Accurate sampling was confirmed by histopathology or successful molecular profiling using next generation sequencing (NGS) approaches. Imaging guided 1-mm biopsy enabled spatial targeting and sampling for *in vivo* diagnosis, feature correlation and predicted depth as confirmed on histopathology. High DNA quantity (680 ng) and quality (DIN: 8) were obtained from imaging-guided high melanoma cell density areas. Subsequent mutational profiling on a 505-gene panel revealed a missense BRAF V600E oncogenic mutations at 0.02 allelic frequency for guiding therapy. Our findings demonstrate accurate sampling of regions of interest that enables downstream diagnosis, molecular analysis and research in both *in vivo* and *ex vivo* settings with broad diagnostic, therapeutic and research potential in dermatological and mucosal settings currently accessible to RCM-OCT imaging.

Introduction

Accurate disease diagnosis and molecular profiling in the presence of spatial heterogeneity require high yield biopsies containing adequate viable pathological tissue with key diagnostic and molecular features. However, the current approach of blind, and thus variable, sampling results in misdiagnoses (1), necessitating multiple biopsies in 17.4–40.5% of cases(2). Furthermore, molecular profiling using processed formalin-fixed, paraffin-embedded (FFPE) specimens with laser-capture microdissection suffers from inadequate DNA and RNA quality and quantity in ~ 20% and 30% specimens, respectively (3, 4). Ultrasound, X-ray, computed tomography, magnetic resonance and fluorescence molecular imaging have been conventionally employed to guide biopsy sites (5, 6); however, the lower resolution impedes visualization of the tissue histomorphology (7). Alternatively, reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) provide cellular- and microstructural-level resolution, respectively (8, 9), enabling visualization of the underlying cellular morphology in neoplasms and inflammatory disorders at the bedside prior to biopsy(10, 11). Thus, RCM/OCT can guide detection of the most diagnostic or prognostic areas directly *in vivo*, allowing for accurate targeted-biopsy and subsequent direct use of fresh tissues for downstream histopathology or molecular profiling (7). Using skin cancer as a model, we demonstrate novel imaging-guided *in vivo* spatial targeting and 1-mm sampling through reflective metallic grids for diagnosis, feature and tumor-depth correlation, and molecular pathology using Memorial Sloan Kettering-Integrated mutation profiling of actionable cancer targets (MSK-IMPACT™) profiling.

Methods

In vivo patient imaging and *ex vivo* studies were conducted on 7 clinically suspicious melanocytic and keratinocytic (basal cell carcinoma [BCC]) tumors in 6 patients under research protocols approved by the Institutional Review Board committee at Memorial Sloan Kettering Cancer Center (MSKCC-IRB). Written informed consent was obtained from all participants in the study. All research was performed strictly in accordance with relevant guidelines and regulations, including Declaration of Helsinki.

Metallic grid design and manufacturing

We designed an optically reflective metallic grid with square-shaped windows of sizes, 0.5, 1.0, and 2.0mm and wall thickness ranging 50–100 μm using computer-aided design software (SolidWorks, Dassault Systèmes, France). The metallic grids were custom manufactured using nickel as the the base through an electroforming process (Shimifrez, Ontario, Canada). Circular notches etched along the X and Y axis ends on the grid served as navigational landmarks.

Grid placement and imaging procedure

The metallic grids, centered over areas of interest, were attached using surgical adhesive (Micropore Surgical Tape [3M, MN, US]) or Mastisol (Eloquest Healthcare, MI, US). Imaging was performed using an RCM (VivaScope 1500 or a handheld VivaScope 3000, Caliber I.D., Rochester, NY) or integrated handheld RCM-OCT prototype. Images were acquired and interpreted in real-time at the bedside to select the targeted biopsy site(s) by 2 investigators (M.C. and A.S.) with greater than 4 years of RCM/OCT reading experience.

Ex vivo proof-of-concept testing

The 0.5-mm and 1.0-mm sampling for morphological or molecular analysis were initially tested on 4 *ex vivo* tissues from debulked keratinocytic tumors obtained from Mohs surgery.

Precision biopsy and histopathology

In the selected grid window (showing features of interest), biopsies were obtained using sterile micro-sampling punches of diameters 1.0 mm or 2.0 mm (Rapid-Core Sampling Tool [Electron microscopy Sciences, PA] or Integra Miltex [Integra Life Sciences, NJ]). The remaining lesion was either excised and processed for routine FFPE histopathology or immediately treated, based on histopathology obtained from the precision biopsy. The biopsy specimens were immediately processed for frozen sectioning. Depending on the clinical question, specimens were embedded either on edge (vertical sections) or *en face* (horizontal sections). Ten-micron thick ribbons of serial sections were prepared and stained with hematoxylin and eosin (H&E).

Quality control and IMPACT analysis

Samples for DNA extraction and quality control assessments were collected in sterile Eppendorf tubes (Eppendorf, Germany) and immediately transferred to -20°C for short-term storage and submitted to

Integrated Genomics Operation (IGO) core. DNA from the frozen tissue was isolated with DNeasy Blood & Tissue Kit (QIAGEN catalog #69504) with 1-hour of incubation at 55°C for digestion. DNA was eluted in 0.5X Buffer AE. The quantity and quality of DNA were estimated using Quant-it PicoGreen and Agilent TapeStation D1000. For MSK-IMPACT, 100ng DNA were used to prepare libraries using KAPA Hyper Prep Kit (Kapa Biosystems KK8504) with 8 PCR-cycles. 100ng of each barcoded library were captured by hybridization in equimolar pools using the IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) assay (Nimblegen SeqCap), designed to capture all protein-coding exons and select introns of 505 commonly implicated oncogenes, tumor suppressor genes, and members of pathways deemed actionable by targeted therapies. Captured pools were sequenced on a HiSeq 4000 in a PE100 run using the HiSeq 3000/4000 SBS Kit (Illumina) producing an average of 500X coverage per tumor.

Results And Discussion

The proof-of-concept for imaging-guided precision targeting was verified with 2-mm biopsies in 4 patients *in vivo.* The representative case shows successful targeting of heart-shaped tumor nests seen *in vivo* on RCM (Fig. 1Ai-iii). Following the successful *ex vivo* testing for 1-mm biopsy (Supplementary Fig. 1), an *in vivo* testing for diagnosis, features and tumor depth correlation was conducted. In the representative case, the lesion clinically suspicious for BCC shows bright cells within tumor nests on RCM which corresponds with the clusters of melanophages and melanocytes within the pigmented BCC on histopathology. This case demonstrates the feasibility of diagnosis and one-to-one correlation of micron-size imaging features with histopathology (Fig. 1Aiv-vii). Another representative case clinically suspicious for BCC highlights the correlation of *in vivo* imaging features with histopathological diagnosis and tumor depth (Fig. 1B). Towards molecular pathology using high-throughput next-generation sequencing (NGS) in small precision biopsies, quantity and quality of DNA were tested *ex vivo* in 0.5-mm and 1-mm biopsies (Supplementary Fig. 2). Subsequently, 1-mm precision-biopsy approach was tested *in vivo* for an invasive melanoma case, where RCM enabled identification of low- and high-melanocyte density areas and targeted biopsy of the high-density area (Fig. 1C, Supplementary video 1). This targeted 1-mm biopsy specimen yielded high DNA quantity (680ng) and quality (DIN: 8), optimal for downstream NGS applications, and was successfully analyzed via an MSK-IMPACT505 panel (12). Missense BRAF V600E oncogenic mutations at 0.02 allelic frequency were found in IMPACT505. The class I activating exon 15 BRAF V600E mutation located in the kinase domain of the BRAF protein renders BRAF constitutively activated in monomeric form and activates the downstream MAPK pathway independent of RAS. Since this mutated BRAF retains sensitivity to RAF monomer inhibitors such as vemurafenib and dabrafenib alone, or in combination with the MEK-targeted inhibitors trametinib and cobimetinib, respectively, the drug combinations can be used as adjuvant therapies for this patient.

Prior studies on *non-targeted* biopsies have demonstrated the feasibility of 0.33–0.5 mm biopsies for histology, ultrastructural analysis, and gene expressions evaluation in a few genes (13, 14), and RCM-guided 2-mm biopsy for histopathology using *paper-rings* (15). The findings from our study demonstrate the ability to perform imaging-guided 1-mm targeted biopsy for downstream diagnosis (by preserving tissue architecture) and next-generation molecular profiling (by ensuring high nucleic acid quality and

quantity) in both *in vivo* and *ex vivo* settings. This novel imaging-guided technique has potential applications for clinical and translational research, and patient care (e.g., monitoring cellular-level treatment response by tracking clonal evolution/tumor mutation burden, and evaluating immune biomarkers such as PD-L1 through *smaller* 1-mm sampling of multiple regions, and at multiple time points, during treatment). Additionally, this approach will allow identification of novel downregulated or upregulated actionable targets in tumor-enriched areas, which may be missed with conventional whole tumor sequencing. In the era of precision medicine, this novel imaging-guided approach has broad diagnostic, therapeutic, and research applications in oncology settings, including primary and metastatic keratinocyte and melanocyte cancers (squamous cell carcinoma, basal cell carcinoma, cutaneous and mucosal melanoma) and lymphoma and beyond.

Declarations

Acknowledgments

Mr. Brandon Possum, Ms. Angela Yepes, Ms. Gil Genna Manuel for assistance with tissue collection and histology and Integrated Genomics Operation Core, funded by the NCI Cancer Center Support Grant (CCSG, P30 CA08748), Cycle for Survival, and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Ms. Cassidy Cobbs for assistance with IMPACT505.

Competing Interest Statement:

Melissa Gill is a consulting investigator on an investigator-initiated study for DBV technologies on a topic unrelated to those described in this study and serves as a research consultant to the Dermatology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. Christi Alessi-Fox reported being employed by and owning equity in Caliber Imaging and Diagnostics Inc (formerly Lucid Inc), the company that manufactures and sells the VivaScope confocal microscope. Dr. Rossi reports travel accommodation from Mavig; he is also a consultant for Merz, DynaMed, Canfield Scientific, Evolus, Biofrontera, QuantiaMD, Lam Therapeutics, and Cutera; is on the advisory board of Allergan. Ashfaq A. Marghoob: received honorarium from 3GEN for dermoscopy lectures, received royalties from publishing companies for books and book chapters, received dermoscopy equipment for testing and feedback, received payment from the American Dermoscopy Meeting for organizing and lecturing at the annual meeting. Chih-Shan Jason Chen: received research funding from Apollo Medical Optics, Inc. Milind Rajadhyaksha is a former employee of and owns equity in Caliber Imaging and Diagnostics. The VivaScope is the commercial version of an original laboratory prototype that he developed at Massachusetts General Hospital, Harvard Medical School. The other authors declare no competing interests.

Author Contributions:

Conceived experiment and interpret data: AS, GP, MC, CN-D, MG, CF, SG, VR, DB, AR AM, CJC, MR

Performed experiments: AS, GP, MC, YO, CN-D, WP, SW, RA, VR, DB, AR, CJC

Sample preparation and histopathological processing: WP, SW, RA.

Manuscript writing: All authors

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Figures

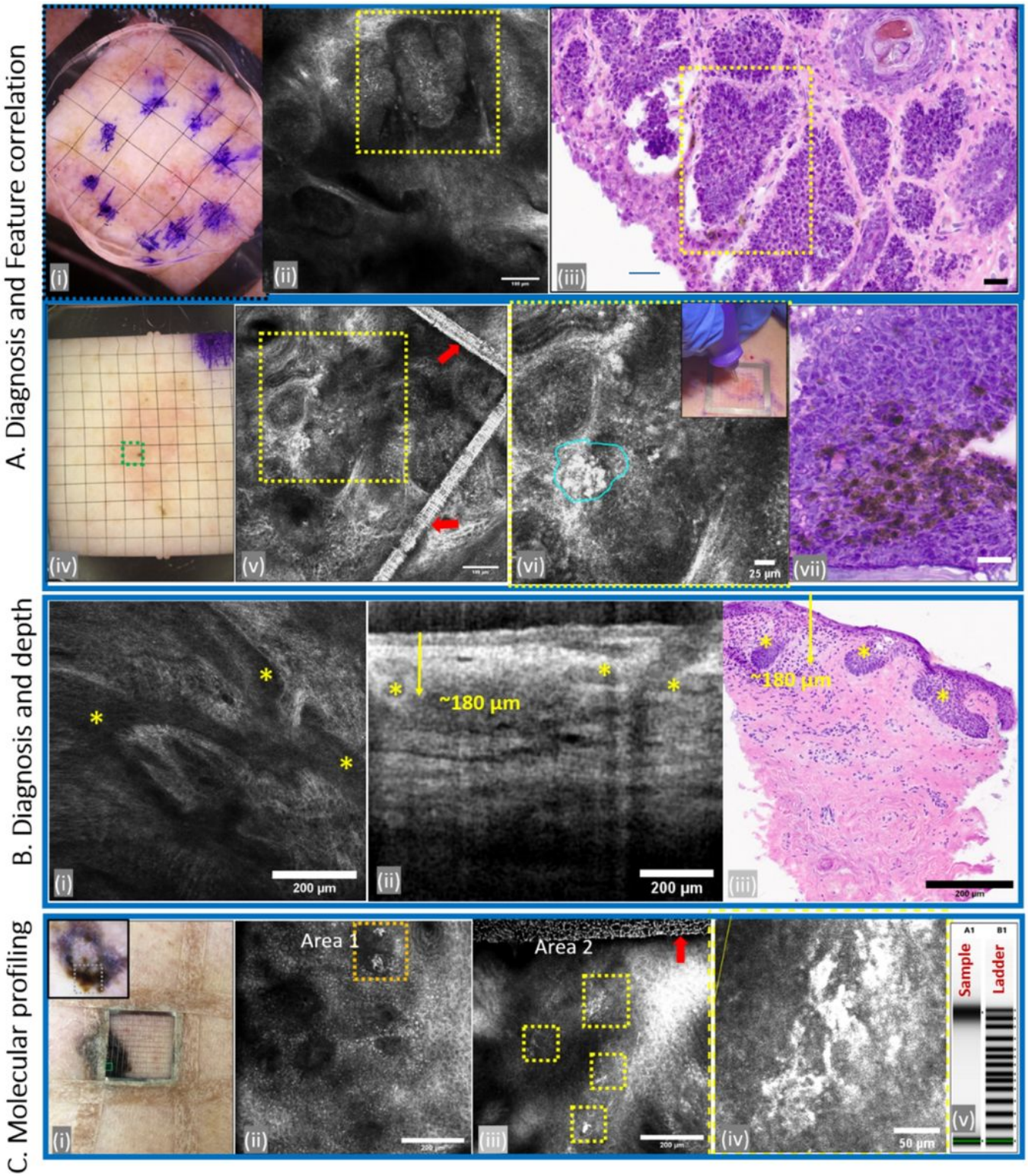

Figure 1

Targeted biopsy enables diagnosis, feature and depth correlation, and molecular pathology A. Diagnosis and feature correlation: 2-mm biopsy. (i) Dermoscopic photograph after placing a 2-mm grid centered on lesion, (ii) RCM shows tumor islands, peripheral palisading and dark rim of mucin (heart-shaped tumor in yellow-dotted rectangle), (iii) 2-mm biopsy through the grid followed by horizontal histopathology confirms the presence of basal cell carcinoma (BCC) and highlights the heart-shaped tumor seen in vivo

(40x, scale =30 μm). This experiment established the proof-of-concept for spatial targeting with 1-mm biopsy. Diagnosis and Feature Correlation-1-mm biopsy. (iv) Clinical photograph after placing 1-mm grid on a suspected pigmented-BCC (green-box shows targeted-biopsy location following RCM), (v) RCM image shows tumor nests with bright cells inside and peripherally confirming BCC diagnosis (metallic gridlines in RCM shown by red-arrows), (vi) magnified view of the yellow-square shows bright cells (within cyan-hoops) inside and peripherally to tumor nests with inset photograph demonstrating 1-mm targeted-biopsy for RCM-histopathology correlation, (vii) histopathology (40x, scale=25 μm) confirms the identity of bright cells as melanophages and melanocytes inside and peripheral to BCC nests. B. Depth correlation. (i) RCM imaging of suspected keratinocytic tumor revealed nests with peripheral palisading (yellow-asterisk) emanating from epidermis, confirming the diagnosis of BCC, (ii) the same nests visualized in the superficial dermis on the spatially-registered OCT image with maximum depth predicted ~180 μm, (iii) precision biopsy followed by vertical sectioning confirmed the RCM-OCT diagnosis and depth. C. Molecular Pathology. (i) Clinical photograph of large invasive melanoma with metallic grid (green-box shows targeted-biopsy location following RCM) and dermoscopic photograph as inset (silver area in inset corresponds to grid placement); (ii) Low density of melanoma cells (orange-boxes) inside tissue volume in area 1, (iii) high density of melanoma cells (yellow-boxes) in area 2, also in magnified RCM image (iv). Targeted 1-mm biopsy from area 2 yielded non-fragmented high-quality DNA (A1, sample) with DIN value of 8.0 and total amount 680 ng which were successfully analyzed with IMPACT505-mutational profiling. These cases illustrate that high-resolution imaging-guided precision biopsy with 1-mm sampling allows correlation of the micron-level features and tumor depth with histopathology and yields adequate quantity and quality of DNA for molecular profiling.

Supplementary Files

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