WinFOLIA

A Leaf Area Meter, Morphology Analyser, Disease and Insect Damage Quantifier

WinFOLIA is a computer image analysis system that accurately do morphological measurements on broad leaves. It comprises hardware for image acquisition (scanner or digital camera and accessories) and a computer program, WinFOLIA, specifically designed for leaf area, morphology and disease analysis. Different configurations are offered in function of measurements, speed, accuracy and portability.

Image Acquisition Hardware

When portability is not required, we recommend WinFOLIA systems based on high resolution desktop scanners. These devices produce high quality images free of illumination problems (such as non-uniform lighting). Unlike cameras, scanners have a built-in permanent calibration and are very easy to use. We sell different models in function of their scan area (the maximum leaf size they can digitise), speed and accuracy.

Note: Desktop scanners cannot be used in the field but are transportable and usable in remote locations where electrical power is available.

Our lightweight portable scanners can be used for in the field leaf image acquisition. Non-destructive acquisition, while leaves are attached to the plant, is a possibility. Some models operate with batteries and do not require a computer to acquire images (they store images on memory cards). Other models take their power from a portable computer and allow to do in field leaf analysis[1] . Both have a very good image quality and ease of use.

WinFOLIA can analyse leaves or foliar disks. One or more samples can be analysed per image.

1 We recommend to do only basic leaf analysis (area, length, width) in field. Disease analysis is possible but requires a fast computer and we strongly recommend to validate the analysis (look at the computer screen to verify the measurements).
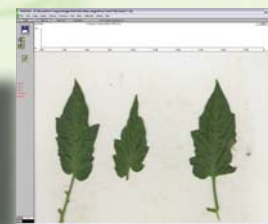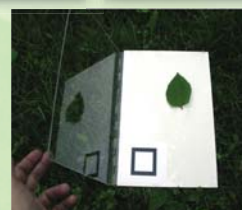
Conventional and inexpensive digital cameras[1] can also be used for leaf image acquisition (in the field or laboratory). Precautions must be taken so that calibration can be done accurately and rapidly. To facilitate this task, we offer the *Leaf image Acquisition Kit (LAK)* for digital cameras. It is made of a plastic background and a translucent anti-reflective fold-up cover that presses the leaves against it to hold them flat. The background can be placed on soil or held in arms for non destructive acquisitions (leaf still on the plant).

The background can also be installed on a base with a camera holder arm which allows to position a camera over it. With this setup it is easier to get the desired field of view and to maintain a constant distance between the camera and the leaves. When this condition is met, the same calibration can be used to analyse a series of images.

The LAK comes with stick-on waterproof removable calibration targets. These targets are pre-defined in WinFOLIA. You enter their dimensions once and calibrating images after is done with a single mouse click.

1 Almost any model can be used. We do not sell or recommend digital cameras.

The WinFOLIA Software Program
It is available in three versions; Basic, Regular & Pro

WinFOLIA Basic is our entry level low cost solution for leaf analysis. This version mimics the functionality of portable leaf area meters. It measures the leaf area, length and width in a simple manner as done with those devices (in the horizontal and vertical directions), perimeter, holes area and some other measurements (see table below).

WinFOLIA Reg has all the *Basic* version's measurements plus some morphological measurements that are specific to leaves. For example, it can differentiate the blade from the petiole so that you can choose to have the petiole included or not in the measurements of area and length. It can measure width in more different ways and lobe angles also. This version also has more features like the possibility of adding observations, doing user defined interactive measurements and a few more.

WinFOLIA Pro has all the *Reg* version's measurements plus some extended morphological measurements (such as Fractals) some of which are specific to leaves (envelope, teeth...). This version also has color analysis that can be used for disease, necrosis and insect damage quantification. It allows you to choose the region of the image to analyse as well.

Measurements per WinFOLIA Software Version

The *Reg* and *Pro* versions can measure the area and length of the blade and the petiole separately or together (as a whole). All versions can produce total and average leaf area (for all leaves in the image) as well as individual areas (per leaf). All versions can measure the leaf perimeter (blade+petiole) but only the *Reg* and *Pro* versions can measure the blade perimeter without the petiole. The analysed region area is also available in the Pro version (this allows to measure the area of any object manually simply by tracing it in the image).

Measurements	Version		
	Basic	Reg	Pro
• Total Leaf Area	•	•	•
• Individual Leaf Area	•	•	•
• Leaf Length	•	•	•
• Maximum & Average Width (horiz. & vertical)	•	•	•
• Leaf Perimeter	•	•	•
• Aspect Ratio	•	•	•
• Form Coefficient	•	•	•
• Holes Area (Total & Individual)	•	•	•
• Individual Blade Area	–	•	•
• Blade & Petiole Length	–	•	•
• Maximum & Average Width (perp. to length)	–	•	•
• Blade Perimeter	–	•	•
• Blade Width at User Definable Positions	–	•	•
• Lobe Angle at User Definable Positions	–	•	•
• User Definable Lengths & Angles (Interactive)	–	•	•
• Analysed Region Area	–	–	•
• Envelope Area	–	–	•
• Teeth Height, Width and Count	–	–	•
• Fractals (for leaf shape analysis)	–	–	•
• Color Analysis (Area per Color, Diseases)	–	–	•

• = yes, – = no

Leaf or blade Maximum Length and Width can be measured in different ways. The simplest method is the one used by hand-held leaf area meters and is available in all versions of WinFOLIA. Leaf length is measured as the largest distance between the highest and lowest points belonging to the leaf in the vertical direction (on the same vertical line) and width is the distance between the leftmost and rightmost points in the horizontal direction (on the same horizontal line). Length and Width can also be measured as the vertical and horizontal size of the leaf bounding box (distance between the leaf leftmost and rightmost boundary points), but unlike the above method, these do not have to be on the same line. Average width is the average of all widths measured along the leaf.

With the *Reg* and *Pro* versions, Length can be measured as the distance between the apex and petiole-blade junction (any orientation close to vertical) and Width as the largest width perpendicular to blade length. These versions can also measure width at two different positions that you specify along the blade length.

Aspect ratio is the ratio of horizontal width to vertical length (it is sometimes called elongation factor).

Form coefficient is a numerical value which grade the leaf shape as between circular (shortest perimeter for a given area) and filliform (longest perimeter for a given area).

Total and individual holes areas. Holes completely inside the blade (enclosed by it) are automatically measured. Those on the edge of it must be manually closed with image edition (as illustrated on next page) to be measured. You can fill the hole with a different color or close the blade edge's boundary.

Blade lobes angle can be measured by the *Reg* and *Pro* versions at two different positions along the blade length.

Interactive user-definable lengths and angles allow to define custom measurements (that WinFOLIA cannot do automatically). As an example, it can be used for measuring veins angle.

The envelope is the length of a line that goes from tooth to tooth on their apex. The area covered by the envelope can be measured by WinFOLIA Pro.

Teeth height, width and count.

Tooth (*yellow*)

Envelope (*magenta*)

Fractals are a mean of quantifying the complexity a leaf shape.

WinFOLIA Pro can quantify the area per color, measure its dominant one and classify objects in function of it or other criteria (it can classify a leaf as diseased if the diseased color group has the largest area or is larger than a percentage that you specify). The leaf color classification can be seen on screen. Colors of interest are specified to WinFOLIA simply by clicking the image. Pixels of the image are classified into classes in function of their color. Classes can be grouped in order to measure areas with more than one color (for example diseased area may contain brown and yellow colors). The classification is done by true color analysis in 24 bits color space and not using a simple color threshold as in some other leaf analysis programs.

*Note: For color analysis without leaf morphology, see our WinCAM program.*

Features per WinFOLIA Software Version

•Image edition allows to modify the image content to remove artifacts, debris or, as illustrated below, to fill in holes. You can choose the color to edit with by picking a color already present in the image or define your own.

In this example a damaged leaf boundary is closed with image edition using the pen tool so that holes on its edge can be measured automatically by WinFOLIA.
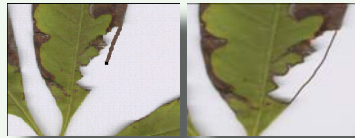
In this example a damaged leaf area is filled in brown using image edition with the brush tool (a lasso is also available). Drawing a region the same color as the leaf allows to include it in leaf area while drawing it a different color allows to measure it in the holes or background area.
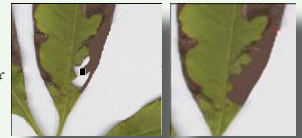
•An observation is a mean of adding measurements not programmed in WinFOLIA or to indicate irregularities observed during measurement. A simple observation could be something like: *Is Damaged*.

•Although WinFOLIA is optimised for leaves, it can also analyse the area and shape of other objects such as foliar disks. For morphology or color analyses of generic objects (other than leaves) see our WinCAM program.

•Debris filtering is available to prevent small particles, such as dust, from being considered as leaves during automatic analyses. Objects with an area larger or smaller than specified values can be removed from the analysis.

•Batch acquisition optimizes speed and minimizes operator intervention during the image acquisition process. Batch analysis allows to analyse series of images without operator supervision. *Note*: Not all measurements can be done in batch.

Features	Version		
	Basic	Reg	Pro
• Image edition	•	•	•
• Observations	-	•	•
• Leaf or Foliar disk analysis	•	•	•
• Debris filtering	•	•	•
• Interactive or batch analysis	•	•	•
• Interactive or batch image acquisition	•	•	•
• Leaf distribution graphic	-	•	•
• Remove background light variations	-	-	•
• User can override pixels classification	-	•	•
• Select the color channel to analyse	-	-	•
• Analyse whole images/sub regions	•/-	•/-	•/•
• Exclusion regions	-	-	•

• = yes, - = no

•View during the analysis the leaf area (or other morphological parameters) distribution into classes that you choose in a graphic above the image. You can also view the color area distribution (*Pro* version).
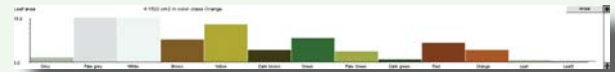
•Background light variations occur when lighting is not uniform, a problem that is encountered with camera based images not with scanners. These variations can cause some wrong pixels classifications (see below). A function is provided to minimise or eliminate them but is effective in laboratory situations. It is more efficient and better to eliminate light variations using proper lighting techniques during image acquisition.

•The possibility of analysing only a part of the images adds flexibility (*Pro* version). It allows to select specific regions of interest to be analysed and to ensure that only leaves are analysed (no debris, text written close to leaves or objects other than leaves).
These regions can have any shape (circular, rectangular or any irregular shape made with the lasso tool). Similarly, you can define regions to be excluded from the analysis. Analysed and exclusion regions can be resized, moved and recreated (to analyse the same region over time or for different

•The Pixels classification is the separation of image pixels (picture elements) into the leaf and background groups (a prerequisite for accurate measurements). This process can be done automatically (with user validation using different methods in function of the WinFOLIA version) or manually. If there are wrong classifications in some image areas, the operator can override it interactively by first selecting that area (outlining it) and changing the classification parameters.

•Some features can be more easily detectable (have higher contrasts) in a specific color channel (Red, Green or Blue) of a color image. This channel, rather than all of them, can then be used to make the pixels classification and the measurements.

Miscellaneous

Data produced by WinFOLIA are saved in standard ASCII text files. They can easily be opened by statistical or spreadsheet programs such as Microsoft Excel. The analysis settings are saved with the measurements data (useful to make verifications or quality control on the measurements). Furthermore, when an image is saved to a *tiff* file after it has been analysed, the analysis is also saved in it and when such file is later opened from WinFOLIA, the analysis is recreated as it was at saving time.

WinFOLIA comes with printed color manuals which are abundantly illustrated. Like all of Regent Instruments' products, it is backed with prompt and competent technical support by email (messages are typically answered within one hour when they are received on working hours Eastern time America). WinFOLIA is updated regularly (typically once a year) to add new features or simply to keep it on par with new operating systems as they become available.

WinFOLIA has a companion program called WinSEEDLE for conifer needles and seeds analysis. Both programs can share the same image acquisition hardware (you must specify it at purchase time). A rebate is offered when both products are purchased together. WinFOLIA and WinSEEDLE are members of a family of products sold by Regent Instruments for plant science research and analysis. Among the other products you will find WinRHIZO for root analysis and WinDENDRO for tree ring analysis. See our web site for more information.
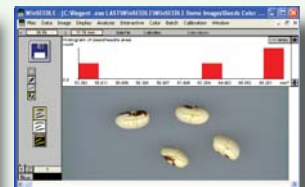
WinFOLIA is Available as Software Alone or as Complete System

Scanners; Model Selection and Image Transfer to WinFOLIA

WinFOLIA uses the TWAIN[1] protocol to communicate with scanners and get images directly from them (without saving them to disk) in a very fast process. There are many versions of TWAIN and some features are not mandatory for a scanner to be TWAIN compliant. Regent Instruments cannot test all scanners and drivers available on the market so we do not guarantee that they will be compatible with all features of WinFOLIA. We have tested and selected a few models that are representative of their categories and we offer them for sale with WinFOLIA as complete systems (WinFOLIA can also be purchased as software alone). These models meet the requirements for precise scientific work and we give them a speed rating to help you choose one. There are important differences in terms of image quality and speed among different models. We retain those that are robust, reliable, will last long and which come with good warranty support from their manufacturer. With all scanner models it is possible to scan leaves using the scanner manufacturer program, save the image to disk and then open and analyse those images in WinFOLIA[2]. This process is slower than TWAIN because it involves two extra steps.

Although WinFOLIA can obtain and analyse images directly from many scanners, it is advantageous to purchase one from Regent for the reasons given here.

We calibrate the scanners we sell for precise measurements. Scanners are mainly made for artistic applications. When you set it to scan at 400 dpi, it might in reality scan at 392 or 410 dpi. This is what our calibration compensates for. Our calibration is permanent and is automatically used by our programs to compensate for differences among scanners so that data produced by different models or different

1 *TWAIN drivers are installed in your Windows operating system when you install the scanner manufacturer software programs.*
2 *Files must be in tiff uncompressed grey levels (8 bits/256 grey levels) or colors (24 bits/millions of colors) or jpeg format.*

units of the same model will give similar results. Our calibration also compensates for differences when using a single scanner under different conditions such as when measuring an object at various orientation on its scan area and, for dual lenses scanners, when using different lenses.

Our scanners come with additional instruction manuals that explain how to install the scanner with our accessories and how to scan biological samples for analysis with our programs. These help you to get the best images from it and to save time by giving some tips that are specific to the scanner that you have purchased (optimising it for speed vs. accuracy or avoiding frequent beginners errors).

We know the scanners we sell for using them in the same conditions as you with WinFOLIA so we can easily help in case of problems. Because testing scanners is a continuous non-neglectable time and money investment, we do not disclose the scanner models we sell, neither do we give our opinion on specific models. If you prefer to buy a scanner locally, we suggest to test it before purchase and to select a reseller that will be able to assist you after as Regent Instruments will not provide technical assistance for scanners not purchased from us.

Our scanners also come with their manufacturer's software program(s) which allow to use them for other generic applications (object, photo, slide or document scanning).

Digital Cameras; Model Selection and Image Transfer to WinFOLIA

Some reasons for using cameras instead of scanners are that they can take images of larger objects (albeit at lower resolution), are (most of the time) more portable or simply because you might already have some at hand (they are quite cheap and common these days). WinFOLIA can open and analyse *jpeg* images files produced by almost any digital camera (*RAW* files do not work).

The fastest way to measure leaf area and morphology in the field is when the computer can get instantaneous image transfer from the camera to it. This is possible if your camera has a TWAIN driver (this is rare) or that it can be remotely controlled from the computer (with the camera manufacturer software) and transfer images directly to it via a wire (such as USB2) or a wireless (WiFi) connection. In those cases, WinFOLIA can analyse images as soon as they are saved to disk (or directly from the camera if a TWAIN driver is available).

It is also possible to transfer one or more images at a time using the following methods:

1) Remove the memory card from the camera and insert it in a card reader connected to the computer (some computers have built-in card readers). The memory card appears as a removable disk on Windows computers. The image files they contain can be opened from WinFOLIA or they can be transferred from the card to the computer hard disk prior to this.
2) Many digital cameras can be mounted as a removable disk simply by connecting them to the computer (after installation of their manufacturer drivers). WinFOLIA can access these images directly or you can transfer them to the computer and open them from there.
3) You can use the software program that came with the camera to transfer images in lot and analyse them later in WinFOLIA.

WinFOLIA can analyse images interactively (one after the other) or in batch (without operator supervision)[1]. This will work if you have the right calibration setup and no unwanted objects in the image (feet, debris, etc....). This allow to acquire images rapidly in the field and to analyse them later in the lab (with or without supervision).

1 Not all analyses can be done in batch (envelope and teeth for example).

For a list of scientific publications in which WinFOLIA was used or for application examples, please refer to:
www.regentinstruments.com/pages/References.html

To get the latest technical information, visit our web site at: www.regentinstruments.com
To place an order or for more information, please contact: sales@regentinstruments.com

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