

Final Report BW Sample Collection & Preparation Device

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January 31, 2002

U.S. Department of Energy

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Attached are the following documents:

- 1) Cepheid Operator's Manual
- 2) Memo from Ron Koopman, dated 12/17/99 – "Cepheid Sample Preparation Module Design Review, December 9, 1999"
- 3) UCRL-ID-141153 – Long Wavelength GaN Blue Laser (400-490nm) Development - Final Report 2000 for LLNL Project

Final Report

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Ronald P. Koopman, Phil Belgrader, Glenn Meyer, William J. Bennett, James B. Richards,
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Project Objective

The objective of this project was to develop the technique needed to prepare a field collected sample for laboratory analysis and build a portable integrated biological detection instrument with new miniaturized and automated sample purification capabilities. The device will prepare bacterial spores, bacterial vegetative cells, and viral particles for PCR amplification.

Project Context

This project is part of a more comprehensive effort to build an automated, portable biological detection instrumentation capability. Other critical areas of development, not covered by this current project, are: PCR detection instrumentation, and integration of the instrument components into other instrument systems under development by other organizations. We anticipate that all of the critical components will come together into a comprehensive system through additional projects being initiated, and through close collaborative efforts among the participating organizations.

Background

This project is a follow-on to eight years of technology development that began with LLNL internal funding under which the micro-fabricated PCR thermal chamber was conceived and developed. This was followed by a three year DARPA funded project (1993-96) that resulted in a suitcase-sized miniature PCR instrument that was successfully demonstrated at JFT III in Sep 1996. In 1998 CMO funded design and construction of a field hardened 10 chamber PCR instrument with 2-plex capability (ANAA) which was delivered to NMRI. In 1999 CMO funded design and construction of a four chamber, handheld instrument (HANAA), a suitcase sized 24 chamber instrument, and initial work on multiplex PCR flow analysis using the Luminex 100 and multiplex assay reagents.

This project continued to draw upon the resources and personnel (scientists, engineers and technicians) from the previous work in this area. The facilities to perform the tasks described herein include static and dynamic testing chambers, a state of the art microfabrication facility, a complete electronic design and fabrication facility, mechanical fabrication capabilities including plastic extrusions, design modeling, system engineering, PCR and nucleic acid sequencing expertise, packaging and extensive field test experience.

Tasks

Task 1. Integrate a DNA purification chip into the autonomous fluidic cartridge-based sample preparation system.

Task 2. Evaluate a combined chemical/heat treatment technique to lyse spores and allow the direct amplification of bacterial DNA.

Task 3. Support development of a blue-diode laser to provide laser options for the nucleic acid systems and flow cytometers.

Task 4. Construct three 4-chamber devices and provide the devices to organizations designated by CM/TCO.

Results

Task 1.

This task was a continuation of work begun the previous year. The contract with Cepheid for this work was signed on August 24, 1999. The first year of development of this sample preparation instrument is described in reference 5. Cepheid's progress on design was rapid, and a formal design review was held at Cepheid on December 9, 1999. The results of that review were written up and are included as an attachment and listed as reference 8. The instrument was completed in October and demonstrated at LLNL on October 6, 2000. During that demonstration, a 1 ml suspension of spores was automatically processed and lysate was added to PCR master mix. This was completed in about 5 minutes and was performed using the internal battery. The lysate was successfully detected on the HANAA. Unprocessed spores were not detected. After final modifications to control software, the instrument was officially transferred to LLNL in late October for preliminary evaluation. An LLNL biologist was trained on the instrument at Cepheid and exercised it as part of our acceptance testing. By direction from CMO, the instrument was then delivered to Mike Goode, ECBC, for evaluation in the Multi-Center Evaluation Project. Documentation of the instrument is provided in the Operator's Manual, reference 6, included as an attachment to this report. The results of this task (as well as the other three tasks) were presented during the CMO review at LLNL on December 5, 2000.

Task 2.

Sonication with glass beads was determined to be a highly effective mechanism to lyse spores in as little as 5 seconds. Results were similar to the gold standard method of germination/heat lysis. In addition, scanning electron microscopy confirmed that this treatment broke open the spores. Cepheid delivered stand-alone sonication systems to USAMRIID, AFIP, and the CDC. All three organizations have had success rapidly lysing spores, and thus provided outside verification to the approach. Another system was sent to Midwest Research Institute for Dr Winegar to use.

Initially, we were concerned that the glass beads could interfere with the flow of fluids in the integrated, microfluidic cartridge system that is being built by Cepheid in Task 1. Therefore, a study to develop an alternative, fallback method that did not utilize glass beads was proposed. The purpose of Task 2 was to pursue further developments in the disruption of spores using a combination of NaOH and either heat or sonication treatments. Preliminary data using the combination protocols on spores resulted in the enhancement of the PCR signal

compared to untreated spores. However, the signal was not as strong as the sonication with beads treatment. In addition, a potential problem with the NaOH method was the possibility that real-world samples (i.e. buffered samples from an aerosol collector) would neutralize the NaOH, reducing its effectiveness.

Many experiments using testbed microfluidics bead-based devices built by Cepheid (filter fixtures, prototype cartridge systems, and disposable filter bodies) resulted in spore lysis without interference with fluid flow by the beads. Therefore, we have adopted the sonication with beads method for Task 1 based on the following rationale. 1) The sonication with beads treatment has proven to be very effective. 2) The beads can be well-contained and do not interfere with fluid flow. 3) The long lead time to complete Task 1 required an early decision on a spore lysis method. 4) The sonication method has been verified by outside groups. 5) We were unable to demonstrate that the NaOH/heat method was as effective as sonication with beads. 6) We were concerned that environmental samples could neutralize the NaOH.

For the reasons cited above, we believed that there was no benefit to proceeding further with Task 2 and requested that Task 2 be terminated, with the remaining funding transferred to Task 1, which was done. In addition, Cepheid conducted experiments testing the ability of the cartridge system to remove inhibitors from samples, which were successful.

Task 3.

Excellent progress towards fabrication of a 490 nm laser diode was accomplished this year. High quality InGaN active regions emitting photoluminescence at 490nm was demonstrated and in addition, significant progress was accomplished in the growth of low defect density devices. The results of this work are documented in reference 7 and included as an attachment to this report. Dr. Den Baars at UCSB is confident that a working 490 nm laser diode could be demonstrated as early as next year with continued funding.

Program Highlights and Achievements 1999-2000 include:

Blue lasers emitting from 400 to 425nm were achieved. Atmospheric pressure MOCVD was used to obtain high quality InGaN active regions emitting photoluminescence at 490nm demonstrate feasibility of shifting wavelength into the longer wavelength region. Reduction of defects by LEO technique improves laser efficiency and will be key to getting longer life CW lasers at blue/green spectral region

Using laterally epitaxially overgrown (LEO) technique UCSB researchers have drastically reduced defect levels from $1E+10cm^{-2}$ to $1E+5cm^{-2}$. The laterally overgrown 'wing' regions as well as the coalescence fronts contained few or no threading dislocations. Laser diodes fabricated on the low-dislocation-density wing regions showed a reduction in threshold current density from 8 kA/cm² to 3.7 kA/cm² compared to those on the high-dislocation 'window' regions. Laser diodes also showed a two-fold reduction in threshold current density when comparing those on the wing regions to those fabricated on conventional planar GaN on sapphire. The internal quantum efficiency also improved from 3% for

laser diodes on conventional GaN on sapphire to 22% for laser diodes on LEO GaN on sapphire.

Electrically pumped distributed feedback nitride laser diodes employing embedded dielectric third order gratings (EDG) were demonstrated for the first time during this program. The distributed feedback laser diodes showed considerable spectral narrowing over the etched facet cavity device. At 1.25 times threshold, the spectrum shows a single mode with full width half maximum of 0.2 nm. An etched facet cavity device operating under similar conditions shows many spectral modes spread over approximately 6 nm.

Task 4.

Three more HANAAs were built and provided to evaluators selected by CMO for this task. The evaluators provided valuable oral and written feedback that was used to create the commercial design of the next generation instrument. A one day training session was held on June 1, 2000 at LLNL for Dr. Peter Emanuel, ECBC, and Lt. Col. Ted Hadfield, AFIP, recipients of the first two instruments. The third HANAA was delivered to Mike Goode, ECBC, for evaluation in the Multi-Center Evaluation Project. Another training session occurred at LLNL on October 13 for Goode's people. The HANAA is described in references 1 and 2. Development of pathogen assays and use of those assays on the HANAA is described in reference 3. The evaluation of HANAA by both civilian and defense evaluators is described in reference 4. These references are available upon request. HANAA is currently being commercialized by Environmental Technologies Group (ETG). A redesigned commercial HANAA prototype (called Bio-Seq), addressing the short-comings identified in the evaluation project, is now available from ETG.

Deliverables

a. Final briefing describing the results of tasks 1 through 3

A briefing on Task 1 and 2 was held at LLNL on October 6, 2000, prior to delivery of the Sample Preparation device to LLNL. The results of the work on all four tasks were presented during the CMO review at LLNL on December 5, 2000.

b. Three 4-chamber devices

Three Hand-held Advanced Nucleic Acid Analyzers (HANAA) were built and delivered to organizations identified by CM/TCO.

c. Quarterly financial and technical reports

Monthly financial and technical reports were provided. Design reviews were held as needed.

Attachments and/or References

1. J.B. Richards, W.J. Bennett, P.L. Stratton, D.R. Hadley, S.L. Nasarabadi, F.P. Milanovich, *Miniaturized Detection System for Handheld PCR Assay*, SPIE International Symposium on Environmental and Industrial Sensing, Boston, MA, November 5-8, 2000, UCRL-JC-138399, not included as an attachment.

2. W.J. Bennett, J.B. Richards, P.L. Stratton, D.R. Hadley, B.H. Bodtker, S.L. Nasarabadi, F.P. Milanovich, R.P. Mariella Jr., R.P. Koopman, P. Belgrader, *Handheld Nucleic Acid Analyzer*, SPIE International Symposium on Environmental and Industrial Sensing, Boston, MA, November 5-8, 2000, UCRL-JC-136587, not included as an attachment.
3. P. Emanuel, T. Hadfield, R.P. Koopman, J.B. Richards, W.J. Bennett, P.L. Stratton, D.R. Hadley, F.P. Milanovich, J.J. Valdes, *Detection of Pathogens Using a Handheld PCR Thermocycler*, NATO Symposium on Operational Medical Issues in Chemical and Biological Defense, Lisbon, Portugal, May 14-17, 2001, UCRL-JC-143868, not included as an attachment.
4. R.P. Koopman, *HANAA Evaluation Summary*, Draft, April 3, 2001, not included as an attachment.
5. P. Belgrader, *A Miniature Sample Preparation Instrument (Phase1)*, FY99 Final Report, UCRL-ID-134506, May 1999, not included as an attachment.
6. Cepheid, *Operators Manual, LLNL Briefcase GeneXpert System*, attached.
7. S. DenBaars, A. Abare, K. Sink, P. Kozodoy, M. Hansen, J. Bowers, U. Mishra, L. Coldren, G. Meyer, *Long Wavelength GaN Blue Laser (400-490nm) Development*, UCRL-ID-141153, October 2000, attached.
8. R.P. Koopman, *Memo: Cepheid Sample Preparation Module Design Review*, December 9, 1999, attached





Operator's Manual

LLNL Briefcase GeneXpert System

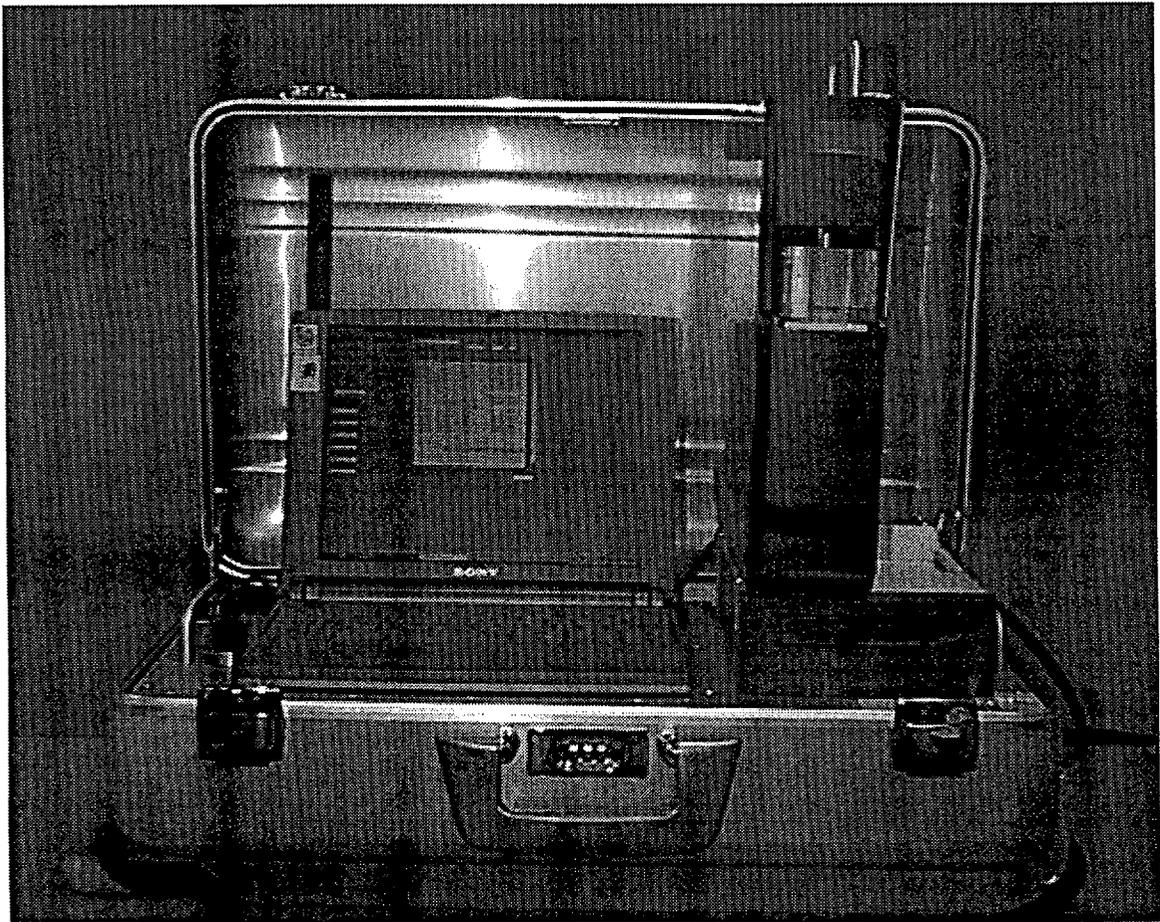


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1.0 Introduction

The briefcase GeneXpert is designed for the development and evolution of protocols in biowarfare defense, where automation and hands-off preparation are required. The GeneXpert system revolutionizes the process of bench top sample prep and reagent handling into a self-contained and automated device. The system also contains a sophisticated power management system that allows for the briefcase to run off AC power, an internal battery, or 12V automobile power.

An assembled cartridge is filled with a sample, wash and lysing reagents, and master mix and then loaded into the instrument. The small laptop contains original software that is simple to use and designed to facilitate the editing and development of protocols. The combination of syringe and valve travel controls the movement of the fluid in the cartridge. By filtering and applying ultrasonic energy, the sample is captured, cleaned and lysed. The DNA is then eluted to a chamber where the master mix has been loaded. At the conclusion of the protocol, the cartridge is removed from the instrument and the sample is removed from the master mix chamber. The sample can then be loaded into a tube for PCR processing. The cartridge body is composed of disposable valve bodies and gaskets as well as the reusable cartridge body, which is decontaminated for future use.

Verification of the instrument was performed using the BG assay described in the manual. Various concentrations were tested and samples of 10^2 CFUs per 1 mL were detectable. The protocols may be edited or amended for further optimization of the protocol. Prior to the operation of the GeneXpert unit, and the editing and development of the protocols, the operator should become familiar with the GeneXpert unit and thoroughly read all of the safety warnings.

2.0 Safety Issues / Warnings - Read Before Use

As with all lab equipment, it is important that the user familiarize themselves with the GeneXpert unit prior to operation. Care should be taken in the operation and transport of the instrument. The briefcase should only be transported by someone who is comfortable lifting up to 40 lbs. The unit is mobile and can be used in both an outdoor environment and as a bench-top laboratory unit.

BG is a biological agent. Therefore, eye protection, gloves, and protective clothing should be worn at all times. In the event of a rupture to the valve body seal, it is possible that a sample could become aerosolized. Appropriate precautions should be taken when working with dangerous biological materials.

The ultrasonic horn is the most significant danger area for the operator. If a protocol is running while a cartridge is loaded, there is no danger of interacting with the horn. However, if a cartridge is not loaded, and the user is operating the software, the ultrasonic horn could be enabled. Touching the horn could result in a significant burn. NEVER TOUCH THE ULTRASONIC HORN.

The cartridge body is reusable, and should be decontaminated as per the decontamination instructions. However, the valve bodies and gaskets are disposable and designed for onetime use ONLY! The valve bodies and gaskets should be placed in a biohazard bag at the conclusion of each run.

Never over-pressurize the valve body. This can only be done by changing the "Dispense to Home Block" value. The value was set to correctly pressurize the valve body for sonication (170 uL). If the value is changed, it could result in the rupture of the valve body film and the contents of the valve body to be released into the instrument.

The majority of the electrical components are contained inside the bottom of the briefcase. Therefore, any spills should be properly cleaned up immediately. Also, if any items are dropped into the bottom of the briefcase (through openings in the sheet metal), they should be removed prior to use of the instrument.

When the instrument is running off the internal battery, there is a slight possibility of receiving a device time out error. If this occurs

during a run, press the "Unload Cartridge" button and remove the cartridge.

Common sense and good laboratory practices will insure a good working relationship with the GeneXpert system and a long system life.

3.0 System Overview / Description

The GeneXpert briefcase instrument is a bench top computer controlled instrument that is used primarily for automated sample preparation. This instrument is intended to develop protocols that perform functions such as Sample Filtration, Washing, Spore Lysing, and Elution.

The instrument contains the following major components:

Briefcase Enclosure: Provides overall structure, mounts, and protection for all of the system components.

Power Supply System: Supplies power to the electronics, laptop, motors, and ultrasonic horn. Power can be supplied via AC, the internal battery, or a 12V Auto battery.

Cartridge Assembly: Consists of the cartridge body (polished polycarbonate), valve body, gasket, retainer and lid. The cartridge assembly houses all of the reagents as well as the filter processing area.

Fluidic Module: Contains syringe and valve drivers that engage with the cartridge assembly for sample processing.

An overall system picture is shown in Figure 3.1.

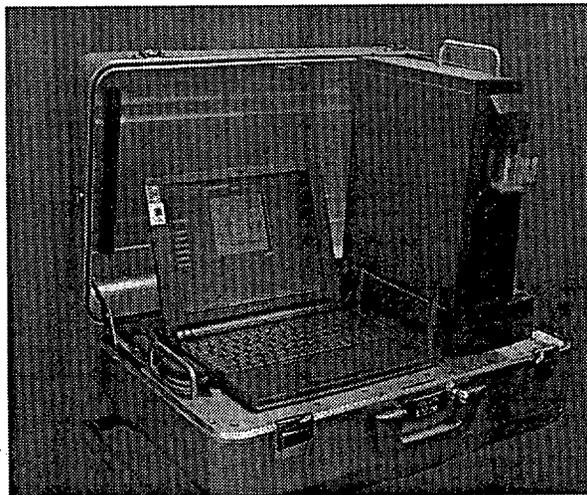


Figure 3.1 The Briefcase GeneXpert System

4.1 GeneXpert Briefcase Enclosure Description

The GeneXpert Enclosure mounts all of the system components for ease of transport and mobility. The top half of the briefcase contains the fluidic module, laptop, zip drive, and power connectors and the on/off switch. Located in the bottom half of the briefcase are the battery, power supplies, control boards, and ultrasonic board.

The briefcase has a handle and wheels for ease in transport. However, the briefcase is used for containment, not protection of the instrument. Dropping the briefcase or rolling it over bumpy surfaces could result in damage to the instrument.

Figure 4.1 Shows a front view of the Briefcase Enclosure with the fluidic module folded down for transport.

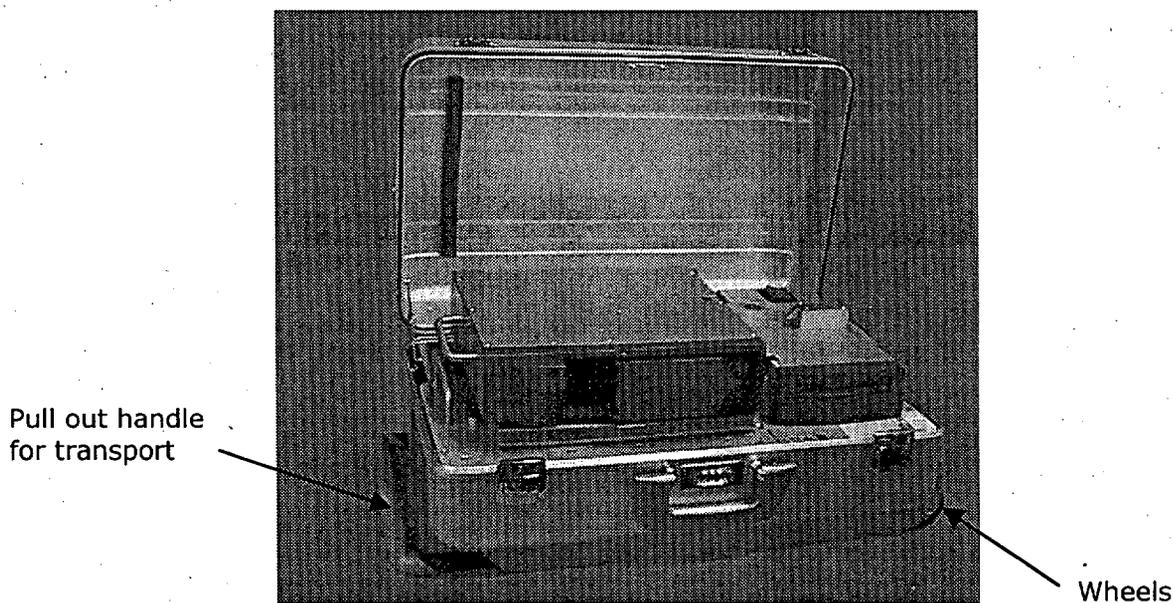


Figure 4.1 Briefcase Enclosure

4.2 Power Supply System Description

The Power Supply System contains the components and control necessary for the unit to run off AC, an internal battery, or a 12V Auto battery. If multiple power sources are connected to the instrument they are used in the following order: AC > auto battery > internal battery. The GeneXpert should be connected to a power source before launching either the deBrief software or the GX_LLNL1.0 software.

When the GeneXpert is connected to AC, that will be the selected power source, and the internal battery will be continuously charged. If the GeneXpert is connected only to an auto battery, the instrument will run off of the auto battery, but the internal battery will not be charged. The laptop runs off its own battery if it isn't connected to the GeneXpert by its power adapter cable. If it is connected to the GeneXpert and deBrief is running, it will select a power source as follows:

- If the GeneXpert is using an AC power source, the laptop will draw power from the GeneXpert's power supply and the laptop battery will be charged.
- If the GeneXpert is using an auto battery, the laptop draws power from the auto battery and the laptop battery will be charged.
- If the GeneXpert is using its own internal battery, the deBrief software will automatically direct the laptop to use either the GeneXpert battery or its own battery, as needed to maximize runtime.
- If deBrief is **not** running, the laptop will draw its power and charge its battery from the GeneXpert's selected power source.

4.3 Cartridge Assembly

The Cartridge Assembly includes the cartridge body, valve body, gasket, retainer and lid. All of the components of the cartridge assembly are depicted in the exploded view illustrated in Figure 4.3.

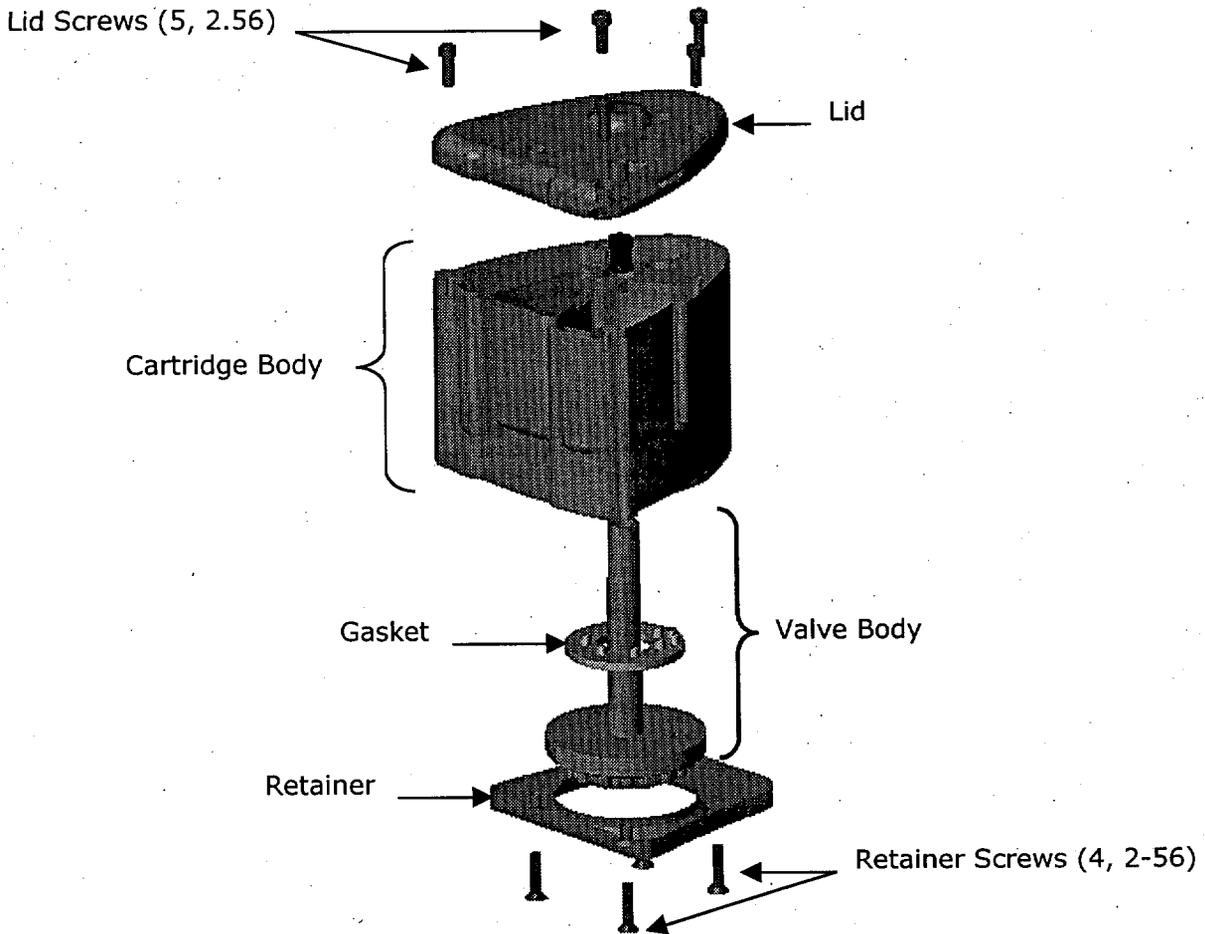


Figure 4.3 – Cartridge Assembly - Exploded View

The following steps explain the assembly process for the cartridge body.

4.3.1 Cartridge Body

- Ensure that cartridge body is completely dry (use Kimwipes to wipe away residual moisture inside chambers)
- Grease outer edge of cartridge body (see Figure 4.3.1) with small amount of silicone grease.

Apply thin coat of silicone grease to the area marked in blue.

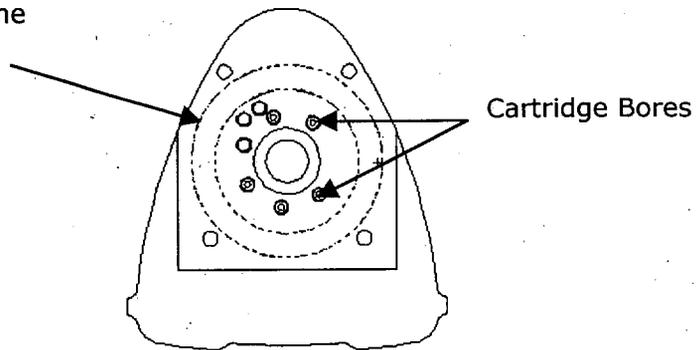


Figure 4.3.1 - Cartridge Body - Bottom View

4.3.2 Gasket

- Lightly wipe the bottom of gasket with silicone grease
- Wipe with Kim-wipe to remove excess grease
- Visually ensure that none of the ports are clogged with grease
- Place in bottom of cartridge body (line up bosses with bores)

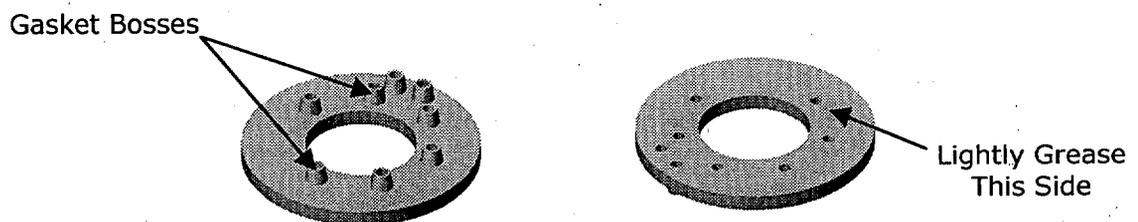


Figure 4.3.2 Gasket (Top view on left, Bottom view on right)

4.3.3 Assembly

- After gasket is in place, insert valve body into cartridge body
- Place the retainer over the valve body
- Begin tightening the four 2-56 stainless steel screws
- Before the retainer is tightened down all the way, line up the valve body inlet with the score mark on the bottom of the shoe (setting to HOME position – see Figure 4.3.3).
- Tighten screws with a torque wrench (set to 12 in.oz.) until the wrench clicks.

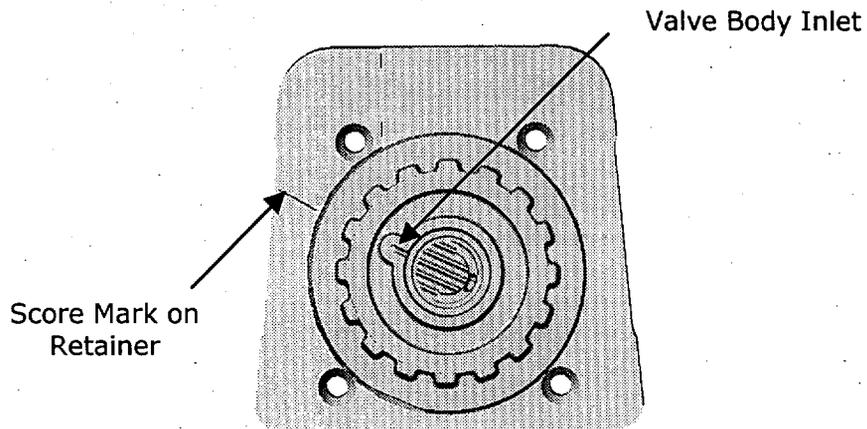


Figure 4.3.3 Setting the Valve Body to the Home Position

4.4 Fluidic Module Description

The Fluidic Module is the instrumentation that drives the valve and syringe and creates fluid movement in the cartridge. The module consists of the following:

- Two stepper motors (one controlling the syringe, and the other controlling the valve)
- An electronic control board
- An ultrasonic horn (lyses spores)
- A sensor that detects the cartridge is loaded properly
- A handle that lifts the valve block to engage with the cartridge
- Cabling to the power supplies and laptop

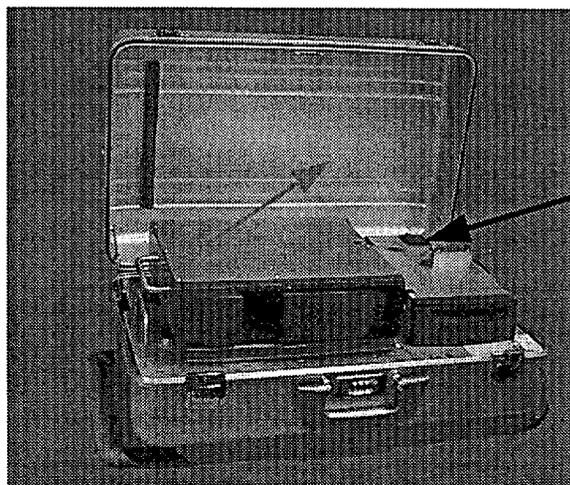
5.0 Operating Instructions

Getting Started

5.0.1 Lift the briefcase lid up and move the fluidics module into the upright position.

5.0.2 Turn on Power to Instrument (red button See Figure 5.0.2).

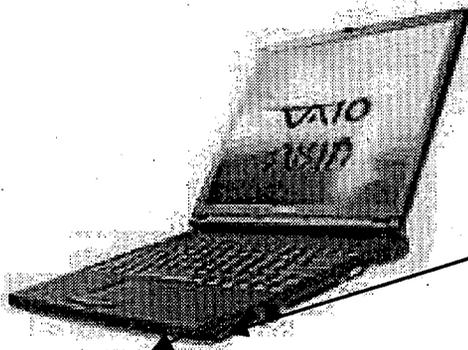
Flip Fluidics Module Up in the direction of the green arrow.



On / Off Button

Figure 5.0.2. On / Off Button

5.0.3 Boot Computer by pulling tab on right side towards user (see Figure 5.0.3).



The on switch is the silver tab located on left hand side of laptop. Pull in the direction of the red arrow.

Figure 5.0.3 Sony Vaio - On Button

5.0.4 Double click the briefcase icon (deBrief) on the desktop (Figure 5.0.4). This opens the power management software (See Section 6.2).

5.0.5 Double click the Icon (GX_LLNL1.0) on the desktop. This opens the GeneXpert software (Figure 5.0.4).

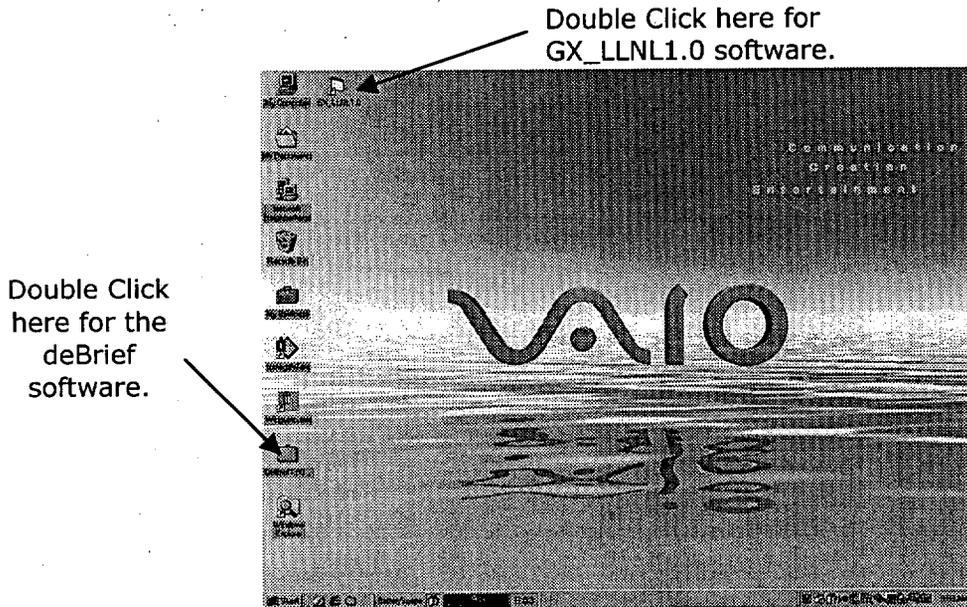


Figure 5.0.4 Sony Vaio – Desktop

5.0.6 Press the **Initialize Device** button (Figure 5.0.6). The syringe will move downward and the valve turns to home.

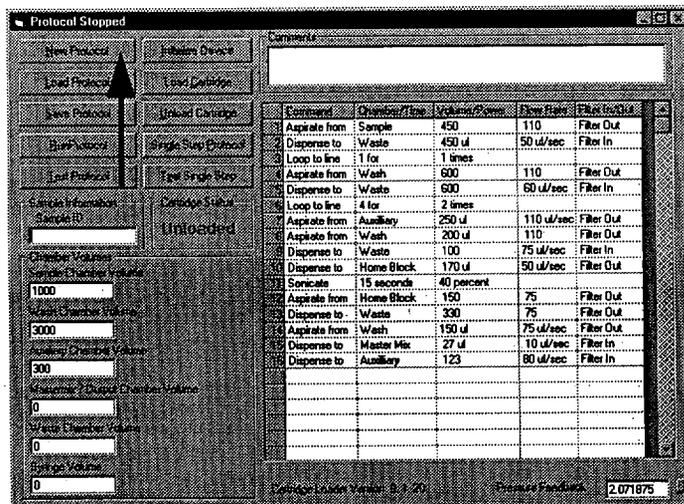


Figure 5.0.6 – Sony Vaio – On Button

5.0.7 Press the **Load Cartridge** button (Figure 5.0.7). The syringe will move back upwards.

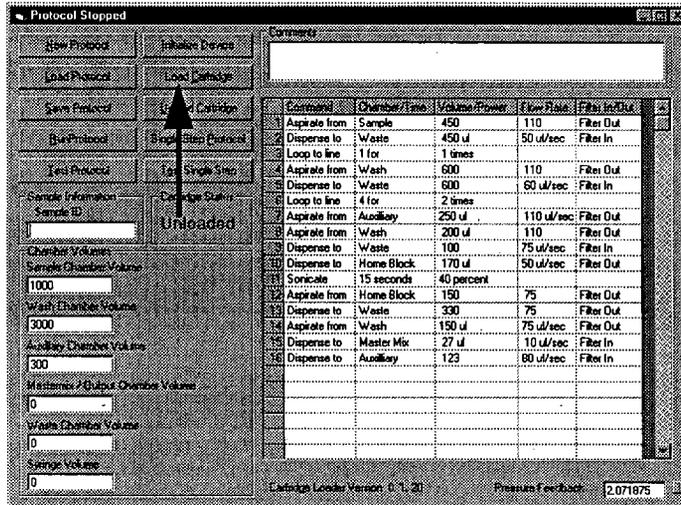
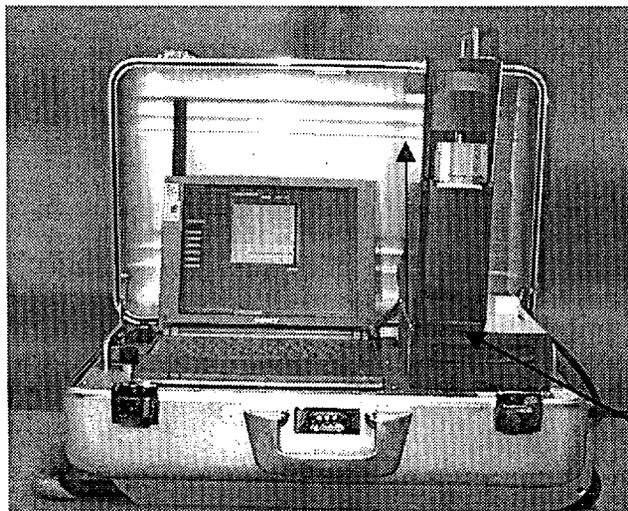


Figure 5.0.7 – GX_LLNL1.0 Software Interface

5.0.8 Insert assembled cartridge (See 4.3 Cartridge Assembly)

5.0.9 Move the handle up to lock the cartridge into place (Figure 5.0.9).



Move the black handle up, in the direction of the red arrow to load the cartridge.

Figure 5.0.9 – GeneXpert - Handle

5.0.10 Press the **Load Cartridge** button again. If the indicator reads "LOADED" (as in Figure 5.0.8 below), then the instrument is prepared to run a protocol. Otherwise, the indicator will read "UNLOADED." Remove the cartridge and check the home position (i.e. that the valve body is aligned with the score mark on the retainer Figure 4.3.3). Replace the cartridge into the instrument and lift the handle. Press the **Load Cartridge** button again. If the indicator still reads "UNLOADED", press the **Initialize Device** button. The syringe will move down into the barrel of the cartridge and the valve will turn a few steps counterclockwise and then clockwise (this valve move will help the instrument to engage the cartridge in the case that the home position is slightly off). A snap sound may be heard, indicating that the instrument has now engaged the cartridge. To verify, press the **Load Cartridge** button to see the indicator change to "LOADED."

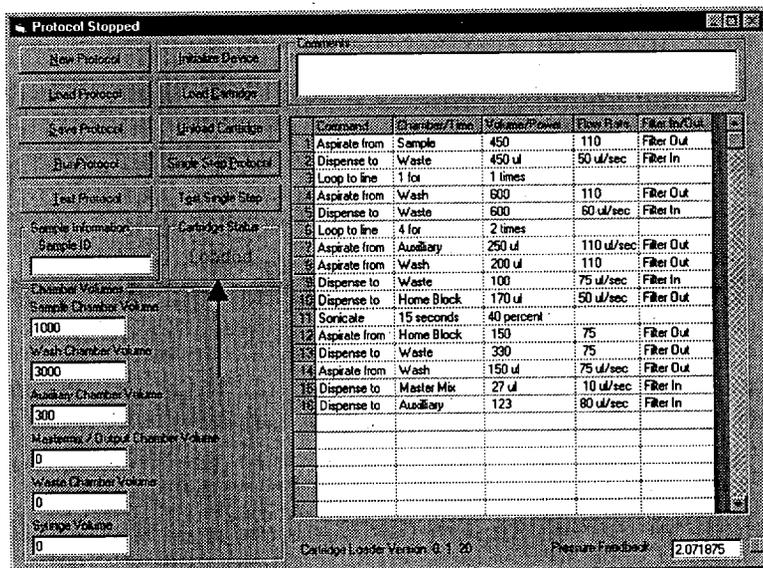


Figure 5.0.10 – GX_LLNL1.0 Software – Cartridge Loaded

5.0.11 Once the cartridge is loaded, the instrument is ready to run a protocol.

5.0.12 Press either the **New Protocol** button, to write a new protocol, or the **Load Protocol** button, to run an existing protocol (e.g. LLNL_1mLSample). If the cartridge is not loaded properly (i.e. UNLOADED), the protocol will not execute.

5.0.13 After the protocol is complete, press the **Unload Cartridge** button. This button vents to waste, strips the plunger off of the syringe driver, pushes the plunger to the bottom of the valve body, and then returns the valve to the home position.

5.0.14 Move the handle down and remove the cartridge body from the instrument.

5.1 Running Protocols

There is a template protocol written that processes 1 mL of sample, a 2 mL wash, sonicates, and elutes 25 uL to the master mix chamber. The protocol can be used for training purposes, as well as for running a 1 mL sample.

- 5.1.1 The protocols are saved in a folder labeled Protocol Library, that can be found on the Desktop as well as in the "My Documents" folder on the C: drive.
- 5.1.2 Click on the Load Protocol button and select a file from the Protocol Library (e.g. LLNL_1mLSample).
- 5.1.3 Click OPEN and the file is loaded.
- 5.1.4 The protocols will be displayed as lines of text. Also, the chamber volumes will be entered.
- 5.1.5 Press the Run Protocol button to begin the protocol. If at anytime you wish to stop the protocol, click anywhere on the screen and then press the unload button.

NOTE: DO NOT click on the screen while a protocol is running unless you wish to stop the protocol.

6.0 Software

There are two software packages loaded on the briefcase. The first is the Cepheid software that controls the fluidic module. The second is the deBrief software that monitors power management.

6.1 GX_LLNL1.0 Software

The user interface can be divided into three main sections, including the 10 user buttons, chamber volumes and the text editor (see Figure 5.2). The 10 user buttons are defined below:

- New Protocol – Clears the Text Editor for a new protocol.
- Initialize Device – Moves the syringe down to home position, and turns the valve to home position
- Load Protocol – Loads a previously written protocol into the Text Editor
- Load Cartridge – Moves the syringe all the way up so that a cartridge can be loaded into the instrument
- Save Protocol – Saves a new protocol or saves changes to an edited protocol
- Unload Cartridge – At the conclusion of a protocol, the valve is vented to waste, the syringe is raised to strip the plunger, the plunger is pushed to the bottom of the valve body and then the valve is re-homed
- Run Protocol – Executes the protocol listed in the Text Editor (cartridge state must be “Loaded”)
- Single Step Protocol – Executes the protocol listed in the Text Editor one step at a time
- Test Protocol - Runs through all of the steps of the protocol in the text editor without actually moving the valve or the syringe (useful in verifying new or edited protocols, prior to execution)

- Test Single Step – Steps through all of the steps of the protocol in the text editor without actually moving the valve or the syringe

The chamber volumes (see Figure 5.2) track the volumes of fluids that are in the cartridge. Before loading the cartridge, ensure that the volumes loaded in the cartridge chambers, correlate to the chamber volumes entered (e.g. according to the chamber volumes in Figure 5.2, the cartridge should be loaded with 1 mL of sample, 2 mL of wash, etc). If the values do not correlate, the software sends an error message and halts the protocol.

The text editor is where the protocol is displayed. The protocol can be edited directly in the text editor as described in section 5.2. If the protocol does not require editing, pressing the Run Protocol button will begin executing the protocol. The instrument will proceed sequentially through the commands and highlight the current step.

6.2 deBrief Software

When the user double-clicks on the deBrief briefcase icon (see Figure 6.2), the primary window, or the Overview window opens. By clicking on the laptop or briefcase A icons, more details ensue.

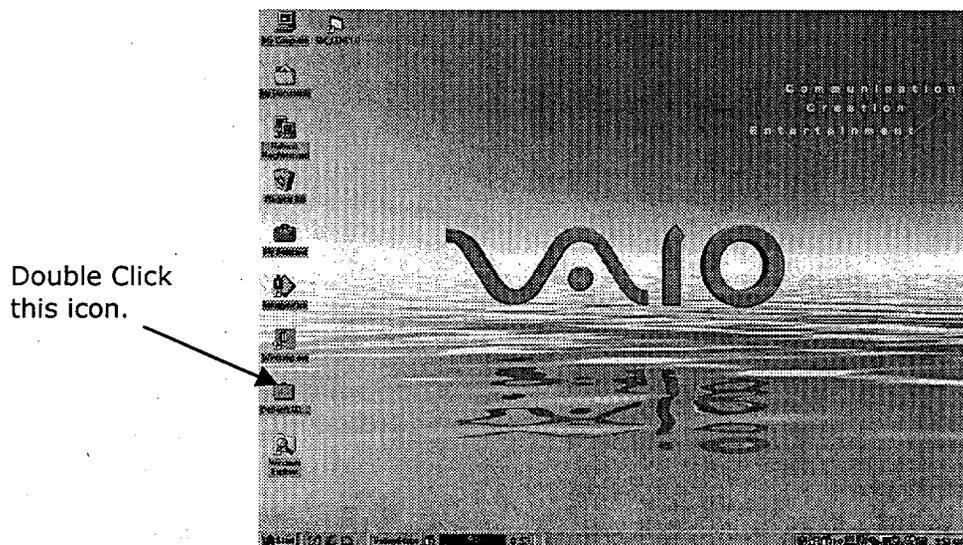
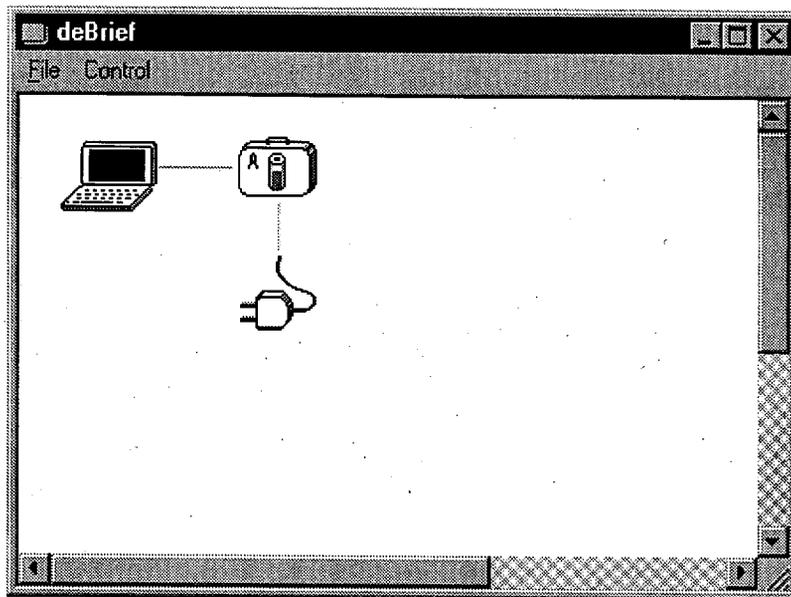


Figure 6.2 – Sony Vaio – Desktop

Overview

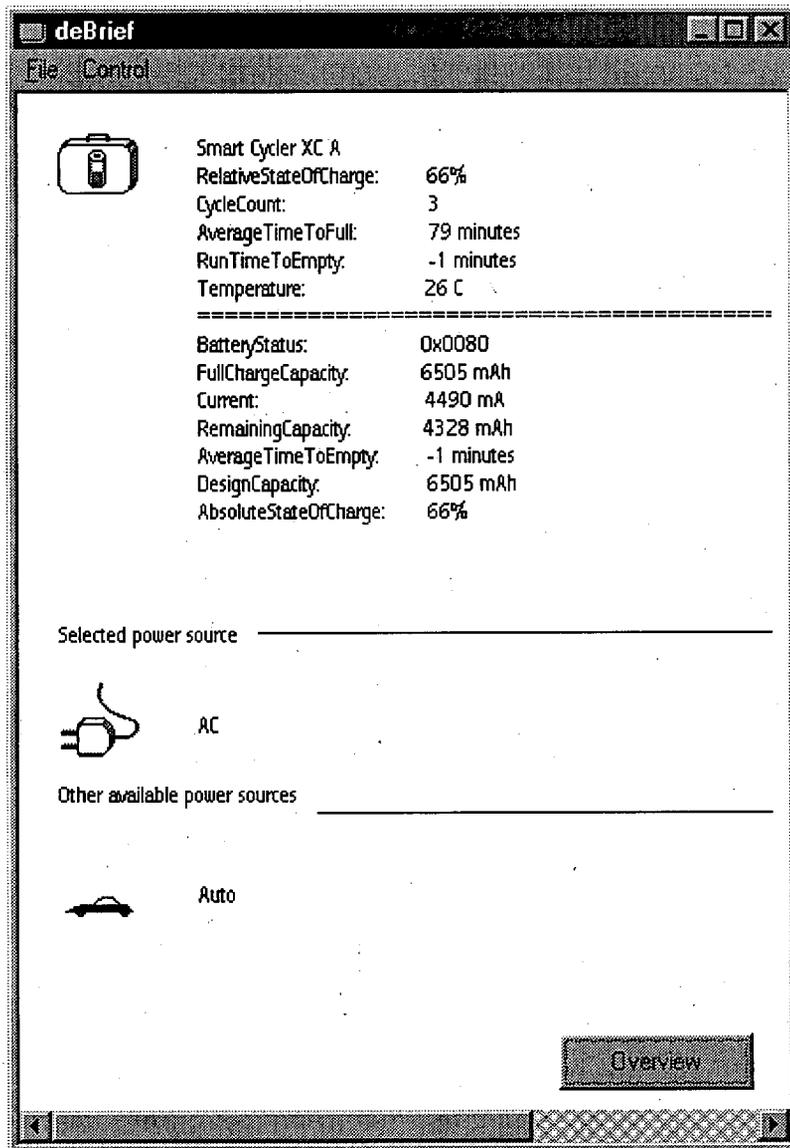
This window gives a general picture of the system. This window shows an icon of the laptop with the attached GeneXpert system shown as a briefcase. Below the briefcase, an icon shows the power selected for the GeneXpert unit. In each briefcase a small battery shows the approximate percent charge of the internal battery.



This Overview window shows that there is one GeneXpert unit attached to the system. The internal battery in this system is approximately 2/3 full and the selected power source is AC line power. When the briefcase icon is clicked, the detail window is opened to show detailed information about this device module. When the laptop icon is clicked, the laptopdetail window is opened to show power information about the laptop.

Detail Window

The Detail window shows internal battery information as well as selected and available power sources.



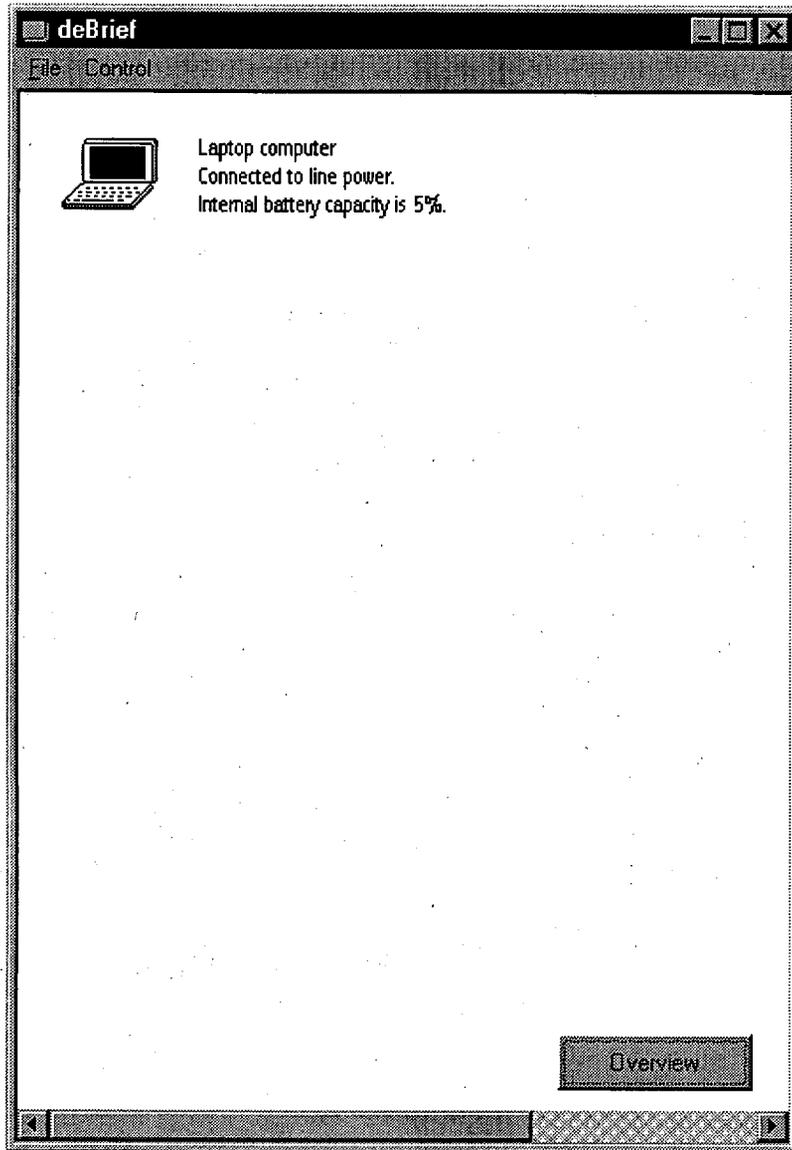
The detail window displays detailed information on the status of the internal battery and the other available power sources. The items listed above the dashed double line are used to monitor power use by the system. The items below the dashed double line provide information used by Technical Support when troubleshooting power management problems.

- RelativeStateOfCharge — a measure of battery charge, based on a dynamically calculated full battery capacity value.

- CycleCount — the number of times the internal battery has been recharged. The battery can be recharged approximately 500 times before it will need replacing.
- AverageTimeToFull — the number of minutes it will take to fully charge the battery. It will be set to "-1 " when the battery is in use.
- RunTimeToEmpty — the number of minutes it will take to completely empty the battery based on the average power consumption when running. It will be set to "-1 " when the battery is charging.
- Temperature — the temperature of the internal battery.

Laptopdetail Window

This window gives detailed information about the laptop. The Laptopdetail Window lists the percent charge of the laptop battery and indicates whether the laptop is connected to line power. When the laptop is connected to line power, this means that the Power Module has enabled power to the laptop.



The software periodically checks status to see if pop-up warning windows should be displayed in the deBrief GUI. The following warning windows are displayed to indicate system device or battery problems:

Bad Battery – the internal battery is returning invalid battery data.

Low Battery – the internal battery has a capacity lower than 10%.

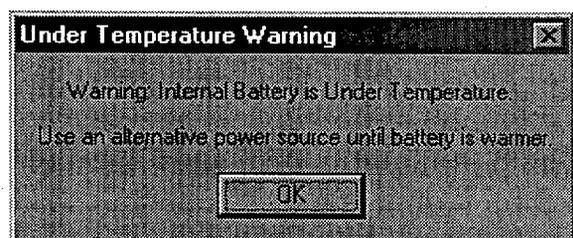
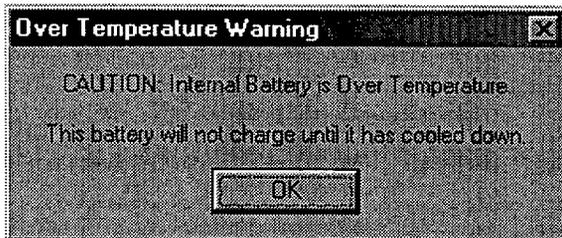
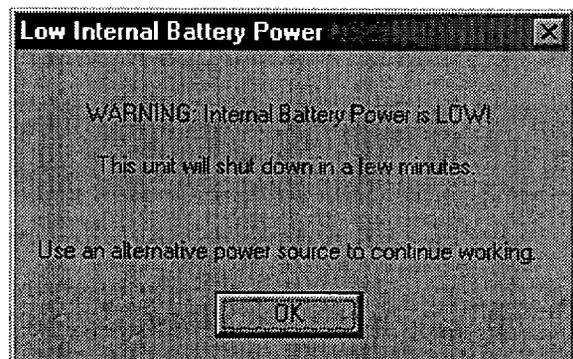
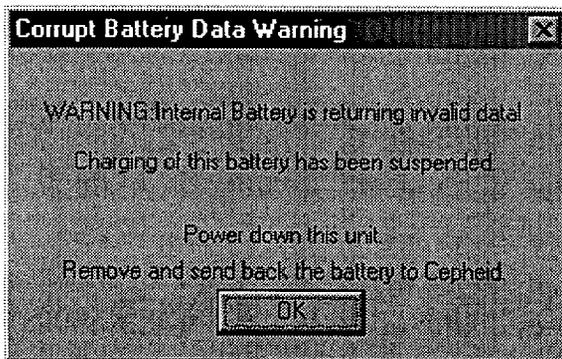
Battery Under Temperature – the internal battery temperature is below 15° C.

Battery Over Temperature – the internal battery Over Temperature flag is set.

Device Blown Mosfet – the system BlownMosfet flag is set.

Device Over Temperature Fault – the system TempFault Flag is set.

Below are a few examples of the pop-up warning windows:



7.0 Technical Support

There are no user-serviceable components inside the GeneXpert unit. Should servicing become necessary, please contact Cepheid.

For technical support, please contact Cepheid at:

Cepheid
1190 Borregas Avenue
Sunnyvale, CA 94089-1302
Phone: (408) 541 – 4191
Fax: (408) 541 – 4192

The technical point-of-contact is:

Phil Belgrader
Phone Extension: 345
Email: belgrader@cepheid.com

The secondary point-of-contact is:

Allen Northrup
Phone Extension: 227
Email: northrup@cepheid.com

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Although the GeneXpert is packaged in a sturdy briefcase, it has not been shock tested and abuse handling could result in damage. Should it become necessary to ship the unit to Cepheid the unit should be carefully packaged to minimize jostling.

8.0 Current BG Protocol

Reagents:

3 mL DEPC water

1 mL of 10^2 CFUs in water (or other concentration)

25 uL of Master Mix

8.0.1 Assemble cartridge as per Cartridge Assembly instructions (Section 4.3).

8.0.2 Fill Wash Chamber with DEPC water (see Figure 8.0).

8.0.3 Vortex the sample and then place 1 mL into the Sample chamber (Figure 8.0).

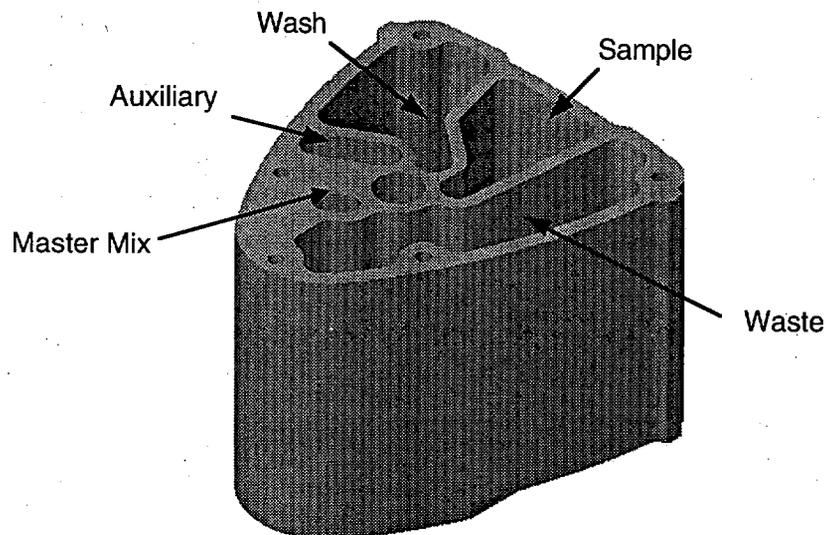


Figure 8.0. Cartridge Body

8.0.4 Pipette 25 uL of master mix into the master mix chamber (see Figure 8.0).

8.0.5 Load cartridge into instrument and run the protocol labeled "LLNL_1mL sample" (C:/MyDocuments/).

8.0.6 After the cartridge has been processed, remove it from the instrument.

8.0.7 Remove the sample from the master mix chamber.

8.0.8 Place sample into a tube and thermal cycle.

8.0.9 As a control, 12.5 uL of the lysate (pipetted out from the Auxiliary Chamber) can be mixed with 12.5 uL offline and also loaded into an Icore tube.

- 8.0.10 The remainder of the fluid left in the cartridge should be discarded in a sink.
- 8.0.11 Disassemble the cartridge body and clean according to the decontamination protocol.

8.1 Decontamination

- 8.1.1 Disassemble the cartridge by first removing all four screws from the retainer.
- 8.1.2 Put the retainer aside and remove the valve body and gasket. Throw them both into the appropriate biohazard receptacle.
- 8.1.3 Place the cartridge body in a fresh 10% bleach solution and allow to soak for 10 minutes. Ensure that there are no air bubbles and all of the chambers fill with the bleach solution.
- 8.1.4 Remove the cartridge(s) from the bleach and rinse with DI water.
- 8.1.5 Allow the cartridges to soak in DI water for 15 minutes.
- 8.1.6 Rinse for another minute and then place the clean cartridges on a clean surface to dry.



December 17, 1999

TO: Distribution

FROM: Ron Koopman *RK*

SUBJECT: Cepheid Sample Preparation Module Design Review, December 9, 1999

Based on accelerated progress by Cepheid, an early design review was held at their facility in Sunnyvale. They expect to accelerate delivery of their sample preparation unit, the Briefcase Lysis System, to March, 2000. A copy of their presentation material is attached, including detailed design requirements information.

The core of this development project continues to be a modification of Cepheid's commercial cartridge system, but with reduced weight (<30lb.) and briefcase size. They showed us a model of the unit with a laptop computer in a commercial aluminum briefcase-size container.

The cartridge appears to be a variant of their commercial cartridge and is designed to interface with the SmartCycler. The ten cartridges to be produced under this contract are all precision machined and cost approximately \$100 each. It is intended that in the next phase of the contract, when large numbers of the cartridges are anticipated, the cartridges would be injection molded at much less cost and would be disposable. The central piece of the current cartridge system, containing the sample cell with beads, filter, sonication membrane, and the syringe pump body, is disposable, with a cost of about \$12 each. The cartridge is designed to accommodate a raw sample (spores in water) of 0.5 to 5 ml and produce a lysate sample of 5-20 microliter for mixing with 5-20 microliter of PCR reagents. Wash reagent volume is 10x lysis chamber volume. PCR product output is 25 microliters with a maximum of 10 microliter residual. The current cartridge will accommodate lyophilized reagents but cannot be preloaded with liquid reagents for fly-away lab applications because of leakage. The injection molded cartridges could be leak tight, but will not be available on this contract.

Results were shown for 1000 spores/rxn B.g. with 10 sec lysis using sonication with detection in 10 minutes. Detection was also demonstrated for 100 B.g. spores/ml washed with 3 ml water through a 1/4 inch filter in a 100 microliter chamber, sonicated for 30 sec and eluted with 0.4 ml water. The 100 microliter chamber will be adopted for this design. Results were also shown to demonstrate the ability to remove PCR inhibitors with the system.

The protocol for spores, using the cartridge system is:

1. Inject 0.5-5 ml B.g. spores at 10^2 - 10^5 /ml. Spores will be trapped by filter.
2. Inject 1-5 ml water to remove untrapped particles. Spores retained.
3. Sonicate for 10-30 sec at about 10 watts. Spores lysed.
4. Inject 0.1-0.5 ml water. Collect filtrate. DNA passes through filter.
5. For 25 microliter reaction volume, mix 5-20 microliter filtrate with 5-20 microliter PCR reagent.



Dist: Lt. Col. Holly Franz, CMO
Scott Smith, CMO
Kelly Weirick
Robin Miles
Bill Benett wo attachment
Rich Langlois wo attachment
Paula McCready wo attachment
Fred Milanovich wo attachment
Phil Belgrader, Cepheid wo attachment
Allen Northrup, Cepheid wo attachment
Farzad Pourahmadi, Cepheid wo attachment



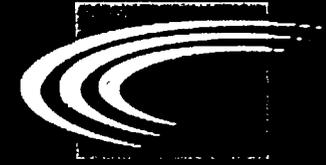
Spore Lysis Cartridge Project Review

December 9, 1999

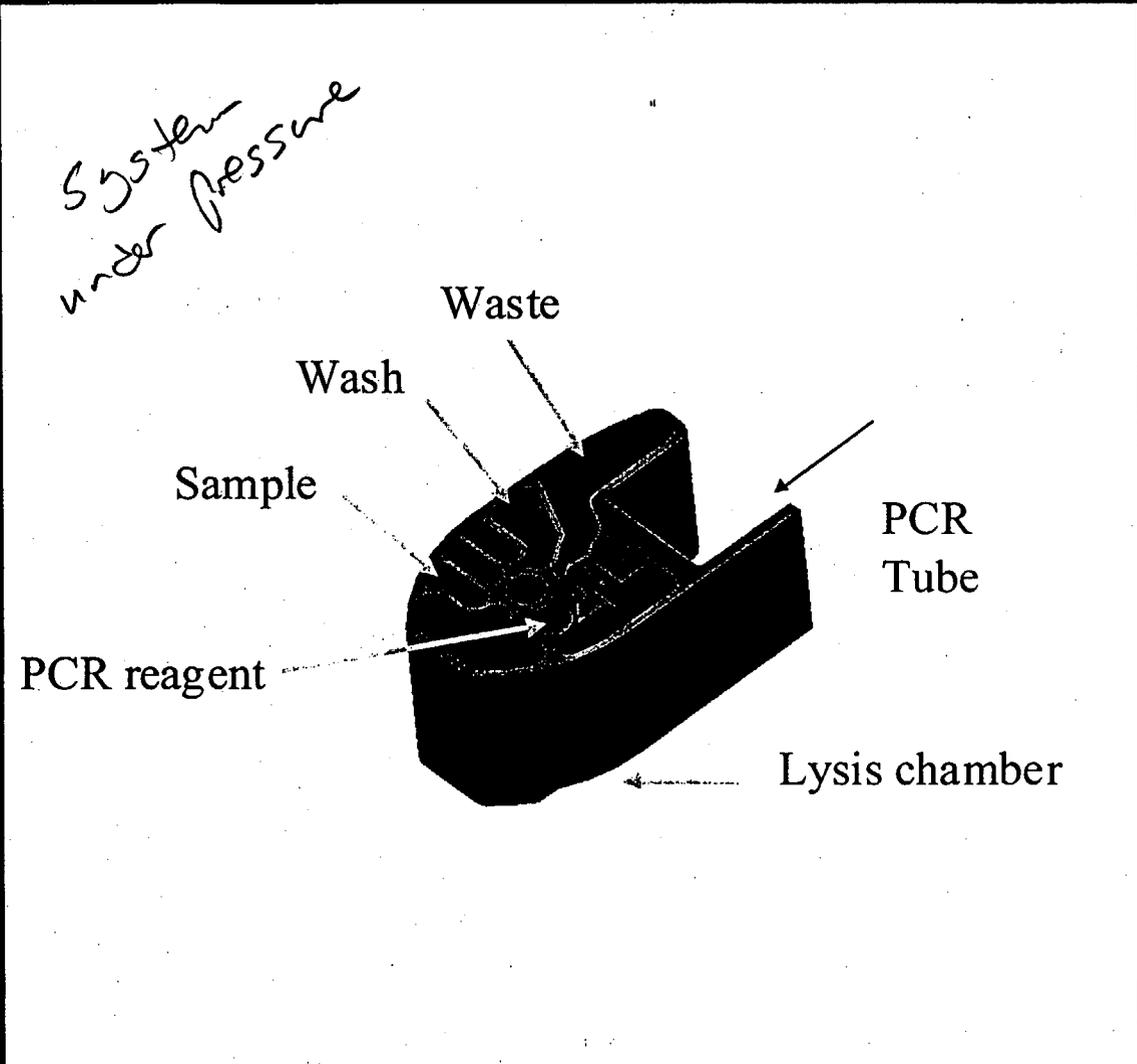
Phil Belgrader, Farzad Pourahmadi, and
Allen Northrup

Cepheid

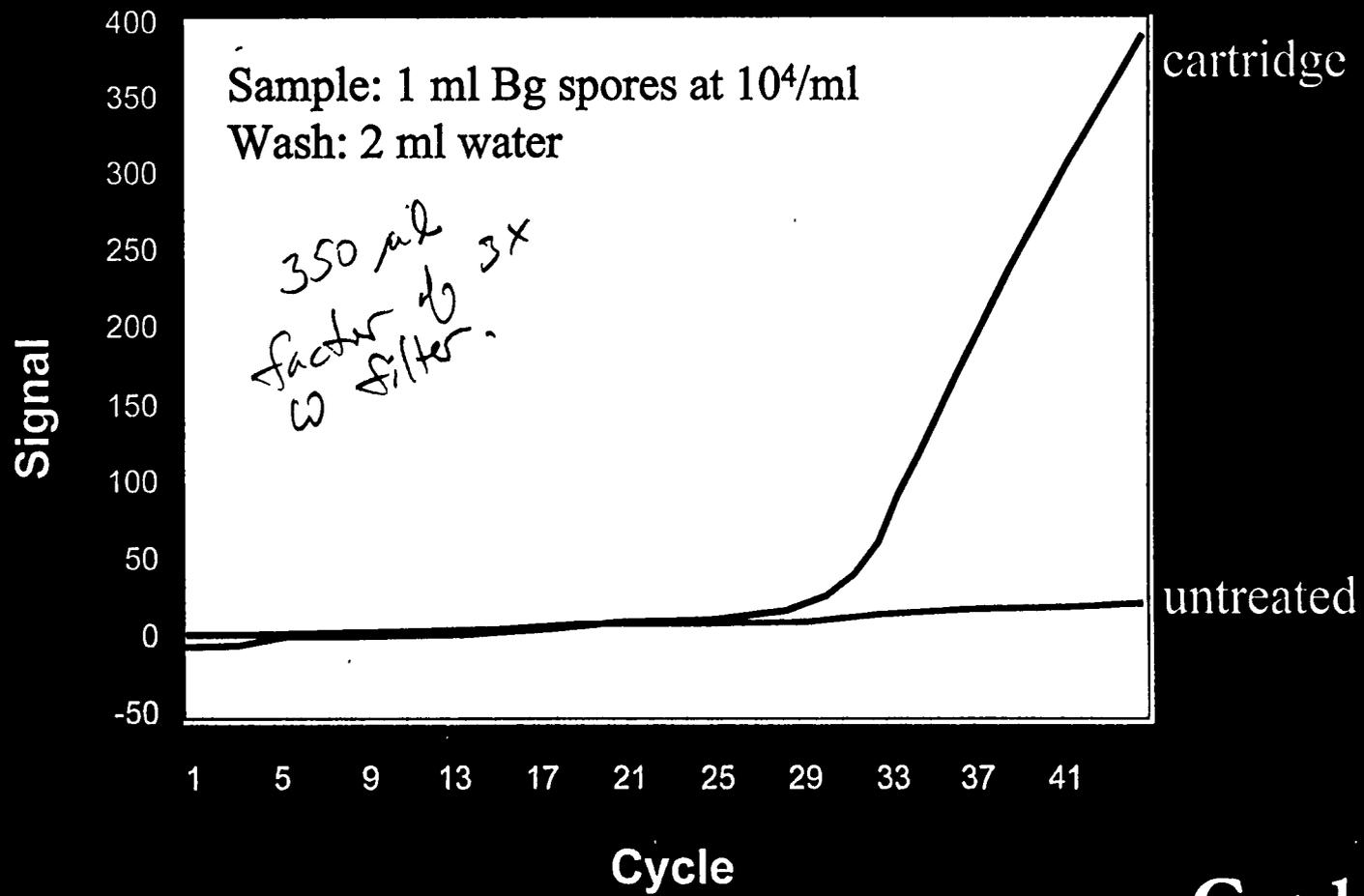
The cartridge concept for automatic spore processing



- Sample is added to the cartridge
- Spores are collected and concentrated in the lysis chamber
- The trapped spores are washed with water to remove potential inhibitors
- The spores are lysed by sonication
- DNA in the lysate is collected in an empty chamber
- A portion of the DNA is mixed with PCR reagent



SmartCycler analysis of Bg spores automatically processed on the cartridge system

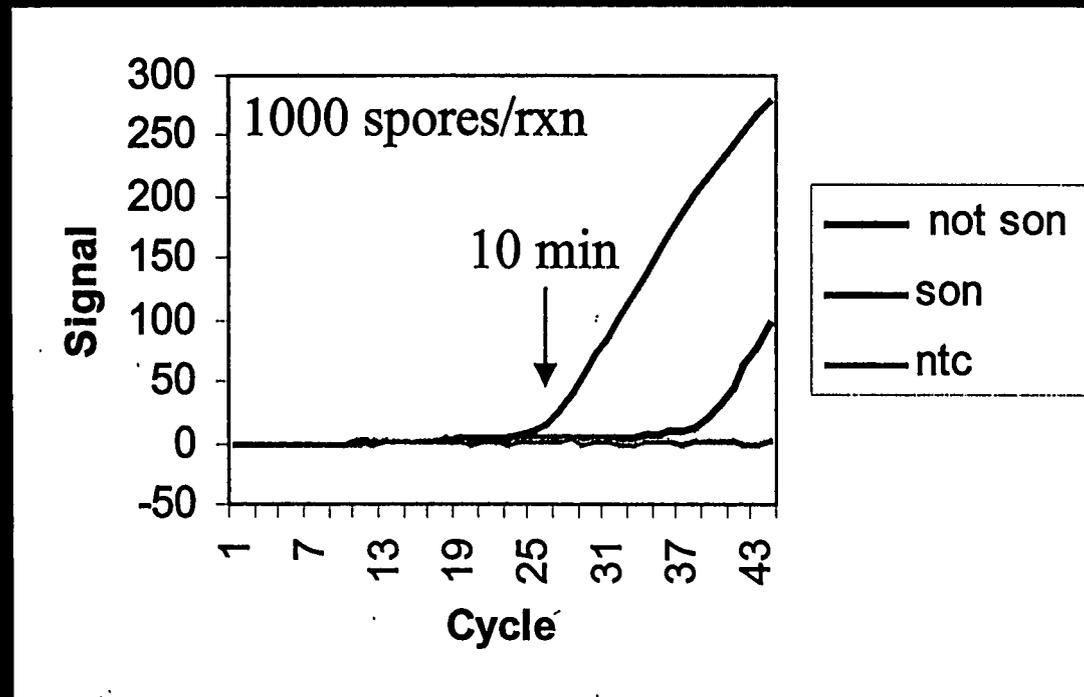


Cepheid



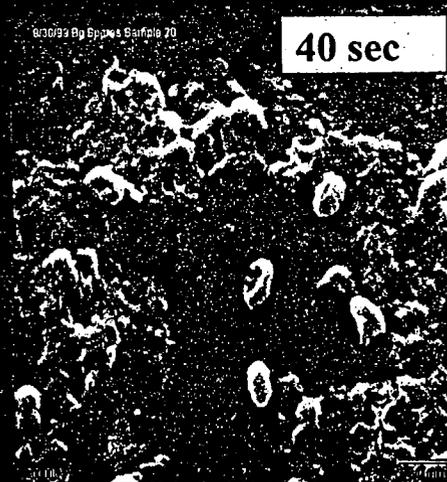
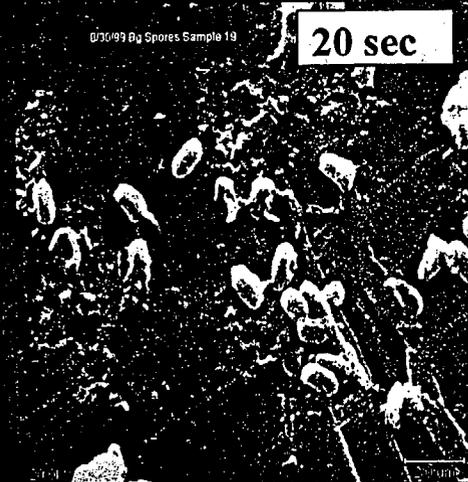
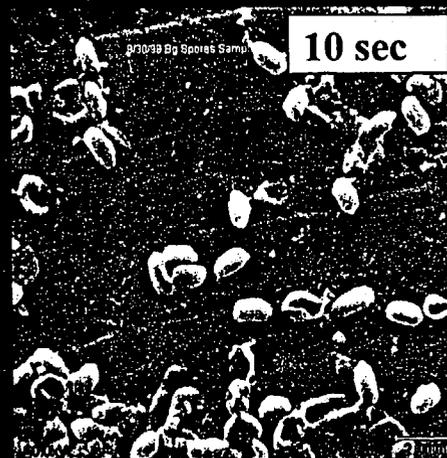
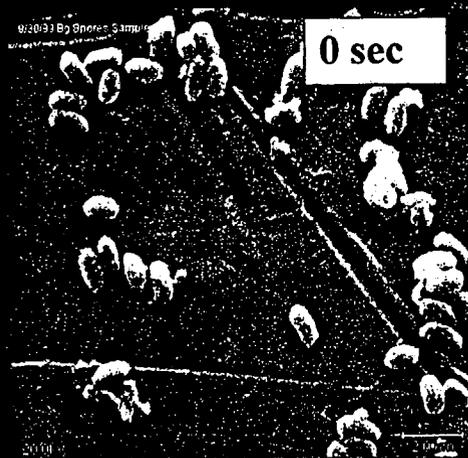
Rapid Lysis of Bg Spores Using the Ultrasonic Tube Fixture

Lysis in 10 sec using sonication
Real-time detection on the SmartCycler



Cepheid

Scanning electron micrographs of sonicated Bg spores



3 watts

SEM performed by Peter Scholl and Brent Baragon
Johns Hopkins Applied Physics Lab

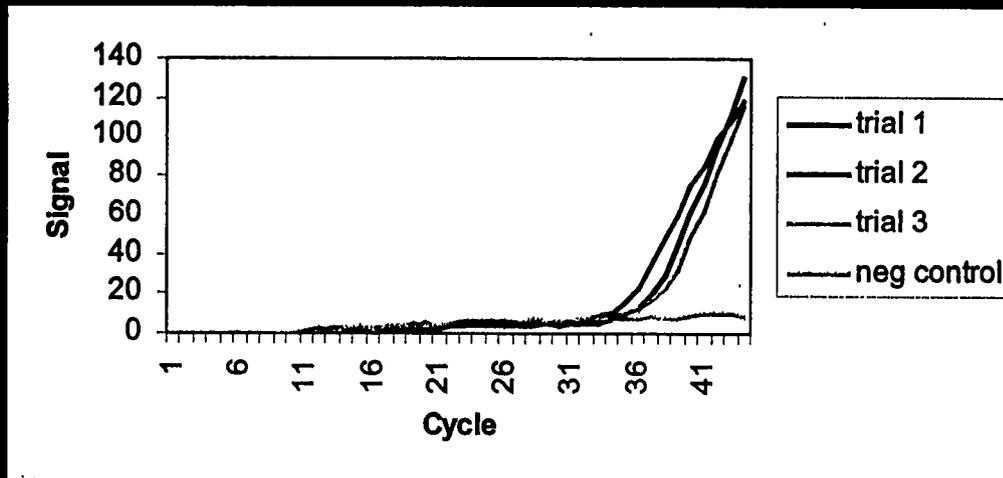
Cepheid

Will go w this 100 µl chamber



Tests using a fixture with a 1/4 " filter

1. 1 ml 1e2 Bg spores/ml
2. Washed with 3 ml water
3. Sonicated for 30 sec at 10 w
4. Eluted with 0.4 ml water

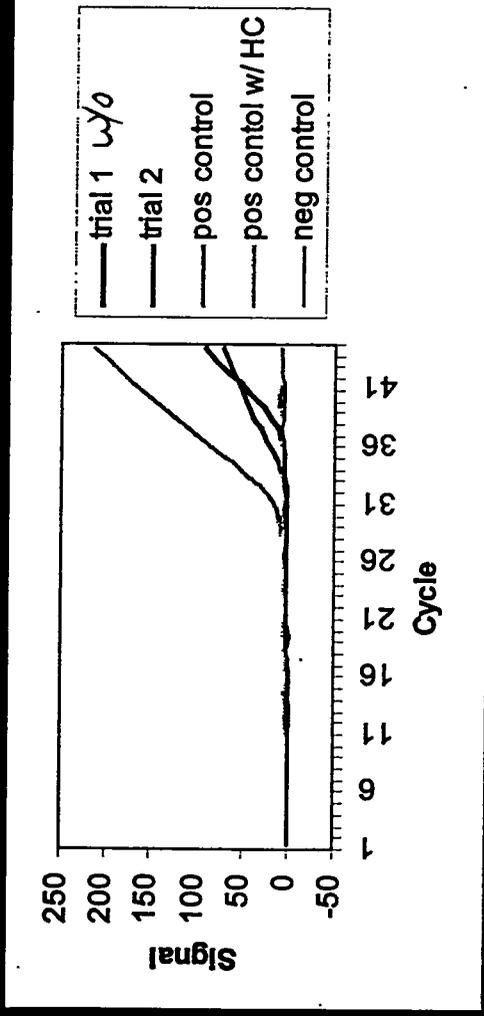


Cepheid

Testing the ability of the filter to remove PCR inhibitors



1. 1 ml 1×10^3 Bg spores/ml with
or without 0.003% hemin chloride
2. Washed with 4 ml water
3. Sonicated for 30 sec at 10 w.
4. Eluted with 0.4 ml of water



Cepheid

Pipette out



Protocol for spores on the cartridge

1. Inject 0.5-5 ml BG spores at 10^2 - 10^5 /ml.
[Spores should be trapped in the chamber.]
2. Inject 1-5 ml of water to remove untrapped particles.
[Spores should be retained]
3. Sonicate for 10-30 sec at about 10 watts.
[Spores should be lysed]
4. Inject 0.1-0.5 ml of water. Collect and save the filtrate.
[DNA should pass through filter]
5. For 25 ul reaction: Mix 5-20 ul of filtrate with 5-20 ul of PCR reagent.

Cepheid

1	A	B	C	D	E	F	G	H
2		Cartridge Technology Platform	Instrument will be universal for all cartridges. Design will be modular				Shared functionality should exist in a common base unit. Expensive components made common. Modules will be plug in replaceable.	
3								
4			Cartridges can be filled with dry reagents				Mixing and reconstitution possible within cartridge	
5			Cartridges can be filled with wet reagents					
6			Some sample processing may be required prior to introduction into cartridge					
7								
8								
9								
10								
11								
12		Product Concept: The Briefcase Lysis System is an instrument that integrates ultrasonic-based lysis, reagent mixing and aliquoting for nucleic acid amplification in a portable format. It requires a PC to drive setup, execution and handle data output.						
13		Intended Use: The Briefcase Lysis System will be used for conducting spore lysis, and reagent manipulation for nucleic acid amplification methods in field environments for LLNL.						

Briefcase size system

Design Input Requirements

	A	B	C	D	E	F	G	H
14	ATTRIBUTE		TARGET	MARKET		RANK	ERS	
15				Test				
16	A Physical Characteristics							
17	A-1	Configuration	1-site unit including computer completely self contained in briefcase.	a		1	Same	
18	A-2	Size and Weight	Unit size and weight will allow carry-on transportation on commercial airlines. Unit does not need to be ruggedized.			1	No bigger than 21" x 14" x 9" System less than 30lbs including case, laptop computer and batteries.	
19								
20	A-3	Footprint	Needs to be one person transportable	a		1	Same as size and weight	
21				a		2		
22	A-4	Impact of Spills	Performance not degraded under normal usage conditions, including leakage of cartridge fluids.	a		2	No impact to mechanical components. No safety danger due to spill. Easily decontaminated without disassembly.	module resistant to spills, performance not degraded under normal usage conditions. Mechanical parts resistant to spills and will be designed to contain any leakage into interior of instrument for decontamination. Cartridge is user replaceable. Electronics will be separate from fluid path. Anything in contact with fluids will be resistant.
23	A-5	Transportability	Easily transportable by one person without the need for an external cart.	a		3	Integrated wheels and extension handle for one person transport	
24	A-6	Human Factors	System will be capable of set up, operation, testing and tear down while in Mission Oriented Protective Posture (MOPP) level IV Nuclear Biological (NBC) protection gear.	b		2	Nothing which tears gloves. No restricted vision. Big buttons.	
25	B Computer Hardware							
26	B-1	Operating System	PC with Windows 98. Operates with Windows 2000.	b		1	See the Smart Cycler DIR (Document #D0169)	
27	B-2	Networking	Must have network and modem connectivity to download data and software.	b		1	Include combined network/modem card	
28	B-3	Mass Storage	Must have 100 mB ZIP Drive				Include ZIP drive	
29								
30	C Internal Battery		Battery gauge on PC	a		2	Use briefcase UPS	
31								
32	C-1	Battery Life	6-10 runs	c		1	Use briefcase UPS	

need to avoid cross contamination cartridges each pre loaded with own reagents

1 - comment
2 - could change
- variable
Cepheid Confidential

Design Input Requirements

	A	B	C	D	E	F	G	H
33	C-2	Recharge Time Desired	Recharge within 3 hours	a		3	Use briefcase UPS	
34	C-3	Recharge Time Acceptable	Overnight rechargeable	a		1	Use briefcase UPS	
35	C-4	Battery Service	Replacable by user				Use briefcase UPS	

Design Input Requirements

	A	B	C	D	E	F	G	H
36	D Power System							
37	D-1	Power Sources	System accepts power from International AC, internal rechargeable batteries.	a		1	Complies with std intl power spec	
38	D-2		System accepts power from external battery pack, and automobile power.			2	Use briefcase UPS	
39	D-3		Uninterruptable Power Supply (UPS) interface to prioritize power source usage and automatically switch between available sources.	b		2	Use briefcase UPS	
40	D-4		All connectors will be keyed to prevent users from plugging into the wrong location.	a		1	Use briefcase UPS	
41								
42								
43	E System Performance							
44								
45	E-1	Cartridge Performance						
46	E-1-1	Sample to Sample carryover	No detectable sample-to-sample cross contamination in cartridge or platform			1	After five runs at maximum input concentration, a negative control will not trigger false positive	
47	E-1-2	Sample labelling	Must be able to be labelled by user			1	Cartridge will have flat surface area 0.5 by 1.0 cm	
48	E-1-3	Cartridge Use and operation	User should be able to easily insert and remove cartridge in 2 steps or less.			1	Insertion mechanism must engage valve, syringe, and horn in one step	
49								
50	E-1-5	PCR reaction volume	minimum of 25ul.			1		
51	E-1-6	Fluid System - Cartridge	1-time use (disposable),			1	5-8 chambers, pre-filtering capability, must contain and retain beads in lysis chamber, no external nor internal leaks, biocompatible material for PCR, must be injection moldable with minimal assembly or testing required, and must be low cost.	

disposable cartridge ing
 low cost is
 < \$500
 10 cartridge body,
 machined \$100 ea
 central piece molded
 & disposable \$12

Design Input Requirements

	A	B	C	D	E	F	G	H
52	E-2	Cartridge						
53	E-2-1	Sample Size (LLNL)	Raw sample (spores in water) input of 0.5-5 mL.				A single cartridge chamber design must accommodate the range 0.5-5.0 mL input volume.	
54	E-2-2	Time to Result	Protocol can be processed to completion within 5 minutes. A test report will be generated in less than 2 minutes.	c		2		
55	E-2-3	Sensitivity	100 CFUs/mL water sample			1	99% confidence in assay Sx implies x capture efficiency	
56	E-2-4	Reagent Usage	25 ul Master Mix LLNL 1-4 ml wash solution			1	5-20 microliter lysate sample mixing with 5-20 microliter PCR reagents. Wash reagents volume is 10X lysis chamber volume. Target (PCR product) output is 25 microliters with a max. of 10 microliter residual	
57	E-2-5	Sample Archive	Pre-PCR sample must be archivable for 1 week at -20C.			1	Sample processed at lowest input concentration will yield positive PCR result after 1 week storage at -20C with 99% confidence.	
58	E-2-6	Sample Archive	Pre-PCR sample must be archivable for 1 year at -20C.			3		
59	E-2-7	Sample Archive	Stackable for storage			1	Cartridges must stack 4 high	
60	E-2-8	Cartridge reagent carryover	Acceptable level of cross contamination for specific target assay			1	Covered in general section	
61								
62	G	Software						
63	G-1	Platform	PC 98 compliant					
64								
65	G-2	Lysis Cartridge specific GUI	Must be able to program protocols, including fluid handling, US horn control, and timing Functions: Rotate RV to any position Aspirate fluids of differing volumes in any order Control Horn (time/power) Program delay times	c		1		

Visual Basic or C

Design Input Requirements

	A	B	C	D	E	F	G	H
66	G-3	Sample Prep GUI	User friendly command structure related to desired actions, e.g. Set flow rate = x uL/s, Set path = through filter, Move x uL from Sample to Waste. Move x uL wash to waste. Horn power = x W, Horn ON, Horn OFF, Delay xx msec.				GUI will translate user level commands to standard Kloehe command sets related to cartridge design.	Or do we accept Kloehe command set as is.
67	G-4	Protocol Storage	Must be able to store protocols				1 Allow storage and recall of sample prep protocols up to 200 steps long.	
68	G-5	Process Monitoring	GUI should give feedback to user on status of protocol execution including error reporting				1 GUI will highlight protocol step in progress. System will display syringe and valve positions. 1 System will sense overpressure condition on syringe and warn user. System will read horn power and display to user.	
69	G-6	Process Logging	User has access to programmed and actual values after run is completed.				2 A log file will be generated containing all commands and actual values, plus any error conditions that occur.	

Design Input Requirements

	A	B	C	D	E	F	G	H
70	G-7	Battery monitor	Battery monitor will be integrated into the application. Will monitor internal battery pack and external battery pack available power. For GUI, will notify user when there is not enough energy in the available sources to complete the selected assay. Prompt to connect to AC power or external battery source.	c			2 Use briefcase UPS	
71	G-8	Communication interface	USB and RS-232	a			2	
72	H Operations							
73	H-1	Set up time	System will be operational within 10 minutes of arrival on site (including setup, warm-up time, and automated engineering check)	b			2 System warmup and self test must require less than 10 minutes.	
74	H-2	LED indicators on instrument	AC power available	a			3 Use briefcase UPS	
75			external battery available	a			3 Use briefcase UPS	
76			auto power available	a			3 Use briefcase UPS	
77			battery charging	a			3 Use briefcase UPS	
78			Power source currently in use	a			3 Use briefcase UPS	
79			Internal battery charge (25%, 50%, 75%, 100%)	a			3 Use briefcase UPS	
80			If can't power up to remove cartridge a procedure must be provided to disengage piston				Procedure must be written. Design for ease of disassembly in case of stuck cartridge.	
81	H-3	System Ready	System should prompt user when cartridge is in place and ready to "go"				2 Must have cartridge detect mechanicsm	
82	I System Powerup Self Tests							
83								
84	I-1	Mechanical preregistration	Instrument prepares to accept cartridge at power up				1 At power up, instrument rotates valve to home position and withdraws syringe in preparation for cartridge insertion.	
85	I-2	Syringe System	Instrument should detect failed syringe motor				At power up, a syringe test will be done to verify operation.	

Design Input Requirements

	A	B	C	D	E	F	G	H
86	I-3	Rotary Valve System	Instrument should detect failed rotary valve motor				At power up, a valve test will be done to verify operation.	
87	J Accessories							
88	J-1	Cartridge Rack	Any kind of racks required to hold cartridge while loading reagents					
89								
90	J-2	External Battery Pack	30-50 samples. Can be used in parallel with internal batteries, auto power or AC power.	c		2	Depends on success of Briefcase system results	
91								
92								
93	L Reliability						Define duty cycle or continuous operation	
94	L-1	Expected Life	Minimum 1 year life			2	System must do 1000 cycles without failure	
95								

Design Input Requirements

	A	B	C	D	E	F	G	H
96	L-2	Horn and related electronics	Minimum 1 year life			1	System must do 1000 cycles without failure	
97	L-3	Mechanical, motors	Minimum 1 year life			1	System must do 1000 cycles without failure	
98	M Maintenance					1		
99	M-1	Cleanability	Outer surfaces must be resistant to 10% bleach and 70% ethanol for decontamination by wiping down.	b		1	All external materials will show no signs of degradation after 24 hour immersion in stated solutions.	
100	M-2	Calibration						
101	M-3	User Notification	The user will be notified when the instrument calibration period has expired.	a		2	Calibration date and next due date will be stored in the instrument and accessed by the GUI. The GUI will notify the user when the instrument calibration period has expired based on the computer system clock.	
102	M-4	Thermal Calibration	To be done at no more than 12 month frequency to maintain performance.	c		2	Temperature to be periodically calibrated by user using probe	
103	M-5	Optical Calibration	To be done at no more than 12 month frequency to maintain performance.	a		2	Software Utility will allow user to use dyes to calibrate system.	
104	M-6	Horn and mechanical functions	No user maintenance of mechanical parts required			2	Mechanical systems must not require lubrication or other maintenance.	
105	Warranty/Service							
106	N-1	Servicability	Servicability will be simple and cost effective	a		1	Instrument design will be modular. Modular components will be: Sample Prep Module, Baseunit, Power system, Battery Pack	
107	N-2	Battery Replacement	User can change internal battery in less than 5 minutes with one standard tool				Battery must be easily accessible for replacement. Use simple fasteners, ideally no tools required.	
108	N-3	Warranty	Warranty will be 90 days from date of purchase.	a				
109								
110	O Regulatory Requirements							
111		Agency Approvals	N/A					
112								
113								
114	P Environmental							
115	P-1	Ambient conditions (operation)	5-49C; 10-95% relative humidity (non condensing)	E1		2		
116	P-2	Ambient conditions (storage / transport)	-31C to 71C; 10-95% relative humidity (non condensing)	E1		2		
117	P-3	Power	90-264V, 50-60 Hz AC.			2	Use universal power supply.	
118	P-4	Vibration (operation)	None			2		

	A	B	C	D	E	F	G	H
119	P-5	Altitude	Operational at 1500 meters	E5		2		
120			Storage at 6000 meters			2		
121	P-6	EMI	CE standard EN55011 class B			2		
122	P-7	Light Levels	Operable in a full-daylight environment			1	Less than 1% effect of full sunlight on optical backgrounds and full scale values.	
123	Q Safety							
124	Q-1	Cepheid	No components requiring special hazard handling during development or production will be used.	S1		1		
125	Q-2	Disposal	Instrument can be thrown in the trash and /or recycled worldwide without special procedures.	S2		1	No CFCs or other toxins to be used.	
126	Q-3	Labelling	Appropriate labeling for hazards				CE Standards	
127	R Packaging		Instrument meets European packaging requirements.					
128	R-1	1a. Instrument Outer Box						
129	R-1-1	Packaging	Able to be shipped,unpacked and packed 3 times each	b		1	Reusable packaging required	
130	R-1-2	Shipping	Able to withstand standard shipping conditions				International Airfreight Transport Standards	
131								
132		Test Method		Rank				
133	a	Self evident						
134	b	Existing standard						

Design Input Requirements

	A	B	C	D	E	F	G	H
135	c	Cepheid Procedure #			plan			
136	d	Cepheid Procedure #		3 Moderate - Not Met will change labeling				
137	e	Cepheid Procedure #		4 Preference - Not met has no impact				
138	etc							

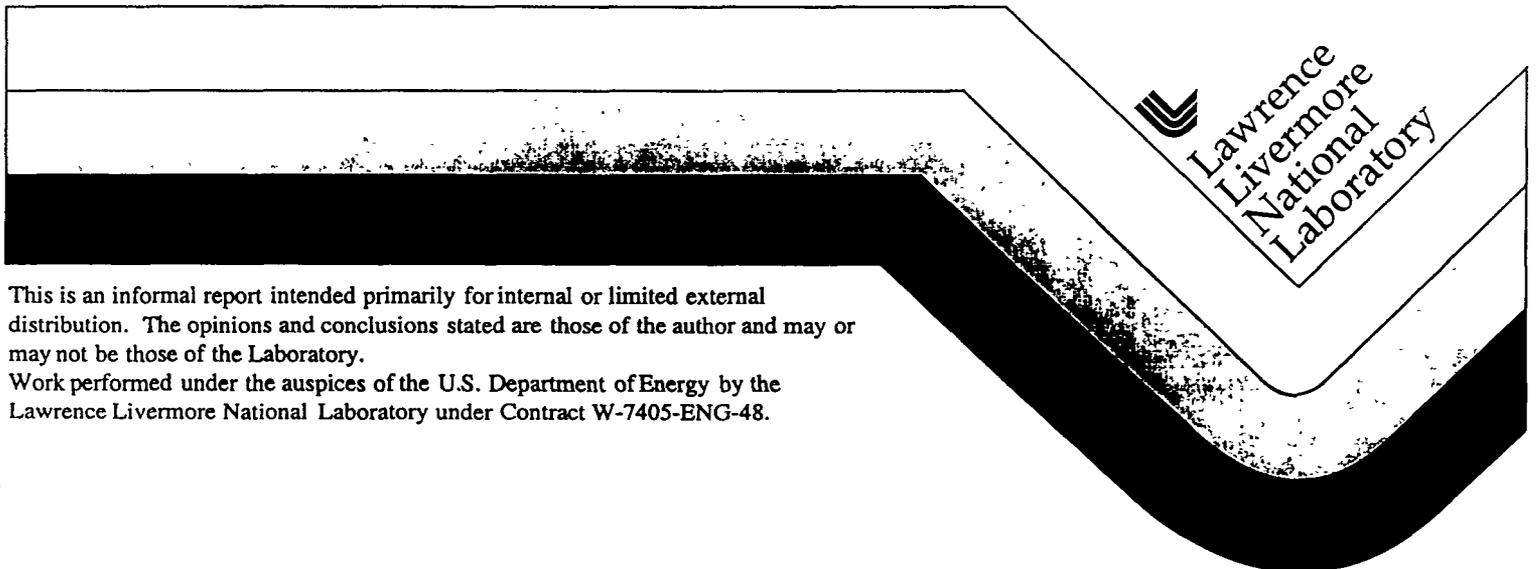
Long Wavelength GaN Blue Laser (400-490nm) Development

Final Report 2000 for LLNL Project

G. Meyer, LLNL

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University of California, Santa Barbara, CA

October 2000



This is an informal report intended primarily for internal or limited external distribution. The opinions and conclusions stated are those of the author and may or may not be those of the Laboratory.

Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-ENG-48.

BW Sample Collection and Preparation Device

Deliverable

Task 3 Blue Laser Diode Final Report

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Prepared for:
Central MASINT Organization
Technology Coordination Office
Lt. Col. Holly Franz and Scott Smith

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Long Wavelength GaN Blue Laser (400-490nm) Development

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EXECUTIVE SUMMARY

Room temperature (RT) pulsed operation of blue nitride based multi-quantum well (MQW) laser diodes grown on c-plane sapphire substrates was achieved. Atmospheric pressure MOCVD was used to grow the active region of the device which consisted of a 10 pair $\text{In}_{0.21}\text{Ga}_{0.79}\text{N}$ (2.5nm)/ $\text{In}_{0.07}\text{Ga}_{0.93}\text{N}$ (5nm) InGaN MQW. The threshold current density was reduced by a factor of 2 from 10 kA/cm^2 for laser diodes grown on sapphire substrates to 4.8 kA/cm^2 for laser diodes grown on lateral epitaxial overgrowth (LEO) GaN on sapphire. Lasing wavelengths as long as 425nm were obtained. LEDs with emission wavelengths as long as 500nm were obtained by increasing the Indium content. These results show that a reduction in nonradiative recombination from a reduced dislocation density leads to a higher internal quantum efficiency. Further research on GaN based laser diodes is needed to extend the wavelength to 490nm which is required for numerous bio-detection applications. The GaN blue lasers will be used to stimulate fluorescence in special dye molecules when the dyes are attached to specific molecules or microorganisms. Fluorescein is one commonly used dye molecule for chemical and biological warfare agent detection, and its optimal excitation wavelength is 490 nm. InGaN alloys can be used to reach this wavelength.

I. INTRODUCTION

The development of blue lasers offers great potential for high density information storage, medical devices, and full-color displays. Since the report of the first RT pulsed operation of nitride based laser diodes by researchers at Nichia Chemical Industries two years ago [1] a handful of research groups in Japan and the United States have reported blue laser operation. Despite the significant progress by Nichia and others, the actual lasing mechanism and its relationship to the structural and electrical properties of these materials is not well understood. In this study we report on the growth of InGaN MQW and the properties of laser diodes made with InGaN MQW active regions.

RESULTS

InGaN multiple-quantum-well (MQW) laser diodes were grown by MOCVD in a two-flow horizontal reactor at both atmospheric and low pressure. In preparation for patterning a subsequent regrowth, a 2 μm thick GaN seed layer was grown on a c-plane sapphire substrate. A 2000 \AA SiO_2 mask was patterned into stripes, oriented in the $\langle 1\bar{1}00 \rangle_{\text{GaN}}$ direction, defining a 5 μm mask opening with a periodicity of 20 μm . After ~ 6 μm of lateral epitaxial overgrowth (LEO) GaN growth on the SiO_2 mask, the GaN stripes grew laterally and coalesced, forming a flat surface. The conditions for growth and coalescence of the LEO GaN are described elsewhere.²⁰ Next, the InGaN MQW laser structure was grown on both LEO GaN and on 2 μm GaN on sapphire. The structure had an active region consisting of a 3 period $\text{In}_{0.13}\text{Ga}_{0.87}\text{N}$ (40 \AA) / $\text{In}_{0.04}\text{Ga}_{0.96}\text{N}:\text{Si}$ (85 \AA) MQW followed by a 200 \AA $\text{Al}_{0.2}\text{Ga}_{0.8}\text{N}:\text{Mg}$ cap. The n and p-type cladding regions surrounding the active region consisted of 25 \AA $\text{Al}_{0.2}\text{Ga}_{0.8}\text{N}$ / 25 \AA GaN superlattices with a total thickness of 0.45 μm . The cladding regions were Si-doped for the n-cladding and Mg-doped for the p-cladding. A 0.1 μm GaN:Mg layer was used as a contact layer and a 0.1 μm $\text{In}_{0.05}\text{Ga}_{0.95}\text{N}:\text{Si}$ layer was used beneath the lower n-type cladding as a compliance layer.

Laser diodes were fabricated above the SiO_2 mask in the nearly dislocation-free wing regions, as well as above the coalescence fronts of the LEO GaN stripes. The laser cavity was oriented parallel to the direction of the SiO_2 stripes. Laser facets were formed by Cl_2 reactive ion etching (RIE) of 45 μm wide mesas of various lengths ranging from 400 μm to 1600 μm and p-contact stripes were patterned on these mesas with widths ranging from 5 μm to 15 μm . The structure was etched around the p-contact stripe through the p-cladding for index guiding. The n and p-contacts were formed by electron beam evaporation of Ti/Al and Pd/Au, respectively.

Figure 1 shows the PL from various QW active regions. The longest emission wavelength obtained was 500nm. LEDs were also fabricated from these active regions. Lasing was only obtained for wavelenths shorter than 420nm. The cause of the high threshold currents is most likely due to the alloy segregation in the higher indium containing alloys.

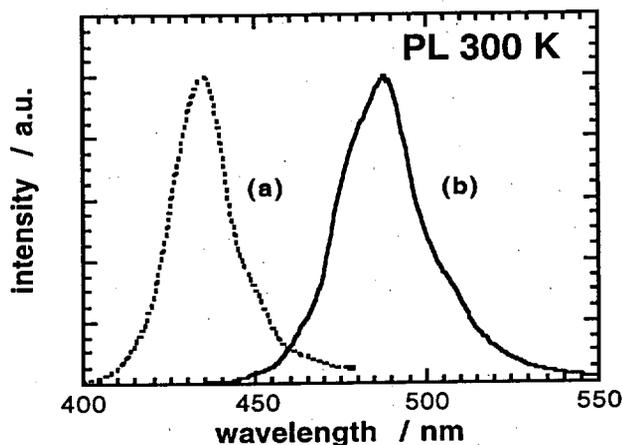


Figure 1 PL from InGa_N quantum well active regions.

Lateral Epitaxial Overgrowth (LEO)

There are few or no threading dislocations generated at the coalescence fronts. Figure 2 shows cross-section TEM micrographs of the coalescence region. This high quality coalescence results from low wing tilts of the laterally growing stripes. The LEO wings have a tilt of 0.1° relative to the underlying seed material, which was measured using x-ray diffraction as described earlier.¹⁸

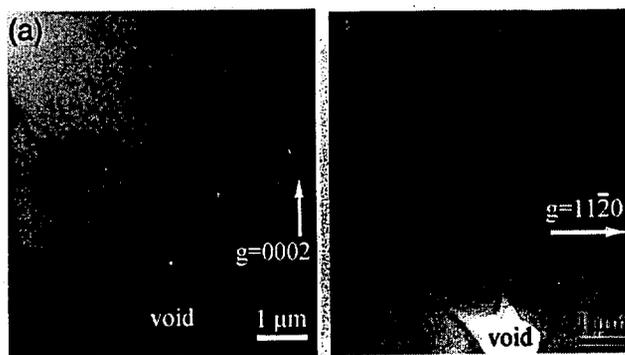


Figure 2: Bright-field cross-section TEM micrographs of a coalescence front viewed with (a) $g = 0002$ and (b) $g = 11\bar{2}0$ two-beam conditions.

The threading dislocations in LEO GaN on sapphire are predominantly located in 'window' stripes every 15 μm , whereas structures on sapphire substrates have a more uniform dislocation distribution over the wafer. The dislocation distribution dominates the size of features in the surface morphology. Atomic force microscopy (AFM) was used to investigate the surface morphology for lasers grown on LEO GaN and laser grown on sapphire as seen in Figure 3. The surface morphology is drastically different for the two structures. The lasers on sapphire show small spirals uniformly distributed whereas on the LEO GaN the laser structure exhibits large spirals. These spirals grow in size until they meet another spiral. Each of these spirals is formed around a threading dislocation with a screw component. In the case of the LEO GaN, the threading dislocations are contained in the window region and are absent in the wing region. This is why all the spirals in the LEO case initiate in the narrow window regions and can grow quite large over the wing until it meets another spiral associated with a screw component threading dislocation from an adjacent window region forming a flat "trench-like" feature. In the case of the laser on sapphire, the spiral remain small because they meet neighboring spirals much more quickly due to the higher and more uniform dislocation distribution.

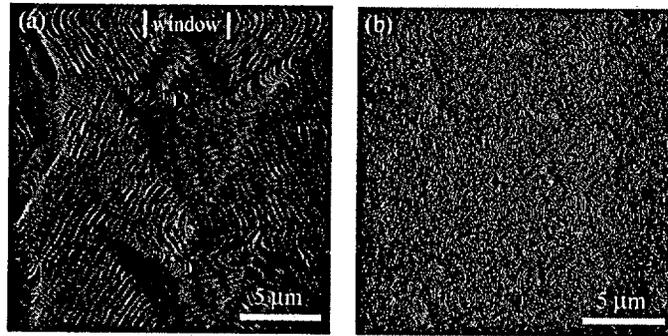


Figure 3: 20x20 μm^2 amplitude AFM images of (a) a laser structure on LEO (b) a laser structure on sapphire. Note: The LEO stripes are running vertically.

Figure 4 shows the typical light output per uncoated facet of a laser diode grown on LEO GaN and a laser diode grown on GaN/sapphire as a function of forward current under pulsed operation. The minimum threshold current density was reduced by a factor of 2 from 10 kA/cm^2 for laser diodes grown on sapphire to 4.8 kA/cm^2 for laser diodes grown on LEO GaN on sapphire. The laser diodes on the LEO GaN showed this low threshold current density of 4.8 kA/cm^2 both above the SiO_2 mask regions and above the coalescence fronts of the LEO GaN. This reduction in threshold current density is attributed to a reduction in nonradiative recombination due to the lower dislocation density in the LEO GaN.

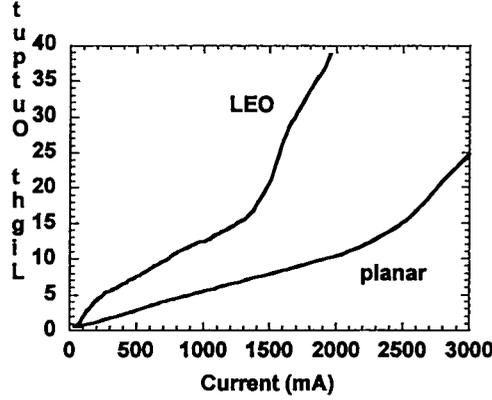


Figure 4: Typical light output per uncoated facet as a function of current for a laser diode grown on LEO GaN and a laser diode grown on sapphire.

Figure 5 shows the reciprocal of external differential quantum efficiency as a function of length for laser diodes grown on sapphire and LEO GaN. The external differential quantum efficiency of the laser diode increases with increasing internal quantum efficiency or decreasing internal optical loss as seen in the following relationship²¹,

$$\eta_d = \eta_i \frac{\alpha_m}{\langle \alpha_i \rangle + \alpha_m} \quad (1)$$

where η_d is the external differential quantum efficiency, η_i is the internal quantum efficiency, α_m is the mirror loss and α_i is the internal optical loss of the laser. The mirror loss can be defined as

$$\alpha_m = \frac{1}{L} \ln \left(\frac{1}{R} \right) \quad (2)$$

where L is the length and R is the facet reflectivity. R is estimated to be approximately 0.053 for RIE etched facets.²² Substituting Eqn. (2) into (1) and rearranging gives

$$\frac{1}{\eta_d} = \frac{1}{\eta_i} + \frac{\langle \alpha_i \rangle}{\eta_i \ln \left(\frac{1}{R} \right)} L \quad (3)$$

The internal quantum efficiency can be extracted from the y-intercept of Fig. 5 using Eqn. (3). The increase in external differential quantum efficiency seen in the lasers on LEO GaN compared to those on sapphire is due to an increase in the internal quantum efficiency from 3% to 22%. As mentioned, the reduced reverse bias leakage current in p-n junction diodes suggests the presence of mid-gap states due to threading dislocations.²³ These mid-gap states provide nonradiative recombination centers thereby decreasing the internal quantum efficiency. Reducing the dislocation density, and hence the mid-gap states, will result in an increased internal quantum efficiency. The same effect is also seen in the spontaneous emission portion of L-I curve below threshold in Fig. 2 as well as in LEDs fabricated on LEO GaN,^{11,24} where the radiative efficiency increases with decreasing dislocation density.

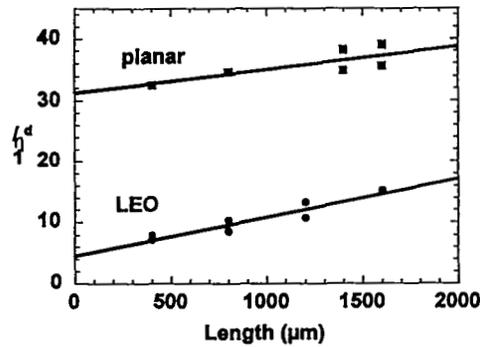


Figure 5: Inverse external differential quantum efficiency as a function of device length.

SUMMARY AND ACKNOWLEDGEMENTS

In summary, InGaN multi-quantum well laser diodes have been fabricated on fully coalesced laterally overgrown GaN on sapphire. The wing regions as well as the coalescence regions of the LEO GaN contain few or no threading dislocations. The threshold current density was reduced by a factor of 2 from 10 kA/cm² for laser diodes grown on sapphire substrates to 4.8 kA/cm² for laser diodes grown on LEO GaN on sapphire. These results show that a reduction in nonradiative recombination from a reduced dislocation density leads to a higher internal quantum efficiency. Lasing wavelengths as long as 425nm were obtained. LEDs with emission wavelengths as long as 500nm were obtained by increasing the Indium content. Further research on laser diodes is needed to extend the wavelength to 490nm which is required for numerous GaN based bio-chemical sensing applications. This work was performed under the auspices of the U.S. Department of Energy by the University of California Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

References

- ¹ S. Nakamura, M. Senoh, S. Nagahama, N. Iwasa, T. Yamada, T. Matsushita, H. Kiyoku, Y. Sugimoto, T. Kozaki, H. Umemeto, M. Sano, and K. Chocho, Proc. Int. Conf. on Nitride Semicond., S-1, p. 444 (1997).
- ² S. Nakamura, M. Senoh, S. Nagahama, N. Iwasa, T. Yamada, T. Matsushita, H. Kiyoku, Y. Sugimoto, T. Kozaki, H. Umemeto, M. Sano, and K. Chocho, Appl. Phys. Lett. **72**, 211 (1998).
- ³ K. Ito, K. Hiramatsu, H. Amano, I. Akasaki, J. Cryst. Growth **104**, 533 (1990).
- ⁴ D. Kapolnek, S. Keller, R. Vetry, R. D. Underwood, P. Kozodoy, S. P. DenBaars, and U.K. Mishra, Appl. Phys. Lett. **71**, 1204 (1997).
- ⁵ T. S. Zheleva, O.-H. Nam, M. D. Bremser, and R. F. Davis, Appl. Phys. Lett. **71**, 2472 (1997).
- ⁶ O.-H. Nam, M. D. Bremser, T. S. Zheleva, and R. F. Davis, Appl. Phys. Lett. **71**, 2638 (1997).
- ⁷ H. Marchand, J. P. Ibbetson, P. T. Fini, P. Kozodoy, S. Keller, J. S. Speck, S. P. DenBaars, and U. K. Mishra, MRS Internet J. Nitride Semicond. Res. **3**, 3 (1998).
- ⁸ A. Usui, H. Sunakawa, A. Sakai, and A. A. Yamaguchi, Jpn. J. Appl. Phys. **36**, L899 (1997).
- ⁹ A. Sakai, H. Sunakawa, and A. Usui, Appl. Phys. Lett. **71**, 2259 (1997).
- ¹⁰ P. Kozodoy, J. P. Ibbetson, H. Marchand, P. T. Fini, S. Keller, S. Keller, J. S. Speck, S. P. DenBaars, and U. K. Mishra, Appl. Phys. Lett. **73**, 957 (1998).
- ¹¹ C. Sasaoka, H. Sunakawa, A. Kimura, M. Nido, A. Usui, and A. Sakai, J. Crystal Growth **189/190**, 61 (1998).
- ¹² S. Nakamura, M. Senoh, S. Nagahama, N. Iwasa, T. Matsushita, and T. Mukai, MRS Internet J. Nitride Semicond. Res. **4S1**, G1.1 (1999).
- ¹³ R. Vetry, H. Marchand, J. P. Ibbetson, P. Fini, S. Keller, J. S. Speck, S. P. DenBaars, and U.K. Mishra, Proceedings of the 25th Int. Symp. Comp. Semicond., Nara, Japan, 1998.
- ¹⁴ G. Parish, S. Keller, P. Kozodoy, J. P. Ibbetson, H. Marchand, P. T. Fini, S. B. Fleischer, S. P. DenBaars, U. K. Mishra, and E. J. Tarsa, Appl. Phys. Lett. **75**, 247 (1999).
- ¹⁵ H. Marchand, J. P. Ibbetson, P. Fini, S. Chichibu, S. J. Rosner, S. Keller, S. P. DenBaars, J. S. Speck, and U.K. Mishra, Proc. 25th Int. Symp. Comp. Semicond., Nara Japan, 1998.
- ¹⁶ K. Tsukamoto, W. Taki, N. Kuwano, K. Oki, T. Shibata, N. Sawaki, and K. Hiramatsu, Proc. 2nd Int. Symp. On Blue Laser and Light Emitting Diodes, Kisarazu, Chiba, Japan, 1998, p. 488-491.
- ¹⁷ P. Fini, J. P. Ibbetson, H. Marchand, L. Zhao, S. P. DenBaars, and J. S. Speck, unpublished (1999).
- ¹⁸ S. Keller, U. K. Mishra, S. P. DenBaars and W. Seifert, Jpn. J. Appl. Phys. **37**, L431 (1998).
- ¹⁹ T. Sugahara, M. Hao, T. Wang, D. Nakagawa, Y. Naoi, K. Nishino and S. Sakai, Jpn. J. Appl. Phys. **37**, L1195 (1998).
- ²⁰ P. Fini, L. Zhao, B. Moran, M. Hansen, H. Marchand, J. P. Ibbetson, S. P. DenBaars, U.K. Mishra, and J. S. Speck, Appl. Phys. Lett. **75**, 1706 (1999).
- ²¹ L. A. Coldren and S. W. Corzine, *Diode Lasers and Photonic Integrated Circuits* (John Wiley & Sons, Inc., New York, 1995).
- ²² M. P. Mack, G. D. Via, A. C. Abare, M. Hansen, P. Kozodoy, S. Keller, J. S. Speck, U. K. Mishra, L. A. Coldren, and S. P. DenBaars, Electron. Lett. **34**, 1315 (1998).
- ²³ For a review on physical properties of threading dislocations in GaN please see J.S. Speck and S. J. Rosner, to be published in Physica B (1999).
- ²⁴ M. Hansen, P. Fini, A. C. Abare, L. A. Coldren, J. S. Speck, and S. P. DenBaars, unpublished (1999).