

Guidelines For Preparation and Sending BIOPTONICS Specimens

General information on Specimens

Specimens with a diameter of 1-10mm and length of up to 15mm can be processed.

All excess membranes should be removed before shipping, as BIOPTONICS will do no further preparation of your specimen prior to OPT scanning. Excess membranes interfere with scanning which results in poor quality movies.

Unstained Specimens

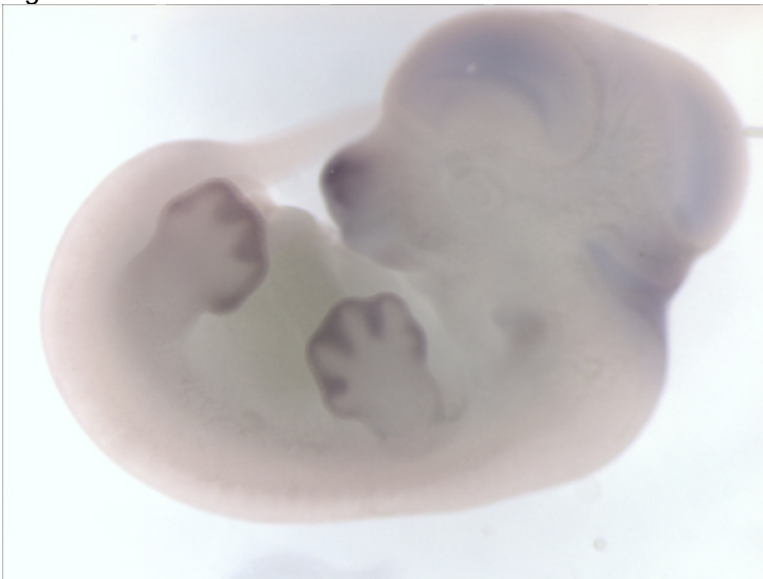
These can be scanned to show anatomy and morphology if there is sufficient autofluorescence in the specimen.

Coloured Specimens

DAB stained specimens should be pale brown **not** dark brown/black. Specimens, which are too darkly stained, cannot be scanned.

NBT/BCIP staining should not be overly dark, see attached photo (Fig 1) of a suitable stain intensity. Non-specific background signal should be as low as possible. NBT/BCIP stain is soluble in BABB which is used in OPT process; therefore very pale staining may disappear before the specimen can be scanned. We have found that an NBT final concentration of **10-45ug/ml** is optimum for OPT. Higher concentrations can lead to background colour developing during scanning process. This can result in poor quality movies. It is **IMPERATIVE** to wash specimen thoroughly after stopping NBT/BCIP reaction. We recommend washing in large volumes of PBS (50mls) with agitation, for 48hours, changing PBS at least 4 times before fixing in 4% PFA.

Figure 1

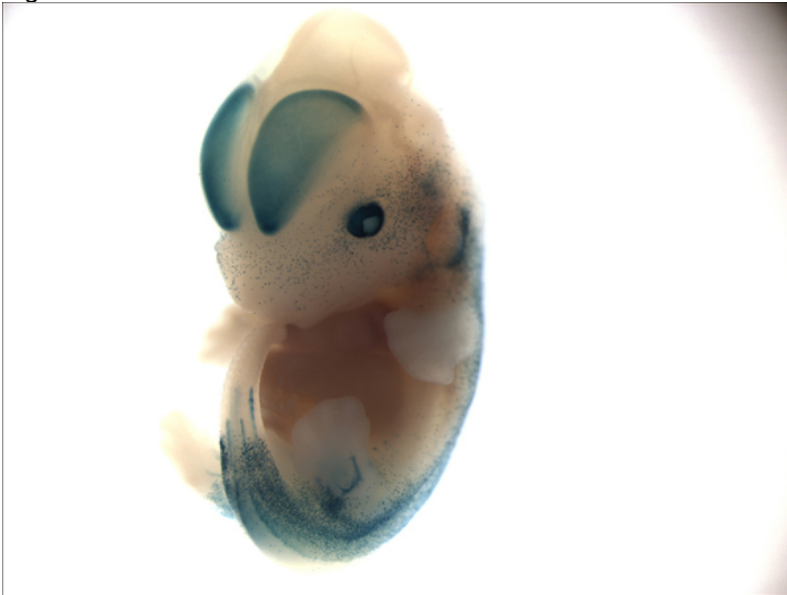


X-Gal staining should be relatively dark. It is very soluble in BABB (more so than NBT/BCIP) therefore very pale staining will disappear before specimen can be scanned.

X-Gal/NBT Addition of NBT to X-Gal reaction stabilises the X-gal signal making it less soluble in BABB. Replace iron in X-Gal stain buffer with NBT final concentration of **50-100ug/ml**. For the best OPT results, the resulting stain intensity should be similar to that shown in Fig 2. Note that addition of NBT will produce a different shade of blue to that obtained with X-Gal alone.

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Figure 2



It is **IMPERATIVE** to wash specimen thoroughly after stopping X-Gal-NBT reaction. We recommend washing in large volumes of PBS (50mls) with agitation, for 48hours, changing PBS at least 4 times before fixing in 4% PFA.

It is possible to scan X-Gal or NBT/BCIP-stained specimens, which have subsequently been fluorescently labelled but not possible to scan specimens stained with more than one visible or coloured stain.

Fluorescent Specimens

OPT can be used for imaging fluorescent dyes, for example in Fluorescent Immunohistochemistry or Fluorescent wholemount *in situ* hybridisation.

GFP expressing specimens cannot be directly viewed using OPT due to the pre-scanning processing required. Specimens immuno stained using fluorescently tagged antibodies against GFP can be scanned with OPT. Please contact us for protocols.

Fluorescent Antibodies. Either single or double-labelled specimens can be scanned. We currently use GFP2 and CY3 filters for scanning signal see table for filter properties. Other filters may be available on request. N.B. Cy3 signal will bleed through into GFP2 channel; it will not be distinguishable from GFP2 signal (e.g. Alexafluor 488) in section & 3D movies.

Tyramide Specimens should have as low background signal as possible. Signal may fade when exposed to light during OPT scanning. GFP2 and CY3 filters are used for scanning

Post-Staining

Specimens should be fixed in 4% PFA overnight. Wash well in PBS, before shipping.

Specimen Info

Please complete all the details on attached form & send with the specimens.

Shipping

Specimens should be shipped in 2ml cryotubes, or similar. Please ensure there is only 1 specimen per tube. Fill to brim with PBS. There should be no air bubbles, as this can lead to specimens being damaged in transit. Label each tube clearly with your unique ID of up to 12 letters & numbers (starting with a letter) Tubes should be sealed with parafilm then wrapped in absorbent material, sufficient to absorb any leakage. Fluorescent & NBT/BCIP specimens should be sealed in a light-tight container.

Ensure all packaging is compliant with IATA DGR packing instruction 650, and that UN3373 label is clearly displayed. Contact your courier company for further details.

Please inform us by email info@bioptonics.com when specimens are posted.

Guidelines For Preparation and Sending BIOPTONICS Specimens

Shipping from outside the UK

Please contact us in advance of shipping your specimens with the details of the species and country of origin. We will then send you the appropriate import authorisation document.

It is **vital** that all the conditions attached to the import authorisation are met, these may vary between authorisations.

Carefully read the conditions and attach all the required documents and information as detailed the authorisation we send you.

Failure to do so will result in the confiscation of your samples by customs authorities.

Payment

A Purchase Order **MUST** be sent with specimens. Contact Kevin Smith on +44 (0) 131 311 7029 Kevin.Smith@tech.mrc.ac.uk with any queries regarding purchase orders.

SUBMISSION OF PURCHASE ORDER INDICATES ACCEPTANCE OF MRC TECHNOLOGY'S STANDARD TERMS AND CONDITIONS.

If VAT exempt, please provide a copy of your certificate from Customs and Excise

Receipt of Specimens

All specimens will be reviewed for suitability.

Any damaged specimens will be reported via email before scanning commences.

Scanning Process

Day 1

Wash sample in PBS.

Embed sample in a deep petri dish containing 1% LMP agarose.

Trim excess agarose so that sample is suspended vertically in the centre.

Attach the agarose block with super glue to a metal mount.

Trim agarose to form an octagon with each face angled outwards.

Start methanol dehydration.

Day 2

Dehydrate in methanol with 2-3 changes. Clear sample in Benzyl Alcohol : Benzyl Benzoate (Murray's Clear) overnight. Large samples may require 24+ hours to clear completely.

Day 3

OPT scan

Day 3 - 7

Process dataset through Bioptonics pipeline to produce an OPT Imaging Package.

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Results

An **OPT Imaging Package** is the Product resulting from the OPT imaging of one Specimen.

Each **Stained Specimen OPT Imaging Package** consists of:

- Series of virtual sections through the specimen in 3 orientations. These include 1 monochrome series for each channel (stacks of png files [for sagittal orientation only] and an mpeg movie for each orientation) and a colour series for the merged channels (stacks of png files and an mpeg movie for each orientation).
- 3D movies and their associated png files. These are colour movies of merged data. These will be based on our experience and expertise in 3D visualisation of biological specimens to provide a series of high-quality movies. Movies will consist of standard trajectories and will include surface rendering, volume rendering and cut-aways. 12 movies will be provided for each specimen, along with their associated png files.
- Original Projection Images. The original projection images for each channel are supplied as a TIFF stack and in mpeg format.

Each **Anatomy Only OPT Imaging Package** consists of:

- Series of virtual sections through the specimen in 3 orientations. These include merged monochrome series (stacks of png files and an mpeg movie for each orientation) and unmerged monochrome series (stacks of png files [for sagittal orientation only] and an mpeg movie for each orientation)
- 3D movies and their associated png files. These are monochrome movies of merged data. These will be based on our experience and expertise in 3D visualisation of biological specimens to provide a series of high-quality movies. Movies will consist of standard trajectories and will include surface rendering and cut-aways. At least 5 movies will be provided for each specimen, along with their associated png files.
- Original Projection Image. The original projection image is supplied as a TIFF stack and in mpeg format.

Return of Specimens

Specimens are not normally returned.

Address to send Specimens:

BIOPTONICS
MRC Technology
Crewe Road South
Edinburgh EH4 2SP
United Kingdom

Tel: +44 (0)131 311 7029

Fax: +44 (0)131 311 7025

email: info@bioptonics.com

Website: www.bioptonics.com

Please fill in, print out and return hard copy with your Specimens:

Contact Name For specimen queries	
Address	
Email address	
Telephone Number	

Contact Name For sending CD's (If different from above)	
Address	
Email address	
Telephone Number	

Purchase Order No.		Total Cost	
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Any Additional Relevant Information-
