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## Dynamic Full-Field Optical Coherence Tomography as Complementary Tool in Fungal Diagnostics

1 **Thomas Maldiney<sup>1,2\*</sup>, Jean-Marie Chassot<sup>3</sup>, Claude Boccara<sup>3</sup>, Mathieu Blot<sup>2,4</sup>, Lionel**  
2 **Piroth<sup>4,5</sup>, Pierre-Emmanuel Charles<sup>2,6</sup>, Dea Garcia-Hermoso<sup>7</sup>, Fanny Lanternier<sup>7,8</sup>,**  
3 **Frédéric Dalle<sup>9,10</sup>, Marc Sautour<sup>9,10</sup>**

4 <sup>1</sup>Department of Intensive Care Medicine, William Morey General Hospital, Chalon-sur-  
5 Saône, France

6 <sup>2</sup>Lipness team, INSERM Research Center LNC-UMR1231, University of Burgundy, Dijon,  
7 France

8 <sup>3</sup>Institut Langevin, ESPCI Paris, PSL University, CNRS, 75005 Paris, France

9 <sup>4</sup>Infectious Diseases Department, Dijon Bourgogne University Hospital, Dijon, France

10 <sup>5</sup>INSERM, CIC1432, Clinical Epidemiology unit, Dijon, France

11 <sup>6</sup>Department of Intensive Care, Dijon Bourgogne University Hospital, Dijon, France

12 <sup>7</sup>Institut Pasteur, Université de Paris, Molecular Mycology Unit, National Reference Center  
13 for Invasive Mycoses and Antifungals (NRCMA), UMR 2000, CNRS, Paris, France

14 <sup>8</sup>Department of Infectious Diseases and Tropical Medicine, Necker-Enfants Malades Hospital,  
15 Assistance Publique-Hôpitaux de Paris, Paris, France

16 <sup>9</sup>Department of Parasitology/Mycology, Dijon Bourgogne University Hospital, 21000 Dijon,  
17 France

18 <sup>10</sup>UMR PAM A 02.102 Procédés Alimentaires et Microbiologiques, Univ. Bourgogne  
19 Franche-Comté, AgroSup Dijon, Dijon, France

20 **\* Correspondence :**  
21 [thomas.maldiney@ch-chalon71.fr](mailto:thomas.maldiney@ch-chalon71.fr)

22 **Keywords: Full-Field Optical Coherence Tomography, Fungal Diagnostics, Invasive**  
23 **Fungal Infections, Dynamic Contrast, Fungal Metabolism.**

### 24 **Abstract**

25 Histopathology and microscopic examination of infected tissue are the gold standards to  
26 prove the diagnosis of invasive fungal infection (IFI). Yet, they suffer from essential  
27 limitations that hamper rapid diagnosis and require the future development of new imaging  
28 tools dedicated to fungal diagnostics. To this end, the present work introduces the first use of  
29 dynamic full-field optical coherence tomography (D-FF-OCT) for the visualization of  
30 microscopic filamentous fungi. Data collected from the observation of three different fungal  
31 species (*Nannizzia gypsea*, *Aspergillus fumigatus* and *Rhizopus arrhizus*) confirm the ability  
32 of D-FF-OCT to visualize not only the main structures of all selected fungal species (hyphae,  
33 spores, conidia, sporulating structures), but also the metabolic activity of the organisms,  
34 which could provide additional help in the future to better characterize the signature of each  
35 fungal structure. These results demonstrate how D-FF-OCT could serve as potential  
36 complementary tool for rapid diagnosis of IFI in both intensive and non-intensive care units.

## 37 1 Introduction

38 Invasive fungal infections (IFI) are associated with a large morbi-mortality in both  
39 immunocompromised and critically ill patients [1,2]. According to a recent update from the  
40 European Organization for Research and Treatment of Cancer and the Mycoses Study Group  
41 Education and Research Consortium (EORTC/MSGERC), IFI diagnosis mostly relies on the  
42 combination of host factors, clinical features, as well as mycological evidence [3]. Indeed,  
43 despite tremendous progress regarding the validation and implementation of polymerase chain  
44 reaction (PCR)-based methods as essential criteria for probable invasive fungal disease,  
45 histopathology or microscopic examination of infected tissue remain gold standards for the  
46 diagnosis of proven IFI [4,5]. Yet, the absolute necessity to obtain a timely and proven  
47 diagnosis in specific cases of IFI requires to address many challenges and pitfalls in  
48 histopathology or other culture-independent solutions dedicated to the diagnosis of fungal  
49 diseases [6]. As an example, a convenient tool to allow a rapid and easy differential diagnosis  
50 between invasive aspergillosis and mucormycosis *in situ* would help both infectious disease  
51 specialists and surgeons to choose the best therapeutic option between exclusive medical  
52 treatment and the adjunction of urgent extensive surgery. One of the solutions would be to  
53 uncover a technology able to assess the invasive nature of a fungal species within one  
54 superficial or deep tissue sample in a few minutes only, and without any coloration step.

55 As full-field optical coherence tomography (FF-OCT), dynamic full-field optical coherence  
56 tomography (D-FF-OCT) relies on broad band interference microscopy and provides ultra-  
57 high resolution structure images of biological tissues [7] as well as subcellular metabolic  
58 contrast within a few minutes [8]. In comparison to standard OCT or confocal microscopy, D-  
59 FF-OCT was shown to improve spatial resolution up to ten times [9]. Apart from recent  
60 medical applications [7,10,11], most studies have considered the potential importance of D-  
61 FF-OCT as imaging alternative for extemporaneous analysis in oncologic interventions and  
62 future way to easier and quicker surgical pathology [12]. However and excluding previous  
63 attempts to visualize *Malassezia* Folliculitis [13], cutaneous larva migrans [14] and even  
64 *Sarcoptes scabiei* infestation [15] with OCT systems displaying neither high spatial resolution  
65 nor the ability to follow dynamic contrast, there has been to our knowledge no reported  
66 attempt to exploit high-definition D-FF-OCT in fungal diagnostics. The present work  
67 introduces the first application of D-FF-OCT for the observation of different filamentous  
68 fungal species *in vitro*. We address the hypothesis that D-FF-OCT could help both physicians  
69 and pathologists to circumvent common pitfalls and develop timely and automated  
70 histopathology analysis dedicated to the diagnosis of IFI.

## 71 2 Materials and Methods

### 72 2.1 Fungal strains

73 *Nannizzia gypsea* (NG), *Aspergillus fumigatus* (AF) and *Rhizopus arrhizus* (RA) were  
74 isolated from patients and identified by mass spectrometry (MALDI-TOF) in the laboratory of  
75 Parasitology-Mycology of Dijon Bourgogne University Hospital. The strains were cultivated  
76 on Sabouraud dextrose agar (BioMérieux SA, Marcy-l'Etoile, France) and incubated at 28°C  
77 for proper growth.

### 78 2.2 FF-OCT standard and dynamic contrast imaging

79 As described elsewhere [10] and presented in Figure 1, D-FF-OCT images were acquired with  
80 a commercially available D-FF-OCT apparatus (Light-CTScanner, LLTech - Aquyre

81 Bisciences, Paris, France) [16]. Briefly, a conventional halogen source with short temporal  
82 coherence length was used for illumination to ensure an axial resolution of 1  $\mu\text{m}$  (Figure 1A).  
83 As for the previously reported D-FF-OCT set-up, 10x water immersion microscope objectives  
84 were placed in the interferometer arms (Linnik configuration) to reach a transverse resolution  
85 of approximately 1.5  $\mu\text{m}$ . Solid cultures (Figure 1B) were sampled and deposited on the upper  
86 slide of the apparatus sample holder according to an adapted protocol based on the tape touch  
87 method described elsewhere for fungal slide mounts [17]. The resulting fungal slides were  
88 then acquired following full-field illumination and subsequent dynamic contrast imaging with  
89 a complementary metal oxide semiconductor camera (Figure 1C). The penetration depth was  
90 adjusted to be approximately 100  $\mu\text{m}$  and series of D-FF-OCT images with 1.5  $\mu\text{m}$  spacing  
91 were recorded in depth.

## 92 **2.3 Image analysis**

93 ImageJ 1.49k software was used for axial z-stacking of multiple images and three-  
94 dimensional reconstruction (3D viewer plugin). Both FF-OCT standard and dynamic contrast  
95 image analysis with length measurements of different fungal compartments for each selected  
96 species were accessible using a contrast-based ImageJ 1.49k protocol called the “Plot Profile”  
97 Function. Regarding the specific analysis of dynamic contrast images, the time series  
98 associated to each pixel behavior was analyzed by a fast Fourier transform program. Then the  
99 spectrum was divided in three parts respectively associated to the three colors of the  
100 composite RGB images so that the highest frequencies (fast movements) correspond to the red  
101 color and the lowest frequencies to the bluer colors.

## 102 **3 Results**

### 103 **3.1 D-FF-OCT observations of *Nannizzia gypsea***

104 The different aspects of NG are displayed in Figure 2. The two-dimensional structural FF-  
105 OCT image of NG from Figure 2A returns multiple thin and septate hyphae, most of the time  
106 longer than 200  $\mu\text{m}$ , with a diameter ranging from 2 to 4  $\mu\text{m}$ . Except for septa, the intensity of  
107 the FF-OCT signal is relatively comparable on the whole hyphae. The dynamic D-OCT image  
108 from Figure 2B confirms a similar shape of the NG hyphae with a homogenous and rather low  
109 dynamic contrast, from blue to pale green, no matter which septum of the septate hyphae.  
110 Three dimensional FF-OCT image of both hyphae and multicelled macroconidia are shown in  
111 Figure 2C. Macroconidia appear as fusiform structures approximately 50  $\mu\text{m}$ -long and 15  $\mu\text{m}$ -  
112 large with distinguishable septa (Figure 2C, white arrow). When compared to the bluish  
113 dynamic aspect of NG hyphae from Figure 2B, dynamic D-OCT image from Figure 2D shows  
114 intense orange dynamic contrast within the macroconidia of NG and outlines its multicelled  
115 structure.

### 116 **3.2 D-FF-OCT observations of *Aspergillus fumigatus***

117 Acquisitions of AF solid cultures with D-FF-OCT are shown in Figure 3. As demonstrated by  
118 the two-dimensional image from Figure 3A, AF is composed of several septate conidiophores  
119 with length ranging from 200 to 300  $\mu\text{m}$  and a diameter of 4-6  $\mu\text{m}$ , slightly larger than the one  
120 from NG. When compared to the different compartments of NG, Figure 3A confirms a  
121 different FF-OCT signal between conidiophores, vesicles, phialides and conidia. The latter,  
122 with the smallest diameter of 2-4  $\mu\text{m}$ , clearly show the brightest FF-OCT signal in  
123 comparison to the other parts of AF, notably vesicles. The two-dimensional dynamic D-OCT  
124 image displayed in Figure 3B highly contrasts with the corresponding structure image  
125 acquired with FF-OCT. Although the different sub-compartments display a morphology

126 comparable to the one from Figure 3A, both dynamic contrast and intensity enlighten  
127 complementary signature of each structure. First, the dynamic contrast can vary from pale  
128 blue to green or even yellow from one side of a conidiophore to another. In addition, vesicles  
129 most often display bright green-orange dynamic contrast, as compared to the pale bluish  
130 colors of the conidial heads or phialides. Finally, we see from Figure 3C that three-  
131 dimensional dynamic D-OCT image confirms the whole structure and dynamic contrast of  
132 both the conidiophores and conidial heads, approximately 50  $\mu\text{m}$ -large.

### 133 3.3 D-FF-OCT observations of *Rhizopus arrhizus*

134 Results from D-FF-OCT observations of RA are presented in Figure 4. The three-dimensional  
135 FF-OCT view of RA sporangiophores from Figure 4A confirms a length above 300  $\mu\text{m}$  for  
136 the longest and a diameter between 10 and 12  $\mu\text{m}$ , without any septum. As for NG, the  
137 dynamic D-OCT image from Figure 4B confirms a similar shape of the RA sporangiophores  
138 with a homogenous and rather low dynamic contrast, from blue to pale green. Figure 4C  
139 precisely shows the dotted spherical shape and organization of RA sporangium, with a total  
140 diameter exceeding 100  $\mu\text{m}$ . As for AF, the two-dimensional dynamic D-OCT image showing  
141 RA sporangiospores seems to highlight two compartments within the sporangium, each with a  
142 different contrast (Figure 4D): one in the center corresponding to the columellae, brighter and  
143 with enhanced contrast when compared to a second more peripheral area unfolding all  
144 sporangiospores.

## 145 4 Discussion

146 Except for a few reports describing the attempt to use standard OCT for the diagnosis of  
147 onychomycosis [11], dermatophytoma [18] as well as other applications in ophthalmology  
148 intended for the imaging of fungal keratitis [19] and chorioretinitis [20], there is to our  
149 knowledge no report of high definition D-FF-OCT imaging in fungal diagnostics. Indeed, the  
150 present study introduces the first observations of filamentous fungal species with D-FF-OCT,  
151 demonstrating the complementary potential of both high-definition structure (Figures 2A, C,  
152 Figure 3A and Figures 4A, C) and dynamic contrast (Figures 2B, D, Figures 3B, C and  
153 Figures 4B, D) images for the identification of fungi.

154 First, FF-OCT acquisitions return comparable information regarding the global structure of all  
155 three selected fungal species. In particular, the main characteristics of AF and RA with  
156 conventional microscopy largely correspond to the one obtained with FF-OCT: the thin  
157 branched and septate hyphae of AF (Figure 3A) are distinguishable from the larger pauci-  
158 septate RA sporangiophores displaying a ribbon-like structure (Figure 4A). A similar  
159 conclusion can be drawn from the comparison of AF conidial heads (Figure 3A) and the  
160 dotted spherical organization of RA sporangiospores (Figure 4C) hiding two compartments  
161 within the sporangium [6]. The observation of FF-OCT images of NG also confirms the  
162 typical ellipsoidal and thin-walled structure of NG multicelled macroconidia (Figure 2C), as  
163 for the usually reported microscopic examination and identification criteria [21]. Obviously,  
164 these results need to be confirmed on other fungal species to test the global performances and  
165 limits of such FF-OCT system for the identification of more precise morphological criteria  
166 usually dedicated to microbiological diagnostics: notably information such as the number of  
167 septa composing macroconidia or hyphae pigmentation.

168 In addition to such structural information, D-FF-OCT gave access to dynamic contrast  
169 imaging of fungal cells. Briefly, this technology was previously shown to provide  
170 complementary subcellular contrast to structural images obtained with FF-OCT. On the

171 suspected basis of mitochondria or cellular organelles movements, dynamic contrast has  
172 already demonstrated its ability to reflect both cell activity and metabolism [8]. Interestingly,  
173 the present data illustrate for the first time the exploitation of D-OCT to probe fungal  
174 subcellular dynamics. As shown in Figures 2B and D, the comparison of hyphae and  
175 macroconidia dynamic contrasts for NG very certainly reflects a different metabolic state of  
176 both structures [22]. In the case of NG, the intense orange dynamic contrast within the  
177 macroconidia may be the reflection of macroconidia metabolic activity (Figure 2D). Dynamic  
178 D-OCT images of AF show the same kind of opposition between vesicles or certain  
179 conidiophore segments displaying bright green-orange contrast and conidial heads or  
180 phialides associated with a paler bluish signal (Figures 3B, C). As for MF, these dynamic  
181 discrepancies in AF compartments very certainly correspond to a changing metabolic activity  
182 and signature of each fungal structure, also possibly occurring in the center of RA sporangium  
183 (Figure 4D). Still, additional experiments will be needed to better understand the exact nature  
184 of such dynamic contrast. Indeed, live-cell imaging of filamentous fungi mainly relies on the  
185 use of either cell wall or membrane selective dyes coupled with confocal laser scanning  
186 microscopy to analyze subcellular compartments dynamics and provide a better understanding  
187 of fungal morphogenesis [23,24]. A comparison of the signal obtained with D-OCT and live-  
188 cell imaging would help to better understand the origin of D-OCT dynamic contrast in fungi.  
189 Finally, given well known morphological discrepancies between *in vitro* and *in vivo* structural  
190 or even metabolic characteristics of fungi, the present D-FF-OCT-based visualization of non-  
191 dermatophytes filamentous species *in vitro* only constitutes the first attempt towards future  
192 works to better appreciate the morphological aspect of the same molds *in vivo*, throughout  
193 their parasitic phase, with deep-tissue D-FF-OCT imaging of direct clinical samples such as  
194 cutaneous, mucosal or lung biopsy.

## 195 **5 Conclusion**

196 Overall, this study introduces a novel application of D-FF-OCT for fungal diagnostics. Based  
197 on unprecedented structure and dynamic contrast imaging of three different fungal species, we  
198 bring conclusive evidence that such technology not only allows rapid identification of fungal  
199 compartments with a definition approaching conventional microscopy but also returns  
200 potential precious metabolic information regarding fungal subcellular dynamics, without any  
201 sample preparation, coloration, or any selective dye. Regarding clinical perspectives, it could  
202 serve rapid bedside extemporaneous analysis following nail scraping, skin biopsy as well as  
203 direct noninvasive *in vivo* histopathological examination of any skin or mucosal lesion within  
204 a few minutes, potentially helpful when compared to the actual time constraints associated  
205 with conventional preparations of histological sections (fixation, embedding, sectioning,  
206 staining) that sometimes require days of technical procedures and lead to significant delay in  
207 final diagnosis. Thus, we believe D-FF-OCT may serve as complementary tool for rapid IFI  
208 diagnosis in future microbiological laboratory and clinical routine practice. Further studies are  
209 needed to assess D-FF-OCT accuracy if applied to clinically relevant samples such as fresh  
210 tissues.

## 211 **Figure captions**

212 Fig. 1. D-FF-OCT set up for the acquisition of fungal solid cultures. (A) Global view of both  
213 hardware and software set up for fungal observations. (B) Fungal solid cultures exploited for  
214 D-FF-OCT observations. (C) Frontal view of the D-FF-OCT acquisition system with sample  
215 holder in the center. White arrow points the halogen source.

216 Fig. 2. D-FF-OCT observations of *Nannizzia gypsea*. (A) Two dimensional FF-OCT image  
217 showing *Nannizzia gypsea* hyphae. (B) Two dimensional D-OCT image showing *Nannizzia*  
218 *gypsea* hyphae. (C) Three dimensional FF-OCT image showing *Nannizzia gypsea* hyphae and  
219 macroconidia. (D) Three dimensional D-OCT image showing *Nannizzia gypsea* hyphae and  
220 macroconidia. Scale bar represents 50  $\mu\text{m}$ . White arrow points the multicelled macroconidia.

221 Fig. 3. D-FF-OCT observations of *Aspergillus fumigatus*. (A) Two dimensional FF-OCT  
222 image showing *Aspergillus fumigatus* conidiophore and conidial heads. (B) Two dimensional  
223 D-OCT image showing *Aspergillus fumigatus* conidiophore and conidial heads. (C) Three  
224 dimensional D-OCT image showing *Aspergillus fumigatus* conidiophore and conidial heads.  
225 Scale bar represents 50  $\mu\text{m}$ . White arrow points a conidial head of *Aspergillus fumigatus*.

226 Fig. 4. D-FF-OCT observations of *Rhizopus arrhizus*. (A) Three dimensional FF-OCT image  
227 showing *Rhizopus arrhizus* sporangiophores. (B) Three dimensional D-OCT image showing  
228 *Rhizopus arrhizus* sporangiophores. (C) Two dimensional FF-OCT image showing *Rhizopus*  
229 *arrhizus* sporangium and sporangiospores. (D) Two dimensional D-OCT image showing  
230 *Rhizopus arrhizus* sporangium and sporangiospores. Scale bar represents 50  $\mu\text{m}$ .

231

## 232 **Conflict of Interest**

233 *The authors declare that the research was conducted in the absence of any commercial or*  
234 *financial relationships that could be construed as a potential conflict of interest.*

## 235 **Author Contributions**

236 TM and MS designed the study, conducted experiments, performed experimental works and  
237 wrote the manuscript. JMC, CB, DGH, FL and MS performed experimental works. JMC, CB,  
238 MB, LP, DGH, FL, MS and PEC contributed to the manuscript revision. TM, MS and FD  
239 supervised the project.

240 All authors contributed to the article and approved the submitted version.

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## 248 **Data Availability Statement**

249 All data from the study are included in the article. Further inquiries can be directed to the  
250 corresponding author.

## 251 **References**

- 252 [1] Chowdhary A, Sharma C, Meis JF. Azole-Resistant Aspergillosis: Epidemiology,  
253 Molecular Mechanisms, and Treatment. *The Journal of Infectious Diseases*  
254 2017;216:S436–44. <https://doi.org/10.1093/infdis/jix210>.
- 255 [2] Danion F, Duréault A, Gautier C, Senechal A, Persat F, Bougnoux M-E, et al.  
256 Emergence of azole resistant-*Aspergillus fumigatus* infections during STAT3-  
257 deficiency. *Journal of Medical Microbiology* 2020;69:844–9.  
258 <https://doi.org/10.1099/jmm.0.001200>.
- 259 [3] Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al.  
260 Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the  
261 European Organization for Research and Treatment of Cancer and the Mycoses Study  
262 Group Education and Research Consortium. *Clinical Infectious Diseases* 2020;71:1367–  
263 76. <https://doi.org/10.1093/cid/ciz1008>.
- 264 [4] Pruskowski KA, Mitchell TA, Kiley JL, Wellington T, Britton GW, Cancio LC.  
265 Diagnosis and Management of Invasive Fungal Wound Infections in Burn Patients. *EBJ*  
266 2021;2:168–83. <https://doi.org/10.3390/ejb2040013>.
- 267 [5] Dekio F, Bhatti TR, Zhang SX, Sullivan KV. Positive Impact of Fungal Histopathology  
268 on Immunocompromised Pediatric Patients With Histology-Proven Invasive Fungal  
269 Infection. *Am J Clin Pathol* 2015;144:61–7.  
270 <https://doi.org/10.1309/AJCPEMVYT88AVFKG>.
- 271 [6] Antinori S, Corbellino M, Parravicini C. Challenges in the Diagnosis of Invasive Fungal  
272 Infections in Immunocompromised Hosts. *Curr Fungal Infect Rep* 2018;12:12–22.  
273 <https://doi.org/10.1007/s12281-018-0306-0>.
- 274 [7] Quénehervé L, Olivier R, Gora MJ, Bossard C, Mosnier J-F, Benoit a la Guillaume E, et  
275 al. Full-field optical coherence tomography: novel imaging technique for  
276 extemporaneous high-resolution analysis of mucosal architecture in human gut biopsies.  
277 *Gut* 2021;70:6–8. <https://doi.org/10.1136/gutjnl-2020-321228>.
- 278 [8] Apelian C, Harms F, Thouvenin O, Boccara AC. Dynamic full field optical coherence  
279 tomography: subcellular metabolic contrast revealed in tissues by interferometric signals  
280 temporal analysis. *Biomed Opt Express* 2016;7:1511.  
281 <https://doi.org/10.1364/BOE.7.001511>.
- 282 [9] Dalimier E, Salomon D. Full-Field Optical Coherence Tomography: A New Technology  
283 for 3D High-Resolution Skin Imaging. *Dermatology* 2012;224:84–92.  
284 <https://doi.org/10.1159/000337423>.
- 285 [10] Maldiney T, Greigert H, Martin L, Benoit E, Creuzot-Garcher C, Gabrielle P-H, et al.  
286 Full-field optical coherence tomography for the diagnosis of giant cell arteritis. *PLoS*  
287 *ONE* 2020;15:e0234165. <https://doi.org/10.1371/journal.pone.0234165>.
- 288 [11] Olsen J, Lindsø Andersen P, Themstrup L, Jemec GBE, Saunte DML. Optical coherence  
289 tomography of onychomycosis: proposed terminology and a suggestion of practical  
290 usage. *Arch Dermatol Res* 2020;312:51–8. <https://doi.org/10.1007/s00403-019-01989-8>.
- 291 [12] van Manen L, Dijkstra J, Boccara C, Benoit E, Vahrmeijer AL, Gora MJ, et al. The  
292 clinical usefulness of optical coherence tomography during cancer interventions. *J*  
293 *Cancer Res Clin Oncol* 2018;144:1967–90. <https://doi.org/10.1007/s00432-018-2690-9>.
- 294 [13] Andersen AJB, Fuchs C, Ardigo M, Haedersdal M, Mogensen M. In vivo  
295 characterization of pustules in *Malassezia* Folliculitis by reflectance confocal

- 296 microscopy and optical coherence tomography. A case series study. *Skin Res Technol*  
297 2018;24:535–41. <https://doi.org/10.1111/srt.12463>.
- 298 [14] Morsy H, Mogensen M, Thomsen J, Thrane L, Andersen PE, Jemec GBE. Imaging of  
299 cutaneous larva migrans by optical coherence tomography. *Travel Medicine and*  
300 *Infectious Disease* 2007;5:243–6. <https://doi.org/10.1016/j.tmaid.2006.12.004>.
- 301 [15] Banzhaf CA, Themstrup L, Ring HC, Welzel J, Mogensen M, Jemec GBE. In vivo  
302 Imaging of *Sarcoptes scabiei* Infestation Using Optical Coherence Tomography. *Case*  
303 *Rep Dermatol* 2013;5:156–62. <https://doi.org/10.1159/000352066>.
- 304 [16] Ghouali W, Grieve K, Bellefqih S, Sandali O, Harms F, Laroche L, et al. Full-Field  
305 Optical Coherence Tomography of Human Donor and Pathological Corneas. *Current*  
306 *Eye Research* 2015;40:526–34. <https://doi.org/10.3109/02713683.2014.935444>.
- 307 [17] Harris JL. Safe, low-distortion tape touch method for fungal slide mounts. *J Clin*  
308 *Microbiol* 2000;38:4683–4. <https://doi.org/10.1128/JCM.38.12.4683-4684.2000>.
- 309 [18] Verne SH, Chen L, Shah V, Nouri K, Tosti A. Optical Coherence Tomography Features  
310 of Dermatophytoma. *JAMA Dermatol* 2018;154:225.  
311 <https://doi.org/10.1001/jamadermatol.2017.4590>.
- 312 [19] Sharma N, Singhal D, Maharana PK, Agarwal T, Sinha R, Satpathy G, et al. Spectral  
313 Domain Anterior Segment Optical Coherence Tomography in Fungal Keratitis. *Cornea*  
314 2018;37:1388–94. <https://doi.org/10.1097/ICO.0000000000001715>.
- 315 [20] Adam MK, Rahimy E. Enhanced Depth Imaging Optical Coherence Tomography of  
316 Endogenous Fungal Chorioretinitis. *JAMA Ophthalmol* 2015;133:e151931.  
317 <https://doi.org/10.1001/jamaophthalmol.2015.1931>.
- 318 [21] Mohamed Shalaby MF, Nasr El-din A, Abu El-Hamd M. Isolation, Identification, and In  
319 Vitro Antifungal Susceptibility Testing of Dermatophytes from Clinical Samples at  
320 Sohag University Hospital in Egypt. *Electron Physician* 2016;8:2557–67.  
321 <https://doi.org/10.19082/2557>.
- 322 [22] Dill BC, Leighton TJ, Stock JJ. Physiological and biochemical changes associated with  
323 macroconidial germination in *Microsporum gypseum*. *Appl Microbiol* 1972;24:977–85.  
324 <https://doi.org/10.1128/am.24.6.977-985.1972>.
- 325 [23] Lichius A, Zeilinger S. Application of Membrane and Cell Wall Selective Fluorescent  
326 Dyes for Live-Cell Imaging of Filamentous Fungi. *JoVE* 2019:60613.  
327 <https://doi.org/10.3791/60613>.
- 328 [24] Pfister J, Lichius A, Summer D, Haas H, Kanagasundaram T, Kopka K, et al. Live-cell  
329 imaging with *Aspergillus fumigatus*-specific fluorescent siderophore conjugates. *Sci Rep*  
330 2020;10:15519. <https://doi.org/10.1038/s41598-020-72452-2>.
- 331

**(A)**

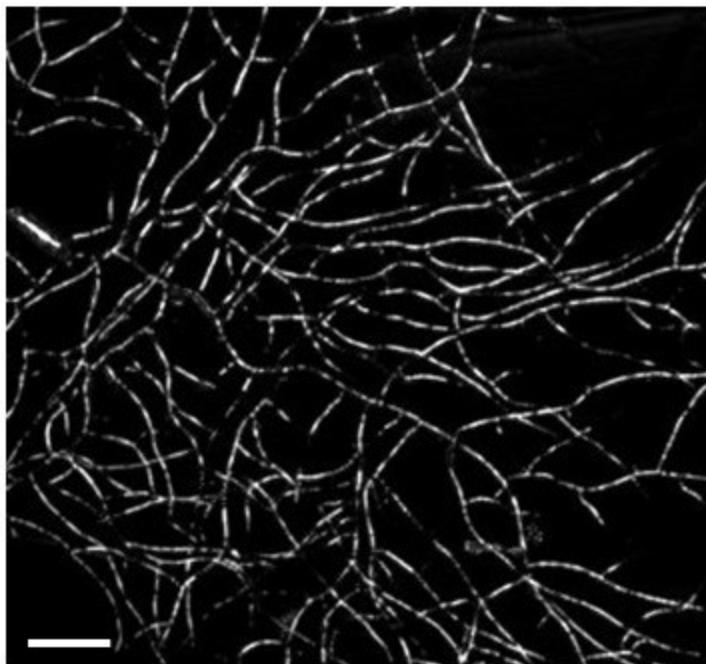
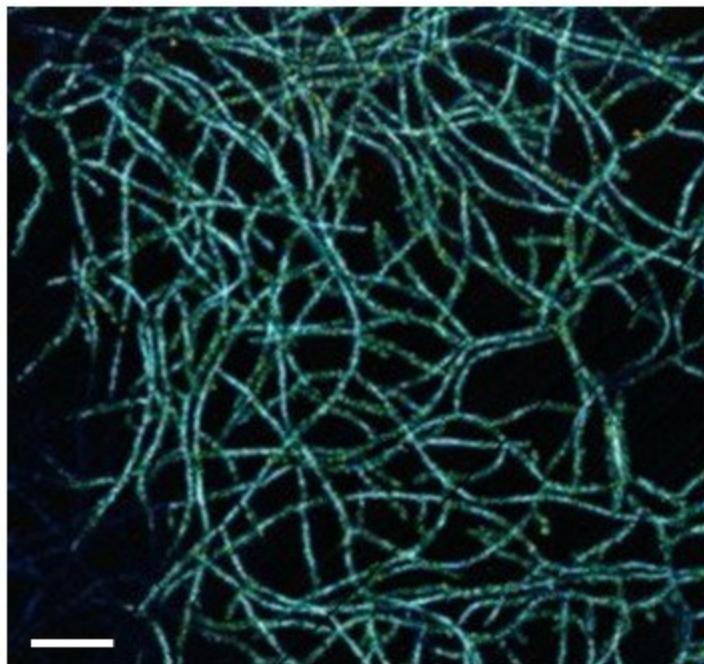
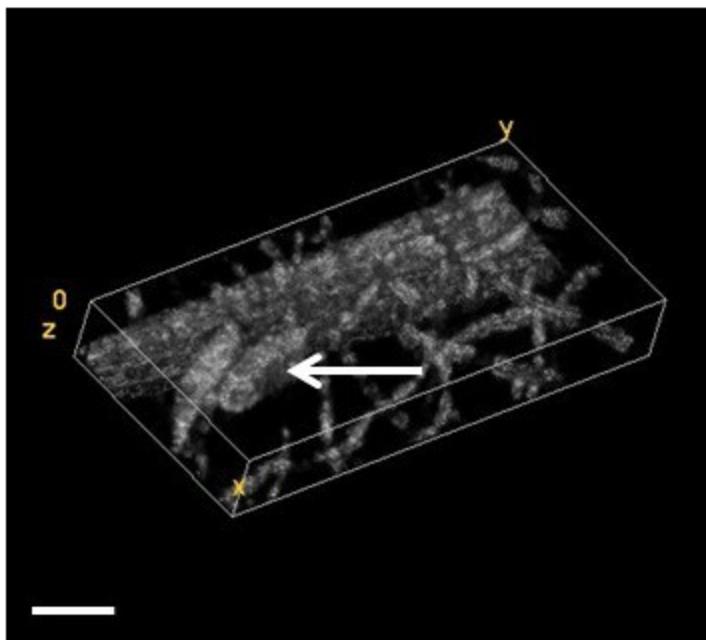
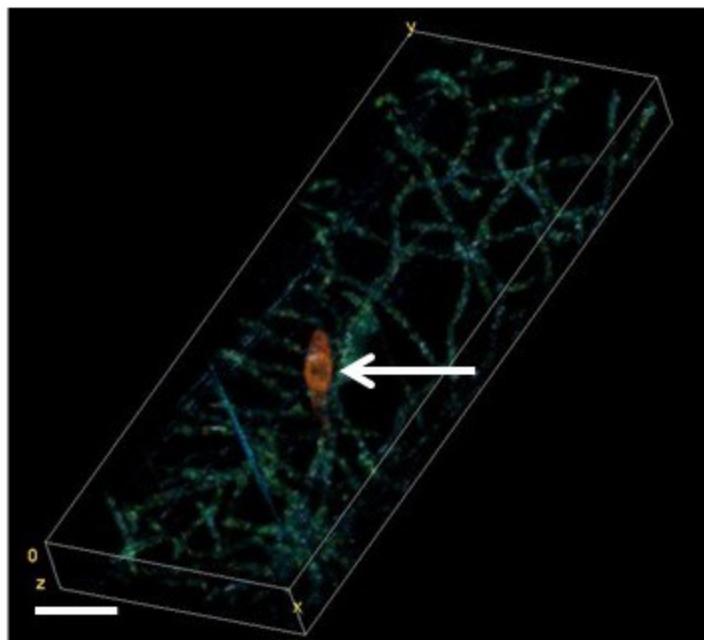


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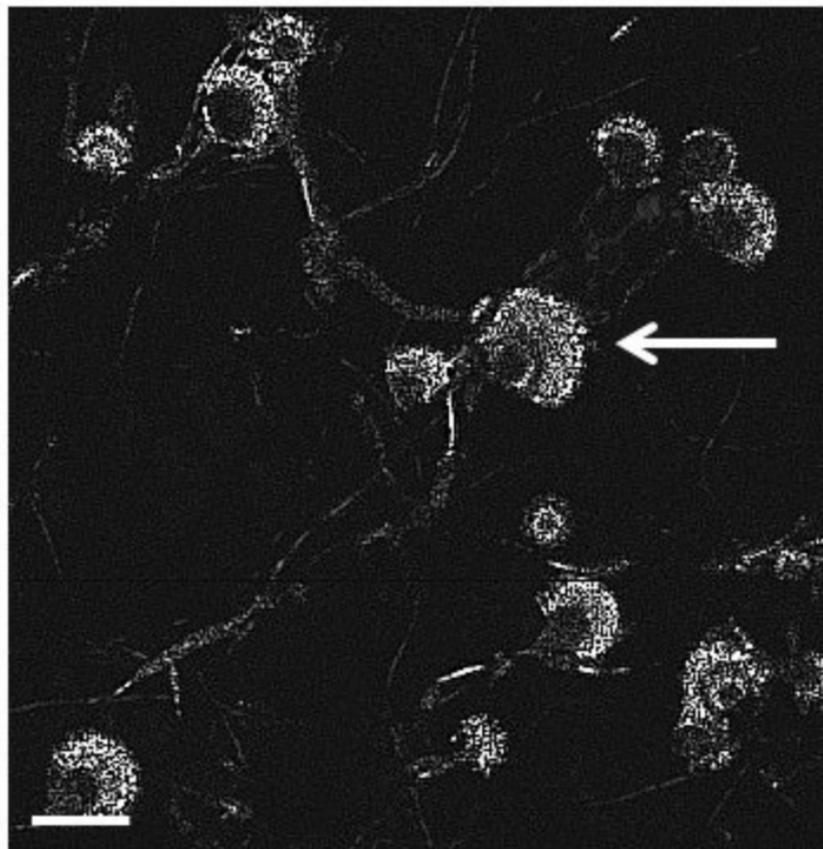


**(C)**

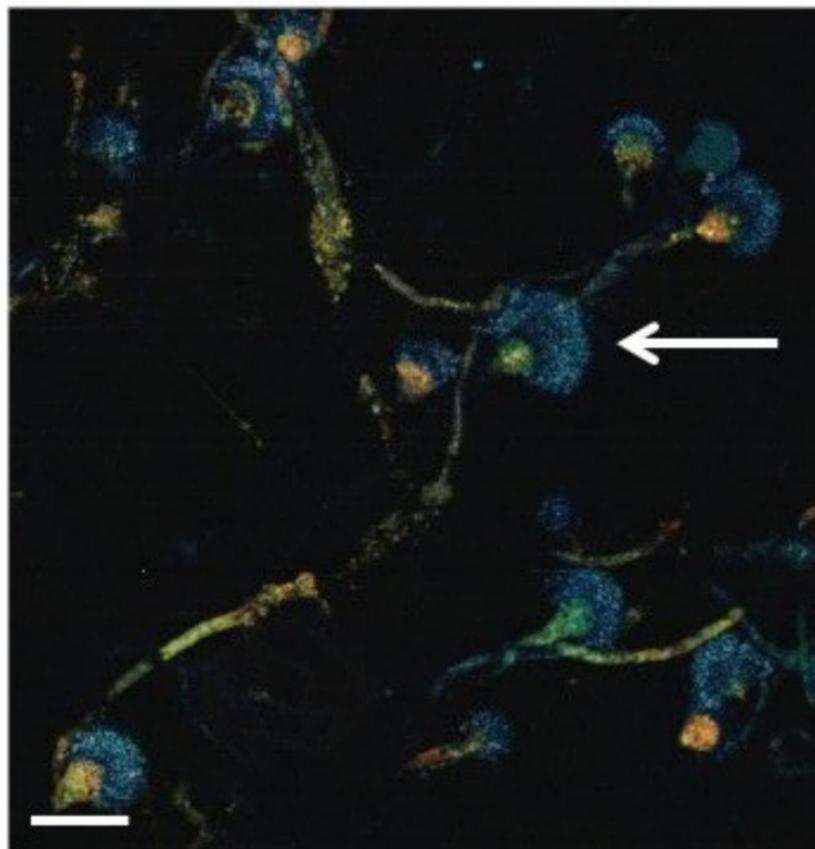


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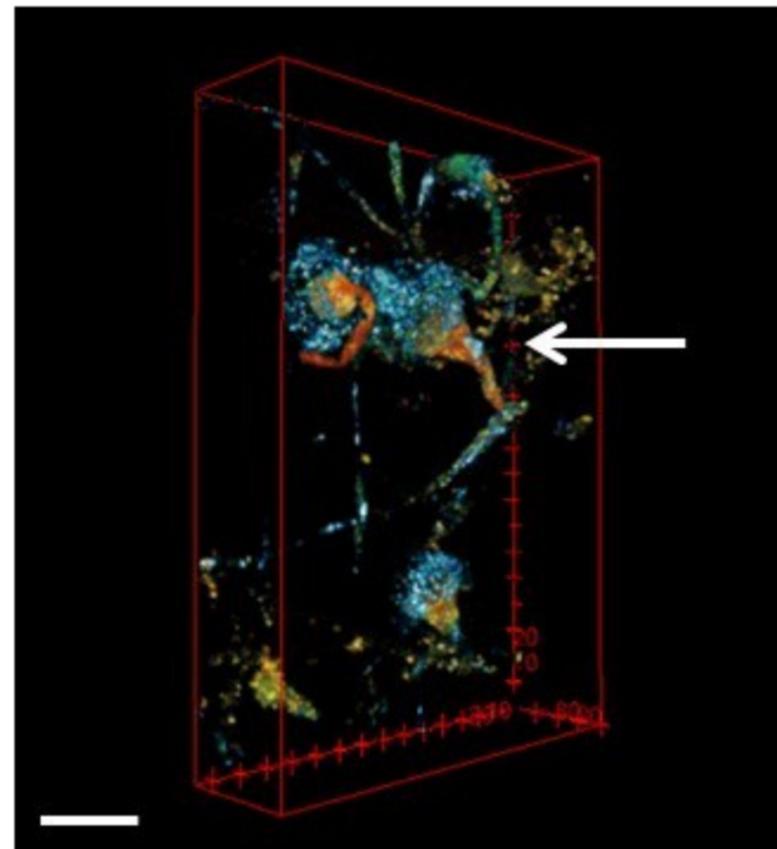
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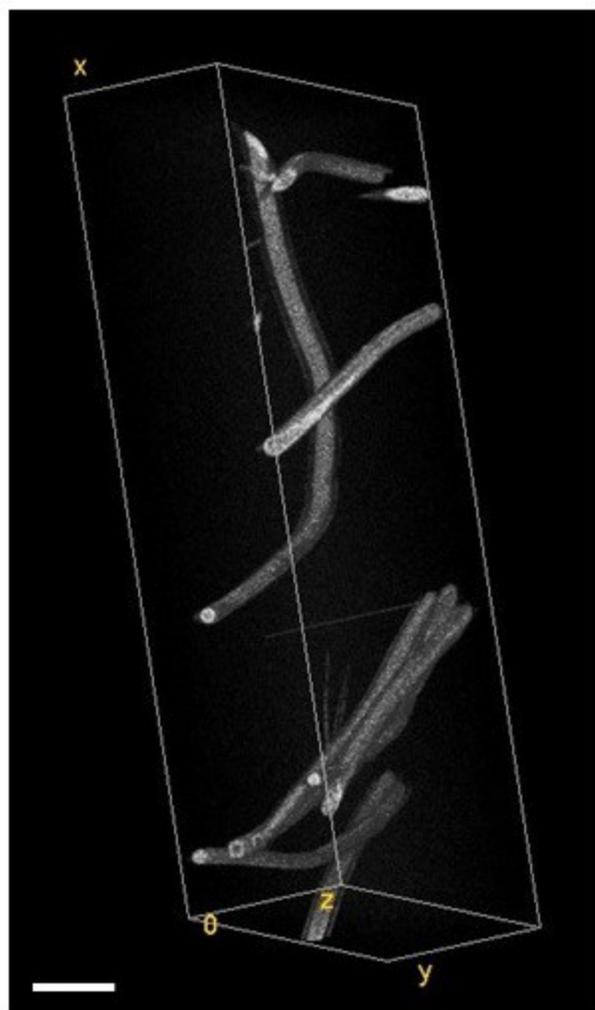
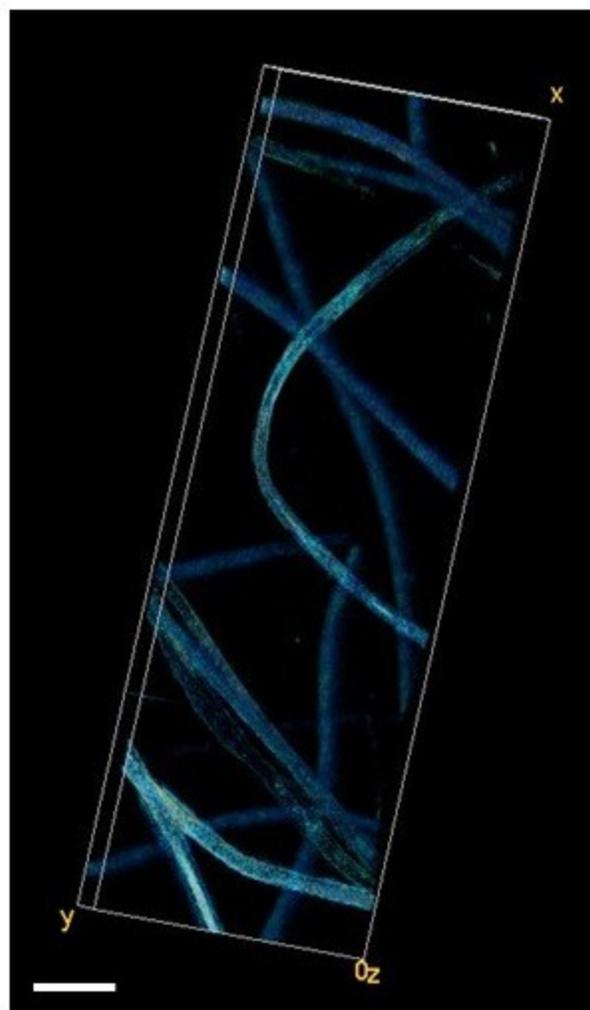
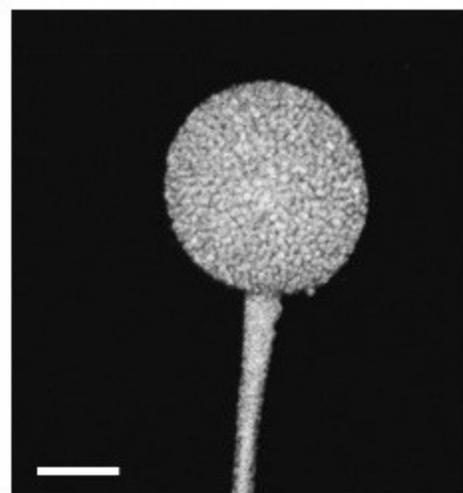


**(B)**



**(C)**



**(A)****(B)****(C)****(D)**