Real-Time 3D Sensing and Identification of Microorganisms

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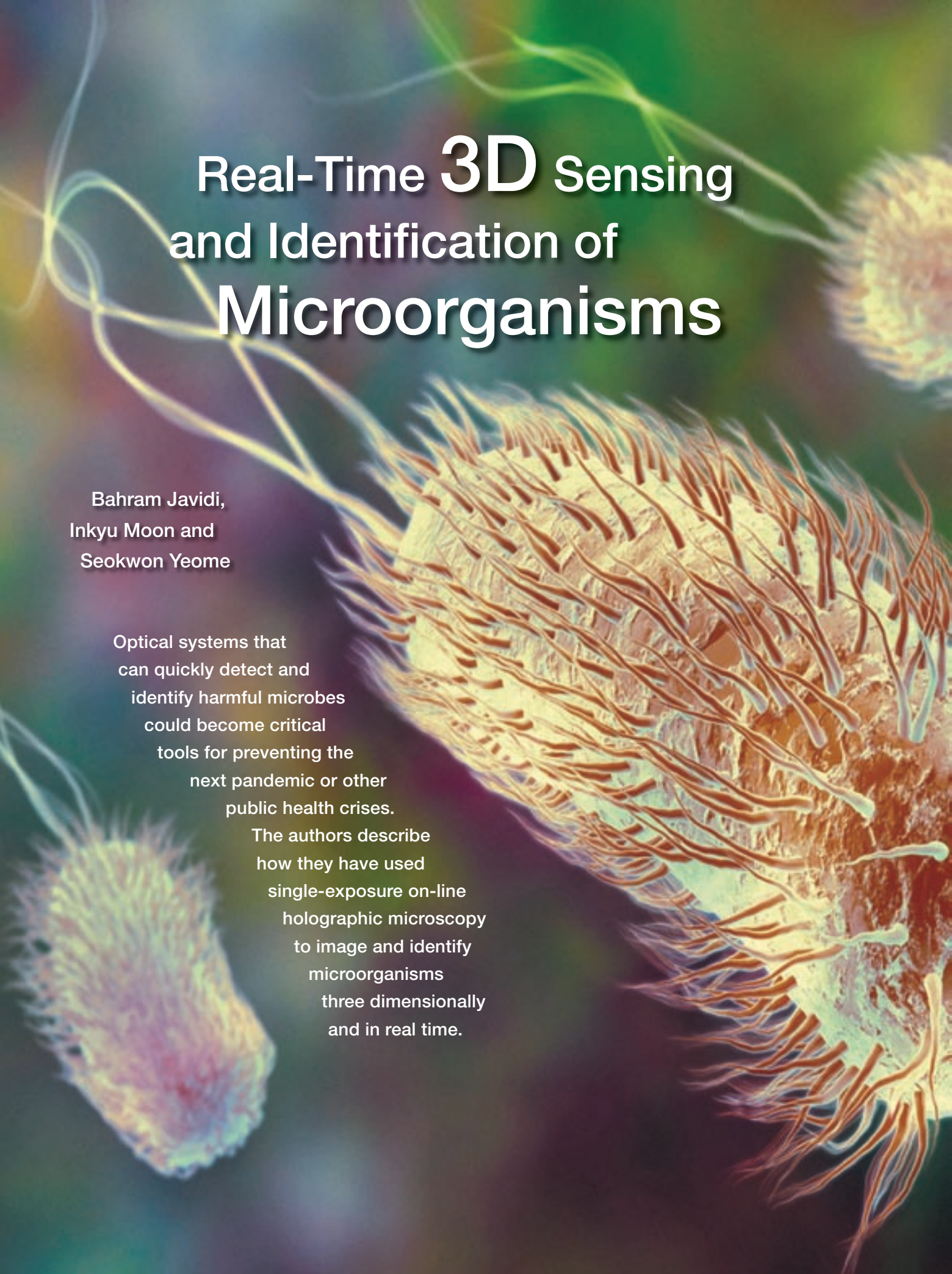
Optical systems that can quickly detect and identify harmful microbes could become critical tools for preventing the next pandemic or other public health crises. The authors describe how they have used single-exposure on-line holographic microscopy to image and identify microorganisms three dimensionally and in real time.



Computer 3D illustration of *E. coli* bacteria.

Recently, public health officials around the world have raised concerns that an avian flu virus could trigger the next pandemic. Some experts fear that the death toll could be in the millions, far greater than the casualties that would be expected due to any wars or terrorist attacks. Indeed, the last influenza pandemic, which took place in 1918, killed an estimated 25 to 50 million people—more than twice the total number of casualties from World War I.

These statistics underscore the urgent need to develop fast, reliable, automated and low-cost methods for detecting and identifying harmful microorganisms. Many countries have dedicated vast resources to conventional military and defense capabilities, but it is not clear that they have allocated sufficient funds to combating humankind's ancient nemesis: catastrophic disease.

An outbreak could occur anywhere in the world, and thus effective inspections systems would need to be in widespread use. They must also be low cost and manufacturable with commercially available off-the-shelf components. Most of the conventional methods that are currently used to inspect bacteria or other microorganisms involve biochemical processing—which is not real-time, may be labor-intensive, and requires special skills.

Systems that perform real-time sensing, imaging and recognition of microbes could have myriad applications beyond preventing or controlling outbreaks of disease. For example, they could be used for waste water treatment, environmental sensing, food safety monitoring and preventing bioterrorist attacks.

There are many challenges associated with developing real-time systems, however. Biological microorganisms are not rigid objects: They differ in size and shape, and they can move, grow and reproduce at varying rates. Bacteria and viruses can be particularly complicated to image because they are very tiny and may occur as either a single cell or associations of varying complexity, depending on their environmental conditions. They have relatively simple morphological traits that do not lend themselves well to image intensity-based recognition and identification.

Most research and development in this field has been targeted at the recognition of specific features of microorganisms based on captured two-dimensional intensity images. The

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microbes have been identified based on their colors, 2D shapes, aggregation size and reaction times. Recently, we have investigated optical systems capable of three-dimensional recognition of biological microorganisms using holographic microscopy. One of the advantages of this type of microscopy is that it automatically produces focused volume images of objects from a single hologram without any mechanical scanning, as is needed in conventional microscopy. This form of microscopy has been well studied because it has broad applications.

The SEOL holographic microscopy system and 3D morphology-based recognition

Our focus in this article is on real-time automated optical systems that use single-exposure on-line (SEOL) digital holographic microscopy to sense, visualize and recognize microorganisms in three dimensions. In SEOL holography, 3D images are reconstructed using signal processing and then segmented to separate the microorganisms of interest from the background.

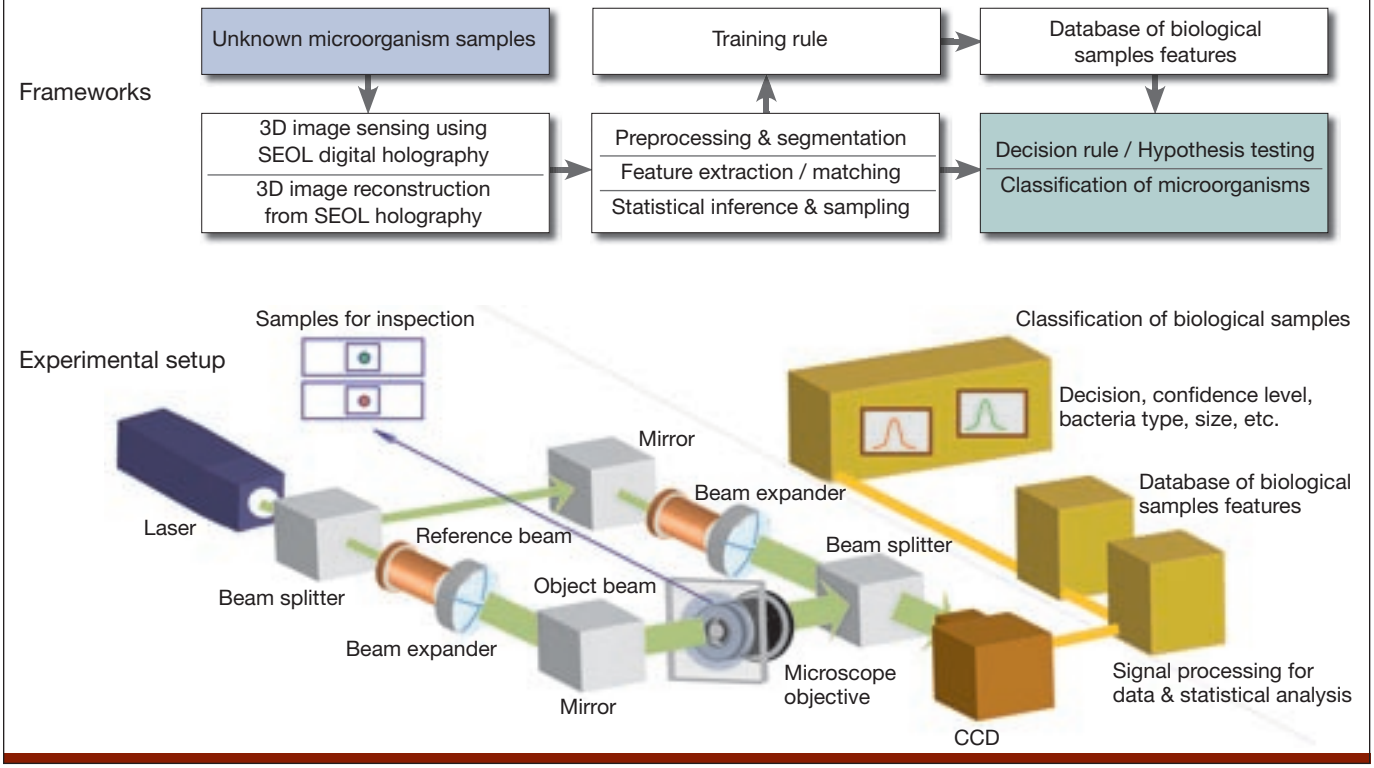
As shown in the figure below, we use a charge-coupled device array to record the interference pattern caused by a microorganism in the Fresnel diffraction domain. A beam splitter divides the laser beam into object and reference waves. Each spatial filter and collimating lens provides a plane-parallel wave. The object wave illuminates the specimen magnified by the microscope object. A digital hologram of a microorganism can then be generated by the reference wave and the diffracted wave-fronts of the specimen. Our system requires only a single exposure, and can therefore recognize a moving 3D object. The reconstruction of the microorganism is performed digitally on a computer; the field distribution is calculated numerically using the inverse Fresnel transformation.

Segmentation is performed by histogram analysis. Then, the salient features of the microorganisms are extracted using Gabor-based wavelets, which decompose the images in the spatial frequency domain. The multi-scaled and multi-oriented Gaussian-form kernels of the wavelets are suitable for local spectral analysis. The wavelets perform band-pass filtering to extract local features with high frequency bandwidth kernels and global features with low frequency bandwidth kernels.

Once the features are extracted, we use the rigid graph matching (RGM) technique to compare 3D complex morphologies in an unknown input sample with those found in known "reference" microorganisms. In other words, the shapes in the sample are measured to see how closely they match those in a predetermined reference graph that represents the unique features of a certain microorganism. In our database, the reference graph can be predetermined in order to represent unique shape features of the microorganism.

Assuming a reference graph *R* covers a designated shape of the representing characteristic in the reference microorganism, we search for a similar local shape by translating and rotating the graph *S* on unknown input images. A similarity cost

SEOL Digital Holography for 3D Imaging of Microorganisms



function and a difference cost function between graphs R and S are defined to measure the similarity of 3D geometrical shapes comprising the complex magnitude between a reference microorganism and unknown input samples.

Shape-independent recognition of biological microorganisms

Because different types of microorganisms may appear similar in shape, morphology-based recognition may not be effective. Therefore, we have also developed an approach for shape-independent 3D recognition of microorganisms by using statistical estimation and inference. In this approach, a number of sample segments are extracted from the reconstructed 3D image and processed using cost functions. A statistical estimation is used to calculate the sampling distributions for the difference in parameters between the sample segment features of reference and input images.

First, we reconstruct a 3D image of the reference microorganism using the SEOL digital hologram. Then, we randomly extract N pixels n times in the reconstructed 3D image and repeat the steps for S specimens of the same microorganism. Each sample segment consists of N by S complex values. We denote each pixel value in the trial sample patch as X_N^S. Similarly, we produce n trial sample patches of unknown input microorganisms. For recognition, we first use metrics of mean-square-distance (MSD) and mean-absolute-distance (MAD) to estimate quantitatively the performance of the recognition system.

The cost functions measure the statistical distances between a training sample of a microorganism and an input microorganism under observation. Also, we perform hypothesis testing for the equality of the parameters between two independent statistical populations of microorganisms using statistical sampling and estimation theory. It is based on comparing the statistical means and variances of the samples taken from the microorganisms. If the microorganisms belong to the same class, their statistical parameters tend to be similar. The T-test and F-test are calculated using statistical estimation and inference algorithms. A statistical decision about the populations can then be made on the basis of the sampling distribution information.

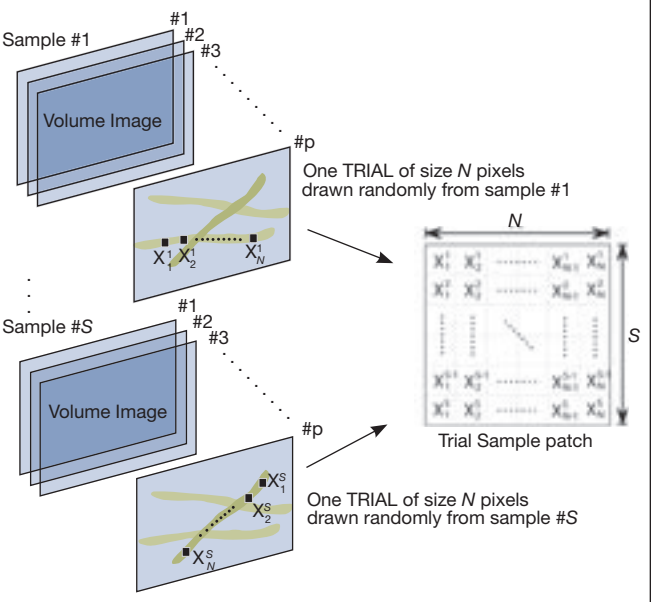
The shape-independent technique is well suited for recognizing microorganisms that do not have well defined shapes or profiles. It could also be applied to cells that can rapidly vary their shape and profile.

Experimental results

> 3D imaging with SEOL digital holography

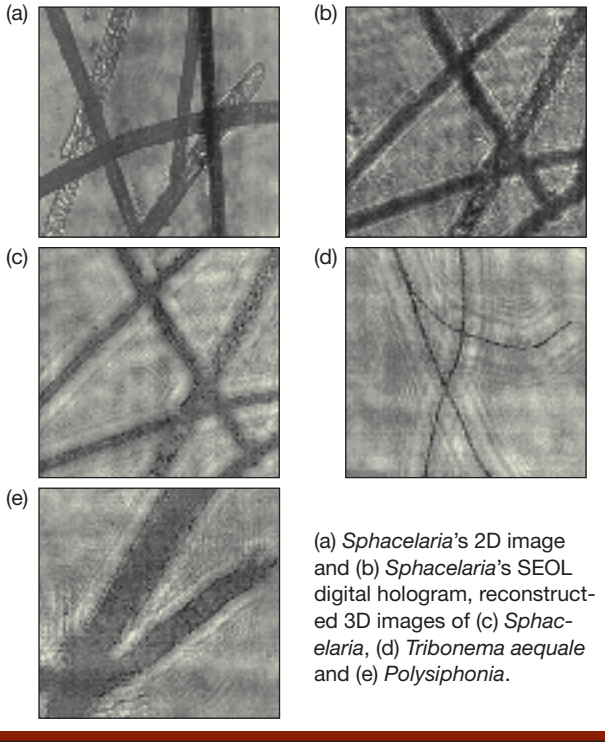
The figure at right shows images of several types of algae. Part (a) is a 2D image of *Sphacelaria* and (b) is the SEOL digital hologram of *Sphacelaria*. Reconstructed images from SEOL digital holograms of *Sphacelaria*, *Tribonema aequale* and *Polysiphonia* appear in (c), (d) and (e), respectively. The reconstructed holograms are at a distance of 180 mm.

Design Procedure for Shape-Independent 3D Recognition of Microorganisms



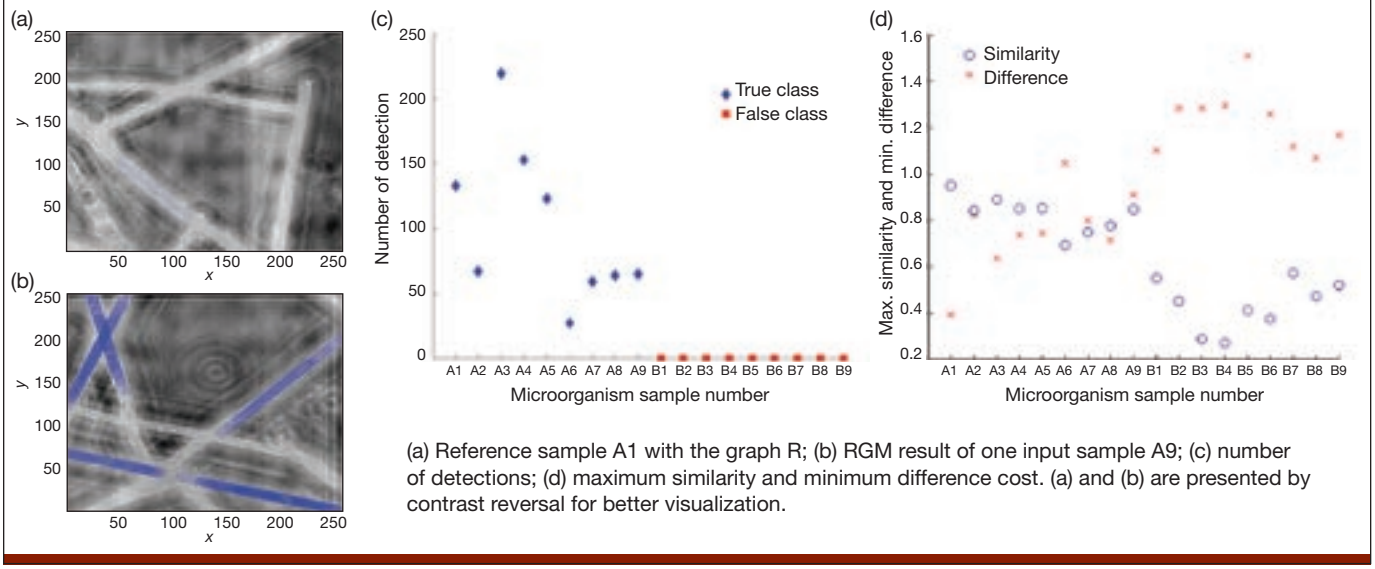
For this technique, we produce n trial sample patches from an unknown input microorganism. We denote each pixel value in the trial sample patch as X_N^S. For recognition, we first use metrics of mean-square-distance and mean-absolute-distance to estimate quantitatively the performance of the 3D recognition system. The cost functions measure the statistical distances between a training sample of a microorganism and an input microorganism under observation.

Experimental Results by Use of a 10× Microscope Objective



(a) *Sphacelaria*'s 2D image and (b) *Sphacelaria*'s SEOL digital hologram, reconstructed 3D images of (c) *Sphacelaria*, (d) *Tribonema aequale* and (e) *Polysiphonia*.

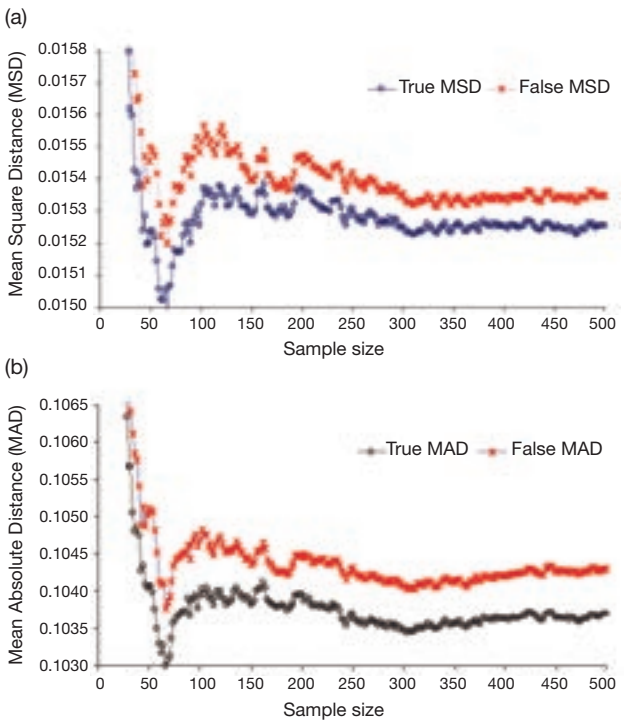
Recognition of *Sphacelaria* Alga



(a) Reference sample A1 with the graph R; (b) RGM result of one input sample A9; (c) number of detections; (d) maximum similarity and minimum difference cost. (a) and (b) are presented by contrast reversal for better visualization.

Real-time 3D imaging systems using SEOL digital holography can provide fast, non-destructive and automatic 3D image-based recognition and monitoring of living microorganisms in dynamic scenes.

Average MSD and MAD Between Sample Segments



(a) Average mean square distance (MSD), (b) average mean absolute distance (MAD). The cost functions are calculated by the complex amplitude of the reference and input segments, and plotted versus the sample size of selected segments.

> Preprocessing and feature extraction

We generated holograms for nine samples of *Sphacelaria*, denoted as A1,…A9, and nine samples of *Tribonema aequale*, labeled as B1,…B9. We cropped and reduced the magnitude and phase parts of computationally reconstructed complex images into images with 256 × 256 pixels. During segmentation, we assumed that less than 20 percent of the lower magnitude region of the complex image was occupied by the microorganisms, and that the magnitude of the microorganisms was less than 45 percent of the background diffraction field.

> Graph matching recognition

We selected a rectangular grid as a reference graph for the *Sphacelaria* alga, which shows regular thickness in the reconstructed images. The reference graph is composed of 25 × 3 nodes, and the distance between nodes is four pixels in the x and y directions. Therefore, the total number of nodes in the graph is 75. The reference graph R is placed in the sample A1, as shown in part (a) of the figure above. Another sample of *Sphacelaria* (A9) is shown in (b) for the input image with the graph matching process. The reference shapes are detected 65 times along the filamentous objects. Part (c) shows the number of detections for nine "true-class" samples (microorganisms in the same class) and nine false-class samples.

The detection number for A1-A9 varies from 27 to 220, showing strong similarity between the reference sample (A1) and the input samples (A2-A9) of the true-class microorganisms. There is no detection found in the samples B1-B9,

which are the false-class microorganisms. The maximum similarity and the minimum difference cost for all samples is pictured in (d).

> Shape-independent 3D recognition

We performed experiments to evaluate the performance of the shape-independent 3D microorganism recognition technique. First, we produced 100 trial sampling segments by randomly selecting the pixel values in the segmented *Sphacelaria* alga 3D image as the true class training (reference) data. Similarly, we randomly selected 100 trial sampling segments in the *Sphacelaria* alga 3D image used as the true-class non-training inputs, and in the *Polysiphonia* alga image used as the false-class inputs, respectively.

We changed the size of each trial sampling segment from 2 to 500 and applied an edge-detection algorithm to the segmented 3D image for fast and efficient recognition. The figure to the lower left on the facing page shows the experimental results of the average MSD and MAD between reference and input sampling segments. The average MSD and MAD for the true-class input patch were calculated to be around 0.01525 and 0.10355, respectively. The average MSD and MAD for the false-class input patch was more than 0.01535 and 0.10425, respectively.

The figure to the upper right shows the results of hypothesis testing for the difference in location parameters between the reference and input sampling segments, using a statistical T-test at the 0.05 confidence level. As we discussed earlier, these tests indicate the similarities between statistical properties of the samples taken from the reference (true-class) microorganisms versus the unknown input microorganism.

The percentages of the correct matched segments for the true-class input segments were around 80 percent. For the false-class input patches, the percentages of the correct matched segments decreases rapidly as sample size increases. We also conducted the hypothesis testing for the ratio of dispersions between the reference and input sampling segments using statistical F-test at the 0.10 confidence level. We have computed the ratio of the dispersions between two sampling segments with a sample size of 500. The number of the correct matched segments for 100 true-class inputs was 87, and only 2 for 100 false-class input samples.

Conclusion

Real-time 3D imaging systems using SEOL digital holography can provide fast, non-destructive and automatic 3D image-based recognition and monitoring of living microorganisms in dynamic scenes. They produce automatically obtained focused volume images of microorganisms from only a single digital hologram without any mechanical scanning, as is needed in conventional microscopy. The system is tolerant to environmental vibration and fluctuations because it requires a single exposure.

These optical systems can be compact and manufactured with commercially available off-the-shelf components. If successful, they can yield substantial benefits in real-time detection and identification of harmful biological microorganisms.

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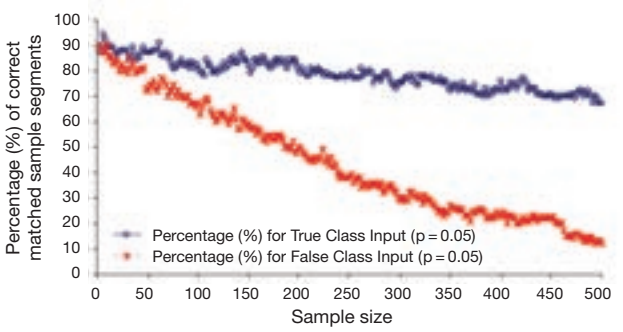
T-test for the Equality of Location Parameters



T-test for the equality of location parameters between two sampling segments versus a sample size.

[References and Resources]

>> J.W. Goodman, *Introduction to Fourier Optics 2nd*. Boston: McGraw Hill, 1996.

>> S.-K. Treskatis et al. Biotech. and Bioeng., **53**, 191 (1997).

>> T. Zhang and I. Yamaguchi, Opt. Lett. 23, 1221 (1998).

>> J.W. Lengeler et al. *Biology of the Prokaryotes*, New York: Blackwell Science, 1999.

>> J.M.S. Cabral, M. Mota and J. Tramper, eds., *Multiphase Bioreactor Design*, "Chapter 2, Image Analysis and Multiphase Bioreactor," Taylor & Francis, London (2001).

>> B. Javidi and F. Okano, eds., *Three Dimensional Television, Video, and Display Technologies*, Springer Verlag Berlin, 2002.

>> J. Alvarez-Borrego et al. Opt. Eng. **41**, 872 (2002).

>> B. Javidi, ed., *Image Recognition and Classification: Algorithms, Systems, and Applications*, New York: Marcel Dekker, 2002.

>> M.G. Forero et al. Real-time Imag. **10**, 251 (2004).

>> A.L. Amaral et al. Environmentrics, **15**, 381 (2004).

>> T. Luo et al. IEEE Trans. on Systems, Man, and Cybernetics Part B, **34**, 1753 (2004).

>> Y. Frauel et al. Applied Optics-Information Processing, **43**, 452, 2004.

>> P. Ferraro et al. Opt. Exp. **13**, 6738 (2005).

>> T. Kreis, ed., *Handbook of Holographic Interferometry*. Wiley, VCH, 2005.

>> I. Moon and B. Javidi. Opt. Exp., **13**, 9612 (2005).

>> B. Javidi et al. Opt. Exp., **13**, 4492 (2005).

>> B. Javidi. *Optical Imaging Sensors and Systems for Homeland Security*, Springer, 2005.

>> S. Yeom et al. Proceedings of the IEEE, **94** (2006).