Dual instrument for \textit{in vivo} and \textit{ex vivo} OCT imaging in an ENT department

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Abstract: A dual instrument is assembled to investigate the usefulness of optical coherence tomography (OCT) imaging in an ear, nose and throat (ENT) department. Instrument 1 is dedicated to \textit{in vivo} laryngeal investigation, based on an endoscope probe head assembled by compounding a miniature transversal flying spot scanning probe with a commercial fiber bundle endoscope. This dual probe head is used to implement a dual channel nasolaryngeal endoscopy-OCT system. The two probe heads are used to provide simultaneously OCT cross section images and \textit{en face} fiber bundle endoscopic images. Instrument 2 is dedicated to either \textit{in vivo} imaging of accessible surface skin and mucosal lesions of the scalp, face, neck and oral cavity or \textit{ex vivo} imaging of the same excised tissues, based on a single OCT channel. This uses a better interface optics in a hand held probe. The two instruments share sequentially, the swept source at 1300 nm, the photo-detector unit and the imaging PC. An aiming red laser is permanently connected to the two instruments. This projects visible light collinearly with the 1300 nm beam and allows pixel correspondence between the \textit{en face} endoscopy image and the cross section OCT image in Instrument 1, as well as surface guidance in Instrument 2 for the operator. The dual channel instrument was initially tested on phantom models and then on patients with suspect laryngeal lesions in a busy ENT practice. This feasibility study demonstrates the OCT potential of the dual imaging instrument as a useful tool in the testing and translation of OCT technology from the lab to the clinic. Instrument 1 is under investigation as a possible endoscopic screening tool for early laryngeal cancer. Larger size and better quality cross-section OCT images produced by Instrument 2 provide a reference base for comparison and continuing research on imaging freshly excised tissue, as well as \textit{in vivo} interrogation of more superficial skin and mucosal lesions in the head and neck patient.

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References and links

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1. Introduction

Head and neck cancer is primarily a mucosal disease of the upper aerodigestive tract with 90% of tumors arising as squamous carcinomas from epithelial membranes of the oral and nasal cavities, the pharynx and larynx. Squamous cell carcinoma of the larynx has been the...
most frequent malignant tumor of the upper aerodigestive tract in Europe. It is a preventable disease resulting from interplay of numerous etiological factors such as chronic consumption of tobacco and/or alcohol, environmental carcinogens, socioeconomic status, occupational hazards, dietary factors and genetic susceptibility. For early-stage laryngeal cancer, both surgery and radiotherapy are effective treatment modalities, offering a high rate of local control and cure. The introduction of new fiber-optic and rigid endoscopic techniques with stroboscopy has greatly enhanced the diagnostic and dynamic assessment of tumors of the upper aerodigestive tract, particularly the larynx [1].

Optical Coherence Tomography (OCT) is an established non-invasive optical biopsy method, capable of imaging ranges of 2-3 mm into tissue [2–5]. By using principles of low coherence light interferometry, OCT can be used to distinguish normal from unhealthy laryngeal mucosa in patients [6–8]. Miniaturized fiber-optic probes are a key component for emerging clinical applications of OCT and offer new possibilities to image diseased tissue deep within the body. Single-mode optical fibers are a base requirement for endoscopic or catheterized deployment of biomedical fiber sensors and imaging systems [9]. Fiber imaging bundles have been incorporated into different OCT systems using a variety of optical configurations that eliminate mechanical scanning components required for endoscopic OCT applications [10–12].

Forward imaging OCT probes have been used, with mechanisms such as scanning microelectromechanical system (MEMS) to redirect the light beam [13–16], or piezoelectric cantilevers to deflect the fiber [17–19]. Different office-based miniaturized OCT probe configurations have been developed and implemented for in vivo examination of the human larynx. Systematic OCT imaging of laryngeal structures has provided information on the thickness of the epithelium, integrity of the basement membrane and structure of the lamina propria. Microstructural features identified included glands, ducts, blood vessels, fluid collection/edema and transitions between pseudostratified columnar and stratified squamous epithelium. Office-based OCT systems as imaging modalities to study the larynx have the potential to guide biopsies, direct therapy and to monitor disease [7,20,21,22].

Most clinical OCT studies outside ophthalmology have involved the use of systems designed and built by research groups focused on enhancing the resolution, image acquisition rates and functionality. Until recently, there have been no commercially available “turn-key” OCT systems for use in the head and neck and furthermore most studies to date have used research devices designed and constructed by associated university optics research laboratories. A commercially available OCT system for use in the head, neck, and upper aerodigestive tract (Niris, ImaluX, Cleveland, OH) using a flexible probe (placed in contact or near contact with the area of interest) and inserted through a rigid laryngoscope has been reported [23]. The Niris imaging probe is 2.7 mm in diameter and can work through endoscopes or independently depending on the procedure. The probe can be inserted into the working port of a variety of rigid and flexible endoscopes. The system has a maximum imaging speed of 8 frames per second and a limited useful life-span of approximately 200 patient procedures [24].

Another report refers to an in vivo imaging study of the human larynx in awake patients using a commercially available flexible fiber-optic naso-laryngoscope simultaneously with a flexible OCT probe, where a “slide-on” channel endosheath was used [25].

To overcome the size, speed and limited life time issues, we propose and demonstrate the use of a 1.9 mm diameter forward-viewing flexible OCT endoscope probe in a 1300 nm swept source (SS) - OCT configuration for in vivo endoscopic imaging of human laryngeal mucosa. The OCT probe was designed and fabricated at the Institute of Applied Physics, Russia and consists of, at the distal end, a 13 mm long cylinder made of stainless steel. The OCT probe is housed together with a commercial fiber-bundle endoscope inside a single channel endosheath. The endosheath is supplied by Standard Medical Ltd., UK and is closed with a transparent optic window at its tip (CAG 0266, length 22.5 cm, shaft inner diameter 5.5 mm, distal end inner diameter 5.4 mm).
To our knowledge, this is the first report for the use of a single channel endosheath with an optically transparent window at the tip in a dual channel nasolaryngeal endoscopy-OCT assembly for in vivo human larynx investigation. Because the in vivo investigation requires a sufficiently small diameter OCT probe, the transversal resolution is larger than 20 microns laterally while the image size is less than 1 mm. In order to investigate the possible detrimental effect on diagnosis utilizing images provided by such a thin OCT probe, a hand-held probe single channel instrument is produced to provide comparatively, images from excised tissue. The hand-held probe uses a galvo-scanner and can collect OCT cross sections with a lateral size larger than 5 mm, however with better transversal resolution (<10 μm).

2. Patients and methods

2.1. OCT imaging instrumentation

The system is schematically presented in Fig. 1. Light from a swept source (Axsun Technologies Ltd, 1310 nm center wavelength, 12 mm coherence length, 106 nm FWHM bandwidth in the range (1256.6 nm–1362.8 nm) with an average output power of 18 mW and a scanning rate of 50 kHz) is provided at its FC/APC receptacle. Either the FC/APC fiber input, FC/APC1 of the OCT1 channel (Instrument 1), or the FC/APC2 of the OCT2 in the single channel hand held probe (Instrument 2) can be connected to the swept source. The fiber from the FC/APC1 end leads to a fiber optical circulator (C) coupled with a 2 x 2 directional coupler (DC) having 95/5 splitting ratio, with 95% of the power from the swept source directed to the sample arm where the endoscope OCT probe connects to.

Because the OCT head in the OCT channel of the dual channel Instrument 1 operates based on principles of common path interferometry, only a single output fiber is sufficient, FC/APCoutOCT1 connected to the photo-detector unit, PhD. The fiber from the FC/APC2 end leads to a triple splitter coupler array, where the first splitter is a 660/1310 nm Wavelength Division Multiplexer (WDM) coupler. The other input from the WDM of the three splitter array is connected to the red aiming beam. The output of the WDM is connected to the 2 x 2 optical coupler architecture (first coupler 80/20 splitting ratio, with 20% of the power directed to the sample arm, where the OCT probe is connected, and 80% to the reference arm and second coupler 50/50 splitting ratio for balance detection).

The signal collected by the photo-detector, PhD (Santec Model BPD-200 DC 80 MHz) is digitized by a 12 bit waveform digitizer (Alazar ATS9350 - 500 MS/s, 12 bit PCI Express Digitizer) while “in-house” created software in Labview (National Instruments, Austin, Texas) has been used to produce, display and record the images. For our study on the human larynx, which has similar thermal and photochemical properties to skin, the optical power directed onto the tissue was around 7 mW at 1300 nm, and was achieved by using an extra patch cord and two fixed in-fiber attenuators (20 dB). The optical power of 7 mW is below the American National Standards Institute (ANSI Z136.1) safe occupational exposure level for the skin. Similar power levels for in vivo imaging of the human epiglottis and human vocal fold were used by other groups [19,26,27].

2.2. Problems connected with guidance

The image to be collected with a probe of less than 2 mm diameter is less than 2 mm lateral size. By simply looking at the OCT image it is difficult to orientate and identify the specific area/sub-site of tissue imaged. Therefore, for reference a guidance image needs to be provided to the OCT channel and this is enabled using the camera in a commercial endoscope system paired with a visible illuminating source. The 1300 nm beam is scanned together with the visible beam such that the lateral scanner projects a visible line on to the tissue, which is then picked up by the camera in the commercial endoscope. A red diode laser at 635 nm operational wavelength was used to illuminate the object under investigation. This provided less than 0.5 mW on the tissue. Figure 1(b) shows a photograph of the distal end of the OCT endoscope probe unit (diameter of 1.9 mm, length of the rigid part of 13 mm).
The OCT endoscope probe has a cylindrical form and is composed of stainless steel containing copper wire, NdFeB magnets and a GRIn lens, with an output window composed of quartz, followed by a Teflon tubing, which leads and connects to the OCT engine. The OCT probe is built in a common path configuration where reference light is derived from the output quartz window. Detailed description of the probe operation can be found in [28]. Figure 1(c) shows a photograph of the entire OCT probe consisting of the optical fiber and driver for the magnetic scanner in the OCT probe head.

A Karl Storz flexible fiber-optic nasendoscope of 3.5 mm outer diameter (Karl Storz, 11101 RP2 Rhino-Pharyngo-Laryngo-fiberscope) was considered fit for this purpose. The endoscope provides an en face image while the OCT channel in the dual channel instrument delivers a cross-section image. In order to simplify image display arrangements, a software program was devised to allow simultaneous monitor display of the two channel images, one produced by SS-OCT and the other by the commercial endoscope.
In order to obtain *ex vivo* OCT images on tissue specimens immediately following excision, a second system was co-assembled (Instrument 2), equipped with a “hand-held” OCT probe imaging assembly. The handheld probe is composed of a rigid, hollow plastic tube assembly, measuring ~20 cm in length, with an outer diameter of 15 mm and contains a Cambridge Technology 6110 transversal scanner with a lens-associated collimator, of 30 mm focal length. These are connected via Thorlab microbench devices with a hand-held platform assembly, shown in Fig. 1(d), and then via a protected fiber to the OCT engine.

The two instruments remain permanently connected to the red laser, whilst the swept source, photo-detector unit and imaging computer are all sequentially switched from one instrument to the next. To switch from the dual channel OCT/endoscope to the “hand-held” OCT channel, the input fiber FC/APC1 or FC/APC2 is inserted into the FC/APC output of the Axsun source and the output fiber FC/APCoutOCT1 is connected to an input of the balanced detector. When using Instrument 2, the two output fibers of the hand held probe OCT2 channel, FC/APC01OCT2 and FC/APC02OCT2, are inserted into the FC/APC connectors of the photo-detector unit. The switch from one instrument to the other only requires for fiber connections to be changed. The two instruments are all assembled inside a rigid metallic box with a locking lid, which in turn is placed on a trolley with castors to allow rolling and portability of the dual instrument arrangement as required in the busy ENT clinic.

Two different Lab software programs have been created for the two instrument arrangements; to serve either the dual channel instrument, or the single channel “hand-held” OCT instrument. The dual channel instrument produces simultaneous *en face* endoscopic images with cross section SS-OCT images *in vivo* whilst the “hand-held” OCT instrument produces SS-OCT images only, either *ex vivo* of excised tissue or *in vivo* from accessible surface skin and mucosal lesions on the scalp, face, neck and oral cavity.

### 2.3. Imaging procedure of the dual channel instrument

The platform used for the nasendoscope OCT application (via nasopharyngeal tract) involves a slight modification of the current standard nasendoscopy procedure practiced by clinicians in the ear, nose and throat (ENT) clinic under topical local anesthesia. The current procedure uses an endoscope consisting of a multi-fiber bundle producing an image on a proximal camera (Xion Medical, Compact Camera). The bundle is introduced into a disposable, single-use protective medical grade endosheath (consisting of a single closed channel and optically clear window at the distal tip), in order to cover almost the entire insertable working tube length of the flexible endoscope. Flexible nasendoscopy is performed routinely this way on patients presenting for ENT consultation and is a simple procedure completed usually within 1-2 minutes once topical local anesthesia has taken effect and requiring no more than verbal consent from the patient. The nasendoscopy modification involves placement of our own assembled novel miniaturized OCT probe to be run in parallel with a 3.5 mm outer diameter commercially available multi-fiber endoscope (typically used for this investigation). At present, commercially available and routinely used flexible nasendoscopes vary widely in their outer diameters from 2.2 mm to 6 mm or more.

The rigid part of the OCT probe is 13 mm long and 1.9 mm wide. The OCT probe is placed distally alongside the standard endoscope bundle inside a single channel, closed system endosheath (Fig. (2)). The use of endoscope sheath covers is generally popular amongst many ENT clinicians as the sheath are quick to change between patients (allowing quick patient turnaround in busy clinics) and easy to use. There is a very small risk of endoscopic contamination if the endosheath is breached *in vivo* which is an exceptionally rare event. The disposable endosheath has been advocated by manufacturers and national specialty bodies (ENT-UK) to be a safe and effective alternative to chemical disinfection systems for simple diagnostic nasendoscopy, effective against bacterial and viral contamination and having been shown to maintain their integrity after patient use [29]. A photo of the disposable endosheath closed with a transparent optic window at its tip is shown in Fig. 3(a). The two combined probe heads sit enclosed within the medical-grade disposable endosheath, as presented in the photos of Fig. 3(b).
Essential for the operation of the system was the correct orientation of the scanning direction of the OCT probe. Looking at the projected line on a target, the OCT probe was rotated inside the endosheath until the line crossed the projection of the endoscope end. The correct orientation is shown in Fig. 2(e). This ensured that the visible red reference line is seen by the endoscope camera for a wider range of distances between the tissue and the cap.

The two probe heads cannot be pushed together into the endosheath with tips flush aligned, as the sum of their external diameters is exactly the internal diameter of the cap. We have found that the most suitable technique was to insert the endoscope along the sheath with the OCT probe lagging behind by a distance longer than the cap length, usually 5 mm to 2 cm (Fig. 3(b) top). With the fiber-optic endoscope inserted almost flush with the cap, the OCT probe head is then maneuvered/pushed forward with slight bending of the distal endosheath so that the two probe heads then sit together flush inside the cap (Fig. 3(b) bottom).

3. Experimental results

3.1 Ex vivo testing

Ex vivo OCT images of human larynx taken in a laboratory-based 1310 nm SS-OCT bench-top system were obtained and compared with those provided by the single channel Instrument
and are presented in Fig. 4. It is clearly seen that comparable OCT images of fine tissue-related structures are possible using both the bench top and portable endoscopic systems.

Following NHS R&D/NRES Research and Ethics Committee approval (Central London REC1, Reference no: 10/H0718/55) and informed patient consent, formalin-fixed laryngeal tissue biopsies taken from laryngectomy patients at NWLH were examined \textit{ex vivo} using a desktop OCT imaging system \cite{30} and comparison made to images taken of the same (as well as other fresh non-formalin fixed laryngeal tissue specimens) with the portable single channel Instrument 2. For laboratory imaging, laryngeal tissue samples were fixed in 10\% formalin solution, dissected into small portions of about 3 mm maximum dimension and then sealed in custom-made plastic imaging chambers containing 10\% formalin solution. The samples were sealed in the chamber by a 170 microns thick flat sheet of borosilicate glass with the seal being provided by a layer of high vacuum silicone grease between the plastic and the coverslip, then oversealed with DPX mounting medium.

3.2. \textit{Phantom model testing of dual channel instrument}

\textit{Ex vivo} testing of the OCT probe/nasendoscope assembly on an anatomically correct, adult human airway model (utilized for training junior doctors and simulating a standard ENT nasendoscopy office procedure) was performed as a prelude to the \textit{in vivo} investigations and laryngeal application.

The AirSim Advance Larynx model, (Trucorp Ltd) acted as an appropriate phantom model for \textit{ex vivo} testing of the dual channel endoscopic OCT arrangement. This model allows the necessary nasendoscopy simulation exercise in safety and the phantom exhibits an external “laryngeal” window opening that allows a direct external view through the “neck tissue” into the “endolarynx.”

Initially, there was a learning curve with some operator exercise necessary for appropriate handling of the combined endoscopic OCT probe head and sheathed fiber optic endoscope assembly. The two probe heads, not being collinear, resulted in the projected red reference line for OCT incident imaging at the target tissue, going out of the endoscopic field of view when the capped tip of the arrangement was placed too close to the tissue. In order to reduce the minimum distance at which the red line would disappear from view, the scanning OCT probe head was rotated inside the endosheath so that the scanned line crossed the projection line of the endoscope bundle, as explained above.
The sheathed combined dual channel probe was passed along the floor of the nasal cavity, navigated around the contours of the soft palate and nasopharynx (taking a maximal 30 degree arc) to enter the larynx and hover a few mm above the vocal cords (Figs. 5(a) and 5(b)). The dual channel OCT probe/endoScope inserted into the disposable endosheath for in vivo investigation on a volunteer is shown in Fig. 5(c).

The transparent cap closing the endosheath is approximately 100 microns thick. This comes up in the OCT image, so the probe has to be placed at a sufficient distance from tissue to display the useful part of the tissue in the image below the lines due to the cap surface. This was not difficult as the working distance of the OCT probe was ~3 mm, much larger than the cap thickness. During our preliminary ex vivo tests, the reflection from the endosheath tip in the OCT image was deemed useful, as it has indicated the distance between the OCT probe and the cap. Several times, the OCT probe had slipped axially inside the sheath and this could immediately be seen by the surface in the image corresponding to the cap, moving to larger depths in the OCT cross-section image. In such situations, the OCT probe was pushed back inside the endosheath until the reflections were moved to the top of the image.

Initial tests on the anatomically correct rubber phantom, have shown that the integral distal endoscope tip tilting/leverage mechanism is still able to function with the OCT probe sitting adjacent to the commercial endoscope probe head. Our tests have shown that this is sufficient to allow the OCT probe to be inserted together with the nasendoscope housed within the endosheath, the camera providing guidance as the tip is progressed through the nasal cavity and flexed around the contour of the soft palate and nasopharynx to visualize the larynx below.

3.3. Preliminary in vivo investigations

Following NHS R&D/NRES Research and Ethics Committee approval (Central London REC1, Reference no: 10/H0718/55) a feasibility in vivo study was performed using the novel dual channel OCT endoscopy arrangement. Clinical investigation of the optical imaging arrangement was performed on 2 volunteers initially (Fig. 5(c)) followed by clinical recruitment of 3 patients attending Northwick Park Hospital ENT-Head & Neck service who were noted to have suspicious larynx appearances on endoscopy. Following informed consent, these patients had endoscopic OCT images taken in the ENT out-patient department under topical local anesthesia before going on to have biopsies of the same larynx tissue under general anesthesia.

In vivo B-scan OCT images of laryngeal lesions were acquired using Instrument 1 (commercial fiber bundle endoscope appearance of left vocal cord lesion with simultaneous
acquisition of B-scan images for the same lesion through the novel endoscopic OCT probe) (Figs. 6 (a) and 6(b)).

After use of the tool, between each patient, the endosheath was disposed of and a new one used to cover the dual probe head assembly before its use on the next patient. Figure 6(c) demonstrates the histology for the same laryngeal lesion (invasive squamous cell carcinoma (SCC) of the left true vocal cord; stain is H&E). Figure 6(d) displays an ex vivo OCT B-scan image of the same left true vocal cord lesion freshly laser excised 20 minutes earlier in the operating theatre under general anesthesia, but the image having been taken using Instrument 2 in the assembly (i.e. with the hand-held probe) so as to allow comparison with the in vivo endoscopic OCT B-scan image taken with Instrument 1.

![Fig. 6. (a), (b) In vivo investigations performed on a patient with a suspect laryngeal lesion using Instrument 1 (a: endoscopic white lesion itself measured approx 1 cm in length, with diameter of field of view no greater than 1.5 cm across) and b: endoscopic OCT image size 0.9 mm x 0.75 mm) followed by (c): histology of the same lesion once excised under general anesthesia ulcerated surface (top) with underlying islands of invasive squamous cell carcinoma in a desmoplastic stroma (middle) and with pale connective tissue uninvolved by carcinoma (bottom). (image size 7 mm x 5.6 mm) and Instrument 2 (d) ex vivo OCT image of the same freshly excised larynx tissue removed 20 minutes earlier under general anesthesia (image size 3 mm x 2.55 mm). Histology confirmed as grade 1 to grade 2 invasive squamous cell carcinoma (SCC) of left glottis (true vocal cord).](image)

Comparing the in vivo endoscopic OCT image using the dual channel Instrument 1 of the same lesion shows a subjectively improved contrast over ex vivo Instrument 2. Although the spatial pixel resolution appears worse with the in vivo Instrument 1 than with ex vivo Instrument 2, this is compensated to some degree by the improved tissue contrast and interesting patterns of light and dark areas that are seen. These interesting complex contrast features present in the in vivo images, together with surface keratin formations appear visible occasionally depending on where the probe has approached or touched the target area.

A significant problem noted in this preliminary work with the in vivo image acquisition is the lack of stability when maneuvering the distal endoscopic tip assembly towards a lesion of interest, particularly in the context of an awake, moving and swallowing patient. This is compounded by a lack of orientation for the operator when the red reference light beam disappears out of the distal endoscope direct field of view. It was not possible, even approximately, to correlate the position of various aspects of the lesion to the OCT B-scan image and it was entirely taken on trust that the lesion itself was imaged at the time the capped tip approached and was directed onto it.

Furthermore the glimpses of the vocal cord lesion seen in the OCT video sequence are fleeting and freeze-frame analysis of the video post-procedure was necessary to try and discern the contrast features of relevance. This has adverse implications for real-time in vivo OCT interpretation. Although there was potentially relevant structural contrast seen, the lack of ability to accurately correlate this to the excised lesion plus the lack of a reference in vivo endoscopic OCT data set (normal vocal cords) mean that it is not possible at this stage, to be able through endoscopic OCT, diagnose the vocal cord lesion as an invasive carcinoma. Further work shall be required for acquisition of a large and thoroughly studied in vivo reference data set prior to any definite conclusions being drawn about the overall usefulness of OCT as a form of histo-diagnostic tool.
4. Conclusions

A portable dual-function instrument was assembled and deployed in a busy ENT department to serve two goals: (a) to perform dual-channel simultaneous in vivo nasendoscopic white light and OCT tissue investigations and (b) to provide a single channel “hand-held” OCT system, to provide in vivo OCT images of accessible skin and mucosal lesions in the head and neck region, as well as demonstrate ex vivo images of tissue biopsies immediately following their excision. The two elements to the assembly complement each other in terms of functionality and portability to the clinical environment. In instrument 1, due to its small size the novel nasendoscopic OCT probe exhibits lower transversal resolution and smaller image size. Instrument 2 has better transversal resolution and larger image size, useful for both ex vivo OCT imaging of excised tissue and in vivo OCT imaging of directly and superficially accessible areas of skin and mucosa; on the scalp, face, neck and oral cavity.

The performance of the sheathed dual channel nasendoscopy-OCT instrument for in vivo endoscopic human laryngeal imaging is presented as a pilot study and preliminary step towards the development of endoscopic OCT as a laryngeal cancer screening tool.

Together, the two instruments satisfy the needs of a busy ENT clinic engaged in both translatable clinical research and advancement of clinical care. The swap of the 5 fiber ends and the change of the software program takes less than 5 minutes to enact and may also be implemented using three optical switches with little penalty in terms of power loss, due to the high transmittance of modern optical switches (such as Thorlabs 1x2 Switch, OWS12-1310-SM, insertion loss <0.7 dB, back-reflection 55 dB). Alternative arrangement possibilities could include the use of an optical switch only for connecting the swept source to either of the two systems, individual photo-detectors on the two instruments and an electronic switch between the two photo-detector units.

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