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Author(s)	Alexandrov, Sergey; McGrath, James; Hrebesh, Subhash; Leahy, Martin
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Novel approach for label free super-resolution imaging in far field

Sergey A. Alexandrov[1]*, James McGrath[1], Francesca Boccafoschi,[2] Cinzia Giannini[3] and Martin Leahy[1]

[1]Tissue Optics & Microcirculation Imaging Group, School of Physics, National University of Ireland, Galway, Ireland

[2]Department of Health Sciences, University of Piemonte Orientale "A. Avogadro", 28100 Novara, Italy

[3]Institute of Crystallography, National Research Council, via Amendola 122/O, Bari 70126 Italy

* Contact author: Sergey Alexandrov, email: sergey.alexandrov@nuigalway.ie

Phone 353 91 495350. Fax: 353 91 495529

James McGrath email: j.mcgrath3@nuigalway.ie

Cinzia Giannini email: cinzia.giannini@ic.cnr.it

Francesca Boccafoschi email: francesca.boccafoschi@med.unipmn.it

Martin Leahy email: martin.leahy@nuigalway.ie

ABSTRACT

Progress in the emerging areas of science and technology, such as bio- and nano-technologies, depends on development of corresponding techniques for imaging and probing the structures with high resolution. Recently the far field diffraction resolution limit in the optical range has been circumvented and different methods of super-resolution optical microscopy have been developed. The importance of this breakthrough achievement has been recognized by Nobel Prize for Chemistry in 2014. However, the fluorescence based super-resolution techniques only function with

fluorescent molecules (which are often toxic and can destroy or lead to artificial results in living biological objects) and suffer from photobleaching. Here we show a new way to break the diffraction resolution limit, which is based on nano-sensitivity to internal structure. Instead of conventional image formation as 2D intensity distribution, in our approach images are formed as a result of comparison of the axial spatial frequency profiles, reconstructed for each image point. The proposed approach dramatically increases the lateral resolution even in presence of noise and allows objects to be imaged in their natural state, without any labels.

INTRODUCTION

Most of the fundamental pathological processes in living tissues, such as cancer, exhibit changes at the nanolevel. Existing high resolution microscopy techniques, including near field imaging (Near Field Scanning Optical Microscopy (NSOM or SNOM)) which breaks the resolution limit by exploiting the properties of evanescent waves,[1-3] electron and atomic force microscopy,[4,5] impose strong limitations on the imaged sample and are unsuitable for the study of live biomedical objects. The best modality for realization of the super-resolution imaging in optical range in far zone is fluorescence microscopy, where the sample acts as a light source itself, providing a very high signal-to-noise (SNR) ratio. Different super-resolution microscopy techniques using fluorescent molecules have been proposed,[4,6-14] but all of them are based on intrinsic marker properties and require labeling which limits their ability for imaging of living objects *in vivo*. [15,16]

Different techniques for label free super-resolution imaging have been also proposed, including synthetic aperture microscopy,[17-19] optical nanoscopy using optically transparent microspheres as far-field superlenses (FSL),[20,21] methods based on the use of a special optical mask to remove the need for evanescent fields,[22] coherent total internal reflection dark-field microscopy,[23] far-field vibrational infrared (IR) absorption microscopy,[24] image scanning microscopy which uses point scanning in combination with wide field detection.[25] Most of the published label free super-resolution methods permit extended resolution, but resolution is still limited to a finite value and theoretically unlimited resolution cannot be achieved.[4] Existing techniques are complicated, expensive, and can hardly be used for in vivo imaging of live objects. In spite of numerous efforts and great achievements in super-resolution microscopy, the challenge now is to make high resolution imaging more accessible and more usable in vitro and in vivo. There remains a great need for further development and creation of new alternative approaches for label-free super-resolution imaging for investigation of biological objects in their natural environment.

A new approach to probe three-dimensional (3D) structure in far field at nanoscale, based on spectral encoding of spatial frequency (SESF), has been developed recently[10,26-28]. Transmittance of instantaneous lateral spatial frequency bandwidth wider than the optical system's diffraction limit through a low numerical aperture (NA) optical system,[26] and high resolution imaging[27], based on spectral encoding of the lateral spatial frequency, have been demonstrated. The ability to reconstruct the axial (along depth) spatial frequency profiles for each point of the image with nanoscale sensitivity to structural changes has been shown[10] and adaptation of the SESF approach for depth resolving imaging has been published.[28,29] Here we report a novel approach, based on spectral encoding of the axial spatial frequency, to break the diffraction limit in far zone and dramatically improve resolution in the lateral direction.

SUPER-RESOLUTION SESF APPROACH

All biomedical objects are three dimensional, including cell cultures, single cells and cell constituents, collagen, etc. Indeed, samples which are used for light microscopy imaging have a thickness at least a few microns (the tissue is cut in the microtome at thicknesses varying from 2 to 50 µm). Even the smallest thickness corresponds to a few wavelengths in visible range. Reflected or transmitted light is a result of interaction of illuminating light with the internal structure. In conventional microscopy each point of image is formed as a superposition of all light waves after interaction with the internal structure at corresponding object's point. Conventional images are two-dimensional (2D) intensity distributions in the image plane where each image point corresponds to one intensity value. The resolution can be defined as the shortest distance between two image points that results in sufficient contrast to allow them to be distinguished. Two features within the object, which are too close and cannot be resolved using conventional microscopy, and area between them, usually have different internal structures in the depth direction. The idea of the srSESF approach, presented here, is to use this additional information about internal structure to resolve features in the lateral direction. We show that it is possible to detect the difference between fine features within the object, separated in lateral direction, via determination of a difference in the axial (in depth direction) spatial frequency (or periods) profiles at points we want to resolve and points between them.

If the lateral structure sizes are too small to be resolved, then light, diffracted on this lateral structure, cannot pass through the optical imaging system. However, in the srSESF approach information about this fine lateral structure is encoded into the axial structure. Indeed, if there are no differences in structure, then the sample is uniform and there are no features to resolve. The lateral spatial separation between features we want to resolve is the separation between corresponding axial spatial frequency profiles. In turn, information about axial spatial frequency profiles is spectrally encoded and can be passed through the optical system as different wavelengths.[10,28] So, the high spatial frequency information of lateral structure will be passed through the optical system as a difference in axial structure at different lateral locations and as a result, the fine lateral structure, unresolved by conventional microscopy, can be resolved.

Super-resolution images are formed as differences between corresponding axial spatial frequency profiles. Different methods can be used for comparison of the axial spatial frequency profiles. For example, the srSESF image can be formed as a map of correlation coefficients between axial spatial frequency profile at a given image point, or profile of the numerically synthesized structure, and profiles at all the other points, etc.

In information theory the fundamental resolution limit is set by the information capacity of the detected signal.[30] The srSESF approach dramatically increases the information capacity. Indeed, for each image point, instead of just one intensity value as in conventional microscopy, we will have axial spatial frequency profiles with hundreds or even thousands points.

The srSESF approach is realized in reflection configuration which facilitates *in vivo* tissue imaging. It is known that, in reflection configuration, backscattered light provides information about high axial spatial frequency content of the object.[28,29,31] The corresponding dominant axial spatial periods of the structure which scatters light are about half the wavelength. It means that, whenever the srSESF approach is applied, even „thin“ specimens with thickness of about a few wavelengths will produce axial spatial frequency profiles encoding nano-sensitivity to structural changes.

RESULTS

Numerical simulation

To validate the novel super-resolution SESF (srSESF) approach we performed numerical simulation of the imaging process (see METHODS for details). The sample of about 1.2 micron thickness is displayed in Fig. 1a. It consists of groups of lines, separated by distance *d3*. Each group is split into two elements of size *d1* separated by distance *d2* (Fig. 1a). The sample was illuminated by a broadband source, with a wavelength range 450 nm-750 nm. An objective lens with NA = 0.9 was used to form images. The initial intensity profile immediately after reflection from the sample is presented in Fig. 1b. Reconstruction of the initial intensity profile on the sample via conventional microscopy is presented in Fig. 1c-f. These results demonstrate that the groups of two areas separated by distance *d3* = 250 nm can be resolved using conventional microscopy, but areas within each group separated by distance *d1* = 50 nm remain unresolvable even without noise addition (Fig. 1c,d).

The srSESF images are presented in Fig. 1g-j as a map of correlation coefficients between the axial spatial period profile at a given pixel and profiles at all other pixels versus lateral coordinate. In contrast to conventional microscopy, using the same objective lens with the resolution limit 400 nm, the fine lateral sample structure within groups, areas of 50 nm size separated just by 50 nm, are resolved using the srSESF approach (Fig. 1g,h). Utilizing information about internal structure of the sample, axial spatial period profiles for each image point, instead of just one intensity value, also permits us to dramatically suppress the noise. Even after noise addition, at signal-to-noise ratio (SNR) 45 dB, the areas within groups can be clearly distinguished (Fig. 1i, j).

If the sizes of the lateral structures are increased (Fig. 2a), then it becomes possible not just to resolve two small features, but to accurately reconstruct the fine profile of the initial intensity on the sample using srSESF approach. We can see that conventional microscopy resolves the fine structure in the absence of noise (Fig. 2b), but this structure cannot be resolved after noise addition (Fig. 2c). The srSESF microscopy clearly resolves the fine structure of the sample (Fig. 2d), providing much better image contrast, and accurately reconstructs the profile of the initial intensity on the object (before convolution). Even in presence of noise, the srSESF approach accurately reconstructs the fine structure of the sample (Fig. 2e). If the sizes of the lateral structure are increased further (Fig. 2f), srSESF microscopy reconstructs the profile of the initial intensity on the object more accurately, as can be seen from comparison of the reconstructed using srSESF microscopy intensity profiles before noise addition Fig. 2i and with noise Fig. 2j, and initial intensity profile Fig. 2f.

Experiments

The custom built scanning microscope was used for experiments (see METHODS for details). Conventional scanning microscopy and srSESF images of the sample, made of 400 nm diameter polymer spheres , are presented in Fig. 3a and Fig. 3b correspondingly. Both conventional scanning microscopy and srSESF images were formed simultaneously for the same area of the sample using the same objective lens with NA = 0.5. These images look different because they were formed using different contrast mechanisms (conventional intensity based image in Fig. 3a and correlation coefficients map in Fig. 3b). Improvement in resolution in image Fig. 3b in comparison with image Fig. 3a can be clearly seen. In magnified portions Fig. 3e,f of the image in Fig. 3b two spheres can be seen separately, but in the corresponding portion Fig. 3d of the conventional image Figure 3a these spheres are totally indistinguishable. The contrast of the srSESF image can be changed by selection of different ranges of the correlation coefficients to better visualize the fine local structure, as demonstrated on the right magnified portion - Fig. 3f - of the image in Fig. 3b. For reference, a conventional high resolution bright field image of the sample, formed in reflection configuration in the visible wavelength range at the same magnification, is also shown in Fig. 3c. The experimental results show that the srSESF approach provides significant improvement in resolution. The resolution obtained is $\lambda/3.3$ which is about 3 times better than diffraction limit of the imaging system used and even 1.3 times better than ultimate diffraction resolution limit for central wavelength 1300 nm.

As an example of the application of the srSESF approach to biomedical objects the images of two different collagen tissues (Fig. 4a-c and Fig. 4d-g) are presented, where the two investigated tissues are obtained with (Fig. 4a-c) or without (Fig. 4d-g) dynamic stimulation, resulting in a different degree of fiber orientation (see details in METHODS for the tissue preparation). Fig. 4a and Fig. 4d are scanning microscopy images, formed using objective lens with NA = 0.5, which are dominated by interference noise and do not clearly image the fiber with the exact orientation. The srSESF image Fig. 4b was formed for the same area as the conventional scanning microscopy image Fig. 4a and the srSESF images Fig. 4e,f were formed for the same area as conventional scanning microscopy image Fig. 4d. The srSESF images in Fig. 4b,e were formed for the same correlation range. The srSESF image in Fig. 4f was formed with reduced correlation range. Fig. 4c and Fig. 4g are conventional high resolution bright field images in reflection configuration formed using visible light.

The improvement in resolution is obvious if we compare conventional and srSESF images. Even from comparison between srSESF images Fig. 4b,e and the high resolution conventional bright field images (Fig. 4c,g) we can much better appreciate the structural heterogeneity, especially along the fiber. The correlation coefficients for most image points in image Fig. 4e are increased and the range of correlation reduced in comparison with image Fig. 4b. Another important advantage of the proposed new contrast mechanism, as it was also demonstrated above using numerical simulation, is the ability to reduce noise and remove image artifacts. For example, interference noise which can be seen in conventional images Fig. 4a,d causing image artifacts such as apparent mis-orientation of the fiber (particularly evident in Fig. 4d), was removed in srSESF images Fig. 4b, e, f.

DISCUSSION

The proposed approach demonstrates significant improvement in resolution. Negligible changes in intensity profiles, which are totally lost after convolution with the PSF of the imaging system even without noise, are here recovered, allowing detection of the lateral sample structure from the axial spatial period profiles. In Supplementary information (Supplementary Figs. S1 and S2) we provide results of analysis of the axial spatial period profiles for two close lateral areas with similar internal structures and for two areas at the same separation with different internal structures, in the image plane, after convolution with the PSF of the imaging system. We used the same optical imaging system which was used to form images in Figs. 1 and 2. The results are presented with and without noise addition. The sizes of the sample lateral and axial structures used to form Figs. S1 and S2 are the same as for Figs. 1 and 2 correspondingly. The results in Fig. S1 confirm that it is possible to clearly distinguish the difference in axial spatial period profiles for two areas, separated by just 25 nm in lateral direction, after convolution with PSF of imaging system (resolution limit 400 nm) and in presence of noise. The Fig. S2 demonstrates accurate reconstruction of the axial spatial period profiles when the size of the sample structure increased.

The proposed approach can be also used to detect the areas with some specific structure, for example, to detect areas with different morphology. To select such areas the corresponding axial spatial frequency profile, taken from such structure or numerically synthesized, should be used to compare with profiles at all areas of the sample. The formed images will also provide sub-wavelength resolution.

Numerical simulation shows that resolution better than 1/6 of central wavelength 600 nm at 45 dB SNR, for thickness of the object of about 1 micron, can be achieved using the srSESF approach. The demonstrated resolution is about 4 times better than the diffraction limit of the imaging system used and more than two-fold better than the ultimate diffraction resolution limit for visible light. The resolution depends on the difference in internal structures between two points in the object (just 30 nm in our case) and, for the given axial structures, can be further improved by increasing SNR, the wavelength range and the thickness of the object. There is no principal limitation on attainable resolution. Generally the approach can be extended to a broad class of objects, including absorbing media. Of course, the approach does not work for samples fully opaque for the whole wavelength range, but optical radiation can penetrate into all biomedical objects, including highly scattering media, like skin.

In summary, we proposed and demonstrated a new contrast mechanism for far field label free super-resolution imaging, srSESF microscopy. Instead of conventional image formation as 2D intensity distribution, srSESF microscopy forms images as a result of comparison of the axial spatial frequency (period) profiles, reconstructed for each image point. The nano-sensitivity of these profiles to structural alterations provides dramatic improvement in resolution. Potentially, the srSESF approach can be realized with high frame rate using, for example, snapshot image mapping spectrometer (IMS)[32] or swept wavelength light source. Improvement of resolution of 4 times in the presence of noise by numerical simulation and of about 3 times experimentally, relative to diffraction limit of the imaging system used, has been shown.

METHODS

Numerical simulation

Usually targets with periodic structure (Ronchi rulings, USAF resolution test charts) are used to test the resolving power of optical imaging systems such as microscopes. Following this principle we numerically constructed a sample, which consists of lateral groups separated by distance *d3*. Each group consists of two lateral areas with similar axial structure (the five reflectors with similar axial spatial periods for two lateral areas) and area between them with different axial structure (the five reflectors with axial spatial periods which are different from axial spatial periods for two lateral areas we want to resolve). The group of two lateral areas with the same axial structure (230 nm axial period) and lateral size *d1* each are separated by an area *d2* which has a different axial structure with 200 nm axial period (Fig. 1a). Thickness of the sample is about 1.2 microns and the refractive index n = 1.35 which is typical for biomedical objects (cells).

A broadband plane wave with spectral range 450-750 nm was simulated for illumination. Images were formed as lateral intensity distributions after convolution of the reflected light with the point spread function (PSF) of the numerically simulated imaging system with numerical aperture NA=0.9, resolution limit 400 nm. Namely, the lateral profiles of intensity $I(x)$ of the reflected light for each wavelength were calculated in the sample plane. These profiles were convolved with the point spread function (PSF) of the numerically simulated imaging system to form images: $I_{im} = |U|^2 \otimes |h|^2$, where I_{im} – intensity distribution in image plane, U – complex amplitude of the reflected light wave, h - PSF. The PSF was simulated as $h(r) = 2J_1(ra)/ra$, where J_1 is a Bessel function of the first kind. The value a is given by $a = 2\pi NA/\lambda$, NA - numerical aperture, r – lateral coordinate, and λ - wavelength. The example of a PSF for NA = 0.9 is presented in Fig. 2. Conventional images were formed as superposition of lateral intensity distributions for all wavelengths after convolution with PSF of the imaging system.

To form srSESF images the wavelengths were converted into the spatial periods according to the relation between wavelengths and spatial frequencies in K-space.[28,31] Profiles of axial spatial periods were reconstructed by taking the intensity at a given pixel for all lateral intensity distributions at all spatial periods. The srSESF images were formed as correlation maps between the axial spatial period profile (intensity versus spatial period) at the given pixel and axial spatial period profiles at all other pixels. An imaging spectrometer or swept light source can be used for recording the spectra. A linear array of detectors was simulated for detection. To simulate the real experimental situation, noise was added and SNR is 45 dB.

Numerical simulation was done for different sizes of the object structure: $d_1 = d_2$=50 nm d_3=250 nm, $d_1 = d_2$=310 nm d_3=1550 nm, and $d_1 = d_2$=560 nm d_3=2800 nm.

Experiments

Scanning IR microscope

The scanning microscopy and srSESF images were acquired for the same areas of the samples using custom built scanning IR microscope (schematic is presented in Fig. 5a). A broadband superluminescent diode (SLD) light source, wavelength range 1230 nm-1370 nm, was used for illumination. The light beam from SLD was sent into the sample arm. The sample arm consisted of a pair of galvanometric driven mirrors and an objective lens with NA = 0.5. Galvanometric mirrors provided 2D lateral scan of the sample. The light, scattered from the sample, was collected in the spectrometer. The spectrometer setup had a 1024 pixels InGaAs line scan camera (SU1024LDH2, Goodrich Ltd. USA) with a maximum acquisition rate of 91 kHz, spectral resolution 0.14 nm. This microscope is much simpler and cheaper than equipment usually used for super-resolution imaging. For each point of the sample the whole spectrum was recorded and converted into axial spatial period profiles (Fig. 5b).[28,31] After that the srSESF images Fig. 5c were formed as correlation coefficient distributions between axial spatial period profiles at different locations (see supplementary information for details).

Samples

Phantom.

To experimentally demonstrate improvement in resolution in comparison with conventional techniques the sample with polymer sphere aggregates was made. The polymer spheres from Bangs Laboratories, Inc., diameter of spheres is 400 nm, were used to make the sample. An aliquot of about 10μl of diluted monodispersed polystyrene nanosphere suspension (n = 1.59) was smeared uniformly onto a glass slide and dried, forming nanosphere aggregates.

Collagen scaffold tissue preparation:

C2C12 cells (ATCC CRL-1772) were plated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 mg/ml streptomycin at 37°C. Cells were grown to approximately 70-80% confluence and used for the experiments.

Collagen type I was extracted from rat tails and processed as previously described[33]. Briefly, 1 g of air-died and ultraviolet-sterilized collagen type I tendons extracted from rat tails were solubilized in 300 mL 0.1% acetic acid, obtaining a collagen acid solution at a concentration of 2 mg/ml, as quantified with BCA assay.

Collagen gels were then processed by mixing the sterile solution with a suspension of C2C12 murine myoblast (2*106). Collagen acidity was neutralized with NaOH (1 M) and NaHCO3 (0.26 M). This solution was then poured into a mold to obtain vessel-like scaffolds. After 24 h at 37°C the collagen acid solution was jellified in the tube, with cells trapped within, and DMEM 10% FBS was placed as nutrient supplement for the cells (all from Lonza, Belgium).

Samples were kept in static condition for 21 days and then subjected where required to dynamic stimulation for 7 days in bioreactor using ElectroForce® BioDynamic™ Test Instruments. Assuming Poiseuille flow, the fluid speed used corresponds to a wall shear stress of 5 dyne/cm^2. Pieces of collagen tissues, fixed in formaldehyde 4% water solution, were placed in a small dish and images were taken.

References

1 Hecht, B. *et al.* Scanning near-field optical microscopy with aperture probes: Fundamentals and applications. *J. Chem. Phys.* **112**, 7761-7774 (2000).

2 Oshikane, Y. *et al.* Observation of nanostructure by scanning near-field optical microscope with small sphere probe. *Sci. Technol. Ad.v Mat.* **8**, 181-185 (2007).

3 Kawata, S., Inouye, Y. & Verma, P. Plasmonics for near-field nano-imaging and superlensing. *Nat. Photonics* **3**, 388-394 (2009).

4 Lauterbach, M. A. Finding, defining and breaking the diffraction barrier in microscopy – a historical perspective. *Opt. nanoscopy* **1**, 1-8 (2012).

5 Pyne, A., Thompson, R., Leung, C., Roy, D. & Hoogenboom, B. W. Single-Molecule Reconstruction of Oligonucleotide Secondary Structure by Atomic Force Microscopy. *Small* **10**, 3257-3261 (2014).

6 Chi, K. R. Super-resolution microscopy: breaking the limits. *Nat. Methods* **6**, 15-18 (2009).

7 Huang, B., Bates, M. & Zhuang, X. W. Super-Resolution Fluorescence Microscopy. *Annu. Rev. Biochem.* **78**, 993-1016 (2009).

8 Gustafsson, M. G. Nonlinear structured-illumination microscopy: wide-field fluorescence imaging with theoretically unlimited resolution. *Proc. Nat. Acad. Sci. USA* **102**, 13081-13086, (2005).

9 Westphal, V. & Hell, S. W. Nanoscale resolution in the focal plane of an optical microscope. *Phys. Rev. Lett.* **94**, 143903 (2005).

10 Laporte, G. P. J., Stasio, N., Sheppard, C. J. R. & Psaltis, D. Resolution enhancement in nonlinear scanning microscopy through post-detection digital computation. *Optica* **1**, 455-460 (2014).

11 Barsic, A., Grover, G. & Piestun, R. Three-dimensional super-resolution and localization of dense clusters of single molecules. *Sci. Rep.* **4**, 5388 (2014).

12 Cho, S. *et al.* Simple super-resolution live-cell imaging based on diffusion-assisted Forster resonance energy transfer. *Sci. Rep.* **3**,1208 (2013).

13 Kuang, C. F. *et al.* Breaking the Diffraction Barrier Using Fluorescence Emission Difference Microscopy. *Sci. Rep.* **3**, 1441 (2013).

14 Hao, X. *et al.* From microscopy to nanoscopy via visible light. *Light Sci. Appl.* **2**, e108 (2013).

15 Marrison, J., Raty, L., Marriott, P. & O'Toole, P. Ptychography - a label free, high-contrast imaging technique for live cells using quantitative phase information. *Sci. Rep.* **3**, 2369 (2013).

16 Rappaz, B., Breton, B., Shaffer, E. & Turcatti, G. Digital Holographic Microscopy: A Quantitative Label-Free Microscopy Technique for Phenotypic Screening. *Comb. Chem. High T Scr.* **17**, 80-88 (2014).

17 Alexandrov, S. A., Hillman, T. R., Gutzler, T. & Sampson, D. D. Synthetic aperture Fourier holographic optical microscopy. *Phys. Rev. Lett.* **97**, 168102 (2006).

18 Mico, V., Zalevsky, Z., Ferreira, C. & Garcia, J. Superresolution digital holographic microscopy for three-dimensional samples. *Opt. Express* **16**, 19260-19270 (2008).

19 Hillman, T. R., Gutzler, T., Alexandrov, S. A. & Sampson, D. D. High-resolution, wide-field object reconstruction with synthetic aperture Fourier holographic optical microscopy. *Opt. Express* **17**, 7873-7892 (2009).

20 Wang, Z. *et al.* Optical virtual imaging at 50 nm lateral resolution with a white light nanoscope. *Nat. Commun.* **2**, 218 (2011).

21 Li, L., Guo, W., Yan, Y. Z., Lee, S. & Wang, T. Label-free super-resolution imaging of adenoviruses by submerged microsphere optical nanoscopy. *Light Sci. Appl.* **2**, e104 (2013).

22 Huang, F. M. & Zheludev, N. I. Super-Resolution without Evanescent Waves. *Nano Lett.* **9**, 1249-1254 (2009).

23 von Olshausen, P. & Rohrbach, A. Coherent total internal reflection dark-field microscopy: label-free imaging beyond the diffraction limit. *Opt. Lett.* **38**, 4066-4069 (2013).
24 Silien, C., Liu, N., Hendaoui, N., Tofail, S. A. M. & Peremans, A. A framework for far-field infrared absorption microscopy beyond the diffraction limit. *Opt. Express* **20**, 29694-29704 (2012).
25 Muller, C. B. & Enderlein, J. Image Scanning Microscopy. *Phys. Rev. Lett.* **104** (2010).
26 Alexandrov, S. A. & Sampson, D. D. Spatial information transmission beyond a system's diffraction limit using optical spectral encoding of the spatial frequency. *J. Opt. A-Pure and Appl. Opt.* **10**, 025304 (2008).
27 Alexandrov, S. A., Uttam, S., Bista, R. K. & Liu, Y. Spectral contrast imaging microscopy. *Opt. Lett.* **36**, 3323-3325 (2011).
28 Uttam, S., Alexandrov, S. A., Bista, R. K. & Liu, Y. Tomographic imaging via spectral encoding of spatial frequency. *Opt. Express* **21**, 7488-7504 (2013).
29 Alexandrov, S. A., Subhash, H. M., Zam, A. & Leahy, M. Nano-sensitive optical coherence tomography. *Nanoscale* **6**, 3545-3549 (2014).
30 Sheppard, C. Fundamentals of superresolution. *Micron* **38**, 165-169 (2007).
31 Born, M. & Wolf, E. *Principles of optics : electromagnetic theory of propagation, interference and diffraction of light*. 7th (expanded) edn, (Cambridge University Press, 1999).
32 Gao, L. A., Kester, R. T., Hagen, N. & Tkaczyk, T. S. Snapshot Image Mapping Spectrometer (IMS) with high sampling density for hyperspectral microscopy. *Opt. Express* **18**, 14330-14344 (2010).
33 Rajan, N., Habermehl, J., Cote, M. F., Doillon, C. J. & Mantovani, D. Preparation of ready-to-use, storable and reconstituted type I collagen from rat tail tendon for tissue engineering applications. *Nat. Protoc.* **1**, 2753-2758 (2006).

Author contributions

SAA proposed the concept, conducted the experiments, analysed the data and wrote the paper. JMcG developed MATLAB codes for simulation and data processing and calibrated experimental setup. FB and CG provided the collagen tissues, wrote the paper. ML provided overall guidance to the project, discussed the results and wrote the paper.

Competing financial interests

The authors declare that they have no competing financial interests.

Figure Legends

Figure 1. Results of numerical simulation. (a) Simulated object, d_1 = 50 nm, d_2 = 50 nm, d_3 = 250 nm. (b) Lateral intensity distribution of the reflected light on the object before convolution. (c) – (f) Intensity distributions in the image plane using conventional microscopy. (g) – (j) Correlation coefficient distributions in the image plane using the srSESF approach; (c), (d) and (g), (h) – without noise; (e), (f) and (i), (j) - with noise. (d), (f), (h), (j) Magnified portions of (c), (e) (g), (i).

Figure 2. Results of numerical simulation. (a) - (e) for d_1 = 0.31 μm, d_2 = 0.31 μm, d_2 = 1.55 μm and (f) – (j) for d_1 = 0.56 μm, d_2 = 0.56 μm, d_2 = 2.8 μm. (a), (f) Lateral intensity distributions of the reflected light on the object. (b), (c), (g), (h) Intensity distributions in the image plane using conventional microscopy. (d), (e), (i), (j) Correlation coefficient distributions in the image plane using srSESF approach; (b), (d), (g), (i) – without noise and (c), (e), (h), (j) - with noise. PSF - point spread function for objective lens with NA = 0.9, wavelength 600 nm.

Figure 3. Images of the nanosphere aggregates: (a) scanning microscopy and (b) srSESF microscopy. Images (a) and (b) were formed using the wavelength range 1230 nm - 1370 nm, NA = 0.5. Images (d), (e) and (f) are magnified portions of the images (a) and (b). Image (f) was formed for a different range of correlation coefficients. Size of magnified portions in the images (a) and (b) is 1000 nm x 1000 nm. (c) Conventional bright field image using visible light, NA = 0.9. Scale bar is 2 microns.

Figure 4. Images of collagen fibers. (a), (d) Scanning microscopy images with interference fringe noise. (b), (e), (f) srSESF microscopy images formed using the wavelength range 1230 nm - 1370 nm, NA = 0.5, reveal the horizontal fibers. (c), (g) High resolution conventional bright field images using visible light, NA = 0.8.The scale bar is 2 microns.

Figure 5. Schematic of the scanning microscope experimental setup with image acquisition. (a) – microscope, where SLD - superluminescent diode 1230 nm - 1370 nm, OC – optical coupler, DG – diffraction grating. (b) – axial spatial period profiles for different lateral locations, (c) – srSESF image.

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