*Biological Sciences*

# Touch imprint cytology of leaves (Vigna radiata) with and without fine iron particles and Prussian Blue staining

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**Touch Imprint Cytology, in which tissues are applied to glass slides and stained, has become a useful method for the examination of various types of tumors that need to be studied and diagnosed peri-operatively. In the present report, we used touch imprinting applied to leaves from the common Mung bean plant (Vigna radiata).Isolated leaves (n=100) were immersed in nano-sized iron particle solutions for 1 hour and the placed between 2 glass slide containing a small amount of aliquots of the iron particle solution plus a specific iron stain Prussian Blue. For comparison, leaves were immersed in deionized water and "sandwiched" between glass slides containing the same medium (n=10). After 12- 24 hour the tissues were peeled off the glass surfaces and examined by optical microscopy. The experimental set, consistently showed outlines of cell types specific for the epidermal and underside of leaves. Specifically, guard cell surrounding stoma were prevalent on the underside of the leaf, whereas trichomes and cell walls of epidermal cells were noted on the upper surface. The control set showed only imprints of the leaf edges. Using iron particles and iron staining solutions, touch imprints of several cell types found on the two leaf surfaces of the Mung bean plant were imaged. Evidence was presented that the lignin in plant cell walls have a strong affinity for iron and that inherent electromagnetic energy derived from metabolism may also contribute to the attraction of iron particles to imaging leaf cytology.** *Journal of Nature and Science, 1(1):e32, 2015.*

Touch imprint cytology | iron particles | Prussian Blue

Touch imprint cytology (TIC) is a well known pathological technique, which is used to provide a rapid intraoperative diagnosis of biopsied tissues. TIC has been used extensively as an intraoperative diagnostic method for parathyroid tissue, sentinel lymph nodes in malignant melanoma, and intraoperative evaluation of brain tumors [1-3]. To date, controversial reports are available about the usefulness of touch imprint cytology (TIC) in the diagnosis of these lesions [4].

In the present report, we used touch imprinting applied to leaves from the common mung bean plant (Vigna radiata). We compared the images obtained after leaves were immersed in a solution containing a mixture of Prussian Blue stain (PBS) and iron (Fe3+) nano- sized particles (average diameter, 2000 nM, experimental set); and when leaves were immersed in deionized water for 1 hour.

## Methods

### Preparation of Iron containing Solution

An iron nanoparticle solution (250 cc) was prepared by mixing several grams of powdered iron filings (Edmond Scientific, Co,Tonawanda, NY) in deionized water (resistivity, 18.2 MΩ.cm). After standing for several hours aliquots of the supernatant were subjected to homogenization (Brinkmann Instruments Co., Sybron Corporation Westbury, NY) for 2 min. These samples were centrifuged for 20-25 min and the upper third of the supernatant was carefully decanted for sizing of the iron nanoparticles.

The particle size and distribution of the nanoparticles were determined using dynamic light scattering (DLS) and the zeta potential of the same preparations were measured using phase analysis light scattering by a Zeta potential analyzer (ZetaPALS, Brookhaven Instruments Corp, Holtsville, NY). For sizing, 1.5 ml of the iron particle solution (de-ionized water, 18.2 MΩ cm, resistivity) was scanned at 25 °C and the values obtained as nM. A similar aliquot of the iron particle solution was scanned for 25 runs at 25 °C. for determining zeta potentials. Zeta potential values were displayed as millivolts (mV).

### Imaging Procedures

### Protocol 1. Direct application of Iron containing solutions to Leaves

Seeds of the Genus and species, Vigna radiata were germinated in tap water for 2 weeks. The first set of mature green leaves (first pair, n=10)) were carefully cut from the plants. and transferred to clean glass slides. Using a transfer pipette, several drops of a solution containing aliquots of the iron containing solution (particle size range, 180-215 nM) and Prussian Blue staining solution (PBs, 2.5% potassium ferrocyanide, 2.5% hydrochloric acid) was added to the two leaves on the slide. One leaf was placed with the epidermal surface in contact with the glass slide and the other leaf with the underside in contact with the glass slide. A second glass slide was applied to hold the leaves in place.

### Protocol 2. Controls

A second set of two leaves (n=10) was prepared by immersing the leaves in deionized water and some of the bathing solution was used to serve as the medium applied between the slides containing the leaf pairs.

After 48 hours or when the liquid between the slides had dried the specimens were reviewed microscopically and photomicrographs made, before and after the leaves were carefully lifted away from the under slide surface.

## Results

Figure 1 shows a typical section of the underside of a leaf treated with a combined solution containing the iron particles and Prussian Blue stain. The vertical arrow points to the hair cell, known as a trichome, which are prevalent along the leaf edge of the Mung bean plant. Note the very dark staining of the interior trichome and the leaf edges from which they extend. Also, note the dark particles at the leaf edges and the double outlines of each of the trichomes. On the other hand, the interior of the leaf is populated by cells showing the characteristic morphology associated with stomata (horizontal arrows).Stomata are cell composed of two guard cells surrounding an opening, viz., stoma. Their function is to regulate oxygen and carbon dioxide exchange.

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Conflict of interest: No conflicts declared.

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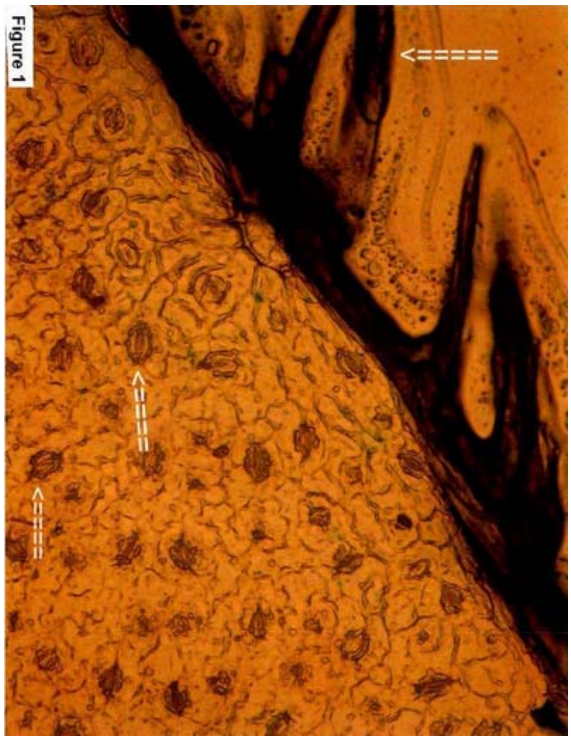


Figure 1. Touch Imprint Cytology image of the underside of the leaf of the Mung bean plant. The leaf was pretreated with a solution containing aliquots of iron particle solution and Prussian Blue Stain. The vertical arrow points to the hair cell, known as a trichome, which are prevalent along the leaf edge. The interior of the underside of the leaf is populated by cells showing the characteristic morphology associated with stomata (horizontal arrows). Magnification 40X

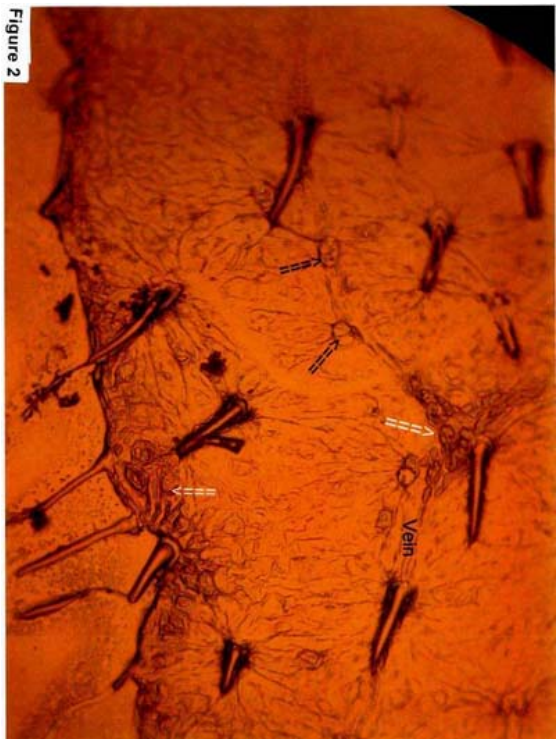


Figure 2. The leaf was treated as described in Figure 1. The epidermal surface shows darkly outlined trichomes at the leaf edge and the surrounding aggregated iron particles. The interior is characterized by numerous trichomes and their basal epidermal cells (white arrows) as well unidentified cell types (black arrows). Also note the cell wall outlines of pavement cells that comprise most of the epidermal surface. Magnification 40X

Figure 2 illustrates the images we found when removing the treated leaf as above but when the epidermal surface of the leaf was in contact with the underneath slide. Again the outlined trichomes are surrounded by dark particles; whereas within the interior there are numerous well delineated trichomes including those associated with veins. Also some unknown types of cells were seen scattered in the interior (arrows) as well as along the inside of the leaf edge. This illustration was typical of the epidermal surface closer to the leaf tip.

Figure 3. Another area of the epidermal surface close to the vein entrance to the leaf. Note the area is devoid of interior trichomes but shows numerous unidentified cell types (arrows). Also faint images of the cell walls of pavement cells and veins are also imaged.

For comparison, in figure 4 leaves which were immersed in deionized water were similarly examined microscopically. Note that the touch imprint images showed only the trichomes at the leaf edges.

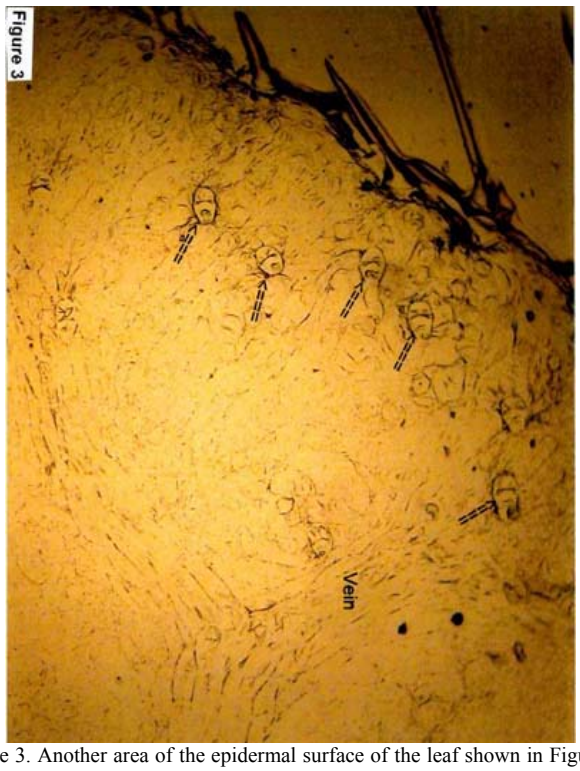


Figure 3. Another area of the epidermal surface of the leaf shown in Figure 2. The area close to the vein entrance is relatively devoid of interior trichomes but shows several of the unidentified cell types. Magnification 40X

## Discussion

### Major findings

The leaves of the Mung bean plant, when isolated and exposed to nano-sized iron particle solutions containing a Prussian Blue stain provided a touch imprint showing images of the cytology associated with the generally known structures of plant tissues. Specifically, we found numerous stomata along the underside of the leaf and other cell types on the epidermal leaf surface.

### Background

The methodology known as Touch Imprint Cytology has become an adjunctive technique competing with frozen section methods for the examination of various types of tumors that need to be studied and diagnosed peri-operatively [1-4]. For comparison with standard histological procedures, for example, "lymph nodes were cut into 3 mm slices… placed on the touch imprint slide, fixed immediately in 95% alcohol and subsequently stained with Hematoxylin and eosin…the corresponding portion…was fixed in formalin and subjected to routine paraffin section and staining…[5].

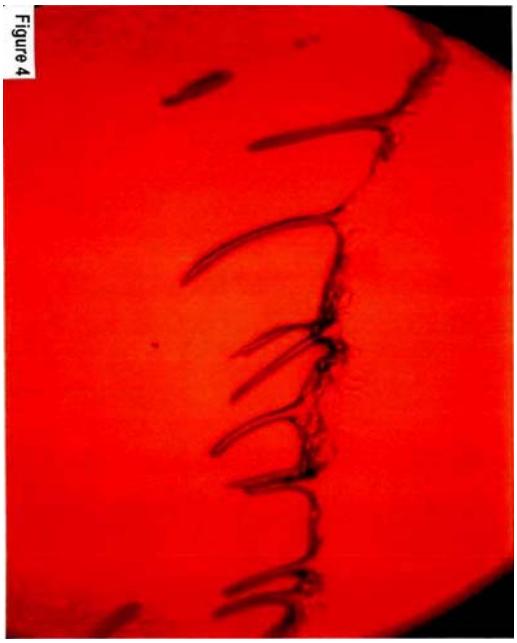


Figure 4. In the control group treated with deionized water, only faint images of the trichomes and leaf edge were seen in the touch imprint. Magnification 40X

In the present study we used aliquots of solutions containing fine iron particles (average size, 2000 nanometers) and PBS. The rationale for this combination was based on our previous studies (unpublished observations, in review). It was demonstrated, both qualitatively and quantitatively, that leaves taken from the Mung bean plant have an affinity for nano-sized iron particles due to inherent electromagnetism related to the electron transport system associated with its metabolism, i.e., photosynthesis and or cellular respiration. Moreover, other reports have emphasized that iron has a particular affinity to plant fiber, specifically to the lignin found in plant cell walls [6-9]. We hypothesize that these two factors account for the aggregated iron particles and wave-like images surrounding the leaf edges and trichomes and the outlines of the guard cells surrounding stomata on the underside of the leaf (figure 1). Similarly, in response to the applied Fe 2000 +PBS, the epidermal surface disclosed heavily outlined trichomes in the interior, their basal epidermal cells as well as the cell wall outlines of the pavement cells of the leaf. In the areas covered by trichomes there were only a few unidentified cell types (arrows, figure 2). However in areas of the epidermal surface where trichomes were sparsely located, these unidentified cell types were prevalent (figure 3, arrows).

Supporting our iron affinity hypothesis, leaves prepared by 1 hour immersion in deionized water and similarly sandwiched between glass slides (control set), as with the experimental set, only showed outlines of the leaf edges and dim images of the associated trichomes (Figure 4). These images were the same from either leaf surface.

**Implications**

This specific method for touch imprint cytology using iron particles and PBS may have potential usefulness in detection and diagnoses of various plant diseases. For example, Blanke and Belcher [10] studying apple leaves grown in vitro, were able to detect deformed stomata which remained open all the time thereby allowing excessive transpiration, i.e., loss of water, resulting in severe dehydration. Although the investigators used scanning electron microscopy to document their finding, a simpler method of touch imprint cytology could be employed for detection of this type of plant pathology. Obviously, the cost and time required would be much reduced.

**Limitations**

We did not apply other types of materials or staining techniques to determine the possible advantages of other methodologies for improving the touch imprint cytological approach. Whether the use of iron nanoparticles (average diameter,≤ 100 nanometers) would affect the image resolution remains a question of interest for those involved in the emerging field of nanotechnology. Also we reviewed the results of the methodology, in the present study, at least 12-24 hour after putting together the glass slide sandwiches containing the iron particle and PBS solutions. It would have to be determined if the images would develop over a shorter time period so that the touch imprint technology could be used in the field.

**Conclusions**

Using leaves from the Mung bean plant which were immersed in solutions of nano-sized iron particles (average diameter, 200 nanometers) for 1 hour and then sandwiched between 2 glass slides. The latter contained a small amount of solution with aliquots of the iron solution and Prussian Blue stain. After 12 to 24 hours, when the solution had dried the leaf was peeled away from the glass slides and the touch imprint images examined under light microscopy and documented as microphotographs. Consistent and distinct images of cytological structures were imaged, such interior and exterior hair cells or trichomes, cell wall outlines of pavement cells and uidentified cell types on the epidermal surface and guard cells surrounding stomata on the underside of the leaf. These images appear to be the result of aggregating iron particles attracted to the inherent electromagnetic energy provided by the metabolizing leaf. Also evidence is presented showing the strong affinity of iron to the lignin comprising the cell walls of the various plant structural components. Controls treated with deionize water showed only dim outlines of the leaf edges and trichomes.

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