J. Biophotonics 5, No. 8–9, 650–660 (2012) / DOI 10.1002/jbio.201200041

Journal of BIOPHOTONICS

REVIEW ARTICLE

Lab-on-a-chip biophotonics: its application to assisted reproductive technologies

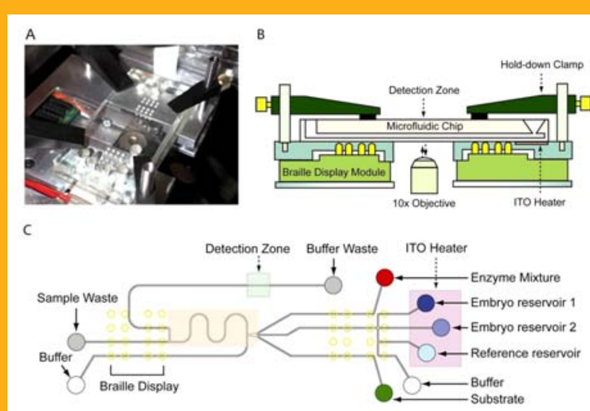
David Lai[1], *Gary D. Smith*[2,3,4], *and Shuichi Takayama*[*,1,5,6]

[1] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA
[2] Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI 48109, USA
[3] Department of Urology, University of Michigan, Ann Arbor, MI 48109, USA
[4] Department of Molecular and Integrated Physiology, University of Michigan, Ann Arbor, MI 48109, USA
[5] Macromolecular Science and Engineering, University of Michigan, Ann Arbor, MI 48109, USA
[6] Division of Nano-Bio and Chemical Engineering WCU Project, UNIST, Ulsan, Republic of Korea

Received 6 March 2012, revised 15 May 2012, accepted 17 May 2012
Published online 12 June 2012

Key words: Lab on a chip, microfluidics, assisted reproductive technology

With the benefits of automation, sensitivity and precision, microfluidics has enabled complex and otherwise tedious experiments. Lately, lab-on-a-chip (LOC) has proven to be a useful tool for enhancing non-invasive assisted reproductive technology (ART). Non-invasive gamete and embryo assessment has largely been through periodic morphological assessment using optical microscopy and early LOC ART was the same. As we realize that morphological assessment is a poor indication of gamete or embryo health, more advanced biophotonics has emerged in LOC ART to assay for metabolites or gamete separation via optoelectrical tweezers. Off-chip, even more advanced biophotonics with broad spectrum analysis of metabolites and secretomes has been developed that show even higher accuracy to predicting reproductive potential. The integration of broad spectrum metabolite analysis into LOC ART is an exciting future that merges automation and sensitivity with the already highly accurate and strong predictive power of biophotonics.



Integration of LOC with simple biophotonics [35]. Currently most advanced LOC requires a large amount of supporting equipment, limiting its integration into complex photonics. Progress in implementation of microfluidic operations without the addition of supporting equipment will make LOC integration with highly complex photonics practical.

1. Introduction

Microfluidics is a relatively new field studying the physical principles of fluid behaviour that have already been extensively applied in chemistry [1] and molecular biology [2]. More recently, microfluidics has also become increasingly applied to cellular behaviour and interaction studies [3–5]. The scalability

* Corresponding author: e-mail: takayama@umich.edu

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

of microfluidics allows for unprecedented cost efficiency due to the minute amount of reagents necessary allowing for the execution of otherwise expensive experiments and automation provided by embedded microfluidic features can allow for high sensitivity without user variability. For cell culture applications, the small volumes can enhance autocrine effects although nutrient depletion and waste accumulation also becomes faster. At the microscale, the flow of even familiar fluids like water and aqueous solutions also becomes dramatically different due to a low Reynolds number, the ratio between inertial forces and viscous forces. When viscous forces dominate, a phenomenon called laminar flow results that where the fluid quickly and reliably moves in response to external forces. When two or more streams converge in laminar flow conditions, they do not mix save for the effects of diffusion due to a lack of turbulence.

These small volume and low Reynolds number flow conditions can be utilized to more closely resemble *in vivo* conditions of insemination and embryo culture than classical *in vitro* methods using culture dish, test tube or drops of media. In 1979, it was first reported that the microenvironment holds a dramatic importance in embryo handling and culture [6]. More recently, it has been shown that dynamic stimulation of oocytes increase fertilization rates [7] as well as increase in oocyte health [8].

Microfluidic devices are typically composed of a transparent elastomer and plastics such as PDMS and polystyrene. This allows many lab-on-a-chip (LOC) applications to use biophotonics for data acquisition. For assisted reproductive technologies (ART), microfluidics provides a tool for non-invasive cell quality assessment especially for precious cell samples such as oocytes and embryos. The speed at which samples can be sorted using microfluidic operations also allow for high-throughput screening in ART particularly for sperm samples.

2. Basic use of biophotonics in LOC ART

Less than half of *in vitro* fertilization (IVF) cycles result in a live birth even for women below the age of 35 that causes severe physical, emotional and financial stress [9]. The success decreases even more with frozen embryos and age. Thus embryo, oocyte and sperm quality assessment is an important procedure to identify the healthiest oocytes and sperm for IVF and the embryos with the highest reproductive potential for implantation. However even now, embryo morphology and cleavage rates are the most common form of embryo quality assessment. Early LOC ART technology also involved direct quality assessment with periodic morphology assessment mostly based on optical and at times fluorescence imaging.

2.1 LOC morphology assessment of oocytes and embryos

It was first shown in 2000 that microfluidic channels supported normal embryo development [10] and later in 2001 to show that many microfabrication materials showed good biocompatibility with murine

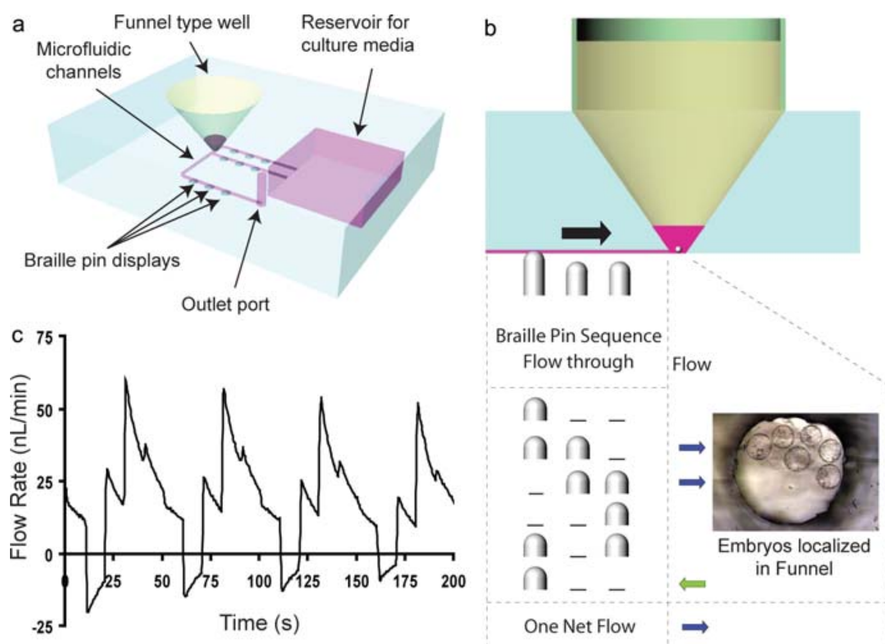


Figure 1 (online color at: www.biophotonics-journal.org) (**A**, **C**) Dynamic microfunnel architecture provides both autocrine factor retention and mechanical stimulation. (**B**) The accessibility of the funnel allows for easy embryo introduction into and extraction from the device via simple pipetting. Outlet channel also provided an effective method to prevent the accumulation of toxins from metabolic waste [14].

 © 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Journal of
BIOPHOTONICS

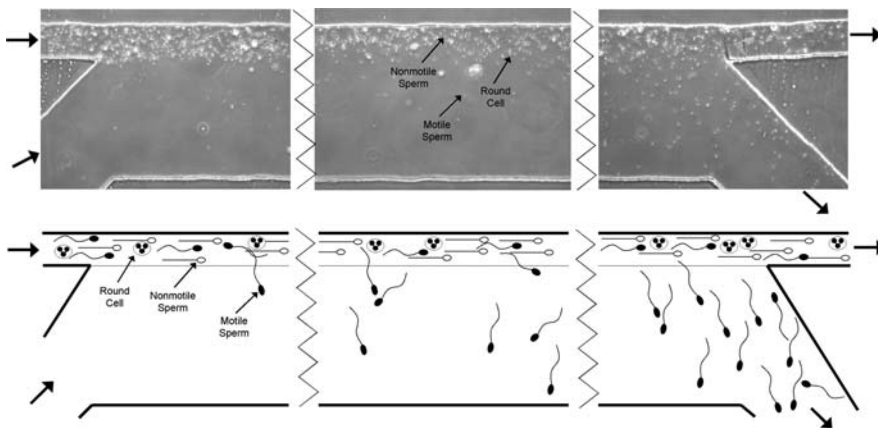


Figure 2 A passively driven sperm sorter was built using surface tension and gravity as a driving force and laminar flow regime. Flow is from left to right. Sperm sample is loaded in the reservoir at the top left. Dead sperm, non-motile sperm and debris are carried straight across the top stream while motile sperm are able to swim under their own power and cross streamlines into the collection reservoir in the bottom right [19].

embryos [11]. Both studies used periodic optical microscopy to assess embryo morphology. Degree of biocompatibility was reported as the percentage of two-cell embryos developing into blastocysts by 96 hours compared to petri dish control.

Microfluidic devices later showed functionality of high-throughput single oocyte trapping, fertilization and embryo culture. Allowing multiple embryos to be individually monitored and tracked during their development showing comparable fertility rates and embryo development to petri dish control [12]. The device improved upon classic embryo culture methods with ease of use through automation that simplified an otherwise labour intensive media changing process in IVF. Due to the scale at which the microfluidic device operates, it only required a small amount of sperm sample for successful fertilization as opposed to its macro-scale counterparts [13].

Meanwhile with specialized channel architecture to produce dynamic fluid mechanical stimulation while maintaining autocrine factor distribution that mimic *in vivo* microenvironments (Figure 1), embryos cultured in microenvironments more resembling *in vivo* conditions had more reproductive potential than petri dish controls [14]. This too used periodic optical microscopy and morphology assessment for measure showing an increase in blastocyst development as well as blastocyst cell number as determined by terminal invasive evaluation on fixed and stained embryos. Rare among LOC ART, this study used viability assessment such as implantation percentage and ongoing pregnancy as a primary outcome and showed over 20% improvement by using dynamic microfluidic culture. The device was driven using peristaltic actuation of Braille pins allowing for portability and convenience without the need for extensive syringe pumps and lines connected to the device common to many microfluidic devices. The microfunnel architecture also provided accessibility of the oocytes in both insertion into and extraction from the device via simple pipetting. As opposed to

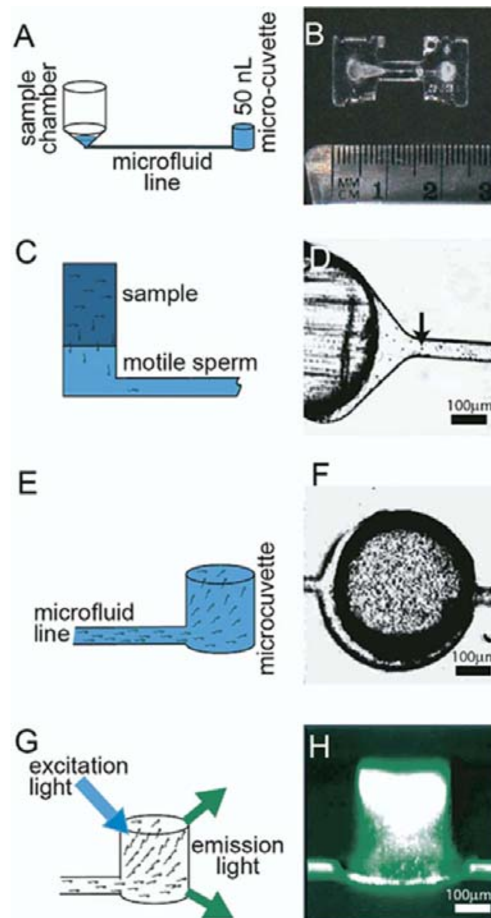


Figure 3 (online color at: www.biophotonics-journal.org) (**A**–**B**) A microfluidic line connects the sample reservoir to a 50 nL micro-cuvette. (**C**–**D**) The microfluidic line which serves as a selective filter to separate motile sperm from dead sperm, non-motile sperm and debris is 52 μm^2 in cross section and 6 mm in length. (**E**–**H**) Sperm is labelled with Calcein-AM and upon reaching the micro-cuvette are excited by excitation light and the fluorescent light emission is detected to assess the number of motile sperm reaching the micro-cuvette over a 50 minute period [20].

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

culture in small droplets in a petri dish, LOC ART devices are capable of extracting media during culture through the outlet channel to prevent accumulation of toxic metabolic waste such as ammonia [15] which may harm embryos [16].

2.2 LOC sperm morphology assessment

Currently, the most successful treatment for male infertility is using intracytoplasmic sperm injection (ICSI) where oocytes are fertilized via direct injection of sperm. Requiring only a small amount of viable sperm, ICSI drastically increases chances of contraception even for severe cases of sperm-based infertility. While theoretically only one viable sperm is required per harvested oocyte, identifying and isolating the most viable sperm is challenging [17]. Typically samples are purified using centrifugation methods but for difficult samples, laboratories may even hand-sort semen samples consisting of mostly dead sperm and debris to isolate the most motile sperm with healthy morphology. In some cases of extremely low sperm counts, the procedure could take hours making automated sperm sorting a welcome technology in ARTs.

Using microfluidic channels to produce mild, biomimetic sorting mechanism based on sperm motility avoids centrifugation reported to cause sublethal damage to sperm [17, 18]. Fluid flow using laminar flow regime consists of multiple streamlines. These streamlines are parallel to one another according to channel geometry with the absence of turbulent mixing. Dead sperm, nonmotile sperm and debris are unable to move on their own and thus are simply carried by the streamline they reside in towards the waste reservoir. However, motile sperm are able to swim under their own power and are capable of moving across streamlines into a secondary reservoir designated to contain only motile sperm for extraction [19]. With surface tension and gravity as the driving force, the channel geometry or loading conditions can be controlled to adjust initial surface tension to vary the motility threshold of sperm collected and purity.

A device using fluorescence was designed using a built-in micro-cuvette for male subfertility screening. A long microfluidic channel connected the sample chamber to the micro-cuvette produced a barrier that allowed only highly motile sperm to reach the micro-cuvette. Calcein-AM dyed sperm can be quantified using a microfluorometer to assess the level of sperm motility [20] where higher fluorescent signal indicates higher motile sperm count. With signal recorded every 30 s for 50 minutes, a profile of sperm motility can be deduced.

2.3 Pitfalls of morphology assessments

It cannot be ignored that morphology is a poor indicator of viability such as viable fetus and live birth. A major pitfall for basic use of biophotonics in LOC ART applications that limits their capability for translational human ART research, it opens a large demand for much more sophisticated biophotonics to provide more accurate primary outcome assessments. An issue spanning all fields of ART, not just LOC, it has allowed for an opportunity in ART for advanced predictive algorithms for long term viability assessment using data from simple photonics equipment that can eventually be integrated into LOC to improve primary outcome assessments.

3. Advanced use of biophotonics in LOC ART

With the increasing popularity of biophotonics, the integration of this technology into LOC ART has been exciting. In recent years, the use of continuous imaging, optical tweezers, traps and broad spectrum spectroscopy has widened the amount of non-invasive analysis for ART purposes that provide quantitative information instead of qualitative morphology assessments.

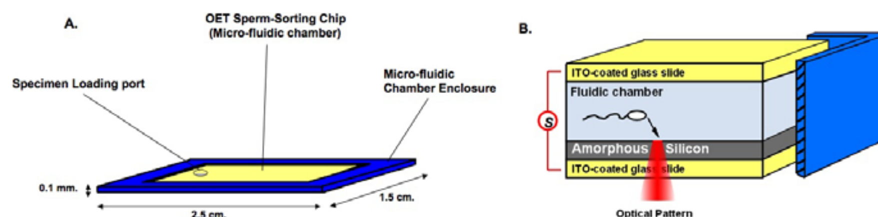


Figure 4 (online color at: www.biophotonics-journal.org) Live cells due to the ability to maintain an ionic gradient to the extracellular space are highly polarized in an electric field. While dead cells have no gradient are not polarized. (**A**) sample is loaded into a microfluidic chamber for OET sorting. (**B**) Live cells experience attractive forces towards regions of high electric field while dead or dying cells will have weak or no repulsive forces [23].

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

3.1 Sperm quality assessment and sorting

In cases of extreme male infertility with the absence of motile sperm, ICSI is still capable of producing live births with non-motile but live sperm. This leaves a great need for the identification of live sperm, even if they are non-motile; a task that is labour intensive and currently requires single sperm isolation and exposure to hypo-osmotic solutions and visualization of membrane swelling [21]. Devices were coated with a polyethylene glycol (PEG) antifouling layer and layered with a photosensitive layer to be used for optoelectric tweezer (OET) applications. Projected light reduces the impedance on the photosensitive layer to create regions of high electric field. The field generated produces dielectrophoretic force to manipulate micro- and nanoparticles. It was shown that OET is capable of non-invasive sorting of viable non-motile sperm from non-viable sperm without introducing DNA damage [22]. Due to a live cell's ability and a dead cell's inability to keep intact a charge gradient between its cytoplasm and extracellular microenvironment, they respond differently to an electric field. Since live cells have a higher dipole moment by maintaining an ionic gradient across its cell membrane, live cells experience attractive forces toward regions of high electric field while dead or dying cells have no or repulsive forces to the electric field (Figure 4) [23]. This OET selection process improves upon existing technologies to retrieve viable sperm independent of its motility overcoming significant challenges in the ICSI sperm selection process particularly for those with extreme infertility complications.

In situations with an abundance of motile sperm, the selection process for the ideal sperm for ICSI is often arbitrary. A group in 2008 developed an automatic microscope system for high-throughput sperm analysis and sorting using an annular laser trap [24] that previously required laborious joystick control. In addition to recording sperm swim speed using automated cell tracking technology, the system is capable of relocating sperm and releasing them at normal or tangential enter angles against an annular laser trap to which the swim behaviour in response to annular trap is assessed and categorized into three major groups: speeding up in response to and penetrating the annular trap, sperm changing its swimming trajectory and slides briefly along the annular trap, and sperm changing its swimming trajectory and slides along the annular trap for an extended period of time (20 s) showing signs of fatigue.

Although not used routinely for human ART, biophotonics combined with microfluidic flow cytometry is now routinely applied for sex preselection of sperm in the agricultural industry. Sperm is extracted from the bull and its DNA stained with Hoescht 33342. Using an argon laser for excitation,

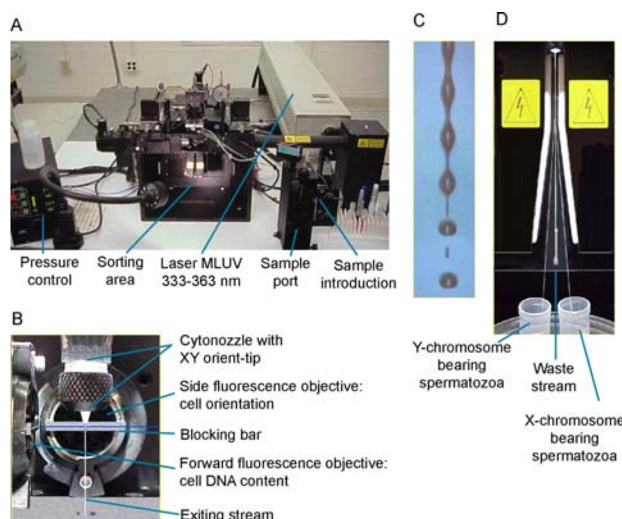


Figure 5 (online color at: www.biophotonics-journal.org) (**A**) Overview of sperm sorter set up. Computer not included. (**B**) Integration of optics and hydrodynamics. (**C**) Instabilities in the stream are applied via mechanical vibrations. (**D**) Droplets are given either a negative or positive charge depending on their sex. Under an electric field, the droplets experience a horizontal force that directs them into the collection tubes [25].

the resulting fluorescence signal allow accurate and high-throughput identification resulting fetal sex according to difference in DNA content, and therefore difference in fluorescence intensity between X- and Y-chromosome carrying sperm (approximately a 3.8% increase in signal due to larger X-chromosome). After identification of sex, the droplet containing the sperm is charged according to the sex and deflected using a charged plate into collection tubes (Figure 5). Flow cytometry combined with fluorescence detection and downstream cell sorting were capable of preselecting the sex of calves with 90% accuracy and produced live calves without anatomical or neurological abnormalities. This system is capable of high-throughput sperm sorting up to 25,000 spermatozoa per second [25]. Although both cytometer and detector are capable of much higher speeds, 25,000 spermatozoa per second was empirically determined as optimal due to limitations stemming from the need to individually encase each spermatozoa in its own droplet for accurate DNA assessment.

3.2 Direct oocyte or embryo quality assessment

Like morphology assessment of spermatozoa, selection of mature oocytes is also often subjective. A quantifiable measure is needed to eliminate subjec-

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

tivity for optimal selection of the healthiest oocytes for IVF. However the need for non-invasive tests limits the number of biophotonic spectroscopies appropriate for direct analysis of precious cell samples.

Oocytes are positioned in the middle of two optical fibres within a microfluidic device by holding micropipettes commonly used in IVF micromanipulators for ICSI. With a white light illumination fibre and a collection fibre, the absorption spectra can be measured. The absorption spectra provided a quantifiable measure of oocyte maturity by comparing different spectra maxima and width. A fully immature oocyte was found to have maxima of ~630 nm wavelength and a width of ~270 nm while a fully mature oocyte was found to have maxima of ~580 nm and a width of ~210 nm (Figure 6) [26]. Using the absorption spectra, the subjectivity of determining maturity has been eliminated with quantifiable measures.

Optoelectric tweezers were also used to separate normal oocytes from abnormal oocytes. Via the same mechanism as for sperm, normal oocytes are able to move against gravity under OET induced electric field while abnormal oocytes stay stationary at their initial starting point [27, 28]. With selective discrimination of healthy oocytes, OET induced electric fields provide another non-subjective mechanism for selecting oocytes with the best developmental potential.

The advancement of LOC ART optical microscopy from periodic to continuous imaging has enabled the control of osmotic shock associated with oocyte cryopreservation. Vitrification is the transformation of a substance into glass and the vitrification of oocytes has recently been shown to be more successful than classical slow-freezing cryopreservation in establishing pregnancy [29]. However to prepare

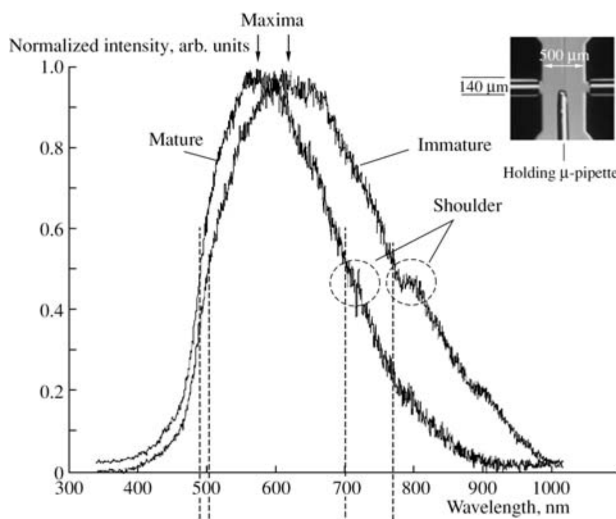


Figure 6 The degree of oocyte maturity can be analysed using the absorption spectra by assessing the location of maxima and spectral width [26].

oocytes into vitrification solution, a highly hyperosmotic solution, oocytes are transferred stepwise into ever increasing concentrated CPA solutions while suffering from an osmotic shock on each step. Studies have been conducted to show that using extra transfer steps with less change in concentration on each step decreases osmotic shock and increases cryopreservation quality [30] but at the cost of practicality as the transfer of oocytes is delicate, prone to accidents and requires extensive training [31]. With the precision and sensitivity afforded by microfluidics, LOC is capable of exposing oocytes to a continuous profile of gradually increasing CPA concentrations. With a continuous profile, essentially an infinite amount of steps, and microfluidic automation and continuous optical microscopy, the oocytes were shown to be successfully exposed to vitrification solutions with dramatically less osmotic shock and labour than manual controls [32].

3.3 Indirect oocyte or embryo quality assessment

As understanding of reproduction and associated technologies become more advanced, simple measures of oocyte quality such as morphology and maturity become insufficient [33]. Indirect oocyte or embryo quality assessment opens the full field of biophotonic spectroscopies to quantify cell quality. Indirect assessments typically perform spent media analysis to calculate oocyte or embryo metabolism. Embryo metabolism provides a much clearer indication of embryo health. For example, mouse blastocysts with low glycolytic activity (closer to *in-vivo* levels) have been shown to indicate high viability [34] and human blastocysts with the highest glucose uptake were correlated with the highest reproductive potential [35].

An automated, computer-controlled microfluidic device was designed for continuous embryo culture with integrated spent media analysis on-chip. Using a versatile Braille display for pumping and valving, the chip is capable of automated glucose measurement assays with fluidic sampling, pumping, mixing and washing steps (Figure 7) [36]. The glucose assay uses coupled glucose oxidase-peroxidase reaction to produce fluorescent resorufin from colourless amplex red reagent at a 1:1 stoichiometry. The long emission wavelengths of resorufin (587 nm) allow for sensitive fluorescent quantification of glucose. The chip is capable of real time and continuous culture with measurements every 5 seconds for over 6 hours. Use of a glucose assay that utilizes long excitation wavelengths (488 nm) are safer for embryos than typical ultraviolet light used in NADPH-linked glucose assays previously used for off-chip embryo

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Journal of
BIOPHOTONICS

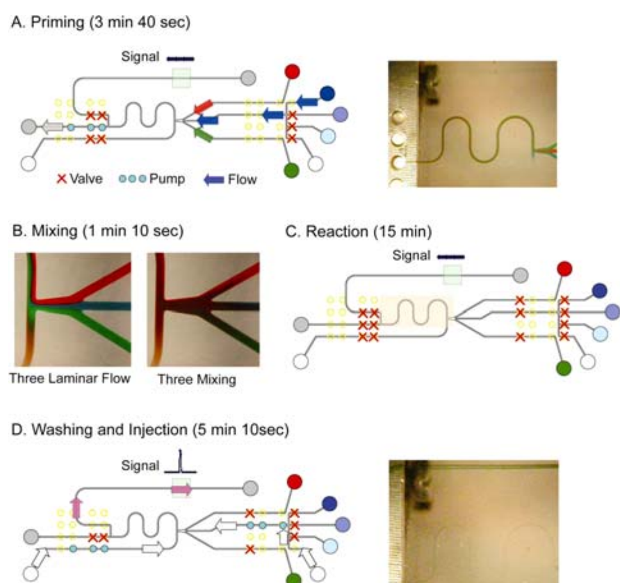


Figure 7 (online color at: www.biophotonics-journal.org) Braille-driven device is highly programmable capable of complex operations such as (**A**) priming, (**B**–**C**) mixing and reactions, and (**D**) washing and injection with minimal support from external sources unlike typical syringe-driven devices that require additional syringe pumps for complex operations [36].

metabolism measures. This was important to prevent exposure of embryos to damaging UV light.

The need of shielding embryos from harmful UV light was the basis of other groups using two devices to perform LOC embryo metabolism measurements to separate embryo cultures from the metabolite assay device later to be subject to UV light. This method was capable of measuring more metabolites using shorter excitation wavelengths but required periodic manual transfer of spent media from embryo culture to microfluidic device. Spent media was extracted via pipetting from microdrops containing embryos on a petri dish and transferred into a microfluidic device. Upon manual loading of spent media sample and reagents necessary for performing glucose, pyruvate, and lactate enzymatic assays in full automation involving enzyme cocktail aliquotting, mixing of reagents, data acquisition, and data analysis [37]. The device is also capable of serial measurements of metabolites within 5 minutes and full automated analysis of 10 samples with intermittent calibration in a 3 hour period.

4. Future of biophotonics in LOC ART

Due to low pregnancy rates per embryo transferred in IVF, IVF treatments typically transfer multiple embryos simultaneously to increase pregnancy potential. However with each addition of an embryo, the chance of multiple births dramatically increases. Single embryo transfer (SET) is an effective technique to minimize risks of multiple pregnancies but its acceptance is limited due to the inaccuracy of embryo reproductive potential assessments [38]. For SET to gain popularity, a more selective process for the embryo with highest reproductive potential is needed. Biophotonics has made significant advances particularly in metabolic profiling for ART. However current LOC metabolic profiling requires assaying for a specific metabolite of interest. When multiple metabolites of interest are involved, the LOC quickly becomes convoluted in design. The integration of LOCs with external optics also becomes more difficult due to the need to place supporting optics and syringe pumps within close proximity to the device. However groups within the biophotonics community, although not on LOC devices, have shown the use of spectroscopy capable of capturing a broad spectrum of metabolites without the need to assay for a specific metabolite.

Although not photonics, nuclear magnetic resonance (NMR) spectroscopy is a powerful technique for non-invasive examination of biological fluids. Based an atomic nuclei's magnetic properties, NMR provides data rich in simultaneous structural and quantitative information capable of screening urine samples for hundreds of pathophysiological conditions in a single spectral measurement [39]. However the technique is expensive in both time and financial costs, although some measurements can take less than a minute, some may take a few hours. NMR also requires highly specialized personnel not always available in a clinical setting to operate equipment and analyse results.

Mass spectroscopy (MS) has also emerged as a popular tool for metabolic profiling providing advantages over other spectroscopies such as the ability to detect many metabolites at physiological conditions from μM concentrations with a high mass accuracy in parts per million [40]. MS was shown to not only be able to detect metabolites consumed by embryos, but also the secretomes of mouse and human embryo. Noting a dramatic difference in secretomes of degenerating embryos and a developing blastocyst [41], MS is providing a new field of opportunities to understand embryonic physiology.

Although not as specific and detailed in information provided compared to NMR, a group used spent embryo media with near-infrared (NIR) spectroscopy to distinguish viable embryos from non-viable embryos independent of morphology. Spectral regions (ROH, –SH, C=C, –OH and –NH groups) of metabolic profiles were quantified using inverse least-squares regression and leave-one-out cross validation to produce a relative embryo viability score

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

to assess embryo reproductive potential [42]. NIR broad spectrum metabolic profiling was shown to be superior to morphology assessment for selective embryos for SET to produce an on-going pregnancy [43]. At day 2 after SET, metabolic profiling was shown to be 40% more accurate in assessing viable embryos than morphology (69% and 27.6%). At day 3 after SET, it was 15% more accurate with 53.6% accuracy with NIR metabolic profiling as opposed to 38.5% for morphology. The study showed a large inter-patient or inter-embryo variability with regards to the calculated viability score and more studies are needed to identify the causes of this variability. Nevertheless, a low viability score was highly predictive of poor pregnancy. In another study, NIR was shown to have 75% sensitivity at predicting embryo implantation and resultant delivery between binary groups of patients (0 or 100% implantation rates) [42]. With each study, NIR metabolic profiling proves to be a powerful analysis to be used in embryo selection for SET.

Raman spectroscopy uses a laser of monochromatic light that provides similar but complimentary results to NIR spectroscopy. Raman spectroscopy is superior to NIR for aqueous samples due to water producing very weak Raman signals. Raman spectroscopy based metabolic profiling by itself was capable of 80.5% accuracy for predicting (100%) implantation and associated delivery or (0%) failed implantation [44]. In agreement with previously described studies of metabolic profiling, there is a clear correlation between metabolism and reproductive potential for human embryos leaving no question that metabolic profiling is a powerful tool for clinical screening for the best embryos for procedures such as SET in the future.

Currently, embryo cultures were done in microdrops consisting of 50 μL media. Raman and NIR spectroscopy can be performed with 15 μL of spent media in approximately one minute [42]. Its integration into LOC technology will provide quantitative information on how recreating physiological conditions during culture increase embryo reproductive potential. Using only a fraction of media necessary with conventional microdrop embryo culture, spent media from LOC devices will have a higher ratio between embryos and surrounding media allowing for higher concentrations of secretomes for heightened detector sensitivity to embryo secretion rates. Automation provided by LOC ART promises a less laborious process as well as elimination of operator variability.

The advancement from periodic to continuous imaging alone delivered incredible insight on human embryo development. Continuous monitoring with dark field microscopy is capable of predicting human embryo development into the blastocyst stage with 93% sensitivity by measuring only three parameters by day 2 after fertilization (Figure 8) [45]. This study used an automatic computer algorithm for real-time embryo development monitoring and prediction showing that with continuous imaging, promise of advanced diagnosis of embryo potential. The integration of this highly accurate predictive technology to LOC ART applications will greatly increase its viability assessment power and address one major pitfall to LOC ART applications translational human research.

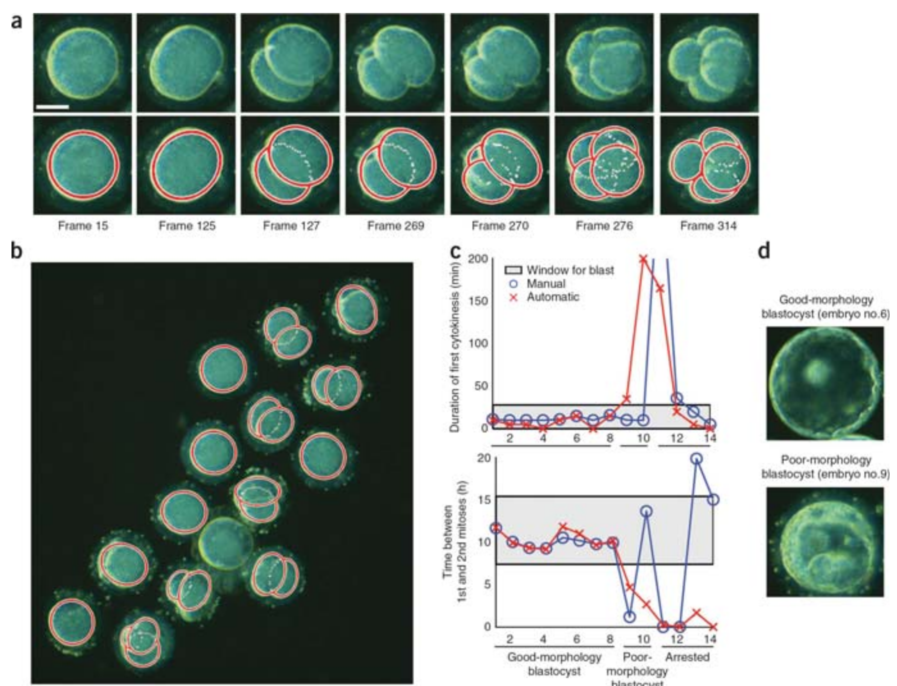


Figure 8 (online color at: www.biophotonics-journal.org) (**A**–**B**) Continuous imaging combined with automatic monitoring algorithm. (**C**–**D**) By analysing parameters such as duration of first cytokinesis and time between 1st and 2nd mitoses, the algorithm is capable of predicting whether embryos will develop with good and bad morphology [45].

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Figure 9 With each additional operation in syringe-driven LOC devices, more supportive equipment is necessary (each tube is typically connected to a syringe pump not pictured). With complicated LOCs, it becomes increasingly difficult to integrate with also complicated optics due to spatial limitations [37].

Integration of spectroscopy with microfluidics that allows for broad spectrum analysis of metabolites or secretomes poses significant challenges. LOC automation typically necessitates microfluidic operations embedded into the device. With each operation (mixing, diluting, valving), the device often becomes much more complicated requiring supporting equipment off-chip such as additional syringe pumps (Figure 9). For this reason, LOC is often a lab-on-a-chip-in-a-lab of specialized equipment, a major challenge to many biology and chemistry labs adopting the technology. Flexibility of operations such as valve timings is limited due to the built-in automation nature of LOC. So often when rather small features need adjustment, entirely new masters need to be prepared using photolithography, a specialized technique involving equipment also not available to most chemistry and biology labs. To address these issues to increase the usability of LOC technologies to a broader scientific community, many groups make progress in developing easy fabrication techniques without the use of photolithography [46] and using intelligent design built-in micro geometries to implement microfluidic operations without the use of additional supporting equipment [47].

Acknowledgements We thank the NIH (GM096040-01), the NSF (DBI0852802), and the WCU (World Class University) program (No. R322008000200540) through the National Research Foundation of Korea (NRF) as funded by the Ministry of Education, Science and Technology (MEST) for support.

David Lai received his B.S. from the University of California, Irvine in Biomedical Engineering and M.S. from the University of Michigan. He is currently working on his Ph.D. at the University of Michigan under the advisement of Dr. Shuichi Takayama in the Department of Biomedical Engineering. His dissertation work is on automated and optimal ART vitrification through elegant design in microfluidics.

Gary D. Smith is currently Director of the Reproductive Sciences Program and Co-Director of the Consortium for Stem Cell Therapies at the University of Michigan. He is a Professor in the Departments of Obstetrics and Gynecology, Molecular and Integrated Physiology, and Urology. In addition, he is Scientific Director of Huntington Center for Reproductive Medicine of Brazil and visiting Professor at the University of Sao Paulo. He completed his Ph.D. and Postdoctoral training at Washington State University and the Oregon Regional Primate Research Center, respectively. Within his research laboratory, investigations focus on regulation of oocyte meiosis and chromatin segregation, male and female gamete cryopreservation, preimplantation embryo development, and integration of new biotechnologies (microfluidics and artificial matrices/scaffolds) in gamete/embryo/ human embryonic stem cell isolation, culture, and selection. Studies in his laboratory range from very basic in nature to translational research aimed at introducing new, or improving existing, means of preserving fertility or treating infertility.

Shuichi Takayama's research interests (B.S. & M.S. from the University of Tokyo, Ph.D. from the Scripps Research Institute) started with organic synthesis. Subsequently he pursued postdoctoral studies in bioengineered microsystems at Harvard University as a Leukemia and Lymphoma Society Fellow. He is currently Professor at the University of Michigan in the Biomedical Engineering Department. He constructs microfluidic

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

models of the body such as the oviduct, lung, and cancer metastasis. He also develops aqueous two phase system micropatterning technologies, studies timing and rhythms of cell signaling, constructs self-switching fluidic circuits, and performs nanofluidic single molecule DNA analysis.

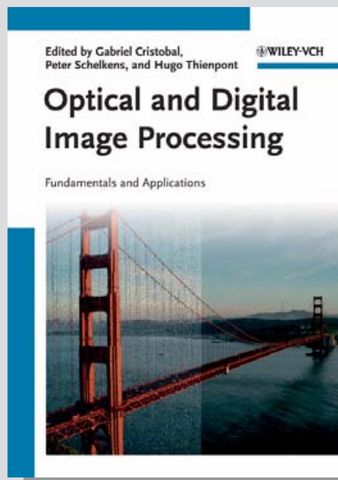
References

[1] A. J. Tomlinson, N. A. Guzman, and S. Naylor, J. Capillary Electrop. **6**, 247–66 (1995).
[2] D. Beebe, M. Wheeler, H. Zerinque, E. Walters, and S. Raty, Theriogenology **57**, 125–135 (2002).
[3] J. Shim, T. F. Bersano-Begey, X. Zhu, A. H. Tkacyk, J. J. Linderman, and S. Takayama, Curr. Top. Med. Chem. **3**, 687–703 (2003).
[4] S. Chung, R. Sudo, V. Vickerman, I. K. Zervantonakis, and R. D. Kamm, Ann. Biomed. Eng. **38**, 1164–1177 (2010).
[5] A. Folch and M. Toner, Ann. Biomed. Eng. **2**, 227–256 (2000).
[6] S. M. Willadsen, Nature **277**, 298–300 (1979).
[7] A. Nir, J. Theor. Biol. **214**, 171–179 (2002).
[8] H. B. Croxatto, Reprod. Biomed. Online **4**, 160–169 (2002).
[9] Society for Assisted Reproductive Technologies. ART Success Rates report (2009).
[10] J. A. Davis, S. Raty, D. T. Eddington, I. K. Glasgow, H. C. Zeringue, M. B. Wheeler, and D. J. Beebe, in: Microtechnologies in Medicine and Biology, 1st Annual International Conference, Lyon, France, 2000 (IEEE, Lyon, 2000), pp. 307–310.
[11] I. K. Glasgow, H. C. Zeringue, D. J. Beebe, S. J. Choi, J. T. Lyman, N. G. Chan, and M. B. Wheeler, IEEE T. Bio-Med. Eng. **48**, 570–578 (2001).
[12] C. Han, Q. Zhang, R. Ma, L. Xie, T. Qiu, L. Wang, K. Mitchelson, J. Wang, G. Huang, J. Qian, and J. Cheng, Lab Chip **10**, 2848–2854 (2010).
[13] R. S. Suh, N. Phadke, D. A. Ohl, S. Takayama, and G. D. Smith, Hum. Reprod. **21**, 477–483 (2006).
[14] Y. S. Heo, L. M. Cabrera, C. L. Bormann, C. T. Shah, S. Takayama, and G. D. Smith, Hum. Reprod. **25**, 613–622 (2010).
[15] D. K. Gardner and M. Lane, Biol. Reprod. **48**, 377–385 (1993).
[16] M. Lane and D. K. Gardner, Biol. Reprod. **69**, 1109–1117 (2003).
[17] S. Smith, S. Hosid, and L. Scott, Fertil. Steril. **63**, 591–597 (1995).
[18] J. G. Alvarez, J. L. Lasso, L. Blasco, R. C, Nunez, S. Heyner, P. P. Caballero, and B. T. Storey, Hum. Reprod. **8**, 1087–1092 (1993).
[19] B. S. Cho, T. G. Schuster, X. Zhu, D. Chang, G. D. Smith, and S. Takayama, Anal. Chem. **75**, 1671–1675 (2003).
[20] M. C. McCormack, S. McCallum, and B. Behr, J. Urology **175**, 2223–2227 (2006).
[21] R. S. Jeyendran, H. H. van der Ven, M. Perez-Palaez, B. G. Crabo, and L. J. D. Zaneveld, J. Reprod. Fertil. **70**, 219–228 (1984).
[22] A. T. Ohta, M. Garcia, J. K. Valley, L. Banie, H. Y. Hsu, A. Jamshidi, S. L. Neale, T. Lue, and M. C. Wu, Lab Chip **10**, 3213–3217 (2010).
[23] M. M. Garcia, A. T. Ohta, T. J. Walsh, E. Vittinghof, G. Lin, M. C. Wu, and T. F. Lue, J. Urology **184**, 2466–2472 (2010).
[24] L. Shi, B. Shao, T. Chen, and M. Berns, J. Biophotonics **2**, 167–177 (2009).
[25] G. E. Seidel and D. L. Garner, Reproduction **124**, 733–743 (2002).
[26] R. Zeggari, B. Wacogne, C. Pieralli, C. Roux, and T. Gharbi, Laser Phys. **16**, 294–302 (2006).
[27] H. Hwang, D. H. Lee, W. Choi, and J. K. Park, Biomicrofluidics **3**, 014103 (2009).
[28] J. K. Valley, P. Swinton, W. J. Boscardin, T. F. Lue, P. F. Rinaudo, M. C. Wu, and M. M. Garcia, Plos One **5**, e10160 (2010).
[29] G. D. Smith, P. C. Serafini, J. Fioravanti, I. Yadid, M. Coslovsky, P. Hassun, J. R. Alegretti, and E. L. Motta, Fertil. Steril. **94**, 2088–2095 (2010).
[30] M. Kuwayama, S. Fujikawa, and T. Nagai, Cryobiology **31**, 415–422 (1994).
[31] M. Antinori, E. Licata, G. Dani, F. Cerusico, C. Versaci, and S. Antinori, Reprod. Biomed. Online **14**, 72–79 (2007).
[32] Y. S. Heo, H. J. Lee, B. A. Hassell, D. Irimia, T. L. Toth, H. Elmoazzen, and M. Toner, Lab Chip **11**, 3530–3537 (2011).
[33] A. Borini, C. Lagalla, M. Cattoli, E. Sereni, R. Sciajno, Carlo Flamigni, and G. Cotcchio, Reprod. Biomed. Online **10**, 653–668 (2005).
[34] M. Lane and D. K. Gardner, Hum. Reprod. **11**, 1975–1978 (1996).
[35] D. K. Gardner, D. M. Lane, J. Stevens, and W. B. Schoolcraft, Fertil. Steril. **76**, 1175–1180 (2001).
[36] Y. S. Heo, L. M. Cabrera, C. L. Bormann, G. D. Smith, and S. Takayama, Lab Chip **12**, 2240–2246 (2012).
[37] J. P. Urbanski, M. T. Johnson, D. D. Craig, D. L. Potter, D. K. Gardner, and T. Thorsen, Anal. Chem. **80**, 6500–6507 (2008).
[38] D. R. Brison, F. D. Houghton, D. Falconer, S. A. Roberts, J. Hawkhead, P. G. Humpherson, B. A. Lieberman, and H. J. Leese, Hum. Reprod. **19**, 2319–2324 (2004).
[39] J. K. Nicholson, J. Connelly, J. C. Lindon, and E. Holmes, Nat. Rev. Drug Discov. **1**, 153–161 (2002).
[40] W. B. Dunn, Phys. Biol. **5**, 011001 (2008).
[41] M. G. Katz-Jaffe, W. B. Schoolcraft, and D. K. Gardner, Fertil. Steril. **86**, 678–685 (2006).
[42] E. Seli, D. Sakkas, R. Scott, S. C. Kwok, S. M. Rosendahl, and D. H. Burns, Fertil. Steril. **88**, 1350–1357 (2007).
[43] C. G. Vergouw, L. L. Botros, P. Roos, J. W. Lens, R. Schats, P. G. A. Hompes, D. H. Burns, and C. B. Lambalk, Hum. Reprod. **23**, 1499–1504 (2008).

© 2012 by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[44] R. Scott, E. Seli, K. Miller, D. Sakkas, K. Scott, and D. H. Burns, Fertil. Steril. **90**, 77–83 (2008).
[45] C. C. Wong, K. E. Loewke, N. L. Bossert, B. Behr, C. J. De Jonge, T. M. Baer, and R. A. Reijo Pera, Nat. Biotechnol. **28**, 1115–1121 (2010).
[46] C. S. Chen, D. N. Breslauer, J. I. Luna, A. Grimes, W. C. Chin, L. P. Lee, and M. Khine, Lab Chip **8**, 622–624 (2008).
[47] B. Mosadegh, C. H. Kuo, Y. C. Tung, Y. Torisawa, T. Bersano-Begey, H. Tavana, and S. Takayama, Nat. Phys. **6**, 433–437 (2010).

+++ Suggested Reading +++ Suggested Reading +++ Suggested Reading +++



2011. 900 pages, 266 figures, 35 in color, 28 tables. Hardcover.
£105.00 / €126.00
ISBN: 978-3-527-40956-3

GABRIEL CRISTOBAL, PETER SCHELKENS, and HUGO THIENPONT (eds.)

Optical and Digital Image Processing

Fundamentals and Applications

In recent years, Moore's law has fostered the steady growth of the field of digital image processing, though the computational complexity remains a problem for most of the digital image processing applications. In parallel, the research domain of optical image processing has matured, potentially bypassing the problems digital approaches were suffering and bringing new applications. The advancement of technology calls for applications and knowledge at the intersection of both areas but there is a clear knowledge gap between the digital signal processing and the optical processing communities. This book covers the fundamental basis of the optical and image processing techniques by integrating contributions from both optical and digital research communities to solve current application bottlenecks, and give rise to new applications and solutions. Besides focusing on joint research, it also aims at disseminating the knowledge existing in both domains. Applications covered include image restoration, medical imaging, surveillance, holography, etc...

Register now for the free
WILEY-VCH Newsletter!
www.wiley-vch.de/home/pas

WILEY-VCH • P.O. Box 10 11 61 • 69451 Weinheim, Germany
Fax: +49 (0) 62 01 - 60 61 84
e-mail: service@wiley-vch.de • http://www.wiley-vch.de