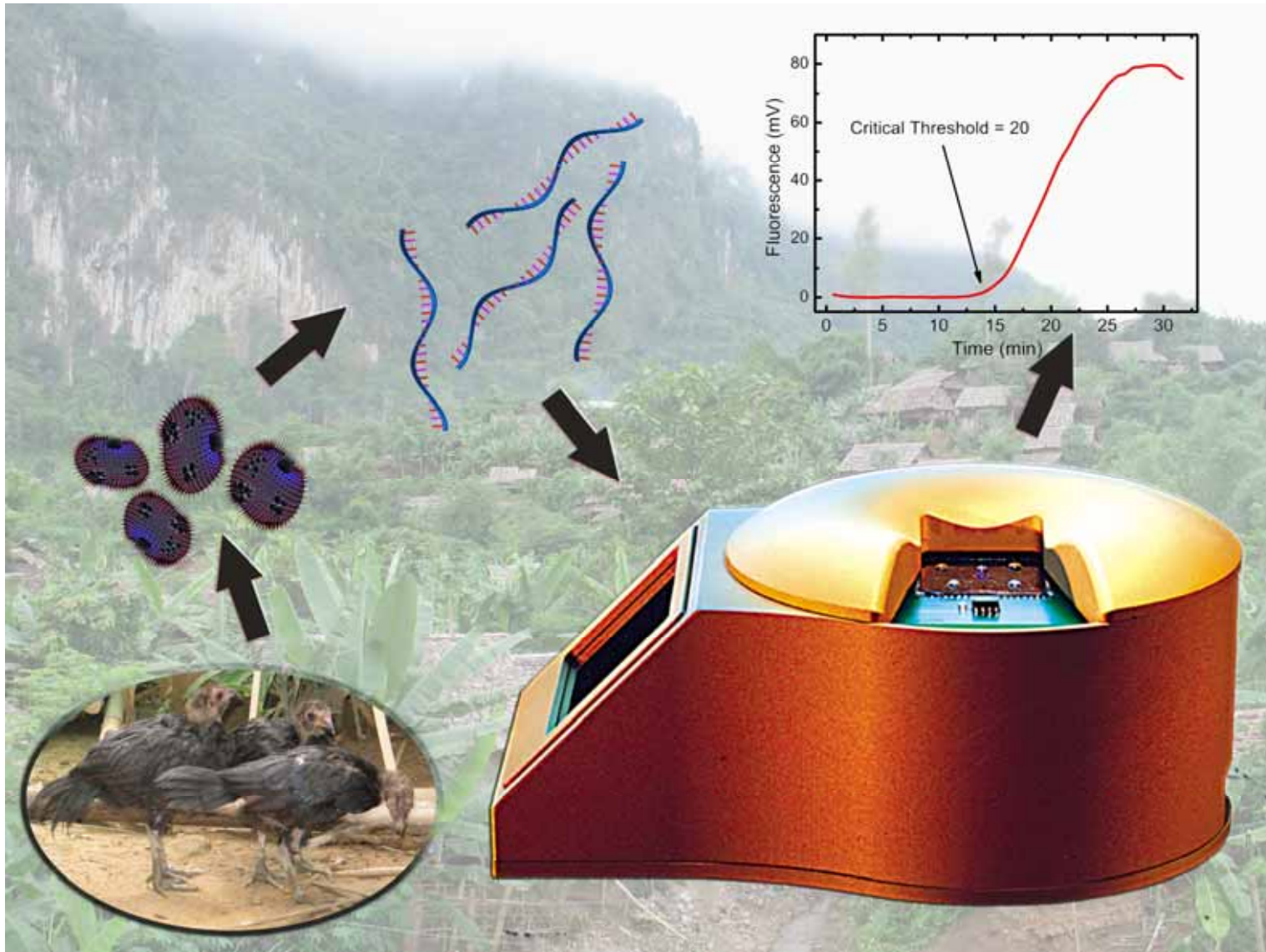


Catching Bird Flu in a Droplet

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(CTU), Lee Shinhan (NP), Lisa Ng (GIS),
Tomas Karasek (IHPC)

Infection disease detection



Outline

- Introduction to infection disease detection
- Molecular biology methods
- Current status, real-time PCR, flow cytometer, capillary electrophoresis
- Chip-in-a-Lab or Lab-on-a-Chip?
- Palm-sized Real-time (RT) PCR system
- Applications, such as rapid detection of Avian Influenza, Dengue or SARS virus.
- Integrated modular portable system

Infection disease detection

- Infection disease based on virus or bacteria
- Detection of the disease is equivalent of detection the presence of the virus or bacteria
- Virus often contains RNA while bacteria DNA
- Performed by detection specific sequence of their RNA/DNA
- Sample preparation - release and purification of RNA/DNA
- DNA/RNA amplification – PCR or RT-PCR
 - PCR and RT-PCR explanation
- PCR Product detection
 - Real-time PCR, electrophoresis or hybridization

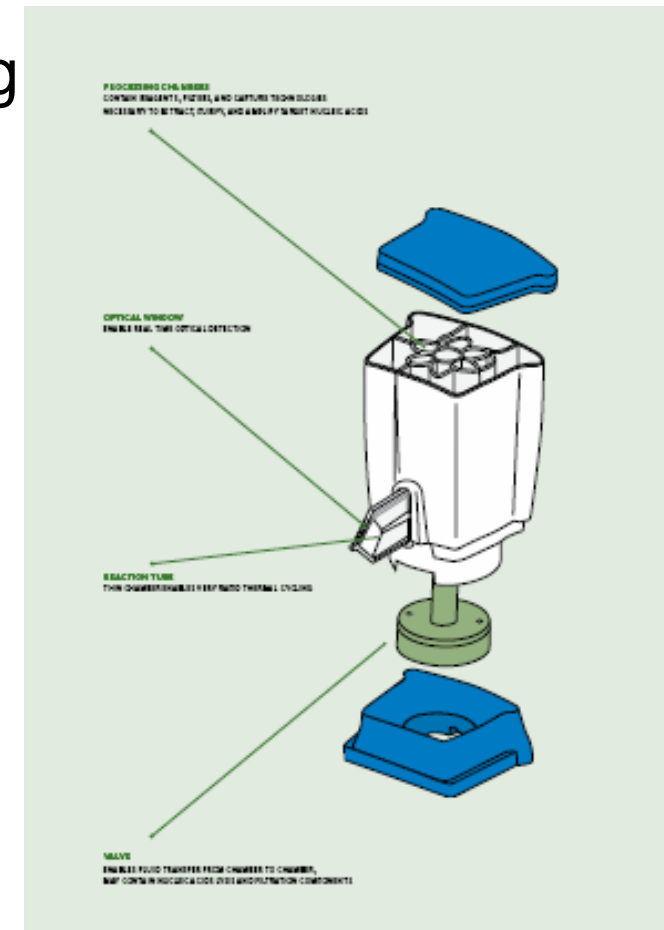
GeneXpert by Cepheid



- Fully integrated system for bacteria/virus detection
- 30 – 40 k USD per system

GeneXpert cartridge

- Made by economical injection moulding technology
- Contains all required chemicals
- Contains ultrasonic horn for shell breaking
- Sample in, result out system
- Cost per cartridge is 6 to 7 USD



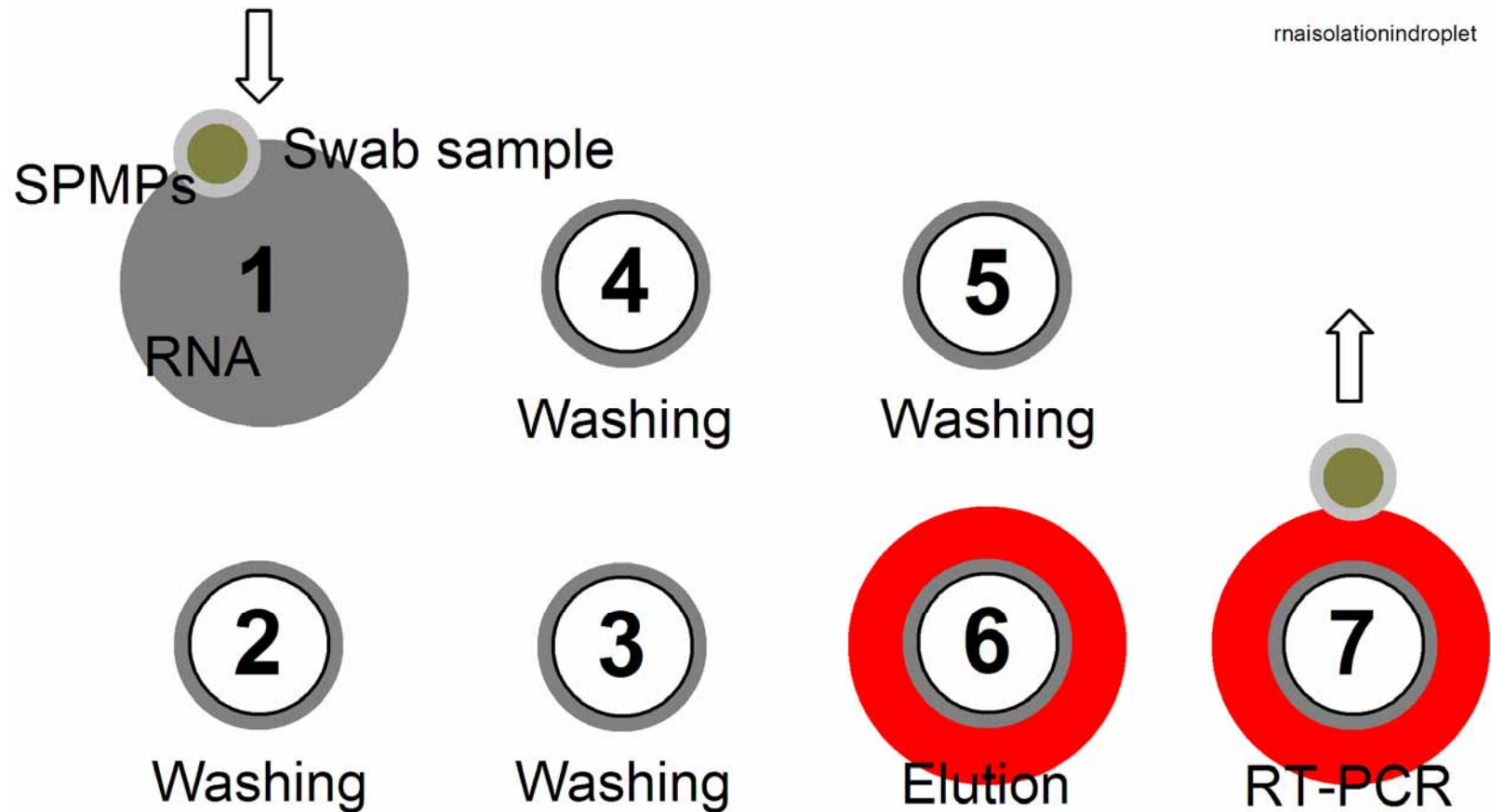
System requirements

- GeneXpert cost is still far too high for developing countries where the outbreak is expected.
- System cost should be below 1000 USD.
- Cartridge is too costly
- Cost per test only a few cents.
- Power efficient system required
- System should be rechargeable battery operated
- Charging by car battery
- Portability

Lab-on-a-chip based systems

- LOC are promising, cheap manufacturing of basic components.
- Unfortunately, currently available LOCs are more Chip-in-a-Lab.
- Expensive and bulky instrumentation.
- Required blocks
 - Sample preparation and handling
 - Heating and temperature controlling system
 - Optical detection system
 - Signal processing
 - Power supply

System design



Virtual Reaction Chamber

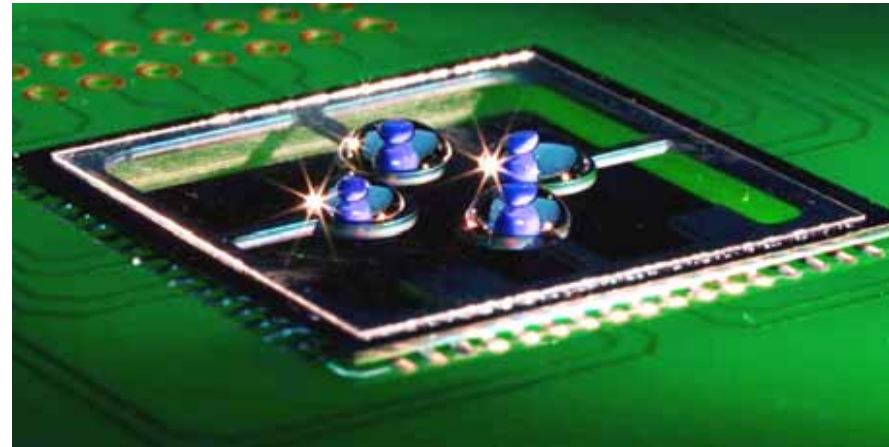
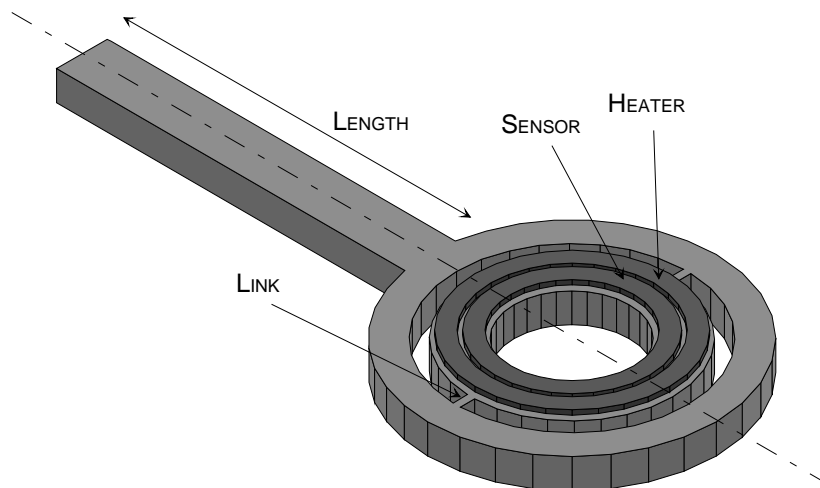
Key properties

- Water-based sample with volume below 1 μL encapsulated by mineral oil.
- PCR or RT-PCR conducted on a DISPOSABLE glass cover slip.
- Economical system due to small volume and cheap substrate.
- 3 – 5 cents per test

Micro PCR systems

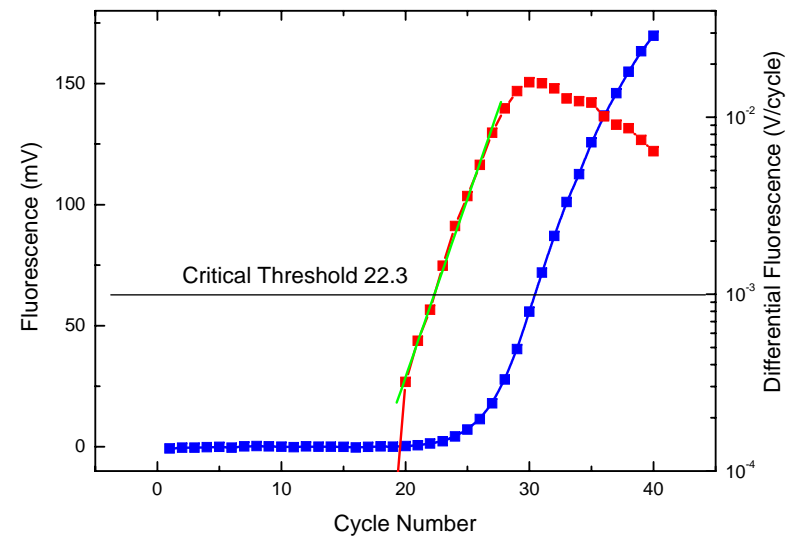
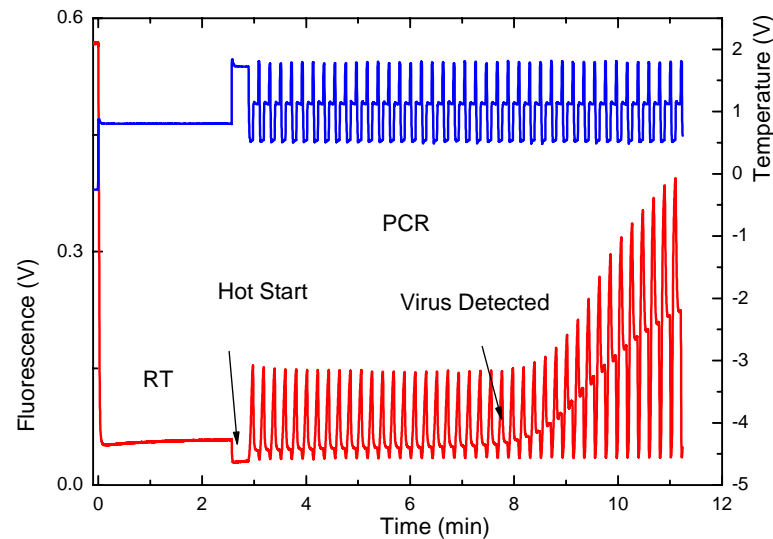
Key properties

- PCR chips directly soldered to a PCB
- Heater integrated with temperature sensor placed on a micromachined silicon brings down the cost of the system



Avian Influenza Virus Detection by RT-PCR

- Economical SYBR-Green Real-Time RT-PCR
- Followed by Melting Curve Analysis
- 8 minutes for virus detection



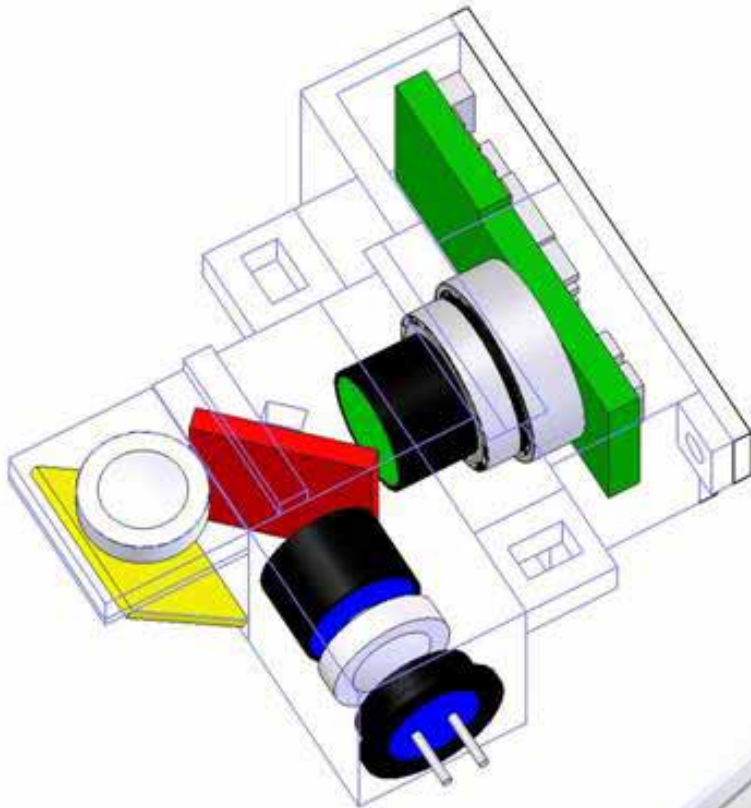
Optical Detection



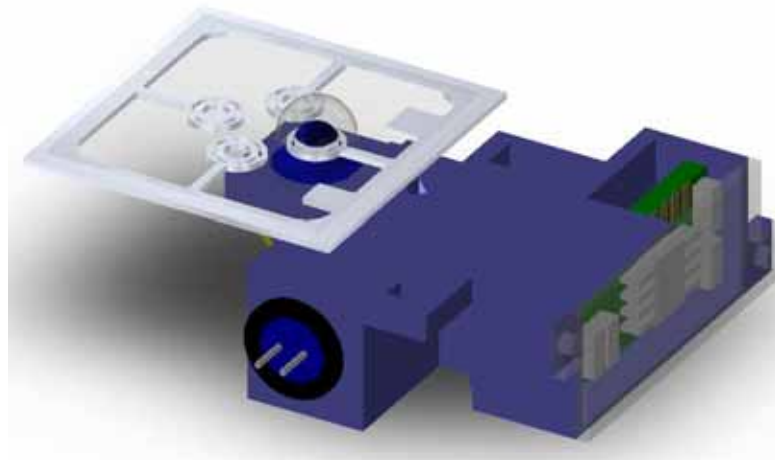
Fluorescent system

- Microscope
- PMT
- Metal halide light source
- Filter cube

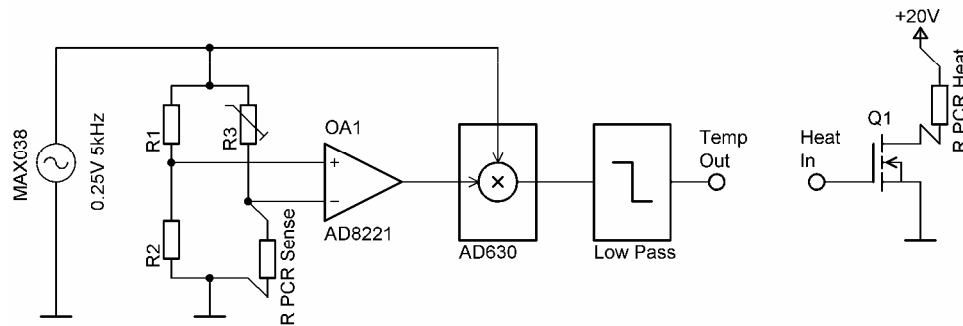
Integrated fluorescent unit



- Located underneath PCR sample
- Blue LED as exciter
- FITC filter set
- Photodiode with amplifier
- Derived from a DVD pickup head

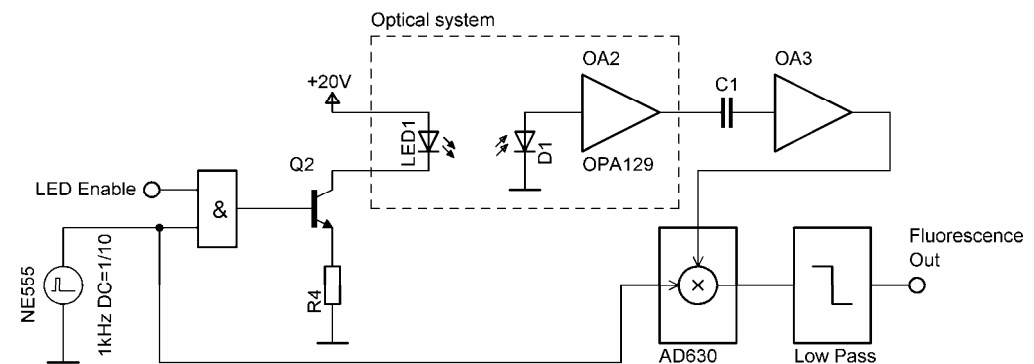


Signal processing



- Thermal management
 - AC Wheatstone bridge
 - demodulator

- Fluorescence processing
 - AC powered triple LED
 - signal amplification 10^8
 - triple demodulator

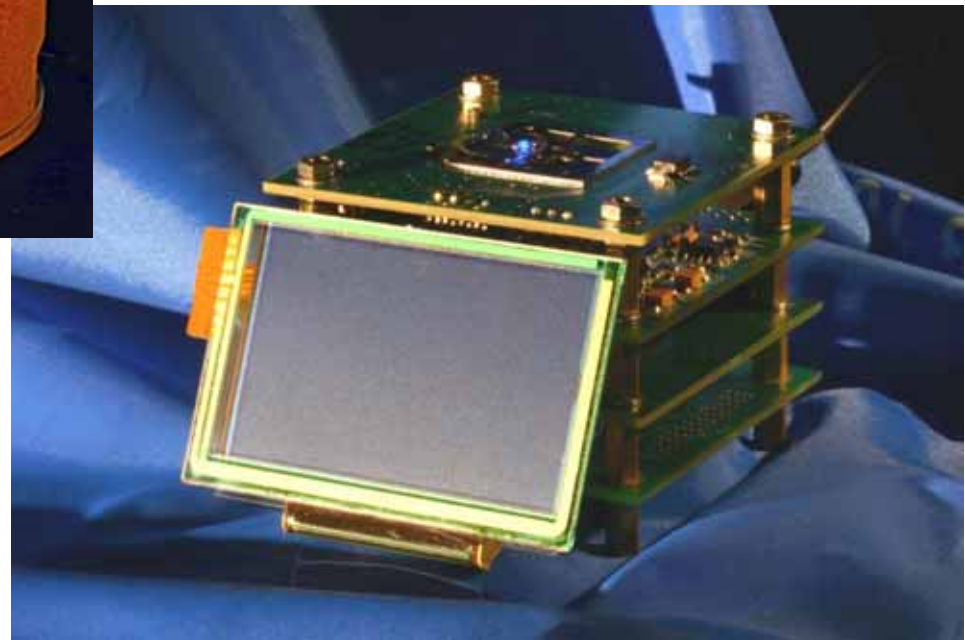
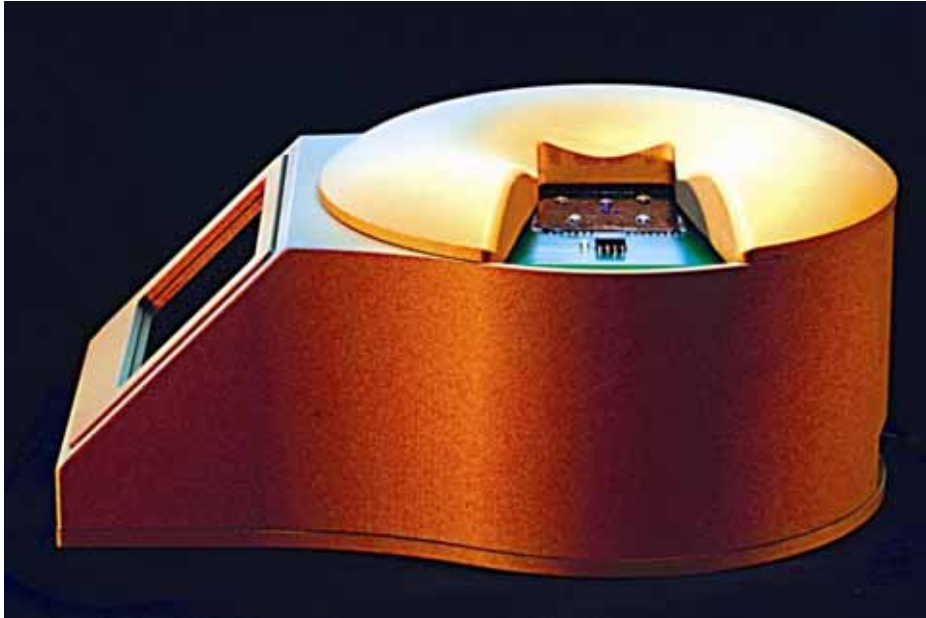


Real-Time PCR

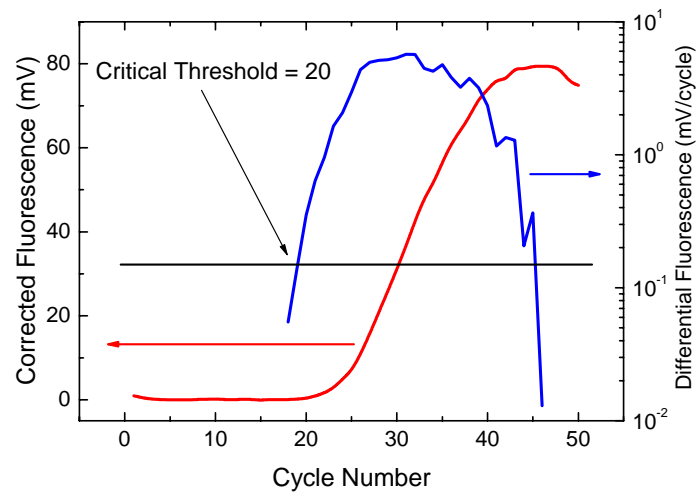
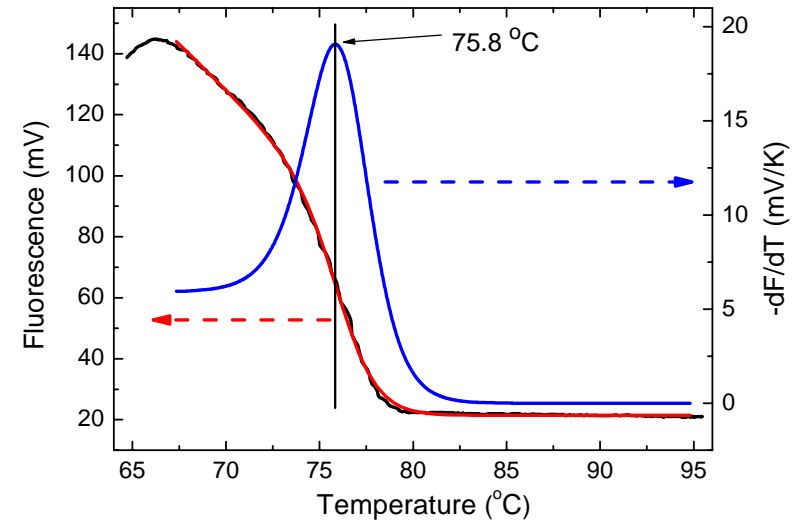
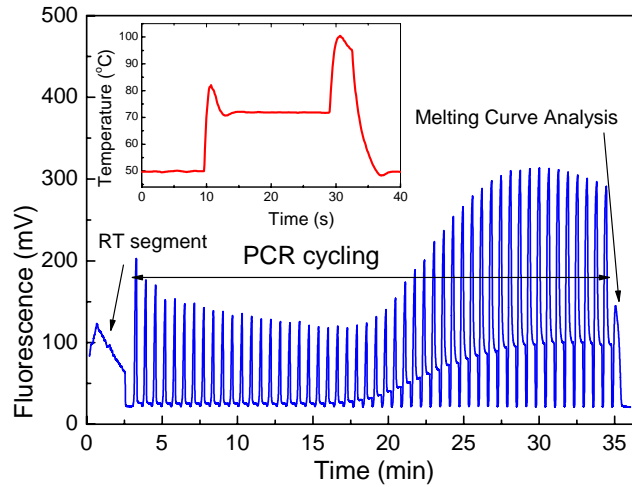
Features

- Silicon PCR chip with heater and temperature sensor
- Integrated optical unit for fluorescence detection
- 3 PC boards carrying
 - PCR chip
 - signal processing electronic
 - power supply

Palm-sized (RT) PCR



Real-Time RT-PCR results



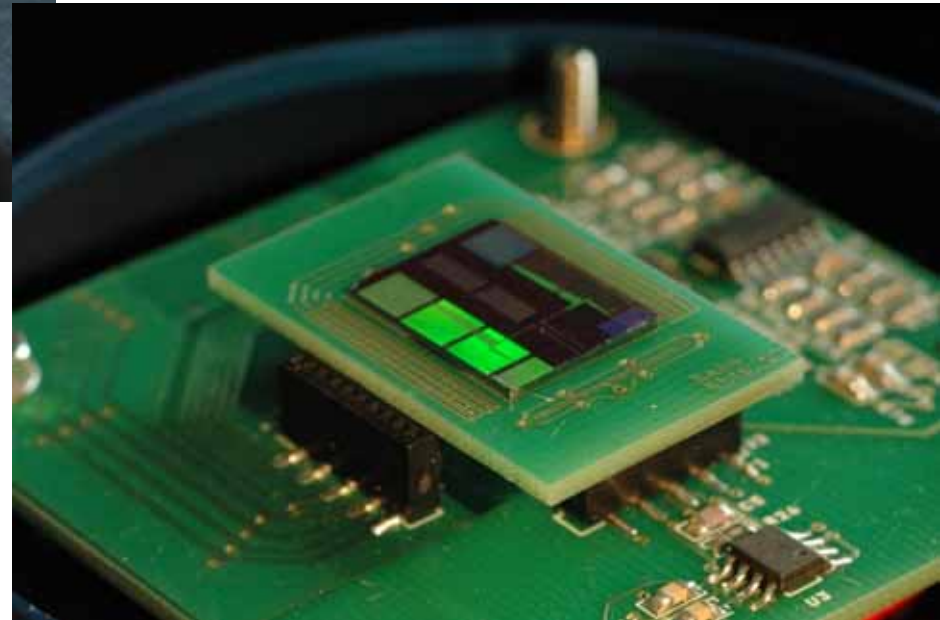
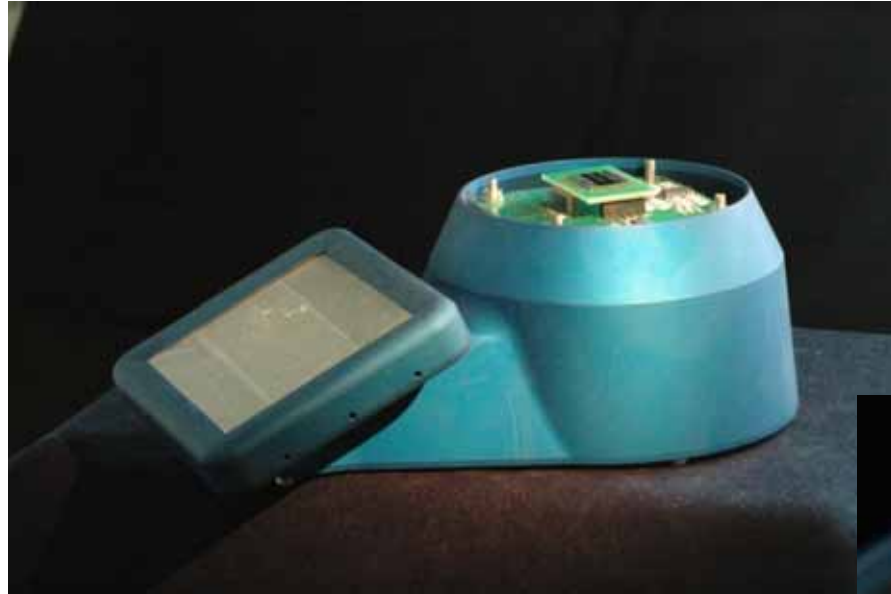
Towards modular LOC system

- The system consists of 5 boards in total
- Top board - customized unit for the particular application and interconnection with the LOC subsystem
- Second board – current detecting system for 4 channels
- Third board – temperature controlling system for 4 channels
- Fourth board – single chip controller to control the boards and LCD display. It includes motor controller and regulated 1000V power supply.
- Fifth board is a power supply connected to the Li-Ion battery.

Possible applications of modular system

- (RT) PCR and real-time (RT) PCR for up to 4 channels
- Stationary or flow through PCR systems
- Complete DNA/RNA detection system with sample preparation.
- Nanowire based sensors.
- Electrochemical systems for bio applications.
- Flow cytometry for up to 4 colours.
- DEP based system.
- Electrophoretic system.

Modular LOC System



- Nanowire configuration
- Label-free detection system

Conclusions

- All functions controlled from touch screen display
- Flash card for data recording
- Autonomous systems with USB connection
- Powered by Li-Ion battery
- Rechargeable via USB port
- Target manufacturing cost below 500 USD
- Low operation cost below 10 c per sample
- Suitable for remote areas in developing countries

Acknowledgments

- Masafumi Inoue (IMCB)
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