REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

I. REPORT DATE (DD-MM-YYYY) 2. REPORT TYPE 3. DATES COVERED (From - To) IG-11-2015 9-Jul-2012 - 8-Apr-2013 A. TITLE AND SUBITILE Sa. CONTRACT NUMBER Final Report: Depth Resolved Nanospray Desorption Sa. CONTRACT NUMBER Electrospray Ionization Mass Spectrometry in Biofilms Sa. CONTRACT NUMBER 6. AUTHORS 5d. PROJECT NUMBER 6. AUTHORS 5d. PROJECT NUMBER 9. SPONSORING ORGANIZATION NAMES AND ADDRESSES Se. TASK NUMBER 9. SPONSORING MONITORING AGENCY NAME(S) AND ADDRESS S. PERFORMING ORGANIZATION REPORT Vulnear 9164 -3140 90164 -3140 9. SPONSOR/ING/MONITORING AGENCY NAME(S) AND ADDRESS III. SPONSOR/MONITOR'S ACRONYM(S) (ES) U.S. Army Research Office NUMBER 9. SPONSOR/ING/MONITOR'S ACRONYM(S) 62140-1.S-II.1 11. SPONSOR/MONITOR'S REPORT 10. SPONSOR/MONITOR'S REPORT NUMBER 62140-1.S-II.1 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ANS IRACT <	The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggesstions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any oenalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.							
4. TITLE AND SUBTITLE Johnson of the second of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved and proteins from biofilms 5a. CONTACT NUMBER 4. TITLE AND SUBTITLE Sa. CONTACT NUMBER 5. ERFORMING ORGANIZATION MARS Spectrometry in Biofilms 5c. PROGRAM ELEMENT NUMBER 6. AUTHORS 611102 6. AUTHORS 5c. PROGRAM ELEMENT NUMBER 6. AUTHORS 5c. TASK NUMBER 7. PERFORMING ORGANIZATION NAMES AND ADDRESSES 8. PERFORMING ORGANIZATION REPORT Washington State University 9164 - 3140 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS 10. SPONSOR/MONITOR'S ACRONYM(S) (ES) 11. SPONSOR/MONITOR'S ACRONYM(S) US. Army Research Office 11. SPONSOR/MONITOR'S REPORT P.O. Box 12211 11. SPONSOR/MONITOR'S REPORT Research Triangle Park, NC 27709-2211 10. SPONSOR/MONITOR'S REPORT 13. SUPPLEMENTARY NOTES 11. SPONSOR/MONITOR'S REPORT The views, options and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The views, options of microcapillary system was developed and tested for this goal. The microcapillary system son optimizer follow rates and its operation. The project	1. REPORT	DATE (DD-MM-	-YYYY)	2. REPORT TYPE			3. DATES COVERED (From - To)	
Final Report: Depth Resolved Nanospray Desorption W911NF-12-1-0254 Electrospray Ionization Mass Spectrometry in Biofilms Sb. GRANT NUMBER 6. AUTHORS 5c. PROGRAM ELEMENT NUMBER Haluk Beyenal, Tim Ewing, Ethan Atci 5c. TASK NUMBER 7. PERFORMING ORGANIZATION NAMES AND ADDRESSES S. PERFORMING ORGANIZATION NAMES AND ADDRESSES Washington State University 423 Ncill Hall Pullman, WA 99164 -3140 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS S. PERFORMING ORGANIZATION REPORT NUMBER 7. Disposed for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES ARO 11. SPONSOR/MONITOR'S ACRONYM(S) ARO 13. SUPPLEMENTARY NOTES Sensor findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully damagnated that a dual microapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrosmeter inlet. We tried different conflow rates and its operation. The project s	16-11-201	5		Final Report			9-Jul-2012 - 8-Apr-2013	
Electrospray Ionization Mass Spectrometry in Biofilms 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER 6. AUTHORS Haluk Beyenal, Tim Ewing, Erhan Atei 5c. TASK NUMBER 7. PERFORMING ORGANIZATION NAMES AND ADDRESSES 8. PERFORMING ORGANIZATION REPORT Vulman, WA 99164 -3140 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (FS) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211 12. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolities and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system decivered a solvent (estanol). The project successfully devended duce docue and tested for this goal. The microcapillary system date developed and tested for this goal. The microcapillary system date developed and tested for t	4. TITLE A	ND SUBTITLE	1		5a. CC	NTF	RACT NUMBER	
6. AUTHORS 6. AUTHORS Haluk Beyenal, Tim Ewing, Erhan Atci 5c. PROGRAM ELEMENT NUMBER 6. AUTHORS Haluk Beyenal, Tim Ewing, Erhan Atci 5c. TASK NUMBER 5c. PROFORMING ORGANIZATION NAMES AND ADDRESSES St. WORK UNIT NUMBER 9. SPONSORING/MONTIORING AGENCY NAME(S) AND ADDRESS (ES) US. Army Research Office P. O. Box 12211 Research Triangle Park, NC 27709-2211 10. SPONSOR/MONITOR'S ACRONYM(S) ADProved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES The yoas ondor in diving contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless or designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary systems and optimized follow rates and its operation. The project successfully distribute the intermetion and the camped liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully distributene tastes neactive a	Final Repo	rt: Depth Resc	lved Nanospra	ay Desorption	W911	INF-12-1-0254		
6. AUTHORS Haluk Beyenal, Tim Ewing, Erhan Atci 5d. PROJECT NUMBER Se. TASK NUMBER 5f. WORK UNIT NUMBER 7. PERFORMING ORGANIZATION NAMES AND ADDRESSES Washington State University 423 Neill Hall Pullman, WA 99164 -3140 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) U.S. Army Research Office P.O. Box 12211 Research Trangle Park, NC 27709-2211 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this groject was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyscs of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully documented text and text and text and text andea text andea text a					5b. GF			
6. AUTHORS Sd. PROJECT NUMBER Haluk Beyenal, Tim Ewing, Erhan Atci Sd. PROJECT NUMBER 5. WORK UNIT NUMBER Se. TASK NUMBER 7. PERFORMING ORGANIZATION NAMES AND ADDRESSES S. PERFORMING ORGANIZATION REPORT Washington State University 423 Neill Hall Pullman, WA 99164 -3140 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS 10. SPONSOR/MONITOR'S ACRONYM(S) (ES) NUMBER U.S. Army Research Office NUMBER(S) P.O. Box 12211 Research Triangle Park, NC 27709-2211 Research Triangle Park, NC 27709-2211 62140-L.S-II.1 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release: Distribution Unlimited 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolities and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary systems and optimized follow rates and it is operation. The project successfully developed and tested for this goal. The microcapillary systems and optimized follow rates and it is operation. The project successfully develope					5c. PR	OGRAM ELEMENT NUMBER		
Haluk Beyenal, Tim Ewing, Erhan Atci 5c. TASK NUMBER 5c. TASK NUMBER 5f. WORK UNIT NUMBER 7. PERFORMING ORGANIZATION NAMES AND ADDRESSES 8. PERFORMING ORGANIZATION REPORT NUMBER Washington State University 423 Neill Hall Pullman, WA 99164 -3140 0. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS 10. SPONSOR/MONITOR'S ACRONYM(S) (ES) U.S. Army Research Office 11. SPONSOR/MONITOR'S ACRONYM(S) (2140-LS-II, 1 P.O. Box 12211 NUMBER(S) Research Triangle Park, NC 27709-2211 10. SPONSOR/MONITOR'S REPORT NUMBER(S) (2140-LS-II, 1 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES 11. SPONSOR/MONITOR'S acronyment of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inflet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully <u>Amateria developed</u> , mass spectroscepy, biofilm, protein, microcapillary, microsensor, and microelectrode.					61110)2		
Se. TASK NUMBER 5f. WORK UNIT NUMBER 7. PERFORMING ORGANIZATION NAMES AND ADDRESSES Washington State University 423 Neill Hall Pullman, WA 99164 -3140 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system said optimized follow rates and its operation. The project successfully due constant data tabol anisons of microcapillary systems and optimized follow rates and its operation. The project successfully due constant data tabol to be biofilms at different depth. A microcapillary system was developed and tested for this project successfully and optimized follow rates and its operation. The project successfully due constant data tabol mismed a charged liquid to the mass spectrometer inlet. We tried different configurations of microca			Erhan Atoi		5d. PR	OJE	CT NUMBER	
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES 8. PERFORMING ORGANIZATION REPORT Washington State University 423 Neill Hall Pullman, WA 99164 -3140 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS 10. SPONSOR/MONITOR'S ACRONYM(S) (ES) 11. SPONSOR/MONITOR'S ACRONYM(S) U.S. Army Research Office 11. SPONSOR/MONITOR'S ACRONYM(S) P.O. Box 12211 62140-LS-IL 1 Research Triangle Park, NC 27709-2211 62140-LS-IL 1 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary systems and optimized follow rates and its operation. The project successfully demeastered by the documentation are author accessible for a busice the biofilms at 15. SUBJECT TERMS Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON		liai, Tim Ewing,			5e. TA	SK N	NUMBER	
Washington State University 423 Neill Hall NUMBER Pullman, WA 99164 - 3140 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) 10. SPONSOR/MONITOR'S ACRONYM(S) ARO U.S. Army Research Office P.O. Box 12211 11. SPONSOR/MONITOR'S ACRONYM(S) ARO Research Triangle Park, NC 27709-2211 62140-LS-II.1 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 62140-LS-II.1 13. SUPPLEMENTARY NOTES 62140-LS-II.1 The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a the bale misrocapillary system sense he used to callest more litere consult from one capillary built to able misrocapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a bale misrocapillary system sense he used to callest more litere consult from the bis of themantheman in the callest more litere consult f					5f. WC	ORK UNIT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) 10. SPONSOR/MONITOR'S ACRONYM(S) ARO U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211 11. SPONSOR/MONITOR'S REPORT NUMBER(S) 62140-LS-II.1 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 62140-LS-II.1 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary system see has used to callest measure the histilize at 15. SUBJECT TERMS Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 15. NUMBER [19a. NAME OF RESPONSIBLE PERSON Haluk Bevenal 10. 10.1 10. 11. LIMITATION OF ABSTRACT 15. NUMBER [19a. NAME OF RESPONSIBLE PERSON Haluk Bevenal	Washington State University							
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) 10. SPONSOR/MONITOR'S ACRONYM(S) ARO U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211 11. SPONSOR/MONITOR'S REPORT NUMBER(S) 62140-LS-II.1 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 62140-LS-II.1 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary system see has used to callest measure the histilize at 15. SUBJECT TERMS Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 15. NUMBER [19a. NAME OF RESPONSIBLE PERSON Haluk Bevenal 10. 10.1 10. 11. LIMITATION OF ABSTRACT 15. NUMBER [19a. NAME OF RESPONSIBLE PERSON Haluk Bevenal	Pullman, W	V A	9916	4 -3140				
P.O. Box 12211 NUMBER(S) Research Triangle Park, NC 27709-2211 62140-LS-II.1 12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary sources on based to collect parablitics complement by hiefflues at 15. SUBJECT TERMS Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 16. SECURITY CLASSIFICATION OF: 17. LIMITATION OF ABSTRACT 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON Haluk Beyenal 10. UU UU 101 UU 19b. TELEPHONE NUMBER	9. SPONSO				S			
12. DISTRIBUTION AVAILIBILITY STATEMENT Approved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully downard to dual microcapillary systems and optimized follow rates and its operation. The project successfully downard to dual microcapillary microsensor, and microelectrode. 16. SECURITY CLASSIFICATION OF: 17. LIMITATION OF 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON 16. SECURITY CLASSIFICATION OF: 17. LIMITATION OF 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON 10U UU UU UU 101 101								
Approved for Public Release; Distribution Unlimited 13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully domenant to the dual microcapillary system can be used to collect non-Different the biofilms at 15. SUBJECT TERMS Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 16. SECURITY CLASSIFICATION OF: 17. LIMITATION OF ABSTRACT 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON Haluk Bevenal UU UU UU UU 101 UU 111	Research T	riangle Park, NC	27709-2211			62140-LS-II.1		
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully domeant that a dual microcapillary evolution can be used to callect nanolites comple from the biofilms at 15. SUBJECT TERMS Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 16. SECURITY CLASSIFICATION OF: a. 17. LIMITATION OF ABSTRACT UU 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON Haluk Bevenal 10. UU UU UU 10. 11. 19b. TELEPHONE NUMBER	12. DISTRIE	BUTION AVAIL	IBILITY STATE	MENT	ı			
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation. 14. ABSTRACT The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully domeant that a dual microcapillary evolution can be used to callect nanolites comple from the biofilms at 15. SUBJECT TERMS Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 16. SECURITY CLASSIFICATION OF: a. 17. LIMITATION OF ABSTRACT UU 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON Haluk Bevenal 10. UU UU UU 10. 11. 19b. TELEPHONE NUMBER								
The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary eventue are be used to called meaning the biofilms at 15. SUBJECT TERMS Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 16. SECURITY CLASSIFICATION OF: 17. LIMITATION OF 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON ABSTRACT UU UU UU 19a. NAME OF RESPONSIBLE PERSON	13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department							
Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode. 16. SECURITY CLASSIFICATION OF: 17. LIMITATION OF 16. SECURITY CLASSIFICATION OF: 17. LIMITATION OF 17. LIMITATION OF 15. NUMBER 19a. NAME OF RESPONSIBLE PERSON Haluk Beyenal UU UU	The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary system can be used to callect nanoliter cample from the biofilms at							
a. REPORT b. ABSTRACT c. THIS PAGE ABSTRACT OF PAGES Haluk Bevenal UU UU UU UU 19b. TELEPHONE NUMBER								
UU UU 19b. TELEPHONE NUMBER							Haluk Beyenal	
				UU				

Report Title

Final Report: Depth Resolved Nanospray Desorption Electrospray Ionization Mass Spectrometry in Biofilms

ABSTRACT

The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary system can be used to collect nanoliter sample from the biofilms at different depth. However, the microcapillary system also collected cells which failed its use for MS.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received

TOTAL:

Number of Papers published in peer-reviewed journals:

Paper

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Erhan Atci our graduate student who build nano-DESI sensor will include Nano-DESI in his thesis and plans to submit an abstract to present it.

	Non Peer-Reviewed Conference Proceeding publications (other than abstracts):
Received	Paper
TOTAL:	
Number of Non	Peer-Reviewed Conference Proceeding publications (other than abstracts):
	Peer-Reviewed Conference Proceeding publications (other than abstracts):
Received	Paper
TOTAL:	
Number of Peer	-Reviewed Conference Proceeding publications (other than abstracts):
	(d) Manuscripts
Received	<u>Paper</u>
TOTAL:	

Books

 Received
 Book

 TOTAL:

 Received

 Book Chapter

Patents Submitted

Patents Awarded

Awards

Graduate Students				
NAME	PERCENT_SUPPORTED	Discipline		
Tim Ewing	0.50			
Erhan Atci	0.40			
FTE Equivalent:	0.90			
Total Number:	2			

Names of Post Doctorates

<u>NAME</u>

PERCENT_SUPPORTED

FTE Equivalent: Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Haluk Beyenal	0.13	
FTE Equivalent:	0.13	
Total Number:	1	

Names of Under Graduate students supported

NAME

PERCENT_SUPPORTED

FTE Equivalent: Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period
The number of undergraduates funded by this agreement who graduated during this period: 0.00 The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields: 0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields: 0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale): 0.00 Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering: 0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

<u>NAME</u>

Total Number:

Names of personnel receiving PHDs

<u>NAME</u>

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED	
Erhan Atci	0.40	
Tim Ewing	0.50	
FTE Equivalent:	0.90	
Total Number:	2	

Sub Contractors (DD882)

Scientific Progress

See attached.

Technology Transfer

We submitted the following proposal which plans to use the technology developed in this proposal.

Development of in vitro biofilm and planktonic culture of "Ca. Liberibacter asiaticus": a game change in HLB research. USDA, PI: David Gang of WSU. \$5,000,000. ~ 1,000,000 to Beyenal group for 5 years.

We proposed to use nano-DESI (nano-capillary system) sensor to collect cells at different depth in the natural biofilms in addition to MS work.

Depth-Resolved Nanospray Desorption Electrospray Ionization Mass Spectrometry in Biofilms

Award no: W911NF1210254

FINAL PROGRESS REPORT

Statement of the problem studied

It is known that there is a wide spectrum of applications in which mass spectroscopy (MS) can provide information from a biofilm sample, and these applications can easily translate to all areas of biofilm research. The diversity of functions within the growing community of a biofilm cannot be displayed because all of the analyses are done in bulk and have no spatial resolution with respect to the biofilm. Separation techniques can be combined with MS to present a more focused data set with regard to composition; however, MS analysis still lacks the capacity to investigate variation with biofilm depth. Depth profiles will have the potential to elucidate the mechanisms of the surrounding matrix and the roles of the microorganism with respect to the biofilm layers and the growth interface. However, it has not been used for depth profiling in biofilms. This is mostly because there was no tool available to conduct these measurements in a biofilm. Our research group specializes in the development of microelectrodes and their application to biofilm systems for the depth profiling of selected chemicals. The microelectrodes we make have a several-micrometer tip diameter and can be used in a biofilm without damaging its structure. The proposed research used the techniques developed for microelectrodes to build a device that collects samples from biofilms for MS analysis. Ambient pressure surface ionization mass spectrometry is used to obtain a chemical analyte for sampling from interfaces without special sample preparation (Roach et al., 2010). Desorption electrospray ionization (DESI) is an ambient ionization technique in which charged droplets from an electrosonic spray ionization source are aimed towards a surface with a proximal atmospheric pressure mass spectrometer inlet. In this technique analyte molecules are collected from flat surfaces followed by ionization using a self-aspirating nanoelectrospray. This technique directly transports and ionizes an analyte that is desorbed from a surface into a liquid. It is called nanospray DESI (nano-DESI). The nanospray capillary transports the charged liquid to the mass spectrometer inlet directly, eliminating splashing while minimizing analyte transport distance. The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins. We proposed three tasks to achieve our goal. Task 1: Develop technology to construct dual-barrel microcapillaries and connect them to solvent and MS lines. Task 2: Optimize the solvent and MS line flow rates for biofilm applications. Task 3: Test the system for monitoring and obtaining depth-resolved mass spectra.

Summary of the most important results

Figure 1 shows developed nano-DESI sensor. This sensor addressed work related to task 1. The final configuration was slightly different than originally proposed. When we tested dual capillary system (originally proposed), we could not deliver and collect solvent at the same rates. Therefore, we tested single capillary system as shown in Figure 1. In this system the solvent and its collection was made from the same capillary. Figure 2 (left) shows a photograph of the tip of nano-DESI sensor. Figure 2 (right) shows a photograph of the nano-DESI sensor with connectors while it was operating after optimization (Task 2). We managed to operate it between 10 nL/min and 100 μ L/min flow rates. After optimization of the flow rates, we tested it in biofilms. The biofilms were grown according to our previously published paper and book (Babauta and Beyenal, 2014; Lewandowski and Beyenal, 2013). However the microcapillary system was plugged by cell-clusters and stopped working (Task 3). In conclusion, we had succeeded in developing nano-DESI sensor but could not operate it with MS due to its unexpected ability to pick up cells in the biofilms. As a result, we used the nano-DESI sensor as a tool to collect cells

from the biofilms. Moreover, the microcapillary system developed for this project enabled us to develop a similar system to quantify electron transfer rates in biofilms (Babauta and Beyenal, 2014).



Figure 1. The configuration for nano-DESI sensor.



Tip of the capillary

Operating in the lab

Figure 2. Left: A photograph of the tip of nano-DESI sensor. Right: A photograph of the nano-DESI sensor with connectors

References

- Babauta, J.T., Beyenal, H., 2014. Local Current Variation by Depth in Geobacter Sulfurreducens Biofilms. Journal of the Electrochemical Society 161, H3070-H3075.
- Lewandowski, Z., Beyenal, H., 2013. Fundamentals of Biofilm Research, Second Edition Edition. CRC Press, Boca Raton, FL.
- Roach, P.J., Laskin, J., Laskin, A., 2010. Nanospray desorption electrospray ionization: an ambient method for liquid-extraction surface sampling in mass spectrometry. Analyst 135, 2233-2236.