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Assessing Breast Cancer Margin Using an Aqueous Quantum Dot (AQDs) Enabled Molecular Probe

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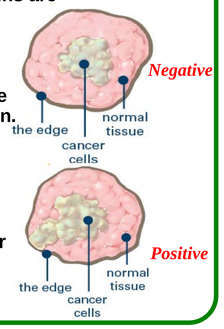
Motivation

- Breast cancer is one of the most common cancers in women, about 30% of all cancers in women. In 2012, there is an estimate of 226,870 new invasive breast cancer cases and 63,300 new ductal carcinomas *in situ* (DCIS) cases.
- Lumpectomy or breast-conserving surgery, in which only the tumor and surrounding tissue are removed, is the most common treatment for breast cancer today. Pathology examination is done following l umpectomy to ensure the margins are free of cancer cells.
- However, about 15-60% of lumpectomy surgeries require a second surgery or re-excision due to unclean margin.
- It would be desirable to have an imaging tool to help surgeons decide if the margin is clean during surgery to reduce re-excision rate, morbidity associated with incomplete resection.



Objective

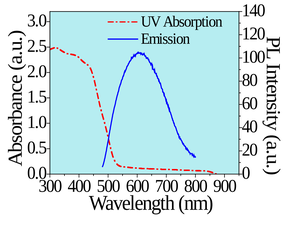
We investigated *aqueous quantum dots (AQDs) conjugated with cancer markers antibodies to image cancer cells in excised tissues*. The ultimate goal is to further develop this technology to help surgeons assess tumor margin during surgery.

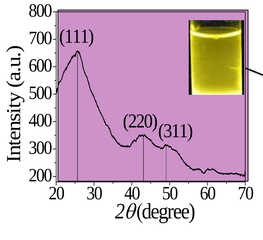
Competitive Matrix

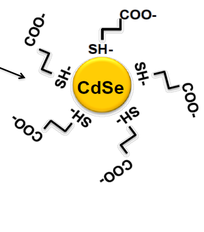
Technology	Entire surface	Molecular specificity	Effective with Heterogeneous Tissue	< 1 mm spots	No Need Pathologist
Touch-prep	Yes	No	Yes	No	No
Frozen section	No	No	Yes	No	No
Duke University[1]	Yes	No	No	No	Yes
MarginProbe[2]	No	No	No	No	Yes
AQD Technology	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**

Aqueous Quantum Dots

- Quantum Dots are semiconducting nanocrystals that exhibit bright photoluminescence (PL) and better photo-stability than fluorescent molecules, ideal for *in vivo* imaging of diseased tissues or monitoring biological process.
- Current organic QDs often are expensive and their synthesis involves environmentally hazardous organic solvent tri-n-octylphosphine oxide (TOPO). Also, ligands exchange are required to make the QDs water soluble.

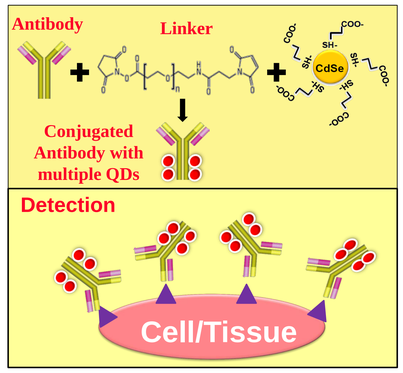


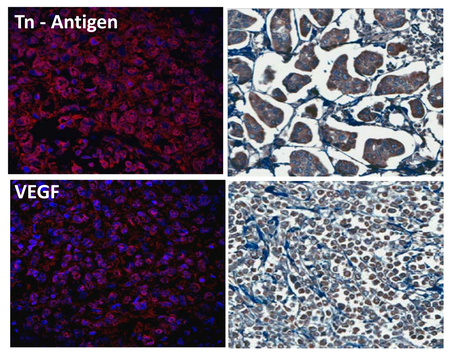




On the other hand, aqueous QDs have been synthesized using 3-mercaptopropionic acid (MPA) without TOPO in one single step. This synthesis route has produced highly luminescent QDs.

Breast Tissue Imaging

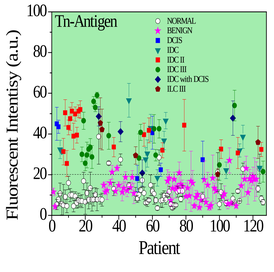


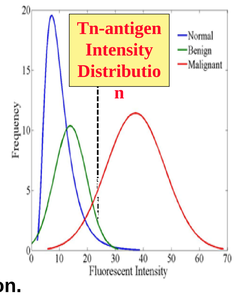


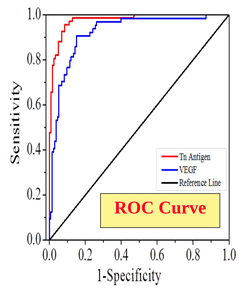
QD Staining *IHC Staining*

- AQDs were conjugated directly to antibodies (Ab) for cancer detection
- Aqueous QDs can be conjugated with antibodies effectively.
- *Cost effective method : 1¢ for AQDs compared to $1.20 for commercial QDs*
- Excised breast tissues were paraffin-embedded and cut in 5 µm sections
- Tn-antigen and Vascular Endothelial Growth Factor (VEGF) could be detected at single cancer cell level using QD-labeled antibodies with better sensitivity and specificity than immunohistochemical (IHC) staining.

94% Sensitivity and 92%Specificity for Cancer Detection





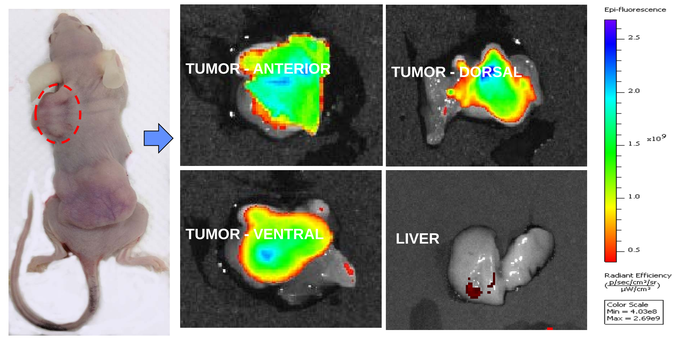


- Fluorescent signal quantification.
- Cut-off point is 20 pixel for non-cancerous cells.
- The dash line indicates the threshold intensity for a cell to be identified as a cancer cell for the statistics.
- A total of 483 paraffin-embedded tissue blocks from 126 patients were examined.
- Statistics of 126 cases for both QD conjugated antibodies and horseradish peroxidase (HRP) conjugated antibodies using VEGF and Tn-antigen as markers.

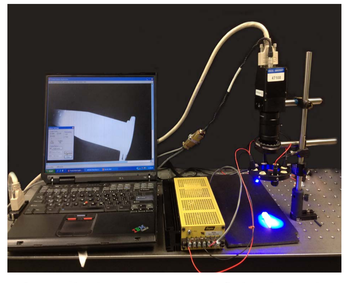
	VEGF-HRP	VEGF-QD	Tn-HRP	Tn-QD
Sensitivity	92%	94%	90%	94%
Specificity	71%	77%	86%	92%

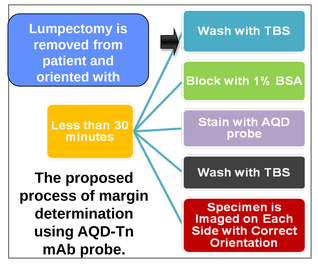
- *QD staining resulted in better sensitivity and specificity than IHC for both Tn-antigen and VEGF.*
- Tn-antigen is a sutiable marker for cancer detection

Cancer Imaging In Excised Tumor And Organ



- Nude mice were xenografted with HT29 colon cancer cells.
- Tumors were harvested and stained with AQDs for Tn-antigen expression.
- Different view of the tumors were imaged with dorsal vand ventral side showing no staining for muscle tissue.
- Liver was used as a negative control.
- Total procedure time < 30 minutes, no interference with standard pathological examinations.





- A preliminary set-up of the imaging system.
- A ring of LEDs shines on the specimens (a tube of AQD suspension) and the fluorescence of AQDs was recorded by a NIR CCD camera and shown on computer screen.

Summary

- AQDs can be conjugated with primary and secondary antibodies to image caner cells at a single cell level with strong fluorescent signals.
- Tn-antigen is a good cancer marker with 94% sensitivity and 92% specificity for cancer detection using QD staining.
- Tn antigen was clearly imaged on human tumors harvested in mice
- Further inference study is needed to establish the protocol.

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Coulter-Drexel Translational Research Partnership Program