Coffee Ring Diagnostics

NCIIA Grant Proposal

Stephanie Anderson
Scott Palmese
Stephanie Preston
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According to the World Health Organization (WHO), malaria affected 216 million people and caused 655,000 deaths in 2010. Despite increased funding and improved preventative measures (pesticides, mosquito nets), malaria claimed 781,000 lives in 2011 (WHO).

The WHO has outlined the need for improved access to malaria diagnostic tests. Diagnosis typically involves manually counting the number of parasites per unit volume in a thick blood smear. This method is time-intensive, highly susceptible to human error, and requires a microscope. In a majority of the regions affected by malaria, laboratory equipment and trained professionals are unavailable or uncommon.

Rapid diagnostic tests (RDTs) represent a relatively cheap, user-friendly alternative to blood smear microscopy. However, current RDTs are unable to withstand harsh conditions. RDTs use a nitrocellulose membrane containing the antigen of interest; presence of the malarial antibody in the patient’s blood produces a color change in the membrane. Since proteins are denatured at high temperatures, antigen storage is unsuitable for extreme temperatures (WHO).

Mavandadi et al. recently advocated for crowd sourcing in the diagnosis of malaria. The team uploaded microscopic blood smear images to a “biogame” for undergraduate students. These students were asked to select each red blood cell that was infected with malaria. Combining the responses from 1,000 individuals produced a diagnostic accuracy only 1.25% less than that of trained pathologists. However, relying on the voluntary participation of the public could produce wide variations in time to diagnosis. Consistent and rapid diagnostic time is particularly crucial in developing countries, where patients struggle to keep follow-up appointments due to lack of time or transportation (Hart et al. 2011).

The many disadvantages to these existing diagnostic methods (inadequate equipment and personnel, lengthy time to diagnosis, inconsistencies due to environmental conditions) are directly associated with the over-diagnosis and overtreatment of malaria in non-infected
individuals (Masika et al. 2006), and inefficiency in treating affected individuals. A robust point-of-care method, wherein the patient is tested, diagnosed, and treated in a single visit, is essential.

Individuals in resource-constrained environments need a cheap, oral point-of-care tool for the diagnosis of malaria that can withstand extreme temperatures. A test that produces a visible color change in the presence of the malaria biomarker, similar to that of the existing RDTs, but does not rely on the storage of proteins, could function in extreme temperatures. Furthermore, using saliva as opposed to blood eliminates the need for a healthcare professional. The most accurate biomarker for malaria, histidine-rich protein II (HRPII), is present in different biological fluids, including plasma and saliva (Wilson et al. 2008); Fung et al. quantified salivary HRPII concentrations at 0.017 – 1.167 ng/ml.

We are proposing a diagnostic device inspired by the microfluidics of an evaporating coffee drop, wherein the binding of a biomarker with functionalized particles in colloidal suspension produces visible changes in the drying pattern (Figure 1).

Technology—When a drop of coffee dries, it leaves behind a ring of particles. Around the edges of the drop, fluid evaporates more quickly due to a fewer number of intermolecular interactions. At the same time, the drop’s edges cannot migrate toward the center due to adhesion. As a result of these two phenomena, fluid from the center migrates to the edges, forming an outward radial velocity profile. These currents carry the solute particles to the edges of the drop, forming the characteristic ring pattern.

Figure 1: Drying patterns of fluorescent particles with poly-L-histidine (left) and poly-L-aspartate (right). A magnetic particle and one of two fluorescent particles were functionalized with Ni-NTA, which binds poly-L-histidine (PLH), an HRPII mimic. In the presence of PLH, the functionalized magnetic and fluorescent particles colocalize above a magnet placed below the center of the drop (Trantum et al. 2011).

+ Clinics that do not have access to diagnostic tests for malaria prescribe medication (artemisinin-based combination therapy, ACT) to any patient presenting with a fever. Aside from misappropriating resources, this practice encourages the development of artemisinin-resistant malaria, and neglects other possible causes of fever (WHO).
Previous studies have exploited the interaction of nickel-nitrilotriacetic acid (Ni-NTA) functionalized magnetic particles, fluorescent particles, and an HRPII mimic (poly-L-histidine) to disrupt ring formation and produce a visible change in solute deposition (Trantum et al. 2011). Ni-NTA is temperature insensitive and could be stored for extended periods of time without refrigeration. However, this covalent functionalization protocol is still being refined, so we will adopt an antibody functionalization for proof of concept of our device.

Since HRPII is present in saliva, we have produced a saliva mimic (simulated saliva buffer, 0.021 M NaH$_2$PO$_4$/Na$_2$HPO$_4$, 36 mM NaCl, 0.96 mM CaCl$_2$) containing 1 mg/ml 1630 kDa hyaluronic acid to simulate viscosity while developing our diagnostic tool (Park et al. 2010). We are using colorimetric beads instead of fluorescent beads, so the user can view the result directly. Storage of the functionalized particles in the device will tentatively be accomplished by dehydration. If we discover that dehydration or rehydration of the particles is problematic, we will investigate preserving the particles in suspension.

Our prototype includes a funnel to guide saliva onto dehydrated, functionalized particles (Figure 2). The user will discharge his/her saliva into the base of the cone, and then close a hinged lid. The lid contains a deformable plastic diaphragm, which when depressed will displace a fixed volume of air and deposit a standardized volume of saliva onto the particles, resuspending them. Once sealed, this prototype will be airtight, creating an isolated environment and guarding against contamination. We will investigate the use of desiccant materials and the application of heat to increase evaporation rate and decrease time to diagnosis.
Our device represents a powerful method by which to promote the availability of all diagnostic tools in resource-constrained environments. In *Universal Access to Malaria Diagnostic Testing*, the WHO identified another challenge in the control of malaria:

“Clinicians are more likely to adhere to the results of a malaria test when they can perform comprehensive assessment of the patient to identify and treat other diseases. For this to be effective, additional diagnostic tools must be available to health workers, which are appropriate to the setting, including clinical diagnostic equipment and additional laboratory tests such as urine dipsticks.”

While our device was originally designed to interface with the coffee ring microfluidics for the detection of malaria described by Trantum et al., it can actually be used to diagnose any disease with an established salivary biomarker. For example, the dehydrated particles could be replaced with microarray or “lab-on-a-chip” technology.

*Business Model and Market*— Our proposal is the first to truly prioritize the needs of a resource-constrained environment. Unlike existing methods, our device can function without a healthcare professional or a sample of blood and doesn't require protection from extreme environmental temperatures. Furthermore, a study in sub-Saharan Africa found that the cultural stigma associated with drawing blood could discourage the use of blood-based RDTs (Mukanga et al. 2010). By using saliva, we avoid this cultural constraint and broaden our customer base. Untrained individuals could test themselves and present the result to the nearest pharmacy for treatment.

![Figure 3: Gantt chart illustrating expected outcomes for Spring 2013.](image-url)
We estimate the cost of our device to be under twenty dollars, which is comparable to existing devices. However, our device eliminates the cost of laboratory equipment, healthcare professionals, and maintaining controlled conditions during transport and storage. We will market our product to global malaria initiatives, as our device could be delivered directly to developing communities by governments or relief organizations. We intend to localize as much production and distribution as possible to malaria endemic regions. Domestic manufacturing and sales will be accomplished by a non-profit organization.

The WHO considers malaria “entirely preventable and treatable” and calls for improved diagnostic methods at the community level to eradicate the disease. Our proposal answers this need. Successfully implementing our device in malaria endemic countries could save hundreds of thousands of lives, and lead to the eventual elimination of the disease.

*Team members*—Our team consists of five members, Stephanie Anderson (cultural liaison), Scott Palmese (team leader), Stephanie Preston (laboratory specialist), Erica Von Stein (web design), and Jiemi Zhu (imaging/programming).

Stephanie Anderson worked in Tanzania repairing medical equipment for a local hospital in the summer of 2012. Scott Palmese has been employed as a clinical research coordinator and has led teams of people in conducting research trials. Stephanie Preston has volunteered in a clinic in rural Costa Rica, and has experience in designing novel experimental techniques. Erica Von Stein has consistently conducted research and developed numerous refined oral and written presentations. Jiemi Zhu is proficient in Matlab and has a working knowledge of C++ and visualization toolkit (VTK).

We are advised by Rick Haselton, PhD. He specializes in developing technologies for diagnostic and research applications at the nano and molecular levels using *in vivo* and *in vitro* systems. Specifically, he has worked on developing technology for resource-constrained environments.
References


February 1, 2013

To Whom It May Concern:

I am pleased to write in support of the Coffee Ring Diagnostics proposal submitted to the NCIIA by Vanderbilt BME senior Scot Palmese et al. The fundamental microfluidic and biochemical aspects of their design has been a focal point of my research over the last several years and I am happy to report that the project has been partially funded by the Gates Foundation because of its potential application as a diagnostic tool in a resource-constrained environment.

The prototype that the team has come up with is a novel implementation that has the potential to make the technology commercially viable. The team aims to develop an inexpensive, easy-to-use diagnostic test for malaria and other infectious diseases by detecting the presence of biomarkers in patient samples. Coffee Ring Diagnostics has been designed to overcome many of the challenges present when attempting to serve a resource-constrained community. For example, in order to avoid cultural stigma and the difficulties inherent in obtaining blood samples, they are advocating the use of saliva instead of blood. Their device will also act as a controlled micro-fluidic environment, an important consideration since many resource-constrained regions are prone to extreme temperatures and other external conditions.

Each of the members of Coffee Ring Diagnostics has played an integral role in the development of this project. Over the summer of 2012, Stephanie Anderson lived in Tanzania and became familiar with many of the needs of a resource-constrained environment. Her insight into the needs of the target market has shaped the development of the team’s prototype. Stephanie Preston and Erica Von Stein have extensive laboratory experience and are responsible for refining much of the chemistry that has been integrated into the prototype. They also were in charge of synthetic saliva production. Jiemi Zhu is proficient in C++ and AutoCAD programming and has been instrumental in developing computer models for the prototype during development. Scott Palmese, the team leader, tells me he has extensive clinical experience and apparently has participated in leading clinical trials. This experience has helped him to understand patient and user needs, which will be helpful when considering the team’s target market.

I am fully prepared to support Coffee Ring Diagnostics as they develop their technology. Already, I have made bench space available and allowed them to use the resources of my lab, including many of my instruments. I am looking forward to continuing our lively discussions about development roadblocks as they work to resolve unanticipated issues. It is a pleasure to work with such a motivated and resourceful group and I am confident that the team will have a great deal of success as they continue to refine and develop their prototype.

Sincerely,

Rick Haselton
Professor of Biomedical Engineering
rick.haselton@vanderbilt.edu
Appendix A – Group Resumes
**STEPHANIE S. ANDERSON**
stephanie.s.anderson@vanderbilt.edu
3211 Long Boulevard • Nashville, TN 37203 • (713) 822-0277

**EDUCATION**

**Vanderbilt University**
Bachelors of Engineering Biomedical Engineering, expected May 2013
Minor: Studio Art
Cumulative GPA: 3.317
Major GPA: 3.5

**WORK EXPERIENCE**

**Engineering World Health**
Arusha and Machame, Tanzania
Summer Institute 2012
- Completed a short course in Swahili and medical device troubleshooting/repair upon arrival in Tanzania.
- Repaired broken medical equipment in Mt. Meru and Machame hospitals.
  - Used troubleshooting and design skills to come up with creative solutions to repair a variety of medical devices ranging from theatre lights to stethoscopes in a rural low resource environment.
  - Created a hospital inventory to track donated medical devices.
  - Conducted interviews for engineering world health in order to help define design problems faced in the Machame hospital.
- Engineering World Health Conference, Presenter – Designed and delivered summer internship accomplishment summary to dignitaries including the Health Minister of Zanzibar.

**Circle Lake Ranch Inc. 2008-Current**
Katy, TX
- Ranch Hand
  - Teach private and group horse riding lessons.
- Dog Handler
  - Work in the office handling customer interactions, bookings and payments while overseeing 40 dogs at the dog-boarding facility.

**Vanderbilt Equestrian Team 2009-Current**
Intercollegiate Horse Show Association (IHSA) Region 1 2nd place winner 2011
IHSA Zone 5 Competitor 2011
Nashville, TN

**WRVU Vanderbilt University Radio Station**
DJ Co-host 2009-Current
Underwriting and Promotions Director 2010
Nashville, TN
- Managed the development, implantation and reading of underwriting, promotions and public service announcements for WRVU radio station.
- Worked with the events and promotions directors to create the graphic designs for WRVU.

**Vanderbilt Alternative Spring Break 2012**
Kyle, SD
- Volunteered as a teacher’s assistant mentoring elementary school children on the Lakota reservation.

**COMPUTER SKILLS:** Excel, Word, Access, Publisher, Mathematic, MatLab and LabView.
**LANGUAGE SKILLS:** Limited working proficiency: Swahili and French.
Scott Joseph Palmese
Senior – Vanderbilt University: Nashville, TN
Home Address: 12 Heather Green Court, Ocoee, FL 34761
School Address: Station B 6098, Nashville, TN 37235
Phone: (407) 620-4491
Email: scott.j.palmese@vanderbilt.edu

Education
- Bachelor of Engineering, Vanderbilt University, Nashville TN (May 2013)
  - Major: Biomedical Engineering
  - Minor: Neuroscience

Awards and Honors
- Dean’s List, Vanderbilt University (Spring 2011 – Present)

Extracurricular Activities
- Volunteer, Alzheimer’s Association Mid-South Chapter, Nashville, TN (October 2012-Present)
  - Helped run special events and led advocacy initiatives
- Mentor for Incoming Freshmen in Biomedical Engineering, Vanderbilt University (2012-2013)
- Studied abroad in Galway, Ireland at the National University of Ireland, Galway (Fall 2011)
  - Member of Biomedical Student Society
- Shadowed Dr. Ira Goodman, Neurologist, Orlando, FL (Summer 2011)
  - Saw patients with a variety of neurological complaints, primarily those with memory disorders
- Biomedical Engineering Society, Vanderbilt Chapter (2010-Present)
  - Member of Executive Board (2012-2013)
  - Historian (2012-2013)
- Dyer House President, Vanderbilt University (2010-2011)
  - Led programming initiatives for dormitory and quad
  - Served as representative for over 100 peers
- Vanderbilt Student Government (2009-2011)
- AED Pre-Medical Society, Vanderbilt Chapter (2009-2011)
- North House Floor Representative, Vanderbilt University (2009-2010)

Work/Research Experience
- Research Assistant – SNARL Laboratory at Vanderbilt University (September 2012 – Present)
  - Developed software utilized for image-guided surgery
  - Shadowed ophthalmologist who utilizes SNARL technology in practice
- Compass Research – Clinical Research Coordinator, Orlando, FL (July 2011 – Present)
  - Conducted my own research trials as lead coordinator – indications were Alzheimer’s disease, Parkinson’s disease, and Multiple Sclerosis
  - Saw patients, conducted lab work, and served as the main point of correspondence for the pharmaceutical companies and Compass Research
  - Performed rating scales, including the Mini-Mental State Examination (MMSE), ADAS-Cog, and Clinical Dementia Rating (CDR)
  - Assisted physicians with medical procedures, including lumbar punctures
- Compass Research – Business Development Assistant, Orlando, FL (May 2011 – Present)
  - Gained experience with the business side of clinical research
  - Submitted clinical protocols for review to IRB, served as contact for legal bodies regarding submissions of new clinical trials
- Compass Research – Interned as Research Assistant, Orlando, FL (May 2010 – August 2010)
  - Learned how to understand clinical protocols and the practice of clinical research
  - Developed new procedures for the intake and analysis of data
  - Trained staff members on new procedures
- Reeve Front Desk Assistant, Vanderbilt University (August 2009 – May 2011)
  - Served as a resource for residents, guests, and visitors to various dormitories at Vanderbilt
  - Monitored traffic in the halls and assisted the Resident Advisors in keeping order in the dorms

Professional Training
- HIPAA Certification – Completed July 2011, Renewed July 2012
- Good Clinical Practice (GCP) Training – Completed July 2011, Renewed November 2012
- Investigator Meetings for New Clinical Protocols
  - Aug. 11, 2012: Development of a New Biosimilar Drug for Rheumatoid Arthritis, San Francisco, CA

Skills/Expertise
- Proficient in Microsoft Office Suite and MATLAB
- Working Knowledge of Wolfram Mathematica, C++, and Visualization Toolkit (VTK)
Erica L. Von Stein

Current Address:
Vanderbilt University
PMB 352925
Nashville, TN 37235-2925

Permanent Address:
835 Bay Point Dr.
Madeira Beach, FL 33708

erica.l.von.stein@vanderbilt.edu

Education

Vanderbilt University, Nashville, TN
Bachelor of Engineering in Biomedical Engineering
Cumulative GPA: 3.98/4.00
MCAT: 12 Biological Sciences, 13 Verbal Reasoning, 12 Physical Sciences (37)
May 2013

Center for Advanced Technologies, St. Petersburg, FL
Cumulative GPA: 4.00/4.00
June 2009

Honors

Phi Eta Sigma, Alpha Lambda Delta, and Lotus Eaters (President) Honor Societies 2010-2013

Cornelius Vanderbilt Scholarship
Awarded 4-year full tuition merit scholarship in the School of Engineering 2009-2013

National Merit Scholarship 2009-2013

Intel International Science and Engineering Fair Finalist
Presented Project: The Inhibitory Effects of Taste on Pain 2008

International Youth Fuel Cell Competition
Designed model hydrogen fuel cell vehicle and fork-lift 2008

Activities & Leadership

Shadowing Neurologist, Dr. Daniel Claassen
Fall 2012- present

All Children's Hospital Volunteer
Volunteered in surgery waiting room and as part of Child Life Services Summer 2012

Shadowed Pediatric Neurosurgeon, Dr. Noel Tulipan
Spring 2012-present

WRVU 91.1 FM Radio DJ
DJ one hour weekly show titled BRAiNWAVES and review albums prior to rotation Spring 2011-present

Phi Sigma Pi National Honor Fraternity
Scholarship Chair, Oversees committee and coordinate Learn from a Brother event Fall 2010-present

AMSA Premedical Chapter at Vanderbilt
Co-founder, Vice President for Policy and Internal Affairs Spring 2010 - present

BMEpulse Student Newsletter
Editor-in-chief, Previously Assistant Editor, Writer Fall 2009 - present

Vanderbilt Student Volunteers for Science
Team Leader, Teach weekly science lessons at local elementary schools Fall 2009 - present

Research

Vanderbilt University Department of Chemical and Biomolecular Engineering
Developing resorbable biomaterials for use in healing of critical bone defects Spring 2010 - present

➢ PI: Dr. Scott Guelcher
➢ First authored abstract accepted by 2011 international AIChE annual conference
   Presented poster: In Vitro Osteoclast Differentiation on Mineralized Bone Particle/Polyurethane Composites

➢ Awarded salary via VU School of Engineering Summer 2011
➢ Applied Cornelius Vanderbilt Research Stipend Summer 2010

University of Florida Department of Community Dentistry & Behavioral Sciences
Investigated the hypothesis that pain response is normally inhibited by taste input Summer 2007

➢ PI: Dr. Henrietta H. Logan
➢ Participated in University of Florida Summer Student Research Program

Additional Skills

Public Speaking, Editing, Writing, MATLAB, Wolfram Mathematica, MS Word, PowerPoint, Excel
Jiemi Zhu
PMB 353656
2301 Vanderbilt Place
Nashville, TN
(615)293-9019

EDUCATION
Vanderbilt University, Nashville TN
Bachelor of Engineering
Major: Biomedical Engineering
May 2013

RELATED EXPERIENCE
Undergraduate Researcher
Dr. Michael Miga, Vanderbilt University, Nashville, TN
May 2011-August 2011

- Accomplished individual research project: Development and Assessment of a Wireless Handheld Preoperative Planning Tool for Neurosurgical Interventions. Programmed in C++ in combination with VTK
- Performed assessment of the tool using head phantom, laser range scan and Polaris (optical measurement system)
- Drastically reduced the computation time required for atlas generation used in image guided surgery from weeks to hours

Sales Associate
Campus Bookstore, Follett/Barnes and Noble, Nashville, TN
August 2011- present

- Worked in a team and adjusted processing methods to speed up the shelving time in order to meet the deadline in the textbook department at the new Barnes and Noble bookstore

APPLICABLE EXPERIENCES
Alternative Winter Break, Huntsville, AL
Jan 2011

- Actively engaged in a week of service as a participant working with poverty and education
- Gained an insider view of how to start a non-profit and what are the hurdles in serving a community in poverty

Alternative Winter Break, Joplin, MO
Jan 2012

- Site Leader: Led and mentored 10 undergraduate students and devoted a week to tornado rescue and learning about community rebuilding in Joplin
- Raised awareness of the needs of Joplin in recovery through interviews by local TV station and newspapers

Concert Choir
Aug 2009 - present

- Alumni Historian: Hosted alumni reunion and was responsible for alumni relations of more than 600 choir alumni

SKILLS
ProE, Matlab, LabVIEW, Excel, Powerpoint, Word

LICENSED / CERTIFICATIONS
Hold interpreter/translator's license for Chinese and Japanese since 2006