HSMS High-speed microscope options, considerations and details

Image sensor:

Sensor options, resolution and rates:

- 1456-226 (best technical choice) – fast, global shutter
- 1984-138 (most adaptable) – fast, global shutter, full HD, gains speed with reduced width as well
- 3088-59 (high resolution) – rolling shutter, 6+ Mpix @59fps

The recommended sensors exhibit frame rate gains when cropping into frames and thereby reducing the acquisition resolution. Most sensors enable such gains only by cropping in vertically. Select sensors enable gains by horizontal cropping as well, however these gains may be lesser.

Estimated sensor rates at reduced resolutions are given in Tables Table 1, Table 2 and Table 3. Note that intermediate resolutions affect the frame rate as well.

Table 1 - Frame rates for sensor 1984-138

Resolution [pixel]	1984x1264 (max)	1920x1200	1440x1080	640x480	320x240	100x100	50x50	1440x50
Frame rate [fps]	138	150	215	817	1392	2364	3144	2398

Table 2 - Frame rates for sensor 1446-226

Resolution [pixel]	1456x1088 (max)	Reduced width	1440x1080	1440x520	1440x208	1440x100	1440x50
Frame rate [fps]	226	No gains	227	453	1015	1776	2717

Table 3 - Frame rates for sensor 3088-59

Resolution [pixel]	3088x2064 (max)	Reduced width	3088x1000	3088x500	3088x320	*Please note that this sensor uses a rolling and not a global shutter*
Frame rate [fps]	59	No gains	121	235	355	

The sensors are capable of very short exposures (8-110 μs depending on the sensor) and, combined with the given high-intensity illumination, one is able to capture phenomena far beyond the frame rate limitations. *In order to achieve higher rates at higher resolutions, a dedicated high-speed camera with internal RAM is required, as the USB3.1 bandwidth can only manage so much. For more information about such an option contact us at info@motion-scope.com.*

Image quality:

All sensor options were chosen with regards to an uncompromised sensitivity and dynamic range at the highest frame rates available for normal computers. The sensors capture fully raw (uncompressed) images, meaning there are no compression artefacts and the image quality is best (see Figure 1 for compression artefacts).

Figure 1 - compression artefacts (left - raw, right - compressed)

1984-138 and 1446-226 sensors use a global shutter. A global shutter acquires the entire image simultaneously. This is different to most all, even high end, consumer cameras, which use a rolling shutter. The rolling shutter acquires lines of the image sequentially causing shifts in the image (see Figure 2).

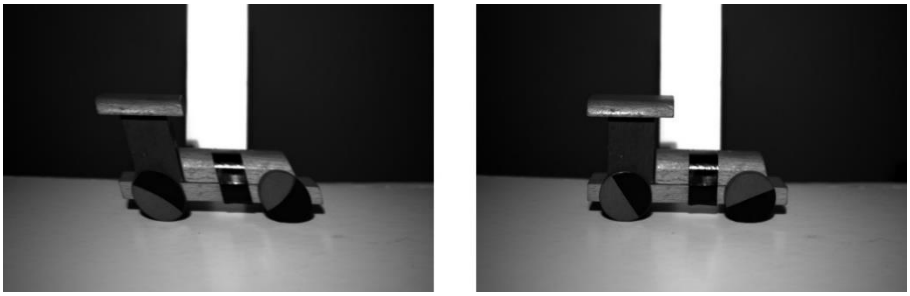


Figure 2 - Rolling (left) vs global (right) shutter. The effect is caused by the object moving across the frame.

Color options:

- B&W mono - (recommended)
- RGB color

Color images are produced by a (non-removable) Bayer filter on top of a sensor (see Figure 3). This filter covers up different pixels with different color filters, transforming e.g. a 1 Mpix (mega pixel) B&W sensor into a 0.5 MPix green + 0.25 MPix red + 0.25 MPix blue sensor, referred to as a 1 MPix color sensor. The image then gets demosaiced (upscaled to 1 MPix). This upscaling somewhat reduces image clarity compared to a true B&W sensor.

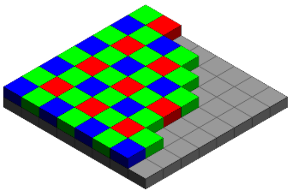


Figure 3 - a typical Bayer pattern

The second advantage of B&W sensors is their sensitivity. Since the light is not color filtered, a B&W sensor captures approx. 4x more white light (Figure 4).

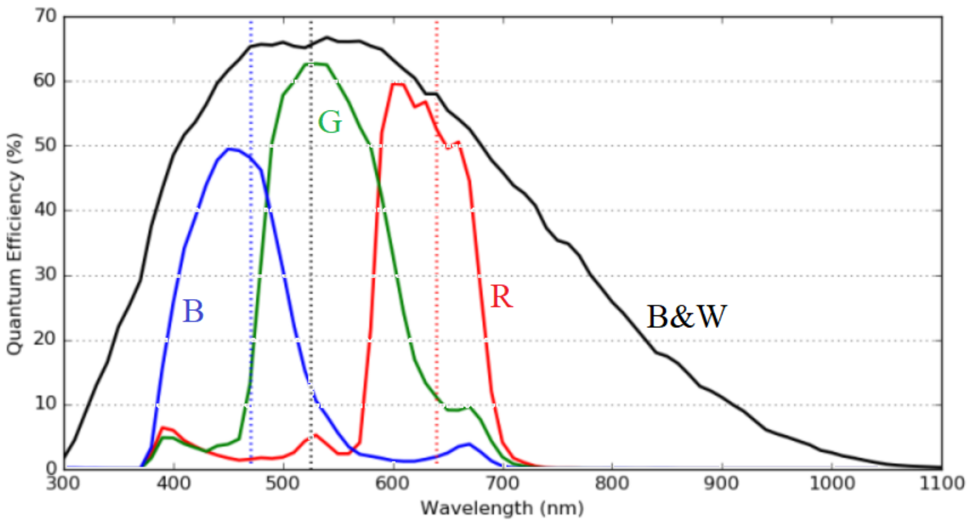
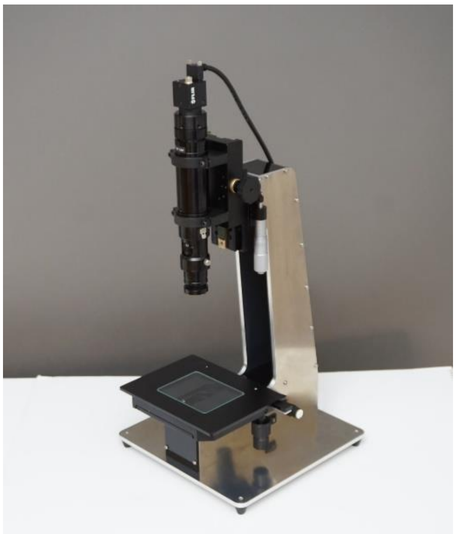


Figure 4 – 1446-226 image sensor spectral sensitivity

Mechanical details:



The microscope base is designed and built with structural dynamics in mind. Structural dynamics is the field of mechanical engineering concerned with vibrations and is also a field of Motion Scope expertise. This results in a sturdy structure that prevents vibrations on the image, when the stages are being manually manipulated.

The microscope uses manual stages, because high-speed photography is too fast for in time focusing, also tactile feedback is preferred for fast, precise stage manipulation. All mechanical motion is achieved with precision linear rails. The XY stage (table) has a motion range of 8 cm by 5 cm. The motion is finely damped and does not stick. The macro stage allows for rough positioning (±4 cm), followed by a very precise micro positioning rail that allows for very fine tuning under loads (±15 mm travel, @ 0.5mm/revolution). The combined macro and micro stages allow for a 9+ cm height accommodation of various complex micro-fluidic chips and all the connections for most all optical setups.

Optics:

The recommended optical setup (1x0.7-4.5x2x) uses a zoom lens, producing a variable magnification range from 0.7x to 4.5x, resulting in a 6.4x variable zoom range. The setup offers a large 4-4.3 cm working distance *WD*. This is the distance from the bottom of the lens to the plane of focus. This is again in order to accommodate complex microfluidic chips and other samples without much hassle. By removing the 2x bottom lens, the user achieves a doubling of the working distance and a halving of the magnification.

Table 4 shows the magnifications for various sensor options at minimal and maximal zoom settings. Since determining a factor of magnification for digital microscopes is ambiguous, often encompassing the size of the screen used to display the image, the magnification here is given as the image size in µm, instead.

The chosen magnification is believed to be an optimal and most practical range for microfluidics. For some reference to scale, note the frames in Figure 5 were acquired at minimal magnification and span slightly over 4000 µm in width.

Magnifications higher than those given in Table 4 don't make much sense, since the pixel size ends up unnecessarily exceeding the optical resolution limit for visible light microscopes, which is determined by:

$$r = \frac{0.61\,\lambda}{NA}$$

Where λ is the wavelength of light (a mid-spectrum wavelength of 550 nm = 0.55 µm is typically used for visible light) and *NA* is the numerical aperture. By having the pixel size slightly below the optical limit, the maximal optical resolution is achieved by digitally zooming into the image.

A note on the numerical aperture; The highest numerical aperture NA that an optical microscope is expected to be able to reach is at about NA=1, producing an optical resolution limit of r ≈ 0.33 µm. However, such optics have miniscule working distances and are not suitable for microfluidics. Numerical aperture is also inversely proportional to the depth of field DOF. A large NA results in a shallow depth of field, which is, again, not desired in case of microfluidics.

Table 4 – Image dimensions for the recommended sensor options when using the recommended optical setup (1x0.7-4.5x2x)

Sensor choice	1446-226 (1456x1088)	1984-138 (1984x1264)	3088-59 (3088x2064)
Zoomed out image scale *NA*= 0.066 *DOF*=380 µm *WD*=43 mm	2.5 µm/pixel 3600 µm 2700 µm	3.4 µm/pixel 6580 µm 4120 µm	1.7 µm/pixel 5300 µm 3540 µm
Zoomed in image scale *NA*= 0.172 *DOF*=25 µm *WD*=40 mm	0.39 µm/pixel 560 µm 420 µm	0.53 µm/pixel 1020 µm 635 µm	0.27 µm/pixel 830 µm 560 µm

Other optical options:

The microscope can be provided with an optical system for interchangeable bottom objectives, particularly for an increase in numerical aperture and magnification. This option raises some considerations and costs depending on the objectives used. For more about such options contact us at info@motion-scope.com.

Illumination:

A major concern with high-speed photography is high luminosity requirements and problems with light flickering caused by inappropriate power regulation. The HSMS microscope uses a custom high-intensity flicker-free light source for illumination. The intensity is manually adjustable.

Illumination options:

- Bottom illumination. The bottom light source is set to produce a high optical contrast images (see Figure 5 right). By using a provided diffusor, the light can be reconditioned to pierce through chips with curved walls that act as lenses (see Figure 5 left).

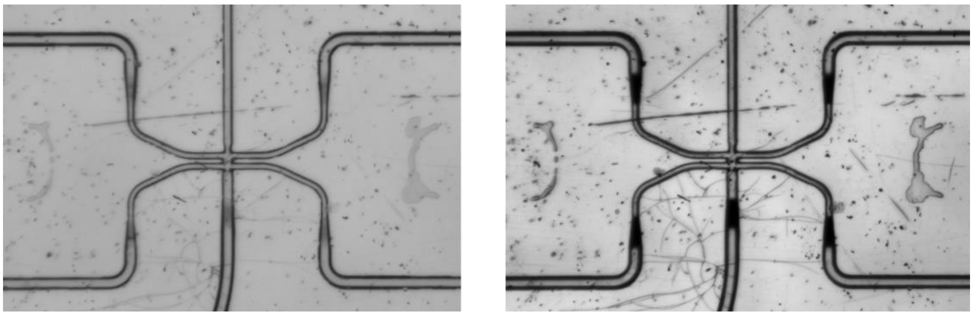


Figure 5 varying phase contrast demonstrated on a low magnification cracked chip.

- Coaxial (top) illumination can be combined with bottom illumination. *Note that coaxial illumination decreases the amount of light getting into the lens, due to the physics of the mirror prism within. The loss is more than 50% in each direction (Figure 6). Since microfluidic chips are transparent, the top illumination does not produce a significant benefit, and is usually omitted.*

Figure 6 - coaxial illumination

Additional requirements:

Computer:

The microscope requires a mid-range computer (e.g., laptop) to connect to and run the supplied 'Motion Scope viewer' software.

Computer minimal requirements:

- Operating system: Windows 10
- Processor: intel i3 / AMD r3 or better
- RAM: 4+ GB – *(recommended 8 GB) RAM serves as a buffer, allowing for longer acquisition times.*
- Solid state drive 'SSD' 250+ GB – *normal hard drives will work as well, however SSDs provide a favourably lower latency, which is particularly useful at playback.*
- USB 3.1 port (aka. USB SuperSpeed) –*All modern PCs use USB 3.1.*
- Integrated intel graphics card or better

The microscope may possibly function with lower specs in but performance will suffer.

Power:

The microscope requires access to mains power supply for the illumination.