Dr Pawel Gaj, post-doc researcher at the Department of Immunology Medical University of Warsaw, participated in the Twinning Programme of the BASTION project held between 3rd and 17th of July. The activities were conducted at the Conway Institute, University College Dublin (UCD). During his stay at UCD he joined the research group of the School of Biomolecular & Biomedical Science led by Professor William Gallagher. The main purpose of Dr Gaj's visit in UCD was to get familiar with two alternative imaging systems used for acquiring images of immunohistochemically stained tissue samples. Importantly, he has had an opportunity to scan IHC slides stained in Poland, and perform basic measurements of staining intensities using color-deconvoluted channels representing individual components of tissue staining image.

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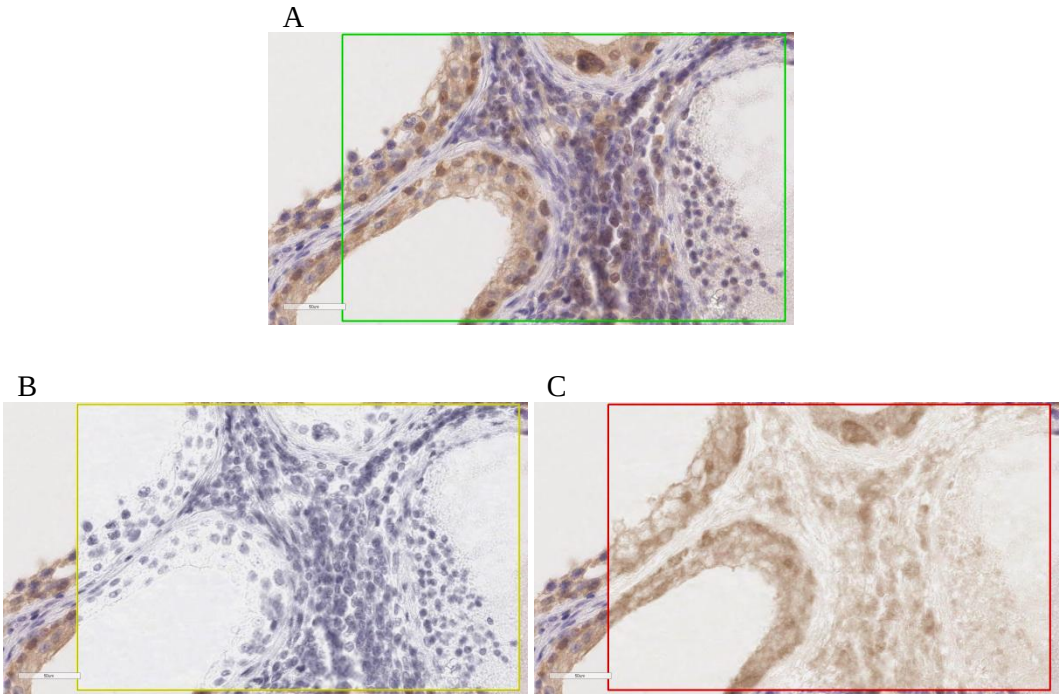
Prior to performing the *Color Deconvolution* analysis of an IHC stained image it is necessary to create a vector of values characteristic for each staining color in the form of three values representing Red, Green and Blue components of the color. Since the IHC staining procedures are difficult for a precise control; often the colors representing the nuclear (Hematoxyline) and specific antibody (DAB) staining can differ between the tissues stained in different batches or even between tissues stained simultaneously as the final staining can also depend on the way a tissue has been acquired, fixed, cut and stored prior to the IHC staining. This is why it is necessary to precisely define RGB vectors for each stained tissue image and for each color channel subjected for the *Color Deconvolution* analysis. During his stay at UCD Dr Gaj has measured the RGB values of both Hematoxylin and DAB channels for each of the 25 IHC slides stained in Poland. Each staining color for each slide has been measured picking at least three regions from each of the slides. Finally, mean vectors of each staining color was drawn from the measurements of each of the individual slides.

Table 1 Nuclear staining using Hematoxylin (blue) and Diaminobenzidine DAB (brown).

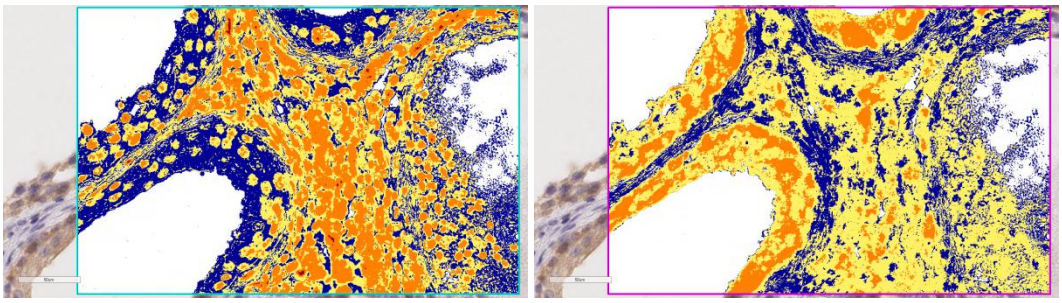
Slide ID	**Negative nuclear staining (blue)**			**Antibody specific staining (brown)**		
	Red	**Green**	**Blue**	**Red**	**Green**	**Blue**
106B_13	0.576	0.667	0.472	0.365	0.562	0.742
10816_12	0.590	0.623	0.514	0.331	0.553	0.765
12_5127	0.587	0.666	0.461	0.331	0.560	0.760
123_13	0.553	0.644	0.529	0.333	0.557	0.761
13890_12	0.604	0.642	0.472	0.357	0.555	0.752
1391_13	0.655	0.648	0.390	0.398	0.593	0.700
13962	0.592	0.692	0.413	0.388	0.586	0.712
14213_12	0.604	0.627	0.493	0.341	0.563	0.752
14550_12	0.630	0.644	0.434	0.324	0.550	0.769
14757	0.640	0.679	0.360	0.322	0.554	0.768
1593_13	0.645	0.676	0.357	0.304	0.549	0.779

17575_12	NA	NA	NA	NA	NA	NA
2314_13	0.636	0.655	0.407	0.401	0.579	0.710
251_13	NA	NA	NA	NA	NA	NA
2740_13	0.656	0.656	0.373	0.335	0.546	0.768
33_13	0.653	0.662	0.368	0.306	0.543	0.782
3361_13	0.601	0.668	0.439	0.292	0.548	0.784
382_13	NA	NA	NA	NA	NA	NA
3840_13	0.582	0.635	0.508	NA	NA	NA
38650-9	NA	NA	NA	NA	NA	NA
458_13	0.605	0.670	0.430	0.362	0.580	0.730
489_13	0.642	0.652	0.405	0.397	0.594	0.700
7294_13	0.584	0.679	0.444	0.429	0.602	0.673
878_13	0.608	0.668	0.428	0.349	0.550	0.759
917_13	0.589	0.649	0.481	0.320	0.553	0.769
Average	**0.611**	**0.657**	**0.437**	**0.349**	**0.564**	**0.747**

Figure 1 Example of the *Color Deconvolution* analysis done using the Aperio ScanScope System®. A: Source IHC stained image B: Deconvoluted nuclear staining - Hematoxylin (blue); C: PRDX1 antibody specific DAB staining; D: visual representation of nuclear staining intensity (blue); E: visual representation of the DAB staining intensity (brown).



D E



Both image scanning systems available at UCD manufactured by either Aperio, or 3DHISTECH (Figure 2) come with a suite of software applications and algorithms. These algorithms allow not only for analysis of either staining intensities within whole sections of the tissue but also facilitate different types of targeted analysis designed for analysis of *Nuclear* staining, *Membrane* staining as well as *Colocalization* of the staining signal. However as opposed to the *Color Deconvolution* other algorithms seem to be rather demanding in terms of the tissue and staining quality and therefore may not be easy for implementation on a large scale.

Figure 2 High resolution IHC scanners. Aperio's ScanScope System® (A) and 3DHISTECH's Pannoramic 250® (B).

A B

Both manufacturers have also implemented web-based interfaces for remote annotations of regions of the stained tissues. This feature could be potentially very useful considering the fact that large amounts of data produced by the scanner are difficult to transfer between different medical facilities and should be stored

on a central storage device. Implementation of web-based tools would be very helpful for remote collaboration between the research group of WUM and histopathology specialists from other centers.

Collaboration between Dr Gaj and researchers from the Conway Institute was initiated prior to his visit in UCD. It concerned identification of different types markers, both mRNA and proteomic, involved in differential molecular and clinical characterization of patients diagnosed with ER positive breast cancer. During Dr Pawel Gaj's visit at UCD Conway Institute not only results of statistical analyses prepared by Dr Gaj were presented and discussed, but also follow-up research directions were proposed.