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Structured illumination microscopy using a photonic chip

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Supplementary Information

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Supplementary note 1: Simulation of chip-based interference patterns

The cSIM interference patterns were simulated using finite element method in Comsol Multiphysics. Figure 1(a) shows two waveguides crossing at an angle of 50° . The red arrows indicate the directions of the straight waveguides along which the light propagates. Since the presented model is limited to a two-dimensional representation of the actual waveguide, the effective refractive indices along the third dimension have been calculated approximating those regions as planar waveguides [1]. Given the fundamental transversal electric (TE) mode being excited in each waveguide, the resulting intensity distribution shows the intended sinusoidal structure as well as a Gaussian envelope due to the shape of the waveguide modes. The mode index in the simulated Si_3N_4 structures is found to be $n = 1.7552$ at a vacuum wavelength of $\lambda = 660 \text{ nm}$. In the presented model only the fundamental modes of the waveguides are excited. As it is indicated in Fig. 1(a), in order to estimate the periodicity of the intensity pattern, a sinusoidal function of the form $I(x) = \exp\left(-\left(\frac{x}{a}\right)^2\right) \sin^2(bx)$ has been fitted; the pattern spacing is found to be 445 nm .

Figure 1(b) shows respective simulation results for counter-propagating waveguides, i.e. interfering at an angle of 180° . As for the case of an interference angle of 50° , the intensity distribution was fitted as $I(x) = \sin^2(ax)$ and a period of 188 nm is found.

Although the pattern spacing can be calculated from the effective mode index without running a full numerical simulation of the structure, here the simulation results are used to illustrate the expected intensity distribution over the whole waveguide geometry.

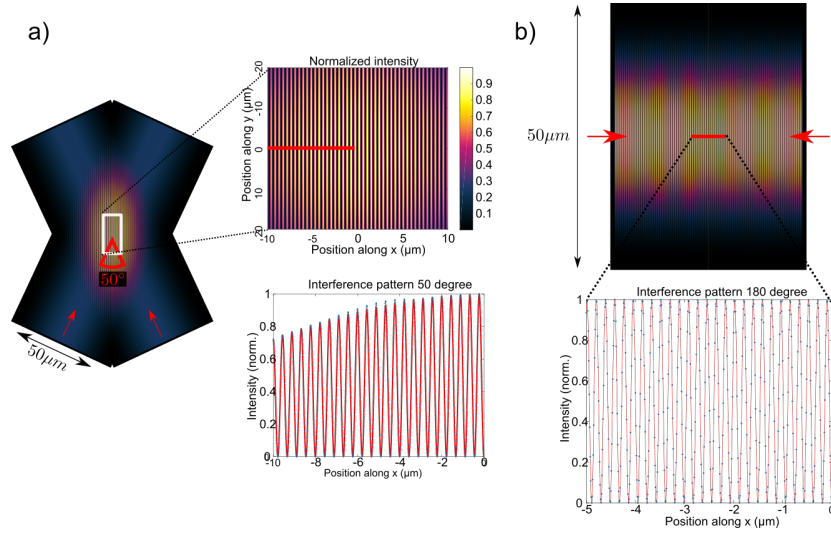


Figure 1: Numerical simulations of the proposed waveguide geometries to generate the illumination patterns for cSIM. The configurations of two waveguides crossing at an angle of 50° (a) generates a pattern with a periodicity of 445 nm. The counterpropagating waveguides (b) create a pattern with a period of 188 nm.

Supplementary note 2: Resolution of cSIM

The microscope objective is inherently a low pass filter, stopping frequency content above a certain value k_0 , often referred to as the diffraction limited. Structure illumination microscopy relies on a sinusoidal excitation light to excite fluorescence in the sample. For a sample having frequency $\mathbf{k}$, the multiplication of structured illumination and sample yields a moirè pattern with observable frequencies $|\mathbf{k} - \mathbf{k}_1|$, if these are within the pass-band of the OTF (k_0) [2]. So as long as $\mathbf{k}$ lies within a circle of radius k_0 centered around $\mathbf{k}_1$, it will be indirectly observed in the moirè fringes. By going through the SIM reconstruction using phase stepping the achievable resolution of the reconstructed image is then $k_0 + k_1$. In this sense, it is desirable to have a large k_1 , but since k_1 is formed using lenses, the method is also limited by diffraction, with the highest possible value of k_1 being k_0 . Finally this would yield a maximum resolution enhancement of $2k_0$. By generating the structured illumination using waveguides the formation of k_1 is no longer depending on lenses, and is thus not diffraction limited but instead relies on the effective refractive index of the waveguide materials, as outlined below.

The resolution of cSIM is governed by the fringe-spacing of the standing wave interference pattern in addition to the N.A. of the collection optics. By interfering waveguides with changing angles θ the fringe spacing f_s can be changed as

$$f_s = \frac{\lambda_{ex}}{2n_{eff} \sin \theta/2}, \quad (1)$$

where n_{eff} is the effective refractive index of the guided mode. Generating the SIM pattern with a planar waveguide thus contributes n_{eff} in to the achievable resolution Δ_{xy} of the method, so the equation describing the theoretical best resolution for cSIM takes the form of

$$\Delta_{xy} = \frac{\lambda_{ex}}{2(N.A. + n_{eff} \sin \theta/2)}, \quad (2)$$

where N.A. is the numerical aperture of the imaging objective lens. Since n_{eff} is larger than N.A. the resolution of the method can exceed that of conventional SIM, which would have $4N.A.$ in the denominator.

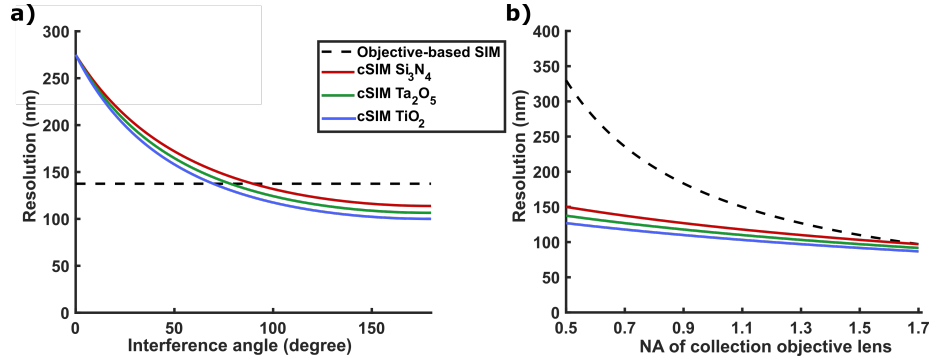


Figure 2: Graphical interpretation of eq. 2 for three different materials is shown. a) The angle of interference is dominating the achievable resolution for cSIM, which for interference angles between 70-100° surpass the resolution of conventional objective based SIM, using N.A.=1.2. b) The maximum achievable resolution for different N.A. objective lens, and thus different field of view size is shown for both cSIM (using 180° interference) and objective based SIM. The potential for cSIM to yield high resolution over large fields of view can be harnessed by acquiring several images with different interference angles as shown in Fig.4. The simulation wavelength is 660 nm

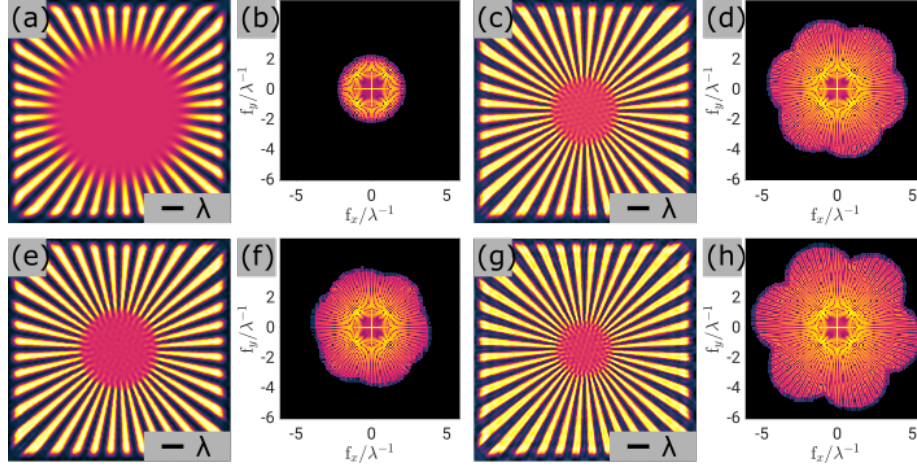


Figure 3: Simulation of SIM using the Siemens star sample. Spatial and frequency images are shown sequentially. a,b) Diffraction limited image. c,d) Conventional SIM image, e,f) cSIM using 60 degree interference angle. g,h cSIM using 180 degree interference angle)

Supplementary note 3: Simulation of chip-based SIM

The effect of structured illumination using planar waveguides is presented in Figs. 3 and 4 for two different imaging objective lenses using the Siemens star sample.

In Fig. 3, imaging using an objective lens with N.A. = 1.2 is simulated. Figure 3(a) shows the spatial result using plain illumination, and Fig. 3(b) shows the logarithmic values of the Fourier spectrum. It is visible in the figure that the highest frequency is limited to $2\text{N.A.}/\lambda = 2.4\lambda^{-1}$. Fig. 3 (c) and (d) show the effect of SIM imaging using the conventional objective based approach, having a spatial frequency of the SIM pattern at the OTF cutoff frequency $2\text{N.A.}/\lambda$, yielding a 2x resolution enhancement. For a sample that is illuminated with a sinusoidal stripe pattern using planar waveguides with an effective refractive index of $n_{\text{eff}} = 1.7$ at an angle between the waveguides of 60° the expected SIM result is shown in Fig. 3(e)-3(f). Increasing the interference angle to 180° increases the modulation frequency of the illumination pattern and thus the expected resolution improvement as shown in Figs. 3(g)-3(h).

In a similar fashion cSIM imaging is simulated in Fig. 4 for an objective lens with N.A. = 0.6. Figures 4(a)-4(b) show the result from using plain illumination. It shows a clear reduction in resolution as compared to the result using an imaging objective with a N.A. = 1.2 as in Figs. 3(a)-3(b).

Using an illumination pattern generated by waveguides with an interfering angle of 30° will improve the resolution as presented in Figs. 4(c)-4(d). For cSIM using patterns generated with interference angles of 120° and 180° it is possible to obtain sample information of high spatial frequency as illustrated in Figs.

4(e)-4(f) and Fig. 4(g)-4(h) respectively. Since the Fourier space is not filled evenly in this approach, a combined use of illumination patterns generated at interference angles of 60° , 120° and 180° as shown in Figs. 4(i)-4(j) will improve the resolution without the loss of frequency bands. Thus, cSIM supports the use of low magnification/N.A. objective lens to enable super-resolution imaging over large field of view (FOV), while the maximum attainable resolution will be supported by the illumination provided by the waveguide as demonstrated in Figs. 4(i)-4(j). In conventional SIM, the illumination and the collection light paths are coupled, thus a use of low. N.A. objective lens to image large FOV will significantly reduce the supported optical resolution.

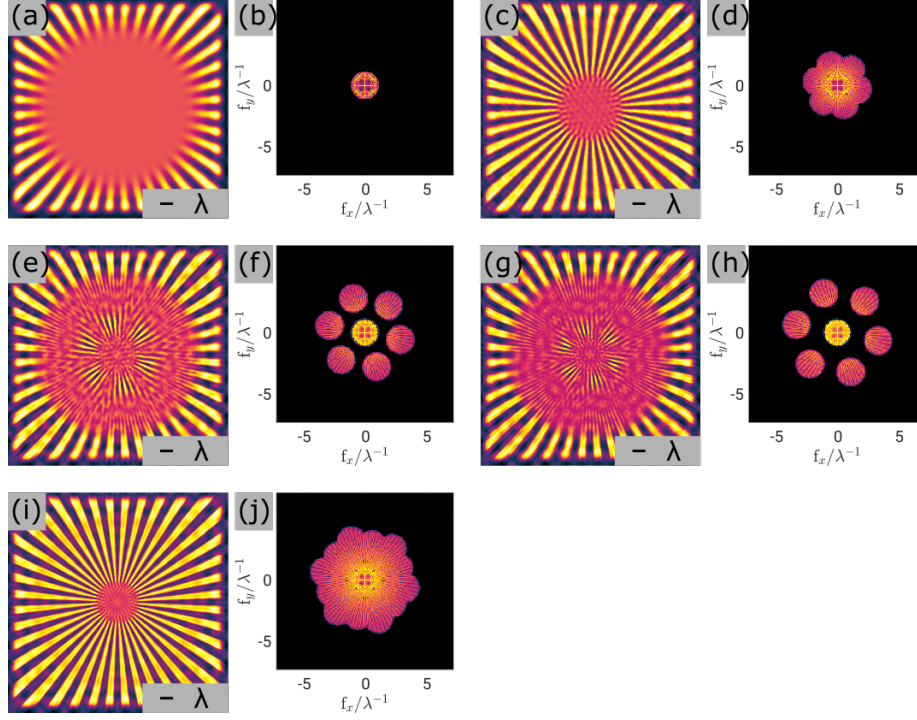


Figure 4: The resolution improvement using chip based SIM is simulated for an imaging objective with $N.A. = 0.6$. (a) and (b) show the result of conventional deconvolution imaging at the indicated wavelength λ and the representation in Fourier space respectively. (c) and (d) show the SIM results for patterns generated at an waveguide interference angle of 60° . (e)-(f) and (g)-(h) show the SIM result using interference patterns generated at angles of 120° and 180° respectively. In (i) and (j) the SIM reconstructions using all interference angles (60° ; 120° ; 180°) are shown, with pattern rotations corresponding to that which can be achieved using the design shown in Fig. 2d in the main paper. In this design, the initial pattern rotation can be different for each of the interference angles, thus filling the frequency space as shown in (j).

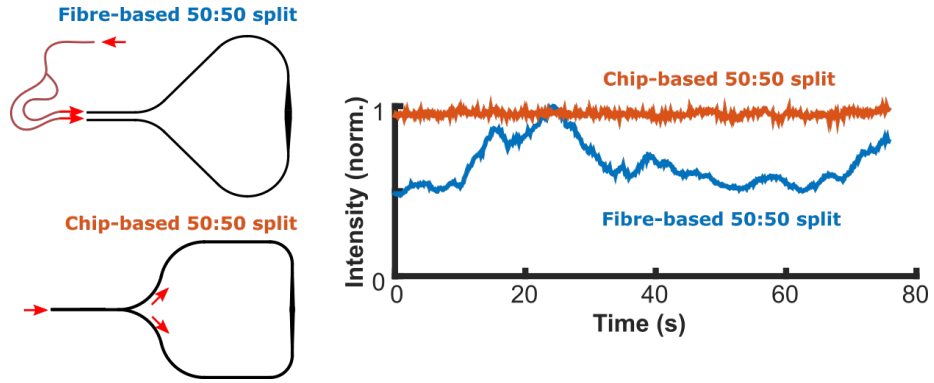


Figure 5: The phase stability of the standing wave SIM interference pattern is shown indirectly by tracking the fluorescence from a bead over time. By splitting the light on the optical table using a 50:50 fibre split the phase of the standing wave is drifting in time, seen with the blue curve. When splitting the light on-chip, the phase is observed to be stable over time (red curve).

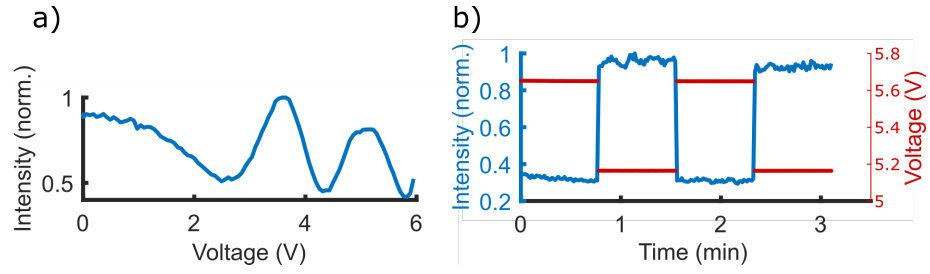


Figure 6: a) By applying current to the thermo-optical element the phase of the SIM interference pattern was changed. A fluorescent bead was tracked while changing the current, thus shifting the SIM pattern phase. b) By selecting the voltages corresponding to a π change in the SIM pattern, the phase was stepped between 0 and π over 3 minutes to demonstrate the reproducibility of the phase stepping method.

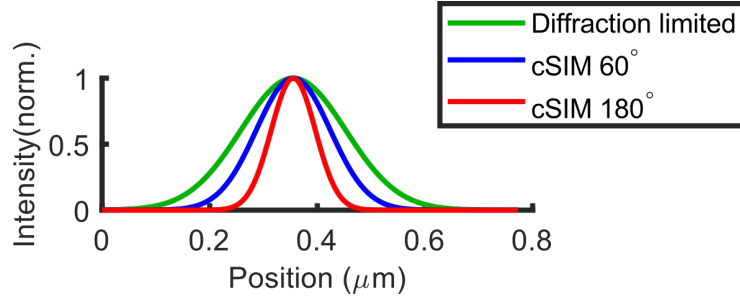


Figure 7: Gaussian fit using the average values from 10 different line-profile measurements from figure 3 and 5, i.e for diffraction limited, 60° cSIM and 180° cSIM. The full width at half maximum gives an estimate for the resolution of the method. The result shows that beads shrink on average 1.63x over the diffraction limit using 60° interference, and 2.39x over the diffraction limited for 180° interference. The theoretical values (eq. 2 of the main manuscript) yields 1.71x for 60° and 2.42x for 180° interference

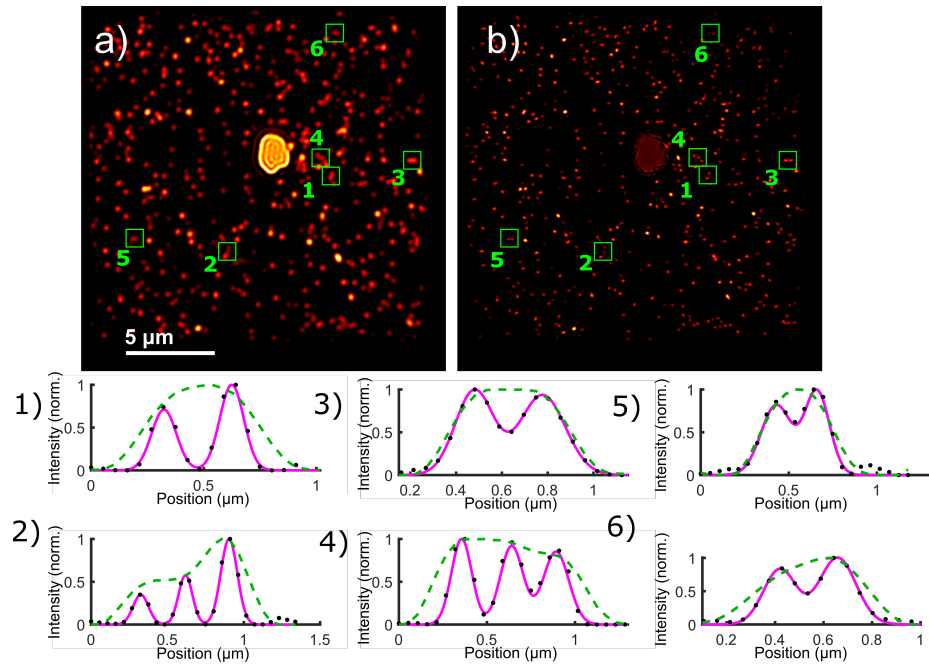


Figure 8: The cSIM result presented in figure 3, using 60° interference angle is shown with several intensity distributions with overlaid line profiles. The resolution enhancement of the cSIM image (b) over the diffraction limited image (a) is shown for 6 different intensity distributions.

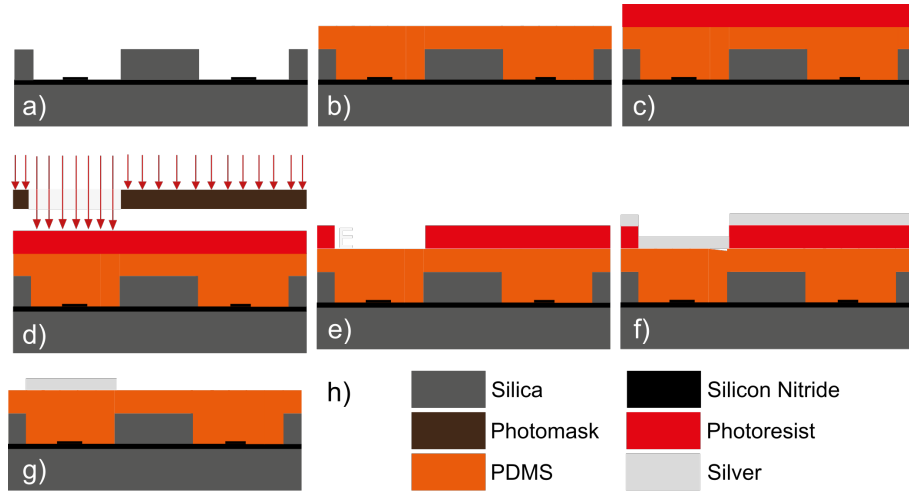


Figure 9: Fabrication of thermo-optic heating element. (a) Both arms of the cSIM structure incorporates opening in the cladding. (b) A cladding layer made from either PDMS or Su-8 covers the entire chip, followed by a photo-resist (c). (d) One of the openings is exposed and the photo-resist was removed. (e) A layer of Ag is sputtered on the entire chip, before the photo-resist is removed leaving only silver on one of the arms.

Supplementary note 4: Characterization of the rise and fall time of thermo-optical phase modulation

We used photonics chip based Mach-Zehnder interferometer (Supplementary Fig. 10) to characterize repeatability and rise and fall time of our polymer-based thermo-optical phase shifter. The light at the output of the waveguide is focused into a photo-detector which is then connected to an oscilloscope. A signal generator is used to deliver the voltage to the heating element. In this measurement, we have deposited 2 μm thick polydimethylsiloxane (PDMS) polymer on the active arm of the Mach-Zehnder interferometer and 120 nm thick and 100 μm wide silver were sputtered on top of the polymer. The resistance in the electrode is 20 ohm. As shown in figure 1b, a change of 6 voltage was needed to achieve a π phase shift. The rise and fall of the thermo-optical phase shifter for a π phase shift take 200 and 100 μs respectively. A significant drift is observed in our measurements when the voltage is stable, which is mainly due to the asymmetric nature of the interferometer used in this experiment. In this design, certain length of opening for polymer deposition is created only on one arm of the interferometer (Supplementary Fig. 10a). To overcome these drift issues, we have implemented symmetric interferometers, as depicted in Fig. 2c,f,h of the main paper. In our symmetric interferometer design, the same opening length for polymer deposition is created on both arms of the interferometer, but the electrode is deposited only on one of the arm.

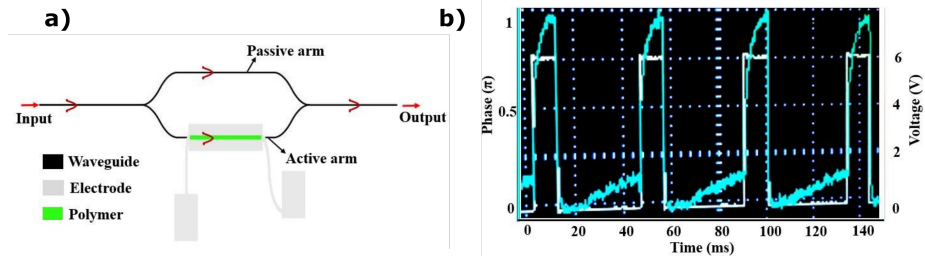


Figure 10: Thermo-optical phase shifter. The sketch of Mach-Zehnder interferometer integrated with thermo-optical phase shifter (a and b) Applied voltage and measured phase with one arm covered with PDMS polymer

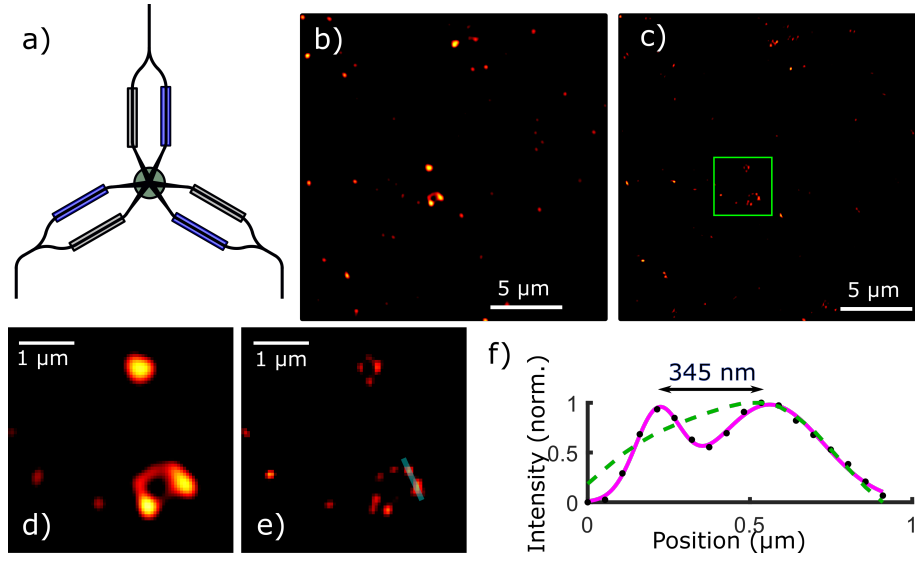


Figure 11: a) cSIM using thermo-optics phase modulation with 50° interference angle. b) Show the diffraction limited image of 100nm fluorescence beads under plain 660nm illumination yielding a diffraction limited image. c) Using the structure shown in a), the cSIM reconstruction shows a clear resolution enhancement. d) and e) shows the zoom indicated with a green box in c). f) Show a line-profile over an intensity distribution comparing the diffraction limited (dotted line) and cSIM reconstruction (solid line). The cSIM image shows two beads separated by 345nm, which is not resolved in the diffraction limited image.

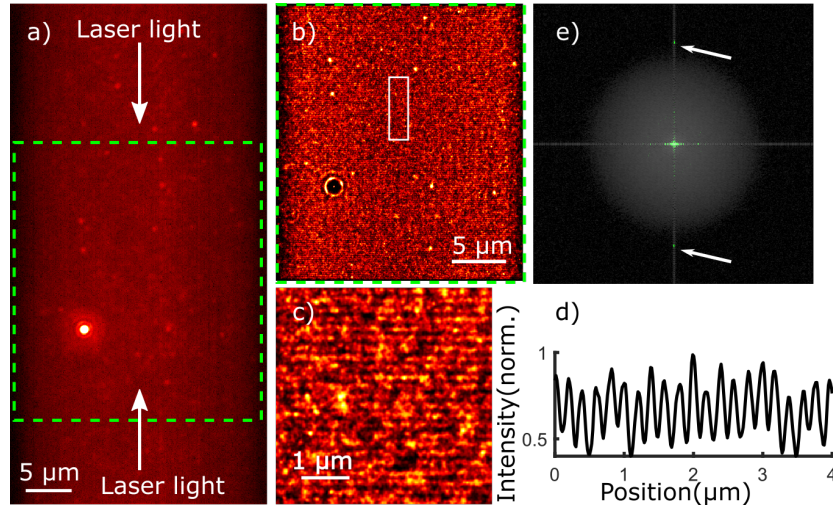


Figure 12: The waveguide surface was stained with a fluorescent dye. a) The standing wave interference pattern is not visible under diffraction limited imaging. b) By using *d*STORM imaging the sub-diffraction limited standing wave pattern was visualized, as better seen in the zoomed image (c). d) A line-profile shows the modulation with a fringe spacing of around 195 nm. e) The power-spectrum of the diffraction limited image (a) and the *d*STORM reconstruction (b) is overlaid, with the green peaks showing the frequency peak of the standing wave at its position outside the span of the OTF.

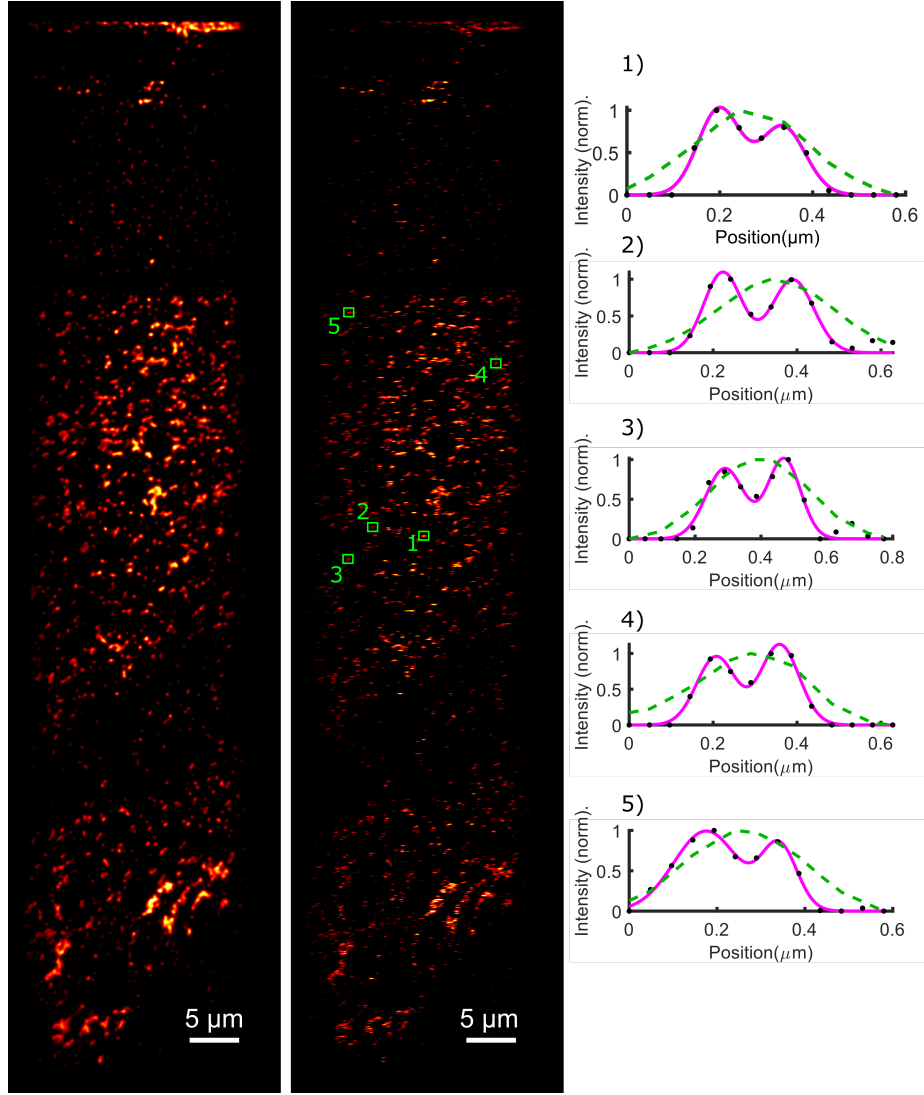


Figure 13: The cSIM result presented in figure 5, using 180° interference angle is shown with several intensity distributions with overlaid line profiles. The resolution enhancement of the cSIM image (b) over the diffraction limited image (a) is shown for 5 different intensity distributions.

Supplementary note 5: Measurement of the evanescent field penetration depth.

The penetration depth of our cSIM structures was measured indirectly by first measuring the fringe spacing f_s of the standing wave pattern, and then calculating the effective refractive index n_{eff} which is related to the penetration depth d by

$$d = \frac{\lambda}{2\pi \sqrt{(n_{eff}^2 - n_s^2)}}, \quad (3)$$

where n_s is the refractive index of the top cladding (water or cell, $n=1.33-1.38$). The effective index is related to the fringe spacing by

$$f_s = \frac{\lambda}{2n_{eff} \sin\left(\frac{\theta}{2}\right)}, \quad (4)$$

where θ is the angle between the interfering waveguides.

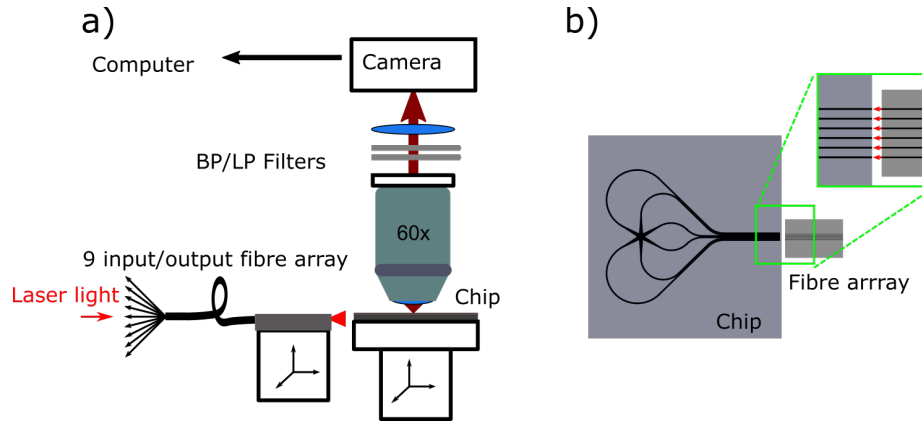


Figure 14: Experimental setup. a) An upright microscope detects the emitted fluorescence. The waveguide chip is held by a vacuum chuck while light is coupled on to the chip via a nine-fibre array adapter. Both sample and coupling stages have xyz movement. b) The fibre array adapter holds 9 optical fibers with equidistant spacing ($127\ \mu\text{m}$), which corresponds to the waveguide spacing on the chip. Each of the 9 optical fibers can be individually activated, according to the chip designs being used.

Supplementary note 6: Scaling the image area for cSIM

Adiabatically tapering a single mode waveguide can provide a uniform excitation over large areas. In our design, a $1.5\ \mu\text{m}$ wide waveguide was adiabatically tapered to a $25\ \mu\text{m}$ wide waveguide using a tapering length of 2 mm. A larger FOV imaging using adiabatic taper can be done but it has some challenges. For example, achieving an adiabatically tapered $300\ \mu\text{m}$ wide waveguide requires 24 mm taper length, which results in complicated design and higher propagation loss.

To overcome the limitation of adiabatic tapering, here we have proposed a different design where a single mode waveguide ends in a slab and allow the light to diverge in the slab. This idea is depicted in Fig. 15a,b, where a waveguide interferometer is shown. A single mode waveguide feed the light to a slab waveguide. By letting light diverge freely in the slab region, the resulting interference pattern can be made available over an area with a size depending on parameters like initial beam waist, which is given by the waveguide width. The angle of light divergence (θ) in the slab depends on the initial beam width (w_0) and wavelength (λ_0), as expressed by eq. 5

$$\theta = \frac{\lambda_0}{n_{eff}\pi w_0} \quad (5)$$

For two lights diverging and interfering in the slab the overlapping region depends on the spacing and angle between the interfering lights. This is illustrated in the waveguide-based interferometer we propose in Fig. 15a. The angle between interfering waveguides (α) is 20° and separation (h) of $375\ \mu\text{m}$, as shown in Fig. 15b. The expected beam diameter from each arm at a distance of around 1.5 mm is around $300\ \mu\text{m}$. Fig. 15c is the experimental fluorescent image of this interferometer. Here, to observe the interference fringes in the slab region we labeled the slab region with cell mask deep red (CMDR) fluorophore, and 660 nm wavelength laser light was coupled into the interferometer. The resulting interference fringes are available over a large area of around $300 \times 400\ \mu\text{m}^2$. The interference pattern is image using a 60x/n.a.1.2 objective shown in the zoomed image.

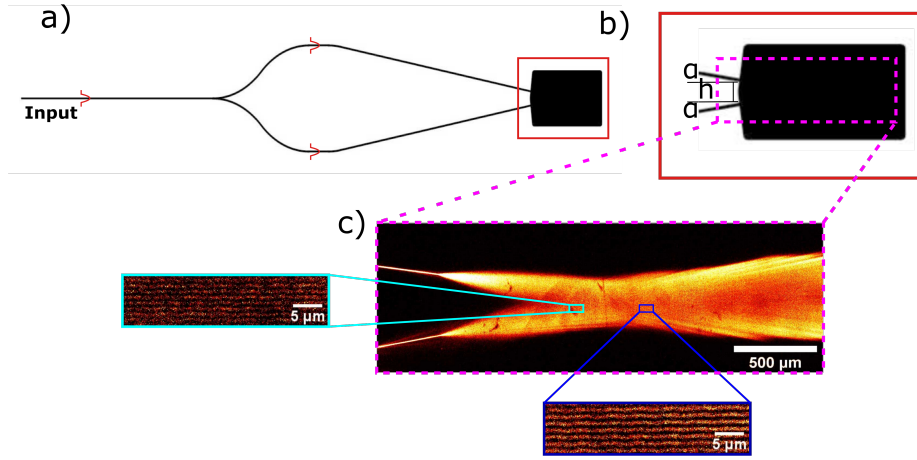


Figure 15: a) Design of a waveguide interferometer ending in slab .b) show the zoomed image marked by a red square in (a). c) The same structure depicted in (a) is used in an experiment to show the presence of interference fringes. The surface of the waveguide is labelled with a fluorescent dye layer (CMDR) and imaging using a 4x objective lens. The pattern becomes visible when using a 60x/1.2n.a. objective as shown in the zoomed image. The pattern is present all over the field-of-view ($300 \times 400 \mu\text{m}^2$).

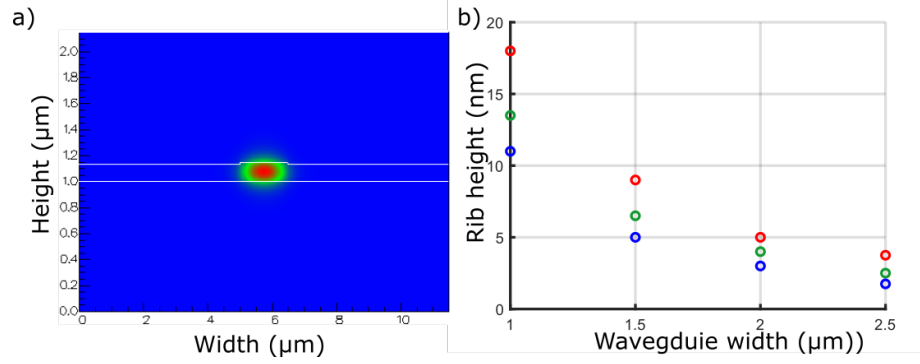


Figure 16: a) Simulation showing the fundamental transverse electric (TE) mode guided in waveguide core, with parts of the light outside the top surface as an evanescent field. b) Simulated threshold rib height values for single mode operation for different waveguide widths and wavelengths (660 nm, 561 nm and 488 nm are shown in the appropriate colors.)

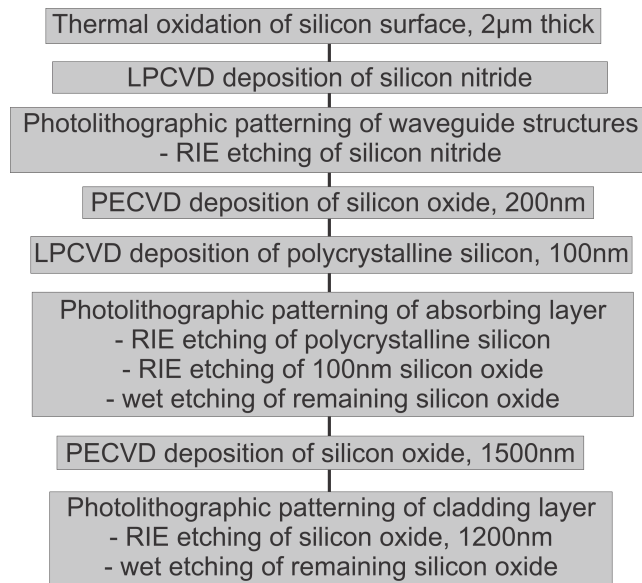


Figure 17: Flow-chart showing the fabrication steps performed in the production of cSIM waveguide chips

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- [2] M. G. L. Gustafsson, “Nonlinear structured-illumination microscopy: Wide-field fluorescence imaging with theoretically unlimited resolution,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 37, pp. 13081–13086, 2005.