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TECH NOTE

HiCAM Fluo

for Microfluidics

The camera that has the ability to overcome the extreme challenge of high-speed imaging at low light intensity in Microfluidics

A challenge that occurs in many different research areas is the point where a process with low luminescence has to be captured at high-speed. The HiCAM Fluo is a camera that has proved to be able to overcome this challenge in a study performed by the Max Planck institute. Jahnke, Weiss, Frey et al. (2019) published an article in which they released an alternative method for the functionalization of microfluidic droplets by self-assembly of cholesterol tagged DNA at the droplet periphery (Figure 1). In their research the kinetic interaction between the cholesterol tagged DNA and the periphery of the droplet was studied by recording videos of the generation of these droplets.

In the research two challenges meet and form one extreme challenge. First of all, the process of generating droplets occurs very fast. One single droplet is generated in less than 0.003 second. In order to create a series of images of the formation of one single droplet, several frames should be captured within the time that one single droplet is formed. Let's assume that 10 frames is the least amount of images one would like to have of the generation of one single droplet, then a frame rate of at least 3334 frames per second is required. Only high-speed cameras are capable of such high frame rates.

The second challenge is the low light intensity. In this experiment the light that is to be detected originates from a Cy3-dye. It is generally known that the light intensity of a fluorescence signal is rather low. Therefore, a very sensitive camera is required for this experiment. The sensitivity needed in this experiment originates from an image intensifier. The inner workings of image intensifiers are explained on pages 7-10. The combination of these two requirements makes it an extreme challenge for cameras, as it should be very sensitive at high-speed.

"With conventional high-speed cameras it is difficult to image fluorescence processes because of the low intensity of the fluorescence signal" to quote Frey (comparison with conventional high-speed camera). Of course there are rather sensitive cameras, but those would be too slow to capture the generation of the droplets.

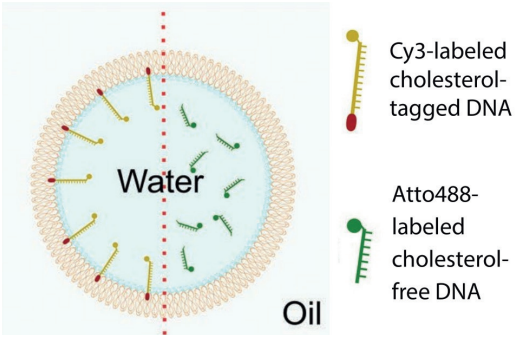

Figure 1: Schematic illustration of a water-in-oil droplet. Left of the red dotted line the droplet is functionalized by cholesterol-tagged DNA. Right of the dotted line cholesterol-free DNA is added but the droplet is not functionalized (Jahnke et al. 201 9).

Comparison with conventional high-speed camera

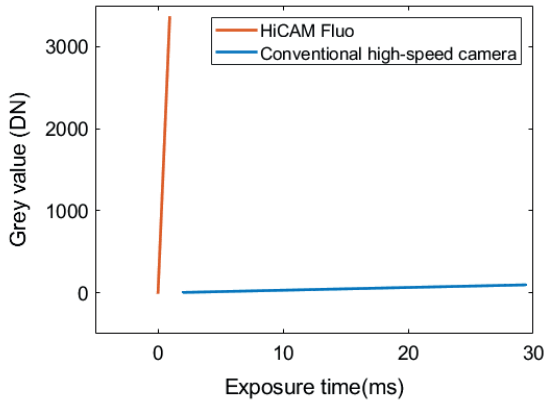

Figure 2: Sensitivity plot of the HiCAM Fluo (orange) and a conventional highspeed camera (blue). The steeper the line, the more sensitive the camera is.

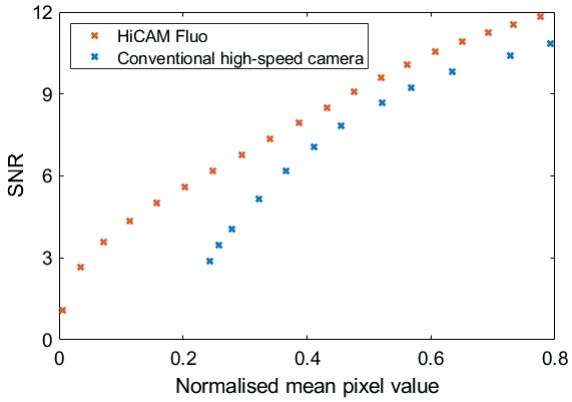

Figure 3: Signal to noise ratio of the HiCAM Fluo (orange) and a conventional high-speed camera (blue). The HiCAM Fluo has a slightly higher SNR than a conventional high-speed camera.

To get an idea of the difference in sensitivity between the HiCAM Fluo and a conventional camera, the sensitivity of a HiCAM Fluo (single-stage intensifier) is compared to a conventional high-speed camera.

A sensitivity diagram is shown in **Figure 2**. In this figure, the average pixel value is plotted as a function of the exposure time (for the intensity of the incoming light). The sensitivity (slope of the graph) is 3599 and 3.37 DN/ms for the HiCAM Fluo and conventional high-speed camera, respectively.

In other words: The HiCAM Fluo is a 1000 times as sensitive as a conventional high-speed camera. The sensitivity makes it clear whether the signal is detectable, but it does not distinguish the signal from noise. Using the M100 method, the SNR was determined using the same data as used for the sensitivity plot. Under these low light conditions, the HiCAM Fluo has a higher signal to noise ratio than a conventional high-speed camera, see **Figure 3**.

Microfluidic imaging experimental setup

The kinetic interaction between the cholesterol-tagged DNA and the periphery of the droplet was studied using the HiCAM Fluo. In figure 4 an overview of the set up is shown. The HiCAM Fluo is easily lens coupled to the camera port of the microscope, as the back focal distance of the input lens mount is already pre-set. The camera is connected to a frame grabber in a computer by a CoaXPress connection, allowing direct data streaming to the hard disc with a transfer speed up to 25 Gbps.

In order to image the Cy3-labeled DNA (M = 550 nm) a fluorescence illuminator (HBO 1 00, Carl Zeiss AG, Germany) and FS43HE filter (Carl Zeiss AG, Germany) were used. The droplets were enlarged using a 40x objective (LD Plan-Neofluar 40x/0.6 Korr, Carl Zeiss AG, Germany).

The photocathode determines the spectral sensitivity of an intensified camera. When fabricating the camera, the photocathode was selected based on the range of dyes expected to be used. Based on the Cy3-dye, the GaAsP photocathode was selected, as this photocathodes has the highest quantum efficiency at a wavelength of 550 nm.

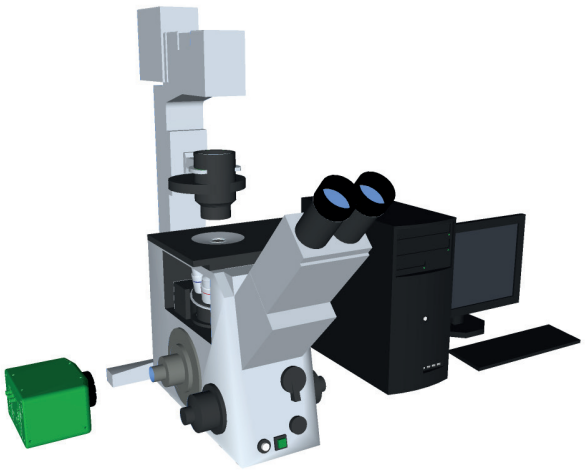

Figure 4: Simplified overview of the set-up; excisting of a HiCAM Fluo camera (green), a microscope and a computer.

Photocathode selection

The photocathode determines the spectral sensitivity of an intensified camera. When fabricating the HiCAM Fluo, the photocathode is selected based on the dye used in the experiment.

In **Figure 5** several photocathodes that can be selected are shown. In this experiment the Cy3-dye emits light with a wavelength of 550 nm. The photocathode that has the highest quantum efficiency at this wavelength is the GaAsP and was therefore selected for the HiCAM Fluo used in this experiment.

Selection of the photocathode should be based on the range of dyes expected to be used.

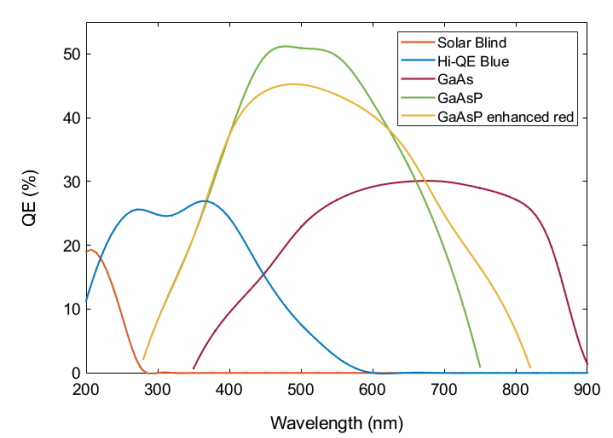

Figure 5: Quantum efficiency of the possible photocathodes of a HiCAM Fluo

Microfluidic device

In the experiment, microfluidic DNA functionalized surfactant-stabilized water-in-oil droplets were formed using a Microfluidic PDMS-based device (Sylgard 184, Dow corning, USA).

In **Figure 6** a schematic representation of this microfluidic device is presented. The device consists of one oil inlet and one aqueous inlet. The water inlet contains 10 µM Cy3-labeled cholesterol-tagged or cholesterol-free DNA and is set to a volume flow rate of 30 µLh-1 .

The oil inlet contains commercial surfactant and is set to a volume flow rate of 120 µLh-1. The oil inlet splits up into two oil phases. The aqueous inlet passes into a single aqueous phase. These three phases come together in flow-focusing cross junction. At the flowfocusing cross junction water droplets in oil are generated and the droplets flow to the outlet.

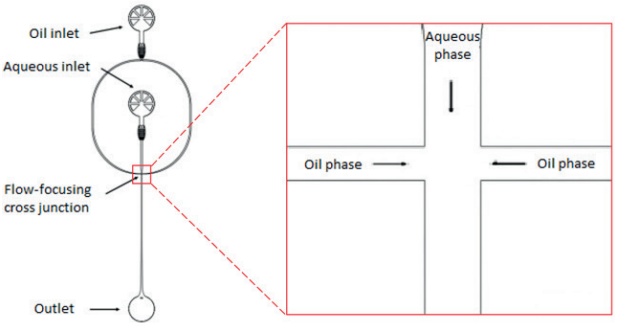

Figure 6: Schematic overview of a micro fluidic PDMS-based device. At the left side an overview of the device is shown, starting at the top with an oil inlet which splits up into two oil phases. The aqueous inlet passes into a single aqueous phase. These three phases come together in flow-focusing cross junction, shown enlarged on the right side. At the flow-focusing cross junction water droplets in oil are generated and flow to the outlet (Jahnke et al. 2019).

Camera settings

In the experiment three videos are captured: One at the flow-focusing cross junction and two at the outlet. In order to be able to capture the generation of one single droplet, a frame rate of 4086 frames per second was used for the first video. The cost of this high frame rate is a down scale of the resolution to 640 x 480 pixels.

For the second and third video a lower frame rate of 1000 frames per second was enough. Although the researcher kept the resolution at 480 x 640 pixels, the HiCAM Fluo is capable to run at 1000 frames per second at its full resolution of 1024 x 1280 pixels. With these settings the required high-speed is met.

The intensifier of the HiCAM Fluo played a crucial role in meeting the required high sensitivity. In this experiment a single stage intensifier with dual MCP was placed in the HiCAM Fluo. During the experiment the MCP gain was set to a gate width of 4 ms and MCP gain of 1.1 kV (MCP gain).

Intensifier Working Principle

The difference in sensitivity between a conventional high-speed camera and the HiCAM Fluo is caused by the image intensifier of a HiCAM Fluo. The single-stage intensifier boost the incoming light signal a thousand times. An image intensifier is a vacuum tube that contains several elements. A schematic picture of a single-stage intensifier is shown in **Figure 7**. Every element will be described briefly below.

Photocathode

The first element of the image intensifier is a photocathode. The aim of the photocathode is to transfer the energy of a photon to an electron (**Figure 8**). When a photon hits the photocathode, an electron is released with a certain possibility. This possibility is also known as the quantum efficiency; the percentage of photons that causes a release of an electron.

Micro channel plate

The electrons emitted from the photocathode accelerate to the second element of the intensifier: the micro-channel plate (MCP). The aim of the MCP is to multiply the number of electrons. The MCP is a glass plate with millions of channels. The channels are placed under an angle of a few degrees, so the incoming electron will have a higher change to hit the inside wall of the channel. The collision induces the emission of secondary electrons. The secondary electrons are accelerated in a parabolic pathway due to the electrical field across the MCP and therefore will hit the inside wall of the channel, inducing secondary electrons. This process repeats itself several times until the electrons leave the channel (**Figure 9**).

Anode

The electrons that leave the MCP are accelerated to the third and last element of the intensifier: an anode screen. The anode screen closes the vacuum tube. The aim of the anode screen is to transfer the energy of incoming electrons to photons (**Figure 10**). This process should be as efficient as possible and occur within one frame. If the process takes longer than one frame, the signal of the previous frame will interfere with the current frame.

Fiber optical coupling

The anode is coupled to the sensor of the camera. This can be done by using lenses or fibers. Fibers are used in the HiCAM Fluo, as they have a higher efficiency of transporting the signal from the anode to the sensor and they are less bulky. Optical fibers are fibers of pure glass, slightly thicker than a human hair, that can transmit light signals from one place to another. The shapes of the optical fibers are tapered in order to be able to fit the area of the anode on the smaller sensor. The consequence of this difference in size is a magnification of 1 .5 time

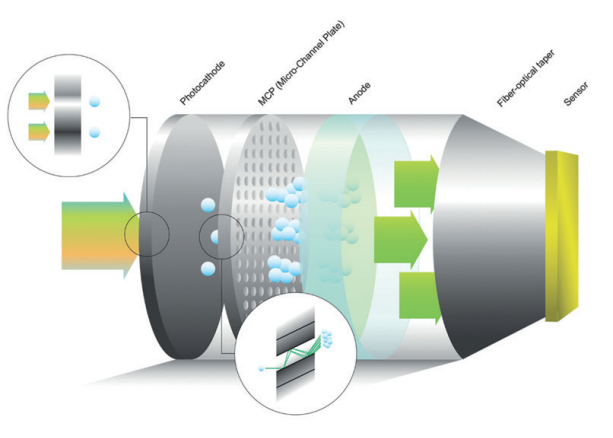

Figure 7: Schematic overview of a single-stage image intensifier.

Figure 8: Schematic presentation of a photocathode.

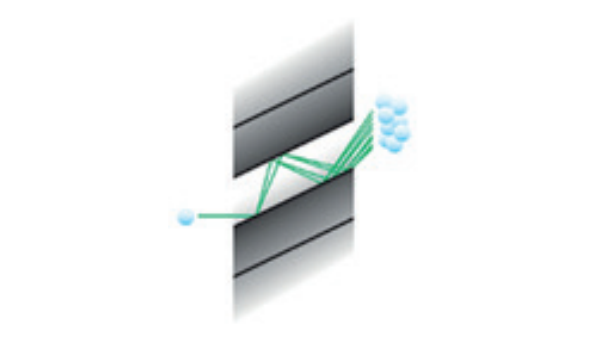

Figure 9: Schematic representation of the electron multiplication in a single channel.

Figure 10: Schematic presentation of an anode screen.

Advanced configurations

The described intensifier is also known as a single stage intensifier. A pro of a single-stage intensifier is the relative high resolution. However, in case that the single-stage intensifier cannot detect the light signal, there are two other configuration that can help: a single-stage, dual MCP or a dual. In the **Table 1** and **Figure 13** an overview of the relative pros and cons of these three configuration is shown.

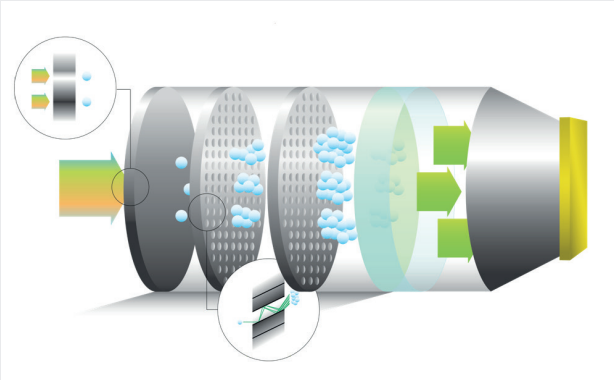

Figure 11: Schematic representation of a single-stage, dual MCP.

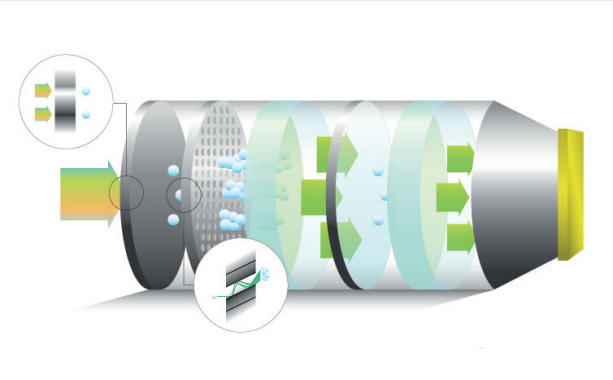

Figure 12: Schematic representation of a dual-stage intensifier.

The **single-stage, dual MCP** intensifier is a single-stage intensifier with an additional MCP, placed directly behind the MCP of the single-stage intensifier (**Figure 11**). As the MCP gain can be increased up to 1800 kV, the signal can be magnified by a factor of 30.000 (compared to a conventional high-speed camera).

The **dual-stage intensifier** consist of a single-stage intensifier with an extra photocathode and anode between the anode of the singel-stage intensifier and the optical fibers (**Figure 12**). The dual-stage intensifier is capable of generating a magnification factor of 10,000. But compared to the other configurations, depletion plays a less important role because the signal is multiplied after the MCP.

	SS	SSDM	DS
Gain	0	++	+
Resolution	++	0	+
Depletion compensation	0	0	+
When to use	High Resolution	Low Flux	High Flux

Table 1: Relative difference between a single-stage (SS), single-stage dual MCP (SSDM), and dual-stage (DS) intensifier.

Table key:
++ Very good + Good 0 Adequate

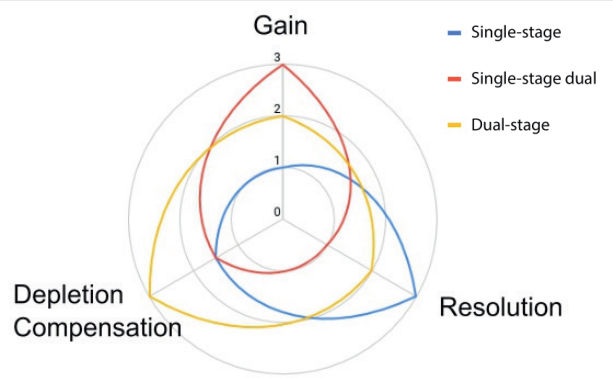

Figure 13: Chart of the relative difference between the possible configurations of an intensifier.

Results

In order to be able to study the kinetics between the cholesterol-tagged DNA and the periphery of the droplet, three videos were captured by the HiCAM Fluo. The first video was captured at the flow-focusing cross junction.

A series of 12 images were captured of the generation of one single droplet. In **Figure 14** the last 8 images of the series is shown. At the bottom of each image, the three phases come together. The two oil phases are black, while it is not fluorescently marked. The water phase contains the Cy3-labeled cholesterol-tagged DNA and are therefore detected as white. At the last image the droplet is totally surrounded by the oil. Both the droplet and aqueous phase are homogenous white in the video. So, the cholesterol-tagged DNA is homogenously spread throughout the droplet, at the moment the droplet is generated.

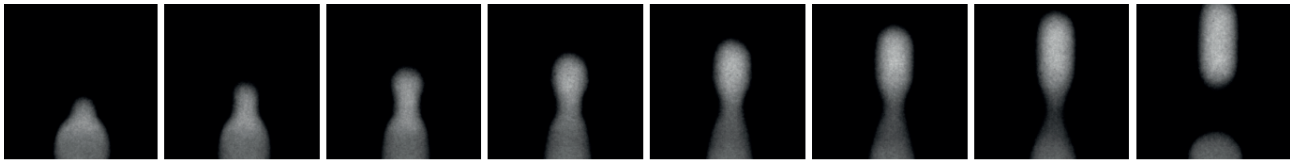

Figure 14: Last 8 images, out of a series of 1 2 images, of the generation of one single droplet (0.003 s) containing Cy3-labeled cholesterol-tagged DNA.

In **Figure 15** a screenshot of video 2 is shown. Video 2 is captured at the output, using Cy3-labeled cholesterol-tagged DNA at the aqueous inlet. A droplet is shown as a white circle. Looking closely at the image of a droplet, the white appears brighter at the edges of the circle. The brightness at the edges of the circle is caused by an increase of concentration Cy3-labeled cholesterol-tagged DNA at the droplet's periphery.

In **Figure 16** a screenshot of video 3 is shown. Video 3 is also captured at the output, but now the aqueous inlet contained Cy3-labeled cholesterol-free DNA. Again the aqueous droplets are imaged as white circles. In this case the droplet appears homogenous white, which means that the cholesterol-free DNA is evenly spread throughout the whole droplet.

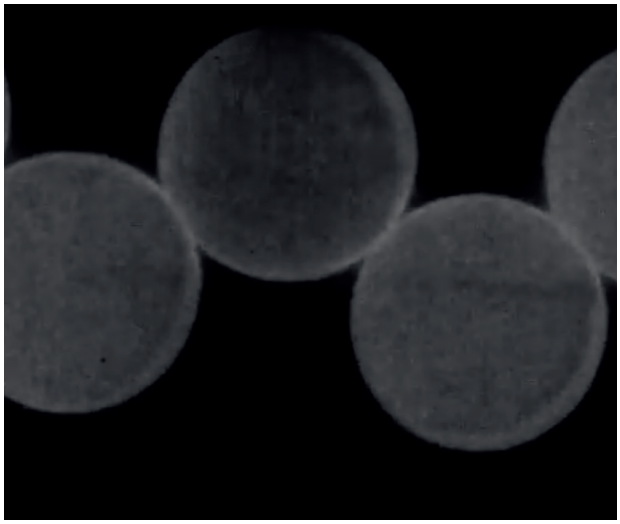

Figure 15: Droplets at the output contaning Cy3-labeled cholesterol-tagged DNA. The DNA concentration increases at the periphery of the droplet.

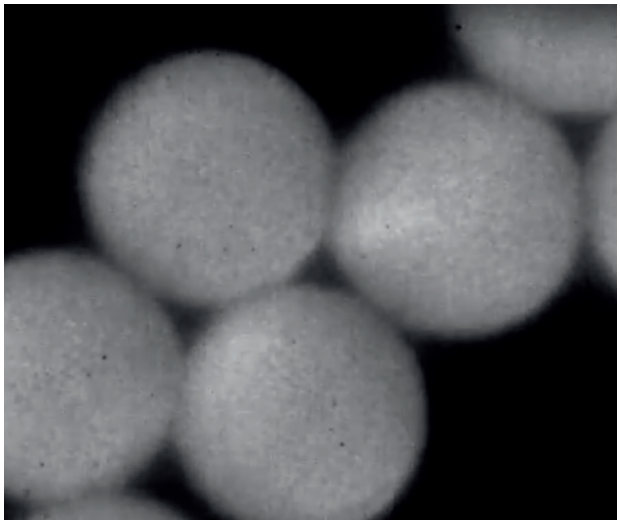

Figure 16: Droplets at the output contaning Cy3-labeled cholesterol-free DNA. The DNA is homogenously spread throughout the droplet.

Videos 1 and 2 prove that this process takes place in the time the droplets flow from the focusing junction to the outlet. The time this process take can be calculated based on the flow rate and the dimensions of the microfluidic device (30 µm x 30 µm x 7 mm):

$$\Delta t = \frac{\Delta V}{Q_{tot}} = \frac{30 \cdot 30 \cdot 7 \cdot 10^3}{120 + 30} \approx 150ms$$

Results using a convential high-speed camera

Using a conventional high-speed camera in this experiment, the challenge of capturing low light intensities at such a high frame rate could not be overcome. “It is just not possible to image the fluorescence dye at such low concentration under such high production frequencies with a conventional high-speed camera. The intensity from the fluorescent signal (excited photons) is not strong enough for the sensor of a conventional high-speed camera, therefore we used the HiCAM Fluo.” to quote Frey.

In order to be able to detect the light with a conventional high-speed camera, the exposure time has to be increased. To be able to increase the exposure time, the frame rate should be lowered. Lowering the frame rate would mean that the formation of the droplet could not be detected anymore, as there are not enough images captured per generation of one single droplet. The only way to make the generation of droplets visible with a conventional camera is by reducing the flow rate of the oil and water inlet of the microfluidic device. And thereby changing the challenge instead of overcoming it.

“It is just not possible to image the fluorescence dye at such lowconcentration under such high production frequencies with a conventional high-speed camera. The intensity from the fluorescent signal (excited photons) is not strong enough for the sensor ofa conventional high-speed camera, therefore we used the HiCAM Fluo.”

Christoph Frey

Conclusion

In this technical note the performance of the HiCAM Fluo was presented based on an experiment performed by the Max Planck Institute. The experiment formed an extreme challenge for a camera as it should be able to record a process with low luminescence at high-speed. Although conventional high-speed cameras were capable of the high frame rate, they lacked the high sensitivity and therefore could not overcome this challenge. The HiCAM Fluo has proved to be able to overcome this extreme challenge; having the high frame rate due to its integrated high-speed camera and the extreme sensitivity due to its integrated single-stage dual MCP intensifier.

References

Jahnke, K.,Weiss, M., Frey, C., Antona, S., Janiesch, J., Platzman, I., Göpfrich, K., & Spatz, J.P. (201 9). Programmable Functionalization of Surfactant-Stabilized Microfluidic Droplets via DNA-Tags. Advanced Functional Materials, 29(23).

Further reading

In this case the HiCAM Fluo was used for high-speed fluorescence detection in the area of microfluidics.

Other applications of the HiCAM Fluo includes:

High-speed fluorescence detection for in-vivo imaging

Time-resolved imaging in plasma physics research

Single-photon imaging for astronomy

Flow visualization using Particle Image Velocimetry (PIV)

Slow motion combustion research for the automotive industry

Lambert Instruments BV
Leonard Springerlaan 19
9727 KB Groningen
The Netherlands

T: +31 50 501 8461
E: sales@lambertinstruments.com

Part of Tibidabo Scientific Industries

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