

Navigating the Evolution of Light-Sheet Microscopy Through Time and Technology

Journey through the history of scientific exploration, where light-sheet microscopy meets the complexities of life sciences. Over the past three decades, microscopic techniques have advanced remarkably, fueled by the innovation of scientific CMOS cameras.

About light-sheet microscopy

Light microscopy is a core technology in life science research. Since its early days, it has evolved with a wide range of microscopic techniques, driven by the rapid development of optoelectronic detectors and the widespread availability of laser-based light sources.

This development is clear when observing the distinctive design of light-sheet microscopes, which varies greatly from the standard microscope design that has remained for centuries.

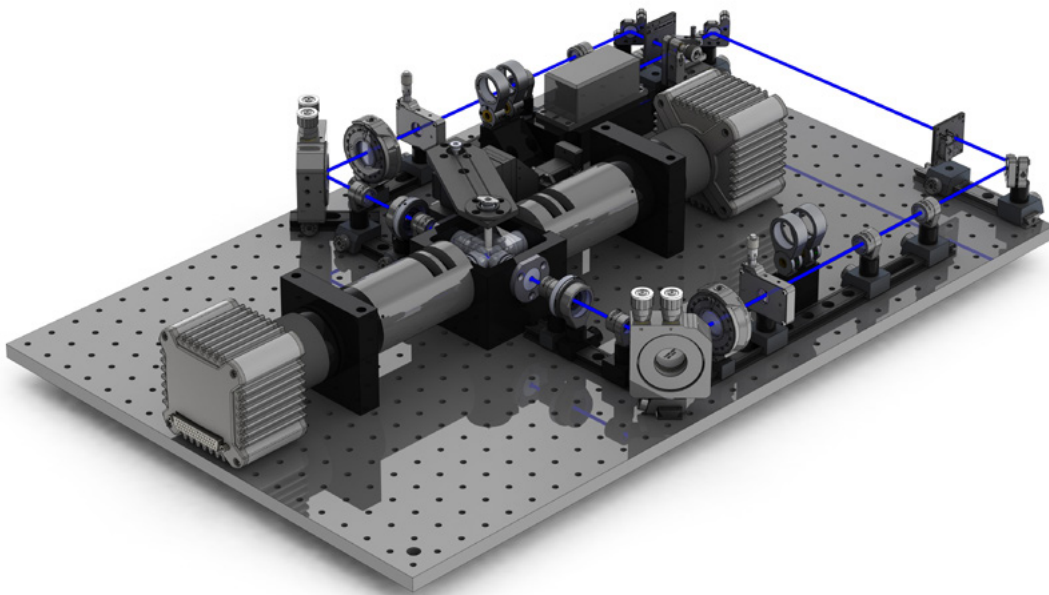


Fig. 1: Light-sheet microscope, OpenSPIM concept configurations. Source: https://openspim.org/Gallery#OpenSPIM_data

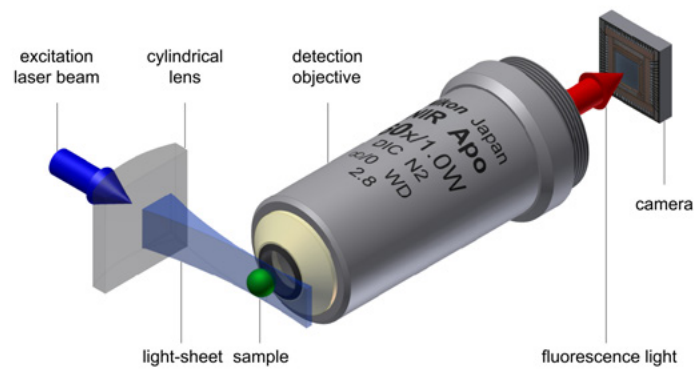


Fig. 2: Light-sheet microscopy principle, based on Jan Krieger, CC BY-SA 3.0. Source: commons.wikimedia.org/w/index.php?curid=22333698

The fundamental concept underlying all light-sheet microscopes is that the sample is illuminated with a thin sheet of light (hence the name), typically perpendicular to the observation axis. This decoupling of the illumination and the detection offers greater freedom in design compared to typical epi- or transillumination designs.

When combined with fluorescence staining, light-sheet microscopy offers several advantages, including 3D resolution and enhanced contrast as there is no out of-focus light generated. This gentle technique preserves both the signal thanks to low photobleaching and the sample integrity due to low phototoxicity. With camera-based detection, acquisition can be swift, limited only by the maximum frame rate of the camera. The flexible design of light-sheet microscopes allows specialization for specific samples, ranging from a few centimeters to the single-cell level (Girkin, 2018 ^[1]).

While those advantages encourage the replacement of standard microscopes, it must be noted that they come with some disadvantages, such as sample mounting, which may hinder certain applications with established protocols. The flexibility in instrument design, which certainly is an advantage on many occasions, might add unwanted complexity to some experiments. Additionally, while the resolution can be diffraction-limited, the highest resolution is still achieved with standard microscopes or with super-resolution approaches, despite demonstrations of combining light-sheet microscopy with super-resolution techniques.

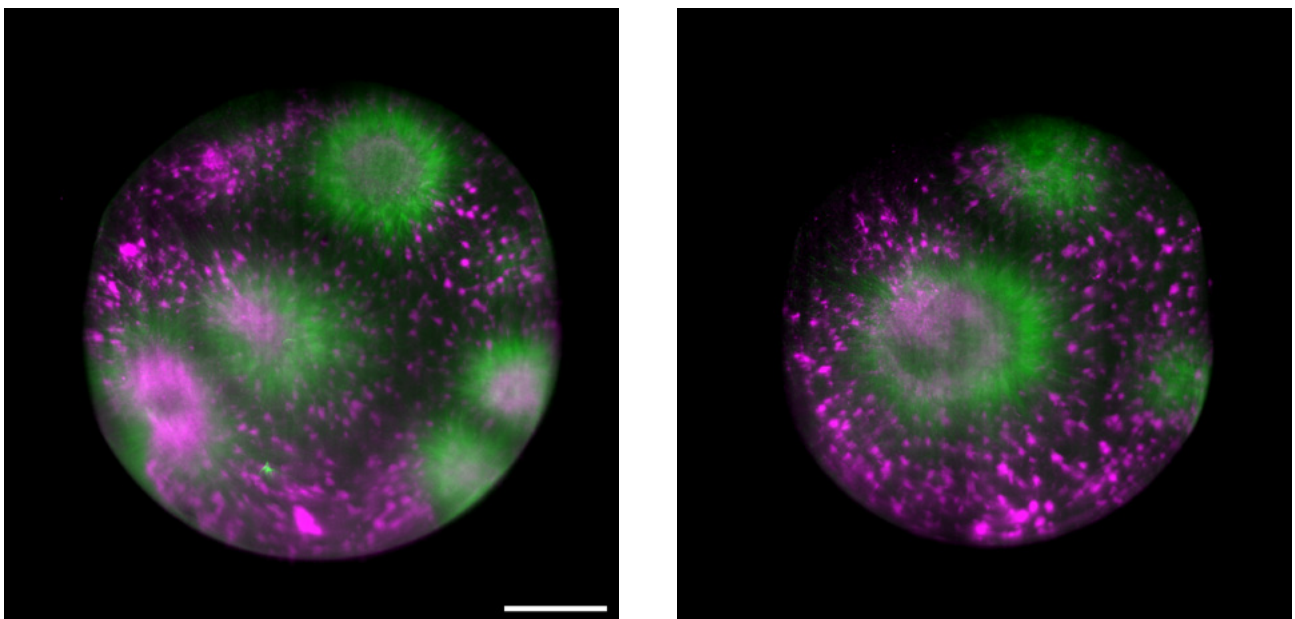


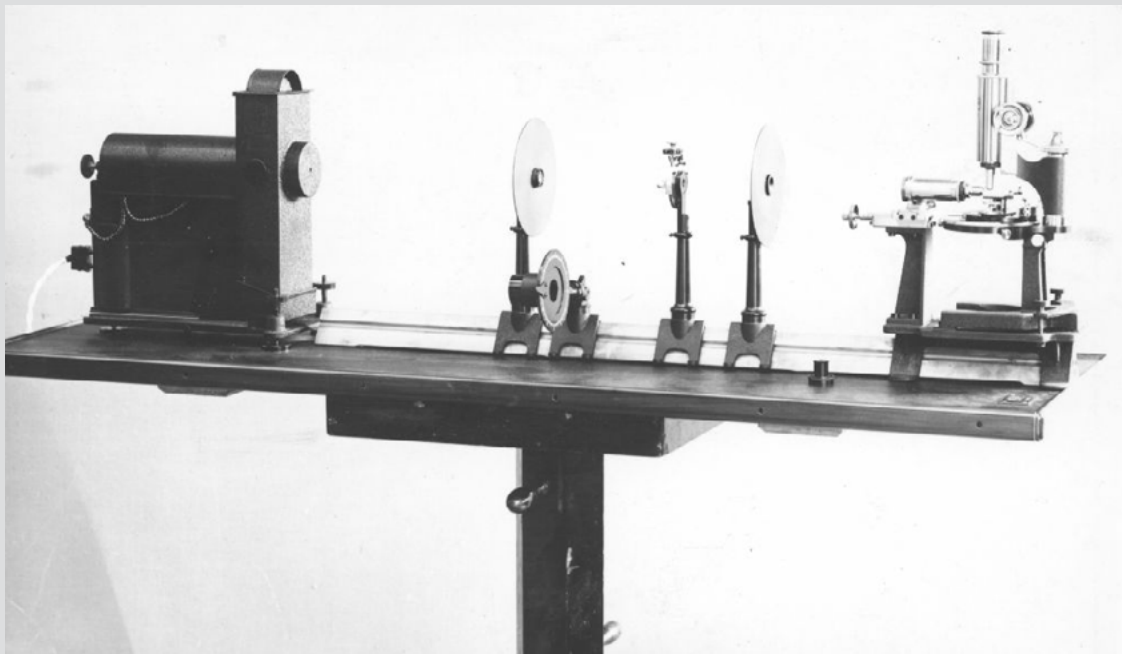
Fig. 3: Tubulin assembly in cytoplasmic droplet, acquired on Viventis LS1 light-sheet microscope with Orca Fusion BT camera, (scale 100µm). Source: Dr. Michael Riedl (Jan Bruges Group) and Dr. Vanessa Carlos (PoL Microscopy), Cluster of Excellence "Physics of Life", TU Dresden.

The origins of light-sheet microscopy

While the basic idea of illumination with a light-sheet is more than a century old, the technique only gained prominence 20 years ago. The success is attributed not only to the widespread availability of laser illumination but also to the advent of fast digital cameras enabling 3D reconstruction of images.

Delving into history, we trace the origins back to 1903 when Siedentopf & Zsigmondy pioneered the first light-sheet microscope (Siedentopf, H. and Zsigmondy, 1903 ^[2]). Their objective, analyzing sub-resolution gold particles, led to a groundbreaking Nobel prize in 1925, the first awarded for a microscopy technique. Their so called “Ultramicroscope” (see below) was a dark field light-sheet setup, designed to visualize particles as small as 4 nm. Apart from being the first demonstrated light-sheet microscope, they also started colloid research, which some view as the foundation of the field of nanotechnology (Mappes, 2012 ^[3]).

Invention of the ultramicroscope



Henry Siedentopf and Richard A. Zsigmondy; ZEISS Microscopy

In 1903 Ernst Abbe retires from the management, handicapped by severe health problems. He lives to see in this year the limits of classical microscopy finally exceeded by the ultramicroscope, an invention by Henry Siedentopf and Richard Zsigmondy, which makes submicroscopical colloids visible. This is actually the first published account of a very simple version of a light sheet fluorescence microscope (or LSFM) in which light of an arc lamp was projected through a slit aperture to observe gold particles. Source: www.flickr.com/photos/zeissmicro/6892932822

Nearly a century later, Voie et al. revived the technique and applied it for the first time to a biological sample, the inner ear cochlea of a guinea pig, using tissue-clearing methods to reveal the inner structure of the tissues (Voie, 1993 ^[4]). Interestingly, they chose an application (imaging of cleared tissue) which blossomed into a major field 20 years later with the advent of modern tissue-clearing protocols.

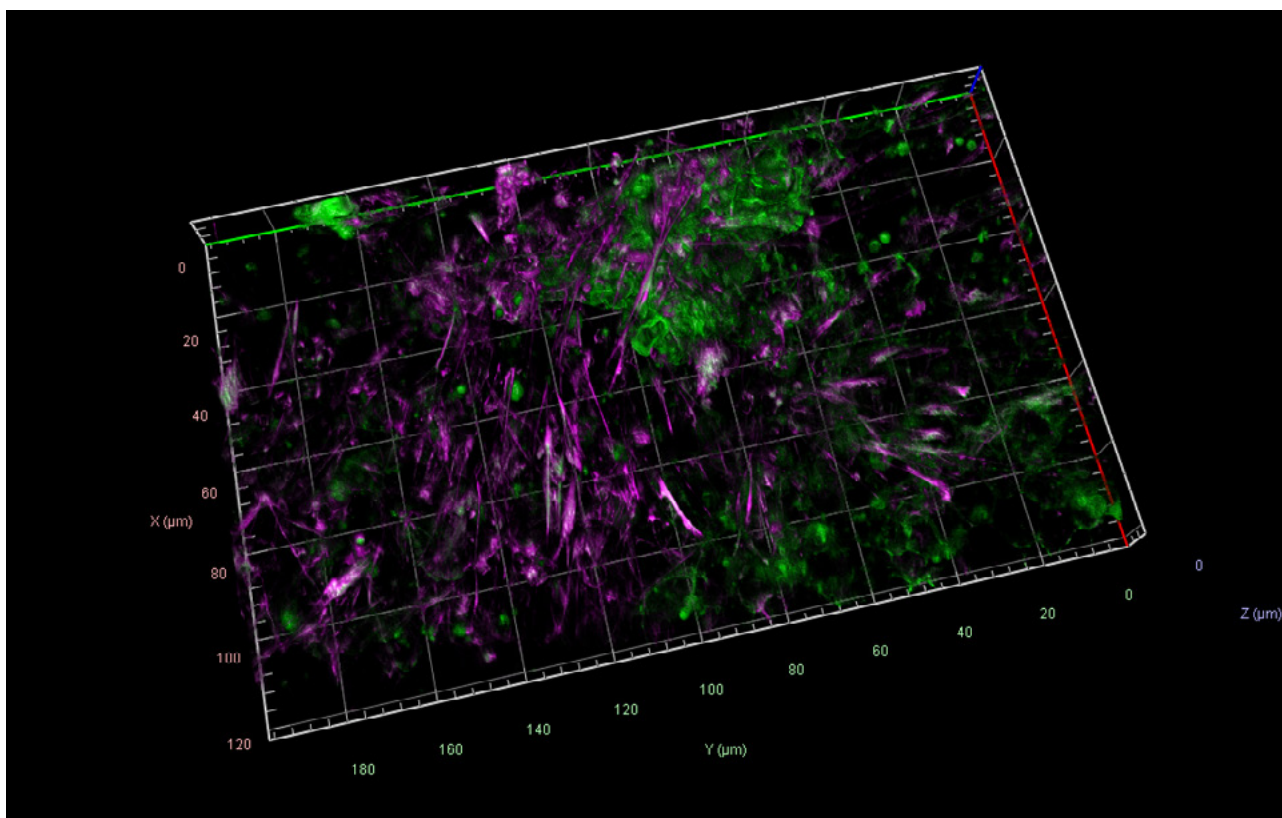


Fig. 4: Epithelial cells stained for E-Cadherin and Actin, imaged on Zeiss Lattice Light Sheet 7 with ORCA® Fusion cameras. Source: Dr. Anastasiia Gabrielyan (Natalie Dye Group) and Dr. Bert Nitzsche (PoL Microscopy), Cluster of Excellence “Physics of Life”, TU Dresden

Modern-day light-sheet microscopy

The year that light-sheet microscopy finally took off was 2004: Jan Huisken et al. developed selective plane illumination microscopy (SPIM), enhancing earlier light-sheet microscopes by incorporating a microscope objective to the illumination arm and a Hamamatsu Photonics ORCA-ER B/W CCD digital camera. This innovative approach improved the 3D resolution and reduced photobleaching, showcasing low phototoxicity and high speed in observing *Drosophila melanogaster* (fruit fly) embryogenesis and medaka (Japanese rice fish) embryos’ heartbeat (Huisken, 2004 ^[5]).

During the next decade, light-sheet microscopy became a recognized tool in developmental biology, witnessing advancements like digital scanned light-sheet and creative implementations like “oblique plane microscopy”. It combined the advantages of light-sheet microscopy with classical microscope bodies and hence brought the light-sheet advantages to standard samples on glass slides (Dunsby, 2008 ^[6]).

Technological acceleration in the early 2010s saw the introduction of scientific CMOS cameras (sCMOS), surpassing previous EMCCDs with more pixels at higher speeds and increased sensitivity – an ideal combination for large, sensitive specimens. Hamamatsu’s sCMOS cameras, particularly Flash 2.8 and 4.0, played a significant role in this shift. Even the initially perceived drawback of CMOS cameras, the rolling shutter, had been converted into an advantage in light-sheet microscopy. The synchronization of the excitation beam in digitally scanned light-sheets with the rolling shutter contributed to reduced stray light, increased contrast, and axial resolution (Baumgart, 2012 ^[7]). The rolling shutter could also be synchronized with a tunable lens in ‘axially swept light-sheet microscopy’ for a uniform light sheet across large field of views (Dean K. M., 2015 ^[12]).

To simplify the synchronization of the rolling shutter with external equipment, Hamamatsu developed the patented 'Light-sheet readout mode'. With the release of the Flash 4.0 V2 in 2013, sCMOS technology reached its maturity. Since this technology appeared – and potentially sparked the development burst of light-sheet microscopy, the Flash 4.0 found widespread use in high-end light-sheets, including those by Nobel laureate Eric Betzig (Chen B. C., 2014 ^[8]) (Liu, 2018 ^[9]), Philip Keller's sophisticated setups (MuVi SPIM, IsoView) (Krzic, 2012 ^[10]) (Chhetri, 2015 ^[11]), Reto Fiolka's innovative microscopy techniques - axially swept light-sheet microscope and many more, (Dean K. M., 2015 ^[12]), Illaria Testa's super resolution and light-sheet combinations (Bodén, 2024 ^[13]) and various others.

Subsequent developments brought forth a whole range of sCMOS cameras, with numerous research projects utilizing Hamamatsu's ORCA® series. In fact, over the last four years, more than 1000 scientific papers* have been published using an ORCA® sCMOS camera. Each camera offers specific features such as different dynamic ranges, shutter functionalities, and sensor dimensions, catering to the unique demands of diverse research endeavors.

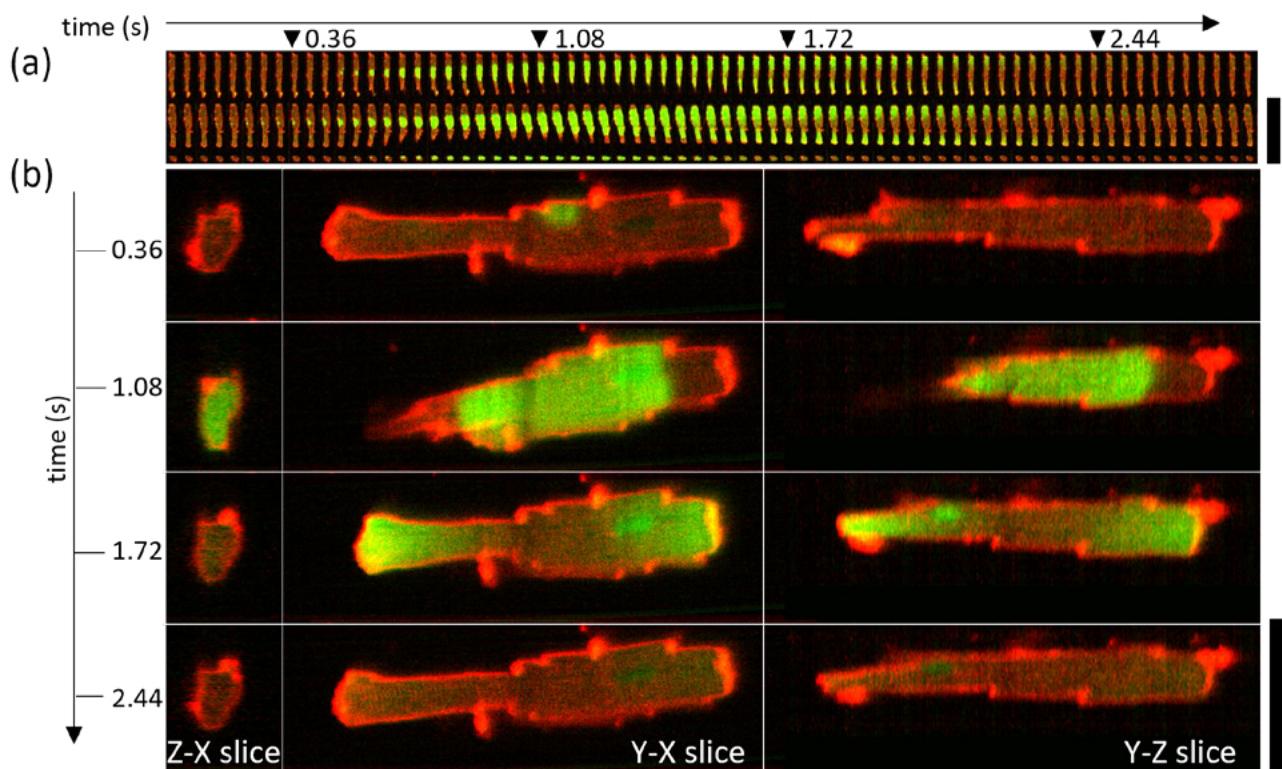


Fig. 5: Spontaneous calcium wave in a live cardiomyocyte cell imaged at 25 volumes per second. (a) shows central volume planes in xy, xz and yz orientation, while (b) shows the expanded views of the datapoints indicated with triangles in (a). Source: Dr. Hugh Sparks and Dr. Christopher Dunsby, Imperial College London, as published in ^[14].

The synergy of scientific research and technological advancements

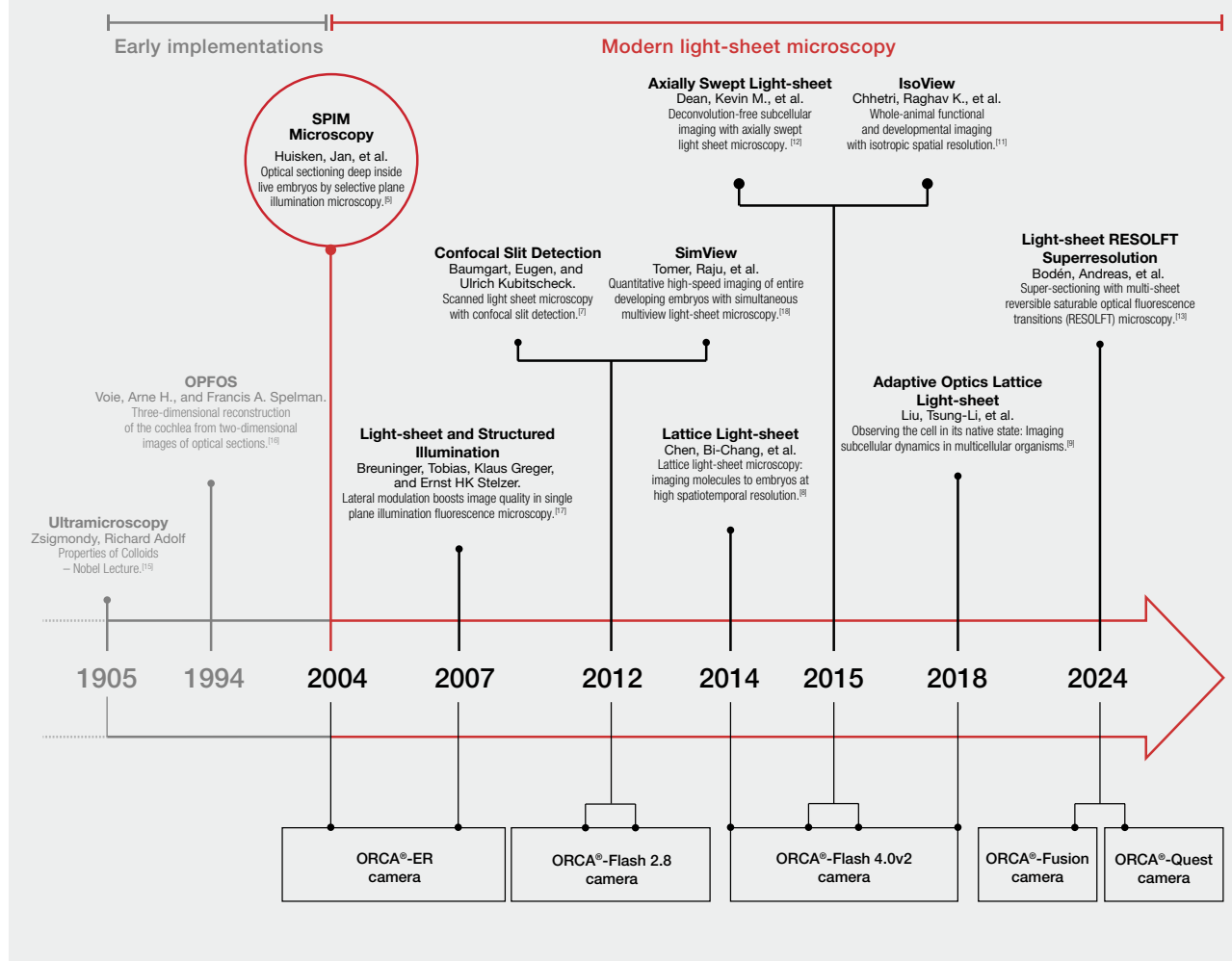
As we navigate the rich history of light-sheet microscopy, a story of constant innovation unfolds. The technique, revolutionized by pioneers like Siedentopf & Zsigmondy in 1903 and revitalized by Voie et al. in 1993, reached a transformative moment in 2004 with the advent of selective plane illumination microscopy

(SPIM). At the heart of SPIM's success was Hamamatsu Photonics' ORCA®-ER B/W CCD digital camera, bringing forth 3D resolution, low photobleaching, and high speed to the forefront. The early 2010s marked a technological peak with the maturity of sCMOS cameras, which was encapsulated by Hamamatsu's Flash 4.0 V2, propelling light-sheet microscopy to new heights. This evolution paved the way for diverse research projects utilizing Hamamatsu's ORCA® series.

Additionally, today, there is a wide range of options for light-sheet setups, ranging from very basic Do-It-Yourself projects like OpenSPIM with step-by-step tutorials to more advanced open hardware projects such as the mesoSPIM project, along with commercial solutions. Remarkably, the flexibility of some of these approaches allows for building the microscope around the sample, rather than fitting the sample to the microscope.

As we anticipate the future of light-sheet microscopy, we predict the continuous collaboration between technology and scientific discovery, with each milestone marking a step closer to unraveling the mysteries of life sciences.

20 years of light-sheet microscopy innovation with Hamamatsu



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*The research was made on Meltwater Media Monitoring from the year 2000 to 2023.