

## Micro- to Nano-scale Chemical and Mechanical Mapping of Antimicrobial-Resistant Fungal Biofilms

Duy Quang Pham,<sup>1</sup> Saffron J. Bryant,<sup>2</sup> Samuel Cheeseman,<sup>2</sup> Louisa Z. Y. Huang,<sup>2</sup> Gary Bryant,<sup>2</sup> Madeleine F. Dupont,<sup>2</sup> James Chapman,<sup>2</sup> Christopher C. Berndt,<sup>1</sup> Jitraporn (Pimm) Vongsvivut,<sup>3</sup> Russell J. Crawford,<sup>2</sup> Vi Khanh Truong,<sup>2,\*</sup> Andrew S. M. Ang,<sup>1,\*</sup> and Aaron Elbourne<sup>2,\*</sup>

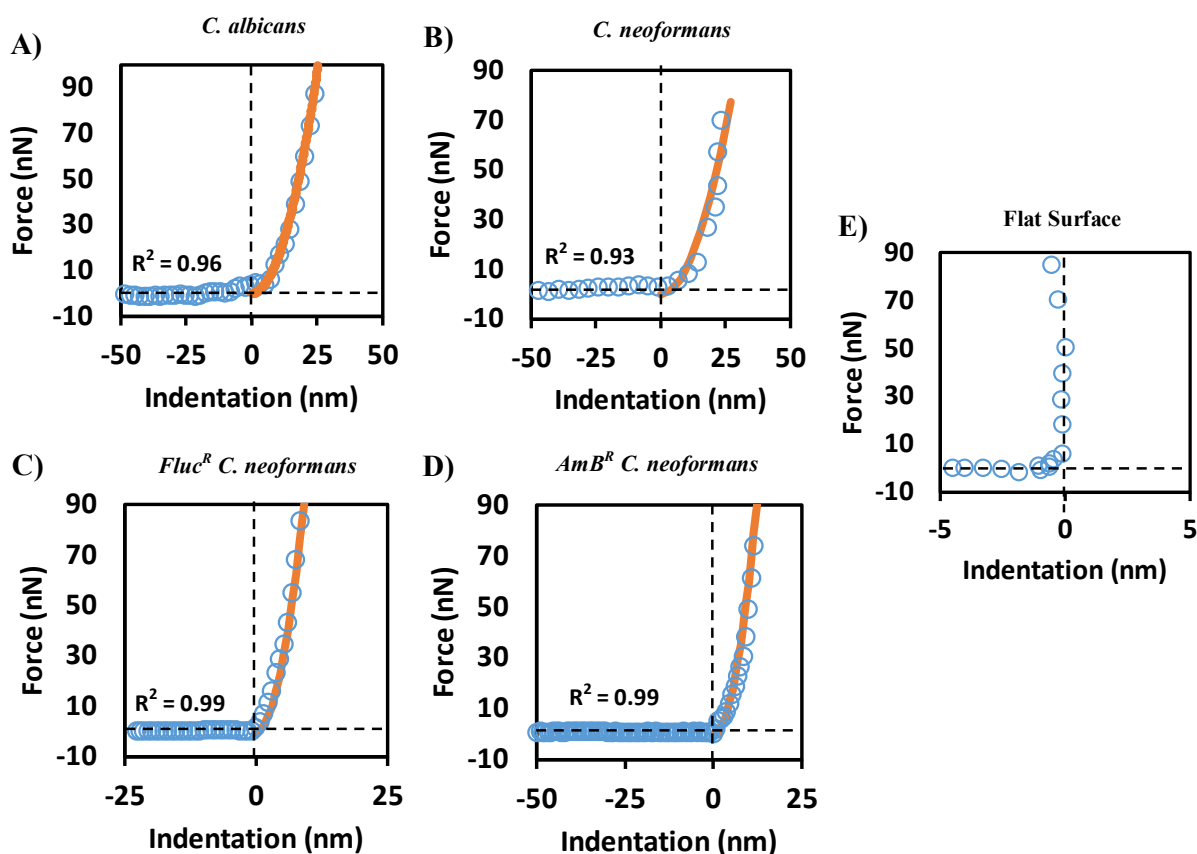
<sup>1</sup>Surface Engineering for Advanced Materials (SEAM), Department of Mechanical and Production Design Engineering, Swinburne University of Technology, Hawthorn, Australia.

<sup>2</sup>School of Science, College of Science, Engineering and Health, RMIT University, Melbourne, Australia.

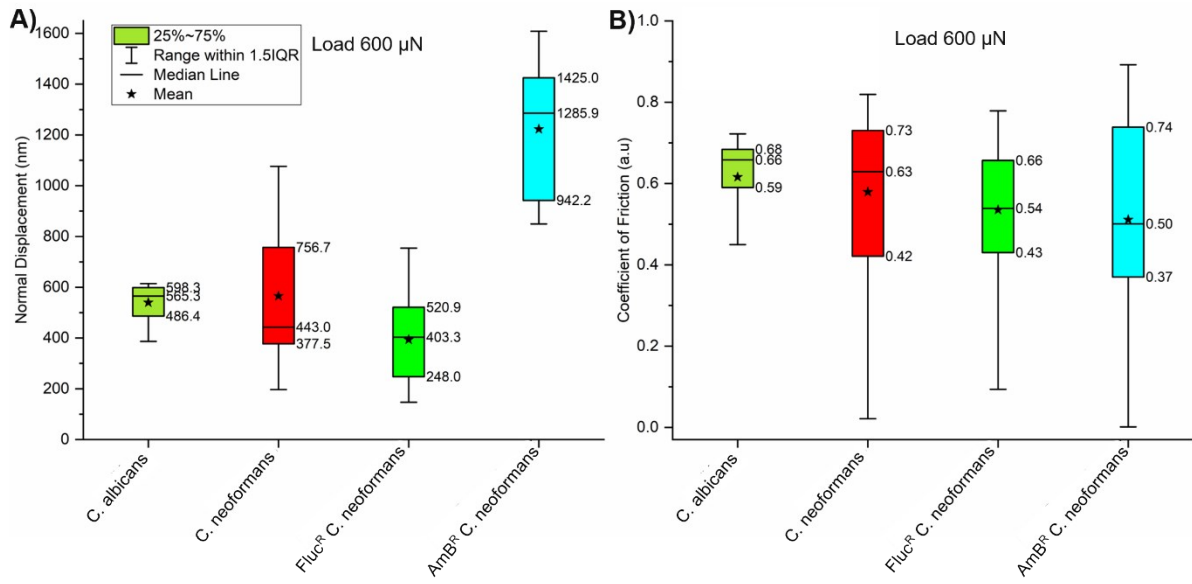
<sup>3</sup>Infrared Microspectroscopy Beamline, ANSTO Australian Synchrotron, Clayton, Victoria 3168, Australia

\*Corresponding authors.

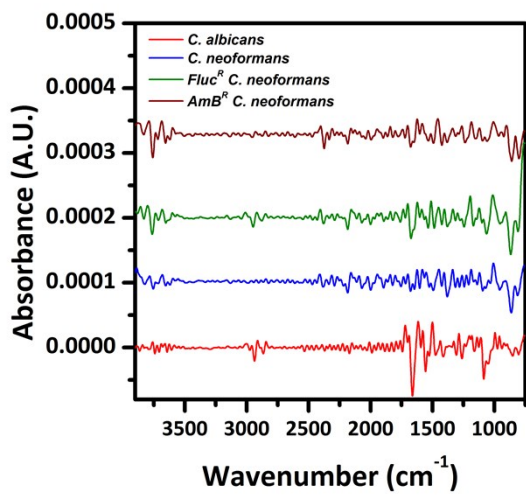
### Supplementary Information



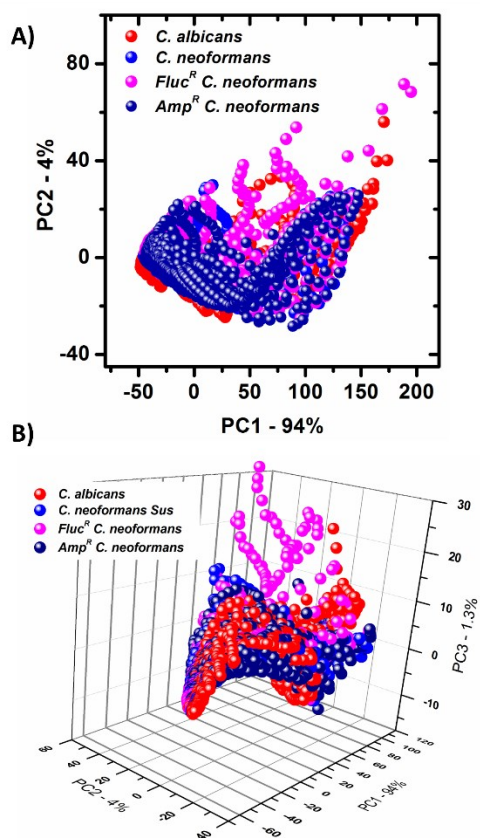
**Figure S1.** A-D) Fitting of AFM indentation curves to determine Young's elastic modulus ( $E$ ) for all fungal cells investigated, and E) that obtained when the cantilever is engaged with a flat surface. Mathematical fitting protocol employed the Hertz/Sneddon equation to convert experimentally derived force versus distance curves into indentation data (blue circles) and to fit the elastic deformation (non-linear) region to calculated  $E$  values (orange line).



**Figure S2.** Results of scratch tests on biofilm of *C. albicans*, *C. neoformans*, *flucR C. neoformans*, and *AmpR C. neoformans* on at a load of 600  $\mu$ N.



**Figure S3.** Representative second derivative of the fungal biofilms FTIR spectra.



**Figure S4.** PCA analysis of fungal biofilms ATR-FTIR data.

**Table S1.** The occurrence of biofilm-related infections caused by *Candida albicans* and *Cryptococcus neoformans*

Fungal species	Biofilm-related infections	References
<i>Candida albicans</i>	Urinary catheter tube	8
	Pacemaker	9
	Bloodstream	1, 2
<i>Cryptococcus neoformans</i>	Prosthetic hip	10
	Ventriculoarterial shunt	11
	catheters	

## References

1. C. J. Nobile and A. D. Johnson, *Annual review of microbiology*, 2015, **69**, 71-92.
2. M. Gulati and C. J. Nobile, *Microbes and infection*, 2016, **18**, 310-321.
3. E. M. Kojic and R. O. Darouiche, *Clinical microbiology reviews*, 2004, **17**, 255-267.
4. L. R. Martinez and A. Casadevall, *Applied and environmental microbiology*, 2007, **73**, 4592-4601.
5. R. Garcia-Rubio, H. C. de Oliveira, J. Rivera and N. Trevijano-Contador, *Frontiers in Microbiology*, 2020, **10**, 2993.
6. K. F. Mitchell, R. Zarnowski and D. R. Andes, in *Fungal Biofilms and related infections: Advances in Microbiology, Infectious Diseases and Public Health Volume 3*, ed. C. Imbert, Springer International Publishing, Cham, 2016, DOI: 10.1007/5584\_2016\_6, pp. 21-35.
7. O. Zaragoza and K. Nielsen, *Curr Opin Microbiol*, 2013, **16**, 409-413.
8. D. G. Maki and P. A. Tambyah, *Emerging infectious diseases*, 2001, **7**, 342.
9. G. Ramage, J. P. Martínez and J. L. López-Ribot, *FEMS yeast research*, 2006, **6**, 979-986.
10. B. Johannsson and J. J. Callaghan, *Diagnostic microbiology and infectious disease*, 2009, **64**, 76-79.
11. T. J. Walsh, R. Schlegel, M. M. Moody, J. W. Costerton and M. Salzman, *Neurosurgery*, 1986, **18**, 376-382.