Helle, Ø.I., Dullo, F.T., Lahrberg, M. Tinguely, J-C., Hellesø, O.G., Ahluwalia, B. S. Structured illumination microscopy using a photonic chip. *Nat. Photonics* **14,** 431–438 (2020). https://doi.org/10.1038/s41566-020-0620-2

Structured illumination microscopy using a photonic chip

Øystein Ivar Helle[1], Firehun Tsige Dullo[1], Marcel Lahrberg[1], Jean-Claude Tinguely[1], Olav Gaute Hellesø[1] and Balpreet Singh Ahluwalia[1]

1 Department of Physics and Technology, UiT-The Arctic University of Norway, 9037 Tromsø, Norway.

Correspondence should be addressed to BSA (balpreet.singh.ahluwalia@uit.no)

Structured illumination microscopy (SIM) enables live-cell super-resolution imaging of sub-cellular structures at high speeds. At present, linear SIM uses free-space optics to illuminate the sample with the desired light patterns, however such arrangements are prone to miss-alignment and add cost and complexity to the microscope. Here, we present an alternative photonic-chip based 2D SIM approach in which the conventional glass sample slide in a microscope is replaced by a planar photonic chip that importantly both holds and illuminates the specimen. The photonic-chip reduces the footprint of the light illumination path of the SIM to around 4x4 cm^2. An array of optical waveguides on the chip create standing-wave interference patterns at different angles, which illuminate the sample via evanescent fields. High refractive index silicon nitride waveguides allow a 2.3x enhancement in imaging spatial resolution, exceeding the usual 2x limit of SIM. In summary, cSIM offers a simple, stable and affordable approach for performing 2D super-resolution imaging over a large field of view.

The spatial resolution of optical microscopy is bound by diffraction effectively limiting the achievable resolution to around 250 nm laterally, and 500 nm axially[1,2]. The advent of super-resolution fluorescence microscopy, commonly known as nanoscopy, has proven the ability to trick the diffraction limit extending the lateral resolution of the microscope down towards just a few nanometre[3]. Among the existing optical nanoscopy methodologies[4-8], structured illumination microscopy (SIM)[9,10] works for most bright fluorophores. Instead of illuminating a sample with a uniform field, in SIM, a sinusoidal excitation pattern illuminates the sample, and the fluorescence emission is captured on a camera. The sinusoidal excitation light is typically generated using interference of two or three beams at the sample plane. The illumination and object functions combine at the sample plane via a multiplication, which in frequency space represents a convolution, mixing the spatial frequencies of the two functions. In this manner, high frequency, un-resolved, content is made available within the passband of the objective lens due to a frequency down-conversion with the resulting fluorescence emission observed as a Moiré fringe pattern. To extract the high frequency content from the Moiré pattern, three or five phase-shifts of the structured illumination is needed for improving the resolution along one axis. For isotropic resolution, the process must be repeated for 3 orientations (angles) of the excitation pattern, for a total of 9 (15) images in case of a 2D (3D) SIM reconstruction. Since SIM only needs 9 (15 for 3D) images to create a super-resolution image over a wide-field of view, this method is inherently fast, making it one of the most popular methods for live cell optical nanoscopy.

The development of high-resolution methods like stimulated excitation depletion (STED) microscopy techniques[4,5] and single-molecule localization microscopy (SMLM)[6-8], yielding resolution down to a few tens of nanometres, has opened new possibilities for discovery within life sciences[11]. Although, STED and SMLM methods provide superior images on a single cell level, these techniques suffer from low throughput when high-speed images over large areas with many cells are needed to build statistical impact. Thus, the next leap for super-resolution imaging may come by increasing the throughput of the nanoscopy methods. Among, the existing super-resolution microscopy techniques, SIM provide the fastest temporal resolution and with its compatibility with standard labels and low photo-toxicity the SIM method points in the direction of real high-throughput nanoscopy. To fully harness the utility of fast SIM imaging technique, the achievable spatial resolution, throughput of the method and the system complexity of SIM would need improvement. In conventional SIM, the illumination and

collection light paths are coupled (Fig 1a), which limits the resolution and hinders the use of low numerical aperture (N.A.) objectives to be used for large area imaging. Furthermore, the generation of the necessary standing wave patterns is usually done with free-space optics, with components to control and maintain the pattern orientation, phase, and polarization state. The resulting SIM microscopes are thus bulky, expensive and prone to misalignment needing highly qualified personnel which further adds to maintenance costs. The resolution of SIM can be improved by using speckle-illumination[12] and blind-SIM[13-15] reconstruction approaches but it comes with a cost of low-temporal resolution due to the need of a large number of images (100s). The speckle illumination methods take away the main concept of "structured" illumination which helps in minimizing the number of frames needed (9-15) by illuminating the sample with pre-determined and highly structured illumination patterns. Non-linear effects can also be harnessed for increased SIM resolution[16,17], but at the cost of photo-toxic light intensities or the need for specialized fluorescent proteins[18,19]. Thus, efforts to enhance the resolution of linear SIM keeping well-defined structured illumination is worth pursuing.

SIM on a chip

In this work, we propose a method that will allow a standard optical microscope to acquire total internal reflection fluorescence (TIRF) SIM[20-22] images using a mass-producible, photonic chip, with better resolution and possibly increased field-of-view (FOV) over conventional SIM. Chip-based fluorescence microscopy relies on total internal reflection of the excitation light inside planar waveguides on a chip[23-26 27,28], with a part of the excitation light available as an evanescent field exponentially decaying from the surface of the waveguide. In chip-based SIM (cSIM), a low-cost upright microscope can gather 2D super-resolved images of the specimen placed directly on top of a planar waveguide surface (Fig. 1b), within the 100-150 nm reach of the evanescent field. The waveguide is made from a dielectric material with high index contrast (HIC) (silicon nitride (Si_3N_4) in this case). By using the interference of spatially coherent light inside single-mode waveguides, standing wave interference fringes are generated on top of the waveguide surface (Fig. 1, Supplementary note 1, Supplementary Fig. 1), which are used to excite fluorescence in the labelled sample at a pre-determined location. An array of single-mode planar waveguides, as shown in Fig. 1c enables three rotational angles at the overlapping imaging area. By maneuvering the phase of the standing wave of the respective waveguide arm, the necessary data for a TIRF-SIM reconstruction, i.e. 9 images (3 angles and 3 phases steps) can be acquired. At the imaging location, the single-mode waveguides are adiabatically tapered[28,29] to a large area, 400 μm^2 in the present study but can be made over several mm^2 (Supplementary Fig 15).

The proposed cSIM methodology opens up the possibility of pushing the resolution enhancement of the SIM technique beyond 2X and enables scalable resolution over a scalable large field of view. This can be seen if we first consider the fringe period f_s generated by interference in a waveguide as given by

$$f_s = \frac{\lambda_{ex}}{2n_f \sin\frac{\theta}{2}} \qquad (1)$$

where λ_{ex} is the excitation wavelength, n_f is the effective refractive index of the guided mode and θ is the angle of interference. Generating the SIM pattern with a planar waveguide thus contributes n_f to the achievable resolution Δ_{xy} of the method, so the equation describing the theoretical resolution limit for cSIM takes the form of

$$\Delta_{xy} = \frac{\lambda_{ex}}{2(N.A. + n_f \sin\frac{\theta}{2})} \qquad (2)$$

where N.A. is the numerical aperture of the imaging objective lens. If n_f is larger than the N.A., the resolution of the method exceeds the resolution of conventional SIM, which would have 4N.A. in the denominator (Supplementary note 2, Supplementary figure 2). In cSIM, the resolution enhancement can be scaled by changing the fringe spacing of the interference pattern using different pairs of waveguides interfering at different angles (Fig. 1d, Supplementary figure 3, Supplementary note 3). A small interference angle between two waveguides produces a large fringe period and thus a low-resolution enhancement while

opposing pair of waveguides (180° between them) yields a very small fringe period and thus a high-resolution enhancement (Fig. 1d-f). Moreover, by using waveguides made of HIC material (n=1.7-2.6), the fringe spacing of the standing wave pattern can be made much smaller than what can be achieved by a high N.A. oil immersion objective lens (N.A.=1.49) in conventional SIM. For Si_3N_4 (n=2) waveguides interfering at 180°, the theoretical resolution enhancement possible for cSIM (Supplementary note 2, Supplementary figure 2) is around 2.4x above the Abbe resolution limit using an N.A. of 1.2. This can be further increased by the use of other HIC dielectric materials with an even higher refractive index such as tantalum pentoxide (n=2.1) or titanium dioxide (n=2.6) (Fig. 1g).

In conventional SIM, a high N.A./magnification objective lens is desirable for high-resolution imaging (to generate small fringe pattern); while a low magnification/N.A. objective lens is required for increasing the FOV and the throughput. Thus, the present solution limits either the resolution or the FOV. In the case of cSIM, since the contribution of n_f in Eq.2 is independent of the objective lens the method can be extrapolated to work with low N.A. and low magnification objectives (Supplementary note 3, Supplementary Figure 4). The shift in frequency space is dictated by n_f but the passband of the objective lens is still governed by N.A., thus the frequency shift would remain the same but more interference angles would be needed to fill any gaps in frequency space (Fig. 1h-j, Supplementary Figure 4). In this sense, the output of the method would show the same resolution enhancement as the high N.A. case (supported by n_f), but over a FOV supported by the lower N.A. imaging objective lens.

Results

Since the method relies on interference, the laser light must be split into two paths. This can be done either off-chip splitting the light in a 50:50 fibre split and by using two waveguides simultaneously (Fig. 2a), or on-chip using a waveguide y-branch (Fig. 2b,c). The on-chip light split is less prone to any phase vibration originating from outside the chip and thus produces less phase-noise as opposed to the off-chip fibre split (Supplementary Figure 5). A key parameter of any SIM imaging method is the accurate control of the illumination patterns phase. For cSIM, three routes are proposed. Firstly, for the design proposed in Fig. 2a, off-chip phase shifts can be achieved by local heating of one arm of the fibre split. Alternatively, by using three discrete waveguides y-branches illuminating the same imaging area, but each with a different optical path length creates a relative phase shift between the three waveguides (Fig. 2b). Finally, using on-chip thermo-optics phase shifter concept[30] (Fig. 2c, Supplementary Figure 6) a pre-determined phase shift can be imparted. This describes resolution enhancement along one axis (Fig 2 a-c), however, to achieve isotropic resolution the structures need to be copied and rotated to have three equally spaced orientations of the cSIM pattern (Fig. 2d-h).

For light splitting off-chip (Fig. 2a), the fringe spacing can be scaled by coupling into different waveguides on the same structure (Fig. 2d) to create interference at different angles. For light splitting on-chip (Fig. 2b, c) separate structures can be used for cSIM imaging to achieve interference at different angles (Fig. 2e-h). The method was experimentally verified by imaging a sample of 100 nm fluorescent beads using the cSIM structure depicted in Fig. 2d, having an interference angle of 60^o. Here, an excitation wavelength of 660 nm was used which for this structure gives a fringe spacing around 400 nm. The light was coupled on to the waveguides via a fibre array, and sequentially guided from different rotational angles towards the central area where the cSIM interference pattern is overlapping for all orientations (Fig.3a-c). The presence of the standing wave with phase shifts was directly observed using a fluorescent dye layer (Fig. 3d). The standing wave fringe spacing and orientation were further confirmed by the cross-correlation power spectrum showing a distinct peak at the standing wave frequency (Fig. 3e-g) with the orientation of the peaks in Fig. 3e-g corresponding to Fig. 3a-c, respectively. By comparing the diffraction-limited images (Fig. 3h,j) with the cSIM reconstruction (Fig. 3i,k) the resolution enhancement is clearly visible. By drawing a line-profile (Fig. 3l) across the intensity distribution marked with a green box in Fig. 3k, what appears as

a continuous distribution in the diffraction-limited image (Fig. 3j) is clearly observed as two beads in the cSIM reconstruction (Fig. 3k). The line-profile shows that the two beads are separated by 209 nm and resolved according to the Abbe criteria. Note that this is obtained using an imaging objective lens of N.A. 1.2. The resolution enhancement is further backed up by full-width at half maxima (FWHM) measurements (Supplementary Figure 7). More line profile measurements are shown in Supplementary Figure 8.

Similarly, thermo-optical phase change using the chip designs shown in Fig 2h. was demonstrated. For this design, the light was coupled on to the three waveguides from free space using an objective lens. A sputtered silver circuit acting as a resistor locally heated a polymer on top of one of the arms leading towards the interference region (Supplementary Figure 9). The resulting change in the effective refractive index yields a phase change in the standing wave interference pattern. To achieve a π-change in the phase of the interference pattern a temperature change of around 0.25 degrees is required for the chosen chip geometry. Using this method, the phase can be accurately controlled using small increments in the voltage supplied to the silver circuit (Supplementary Figure 6). The rise and fall times to achieve a π shift in the phase of the interference pattern were found to be on the 100-200 microsecond range respectively (Supplementary Figure 10), which would allow high-speed cSIM imaging. We confirmed this method by imaging a 100 nm bead sample and the results are shown in Supplementary Figure 11.

Furthermore, we demonstrate the feasibility of using the cSIM platform to image biological specimens. For biological experiments off-chip phase shifting strategy as depicted in Fig. 2d was used and by using waveguides interfering at an angle of 60°. Primary liver sinusoidal endothelial scavenger cells were deposited on the waveguide surface following established methods[26]. The cells were fluorescently labelled for the actin filaments using Atto 647N phalloidin. Figure 4(a) shows the diffraction-limited image and Fig. 4(b) the cSIM reconstruction. Observing the zoomed image in Fig. 4c,d there is a clear improvement in resolution between the diffraction-limited (Fig. 4c) and the cSIM image (Fig. 4d). Figure 4e shows a line profile across the actin filaments, where the cSIM image visualizes the separation of actin filaments not resolved in the diffraction-limited image.

Finally, we demonstrate that cSIM can surpass the resolution enhancement of conventional linear SIM, achieved using opposing pairs of waveguides (interfering at 180º). This will generate interference fringes (see Eq. 1) with fringe-spacing below the diffraction limit, which is smaller than what is presently possible using a high N.A. objective lens (e.g. 1.49 N.A.) in conventional TIRF-SIM. Such a chip design would enable a resolution enhancement beyond a factor of 2x (as in the case of linear SIM). Using the Si_3N_4 waveguide platform and an imaging objective lens of 1.2 N.A. a theoretical resolution enhancement of 2.4x (Supplementary note 1) is possible. 1D counter-propagating waveguides (Fig. 2b) were used to investigate this idea. For this experiment, the laser light was coupled to the waveguides using a fibre array. As the fringe patterns were smaller than the diffraction limit of the imaging objective lens, they could not be observed directly. To verify the presence of a sub-diffraction limited standing wave pattern, the surface of the chip was stained using a fluorescent dye and brought in to a blinking state following *d*STORM imaging protocols[31]. This allows a super-resolution image of the evanescent field intensity distribution to be formed, visualizing the standing wave (Fig. 5a-c, Supplementary Figure 12). This method both verifies that there is a standing wave present, and more importantly confirms the phase stability of the pattern. Since a *d*STORM measurement consists of thousands of frames over tens of minutes, any phase drift during the measurement would render an image of smeared out fringes. A fluorescent bead sample was imaged using both diffraction-limited (Fig. 5 d,f) and cSIM (Fig. 5e,g) showing a clear resolution enhancement along one direction. Two beads separated by 117 nm are resolved according to the Abbe criteria as shown in the line-profile (Fig. 5h), corresponding to a 2.3x resolution enhancement while using an imaging objective lens of 1.2 N.A. and 660

nm excitation light, which is further backed up with FWHM measurements (Supplementary Figure 7). More line profile measurements are shown in Supplementary Figure 13.

Discussion and Conclusion

In this work, we have demonstrated a new concept of performing SIM imaging. The main idea is to harness photonic integrated circuits (PICs) to generate the illumination patterns required in SIM instead of using the conventional approach of the objective lens. We outline several advantages of this novel proposed route. Firstly, the photonic chip enables easy generation of user-defined, highly stable on-chip illumination patterns, available up to 100-150 nm away from the chip surface (Supplementary note 5) without needing any free-space optical components. Secondly, by using a photonic-chip the effective refractive index of the guided mode dictates how tightly the light is confined inside the waveguide, and by using large interference angles (e.g. 180^{o}) the fringe spacing of cSIM will be smaller than what can be generated using the high N.A. oil-immersion objective lens commonly used. Thirdly, photonic chips made of dielectric materials can generate uniform illumination over arbitrarily large areas which are determined by the dimensions (width and length) (Supplementary Figure 15) of the waveguides[26,27]. This is in contrast to objective lens-based illumination that suffers from field-flatness and generates an illumination pattern with a Gaussian profile over a limited FOV, determined by its magnification. Finally, our proposed cSIM is inherently compact and since it is an integrated technology, replying on PICs it will open the avenues for parallelization, automation, high-throughput, and miniaturization.

In our proposed method, the entire illumination light path, i.e. light delivery, pattern generation, and beam steering are provided using a photonic chip, which can be easily retrofitted allowing any standard microscope (Supplementary Figure 14) to acquire super-resolution SIM images (Fig. 3h-k, 4, 5f-g, Supplementary Figure 11). Moreover, the proposed setup, together with on-chip light splitting and phase modulation (Supplementary Figure 5, Fig. 5a-c) is inherently very stable with minimum bulk optical elements and therefore free from miss-alignment issues. Besides, being compact, we have shown that cSIM has the potential to surpass the resolution enhancement provided by conventional objective-based SIM. cSIM imaging with up to 2.3x resolution enhancement (Fig. 1, Fig. 5, Supplementary note 1, Supplementary Fig. 12) was demonstrated.

Compared to previous approaches where the formation of illumination fringes was shown using surface plasmon interference and localized plasmons[32,33] with a metal interface, here we demonstrate the advantages of using PICs made of dielectric material. Dielectric materials can propagate and deliver light to much longer distances (centimetres, Fig. 1 c) as opposed to plasmons based on metals. The cSIM imaging area can easily be expanded using wider adiabatically tapered single-mode waveguides[34], or by harnessing divergence in slab waveguides as shown in Supplementary note 6 and Supplementary Figure 15 where interference fringes are formed over an area of 300 x 400 μm^2. Compared with on-chip label-free imaging methods[35], the proposed cSIM method inherits the advantages of fluorescence microscopy which are high-specificity, high signal to noise, and do not rely on scattering from high refractive index contrasts between the sample and the surroundings.

PICs enables easy manipulation of the light on the chip, opening avenues for user-defined illumination pattern and phase stepping strategies as outlined in Fig. 1c-d and Fig. 2. Using PICs we proposed different waveguide designs where light splitting and phase stepping were implemented both off-chip (Fig. 2a, d) and on-chip (Fig 2. b,c & e-h). To maintain stable and repeatable phase steps, the on-chip methods are preferred due to the inherently low phase noise achieved when splitting the light on-chip with waveguides covered by a protective silica top-cladding (Supplementary figure 5). While all the methods for phase-stepping finally worked (Fig. 3, Fig. 4, Fig. 5, Supplementary figure 11), the methods holding the potential for high-speed imaging are using thermo-optics (Fig. 2c,f,h), or on-chip path-length stepping (Fig. 2b,e,g). Both of these methods can be made to switch phase very fast, using a voltage supply

or fibre-optical switch, respectively. The thermo-optical phase modulation can switch phases on the order of 100-200 μs (Supplementary note 4), while commercially available fibre optical switches can switch the light between waveguides, and thus change the pattern rotation, at MHz speeds. Thus, the final temporal resolution of the method would then be dominated by the camera exposure time, camera imaging speed, and the brightness of the fluorophores used which determines the sufficient signal to noise ratio necessary for a successful SIM reconstruction.

The relatively new field of on-chip nanoscopy would benefit from future improvements. The present implementation of cSIM is done using opaque silicon substrates and with an upright microscope equipped with a water immersion objective lens (Olympus UPLSAPO60XW). Future, development of cSIM will be done using thin transparent substrates. This will enable using an inverted optical microscope configuration equipped with a high-N.A. oil immersion objective lens (e.g. 1.4-1.7[19] instead of 1.2 used in the present study). This will further extend the resolution of cSIM, while at the same time keeping the scalability of the method without affecting the benefits of the inverted microscope configuration.

cSIM decouples the illumination and the collection light paths which although is beneficial for higher resolution opens new imaging challenges. The imaging area where the fringe illumination is formed is pre-determined by the waveguide design. Moreover, in cSIM the entire waveguide regions are illuminated, thus area outside the FOV of the objective lens (i.e. areas not being imaged) is also exposed to the photo-bleaching. In cSIM, it is therefore desirable to use a low N.A./ magnification objective lens which provides a larger FOV while still maintaining the resolution improvement provided by the waveguide generated illumination pattern (Supplementary Figure 4). This represents an improvement over conventional SIM, where the use of a low. N.A. objective lens to image large FOV will significantly reduce the supported optical resolution (Supplementary note 2, 3 and, Supplementary Figure 4.

The chip-based illumination strategy is a lucrative route, although the focus of this work is to report chip-based SIM, it can be foreseen that PICs provide new research directions of performing complex light beam shaping for other nanoscopy methodologies such as stimulated emission depletion microscopy[5,36] by replacing donut beam with interference fringes formed either by two or more waveguides; light-sheet microscopy[37] by designing on-chip axicon; and the idea can also be extended towards SIM-FLUX[38]. Intricate waveguide geometries are capable of generating a range of exotic intensity distributions by harnessing multimode light guiding and interference for altogether new illumination strategies within optical microscopy and nanoscopy. Due to the widespread use of microfabrication techniques within mainstream CMOS technology, the fabrication facilities for PICs are already existing, making mass production of photonic chips feasible, with the potential cost per chip could be reduced to just a few Euros.

Furthermore, on-chip optical nanoscopy could also benefit from the development of on-chip integration of light sources[39] and frequency combs[40], further miniaturizing the optical set-up. Although the on-chip illumination strategy enables excitation over large areas, the light collection is still presently limited by the objective lens. In the future, the marriage of on-chip illumination with micro-lens arrays[41] for light collection could potentially open the avenues for extra-ordinary high-throughput, where illumination and collection both can be done parallel over large areas, completely removing the dependency on a bulky objective lens. On-chip optical nanoscopy makes integration with other on-chip optical functions straightforward, such as on-chip Raman spectroscopy[42], waveguide trapping[43,44], micro-fluidics[45] , optical phase tomography[46] and others.

Figure 1 – The concept. (a) Conventional SIM relies on a high N.A. objective lens for both excitation and collection. (b) cSIM harnesses interference in a waveguide to excite the specimen via evanescent fields, decoupling excitation and collection light paths. (c) Three pairs of waveguide chip interferer at different rotational angles (pseudo-color). (d) A low interference angle creates an interference pattern with a high fringe spacing, while counter-propagating light yields the smallest fringe spacing. (e) The microscope resolution is represented by the optical transfer function (OTF) (solid circle). The maximum OTF shift in conventional SIM yields a 2x resolution enhancement (dotted circle). (f) The OTF shifts with the fringe spacing, where cSIM surpass 2x resolution for large interference angles. g) Using higher refractive index materials scale the resolution more, e.g. Si_3N_4 ($n \approx 2.0$), Ta_2O_5 ($n \approx 2.05$) and TiO_2 ($n \approx 2.6$). (h) Using a low N.A. objective lens in conventional SIM is not viable due to the compromised fringe spacing. (i) In cSIM, the shift of the OTF is unaffected by the low N.A., however, more interference angles are needed to fill the gaps.

Figure 2 – Waveguide designs for cSIM. (a) Light is split in fibres on the bench supplying two input waveguides on the chip that interfere at the image region (marked with a circle). This structure will create a 1D cSIM image. For the 2D case, three rotational angles of the cSIM pattern is needed as shown in (d). The structure in (d) can be used to scale the cSIM resolution, depending on which waveguides are active, i.e. interfering at 60° or 180°. b) Light splitting on-chip yields a more phase-stable interference pattern. Three different waveguides merging at the image area yields phase stepping by the difference in optical path length. To get isotropic resolution the structure must be rotated as shown in (e) for 180°, or (g) 60° interference. c) With thermo-optics, the phase can be controlled using a resistive circuit. A polymer in contact with the waveguide thermally expands changing the refractive index which causes the phase change. (f, h) The structures are rotated in three angles to yield an isotropic resolution.

Figure 3 – cSIM imaging. (a) The structure depicted in Fig. 2d is used with 60° interference angle. (b, c) Rotation of the cSIM pattern by using different waveguides is necessary for isotropic resolution enhancement. d) The cSIM pattern, at three phase steps, is imaged via a fluorescent dye (20° interference is used for increased contrast). (e-g) The power spectrum of the overlapping bands used for the reconstruction show peaks corresponding to the modulation frequency and orientation in (a-c). h) A 100 nm fluorescent bead sample is imaged with diffraction limited resolution, while (i) shown the cSIM reconstruction of the same region. (j-k) Show a zoom indicated with a white box in (i) with the green box indicating the position of the line-profile shown in (l). Two beads located 209 nm apart are resolved using cSIM, but not in the diffraction-limited image. The excitation/emission wavelengths are 660nm/690nm. A cluster of beads saturates the camera in the central part the image, observed in both the diffraction limited (h) and the cSIM reconstruction (i) as an artifact.

Figure 4 – cSIM imaging of biological specimens. Liver sinusoidal scavenger endothelial cells are imaged with cSIM. The cells are stained for the actin filaments using Atto647N phalloidin and imaged in an oxygen-depleted buffer to reduce photobleaching. (a) Shows the filtered diffraction-limited image of the actin filaments. (b) The cSIM image shows a clear resolution

enhancement compared to (a). The cSIM waveguide depicted in Fig. 4(d) was used with a 60° interference angle. (c, d) Show the zoomed image marked with a white box in (a) for the diffraction-limited and cSIM image, respectively. (e) A line profile, indicated with a green line in (c), shows filaments that are not resolved in the diffraction-limited image (dotted green line) is clearly resolved in the cSIM image (black dots show the experimental measurements, and the magenta line is a fitted curve).

Figure 5 – cSIM with increased resolution. (a) Counter-propagating light sets up a sub-diffraction limit interference pattern not visible using a fluorescence dye layer. (b) The presence of the standing wave is confirmed using *d*STORM imaging of the fluorescent dye layer, visualizing the standing wave having a fringe spacing of 195 nm (c). (d, e) The same 1D structure was used for cSIM, with diffraction-limited and 1D cSIM images shown respectively. (f, g) Show the zoom indicated with a white box in (d), with a line profile (h) demonstrating two beads separated by 117nm which corresponds to 2.3x resolution enhancement using cSIM. The green dotted line is the diffraction-limited result while the magenta solid line is the Gaussian fit of the cSIM result, with the measurement values displayed with black dots. Excitation and emission wavelengths are 660 nm/690 nm.

REFERENCES

1 Goodman, J. W. *Introduction to Fourier Optics*. (W. H. Freeman, 2005).

2 Pawley, J. *Handbook of Biological Confocal Microscopy*. (Springer US, 2010).

3 Eggeling, C., Willig, K. I., Sahl, S. J. & Hell, S. W. Lens-based fluorescence nanoscopy. *Quarterly Reviews of Biophysics* **48**, 178-243, doi:10.1017/S0033583514000146 (2015).

4 Hell, S. W. & Wichmann, J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Opt. Lett.* **19**, 780-782, doi:10.1364/OL.19.000780 (1994).

5 Willig, K. I., Rizzoli, S. O., Westphal, V., Jahn, R. & Hell, S. W. STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis. *Nature* **440**, 935-939, doi:10.1038/nature04592 (2006).

6 Rust, M. J., Bates, M. & Zhuang, X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nature Methods* **3**, 793, doi:10.1038/nmeth929 (2006).

7 Heilemann, M. *et al.* Subdiffraction-resolution fluorescence imaging with conventional fluorescent probes. *Angewandte Chemie (International ed. in English)* **47**, 6172-6176, doi:10.1002/anie.200802376 (2008).

8 Betzig, E. *et al.* Imaging intracellular fluorescent proteins at nanometer resolution. *Science (New York, N.Y.)* **313**, 1642-1645, doi:10.1126/science.1127344 (2006).

9 Gustafsson, M. G. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *Journal of microscopy* **198**, 82-87 (2000).

10 Heintzmann, R. & Cremer, C. G. *Laterally modulated excitation microscopy: improvement of resolution by using a diffraction grating*. Vol. 3568 EBO (SPIE, 1999).
11 Sahl, S. J., Hell, S. W. & Jakobs, S. Fluorescence nanoscopy in cell biology. *Nature Reviews Molecular Cell Biology* **18**, 685, doi:10.1038/nrm.2017.71 (2017).
12 Yeh, L.-H., Chowdhury, S., Repina, N. A. & Waller, L. Speckle-structured illumination for 3D phase and fluorescence computational microscopy. *Biomed. Opt. Express* **10**, 3635-3653, doi:10.1364/BOE.10.003635 (2019).
13 Mudry, E. *et al.* Structured illumination microscopy using unknown speckle patterns. *Nat Photonics* **6**, 312, doi:10.1038/nphoton.2012.83 (2012).
14 Ayuk, R. *et al.* Structured illumination fluorescence microscopy with distorted excitations using a filtered blind-SIMalgorithm. *Opt. Lett.* **38**, 4723-4726, doi:10.1364/OL.38.004723 (2013).
15 Jost, A. *et al.* Optical Sectioning and High Resolution in Single-Slice Structured Illumination Microscopy by Thick Slice Blind-SIM Reconstruction. *Plos One* **10**, e0132174, doi:10.1371/journal.pone.0132174 (2015).
16 Heintzmann, R., Jovin, T. M. & Cremer, C. Saturated patterned excitation microscopy—a concept for optical resolution improvement. *J. Opt. Soc. Am. A* **19**, 1599-1609, doi:10.1364/JOSAA.19.001599 (2002).
17 Gustafsson, M. G. L. 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* **102**, 13081-13086, doi:10.1073/pnas.0406877102 (2005).
18 Rego, E. H. *et al.* Nonlinear structured-illumination microscopy with a photoswitchable protein reveals cellular structures at 50-nm resolution. *Proc Natl Acad Sci U S A* **109**, E135-143, doi:10.1073/pnas.1107547108 (2012).
19 Li, D. *et al.* Extended-resolution structured illumination imaging of endocytic and cytoskeletal dynamics. *Science (New York, N.Y.)* **349**, aab3500, doi:10.1126/science.aab3500 (2015).
20 Cragg, G. E. & So, P. T. C. Lateral resolution enhancement with standing evanescent waves. *Opt. Lett.* **25**, 46-48, doi:10.1364/OL.25.000046 (2000).
21 Chung, E., Kim, D. & So, P. T. Extended resolution wide-field optical imaging: objective-launched standing-wave total internal reflection fluorescence microscopy. *Opt. Lett.* **31**, 945-947, doi:10.1364/OL.31.000945 (2006).
22 Kner, P., Chhun, B. B., Griffis, E. R., Winoto, L. & Gustafsson, M. G. L. Super-resolution video microscopy of live cells by structured illumination. *Nature Methods* **6**, 339, doi:10.1038/nmeth.1324 (2009).
23 Grandin, H. M., Stadler, B., Textor, M. & Voros, J. Waveguide excitation fluorescence microscopy: A new tool for sensing and imaging the biointerface. *Biosensors & Bioelectronics* **21**, 1476-1482, doi:10.1016/j.bios.2005.06.011 (2006).
24 Agnarsson, B., Ingthorsson, S., Gudjonsson, T. & Leosson, K. Evanescent-wave fluorescence microscopy using symmetric planar waveguides. *Opt Express* **17**, 5075-5082, doi:10.1364/Oe.17.005075 (2009).
25 Agnarsson, B., Jonsdottir, A. B., Arnfinnsdottir, N. B. & Leosson, K. On-chip modulation of evanescent illumination and live-cell imaging with polymer waveguides. *Opt Express* **19**, 22929-22935, doi:10.1364/Oe.19.022929 (2011).
26 Diekmann, R. *et al.* Chip-based wide field-of-view nanoscopy. *Nat Photonics* **11**, 322-+, doi:10.1038/Nphoton.2017.55 (2017).

27 Helle, Ø. I., Coucheron, D. A., Tinguely, J.-C., Øie, C. I. & Ahluwalia, B. S. Nanoscopy on-a-chip: super-resolution imaging on the millimeter scale. *Opt Express* **27**, 6700-6710, doi:10.1364/OE.27.006700 (2019).
28 Tinguely, J.-C., Helle, Ø. I. & Ahluwalia, B. S. Silicon nitride waveguide platform for fluorescence microscopy of living cells. *Opt Express* **25**, 27678-27690, doi:10.1364/OE.25.027678 (2017).
29 Fu, Y., Ye, T., Tang, W. & Chu, T. Efficient adiabatic silicon-on-insulator waveguide taper. *Photon. Res.* **2**, A41-A44, doi:10.1364/PRJ.2.000A41 (2014).
30 Harris, N. C. *et al.* Efficient, compact and low loss thermo-optic phase shifter in silicon. *Opt Express* **22**, 10487-10493, doi:10.1364/OE.22.010487 (2014).
31 van de Linde, S. *et al.* Direct stochastic optical reconstruction microscopy with standard fluorescent probes. *Nature protocols* **6**, 991, doi:10.1038/nprot.2011.336 (2011).
32 Wei, F. & Liu, Z. Plasmonic Structured Illumination Microscopy. *Nano Letters* **10**, 2531-2536, doi:10.1021/nl1011068 (2010).
33 Wei, F. *et al.* Wide Field Super-Resolution Surface Imaging through Plasmonic Structured Illumination Microscopy. *Nano Letters* **14**, 4634-4639, doi:10.1021/nl501695c (2014).
34 Archetti, A. *et al.* Waveguide-PAINT offers an open platform for large field-of-view super-resolution imaging. *Nature Communications* **10**, 1267, doi:10.1038/s41467-019-09247-1 (2019).
35 Liu, X. *et al.* Fluorescent Nanowire Ring Illumination for Wide-Field Far-Field Subdiffraction Imaging. *Physical Review Letters* **118**, 076101, doi:10.1103/PhysRevLett.118.076101 (2017).
36 Schneider, J. *et al.* Ultrafast, temporally stochastic STED nanoscopy of millisecond dynamics. *Nature Methods* **12**, 827, doi:10.1038/nmeth.3481 (2015).
37 Santi, P. A. Light Sheet Fluorescence Microscopy: A Review. *Journal of Histochemistry & Cytochemistry* **59**, 129-138, doi:10.1369/0022155410394857 (2011).
38 Cnossen, J. *et al.* Localization microscopy at doubled precision with patterned illumination. *bioRxiv*, 554337, doi:10.1101/554337 (2019).
39 Otterstrom, N. T., Behunin, R. O., Kittlaus, E. A., Wang, Z. & Rakich, P. T. A silicon Brillouin laser. *Science (New York, N.Y.)* **360**, 1113-1116, doi:10.1126/science.aar6113 (2018).
40 Gaeta, A. L., Lipson, M. & Kippenberg, T. J. Photonic-chip-based frequency combs. *Nat Photonics* **13**, 158-169, doi:10.1038/s41566-019-0358-x (2019).
41 Orth, A. & Crozier, K. Gigapixel fluorescence microscopy with a water immersion microlens array. *Opt Express* **21**, 2361-2368, doi:10.1364/OE.21.002361 (2013).
42 Løvhaugen, P., Ahluwalia, B. S., Huser, T. R. & Hellesø, O. G. Serial Raman spectroscopy of particles trapped on a waveguide. *Opt Express* **21**, 2964-2970, doi:10.1364/OE.21.002964 (2013).
43 Helle, Ø. I., Ahluwalia, B. S. & Hellesø, O. G. Optical transport, lifting and trapping of micro-particles by planar waveguides. *Opt Express* **23**, 6601-6612, doi:10.1364/OE.23.006601 (2015).
44 Ahluwalia, B. S., Løvhaugen, P. & Hellesø, O. G. Waveguide trapping of hollow glass spheres. *Opt. Lett.* **36**, 3347-3349, doi:10.1364/OL.36.003347 (2011).

45 Wang, X. *et al.* Enhanced cell sorting and manipulation with combined optical tweezer and microfluidic chip technologies. *Lab Chip* **11**, 3656-3662, doi:10.1039/c1lc20653b (2011).
46 Yurtsever, G., Weiss, N., Kalkman, J., van Leeuwen, T. G. & Baets, R. Ultra-compact silicon photonic integrated interferometer for swept-source optical coherence tomography. *Opt. Lett.* **39**, 5228-5231, doi:10.1364/OL.39.005228 (2014).

Data availability
The data that support the plots within this paper and other finding of this study are available from the corresponding author upon reasonable request.

Acknowledgments
The authors thank Rainer Heintzmann and Sourov Das, for help with the SIM reconstruction algorithms and design of fibre-optical phase modulator, respectively. We further acknowledge Cristina I. Øie and Deanna Wolfson for help with biological specimens, and Thomas Huser (T.H.) for fruitful conceptual discussions. This work was supported by the European Research Council (grant no. 336716 to B.S.A.).

Competing financial interests
B.S.A., O.G.H and T.H. have applied for patent GB1705660.7 on SIM-on-chip. The other authors declare no competing financial interest.

Author contributions
B.S.A. supervised and conceived this project and provided funding for the project. Ø.I.H. built the setup, performed the experiments and analysed the data. J.C.T, F.T.D and O.G.H. designed the waveguide chip and the mask for the fabrication. All authors contributed towards chip designs. Ø.I.H., F.T.D. and J.C.T characterized the waveguides. F.T.D. and Ø.I.H. fabricated the on-chip thermo-optics for phase modulation. M.L. performed all the simulation of cSIM. Ø.I.H. and B.S.A. mainly wrote the manuscript and all authors commented to the manuscript.

Additional information
Correspondence and request for materials should be addressed to B.S.A.

METHODS
Chip Design.
The key parameters in SIM are interference fringe spacing, phase modulation, and fringe contrast. For chip-based SIM the dimensions of the active region are also crucial, to ensure that biological specimens are evenly illuminated by the structured illumination. The lateral size of cells is often from 10 μm and upwards.

The waveguide platform has been developed using the high-refractive-index material Si_3N_4. High-index contrast materials enable tight confinement of the light inside the waveguide which entails a compact bend radius. This allows for ultra-compact and dense waveguide structures with a small footprint benefiting from high intensity in the evanescent field. In the visible range, strong evanescent fields can be achieved by designing waveguides with core thickness between 100-200 nm. For a 150 nm thick core material; single-mode condition, adiabatic taper condition, and bending losses were previously investigated[28]. It was found that 150 nm thick waveguide core gives a strong evanescent field intensity without sacrificing much on the coupling efficiency. For this work single-mode condition (Supplementary figure 16(a)) is necessary to generate a uniform fringe pattern, which can be formed by interfering fundamental mode light guided inside the waveguide. The presence of any residual higher-order modes will generate a mode-beat pattern, which is undesirable. Thus, shallow rib

waveguides were preferred over strip waveguides. For a shallow rib, fundamental mode guiding can be achieved using waveguide widths (1-1.5 μm wide) (Supplementary figure 16(b)) within the reach of fabrication using standard photolithography techniques. Waveguides with 4 nm rib height (total height of 150 nm) and around 1-1.5 μm width were adiabatically tapered out to 25 μm or 50 μm with a tapering length of 2 mm or 4 mm, respectively. A significantly low bending loss for a shallow rib waveguides was achieved for a bend radius of 2 mm and more. The optimization of the designed parameters can be found in the previous literature[28].

Chip fabrication.
The production of waveguide chips was performed at the Institute of Microelectronics Barcelona (IMB-CNM, Spain). First, an oxide layer of ca. 2 μm was thermally grown on a silicon slab, followed by low-pressure chemical vapor deposition (LPCVD) of 150 nm Si_3N_4 at 800°. Conventional photo-lithography imprinted the waveguide geometries on a layer of photo-resist. 4 nm rib waveguides were realized using reactive-ion-etching (RIE). In order to prevent cross-talk of light into adjacent waveguide structures, an absorption layer consisting of 200 nm SiO_2 (deposited by plasma-enhanced chemical vapor deposition (PECVD)) followed by 100 nm poly-crystalline silicon (deposited by PECVD at 300°) was designed as a negative image of the first mask with 10 μm added to the waveguide width. RIE was used to etch until 100 nm of SiO_2 remained, with wet etching (using hydrofluoric acid) removing the remaining material from the waveguide surface. Finally, a 1.5 μm SiO_2 layer (deposited by PECVD) built the cladding layer. The chip fabrication workflow is shown in supplementary figure 17. Imaging areas were patterned by a 3rd photo-lithography step. The window openings were realized using RIE until 100 nm SiO_2 was remaining, followed by wet etching to completely remove the oxide layer while preserving the Si_3N_4. Further details of the fabrication optimization and process can be found elsewhere[47].

Experimental setup.
A microscope modified from an Olympus modular microscope (BXFM) was mounted on an XY motorized translation stage. The microscope was fitted with a Hamamatsu Orca flash sCMOS camera. cSIM images were acquired using either Olympus x60/1.3SiO or Olympus x60/1.2W objective lenses. The waveguide chip was mounted on a micrometre XYZ-stage using a vacuum chuck to hold the chip. The light was coupled on to the chip using a nine-fiber array adapter. The fibre array adapters are commercially available with fixed spacing (e.g. 127 μm). The waveguides were separated by the same space (127 μm) as the fibre array, allowing for multiple waveguides to have light coupled at the same time (supplementary figure 7b). For off-chip cSIM (Fig. 2a,d), an additional 50:50 fused fibre-split (OZ optics) was used to split the light. Thermo-optical phase stepping was achieved using a 6V voltage supply coupled to the resistive on-chip electro-optical heating elements. A schematic of the setup is shown in supplementary figure 14. The image data were analyzed using Fiji open-source image processing software and FairSIM[48], an open-source SIM reconstruction plugin. cSIM data from the 180° interference waveguides were analyzed with custom software.

Near-field mapping using dSTORM.
dSTORM near field mapping was performed by staining the waveguide surface with a layer of fluorescent molecules. A 1/1000 solution of CellMask deep red (Thermofisher, C10046) in phosphate buffered saline (PBS) was incubated for 20 minutes on the waveguide surface at room temperature. The solution was aspirated and a PDMS micro-chamber positioned on top of the chip was filled with dSTORM switching buffer consisting of an enzymatic oxygen scavenging system and 100 mM β-Mercaptoethylamine (MEA). The surface was excited with the 660 nm laser with sufficiently high power to achieve photo-switching. A stack of 100000 images was acquired at 30 ms exposure time and reconstructed using the Fiji plugin ThunderStorm[49].

Sample preparation.

The chip surface was coated with a 0.5% Poly-l-lysine (PLL) solution, aspirated and dried with compressed Nitrogen. Depending on the desired distribution of fluorescent beads the chip would be plasma treated at a high setting for 30s, ensuring hydrophilic surface properties. A 0.5 µl drop of 100 nm diameter fluorescent bead stock (Tetraspeck, Thermofisher T7279) was put at the cSIM image region and allowed to dry completely. The chamber was filled with dH2O and the chip brought to the microscope for imaging.

Frozen liver sinusoidal scavenger cells[50] were attached to the waveguide surface. The cells were permeabilized using 0.1% triton x-100 for 4 minutes and stained using 3/100 Atto647N phalloidin (Sigma-Aldrich #65906) in 1% bovine serum albumin (BSA) for 1H at room temperature. The cell layer was carefully washed with phosphate-buffered saline (PBS) several times and left submerged in PBS in steps of 5 min, 20 min, and 30 min to let unbound dye seep out of the cells.

References for methods

47 Prieto, F. *et al.* An integrated optical interferometric nanodevice based on silicon technology for biosensor applications. *Nanotechnology* **14**, 907 (2003).

48 Müller, M., Mönkemöller, V., Hennig, S., Hübner, W. & Huser, T. Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ. *Nature Communications* **7**, 10980, doi:10.1038/ncomms10980 (2016).

49 Ovesný, M., Křížek, P., Borkovec, J., Švindrych, Z. & Hagen, G. M. ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging. *Bioinformatics* **30**, 2389-2390, doi:10.1093/bioinformatics/btu202 (2014).

50 Mönkemöller, V. *et al.* Primary rat LSECs preserve their characteristic phenotype after cryopreservation. *Sci Rep-Uk* **8**, 14657-14657, doi:10.1038/s41598-018-32103-z (2018).