Adaptive Measurement Method for Area Chromatic Confocal Microscopy

Ding Luo

Vision and Fusion Laboratory
Institute for Anthropomatics
Karlsruhe Institute of Technology (KIT), Germany
ding.luo@kit.edu

Technical Report IES-2017-03

Abstract: Although well known for its depth discerning capability, conventional confocal microscopy has limited application due to its slow scanning speed. From Nipkow disk to various programmable spatial light modulator, various research has been conducted with the aim of improving the speed of confocal microscopy. Nevertheless, the fundamental conflict between axial sensitivity and lateral density remains unsolved. In this report, a novel adaptive measurement method is proposed based on iterative refinement of the axial measurement as well as condensation of the lateral measurement grid. Initial experimental investigation has shown overall good measurement result with a specific type of artifacts due to inaccurate estimation in earlier measurement stages. Despite of this problem, the proposed system and the accompanying algorithms have shown great potential in improving the measurement speed of area chromatic confocal microscopy.

1 Introduction

Conventional scanning confocal microscopy suffers from a relatively slow measurement speed due to its requirement for mechanical scanning, which largely limits its application in various fields. To tackle this problem, Egger et al. first proposed to utilize the Nipkow disk to generate a moving array of measurement

locations in order to accelerate the scanning process [EP67]. Recently, more advanced disk pattern has been designed to be coupled with structured illumination technology, in order to achieve superresolution imaging [HO15].

With the development of new optical components and computer technology, this idea of using an array of measurement locations has transformed into an important field of research, i.e. programmable array microscopy (PAM). Programmable array microscope refers to a family of imaging systems where a spatial light modulator (SLM) is applied to dynamically change the patterns of illumination and/or detection. With the target of eliminating lateral mechanical scanning, different SLMs have been applied, including digital mirror device (DMD) [HVG+99, CRS15], liquid crystal on silicon (LCoS) [HCT+07, KDP+14], and polymer-dispersed liquid crystal (PDLC) [CS17].

Despite the improvement achieved through these developments, one fundamental problem remains unsolved. The unique depth discerning capability of the confocal technology originates from the fact that the light which is not focused on the object is distributed to the adjacent area, thus dramatically reducing the reflected light collectable to the confocal pinhole. Such a principle intrinsically demands larger numerical aperture (NA) to generate highly focused spot, in order to achieve better axial sensitivity and lateral resolution, which has not been a problem for conventional scanning confocal microscopy. Nevertheless, for array scanning microscopy, realized through whether mechanically scanned disk or spatial light modulator, the blurred illumination spot of one measurement location quickly generates crosstalk over its adjacent measurement locations. This leads to an inverse relationship between the minimum allowable pitch of the measurement array and the NA of the system as well as the axial measurement range.

This report aims to provide a potential solution for this problem through an adaptive measurement strategy based on a particularly dynamic hardware setup, which will be discussed in details in the following sections.

2 System Setup and Calibration

The proposed system is composed of two components, i.e. a programmable light source and a DMD-based programmable array chromatic confocal microscope. Due to its nature of adaptability, the system is denoted as AdaScope.

2.1 Programmable Light Source

The programmable light source is based on two-dimensional dispersion of a white light laser. As illustrated in Fig. 2.1, the laser is firstly dispersed horizontally by a prism and then dispersed vertically by the echelle grating to achieve very high overall dispersion. A digital mirror device is used to select the desired wavelengths, which is collected by the output liquid light guide. The system is capable of generating light spectrum in the range of $480\,\mathrm{nm}$ to $680\,\mathrm{nm}$. More details regarding the programmable light source can be found in the corresponding paper [LTLB17].

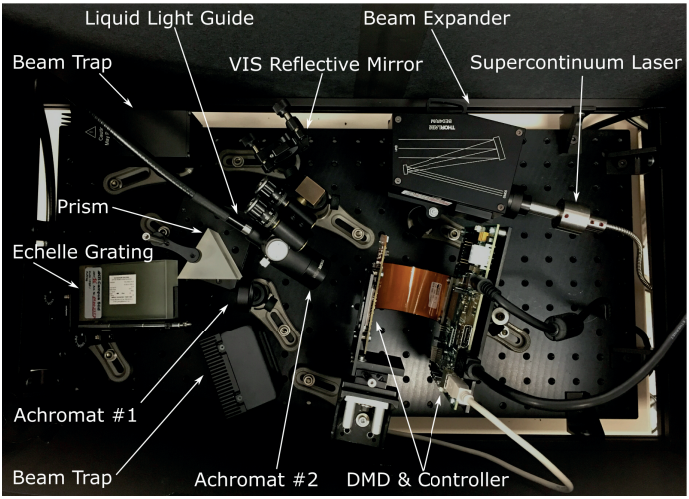

Figure 2.1: Setup of programmable light source.

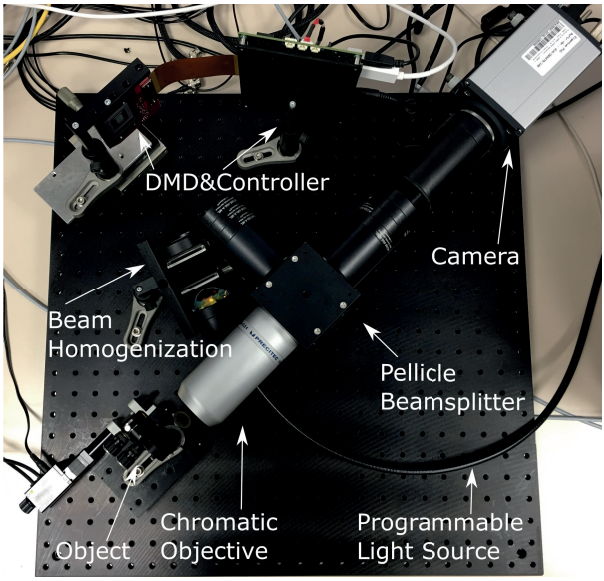

Figure 2.2: Setup of programmable array microscope.

2.2 DMD-based Programmable Array Microscope

In the microscope setup, light coming from the programmable light source is first homogenized and projected onto the DMD, which acts as an array of secondary sources. Illumination light is projected onto the object using an objective (Precitec CLS4) with designed chromatic separation along the optical axis. The reflected light travels through the same objective and is collected by the camera. The sCMOS camera (Andor Zyla 5.5) in the system has very good signal to noise ratio and color depth (dynamic range) but not a very fast speed. As will be later discussed, since the speed of the programmable light and the DMD in the microscope are both very fast, the frame rate of the camera will be a major limiting factor that has to be considered when designing the measurement algorithm. The measurement area is $5.4\,\mathrm{mm}$ by $3.0\,\mathrm{mm}$ laterally and the depth measurement range is $4.6\,\mathrm{mm}$ for a wavelength range of $200\,\mathrm{nm}$.

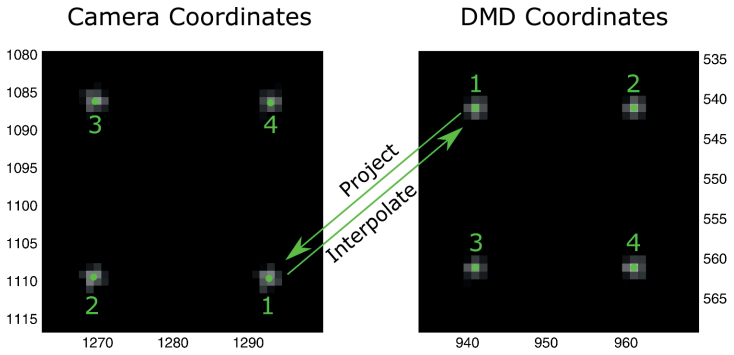

Figure 2.3: Camera calibration for AdaScope.

2.3 Camera Calibration

As a microscope system, the illumination homogenization and projection system, the DMD and the camera arm all have to be aligned very accurately for the confocal principle to work. After the alignment, camera calibration is implemented. Since the system is designed to be telecentric, the camera is only calibrated for one wavelength ($555\,\mathrm{nm}$) where a registration is made between the camera coordinates and the DMD coordinates. The registration toward the object / world coordinate system is not considered at the moment. All measurements with AdaScope shown in this report are in the DMD coordinate system by first projecting the DMD coordinate to the camera coordinate system and then making an interpolation, as shown in Fig. 2.3.

As demonstrated in Fig. 2.4, due to the large NA of the microscopic objective, when a flat mirror serves as the target object, the blurred spot due to defocus covers a large area even at a small distance. The image demonstrates the burred spot when the mirror is located at a distance of $95.25\,\mu\mathrm{m}$ from the focal plane. This corresponds roughly to a wavelength shift of $4\,\mathrm{nm}$ in the chromatic confocal scan. It can be seen that the crosstalk reaches more than a distance of 10 pixels already. To fully suppress the effect of crosstalk, a minimum pitch of 20 pixels is required. To scan through 200 wavelength steps, 80000 frames have to be taken, which costs roughly $0.75\,\mathrm{h}$ of acquisition time. If certain levels of crosstalk can be tolerated, a pitch of 10 pixels can be taken, which leads to an acquisition

time of 11 minutes. Even in this case, the speed of measurement still cannot be considered to be practical for real industrial applications.

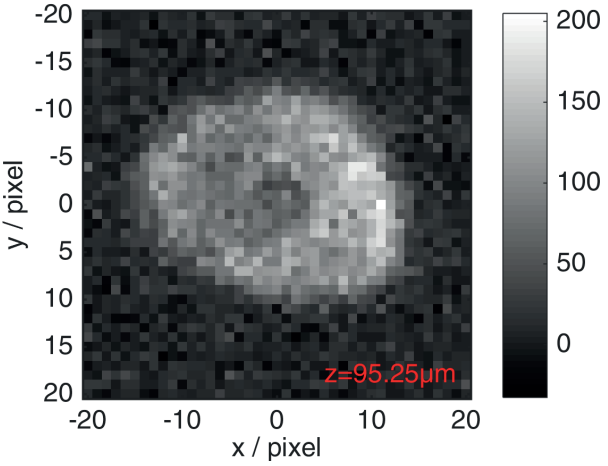

Figure 2.4: Defocus blurring.

3 Adaptive Measurement Method

To further accelerate the speed of area chromatic confocal measurement, an adaptive grid resizing algorithm has been developed. The idea originates from the observation that the uncertainty of the chromatic confocal measurement is in fact coupled with the lateral density of measurement locations, as shown in Fig. 3.1. When little information of the measurement locations is gathered, the crosstalk could potentially be very large and therefore a larger distance between adjacent points is required. As the measurements at each point become more and more accurate, the possibly generated crosstalk also gets smaller which allows for a denser measurement array.

Based on this observation, the measurement is conducted in several iterations. In each iteration, measurements with limited accuracy are made for all positions through array scanning with a fixed pitch distance. Based on the result from one iteration, more refined measurements are made with a denser grid in the next iteration.

3.1 Axial Measurement Refinement

In this iteration, a two-channel linear measurement system is applied to each measurement location. The two measurement functions are two ramp-shaped functions in opposite directions. To measure the axial location of the corresponding chromatic confocal peak, illuminations with spectra in the shape of the measurement functions are applied and the corresponding images are captured. As 1st order Bernstein polynomials, these functions have the nice property that the corresponding linear transformation maintains the centroid of the original signal. Therefore, the centroid of the chromatic confocal peak can be estimated with very fast computation:

$$\mathbf{m} = \begin{bmatrix} m_1 \\ m_2 \end{bmatrix} = \begin{bmatrix} \mathbf{f_1}^T \\ \mathbf{f_2}^T \end{bmatrix} \mathbf{g}$$

$$centroid(\mathbf{g}) = centroid(\mathbf{m}) = \frac{m_2}{m_1 + m_2}$$

where $\mathbf{g}$ represents the original confocal signal, $\mathbf{m}$ denotes the measurement, $\mathbf{f_1}$ and $\mathbf{f_1}$ represent the illumination spectra.

There are several reasons for using such a linear measurement system. Firstly, since more than one iterations are performed, each iteration must be very efficient in terms of the number of frames taken. Secondly, the crosstalk at a fixed distance should be proportional to the measurement range. This means that as the location of the object becomes more certain, the crosstalk should become smaller. Lastly, the uncertainty should be inversely proportional to the measurement range. This means that for a smaller measurement range, the sensitivity should be higher.

All these properties are achieved by iteratively reducing the wavelength range of the illumination according to the previous estimation, such as illustrated by

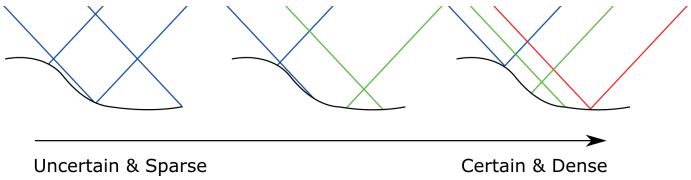

Figure 3.1: Coupling of axial measurement uncertainty and lateral measurement density.

Fig. 3.2. Suppose the position of the object is represented by the arrow. In the first iteration, the camera takes two frames with the two illumination spectra covering the complete wavelength range. Based on estimation result from the first iteration, which is not extremely accurate, the object is determined to be in the top half of the measurement range. In the second iteration, the AdaScope makes measurement in the new measurement range with two similar illumination spectra. This appears to be like a binary search, but if the measurement in each iteration is accurate enough, the search process can be much faster. For example, a direct jump from iteration #1 to iteration #3 will also be possible.

Apparently this method is not very sensitive and is not robust against the noise due to the limited number of linear measurement channels, but it should be sufficient to bound the measurement range to a certain level for the next iteration.

3.2 Lateral Grid Condensation

As mentioned previously, in each iteration, the measurement density is also increased accordingly. As shown by the example in Fig, 3.3, in iteration #1 with a pitch of 20 pixels, grid has to be scanned 20 by 20 times, and in each time, the system makes two measurements using the corresponding illumination spectra. In the next iteration, the density of the grid can be increased depending on how much the new measurement range is bounded.

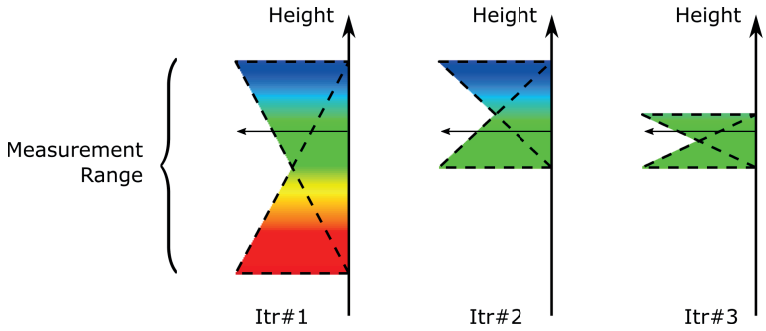

Figure 3.2: Iterative refinement of axial measurement.

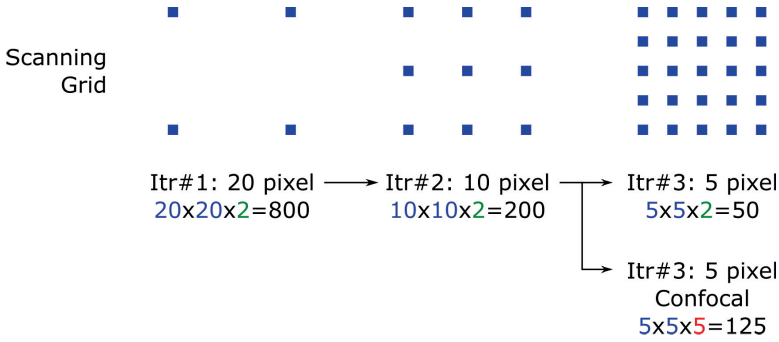

Figure 3.3: Iterative condensation of lateral grid.

At a certain iteration, based on the estimation uncertainty from previous iteration, the measurement process can be switched to a localized chromatic confocal measurement centered around the previous estimation result, in order to get more accurate measurement result.

3.3 Hardware Triggering

As an example, the triggering diagram for the second iteration as well as the corresponding illumination spectra are illustrated in Fig. 3.4. Since the camera is the slowest component, it serves as the master which triggers the spectral DMD in the programmable light source. This DMD displays several patterns corresponding to several illumination spectra. Each spectral DMD patterns triggers its corresponding spatial DMD pattern in the microscope. Based on estimation from the first iteration, all points are already bounded to either the top half or the bottom half of the complete measurement range. For each measurement grid, two frames are captured. Within each frame, two spectra are projected to two different spatial patterns. In the second frame, the spatial patterns are repeated but the spectra are different. This process is then repeated pitch^2 times for complete measurement of this iteration.

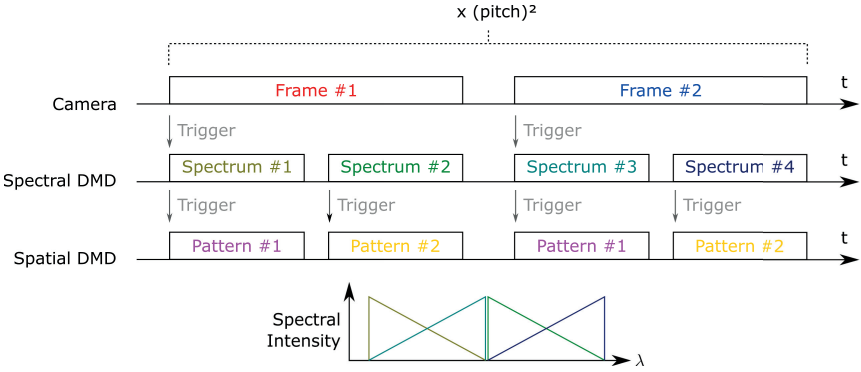

Figure 3.4: Exemplary triggering diagram of iteration #2.

4 Experiment and Analysis

To investigate the feasibility of this adaptive measurement method, a test measurement is conducted with a one euro coin serving as the target. For proof of concept, the wavelength range is limited to $530\,\mathrm{nm}$ to $580\,\mathrm{nm}$, which corresponds to a measurement range of $1.15\,\mathrm{mm}$.

For the adaptive scan, in each iteration, two images are captured with different illumination spectra and spatial patterns. Fig. 4.1 shows the raw measurement data from the first iteration with their corresponding illumination spectra. It can be seen that the image with first illumination spectrum is much brighter than the second one. This indicates that the centroid of the chromatic confocal peak is closer to the focus position of $530\,\mathrm{nm}$.

After the first iteration, a rough estimation of the height can be performed based on the two-channel linear measurement principle, which is then binarized into two levels. As can be seen in Fig. 4.2, for most of the area, the height lies in the upper half of the current measurement range.

After the second iteration with a denser grid, more accurate estimation is performed, which generate four measurement range divisions (Fig. 4.3).

After iteration #2, the system directly makes localized chromatic confocal measurement with five wavelength steps with a step size of $1\,\mathrm{nm}$. These five steps

are centered around the estimation result from iteration #2. In this iteration, the pitch is further reduced to 5 pixels. As shown in Fig. 4.4, estimation of the height and the intensity (texture) is performed through Gaussian fitting on the localized measurements.

Although the overall estimation is correct, there are apparent artifacts all across the measurement area. The major reason for this kind of artifact is the inaccurate estimation result before the localized chromatic scanning. Since the iteration of the localized scan is initialized based on previous estimation, when the starting point is already too far away from the actual chromatic peak, two adverse effects could happen. On one hand, the five wavelength scanning steps are not enough to cover the peak position of the chromatic confocal signal. On the other hand, when the scanning wavelength steps are too far from the peak, the generated crosstalk for the adjacent locations will no longer be tolerable for the selected pitch distance of the measurement grid. To avoid such artifacts, more accurate estimation from the linear measurement stage must be achieved in order to correctly initialize the localized chromatic scanning, which will be the key part in future research.

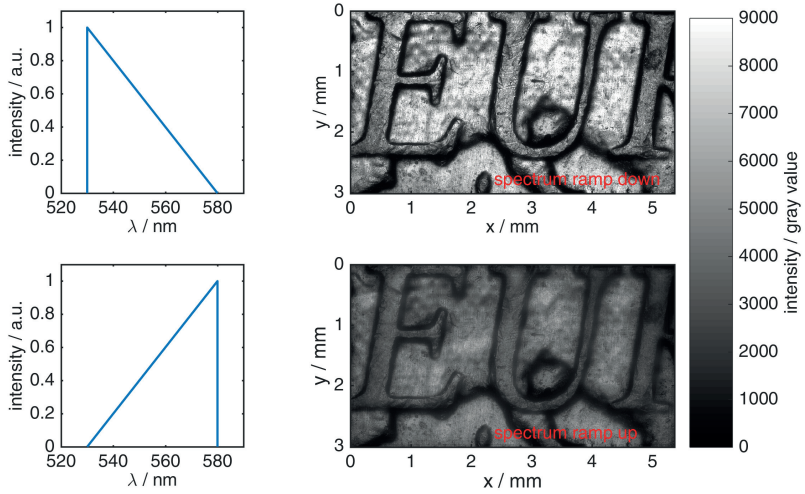

Figure 4.1: Illumination spectra (left) and raw measurements (right) from iteration #1.

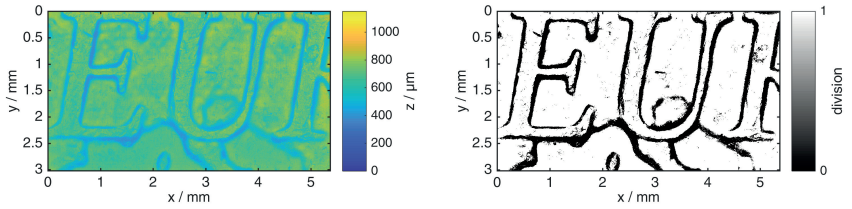

Figure 4.2: Estimation (left) and binarized levels (right) from iteration #1.

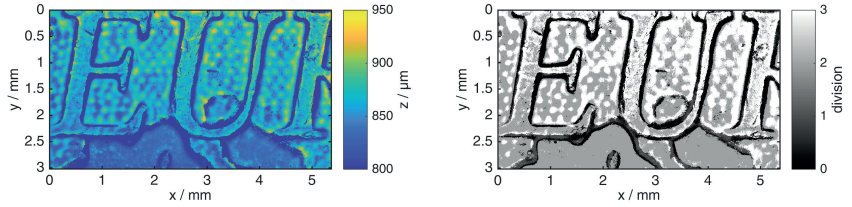

Figure 4.3: Estimation (left) and binarized levels (right) from iteration #2.

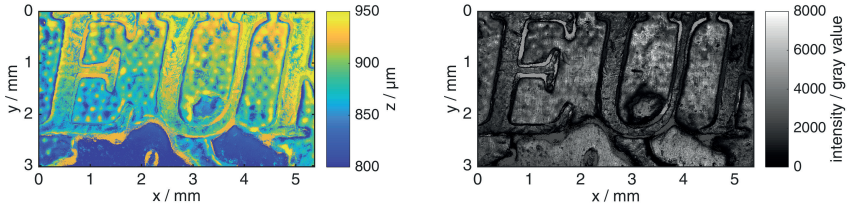

Figure 4.4: Height estimation (left) and intensity (right) from iteration #3.

5 Conclusion

This report presents a novel approach to the problem of area chromatic confocal microscopy. The hardware setup consists of two fundamental components. One part is a programmable light source based on a white light laser. The other part is a DMD-based programmable array chromatic confocal microscope. The combination of both, namely the AdaScope, provides an adaptive platform which

can be utilized for 3D measurement of reflective/defusing samples. An adaptive measurement method is developed based on this system setup, which iteratively improves the axial measurement uncertainty and lateral measurement density. Although initial experimental investigation has revealed certain artifacts in the measurement result mainly due to inaccuracy in the linear measurement iterations, it is believed that such approach has great potential in increasing the measurement speed compared to a naive fixed grid scanning. This could lead to applications of the area chromatic confocal measurement technology in real industrial settings.

Acknowledgement Research conducted in this report is financed by the Baden-Württemberg Stiftung gGmbH.

Bibliography

[CRS15] Nadya Chakrova, Bernd Rieger, and Sjoerd Stallinga. Development of a DMD-based fluorescence microscope. *Proc. SPIE*, 9330:9330–9330–11, 2015.

[CS17] Ting-Jui Chang and Guo-Dung J. Su. A confocal microscope with programmable aperture arrays by polymer-dispersed liquid crystal. *Proc. SPIE*, 10376:10376–10376–7, 2017.

[EP67] M. David Egger and Mojmir Petran. New reflected-light microscope for viewing unstained brain and ganglion cells. *Science*, 157(3786):305–307, 1967.

[HCT+07] Guy M. Hagen, Wouter Caarls, Martin Thomas, Andrew Hill, Keith A. Lidke, Bernd Rieger, Cornelia Fritsch, Bert van Geest, Thomas M. Jovin, and Donna J. Arndt-Jovin. Biological applications of an LCoS-based programmable array microscope (PAM). *Proc. SPIE*, 6441:6441–6441–12, 2007.

[HO15] Shinichi Hayashi and Yasushi Okada. Ultrafast superresolution fluorescence imaging with spinning disk confocal microscope optics. *Molecular Biology of the Cell*, 26(9):1743–1751, 2015.

[HVG+99] Q. S. Hanley, P. J. Verveer, M. J. Gemkow, D. Arndt-Jovin, and T. M. Jovin. An optical sectioning programmable array microscope implemented with a digital micromirror device. *Journal of Microscopy*, 196(3):317–331, 1999.

[KDP+14] Sharon V. King, Ana Doblas, Nurmohammed Patwary, Genaro Saavedra, Manuel Martínez-Corral, and Chrysanthe Preza. Implementation of PSF engineering in high-resolution 3D microscopy imaging with a LCoS (reflective) SLM. *Proc. SPIE*, 8949:8949–8949–7, 2014.

[LTLB17] Ding Luo, Miro Taphanel, Thomas Längle, and Jürgen Beyerer. Programmable light source based on an echellogram of a supercontinuum laser. *Applied Optics*, 56(8):2359–2367, 2017.