united nations educational, scientific and cultural organization

international atomic energy agency

the
abdus salam
international centre for theoretical physics

SMR 1495 - 4

WINTER COLLEGE ON BIOPHOTONICS:
Optical Imaging and Manipulation of Molecules and Cells
(10 - 21 February 2003)

Coherence-gated Imaging through Scattering Media

P.M.W. FRENCH
Head of Photonics Group, Physics Department,
Imperial College London, U.K.

These are preliminary lecture notes, intended only for distribution to participants.

Biomedical optical diagnostics

Spectroscopy
Detect/quantify variations in tissue properties
(chemically-specific)

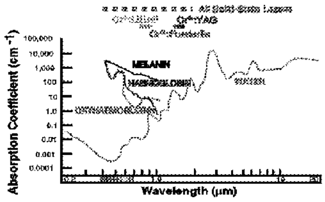
Imaging
Detect/monitor localised heterogeneity
(physical structure)

Absorption
Fluorescence
Raman

Optical biopsy *in vitro* and *in vivo*
Functional imaging
Monitoring laser surgery/therapy

Problems: heterogeneity absorption scattering

Imaging through biological tissue

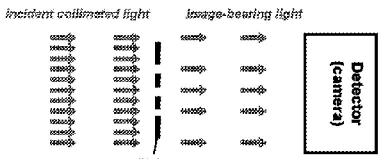


Optical transmission window ~ 600-1100 nm

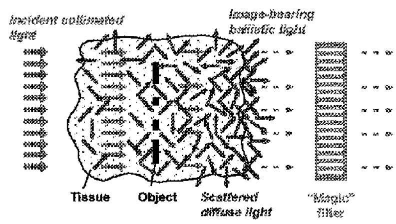
Absorption: μ_{abs} ~ 0.003 - 0.07 mm^{-1}

Scattering: μ_{scat} ~ 1 - 40 mm^{-1}

Imaging in the absence of scattering



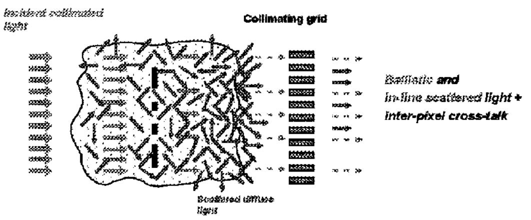
Imaging objects through scattering media



$$I_{ballistic} = I_{incident} \cdot e^{-(\mu_{abs}+\mu_{scat})L}$$

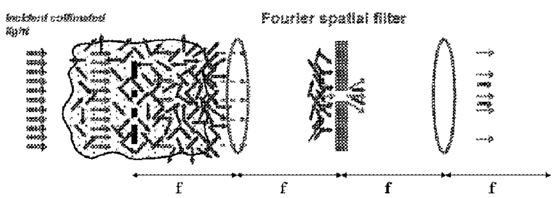
Attenuation length ~ scattering MFP = 1/μ_{scat} ~ 100 µm

Imaging through scattering media using whole-field spatial filtering



Suitable for weak scattering – applied to X-ray imaging

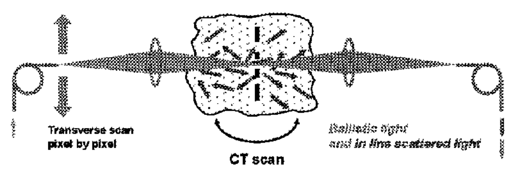
Imaging through scattering media using Fourier filtering



Wide-field spatial filter – used in microscopy

Spatial filtering: confocal imaging in transmission

Image along a line of sight

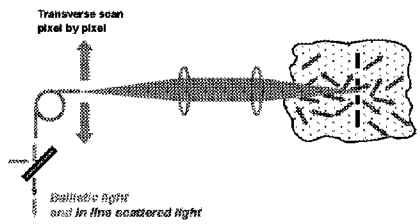


No depth discrimination in transmission

Use computer tomography techniques for 3-D imaging

Scattered light still a problem for thick (>~300 μm) samples

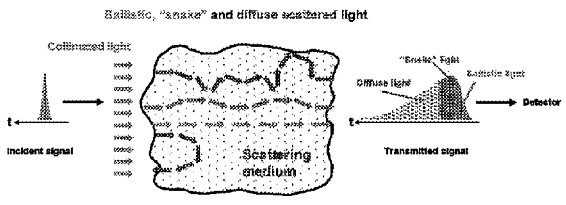
Spatial filtering: confocal imaging in reflection



***Depth-resolved (3-D) imaging* obtained in reflection mode**

Scattered light still a problem for thick (>~300 μm) samples

Time-resolved propagation through scattering media

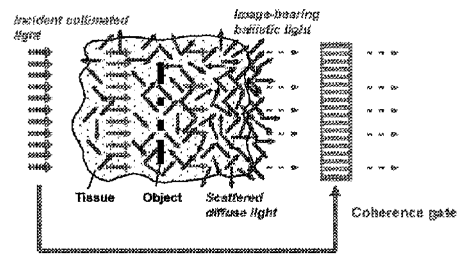


earliest arriving *ballistic* light ⇒ diffraction-limited resolution (μm)

next arriving *snake* light ⇒ degraded resolution (10's – 100's μm)

later arriving *diffuse* light ⇒ severely degraded resolution (cm)

Low coherence interferometry ⇒ sub-ps time gates



Coherence-gated imaging

For *monochromatic* light:

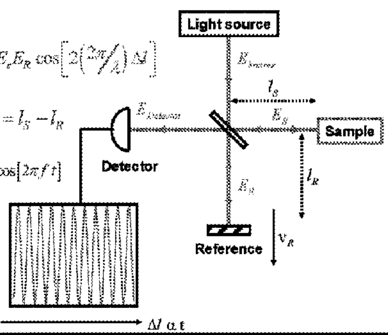
$$I_{Detector} = \frac{1}{4}|E_s|^2 + \frac{1}{4}|E_R|^2 + \frac{1}{2}E_s E_R \cos\left[2\left(\frac{2\pi}{\lambda}\right)\Delta l\right]$$

Scanning reference arm: $\Delta l = l_S - l_R$

$$I_{Detector} = \frac{1}{4}|E_s|^2 + \frac{1}{4}|E_R|^2 + \frac{1}{2}E_s E_R \cos[2\pi f t]$$

where $f = \frac{2v_R}{\lambda}$

(Doppler frequency)



Confocal coherence-gated imaging: Optical Coherence Tomography (OCT)

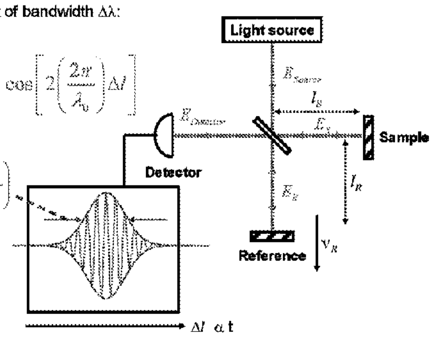
For *low coherence* light of bandwidth Δλ:

$$I_{Detector} \propto e^{\left[\frac{4\ln 2\,\Delta l^2}{(\Delta l_{FWHM})^2}\right]} \cos\left[2\left(\frac{2\pi}{\lambda_0}\right)\Delta l\right]$$

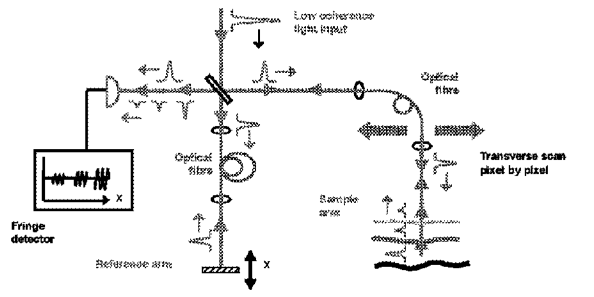
where

$$\Delta l_{FWHM} = \frac{2\ln 2}{\pi}\left(\frac{\lambda_0^2}{\Delta\lambda_{FWHM}}\right)$$

(Gaussian spectrum)

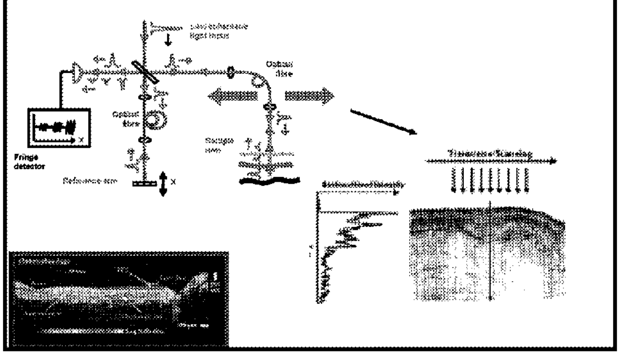


Coherence gated confocal microscopy: Optical Coherence Tomography (OCT)



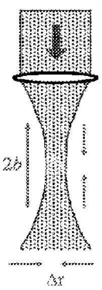
Envelope detection of OCT signal ⇒ depth information

Confocal coherence gated imaging: Optical Coherence Tomography (OCT)



OCT & Optical Coherence Microscopy (OCM)

OCT $\Delta z < 2b$



For rapid axial scanning, use low NA objectives with long confocal parameter

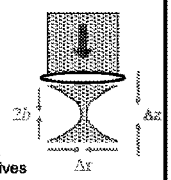
$$2b = 0.44\pi\lambda / (NA)^2$$

$$\Delta z = \frac{2\ln 2}{\pi}\frac{\lambda^2}{\Delta\lambda}$$

Axial resolution depends mostly on $\Delta\lambda$

$$\Delta r = 0.44\lambda / NA$$

OCM $\Delta z > 2b$

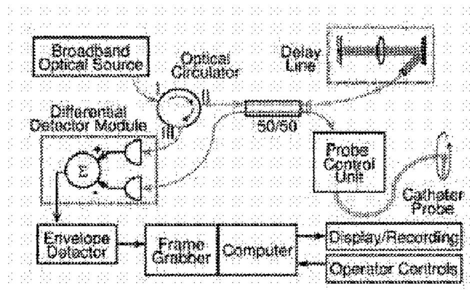


For high resolution, use high NA objectives

Axial resolution depends mostly on NA

Resulting sectioning strength is a *convolution* of coherence-gated PSF and confocal PSF

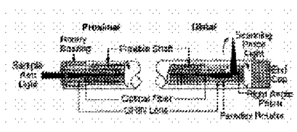
OCT: integrated fibre-optic implementation



From Opt. Lett. 24 (1999) 1358

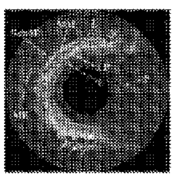
OCT: endoscopic application

Transverse radial scanning endoscopic OCT probe



Typically: 4 frames/second, 2.5 mm scan depth

Individual captured EOCT frame in the normal human esophagus



From ..Izatt et al. Opt. Lett. 24 (1999) 1358

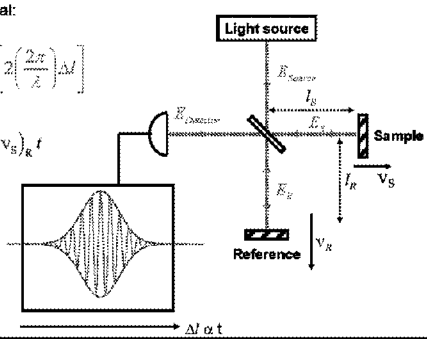
OCT: Doppler imaging of moving sample

Interferometric OCT signal:

$$I_{Detector} \propto e^{-\left[\frac{4\ln 2\,\Delta l^2}{(\Delta l_{FWHM})^2}\right]}\cos\left[2\left(\frac{2\pi}{\lambda}\right)\Delta l\right]$$

$$\Delta l = l_R - l_S$$

$$\Delta l = l_{R,t=0} - l_{S,t=0} + (v_R - v_S)_R\, t$$



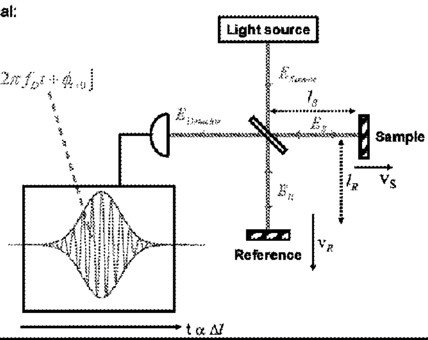
OCT: Doppler imaging

Interferometric OCT signal:

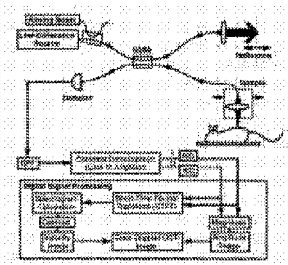
$$I_{Detector} \propto e^{\left[-\frac{4\ln 2\,\Delta l^2}{(\Delta l_{FWHM})^2}\right]}\cos[2\pi f_D t + \phi_{l,0}]$$

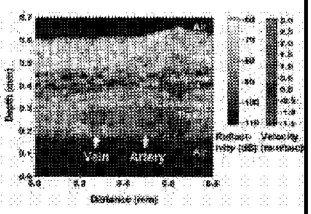
where $f_D = \frac{2v_S - v_R}{\lambda_0}$

(Doppler frequency)



Doppler OCT: imaging blood flow

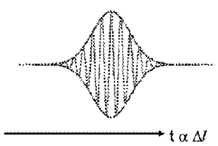




From ..Izatt et al. Opt. Lett. 22 (1997) 1439

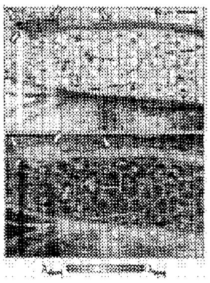
OCT: Spectroscopic imaging

Interferometric OCT signal



Interferometric OCT signal encodes spectral information

⇒ extract using Fourier or wavelet transform

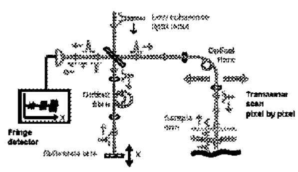


From ..Fujimoto et al. Opt. Lett. 25 (2000) 111

Optical Coherence Tomography (OCT)

Combines confocal scanning with low coherence interferometry
- excellent rejection of scattered light

Single pixel channel acquisition
- high dynamic range detection
- sophisticated lock-in detection and signal processing e.g. to resolve λ, velocity, polarization...



But
- *requirement for spatially coherent source keeps price high if high power and broad bandwidth are needed (e.g. mode-locked lasers, fibre ASE sources..)*
- *sequential pixel scanning limits frame rate – and high frame rates require higher power sources*

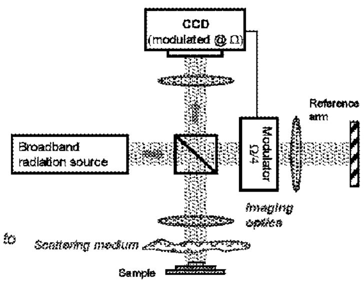
OCT ⇒ wide-field ?

Wide-field coherence-gated imaging using optical heterodyne detection: optical coherence microscopy

Coherent heterodyne detection provides optical sectioning with high speed parallel pixel acquisition

But
- *CCD detector offers reduced dynamic range compared to OCT (more scattered light is detected and detector saturates more easily)*
- *interpixel cross-talk is an issue*
- *temporal modulation requires multiple (4) image acquisitions to extract coherent image (over which sample must be stable)*

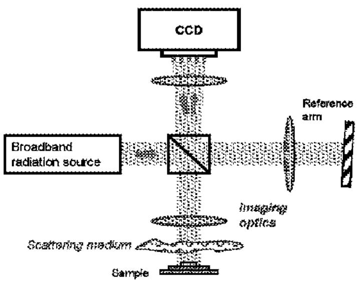
Vabre et al., OL 27 (2002) 530

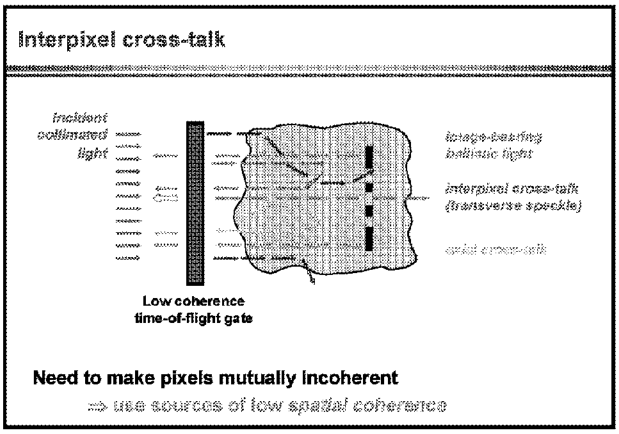


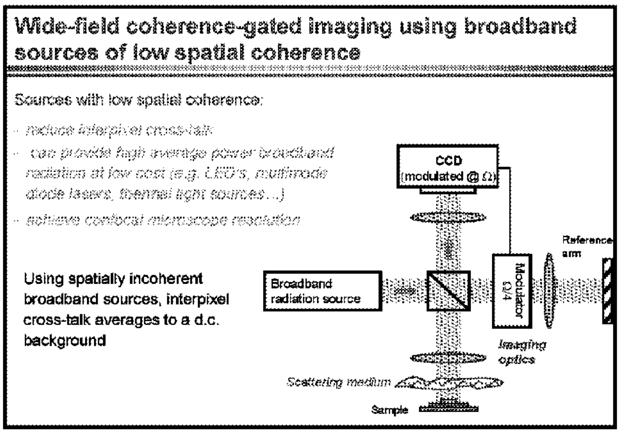
Wide-field coherence-gated imaging using digital (electronic) holography

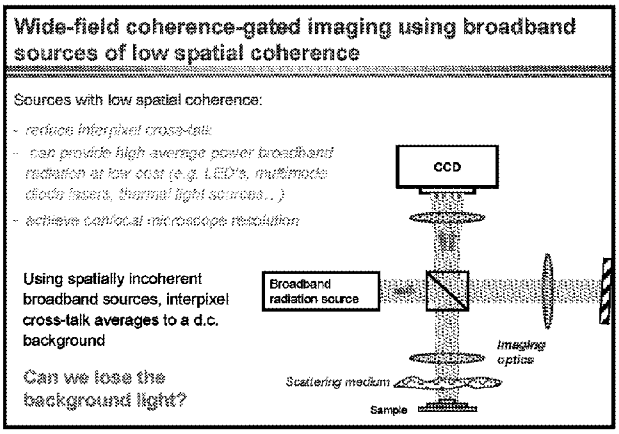
Spatial modulation requires only single image acquisition to extract coherent image with high speed parallel pixel acquisition

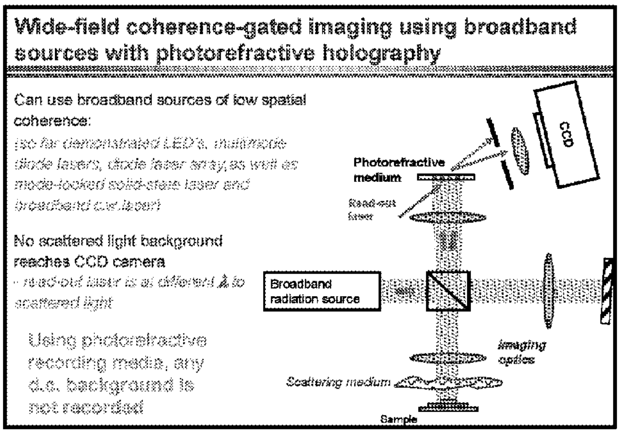
But
- *CCD detector offers reduced dynamic range compared to OCT (more scattered light is detected and detector saturates more easily)*
- *interpixel cross-talk is an issue*

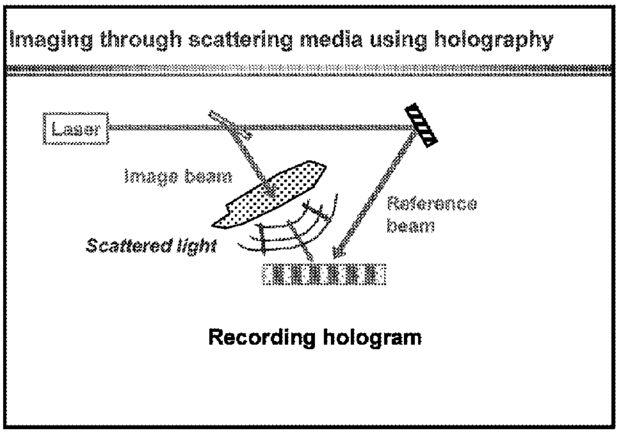


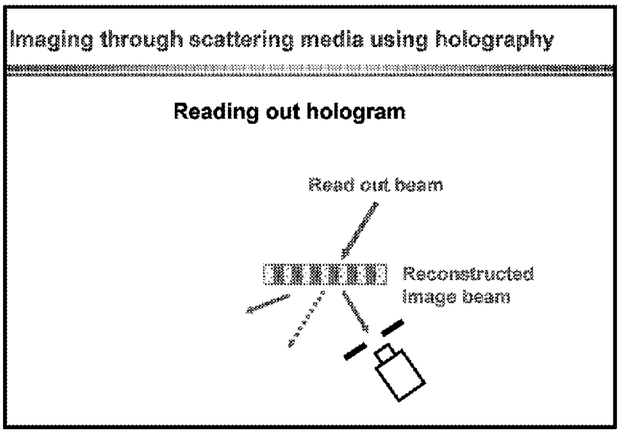

Interpixel cross-talk
Incident collimated light
Image-bearing ballistic light
Interpixel cross-talk (transverse speckle)
axial cross-talk
Low coherence time-of-flight gate
Need to make pixels mutually incoherent
⇒ use sources of low spatial coherence

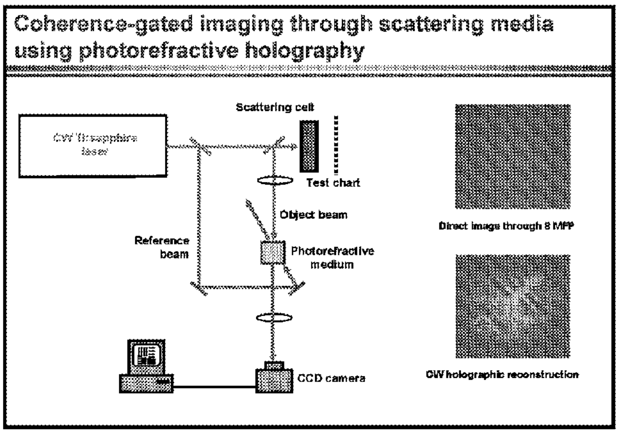

Wide-field coherence-gated imaging using broadband sources of low spatial coherence
Sources with low spatial coherence:
- reduce interpixel cross-talk
- can provide high average power broadband radiation at low cost (e.g. LED's, multimode diode lasers, thermal light sources…)
- achieve confocal microscope resolution
Using spatially incoherent broadband sources, interpixel cross-talk averages to a d.c. background
CCD (modulated @ Ω)
Reference arm
Broadband radiation source
Modulator Ω/4
Imaging optics
Scattering medium
Sample

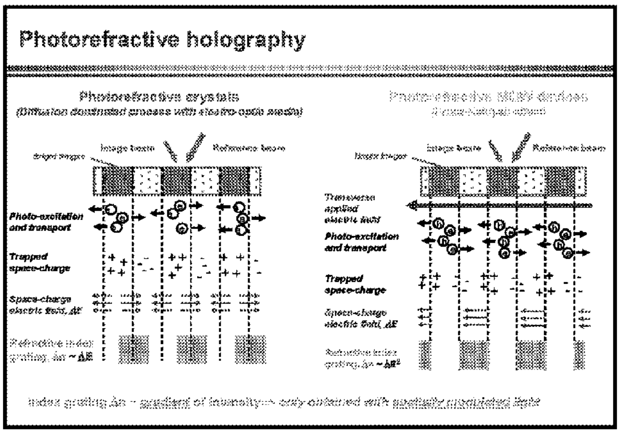

Wide-field coherence-gated imaging using broadband sources of low spatial coherence
Sources with low spatial coherence:
- reduce interpixel cross-talk
- can provide high average power broadband radiation at low cost (e.g. LED's, multimode diode lasers, thermal light sources…)
- achieve confocal microscope resolution
Using spatially incoherent broadband sources, interpixel cross-talk averages to a d.c. background
Can we lose the background light?
CCD
Broadband radiation source
Imaging optics
Scattering medium
Sample

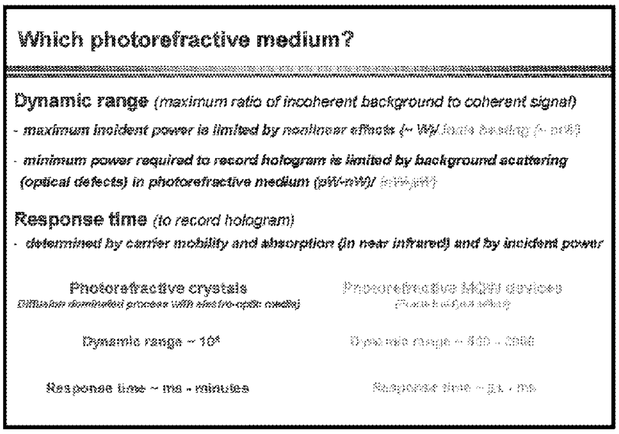

Wide-field coherence-gated imaging using broadband sources with photorefractive holography
Can use broadband sources of low spatial coherence:
(so far demonstrated LED's, multimode diode lasers, diode laser array, as well as mode-locked solid-state laser and broadband c.w. laser)
No scattered light background reaches CCD camera
- read-out laser is at different λ to scattered light
Using photorefractive recording media, any d.c. background is not recorded
CCD
Photorefractive medium
Read-out laser
Broadband radiation source
Imaging optics
Scattering medium
Sample

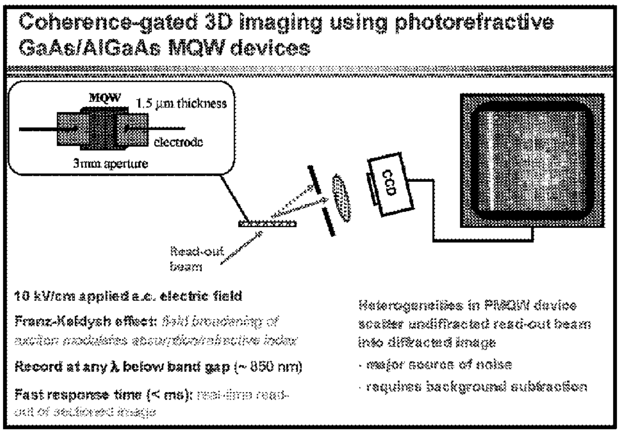

Imaging through scattering media using holography
Laser
Image beam
Reference beam
Scattered light
Recording hologram

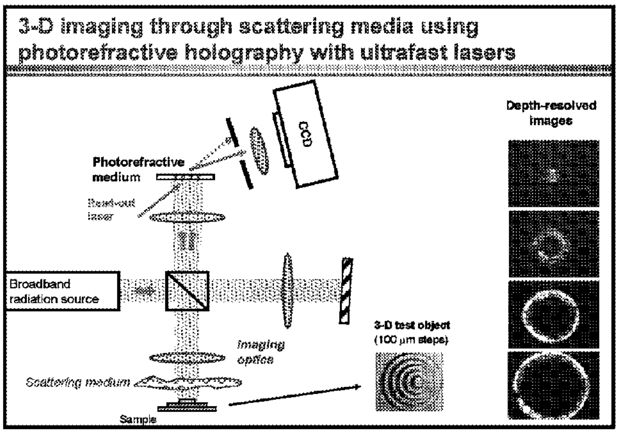

Imaging through scattering media using holography
Reading out hologram
Read out beam
Reconstructed image beam

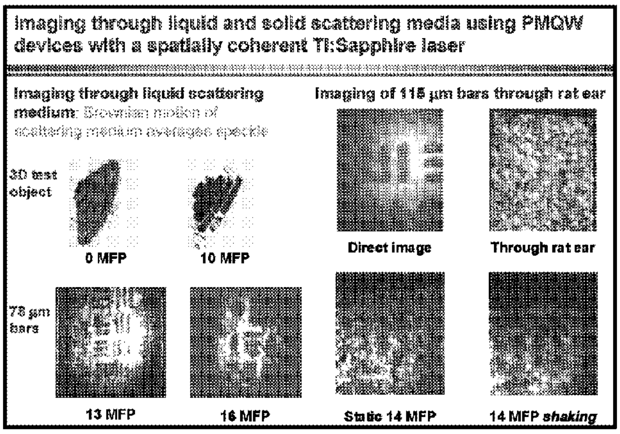

Coherence-gated imaging through scattering media using photorefractive holography
Scattering cell
Test chart
Object beam
Reference beam
Photorefractive medium
CCD camera
Direct image through 8 MFP
CW holographic reconstruction

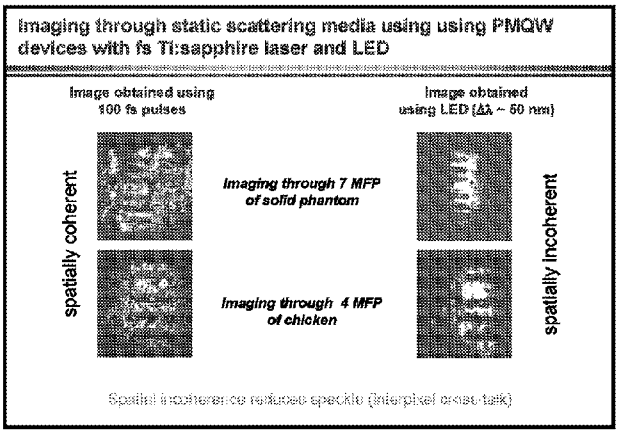

Photorefractive holography
Photorefractive crystals
Photo-excitation and transport
Trapped space-charge
Space-charge electric field, ΔE
Photo-excitation and transport
Trapped space-charge

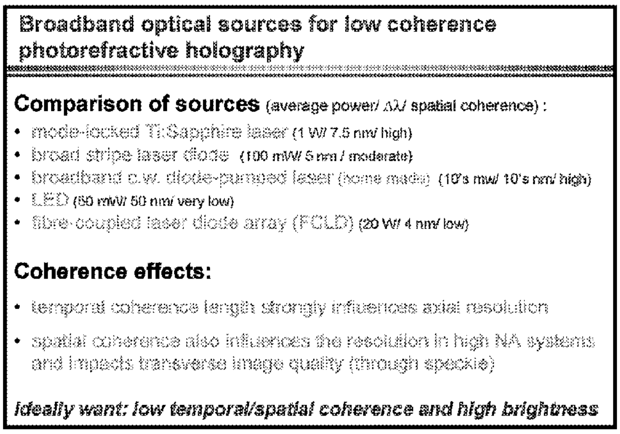

Which photorefractive medium?
Dynamic range (maximum ratio of incoherent background to coherent signal)
- maximum incident power is limited by nonlinear effects (~ W)/
- minimum power required to record hologram is limited by background scattering (optical defects) in photorefractive medium (pW-nW)/
Response time (to record hologram)
- determined by carrier mobility and absorption (in near infrared) and by incident power
Photorefractive crystals
Response time ~ ms - minutes

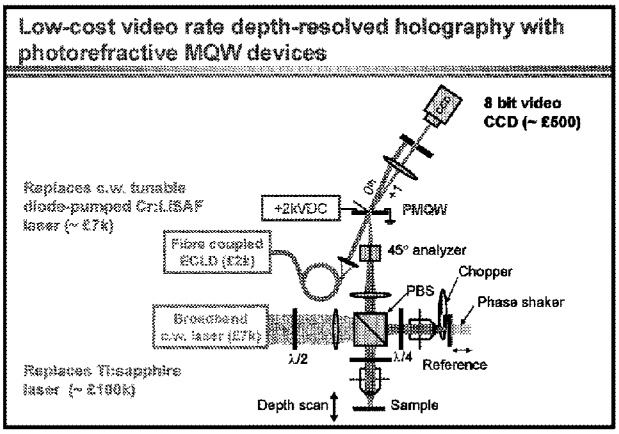

Coherence-gated 3D imaging using photorefractive GaAs/AlGaAs MQW devices
MQW
1.5 μm thickness
electrode
3mm aperture
CCD
10 kV/cm applied a.c. electric field
Franz-Keldysh effect:
Record at any λ below band gap (~ 850 nm)
Fast response time (< ms):

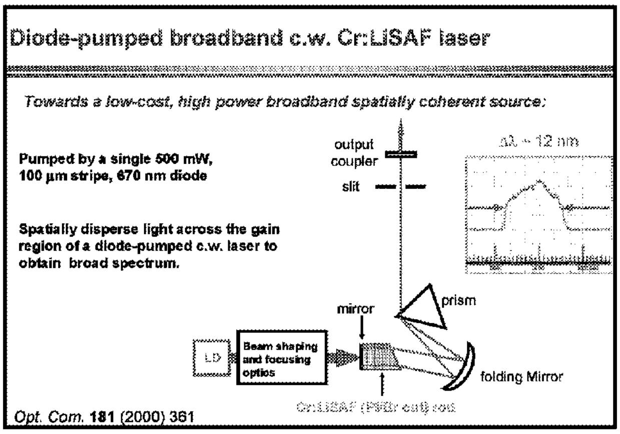

3-D imaging through scattering media using photorefractive holography with ultrafast lasers
CCD
Depth-resolved images
Photorefractive medium
Broadband radiation source
3-D test object (100 μm steps)
Imaging optics
Scattering medium
Sample

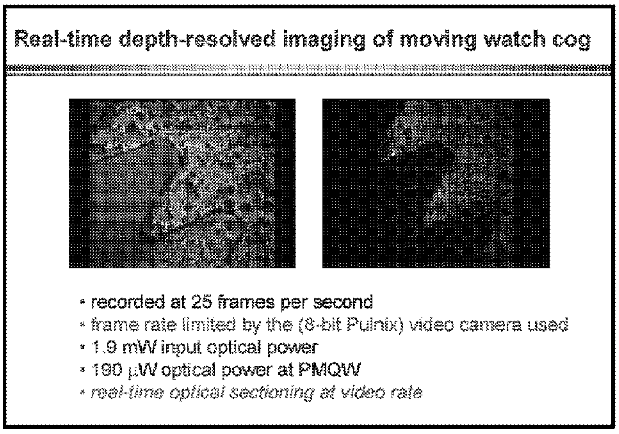

Imaging through liquid and solid scattering media using PMQW devices with a spatially coherent Ti:Sapphire laser
Imaging through liquid scattering medium:
Imaging of 115 μm bars through rat ear
3D test object
0 MFP
10 MFP
Direct image
Through rat ear
13 MFP
16 MFP
Static 14 MFP
14 MFP shaking

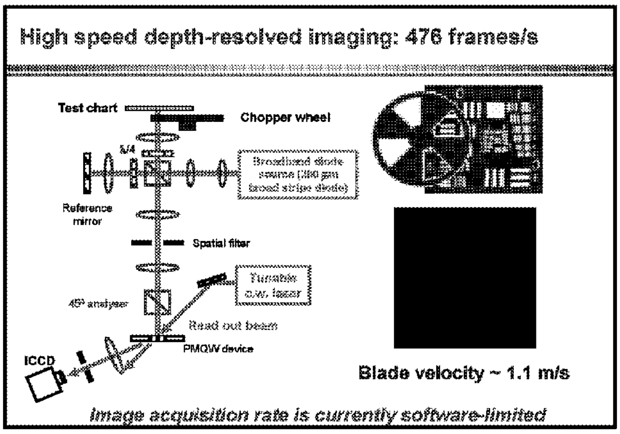

Imaging through static scattering media using using PMQW devices with fs Ti:sapphire laser and LED
Image obtained using 100 fs pulses
Image obtained using LED (Δλ ~ 50 nm)
spatially coherent
Imaging through 7 MFP of solid phantom
Imaging through 4 MFP of chicken
spatially incoherent
Spatial incoherence reduces speckle (interpixel cross-talk)


Broadband optical sources for low coherence photorefractive holography
Comparison of sources (average power/ Δλ/ spatial coherence) :
• mode-locked Ti:Sapphire laser (1 W/ 7.5 nm/ high)
• broad stripe laser diode (100 mW/ 5 nm / moderate)
• broadband c.w. diode-pumped laser (home made) (10's mw/ 10's nm/ high)
• LED (50 mW/ 50 nm/ very low)
• fibre-coupled laser diode array (FCLD) (20 W/ 4 nm/ low)
Coherence effects:
• temporal coherence length strongly influences axial resolution
• spatial coherence also influences the resolution in high NA systems and impacts transverse image quality (through speckle)
Ideally want: low temporal/spatial coherence and high brightness

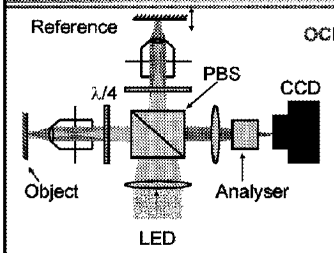

Low-cost video rate depth-resolved holography with photorefractive MQW devices
8 bit video CCD (~ £500)
Replaces c.w. tunable diode-pumped Cr:LiSAF laser (~ £7k)
+2kVDC
PMQW
Fibre coupled ECLD (£2k)
45° analyzer
Chopper
PBS
Phase shaker
Broadband c.w. laser (£7k)
λ/2
λ/4
Reference
Replaces Ti:sapphire laser (~ £100k)
Depth scan
Sample


Diode-pumped broadband c.w. Cr:LiSAF laser
Towards a low-cost, high power broadband spatially coherent source:
Pumped by a single 500 mW, 100 µm stripe, 670 nm diode
Spatially disperse light across the gain region of a diode-pumped c.w. laser to obtain broad spectrum.
output coupler
slit
Δλ ~ 12 nm
prism
mirror
LD
Beam shaping and focusing optics
folding Mirror
Opt. Com. 181 (2000) 361


Real-time depth-resolved imaging of moving watch cog
• recorded at 25 frames per second
• frame rate limited by the (8-bit Pulnix) video camera used
• 1.9 mW input optical power
• 190 µW optical power at PMQW
• real-time optical sectioning at video rate


High speed depth-resolved imaging: 476 frames/s
Test chart
Chopper wheel
λ/4
Reference mirror
Spatial filter
Tunable c.w. laser
45° analyser
Read out beam
PMQW device
ICCD
Blade velocity ~ 1.1 m/s
Image acquisition rate is currently software-limited

High speed wide-field coherence-gated imaging using optical heterodyne detection: Single-shot OCM



OCM: phase-stepping holography

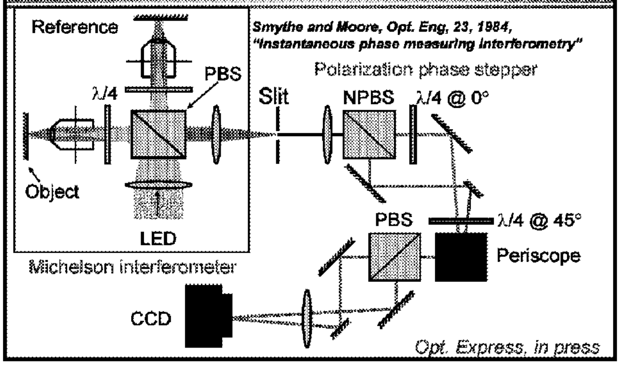
Usually acquire a series of (typically 4) images sequentially, corresponding to different positions of the reference arm.

Single-shot OCM

Acquire 4 phase-stepped images simultaneously

Four channel polarization phase stepper

Smythe and Moore, Opt. Eng, 23, 1984, "Instantaneous phase measuring interferometry"

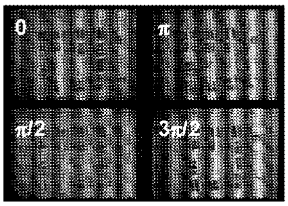


Opt. Express, in press

Modified phase-stepping algorithm

Standard 4 phase algorithm

$$S=\sqrt{(I_{3\pi/2}-I_{\pi/2})^2+(I_\pi-I_0)^2}$$

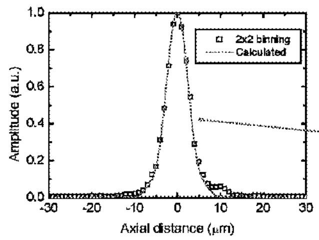


Modified 4 phase algorithm

$$S\propto\sqrt{\frac{1}{L_1^2}\left(\frac{I_{3\pi/2}}{A_{3\pi/2}}-\frac{I_{\pi/2}}{A_{\pi/2}}-K_1\right)^2+\frac{1}{L_2^2}\left(\frac{I_\pi}{A_\pi}-\frac{I_0}{A_0}-K_2\right)^2}$$

LED axial sectioning

$\Delta\lambda_{FWHM}$ = 44 nm

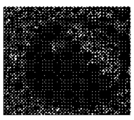


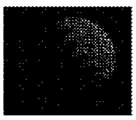
Δz_{FWHM} = 6.2 μm

curve calculated from measured LED spectrum

Results: moving watch cog

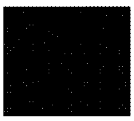
Conventional image

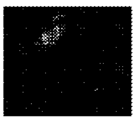




z = 0.55 mm

z = 0.97 mm



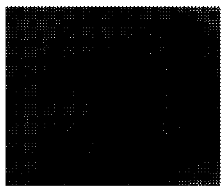


z = 2.54 mm

Hardware 2×2 binning, 16.5 frames per second, Exposure time = 1 ms, FOV = 1.8×1.7 mm, Optical power = 0.4 mW.

Results: sample of onion

x-y slice



FOV = 270×250 μm

x-z slice

FOV = 270×210 μm

2×2 software binning, 8 frames per second, Exposure time = 3.6 ms, Optical power = 1.5 mW.

Further reading

http://www.imperial.ac.uk/research/phot

1. *Depth-Resolved Holographic Imaging through Scattering Media using Photorefraction*
S. C. W. Hyde, N. P. Barry, R. Jones, J. C. Dainty, P. M. W. French, M. B. Klein and B. A. Wechsler, Opt Lett, 20 (1995) 1331
2. *Real-time 3-D imaging through turbid media with ballistic light using time-gated holography*,
S. C. W. Hyde, N. P. Barry, R. Jones, J. C. Dainty, P. M. W. French, K. M. Kwolek, D. D. Nolte and M. R. Melloch, IEEE JSTQE Special Issue on Lasers in Medicine and Biology, 2 (1996) 965-975
3. *Direct-to-video holographic read-out in quantum wells for 3-D imaging through turbid media*,
R. Jones, N. P. Barry, S. C. W. Hyde, P. M. W. French, K. M. Kwolek, D. D. Nolte and M. R. Melloch, Opt Lett, 23 (1998) 103-105
4. *Biomedical Optics in the 21st Century*, P. M. W. French, Physics World, (June 1999) 41-46
5. *High frame-rate, 3 D Photorefractive Holography through turbid media with arbitrary sources, and Photorefractive Structured Illumination*
Z. Ansari, Y. Gu, J. Siegel, D. Parsons-Karavassilis, C. Dunsby, M. Itoh, M. Tziraki, R. Jones and P. M. W. French, D. D. Nolte, W. Headley and M. R. Melloch,
IEEE JSTQE Special Issue on Lasers In Medicine and Biology, 7 (2001) 878-887
6. *Single-shot phase-stepped wide-field coherence-gated imaging*, C. Dunsby, Y. Gu, P. M. W. French,
Opt. Express, 11 (2003) 105-115