Design of Real-time Advanced Lens Free Imager

Masashi Takemoto
Tokyo Univ. of Agriculture and
Technology/BeatCraft, Inc.
lesser@beatcraft.com

Masayuki Yashiro
BeatCraft, Inc.
yas@beatcraft.com

Ryota Suzuki
Tokyo Univ. of Agriculture and
Technology
suzuki@nj.cs.tuat.ac.jp

Tetsuya Ryuchi
BeatCraft, Inc.
ryuchi@beatcraft.com

Katsuhiko Umeno
BeatCraft, Inc.
umeno@beatcraft.com

Kohta Ohshima
Tokyo Univ. of Marine Science
and Technology
Koto-ku, Tokyo Japan
kxoh@kaiyodai.ac.jp

Naoya Kitagawa
Tokyo Univ. of Agriculture and
Technology
Koganei-shi, Tokyo Japan
nakit@cc.tuat.ac.jp

Hironori Nakajo
Tokyo Univ. of Agriculture and
Technology
Koganei-shi, Tokyo Japan
nakajo@cc.tuat.ac.jp

ABSTRACT

A Lens Free Imager (LFI) is one of promising candidates for massive visual inspection systems in biology and medical science. In a practical application, millions of samples need to be inspected in a short period of time, which a single Lens Free Imager (LFI) system cannot accomplish. Therefore, we propose a high performance parallel LFI system called RALFIE (Real-time Advanced LFI Evaluation system). In this paper, we introduce the concept and detail of the design of RALFIE and a target application.

CCS Concepts

•Computer systems organization → Embedded hardware; Real-time system architecture;

Keywords

Lens Free Imager; Embedded system; Digital holography; Reconfigurable computing; FPGA; Hardware acceleration

1. INTRODUCTION

1.1 What is LFI?

Lens Free Imaging is one of digital holographic technologies with no optical components such as lenses, mirrors and beam splitters, which are the most significant characteristics in a conventional optical microscope.

An LFI can be simply configured with only two major components: a light source and a camera sensor as shown in

Figure 1: Principle of an LFI.

Figure 1.

As constant light goes through a transparent material, it changes its phase and interferes with the diffraction light around the material. A latest CMOS camera in the market can capture the phenomena as waves spread horizontally as shown in Figure 2. Thus the interference can be simulated to be traced back where it occurs, as the result the original image can be drawn by a computing process called reconstruction as shown in Figure 3.

1.2 Towards massive samples

Recently, high demands for massive visual inspection systems are rising among biology and medical science as expectations to be industrialized grow rapidly, especially after emerging iPSC and cell therapy have been introduced to the public.

For many years, a phase contrast microscope, which converts phase shifts in constant light passing through a transparent material to brightness changes in the image, has been recognized as the standard tool for researchers. One reason is that cells and many other living objects for biologists are transparent, thus a conventional optical microscope cannot

Permission to make digital or hard copies of all or part of this work for personal or classroom use is granted without fee provided that copies are not made or distributed for profit or commercial advantage and that copies bear this notice and the full citation on the first page. Copyrights for components of this work owned by others than ACM must be honored. Abstracting with credit is permitted. To copy otherwise, or republish, to post on servers or to redistribute to lists, requires prior specific permission and/or a fee. Request permissions from permissions@acm.org.

soICT '16, December 08-09, 2016, Ho Chi Minh City, Viet Nam

© 2016 ACM. ISBN 978-1-4503-4815-7/16/12...\$15.00

DOI: <http://dx.doi.org/10.1145/3011077.3011097>

Figure 2: Captured raw image.

Figure 3: Reconstructed image.

be well applied to them.

However, it is not realistic to build a massive inspection system based on a phase contrast microscopy, which is significantly expensive and large in size due to complex optical components inside such as lenses, mirrors, and beam splitters. On the other hand, an LFI can be simply configured with a few components.

Moreover, inspection time of a single LFI unit is much shorter than that of a phase contrast microscope because of having no physical focus. Furthermore, sensitive biomaterials have to be less damaged by an inspection and also more samples can be handled in a certain time of period. Thus an LFI is one of promising candidates for massive visual inspection systems.

We have designed, implemented and evaluated a prototype of an LFI image capturing system as shown in Figure 4 and 5. We focus on laser diode as a light source and a camera sensor with an existing reconstruction algorithm to design the capturing system as a general purpose device in order to correspond to various kinds of requirements. We also have evaluated parameters related among mechanical accuracy, capturing mechanisms as well as parameters in

Figure 4: Prototype of the LFI system.

Figure 5: Block diagram of the prototype.

reconstructing for the future LFI system.

A few millions of specimen requires inspections for cell therapy using iPSC in a short period of time, probably within 15 minutes, in order to avoid damaging cells. A method of flow cytometry in micro fluidics will be used to guide floating cells in liquid medium to an LFI through a thin transparent tube. Qualified cells are stored back in the culture plate, and unqualified ones are guided to a tube to dispose or further inspections such as DNA checking. Cancer inspection from sampled blood will be investigated as well. Moreover, a future cell sorter will be built in the same system structure; however, faster processing speed will be required in order for sorted qualified cells to be sent back to patient's blood stream. As expected results, a cell sorter decreases cancer recurrence rate and reduce chemotherapy.

In the rest of paper, we show related works and our approach in section 2. In section 3, we introduce the new LFI system called RALFIE (Real-time Advanced LFI Evaluation system) as a massive visual inspection system. Section 5 describes designed detail for the RALFIE. Finally, we conclude section 6.

2. RELATED WORKS AND OUR APPROACH

Some LFI systems have been developed to be applied to limited materials. A research team at CALTECH shows that algorithms of LFI are feasible and generate images with fine resolutions[1]. Their system uses a micrometer sized pin hole for aperture and multiple light sources to enhance resolutions. At IMEC, reconstructed images from 4 captured raw images with different wavelength lasers show images to be sufficient for biological and medical inspections, using iPS cells as samples[2]. Later, another team at IMEC also shows that an LFI can be used to observe cardiac cells in a drug screening method[3]. These researches are still ongoing; however, their achievements have been expected for the advance of LFI technologies.

The prototype system shown in Figure 4 and Figure 5 has two capturing modes, which are full screen mode and clipping mode. With a full screen mode, it can capture by $4,208 \times 3,120$ at 22.5 Frames Per Second (FPS), and the size of $4,208 \times 3,120$ is all of pixels on the sensor. From our previous examination, this is as fast as the prototype device can handle. With a clipping mode, a rectangle area to be captured within the sensor can be assigned. For example, when some of rectangle is $1,024 \times 1,024$, it can capture 60 FPS, and its field of view is about a square of 1mm. This mode is useful for flow cytometry which is used to analyze physical characteristics of particles passing through in a micro tube filled with liquid medium. The field of view is wide enough to observe a micro tube. The prototype system also has precise control of laser system. It can control emitting timing and duration in real-time manner with the granularity of less than 5 microseconds, which is the most important factor to capture images in reasonable quality.

Our previous LFI prototype system consists of only 2 major components, laser and camera sensor without special mechanics. Distance and position between a laser light source and a camera sensor are not so sensitive to capture an image of a sample target. Therefore, adjustment is very easy to handle and its building cost is reasonable comparing to a generic optical microscope.

3. REAL-TIME ADVANCED LENS FREE IMAGER FOR MICRO FLUIDICS

In order to realize the target application, the proposed system has been designed to work fast and robust as a biomedical equipment, as well as easy to handle enough to be operated in a small clinic as an embedded appliance rather than PC based system. Also, we have configured multiple LFI units together to process a great number of samples in a short period of time, as a single LFI system.

Based on knowledges from various experiments with the LFI prototype, we have designed the system and a capturing device as a building block for the target applications.

3.1 System Design for Flow Cytometry

In flow cytometry, a sample is guided into a micro tube, whose diameter is less than 1 mm, one by one. In a tube, different flowing speeds around center and outer of the stream are manipulated so that the sample can be always observed in the center of the tube. It is very convenient for capturing and processing image however the way of keeping distance between samples constant is still under consideration and not commonly available yet. Especially, in our target appli-

Figure 6: Multi-LFI system for targeted applications.

Figure 7: Pair of LFI units for flowing cultural medium.

cations, a stream is designed to be split into multiple streams to increase the numbers of samples to inspect within a limited time.

As shown in the Figure 6, LFIs are set above the parallel tubes in which distances of flowing samples are different. Therefore, capturing timings of LFIs should be set differently. Theoretically, though it is possible to adjust tubes for all the LFIs to be synchronized by mechanically, it is significantly complex to adjust them accurately. Especially,

the system is expected to be equipped with many parallel tubes, which brings a disadvantageous cost for an LFI system against its simplicity and low-cost. Such issue should be overcome to realize a practical LFI system.

Though configuration with multiple tubes is essential to process a great amount of samples in a short period of time, data should be acquired from each tube independently. Some applications may need to keep informations on sample's origin, forked tube ID and a timestamp as attributes of captured data. Those attributes can be helpful to the system for adequate maintainability and traceability which are required regularly.

In a stream, the first LFI unit detects a cell and sends a signal to capture to the second unit. Thus severe real-time control is needed in two part. One is latency for the signal between 2 LFIs, and the other is timing to emit laser for capturing. Only two units in the same stream is tightly-coupled, and nodes between streams are loosely-coupled. This gives the system flexibility as shown in Figure 7.

Figure 8: Prototype system with a pair of camera sensors.

Figure 9: Block diagram of the prototype with dual camera sensors.

3.2 Dual Camera System

We have designed a prototype to explore the possible architecture for target applications as shown in Figure 8 and 9.

Basically, though the prototype has the same function as the first one shown in Figure 4, it has a MIPI CSI-2 switch which connects 2 camera sensors and selects one of them to acquire data by electronically switching data bus in the same way in which a smart phone switches a front and a back camera. Its switching speed is quite fast, and it can switch one to another within a period of a single switching frame at 60 FPS, 1/60 second, while both cameras are initialized properly and kept powered on.

The switching speed is so fast that the system can detect a sample by the first camera and capture the image of the sample by the second camera.

However, we realized that advantages of this dual camera system can be emulated by run a micro tube twice across a view field of a single camera. The way of the configuration is much cheaper than one with an extra camera in the system.

3.3 System Requirements

From the viewpoint of a medical equipment for the target application, though captured data must have only correct reference to its origin which is a sample contributor or an identifier for management, some other extra information needs to be recorded in order to maintain samples as well as shoot troubles.

- Maintenance

Although a generic CMOS camera sensor is packaged with protective glass cover above the surface of the sensor, we have a bare chip wire-bonded to a PCB to avoid interferences with cover glass. So, the surface cannot be touched or wiped off to clean micro dusts and water drops. Micro dusts are projected into a captured image as a black spot, so the system should keep tracks of those spots and issue an alert message.

- Trouble Shooting

The numbers of samples detected in each forked micro tubes is designed to be around the same. If the detected number of samples differs from another one significantly, the system would be suspicious with some trouble in tubing. Such trouble should be detected and predicted in order for the system to keep its availability.

- Traceability

In general, a regulatory requires an equipment to have reliable traceability from the origin of sample to examined results including pre and post cleaning process. Those recorded data and logs of processes are used to decide whether any part of tubing should be replaced when the system encounters a contamination. Moreover, for a critical application like a cell sorter, it is very important that the system can apply its critical action and take the responsibility.

Though these issues should be considered in the system, such a trouble doesn't have to be fixed in operating real-time. Therefore, the LFI system consisting of multiple paired LFI units is designed as a loosely coupled distributed parallel system.

4. SAMPLE DETECTION IN FLUID

In the target application, since the LFI system detects or counts only known particles, no unknown particles are assumed in medium. Detecting an unknown particle is handled as a fatal error called contamination.

However, instead of a real blood sample with a custom micro tube structure, we have adopted a substitutes for blood cells with simulating tubings in these experiments.

We have prepared two kinds of samples which can be commonly available. One is micro beads which are extracted from a toothpaste as shown in Figure 10, and the other is pollen of Japanese cedar as shown in Figure 11. The left side is captured raw image, and the right one is its reconstructed image.

There is the obvious size difference between a bead and pollen. It is similar to the relativity between a red blood cell and a white blood cell in a blood sample.

An LFI catches waves of inference which occurs around the boundary of two materials with different reflective index, and how liquid medium appears the same regardless of its

Figure 10: Captured and reconstructed images of micro beads extracted from a toothpaste.

Figure 11: Captured and reconstructed images of pollen of Japanese cedar.

speed. Thus simulated images in a fluid can be created from images in stilled liquid medium. We suppose that LFI is set to the clipping mode of 1024×1024 in the center of the sensor, and samples are flowing from left to right to discuss how to detect a sample in a fluid.

Series of 128×512 rectangles moving from left to right 1 pixel by 1 pixel are cropped from an image of a micro bead with a large empty area as shown in Figure 12. It is equivalent to what LFI gets when it is crossing the field of view. The difference is calculated from every 2 of them pixel by pixel and is added to represent amount of transiting intensity of brightness as shown in the graph in Figure 12.

Since a micro bead is solid and opaque, a large dark spot of the sample appear in the image reconstructed by an LFI. Thus the significant decrease of the intensity of brightness is shown in Figure 12 apparently.

Figure 13 is a reconstructed image for pollen of Japanese cedar. Since the sample is translucent, the reconstructed image shows a round shell. Since the sample has complex structure comparing to a bead, some peaks appear in the graph. However, the reconstructed target image is slightly obscure due to background noise brought by dusts on the surface of the sensor. These microscopic dusts are electrostatically clung to a surface of a sensor and are hard to be removed completely in a usual space except for a clean room. Despite noises appearing in the captured image, characteristics of flowing pollen are well detected by the system.

Characteristics of samples can be retrieved from raw images; however, they are not always clear to understand for us. A supervised machine-learning algorithm may be applied to the system. Samples of training data can be created eas-

Figure 12: Transiting intensity of brightness of a micro bead.

Figure 13: Transiting intensity of brightness of pollen.

ily by filtering samples in advance and inputting them into the system.

5. DESIGN OF CORE UNIT FOR REAL-TIME ADVANCED LFI

An FPGA SoC, Xilinx Zynq, has been adopted for core of a single board computer as a building block.

5.1 Key Features

In order to implement a high performance real-time LFI system, the following features are required for the target applications.

- Real-time synchronization mechanism
 - The camera sensor and laser diode controller to capture qualified samples
 - The flowing speed of a single stream can be stabilized as constant; however, the distance between flowing samples cannot be easily kept.

Figure 14: Block diagram of Zynq based prototype.

- Fast I/O to store and transfer data
As a bio-medical equipment, it is required to save original data. Building a huge storage system is not our concern; however, the system must have capability to send data to a backend storage without any fail.
- Standard developing environment for parallel and distributed computing
We expect that the system needs to be integrated to correspond to many of applications.

5.2 Zynq Based Single Board Computer

A MIPI bridge for a camera sensor and DRAM is attached to Programmable Logic (PL), and a laser controller as well as real-time syncing mechanism will be implemented as hardware logic. So, the system will have benefits from both of hardware real-time capabilities and Linux on cortex as shown in Figure 14.

5.3 Expected Performances

It is supposed that a single LFI node can capture and process data in 60 samples per second thus 36,000 samples would be reconstructed within 10 minutes. It means that the system needs to consist 28 sample streams to process one million samples in 10 minutes. Thus the system has fifty six LFI units altogether when a node has a pair of LFI unit per a stream.

5.4 Network Based Large System and Security

Since the proposed machine can learn characteristics of iPS cells and cancer cells, an LFI embedded with the knowledge will be able to evaluate cells. Moreover, connecting all of LFIs to a cloud computing system can extend the knowledge to higher level. One of our goals is to establish an expert knowledge system that can be accessed from anywhere to provide professional services available from anyone by information technologies.

Any data related to iPS cells, especially an image of culturing process should be kept confidential and also information of cancer treatment is significantly private. Since these sensitive information must be handled securely, we proceed following steps to embedded security technologies into the architecture of RALFIE.

- Evaluation of automatically judging a sample by machine learning:
We will implement a knowledge-based qualification algorithm, using result of machine learning to an LFI and evaluate its performance.
- Sharing data and knowledge by cloud technologies:
We will create a cloud system to aggregate all of evaluation results and acquire images from many LFIs via network and try to find a greater knowledge by machine learning. At the same time, we will reinforce security of the LFI system.

6. CONCLUSION

RALFIE is a parallel processing system, which consists of many paired tightly-coupled LFIs. The size of a practical system will be decided by an amount of samples and required time to process in a specific application. In the current target application, the whole system consists of RALFIE and micro fluidics for the bio-medical field, especially, for real-time cell inspection. From our estimation, fifty six processing nodes might be required to capture a million of images within ten minutes. RALFIE will contribute emergence of practice for regenerative medicine and cell therapy. In the future, we are going to build the RLAFIE system to evaluate the performance as well as reliability to utilize it for iPSC and cell therapy with comparing other optical imagers.

7. ACKNOWLEDGMENTS

This work was partially supported by MEXT Fund for Smart Space Technology toward Sustainable Society and by JSPS Grant-in-Aid for Scientific Research (C) Number 25330067 and 16K00078.

8. REFERENCES

- [1] Sang Bok Kim, Hojae Bae, Kyo-in Koo, Mehmet R. Dokmeci, Aydogan Ozcan, Ali Khademhosseini: "Lens-Free Imaging for Biological Applications", Jala-J. Lab Autom. 2012;17: pp. 43-49 (2012)
- [2] Richard Stahl, Geert Vanmeerbeeck, Gauthier Lafruit, Roeland Huys, Veerle Reumers, Andy Lambrechts, Chao-Kang Liao, Chin-Chun Hsiao: "Lens-free digital in-line holographic imaging for wide field-of-view, high-resolution and real-time monitoring of complex microscopic objects", SPIE 8947, Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XII, 89471F (2014).
- [3] Thomas Pauwelyn, Veerle Reumers, Geert Vanmeerbeeck, Richard Stahl, Stefan Janssens, Liesbet Lagae, Dries Braeken, Andy Lambrechts: "Label-free cardiac contractility monitoring for drug screening applications based on compact high-speed lens-free imaging", SPIE 9328-43, Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XIII (2015)