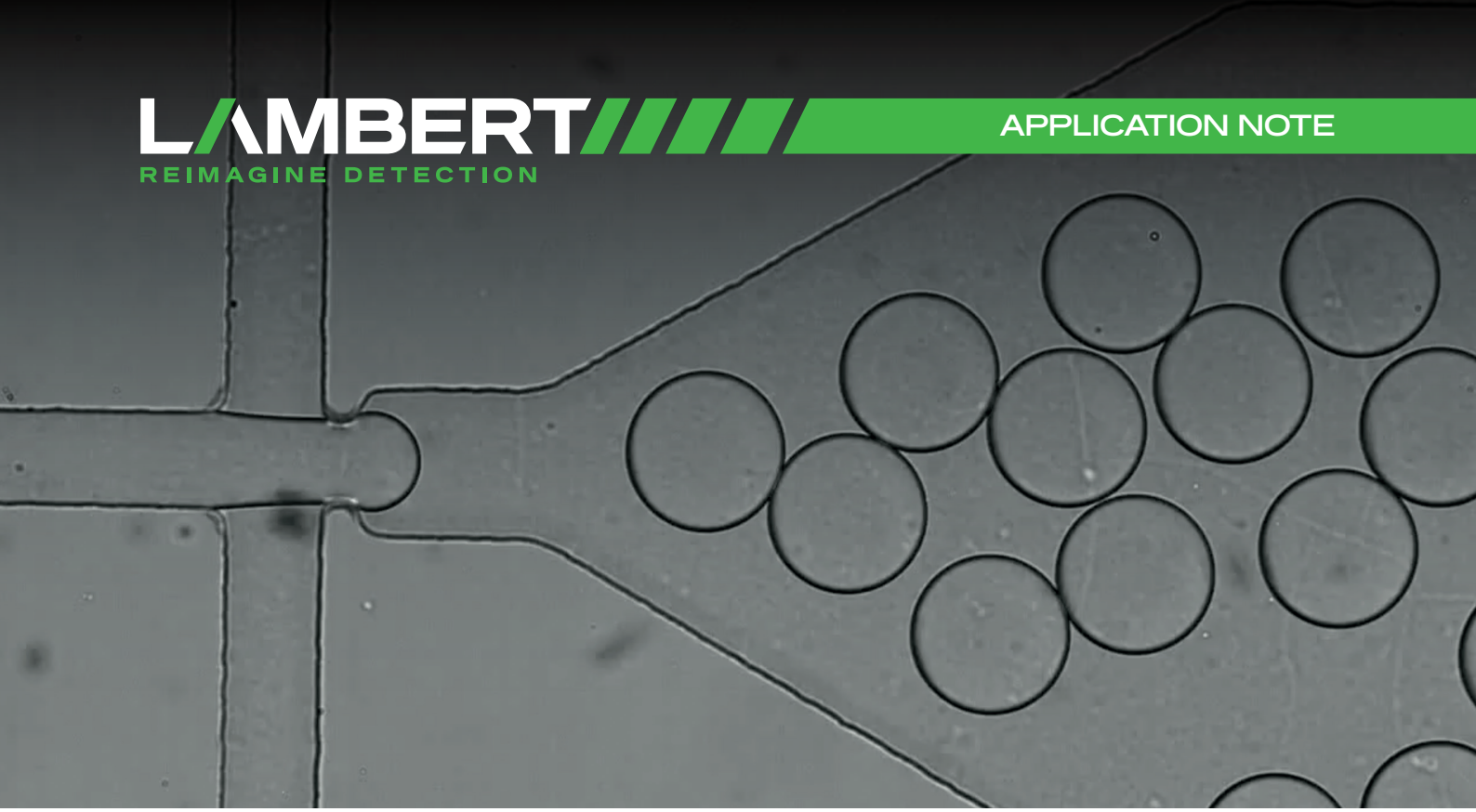
LAMBERT
REIMAGINE DETECTION

APPLICATION NOTE



Microfluidics

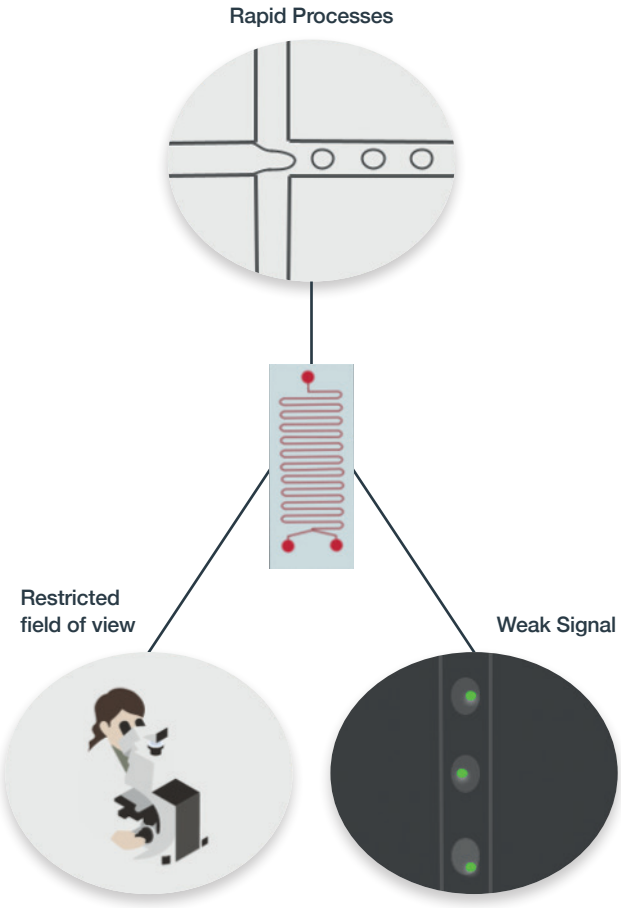
Introduction

Imaging in microfluidics is challenging for several reasons...

First, the features and objects introduced in a microfluidic system range from a few micrometers to hundreds of micrometers in size. This necessitates the use of a microscope for imaging, which reduces the field of view and restricts the view of the chip to only one section at a time (Wu et al., 2012). Furthermore, capturing sufficient light from tiny components while limiting noise can also be a challenge.

Second, fluid flow and reactions in microfluidic channels can be extremely rapid (Zhou et al., 2022). This requires short exposure times to capture sharp images without motion blur, which is the smearing of an image due to the relative motion between the camera and the subject during the exposure time.

This is further complicated by the fact that shorter exposure times require brighter illumination sources to detect the required signal. However, the resulting heat from these brighter sources can alter fluid properties or damage biological samples commonly used in microfluidics (Thoroddsen et al., 2008).



Graphical summary of the challenges faced in imaging of microfluidics

Droplet microfluidics

A subset of microfluidics is droplet microfluidics. Droplet microfluidics involves generation and manipulation of individual droplets within microdevices (Teh et al., 2008). It has several applications in the biomedical field as it allows precise manipulation and analysis of single cells or biological molecules in small droplets with high throughput.

This study by (Barcaru, 2023) highlights the use of the **HiCAM Fluo** for detection of droplets in a microfluidic chip for cell-counting applications. A flow-focusing device was manufactured and used to generate oil-in-water droplets using syringe pumps for flow control. The higher the flow rate, the faster the speed of the droplets. As previously mentioned, fast-moving objects are typically difficult to image due to motion-blur.

This study also involves the use of a fluorescent dye for imaging - erythrosine B - as this can aid in cell detection. Fluorescent dyes absorb light at one wavelength (excitation) and emit light at a different, typically longer wavelength (emission). Fluorescent dyes are widely used in cell biology due to their superior sensitivity, specificity, and versatility compared to other, non-fluorescent dyes (Drummen, 2012). As fluorescent dyes emit their own light, low-light conditions are generally desirable for fluorescence imaging in order to limit background noise.

So the imaging challenge here is two-fold: it requires the use of a camera that **(1)** is able to operate at sufficiently high frame rates to avoid motion blur and **(2)** is able to capture images in low-light conditions.

The **HiCAM Fluo**[1] is a high-speed intensified camera specialized for fluorescence imaging. The camera has a frame rate of 2.000 fps at the maximum resolution of 1920 x 1080 (and over 248,000 fps at a resolution of 1400 x 4 pixels).

In brief, an image intensifier is a device that amplifies light signals. It achieves this by converting the photons that make up the incoming light signal to electrons, rapidly multiplying the number of electrons, and then converting them back to photons, so that the light that ultimately reaches the camera's sensor has a much higher intensity than the light that enters the camera. This broadens the light conditions in which images can be recorded and allows for ultra-fast imaging in low-light conditions, as the intensifier negates the need for longer exposure times for visibility in low-light conditions. Detailed information on the working of the intensifier can be found at: https://lambertinstruments.com/knowledge-base.

Droplet movement in the microfluidic setup was recorded/imaged using the **HiCAM Fluo** in comparison with a color digital camera and the quality of the resulting recordings was evaluated. Details of the experimental set-up, microchip design, selection of appropriate camera settings, and an analysis of the obtained images is described in the following sections.

[1] The HiCAM Fluo and its intensifier are customizable. This study makes use of the HiCAM Fluo 2000 FPS with a single-stage intensifier. For more information about potential customisation, please get in touch with us.

Methods

The experimental set-up consisted of a microfluidic system which was imaged using a microscope, attached camera, and attached light source. The microscope was further connected to a computer to enable data acquisition and analysis.

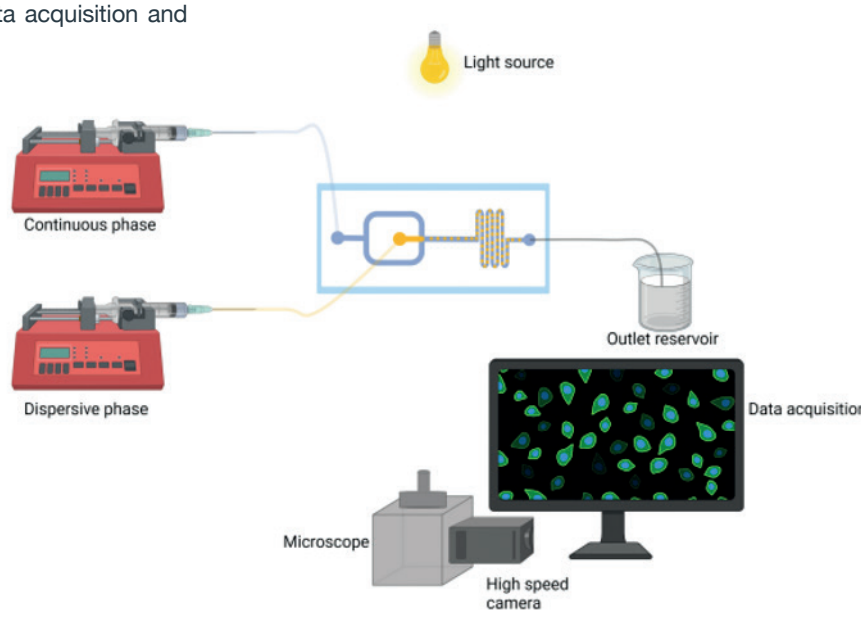
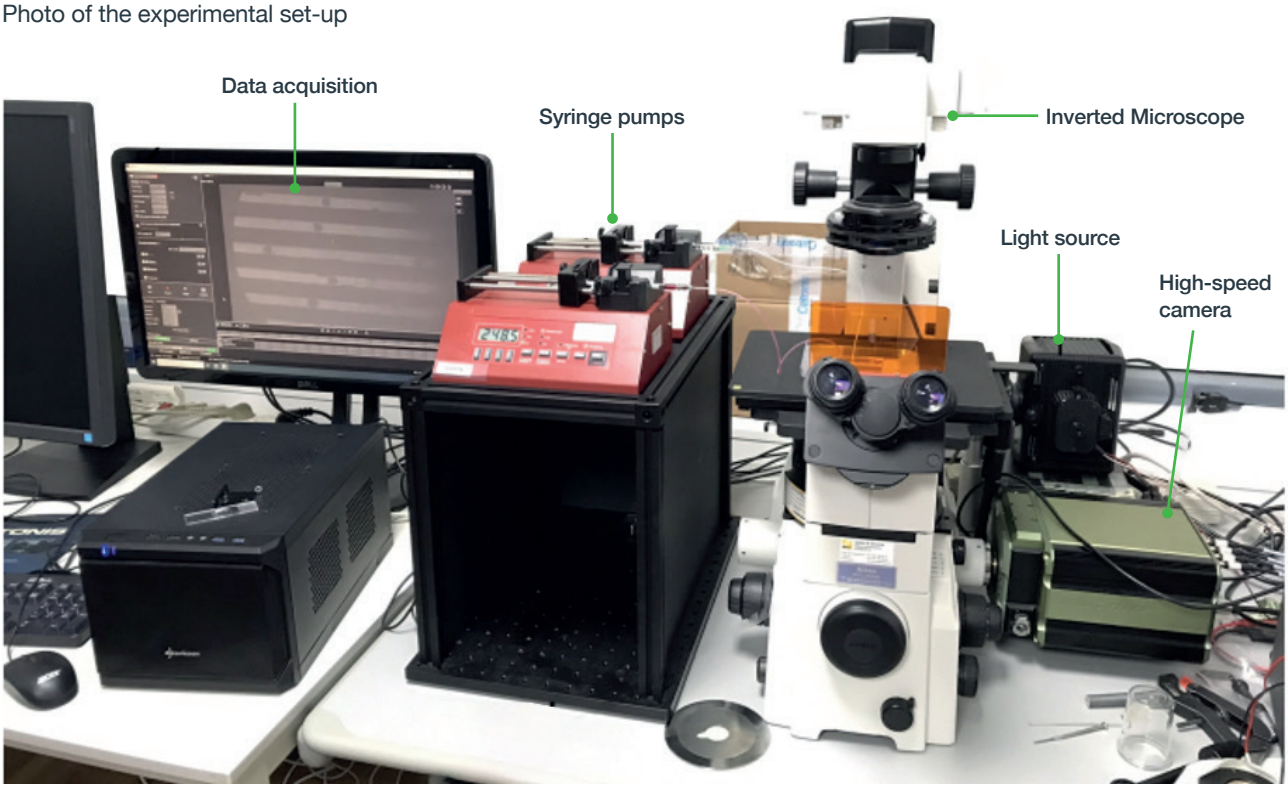


Figure 1:
Schematic representation of the experimental set-up

Figure 2:
Photo of the experimental set-up



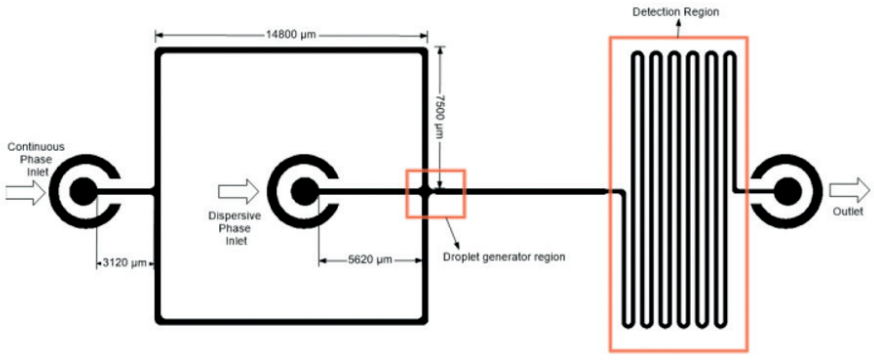
Microfluidic system

To achieve this, a PDMS-based microfluidic chip was manufactured using soft lithography. A schematic overview of the device can be seen below in Figure 3. The device consists of two inlets: one for the continuous phase (demiwater) and one for the dispersive phase (mineral oil). Two ProSense B.V. NE-1000 syringe pumps were employed for liquid pumping. Both fluids were dispensed using 1 mL syringes. The chip design achieved droplet formation in two ways. One, through the incorporation of a flow-focusing junction (As you can see in Fig 3. the continuous phase flows perpendicular to the dispersive phase, thereby pinching the dispersive phase and supporting droplet formation). And two, the flow of the dispersive phase is interrupted by a constriction in the channel, creating a geometric constraint and further supporting droplet formation. Formed droplets flow through the outlet into the reservoir. On their way to the reservoir, they pass through a serpentine, which is used for image acquisition.

Erythrosine, a pink food dye, was added to the aqueous phase. It has an absorption maximum at 526 nm and an emission peak at 546 nm. This makes it suitable for detection with the HiCAM Fluo with a GaAsp photocathode having a maximum quantum efficiency of over 50% at a wavelength of 550nm.

While in this instance the dye was added to the aqueous phase, it could also be added to the dispersive phase or used to stain the cell preparation directly depending on the end goal. Fluorescent dyes have excellent sensitivity, and are designed to be detectable even at extremely low concentrations, allowing detection of the smaller cells or droplets against the channels of the chip.

Figure 3:
Design of the microfluidic chip



Imaging Set-up

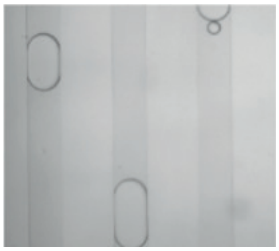
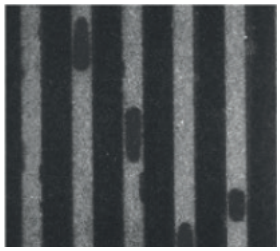
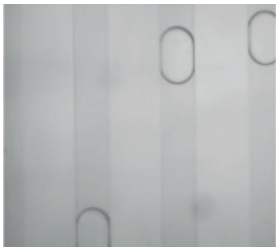
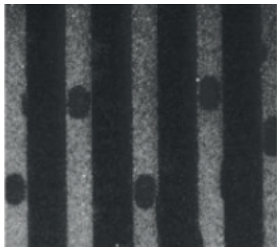
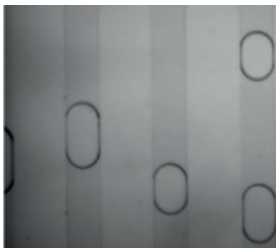
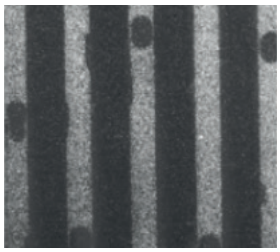
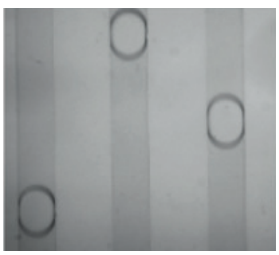
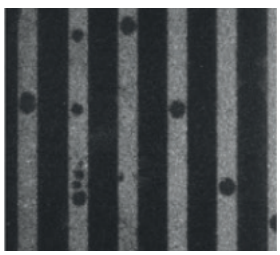
The image acquisition system consisted of the **HiCAM Fluo** intensified high speed camera from Lambert Instruments, coupled with a Nikon inverted microscope and a NIkon HMX3A light source. The camera was easily connected to the microscope's camera port using a lens mount. The magnification was set to 4X. The recordings were captured at a frame rate of 1500 fps, with a resolution of 1920x1080 pixels. The exposure time was set to 661 microseconds, and the MCP voltage was adjusted to 701 V.

The camera is connected to a frame grabber in a computer via a CoaXPress 2.0 connection, which facilitates direct data streaming to the hard disk at transfer speeds of up to 50 Gbps. This setup enables efficient and high-speed data capture and storage.

For comparison, the same experiments were recorded using a Leica DFC365 FX color digital camera, along with a Leica DM IL inverted microscope and an EL6000 fluorescence excitation source. The droplets were photographed in full frame mode (1392x1040 pixels) in both visible (exposure time 1000 ms) and fluorescent light (exposure time 1 ms).

Results

In order to detect the migration of the oil droplets through the microfluidic device, the device was imaged using the **HiCAM Fluo** and the color digital camera Leica DFC 365 FX. The results can be seen below in **Figure 4** with images procured using both cameras with increasing flow rates of the continuous phase.

Flow Rate	Colour Digital Camera **Leica DFC 365 FX** visible light	Colour Digital Camera **Leica DFC 365 FX** fluorescent light	High-speed Camera **HiCAM Fluo** fluorescent light
Qcontinuous = 200 µl/h Qdispersive = 40 µl/h Flow rate ratio = 5			
Qcontinuous = 400 µl/h Qdispersive = 40 µl/h Flow rate ratio = 10			
Qcontinuous = 600 µl/h Qdispersive = 40 µl/h Flow rate ratio = 15			
Qcontinuous = 800 µl/h Qdispersive = 40 µl/h Flow rate ratio = 20			

Results cont.

In visible light, the droplets can be seen in the channels of the chip as light ovals with a dark outline. In fluorescent light, in images captured using the Leica DFC 365 FX, the channels appear as bright lines due to the presence of erythrosine B in the aqueous phase while the oil droplets appear as dark smudges against the bright channels and are not clearly discernible. In images captured using the **HiCAM Fluo**, the droplets can be seen as dark ovals inside the bright channels.

While the Leica DFC 365 FX was capable of successfully detecting droplet motion in visible light at lower flow rates, at a higher flow rate of 800ul/h the droplets appear blurred due to the increased velocity of the droplets. The Leica DFC 365 FX also failed at distinguishing between the channel and droplets in low light.

On the other hand, the **HiCAM Fluo** was able to capture images in both low light and high flow speed conditions, demonstrating its ability to overcome two of the main challenges faced in microfluidic imaging.

Conclusion

This application highlights the performance of the **HiCAM Fluo** for droplet detection in a microfluidic system. The **HiCAM Fluo** was able to successfully detect the droplets even under high speed and low light conditions because of **(1)** its high frame rate and **(2)** its in-built image intensifier capable of amplifying incoming light.

The relatively simple set-up used in this study makes this a useful resource for rapid, automated counting of cells in low-light conditions, requiring minimal training and simple equipment compared with traditional cell-counting and sorting methods. A set-up such as this could also be used for drug screening, where high-throughput and accurate detection are essential.

Generally speaking, being able to image microfluidics opens up applications in several areas such as: studying fluid and droplet dynamics, detection of pollutants and contaminants for environmental and health monitoring, and analysis of biological molecules such as DNA.

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Further reading

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Other applications of the HiCAM Fluo

High-speed fluorescence imaging, bioluminescence and chemiluminescence detection for in-vivo imaging

Particle Image Velocimetry (PIV)

Time-resolved imaging and spectroscopy using ultra-short exposures

Laser-Induced Fluorescence (LIF)

Super-slow motion combustion research

Plasma physics research

Single-photon imaging for astronomy

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Lambert Instruments is dedicated to development, production and worldwide sales of products for **time resolved imaging at low-light levels**.

Our mission is to enable our users to **reveal previously unseen phenomena**. Our products provide a possibility to record fast events at low-light conditions. Together with our software, we **reimagine detection** to offer complete solutions to challenging imaging problems.